



Responses of gut microbiomes to commercial polyester polymer biodegradation in *Tenebrio molitor* Larvae

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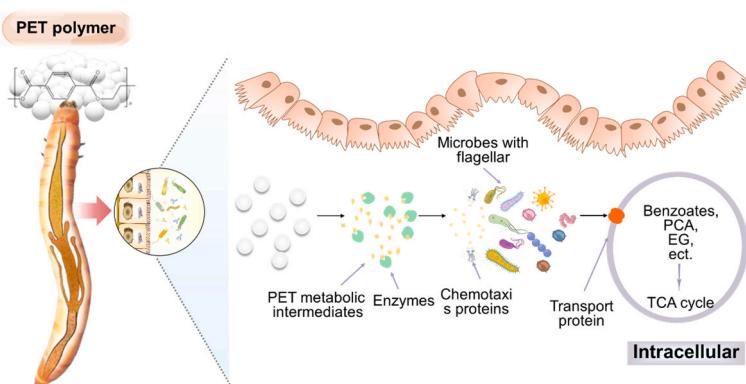
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HIGHLIGHTS

- Confirmation of commercial PET biodegradation by *Tenebrio molitor*.
- Minor difference in degradation performance of two PET polymers.
- Spiroplasma*, *Dysgonomonas* and *Hafnia-Obesumbacterium* associated with PET degradation.
- Both host and gut microbiota contributed enzyme repertoire to PET degradation.
- A plausible mechanism was proposed based on 16 S rRNA and metabolome analyses.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyethylene terephthalate (PET) is a mass-produced fossil-based plastic polymer that contributes to catastrophic levels of plastic pollution. Here we demonstrated that *Tenebrio molitor* (mealworms) was capable of rapidly biodegrading two commercial PET resins (microplastics) with respective weight-average molecular weight (M_w) of 39.33 and 29.43 kDa and crystallinity of $22.8 \pm 3.06\%$ and $18 \pm 2.25\%$, resulting in an average mass reduction of 71.03% and 73.28% after passage of their digestive tract, and respective decrease by 9.22% and

Abbreviations: FA, Fatty acid; FC, fold change; GO, gene ontology; GPC, gel permeation chromatography; HFIP, 1,1,1,3,3-hexafluoroisopropanol; MCPs, methyl-accepting chemotaxis proteins; MPs, microplastics; M_n , number-average molecular weight; M_w , weight-average molecular weight; M_z , size-average molecular weight; MW, molecular weight; NPs, nanoplastics; OUT, operational taxonomic unit; PCA, principal component analysis; PDI, polydispersity index; PE, polyethylene; PET, polyethylene terephthalate; PICRUSt2, phylogenetic investigation of communities by reconstruction of unobserved states; PP, polypropylene; PS, polystyrene; PUR, polyurethane; PVC, polyvinyl chloride; SPCR, specific PET consumption rate; SR, survival rate; TCA, tricarboxylic acid; WB, wheat bran.

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Metabolome
Gut microbiota

11.36% in M_w of residual PET polymer in egested frass. Sequencing of 16 S rRNA gene amplicons of gut microbial communities showed that dominant bacterial genera were enriched and associated with PET degradation. Also, PICRUSt prediction exhibited that oxidases (monooxygenases and dioxygenases), hydrolases (cutinase, carboxylesterase and chitinase), and PET metabolic enzymes, and chemotaxis related functions were up-regulated in the PET-fed larvae. Additionally, metabolite analyses revealed that PET uptake caused alterations of stress response and plastic degradation related pathways, and lipid metabolism pathways in the *T. molitor* larvae could be reprogrammed when the larvae fed on PET. This study provides new insights into gut microbial community adaptation to PET diet under nutritional stress (especially nitrogen deficiency) and its contribution to PET degradation.

Environmental Implication

Improper disposal of plastics can release toxic chemicals and pollutants into nature. It leads to the formation of microplastics and nanoplastics, which accumulate in the environment, impact natural habitats, and induce reproductive, neuro, and immune toxicities in all life forms.

For the first time, we characterized and found PET degradation efficiency by mealworms was faster than other known microorganisms. Through omics analyses, we explored how the larvae survive and utilize PET as carbon sources under nutritional stress of PET feeding. This breakthrough opens a new avenue for rapid and low-carbon biodegradation of petroleum-based plastics.

Data availability

Data will be made available on request.

1. Introduction

Polyethylene terephthalate (PET), a semicrystalline thermoplastic polymer of aliphatic–aromatic polyester group with the chemical formula $[C_{10}H_8O_4]_n$ [1,2]. PET incorporates into our daily life mainly in manufacturing materials for textile fibers, packaging, household, etc. [3] and is well known as “Polyester” in the textile industry [4]. Global PET production is raised continuously by an estimated rate of 8.4% in 2021 [3]. However, recycle of it to bottles, fiber, films, and sheets is just 28.4% [5]. The majority is dumped into nature, disrupting ecosystems due to ultimate weathering over time and release of toxic constituents [6].

At present, countermeasures are developed via appropriate mechanical (collected for pelletting), biological, chemical (such as methanolysis, hydrolysis, glycolysis, aminolysis and ammonolysis), and physical treatments [4,7–9]. Therein, biological processing is considered as eco-friendly and cost-effective way to manage plastic/PET waste. Different from non-hydrolysable polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC), PET belongs to hydrolysable polymers. PET has hydrolysable ester backbones and thus more susceptible to microbial degradation and enzymatic attack than petrochemical vinyl plastics with solely carbon–carbon backbones [10]. Enzymatic recycling of PET has been investigated for more than 20 years [11,12]. However, as separate polymer chains of PET are held together by van der Waals forces and H-bonding and the crystalline structures of PET polymers have hydrophobic surfaces, efficient methods for bio-depolymerization of PET are yet considered a bottle neck [13,14].

In recent years, insect larvae belonging to darkling beetle family (Coleoptera: Tenebrionidae) [15–23], pyralid moths family (Lepidoptera: Pyralidae) [24–29], and other macroinvertebrates [30,31] have been identified to biodegrade major plastic polymers including PS, PE, PP, PVC and PUR. *Tenebrio molitor* (*T. molitor*, mealworms), which is commercially available as pet feed and easily breed, is able to degrade plastics within short digestive time (about 12–15 hrs in intestine) and has been well-studied [32–37]. The ingested plastic polymers were

degraded rapidly, resulting in short half-life between 3.4 and 16.6 hrs, depending on polymer type and molecular weight (Table S1). The half-life of plastics in the larvae was only 0.015–1.79% of that of bacterial cultures tested *in vitro* of 924–21789 hrs (Table S1). Polymer molecular weight, which is presented as number-average molecular weight (M_n), weight-average molecular weight (M_w) and size-average molecular weight (M_z), has strong impact on depolymerization, biodegradation and gut microbiome during plastics degradation by *T. molitor* [38,39]. Although biodegradation of above major plastics in *T. molitor* larvae has been confirmed [19,40–42], the biodegradation and depolymerization of polyester or PET plastics (*i.e.* PET) in *T. molitor* larvae have not been characterized and the microbiome has not yet been well investigated.

In this research report, we investigated PET degradation by *T. molitor* larvae with two commercial PET polymer resins with M_w of respective 29.43 kDa and 39.58 kDa, compared the PET biodegradation performance, and characterized gut microbiome and metabolome of the larvae degrading the commercial PET polymers. The results indicated that the biodegradation of PET polymers resulted in different gut microbiomes and metabolomes from the larvae fed high purity PET MPs with defined molecular weights.

2. Materials and methods

2.1. *T. molitor* species and feedstocks

T. molitor larvae (weighting 27.13 ± 0.5 mg/larvae) tested for biodegradation were purchased from a breeding farm in Dezhou, Shandong, China. The larvae were fed with wheat bran (WB) prior to testing.

Commercial PET resin in form of microplastic (MP) powders (< 75 μm) with M_n of 24.83 kDa and M_w of 39.33 kDa and crystallinity degree of 22.8 ± 3.06 was purchased from Hongyuan Plastic Factory (Shanghai, China) and another PET MP powders with M_n of 18.59 kDa and M_w of 29.43 kDa and crystallinity degree of $18 \pm 2.25\%$ from Zhongxin Plastic Factory (Guangdong, China) (Fig. S1a and b). The two commercial plastics were tested and compared for biodegradation. According to the manufacturers and our analysis (Supporting Information, Text S1), they did not contain toxic and organic additives or catalysts and were named in this study as PET-1 and PET-2, respectively, for comparison. They had trace ash components (<0.02%, w/w) including Na, Mg, P, S, Ca, Fe and Zn but well below the levels supporting microbial growth.

WB were purchased from a breeding farm in Dezhou, Shandong, China. Agar powder was biological reagent and was purchased from Aoboxing Biotechnology Co., Ltd (Beijing, China). It was used as adhesive to make agar-PET gel to feed the larvae. 1,1,1,3,3-hexafluoroisopropanol solvent (HFIP, 99.5%) was obtained Aladdin Reagent Int., (Shanghai, China). All other chemicals were purchased from Macklin (Shanghai, China).

2.2. Biodegradation of PET polymers

Prior to the experiment, the larvae were starved for 24 h to digest

their intestinal contents from previous feedstock and egest frass. Then, they were reared with different diets in food-grade PP containers as incubator under controlled conditions ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $70 \pm 5\%$ relative humidity and dark environment). As dry PET MPs powders tend to stick to the larval body, which could influence the results of test, and the larvae do not favor eating plastic powders, the PET MPs were mixed and wrapped in agar to form solidified PET-agar gel as feedstock for the larvae. Agar does not supply minerals and nutrients even though it was digested by the gastrointestinal tract [43]. For making up PET-agar gel, 5 g of PET powders were mixed with 100 ml of boiled agar solution (2%, w/w) and then solidified prior to feeding the larvae. In each incubator ($16 \times 11 \times 5$ cm), 500 larvae were reared and fed PET-1 or PET-2 to evaluate biodegradation performance for 36 days. Control groups included unfed (starvation) larvae, and the larvae fed on the agar gel only and WB-agar gel. Each test was conducted in triplicate. Specific PET consumption rate (SPCR, mg PET•100 larva $^{-1}$ d $^{-1}$) was calculated on the basis of the mass of PET consumed (or ingested) over the test period (36 days). Specific removal rate (mg PET 100 larvae $^{-1}$ d $^{-1}$) was obtained on the basis of the subtraction of the mass of PET consumed by the mass of PET remaining in frass per 100 larvae over the test period [44].

2.3. Collection of frass and analytical methods

Larvae were cleaned with compressed air to remove the exuviate and the residues attached to the body, and moved into clean containers to collect frass. The collected frass (Fig. S1c and d) was stored at -80°C prior until analysis.

Molecular weights and polydispersity index (PDI) of PET were determined by gel permeation chromatography (GPC) using Agilent PL-GPC-220 coupled with 1100 refractive index detector. The PET polymers were extracted with HFIP. The analytical procedures are described in details in Supporting Information (Text S2).

Fourier-transform infrared spectroscopy (FTIR, Thermo Scientific Nicolet iS20 FT-IR Spectrometer, Pittsburgh, PA, the USA) was used to detect changes in the molecular structure and chemical bonds on the polymer surface in the frass and the plastics. Proton nuclear magnetic resonance (^1H NMR, Bruker 400 M, Bruker Biospin GmbH, Germany) spectra was recorded to confirm the PET oxidation and biodegradation with dimethyl sulfoxide-d₆ (DMSO, 99.9%, Cambridge Isotope Laboratories, Inc., Andover, U.S.A.) as solvent. The ^1H -spectra [16 scans, delay time (d_1) = 1.0 s] were referenced to the residual deuterated-DMSO peak [2.5 ppm]. Spectra were analyzed using MestReNova software (version 14.0.0). The crystallinity degree of the PET polymer was determined using an X-ray diffraction instrument (Bruker D8 Advance, Germany). Plastic remnants in frass that passed through larvae digestive tract were calculated as described in Supplemental Text S3.

2.4. Gut microbiome analysis

Intestinal tissue samples were collected from the larvae fed with PET-2 and WB and unfed larvae. The collected samples were immediately rinsed with sterile water to remove soluble organic contaminates from host. High-throughput sequencing of bacterial 16 S rRNA gene amplicons was performed on the Illumina MiSeq platform (Illumina MiSeq PE300, Illumina Inc., San Diego, CA, USA) with detailed information described in Supplemental Text S4. On the online platform of Majorbio Cloud Platform (www.majorbio.com), to ensure accuracy of data analysis. the data was processed by removing contaminating sequences (mitochondrial), normalizing the sample sequencing depth before proceeding with bioinformatic analysis. PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states, version 2.2.0) (<https://github.com/picrust/picrust2/>) was carried out to predict the functional composition of gut microbiota of each group using 16 S OTU table. BugBase (<http://github.com/danknights/bugbase>) was used to calculate differences among groups in terms of microbial phenotypes.

2.5. Metabolome analysis

Untargeted metabolomic profiling was performed by the ultrahigh-performance liquid chromatography coupled with quadrupole exactive mass spectrometry (UPLC-Q Exactive system, Thermo Fisher Scientific, USA). Digestive tract samples were collected from the larvae fed PET-2 and agar. Detailed experimental procedure described in Supplemental Text S5. The generated raw data were preprocessed by Progenesis QI (Waters Corporation, Milford, USA) and searched in the main database of HMDB (<http://www.hmdb.ca/>), Metlin (<https://metlin.scripps.edu/>) and Majorbio Database. During the data analysis, metabolic features detected at least 80% in any sample set were retained. In the normalized data matrix, variables with relative standard deviation $> 30\%$ of QC samples were removed, and log₁₀ logarithmization was performed to obtain the final data matrix for subsequent analysis.

3. Results and discussion

3.1. Consumption of PET microplastics by *T. molitor* larvae

The *T. molitor* larvae actively ate the PET-agar-gels made of PET-1 MPs and PET-2 MPs (Fig. 1a). PET-agar gels of 30.68 ± 0.11 g PET-1 and 33.86 ± 0.54 g PET-2 were respectively ingested by the group of 500 larvae over a 36-day period, and the respective average SPCR were 190 ± 3.0 mg PET 100 larvae $^{-1}$ d $^{-1}$ and 203 ± 6.0 mg PET 100 larvae $^{-1}$ d $^{-1}$ with specific removal rate more than 136 mg PET 100 larvae $^{-1}$ d $^{-1}$ and 152 mg PET 100 larvae $^{-1}$ d $^{-1}$ over 36 days, indicating that the consumption rate of PET-1 MPs in this study was slightly lower than PET-2 but still within the similar range.

Sharp increase in averaged weight of the larvae fed with different diets was observed during initial 8 days (Fig. 1b). This is likely due to recovery from previous starvation by digestion ingested carbon sources (WB, PET and agar). As expected, the unfed larvae continued weight loss. After then, the larval weight of PET-1, PET-2 and agar only-fed groups had little changed and slightly dropped to reach final weight within similar range i.e., 38.00 ± 0.79 mg-larvae $^{-1}$ for PET-1, 38.77 ± 1.35 mg-larvae $^{-1}$ for PET-2, and 38.99 ± 2.07 mg-larvae $^{-1}$ for agar on day 36 (Fig. 1c and data in Table S2). The WB-fed group increased to 46.63 ± 0.40 mg-larvae $^{-1}$, as expected while the unfed larvae decreased their weight to 20.35 ± 1.18 mg-larvae $^{-1}$. The similar change in averaged weight of the larvae fed on PET-agar gel and agar only suggested that the larvae likely digested both agar and PET as energy source to support their life activities in comparison with the unfed groups that live on consumption of their body biomass with significant weight loss by $21.92 \pm 3.14\%$. Compared with WB fed larvae, no weight increase or growth was observed in the PET and agar only-fed groups. This is because either agar or PET does not contain important nutrition, such as vitamin, minerals, and amino acids and efficient minerals for cell growth.

As expected, the lowest survival rate was observed in unfed group ($44.4\% \pm 0.52\%$) while the highest in WB-fed group ($91\% \pm 0.72\%$) (Fig. 1d). The lower survival rate of agar only gel-fed larvae (65.67 ± 1.63) compared to the PET agar gel-fed larvae (69.93 ± 1.92 and 76.07 ± 0.90 for PET-1 and PET-2, respectively) suggested that the larvae fed with PET-agar gel received more energy source than agar only for survival (Fig. 1d and S2, data in Table S2). Because PET-agar gel fed and agar only gel-fed groups received same amount of agar, the higher survival rates of PET-agar-fed groups than agar only-fed group supported that the larvae received energy from digestion or biodegradation of PET. This observation was similar to those observed previously when the larvae were fed with other plastic foams in comparison with the unfed control or the WB-fed control, and weight loss and SR decline were observed as *T. molitor* larvae were fed PS, LDPE, PVC, and PP as sole diet, indicating that plastics did not have adequate nutrition sources for larvae although the ingested plastics were able to contribute to energy and even assimilated as carbon source of the larval biomass [22].

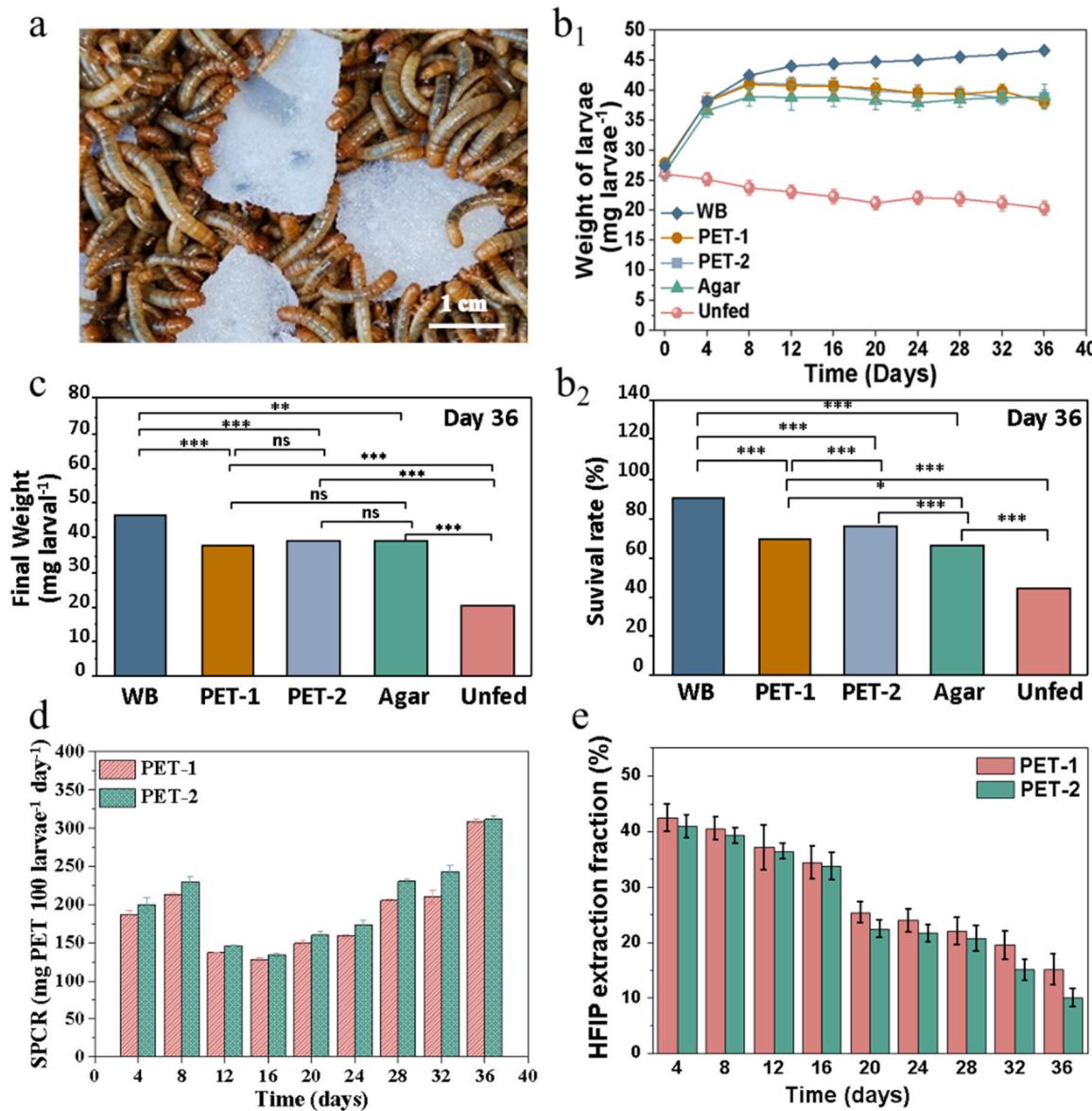


Fig. 1. Physiological response of *Tenebrio molitor* larvae to PET consumption and degradation. (a). *T. molitor* larvae fed with PET-agar gel. (b) The changes of averaged weight of larvae fed with different diets over a 36-day experiment period. (c) The final average weights of the larvae fed different diets (d) Survival rates of larvae fed with different diets at the end of the test. (e) PET consumption rates of PET-1 and PET-2 as test period elapsed. (f) Progressive decrease in the HFIP-extractable fraction in frass indicating decrease in residual PET in frass and improved PET degradation. All measurements were conducted in triplicate ($n = 3$). Student's t-tests: $p < 0.05$ indicated by *, $p < 0.01$ indicated by **, $p < 0.001$ indicated by ***, and no statistical significance indicated by ns.

The results implied that PET did not provide necessary nutrition and efficient minerals for the larvae growth and development for a long term although its digestion sustained the life and activity of larvae.

In addition, we measured the water content of mealworms supplemented with PET agar gel and agar gel alone, and found that the water content was in the similar range i.e. $58.3 \pm 0.98\%$ and $62.1 \pm 1.04\%$, respectively. Since *T. molitor* larvae have the ability to store water in their body [45,46], the water content in agar gel helped their higher survival rates than unfed larvae.

The SPCRs were calculated every four days over 36-day test period (Fig. 1e, data in Tables S2 and S3). The larvae exhibited slightly higher consumption rates for PET-2 than PET-1 during initial 8 days, then declined on day 12 and day 16 then gradually elevated (Fig. 1e). The initial higher rates could be forced by previous starvation. The decrease in SPCRs within the first 16 days could be due to the inadaptation of the larval metabolism to PET diets after initial forced eating behavior. The gradual increase after day 16 suggested that the larvae and their gut

microbiota adapted new feedstock and stabilized their digestive system to PET-agar gel. According to previous studies [33,47], plastic-degrading bacterial strains colonized and showed signs of respiration on plastics (e.g., PS and PE) after 2 weeks of *in vitro* cultivation. The gut microbes for PET degradation could adapt PET more rapidly *in vivo* but still takes time. The increase in the PET consumption rate by the larvae was possibly helped by the shift of the gut microbiota to accommodate the PET diet as well as agar. The averaged SPCRs of both PET polymers over 36 days (190 ± 3.0 mg PET-1•100 larva⁻¹ d⁻¹ and 203 ± 6.0 mg PET-2•100 larva⁻¹ d⁻¹) were generally higher than the those of other plastic materials consumed by *T. molitor* larvae published before, i.e., PS at 8.46 ± 0.14 – 20.71 ± 0.51 mg•100 larvae⁻¹•day⁻¹ as tests [15,37], LDPE foam at 4.9 ± 0.3 mg•100 larvae⁻¹ d⁻¹ day⁻¹ to about 23.5 mg•100 larvae⁻¹ d⁻¹ day⁻¹ [15,41], PVC MPs at 36.62 ± 6.79 mg•100 larvae⁻¹ d⁻¹ [19], PLA powder at 45.2 – 202.7 mg•100 larvae⁻¹ d⁻¹ [48], PP foam at 1.0 – 1.6 mg•100 larvae⁻¹ d⁻¹ [42], PUR foam at 18 mg•100 larvae⁻¹ d⁻¹ [17] using the

larvae from different sources in the USA, China, and the United Kingdom (Table S4). The results indicated that PET consumption rates by *T. molitor* larvae were much higher than other major plastics including PE, PP, PS, PVC and PUR.

The residual PET polymers in the egested frass were determined using the HFIP extractable fraction in the frass of the larvae fed the PET MPs. The HFIP extractable fraction (% w/w) decreased gradually over the 36-day test period (Fig. 1f and Table S3). The undigested and partially digested PET residues in the frass of the larvae fed with both PET-1 and PET-2 appeared to be insignificantly different. For PET-1 and PET-2, the polymer residues in the frass decreased gradually from initial $42.45 \pm 2.53\%$ and $40.92 \pm 2.09\%$ on day 4– $34.36 \pm 2.91\%$ and $33.70 \pm 2.39\%$ on day 16, respectively. Thereafter, they decreased continually, and reached only $15.24 \pm 2.79\%$ and $10.11 \pm 1.61\%$, on day 36 with 84.76% and 89.89% mass reduction for the PET-1 and PET-2, respectively. The consumed PET and agar could be converted to CO_2 , assimilated to larval biomass, and egested as degraded intermediates in frass residues as described during PS biodegradation by *T. molitor* larvae by feeding PS only previously [22]. Due to the feeding mixture of PET

and agar together in this study, it was impossible to determine stoichiometric carbon mass balance. However, future research is still needed to overcome the technical challenge.

Over 36 days, the averaged mass reduction was 71.03% and 73.28% for the ingested PET-1 and PET-2. Recent study indicated that passage through digestive tract, no nanoplastics (NPs) appeared in the frass of PE-fed and PS-fed *T. molitor* larvae [49]. With much higher removal efficiency of PET than PS and PE, we expected no PET NP accumulation in the frass. Future study is needed to verify this hypothesis.

Similar to consumption rates discussed above, the degradation rates and mass reduction efficiencies of PET by *T. molitor* larvae are higher than those reported on other persistent plastics (*i.e.*, PS, PE, and PVC) but are slightly lower than those of biodegradable polymer PLA [48]. This is basically due to polymer biodegradability as described during plastic degradation in environment [50]. In addition, the half-life of PET polymers (3.36 and 4.32 hr for respective PET-1 and PET-2) during biodegradation by *T. molitor* larvae is much shorter than that (924 hr) of *in vitro* PET-degrading bacterium *Ideonella sakaiensis* 201-F6, which had the highest PET degrading capacity [51].

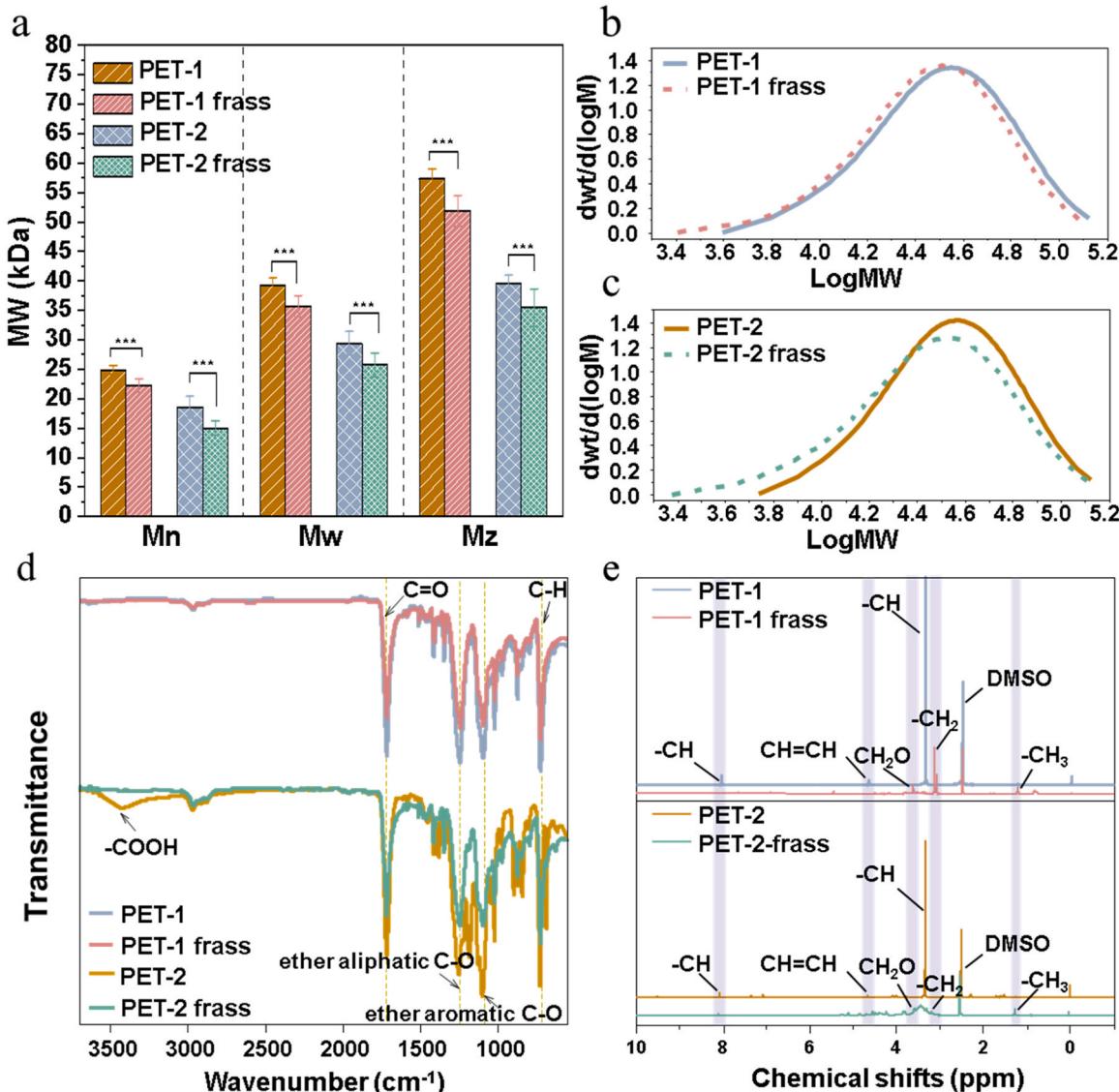


Fig. 2. Characterization of biodegradation of PET polymers. (a) Comparison of M_w , M_n , and M_z of PET-1 and PET-2 before and after biodegradation (Significant correlations are indicated by asterisks, where * represents $p < 0.05$, ** indicates $p < 0.01$, and *** marks $p < 0.001$). (b) MW distribution shift of PET-1 before and after biodegradation. (c) MW distribution shift of PET-2. (d) FTIR spectra of residual polymer from the frass of PET-1 and PET-2 fed mealworms. (e) ^1H NMR spectra of residual polymer from the frass of PET-1 and PET-2 fed mealworms.

3.2. PET depolymerization and oxidation/biodegradation

M_n , M_w and M_z are key molecular weight (MW) parameters and provide information on the changes in molecular properties and polymer structures. The results of GPC analysis indicated that MWs of residual polymers from the frass of larvae fed with both PETs decreased significantly ($p < 0.001$) (Fig. 2a, data in Table S2), i.e., M_n values of PET-1 and PET-2 decreased by 10.49% and 19.32%, respectively; M_w declined by 9.22% and 12.5%, respectively; M_z dropped by 9.37% and 10.4%, respectively, implying that significant reduction of PET polymer chains with a broad depolymerization pattern (decrease in all the M_n , M_w , and M_z) [19,44]. MW distribution of the two PET MPs indicated a clear shift to small MW side after PETs were ingested (Fig. 2b and c). PDI gives an indication of the broadness of the polymer distribution. After passing through the larval gut, PDI of PET-1 and PET-2 increased from 1.58 to 1.60 and 1.48–1.72, respectively, indicating broaden in the MW of residual polymer due to decomposition. The depolymerization extent or molecular weight reduction of PET-2 was slightly higher than PET-1 that had slightly higher MW, indicating that the PET polymer with lower MW can be depolymerized at the higher extent. The same trend was observed during biodegradation of PE by *T. molitor* larvae previously [38,39].

FT-IR and ^1H NMR analysis confirmed the biodegradation of both PET MPs (Fig. 2d and e). Based on the FT-IR spectra (Fig. 2d), four main characteristic peaks of raw PET MPs at wavenumbers 725, 1090, 1240, and 1713 cm^{-1} , corresponding to aromatic (C–H), ether aliphatic (C–O), ether aromatic (C–O), and carbonyl (C = O) bond were dampened or even disappeared after passage through the larval gut. Similar ATR-FTIR spectra were observed on the surface of PET bottles after significant degradation in marine environment, reported by Loakeimidis et al. [52].

^1H NMR spectra of the two PETs displayed one characteristic peak at δ 3.31 ppm (Fig. 2e), which is assigned to the –CH in the main chain. After biodegradation, the peaks of –CH were absent in both of the residue polymers due to the breakage of benzene ring. Furthermore, the formation of new peaks, i.e., methyl (–CH₃), methylene (–CH₂), methylene protons of CH₂O [53] and other small peaks, in residual PET polymers indicated the breakdown of long chain polymers during degradation.

The results of FTIR and ^1H NMR provided qualitative evidence of PET degradation and oxidation. Based on our analyses by GPC, FTIR and ^1H NMR of the frass of the larvae fed two commercial PET MPs, a general conclusion is that *T. molitor* larvae are capable of decomposing PET polymers.

The results also showed the impact of polymer MW and crystallinity on PET degradation. PET-1 had higher crystallinity degree and MW than PET-2, the lower SR of the PET-1-fed larvae, and lower PET consumption and degradation rate, and lower depolymerization extent were likely attributed to the impact of crystallinity and MW on biological attack. Increased crystallinity limits the movement of polymer chains and regularly-arranged crystalline region increase the hydrophobicity of the polymer [54]. The higher crystallinity and higher MW decrease the availability of polymer chains in enzymatic attack and cause lower degradation rates [50,55].

3.3. Microbial communities associated to PET biodegradation

The research on gut microbial communities was conducted using the larvae fed with PET-2 because the biodegradation of PET-1 and PET-2 showed a similar performance. We examined the role of gut microbiomes associated to PET degradation using the larvae fed PET-2.

Antibiotic suppression test was conducted to examine the depolymerization and biodegradation of PET in the presence of five antibiotics (gentamicin sulfate, erythrocine, chloramphenicol, tetracycline, ampicillin, and kanamycin at 0.075 mg/mg PET) in feed. This antibiotic mixture inhibits gut microbes belonging to Gram-positive and Gram-negative bacteria. After a 5-day antibiotic treatment, no gut bacteria

were detected in the bacteria counting media (Fig. S2a and b), indicating suppression of gut bacteria by the antibiotics. The M_n , M_w and M_z of residual PET in the frass of the larvae under antibiotic suppression were similar to those without antibiotic treatment (Fig. S2c). The results indicated that the larvae biodegraded PET under suppression of gut bacteria, i.e., PET biodegradation was independent of gut-bacteria. This observation is different from previous observations of PS and LDPE biodegradation i.e., PS or LDPE depolymerization was almost completely stopped or severely inhibited under antibiotic suppression [56,57]. It is noticed that the antibiotic treatment ignored the role of archaea and eukaryote because their abundance (<1%) was too low to be considered to play a significant role in PET biodegradation in the larvae (Text S6 and Text S7).

At the end of the experimental period of 36 days, 16 S rRNA gene amplicons were sequenced and gut microbial communities of *T. molitor* larvae of three diet groups were analyzed: WB-reared (normal diet), PET-fed and unfed group. The rarefaction curves with a Shannon index of all samples reached a plateau at approximately 4000 reads, implying that the number of sequences were sufficient for further analysis (Fig. S3a). The α diversity (Fig. S3b and c) of PET- and WB-fed samples and their OTU numbers (Fig. S3d) were not significantly different ($p > 0.05$). Analysis of similarities (ANOSIM) boxplot showed that the difference among groups was greater than that within groups ($R^2 > 0$), indicating that the sample grouping was statistically significant (Fig. S3e).

Changes in the relative abundance of OTUs in the larvae fed with PET diet were remarkable compared with the WB-fed and unfed groups at the family level (Fig. 3a). Ingestion of PET led to significant increase in the relative abundance of families Spiroplasmataceae, Enterococcaceae and Dysgonomonadaceae. According to the abundance bubble diagram (Fig. 3c and data in Table S5), OTUs at the genus level included *Spiroplasma* sp. (41.63%), *Acinetobacter* sp. (12.28%), *Hafnia-Obesumbacterium* sp. (6.50%), *Citrobacter* sp. (5.70%), *Enterococcus* sp. (5.22%), *Lactococcus* sp. (5.04%), and *Dysgonomonas* sp. (2.4%) as dominant genera (abundance > 2%). *Enterococcus* sp. was reported as a plastic-degrading genus enriched or isolated from environments as plastic-degrading bacteria [18,58,59]. Members of *Spiroplasma* sp. are well known as common gut-associated bacteria in *T. molitor* larvae gut microbiota associated with plastic-degradation, e.g., LDPE, PS, and PLA [21,41,48]. Venn analysis reflects the correspondence and abundance information among different groups of species. As shown in Fig. 3b, only respective 47.17% and 55.35% of bacteria in PET-fed larvae had overlaps with the microbiota of unfed and WB-fed larvae, indicating that a majority of gut bacteria from the PET-fed larvae did not exist at a significant level in the larval guts fed with WB or unfed although these three groups had the same gut microbial sources. The results indicated that PET diet shifted gut microbial communities remarkably.

To analyze dominant bacterial genera for PET metabolism and possible functions in PET depolymerization, two-factor correlation network analysis was used to find potential correlations of PET biodegradation and associated species represented by the Spearman's correlation ($p < 0.05$) (Fig. 3d). The result exhibited that three genera could be related to PET biodegradation, i.e., *Spiroplasma* sp. (41.63%), *Dysgonomonas* sp. (2.4%), and *Hafnia-Obesumbacterium* sp (6.5%). Previous studies indicated that *Spiroplasma* sp. was strongly associated with PS diet in *T. obscurus* [21], and *Dysgonomonas* sp. was correlated with lignin degradation, inferring that they may participate in biodegradation of aromatic rings in PET [60]. However, further studies are still needed to identify whether the contribution of the two genera was to directly break down (or depolymerize) PET chains, or degrade intermediates, or perform both reactions. The heatmap diagram of species correlation analysis (Fig. S4) of microbial communities in PET-fed samples ($p < 0.05$), *Dysgonomonas* sp. ($r = 0.866$) and *Hafnia-Obesumbacterium* sp. ($r = 0.866$) co-occurred. Interestingly, as exhibited by the PICRUSt analyses (Fig. 4a and Data S1), there were more flagellar biosynthetic protein-related enzymes over-expressed in the PET fed

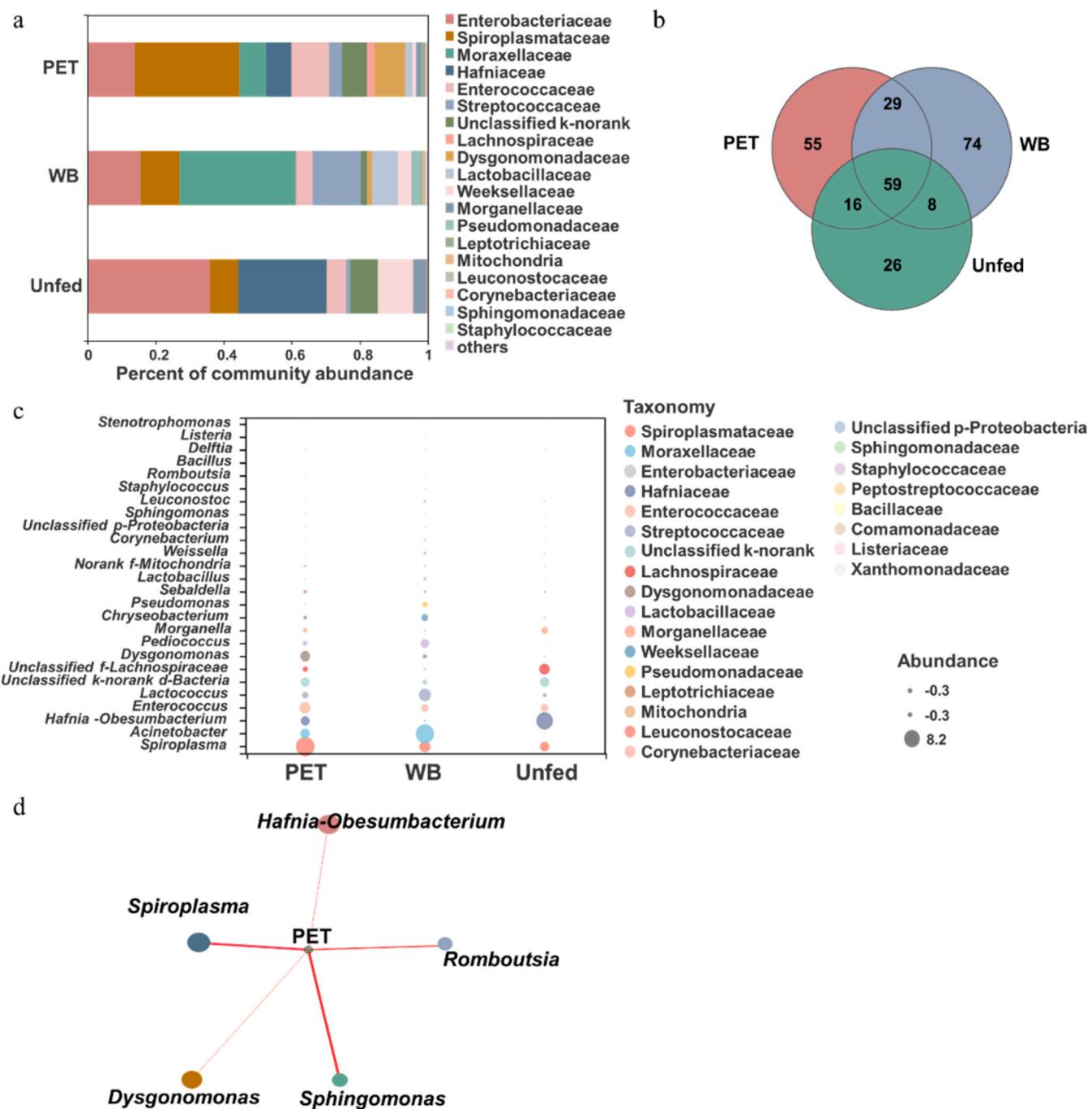


Fig. 3. Analyses of the gut microbial communities of *T. molitor* larvae. (a) Relative bacterial abundances of dominant populations in the gut microbial communities of larvae under three different diets at the family level (bacteria < 0.001 in abundance were categorized as others). (b) Venn diagram on the shared and unique microbial operational taxonomic units among PET, WB, and unfed groups. (c) Abundance bubble diagram in the genera level (bacterial species <0.001 in abundance were categorized as others). (d) Correlation-based network analysis of bacterial species co-occurrence patterns for PET feeding.

group than in the WB fed group. This likely reflected that more flagellar bacteria were developed with PET diet than with WB diet. In addition, connection among cells was observed among an effective PET degrading bacterium-*Ideonella sakaiensis* 201-F6 when they degraded PET [51]. However, the results of PICRUSt analyses are only predictions, providing a hypothesis on PET biodegradation. Further study is still needed to understand flagellar physiology with PET and WB diets in relation to metabolism and biodegradation.

Notably, predominated bacteria *Hafnia-Obesumbacterium* sp. and *Spiroplasma* sp. are facultatively anaerobic commensals and *Dysgonomonas* sp. are anaerobic bacteria. A decreased oxygen and redox gradient

are likely present along the intestinal track of the larvae, resulting in intestinal environments for heterotrophic aerobic, facultative and anaerobic microbes. Oxygen in air enters the intestinal tract as the larvae chew food (PET, WB, etc.) and gradually decreases in foregut and midgut, and likely is consumed in hindgut where it is generally under anaerobic conditions. N₂ in air also enters the intestinal tract and serves as N source by nitrogen fixing microbes which were found in the gut of *T. molitor* larvae [61,62]. Nitrogen is an essential element for all living things growing and developing. Insects living on diets with low N content rely on N-fixing bacteria to mediate nutritional nitrogen requirements [63]. In this study, *Sebaldella* sp., which was reported to be

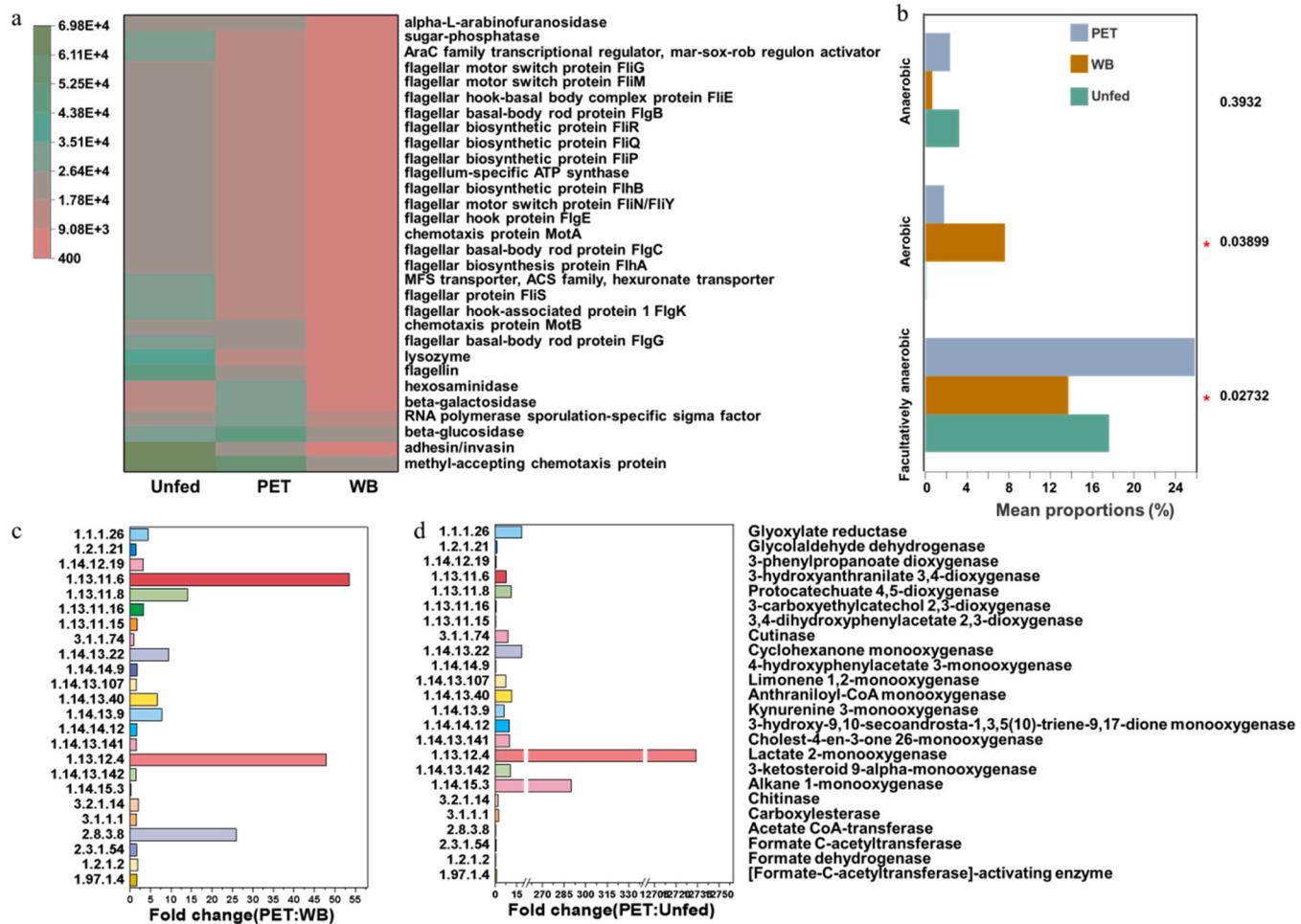


Fig. 4. PICRUSt prediction of the gut microbiome. (a) Heatmap plot of the GO of the gut microbiome during PET utilization by larvae predicted by PICRUSt analysis. (b) Kruskal-Wallis H test on phenotype differences prediction based on Bugbase analyses, where * represents $p < 0.05$, ** indicates $p < 0.01$, and *** marks $p < 0.001$. Fold change of differential PET-metabolism related enzymes between PET-fed and WB-fed groups (c) and between PET-fed and unfed groups (d) predicted by PICRUSt analysis.

related to nitrogen fixation [64], was detected at higher abundance in the larvae fed PET (abundance of 1.22%) than those fed WB (0.39%) and unfed (0.18%). Biodegradation of PET and intermediates as well as nitrogen-fixation could involve more facultative and anaerobic bacteria. The BugBase Phenotypic prediction further confirmed the concept (Fig. 4b and S5), which exhibited that the relative abundance of facultatively anaerobic bacteria is significantly more ($p = 0.027$) in PET-fed group ($25.86 \pm 8.83\%$) compared to those of WB-fed group ($13.75\% \pm 2.93\%$). In contrast, aerobic bacteria were significantly less ($p = 0.039$) in PET-fed samples than in WB-fed samples.

In environment (e.g., wastewater treatment), facultative anaerobic/anaerobic microbes mainly contribute to biodegradation of various organics [60]. In the larval gut, they could degrade PET depolymerized intermediates via hydrolysis, fermentation, and final mineralization. Therefore, in this study, PET was oxidized and/or hydrolyzed to intermediates, and then decomposed into small molecular of organic intermediates such as formic acid and acetic acid, which can be utilized by various bacteria (such as *Dysgonomonas* sp.) as electron donor to convert to CO_2 . This hypothesis was supported by the observation of the over-expressed enzymes for formate formation (EC 1.97.1.4, EC 1.2.1.2), and metabolism of formate and acetate (EC 2.3.1.54, EC 2.8.3.8) in the PET-fed samples (Fig. 4c). On the other hand, the over-expression of many flagella biosynthesis-related proteins suggested that the possibility of degradation of PET intermediates could involve in transferring electrons through conductive flagella. Further investigation

is needed to confirm these hypotheses. During this process, N-fixing bacteria which belong to anaerobic microbes in the plastic-eating larvae supplemented nitrogen source under nitrogen limitation feeding condition.

3.4. Functional prediction of the gut microbial community

The results of PICRUSt analyses revealed the functions of gut microbial communities of *T. molitor* larvae during PET degradation. The larvae fed with normal food of WB was used as the control group. From previous studies on enzymatic PET degradation, enzymes associated with PET biodegradation or/and modification include hydrolases (i.e. cutinase, carboxylesterase, chitinase) [65,66] and oxidase (i.e. alkane 1-monooxygenase) [12]. The results showed that carboxylesterase and chitinase were up-regulated in PET-fed larvae compared with the control group with FC of 1.53 and 2.06 (Fig. 4c). Carboxylesterase was reported to be associated to PET depolymerization and exhibited especially high active toward PET oligomers [12,55,66]. Chitinase identified in *Streptomyces thermophilus* OPC-520 was reported to have poly(ϵ -caprolactone)-degrading activity [65], and *Dysgonomonas* sp. (2.4% abundance in OTU), one of dominated gut microbes, was predicted to produce carboxylesterase and chitinase for PET in this study. The results implied that the larvae could benefit from symbiotic bacteria due to their ability to production of enzymes to support PET biodegradation, as Urbanek et al. discussed previously [67].

Oxidases, a total 24 of monooxygenases and dioxygenases, were also over-expressed ($FC \geq 1.5$) in PET-fed group (Fig. 4c and Data S1). The significantly enriched various oxidases in PET-fed samples implicated that these enzymes could contribute to PET oxidization in the larval gut. PET can be hydrolyzed into bis (2-hydroxyethyl) terephthalate and mono-(2-hydroxyethyl) terephthalate [68]. Mono-(2-hydroxyethyl) terephthalate can be further hydrolyzed to terephthalic acid and ethylene glycol (EG). Terephthalic acid is usually metabolized to protocatechic acid by dioxygenase [68]. In this study, the gut microbiome was predicted to produce oxidases that metabolize 4-hydroxyphenylacetate (EC 1.14.14.9), 3,4-dihydroxyphenylacetate (EC 1.13.11.15), 3-phenylpropanoate (EC 1.14.12.19), 3-hydroxyanthranilate (EC 1.13.11.16), and protocatechic acid (EC 1.13.11.8) because these enzymes were more abundant in the PET-fed samples than WB-fed groups, indicating they could be related to degradation of PET intermediates. Similarly, PET metabolism could occur through a sequential oxidation of EG to glyoxylate via the intermediates glycolaldehyde and glycolate [69] by gut microbes. In comparison with WB-fed and unfed groups, glycolaldehyde dehydrogenase (EC 1.2.1.21) and/or glyoxylate reductase (EC 1.1.1.26) were up-regulated in the PET-fed group (Fig. 4c), suggesting metabolism of EG. These results indicated that the gut bacterial community of PET-fed larvae was capable of decomposing PET into intermediate metabolites of aromatic organic acids and EG, which could be further transformed and enter the tricarboxylic acid cycle (TCA cycle) prior to mineralization. Other enzymes (i.e., decarboxylase, depolymerase, oxidoreductase, and dehydrogenase) were also over-represented in PET-fed samples compared with WB-fed samples ($FC \geq 1.5$). Their contributions to PET biodegradation should be further identified.

Remarkably, the genes functioned as methyl-accepting chemotaxis proteins (MCPs), tightly adherence-related proteins, and flagellin-related proteins were significantly enriched in the PET-fed group according to the gene ontology (GO) enrichment analysis (Fig. 4a). Similar over-expression of such proteins was observed during PET [70] and aromatic-aliphatic copolyester [71] degradation by marine bacterial cultures. The functional characteristics of the proteins secreted by the gut microbes is proposed as follows. First, bacteria develop chemotaxis towards aromatic compounds [72]. For example, Ni et al. proposed that a MCP triggers taxis towards aromatic compounds by sensing TCA cycle intermediates [73]. Secondly, the adhesion of microbes onto the surface of PET is ready for plastic biodegradation. For the bacteria with flagella, the adhesion capacity of bacteria is directly proportional to the number of flagella [74], and by reaching into crevices of the target objective, the flagella can grasp onto its surface to enhance bacterial adhesion [75]. Also, the flagella can facilitate electron transport to aid metabolism of organic matter [76]. As a result, bacterial cells with flagellar and developed chemotactic responses could be selected so that they would efficiently seek carbon source under low nutrient availability [77]. Therefore, the above functions were highly expressed in the larvae under starvation condition in this study.

In fact, only PET degraded products with small molecules can enter microbial cells with the help of dedicated transport systems, as reported in PE degradation by *Rhodococcus ruber* [78,79]. Not surprisingly, predicted GO, such as ATP-binding cassette (K06147), peptide/nickel transport system substrate-binding protein (K02035), and multiple sugar transport system permease protein (K02025) were identified to be the remarkably differential GO in this study (Fig. S6).

3.5. Metabolome analysis

To comprehensively and systematically understand the pathways of PET biodegradation, non-targeted metabolites were analyzed by LC-MS. These metabolites were results of syngeneic bioreactions by the gut microbiota and the larval host. To exclude the influence of metabolites from agar gel, samples of agar-fed larvae were used as a control. The principal component analysis (PCA) results indicated that the sample of

PET diet was fully differentiated from the control (Fig. S7a and b). This was consistent with the results of the hierarchical clustering analysis with a heatmap, which showed a markedly distinction in metabolites between the PET agar gel fed and agar only gel-fed groups. (Fig. S7c).

Compared with the sample of the agar-fed larvae, 103 metabolites were differentially accumulated in the PET-fed samples (fold change ($FC \geq 1.0, p < 0.5$) ((Fig. 5a, Data S2). According to the HMDB classification results, the identified significantly up-regulated differentially metabolites were classified into 10 HMDB superclass and 20 subclass (Fig. 5b and d). Of these, 41 metabolites were included in the “lipid and lipid-like molecules” (Fig. 5b). The enrichment of lipid metabolites was also observed in plastic-eating insect *Galleria mellonella* larvae in response to PE and PS feeding [34,80]. *G. mellonella* larvae compensated for the lack of nutrients in plastic by augmenting lipid metabolic pathways [34]. Gravouil et al. [78] verified that PE fragments could incorporate within phospholipid of degrading microbes, indicating that PE could be assimilated as carbon source. However, besides energy storage and assimilation, there is also possibility that the upregulated lipid metabolic pathways were a partially result of oxidative stress defense. Disruption of lipid homeostasis by MPs has been reported as normal responses of various non-plastics-degrading organisms e.g., earthworms, zebrafish living on plastics [81–83]. However, no research has been done with plastics-degrading insects e.g. *T. molitor*. Further studies are needed to verify whether PET degraded products can be transformed to lipid and assimilated by *T. molitor* larvae.

On the other hand, we detected 12, 7, 7, and 3 metabolites in the PET-fed samples belonging to respective organic acids and derivatives, organic oxygen compounds, organoheterocyclic compounds, and benzenoids (Data S3), which were under detection limit in agar-fed samples. These compounds were likely produced from PET degradation as intermediates. The main classes of differentially accumulated metabolites in the commercial PET-fed samples suggested that benzenoids, lipids and lipid like molecules, organic acids and derivative, organic nitrogen compounds, organic oxygen compounds, and organoheterocyclic compounds were PET metabolizing products by the larvae.

KEGG pathways of the 103 differentially expressed metabolites were further analyzed based on topological analysis (TA) (Fig. 5c). Among the enriched KEGG pathways, glycerophospholipid metabolism, arginine and proline metabolism, and amino sugar and nucleotide sugar metabolism were significantly enriched ($p < 0.05$). Glycerophospholipid metabolism was associated with maintaining optimal physical properties and functions of cell membranes as well as correlated with normal mitochondrial functions in the gut microbiome [38,84]. Arginine and proline metabolism is critical for maintaining osmotic equilibrium and interactions with enzymes to protect the structure of proteins. The above two pathways as well as glutathione metabolism could be stress response caused by microplastics [38,85]. In addition, plastic degradation related pathways involving alpha-linolenic acid metabolism, ether lipid metabolism, and fatty acid degradation were enriched. Amino sugar and nucleotide sugar metabolism, which is key carbohydrate metabolism, was also significantly altered in this study. Accordingly, we hypothesized that the biodegradation of PET polymer by the *T. molitor* larvae could be concorded with induction of oxidative stress related metabolic pathways by both gut microbiota and larval host due to biofragmentation of ingested plastic materials into microplastics within the intestinal environment [38]. Nevertheless, more solid evidence is needed to understand the physiological and toxicological impacts on the gut microbiota and the larvae during the biodegradation process.

3.6. The link between microbes and metabolism

Most functions in insect digestive tract are performed with the intervention of gut symbionts [86]. Therefore, the correlations between dominant bacteria and differential metabolites provide the relationship between gut microbes and metabolites (Fig. 6). Based on the Pearson algorithm, the correlation calculation showed that different metabolites

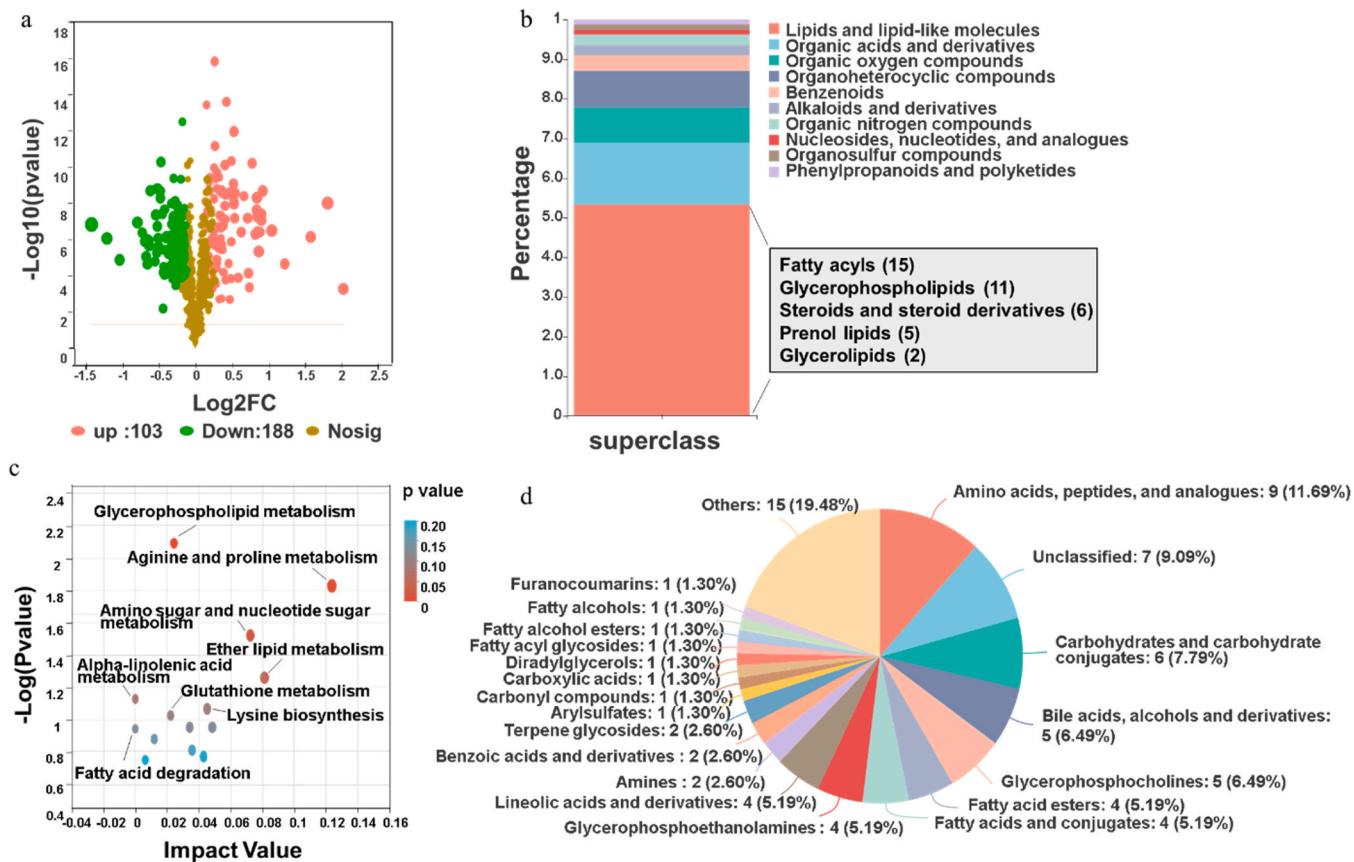


Fig. 5. Intestinal metabolic expression in *T. molitor* larvae fed PET for 36 days. (a) Volcano map of the metabolites of the PET-fed and the control (agar-fed) groups. Identified significantly up-regulated differentially metabolites were classified according to the HMDB database at superclass level (b) and subclass level (d). (c) KEGG pathway analysis of the up-regulated differentially metabolites between of PET- and agar-fed gut according to topology analysis. Data were shown as the mean \pm SD ($n = 6$).

and bacteria formed two correlation groups. Similar with the results of bacterial species correlation analysis, *Dysgonomonas* sp. and *Hafnia-Obesumbacterium* sp. co-occurred, and they formed one correlation group, which was correlated with lipid and lipid-like molecules. The results suggested that *Dysgonomonas* sp. and *Hafnia-Obesumbacterium* sp. could exert similar functions during PET degradation and showed positive correlations with pathways of lipid metabolism (i.e. ether lipid metabolism and glycerophospholipid metabolism) and carbohydrate metabolism. Particularly, PICRUSt prediction of the gut microbiome did not show significant enrichment of lipid metabolism-related genes, indicating alteration of lipid metabolic pathways was more of a host-specific response on PET uptake. The results suggested that the developed dominant bacteria in the larval gut reprogrammed the lipid metabolism pathways in the host. Plastic diets could not only lead to alterations in dominant intestinal bacteria but also in the physiological and metabolic functions of the host larvae.

3.7. A PET degradation mechanism contributed by the gut microbiota

As for the role of gut microbiota during PET biodegradation, a plausible mechanism was proposed on the basis of the results in this study and published literatures. Aerobic bacteria, and facultative anaerobic bacteria act together for PET oxidation and depolymerization, while facultative anaerobic and anaerobic bacteria contribute mainly to hydrolysis, fermentation and mineralization. After adhering onto the surface of PET polymer, the microbes secrete extracellular PET hydrolases and oxidases, along with the similar degradation enzymes released from the host, to initiate PET degradation process and generate degraded products for further metabolism, assimilation, and

mineralization. Under nutrient deficient conditions (PET-fed), the gut microorganisms that develop chemotaxis and are equipped with flagella for seeking carbon source are selected or enriched, and nitrogen fixing microbes convert dissolved N_2 to ammonium as N source to mitigate nitrogen deficiency. The intermediate products of PET are utilized by various gut microbes and further metabolized into the TCA cycle. With the help of O_2 by oxidases, the degraded intermediates are transformed into compounds such as 4-hydroxyphenylacetate, 3,4-dihydroxyphenylacetate, 3-phenylpropanoate, 3-hydroxyanthranilate and protocatechuic acid as major intermediates by aerobic decomposition. As oxygen is consumed in midgut, depolymerized PET polymer is also further decomposed into formic acid via facultative anaerobic/anaerobic decomposition, which can be further utilized by *Dysgonomonas* sp. and *Hafnia-Obesumbacterium* sp. as electron donor source for mineralization.

4. Conclusions and future studies

This study has first detailed the biodegradation of commercial PET (or polyester) resin powders in *Tenebrio molitor* larvae. The results demonstrated that the larvae are capable of rapidly biodegrading PET with the mass reduction efficiency of 71.03% and 73.28%, or PET half-life of 0.14 day and 0.26 day for two commercial PET with molecular weight (M_w) of 39.33 and 29.43 kDa. *T. molitor* larvae consumed and biodegraded PET more rapidly than other major non-hydrolysable plastics e.g., PE, PS, PP and PVC as well as hydrolysable PUR polymers reported in literatures. Although PET biodegradation can occur independently under antibiotic suppression of gut bacteria, the gut microbiota has a significant contribution to PET biodegradation. Feeding PET

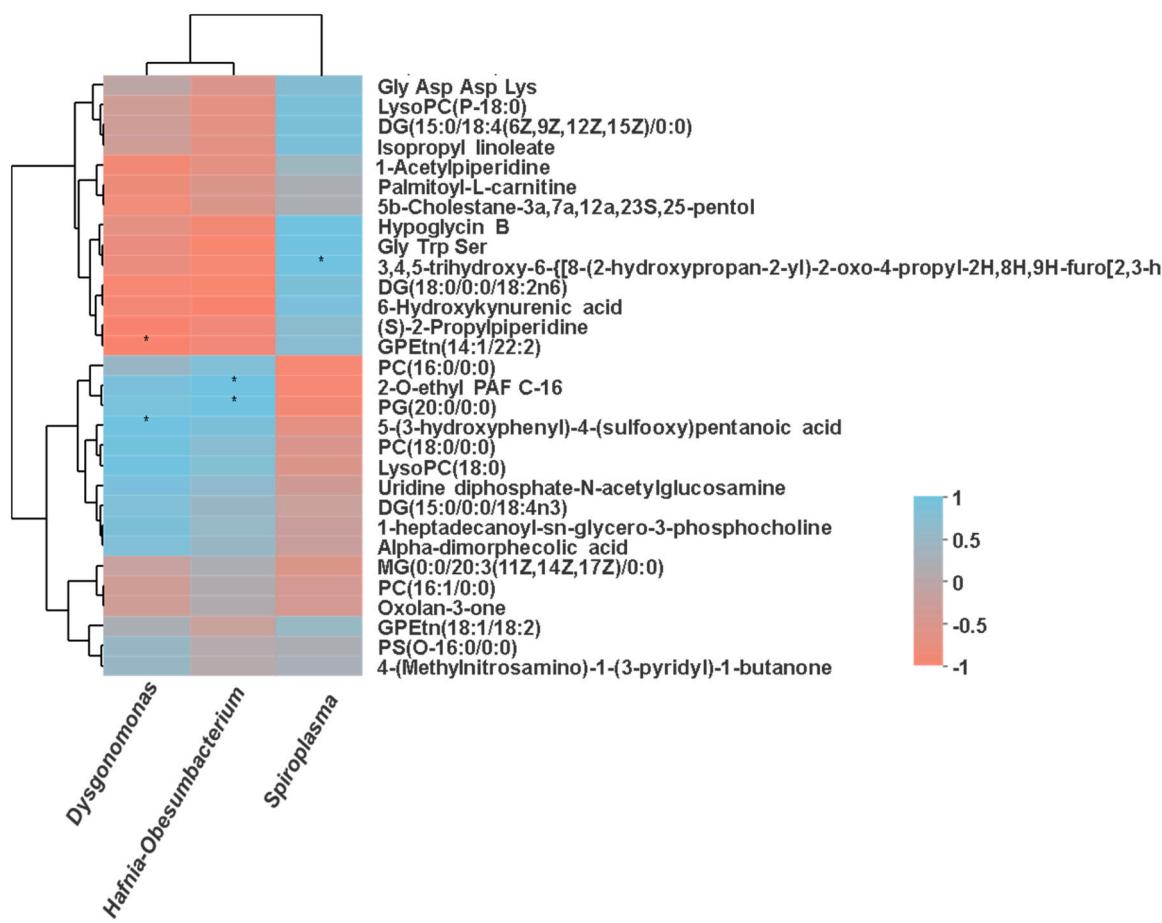


Fig. 6. Association between microbial genus abundance and metabolite abundance. Heatmap showing the Pearson correlation coefficient between the relative abundance of individual microbial genera and the relative abundance of differential metabolites. where * represents $p < 0.05$, ** indicates $p < 0.01$, and *** marks $p < 0.001$.

diet altered gut microbiome and host metabolites. Dominant bacterial genera *Spiroplasma* sp., *Dysgonomonas* sp. and *Hafnia-Obesumbacterium* sp. were observed to be related to PET biodegradation. Bacterial enzymes and GOs related to plastic degradation were enriched or upregulated in the gut microbiome, including PET polymer degrading enzymes (hydrolases, oxidases, decarboxylase, depolymerase, oxidoreductase, and dehydrogenase), chemotaxis proteins, transport proteins, and flagella formation-related proteins. Metabolome analysis indicated the synergistic metabolic pathways of biodegradation, mineralization and assimilation by the gut microbiota and host larvae. During PET degradation process, aerobic bacteria, facultative bacteria, and anaerobic bacteria contributed to biodegrade PET and degraded intermediates. The pathways of lipid metabolism of the host were reprogrammed during PET degradation.

Overall, this study has provided new insights into the biodegradation of plastics by the *T. molitor* larvae and its associated gut microbial communities. Specially, we proposed possible mechanism and pathways for PET biodegradation in the insect larvae, which open an avenue for further studies, such as isolation and purification of PET degradation keys species, optimization of degradation capacity *in vitro*. Also, we will examine whether the gut species in larvae exhibit interspecific interactions during degradation of recalcitrant plastics to enable their applications of plastic degradation in the future.

CRediT authorship contribution statement

Lei He: Conceptualization – experimental design, Formal analysis, Sample collection, Methodology, Supervision, Validation, Writing –

original draft, Writing – review & editing. **Shan-Shan Yang:** Methodology, Formal analysis, Investigation, Writing – review & editing, Funding acquisition. **Jie Ding:** Conceptualization, Supervision. **Zhi-Li He:** Conceptualization – experimental design, Writing – review & editing. **Ji-Wei Pang:** Methodology. **De-Feng Xing:** Conceptualization – experimental design. **Lei Zhao:** Methodology. **He-Shan Zheng:** Formal analysis. **Nan-Qi Ren:** Conceptualization – experimental design. **Wei-Min Wu:** Conceptualization, Methodology, Validation, Writing – drafting, Writing – review & editing, Funding acquisition. All authors contributed to manuscript reviewing & editing.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131759.

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