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Nutrient characteristics and 16S rRNA gene-based bacterial diversity of three laboratory-prepared composting systems

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ABSTRACT

Composting converts organic waste into soil amendments containing nutrients and diverse microbial communities. This study descriptively characterized the nutrient properties and bacterial community profiles of three laboratory-prepared composting systems consisting of household organic waste mixed with soil (C1), leaf litter supplemented with EM4 and palm sugar (C2), and goat manure supplemented with EM4 and palm sugar (C3). Each composting system was prepared as a single independent batch and processed for 3 months. Five subsamples collected from different positions within each batch were pooled into one composite sample; therefore, each composting system was represented by one sample for nutrient analysis and one composite sequencing library. The moisture content, pH, organic carbon, total nitrogen, P_2O_5, K_2O, Fe, Mg, Mn, and Cu were analyzed using standard laboratory methods. Bacterial communities were characterized by Illumina MiSeq sequencing of the 16S rRNA gene (V3–V4) amplicons, followed by data processing using QIIME2 and DADA2. Across the three composting systems, moisture content ranged from 10.40% to 17.76%, pH from 7.67 to 8.32, organic carbon from 17.69% to 20.80%, C/N ratio from 7.07 to 10.40, combined N + P_2O_5 + K_2O from 6.99% to 7.47%, and Fe from 454 to 1,578 ppm. These physicochemical parameters generally fell within the reference ranges established by the Indonesian compost quality standards, although full regulatory compliance could not be confirmed because several safety and quality parameters were not assessed. A total of 36 bacterial phyla were detected across the three composite sequencing libraries. Proteobacteria and Actinobacteria were the dominant bacterial phyla, although differences in taxonomic composition were observed among the composting systems. Because the study lacked independent biological replication, the findings should be interpreted as preliminary descriptive profiles rather than evidence of substrate effects or reproducible treatment differences.

1. INTRODUCTION

The agricultural sector (including forestry and fisheries) contributes approximately 4% to global gross domestic product [1]. With the continuous growth of the global population, which is projected to reach 9.5 billion by 2050, food demand is expected to increase substantially [2]. Soil quality and health, nutrient availability, environmental conditions, and soil biological activity play critical roles in determining plant growth and crop yield per unit area [3].

Conventional agricultural systems have relied heavily on the excessive use of chemical fertilizers, particularly those containing nitrogen (N), phosphorus (P), and potassium (K), to meet crop nutritional requirements and increase agricultural productivity. Composting represents a sustainable approach to managing organic waste by producing valuable soil amendments while reducing the need for chemical fertilizers [4]. Compost derived from organic substrates offers an excellent alternative for improving soil health and promoting sustainable crop production. Ravindran *et al*. [5] reported that composting can reduce greenhouse gas emissions and enhance soil quality. Compost produced from kitchen waste, the organic fraction of municipal solid waste, and vermicompost have been reported to improve soil properties and promote plant growth [6,7]. Compost applications can improve soil health, support plant growth and development, and enhance the soil's ability to suppress soil-borne pathogens [8], thereby supporting sustainable agriculture.

The application of beneficial microbiomes as biofertilizers has emerged as an innovative and environmentally friendly strategy for enhancing soil fertility and promoting plant growth within the framework of sustainable agriculture [9,10]. Biofertilizers offer cost-effective and eco-friendly alternatives to chemical fertilizers. Continuous mapping, identification, and screening of efficient microbial strains with biofertilization potential are therefore required [11]. Various microbial taxa associated with plant growth promotion (PGP) can decompose

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organic matter, enhance nutrient availability, produce phytohormones, and contribute to mitigating abiotic and biotic stresses [12,13]. Each microorganism within a biofertilizer microbiota possesses a distinct taxonomic composition and metabolite profile.

The composition and structure of bacterial communities in compost, including their taxonomy, ecological functions reported in the literature, and interaction dynamics, provide valuable considerations for future applied studies. The diversity and abundance of microbial communities in compost depend largely on the substrate type and composting process [14]. Interactions between bacteria and their environment play essential roles in shaping microbial dynamics in compost derived from organic waste [15]. Yetgin *et al.* [16] reported that bacterial communities are the primary drivers of the composting process, being responsible for the decomposition and transformation of organic matter. Throughout the composting process, bacteria play essential roles in degrading complex compounds into simpler, beneficial products. Proteobacteria are known to function as anaerobic degraders of organic matter [17] and play important roles in nitrogen, sulfur, and carbon cycling [18]. The ecological significance of Actinobacteria in compost is largely attributed to their strong ability to degrade plant-derived biomass. Members of the phylum Actinobacteria produce a wide range of hydrolytic enzymes involved in the degradation of lignocellulose and cellulose [19], including α-amylase, glucoamylase, glucose isomerase, proteases, and lignin peroxidase [20]. Actinobacteria are also known to synthesize antibiotics that suppress pathogenic microorganisms [21], participate in nitrogen cycling, solubilize phosphate, and promote plant growth [22].

Previous studies have examined bacterial communities and specific microbial taxa involved in composting processes [19,23]. However, most previous studies have focused on individual composting materials, defined experimental treatments, or specific microbial processes, while comparative information integrating nutrient characteristics and bacterial community profiles across locally practiced composting systems remains limited, particularly in the Indonesian context. Household organic waste, leaf litter, and livestock manure are commonly processed into compost by farmers and households using locally adapted practices that may differ in substrate composition and amendment inputs. Therefore, C1, C2, and C3 were selected to represent three locally relevant composting systems consisting of household organic waste mixed with soil, leaf litter supplemented with EM4 and palm sugar, and goat manure supplemented with EM4 and palm sugar, respectively.

The novelty of this study lies in the integrated characterization of nutrient properties and bacterial community profiles across these three distinct composting systems using 16S rRNA gene amplicon sequencing. Rather than testing the causal effects of individual substrates or amendments, this study provides a comparative baseline of the physicochemical and bacterial characteristics associated with these locally practiced composting systems. Some information may help contextualize compost-associated bacterial community patterns within the specific materials and processing practices used in small-scale compost production in Indonesia. The study aims to characterize the nutrient properties and bacterial community profiles of three laboratory-prepared composting systems consisting of household organic waste mixed with soil (C1), leaf litter supplemented with EM4 and palm sugar (C2), and goat manure supplemented with EM4 and palm sugar (C3) and to describe the major bacterial taxa associated with each composting system. With advances in sequencing technology, molecular characterization of bacterial communities in compost using next-generation sequencing has been reported by Yetgin *et al*. [16], Yang *et al*. [23], and Varma *et al*. [24]. Given the descriptive nature of the study, the observed differences are interpreted as system-level patterns rather than evidence of causal effects of substrate type or microbial inoculation.

2. MATERIALS AND METHODS

2.1. Study Site and Laboratory Work

Compost preparation and microbial analyses were performed at the Biology Laboratory, Universitas Negeri Semarang, Central Java, Indonesia. Macro- and micronutrient analyses of the compost samples were conducted at the Testing Laboratory of the Agricultural Instrument Standardization Centre, Yogyakarta. The 16S rRNA gene amplicon sequencing analyses were performed at PT Genetika Science Indonesia.

2.2. Compost Preparation

Three types of compost were prepared using substrate combinations commonly employed by local farmers. The first type (C1) was produced from household organic waste consisting of chopped vegetable and fruit residues mixed with soil at a 1:1 ratio. The second type (C2) was prepared by combining 10 kg of dried leaf litter with 1 l of water, 250 g of palm sugar, and 100 ml of EM4, corresponding to a ratio of 10:1:0.25:0.1. The third type (C3) consisted of 10 kg of goat manure supplemented with 700 ml of water, 100 g of palm sugar, and 70 ml of EM4, with a final ratio of 10:0.7:0.1:0.07.

EM4 was applied to C2 and C3 as a microbial starter following common farmer composting practices. In contrast, C1 was prepared without EM4 because household-waste composting typically relies on soil as a natural microbial inoculum. Thus, the soil component in C1 functioned as an indigenous microbial starter. This study was designed to reflect farmer-derived composting systems.

2.2.1. Preparation of C1 compost

The composting process began with the preparation of organic waste and the compost bag. Soil was collected from a garden, manually crushed into a fine texture, and weighed 10 kg. Household organic waste, consisting of spinach, water spinach, carrots, cabbage, long beans, bean sprouts, spring onion leaves, garlic peels, shallot peels, papaya peels, banana peels, and dragon fruit peels, was chopped into 2–3 cm pieces and weighed to 10 kg. Each type of household waste weighed 833.3 g.

A 2 cm layer of soil was spread at the bottom of a high-density polyethylene woven compost bag, followed by a 2 cm layer of chopped household waste. The layers were alternated (soil–waste–soil–waste) until all materials had been used. The compost bag was sealed and allowed to ferment for 3 months. The compost was mixed and turned weekly to ensure aeration before being sealed again.

2.2.2. Preparation of C2 compost

Dried leaves, including banana, mango, guava, mangosteen, avocado, and citrus leaves, were chopped into 2–3 cm pieces. Each type of dried leaf weighed 1,666.7 g. Palm sugar (250 g) was sliced and dissolved together with 100 ml of EM4 in 1 l of water. The EM4 solution was sprayed evenly onto the chopped leaf litter. The mixture was placed in a compost bag, sealed, and fermented for 3 months. The compost was mixed and turned weekly.

2.2.3. Preparation of C3 compost

Goat manure (10 kg) was prepared, and palm sugar (100 g) and EM4 (70 ml) were dissolved in 700 ml of water. The solution was sprayed evenly onto the manure. The mixture was placed in a compost bag, sealed, and fermented for 3 months with weekly turning.

2.3. Sample Preparation

After 3 months of fermentation, the compost was sieved to obtain fine particles. Samples were collected from five different positions within each compost using a spoon, with 250 g collected from each position. The subsamples were then thoroughly combined (pooled) until homogeneous. Because the five subsamples were pooled into a single composite sample, they did not represent independent biological replicates; consequently, each composting system was represented by a single batch and one sequencing library.

Each compost type (C1, C2, and C3) was divided into two portions: [1] 1,200 g for macro- and micronutrient analysis and [2] 50 g for 16S rRNA gene amplicon sequencing analysis. Thus, the composting process itself was not biologically replicated across independent batches, with only one batch representing each treatment.

2.4. Macro- and Micronutrient Analysis

Nutrient analyses were performed following standard procedures at the Agricultural Instrument Standardization Laboratory [25]. Moisture content was determined using the oven-drying method at 105°C, whereas pH was measured electrometrically using a calibrated pH meter. Organic carbon (C-organic) was quantified by ashing at 600°C for 4 hours. Total nitrogen (N) was analyzed using the Kjeldahl digestion method followed by titration. Available phosphorus (P_2O_5) was measured by wet oxidation using a mixture of HNO_3 and $HClO_4$ and quantified by spectrophotometry. Concentrations of potassium (K_2O) and the micronutrients iron (Fe), magnesium (Mg), manganese (Mn), and copper (Cu) were determined by wet oxidation (HNO_3 + $HClO_4$) followed by atomic absorption spectrophotometry (AAS) [26].

2.5. 16S rRNA Gene Amplicon Sequencing

2.5.1. DNA extraction

One gram of compost suspension was used for DNA extraction using the Quick-DNA™ MagBead Plus Kit (Zymo Research, D4082) according to the manufacturer's instructions. DNA quality was verified by 1% agarose gel electrophoresis. DNA concentrations were measured using a NanoDrop™ 2000 spectrophotometer and Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). The minimum DNA concentrations required for polymerase chain reaction (PCR) amplification were 2 ng/µl as measured by NanoDrop or 0.1 ng/µl as measured by Qubit.

2.5.2. PCR amplification of 16S rRNA (V3–V4)

Bacterial 16S rRNA gene amplicon profiling was carried out by targeting the V3–V4 region using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) to generate paired-end raw reads [27]. PCR amplification was performed using the KOD Multi & Epi™ Kit (Toyobo, KME-101) under the following cycling conditions: an initial pre-denaturation at 96°C for 3 minutes, followed by 27 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. A negative control (no-template control) was also included in the PCR amplification. The resulting PCR product, with an expected size of approximately 470 bp, was verified by 1% agarose gel electrophoresis and quantified using the Qubit dsDNA HS Assay.

2.5.3. Sequencing of 16S rRNA (V3-V4)

PCR products showing an approximately 470 bp band on agarose gel electrophoresis were subjected to PCR product clean-up using the Qubit system before library preparation for 16S rRNA gene (V3-V4) sequencing. Amplicon sequencing was subsequently performed using the Illumina MiSeq PE300 (Illumina, San Diego, CA, USA) [28] at PT Genetika Science Indonesia, with a required library concentration of 10 ng/µl. The primer sequences targeting the V3–V4) region of the 16S rRNA gene were the same as those used for PCR amplification (i.e., 341F and 806R).

2.6. Data Analysis

Microbial 16S rRNA gene sequencing data were processed using QIIME2 (Quantitative Insights into Microbial Ecology) version 2024.10 [29]. Adapter and primer sequences were removed using Cutadapt with the default settings applied by the sequencing provider. Raw reads were first demultiplexed using the *demux* plugin, followed by quality filtering and denoising with DADA2 version 1.34.0 to remove low-quality sequences and chimeras with the provider's default parameters [30]. The resulting amplicon sequence variant (ASV) data were used for taxonomic classification against the SILVA (silva_nr99_v138.2) 16S database. All reference sequences were retrieved from the SILVA version 138 database using a 99% sequence similarity threshold. The relative abundance of each sample was calculated based on the total taxa identified at the phylum and genus levels. Downstream analyses and data visualization were performed using packages in RStudio (R version 4.2.3) (http://www.R-project.org/). Taxonomic bar plots were generated using Microsoft Excel version 2601 from Microsoft 365 Apps.

A no-template control was included during PCR amplification, and no visible amplification was observed in the no template control lane by agarose gel electrophoresis. No fixed-depth rarefaction was applied to normalize the ASV feature table. Following taxonomic assignment, unclassified reads were excluded, and the remaining classified reads were used for downstream analyses. Rarefaction curves of bacterial ASV richness were constructed to assess sampling adequacy before diversity comparisons. All curves approached saturation, indicating sufficient sequencing depth. As shown in Figure 1, C1, C2, and C3 exhibited clear leveling-off patterns, confirming that the sequencing depth was sufficient to adequately characterize the bacterial diversity of each library. Alpha diversity indices were calculated for each sample using taxonomic assignment and abundance data. Six diversity indices were generated, including Observed ASVs, Shannon, Simpson, Chao1, abundance-based coverage estimator (ACE), and Fisher.

3. RESULTS AND DISCUSSION

3.1. Nutrient Profiles and Physicochemical Characteristics of Compost Produced by Three Composting Systems

The physicochemical parameters measured—including pH, moisture content, organic carbon, total nitrogen, C/N ratio, phosphorus, and potassium—generally fell within the reference ranges established by the Indonesian compost quality standards (Table 1). However, the composts could not be fully confirmed to comply with the national standards because other criteria, such as microbiological safety,

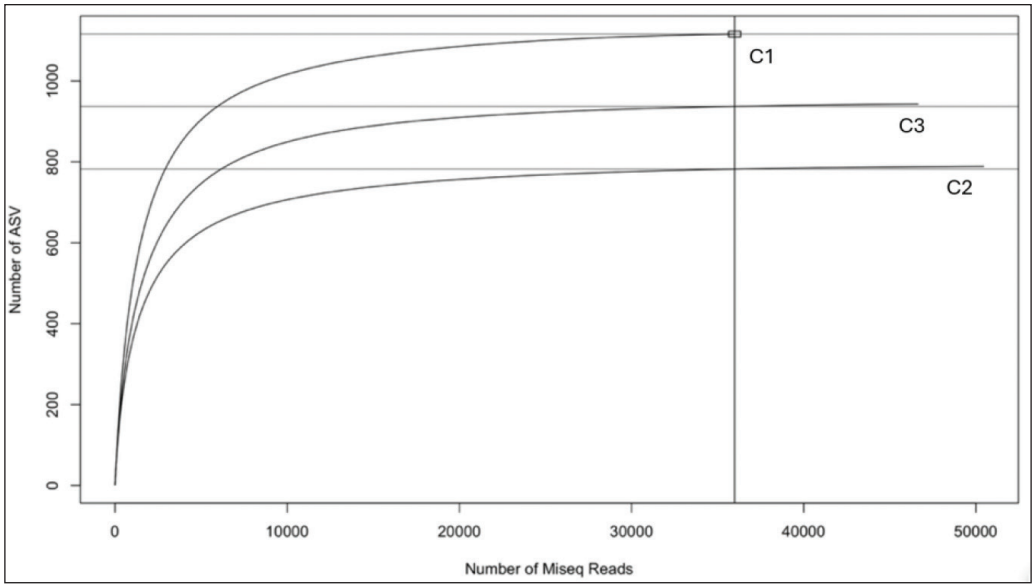


Figure 1. Rarefaction curves of bacterial ASV richness in C1, C2, and C3 compost.

Table 1. Nutrient data of compost.

Parameter	Unit	Compost type			Standard*
		C1	C2	C3	
Moisture content	%	17.76	10.40	14.98	8–20
pH (H_2O)		7.67	8.32	7.85	4–9
C-organic	%	17.69	19.59	20.80	Minimum 15%
C/N ratio		7.79	7.07	10.4	≤25
Total N + P_2O_5 + K_2O	%	7.02	7.47	6.99	Total Minimum 2%
Fe	ppm	1,578	721	454	Maximum 15,000
Mg	%	0.20	0.41	0.39	No requirement
Mn	ppm	808	313	216	No requirement
Cu	ppm	69	16	12	No requirement

*Standards based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 261/KPTS/SR.310/M/4/2019 [25].

heavy metal concentrations, the presence of foreign matter, compost stability, and sanitation, were not assessed in this study. The C/N ratio values varied among the compost types, with C3 exhibiting the highest value. Nevertheless, the C/N ratios of all composts were within the range specified for compost according to the Indonesian compost standard [25].

Nutrient availability directly shapes the composition of bacterial communities at the phylum level. Previous studies have reported associations between nutrient availability and bacterial community composition in compost [17]. High nutrient availability promotes the dominance of copiotrophic bacterial groups such as Proteobacteria. Members of this phylum have been reported to include taxa associated with carbon and nitrogen cycling and the decomposition of simple organic matter [31]. Actinobacteria and Bacteroidetes have commonly been reported in composting systems containing diverse and more complex organic substrates, including cellulose- and polysaccharide-rich materials. Members of the phylum Firmicutes have frequently been detected during the decomposition of organic waste. Members of the phylum Myxococcota have been reported to act as predators that help maintain the balance of microbial populations [17]. Members of the phylum Planctomycetota are widely recognized to include species that contribute to nitrogen transformation and the utilization of dissolved organic compounds [32].

This study assessed only the taxonomic composition of bacterial communities using 16S rRNA gene amplicon sequencing. Therefore, the evaluation of functional genes, metabolic activities, and biofertilizer performance should be considered in future research.

3.2. Bacterial Richness and Diversity Across Composite Libraries Using Alpha Diversity Indices

A total of high-quality reads clustered into ASVs were obtained from all composite samples in this study. Paired-end 16S rRNA gene sequences were processed using QIIME 2 version 2024.10 and the DADA2 denoising workflow. Following primer removal and quality trimming, 99,811, 99,794, and 99,811 trimmed reads were obtained for C1, C2, and C3, respectively. Quality filtering remained at 79,818 reads in C1, 79,906 reads in C2, and 80,706 reads in C3. After denoising, paired-end merging, and chimera removal, 45,093, 54,283, and 55,717 non-chimeric reads remained for C1, C2, and C3, corresponding to retention rates of 45.18%, 54.40%, and 55.82%, respectively. These non-chimeric reads were used to generate the ASV

feature table (Table 2). Following taxonomic assignment, unclassified reads were excluded, and the remaining classified reads were used for alpha-diversity and relative-abundance analyses.

The number of observed taxa was numerically higher in C1 (1,116) than in C3 (943) and C2 (789). This pattern was consistent with the Chao1 and ACE richness estimators, both of which closely reflected the observed taxon counts. Fisher's alpha index also indicated that C1 exhibited the highest observed richness (218.36), followed by C3 (167.39) and C2 (132.76). Shannon diversity values likewise showed that C1 had the highest microbial diversity (6.38), followed by C3 (5.92) and C2 (5.65). Similarly, Simpson's index indicated that C1 exhibited the highest evenness and the lowest dominance (0.997), followed by C3 (0.992) and C2 (0.989) (Table 2).

The observed ASV richness and alpha diversity indices are reported here as descriptive characteristics of each composite sequencing library. C1 contained 1,116 observed ASVs, C2 contained 789, and C3 contained 943, with corresponding Shannon diversity indices of 6.38, 5.65, and 5.92, respectively. The observed differences in microbial richness and diversity among the sampled compost types were descriptive and may reflect differences in substrate composition and other composting conditions. However, these patterns cannot be attributed specifically to substrate composition because each compost type was represented by a single compost batch. Independent biological replication is required to evaluate the reproducibility of these observations and determine the factors underlying the observed microbial patterns.

Previous research has indicated that compost feedstock and physicochemical characteristics can influence microbial communities during the composting process. Matheri *et al.* [33] reported differences in bacterial diversity among composts produced from different plant substrates and across composting stage. Similarly, Song *et al.* [34] demonstrated that substrate composition affected bacterial diversity in manure-based compost, with compost produced from a mixture of chicken and pig manure showing a higher Shannon diversity index than compost produced from the individual manure substrates. On the other hand, previous studies have reported that the influence of raw materials on the compost microbiome may diminish as the composting process progresses. Aigle *et al.* [35] found that composting using various organic waste sources exerted a stronger influence on bacterial community composition than the origin of the organic waste itself, resulting in convergence of the compost microbiome despite the initial differences in raw materials. This study was designed as an exploratory investigation. Therefore, the alpha diversity patterns among the compost types were interpreted descriptively without inferential statistical comparisons. Given the exploratory design of this study, alpha diversity indices were summarized descriptively for each composite sequencing library, and no inferential comparisons among composting systems were performed [36].

3.3. The Composite Libraries Shared Only a Limited Number of Bacterial ASVs

The Venn diagram illustrates the distribution of bacterial taxa detected across the three compost types. Six taxa were shared among C1, C2, and C3, while the largest overlap was observed between C1 and C2 (67 taxa), followed by C1 and C3 (14 taxa) and C2 and C3 (8 taxa) (Fig. 2). The overlaps describe the distribution of detected ASVs among the three composite libraries and provide a descriptive view of the shared and unique taxa in the sampled compost batches. However, because each composting system was represented by a single batch, this pattern cannot be used to infer ecological filtering, treatment effects, or the presence of a core microbiome.

Differences in substrate composition and composting conditions may contribute to the observed variation in microbial taxonomic composition; however, this possibility requires confirmation using independently replicated composting experiments. Yetgin *et al.* [16] highlighted the important role of bacterial communities in composting, particularly in the decomposition and transformation of organic matter. However, Aigle *et al.* [35] reported that the composting process itself may exert a stronger influence on bacterial community composition than differences in the raw materials.

3.4. Bacterial Community Composition at the Phylum and Genus Levels

At the phylum level, a total of 36 bacterial phyla were identified across all compost samples. Compost C1 contained 25 phyla, C2 contained 17 phyla, and C3 contained 30 phyla. In C1, the identified phyla were Proteobacteria (33.48%), Actinobacteria (23.98%), Planctomycetes (11.64%), and Myxococcota (6.55%), whereas the remaining 21 phyla were present at relative abundances ranging from 4.64% to 0.01%. Compost C2 was dominated by Proteobacteria (39.26%), Actinobacteria (22.79%), Bacteroidetes (18.29%), and Planctomycetes (7.37%), whereas the remaining 13 phyla were detected at relative abundances ranging from 2.59%–0.01%. In C3, the identified phyla included Proteobacteria (38.39%), Actinobacteria (14.96%), Bacteroidetes (14.45%), Planctomycetota (6.34%), and Firmicutes (6.27%), whereas the remaining 25 phyla were detected at relative abundances ranging from 5.57% to 0.01% (Fig. 3; Appendix 1).

At the phylum level, Proteobacteria and Actinobacteria were among the most abundant groups detected in the three sampled compost types, together accounting for 57.46% of the community in C1, 62.05% in C2, and 53.35% in C3 (Fig. 3). This observed pattern is broadly consistent with the findings of Aguilar-Paredes *et al.* [17], who reported that Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes are commonly detected in compost derived from leaf litter, household waste, and manure. The relative proportions of these phyla may vary among composts depending on substrate characteristics and composting conditions. Given that each compost type in the present study was represented by a single batch, these observations should be interpreted descriptively rather than as evidence of consistent treatment-specific patterns.

The bacterial communities detected in the three composite compost samples showed differences in taxonomic composition, providing a descriptive overview of the microbial profiles of the sampled compost batches. However, because the experimental systems differed simultaneously in multiple factors—including raw material

Table 2. Non-chimeric read counts, observed ASVs, and alpha diversity indices of the three composite sequencing libraries.

Compost	Non-Chimeric reads	Observed ASVs	Chao1	ACE	Shannon	Simpson	Fisher
C1	45,093	1,116	1,120.29	1,124.25	6.38	0.997	218.36
C2	54,283	789	789.88	791.99	5.65	0.989	132.76
C3	55,717	943	944.69	946.60	5.92	0.992	167.39

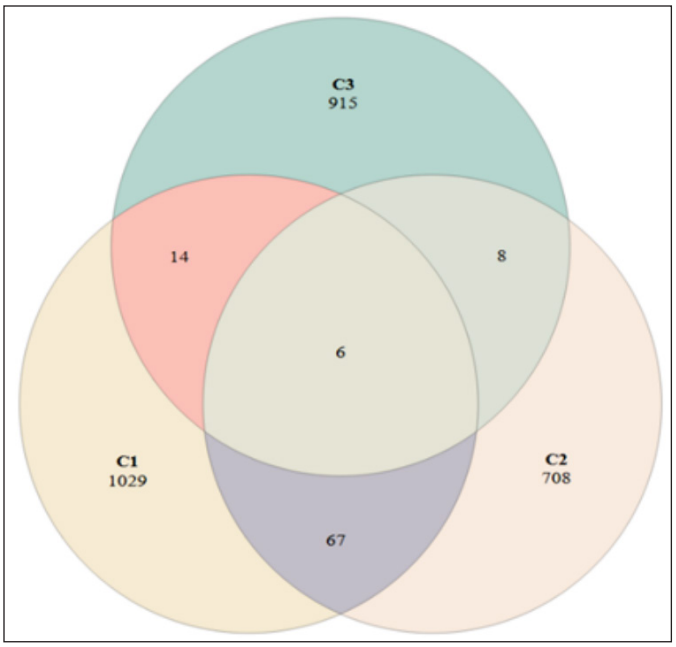


Figure 2. Venn diagram showing the overlap of observed ASVs among the three composite sequencing libraries.

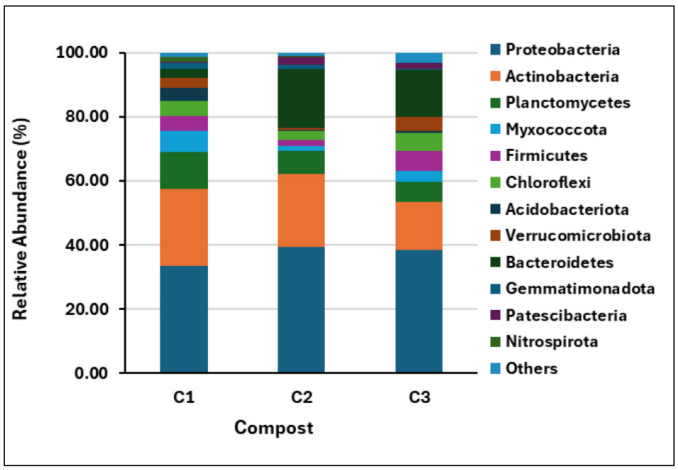


Figure 3. Relative abundance of the top 11 bacterial phyla in compost derived from three composite sequencing libraries (C1, C2, and C3) based on 16S rRNA gene (V3–V4) amplicon sequencing. Relative abundance was calculated after removing unclassified sequences. Phyla with a relative abundance of <1.0% were grouped under Others.

composition, the presence or absence of EM4 inoculum, the addition of palm sugar, the use of garden soil, and water content—the observed microbial variations cannot be attributed specifically to substrate type. The differences in bacterial community composition observed in C1, C2, and C3 therefore represent descriptive differences among the sampled compost batches rather than specific microbial responses attributable to the substrate. The presence of EM4 in C2 and C3 likely introduced additional microbial populations, whereas variations in moisture content could have influenced oxygen availability, microbial activity, and decomposition dynamics. Similarly, the addition of garden soil to C1 may have contributed indigenous soil microorganisms that were absent from the other composting systems.

Previous research has indicated that physicochemical characteristics can influence microbial communities during the composting process [33,34]. Conversely, microbial communities originating from different feedstocks can become more similar as composting progresses, suggesting that the composting process itself may exert a stronger influence than the initial substrate [35]. Therefore, the taxonomic differences observed in this study should be interpreted as descriptive observations rather than evidence of specific microbial responses to substrate type, pending validation through controlled experimental designs with adequate biological replication.

The composting process can support the development of diverse microbial communities, with their composition varying according to the type and composition of the raw materials used [14]. Microbial community structure is also shaped by interactions among microorganisms, substrate characteristics, and composting conditions [37–39]. In the present study, 16S rRNA gene sequencing revealed differences in bacterial taxonomic composition among the three composting systems. However, functional characterization using approaches such as functional gene analysis, enzyme assays, viable-cell enumeration, or the isolation and characterization of representative microorganisms would be required to substantiate these potential functions. Similarly, pot, greenhouse, or field experiments are needed to determine whether the observed microbial differences translate into measurable effects on soil properties, nutrient availability, or plant growth.

At the genus level, a total of 232 genera were identified in compost C1, 209 genera in C2, and 303 genera in C3. The identified genera varied considerably among the compost types. In C1, the most abundant genera were *Rhodococcus* (4.17%), *Kineobactrum* (3.81%), Pir4 lineage (3.70%), *Mycobacterium* (3.53%), and *Agromyces* (2.79%) (Fig. 4A). Compost C2 was dominated by *Arachidicoccus* (10.65%), *Edaphocola* (5.06%), *Mycobacterium* (4.55%), *Pseudolabrys* (4.06%), and *Devosia* (3.47%) (Fig. 4B). Meanwhile, compost C3 was dominated by *Hydrogenophaga* (7.24%), *Planktosalinus* (6.64%), *Pseudaminobacter* (3.20%), *Aquamicrobium* (2.84%), and *Chryseolinea* (2.83%) (Fig. 4C).

The detection of *Rhodococcus* and *Arachidicoccus* in the compost samples is noteworthy because members of these genera have previously been associated with diverse metabolic capabilities. *Rhodococcus* species have been reported to degrade a wide range of complex organic compounds, including aliphatic, aromatic, and xenobiotic hydrocarbons [40], and some strains have demonstrated the ability to utilize petroleum hydrocarbons [41]. Similarly, members of the genus *Arachidicoccus* have been reported to exhibit several plant-associated traits, including the production of indole-3-acetic acid, ammonia production, phosphate solubilization, and the utilization of 1-aminocyclopropane-1-carboxylate (ACC) through ACC deaminase activity [42].

Proteobacteria were detected at relatively high proportions in the three sampled compost batches examined in this study. Members of this phylum have previously been associated with the decomposition of organic matter and the biogeochemical cycling of carbon, nitrogen, and sulfur [17,18]. Proteobacteria have also been reported to occur during different stages of composting, including the later stages, although their relative abundance can vary depending on substrate composition and composting conditions. In addition, some thermophilic bacteria belonging to this phylum have been reported to possess lignocellulose-degrading capabilities [43].

After 3 months of composting, several Proteobacteria-associated genera were detected in C1, including *Kineobactrum* (3.81%), *Pedomicrobium*

Appendix 1. Classified read counts and relative abundance of bacterial phyla in C1, C2, and C3 compost.

No	Phylum	Absolute Abundance in Compost			Relative Abundance (%) in Compost		
		C1	C2	C3	C1	C2	C3
1	Abditibacteriota	0	57	0	0.00	0.11	0.00
2	Acidobacteriota	1,499	201	381	4.17	0.40	0.82
3	Actinobacteria	8,630	11,498	6,976	23.98	22.79	14.96
4	Armatimonadota	26	0	61	0.07	0.00	0.13
5	Bacteroidetes	988	9,227	6,738	2.75	18.29	14.45
6	Balneolota	0	0	23	0.00	0.00	0.05
7	Bdellovibrionota	107	123	209	0.30	0.24	0.45
8	Campylobacterota	0	0	11	0.00	0.00	0.02
9	Candidatus Kryptonia	8	0	0	0.02	0.00	0.00
10	Chlamydiota	137	186	232	0.38	0.37	0.50
11	Chloroflexi	1,619	1,309	2,596	4.50	2.59	5.57
12	Cyanobacteriota	10	26	0	0.03	0.05	0.00
13	Dadabacteria	11	0	6	0.03	0.00	0.01
14	Deferribacterota	0	0	13	0.00	0.00	0.03
15	Deinococcota	0	0	207	0.00	0.00	0.44
16	Dependentiae	6	89	202	0.02	0.18	0.43
17	Entotheonellaeota	21	0	0	0.06	0.00	0.00
18	Fibrobacterota	10	0	31	0.03	0.00	0.07
19	Firmicutes	1,668	936	2,925	4.64	1.86	6.27
20	Gemmatimonadota	766	606	156	2.13	1.20	0.33
21	Halanaerobiaeota	0	0	8	0.00	0.00	0.02
22	Hydrogenedentes	28	0	162	0.08	0.00	0.35
23	Ignavibacteriota	0	0	31	0.00	0.00	0.07
24	Methylomirabilota	154	0	0	0.43	0.00	0.00
25	Myxococcota	2,357	811	1,552	6.55	1.61	3.33
26	Nitrospirota	559	219	0	1.55	0.43	0.00
27	Patescibacteria	18	1,309	887	0.05	2.59	1.90
28	Planctomycetes	4,188	3,719	2,955	11.64	7.37	6.34
29	Proteobacteria	12,049	19,810	17,907	33.48	39.26	38.39
30	Rhodothermota	0	0	240	0.00	0.00	0.51
31	SAR324 clade (Marine group B)	13	0	26	0.04	0.00	0.06
32	Spirochaetota	0	0	23	0.00	0.00	0.05
33	Sumerlaeota	15	0	18	0.04	0.00	0.04
34	Synergistota	0	0	5	0.00	0.00	0.01
35	Thermodesulfobacteriota	0	0	30	0.00	0.00	0.06
36	Verrucomicrobiota	1,099	328	2,034	3.05	0.65	4.36
Total		35,986	50,454	46,645	100.00	100.00	100.00

(2.17%), Devosia (0.71%), *Pseudaminobacter* (0.66%), and *Hydrogenophaga* (0.16%). In C2, the identified Proteobacteria genera included *Pseudolabrys* (4.06%), *Devosia* (3.47%), and *Luteimonas* (3.23%). Compost C3 contained *Hydrogenophaga* (7.24%), *Pseudaminobacter* (3.20%), *Aquamicrobium* (2.84%), *Devosia* (2.44%), *Luteimonas* (1.32%), and *Pseudolabrys* (0.52%). Similar findings were reported by Xu *et al*. [31], who observed the genera Luteimonas during the final stage of composting of saline soil substrates.

Actinobacteria were relatively abundant in the household-waste compost (C1) and leaf-litter compost (C2) but occurred at a lower relative abundance in the sampled manure-based compost (C3). This observed variation may reflect differences in substrate composition and other composting conditions. However, given the absence of biological replication, these patterns should be interpreted as descriptive and cannot be attributed specifically to substrate type. Members of this phylum have previously been reported to possess diverse metabolic capabilities related to the decomposition of plant-derived organic matter, including lignocellulosic substrates [19,20]. Some Actinobacteria have also been reported to exhibit activities

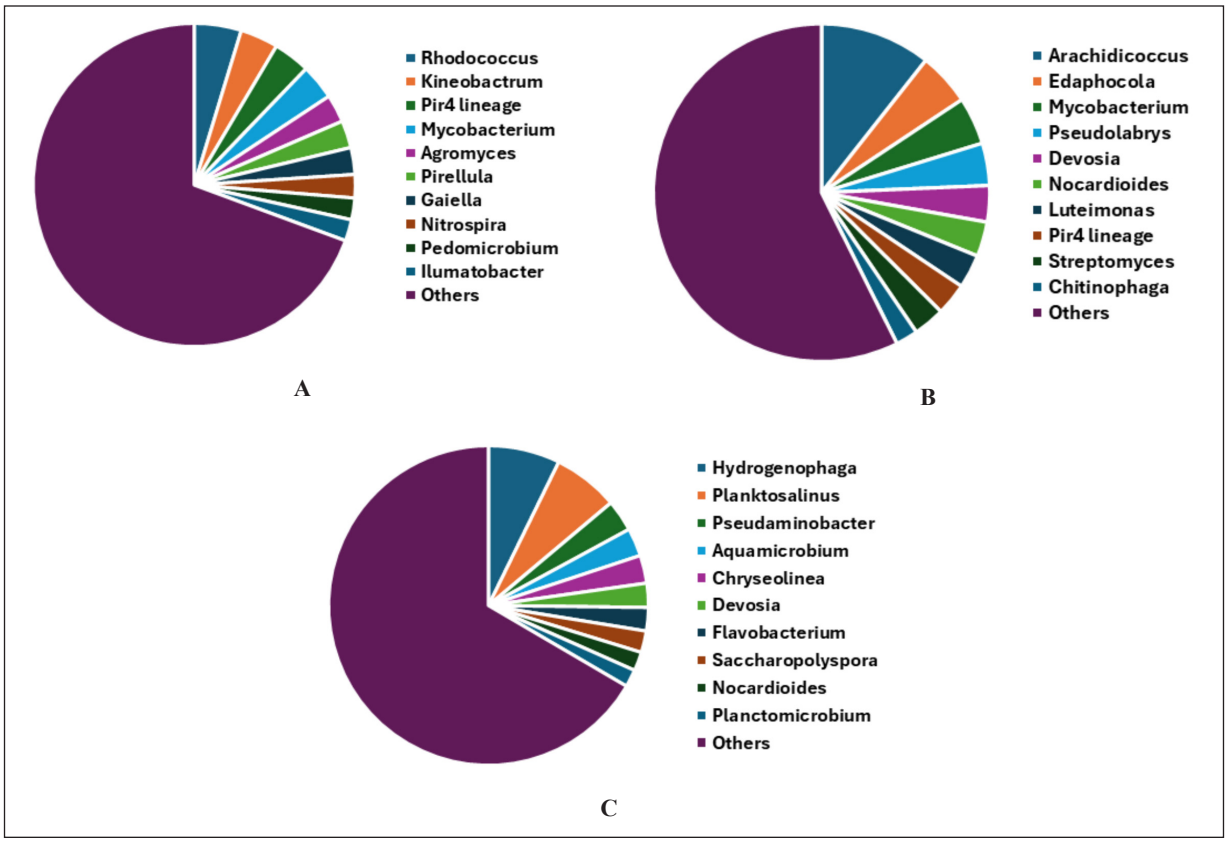


Figure 4. Relative abundance of the top 10 bacterial genera in compost derived from three composite sequencing libraries (C1, C2, and C3) based on 16S rRNA gene (V3–V4) amplicon sequencing. Relative abundance was calculated after removing unclassified sequences. Genera with relative abundances of <2.13% in C1 and C2 and <1.77% in C3 were grouped under Others. A: C1 compost; B: C2 compost; C: C3 compost.

related to nitrogen cycling, phosphate solubilization, and interactions with plants or other microorganisms [21,22]. Similarly, functional metagenomic analyses have suggested potential contributions of Actinobacteria to nitrogen cycling in agricultural ecosystems [44].

The genus *Streptomyces*, which has frequently been detected in compost environments, includes species previously reported to possess antibiotic-producing and plant-associated traits [45–47]. In this study, *Streptomyces* were detected at 0.33% in C1, 3.03% in C2, and 0.45% in C3. The genus *Microbacterium* was also detected in all compost types, with relatively similar abundances (0.16%–0.20%). Other notable Actinobacteria genera detected in C1 included *Rhodococcus* (4.17%), *Agromyces* (2.79%), *Gaiella* (2.69%), *Ilumatobacter* (2.14%), and *Micromonospora* (0.26%). In C2, *Nocardioides* (3.36%), *Agromyces* (0.47%), *Gaiella* (0.23%), *Rhodococcus* (0.07%), and *Ilumatobacter* (0.07%) were detected, whereas C3 contained *Nocardioides* (1.91%), *Saccharopolyspora* (2.18%), *Saccharomonospora* (0.19%), and *Thermoactinomyces* (0.50%).

Firmicutes showed the highest relative abundance in the sampled manure-based compost batch (C3), followed by C1 and C2. The variation may be associated with differences in substrate composition and composting conditions, although the absence of biological replication limits attribution of this pattern to substrate type. Members of this phylum have frequently been reported in composting systems and include taxa associated with the decomposition of organic matter, particularly carbohydrate-rich and lignocellulosic substrates [17]. Their relative abundance has also been reported to vary across composting stages, with Firmicutes often being more prevalent during the early stages and declining toward the later stages [23]. In addition, some Firmicutes exhibit broad physiological and thermal tolerances, allowing them to persist under variable temperature conditions during composting [48]. Genera such as *Bacillus*, *Brevibacillus*, and *Geobacillus* include species reported to grow across relatively broad temperature ranges, including approximately 20°C–60°C [43]. In this study, *Bacillus* was detected in all compost types (0.22%–0.57%), whereas *Brevibacillus* and *Geobacillus* were detected only in C3.

Genera such as *Thermoanaerobacterium*, *Pseudoclostridium*, *Caldibacillus*, and *Thermohydrogenium*, which have previously been reported in association with the degradation of lignocellulosic and cellulose-rich substrates and the production of hydrolytic enzymes such as proteases, pectinases, endoglucanases, and exoglucanases [49], were not detected in the present study. Their absence may be associated with differences in substrate composition, environmental conditions, or other ecological factors among the composting systems. However, the underlying mechanisms cannot be determined from the taxonomic data alone.

Cyanobacteriota were detected at low relative abundances in C1 and C2 but were not detected in the sampled C3 compost batch. Previous studies have reported the occurrence of cyanobacteria in composts derived from kitchen waste and leaf litter. Members of this phylum possess diverse physiological characteristics, including photosynthetic metabolism, and some taxa have been reported to exhibit nitrogen-fixing or plant-associated capabilities [50]. However, these functional characteristics cannot be directly attributed to the Cyanobacteriota

detected in C1 and C2 because the present study did not assess nitrogen-fixation activity, functional genes, or plant-growth responses.

Bacteroidetes were also detected across the composting systems. This phylum is widely distributed in soil and other environments [51], and its members have frequently been associated with the transformation of polymeric organic matter, particularly carbohydrate-rich substrates. Bacteroidetes have also been reported in composting systems, and their relative abundance may vary according to substrate characteristics and composting conditions [17]. Several soil-associated genera, including *Flavobacterium*, *Pedobacter*, *Cytophaga*, and *Spirosoma*, have been reported within this phylum [52]. In this study, *Flavobacterium* was detected at 0.20%–2.36% across all three sampled compost batches, whereas *Pedobacter* was detected only in C2 (0.23%) and *Cytophaga* only in C3 (0.32%). C2 also contained several abundant Bacteroidetes genera, including *Arachidicoccus* (10.65%), *Edaphocola* (5.06%), and *Chitinophaga* (2.13%). In C3, the identified Bacteroidetes genera included *Planktosalinus* (6.64%) and *Chryseolinea* (2.83%).

Chloroflexi were detected in C1, C2, and C3 sampled compost batches. Members of this phylum have previously been reported in composting systems and have been associated with the transformation of complex organic matter, including hemicellulose, particularly under thermophilic conditions [53]. Other studies have also associated Chloroflexi with diverse biogeochemical processes and the transformation of carbon- and nitrogen-containing compounds [54]. Other phyla detected in this study, including Planctomycetota, Acidobacteria, Verrucomicrobia, and Gemmatimonadota, have also been reported in soil- and compost-associated environments [55].

The detection of various bacterial phyla and genera in this study primarily provides a description of the taxonomic composition of the microbial communities in each sampled compost batch. Although previous studies have reported that some of these taxa play roles in organic matter decomposition, nutrient transformation, biogeochemical cycling, pathogen suppression, or PGP, these functions cannot be directly attributed to the specific taxa detected in this study. Analysis based on the 16S rRNA gene provides information only on the presence and relative abundance of bacterial taxa and does not provide information on functional genes, gene expression, enzymatic activity, or metabolic activity.

The nutrient characteristics and bacterial diversity profiles showed no consistent descriptive pattern across the three composting systems. C1 exhibited the highest observed ASV richness and Shannon diversity, despite having the lowest organic carbon content among the three batches. In contrast, C2 had the highest total nitrogen content and the lowest C/N ratio but showed the lowest observed richness and Shannon diversity. C3, which had the highest organic carbon, K_2O, and C/N ratio, exhibited intermediate richness and diversity values. However, the bacterial community was characterized only at the final stage of the composting period, which lasted approximately 3 months. Since the microbiome of the starting materials was not analyzed and no intermediate sampling points were included, the study could not reveal microbial succession or determine whether specific taxa introduced via EM4, soil, or manure subsequently underwent population increases or decreases during composting. The community profile observed at the final stage reflects the bacterial configuration detected at the time of sampling, rather than the dynamics of community development throughout the composting process.

A limitation of this study is that each composting system was represented by a single independently prepared compost, with five subsamples pooled into a single composite sample for microbiome characterization. Microbial profiling was performed only at the final stage of the composting using 16S rRNA gene amplicon sequencing. Consequently, the observed physicochemical and taxonomic pattern should be interpreted as descriptive observations specific to the sample batches and cannot be used to estimate between-batch variability, establish treatment effects, or assess reproducibility. Nevertheless, these findings provide baseline data to guide the design of future studies incorporating independent biological replicates, longitudinal sampling throughout the composting process, and complementary functional approaches such as shotgun metagenomics or targeted functional gene analysis.

Another limitation of this study is that compost maturity and hygienic safety were not directly evaluated. Although several physicochemical properties were characterized, these measurements alone are insufficient to establish compost maturity, stability, or sanitization. Parameters commonly used to assess compost maturity include temperature, dissolved organic carbon, humification index, respiration rates, germination index, and ammonium-to-nitrate ratio [56]. Microbiological safety was also not assessed through pathogen testing, which is particularly relevant for compost containing goat manure intended for agricultural application. Therefore, the physicochemical characteristics and bacterial taxonomic composition reported in this study should be regarded as preliminary descriptive observations specific to the sampled compost batches and final sampling stage. Future studies should therefore incorporate longitudinal monitoring of composting conditions, maturity and stability indicators, and targeted pathogen testing in replicated composting experiments.

4. CONCLUSION

In conclusion, this study provides a descriptive characterization of the nutrient properties and bacterial taxonomic profiles of three laboratory-prepared composting systems. Among the three composite sequencing libraries, Proteobacteria and Actinobacteria were the dominant bacterial phyla, although differences in taxonomic composition were observed among the composting systems. Because each composting system was represented by only a single independently prepared batch and one composite sequencing library, these findings should be interpreted as descriptive observations rather than treatment-level conclusions. Independent biological replication and appropriate statistical analyses are essential to determine whether the observed patterns are reproducible and attributable to differences among the composting systems.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors declare that they have no financial or other conflicts of interest related to this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

The 16S rRNA gene sequencing data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject accession number PRJNA1427732 and are publicly available via https://www.ncbi.nlm.nih.gov/sra/PRJNA1427732.

10. PUBLISHER'S NOTE

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The author used ChatGPT (GPT-5.6, OpenAI; website link: https://chatgpt.com/) solely to assist with language editing and to improve the clarity and readability of the manuscript. ChatGPT was not used to generate or analyze research data, produce scientific findings or interpretations, or generate references. All AI-assisted revisions were reviewed and verified by the author, who assumes full responsibility for the accuracy, integrity, and final content of the manuscript.

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