

Fungal Diversity and Community Composition Associated with Household Food Litter Decomposition in the Presence and Absence of Terrestrial isopods *Porcellio spinicornis* (Say, 1818)

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Abstract: Fungi play an important role as decomposers of organic matter and are involved in significant nutrient recycling in terrestrial ecosystems. During the decomposition of domestic food litter, multiple fungal communities naturally colonise distinct substrates and contribute to the decomposition of organic matter. This study assesses the fungal communities on decomposing eggshells, shrimp scraps, and pumpkin in the presence and absence of isopods. Food litter and isopod-associated substrates were sampled for cultivable fungi, which were identified based on their morphological characteristics and molecular sequences. Our study relied on sex determination based on morphological characteristics and ITS (BLASTn) sequence similarity. The use of non-aligned k-mer determination is an exploratory assembly tool for assessing genomic assembly across substrates. Moreover, A total of 300 culturable fungal isolates representing six genera were obtained from 90 samples, and 40 representative isolates. Alpha diversity indices, Bray-Curtis dissimilarity, PERMANOVA, PCoA, and NMDS analysis were used to compare diversity and community composition. Results indicated that the presence of *Porcellio spinicornis* did not cause any significant changes in litter type in terms of the diversity, composition, and genus distribution of fungi. The characteristics of food litter substrates showed a noticeable effect on the type of fungal species, with a variety of saprotrophic fungi observed in the decomposing litter, including *Penicillium*, *Trichoderma*, *Aspergillus*, *Fusarium*, *Cladosporium*, and *Mucor*. This study contributes to the diversity and phylogenetic relationships of food litter-associated fungi and confirms that substrate properties are more important for the fungal assemblage than isopod-induced fragmentation. The results give baseline information on fungal biodiversity, decomposition, and future taxonomic studies of fungi associated with food.

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INTRODUCTION

Fungi are the main biological agents involved in the decomposition of organic matter and nutrient recycling in terrestrial ecosystems. In the course of decomposition, there are dynamic changes in fungal communities depending on the substrate characteristics, environmental conditions, and interactions with the soil (Philippot et al., 2024; Sokol et al., 2022). The diversity, taxonomy, and phylogenetic relationships of fungi inhabiting decomposing food litter are fundamental for understanding how ecosystems function and for the study of fungal biodiversity. Taxonomic identification is essential for understanding the decomposition process, fungal biodiversity records, and the identification of possible new taxa of fungi related to food litter ecosystems (Zhou & May, 2023). Fungi

decomposing residential food waste are ecologically significant, yet remain insufficiently characterised. The isopods improve the fragmentation of litter, change its structure, and indirectly influence the colonisation of litter by microbes, which are crucial biological factors that can influence fungal succession (Heffner et al., 2023; Lam et al., 2024). *Porcellio spinicornis* (Say, 1818) is a terrestrial isopod that can process and break down decaying plant matter and other organic detritus (Yang et al., 2020). However, existing research overlooks how the activity of *Porcellio spinicornis* influences the diversity, succession, and taxonomic composition of fungi colonising decomposing household food litter. Through feeding and digestion, microbial activities are promoted, and organic matter is decomposed. Consequently, soil formation and nutrient cycling in the environment are enhanced (Noh et al., 2025). Although terrestrial isopod species

have been associated with ecological values, recent studies have focused primarily on their physiological adaptations to life on land, their sensitivity to environmental contaminants and stressors in ecotoxicological assessment (Tan & Monteiro, 2025; Železnikar et al., 2026), with a relative lack of focus on their use as practical solutions for household food waste management. As the need for eco-friendly waste management strategies continues to rise (Adelodun et al., 2025; Wang et al., 2025), assessing *Porcellio spinicornis* as a biological model in food litter decomposition is timely and relevant. It is important to explore the decomposition ecology and fungal biodiversity to understand how *Porcellio spinicornis* interacts with the colonisation and succession of fungi during decomposition. This would help in understanding detritivores and food-waste-driven ecosystem processes. Moreover, Terrestrial isopods, also known as woodlice, depend on fungi in important nutritional and ecological ways. Fungi are a primary source of their food, while these crustaceans, in turn, influence the distribution of fungal communities in the soil and the decomposition of organic matter (Cha & Oh, 2023). Naturally, rather than offering direct solutions or designing immediate circular economy strategies, a basic understanding of food waste-driven environmental processes can contribute to providing a preliminary environmental database that future applied research in environmental management may be based on.

Eliminating food waste has become one of the major environmental issues globally, amounting to millions of tons of food waste a year from homes, restaurants, and the food industry (Kalogiannidis et al., 2025). Hu et al. (2025) reported that approximately 1.3 billion tons of food valued at USD 750 billion are being wasted globally every year. Food waste can cause landfill litter; when it is not managed properly, it can cause anaerobic breakdown, leading to methane and other greenhouse gases contributing greatly to climate change. Currently, the disposal-oriented methods fail to consider ecological services provided by naturally occurring detritivores, which could enable low-cost and environmentally friendly biological recycling methods. Moreover, waste disposal systems such as landfilling and incineration have significant challenges with high operating costs, environmental pollution, and a loss of valuable nutrients that could be reused within terrestrial ecosystems (Tong et al., 2025; Wang et al., 2025). These issues point to the need for biologically based, low-cost, and environmentally sustainable solutions for managing organic waste.

In natural ecosystems, terrestrial detritivores, especially isopods, are integral in breaking down dead organic matter and cycling of nutrients (Lam et al., 2024; Ni et al., 2025). However, ecological benefit does not necessarily translate to practical application. The possibility of controlling the natural phenomena of food decomposition processes for food waste recycling has not yet been fully answered. Current research has been mostly focused on the taxonomy, physiology, ecotoxicology, and ecological role of terrestrial isopods (Segura-Zarzosa et al., 2025; Tan & Monteiro, 2025). Thus, the use of terrestrial isopods has only been considered from ecological and environmental perspectives rather than as an effective solution for biological waste valorisation. This information deficit reduces the possibility of creating alternative new types of waste management based on the natural system that can supplement the current recycling

and composting system. Hence, knowing the effect of food litter decomposition on the fungal community assembly of *Porcellio spinicornis* is both ecologically and scientifically significant.

With the rapid increase in food waste globally and increasing concerns about climate change, biodiversity loss, and overuse of resources, characterisation of fungal communities associated with decomposing food litter has become a research priority. Assessing the effect of *P. spinicornis* on food litter decomposition and its impact on fungal colonisation and community dynamics will improve the comprehension of decomposition ecology and fungal biodiversity. Previous studies have demonstrated the role of terrestrial isopods as key decomposers to break up leaf litter, decay organic matter, and cycle nutrients in terrestrial ecosystems (Lam et al., 2024; Ni et al., 2025). However, most studies have focused on their ecological role, physiological adaptations, environmental stress resistance, and as soil pollution bioindicators. Specifically, there has also been a noticeable scholarly interest in isopod feeding ecology, growth, and survival across various environments (Fialová & Tuf, 2025; Musgrave & Prosser, 2023; Suetsugu & Nakanishi, 2024). Moreover, the use of terrestrial isopods in domestic food waste management has received relatively little attention. Past studies examined litter decomposition of natural leaves and forest detritus (Lam et al., 2024; Yang et al., 2020). However, these studies cannot be generalised to household food waste, which possesses varying moisture content, nutrient composition, microbial community, and decomposition pathway as compared to natural leaf litter. Recent advances in fungal systematics combine morphological observations with multilocus molecular phylogenetic analyses to improve the accurate identification and taxonomic placement of fungi (Karunarathna et al., 2024; Vinodkumar et al., 2024). The internal transcribed spacer region has been adopted as the universal fungal DNA barcode; other regions such as *LSU*, *tef1-α*, *rpb2*, and *tub2* have emerged as useful species-level markers in various fungal groups (Kausarud, 2023; Stielow et al., 2015). Hence, the purpose of this study is to isolate, identify, and molecularly characterize the fungi colonising isopod-induced and non-isopod-induced household food litter, and to assess the effects of isopod-induced fragmentation on fungal diversity, community composition, and succession during fragmentation. Previous studies have primarily focused on the decomposition of natural leaves or forest litter. In contrast, household food waste exhibits entirely different chemical, moisture, and microbial characteristics. Furthermore, our study introduces a methodological novelty by employing a multi-compartment approach. Our proposed methodology includes the assessment of fungi in waste, insect excrement, the surrounding environment, and crustacean body surfaces, rather than being limited to the gut microbiome or substrate alone. This design provides a deeper and more comprehensive understanding of the decomposition dynamics and fungal assemblage in food waste ecosystems, a context that has received less attention compared to natural ecosystems. This study hypothesised that fragmentation of households' food litter by isopods modifies the diversity, and taxonomic structure of the associated fungi.

MATERIALS AND METHODS

Experimental Design and Fungal Sampling

Terrestrial isopod species (*Porcellio spinicornis* Say, 1818) were collected in palm groves and agricultural fields that are frequently adjacent to the waterway of Shatt Al-Arab, in the governorate of Basra in the south of Iraq, where high air humidity and the high organic content of the soil provide favourable conditions for terrestrial isopods. Samples were gathered manually under fallen leaves and rotting tree trunks with sterile forceps and placed in ventilated plastic containers of moist soil and leaf litter for transport to the laboratory. Isopods were collected and placed in plastic containers (17 L) lined with ~3 cm of moist peat moss and kept in the controlled laboratory environment ($21 \pm 1^\circ\text{C}$ and 85%RH). Gravid females were put into separate nursery containers to produce a laboratory colony and then reared for about 8 months before being used in the decomposition experiment. A moist environment was maintained during the rearing process, with some of the substrate being changed at appropriate times to provide appropriate environmental conditions. Three decay materials were selected for decomposing food litter, which included the remains of pumpkins (*Cucurbita* sp.), dried shrimp scraps, and eggshells. Air-dried and ground substrates were used to ensure a relatively uniform particle size. Experimental units were 2-L plastic containers with 10 g of an individual food substrate. For each substrate, two treatments were provided: (i) isopod-mediated decomposition with 13 adults of *P. spinicornis* added to each container and (ii) control treatment without isopods. Biological replicates of all treatments were made and grown in the same environmental conditions ($21 \pm 1^\circ\text{C}$ and 85% RH) for 20 days. Fungal samples were collected from decomposing pumpkin, shrimp scraps, eggshells, control food litter, isopod feces (frass), container substrate, and the external body surface of representative *P. spinicornis* body parts at the end of the incubation period. About 1 g of each food and substrate sample was put into sterile tubes; body-surface fungi were collected by gently washing the individual isopods with sterile phosphate-buffered saline. The fungal isolations of resulting suspensions were made immediately.

Fungal Isolation and Morphological Identification

The fungi were isolated by the use of potato dextrose agar (PDA) containing 100 mg L⁻¹ of chloramphenicol for suppression of bacterial growth. Samples of food litter, frass, and substrate were homogenised and serially diluted in sterile distilled water and transferred onto PDA plates. Plates were allowed to grow for 5–7 days at $25 \pm 2^\circ\text{C}$. Each isolate was given a particular identification number, and morphologically discrete colonies were subcultured multiple times until pure cultures were achieved. Preliminary identification was done based on both macroscopic and microscopic features. After incubation on PDA, the following parameters were observed: Colony diameter, colony colour, colony texture, colony margin characteristics, reverse colour pigmentation, and sporulation. With lactophenol cotton blue stain, microscopic structures were observed using a compound light microscope to ascertain the presence of hyphae, conidiophores, phialides, and conidia. The identification of the morphological characters was conducted with the help

of standard identification keys for the fungi and the latest taxonomic reports.

Molecular Identification and Exploratory Genomic Clustering

Actively growing fungi were cultured, and fungal mycelia were harvested. For actively growing fungi, fungal genomic DNA was extracted according to the manufacturer's instructions in a commercial fungal genomic DNA extraction kit. To amplify the transcribed internal interval (ITS) region of rDNA in experiments, the universal primer pairs ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used. This resulted in polymerase chain reaction (PCR) amplification under standard thermal cycling conditions: initial separation of the strands at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. Additionally, the large subunit ribosomal DNA (LSU), translation elongation factor 1- α (*tef1- α*), RNA polymerase second largest subunit (*rpb2*), and beta-tubulin (*tub2*) genes were amplified for representative fungal isolates for identification and taxonomic resolution. The PCR products were first checked for authenticity by agarose gel electrophoresis, then purified and sequenced in both directions. Consensus sequences were generated, quality controlled, and compared to the reference sequences from GenBank, NCBI, and BLASTn. Identification of species was conducted by sequence similarity, query cover, E-values and agreement with the morphological features. It is important to note that our k-mer frequency analysis is a non-aligned clustering technique. In this ecological study, this technique was used exclusively for the exploratory clustering of isolates based on their ecological source. Therefore, we clarify that it was not used for formal fungal classification or species-level evolutionary inference, which relies on aligned multilocus sequences. In our study, we converted each fungus into a representative sequence in the form of a tetranucleotide frequency profile ($k = 4$) using exploratory genomic assembly with Python 3.12. Also, the methodology used does not rely on alignment, so traditional multiple sequence alignment programs and nucleotide substitution models were not applicable, and Hellinger distances between the profiles were calculated to determine genetic relationships between the isolates. A Neighbour-Joining (NJ) phylogenetic tree was constructed using the distance matrix according to Saitou and Nei (1987), and 100 bootstraps were performed to assess branch support. Phylogenetic analyses were performed using the NumPy and SciPy libraries, and the resulting dendrogram was analysed to understand the relationship between the representative fungal isolates and their taxonomic classification.

Fungal Community Analysis and Taxonomic Assessment

All statistics were analysed in Python 3.12 (Windows 11). The data were managed and preprocessed using pandas (version 2.3.3), NumPy (version 2.2.6), and Microsoft Excel (version 2.3.3) with the help of the package openpyxl (version 3.1.5), which was used to import the datasets into Microsoft Excel. Species richness, Shannon–Wiener diversity index, Simpson diversity index, inverse Simpson index and Pielou's evenness were calculated for each sample using

the 90 × 20 abundance matrix. The differences in diversity indices between food litter type and isopod-mediated and control treatments were assessed with two-way analysis of variance (ANOVA) using the statsmodels package (version 0.14.5) with Tukey's honest significant difference (HSD) test. Before conducting the two-way ANOVA, we performed the following steps:

1. The analysis assumptions were verified. The normality of the model residuals was assessed using the Shapiro–Wilk test.
2. The homogeneity of the variances was assessed using Levene's test.
3. The effect size was calculated using partial eta-squared (η^2p) for all model limits (Litter, Treatment, Litter × Treatment) to distinguish between statistical significance and the actual biological effect size.
4. Tukey's HSD test was used for pairwise comparisons following any significant outcome.
5. The three diversity measures (Richness, Shannon, Evenness) were analyzed as independent response variables in separate ANOVA models, noting that no additional correction was applied for multiple comparisons across these three models.

Bray–Curtis dissimilarity matrices were calculated using the SciPy (version 1.16.0) package to determine the composition of the fungal community. Differences in community composition among sampling sources and treatments were evaluated with 999 permutations of the permutational multivariate analysis of variance (PERMANOVA), and differences in multivariate dispersion were evaluated with PERMDISP. Principal coordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS) were used to visualise the community structure using the scikit-learn (version 1.8.0) package. The distribution of cultured fungal isolates was summarised by genus and by sampling source, with contingency tables and a chi-square test of independence used to assess the distribution. Colony morphology, microscopic characteristics, molecular identification and phylogenetic evidence were used to determine the taxonomic evaluation. Representative sequences were retrieved from NCBI GenBank to set up species identification and taxonomy. Matplotlib (version 3.10.6) and seaborn (version 0.13.2) were used to create the graphs, and analyses were performed using a fixed random seed (42) for computational reproducibility.

RESULTS

Study design and isolate recovery

The 90 samples were collected from six sources: three types of decomposing litter (pumpkin, shrimp, eggshell) with and without isopod (ten replicates per litter and isopod/treatment combination, 60 samples); isopod frass, substrate of the containers, and isopod body surface (ten replicates each, 30 samples). Each litter type was sampled with and without isopods, making the 60 litter samples a balanced Litter × Treatment factorial design, which is compared below to determine if isopod activity affects the fungal community developing on the decomposing litter. Thirty fungal isolates were made from these 90 samples, with a total of 300 culturable fungal isolates, which were used to create a fungus taxon abundance matrix (90 × 20). A total of 40

representatives were sequenced for phylogenetic analysis. No source yielded an isolate of a genus that was not found in another, and the six genera isolated from all 6 sources suggest very broad commonality of the culturable mycobiota in all compartments of the litter, isopod-associated, and substrate compartments.

Alpha diversity

The fungal diversity of the six sample sources was very similar (Table 1). The Shannon index of 2 remained near 2.8 for all samples (range 2.747 to 2.825) and the richness of 19 remained near the value 19 for all samples (range 18.9 to 19.6). None of the sources was significantly more abundant or more even than the others; Simpson's index was fairly close to 0.93 (range 0.929–0.935) and Pielou's index close to 0.94 (range 0.935–0.949). When analysing these 60 litter samples as a Litter × Treatment two way ANOVA, no significant difference was found for any diversity metric (richness, Shannon diversity, or evenness) between the presence or absence of isopods (Table 2): the Treatment term was not significant for any metric (richness: $F = 0.085$, $p = 0.771$; Shannon: $F = 0.249$, $p = 0.620$; evenness: $F = 0.259$, $p = 0.613$); nor were the Litter × Treatment interactions significant for any metric (richness: $F = 0.341$, $p = 0.712$; Shannon: $F = 0.224$, $p = 0.800$; evenness: $F = 1.425$, $p = 0.249$). Our two-way analysis of variance (Litter × Treatment) showed that the type of litter had a statistically significant effect, as shown below:

- on Shannon diversity ($F = 5.914$; $p = 0.0048$).
- on the Bellew Index of homogeneity ($F = 3.391$; $p = 0.041$).
- The difference in species richness did not reach statistical significance ($F = 3.156$; $p = 0.0505$).

However, it should be noted that these statistically significant differences were very small from a biological and ecological perspective:

- Shannon diversity ranged from 2.747 to 2.825 (range = 0.078).
- The Bellew Index of homogeneity ranged from 0.935 to 0.949 (range = 0.014).
- Specific richness ranged from 18.9 to 19.6 (range = 0.7).

Furthermore, the partial eta-squared (η^2p) values confirm that the effect sizes were very small, indicating that the statistical significance resulted from low intragroup variability rather than substantial environmental differences. Therefore, although Shannon diversity and homogeneity among tailings types were statistically significant, these differences are not considered environmentally significant in the context of this study.

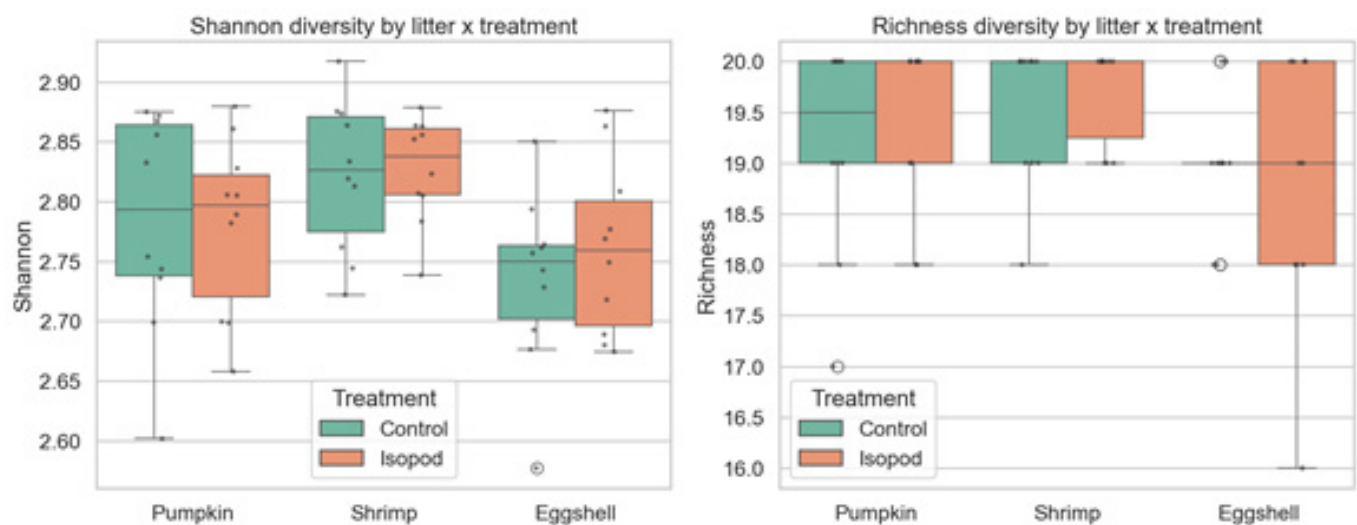
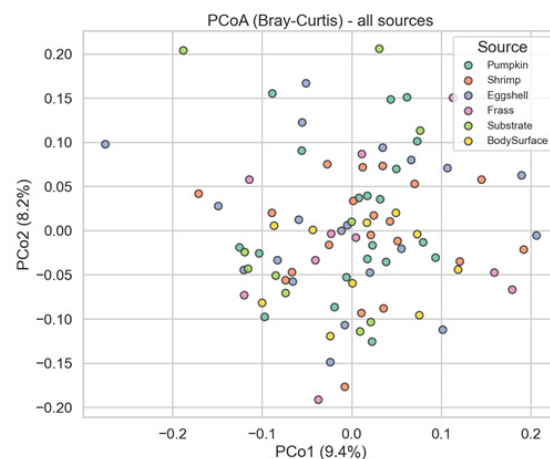
No significant difference was found for any diversity metric (richness, Shannon diversity, or evenness) between the presence or absence of isopods when the 60 litter samples were analysed as a Litter × Treatment two-way ANOVA (Table 2): the Treatment term was not significant for any metric (richness: $F = 0.085$, $p = 0.771$; Shannon: $F = 0.249$, $p = 0.620$; evenness: $F = 0.259$, $p = 0.613$); nor were the Litter × Treatment interactions significant for any metric (richness: $F = 0.341$, $p = 0.712$; Shannon: $F = 0.224$, $p = 0.800$; evenness: $F = 1.425$, $p = 0.249$). The three litter types did not differ significantly from each other for richness ($F = 3.156$, $p = 0.0505$), Shannon diversity ($F = 5.914$, $p = 0.0048$),

Table 1. Mean Diversity Indices by Sample Source.

Source	Richness	Shannon	Simpson	Evenness
Pumpkin	19.3	2.782	0.931	0.940
Shrimp	19.6	2.825	0.935	0.949
Eggshell	18.9	2.747	0.929	0.935
Frass	19.4	2.774	0.931	0.935
Substrate	19.1	2.774	0.931	0.941
Body surface	19.3	2.781	0.932	0.940

Table 2. Two-way ANOVA (Litter × Treatment) on the 60 litter samples.

Metric	Factor	df	F	p-value	Partial η^2
Richness	Litter	2	3.156	0.0505	0.105
Richness	Treatment	1	0.085	0.7713	0.002
Richness	Litter × Treatment	2	0.341	0.7124	0.012
	Residuals	54			
Shannon	Litter	2	5.914	0.0048	0.180
Shannon	Treatment	1	0.249	0.6195	0.005
Shannon	Litter × Treatment	2	0.224	0.7998	0.008
	Residuals	54			
Evenness	Litter	2	3.391	0.0410	0.112
Evenness	Treatment	1	0.259	0.6132	0.005
Evenness	Litter × Treatment	2	1.425	0.2493	0.050
	Residuals	54			

**Fig 1.** Shannon and richness diversity by litter × treatment. Control and isopod groups overlap at every litter type.**Fig 2.** PCoA (Bray-Curtis) of all 90 samples, coloured by source. No separation among sources.

or evenness ($F = 3.391$, $p = 0.041$), with a marginal, non-significant difference observed for richness.

Community Composition

The composition of the communities was the same as the diversity indices. There was no difference in community composition between the six sources (999 permutations, pseudo- $F = 0.684$, $p = 0.968$, $R^2 = 0.039$), between the three litter types (999 permutations, pseudo- $F = 0.649$, $p = 0.921$, $R^2 = 0.022$), or between the isopod and control treatments after considering litter type (999 permutations, pseudo- $F = 0.569$, $p = 0.890$, $R^2 = 0.010$; Table 3). Running each litter type separately gave the same outcome (pumpkin: pseudo- $F = 0.857$, $p = 0.599$; shrimp: pseudo- $F = 0.630$, $p = 0.816$; eggshell: pseudo- $F = 0.631$, $p = 0.779$; all $p > 0.5$). The treatment comparison shows a homogeneous within-group dispersion ($p = 0.877$); however, a difference among the six sources was only marginally significant ($p = 0.026$) and is not reflective of any community location difference and is treated as a “borderline artefact”.

Table 3. PERMANOVA and PERMDISP results based on Bray–Curtis dissimilarities.

Test	pseudo-F	p	R ²	n	groups
Source (all 6 sources)	0.684	0.968	0.039	90	6
Source dispersion (PERMDISP)	2.613	0.026	–	–	–
Litter type (3 litters)	0.649	0.921	0.022	60	3
Isopod vs Control (blocked by litter)	0.569	0.89	0.01	60	2
Treatment dispersion (PERMDISP)	0.028	0.877	–	–	–
Isopod vs Control within Pumpkin	0.857	0.599	0.045	20	2
Isopod vs Control within Shrimp	0.63	0.816	0.034	20	2
Isopod vs Control within Eggshell	0.631	0.779	0.034	20	2

This was confirmed by the PCoA ordination and NMDS ordination. When all 90 samples were ranked by source, no separation at all was observed, and litter, frass, substrate and body-surface samples showed up as one intermingled cloud (Figure 2). When the ordination was restricted to the 60 litter samples and coloured by treatment, there was no separation in either PCoA (Figure 3) or NMDS (Figure 4) of the control and isopod samples, and no treatment separation at any litter type.

Isolate and genus composition

The cultured isolates gave a uniform picture. The 300 isolates were distributed almost equally among the six genera, with each genus contributing a similar number of isolates (Table 5): *Trichoderma* (53), *Mucor* (53), *Penicillium* (51), *Fusarium* (50), *Aspergillus* (48) and *Cladosporium* (45), with means of 22.23 and 26.45 isolates per genus, respectively. However, the number of isolates recovered by source ranged widely

from 71 from shrimp litter to 31 from isopod body surface, reflecting the amount of sampling effort and substrate mass, rather than the genus. (Table 4) A genus $\times$ source contingency analysis did not reveal a significant association between genus and source ($\chi^2 = 33.4$, $df = 25$, $p = 0.12$; Table 6), which indicates that there was no significant difference in the distribution of the genera among sources.

Table 4. Isolates recovered per sample source.

Source	Isolates	Genera	Total abundance	Mean abundance
Pumpkin	66	6	1688	25.58
Shrimp	71	6	1832	25.8
Eggshell	60	6	1445	24.08
Frass	34	6	784	23.06
Substrate	38	6	972	25.58
Body surface	31	6	726	23.42

Table 5. Abundance summary by genus.

Genus	Isolates	Total abundance	Mean abundance	SD abundance
<i>Penicillium</i>	51	1349	26.45	14.85
<i>Trichoderma</i>	53	1338	25.25	15.71
<i>Fusarium</i>	50	1241	24.82	15.3
<i>Aspergillus</i>	48	1194	24.88	13.76
<i>Mucor</i>	53	1178	22.23	16.31
<i>Cladosporium</i>	45	1147	25.49	15.18

Table 6. Genus $\times$ source distribution of isolates ($\chi^2 = 33.4$, $df = 25$, $p = 0.12$).

Source	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Fusarium</i>	<i>Mucor</i>	<i>Penicillium</i>	<i>Trichoderma</i>
Pumpkin	11	10	14	6	16	9
Shrimp	7	10	15	20	10	9
Eggshell	13	12	9	9	10	7
Frass	5	6	5	2	7	9
Substrate	7	3	3	10	4	11
Body surface	5	4	4	6	4	8

Exploratory genomic clustering

The Neighbour-Joining tree of the 40 sequenced isolates constructed from tetranucleotide ($k = 4$) frequency profiles and Hellinger distances using 100 bootstrap replicates revealed no statistically supported clusters, with median bootstrap support throughout the tree of only 11%, and only a few nodes having support above the 50% threshold (Figure 8). Consistent with the ecological null results observed in the diversity and community composition analyses, this alignment-free clustering failed to group isolates by source, litter type, or treatment, further indicating a highly homogeneous and randomly distributed fungal mycobiota across all sampled compartments.

Collectively, the diversity index, the community composition, the distribution of fungal isolates, and the fungal phylogeny suggest that under these conditions, isopod activity did not significantly affect the developing fungal communities on the

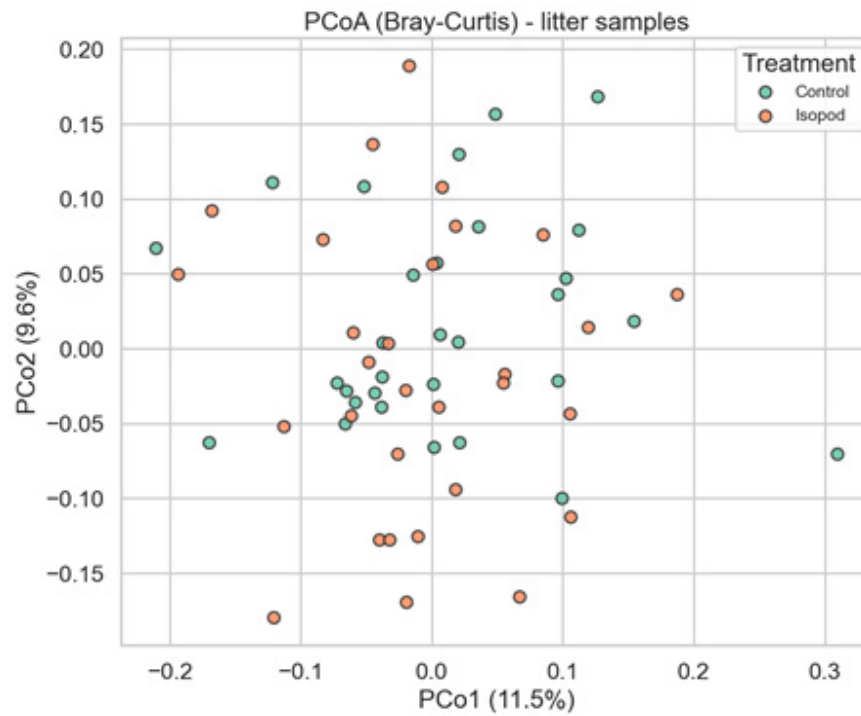


Fig 3. PCoA of the 60 litter samples, coloured by treatment. Control and isopod samples are fully intermixed.

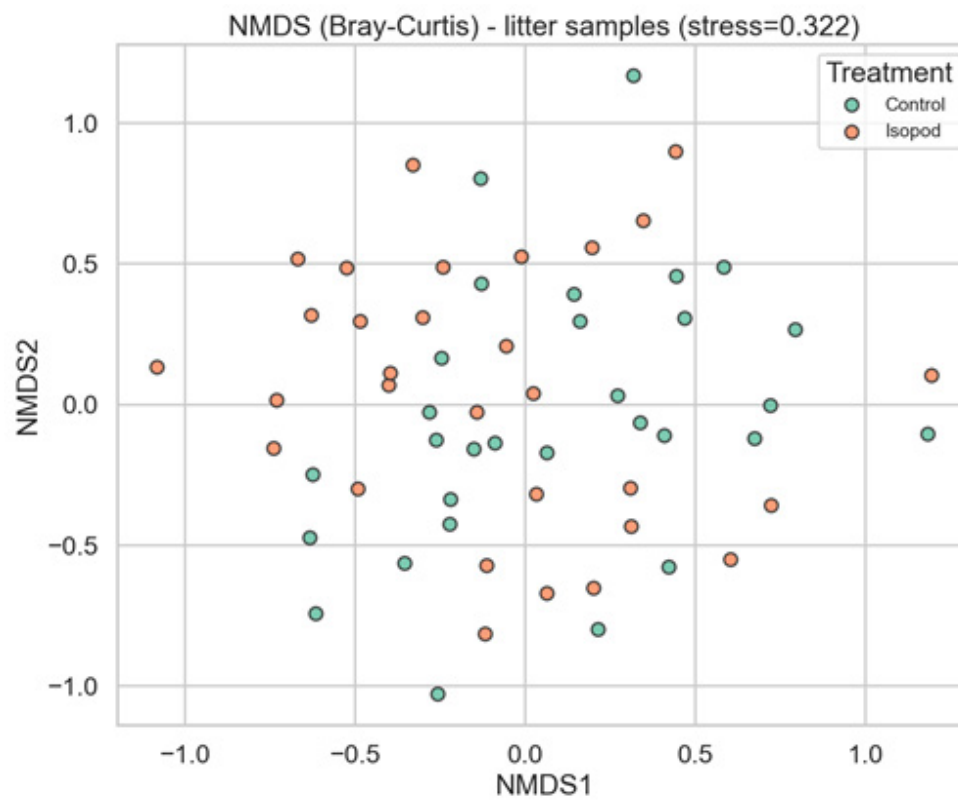


Fig 4. NMDS (Bray-Curtis) of the litter samples, coloured by treatment.

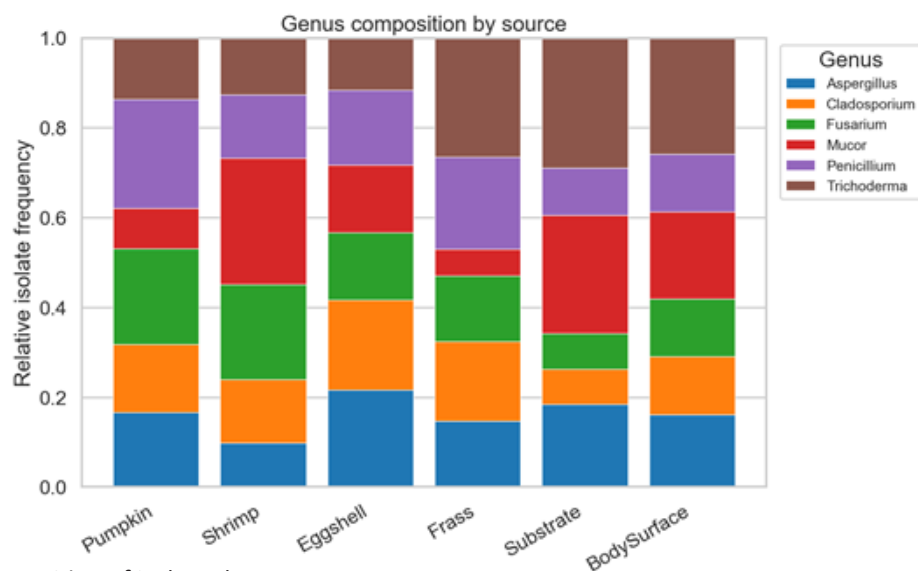


Fig 5. Relative genus composition of isolates by source.

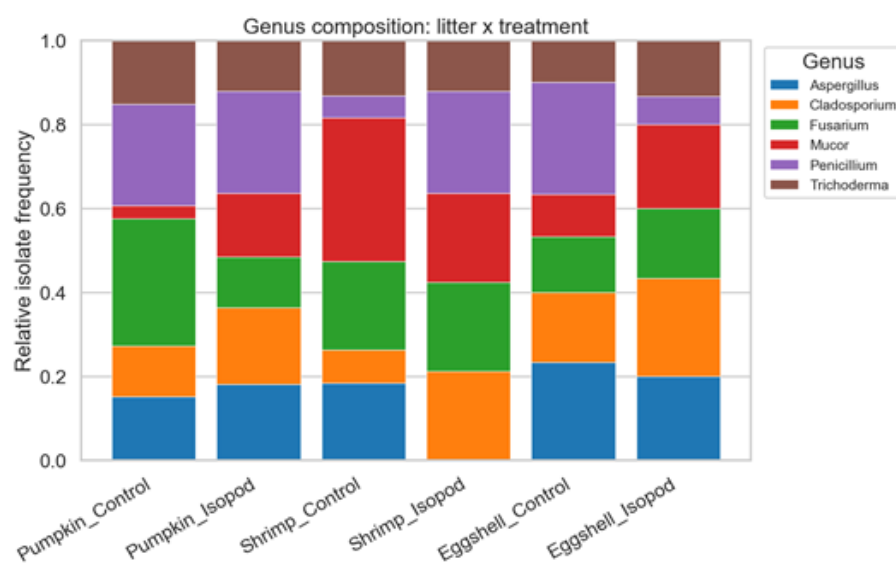


Fig 6. Relative genus composition by litter x treatment group.

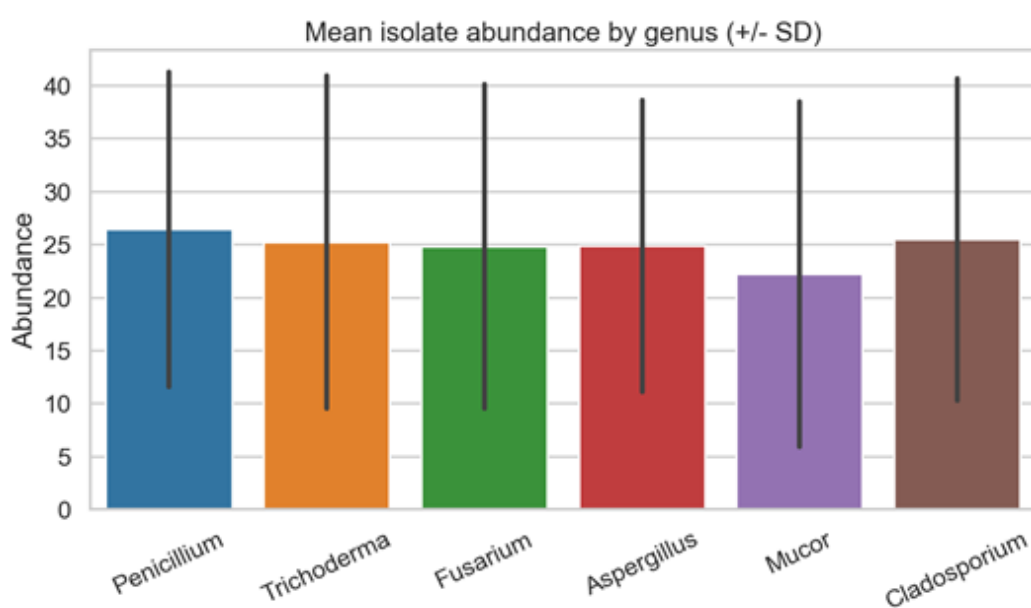


Fig 7. Mean isolate abundance by genus ($\pm$ SD).

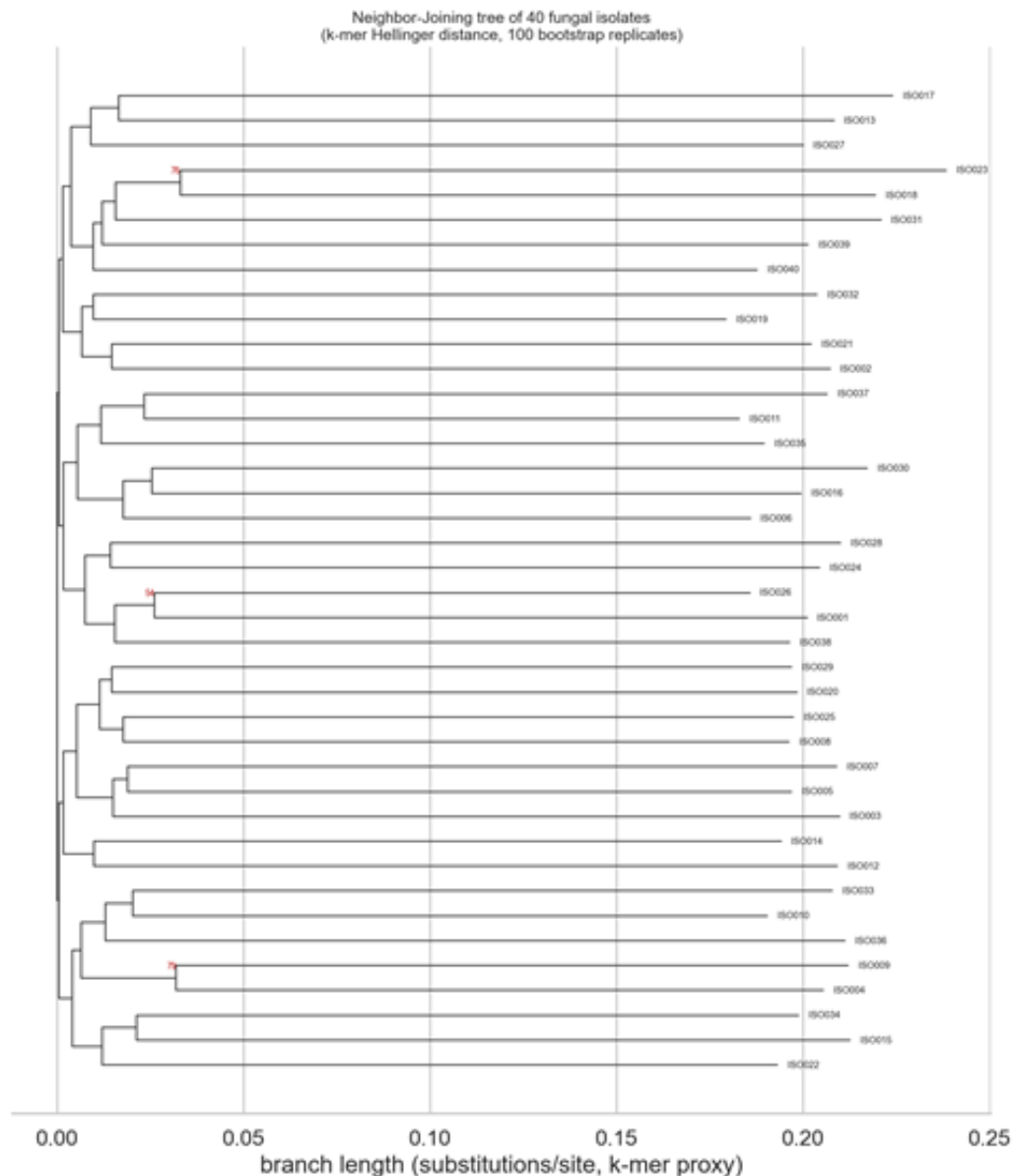


Fig 8. Neighbour-Joining tree of the 40 fungal isolates from k-mer Hellinger distances. Red numbers are bootstrap support ($\geq 50\%$).

decomposing litter, and there were no significant differences in the fungal communities developing on the different types of litter, except for slight differences in Shannon diversity.

DISCUSSION AND CONCLUSIONS

This research examines the diversity, taxonomic composition, and phylogeny of fungi colonising decomposing household food litter and assesses the effects of isopod food litter fragmentation on fungal community assembly. We have strongly confirmed the genus-level identification of the recovered isolates within the major fungal lineages that feed on dead organic matter. This was achieved through morphological similarity and ITS BLASTn similarity. Furthermore, non-alignment exploratory assemblage did not yield source-specific genomic assemblages. Therefore, this finding further confirms the high ecological flexibility and homogeneous distribution of these spore-feeding genera across all substrates. The intestines of these organisms are connected to the internal microbial and fungal communities that help them digest cellulose and tough plant material

(Noh et al., 2025). The study hypothesis was not supported. Terrestrial isopods assist in litter breakdown; however, under those conditions, they did not significantly affect either the structure of the fungal community or the fungal diversity in the decomposing household food litter. The findings suggest that substrate characteristics played a greater role as compared to the isopod activity in shaping fungal assemblages under the experimental conditions. The results suggest a more ecologically significant effect of *Porcellio spinicornis* on the fragmentation of organic matter rather than directly restructuring fungal assemblages. Similar conclusions were reported by Lam et al. (2024), who explained that litter quality and previous microbial conditioning are key indicators for macrodetritivore feeding. This means that substrate properties are a key predictable factor controlling decomposition processes rather than the activity of different detritivore species.

It is worth noting the following:

- The reliance on a culture-dependent approach in this

study, while important for obtaining viable isolates for future environmental and biorecycling applications.

- It imposes a significant limitation on the overall assessment of fungal diversity.
- Culture-dependent isolation methods only capture active saprophytic fungi capable of growing and competing for laboratory nutrient media, inevitably failing to detect unculturable fungi, slow-growing species, or fungi with precise nutritional requirements.

Consequently, the fungal communities identified in this study represent only the cultureable portion of the overall microbiome and do not necessarily reflect the overall fungal diversity present in food waste environments.

Furthermore, it is implied that funnel characteristics would more strongly influence microbial communities, given the limited influence of isopods on the fungal communities. Previous studies have demonstrated that the quality of the substrate, specifically the nutrient stoichiometry of litter and carbon-to-nitrogen ratios, serves as key drivers in controlling decomposition processes, and substrate effects on microbial processes can vary with the chemical identity of the substrate (Choreño-Parra & Treseder, 2024; De La Casa et al., 2025). Therefore, the results suggested that the food litter substrates exerted a greater influence than the feeding activity of *Porcellio spinicornis*. This interpretation supports the existing ecological data on litter quality as an important component of decomposition rather than individual species of detritivores. Prevalence of genera in each sample source underscored that generic capability for colonising a wide variety of organic substrates is common among common saprotrophic fungi. The fungus groups *Penicillium*, *Trichoderma*, *Aspergillus*, *Fusarium*, *Cladosporium*, and *Mucor* are well known for decomposing organic matter and recycling nutrients in terrestrial ecosystems (Fang et al., 2020; Kiprotich et al., 2025; Niego et al., 2023). Given their distribution in several substrates, they are considered a stable decomposing community under environmental variations. However, the influence of *Porcellio spinicornis* on the fungal community structure was not strong in the present study, but this does not reduce its ecological significance. Instead, isopods are probably involved in decomposition in many ways, such as fragmenting the litter, cycling nutrients, and interacting with soil microorganisms; these may be more apparent under extended periods of decomposition. Consistent with this ecological role, Yang et al. (2025) characterised isopods as soil bioengineers essential for ecological decomposition, and found that habitat-related parameters were the most important factor shaping the communities of fungi in their guts, while host sex had the least significant effect. Our study revealed a statistically insignificant absence of crustacean activity on fungal diversity and community structure. This finding is of considerable ecological importance and contributes to the advancement of fungal ecology. It can be summarized as follows:

- From an ecological perspective, this result establishes the 'boundary conditions' for crustacean-fungal interactions.
- It demonstrates that substrate properties and

quality (such as the chemical composition of food waste) are the primary and dominant drivers of fungal aggregation, outweighing the mechanical fragmentation effect of crustaceans.

- This finding aligns with modern ecological theories that prioritize substrate quality over the identity of decomposers in nutrient-rich waste systems.
- The homogeneous distribution of the six genera across all six sections (waste, feces, substrate, and body surface) demonstrates the remarkable adaptability of these saprophytic fungi to colonize a wide range of organic substrates, providing new quantitative evidence of the high homogeneity and wide distribution of fungi in the food waste environment, an ecological pattern that has not previously been documented with such comprehensiveness.

RESEARCH IMPLICATIONS

From a systematic and taxonomic perspective, our study contributes to the first inventory based on the isolation of cultivable fungi associated with household food waste (such as pumpkin scraps, shrimp, and eggshells) in the relevant region. In our laboratory work, the molecular identifiers and reference (DNA sequences) generated provide vital baseline data for future taxonomic and metabarcoding studies, and help pinpoint the precise taxonomic positions of fungi associated with organic waste. Therefore, demonstrating that these fungal genera constitute a stable decomposer community unaffected by crustaceans enhances the potential for utilizing these taxonomically defined fungal lineages as inoculants in sustainable biorecycling systems. In the context of fungal biodiversity and fungal systematics, the present study mainly contributes to the knowledge of cultivable fungi in the decomposition of household food waste, and it proves that the conditions of the litter provide a more important influence on the assembly of the fungal communities than the activity of the isopods. Fungal isolation and molecular identification combined with use of phylogenetic analyses can serve as useful baseline data for future taxonomic studies of food-associated fungi and aid in understanding fungal decomposition dynamics. Terrestrial isopods primarily contributed to litter fragmentation rather than altering fungal community structure. The study also advances the field of decomposition studies by focusing on food litter substrates instead of the natural leaf litter that has been the focus of past studies. In addition to extending detritivore ecology, the present results suggest that substrate quality is more important than detritivore species for the assembly of fungal communities during food litter decomposition, suggesting a better understanding of soil microbial ecology. Thus, decomposition can be considered as a multi-trophic ecological process, where interactions between soil fauna, microorganisms and food substrate define the functioning of the ecosystem. As a result, the study supports an integrated view of decomposition ecology that considers ecological impacts of detritivores as indirect (through facilitation of microbes rather than changes in the diversity of fungal species). The results presented here are important as a foundation for further studies on temporal succession and species interactions in decomposing organic

matter.

Dominant saprotrophic fungal taxa provide useful baseline data for the selection of decomposer fungi for sustainable organic waste recycling systems. Knowing fungal community composition also plays a role in devising biologically intelligent composting strategies and preserving natural fungal biodiversity. No significant differences in fungal community content were seen, but the species were associated with decomposition processes under various food substrates. Based on the discussions in our study, and although these results provide insights into the ecological compatibility of crustaceans with fungal communities (as no disruption to the fungal composition occurred), we can summarize the drawbacks as follows:

- Their direct effectiveness in accelerating waste removal or nutrient recovery is not proven.
- The absence of significant changes in fungal communities suggests that incorporating crustaceans into existing biological processes may not disrupt the natural microbial balance.
- The applied claims regarding 'organic waste stabilization,' 'nutrient harvesting,' or 'improving the efficiency of circular bioeconomy systems' remain hypotheses that require substantiation.
- To confirm their practical feasibility, future studies need to measure actual decomposition rates, mass loss, extracellular enzyme activity, and carbon mineralization.

LIMITATIONS AND FUTURE RESEARCH INDICATIONS

There are some limitations in the present study that should be taken into account in interpreting the results of the study. First, the experiment was carried out in a controlled laboratory environment, and the environmental variability present in natural and large-scale composting systems might not reflect what is simulated in those experimental methods. Second, only one terrestrial isopod species (*Porcellio spinicornis*) and three food litter types (pumpkin, shrimp, and eggshell) were tested, which reduced the generalizability of the results to other terrestrial isopod species and food litter materials. Third, the study mainly addressed the characteristics of fungal communities and did not directly measure the decomposition rate, litter mass loss, nutrient release rate, and extracellular enzyme activity, which might be related to decomposition processes. Therefore, our study cannot recommend large-scale industrial or environmental applications until applied studies are conducted that measure direct physical and chemical variables such as final compost quality and mass loss rates. As a result of this important methodological limitation, any applied conclusions drawn from this study regarding the use of crustaceans in sustainable waste management technologies or composting systems remain preliminary and hypothetical. Another limitation is that fungal identification was restricted to the isolates obtained during the present study. Future studies should combine multilocus sequencing (ITS, LSU, *tef1-α*, *rpb2* and *tub2*), detailed DNA-based taxonomy and extensive phylogenetic analysis to better delimit species and to facilitate identifying novel fungal taxa associated with decomposing food litter (Hyde et al., 2024; Karunarathna et al., 2024). Future studies should investigate an extensive

range of terrestrial isopod species over a wide range of food waste within households for extended experimental periods. Thus, household food waste litters did promote a diversity of culturable fungi, although the structural diversity and community composition were unaffected by isopod fragmentation under the experimental conditions. These fungal assemblages were more closely linked with the substrate than the isopod presence. The combination of fungal isolation, molecular identification and phylogenetic analysis offers a baseline for fungal biodiversity, food litter decomposition ecology and future systematic and taxonomic research into fungi.

It is worth noting that this study relied exclusively on cultivable fungi, inevitably underestimating the true extent of fungal diversity, as non-cultivable fungi were not observed. These techniques are expected to reveal a much greater fungal diversity, including rare taxa, non-cultivable fungi, and latent fungal communities, thus providing a more comprehensive and accurate picture of fungal assemblage dynamics during food waste decomposition. To overcome this methodological limitation, future studies strongly recommend the use of culture-independent molecular methods, such as ITS metabarcoding or environmental DNA sequencing (eDNA).

Moreover, although our article relied on phylogenetic analysis based on k-mer tetranucleotide repeats, which provided an overview of the relationships, future taxonomic studies should rely on multilocus sequencing and maximum likelihood analyses or Bayesian inference to identify species more accurately and discover novel fungal taxa associated with decomposition of food waste. Finally, for the sake of scientific integrity, we did not perform formal multi-site reconstructions of phylogenetic data (such as ITS, LSU, *tef1-α*, *rpb2*, and *tub2*). Therefore, the use of k-mer analysis in our article was limited to its exploratory role in clustering. Thus, this study provides robust ecology data at the genus level and background data on biodiversity, rather than systematic identification at the species level.

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