https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Fertilizer regimes reshape microbial interaction networks without altering sugarcane rhizosphere diversity

Leonardo Zamora-Leiva[1], Jorge Soto[1], Ignacio Ramos-Tapia[2], Manuel Paneque[3]*

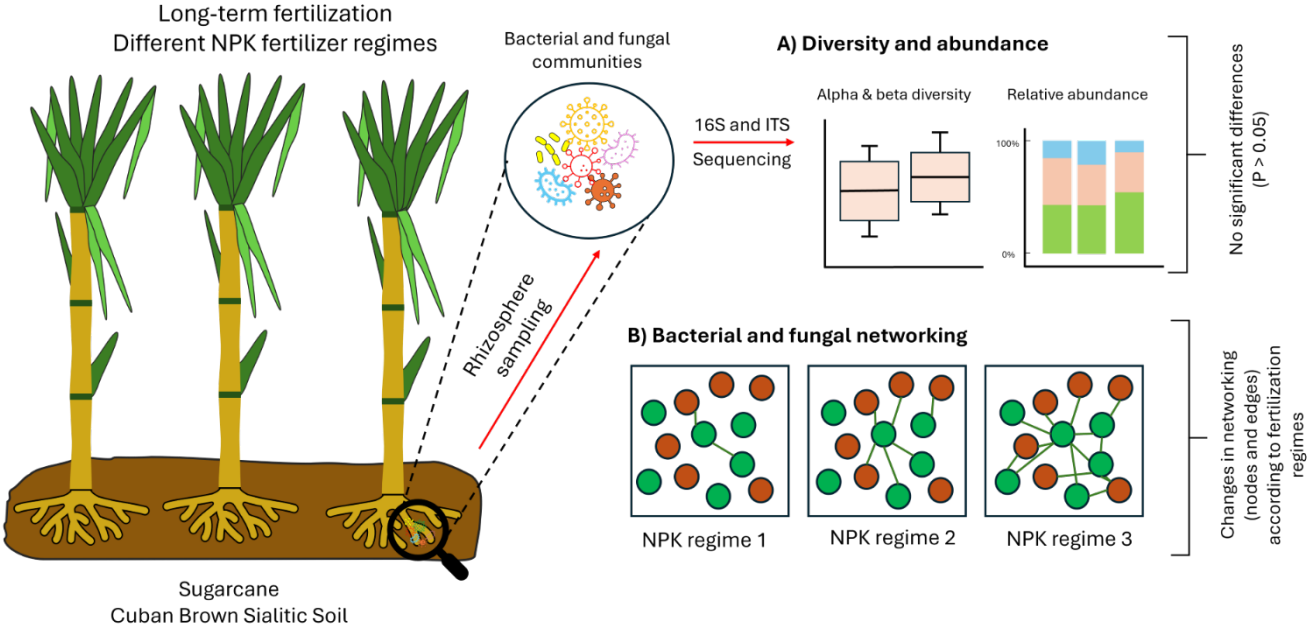
[1]Laboratory of Metagenomics, Bionostra Chile Research Foundation, San Miguel, Santiago, Chile; lzamo-ra@bionostra.com; jsoto@bionostra.com
[2]Center for Bioinformatics and Integrative Biology, Facultad de Ciencias de la Vida, Universidad Andrés Bello, República 330, Santiago, Chile; ramostapiai@gmail.com
[3]Laboratory of Bioenergy and Environmental Biotechnology, Department of Environmental Sciences and Natural Resources, Faculty of Agricultural Sciences, University of Chile, La Pintana, Santiago, Chile

Correspondence to: Manuel Paneque (mpaneque@uchile.cl); Tel.: +56-22-978-5863

Abstract. Sugarcane (*Saccharum* spp.), an economically important crop in the food and bioenergy industries, has historically played a central role in the economy of Cuba, shaping its agricultural landscape and international relations. Although production has declined in recent decades, sugarcane remains a strategic crop, with its byproducts contributing to national energy and industrial outputs. However, the way in which long-term fertilization interacts with soil microbial communities under varying edaphoclimatic conditions remains largely unknown. Here, we investigated a traditional sugarcane plantation to evaluate how distinct fertilizer formulations varying in nitrogen, phosphorus, and potassium affect the soil and rhizosphere microbiota. Using high-throughput sequencing of 16S rRNA (bacterial) and internal transcribed spacer (fungal) genes, we identified 421 bacterial and 471 fungal genera from 5,741 amplicon sequence variants across different fertilization regimes. While microbial composition and diversity did not differ significantly between treatments, co-occurrence network analysis showed clear nutrient-specific patterns. This indicated that each fertilizer regime shaped distinct interaction networks among microbial taxa. These shifts suggest modifications in soil and rhizosphere functioning linked to nutrient availability rather than to taxonomic turnover alone. The findings provide a detailed characterization of the rhizosphere microbiome of *Saccharum* spp. in brown sialitic soils (inceptisol), offering ecological insights into its bacterial–fungal associations. This highlights the importance of understanding how long-term fertilization regimes influence rhizosphere microbial dynamics, which is key to designing more sustainable soil management and fertilization practices in sugarcane production systems.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Graphical Abstract



1 Introduction

Cultivated across tropical and subtropical regions for sugar and bioethanol production, sugarcane (*Saccharum officinarum* L.) is one of the most important industrial crops worldwide (Cherubin et al., 2018; Otto et al., 2022). Sugarcane is predominantly cultivated as a monoculture under intensive management systems that rely on long-term fertilization practices (Cherubin et al., 2018; Dinesh Babu et al., 2022; Khan et al., 2023) due to its high biomass productivity and nutrient demand. This results in cultivation that requires substantial inputs of nitrogen, phosphorus, and potassium fertilizers (Cherubin et al., 2019). Sugarcane is primarily produced in Brazil, accounting for 38% of global production, followed by India (23%) and China (5%) (Ritchie et al., 2023). Farmers worldwide have indiscriminately applied nitrogen fertilizers to ensure high crop yields, thereby emitting nitrous oxide into the environment (Tian et al., 2020). To increase nutrient availability, accumulation, and use efficiency in the current sugarcane crop system, new fertilization management strategies are needed. In several production systems, fertilizer application is one of the highest economic and environmental costs of sugarcane farming. This has strong implications for greenhouse gas emissions, soil acidification, and nutrient leaching (Wang et al., 2024). Therefore, assessing the interactions between fertilizer regimes and soil microbial communities in sugarcane agroecosystems has become a key priority (Y. Liu et al., 2024). In Cuba, sugarcane cultivation has historically relied on fixed-dose fertilization schemes, primarily involving nitrogen, phosphorus, and potassium (FAO, 2003). Even when these schemes account for soil heterogeneity and plant nutrient demands, sugarcane productivity in Cuba has declined due to inadequate attention to the crop, shortages of fuel and herbicides,

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

reduced water availability, and other limitations (FAO, 2003, 2025). Fertilization regimes often do not consider microbial interactions, which can influence ecosystem functioning (Z. Guo et al., 2020). Nutrient over- or under-application may occur, leading to reduced yields or environmental problems. This can include soil contamination through nutrient leaching and a loss of ecosystem services due to reduced microbial diversity (Berger et al., 2024; Du et al., 2025).

Closely related to fertilization systems, soil microbial communities are central regulators of terrestrial ecosystem functioning. They play essential roles in nutrient cycling, organic matter turnover, soil structure, and plant health (Du et al., 2025). Different ecosystems, including tropical agroecosystems, can host highly diverse microbial communities adapted to specific edaphoclimatic environments (Tripathi et al., 2016; J. Guo & Chen, 2022; Zamora-Leiva et al., 2025; Ramos-Tapia et al., 2023). These microbial consortia are particularly relevant because they directly influence nutrient availability, plant productivity, and resilience to environmental stress (Antoszewski et al., 2022; Kumar et al., 2023; Nuñez-Salazar et al., 2020; Ramos-Tapia et al., 2022; Salazar et al., 2020; Souza et al., 2025). Agricultural practices, including tillage, crop rotation, irrigation, and fertilization regimes, can have strong selective pressures on soil microbiomes. This can lead to shifts in microbial community composition and functionality (Q. Liu et al., 2021; Orrù et al., 2021; Sui et al., 2023). Climatic conditions, such as high rainfall and intense weathering, which are common in tropical ecosystems, often result in soils that are nutrient-poor, acidic, and highly leached, with low cation exchange capacity (Oishy et al., 2025). Therefore, understanding how soil microbial communities respond to different fertilization strategies is crucial to optimizing sustainable crop management and maintaining soil health.

Although continuous monocropping, combined with sustained fertilizer inputs, can strongly influence soil health and microbial community structure, the effects are not easily predictable. Long-term fertilization can produce contrasting outcomes, ranging from enhanced nutrient cycling to reduced microbial diversity and ecosystem resilience (Pan et al., 2014; Kracmarova et al., 2022; S. Chen et al., 2023; Akter et al., 2025). Nitrogen fertilization has been associated with decreases in microbial alpha diversity, community shifts toward copiotrophic taxa, and reductions in nitrogen-fixing microorganisms (Sui et al., 2023). Phosphorus and potassium amendments affect fungal communities, particularly arbuscular mycorrhizal fungi, which are highly responsive to changes in nutrient availability (Semenov et al., 2022; Yang et al., 2025). This indicates that fertilizer regimes can shape soil microbial assemblages in ways that alter nutrient cycling processes, soil–plant interactions, and ecosystem sustainability (de Castro et al., 2022; Mitter et al., 2021). However, the response of soil microbial communities to fertilization is not uniform across ecosystems and is highly context-dependent (Kracmarova et al., 2022; Moneda et al., 2022). Factors, such as soil type can influence it (Huang et al., 2025), climate (Du et al., 2025), cropping history (Pan et al., 2014), and management intensity (Suman et al., 2022). In certain cases, microbial communities show considerable resilience, maintaining similar diversity and composition across a wide range of fertilization treatments (Philippot and Langenheder, 2021). This variability complicates extrapolating results from one agroecosystem to another, underscoring the need for site-specific studies (Souza et al., 2025).

Microbial communities are commonly studied through high-throughput sequencing techniques (Lahlali et al., 2021). These technologies have substantially expanded our ability to explore the structure and function of microbial communities in

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

environmental samples, describing the taxonomic composition and relative abundance of microbial taxa across diverse ecosystems (Lahlali et al., 2021). However, while attention has been traditionally focused on how community composition and diversity shift under different environmental or management conditions, an equally important aspect lies in the relationships among microbial taxa (Zamkovaya et al., 2021). These interactions, whether cooperative, competitive, or neutral, shape the functional stability and resilience of microbial ecosystems (Freilich et al., 2011). Network-based approaches are powerful tools to explore these relationships, allowing the identification of co-occurrence patterns, ecological clusters, and potential keystone taxa that play central roles in ecosystem functioning (Shaw et al., 2020). Integrating network analysis with community profiling can provide a deeper understanding of how microbial communities respond to environmental factors, such as fertilization regimes (Gao et al., 2022).

In the context of Cuban agriculture, sugarcane is largely cultivated on brown sialitic soils, which are mineral-rich soils with relatively underexplored microbiological dynamics. Understanding how fertilization interacts with local edaphoclimatic conditions is essential for improving sustainable management strategies and maintaining soil function in tropical agroecosystems. However, traditional fertilization recommendations in Cuba, such as those from SERFE (Servicio de Recomendaciones de Fertilizantes), primarily focus on crop nutritional requirements and do not explicitly account for soil microbial processes. Notably, available yield records from the long-term fertilization trial, established in 1998 at the Sugar Cane Research Institute in Santiago de Cuba, indicate that while fertilized plots consistently outperform non-fertilized controls, sugarcane yield tends to plateau across a wide range of NPK fertilization regimes, with limited differences among moderate and high input treatments (Supplementary table 1, Tamayo-Isaac et al., 2024). This apparent decoupling between fertilizer dose and yield response suggests that factors other than nutrient availability alone may regulate system functioning under long-term management. We therefore hypothesized that sustained fertilization under tropical conditions leads to physicochemical homogenization of these soils, generating changes in microbial community composition and promoting nutrient-specific reorganization of microbial interactions within the rhizosphere. To test this hypothesis, we leveraged the long-term fertilization trial established in 1998 at the Sugar Cane Research Institute in Santiago de Cuba, which provides a rare opportunity to assess the legacy effects of sustained fertilization on soil microbial network structure. Using high-throughput sequencing of bacterial (16S rRNA gene) and fungal (internal transcribed spacer; ITS region) markers, we evaluated how gradients of nitrogen, phosphorus, and potassium (NPK) inputs shape both microbial composition and co-occurrence networks in rhizosphere and bulk soil samples.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

2 Materials and methods

2.1 Experimental design and sample collection

2.1.1 Profile of the experimental site

The long-term experiment, encompassing different mineral fertilization rates, was established in 1998 on sialitic brown soil at the *Estación Territorial de Investigaciones de la Caña de Azúcar* (ETICA) *Oriente Sur*. It is located in the municipality of Palma Soriano in the province of Santiago de Cuba (Sieiro & Pablos, 2021). Sialitic brown soils have moderate organic matter content, high clay fraction, and a dominance of exchangeable Ca^{2+} and Mg^{2+}. These properties contribute to a relatively stable pH and high cation exchange capacity. The area is characterized by a humid tropical climate, with an average annual rainfall of 1,375 mm, and two well-defined periods: rainy (May–October) and dry (November–April) (Hernández-Jiménez et al., 2019). The study reproduced the traditional sugarcane cultivation system used in Cuba, with manual planting and harvesting, as well as replenishment cycles, after five cuts. In the present study, the third cycle was underway, and the second ratoon vine was planted in spring with cultivar C86-12.

2.1.2 Experimental design

The completely randomized block experiment included 11 treatments, with different co-concentrations of nitrogen, phosphorus, and potassium, all expressed in kg/ha (Table 1): (i) N0 (N0P25K50), no nitrogen application; (ii) N1 (N50P25K50), as a basal application of nitrogen, phosphorus and potassium; (iii) N2 (N100P25K50), medium nitrogen application; (iv) N3 (N150P25K50), high nitrogen application; (v) N4 (N200P25K50), very high nitrogen application; (vi) N5 (N50P0K50), no phosphorus; (vii) P1 (N50P75K50), medium phosphorus application; (viii) P2 (N50P100K50), high phosphorus application; (ix) K1 (N50P25K100), medium potassium application; (x) K3 (N50P25K200), high potassium application; and (xi) K4 (N50P25K300), very high potassium application.

Table 1. Concentration of fertilization for the treatments

Sample	Characteristic	Nitrogen	Phosphorus	Potassium
N0	Non-nitrogen	0	25	50
N1	Basal treatment	50	25	50
N2	Medium nitrogen	100	25	50
N3	High nitrogen	150	25	50
N4	Very high nitrogen	200	25	50
N5	Only nitrogen	50	0	0
P1	High phosphorus	50	75	50
P2	Very high phosphorus	50	100	50

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

K1	Medium potassium	50	25	100
K3	High potassium	50	25	200
K4	Very high potassium	50	25	300

Derived from the experimental design, three comparison groups were generated to evaluate different parameters (Table 2): (i) effect of the increase in nitrogen, (ii) effect of the increase in phosphorus, and (iii) effect of the increase in potassium.

Table 2. Fertilization regimes and comparison groups

Sample	Characteristic	Nitrogen (N)	Phosphorus (P)	Potassium (K)
N changes – P+K do not change				
N0	Non-nitrogen	0	25	50
N1	Basal treatment	50	25	50
N2	Medium nitrogen	100	25	50
N3	High nitrogen	150	25	50
N4	Very high nitrogen	200	25	50
P changes – N+K do not change				
N1	Basal treatment	50	25	50
P1	High phosphorus	50	75	50
P2	Very high phosphorus	50	100	50
K changes – N+P do not change				
N1	Basal treatment	50	25	50
K1	Medium potassium	50	25	100
K3	High potassium	50	25	200
K4	Very high potassium	50	25	300

Two controls were considered, namely, soil without planting (control 1) and rhizosphere without fertilization (control 2). Each treatment was repeated three times, and each replicate was 48 m^2. The chemical fertilizers applied were urea (46% N), calcium superphosphate (12% P_2O_5), and potassium chloride (60% K_2O). All fertilizers were applied at the bottom of the furrow before planting and 20 d post-harvest, manually and without fractioning doses (INICA, 1990). The evaluated plot had received three fertilizer applications by the sampling date, with planting in spring 2020 and sampling in March 2022, corresponding to the initial application plus those made after each cutting/ratoon.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

2.1.3 Soil sampling

These were shaken to remove excess soil not adhering to the roots. The samples were stored in 50 mL Falcon™ tubes (Thermo Fisher Scientific, Waltham, MA, USA) and transported to the laboratory (Zamora-Leiva et al., 2025). The tubes were immediately sealed to avoid environmental contamination, labeled, and stored at 4 °C during the laboratory transport (Ramos-Tapia et al., 2022). Enriched soil samples were obtained from each experimental plot for chemical analysis and soil respiration, developed by ETICA (Tamayo-Isaac et al., 2023, 2024). The sampling was conducted in March, which is one of the driest months (Centella et al., 2006; Durán, 2012). For physicochemical parameters, pH in water and KCl was measured with potentiometric method (soil:solution ratio 1:2.5); organic matter with Walkey–Black colorimetric method, digestion with H_2SO_4, $K_2Cr_2O_7$ 1N (ox.) and $FeSO_4$ x7 H_2O 0.5 N (red.), using Vam Bemmen's factor (1.724) to convert carbon into organic matter; assimilable phosphorus as P_2O_5 mg/100g with Oniani method, extraction with 0.1 N H_2SO_4, soil:solution ratio 1:2.5, determination by colorimetry; assimilable potassium as K2O mg/100g with Oniani method, extraction with 0.1 N H2SO4, soil:solution ratio 1:2.5, by flame photometry; and exchangeable bases (K+, Ca2+, Mg2+, Na+) as cmol(+)/kg with extraction with 1N NH_4OAc at pH 7, determining calcium and magnesium by EDTA titration, sodium and potassium by flame photometry (INICA, 1990; Tamayo-Isaac et al., 2024).

2.1.4 DNA extraction, PCR, and sequencing

For each sample, genomic DNA was extracted from a total of 0.5 g of fresh soil using the DNeasy Powersoil DNA isolation kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA concentration and purity were checked with a Qubit dsDNA HS Assay kit and Qubit 4.0 fluorometer (Thermo Fisher Scientific). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified with the primers 5'-CCTACGGGGGNGGCWGCAG-3' and 5'-GACTACHVGGGTATCTAATCC-3' (Klindworth et al., 2013). Meanwhile, for the fungal ITS1 and ITS2 regions, the primers 5'-CTTGGTCATTTAGAGGAAGTAA-3' and 5'-GCTGCGTTCTTCATCGATGC-3' were used (Orgiazzi et al., 2012). Amplification and bulk sequencing were performed with Illumina MiSeq technology by Austral-Omics (Universidad Austral de Chile). The sequencing process was performed with the Miseq Reagent v3 600-cycle kit (2× 250 pair-end). The sequences were imported as FASTQ files with forward and reverse for each sample.

2.1.5 Bioinformatic analysis

The FASTQ files were processed using QIIME 2 v2025.7 (Caporaso et al., 2010). Read quality was evaluated using FastQC v0.11.9 (Andrews, 2010). Based on this evaluation, the adapters were removed, and the lengths of the forward and reverse sequences were trimmed for bacterial and fungal sequences at 260 and 205 bases, and 250 and 220 bases, respectively, using the DADA2 denoise-paired plugin (Callahan et al., 2020). Quality was maintained using PHRED 20 (Callahan et al., 2020). Taxonomic assignment was performed using the classify-sklearn plugin in the SILVA database version 138.2 (Quast et al., 2013) and UNITE database version 10.0 (Abarenkov et al., 2024), for bacterial and fungal classification, respectively, both

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

preprocessed with RESCRIPt (Robeson et al., 2021). New unified Amplicon Sequence Variant (ASVs) with 99% identity were aligned using MAFFT (Katoh & Standley, 2013). The maximum likelihood was inferred using FastTree v2.2.0 (Price et al., 2009). The abundance, taxonomy, phylogeny, and metadata of the ASVs were integrated into a *Microeco* object for subsequent analyses using the *Microeco* v1.15.0 R package (C. Liu et al., 2021), in R v4.3.2 (https://www.r-project.org). ASVs assigned to archaea, mitochondria, chloroplasts, metagenomes and ambiguous taxa were deleted, as well as fungal ASV on bacterial analysis, and bacterial ASVs for fungal analysis. Samples with <1,000 reads were excluded. ASVs that were not observed more than twice in at least 10% of the samples were excluded (Callahan et al., 2017). Samples were analyzed with both unassigned ASVs removed and retained. The *Microeco* object was used to calculate alpha and beta diversities and for constructing relative abundance plots (C. Liu et al., 2021). Alpha diversity was calculated using Chao1, that is, an estimate of richness by species, observed features—species richness on samples, Shannon—entropic information on abundance of observed ASVs, and Simpson (1-dominance) indices. To determine beta diversity, all samples were compared across sampling points using the Bray–Curtis, Canberra, Jaccard, Robust Aitchison, Unifrac, and weighted Unifrac matrices. Significant differences were evaluated with Permutational Multivariate Analysis of Variance (PERMANOVA) and Kruskal–Wallis tests. ASVs were transformed into proportions, and relative abundance was used to measure the composition of prokaryotic communities at the phylum, class, order, family, and genus levels. All the samples were rarefied according to the minimum sample size of 11,248 and 18,829 for bacteria and fungi, respectively. The differential abundance of taxa was analyzed by implementing linear discriminant analysis effect size (LEfSe, LDA value > 2, $\alpha = 0.05$), supported with an Analysis of Compositions of microbiomes with Bias Correction 2 (ANCOM-BC2) analysis.

For the co-occurrence network analysis, the bacterial and fungal datasets were merged. To account for differences in sequencing depth, the complete dataset was rarefied to the lowest sample size, i.e., 11,248 reads per sample, using the trans_rarefy function from the *Microeco* R package. Co-occurrence networks were constructed using the cal_network function in *Microeco*, an R package that implements the *igraph* v2.1.4 R package. The Spearman correlation method was used to compute pairwise correlations between taxa (Looney & Hagan, 2011). Network modularity was defined as the extent to which a network is organized into cohesive subgroups of nodes (modules), with higher within-module connectivity than between modules. Modules were detected using the cluster_fast_greedy algorithm, and modularity values were computed with the modularity function in the *igraph* R package. Network hubs were identified based on within-module connectivity (Zi) and among-module connectivity (Pi) using the get_node_table function from the *Microeco* R package. The network was constructed by retaining only significant positive co-occurrences between taxa and visualized using Gephi v0.10.1 (Bastian et al., 2009).

To compare the structure and composition of microbial co-occurrence networks among fertilization regimes, individual networks were constructed for each treatment (Table 2). Each network was built following the same rarefaction, correlation, and filtering criteria described above, using the cal_network function from the *Microeco* R package. The networks were constructed as undirected graphs using the cluster_fast_greedy modularity optimization algorithm to ensure comparability across treatments. Overlap and unique taxa among treatments were assessed using the *meconetcomp* v0.6.1 R package. The

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

node_comp and edge_comp functions from the *Microeco* R package were used to identify shared and exclusive nodes and connections (edges) among ASVs across treatment-specific networks. The resulting intersections were summarized and visualized as a Venn diagram.

3 Results

3.1 Physicochemical characterization of brown sialitic soils under different fertilization regimes

The physicochemical properties of soils across fertilization treatments are summarized in Table 3. The data showed minimal variation among treatments, suggesting strong homogenization of soil conditions across the experimental site. Soil pH values remained nearly constant, ranging from 6.4 to 6.5 (HCl) and 7.7 to 7.9 (H_2O). This indicates a slightly acidic to neutral reaction typical of sialitic soils. This stability is consistent with the buffering capacity conferred by high levels of exchangeable calcium (58–69 cmol(+)/kg) and magnesium (0.4–11 cmol(+)/kg). The Ca/Mg ratios fluctuated only slightly among treatments, reinforcing the notion of a chemically equilibrated soil system. Organic matter (OM) content varied between 2.9 and 3.9%, without clear trends associated with fertilization. Similarly, available phosphorus (P_2O_5) and potassium (K_2O) concentrations exhibited broad ranges (P_2O_5: 63–139 mg/100g; K_2O: 25–88 mg/100g). However, these variations did not translate into distinct patterns of nutrient enrichment in response to applied fertilizers. This lack of correlation suggests that rapid nutrient turnover or leaching, potentially driven by intense rainfall and the clay-rich matrix of sialitic soils, may diminish the effect of fertilization inputs.

Table 3. Physicochemical characterization of sites

Treatment	Characteristic	Microbial respiration (mg CO_2)	P_2O_5 (mg/100g)	K_2O (mg/100g)	pHCl	pH H_2O	Ca	Mg
Control	Bulk soil	1,650.70	78.13	32.4	6.51	7.94	49.6	11.2
	(no	1,655.60	83.05	33.12	6.55	7.85	49.8	11.1
	rhizosphere)	1,657.90	80.59	32.76	6.53	7.89	49.4	11.1
Blank	Rhizosphere	1,914.18	63.77	29.88	6.5	7.92	50.6	10.2
	(no	1,912.75	63.57	32.76	6.45	7.78	50.3	10.2
	fertilization)	1,911.51	71.24	34.21	6.47	7.79	50.7	10.3
N0	$N_0P_{25}K_{50}$	1,933.42	93.48	38.34	6.44	7.76	64.9	2.55
		1,930.74	76.95	32.04	6.48	7.79	64.4	2.56
		1,931.43	73.41	29.88	6.52	7.87	64.3	2.54
N1	$N_{50}P_{25}K_{50}$	1,667.17	92.5	35.51	6.44	7.76	62.15	7.68

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

		1,908.56	98.99	40.46	6.49	7.78	62.1	7.65
		1,912.36	87.97	43.3	6.52	7.79	62.4	7.69
N2	$N_{100}P_{25}K_{50}$	2,076.42	97.62	37.63	6.5	7.71	62.15	4.83
		2,075.10	86.6	25.57	6.52	7.83	62.15	4.81
		2,076.57	98.6	27.01	6.51	7.82	62.1	4.83
N3	$N_{150}P_{25}K_{50}$	2,097.77	68.1	33.85	6.47	7.61	58.3	4.4
		2,100.24	62.39	29.52	6.43	7.66	58.5	4.15
		2,098.69	65.93	29.88	6.48	7.79	58.7	4.3
N4	$N_{200}P_{25}K_{50}$	1,913.86	108.83	33.49	6.5	7.76	62.7	0.48
		1,914.70	108.05	29.52	6.45	7.79	62.8	0.48
		1,913.74	112.57	37.63	6.5	7.68	62.4	0.48
N5	$N_{50}P_{0}K_{0}$	1,990.90	96.44	29.88	6.49	7.72	56.1	7.08
		1,989.55	86.79	28.08	6.47	7.73	56	7.1
		1,992.67	67.7	31.32	6.46	7.72	56.3	7.05
P1	$N_{50}P_{75}K_{50}$	1,889.90	80.49	29.88	6.51	7.79	69.3	2.9
		1,887.51	83.45	30.24	6.49	7.82	69.5	2.91
		1,899.67	93.48	39.04	6.46	7.81	69	2.9
P2	$N_{50}P_{100}K_{50}$	2,096.22	119.27	39.75	6.5	7.85	60	0.76
		2,095.27	132.84	43.3	6.42	7.82	60	0.77
		2,098.32	114.15	43.3	6.45	7.75	60	0.76
K1	$N_{50}P_{25}K_{100}$	1,562.32	106.28	40.46	6.44	7.74	56.7	6.87
		1,564.90	93.68	40.46	6.46	7.81	56.5	6.85
		1,563.25	105.88	44.72	6.47	7.8	56.1	6.86
K3	$N_{50}P_{25}K_{200}$	1,747.80	138.95	73.5	6.48	7.79	68	4.1
		1,743.30	138.16	88.02	6.52	7.83	68.1	4.15
		1,745.90	121.43	49	6.51	7.82	68.3	4.13
K4	$N_{50}P_{25}K_{300}$	1,440.90	100.96	34.21	6.5	7.77	67.6	1.3
		1,443.60	106.47	33.49	6.46	7.8	67.4	1.29
		1,444.88	86.2	39.75	6.47	7.75	67.2	1.27

Soil respiration, i.e., microbial CO_2 release after 9 d of incubation, ranged between 1,440 and 2,100 mg CO_2, with no significant differences among treatments. However, slightly higher activity was observed in plots with intermediate and high nitrogen, and medium phosphorus fertilization (>2,000 mg CO_2 in each case). This may reflect a relatively uniform microbial metabolic

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

activity across the experimental field, consistent with the observed chemical homogeneity. Crop yield also showed minor fluctuations, ranging from 87.7 to 99.8 t/ha, and appeared to be negatively affected by high nitrogen fertilizer levels, with the high (N150) and very high (N200) treatments yielding the lowest among the fertilized plants. Despite the application of nutrient gradients, soil and plant responses remained stable.

3.2 Characterization of bacterial and fungal communities

Microbial DNA was obtained from the 33 rhizosphere samples, including 11 treatments, three control (non-fertilized), and three bulk soil (non-rhizosphere) samples. For the bacterial and fungal analysis, we obtained 3,034 and 2,707 ASVs identified post quality filters among all the samples, with a median of 16,883 and 48,883 reads per sample, respectively. Taxonomic assignments were made at the phylum (98.26% and 91.28%), class (96.94% and 85.52%), order (89.46% and 84.97%), family (68.98% and 73.59%), and genus (34.46% and 66.89%) levels, in bacteria and fungi, respectively. The percentage of classified ASVs diminished substantially for the bacterial sequences. Meanwhile, the fungal sequences had almost twice the classified ASVs at the genus level.

Across all the fertilization treatments, the bacterial communities associated with the rhizosphere of *Saccharum* spp. were dominated by a limited number of major phyla. This reflected a relatively stable community structure. The most abundant bacterial phyla were Actinomycetota, Pseudomonadota, and Acidobacteriota, which together accounted for approximately 60.4% of the total relative abundance. Subdominant groups included Bacillota and Planctomycetota, each representing less than 16.6% of the total sequences (Figure 1A). Within these major lineages, members of Bacilli (Bacillota), Alphaproteobacteria (Pseudomonadota), Vicinamibacteria (Acidobacteriota), and Thermoleophilia (Actonomycetota) were present across all treatments with relative abundance higher than 11%. This suggests that the fertilization regime did not substantially alter the core microbiota composition (Figure 1B). Minor variations were observed in the relative abundance of Actinomycetota, which increased in soils receiving higher nitrogen inputs. However, these differences were not statistically significant (Kruskal–Wallis, $P > 0.05$). While Actinomycetota is the dominant phylum, from class to genus levels, the most represented taxa belonged to Bacillota. This demonstrated that most ASVs in this phylum were successfully classified. At the order level, the number of incertae sedis and unclassified taxa increases, representing the fifth and sixth in relative abundance, respectively, with 7.4 and 3.1% of the total relative abundance (Figure 1C). Relative abundances at the genus level are detailed in Supplementary Figure 1.

Among fungal communities, the phylum Ascomycota was dominant across all treatments, with a relative abundance exceeding 69%. This was followed by Basidiomycota and Chytridiomycota, which together represented almost 18% of relative abundance (Figure 2A). Six minor groups, such as Kickxellomycota, Glomeromycota, Calcarisporiellomycota, Mucoromycota, Neocallimastigomycota, and Zoopagomycota, were present in lower abundances (<1%) and did not show significant treatment-related variation (Figure 2A). At the class level, even when several taxa appeared to be involved in the ecosystem, only three classes represent more than 65.4% (Sordariomycetes, Eurotiomycetes, and Leotiomycetes), which belong to the Ascomycota phylum (Figure 2B). This behavior repeats at the order level, where six taxa (Eurotiales, Helotiales,

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Hypocreales, Sordariales, Chaetothyriales, and Pleosporales) that belong to the phylum Ascomycota represent more than 54% of the total (Figure 2C). This result underscores the importance and prevalence of a few representatives of the phylum Ascomycota in sugarcane communities. Relative abundances at the genus level are detailed in Supplementary Figure 2.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

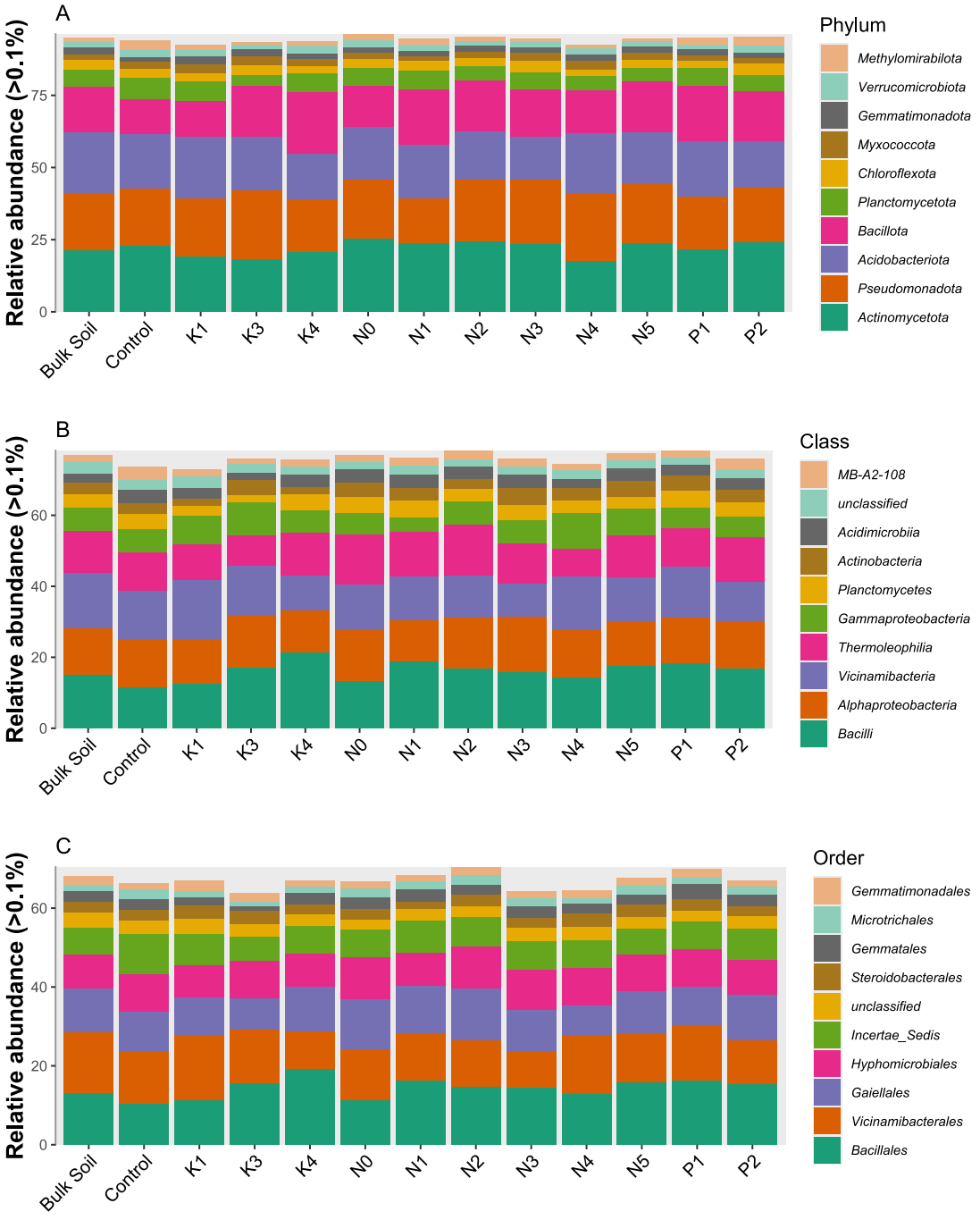


Figure 1: Bacterial relative abundance at three different taxonomic levels. (A) 10 most abundant bacteria per sample at the phylum level, (B) 10 most abundant bacteria per sample at the class level, and (C) 10 most abundant bacteria per sample at the order level. Sample abbreviations: K1, medium potassium application; K3, high potassium application; K4, very high potassium application; N0, no nitrogen application; N1, low nitrogen application; N2, medium nitrogen application; N3, high nitrogen application; N4, very high N application; N5, low nitrogen application; P1, medium phosphorus application; P2, high phosphorus application.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

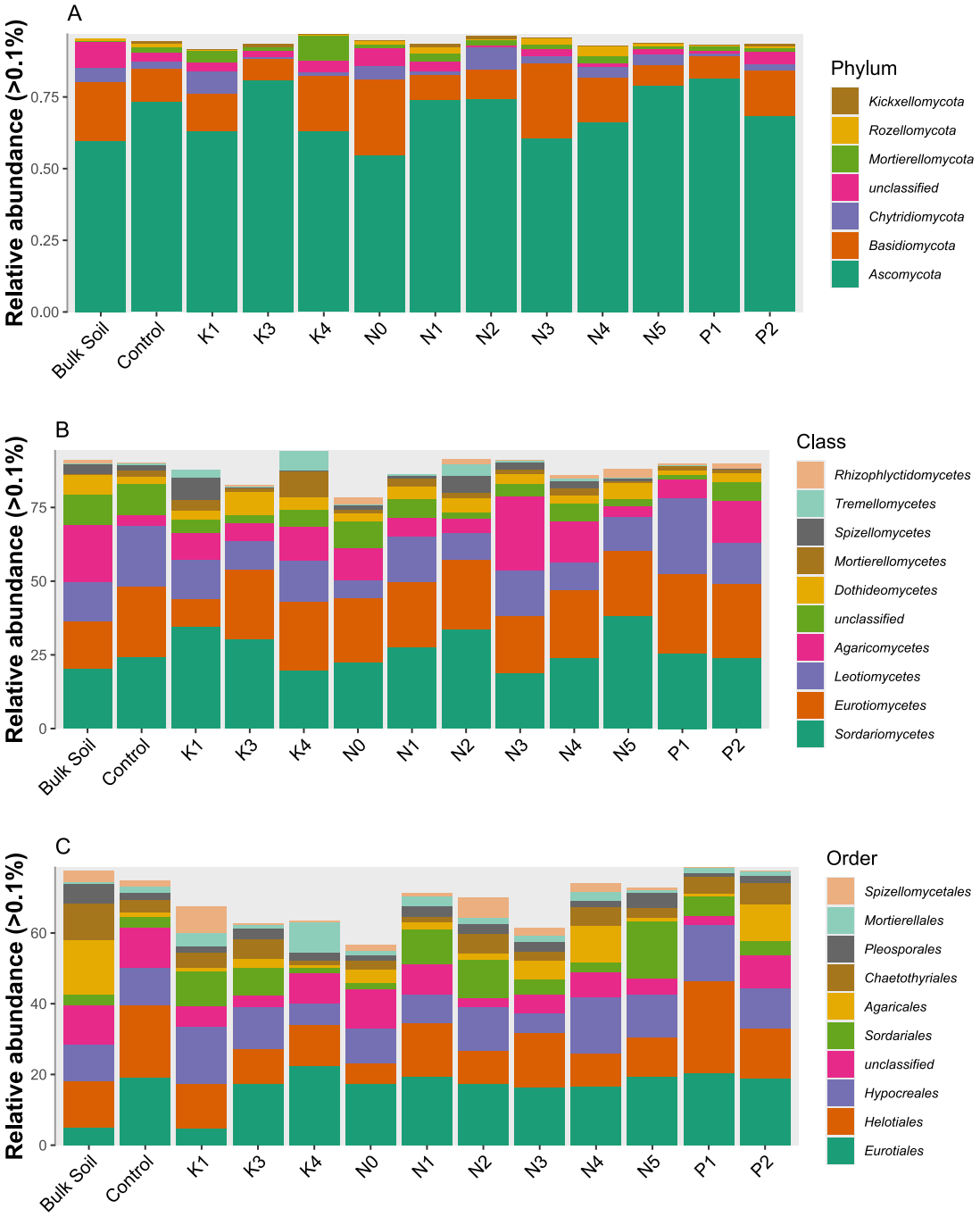


Figure 2. Fungal relative abundance at three different taxonomic levels. (A) 10 most abundant fungi per sample at the phylum level, (B) 10 most abundant fungi per sample at the class level, and (C) 10 most abundant fungi per sample at the order level. Sample abbreviations: K1, medium potassium application; K3, high potassium application; K4, very high potassium application; N0, no nitrogen application; N1, low nitrogen application; N2, medium nitrogen application; N3, high nitrogen application; N4, very high N application; N5, low nitrogen application; P1, medium phosphorus application; P2, high phosphorus application.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

3.4 Alpha and beta diversity analysis

Alpha diversity indices, namely, Observed, Chao1, Shannon, and Simpson, showed no significant differences among fertilization treatments for either the bacterial or fungal communities (Kruskal–Wallis, $P > 0.05$, Supplementary Figures 3 and 4, respectively). This pattern indicates that nutrient fertilization did not significantly affect within-sample diversity. Microbial communities remained compositionally stable despite long-term exposure to different NPK regimes (Figure 3). Even so, bacterial communities exhibited relatively high species richness and evenness, with average Shannon indices ranging from 4.9 to 5.9. Meanwhile, fungal diversity indices were lower, ranging from 2.9–4.3, suggesting a more specialized community structure in the rhizosphere.

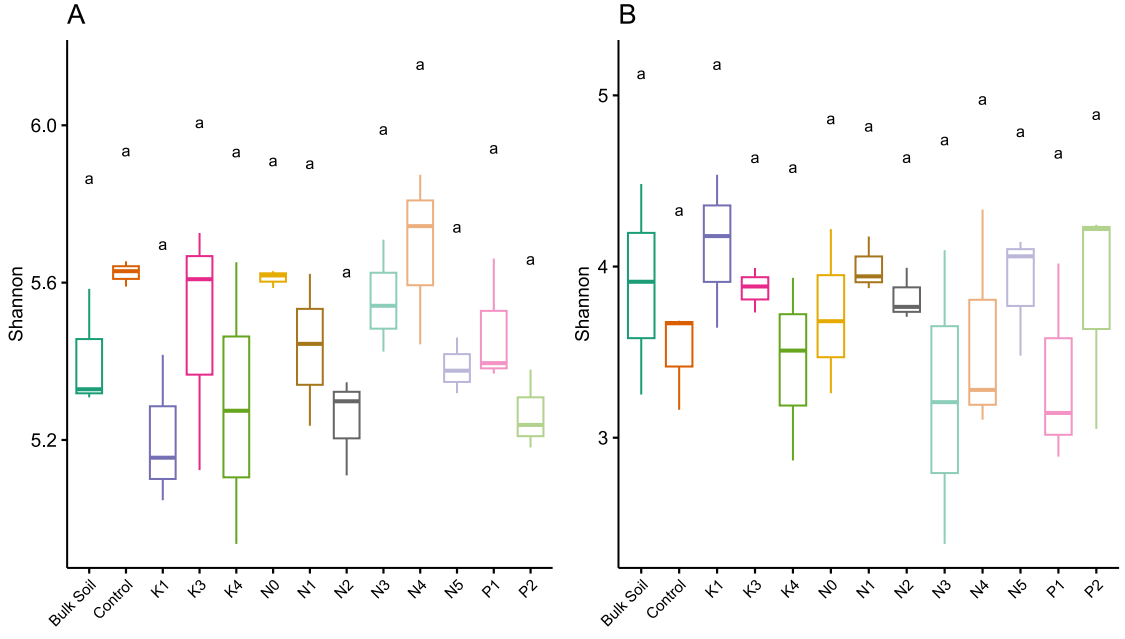


Figure 3. Alpha diversity for rhizosphere and soil samples based on the Shannon index. (A) Bacterial and (B) Fungal. Dunn's Kruskal–Wallis test, non-significant. Sample abbreviations: K1, medium potassium application; K3, high potassium application; K4, very high potassium application; N0, no nitrogen application; N1, low nitrogen application; N2, medium nitrogen application; N3, high nitrogen application; N4, very high N application; N5, low nitrogen application; P1, medium phosphorus application; P2, high phosphorus application.

Based on six indices, beta diversity analysis showed no clear clustering of samples according to fertilization treatment (PERMANOVA, $P > 0.05$; Figure 4). This implies that other environmental factors, such as soil texture, OM, or rainfall regime, may have a stronger influence on microbial assemblages than the applied fertilization treatments. Principal Coordinate Analysis (PCoA) does not reveal clustering among samples across different fertilization treatments. The microbial communities associated with *Saccharum* spp. in brown sialitic soils are compositionally rich, yet functionally stable, showing limited responsiveness to changes in nutrient fertilization.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

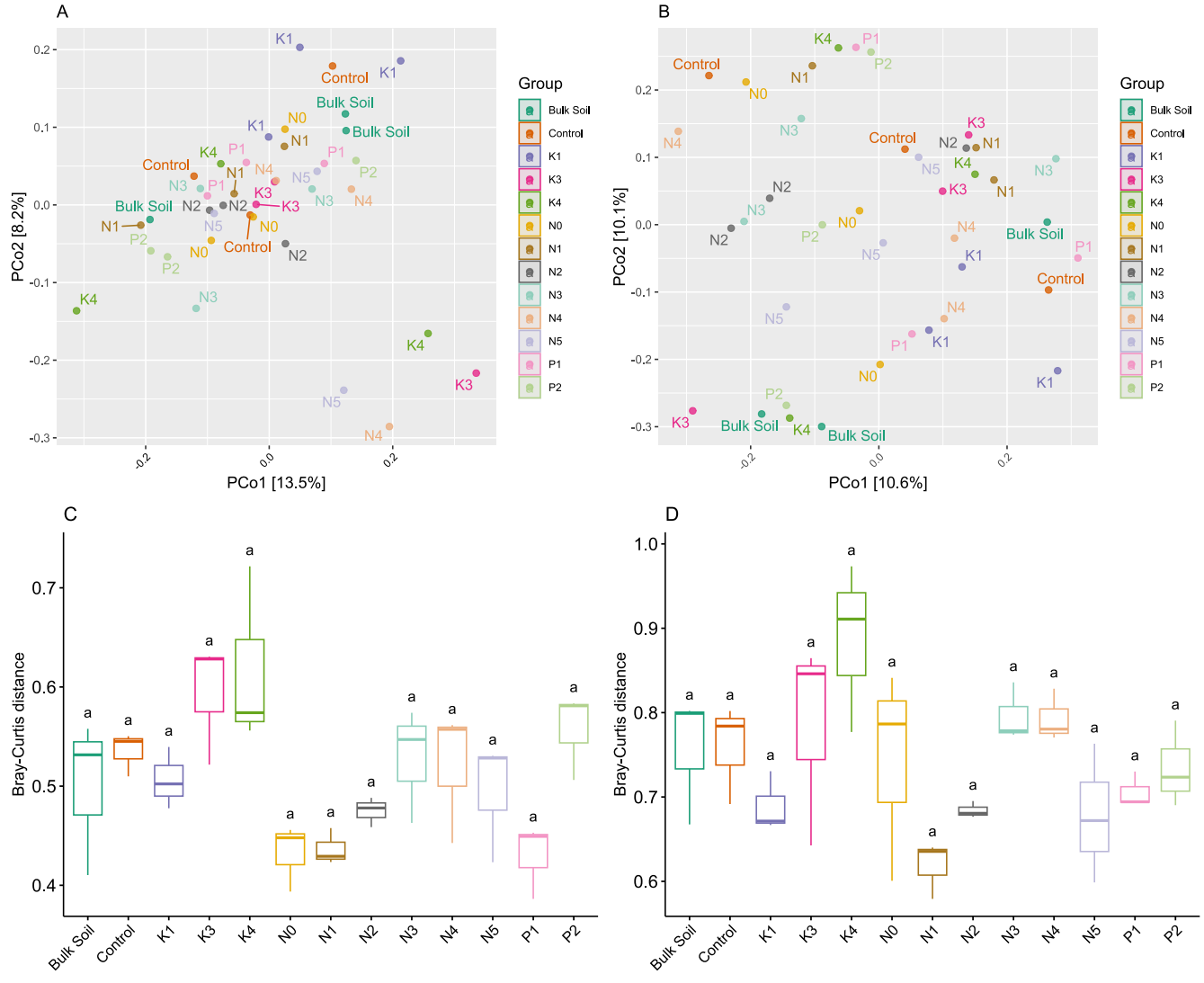


Figure 4. Beta diversity for rhizosphere and soil samples. Principal coordinate analysis determined by using the Bray–Curtis method test for (A) Bacterial and (B) Fungal communities; and group distances calculated with Dunn's Kruskal–Wallis test for (C) Bacterial and (D) Fungal communities, with non-significant differences.

3.5 Co-occurrence between bacterial and fungal taxa

The co-occurrence network for the bacterial and fungal datasets indicated a complex and heterogeneous microbial structure, encompassing a total of 371 nodes and 747 edges, represented as bacterial and fungal (Figure 5) and by the phylum of each node (Supplementary Figure 5). Taxonomic annotations showed a dominance of fungal taxa, with Ascomycota being the most abundant phylum in the network. This was followed by bacterial groups, such as Basidomycota, Pseudomonadota, Bacillota,

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Actinomycetota, and Planctomycetota. This indicates that fungi constitute the structural backbone of the rhizosphere-associated microbiome in sugarcane cultivated in brown sialitic soils. Meanwhile, bacterial taxa contribute to peripheral but functionally diverse connections. The genus *Methylomirabilota* appears among the ASVs with the highest node numbers, including when it represents 1.8% of the relative abundance.

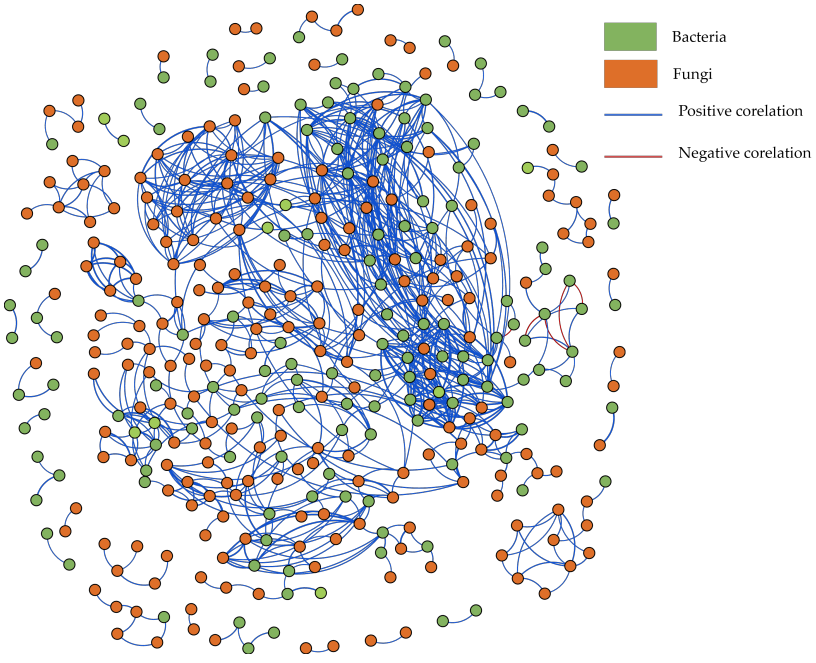


Figure 5. Microbial interaction network. Bacterial and fungal ASVs are graphed as nodes (bacteria, green dots; fungi, orange dots), while the relationship between nodes is displayed as edges (positive relations, blue line; negative correlations, red). Significant positive Spearman correlations are graphed as edges ($\rho > 0.6$, false discovery rate adjusted $P < 0.01$, two-sided).

The network was constructed from 48 modules, indicating that independent clusters of microorganisms coexist rather than a few groups with high correlations. Among these, one ASV was classified as a potential hub or keystone taxon**,** belonging to the Pseudomonadota phylum, but with no classified genus (Supplementary Figure 6). Among the bacteria, highly connected taxa included members of Pseudomonadota, such as *Pseudomonas* and *Steroidobacter*, and Actinomycetota (*Gaiella*, *Streptomyces*). Meanwhile, fungi showed greater connectivity than bacteria, with higher degrees within the network. Even though the largest number of nodes belonged to the phylum Ascomycota, Basidiomycota, and Mortierellomycota also showed higher connectivity than bacteria with higher node numbers. The interactions seem to be even between bacteria and fungi. (Supplementary Figure 7).

More than half of the network nodes corresponded to unclassified taxa from both fungal and bacterial groups. This indicates that a substantial portion of the microbial diversity in this soil remains taxonomically unresolved. The network topology was dominated by positive correlations, with only five negative correlations, all between Pseudomonadota and Bacillota ASVs.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

This result reflects potential mutualistic or commensal relationships among microorganisms rather than competitive exclusion. This predominance of cooperative interactions may contribute to the observed ecological stability of these soils, where community composition remained largely unchanged across fertilization regimes.

To evaluate the effect of fertilization regimes on microbial co-occurrence, we constructed ASV-level networks for each treatment (Table 2) and compared their structure across conditions (Figure 6 and Supplementary Figure 8). These analyses enabled us to examine both the compartment-specific effects on the soil, control, and fertilized rhizosphere (Figure 6) and the variation along nutrient gradients (Supplementary Figure 8).

The nitrogen treatments induced shifts in microbial co-occurrence patterns (Figure 6A, 6C). Under nitrogen-only conditions ($N_{50}P_0K_0$; Figure 6A), the rhizosphere network shared few nodes with the control or bulk soil. This indicates that nitrogen addition alone promotes a distinct set of microbial interactions. The basal fertilization regime ($N_{50}P_{25}K_{50}$; Figure 6B) showed slightly greater overlap between compartments; however, each retained several unique nodes. This suggests that microbial associations remain strongly compartmentalized, even under balanced fertilization. When nitrogen concentration increased (100–200 kg N ha^{-1}; Figure 6C), the number of unique nodes in the rhizosphere networks increased, particularly under high nitrogen conditions. The comparison among nitrogen treatments (N1–N4) confirmed this pattern. High nitrogen conditions (N3–N4) harbored most of the unique nodes, while low-nitrogen treatments (N1–N2) exhibited greater overlap. This indicates limited microbial restructuring under low nitrogen input (Supplementary Figure 8A). These findings suggest that nitrogen availability is a major ecological driver influencing microbial network assembly and favoring nutrient-specific co-occurrence patterns.

In contrast, phosphorus variation generated a more complex and non-linear response in network topology (Figure 6D). From the basal condition (25 kg P ha^{-1}), the total number of nodes decreased at moderate P levels (75 kg P ha^{-1}), from 2,116 to 1,450 nodes, indicating a loss of microbial connectivity under intermediate phosphorus availability. However, at high-P input (100 kg P ha^{-1}), the network expanded to 4,616 nodes, the highest among all the treatments. This suggests a strong stimulation of microbial associations under phosphorus abundance. This indicates that phosphorus has a threshold-dependent effect on the rhizosphere microbiota, where moderate enrichment may restrict certain microbial interactions. Meanwhile, higher concentrations could favor the proliferation of specialized or opportunistic taxa, leading to a dense and highly connected network. The comparison among phosphorus regimes further supports this view (Supplementary Figure 8B). This shows that high-P conditions (P2) harbored the largest fraction of exclusive nodes, implying that phosphorus enrichment favored distinct microbial groups rather than gradually modifying the community structure.

Potassium variation (Figure 6E) generated a more consistent response, with a moderate increase in unique nodes and connections at higher potassium concentrations. The networks from the high-potassium treatments (K3–K4) overlap (Supplementary Figure 8C). This suggests that microbial assemblages may stabilize under conditions of potassium abundance. Although K appears to exert less influence than phosphorus, its consistent effect on node uniqueness and overlap suggests a role in modulating rhizosphere network stability under nutrient-enriched conditions. The response of microbial communities

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

to potassium does not decrease significantly, unlike nitrogen at its highest concentration and phosphorus at its intermediate concentration, which together exert a positive modulation effect on the microbiota up to the highest concentration evaluated.

Figure 6. Venn diagram of shared nodes on ASVs networks of different fertilization regimes vs non-fertilization conditions (Soil and Control Rhizosphere). A) Nitrogen-only effect (N50P0K0); B) Basal condition, N50P25K50 (kg/ha); C) Nitrogen variation (100–200 kg/ha) with phosphorus and potassium identical to basal condition; D) Phosphorus variation (75–100 kg/ha) with nitrogen and potassium identical to basal condition; E) Potassium variation (100–300 kg/ha) with nitrogen and phosphorus identical to basal condition. The number of nodes is represented as a number inside the circle. The edges are represented as a number in parentheses below the number of nodes.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

4 Discussion

4.1. Sialitic soils have homogeneous physicochemical properties

As Cuba is one of the few countries that uses a nationally developed soil classification system (Hernández-Jiménez et al., 2019), brown sialitic soils are rarely referenced in the literature. Therefore, in this study, we have provided one of the first integrative characterizations of rhizosphere-associated microbial communities of *Saccharum* spp. cultivated on this soil type. However, the Cuban classification can be linked to international systems. Brown sialitic soils correspond to inceptisols in Soil Taxonomy (USDA) and cambisols in the World Reference Base (WRB, FAO) system (Sanchez, 2019).

Inceptisols are young soils with weak horizon development, widely distributed across diverse landscapes (USDA, 2015). They typically have low natural fertility (Syamsiyah et al., 2018), characterized by limited availability of nitrogen, phosphorus, and potassium; low OM content and slightly acidic pH (Sofyan et al., 2024). These conditions contribute to nutrient leaching, with most N losses in inceptisols occurring within the first week after fertilization (Sofyan et al., 2024). When nutrient uptake by plants during that time is insufficient, crops may experience long-term nutrient limitation (Rashid et al., 2024). Slow-release fertilizers are an effective strategy to mitigate nutrient leaching in inceptisols (Hakim et al., 2022). Therefore, this may be relevant for improving fertilizer efficiency in Cuban sugarcane production systems.

Although fertilization has a substantial effect on soil and rhizosphere microbial composition in agricultural crops (de Castro et al., 2022; Lopes et al., 2021; Pan et al., 2014; Y. Zhang et al., 2023), relatively few studies have examined how fertilization reshapes the interactions and co-occurrence patterns among microorganisms (Moneda et al., 2022). This knowledge gap is especially relevant in Cuban sugarcane systems, as the crop is predominantly cultivated as a monoculture (Monzote, 2016), a management practice that intensifies nutrient depletion and reduces microbial diversity over time (Hu et al., 2025; Tayyab et al., 2021). Understanding how microbial interactions respond to fertilization is crucial for sustaining long-term soil productivity.

As hypothesized, our results indicate high physicochemical homogeneity across all samples, including both the soil and the rhizosphere. This likely results from intrinsic soil properties, agricultural practices, such as tilling of sugarcane, and external climatic influences (McDonald et al., 2024; Peng et al., 2024; Skidmore et al., 2023). Brown sialitic soils have mid-to-high nutrient loss rates (Hernandez et al., 2015; Kaur et al., 2024), and under long-term cultivation and repeated fertilizer inputs, nutrient redistribution via rainfall and leaching can lead to chemical homogenization within the soil matrix. In tropical systems with high precipitation and sediment movement, fertilization gradients can be rapidly diluted or redistributed (Oishy et al., 2025). This may explain the absence of significant differences in microbial taxonomic composition observed in this study.

Inceptisols, including sialitic soils, often favor the formation of stable soil aggregates due to their mineralogical properties and clay activity (Liao et al., 2020). This is especially relevant to long-term fertilization, as the addition of organic and inorganic fertilizers increases the amounts of organic and inorganic carbon and cation exchange capacity in macroaggregates (Meena et al., 2022; Niu et al., 2022). These aggregates become physically protected microhabitats. Here, plant roots, fungal hyphae, and bacterial extracellular polymers bind particles together and create localized niches with steep gradients of oxygen, carbon

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

availability, moisture, and nutrients (Wilpiszeski et al., 2019; Zhong et al., 2017). The distribution and relative abundance of micro- and macroaggregates also influence the bulk properties of soil, including organic carbon content, water content, and niche availability. The interiors of aggregates can exhibit properties distinct from the surrounding matrix (Wilpiszeski et al., 2019). The rhizosphere promotes soil aggregate stability where microorganisms coexist (Li et al., 2020). In these protected environments, microbial communities can assemble structured and persistent interaction networks, including when broader soil conditions appear uniform. This is consistent with the results of the present study, in which microbial co-occurrence networks showed nutrient-dependent restructuring despite the absence of substantial changes in community composition. Such findings support the hypothesis that the rhizosphere functions as a microenvironment that maintains biological organization, even when the surrounding soil has been homogenized by long-term fertilization and environmental processes.

4.2 Long-term fertilization does not change the composition of microbial communities

All fertilization treatments in the study exhibit similar patterns in bacterial and fungal abundance. Long-term fertilization can directly and indirectly impact the soil microbiota, either decreasing or increasing the abundance of certain taxa (S. Zhang et al., 2022). Alpha and beta diversity analyses showed no significant differences among fertilization treatments, indicating that microbial community composition remained relatively stable across nutrient gradients (Supplementary Figures 3 and 4). This could reflect the adaptation of microbial communities to persistent management regimes, as seen in other intensively cultivated soils (Kostin et al., 2021). In such systems, microbial assemblages may reach a resilient equilibrium, maintaining taxonomic structure despite environmental or nutritional variations. Similar results have been reported in long-term agricultural trials (Lori et al., 2023; Luo et al., 2023). Here, nutrient additions did not produce substantial shifts in richness or evenness but influenced community function and metabolic potential. Furthermore, the results showed no difference relative to non-fertilized soil or bulk soil, suggesting that the microbial consortia associated with sugarcane in brown silty soils are resilient to variations in nitrogen, phosphorus, and potassium availability. Such resilience may be linked to the soil physicochemical properties and climatic conditions in eastern Cuba, characterized by high rainfall and seasonal variability (Centella et al., 2006). These conditions could homogenize nutrient availability and microbial niches across treatments. Another possible explanation is that natural conditions, including heavy rain, could mobilize nutrients through leaching and translocation (lateral movement), thereby homogenizing soil physicochemical properties and microbial communities (Stover & Henry, 2018).

When the bacterial and fungal communities were analyzed at the genus level, a considerable fraction of the identified ASVs remained taxonomically unresolved and were either unclassified or incertae sedis. In the bacterial datasets, approximately 65% of all sequences could not be assigned to any known genus. Meanwhile, for fungi, this proportion reached 33%. The unclassified ASVs at the genus level spanned multiple bacterial and fungal phyla, with representation from Actinomycetota, Acidobacteriota, and Bacillota among bacteria, and Ascomycota, Basidiomycota, and Chytridiomycota among fungi. These groups are typically associated with key ecological processes in soil systems, including carbon decomposition (Actinomycetota, Ascomycota, Basidiomycota) (Singavarapu et al., 2023), nutrient cycling under oligotrophic conditions (Acidobacteriota) (Gonçalves et al., 2024), stress tolerance and sporulation (Bacillota) (Filippidou et al., 2016), lignin decay

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

and soil aggregate formation (Basidiomycota) (Datta et al., 2017), and the degradation of complex biopolymers (Chytridiomycota) (Zeghal et al., 2021). The presence of numerous unclassified taxa within these ecologically important groups suggests that brown sialitic soils may harbor poorly described or potentially novel microbial lineages that contribute to the unique functioning of these tropical, nutrient-leached systems. This taxonomic ambiguity is likely related to two major factors: (i) the limited representation of tropical and Caribbean soil microorganisms in public reference databases, such as SILVA and UNITE, and (ii) the physicochemical characteristics of sialitic soils, which may select for endemic or highly specialized microbial taxa, such as *Stretpomyces* (Andam et al., 2016; Martiny, 2016). Despite the classification limitations, these unassigned ASVs are an important component of the microbial diversity in this agroecosystem. This highlights the need to expand genomic reference databases to better represent tropical soil microbiomes. The proportion of incertae sedis taxa remained relatively constant across all fertilization treatments. This further supports the observation that nutrient inputs had minimal effect on the composition and diversity of the microbial community. However, their consistent presence across samples reflects the potential of these soils as reservoirs of unexplored microbial diversity, with implications for biotechnological discovery and sustainable agriculture in tropical systems.

Bacteria have a lower percentage of unclassified taxa at the phylum level than fungi at 1.7% and 8.8%, respectively. However, at lower taxonomic levels, bacteria could be classified to a lesser extent than fungi at 65.5% and 33.1%, respectively. This further emphasizes the limited reference coverage for bacterial taxa from tropical soils. Both bacterial and fungal community structures showed taxonomic stability across the nutrient fertilization gradient, suggesting that the microbiota inhabiting these soils are resilient to moderate variations in nutrient inputs.

4.3 Insights from co-occurrence networks

Although long-term fertilization did not substantially shift community composition or diversity, network topology showed pronounced changes in microbial co-occurrences. While diversity metrics were relatively stable, the co-occurrence network analysis indicated strong nutrient-specific effects on microbial interactions. Network topology, modularity, and node distribution differed across fertilization regimes. This finding highlights that even when composition remains unchanged, community connectivity and potential functional relationships can shift. This is consistent with nutrient enrichment homogenizing bulk soil conditions over time through leaching, repeated inputs, and microbial convergence. Meanwhile, the rhizosphere remains structured by plant-driven microenvironments. Root exudates and microbial extracellular polymers promote soil aggregation (Liao et al., 2020; Wilpiszeski et al., 2019), generating microsites with distinct nutrient, carbon, and oxygen dynamics. These can harbor highly structured and functionally specialized microbial associations (Wilpiszeski et al., 2019). Such soil aggregates act as ecological refuges that stabilize microbial communities and promote network-level interactions, even when taxonomic profiles remain similar (Akter et al., 2025; Li et al., 2020; Meena et al., 2022; Wilpiszeski et al., 2019). Therefore, the observed reconfiguration of bacterial–fungal co-occurrence networks may reflect functional reorganization within rhizosphere microhabitats rather than changes in species presence alone.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Only a minor fraction of ASVs was shared among all fertilization regimes, comprising 7.4% of bacteria and 14% of fungi, suggesting a limited core microbiome in these soils. This implies that network stability is primarily maintained through dynamic, treatment-specific microbial associations rather than a fixed set of taxa. Fungal nodes, particularly those from Ascomycota, dominated the central modules, underscoring their potential keystone role in structuring soil microbial networks. The presence of bacterial phyla such as Pseudomonadota, Actinomycetota, and Bacillota in co-occurrence cores points to functional interdependence between bacteria and fungi in nutrient turnover and soil structure maintenance (Fabian et al., 2017). Notably, similar patterns have been reported across diverse ecosystems, where functional conservation is often stronger than taxonomic conservation, and ecosystem processes are maintained by different taxa performing equivalent ecological roles (Hou et al., 2021). In this context, microbial taxa with unique or specialized functions may exert a disproportionate influence on ecosystem functioning, promoting shifts in microbial interactions and network organization even in the absence of major taxonomic changes (Tang et al., 2023). This functional redundancy and specialization likely underpins the dynamic, treatment-specific microbial networks observed in these soils.

Nitrogen variation resulted in clear differentiation among treatments, with the highest nitrogen levels (150–200 kg/ha) harboring the greatest number of unique nodes. This suggests that high nitrogen promotes specialized microbial associations that are potentially linked to copiotrophic taxa and nitrogen cycling microorganisms (Nie et al., 2018). Lower nitrogen levels showed greater overlap, indicating functional redundancy at low nitrogen inputs. Conversely, phosphorus showed a non-linear response. Connectivity decreased at moderate concentrations (75 kg/ha), but increased substantially at high levels (100 kg/ha). This suggests a threshold effect, where intermediate phosphorus availability may constrain certain microbial interactions. Meanwhile, higher availability promotes the proliferation of phosphorus-tolerant or phosphorus-responsive taxa, including arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria (Mu et al., 2024). This nonlinear response may reflect differential nutrient limitation dynamics in sialitic soils. Potassium variation exhibited a more gradual and consistent effect, with higher potassium levels (200–300 kg/ha) increasing the number of nodes and edges. This implies that potassium enrichment supports microbial diversification and enhanced co-occurrence. The overlap between high-potassium treatments indicates a potential stabilization or saturation effect under nutrient-rich conditions. These results suggest that microbial co-occurrence patterns in sugarcane rhizosphere soils are strongly structured by nitrogen availability. The absence of unique taxa in low-nitrogen treatments (N1, N2) may indicate a homogenization effect under nutrient limitation. Meanwhile, the emergence of exclusive modules in high nitrogen soils (N3, N4) reflects the enrichment of specialized bacterial and fungal groups adapted to nitrogen-rich conditions. When comparing nutrient-specific effects, nitrogen showed the strongest filtering influence on microbial network composition. Meanwhile, phosphorus and potassium induced more subtle, yet detectable, shifts. The lower proportion of shared ASVs across all treatments for each element indicates that, regardless of type, nutrient enrichment leads to community specialization and microbial co-occurrence network fragmentation. This is relevant because soils in tropical zones, and inceptisols in particular, exhibit significant N deficiencies, which are typically lost through leaching or evaporation, depending on the fertilization method. In areas with abundant clay, N can become adsorbed to soil colloids (Harahap et al.,

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

2021; Motasim et al., 2021). N and K are the main nutrients required by *Saccharum* spp., both for biomass production and for various physiological processes (da Silva et al., 2018; Zeng et al., 2020).

4.4 Ecological and agronomic implications

The observed resilience in diversity but plasticity in network structure indicates that microbial ecosystems in brown sialitic soils maintain compositional stability while adapting interaction patterns under changing fertilization regimes. Fungal–bacterial networks emerge from trophic exchanges, metabolite fluxes, and the physical architecture of hyphae, biofilms, and soil aggregates. Their structure and functioning vary with environmental factors, such as pH, soil moisture, aggregate type and size, and management practices (Gao et al., 2024; Matazimov et al., 2025). These networks can coordinate biogeochemical cycles and influence plant nutrition, and contribute to soil structure and system-level resilience. Within biogeochemical cycles, these networks regulate nitrogen and phosphorus mobilization, influencing nutrient retention or leaching (Tedersoo & Bahram, 2019). Their enhancement can increase functional enzyme activity, enabling a 25–50% substitution of synthetic nitrogen with organic nitrogen, while also supporting carbon, nitrogen, and phosphorus cycling (Gao et al., 2024). Likewise, biocrusts formed by fungal–bacterial networks improve the connectivity of carbon pools, stimulating or suppressing carbon uptake depending on the dominant guild, and modulating soil organic carbon gains (Naylor et al., 2020; Tedersoo & Bahram, 2019). Common mycorrhizal networks connect plant roots and facilitate the movement of key nutrients, such as carbon and nitrogen (Matazimov et al., 2025). Fungal hyphae bind soil particles, while bacterial extracellular polymeric substances contribute to aggregate stabilization, influencing pore connectivity and water retention. Fungal–bacterial networks can help suppress pathogens in the rhizosphere through antibiosis, resource competition, and the induction of plant defenses, enhancing system resilience (Matazimov et al., 2025).

From an agronomic perspective, these findings suggest that further intensification of nitrogen and phosphorus fertilization may not significantly alter microbial diversity but can reorganize microbial connectivity. This potentially affects functional stability and soil health. Therefore, sustainable fertilization strategies should aim to preserve network connectivity while avoiding nutrient thresholds that disrupt microbial cooperation.

5 Conclusions

This study demonstrates that long-term fertilization in brown sialitic soils can profoundly influence the organization of rhizosphere microbial communities by reshaping their interaction networks, even in the absence of significant changes in taxonomic composition or diversity metrics. Contrary to our initial expectation that fertilization would primarily alter community structure through shifts in abundance, our results support the hypothesis that nutrient inputs act mainly by reorganizing microbial associations, revealing hidden ecological responses that are not captured by conventional diversity-based approaches.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

The distinct network responses observed for nitrogen, phosphorus, and potassium indicate that each nutrient imposes a specific ecological constraint on microbial community assembly. Nitrogen promoted a gradual differentiation of microbial interactions, consistent with its role as a primary growth-limiting nutrient. In contrast, phosphorus exhibited a non-linear effect, reducing network connectivity at intermediate concentrations but strongly enhancing it at high levels, suggesting a threshold-driven reorganization of microbial interactions. Potassium, meanwhile, led to progressive network stabilization, indicating a buffering or saturation effect under increasing availability. Together, these patterns suggest that fertilization modifies the functional architecture of microbial communities rather than their taxonomic identity.

Importantly, the stability of soil physicochemical properties and microbial composition across treatments highlights the rhizosphere as a microscale ecological niche, in which microbial interactions respond to nutrient availability independently of bulk soil homogenization. In highly weathered tropical soils such as sialitic soils, microbial networks may therefore represent a key mechanism for maintaining ecosystem functioning under long-term agricultural management.

Overall, our findings emphasize that microbial interaction networks provide crucial insights into soil ecosystem responses to fertilization that are not evident from diversity or abundance data alone. Incorporating network-based perspectives is thus essential for understanding the ecological consequences of fertilization practices and for designing more sustainable nutrient management strategies in tropical agroecosystems.

Author contributions

I.R-T., J.S. and M.P.: conceptualization, methodology, investigation, and formal analysis; L.Z.: formal análisis, writing—original draft and visualization; J.S., I.R-T. and M.P.: writing—review and editing, M.P. resources and supervision.

Competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Disclaimer

Copernicus Publications adds a standard disclaimer: “Copernicus Publications remains neutral with regard to jurisdictional claims made in the text, published maps, institutional affiliations, or any other geographical representation in this paper. While Copernicus Publications makes every effort to include appropriate place names, the final responsibility lies with the authors. Views expressed in the text are those of the authors and do not necessarily reflect the views of the publisher.”

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Please feel free to add disclaimer text at your choice, if applicable.

Acknowledgements

We extend our gratitude to the researchers from the Instituto de Investigaciones de la Caña de Azúcar in Santiago de Cuba, Cuba—Dolores del Rosario Piñón-Gómez, Mónica Tamayo-Isaac, Pablo D. Pablos-Reyes, and René Barbosa-García—for generously providing physical soil samples, which made our soil metagenomic studies possible.

Financial support

This work was funded by Bionostra Chile Research Foundation and Agroenergía Ingeniería Genética S.A

Declaration of generative AI in scientific writing

The authors declare that they did not use generative artificial intelligence (AI) or AI-assisted technologies in the writing process.

Data availability

All raw sequences have been deposited in the National Center for Biotechnology Information (NCBI) database (Bethesda, MD, USA) under BioProject accession code PRJNA1380633.

References

Abarenkov, K., Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., May, T. W., Frøslev, T. G., Pawlowska, J., Lindahl, B., Põldmaa, K., Truong, C., Vu, D., Hosoya, T., Niskanen, T., Piirmann, T., Ivanov, F., Zirk, A., Peterson, M., Cheeke, T. E., Ishigami, Y., Jansson, A. T., Jeppesen, T. S., Kristiansson, E., Mikryukov, V., Miller, J. T., Oono, R., Ossandon, F. J., Paupério, J., Saar, I., Schigel, D., Suija, A., Tedersoo, L., Kõljalg, U.: The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered, Nucleic Acids Res., 52, D791–D797, https://doi.org/10.1093/nar/gkad1039, 2024.

Akter, S., Mahmud, U., Shoumik, B. A. A., and Khan, M. Z.: Although invisible, fungi are recognized as the engines of a microbial powerhouse that drives soil ecosystem services, Arch. Microbiol., 207, 79, https://doi.org/10.1007/s00203-025-04285-4, 2025.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Andam, C. P., Doroghazi, J. R., Campbell, A. N., Kelly, P. J., Choudoir, M. J., and Buckley, D. H.: A latitudinal diversity gradient in terrestrial bacteria of the genus *Streptomyces*, mBio, 7, 2, https://doi.org/10.1128/mbio.02200-15, 2016.

Andrews, S.: FastQC: a quality control tool for high-throughput sequence data, Babraham Bioinformatics, http://www.bioinformatics.babraham.ac.uk/projects/fastqc/, 2010.

Antoszewski, M., Mierek-Adamska, A., and Dąbrowska, G. B.: The importance of microorganisms for sustainable agriculture – a review, Metabolites, 12, 1100, https://doi.org/10.3390/metabo12111100, 2022.

Bastian, M., Heymann, S., and Jacomy, M.: Gephi: an open source software for exploring and manipulating networks, in: Proceedings of the International AAAI Conference on Web and Social Media, 3, 361–362, 2009.

Berger, A., Gatt Harari, M., Gross, A., and Erez, A.: Diversity loss in microbial ecosystems undergoing gradual environmental changes, Cell Rep. Sustain., 1, 100242, https://doi.org/10.1016/j.crsus.2024.100242, 2024.

Callahan, B. J., McMurdie, P. J., and Holmes, S. P.: Exact sequence variants should replace operational taxonomic units in marker-gene data analysis, ISME J., 11, 2639–2643, https://doi.org/10.1038/ismej.2017.119, 2017.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.: DADA2: high-resolution sample inference from Illumina amplicon data, Nat. Methods, 13, 581–583, https://doi.org/10.1038/nmeth.3869, 2020.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R.: QIIME allows analysis of high-throughput community sequencing data, Nat. Methods, 7, 335–336, https://doi.org/10.1038/nmeth.f.303, 2010.

Centella, A., Lapinel, B., Solano, O., Vázquez, R., Fonseca, C., Cutié, V., Baéz, R., González, S., Sille, J., Rosario, P., and Leoncio, D.:
La sequía meteorológica y agrícola en la República de Cuba y la República Dominicana,
Issuing Organization, Location (optional), Persistent identifier or URL, 2006.

Chen, S., Gao, J., Chen, H., Zhang, Z., Huang, J., Lv, L., Tan, J., and Jiang, X.: The role of long-term mineral and manure fertilization on P species accumulation and phosphate-solubilizing microorganisms in paddy red soils, Soil, 9, 101–116, https://doi.org/10.5194/soil-9-101-2023, 2023.

Cherubin, M. R., Márcio, D., Feigl, B. J., Pimentel, L. G., Lisboa, I. P., Gmach, M. R., Varanda, L. L., Morais, M. C., Satiro, L. S., Popin, G. V., Paiva, R. D., Klebson, A., Luisa, A., Vasconcelos, S. D., Lineker, P., Melo, A. D., Cerri, E. P., and Cerri, C. C.: Crop residue harvest for bioenergy production and its implications on soil functioning and plant growth: A review, Scientia Agricola, 75, 255–272, 2018.

da Silva, V. S. G., de Oliveira, M. W., Ferreira, V. M., de Oliveira, T. B. A., Galvão, E. R., da Silva, A. F., and Machado, P. A. S.: Nutritional requirement of sugarcane cultivars, J. Agric. Sci., 10, 361, https://doi.org/10.5539/jas.v10n4p361, 2018.

Datta, R., Kelkar, A., Baraniya, D., Molaei, A., Moulick, A., Meena, R. S., and Formanek, P.: Enzymatic degradation of lignin in soil: a review, Sustainability, 9, 1163, https://doi.org/10.3390/su9071163, 2017.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

de Castro, S. G. Q., Magalhães, P. S. G., de Castro, S. A. Q., Kölln, O. T., and Franco, H. C. J.: Optimizing nitrogen fertilizer rates at distinct in-season application moments in sugarcane, Int. J. Plant Prod., 16, 137–152, https://doi.org/10.1007/s42106-021-00175-z, 2022.

Dinesh Babu, K. S., Janakiraman, V., Palaniswamy, H., Kasirajan, L., Gomathi, R., and Ramkumar, T. R.: A short review on sugarcane: its domestication, molecular manipulations and future perspectives, Genet. Resour. Crop Evol., 69, 2623–2643, https://doi.org/10.1007/s10722-022-01430-6, 2022.

Du, J., Gao, Q., Sun, F., Liu, B., Jiao, Y., and Liu, Q.: Agricultural soil microbiomes at the climate frontier: nutrient-mediated adaptation strategies for sustainable farming, Ecotoxicol. Environ. Saf., 295, 118161, https://doi.org/10.1016/j.ecoenv.2025.118161, 2025.

Durán, I.: Comportamiento de la precipitación en estaciones meteorológicas seleccionadas de Cuba, thesis, Universidad de La Habana, Cuba, 2012.

Fabian, J., Zlatanovic, S., Mutz, M., and Premke, K.: Fungal–bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality, ISME J., 11, 415–425, https://doi.org/10.1038/ismej.2016.131, 2017.

FAO: Fertilizer use by crop in Cuba, Food and Agriculture Organization of the United Nations, Rome, Italy, 2003.

FAO: Sugar cane production, Food and Agriculture Organization of the United Nations, https://archive.ourworldindata.org/20251014-161652/grapher/sugar-cane-production.html (last access: 14 October 2025), 2025.

Filippidou, S., Wunderlin, T., Junier, T., Jeanneret, N., Dorador, C., Molina, V., Johnson, D. R., and Junier, P.: A combination of extreme environmental conditions favor the prevalence of endospore-forming firmicutes, Front. Microbiol., 7, 1707, https://doi.org/10.3389/fmicb.2016.01707, 2016.

Freilich, S., Zarecki, R., Eilam, O., Segal, E. S., Henry, C. S., Kupiec, M., Gophna, U., Sharan, R., and Ruppin, E.: Competitive and cooperative metabolic interactions in bacterial communities, Nat. Commun., 2, 589, https://doi.org/10.1038/ncomms1597, 2011.

Gao, C., Bezemer, T. M., de Vries, F. T., and van Bodegom, P. M.: Trade-offs in soil microbial functions and soil health in agroecosystems, Trends Ecol. Evol., 39, 895–903, https://doi.org/10.1016/j.tree.2024.05.013, 2024.

Gao, C., Xu, L., Montoya, L., Madera, M., Hollingsworth, J., Chen, L., Purdom, E., Singan, V., Vogel, J., Hutmacher, R. B., Dahlberg, J. A., Coleman-Derr, D., Lemaux, P. G., and Taylor, J. W.: Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities, Nat. Commun., 13, 3867, https://doi.org/10.1038/s41467-022-31343-y, 2022.

Gonçalves, O. S., Fernandes, A. S., Tupy, S. M., Ferreira, T. G., Almeida, L. N., Creevey, C. J., and Santana, M. F.: Insights into plant interactions and the biogeochemical role of the globally widespread Acidobacteriota phylum, Soil Biol. Biochem., 192, 109369, https://doi.org/10.1016/j.soilbio.2024.109369, 2024.

Guo, J. and Chen, J.: The impact of heavy rainfall variability on fertilizer application rates: evidence from maize farmers in China, Int. J. Environ. Res. Public Health, 19, 15906, https://doi.org/10.3390/ijerph192315906, 2022.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Guo, Z., Wan, S., Hua, K., Yin, Y., Chu, H., Wang, D., and Guo, X.: Fertilization regime has a greater effect on soil microbial community structure than crop rotation and growth stage in an agroecosystem, Appl. Soil Ecol., 149, 103510, https://doi.org/10.1016/j.apsoil.2020.103510, 2020.

Hakim, F. K., Soyfan, E. T., Sudirja, R., Joy, B., Yuniarti, A., and Herdiyantoro, D.: The application of slow-release NPK fertilizer on inceptisols to changes of soil chemical properties and growth of sweet corn (*Zea mays* L. saccharata), Int. J. Environ. Agric. Biotechnol., 7, 18–24, https://doi.org/10.22161/ijeab, 2022.

Harahap, F. S., Oesman, R., Fadhillah, W., and Rafika, M.: Chemical characteristics of inceptisol soil with urea and goat manure fertilizer, J. Agron. Tanaman Trop., 3, 117–127, https://doi.org/10.36378/juatika.v3i2.1398, 2021.

Hernández, A., Pérez, J., Bosch, D., and Castro, N.: *Clasificación de los suelos de Cuba 2015*, Instituto Nacional de Ciencias Agrícolas (INCA) and Instituto de Suelos, Cuba, 2015.

Hernández-Jiménez, A., Pérez-Jiménez, J., Bosch-Infante, D., and Castro, N.: La clasificación de suelos de Cuba: énfasis en la versión de 2015, Cultivos Trop., 40, 31, 2019.

Hou, Z., Lv, G., and Jiang, L.: Functional diversity can predict ecosystem functions better than dominant species: the case of desert plants in the Ebinur Lake Basin, Sustainability, 13, 2858, https://doi.org/10.3390/su13052858, 2021.

Hu, J., Wan, L., Wang, Y., Dai, K., Butterbach-Bahl, K., and Lin, S.: The decrease of soil microbial community diversity and network complexity results in the increase of soil-borne diseases with monocultural years in greenhouse tomato production systems, Environ. Microbiol. Rep., 17, e70165, https://doi.org/10.1111/1758-2229.70165, 2025.

Huang, R., Li, W., Qiu, S., Long, Y., Zeng, Z., Tang, J., and Huang, Q.: Impact of land use types on soil microbial community structure and functional structure in Baihualing Village, China, Glob. Ecol. Conserv., 57, e03379, https://doi.org/10.1016/j.gecco.2024.e03379, 2025.

INICA: Normas metodológicas del departamento de suelos y agroquímica, Tomo 1, p. 100, 1990.

Katoh, K. and Standley, D. M.: MAFFT multiple sequence alignment software version 7: improvements in performance and usability, Mol. Biol. Evol., 30, 772–780, https://doi.org/10.1093/molbev/mst010, 2013.

Kaur, S., Bedi, M., Singh, S., Kour, N., Bhatti, S. S., Bhatia, A., Kumar, M., and Kumar, R.: Monoculture of crops: a challenge in attaining food security, in: Environmental Challenges in Attaining Food Security, Vol. 9, edited by: Sharma, A., Kumar, M., and Sharma, P., Elsevier, https://doi.org/10.1016/bs.af2s.2024.07.008, 2024.

Khan, A., Wei, Y., Adnan, M., Ali, I., Zhang, M.: Dynamics of rhizosphere bacterial communities and soil physiochemical properties in response to consecutive ratooning of sugarcane, Front. Microbiol., 14, 1197246, https://doi.org/10.3389/fmicb.2023.1197246, 2023.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F. O.: Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, Nucleic Acids Res., 41, e1–e1, https://doi.org/10.1093/nar/gks808, 2013.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Kostin, J. E., Cesarz, S., Lochner, A., Schädler, M., Macdonald, C. A., and Eisenhauer, N.: Land-use drives the temporal stability and magnitude of soil microbial functions and modulates climate effects, Ecol. Appl., 31, e02325, https://doi.org/10.1002/eap.2325, 2021.

Kracmarova, M., Uhlik, O., Strejcek, M., Szakova, J., Cerny, J., Balik, J., Tlustos, P., Kohout, P., Demnerova, K., and Stiborova, H.: Soil microbial communities following 20 years of fertilization and crop rotation practices in the Czech Republic, Environ. Microbiome, 17, 13, https://doi.org/10.1186/s40793-022-00406-4, 2022.

Kumar, V., Raghuvanshi, N., Pandey, A. K., Kumar, A., Thoday-Kennedy, E., and Kant, S.: Role of halotolerant plant growth-promoting rhizobacteria in mitigating salinity stress: recent advances and possibilities, Agriculture, 13, 168, https://doi.org/10.3390/agriculture13010168, 2023.

Lahlali, R., Ibrahim, D. S. S., Belabess, Z., Kadir Roni, M. Z., Radouane, N., Vicente, C. S. L., Menéndez, E., Mokrini, F., Barka, E. A., Galvão de Melo E Mota, M., and Peng, G.: High-throughput molecular technologies for unraveling the mystery of soil microbial community: challenges and future prospects, Heliyon, 7, e08142, https://doi.org/10.1016/j.heliyon.2021.e08142, 2021.

Li, J., Yuan, X., Ge, L., Li, Q., Li, Z., Wang, L., and Liu, Y.: Rhizosphere effects promote soil aggregate stability and associated organic carbon sequestration in rocky areas of desertification, Agr. Ecosyst. Environ., 304, 107126, https://doi.org/10.1016/j.agee.2020.107126, 2020.

Liao, H., Zhang, Y., Wang, K., Hao, X., Chen, W., and Huang, Q.: Complexity of bacterial and fungal network increases with soil aggregate size in an agricultural inceptisol, Appl. Soil Ecol., 154, 103640, https://doi.org/10.1016/j.apsoil.2020.103640, 2020.

Liu, C., Cui, Y., and Li, X., Yao, M.: Microeco: an R package for data mining in microbial community ecology, FEMS Microbiol. Ecol., 97, fiaa255, https://doi.org/10.1093/femsec/fiaa255, 2021.

Liu, Q., Zhao, X., Liu, Y., Xie, S., Xing, Y., Dao, J., Wei, B., Peng, Y., Duan, W., and Wang, Z.: Response of sugarcane rhizosphere bacterial community to drought stress, Front. Microbiol., 12, 716196, https://doi.org/10.3389/fmicb.2021.716196, 2021.

Liu, Y., Liang, D., Xing, J., Xue, Z., and Zhang, Z.: Interactions between sugarcane leaf return and fertilizer reduction in soil bacterial network in Southern China red soil, Microorganisms, 12, 1788, 2024.

Looney, S. W., and Hagan, J. L.: Statistical methods for assessing biomarkers and analyzing biomarker data, in:*Essential Statistical Methods for Medical Statistics*, edited by: Rao, C. R., Miller, J. P., and Rao, D. C., Elsevier, 27–65, https://doi.org/10.1016/B978-0-444-53737-9.50005-0, 2011.

Lopes, M. J. dos S., Dias-Filho, M. B., and Gurgel, E. S. C.: Successful plant growth-promoting microbes: inoculation methods and abiotic factors, Front. Sustain. Food Syst., 5, 1–13, https://doi.org/10.3389/fsufs.2021.606454, 2021.

Lori, M., Hartmann, M., Kundel, D., Mayer, J., Mueller, R. C., Mäder, P., and Krause, H. M.: Soil microbial communities are sensitive to differences in fertilization intensity in organic and conventional farming systems, FEMS Microbiol. Ecol., 99, fiad046, https://doi.org/10.1093/femsec/fiad046, 2023.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Luo, J., Liao, G., Banerjee, S., Gu, S., Liang, J., Guo, X., Zhao, H., Liang, Y., and Li, T.: Long-term organic fertilization promotes the resilience of soil multifunctionality driven by bacterial communities, Soil Biol. Biochem., 177, 108922, https://doi.org/10.1016/j.soilbio.2022.108922, 2023.

Martiny, J. B.: History leaves its mark on soil bacterial diversity, *mBio*, **7**, 3, https://doi.org/10.1128/mBio.00784-16, 2016.

Matazimov, M. T., Sidametova, Z. E., Olimov, N. K., Abdullaeva, M. U., Rakhimova, D. O., Rustamov, I. X., and Umurzakova, N. S.: Ecological roles of fungal networks: integrating nutrient cycling, soil health, and climate resilience—A systematic review, Microbial Bioactives, 8, 1–8, 2025.

McDonald, M. D., Lewis, K. L., Blazier, J. C., and Gentry, T. J.: Semi-arid soil bacterial communities are refined by altered plant selection pressure under conservation management practices, Appl. Soil Ecol., 194, 105191, https://doi.org/10.1016/j.apsoil.2023.105191, 2024.

Meena, S. K., Dwivedi, B. S., Meena, M. C., Datta, S. P., Singh, V. K., Mishra, R. P., Chakraborty, D., Dey, A., and Meena, V. S.: Impact of long-term nutrient supply options on soil aggregate stability after nineteen years of rice–wheat cropping system, Land, 11, 1465, https://doi.org/10.3390/land11091465, 2022.

Mitter, E. K., Tosi, M., Obregón, D., Dunfield, K. E., and Germida, J. J.: Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies, Front. Sustain. Food Syst., 5, 1–23, https://doi.org/10.3389/fsufs.2021.606815, 2021.

Moneda, A. P. C., de Carvalho, L. A. L., Teheran-Sierra, L. G., Funnicelli, M. I. G., and Pinheiro, D. G.: Sugarcane cultivation practices modulate rhizosphere microbial community composition and structure, Sci. Rep., 12, 19174, https://doi.org/10.1038/s41598-022-23562-6, 2022.

Monzote, R. F.: Sugar cane and agricultural transformations in Cuba, in: Oxford Research Encyclopedia of Latin American History, Oxford University Press, https://doi.org/10.1093/acrefore/9780199366439.013.4, 2016.

Motasim, A. M., Samsuri, A. W., Abdul Sukor, A. S., and Adibah, A. M.: Nitrogen dynamics in tropical soils treated with liquid and granular urea fertilizers, Agriculture, 11, 6, https://doi.org/10.3390/agriculture11060546, 2021.

Mu, P., Ding, G., Zhang, Y., Jin, Q., Liu, Z., Guan, Y., Zhang, L., Liang, C., Zhou, F., and Liu, N.: Interactions between arbuscular mycorrhizal fungi and phosphate-soluble bacteria affect ginsenoside compositions by modulating the C:N:P stoichiometry in Panax ginseng, Front. Microbiol., 15, 1426440, https://doi.org/10.3389/fmicb.2024.1426440, 2024.

Naylor, D., Sadler, N., Bhattacharjee, A., Graham, E. B., Anderton, C. R., McClure, R., Lipton, M., Hofmockel, K. S., and Jansson, J. K.: Soil microbiomes under climate change and implications for carbon cycling, Annu. Rev. Environ. Resour., 45, 29–59, https://doi.org/10.1146/annurev-environ-012320-082720, 2020.

Nie, Y., Wang, M., Zhang, W., Ni, Z., Hashidoko, Y., and Shen, W.: Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment, Sci. Total Environ., 624, 407–415, https://doi.org/10.1016/j.scitotenv.2017.12.142, 2018.

Niu, Z., An, F., Su, Y., Liu, T., Yang, R., Du, Z., and Chen, S.: Effect of long-term fertilization on aggregate size distribution and nutrient accumulation in aeolian sandy soil, Plants, 11, 909, https://doi.org/10.3390/plants11070909, 2022.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Núñez Salazar, R., Aguirre, C., Soto, J., Salinas, P., Salinas, C., Prieto, H., and Paneque, M.: Physicochemical parameters affecting the distribution and diversity of the water column microbial community in the high-altitude Andean lake system of La Brava and La Punta, Microorganisms, 8, 1181, https://doi.org/10.3390/microorganisms8081181, 2020.

Oishy, M. N., Shemonty, N. A., Fatema, S. I., Mahbub, S., Mim, E. L., Hasan Raisa, M. B., and Anik, A. H.: Unravelling the effects of climate change on the soil-plant-atmosphere interactions: a critical review, Soil Environ. Health, 3, 100130, https://doi.org/10.1016/j.seh.2025.100130, 2025.

Orgiazzi, A., Lumini, E., Nilsson, R. H., Girlanda, M., Vizzini, A., Bonfante, P., and Bianciotto, V.: Unravelling soil fungal communities from different Mediterranean land-use backgrounds, PLoS ONE, 7, e34847, https://doi.org/10.1371/journal.pone.0034847, 2012.

Orrù, L., Canfora, L., Trinchera, A., Migliore, M., Pennelli, B., Marcucci, A., Farina, R., and Pinzari, F.: How tillage and crop rotation change the distribution pattern of fungi, Front. Microbiol., 12, 634325, https://doi.org/10.3389/fmicb.2021.634325, 2021.

Otto, R., Ferraz-Almeida, R., Sanches, G. M., Lisboa, I. P., and Cherubin, M. R.: Nitrogen fertilizer consumption and nitrous oxide emissions associated with ethanol production—A national-scale comparison between Brazilian sugarcane and corn in the United States, J. Clean. Prod., 350, 131482, https://doi.org/10.1016/j.jclepro.2022.131482, 2022.

Pan, Y., Cassman, N., de Hollander, M., Mendes, L. W., Korevaar, H., Geerts, R. H. E. M., van Veen, J. A., and Kuramae, E. E.: Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil, FEMS Microbiol. Ecol., 90, 195–205, https://doi.org/10.1111/1574-6941.12384, 2014.

Peng, Z., Qian, X., Liu, Y., Li, X., Gao, H., An, Y., Qi, J., Jiang, L., Zhang, Y., Chen, S., Pan, H., Chen, B., Liang, C., van der Heijden, M. G. A., and Wei, G.: Land conversion to agriculture induces taxonomic homogenization of soil microbial communities globally, Nat. Commun., 15, 3624, https://doi.org/10.1038/s41467-024-47348-8, 2024.

Philippot, L., Griffiths, B. S., and Langenheder, S.: Microbial community resilience across ecosystems and multiple disturbances, Microbiol. Mol. Biol. Rev., 85, 1–24, 2021.

Price, M. N., Dehal, P. S., and Arkin, A. P.: FastTree: computing large minimum evolution trees with profiles instead of a distance matrix, Mol. Biol. Evol., 26, 1641–1650, https://doi.org/10.1093/molbev/msp077, 2009.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.: The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Res., 41, D590–D596, https://doi.org/10.1093/nar/gks1219, 2013.

Ramos Tapia, I., Nuñez, R., Salinas, C., Salinas, P., Soto, J., and Paneque, M.: Study of wetland soils of the Salar de Atacama with different azonal vegetative formations reveals changes in the microbiota associated with hygrophile plant type on the soil surface, Microbiol. Spectrum, https://doi.org/10.1128/spectrum.00533-22, 2022.

Ramos Tapia, I., Salinas, P., Nuñez, R., Cortez, D., Soto, J., and Paneque, M.: Compositional changes in sediment microbiota are associated with seasonal variation of the water column in high-altitude hyperarid Andean lake systems, Microbiol. Spectrum, https://doi.org/10.1128/spectrum.05200-22, 2023.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Rashid, H., Sun, X., Wu, F., Ni, X.: Nutrient leaching after subtropical forest conversions and its implications for soil fertility, For. Ecol. Manage., 566, 122044, https://doi.org/10.1016/j.foreco.2024.122044, 2024.

Ritchie, H., Rosado, P., and Roser, M.: Agricultural production, Our World in Data, 2023.

Robeson, M. S., O'Rourke, D. R., Kaehler, B. D., Ziemski, M., Dillon, M. R., Foster, J. T., and Bokulich, N. A.: RESCRIPt: reproducible sequence taxonomy reference database management, PLoS Comput. Biol., 17, e1009581, https://doi.org/10.1371/journal.pcbi.1009581, 2021.

Sanchez, P. A.: Soils of the tropics, in: Properties and Management of Soils in the Tropics, Cambridge University Press, Cambridge, 82–119, https://doi.org/10.1017/9781316809785.006, 2019.

Semenov, M. V., Krasnov, G. S., Semenov, V. M., and van Bruggen, A.: Mineral and organic fertilizers distinctly affect fungal communities in the crop rhizosphere, J. Fungi, 8, 251, 2022.

Shaw, G. T.-W., Liu, A.-C., Weng, C.-Y., Chen, Y.-C., Chen, C.-Y., Weng, F. C.-H., Wang, D., and Chou, C.-Y.: A network-based approach to deciphering a dynamic microbiome's response to a subtle perturbation, Sci. Rep., 10, 19530, https://doi.org/10.1038/s41598-020-73920-5, 2020.

Sieiro, G. L., and Pablos, P.: Indice de calidad para un suelo Sialitico Monocultivado con Saccharum officinarum L., Rev. Agric. Trop., 7, 1–11, 2021.

Singavarapu, B., Du, J., Beugnon, R., Cesarz, S., Eisenhauer, N., Xue, K., and Wang, Y., Bruelheide, H., and Wubet, T.: Functional potential of soil microbial communities and their subcommunities varies with tree mycorrhizal type and tree diversity, Microbiol. Spectrum, 11, 1–19, https://doi.org/10.1128/spectrum.04578-22, 2023.

Skidmore, M., Andarge, T., and Foltz, J.: The impact of extreme precipitation on nutrient runoff, J. Agric. Appl. Econ. Assoc., 2, 769–785, https://doi.org/10.1002/jaa2.90, 2023.

Sofyan, E. T., Yuniarti, A., and Alparidzy, M.: Effect of liquid and single compound NPK fertilizer on soil and plant nitrogen content and yield of sweet corn (*Zea mays* L. saccharata) in inceptisols of Jatinangor, Asian J. Soil Sci. Plant Nutr., 10, 407–414, https://doi.org/10.9734/ajsspn/2024/v10i3352, 2024.

Stover, H. J., and Henry, H. A. L.: Soil homogenization and microedges: perspectives on soil-based drivers of plant diversity and ecosystem processes, Ecosphere, 9, 6, https://doi.org/10.1002/ecs2.2289, 2018.

Souza, V. S., Canisares, L. P., Schiebelbein, B. E., Santos, D. de C., Menillo, R. B., Pinheiro, C. R., and Cherubin, M.: Cover crops enhance soil health, crop yield and resilience of tropical agroecosystem, Field Crops Res., 322, 109755, https://doi.org/10.1016/j.fcr.2025.109755, 2025.

Sui, B., Wang, L., Wang, H., Zhao, X., Jin, F., Wang, H., Guo, J., and Xu, Q.: Deep tillage inhibits microbial species interactions and exhibits contrasting roles in bacterial and fungal assembly, Agric. Ecosyst. Environ., 357, 108679, https://doi.org/10.1016/j.agee.2023.108679, 2023.

Suman, J., Rakshit, A., Ogireddy, S. D., Singh, S., Gupta, C., and Chandrakala, J.: Microbiome as a key player in sustainable agriculture and human health, Front. Soil Sci., 2, 1–13, https://doi.org/10.3389/fsoil.2022.821589, 2022.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Syamsiyah, J., Sumarno, S., Suryono, S., Sari, W., and Anwar, M.: Chemical properties of inceptisol and rice yields applied with mixed source fertilizer (MSF), *J.* Trop. Soils, 23, 1–9, https://doi.org/10.5400/jts.2018.v23i1.1-9, 2018.

Tamayo-Isaac, M., Piñón-Gómez, D. R., Ramos-Tapia, I., Soto-Winckler, J., and Reynosa-Rodríguez, G., Paneque, M.: Diversidad microbiana en estudios de fertilización mineral de larga duración en caña de azúcar, Rev. U.D.C.A Actualidad Divulg. Cient., 26, 2, https://doi.org/10.31910/rudca.v26.n2.2023.2511, 2023.

Tamayo-Isaac, M., Piñón-Gómez, D., Puchades-Izaguirre, Y., and Pablos-Reyes, P., Pérez-Silva, R.: Biological activity of the microbial population associated with the rhizosphere of Saccharum officinarum L., Rev. Cubana Quím., 36, 2, 1–12, 2024.

Tang, R., Li, S., Lang, X., Huang, X., and Su, J.: Rare species contribute greater to ecosystem multifunctionality in a subtropical forest than common species due to their functional diversity, For. Ecol. Manage., 538, 120981, https://doi.org/10.1016/j.foreco.2023.120981, 2023.

Tayyab, M., Yang, Z., Zhang, C., Islam, W., Lin, W., and Zhang, H.: Sugarcane monoculture drives microbial community composition, activity and abundance of agricultural-related microorganisms, Environ. Sci. Pollut. Res. Int., 28, 48080–48096, 2021.

Tedersoo, L., and Bahram, M.: Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes, Biol. Rev., 94, 1857–1880, https://doi.org/10.1111/brv.12538, 2019.

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P., Jackson, R. B., Janssens-Maenhout, G., Prather, M. J., Regnier, P., Pan, N., Pan, S., Peters, G. P., Shi, H., Tubiello, F. N., Zaehle, S., Zhou, F., Arneth, A., Battaglia, G., Berthet, S., Bopp, L., Bouwman, A. F., Buitenhuis, E. T., Chang, J., Chipperfield, M. P., Dangal, S. R. S., Dlugokencky, E., Elkins, J. W., Eyre, B. D., Fu, B., Hall, B., Ito, A., Joos, F., Krummel, P. B., Landolfi, A., Laruelle, G. G., Lauerwald, R., Li, W., Lienert, S., Maavara, T., MacLeod, M., Millet, D. B., Olin, S., Patra, P. K., Prinn, R. G., Raymond, P. A., Ruiz, D. J., van der Werf, G. R., Vuichard, N., Wang, J., Weiss, R. F., Wells, K. C., Wilson, C., Yang, J., and Yao, Y.: A comprehensive quantification of global nitrous oxide sources and sinks, Nature, 586, 248–256, https://doi.org/10.1038/s41586-020-2780-0, 2020.

Tripathi, B. M., Song, W., Slik, J. W. F., Sukri, R. S., Jaafar, S., and Dong, K., Adams, J. M.: Distinctive tropical forest variants have unique soil microbial communities, but not always low microbial diversity, Front. Microbiol., 7, 376, https://doi.org/10.3389/fmicb.2016.00376, 2016.

USDA: Illustrated guide to soil taxonomy, version 2, https://doi.org/10.2134/jae.1982.0003, 2015.

Wang, C., Miao, Q., Wei, Z., Guo, Y., Li, J., Fan, Z., Hu, Y., Zhang, H., Sun, J., and Cui, Z.: Nutrient runoff and leaching under various fertilizer treatments and pedogeographic conditions: a case study in tobacco (*Nicotiana tabacum* L.) fields of the Erhai Lake basin, China, Eur. J. Agron., 156, 127170, https://doi.org/10.1016/j.eja.2024.127170, 2024.

Wilpiszeski, R. L., Aufrecht, J. A., Retterer, S. T., Sullivan, M. B., Graham, D. E., Pierce, E. M., Zablocki, O. D., Palumbo, A. V., and Elias, D. A.: Soil aggregate microbial communities: towards understanding microbiome interactions at biologically relevant scales, Appl. Environ. Microbiol., 85, e00324-19, https://doi.org/10.1128/AEM.00324-19, 2019.

https://doi.org/10.5194/egusphere-2026-629
Preprint. Discussion started: 18 February 2026

Yang, H., Yang, Y., Zhu, H., Xiong, H., Zhang, Y., Liu, Y., Huang, X., Li, Y., and Jiang, T.: Short-term phosphorus fertilization alters soil fungal community in long-term phosphorus-deprived yellow soil paddy fields, Agriculture, 15, 280, https://doi.org/10.3390/agriculture15030280, 2025.

Zamora-Leiva, L., Soto, J., Román-Figueroa, C., Peña, F., Univaso, L., and Paneque, M.: Characterization of bacterial diversity associated with a Salar de Atacama native plant Nitrophila atacamensis, Environ. Microbiome, 20, 100, https://doi.org/10.1186/s40793-025-00766-7, 2025.

Zamkovaya, T., Foster, J. S., de Crécy-Lagard, V., and Conesa, A.: A network approach to elucidate and prioritize microbial dark matter in microbial communities, ISME J., 15, 228–244, https://doi.org/10.1038/s41396-020-00777-x, 2021.

Zeghal, E., Vaksmaa, A., Vielfaure, H., Boekhout, T., and Niemann, H.: The potential role of marine fungi in plastic degradation – a review, Front. Mar. Sci., 8, 738877, https://doi.org/10.3389/fmars.2021.738877, 2021.

Zeng, X., Zhu, K., Lu, J., Jiang, Y., Yang, L., and Xing, Y.: Long-term effects of different nitrogen levels on growth, yield, and quality in sugarcane, Agronomy, 10, 353, https://doi.org/10.3390/agronomy10030353, 2020.

Zhang, S., Li, X., Chen, K., Shi, J., Wang, Y., Luo, P., Yang, J., Wang, Y., and Han, X.: Long-term fertilization altered microbial community structure in an aeolian sandy soil in northeast China, Front. Microbiol., 13, 979759, https://doi.org/10.3389/fmicb.2022.979759, 2022.

Zhang, Y., Cheng, Z., Li, Q., Dai, Q., and Hu, J.: Responses of rhizosphere bacterial communities in newly reclaimed mudflat paddies to rice genotype and nitrogen fertilizer rate, Environ. Sci. Pollut. Res. Int., 30, 38761–38774, https://doi.org/10.1007/s11356-022-25020-2, 2023.

Zhong, X., Li, J., Li, X., Ye, Y., Liu, S., Hallett, P. D., and Ogden, M. R.: Physical protection by soil aggregates stabilizes soil organic carbon under simulated N deposition in a subtropical forest of China, Geoderma, 285, 323–332, https://doi.org/10.1016/j.geoderma.2016.09.026, 2017.