

Molecular-Weight-Dependent Degradation of Plastics: Deciphering Host–Microbiome Synergy Biodegradation of High-Purity Polypropylene Microplastics by Mealworms

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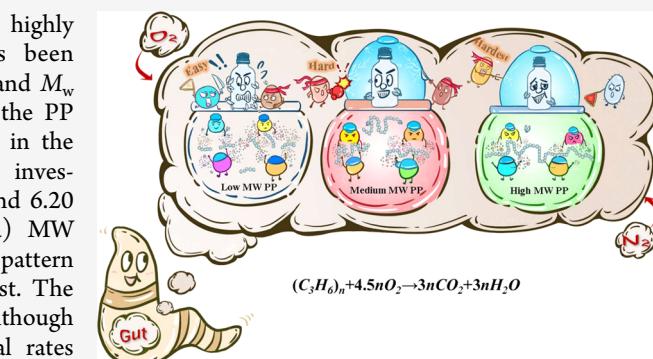
ABSTRACT: The biodegradation of polypropylene (PP), a highly persistent nonhydrolyzable polymer, by *Tenebrio molitor* has been confirmed using commercial PP microplastics (MPs) (M_n 26.59 and M_w 187.12 kDa). This confirmation was based on the reduction of the PP mass, change in molecular weight (MW), and a positive $\Delta\delta^{13}\text{C}$ in the residual PP. A MW-dependent biodegradation mechanism was investigated using five high-purity PP MPs, classified into low (0.83 and 6.20 kDa), medium (50.40 and 108.0 kDa), and high (575.0 kDa) MW categories to access the impact of MW on the depolymerization pattern and associated gene expression of gut bacteria and the larval host. The larvae can depolymerize/biodegrade PP polymers with high MW although the consumption rate and weight losses increased, and survival rates declined with increasing PP MW. This pattern is similar to observations with polystyrene (PS) and polyethylene (PE), *i.e.*, both M_n and M_w decreased after being fed low MW PP, while M_n and/or M_w increased after high MW PP was fed. The gut microbiota exhibited specific bacteria associations, such as *Kluyvera* sp. and *Pediococcus* sp. for high MW PP degradation, *Acinetobacter* sp. for medium MW PP, and *Bacillus* sp. alongside three other bacteria for low MW PP metabolism. In the host transcriptome, digestive enzymes and plastic degradation-related bacterial enzymes were up-regulated after feeding on PP depending on different MWs. The *T. molitor* host exhibited both defensive function and degradation capability during the biodegradation of plastics, with high MW PP showing a relatively negative impact on the larvae.

KEYWORDS: polypropylene, biodegradation, molecular weight, *Tenebrio molitor*, gut microbiome, transcriptome

INTRODUCTION

Polypropylene (PP, $(\text{C}_3\text{H}_6)_n$) ranks as the second most widely used plastic, contributing to 18.9% of global plastic production in 2022¹ and ranking as a major waste plastic following polyethylene (PE).² The PP polymer is characterized by a high molecular weight (MW) (10–550 kDa) and long carbon chains, which hinder atmospheric oxidation and degradation. The accumulation of PP or other plastic wastes exerts adverse biological effects on various cell lines and human health.³

As a nonhydrolyzed polyolefin plastic,^{4,5} the iso-paraffin structure with side chains ($-\text{CH}_3$) in PP makes it more resistant to biodegradation compared to the linear *n*-paraffin in PE. To date, only a limited number of microorganisms have been reported to degrade PP, typically requiring pretreatment and exhibiting a slow degradation rate.^{6,7} Recent researchers have identified certain macroinvertebrates capable of biodegrading major plastics,⁸ such as insect larvae belonging to darkling beetles (Coleoptera: Tenebrionidae) including *Tenebrio molitor* (*T. molitor*),^{9–13} *Tenebrio obscurus*,¹⁴ *Zophobas atratus*,^{15,16} *Uloma* sp.,¹⁷ and *Plesiophthalmus*



davidis,¹⁸ pyralid moths (Lepidoptera: Pyralidae) including *Plodia interpunctella*,^{19,20} *Galleria mellonella*,^{21–27} and *Achroia grisella*,²⁸ as well as other macroinvertebrates *e.g.*, land snails *Achatina fulica*.²⁹ Among these, *T. molitor* larvae have been extensively studied for their ability to degrade a wide range of materials, including wood residues in the environment,³⁰ lignocellulosic agricultural residues,^{31,32} and various plastics including polystyrene (PS),^{7–9,31–35} low-density polyethylene (LDPE) and high-density polyethylene (HDPE),^{12,15,36,37} polyvinyl chloride (PVC),¹⁵ polyethylene terephthalate (PET),³⁸ polyurethane (PUR),³⁹ polylactic acids (PLA),⁴⁰ and even rubber⁴¹ and graphene oxide.⁴² Our previous study

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first confirmed the biodegradation of commercial PP foam by the larvae of *T. molitor* and *Z. atratus*.⁴³

The processes of plastic degradation depend on the polymer chemical and physical properties.^{5,33,44} Since a large amount of data regarding plastic properties have been measured along with changes in MW during the biodegradation of plastics, MW is considered a dominant property that impacts PP biodegradation.⁴⁴ Peng et al.³³ found that the gut microbiome and metabolome responded to PS polymers with a wide MW range from 6.70 to 1346 kDa. Yang et al. examined the biodegradation of LDPE microplastics (MPs) with M_w from 0.84 to 106.8 kDa and HDPE with M_w from 52 to 132.7 kDa, finding that PE with lower M_w underwent a greater extent of depolymerization. Furthermore, factors, such as MW, PE type, branching, and crystallinity, significantly influence the larvae's biodegradation capabilities.³⁷ These studies indicated that the MW of PS and PE impacts depolymerization patterns and microbiome development, but the impact on host gene expression was not reported.

Among commonly used plastics, PP exhibits the highest resistance to degradation.⁴⁴ To date, the degradation mechanism of PP polymers in *T. molitor* larvae remains largely unknown. Based on previous findings related to the biodegradation of PS and LDPE,^{33,37,45} we hypothesized that (a) a synergistic effect might exist between the insects and their microorganisms during PP degradation; (b) the insect's digestive function and its gut microbiota would be altered to facilitate their adaption to a plastic-rich diet;^{46,47} and (c) physical properties, especially MWs, significantly impact PP biodegradation influencing both the gut microbiome and host gene expression for enzyme synthesis. However, the response of *T. molitor* larvae to different MW portions of PP has not yet been investigated. It is interesting to determine whether the MW of PP exhibited a similar or different impact on plastic depolymerization/biodegradation and the gut microbiome compared to other polyolefins by the larvae. Consequently, understanding the host gene expression response to various MWs of PP is crucial for elucidating the degradation mechanism of *T. molitor* larvae.

The aim of this study was to explore the synergistic effect of the host digestive system and the diverse symbiotic gut microbes harbored within it on the biodegradation of PP polymers across a range of low to high MWs, which encompass the MW range of commercial products. This study was based on the three hypotheses mentioned above to investigate the knowledge regarding the factors affecting PP degradation, including the physical and chemical properties of PPs, host gene expression, and interactions among microbial species in *T. molitor* larvae. 16S rRNA gene amplicon sequencing and transcriptome profiling were utilized to comprehend PP metabolism and energy supply by digesting the nutrient-limited PP polymer.

MATERIALS AND METHODS

Mealworms and Feedstocks. *T. molitor* larvae (average weight: 27 ± 0.5 mg/Larva; length: approximately 1.5 cm; instar: 4–5) were obtained from a breeding farm in Dezhou, China. Commercial PP MPs (10 μm) with a number-average molecular weight (M_n) of 26.59, a weight-average molecular weight (M_w) of 187.12, and a z-average molecular weight (M_z) of 731.6 kDa were purchased from Kexinda Plastic Factory (Shanghai, China). Five high-purity PP samples were acquired from American Polymer Standards Corporation, classified by

their respective M_w of 0.83, 6.20, 50.40, 108.00, and 575.00 kDa, and designated as PP_{0.83}, PP_{6.2}, PP_{50.4}, PP₁₀₈, and PP₅₇₅ (Figure S1). PP_{0.83} was in a viscous liquid form, and PP_{6.2} was presented as a gel-like soft solid. PP_{50.4} and PP₁₀₈ were nonuniform solid bulk materials, while PP₅₇₅ consisted of pellets measuring 2–4 mm. The three solid PP samples were ground under freezing conditions using a stainless-steel high-throughput tissue grinder (Wonbio-L, Shanghai Wonbio Biotechnology Co., Ltd., Shanghai) to reduce the particulate size to less than 75 μm for testing. Agar was purchased from Beijing Aoboxing Biotech Co., Ltd. Wheat bran (WB) was procured from a breeding farm in Dezhou, Shandong, China.

Mealworm Rearing and Biodegradation of PP Polymers.

Prior to testing, the larvae were kept unfed for 24 h and then reared under controlled conditions (25 °C, 60% relative humidity and dark conditions).

The commercial PP MPs, typically used for PP products with lower MW than the previously tested PP foam,⁴³ were used to examine consumption and degradation of PP MPs with broad MW distribution. Each incubator (16 × 11 × 5 cm) contained 500 randomly selected larvae. PP polymers (5 g) were mixed with a boiled 2% (w/w) agar solution (100 mL) and solidified as PP-agar gel (5:2, w/w) to feed the larvae. Control groups included larvae fed on agar gel and those left unfed.

The test to examine the effect of MW on PP degradation involved mixing each high-purity PP polymer (400 mg) with a 2% (w/w) agar solution (Supporting Information M1). The experiment ended when the feedstocks were completely consumed, with all treatments conducted in triplicate ($n = 3$).

Collection of Frass and Analytical Methods. At the end of the test, *T. molitor* larvae were cleansed with compressed air to remove residual PP debris from the body and then transferred to a clean box for approximately 12 h to collect frass. The MW (M_n , M_w , and M_z) of PP was determined using high-temperature gel permeation chromatography (HT-GPC). Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (¹H NMR) spectroscopy were conducted to characterize the polymer's functional groups, as detailed in SI M2.⁴⁸ The polydispersity index (PDI) was calculated as $PDI = M_w/M_n$. The ¹³C stable isotope signatures of the residual plastics from the frass, relative to the pristine PP plastics, expressed in delta (δ) notation in parts per thousand (‰), were calculated as outlined in SI M3

$$\delta^{13}\text{C}(\text{\textperthousand}) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

Gut Microbiome and Transcriptome Analyses. At the end of the experiment, 20 larvae were randomly selected from each parallel group. The guts were extracted from these selected larvae and combined into one sample. This process was conducted in triplicate for the three parallel groups of each treatment. Second-generation sequencing was used to analyze changes in the gut microbiome and differences in transcriptome expression. High-throughput sequencing of bacterial 16S rRNA was performed on the Illumina NovaSeq PE250 platform (Illumina NovaSeq PE250, Illumina Inc., San Diego, CA) (SI M4). The sequencing data of 16S rRNA have been deposited at NCBI with the BioProject ID PRJNA1070369 (BioSample ID SAMN39643795–SAMN39643809 and SAMN39643777–SAMN39643779). The *T. molitor* transcriptome was conducted by performing the paired-end

Table 1. Characteristics of Consumption of Different Diets (PP, Agar, WB, and Unfed Groups) by *T. molitor*^a

feedstocks	initial weight, mg worm ⁻¹	final weight, mg worm ⁻¹	cumulative consumption, g	digested PP, g	SR, %	weight change, %
PP	51.20 ± 1.14	72.62 ± 2.13	7.0	2.04 ± 0.09	78.64 ± 3.76	41.84
Agar	51.56 ± 1.09	57.60 ± 2.65	1.5	nd	56.20 ± 4.52	11.71
WB	50.72 ± 1.27	81.70 ± 2.2	35	nd	87.60 ± 1.53	61.08
Unfed	51.06 ± 1.32	44.02 ± 1.16	nd	nd	46.35 ± 1.87	-13.79

Note: The initial larval number: 500. nd = Not determined. (Pristine PP: $M_n = 26.59 \pm 3.86$ kDa, $M_w = 187.12 \pm 0.26$ kDa, $M_z = 731.61 \pm 31.09$ kDa, PDI = 7.04; after biodegradation: $M_n = 36.21 \pm 4.17$ kDa, $M_w = 159.13 \pm 9.76$ kDa, $M_z = 523.12 \pm 65.56$ kDa, PDI = 4.39) ^aSPCR stands for specific PP consumption rate, which was calculated based on the weight of digested polymer over the test period (32 days) divided by consumption days and the initial number of larvae.

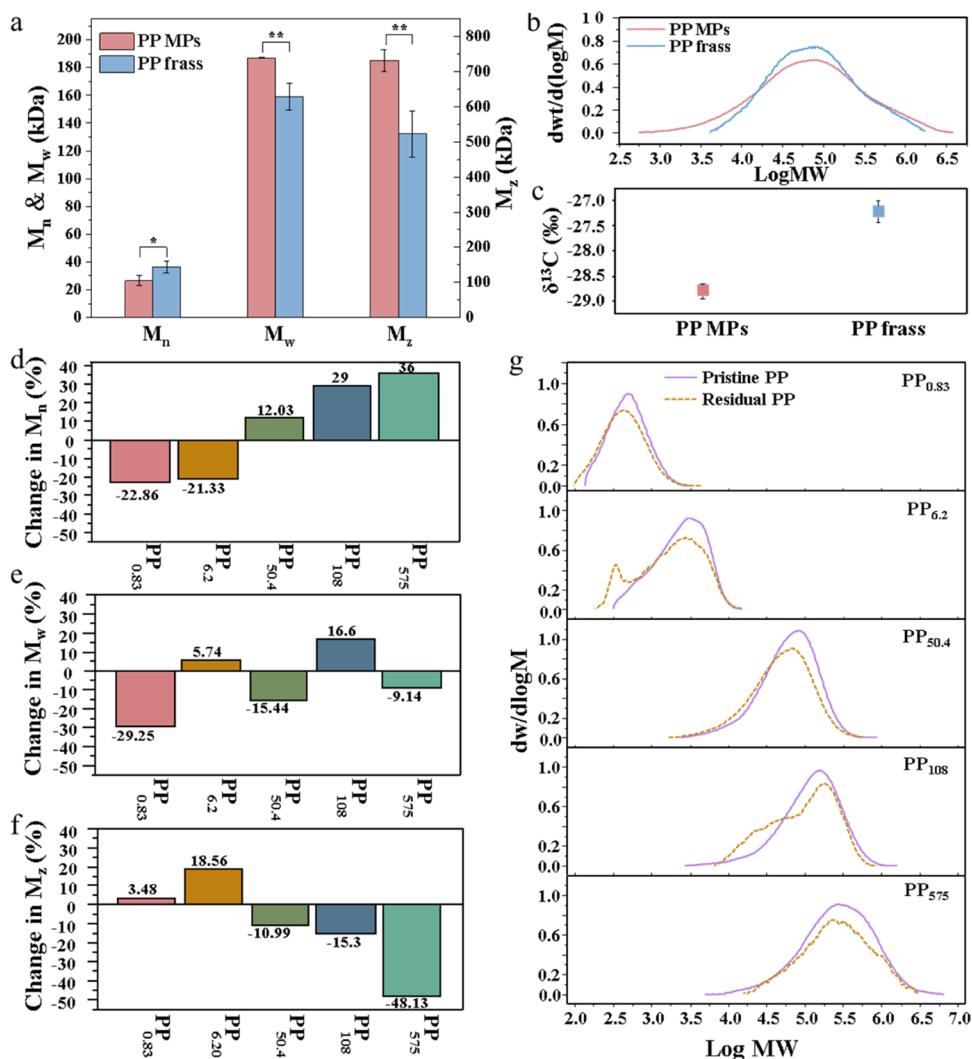


Figure 1. Characterization of PP biodegradation. (a) MW changes of commercial PP MPs before and after biodegradation; MW distribution (b) and $\delta^{13}\text{C}$ stable isotope signature (c) of pristine PP MPs versus PP residues in frass; changes in M_n (d), M_w (e), and M_z (f) of high-purity PP before and after biodegradation; and (g) shift of the MWD of the ingested PP with different MWs.

sequencing on an Illumina Novaseq 6000 (LC Sciences). The sequencing data of the transcriptome have been deposited at NCBI with the BioProject ID PRJNA1071745 (BioSample ID SAMN39711160–SAMN39711174 and SAMN39711143–SAMN39711145). Additional methods are listed in SI MS.

RESULTS AND DISCUSSION

Consumption and Biodegradation of Commercial PP MPs with a Wide MW Distribution. The biodegradation of commercial PP MPs with a broad MW distribution (PDI = 7.04) was confirmed by *T. molitor* larvae. Over a 36 day period,

the larvae actively chewed and ingested the PP gel (Figure S2a) and consumed 7 g of PP, resulting in an average mass reduction of 29.14% and a specific removal rate of 13.72 mg 100 larvae⁻¹ d⁻¹ (Table 1). The survival rates (SRs) and weight change of the larvae fed with the commercial PP polymers plus agar were higher than those of the agar-fed larvae, much higher than those of the unfed larvae, and slightly lower than those fed with WB, as expected (Figure S2b,c, data in Table 1). This indicated that PP served as a source of energy and carbon for *T. molitor* larvae in addition to agar during the period of 36 days (SI R1). The lower SR and weight increase

of the larvae fed with PP plus agar, compared to those fed with WB, were attributed to the lack of nutrients and minerals, as observed previously during PS degradation tests.⁴⁹

After digestion, the MW of residual PP polymers changed, with M_n increasing by 36.19%, and M_w and M_z reducing by 14.96 and 25.50%, respectively (Figure 1a). The depolymerization pattern of the commercial PP MPs appeared to show a limited extent of depolymerization (LD),⁴⁸ which was similar to that observed in our previous study when mealworms biodegraded PP foam with a higher MW (M_n of 356.20 kDa, M_w of 109.80 kDa, and M_z of 765.00 kDa). The LD pattern, i.e., an increase in M_n and/or M_w , suggested a selective breakdown of low MW polymers or a relatively poor ability to cleave longer molecular chains.^{33,37,50} The obvious change in the MW distribution (MWD) was also similar to that observed during the biodegradation of PP foam by *T. molitor* previously⁴³ (Figure 1b), confirming the biodegradation of the PP MPs. The PDI of PP, after passing through the gut of larvae, decreased from 7.04 to 4.39, indicating a narrowed MWD of the residual polymer due to the biodegradation of both lower and higher MW polymers.

Stable isotope analysis was utilized to validate the biodegradation of PP polymer by *T. molitor* larvae, which is a novel methodology based on isotopic fractionation utilizing the natural labeling of stable isotopes in the environment. The isotopic values for the pristine PP and the extracted residue PP from the frass were -28.80 and -27.22‰ , respectively, with a $\Delta\delta^{13}\text{C}$ of 1.58‰ (Figure 1c). This indicates a ^{13}C enrichment in the residual PP polymers following the passage of PP polymer through the larval gut. During biochemical reactions, the microbial loop preferentially utilizes lighter isotopes (^{12}C), leading to an enrichment of heavier isotopes (^{13}C) in the remaining substrate pool,^{51,52} thereby increasing the ^{13}C in the residual PP left in frass. Although the observed $\Delta\delta^{13}\text{C}$ might appear small, the high precision of isotope ratio mass spectrometers (0.2‰) enables reliable measurement of minor differences.⁵² Similar $\delta^{13}\text{C}$ values have been reported previously for HDPE and PET, with $\Delta\delta^{13}\text{C}$ values ranging from approximately 0.20 to 0.70‰ over a 32 day exposure in soil and sludge,⁵² confirming that PP MPs undergo biodegradation in *T. molitor* larvae. This demonstrated that stable isotope analysis offers an insightful approach to assessing the biodegradation of petroleum-derived plastics.

Impact of MW on Consumption and Biodegradation of PP. High-purity PP polymers of varying MWs were examined to determine the influence of MW on degradation. The larvae consumed all five PP-agar gels at the end of the test. An increased duration for consumption, reduced average weights, and decreased SRs were noted in larvae fed with PP (Table S1). Polymers with low MW are generally less rigid than those of high MW,⁵³ rendering them more susceptible to being chewed and ingestion by the larvae. These characteristics elucidate the observed PP consumption rates and SRs. Notably, the gel-like PP_{6,2}, being a little sticky, contributed to a longer consumption time (16 days) and a lower SR (64.32 \pm 2.43%) (Table S1). WB, the larvae's normal food, containing rich nutrition was consumed the quickest (8 days) and resulted in an average weight increase of $56.07 \pm 5.80\%$ with the highest SR of $92.05 \pm 2.08\%$ among the six groups (Table S1). Larvae fed with PP polymers exhibited SRs ranging from 76.63 to 63.33% and lost weight by 4.25 to 10.89% as the MW increased from 0.83 to 575.00 kDa. In contrast, the unfed control group lost an average weight of $27.01 \pm 4.55\%$, with

the lowest SR ($40.26 \pm 5.02\%$). The agar-fed control group lost an average weight of $14.36 \pm 2.50\%$ with an SR of $69.63 \pm 2.80\%$. The impact of PP MW on PP consumption and larval weight was similar to that observed in the degradation of different MW PE or PS polymers,^{33,37} suggesting that the polymer MW in polyolefins exhibited similar trends in *T. molitor* larvae.

Chemical modifications and the formation of oxidized groups in the residues were confirmed using FT-IR (Figure S3) and ^1H NMR (Figure S4). The spectra indicated oxygen incorporation and chemical modifications of the PP chain resulting from the biodegradation of polyolefin plastic by *T. molitor*⁴³ (SI R2). The MW change results for high-purity PP confirmed that the larvae biodegraded all PP from low to high MW, with different depolymerization patterns (Figure 1d–f). Except for the low MW PP_{0,83}, which showed a broad extent of depolymerization (BD), i.e., a decrease in both M_n and M_w (Figure 1d), the other samples exhibited an LD pattern (Figure 1d–e). This phenomenon is attributed to the variation in the biodegradability of PP with different MW, where lower MW PP can be more rapidly broken down than higher MW PP. However, the residues of PP polymers with high MW, such as PP_{50,4}, PP₁₀₈, and PP₅₇₅, showed a significant reduction in M_z values by 10.99, 15.31, and 48.13%, respectively (Table S2), indicating effective depolymerization of the high MW portions. This is consistent with the results of biodegradation of the commercial PP MPs, where the PDI of PP after passing through the gut of larvae decreased and a shift in the MWD (Figure 1b), indicating a narrowed MWD of the residual polymer due to the biodegradation of both lower and higher MW polymers. Similar limited extent depolymerization has been observed in the biodegradation of other plastic materials by plastic-eating invertebrates, e.g., LDPE foam degradation by *T. molitor*,^{36,43} EPS, PP, and LDPE biodegradation by *Zophobas atratus*,^{15,43} and PS biodegradation by land snails *Achatina fulica* and *G. mellonella*.^{27,29}

These observations likely result from differences in the biodegradability of various MW PP by *T. molitor* larvae. For PP with low MW (PP_{0,83} and PP_{6,2}), the more rapid degradation of the lower MW portion than the higher MW portion led to an increase in M_w in the residual polymers.⁵⁴ In contrast, when polymers with higher MW, i.e., PP_{50,4}, PP₁₀₈, and PP₅₇₅, were degraded, the long-chain polymers were cleaved or broken into relatively short-chain polymers via depolymerization. M_z , representing the long-chain portion, was reduced, but M_n , presenting the low-chain polymer, increased (Figure 1d–f). The GPC results indicated that the MWD of all tested PP polymers shifted toward the lower MW direction (Figure 1g) and demonstrated that the larvae are capable of biodegrading PP across a broad MW range (0.83 to 575.00 kDa), covering the MW of commercial PP; and larger MW negatively impacts biodepolymerization/biodegradation as expected.

Compared with the other two majorly produced polyolefin polymers (i.e., PS and PE) that lack heteroatoms in the main carbon chain, the biodegradability of PP by *T. molitor* larvae was relatively lower than that of PS and PE (Figure S5, Table S2) as expected, due to physical properties and molecular structure.⁴⁴ However, the trend of the impact of MW was similar among PP, PS, and LDPE polymers. M_n and M_w declined during the biodegradation of low MW polymers. As MW increased to certain levels, M_n and/or M_w of the residual polymers increased, indicating an LD pattern. This analysis indicates that the depolymerization pattern of polymers,

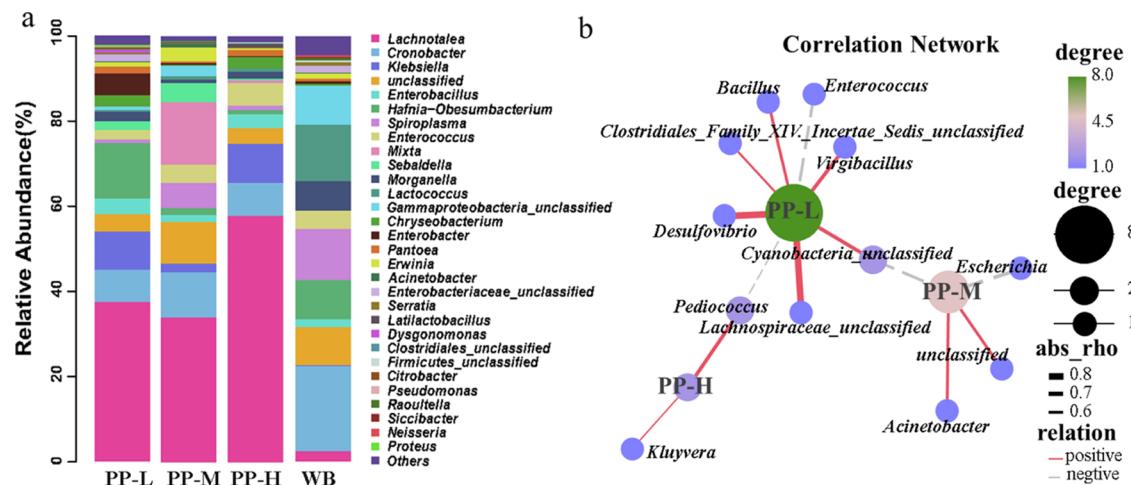


Figure 2. (a) Analysis of gut microbial community. Relative bacterial abundances of dominant populations (top 30) in *T. molitor* larvae at the genus level; (b) Correlation-based network analysis of top 50 genera co-occurrence patterns for PP-L, PP-M, and PP-H rearing.

regardless of the plastic types in polyolefin plastics, is strongly influenced by polymer MW.

Gut Microbiome Analyses. 16S rRNA sequencing was utilized to analyze changes in the gut microbial community composition of *T. molitor* larvae under different feeding conditions (five PP polymers with different MW and WB). Gut community richness and diversity declined with PP feeding (SI R3-1, Figure S6a,b). A PCA based on genera (Figure S6c) and a heatmap based on Euclidean distances (Figure S7) revealed that the gut microbiota of *T. molitor* larvae fed with lower MW PPs were closer, and similarly, as did those fed with higher MW PPs. This indicated that the composition of intestinal microbes varied based on MW. Consequently, the PP-fed groups were categorized into three subgroups: PP-L (PP_{0.83} and PP_{6.2}), PP-M (PP_{50.4} and PP₁₀₈), and PP-H (PP₅₇₅), to further analyze the contribution of gut microbiota to PP degradation.

Microbial community analysis at the genus level indicated a significant shift in gut microbiomes after PP was fed (Figure 2a), revealing the association of other polyolefin-degrading-related bacteria with PP degradation in the larval gut, despite the scarcity of microbes capable of biodegrading unpretreated PP as a sole carbon source. The dominant species in PP-L were *Lachnotalea* sp. (37.49%), *Hafnia-Obesumbacter* sp. (13.16%), and *Klebsiella* sp. (9.04%); in PP-M, they were *Lachnotalea* sp. (33.88%), *Mixta* sp. (14.79%), and *Cronobacter* sp. (10.68%); and in larvae fed with PP-H, they were *Lachnotalea* sp. (57.75%), *Klebsiella* sp. (9.18%), and *Cronobacter* sp. (7.74%) in larvae fed with PP-H, respectively. Among these, *Klebsiella* sp., *Cronobacter* sp., and *Mixta* sp. are commonly reported genera associated with plastic degradation or nitrogen fixation in *T. molitor*.^{47,55,56} A bacterium belonging to *Klebsiella* sp. (*Klebsiella pneumoniae* Mk-1) has been isolated from soil and confirmed to degrade PE.⁵⁷ *Lachnotalea* sp. was isolated from a lignocellulose-rich microbial community and shows potential for degrading natural organic polymer.⁵⁸ *Hafnia-Obesumbacter* sp. has been reported to be associated with PET degradation in *T. molitor*³⁸ and PS degradation in *Z. atratus*.⁵⁹ However, further research is needed to investigate their ability to degrade PP.

Correlation network analysis of the top 50 bacteria revealed distinct keystone species recognized for the biodegradation of low-, medium-, to high MW PP (Figure 2b). PP-L mastication

resulted in the enrichment of *Bacillus* sp. (0.11%), *Clostridiales Family XIV*, *Incertae Sedis unclassified* (0.14%), *Lachnospiraceae unclassified* (0.05%), and *Virgibacillus* sp. (0.05%) in *T. molitor* larvae. These species were possibly associated and/or involved in PP degradation in the gut of *T. molitor* larvae, albeit with low abundance (<1%). A detailed discussion of their role can be found in SI R3-2. Correlation network analysis that included WB is also exhibited in Figure S8 and discussed in SI R3-3.

Acinetobacter sp. (0.71%) was enriched in the gut of PP-M-fed larvae as a potential candidate for the degradation of PP-M. Although *Acinetobacter* sp. has not been identified in PP degradation, it has been strongly associated with LDPE and PS biodegradation in *T. molitor* and *G. mellonella* larvae.^{60–63} This genus encompasses strictly aerobic species capable of oxidizing and depolymerizing PP carbon chains.

Kluyvera sp. (0.36%) and *Pediococcus* sp. (0.11%) were highly correlated in larvae fed with PP-H, suggesting their strong association with high MW PP degradation. In our previous study of the biodegradation of PP foam (Mw 356.00 kDa) by *T. molitor*, *Kluyvera* sp. associated with PP-H biodegradation was identified.⁴³ The PP foam had a relatively high MW, albeit slightly lower than that of the PP-H in this study ($M_w = 575.00$ kDa). *Pediococcus* sp., previously found to be associated with the production of organic acids from water-soluble carbohydrates during the fermentation of whole-plant corn silage,⁶⁴ showed a high correlation with PP₅₇₅. This suggests that this genus may utilize the intermediates of PP degradation as substrates.

The 16S rRNA data suggested that different bacteria could synergistically degrade PP in the larval gut, akin to the cooperation observed between *Acinetobacter* NyZ450 and *Bacillus* spp. NyZ451 for the biodegradation of a PE mulching film.⁶¹ Specific bacterial dominance was observed in larvae fed with different MW PPs, with cooperative interactions hinted at for the utilization of PP degradation intermediates. Notably, some of the key microorganisms involved in the degradation of different MW PP were also identified as key microorganisms in lignocellulose degradation,^{31,64–66} such as *Bacillus* sp., *Spiroplasma* sp., *Pediococcus* sp., and *Enterococcus* sp. This suggests that the ability of *T. molitor* to degrade PP can be traced back to its ability to degrade natural lignocellulosic polymers.^{31,32,67}

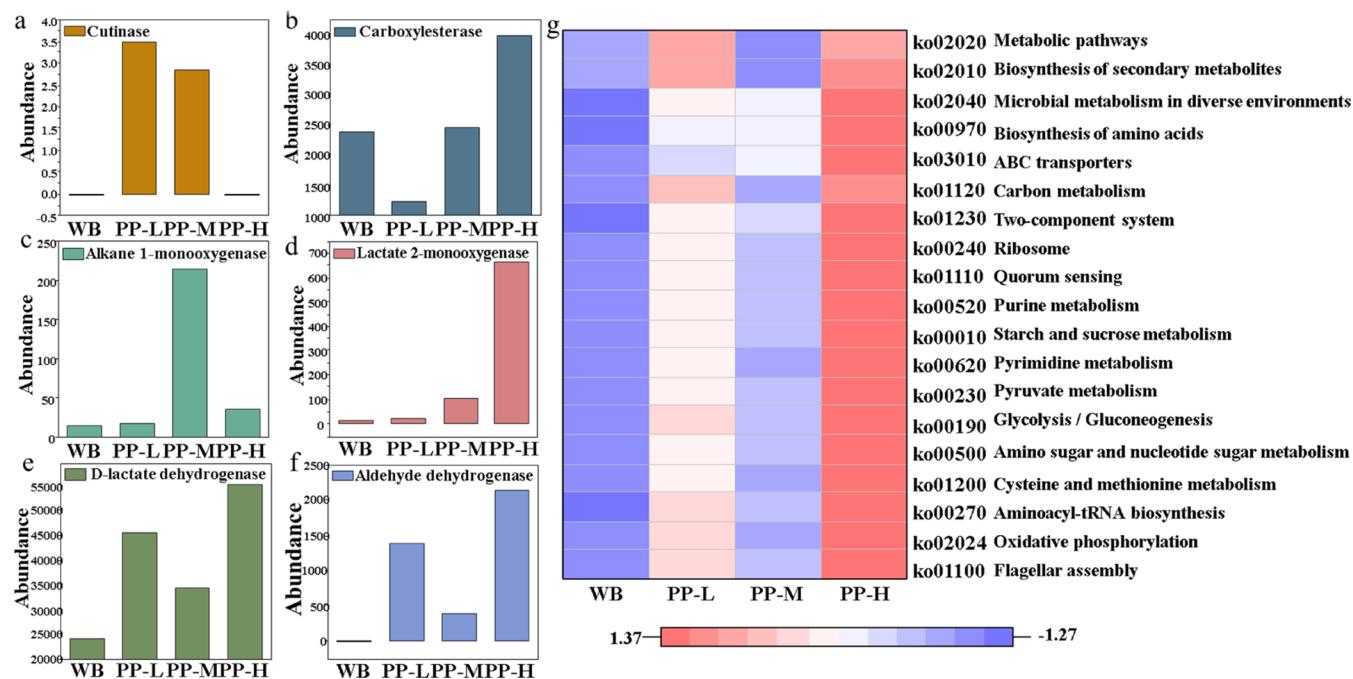


Figure 3. PICRUSt prediction of the gut microbiome. (a–f) PP degradation-related enzymes produced in gut microbiome from larvae fed on different MW PP and WB and (g) heatmap plot of top 20 KEGG pathways of the gut microbiome during PP utilization.

Functional Prediction of the Gut Microbial Communities. Functional prediction by PICRUSt analysis revealed a higher abundance of specific enzymes, i.e., alkane-1-monoxygenase, carboxylesterase, cutinase, and dehydrogenase in PP-fed larvae compared to those fed with WB (control) (Figure 3a–f). This indicates their potential functions in plastic degradation.

Among the top 20 KEGG pathways, metabolic pathways were the most enriched (Figure 3g). The gene expression of gut microbiota in the PP-M and WB groups was similar and lower than that in the PP-L and PP-H groups. Specifically, larvae fed on different PPs showed up-regulation in genes related to the biosynthesis of secondary metabolites, microbial metabolism in diverse environments, biosynthesis of amino acid metabolism, and carbon metabolism compared to the WB control. Rather, ATP-binding cassette (ABC) transporter pathways were highly expressed in the larvae fed on distinct MW of PP, facilitating intracellular importation of intermediate products following the extracellular depolymerization of PP in bacteria, as previously reported.⁶⁸ These observations indicate the assimilation of PP intermediates via cellular carbon metabolism by the microbiota. Notably, pathways related to chemotaxis, such as two-component system, quorum sensing, bacterial chemotaxis, and flagellar assembly, were enriched in PP-fed microbiota, similar to the degradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium.⁶⁹ These functions aid in bacterial community colonization, motility, and the transfer of electrons to electron acceptors outside bacterial cells. The phenomenon of quorum sensing suggests coordination among various gut microbes during PP degradation. The nitrogen metabolism pathway (KO00910) was enriched in PP-fed groups (Figure S9), indicating that, besides the plastic degradation, nitrogen fixation was also performed by intestinal bacteria when the larvae received a nitrogen-deficient diet of PP as a food source. As observed, many insects that feed on a low-nitrogen diet

(such as wood-feeding beetles, fruit flies, termites, and a wood wasp) rely on nitrogen-fixing bacteria (diazotrophs) to supplement their nitrogen supply.⁷⁰ Recently, evidence for nitrogen fixation has been found during the degradation of PS, LDPE, and PET by *T. molitor* larvae.^{38,56,71}

The analysis of gut microbial communities showed that various microorganisms in *T. molitor* larvae degrade polymers with different MW of PP polymers, yet the main functions of intestinal microorganisms in the process of plastic degradation are unified. This finding implies that the intestinal microbial community of *T. molitor* larvae contributes to PP biodegradation by forming a system conducive to plastic degradation domesticated through ingesting polymers. Specifically, after plastics enter the digestive tract, gut microorganisms utilize chemotaxis-related pathways (such as two-component systems, quorum sensing, bacterial chemotaxis, and flagellar assembly) to navigate toward PPs, aiding in the establishment and movement of bacterial communities. Through enzymes secreted collaboratively by gut microorganisms and the larval host, polymers are initially degraded. Intermediate products are then transported into microbial cells via transport systems for further metabolism.

Host Transcriptome Response to PP Polymers. To reflect the function of the *T. molitor* larval host during *in vivo* degradation of PP, changes in gene expression in the intestines were evaluated via the host transcriptome. PCA (Figure S10a) and Spearman correlation (Figure S10b) analyses revealed clusters associated with different diets (PP-L, PP-M, PP-H, and WB), indicating that the larvae developed distinct host gene expression during the biodegradation of PP polymers with three different MW groups.

Compared to WB-fed larvae, there were a total of 296, 820, 451, 714, and 430 differentially expressed genes (DEGs) up-regulated, and 218, 372, 174, 297, and 410 DEGs down-regulated in the larvae fed with PP_{0.83}, PP_{6.2}, PP_{50.4}, PP₁₀₈, and PP₅₇₅, respectively (Figure 4a). The PP₅₇₅, with the largest

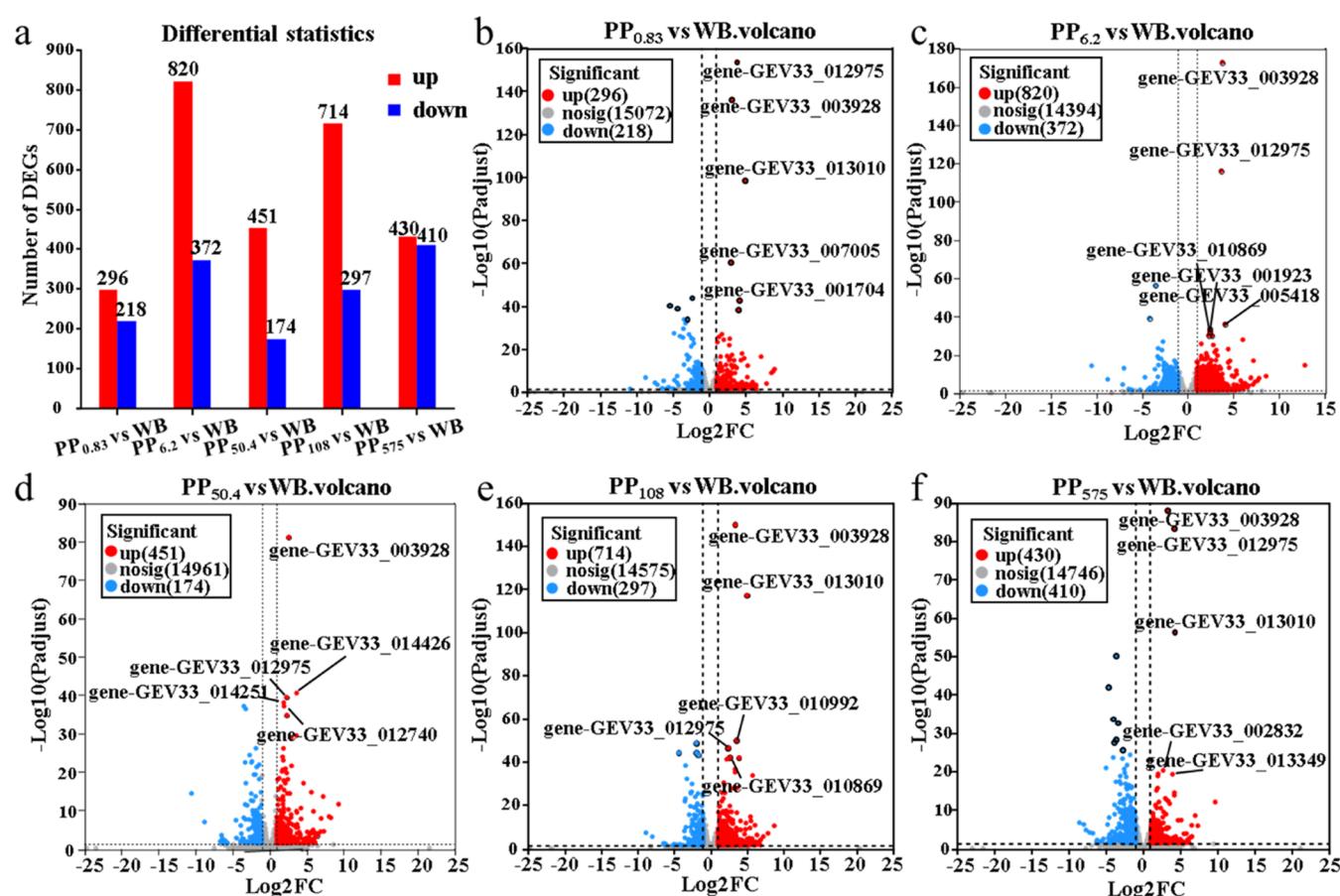


Figure 4. Transcriptomic analyses of *T. molitor* larvae host. (a) Difference statistics of genes expression between PP polymers fed group and WB-fed group; volcano plot of DEGs of PP_{0.83} vs WB (b), PP_{6.2} vs WB (c), PP_{50.4} vs WB (d), PP₁₀₈ vs WB (e), and PP₅₇₅ vs WB (f) with top 5 significantly up-regulated DEGs marked.

MW, showed the most down-regulated DEGs, suggesting that high MW PP had reduced enzymatic activities for certain substrates in the host. Volcano plots displayed DGE distribution between PP-fed and WB-fed groups, with the top 5 significantly up-regulated (cutoff >1.50 folds, $p < 0.05$) DEGs marked (Figure 4b–f, data in Table S3). Similarly, in all PP-fed larvae samples, genes encoding serine-type endopeptidase (GEV33-012975, GEV33-010869) and cysteine-type endopeptidase (GEV33-003928) were significantly enriched. Serine-type endopeptidase is affiliated with the class of endopeptidases, which are involved in cell differentiation, protein degradation, and processing.⁷² They are the major enzymatic components of the larval midgut of Tenebrionidae beetles and are involved in digestion, immune defense, development, and other processes.⁷³ Cysteine-type endopeptidase belongs to cysteine proteases, which also accounts for most of the digestive activity in Coleopteran insects.⁷⁴ In a previous study, serine endopeptidase was reported to be capable of degrading PLA.⁷⁵ Cysteine-rich proteins have been reported to increase the enzymatic hydrolysis of PET by facilitating easy accessibility to PET polymers.⁷⁶ Cysteine protease can also be used for polyamide textile modification.⁷⁷ This finding implies that innate digestive enzymes could possibly contribute to the biodegradation of PP polymers with different MW. Further analyses should be conducted to verify this hypothesis.

Other DEGs related to coding multidrug resistance-associated proteins (MRPs) (GEV33-001923), gustatory

receptor of trehalose (GEV33-014251), Chitinase (GEV33-013010), lipase (GEV33-002832), lipid metabolic process (GEV33-001704), and carbohydrate transport and metabolism (GEV33-001704, GEV33-010992) were also among the top five significantly up-regulated genes in certain sample (Table S3). These genes help the host adapt to nutrient limitations and optimize resource utilization. MRPs are transporters that effectively remove metabolites and harmful byproducts from cells toward areas of excretion,⁷⁸ serving as a defense mechanism to reduce the metabolic burden of plastics on the host. In addition to the genes listed above, some other genes related to MRPs were also found to be remarkably up-regulated among the five PP-fed groups. Gustatory information is considered vital for animals to regulate their behavior when searching for food or mates.⁷⁹ Lipases, which are triacylglycerol acyl hydrolases, can break ester bonds in insoluble substrates of tri-, di-, and monoglycerides into free fatty acids and glycerol. Chitinase, a hydrolase, is essential for the periodic shedding of the old cuticle ecdysis.⁸⁰ In the PP₅₇₅-fed sample, both genes were among the top up-regulated genes. Previous studies have reported the involvement of these two enzymes in plastic degradation.^{46,81} However, their function on the nonhydrolyzable polyolefin plastics should be further investigated. Cannibalism is a common phenomenon in *T. molitor*. Therefore, we suppose that these two genes, as well as the gene related to carbohydrate transport and metabolism and lipid metabolic process, originate from the host's consumption

of its own fat and digestion of agar and self-cannibalism products due to nutrient deficiency.

Specifically, in addition to the DEGs with the highest *p*-values, known polyolefin degradation oxidases,⁸² including various monooxygenase (GEV33-009675, gene-GEV33-005371, gene-GEV33-014143, gene-GEV33-006993, gene-GEV33-013368, gene-GEV33-009986, gene-GEV33-001449, gene-GEV33-001856), laccase (GEV33-001044), and peroxidase (gene-GEV33-002024, gene-GEV33-013532), showed substantial enrichment in the PPs-fed groups compared to the control group. This finding indicates that the host can produce these three types of oxidases for PP oxidation.

The above observations indicate that *T. molitor* host possesses both defensive functions and degradation capabilities toward plastics. Compared to the WB-fed group, larvae fed PP ranging from low- to high MW demonstrated up-regulation of genes associated with innate digestive enzymes and plastic degradation-related enzymes. Genes encoding other functions such as MRPs and signal transduction represent an adaptive response to nutrient-deficient conditions induced by PP diets. When the MW reached a high level (*i.e.*, PP₅₇₅), the most down-regulated genes were observed among the five MWs of PP-fed samples (Figure 4a), indicating a potential depression of certain bodily functions due to the deteriorated biodegradability of high MW PP.

Plausible Biodegradation Mechanisms of PP Polymers. The metabolic pathways of PP are complicated and could be influenced by many factors, including multiple enzymes secreted by both gut microbiota and the host. Based on the results of this study, hydroxylation or carboxylation, which are crucial for activating the inert C–C bond in the PP backbone for scission, poses a challenging task for a single strain to carry out. The results of the present study point to a dual contribution from the host and gut microbiota in the biodegradation of PP from low to high MW. Within the larval gut, multiple enzymes, as well as bioemulsifying factors⁴⁷ secreted by the host and microbes, accelerate the biodegradation of plastics such as PP. Combining the results from previous reports⁴³ and our study, we hypothesize plausible PP biodegradation pathways as follows. Initially, the host larvae defragment PP particles by chewing and ingesting them. Digestive enzymes secreted by the larvae and gut bacteria move around the plastic particles and attach to the surface of polymers with the help of bioemulsifying factors. The initial depolymerization of nonhydrolyzable PP is performed by multiple extracellular enzymes secreted from the bacteria and the host (*e.g.*, monooxygenase, laccase, and peroxidase) to attack and oxidize the PP polymer, resulting in the formation of oxygen-containing functional groups on the surface and chain breaking. This alteration renders the surface hydrophilic, allowing for further disintegration and fragmentation of PP particles into short chains or small molecules. Transmembrane proteins then transport these molecules into the microbial cells to enter diverse metabolic pathways for the complete metabolism. During this process, gut microbes utilize different MW fractions of PP and fix ingested nitrogen gas for the synthesis of proteins needed by the larvae and gut microbiota. The host exhibited a dietary adaptive regulatory mechanism to survive and reproduce in environments with nutrient restrictions. The host activates MRPs, transporters involved in the efflux of plastics within cells, reducing the metabolic pressure exerted on the plastics. The host also activates plastic-biodegradation-related pathways to accelerate PP degradation

and metabolism, thus receiving energy and carbon sources for their living activities.

Environmental Implications. This is the first report to confirm the inherent capability of *T. molitor* larvae to biodegrade PP polymers spanning from low to high MWs, ranging from M_w of 0.83 to 575.00 kDa. This indicates that most commercial PP products (M_w from 120.00 to 550.00 kDa) could be biodegraded by the larvae. The polymer MW affects depolymerization patterns and the degradation process. The PP biodegradation can be effectively accessed by determining the $\Delta\delta^{13}\text{C}$ of the PP polymers before and after biodegradation.

The results reveal that the larvae altered gene expression of both gut bacteria and host to form a system conducive to plastic degradation when they received PP diets, contingent on PP MWs for utilizing them as a carbon source and adapting to nutritional stress. The larval gut functions as an effective bioreactor, activating innate digestive enzymes, oxidases, defensive functions, signal transduction functions, and carbohydrate transport and metabolism-related functions for synergistic PP degradation. This explains why *in vitro* biodegradation of plastics like PP, PS, and PE by gut bacterial strains isolated from the *T. molitor* and other plastic-degrading larvae is much less efficient than *in vivo* biodegradation. These findings offer new dimensions for understanding the biodegradation process of polyolefins in *T. molitor* larvae, providing a theoretical basis for strengthening plastic degradation by harnessing the physiological characteristics of plastic-eating insects. Additionally, the findings that different predominant bacteria are responsible for biodegrading PP with different MWs provide information on the selection of targeted microbes and enzymes for distinct types of PP waste. However, besides MW, the physical properties of the polymer such as particle size will also affect its biodegradation, and further experiments are needed to investigate it.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.3c06954>.

Sample preparation, analysis methods, characterization of pristine commercial PP MPs and polymers from frass, and omics analysis processes; and discussion details on results of 16S rRNA analyses, tables, and figures supporting the experimental and data analysis materials (PDF)

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L.H.: Conceptualizations—experimental design, formal analysis, sample collection, methodology, supervision, and validation; Writing—original draft, review, and editing. J.D.: conceptualization and funding acquisition. S.—S.Y.: Methodology, formal analysis, and funding acquisition. Y.-N.Z.: Conceptualization—experimental design. J.-W.P.: Conceptualization—experimental design. D.X. and L.-Y.Z.: Methodology. N.R.: Conceptualization—experimental design. W.-M.W.: Conceptualization, methodology, supervision, validation; Writing—drafting, review, and editing.

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Notes

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NOMENCLATURE

ABC	ATP-binding cassette
BD	broad extent of depolymerization
DEGs	differentially expressed genes
GO	gene ontology
FT-IR	Fourier transform infrared spectroscopy
GPC	gel permeation chromatography
HDPE	high-density polyethylene
^1H MNR	proton nuclear magnetic resonance
HT-GPC	high-temperature-gel permeation chromatography
KEGG	Kyoto encyclopaedia of genes and genomes
LD	limited extent depolymerization
LDPE	low-density polyethylene
LD	limited extent of depolymerization
M_n	number-average molecular weight
MPs	microplastics
M_w	weight-average molecular weight
M_z	size-average molecular weight
MW	molecular weight
MWD	molecular weight distributions
PCA	principal component analysis
PDI	polydispersity index
PE	polyethylene
PET	polyethylene terephthalate
PLA	polylactic acid
PP	polypropylene
PS	polystyrene
PUR	polyurethane
PVC	polyvinyl chloride
SRs	survival rates
<i>T. molitor</i>	<i>Tenebrio molitor</i>
TCB	1,2,4-trichlorobenzene
TGA	thermogravimetric analysis
WB	wheat bran

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