

Article

The Diversity Indices of Culturable Bacteria from the Rhizosphere of *Pennisetum clandestinum* and *Pseudelephantopus spicatus* in Urban Soil

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Abstract

Urban soils are subject to intense anthropogenic disturbance, often resulting in biodiversity loss and reduced ecosystem functionality. However, rhizospheric microbial communities help maintain critical soil-ecosystem services, supporting urban soil resilience. This study evaluated the diversity of culturable bacteria associated with the rhizospheres of *Pennisetum clandestinum* and *Pseudelephantopus spicatus* in green areas of Medellín, Colombia, under contrasting levels of anthropic pressures. Rhizospheric and non-rhizospheric soils were sampled near automotive mechanic sites, and bacterial communities were assessed through plate counting and morphological characterization. Alpha, beta, and rarefaction diversity indices were applied to evaluate culturable morphotypes. *P. clandestinum* supported a more diverse and complex rhizospheric microbiome, particularly in non-exposed soils, while *P. spicatus* hosted less diverse communities under similar conditions. Diversity indices effectively distinguished microbial patterns, demonstrating the utility of culture-based methods for microbial community assessment. As a first step in microbial bioprospecting workflows, these methods allow for the rapid screening of culturable diversity and support decision-making for the selection of promising environments, plant species, and microbial isolates. This approach can inform urban soil threats, the promotion of beneficial plant-microbe interactions, and the identification of bioindicator species for soil health monitoring in a framework for the management of green areas.

Keywords: bioprospection; environmental monitoring; ecosystem services; functional redundancy; rarefaction; soil quality; urban biodiversity



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1. Introduction

Industrial operations, farming practices, and urban growth modify the soil's properties and its functionality, which are relevant for providing ecosystem services [1,2]. As the richness and complexity of its ecological structure expand, the biota engages in key soil

processes and contributes to environmental resilience [3]. A higher diversity of species increases the possibility of balancing soil processes under changing conditions. Soils retain their ecosystem activity balance within a wider range of tolerance to environmental changes as a result of functional redundancy [4]. Microorganisms, in particular, play a vital role in organic matter transformations, plant growth, nutrition cycles, pest control, pollutant attenuation, and the physicochemical development of soils [5]. Recent studies show that microorganisms play an important role in sustaining soil-ecosystem services in extreme environments [6] under intensive use conditions and climate-change scenarios [7]. This role extends to microbial communities in urban soils [8], degraded environments [9], locations with hazardous trace elements, including heavy metals [10], hydrocarbons [11], or pesticides [12], and during the implementation of remediation processes [13].

Microbial activity is assessed using respiration and biomass measurements, biochemical studies, and molecular approaches [14], varying in sensitivity and scope due to limitations such as edaphic medium heterogeneity, the spatial and temporal variability of soil properties, and difficulties in taxonomic classification levels in some categories [15]. In recent decades, modern molecular techniques, particularly next-generation sequencing, have greatly increased the study possibilities of soil microbiological features, allowing for the identification of many taxonomic groups connected with various biochemical processes [16]. Yet, the isolation and purification of many species continue to present challenges, making plate-counting techniques still necessary [17]. Each method is chosen based on the aims of the study, although in most cases, several or combinations of methods are required to obtain a broader interpretation of microbial diversity and activity [18].

Biological diversity is generally evaluated using indices based on species richness (alpha diversity), species dominance and relative abundance at a community level (beta diversity), models of species abundance, or combinations thereof [19,20]. Most microbiological studies have focused on rhizosphere activity, as the root–soil interface constitutes a dynamic habitat where most symbiotic associations between microorganisms and plants occur [21,22]. Plants provide organic matter to the soil while also releasing exudates rich in carbohydrates, amino acids, and organic acids through their roots, which stimulate the activity of microorganisms [23]. In this approach, vegetation promotes microbial variety, while distinct microbial species contribute to nutrition and water absorption, disease control, and tolerance to abiotic stressors via specialized mechanisms [24,25]. Understanding interactions between plants and microorganisms is essential for proper soil management, both from the perspective of microbial ecology and environmental quality [26].

Given the importance of symbiotic interactions for plants and their impact on soil qualities, the study of microbial communities in terms of richness, structure, and function is employed as an indicator of soil health and a tool to find potential for biological prospecting [27]. Molecular techniques have revealed a vast array of previously unculturable microorganisms, significantly enhancing biodiversity indices and improving our understanding of community structure. However, culture-based methods remain essential for targeting specific functional groups and recovering viable strains, especially during critical phases of microbial bioprospecting and the design of biotechnological applications that depend on pure culture isolation. Culture-based methods enable the direct assessment of morphological, physiological, and functional traits, aiding the identification of microorganisms [28,29]. Though culturing underestimates total diversity, it recovers viable organisms for further use like bioremediation or plant growth promotion, where the assessment of culturable strains remains essential for microbial consortia and bioinoculant development [30,31]. Following the exploration of culturable diversity, molecular tools provide a complementary approach to refine phylogenetic classification, assess functional potential through metagenomics, and link morphological traits to ecological roles, reinforcing

ing the integrative value of combining culture-dependent and molecular techniques in soil microbiology [32,33].

Soils in green areas such as parks, residential gardens, and recreational fields located within cities constitute an essential component of the urban ecological system, considered an indicator of residents' quality of life [34]. These areas provide ecosystem services such as water retention, runoff control, carbon sinks, and pollutant immobilization [35]. However, urban soils often exhibit reduced microbial diversity due to deteriorated physico-chemical properties, vegetation homogeneity, limited connectivity between green patches, and contamination by anthropogenic pollutants [36,37]. In tropical Andean cities like Medellín—Colombia's second most populated city—urban expansion and densification have led to a notable decline in grass and tree cover, fragmenting natural structures and intensifying the need to manage urban ecosystem services more effectively [38–40]. Grass species play a crucial role in the functionality of these green spaces, contributing to soil stabilization, biodiversity conservation, and microclimate regulation [41,42].

Pennisetum clandestinum and *Pseudelephantopus spicatus* are frequently found in urban lawns, road margins, and disturbed green spaces. Both species are valued for their fast growth, soil coverage, and resilience to anthropogenic stressors. *P. clandestinum*, commonly used in landscaping and soil cover, is a widespread grass in the Andean and tropical regions, while *P. spicatus* is a short-lived perennial herb native to the Americas and often found in Andean grasslands [43–45]. Recent studies suggest that urban rhizospheric diversity holds potential for discovering microorganisms relevant to environmental and pharmaceutical biotechnology applications and monitor soil quality [46–49]. Given their ecological roles and exposure to varying urban conditions, it is reasonable to expect that the rhizospheres of *P. clandestinum* and *P. spicatus* support distinct microbial communities influenced by environmental stressors and site-specific disturbances.

In microbial bioprospecting, the initial selection of promising environments is often guided by diversity patterns—either richness, evenness, or functional dominance—that suggest the influence of specific natural or anthropogenic factors fostering distinct microbial traits [50]. Thus, the presence of high microbial diversity or unique community structures in the rhizosphere can indicate selective niches where microorganisms with differentiated metabolic or functional capacities may reside [21]. Bioprospecting workflows typically proceed through microbial isolation, purification, and functional screening. Culture-dependent techniques play a central role in this pipeline, not only by enabling the recovery of viable organisms for subsequent characterization, but also by supporting taxonomic classification and the development of microbial inoculants [51,52]. While molecular methods such as high-throughput sequencing have advanced our ability to analyze uncultured microbial diversity, they do not substitute the need for culturable isolates in downstream applications like metabolite extraction, functional testing, or genomic manipulation [53]. Moreover, culture-based approaches offer the dual advantage of assessing diversity while simultaneously providing the basis for microbial isolation and downstream biotechnological development.

In this context, *Pennisetum clandestinum* and *Pseudelephantopus spicatus* are selected as representative models of urban plant–soil systems due to their ecological resilience and frequent presence in public green spaces. The hypothesis is that selecting appropriate diversity indices can enhance the detection of shifts in rhizospheric microbial composition, reflecting either plant-associated influences or responses to environmental stressors. Therefore, this study aims to describe the diversity of culturable bacterial communities in the rhizospheres of *P. clandestinum* and *P. spicatum* using plate-counting methods and ecological diversity indices. This integrative approach—combining culture-dependent techniques with ecological metrics—enables a rapid and informative assessment of microbial communities. As a tool for microbial characterization and the selection of biotechnological prospects, it supports

early-stage bioprospecting by identifying environments or host plants with high potential to harbor functionally significant microorganisms.

2. Materials and Methods

2.1. Study Area and Sampling

The study area is located in Medellín, Colombia (Figure 1). Green areas were selected at the coordinates $6^{\circ}16'43.50''$ N, $75^{\circ}35'53.40''$ W and an elevation of 1645 m above sea level. The area receives an average annual precipitation of 1685 mm with a bimodal distribution, where monthly means range from 70 to 210 mm, and the average annual temperature is 22°C , with mean monthly minimum and maximum temperatures of 17°C and 28°C , respectively [54]. This location falls within the humid montane forest life zone. According to the USDA Soil Taxonomy, these soils are classified within the Inceptisols order as Typic Dystrudepts and display features typical of mid- and upper-slope mountain landscapes, belong to the iso-thermic temperature regime and exhibit a udic moisture regime [55,56]. Due to the stable tropical climate, a single sampling was carried out and seasonality was not considered. Two sampling sites were established to represent contrasting land-use pressures on the soil, enabling comparison between areas with differing levels of urban impact. The first sampling site (exposed) was located near informal automotive mechanical workshops, where daily activities often result in liquid waste discharge and diffuse soil contamination. In contrast, the second sampling site (non-exposed) was situated in a nearby residential area with minimal influence from such anthropogenic activities, serving as a reference for lower urban impact. Soil samples were collected from both rhizospheric and non-rhizospheric zones for the plants *Pennisetum clandestinum* and *Pseudelephantopus spicatus*, as *P. clandestinum* is commonly used for urban green areas, and *P. spicatus* is a frequently occurring weed in these environments.

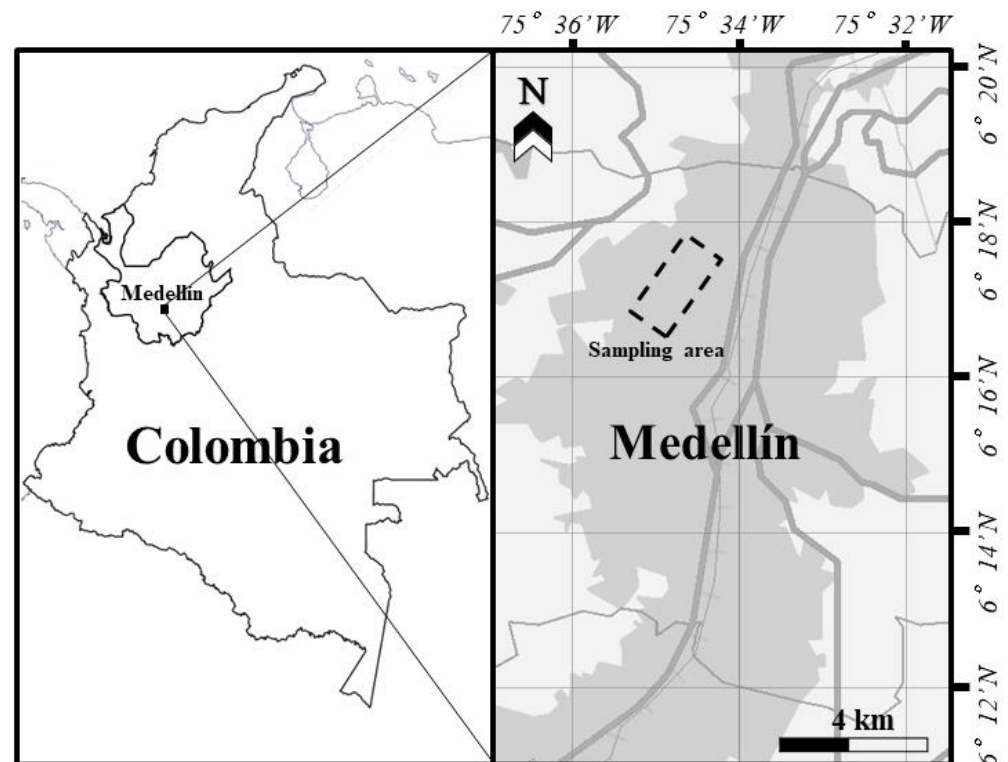


Figure 1. Location and study area.

Healthy individuals of *Pennisetum clandestinum* and *Pseudelephantopus spicatus* were randomly identified, and standard protocols were followed to obtain rhizospheric soil

subsamples from the two selected sites, respectively [57]. Samples were carefully extracted using a shovel to maintain its integrity as much as possible. The soil adhering to the roots of each individual ($n = 5$) was then collected, resulting in a composite sample of rhizospheric soil of each species. Subsequently, non-rhizospheric soil subsamples were collected from a depth of 20 to 40 cm in areas where root growth was not observed. Samples from each treatment were placed in clean plastic bags, stored in darkness at a temperature of 4 °C, and transported to the laboratory for immediate microbiological analysis.

2.2. Soil Analyses and Microorganisms Culture

Sub samples of soil were dried at 105 °C during 4 h and prepared for physical and chemical analysis following standard methods [58]. The samples were grinded and sieved with meshes 2 mm and 0.149 mm size. The hydrometer method was used to analyze soil texture, bulk density (D_b) was measured by the clod method, and pH measurements were determined with a glass electrode in water and 1.0 M KCl 1:1 (w/v) saturated paste, respectively. Soil organic carbon (OC) was determined by the Walkley Black method [59]. Olsen sodium-bicarbonate extraction followed by colorimetric analysis were utilized to determine extractable P [25]. The major elements Ca, K, and Mg were extracted with 1.0 N ammonium acetate at pH 7.0 and their concentrations were quantified by atomic absorption spectroscopy (iCE 3000 series, ThermoFisher Scientific Waltham, MA, USA).

Serial dilutions, plate counting, and morphological descriptions were used to analyze the microbial communities present in the soil samples. In brief, 10 g of soil from each composite sample was added to an Erlenmeyer flask containing 90 mL of sterile water and homogenized using an orbital shaker. Subsequently, using a sterile pipette, 1 mL of this suspension was transferred to a test tube with 9 mL of sterile water and agitated with a vortex mixer, resulting in a 10^{-2} dilution. The above was repeated from this tube to complete successive dilutions until reaching 10^{-6} . Dilutions of 10^{-3} and 10^{-6} were used for bacterial growth in culture medium. From each dilution, 0.1 mL was transferred to the center of a Petri dish containing Merk® nutrient agar culture medium and evenly spread using Drigalski loops. The procedure was carried out in triplicate for each treatment. Then, the seeded Petri dishes were incubated for 24 h at 28 °C [22].

Microbial colonies were described based on standard descriptors of their external morphological characteristics and colony-forming units (CFU g^{-1} soil $\times 10^3$) were counted. A difference in at least one characteristic was considered a morphotype. The visible features included color, shape, surface texture, and border [28] (Table 1). Color refers to the pigmentation of the colony, with observed colors including beige, yellow, light yellow, and white. Shape describes the overall form of the colony from a top view and was classified as circular (symmetrical and round), irregular (asymmetrical with uneven edges), filamentous (with thread-like extensions), or rhizoid (exhibiting root-like branching). Surface texture was categorized as rough (uneven or granular), smooth (even and flat), opaque (non-translucent), or bright (shiny or glossy). The border describes the appearance of the colony edge and was recorded as whole (smooth and continuous), curly (wavy or undulating), lobed (with lobe-like extensions), or filiform (thread-like or fringe-like).

Table 1. Morphological description criteria for identified bacterial colonies.

Class	Color	Shape	Surface	Border
Subclass	Beige	Circular	Rought	Whole
	Yellow	Irregular	Smooth	Curly
	Light yellow	Filamentous	Opaque	Lobed
	White	Rhizoid	Bright	Filiform

2.3. Statistics and Microbial Diversity Analyses

The counts of morphotypes were reported for each soil sample and plant as the mean colony-forming units (CFUs) $\pm$ standard error of the mean ($\bar{X} \pm \text{SEM}$), in terms of CFU g⁻¹. A hypothesis testing was developed in order to identify significant differences between the mean abundance values found between exposed and non-exposed soils, as well as for each morphotype. Data were assessed using Kolmogorov–Smirnov test and Bartlett tests and differences in mean abundance among all morphotypes found for each sample type were evaluated using ANOVA followed by Tukey’s HSD multiple-comparisons test. Alternatively, the Kruskal–Wallis method and Dunn’s comparison test were used as nonparametric analysis. Significance was set at $p < 0.05$ for all statistics. Statistical analyses and graph plotting were performed using Statgraphics Centurion XVI.I and GraphPad Prism 8 software, with a significance level of 95% for all tests.

Bacterial communities were compared using diversity indices, calculated using data corresponding to the number of morphotypes identified in the rhizospheric soil samples of *Pennisetum clandestinum* and *Pseudelephantopus spicatus*, as well as non-rhizospheric soil samples, in both the exposed and non-exposed soil sampling area. Alpha diversity was analyzed using the index of species richness, dominance, and evenness [18,60–64]. The software EstimateS 9.1.0 [65] was employed to generate abundance models using rarefaction curves, considering the number of morphotypes and individuals for each sample type, with a 95% confidence level for all observations. Beta diversity was estimated using Jaccard and Ochiai similarity coefficients [66]. Additionally, common morphotypes among samples were analyzed using Venn diagrams in the software Venny [67]. The calculation models for the diversity indices and used coefficients are presented in Table 2.

Table 2. Microbial diversity indices.

Index	Calculation	Description
Richness	S	S : Total number of morphotypes identified in a sample
Margalef	$D = \frac{(S-1)}{\ln N}$	N : Sum of the frequencies of the morphotypes observed in a sample
Menhinick	$D = \frac{S}{\sqrt{N}}$	
Shannon	$H' = - \sum_{i=1}^S p_i \log_2 p_i$	n_i : i Morphotype frequency $p_i = \frac{n_i}{N}$; Proportional abundance of the i morphotype
Simpson	$D = \sum_{i=1}^S \frac{n_i(n_i - 1)}{N(N - 1)}$	
Berger-Parker	$d = \frac{n_{max}}{N}$	n_{max} : Frequency of the most abundant morphotype.
Pielou’s equity	$J = \frac{H'}{\log_2 S}$	
Jaccard similarity coefficient	$I_J = \frac{c}{a+b-c}$	a : 1 On-site morphotypes. b : 2 On-site morphotypes. c : Common morphotypes at sites 1 and 2.
Ochiai coefficient	$I_O = \frac{c}{\sqrt{(a+c)(b+c)}}$	

The results of the Margalef and Menhinick indices, which are based on species count (richness), were interpreted using the following classification: values of $D < 1$ indicate very low diversity; $1 \leq D < 2$, low diversity; $2 \leq D < 3$, moderate to high diversity; and $D \geq 3$, very high diversity. The Shannon index (H'), which integrates both species richness and evenness, was interpreted as follows: $H' < 1$ reflects very low diversity; $1 \leq H' < 1.8$, low diversity; $1.8 \leq H' < 2.1$, moderate diversity; $2.1 \leq H' < 2.3$, high diversity; and $H' \geq 2.3$, very high diversity. Dominance indices, including Simpson’s index (reflecting the probability of randomly selecting individuals from the same species) and the Berger–

Parker index (representing the proportion of the most abundant species), were interpreted such that values close to 0 indicate highly diverse communities with no dominant species, while values near 1 suggest dominance by a few species and therefore low diversity. Pielou's evenness index ranges from 0 to 1, with values closer to 0 indicating uneven species distribution and values near 1 indicating uniformity in species abundance. Finally, similarity coefficients (I) were categorized as follows: $I < 20\%$ indicates very low similarity; $20\% \leq I < 40\%$, low; $40\% \leq I < 60\%$, moderate; $60\% \leq I < 80\%$, high; and $I \geq 80\%$, very high similarity.

3. Results and Discussion

3.1. Soil Characteristics

General properties of soils are presented in Table 3. Soils had a moderately to slightly acid reaction, sandy loam texture, and low organic carbon content, which varied slightly between samples. Bulk density is near the upper limit of the acceptable range for sandy loam soils ($<1.40 \text{ Mg/m}^3$) [68]. This type of alteration may be related to the low content of organic matter and some degree of compaction [69].

Table 3. General properties of soils.

Property	Units	Non-Exposed Soil	Exposed Soil
Sand	%	73	76
Clay	%	19	8
Lime	%	8	16
Texture		Sandy loam	Sandy loam
D_b	Mg/m^3	1.4	1.6
pH H_2O		6.3	5.9
pH KCl		5.5	5.6
OC	%	1.4	1.3
Ca	cmol/kg	11.9	12.7
Mg	cmol/kg	7.6	6.1
K	cmol/kg	0.3	0.4
P	mg/kg	8.7	5.8

The physicochemical characteristics of urban soils under different uses are important for plant growth and microbial activity [37]. The soil properties are slightly different; however, both showed low levels of nutrients K and P and excess Ca and Mg, taking as a reference the interpretation ranges for tropical mountain soils [70]. *P. clandestinum* is known to tolerate acidic soils but is sensitive to deficiencies in Mg, P, and especially K—an element that influences nitrogen uptake, which is itself a limiting factor for plant growth [71]. The imbalance in Ca/Mg and Mg/K ratios may negatively affect the development of plant species commonly used for green-space establishment in tropical cities like Medellín [72]. Additionally, soil organic carbon was lower than that recorded in soils with grass cover in a natural parks near the study area [41], suggesting that the influence of not only land cover but also land use can alter this property in urban areas.

3.2. Description of Culturable Microorganisms

A total of 16 bacterial morphotypes were identified, isolated from rhizospheric and non-rhizospheric soil samples of urban soil in both studied areas. The morphotypes were classified based on their morphological features, as presented in Table 4.

Table 4. Description of isolated bacterial morphotypes.

Morphotype	Color	Shape	Border	Surface
1	White	Rhizoid	Filiform	Flat
2	Light yellow	Irregular	Curly	Bright curly
3	White	Filamentous	Filiform	Flat
4	White	Irregular	Lobed	Wrinkled
5	White	Circular	Complete	Flat
6	Yellow	Irregular	Curly	Bright flat
7	White	Filamentous	Filiform	Wrinkled opaque
8	White	Irregular	Lobed	Flat
9	Beige	Filamentous	Filiform	Wrinkled
10	White	Irregular	Curly	Wrinkled opaque
11	Light yellow	Filamentous	Filiform	Flat
12	Beige	Filamentous	Filiform	Wrinkled
13	White	Filamentous	Filiform	Bright flat
14	White	Rhizoid	Lobed	Flat
15	Beige	Rhizoid	Filiform	Wrinkled
16	White	Irregular	Curly	Flat

Upon an analysis of the rhizospheric samples from *P. clandestinum*, a total of 12 morphotypes were identified (Figure 2). Of these, 11 were present in the non-exposed soil samples and 8 in the exposed samples, with 7 morphotypes shared between both conditions. No significant differences in abundance were observed for most morphotypes when comparing the two soil types. However, morphotypes 2 and 5 were significantly more abundant in the non-exposed rhizospheric samples ($p = 0.0394$). A similar trend was observed in the exposed samples, although it was not statistically significant ($p = 0.0754$).

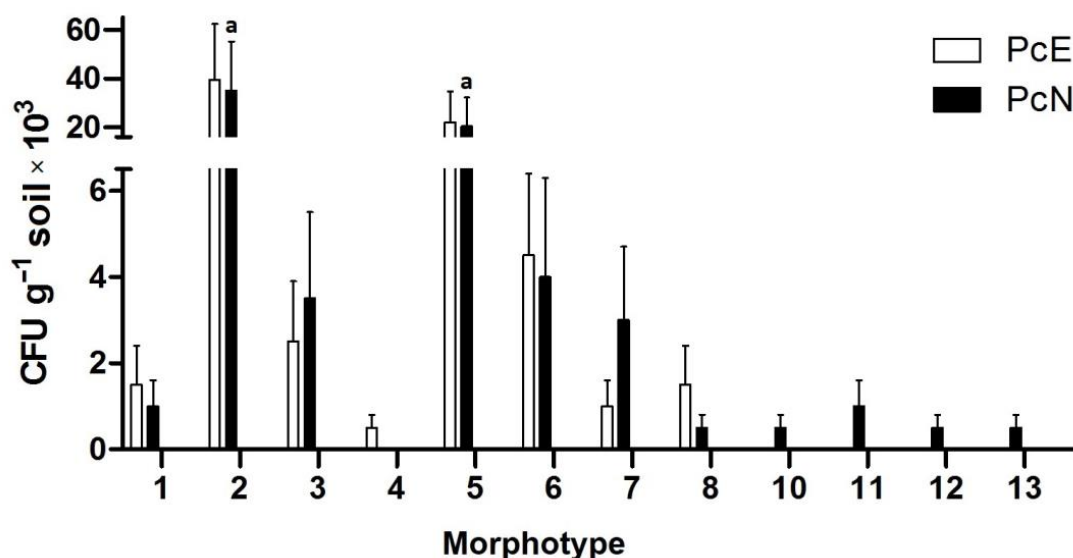


Figure 2. Bacterial count in rhizospheric soil samples of *Pennisetum clandestinum* (Pc); E: exposed soil; N: unexposed soil. Lowercase letters represent homogeneous groups of one-way ANOVA and Tukey's HSD multiple-comparisons test between morphotypes of each treatment, respectively.

In rhizospheric soil samples from *Pseudelephantopus spicatus*, eight morphotypes were detected in both non-exposed and exposed conditions. Four of these morphotypes were common to both sample types and showed similar abundances (Figure 3). As observed in *P. clandestinum*, morphotypes 2 and 5 were significantly more abundant in the exposed samples of *P. spicatus* ($p = 0.0054$). A similar trend was noted in the non-exposed samples; however, the difference was not statistically significant ($p = 0.1026$).

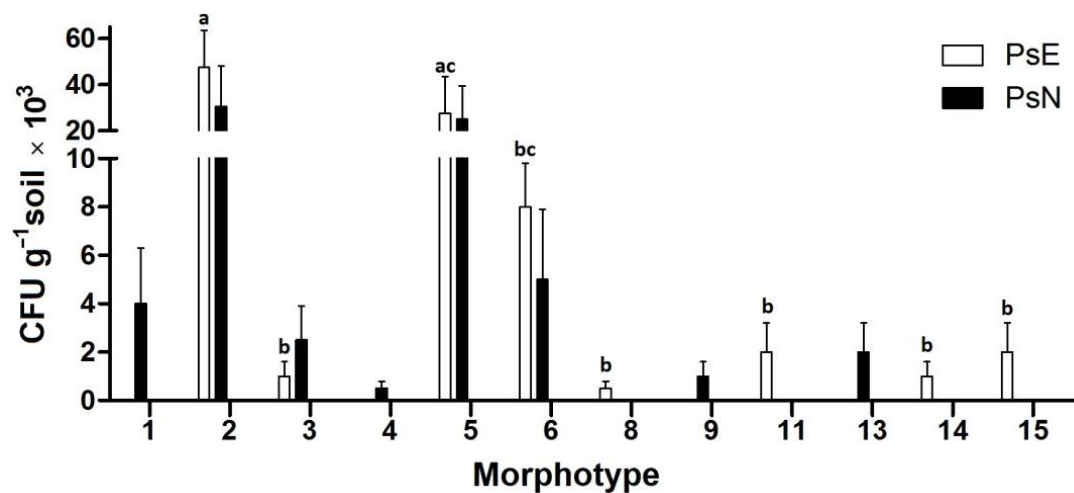


Figure 3. Bacteria count in rhizospheric soil samples of *Pseudelephantopus spicatus* (Ps), E: exposed soil; N: unexposed soil. Lowercase letters represent homogeneous groups of one-way ANOVA and Tukey's HSD multiple-comparisons test between morphotypes of each treatment, respectively.

A total of 11 morphotypes were found in the non-rhizospheric samples, with 10 of them being found in exposed soil, whereas 8 of them were observed in non-exposed samples (Figure 4). Morphotypes 2, 3, 5, 6, 9, 10, and 13 were common to both conditions and exhibited similar abundances ($p > 0.05$). Among them, morphotype 5 was significantly more abundant in both exposed ($p = 0.0182$) and non-exposed soils ($p = 0.0013$), followed by morphotype 2, which also showed higher abundance, though not statistically significant.

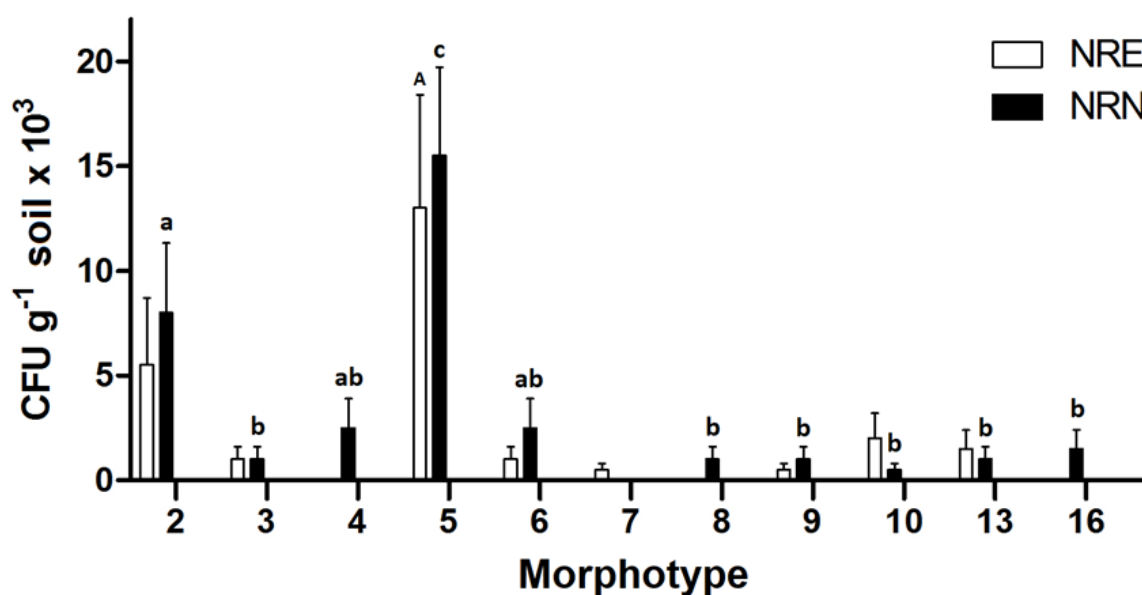


Figure 4. Bacterial counts in non-rhizospheric soil samples. NR: Non-rhizospheric. E: Exposed soil; N: Unexposed soil. Capital and lowercase letters represent homogeneous groups of one-way ANOVA and Tukey's HSD multiple-comparisons test between morphotypes of each treatment, respectively.

3.3. Rarefaction Assessment

Rarefaction analysis was used to establish comparisons between samples based on richness. The curves depict a trend based on the number of observed morphotypes relative to the total number of individuals (CFUs) in the analyzed sample. The curves obtained using the EstimateS software are presented in Figure 5.

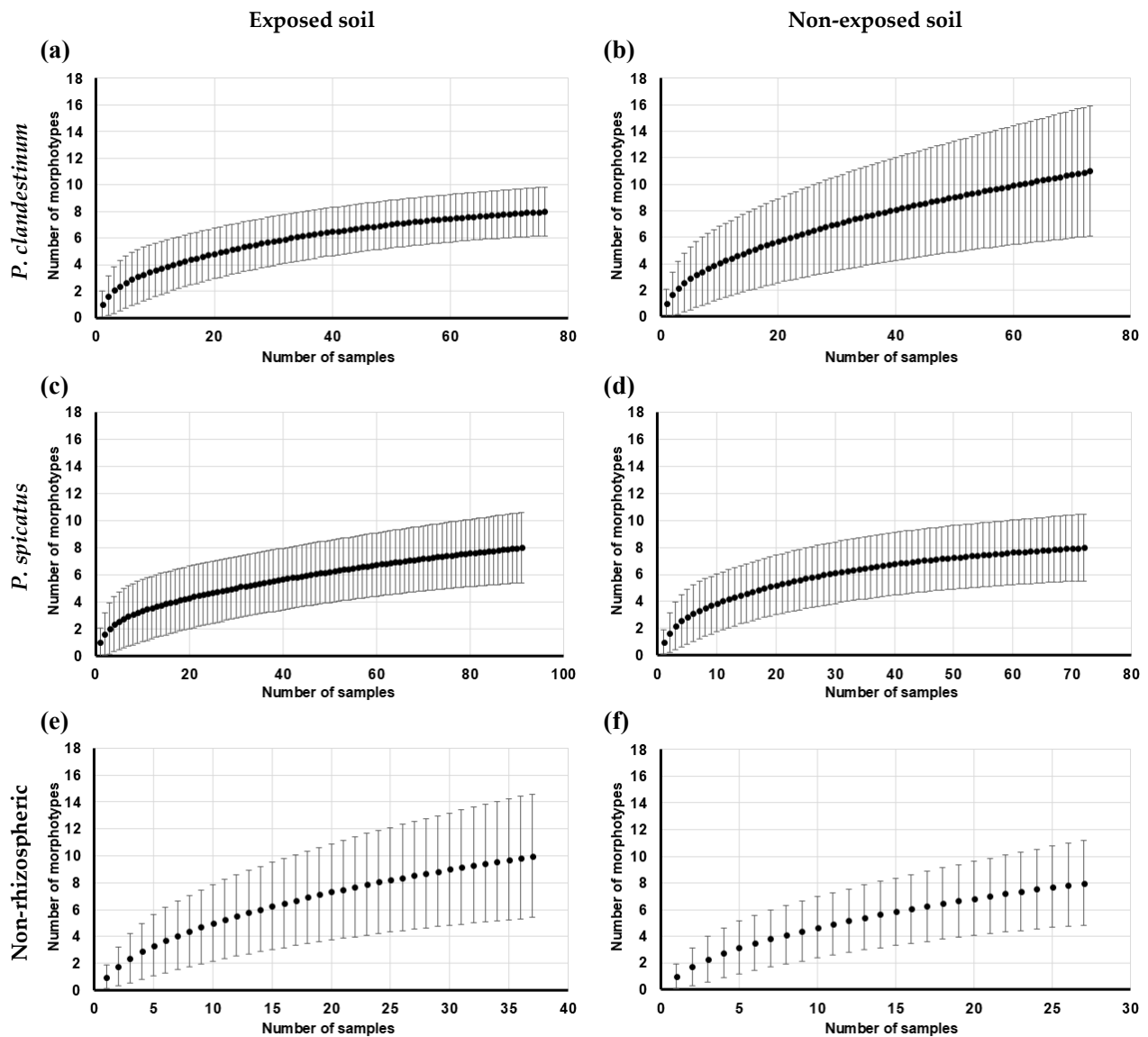


Figure 5. Rarefaction curves for species richness of (a) *P. clandestinum* in exposed soil; (b) *P. clandestinum* in non-exposed soil; (c) *P. spicatus* in exposed soil; (d) *P. spicatus* in non-exposed soil; (e) non-rhizospheric exposed soil; (f) non-rhizospheric non-exposed soil. The bars around the points represent a 95% confidence interval, obtained from 1000 permutations.

The highest species richness was observed in the non-exposed rhizospheric soil associated with *P. clandestinum* (Figure 5b). In contrast, the rarefaction curves for *P. spicatus* (Figure 5c,d) and the exposed *P. clandestinum* samples (Figure 5b) displayed similar patterns in terms of slope and asymptote. Under the applied sampling effort, a greater number of culturable organisms is expected in the rhizosphere of *P. clandestinum*. Additionally, species richness in the exposed non-rhizospheric soil was higher than in the non-exposed non-rhizospheric soil. However, both non-rhizospheric curves reached their asymptotes with fewer samples, confirming a lower overall abundance and richness of culturable organisms compared to rhizospheric soils and highlighting the influence of land-use pressure on microbial diversity.

3.4. Microbial Diversity Indices and Community Comparisons

The estimated alpha diversity indices for the culturable microbial communities associated with the studied plants showed clear differences in relation to soil type and

rhizospheric environment (Table 5). The non-exposed rhizospheric soil of *P. clandestinum* exhibited the highest species richness, as indicated by both the Margalef and Menhinick indices ($p = 0.0004$; $F = 11.05$ and $p = 0.0117$; $F = 4.85$, respectively). These results suggest that *P. clandestinum* promotes greater microbial diversity in less disturbed conditions, potentially due to more favorable soil characteristics or root exudate profiles. In contrast, no significant differences were detected for *P. spicatus* between soil exposure conditions, suggesting a more stable or restricted microbial association regardless of environmental disturbance.

Table 5. Diversity indices for bacterial morphotypes in rhizospheric and non-rhizospheric soil in exposed (E) and non-exposed (NE) conditions.

Index	Rhizospheric Soil				Non Rhizospheric Soil		<i>p</i> -Value *
	<i>P. clandestinum</i>		<i>P. spicatus</i>		E	NE	
	E	NE	E	NE			
S	8	11	8	8	10	8	
Margalef	1.64 ab	2.45 c	1.62 ab	1.75 ab	1.46 a	1.80 b	0.0004
Menhinick	0.95 ab	1.42 d	0.92 a	1.08 abc	1.21 bcd	1.32 cd	0.0117
Shannon	1.77	1.86	1.71	1.77	1.83	2.05	0.5313
Simpson	0.63	0.63	0.62	0.61	0.64	0.66	0.9534
Berger–Parker	0.49	0.51	0.53	0.53	0.52	0.51	0.9967
Pielou	0.59 a	0.54 a	0.57 a	0.59 a	0.76 b	0.73 b	0.0101

* One-way ANOVA and Tukey's HSD multiple-comparisons test. Lowercase letters represent homogeneous groups.

In non-rhizospheric samples, higher richness was also observed in the non-exposed soil according to the Margalef index, supporting the idea that disturbance reduces culturable diversity in bulk soils. However, the Shannon, Simpson, and Berger–Parker indices—reflecting a combination of richness and evenness, and dominance—did not show significant differences among samples, indicating that overall community structure in terms of abundance distribution remained relatively stable across non-rhizospheric samples. One possible explanation is the effect of soil depth: many alterations associated with urban land use tend to occur in the uppermost centimeters of the soil, where plant roots and the rhizosphere are more directly impacted, while deeper bulk soil layers may be less affected by surface disturbances [37,73,74].

Notably, the Pielou's evenness index showed significant differences ($p = 0.0101$; $F = 5.05$), with lower values observed in rhizospheric samples across both species and soil types. This suggests that rhizospheric communities, while richer in species, tend to have less uniform distributions, possibly due to the selective enrichment of specific taxa favored by root exudates or microhabitat variability [75].

The distribution of bacterial morphotypes across different soil samples was analyzed using Venn diagrams to visualize shared and unique taxa between rhizospheric and non-rhizospheric environments. Figure 6 illustrates the overlap of microbial morphotypes between the rhizosphere of *Pennisetum clandestinum* and *Pseudelephantopus spicatus* in both exposed and non-exposed soils, as well as their relation to non-rhizospheric soils. Morphotype 12 was only identified in non-exposed rhizospheric soil samples of *Pennisetum clandestinum*, whereas morphotypes 1, 11, 12, 14, and 15 were only found in rhizospheric soil samples. Furthermore, morphotype 16 was only found in non-rhizospheric soil. When comparing the distribution of shared morphotypes among rhizospheric samples, the Venn diagram reveals differential microbial establishment patterns influenced by both plant-host and land-use conditions. The highest number of shared morphotypes was observed in the rhizosphere of *Pennisetum clandestinum* in non-exposed soil. Of the 11 morphotypes identified in this condition, 9 were shared with at least one other sample type, suggesting a

higher capacity of this plant to support a diverse and overlapping microbial community. Interestingly, the exposed rhizosphere of *P. clandestinum* displayed complete overlap, with 100% of its morphotypes shared across samples. In contrast, *Pseudelephantopus spicatus* in exposed soil exhibited greater exclusivity, with 2 unique morphotypes and 6 shared ones, while in non-exposed soil, 1 exclusive and 7 shared morphotypes were observed. These patterns suggest that both plant species and soil exposure levels influence the composition and distribution of culturable bacterial communities in urban rhizospheres.

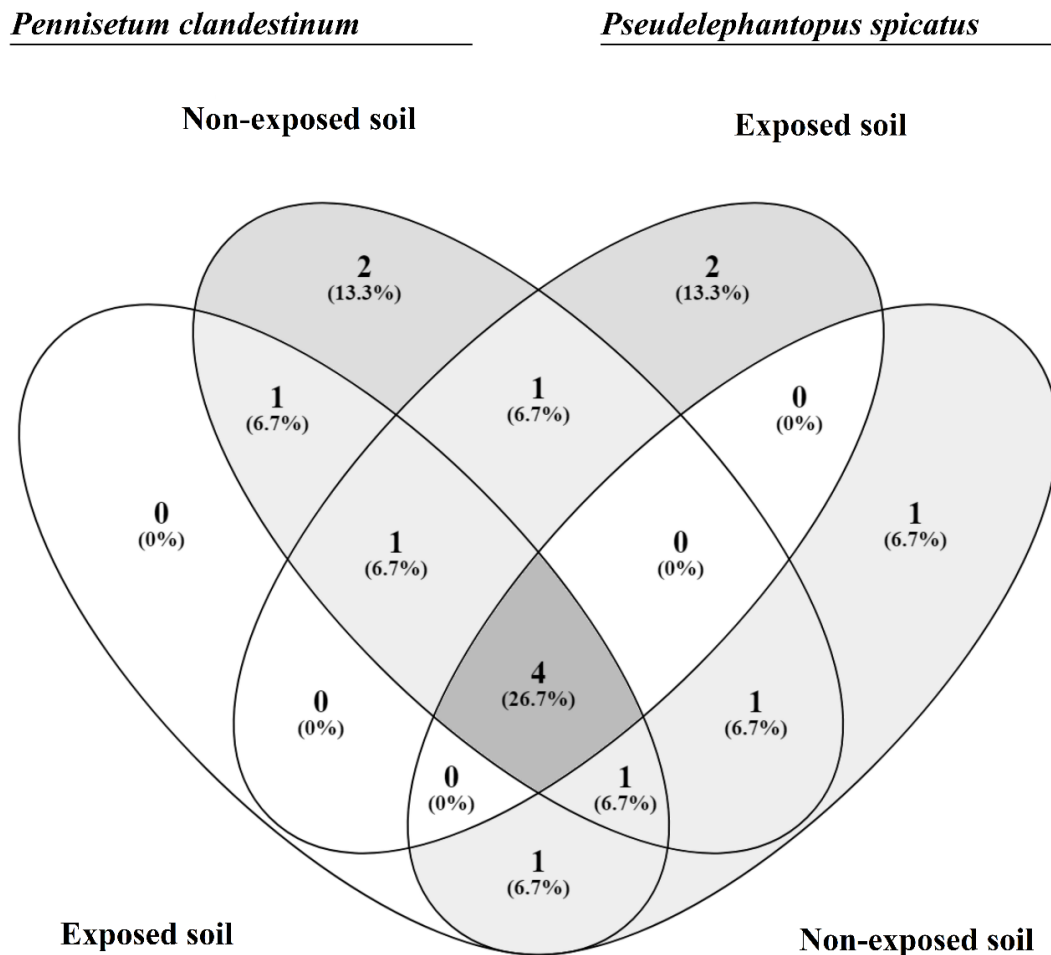


Figure 6. The distribution of rhizospheric morphotypes in the Venn diagram.

The similarity indices (Table 6) further support the distribution of communities in Figure 6, with the highest Jaccard and Ochiai coefficients found between non-rhizospheric samples and the rhizosphere of *Pseudelephantopus spicatus* in non-exposed soil. These coefficients fall within the moderate to high similarity category, suggesting partial overlap in microbial composition between these environments. This pattern aligns with previous findings highlighting the impact of plant species and soil conditions on microbial diversity [21,22]. Notably, the highest similarity was observed between *P. spicatus* in non-exposed soil and the non-rhizospheric soil under the same condition (Jaccard = 0.64), indicating that lower disturbance may foster microbial communities with greater compositional consistency across rhizospheric and bulk soil compartments. In contrast, lower coefficients involving *P. spicatus* in exposed soil (Jaccard ≤ 0.38) reflect a more distinct microbial profile, likely shaped by urban soil stressors and plant-specific responses. Additionally, the presence of exclusive morphotypes in rhizospheric soils reinforces the role of plant–microbe interactions in shaping microbial assemblages. These differences support the idea that

vegetation contributes to niche differentiation by selectively enriching microbial groups through root exudation, microhabitat heterogeneity, and competitive interactions [23,24].

Table 6. Jaccard and Ochai similarity coefficient matrix.

Jaccard		P.c.-E	P.c.-NE	P.s.-E	P.s.-NE	NR-E	NR-NE
Ochai							
	P.c-E		0.58	0.45	0.60	0.50	0.45
	P.c.-NE	0.43		0.46	0.46	0.50	0.58
	P.s.-E	0.38	0.39		0.33	0.38	0.33
	P.s.-NE	0.43	0.39	0.33		0.64	0.60
	NR-E	0.40	0.40	0.36	0.44		0.64
	NR-NE	0.38	0.43	0.33	0.43	0.44	

P.c.-E: *Pennisetum clandestinum* in exposed soil; P.c.-NE: *Pennisetum clandestinum* in non-exposed soil; P.s.-E: *Pseudelephantopus spicatus* in exposed soil; P.s.-NE: *Pseudelephantopus spicatus* in non-exposed soil; NR-E: non-rhizospheric soil in exposed conditions; NR-NE: non-rhizospheric soil in non-exposed conditions.

Diversity indices are important tools for bioprospection, as they allow for the selection and classification of microbial communities with potential biotechnological applications. Species richness indices, such as Margalef and Menhinick, are particularly useful for identifying environments with a higher number of unique microbial morphotypes, which increases the probability of discovering strains with novel metabolic capabilities [18,22]. Dominance-based indices like Simpson and Berger–Parker help highlight the prevalence of specific morphotypes, which may indicate ecological competitiveness and resilience—valuable traits for biotechnological applications such as bioremediation [76,77]. Evenness metrics, including Pielou’s index, provide insight into the distribution of microbial populations, ensuring that highly abundant but ecologically significant taxa are not overlooked. Rarefaction curves further support the evaluation process by estimating the completeness of sampling, guiding efforts to capture a representative microbial community [78,79].

Herbaceous plants have demonstrated tolerance to stress conditions induced by soil contamination and altered physicochemical properties [80–83], making them valuable in the context of urban soil resilience. Most of these functions are related to rhizospheric microbial communities shaped by root exudation profiles [84]. Root exudates—comprising amino acids, low-molecular-weight organic acids, sugars, and secondary metabolites—serve dual roles as nutrient sources and chemical signals, influencing microbial community composition and activity in response to stressors such as contamination and nutrient scarcity [21,84,85]. In this study, the higher bacterial diversity observed in the rhizosphere of *P. clandestinum*—particularly in non-exposed soils—may be attributable to its more robust exudation activity, which supports a greater variety of microbial niches. *P. clandestinum*, widely used as turfgrass in urban settings, has demonstrated remarkable adaptability to compacted, nutrient-poor or saline soils [86]. Its extensive, fibrous root system not only facilitates soil stabilization but also enhances microbial colonization [87], modulates nitrification-associated microbial populations [88], and has demonstrated phytoremediation potential through active microbial recruitment [83,89]. In contrast, results suggest that *P. spicatus* may exert a more selective pressure on microbial colonization due to differences in root chemistry or lower exudate diversity. This plant has shown resilience in contaminated environments and has been studied for its potential in the phytoremediation of heavy metals [90]. Additionally, *P. spicatus* is known to produce a wide array of secondary metabolites in response to environmental adaptations [91,92], suggesting a distinct profile of root exudates.

The culture-dependent techniques provide an essential starting point for identifying microbial strains with ecological and biotechnological relevance, such as those involved in biofertilization, bioremediation, or antibiotic production [25,93,94]. In the context of targeted microbial bioprospecting, the combined use of richness indices—to maximize candidate selection—and dominance indices—to highlight robust and potentially competitive strains—offers an efficient strategy for prioritizing isolates with functional potential. Although colony morphotypes represent a preliminary level of classification, they offer valuable phenotypic cues associated with functional traits. Characteristics such as pigmentation, mucous texture, filamentous edges, and surface wrinkling are frequently linked to microbial genera capable of producing biosurfactants, exopolysaccharides, or other metabolites of interest in environmental remediation and plant growth promotion [9,95,96]. Integrating morphotype-based assessments with diversity indices and culture-dependent methods thus enables rapid community differentiation and functional screening. This combined approach provides a practical and informative framework to guide the initial stages of microbial bioprospecting, particularly in complex environments such as urban soils.

In Colombian cities, urban soils are subjected to multiple environmental pressures, including construction expansion, solid waste accumulation, air pollution, and urban drainage, all of which can significantly impact soil quality and functionality [38,97–99]. In this context, the association of *Pennisetum clandestinum* and *Pseudelephantopus spicatus* with diverse rhizospheric microbial communities highlights their potential not only for microbial bioprospecting but also for enhancing soil health in urban green spaces. Microbial diversity acts as a key indicator of ecosystem resilience, as it supports essential soil functions such as nutrient cycling, pollutant degradation, and pathogen suppression, buffering urban soils against environmental stress [5,8]. Biodiversity indices provide a quantitative framework to evaluate these ecological functions and detect degradation before visible impacts emerge [1,3,100]. These approaches could be incorporated into environmental monitoring tools to guide soil restoration, inform greening strategies, and support public health initiatives [42,49,101–103]. Prioritizing microbial diversity assessments can thus ensure more sustainable and ecologically sound urban development.

4. Conclusions

This study compared the diversity of potentially culturable bacteria in the rhizosphere of plant species *Pennisetum clandestinum* and *Pseudelephantopus spicatus* in urban soils. The combined use of rarefaction, richness, dominance, and evenness indices provided complementary insights into microbial communities. Richness indices were more sensitive to plant and soil differences, particularly in rhizospheric samples. In contrast, dominance indices showed limited resolution, while Pielou's index captured uneven distributions, supporting its relevance in rhizosphere analyses. These methods provide information about active and culturable segment of the microbial community. Notably, *Pennisetum clandestinum* exhibited a more pronounced promotion of complex microbial establishment. The findings underscore the impact of urban pressures on rhizospheric microbial communities and highlight the potential diversity index and culture methods as both a soil-quality indicator and a tool for prospecting culturable microorganisms.

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References

1. FAO; ITPS. *Status of the World's Soil Resources (SWSR)—Main Report*; FAO: Rome, Italy, 2015; ISBN 9789251090046.
2. FAO; UNEP Global Assessment of Soil Pollution; FAO; UNEP: Rome, Italy, 2021; ISBN 978-92-5-134469-9.
3. FAO; ITPS; GSBI; SCBD; EC. *State of Knowledge of Soil Biodiversity—Status, Challenges and Potentialities*; FAO: Rome, Italy, 2020; ISBN 978-92-5-133582-6.
4. Maron, P.A.; Sarr, A.; Kaisermann, A.; Lévêque, J.; Mathieu, O.; Guigue, J.; Karimi, B.; Bernard, L.; Dequiedt, S.; Terrat, S.; et al. High Microbial Diversity Promotes Soil Ecosystem Functioning. *Appl. Environ. Microbiol.* **2018**, *84*, e02738-17. [[CrossRef](#)] [[PubMed](#)]
5. Fierer, N. Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [[CrossRef](#)] [[PubMed](#)]
6. Eida, A.A.; Ziegler, M.; Lafi, F.F.; Michell, C.T.; Voolstra, C.R.; Hirt, H.; Saad, M.M. Desert Plant Bacteria Reveal Host Influence and Beneficial Plant Growth Properties. *PLoS ONE* **2018**, *13*, e0208223. [[CrossRef](#)] [[PubMed](#)]
7. Dhankher, O.P.; Foyer, C.H. Climate Resilient Crops for Improving Global Food Security and Safety. *Plant Cell Environ.* **2018**, *41*, 877–884. [[CrossRef](#)]
8. Yan, B.; Li, J.; Xiao, N.; Qi, Y.; Fu, G.; Liu, G.; Qiao, M. Urban-Development-Induced Changes in the Diversity and Composition of the Soil Bacterial Community in Beijing. *Sci. Rep.* **2016**, *6*, 38811. [[CrossRef](#)]
9. Beltrán-Pineda, M.E.; Rocha-Gil, Z.E.; Bernal-Figueroa, A.A.; Pita-Morales, L.A. Functional Microorganisms in Soil with and without Revegetation in the Municipality of Villa de Leyva-Boyaca. *Colomb. For.* **2017**, *20*, 158–170. [[CrossRef](#)]
10. Zhao, X.; Huang, J.; Lu, J.; Sun, Y. Study on the Influence of Soil Microbial Community on the Long-Term Heavy Metal Pollution of Different Land Use Types and Depth Layers in Mine. *Ecotoxicol. Environ. Saf.* **2019**, *170*, 218–226. [[CrossRef](#)]
11. Yrjälä, K.; Keskinen, A.K.; Åkerman, M.L.; Fortelius, C.; Sipilä, T.P. The Rhizosphere and PAH Amendment Mediate Impacts on Functional and Structural Bacterial Diversity in Sandy Peat Soil. *Environ. Pollut.* **2010**, *158*, 1680–1688. [[CrossRef](#)]
12. Jacobsen, C.S.; Hjelmsø, M.H. Agricultural Soils, Pesticides and Microbial Diversity. *Curr. Opin. Biotechnol.* **2014**, *27*, 15–20. [[CrossRef](#)]
13. Guo, M.; Gong, Z.; Miao, R.; Rookes, J.; Cahill, D.; Zhuang, J. Microbial Mechanisms Controlling the Rhizosphere Effect of Ryegrass on Degradation of Polycyclic Aromatic Hydrocarbons in an Aged-Contaminated Agricultural Soil. *Soil Biol. Biochem.* **2017**, *113*, 130–142. [[CrossRef](#)]
14. Hill, G.T.; Mitkowski, N.A.; Aldrich-Wolfe, L.; Emele, L.R.; Jurkonie, D.D.; Ficke, A.; Maldonado-Ramirez, S.; Lynch, S.T.; Nelson, E.B. Methods for Assessing the Composition and Diversity of Soil Microbial Communities. *Appl. Soil Ecol.* **2000**, *15*, 25–36. [[CrossRef](#)]
15. Kirk, J.L.; Beaudette, L.A.; Hart, M.; Moutoglis, P.; Klironomos, J.N.; Lee, H.; Trevors, J.T. Methods of Studying Soil Microbial Diversity. *J. Microbiol. Methods* **2004**, *58*, 169–188. [[CrossRef](#)] [[PubMed](#)]
16. Alain, K.; Querellou, J. Cultivating the Uncultured: Limits, Advances and Future Challenges. *Extremophiles* **2009**, *13*, 583–594. [[CrossRef](#)]
17. Stefani, F.O.P.; Bell, T.H.; Marchand, C.; De La Providencia, I.E.; El Yassimi, A.; St-Arnaud, M.; Hijri, M. Culture-Dependent and -Independent Methods Capture Different Microbial Community Fractions in Hydrocarbon-Contaminated Soils. *PLoS ONE* **2015**, *10*, e0128272. [[CrossRef](#)]
18. Hill, T.C.J.; Walsh, K.A.; Harris, J.A.; Moffett, B.F. Using Ecological Diversity Measures with Bacterial Communities. *FEMS Microbiol. Ecol.* **2003**, *43*, 1–11. [[CrossRef](#)]
19. Hughes, J.B.; Hellmann, J.J.; Ricketts, T.H.; Bohannan, B.J.M. Counting the Uncountable: Statistical Approaches to Estimating Microbial Diversity. *Appl. Environ. Microbiol.* **2001**, *67*, 4399–4406. [[CrossRef](#)]
20. Orlóci, L.; Anand, M.; Pillar, V.D. Biodiversity Analysis: Issues, Concepts, Techniques. *Community Ecol.* **2002**, *3*, 217–236. [[CrossRef](#)]
21. Li, H.; Su, J.Q.; Yang, X.R.; Zhu, Y.G. Distinct Rhizosphere Effect on Active and Total Bacterial Communities in Paddy Soils. *Sci. Total Environ.* **2019**, *649*, 422–430. [[CrossRef](#)]
22. Chandra, P.; Dhuli, P.; Verma, P.; Singh, A.; Choudhary, M.; Prajapat, K.; Rai, A.K.; Yadav, R.K. Culturable Microbial Diversity in the Rhizosphere of Different Biotypes under Variable Salinity. *Trop. Ecol.* **2020**, *61*, 291–300. [[CrossRef](#)]
23. García-Villaraco Velasco, A.; Probanza, A.; Gutierrez Mañero, F.J.; Ramos, B.; Lucas García, J.A. Functional Diversity of Rhizosphere Microorganisms from Different Genotypes of *Arabidopsis Thaliana*. *Community Ecol.* **2009**, *10*, 111–119. [[CrossRef](#)]

24. Aislabie, J.; Julie, R. Deslippe Soil Microbes and Their Contribution to Ecosystem Services. In *Ecosystem Services in New Zealand—Conditions and Trends*; Dymond, J., Ed.; Manaaki Whenua Press: Lincoln, New Zealand, 2013; pp. 143–161. ISBN 9780478347364.
25. López, J.E.; Gallego, J.L.; Vargas-Ruiz, A.; Peña-Mosquera, A.L.; Zapata-Zapata, A.D.; López-Sánchez, I.J.; Botero-Botero, L.R. *Aspergillus tubingensis* and *Talaromyces islandicus* Solubilize Rock Phosphate Under Saline and Fungicide Stress and Improve Zea Mays Growth and Phosphorus Nutrition. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 2490–2501. [[CrossRef](#)]
26. Wozniak, G.; Markowicz, A.; Borymski, S.; Piotrowska-Seget, Z.; Chmura, D.; Besenyi, L. The Relationship between Successional Vascular Plant Assemblages and Associated Microbial Communities on Coal Mine Spoil Heaps. *Community Ecol.* **2015**, *16*, 23–32. [[CrossRef](#)]
27. Arias, M.E.; González-Pérez, J.A.; González-Vila, F.J.; Ball, A.S. Soil Health—A New Challenge for Microbiologists and Chemists. *Int. Microbiol.* **2005**, *8*, 13–21.
28. Sousa, A.M.; Machado, I.; Nicolau, A.; Pereira, M.O. Improvements on Colony Morphology Identification towards Bacterial Profiling. *J. Microbiol. Methods* **2013**, *95*, 327–335. [[CrossRef](#)]
29. Overmann, J.; Abt, B.; Sikorski, J. Present and Future of Culturing Bacteria. *Annu. Rev. Microbiol.* **2017**, *71*, 711–730. [[CrossRef](#)]
30. Alexandrino, D.A.M.; Mucha, A.P.; Tomasino, M.P.; Almeida, C.M.R.; Carvalho, M.F. Combining Culture-Dependent and Independent Approaches for the Optimization of Epoxiconazole and Fludioxonil-Degrading Bacterial Consortia. *Microorganisms* **2021**, *9*, 2109. [[CrossRef](#)]
31. Seyedpour Layalestani, S.S.; Shavandi, M.; Haddadi, A.; Amoozegar, M.A.; Dastgheib, S.M.M. Analysis of Bacterial Diversity during Bioremediation of Gasoil-Contaminated Soils with Different Salinities. *Soil Sediment Contam.* **2021**, *30*, 622–638. [[CrossRef](#)]
32. Hinsu, A.; Dumadiya, A.; Joshi, A.; Kotadiya, R.; Andharia, K.; Koringa, P.; Kothari, R. To Culture or Not to Culture: A Snapshot of Culture-Dependent and Culture-Independent Bacterial Diversity from Peanut Rhizosphere. *PeerJ* **2021**, *9*, e12035. [[CrossRef](#)]
33. Thrash, J.C. Culturing the Uncultured: Risk versus Reward. *mSystems* **2019**, *4*, e00130-19. [[CrossRef](#)]
34. Ajmone-Marsan, F.; Certini, G.; Scalenghe, R. Describing Urban Soils through a Faceted System Ensures More Informed Decision-Making. *Land Use Policy* **2016**, *51*, 109–119. [[CrossRef](#)]
35. Birt, H.W.G.; Bonnett, S.A.F. Microbial Mechanisms of Carbon and Nitrogen Acquisition in Contrasting Urban Soils. *Eur. J. Soil Biol.* **2018**, *88*, 1–7. [[CrossRef](#)]
36. Thompson, G.L.; Kao-Kniffin, J. Diversity Enhances NPP, N Retention, and Soil Microbial Diversity in Experimental Urban Grassland Assemblages. *PLoS ONE* **2016**, *11*, e0155986. [[CrossRef](#)] [[PubMed](#)]
37. Christel, A.; Dequiedt, S.; Chemidlin-Prevost-Bouré, N.; Mercier, F.; Tripied, J.; Comment, G.; Djemiel, C.; Bargeot, L.; Matagne, E.; Fougeron, A.; et al. Urban Land Uses Shape Soil Microbial Abundance and Diversity. *Sci. Total Environ.* **2023**, *883*, 163455. [[CrossRef](#)] [[PubMed](#)]
38. Paniagua-Villada, C.; Garizabal-Carmona, J.A.; Martínez-Arias, V.M.; Mancera-Rodríguez, N.J. Built vs. Green Cover: An Unequal Struggle for Urban Space in Medellín (Colombia). *Urban Ecosyst.* **2024**, *27*, 1055–1065. [[CrossRef](#)]
39. Montoya-Tangarife, C.; Villamizar Duarte, N.; Jorquera Guajardo, F.; Cardenas, M.F.; Giraldo-Ospina, T. Accessibility to Public Spaces: Boosting Ecosystem Services in Urban Areas in Four Latin American Cities. *Front. Sustain. Cities* **2022**, *4*, 796122. [[CrossRef](#)]
40. Garcia Ferrari, S.; Smith, H.; Coupe, F.; Rivera, H. City Profile: Medellín. *Cities* **2018**, *74*, 354–364. [[CrossRef](#)]
41. Mondragón, V.; Hurtado, F.M.; Jaramillo Jaramillo, D.F. Soil Organic Carbon Stocks and Properties Are Affected by Plant Cover Types in an Urban Ecosystem in Colombia. *S. Afr. J. Plant Soil* **2022**, *39*, 322–330. [[CrossRef](#)]
42. Zhang, F.; Qian, H. A Comprehensive Review of the Environmental Benefits of Urban Green Spaces. *Environ. Res.* **2024**, *252*, 118837. [[CrossRef](#)]
43. Canizales, S.A.; Celemin, J.S.; Mora-Delgado, J. Diversity and Uses of Weeds on Pastures of Livestock Farms in the Department of Tolima (Colombia). *Zootec. Trop.* **2010**, *28*, 427–437.
44. Jimenez Usuga, N.D.S.; Malafronte, N.; Durango, E.J.O.; Braca, A.; De Tommasi, N. Phytochemical Investigation of *Pseudelephantopus spiralis* (Less.) Cronquist. *Phytochem. Lett.* **2016**, *15*, 256–259. [[CrossRef](#)]
45. Sánchez, D.B.; Gómez, R.M.; García, A.M.; Bonilla, R.R. Phosphate Solubilizing Bacteria Isolated from *Pennisetum clandestinum* Associate to Livestock Systems in the Andean Area. *Rev. U.D.C.A Actual. Divulg. Científica Divulg. Científica* **2014**, *17*, 423–431.
46. Schmidt, D.J.E.; Pouyat, R.; Szlavecz, K.; Setälä, H.; Kotze, D.J.; Yesilonis, I.; Cilliers, S.; Hornung, E.; Dombos, M.; Yarwood, S.A. Urbanization Erodes Ectomycorrhizal Fungal Diversity and May Cause Microbial Communities to Converge. *Nat. Ecol. Evol.* **2017**, *1*, 123. [[CrossRef](#)] [[PubMed](#)]
47. Gill, A.S.; Lee, A.; McGuire, K.L. Phylogenetic and Functional Diversity of Total (DNA) and Expressed (RNA) Bacterial Communities in Urban Green Infrastructure Bioswale Soils. *Appl. Environ. Microbiol.* **2017**, *83*, e00287-17. [[CrossRef](#)]
48. Charlop-Powers, Z.; Pregitzer, C.C.; Lemetre, C.; Ternei, M.A.; Maniko, J.; Hover, B.M.; Calle, P.Y.; McGuire, K.L.; Garbarino, J.; Forgione, H.M.; et al. Urban Park Soil Microbiomes Are a Rich Reservoir of Natural Product Biosynthetic Diversity. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 14811–14816. [[CrossRef](#)]

49. Wang, H.; Cheng, M.; Dsouza, M.; Weisenhorn, P.; Zheng, T.; Gilbert, J.A. Soil Bacterial Diversity Is Associated with Human Population Density in Urban Greenspaces. *Environ. Sci. Technol.* **2018**, *52*, 5115–5124. [\[CrossRef\]](#)
50. Xie, F.; Pathom-aree, W. Actinobacteria from Desert: Diversity and Biotechnological Applications. *Front. Microbiol.* **2021**, *12*, 765531. [\[CrossRef\]](#)
51. Díaz-Rodríguez, A.M.; Chávez-Luzanía, R.A.; Rojas Anaya, E.; Villalobos, S.; de los, S. Bioprospection of Beneficial Bacteria for Bioproducts Innovation in Agriculture. In *New Insights, Trends, and Challenges in the Development and Applications of Microbial Inoculants in Agriculture*; Academic Press: Cambridge, MA, USA, 2024; pp. 53–58. [\[CrossRef\]](#)
52. Sprocati, A.R.; Alisi, C.; Tasso, F.; Fiore, A.; Marconi, P.; Langella, F.; Haferburg, G.; Nicoara, A.; Neagoe, A.; Kothe, E. Bioprospecting at Former Mining Sites across Europe: Microbial and Functional Diversity in Soils. *Environ. Sci. Pollut. Res.* **2014**, *21*, 6824–6835. [\[CrossRef\]](#)
53. Baltz, R.H. Natural Product Drug Discovery in the Genomic Era: Realities, Conjectures, Misconceptions, and Opportunities. *J. Ind. Microbiol. Biotechnol.* **2019**, *46*, 281–299. [\[CrossRef\]](#)
54. Hurtado Moreno, G.; González, O.C.; Cadena, M.; Benavides, H.; Rúiz, F.; Montealegre, E.; Ortiz Royero, J.C.; Montoya Ramírez, R.D. *Atlas Climatológico de Colombia*, 2nd ed.; Instituto de Hidrología Meteorología y Estudios Ambientales IDEAM: Bogotá, Colombia, 2017; ISBN 978-958-8067-95-7.
55. IGAC. *Suelos y Tierras de Colombia*, 1st ed.; Instituto Geográfico Agustín Codazzi: Bogotá, Colombia, 2016; ISBN 9789588323831.
56. García Castellanos, L.E.; Díaz Ávila, J.H.; Burgos Revelo, L.A.; Ortíz, Peña, L.E.; González Nivia, J.; Vera Raigosa, D.F.; Martínez Burgos, R.; Peña Hernández, G.; Fabían, R.; et al. *Estudio General de Suelos y Zonificación de Tierras: Departamento de Antioquia*, 1st ed.; Codazzi, I.G.A., Ed.; Imprenta Nacional de Colombia: Bogotá, Colombia, 2007; ISBN 9789588323077.
57. Barillot, C.D.C.; Sarde, C.O.; Bert, V.; Tarnaud, E.; Cochet, N. A Standardized Method for the Sampling of Rhizosphere and Rhizoplane Soil Bacteria Associated to a Herbaceous Root System. *Ann. Microbiol.* **2013**, *63*, 471–476. [\[CrossRef\]](#)
58. Gallego, J.L.; Olivero-Verbel, J. Cytogenetic Toxicity from Pesticide and Trace Element Mixtures in Soils Used for Conventional and Organic Crops of *Allium cepa* L. *Environ. Pollut.* **2021**, *276*, 116558. [\[CrossRef\]](#)
59. Walkley, A.; Black, I.A. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37*, 29–38. [\[CrossRef\]](#)
60. Margalef, R. Information Theory in Ecology. *Gen. Syst.* **1958**, *3*, 36–71.
61. Berger, W.H.; Parker, F.L. Diversity of Planktonic foraminifera in Deep-Sea Sediments. *Science* **1970**, *168*, 1345–1347. [\[CrossRef\]](#)
62. Pielou, E.C. The Measurement of Diversity in Different Types of Biological Collections. *J. Theor. Biol.* **1966**, *13*, 131–144. [\[CrossRef\]](#)
63. Simpson, E.H. Measurement of Diversity. *Nature* **1949**, *163*, 688. [\[CrossRef\]](#)
64. Menhinick, E.F. A Comparison of Some Species-Individuals Diversity Indices Applied to Samples of Field Insects. *Ecology* **1964**, *45*, 859–861. [\[CrossRef\]](#)
65. Colwell, R. *EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples*; Version 8.2.0; University of Connecticut: Storrs, CT, USA, 2009.
66. Sanjerehei, M.M.; Rundel, P.W. A Comparison of Methods for Detecting Association between Plant Species. *Ecol. Inform.* **2020**, *55*, 101034. [\[CrossRef\]](#)
67. Oliveros, J.C. Venny. An Interactive Tool for Comparing Lists with Venn Diagrams. 2007. Available online: <https://bioinfo.gp.cnb.csic.es/tools/venny/> (accessed on 17 January 2025).
68. Håkansson, I.; Lipiec, J. A Review of the Usefulness of Relative Bulk Density Values in Studies of Soil Structure and Compaction. *Soil Tillage Res.* **2000**, *53*, 71–85. [\[CrossRef\]](#)
69. Reynolds, W.D.; Bowman, B.T.; Drury, C.F.; Tan, C.S.; Lu, X. Indicators of Good Soil Physical Quality: Density and Storage Parameters. *Geoderma* **2002**, *110*, 131–146. [\[CrossRef\]](#)
70. Osorio, N.W. Como Interpretar Los Resultados Del Análisis de Fertilidad Del Suelo. *Boletín Manejo Integr. Suelo Nutr. Veg.* **2012**, *1*, 1–3.
71. Marais, J.P. Factors Affecting the Nutritive Value of Kikuyu Grass (*Pennisetum clandestinum*)—A Review. *Trop. Grassl.* **2001**, *35*, 65–84.
72. Herrera Ramírez, D.A.; León Peláez, J.D.; Ruiz Rendón, M.; Osorio Vega, N.W.; Correa Londoño, G.; Ricardo, R.E.; Uribe Bravo, A. Evaluation of Nutritional Requirements in Nurseries of Tropical Species Used in Urban Greening. *Rev. EIA/Engl. Version* **2014**, *11*, 41–54.
73. Stoma, G.V.; Manucharova, N.A.; Belokopytova, N.A. Biological Activity of Microbial Communities in Soils of Some Russian Cities. *Eurasian Soil Sci.* **2020**, *53*, 760–771. [\[CrossRef\]](#)
74. Curd, E.E.; Martiny, J.B.H.; Li, H.; Smith, T.B. Bacterial Diversity Is Positively Correlated with Soil Heterogeneity. *Ecosphere* **2018**, *9*, e02079. [\[CrossRef\]](#)
75. Bach, E.M.; Williams, R.J.; Hargreaves, S.K.; Yang, F.; Hofmockel, K.S. Greatest Soil Microbial Diversity Found in Micro-Habitats. *Soil Biol. Biochem.* **2018**, *118*, 217–226. [\[CrossRef\]](#)

76. Hamood Altowayti, W.A.; Almoalemi, H.; Shahir, S.; Othman, N. Comparison of Culture-Independent and Dependent Approaches for Identification of Native Arsenic-Resistant Bacteria and Their Potential Use for Arsenic Bioremediation. *Ecotoxicol. Environ. Saf.* **2020**, *205*, 111267. [\[CrossRef\]](#)
77. Ukaegbu-Obi, K.M.; Mbakwem-Aniebo, C.C. Bioremediation Potentials of Bacteria Isolated from Rhizosphere of Some Plants of Oil Contaminated Soil of Niger Delta. *J. Appl. Environ. Microbiol.* **2014**, *2*, 194–197. [\[CrossRef\]](#)
78. Hughes, J.B.; Hellmann, J.J. The Application of Rarefaction Techniques to Molecular Inventories of Microbial Diversity. *Methods Enzymol.* **2005**, *397*, 292–308. [\[CrossRef\]](#)
79. Kim, B.R.; Shin, J.; Guevarra, R.B.; Lee, J.H.; Kim, D.W.; Seol, K.H.; Lee, J.H.; Kim, H.B.; Isaacson, R.E. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. *J. Microbiol. Biotechnol.* **2017**, *27*, 2089–2093. [\[CrossRef\]](#)
80. Morales, G.E.; Gallego, J.L. Determinación de Los Efectos Tóxicos Del Mercurio En La Especie *Brachiaria dictyoneura* (Fig. & De Not.) Stapf. *Av. Cienc. Ing.* **2013**, *4*, 1–17.
81. Cruz, Y.; Carmago, G.; Gallego, J.L.; Saldarriaga, J.F. A Kinetic Modelling of the Growth Rate of *Lolium perenne* for Phytotoxicity Bioassays. *Chem. Eng. Trans.* **2019**, *74*, 1441–1446. [\[CrossRef\]](#)
82. Cruz, Y.; Villar, S.; Gutiérrez, K.; Ruiz, C.M.; Gallego, J.L.; Delgado, P.; Saldarriaga, J.F. Gene Expression and Morphological Responses of *Lolium perenne* L. Exposed to Cadmium (Cd^{2+}) and Mercury (Hg^{2+}). *Sci. Rep.* **2021**, *11*, 11257. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Chang Kee, J.; Gonzales, M.J.; Ponce, O.; Ramírez, L.; León, V.; Torres, A.; Corpus, M.; Loayza-Muro, R. Accumulation of Heavy Metals in Native Andean Plants: Potential Tools for Soil Phytoremediation in Ancash (Peru). *Environ. Sci. Pollut. Res.* **2018**, *25*, 33957–33966. [\[CrossRef\]](#)
84. Yetgin, A. The Dynamic Interplay of Root Exudates and Rhizosphere Microbiome. *Soil Stud.* **2023**, *12*, 111–120. [\[CrossRef\]](#)
85. Vives-Peris, V.; de Ollas, C.; Gómez-Cadenas, A.; Pérez-Clemente, R.M. Root Exudates: From Plant to Rhizosphere and Beyond. *Plant Cell Rep.* **2020**, *39*, 3–17. [\[CrossRef\]](#)
86. Muscolo, A.; Panuccio, M.R.; Eshel, A. Ecophysiology of *Pennisetum clandestinum*: A Valuable Salt Tolerant Grass. *Environ. Exp. Bot.* **2013**, *92*, 55–63. [\[CrossRef\]](#)
87. Cheung, P.K.; Nice, K.A.; Livesley, S.J. Impacts of Irrigation Scheduling on Urban Green Space Cooling. *Landsc. Urban Plan.* **2024**, *248*, 105103. [\[CrossRef\]](#)
88. Zhou, Y.; Lambrides, C.J.; Li, J.; Xu, Q.; Toh, R.; Tian, S.; Yang, P.; Yang, H.; Ryder, M.; Denton, M.D. Nitrifying Microbes in the Rhizosphere of Perennial Grasses Are Modified by Biological Nitrification Inhibition. *Microorganisms* **2020**, *8*, 1687. [\[CrossRef\]](#)
89. Singh, N.; Megharaj, M.; Kookana, R.S.; Naidu, R.; Sethunathan, N. Atrazine and Simazine Degradation in *Pennisetum* Rhizosphere. *Chemosphere* **2004**, *56*, 257–263. [\[CrossRef\]](#)
90. Lu, A.J.D.T.; Navarro, F.J.B.; Pascual, R.Z.; Clemen-Pascual, L. Phytoremediation Potential of Dilang-Aso (*Pseudelephantopus spicatus* (Juss.) Rohr) in Lead-Contaminated Soil. *J. Agric. Res. Dev. Ext. Technol.* **2020**, *2*, 40–52.
91. Ali, H.; Shariful, I.; Mohammad, M.H.; Rahman, H.; Asheka, R.; Mohammad, A.T.; Tanvir, M. Studies of Phytochemical Analysis, In Vitro and In Vivo Evaluation of the Local *Pseudelephantopus spicatus* Plant. *Afr. J. Pharm. Pharmacol.* **2024**, *18*, 114–126. [\[CrossRef\]](#)
92. Odonne, G.; Herbette, G.; Eparvier, V.; Bourdy, G.; Rojas, R.; Sauvain, M.; Stien, D. Antileishmanial Sesquiterpene Lactones from *Pseudelephantopus spicatus*, a Traditional Remedy from the Chayahuita Amerindians (Peru). Part III. *J. Ethnopharmacol.* **2011**, *137*, 875–879. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Acevedo-Barrios, R.; Tirado-Ballesteros, I.; Bertel-Sevilla, A.; Cervantes-Ceballos, L.; Gallego, J.L.; Leal, M.A.; Tovar, D.; Olivero-Verbel, J. Bioprospecting of Extremophilic Perchlorate-Reducing Bacteria: Report of Promising *Bacillus* spp. Isolated from Sediments of the Bay of Cartagena, Colombia. *Biodegradation* **2024**, *5*, 601–620. [\[CrossRef\]](#)
94. Das, R.; Kazy, S.K. Microbial Diversity, Community Composition and Metabolic Potential in Hydrocarbon Contaminated Oily Sludge: Prospects for In Situ Bioremediation. *Environ. Sci. Pollut. Res.* **2014**, *21*, 7369–7389. [\[CrossRef\]](#)
95. Das, S.; Nurunnabi, T.R.; Parveen, R.; Nur Mou, A.; Emdadul Islam, M.; Didarul Islam, K.M.; Rahman, S.M.M. Isolation and Characterization of Indole Acetic Acid Producing Bacteria from Rhizosphere Soil and Their Effect on Seed Germination. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 1237–1245. [\[CrossRef\]](#)
96. Alexandrino, D.A.M.; Ribeiro, I.; Pinto, L.M.; Cambra, R.; Oliveira, R.S.; Pereira, F.; Carvalho, M.F. Biodegradation of Mono-, Di- and Trifluoroacetate by Microbial Cultures with Different Origins. *New Biotechnol.* **2018**, *43*, 23–29. [\[CrossRef\]](#)
97. Saldarriaga, J.F.; Gallego, J.L.; López, J.E. Determination of Kinetics Parameters for Composting Process of the Organic Fraction of Municipal Solid Waste Separated at Source. *Chem. Eng. Trans.* **2018**, *70*, 217–222. [\[CrossRef\]](#)
98. Romero-Murillo, P.; Gallego, J.L.; Leignel, V. Marine Pollution and Advances in Biomonitoring in Cartagena Bay in the Colombian Caribbean. *Toxics* **2023**, *11*, 631. [\[CrossRef\]](#)
99. Narváez-Valderrama, J.F.; Alzate-B, S.V.; Correa-Gil, V.; García-L, J.J.; Bedoya-Soto, J.M.; Molina-P, F.J.; Pauta-Calle, G.G.; Vázquez-Guillén, G.B.; Ramos-Contreras, C.D. Traffic and Industrial Contributions of Particle-Bound PAHs during an Air Pollution Event in the Metropolitan Area of Medellín-Colombia: Inhalation Intake Risk during Pregnancy. *Atmosphere* **2024**, *15*, 173. [\[CrossRef\]](#)

100. Calzolari, C.; Tarocco, P.; Lombardo, N.; Marchi, N.; Ungaro, F. Assessing Soil Ecosystem Services in Urban and Peri-Urban Areas: From Urban Soils Survey to Providing Support Tool for Urban Planning. *Land Use policy* **2020**, *99*, 105037. [[CrossRef](#)]
101. González-Méndez, B.; Chávez-García, E. Re-Thinking the Technosol Design for Greenery Systems: Challenges for the Provision of Ecosystem Services in Semiarid and Arid Cities. *J. Arid Environ.* **2020**, *179*, 104191. [[CrossRef](#)]
102. Anne, B.; Geoffroy, S.; Cherel, J.; Warot, G.; Marie, S.; Noël, C.J.; Louis, M.J.; Christophe, S. Towards an Operational Methodology to Optimize Ecosystem Services Provided by Urban Soils. *Landsc. Urban Plan.* **2018**, *176*, 1–9. [[CrossRef](#)]
103. O’Riordan, R.; Davies, J.; Stevens, C.; Quinton, J.N.; Boyko, C. The Ecosystem Services of Urban Soils: A Review. *Geoderma* **2021**, *395*, 115076. [[CrossRef](#)]

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