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Dual Effect of Microplastics and Cadmium on Stream Litter Decomposition and Invertebrate Feeding Behavior

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Abstract: This study investigates the combined effect of microplastics and cadmium on the decomposition of litter, the structure of fungal communities, and the feeding behavior of invertebrates in an aquatic ecosystem. Through a series of microcosm experiments, we demonstrate that exposure to MPs and Cd significantly reduced the decomposition of leaf litter. Notably, the cumulative impact of combined MP and Cd exposure was found to be greater than their individual effects. During this process, the carbon–nitrogen ratio of the litter increased, while dehydrogenase activity and fungal biomass were inhibited. Additionally, the relative abundance of Ascomycota and Basidiomycota fungi decreased, weakening their role in the decomposition of leaf litter. Conversely, MPs and Cd reduced the relative content of leaf litter lignin, improving its quality as food, thereby leading to an increase in the feeding rate of invertebrates. This dual effect indicates that micropollutants suppress the decomposition of litter by regulating microbial metabolic activity and fungal community structure but promote invertebrate feeding. Our findings provide crucial insights into the adverse effects of MPs and Cd on the structure and diversity of aquatic fungal communities, which could have long-term impacts on the food webs and nutrient cycling progress of aquatic ecosystems.

Keywords: microplastics; cadmium; litter decomposition; fungal communities; invertebrate feeding

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1. Introduction

The burgeoning presence of microplastics (MPs) and heavy metals like cadmium (Cd) in aquatic ecosystems has emerged as a focal concern for environmental scientists worldwide [1–3]. Recent studies have underscored their ubiquitous distribution, from the deepest ocean trenches to the most remote freshwater systems, and have begun to unravel the complex ramifications of these pollutants on aquatic life and ecosystem health [4,5]. Despite significant advancements in our understanding, the interplay between such contaminants and aquatic ecosystem processes, particularly those fundamental to ecological function and resilience, remains inadequately explored [6,7].

Microplastics, defined as particles smaller than 5 mm, are omnipresent in marine and freshwater systems, originating from cosmetics, clothing, and the decomposition of larger plastic debris [8–10]. Similarly, cadmium (Cd), a heavy metal causing significant environmental concern, enters water bodies through agricultural runoff, industrial emissions, and atmospheric deposition, often attaching to particulates and sediments [11,12]. The coexistence of micropollutants in aquatic environments suggests potential interactive or

additive effects on the health and function of aquatic ecosystems, necessitating research into their combined impacts [13–15]. However, existing studies primarily focus on individual pollutants, with less research on the interactions and impacts of micropollutants in aquatic ecosystems [16,17].

Research indicates that microplastics can disrupt the feeding, growth, and reproductive functions of aquatic invertebrates [18,19]. Moreover, microplastics can act as vectors for other pollutants, particularly heavy metals, exacerbating their potential harm [20,21]. The adverse effects of microplastics and heavy metals on aquatic fungi are well documented in the literature [22]. For example, a study explored the impact of heavy metals, including cadmium, on the growth and sporulation of several aquatic fungi [23]. This research highlighted that heavy metals like cadmium could inhibit the sporulation of aquatic fungi, affecting their role as mediators between plant litter and invertebrates in aquatic ecosystems, thereby impairing ecosystem function. Yet, studies on the combined effects of microplastics and heavy metals are scarce, indicating a gap in current scientific knowledge [24,25]. Furthermore, the impact of these pollutants on the feeding behavior of aquatic invertebrates in co-polluted scenarios has largely been overlooked [26].

Recent research has highlighted that the presence of micropollutants in aquatic environments can cause severe disruptions to cellular processes by binding to proteins and replacing essential metals in enzymes, leading to impaired enzymatic activity [27]. This interference with biochemical pathways can lead to systemic toxicity in aquatic organisms, affecting growth, reproduction, and overall ecosystem health [28]. These interactions not only stress individual organisms but also lead to broader ecological impacts, such as changes in community composition and function, which are critical for the overall resilience and sustainability of aquatic ecosystems [29].

The decomposition of plant litter in aquatic ecosystems is not just a crucial indicator of ecological health; it is a fundamental process that influences broader ecological dynamics [30–32]. By breaking down organic material, this process not only recycles nutrients but also supports higher trophic levels within the food web [33,34]. Macroinvertebrates, for example, play a pivotal role in this context, as they feed on decomposed matter and the fungi that colonize it, effectively transferring energy up the food chain [35–37]. The study of factors affecting litter decomposition, therefore, has implications that extend well beyond decomposition rates, impacting food web structures and ecosystem resilience [38].

We sought to uncover the complex dynamics of micropollutants in aquatic ecosystems by studying the interactions between microplastics, heavy metals, and riverine invertebrates and their potential ecological effects, filling the knowledge gap on the impact of micropollutants on aquatic ecosystem functions. We hypothesized that the combined pollution of MPs and Cd negatively affects litter degradation and the composition of aquatic fungal communities while altering the nutritional quality of leaves, thereby affecting the feeding behavior of invertebrates.

This study aimed to address the following scientific research areas:

(1) The impact of combined pollution on litter decomposition: Investigating how the combined pollution of MPs and Cd affects the decomposition of litter, a process crucial for nutrient cycling and maintaining the health of aquatic ecosystems [39,40].
(2) The impact of combined pollution on aquatic microbial communities: Examining how the combined pollution of MPs and Cd affects the metabolic activity and community structure of aquatic microbes, especially aquatic fungi, as they are essential for litter decomposition [41,42].
(3) The impact of combined pollution on the feeding behavior of aquatic invertebrates: Investigating whether the combined pollution of MPs and Cd alters the nature of litter, thereby causing changes in invertebrate feeding behavior. We speculated on the ecological consequences of this behavior change and its feedback effects on nutrient and pollutant dynamics within the ecosystem [43,44].

2. Materials and Methods

2.1. Collection and Pre-Treatment of Litter

In this experiment, *Pterocarya stenoptera* C. DC., a tree species widely distributed along water edges in the southern regions of China, was chosen as the source of litter. *Pterocarya stenoptera* is a dominant species at the sampling site, making its leaves particularly representative of the dominant vegetative input and serving as a primary resource for detritivores in aquatic ecosystems. Fresh fallen leaves were manually collected between October and November 2022 along the streams of Zijin Mountain in Nanjing, Jiangsu Province (32°3′3.30″ N, 118°51′58.32″ E), an area distant from urban centers to ensure no interference from other pollution sources. The litter was brought back to the laboratory, air-dried naturally, and stored at room temperature for further use.

The collected leaves were soaked in sterilized (121 °C, 20 min), deionized water for 24 h prior to the experiment to remove soluble compounds [45]. Using a punch, discs approximately 12 mm in diameter were cut, avoiding the base of the veins, and then dried in an oven at 40 °C for 72 h to a constant weight [46]. In May 2023, using a precision balance, the dried leaf discs were weighed, with each set being 0.5 ± 0.003 g, and placed into a total of 72 nylon mesh bags (12 × 16 cm, 0.5 mm mesh size to minimize contact with benthic macroinvertebrates). The litter bags were placed in the streams of Zijin Mountain for 7 days to allow microbial colonization [47]. During the microbial colonization period, stream water was collected to measure its physicochemical properties (Table S1).

After a week, the litter bags were retrieved and stream water was collected and stored at 4 °C and immediately transported back to the laboratory. Stream water was first filtered through a 0.45 μm membrane filter (HAWP04700, Millipore, Boston, MA, USA) to remove debris and fungi and then sterilized (120 °C, 20 min) and stored. Leaf disc surfaces were gently wiped with sterile paper towels, and the wet weight of the leaf discs was recorded. The initial wet weight of each set of leaf discs was used to calculate the mass loss during the microbial colonization period [48]. Furthermore, three bags were selected to estimate the initial dry weight of each set of leaf discs, multiplying the wet weight of each set by a conversion factor. The conversion factor was calculated as DM/WM, where WM is the average wet weight of leaf discs taken from three litter bags and DM is the average dry weight of the same discs dried in an oven (105 °C, 24 h).

2.2. Preparation of Micropollutant Solutions

Polystyrene (PS) particles were used as the experimental material in this experiment, representing a common type of plastic product [49]. The PS particles had a diameter of 1 μm initially in suspension without any stabilizers or additives (100,000 mg/L, Sigma-Aldrich, Saint Louis, MO, USA). The suspension was ultrasonicated in the dark for 30 min (40 kHz, KQ-500DE, ShuMei, Kunshan, China) to ensure uniform distribution and then diluted with ultrapure water to prepare the stock solution, which was stored in a refrigerator (4 °C) for future use [50]. Since the plastic particles had sulfate groups on their surface, a zeta potential analyzer (Zeta Plus 90, Brookhaven Inc., New York, NY, USA) was used to measure the zeta potential of the plastic particles (−30 to −40 mV), reflecting their aggregation and stability in the microecosystem.

Based on predictions from 2015, global plastic waste inputs into aquatic systems are expected to increase tenfold by 2025, reflecting a significant and rapid escalation in environmental plastic pollution [51]. The concentration of microplastics added in this experiment was set at 30 μg/L, obtained by diluting the PS suspension with sterilized, filtered stream water [45,50,52]. According to Batista et al. [53], the concentration of Cd added in this experiment was set at 15 μg/L. The cadmium (Cd) solution was prepared by dissolving analytical-grade cadmium chloride (CdCl2, 10108-64-2, Aladdin, Shanghai, China) in sterilized, filtered stream water.

2.3. Microcosm Experiment Setup

We used seventy-two 150 mL conical flasks, each containing 60 mL of sterilized stream water, to serve as a microcosm. The leaf discs from each retrieved litter bag were gently rinsed with distilled water and transferred into each microcosm. Experimental setups included a control group (Group A), MP treatment group (Group B), Cd treatment group (Group C), and combined treatment group (Group D). All microcosms were cultured on a constant temperature shaker for 60 days at a temperature of 25 °C and a rotation speed of 150 rpm, with a cycle of light and dark periods alternating every 12 h. The sterilized water and micropollutants in the microcosms were replaced every 5 days. On the 15th, 30th, 45th, and 60th days after exposure, a set of microcosms was collected to measure relevant indicators [54]. For specific experimental setups and procedures, see Figure S1 and Table S2.

Forty-eight microcosms (4 treatments × 3 replicates × 4 sampling times) were selected to test the impact of micropollutants on litter decomposition. Another twenty-four microcosms (4 treatments × 3 replicates × 2 sample sets) were used for the invertebrate feeding experiment. Physiological and biochemical indicators of the leaves were measured, including remaining mass and nutrient content of litter, lignin and cellulose content, microbial metabolic activity, fungal biomass, and fungal community structure. Additionally, the morphology of litter was characterized using FE-SEM (Figure S2).

2.4. Determination of Remaining Mass and Nutrient Content of Litter

Leaf discs collected from each microcosm were rinsed with distilled water to remove surface chemicals, dried in an oven to a constant weight (40 °C, 72 h), and finally weighed using a precision balance (AS 220/C/2, Radwag, Radom, Poland) to the nearest 0.001 g to calculate the remaining mass of litter. The decomposition rate k was calculated using Olson's Formula (1):

$$X_t = X_0 e^{-kt} \quad (1)$$

where X_0 and X_t represent the initial mass at time 0 and the remaining mass at time t, respectively [55].

The dried leaf discs were ground into powder using a ball mill, weighed in tin foil cups to the nearest 0.001 g, and analyzed for carbon (C) and nitrogen (N) content, i.e., the nutrient content of litter [56], using an organic element analyzer (vario MACRO cube, Elemental Analysensysteme GmbH, Frankfurt, Germany).

2.5. Determination of Lignin and Cellulose Content

Lignin and cellulose are the main structural components of plant cell walls, directly affecting the degradability of litter and the feeding preferences of aquatic invertebrates [57,58]. Lignin content was determined using a lignin content assay kit (BC4200, Solarbio, Beijing, China), following the instructions to acetylate the phenolic hydroxyl groups in lignin; then, the absorbance was detected at wavelength 280 nm with a UV spectrophotometer and the content was calculated using the formula. Cellulose content was determined using a cellulose content assay kit (BC4280, Solarbio, Beijing, China), following the instructions to decompose cellulose into β-D-glucose under acidic conditions; then, it was colorized with anthrone under strong acidic conditions, the absorbance was detected at wavelength 620 nm with a visible spectrophotometer, and the content was calculated using the formula.

2.6. Microbial Metabolic Activity Assessment

To evaluate overall microbial activity, dehydrogenase activity on leaf discs was measured using a spectrophotometric method [59]. For each sample group, three leaf discs were incubated in 400 µL of triphenyltetrazolium chloride (TTC) solution (pH 7.6) in the dark at 30 °C for 24 h, followed by incubation in 4 mL of acetone for 2 h. Three autoclaved leaf discs served as a negative control. The absorbance of the mixture was measured using a UV–Vis spectrophotometer (T3200s) at 485 nm.

2.7. Fungal Biomass Measurement

Ergosterol, also known as ergosta-5,7,22-trien-3β-ol, is an important component of fungal cell membranes that is stable in structure and specific, making it more representative than glucosamine for biomass measurement. Ergosterol content can be used to measure fungal biomass [60,61]. The specific experimental procedure was as follows: 5 freeze-dried leaf discs were added to 10 mL of KOH–methanol solution (0.8%) and heated at 80 °C for 30 min to extract the lipids by solid-phase extraction. The extract was saved and measured using a high-performance liquid chromatography (HPLC) system (Thermo Ultimate 3000, Thermo Scientific, Waltham, MA, USA). Methanol of HPLC grade was used, with the wavelength set at 282 nm, flow rate at 1.4 mL/min, and temperature at 33 °C. The ergosterol content in aquatic fungi is usually equivalent to 5.5 mg/g of the dry weight of the fungi.

2.8. Invertebrate Feeding Experiment

The Chinese round snail (*Cipangopaludina chinensis*) was selected as the model animal for this experiment as it dominates the streams of Zijin Mountain and represents the stream's invertebrates. It is an omnivorous animal that feeds on plants and litter, playing the role of both a grazer and a decomposer in the ecosystem [62,63], which is essential for nutrient cycling and the overall productivity of aquatic ecosystems [64].

In May 2023, healthy snails (*Cipangopaludina chinensis*) were collected from the streams near the litter collection site and transported back to the laboratory with stream water. They were acclimatized in filtered sterile stream water for one week, fed with pre-processed leaf discs, and aerated regularly to prevent hypoxia. To observe the snail's feeding process on litter, a preliminary experiment was conducted with a randomly selected snail. The results showed that snails could normally feed on litter (Figure S3).

Before the official experiment started, feeding was stopped, and the snails were purged for 24 h to induce a state of hunger. Healthy, uniformly sized snails (6.8 ± 1.2 mm) were selected for the official experiment through observation. After 15 days of exposure, the litter from the microcosm was collected and placed in 150 mL beakers, each with 60 mL of sterile, filtered stream water. One group of 12 beakers, each with one Chinese round snail, served as the experimental group. Another group of 12 beakers, without Chinese round snails, served as the control group to evaluate the decomposition quality of litter during the experiment. All beakers were aerated regularly to prevent the snails from suffocating. On the 3rd day after the experiment started, all snails and litter were collected, and the mass loss of the litter was measured [65].

The remaining leaf discs and animals were freeze-dried (−50 °C, 12 h, Lablyo mini, Coolvacuum, Barcelona, Spain) and weighed (d = 0.1 μg, UMX2, Mettler Toledo, Zurich, Switzerland) to obtain the final dry weight of leaf discs (mg) and invertebrates (mg). The feeding rate f of the Chinese round snail was calculated using the following Formula (2):

$$f = \frac{(L_i - L_f) - (C_i - C_f)}{I_f \times t} \quad (2)$$

where L_i is the initial weight of leaves fed to the animals (mg), L_f is the final weight of leaf discs after feeding (mg), C_i and C_f represent the initial and final dry weight of the control leaf discs (without animals) (mg), I_f is the dry weight of the animal (mg), and t is the feeding time. The feeding rate was expressed in mg leaf DM mg^{-1} animal DM d^{-1} [66].

2.9. Fungal Community Structure Analysis

After the final sampling, three freeze-dried leaf discs were randomly selected from each group for fungal community structure analysis. The highly variable region of the fungal genome rDNA ITS was amplified by PCR using the primer pair ITS1F/ITS2R. For primer information, see Table S3, and, for gel electrophoresis, see Figure S4. The specific process was as follows: total microbial DNA from each sample was extracted using the MagAtrract PowerSoil Pro DNA Kit (4898129, Qiagen, Hilden, Germany), following the manufacturer's

protocol. The purity (NanoDrop2000) and integrity (1% agarose gel electrophoresis) of the extracted DNA were tested. After a preliminary experiment, the official PCR experiment used TaKaRa rTaq DNA Polymerase in a 20 μL system. The amplification program was as follows: 95 °C for 3 min, 35 cycles (95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s), and 72 °C for 10 min, finally storing at 4 °C (PCR instrument: ABI GeneAmp® 9700). All samples were processed under official experimental conditions, with three repeats for each sample. PCR products of the same sample were mixed and detected by 2% agarose gel electrophoresis (Agarose75510019, Thermo Scientific, Waltham, MA, USA), and the PCR products were recovered by gel cutting using the AxyPrepDNA Gel Recovery Kit (AXYGEN, San Francisco, CA, USA) and eluted with Tris_HCl. The PCR products were purified (AxyPrep DNA Gel Extraction Kit) and quantified (Quantus Fluorometer, Promega, Madison, WI, USA). Libraries were constructed using the NEXTFLEX Rapid DNA-Seq Kit and sequenced on the Miseq PE300 platform (Illumina, San Diego, CA, USA). Sequencing results were first optimized, and the original sequencing sequences were quality-controlled using fastp software (version 0.20.0) and assembled using FLASH software (version 1.2.7). OTU clustering was performed based on 97% similarity using UPARSE software (version 11). OTU taxonomic annotation was conducted using the RDP classifier (version 2.11) against the Silva 16S rRNA gene database (v138), with a confidence threshold of 70%, and the community composition of each sample was analyzed at different taxonomic levels. Functional prediction analysis of 16S was performed using PICRUSt2 software (version 2.2.0).

2.10. Data Analysis

Data analysis and plotting were performed using RStudio software (version 4.3.2). Data were first subjected to a normality test (Shapiro–Wilk test) and a homogeneity of variances test (Bartlett's Test). If the data were normally distributed, ANOVA was used for within-group variance analysis and the Tukey test was used for intergroup multiple comparisons. If the data were not normally distributed, non-parametric tests (Kruskal–Wallis test) were used for within-group variance analysis and Dunn's test with Bonferroni correction was used for intergroup multiple comparisons.

All figures were created using Rsudio software (version 4.3.2) and its relevant packages (tidyverse, reshape2, ggpubr, ggrepel, ggcorrplot, corrplot, agricolae, vegan, patchwork, readxl, etc.). One-way ANOVA was used to compare the rates of litter decomposition, nutrient content, lignin, cellulose, dehydrogenase activity, and fungal biomass between different treatment groups. Correlation analysis was used to examine the relationships between litter decomposition rate, invertebrate feeding rate, and various properties of litter. Additionally, the differences in fungal community structure between different treatment groups and the variation scale under multidimensional environmental stress were analyzed.

3. Results

3.1. Changes in Mass Remaining and Decomposition Rate of Litter

This study assessed the changes in litter mass and decomposition rate under exposure to MPs, Cd, and their combination (Figure 1). Figure 1A indicates that the control group (A) showed the most significant reduction in litter mass over 60 days, with a significantly lower percentage of remaining mass compared to the treated groups ($p < 0.05$). In contrast, the combined treatment group (Group D) showed a reduced decomposition rate, with the highest percentage of remaining mass at all time points. Additionally, the decomposition rate (Figure 1B) was significantly higher in the control group (A) than in the groups treated with MPs (Group B), Cd (Group C), and their combination (Group D) ($p < 0.001$). Group D exhibited the lowest decomposition rate, indicating the cumulative effect of the pollutants.

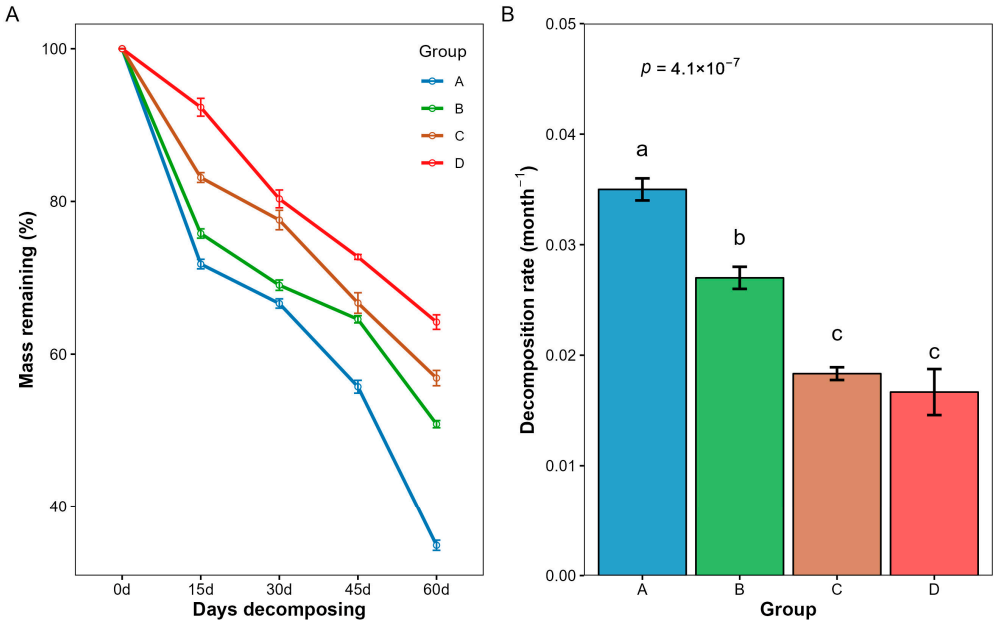

Figure 1. Mass remaining and decomposition rate of litter in different treatment groups. (**A**) Mass remaining. (**B**) Decomposition rate. Different letters at the top of the bar chart indicate significant differences between treatments. Error bars = mean ± standard deviation, $n = 3$.

3.2. Changes in Nutrient Content and Carbon–Nitrogen Ratio of Litter

Figure 2 presents changes in nutrient content and the carbon–nitrogen (C:N) ratio in different treatment groups. Figure 2A reports the carbon content of the litter. The control group (A) had the lowest carbon content, while the Cd treatment group (C) had the highest. The difference between groups was significant ($p < 0.001$). Figure 2B shows the nitrogen content in the litter, with the highest values for the control group (A). The nitrogen content in the MPs treatment group (B) was slightly reduced compared to the control group (A) but was not statistically significant, while Groups C and D showed a more pronounced decrease, with significant differences observed ($p < 0.01$). Figure 2C displays the C:N ratio, with the control group (A) having the lowest ratio, indicating higher-quality litter that was more suitable for microbial decomposition. The ratio gradually increased in the treatment groups, with Group C having the highest C:N ratio, indicating poorer-quality litter due to the presence of MPs and Cd that was less favorable for microbial decomposition ($p < 0.001$).

3.3. Changes in Cellulose and Lignin Content of Litter

Analysis of litter components revealed significant changes in cellulose and lignin content due to the presence of MPs, Cd, and their combination (Figure 3). In the combined treatment group (D), the cellulose content was significantly higher, followed by the Cd group (C), the control group (A), and the MPs group (B). The analysis of lignin content (Figure 3B) showed the lowest percentage of lignin in the combined treatment group (D), indicating a slower decomposition of other litter components. Individual treatments with MPs (B) and Cd (C) led to medium levels of lignin content, with the control group (A) having the highest. These findings suggest that combined micropollutant stress significantly hindered the microbial decomposition of other litter components compared to single pollutant exposure ($p < 0.001$).

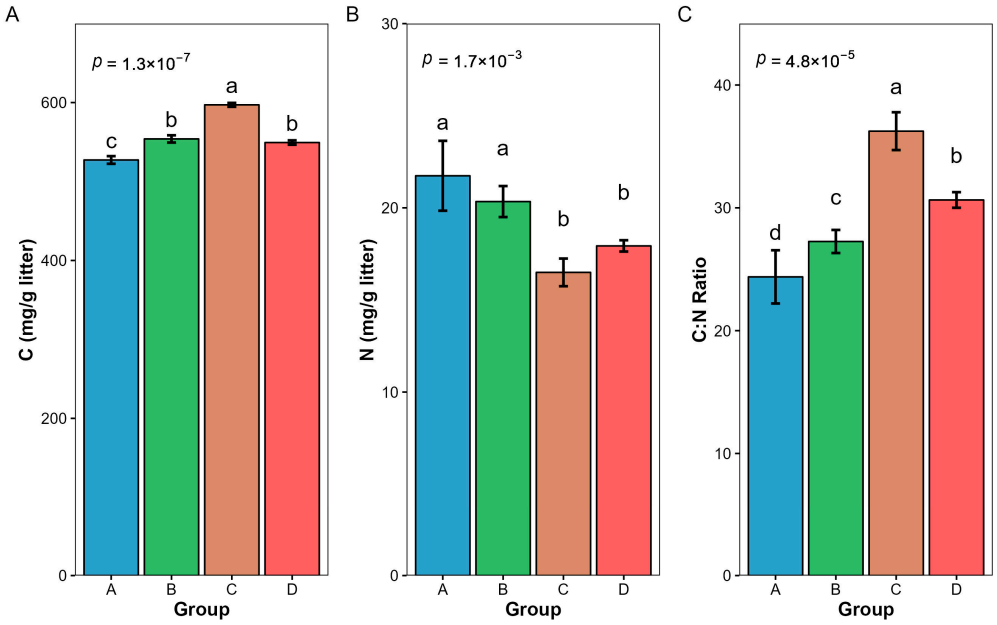

Figure 2. Nutrient content and carbon–nitrogen ratio of litter in different treatment groups. (**A**) Carbon content. (**B**) Nitrogen content. (**C**) Carbon–nitrogen ratio.

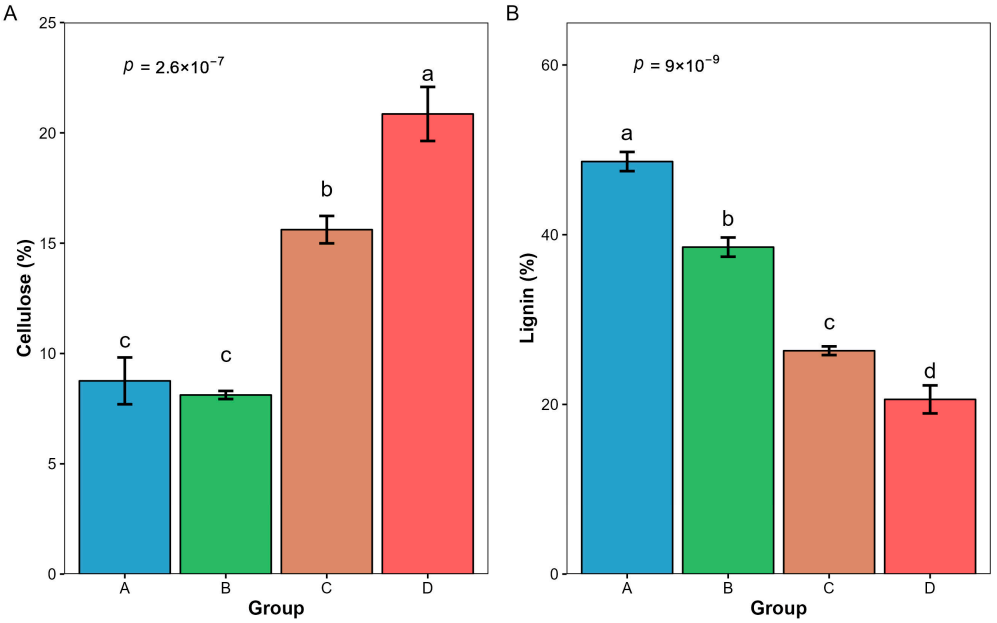

Figure 3. Cellulose and lignin content of litter in different treatment groups. (**A**) Cellulose content. (**B**) Lignin content.

3.4. Changes in Microbial Activity

Microbial dehydrogenase activity, a key indicator of microbial oxidative activity in litter, was significantly affected by the application of MPs, Cd, and their combination (Figure 4A). During the 60-day decomposition period, the control group (A) showed a peak in dehydrogenase activity at 45 days, which then stabilized, indicating a temporary enhancement of microbial activity corresponding to active litter decomposition. Compared to the control group, the MPs treatment group (B) showed a moderate decrease in the microbial activity peak, while Cd exposure (C) led to a further reduced peak. Notably, the combined treatment (D) resulted in the lowest dehydrogenase activity throughout the decomposition period, highlighting the compounded negative impact of mixed pollutants on microbial function. The observations of average dehydrogenase activity (Figure 4B)

were consistent with these observations, with the control group (A) displaying the highest activity. Groups B and C showed lower activity in succession, while Group D had the lowest average activity, with significant differences between groups ($p < 0.001$). These results clearly demonstrate a trend of inhibition of microbial oxidative functions with increasing micropollutant stress, emphasizing the critical role of pollutant interactions in affecting microbial ecosystem services.

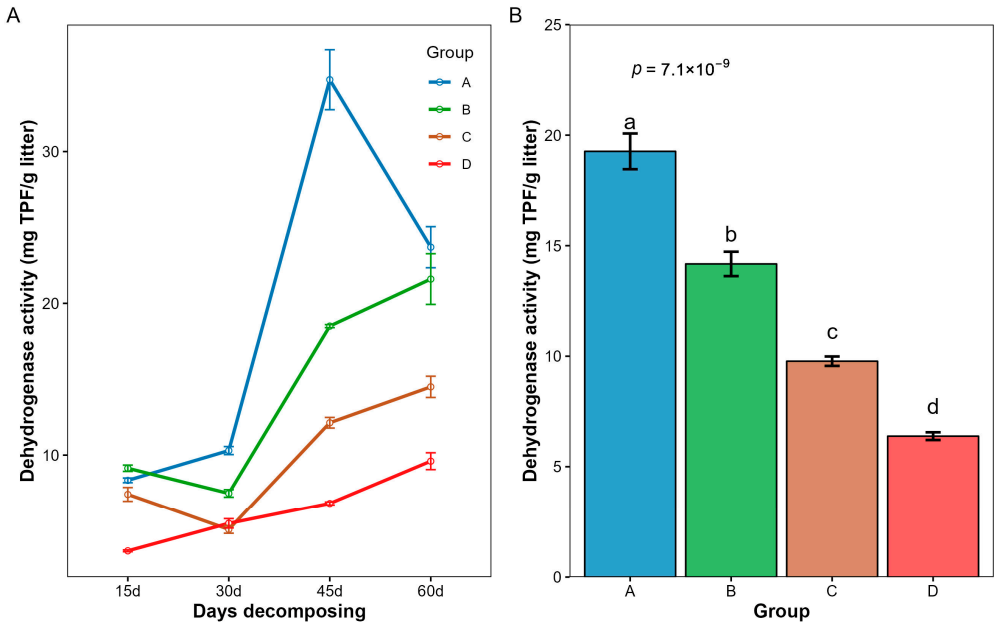

Figure 4. Dehydrogenase activity of litter in different treatment groups. (**A**) Dynamic values. (**B**) Mean values.

3.5. Changes in Fungal Biomass

Fungal biomass in litter was significantly influenced by treatment conditions, as observed in changes during the decomposition process (Figure 5). The control group (A) showed a substantial increase in fungal biomass, peaking at around 45 days before stabilizing, consistent with the expected growth curve of fungi in natural litter decomposition. The fungal biomass in the combined treatment group (D) followed a similar pattern to that of the control group but with reduced magnitude, reaching a lower peak. The MPs treatment group (B) led to further inhibited growth of fungal biomass, with a peak significantly lower than that of the control group. The most significant reduction in fungal biomass was observed in the Cd treatment group (C), where fungal biomass remained the lowest throughout the study period. The quantification of average fungal biomass clearly showed that the control group maintained the highest level (Figure 5B). The MPs group (B) and the Cd group (C) displayed reduced biomass, but the combined treatment group (D) did not have the lowest biomass, with significant differences between treatments ($p < 0.001$). These results showcase the adverse effects of micropollutants on the growth of fungal communities crucial for litter decomposition.

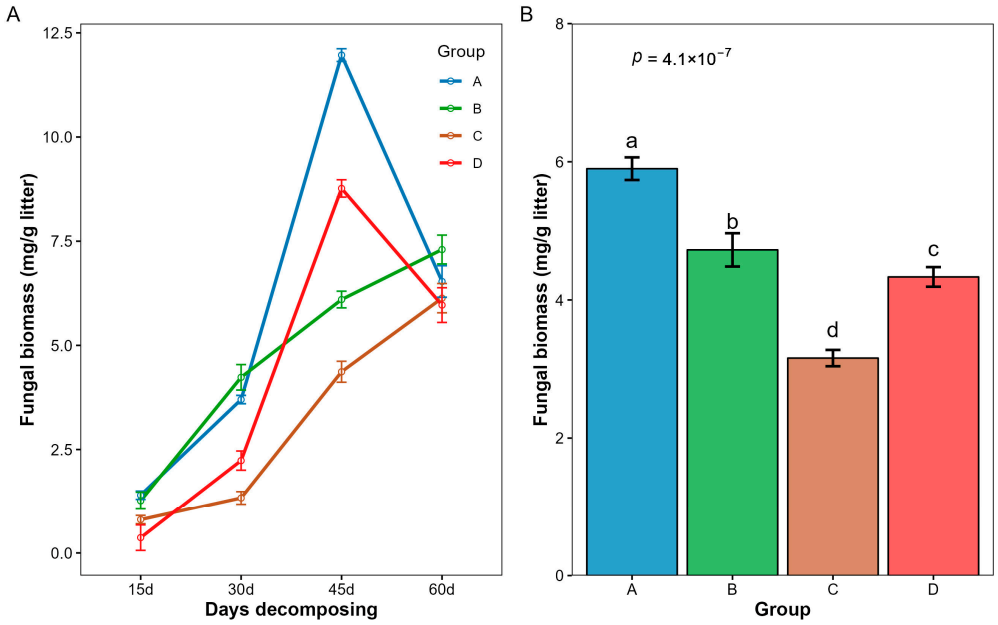

Figure 5. Fungal biomass of litter in different treatment groups. (**A**) Dynamic values. (**B**) Mean values.

3.6. Changes in Invertebrate Feeding Rate

Contrary to the observed reductions in microbial activity and fungal biomass, invertebrate feeding rates showed different responses to micropollutant stress (Figure 6). The control group (A) had the lowest feeding rate, while exposure to MPs (B) led to an increase. The Cd group (C) showed a slight reduction in feeding rate compared to the MPs group but still higher than the control. Notably, the combined treatment group (D) displayed the highest feeding rate, with significant differences compared to the control group ($p < 0.001$). Despite reductions in microbial and fungal activity, the increase in feeding rates suggests that the presence of micropollutants changed the quality or palatability of the litter, potentially making it more attractive or less defensive to invertebrate consumption.

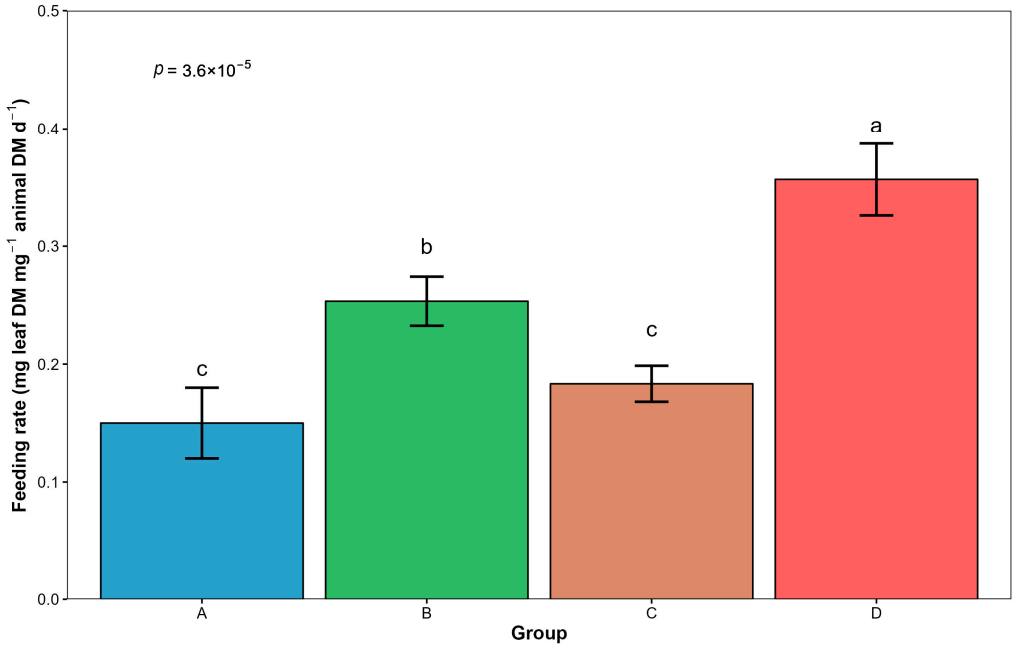

Figure 6. Invertebrate feeding rate on the litter of different treatment groups.

3.7. Relationships between Litter Decomposition Rate, Invertebrate Feeding Rate, and Environmental Factors

Correlation analysis enhanced the understanding of ecosystem responses to micropollutant stress, integrating various factors and their interactions (Figure 7). Decomposition rate (k) showed a strong positive correlation with dehydrogenase activity (DH) and fungal biomass, highlighting the role of microbial activity in litter decomposition. Invertebrate feeding rate (f) correlated with carbon content (C), indicating that a higher carbon content (carbohydrates) might increase the palatability of litter to invertebrates.

Figure 7. Heatmap of correlations between litter decomposition rate, invertebrate feeding rate, and environmental factors. Asterisks indicate the level of significance of the correlation, * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$. Numbers in the lower left corner represent correlation coefficients, × indicates a lower coefficient.

Fungal biomass showed a strong positive correlation with nitrogen content (N) and dehydrogenase activity (DH), indicating that fungal growth is closely related to nutrient availability and microbial activity in the ecosystem. Conversely, cellulose content showed a strong negative correlation with dehydrogenase activity (DH) and lignin, suggesting that increased cellulose may be associated with the inhibition of microbial processes.

The observed negative correlation between lignin content and feeding rate (f) suggests that lignin may hinder invertebrate consumption ($p < 0.05$). Overall, these results elucidate the complex relationships between multiple environmental factors, where micropollutants affected the quality of litter, microbial functions, and invertebrate feeding behaviors.

3.8. Changes in Fungal Community Structure

The presence of micropollutants significantly influenced the fungal community structure in the litter, as shown by phylum-level community bar charts (Figure 8). The control group displayed a diverse fungal community, dominated by Ascomycota, with a smaller proportion of Basidiomycota and other fungal phyla. Exposure to MPs caused a significant shift, with a decreased abundance of Ascomycota and Basidiomycota and an increase in less abundant phyla, such as Chytridiomycota. Cd treatment led to further changes in community structure, with a noticeable reduction in fungal diversity and an increase in unidentified fungi. This change suggests that Cd negatively impacts fungal diversity and the ecological functions that these fungi perform in litter decomposition. Combined exposure produced the most significant effect, with a notable decrease in Ascomycota and

an increase in unidentified fungi, possibly indicating the loss of functional groups critical to effective decomposition. Other phyla, such as Chytridiomycota and Rozellomycota, were also affected, showing variability across different treatments. Overall, micropollutants not only reduced the abundance of key fungal taxa but also led to less diverse fungal communities, potentially disrupting these communities' ability and balance within aquatic ecosystems, thus affecting ecosystem functions like litter decomposition and nutrient cycling.

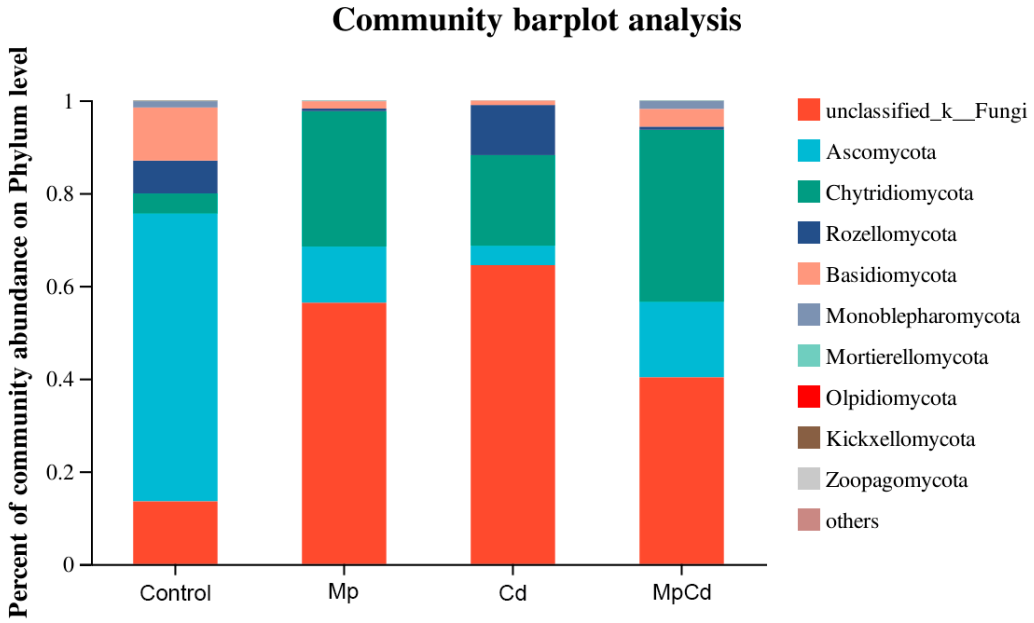

Figure 8. Differences in fungal community structure at the phylum level.

The Venn diagram reveals the distribution and overlap of operational taxonomic units (OTUs) between the four treatment groups, providing insights into the diversity and uniqueness of microbial communities within each treatment (Figure 9). All treatment groups shared some OTUs with the control group, indicating that, despite pollution, there was some commonality among microbial components. The control group (A) had a large number of unique OTUs (1213), indicating a diverse microbial community in the absence of micropollutants. Groups B (MPs) and C (Cd) showed fewer unique OTUs, with 126 and 123, respectively, suggesting that each micropollutant preferred specific microbial taxa, reducing fungal diversity to some extent. The combined treatment group (D) displayed a moderate number of unique OTUs (307), indicating that combined pollution alters community composition differently from individual pollutants. The area of overlap between groups revealed shared OTUs, with the central intersection containing 275 OTUs shared by all four treatments, highlighting a core fungal community resilient to micropollutant stress. Additionally, the unique intersection between the Cd group (C) and the combined treatment group (D), containing 33 OTUs, might be taxa-specifically favored or unaffected by combined micropollutant stress. These results demonstrate that MPs and Cd, whether individually or in combination, have adverse effects on the structure and composition of microbial communities in aquatic ecosystems.

Hierarchical clustering further elucidated the impact of micropollutants on fungal community structure (Figure 10). The control group (A) clustered alone, indicating a unique community profile. Groups B (MPs) and C (Cd) formed one cluster, while the combined treatment group (D) was more closely related to group C (Cd), suggesting that the impact of Cd on fungal community structure was more dominant than that of MPs. The taxa bar shows the relative abundance of specific OTUs within each treatment, with some OTUs dominating in certain treatments, reflecting the selective pressure imposed by micropollutants. Together, these analyses reveal that pollution by MPs and Cd not only

reduces fungal biodiversity but also induces changes in community structure at the OTU and higher taxonomic levels.

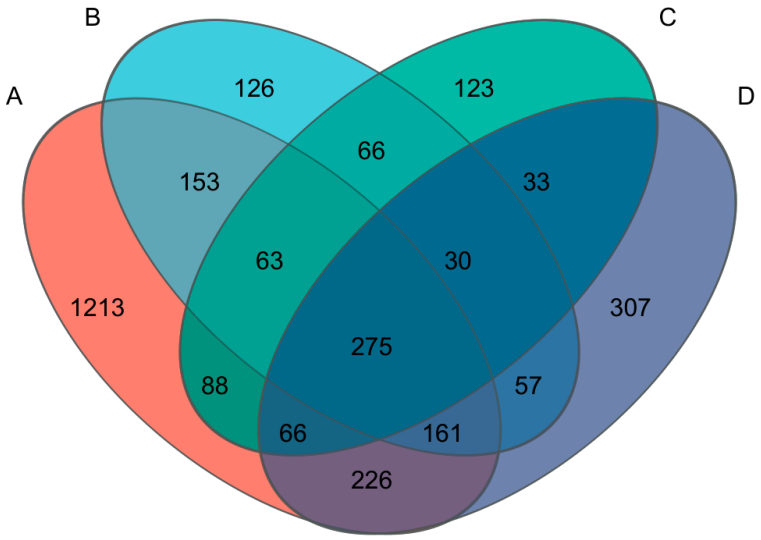

Figure 9. Venn diagram of fungal OTUs. Different colors represent different groups, with numbers in overlapping parts indicating the number of species shared among multiple groups and numbers in non-overlapping parts indicating species unique to corresponding groups.

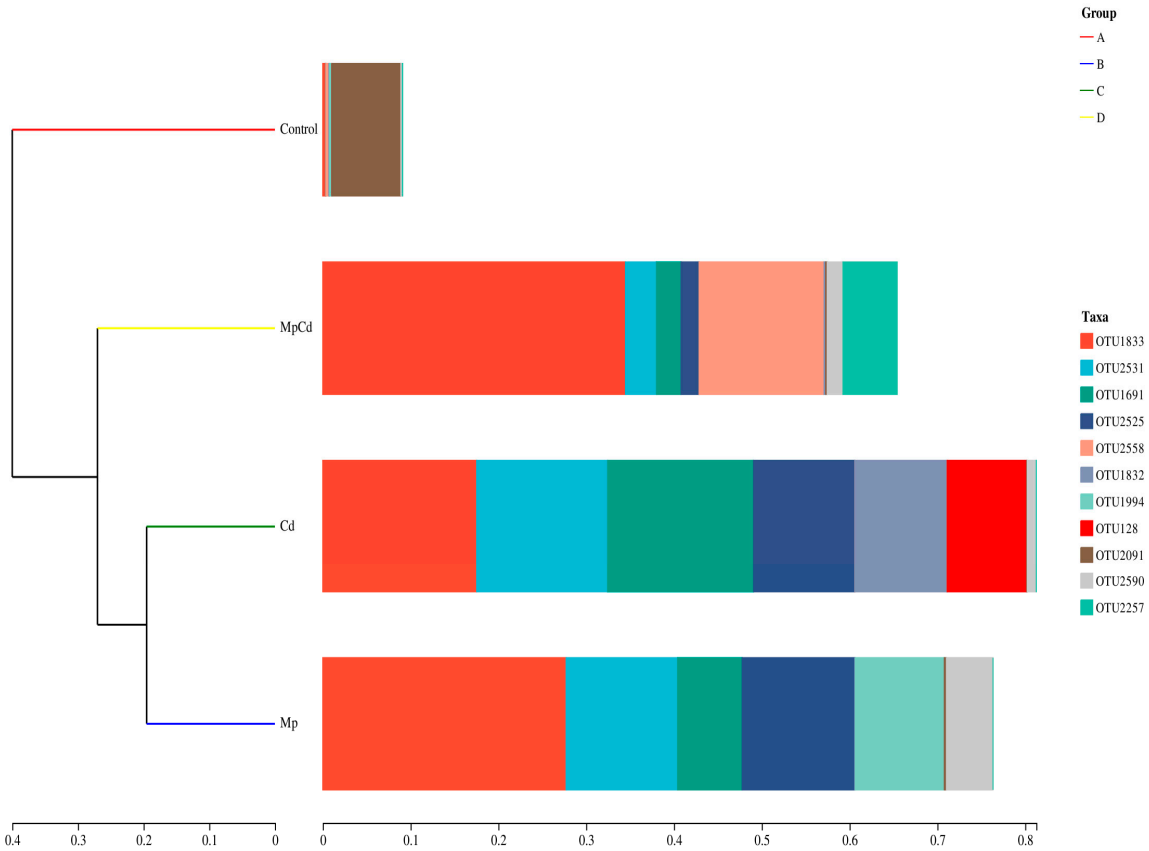

Figure 10. Hierarchical clustering tree of fungal community structure at the OTU level. The length between branches represents the distance between samples, with different groups presented in different colors. On the right side of the clustering tree, the composition of the dominant species in each sample is shown.

3.9. Relationship between Fungal Diversity and Environmental Factors

Canonical correspondence analysis (CCA) was conducted to explore the impact of MPs and Cd on fungal community composition at the OTU level (Figure 11). The analysis accounted for 40.25% of the community variance on the CCA1 axis and 19.74% on the CCA2 axis. The CCA plot positioned the microbial communities of each treatment group against these two principal gradients. The CCA1 axis clearly separated the control group (A) from the treatment groups, indicating a significant shift in the community upon exposure to MPs and Cd. The arrow pointing toward the Cd vector showed a strong association with changes along the CCA1 axis, highlighting the significant impact of Cd on microbial community structure. Additionally, Group B (MPs) was positioned close to the MPs vector and away from Group A, emphasizing the unique impact of MPs on microbial OTUs. Group D (MPs + Cd) was located near the center of the CCA plot, suggesting that the combined effects of MPs and Cd might not be simply additive and could interact in complex ways not fully captured by two axes of variation. The CCA plot emphasized the significant impact of micropollutants on microbial community structure, with each pollutant contributing differently to the observed changes in community composition. CCA provided a nuanced perspective on the relationship between different environmental stressors and microbial community changes, laying a foundation for interpreting the potential ecological impacts of micropollutants.

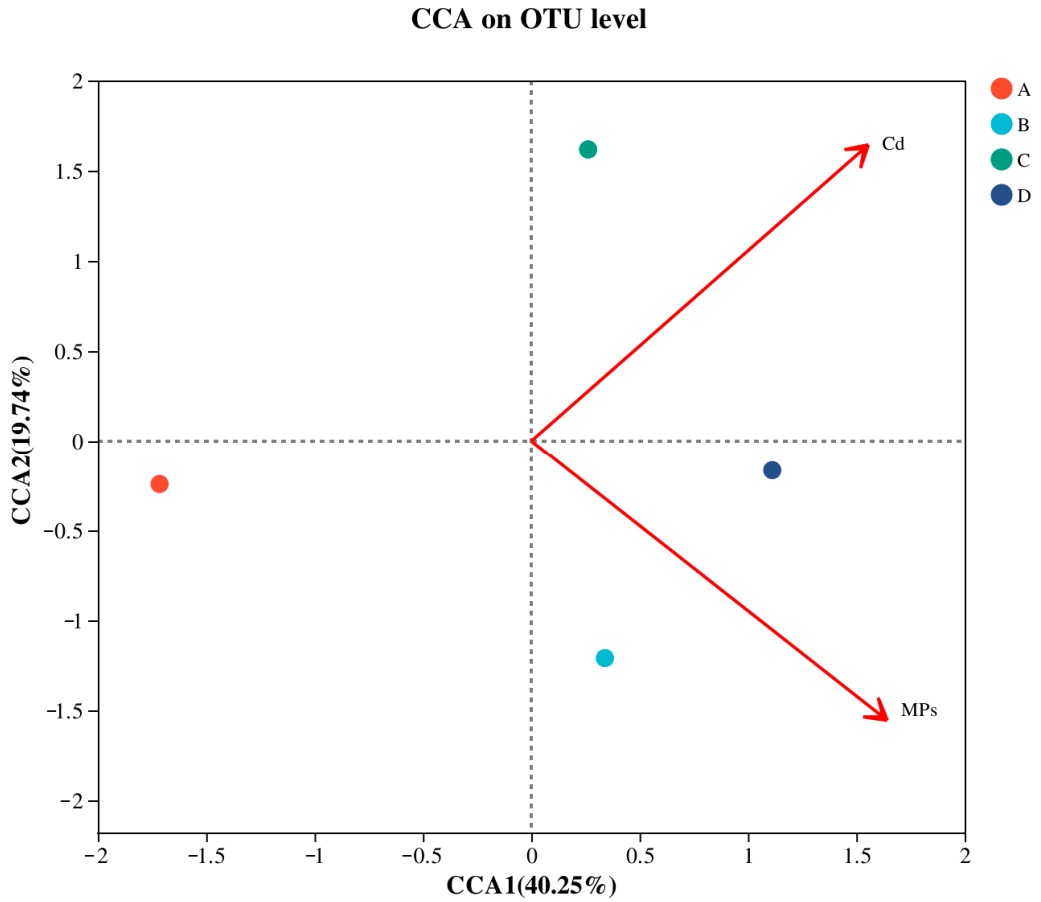

Figure 11. Canonical correspondence analysis (CCA) of fungal community structure. Points in the diagram represent samples, with different colors indicating different groups. The distance between points represents the similarity and differences in species composition between samples. Arrows represent environmental factors, with the projection distance of sample points to quantitative environmental factor vectors indicating the extent to which samples were influenced by environmental factors.

A Spearman correlation heatmap displays the correlation between MPs and Cd with different fungal phyla (Figure 12). With MP exposure, phyla like Chytridiomycota showed a strong positive correlation, indicating that these phyla may thrive or maintain their presence when exposed to MPs. Conversely, Rozellomycota displayed a strong negative correlation, suggesting an inhibitory effect of microplastics on this phylum. Ascomycota and Basidiomycota showed less pronounced negative correlations, which may reflect some degree of tolerance or adaptation to MPs. Similarly, under Cd exposure, Ascomycota and Basidiomycota exhibited weak negative correlations, indicating the potential vulnerability of these phyla to heavy metal stress. Rozellomycota and Chytridiomycota showed positive correlations, differing from the impact of MPs.

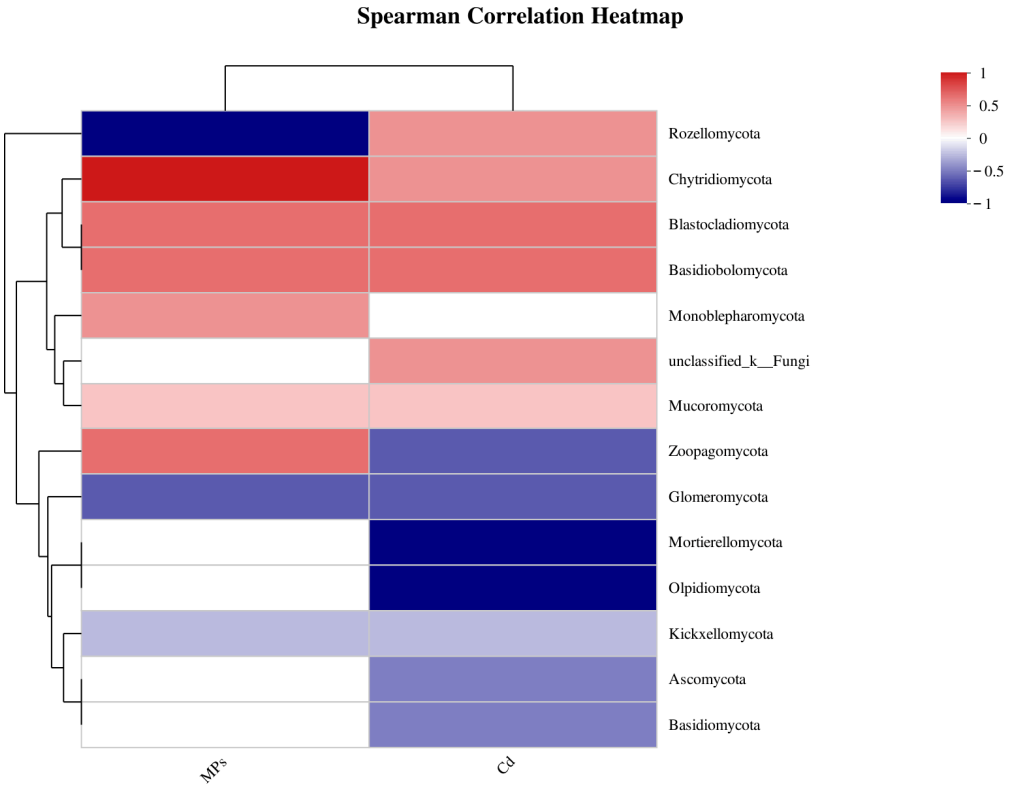

Figure 12. Spearman correlation heatmap of environmental factors and different species. The legend on the right is the color interval for different r values.

The hierarchical clustering on the side of the heatmap revealed phylogenetic relationships between phyla and similarities in their response patterns to these micropollutants. Closely clustered phyla may have similar ecological niches or functional roles within the ecosystem, which might explain their parallel responses to micropollutant exposure. For instance, Ascomycota and Basidiomycota are both key fungi in the decomposition process of litter. This heatmap analysis not only highlights the differential impacts of MPs and Cd on different fungal phyla but also offers insights into potential adaptation mechanisms or vulnerabilities of fungal communities when facing stress factors.

Circos relationship diagrams detail the correlations between different treatments (MP, Cd, and MPs + Cd) and fungal species (Figure 13). This visualization technique displays the relative abundance of various fungal phyla and their relationships within and across different treatment groups. In the control group, a broad diversity of fungal phyla was evident, with Ascomycota and Basidiomycota showing considerable representation, reflecting a typical, balanced fungal community. However, preferences emerged after exposure to MPs, such as a shift toward Chytridiomycota and Rozellomycota, while the representation of Ascomycota declined. Similar shifts were observed with the presence of

Cd, with unique patterns of specific fungal group abundances indicating unique responses to heavy metal stress.

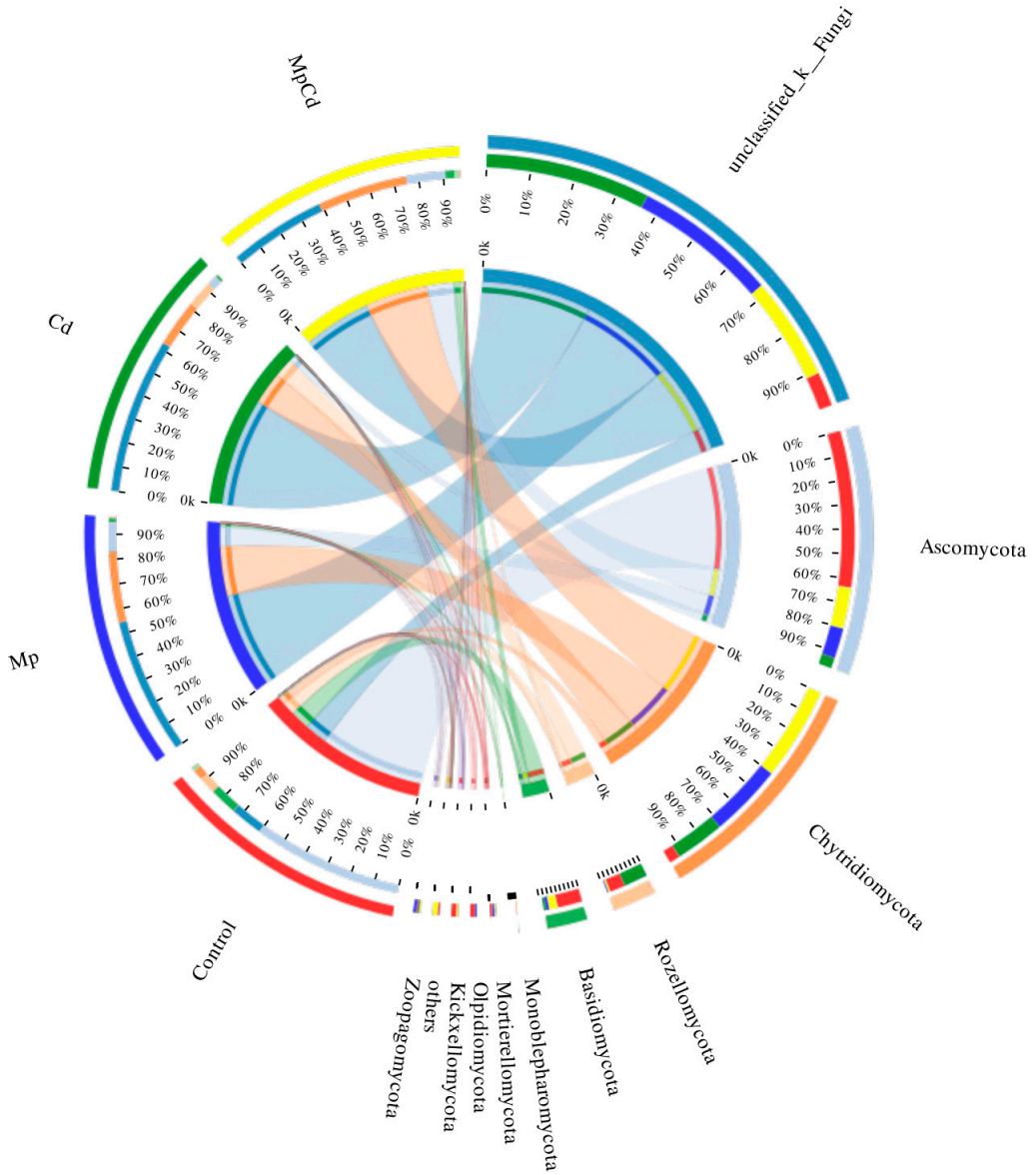

Figure 13. Circos diagrams of correlations between different treatments and species. The small semicircle (left half-circle) represents the composition of species in samples, with the color of the outer ribbon representing the group it comes from and the color of the inner ribbon representing the species, with the length representing the relative abundance of that species in the corresponding sample. The large semicircle (right half-circle) represents the distribution proportion of species at that taxonomic level in different samples, with the outer ribbon representing species, the color of the inner ribbon representing different groups, and the length representing the distribution proportion of that sample in a certain species.

The combined treatment showed the most profound changes, with usually dominant phyla decreasing and uncommon phyla increasing, suggesting a synergistic effect of combined pollutants. The interconnections between treatment groups represented by ribbons in the Circos relationship diagram showed shared taxa among groups and unique taxa to each treatment, emphasizing the complex responses of fungal communities to different micropollutant stresses. The Circos relationship diagram highlighted the complex dynamics

of fungal community composition in response to exposure to MPs and Cd, indicating that the ecosystem functions these key microbes participate in might also change.

4. Discussion

4.1. Inhibitory Effects of Micropollutants on Litter Decomposition

The impact of MPs and heavy metals like Cd on litter decomposition represents a critical area of research due to their significant implications for nutrient cycling and ecosystem health [67]. The presence of these pollutants in aquatic ecosystems can alter the structure and function of microbial communities and the feeding behaviors of invertebrate detritivores, which play key roles in litter decomposition [33].

The observed reduction in litter decomposition rates in the presence of MPs and Cd, whether used individually or in combination, aligns with the results of existing studies that highlight the potential of such micropollutants to hinder microbial enzymatic activity and alter community composition [68]. For instance, MPs have been shown to affect the structure of microbial biofilms and reduce the abundance of key decomposers like fungi and bacteria [69,70]. Similarly, heavy metals like Cd are known to exert toxic effects on microbial communities, inhibiting the growth and enzymatic activity of fungi crucial for litter decomposition [71].

The reduction in dehydrogenase activity and fungal biomass found in this study under MP and Cd treatments indicates the inhibition of microbial and fungal processes essential for the decomposition of organic matter [72–74]. This is consistent with the work of Huang et al. [75], who reported a decrease in microbial activity in soils contaminated with MPs, likely due to physical disturbances and changes in microhabitats. Moreover, the observation of Tan, Wang [76] of Cd's inhibitory effects on soil dehydrogenase can be extended to aquatic ecosystems, further supporting the results seen for the combined treatment group.

The complex relationship between the presence of micropollutants and litter quality is highlighted by the increase in the C:N ratio, reflecting the quality of organic matter available for decomposition [77]. A higher C:N ratio indicates poorer-quality litter, which is usually more difficult to decompose [78]. The finding of an elevated C:N ratio in the presence of MPs and Cd emphasizes the potential of these pollutants to exacerbate the accumulation of hard-to-degrade organic matter, thereby slowing nutrient cycling processes [79].

The counterintuitive result of increased invertebrate feeding rates in the presence of pollutants, especially with the combined MP and Cd treatment, suggests indirect effects of altered litter properties, potentially making litter more palatable or less toxic to invertebrates [80]. The increase in invertebrate feeding rates could partially offset the negative impacts of micropollutants on decomposition rates [81]; however, this compensation is insufficient to restore the decomposition process to its natural rate, as evidenced by the overall reduced nutrient release [82].

4.2. Regulatory Effects of Micropollutants on Aquatic Fungal Community Structure

The multifaceted impacts of MPs and Cd on aquatic fungal communities represent an emerging field of environmental research with significant implications for ecosystem dynamics and functions [83]. The results of this study provide valuable insights into the regulatory effects of these micropollutants on the structure of aquatic fungal communities, showcasing identifiable shifts in fungal diversity and community composition.

Aquatic fungi with reproductive structures (sporangia) are adapted to flowing waters, capable of rapidly colonizing litter that falls into streams [84]. Additionally, aquatic fungi release extracellular enzymes to soften leaves and enhance the quality and palatability of leaves for invertebrates [85]. Thus, aquatic fungi play a crucial role in transferring nutrients and energy from riparian leaf litter to higher trophic levels [86]. Reports indicate that various pollutants, including heavy metals, organics, pesticides, and metal nanoparticles, affect the biomass and sporulation rates of aquatic hyphomycetes in litter decomposition [87].

The observed reduction in the diversity and abundance of certain fungal phyla, such as Ascomycota and Basidiomycota, in the presence of MPs and Cd, aligns with literature views suggesting that pollutants may attract certain tolerant species, thereby reducing overall diversity [88]. For example, the study by Kettner, Rojas-Jimenez [83] emphasized that MPs could bind to fungal spores, potentially interfering with spore dispersal mechanisms and indirectly affecting community structure. The observed shifts could also result from Cd's known direct toxic effects in disrupting cellular processes, leading to decreased fungal biomass and altered community structure [89].

The selective pressure exerted by these pollutants further evidenced the resilience of phyla like Rozellomycota and Chytridiomycota. Such shifts might reflect not only direct tolerance to MPs and Cd but also indirect effects through changes in interspecific fungal interactions, as indicated by the persistence of groups typically associated with tolerance to stress or opportunistic strategies, such as Mortierellomycota [73].

Interestingly, this study revealed an increase in the relative abundance of less common phyla, such as Monoblepharomycota, in the combined treatment group. This suggests that the combined treatment might create niches favoring these groups or alter environmental conditions, potentially leading to new ecological dynamics within the fungal community [90]. This finding is supported by recent work by Koh, Bairoliya [91], which showed that the accumulation of MPs could lead to the establishment of "plastisphere" communities with unique compositions and ecological interactions.

Moreover, the observation of altered fungal community dynamics with a micropollutant presence echoes the concept of "ecotoxicological effects cascades," where pollutants impact not only species abundance but also the interactions connecting them, potentially leading to ecosystem-level consequences [92]. The reduced fungal biomass and enzymatic activity suggest that MPs and Cd may disrupt fungal-mediated processes such as litter decomposition and nutrient cycling, with profound implications for ecosystem health and function [93].

4.3. Promoting Effects of Micropollutants on Feeding by Aquatic Invertebrates

The observed changes in feeding behavior of aquatic invertebrates due to pollution by MPs and Cd, as demonstrated in this study, contrast with the adverse impacts on microbial communities and decomposition processes. The enhanced feeding rates of invertebrates in the presence of these micropollutants highlight the complex interactions within aquatic ecosystems.

The increase in feeding rates in the presence of these toxic pollutants, MPs and Cd, might initially seem counterintuitive [94]. However, the literature suggests that micropollutants can alter the palatability of litter or trigger stress responses in invertebrates, leading to compensatory feeding—a phenomenon where organisms increase their intake to counteract reduced food quality or meet metabolic demands increased by stress [95]. Moreover, MPs have been found to affect the physical structure of litter, potentially making it easier for invertebrates to consume [68].

In this study, the presence of MPs and Cd led to a relative increase in cellulose content while decreasing the lignin content of litter. As the primary structural component of plant leaves, the reduction in lignin could decrease leaf toughness, thereby facilitating consumption by detritivores like the Chinese round snail (*Cipangopaludina chinensis*), resulting in increased feeding rates.

Research also indicates that exposure to nanoplastics can increase the content of unsaturated fatty acids in litter, thereby improving food quality and making invertebrates more inclined to consume contaminated leaves [96]. This corroborates the findings of this study, where micropollutants, by altering litter food quality, influenced invertebrate feeding preferences.

Furthermore, some invertebrates might ingest MPs due to their size and shape, mistaking them for food [97]. This non-selective feeding behavior could lead not only to physical

harm and energy dilution but also serve as a vector for MPs and associated pollutants into the food web.

In the context of Cd, previous research has shown that heavy metals can accumulate in litter, altering its chemical properties and potentially affecting its consumption by detritivores [98]. However, the balance between avoidance behavior due to toxicity and the urgent need for nutrients needs to be considered, which can lead to varying feeding responses [99].

Promoting invertebrate feeding in environments with elevated concentrations of micropollutants may not be beneficial in the long run. The increased ingestion of contaminated substances could lead to the bioaccumulation of toxic substances, posing significant risks to invertebrates themselves and higher trophic levels through biomagnification [100]. Moreover, although increased invertebrate feeding might initially seem to compensate for reduced microbial decomposition, if invertebrates are affected by toxins, the potential for efficient nutrient cycling could be compromised [101].

Therefore, the dual impact of MPs and Cd on the feeding behavior of aquatic invertebrates and its subsequent effects on ecological processes highlight the necessity of a comprehensive approach to assessing environmental risks. These findings emphasize the complexity of micropollutants' impact on freshwater ecosystems, extending beyond direct implications for organism health to broader ecological significance.

5. Conclusions

Our comprehensive study of the interactions between microplastics and cadmium in aquatic ecosystems provides significant insights into the complex dynamics controlling litter decomposition. This research found that the presence of these micropollutants, whether individually or in synergy, significantly hinders the decomposition process by adversely affecting microbial activity, particularly the structure of aquatic fungal communities. Moreover, the combined effects were stronger than the individual ones, exhibiting cumulative impacts. An intriguing contrast was revealed in the feeding behavior of aquatic invertebrates, showing an increase despite a decrease in litter decomposition rates. This counterintuitive finding suggests that microplastics and cadmium alter the properties of litter, making it more attractive to invertebrates, a novel observation warranting further investigation. These findings contribute key data to the growing body of evidence on the widespread impact of environmental pollutants on aquatic food webs and nutrient cycles.

Nevertheless, our study is not without limitations. One of the primary constraints is the controlled laboratory setting of our microcosm experiments, which may not have fully captured the complexity of natural aquatic ecosystems. For instance, the static conditions of the microcosms did not entirely mimic the dynamic interactions and environmental variability found in natural settings, such as fluctuations in water flow, temperature, and interactions with other chemical and biological factors. Additionally, our focus on a limited number of microbial and invertebrate species may not have reflected the broader ecological interactions and biodiversity present in natural environments. Future studies should aim to address these limitations by incorporating more complex ecological models and extending research to field-based experiments. This approach would help in validating the laboratory findings under more variable and realistic conditions.

Looking ahead, this research underscores the urgency of addressing the widespread dissemination of microplastics and heavy metals in aquatic habitats. As it becomes increasingly clear that these pollutants have the capacity to fundamentally alter ecosystem processes, further research is crucial for fully understanding their long-term ecological consequences. Future studies should aim to unravel the complex interactions between pollutants and the biological components of various trophic levels. Moreover, there is an urgent need to develop innovative remediation strategies to mitigate the presence of these pollutants and protect the integrity and functionality of aquatic ecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16091295/s1: Figure S1: Experimental setup and flow; Figure S2: SEM images of litter surface structure; Figure S3: Feeding process of *Cipangopaludina chinensis*; Figure S4: Agarose gel electrophoresis detection and band distribution diagram; Table S1: Physicochemical properties of Zijin Mountain stream water; Table S2: Experimental setup; Table S3: PCR primer information.

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