

Research Paper

Unveiling the residual plastics and produced toxicity during biodegradation of polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) microplastics by mealworms (Larvae of *Tenebrio molitor*)



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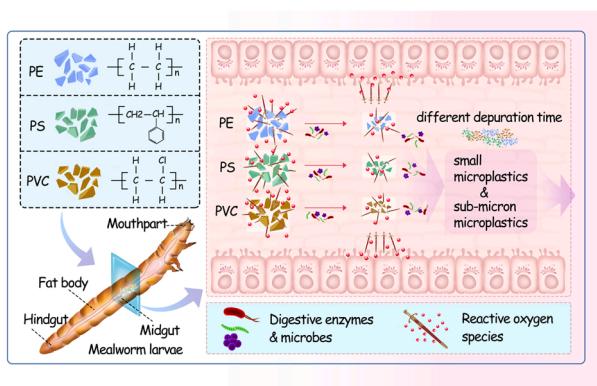
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HIGHLIGHTS

- PE, PS, and PVC microplastics were rapidly ingested and efficiently biodegraded by mealworms.
- The PVC-fed mealworms had the worst physiological performance during the 24-day test.
- Undigested microplastics remained in mealworm intestines for a longer time than normal food.
- Oxidative stress responses were discovered in the mealworms fed with PE, PS, and PVC microplastics.
- Sub-micron microplastics and small microplastics were found in the frass of the mealworms.

GRAPHICAL ABSTRACT



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ABSTRACT

Evidence for plastic degradation by mealworms has been reported. However, little is known about the residual plastics derived from incomplete digestion during mealworm-mediated plastic biodegradation. We herein reveal the residual plastic particles and toxicity produced during mealworm-mediated biodegradation of the three most common microplastics, i.e., polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC). All three microplastics are effectively depolymerized and biodegraded. We discover that the PVC-fed mealworms exhibit the lowest survival rate ($81.3 \pm 1.5\%$) and the highest body weight reduction ($15.1 \pm 1.1\%$) among the experimental groups by the end of the 24-day experiment. We also demonstrate that the residual PVC microplastic particles are more difficult to depurate and excrete for the mealworms compared to the residual PE and PS particles by using laser direct infrared spectrometry. The levels of oxidative stress responses, including reactive oxygen species, antioxidant enzyme activities, and lipid peroxidation, are also highest in the PVC-fed

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mealworms. Sub-micron microplastics and small microplastics are found in the frass of mealworms fed with PE, PS, and PVC, with the smallest particles detected at diameters of 5.0, 4.0, and 5.9 μm , respectively. Our findings provide insights into the residual microplastics and microplastic-induced stress responses in macroinvertebrates under micro(nano)plastics exposure.

1. Introduction

Global plastic production reached near 370 million metric tons (Mt) in 2021, with Asia accounting for 51% of this production [17,34], and is even projected to increase to 12,000 Mt globally by 2050. However, only 18% of the waste plastics are recycled or upcycled, and 24% of the waste plastics are incinerated, with the remaining 58% entering landfills or the natural environment [5]. Unfortunately, these plastic wastes take from tens to hundreds of years to decompose and seriously harm ecosystems and animals, including by entangling animals, transferring pathogens, disrupting food chains, etc. Plastic waste accumulation and the associated pollution have been serious environmental and socioeconomic problems [36,64]. Polyethylene (PE), chemically expressed as $[-\text{CH}_2-\text{CH}_2-]_n$, polystyrene (PS), chemically expressed as $[-\text{CH}(\text{C}_6\text{H}_5)\text{CH}_2-]_n$, and polyvinyl chloride (PVC), chemically expressed as $[-\text{CHClCH}_2-]_n$, are three of the most commonly used petroleum-based polymers, accounting for near 50% of the total market share [34]. The long-term persistence of the three plastics has greatly contributed to accumulation challenges for waste management and recycling industries [8,22].

Plastic waste from anthropogenic sources experiences disintegration and fragmentation through weathering, photolysis, cracking, abrasion, and microbial decomposition in the natural environment, resulting in the ubiquity and persistence of microplastics (MPs, 100 nm–5 mm in size) and nanoplastics (NPs, < 100 nm in size) all around the globe in the biosphere, atmosphere, and hydrosphere [1,12,37]. Microplastics can be further subdivided into large microplastics (1–5 mm in size), small microplastics (20–999 μm) in size, and sub-micron microplastics (1–20 μm in size) [21]. Due to the unique physiochemical properties of microplastics (e.g., large specific surface area, tiny size, and strong adsorption capacities), they experience more complex fates in environments than conventional plastic waste and have emerged as particulate anthropogenic contaminants, arousing growing scientific and public interest [2,40,42,43,49,9]. Beyond environmental health, microplastics can also be directly ingested and inhaled, raising concerns about their impacts on human health [1,37].

Discarded plastic products in the environment are readily ingested by living creatures and are typically disintegrated into smaller-sized plastic debris (e.g., microplastics) after biofragmentation [48,50,51]. For instance, terrestrial snails (*Achatina fulica*) fragmented the commercial PS foam into large microplastics (1.34 mm in size) through digestive fragmentation [41]. Adult earthworms (*Eisenia fetida*) exposed to microplastics-contaminated soil also disintegrated bio-based poly-lactic acid (PLA) microplastics into sub-micron microplastics in their digestive tracts [48]. In the short term, these plastic fragments as exogenous substances could bioaccumulate in organisms, cause intestinal blockage and gut epithelial damage, and elicit gut dysbiosis, oxidative damage, and inflammatory responses in individuals [25]. In the long term, the microplastic particles would be excreted with excrement and eventually enter ecosystems, causing adverse effects on other species and populations as secondary microplastics [2,23,48]. Therefore, the biofragmentation of plastic waste into micro- and nano-plastics has become an emerging environmental concern. There is a general consensus that plastic pollution urgently needs not only strategies for waste management but also comprehensive knowledge about the toxic risks of particulate plastic residue to environmental and human health.

Researchers have been contemplating technologies for sustainable treatment of plastic waste to reduce the burden of global plastic pollution, and biodegradation is considered a promising green strategy. Some lepidopteran and coleopteran insect larvae were found to consume and

degrade untreated commercial plastic products [18,27,55,61]. The larvae of *Tenebrio molitor* L., also known as mealworms, yellow mealworms, or meal moths, can rapidly ingest and oxidize PE (including linear low-density PE; LLDPE, low-density PE; LDPE, and high-density PE; HDPE) [4,56,60], PS [29,31,44–46,58,61,66,67], and PVC [13,26] within a fairly short retention time (about 12 h). The ingested plastics could be efficiently depolymerized, assimilated, and mineralized in the gut microenvironments of mealworms via different gut microbiome-related mechanisms [19,65], making it the most highly efficient known plastic-degrading macroinvertebrate. Mealworms can also degrade both bio-based PLA and petro-based Poly(butylene adipate-co-terephthalate) (PBAT) and use the intermediates and degradation products as energy supplements, indicating a promising method for converting plastic waste into valuable insect biomass [27,32].

Previous reports on plastic biofragmentation primarily focused on target macroinvertebrates without plastic-degrading abilities but seldom shed light on the residual plastic particles generated by plastic-degrading macroinvertebrates, especially after the ingestion and biodegradation of microplastics. To the best of our knowledge, the stress responses and physiological performance of plastic-degrading macroinvertebrates after digestive biofragmentation and biodegradation also remain unknown. Our work here begins with an initial observation that the plastic-degrading macroinvertebrate mealworms can eat PE, PS, and PVC microplastics at different rates. We then measure the survival and growth of mealworms fed with the PE, PS, and PVC microplastics during the 24-day incubation and perform gel permeation chromatography (GPC) analysis to confirm the depolymerization and biodegradation. We determine the number of the PE, PS, and PVC microplastics in the intestines of the plastic-degrading mealworms and the differences in depuration time using laser direct infrared spectrometry (LDIR). Motivated by the results and our curiosity, we identify the microplastic-induced oxidative stress responses by testing the reactive oxygen species (ROS), superoxide dismutase (SOD), and malondialdehyde (MDA) in the mealworms since these indexes rise in organisms in resistance to environmental stress when exposed to microplastic contaminants [53]. Furthermore, we explore the residual plastic particles in the frass of mealworms after the biodegradation of microplastics via highly sensitive laser light scattering. The results of this study advance our conceptual understanding of plastic biofragmentation by macroinvertebrates with plastic biodegradation capacities, and also provide valuable methodological insights into assessing microplastics in both biological and environmental samples.

2. Material and methods

2.1. Chemicals and laboratory cultivation

The 4th-instar mealworm larvae (*Tenebrio molitor* L.) were collected from Binzhou Mealworm Co., Shandong Province, China. They were then shipped to our laboratory in Yangpu, Shanghai, China, and cultured indoors with their common food bran (C: H: O: N ratio of 40.1: 7.7: 47.3: 3.4 according to the elemental analysis). The 4th-instar mealworms were in a rapid growth period during which they had the highest feedstock consumption capacities and were also sensitive to external environmental stress. The initial average weight of the mealworms was 72.2 ± 2.7 mg with an average length of 1.7 ± 0.1 cm. Standard PE, PS, and PVC microplastics (Fig. S1), the three most common polymers, were purchased from Zhonglian Petrochemical Materials in Guangdong Province, China. All microplastics (size: < 300 μm) were selected and

obtained by sieving using stainless steel mesh. The chemical reagents used in this study were obtained from Sigma-Aldrich.

To enable the ingestion, biofragmentation, and biodegradation of the three tested microplastics, four rearing conditions ($n = 3$ trials) were prepared for the mealworms: a) PE-fed; b) PS-fed; c) PVC-fed; d) bran-fed; and e) unfed. Each experimental group was started with 100 mealworm larvae with the same growth status and the respective microplastics (3.0 g PE, PS, or PVC) in glass containers (15 cm \times 15 cm \times 6 cm) with smooth insides. The bran-fed control group was also started with 100 mealworm larvae and 3.0 g bran and was supplemented with an additional 3.0 g bran every 4 days to ensure a sufficient supply of the feedstocks as in the mealworm breeding. The survival rates and average body weights of mealworm larvae were measured every 4 days by counting and weighing the survivors. The incubation of mealworms lasted for 24 days. All glass containers were kept in a thermostatic incubator at 25 ± 0.5 °C with constant humidity of $70 \pm 5\%$ and dark conditions.

2.2. Plastic biodegradation and analytical methods

At the end of the test, the fresh frass of each experimental group was collected to confirm the biodegradation of the three microplastics [4,31, 60]. Gel permeation chromatography (GPC; 1260 Infinity II GPC/SEC, Agilent Technologies Inc., U.S.A.) was used to characterize the changes in molecular weights of the ingested PS and PVC polymers. The depolymerization of the ingested PE polymer was identified by using high-temperature gel permeation chromatography (HT-GPC; 1260 Infinity II HT GPC, Agilent Technologies Inc., U.S.A.). The polymer depolymerization patterns were determined by comparing the weight-average molecular weight (M_w), number-average molecular weight (M_n), and size-average molecular weight (M_z) values of the residual polymers versus the original microplastics. The extraction solvent for PS and PVC was tetrahydrofuran (THF), and that for PE was 1, 2, 4-trichlorobenzene (TCB). More details on the GPC analyses are described in Text S1.

2.3. Characterization of residual plastics in mealworm intestines

More containers were prepared under the same incubation conditions to obtain enough mealworms and frass samples for the analysis of residual plastics after biodegradation. By the end of the test (day 25), the mealworms in each experimental group were moved to stainless-steel sieves. Before being transferred to clean glass containers for excretion, they were gently cleaned with streaming air to remove molted exoskeletons. Mealworms were dissected to retrieve the intestine tissues after different depuration times (12, 24, 36, 48, and 60 h). In brief, the mealworms were first sterilized with 70% alcohol and rinsed three times with sterile Milli-Q ultrapure water. Subsequently, the head of the mealworm larva was removed with a sterile scalpel, and the intestine of the larva was gently removed with tweezers and immediately transferred to a sterile centrifuge tube (1.5 mL). Each replicate sample contained one mealworm intestine, and five replicates were prepared for each experimental condition for the analysis of residual plastics. The residual particle number was adjusted to an integer to indicate the results.

The obtained intestine samples were first crushed under liquid nitrogen conditions and then thoroughly digested using KOH, cellulase, and 30% H₂O₂, following previously established procedures [14,20,54]. After the supernatant was clear, we let the digestion solution containing residual plastic particles stand for 1 h. The solution was then filtered through a metal membrane filter (pore size: 10 µm, Yibo Filter Material Co., Zhejiang, China), and the filter was carefully rinsed with Milli-Q ultrapure water. Finally, the attached residual “small microplastics” (nominally 20–999 µm) [21] were collected, dried, and identified using laser direct infrared spectrometry (LDIR, Agilent 8700 LDIR, Agilent Technologies Inc., California, U.S.A.), with a minimum detection limit of

20 µm [24,33]. Infrared spectroscopy analysis was performed using Agilent Clarity software with the customized spectrum library in the microplastic test module. The material was determined when the match degree with the standard spectrum was greater than 80%. The sample was scanned 64 times with a resolution of 8 cm⁻¹ and repeated three times.

2.4. Characterization of the size distribution of residual plastics in frass

After the 24-day test period, the frass samples from the PE-fed, PS-fed, and PVC-fed mealworms were first mixed with a 30% H₂O₂ solution (wt/wt) under gentle heating (70 °C) for 24 h to thoroughly digest the organic matters. After the supernatant was clear, the solution after digestion was then filtered through a PA filter (pore size: 100 nm, Yibo Filter Material Co., Zhejiang, China). The size distribution of the attached plastic particles was measured via laser light scattering (Malvern Mastersizer 3000, Malvern Panalytical, UK) based on the polymer volume with a particle measurement range of 0.01–3500 µm [9,30,41]. The accuracy of the automatic measurement is better than 0.6% variable, with 100 size classes for each measurement and a data acquisition rate of 10 kHz. The repeatability of the particle measurement is higher than 99.5%.

2.5. The oxidative damage and antibiotic performance in mealworms

To identify whether oxidative stress and damage could be induced in mealworms, which had preeminent capacities for plastic biodegradation, the levels of reactive oxygen species (ROS), superoxide dismutase (SOD), and malondialdehyde (MDA) in mealworms were determined at the end of the test (day 25).

The ROS level was analyzed using a dihydroethidium (DHE) assay kit obtained from Biolab Technology Co., Ltd. (Beijing, China). The mealworms were first cleaned and ground with homogenate buffer, followed by cell disruption using a cell disruptor [17,40]. Subsequently, the homogenate was centrifuged at 12000 rpm for 20 min at 4 °C, and the supernatant was incubated at 37 °C for 30 min. The bicinchoninic acid (BCA) protein quantification and ROS fluorescence detection were conducted with an excitation wavelength of 488–535 nm and an emission wavelength of 610 nm [17]. The lipid peroxidation level could be indicated by the malondialdehyde (MDA) content and was determined using MDA assay kits from Abbkine Scientific Co., Ltd. (Shanghai, China). A microplate reader (SpectraMax iD3, Molecular Device, San Jose, CA, U.S.A.) with absorbances of 532 nm and 600 nm was used to measure the concentration [40,57].

The antibiotic performance could be reflected by the level of SOD activities in tissues [15,17,68]. The content of SOD in the mealworms under different feeding conditions was analyzed using the SOD enzyme assay kit from Abbkine Scientific Co., Ltd. (Shanghai, China), following the instructions of the manufacturers. A microplate reader with an absorbance of 450 nm was used to measure the SOD concentration. The overall physiological stress responses and antioxidant performance of the mealworms were further calculated and evaluated, with the results presented with the ternary diagram.

2.6. Statistical analysis and quality control

In this study, the significant differences in the survival indices of mealworms and the M_w , M_n , and M_z values were evaluated by ANOVA and the student's t test with Tukey's correction. The statistical analyses were conducted on Origin Pro 2021 software (OriginLab Corp., MA, U.S.A.). For statistical significance, we used adjusted p -values < 0.05 . All error values were reported as the mean \pm standard deviations (mean \pm SDs).

All experimental containers, vessels, and flasks were rinsed three times with Milli-Q ultrapure water and methanol to remove any potential background of microplastics. After thorough drying in the fume

hood, the test materials and lab equipment were wrapped in aluminum foil and prepared for use. Before filtration, Milli-Q ultrapure water was filtered through the membranes. To avoid deviations caused by indoor dust particles, all procedures of separation and particle identification were carried out in the fume hood in a dust-free clean room (Liu, J. et al., 2022; Wang, L. et al., 2022).

3. Results and discussion

3.1. Mealworm larvae can survive on PE, PS, and PVC microplastics

A 24-day cultivation test was designed and conducted to verify whether the mealworms could effectively survive on the three microplastics (PE, PS, and PVC). The key physiological indices of mealworms, including the survival rate and average body weight, were measured every 4 days during the experiment (Fig. 1a-c). Overall, the survival rates of mealworms fed with microplastics were all significantly lower than those of the bran-fed control during the test period (p value < 0.001) but remarkably higher than those of the starvation group

(Fig. 1a). The final survival rates were $91.3 \pm 0.6\%$, $90.7 \pm 1.5\%$, $81.3 \pm 1.5\%$, $96.3 \pm 0.6\%$, and $72.0 \pm 2.0\%$ for the PE-fed, PS-fed, PVC-fed, bran-fed, and unfed mealworms, respectively, by the end of the experiment (Fig. S2 and Table S1). The results were consistent with the changes in the body weights of the mealworm groups over the period (Figs. 1b and 1c). These findings implied that ingesting microplastics as an exogenous food source could provide energy for the survival of mealworm larvae and sustain their growth, although this specialized energy source was not as efficient as the normal food (wheat bran). A plastic diet alone could cause nutrient exhaustion and a lack of trace elements required for the production of both digestive enzymes and transporters [31,38], leading to decreased nutrient uptake and, as a result, negative growth of the mealworms.

Interestingly, the survival curves of the PE-fed and PS-fed mealworms over the period were not statistically different, while those were both significantly different from that of the PVC-fed mealworms (Fig. 1a). Similarly, a relatively lower body weight of mealworms was also found in the PVC-fed group compared with those fed with PE and PS microplastics after 24 days (Fig. 1c, Table S2 and S3). The degradation of

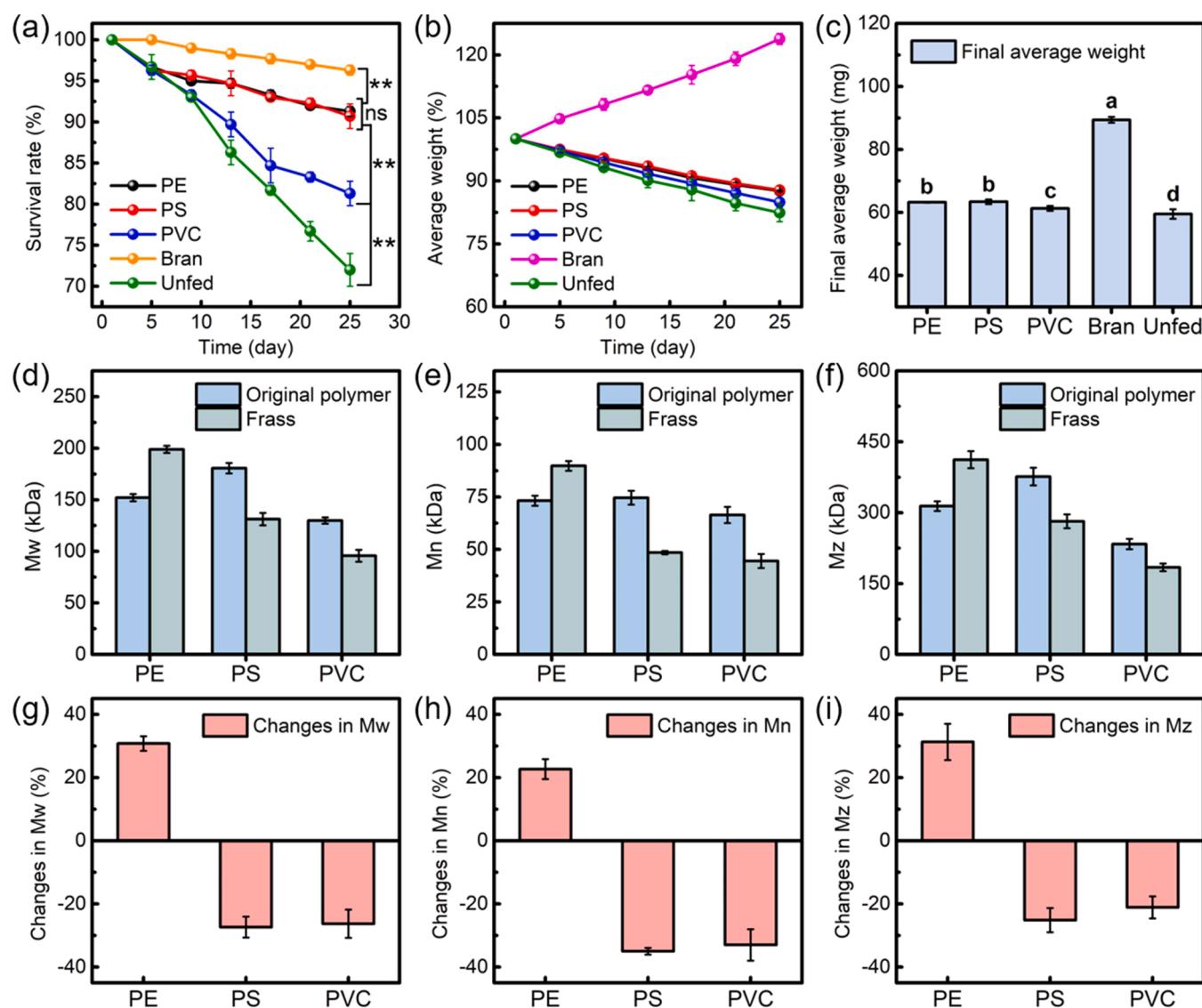


Fig. 1. Survival indices of mealworms and biodegradation of PE, PS, and PVC in the mealworms (a) Changes in survival rates (%) of mealworms over the test; statistical significance between survival curves was determined by Kaplan-Meier survival analysis. The symbols ** and ns indicate statistical significance ($p < 0.001$) and no significant difference, respectively. (b) Changes in average weights of mealworms over the test (%). (c) Final average weights of mealworms (mg) at the end of the test (day 25). The (d) M_w , (e) M_n , and (f) M_z values (kDa) of the frass versus the original polymers (PE, PS, and PVC). Changes in (g) M_w , (h) M_n , and (i) M_z of the ingested microplastics after biodegradation.

PVC was partial, and the extent of PVC mineralization was comparatively limited during the insect-mediated biodegradation [13,26,64], which supplied less available energy and carbon sources. Toxic substances, e.g., chlorinated intermediates (up to 63%) and hydrogen chloride, were also produced during the PVC depolymerization process [26], which might impair the intestinal epithelium and damage the homeostasis of mealworms. The combined factors resulted in the poor survival status and physiological responses of the PVC-fed mealworms during the experiment (Fig. 1a-c and Table S1-3). Future studies on invertebrate-mediated plastic biodegradation should identify the impact of degradation intermediates and mineralization products on the invertebrate and degradation processes.

3.2. Evidence of biodegradation of the microplastics

GPC analysis was applied to obtain evidence of polymer depolymerization and confirm the biodegradation of the ingested plastics by mealworms. The M_w , M_n , and M_z of the ingested PS significantly decreased from 180.7 ± 5.1 kDa, 74.6 ± 3.3 kDa, and 376.4 ± 18.7 kDa to 131.3 ± 6.0 kDa (by $27.34 \pm 3.32\%$), 48.5 ± 0.8 kDa (by $34.99 \pm 1.07\%$), and 281.8 ± 14.5 kDa (by $25.13 \pm 3.85\%$) after mealworm digestion (Fig. 1d-i and Table 1), suggesting a typical broad depolymerization pattern as shown in previous studies [28,56,59]. The ingested PVC exhibited a similar depolymerization pattern as that of the ingested PS microplastics, i.e., the M_w , M_n , and M_z were reduced by $26.27 \pm 4.47\%$, $32.98 \pm 4.97\%$, and $21.10 \pm 3.47\%$, respectively (Fig. 1g-i). On the other hand, the PDI of the recovered PS and PVC residue in frass (2.707 and 2.151) was both higher than that of the original PS and PVC microplastics (2.422 and 1.955) (Table 1). The increase in the PDI value suggested the random internal scission (or endo-type depolymerization) of polymer macromolecules [10], yielding mid- and short-chain polymers with heterogeneous molecular weight distributions in the intestine of mealworms. Taken together, these data indicated that the polymer chains of the ingested PS and PVC were effectively shortened and reduced after passage through the gut of mealworms, demonstrating the biodegradation of the microplastics.

The molecular weights of the ingested PE microplastics also significantly changed after mealworm digestion (Fig. 1d-i). The M_w , M_n , and M_z values of the recovered PE residue increased by $30.77 \pm 2.30\%$, $22.68 \pm 3.14\%$, and $31.28 \pm 5.73\%$, respectively, compared to those of the original PE microplastics (Table 1), revealing a limited-extent depolymerization pattern of the ingested PE polymers [28,56,59]. The limited-extent depolymerization pattern could be attributed to the functional enzymes and gut bacterial consortiums of mealworms reacting slowly on the long-chain PE polymers with high molecular weights but attacking the short-chain and side-chain PE polymers more efficiently, resulting in the accumulation of the long-chain portion and an increase in the M_w , M_n , and M_z values. The difference in the depolymerization patterns of the polymers could also be associated with macromolecular structure, material crystallinity, and polymer degradability [5]. The atactic structure precludes PS polymer from forming any crystallinity, and the crystalline structure in PVC polymer is also inadequate, while the PE polymer, which contains side chains, belongs to crystalline plastics [10,60,62]. Since the endo-type depolymerization

occurred more readily in the amorphous fractions of polymers than the crystalline fractions, the PS and PVC polymers were more likely to be attacked and depolymerized than the PE polymers in the gut microenvironment of mealworms, resulting in the difference in the depolymerization patterns. More research is needed to determine the reaction order, depolymerization rate, and digestive model in the mealworm-mediated plastic-degrading process, as overcoming this barrier will be the key to practical applications of biodegradation. Techno-economic analysis will also be necessary to guide future progress toward economical and sustainable processes.

3.3. Quantity of residual microplastics in the mealworm intestine

Mealworms can rapidly digest the ingested feedstocks (e.g., wheat bran, straw powder, and vegetables) and excrete mealworm frass residue within about 12 h [3,4,47]. To identify the undigested residual microplastics in the mealworm intestines after the biodegradation and the time required for complete depuration, the LDIR technique was performed to count the number of the residual microplastics after different time of depuration (i.e., 12, 24, 36, 48, and 60 h) at the end of the experiment.

After 12 h of depuration, there were still a number of residual plastic particles of different sizes in the mealworm intestine, i.e., 79 items for the PE-fed mealworm, 74 items for the PS-fed mealworm, and 91 items for the PVC-fed mealworm (Fig. 2a-c and S3). Interestingly, the number of residual PVC particles in the intestine was significantly higher, and the proportion of the large PVC microplastic particles (i.e., the 100–300 μm particles and 50–100 μm particles) was much higher than those of the PE and PS microplastics. Unlike normal feedstocks, the undigested microplastic particles, particularly the PVC microplastics, likely remained in the intestinal tract of mealworms for a much longer time. Previous studies indicated that the ingestion of microplastics by organisms without plastic-degrading capacities (e.g., copepods, crabs, koi carp, zebra fish, etc.) would lead to intestinal blockage and physical damage, eliciting decreased nutrient uptake and inflammatory responses [7,25,35]. Therefore, the low mobility and relatively poor physiological performances of the mealworms fed with microplastics alone during the experiment (Fig. 2a-c) could be related to the retention of undigested microplastics and the microplastic-induced damage in the gut microenvironment.

On the other hand, the time needed for complete depuration was also different for the PE-fed, PS-fed, and PVC-fed mealworms. Overall, the number of residual microplastic particles in the intestinal tracts of mealworms all decreased progressively (Figs. 2a-2c). After 48 h of depuration, no microplastics appeared in the intestines of the PE-fed and PS-fed mealworms, as indicated by the LDIR analysis (Figs. 2a and 2b), indicating complete digestion and depuration of the microplastics. In contrast, there were still 9 items, i.e., 2 items of 50–100 μm particles, 4 items of 30–50 μm particles, and 3 items of 0–30 μm particles, observed in the intestine of the PVC-fed mealworm (Fig. 2c), suggesting that the grinding, digestion, and excretion of the PVC microplastics could be more difficult than those of the PE and PVC microplastics by the plastic-degrading mealworm larvae. Similarly, the disappearance of PS particles in the gut of the great wax moth (*Galleria mellonella* larvae, Lepidoptera:

Table 1

Characterization of molecular weight changes and polydispersity of the ingested microplastics ($n = 3$). M_w = weight-average molecular weight; M_n = number-average molecular weight; M_z = size-average molecular weight; PDI = Polydispersity index.

	M_w (kDa)	M_w change (%)	M_n (kDa)	M_n change (%)	M_z (kDa)	M_z change (%)	PDI
PE	152.1 ± 3.6	—	73.2 ± 2.4	—	313.9 ± 10.2	—	2.078
PE frass	198.9 ± 3.5	$+ 30.77 \pm 2.30$	89.8 ± 2.3	$+ 22.68 \pm 3.14$	412.1 ± 18.0	$+ 31.28 \pm 5.73$	2.215
PS	180.7 ± 5.1	—	74.6 ± 3.3	—	376.4 ± 18.7	—	2.422
PS frass	131.3 ± 6.0	-27.34 ± 3.32	48.5 ± 0.8	-34.99 ± 1.07	281.8 ± 14.5	-25.13 ± 3.85	2.707
PVC	129.8 ± 3.1	—	66.4 ± 3.9	—	233.6 ± 10.9	—	1.955
PVC frass	95.7 ± 5.8	-26.27 ± 4.47	44.5 ± 3.3	-32.98 ± 4.97	184.3 ± 8.1	-21.10 ± 3.47	2.151

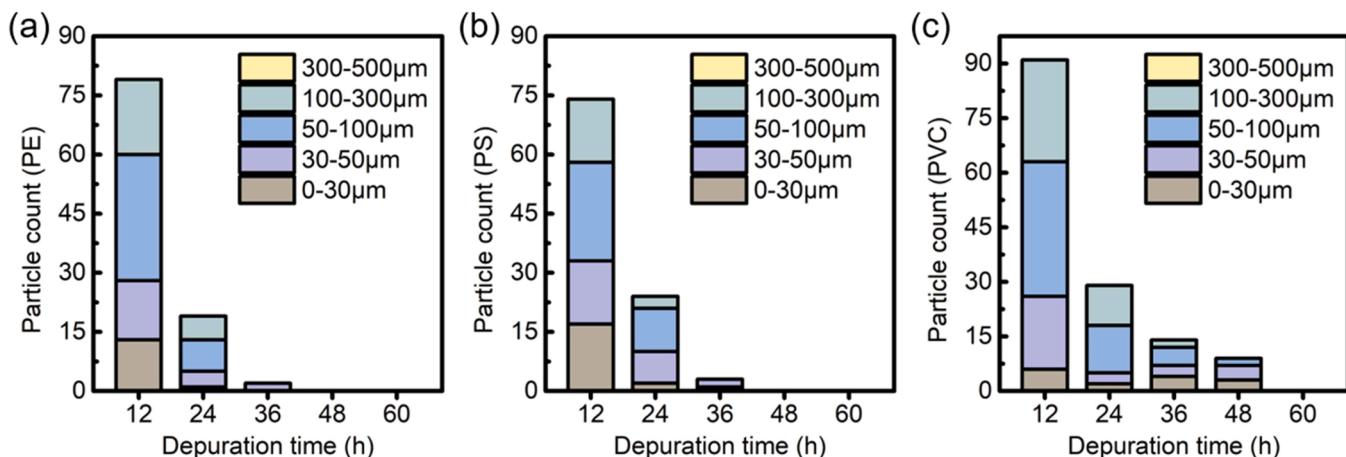


Fig. 2. The number of residual plastic particles in the intestine of (a) PE-fed, (b) PS-fed, and (c) PVC-fed mealworms after different depuration time (12, 24, 36, 48, and 60 h). The particles were identified using the Laser Direct Infrared Spectrometry (LDIR, Agilent 8700 LDIR). The results depict the average of five replicates (adjusted to an integer).

Pyralidae) was also observed after 48 h of force feeding with PS microplastics (< 75 μm in size) (Wang, S. et al., 2022). We speculate that the time for complete digestion and depuration of the ingested plastics by plastic-degrading invertebrates may be associated with the physico-chemical properties of polymers, the extent of polymer degradation and mineralization, as well as the toxicity of the degradation intermediates. Future follow-up research should help verify these hypotheses with other major petro-based polymers and degradable plastics (e.g., polypropylene (PP), polyethylene terephthalate (PET), polyurethane (PUR), PLA, PBAT, etc.).

3.4. The oxidative stress in mealworm larvae after plastic biodegradation

The ingestion and biofragmentation of micro(nano)plastics would cause endogenous oxidative damage in invertebrates [7,49]. To identify whether similar oxidative stress was induced in the plastic-degrading mealworms, we determined related biochemical indicators by the end of the experiment. After 24 days of continuous plastic feeding, elevated ROS levels were observed in the mealworm groups fed with microplastics alone (Fig. 3a), i.e., 4.85×10^6 , 4.72×10^6 , and 5.81×10^6 fluorescence intensity per mg protein for the PE-fed, PS-fed, and PVC-fed mealworms, respectively. As expected, the highest ROS level was detected in the PVC-fed mealworms, which was 38.1% higher than that of the bran control (4.20×10^6 fluorescence intensity/mg protein). The result of ROS levels revealed that the ingestion and biodegradation of the PE, PS, and PVC microplastics elicited oxidative stress in the mealworms, while that in the PVC-fed mealworms was the most serious, which confirmed our hypothesis that microplastics could cause an increase in ROS production in invertebrates with strong plastic biodegradation abilities.

Excessive production of ROS could result in oxidative damage and metabolic dysbiosis in organisms [35,39]. To further examine the activity of antioxidant enzymes against ROS in mealworms, the concentrations of SOD, the first-line antioxidant defense, were measured. After the 24-day incubation, the SOD concentrations in the PE-fed, PS-fed, and PVC-fed mealworms (i.e., 109.88 ± 1.91 , 102.45 ± 4.62 , and 138.17 ± 7.09 U per g fresh weight, respectively) were all significantly higher than the bran-fed control (i.e., 56.51 ± 2.12 U per g fresh weight) as expected (Fig. 3b). Notably, the SOD concentration was highest in the PVC-fed mealworms, which could be attributed to the limited extent of mineralization and the toxicity of the degradation products (e.g., chlorinated intermediates and hydrogen chloride) [13,26]. SOD is indispensable for the elimination of the superoxide anion (O_2^-) in organisms [11,52]. Therefore, these observations corroborated that digestion and

biodegradation of microplastics would also initiate the defenses of antioxidant systems to scavenge free radicals in plastic-degrading invertebrates. However, the increase in the activities of SOD and other antioxidant enzymes could not counteract the excessive ROS production [16,48], resulting in relatively increased oxidative stress in mealworms (Fig. 3).

The increase in the concentration of MDA could be used to monitor the occurrence of oxidative damage in organisms, as excessive free radicals would attack biomacromolecules like membrane lipids and cause rises in the MDA content [16,39,48]. Thus, we analyzed the changes in MDA content to better understand the oxidative damage in the mealworms. As with the increase in ROS, the concentrations of MDA were also elevated in the mealworm groups fed on plastic diets (Fig. 3c). The MDA content was 9.19 ± 0.11 , 9.08 ± 0.23 , and 10.97 ± 0.80 nmol per g fresh weight for the PE-fed, PS-fed, and PVC-fed mealworms, which was significantly higher than that in the bran-fed mealworms (8.13 ± 0.31 nmol per g fresh weight). The results indicated lipid peroxidation and oxidative damage in the plastic-fed mealworms. Likewise, the accumulation of MDA content was also found in *Eisenia fetida* (earthworms) under exposure to PE microplastics when the production rates of antioxidant and detoxification enzymes could not accommodate the increases in free radicals [6,49]. Microplastic-induced oxidative damage could further trigger mitochondrial dysfunction and apoptosis, leading to the abnormal development of organisms [63,68]. Hence, the performance of the mealworms in the key physiological indices (including survival rate and body weight of mealworms) could be associated with the oxidative damage induced by the ingested microplastics. Taken together, the overall evaluation of the oxidative stress and antioxidant performance of mealworms under exposure to PE, PS, and PVC microplastics is shown in the ternary diagram (Fig. 3d and Table S4). Compared with the bran-fed control (light red color), global increases in oxidative stress and damage were found for all mealworm groups fed with microplastics alone, with the highest increase observed in the PVC-fed mealworms. These results demonstrated that the oxidative damage and negative physiological responses would also be induced in plastic-degrading insect larvae. Follow-up research should examine the microplastic-induced oxidative stress in other plastic-degrading invertebrates, including *Zophobas atratus* larvae [30,51,59], *Galleria mellonella* larvae [18,50], and *Spodoptera frugiperda* larvae [64], with additional petro-based major polymers and bio-based degradable plastics.

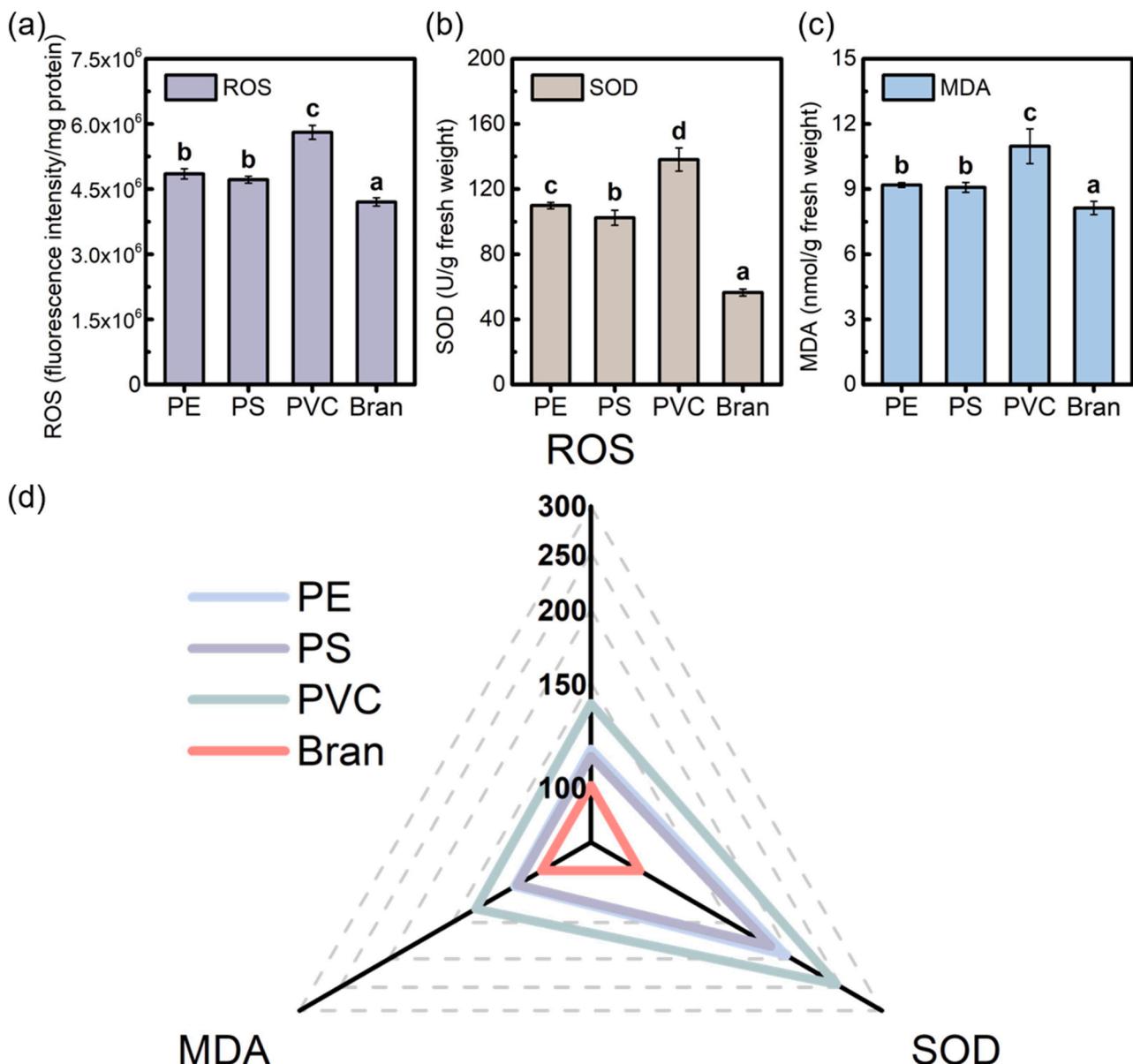


Fig. 3. Characterizations of the levels of oxidative stress in mealworms after biodegradation of PE, PS, and PVC. The levels of (a) reactive oxygen species (ROS, fluorescence intensity/mg protein), (b) superoxide dismutase (SOD, U/g fresh weight), and (c) malondialdehyde (MDA, nmol/g fresh weight) in mealworms at the end of the test (day 25). Different letters above the columns indicate significant differences. (d) The assessment of overall physiological stress responses (ROS, SOD, and MDA) of mealworms to the biodegradation of PE, PS, and PVC. The oxidative stress levels could be represented by the relative area on the ternary diagram.

3.5. The size distribution of residual microplastics in frass

To determine the undigested plastic particles in frass, highly sensitive laser light scattering was used to obtain the accurate size distribution of residual microplastics on a volume basis after biodegradation (size measurement range of 0.01–3500 µm). Plastics ingested would be physically biofragmented first by the grinding actions of mealworm mouthparts and intestinal peristalsis, then biodegraded by the gut microbiome and digestive enzymes. As expected, the overall size-frequency distribution of the PE, PS, and PVC microplastics all shifted towards a smaller particle size direction after digestive biofragmentation and biodegradation by mealworms (Fig. 4). The maximum volume percentages of the residual PE, PS, and PVC microplastics in frass were achieved at 86.4 µm (ratio: 8.97%), 76.0 µm (ratio: 7.82%), and 66.9 µm (ratio: 8.93%), respectively. On the other hand, the smallest residual plastic particles in frass were detected at around 5.0 µm (ratio: 0.18%), 4.0 µm (ratio: 0.14%), and 5.9 µm (ratio: 0.05%)

for the PE-fed, PS-fed, and PVC-fed mealworms, respectively. The smallest particles fell in the range of “sub-micron microplastics”, and their proportions were almost negligible [21], indicating that nano-sized plastics were not generated and accumulated in the gut microenvironments of the plastic-degrading mealworms during the biodegradation of microplastics. Since nano-sized plastics have very large specific surface areas and high surface reaction energies [1,21], digestion biodegradation reactions would be accelerated exponentially. The nano-sized plastics would be rapidly and instantaneously eliminated in the intestines without any accumulation. Hence, the results suggest that the mealworm-mediated biodegradation of major microplastics (e.g., PE, PS, and PVC) is a relatively environmentally safe recycling and treatment method.

4. Concluding remarks

The results of this study first demonstrate that the three most

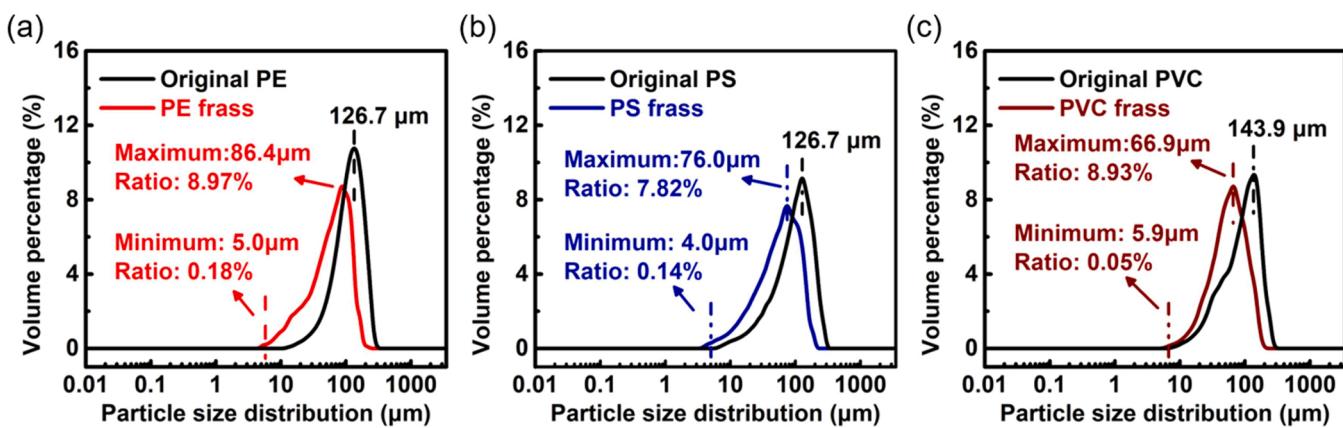


Fig. 4. Characterization of the size distribution of microplastics in frass after plastic biodegradation. The size-frequency distribution of residual (a) PE, (b) PS, and (c) PVC particles in frass versus the original microplastics. The particle size distribution was based on volume percentage.

common microplastics, i.e., PE, PS, and PVC, can be rapidly ingested and efficiently biodegraded by the invertebrate *Tenebrio molitor* (yellow mealworms). The PS and PVC microplastics were depolymerized in the mealworm intestines via the broad depolymerization pattern, while the PE microplastics were depolymerized via the limited-extent depolymerization pattern. The ingestion of microplastics can negatively influence the key physiological indices of the mealworms, including their survival rates and body weights, despite their remarkable capacities for plastic biodegradation. At the end of the 24-day experiment, the mealworms fed with PVC microplastics showed the lowest survival rate ($81.3 \pm 1.5\%$) and the highest body weight reduction ($15.1 \pm 1.1\%$), which could be attributed to the limited extent of mineralization and the toxicity of the degradation products.

Undigested microplastic particles remained in mealworm intestinal tracts for a longer period of time than normal food bran, taking up to 60 h to depurate. The undigested PVC microplastic particles, in particular, were more difficult to depurate and excrete for the mealworms in comparison to the residual PE and PS microplastic particles. Microplastic-induced oxidative stress responses, including the significant increase in ROS, antioxidant enzyme activities (i.e., SOD), and lipid peroxidation (i.e., MDA content), were discovered in the mealworms fed with microplastics alone, with the highest levels found in the PVC-fed mealworms. Sub-micron microplastics and small microplastics are

found in the frass collected from the mealworms fed with PE, PS, and PVC microplastics, with the smallest particles detected at diameters of 5.0 μm , 4.0 μm , and 5.9 μm , respectively. During the biodegradation of microplastics by mealworms, no nano-sized plastic particles ($< 1 \mu\text{m}$) were formed and accumulated in frass. Based on the results and the data of this study, we propose a conceptual schematic diagram for the biodegradation of PE, PS, and PVC microplastics by mealworms (Fig. 5), which highlights the need for assessing plastic residues in macro-invertebrates with plastic biodegradation or biofragmentation capacities and their physiological responses under environmental exposure to microplastics and nanoplastics. Overall, plastic waste is a low-cost, carbon-rich, and easily obtained feedstock. Understanding the plastic degradation processes by plastic-degrading invertebrates is critical for developing green and sustainable plastic waste treatment and recycling strategies, as well as addressing the overall plastics dilemma.

Environmental implications

This work comprehensively investigates the biodegradation of the three most common microplastics, i.e., PE, PS, and PVC, by *Tenebrio molitor* larvae and assesses the fate of the residual plastics as well as the microplastic-induced stress responses. We demonstrate that residual microplastic particles remain in intestines for a longer time than normal

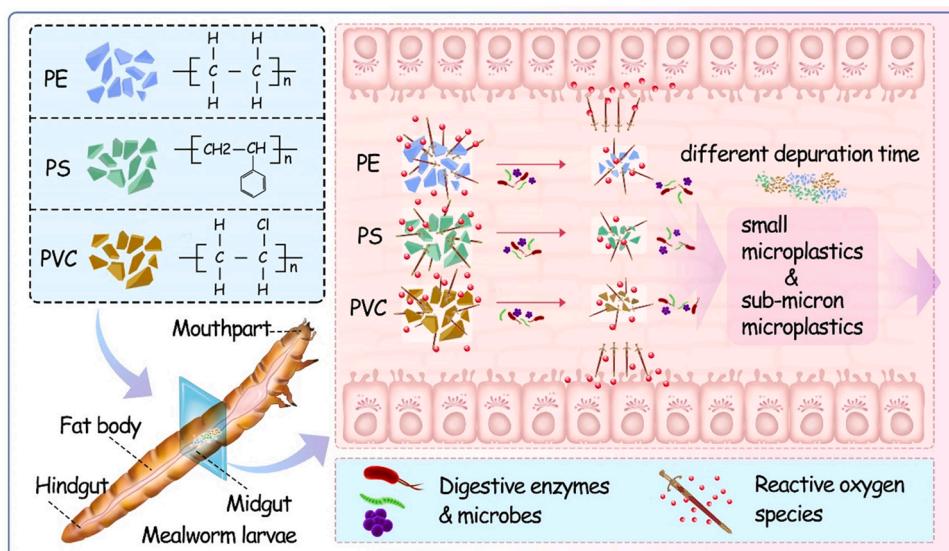


Fig. 5. Conceptual schematic diagram for the biodegradation of PE, PS, and PVC microplastics by mealworms.

food, which induces oxidative stress and negative physiological responses in mealworms. Importantly, nanoplastics could not form and accumulate in the excrement due to the prominent plastic-degrading abilities of mealworms. Our findings provide insights into the residual microplastics and microplastic-induced stress responses in macro-invertebrates under micro(nano)plastics exposure.

CRediT authorship contribution statement

Bo-Yu Peng: Conceptualization, Methodology, Formal analysis, Investigation, Methodology, Writing – original draft. **Ying Sun:** Formal analysis, Validation. **Jingjing Sun:** Validation. **Yazhou Xu:** Formal analysis. **Shaoze Xiao:** Conceptualization, Methodology. **Xu Zhang:** Formal analysis. **Jaibin Chen:** Validation, Funding acquisition. **Xuefei Zhou:** Methodology, Funding acquisition. Validation, Writing – review & editing, Funding acquisition. **Yalei Zhang:** Conceptualization, Methodology, Validation, Project administration, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

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

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2023.131326](https://doi.org/10.1016/j.jhazmat.2023.131326).

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