

Article

Sustainable Bioremediation of Plastic Waste: How the Flame Retardant TCPP Affects Polyurethane Foam Biodegradation by *Galleria mellonella* Larvae

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Abstract

As a common substitute for brominated flame retardants (BFRs), organophosphate flame retardants (OPFRs) have been insufficiently studied in terms of their ecotoxicological impacts on plastic biodegradation processes in invertebrate systems. This study investigated the impact of an OPFR, tris (1-chloro-2-propyl) phosphate (TCPP), on the dietary behavior and gut microbiota of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) larvae during the biodegradation of rigid polyurethane (RPU), as well as the fate of TCPP. The results show that TCPP interfered with larval feeding activity, hindered the nutritional conversion of food, and triggered metabolic compensation through lipid reserve catabolism. Notably, mass balance analysis revealed that bioaccumulation of TCPP was negligible, with most of it excreted through frass, indicating limited biodegradation of this organophosphate ester. 16S rRNA sequencing indicated that TCPP drove the reconstruction of gut microbiota in larvae and identified three dominant bacteria of *Morganellaceae*, *Enterobacteriaceae*, and *Staphylococcaceae* families, as well as non-dominant bacteria of *Klebsiella* and *Vagococcaceae* families, as characteristic microbiota contributing to RPU and TCPP biotransformation. This study serves as a reminder to pay attention to the toxicity, migration, and transformation of OPFRs in biodegradable plastics. Notably, TCPP, a dominant chlorinated OPFR, exhibits environmental persistence with limited biodegradability and low bioaccumulation, traits which hinder the spontaneous attenuation of plastic waste in ecosystems and undermine the sustainability of the plastic lifecycle. This work emphasizes the need to integrate risk assessments of specific additives into the plastic waste management framework and to develop targeted detoxification strategies for promoting a sustainable material lifecycle.



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Keywords: organophosphate flame retardants; plastics; invertebrates; gut microbiota; host-mediated biodegradation; sustainability

1. Introduction

Brominated flame retardants (BFRs, such as polybrominated diphenyl ethers (PBDEs), Hexabromocyclododecane (HBCD), and tetrabromobisphenol A (TBBPA)) decompose at high temperatures to produce harmful gases (such as HBr), which capture free radicals (such as OH·, O·) in the combustion chain reaction to terminate flame propagation [1,2]. With the European Union's restriction on typical BFRs, they have faced declining usage. Organophosphate flame retardants (OPFRs) isolate oxygen and heat by forming a polyphosphate isolation layer on the material surface and promoting the formation of a carbonization

layer, without producing harmful gases. They are considered less toxic than BFRs. Their global production reached ~2.8 million tons in 2018 [3]. However, the toxicity of OPFRs gradually emerges over time, and they are classified as emerging contaminants, which now raises significant concerns regarding environmental persistence and ecological safety [4].

Rigid polyether polyurethane foam (RPU), characterized by its cross-linked polymeric structure dominated by ether bonds ($-C-O-C-$) in the soft segments and aromatic isocyanate-derived hard segments, is extensively utilized as thermal insulation in refrigerators due to its low thermal conductivity and mechanical robustness [5,6]. Conventional disposal methods for RPU waste, including landfill, incineration, and chemical recycling, face significant limitations. Landfill exacerbates microplastic leakage and occupies valuable land resources, while incineration above 400 °C releases toxic volatiles (e.g., hydrogen cyanide, isocyanates) and carcinogenic dibenzofurans, posing severe environmental and health risks [6,7]. Chemical techniques such as glycolysis or hydrolysis can recover polyols. However, they demand high energy inputs, generate acidic/alkaline wastewater, and struggle with additive-contaminated RPU (e.g., FRs), yielding impure recyclables unsuitable for high-performance reapplication [5,8].

In contrast, biodegradation offers an eco-efficient alternative by utilizing enzymatic catalysis under ambient conditions, minimizing secondary pollution. Microbial communities (e.g., *Pseudomonas*, *Acinetobacter*) and fungi (*Cladosporium pseudocladosporioides*) partially degrade polyester PU via esterase-mediated hydrolysis, but exhibit negligible activity against ether-bond-rich RPU [9,10]. Enzymatic depolymerization—mediated by cutinases, esterases, and other serine hydrolases—remains confined to the outermost micrometers of RPU thermosets because the densely cross-linked aromatic–aliphatic network sterically excludes high-molecular-weight catalysts. Diffusion constraints are compounded by reversible inhibition through phosphate-based flame retardants such as tris(1-chloro-2-propyl) phosphate (TCPP), which competitively acylates catalytic serine and suppresses turnover by two orders of magnitude, rendering bulk biocatalytic erosion unattainable [11,12]. Recent breakthroughs in plastic biodegradation mediated by invertebrates have revealed unprecedented efficiency. *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) depolymerized polyether PU with a mass reduction of 35–40% within 15–20 days, enriching *Enterobacteriaceae* and *Streptococcaceae* in their gut microbiomes [13]. Notably, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) larvae achieved 35.08% RPU mass loss within seven days, attributed to the activity of esterases and urethanases, and dynamically remodeled their gut microbiota towards *Morganellaceae* dominance [14].

However, our previous study found that typical BFRs-TBBPA inhibited the degradation of RPU by altering the structure of the *Galleria mellonella* microbiota and stain growth [15]. OPFRs and BFRs have different flame retardant mechanisms, structures, and toxicities. Among chlorinated OPFRs, TCPP diverges from other OPFRs (e.g., triphenyl phosphate, tris(2-ethylhexyl) phosphate) [16]. The chlorinated propyl moieties in TCPP modify its interactions with biological membranes and enzymatic systems, potentially disrupting larval neurobehavioral and nutrient assimilation processes in manners distinct from OPFRs with alkyl or aryl substitutions [17]. Further, TCPP, like TBBPA, is a widely used additive in polymers, electronics, and construction materials, which is integrated into rigid polyurethane (RPU) foams as refrigerator insulation material, improving fire resistance [18]. Elucidating TCPP's mechanistic impacts on *Galleria mellonella*-mediated RPU biodegradation is indispensable for addressing the gap in understanding halogenated OPFRs' involvement in the insect–microbe plastic cycle.

TCPP is a persistent organic pollutant, and its microbial degradation has been extensively researched. However, current reports on biodegradation are largely confined to the studies of aromatic OPFRs in activated sludge [19]. Purified microorganisms capable

of degrading OPFRs remain scarce, with only a few bacterial species reported, including *Roseobacter* [20], *Sphingomonas* sp. TDK1, *Sphingobium* sp. TCM1, and *Brevibacillus brevis* [20–23]. Two strains identified as members of the *Sphingomonas* were found to effectively degrade Tris(2-carboxyethyl)phosphine and Tri(1,3-dichloropropyl)phosphate [24]. Three strains classified as *Xanthobacter* have recently been confirmed as effective degraders of Tris(2-carboxyethyl)phosphine [25]. The strain *Amycolatopsis* sp. FT-1 has been identified as a highly efficient degrader of TCPP, offering valuable insights into the proteomic mechanisms underlying the microbial degradation of this compound [17]. Denitrifying bacteria can mineralize TCPP to produce PO_4^{3-} and Cl^- under specific conditions [26]. However, microorganisms often exhibit low resistance to environmental disturbances, resulting in limited efficiency in degrading TCPP, which hampers their practical application. In contrast, the gut of invertebrates can serve as an effective bioreactor, significantly addressing the limitations of microbial methods. Therefore, it is valuable to study the potential reactions that occur after ingestion of TCPP. Unfortunately, most biodegradation studies have utilized pure reagents. To our knowledge, no research has explored how TCPP influences the biodegradation of RPU in invertebrates. Although *Galleria mellonella* has demonstrated substantial plastic degradation potential, the specific effects of its interaction with plastics containing OPFRs remain unclear.

This study employs the established plastic-degrading invertebrate model *Galleria mellonella* to investigate interactions between RPU and TCPP. We aim to quantify TCPP's impact on larval growth and RPU consumption dynamics, evaluate its perturbations on gut microbiome structure and function, and assess its bioaccumulation potential and metabolic fate within the larvae. Through comparative analysis with other FRs, we describe the mechanistic distinctions in how these additives modulate plastic biodegradation pathways. This work advances the fundamental understanding of the biophysiological interactions governing additive-laden plastic fate in biological systems.

2. Methods and Materials

2.1. Sources of *Galleria mellonella* and Test Materials

Galleria mellonella larvae (25–30 mm body length) and beeswax (BW) were purchased from a commercial apiary in Yancheng, Jiangsu Province, China, where the larvae were maintained on a BW diet before the experiment (Figure S1). TCPP was purchased from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China).

RPU and TCPP-containing RPU (RPUP) were synthesized according to our previous study [15], as shown in Figure S2a. Covaci et al. pointed out that TBBPA was typically added in a ratio of 10–20% (wt%) due to the differences in polymers [27]. For example, Ülkü et al. added 9 wt% of the TCPP microcapsules to prepare HB grade flame retardant RPU [28]. Thus, RPU loaded with 10% TCPP was synthesized. For larval feeding experiments, RPU and RPUP were cut into $2 \times 2 \times 1 \text{ cm}^3$ cubic pieces (Figure S2b) and characterized using X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Fisher Scientific, Waltham, MA, USA) [29]. As shown in Figure S3, RPU consists of C, N, and O, and RPUP contains C, N, O, Cl, and P, indicating that TCPP was successfully incorporated into the polyurethane matrix.

2.2. Experiment of *Galleria mellonella* Larvae Ingestion

Galleria mellonella larvae underwent a 72 h starvation period to eliminate intestinal residues before the experiment. To analyze the impact of TCPP on larval behavior, growth, and RPU degradation, the *Galleria mellonella* larvae were fed under five conditions: starvation (ST, control diet), beeswax (BW, control diet), TCPP-containing beeswax (BWP), RPU, and RPUP. Each diet condition was carried out in triplicate. For each experiment, 40 larvae

were housed in glass chambers (20 × 10 × 5 cm; 2 mm wall thickness) containing 2.0 g of the initial substrate. Supplemental feed (0.5 g) was provided every two days to maintain substrate availability. All trials were conducted under controlled conditions (25.0 ± 1.0 °C, 70 ± 5% humidity, continuous darkness) [30,31].

RPU and RPUP consumption, larval mass variations, survival rates, and cumulative pupation numbers of the *Galleria mellonella* larvae were systematically recorded under distinct feeding conditions. The larvae were incubated for 7 days. During this period, the larvae underwent airstream cleaning and were transferred to a clean container for data collection every day.

2.3. Biochemical Characteristics of Larvae

After the incubation period, 10 *Galleria mellonella* larvae from each distinct feeding condition were analyzed individually to assess their biochemical characteristics. Protein, lipid, and total water-soluble carbohydrate contents were quantified using a UV spectrophotometer (UV-6100S, Shanghai Mai Shi Instrument Co., Ltd., Shanghai, China) at 595 nm, 525 nm, and 625 nm, respectively, employing the Coomassie Brilliant Blue G-250 (CBB G-250) method [32], sulfuric acid-vanillin phosphate reaction method [33], and anthrone assay [34]. Comprehensive descriptions of the larval biochemical characteristics are detailed in the Supplementary Materials, Method S1.

2.4. Instrumental Analysis

To assess the effects of RPU and RPUP on *Galleria mellonella* larvae during the feeding period, *Galleria mellonella* larvae were fed with ST, BW, BWP, RPU, and RPUP diets for 2, 4, and 6 days, followed by a 2-day fasting period, and collected for fluorescence imaging using an IVIS S LUMINA spectrum system (PerkinElmer Instruments, Inc., Waltham, MA, USA, excitation 440 nm, emission 620 nm) [14]. Excitation–emission matrix (EEM) spectroscopy (F-7000, Hitachi, Tokyo, Japan) was utilized to characterize changes in dissolved organic matter (DOM) within the gut of *Galleria mellonella* larvae during RPU and RPUP feeding periods [35]. The intestinal tracts were harvested on 0, 2, 4, and 6 days for EEM analysis. Detailed methodologies are provided in the Supplementary Materials, Method S2.

Scanning electron microscopy (SEM, JSM-IT 800, Royal Philips Electronics, Eindhoven, The Netherlands) at 5.0 kV was used to analyze the micromorphology of RPU and RPUP in the gut and frass of *Galleria mellonella* larvae. To improve sample conductivity, a conductive layer was applied via an ACE-600 high-vacuum ion sputter coater [9]. Comprehensive procedures of larval intestine dissection and extracting RPU/RPUP from intestinal contents and frass are detailed in the Supplementary Materials, Methods S3 and S4 [36].

Gas chromatography–mass spectrometry (GC-MS, 7890A/5975C, Agilent, Santa Clara, CA, USA) was employed to analyze the degradation of BWP and RPUP in larval intestinal contents and frass after a 7-day incubation period [37]. This analysis aimed to investigate the migration and transformation of TCP during RPU degradation by *Galleria mellonella* larvae [38]. Detailed methodologies are provided in the Supplementary Materials, Method S5.

2.5. Microbial Community Analysis

To investigate the differential impacts of TCP on the gut microbiota of *Galleria mellonella* larvae during ingestion, 16S rRNA high-throughput sequencing analysis was performed [39]. The larvae were fed ST, BW, BWP, RPU, and RPUP diets during the 7-day incubation period followed by a 2-day fasting period. Intestines from 20 larvae were collected on days 0, 2, 4, 6, and 8 and stored at −80 °C for subsequent DNA analysis [40]. Detailed procedures are provided in the Supplementary Materials, Methods S6 and S7.

2.6. Statistical Analysis

In the present study, all data are presented as the mean values with the standard deviation (SD) from replicate experiments. Statistical analyses were carried out using IBM SPSS Statistics (Version 25.0). Changes in larval mass of *Galleria mellonella*, plastic mass loss, and cumulative pupation rates were assessed by one-way analysis of variance (ANOVA). Where significant effects were detected, pairwise comparisons were performed using Student's t-test with the least significant difference (LSD) correction to control for multiple comparisons. Community divergence was confirmed by PERMANOVA and corroborated by NMDS.

3. Results and Discussion

3.1. Biophysiological Responses of *Galleria mellonella* Larvae to RPUP Diet

The effects of TCPP on the biophysiological characteristics of the *Galleria mellonella* larvae are shown in Figure 1. Figure 1a shows similar consumption rates for RPU and RPUP ($p > 0.05$), indicating that TCPP has minimal impact on RPU intake. However, the anti-nutritive effect of TCPP appears in the *Galleria mellonella* larvae mass change [15]. Only the larvae fed with BW show an increase in mass, while the larvae fed with BWP and RPUP show a decrease in mass, suggesting that TCPP disrupts food conversion [41]. Considering that insect gut microbiota can convert food into nutrients [42,43], Lu et al. indicated that TCPP is a typical endocrine-disrupting chemical that can exert its biological toxicity by interfering with hormonal functions and disrupting hormone signaling pathways [18]. On the other hand, it can be observed from the fluorescence imaging of the larval gut in Figure 1b that only the intake of BW gradually increases over time, while the intake of BWP, RPU, and RPUP gradually decreases over time, and the intake of BWP and RPUP is obviously lower than that of BW and RPU [44], implying that RPU and TCPP are both foods that the larvae dislike, and that their interactions systemically interfere with larval feeding, resulting in a decrease in larvae mass, as shown in Figure 1a. Moreover, the absence of TCPP effects on RPU consumption suggests that plastic avoidance by larvae masks the chemical impacts [15].

During the 7-day incubation period, the survival rates of the BWP and RPUP groups were maintained at over 80% (Figure S4), indicating that TCPP has little effect on the survival status of the larvae in the short term. However, the sustained decrease in mass under feeding with BWP and RPUP decreased the long-term viability of larvae (Figure 1a). Meanwhile, TCPP had a visible impact on the cumulative pupation number of the BWP group ($F(1, 4) = 264.58, p < 0.001$) (Figure 1c), suggesting that compared to the RPUP group, TCPP disturbed food conversion more obviously in the BWP group due to the larvae ingesting more BWP. Pupation demands a large number of nutritional reserves [45], and TCPP reduced the conversion rate of BW into nutrients, resulting in a low pupation rate in the BWP group. However, the pupation rate of the RPUP group is similar to that of the RPU group ($p > 0.05$), inferring that the low intake of the RPUP group may have reduced the impact of TCPP [46], allowing pupation to be achieved through both the food conversion of RPU and larval nutritional consumption [47].

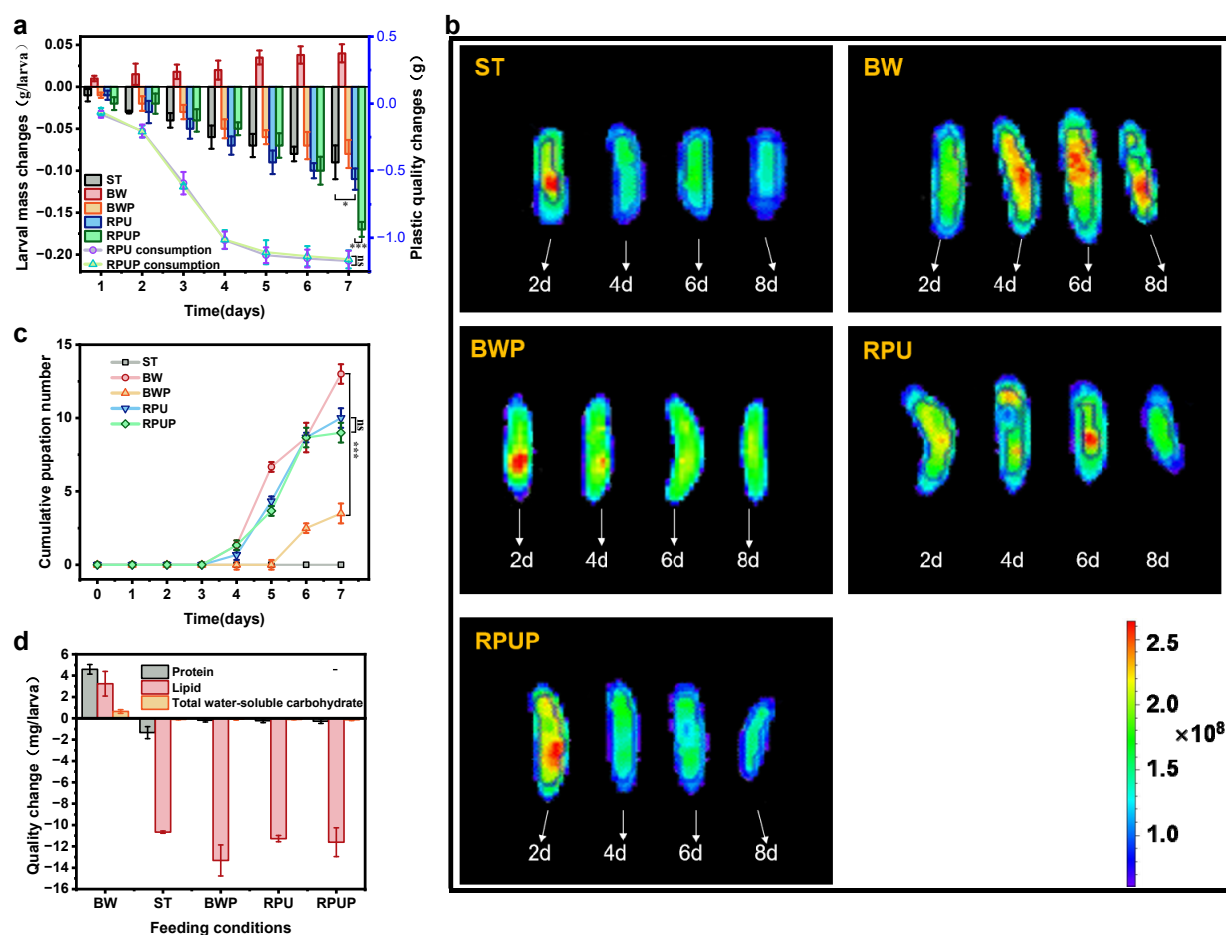


Figure 1. Feeding of larvae. The mass changes of *Galleria mellonella* larvae and plastic quality changes (a); small animal imaging pictures of *Galleria mellonella* larvae in different feeding conditions over time (b); the cumulative pupation number of *Galleria mellonella* larvae (c); biochemical substance content for *Galleria mellonella* larvae in different feeding conditions (d). The bars represent the mean $\pm$ SD of three biological replicates. One-way ANOVA was performed with LSD/Duncan pairwise comparison testing. * $p < 0.05$, and *** $p < 0.001$. ns: not significant, $p > 0.05$.

As shown in Figure 1d, metabolic analysis reveals that TCPP induced biochemical dysregulation, and only the protein, lipid, and total water-soluble carbohydrate content increased in the BW group, corresponding to the data in Figure 1a. This indicates that TCPP interfered with the metabolic processes of these biochemical substances in the larvae. Furthermore, the strong reduction in lipid content in the *Galleria mellonella* larvae is due to the fact that insects typically survive during periods of nutrient scarcity or dry feeding conditions through lipid metabolism [48]. Larvae do not have sufficient time to absorb adequate nutrients prior to initiating pupation, forcing them to rely on their lipid reserves to obtain the nutrients essential for successful pupation.

Overall, TCPP exacerbates larval biomass loss, pupation failure, and metabolic dysfunction, all of which are modulated by substrate nutrient value and incubation duration. This result is consistent with our previous research on the effect of TBBPA on RPU degradation by *Tenebrio molitor* and *Galleria mellonella* larvae [15], as well as Brandon's research on the effect of HBCD on PS degradation by *Tenebrio molitor* larvae [46], indicating that FRs have a similar effect on invertebrates fed with plastics containing FRs. The ability of FRs to disrupt nutrient absorption highlights the subcellular toxicity associated with plastic degradation in ecosystems. In the future, it is necessary to research the mechanisms by which FRs affect the gut microbiome and endocrine pathways.

3.2. Analysis of Gut Secretions Due to RPUP Degradation by *Galleria mellonella* Larvae

The morphology of RPU and RPUP in the digestive tract and frass of *Galleria mellonella* larvae is clearly observed in Figure 2a,b. As can be seen, there is a transition from pristine smoothness to erosion in the plastic surface, with the textures becoming increasingly rough and forming microcracks, indicating that enzymes mediated by the gut microbiota have degraded the plastics [15]. Additionally, the surface of RPUP is visibly less eroded than that of RPU, implying that TCPP interfered with the degradation of RPU by the gut microbiota.

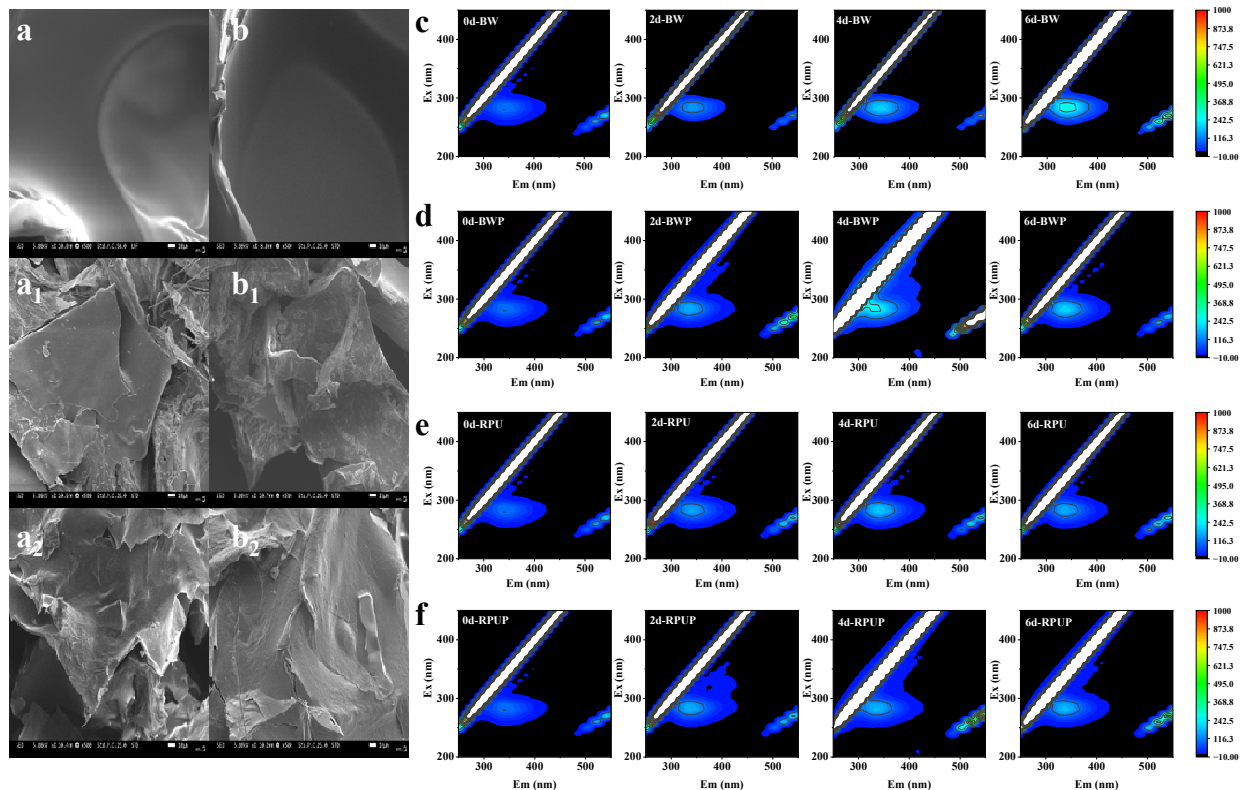


Figure 2. SEM analysis of the intestine and frass of *Galleria mellonella* larvae fed with RPU and RPUP: raw RPU (a) and RPUP (b); RPU (a₁) and RPUP (b₁) in the intestines; RPU (a₂) and RPUP (b₂) in the frass (all at a magnification of $\times 500$). The EEM of DOM in the intestine of *Galleria mellonella* larvae under various feeding conditions and at different time points: BW (c), BWP (d), RPU (e), and RPUP (f).

It is clear that plastic degradation can produce DOM composed of additives, oligomers, and monomers, which constitute the basic components of plastic polymers [49]. EEM can characterize the degree of plastic degradation by detecting changes in protein-like fluorescence peaks generated by DOM [50]. Therefore, we conducted longitudinal monitoring of the dynamic changes in DOM in the gut of *Galleria mellonella* larvae fed with RPU and RPUP over time. As shown in Figure 2c–f, there is a protein-like fluorescence peak at the excitation/emission wavelengths of 283/339 nm under all feeding conditions [51,52]. Compared with the baseline value on day 0, the fluorescence intensity of these protein-like peaks is closely related to microbial metabolic activity, which visibly strengthens with increased feeding time, highlighting the dynamic correlation between microbial community activity and the expression of fluorescent biomolecules in the intestinal environment of *Galleria mellonella* larvae [53]. This result indicates that the gut microbiota of *Galleria mellonella* larvae actively participate in metabolic processes [54], demonstrating their ability to convert BW, BWP, RPU, and RPUP into protein-like substances at different efficiencies. Interestingly, the fluorescence intensities of BWP, RPU, and RPUP reached their peak on

the 4th day, which may be due to the discovery by *Galleria mellonella* larvae that plastics and substances containing TCPP are difficult to consume as food over time, and the larvae gradually entered the pupation stage, corresponding to the trends seen in Figure 1a,c. The increased fluorescence intensity offers strong evidence for the partial degradation of RPU and RPUP, emphasizing the biotransformation potential of *Galleria mellonella* larvae in terms of plastic materials. It also strongly suggests that microorganisms produce extracellular enzymes essential for polymer degradation. Specifically, these fluorescence intensities are probably linked to hydrolytic enzymes such as esterases and urethanases, which are known to break down the ester and urethane bonds in PU [55,56]. They may also relate to oxidoreductases (e.g., laccases) involved in degrading aromatic intermediates formed during PU degradation [57]. This enzymatic inference aligns with the observed temporal dynamics of fluorescence intensity. With the passage of feeding time, the fluorescence intensity of protein-like peaks shows a clear order: BW > BWP > RPU $\approx$ RPUP. The temporal variation of fluorescence intensity indicates that under BWP/RPUP feeding conditions, the intestinal microbial activity of *Galleria mellonella* larvae is obviously lower than that of the BW/RPU group. This phenomenon is in accordance with our previous research on the effect of TBBPA on RPU degradation by *Tenebrio molitor* and *Galleria mellonella* larvae [15]. TCPP and TBBPA disrupted the normal activity of the gut microbiota of *Galleria mellonella* larvae, thereby hindering the biotransformation of protein components. Such microbial dysfunction may be due to the toxic effects of these FRs, which can disrupt the delicate ecological balance in the gut and interfere with enzyme activity, which is crucial for plastic degradation.

This study visually demonstrates the inhibitory effect of TCPP on the biodegradation kinetics of RPU mediated by *Galleria mellonella* larvae and analyzes the interaction relationship between plastic additives, polymer matrices, and gastrointestinal metabolic processes.

3.3. Fate of TCPP During Feeding on RPUP by *Galleria mellonella* Larvae

The above study found that TCPP interferes with the metabolic processes of the gut microbiota of *Galleria mellonella* larvae. The influence of the larval gut microbiota on TCPP migration was investigated. TCPP is a flame retardant composed of three isomers. As shown in Figure 3a,b, GC-MS analysis of BWP and RPUP reveals that both have a characteristic spectrum with two prominent adjacent peaks at retention times of 12.20 min and 12.33 min, representing the two TCPP isomers. However, the low intake of BWP and RPUP by *Galleria mellonella* larvae resulted in relatively low concentrations in the gut, resulting in the third isomer failing to produce a distinguishable peak in the GC-MS chromatogram, which is consistent with Truong, J.W. et al.'s GC-MS analysis of TCPP [58].

After 7 days of feeding, TCPP was detected in the intestine and frass of *Galleria mellonella* larvae in the BWP and RPUP groups, as shown in Figure 3c–f. Compared with the frass, the peak intensity of TCPP in the intestine is visibly weaker, which was analyzed by the changes in TCPP content in the larval intestine over time under BWP feeding conditions. As shown in Figure S5, due to the accumulation of BWP in the first two days, TCPP content is the highest. Over time, the intake of the larvae decreased, and their sustained excretion led to a gradual decrease in TCPP content. After a 2-day fasting period (the 8th day), the TCPP content in the intestine decreased by 70% and over half of the TCPP was excreted through frass. Brandon et al.'s study also indicated that *Tenebrio molitor* larvae fed with PS containing HBCD excreted the majority of HBCD in the form of frass after 48 h [46]. This result is also consistent with our previous research, which showed that the majority of TBBPA was excreted through the frass of *Tenebrio molitor* and *Galleria mellonella* larvae, indicating that FRs do not have substantial bioaccumulation in

invertebrates [15]. Furthermore, two TBBPA degradation products, Tribromobisphenol A and 4-(2-Hydroxyisopropyl)-2,6-dibromophenol, were detected by Orbitrap-HRMS in the gut of the BWT and WBT groups of two larvae. But Orbitrap-HRMS did not detect TCPD degradation products in the larval gut of the BWP group in this study, inferring that TCPD was primarily excreted without substantial metabolism under our experimental conditions. Another possibility is that the high viscosity of TCPD is different from that of TBBPA, affecting larval feeding of BWP, leading to the concentrations of any potential metabolites falling below our MDL or being quickly further transformed, and making it difficult to detect degradation products in the gut [15].

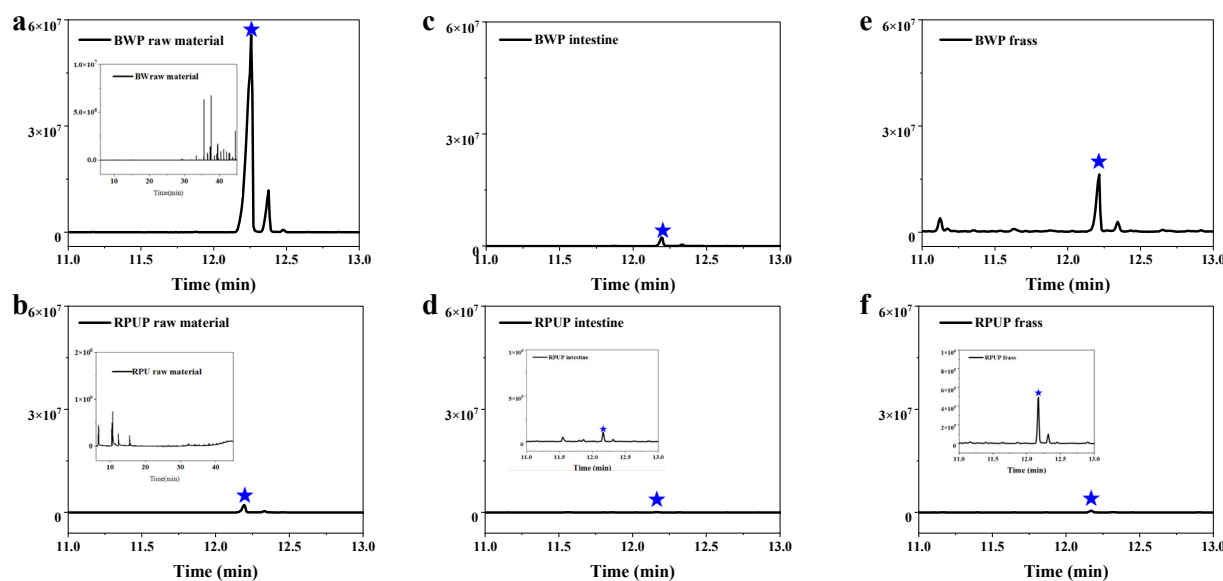


Figure 3. GC-MS analysis of TCPD in the intestine and frass of *Galleria mellonella* larvae: BWP raw material (a); RPUP raw material (b); BWP intestine (c); RPUP intestine (d); BWP frass (e); and RPUP frass (f). The blue star represents TCPD.

This convergent discovery of larval insect species highlights a conserved mechanism for eliminating flame retardant additives in plastics, implicating that the gut microbiota plays an important role in mediating xenobiotic transport and detoxification pathways. Notably, the TCPD peak intensity in the gut and frass of *Galleria mellonella* larvae fed with BWP is obviously higher than that of the RPUP group, indicating that the consumption of BWP was higher than that of RPUP, which is consistent with the results presented in Figure 2d,f.

3.4. Gut Microbiota Response to RPUP Degradation in Larvae

The effect of TCPD on the gut microbiota of larvae over time was investigated using 16S rRNA high-throughput sequencing analysis. To characterize the species richness and diversity of intestinal microbial communities at different time points, the Sobs index was evaluated at the ASV level using intestinal samples from *Galleria mellonella* larvae. As illustrated in Figure S6 and Table S1, rarefaction curves exhibit plateauing patterns, demonstrating that the sequencing depth was sufficient to capture the full spectrum of microbial diversity within larval intestines. This validation of the sequencing methodology ensures accurate characterization of temporal dynamics in microbial community structures.

Figure 4a–e illustrate the dynamic changes in the gut microbiota of *Galleria mellonella* larvae over time under different feeding conditions. At the family level, *Enterococcaceae*, *Acetobacteraceae*, *Morganellaceae*, and *Enterobacteriaceae* are the top four dominant bacterial families in the ST state (Figure 4a). With the feeding of different foods and the passage

of time, the gut microbiota of larvae undergoes large changes. The relative abundance of some microbial communities decreases or increases, and some microbial communities disappear or appear (Figure 4b–e). The relative abundance of *Enterococcaceae* decreases with feeding time for BW, remains relatively stable with feeding time for BWP and RPUP, and increases with feeding time for RPU, indicating that BW is not suitable for the growth of *Enterococcaceae*. Nevertheless, *Enterococcaceae* has good adaptability to BWP and RPUP containing TCPP, and shows a trend of growth with feeding time for RPU, inferring that *Enterococcaceae* may have the ability to degrade RPU [59]. The relative abundance of *Acetobacteraceae* increases notably on the 6th day of feeding with BW, and Figure 2c shows that the fluorescence intensity of protein-like peaks in the BW group is the strongest on the 6th day, indicating that BW is beneficial for the growth of *Acetobacteraceae*, while *Acetobacteraceae* digested and decomposed BW, which is consistent with our previous research. Meanwhile, under other feeding conditions, the relative abundance of *Acetobacteraceae* does not fluctuate considerably, implying that *Acetobacteraceae* has strong adaptability and survival ability in different environments. The relative abundance of *Morganellaceae* and *Enterobacteriaceae* disappears under feeding with BW, but remains stable under other feeding conditions, and the relative abundance of *Enterobacteriaceae* increases over time under feeding with RPUP, indicating that BW inhibited the growth of *Morganellaceae* and *Enterobacteriaceae*. According to data, *Morganellaceae* maintains a dominant position even in intestinal environments contaminated with pesticides, underscoring its strong adaptability to toxic stress [60]. *Morganellaceae* in aphids may endow them with the metabolic capability to utilize pesticides as their sole carbon and nitrogen sources [61], suggesting that *Morganellaceae* are more adaptable to these adverse environments of BWP, RPU, and RPUP. While on the 4th day the relative abundance of *Morganellaceae* is the highest, consistent with Figure 2d–f, the fluorescence intensity of protein-like peaks is the strongest also on the 4th day. The enrichment of *Morganellaceae* under TCPP stress is correlated with an alleviation of its inhibition on the intestinal microbiota, which coincides with improved RPU degradation efficiency. Meanwhile, *Enterobacteriaceae* secretes esterases and lipases, which may directly hydrolyze the amino ester bonds in RPU [62]. This is a rate-limiting step that initiates the breakdown of polymer chains into smaller metabolizable fragments. Therefore, *Morganellaceae* and *Enterobacteriaceae* may degrade RPU and TCPP. In addition, *Staphylococcaceae* appeared in the gut microbiota of larvae fed with RPU and RPUP (Figure 4d,e), implying that *Staphylococcaceae* prefer this environment, can feed on RPU and RPUP, and may degrade them. *Staphylococcaceae* contributes to the downstream catabolism of RPU, which produces extracellular proteases that further break down RPU-derived oligomers into small, utilizable metabolites [63]. Collectively, these microbial communities in *Galleria mellonella* may form a functionally complementary consortium to modulate RPU biodegradation, with each group targeting distinct steps of the RPU degradation process, from polymer initiation to oligomer catabolism, while accounting for TCPP-mediated perturbations. Our previous studies on the influence of BWT and RPUT feeding on the intestinal microbiota of *Galleria mellonella* larvae revealed that *Enterococcaceae* and *Enterobacteriaceae* have the ability to degrade TBBPA [15], which is divergent from the dominant microbial groups under TCPP feeding conditions, indicating that different FRs have different mechanisms of impact on invertebrate gut microbiota.

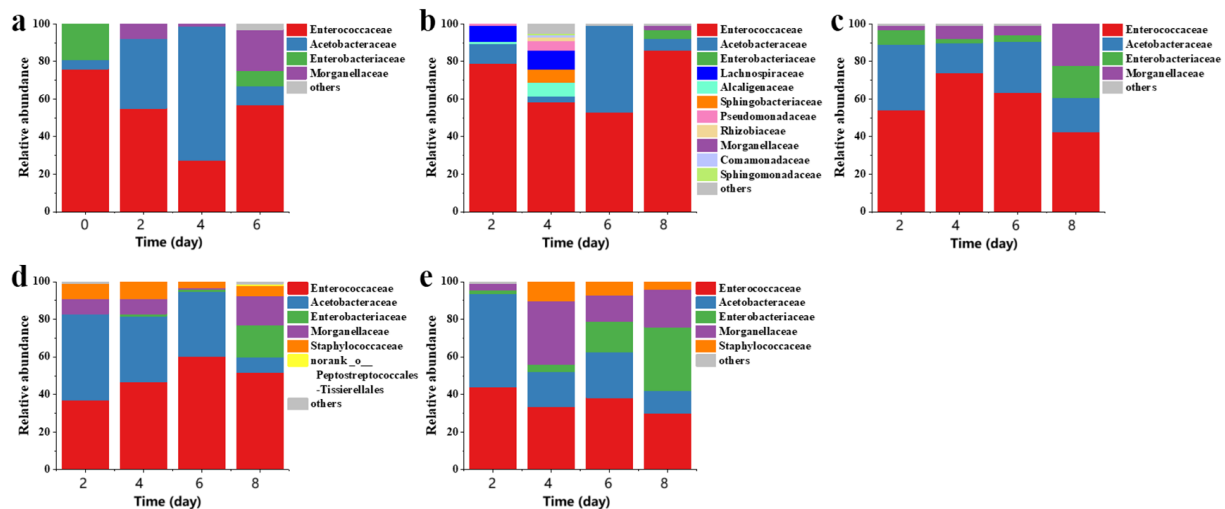


Figure 4. Composition analysis of intestinal microbial communities of the *Galleria mellonella* larvae at the family level under different feeding conditions and at different time points: ST (a), BW (b), BWP (c), RPU (d), and RPUP (e) at 2, 4, 6, and 8 days.

Non-metric multidimensional scaling (NMDS) employing Bray–Curtis dissimilarity reveals the effects of different feeding regimes on the intestinal microbial communities of *Galleria mellonella* larvae. As shown in Figure 5a, only the BW group moved away from the other groups. In contrast, the BWP, RPU, and RPUP groups overlapped with the ST group in the first two days, inferring that their gut microbiota had not yet adapted to non-traditional foods in the initial feeding regime, so it is similar to the ST group and considerably different from the gut microbiota of the BW group. This result corresponds to the data in Figure 1a, which only show the increase in larval mass of the BW group, indicating that the gut microbiota of the larvae had not yet adapted to unfavorable dietary conditions in the early stages. As time passed, the RPU/RPUP groups became closer to each other and farther away from the BWP/ST groups on the 4th day (Figure 5b), suggesting that RPU began to affect the gut microbiota of larvae. At this point, the gut microbiota of the RPU group and the RPUP group were similar, with marked differences compared to the BWP/ST groups. All five groups moved away from each other by the 6th day (Figure 5c), inferring that the pronounced displacement of the RPUP cluster from the BW centroid implies severe disruption of the microbial community, characterized by a surge in tolerant taxa and a parallel loss of key functional guilds, pointing to the most acute ecological deterioration. Overall, the effects of RPU and TCPP on the gut microbiota gradually deepened over time.

Linear discriminant analysis effect size (LEfSe) and linear discriminant analysis (LDA) were employed to analyze marked variations in the abundance of *Galleria mellonella* gut microbiota under different feeding conditions (Figure 5d,e). LEfSe can identify the enrichment and decay of gut microbiota in larvae, revealing unique microbial communities associated with different feeding conditions. LDA can quantify the degree of influence of these different abundance microbial communities and conduct systematic cross-comparisons of gut microbiota. This elucidates how different feeding conditions reconfigure the composition and structure of the gut microbiota, emphasizing the ecological impact of feeding behavior. Pronounced enrichment of *Morganellaceae*, *Enterobacteriaceae*, and *Staphylococcaceae* was observed in the BWP, RPU, and RPUP groups, respectively (Figure 5d), indicating that they may be related to the degradation of RPU and TCPP, corresponding to Figure 4. Additionally, *Klebsiella* and *Vagococcaceae* were also enriched in the BWP and RPU groups, respectively (Figure 5e), suggesting that they may contribute to the degradation of RPU and TCPP, although they are not dominant bacterial species. In the future, these microbial

communities can be specifically cultivated to explore their potential for degrading plastics and BFRs.

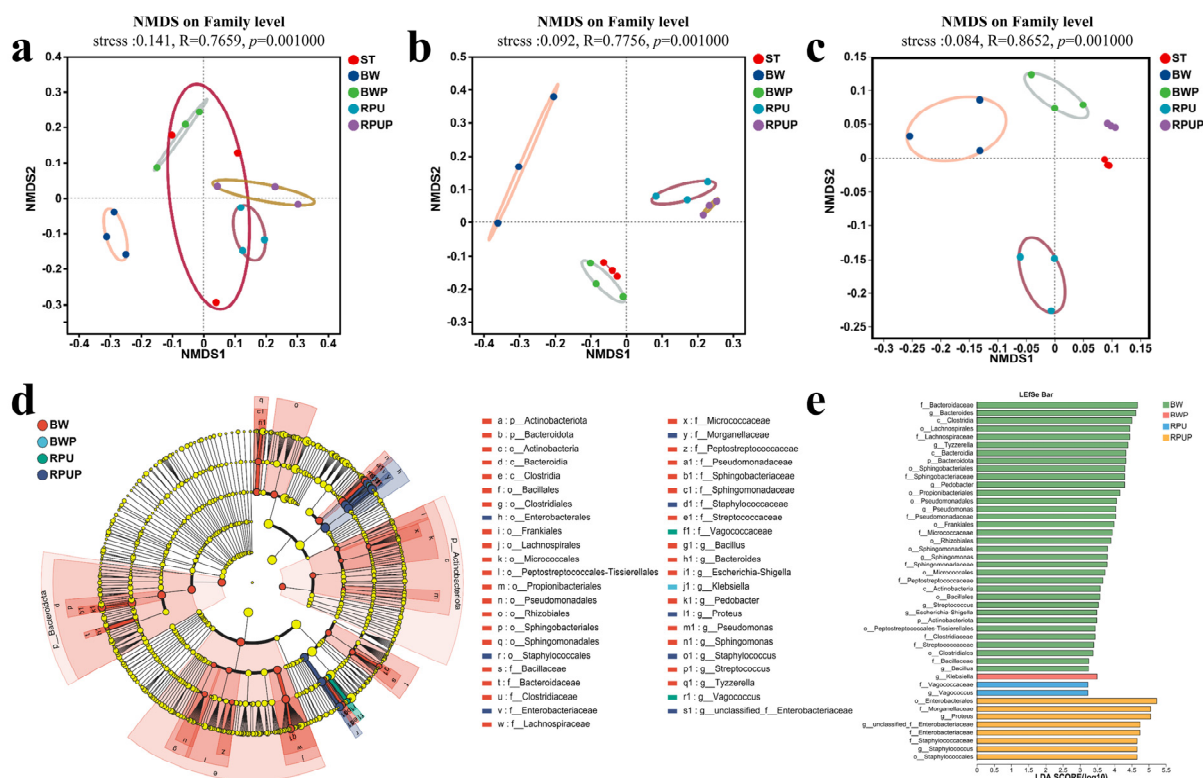


Figure 5. NMDS analysis of *Galleria mellonella* larvae at 2 (a), 4 (b), and 6 (c) days. Discriminant analysis of intestinal taxa enrichment of *Galleria mellonella* larvae (d). LDA effect size analysis of significant differences in the relative abundance of bacteria in the intestines of *Galleria mellonella* larvae (e) under different feeding conditions.

4. Conclusions

This study provides a mechanistic insight into the environmental fate of OPFRs during plastic biodegradation, extending prior work centered on BFRs such as TBBPA and HBCD. While BFRs undergo microbial debromination and aromatic ring cleavage, the chlorinated OPFR TCPP was negligibly degraded in *Galleria mellonella* larvae and was excreted essentially unaltered, underscoring a pronounced disparity in persistence and biological processing between OPFRs and BFRs.

We have discovered a triple physiological interference mechanism of TCPP inhibition of host-mediated plastic degradation, which includes (1) neurobehavioral feeding inhibition, (2) nutrient assimilation disruption, and (3) metabolic energy diversion toward lipid catabolism. Furthermore, *Morganellaceae*, *Enterobacteriaceae*, and *Staphylococcaceae* families in the gut of *Galleria mellonella* exhibit certain biodegradation potential for RPU and TCPP, and TCPP exposure selects for low abundance, stress-resistant genera (*Klebsiella*, *Vagococcaceae*) capable of partial dechlorination, suggesting a distinct detoxification strategy for OPFRs. These results are different from those found for the microbial community of degrading TBBPA. Moreover, BFR degradation often relies on specialized dehalogenases from dominant gut taxa.

These findings reframe the perceived hazard of organophosphate flame retardants: risk arises not through classic bioaccumulation but via indefatigable environmental persistence and sustained dissemination by invertebrate vectors. Operationally, waste management frameworks must embed additive–polymer interdependencies, and circular economy

directives should expedite the phase-out of non-degradable, biologically active congeners such as TCPP and promote the invention of fully biodegradable material platforms that prevent the formation of refractory residues.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/su17209203/s1>: Figure S1: Pictures of experimental materials; Figure S2: Foamed RPU (RPUP) and $2 \times 2 \times 1 \text{ cm}^3$ RPU (RPUP) lumps; Figure S3: XPS plot of the RPU, 10% RPUP; Figure S4: Larval survival rate under different feeding conditions; Figure S5: Intestinal TCPP content of *Galleria mellonella* under BWP feeding conditions over feeding times; Figure S6: Sobs index of the *Galleria mellonella* larvae at the ASV level; Table S1: Statistical table of sample information of *Galleria mellonella*.

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Nomenclature

EEP	electrical plastic
OPFRs	organophosphate flame retardants
FRs	flame retardants
RPU	rigid polyurethane
TCPP	Tris(1-chloro-2-propyl) phosphate
PU	polyurethane
HDPE	high-density polyethylene
BW	beeswax
BWP	TCPP-containing beeswax
RPUP	TCPP-containing rigid polyurethane
ST	starvation
CBB G-250	Coomassie Brilliant Blue G-250
EEM	excitation–emission matrix
DOM	dissolved organic matter
ANOVA	analysis of variance
LSD	least significant difference
PS	polystyrene
PVC	polyvinyl chloride
DEHP	di(2-ethylhexyl) phthalate
ASV	amplicon sequence variant
NMDS	non-metric multidimensional scaling
LEfSe	linear discriminant analysis effect size
LDA	linear discriminant analysis
SD	standard deviation

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