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Characterization of the Bacterial Microbiome Structure and Identification of the Beneficial Genera in the Leaf Litter Compost for its Potential Application as a Bioorganic Fertilizer

Sophayo Mahongnao*, Pooja Sharma*, Arif Ahamad, Neeraj Dohare*, Neeru Dhamija*, Anita Garg Mangla* and Sarita Nanda**†**

*Department of Biochemistry, Daulat Ram College, University of Delhi, Delhi-110007, India
**Department of Environmental Science, Jamia Millia Islamia University, New Delhi-110025, India
†Corresponding author: Sarita Nanda; saritananda123@gmail.com

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ABSTRACT

This study investigates the potential of leaf and various organic waste composts as bio-organic fertilizers using 16S rRNA metagenomics. The microbial richness and diversity analysis, employing alpha and beta diversity indices, reveal substantial variations influenced by organic substrates during composting. The leaf compost had a high total OTU (70,554) but low microbial diversity (Chao 1 index = 272.27). The kitchen waste compost had the highest microbial diversity (Chao 1 index = 429.18). Positive correlations between microbial biomass, diversity, and compost quality highlighted the pivotal role of microbial activity. The beneficial genera identified across all the bio-composts were *Lactobacillus, Leuconostoc, Sphingobacterium, Paenibacillus, Pseudomonas,* and *Clostridium*. Some pathogenic genera were also detected in all the composts analyzed, viz. *Prevotella, Agrobacterium, Fusobacterium,* and *Streptococcus*. Nonetheless, the ratio of beneficial to the pathogenic genera was generally high in all compost, highlighting the enrichment with beneficial microorganisms. The leaf compost demonstrated the highest proportion of beneficial genera, about 92%, indicating significant bio-fertilizing potential, with a low % level of pathogenic genera of about 3%. Thus, the leaf compost has excellent potential to be used as a bio-organic fertilizer. Understanding the microbial composition of organic waste composts is crucial for its application as bio-fertilizer for promoting sustainable agriculture.

INTRODUCTION

Composting is a widely recognized sustainable method for managing organic waste, producing valuable soil amendments, and reducing the need for synthetic fertilizers in agriculture and horticulture (Bustamante et al. 2021, Fertiplus et al. 2019). Alternative means to chemical fertilizer for sustainable productivity have become paramount since the disproportionate use of chemical fertilizer has caused extensive environmental contamination (Chauhan 2016, Zhang et al. 2018). Composts produced from organic wastes could be an excellent alternative to chemical fertilizers for soil health and sustainable productivity. Ravindran (2022) noted that composting can help reduce greenhouse gas emissions and improve soil health. Various composting processes are available, each with unique advantages and disadvantages. Leaf litter can be a good substrate source for composting to produce valuable compost. Application of the composts generated from a different substrate, such as kitchen waste, organic fraction of the municipal solid waste (OFMSW), and vermicompost, as soil amendments have been shown to improve soil quality and increase plant growth (Eifediyi et al. 2015, Gupta et al. 2014, Horz & Conrads 2010, Machado et al. 2021, Pathak et al. 2020). However, the leaf compost produced from the leaf litter has yet to be studied comprehensively. It is essential to check the total microbiota, beneficial, and pathogenic microorganisms present in the leaf compost to understand the bio-fertilizing potential of the leaf-based compost compared to other organic waste composts.

The microbial communities present in different composts can vary depending on the type of feedstock and composting process used (Siles et al. 2021, Vishan et al. 2014). It has been reported that *Firmicutes, Proteobacteria, Actinobacteria,* and *Bacteroidetes* were the most abundant phyla in various organic waste composts such as green waste compost, food waste compost, manure compost, and vermicompost of multiple substrates such as cow manure and kitchen waste (Aguilar-Paredes et al. 2023, Wang et al. 2022). A study by Wan (2021) also reported that *Proteobacteria* and

Chloroflexi were the major phyla in sheep and cattle manure composts, and *Firmicutes* dominated pig and chicken manure composts. The phyla were detected in varied proportions in different organic waste composts. Still, a comprehensive analysis of the total microbiota and beneficial and pathogenic microorganisms in different organic waste composts has yet to be done and reported.

Recent advances in metagenomics have revolutionized the study of microbial communities in various environments, including organic waste composts (Horz & Conrads 2010). The16S rRNA metagenomic profiling has emerged as a powerful tool to study microbial communities in environmental samples such as composts. Using this technique, it is possible to identify the bacterial and archaeal taxa present with their relative abundance in different composts. Understanding the microbial ecology of organic waste composts can help identify the beneficial and pathogenic microorganisms in different composts. It could help us realize the potential benefits of composts and their suitability for application as bio-organic fertilizer for soil health and plant growth (Zhang et al. 2019).

The present study was designed to use 16S rRNA metagenomics to investigate the bacterial communities present in the leaf compost to identify the microbiome richness and presence of beneficial and pathogenic microbes in the compost. The fertility index and clean index, which indicate the fertilizing potential and level of potentially toxic elements, have been reported to be high for the leaf litter compost (Mahongnao et al. 2023). We further hypothesized that the leaf-based bio-compost is rich in beneficial microorganisms and can be used as a bio-organic fertilizer. Deciphering the intricate interplay between bacteria and their environment is paramount in unraveling the microbial dynamics inherent to leaf litter and diverse organic waste composts (Faust et al. 2015). This comprehension is pivotal for leveraging the intrinsic benefits of compost in bolstering soil health, promoting plant growth, and suppressing pathogens (Mahapatra et al. 2022, Wang et al. 2022). Against the backdrop of escalating concerns regarding sustainable waste management and an acknowledgment of the integral role bacteria play in ecosystem functionality. Our study seeks to furnish a comprehensive elucidation of bacterial microbiome structures within leaf litter and a spectrum of organic waste composts. These encompass cow dung manure, kitchen waste compost, municipal organic waste compost, vermicompost, and neem cake compost, with our methodology leveraging 16S metagenomic profiling.

This investigation also delved into the formulation of innovative approaches for leaf litter composts with the potential to function as biofertilizers, thereby enhancing plant health and soil quality. Our study systematically scrutinizes diverse formulations of leaf litter composts, encompassing variations in composting inoculum, initial leaf litter substrate, and including neem and castor leaves.

Beyond the meticulous evaluation of bacterial diversity and composition, our research adopts a holistic approach by integrating temporal monitoring of bacterial communities at different maturation time points during the composting processes of leaf litter. This comprehensive methodology elucidates dynamic changes in bacterial populations as compost maturation and transformation unfold. By shedding light on these critical facets, our work aspires to yield valuable insights capable of optimizing leaf litter composting protocols, thereby fostering more sustainable and ecologically responsible organic waste management practices, ultimately producing high-quality organic compost.

We also conducted a comprehensive assessment of bacterial microbiome richness and diversity within matured leaf litter compost, juxtaposed with analogous matured organic waste composts, namely kitchen waste compost, cow dung manure, municipal organic waste compost, vermicompost, and neem cake compost. Our investigation extended to discerning the presence of both beneficial and pathogenic fungal genera in these organic composts. This comparative analysis serves as a valuable tool in comprehending the aptness of these composts for utilization as bio-organic fertilizers (De Corato 2020, González-González et al. 2021).

MATERIALS AND METHODS

Samples Collection and Preparation

Leaf litter from trees such as Indian beech (*Pongamia pinnata*), Krishna kadamb (*Mitragyna parviflora* (Roxb.) Korth), mulberry (*Morus alba*), scholar tree (*Alstonia spp*.), frangipani (*Plumeria rubra*), and fig (*Ficus* spp.) was systematically collected within the premises of the institutional campus and subjected to shredding for composting.

During the autumnal season, a monthly accumulation of approximately 450 kg, equating to 15 kg per day, of leaf litter waste was thoroughly gathered. Mechanical shredders were employed to shred the leaf waste. Subsequently, composting was done within experimental-scale bins measuring 3 feet × 1.5 feet × 0.5 feet (length × breadth × height) at ambient environmental conditions, employing the Effective Microorganisms (EM) method (Mahongnao et al. 2023). Four sets of leaf litter composting were set up in parallel with different inoculums. Each bin was initially loaded with 4 kg (dry weight) of shredded leaf waste and subjected

to a composting duration of twelve weeks, with periodic mixing occurring every 3 to 4 days. Sampling events were conducted at three and twelve weeks into the composting duration to assess the progression of the composting process. Specifically, the leaf compost sample denoted as DRCC20 underwent a three-week composting cycle employing water as the sole inoculum. DRCTI10 was subjected to a three-week composting regimen utilizing a waste decomposer. This particular decomposer is a consortium of microorganisms extracted from cow dung developed by the National Centre of Organic Farming, Government of India.

In contrast, DRCTI140D underwent a more extended twelve-week composting cycle using the same waste decomposer developed by the National Centre of Organic Farming. Another batch, DRCTB10, was composted for three weeks, employing a stimulated sludge derived from landfill soil collected from a landfill site in New Delhi. The final compost sample, DRCLC36W, underwent a three-week composting process utilizing a combination of microorganisms extracted from fresh cow dung. Additionally, neem and castor leaves were incorporated into the composting material at a ratio of approximately 20% (w/w).

In addition to the experimental compost samples, various matured organic waste composts, including kitchen compost (DRCK), municipal organic waste compost (DRCM), cow dung manure (DRCCD), vermicompost (DRVM), and neem cake compost (DRCNM), were sourced from local producers in the Delhi-National Capital Region, India, for comparative analysis.

DNA Extraction and PCR Amplification of V3-V4 Region of 16S gene

DNA extraction was done using the suitable method for the sample type from commercially available kits such as QIAGEN (Qiagen India Pvt Ltd**,** Delhi India), ZYMO RESEARCH (California, USA), and Thermo-Fisher (Massachusetts, USA). DNA extraction was done as per the manufacturer's recommendation. Extracted DNA from the samples was subjected to NanoDrop and gel Check before being taken for PCR amplification: The NanoDrop readings of 260/280 at a value of 1.8 to 2 were used to determine the DNA's quality (Devi et al. 2015, García-Alegría et al. 2020).

For the metagenomic analysis, the extracted DNA was amplified and sequenced to obtain the DNA sequence of the V3-V4 region of the 16S rRNA bacterial gene. The amplification was performed using a PCR mix containing High-Fidelity DNA Polymerase, 0.5mM dNTPs, 3.2mM $MgCl_2$, and PCR Enzyme Buffer. The primers used were 16sF 5' AGAGTTTGATGMTGGCTCAG 3' and 16sR 5' TTACCGCGGCMGCSGGCAC 3'. The conditions for the polymerase chain reaction (PCR) amplification were that 40ng of Extracted DNA was used for amplification along with 10 pM of each primer. The initial denaturation was set at 95°C. The 25 Cycles were set with the following conditions: denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, elongation at 72°C for 2 minutes, and final extension at 72°C for 10 minutes, and hold at 4°C. The amplified 16s PCR Product is purified and subjected to gel check and Nanodrop Quality Control. The NanoDrop readings of 260/280 at a value of 1.8 to 2 were used to determine the DNA's quality (Martins et al. 2013).

Overview of Sequencing and Bioinformatics Protocol

The amplicons from each sample were purified with Ampure beads to remove unused primers, and an additional 8 cycles of PCR were performed using Illumina barcoded adapters to prepare the sequencing libraries. Libraries were purified using Ampure beads and quantitated using a Qubit dsDNA High Sensitivity assay kit (Invitrogen, California, USA). Sequencing was performed using Illumina Miseq with a 2x300PE v3 sequencing kit (Illumina, Portland, USA). Raw data quality control (QC) was done using FASTQC and MULTIQC, followed by trimming of adapters and low-quality reads by TRIMGALORE. The trimmed reads are further taken for processing, including merging of paired-end reads, chimera removal, and OTU abundance calculation and estimation correction.

The binary base call (BCL) data acquired from the sequencer underwent demultiplexing, resulting in the generation of Fastq raw data. After this, the demultiplexed data quality was evaluated using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. Samples that successfully passed the quality control assessment were deemed eligible for further analysis. Our proprietary metagenomics pipeline, the Biokart Pipeline, designed for 16S, was employed for subsequent analysis. Following the completion of the sequencing run, the final raw Operational Taxonomic Unit (OTU) table was obtained, serving as the foundational dataset for subsequent analytical visualization. This was achieved using QIIME/MOTHUR/KRAKEN/BRACKEN workflows (Ramírez-Guzmán et al. 2004).

The Table 1 shows the code of each sample, their description, and the International Nucleotide Sequence Database Collaboration (INSDC) accession number.

The construction of abundance feature tables outlining the prevalence of organisms in each sample was executed utilizing Microsoft Excel (2021). Additional analyses, such as Heatmap generation, identification of the core microbiome, Dendrogram construction, Alpha diversity assessment, Beta diversity analysis, Principal Coordinates Analysis (PCOA)

Table 1: Compost sample code and description.

Sample code	Initial Substrate	Inoculum used in composting	Maturity time	The International Nucleotide Sequence Database Collaboration (INSDC) accession number.
DRCC20	Leaf litter	Water	3 Weeks	ERS15510559
DRCTI10	Leaf litter	Waste decomposer	3 Weeks	ERS15529905
DRCTB10	Leaf litter	Sludge of landfill soil	3 Weeks	ERS15529906
DRCLC36W	Leaf litter with neem and castor leaves	Waste decomposer	3 Weeks	ERS15542713
DRCTI140D	Leaf litter	Waste decomposer	12 weeks	ERS15542763
DRCCD	Cow dung	Waste decomposer	14 weeks	ERS15529931
DRCK	Kitchen organic waste	Waste decomposer	10 weeks	ERS15529907
DRCM	Municipal organic waste	Waste decomposer	10 weeks	ERS15529930
DRCV	Cow dung manure	Waste decomposer	10 Weeks	ERS15529908
DRCNM	Neem cake	Waste decomposer	10 weeks	ERS15529940

plot generation, and Rarefaction curve assessment, were conducted through the utilization of Microbiomeanalyst, an online tool accessible at https://www.microbiomeanalyst.ca.

This workflow enables highly accurate investigations at the genus level. The microbial diversity of different biocomposts was analyzed through Alpha and Beta diversity indices. The databases used were SILVA/ GREENGENES/ NCBI. Each read was classified based on % coverage and identity. The 16S workflow helps identify pathogens in a mixed sample or understand microbial community composition (Mbareche et al. 2017).

The raw data of the Illumina Miseq sequencing of all the samples were deposited at the Indian Nucleotide Data Archive (INDA) of the Indian Biological Data Centre with the referenced INDA (Study/Bioproject) Accession number INRP000063. The International Nucleotide Sequence Database Collaboration (INSDC) Bioproject has the accession number of this study as PRJEB62440 (Table 1).

Statistical Analysis

A systematic categorization into three groups was undertaken to conduct an in-depth analysis of metagenomic data, including assessments of alpha and beta diversity, as well as the statistical evaluation of microbiome levels across distinct compost samples. Group 2 comprised leaf litter compost samples, namely DRC20, DRCTI10, and DRCTB10. Group 3 encompassed other organic waste compost samples, specifically DRCCD, DRCK, DRCM, DRCV, and DRCNM. Group 8 included leaf compost samples DRCTI140D and DTCLC36W. Subsequently, data pertaining to these sample groups underwent filtration, and alpha diversity was quantified utilizing four methods, Shannon-Weiner, Fisher, and Simpson, accompanied by statistical analyses employing T-test/ANOVA methodology (Willis 2019). Furthermore, beta diversity, constructed at the Genus taxonomic level, employed the Bray-Curti's index distance method, with statistical significance determined through the Permutational MANOVA (PERMANOVA) approach (Maziarz et al. 2018).

RESULTS AND DISCUSSION

The Microbiome Richness and Diversity

The number of reads for each compost type provides an estimate of the amount of microbial DNA present in the sample. The leaf compost sample DRC-C20 had the highest read count at 0.8 million and total OTU (253,373), followed by the treatment leaf compost samples DRC-TI10 (reads, 0.4 million, OTU, 70,554) and DRC-TB10 (reads, 0.2 million, OTU, 96,328). The leaf compost sample LC36W had the lowest read count of 0.2 million and OTU of 9,057 (Fig. 1).

Leaf waste compost, DRCC20, had the largest library size, followed by cow dung manure (DRCCD)

Among the other organic waste composts analyzed, the cow dung manure, DRCCD, had the highest reads at 0.8 million and OTU of 230,466. Whereas the neem cake compost, DRCNM, had the lowest reads and OTU at 0.05 million reads and 18,133. Interestingly, despite having fewer reads than some of the other composts, vermicompost, and kitchen-waste compost had a relatively higher total number of OTUs of 86328 and 75708, respectively.

Alpha and Beta Diversity

All the compost samples were rarefied or normalized to even sequencing depth based on the lowest sequencing depth sample. The analysis was plotted with the filtered data source. The rarefaction curve indicated that the kitchen

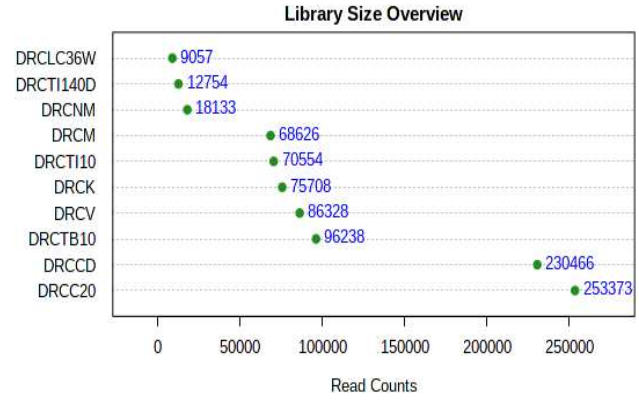

Fig. 1: Library-size overview of the leaf composts and the other organic waste composts.

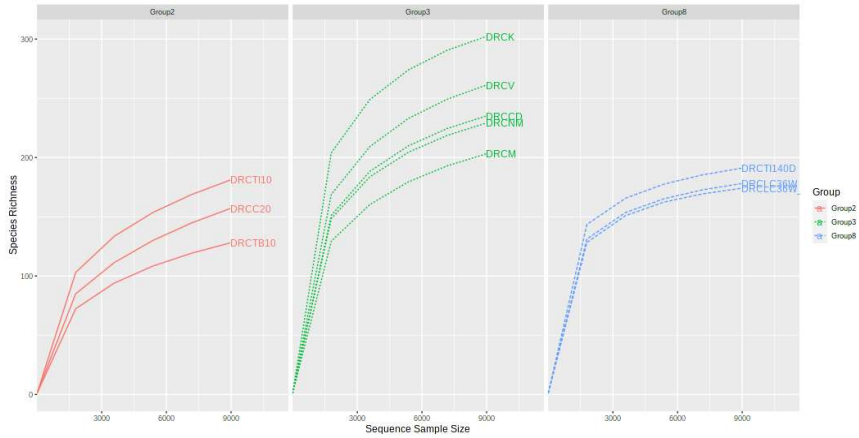

Fig. 2: Rarefaction curve of the leaf composts and the other organic waste composts.

waste compost had the highest species richness, followed by vermicompost, cow dung manure, neem cake compost, and municipal organic waste compost. The leaf compost had relatively lower species richness than the other composts analyzed in this study (Fig. 2).

The results of the alpha diversity analysis measured through diversity indices of Chao 1, Shannon, Fisher, and Simpson revealed that leaf composts and other organic waste composts had varied microbial richness and diversity (Table 2). The kitchen waste compost, DRCK, had the highest alpha diversity indices (Chao 1 index = 429.184, Shannon index = 4.452, Fisher index = 72.994, Simpson index = 0.976). Generally, the leaf composts had relatively lower alpha diversity indices than the compost produced from other organic waste substrates. Among all the samples analyzed, the leaf compost sample, DRCTB10, had the lowest alpha diversity indices (Chao 1 index = 272.276, Shannon index = 2.924, Fisher index = 35.886, Simpson index = 0.888). The *p-values* in Chao1, Shannon, Fisher, and Simpson's alpha diversity were measured to be 0.045858, 0.00055648, 0.015549, and 0.0058932, with the ANOVA *F*-value of 4.9435, 26.281, 8.0009, and 11.674, respectively (Fig. 3).

Table 2 shows the Alpha diversity indices of different bio-composts, which reveal that the different bio-composts have varying levels of bio-diversity.

Beta diversity was used to evaluate the sample diversity across the bio-compost samples. The beta diversity was constructed at the Genus taxonomic level with the Bray-Curtis index distance method based on Permutational MANOVA (PERMANOVA) statistical method. The *p*-value was < 0.002 with the PERMANOVA *F*-value of 4.8217. As expected, all the leaf composts clustered together, signifying the presence of common microorganisms. The other bio-composts spread out from leaf compost and each other, representing a different microbial diversity (Fig. 4).

Heat Mapping

The heat map was constructed at the Genus taxonomic level. The samples are clustered using the Ward cluster algorithm

Table 2: Alpha diversity indices of the bacterial microbiome in the leaf and other organic waste composts.

Samples	Chao 1 index	Shannon index	Fisher index	Simpson index
DRCC20	235.17 ± 31.79	2.92	27.25	0.887
DRCTI10	272.27 ± 26.76	2.92	35.88	0.888
DRCTB10	193.71 ± 29.27	2.83	21.25	0.898
DRCTI140D	232.71 ± 12.06	4.20	39.92	0.969
DRCLC36W	205.9 ± 9.42	3.96	35.42	0.959
DRCV	294.37 ± 14.19	4.08	52.08	0.965
DRCCD	306.78 ± 18.88	3.91	50.54	0.959
DRCM	298.19 ± 22.96	3.79	45.03	0.955
DRCK	429.18 ± 27.72	4.45	72.99	0.975
DRCNM	298.57 ± 17.17	3.75	50.29	0.909

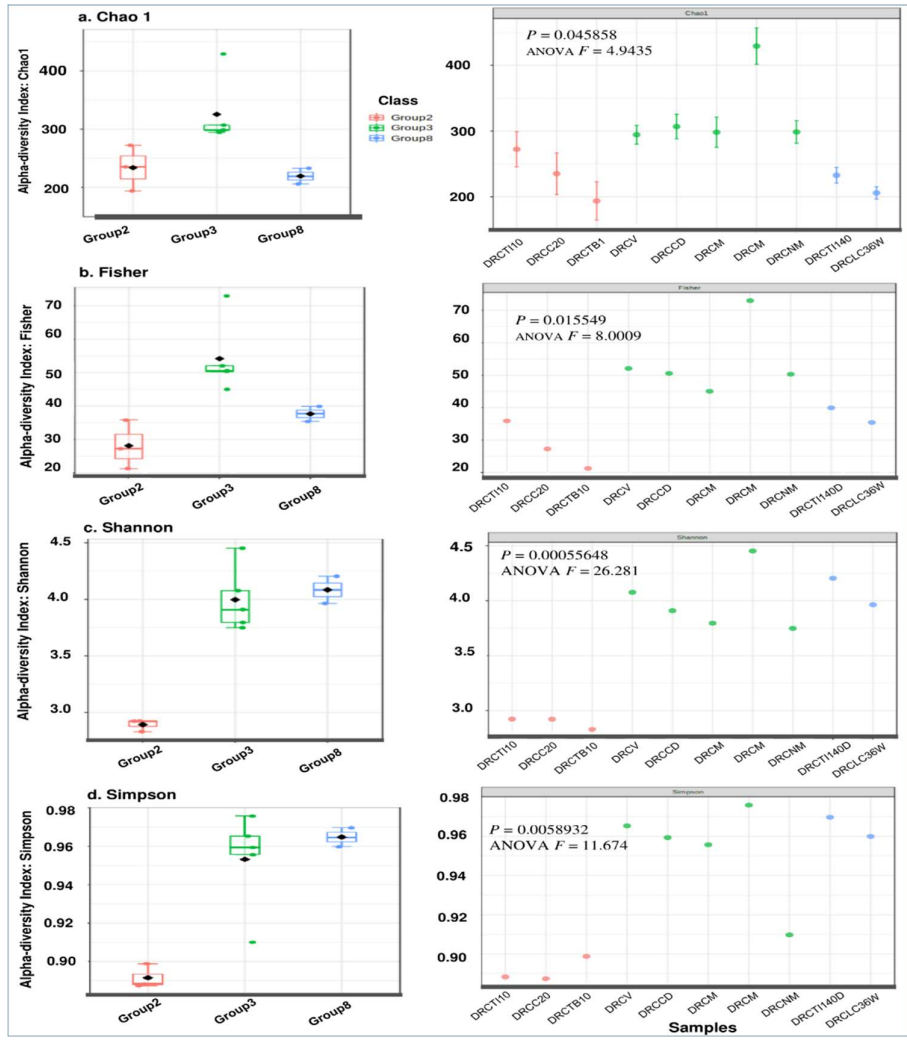

Fig. 3: Alpha diversity analysis of the leaf composts and the other organic waste composts.

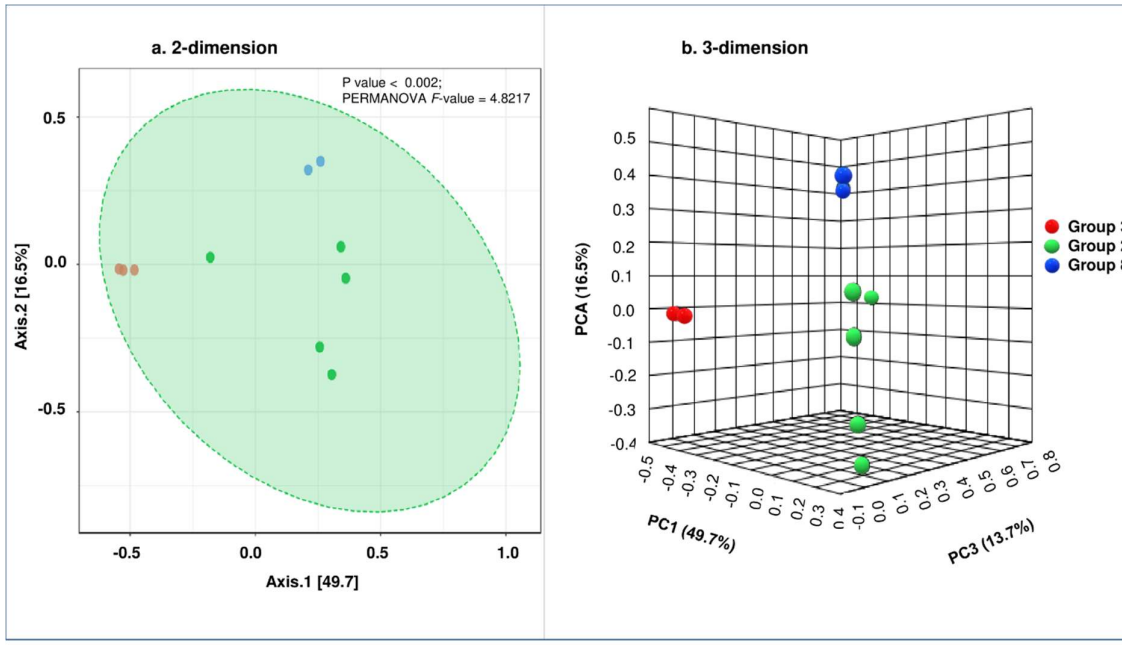

Fig. 4: Beta diversity analysis of the leaf composts and the other organic waste composts.

based on the Euclidean distance measure. About 30 bacterial genera could be classified as the core microbiome, a set of taxa detected in a high fraction of the population above a given abundance threshold. The genera detected across different composts varied according to the nature of the compost, which includes *Lactobacillus, Leuconostoc, Sphingobacterium, Paenibacillus, Pseudomonas, Clostridium, Planctomyces, Achromobacter, Stenotrophomonas,* and others. After three weeks of composting using different inoculums, namely DRDC20 and DRCTI10, the leaf composts showed changes in the bacterial community.

At the same time, there were some similarities in the bacterial community between the leaf composts, DRCC20 and DRCTB10. The heat map also showed that cow dung manure (DRCCD) and vermicompost (DRCV) had some common bacteria. The neem cake compost, DRCNM, had high amounts of bacteria genera such as Prevotella, Pseudomonas, Bacillus, and Lactococcus. At the same time, some bacterial communities in the neem cake compost were similar to the leaf litter compost. The municipal (DRCM) and kitchen waste compost (DRCK) had some common bacteria, which are present in high amounts (Fig. 5).

Taxonomic Classification, Identification of Beneficial and Pathogenic Microbes

Phylum level: The microbial composition of compost samples was characterized, revealing the presence of twenty-one phyla, with the top ten species accounting for 98-99% coverage. Predominant phyla included *Proteobacteria, Firmicutes, Chloroflexi, Actinobacteria,* and *Bacteroidetes,* exhibiting divergent proportions in leaf and other organic waste composts**.** Notably, *Proteobacteria* levels were higher in leaf compost DRCC20 compared to other leaf compost treatments (DRCTI10 and DRCTB10) and comparable to cow dung manure (DRCCD), neem cake compost (DRCNM), and vermicompost (DRCV). *Firmicutes* were consistently abundant, particularly in leaf composts, with DRCC2 having higher levels. *Chloroflexi*, *Actinobacteria,* and *Acidobacteria* were present in all composts, with leaf compost showing elevated levels. *Bacteroidetes* levels were relatively lower in leaf composts than in other organic waste composts. *Planctomycetes* and *Verrucomicrobia* were more abundant in different organic waste composts such as cow dung manure, kitchen compost, and vermicompost than leaf compost. *Euryarchaeota* and *Cyanobacteria* were present in various composts, notably with higher abundance in the leaf compost than the other organic waste compost analyzed. Pathogenic phyla such as *Spirochaetes, Fusobacteria,* and *Chlamydiae* were identified across composts but had lower OTU counts than non-pathogenic phyla. *Spirochaetes* were relatively higher in municipal organic waste compost (DRCM), kitchen waste compost (DRCK), and cow dung manure (DRCCD). *Fusobacteria* were elevated in neem cake compost (DRCNM) and leaf compost DRCLC36W. *Chlamydiae* was higher in cow dung manure (DRCCD), vermicompost (DRCV), and kitchen waste compost (DRCK).

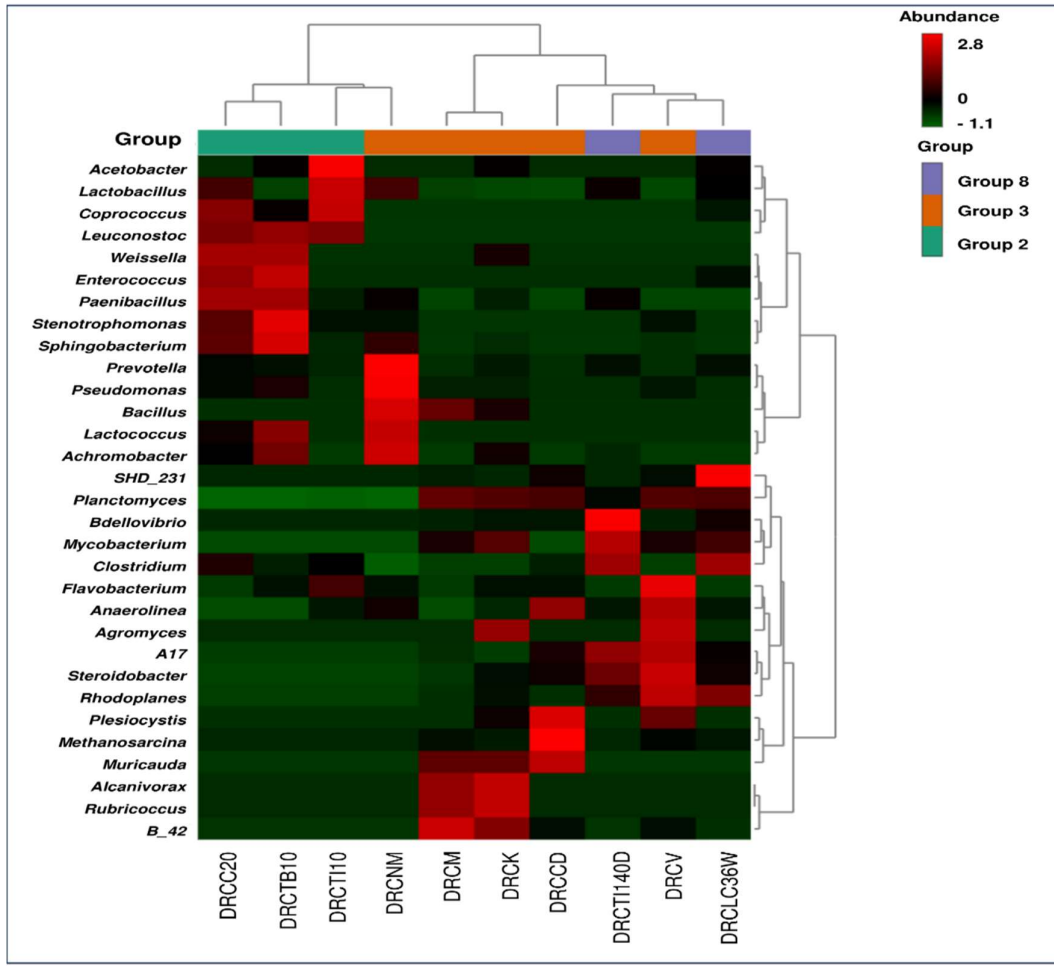

Fig. 5: Heat map of the core microbiome detected in the leaf and other organic waste composts.

These findings provide valuable insights into the microbial dynamics of diverse compost samples at the phylum level.

Genus and species level: Some bacterial genera identified were shared in different composts, while others were unique to each type of compost analyzed. The bacterial genera detected across the leaf litter composts and other different organic waste composts varied according to the nature of the substrate used to produce the compost. The primary beneficial bacterial genera identified across all the composts include *Lactobacillus, Leuconostoc, Sphingobacterium, Pseudomonas, Clostridium, Flavobacterium, Planctomyces, Stenotrophomonas, Achromobacter, Coprococcus, Paenibacillus, Weissella,* and *Acetobacter.*

Lactobacillus counts were much higher in the leaf compost than in the other organic waste composts. Among the leaf composts, the sample DRCC20 had a higher count of *Lactobacillus* than the treatment samples like DRCTI10 and DRCTB10. At the same time, the presence of *Lactobacillus* was drastically reduced in the leaf compost samples, DRCTI140D and DRCLC36W. Lactobacillus counts were relatively low in the other composts analyzed, though high only in the neem cake compost sample, DRCNM. *Leuconostoc* and *Sphingobacterium* were detected with relatively higher levels in the leaf composts than in the other organic waste composts. Among the leaf compost, the sample DRCC20 had a higher genera count than the other treatments. Remarkably, *Leuconostoc* and *Sphingobacterium* were not detected in the leaf compost samples, DRCTI140D and DRCLC36W. In the additional organic waste composts analyzed, the levels of *Leuconostoc* and *Sphingobacterium* were relatively deficient. The vermicompost (DRCV) had the lowest count of these genera among all the organic waste composts analyzed.

Pseudomonas was found across all the composts analyzed. The leaf compost had a higher richness of *Pseudomonas* than other organic waste composts analyzed,

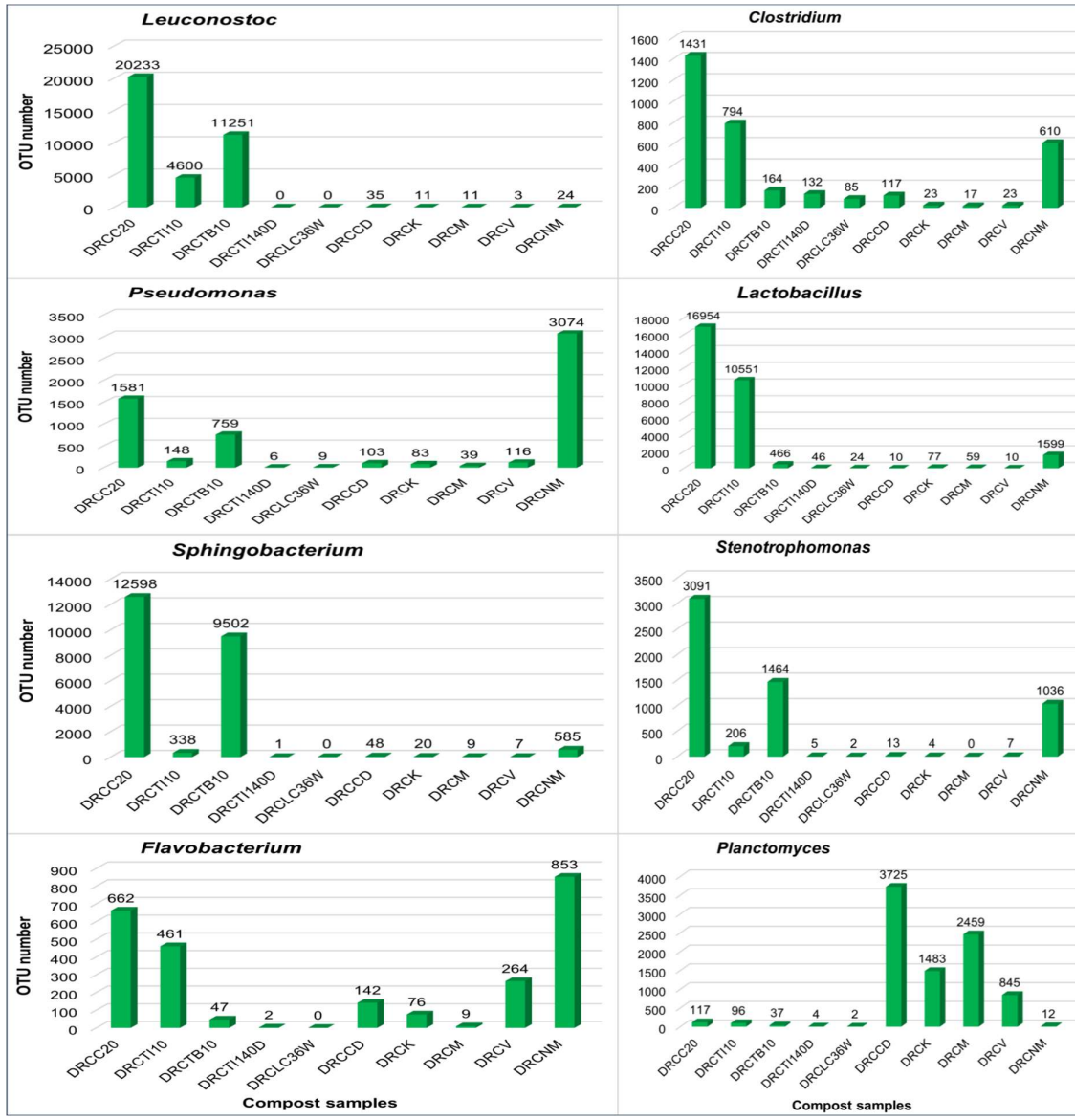

Fig. 6: The prominent beneficial bacterial genera identified in the leaf litter compost and other organic waste composts.

though lower than neem cake compost (DRCNM). Among the leaf composts, the sample DRCC20 had a higher count of *Pseudomonas* than the other treatments. Meanwhile, the municipal organic waste compost (DRCM) had the lowest count of *Pseudomonas* among all the organic waste composts analyzed. *Clostridium* enrichment was found in all the composts analyzed in this study, with relatively higher counts in leaf and neem cake composts. The counts of *Clostridium* were relatively low in the kitchen waste compost (DRCK) and vermicompost (DRCV). The municipal organic waste compost (DRCM) had the lowest level of *Clostridium* among all the composts analyzed (Fig. 6).

Planctomyces were also detected in all the composts but with a relatively lower level in the leaf composts. The enrichment of *Planctomyces* was highest in cow dung manure (DRCCD), followed by municipal organic waste compost (DRCM). The kitchen waste compost and vermicompost were also seen with high enrichment of *Planctomyces*. Conversely, the neem cake compost (DRCNM) had a very low level of *Planctomyces* enrichment, the lowest among all the composts. The levels of *Flavobacterium* were relatively higher in the neem cake compost, leaf compost, and vermicompost. While their counts were low in the municipal organic and kitchen waste compost

The richness of *Paenibacillus* was low in all the composts but relatively higher in the leaf composts and the neem cake compost. The cow dung manure (DRCCD) and vermicompost (DRCV) had the lowest count of *Paenibacillus* among all the organic waste composts. *Achromobacter* was also present in all the composts, with a relatively higher abundance in the leaf and neem cake composts. The level of *Achromobacter* was relatively low in the cow dung manure, municipal organic waste compost, and vermicompost. The bacterial genera such as *Stenotrophomonas, Acetobacter,* and *Enterococcus* were relatively high only in the leaf and neem cake compost.

Some bacterial genera, such as *Coprococcus, Gluconobacter, Haloarcula,* and *Haloferax*, were found to be exclusively high in leaf compost. Such bacterial genera were either very low or absent in the other organic waste composts analyzed in this study. *Methanosarcina,* a methane-producing bacterial genus, was found in high abundance in cow dung manure (DRCCD). At the same time, its presence was very low in other composts and completely absent in the leaf compost and neem cake compost. *Rhodoplanes* were also found with higher abundance in the cow dung manure and vermicompost. *Bacillus*, a biological pest control agent, was found to be present in all the composts but with low abundance in all the composts except in the neem cake compost. The neem cake compost was seen to be highly enriched with *Bacillus*.

Some bacterial genera that can be detrimental to soil and pathogenic to humans and plants were also found in different composts analyzed in this study. However, their level of counts was much lower than the beneficial genera. Such bacterial genera identified were *Agrobacterium, Fusobacterium, Prevotella, Steroidobacter, Streptococcus, Neisseria,* and *Mycobacterium*. The level of counts of each genus varied depending on the types of composts. The neem cake and leaf compost had a relatively higher count of *Prevotella*. The cow dung manure had the highest count of *Steroidobacter* among all the composts. The vermicompost

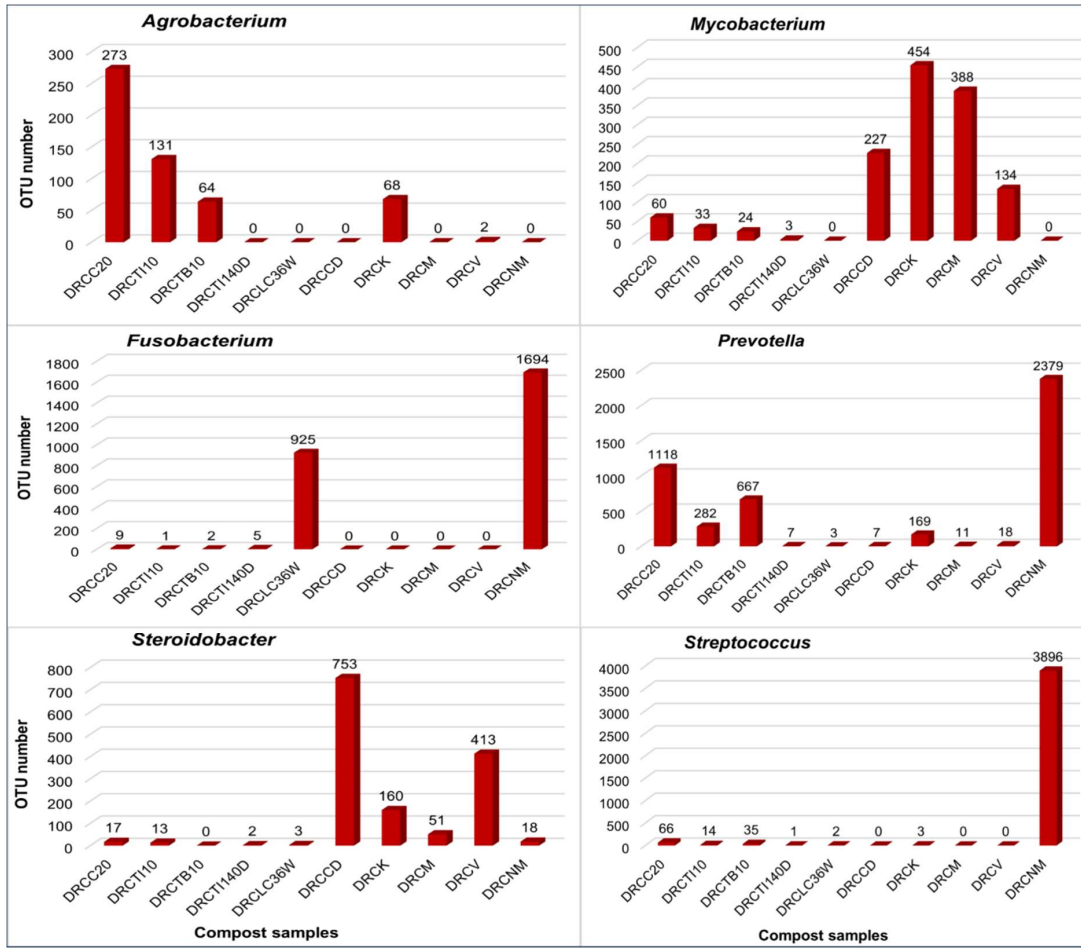

Fig. 7: The prominent bacterial pathogenic genera detected in the leaf litter compost and other organic waste composts.

and kitchen waste compost also had a relatively high level of *Steroidobacter*. A plant pathogenic genus, *Agrobacterium,* was detected only in leaf and kitchen waste compost but with relatively low abundance. The neem cake compost had a higher level of *Streptococcus, Neisseria,* and *Fusobacterium*. Such bacterial genera were either very low or absent in the other composts analyzed. *Mycobacterium, a* common human pathogen, was found with a relatively high level in kitchen waste compost, municipal organic waste compost, cow dung manure, and vermicompost. *Mycobacterium* was also detected in the leaf compost but with very low counts, and it was completely absent in the neem cake compost (Fig. 7).

The resolution of the metagenomic analysis through 16S rRNA profiling at the species level could have been better**.** However, we could identify some beneficial and pathogenic bacterial species across all the compost samples**.** The leaf compost was high in bacterial species such as *Sphingobacterium zeae, Lactobacillus intestinalis, Sphingobacterium multivorum,* and *Prevetolla copri*. Their contents were higher in the sample DCRC20 than the other treatment samples like DRCTI10 and RDCTB10. The bacterial species such as *Kuenenia stuttgartiensis, Flavobacterium defluvii, Sorangium cellulosum,* and *Ignavibacterium album* were high in the leaf compost samples, DRCTI140D and DRCLC36W. The presence of various bacterial species in different composts was also detected. The bacterial species were present in varied proportions in different bio-composts. Cow dung manure (DRCCD) was high in bacterial species such as *Sphingobacterium multivorum, Sorangium cellulosum, Legionella pneumophila*, and *Bdellovibrio bacteriovorus*. Kitchen waste compost (DRCK) had a high content of *Nocardia vinacea, Methylocystis hirsuta, Prevetolla copri, Acholeplasma ladlawii*, and *Brevundimonas diminuta*. The municipal organic waste compost (DRCM) also had an increased range of *Nocardia vinacea* and *Acholeplasma Acholeplasma ladlawii,* along with other species such as *Bacillus thermoamylovorans, Bacillus rugosus*, and *Corynebacterium stationis*. Vermicompost (DRCV) was also seen high of *Cladosporium fulvum, Nocardia vinacea, Myconacterium arupense, Prevetolla copri*, and *Halomonas campisalis*. The neem cake compost (DRCNM) had a high content of different species, such as *Streptococcus mutans, Bifidobacterium animalis, Treponema denticola, Fusobacterium nucleatum*, and *Rothia mucilaginosa*.

The Proportion of Beneficial and Pathogenic Microbiome

The leaf compost has an aptly high proportion of beneficial genera both in the control and the experimental samples. The proportion of beneficial and pathogenic genera varied from sample to sample, depending on the nature of the compost. The leaf compost, DRCTI10, had the highest proportion of the beneficial genera, followed by cow dung manure (DRCCD). The leaf composts had a proportion of about 92% to 95% beneficial genera. The neem cake compost, DRCNM, had the lowest percentage of the beneficial genera at 13%. The kitchen waste compost (DRCK) and municipal organic waste compost (DRCM) also had a relatively lower proportion of the beneficial genera at 21% and 24%, respectively. The cow dung manure (DRCCD) and vermicompost (DRCV) had a relatively high proportion of beneficial genera at 54% and 37%, respectively.

Table 3 shows the percentage of beneficial and pathogenic microbes in different bio-composts. The leaf waste compost and cow dung manure had a high proportion of beneficial microbes with low pathogenic microbes.

The proportion of the pathogenic genera in the leaf compost DRCTI10 was low, about 3%. The municipal organic waste and cow dung manure had a comparatively similar fraction of the pathogenic genera with that of the leaf waste compost, at 3% and 4%, respectively. The neem cake compost, municipal organic waste compost, and kitchen waste compost had a relatively higher fraction of pathogens with respect to the leaf waste compost, at about 13%, 9 %, and 6%, respectively. The leaf compost and cow dung manure had the best ratio of beneficial to pathogenic genera at 34.61 and 14.97. The neem cake compost, kitchen waste compost,

Table 3: Percentage of beneficial and pathogenic microbes in leaf compost and other organic waste composts.

	DRC C20	DRC TI10	DRC TB10	DRC TI140D	DRC LC36W	DRC CD	DRCK	DRCM	DRCV	DRCNM
Total OTU of all the bacterial genera	84458	22084	38200	5044	4495	27653	15134	15071	6426	97541
Total OTU of beneficial genera	79761	20422	36167	3572	2540	15026	3169	3669	2392	12332
Percentage of beneficial genera	**94%**	**92%**	**95%**	**71%**	**57%**	**54%**	**21%**	**24%**	**37%**	**13%**
Total OTU of pathogenic genera	9664	590	5928	18	974	1004	855	450	568	12216
Percentage of pathogenic genera	**11%**	**3%**	**16%**	**0.35%**	**22%**	**4%**	**6%**	**3%**	**9%**	**13%**
Ratio of beneficial and pathogenic genera	8.25	34.61	6.10	198.44	2.61	14.97	3.71	8.15	4.21	1.01

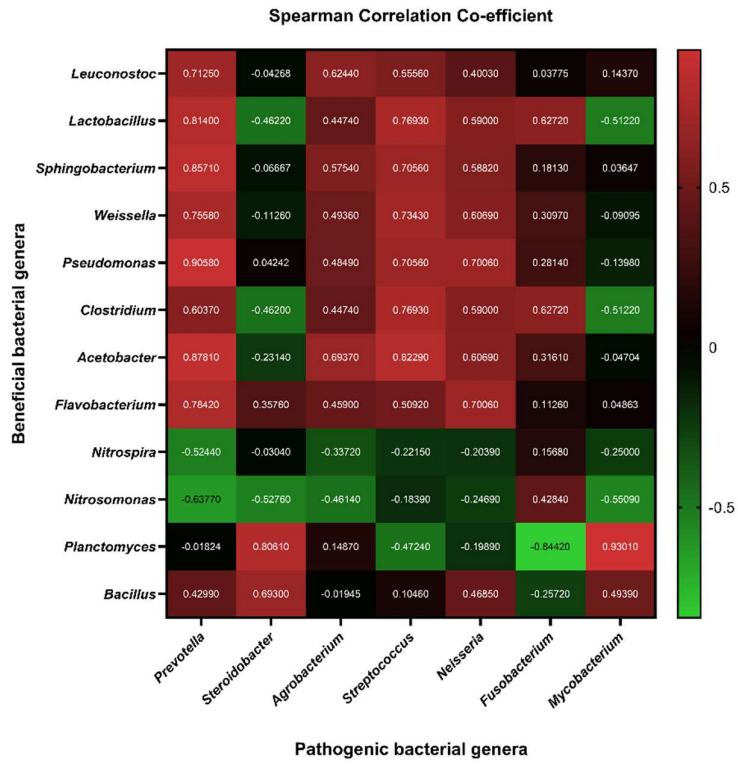

Fig. 8: The Spearman correlation coefficient between the beneficial and pathogenic bacterial genera detected in the composts.

vermicompost, and municipal organic waste compost had a lower ratio of beneficial to pathogenic genera than the leaf compost (Table 3).

The Spearman correlation coefficients revealed associations between various bacterial genera. Notably, Leuconostoc exhibited strong positive correlations with several genera, including *Lactobacillus, Sphingobacterium, Weissella, Pseudomonas, Clostridium, Acetobacter,* and *Flavobacterium*, while showing negative correlations with *Nitrospira* and *Nitrosomonas*. *Steroidobacter* demonstrated positive correlations with *Planctomyces* and *Bacillus* and negative correlations with multiple genera. *Agrobacterium* was positively correlated with numerous genera but negatively correlated with *Nitrospira* and *Nitrosomonas*. *Streptococcus* and *Neisseria* exhibited positive correlations with various genera and negative correlations with *Nitrospira* and *Nitrosomonas*. *Fusobacterium* displayed positive correlations with multiple genera and negative correlations with *Nitrospira* and *Nitrosomonas*. *Mycobacterium* was positively correlated with *Planctomyces, Bacillus,* and *Nitrosomonas*, with negative correlations observed with several other genera. These findings highlighted microbial communities' interconnectedness and potential ecological relationships, providing insights into co-occurrence patterns among different bacterial genera (Fig. 8).

Bacterial Microbiome Diversity of Leaf and Other Organic Waste Composts

This study was conducted to understand the potential of leaf and different organic waste composts as bio-organic fertilizers through the 16S rRNA profiling method. This method is one of the most adopted approaches to analyze the whole bacterial and archaeal communities present in environmental samples like compost since it is a low-cost method with high computational and analytical speed, having a curated and extensive reference database with well-established bioinformatic analytical pipelines (Callahan et al. 2016, Quast et al. 2013, Marcelino et al. 2020). The investigation into microbial richness and diversity within these diverse compost types yielded substantive insights into the intricacies of composting processes and the resultant quality of matured compost. Notably, the enumeration of reads and operational taxonomic units (OTUs) serves as proxy indicators for microbial biomass and diversity, respectively (Mysara et al. 2017). The observed variations in microbial diversity, as discerned through alpha diversity indices (Chao 1, Shannon, Fisher, and Simpson), underscore the profound influence of organic substrates on microbial communities during composting. This phenomenon finds corroboration in existing literature, elucidating the substantive role of substrate composition in shaping

microbial consortia during composting (Franke-Whittle et al. 2014, Matheri et al. 2023, Wang et al. 2022, Willis 2019). Moreover, the inferred positive correlation between microbial biomass, as reflected by read counts, and compost quality aligns with precedent studies, substantiating the pivotal role of microbial activity in the composting process (Tiquia 2005). The alpha diversity indices, encapsulating richness and evenness, signify implications for compost quality and maturity.

Furthermore, the beta diversity analysis, conducted at the taxonomic genus level using the Bray-Curtis index distance method, affords insights into the unique microbial communities characterizing distinct compost types. The collective clustering of leaf litter composts and the discernible dispersion of other types of organic waste composts underline the divergence in microbial diversity and highlight the potential avenues for optimizing composting processes. Integrating these outcomes with other studies that elucidated the augmenting influence of diverse organic materials on microbial diversity and composting acceleration unveils prospective strategies for refining compost production practices (Chandna et al. 2013, Wang et al. 2023). The refined understanding of microbial dynamics within various compost types offers a scientific basis for adapting feedstock selection and management protocols, thereby contributing to the realization of targeted objectives of compost quality production. However, it is imperative to acknowledge the study's limitations and advocate for future research endeavors exploring the relationship between compost microbial diversity and specific indicators of plant growth and soil health. In consolidation, this investigation advances our scientific understanding of composting intricacies and their implications, laying a basis for refined practices in sustainable compost production.

Bacterial Microorganisms in Composts Could Have Potential Benefits for Soil Health and Plant Growth

Different organic-waste composts were seen to be enriched with various beneficial microbes that could play important roles in soil fertility and plant growth. Beneficial phyla identified in different composts include *Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria, Euryarchaeota,* and others. *Firmicutes* are known to be probiotics, which can degrade carbohydrates. It has also been reported that they could facilitate the bioremediation of contaminated soil (Gupta et al. 2018, Li et al. 2020, Wieczorek et al. 2019). Their presence was detected in all the composts analyzed, with relatively high in the leaf compost and neem cake compost. *Proteobacteria* is another phyla of interest present in all the composts studied with relatively high amounts since they have the potential for nitrogen fixation. However, some species can cause opportunistic infection in humans (Delmont et al. 2018). *Cyanobacteria* is a photosynthetic bacterium with the potential to fix nitrogen and promote plant growth (Rai et al. 2018, Singh et al. 2016). *Cyanobacteria* were detected in all the analyzed composts, with relatively higher counts in kitchen waste and leaf compost, indicating their presence in foliage compost. *Actinobacteria* are decomposers that could play an important role in the nitrogen cycle and phosphate solubilization, promoting plant growth (Vargas Hoyos et al. 2021, Zhang et al. 2019). *Actinobacteria* were detected in all the composts analyzed. Their content was high in the cow dung manure and neem cake compost while relatively poorer in the leaf compost. *Euryarchaeota*, which is a methanogenic halophile and thermophilic decomposer (Castro-Fernandez et al. 2017, Horz & Conrads 2010), was also detected in higher concentration in the cow dung manure as compared to the leaf compost and other organic-waste composts analyzed. The other phyla that could benefit soil fertility and plant growth were also detected, viz. *Planctomycetes, Chloroflexi, Acidobacteria, Verrucomicrobia, Fibrobacteres, Chlorobi, Gemmatimonadota,* and *Nitrospirae*. However, such phyla were detected in the leaf compost with a relatively lower concentration than the other composts analyzed. The presence of such phyla can render beneficial potential to the bio-composts like organic matter decomposition, nitrification, and plant growth promotion.

The prominent beneficial bacterial genera identified in different composts were *Leuconostoc*, *Lactobacillus, Sphingobacterium, Coprococcus, Clostridium, Acetobacter, Gluconobacter,* and *Haloarcula*. These genera were detected at a relatively higher level in the leaf compost than in the other organic waste composts. Such genera could impart bio-fertilizing and bio-control potential to the compost since such genera are known to be plant growth promoters, nitrogen-fixing, nutrients solubilizing, toxins degrading, salt tolerant, anti-oxidant, and anti-microbial (Horz & Conrads 2010, Mohamed & Abd-el salam 2021, Tóth et al. 2021, Vaishnav et al. 2020).

Biological pest control agents such as *Pseudomonas* and *Bacillus* were relatively higher in the neem cake compost than in the leaf compost and other composts. Methane-producing bacteria, *Methanosarcina*, was identified in cow dung manure with much abundance compared to others, and their presence was almost absent in the leaf compost. *Planctomyces*, an anammox metabolizing bacteria, were detected relatively higher in abundance in cow dung manure, municipal organic waste compost, kitchen waste compost, and vermicompost but were almost absent in the leaf and neem cake compost. Another nitrogen-fixing bacteria, *Rhodoplanes*, was identified in higher concentrations in

cow dung manure and vermicompost. While their content was almost negligible in the leaf compost and kitchen-waste compost.

At the species level, the resolution of 16s rRNA profiling of bacterial microbiome becomes very ambiguous since numerous OTUs could not be classified. However, the presence of some beneficial and pathogenic bacterial species was detected. The beneficial species detected across samples include *Sphingobacterium zeae, Prevetolla copri,* Pseudomonas *geniculata, Faecalibacterium prausnitzii, Stenotrophomonas acidaminiphila, Bdellovibrio bacteriovorus, Pseudomonas stutzeri, Flavobacterium defluvii, Lactobacillus intestinalis*, and *Blastococcus endophyticus*. These species can render beneficial characteristics to soil fertility and plant health since they can solubilize nutrients, fix nitrogen in the soil, promote plant growth, and bioremediate toxins (Gopalakrishnan et al. 2015, Lalucat et al. 2006, Yeoh et al. 2022).

Bacterial Microorganisms in the Composts Could Pose Potential Risks to Soil Health and Plant Growth

The presence of some pathogenic species was detected in low amounts in all the composts analyzed, with the lowest counts in the leaf compost. Some pathogenic phyla identified were *Spirochaetes, Fusobacteria,* and *Chlamydiae*. *Spirochaetes*, which is a human pathogen, was detected relatively higher in cow dung manure, kitchen waste compost, and municipal organic waste compost. *Fusobacteria*, which is also a human pathogen, was detected relatively higher in the neem cake compost and the leaf compost that was composted long with neem and castor leaves. Another human pathogenic species, *Chlamydiae*, was detected with a relatively higher content in cow dung manure, vermicompost, and kitchen waste compost than leaf compost.

Some pathogenic genera were also detected with dissimilar proportions in different composts analyzed, although their presence was very low compared to the beneficial genera. The pathogenic genera identified across the samples include *Prevotella, Agrobacterium, Streptococcus, Neisseria, Fusobacterium, and Mycobacterium*. *Prevotella,* a human pathogen (Tett et al. 2021), was detected relatively high in the neem cake compost. Its presence was also comparatively high in the leaf and kitchen waste compost with respect to vermicompost, municipal organic waste compost, and cow dung manure. *Agrobacterium*, a common plant pathogen, was detected in trace amounts in the leaf compost, kitchen waste compost, and vermicompost. However, its presence could not be detected in other bio-compost analyses. *Streptococcus*, a human pathogen, and *Neisseria*, a human pathogen and denitrifying bacteria, were detected with relatively higher abundance in the neem cake compost. Their presence was almost absent in the other composts analyzed. *Mycobacterium*, a potential human pathogenic bacterial genus, was identified in the kitchen waste compost, municipal organic waste compost, cow dung manure, and vermicompost with higher abundance than the leaf compost. *Steroiderbacter*, a denitrifying bacteria that can cause loss of nutrients in the soil (Fahrbach et al. 2008), was another non-beneficial genus detected with higher abundance in the cow dung manure, vermicompost, and kitchen waste compost.

Non-beneficial and pathogenic bacterial species identified across the samples were *Enterococcus faecium, Clostridioides difficile, Legionella pneumophila, Halomonas campisalis, Mycobacterium arupense,* and *Treponema denticola*. Many of them are pathogenic to humans, whereas some species, such as *Halomonas campisalis,* can cause de-nitrification in the soil (Elsayed & Zhan 2004, Zhou et al. 2020). However, the level of such species was relatively low compared to the beneficial species identified across the bio-composts.

The Bio-Fertilizing Potential of Leaf and Other Organic Waste Composts

The ratio of the beneficial genera to the pathogenic genera and total microbiome can give some insights into the bio-fertilizing potential of the compost. The results revealed that leaf compost has a high proportion of beneficial genera, making it a potentially valuable bio-fertilizer. The high percentage of beneficial genera, ranging from 92% to 95%, indicates that the leaf compost is rich in microorganisms that could contribute to the enhancement of soil fertility and plant growth (Ho et al. 2022, Ventorino et al. 2019). The relatively low proportion of pathogenic genera in the leaf compost, around 3%, is promising for its bio-fertilizing potential. A low presence of pathogenic microorganisms suggests that the compost is less likely to cause harm to plants or the soil ecosystem. This is crucial for bio-fertilizers, as they should ideally promote plant health without introducing harmful pathogens (Ahmed et al. 2023, Daniel et al. 2022). Comparatively, cow dung manure also demonstrated a high proportion of beneficial genera at 54%, making it another promising source of bio-fertilizer. The low percentage of pathogenic genera (4%) in cow dung manure further supports its potential as a beneficial soil amendment (Tagele et al. 2023).

On the other hand, neem cake compost exhibited a low percentage of beneficial genera (13%) and a relatively higher fraction of pathogenic genera (13%). This suggests that while neem cake compost may still have some bio-fertilizing potential, its effectiveness might be limited compared to

leaf compost or cow dung manure. Neem cake is known for its pesticidal properties, and its use in composting may impact the microbial composition (Del Serrone & Nicolett 2013). Kitchen waste compost and municipal organic waste compost showed lower proportions of beneficial genera (21% and 24%, respectively) and a higher fraction of pathogenic genera (6% and 9%, respectively). These composts may still contribute to soil fertility, but their bio-fertilizing potential might be somewhat compromised due to the higher presence of potentially harmful microorganisms unless well-segregated organic sources are used in composting. Vermicompost had a moderate proportion of beneficial genera (37%) and a relatively low percentage of pathogenic genera, suggesting it could be a reasonably effective bio-fertilizer, although not as high as leaf compost or cow dung manure.

In general, the leaf compost, especially DRCTI10 and cow dung manure, appear to be promising bio-fertilizers due to their high proportion of beneficial genera and low presence of pathogens. These findings emphasize the importance of understanding the microbial composition of composts to optimize their bio-fertilizing potential and promote sustainable agriculture practices (Ahmed et al. 2023, Kumar et al. 2022). The leaf litter compost was also reported to have high nutrients and low potentially toxic elements (Mahongnao et al. 2023). Thus, with these results that leaf compost has a high nutrient level and a high proportion of beneficial microorganisms with low pathogenic microorganisms and potentially toxic elements, we recommend that leaf litter compost could be suitably used as bio-organic fertilizer for sustainable agricultural productivity.

CONCLUSION

We can conclude that leaf-based composts and other types of composts produced from different organic wastes have varied microbiome richness and diversity. Various beneficial microorganisms viz. *Lactobacillus, Leuconostoc, Sphingobacterium, Paenibacillus, Pseudomonas,* and *Clostridium* were present in divergent proportions in the leaf compost and other organic waste composts. All the composts analyzed have a high proportion of beneficial and low pathogenic organisms. The best proportion of the beneficial to the pathogenic genera was found in the leaf litter compost. Thus, it could be used as a valuable bio-organic fertilizer in agricultural systems for promoting soil health and plant growth.

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ORCID DETAILS OF THE AUTHORS

Sophayo Mahongnao: https://orcid.org/0000-0001-7222-5987
Sarita Nanda: https://orcid.org/0000-0003-3684-606X