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Fungal community dynamics and pathogenic risk in composting, vermicomposting and leachate systems amended with rabbit manure ☆

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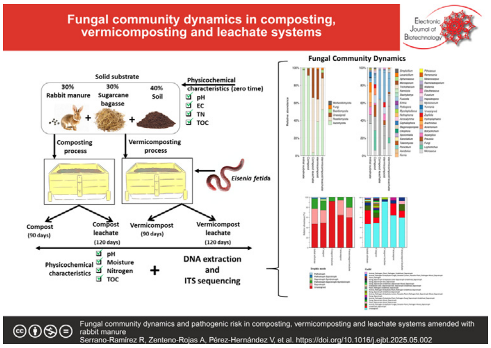
GRAPHICAL ABSTRACT

Fungal community dynamics and pathogenic risk in composting, vermicomposting and leachate systems amended with rabbit manure



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ABSTRACT

Background: Understanding the dynamics of fungal communities in composting and vermicomposting systems is essential for optimizing waste management practices and minimizing pathogen risks. For this reason, this study assessed the fungal community structure and potential pathogenic risks in composting, vermicomposting and leachate systems amended with rabbit manure, using Illumina's MiSeq platform for internal transcribed spacer (ITS) sequencing and FUNGuild analysis.

Abbreviations: ALDEx2, Analysis of Differential Abundance taking Sample Variation into Account; AOAC, Association of Official Analytical Chemistry; ASVs, Amplicon Sequence Variants; CAP, Constrained Analysis of Principal Components; CLR, Centered Log-Ratio; DESeq2, Differential Expression Analysis for Sequence Count Data; DNA, Deoxyribonucleic Acid; EC, Electrical Conductivity; FUN-Guild, Fungal Guild (tool for categorizing fungal taxa); ITS, Internal Transcribed Spacer; NCBI, National Center for Biotechnology Information; PCR, Polymerase Chain Reaction; pH, Potential of Hydrogen; QIIME2, Quantitative Insights Into Microbial Ecology version 2.0; SRA, Sequence Read Archive; TN, Total Nitrogen; TOC, Total Organic Carbon; UNITE, UNITE is a database for the molecular identification of fungi (it serves as a specific name for a resource).

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Results: Phylotypes from Basidiomycota were predominant in all treatments, while the pathogenic genus *Microascus*, initially abundant (37%), showed a significant reduction to 5% and 7% following composting and vermicomposting, respectively, and to 1% and 13% in their leachates. Given *Microascus*'s association with human skin diseases, proper handling of organic waste is critical before its agricultural use. In contrast, the FUNGuild analysis revealed a high abundance of saprotrophic fungi such as *Aspergillus*, *Preussia, Botryotrichum*, and *Acremonium* known for producing enzymes that promote nutrient cycling and soil fertility.

Conclusions: These findings highlight the potential for vermicomposting to reduce pathogen risks while enhancing fungal-driven nutrient recycling, offering practical insights for sustainable agriculture and organic waste management.

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1. Introduction

The sustainable management of animal organic waste has become a critical issue in modern agriculture, as the increasing reliance on chemical fertilizers is contributing to soil degradation, environmental contamination, and reduced biodiversity [1]. Composting and vermicomposting are effective biotechnological solutions for converting organic waste into valuable soil amendments. Composting, a process driven by microbial degradation [2], and vermicomposting, which involves the action of earthworms, both enhance nutrient availability, support microbial life, and improve soil structure [3,4]. These processes are essential for sustainable agricultural practices [5]. Among the byproducts of these processes, worm leachates have gained particular attention due to their ability to supply essential plant nutrients and promote soil health [6,7].

Fungal communities are pivotal to these processes due to their ability to degrade recalcitrant organic compounds, such as cellulose and lignin, which are difficult for bacteria and other microorganisms to break down [8,9]. Saprotrophic fungi are critical in producing enzymes that drive the breakdown of organic matter, releasing essential nutrients such as nitrogen and phosphorus into the soil, thus supporting plant growth and increasing crop yield [10]. Moreover, these fungi contribute to long-term soil health by improving nutrient cycling and organic matter stabilization [11]. The presence of a rich and diverse fungal community in vermicompost is therefore associated with improved soil fertility and enhanced crop productivity [12,13]. In addition to their role in nutrient cycling, fungal communities also contribute to the ecological balance of the soil microbiome by interacting with other organisms, including bacteria and plants. Their ability to produce a variety of secondary metabolites and enzymes enables them to play an essential role in suppressing plant pathogens and promoting plant growth [14,15]. Composting and vermicomposting, therefore, create environments where beneficial fungi thrive, facilitating soil resilience and reducing the need for chemical inputs in agriculture.

However, not all fungi present in organic waste are beneficial. Certain pathogenic fungi, such as species within the genus *Microascus*, can pose health risks when raw animal waste is applied directly to agricultural fields. *Microascus* is known to cause human skin diseases, particularly in environments with high exposure to untreated excreta [16]. This underscores the importance of managing organic waste properly before it is used as a soil amendment, to reduce the risk of pathogen transmission [12,13].

Despite the recognized importance of fungi in organic waste degradation and soil fertility, there remains a significant gap in our understanding of fungal community dynamics in composting and vermicomposting systems, particularly when using specific animal manures such as rabbit manure. Most studies have focused on bacterial communities or have used cattle or pig manure as substrates [17], leaving the role of fungi underexplored. Rabbit manure represents a unique and underutilized resource due to its high content of nitrogen, phosphorus, and organic matter, as well as its relatively dry consistency and low odor compared to other types of animal waste. Its physicochemical properties make it particularly suitable for composting and vermicomposting, yet few studies have investigated how it shapes fungal community composition and function.

To improve the efficiency of these bioconversion processes, rabbit manure was combined with sugarcane bagasse, a major agro-industrial byproduct in Mexico, widely available in sugar-producing regions. Sugarcane bagasse was selected as a co-substrate due to its high lignocellulosic content, which serves as a carbon source and structural bulking agent, enhancing aeration, moisture retention, and microbial colonization. The combination of rabbit manure and sugarcane bagasse not only reflects their availability and complementary properties but also provides an ideal matrix for studying fungal succession and functionality during the transformation of organic waste through these biotechnological approaches. However, the influence of this substrate combination on fungal community dynamics in composting and vermicomposting systems remains poorly understood. For this reason, this study aims to fill this knowledge gap by comparing the fungal communities in composting and vermicomposting systems amended with rabbit manure, focusing on their composition, diversity, and potential implications for pathogen control and sustainable agricultural practices. Understanding the dynamics of these fungal communities is crucial for improving organic waste management strategies and maximizing their agricultural benefits.

2. Materials and methods

2.1. Experimental setup and sample collection

The composting and vermicomposting experiments were conducted using rabbit manure as the primary substrate, mixed with sugarcane bagasse and soil in a ratio of 30:30:40% (w/w/w). For the vermicomposting process, a total of 10 mature earthworms were introduced into each experimental unit. In contrast, the composting process was carried out without earthworms. The rabbit manure and earthworms (*Eisenia fetida*) were sourced from a local farm "Rancho Luanda" in the municipality of Ocozocoautla de Espinosa (16°45'36.7" N, 93°22'32.1" W), Chiapas, Mexico. The soil was collected from an experimental field at the Tuxtla Gutierrez Institute of Technology", and sugar cane from a local producer. The experimental units were carried out in polyvinyl chloride (PVC) containers measuring 40 cm in long, 30 cm in wide and 20 cm in deep, with a 15° inclination to collect the leachates [16].

Four treatments were set up ($N = 4$ for each treatment): compost, compost leachate, vermicompost, vermicompost leachate. The treatments were conducted over a period of 90 d in shaded conditions, with periodic homogenization every 7 d to prevent anaerobic conditions [18]. The room temperature during the composting and vermicomposting processes ranged between 23 and 28°C. Moisture content was maintained at 50% of the soil's water retention capacity by adding water every third day, with monitoring conducted using an IRROMETER SR tensiometer to ensure consistent moisture levels and prevent desiccation. Following this period, only the leachates were stored in complete darkness for an additional 30 d for use as organic fertilizer.

2.2. Physicochemical analysis

The physicochemical properties of the initial substrate, compost, vermicompost, and their respective leachates were analyzed to assess changes in pH, electrical conductivity (EC), total nitrogen (TN) and total organic carbon (TOC), according to the Official Association of Analytical Chemistry [19]. The pH and EC were measured using a Mettler Toledo® Model S220 (New York, USA) digital pH meter.

2.3. DNA extraction and ITS sequencing

Samples of the initial substrate (zero time), compost (90 d), vermicompost (90 d), and their respective leachates (120 d) were collected at their respective time points for molecular analysis. Total DNA was extracted from 1.0 g of solid samples (compost and vermicompost) and 1.5 g of sediment from the leachates [16] using chemical [20], thermal [21], and lysozyme lysis [22] methods. The twelve metagenomic DNA samples were stored at –21°C until further PCR amplification.

The internal transcribed spacer (ITS) region was amplified using ITS1F (5′- CTA CGG GIG GCW GCA G -3′) and ITS2R (5′- GAC TAC HVG GGT ATC TAA -3′) primers, with an Illumina adapter and an 8 bp unique barcode for each sample [23,24]. The amplification protocol followed Illumina's two-step PCR procedure (15044223 Rev. B). The PCR reactions were carried out in triplicate, purified using FastGene™ columns (Nippon Genetics, Co., Ltd), and quantified using a Nanodrop™ 3300 spectrophotometer (Thermo Fisher Scientific Inc., Suwanee, CA). Equimolar concentrations of amplicons were pooled and sequenced using Illumina's MiSeq 2 × 300 paired-end platform at Macrogen Inc. (DNA Sequencing Service, Seoul, South Korea). All sequencing data generated in this study were deposited in the NCBI Sequence Read Archive (SRA) under BioProject ID PRJNA1077090.

2.4. Bioinformatic data analysis

The raw sequencing reads were quality-checked using FastQC tool [25] and processed using the "Quantitative Insights Into Microbial Ecology v2.0" pipeline (QIIME2 v2022.2; [26]). Demultiplexing procedure was done using the 8-pb barcodes used during PCR-amplification. Only the forward reads (R1) were used for further analysis due to the low quality of the reverse reads. Reads were denoised, trimmed, and dereplicated using the DADA2 algorithm [27], with trimming parameters set at --p-trim-left 13 and --p-trunc-len 260.

Taxonomic classification was performed using the UNITE database for fungal ITS sequences [28]. A total of 27,294 high-quality sequences were obtained, representing 234 amplicon sequence variants (ASVs). Fungal community functionality was predicted using the FUNGuild v1.1 tool [29] to classify the fungal taxa into trophic modes based on their nutrient acquisition strategies.

2.5. Statistical data analyses

All statistical analyses were performed in the R environment (v4.2, R Core Team, [30]) using the RStudio interface (version 2023.03.0, Posit Software, PBC). All physicochemical parameters were statistically analyzed using a one-way ANOVA followed by Tukey's HSD test to determine significant differences between treatments ($p < 0.05$). The artifacts generated from the QIIME2 pipeline (i.e., *qza* output files) were imported into R using the qiime2R package (v0.99, [31]) and converted into a phyloseq object [32]. The Phyloseq package (v1.46) was used for taxonomic summaries, relative abundance transformations per sample or treatment, and alpha diversity estimations through the tax_glom, merge_samples, and estimate_richness functions [32]. Data visualization was carried out using the ggplot2 package (v3.5; [33]).

A constrained analysis of principal components (CAP) was used to assess differences in the fungal community composition across treatments and to evaluate their relationship with physicochemical characteristics of vermicompost and leachates. CAP was determined using the vegan package (v2.6-4, [34]) with datasets transformed via centered log-ratio (clr) using the ALDEx2 package to mitigate bias inherent in compositional sequencing data [13,35]. Differential abundance analysis between treatments was performed using DESeq2 [36] and visualized using UpSetR plots to highlight the shared and unique fungal ASVs across treatments [37]. The relationship between fungal communities and physicochemical parameters was evaluated using CAP and correlation analysis.

3. Results

3.1. Physicochemical characteristics

The physicochemical analysis of the initial substrate, compost, vermicompost, and their respective leachates revealed significant changes in key parameters ($p < 0.05$, Table 1), including pH, EC, TN and TOC, which are crucial for determining the quality and potential applications of the final compost and vermicompost products as organic fertilizers. The pH of the initial substrate was nearly neutral, with a value of 7.0 ± 0.08, which is typical for raw animal manure mixed with organic material. After 90 d of composting and vermicomposting, the pH increased significantly ($p < 0.05$) to alkaline levels, reaching 7.9 ± 0.19 in compost and 8.3 ± 0.14 in vermicompost. Notably, the leachates showed similar pH, with values of 8.0 ± 0.05 and 8.0 ± 0.09 for compost and vermicompost leachates, respectively. The higher alkalinity in the compost, vermicompost and leachates suggests a more advanced stage of degradation, where microbial activity continues to break down organic compounds, releasing minerals that contribute to pH elevation.

The EC showed notable changes throughout the process. The initial substrate exhibited a moderate EC value of 2.75 ± 0.1 dS/m. However, during composting and vermicomposting, EC decreased to 0.75 ± 0.06 dS/m in compost and 0.73 ± 0.21 dS/m in vermicompost, indicating a reduction in soluble salts and a stabilization of the organic material. Interestingly, the EC in

Table 1
Physicochemical characterization of initial substrate and final products.

Treatment	pH	EC	TN	TOC
Initial substrate	7.0 ± 0.08^{b}	2.75 ± 0.01^{b}	4.77 ± 0.09^{e}	307.66 ± 0.57^{c}
Compost	7.9 ± 0.19^{a}	0.75 ± 0.06^{c}	8.16 ± 0.00^{d}	156.33 ± 0.57^{d}
Compost leachate	8.0 ± 0.05^{a}	8.02 ± 0.05^{a}	49.28 ± 0.38^{b}	411.49 ± 0.44^{b}
Vermicompost	8.3 ± 0.14^{a}	0.73 ± 0.21^{c}	9.20 ± 0.01^{c}	156.66 ± 0.57^{d}
Vermicompost leachate	8.0 ± 0.09^{a}	8.04 ± 0.09^{a}	92.41 ± 0.37^{a}	860.12 ± 2.74^{a}

Electrical conductivity (EC) is measured in dS/m; total nitrogen (TN) and total organic carbon (TOC) values are expressed on a dry weight basis in g/kg.
Values indicate the mean ± standard deviation ($n = 4$).
Values in the same row followed by different superscript letters (a, b, c, d or e) are significantly different ($p < 0.05$) among treatments.

leachates increased significantly, reaching 8.02 ± 0.05 dS/m for compost leachate and 8.04 ± 0.09 dS/m for vermicompost leachate, indicating a concentration of dissolved nutrients in the liquid phase.

The TOC in the initial substrate was 307.66 ± 0.57 g/kg, reflecting the high organic matter load typical of raw animal manure mixed with organic residues. During composting and vermicomposting, the TOC decreased significantly to 156.33 ± 0.57 g/kg and 156.66 ± 0.57 g/kg, respectively ($p < 0.05$), which can be attributed to the microbial degradation of organic matter and the respiration of carbon as CO_2. Conversely, TN increased from 4.77 ± 0.09 g/kg in the initial substrate to 8.16 ± 0.0 g/kg in compost and 9.20 ± 0.01 g/kg in vermicompost, likely due to the concentration of TN as organic matter is mineralized and carbon is lost as CO_2. Finally, the leachates showed even higher TN levels, with 49.28 ± 0.38 g/kg in compost leachate and 92.41 ± 0.37 g/kg in vermicompost leachate. These results demonstrate that leachates are rich in nitrogen and hold significant potential as liquid biofertilizers for agricultural use. However, due to their elevated nitrogen concentration, it is advisable to dilute them prior to application in order to minimize the risk of phytotoxic effects. A standard dilution of 1:10 (v/v) with water is commonly used for fertigation or foliar spraying.

3.2. Alpha diversity of fungal community

The alpha diversity of fungal communities, as indicated by richness and diversity indices, revealed significant variations across treatments (Table 2). The observed species and Chao1 indices showed the highest richness in the initial substrate (45.3 ± 3.3), which was significantly reduced in compost (24.7 ± 11.9), compost leachate (11.3 ± 5.4), vermicompost (5.1 ± 4.2), and vermicompost leachate (7.5 ± 2.5).

Regarding diversity, the Shannon index also indicated the highest diversity in the initial substrate (2.6 ± 0.1), which was significantly reduced in compost (1.9 ± 1.0), compost leachate (1.5 ± 0.7), vermicompost (1.0 ± 0.7) and vermicompost leachate (1.7 ± 0.2). In terms of evenness, as measured by the Simpson index (Table 2), the initial substrate exhibited the highest value (0.90 ± 0.0), indicating a more even distribution of fungal taxa. In contrast, compost (0.6 ± 0.3), vermicompost (0.5 ± 0.0), and compost leachate (0.6 ± 0.3) showed lower evenness, reflecting the dominance of certain fungal groups during the decomposition process. Interestingly, vermicompost leachate presented a Simpson index of 0.8 ± 0.0, suggesting a fungal community structure with greater evenness compared to the other processed treatments. This may indicate that the liquid environment of the leachate favors a more balanced distribution of fungal taxa, potentially due to different ecological pressures or nutrient availability.

The ACE index, used to estimate species abundance, mirrored the richness trends observed in the other indices, with the highest value in the initial substrate (45.3 ± 3.3) and a marked decrease across compost, vermicompost, and their respective leachates. These results indicate that the composting and vermicomposting processes significantly impacted the fungal community composition, reducing both species richness and evenness, particularly in the leachates. Vermicompost treatments generally showed slightly higher diversity compared to compost ones, especially in terms of species evenness.

3.3. Fungal community dynamics

The composition of the fungal communities varied significantly across treatments, as revealed by the relative abundances of different fungal phyla and genera. At the phylum level, Basidiomycota was the dominant group, being present in all five treatments with relative abundances of 98%, 92%, 3%, 38%, and 53% for the initial substrate, compost, compost leachate, vermicompost, and vermicompost leachate, respectively. Rozellomycota, another prominent phylum, showed variability in abundance, with values of 62%, 25%, and 36% in compost leachate, vermicompost, and vermicompost leachate, respectively. Unassigned fungal phylotypes were notable in compost leachate (33%) and vermicompost (32%), while other phyla contributed less than 5% to the total fungal community across all treatments (Fig. 1a).

At the genus level (Fig. 1b), the most abundant fungal phylotypes belonged to *Microascus*, which accounted for 37% of the initial substrate's community. However, its relative abundance

Table 2
Alpha diversity results of fungal community structure based on traditional indices.

Treatment	Observed	Chao1	Shanon	Simpson	ACE
Initial substrate	45.3 ± 3.3	45.3 ± 3.3	2.6 ± 0.1	0.9 ± 0.0	45.3 ± 3.3
Compost	24.7 ± 11.9	24.7 ± 11.9	1.9 ± 1.0	0.6 ± 0.3	24.7 ± 11.9
Compost leachate	11.3 ± 5.4	11.3 ± 5.4	1.5 ± 0.7	0.6 ± 0.3	16.5 ± 2.0
Vermicompost	5.1 ± 4.2	5.1 ± 4.2	1.0 ± 0.7	0.5 ± 0.0	8.0 ± 4.9
Vermicompost leachate	7.5 ± 2.5	7.5 ± 2.5	1.7 ± 0.2	0.8 ± 0.0	7.5 ± 2.5
F value	5.889	5.889	0.789	0.460	4.372
p value	0.013	0.013	0.560	0.762	0.044

Observed species (richness estimator).
ACE abundance-based coverage estimator.
Values indicate the mean ± standard deviation ($n = 4$).

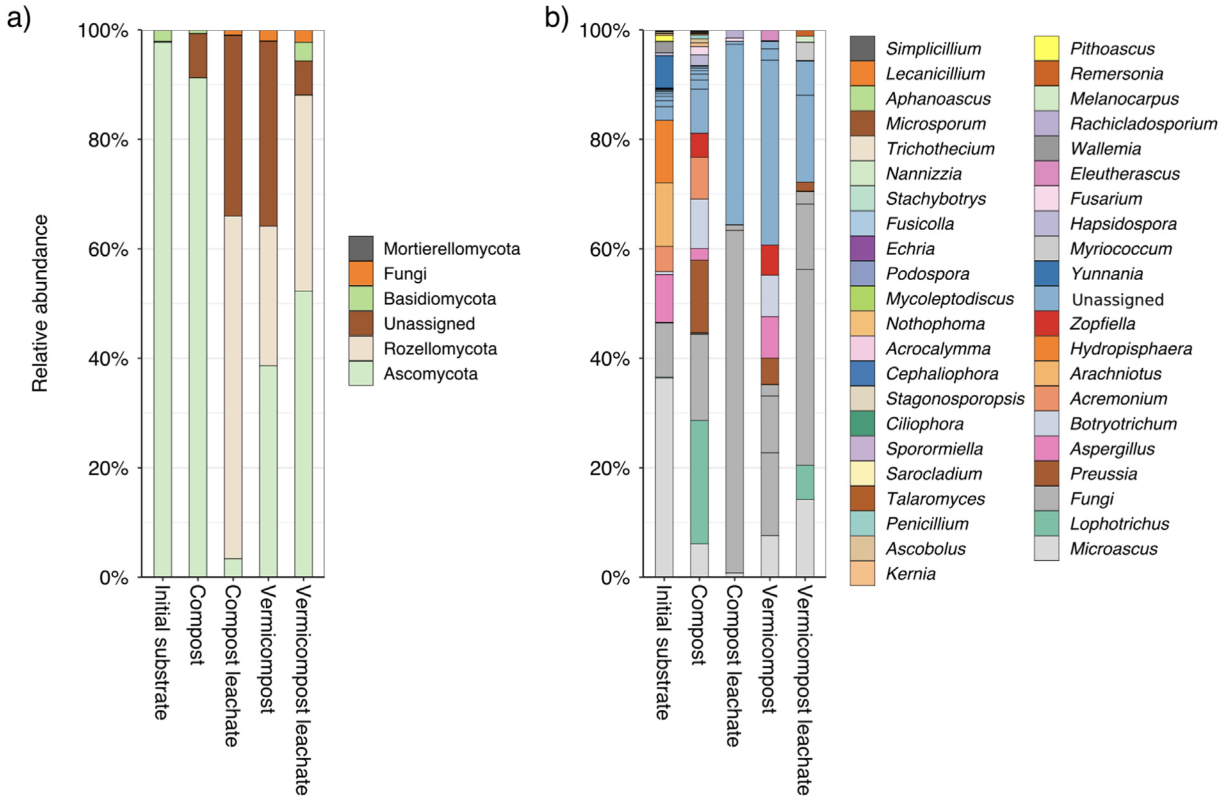

Fig. 1. Fungal community structure based on the relative abundance of amplicon sequence variants assigned up to a) the taxonomic level of phylum, and up to b) the taxonomic level of genus. Taxonomic groups and their corresponding colors are ordered from the most to the least abundant on average of all treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dropped to 5% in compost, 7% in vermicompost, and 13% in vermicompost leachate. Other genera such as *Aspergillus* showed 9% abundance in the initial substrate, reducing to 2.5% in compost but increasing to 7% in vermicompost. Additional dominant genera included *Hydropisphaera, Arachniotus, Acremonium, Lophotrichus*, *Zopfiella*, and *Preussia*, which displayed variable abundances across treatments.

The constrained analysis of principal components (CAP) highlighted distinct clustering patterns. The initial substrate and compost grouped together with negative CAP1 values, whereas the vermicompost leachates grouped with positive values (Fig. 2a). Additionally, members of *Acremonium* and *Myriococcum* correlated positively with carbon and nitrogen content, while *Preussia* displayed an inverse relationship. *Microascus* and *Aspergillus*

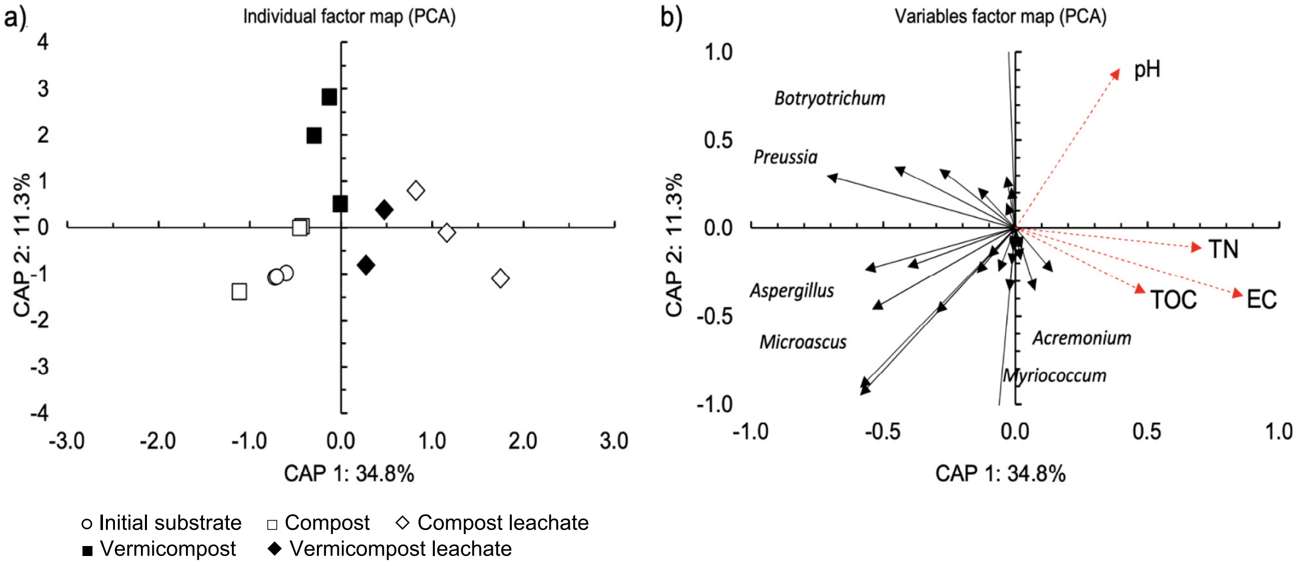

Fig. 2. Constrained principal component analysis (PCA) based on clr-transformed counts of fungal phylotypes at the genus level. (a) Ordination of samples according to their fungal community composition. Different symbols represent distinct sampling locations. (b) Biplot showing the correlation between fungal genera and environmental variables (red arrows): pH, total nitrogen (TN), total organic carbon (TOC), and electrical conductivity (EC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

separated along negative CAP2 values and were negatively correlated with the pH of the treatments (Fig. 2b).

3.4. Core fungal community and their effect

The core fungal community analysis showed a notable variation in the number of unique and shared amplicon sequence variants (ASVs) among treatments (Fig. 3). The initial substrate exhibited the highest number of unique ASVs ($n = 59$), followed by compost and compost leachate. The vermicompost and vermicompost leachate contained the lowest number of unique ASVs, suggesting a significant reduction in fungal diversity during the vermicomposting process.

The Upset plot (Fig. 3) highlights the shared ASVs among treatments, where the initial substrate and compost shared the greatest number of common ASVs, followed by their respective leachates. In contrast, vermicompost and leachate treatments had fewer shared ASVs with the initial substrate, indicating that the vermicomposting process selectively enriched or depleted specific fungal taxa.

Differential abundance analysis (Fig. 4) demonstrated that the composition of fungal communities was significantly influenced by the composting and vermicomposting processes. Compared to the initial substrate, composting led to a reduction in the relative abundance of genera such as *Preussia, Fusarium*, and *Penicillium*, while vermicomposting enriched genera including *Aspergillus, Acremonium, Pithoascus*, and *Hydropisphaera*. When comparing composting with vermicomposting (Fig. 4e–g), it was observed that vermicomposting significantly increased the relative abundance of genera such as *Aspergillus, Preussia, Botryotrichum*, and *Acremonium*, further highlighting the impact of vermicomposting on fungal community composition.

Overall, these results underscore the distinct effects of composting and vermicomposting processes on fungal diversity and community structure, and with vermicomposting fostering, a unique set of fungal species do not present in the composting treatments.

3.5. Functional prediction analysis of fungal community

The functional prediction analysis, based on the FUNGuild tool, revealed distinct nutrient acquisition strategies among the fungal communities across treatments (Fig. 5a). Saprotrophic fungi represented the most abundant functional group in all treatments, with relative abundances ranging from 20% to 29%, except in the compost leachate, where they were not detected. In all the samples, the pathotroph-saprotroph-symbiotroph fungi were consistently present, ranging between 5 and 20% across treatments. Conversely, saprotroph-symbiotroph, pathotroph-saprotroph and pathotroph categories were identified exclusively in the initial substrate and compost, where they represented less than 7% of the total fungal community (Fig. 5a).

Further analysis of the fungal guilds (Fig. 5b) indicated that saprotrophs were consistently abundant, ranging from >10% to <35% across all treatments. Fungi classified as Animal_Pathogen-Endophyte-Plant_Pathogen-Dung_Saprotroph-Undefined_Saprotroph were present at lower abundances (3–8%) across treatments. Interestingly, dung saprotrophs were identified exclusively in the vermicompost treatments, albeit at less than 5% relative abundance. Additionally, fungi classified as Plant_Saprotroph-Wood_Saprotroph were uniquely identified in the vermicompost leachate. These findings highlight the functional diversity of fungal communities across treatments and suggest a shift in the ecological roles of fungi in response to compost and vermicomposting processes, with vermicomposting fostering specific guilds, particularly those involved in plant and dung degradation.

4. Discussion

Soil fungi play a crucial role in various ecological processes, including carbon sequestration through interactions with plant life and the mineralization of nutrient [38]. In this study, the physicochemical characterization of the final products revealed significant changes in several key parameters. The pH significantly increased in the compost and vermicompost treatments compared to the initial substrate, which could be related to the degradation of organic matter and the release of basic nutrients, as reported in previous studies [39,40]. This increase in pH is a positive indicator of the stabilization of organic material, facilitating the biological activity of microorganisms involved in the process [41]. Notably, *Botriotrichum sp*., a fungal genus phylogenetically related to *Chaetomium sp*., includes species adapted to alkaline conditions [42], which both could benefit from the pH increase during composting

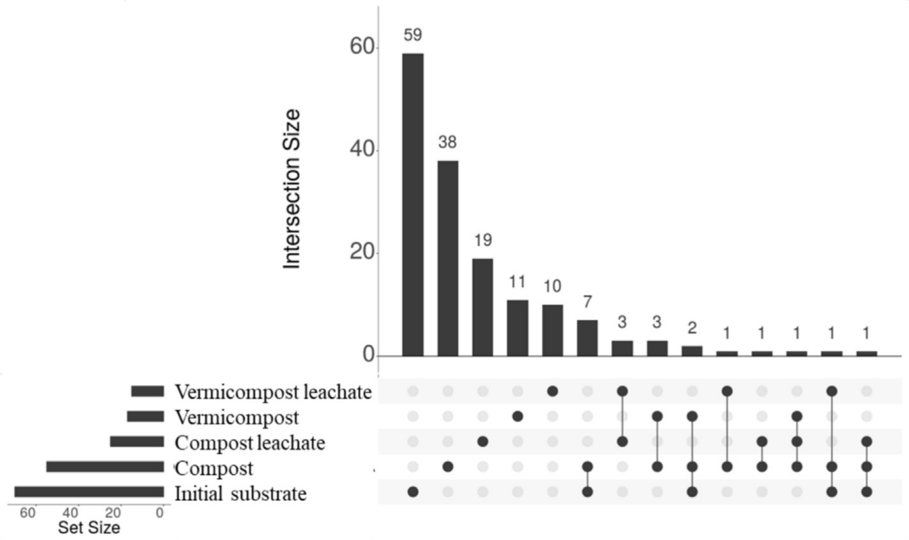

Fig. 3. Upset plot showing the number of unique and shared fungal amplicon sequence variants (fungal ASVs) between sets (treatments, $n = 5$). Set size (left-bar plot) shows the total fungal ASVs per treatment ($n = 5$), whereas the "Intersection size" ("y-axis", upper-bar plot) shows the number of unique/shared ASVs per treatment. Unique ASVs are shown in black bars with a single and black circular shape, whereas the number of shared ASVs between treatments is shown with black circular shapes connected with a vertical straight line.

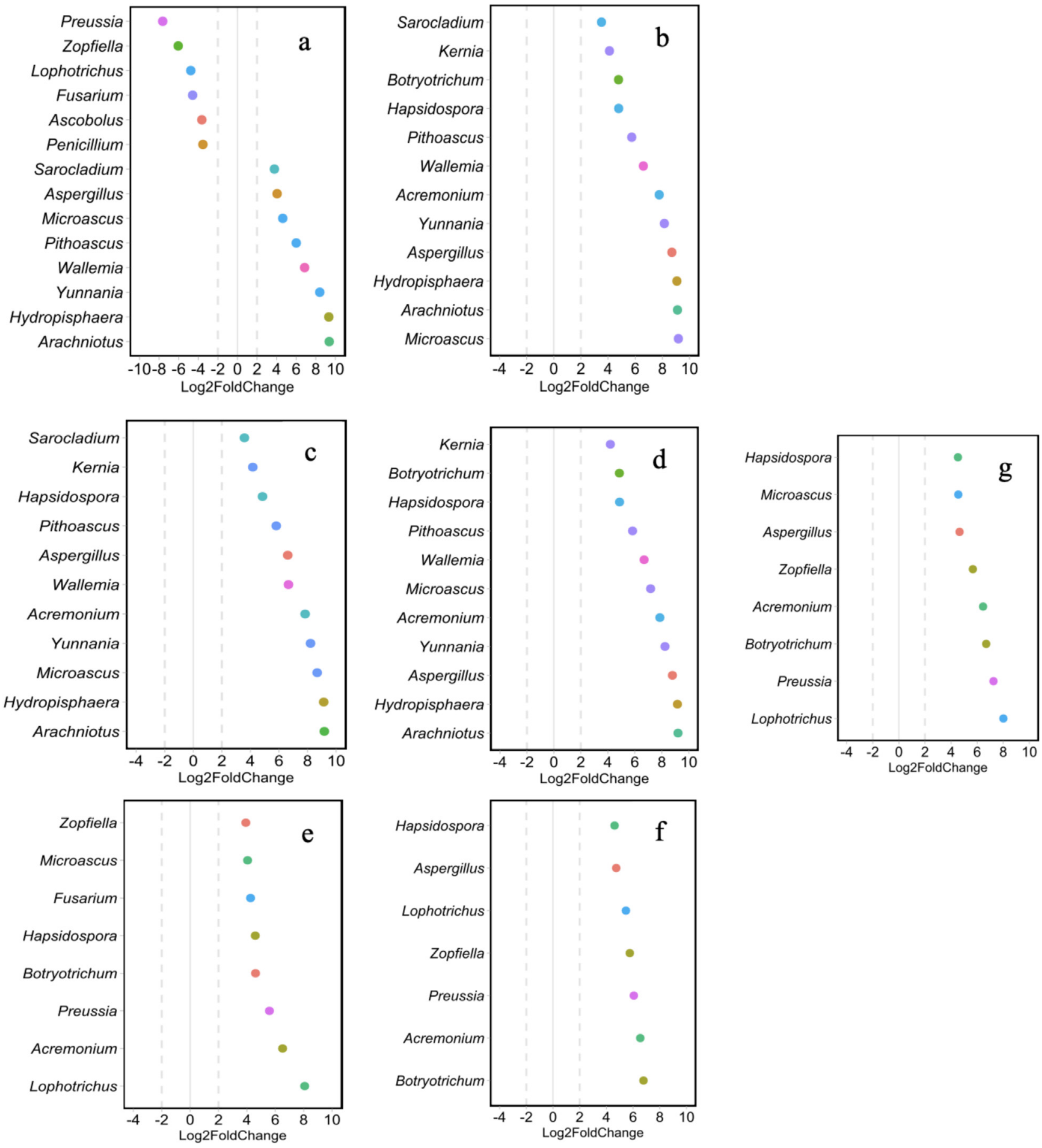

Fig. 4. Differential abundance analyses of fungal genera that were significantly affected by treatment application. Positive "Log2FoldChange" values indicate a significantly enriched group by treatment application, and those with a negative one showed the opposite, based on DESeq2 results. Comparisons made were a) initial substrate vs compost; b) initial substrate vs. compost leachate; c) initial substrate vs vermicompost; d) Initial substrate vs vermicompost leachate; e) compost vs vermicompost; f) compost vs vermicompost leachate; g) compost vs compost leachate.

and vermicomposting processes, as reflected in their increased abundance in our study. Conversely, the EC decreased in compost and vermicompost compared to the initial substrate. This reduction can be attributed to the leaching of soluble mineral salts into the leachate during the composting and vermicomposting processes. As water percolates through the substrate, it dissolves and carries away ions such as nitrates, potassium, and other soluble nutrients, reducing their concentration in the solid fraction. As a result, the leachate generated during composting and vermicomposting increased the EC content from 2.75 dS/m to 8.04 dS/m. This increase in EC may be related to the mineralization of soluble salts during the degradation of organic matter, which is a common phenomenon in composting and vermicomposting processes [43]. The high EC levels in the leachates indicate an enrichment in mineral nutrients, which could enhance their value as liquid biofertilizers.

During vermicomposting, nitrogen levels increase due to the secretion of body fluids and enzymes (e.g., proteases, lipases, cellulases, amylases, alkaline phosphatases, dehydrogenases, and ureases) during ingestion inside the worm's intestine [44,45,46].

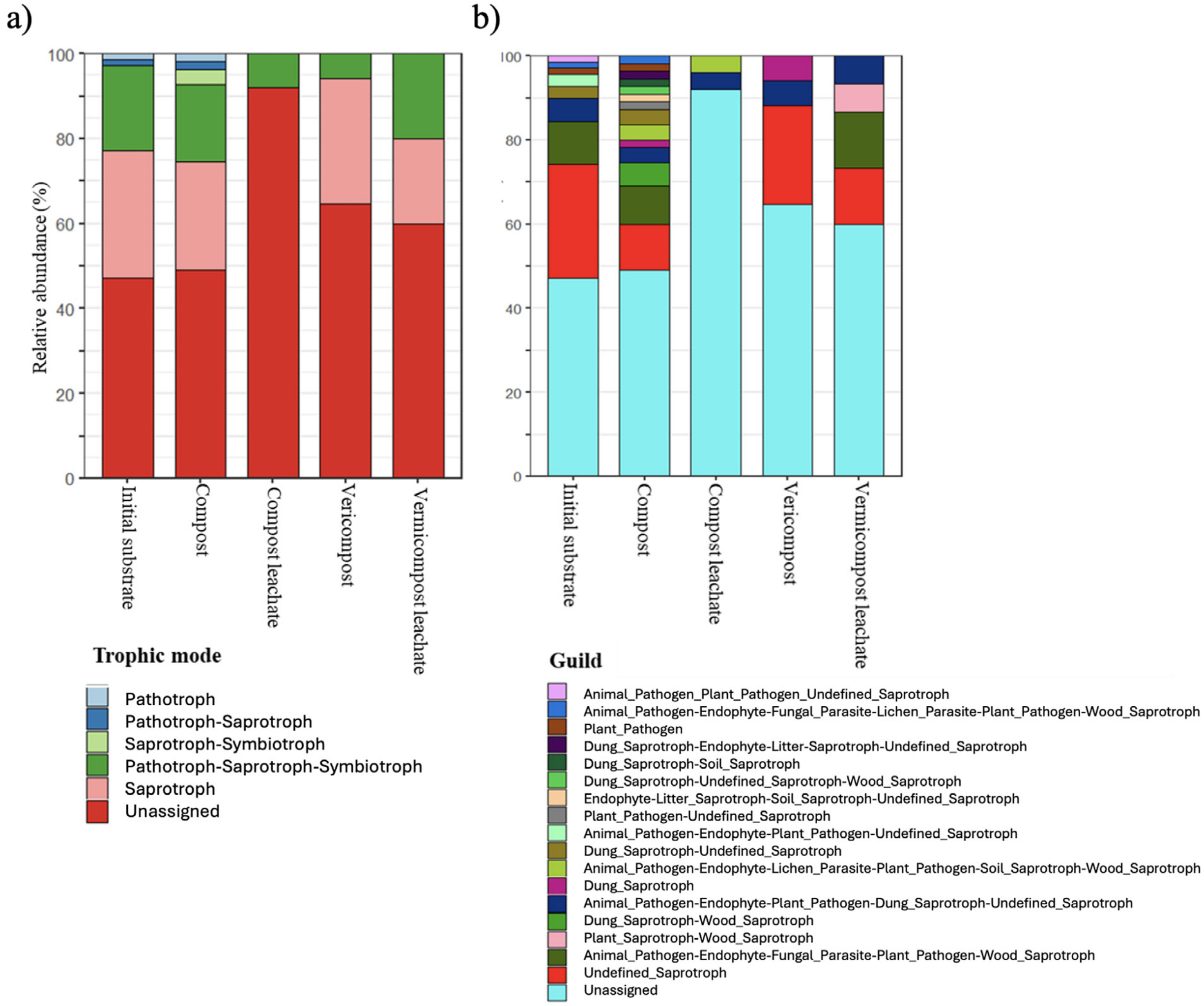

Fig. 5. Functional prediction analysis of the fungal community as inferred using FUN-Guild tool. (a) Barplot showing the relative abundance of annotated trophic modes (based on different fungal forms of receiving nutrients: pathotroph, saprotroph, symbiotroph) and (b) relative abundance of the guild mode based on fungal habitat source preference.

Microorganisms like *Aspergillus* sp. and *Acremonium* sp. secrete protease enzymes that aid in degrading organic material in the vermicomposting process, contributing to soil fertility through a mutualistic relationship with earthworms. This process generates compounds that enhance soil fertility [47]. Results indicated a significant increase in nitrogen content in both solid fractions (compost and vermicompost) and the liquid fractions (compost leachate and vermicompost leachate) compared to the initial substrate, with leachates exhibiting the highest nitrogen concentrations. Similar findings were reported by Serrano-Ramírez et al. [16] for leachates obtained from cow wastes, where substantial increases in nitrogen content were observed in leachate samples. Consequently, leachates have been utilized as organic and suitable biofertilizers in agriculture [7,45,48]. Conversely, the TOC content decreased during the composting and vermicomposting processes compared to the initial substrate. This decline could be attributed to the significant role that worms play in the mineralization of organic material [47]. Fungi such as *Preussia* sp. and *Myriococcum sp*., known for their cellulase production [49,50], actively contribute to microbial leading to the degradation of organic material respiration [51,52].

A particularly important finding demonstrates that the composting and vermicomposting processes not only significantly modify the composition of fungal communities but also promote the proliferation of fungi with key functional roles in organic matter degradation and nutrient recycling. The analysis of alpha and beta diversity showed a clear decrease in species richness and diversity as the composting and vermicomposting processes progressed. This is consistent with previous studies suggesting that the treatment of organic waste, particularly when composting and vermicomposting are involved, tends to select for specialized microbial communities adapted to the specific degradation conditions [53,54]. Despite the reduction in diversity observed in the leachates, the presence of specific saprotrophic fungi species, such as *Aspergillus*, *Acremonium*, and *Preussia*, suggests that these communities play a crucial role in the degradation of organic matter, particularly through the production of enzymes like cellulases and proteases, which facilitate the breakdown of recalcitrant compounds such as cellulose and lignin [50,55]. Moreover, members of *Aspergillus* are recognized as cellulolytic fungus that enhances polymer degradation when introduced into composting and vermicomposting processes [55]. Members of *Preussia* are involved in nitrogen transformation within composting and vermicomposting processes, regulating the conversion of NO_3^- and N in the middle layer [56], whereas members of *Acremonium* are commonly found in waste with high organic matter content [57].

Also, the increase in the abundance of genera such as *Aspergillus* and *Acremonium* in vermicompost highlights the importance of these fungi in improving soil fertility through the degradation of organic residues. These genera are well-known for their ability to secrete a wide variety of hydrolytic enzymes that break down complex plant matter, releasing essential nutrients that can be taken up by plants [8]. These findings are consistent with previous studies [16]. Vermicomposting not only accelerates the degradation of organic matter but also promotes the establishment of fungal communities. These fungi actively contribute to nutrient recycling and help improve soil structure. Additionally, the enrichment of fungi from the genus *Preussia*, known for its ability to participate in nitrogen transformation, suggests a positive impact on the stability and availability of this element in the final vermicompost products [56].

One of the most noteworthy aspects of the study was the identification of *Botryotrichum* in the vermicompost leachates, a genus previously associated with alkaline environments. This finding is particularly relevant given the tendency for an increase in pH in the leachates observed in this study. *Botryotrichum* is known for its ability to break down complex organic materials in environments with elevated pH levels, suggesting that vermicomposting not only effectively reduces pathogenicity but also promotes the proliferation of fungi adapted to specific chemical conditions [42]. This could be of particular interest in the management of organic waste in alkaline soils or in areas where pH adjustment is required. The presence of *Botryotrichum* and other saprotrophic fungi in the vermicompost not only demonstrates their ability to thrive under these conditions but also underscores their potential in the biotransformation of organic matter into high-value agricultural products.

Furthermore, a large number of phylotypes belonging to Ascomycota are saprophytes, known for degrading plant residues and soil organic matter [58]. Fungi within Basidiomycota exhibit a strong ability to degrade lignocellulose [11], representing unique groups within each evaluated treatment. *Acremonium* was found in all composting and vermicomposting treatments but not in the initial substrate. Their members are geographically widespread and ecologically diverse, e.g., they colonize various substrates, serve diverse as a biological control improving drought tolerance in grasses, enhancing nectar production in beans, and boosting plant resistance to pathogens [59,60,61,62].

A particularly important finding is the significant reduction in pathogenic fungi such as *Fusarium* during the vermicomposting process. This suggests that this method could be an effective tool to reduce the risks associated with the presence of pathogens in animal waste before its agricultural application, thereby improving the safety of the final products. Previous studies have also noted the capacity of vermicomposting to mitigate the presence of pathogens, reinforcing its value as a sustainable technology for organic waste management and the production of safe biofertilizers [52,63].

The FUNGuild analysis revealed a higher relative abundance of saprotrophic fungi in all samples, except in the compost leachate, reaffirming their key role in organic matter degradation and nutrient recycling. Additionally, the identification of functional categories such as "Pathotroph-Saprotroph-Symbiotroph" in all treatments indicates that these communities not only participate in the degradation of organic residues but may also play important roles in symbiotic interactions with plants and other organisms, which could have direct implications for improving agricultural productivity [64]. The higher abundance of saprotrophs in the vermicompost treatments suggests that this process promotes functionally active fungal communities that can offer additional benefits for soil fertility, not only through nutrient recycling but also by producing secondary metabolites and enzyme complexes that promote the biotransformation of recalcitrant compounds [15].

Fungi classified as Animal_Pathogen-Endophyte-Fungal_Parasite-Plant_Pathogen-Wood_Saprotroph exhibited considerable relative abundance in the initial substrate but were not detected in the compost and vermicompost leachates. This suggests that the composting and vermicomposting processes may be effective in reducing the presence of potentially pathogenic or undesirable fungal groups, likely due to competition with beneficial microbes and changes in physicochemical conditions. The elimination of these functional groups from the final products may contribute positively to soil health and agricultural safety by minimizing the risk of pathogen transmission to crops and soil ecosystems [65]. The broad classification of fungi into saprophytes, which degrade organic remains for nutrients, and symbionts, which live in symbiosis with plants, is essential in agricultural production [14]. Fungi play a crucial role not only due to their beneficial attributes but also because of their potential pathogenicity in crop chains. Understanding the dynamics between saprophytic and pathogenic fungi is vital [63].

Therefore, composting and vermicomposting processes generate fungal biomass suitable for environmentally beneficial applications such as bioremediation and agriculture by utilizing the solid or liquid products obtained [66]. Composting facilitates the controlled conversion of degradable organic materials and waste into stable products with the assistance of fungal communities and other microorganisms [67]. Recycling residual biomass *in situ* into high-value products, such as compost for soil amendment and plant treatment, is currently a feasible strategy. This is largely due to the benefits provided by the microorganisms within fungal communities [68], particularly their role in enhancing soil fertility through compost application [1]. Moreover, fungal microbial activity and composition play a crucial role in the manure stabilization process, facilitating nitrogen retention and the gradual release of phosphorus, along with a reduction in EC, thereby producing high-quality substrates for agricultural use [69].

Furthermore, vermicomposting has been shown to enhance the production of pharmaceutically important secondary plant metabolites, such as with anolides and polyunsaturated fatty acids. Microbial supplementation with various fungal strains during vermicomposting proves beneficial in increasing nitrogen content, degrading organic waste, providing aeration, and stabilizing vermicompost [15]. Consequently, several studies have indicated that vermicompost exhibits superior effects on plant growth compared to compost, highlighting that traditional chemical analyses may not fully capture the potential of organic amendments/fertilizers. Indeed, field applications of vermicompost and compost result in increased carbon storage, water retention, enzymatic and microbiological activity, as well as the abundance and diversity of soil fauna [70], thereby exerting positive effects on agricultural processes.

5. Conclusions

The findings of this study might provide a clearer understanding of the dynamics of the fungal communities present in composting and vermicomposting processes. Composting or vermicomposting animal organic waste stabilizes the manure, thereby fostering improvements in fungal communities beneficial for agricultural applications. At the outset of the experiment, fungal genera pathogenic to human were identified; however, through composting and vermicomposting processes, their presence was minimized. The pathotroph, saprotroph, and symbiotroph fungal communities identified serve distinct functions depending on their habitat. These functions include enhancing available nitrogen and phosphorus, as well as producing metabolites and enzymatic complexes that facilitate the breakdown of macromolecules and increase nutrient availability in the final products. These finished products can subsequently be applied in bioremediation processes and sustainable agriculture practices.

CRediT authorship contribution statement

Rocío del Pilar Serrano-Ramírez: Writing – original draft, Validation, Methodology, Funding acquisition, Conceptualization. **Adalberto Zenteno-Rojas:** Writing – original draft. **Valentín Pérez-Hernández:** Visualization, Software, Formal analysis, Data curation. **Odín Reyes-Vallejo:** Supervision. **Sugey Vásquez-Hernández:** Writing – review & editing. **Héctor Hiram Torres-Ventura:** Writing – review & editing. **Mayram Margarita González-Reyes:** Visualization, Software, Formal analysis, Data curation. **Wilber Montejo-López:** Writing – review & editing. **Abumalé Cruz-Salomón:** Writing – review & editing, Supervision. **Joaquín Adolfo Montes-Molina:** Validation, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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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.

Supplementary material

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Data availability

Data will be made available on request.

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