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Different plastics ingestion preferences and efficiencies of superworm (*Zophobas atratus* Fab.) and yellow mealworm (*Tenebrio molitor* Linn.) associated with distinct gut microbiome changes

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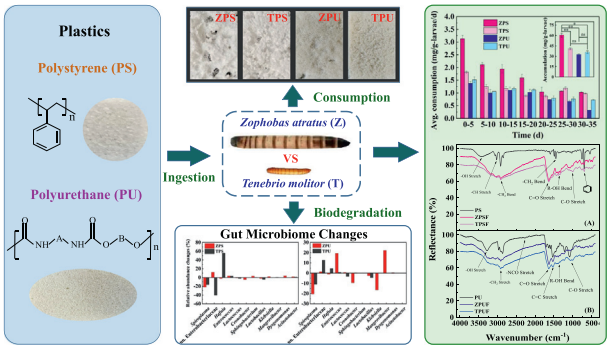
HIGHLIGHTS

- Higher PS consumption rates (mg/g-larvae) were obtained in superworms *Z. atratus* compared to yellow mealworms *T. molitor*.
- PU consumption rates (mg/g-larvae) were similar in superworms and yellow mealworms.
- Similar changes of PS or PU functional groups were detected in frass of both species.
- Changes of gut-microbiomes were associated with plastic feedstocks types and larvae species.
- Biodegradations of PS and PU were associated with distinct gut-microbes in both species.

GRAPHICAL ABSTRACT



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ABSTRACT

Larvae of superworms (*Zophobas atratus* Fab.) and yellow mealworms (*Tenebrio molitor* Linn.) can survive on sole plastic diets. However, no side-by-side comparison of plastics degradation by both species is available yet. Here, superworms and yellow mealworms were fed with polystyrene (PS) or polyurethane (PU) foam plastics as sole diets for 35 days with bran as control. Superworms survived 100% on all diets but decreased weights were observed after 20 days with sole plastic diets. In contrast, yellow mealworms survived 84.67% or 62.67% with PS or PU diet, respectively, both plastics diet groups showed increased weights. Cumulative consumption of plastics by superworms were 49.24 mg-PS/larva and 26.23 mg-PU/larva, which were 18 and 11 folds of that of yellow mealworms, respectively. When converted into mg/g-larvae, superworms had a higher PS consumption rate but both species had similar PU consumption rates. Similar changes of the plastic chemical functional groups in frass indicated occurrences of oxidation and biodegradation of plastics in the guts of both species. Changes of gut microbial communities were found associated with plastics feedstocks and larvae species. The increased relative abundances of unclassified Enterobacteriaceae, *Klebsiella*, *Enterococcus*, *Dysgonomonas* and *Sphingobacterium* were strongly associated with PS diet in superworms, while *Hafnia* was strongly associated with PS diet in yellow mealworms. *Enterococcus* and *Mangrovibacter* were dominant

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in PU-fed superworm guts, while unclassified Enterobacteriaceae and *Hafnia* were strongly associated with PU feeding in yellow mealworms. The results demonstrated that different plastics ingestion preferences and efficiencies of both species were associated with distinct dominant microbiomes although similar changes of chemical groups in plastics were observed.

1. Introduction

Plastic wastes have become a global environmental concern due to its ever-increasing accumulation in natural environment (Barnes et al., 2009; Sutherland et al., 2019). By the end of 2018, the plastics production around the world has reached nearly 359 million tons (PlasticsEurope, 2019). The degradation rates of plastics in natural environments are very low due to the macromolecular structure, leading to not only increased impacts to the ecosystem, soil, waterbody by plastics accumulation, but also the survival risk of wildlife and even human health by plastic nanoparticles (Thompson et al., 2009; Ivleva et al., 2017; Wang et al., 2019; Wu et al., 2017). The ever-increasing production of plastics and low recycling rates of plastic wastes inevitably impose burdens to the environment and challenges to the waste management (Geyer et al., 2017; Jambeck et al., 2015). How to mitigate the plastic waste problem and remediate the plastic pollution has become an urgent environmental issue in the global scale.

Discoveries of ingestion and degradation of plastics by certain kinds of insect larvae provided new directions for plastics biodegradation. Yang et al. (2015) firstly reported that yellow mealworms (*Tenebrio molitor*) larvae could chew and eat polystyrene (PS) foam as sole diet with a PS mass loss of 0.12 mg/larva/d. PS biodegradation by superworms (*Zophobas atratus*) larvae were also reported with a plastic mass loss of 0.58 mg/larva/d (Yang et al., 2020). Peng et al. (2019) compared the PS degradation efficiencies of yellow mealworms and dark mealworms (*T. obscurus*) larvae and demonstrated that dark mealworms had a higher PS foam consumption rate of 0.32 mg/larva/d while the yellow mealworms had a lower PS foam consumption rate of 0.24 mg/larva/d. Polyethylene (PE) foam plastics consumption and biodegradation by yellow mealworms was reported by Yang and Wu (2020), Lou et al. (2021) as well as Yang et al. (2021c). Biodegradation of low-density PE (LDPE) foam by superworms were demonstrated with the mass loss of 0.30 and 0.59 mg/larva/d, respectively (Luo et al., 2021; Peng et al., 2020a). Besides PS and PE, biodegradation of other types of plastics, such as polypropylene (PP), polyurethane (PU), polylactic acid (PLA) and polyvinyl chloride (PVC), by yellow mealworms and superworms were also reported (Bulak et al., 2021; Peng et al., 2020b, 2021; Yang et al., 2021a; Zhu et al., 2022).

Both superworm *Z. atratus* Fab. and yellow mealworm *T. molitor* Linn. belong to Coleoptera within the cosmopolitan family Tenebrionidae, which comprises more than 20,000 species. They have an average genetic similarity of 79.1% and dramatic body size difference (Park et al., 2013). Superworms are larger in size and heavier in body weights. Generally, body sizes and weights of superworms were about 3– 4 folds and 10– 15 folds of yellow mealworms, respectively. In our previous study, consumptions of PS, PU, and PE foam plastics by superworms were monitored and characterized (Luo et al., 2021). Although both yellow mealworms and superworms were capable of PS and PU foam ingestion and degradation, different larval geological sources and feeding environment may lead to differences in plastic ingestion efficiencies, biodegradation capacities, depolymerization patterns, and functional gut microbiomes. Therefore, a side-by-side comparison of both species under the same conditions is necessary to uncover the differences in plastics consumption efficiencies and degradation preferences by both species.

In this study, we aimed to uncover the differences between biodegradation of PS and PU by superworm and yellow mealworm, changes of plastic functional groups, and the associated gut microbiome changes. Towards this purpose, the sole PS or PU foam plastics feeding experiment of yellow mealworm larvae was compared with the parallel experiment of sole PS or PU foam plastics feeding experiment of superworms (Luo et al., 2021). When plastic consumption rate was calculated as plastics mass loss per unit mass of larvae, higher PS consumption rates by superworms but similar PU consumption rates by both superworms and yellow mealworms were obtained. Analysis of frass samples demonstrated similar changes of the functional groups derived from oxidation and biodegradation of PS and PU in guts of both larvae. But the biodegradation of PS and PU was associated with significantly different gut microbiomes in both larvae species.

2. Materials and methods

2.1. Larvae and feed stocks

Superworms and yellow mealworms larvae were purchased from Wuxi Insects Breeding Plant, Jiangsu, China. Superworms were approximately 5.0 ± 0.3 cm in length and 0.80 ± 0.03 g/larva in weight. Yellow mealworms were approximately 1.52 ± 0.03 cm in length and 0.06 ± 0.07 g/larva in weight. Natural wheat bran was purchased from specialty stores and foam plastics of PS (0.008 g/cm^3, highly expanded polystyrene) and PU (0.012 g/cm^3, polyurethane sponge, flexible polyurethane foam) were purchased from Yinlong Plastic Company, Zhejiang, China (Luo et al., 2021). Before the tests, the PS and PU 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. All larvae were fed with bran and starved for 72 h before switching to experimental diets.

2.2. Larvae growth and plastics consumption

Six experiment groups including three groups of bran-, PS-, or PU-fed superworms and three groups of bran-, PS-, or PU-fed yellow mealworms with triplicates in each group were set up. The bran-fed groups were used as controls. To keep a similar initial larvae biomass, 15 superworms or 100 yellow mealworms (Przemieniecki et al., 2019) were included in each group and placed in an incubate container (15 × 10 × 7 cm). At the beginning of the experiment, bran (5 g) or foam plastic pieces were added into each container, and the same amounts of feedstocks were supplemented every 5 days. All containers were maintained under the same condition for 35 days with temperature at 25 ± 1 °C and humidity of 60 ± 5% (Luo et al., 2021). At every 5 days, big pieces of PS or PU foam and plastic debris derived from larvae gnawing were collected as much as possible using forceps and weighed together to calculate the PS or PU consumption, the numbers of living larvae were counted to calculate the survival rates, the weights of larvae were measured to calculate the larvae weight changes. Accumulative changes were calculated by comparing the end-point values with the initial values, changes of every 5 days were calculated as the differences between the 5-day periods. Dead larvae and molting exoskeleton were checked every day and removed immediately to prevent the mass consumed by cannibalism.

2.3. Frass collection and characterization

Collection of frass samples was conducted by transferring larvae to a clean container to avoid contamination of un-ingested plastic and egested frass. Frass samples were collected pellet by pellet at 5-day interval and pooled and stored in aseptic tubes at −20 °C till further analysis (Luo et al., 2021). The attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) (vertex70, Brock instruments, Germany) was employed to identify changes of the major functional groups in frass samples in comparison to the original plastics (Rajandas et al., 2012). The wavenumber range of 4000–550 cm^{-1} was used. Prior to the ATR-FTIR test, the frass samples were thawed and air-dried for 5 h in a 40 °C incubator. The peak values were analyzed with the OMNIC software and confirmed

based on the best expert judgment by the presence of specific absorption bands (Hummel, 2002). Thermal gravimetric analysis (SDT Q600, TA Instruments, USA) was conducted to characterize thermal stability changes in frass compared to plastics feedstock. The sample was heated from 40 °C to 800 °C at a rate of 20 °C/min under a high-purity argon ambience (Peng et al., 2020a; Luo et al., 2021).

2.4. Gut microbiome analysis

At the end of the 35-day experiment, the larval guts of each experiment group (randomly selected 6 superworms or 30 yellow mealworms in each treatment group) were collected and pooled into six 10 mL sterile centrifuge tubes with 5 mL sterile normal saline, respectively (Yang et al., 2014). After a 5 min vortex, the samples were centrifuged at 10000 rpm for 2 min, and the pellets were stored at −20 °C for the microbiome analysis (Luo et al., 2021). Genomic DNA extraction, PCR amplification, and Illumina MiSeq analysis of guts microbiome were performed as described previously (Luo et al., 2021). The V3–V4 regions of 16S rRNA genes were amplified using the universe primer pairs of 341F (CCT ACG GGN GGC WGC AG) and 805R (GAC TAC HVG GGT ATC TAA TCC). The purified PCR products were sequenced on an Illumina MiSeq platform. The low-quality sequences were filtered according to the criteria described by Gill et al. (2006). Operational taxonomic units (OTUs) were clustered using UPARSE with 0.97 identity threshold (Edgar, 2010), and chimeric sequences were removed using UCHIME (Edgar et al., 2011). Taxonomies of 16S rRNA gene sequences were analyzed by Ribosomal Database Project (RDP) Classifier against the Silva 16S rRNA database (James et al., 2014). Alpha diversity was represented by the Shannon diversity index. Principal component analysis (PCA) and hierarchical clustering analysis were conducted by R packages (Ramette, 2007).

2.5. Statistical analysis

Statistical analysis was performed in Prism (version 8) (Brandon et al., 2018). The differences in PS and PU consumption rates, larvae survival rates, and gut microbial diversities between two larvae species were assessed by ANOVA (Yang et al., 2021a). The pairwise comparisons were performed with the Student's *t*-test (Yang et al., 2021a). 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 discussion

3.1. Effects of sole plastic diets on growth and development of larvae

Survival rates, pupation, and weight changes were used to assess the influences of sole plastic diets on growth and development of larvae. Survival rates of larvae over 35 days were significantly different between superworms (Z) and yellow mealworms (T) (Fig. 1). All superworms in both plastic-fed and control bran-fed groups survived (survival rates 100%) (Fig. 1A). On the contrary, the survival rates of PS-fed and PU-fed yellow mealworms were 84.67 ± 2.42% and 62.67 ± 1.62%, respectively, which were significantly ($p < 0.01$) lower than that of bran-fed group (90.67 ± 0.88%) (Fig. 1B). The life cycles of superworms or yellow mealworms include four stages: egg, larva, pupa and adult (Miao, 2010; Shen, 2011). A variety of factors could affect the pupation. The pupation can be used as one of the indicators of the health and development of larvae. In this experiment, only one superworm larva in PU-fed group pupated (Fig. 1 A). For yellow mealworms, a total of 22 bran-fed yellow mealworm larvae pupated, which was much greater ($p < 0.01$) than that of PS-fed group (only 1) and PU-fed group (none) (Fig. 1 B). Bran is commonly used as feedstocks for yellow mealworms and superworms, which provides enough nutrients to complete their life cycle. Bran-fed superworms did not pupate during the experiment, possibly due to their longer life cycle than yellow mealworms (Miao, 2010; Shen, 2011). While the significantly decreased pupation rates in PS-fed or PU-fed yellow mealworms indicated that sole PS or PU plastic diet could not meet the nutrients requirements to complete their life cycle.

In addition to the survival rates and pupation rates, compared to the initial average weight, the cumulative net weight change trends over 35 days

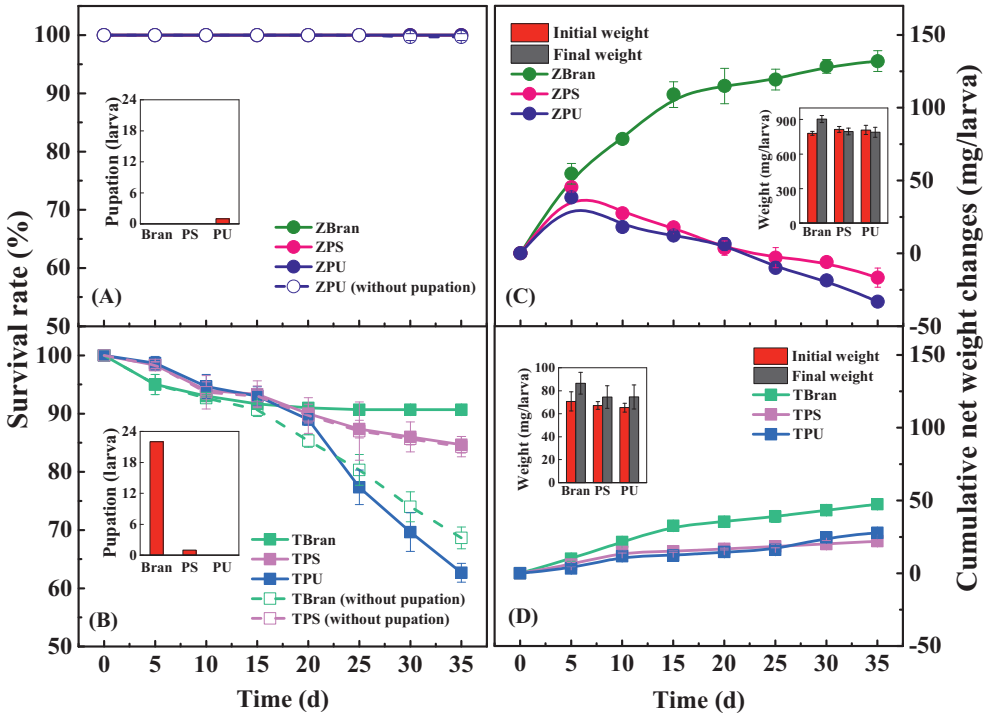

Fig. 1. Survival rates, pupation, and cumulative net weight changes of superworm *Z. atratus* and yellow mealworm *T. molitor* with different diets. (A) Survival rates and pupation of superworms; (B) Survival rates and pupation of yellow mealworms; (C) Weight changes of superworms; (D) Weight changes of yellow mealworms. Z = *Z. atratus*; T = *T. molitor*; PS = polystyrene foam; PU = polyurethane foam.

of sole plastic diets were different between superworms and yellow mealworms. Weight gain of superworms was the highest on the fifth day, gradually reduced afterwards, and became weight loss after 20 days (Fig. 1C). In contrast, yellow mealworms had relatively constant weight gains over 35 days (Fig. 1D). At the 35th day of the experiment, the bran-fed control superworm group had a net weight gain of 132 ± 7.17 mg/larva, while PS- and PU-fed superworm groups had 16.7 ± 4.55 and 33.21 ± 0.18 mg/larva weight loss, respectively (Fig. 1C). Yellow mealworms groups had weight gains of 47.36 ± 1.45, 22.01 ± 0.68, and 27.78 ± 2.01 mg/larva for bran-, PS-, and PU-fed groups, respectively (Fig. 1D). The weight gain rates of bran-fed superworms and yellow mealworms were 16.25% and 78.90%, respectively, indicating a higher metabolism demand of superworms than yellow mealworms under the condition of adequate bran diet supplement. The overall trends of weight loss of plastic-fed superworms provided evidence for the undersupply of nutrient with sole plastic diets.

3.2. Plastics consumption

Hollows and pits were observed in PS and PU foams after incubation with superworm or yellow mealworm larvae. Fig. 2 showed the morphology of plastics on the fifth day of the experiment. Much larger hollows and pits were observed in plastic-fed superworm groups than that of yellow mealworm groups (Fig. 2). Over the 35-days experiment, PS or PU consumptions in superworm and yellow mealworm groups decreased over time (Fig. 3A and B and Fig. S1). The cumulative consumptions of 49.24 ± 1.4 mg PS/larva and 26.23 ± 1.03 mg PU/larva by superworms, which were 17.9 and 11.2 folds of that of 2.75 ± 0.12 mg PS/larva and 2.34 ± 0.12 mg PU/larva by yellow mealworms, respectively (Fig. 3C). The average PS foam consumption rates of superworms and yellow mealworms could be calculated as approximately 1.41 mg/larva/d and 0.08 mg/larva/d, which were about 2.3 and 2 folds more than that of 0.62 mg/larva/d by superworms (Peng et al., 2020a) and 0.04 mg/larva/d by yellow mealworms (Yang et al., 2021c), respectively. The higher PS consumption efficiency of superworms might be related to the larvae size, the average initial weight of 800 ± 30 mg/larva in this study vs. 137 ± 4 mg/larva in Peng et al. (2020a). The higher PS consumption efficiency of yellow mealworms may be related to the higher PS foam supply, 3.2 g/100 larvae in this study vs 3.0 g/300 larvae in Yang et al. (2021c). In addition, the different geographic sources (Superworm: Wuxi vs. Guangzhou, China; yellow mealworm: Wuxi vs. Beijing, China) of the test larvae could contribute to the difference of foam plastics ingestion. The PU consumption rate of yellow mealworms was 0.07 mg/larva/d, which was higher than the previous report of 0.04 mg/larva/d (Bulak et al., 2021). To eliminate the effect of body size difference between two species, foam consumption rates were also calculated as total plastic mass loss per initial average larvae weight (Fig. 3D). At the 35th day, superworms had a cumulative PS foam consumption of 60.49 ± 1.94 mg/g larvae, which was about 1.5 folds of that of 40.89 ± 1.37 mg/g yellow mealworm larvae. In contrast, superworms had a cumulative PU foam consumption of 32.44 ± 1.31 mg/g larvae, which was a little lower than that of 35.80 ± 1.13 mg/g yellow mealworm larvae. The results indicated that superworms consumed PS foam plastics at rates significantly higher than that of yellow mealworms ($p < 0.01$) under the same condition, suggesting a PS foam ingestion preference of superworms. But there was no significant difference in PU foam consumptions between the two species when calculated as per unit mass of larvae, suggesting a similar PU foam ingestion preference in both superworms and yellow mealworms.

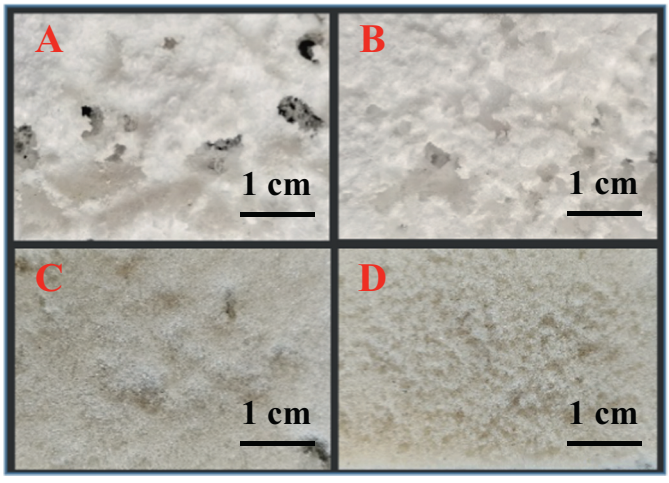

Fig. 2. Hollows and pits on PS (top panel, A & B) and PU (bottom panel, C &D) foam plastics after 5 days of incubation with superworm (A, C) or yellow mealworm (B, D) larvae.

3.3. Changes of function groups in PS and PU foam plastics

ATR-FTIR of foam plastics and egested frass of both species were conducted and compared to identify the chemical structure and composition changes derived from plastics consumption in larvae guts (Fig. 4). In PS-fed groups (Fig. 4A and Table S1 in the Supplementary), spectra of superworms frass were similar to that of yellow mealworms frass, both of which were significantly different from PS feedstock. In frass samples of both species, characteristic peaks of PS benzene ring skeleton vibrations (755 and 696 cm^{-1}) disappeared, indicating the cleavage of benzene ring. Peaks at 2880–2972 cm^{-1} ($-CH_2$ stretch) were much weaker compared to PS feedstock, peaks at 3419 cm^{-1} (−OH stretch) and 3024 cm^{-1} (−CH stretch) disappeared, indicating the missing of −CH and −OH structures in the PS main chain in frass. In addition, new functional groups were observed in frass of both species, such as peaks at 1630–1736 cm^{-1} (CO stretch), 1300–1450 cm^{-1} (R − OH bend) and 1000–1200 cm^{-1} (C−O stretch). The addition of oxygen functional groups into the polymer chain was considered as the preliminary and key step in plastic degradation (Singh and Sharma, 2008). The results suggested the occurrence of oxidation and depolymerization of PS in guts of superworms and yellow mealworms through similar degradation processes.

In PU-fed groups (Fig. 4B and Table S2), the ATR-FTIR spectra of frass from both species were also similar. The peaks of PU at 3271 cm^{-1} (−OH stretch) and 2273 cm^{-1} (N−C=O stretch) disappeared. The asymmetric stretching vibration of N − C=O was the characteristic absorption peak of PU (Špírková et al., 2017; Gomez et al., 2014). The appearance of peaks at 1630–1736 cm^{-1} (CO stretch) and 1300–1450 cm^{-1} (−OH bend) in frass of both species, suggesting the occurrence of incorporation of oxygen during PU degradation. The decreased heights of peaks at 2880–2972 cm^{-1} ($-CH_2$ stretch), 1550–1610 cm^{-1} (CC stretch) and 1000–1200 cm^{-1} (C−O stretch) suggested the occurrence of oxidation and de-polymerization of PU in the guts of both species. Similar changes of chemical functional groups in PU foam after passing through guts of both species indicated the occurrences of similar oxidation or depolymerization processes.

To uncover whether different plastics were degraded by the same larvae with similar processes, the spectra of frass of bran-, PS- and PU-fed from the same specie larvae were compared, respectively, which trends were roughly similar with few different characteristic peaks like 2880–2972 cm^{-1} ($-CH_2$ stretch), 1650–1736 cm^{-1} (CO stretch) and 1000–1200 cm^{-1} (C − O stretch) (Fig. 4C and D. and Table S3-S4). The results indicated that larvae of the same species probably have similar processes in different plastics biodegradation. However, due to the components and structures difference of plastics, some characteristic peaks were obtained differently.

Thermal stabilities of plastics and frass samples were assessed using the DSC-TGA (Fig. S2). In PS-fed groups, the weight loss of PS feedstocks occurred at temperature range of 366.7– 431.4 °C with a weight loss rate of 95.5%, while the weight loss rates of frass of PS-fed superworms and PS-fed yellow mealworms were about 71.1% and 87.4%, respectively. In PU-fed groups, the weight loss of PU feedstocks occurred at temperature

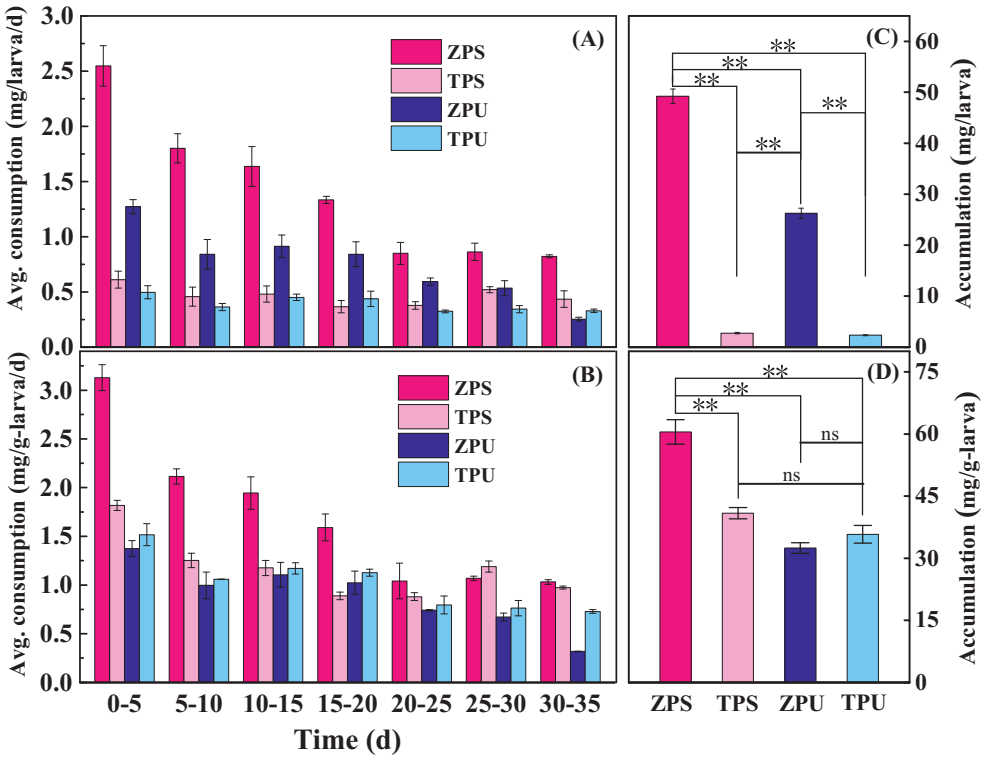

Fig. 3. Average (Avg.) consumptions of plastics every five days (mg) by superworm *Z. atratus* or yellow mealworm *T. molitor* calculated by per larva (A) or per unit (g) larvae biomass (B); Accumulative plastics consumption by larvae of two species by per larva (C) or per unit larvae biomass (D). Student's *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*; PS = polystyrene foam; PU = polyurethane foam.

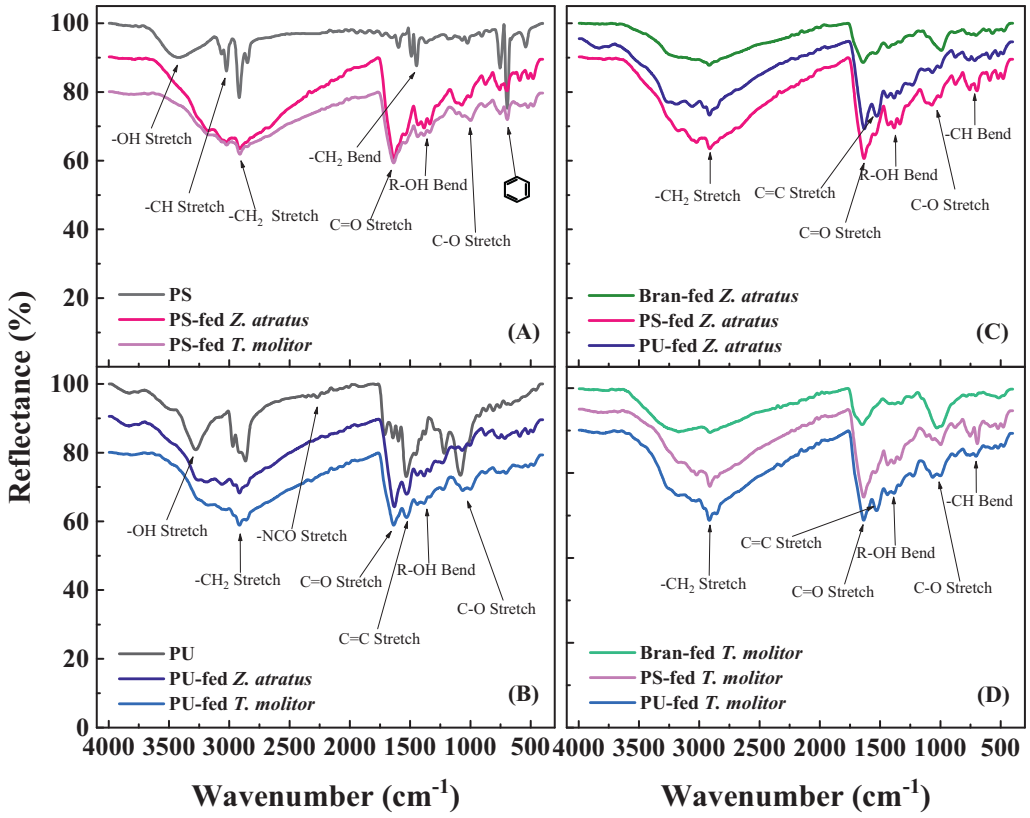

Fig. 4. ATR-FTIR spectra of plastics and frass. (A) PS and frass collected from PS-fed superworms or yellow mealworms; (B) PU and frass collected from PU-fed superworms or yellow mealworms; (C) Frass of bran-, PS-, PU-fed superworms; (D) Frass of bran-, PS-, PU-fed yellow mealworms.

range of 227.4– 419.5 °C with a weight loss rate of 65.4%, while the weight loss rates of frass of PU-fed superworms and PU-fed yellow mealworms frass were about 61.4% and 57.3%, respectively. These results indicated that the frass contained not only residual plastics samples but also new organic intermediates with different thermal properties (Peng et al., 2019; Yang et al., 2021a). In addition, superworms was probably more efficient in PS biodegradation than yellow mealworms, but the biodegradation degree of PU plastic by two larvae species was similar. In summary, the thermal stability changes of frass samples provided evidence for structural changes of PS and PU plastics when passing through the guts of larvae.

3.4. Associations between gut microbiomes and plastic types and species

Illumina MiSeq analysis of PCR amplified 16S rRNA fragments was used to assess the gut microbial community composition changes of both species with different sole plastic diets compared to control bran-fed groups. The Illumina MiSeq sequences of gut microbiome were deposited in the GenBank (accession numbers: SRR18172502–SRR18172507). The Shannon Index at OTU level showed higher species diversities in plastics-fed groups than bran-fed groups, indicating that larval gut microbiome diversities significantly increased under sole plastic feeding conditions (Fig. S3 and Table S5). Among plastic feeding groups, the gut microbiomes of PS-fed groups showed higher community diversities than the PU-fed groups in both larvae species.

Analysis of relative abundance at genus level indicated the significant shift of the gut microbiomes in plastic-fed larvae of both species (Fig. 5). The top 12 abundant genera (relative abundance cutoff of 1%) were shown in Fig. 5A. Among these, *Spiroplasma*, unclassified Enterobacteriaceae, and *Enterococcus*, were also reported common gut-associated genera in yellow mealworms and dark mealworms (Luo et al., 2021; Wang and Zhang, 2015). In bran-fed superworm control group, the dominant OTUs were *Spiroplasma* sp. (35.33%), *Klebsiella* sp. (20.62%), *Lactococcus* sp. (7.33%) and *Cronobacter* sp. (12.3%). The dominant OTUs in yellow mealworm guts were unclassified Enterobacteriaceae (43.44%), *Spiroplasma* (43.39%), *Lactobacillus* (4.54%) and *Enterococcus* (4.24%). The results indicated that both species had different initial gut microbial dominants possibly related to their feed histories and gut eco-physiologies.

Compared to the bran-fed groups, the larval gut microbiomes in PS- and PU-fed groups shifted significantly in both species and the different responses of gut microbiomes in sole plastic diets in both species ($p < 0.05$) (Fig. 5A). In PS-fed superworms, the dominant genera were unclassified Enterobacteriaceae and *Klebsiella*. The relative abundance of unclassified Enterobacteriaceae increased (21.62%) compared to that of the bran-fed control (11.35%), consistent to the changes in the gut of PS fed yellow

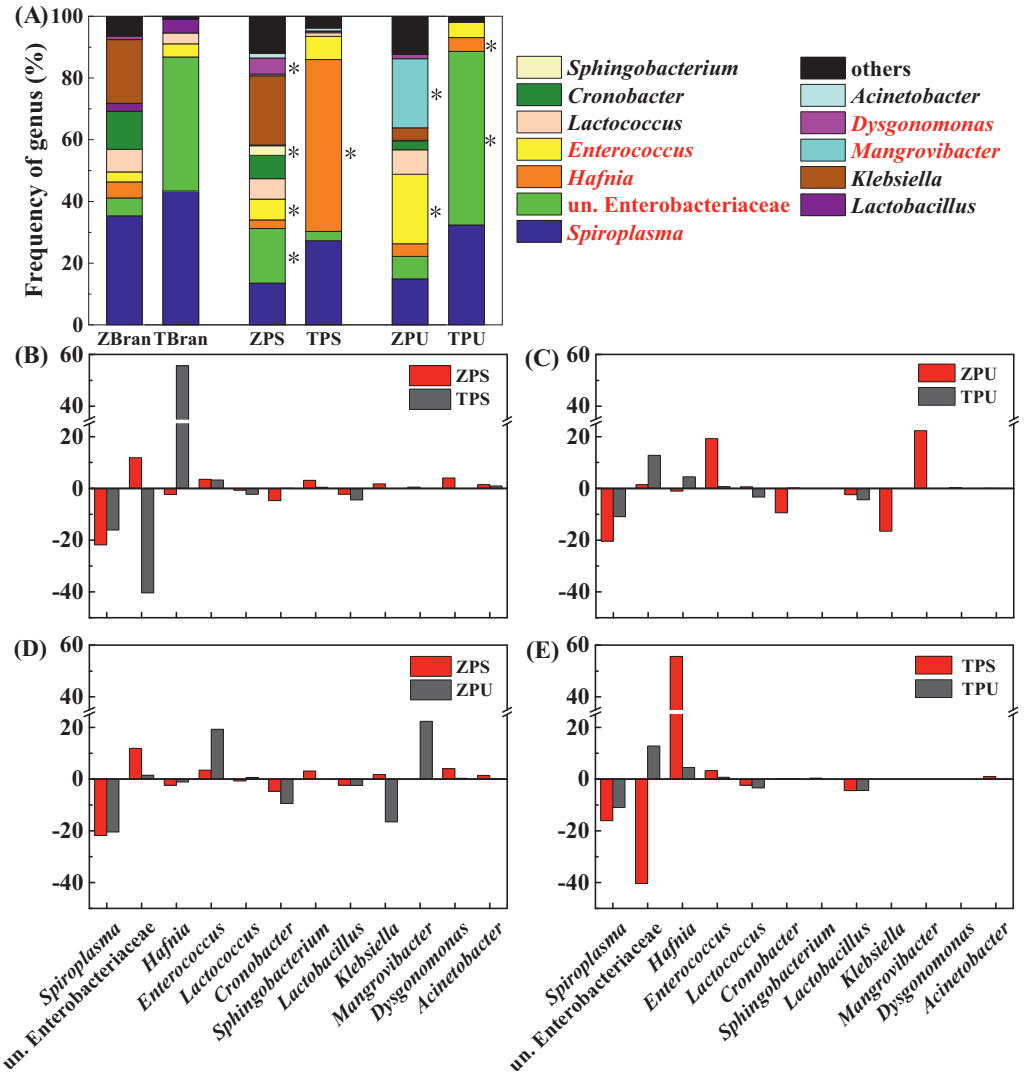

Fig. 5. Changes of gut microbiomes of superworms and mealworms. (A) Relative abundances of gut microbes in superworms and mealworms at the 35th day of the incubation experiment. Relative abundance changes of gut microbiomes at genus level in plastic-fed groups compared with bran-fed groups (PS, B; PU, C), in superworms groups (D), or mealworms groups (E). *: Genus strongly associated with plastic degradation. Z = *Z. atratus*; T = *T. molitor*; PS = polystyrene foam; PU = polyurethane foam.

mealworms (Yang et al., 2021c). Relative abundance of *Klebsiella* remained relatively stable (20.11%) compared to that of the bran-fed control (20.62%), suggesting its importance under both normal growth conditions such as bran feeding and PS feeding conditions in superworms. The strong association of *Klebsiella* with PS degradation was also reported in yellow mealworms (Brandon et al., 2021), and it is suggested that *Klebsiella* is an important nitrogen-fixing gut genus in PS-fed yellow mealworms for the nitrogen nutritional supplementation since PS plastics diet cannot meet the nitrogen requirement (Yang et al., 2022). In addition to unclassified Enterobacteriaceae and *Klebsiella*, the relative abundances of *Enterococcus*, *Dysgonomonas,* and *Sphingobacterium* increased in PS-fed superworms, accounting for 6.09%, 4.75% and 2.92%, respectively. The relative abundance of *Enterococcus* almost doubled in PS-fed superworms compared to the bran-fed control, suggesting its strong association with PS biodegradation in superworms. *Enterococcus* was also found strongly associated with PE and PS degradation in dark mealworms and yellow mealworms (Brandon et al., 2021; Yang et al., 2021b). This is the first report about the association of *Dysgonomonas* and *Sphingobacterium* with plastic degradation. Previous studies found that *Dysgonomonas* was correlated with azo dye degradation (de Almeida et al., 2021) and *Sphingobacterium* could degrade sulfamethoxazole as sole carbon source for growth (Song et al., 2021), inferring that they may participate in the benzene ring degradation process in PS. Future work should assess the role of *Dysgonomonas* and *Sphingobacterium* in the PS degradation. In PS-fed yellow mealworms, *Hafnia* was the dominant genus with relative abundance of 55.65% while not detected in bran-fed group, suggesting its strong association with PS diet in yellow mealworms. In this study, previously reported PS-degrading genera *Citrobacter*, *Kosakonia* and *Pseudomonas* (Brandon et al., 2018; Kim et al., 2020; Przemieniecki et al., 2019) were not detected in both bran-fed control and PS-fed superworms and yellow mealworms gut microbiome, this is possibly due to the different geographic sources of the test larvae.

In PU-fed superworms, the dominant genera were *Enterococcus* and *Mangrovibacter*. The relative abundance of *Enterococcus* increased by 19.26%, *Mangrovibacter* was 21.28% compared to the bran-fed group (not detected), suggesting their importance in PU diet in superworms. In PU-fed yellow mealworms, community shift mainly consisted of increased relative abundances of unclassified Enterobacteriaceae and *Hafnia*, 56.18% and 4.5% respectively, comparing to that of the bran-fed group, 43.64% and 0%, respectively, suggesting that they were strongly associated with PU diet in yellow mealworms.

Differential abundances analysis of gut microbiomes was conducted to assess whether specific OTUs were associated with plastic biodegradation in different larvae species (Fig. 5B and C). The results showed the significant differences of gut microbiome changes between superworms and yellow mealworms under the same plastic diets. In PS-fed groups (Fig. 5B), the relative abundance of unclassified Enterobacteriaceae increased by 10.27% in superworms, but decreased by 40.39% in yellow mealworms, the relative abundance of *Hafnia* decreased by 2.41% in superworms, while increased by 55.65% in yellow mealworms, indicating the significant differences between the gut microbiomes responses of PS-fed superworms and yellow mealworms. Nevertheless, the relative abundances of *Enterococcus* increased in both PS-fed superworms and yellow mealworms, suggesting the importance of *Enterococcus* in PS biodegradation in both superworms and yellow mealworms. In PU-fed groups (Fig. 5C), the relative abundance of unclassified Enterobacteriaceae slightly increased by 1.48% in superworms, while significantly increased by 12.76% in yellow mealworms, the relative abundance of *Hafnia* decreased in superworms but increased in yellow mealworms, indicating that unclassified Enterobacteriaceae and *Hafnia* were more significantly associated to PU diet in yellow mealworms. Dramatic increases of relative abundances of *Enterococcus* and *Mangrovibacter* in PU-fed superworms, indicating their strong associations with PU diet in superworms. Especially *Mangrovibacter* was probably a specific functional group associated with PU diet in superworms. The results showed that the gut microbiomes were shaped by larvae species, and core gut microbiomes between superworms and yellow mealworms were markedly different with ingesting PS and PU foam under the same culture conditions.

To further assess whether specific OTUs were associated with different diets, the relative abundance changes of gut microbiome at genus level were compared within the different plastic feeding groups (Fig. 5D and E). Compared to the bran-fed group, abundances of *Spiroplasma* decreased in PS- and PU-fed groups, similar to that of PS- or PE-fed yellow mealworms in previous studies (Brandon et al., 2018; Yang et al., 2021c), suggesting that *Spiroplasma*, as a common gut genus, may not be involved in the degradation of PS and PU plastics. The relative abundances of *Enterococcus*, *Sphingobacterium*, and *Dysgonomonas* increased in PS-fed superworms, while the relative abundances of *Enterococcus* and *Mangrovibacter* increased in PU-fed superworms, indicating the gut microbiome changes were related to the diet. In yellow mealworms groups, the relative abundance of *Hafnia* increased to 55.65% and 4.5% in PS- and PU-fed yellow mealworms, respectively, in comparison to bran-fed yellow mealworms, suggesting that *Hafnia* probably played significant roles in PS and PU degradation in yellow mealworms. Relative abundance of unclassified Enterobacteriaceae increased by 12.76% in PU-fed yellow mealworm, while decreased in PS-fed yellow mealworms. In conclusion, the gut microbiomes were also shaped by diets.

To further understand the differences in gut microbial communities of both species, PCA analysis of genus and hierarchical analysis based on Bray-Curtis distances were conducted (Fig. 6). The microbiomes were

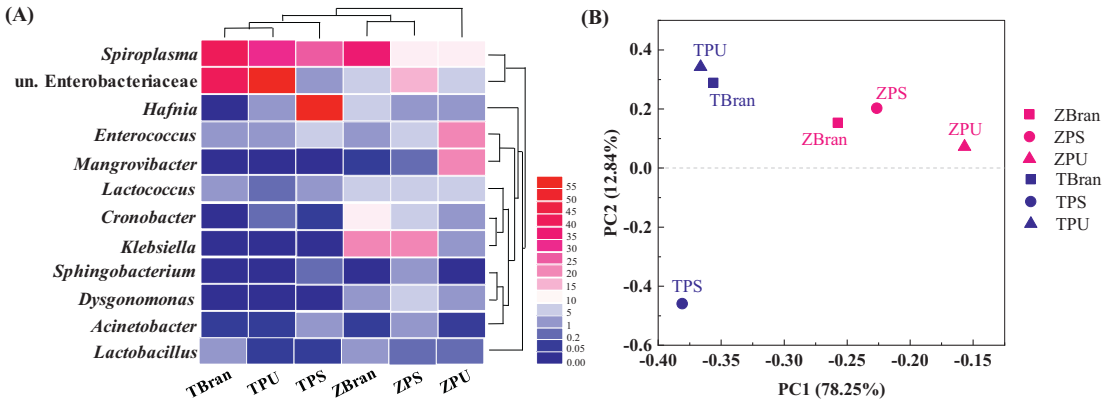

Fig. 6. Associations between gut microbial community compositions and different diets as well as larval species. (A) Hierarchical cluster analysis of gut bacterial communities in bran- or plastics-fed superworms and yellow mealworms; (B) Principal component analysis (PCA) of microbial communities of superworms and yellow mealworms fed with bran or plastics. The top twelve abundant genera in each group were included in the analysis. Z = *Z. atratus*; T = *T. molitor*; PS = polystyrene foam; PU = polyurethane foam.

grouped into four clusters: Cluster I (TBran and TPU), Cluster II (ZBran and ZPS), Cluster III (TPS) and Cluster IV (ZPU) (Fig. 6A). The gut microbiomes of bran- and PU-fed yellow mealworms were similar, and in a different cluster from the PS-fed yellow mealworms. The gut microbiomes of bran- and PS-fed superworms were similar. The bacterial community of PU-fed superworms was in a distinct cluster (Fig. 6A). All three superworm groups clustered together in the PCA plot (Fig. 6B). Yellow mealworm groups with different sole diets were in one group although the PS-fed yellow mealworm group had lower similarities with bran- and PU-fed groups. The PCA and hierarchical cluster analysis results suggested that the gut-microbiome shifts were associated with both plastics sole diets and larval species.

In addition to the distinct dominant gut bacteria, the gut-fungus, the larvae host, and host gut enzyme(s) probably play critical roles in plastics depolymerization and biodegradation. Thus, future studies should focus on isolation and characterization of the key microbial species involved in plastics biodegradation, monitoring changes of gut fungal diversity and the host gut enzymes during plastic feeding. Characterization of the metabolic pathways of various bacteria and fungi will shed insights on the mechanisms of plastics biodegradation in worms. Considering the differences of larvae gut microbiomes of the same species possibly due to different geological sources, comparison of the same larvae species from different geological sources under the same experiment conditions may provide evidence for the key species involved in plastic degradation.

4. Conclusions

Differences of PS and PU foam consumption and biodegradation by superworm *Z. atratus* and yellow mealworm *T. molitor*, and the associated changes of plastics and gut microbiomes were characterized in this study. Under the same condition, superworms had a higher cumulative PS foam plastics consumption rate than that of yellow mealworms, but similar cumulative PU consumption rates by both species when calculated by mg-plastics per unit larval mass. With sole plastics diets, superworms showed the overall trend of decreased weight, while yellow mealworms showed weight gains, indicating a higher metabolism demand of superworms than yellow mealworms. The results of ATR-FTIR analysis indicated that oxidation and biodegradation processes of PS and PU foam plastics in guts of both species were similar. Different from the associations of unclassified Enterobacteriaceae, *Klebsiella*, *Enterococcus*, *Dysgonomonas* and *Sphingobacterium* with PS diet in superworms, *Hafnia* was strongly associated with PS diet in yellow mealworms. *Enterococcus* and *Mangrovibacter* were the dominant OTUs in PU-fed superworm gut microbiome, while unclassified Enterobacteriaceae and *Hafnia* were predominant in PU-fed yellow mealworm gut microbiome. The gut microbiome diversities were significantly associated with foam plastic diets and larvae species.

CRediT authorship contribution statement

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Hongxuan Wang: Investigation.
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Tianfang Han: Writing - Original Draft.
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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155719.

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