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RESEARCH ARTICLE

Diversity and Abundance of Bacterial Community in Pepper, the "King of Spices" *(Piper nigrum)*

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Abstract Pepper (*Piper nigrum* L.) is one of the most widely consumed spices. Indonesia has become a significant producer and exporter of high-quality pepper, known for its unique flavour. The interactions between plants and microbes have been widely studied. However, comprehensive information on Indonesian pepper biotic (microbiome) and abiotic (soil and climate) factors has not been reported in detail. This study investigated bacterial diversity, abundance, and community structure in pepper plantations from three major production regions—Lampung, Bangka, and Belitung—across root endosphere, rhizosphere, and bulk soil compartments using 16S rRNA gene amplicon sequencing of the V3–V4 region. Sequencing generated sufficient high-quality data, with final nonchimeric read counts ranging from 43,972 ± 2,564 to 66,171 ± 819 across sample groups. Soil properties differed among regions, with Lampung showing higher rhizosphere pH (6.00), NH_4 (11.91 ppm), K (106.24 ppm), Ca (2918.73 ppm), Mn (129.12 ppm), Cu (0.57 ppm), and Zn (1.32 ppm), whereas Bangka showed higher organic C (3.27%), total N (0.25%), EC (246.33 µS/cm), Fe (8.98 ppm), and Sn (5.656 ppm). A total of 25 bacterial phyla were detected, with Proteobacteria dominating root endosphere (60–78%) and rhizosphere samples (44–56%), while Acidobacteriota was more abundant in bulk soil (36–41%). At the genus level, *Acinetobacter* was enriched in plant-associated compartments, reaching approximately 68% in Rhizosphere_Lampung, 50% in Rhizosphere_Belitung, and 38% in Root EC_Bangka. Other dominant genera showed compartment- and location-specific patterns, including *Enterobacter* and *Klebsiella* in Root EC_Belitung, *Burkholderia–Caballeronia–Paraburkholderia*, *Bacillus*, and *Chryseobacterium* in Rhizosphere_Bangka, and *Sinomonas* and *Acidothermus* in bulk soil. Alpha diversity showed that the rhizosphere was the main hotspot of diversity, with median Observed ASVs of 810–860 and the highest shared richness among

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regions (258 ASVs). Weighted UniFrac PCoA revealed clear community separation, with PCoA1 and PCoA2 explaining 31.86% and 15.32% of variation, respectively. PCA and RDA further indicated that Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, *Acinetobacter*, *Chryseobacterium*, *Enterobacter*, and *Sinomonas* contributed to bacterial community differentiation. These findings indicate that Indonesian pepper bacterial communities are structured by plant compartment and regional edaphic heterogeneity.

Keywords: Plant-microbe interaction, 16S amplicon sequencing, rhizosphere microbiome, *Piper nigrum*.

Introduction

Pepper (*Piper nigrum* L.), often called the “King of Spices,” holds a significant place in global cuisine and the spice trade for its unique flavor, nutritional value, and medicinal properties [1, 2]. As one of the most widely consumed spices worldwide, pepper has been cultivated for centuries in tropical regions, with Indonesia emerging as a producer and exporter of high-quality peppers. The main areas of *P. nigrum* plantations are found in Lampung, Bangka, and Belitung [3]. Several factors, including weather, soil, agricultural practices, and interactions between *P. nigrum* and the microbes in its immediate environment, influence the plant’s development and yield. Plant health, nutrient uptake, and disease resistance depend on the rhizosphere, the soil zone influenced by the root system. Plant development, nitrogen cycling, and pathogen protection depend on soil bacteria in this zone [4, 5]. A previous study reported that the Avicennia marina rhizosphere harbored bacterial populations that differed considerably from those in the bulk soil, demonstrating that roots may shape these communities. The bacterial communities at these two locations appear to be affected by vegetation and location [6]. The root-associated microbiota is a dynamic and highly diverse community that can vary significantly across niches and soil types [6, 7, 8, 9, 10]. Despite the recognized importance of microbial communities in plant growth and health, knowledge of bacterial diversity and abundance in the root zone of *P. nigrum* remains limited, particularly across different pepper-producing regions in Indonesia.

Molecular approaches now enable culture-independent characterization of microbial communities, revolutionizing our understanding of microbial diversity. The structure and composition of bacterial communities in complex settings, such as bulk soil and the rhizosphere, can now be investigated using high-throughput sequencing. This technique identifies numerous rare microbial species, revealing the ecological roles of the soil microbiome [11, 12]. According to Schreiter et al. [13], soil properties and other local environmental conditions significantly affect the composition of soil microbes and microbiota in plant roots. The structure of root-associated bacterial communities appears to be more influenced by soil microbial community composition and related environmental factors than by plant species or genotypes [14, 15, 16]. This shows that sample type or location affects microbial populations. The diversity and abundance of microbes are being better understood through molecular investigations of bacterial composition in soil, roots, and the rhizosphere across various regions [17, 18].

This study employed 16S rRNA amplicon sequencing to examine the diversity and abundance of bacterial populations in the root zone of *P. nigrum* growing in Lampung, Bangka, and Belitung, Indonesia's three central pepper-producing regions. Studying bacterial communities in the rhizosphere, roots, and bulk soil is necessary to understand how location, soil type, and plant-associated microbiota affect microbial structure and function in the pepper habitat. This research provides new insights into the microbial ecology of *P. nigrum*, which may inform sustainable farming practices and enhance pepper quality through improved microbial control.

Materials and Methods

Sample site and collection

To ensure consistency in plant genetic background across all study locations, the sampling sites were deliberately selected from pepper plantations cultivated with the Lampung Daun Lebar (LDL) cultivar. LDL is an important local pepper cultivar in Indonesia. This cultivar is recognized as part of the major pepper germplasm cultivated in the region and characterized by relatively broad leaves. LDL can be distinguished based on several traits, including leaf shape, leaf venation, lateral branch habit, mature fruit colour, and stem morphology.

Soil samples were collected in bulk at a depth of 25 cm using a drill [19]. To minimize the influence of pepper root exudates, sampling was conducted 5 meters away from the plant or outside the pepper

plantation. Root samples were collected from three healthy, same-age, and same-variety plants at each location. Rhizosphere soil was obtained by gently shaking soil adhering to root surfaces [20]. A multistage hierarchical sampling strategy was used to capture the spatial heterogeneity of pepper agroecosystems across Indonesia. Three major pepper-producing regions (Lampung, Bangka, and Belitung) were selected as the primary sampling units. Within each region, three representative areas were identified, and one to three independent pepper plantations were then selected within each area. To reduce the influence of microscale spatial variation, each plantation sample was a composite of three sampling points collected within the same field. Root, rhizosphere, and bulk soil samples were obtained from each composite and subjected to profiling. This sampling design enabled the evaluation of microbial community variation across multiple spatial scales, from region/island to plantation level. The sampling locations are characterized by distinct climates and soil types. The sampling design, geographic, and agroecological characteristics are summarized in Table 1.

Table 1. The sampling design, geographic, and agroecological characteristics of three major pepper-producing regions (Lampung, Bangka, and Belitung) selected for sample collection

Category		Regions		
		Lampung	Bangka	Belitung
Sampling design	Sampling period	13–14 May 2024	23–25 May 2024	31 May–01 June 2024
	Number of representative areas per region (n)	3	3	3
	Number of plantations per area (composited)	1	1-3	1
	Number of sampling sites per plantation (composited)	3	3	3
Total number of plant samples (root + rhizosphere)		3 x 1 x 3 = 9	3 x 1 x 3 = 9	3 x 1 x 3 = 9
Total number of bulk soil samples		3 x 1 x 3 = 9	3 x 1 x 3 = 9	3 x 1 x 3 = 9
Geographic coordinate	Coordinate range	5°9′26″–5°15′4″ S; 104°42′39″–105°35′37″ E	2°3′34″–2°49′41″ S; 106°3′26″–106°15′20″ E	2°38′56″–2°57′55″ S; 107°38′47″–108°5′9″ E
	Elevation range and aspect	28.9–370.0 m; 111°–307°	10.2–36.5 m; 81°–216°	10.0–44.9 m; 56°–178°
Agroclimatic condition	Temperature, T (°C)	27.10	26.86	26.75
	Rainfall, R (mm month^{-1})	165.06	218.21	285.36
	Relative humidity, H (%)	80.86	87.92	87.24
Soil texture type		Silt loam	Sandy clay loam	Sandy clay

Analysis of chemical soil collection

Bulk and rhizosphere soils were air-dried at room temperature, homogenized, and sieved through a 2-mm mesh prior to physicochemical analysis. Soil pH and electrical conductivity (EC) were measured in a soil-water suspension using calibrated pH and EC meters, respectively. Soil organic carbon (SOC) was measured using the Walkey-Black [21] wet oxidation method, whereas total nitrogen (TN) was determined using the Kjeldahl digestion procedure. Macronutrients (N, P, K, Mg, Ca, and S), micronutrients (Fe, Mn, Zn, and Cu), and heavy metal tin (Sn) were quantified following the standardized analytical procedures described by Wolf [22]. All laboratory analyses were conducted under a quality assurance and control protocol, including instrument calibration, procedural blanks, and analytical standards. Soil property data were analyzed using IBM SPSS Statistics version 29.

Preparation of samples and 16S rRNA extraction

Sample types were pretreated differently before 16S rRNA (DNA) extraction. Bulk soil and rhizosphere samples may be collected without additional procedures. Briefly, 250 mg of each bulk soil and rhizosphere sample was extracted using the DNeasy Power Soil Kit (Qiagen, Hilden, Germany). Root samples were washed in running water and surface-sterilized according to Sessitsch et al. [23], with minor adjustments. The main modification concerned the root surface sterilization procedure and bacterial recovery strategy. Pepper roots were first washed in sterile cold water with shaking at 150 rpm for 30 minutes, and this procedure was repeated two to three times. The roots were thoroughly rinsed with sterile water again. Surface sterilization was then carried out by immersing the roots in 2.25% NaOCl for 3 min, followed by 70% ethanol for 1 min, and rinsing thoroughly with sterile cold water. The sterilized roots were frozen with liquid nitrogen and ground into powder using a sterile mortar and pestle for subsequent DNA extraction. The wash solutions collected before NaOCl and ethanol treatment were pooled, centrifuged at 5.000 rpm for 10 min at 4 °C, and the resulting pellet was used as an additional rhizosphere bacterial fraction. DNA from the pellet of surface-sterilized root tissues, representing the bacterial root endophytic, was extracted using the ZymoBIOMICS 96 Magbeads DNA Kit (Zymo

Research, California, USA), according to the manufacturer's instructions for plant tissue. DNA was extracted three times for each sample type and location. The amount and quality of extracted DNA were determined using a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. The extracted DNA was kept at -80°C until analysis.

16S rRNA sequencing

The collected 16S rRNA (DNA) was transported to Indonesia's Genetika Science Laboratory for Illumina sequencing. The Qubit dsDNA HS Assay Kit from Thermo Fisher Scientific, USA, was used to assess the initial DNA concentration. Illumina's Nextera DNA Flex kit was used to prepare DNA libraries. Library preparation typically used 25–50 ng of DNA per sample. Samples were fragmented, and adapter sequences were added. Primers were used to amplify the V3-V4 region of the 16S rRNA gene for bacterial community profiling. We used the Toyobo KOD Multi & Epi (KME-101) from Japan for PCR. The Qubit dsDNA HS Assay Kit, manufactured by Thermo Fisher Scientific, was used to determine the final library concentration after library preparation. Agilent Technologies' 2100 Bioanalyzer was used to measure the average library size. DNA libraries were sequenced on the Illumina MiSeq (Illumina, San Diego, CA) using 2 × 300 bp paired-end sequencing.

Bioinformatic processing

Sequencing data were processed and analyzed with a series of bioinformatic tools. PCR primers and adapter sequences were removed from paired end reads using Cutadapt [24]. Illumina paired-end FASTQ data were analyzed with the DADA2 pipeline (v.1.12.1) to identify amplicon sequence variants (ASVs). The filter and trim function excluded FASTQ reads with ambiguous nucleotides (maxN=0) and allowed a maximum anticipated error rate of one (maxEE=1). We calculated error rates for the first 1–108 nucleotides of both forward and reverse reads that had been screened [25]. The pseudo-pooling approach in the data function, using learned error rates, predicted ASVs in both filtered read orientations after dereplication. Bimera Denovo eliminated chimeras by consensus, and merge pairs combined the paired reads. ASV taxonomy was assigned using the RDP Naive Bayesian Classifier (minBoot = 50) implemented in the assigned taxonomy function against the SILVA 16S rRNA reference database (Silva _nr99_v138.1).

Diversity, ordination, and statistical analysis

Alpha diversity was evaluated using evenness and richness. All sample sites were assessed in PAST version 3.20, and the Kruskal–Wallis test was used to compare the Shannon and Simpson diversity indices (Observed ASVs, Shannon, Simpson, and inverse Simpson indices). Beta diversity was described using weighted UniFrac (Wunifrac) dissimilarities in a PCoA. Phyla and genus distributions across locations were determined using Principal Component Analysis (PCA) and Redundancy Analysis (RDA). Venn diagrams, PCoA, PCA, and RDA plots were analyzed and visualized using RStudio (R version 4.2.3) (https://www.R-project.org/).

Results and Discussion

To control for potential host genotype effects, all sampling sites were selected from pepper plantations cultivated with a single cultivar, Lampung Daun Lebar (LDL; *Piper nigrum* L.). LDL is an important pepper cultivar documented in regional pepper germplasm collections and is recognized for its distinctive morphological characteristics. Beyond its role as a production cultivar, LDL may serve as a valuable genetic resource for pepper germplasm conservation and breeding programs, particularly for traits related to environmental adaptation, plant morphology, and productivity. Previous phenetic and botanical studies have reported that LDL can be distinguished from other pepper cultivars by quantitative and qualitative morphological traits [26, 27].

The three pepper-growing regions investigated in this study, namely Lampung, Bangka, and Belitung, were sampled using a harmonized field design, with each region represented by three sampling areas and including root-associated, rhizosphere, and bulk soil compartments. This comparable sampling framework reduced methodological bias and allowed differences in bacterial community structure to be interpreted primarily in relation to geographical, agroclimatic, and edaphic gradients rather than cultivar-related variation. Despite using the same cultivar, the three regions exhibited contrasting agroecological and soil conditions, indicating that Indonesian pepper plantations are established across heterogeneous environmental settings. Such regional differences may influence soil development, nutrient retention, root-soil interactions, and microbial habitat structure, thereby providing an important ecological basis for interpreting variation in bacterial communities across pepper-growing systems.

Agroecological and edaphic gradients across major pepper

Lampung, Bangka, and Belitung are in tropical regions suitable for pepper cultivation. However, they differ in geographic position, elevation, agroclimatic conditions, and soil type. Lampung has a broader elevation range (28.9 to 370.0 m above sea level), lower rainfall (165.06 mm per month) and relative humidity (80.86%), and silt loam soil texture, whereas Bangka and Belitung are lower-elevation island regions (ranging from 10.2 to 370.0 m above sea level) with higher rainfall (sequential: 218.21 mm per month and 285.36 mm per month), higher humidity (sequential: 87.92% and 87.24%), and sandier soil textures (Table 1). These agroclimatic differences indicate that Bangka and Belitung provide wetter, more humid growing environments than Lampung. High rainfall in humid tropical agroecosystems can accelerate the leaching of base cations and influence soil acidity, nutrient availability, and redox-related processes. The observed agroclimatic gradient may underlie regional differences in soil chemical properties. Soil texture also differs markedly among regions, with Lampung classified as silt loam, Bangka as sandy clay loam, and Belitung as sandy clay. Ecologically, these textural differences are important because they regulate water retention, aeration, and nutrient leaching under high-rainfall conditions. Therefore, the combined effects of agroclimatic gradients and soil textural variation may influence [28].

Soil chemical properties further confirmed strong regional differentiation (Table 2). In the rhizosphere, Lampung generally showed higher levels of nitrogen (NH_4), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), and acidity/alkalinity (pH), indicating a relatively nutrient-enriched, less acidic soil environment. Rhizosphere NH_4 was highest in Lampung at 11.91 ppm and significantly exceeded values in Bangka and Belitung. Similarly, Lampung showed markedly higher rhizosphere K and Ca concentrations, reaching 106.24 ppm and 2918.73 ppm, respectively. These results indicate that the Lampung rhizosphere was comparatively enriched in ammonium and basic cations. Such nutrient enrichment may be associated with local soil texture, reduced rainfall-driven leaching, parent material, and fertilization history. In contrast, Bangka and Belitung were more acidic and had lower concentrations of several basic cations, although they showed higher concentrations of phosphorus (PO_4) and sulfur (SO_4). Rhizosphere PO_4 was 0.39 ppm in Lampung, 4.40 ppm in Bangka, and 4.98 ppm in Belitung, while SO_4 was 3.17 ppm in Lampung, 6.84 ppm in Bangka, and 7.69 ppm in Belitung. Although several of these parameters did not differ significantly, the numerical trends suggest that nutrient availability varied by element and region. This pattern indicates that soil fertility in Indonesian pepper plantations is not uniform but is structured by specific nutrient gradients.

Based on the sample analysis, Bangka also had higher organic carbon, total nitrogen, electrical conductivity (EC), iron (Fe), and tin (Sn) than Lampung and Belitung. This pattern likely reflects soil formation, management history, and local geochemical characteristics. Micronutrient availability varied markedly among regions. Lampung rhizosphere soils contained significantly higher levels of Mn, Cu, and Zn than those in Bangka and Belitung, whereas Fe was higher in Bangka and Belitung. Mn concentrations in Lampung were particularly high, at 129.12 ppm, compared with 2.05 ppm in Bangka and 2.73 ppm in Belitung. Conversely, Fe was highest in Bangka and Belitung, at 8.98 and 7.53 ppm, respectively, compared with 4.02 ppm in Lampung. These contrasts likely reflect regional differences in soil acidity, redox conditions, mineral composition, and interactions among organic matter. Because micronutrients function as essential enzymatic cofactors and can act as stressors at elevated concentrations, their spatial heterogeneity may shape microbial selection and metabolic potential in the rhizosphere. The heavy metal Sn also showed distinct regional variation, with the highest rhizosphere concentration in Bangka (5.656 ppm), followed by Lampung (1.619 ppm) and Belitung (1.054 ppm). This pattern is consistent with the tin-associated geochemical background of Bangka. However, total or extractable metal concentrations should not be directly interpreted as indicators of toxicity or bioavailability. Nonetheless, such metal gradients may act as environmental filters by selecting bacterial taxa with greater tolerance or functional capacities for metal transformation, sequestration, and detoxification.

Table 2. The soil chemical properties data for three pepper-growing regions locations in Indonesia (Lampung, Bangka, and Belitung), from rhizosphere and bulk soil

Component observations	Rhizosphere	Bulk soil *

		Lampung	Bangka	Belitung	Lampung	Bangka	Belitung
Macronutrient (ppm)	NH4	11.91 **a**	4.6 **b**	3.35 **b**	8.84	2.71	2.79
	PO4	0.39 **a**	4.4 **a**	4.98 **a**	0.73	0.54	3.21
	K	106.24 **a**	83.29 **a**	32.05 **b**	66.86	62.46	11.24
	Ca	2918.73 **a**	1217.13 **b**	736.30 **b**	1968.84	464.02	226.46
	Mg	214.42	168.57	68.95	162.18	27.7	22.21
	SO4	3.17 **a**	6.84 **a**	7.69 **a**	3.81	4.67	5.55
Micronutrient (ppm)	Fe	4.02 **a**	8.98 **b**	7.53 **b**	2.89	9.41	9.95
	Mn	129.12 **a**	2.05 **b**	2.73 **b**	2.07	1.11	2.22
	Cu	0.57 **a**	0.11 **b**	0.07 **b**	0.75	0.02	0.04
	Zn	1.32 **a**	0.23 **b**	0.34 **b**	0.89	0.06	0.05
Heavy metal: Sn (ppm)		1.619 **a**	5.656 **b**	1.054 **a**	0.203	0.799	0.198
C-Organic (%)		2.24 **a**	3.27 **a**	2.34 **a**	1.90	2.35	1.72
Nitrogen Total (%)		0.19 **a**	0.25 **a**	0.17 **a**	0.19	0.13	0.17
C/N		12.00 **a**	13.33 **a**	13.33 **a**	10.00	18.00	10.00
pH		6.00 **a**	4.98 **b**	5,27 **b**	5.45 **a**	4.63 **b**	4.29 **b**
EC (uS/cm)		116.50 **a**	246.33 **b**	142.00 **ab**	135.17 **a**	84.17 **a**	93.67 **a**

Notes: Values followed by the same letter within the same row (for each type of organ, both rhizosphere and bulk soil) are not significantly different according to Duncan's multiple range test ($p < 0.05$). (*) Statistical comparisons were not performed for some bulk soil properties due to missing data; therefore, no values are provided for those properties.

Organic and general soil chemical properties showed more moderate regional variation. Rhizosphere organic C ranged from 2.24% in Lampung to 3.27% in Bangka and 2.34% in Belitung, while total N ranged from 0.17% to 0.25%. Although these variables did not differ significantly among rhizosphere soils, Bangka tended to have higher organic C and N. The C/N ratio was comparable across regions, ranging from 12.00 to 13.33, suggesting broadly similar organic matter decomposition status. These organic substrates may regulate microbial metabolism and influence the balance between copiotrophic and oligotrophic bacterial groups. Soil pH was one of the strongest regional chemical gradients. Rhizosphere pH was highest in Lampung (6.00), whereas Bangka and Belitung were more acidic, at 4.98 and 5.27, respectively. A similar pattern was observed in bulk soil, with pH values of 5.45 in Lampung, 4.63 in Bangka, and 4.29 in Belitung. This pH gradient is likely a key driver of bacterial community structure because pH directly affects microbial physiological tolerance and indirectly regulates nutrient solubility and availability. Previous studies have shown that pH shifts strongly alter bacterial abundance, diversity, and composition in agricultural soils, often more markedly than fungal communities [29], and can reshape rhizosphere microbiomes by altering nutrient availability and plant–microbe interactions [30]. Electrical conductivity also varied across regions and soil compartments. In the rhizosphere, EC was highest in Bangka (246.33 µS/cm), followed by Belitung (142.00 µS/cm) and Lampung (116.50 µS/cm). In bulk soil, EC values were lower in Bangka and Belitung but remained relatively high in Lampung. The elevated rhizosphere EC in Bangka suggests higher soluble ion concentrations, potentially linked to mineral composition, fertilizer inputs, organic matter mineralization, and rhizosphere-driven nutrient mobilization. Although these EC values do not indicate severe salinity, variation in soluble ions may still affect microbial activity, nutrient cycling, and community assembly in root-influenced soil compartments. The rhizosphere generally contained higher nutrient concentrations than the corresponding bulk soils, particularly for NH_4, K, Ca, Mg, Mn, Cu, Zn, C-Organic, and EC. This pattern indicates a clear rhizosphere effect, likely driven by root exudation, nutrient mobilization, microbial turnover, and localized biogeochemical activity around pepper roots. Because the rhizosphere is the primary interface for plant–microbe interactions, these chemical modifications are expected to influence bacterial community assembly.

Among the measured variables, pH, nutrient availability, organic carbon, EC, and metal-related properties are particularly relevant for interpreting bacterial community structure. Soil pH is widely recognized as a strong determinant of bacterial diversity and composition, while nutrient availability can influence the balance between copiotrophic and oligotrophic bacterial groups. Thus, the relatively higher pH and nutrient availability in Lampung may favor copiotrophic and plant-associated taxa, whereas the more acidic and chemically distinct soils of Bangka and Belitung may select for bacterial groups adapted to acidity, lower base cation availability, or metal-associated stress. These agroecological and edaphic gradients provide an important explanatory framework for interpreting variation in bacterial community composition across pepper rhizosphere and bulk soil compartments [31]. According to Carrión et al. and Compant et al. [32, 33], high-throughput amplicon and shotgun metagenome sequencing data indicate

that the plant microbiome is shaped by intricate interactions among the host, microorganisms, and the environment. Biotic and abiotic variables affect the plant endosphere population. These include host plant type, organs present, growth stage, soil type, cultivation methods, and fertilization [34, 35]. The influence of habitat on plant-associated bacterial communities has also been observed in other plant groups, such as rice [36], maize [37], and Cactaceae [38]. Plant genotype, growth conditions, and environmental factors affect root microorganisms [39]. Furthermore, the growing season has a substantial impact on the tea root microbiota [40]. Although bacterial communities are shaped by multiple interacting factors, the observed differences in agroclimatic conditions, soil texture, pH, nutrient availability, and metal-related properties suggest that regional edaphic heterogeneity may act as an important environmental filter for pepper-associated bacterial assemblages.

Sequencing quality and overview of bacterial community profiling

To complement the agroecological characterization of the three regions, sequencing quality-control metrics were evaluated to ensure that the observed bacterial community patterns were supported by reliable sequence data. This step is essential because differences in soil compartments and regional soil properties may affect DNA recovery, read retention, and downstream interpretation of the microbial community. The resulting quality-control summary is presented in Table 3. The bacterial 16S rRNA sequencing quality-control metrics indicated that the dataset was generally reliable for downstream microbiome analysis, although read retention varied across sample compartments and locations. Root samples showed the most consistent sequencing performance, with trimmed reads ranging from 99,809 ± 189 to 99,927 ± 29 and final nonchimeric reads from 63,055 ± 3,087 to 66,171 ± 819. This corresponded to nonchimeric/trimmed read retention of 63.15 ± 3.05% to 66.22 ± 0.83%, indicating stable read recovery and sufficient sequencing depth across root samples from Lampung, Bangka, and Belitung.

Greater variation was observed across rhizosphere and bulk soil samples. Rhizosphere trimmed reads ranged from 78,671 ± 6,281 in Belitung to 99,911 ± 30 in Lampung, while final nonchimeric read retention ranged from 53.35 ± 1.67% to 60.30 ± 1.63%. Bulk soil samples retained 44,673 ± 9,915 to 53,800 nonchimeric reads, equivalent to 55.26–61.54% of trimmed reads. The lower and more variable retention in soil-associated samples may reflect differences in soil matrix complexity, DNA quality, microbial biomass, and the presence of PCR inhibitors such as humic substances, clay particles, or metal ions. The progressive reduction from trimmed reads to filtered, denoised, merged, and nonchimeric reads is expected in 16S rRNA amplicon sequencing. Quality filtering removes low-quality reads, denoising corrects sequencing errors, paired-end merging excludes reads with insufficient overlap or excessive mismatches, and chimera removal eliminates PCR-derived artifacts. These steps are essential for minimizing technical noise and avoiding artificial inflation of microbial richness. DADA2-based denoising is widely used because it enables inference of amplicon sequence variants at single-nucleotide resolution and improves reproducibility compared with conventional OTU clustering approaches [41, 42]. Recent benchmarking studies also emphasize that denoising strategy, read merging, and chimera removal are critical determinants of accurate microbiome profiling [43, 44].

Overall, all sample groups produced enough nonchimeric reads for subsequent analyses of bacterial diversity, taxonomic composition, and community structure. The lowest mean value, observed in Rhizosphere_Belitung at 43,972 ± 2,564 nonchimeric reads, remained adequate for ecological interpretation. Therefore, the quality-control results support the dataset's robustness and highlight that compartment- and location-specific variation in read recovery should be considered when interpreting bacterial community patterns across Lampung, Bangka, and Belitung.

Abundance of bacterial communities and taxonomic composition

Following the quality-control assessment, the retained high-quality, nonchimeric sequences were used to characterize bacterial community composition across the root endosphere, rhizosphere, and bulk soil compartments. This analysis provides taxonomic insight into how bacterial assemblages differ among sampling sites and plant-associated microhabitats. Relative abundance patterns at the phylum and genus levels are presented in Figure 1. The relative abundance profile of the ten most dominant bacterial phyla revealed clear compartment-specific patterns across root endosphere, rhizosphere, and bulk soil samples from Lampung, Bangka, and Belitung. A total of 25 bacterial phyla were detected across all samples, indicating broad taxonomic diversity among bacteria in pepper-associated agroecosystems from Lampung, Bangka, and Belitung. Of these, 11 phyla were consistently detected across all sample types, namely Acidobacteriota, Actinobacteriota, Bacteroidota, Chloroflexi, Dependentiae, Firmicutes, Gemmatimonadota, Myxococcota, Patescibacteria, Planctomycetota, and Proteobacteria, suggesting that these groups represent the core bacterial phyla across the studied soil–plant compartments. However, several phyla showed compartment- and site-specific occurrence, including Fibrobacterota and Hydrogenedentes, which were detected only in the pepper rhizosphere from Belitung; Spirochaetota, which was restricted to root endosphere samples from Belitung; and Sumerlaeota, which was found only in the pepper rhizosphere from Lampung. This selective distribution may reflect microhabitat-specific ecological filtering driven by differences in root-associated niches, soil physicochemical properties,

nutrient availability, and regional agroecological conditions. The greater number of phyla observed in rhizosphere samples, particularly in Lampung and Belitung, further supports the rhizosphere's role as a biologically active interface where root exudates and soil-derived resources promote the coexistence of diverse bacterial taxa.

Variation at the phylum level (Figure 1-a) is shown as a stacked bar chart depicting the relative abundance of the ten most abundant phyla across the nine groups. Proteobacteria were the dominant phylum in root endosphere and rhizosphere samples, contributing approximately 60–78% in roots and 44–56% in rhizospheres, whereas bulk soil samples showed a higher relative contribution of Acidobacteriota, approximately 36–41%. An exception was observed in Bulk Soil_Lampung, where Proteobacteria and Acidobacteriota were nearly equally dominant, each accounting for approximately 36% of the community. This pattern suggests that plant-associated compartments, particularly roots and rhizospheres, selectively enrich copiotrophic bacterial groups such as Proteobacteria, which are commonly associated with rapid utilization of root-derived carbon compounds and plant–microbe interactions. In contrast, the greater dominance of Acidobacteriota in bulk soil may reflect their ecological adaptation to oligotrophic conditions, acidic environments, and soil habitats with more limited availability of labile carbon. Such compartment-based differentiation is consistent with previous studies showing that plant roots act as strong ecological filters shaping microbial community assembly, whereas bulk soil communities are more strongly governed by soil physicochemical properties and environmental filtering. In line with our research findings, Tran et al. [45] reported that Proteobacteria (87.5%) and Actinobacteria (12.5%) were the most prevalent in the root sample of black pepper grown in Vietnam. This suggests that the members of these two phyla may be essential endophytic bacteria in the pepper plant. The rhizosphere and root samples contain more Proteobacteria than bulk soil, suggesting a close association with plant and root exudates. Compared with nearby bulk soils, the rhizospheres of Brazilian pepper plants exhibit much higher bacterial diversity, particularly within Proteobacteria. For instance, the least diverse group of Proteobacteria is found in bulk soil without plants [46].

At the genus level (Figure 1-b), bacterial community composition showed stronger compartment- and site-dependent differentiation than at the phylum level. In the root endosphere, dominant genera varied among regions: Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium accounted for approximately 38% of the community in Root EC_Lampung, whereas Acinetobacter reached about 38% in Root EC_Bangka. Root EC_Belitung displayed a distinct profile, with relatively high proportions of Enterobacter and Klebsiella, contributing approximately 32% and 27%, respectively. These patterns indicate that the internal root compartment may selectively harbor specific plant-associated bacterial taxa depending on geographic and edaphic context. The enrichment of these genera is ecologically relevant because several members of these groups have been associated with nitrogen transformation, nutrient mobilization, phytohormone production, and enhanced plant tolerance to abiotic stress, suggesting potential functional roles in pepper root adaptation [47, 48, 49]. Rhizosphere samples exhibited an intermediate but highly selective bacterial structure, reflecting the combined influence of root exudation and surrounding soil conditions.

Table 3. Summary of bacterial sequencing quality control metrics for three locations in Indonesia (Lampung, Bangka, and Belitung), from root, rhizosphere, and bulk soil

Sample group	n	Trimmed reads	Filter reads	Denoised F	Denoised R	Merger reads	Nonchimeric reads	Filtered/trimmed (%)	Mergered/trimmed (%)	Nonchimeric/trimmed (%)
Root EC_Lampung	3	99,927 ± 29	72,334 ± 649	71,699 ± 513	71,794 ± 634	69,413 ± 343	66,171 ± 819	72.39 ± 0.67	69.46 ± 0.33	66.22 ± 0.83
Root EC_Bangka	3	99,809 ± 189	73,849 ± 930	72,158 ± 1,096	72,457 ± 1,223	66,266 ± 2,135	63,278 ± 1,407	73.99 ± 1.06	66.39 ± 2.25	63.40 ± 1.51
Root EC_Belitung	3	99,846 ± 68	73,536 ± 1,343	72,241 ± 1,858	72,437 ± 1,665	67,727 ± 3,265	63,055 ± 3,087	73.65 ± 1.30	67.83 ± 3.23	63.15 ± 3.05
Rhizosphere_Lampung	3	99,911 ± 30	72,361 ± 300	68,857 ± 255	68,837 ± 411	56,267 ± 1,598	53,297 ± 1,660	72.43 ± 0.28	56.32 ± 1.61	53.35 ± 1.67
Rhizosphere_Bangka	3	89,890 ± 17,193	76,463 ± 14,321	72,539 ± 13,498	72,666 ± 13,647	57,819 ± 10,294	54,038 ± 9,250	85.11 ± 0.39	64.45 ± 1.03	60.30 ± 1.63
Rhizosphere_Belitung	3	78,671 ± 6,281	65,444 ± 5,258	60,896 ± 4,439	61,314 ± 4,902	45,995 ± 2,612	43,972 ± 2,564	83.19 ± 0.20	58.55 ± 2.02	55.96 ± 1.42
Bulk soil_Lampung	1	87,423	72,779	69,302	69,885	56,268	53,800	83.25	64.36	61.54
Bulk soil_Bangka	3	80,254 ± 17,247	66,038 ± 12,765	61,979 ± 12,228	62,633 ± 12,390	47,673 ± 10,337	44,673 ± 9,915	82.54 ± 1.78	59.38 ± 0.50	55.60 ± 0.85
Bulk soil_Belitung	3	83,918 ± 18,372	69,657 ± 15,888	65,648 ± 15,038	66,451 ± 15,477	51,690 ± 12,620	46,510 ± 10,973	82.87 ± 1.07	61.33 ± 2.18	55.26 ± 1.52

Notes: Values represent mean ± standard deviation across replicates. Percent retention was calculated relative to trimmed reads. The bulk soil_Lampung sample was represented by a single replicate (16S rRNA difficult to isolate).

Acinetobacter was particularly dominant in Rhizosphere_Lampung and Rhizosphere_Belitung, representing approximately 68% and 50% of the bacterial community, respectively. In contrast, Rhizosphere_Bangka showed a more even genus-level distribution, with Burkholderia–Caballeronia–Paraburkholderia contributing approximately 17%, Enterobacter about 17%, Bacillus about 13%, and Chryseobacterium about 12%. Bulk soil communities were clearly distinct from plant-associated compartments, characterized by higher proportions of Sinomonas, particularly in Bulk Soil_Bangka and Bulk Soil_Belitung, where it accounted for approximately 35–38% of the community, and by notable contributions of Acidothermus, which reached about 18%, 24%, and 12% in Bulk Soil_Lampung, Bulk Soil_Bangka, and Bulk Soil_Belitung, respectively. Overall, these quantitative patterns demonstrate a clear transition in bacterial community structure from bulk soil to rhizosphere and root endosphere, with increasing host influence toward internal root tissues and stronger edaphic filtering in bulk soil [50, 49, 47].

Overall, phylum- and genus-level patterns indicate that bacterial community structure in pepper agroecosystems is jointly shaped by plant compartment and geographic origin. The enrichment of Proteobacteria and several root-associated genera in the endosphere and rhizosphere supports the concept of host-mediated microbial selection, whereas the dominance of Acidobacteriota and soil-adapted genera in bulk soil reflects stronger environmental control. These findings align with current microbiome frameworks, which suggest that microbial community assembly follows a gradient from bulk soil to the rhizosphere and root endosphere, with the strength of plant selection increasing toward internal root tissues [49, 47]. Therefore, the observed bacterial profiles suggest that pepper plants in Sumatra harbor distinct yet interconnected microbial communities, with potential implications for nutrient cycling, soil adaptation, and plant health under region-specific agroecological conditions.

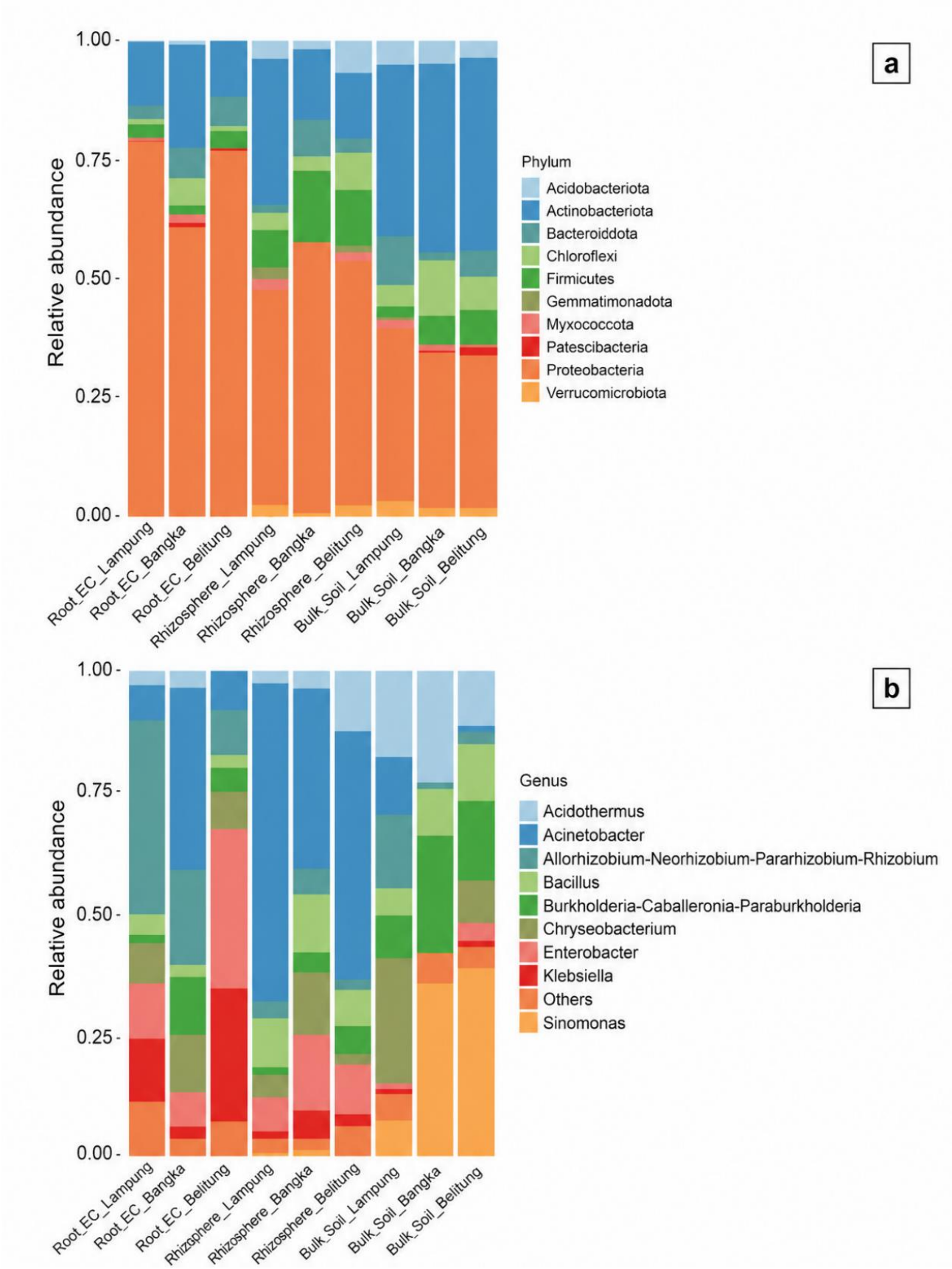

Figure 1: Relative abundance and taxonomic structure of dominant bacterial communities at the 10 most abundant bacterial phyla (a) and the 10 most prevalent bacterial taxa or genus levels (b).

Distribution of bacterial communities across roots EC, rhizosphere, and bulk soil, and their shared richness

Beyond relative abundance patterns, the extent of shared and unique bacterial amplicon sequence variants (ASVs) among regions was further evaluated to clarify spatial variation in community richness within each soil–plant compartment. This approach provides insight into whether bacterial communities are broadly conserved across sites or shaped by region-specific ecological filtering. The distribution of shared and unique bacterial ASVs is summarized in the Venn diagram.

The Venn diagram revealed distinct patterns of shared and unique bacterial richness across Lampung, Bangka, and Belitung in the three compartments. In the root endosphere, 113 bacterial ASVs were shared among all regions, while each site also harbored a substantial number of unique taxa, with Root EC_Bangka having 1,255 unique ASVs, followed by Root EC_Belitung with 727 and Root EC_Lampung with 560. The relatively small shared fraction, despite the high number of site-specific taxa, suggests that the internal root microbiome of pepper is strongly shaped by local environmental conditions, host–microbe filtering, and regional soil characteristics. This aligns with the concept that root endosphere communities represent a selectively assembled subset of soil-derived microorganisms, shaped by both plant traits and external edaphic conditions [50, 47].

The rhizosphere showed the highest shared richness among the three regions, with 258 ASVs detected across Lampung, Bangka, and Belitung. This compartment also contained the greatest number of unique ASVs, with the highest in Rhizosphere_Lampung (1,535), followed by Rhizosphere_Belitung (1,244) and Rhizosphere_Bangka (1,136). These results indicate that the rhizosphere acts as a highly diverse and dynamic microbial hotspot, where root exudates, nutrient gradients, and soil physicochemical heterogeneity promote both common and site-specific bacterial assemblages. The higher overlap in the rhizosphere compared with root endosphere and bulk soil suggests that pepper roots may create a partially conserved rhizosphere niche, while local agroecological factors still drive strong regional differentiation. Similar rhizosphere-driven enrichment and niche differentiation have been reported in recent plant microbiome studies, where root-derived carbon inputs strongly regulate bacterial recruitment and community assembly [49, 29].

In bulk soil, 129 ASVs were shared among all regions, whereas unique ASVs were highest in Bulk Soil_Bangka with 895, followed by Bulk Soil_Belitung with 838 and Bulk Soil_Lampung with 482. The lower shared richness in bulk soil than in the rhizosphere indicates that free-living soil bacterial communities are more strongly shaped by site-specific edaphic factors, such as soil pH, organic matter, nutrient availability, and metal content. Overall, the Venn analysis supports the relative abundance results by showing that bacterial communities in pepper agroecosystems are structured by both compartment-specific selection and geographic variation. The rhizosphere represented the richest and most interconnected compartment, whereas the root endosphere and bulk soil displayed stronger site-specific signatures, reflecting the combined influence of host selection and environmental filtering.

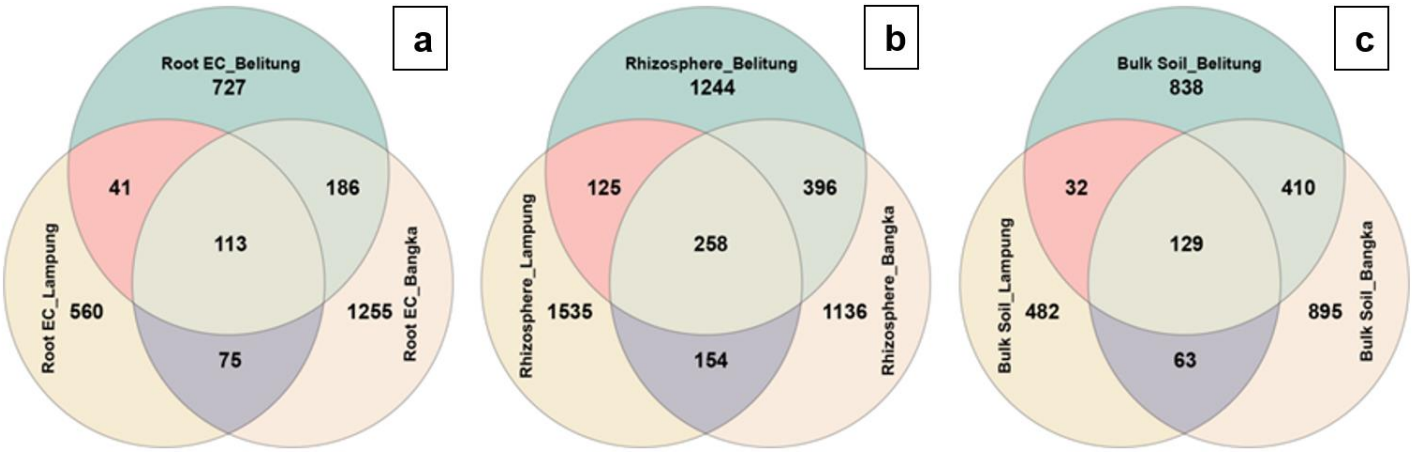

Figure 2. Distribution number of shared and unique bacterial ASVs among sampling sites within each compartment: roots EC (a), rhizosphere (b), and bulk soil (c), including the total shared richness.

Bacterial diversity analysis using alpha diversity

After identifying shared and site-specific bacterial ASVs, alpha diversity was assessed to determine how these ASV patterns translate into within-sample richness, evenness, and dominance across compartments and regions. This analysis provides a quantitative measure of bacterial community complexity beyond taxonomic overlap. The alpha diversity patterns are summarized in Figure 3. Alpha diversity was assessed using complementary indices that capture different dimensions of within-sample bacterial diversity: Observed ASVs quantify taxonomic richness; the Shannon index integrates richness and evenness; and the Simpson and inverse Simpson indices emphasize dominance structure and the effective number of abundant taxa. Together, these metrics provide a more comprehensive evaluation of community complexity than any single diversity measure alone.

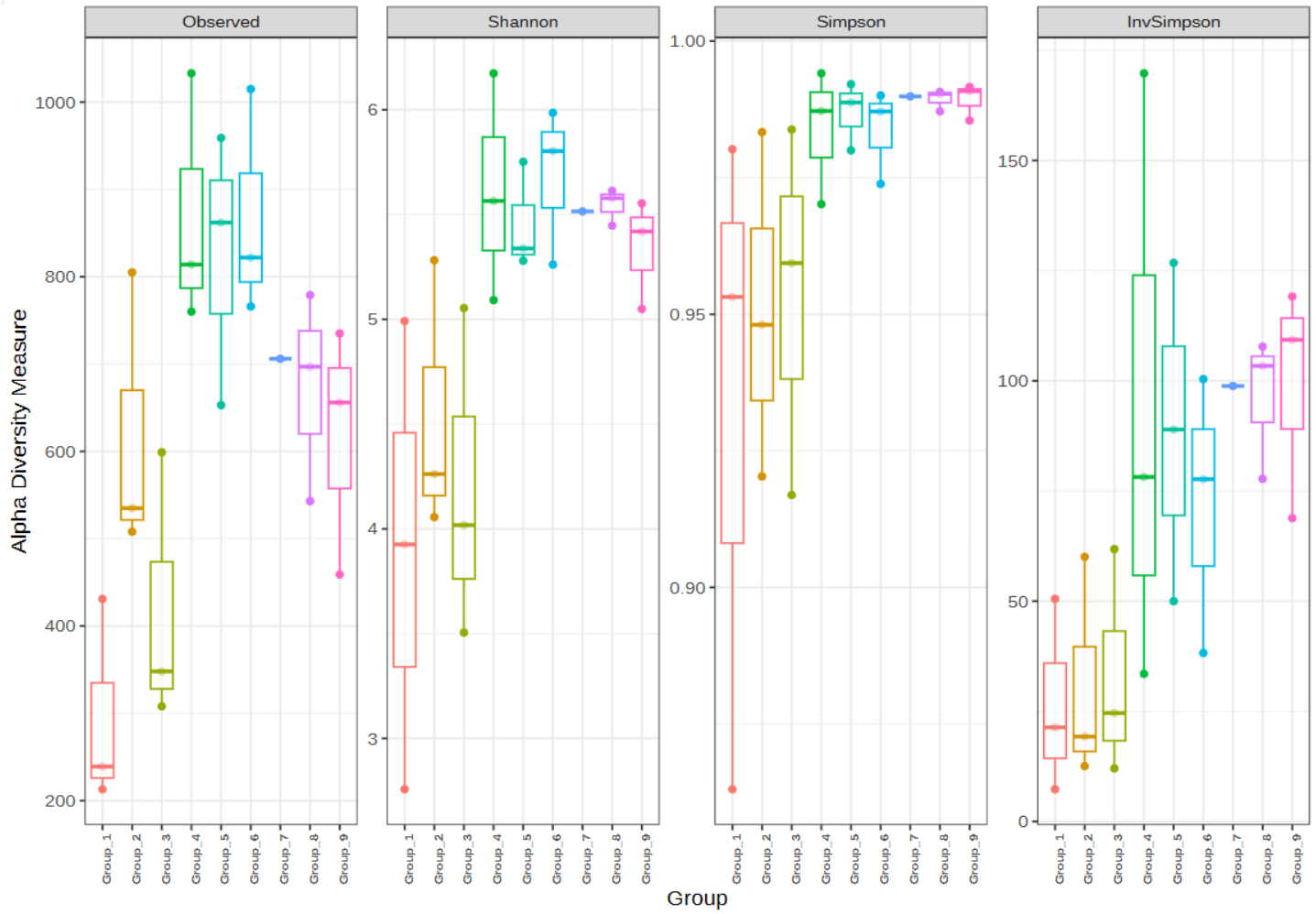

Figure 3. Alpha diversity of bacterial communities across root endosphere, rhizosphere, and bulk soil samples from Lampung, Bangka, and Belitung based on Observed ASVs, Shannon, Simpson, and inverse Simpson indices: Root EC_Lampung (Group 1); Root EC_Bangka (Group 2); Root EC_Belitung (Group 3); Rhizosphere_Lampung (Group 4); Rhizosphere_Bangka (Group 5); Rhizosphere_Belitung (Group 6); Bulk soil_Lampung (Group 7); Bulk soil_Bangka (Group 8); and Bulk soil_Belitung (Group 9).

Alpha diversity analysis revealed clear compartment-dependent differences in bacterial richness and diversity across the nine sample groups. The Observed ASVs index indicated that root endosphere samples had the lowest richness, particularly Root EC_Lampung and Root EC_Belitung, with median values of approximately 240 and 340 ASVs, respectively. By contrast, Root EC_Bangka showed a higher median richness of around 530 ASVs. Rhizosphere samples consistently exhibited the highest richness, with median Observed ASVs of approximately 810, 860, and 820 in Rhizosphere_Lampung, Rhizosphere_Bangka, and Rhizosphere_Belitung, respectively. Bulk soil samples showed intermediate richness, with medians of approximately 710, 690, and 650 ASVs in Lampung, Bangka, and Belitung, respectively. This pattern suggests that the rhizosphere functions as a microbial diversity hotspot, likely due to the combined effects of root exudates, nutrient gradients, and recruitment of soil-derived bacteria, whereas the root endosphere represents a more selectively filtered habitat.

Consistent trends across Shannon, Simpson, and inverse Simpson indices indicate that differences extend beyond richness to include variation in evenness and dominance structure. Shannon diversity was lowest in root endosphere samples, particularly Root EC_Lampung and Root EC_Belitung, with median values of approximately 3.9 and 4.0, whereas rhizosphere and bulk soil samples generally showed higher values, mostly ranging from about 5.3 to 5.8. Simpson values were high across all groups, but root samples showed greater variability and slightly lower medians, whereas rhizosphere and bulk soil samples remained close to 0.98–0.99, indicating more even bacterial assemblages. Similarly, inverse Simpson values were lowest in root samples, particularly Root EC_Lampung, and increased markedly in rhizosphere and bulk soil, with the highest values observed in Bulk soil Belitung and Bulk soil_Bangka. These results support the concept that plant roots impose strong ecological filtering on bacterial communities, reducing internal diversity while enriching specific root-adapted taxa, whereas rhizosphere and bulk soil environments maintain broader and more even bacterial assemblages due to greater niche availability and edaphic heterogeneity.

Overall, the alpha diversity results align with the Venn diagram and relative abundance profiles, indicating that the rhizosphere harbors the most diverse bacterial communities, whereas the root endosphere shows lower richness and stronger taxonomic selection. Such compartmentalized diversity patterns are widely reported in plant microbiome studies, where bacterial diversity typically decreases from bulk soil to rhizosphere and root endosphere as host selection intensifies toward internal plant tissues. The high diversity observed in rhizosphere and bulk soil samples may also reflect the influence of soil physicochemical heterogeneity, organic matter availability, and local agroecological conditions, which are known to regulate microbial community assembly and ecosystem functioning [51].
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Differences in bacterial community structure analyses using beta diversity

While alpha diversity describes within-sample richness and evenness, beta diversity was used to assess differences in bacterial community composition among compartments and geographic locations. Principal coordinate analysis (PCoA) provides an ordination-based visualization of community dissimilarity and helps identify clustering patterns associated with plant compartment and site origin. These compositional differences are presented in Figure 4. The plots are based on an average weighted UniFrac (Wunifrac) distance matrix that incorporates both phylogenetic and abundance data. PCoA showed clear compositional separation among bacterial communities across compartments and sampling locations, with the first two axes explaining 47.18% of the total variation. PCoA1 accounted for 31.86% of the variation and appeared to separate several rhizosphere and bulk soil samples toward the positive axis from most root endosphere samples, which were predominantly positioned on the negative side. PCoA2 explained an additional 15.32% of the variation and further distinguished samples within the same compartment, particularly among rhizosphere and bulk soil groups. This ordination pattern indicates that bacterial community composition was not randomly distributed but structured by both plant-associated compartment and regional origin.

Root endosphere samples tended to cluster toward the negative side of PCoA1, especially Root EC_Lampung, Root EC_Bangka, and part of Root EC_Belitung, suggesting a distinct internal root bacterial assemblage compared with surrounding soil compartments. In contrast, Rhizosphere_Lampung and Bulk soil_Belitung were mostly positioned on the positive side of PCoA1, indicating stronger compositional divergence from root-associated communities. Rhizosphere_Belitung and Bulk soil_Lampung showed intermediate positions, suggesting transitional or site-specific bacterial assemblages influenced by both root-derived inputs and local soil conditions. Dispersion among replicates, particularly in Root EC_Belitung and some rhizosphere samples, also suggests within-group heterogeneity, which may reflect microscale variation in root colonization, soil chemistry, or microbial recruitment processes. Due to root nutrient exudates and signaling chemicals, the plant rhizosphere is more diverse than the bulk soil [52, 53]. This variety is also due to predation shields, mycorrhizal benefits, and the attachment niches of other roots. It is well known that plant roots strongly influence rhizosphere microorganisms. Roots secrete important organic molecules in the form of exudates that attract microbes. However, these chemicals vary across plant species, soil types, developmental stages, concentrations, and chemical nature [54, 55, 56]. Various crop species attract different bacterial populations owing to differences in root exudates [57, 58]. Rhizobacterial microflora may also be affected by soil features when certain plant-associated microorganisms are grown in various soils [57, 59].

Overall, the beta diversity pattern aligns with the alpha diversity and taxonomic abundance results, showing compartment-specific structuring of bacterial communities in pepper agroecosystems. The separation between the root endosphere and soil-associated compartments is consistent with the ecological filtering model, in which plant roots selectively recruit a subset of soil-derived microorganisms, whereas rhizosphere and bulk soil communities are more strongly shaped by edaphic properties and geographic heterogeneity. Similar compartmentalization of plant-associated microbiomes has been

reported across diverse crops, where host selection, root exudation, soil pH, nutrient availability, and environmental filtering jointly shape microbial assembly from bulk soil to the rhizosphere and internal root tissues.

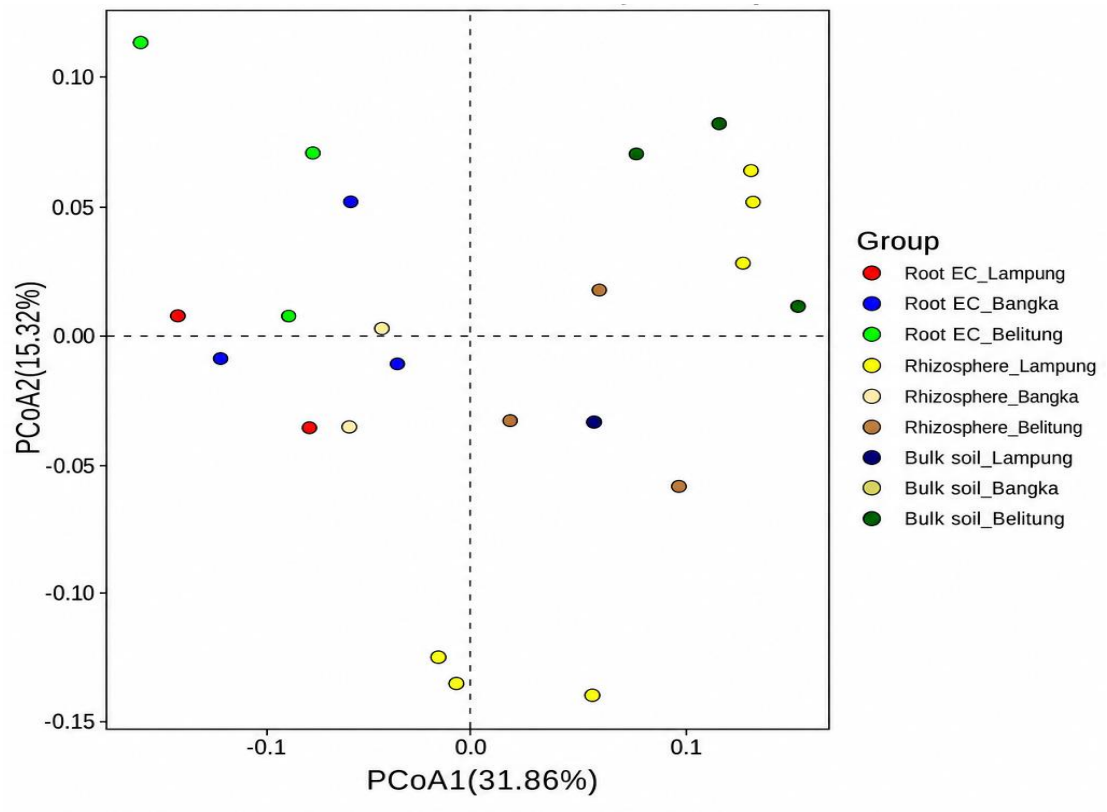

Figure 4. Principal coordinate analysis (PCoA) of bacterial community composition across root, rhizosphere, and bulk soil samples from Lampung, Bangka, and Belitung based on weighted UniFrac.

Taxonomic drivers of bacterial community differentiation based on PCA

To complement the beta-diversity analysis based on weighted UniFrac (Wunifrac) distances, principal component analysis (PCA) was conducted to identify the bacterial taxa most strongly associated with the observed community separation. This approach clarifies which dominant phyla and genera contribute to differentiation among compartments and sampling locations. PCA biplots at the phylum and genus levels are shown in Figure 5. At the phylum level (Figure 5a), the first two axes explained 62.4% of the total variation, with PC1 and PC2 accounting for 36.8% and 25.6%, respectively. The biplot indicated that several dominant phyla contributed differently to community separation among compartments and regions. Acidobacteriota and Verrucomicrobiota were oriented toward the positive side of PC1, suggesting a stronger association with soil-related bacterial assemblages, particularly samples positioned on the right side of the ordination space. In contrast, Proteobacteria, Bacteroidota, and Patescibacteria were positioned toward the negative side of PC1, indicating a greater association with root- and rhizosphere-related communities. Actinobacteriota, Chloroflexi, Firmicutes, Gemmatimonadota, and Myxococcota were located closer to the ordination center, suggesting broader distribution across sample types or weaker discriminatory contribution. These patterns align with the relative-abundance results, in which Proteobacteria dominated plant-associated compartments, whereas Acidobacteriota was more prominent in bulk soil.

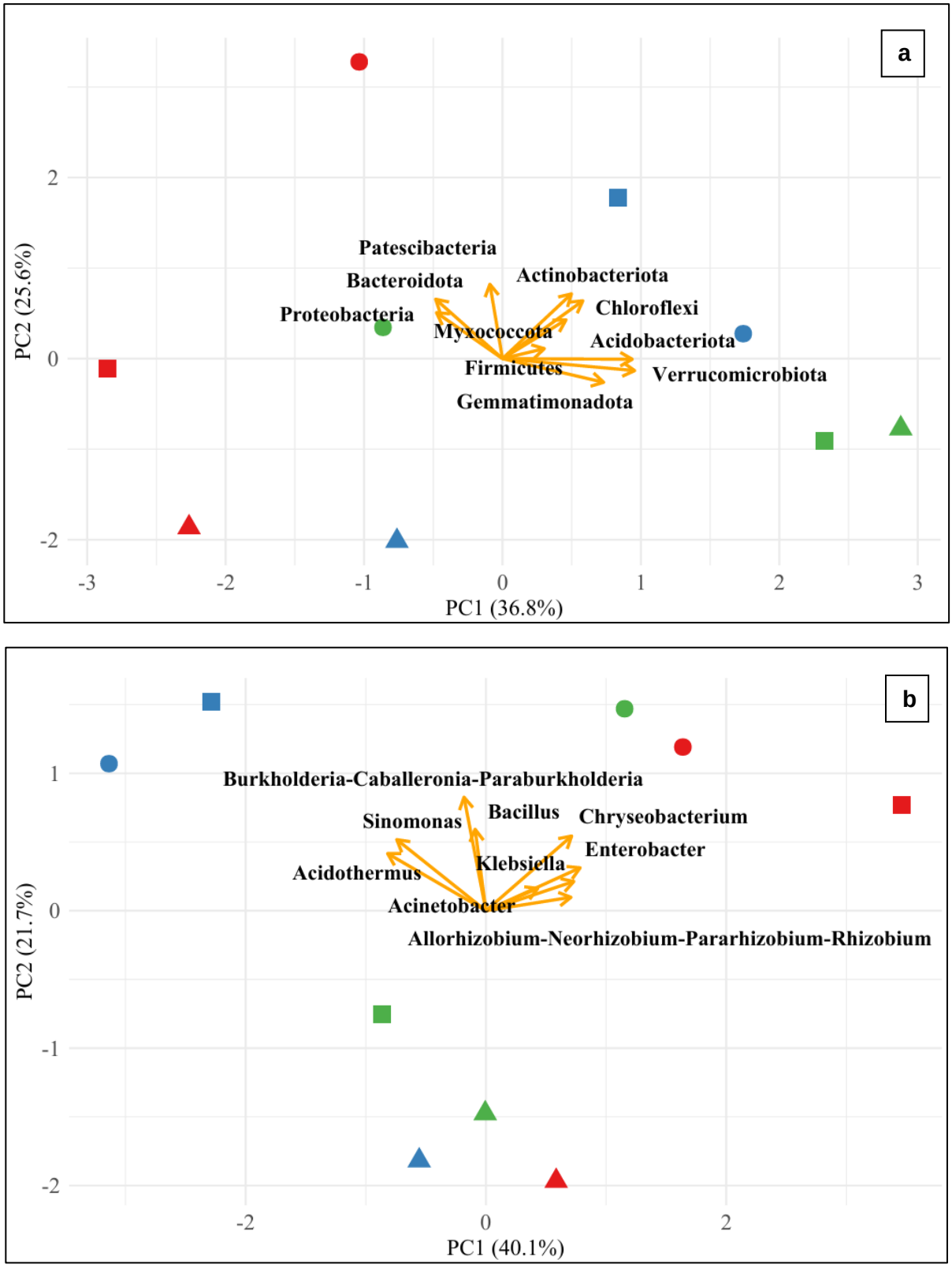

Figure 5. The principal component analysis (PCA) of pepper's bacterial communities: (A) Phyla and (B) Genus (triangle /▲: Lampung; circle / ●: Bangka; and square / ■: Belitung; Red: Root EC; Green: Rhizosphere; and Blue: Bulk soil).

At the genus level (Figure 5b), PC1 and PC2 explained 40.1% and 21.7% of the total variation, respectively, for a cumulative 61.8%. The genus-level biplot showed that Burkholderia–Caballeronia–Paraburkholderia, Sinomonas, Acidothermus, and Acinetobacter were oriented toward the negative side

of PC1, indicating their contribution to the separation of specific root, rhizosphere, or bulk soil communities. Conversely, Chryseobacterium, Enterobacter, Klebsiella, and the Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium group were oriented toward the positive side of PC1, suggesting stronger associations with samples positioned on that side of the ordination. Bacillus occupied an intermediate position, indicating a more generalist distribution across compartments. These results highlight that bacterial community differentiation was not driven by a single taxon but by coordinated shifts across several plant-associated and soil-adapted genera.

Overall, the PCA results support the Wunifrac beta-diversity pattern by identifying the dominant taxa that structure bacterial communities across pepper agroecosystems. The association of Proteobacteria and several root-associated genera with plant compartments aligns with host-mediated microbial selection and the ability of these taxa to utilize root-derived carbon compounds. By contrast, the association of Acidobacteriota and soil-adapted genera with bulk soil reflects environmental filtering driven by edaphic conditions. These patterns are consistent with previous plant microbiome studies showing that root exudation, soil physicochemical properties, host genotype, and local environmental conditions jointly shape bacterial community assembly from bulk soil to the rhizosphere and root endosphere [50, 49, 47, 48].

Environmental and compartmental structuring of bacterial taxa based on redundancy analysis (RDA)

Following PCA-based identification of the dominant taxa driving community differentiation, redundancy analysis was further applied to assess how bacterial assemblages at the phylum and genus levels were constrained by sample compartment and geographic origin. This analysis provides additional insight into the taxa most closely associated with root endosphere, rhizosphere, and bulk soil habitats across pepper-growing regions. The RDA ordination patterns are shown in Figure 6.

Redundancy analysis revealed clear taxonomic structuring of pepper-associated bacterial communities across compartments and locations. At the phylum level (Figure 6a), RDA1 explained the largest proportion of variation (71.4%), while RDA2 explained 6.7%, indicating that most phylum-level differentiation occurred along the primary constrained axis. Proteobacteria showed the strongest vector loading on the positive side of RDA1, suggesting a close association with samples positioned in this direction, particularly root- and rhizosphere-associated communities. In contrast, Acidobacteriota, Verrucomicrobiota, Chloroflexi, and Actinobacteriota were oriented toward the negative side of RDA1, indicating stronger associations with soil-related or site-specific bacterial assemblages. This pattern aligns with previous abundance and PCA results, in which Proteobacteria was more closely linked to plant-associated compartments, whereas Acidobacteriota and other oligotrophic or soil-adapted phyla were more prominent in bulk soil.

At the genus level (Figure 6b), RDA1 and RDA2 explained 36.8% and 20.9% of the variation, respectively, indicating that genus-level community differentiation was more evenly distributed across both axes. *Acinetobacter* was strongly oriented toward the negative side of RDA1, indicating a distinct association with specific root or rhizosphere samples, whereas *Sinomonas*, *Acidothermus*, and *Burkholderia–Caballeronia–Paraburkholderia* were positioned toward the positive side of RDA1. Other genera, including *Enterobacter*, *Klebsiella*, *Chryseobacterium*, *Bacillus*, and the *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium* group, occupied intermediate positions, suggesting variable contributions across compartments and locations. These genera are widely reported as root- or rhizosphere-associated bacteria with potential roles in nutrient mobilization, stress tolerance, and plant growth promotion. However, their functional contributions in the present study should be interpreted with caution because functional assays were not performed.

Overall, the RDA results indicate that bacterial community composition in pepper agroecosystems was shaped by the combined effects of plant compartment and geographic origin, with clear taxonomic signatures at both the phylum and genus levels. The stronger association of Proteobacteria and several plant-associated genera with the root and rhizosphere compartments is consistent with the idea that root exudates and host-mediated filtering enrich copiotrophic and plant-adapted bacterial taxa. Conversely, the association of Acidobacteriota, Verrucomicrobiota, Chloroflexi, and Actinobacteriota with soil-related samples may reflect adaptation to edaphic heterogeneity, nutrient availability, and soil physicochemical conditions. Similar environment- and compartment-driven microbial structuring has been reported in other crop systems, where soil properties, plant compartment, and local management practices jointly regulate rhizosphere and endosphere microbiome assembly [60, 61, 62, 63].

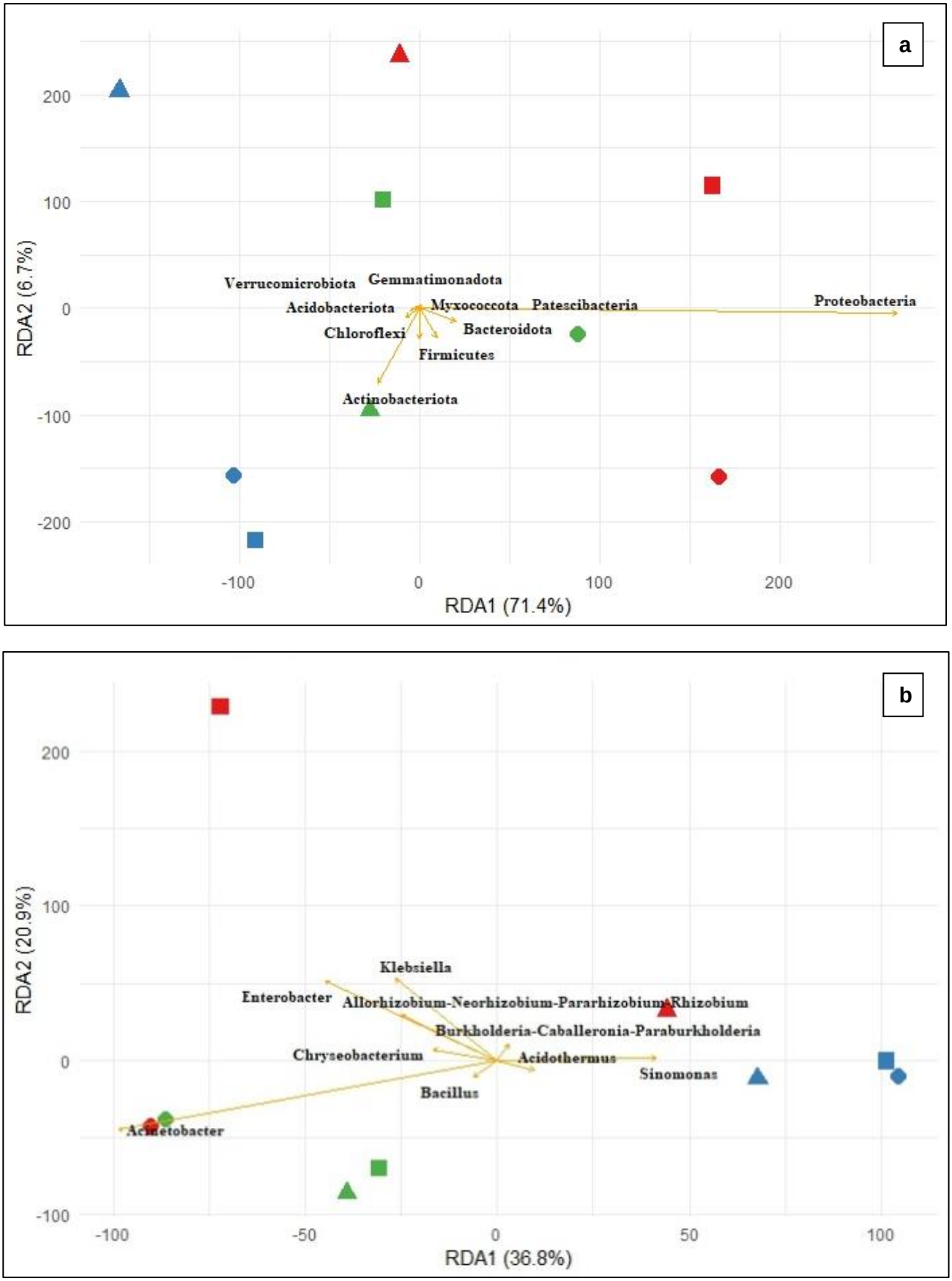

Figure 6. The redundancy analysis (RDA) of pepper's bacterial communities in threshold 0.5%: (A) Phyla and (B) Genus (triangle / ▲: Lampung; circle / ● : Bangka; and square / ■ : Belitung; Red: Root EC; Green: Rhizosphere; and Blue: Bulk soil).

The integrated analysis of soil characteristics, nutrient status, bacterial abundance, and diversity indicates that pepper-associated bacterial communities were structured by the combined effects of plant compartment and local edaphic conditions. Proteobacteria consistently dominated across samples, particularly in the root endosphere and rhizosphere, suggesting strong plant-associated selection for copiotrophic and metabolically versatile bacteria capable of responding to root-derived carbon inputs. Many bacterial phyla identified in the rhizosphere have been shown to support soil and plant growth-promoting activities [52, 64, 65, 66]. In contrast, the presence of Bacteroidota, Firmicutes, Actinobacteriota, Acidobacteriota, and Verrucomicrobiota across compartments indicates that the pepper microbiome was not dominated by a single lineage but rather by a consortium of taxa with distinct ecological strategies. Acidobacteriota and other soil-adapted phyla were more abundant in bulk soil, consistent with their known association with acidic, oligotrophic, and nutrient-regulated soil environments. Therefore, differences in soil pH, nutrient availability, organic matter, electrical conductivity, and site-specific agroecological conditions may partly explain the observed shifts in bacterial relative abundance, although these relationships should be interpreted as ecological associations rather than direct causal effects unless supported by formal multivariate testing.

The diversity patterns further support this compartment-based structuring. Rhizosphere samples generally showed greater bacterial richness and diversity than root endosphere samples, reflecting the rhizosphere as a biologically active interface enriched by root exudates, mucilage, sloughed cells, and nutrient gradients. These substrates create heterogeneous microhabitats that support a broader range of bacterial taxa, whereas the root endosphere is a more selective habitat in which only a subset of soil- and rhizosphere-derived microorganisms can successfully colonize internal tissues. This ecological filtering explains why root endosphere communities often show lower alpha diversity but a higher proportion of specific plant-associated taxa. Beta-diversity, PCA, and RDA patterns also reinforce this interpretation, showing that differences in relative abundance were accompanied by compositional separation among root, rhizosphere, and bulk soil samples. Such compartmentalization is widely reported in plant microbiome studies, where host selection, soil physicochemical properties, nutrient status, and environmental heterogeneity jointly regulate microbial assembly from bulk soil to rhizosphere and endosphere [67, 60, 47, 62, 63].

At the genus level, Acinetobacter predominates across samples, particularly in the root and rhizosphere compartments, suggesting that this genus may be an important component of the pepper-associated bacterial consortium. Several other detected genera, including Enterobacter, Klebsiella, Bacillus, Chryseobacterium, Burkholderia–Caballeronia–Paraburkholderia, and the Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium group, are commonly reported as rhizosphere- or endosphere-associated bacteria with potential roles in nutrient mobilization, nitrogen cycling, phosphate solubilization, stress tolerance, and plant growth promotion. Furthermore, research conducted on wheat revealed that the majority of plant growth-promoting rhizobacteria (PGPR) belong to the Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria of different genera [68]. However, their ecological roles in this study should be interpreted cautiously because taxonomic profiling alone does not directly confirm function. Overall, abundance and diversity results suggest that Indonesian pepper agroecosystems harbor compartment-specific bacterial communities shaped by both plant-mediated recruitment and local soil conditions. These findings provide an important baseline for future functional studies aimed at identifying beneficial pepper-associated bacteria that could support sustainable nutrient management and reduce dependence on synthetic fertilizers and pesticides [69].

All the facts regarding soil properties and climate were discussed and matched with the information provided in the article about the variety and abundance of bacterial communities in Indonesian pepper plants. These findings provide a rational justification for the notable variety and abundance of bacterial communities throughout the various compartments and sites. Furthermore, these data may elucidate the impact of soil conditions and geographical factors on the bacterial population associated with pepper plants. These results are corroborated by our investigation, which found that sample types and locations differed significantly in bacterial community diversity, as measured by alpha diversity and other biodiversity indices. Compared to bulk soil samples, rhizosphere samples had higher Shannon and Simpson indices, indicating a more diversified environment. The greater alpha diversity seen in the rhizosphere samples, for example, suggests that soil type, weather, and farming techniques in the Bangka area could be major factors in determining bacterial community composition. However, further research is needed to fully understand the factors contributing to the greater variety of bacteria in the soil of this region. Our findings emphasize that the rhizosphere is a unique and diverse environment. Additionally, we highlight the necessity for agricultural ecosystems to encompass multi-scale environments with spatial heterogeneity in both the abundance and composition of bacterial communities. This understanding could facilitate the development of effective agricultural practices to enhance soil health and vegetation. The differences in phylum abundance among groups can be attributed to variations in environmental conditions that influence bacterial community composition. The uneven distribution of these phyla reflects the diversity of their functional capabilities within the bacterial

communities. Various phyla are recognized for their distinct ecological roles, including nutrient cycling, carbon fixation, and organic matter decomposition.

Conclusions

This study presents a 16S rRNA amplicon-based assessment of bacterial communities associated with Indonesian pepper plantations across Lampung, Bangka, and Belitung. Bacterial assemblages were strongly structured by plant compartment and regional soil conditions, including pH, nutrient availability, organic carbon, electrical conductivity, and metal-related properties. Across all samples, 25 bacterial phyla were detected, with Proteobacteria dominating the root endosphere and rhizosphere at approximately 60–78% and 44–56%, respectively, whereas Acidobacteriota was more prominent in bulk soil at approximately 36–41%. At the genus level, Acinetobacter was highly enriched in root- and rhizosphere-associated samples, reaching approximately 68% in Rhizosphere_Lampung, 50% in Rhizosphere_Belitung, and 38% in Root EC_Bangka. Other dominant genera showed compartment- and location-specific patterns, including Enterobacter and Klebsiella in Root EC_Belitung, Burkholderia–Caballeronia–Paraburkholderia, Bacillus, and Chryseobacterium in Rhizosphere_Bangka, and Sinomonas and Acidothermus in bulk soil. Diversity analysis confirmed the rhizosphere as the most diverse compartment, with median Observed ASVs of 810–860 and the highest shared richness among regions (258 ASVs), compared with root endosphere (113 ASVs) and bulk soil (129 ASVs). Beta diversity further showed clear compositional separation, with weighted UniFrac PCoA explaining 47.18% of total variation across the first two axes. PCA and RDA confirmed that differences in community structure were associated with coordinated shifts in dominant phyla and genera, particularly Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, Acinetobacter, Enterobacter, Chryseobacterium, and Sinomonas. Overall, pepper-associated bacterial communities are shaped by host-mediated selection and local edaphic filtering. These taxa provide a baseline for future functional validation and microbiome-based strategies for sustainable pepper cultivation.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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