Journal of Environmental Chemical Engineering 10 (2022) 108957

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Different performances in polyethylene or polystyrene plastics long-term feeding and biodegradation by Zophobas atratus and Tenebrio molitor larvae, and core gut bacterial- and fungal-microbiome responses

Jiaming Wang [a,1], Yumeng Wang [a,1], Xin Li [a], Yue Weng [a], Yijing Wang [a], Xiaoyu Han [a], Mu Peng [c], Aifen Zhou [b], Xin Zhao [a,*]

[a] Department of Environmental Engineering, School of Resources and Civil Engineering, Northeastern University, Shenyang 110819, China
[b] Institute for Environmental Genomics, Department of Microbiology & Plant Biology, University of Oklahoma, Norman 73019, USA
[c] College of Biological Science and Technology, Hubei Minzu University, Enshi 445000, China

ARTICLE INFO

Editor: Dr Y. Liu

Keywords:
Plastic biodegradation
Insect larvae
Gut bacterial-microbiome
Gut fungal-microbiome
Polyethylene
Polystyrene

ABSTRACT

The biodegradation of plastics by insect larvae is considered as an emerging strategy for the resistant plastic wastes disposal. To uncover the correlation of insect larval gut-microbiome with plastics biodegradation, two worm species including superworm *Zophobas atratus* and yellow mealworm *Tenebrio molitor* were fed with either polyethylene (PE) or polystyrene (PS) foam as sole diet for a longer period of 45 days, sole bran diet was used as control. Rapid declines of survival rates and plastic ingestions after 30 days were observed, suggesting that long-term sole plastic diet was detrimental to growth and life cycle completion in both larval species. Both PE and PS plastics were found more extensively depolymerized and biodegraded in gut of yellow mealworms than that of superworms by GPC analysis. The larval gut bacterial- and fungal-microbiomes at day 45 were assessed by Illumina MiSeq. The results showed that both gut bacterial and fungal communities shifted upon sole plastic diet ingestion compared to the control. Gut bacterial communities were similar in PS-fed groups but significantly different in PE-fed groups between both species. In contrast, gut fungal communities were clustered by species in general, although they were similar between PE- and PS-fed yellow mealworms but were more divergent between PS- and PE-fed superworms. Analysis of the changes of relative abundances of dominant bacterial and fungal genera demonstrated the strong association between the distinctive gut microbiome and plastic degradation. For instance, *Spiroplasma* and *Rhodotorula* for PE degradation, *Cryptococcus* for PS degradation in mealworms. *Issatchenkia* for both PS and PE degradation, *Pseudomonas* for PS degradation in superworms. In conclusion, changes of both gut bacterial- and fungal-microbiomes diversities were associated with plastic feedstock types and larval species, especially fungal microbiomes were strongly associated with larval species.

1. Introduction

Plastics, such as polyethylene (PE), polystyrene (PS), polyurethane (PU), and polypropylene (PP), are widely used in our daily life, including chemical instrument parts, transparent films, and pipes. In 2019, the global plastic production reached 368 million tons with an increase of nearly 10 million tons compared to the year 2018 [27]. The global plastic waste emissions increased exponentially, but only 10% of plastic waste was recycled [34]. Plastic wastes have become one of the top global environmental concerns due to the ever-increasing accumulation in environment as a result of plastics' mass production and recalcitrance to degradation [14]. The inherent characteristics of plastic polymers, including high molecular weight and high crystallinity, hinder their disintegration and lead to their accumulation in natural environment [1,31]. Accumulation of plastic waste not only affects environmental aesthetic quality but also poses substantial threats to the health and survival of wildlife and human beings [6,18]. Therefore, scientists have been advocating for limiting the use of plastic as well as exploring the ways to effectively degrade plastics.

The discoveries of ingestion and degradation of plastic by insect

* Correspondence to: School of Resources and Civil Engineering, Northeastern University, No. 11, Lane 3, Wenhua Road, P.O. Box 265, Shenyang 110819, China.
E-mail address: zhaoxin@mail.neu.edu.cn (X. Zhao).
[1] Jiaming Wang and Yumeng Wang contributed equally to this work.

https://doi.org/10.1016/j.jece.2022.108957
Received 13 July 2022; Received in revised form 12 October 2022; Accepted 10 November 2022
Available online 11 November 2022
2213-3437/© 2022 Elsevier Ltd. All rights reserved.

larvae provided a new research direction for plastic biodegradation. In current investigations, larvae of six insect species have been found capable of plastic ingestion and degradation, including superworm *Zophobas atratus* [19,26], yellow mealworm *Tenebrio molitor* [4,25,44], dark mealworm *Tenebrio obscurus* [25], greater waxworm *Galleria mellonella* [2], lesser waxworm *Achroia grisella* [16], and Indian-meal moths *Plodia interpunctella* [39]. A variety of analytical methods, such as gel permeation chromatography (GPC) [41,44], attenuated total reflection Fourier transform infrared (ATR-FTIR) [19,30] and thermogravimetric analysis (TGA) [19,25], have been employed to identify the changes of the molecular weights, functional groups, and the associated thermal stabilities of plastics. Results from these studies confirmed the degradation of plastic in the larval gut and revealed the changes of larval gut bacterial communities with sole plastic diets. For instance, *Citrobacter* was associated with PE and PP diets in superworms [19,40] and strongly associated with both PE and PS diets in mealworms [3]. *Sphingobacterium* and *Dysgonomonas* were associated with PS diet in superworms [19], while *Klebsiella* was in mealworms [4]. *Enterobacter* was associated with PP diet in superworms, while *Kluyvera* in mealworms [40]. *Mangrovibacter* was related to PU diet in superworms [19]. In contrast to the frequent reports on the larval gut bacteria association with plastic degradation, associations between fungi and plastics degradation in larval guts were rarely reported. There is only one report about the involvement of *Aspergillus*, *Hyphodermella*, and *Trichoderma* in PS plastic degradation in mealworm guts [7]. Therefore, it is necessary to explore changes of gut fungal communities to reveal their associations with plastic diets.

We hypothesize that the original gut microbial (including bacteria and fungi) communities are different in different worm species, but key OTUs strongly associated with plastic degradation depend on the type of plastics. To test this hypothesis, larvae of superworm *Z. atratus* and yellow mealworm *T. molitor* were fed with PS or PE foam plastics as sole diets with sole bran feeding as controls under the same conditions for 45 days. Analysis of frass samples by ATR-FTIR, TGA and GPC confirmed the degradation of plastics in worm guts. Amplicon sequencing results of 16S rRNA and ITS gene with Illumina MiSeq demonstrated the changes of gut bacterial and fungal microbiome diversities with sole plastic diet. The results indicated that the larval plastic ingestion preferences and efficiencies were strongly associated with larval species. Changes of microbial communities were more complex than we hypothesized. Bacterial community diversities decreased, but fungal community diversities increased. In terms of overall similarities of gut microbiomes, for bacterial community, PS-fed groups had the highest similarities, PE-fed group had the least similarities, and the control group had the intermediate similarities. In contrast, for fungal communities, control groups had the highest similarities, plastic-fed superworm groups had the least similarities, and plastic-fed mealworm groups had the intermediate similarities. The results suggest that plastic type is an important factor for bacterial community change while worm species is an important factor for fungal community changes under sole plastic diet.

2. Materials and methods

2.1. Larvae and feedstocks

Superworms and yellow mealworms larvae were purchased from Wuxi Insects Breeding Plant, Jiangsu, China. Superworm larvae were approximately 5.00 ± 0.25 cm in length and 613.36 ± 19.55 mg/larva in weight. Mealworm larvae were approximately 1.48 ± 0.12 cm in length and 58.28 ± 2.34 mg/larva in weight. Wheat bran was purchased from the specialty stores and used as the feedstock for control groups. Foam plastics of PE (low density polyethylene, LDPE, 0.010 g/cm^3) and PS (highly expanded polystyrene, 0.009 g/cm^3) were purchased from Yinlong Plastic Company (Zhejiang, China) and used as experimental feedstocks, no plastic was replaced during the experiment. The bran was natural without any additives. Larvae were fed with bran and starved for 72 h before initiation of the experimental diets.

2.2. Larval growth and plastics consumption

A total of six groups of larvae were included in the experiment, including three groups of bran-, PE-, or PS-fed superworms, and three groups of bran-, PE-, or PS-fed yellow mealworms, with three replicates in each group. Bran-fed groups were used as controls. For each group, randomly selected 20 superworms or 100 yellow mealworms were incubated in a container (15 ×10 ×7 cm) to maintain the convenient density for good larval growth [28]. All groups were kept under the controlled condition of 25 ± 1 °C, 60 ± 5% humidity, and dark environment [19,45] for 45 days. Before the experiment, the PE and PS foam plastics were cut into pieces with sizes of 8 × 5 × 2 cm, cleaned with distilled water, and dried at 30 °C for 2 days. At the beginning of the experiment, 5 g bran was added in the control groups, and additional 5 g bran was supplemented every 5 days. One piece of PE or PS foam plastic (PE = 800 ± 16 mg; PS = 720 ± 12 mg) was added in each plastic diet group as sole feedstock for 45 days. Dead larvae and molting exoskeleton were removed immediately via daily checking. Weights of plastic and larvae were measured every 5 days. Larval survival rates, accumulative net weight changes, and cumulative foam consumptions were calculated at 5-day intervals by comparing to the amounts or weights at the beginning of the experiment.

2.3. Collection and characterization of frass samples

Collection and analysis of the residual polymers in the larval frass were performed as described previously [19]. Briefly, the frass samples were collected at a 5-day interval. Larvae were transferred to new containers before collecting the frass to avoid carryover of the un-ingested plastic in frass. The frass was collected pellet by pellet using forceps and stored in aseptic tubes at − 20 °C till further analysis.

Major functional groups in frass were characterized by an ATR-FTIR (vertex70, Brock instruments, Germany) with wavenumbers ranging from 4000 to 400 cm^{-1} and compared to the original PE or PS feedstocks [30]. To further demonstrate the degradation of plastic in larval frass, frass pellets were dissolved in water, vortexed and centrifuged at 6000 rpm. After centrifugation, the floating plastic on the water surface and the pellets at the bottom of tubes were collected separately and stored at − 20 °C. The residual plastics in the frass was observed by optical microscope (BX53, U-HGLGPS, Olympus, Japan) and scanning electron microscope (SEM, Apreo 2 C, Thermo Scientific, Inc., USA), and then analyzed respectively by the ATR-FTIR and GPC. The frozen samples were thawed and air-dried in a 40 °C incubator for 5 h before the ATR-FTIR analysis. Peak values were analyzed with the OMNIC software and confirmed based on the best expert judgment by the presence of specific absorption bands in accordance with literatures [15,19], the process for SEM observation was described in Method S1 in Supporting Information.

Thermal gravimetric analysis (SDT Q600, TA Instruments, USA) was conducted to characterize the thermal changes of plastic polymers in frass pellets of plastic-fed superworms or yellow mealworms. The sample was heated from 40°C to 800°C at a rate of 20 °C/min under a high-purity argon ambience [19,26].

The gel permeation chromatography (GPC, PL-GPC50, Agilent Technologies, Inc., USA) analysis was conducted to characterize the depolymerization and biodegradation of residual polymers in the plastics-fed larval frass. The GPC procedures were performed as described in literatures [36,43] and provided in Supporting Information (Method S2, Result and discussion S1).

2.4. Gut microbiome analysis

At the end of the 45-day experiment, 6 superworms or 30 yellow mealworms were randomly picked from each group for the gut

microbiome analysis. Larval guts from each group were pooled into 1 mL sterile normal saline in a 2 mL sterile centrifuge tube [39]. After a 5 min vortex, the samples were centrifuged at 10,000 rpm for 2 min, and the pellets including both the gut tissue and microbiomes were stored at −20 °C before analysis. Genomic DNA was extracted with an E.Z.N.ATM Mag-Bind Soil DNA Kit (Omega Bio-Tek, China) and used as the template (20 ng) for PCR amplification of the V3-V4 region of the 16S rRNA gene and ITS gene, respectively. The universal primer pair of 341F (5′-CCT ACG GGN GGC WGC AG) and 805R (5′-GAC TAC HVG GGT ATC TAA TCC) was used for bacterial 16S rRNA gene PCR amplification ([18,19]; Wang et al., 2022). The universal primer pair of ITS1F (5′-CTT GGT CAT TTA GAG GAA GTA A) and ITS2 (5′-GCT GCG TTC TTC ATC GAT GC) was used for fungal ITS gene amplification [7]. Purified amplicons were sequenced on an Illumina MiSeq platform by Sangon Biotech (Shanghai, China). The sequencing reads were partitioned to sequences represented by OTU using USEARCH [10]. UCHIME [11] and RDP database were used for chimera screening [5]. The UPARSE [12] global alignment algorithm was used to classify high-quality sequences into the corresponding OTU based on 97% similarity threshold.

2.5. Statistical analysis

Statistical analyses of weight changes, plastic consumption rates, etc. were performed in Prism (version 8) [3]. The pairwise comparisons were performed with the Student's t-test [40]. All *p*-values were adjusted *p*-values and all error values were average ± standard deviation. The *p*-value < 0.05 was considered as statistically significant.

3. Results and discussions

3.1. Effects of sole plastic diets on larval growth and plastics consumption

The survival rates of each group during the 45-day experiment were calculated and shown in Fig. 1a. At the end of the experiment, for superworm groups, the bran-fed control group had a survival rate of 100%, the PS-fed groups had intermediate survival rate of 78.33 ± 5.67%, and the PE-fed groups had the lowest survival rate of 68.33 ± 2.88%. Similar trends but generally lower survival rates were observed for mealworm groups, the survival rates for the bran-fed control groups, the PS-fed groups, and the PE-fed groups were 90.67 ± 3.21%, 67.97 ± 2.92%, and 60.66 ± 7.08%, respectively. Comparing to previous studies with 35 days of plastic feeding, the survival rates of both larvae species in this study were lower ([26]; Luo et al., 2020; [18]), indicating that long-term plastic diet negatively affected growth and survival of both superworms and yellow mealworms. Similar trends of survival rates in both types of worms, namely bran-fed > PS-fed > PE-fed, demonstrated the adverse effects of sole plastics diets on the survival of both larvae species. Superworms had higher survival rates than yellow mealworms, possibly due to their higher vitality and resistance to hunger and thirst [26].

In terms of pupation, there were no significant differences between different diets in superworms, but significant differences in yellow mealworms. For superworms, totally two pupae were observed in PS-fed group on the 30th and the 40th day, respectively, one pupa was found in PE-fed group on the 30th day, but no pupa was found in the bran-fed control groups. For yellow mealworms, 50 larvae in bran-fed group pupated, but none in plastic-fed groups. The few pupations in superworms were probably due to their longer life time of 260–300 days in comparison to yellow mealworms of 90–100 days [20,33]. Therefore, we could not conclude that the pupation of superworms was negatively affected by plastic diets. In contrast, the significantly decreased pupation rates in plastic-fed mealworm groups indicated the adverse effects of sole plastic diets on growth and development.

The average accumulative net weight changes in each group were shown in Fig. 1b. In bran-fed groups, the average net weight changes of superworms and yellow mealworms continuously increased by 138.10 ± 16.85 and 108.95 ± 6.86 mg/larva during the 45-day experiment, respectively. The accumulative net weight changes in plastic-fed superworm groups continuously decreased after the initial increases in the first 5 days, suggesting that sole plastic diet could not provide sufficient nutrition to support their growth and development. In contrast, comparing to the bran-fed control group, the accumulative net weight changes of plastic-fed yellow mealworms showed slight increases during the experiment with similar net weight gains of 35.90 ± 0.15 and 35.38 ± 1.04 mg/larva in PS-fed and PE-fed mealworm groups, respectively. Overall, significant weight loss was observed in plastic-fed superworms, while yellow mealworms had a slight increase in body weight change, similar to the results in previous studies (Luo et al., 2020; [37]).

Plastic consumption rates over the 45-day-experiment were shown in Fig. 2. When calculated as mg plastic/larva, the cumulative foam plastics consumption rates were 26.98 ± 1.43 mg PS/larva and 13.56 ± 2.66 mg PE/larva by superworm, 2.51 ± 0.15 mg PS/larva and 2.10 ± 0.53 mg PE/larva by yellow mealworm. Plastic consumption rates by superworms were approximately 11 folds ($p < 0.01$) for PS and 6 folds ($p < 0.05$) for PE comparing to yellow mealworms, respectively, indicating that superworms had much higher plastic consumption rates

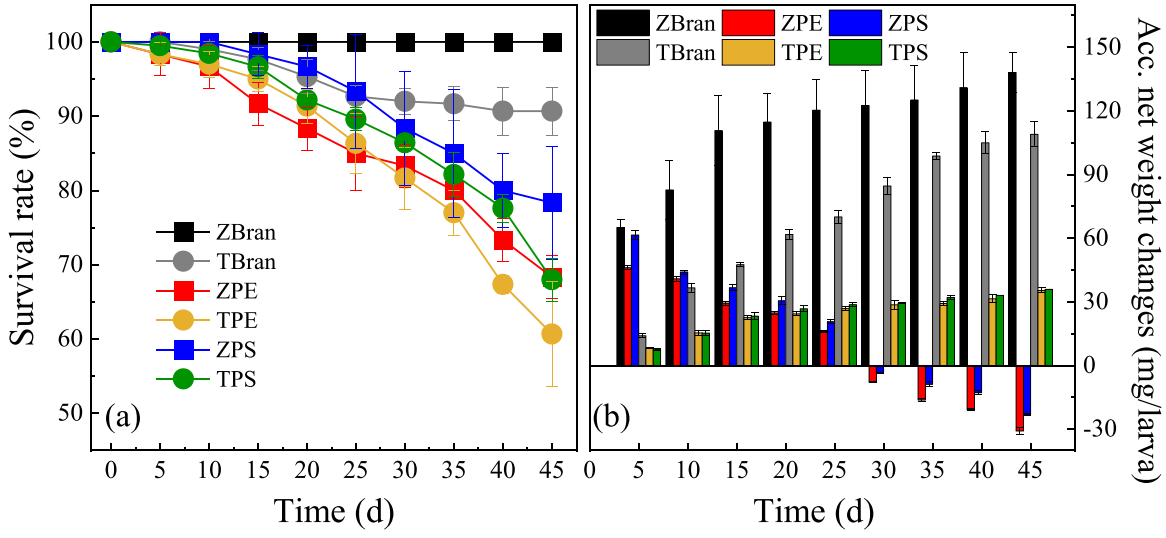

Fig. 1. Survival rates (a) and average accumulative (Acc.) net weight changes (b) of superworm *Z. atratus* and yellow mealworm *T. molitor* larvae with different diets. Z = *Z. atratus*; T = *T. molitor*; PE = polyethylene foam; PS = polystyrene foam.

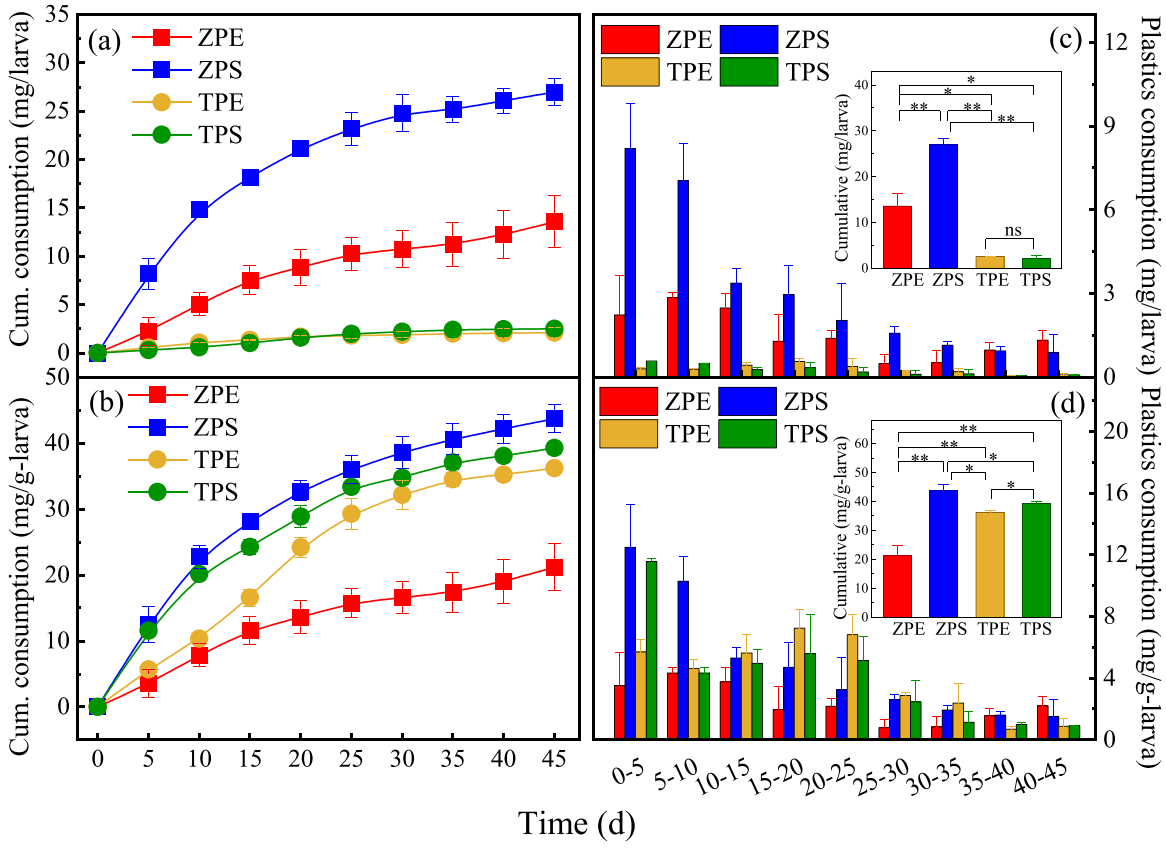

Fig. 2. Cumulative (Cum.) plastics consumption by larvae of superworm *Z. atratus* (Z) and yellow mealworm *T. molitor* (T) calculated by per larva (a) or unit larvae biomass (b); Plastic consumption by larvae of two species by per larva (c) or per unit larvae biomass (d) at 5-day intervals. The inset figure shows cumulative plastic consumption by larvae of two species by per larva (c) or per unit larvae biomass (d). Student's t-tests were used for significance tests of the differences between groups. * , $p < 0.05$; **, $p < 0.01$; ns, not significant. Z = *Z. atratus*; T = *T. molitor*; PE = polyethylene foam; PS = polystyrene foam.

(Fig. 2a). To eliminate the effect of body size difference between two species, foam consumption efficiencies were also calculated as total plastic mass loss per initial larvae average weight. PS foam consumption rates were 43.70 ± 2.13 mg/g superworms and 39.08 ± 0.99 mg/g yellow mealworms, respectively (Fig. 2b). PE foam consumption rates were 21.20 ± 3.58 mg/g superworms and 36.20 ± 0.51 mg/g yellow mealworms, respectively (Fig. 2b). There was significant difference between the consumption rates of PE and PS by superworms ($p < 0.01$), while no significant difference between the consumption rates of PE and PS by yellow mealworms ($p < 0.05$). The difference of consumption rates suggested that the preference and consumption efficiency of plastics by both species were different. Unlike the 30- or 35-day-tests in literatures [18,19,26], the feeding time was extended to 45 days in this study. Although the plastic consumption efficiencies fluctuated slightly during the experiment, there was an overall downward trend (Fig. 2c & 2d), especially in the last 10 days (35 ~ 45 d), which was consistent with the rapidly decreased survival rates in the last 10 d. Therefore, the results indicated that foam plastic cannot be used as sole and long-term feedstocks for both larvae species due to the adverse effects on larval growth, pupation, and completion of life time. Amendment of certain amounts of nutrients such as bran could ameliorate the situation and promote sustained plastic consumption by these worms.

3.2. Degradation and oxidation of plastics

Evidence for oxidation and de-polymerization of PS and PE by both larvae species were obtained and confirmed with ATR-FTIR analysis (Fig. 3, Tables S1 and S2). The ATR-FTIR spectra of the original plastic feedstocks and frass samples of plastics-fed larvae were shown in Fig. 3a and b. For PE-fed groups (Fig. 3a), the appearance of peaks at 1666 cm^{-1} (C═O stretch) and the significant decrease of peak intensity at 2910 cm^{-1} ($-CH_2$ stretch) were observed, suggesting the occurrence of certain level of oxidation and de-polymerization of PE in larval guts of both species. For PS-fed groups (Fig. 3b), the appearance of peaks at 1635 and 1640 cm^{-1} (C═O stretch) and the disappearance of characteristic peaks of PS benzene ring skeleton vibrate (703 and 744 cm^{-1}) were observed, indicating the oxidation and depolymerization and the cleavage of benzene ring of PS occurred in larval guts of both species. Similar to previous reports [3,26], the oxidation of PS and PE occurred in the range of 1700–1100 cm^{-1}, that new functional groups of C═O stretch (1645–1675 cm^{-1}) and C–O stretch (1075–1185 cm^{-1}) were detected in the frass from PS- and PE-fed groups. The addition of oxygen functional groups into the polymer chain was considered as the preliminary and key step in plastic degradation [13,32]. The broad peaks at about 3370 cm^{-1} were assigned to hydroxyl or carboxylic acid groups, suggesting a change of hydrophobic to more hydrophilic surface [25]. The ATR-FTIR results indicated the occurrence of oxidation and degradation of PS and PE plastic after passing through larval guts of both species.

To avoid the interference of larval fat or protein in the frass, the floating portion of frass samples containing partially degraded plastic in the upper layer after centrifugation was observed and analyzed by optical microscope, SEM, ATR-FTIR and GPC, respectively. As shown in Fig. S1, the frass pellets of superworms were larger than that of yellow mealworms, which possibly due to the larger body size and larger mouthparts of superworms than yellow mealworms, and also resulting in larger debris in the frass (shown in Fig. S2). In addition, the frass pellets of PS-fed both larvae species were fluffier than that of PE-fed, which was suggested due to the different polymeric structure of two plastic types, as PE foam was a laminated structure and easier be

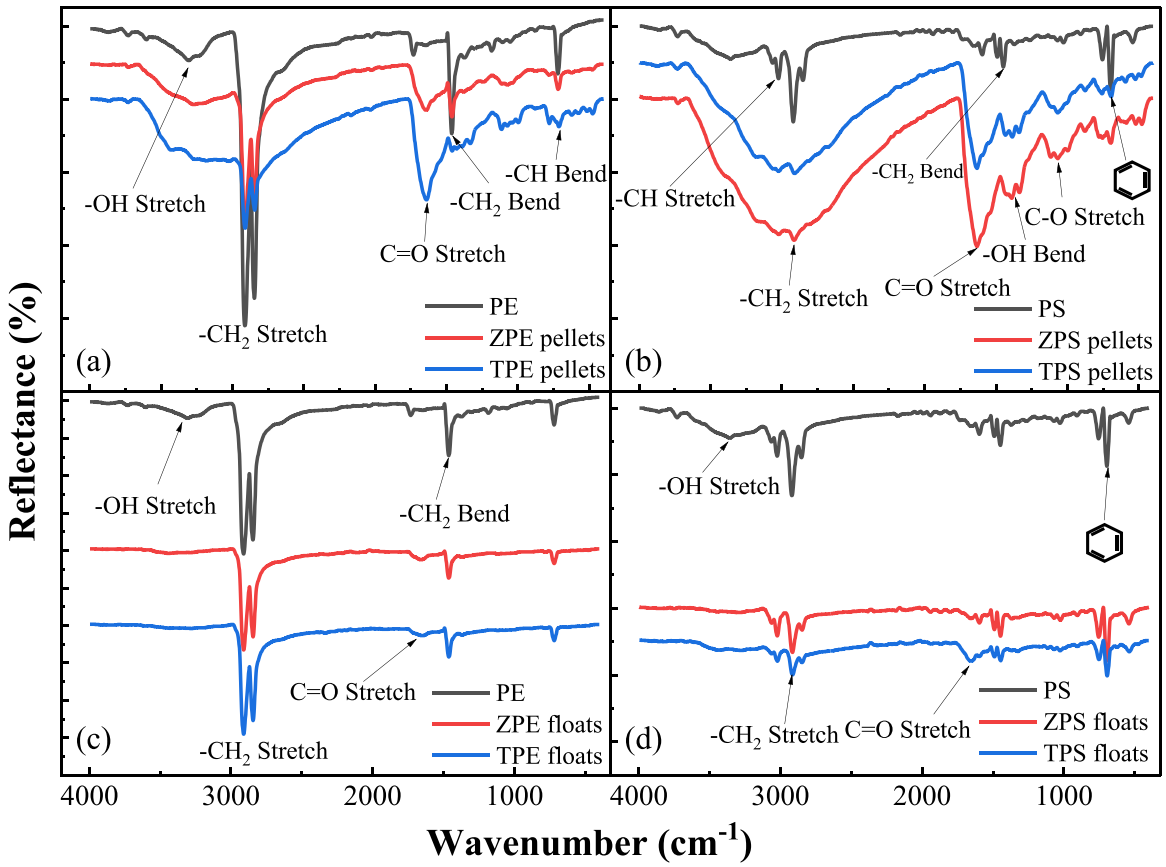

Fig. 3. ATR-FTIR spectra of the original plastics feedstocks and the frass samples of plastic-fed superworms (*Z. atratus*) and yellow mealworms (*T. molitor*) larvae. (a) PE feedstock and pellet frass of PE-fed larvae; (b) PS feedstock and pellet frass samples of PS-fed larvae; (c) PE feedstock and floating plastics in frass samples of PE-fed larvae; (d) PS feedstock and floating plastics in frass samples of PS-fed larvae. Z = *Z. atratus*; T = *T. molitor*; PE = polyethylene foam; PS = polystyrene foam.

compacted. The SEM was used to further compare the morphological differences among original foam plastics, frass of plastics-fed larvae and floating plastics from frass (Fig. S3). Comparing to PE-fed frass and floating PE from frass, PS had a larger bulkiness and showed a clear mass structure with many holes, whereas PE showed a torn sheet shape. Compared to original foam plastics, pits and cracks were observed in the floating plastics and the pellets, demonstrating the degradation of PE and PS plastics did occur. For floating plastic groups (Fig. 3c and d), the characteristic peaks of PE ($-CH_2$ stretch) at 2910 cm^{-1} and PS (benzene ring skeleton vibrate) at 703 and 744 cm^{-1} were slightly weaker than the original foam plastic, indicating partial depolymerization and oxidation of floating plastic in guts of both superworms and yellow mealworms. For bottom sedimentation groups (Fig. S4), partial degradation substances or degradation intermediates were probably present, but it was difficult to conduct in-depth analysis to determine the origin of various peaks due to the complexity of precipitates. Overall, the ATR-FITR analysis of frass, floating plastic and bottom sedimentation demonstrated that oxidation and biodegradation of PE and PS foam plastic did occur.

Thermal stabilities of foam plastic and frass samples were examined using DSC-TGA (Fig. 4a and b). The weight loss occurred and accelerated at temperature range of 400–500 °C. The reduction in the rate of weight loss indicated the emergence of new substances in the frass, such as inorganic substances, partially degraded plastics, and other biodegradation byproducts [26]. The decomposed parts at temperature ranges of 100–370 °C might be contributed by other biological digestive products [40], including cannibalism and plastic biodegradation residues from the guts. For PE-fed superworm frass, the weight loss of about 88.50% occurred and accelerated at temperature range of 400–503 °C. Only two DSC curves in PE-fed frass sample were observed at 122.24 °C and 483.21 °C, which were similar to the endothermic peaks of the original PE plastic, indicating that PE was relatively thermally stable, and the degree of PE degradation by superworms was relatively lower. For PE-fed yellow mealworm frass, the weight loss of about 57.07% was much lower than the control PE foam plastic of 99.02%, indicating that the frass contained not only residual PE polymer but also new biodegraded products [42]. The DSC-TGA results confirmed that PE had undergone more obvious modification and degradation in yellow mealworm guts. For PS-fed groups, the weight losses of about 87.65% in superworm frass and 72.78% in yellow mealworm frass were much lower than that of PS foam plastic (98.15%), and the DSC curves of both larvae species were similar. Overall, the DSC-TGA results suggested that the plastic components were degraded after passaging through the larval gut.

The molecular weights of the ingested plastic polymers decreased significantly based on GPC analysis of the original foams and frass of plastics-fed larvae (Fig. 4c and d). For PE-fed groups, the number average weights (M_n) decreased by 26.18% and 30.67%, respectively (original 35.2 ± 1.8 vs. superworms frass 26.0 ± 2.6, yellow mealworms frass 24.4 ± 3.7 kDa), the weight average weights (M_w) decreased by 20.40% and 50.01%, respectively (original 103.2 ± 2.5 vs. superworms frass 82.2 ± 2.6, yellow mealworms frass 51.6 ± 2.7 kDa). For PS-fed groups, M_n decreased by 32.80% and 36.19%, respectively (original 125.2 ± 1.5 vs. superworms frass 84.1 ± 2.0, yellow mealworms frass 79.9 ± 2.8 kDa), and M_w decreased by 23.19% and 27.52%, respectively (original 234.9 ± 2.5 vs. superworms frass 180.4 ± 2.0, yellow mealworms frass 170.3 ± 1.9 kDa). The results confirmed that PE and PS foams were biodegraded via broad depolymerization in superworms and yellow mealworms as previous reported (Yang et al., 2018; [3,18]; Wu & Criddle 2021). The GPC analysis of the floating

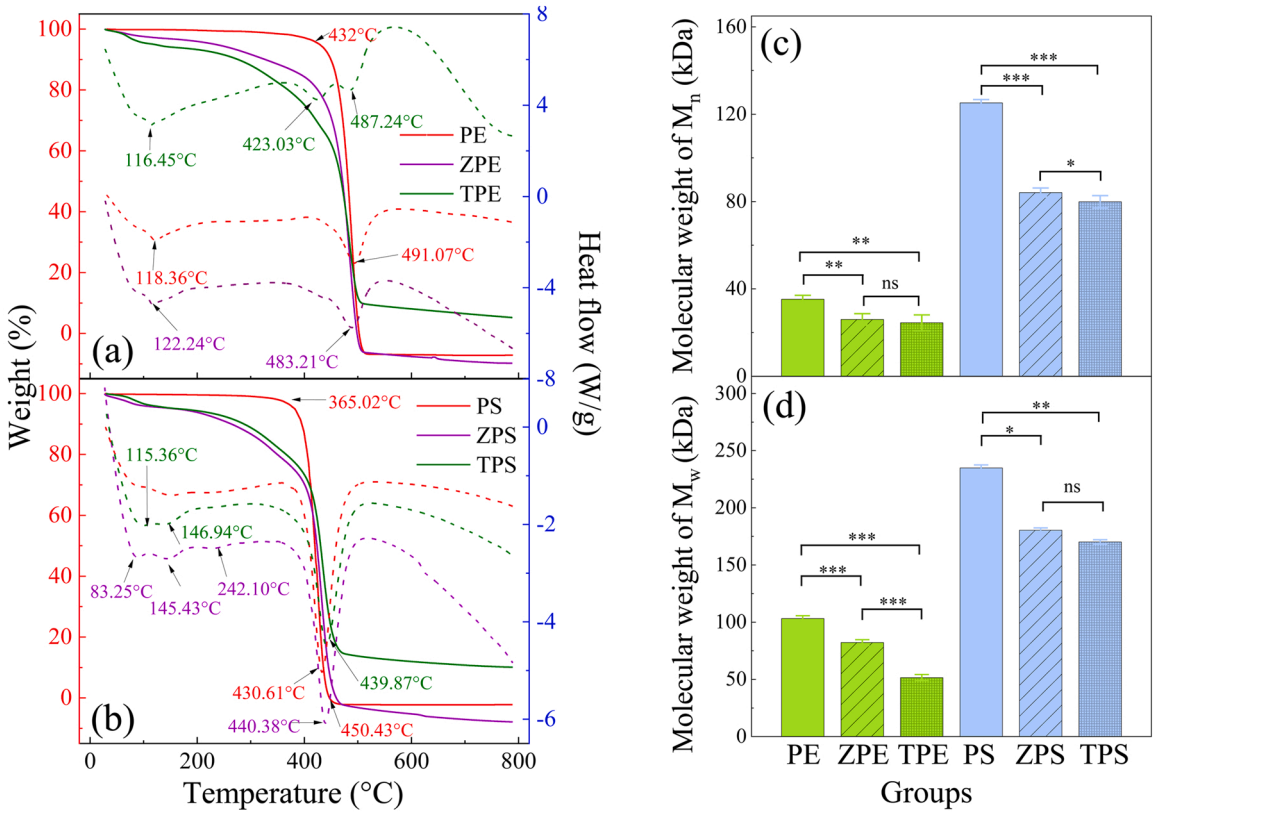

Fig. 4. TGA and GPC analysis of plastic feedstocks and frass of plastics-fed larvae of superworm (*Z. atratus*) and yellow mealworm (*T. molitor*). (a) TGA analysis for PE feedstock and frass of PE-fed larvae; (b) TGA analysis for PS feedstock and frass of PS-fed larvae; (c) GPC analysis of number average weights (M_n) for plastic feedstocks and plastics-fed larval frass; (d) GPC analysis of weight average weights (M_w) for plastic feedstocks and plastics-fed larval frass; Weight curves and heat flow curves were shown in solid and dash lines, respectively. Z = *Z. atratus*; T = *T. molitor*; PE = polyethylene foam; PS = polystyrene foam.

plastics from the frass samples showed similar results (Results and Discussions S1 in the Supplementary). Interestingly, significant decreases of M_n and M_w were more severe in yellow mealworms than that in superworms, suggesting that the plastic depolymerization and biodegradation was more significant in gut of yellow mealworms, which was in contrast to the higher cumulative plastics consumption in superworms than yellow mealworms.

3.3. Response of gut-bacterial microbiome

Illumina Miseq of the 16S rRNA gene was used to investigate the effect of plastic diets on the bacterial community. The Illumina MiSeq sequences of gut bacterial microbiome were deposited in the GenBank (accession numbers: SRR21384725, SRR21384726, SRR21384727, SRR21384728, SRR21517859 and SRR21517861). A total of 368,287 sequences were obtained with an average of 61381, and the sampling coverages were above 99%, suggesting that the sequencing result was capable of detecting most of the reads (Table S3). The gut-bacterial community analysis results demonstrate the significant changes of bacteria communities in guts of both species with sole PE or PS diets compared to the control bran diet. Gut bacterial species richness and diversity estimators including OTUs, Chao, Shannon, Simpson, ACE and Coverage were summarized in Table S4. The OTU numbers of bran-fed control groups were similar with 186 in superworms and 180 in yellow mealworms, decreased significantly in plastic-fed groups with 56 in PE-fed superworms and 112 in PS-fed superworms, 88 in PE-fed yellow mealworms and 172 in PS-fed yellow mealworms. The results indicated that feeding plastic significantly reduced gut bacterial community diversity (Fig. S5a). Shannon and Simpson indices also indicated that bacterial richness significantly decreased in both superworms and yellow mealworms with sole plastic diet, especially PE-fed group (Table S4). The greater changes of OTUs in plastic-fed superworms indicated that the gut-bacteria in superworms were more sensitive to the plastic sole diet than that of yellow mealworms. The OTU numbers of plastics-fed groups significantly decreased, similar to the change of gut bacterial community diversity in the PE degradation [17]. As feedstocks of complex polymers like PS and PE are hard to be utilized for most of bacteria, bacteria species lack of the plastic degradation pathway genes might not survive, leading to the lower bacterial OTUs number in the plastics-fed larval gut.

The NMDS based on Unweighted Unifrac and heatmap based on Bray-Curtis distances revealed clusters associated with the different larval species and diets (Fig. 5a and b). The gut bacterial communities were similar in the control bran-fed groups and PS-fed groups in both species, which was consistent with the more efficient ingestion of PS foam by both larvae species. In contrast, the gut bacterial communities of PE-fed groups were significantly different between two larvae species, which was consistent with the different PE consumption preferences (Fig. 2a) and might contribute to the different appearance of the guts (Fig. S6). In conclusion, changes of larval gut bacterial community were shaped by not only the type of feedstocks, but also larval species.

At phylum level, an analysis of the microbial community compositions revealed that Proteobacteria, Tenericutes and Firmicutes were the dominant phyla (Fig. 5c). Abundances of Proteobacteria were significantly higher in PS-fed superworms and yellow mealworms as well as PE-fed superworms, compared to bran-fed controls. Proteobacteria was also the predominant phyla associated with PS feeding in lesser mealworms *Alphitobius diaperinus* and yellow mealworms *T. molitor* larvae [7, 28] and PE feeding in greater waxworms *G. mellonella* [17]. Firmicutes was the dominant phyla in PE-fed superworms, Tenericutes was the dominated phyla in PE-fed yellow mealworms.

Changes of the relative abundances of the top 15 abundant genera

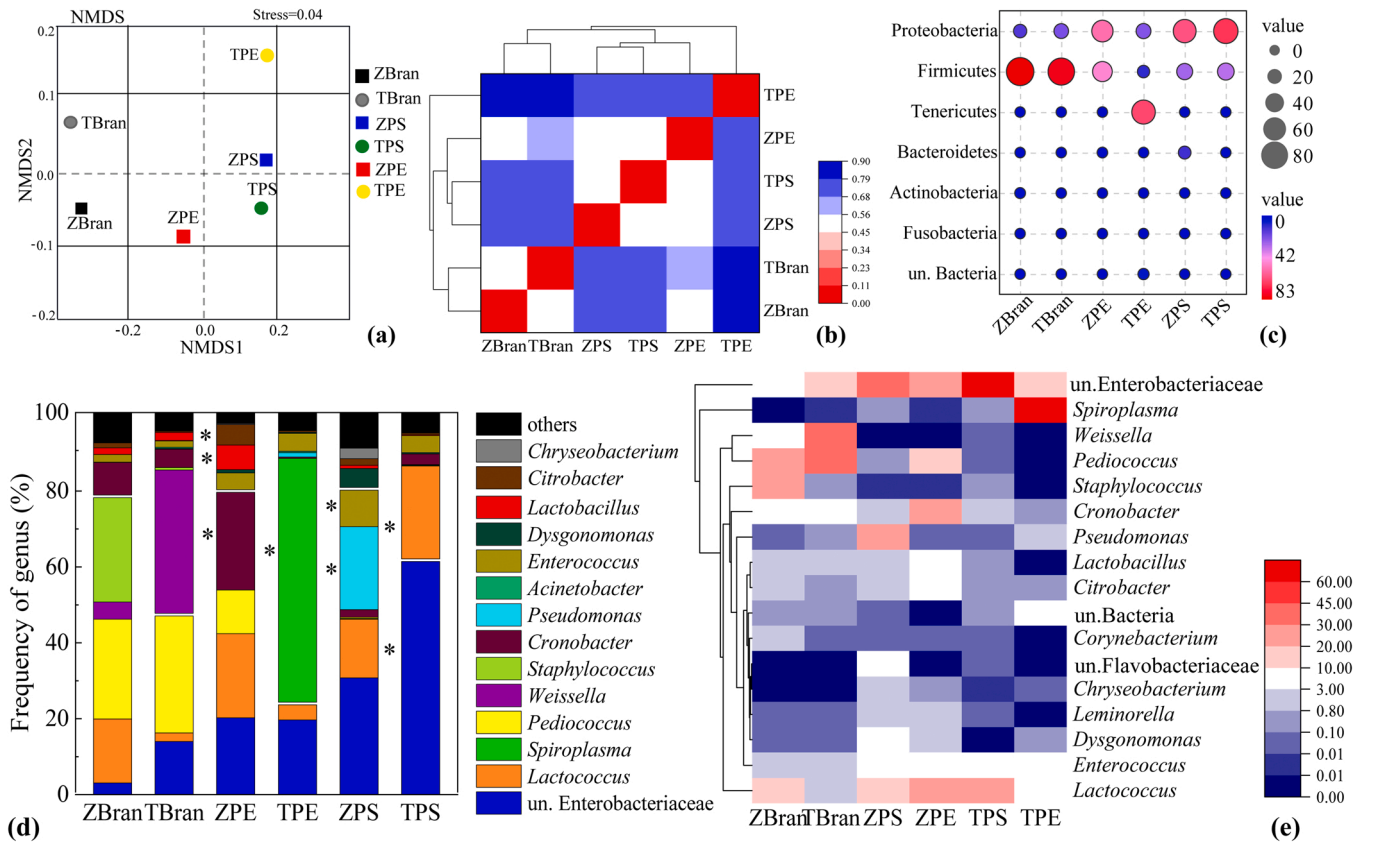

Fig. 5. Analysis of gut bacterial diversities in superworm *Z. atratus* and yellow mealworm *T. molitor* larvae with different diets. (a) Nonmetric multidimensional scaling based on Unweighted Unifrac; (b) Gut bacterial community diversity distance analysis based on Bray-Curtis distance; (c) Relative bacterial abundances at phylum level; (d) Relative bacterial abundances at genus level; (e) Heatmap and hierarchical cluster analysis of predominant genera (Relative abundance cutoff is 1.0%). Z = *Z. atratus*; T = *T. molitor*; PE = polyethylene foam; PS = polystyrene foam.

with 1% cutoff in both species were analyzed and shown in Fig. 5d and e, respectively. In PE-fed superworms, community shift was mainly associated with increased relative abundances of *Cronobacter*, *Lactococcus*, unclassified Enterobacteriaceae, *Lactobacillus* and *Citrobacter* accounting for 25.67%, 22.22%, 20.22%, 6.51% and 5.45%, all increased compared to the relative abundances of 16.84%, 1.32%, 1.76%, 1.77% and 1.32% in bran-fed group, respectively. *Cronobacter* and *Lactococcus* were common insect gut-associated bacteria, and *Lactococcus* and *Lactobacillus* were lactic acid genus, which might contribute to adjusting and maintaining the health of the gut microbiome environment [3,18]. Unclassified Enterobacteriaceae was also found strongly associated with degradation of LDPE, PS and PU in superworms *Z. atratus* ([19]; Wang et al., 2022). The PE-degrading strain *Enterobacter absuriae* YT1, isolated from the PE-fed waxworms gut, belongs to family Enterobacteriaceae [39]. Significantly increased abundances of *Lactococcus*, *Lactobacillus* and *Citrobacter* were probably related to the appearance of bubbles in the gut of PE-fed superworms (Fig. S6), as they could generate biogas during the fermentation process [9,22,23]. The strong association between *Citrobacter* and degradation of PE by superworms *Z. atratus* and yellow mealworms *T. molitor* was also reported previously [3,19]. In PE-fed yellow mealworms, *Pediococcus* and *Weissella* disappeared although they were dominant in the bran-fed control group, and *Weissella* was reported as gut associated bacteria in mealworms [38]. Interestingly, *Spiroplasma* was the dominant in PE-fed mealworm gut bacterial community with a relative abundance of 64.24%, which was significantly higher than that in bran-fed (<0.01%) or PS-fed (0.18%) groups, indicating that *Spiroplasma* was strongly associated with PE diet in yellow mealworms. Previous studies also reported that *Spiroplasma* was a common insect gut-associated genus and known member of the yellow mealworms *T. molitor* gut microbiome [3,18].

In PS-fed superworms, the dominant genera were unclassified Enterobacteriaceae, *Pseudomonas*, and *Lactococcus*, with relative abundances of 30.75%, 21.82% and 15.54%, respectively. Associations of *Pseudomonas* with PS degradation in superworms [29], yellow mealworms [35] and greater wax moth [17] have been reported. In addition, relative abundances of *Enterococcus* and *Dysgonomonas* increased dramatically by 7.67% and 4.95%, respectively, comparing to bran-fed group. *Enterococcus* was reported to be associated with PE diet in the gut of both yellow and black mealworms [18,40], and *Dysgonomonas* was reported in the gut of PS-fed superworms [19], indicating that these genera might play significant roles in PS degradation in the superworm gut. In PS-fed yellow mealworms, comparing to the control, relative abundances of unclassified Enterobacteriaceae and *Lactococcus* increased to 61.40% and 24.53%, respectively, but *Pediococcus* and *Weissella* disappeared, which be probably related to the substances contained in the bran. The results indicated that *Pseudomonas*, unclassified Enterobacteriaceae, *Enterococcus* and *Dysgonomonas* were associated with PS diet in superworms, while unclassified Enterobacteriaceae and *Lactococcus* were associated with PS diet in yellow mealworms.

3.4. Response of gut-fungal microbiome

It has been suggested that the joint metabolic activities of both fungal and bacterial communities play key roles in PS biodegradation within insect larval gut [7,21]. In this study, Illumina MiSeq of the ITS gene was used to investigate the effect of plastic diets on fungal communities. The Illumina MiSeq sequences of gut fungi were deposited in the GenBank (accession numbers: SRR21384721, SRR21384722, SRR21384723, SRR21384724, SRR21517858 and SRR21517860). A total of 327,584 sequences were obtained with an average of 54597, and the sampling

coverages were above 99%, suggesting that the sequencing result was capable of detecting most of the reads (Table S3). Here we found that fungal community diversities of both species also shifted significantly with sole PE or PS diets compared to the control bran diet. Larval gut-fungi species richness and diversity estimators of OTUs, Chao, Shannon, Simpson, ACE and Coverage were summarized in Table S5. The OTU numbers of bran-fed control groups were similar with 18 in superworms and 23 in yellow mealworms, increased significantly in plastic-fed groups with 94 in PE-fed superworms and 73 in PS-fed superworms, 53 in PE-fed yellow mealworms and 54 in PS-fed yellow mealworms. The results indicated that feeding plastic significantly increased gut fungal community diversity, especially in plastic-fed superworm groups (Fig. S5b), which was in contrast to gut-bacteria. Shannon and Chao indices also indicated that fungal richness slightly increased in superworms and yellow mealworms with PS and PE sole diets, opposite to the trends in changes of gut bacterial communities.

The NMDS based on Unweighted Unifrac and heatmap based on Bray-Curtis distances (Fig. 6a and b) were performed to further compare differences in gut fungal microbiomes of two larvae species. The gut fungal community diversities in both superworms and yellow mealworms were strongly related to worm species (Fig. 6a). Both larvae species in control groups were obtained with similar gut fungal microbiome. The gut fungal microbiomes in PS- and PE-fed superworms were significantly different, which was consistent to the plastic ingestion preference and gut bacterial community difference. Unlike larval gut bacteria, the gut fungal communities were similar between PS- and PE-fed yellow mealworms. As shown in Fig. 6b, fungal communities were similar not only in the cluster of bran-fed two species, but also in the plastics-fed yellow mealworms. PE- and PS-fed superworms were obtained in different clusters from each other and the other groups.

As shown in the bubble plot (Fig. 6c), there were four predominant fungal phyla, including Ascomycota, Basidiomycota, Mucoromycota and Mortierellomycota. Ascomycota was the predominant phyla in bran-fed groups of both larvae species and PS-fed superworms, which were also reported associated with PS ingestion in lesser mealworms of *A. diaperinus* larvae [7]. The relative abundance of Basidiomycota increased in PS- and PE-fed yellow mealworm guts, suggesting that Basidiomycota was strongly associated with plastics diets in yellow mealworms.

Changes of relative abundances of the top 15 abundant fungal genera with $>1\%$ relative abundances in both species were analyzed and shown in Fig. 6d and e, respectively. In contrast to bacteria, there was a higher diversity of fungi in plastic-fed groups compared to bran-fed samples. *Hyphopichia* and *Debaryomyces* were predominant fungi in guts of both bran-fed superworms and yellow mealworms. In PE-fed superworms, 3 OTUs including *Issatchenkia*, *Wickerhamomyces* and *Rhizomucor* were the dominant genera, accounting for 31.36%, 26.33% and 13.76%, in comparison to bran-fed group ($<0.01\%$), respectively. In PS-fed superworms, *Issatchenkia* had a relative abundance of 30.95%. The dominance of *Issatchenkia* was unique and its association with plastic-degradation was not reported in literatures yet. In PS- and PE-fed yellow mealworms, *Debaryomyces* and *Hyphopichia* disappeared although they were dominant in bran-fed group. In contrast, *Rhodotorula* and *Cryptococcus* showed higher relative abundances of 27.95% and 65.80% in PS-fed group, as well as 53.45% and 35.12% in PE-fed group, indicating that *Rhodotorula* and *Cryptococcus* were probably specific functional groups associated with PS and PE degradation in yellow mealworms.

As shown in Fig. 6d, in controls with the sole diet of bran, the dominant gut fungi were similar in superworms and yellow mealworms.

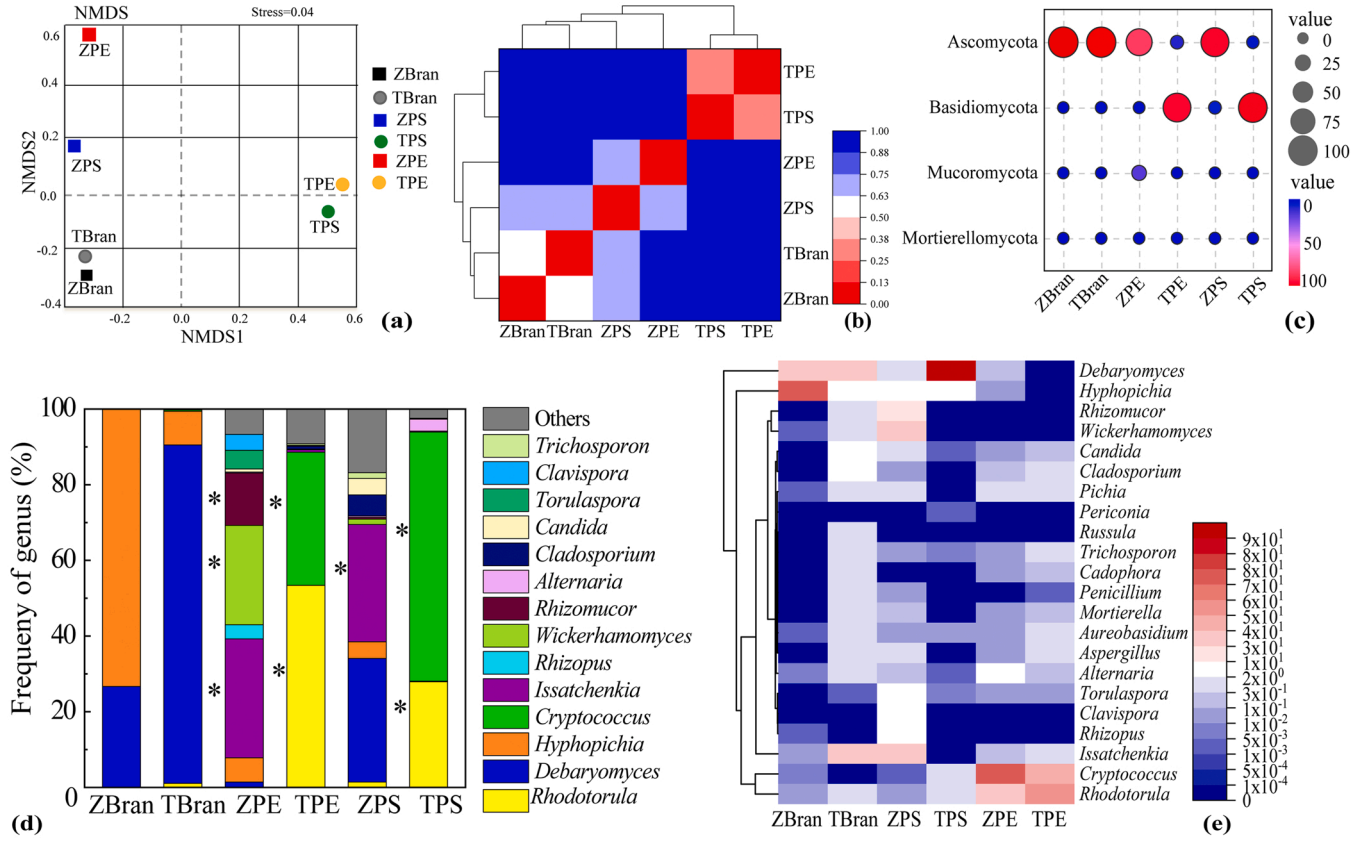

Fig. 6. Analysis of gut fungal diversities superworm *Z. atratus* and yellow mealworm *T. molitor* larvae with different diets. (a) Nonmetric multidimensional scaling based on Unweighted Unifrac; (b) Gut fungal community diversity distance analysis based on Bray-Curtis distance; (c) Relative fungal abundances at phylum level; (d) Relative fungal abundances at genus level; (e) Heatmap and hierarchical cluster analysis of predominant genera (Relative abundance cutoff is 1.0%). Z = *Z. atratus*; T = *T. molitor*; PE = polyethylene foam; PS = polystyrene foam.

In the plastics-fed groups, the dominant fungal OTUs of *Hyphopichia* and *Debaryomyces* probably with high carbohydrate metabolism efficiency decreased or disappeared in both larvae species; some fungal genera that were suggested to be associated to plastic degradation appeared or increased significantly, such as *Issatchenkia*, *Rhodotorula* and *Cryptococcus*. Although some studies on plastic-degrading fungi isolation from gut of insect have been reported [7,46], they were much fewer than that on bacteria. Therefore, studies on plastic biodegradation fungi and the co-culture of bacteria and fungi in the plastics biodegradation process should be further carried out, which will be of great significance to uncover the plastics degradation process in gut of insect larvae.

To our knowledge, until now, there is only one report on the increase abundances of insect larvae gut fungi such as *Aspergillus*, *Penicillium*, *Hyphodermella* and *Trichoderma* in PS-fed lesser mealworms of *A. diaperinus* larvae [7]. However, fungi have been considered as potential candidates for LDPE degradation due to their ability of attaching on the polymer surface by synthesizing hydrophobic proteins [8,24]. *Aspergillus* from the guts of wax moth *G. mellonella* larvae has been reported for the degradation of PE plastic [46]. In our study, *Cryptococcus* and *Rhodotorula* were strongly associated to both PE and PS diet in yellow mealworms, while *Issatchenkia* was associated with both PE and PS diet in superworms.

Overall, the core gut functional microbiome compositions in both superworms and yellow mealworms not only related to feedstocks, but also the species origins. Changes of gut bacterial and fungal communities in response to different plastic diets were observed, providing evidence for their important roles in the plastic degradation process.

4. Conclusions

Long-term foam plastic supply as sole feedstocks had adverse effects on growth, pupation, and generation completion in both superworms and yellow mealworms. The survival rates and plastic consumption rates declined rapidly after 30 days. Plastic consumption rates varied depending on larvae species. The higher cumulative consumptions of PS and PE foam were observed in superworms, while the plastics depolymerization and biodegradation was extensive in yellow mealworms. The gut bacterial communities of PS-fed superworms and yellow mealworms were similar, but significantly different between PE-fed of them. The yellow mealworms gut fungal communities were not significantly different between PE- and PS-fed groups, but superworms gut fungal communities were significantly different between PS- and PE-fed groups compared to the control. To our knowledge, this is the first in-depth study reporting responses of both gut bacterial- and fungal-microbiomes in relation to plastic feedstocks and larvae species. Future studies such as isolation of individual fungi species, and the co-cultures of gut bacteria and fungi in relation to plastic degradation processes will facilitate the understanding of the molecular mechanisms of plastic degradation in larval guts.

CRediT authorship contribution statement

Jiaming Wang: Investigation, Writing – original draft. **Yumeng Wang**: Investigation, Writing – original draft. **Xin Li:** Investigation, Visualization. **Yue Weng:** Investigation. **Yijing Wang:** Investigation. **Xiaoyu Han:** Investigation. **Mu Peng:** Writing – review & editing, Validation. **Aifen Zhou**: Supervision, Writing – review & editing. **Xin Zhao**: Supervision, Writing – review & editing, Conceptualization.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by National Key Research and Development Plan, China (2019YFC1907204), Science and Technology Major Projects of Liaoning, China (2020020307-JH1/103–04). We are grateful to the test services from Analytical and Testing Center of Northeastern University and Sangon Biotech (Shanghai) Co., Ltd.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.108957.

References

[1] A. Amobonye, P. Bhagwat, S. Singh, S. Pillai, Plastic biodegradation: Frontline microbes and their enzymes, Sci. Total Environ. 759 (2021), 143536.
[2] P. Bombelli, C.J. Howe, F. Bertocchini, Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*, Curr. Biol. 27 (8) (2017) R292–R293.
[3] A.M. Brandon, S.H. Gao, R. Tian, D. Ning, S.S. Yang, J. Zhou, W.M. Wu, C. S. Criddle, Biodegradation of polyethylene and plastic mixtures in mealworms (larvae of *Tenebrio molitor*) and effects on the gut microbiome, Environ. Sci. Technol. 52 (11) (2018) 6526–6533.
[4] A.M. Brandon, A.M. Garcia, N.A. Khlystov, W.M. Wu, C.S. Criddle, Enhanced bioavailability and microbial biodegradation of polystyrene in an enrichment derived from the gut microbiome of *Tenebrio molitor* (mealworm larvae), Environ. Sci. Technol. 55 (2021) 2027–2036.
[5] J.R. Cole, Q. Wang, J.A. Fish, B. Chai, D.M. McGarrell, Y. Sun, C.T. Brown, A. Porras-Alfaro, C.R. Kuske, J.M. Tiedje, Ribosomal Database Project: data and tools for high throughput rRNA analysis, Nucleic Acids Res 42 (2014) D633–42.
[6] M. Compa, C. Alomar, C. Wilcox, E. van Sebille, L. Lebreton, B.D. Hardesty, S. Deudero, Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea, Sci. Total Environ. 678 (2019) 188–196.
[7] C. Cucini, C. Leo, M. Vitale, F. Frati, A. Carapelli, F. Nardi, Bacterial and fungal diversity in the gut of polystyrene-fed *Alphitobius diaperinus* (Insecta: Coleoptera), Anim. Gene 17–18 (2020), 200109.
[8] M.P. Das, S. Kumar, J. Das, Fungal-mediated deterioration and biodegradation study of low-density polyethylene (LDPE) isolated from municipal dump yard in Chennai, India *Energy Ecol. Environ.* 3 (4) (2018) 229–236.
[9] S. Ding, T. Tan, l-lactic acid production by *Lactobacillus casei* fermentation using different fed-batch feeding strategies, Process Biochem. 41 (2006) 1451–1454.
[10] R.C. Edgar, Search and clustering orders of magnitude faster than BLAST, Bioinformatics 26 (19) (2010), 2460-1.
[11] R.C. Edgar, B.J. Haas, J.C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection, Bioinformatics 27 (16) (2011), 2194-200.
[12] R.C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads, Nat. Methods 10 (10) (2013), 996-8.
[13] R. Gautam, A.S. Bassi, E.K. Yanful, A review of biodegradation of synthetic plastic and foams, Appl. Biochem. Biotechnol. 141 (2007) 85–108.
[14] R. Geyer, J.R. Jambeck, K.L. Law, Production, use, and fate of all plastics ever made, Sci. Adv. 3 (7) (2017), e1700782.
[15] D.O. Hummel, Atlas of Plastics Additives: Analysis by Spectrometric Methods, Springer,, 2002.
[16] H. Kundungal, M. Gangarapu, S. Sarangapani, A. Patchaiyappan, S.P. Devipriya, Efficient biodegradation of polyethylene (HDPE) waste by the plastic-eating lesser waxworm (*Achroia grisella*), *Environ. Sci. Pollut. Res. Int.* 26 (18) (2019) 18509–18519.
[17] Y. Lou, P. Ekaterina, S.S. Yang, B. Lu, B. Liu, N. Ren, P.F. Corvini, D. Xing, Biodegradation of polyethylene and polystyrene by greater wax moth larvae (*Galleria mellonella* L) and the effect of co-diet supplementation on the core gut microbiome, Environ. Sci. Technol. 54 (2020) 2821–2831.
[18] Y. Lou, Y. Li, B. Lu, Q. Liu, S.S. Yang, B. Liu, N. Ren, W.M. Wu, D. Xing, Response of the yellow mealworm (*Tenebrio molitor*) gut microbiome to diet shifts during polystyrene and polyethylene biodegradation, J. Hazard. Mater. 416 (2021), 126222.
[19] L. Luo, Y. Wang, H. Guo, Y. Yang, N. Qi, X. Zhao, S. Gao, A. Zhou, Biodegradation of foam plastics by *Zophobas atratus* larvae (Coleoptera: Tenebrionidae) associated with changes of gut digestive enzymes activities and microbiome. Chemosphere 282 (2021), 131006.
[20] S. Miao, Study on biological characteristic of *Zophobas morio* and its functions on plastic degradation. *Northwestern University of Agriculture*, For. Technol. (2010).
[21] K. Nam, J.J. Kukor, Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil, Biodegradation 11 (1) (2000) 1–9.

[22] Y. Oh, H. Kim, S. Park, M. KIM, D. RYU, Metabolic-flux analysis of hydrogen production pathway in *Citrobacter* amalonaticus Y19, Int. J. Hydrog. Energy 33 (5) (2008) 1471–1482.
[23] Rodolfo Palomo-Briones, José De. Jesús Montoya-Rosales, Elías Razo-Flores, Advances towards the understanding of microbial communities in dark fermentation of enzymatic hydrolysates: Diversity, structure and hydrogen production performance, Int. J. Hydrog. Energy 46 (54) (2021) 27459–27472.
[24] S. Parte, V.L. Sirisha, J.S. D'Souza, Biotechnological applications of marine enzymes from algae, bacteria, fungi, and sponges, Adv. Food Nutr. Res. 80 (2017) 75–106.
[25] B.Y. Peng, Y. Su, Z. Chen, J. Chen, X. Zhou, M.E. Benbow, C.S. Criddle, W.M. Wu, Y. Zhang, Biodegradation of polystyrene by dark (*Tenebrio obscurus*) and yellow (*Tenebrio molitor*) mealworms (Coleoptera: Tenebrionidae), Environ. Sci. Technol. 53 (2019) 5256–5265.
[26] B.Y. Peng, Y. Li, R. Fan, Z. Chen, J. Chen, A.M. Brandon, C.S. Criddle, Y. Zhang, W. M. Wu, Biodegradation of low-density polyethylene and polystyrene in superworms, larvae of *Zophobas atratus* (Coleoptera: Tenebrionidae): Broad and limited extent depolymerization, Environ. Pollut. 266 (2020), 115206.
[27] PlasticsEurope, Plastics-The Facts 2020, Anal. Eur. Plast. Prod., Demand Waste data (2020). ⟨https://www.plasticseurope.org/en⟩.
[28] S.W. Przemieniecki, A. Kosewska, S. Ciesielski, O. Kosewska, Changes in the gut microbiome and enzymatic profile of *Tenebrio molitor* larvae biodegrading cellulose, polyethylene and polystyrene waste, Environ. Pollut. 256 (2019), 113265.
[29] H.R. Kim, H.M. Lee, H.C. Yu, E. Jeon, S. Lee, J. Li, D.H. Kim, Biodegradation of polystyrene by *Pseudomonas* sp. isolated from the gut of superworms (larvae of *Zophobas atratus*), Environ. Sci. Technol. 54 (2020) 6987–6996.
[30] H. Rajandas, S. Parimannan, K. Sathasivam, M. Ravichandran, L. Su Yin, A novel FTIR-ATR spectroscopy based technique for the estimation of low-density polyethylene biodegradation, Polym. Test. 31 (8) (2012) 1094–1099.
[31] J. Ru, Y. Huo, Y. Yang, Microbial Degradation and Valorization of Plastic Wastes, Front. Microbiol. 11 (2020) 442.
[32] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: a comprehensive review, Biotechnol. Adv. 26 (2008) 246–265.
[33] Shen Y. Isolation of intestinal bacteria from *Tenebrio molitor* and study on the phenomenon of plastic degradation. *East China Normal University*, 2011.
[34] N. Taghavi, N. Singhal, W.Q. Zhuang, S. Baroutian, Degradation of plastic waste using stimulated and naturally occurring microbial strains, Chemosphere 263 (2020), 127975.
[35] A.K. Urbanek, J. Rybak, M. Wróbel, K. Leluk, A.M. Mirończuk, A comprehensive assessment of microbiome diversity in *Tenebrio molitor* fed with polystyrene waste, Environ. Pollut. 262 (2020), 114281.
[36] J. Wang, Y. Wang, X. Li, Y. Weng, X. Dong, X. Zhao, Comparison on the effectiveness of Fourier Transform Infrared (FT-IR) and attenuated total reflection Fourier Transform Infrared (ATR-FT-IR) in characterizing plastics biodegradation by insect larvae, Sci. Total Environ. 839 (2022), 156289.
[37] Y. Wang, L. Luo, X. Li, J. Wang, H. Wang, C. Chen, H. Guo, T. Han, A. Zhou, X. Zhao, Different plastics ingestion preferences and efficiencies of superworm (*Zophobas atratus* Fab.) and yellow mealworm (*Tenebrio molitor* Linn.) associated with distinct gut microbiome changes, Sci. Total Environ. 837 (2022), 155719.
[38] Y. Wang, Y. Zhang, Investigation of gut-associated bacteria in *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae using culture-dependent and DGGE methods, Ann. Entomol. Soc. Am. 108 (5) (2015) 941–949.
[39] J. Yang, Y. Yang, W.M. Wu, J. Zhao, L. Jiang, Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms, Environ. Sci. Technol. 48 (23) (2014) 13776–13784.
[40] S.S. Yang, M.Q. Ding, L. He, C.H. Zhang, Q.X. Li, D.F. Xing, G.L. Cao, L. Zhao, J. Ding, N.Q. Ren, W.M. Wu, Biodegradation of polypropylene by yellow mealworms (*Tenebrio molitor*) and superworms (*Zophobas atratus*) via gut-microbe-dependent depolymerization, Sci. Total Environ. 756 (2020), 144087.
[41] Y. Yang, J. Wang, M. Xia, Biodegradation and mineralization of polystyrene by plastic-eating superworms *Zophobas atratus*, Sci. Total Environ. 708 (2020) 135233.1–135233.7.
[42] S.S. Yang, M.Q. Ding, Z.R. Zhang, J. Ding, S.W. Bai, G.L. Cao, L. Zhao, J.W. Pang, D.F. Xing, N.Q. Ren, W.M. Wu, Confirmation of biodegradation of low-density polyethylene in dark- versus yellow- mealworms (larvae of *Tenebrio obscurus* versus *Tenebrio molitor*) via. gut microbe-independent depolymerization, Sci. Total Environ. 789 (2021), 147915.
[43] S.S. Yang, M.Q. Ding, X.R. Ren, Z.R. Zhang, M.X. Li, L.L. Zhang, J.W. Pang, C. X. Chen, L. Zhao, D.F. Xing, N.Q. Ren, J. Ding, W.M. Wu, Impacts of physical-chemical property of polyethylene on depolymerization and biodegradation in yellow and dark mealworms with high purity microplastics, Sci. Total Environ. 828 (2022), 154458.
[44] Y. Yang, J. Yang, W.M. Wu, J. Zhao, Y. Song, L. Gao, R. Yang, L. Jiang, Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests, Environ. Sci. Technol. 49 (20) (2015) 12080–12086.
[45] J. Zaelor, S. Kitthawee, Growth response to population density in larval stage of darkling beetles (Coleoptera; Tenebrionidae) *Tenebrio molitor* and *Zophobas atratus*, Agric. Nat. Resour. 52 (6) (2018) 603–606.
[46] J. Zhang, D. Gao, Q. Li, Y. Zhao, L. Li, H. Lin, Q. Bi, Y. Zhao, Biodegradation of polyethylene microplastic particles by the fungus *Aspergillus flavus* from the guts of wax moth *Galleria mellonella*, Sci. Total Environ. 704 (2020) 135931.1–135931.8.