



Unveiling the microbial dynamics in vermicomposting with coir pith as earthworm substrate

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ABSTRACT

This study explored the impact of incorporating coir pith, a byproduct of the coconut industry, into the vermicomposting substrate of *Eudrilus eugeniae* earthworms. The groups were compared based on their diets: cow manure only or cow manure mixed with varying amounts of coir pith. The aim was to assess the effects of coir pith on earthworm growth, mortality and the microbial community involved in vermicomposting. Earthworms fed with higher proportions of coir pith (70 % w/w) experienced reduced growth (0.81 g/worm) and increased mortality (24.67 %) after 5 weeks of vermicomposting. These effects were attributed to the high level of total phenolic content in the system. Coir pith required specific bacteria for digestion and detoxification, and excessive intake disrupted the earthworms' digestion, thus hindering nutrient absorption. The study also examined the microbial composition of the vermicast samples and identified variations based on the diet. Bacterial taxa involved in lignocellulose degradation, such as Bacteroidota, *Azospirillum*, Chitinophagaceae, *Marinomonas* and *Pantoea*, exhibited decreased abundances in treatments with coir pith. Conversely, the abundances of potentially harmful bacteria, such as *Aeromonas*, increased with higher coir pith inclusion levels. This pioneering investigation sheds light on the feasibility of coir pith use in vermicomposting and emphasises the importance of optimising earthworm diets to enhance microbial ecological functions and improve vermicompost quality.

1. Introduction

Vermicomposting is a natural process that harnesses the feeding habits of earthworms to convert organic waste into valuable soil amendment. It involves the use of earthworms to decompose organic waste and transform it into nutrient-rich compost [1], which

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makes it an efficient and sustainable method of recycling organic materials while simultaneously improving soil fertility [2]. Whilst earthworms are highly efficient in converting organic waste into vermicompost, ensuring an adequate and appropriate food supply for them is crucial for successful vermicomposting [3,4]. Earthworms require a balanced diet for optimal health and productivity, and it is essential to provide a variety of organic waste materials to meet their nutritional needs [5,6]. A diet that is too heavy in one type of waste, such as an excess of fruit peels or high-nitrogen materials, can lead to imbalances in the vermicompost's nutrient content [7]. When excessive amounts of lignin and phenolic compounds are present in the feed, they negatively affect earthworm activity in forest soils. However, depending on the specific concentrations and composition of these compounds, earthworm species and soil conditions, the effects may vary [8]. It is therefore essential to be selective about the types of waste added to the vermicomposting system.

Coir pith, an agricultural waste product, balances the carbon-to-nitrogen ratio, retains moisture, improves aeration, neutralises the pH and promotes sustainability. It enriches vermicompost and enhances the structure, texture, water-holding capacity, aeration and nutrient availability [9–11]. Cow manure and coir pith are readily available to feed earthworms in vermicomposting fields [12,13]. Whilst cow manure provides nutrients, coir pith retains moisture and improves aeration, promoting earthworm activity and organic matter breakdown. This combination offers a balanced diet for earthworms, promoting their activity and enhancing the breakdown of organic matter [13].

Vermicomposting using a combination of cow manure and agricultural waste can be an effective approach to producing high-quality vermicompost. The combination of these organic materials provides a balanced nutrient composition and enhances the overall decomposition process [3,12]. When coir pith is processed (pre-composted) and adequately prepared, it becomes safe and beneficial for earthworms in vermicomposting [9,14]. However, most of the coir pith in Thailand is a by-product of the coir fibre industry and producing using mechanical defibrating. This raw and untreated coir pith may contain elevated levels of phenolic compounds, which are naturally occurring organic compounds and can potentially be harmful to earthworms, with toxic effects on them [15,16]. Jayakumar et al. [14] highlighted the need for treatment when using coir pith in vermicomposting due to the presence of substances that can adversely affect earthworms. The researchers demonstrated the effectiveness of a treatment process involving the use of *Pleurotus sajor-caju*, *Gliricidia sepium* biomass and cow dung as amendment materials to remove toxins from coir pith during vermicomposting.

The vermicompost microbiota, consisting of bacteria, fungi, protozoa and other microorganisms, plays a significant role in the digestion and decomposition of organic matter [17]. This diverse community of microorganisms contributes to the breakdown of organic materials, nutrient release and soil fertility enhancement. Understanding the composition and dynamics of vermicompost microbiota is therefore crucial for optimising vermicompost production and its application in different agricultural and environmental contexts. Domínguez et al. [18] found that the bacterial communities of vermicompost produced by Scotch broom changed over time during the decomposition process, with different bacterial species dominating at different stages. However, the specific dynamics of bacteria during vermicomposting are less well understood compared to those involved in traditional composting. Budroni et al. [19] observed that earthworms fed brewers' spent grains (FBSG) had an enriched microbiota, including Paenibacillaceae, Enterobacteriaceae, Chitinophagaceae and Comamonadaceae. The FBSG-fed earthworms exhibited a higher predicted abundance of genes associated with cellulose degradation and nitrogen cycling, along with the increased use of ammonia and nitrate.

Feeding earthworms of the species *Eudrilus eugeniae* with different ratios of cow manure and coir pith resulted in varying growth rates. Particularly, earthworms fed a high proportion of coir pith showed lower growth rates. Moreover, earthworm mortality occurred when they were solely fed coir pith [20]. In this study, we used microbiome approaches to examine the bacterial diversity of vermicompost at two time points (0 and 5 weeks) under different feeding ratios of cow manure and coir pith. This allowed us to gain a comprehensive understanding of the temporal changes in microbial communities during vermicomposting. The obtained data were then analysed to describe the bacterial succession patterns during vermicomposting and infer the metabolic functions of the vermicompost microbiome.

2. Materials and methods

2.1. Materials and earthworm preparation

The materials used for vermicomposting consisted of coir pith, cow manure and *E. eugeniae* earthworms. Coir pith was procured from a local plant shop in Nonthaburi Province, Thailand, and the mature cow manure was obtained from a trusted local worm breeder in Samut Sakhon Province, Thailand. Prior to use, the mature cow manure was thoroughly soaked in water, involving three cycles of 24-h soaking periods, to eliminate excess ammonia. The *E. eugeniae* earthworms, African night crawlers, were cultured in plastic trays filled with soaked cow manure. The earthworms used in the experiments were adult worms weighing between 0.9 and 1.0 g, and the culture was maintained at room temperature (28–33 °C) with a moisture content of 70%–80 %, maintained by tap water.

2.2. Vermicomposting process/experimental design

The experiments were conducted in plastic containers measuring 30 × 50 × 17 cm (width × length × height). The substrates used were coir pith and cow manure; two different treatments were prepared, with coir-pith-to-cow manure (weight/weight) ratios of 20:80 (T1) and 70:30 (T2), whereas cow manure alone served as the control. The initial characteristics of the feed materials are detailed in [Supplementary Table 1](#). Fifty earthworms were placed in each plastic container and supplied with 1 kg-feed kg-worm⁻¹ day⁻¹ of feed materials. The moisture content was carefully adjusted and maintained at 70%–80 % throughout the vermicomposting period. All treatments were performed in triplicate to ensure accuracy and reliability. The monitoring of earthworm activities, including

earthworm weight gain and the number of earthworms, was carried out at 0, 2 and 5 weeks. Vermicompost samples were collected at these time points for analysis. To quantify the total phenolic content (TPC) of the vermicompost, the Folin-Ciocalteu method [21] was employed. The vermicast samples at Week 0 (earthworm casts obtained from earthworms fed cow manure on the first day) and Week 5 were kept under aseptic conditions to preserve their integrity. The earthworms from each treatment were placed on wet filter paper at room temperature in the dark for 24 h to purge their gut contents. This procedure facilitated DNA extraction for subsequent analysis.

2.3. Sample DNA extraction

Genomic DNA from the substrates and vermicast was extracted using the DNeasy® PowerSoil® Pro Kit (Qiagen, Germany), a specialised soil DNA kit known for its efficiency. To overcome the challenge posed by the high polyphenol content in coir pith, we employed a modified extraction protocol following Angeles et al. [22]. Briefly, 2 g of coir pith were ground into a fine powder using a mortar and pestle, with the addition of 200 mg of polyvinylpyrrolidone (PVPP) during the grinding process to facilitate the extraction. The resulting coir pith powder was then carefully transferred to sterile 50-mL Falcon tubes preloaded with 20 mL of the DNA extraction buffer (2 M NaCl, 0.2 M Tris-HCl [pH 8.0], 0.07 M EDTA, 0.2 M β-mercaptoethanol). A 1-mL aliquot of 20 % SDS was added to the mixture, which was thoroughly mixed and subsequently incubated at 65 °C for 1 h. Following incubation, the solution underwent centrifugation at 21,000×g and 4 °C for 15 min, allowing the collection of 500 mL of supernatant. The supernatant was then divided into several sterile Eppendorf tubes, each of which received 125 µL of 3 M sodium acetate and 500 µL of absolute isopropanol. After gentle mixing, the tubes were incubated at –80 °C for 15 min, promoting DNA precipitation. Further centrifugation at 10,000×g and 4 °C for 15 min facilitated the removal of the supernatant, leaving behind the DNA pellet. The pellet was subjected to two washes with 70 % ethanol and then air-dried at room temperature. Finally, the DNA pellet was redissolved with 50 µL of sterile nanopure water, ensuring the recovery of genomic DNA from the coir pith. To ensure the purity and integrity of the DNA samples, total genomic DNA from coir pith underwent a supplementary purification step using the same soil DNA kit. The quality of the DNA was rigorously evaluated using agarose gel electrophoresis, and the DNA concentration was accurately quantified using the Qubit® dsDNA HS Assay Kit (Thermo Fisher, USA). Subsequently, the extracted DNA was stored at –80 °C after concentration.

Throughout this study, the DNA samples were designated as follows: CM - DNA extracted from cow manure, CP - DNA extracted from coir pith, Control-0W - DNA extracted from vermicasts obtained from earthworms fed cow manure on the first day, Control-5W - DNA extracted from vermicasts obtained from earthworms fed cow manure on Day 35 (5 weeks), T1-5 W - DNA extracted from vermicasts obtained from earthworms fed a mixture of coir pith and cow manure (20:80 ratio) on Day 35 (5 weeks) and T2-5 W - DNA extracted from vermicasts obtained from earthworms fed a mixture of coir pith and cow manure (70:30 ratio) on Day 35 (5 weeks).

2.4. Amplification, sequencing and analysis of 16S rRNA genes

The sequencing library was constructed using the MetaVX Library Preparation Kit. To achieve this, 20–50 ng of DNA was used to generate amplicons covering the V3 and V4 hypervariable regions of the bacterial 16S rRNA gene. The forward primer sequence 'CCTACGRRRBGCASCAGKVRVGAAT' and the reverse primer sequence 'GGACTACNVGGGTWTCTAATCC' were employed for amplification [23]. The DNA concentration was measured using a microplate reader (Tecan, Infinite 200 Pro), and the fragment size was assessed through 1.5 % agarose gel electrophoresis, where an expected size of approximately 600 bp was observed. The PCR amplification procedure involved two rounds. In the first round, a 25-µL reaction mixture comprising 2.5 µL 10 × TranStart buffer, 2 µL dNTP (2.5 mM), 2.5 µL of each primer, 17.5 µL template DNA and 0.5 µL TransStart Taq (2.5 U/µL) was prepared. The temperature cycling consisted of initial denaturation at 94 °C for 3 min, followed by 14–16 cycles at 94 °C for 10 s, 57 °C for 90 s, 72 °C for 15 s and a final extension at 72 °C for 5 min. The second round involved a 50-µL reaction mixture with 2.5 µL 10 × TranStart buffer, 2 µL dNTP (2.5 mM), 3 µL of each index primer, 4 µL cocktail, 25 µL first-round PCR products, 0.5 µL TransStart Taq (2.5 U/µL) and 10 µL ddH₂O. The temperature cycling for the second round comprised initial denaturation at 94 °C for 3 min, followed by 10–12 cycles at 94 °C for 10 s, 60 °C for 30 s, 72 °C for 15 s and a final extension at 72 °C for 5 min. Subsequently, next-generation sequencing was performed on an Illumina Miseq/Novaseq Platform (Illumina, San Diego, USA). This involved automated cluster generation and 250/300 paired-end sequencing with dual reads, ensuring comprehensive and accurate data generation for further analysis.

The analysis process commenced with double-end sequencing of both positive and negative reads. Subsequently, the initial two reads were merged, and sequences containing the character 'N' were filtered out. Only sequences exceeding a length of 200 base pairs (bp) were retained after applying a quality filter and purifying chimeric sequences. The resulting sequences were used for Operational Taxonomic Unit (OTU) clustering applying VSEARCH clustering (version 1.9.6) with a sequence similarity threshold set at 97 %. These sequences were compared against the Silva 138 16S rRNA reference database (<http://www.arb-silva.de/>) to determine their taxonomic identity, using the RDP (Ribosomal Database Program) classifier (version 2.2) with the Bayesian algorithm [24]. The community composition of each sample was statistically analysed at different species classification levels. To assess alpha diversity indices, such as Shannon and Chao1, and evaluate species abundance and diversity, rarefaction curves and rank-abundance graphs were generated, reflecting species richness and evenness [25]. To explore significant differences in the microbial community among samples, (UN) weighted UniFrac analysis was performed. Additionally, beta diversity visualisation was achieved through PCA (principal components analysis) and PCoA (principal coordinates analysis) using R (version 3.3.1). The PCA used the OTU abundance table, whereas the PCoA was based on the Bray–Curtis distance matrix [26]. In addition, analysis of similarity (ANOSIM) was performed to evaluate the differences in microbiota composition among the samples [27]. To characterise the dissimilarities in microbial community structure and species composition among groups, linear discriminant analysis (LDA) was performed.

2.5. Statistical analysis

All statistical analyses were performed with Minitab 18 (Minitab Inc., United States). Analysis of variance (ANOVA) and Tukey's HSD test, at the confidence level of 95 %, were conducted to test for significant differences among treatments.

3. Results

3.1. Influence of coir pith content on earthworm mortality and body weight dynamics during a 5-week cultivation experiment

Throughout the 5-week earthworm cultivation experiment, varying ratios of cow manure and coir pith were employed as dietary components. Notably, the control group that exclusively received cow manure demonstrated favourable outcomes, with a mortality rate of merely 2 % and an average worm weight of 1.65 g. Conversely, a discernible trend emerged as earthworm mortality rates fluctuated in response to increasing coir pith inclusion levels. Specifically, the 70 % coir pith group (T2) exhibited a higher mortality rate of 24.67 %, whereas the 20 % coir pith group (T1) displayed a lower rate of 4.67 %. Consistently, average individual worm weight exhibited a corresponding pattern, with the 70 % coir pith group (T2) showing the lowest average weight of 0.81 g (Table 1). These findings underscore the noteworthy impact of the coir pith content in the diet on both earthworm mortality and body weight dynamics throughout the 5-week observation period.

3.2. Impacts of coir pith and cow manure on tannic acid contents in earthworm casts

We assessed the tannic acid content in earthworm casts, particularly when the worms were fed a diet containing coir pith and cow manure. Table 2 shows a significant increase in tannic acid levels in casts when earthworms were provided a diet comprising a mixture of coir pith and cow manure, compared to those fed solely cow manure. The initial tannic acid content in vermicasts from the cow manure group was 5.08 mg/g, which decreased to 2.49 and 0.77 mg/g after 2 and 5 weeks, respectively (an 85.07 % reduction). Similarly, the 20:80 ratio group showed an initial tannic acid content of 6.63 mg/g, decreasing to 2.70 and 0.99 mg/g after 2 and 5 weeks, respectively (an 84.10 % decrease). The 70:30 ratio group had an initial tannic acid content of 11.72 mg/g, which decreased to 4.57 and 3.03 mg/g after 2 and 5 weeks, respectively (a 73.49 % decrease). Higher coir pith proportions led to elevated mortality rates among earthworms. The presence of phenolic compounds in coir pith, affecting earthworm growth, warrants further examination of their impacts on vermicomposting and bacterial community composition.

3.3. Characterisation of the bacterial community composition in feeding substrates and earthworm casts using high-throughput sequencing and OTU analysis

A total of 2,523,172 raw reads were obtained from analysing *E. eugeniae* samples at different time points. After applying the filtering criteria, 2,280,631 effective reads (90.38 % of the initial raw reads) were retained. The average number of sequences per sample ranged from 110,500 to 136,489. The analysis identified 6696 operational taxonomic units (OTUs), classified into 44 phyla, 117 classes, 287 orders, 515 families, 1076 genera and 1212 species (Supplementary Data Set S1). The rarefaction curve, which plots the observed OTUs at a 97 % similarity index, serves two main purposes: determining the sample size and assessing species abundance within different groups. This curve is a valuable tool for understanding species composition and predicting abundance and commonly used in biodiversity and community surveys. In our study, the flat OTU species curve suggests that sequencing adequately captured the bacterial community's richness (Supplementary Fig. 1). We also used a petal diagram (Fig. 1) to present the results visually. The unique bacterial OTU counts for each treatment were 1,675, 673, 800, 392, 218 and 274 for CM, CP, Control-0W, Control-5W, T1-5 W and T2-5 W, respectively. Notably, 444 OTUs were shared among all treatments.

Fig. 1B shows the top 30 OTUs with the highest abundances. The results of the analysis revealed that OTUs 5, 4 and 11 were highly abundant in the coir pith food sample. These groups of microorganisms were also found in other experimental samples, albeit at low

Table 1
Mortality and average body weight of earthworms fed different ratios of cow manure and coir pith.

| Treatment | Vermicomposting time (week) | Control | T1 | T2 |
|----------------------------|-----------------------------|------------------------------|-------------------------------|-----------------------------|
| Worm mortality (%) | 0 | 0 ^{a,a} | 0 ^{a,a} | 0 ^{a,a} |
| | 2 | 0.67 ± 1.15 ^{b,a} | 1.33 ± 2.31 ^{b,a} | 20.67 ± 3.06 ^{a,a} |
| | 5 | 2.00 ± 2.00 ^{b,a} | 4.67 ± 3.06 ^{b,a} | 24.67 ± 5.77 ^{a,a} |
| Total worm weight (g) | 0 | 51.53 ± 1.00 ^{a,c} | 49.77 ± 3.09 ^{a,b} | 48.20 ± 2.38 ^{a,a} |
| | 2 | 101.00 ± 2.31 ^{a,a} | 67.80 ± 7.66 ^{b,a} | 34.20 ± 2.31 ^{c,b} |
| | 5 | 81.07 ± 5.62 ^{a,b} | 62.07 ± 7.12 ^{b,a,b} | 30.73 ± 4.38 ^{c,b} |
| Individual worm weight (g) | 0 | 1.03 ± 0.02 ^{a,c} | 1.00 ± 0.06 ^{a,b} | 0.96 ± 0.05 ^{a,a} |
| | 2 | 2.03 ± 0.03 ^{a,a} | 1.37 ± 0.13 ^{b,a} | 0.86 ± 0.03 ^{c,ab} |
| | 5 | 1.65 ± 0.08 ^{a,b} | 1.30 ± 0.11 ^{b,a} | 0.81 ± 0.06 ^{c,b} |

Different superscript first and second letters indicate significant differences within rows and columns, respectively, as determined by ANOVA followed by Tukey's HSD test ($p < 0.05$). T1 = earthworms fed coir pith and cow manure (20:80) and T2 = earthworms fed coir pith and cow manure (70:30).

Table 2
Total phenolic contents in casts of earthworms fed different diets.

| Treatment | TPC (mg as tannic acid/g sample) | | | Reduction (%) | |
|-----------|----------------------------------|----------------------------|----------------------------|------------------------------|-----------------------------|
| | Week 0 | Week 2 | Week 5 | Week 2 | Week 5 |
| Control | 5.08 ± 0.56 ^{a,b} | 2.49 ± 0.41 ^{b,b} | 0.77 ± 0.28 ^{c,b} | 50.95 ± 4.52 ^{b,b} | 85.07 ± 4.06 ^{a,a} |
| T1 | 6.63 ± 0.68 ^{a,b} | 2.70 ± 0.26 ^{b,b} | 0.99 ± 0.00 ^{c,b} | 59.32 ± 0.69 ^{b,a} | 84.10 ± 0.11 ^{a,a} |
| T2 | 11.72 ± 0.76 ^{a,a} | 4.57 ± 0.49 ^{b,a} | 3.03 ± 0.18 ^{c,a} | 54.79 ± 1.82 ^{b,ab} | 73.49 ± 0.42 ^{b,b} |

Different superscript first and second letters indicate significant differences within rows and columns, respectively, as determined by ANOVA followed by Tukey's HSD test ($p < 0.05$). T1 = earthworms fed coir pith and cow manure (20:80) and T2 = earthworms fed coir pith and cow manure (70:30).

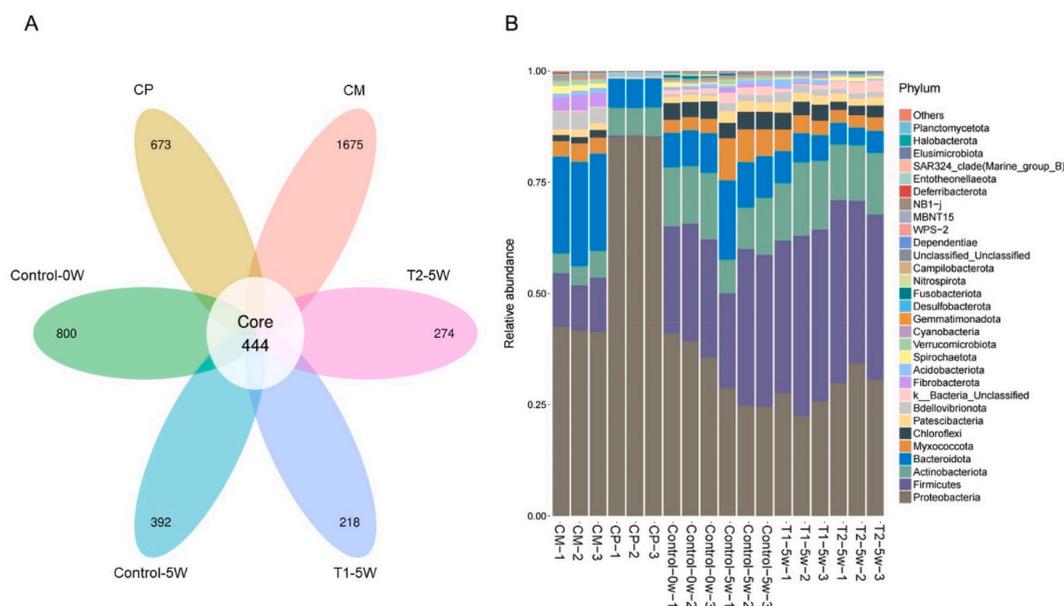


Fig. 1. OTU petal diagram and heatmap analysis. (A) The petal diagram of the OTU cluster analysis indicates the diets and casts from the vermicompost group. The different colours in the petal diagram represent different samples in each group, and the numbers in the figure represent the numbers of OTUs unique or common to each sample. The white circle in the middle represents the number of OTUs shared by all samples and groups. (B) Heatmap analysis showing the abundance of information on selected OTUs as well as the similarities and differences across OTUs and samples by similarity clustering. CM = cow manure, CP = coir pith, Control-0W = vermicasts obtained from earthworms fed cow manure on the first day, Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5W = vermicasts obtained from earthworms fed coir pith and cow manure (20:80) in Week 5 and T2-5W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5. Next-Generation Sequencing (NGS) raw data is available at Mendeley Data [28] doi: <https://doi.org/10.17632/kvfk7mf9vz.3>. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

quantities. Conversely, the OTU 17, which was prevalent in the cow manure sample, was also found in minimal amounts in earthworm casts across various experimental setups. Certain bacterial groups in the food might diminish in the vermicomposting system. In some instances, OTUs 2, 41, 83 and 4658 were scarcely present in both cow manure and coconut coir but were found at higher quantities in earthworm casts, particularly those from the control group and the experimental group where the inclusion level of coir pith was high. Furthermore, OTU 14 was also identified in the earthworm casts from the cow manure-fed vermicomposting system starting from the first day of the experiment. The quantity of this bacterium increased significantly over time in the treatment with cow manure. However, when the earthworms were fed a mixture of coir pith and cow manure, the abundances of bacteria in this group decreased.

3.4. Analysis of bacterial diversity and species richness in casts from earthworms fed different diets

3.4.1. Alpha (α)-diversity analysis

The analysis of Chao1 species richness yielded noteworthy findings on bacterial diversity. Cow manure exhibited a significantly higher species richness compared to coir pith. However, when examining the bacterial communities in earthworm casts at different time points, no significant differences were observed (Table 3). Across all samples, cow manure displayed the highest bacterial diversity, significantly different from that of the other samples. Conversely, coir pith had the lowest diversity, also differing significantly. No significant variations were found in the microbial diversity of casts obtained from earthworms fed either cow manure or 20:80 coir

pith-cow manure. However, a significant difference was observed in the 70:30 coir pith-cow manure casts (Table 3).

Different superscript first and second letters indicate significant differences within rows and columns, respectively, as determined by ANOVA followed by Tukey's HSD test ($p < 0.05$). Control-0W = vermicasts obtained from earthworms fed cow manure on the first day, Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5 W = vermicasts obtained from earthworms fed with coir pith and cow manure (20:80) in Week 5 and T2-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5.

3.4.2. Beta (β)-diversity analysis

Beta (β) diversity analysis, employing techniques such as PCoA and PCA, provides valuable insights into variations and dissimilarities among samples, reflecting the overall diversity. In our study, PCoA revealed that PC 1 accounted for 46.34 % and PC 2 for 27.86 % of the total variation (Fig. 2A). Similarly, PCA explained 24.20 % (PC 1) and 13.59 % (PC 2) of the variation (Fig. 2B). Notably, these analyses demonstrate distinct bacterial diversity patterns associated with cow manure (CM) and coir pith (CP) diets, differentiating them from other groups. The PCoA and PCA results clearly separated the microbiota profiles into cow manure, coir pith and vermicomposting casts. A heatmap, employing weighted and unweighted UniFrac distances, further assessed the dissimilarity coefficients among the samples, indicating minimal variation and reinforcing consistency (Supplementary Fig. 2).

3.5. Microbial community composition and bacterial families in casts of earthworms fed different diets: insights from 16S rRNA sequencing

In the case of the CM diets, the predominant bacterial phyla were Proteobacteria (41.79 %), Firmicutes (11.55 %) and Bacteroidota (22.25 %). Conversely, in the CP feed type, the dominant phyla were Proteobacteria (85.22 %), Actinobacteriota (6.38 %) and Bacteroidota (6.40 %). When earthworms were subjected to various experimental diets, namely cow manure alone, coir pith and cow manure at ratios of 20:80 and 70:30, the relative abundances of bacterial taxa at the phylum level varied in response to the specific characteristics of the diet and the duration of vermicomposting. A noteworthy temporal shift was observed when analysing subsequent feeding types and time points, namely Control-0W, Control-5W, T1-5 W and T2-5 W. Specifically, there was a downward trend in the relative abundance of Proteobacteria, declining from the initial values of 41.79 % and 85.22 % in CM and CP, respectively, to 38.63 %, 26.02 %, 25.24 % and 31.57 % in Control-0W, Control-5W, T1-5 W and T2-5 W, respectively. A similar trend was observed for the relative abundances of Bacteroidota, which also declined over time and with different dietary inputs, reaching levels of 8.17 %, 12.41 %, 6.45 % and 4.53 % in Control-0W, Control-5W, T1-5 W and T2-5 W, respectively. Conversely, certain bacterial taxa experienced an increase in abundance over time and with varying dietary inputs. For instance, Firmicutes displayed a notable increase, reaching proportions of 25.71 %, 30.23 %, 37.82 % and 38.29 % in Control-0W, Control-5W, T1-5 W and T2-5 W, respectively. Similarly, Actinobacteriota demonstrated an upward trend, increasing to 13.75 %, 9.94 %, 15.03 % and 12.99 % in Control-0W, Control-5W, T1-5 W and T2-5 W, respectively (Supplementary Fig. 3). These findings underscore the dynamic nature of bacterial communities during vermicomposting with varying dietary inputs, highlighting substantial fluctuations in the relative abundances of key bacterial phyla.

At the family level, within the CM treatment, several prominent bacterial families were identified, including Comamonadaceae (5.02 %), Cellvibrionaceae (4.57 %), Devosiaceae (3.65 %), Chitinophagaceae (3.69 %), Peptostreptococcaceae (3.4 %), Fibrobacteraceae (3.22 %), Rhizobiaceae (2.98 %), Prolixibacteraceae (2.84 %), Marinilabiliaceae (2.74 %) and Clostridiaceae (2.55 %). In contrast, the bacterial composition of the coir pith samples differed significantly from that of the cow manure samples. Notably, the dominant bacterial families in the coir pith samples were Marinomonadaceae (18.57 %), Solimonadaceae (10.01 %), Erwiniaceae (8.93 %), Rhizobiaceae (7.55 %), Rhodobacteraceae (7.02 %), Rhodanobacteraceae (5.89 %), Xanthomonadaceae (4.11 %) and Chitinophagaceae (4.11 %) (Fig. 3A). In the case of earthworm casts obtained after the initial day of the experiment, from earthworms exclusively fed cow manure, the bacterial composition exhibited marked distinctions from both substrate types. Specifically, the bacterial family Aeromonadaceae (20.77 %) was only present in the cow manure-fed earthworm casts and was absent in both of the two initial feed sources. Additionally, other prevalent bacterial families, including Peptostreptococcaceae (7.57 %), Clostridiaceae (4.75 %), Microscillaceae (3.92 %) and Microbacteriaceae (2.24 %), were also identified.

Following a period of exclusive cow manure feeding for 5 weeks, notable changes in the quantities of certain bacterial families were observed. Some families exhibited an increase in abundance, such as Peptostreptococcaceae (11.71 %), Microscillaceae (7.37 %) and Planococcaceae (5.25 %), whereas others exhibited a decrease, such as Aeromonadaceae (5.04 %), Clostridiaceae (4.05 %) and Lachnospiraceae (2.34 %) (Fig. 3A). These findings highlight the dynamic nature of the bacterial community in response to different dietary inputs and the evolution of the bacterial community over time. Upon feeding the earthworms with a mixture of coir pith and

Table 3

Summary of alpha-diversity results (Chao1 richness and Shannon) for microbiota samples collected from feed and vermicast in each group.

| Sample | Chao1 | Shannon |
|------------|-------------------------------|--------------------------|
| Cow manure | 4410.68 ± 37.87 ^a | 9.47 ± 0.10 ^a |
| Coir pith | 1616.98 ± 36.81 ^c | 6.35 ± 0.03 ^d |
| Control-0w | 3806.96 ± 347.19 ^b | 8.27 ± 0.20 ^b |
| Control-5w | 3648.80 ± 140.36 ^b | 8.34 ± 0.13 ^b |
| T1-5w | 3689.30 ± 172.38 ^b | 8.61 ± 0.09 ^b |
| T2-5w | 3482.64 ± 138.79 ^b | 7.70 ± 0.21 ^c |

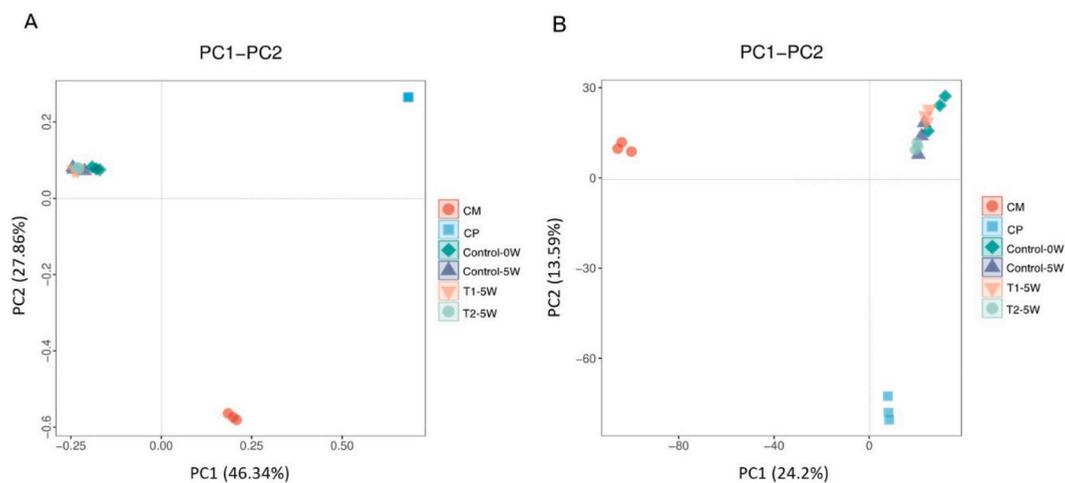


Fig. 2. Beta (β)-diversity analysis. (A) The PCoA score plot displaying PC1_vs_PC2 represents the first and second principal coordinates. The percentage values on the axis labels indicate the contribution of each coordinate to the variation in the samples, indicating how much information is captured by each principal component. The distance between sample points on the plot reflects the similarity of the microbial communities in each sample. Points that are closer together have a higher degree of similarity. Samples that cluster together share similar microbial compositions, suggesting a common microbiota pattern. (B) The PCA score plot, namely PC1_vs_PC2, represents the first and second principal components, respectively. The percentage associated with each principal component indicates its contribution rate to the variation in the samples, indicating how much information each component captures. The distances between the samples on the plot indicate the similarity in the distribution of functional classifications within each sample. A shorter distance reflects a higher degree of similarity. Samples that are closer together share a more similar distribution of functional classifications, suggesting a common pattern in the gut microbiota. CM = cow manure, CP = coir pith, Control-0W = vermicasts obtained from earthworms fed cow manure on the first day, Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5 W = vermicasts obtained from earthworms fed with coir pith and cow manure (20:80) in Week 5 and T2-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5.

cow manure at the ratios of 20:80 and 70:30, notable increases in the abundances of specific bacterial families were observed compared to the control group. For instance, the abundances of Peptostreptococcaceae increased to 12.73 % and 13.35 %, those of Clostridiaceae to 5.53 % and 5.92 % and those of Rhizobiaceae to 3.68 % and 1.69 %, respectively. Conversely, certain bacterial families experienced a decrease in abundance compared to the control group, such as Lachnospiraceae (3.08 % and 3.19 %) and Microscillaceae (2.58 % and 1.03 %) (Fig. 3B). Surprisingly, some bacterial families initially present in the cow manure and coir pith substrates either vanished or significantly decreased in abundance after passing through the digestive and excretory systems of the earthworms. These families included Comamonadaceae, Cellvibrionaceae, Marinomonadaceae, Rhodobacteraceae, Solimonadaceae and Erwiniaceae (Fig. 3B).

Based on the analysis of bacterial families with relative abundances greater than 0.1 %, notable trends of keystone bacterial taxa were discovered. Different bacterial families manifested higher relative abundance profiles in response to different diets. Furthermore, it is noteworthy that certain bacterial taxa exhibited a pronounced prevalence across various gut content groups. For instance, Chitinophagaceae, Rhizobiaceae and Sphingomonadaceae showed higher relative abundances within both the CM and CP diets. Nevertheless, a temporal perspective revealed a diminishing trend in the prevalences of these bacterial taxa, particularly within the gut contents of earthworms that had been exposed to elevated coir pith concentrations. Conversely, specific bacterial assemblages exhibited elevated relative abundances exclusively in the context of the CP diet, such as Rhodobacteraceae and Xanthomonadaceae. Intriguingly, this trend persisted even when the earthworms were sustained on a coir pith-only-diet for a continuous period of 5 weeks, indicating a complex interplay between dietary substrates and microbial dynamics (Fig. 3C and Supplementary Data Set S2).

Interestingly, certain bacterial cohorts that initially presented with limited relative abundances manifested an augmented prevalence following a 5-week dietary exposure regimen. This category encompassed Microbacteriaceae, Mycobacteriaceae, Micromonosporaceae and Xanthobacteraceae, indicating their adaptability and responsive behaviour in the context of dietary shifts. Notably, Pseudomonadaceae, with a high initial relative abundance within the CM diet, exhibited an increase in abundance upon the commencement of the experiment with CM. Nevertheless, in the treatment with CM throughout the 5 weeks, this taxon showed a decline in the relative abundance in the gut content, mirroring the dynamics observed in the group exposed to increased coir pith levels (Fig. 3C and Supplementary Data Set 2). These observations underline the profound influence of dietary substrates and vermicomposting duration on the composition of the bacterial communities within the earthworm gut. The observed trends indicate microbial responses to varying dietary compositions, along with temporal shifts in relative abundances.

These discrepancies in bacterial composition may be attributed to the selective feeding behaviour exhibited by earthworms. Previous research conducted by Bonkowski et al. [29] delved into the feeding preferences of five distinct earthworm species, namely *Lumbricus terrestris*, *L. castaneus*, *Aporrectodea caliginosa*, *A. rosea* and *Octolasion cyaneum*, across nine fungal species. The findings indicated that *Fusarium nivale* and *Cladosporium cladosporioides* were favoured by most earthworm species, followed by *Mucor* sp. and

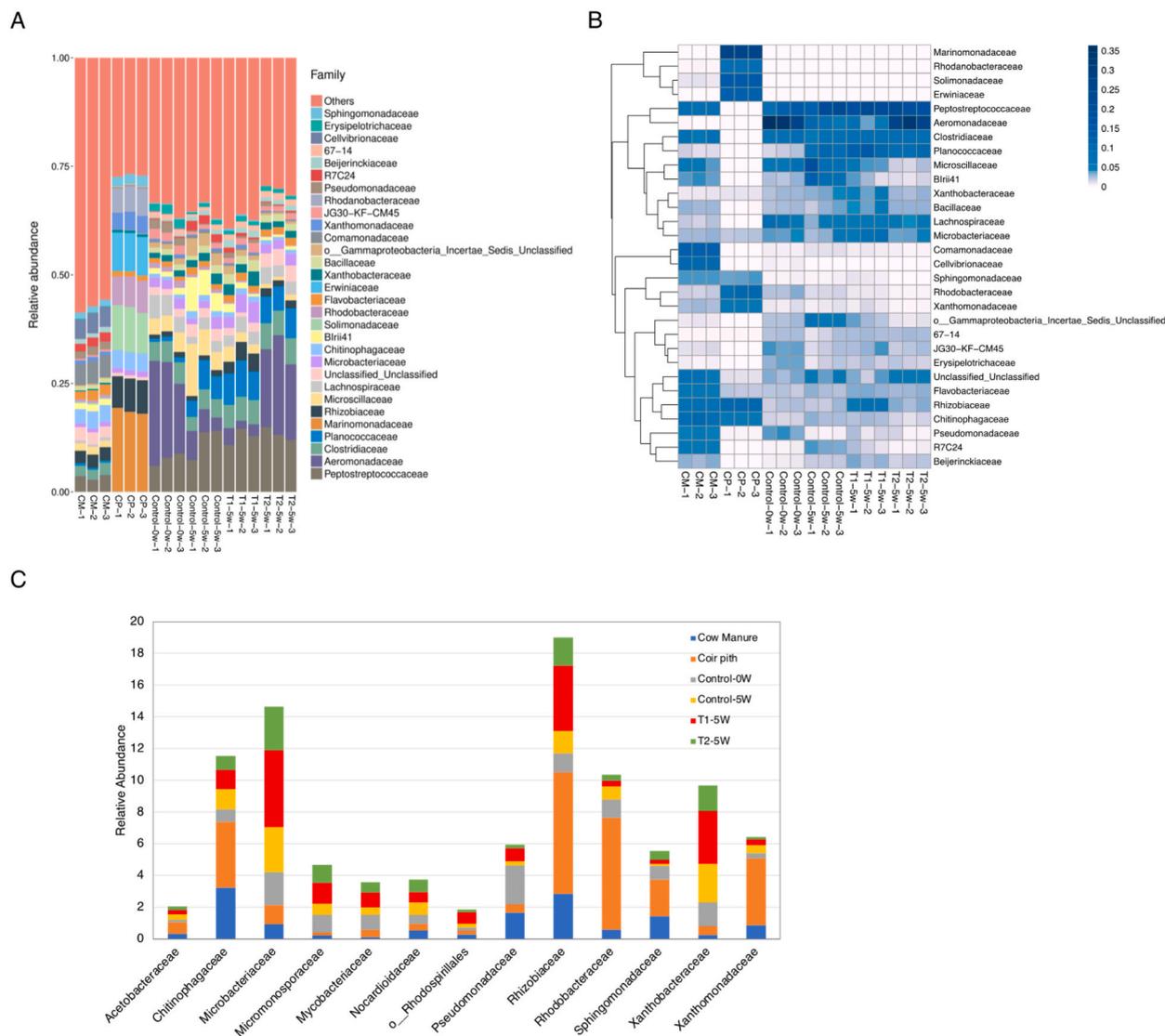


Fig. 3. (A) Relative microbial abundances at the family level for diets and casts of earthworms fed with different diet types on the first day and throughout the experimental period of 5 weeks. Each colour corresponds to a different family. The length of the colour block represents the relative abundance of the bacterial family represented by that colour block. (B) Heatmap showing differences in the abundances of the bacterial families among groups (top 30). The heatmap colour, pink to blue, displays the low to high relative abundance of each taxon across all samples. (C) Relative abundances of the selective keystone bacteria in each treatment. CM = cow manure, CP = coir pith, Control-0W = vermicasts obtained from earthworms fed cow manure on the first day, Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5W = vermicasts obtained from earthworms fed coir pith and cow manure (20:80) in Week 5 and T2-5W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Rhizoctonia solani. Interestingly, the epigeic species *L. rubellus* displayed a strong preference for a particular fungal species, whereas the endogeic species exhibited a more balanced feeding behaviour across various fungal species. These observations underscore the intricate interplay between earthworm feeding habits and the resultant shifts in the bacterial community composition during vermicomposting, shedding light on the selective processes at play.

3.6. Characterisation of the microbial taxa in casts of earthworms fed different diets

Based on the ANOSIM results, there was a substantial influence of vermicomposting duration and dietary factors on the bacterial community composition. We observed significant dissimilarities in the microbiota across all treatments, underlining the impact of food sources and vermicomposting duration. Regardless of dietary regimens, diets containing only cow manure (Fig. 4A and B), cow manure with limited coir pith (Fig. 4C and D) or rich in coir pith (Fig. 4E and F), in addition to feeding duration, significantly affected the

bacterial abundance. The R-values for each experimental set equalled 1, and the associated p-values were less than 0.05, indicating significant differences among the experimental sets. Our findings suggest dynamic shifts in vermicompost microbial composition in response to vermicomposting duration and diet, offering valuable insights into vermicomposting and soil microbiology.

In this study, taxa that exhibited a log LDA (linear discriminant analysis) score exceeding 3.0 were deemed as highly significant. This analysis revealed family-level biomarkers within the earthworm casts fed different diets. To provide a succinct summary of our findings, it is worth noting that Comamonadaceae, Cellvibrionaceae, Devosiaceae, Fibrobacteraceae, Marinilabiliaceae, Prolixibacteraceae, Dysgonomonadaceae, Rhodocyclaceae, Chromatiaceae, Flavobacteriaceae, Bdellovibrionaceae, Spirochaetaceae and Bacteriovoracaceae emerged as biomarkers in the cow manure sample, whereas Marinomonadaceae, Solimonadaceae, Erwiniaceae, Rhizobiaceae, Rhodobacteraceae, Rhodanobacteraceae, Xanthomonadaceae, Chitinophagaceae, Sphingomonadaceae, Alcanivoracaceae, Streptomyetaceae, Halomonadaceae, Gallaecimonadaceae and Methyloigellaceae were biomarkers in the coir pith sample.

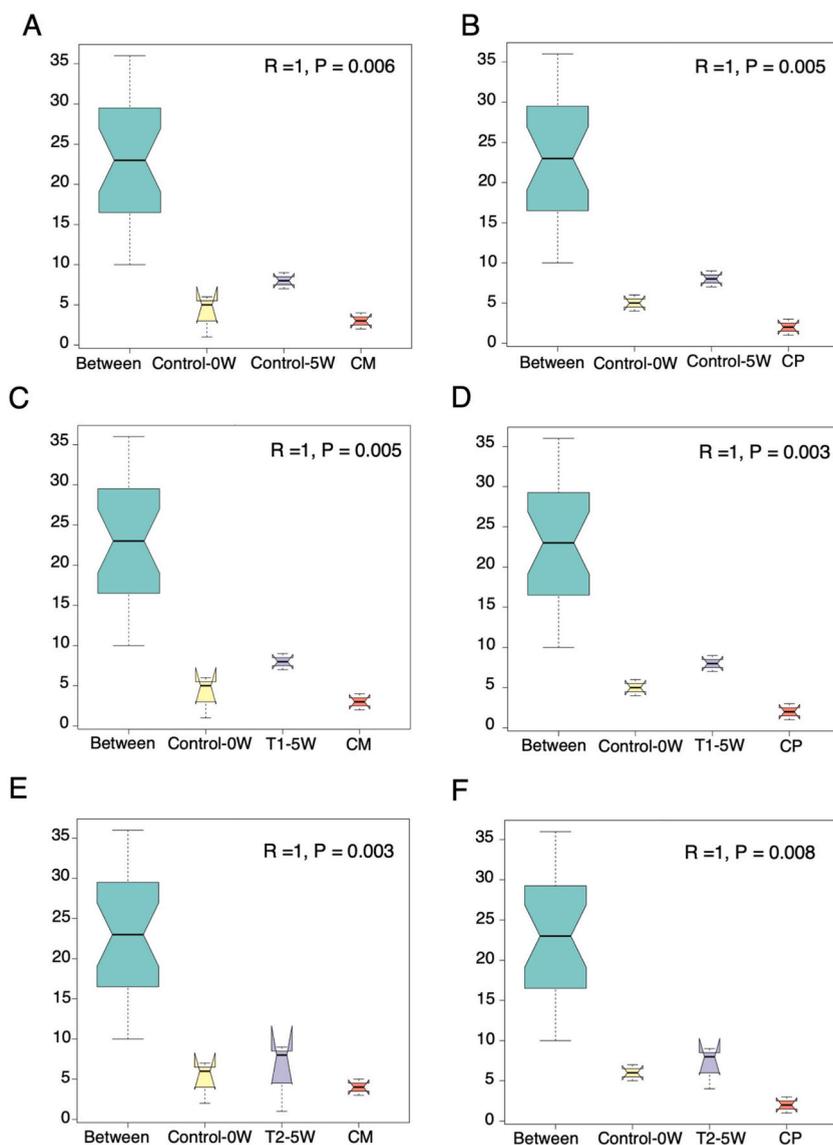


Fig. 4. ANOSIM results regarding the inter- and intra-group differences using R-values ranging from -1 to 1 . The values typically range from 0 to 1 , where an R-value close to 0 indicates no significant differences, whereas a value close to 1 signifies greater inter-group differences. The P-value reflects the statistical significance, with $P < 0.05$ indicating a significant difference. The plot shows the distance rank among samples on the y-axis and the results among groups on the x-axis. Intra-group results are displayed for each group. (A, B) Earthworms fed cow manure only, (C, D) earthworms fed coir pith and cow manure (20:80) and (E, F) earthworms fed coir pith and cow manure (70:30). CM = cow manure, CP = coir pith, Control-0W = vermicasts obtained from earthworms fed cow manure on the first day, Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (20:80) in Week 5 and T2-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5.

In the initial phase of the experiment, casts from earthworms exclusively fed cow manure contained a distinct set of microbial families, including Aeromonadaceae, Lachnospiraceae, Pseudomonadaceae, Erysipelotrichaceae, Mycoplasmataceae, Mycobacteriaceae and JG30_KF_CM45. Over the course of a 5-week period during which earthworms were exclusively fed cow manure, we observed notable alterations in the microbial community within the vermicomposting system. This led to the identification of specific microbial families, namely Microscillaceae, Biri41, R7C24, Halieaceae, Nitrosomonadaceae, KD4_96 and Demequinaceae, as biomarkers. However, when coconut coir was introduced into their diet, there was a discernible reduction in the abundances of these biomarker microbes. In cases where earthworms were fed a blend of coconut coir and cow manure at a 20:80 ratio, we identified other microbial families as

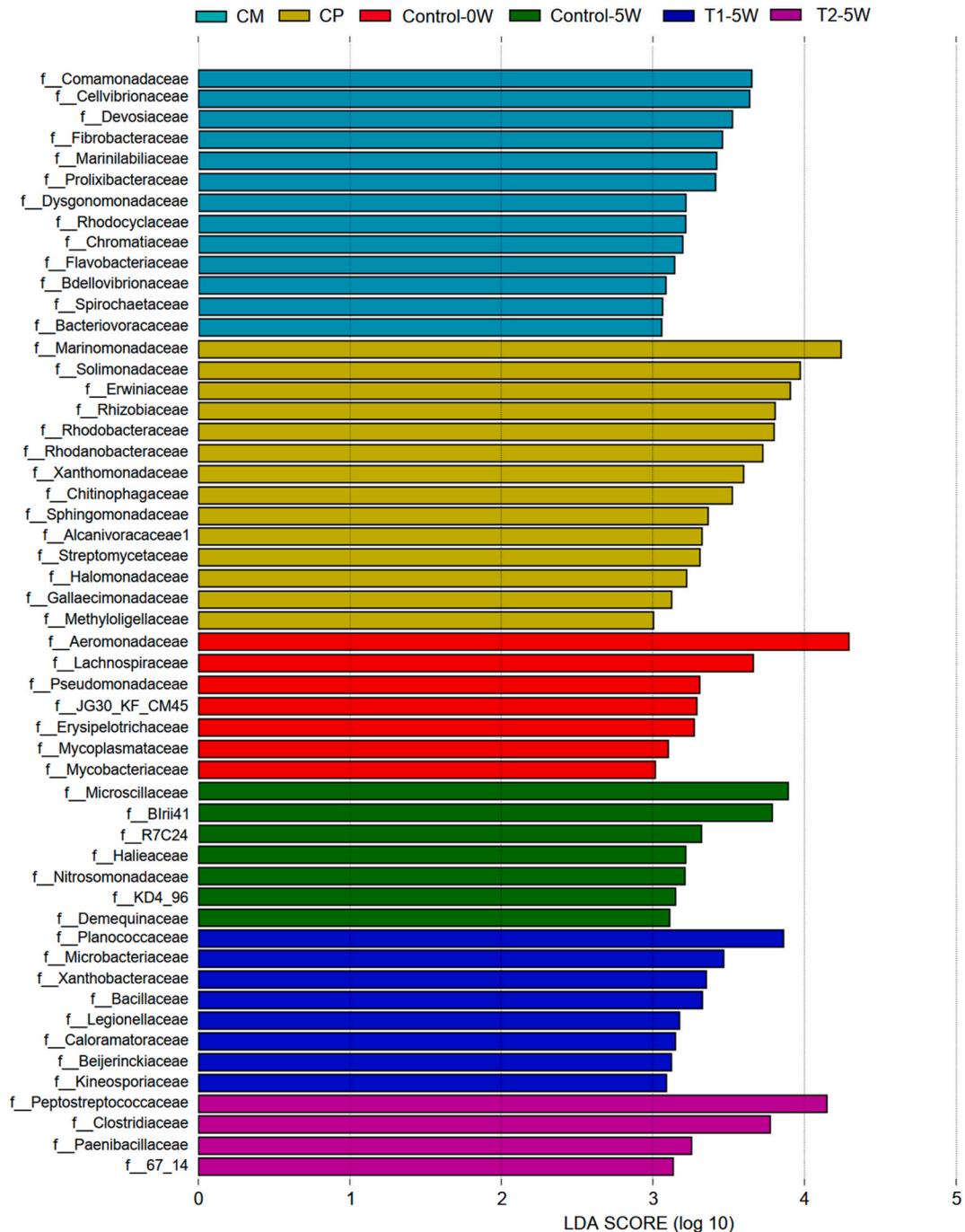
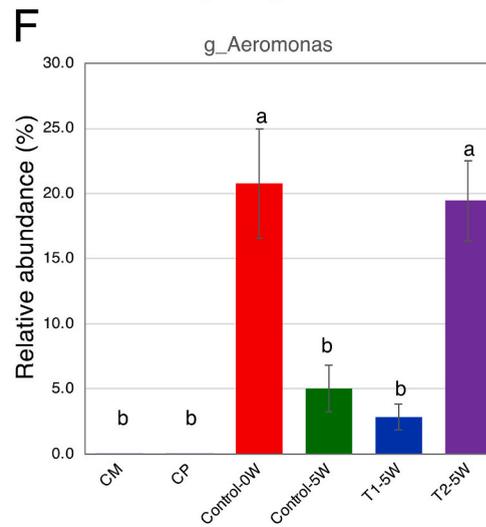
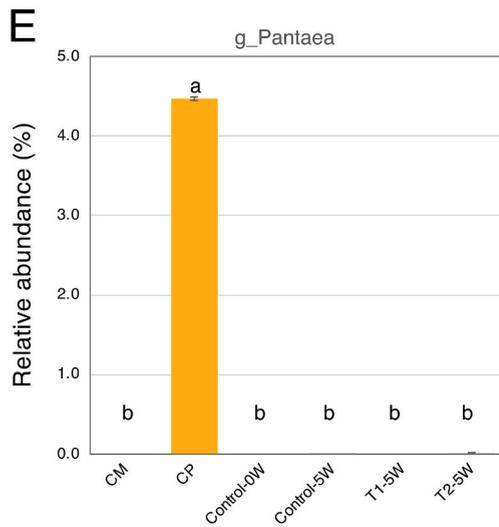
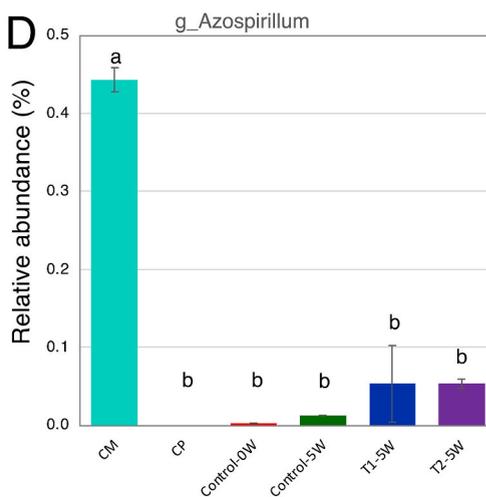
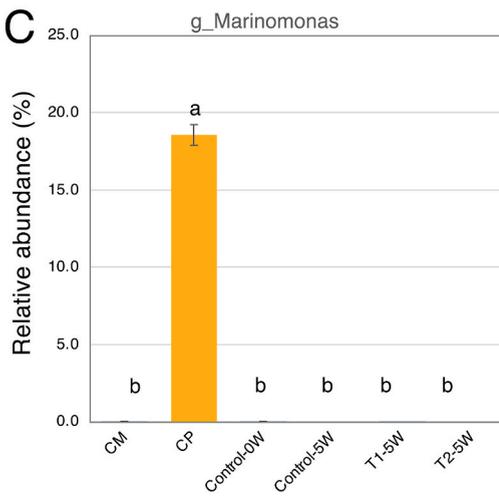
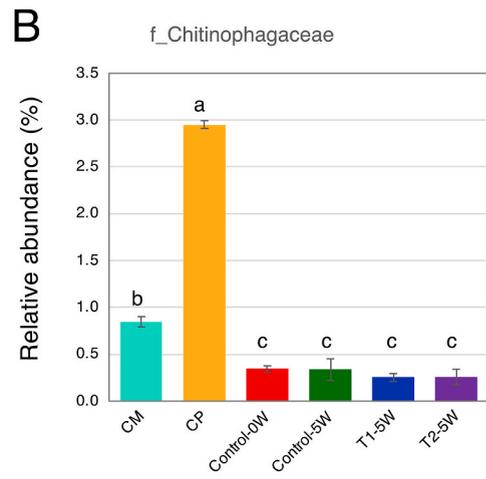
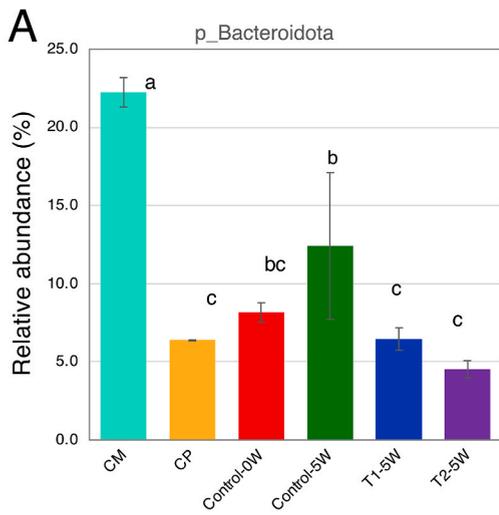


Fig. 5. Histogram of the LDA scores, revealing the most differentially abundant taxa among different treatments. Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (20:80) in Week 5 and T2-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5.



(caption on next page)

Fig. 6. Changes in the abundances of bacteria in casts of earthworms fed different diets. (A) Bacteroidota, (B) Chitinophagaceae, (C) *Marinomonas*, (D) *Azospirillum*, (E) *Pantaea* and (F) *Aeromonas*. Data represent means \pm SD ($n = 3$). CM = cow manure, CP = coir pith, Control-0W = vermicasts obtained from earthworms fed cow manure on the first day, Control-5W = vermicasts obtained from earthworms fed cow manure in Week 5, T1-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (20:80) in Week 5 and T2-5 W = vermicasts obtained from earthworms fed coir pith and cow manure (70:30) in Week 5. Different superscript letters in the same graph indicate significant differences among treatments as determined by ANOVA followed by Tukey's HSD test ($p < 0.05$).

biomarkers, including Planococcaceae, Microbacteriaceae, Xanthobacteraceae, Bacillaceae, Legionellaceae, Caloramatoraceae, Beijerinckiaceae and Kineosporiaceae. Conversely, in earthworms fed a mixture of coconut coir and cow manure at a 70:30 ratio, microbial families such as Peptostreptococcaceae, Clostridiaceae, Paenibacillaceae and 67_14 emerged as biomarkers (Fig. 5). These collective findings underscore the substantial shifts in the microbial community composition within earthworm casts in response to varying dietary conditions.

3.7. Impact of coir pith content on the microbial community in earthworm casts

When comparing the experimental data with previous reports, it was found that certain types of bacteria have the ability to degrade cellulose, hemicellulose and lignin [30–32], which are the main components of coir pith. These bacteria showed a decrease in abundance during vermicomposting. For example, in the sample of vermicompost produced by feeding earthworms with a mixture of coir pith and cow manure at a 70:30 ratio (w/w), a significant reduction in the quantity of bacteria of the phylum Bacteroidota, which produces cellulolytic enzymes, was observed (Fig. 6A). Additionally, bacteria from the family Chitinophagaceae, capable of degrading hemicellulose and abundant in coir pith samples, decreased in abundance after vermicomposting in all experimental sets (Fig. 6B). Similarly, there was a significant decrease in the abundances of *Azospirillum* and *Marinomonas*, which degrade lignin (Fig. 6C and D). Finally, *Pantaea*, lignocellulose degraders, were highly abundant in coir pith, and their abundance was decreased after vermicomposting (Fig. 6E).

Surprisingly, there was an increase in the abundance of bacteria of the genus *Aeromonas*, which are capable of producing toxins such as enterotoxin and haemolysin [33]. These bacteria were found in all vermicompost samples. For the sample where earthworms were fed solely with cow manure on the first day, species of the genus *Aeromonas* had a high relative abundance of 20.77 %. When earthworms were fed different types of feed, including only cow manure, coir pith mixed with cow manure at a 20:80 ratio (w/w) and coir pith mixed with cow manure at a 70:30 ratio (w/w), in the experimental set where worms were fed with coir pith mixed with cow manure at a 20:80 ratio (w/w) (T1-5 W), the relative abundance of *Aeromonas* decreased significantly. The relative abundance of *Aeromonas* at the 70:30 ratio (w/w) set was 19.44 %. In contrast, when earthworms were fed solely with cow manure and coir pith mixed with cow manure at a 20:80 ratio (w/w), the relative abundances of *Aeromonas* were only 5.04 % and 2.83 %, respectively (Fig. 6F).

Overall, the inclusion of a high proportion of coconut coir, specifically at a ratio of 70 % (w/w), in the earthworms' diet affected their growth, as evidenced by the reduced growth rates and increased mortality rates. This observation aligns with the higher presence of phenolic compounds detected in the earthworm casts derived from the experimental group where coconut coir constituted 70 % of the feed mixture. The elevated levels of phenolic compounds in the vermicasts can be attributed to a decline in the abundances of certain bacterial groups effectively degrading lignocellulose. The decreased abundances of bacteria proficient in phenolic compound degradation may impede the optimal breakdown of lignocellulose, thereby exerting detrimental effects on other beneficial bacteria within the worm's digestive system.

4. Discussion

4.1. Impact of coir pith on earthworm growth, survival and phenolic compound accumulation

Coir pith, a fibrous material that binds coir fibres in coconut, is lignocellulosic biomass. Raveendran et al. [34] revealed that coir pith comprises 31.2 % lignin, 28.6 % cellulose and 15.3 % hemicellulose. As a byproduct of the coconut industry, it is produced at high amounts [35]. The inherent moisture retention and aeration properties of coir pith create favourable conditions for earthworm activities. Using coir pith in the diet of earthworms facilitates the recycling of a significant waste material that would otherwise contribute to landfill accumulation [36]. Moreover, it offers a sustainable and cost-effective approach to producing high-quality earthworm casts, commonly known as vermicompost, which can serve as nutrient-rich organic fertiliser [37]. However, it is important to note that coir pith should not be the sole food source for earthworms because of its high phenolic compound content [38]. Pundee et al. [20] demonstrated the adverse effects of these compounds on the growth and survival of the worms.

In this study, *E. eugeniae* earthworms were fed three different diets: cow manure only and coir pith mixed with cow manure (20:80 and 70:30 ratios (w/w)). After 5 weeks, the group fed with 70 % coir pith had the lowest body weight and highest mortality (Table 1). The levels of phenolic compounds in worm casts were lowest in the cow manure-only group and highest in the coir pith mixtures, corresponding to the amount of coir pith used (Table 2). Earthworms fed coir pith showed slower growth and higher mortality compared to the group that only received cow manure. These findings suggest that the diet significantly affected the growth and survival of the earthworms. The decrease in body weight and the increased mortality rate observed for the earthworms fed with coir pith could be attributed to the accumulation of toxic phenolic compounds in their gut. Coir pith contains lignocellulosic compounds,

which can be broken down and detoxified by specific bacteria [39–41]. However, when earthworms consume large quantities of coir pith, this may disrupt their digestive system as certain bacteria capable of degrading these compounds may not function effectively. Consequently, the earthworms experienced difficulties in feed digestion and nutrient absorption.

So far, there are no studies on the effects of phenolic compounds on feed digestion and nutrient absorption in earthworms. Nevertheless, there are several reports discussing this issue in humans. Phenolic compounds attach to various macromolecules found in food, including carbohydrates, lipids and proteins [42]. In a study investigation the addition of purified green tea polyphenols to the diet, starch retrogradation was reduced, which could be attributed to the interaction between the hydroxyl groups of tea polyphenols and the hydroxyl groups on the starch molecules. This interaction restricted the re-association of starch polymers during the retrogradation [42]. Furthermore, phenolic compounds can impede the activity of digestive enzymes such as α -amylase and amyloglucosidase through chemical interactions. This leads to enzyme precipitation, ultimately reducing their effectiveness in breaking down carbohydrate-rich foods. Phenolic compounds can also form covalent bonds with sugars, either through O-glucoside or, albeit less frequently, C-glycoside bonds [43]. This results in the formation of high-molecular-weight polymeric glycoside complexes, which either precipitate under certain pH conditions or remain unabsorbed due to their large molecular size, thus impacting the uptake of phenolics. The interaction between polyphenols and carbohydrates can delay carbohydrate digestion, prolonging the digestion process and potentially reducing glucose release and absorption [44]. Possibly, phenolic compounds have an impact on earthworms' digestive systems, beyond just inhibiting nutrient absorption. Phenolic compounds can directly affect the bacteria capable of producing lignocellulolytic enzymes, which are responsible for the digestion of coir pith within the worms' intestines. This could result in the release of phenolic compounds by undigested coir pith. According to Theisen et al. [45], tannic acid has attracted considerable attention due to its wide range of chemical and biological characteristics. Dabbaghi et al. [46] demonstrated the efficacy of tannic acid against *Staphylococcus aureus* and *Escherichia coli*, with the activity depending on the concentration of the phenolic hydroxyl groups. Tannic acid has antibacterial activity against both Gram-positive and Gram-negative bacteria. The effectiveness of tannins in exerting antibacterial effects can be attributed to their ability to penetrate the bacterial cell wall and reach the internal membrane, leading to interference with the cell's metabolism and subsequent destruction. In Gram-positive bacteria, tannins exhibit rapid activity. However, in Gram-negative bacteria, their action is slower due to the presence of a bi-layered membrane. Given that Gram-negative bacteria are particularly harmful and associated with specific diseases, it is crucial to extensively investigate this bacterial group [47,48].

4.2. Impact of phenolic compounds in coir pith on the microbial diversity of earthworm casts

In this study, we compared the initial microbiota composition with the microbiota found in casts produced by earthworms fed different diets over a 5-week period. Analysis of the bacterial communities in the earthworms and their cast samples revealed notable variations. Rarefaction curve analysis demonstrated that the sequencing depth was adequate to capture the richness of the bacterial community. Furthermore, α -diversity analysis revealed a significant influence of diet on the gut bacterial communities, with cow manure (CM) exhibiting higher diversity compared to coir pith (CP) and the resulting vermicompost casts. The β -diversity analysis, using PCoA and PCA, revealed differences in microbial profiles among the casts produced from cow manure and vermicomposting mixtures. These results underscore the impact of coir pith proportion on the structural composition of the bacterial community within the earthworms and their vermicasts. Moreover, they provide valuable insights into the microbial ecology of the vermicomposting process (Figs. 1 and 2). Further investigations into the microbial community composition at the phylum and family levels through cluster analysis unveiled obvious patterns associated with the different diets and the digestive processes of earthworms. The abundance and distribution of bacterial families exhibited variations depending on the diet, e.g., cow manure, coir pith or their mixtures. Notably, the abundances of certain bacterial families changed over time, suggesting the influence of diet on microbial composition. Intriguingly, specific bacterial families experienced a decrease in abundance or completely disappeared following their passage through the digestive and excretory systems of the earthworms. These findings highlight the dynamic nature of the microbial community residing in the earthworms' gut and its response to varying dietary inputs (Figs. 3–5).

Numerous studies have investigated the impacts of different food types on the composition and abundance of bacteria involved in vermicomposting. Thakuria et al. [49] examined gut wall-associated bacterial communities in different ecological groups of earthworms, namely anecic earthworms (*Lumbricus terrestris* and *L. friendi*) and endogeic earthworms (*Aporrectodea caliginosa* and *A. longa*). The authors found that whilst all identified bacterial members were present in the soil, their relative abundances on the gut walls varied depending on the ecological group and habitat conditions, such as food quality and availability. This indicates the influence of both the earthworms' ecological group and habitat on the composition of the gut wall-associated bacterial community. Aira et al. [50] investigated the influence of earthworm gut transit on the bacterial community in horse, cow and pig manure and found substantial differences in the microbial community composition between the original manure and the resulting earthworm casts. The type of manure and the process of gut transit significantly influenced the presence, abundance and phylogenetic composition of bacterial groups, suggesting the importance of the earthworm-manure interaction and gut transit in shaping the microbial communities involved. In a study by Liu et al. [51], the gut microbiota of *E. fetida* earthworms was examined in relation to the nutrient availability of their habitat. Earthworms in nutrient-poor substrates, such as ore powder or mineral soil, showed higher abundances of Proteobacteria and Bacteroidetes in their gut microbiota compared to those in organic-rich compost soil. These microbial communities potentially aid earthworms in nutrient acquisition and metabolic adaptations, contributing to their survival in nutrient-poor environments.

4.3. Impact of coir pith-based diet and experimental period on bacterial community dynamics in vermicompost

In this comprehensive study, a discernible shift in the relative abundances of bacterial taxa was observed throughout the

vermicomposting period, as illustrated in Figs. 3C and 6. Across all experimental conditions, encompassing diverse food sources such as cow manure and coir pith, a coherent pattern emerged: a notable reduction in the relative abundances of specific bacterial taxa. Remarkably, taxa such as *Bacteroidota*, Azospirillum, Chitinophagaceae, *Marinomonas*, *Pantaea* and Pseudomonadaceae, commonly associated with cow manure, showed decreased abundances over time with different dietary substrates. Furthermore, the vermicomposting period greatly affected these microbial dynamics. Importantly, investigations at the beginning and the end of the experiment point to variations in the relative abundances of bacteria within each treatment. The pH values across all treatments ranged from 6 to 7, suggesting that the microbial communities within each treatment were resilient to pH fluctuations, emphasising the robustness of these communities in the face of varying pH conditions.

The bacteria described in this study can degrade complex organic compounds such as lignocellulose, which is a crucial component of plant cell walls [52,53]. Gharechahi et al. [54] reported *Bacteroidota* as one of the dominant bacterial groups of the rumen microbiota, contributing to the degradation of lignocellulosic polymers in the cattle's diet. The presence of polysaccharide utilisation loci (PULs) suggests their efficient use of polysaccharides in the rumen. Additionally, bacteria commonly found in coir pith, including Chitinophagaceae, *Marinomonas*, and *Pantoea*, exhibited decreased abundances across all treatment groups. Previous studies have highlighted the lignocellulose-degrading ability of Chitinophagaceae bacteria [55,56], whereas the genus *Marinomonas* is known for its production of laccase enzymes, which assist in lignin degradation. Phenotypically, the genus *Marinomonas* exhibits similarities to other aerobic marine bacteria characterised as gamma-proteobacteria with polar flagellation, strict aerobes and having a G + C content below 50 % [57]. In a previous study by Jimenez-Juarez et al. [58], *Marinomonas mediterranea* produced alkali and halide-resistant multipotent oxidase 1 (PPO1). *Pantoea ananatis* Sd-1 exhibited the ability to degrade lignocellulosic polysaccharides and lignin, and this strain has a diverse range of enzymes essential for cellulose and hemicellulose degradation, as evidenced by its lignocellulolytic enzyme repertoire. Genomic analysis of *P. ananatis* Sd-1 identified 154 genes responsible for potential carbohydrate-active enzymes (CAZy), surpassing other cellulolytic and ligninolytic bacteria in quantity [59]. Whilst in the present study, the relative abundance of lignocellulose-degrading bacteria decreased, that of bacteria associated with animal diseases, including *Aeromonas*, increased. Our study revealed a higher abundance of *Aeromonas* in the cast of earthworms fed a diet containing 70 % coir pith. *Aeromonas* species are Gram-negative bacteria with the ability to grow either aerobically or anaerobically, widely distributed in different environments. They can proliferate and produce enterotoxin and haemolysin at temperatures as low as 4 °C [60]. Specifically, *A. hydrophila* poses a significant threat to aquaculture, resulting in mass mortality and substantial economic losses in farm-raised crayfish. A study by Jiravanichpaisal et al. [61] demonstrated that *A. hydrophila* isolated from freshwater crayfish is highly pathogenic, causing rapid and complete mortality when injected with a high bacterial load. Histopathological analysis revealed extensive tissue damage and infiltration of haemocytes in various organs. The extracellular products of *A. hydrophila* were lethal to crayfish, inducing cytotoxicity in haemocytes and haematopoietic cells, both *in vivo* and *in vitro*. The presence of endotoxin genes suggests their involvement in crayfish mortality.

Vermicomposting using a diet containing 70 % coconut coir reduced the abundances of cellulose, hemicellulose and lignin-degrading bacteria. This decrease led to higher phenolic compound levels in worm casts, potentially affecting earthworm growth and survival. Furthermore, all vermicompost samples showed an increase in the abundances of potentially harmful bacteria, including *Aeromonas*. These findings emphasise the significance of considering the earthworm diet's composition and its influence on the microbial community and vermicomposting results. Furthermore, it is advisable to investigate the initial earthworm stocking density in vermicomposting using coir pith as a substrate. Devi et al. [62] highlighted that higher earthworm stocking densities can significantly improve vermicomposting efficiency. Their study also revealed that earthworm activity greatly affected the microbial community structure and fatty acid profiles in vermicompost, with implications for nutrient cycling and soil ecosystem health. The authors emphasise the role of microbial phospholipid fatty acid (PLFA) analysis in regulating metal availability in lignocellulosic vermicompost, offering potential applications for enhanced metal removal in vermicomposting systems.

5. Conclusion

This study examined the use of coir pith in the diet of earthworms in vermicomposting. Coir pith is a byproduct of the coconut industry that can be recycled to produce organic fertilisers, but it should not be the sole food source for earthworms due to its high phenolic compound content. Earthworms fed with coir pith showed a slower growth, higher mortality and increased accumulation of toxic phenolic compounds in their gut. The study also analysed the impacts of phenolic compounds on the diversity and abundance of bacteria in earthworm casts. The different diets greatly affected the microbial composition of the vermicasts. The abundances of lignocellulose-degrading and potentially harmful bacteria, such as *Aeromonas*, were negatively and positively affected, respectively, by the coir pith inclusion level. These findings emphasise the need to consider earthworm diet composition and its effect on the microbial community and vermicomposting outcomes. Further research is necessary to understand how phenolic compounds affect earthworm digestion, nutrient absorption and their interactions with lignocellulolytic bacteria. Such knowledge can serve as a basis for the optimisation of the vermicomposting of agricultural material with a high lignocellulosic content and for the maximisation of the potential of coir pith as a sustainable substrate for earthworm diets.

CRedit authorship contribution statement

Kanokwan Pundee: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Saengchai Akeprathumchai:** Writing – review & editing, Writing – original draft, Conceptualization. **Sударut Tripetchkul:** Writing – review & editing, Conceptualization. **Lakha Salaipeh:** Writing – review & editing, Writing – original draft,

Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e22945>.

Data availability

The authors affirm that the information substantiating the conclusions of this research is included in the article and its supplementary material. Data associated with this study has been deposited at L. Salaipeth, S. Tripetchkul, S. Akeprathumchai, K. Pundee, Unveiling the Microbial Dynamics in Vermicomposting with Coir Pith as Earthworm Substrate, Mendeley Data, V3, 2023, doi: <https://doi.org/10.17632/kvfk7mf9vz.3>.

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