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Evaluation of the Bacterial Diversity of Composts and Vermicomposts Fed with Animal Waste. Data Analysis Based on Illumina High-Throughput Sequencing
Evaluación de la Diversidad Bacteriana de Compostas y Vermicompostas Alimentadas con Desechos Animales. Análisis de Datos Basado en la Secuenciación de Alto Rendimiento de Illumina

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SUMMARY

Animal organic wastes are commonly applied to soil to enhance fertility. Still, before application, they are often composted or vermicomposted to produce a more stabilized, nutrient-rich product with a broad beneficial bacterial diversity. This study was aimed to investigate how composting and vermicomposting influenced the physicochemical properties and bacterial community structure of various organic wastes. Cow manure, goat and rabbit feces, and pig slurry were subjected to either composting or vermicomposting using *Eisenia fetida* for 90 days, during which their physicochemical characteristics and bacterial communities were monitored. Both composting and vermicomposting had similar effects on the physicochemical properties of the animal wastes: pH and total nitrogen (N) content increased. In contrast, electrolytic conductivity and organic carbon (C) content decreased. Although both processes altered the bacterial community structure across all operational taxonomic units (OTUs), significant differences remained between the bacterial communities of the different organic wastes. Vermicomposting also reduced the prevalence of bacterial genera with pathogenic potential, such as *Enterobacterand* and *Acinetobacter*. The introduction of earthworms into organic waste had a similar effect to composting on the structure of the bacterial community.

Index words: *animal feces, Eisenia fetida, miseq, metagenomic study.*

RESUMEN

Los desechos orgánicos animales se aplican comúnmente al suelo para mejorar la fertilidad, pero antes de la aplicación, a menudo se compostan o se vermicompostan para producir un producto más estabilizado, rico en nutrientes y con una amplia diversidad bacteriana beneficiosa. Este estudio tuvo como objetivo investigar cómo el compostaje y el vermicompostaje influyeron en las propiedades fisicoquímicas y la estructura de la comunidad bacteriana de varios desechos orgánicos. El estiércol de vaca, cabra, conejo y cerdo se sometieron a compostaje o vermicompostaje utilizando *Eisenia fétida* durante un período de 90 días, durante el cual se monitorearon sus características fisicoquímicas y comunidades bacterianas. Tanto el compostaje como el vermicompostaje tuvieron efectos similares en las propiedades químicas de los desechos animales: el pH y el contenido de nitrógeno total (N) aumentaron,

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mientras que la conductividad electrolítica y el contenido de carbono orgánico (C) disminuyeron. Aunque ambos procesos alteraron la estructura de la comunidad bacteriana en todas las unidades taxonómicas operativas (UTO), se mantuvieron diferencias significativas entre las comunidades bacterianas de los diferentes desechos orgánicos. El vermicompostaje también redujo la prevalencia de géneros bacterianos con potencial patógeno, como *Enterobacter* y *Acinetobacter*. La inserción de lombrices a los desechos orgánicos tuvo un efecto similar al de compostaje sobre la estructura de la comunidad bacteriana.

Palabras clave: *excretas animales, Eisenia fetida, miseq, estudio metagenómico.*

INTRODUCTION

The demand for environmentally sustainable practices has driven interest in the use of high-value, eco-friendly biofertilizers (Enebe and Erasmus, 2023). Technologies like vermicomposting play a crucial role in the production of these biofertilizers. Vermicomposting is a biotechnological process that leverages various organic wastes to not only disinfect them by reducing the pathogenic microbial load but also to transform them into nutrient-rich biofertilizers (Swati and Hait, 2018). Among the most valuable biofertilizers are those derived from organic animal waste. In vermicomposting, the decomposition process is initiated by microorganisms present in the organic waste, followed by the active ingestion of worms, which help aerate and distribute moisture throughout the decomposing material (Santana, Seminoti, Antoniolli, Martínez, and Domínguez, 2020). During this process, microbial diversity within the substrate can be altered by interactions within the earthworm's intestine (Aira, Pérez, and Dominguez, 2019; Gómez-Brandón, Aira, Lores, and Dominguez, 2011). This is largely due to direct effects such as microbial inhibition caused by enzymatic activity and the secretion of coelomic fluids with antibacterial properties inside the earthworm's gut (Swati and Hait, 2018). Additionally, digestive enzymes like proteases secreted by earthworms can contribute to pathogen reduction during vermicomposting (Ordoñez-Arévalo, Guillén, Huerta, Cuevas, and Calixto, 2018). Several variables, including the raw material, pH, temperature, humidity, aeration, and type of worms, influence the efficiency of these processes. Microbial communities are essential for the decomposition of organic matter, with bacteria, actinomycetes, and fungi playing key roles (Wang *et al.,* 2018; Liu *et al.,* 2020). The composition of these microbial communities largely depends on the initial substrate.

The quality of nutrients in the initial material significantly influences microbial succession, which is characterized by the gradual replacement of certain bacterial groups by others (Aira, Pérez and Dominguez, 2019). Due to the advantages that this process offers, such as increased organic carbon, water retention, porosity, soil aeration, high nutrient content (N, P, K), desirable physicochemical characteristics, and buffer actions (Thirunavukkarasu *et al*., 2023), vermicompost improves plant nutrient absorption and plant growth (Haque and Biswas, 2021; Liu *et al.,* 2020; Wang *et al.,* 2018). Therefore, many studies have demonstrated that vermicompost and its byproducts are superior to compost in enhancing soil quality (Fornes, Mendoza-Hernández, García-de la Fuente, Abad, and Belda, 2012; Romero-Tepal *et al.,* 2014; Lim, Wu, Lim, and Shak, 2014; Serrano-Ramirez, Hernandez, Ruiz, Ruíz, and Montes, 2021).

In this study, cow manure, pig slurry, and rabbit and goat feces were subjected to composting or vermicomposting for 90 days. The study evaluated changes in the characteristics of these organic wastes, as well as earthworm survival. Metabarcoding and high-throughput sequencing techniques were employed to analyze the bacterial community structure within the four animal wastes and to observe how composting and vermicomposting with *Eisenia fetida* impacted it after 90 days (Srivastava, Squartini, Masi, Sarkar, and Pratap, 2021). Furthermore, bacterial groups associated with human pathogenicity were identified through the bacterial community's putative functional profile using the annotation of prokaryotic taxa (Louca, Parfrey and Doebeli, 2016) and next-generation sequencing for determining the diversity of bacterial communities.

MATERIALS AND METHODS

Vermicomposting and Experimental Design

The feces from cows, pigs, goats, and rabbits, as well as *Eisenia fetida* earthworms, were obtained from a local farm, "Rancho Luanda," in Ocozocoautla (16° 45′ 36.7″ N, 93° 22′ 32.1″ W), Chiapas, Mexico. The animal wastes were dried in the dark at ambient temperatures. Soil was collected from an experimental field at the "Instituto Tecnológico de Tuxtla Gutiérrez". The sandy loam soil had a pH of 8.4, an electrolytic conductivity (EC)

of 0.17 dS m^{-1}, an organic carbon content of 6.1 g kg^{-1}, and a total nitrogen content of 0.9 g kg^{-1}. Sugar cane bagasse was obtained from a local producer. For each type of animal waste, three samples (n=3) were collected, resulting in a total of 12 waste samples. Each type of animal waste was mixed with soil and sugar cane bagasse in a ratio of 30:30:40%. This combination, previously found to produce an optimal final product in experiments and practical applications at "Rancho Luanda," was used in the vermicomposting process. Sugarcane bagasse, a byproduct of substantial sugarcane production in Chiapas, is easily vermicomposted and contributes valuable nutrients (Vellaikkannu, Selvakumar, Mohan, and Chathlingathe, 2018). Incorporating it into composting and vermicomposting processes helps reduce organic waste and adds a readily decomposable substrate (Aguilar-Rivera, Rodríguez, Enríquez, and Herrera, 2012). Additionally, the application of sugarcane increases the C–N ratio of the organic waste, stimulating microbial mineral N immobilization (Chowdhury, Neergaard and Jensen, 2014). This limits the possible loss of a valuable nutrient through NH_3 volatilization and NO^{3-} leaching and decreases the emissions of nitrous oxide during the vermicomposting process. Soil was added to animal wastes to increase the microbial diversity in the organic wastes during vermicomposting (Singh, Khare, Bhargava, and Bhattacharya, 2005) Although the animal wastes were mixed with sugar cane and soil, they will be referred to as animal wastes, i.e., cow and goat feces, pig slurry, and cow manure, in the text.

After thoroughly mixing each of the 12 animal waste samples with sugarcane and soil, a sub-sample from each mixture was extracted for DNA analysis to determine the bacterial community at the onset of the experiment. Twenty-four polyvinylchloride (PVC) boxes (40 cm long, 30 cm wide, and 20 cm high) were filled with 400 g of each sample (n=3) of the four animal wastes (n=4) and amended with 50 ml distilled water. Twelve boxes were amended with 10 earthworms for vermicomposting, and the other 12 boxes were left unamended and used for composting. As such, the 24 PVC boxes included four animal wastes (soil + bagasse + cow manure, pig slurry, goat or rabbit feces) and two treatments (composting or vermicomposting) in triplicate (n=3). The boxes were fitted with an outlet so that leachate formed, drained freely, and anaerobic conditions were prevented. The boxes were covered with meshed cloth to prevent insects from entering the boxes. Every day, 50 ml of distilled water was added to maintain the animal wastes at 40-60% moisture and earthworm activity.

Physicochemical Analysis

A waste sample was mixed with distilled water in a 1:2.5 ratio, and pH was determined with a potentiometer Mettler Toledo® Model S220 (New York, USA), while a ratio of 1:5 was used to determine electrolytic conductivity (EC) with a conductimeter Mettler Toledo® Model S220 (New York, USA). Total nitrogen and carbon were determined with a Thermo Scientific™ FlashSmart™ Elemental Analyzer (Waltham, Massachusetts, USA) following the standard protocol given by the manufacturer (Ruíz-Valdiviezo *et al.,* 2010).

DNA Extraction and 16S rRNA Gene Amplification

Metagenomic DNA was extracted using three different cellular lysis methods: i) a chemical and thermal method (Valenzuela-Encinas *et al.,* 2008), ii) an enzyme-based method with lysozyme (Sambrook and Russell, 2006), and iii) a detergent solution combined with mechanical disruption (Hoffman and Winston, 1987). Each method was applied to a 1 g subsample of waste, and the resulting DNA was pooled. Consequently, DNA was extracted from a total of 3 g of each waste type at the beginning of the experiment and after 90 days of composting or vermicomposting. In total, 36 metagenomic DNA samples were collected: four types of organic wastes in triplicate at the start and four types of composted or vermicomposted products in triplicate after 90 days. Triplicate PCR reactions (12.5 µl each) were performed to amplify the hypervariable V3-V4 regions of the 16S rRNA gene from each metagenomic DNA sample.

PCR amplifications were performed using 8-bp barcoded primers: 341-F (5'–CTACGGGIGGCWGCAG–3') and 805-R (5'–GACTACHVGGGTATCTAATCC–3') (Herlemann *et al.,* 2011). The procedure followed a two-step PCR protocol as described in the Illumina Inc. 16S metagenomic sequencing library preparation guide (15044223 Rev. B) (Navarro-Noya *et al*., 2013). The pooled PCR products were purified using FastGene™ columns (Nippon Genetics Co., Ltd., Tokyo, Japan). Amplicon quantification was conducted with a Nanodrop™ 3300 (Thermo Fisher Scientific Inc., Suwanee, CA, USA), and the samples were standardized to equal concentrations for sequencing. Sequencing was performed by Macrogen Inc. (DNA Sequencing Service, Seoul, Korea) using the Illumina® MiSeq 2×300 paired-end platform. The raw sequence datasets have been submitted to the Sequence Read Archive on the NCBI website, under BioProject accession number PRJNA547674.

Bioinformatic Analysis

The raw sequences were analyzed using the QIIME pipeline v.1.9.1 (Caporaso *et al*., 2010). Quality control of the raw reads was examined with FastQC software (Andrews, 2019). Paired-end reads were merged using the fastq join method (Aronesty, 2013) with the join_paired_ends.py QIIME's script with a 100 nt minimum overlap region (-j 100), and then demultiplexed based on the unique-by-sample 8-pb barcodes employed during the gene amplification.

Chimera checking and filtering were done with VSEARCH v2.8 (Rognes, Flouri, Nichols, Quince, and Mahé, 2016). The high quality, chimeras free, and paired end joined sequences were clustered into operational taxonomic units (OTUs) at the 97% identity level with the USEARCH61 algorithm (Edgar, 2010). Taxonomic assignment was based on aligning the most abundant OTU (representative OTU) with the Greengenes 13_8 database using PyNAST (McDonald *et al.,* 2012). Within-sample diversity indices, including Chao1 richness, Shannon, Simpson, and phylogenetic diversity (PD whole tree), were calculated using 1000 randomly selected reads per sample with the alpha_diversity.py script from the QIIME pipeline.

To assess differences and shifts in bacterial diversity among the vermicomposted wastes, overall beta diversity and its components (turnover and nestedness) were calculated. Beta diversity was determined using Jaccard-based dissimilarity with the betapart v1.5 R package (Baselga and Orme, 2012). Bacterial groups shared among treatments with an occupancy of 80% (the percentage of samples that shared given taxa) were considered part of the core microbiome, as identified using the psvenn function from the "MicEco" package v0.9 in R (Russel, 2021). Bacterial groups with potential human pathogenic activity were identified by converting the community profile into a putative functional profile using FAPROTAX v1.2 (Louca, Parfrey, and Doebeli, 2016). The relative abundance of functional modules within FAPROTAX was calculated using the total sum scaling procedure on a per-sample basis.

Statistical Analysis

All statistical analyses were done in R version 4.0.2. (R Core Team, 2020). An ANOVA test (stats: aov function) was used to determine the effect of composting and vermicomposting on the characteristics of the animal wastes. Bar plots of the relative abundances of the bacterial groups were constructed with the phyloseq (v1.40) and the ggplot2 (v2.3) packages (McMurdie and Holmes, 2013; Wickham, 2016). Ordination (principal component analysis (PCA)), multivariate comparison (perMANOVA), and differential abundance (ALDEx2) were done with converted sequence data using the centered log-ratio transform test returned by the aldex.clr argument (ALDEx2 package, version 1.21.1., Gloor, Macklaim, Pawlowsky, and Egozcue, 2017). The PCA was done with the vegan package version 2.5-6 (Oksanen *et al*., 2013). The effect of composting and vermicomposting compared to the initial substrate on the bacterial groups was done using a compositional approach, analysis of differential abundance taking sample variation into account (aldex.kw argument, ALDEx2 package, version 1.21.1., Gloor *et al.,* 2020). The effect of composting and vermicomposting compared to the initial substrate on the bacterial community structure was determined with a per-MANOVA analysis using the adonis2 argument, vegan package, version 2.5-6 (Oksanen *et al*., 2013). Correlations between bacterial groups and soil animal wastes characteristics were determined with the propr argument (propr(counts, metric = c("rho"))) of the propr package in R version 4.2.6 (Quinn *et al.,* 2019). The effect of composting or vermicomposting on the bacterial groups was determined separately in the cow manure, pig slurry, and rabbit and goat feces soil.

The ratio was calculated as: Ratio = (relative abundance of the bacterial group after composting or vermicomposting for 90 days - relative abundance of the bacterial group in the animal waste at the onset of the experiment)/(relative abundance of the bacterial group in the animal waste at the onset of the experiment).

RESULTS AND DISCUSSION

Composting and vermicomposting significantly affected the chemical characteristics of the animal wastes. At the onset of the experiment, the pH values of the four types of animal wastes ranged from 6.9 to 7.3, with no significant differences observed between them (Table 1). Both composting and vermicomposting led to a significant increase in pH compared to the initial substrates ($P < 0.001$). Singh, Khare, Bhargava and Bhattacharya (2005) monitored the pH during the composting and vermicomposting of plant residues with an initial pH ranging from 4.3 to 6.9 by adding different proportions of mango waste. This increased in all treated substrates to a pH of 8.2 and 8.3, neutralizing after 35 days. In this study, the pH of the animal wastes increased to a maximum of 8.3 after 90 days. At the onset of the experiment, the EC of cow manure and pig slurry was significantly higher than that of the rabbit feces but lower than that of the goat feces ($P < 0.001$). Composting and vermicomposting decreased significantly the EC ≥ 3.5 times ($P < 0.001$) compared to the initial substrate.

Table 1. Characteristics of the different substrates at the beginning of the experiment and after 90 days of composting or vermicomposting.

	pH					Electrolytic conductivity				
						dS m^{-1}				
Substrate	Initial	Compost	Vermicompost	F value	P value	Initial	Compost	Vermicompost	F value	P value
Cow manure	7.2 a B	8.3 a A	8.1 a A	14.2	< 0.001	3.4 b A	0.7 ab B	0.3 b C	55	< 0.001
Goat faeces	6.9 a B	8.1 a A	8.0 a A	11.8	< 0.001	4.4 a A	0.9 a B	0.8 a B	344	< 0.001
Pig slurry	7.3 a B	8.2 a A	8.2 a A	14.3	< 0.001	3.1 bc A	0.4 b B	0.6 ab B	20	< 0.001
Rabbit faeces	7.0 a B	8.0 a A	8.3 a A	53.7	< 0.001	2.8 c A	0.8 ab B	0.8 a B	240	< 0.001
F value	0.58	3.07	3.07	-	-	48.32	6.21	5.56	-	-
P value	0.647	0.091	0.091	-	-	< 0.001	0.018	0.023	-	-
	Organic carbon content					Total nitrogen content				
	g kg^{-1}									
Cow manure	310 b A	172 a B	159 c C	37541	< 0.001	7.7 a B	7.5 b B	9.8 a A	4459	< 0.001
Goat faeces	301 b A	129 c C	165 b B	44499	< 0.001	5.0 c C	5.8 d B	8.4 c A	2147	< 0.001
Pig slurry	373 a A	173 a B	169 a B	240	< 0.001	5.6 b B	7.2 c B	9.1 b A	1436	< 0.001
Rabbit faeces	308 b A	156 b B	157 d B	-	< 0.001	4.8 d C	8.2 a C	9.2 b A	14599	< 0.001
F value	22770	2511	194	-	-	3219	1247	169	-	-
P value	< 0.001	< 0.001	< 0.001	-	-	< 0.001	< 0.001	< 0.001	-	-

a = mean of three replicates; b = values with the same letter are not significantly different between the substrate (within the column) ($P < 0.05$), c = values with the same capital letter are not significantly different between the treatment applied to the different substrates (within the rows) ($P < 0.05$).

The organic C content in the pig slurry was significantly higher than in the other substrates at the onset of the experiment ($P < 0.001$) (Table 1). Composting and vermicomposting significantly decreased the organic C content by ≥ 55% (mean of the four wastes) compared to the initial substrate. The C content between composting and vermicomposting was not similar in cow manure and goat feces. The organic C content was greater in the composted than in the vermicomposted waste (cow manure), lower (goat feces), or similar (rabbit feces and pig slurry) ($P < 0.05$). The total N content of the animal wastes was significantly different at the onset of the experiment ($P < 0.001$) (Table 1). Vermicomposting significantly increased the total N content compared to the initial substrate ($P < 0.05$). Quintero-Lizaola (2014) evaluating the microbial populations, enzyme activity and humic substances (HS) during composting and vermicomposting (*Eisenia andrei* Bouché) concluded that, the incorporation of *Eisenia andrei* (Bouché) had an influence on the measured variables that were, amylase, cellulase, lipase, invertase, protease, amidase, urease, nitrogenase, acid and alkaline phosphatase, arylsulfatase and dehydrogenase, as well as proteolytic, ammonifying, nitrifying, cellulolytic, amylolytic, lipolytic microorganisms. The results shows that mineralization in the vermicomposting process is accelerated with respect to compost, which could be reflected in a greater availability of nutrients for crops biofertilized with vermicompost. Mineralization of organic material releases various ions, such as Ca^{+2}, K^{+}, Mg^{+2}, or PO_4^{-3}, so that the EC of the compost or vermicompost increases (Pattnaik and Reddy, 2011; Ramnarain, Ansari, and Ori, 2019). In this study, the liquid drained freely during the composting and vermicomposting, so the salts of the organic wastes were leached out, and the EC dropped from ≥ 2.8 dS m^{-1} to ≤ 0.9 deciSiemens por metro.

However, some plant nutrients, such as NO_3^-, and other minerals may have also been lost during the process. After 90 days, approximately 50% of the organic material in the wastes had decomposed, indicating that the conditions for composting and vermicomposting were optimal (Vellaikkannu, Selvakumar, Mohan and Chathlingathe, 2018). The presence of earthworms often accelerates the decomposition of organic material. By burying and feeding, earthworms enhance the contact between the organic material and heterotrophic microorganisms, thereby accelerating carbon mineralization.

In this study, the total nitrogen (N) content increased in the vermicomposted organic wastes, resulting in a decreased carbon-to-nitrogen (C) ratio after 90 days (Manna, Jha, Ghosh and Acharya, 2003). The total N content in the vermicomposted wastes nearly doubled per kilogram as CO_2 emissions and water loss through leaching reduced the overall weight. Although some inorganic nitrogen, such as NH_3, N_2O, and NO_3^-, might have been lost during vermicomposting, the high total N content suggests a nutrient rich final product (Biruntha *et al.,* 2020).

At the onset of the experiment, the number of bacterial phyla detected in the different animal wastes was similar: 24 in pig slurry, cow manure, and goat feces, and 26 in rabbit feces (Figure 1a). The most abundant bacterial phyla across all treatments were *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, with relative abundances of 44, 15, and 15%, respectively.

The dominant bacterial genus was *Pseudomonas* (5.6±3.2%), followed by *Acinetobacter* (2.5±2.4%) (Figure 1b). The relative abundance of most bacterial genera varied significantly between treatments.

The Principal Component Analysis (PCA) of all operational taxonomic units (OTUs) grouped the bacterial communities of cow manure and goat feces, distinguishing them from those of pig slurry and rabbit feces (10% and 9.5% on average, dimensions 1 and 2, respectively) (Figure 2). The bacterial community structure was highly significantly different among the four animal wastes when considering all OTUs and bacterial groups identified up to the genus level ($P < 0.001$). However, no significant differences were observed when considering the bacterial communities at the phylum level (Figure 2), among 14 phyla and 50 genera approximately found in the analysis.

Although the animal wastes contributed only 30% of the mixture that was vermicomposted, their bacterial community structure remained distinct. The bacterial community in the animal wastes was influenced by factors such as the bacterial gut biome, the characteristics of the digestive tract, and the composition of the feed and digestion processes. For instance, the relative abundance of the OTU belonging to *Paracoccus* (OTU-4165) was notably higher in rabbit feces compared to the feces of the other animals used in this study. Additionally, *Brachybacterium phylotypes*, previously identified in cow manure (Murayama *et al.*, 2010), were more prevalent in cow manure, with OTU-4848 from this genus showing higher relative abundance in cow manure compared to goat feces and pig slurry.

In this study, the relative abundance of OTUs belonging to Turicibacter (New Reference OTU323) and Dietzia (New CleanUp Reference OTU-6618) was higher in rabbit feces and cow manure compared to pig slurry. Although Dietzia was more abundant in rabbit feces than in other animal wastes, another OTU from this genus (New Reference OTU-5593) was more prevalent in pig manure and goat feces than in cow manure and rabbit feces. This suggests that different species within the same genus may have distinct metabolic pathways, which could account for variations in their relative abundance across different animal feces. Zhang, Tan, Deng, and Cao (2015) studied *Actinobacteria* in the feces of the white-tailed rabbit (*Sylvilagus audubonii*, Baird 1858) and identified members of *Brachybacterium* and *Dietzia*. Consistent with these findings, our study found that members of these genera were more abundant in rabbit feces compared to the feces of other animals.

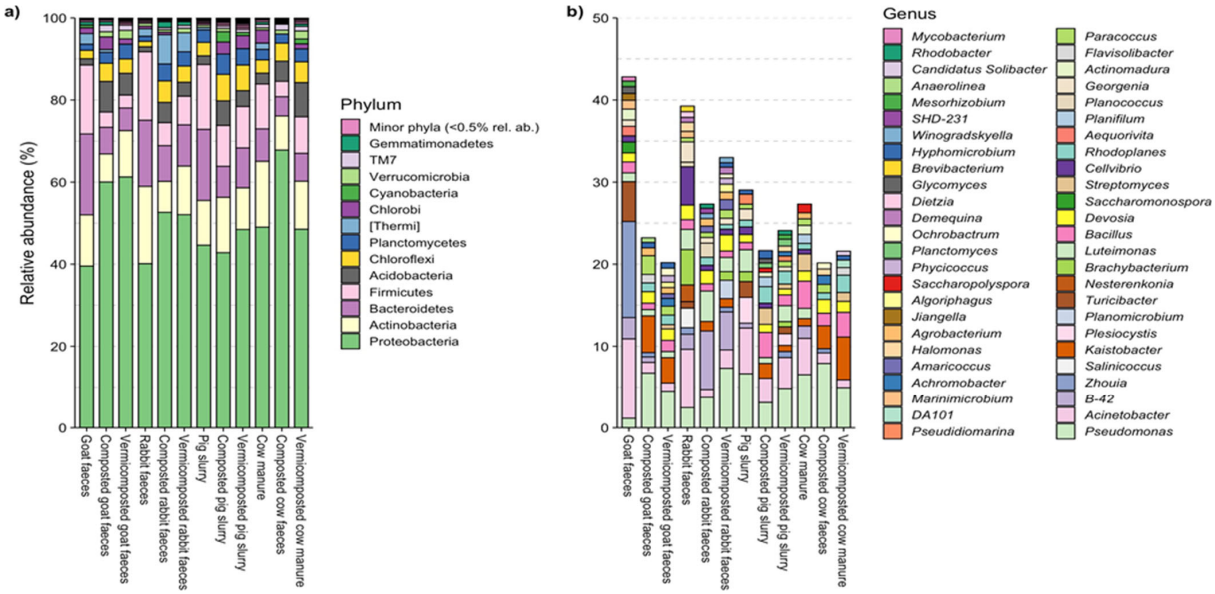

Figure 1. Barplot-graph with the relative abundance (%) of the a) bacterial phyla and b) the 50 most abundant genera across treatments.

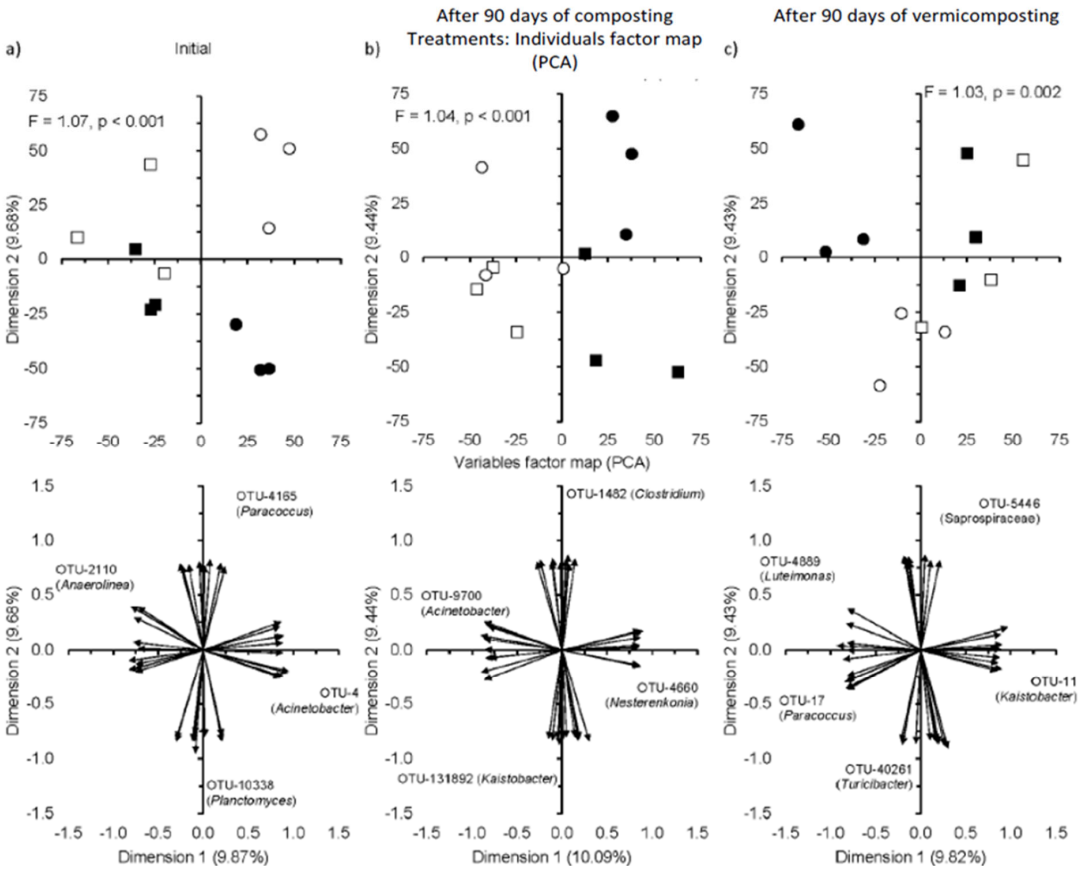

Figure 2. Principal component analysis (PCA) with all operational taxonomic units (OTUs) in the cow manure (□), goat feces (■), pig slurry (○) and rabbit feces (●) a) at the initial substrate, b) composted substrate, and c) vermicomposted substrate. F and p values were obtained after permutational multivariate analysis of variance (perMANOVA).

Several bacterial groups were present in all four types of animal waste, with the most abundant core bacteria (>1% relative abundance) belonging to the phyla *Proteobacteria*, including genera such as *Acinetobacter*, *Pseudomonas*, and *Cellvibrio*, as well as *Firmicutes*, including *Turicibacter* and *Bacillus* (Figure 3a). Unique bacterial genera were also identified in each type of waste (Figure 3b, c). The number of unique bacterial genera varied significantly between composted and vermicomposted animal wastes, as well as compared to the initial substrates (Figure 3). In vermicomposted substrates, the core microbiome primarily consisted of *Proteobacteria* (n=6 genera) and *Acidobacteria* (n=2), with *Pseudomonas* and *Bacillus* being the most abundant genera.

In this study, the core microbiome of vermicomposted animal wastes included members of *Proteobacteria* (*Paracoccus* and *Pseudomonas*) and *Firmicutes* (*Bacillus*). Both *Pseudomonas* and *Bacillus* are well known for their ability to degrade high molecular weight compounds and are found ubiquitously. The functional diversity of these bacteria could potentially work synergistically to enhance the quality of vermicomposted organic materials, resulting in a nutrient-rich product that can be effectively used for organic fertilization (Raza *et al.,* 2020).

During vermicomposting, members of *Acinetobacter, Enterobacter*, and *Enterobacteriaceae* were reduced, though the extent of this reduction varied depending on the type of animal waste used. Therefore, further investigation is needed to determine the time required to effectively decrease pathogen levels in different types of feces under the conditions used in this study. Both *Acinetobacter* and *Enterobacter* have been identified as multidrug-resistant phylotypes in animals (Zordan *et al.,* 2011), posing significant risks to human health (Delahoy *et al.,* 2018). Previous studies, such as Karimi, Mokhtari, Salehi, Sojoudi and Ebrahimi (2017), indicated that removing pathogenic bacteria during vermicomposting can take up to 90 days, depending on the waste type and specific vermicomposting conditions. Swati and Hait (2018) also reported that the effectiveness of vermicomposting depends on factors such as the species of earthworm used, the origin of the organic waste, and the pathogenic group in question. In this study, composting and vermicomposting significantly impacted specific bacterial groups (Figure 4).

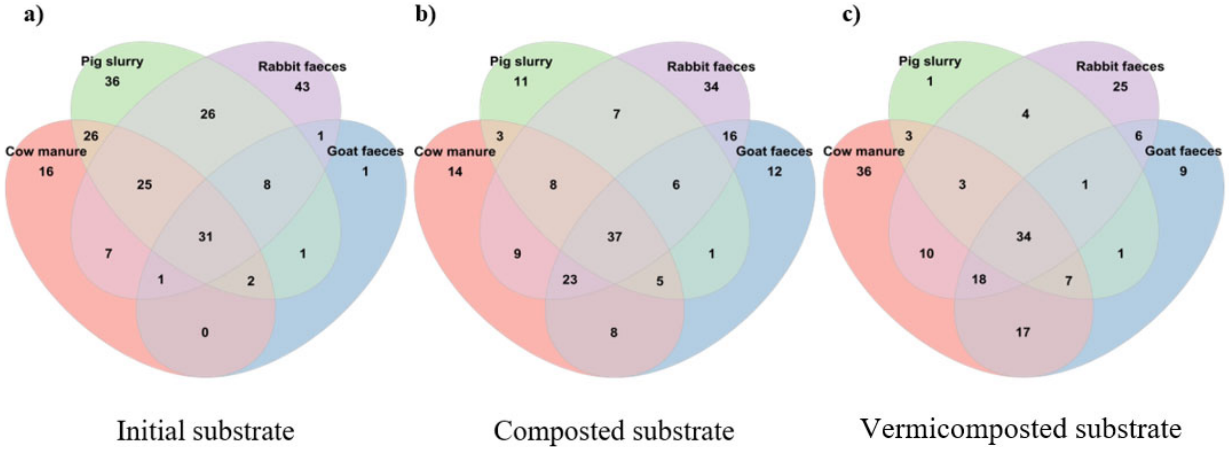

Figure 3. Venn diagram showing the core microbiome between a) initial animal substrates, b) composted animal substrates and c) vermicomposted animal substrates. Core microbiome was considered as the shared bacterial groups up to the genus taxonomic level and a prevalence of 80%.

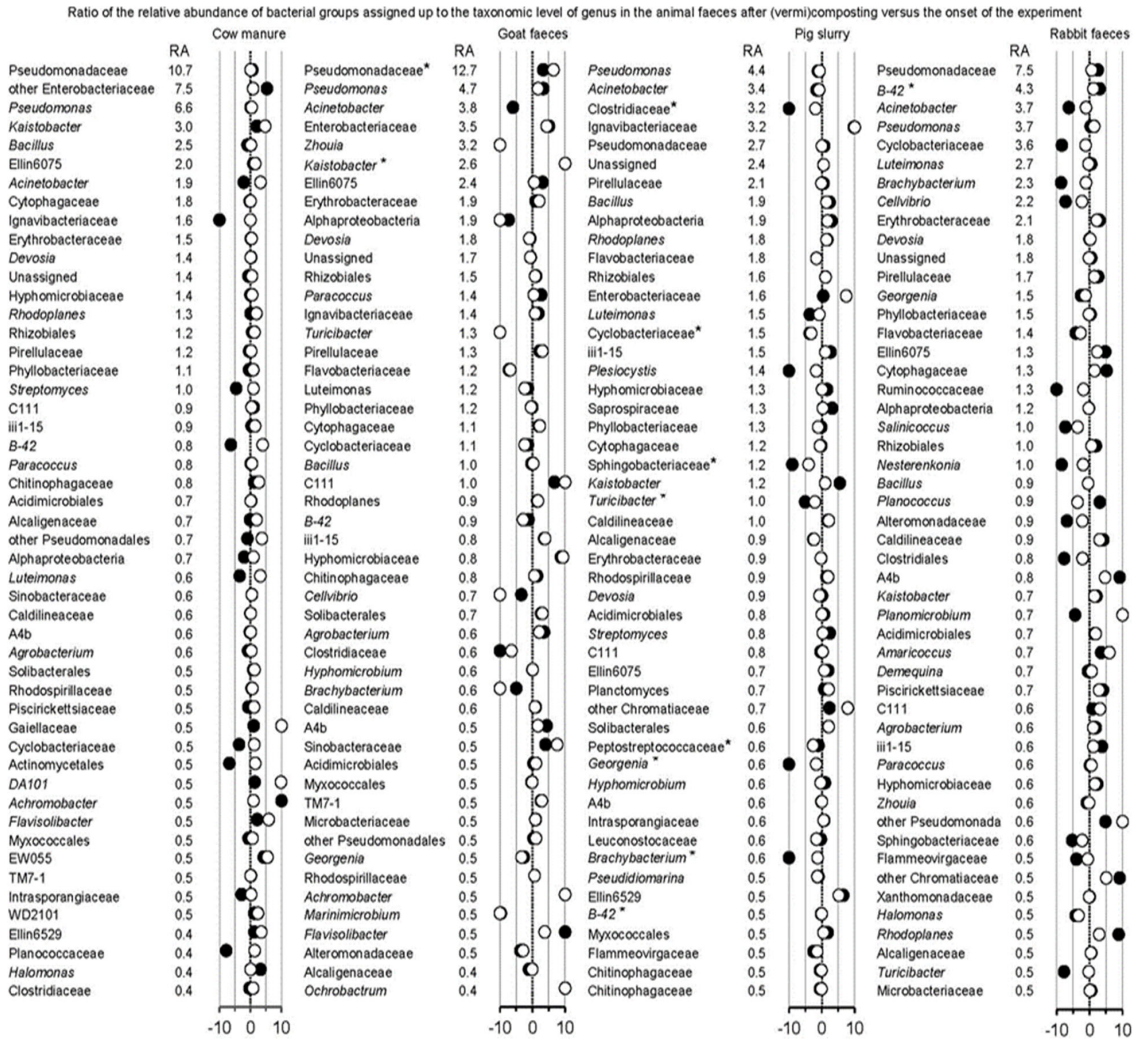

Figure 4. Ratio between the relative abundance of the bacterial groups after composting (○) vermicomposting (●) versus the initial substrate. * Bacterial groups that were significantly affected by composting and vermicomposting of the organic waste ($P < 0.05$).

The PCA separated the bacterial community in the composted from that in the vermicomposted animal wastes most clearly when considering all bacterial groups assigned to the taxonomic level of genus or OTUs, but less so when considering higher taxonomic levels (Figure 5). Composting or vermicomposting had a significant effect on the bacterial community structure of the animal wastes considering all OTUs, but not always when considering the higher taxonomic levels of the genus or phylum (Figure 5). Vermicomposting decreased the relative abundance of bacterial groups with putative human pathogenic activity as determined with FAPROTAX in the pig slurry and the cow manure compared to the initial substrate, but it increased them in the goat feces and the vermicomposted rabbit feces.

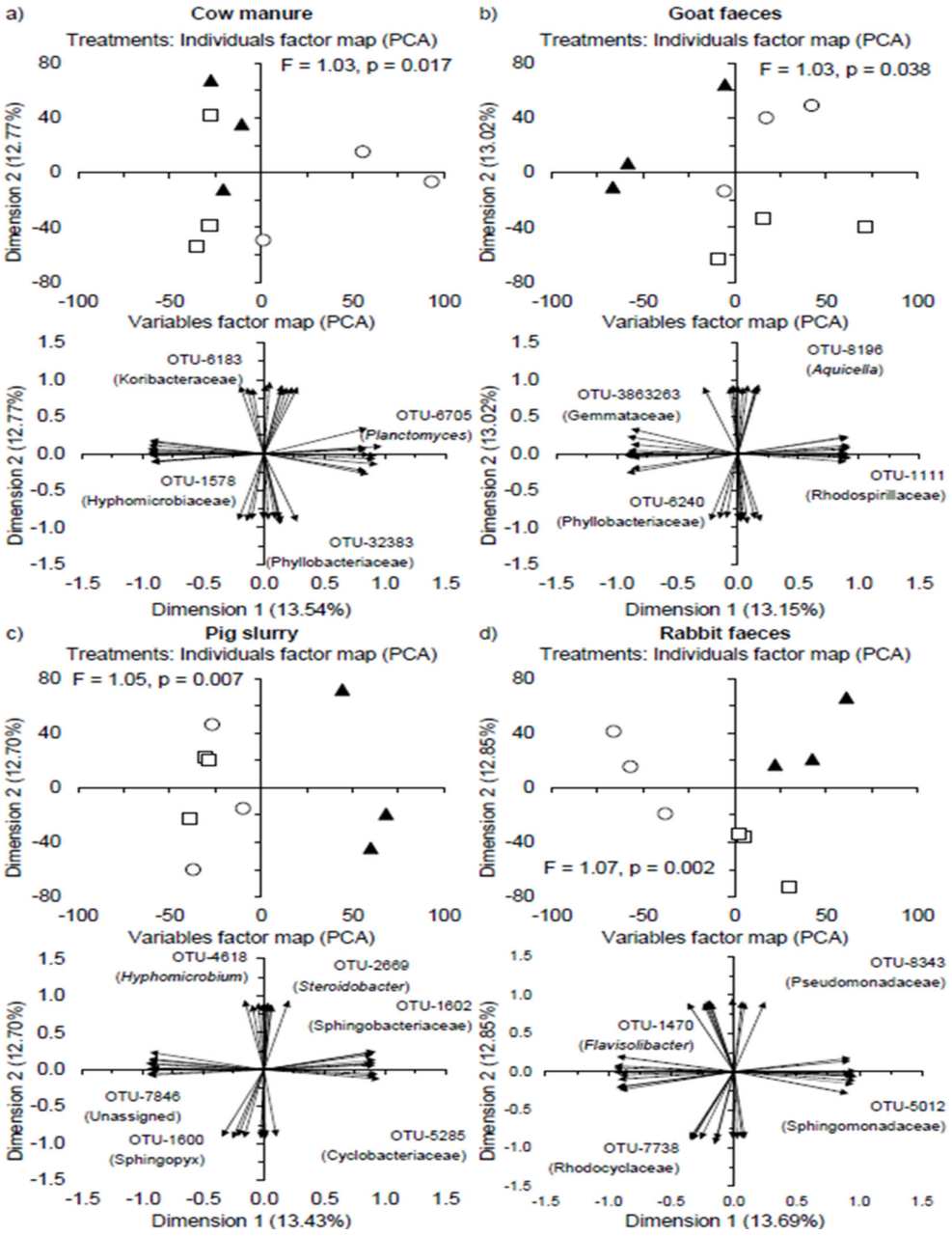

Figure 5. Principal component analysis (PCA) with all bacterial operational taxonomic units (OTUs) in animal wastes: a) cow manure, b) goat feces, c) pig slurry and d) rabbit feces. initial substrate (▲) composted substrate (□) and vermicomposted substrate(○). F and p values were obtained after permutational multivariate analysis of variance (perMANOVA).

CONCLUSIONS

The composting and vermicomposting of animal wastes increased the pH and total N content while reducing organic carbon and EC of the substrates. Alpha diversity indices remained similar between the organic wastes and showed no significant differences between composted and vermicomposted substrates. Changes in beta diversity were driven more by species replacement than by species loss. The bacterial community structure differed across the four animal wastes at lower taxonomic levels, but not at higher ones. Some bacterial groups were notably influenced by the vermicomposting process compared to the initial substrate. The enrichment of bacterial groups such as *Acinetobacter*, *Pseudomonas*, *Planctomyces*, and *Glycomyces* suggested their rapid adaptation to the chemical changes in the substrate. After 90 days of vermicomposting, the bacterial community structure remained distinct between the four animal wastes at lower taxonomic levels but converged at higher levels.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Data sets used or analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FINANCING

Not applicable.

AUTHORS' CONTRIBUTIONS

Project management, fund acquisition: J.A.M.M., and L.D. Research formulation: L.D. Field execution: R.P.S.R. Data analysis: L.D. Original draft preparation, journal submission, and follow-up: L.D, M.H.G., and R.P.S.R. Participated in the field phase, data organization and draft review: R.P.S.R. Responsible for field phase, data organization, and draft review: J.A.M.M. Responsible for soil sampling planning, lab analysis, and draft review: J.A.M.M., and L.D. Responsible for soil sampling planning, lab analysis, and draft review: V.M.R.V. Responsible for analysis and draft review: M.H.G., and AZR. Planning soil sampling, lab analysis, and draft review: V.M.R.V. Assisted in original draft preparation, review and final editing for submission: R.P.S.R., and A.Z.R.

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