

Microbial community profiling of volcanic-affected rice soils near Mount Semeru, Lumajang, Indonesia using Next-Generation Sequencing for biofertilizer development

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Manuscript received: 30 May 2025. Revision accepted: 14 September 2025.

Abstract. Sari DNR, Ni'matuzahroh, Fatimah, Salamun, Affandi M, Junairiah. 2025. *Microbial community profiling of volcanic-affected rice soils near Mount Semeru, Lumajang, Indonesia using Next-Generation Sequencing for biofertilizer development. Biodiversitas 26: 4424-4434.* This study represents the first attempt to apply Next-Generation Sequencing (NGS) based on the 16S rRNA gene to analyze the microbial community structure in paddy soil affected by the eruption of Mount Semeru in Lumajang. This study aims to profile the microbial communities of volcanic-affected rice soils near Mount Semeru. Sequencing was conducted using ONT, with Guppy and NanoFilt for bioinformatic processing. Soil chemical analysis revealed a moisture content of 1.22%, organic carbon at 0.96%, and nitrogen at 0.1%. Available phosphorus was measured at 11 ppm, with a Cation Exchange Capacity (CEC) of 6.22 cmol(+)kg⁻¹. The microbial community was highly diverse, encompassing 100,046 observed species, with a Shannon-Wiener diversity index of 5.528 and estimated species richness values of 2,337.51 (Chao1) and 1,242.00 (ACE). This diversity spanned 44 phyla, 109 classes, and more than 5,000 species. The dominant phylum was Pseudomonadota (46.35% of total OTUs in the sample), with major genera including *Lysobacter* (5.90%) and *Gaiella* (4.44%). Opportunistic pathogens were also detected, underscoring the need to strengthen health and safety measures in post-eruption agricultural areas. These findings provide critical insights into the resilience of microbial communities and highlight their potential application in the development of biofertilizers and environmental biotechnology in Indonesia, offering hope for the future of agriculture in volcanic-affected areas.

Keywords: 16S rRNA, microbiome, Mount Semeru Lumajang, NGS, volcanic soil

INTRODUCTION

Indonesia is one of the countries with the highest levels of volcanic activity in the world (Hidayat et al. 2020; Malawani et al. 2021), making it a region with widespread volcanic soils, which are well known for their fertility. Volcanic soils are rich in nutrients essential for plant development, offering significant potential for the advancement of high-yield agricultural systems (Gani et al. 2021; Vinoth et al. 2025). One region exhibiting these characteristics is the area surrounding Mount Semeru in Lumajang, which is the highest volcano in Indonesia (8°06.5' S and 112°55' E, with its summit, Mahameru, reaching 3,676 meters above sea level) and also one of the most active volcanoes in the country (Kristianto et al. 2023).

The rice agricultural lands in Lumajang District, located around Mount Semeru, are known for their high fertility levels (Ariadi et al. 2024); however, these lands are periodically affected by eruptive activity that deposits volcanic ash and other pyroclastic materials. Although

these materials can enrich the soil with essential mineral nutrients (Rozaki et al. 2022), eruptions also have the potential to cause significant changes in the chemical, physical, and biological properties of the soil (Usharani et al. 2019), including disruption of the soil microbial community balance. Moreover, soil microorganisms are key components in maintaining soil fertility (Musbau and Ayinde 2021) and sustaining agricultural productivity, particularly within paddy field ecosystems (Wang et al. 2019; Wang et al. 2024). Microbial groups such as bacteria, archaea, and fungi play essential roles in various processes, including organic matter decomposition, nitrogen fixation, phosphate solubilization, and the production of bioactive compounds that support plant growth (Tabacchioni et al. 2021; Timofeeva et al. 2023). High soil microbial diversity is directly correlated with soil health and optimal crop yields (Stefan et al. 2021).

In disaster-prone areas, the natural recovery of soil structure and ecosystem restoration heavily depend on the activity of resilient soil microbial communities. Therefore,

understanding how these microbial communities respond to environmental stressors induced by volcanic eruptions is essential. Several previous studies have demonstrated that volcanic eruptions lead to primary microbial succession, which begins with the colonization of fast-growing, stress-tolerant microbial taxa. For example, post-eruption soils from the Hunga Tonga Island showed microbial shifts marked by the emergence of extremotolerant (Dragone et al. 2023). However, to date, it is estimated that approximately 99% of soil microorganisms cannot be cultured using conventional culturing techniques (Luciani et al. 2012). This poses a major challenge in revealing microbial diversity and comprehensively understanding their ecological roles. To address this challenge, Next-Generation Sequencing (NGS) based approaches have emerged as effective modern solutions for studying microbial communities (Wei et al. 2022). This technology enables comprehensive analysis of the genetic sequences of microorganisms, including those that are unculturable by conventional methods (Kristianti and Amalia 2023). Thus, it can provide a deeper insight into the microbial community structure and their potential functional roles in extreme environments such as volcanic soils.

The application of NGS technology in the context of volcanic soils is still relatively new in Indonesia, yet it holds significant potential to accelerate the exploration of local microbial biodiversity. 16S rRNA-based analysis enables microbial identification down to the species level, as well as the assessment of community diversity distributed both spatially and temporally. In this context, exploring the local soil microbiome within paddy agroecosystems impacted by volcanic eruptions is of great importance. One promising practical application is the development of indigenous microbe-based biofertilizers, which can enhance soil fertility (Ranjan et al. 2020), naturally promote plant

growth (Aloo et al. 2022), reduce dependence on chemical fertilizers (Quoreshi et al. 2019; Gani et al. 2021), and help mitigate environmental degradation (Guardiola-Márquez et al. 2023). To date, no studies have investigated the microbial dynamics in actively cultivated volcanic rice soils in Indonesia, despite their critical relevance for biofertilizer development tailored to high-stress environments.

This study investigates the soil microbiome in actively cultivated rice fields near Mount Semeru, aiming to fill the knowledge gap in microbial succession under volcanic stress. Unlike previous research focused on barren post-eruption areas, this study highlights microbial dynamics in productive agroecosystems. Using NGS-based metagenomics, it reveals native microbial structures and functions with implications for sustainable agriculture and localized biofertilizer development.

MATERIALS AND METHODS

Study area

This study was conducted in Sumber Wuluh Village, Candipuro Sub-district, Lumajang District, East Java, Indonesia, at coordinates 8°09'52.5" S and 113°00'48.5" E (Figure 1), an area significantly affected by the volcanic activity of Mount Semeru (Puspaningrum 2021; Ansori et al. 2025). Soil samples were collected from rice paddy fields affected by the deposition of volcanic material following the eruption of Mount Semeru in Lumajang. Although the village has been labeled a “ghost village” due to many residents choosing to leave, agricultural activities remain active in the area, predominantly rice cultivation. However, the soil’s physical and chemical properties have been altered by the volcanic ash deposits (Figure 2).

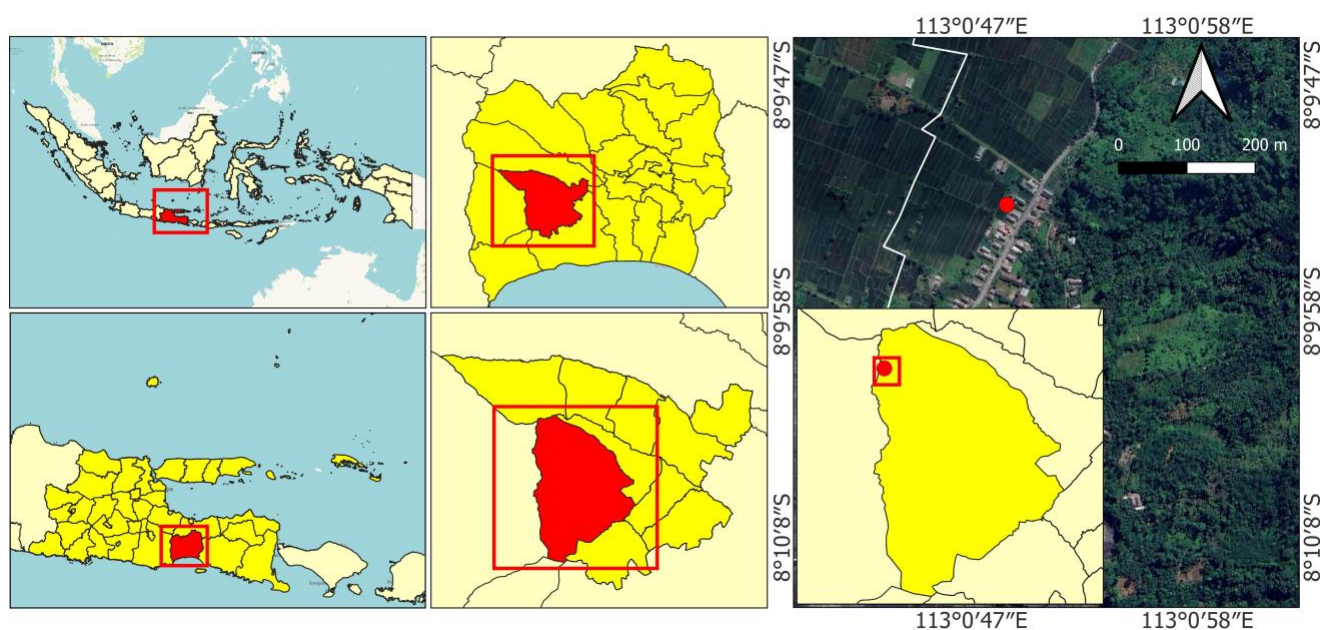


Figure 1. Sampel location Sumber Wuluh, Candipuro, Lumajang, East Java, Indonesia



Figure 2. Soil sample condition

These disturbances have contributed to changes in soil structure, reduced fertility levels, and disrupted soil microbiological activity. Field sampling was conducted with official approval from the Department of Agriculture, Lumajang Regency, and did not involve protected areas requiring SIMAKSI. The primary objective of this study is to identify indigenous microorganisms with potential as biofertilizer candidates. Local microbes that can survive and adapt to post-eruption soil conditions are considered to have significant potential for improving soil fertility and supporting sustainable agricultural systems in the affected regions.

Sample collection procedure

Samples for this study were collected from active rice paddy fields located in one of the areas affected by volcanic ash from Mount Semeru Lumajang, specifically in Sumber Wuluh Village, Candipuro Sub-district, Lumajang District, East Java, Indonesia, at coordinates 8°09'52.5" S and 113°00'48.5" E. Soil samples were taken from five points according to standard soil sampling procedures, each placed in labeled plastic bags. Sampling was conducted at two depths, 20-30 cm (Timofeeva et al. 2023), using sterilized tools before and after each sample collection. Soil sampling was conducted with five biological replicates per location, collected from independent plots. Soil from each depth was pooled into sterile containers, clearly labeled with location, sampling point, and time, and stored under cool conditions to preserve microbial viability prior to laboratory isolation. Samples were promptly transported to the laboratory for further analysis. Temperature and pH were measured in situ, with average values recorded at approximately 26.72°C and pH 6, respectively.

Genomic DNA extraction

Genomic DNA extraction was performed using the Quick-DNA MagBead Plus Kit (Zymo Research, D4082) for 16S rRNA NGS. The DNA extraction process for the TMIE isolate followed the instructions provided in the Quick-DNA MagBead Plus Kit (Zymo Research, D4082). Approximately 35 µL of soil sample and 750 µL of DNA/RNA Shield were added to a lysis tube, followed by

homogenization through centrifugation at $10,000 \times g$ for 1 minute. The resulting supernatant was transferred to a new tube. Each sample was then mixed with Quick-DNA MagBinding Buffer and agitated for 10 minutes. Subsequently, the sample was separated from the solution, transferred, and the supernatant collected. Washing was performed twice using a pre-wash buffer. DNA was centrifuged at $10,000 \times g$ for 1 minute, then the sample was transferred and incubated at 55°C for 10 minutes. Next, the DNA Elution Buffer was added to each sample and centrifuged at room temperature for 5 minutes. This process transferred the sample to a new tube, preparing it for subsequent steps (Sukapiring et al. 2024).

16S rRNA gene amplicon sequencing

DNA concentration was measured using both a NanoDrop spectrophotometer and a Qubit fluorometer; sample library preparation utilized kits from Oxford Nanopore Technology (Kristianti and Amalia 2023). Basecalling was performed using Guppy version 6.5.7 (Bahram et al. 2019). Quality assessment of FASTQ files was conducted with NanoPlot, followed by filtering with NanoFilt (De Coster et al. 2018; Nygaard et al. 2020). The 16S rRNA database from NCBI (<https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/>) served as the reference for indexing bacterial and archaeal sequences. For detailed identification and visualization of bacteria and archaea, Pavian (<https://github.com/fbreitwieser/pavian>), Krona Tools (<https://github.com/marbl/Krona>), and RStudio version 4.2.3 (<https://www.r-project.org/>) were applied (Kristianti and Amalia 2023). While QIIME2 and DADA2 are widely utilized for analyzing short-read data, particularly from Illumina platforms, this research adopted a specialized bioinformatics workflow designed specifically for Oxford Nanopore long-read 16S rRNA sequencing. The analytical process incorporated tools such as Dorado, Guppy, NanoFilt, and Minimap2 to carry out high-accuracy basecalling, sequence filtering, and taxonomic classification following ONT-recommended procedures.

Next Generation Sequencing (NGS) libraries

Bacterial sequencing analysis was conducted using Oxford Nanopore Technologies (ONT). Library preparation

followed the protocol of the Ligation Sequencing Kit (SQK-LSK114), utilizing Flow Cell R10.4.1 to achieve high accuracy. Basecalling was performed using Dorado software (v0.3.2) (available at <https://github.com/nanoporetech/dorado>) with the model “dna_r10.4.1_e8.2_400bps_sup@v4.2.0,” specifically designed for this flow cell type. The command “dorado duplex” was applied to generate duplex reads, thereby improving sequencing accuracy. Duplex yield was calculated by multiplying the number of duplex bases by two and dividing by the total number of simplex bases. After basecalling, reads were demultiplexed based on barcodes using Guppy (v6.4.6) with the “--trim-adapters” option enabled, while other parameters were set to default. The resulting FASTQ files were filtered using the “seq” command from SeqKit (v2.3.1), removing reads with an average quality score below 7 or lengths shorter than 500 bp. Filtered data were then mapped to reference genomes using Minimap2 (v2.24) with the “map-ont” preset to ensure compatibility with ONT sequencing data. The reference genomes used corresponded to internationally recognized bacterial genome databases (Kristianti and Amalia 2023).

Data analysis

Sequencing results obtained from rice field soils influenced by the volcanic activity of Mount Semeru were analyzed using a bioinformatics workflow specifically optimized for Oxford Nanopore Technologies (ONT). This approach revealed a diverse array of microbial taxa with potential ecological functions related to soil fertility enhancement and sustainable rice production in post-eruption environments. To minimize bias due to uneven sequencing depth, read counts were normalized by applying rarefaction to a minimum threshold of 25,000 reads per sample. Samples with read numbers below this threshold were excluded from downstream analysis. Alpha diversity metrics, including Shannon, Chao1, Simpson, and Good's coverage, were calculated using custom R scripts. Taxonomic classification and data visualization were performed using Minimap2 for alignment, Krona Tools and Pavian for interactive taxonomic display, and RStudio (V4.2.3) for statistical graphics.

RESULTS AND DISCUSSION

Soil sample analysis

Soil chemical analysis of the research samples showed a soil moisture content of 1.22%, organic carbon at 0.96%, and total nitrogen at 0.1% (Table 1). Available phosphorus (P-Olsen) was measured at 11 ppm, while the Cation Exchange Capacity (CEC) reached 6.22 cmol(+) kg⁻¹. Among the macronutrients, potassium (K) was relatively low at 0.02 cmol(+) kg⁻¹, whereas calcium (Ca) had the highest concentration at 10.95 cmol(+) kg⁻¹, followed by magnesium (Mg) at 1.46 cmol(+) kg⁻¹ and sodium (Na) at 0.86 cmol(+) kg⁻¹. Micronutrients detected in the soil included iron (Fe) at 4,576 ppm, manganese (Mn) at a high level of 256 ppm, and zinc (Zn) at 35 ppm. Copper (Cu) was not detected. The soil fertility index, measured by

electrical conductivity, was 222.8 ms/cm, indicating an adequate level of soil fertility. Other field physical parameters included a neutral soil pH of 6, ambient temperature of 26.7°C, and relative humidity of 69.2% (Table 1).

Soil microbial diversity based on NGS analysis

Next-Generation Sequencing (NGS) analysis of paddy field soil samples affected by the volcanic activity of Mount Semeru in Lumajang revealed a remarkably high microbial diversity. A total of 44 phyla, 109 classes, 249 orders, 585 families, 1,929 genera, and 5,383 microbial species were identified from the analyzed samples (Figure 4), demonstrating the complexity of the microbial community that continues to thrive despite environmental disturbances caused by volcanic activity. Based on 16S rRNA gene sequencing data, the ten bacterial taxa with the highest relative abundances were identified, reflecting the hierarchical distribution of bacterial communities within the analyzed microbiome.

The volcanic ash-affected paddy soil samples exhibited a very high microbial diversity, with 100,046 observed species detected and a Shannon-Wiener diversity index of 5.53, indicating a complex and evenly distributed microbial community. Species richness estimates, based on Chao1 and ACE methods, reached 2,337.51 and 1,242.00, respectively (Table 2), suggesting the potential presence of rare or undetected taxa within the community. A Good's Coverage value of 100% indicated sufficient sequencing depth to capture nearly the entire microbial diversity present in the samples. The overall prokaryotic community structure was visualized using a Krona chart (Figure 2), providing a comprehensive overview of the taxonomic distribution.

Table 1. Results of soil chemical analysis from the samples

Parameter	Unit	Value
Available Water (AW)	%	1.22
C-Organic	%	0.96
Total Nitrogen (N)	%	0.10
Phosphorus (P)	ppm	11
Cation Exchange Capacity (CEC)	cmol(+).kg ⁻¹	6.22
Potassium (K)	cmol(+).kg ⁻¹	0.02
Calcium (Ca)	cmol(+).kg ⁻¹	10.95
Magnesium (Mg)	cmol(+).kg ⁻¹	1.46
pH	—	6.00
Sodium (Na)	cmol(+).kg ⁻¹	0.86
Iron (Fe)	ppm	4,576
Manganese (Mn)	ppm	256
Zinc (Zn)	ppm	35
Copper (Cu)	ppm	nd
Electrical Conductivity (Fertility)	mS/cm	222.8
Moisture Content	%	75.2
Humidity	%	69.2

Note: nd: Not detected

Table 2. Number of observed species (Shannon-Wiener; Chao1; ACE)

Observed species	Shannon-Wiener index	Chao1	ACE	Good's coverage estimator
100,046	5.53	2,337.51	1,242.00	100

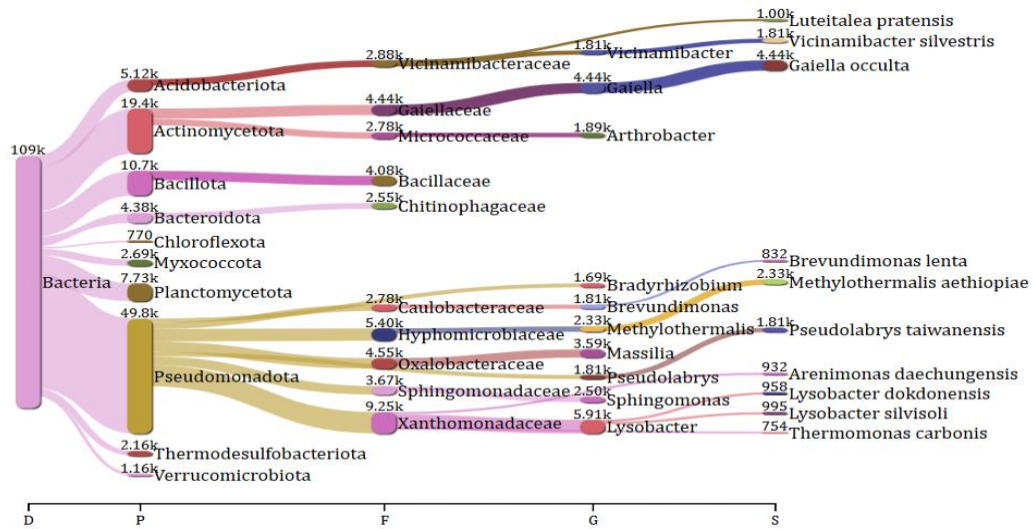


Figure 3. Taxonomic composition of bacterial communities in volcanic paddy soils, visualized using a Sankey diagram. D: Domain, P: Phylum, F: Family, G: Genus, and S: Species, reflecting their hierarchical distribution and relative abundance

Bacterial taxonomic composition

The hierarchical distribution of bacterial communities based on 16S rRNA gene sequencing data is visualized using a Sankey diagram (Figure 3), illustrating the taxonomic flow from the phylum to the order level. The diagram reveals that Actinomyetota, Pseudomonadota, and Bacillota are the three dominant phyla, which flow respectively into the families Xanthomonadaceae (phylum Pseudomonadota), Gaiellaceae (phylum Actinomyetota), and Bacillaceae (phylum Bacillota). Subsequent flows demonstrate the connections between the most abundant genera and their corresponding families, specifically *Lysobacter* and *Gaiella*.

The prokaryotic community visualization through a Krona diagram (Figure 4) indicates a dominance of Gram-negative bacteria in the paddy soils impacted by volcanic eruptions around Mount Semeru. The main phyla identified include Pseudomonadota, Planctomycetota, Actinomyetota, Bacillota, and Acidobacteriota. The classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria emerged as dominant groups with high sequence abundance. At the order level, Burkholderiales, Hyphomicrobiales, and Rhodocyclales predominated the community composition. At the family and genus levels, microorganisms such as Beijerinckiaceae, Geobacteraceae, Bradyrhizobium, Rhodoplanes, and Hyphomicrobium significantly contributed to the structure of the soil microbial community. These results reflect the high diversity and adaptive capacity of microbes to extreme environmental conditions induced by volcanic activity. The absence of family-level classification for Chloroflexota, Myxococcota, and Planctomycetota in Figure 3 is attributed to limitations in the reference annotation database and the low abundance of these phyla in the sample, which precluded further taxonomic resolution (Figure 3).

Although in lower proportions, Gram-positive bacteria were also identified within the soil microbial community of paddy fields affected by volcanic eruptions. The phyla Actinomyetota and Bacillota, along with the class Actinomycetes, were the primary groups detected. Families

such as Micrococcaceae and Bacillaceae, as well as genera including *Arthrobacter* and *Gaiella*, appeared in significant abundance. Analysis of 16S rRNA gene sequences using Next Generation Sequencing (NGS) revealed a complex and diverse microbial community composition across all samples. The data were classified into major taxonomic ranks (phylum, class, order, family, and genus) based on absolute counts and relative abundance. A total of 109,460 high-quality sequences were clustered into 1,200 Operational Taxonomic Units (OTUs) at a 97% similarity threshold and analyzed to determine the microbial composition from phylum to genus levels.

At the phylum level (Figure 5), Pseudomonadota was the dominant group with 49,142 reads, accounting for approximately 46.35% of total OTUs in the sample, followed by Actinomyetota (17.81%) and Bacillota (10.10%). Other phyla detected with significant abundance included Planctomycetota (7.24%), Acidobacteriota (4.81%), and Bacteroidota (4.11%). Phyla with lower abundance comprised Myxococcota (2.53%), Thermodesulfobacteriota (1.86%), Verrucomicrobiota (1.02%), and Gemmatimonadota (0.70%). Taxonomic analysis of microbial communities at the family level (Figure 6) revealed the dominance of Xanthomonadaceae (9.07% of total OTUs in the sample), followed by Hyphomicrobiaceae (5.28%) and Oxalobacteraceae (4.45%), all belonging to Gram-negative groups. In addition, Gram-positive families such as Gaiellaceae (4.35%) and Bacillaceae (4.19%) were also found in relatively high proportions. Other families detected with significant abundance included Sphingomonadaceae (3.59%), Vicinamibacteraceae (2.81%), Caulobacteraceae (2.72%), Micrococcaceae (2.72%), and Chitinophagaceae (2.49%). This distribution reflects a relatively balanced structural diversity of the bacterial community at the family level. The composition indicates that the analyzed soil samples harbor a taxonomically diverse bacterial community, with the presence of several significant Gram-negative and Gram-positive families.

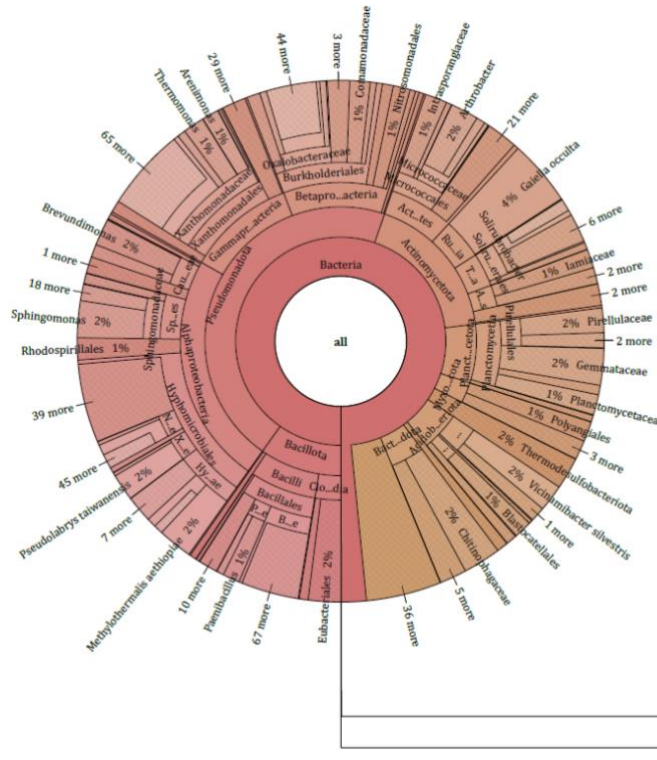


Figure 4. Sunburst diagram illustrating (Krona diagram) the hierarchical composition of bacterial taxa obtained from 16S rRNA sequencing of paddy soils affected by volcanic activity from Mount Semeru. Taxonomic levels are displayed from phylum to genus, with relative abundance (%) indicated

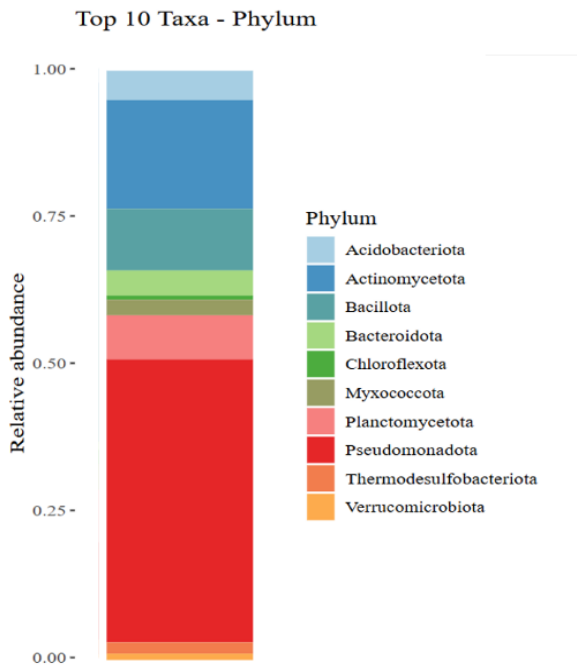


Figure 5. Bacterial Operational Taxonomic Units (OTUs) at the phylum level were identified from paddy soils influenced by volcanic activity from Mount Semeru in Lumajang, East Java, Indonesia. Phyla other than the ten most prevalent were collectively categorized as 'Others' based on their total relative abundance. Among the identified phyla, Pseudomonadota emerged as the most dominant within the samples

At the genus level (Figure 7), the soil microbial community was dominated by *Lysobacter* from the family Xanthomonadaceae (Gram-negative), with relative abundance of 5.90% of total OTUs in the sample. The genus *Gaiella* (Gram-positive) from the phylum Actinomycetota ranked second, representing 4.44%. Other notable Gram-negative genera included *Massilia* (3.58%), *Sphingomonas* (2.50%), and *Methylothermalis* (2.32%), while the Gram-positive genus *Arthrobacter* accounted for 1.89%. Several additional Gram-negative genera, such as *Brevundimonas*, *Pseudolabrys*, and *Vicinamibacter*, were also present at significant levels, each comprising 1.81%. *Bradyrhizobium*, belonging to the order Rhizobiales, was detected with a relative abundance of 1.68%. Overall, the results indicated a predominance of Gram-negative genera within the analyzed soil microbial community.

At the species level (Figure 8), the microbial community was primarily composed of *Gaiella occulta* (Gram-positive) with a relative abundance of 4.44% of total OTUs in the sample. Other abundant species included *Methylothermalis aethiopiae* (Gram-negative, 2.32%), *Pseudolabrys taiwanensis* (1.81%), and *Vicinamibacter silvestris* (1.81%). The species *Luteitalea pratensis* from the genus *Lysobacter* (Gram-negative) accounted for 1.00%, followed by *Lysobacter silvisoli* (0.99%) and *Lysobacter dokdonensis* (0.96%). Other Proteobacteria species present in significant amounts included *Arenimonas daechungensis* (0.93%) and *Brevundimonas lenta* (0.83%). Additionally, *Thermomonas carbonis* (0.75%) was among the ten most abundant Gram-negative species identified in the sample.

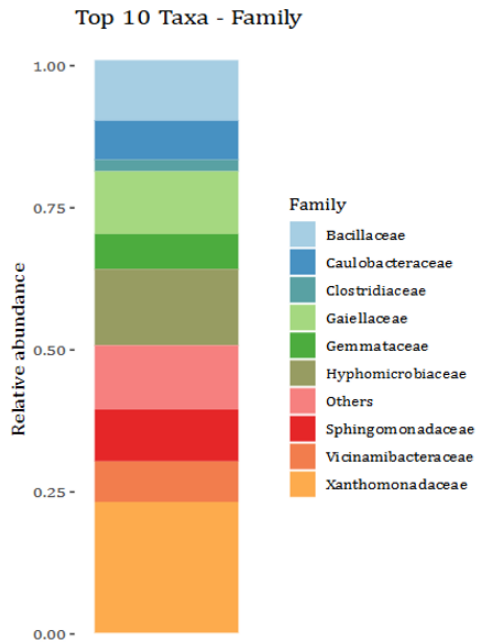


Figure 6. The relative abundance of bacterial Operational Taxonomic Units (OTUs) at the family level from paddy soils influenced by the volcanic activity of Mount Semeru in Lumajang, East Java, Indonesia, is presented. Bacterial families with relative abundance lower than the top ten most abundant families were grouped under the 'Others' category. The family Xanthomonadaceae was recognized as the most dominant group within the samples

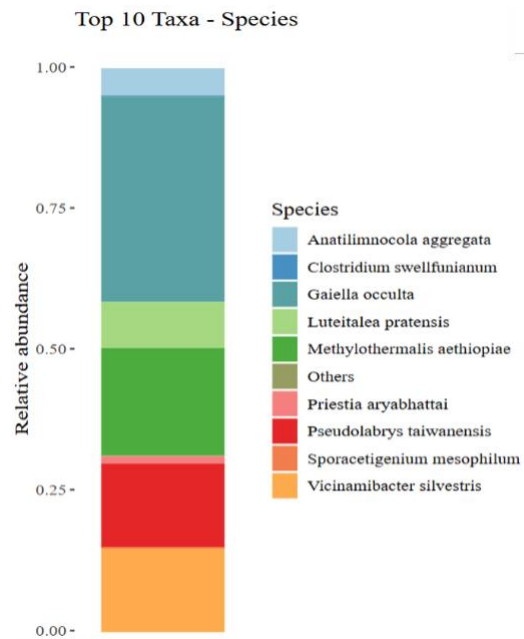


Figure 8. This study presents the relative abundance of bacterial Operational Taxonomic Units (OTUs) at the species level from paddy field soils impacted by volcanic activity from Mount Semeru, Lumajang, East Java, Indonesia. Species with lower relative abundance than the ten most dominant were grouped into the 'Others' category. *Gaiella occulta* was identified as the most prevalent species among the analyzed samples

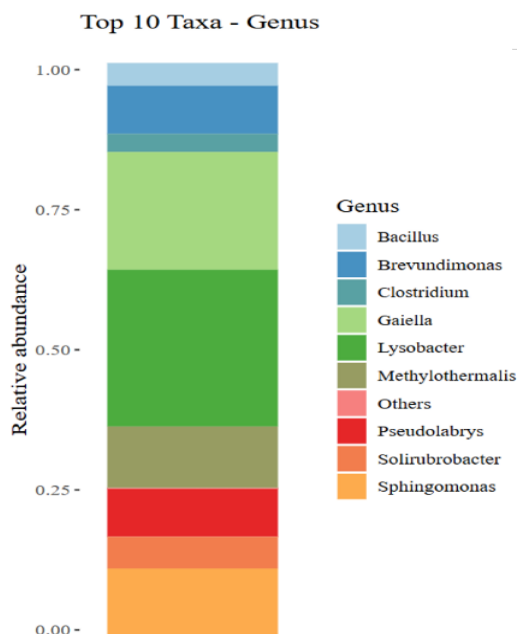


Figure 7. This study presents the relative abundance of bacterial Operational Taxonomic Units (OTUs) at the genus level derived from paddy field soils influenced by volcanic activity from Mount Semeru, Lumajang, East Java, Indonesia. Bacterial genera outside the ten most abundant were classified under the category 'Others.' Among the identified genera, *Lysobacter* was found to be the most dominant in the samples

Discussion

This discussion elaborates on the ecological and functional roles of dominant microbial taxa identified in the post-eruption paddy soils, particularly *Bacillus*, *Pseudomonas*, and Actinobacteria, in relation to nutrient cycling and stress adaptation. Comparisons with similar studies in volcanic and extreme ecosystems are also integrated to contextualize the findings. The findings of this study indicate that paddy soils affected by volcanic eruptions in the vicinity of Mount Semeru, Lumajang, East Java, Indonesia, harbor a distinctive and highly diverse microbial community with significant potential for the development of biofertilizers.

Using a Next-Generation Sequencing (NGS) approach targeting the 16S rRNA gene, this study identified a wide range of bacterial taxa, many of which contribute to nutrient cycling, plant growth promotion, and environmental stress tolerance. The high Shannon-Wiener index and species richness suggest that volcanic disturbance may enhance habitat heterogeneity, promoting microbial diversification (Table 2). These values reflect a complex and relatively even microbial community, suggesting that volcanic disturbances may enhance habitat heterogeneity, which in turn promotes increased microbial diversity in soil ecosystems (Sembiring and Sabrina 2022; Timofeeva et al. 2023).

Several studies have explored functional microorganisms from agricultural soils (Mir et al. 2022; Chen et al. 2024; Salisu and Isiya 2024; Kiprotich et al. 2025) and other extreme ecosystems (Pandey and Sharma 2021), including those in Indonesia (Rakhmawati et al. 2021; Ustiatik et al.

2023). However, to date, there has been limited research specifically examining the structure of microbial communities in agricultural soils affected by volcanic activity from Mount Semeru using advanced sequencing technologies such as Next-Generation Sequencing (NGS). Therefore, this study employed a 16S rRNA-based metagenomic approach to uncover the diversity of microbial communities that have developed in post-eruption paddy soils, with a particular focus on identifying bacterial taxa that may be pathogenic, tolerant to extreme environmental stress, or possess commercial value as biofertilizers and bioremediation agents.

The results of the soil chemical and physical analyses indicate that the paddy field soil affected by volcanic activity from Mount Semeru still possesses characteristics conducive to microbial life, despite certain limitations in soil fertility. The low potassium content ($0.02 \text{ cmol}(+) \text{ kg}^{-1}$), moderate cation exchange capacity ($6.22 \text{ cmol}(+) \text{ kg}^{-1}$), and undetectable copper levels reflect these limitations. Nevertheless, the high soil moisture (75.2%), neutral pH (6.0), and optimal field temperature (26.7°C) provide a relatively stable environment for microbial biological activity. These conditions support the Next Generation Sequencing (NGS) results, which revealed a high level of microbial diversity in the soil, identifying a total of 5,383 species. The dominance of phyla such as Actinomycetota, Pseudomonadota, and Bacillota, along with the presence of adaptive genera such as *Bradyrhizobium*, *Lysobacter*, and *Gaiella*, suggests a robust microbial community capable of withstanding and adapting to environmental stress caused by volcanic eruptions. Microbial activity remains high despite elevated levels of micronutrients such as manganese (Mn) and zinc (Zn); these elements may even support the growth of metal-tolerant species. The combination of favorable physical conditions and high microbial diversity makes this soil a strong candidate for soil microbiome engineering and the development of microbial-based biofertilizers adapted to extreme environments.

Microbial community analysis of rice paddy soil affected by volcanic activity from Mount Semeru in Lumajang, East Java, using a Next-Generation Sequencing (NGS) approach, revealed an exceptionally high level of microbial diversity, even under conditions of extreme environmental disturbance. The identification of a wide range of microbial taxa across multiple hierarchical levels suggests that volcanic soils retain a resilient biological framework capable of supporting nutrient cycling and environmental recovery (Bissett et al. 2013; Philippot et al. 2021). These findings align with the concept of microbial resilience in extreme ecosystems (Pandey and Sharma 2021), where microbial communities can adapt and continue to perform essential ecological roles, including nutrient cycling (Castro-Severyn et al. 2021; Hidalgo-Arias et al. 2023; Wang et al. 2023), nitrogen fixation (Rincón-Molina et al. 2022; Muñoz-Torres et al. 2023; Hadland et al. 2024), and the degradation of complex organic matter (Hidalgo-Arias et al. 2023).

Visualization of the microbial community using Krona and Sankey diagrams revealed a significant dominance of Gram-negative bacteria, particularly those belonging to the

phylum Pseudomonadota. Genera such as *Lysobacter*, *Massilia*, *Sphingomonas*, *Methylothermalis*, *Pseudolabrys*, *Brevundimonas*, and *Bradyrhizobium* were identified with the highest sequence counts, reflecting both their ecological dominance and high functional potential in post-eruption environments. This group is known for its broad metabolic capacity and involvement in key ecological processes such as nitrogen fixation, aromatic compound degradation, and sulfur cycling (Miller et al. 2020; Islam et al. 2021; Wang et al. 2023; Zhang et al. 2023; Semenova et al. 2024; You et al. 2024). Within this phylum, classes Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria were the most abundant, underscoring their central role in soil microbial dynamics (Alain et al. 2012; Zedelius 2012; Liao et al. 2021; Eziuzor et al. 2022; Staninska-Pięta et al. 2022; Ji et al. 2024). In addition, other Gram-negative phyla such as Bacteroidota, Planctomycetota, Myxococcota, Acidobacteriota, Gemmatimonadota, Verrucomicrobiota, and Thermodesulfobacteriota contributed adaptively to environments that are oligotrophic, thermally variable, or rich in complex organic compounds (Storesund et al. 2020; Chen et al. 2024; Ji et al. 2024; Kurashita et al. 2024; Rakitin et al. 2024; Ye et al. 2024; Guo et al. 2025; Kuka et al. 2025).

The dominance of microbial phyla Actinomycetota, Pseudomonadota, and Bacillota in post-volcanic soils, along with the presence of adaptive genera such as *Gaiella*, *Lysobacter*, and *Bradyrhizobium*, highlights a strong potential for the development of locally sourced biofertilisers that are resilient to extreme environmental conditions. The three principal phyla possess key agronomic functions, ranging from nitrogen fixation (Actinomycetota, *Bradyrhizobium*) (Yuan et al. 2023), phosphate solubilisation (Bacillota, Pseudomonadota) (Ducouso-Détréz et al. 2024), to the production of plant hormones and bioactive metabolites (Pseudomonadota, *Lysobacter*) (Brescia et al. 2021; Carolina et al. 2025). The Actinomycetes consortium has been proven to significantly enhance soil microbial activity and increase the availability of nitrogen, phosphorus, and potassium (Al-Maliki et al. 2021; Boubekri et al. 2021). *Lysobacter enzymogenes* LE16 demonstrated the capacity to boost crop yields by up to 70% through organic mineralisation processes. At the same time, *Gaiella* spp., though still under exploratory investigation, shows strong tolerance to extreme pH and drought conditions and contributes to organic carbon decomposition. Meanwhile, *Bradyrhizobium*-based biofertiliser formulations have been widely adopted in leguminous crops, consistently improving nutrient uptake efficiency and seed production. Collectively, this evidence supports the notion that volcanic soil microbial communities are not only ecologically resilient but also represent a promising foundation for the development of adaptive and sustainable biofertilizers tailored to marginal environments.

Meanwhile, the detected Gram-positive groups were primarily associated with the phyla Actinomycetota and Bacillota. Both are well-known for their ability to degrade complex organic matter and produce bioactive compounds such as antibiotics (Lee et al. 2023; Argentino et al. 2025; Helmi 2025; Saez et al. 2025). The presence of Actinomycetota,

which includes genera such as *Arthrobacter* and *Gaiella*, indicates a significant contribution to soil biochemical dynamics, particularly during the ecosystem recovery phase following volcanic eruptions (Gadson et al. 2022; Arsyadi et al. 2023; Lathifah et al. 2024). The Bacillota phylum, particularly *Bacillus* spp., is notable for its spore-forming ability, which enhances survival under harsh environmental conditions (Nicholson et al. 2000; Zammuto et al. 2022), making it highly suitable for biofertilizer formulations. Many *Bacillus* species also contribute to plant growth by solubilizing phosphate and potassium, producing phytohormones such as IAA and cytokinins, and promoting stress tolerance through the production of antimicrobial compounds and enzymes that suppress soil-borne pathogens (Wu et al. 2025). While the dominance of Gram-negative bacteria highlights the high adaptive capacity of the microbial community under geological and environmental stress induced by volcanic activity, the presence of stress-tolerant Gram-positive taxa further enriches the ecological functionality of the soil. Together, these microbial groups represent valuable biological resources for the development of biofertilizers and environmental biotechnology applications.

Members of the genus *Pseudomonas* are widely recognized for their plant growth-promoting abilities, including phosphate solubilization, siderophore production, and synthesis of phytohormones such as Indole-3-Acetic Acid (Singh et al. 2022), which enhance root development and nutrient uptake. In addition, *Pseudomonas* spp. are known for their strong antagonistic activity against soil-borne pathogens through the production of antibiotics and lytic enzymes (Fasusi et al. 2021). Likewise, *Actinobacteria*, particularly genera such as *Micromonospora* and *Streptomyces*, contribute to nutrient cycling, organic matter degradation, and the synthesis of secondary metabolites that support plant health. Their ability to thrive under nutrient-limited and stress-prone conditions makes them excellent candidates for biofertilizer development in volcanic or degraded soils (Kaari et al. 2023).

Furthermore, the identification of 109,460 high-quality sequences clustered into 1,200 Operational Taxonomic Units (OTUs) at a 97% similarity threshold reinforces the idea that volcanic ash-affected soils are not biological dead zones but rather complex, dynamic living systems. Adaptive Gram-negative bacteria, alongside stress-resistant Gram-positive taxa, dominate the microbial community and provide strong evidence that volcanic environments not only support microbial survival but also promote rich biodiversity with significant ecological and agronomic value. These findings open pathways for further research on the exploration of indigenous microbial resources as biofertilizers, bioremediation agents, or biostimulants. This supports the growing recognition of volcanic soils as biodiversity reservoirs rich in functional microbes (King 2007; Timmis and Ramos 2021; Hadland et al. 2024), with strong potential for the development of locally adapted biofertilizer innovations.

In conclusion, this study provides new insights into the structure of microbial communities in paddy soils affected by volcanic activity around Mount Semeru, Lumajang, Indonesia. Using Next-Generation Sequencing (NGS)

targeting the 16S rRNA gene, we revealed a highly diverse bacterial composition, dominated by the phyla Bacillota, Pseudomonadota, and Actinomycetota—groups known for their roles in nutrient cycling and resilience to environmental stress. Notably, this research is among the first to report the relative abundance of genera such as *Lysobacter*, *Gaiella*, *Massilia*, and *Methylothermalis* in agricultural soils impacted by volcanic ash in Indonesia. The presence of beneficial functional microbes, including nitrogen-fixers, organic matter decomposers, and antimicrobial compound producers, demonstrates strong potential for the development of biofertilizers tailored to the physicochemical properties and ecological constraints of volcanic soils, offering a promising avenue for sustainable agriculture. Based on their functional attributes and abundance profiles, genera such as *Lysobacter*, *Gaiella*, *Bradyrhizobium*, and *Arthrobacter* are recommended as potential candidates for future isolation and cultivation. The insights from this study can inform the strategic formulation of microbial consortia designed to enhance nutrient cycling, increase soil resilience, and support sustainable agriculture in post-eruption environments.

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