



Biodegradation of aged polyethylene (PE) and polystyrene (PS) microplastics by yellow mealworms (*Tenebrio molitor* larvae)

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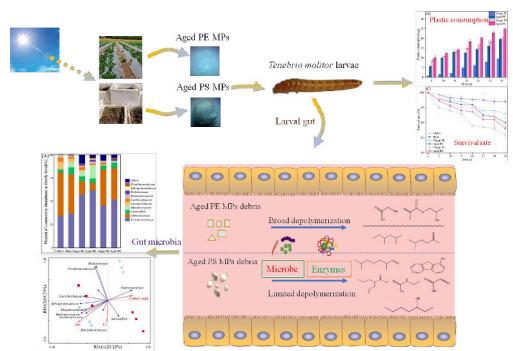
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HIGHLIGHTS

- This paper addresses microplastic (MP) pollution with *Tenebrio molitor* larvae (yellow mealworm).
- The *Tenebrio molitor* larvae were most effective when consuming aged MPs.
- Aged MPs were depolymerized and biodegraded more efficiently than pristine MPs.
- The *T. molitor* aging indexes were highly correlated to the microbial communities.
- T. molitor* study yielded new strategies for MP eradication solutions.

GRAPHICAL ABSTRACT



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ABSTRACT

Globally, over 287 million tons of plastic are disposed in landfills, rivers, and oceans or are burned every year. The results are devastating to our ecosystems, wildlife and human health. One promising remedy is the yellow mealworm (*Tenebrio molitor* larvae), which has proved capable of degrading microplastics (MPs). This paper presents a new investigation into the biodegradation of aged polyethylene (PE) film and polystyrene (PS) foam by the *Tenebrio molitor* larvae. After a 35 – day feeding period, both pristine and aged MPs can be consumed by larvae. Even with some inhibitions in larvae growth due to the limited nutrient supply of aged MPs, when compared with pristine MPs, the aged MPs were depolymerized more efficiently in gut microbiota based on gel permeation chromatography (GPC) and Fourier transform infrared spectroscopy (FTIR) analysis. With the change in surface chemical properties, the metabolic intermediates of aged MPs contained more oxygen-containing functional groups and shortened long-chain alkane, which was confirmed by gas chromatography and mass spectrometry (GC–MS). High-throughput sequencing revealed that the richness and diversity of gut microbes were restricted in the MPs-fed group. Although MPs had a negative effect on the relative abundance of the two dominant bacteria *Enterococcaceae* and *Lactobacillaceae*, the aged MPs may promote the relative abundance of *Enterobacteriaceae* and *Streptococcaceae*. Redundancy analysis (RDA) further verified that the aged MPs are effectively biodegraded by yellow mealworm. This work provides new insights into insect-mediated mechanisms of aged MP degradation and promising strategies for MP sustainable and efficient solutions.

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1. Introduction

Global plastic production reached approximately 430 million tons in 2023 and two-thirds of that were short-live plastics (United Nations, 2023a). The majority of these plastics were dumped into landfills, rivers, and oceans, where chemical additives can leach from plastics, which has been linked to several human diseases, including cancer (Mohammadi et al., 2022; Schmidt et al., 2020; Maes et al., 2023). After decomposed into smaller pieces (< 5 mm), they become microplastics (MPs) and continue to have lethal effects on both humans and wildlife (Peng et al., 2019). The UN General Assembly approved the International Negotiating Committee (INC), which authorized and helped developing an international plan to severely limit the dumping of plastics (United Nations, 2023b). MPs can easily transfer into the human body through the food chain, which threatens both human and wildlife health (Leslie et al., 2022). Because of the small particle size and large surface area, MPs can act as vectors to inorganic and organic pollutants which have complex ecological effects on organisms in soil, freshwater and the marine ecosystem (Li et al., 2022a; Purwiyanto et al., 2020; Goh et al., 2022; Pham et al., 2021). Present research related to MPs mainly focuses on exploring their environmental behavior, including the adsorption of other pollutants (Munoz et al., 2021) and the biological effect on marine or freshwater organisms (Nugnes et al., 2022; Ma et al., 2019; Bargagli and Rota, 2023). However, few studies focus on the disposal and recycling of MPs, which greatly affects the sustainable development of plastics.

With easy operation, low energy consumption, high efficiency and no secondary pollution, biodegradation is considered as an environment-friendly method for degrading MPs (Pham et al., 2021). Auta et al. (2018) revealed that two bacteria, *Bacillus* and *Rhodococcus*, in the mangrove environment could degrade polypropylene (PP) effectively by aggregating on the surface of the polymer and producing extracellular enzymes. Park et al. also found that the most dominant bacterial species of *Bacillus* and *Paenibacillus* in the sediment of landfills could decompose micro-sized polyethylene (PE) efficiently (Park and Kim, 2019). Some Lepidoptera and coleoptera have been observed taking plastics as a food supply, and they were able to decompose them in vivo (Sanchez-Hernandez, 2021). Kundungal et al. (2019) verified that the high-density PE MPs can be depolymerized by *Achroia grisella* in a gut environment. Chen et al. (2023) found that the degradation of polystyrene (PS) by *Zophobas atratus* larvae is caused by the combined effect of reactive oxygen species (ROS) and extracellular oxidase of gut microbes. *Tenebrio molitor* (*T. molitor*), also known as the yellow mealworms, is considered as a useful model organism for plastic biodegradation. The biodegradation ability of MPs by *T. molitor* larvae is mainly due to the depolymerization of intestinal microorganisms and enzymes secreted by larvae (Machona et al., 2022; Mamtimin et al., 2023; Yang et al., 2023; Xu et al., 2023). With the features of low price, widespread availability and easy-to-feed, they are considered as an ideal organism for the degradation of MPs including PE, PP and PS MPs (Brandon et al., 2018).

The biodegradation efficiency can be greatly affected by the physicochemical properties of plastics. Yang et al. (2022) reported that the molecular weight and crystallinity of plastics were two important factors that affect the biodegradation abilities of PE MPs by both *T. molitor* and *Tenebrio obscurus*. The *Tenebrio obscurus* is a darkling beetle with larvae referred to as mini mealworms (Peng et al., 2019). Zhong et al. (2022) determined that the *T. molitor* larvae can depolymerize the high impact PS and low-density PE MPs, but cannot degrade the linear low-density polyethylene (LLDPE) and rigid PP MPs. Moreover, the hydrophilic feature, surface area, and chemical structure of polymers also play crucial roles in biodegradation process (Bacha et al., 2023). When exposed to the environment, the MPs cannot avoid going through the aging effect, such as that caused by UV radiation. UV radiation is considered as an important factor in accelerating the aging process of plastic (Kasmuri et al., 2022; Zhang et al., 2021). After UV aging, the

physicochemical characteristics of MPs were changed obviously with decreased hydrophobicity, increased specific surface area and crystallinity (Sørensen et al., 2021; Binda et al., 2023; Feng et al., 2022). More oxygen-containing group was emerged due to the oxidation reaction on the surface of polymers (Song et al., 2017; Wang et al., 2024). However, research is limited on the environmental aging effect of MP physicochemical properties, which can further affect the acceptance of biotic degradation.

In this study, the degradation of the aged MPs (including PE film and PS foam) is compared with the pristine ones degraded by *T. molitor* larvae. The authors also analyzed the growth trend and survival rate of larvae under different types of MP feeding conditions. The change of the functional groups and the molecular weight of MPs after depolymerization were characterized by Fourier transform infrared spectroscopy (FTIR), gel permeation chromatography analysis (GPC) and high-temperature gel permeation chromatography (HT-GPC) analysis. The biodegradation products of MPs by larvae were also confirmed by using gas chromatography-mass spectrometry (GC-MS) method. The gut microbiomes of the larvae degrading the PE and PS were investigated as well as the correlation with the aging index of MPs. Our studies provided a valuable and effective method for the MP consumption in nature and as well as a prospective understanding of MP biodegradation.

2. Materials and methods

2.1. Plastic materials and *Tenebrio molitor* larvae

The virgin PE film and PS foam were purchased from Daqing Wusu Plastic Products Co., LTD (Daqing, Dongguan, China) and Zhongyuan Foam Products Co., LTD (Xinxiang, Henan, China), respectively. The aged PE film and PS foam were collected from a Ma'anshan vegetable farm in Anhui, China before being washed with distilled water. The fragments of the pristine and aged MPs were prepared by cutting the raw materials into around 3 mm pieces. Both the pristine and aged MPs were purified with 95 % ethanol followed by a 2 % HNO₃ solution to remove organic impurities and metal ions. Then, the MPs were washed twice with distilled water before they were air dried (Wang et al., 2022a; Zhong et al., 2023). The water contact angle of the MP surfaces was measured using an LSA100 contact angle meter (Lauda Scientific GmbH, Lauda-Königshofen, Germany). The PE and PS hardness were determined by a Vickers hardness tester (Falcon 500 Durometer, INNOVATEST, Netherlands) and a GS-701N Durometer hardness tester (TECLOCK, Hanoi, Japan), respectively. The *T. molitor* (mealworm) larvae were obtained from a yellow mealworm farm in Dezhou (Shandong, China). The larvae were fed wheat bran prior to tests. The initial weight of the larvae was between 55 and 75 mg for larvae and the length was 1.5 to 2.5 cm. The chemical reagents used in the study were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Biodegradation of PE film and PS foam

Before testing, the larvae were starved for 72 h to eliminate the previous feedstock. Six feeding groups were prepared for the mealworm larvae: (a) virgin PE film; (b) aged PE film; (c) virgin PS foam; (d) aged PS foam; (e) bran and (f) unfed. The unfed group was also the control group. Each experiment group was set up with 100 larvae reared in a 300-mL volume culture dish; then, they were kept in dark incubators maintained at 25 °C with 70 % humidity (Przemieniecki et al., 2020). The plastic-fed group was supplemented with 0.3 g of plastics while the bran-fed group was fed with 1.5 g of bran initially (Zhong et al., 2023; Jiang et al., 2021). The molten exoskeleton and dead mealworms were removed from the containers to prevent being eaten by living larvae. The survival rate, the weight changes of the larvae and the consumption of plastics were measured every five days during 35 days of testing. All treatments were conducted in triplicate.

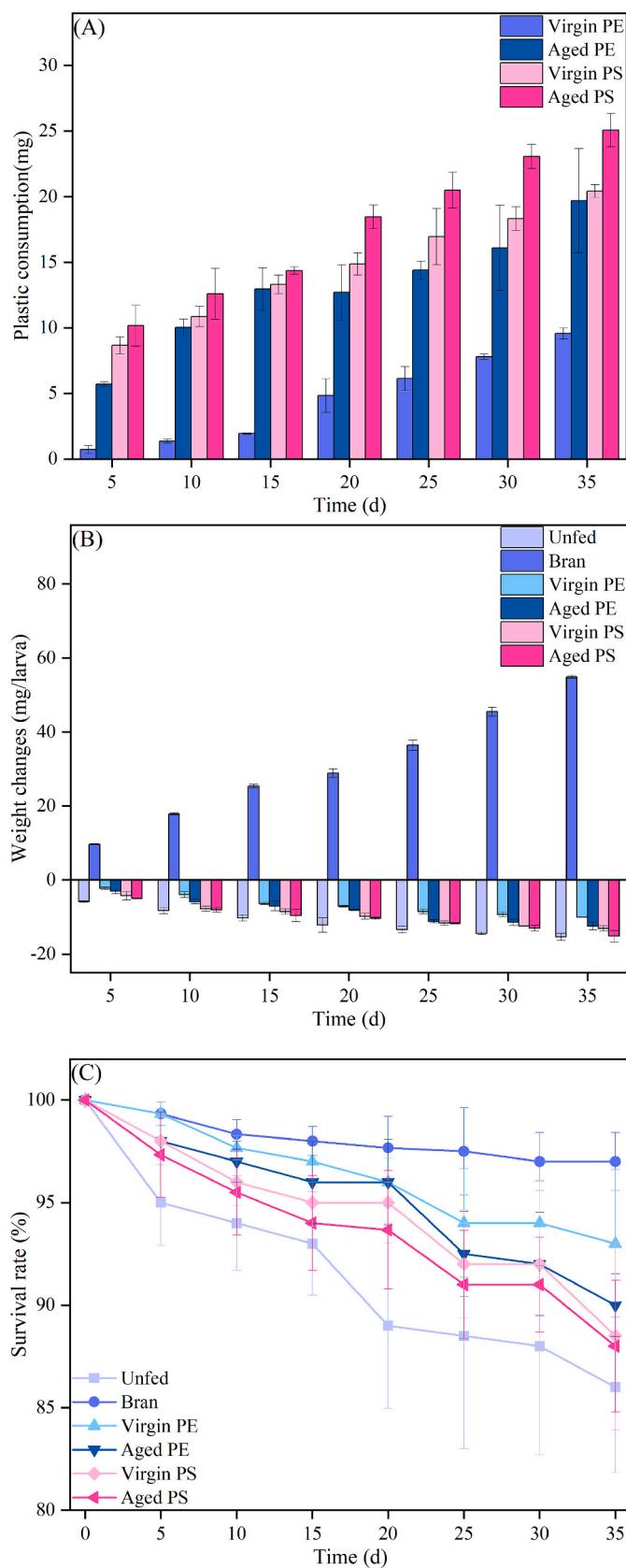


Fig. 1. Plastics consumption (A), weight changes (B) and survival rates (C) by *Tenebrio molitor* larvae under different dietary conditions over 35-day experimental period with unfed group as control.

2.3. Analytical methods

After the experiments, the larvae groups fed with different diets were removed to clean containers while the frass of larvae were collected and then freeze dried for 48 h. The obtained frass was stored under -20°C before further analysis.

A Thermo–Nicolet 6700 Series FTIR spectrometer (Thermo Fisher Scientific, Sunnydale, CA USA) was used to determine the main changes of the functional groups of PS and PE plastics after biodegradation. High temperature gel permeation chromatography (HT-GPC) PL-GPC 220 (Agilent Technologies Inc., Santa Clara, CA, USA) and the GPC 50 (Agilent Technologies Inc., Santa Clara, CA, USA) were used to determine the molecular weight values containing the number-average molecular weight (M_n), weight-average molecular weight (M_w) and size-average molecular weight (M_z) of the plastic samples and the frass of larvae, respectively. A gas chromatography–mass spectrometry quadrupole GC–MS–QP2010 Plus (Shimadzu Co., Kyoto, Japan) was performed to further identify the depolymerization products of plastics. The pretreatment of samples for the analysis of GPC was provided in Supporting Information (SI) as well as the detailed method of GC–MS.

The intestines were dissected out of the *T. molitor* larvae and fixed with 3.5 mL 2.5 % glutaraldehyde for 12 h, then rinsed with PBS solution (pH = 7.4) and dehydrated with 50 %, 70 %, 90 % and 100 % ethanol solutions with a wait time of 10 min for each gradient. 1 mL isoamyl acetate was added for precipitation and also had a 10 min wait time. The treated *T. molitor* larvae intestines were then freeze dried for at least 36 h (Chen et al., 2020). The last step was a final coat of palladium on freeze dried larvae which was observed by a high-performance, scanning electron microscope with a high resolution of 3.0 nm, the JSM-6490LV SEM (JEOL USA, Peabody, MA, USA).

2.4. Microbial community analysis

After the 35-day experiment, 60 larvae from each treatment group were randomly collected and mixed into 10 mL sterile centrifuge tubes for the gut microbiome analysis. They were preserved in 100 % ethanol at -80°C and transported to Shenzhen WeiKe Meng Tech Group Co., Ltd. (Shenzhen, China) for microbial community analysis. DNA extraction, PCR amplification and high-throughput sequencing of intestinal samples were performed, and the results were analyzed based on the Greengenes 16S rRNA database (Wang et al., 2022b).

2.5. Statistical analysis

Microsoft Excel 2019 was used to calculate the averages and standard deviations. A column diagram was drawn using Origin PRO 2021 (Origin PRO, USA). Statistical tests were performed using IBM SPSS Statistics (2016) version 24 with one-way ANOVAs, and the differences were considered significant at $p < 0.05$. All results were replicated in triplicate and given a mean \pm standard deviation (SD).

Table 1
The physiochemical characteristics of MPs.

Parameters	Virgin PE film	Aged PE film	Virgin PS foam	Aged PS foam
Water contact angle($^{\circ}$)	88.5 ± 3.5	79.2 ± 2.3	82.2 ± 2.5	63.1 ± 1.6
Hardness	$7.8 \pm 0.6\text{Kgf}/\text{mm}^2$	$5.6 \pm 0.7\text{Kgf}/\text{mm}^2$	$40.6 \pm 3.6^{\circ}$	$34.1 \pm 1.5^{\circ}$
Carbonyl index	0.34 ± 0.02	0.63 ± 0.04	0.43 ± 0.03	0.67 ± 0.06
M_n (kDa)	99.58 ± 2.15	56.30 ± 0.12	74.61 ± 0.97	78.24 ± 2.34
M_w (kDa)	317.27 ± 0.82	239.67 ± 1.00	193.06 ± 3.46	208.18 ± 3.01
M_z (kDa)	770.44 ± 1.20	727.35 ± 2.17	365.70 ± 2.35	405.54 ± 2.15

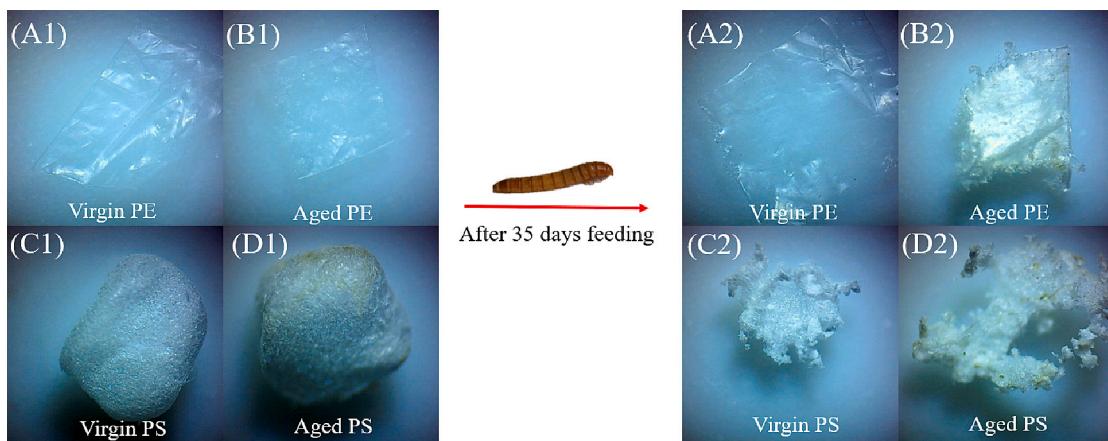


Fig. 2. Changes of PE and PS surface morphology after 35 days feeding by *Tenebrio molitor* larvae.

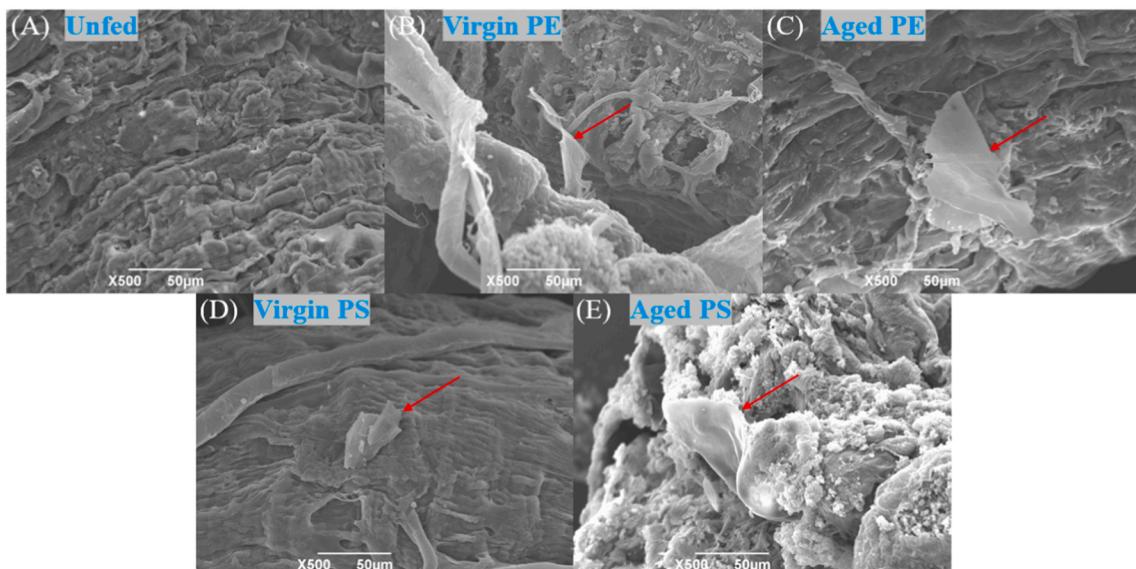


Fig. 3. The guts of *Tenebrio molitor* larvae in the five feeding groups ((A)unfed, (B) virgin PE, (C) aged PE, (D) virgin PS and (E)aged PS) revealed by SEM.

3. Results and discussion

3.1. Microplastics consumption and survival of *T. molitor* larvae

The consumption of aged PE film and PS foam by larvae over a 35-day experimental period was compared with the pristine PE and PS (Fig. 1(A)). Overall, the PS foam showed a higher consumption than the PE film, which may be related to the feeding preference of larvae for PS and the lower density of PS foam (Liu et al., 2022a; Wang et al., 2022b). Compared with the pristine MPs, aged MPs exhibited an obviously increased consumption trend by larvae, which related to the lower hardness of aged MPs (Table 1). Results indicated that the physical characteristics of plastics varied during the aging process, which may have led to changes in the preferred ingestion habits of larvae.

The weight changes of *T. molitor* larvae after MP feeding are also analyzed in Fig. 1(B). After the 35-day feeding period, the weight loss of larvae fed by MPs was 10.05–15.18 mg/larva, while the weight change of larvae fed by bran was 54.90 mg/larva. Compared with the bran-fed group, the growth of larvae fed by MPs was strongly inhibited. However, the weight loss of larvae feeding by MPs was lower than the starvation group (15.35 mg/larva). Moreover, the more polymers the larvae ingested, the more weight the larvae loss. It was attributed to the fact that MPs were only a single source of carbon, which was distinct from

bran, which contained sufficient nutrients, including protein and trace elements (Peng et al., 2022; Yang et al., 2021). At the end of the 35-day feeding period, the survival rate of larvae fed with MPs was between 88 % and 93 %, which was higher than the unfed group (86 %), but lower than the bran-fed group (97 %) (Fig. 1(C)). The aged MP-fed groups showed lower survival rates than the pristine ones with more plastic consumption by larvae. This indicates that the intake of MPs had a certain negative effect on the growth of larvae, which may be related to the toxic additives contained in the MPs (Sanchez-Hernandez, 2021). The results further demonstrated that the more MP intake by larvae, the more adverse effects was reported on the growth of larvae.

3.2. Ingestion of PE and PS microplastics by *T. molitor* larvae

The changes in MP surface morphology after being fed with larvae are shown in Fig. 2. Some irregular bite marks emerged on the surface of the MPs. Compared to PE film, the biting traces on PS foam were more remarkable. Moreover, the aged plastics seemed to be more prone to ingestion by mealworms when compared to MPs in their pristine condition. SEM images of larval intestines after MP feeding were also observed (Fig. 3). The SEM images after MPs had been subjected to larval feeding further confirmed by the effective ingestion of MPs by larvae.

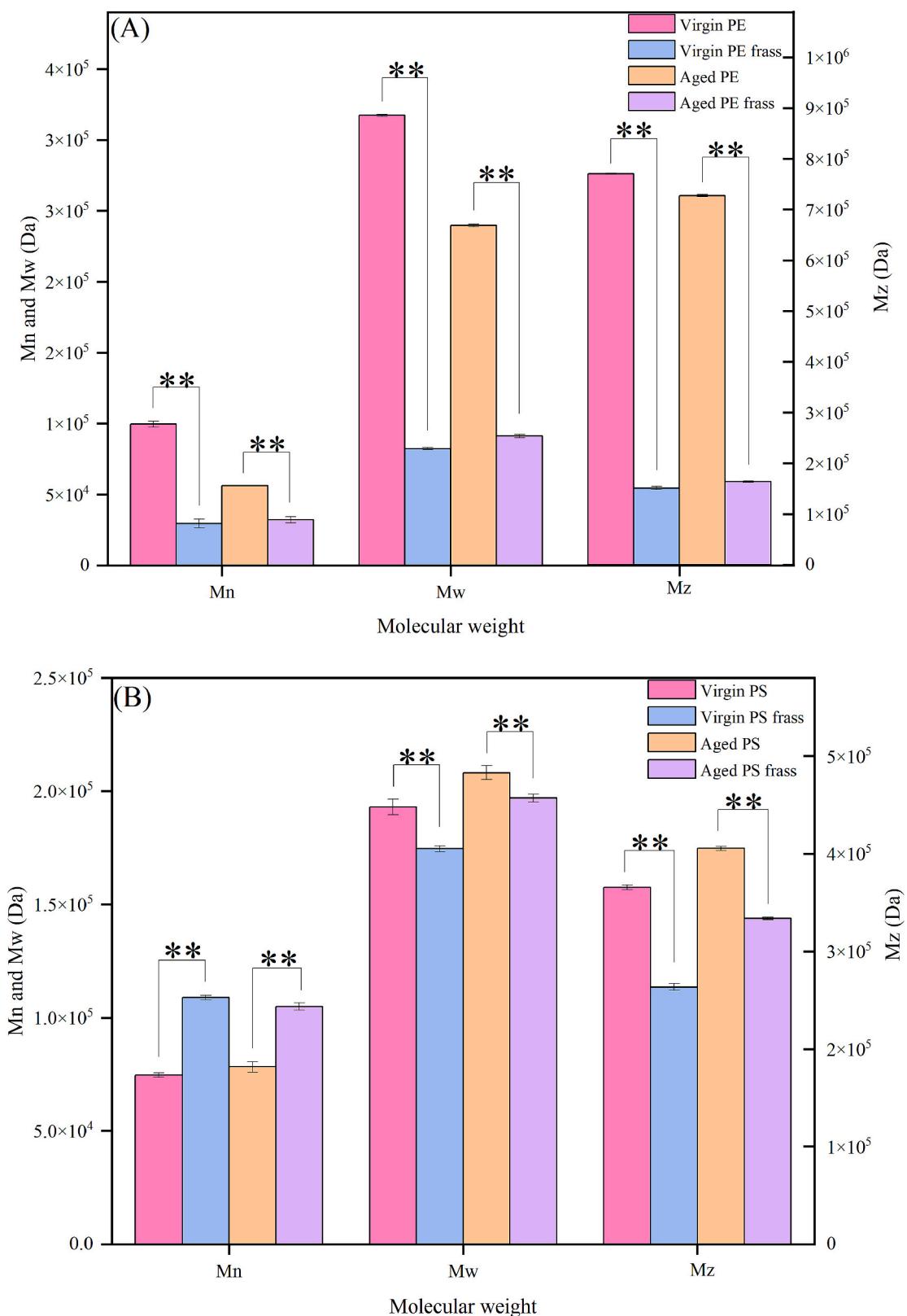


Fig. 4. Molecular weight (M_n , M_w and M_z) of (A) PE and (B) PS microplastic and frass of microplastic-fed larvae. M_n = number-average molecular weight; M_w = weight-average molecular weight; M_z = size-average molecular weight. Statistical significance $p < 0.05$ indicated by *, $p < 0.01$ indicated by **, ns: non-significant (Student's *t*-tests).

Table 2

Changes in molecular weight and polydispersity index (PDI) of virgin and aged PE and PS before and after biodegradation by *Tenebrio molitor* larvae (mean \pm standard deviation, $n = 3$).

Sample	Change in M_n (%)	Change in M_w (%)	Change in M_z (%)	PDI
Virgin PE	–	–	–	3.19 \pm 0.06
Virgin PE frass	-70.43 ± 2.53	-74.05 ± 0.20	-80.39 ± 0.39	2.81 \pm 0.27
Aged PE	–	–	–	4.26 \pm 0.01
Aged PE frass	-42.92 ± 3.70	-61.94 ± 0.35	-77.51 ± 0.10	2.84 \pm 0.15
Virgin PS	–	–	–	2.59 \pm 0.01
Virgin PS frass	45.83 ± 0.57	-9.56 ± 0.92	-27.92 ± 0.50	1.60 \pm 0.00
Aged PS	–	–	–	2.66 \pm 0.04
Aged PS frass	40.58 ± 2.08	-5.38 ± 0.56	-17.69 ± 0.12	1.79 \pm 0.01

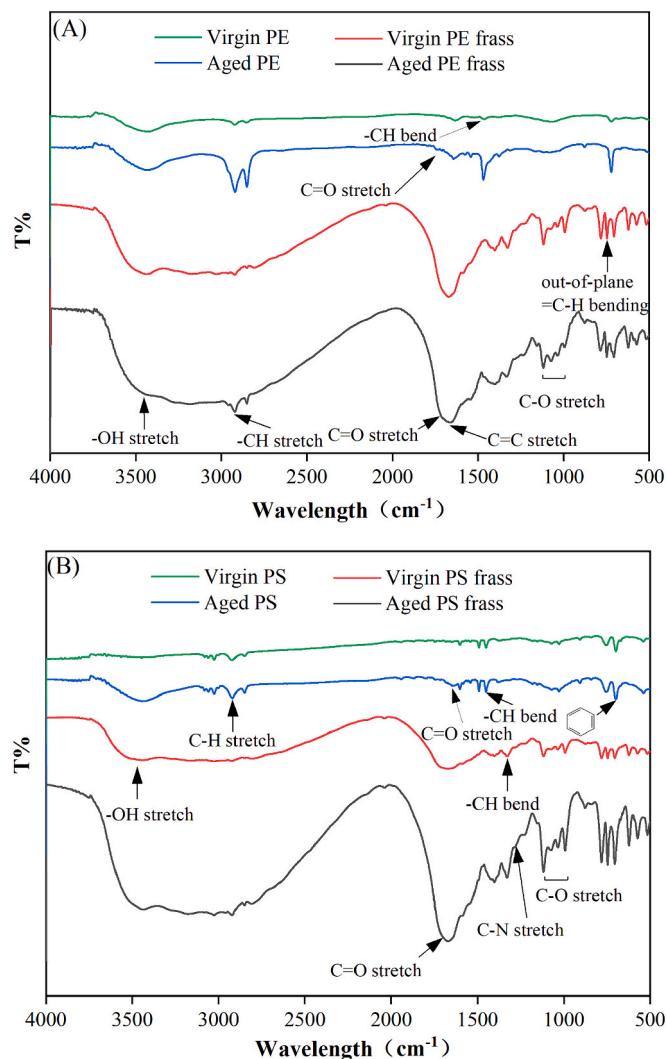


Fig. 5. FTIR spectra for (A) PE and (B) PS microplastic and frass of microplastic-fed larvae.

3.3. Depolymerization of PE and PS microplastics ingested by *T. molitor* larvae

The molecular-weight changes of the MP polymers after biodegradation were identified by GPC analysis (Fig. 4). M_n , M_w , and M_z all decreased significantly based on the residues excreted by larvae after ingestion of PE MPs, which shows a typical pattern of broad depolymerization (BD). However, the M_w and M_z of PS decreased after depolymerization, while the M_n increased. The results demonstrated that the biodegradation of PS was classified to the limited depolymerization (LD) pattern which was distinct from PE (Peng et al., 2020b). This may be related to the fact that enzymes and intestinal microorganisms in the larvae can selectively react quickly with the smaller polymer chain, leading to the accumulation of long-chain polymers (Peng et al., 2022). These results confirm that the molecular chains of polymers can be depolymerized by larvae, which is consistent with previous research by Yang et al. (2022).

The polydispersity index (PDI), represents the heterogeneity of chain length polymer distribution and determines the change in the polymers' molecular weight after depolymerization (Peng et al., 2020a; Peng et al., 2022). The downtrend of the PDI index of the aged PE was more significant than that of the pristine one after biodegradation, which indicates that larvae prefer to depolymerize aged PE into low polymer with uniform molecular weight distribution (Table 2). The PDI of the aged and virgin PS both declined to a certain degree, but the differences between them were not significant. Thus, the PDI revealed that the aging effect on the molecular weight distribution of degraded PS polymer products was limited.

3.4. Degradation of PE and PS microplastics ingested by *T. molitor* larvae

The function groups after MP degradation were determined by FTIR analysis (Fig. 5A). The main chemical groups greatly varied after biodegradation. The aged PE had strong absorption peaks of 1340–1465 cm^{-1} and 1650–1800 cm^{-1} , which correspond to C–H bending and C=O stretching, respectively. And the change of the –OH group revealed that the hydrophobicity of PE MPs decreases after aging which is consistent with the contact angle (Fig. S1). These results confirm that the aging process caused the oxidation reaction of PE plastics (Wang et al., 2022c). After depolymerization, the strong absorption peaks measured from 1100 to 1300 cm^{-1} and 1650 to 1800 cm^{-1} , which corresponded to C–O and C=O stretching, respectively. This indicates that PE biodegraded to oxygen-containing compounds such as ethers, carboxylic acids, aldehydes, and ketones or esters (Soni et al., 2009). Moreover, the absorption peaks at 1640–1680 cm^{-1} represented C=C stretching, illustrating that the oxidation and cleavage of PE MPs was activated by *T. molitor* larvae (Yang et al., 2022). The broader absorption peak between 3200 and 3500 cm^{-1} corresponding to the –OH group indicated oxidation of the chain of polymers and the reduction of PE hydrophobicity (Kowalczyk et al., 2016). However, no obvious differences between virgin and aged PE were present in the main function groups after depolymerization.

The FTIR spectra of the aged PS was confirmed by the appearance of the absorption peaks at 1650–1800 cm^{-1} and corresponds to the C=O stretching vibration (Fig. 5B). The hydrophobicity of PS MPs also decreased after aging (Fig. S1). The disappearance of the vibration peaks at 755 cm^{-1} and 696 cm^{-1} referred to the C–H stretching peak indicating that the benzene ring was dissociated after biodegradation (Wang et al., 2022b). Meanwhile, the oxygenated groups were also certified by the emerging peaks of C–O and C=O (Yang et al., 2022). The broaden absorption peak at 3200–3500 cm^{-1} represents the –OH group, showing how the surface characteristics of both aged and virgin PS can change when going from the hydrophobic to hydrophilic stage after degradation, which benefits the degradation of polymers by larvae (Yang et al., 2018). Moreover, the appearance peak of 1000–1350 cm^{-1} represents the C–N group in the PS samples' frass, which indicates the oxidation

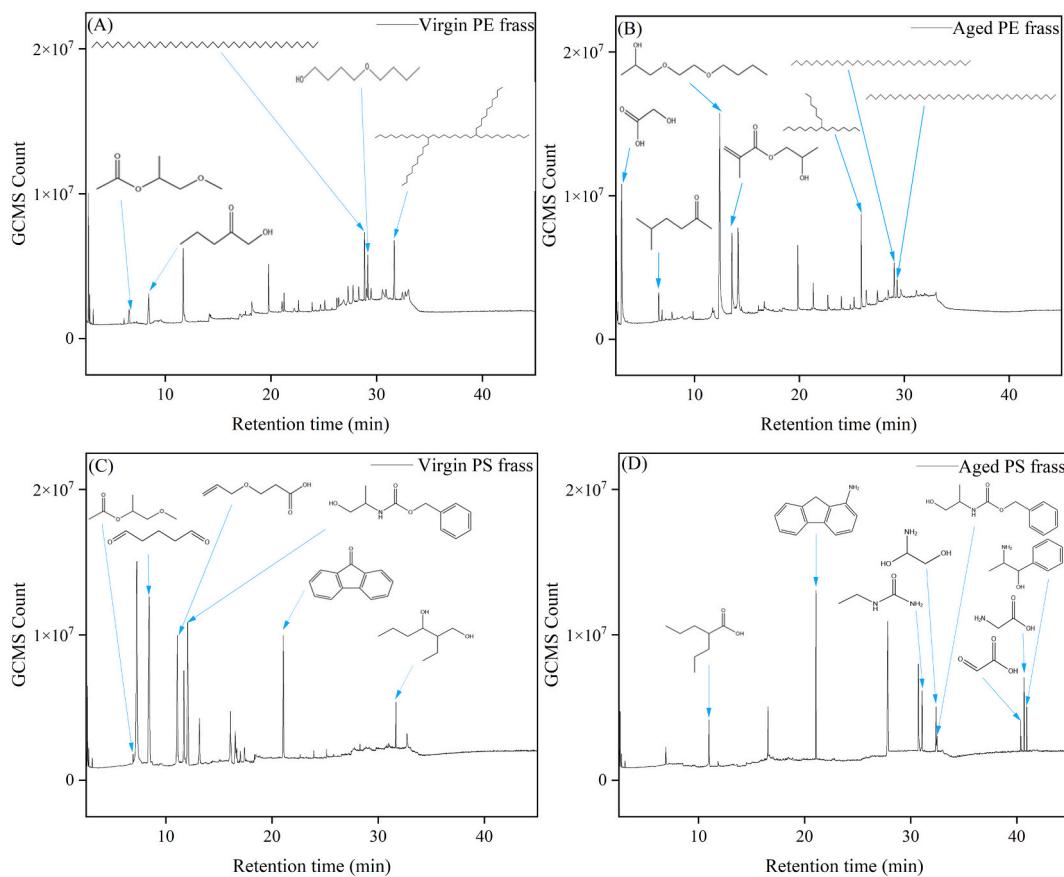


Fig. 6. GC-MS analysis of degradation products from the four frass samples of *T. molitor* larvae fed with (A) virgin PE, (B) aged PE, (C) virgin PS and (D) aged PS diets.

and depolymerization of PS (Wang et al., 2022b). Interestingly, the emergence of the peak at 1250 to 1500 cm⁻¹ was also related to the C—N group in the frass (excrement) of aged PS-fed larvae, which shows that the N element was directly bond to the benzene ring and caused the shift to the higher frequency area due to conjugation (Zhong et al., 2023; Wang et al., 2022b).

3.5. Biodegradation products of PE and PS microplastics by *T. molitor* larvae

The degradation products of MPs by *T. molitor* larvae were also analyzed by GC-MS (Fig. 6). The GC-MS analysis revealed that the formation of long-chain alkane such as hexatriacontane (C₃₆H₇₄) and tetracontane (C₄₀H₈₂) in the frass of aged PE-fed larvae were shorter than tetratetracontane (C₄₄H₉₀) in the frass of pristine PE-fed larvae. This was consistent with the results of the molecular weight change of PE after biodegradation. Moreover, there were more oxygen-containing compounds in the byproduct of the aged PE. These oxygen containing compounds included 2-hydroxyacetic acid (C₂H₄O₃), 5-methyl-2-hexanone (C₇H₁₄O), 1-(2-butoxyethoxy) propan-2-ol (C₉H₂₀O₃) and 2-hydroxypropyl methacrylate (C₇H₉O₃) than that in the virgin one (Table S1). It was attributed to the decrease of the molecular weight of aged PE MPs, as well as the oxidation reactions on the surface of polymers under UV radiation. Moreover, due to the surface oxidation under the effect of aging, the hydrophobicity of the aged PE reduced, and the microbial community and the enzymes secreted in the gut of larvae easily adhered to the polymer. This facilitated the decomposition of the aged MPs into smaller monomers by oxidation and depolymerization (Das and Tiwari, 2018; Bacha et al., 2023).

The GC-MS analysis revealed that 1,3-di-*tert*-butyl-2,5-

dimethylbenzene (C₁₆H₂₆) was found in the frass of PS-fed larvae, which is a normal antioxidant additive in the production of plastics (Tsochatzis et al., 2020). However, there were more types of amino compounds contained in the degradation products of the aged PS such as 9,10-dihydroacridine (C₁₃H₁₁N), 1-ethylurea (C₃H₈ON₂), 1-aminoethane-1,2-diol (C₂H₇O₂N) and 2-amino-1-phenyl-1-propanol (C₉H₁₃ON), which may be related to the higher activity of enzymes associated with stronger catalytic abilities of bioactive substances in the larval intestines (Tsochatzis et al., 2021). The results further demonstrated that the aging effect was more beneficial for the depolymerization of PS MPs and the generation of various degradation products, which is consistent with the authors' FTIR analysis. It was determined that the related enzyme activities secreted by larvae could affect the aged MPs, resulting in different degradation products with more effective ingestion of MPs by larvae.

3.6. Gut microbial community in *T. molitor* larvae supplemented with PE and PS microplastics diets

The microbial community distribution in the intestine of *T. molitor* larvae under different feeding conditions was analyzed using high-throughput sequencing. Intestinal microbes that are highly sensitive to exogenous substances were used as an indicator of the exogenous substance biodegradation (Liu et al., 2022b). The operational taxonomic units (OTUs) of MPs-fed larvae were relatively lower than those of larvae fed with bran (Table S2). The *alpha* diversity of each feeding group was analyzed by the Chao1 and Shannon index (Fig. 7). The species richness and community diversity of gut microbes in the MPs-fed groups was lower than the bran-fed group, which was indicated by the lower Chao1 and Shannon index (Lou et al., 2021). This analysis relates

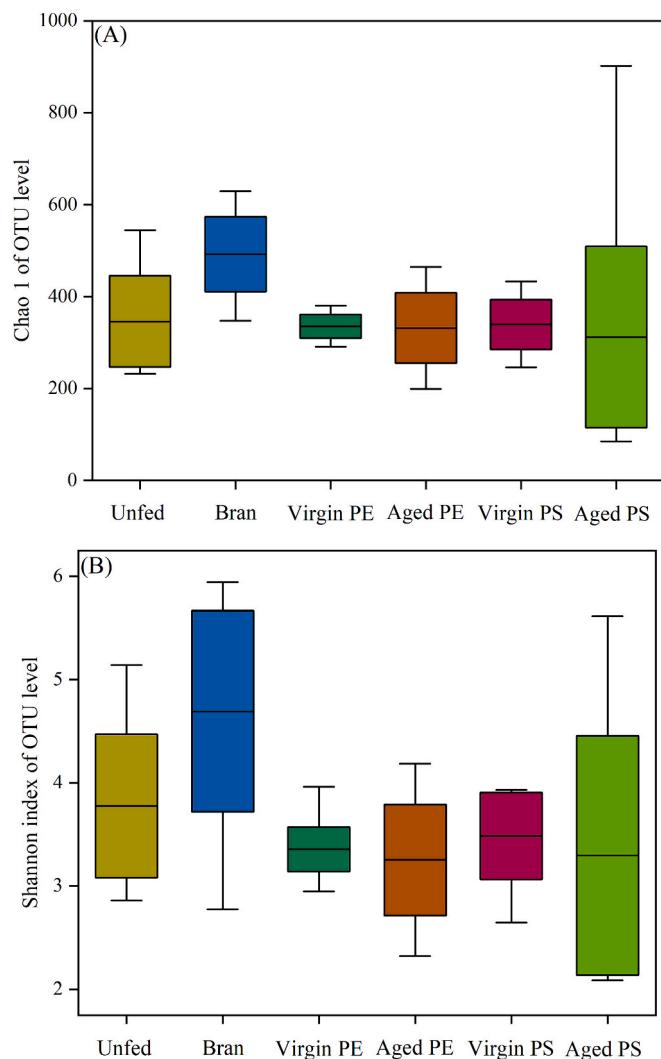


Fig. 7. The alpha diversity of the gut microbes in *Tenebrio molitor* larvae between six experimental diets. (A) Chao1 and (B) Shannon index of OTU level ($p < 0.05$, student's t-tests).

to the limited nutrients provided by the exogenous polymers of MPs (Peng et al., 2019). With the higher consumption of the aged MPs by larvae, the diversity of the gut microbial community was lower than that of pristine MPs.

The relative abundance in gut bacterial communities displayed remarkably distinct features for various feeding conditions after 35 days of cultivation (Fig. S2). The predominant components of microbiota at family level of the six feeding groups are: *Enterobacteriaceae*, *Enterococcaceae*, *Streptococcaceae* and *Lactobacillaceae* (Fig. 8(A)). The relative abundances of *Enterococcaceae* and *Lactobacillaceae* decreased responding to, which may be related to the additives and other components contained in polymers that are known to be harmful to the growth of these bacteria (Flury and Narayan, 2021). However, the relative abundance of *Enterobacteriaceae* in larval intestines increased significantly, which may be related to the utilization of the organic components of plastics as substrates for growth and reproduction, thereby promoting the degradation of MPs (Li et al., 2022b). Compared with the pristine MPs, the uptake of the aged MPs may promote the relative abundance of *Enterobacteriaceae* and *Streptococcaceae*. The family *Enterobacteriaceae* are gram positive bacteria and the family *Streptococcaceae* also include gram positive spherical bacteria growing in chains or pairs are the dominant gut microbes in the biodegradation of plastic (Sanchez-Hernandez, 2021). With the variation of

physicochemical properties under the effect of UV aging, the aged MPs had an inevitable effect on the growth and distribution of intestinal microorganisms (Chai et al., 2020). Fig. 8(B) shows the relationships between aging indexes and the microbial community by a redundancy analysis (RDA). Three aging indexes including the carbonyl index (CI), M_w (the averaged molecular weight of polymers), and the water contact angle were selected, which could be used to represent the aging degree, molecular weight and hydrophilicity of MPs, respectively. First, two axes could explain 55.74 % of the changes in community composition. According to the redundancy analysis, the three aging indexes were highly correlated to the microbial communities and exhibited significant differences in the microbial communities of the four MP feeding groups (Tang et al., 2020). The relative abundance of *Enterobacteriaceae* and *Streptococcaceae* had significant positive correlations with CI and M_w , but their gut microbes in the biodegradation of plastic negatively correlated with the water contact angle. As the degree of aging MPs heightened, the intake of MPs by *T. molitor* larvae increased, which led to changes in the larvae's growth and variations on the community diversity of intestinal health damaged by plastics. Meanwhile, more oxygen-containing groups on the surface of aged MPs are represented by an increased CI value that promotes the growth of *Enterobacteriaceae* and *Streptococcaceae*. In addition, oxygen free radicals can be formed by the reaction of oxygen binding to the polymers, which helps achieve degradation of MPs by the dominant microbiota (Yang et al., 2014). Moreover, the decrease in hydrophobicity of aged MPs represented by water contact angle can also promote the colonization and growth of the dominant microbiota in the degradation of the aged MPs (Kunlere et al., 2019; Maity et al., 2021).

4. Conclusion

This study has investigated the biodegradation of the aged MPs by *T. molitor* larvae. With the surface characteristics changes, the aged MPs were preferred over the pristine ones for larvae ingestion. However, the more consumption of plastics, the greater the adverse effects on the larvae growth. GPC and FTIR analysis confirmed the depolymerization and degradation of MPs by larvae; however, more oxygenated compounds were generated after the biodegradation of the aged MPs compared to the pristine ones. High-throughput sequencing revealed certain shifts in the predominant gut microbial community associated with different MP-fed groups. Redundancy analysis (RDA) was used to further identify the relationship of aging indexes with microbial communities. The results confirmed that the microbial communities were highly correlated to the aging MP indexes. The effect of MPs on the synergy of enzymes and gut microbial communities in the biodegradation process is an important study, that improves the in-depth understanding of the biodegradation mechanism.

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CRediT authorship contribution statement

Qiongjie Wang: Formal analysis, Conceptualization, Project administration, Writing – review & editing. **Huijuan Chen:** Writing – original draft, Methodology, Data curation. **Wanqing Gu:** Supervision, Investigation. **Shurui Wang:** Visualization, Software. **Yinghua Li:** Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

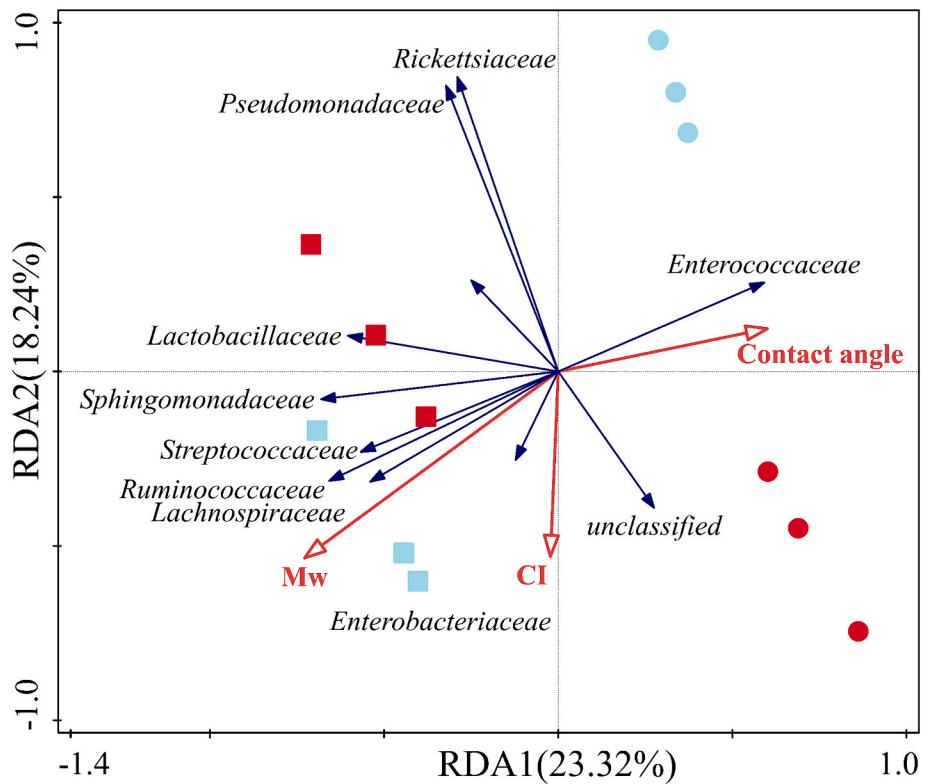
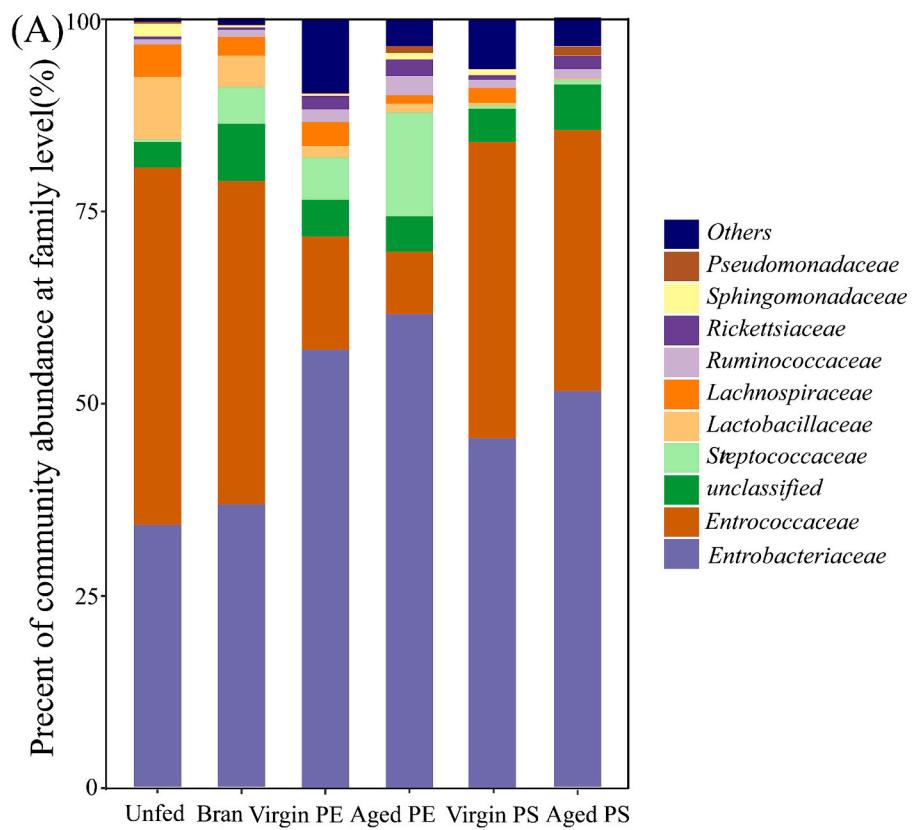


Fig. 8. (A) Relative abundances of gut microbial communities at family level between six experimental diets. The color intensity of the scale indicates the relative abundance. (B) Redundancy analysis (RDA) between the top 10 bacterial and aging indexes of microplastics. CI is abbreviation of carbonyl index.

the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.172243>.

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