Manure amendment and the rhizosphere of *Amaranthus hybridus* soil bacterial communities in a semi-arid irrigated agroecosystem

Enmienda con estiércol y comunidades bacterianas rizosféricas de *Amaranthus hybridus* en un agroecosistema irrigado semiárido

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Scientific article

Received: March 07, 2026
Accepted: August 11, 2026

ABSTRACT. Soil biodiversity is fundamental to nutrient cycling and ecosystem functioning in agroecosystems, yet the combined effects of organic amendments and weed rhizospheres on soil bacterial communities remain insufficiently explored in semi-arid irrigated systems. We evaluated soil physicochemical properties and bacterial diversity inferred from 16S rRNA gene amplicon sequencing in three soil treatments: initial bulk soil (T1), bulk soil amended with cattle manure at 20 Mg ha^{-1} and sampled 11 days after application (T2), and the rhizosphere of *Amaranthus hybridus* sampled 75 days after manure application (T3). Alpha diversity (Faith's phylogenetic diversity, Shannon diversity, and evenness), beta diversity (Bray–Curtis, weighted UniFrac, and unweighted UniFrac), PERMANOVA, and redundancy analysis (RDA) were used to characterize bacterial communities. Manure amendment increased bacterial richness and alpha diversity, whereas the *A. hybridus* rhizosphere exhibited intermediate diversity, a distinct taxonomic composition, and higher micronutrient concentrations. Multivariate analyses further demonstrated clear differences in bacterial community composition among treatments and strong associations between dominant bacterial taxa and soil physicochemical gradients. These findings indicate that manure amendment and rhizosphere-associated processes jointly promote heterogeneous bacterial community configurations in semi-arid irrigated agroecosystems.
Keywords: 16S rRNA, soil bacterial diversity, soil ecological engineering.

RESUMEN. La biodiversidad del suelo es fundamental para el ciclado de nutrientes y el funcionamiento de los agroecosistemas; sin embargo, los efectos combinados de las enmiendas orgánicas y la rizósfera de las malezas sobre las comunidades bacterianas del suelo han sido poco estudiados en sistemas semiáridos bajo riego. En este estudio se evaluaron las propiedades fisicoquímicas del suelo y la diversidad bacteriana inferida mediante la secuenciación de amplicones del gen 16S rRNA en tres tratamientos de suelo: suelo inicial sin enmienda (T1), suelo enmendado con estiércol bovino a razón de 20 Mg ha^{-1} y muestreado 11 días después de su aplicación (T2), y suelo asociado a la rizósfera de *Amaranthus hybridus*, muestreado 75 días después de la aplicación del estiércol (T3). La diversidad bacteriana se caracterizó mediante diversidad alfa (diversidad filogenética de Faith, diversidad de Shannon y equidad), diversidad beta (Bray–Curtis, UniFrac ponderado y no ponderado), PERMANOVA y análisis de redundancia (RDA). La aplicación de estiércol incrementó la riqueza bacteriana y la diversidad alfa, mientras que la rizósfera de *A. hybridus* presentó una diversidad intermedia, una composición taxonómica distintiva y mayores concentraciones de micronutrientes. Los análisis multivariados evidenciaron diferencias claras en la composición de las comunidades bacterianas entre tratamientos y una estrecha asociación entre los principales taxa bacterianos y los gradientes fisicoquímicos del suelo. En conjunto, estos resultados indican que la aplicación de estiércol y los procesos asociados a la rizósfera interactúan para incrementar la heterogeneidad de las comunidades bacterianas en agroecosistemas semiáridos bajo riego.
Palabras clave: 16S rRNA, diversidad bacteriana del suelo, ingeniería ecológica del suelo.

How to cite: Vásquez-Arroyo J, Nava-Reyna E, Cabrera-Rodríguez A, Zapata-Sifuentes G, Valenzuela-Núñez LM, García-De la Peña **C** (2026) Manure amendment and the rhizosphere of *Amaranthus hybridus* soil bacterial communities in a semi-arid irrigated agroecosystem. Ecosistemas y Recursos Agropecuarios Núm. Esp. VI: e5239. DOI: 10.19136/era.a13nVI.5239.

INTRODUCTION

Drought and soil degradation are becoming increasingly widespread worldwide and are projected to intensify as climate change progresses, posing major challenges for food security and the long-term sustainability of agricultural ecosystems. Soil biodiversity underpins essential ecosystem functions, including nutrient cycling, soil structure maintenance, and plant productivity, yet the mechanisms through which agricultural management shapes belowground microbial communities remain incompletely understood, particularly in semi-arid regions (Tsiafouli *et al.* 2015, Schmidt *et al.* 2019). Under these environmentally constrained conditions, developing management practices that maintain soil fertility while conserving microbial diversity has become a key objective for sustainable agriculture (Chen *et al.* 2023).

Agricultural management strongly influences the physicochemical environment in which soil organisms and plant roots interact. Common agronomic practices, including tillage, fertilization, irrigation, and the incorporation of organic amendments, alter soil organic matter (SOM), nutrient availability, pH, and salinity, ultimately affecting the abundance, activity, and composition of soil biota (Meena *et al*. 2017, Zegeye *et al*. 2019). Among these practices, the application of animal manure has been widely adopted as a means of recycling nutrients, increasing SOM, and improving soil structure, particularly in low-input and resource-constrained agricultural systems. Nevertheless, its effects on bacterial diversity and community composition have not been consistent across studies, as responses depend on factors such as manure characteristics, application rates, soil properties, and local climatic conditions (Kushwaha *et al*. 2024). These contrasting observations highlight the importance of evaluating manure effects under specific environmental and management settings rather than relying solely on generalized responses.

In addition to the effects of organic amendments on soil properties, plant roots actively shape the rhizosphere by generating physicochemical and biological gradients that influence microbial colonization. Through root exudation, rhizodeposition, and localized changes in pH and redox potential, plants create heterogeneous microhabitats that favor microbial groups with functional traits involved in nutrient mobilization, stress tolerance, and plant growth promotion (Ofek-Lalzar *et al.* 2014, Ke *et al.* 2021). Although the rhizospheres of cultivated crops have received most research attention, arable weeds also support distinctive microbial assemblages. In several weed species, these communities differ substantially from those inhabiting adjacent bulk soil and have been linked to nutrient cycling, organic matter turnover, and, in some cases, suppression of soil-borne pathogens (Sun *et al*. 2021, Fernández-Huarte *et al.* 2023).

Within semi-arid irrigated agroecosystems, weeds are generally viewed as competitors for water and nutrients. However, their rhizospheres may also represent unique microbial habitats, particularly in soils receiving organic amendments. Changes in soil properties driven by manure application, together with rhizosphere-mediated microbial recruitment, are likely to influence bacterial community composition and promote niche differentiation within agricultural soils (Delgado-Baquerizo *et al*. 2016, Fernández-Huarte *et al.* 2023). Despite growing interest in these interactions, field-based studies simultaneously evaluating manure amendments and weed rhizospheres remain limited, especially under the environmental conditions characteristic of semi-arid regions in Mexico.

Based on these considerations, we hypothesized that cattle manure application together with the rhizosphere of naturally occurring weeds would influence soil bacterial diversity and community composition in semi-arid irrigated agroecosystems. Advances in high-throughput sequencing and bioinformatic approaches, including the QIIME 2–DADA2 pipeline combined with curated 16S rRNA reference databases, now provide robust tools for examining how these management components shape bacterial communities under field conditions (Chen *et al*. 2023). To address this hypothesis, we characterized bacterial communities from bulk soil, manure-amended soil, and the rhizosphere of the dominant weed *Amaranthus hybridus* using 16S rRNA amplicon sequencing in a semi-arid irrigated agroecosystem of northwestern Mexico. Specifically, our objectives were to: (i) determine how cattle manure and the *Amaranthus* rhizosphere influence soil physicochemical properties; (ii) evaluate their effects on bacterial alpha and beta diversity; and (iii) examine the relationships between bacterial community composition and gradients of soil fertility and micronutrient availability. We further expected that manure amendment together with the rhizosphere of *A. hybridus* would produce measurable shifts in bacterial diversity and community composition under these field conditions.

MATERIALS AND METHODS

Study area and experimental design

The field study was carried out during 2023 at the Universidad Autónoma Agraria Antonio Narro, Unidad Laguna, in Torreón, Coahuila, Mexico, within a semi-arid irrigated agroecosystem managed under drip irrigation. The experimental site was located between 25°33′22.4″–25°33′25.1″ N and 103°22′09.9″-103°22′14.3″ W. This region receives an average annual rainfall of approximately 258 mm and has a mean annual temperature of 21 °C, with typical maximum and minimum temperatures of 33.7 °C and 7.5 °C, respectively (Montemayor-Trejo *et al*. 2012). According to its textural classification, the soil was classified as sandy clay loam, whereas its physicochemical properties are summarized in Table 1.

Table 1. Soil physicochemical properties across three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *A. hybridus*). Values represent means ± standard deviation. Biological replicates were n = 4 for T1 and T2, and n = 3 for T3 due to sample loss during processing. Means within the same row followed by different letters indicate statistically significant differences among soil treatments ($P < 0.05$).

Soil treatments	pH	EC	Sand	Clay	Silt	EC	AD
T1	7.93 ± 0.04^{a}	4.34 ± 0.03^{b}	23.54 ± 0.05^{b}	17.56 ± 0.05^{c}	58.72 ± 0.03^{a}	28.25 ± 0.96^{a}	1.16 ± 0.03^{c}
T2	7.84 ± 0.03^{c}	6.335 ± 0.05^{a}	35.86 ± 0.04^{a}	21.67 ± 0.04^{b}	42.45 ± 0.05^{c}	25.25 ± 0.96^{b}	1.22 ± 0.01^{b}
T3	7.91 ± 0.04^{b}	3.76 ± 0.07^{c}	22.37 ± 0.15^{c}	29.42 ± 1.47^{a}	48.19 ± 1.47^{b}	21 ± 1.00^{c}	1.24 ± 0.01^{a}

Before the experiment was established, the field had remained uncultivated for more than six years, thereby minimizing the influence of recent agricultural practices on soil characteristics. The area was subsequently prepared for irrigated maize production using a drip irrigation system; however, limitations in water availability during field establishment affected crop development. Under these conditions, three soil treatments were selected for evaluation: (i) unamended bulk soil collected

before manure application (T1); (ii) bulk soil sampled 11 days after the application of cattle manure at a rate of 20 Mg ha^{-1} (T2); and (iii) rhizosphere soil associated with naturally occurring *A. hybridus* plants collected 75 days after manure application (T3). The organic amendment consisted of aged dairy cattle manure obtained from the dairy facilities of the Universidad Autónoma Agraria Antonio Narro, where it had been stockpiled for more than three years before being incorporated into the experimental field.

Soil and rhizosphere sampling

The experimental field was initially established for irrigated maize cultivation using two native landraces (R1 and R2) inoculated with three microbial inoculants (BUAP, UAAAN, and FCB), together with a non-inoculated soil treatment. To minimize potential cross-contamination among inoculants, the experimental layout consisted of individual plots separated by border rows. Each experimental unit comprised a 14-m row spaced 0.75 m apart, with the central 10 m designated as the effective sampling area after excluding 2 m at each end. Maize plants were established at 0.20-m intervals within rows, and the experiment was arranged into four spatial blocks distributed across the field. Sampling sites within each block were selected according to the original experimental design.

Bulk soil corresponding to the initial treatment (T1) was collected from the upper 0–20 cm soil layer before manure application using a five-point composite sampling strategy within each block. To evaluate the manure-amended treatment (T2), cattle manure was applied at a rate of 20 Mg ha^{-1}, and bulk soil samples were obtained 11 days later to assess early physicochemical changes together with initial bacterial community responses following amendment incorporation. Naturally occurring *A. hybridus* plants growing within the experimental area were used to represent the rhizosphere-associated treatment (T3). Rhizosphere samples were collected 75 days after manure application by carefully uprooting individual plants and gently recovering the soil that remained firmly attached to the root system.

Soil physicochemical analyses

Soil pH and electrical conductivity (EC) were measured in soil–water suspensions prepared at ratios of 1:2 and 1:2.5 (w/v), respectively, using a potentiometer and a conductivity meter with resolutions of 0.01 pH units and 0.01 dS m^{-1} (Luján-Soto *et al.* 2021). Soil organic matter (SOM) was determined following the Walkley-Black procedure (Walkley and Black 1934), whereas total nitrogen (N) was quantified by the Kjeldahl digestion method and available phosphorus (P) by Olsen extraction. Exchangeable Ca, Mg, K, and Na were extracted with ammonium acetate and subsequently quantified by atomic absorption spectrometry (Perkin-Elmer Model 2380, USA). The same analytical platform was used to determine DTPA-extractable micronutrients (Cu, Fe, Zn, and Mn). Soil texture was evaluated using the hydrometer method to estimate the relative proportions of sand, silt, and clay. All physicochemical analyses were performed in triplicate, and the resulting values are expressed on an oven-dry basis.

DNA extraction and 16S rRNA amplicon sequencing

Bacterial community composition was characterized by amplifying the V3-V4 region of the 16S rRNA gene with primers 341F and 785R carrying Illumina overhang adapters. PCR amplifications

were conducted in a final reaction volume of 25 µL containing template DNA, primers, dNTPs, reaction buffer, and Taq DNA polymerase. Amplification was carried out under the following thermal profile: an initial denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 5 min. Amplicons were verified by agarose gel electrophoresis, purified, and subsequently indexed using the Illumina dual-index system.

Sequencing was conducted on an Illumina MiSeq platform (Illumina Inc., USA) using 2 × 250 bp paired-end chemistry according to the manufacturer's recommendations for 16S rRNA amplicon sequencing. The resulting sequence data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1391542.

Bioinformatic processing

Raw sequence processing and diversity analyses were performed in QIIME 2 version 2023.5 (Bolyen *et al.* 2019), which was used for quality control, denoising, and inference of amplicon sequence variants (ASVs) following the DADA2 algorithm (Callahan *et al.* 2016). Paired-end reads underwent demultiplexing, quality filtering, trimming, and denoising, after which chimeric sequences were identified and removed to obtain high-quality ASVs. Taxonomic classification was carried out with a Naïve Bayes classifier trained on the Greengenes2 reference database (version 2025) for the 16S rRNA V3-V4 region. Taxonomic assignments were further verified against the NCBI Taxonomy database (McDonald *et al.* 2024). A phylogenetic tree was subsequently generated within QIIME 2 to support downstream phylogenetic diversity analyses.

Diversity indices and statistical analyses

Alpha diversity was estimated from a rarefied ASV table to minimize the effects of differences in sequencing depth among samples. Richness (Observed ASVs), Shannon diversity, Simpson diversity, and Faith's phylogenetic diversity were calculated for each sample. Comparisons among soil treatments (T1, T2, and T3) were performed using Kruskal–Wallis tests followed by Dunn's post hoc multiple-comparison procedure. Beta diversity was assessed using Bray-Curtis dissimilarity together with weighted and unweighted UniFrac distance matrices. Sample relationships were visualized by principal coordinate analysis (PCoA), whereas differences in bacterial community composition among soil treatments were evaluated by PERMANOVA using 999 permutations.

Associations between bacterial community composition and soil physicochemical variables were further examined using principal component analysis (PCA) and redundancy analysis (RDA). All multivariate analyses were conducted in R with the vegan package, whereas heatmaps and hierarchical clustering were generated using ClustVis version 2.0 (Metsalu and Vilo 2015). Unless otherwise specified, results are presented as mean ± standard deviation, and statistical significance was defined at $P < 0.05$.

RESULTS

Significant differences in soil physicochemical properties were observed among the three soil treatments (T1, T2, and T3; Table 1). The manure-amended soil (T2) was characterized by the highest electrical conductivity (EC), soil organic matter (SOM), and macronutrient concentrations, including available phosphorus and exchangeable K, Ca, Mg, and Na. By comparison, the initial bulk soil (T1) displayed intermediate values for most of these variables. In contrast, rhizosphere-associated soil of *A. hybridus* (T3) contained lower SOM and EC values but greater clay content together with higher concentrations of the micronutrients Cu, Fe, Zn, and Mn. Taken together, these differences illustrate the contrasting edaphic environments generated by manure amendment and rhizosphere-associated soils (Table 2).

Table 2. Soil chemical properties across three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *A. hybridus*). Values represent means ± standard deviation. Biological replicates were n = 4 for T1 and T2, and n = 3 for T3 due to sample loss during processing. Means within the same row followed by different letters indicate statistically significant differences among soil treatments ($P < 0.05$).

Soil treatments	SOM	N	P	K	Ca	Mg	Na	Cu	Fe	Zn	Mn
T1	2.58 ± 0.02^{b}	0.10 ± 0.01^{b}	18 ± 0.08^{b}	0.53 ± 0.03^{c}	27.44 ± 0.05^{c}	4.12 ± 0.02^{b}	9.14 ± 0.03^{b}	0.87 ± 0.02^{b}	0.34 ± 0.03^{c}	1.82 ± 0.03^{c}	6.84 ± 0.03^{b}
T2	2.73 ± 0.05^{a}	0.13 ± 0.02^{a}	42.41 ± 0.01^{a}	0.78 ± 0.02^{a}	41.33 ± 0.03^{a}	6.16 ± 0.04^{a}	15.02 ± 0.01^{a}	0.74 ± 0.02^{c}	0.99 ± 0.01^{b}	2.22 ± 0.01^{b}	2.92 ± 0.03^{c}
T3	1.13 ± 0.13^{c}	0.10 ± 0.00^{b}	17.51 ± 0.23^{c}	0.66 ± 0.01^{b}	29.40 ± 1.30^{b}	3.73 ± 0.15^{c}	4.48 ± 0.78^{c}	1.34 ± 0.05^{a}	1.82 ± 0.11^{a}	2.75 ± 0.01^{a}	18.38 ± 1.13^{a}

Principal component analysis (PCA) integrated these patterns and clearly differentiated soil treatments in multivariate space. Soil T2 was associated with higher SOM, EC, and macronutrient levels, whereas T3 aligned with gradients of clay content and micronutrients (Mn, Fe, Cu, and Zn) (Figure 1). The initial bulk soil (T1) occupied an intermediate position between these two contrasting soil treatments. These patterns suggest that manure-associated soil conditions and the *Amaranthus* rhizosphere were linked to contrasting edaphic characteristics relative to the initial bulk soil treatment.

Redundancy analysis (RDA), constrained by soil physicochemical variables, clearly differentiated the three soil treatments in canonical space (Figure 2). Samples from the manure-amended treatment (T2) were distributed along gradients characterized by elevated SOM, EC, and macronutrient concentrations, together with greater relative abundances of Proteobacteria, Bacteroidota, and Firmicutes_D, consistent with the taxonomic patterns summarized in Table 3. In contrast, the initial bulk soil (T1) was primarily associated with pH, silt content, and intermediate nutrient levels, while its ordination position corresponded closely with the predominance of Actinobacteriota and Planctomycetota. The rhizosphere-associated samples (T3) occupied a separate region of the ordination, corresponding to higher clay content and increased concentrations of Mn, Fe, Cu, and Zn, and were characterized by a greater representation of Gemmatimonadota, Acidobacteriota, and Myxococcota.

Overall, the RDA highlights a strong coupling between edaphic treatments and phylum-level community composition, demonstrating that manure addition and the *Amaranthus* rhizosphere induce contrasting but well-defined shifts in bacterial assemblages relative to the initial soil condition.

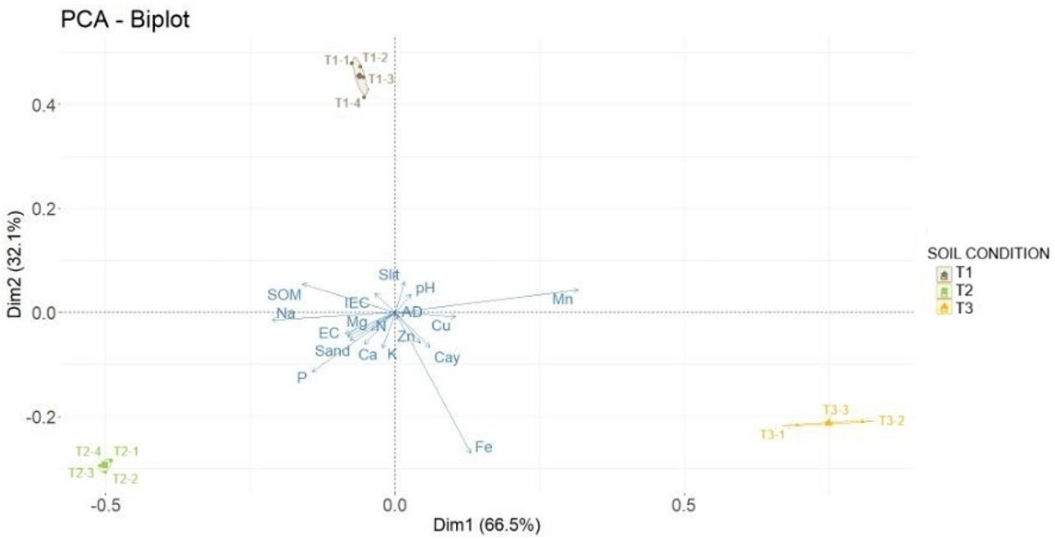

Figure 1. Principal component analysis (PCA) ordination of soil physicochemical properties across three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *A. hybridus*).

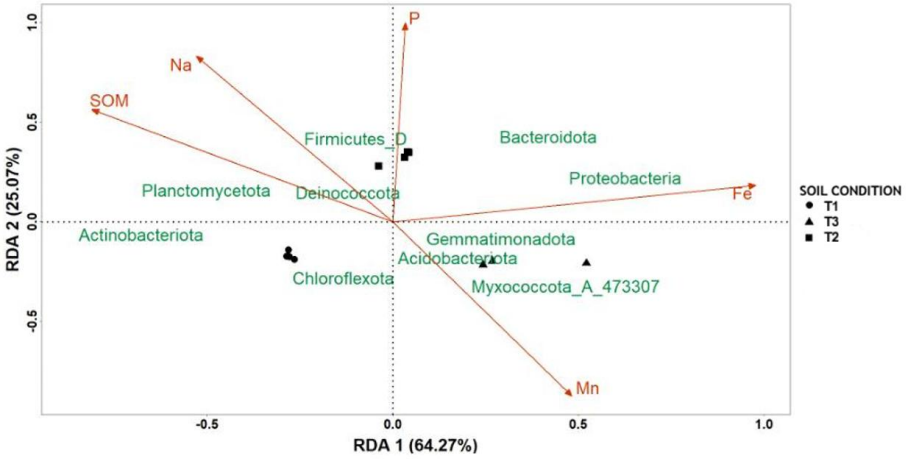

Figure 2. Redundancy analysis (RDA) ordination illustrating the relationships among bacterial community composition at the phylum level, soil physicochemical variables, and the three evaluated soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*).

Taxonomic profiling demonstrated high bacterial richness across all soil treatments, although differences became evident from the order level upward (Table 4). At the phylum level, both T1 and T2 contained 43 phyla, whereas the rhizosphere-associated soil (T3) comprised 40 phyla. Greater taxonomic richness at the lower hierarchical levels was observed in T2, comprising 269 orders, 480 families, 1,035 genera, and 1,391 taxa assigned at the species level. By comparison, T1 contained 241 orders, 414 families, 828 genera, and 1,035 species-level assignments. The rhizosphere-associated soil (T3) showed intermediate richness across most taxonomic ranks, with 264 orders, 445 families, 844 genera, and 1,060 species-level assignments. An exception to this

overall pattern was observed at the class level, where T3 exhibited 90 classes, exceeding the values recorded for T1 and T2 (86 and 84 classes, respectively).

Table 3. Relative abundance (%) of the 20 most abundant bacterial phyla across three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *A. hybridus*).

Phyla	T1	T2	T3
Actinobacteriota	48.58	30.78	19.30
Proteobacteria	17.60	30.15	35.56
Chloroflexota	9.05	5.32	7.76
Gemmatimonadota	6.18	6.35	10.71
Planctomycetota	4.60	4.04	0.58
Acidobacteriota	4.47	3.04	6.56
Firmicutes_D	2.37	6.78	1.69
Bacteroidota	2.24	8.94	6.72
Other	1.33	0.97	1.56
Myxococcota_A	0.99	0.88	5.79
Verrucomicrobiota	0.85	1.20	1.48
Desulfobacterota_B	0.58	0.32	0.37
Methylomirabilota	0.22	0.13	0.18
Deinococcota	0.20	0.34	0.04
Nitrospirota_A	0.20	0.12	0.31
Tectomicrobia	0.10	0.04	0.03
Patescibacteria	0.09	0.23	0.34
Sumerlaeota	0.08	0.05	0.16
Cyanobacteria	0.08	0.08	0.09
Bdellovibrionota_E	0.07	0.07	0.20

Table 4. Taxonomic richness of bacterial communities across major taxonomic ranks under three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*).

Soil condition	Phyla	Classes	Orders	Families	Genus	Species
T1	43	86	241	414	828	1035
T2	43	84	269	480	1035	1391
T3	40	90	264	445	844	1060

Venn diagrams were used to examine patterns of shared and unique bacterial taxa among the three soil treatments. At the phylum level, most phyla were common to all treatments, whereas smaller subsets were detected exclusively in T2 and T3 (Figure 3). A similar pattern was observed at the genus level, where a large proportion of genera was shared across soil treatments, although numerous genera occurred exclusively in the manure-amended soil (T2) or in the rhizosphere-associated soil (T3) (Figure 4). These patterns are consistent with greater taxonomic differentiation associated with manure amendment and the rhizosphere environment.

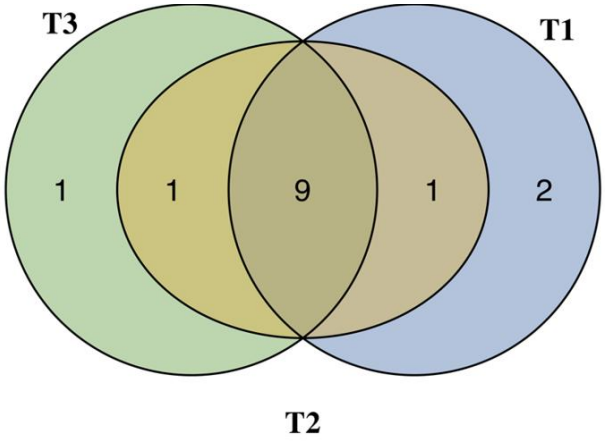

Figure 3. Venn diagram showing the overlap of bacterial phyla among three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*).

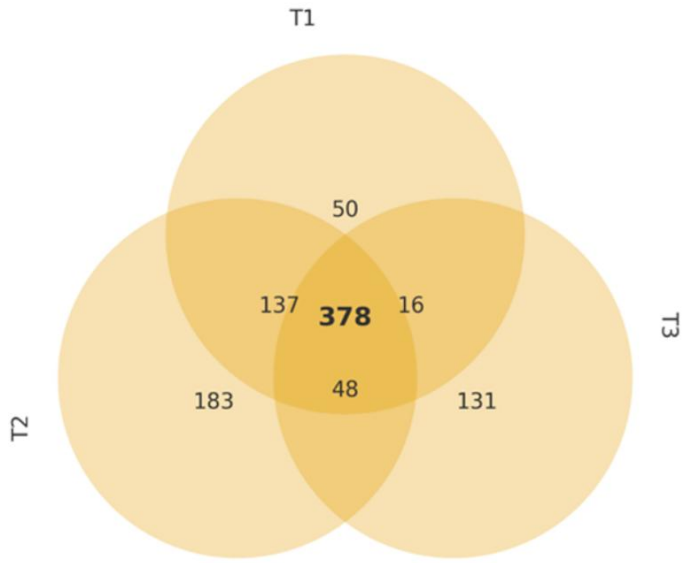

Figure 4. Venn diagram showing the overlap of bacterial genera among three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*).

At the phylum level, bacterial communities were dominated by Actinobacteriota and Proteobacteria, although their relative abundances varied markedly among soil treatments (Table 3). The unamended bulk soil (T1) was characterized by a strong predominance of Actinobacteriota (48.58%), followed by Proteobacteria (17.60%) and Chloroflexota (9.05%). Following manure amendment, the contribution of Actinobacteriota declined to 30.78%, whereas Proteobacteria (30.15%), Bacteroidota (8.94%), and Firmicutes_D (6.78%) became comparatively more abundant. A different community profile was observed in the *A. hybridus* rhizosphere (T3), where Proteobacteria represented the dominant phylum (35.56%), accompanied by increased relative abundances of Gemmatimonadota (10.71%), Bacteroidota (6.72%), and Acidobacteriota (6.56%)

compared with T1. Collectively, these compositional shifts illustrate the marked restructuring of bacterial communities in response to manure amendment and the rhizosphere environment.

Genus-level profiles revealed considerable bacterial diversity across the three soil treatments, although between 35.9% and 41.5% of sequence reads remained unclassified at this taxonomic rank ("Unassigned") (Table 5). Among the identified genera, several exhibited distinct distribution patterns across treatments. The unamended bulk soil (T1) showed the highest relative abundances of genera commonly associated with semi-arid soils, including *Nocardioides*, *Solirubrobacter*, *Pseudonocardia*, *Blastococcus*, and *Streptomyces*. In contrast, manure amendment (T2) was accompanied by an increased representation of *Cellvibrio* (2.8%), together with higher abundances of UBA2421, HRBIN40, and other genera involved in the degradation of readily available organic substrates. The rhizosphere-associated soil (T3) was distinguished by greater abundances of *Sphingomicrobium*, *Microvirga*, and *Longimicrobium*, genera frequently reported from rhizosphere environments and likely favored by root exudation together with micronutrient-enriched microhabitats. Together, these genus-level patterns reinforce the strong influence of manure amendment and rhizosphere-associated processes on bacterial community composition.

Table 5. Relative abundance (%) of the 15 predominant bacterial genera across three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*).

Genus	T1	T2	T3
Unassigned	41.5	35.9	39.9
AC-16	3.3	1.5	1.0
*Sphingomicrobium*_483265	1.6	1.4	2.5
UBA2421	2.6	2.3	0.3
Nocardioides_A_392796	2.9	1.5	0.6
HRBIN40	2.2	1.0	1.4
Microvirga	1.8	1.2	1.6
Solirubrobacter	2.8	1.2	0.5
Pseudonocardia	2.0	1.0	0.9
Longimicrobium	0.7	0.9	2.2
Blastococcus	1.8	1.1	0.8
CF-167	2.1	1.0	0.6
GWC2-73-18	1.5	0.8	0.8
Cellvibrio	0.0	2.8	0.1
*Streptomyces*_G_399870	1.3	0.9	0.6

Alpha diversity metrics varied significantly among the three soil treatments, revealing distinct alpha diversity profiles (Figure 5). The manure-amended soil (T2) consistently showed the highest values of Faith's phylogenetic diversity and Shannon diversity, whereas the lowest values were recorded in the initial bulk soil (T1); the rhizosphere-associated soil (T3) displayed intermediate values for both indices. Kruskal-Wallis analyses confirmed significant differences among soil treatments for Shannon diversity ($P \leq 0.05$), whereas Faith's phylogenetic diversity and evenness did not differ significantly among treatments. This pattern indicates that differences among soil

treatments were primarily associated with changes in richness and phylogenetic breadth rather than with pronounced shifts in community dominance. The alpha diversity results closely paralleled the taxonomic richness patterns, with T2 showing the greatest richness, T3 occupying an intermediate position, and T1 exhibiting the lowest richness.

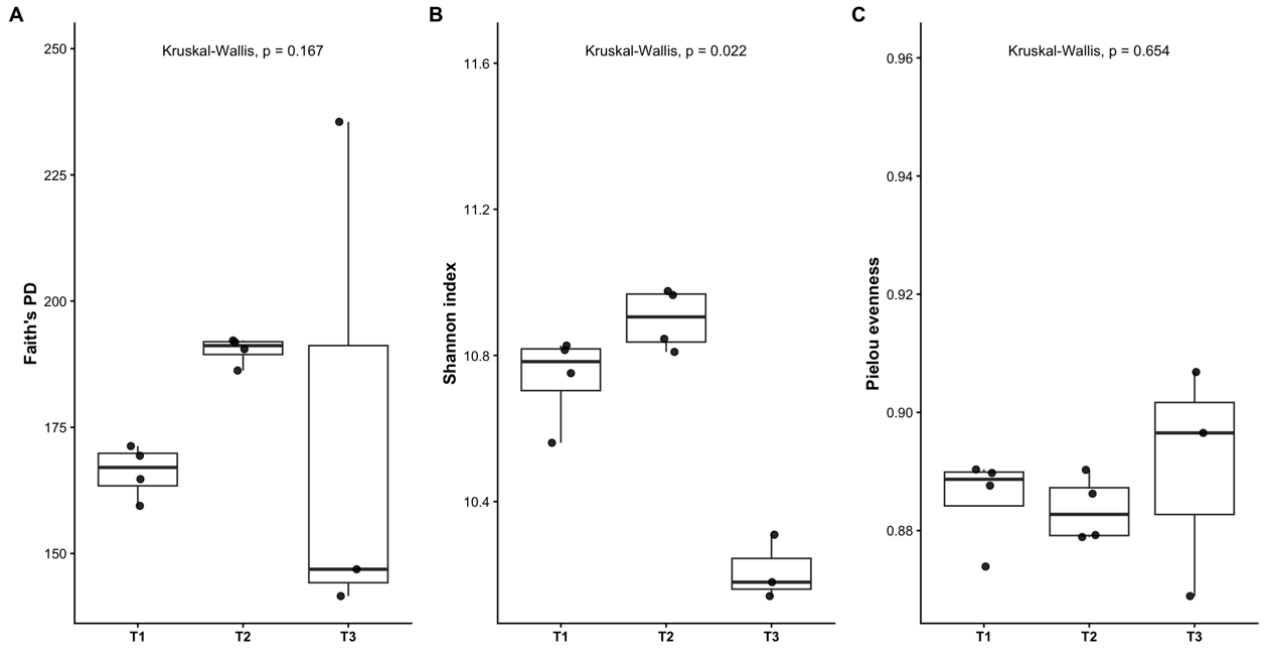

Figure 5. Alpha diversity patterns of bacterial communities under three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*). Box plots illustrate Faith's phylogenetic diversity, Shannon diversity, and evenness.

Beta diversity analyses based on Bray–Curtis dissimilarity together with weighted and unweighted UniFrac distances demonstrated marked differences in bacterial community composition among the three soil treatments (Figure 6). In the principal coordinate analysis (PCoA), samples showed treatment-associated spatial differentiation, with T1, T2, and T3 occupying distinct regions of the ordination despite within-treatment variation. This pattern was consistent with the significant differences detected by PERMANOVA ($P < 0.01$).

DISCUSSION

The present study was carried out under field conditions in a semi-arid irrigated agroecosystem, where soil bacterial communities are shaped by multiple interacting factors in addition to manure amendment and rhizosphere-associated processes. Environmental variables such as irrigation regime, soil moisture dynamics, temperature, and plant developmental stage are all likely to influence microbial recruitment and the physicochemical characteristics of the soil. Consequently, changes in water availability and localized rhizosphere conditions may alter nutrient distribution, salinity, and microbial colonization patterns over time. Because the three soil treatments were sampled at different intervals following manure application, part of the observed variation in

bacterial community composition may also reflect temporal changes associated with crop development, irrigation management, and seasonal environmental conditions.

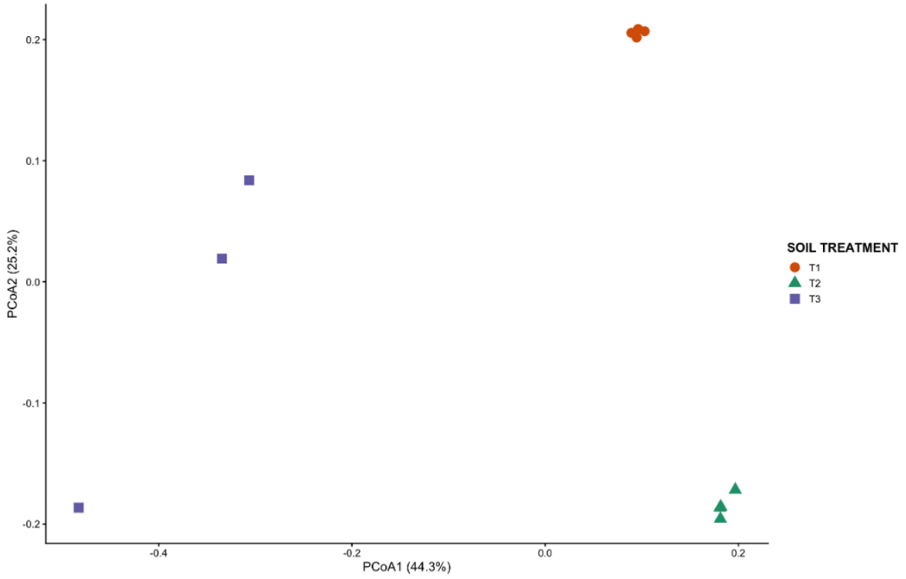

Figure 6. Principal coordinate analysis (PCoA) illustrating beta diversity patterns of bacterial communities under three contrasting soil treatments: T1 (initial bulk soil), T2 (bulk soil amended with cattle manure), and T3 (rhizosphere-associated soil of *Amaranthus hybridus*). Ordinations were generated from Bray–Curtis dissimilarity together with weighted and unweighted UniFrac distance matrices. Separation among soil treatments was supported by PERMANOVA ($P < 0.01$).

PERMANOVA analyses demonstrated significant differences among soil treatments for all distance metrics ($P < 0.01$), with the greatest separation observed between T1 and T2 and between T1 and T3. These compositional differences were accompanied by marked variation in soil physicochemical properties and bacterial taxonomic composition, supporting the view that manure amendment and rhizosphere-associated processes contributed to the development of distinct microbial communities within this semi-arid irrigated agroecosystem. Compared with the initial bulk soil, the manure-amended treatment (T2) contained higher SOM, EC, and macronutrient concentrations, whereas the rhizosphere-associated soil of *A. hybridus* (T3) was characterized by greater clay content together with elevated concentrations of micronutrients. Similar relationships between organic amendments, rhizosphere processes, and shifts in soil physicochemical conditions have been documented previously, with corresponding changes in nutrient availability and microbial habitat heterogeneity that influence bacterial community composition (Hartmann *et al.* 2015, Zegeye *et al.* 2019). Because manure application and rhizosphere development occurred under open-field conditions, it is unlikely that a single factor explains the observed community shifts. Rather, the bacterial assemblages appear to reflect the combined influence of organic inputs, spatial heterogeneity within the soil, and rhizosphere-mediated processes acting simultaneously throughout the experiment.

The greater bacterial richness and alpha diversity observed in the manure-amended soil (T2) support the idea that organic amendments expand both the diversity of available resources and the

range of ecological niches accessible to soil microorganisms, particularly in nutrient-limited or degraded environments. By introducing carbon together with readily available and more recalcitrant nutrient sources, manure creates conditions that favor copiotrophic bacteria while simultaneously allowing the coexistence of microbial groups with contrasting ecological strategies (Lupatini *et al.* 2017, Liu *et al.* 2021). Similar responses have been reported in a variety of cropping systems, where manure or compost applications consistently increased microbial diversity relative to mineral fertilization or unamended soils, especially under dryland and semi-arid conditions. Nevertheless, previous studies also indicate that the magnitude of these responses is strongly influenced by local soil properties, climatic conditions, and the characteristics of the organic amendment, emphasizing the importance of interpreting manure effects within their specific environmental context. Taken together, these results indicate that manure amendment increased not only bacterial diversity but also the ecological opportunities available for microbial colonization under the semi-arid conditions evaluated here.

Compared with both bulk soil treatments, the *A. hybridus* rhizosphere (T3) displayed intermediate alpha diversity together with a clearly differentiated taxonomic composition. This response agrees with the well-established rhizosphere effect, whereby plant roots selectively recruit particular microbial groups while limiting the establishment of others (Bulgarelli *et al.* 2013, Trivedi *et al.* 2020). Rather than representing a simple extension of the manure-associated community, the rhizosphere supported a distinct assemblage that included taxa commonly associated with root-influenced microhabitats together with genera that may benefit from root exudation, localized pH gradients, and increased micronutrient availability. These observations reinforce the view that arable weeds contribute to microbial heterogeneity within agricultural soils by maintaining bacterial assemblages that differ from those of the surrounding bulk soil. Such assemblages may have important implications for nutrient cycling and plant–microbe interactions under field conditions (Sun *et al.* 2021, Fernández-Huarte *et al.* 2023). From an ecological perspective, our findings suggest that the rhizosphere of naturally occurring weeds represents an additional source of microbial heterogeneity within managed agricultural soils.

Differences in beta diversity provided additional evidence that manure amendment and the *A. hybridus* rhizosphere influenced bacterial community composition under field conditions. The clear separation observed in Bray–Curtis and UniFrac ordinations, together with the significant PERMANOVA results, indicates that each soil treatment supported a distinct bacterial assemblage. The pronounced divergence between the initial bulk soil (T1) and the manure-amended soil (T2) is consistent with the well-documented restructuring of microbial communities following organic amendment. By contrast, the partial overlap between T2 and the rhizosphere-associated soil (T3) suggests that the rhizosphere retained bacterial groups shared with manure-amended soil while simultaneously selecting additional taxa associated with root-influenced microhabitats. Collectively, these observations reinforce previous evidence showing that soil management practices and rhizosphere-mediated processes are major drivers of bacterial beta diversity and community differentiation in agricultural soils (Delgado-Baquerizo *et al.* 2016, Fierer 2017).

The transition from Actinobacteriota-dominated communities in the initial bulk soil to assemblages enriched in Proteobacteria, Bacteroidota, Gemmatimonadota, and other phyla following manure amendment and within the *A. hybridus* rhizosphere is consistent with differences in nutrient

availability and organic resource inputs among soil conditions (Fierer *et al.* 2007, Hartmann *et al*. 2015). Members of Actinobacteriota and Chloroflexota commonly dominate semi-arid and desert soils, where they contribute to organic matter turnover and exhibit adaptations to drought and elevated temperatures. In contrast, Proteobacteria and Bacteroidota are widely recognized as responding rapidly to fresh organic inputs, the availability of readily degradable carbon, and plant-associated habitats (Peng et al. 2021). The increased representation of Gemmatimonadota and Acidobacteriota in the rhizosphere-associated soil further suggests that localized gradients generated at the root–soil interface create environmental conditions favorable for these bacterial groups. Taken together, these taxonomic shifts indicate that manure amendment and rhizosphere-associated processes reshape bacterial communities by modifying the availability of ecological niches and soil resources.

Genus-level patterns further reinforced the compositional differences observed among soil conditions following manure amendment and within the *A. hybridus* rhizosphere. The predominance of genera such as *Nocardioides*, *Solirubrobacter*, *Pseudonocardia*, *Blastococcus*, and Streptomyces in the initial bulk soil is consistent with their well-documented occurrence in semi-arid environments characterized by limited nutrient availability together with elevated thermal and desiccation stress. In contrast, manure amendment favored genera commonly associated with the decomposition of readily available organic substrates and nutrient transformations, whereas the rhizosphere supported bacterial groups frequently reported from plant-associated microbiomes, including taxa with potential roles in nitrogen fixation, phytohormone production, and micronutrient mobilization. Although functional traits cannot be confirmed from 16S rRNA gene sequencing alone, the observed taxonomic patterns are compatible with ecologically differentiated bacterial assemblages shaped by organic inputs and rhizosphere-mediated selection (Berendsen *et al.* 2012, Ke *et al.* 2021). Together, these genus-level changes complement the phylum-level patterns, indicating that manure amendment and rhizosphere-associated processes influence not only the overall taxonomic structure but also the ecological specialization of bacterial communities.

The integration of soil physicochemical characteristics with bacterial community structure through PCA and RDA revealed strong relationships between edaphic conditions and microbiome composition. Manure-amended soils were associated with gradients of higher SOM, EC, and macronutrient availability, whereas the *A. hybridus* rhizosphere was distinguished by greater clay content together with elevated concentrations of Mn, Fe, Cu, and Zn. These environmental gradients closely matched the distribution of the dominant bacterial phyla and discriminant genera within the ordination analyses, indicating that variation in soil resources and micronutrient availability was closely linked to bacterial community differentiation (Delgado-Baquerizo *et al.* 2017, Li *et al*. 2024). Rather than acting independently, organic inputs and rhizosphere-mediated processes appear to interact in shaping the soil environment, generating distinct ecological niches that favor different bacterial assemblages. Under the semi-arid irrigated conditions evaluated here, this interaction provides a plausible explanation for the differentiated bacterial community configurations observed across soil treatments.

Several limitations should be considered when interpreting these findings. First, the study was conducted at a single experimental site during one growing season, limiting the extent to which

the observed patterns can be generalized across years, management systems, or soil types. In addition, the use of composite samples within each soil treatment provided a robust characterization of local variability but did not capture broader spatial heterogeneity at the landscape scale. The present work also focused exclusively on bacterial communities inferred from 16S rRNA gene amplicon sequencing and therefore did not address other microbial groups, functional gene expression, or direct measurements of ecosystem processes and crop performance. Future studies would benefit from combining multi-site and multi-year experimental designs with complementary approaches such as enzyme activity assays, shotgun metagenomics, metatranscriptomics, and plant productivity measurements to better understand the functional consequences of the compositional changes documented here.

In summary, the present study demonstrates that manure amendment and the rhizosphere of naturally occurring *A. hybridus* are associated with distinct bacterial community configurations under semi-arid irrigated field treatments. Differences in soil physicochemical properties, bacterial diversity, and taxonomic composition collectively indicate that organic inputs and rhizosphere-mediated processes contribute to the generation of heterogeneous microbial habitats within agricultural soils. These findings expand our understanding of how soil management practices and naturally occurring plant–microbe interactions jointly shape bacterial community assembly in water- and nutrient-limited agroecosystems. More broadly, they provide an ecological framework for future studies exploring the role of beneficial soil microbiomes in promoting resilient and sustainable agricultural production under semi-arid conditions.

CONCLUSIONS

This study demonstrates that cattle manure amendment and the rhizosphere of naturally occurring *Amaranthus hybridus* are associated with distinct bacterial community configurations in semi-arid irrigated soils. Manure application promoted higher soil organic matter, nutrient availability, and bacterial alpha diversity, whereas the *A. hybridus* rhizosphere supported a taxonomically distinct bacterial assemblage associated with greater clay content and elevated concentrations of micronutrients (Cu, Fe, Zn, and Mn). Multivariate analyses consistently showed that differences in soil physicochemical properties were closely linked to shifts in bacterial community composition, including the transition from Actinobacteriota-dominated assemblages toward communities enriched in Proteobacteria, Bacteroidota, Gemmatimonadota, and other bacterial groups commonly associated with organic inputs and plant-influenced environments. Taken together, our results demonstrate that the interaction between organic amendments and naturally occurring rhizosphere processes contributes to the generation of heterogeneous microbial habitats within semi-arid agricultural soils. This ecological heterogeneity is likely to play an important role in the assembly and maintenance of bacterial communities under field conditions and provides a valuable foundation for future studies on sustainable soil management in water-limited agroecosystems.

ACKNOWLEDGMENTS

We thank the technical staff and students of the Departamento de Agroecología, Universidad Autónoma Agraria Antonio Narro, Unidad Torreón, for their support during field sampling and laboratory analyses.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Data availability

Raw sequence data generated in this study were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1391542.

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