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Original Article

Identification and characterization of maize rhizospheric bacterial community by 16S ribosomal RNA in the semi-arid area of East Nusa Tenggara, Indonesia

Identificação e caracterização da comunidade bacteriana rizosférica do milho por RNA ribossômico 16S na área semiárida de Nusa Tenggara Oriental, Indonésia

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Abstract

Maize productivity in semi-arid regions such as East Nusa Tenggara Indonesia is significantly hindered by drought stress and low soil fertility resulting in yields well below the national average. The plant rhizosphere, a critical interface between roots and soil, hosts diverse microbial communities, particularly plant growth-promoting rhizobacteria, which are essential for enhancing plant resilience to abiotic stresses. This study aimed to identify and characterize the rhizospheric bacterial community associated with maize in NTT using 16S rRNA gene sequencing. Our findings revealed significant variations in bacterial abundance and community composition across different sites, reflecting environmental heterogeneity. Sample J1_KP exhibited a high abundance of *Bacillus* and *Ammoniphilus* spp., known for their roles in improving water use efficiency and stress tolerance through endospore formation. In contrast, sample J8_BTP was dominated by *Pseudomonas* spp., which are crucial for maintaining plant water status and root morphology under drier conditions. The presence of extremophiles like *Melghirimyces thermohalophilus* and nutrient cyclers such as *Devosia oryziradicis* and nitrifying bacteria (*Nitrosospira multiformis*, *Nitrospira japonica*) further highlights the adaptive strategies within these communities. This research provides valuable insights into microbial adaptations in challenging semi-arid environments, offering a foundation for developing targeted microbial inoculants to enhance maize resilience and productivity.

Keywords: 16S rRNA, bacterial community, East Nusa Tenggara, maize rhizosphere, semi-arid.

Resumo

A produtividade do milho em regiões semiáridas como Nusa Tenggara Oriental, Indonésia, é severamente prejudicada pelo estresse hídrico e baixa fertilidade do solo, com rendimentos significativamente abaixo da média nacional. A rizosfera da planta, uma interface crucial entre as raízes e o solo, abriga diversas comunidades microbianas, particularmente rizobactérias promotoras de crescimento vegetal, que são vitais para aumentar a resiliência das plantas contra estresses abióticos. Este estudo teve como objetivo identificar e caracterizar a comunidade bacteriana rizosférica associada ao milho em NTT através do sequenciamento do gene 16S rRNA. Nossos resultados revelaram variações significativas na abundância bacteriana e na composição da comunidade em diferentes locais, refletindo a heterogeneidade ambiental. A amostra J1_KP mostrou alta abundância de espécies de *Bacillus* e *Ammoniphilus* spp., conhecidas por seus papéis na melhoria da eficiência do uso da água e tolerância ao estresse por meio da formação de endosporos. Em contraste, a amostra J8_BTP exibiu uma dominância de espécies de *Pseudomonas*, críticas para o estado hídrico da planta e morfologia radicular em condições mais secas. A presença de extremófilos como *Melghirimyces thermohalophilus* e cicladores de nutrientes como *Devosia oryziradicis* e bactérias nitrificantes (*Nitrosospira multiformis*, *Nitrospira japonica*) destaca ainda mais as estratégias adaptativas dentro dessas comunidades. Esta pesquisa fornece informações valiosas sobre as adaptações microbianas em ambientes semiáridos desafiadores, oferecendo uma base para o desenvolvimento de inoculantes microbianos direcionados para aumentar a resiliência e produtividade do milho.

Palavras-chave: 16S rRNA, comunidade bacteriana, Nusa Tenggara Oriental, rizosfera do milho, semiárido.

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1. Introduction

Maize constitutes a fundamental pillar of global caloric security and industrial feedstock. Within the Indonesian archipelago, the stability of maize production is increasingly undermined by climatic volatility, particularly in the marginal agroecosystems of East Nusa Tenggara (Ngongo et al., 2021). This province is characterized by a pronounced dry season spanning eight to nine months, with annual precipitation averaging approximately 1,560 mm concentrated heavily between December and March (Yustiningsih et al., 2026). West Kupang typically experiences a short rainy season of 3–5 months followed by a long dry period of 7–8 months (Ardan, 2016), while Batu Putih in the South Central Timor region often exhibits lower cumulative rainfall and more acute aridity (Kotta et al., 2024). As a representative semi-arid province, NTT faces a staggering yield gap; average productivity in 2024 stagnates at approximately 2.69 t ha^{-1}, a figure markedly below the national average (BPS-Statistics, 2025). This deficit remains fundamentally linked (Seran et al., 2021).

The synergistic impact of drought and nutrient stress fundamentally reshapes maize physiology, primarily by restricting stomatal conductance, compromising membrane integrity, and impairing photosynthetic efficiency during critical reproductive stages (Anjum et al., 2017). Given the limitations of physical interventions, the biological potential of the plant rhizosphere has emerged as a critical frontier for enhancing crop fitness. The root-associated microbiome is increasingly recognized as a second genome for the host, extending the plant's capacity to mitigate environmental extremes (Berendsen et al., 2012). Plant growth-promoting rhizobacteria confer resilience through sophisticated metabolic pathways, including the enzymatic regulation of ethylene via 1-aminocyclopropane-1-carboxylate deaminase and the synthesis of stabilizing exopolysaccharides (Naylor and Coleman-Derr, 2018). Beyond individual taxa, the diversity and structural assembly of these microbial networks are vital indicators of soil health and ecosystem stability (Philippot et al., 2013). Such biological networks provide essential ecosystem services, facilitating nutrient acquisition and pathogen suppression in degraded landscapes. These mechanisms collectively dictate the adaptive trajectory of maize in semi-arid soils.

Despite global efforts to map the maize microbiome, the distinct microbial lineages inhabiting the isolated, semi-arid islands of NTT remain a critical knowledge gap. Climatic factors, particularly the aridity index and precipitation gradients, have been identified as primary drivers of soil bacterial turnover and diversity patterns in semi-arid ecosystems (Karray et al., 2020; Ma et al., 2021). Previous studies indicate that persistent drought and thermal stress can significantly reshape bacterial community profiles, favoring taxa with specialized survival strategies and enhancing community-wide adaptation to drier environments (Reis et al., 2019; Diaz-Garza et al., 2020). The unique selective pressures imposed by NTT's climate likely drive the assembly of indigenous microbial consortia with specialized adaptive traits. Previous surveys in this region have predominantly relied on culture-dependent techniques, which overlook the vast majority of microbial dark matter (Amann et al., 1995). Resolving this necessitates a culture-independent approach; high-throughput 16S rRNA gene sequencing offers the resolution required to decode the taxonomic landscape and niche occupancy within the heterogeneous maize rhizosphere (Thompson et al., 2017).

Based on these premises, the hypothesis is posited that the maize rhizosphere in NTT harbors a distinct core microbiome that has co-evolved with the local environment to facilitate host survival under drought. This study aims to characterize the bacterial community assembly and identify the biological drivers of maize resilience in these challenging dryland ecosystems. These data will provide the requisite scientific framework for the future development of targeted microbial inoculants. By strategically leveraging indigenous microbial assets, the productivity gap currently hindering regional food security in East Nusa Tenggara can be bridged. Understanding the assembly rules of these communities is essential for translating biological insights into practical agricultural tools. This research ultimately seeks to harmonize ecological stability with crop yield objectives, ensuring that semi-arid farming systems remain viable despite intensifying global climate change. Through this exploration, the potential of beneficial bacteria to sustain productivity is uncovered.

2. Materials and Methods

2.1. Study site

The study was conducted across two geographically and ecologically distinct regions in East Nusa Tenggara, Indonesia: West Kupang and Batu Putih. West Kupang is characterized by a coastal lowland topography with predominantly alluvial and calcareous soils. This region experiences a semi-arid climate categorized under Oldeman types D4 and E4, marked by a prolonged dry season of 7–8 months (Ardan, 2016; Sudhiatiningsih et al., 2024). Conversely, Batu Putih, situated in the South Timor Tengah Regency, represents an inland highland environment with a more rugged, hilly topography. While sharing a semi-arid climate, BTP typically exhibits higher environmental stress due to steeper slopes and potentially different soil development compared to the coastal KP sites.

2.2. Sampling strategy and notation

A purposive sampling method was employed to select active maize agricultural plots across both regions. A total of nine rhizosphere soil samples were collected in September 2025: four from West Kupang and five from Batu Putih (Figure 1). To ensure representative sampling and minimize individual plant variability, a common practice in rhizomicrobiome studies, each sample was obtained as a composite from five randomly selected plants at each site. The sample notation follows a structured alphanumeric code: the prefix 'J' denotes *Zea mays*, followed by a site-specific identifier and the regional code (Table 1).

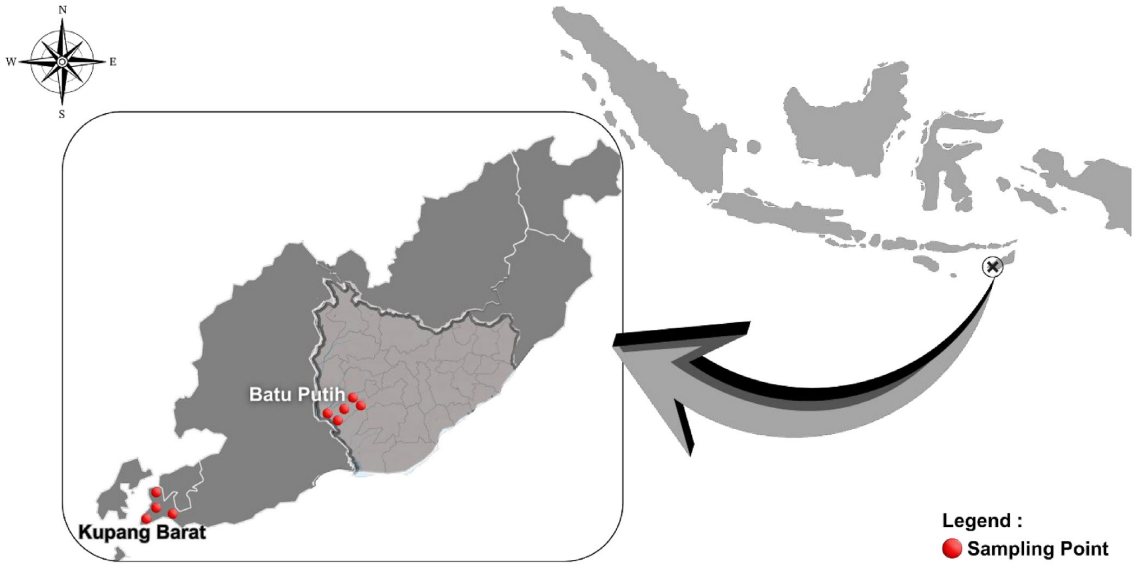

Figure 1. Location of Maize Rhizospheric Soil Sample Collection.

Table 1. Coordinates of Sampling Location.

No	Location	Sample Code	Coordinate
1.	KP	JAS_KP	10°13'28.6"S 123°30'52.9"E
2.	KP	J1_KP	10°17'25.0"S 123°31'06.6"E
3.	KP	J2_KP	10°19'51.3"S 123°29'01.4"E
4.	KP	J3_KP	10°19'42.2"S 123°33'33.0"E
5.	BTP	J4_BTP	9°54'04.1"S 124°13'09.4"E
6.	BTP	J5_BTP	9°55'18.2"S 124°14'03.7"E
7.	BTP	J6_BTP	9°56'25.7"S 124°12'29.9"E
8.	BTP	J7_BTP	9°59'15.7"S 124°09'10.5"E
9.	BTP	J8_BTP	9°57'26.6"S 124°09'06.3"E

All samples were collected during the flowering stage (R1 phenological stage), which represents the peak of root exudation and microbial activity in the maize life cycle.

2.3. Rhizosphere soil collection

Rhizosphere soil was collected following established protocols for field-grown crops. Maize plants were carefully uprooted to maintain the integrity of the root system. Loosely adhering soil was removed by vigorous manual shaking, leaving only the soil tightly bound (1–3 mm) to the root surface, which was defined as the rhizosphere. This tightly adhering soil was carefully brushed into sterile containers. To preserve microbial DNA and RNA integrity, 0.90 g of the collected rhizosphere soil was immediately homogenized in 10 mL of DNA/RNA Shield within 50 mL sterile tubes. All samples were transported in ice-chilled containers (4°C) and stored at –80°C upon arrival at the laboratory for downstream metagenomic analysis.

2.4. DNA extraction and 16S rRNA gene amplification

Total genomic DNA was extracted from approximately 0.25 g of each rhizospheric soil sample utilizing the ZymoBIOMICS DNA Miniprep Kit in accordance with the manufacturer's protocol. Minor modifications were implemented, specifically an extended bead-beating duration, to ensure efficient cell lysis from the complex soil matrices. The quantity and quality of the extracted DNA were subsequently assessed using a NanoDrop 2000 spectrophotometer and a Qubit 3.0 Fluorometer with the Qubit dsDNA HS Assay Kit. A minimum DNA concentration of 0.1 ng/µL (as quantified by Qubit) or 2 ng/µL (as quantified by Nanodrop) was confirmed for subsequent PCR amplification.

The full-length 16S rRNA gene was amplified using the universal primer pair 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Each Polymerase Chain Reaction was performed in a 20 µL reaction volume containing 10 µL of 2X Phusion™ Plus Green PCR Master

Mix, 0.4 µM of each primer, and approximately 20 ng of template DNA. The PCR thermal profile followed a modified 3-step protocol: an initial denaturation at 98°C for 30 seconds, followed by 30 cycles of denaturation at 98°C for 10 seconds, primer annealing at 60°C for 10 seconds (utilizing the master mix's universal annealing feature), and extension at 72°C for 30 seconds. A final extension step was performed at 72°C for 5 minutes. PCR products were verified via electrophoresis on a 1% agarose gel. All procedures were conducted at the Biotechnology Laboratory of Brawijaya University.

2.5. 16S rRNA gene sequencing

Purified PCR products were pooled in equimolar amounts and subjected to full-length 16S rRNA gene sequencing using the Oxford Nanopore Technologies platform. Library preparation was carried out using the Nanopore Ligation Sequencing Amplicons - Native Barcoding Kit 96 V14 (SQK-NBD114.96). Sequencing was performed on a MinION flow cell on a Nanopore GridION platform according to the manufacturer's protocol. Nanopore sequencing operations were managed by MinKNOW software version 25.03.7. Basecalling, which converts raw electrical signals into DNA sequences, was performed using Dorado software version 7.8.3 with a high-accuracy model. Sequencing was performed by Genetika Science Indonesia.

2.6. Data analysis

The data analysis encompassed a multi-step bioinformatic pipeline to characterize the maize rhizospheric bacterial communities. Initially, raw 16S rRNA gene sequencing reads underwent stringent quality control, including adapter trimming and filtering of low-quality sequences. High-quality reads were subsequently processed to derive amplicon sequence variants or operational taxonomic units, followed by taxonomic assignment using established reference databases. Alpha diversity metrics were calculated following the established formulas for Shannon (Shannon, 1948), Simpson (Simpson, 1949), Chao1 (Chao, 1984), and ACE (Chao and Lee, 1992) indices to quantify within-sample richness and evenness. Beta diversity was assessed through multivariate statistical methods such as Principal Component Analysis (PCA) or Principal Co-ordinates Analysis (PCoA) for community clustering, and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrograms to illustrate phylogenetic relationships. Additionally, heatmaps were generated to visualize the relative abundances of key taxa across samples, thereby elucidating community composition and adaptive strategies. All multivariate analysis was performed using R-Studio 4.4.3.

3. Results

3.1. Taxonomic composition of maize rhizospheric bacterial communities

Analysis of the bacterial composition across all samples revealed that the KP samples generally possessed a higher bacterial population than the BTP samples. Metagenomic quantification established a distinct gradient in the total bacterial abundance between the two semi-arid regions of NTT. The West Kupang sites exhibited a superior average microbial population of 145,190, compared to the 140,535 recorded in Batu Putih. At the granular level, J1_KP emerged as the most densely populated niche with a total abundance of 148,110, whereas J8_BTP represented the most constrained environment with only 132,462. This quantitative disparity indicates that the coastal lowland conditions of West Kupang provide a more expansive ecological threshold for bacterial proliferation than the highland terrains of Batu Putih. Furthermore, no bacterial groups identified were found to have a negative or toxic impact on plants.

The Krona plot for sample J1_KP provides a high-resolution visualization of a bacterial community structure predominantly defined by the phylum *Firmicutes* (Figure 2). This specific niche was characterized by a high relative dominance of the genera *Bacillus* spp. (17%), *Ammoniphilus* spp. (13%), and *Niallia* spp. (10%). At the species level, the interpretation reveals a community heavily weighted toward specialized taxa, most notably *Ammoniphilus resinae*, which accounted for 16.5k of the total bacterial abundance, and *Bacillus thaonhiensis*, which contributed 12.3k. The remaining percentage of the population was distributed among a diverse array of minority bacterial groups, indicating a specialized but multi-layered community architecture within the West Kupang rhizosphere.

In contrast, the Krona plot for sample J8_BTP (S9) revealed a distinct taxonomic turnover, characterized by a significant shift toward the phylum *Proteobacteria* (Figure 3). The community in this highland environment was primarily defined by the prevalence of the genus *Pseudomonas* spp. (4%), alongside a substantial representation from the class *Gammaproteobacteria*. Species-level interpretation for J8_BTP identified *Pseudomonas aeruginosa* as the primary dominant taxon, comprising 5.51k of the total abundance. While *Bacillus* spp. remained present in the BTP samples, their relative density and dominance were markedly lower compared to the J1_KP site. This shift signifies a regional specialization where different environmental filters select for divergent microbial lineages between the coastal and highland maize cultivation areas.

3.2. Top 10 maize rhizospheric bacterial communities

The taxonomic analysis of the maize rhizospheric bacterial communities, as detailed in Table 2, reveals a complex and diverse array of species and genera with varying abundances across the different maize rhizosphere samples (S1-S9). This rich microbial composition provides crucial insights into the adaptations and potential functions within the semi-arid environment of East Nusa Tenggara.

Metagenomic resolution reveals a significant biogeographical gradient in total bacterial abundance between the regional extremes of West Kupang and Batu Putih. Sample J1_KP represents the study's highest microbial carrying capacity with a total bacterial abundance of 148,110, characterized by a predominant "Firmicutes-heavy" architecture. This specific niche is

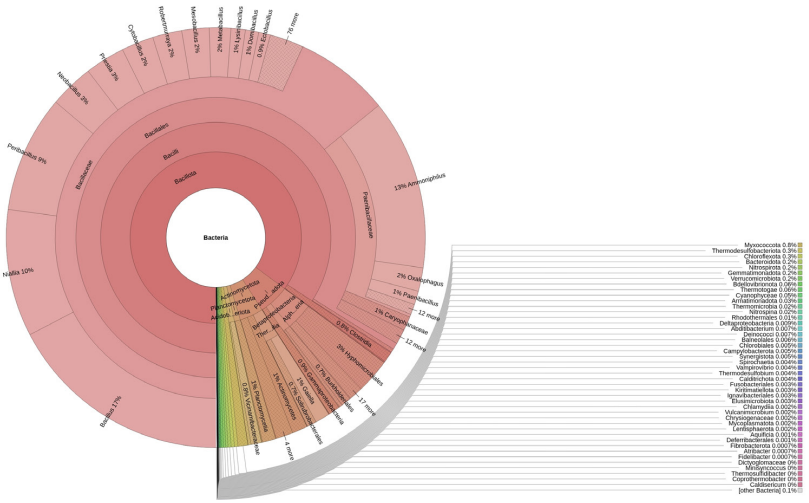

Figure 2. Taxonomic Composition of Maize Rhizospheric Bacterial Communities in J1_KP.

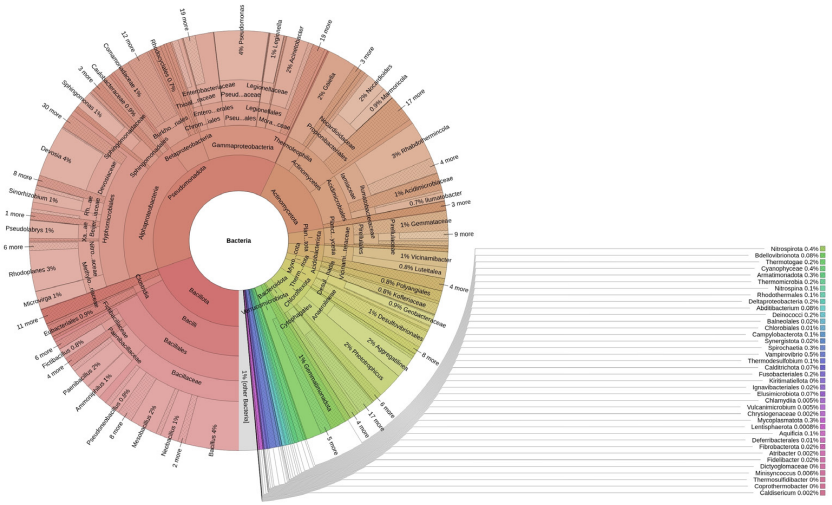

Figure 3. Taxonomic Composition of Maize Rhizospheric Bacterial Communities in J8_BTP.

defined by a high concentration of specialized taxa, including *Ammoniphilus resinae* (16.5k abundance) and a robust *Bacillus* complex comprising *Bacillus thaonhiensis* (12.3k), *Peribacillus asahii*, and *Bacillus songklensis*, alongside *Niallia endozanthoxylica*. In contrast, sample J8_BTP exhibits a significantly more restricted population of 132,462, marked by a distinct taxonomic turnover toward the phylum *Proteobacteria*. This highland niche is primarily defined by the dominance of the *Pseudomonas aeruginosa* group (5.51k abundance) and *Rhabdothermincola sediminis* (3.29k). The quantitative and qualitative divergence between these samples confirms a stark regional specialization in the bacterial community assembly between the coastal lowland and highland maize rhizospheres.

Beyond these regional extremes, the taxonomic analysis identifies a diverse array of functional specialists and a resilient core microbiome across the remaining samples. A critical finding is the ubiquitous presence of *Gaiella occulta*, which functions as a stable core member across the geographical divide, maintaining substantial population densities in J3_KP (4.53k abundance) and J4_BTP (4.13k abundance). Furthermore, the data reveal a sophisticated microbial economy geared toward nutrient acquisition and survival in extreme conditions. This is evidenced by the identification of key nitrogen-cycling drivers such as *Nitrosospira multiformis* in J7_BTP and *Nitrospira japonica* in J4_BTP, alongside phosphate-solubilizing strains of *Devosia*, particularly *Devosia oryziradicis*. The presence of specialized extremophiles, including *Melghirimyces thermohalophilus*

Table 2. Taxonomic Composition of Maize Rhizospheric Bacterial Communities.

Species Name	S1	S2	S3	S4	S5	S6	S7	S8	S9
Gaiella occulta	-	1.68k	1.21k	4.53k	4.13k	2.75k	-	1.22k	2.97k
Bacillus songklensis	-	4.44k	-	-	-	-	-	-	-
Bacillus thaonhiensis	-	12.3k	-	1.32k	2.83k	-	-	-	2.76k
Niallia endozanthoxylica	-	8.77k	-	-	-	-	-	-	-
Niallia oryzisoli	-	2.51k	-	-	-	-	-	-	-
Peribacillus asahii	-	9.01k	-	-	2.02k	-	-	-	-
Priestia abyssalis	-	1.84k	-	-	-	-	-	-	-
Ammoniphilus oxalaticus	-	2.75k	-	-	-	-	-	-	-
Ammoniphilus resinae	-	16.5k	-	1.58k	2.02k	-	-	-	-
Oxalophagus oxalicus	-	2.68k	-	-	-	-	-	-	-
Melghirimyces thermohalophilus	3.95k	-	980	-	-	-	-	-	-
Novibacillus thermophilus	1.13k	-	-	-	-	-	-	-	-
Phototrophicus methaneseepsis	1.61k	-	-	1.45k	-	-	1.25k	-	2.59k
Vicinamibacter silvestris	1.76k	-	2.21k	1.16k	2.12k	1.45k	-	-	-
Chryseosolibacter histidini	2.95k	-	-	-	-	-	-	-	-
Longimicrobium terrae	1.25k	-	-	-	-	-	1.66k	1.49k	-
Schlesneria paludicola	1.42k	-	-	-	-	-	-	-	-
Acidibacter ferrireducens	1.48k	-	959	-	-	-	-	-	-
Methylothermalis aethiopiae	1.34k	-	1.96k	1.12k	-	1.08k	-	-	-
Povalibacter uvarum	1.25k	-	1.16k	-	-	-	-	-	-
Luteitalea pratensis	-	-	1.10k	-	-	-	-	-	-
Cellvibrio mixtus	-	-	899	-	-	-	-	-	-
Sphingobium quisquiliarum	-	-	1.08k	-	-	-	-	-	-
Steroidobacter agaridevorans	-	-	989	-	-	-	-	-	-
Fontisphaera persica	-	-	-	1.05k	-	-	-	-	-
Pseudomonas aeruginosa group	-	-	-	1.20k	1.47k	-	-	-	5.51k
Pseudomonas aeruginosa	-	-	-	1.20k	1.47k	-	-	-	5.51k
Pseudomonas indica	-	-	-	2.69k	-	1.93k	3.11k	4.78k	-
Tumebacillus ginsengisoli	-	-	-	-	1.61k	-	-	-	-
Neobacillus niacini	-	-	-	-	1.41k	-	-	-	-
Nitrospira japonica	-	-	-	-	1.55k	-	-	-	-
Chitinophaga aurantiaca	-	-	-	-	-	1.23k	-	-	-
Chitinophaga filiformis	-	-	-	-	-	1.44k	-	-	-
Chitinophaga tropicalis	-	-	-	-	-	2.90k	-	-	-
Dongia mobilis	-	-	-	-	-	1.44k	-	-	-
Lacibacterium aquatile	-	-	-	-	-	1.93k	-	-	-
Pseudolabrys taiwanensis	-	-	-	-	-	1.16k	-	-	1.81k
Rhabdothermincola sediminis	-	-	-	-	-	-	1.84k	2.13k	3.29k
Dawidia soli	-	-	-	-	-	-	2.76k	-	-
Hylemonella gracilis	-	-	-	-	-	-	2.99k	-	-
Devosia oryziradicis	-	-	-	-	-	-	1.24k	2.65k	-

Note: S1: JAS_KP; S2: J1_KP; S3: J2_KP; S4: J3_KP; S5: J4_BTP; S6: J5_BTP; S7: J6_BTP; S8: J7_BTP; S9: J8_BTP; (-): not included in the top 10 abundance of bacteria in the sample.

Table 2. Continued...

Species Name	S1	S2	S3	S4	S5	S6	S7	S8	S9
Borborobacter arsenicus	-	-	-	-	-	-	2.32k	2.57k	-
Manganibacter manganicus	-	-	-	-	-	-	1.95k	-	-
Ohtaekwangia koreensis	-	-	-	-	-	-	1.20k	-	-
Castellaniella ginsengisoli	-	-	-	-	-	-	-	2.26k	-
Cognatiluteimonas lumbrici	-	-	-	-	-	-	-	1.79k	-
Nitrosospira multiformis	-	-	-	-	-	-	-	1.22k	-
Mesorhizobium retamae	-	-	-	-	-	-	-	1.32k	-
Nocardioides yefusunii	-	-	-	-	-	-	-	-	1.87k
Mesobacillus subterraneus	-	-	-	-	-	-	-	-	2.30k
Aggregatilinea lenta	-	-	-	-	-	-	-	-	2.20k

Note: S1: JAS_KP; S2: J1_KP; S3: J2_KP; S4: J3_KP; S5: J4_BTP; S6: J5_BTP; S7: J6_BTP; S8: J7_BTP; S9: J8_BTP; (-): not included in the top 10 abundance of bacteria in the sample.

(3.95k abundance in JAS_KP) and *Novibacillus thermophilus*, further confirms that the maize rhizosphere in the semi-arid drylands of East Nusa Tenggara harbors highly adapted bacterial taxa capable of maintaining metabolic activity despite acute thermal and osmotic stressors.

3.3. Alpha diversity of maize rhizospheric bacterial communities

Further to the taxonomic insights, an analysis of alpha diversity metrics provides a quantitative measure of bacterial richness and evenness within each maize rhizospheric sample (Table 3). This section explores the variations in microbial diversity across different samples, offering insights into the complexity and variety of bacterial communities present.

The study employed a suite of established alpha diversity metrics, including observed species richness, Shannon index, Simpson index, ACE, and Chao1, to quantitatively characterize these communities. As detailed in Table 3, significant variation in these metrics was observed across the sampled sites, reflecting the heterogeneity of environmental conditions. Notably, sample J2_KP consistently exhibited the highest alpha diversity, with 7444 observed species, a Shannon index of 7.17, and an ACE estimate exceeding 10,000. These robust values signify a bacterial community that is not only highly diverse in terms of the number of distinct taxa but also characterized by a remarkably even distribution of these taxa. Conversely, sample J8_BTP presented the lowest diversity, recording only 3,225 observed species and a Shannon index of 5.97, indicating a less rich and potentially less evenly distributed community, suggesting a more selective environment or a more specialized community structure at this site.

3.4. Beta diversity of maize rhizospheric bacterial communities

Building upon the alpha diversity assessment, beta diversity analyses were conducted to evaluate the compositional dissimilarities and structural variations of bacterial communities between the different maize rhizospheric samples. These analyses reveal how environmental factors influence the clustering and separation of microbial communities across the study sites.

The 3D PCA plot (Figure 4A) accounted for a cumulative variance of 66,11%, with PC1, PC2, and PC3 explaining 31,94%, 18,92%, and 15,25% of the total variation, respectively. Similarly, the 3D PCoA plot (Figure 4B) explained a total of 63,54% of the compositional dissimilarity across the first three principal coordinates (Figure 4). The 3D visualization revealed a more pronounced spatial separation between the KP and BTP samples that was not fully captured in 2D space. Notably, sample J1_KP remained highly distinct, forming an isolated cluster along the PC3/PCoA3 axis, further confirming its unique microbial assemblage dominated by *Bacillus* spp. and *Ammoniphilus* spp.. Meanwhile, samples from the BTP region (especially J6_BTP and J7_BTP) showed tighter clustering in the 3D space, suggesting a high degree of phylogenetic and compositional similarity driven by the arid environmental filtering of the BTP site. The inclusion of the third dimension illustrates that the bacterial community transition from KP to BTP is driven by a complex interplay of environmental factors that require multiple axes of variation to be fully resolved.

The hierarchical topology of the UPGMA dendrogram delineates the compositional architecture of the maize rhizospheric microbiome into four primary phylogenetic clusters, providing robust evidence of deterministic niche partitioning across the semi-arid gradient (Figure 5). Cluster I is exclusively represented by sample J1_KP, which occupies the most basal position in the tree. This profound divergence signifies a highly specialized microbial assembly, likely governed by the unique edaphic conditions and intense environmental filtering at this specific coastal lowland site. Cluster II bifurcates as a standalone lineage comprising sample J8_BTP. Its significant phylogenetic distance from the core clades indicates a unique successional trajectory and high

Table 3. Alpha Diversity of Maize Rhizospheric Bacterial Communities.

Sample	Observed	Shannon	Simpson	ACE	Chao1
S1	6291	6.70	0.996	8716.82	8765.89
S2	3534	4.82	0.964	5361.80	5523.33
S3	7444	7.17	0.998	10007.48	10,155.04
S4	4612	6.68	0.996	6198.54	6261.53
S5	4661	6.61	0.996	6278.87	6558.34
S6	5417	6.84	0.997	7133.86	7287.18
S7	5320	6.65	0.996	7166.39	7302.19
S8	5337	6.53	0.995	7252.02	7406.17
S9	3225	5.97	0.993	4715.83	4850.36

Note: S1: JAS_KP; S2: J1_KP; S3: J2_KP; S4: J3_KP; S5: J4_BTP; S6: J5_BTP; S7: J6_BTP; S8: J7_BTP; S9: J8_BTP.

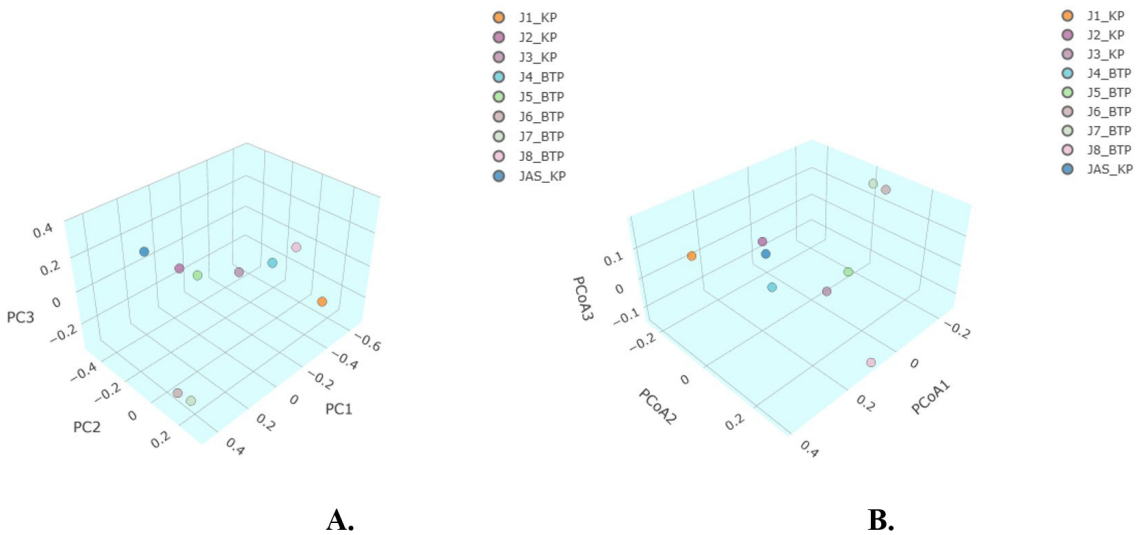

Figure 4. Principal Component Analysis (A) and Principal Coordinates Analysis (B) of Maize Rhizospheric Bacterial Communities samples.

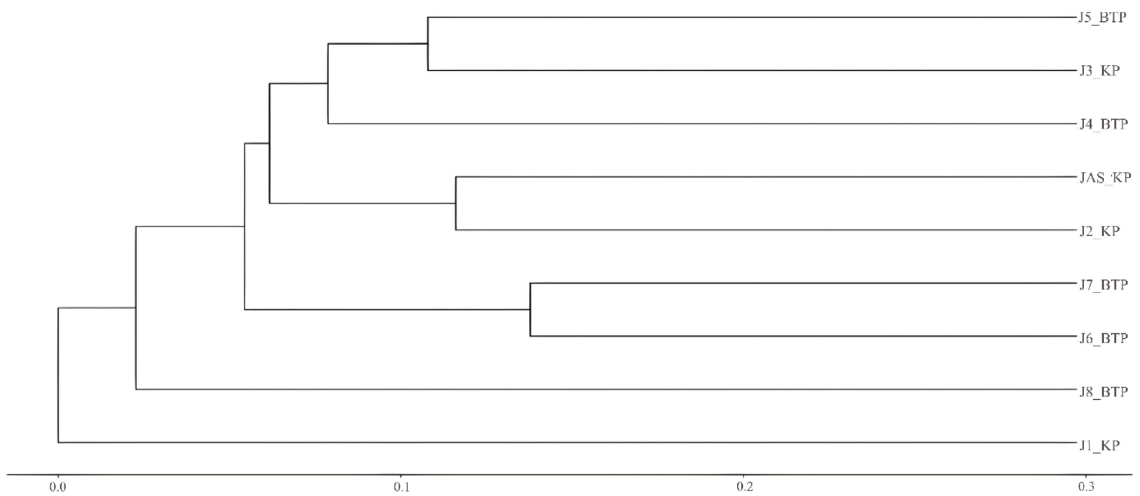

Figure 5. Unweighted Pair Group Method with Arithmetic Mean Dendrogram of Maize Rhizospheric Bacterial Communities samples.
Note: The scale bar represents the Bray-Curtis simmilarity index.

niche specialization within the Batu Putih highland environment. Cluster III forms a discrete regional group containing J6_BTP and J7_BTP, reflecting a convergent highland microbial signature driven by shared environmental filters. Cluster IV encompasses the largest and most heterogeneous assembly, including JAS_KP, J2_KP, J4_BTP, J3_KP, and J5_BTP. Notably, the close sister-clade relationship between J3_KP and J5_BTP within this cluster suggests that localized edaphic commonalities can occasionally override broad regional geography in driving microbial assembly rules.

The heatmap visually represented the top 50 relative abundances of the identified bacterial species across all samples, corroborating the beta diversity analyses (Figure 6). It clearly highlighted intense color corresponding to high relative abundances of *Niallia endozanthoxylica*, *Bacillus songklensis*, *Niallia oryzisoli*, *Ammoniphilus oxalaticus*, *Ammoniphilus resinae*, *Peribacillus asahii*, and *Bacillus thaonhiensis* specifically in sample J1_KP. Conversely, sample J8_BTP exhibited pronounced high abundances of *Pseudomonas aeruginosa*, *Pseudolabrys taiwanensis*, *Rhabdothermincola sediminis*, and *Nocardioides yefusunii*. The heatmap also showed *Gaiella occulta* to be relatively abundant across multiple samples, notably J3_KP, J4_BTP, J5_BTP, and J8_BTP, reflecting its broad ecological niche. Distinct presence patterns of *Melghirimyces thermohalophilus* were observed in JAS_KP and J2_KP, while *Borborobacter arsenicus* showed higher relative abundances in J7_BTP, and *Nitrospira japonica* was prominent in J4_BTP.

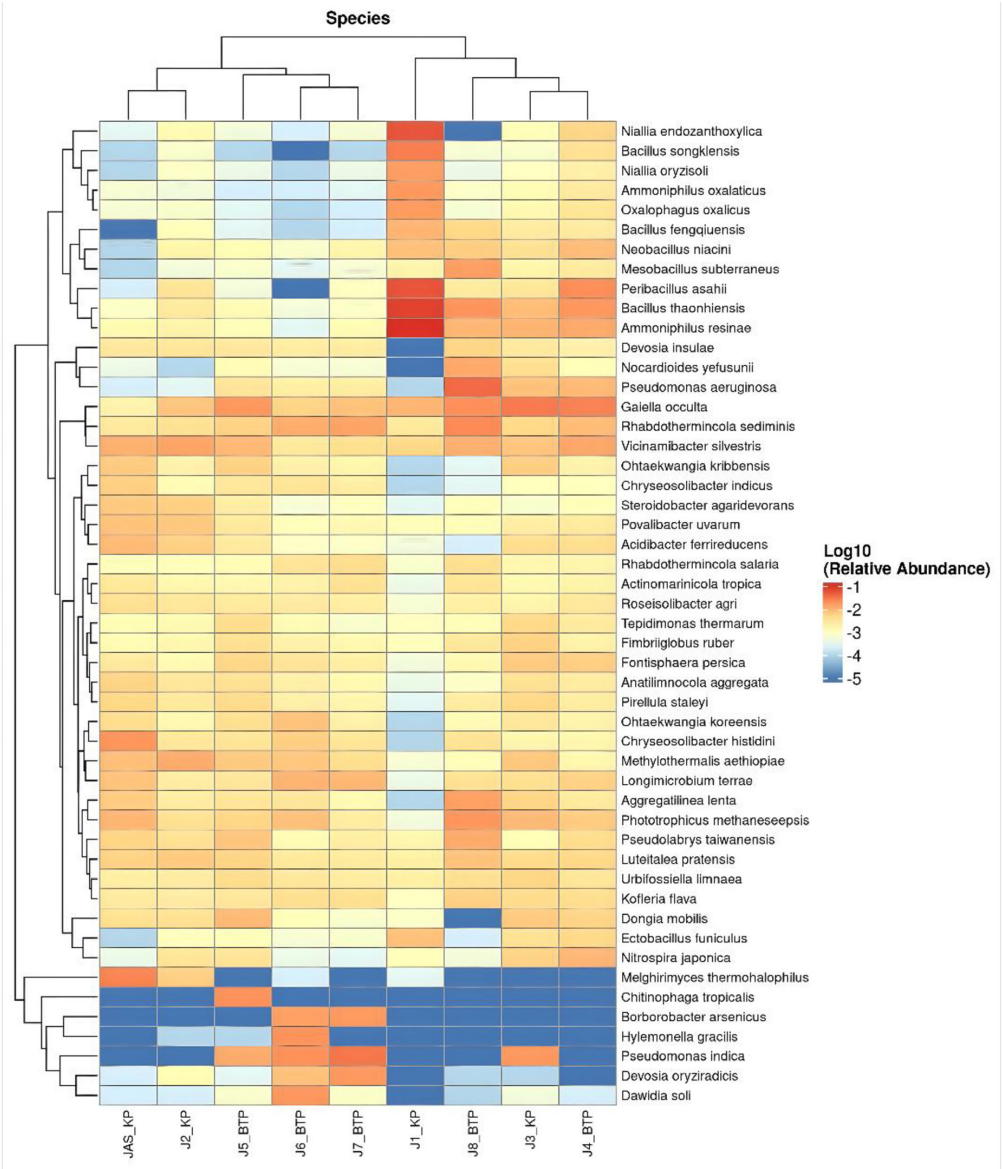

Figure 6. Heatmap of the Top 50 Most Abundant Maize Rhizospheric Bacterial Species.

4. Discussion

The taxonomic resolution in this study was achieved through full-length 16S rRNA sequencing using the 27F and 1492R universal primers. This methodological approach successfully captured the comprehensive diversity of the maize rhizospheric community at the species level. Traditional partial gene sequencing often encounters difficulties in distinguishing between distinct species that share identical sequences in specific hypervariable regions (Chen et al., 2014). While shorter read lengths are common for genus-level assignments, they frequently lack the precision required for species-level identification (Wensel et al., 2022). The use of full-length sequences in this study facilitated more accurate taxonomic distinctions by encompassing the entire 1,500–1,600 bp amplicon (Johnson et al., 2019). This increased resolution is essential for decoding complex microbial interactions within soil matrices. By leveraging advanced molecular techniques and high-accuracy basecalling, this research provides a reliable framework for analyzing the structural assembly and niche occupancy of bacterial lineages in the semi-arid environments of East Nusa Tenggara.

Environmental heterogeneity across the study sites significantly influenced the observed patterns of bacterial population density and community complexity. The West Kupang region maintained a consistently higher carrying capacity and diversity than the highland terrains of Batu Putih. This biogeographical gradient indicates that coastal lowland conditions provide a more hospitable ecological threshold for microbial proliferation. Moisture availability is widely recognized as a fundamental driver of microbial activity and community structure in water-limited ecosystems (Chen et al., 2022; Xiao et al., 2023). The relative reduction in microbial density at the highland sites suggests that acute aridity acts as a stringent environmental filter that constrains biomass accumulation (Maestre et al., 2015; Catania et al., 2022). Such patterns align with ecological models where localized microclimatic variations and soil moisture dictate the baseline of microbial density (Akinola et al., 2021; Wang et al., 2022). Consequently, the higher diversity observed in the lowland niches implies a greater potential for ecological resilience and stable nutrient cycling compared to the more selective highland environments.

The localized dominance of specialized bacterial genera reflects specific life-history strategies for surviving in marginal semi-arid soils. In the West Kupang lowland sites, the community was primarily defined by a high relative abundance of the genera *Bacillus* spp. and *Ammoniphilus* spp. This taxonomic profile suggests a persistence-based strategy geared toward long-term viability during prolonged dry periods. Members of the Bacillaceae family are well-documented for their ability to form long-lived, stress-tolerant endospores that ensure survival under severe desiccation (Lin et al., 2016; Beskrovnaya et al., 2021). These specialized structures allow microbial populations to remain viable and ecologically active when moisture levels are critically low (Beskrovnaya et al., 2021; Ahmad et al., 2022). The prevalence of *Ammoniphilus resinae* further highlights the specialization of the rhizosphere toward endospore-forming taxa that can withstand the physical stressors of NTT's drylands (Lin et al., 2016; Castro-Severyn et al., 2024). Such adaptive traits are vital for maintaining a stable microbial presence in environments characterized by chronic water scarcity and fluctuating soil temperatures.

In contrast, the transition toward *Pseudomonas* spp. and Gammaproteobacteria in the Batu Putih highland sites indicates an alternative adaptive model. This shift in community architecture suggests a reliance on metabolic plasticity to manage acute osmotic stress in drier terrains. *Pseudomonas* spp. are known for their rapid root colonization and the production of protective exopolysaccharides that enhance host plant hydration (Nishu et al., 2022; Pakar et al., 2025). These traits facilitate sustained growth-promoting activity and root morphology modulation even under conditions of severe drought (Xie et al., 2021; Abideen et al., 2022). The co-occurrence of *Pseudomonas* spp. with other stress-tolerant taxa suggests potential synergistic interactions that collectively bolster maize resilience against desiccation (Pakar et al., 2025). By prioritizing immediate physiological protection through extracellular matrix production, these bacterial lineages mitigate the negative impacts of water stress on plant development. This metabolic strategy complements the persistence-based approach observed in other regions, demonstrating a diverse array of microbial adaptations tailored to specific environmental pressures.

The functional landscape of the maize rhizosphere is further enriched by specialized functional drivers and extremophilic taxa. Metagenomic detection of *Melghiribacillus thermohalophilus* highlights the presence of highly adapted thermophilic and halophilic bacteria in NTT's marginal soils. This taxon possesses specialized mechanisms for maintaining metabolic activity under conditions of high evaporation and thermal stress (Addou et al., 2015; Anuoluwa et al., 2024). Additionally, the identification of *Devosia oryziradicis* signifies the presence of microbes capable of essential nutrient transformations. Members of this genus, alongside other rhizobacteria, are recognized for their multifunctional plant growth-promoting activities, including potential phosphate solubilization and the production of phytohormones like indole-3-acetic acid (Agnolucci et al., 2019; Rivera-Hernández et al., 2024). These processes are critical for enhancing nutrient acquisition in phosphorus-limited arid soils (Kumarapeli et al., 2018). Furthermore, the detection of nitrifying bacteria like *Nitrosospira multiformis* confirms that indigenous consortia are actively involved in converting ammonia to plant-available nitrate (Fudjoe et al., 2023; Wang et al., 2023). Such functional specialization is vital for supporting maize productivity in the nutrient-depleted agroecosystems of the semi-arid Indonesian archipelago.

The stability and resilience of the rhizospheric ecosystem are maintained through a combination of a stable core microbiome and functional redundancy. *Gaiella occulta* emerged as a ubiquitous core member across the geographical divide, indicating its broad adaptability

and fundamental role in NTT's maize cultivation areas. As a persistent Actinobacteria, this species is associated with maintaining carbon metabolism and community stability under thermal stressors (Akinola et al., 2021; Vásquez-Arroyo et al., 2023). The consistency of this core assembly suggests that the maize rhizosphere retains essential microbial functions regardless of localized variations in population density. High functional redundancy within these communities is vital for ensuring that biogeochemical processes continue despite environmental perturbations (Anuoluwa et al., 2024; Ramond et al., 2025). Such structural resilience allows the soil-plant system to recover from extreme drought events more effectively (Swift et al., 2025). Collectively, these findings underscore the importance of indigenous microbial networks in providing ecological services that facilitate crop survival and productivity in challenging, water-limited environments.

5. Conclusion and Recommendations

In conclusion, this study successfully characterized maize rhizospheric bacterial communities in the semi-arid region of East Nusa Tenggara, Indonesia, revealing a highly diverse and complex microbial ecosystem actively associated with maize roots. Distinct community structures were identified, with a prevalence of bacterial groups such as *Bacillus* spp., *Ammoniphilus* spp., *Pseudomonas* spp., and *Nitrosospira* spp., suggesting specific adaptations to drought stress and low soil fertility. This research contributes significantly to the ecological understanding of semi-arid maize rhizosphere systems by elucidating the taxonomic composition and diversity patterns in a previously underexplored region. Future research should prioritize functional validation using advanced multi-omics approaches, investigate specific adaptation mechanisms through controlled studies, and analyze complex microbe-plant-environment interactions to fully comprehend the ecological roles of these communities.

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Data Availability Statement

The raw 16S rRNA gene sequencing datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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