

Differences in the properties of polyolefin and polyester plastics biodegradation by insects and gut microbiome responses

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Abstract. The issue of plastic pollution is becoming increasingly severe, and the potential of insects to biodegrade plastics offers new insights for the development of harmless and resource-efficient plastic waste treatment technologies. However, plastic wastes discarded in the environment are complex and diverse. There is limited knowledge regarding the feeding preferences, degradation efficiency, and impact on growth performance of different insects for various types of plastics, all of which would affect the plastics degradation effectiveness in practical applications involving insects. This study compared the degradation efficiency and growth index of two typical insects (mealworm *Tenebrio molitor* and superworm *Zophobas atratus*) on two types of typical foam plastics, i.e., a polyolefin plastic of polyethylene (PE), and a polyester plastic of polyurethane (PU). The results indicated that: (1) Insects exhibited higher feeding efficiency and degradation degree on polyester plastics than polyolefin plastics. Superworms showed higher plastic feeding efficiency than mealworms, while mealworms achieved a higher degradation degree, with their degradation products exhibiting lower toxicity than superworms; (2) Significant changes occurred in the microbial community of insects during the ingestion and degradation of plastics. Key microbial species involved in PE and PU biodegradation were distinct, with un_Enterobacteriaceasp., *Hafnia-Obesumbacterium* sp. and *Acinetobacter* sp. predominating PE degradation, while *Morganella* sp., un_Lachnospiraceasp. and *Providencia* sp. predominating PU degradation; (3) Potential plastic-degrading enzymes were enriched, with dehydrogenase (betB), monooxygenase (ssuD), and peroxidase (EfeB) playing significant roles in PE degradation, while dehydrogenase (dapH), laccase (aes), and hydroxylase (mhpA) being crucial for PU degradation. These findings provided essential guidance for the development of insect-based plastic biodegradation processes and harmless treatment of plastic wastes.

Keywords: *T. molitor* larvae, *Z. atratus* larvae, Plastics degradation, Gut microbiome, Function prediction

Received on 15 March 2023, Accepted on 15 Apr 2023, Published on 28 June 2023

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1 Introduction

The relentless proliferation of plastic waste constitutes one of the most pressing environmental challenges of the Anthropocene. Global plastic production has surged exponentially since the mid-20th century, yet dismal recycling rates—around 9%—mean the vast majority of this durable material accumulates in landfills and natural ecosystems [4]. Among the plethora of synthetic polymers, polyethylene (PE) and polyurethane (PU) are quintessential representatives of two major classes with widespread use and persistence. PE, a polyolefin with a backbone of robust carbon-carbon (C-C) bonds, is the world's most produced plastic, valued for its inertness and durability, which ironically are the very properties that render it highly recalcitrant in the environment [3]. PU, a polyester containing more labile carbamate (urethane) and ester (C-O) linkages, is another high-volume polymer extensively used in foams, coatings, and elastomers [3]. The environmental fate of these materials is alarming; they slowly fragment through weathering and mechanical action into micro- and nano-plastics, pervasive pollutants that infiltrate every trophic level, posing direct and indirect threats to biodiversity and potentially human health [5]. Conventional waste management strategies, primarily landfilling and incineration, are fraught

with shortcomings, including long-term ecological footprint, greenhouse gas emissions, and potential for secondary pollution, thus standing at odds with global sustainability and "carbon neutrality" objectives [6]. This dire context has catalyzed an urgent search for innovative, eco-compatible solutions for plastic waste remediation, with biological degradation emerging as a particularly promising avenue.

In this quest, insect-mediated biodegradation has surfaced as a remarkable and surprising frontier. Over the past decade, larvae of certain insect species, notably from the Coleoptera order such as the yellow mealworm (*Tenebrio molitor*) and the superworm (*Zophobas atratus*), have been empirically demonstrated to ingest and degrade a spectrum of plastics, including PS, PE, PP, PU, and PVC [6, 8-17]. This capability is not a mere physical fragmentation but involves genuine biodegradation, evidenced by depolymerization, chemical modification (e.g., oxidation, hydrolysis), and assimilation of plastic carbon into insect biomass or its mineralization to CO₂ [7, 14]. The central mechanism underpinning this process is a symbiotic partnership between the insect host and its gut microbiome. The insect's digestive tract provides a unique bioreactor—a confined, moist, and enzymatically active environment—where ingested plastic particles are mechanically fragmented and exposed to a consortium of microbial symbionts. These gut microbes, believed to be the primary agents of plastic depolymerization, harbor and express catabolic enzymes capable of breaking down polymeric chains [18-19]. Research has shown that shifts in diet (e.g., from bran to plastic) induce significant restructuring of the gut microbial community, selectively enriching for bacterial taxa and functional genes associated with polymer degradation [13, 16]. This host-microbiome synergy transforms the insect gut into a dynamic site for plastic bioprocessing, offering a novel pathway for waste valorization and a rich resource for discovering novel plastic-degrading enzymes and microbial consortia.

Despite these promising advances, critical knowledge gaps impede the translation of this phenomenon from laboratory curiosity to a scalable, practical biotechnology. Real-world plastic waste is immensely diverse, and the efficiency of biodegradation is intrinsically linked to polymer chemistry. While both *T. molitor* and *Z. atratus* can degrade various plastics, a systematic, side-by-side comparison of their performance on structurally distinct polymers—specifically, the inert polyolefin PE versus the more hydrolysable polyester PU—is lacking. Fundamental questions remain unanswered: Do these insects exhibit a feeding preference or differential degradation efficiency between these two major plastic classes? How do their growth, survival, and reproductive fitness compare when subsisting solely on these different plastics, a crucial parameter for assessing the feasibility of long-term, continuous biodegradation processes? Furthermore, the distinct chemical structures of PE and PU likely engage different microbial metabolic pathways and enzymatic machineries within the insect gut. A comparative analysis of the gut microbiome's response—identifying the key microbial taxa, enriched functional pathways, and putative degrading enzymes (e.g., oxygenases for PE vs. esterases/laccases for PU)—is essential to decipher the underlying biochemical mechanisms. Therefore, this study was designed to conduct a comprehensive comparative investigation, evaluating the ingestion efficiency, biodegradation extent (via GPC, FTIR, TGA), and impact on growth metrics of *T. molitor* and *Z. atratus* larvae fed exclusively on PE and PU foams. Concurrently, we employed 16S rRNA gene sequencing coupled with PICRUSt2 and FAPROTAX analyses to profile the associated shifts in gut microbiota structure and function. The findings aim to provide a mechanistic groundwork for optimizing insect-based biodegradation processes and advancing the development of targeted, synthetic microbial consortia for the sustainable management of complex plastic waste streams.

2 Materials and Methods

2.1 Insect Rearing and Plastic Sources

Mealworm and superworm larvae were purchased from an agricultural farm in Wudi County, Binzhou City, Shandong Province. Wheat bran was purchased from Zhejiang Jinhua Chenxi Organic Feed Company. Polyethylene (PE) and polyurethane (PU) foam plastics were purchased from Jiangsu Suzhou Jianai'er Packaging Material Factory. Mealworms and superworms were fed with wheat bran as food for 3 days and then starved for 2 days under dark conditions in an artificial incubator at 26°C and 60% humidity before starting the experiment [7].

2.2 Feeding Plastics to Mealworms and Superworms

Mealworm larvae with a body length of about 2.0-2.2 cm and superworm larvae with a body length of 4.0-4.2 cm were selected. Typical polyolefin foam plastic PE and typical polyester foam plastic PU were used as the sole food source for mealworm and superworm larvae. The foam plastics were cut into small pieces of 8 cm × 5 cm × 1 cm, washed with distilled water, dried, and UV-sterilized before the experiment. The group fed with wheat bran was set as the control group. About 10 g of larvae were placed in each group, with 3 replicates. Every 7 days, the growth and development of the larvae, including body length, body width, body weight, survival rate, and pupation rate, were measured. Simultaneously, the weight loss of the plastic was measured, dead insects and molted skins were cleaned, and frass (excrement) was collected. The entire experimental period was 42 days.

2.3 Collection and Characterization of Original Plastics and Residual Plastics in Frass

Frass samples from each group were collected every 7 days and stored in a -20°C freezer for further analysis. Residual microplastics in the frass were ultrasonicated in 5% SDS (Sodium dodecyl sulfate) solution for 30 min and then washed three times with sterilized ultrapure water to remove biomolecular contamination and reduce the possible influence of plastic additives [20]. The recovered residual plastics were dried in an oven at 60°C for 48 h for further analysis. Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the material composition of the plastics and residual plastics in superworm frass, with the spectral range set at 4000-500 cm⁻¹. A Thermogravimetric Analyzer (TGA) was used to characterize the thermal stability of the original plastics and residual plastics in frass. The heating temperature range was 0-600°C, with a heating rate of 20°C·min⁻¹, under an Ar protective atmosphere. Gel Permeation Chromatography (GPC) (Agilent 1260, Waters, USA) was used to analyze the molecular weight changes of PE and PU polymers. PE and PU plastic samples were mixed with 1,2,4-trichlorobenzene and N,N-dimethylformamide solutions, respectively, heated slowly at 60°C, then precipitated, allowed to stand, and the supernatant was taken and filtered through a 0.22 μm PVDF (Polyvinylidene fluoride) filter. 20 μL was injected at a rate of 0.8 mL·min⁻¹ for GPC analysis [7, 14, 21-23]. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) equipped with an HESI source (Thermo Scientific Q Exactive, USA) was used to analyze the biodegradation products of the plastics [24]. Toxicity analysis of the degradation products measured by LC-MS/MS was performed using the T.E.S.T. toxicity prediction tool developed by the USEPA. The molecular formulas or chemical names of the measured degradation products were input into the software, which automatically predicted the corresponding toxicity values based on indicators such as developmental toxicity, teratogenic factor, and bioconcentration factor.

2.4 Extraction of Mealworm and Superworm Guts

After the experiment, the guts of mealworm and superworm larvae were extracted for subsequent analysis: first, the larvae were immersed in 95% alcohol for 5 min, then soaked in sterilized physiological saline for 5 min [18]. The larvae were placed on a sterilized wax dish, and the guts were dissected out with a dissecting needle and placed in 1.5 mL centrifuge tubes. 200 μL of PBS (Phosphate buffer saline) was added, and the samples were homogenized using an electric homogenizer and stored at -80°C.

2.5 Analysis of Gut Microbial Community Structure

Total DNA was extracted from gut samples using the DNeasy Blood and Tissue Kit (QIAGEN, Germany) and sent to Shanghai Majorbio Bioengineering Co., Ltd. for PCR (Polymerase chain reaction) amplification and sequencing [25]. The V4 region of the 16S rRNA gene was amplified using the primer pair 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') [26]. Paired-end sequencing (PE250) was performed on the Illumina HiSeq2500 platform. Finally, the online Majorbio Cloud Platform (Shanghai, China) was used for gut microbial community analysis. The effective sequence data from each sample were rarefied (normalized) before subsequent analysis. PICRUSt2 and FAPROTAX were used to compare the ASV sequences with their internal reference sequences to obtain the corresponding functional information and abundance information for each sample. Abundance was expressed as lg(abundance+1).

2.6 Statistical Analysis Methods

All experiments were performed with 3 replicates. SPSS 25.0 software (SPSS, Chicago, USA) was used for statistical comparison of the data. Results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed for differences among multiple groups, followed by Tukey's post hoc test. P <

0.05 was considered statistically significant.

3 Results and Discussion

3.1 Differences in the Characteristics of Mealworms and Superworms Feeding on PE and PU Foam Plastics

3.1.1 Differences in the Feeding Efficiency of Mealworms and Superworms on Foam Plastics

Both mealworm and superworm larvae showed active feeding on PE and PU foam plastics, mainly in the form of boring holes (Fig. 1A). Their feeding efficiency on the two types of foam plastics differed within the 42-day experiment. On average, per gram of mealworms consumed 31.29 mg of PE and 61.20 mg of PU plastic; per gram of superworms consumed 68.90 mg of PE and 80.38 mg of PU (Fig. 1B). It can be seen that compared to the polyolefin plastic PE, both superworms and mealworms tended to feed more on the polyester plastic PU. The feeding efficiency of mealworms and superworms on polyester plastics was 1.17-1.96 times that on polyolefin plastics. At the same time, under the same conditions, the plastic feeding efficiency of superworm larvae was significantly higher than that of mealworm larvae (ANOVA, $P < 0.05$), about 1.3-2.20 times that of mealworms. The feeding efficiency of superworms on the polyolefin plastic PE was even slightly higher than that of mealworms on the polyester plastic PU. Considering the ecological sustainability of the process, it is also necessary to explore the growth and reproduction of insects after feeding on plastics.

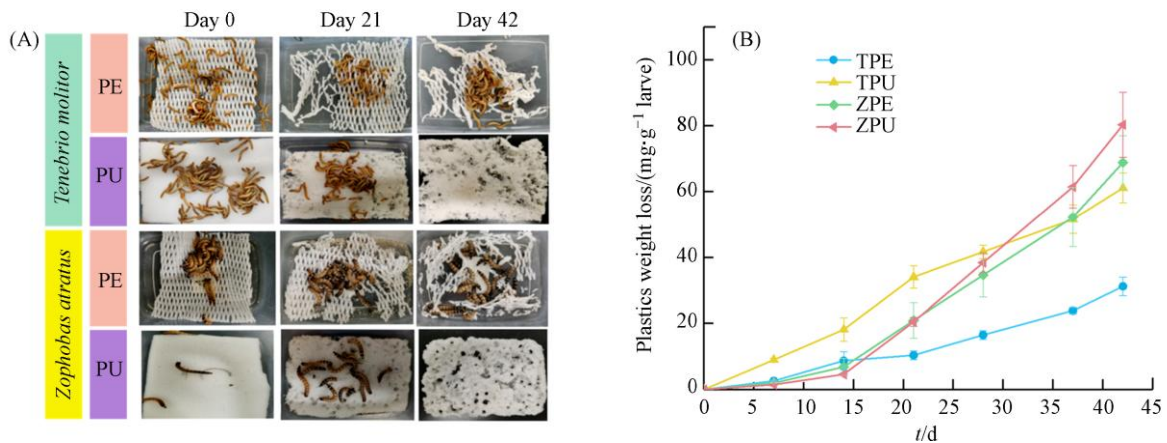


Figure 1 Ingestion diagram (A) and plastics quality change diagram (B) of PE and PU foam plastics by *T. molitor* and *Z. atratus* larvae

3.1.2 Differences in Growth and Development Indices of Mealworms and Superworms After Feeding on Foam Plastics

Table 1 shows the differences in growth and development indices of mealworm and superworm larvae after feeding on the two types of plastics. It can be seen that the body weight or body length of mealworms feeding on PE (TPE), mealworms feeding on PU (TPU), superworms feeding on PE (ZPE), and superworms feeding on PU (ZPU) increased to some extent, indicating that PE and PU foam plastics as the sole food can provide the energy needed to maintain normal growth. Compared to the wheat bran control groups (TBran, ZBran), the growth was poorer, with lower weight gain rates, lower survival rates, and almost 0% pupation rate. This indicates that feeding solely on plastics cannot ensure their normal growth and reproduction, which is unfavorable for the practical application of using mealworms and superworms for long-term plastic degradation. The survival rate of the superworm groups was significantly higher than that of the mealworm groups (ANOVA, $P < 0.05$). At the same time, the plastic degradation efficiency of superworms was also higher than that of mealworms. This suggests that superworms are more suitable for practical insect plastic degradation processes. Relevant literature points out that adding wheat bran or adopting alternate feeding of plastics and wheat bran can not only improve foam plastic degradation ability but also reduce the impact of plastic feeding on insect growth and development [7, 13]. Therefore, in practical insect-based plastic biodegradation processes, co-feeding with wheat bran may

be an efficient and ecologically sustainable method.

Table 1 Growth indexes of *T. molitor* larvae and *Z. atratus* larvae after feeding on foam plastics

Index	TBran	TPE	TPU	ZBran	ZPE	ZPU
Pupation Rate /%	8.69±2.35 a	0.91±1.01 bc	2.19±1.20 b	0±0 c	0±0 c	0±0 c
Survival Rate /%	64.41±4.91 a	52.04±14.28 a	44.41±19.66 a	96.67±5.77 b	95±5 b	90±10 b
Weight Gain Rate /%	81.53±10.09 a	30.39±8.01 b	25.15±9.59 b	9.10±4.10 cd	0.87±11.70 d	-2.83±6.98 d
Body Length Gain Rate /%	15.90±6.67 a	10.58±8.77 ab	3.14±8.99 bc	6.48±4.84 bc	0±0 c	2.78±6.18 bc
Body Width Gain Rate /%	20.63±0.05 a	4.22±0.02 bc	1.76±0.02 c	7.87±0.03 b	3.78±0.024 bc	5.06±0.06 bc

Note: Different letters (a, b, c, d) in the table indicate significant differences between groups for each index (ANOVA, $P < 0.05$).

3.2 Differences in the Characteristics of Mealworm and Superworm Degradation of PE and PU Foam Plastics

3.2.1 Differences in Molecular Weight Changes of PE and PU Foam Plastics After Feeding by Mealworms and Superworms

After feeding on foam plastics for 42 days, residual plastic fragments were recovered from the larval frass for molecular weight change testing (Fig. 2). GPC analysis revealed that after mealworms and superworms fed on PE foam plastic, the molecular weights (number-average molecular weight (M_n) and weight-average molecular weight (M_w)) of the residual PE significantly decreased compared to the original PE plastic (ANOVA, $P < 0.05$), indicating that depolymerization of the PE plastic occurred during the feeding process by mealworm and superworm larvae. After mealworms and superworms fed on PU foam, the molecular weights of the residual plastic also significantly decreased (ANOVA, $P < 0.05$). The simultaneous decrease in both M_n and M_w indicates that insect degradation of plastics is relatively complete, with polymer chains breaking into small molecules and being completely decomposed [19]. Comparing the extent of molecular weight decrease for the two plastics, it can be seen that mealworm and superworm larvae had the highest degradation degree and most complete degradation for the polyester plastic PU. This is speculated to be related to the molecular structure of the plastic itself. PE is a polyolefin plastic, mainly containing C-C single bonds, which are not easily hydrolyzed by microorganisms and enzymes, while PU is a polyester plastic containing ester bonds that are easily hydrolyzed by hydrolases [27]. The degradation extent of the two insects for the same plastic was similar, with no significant difference in the degree of molecular weight change of the plastic (ANOVA, $P > 0.05$). Under the same cultivation conditions and time, the M_n and M_w molecular weight change degree of PE degraded by an equivalent biomass of mealworms was slightly higher than that by superworms, and the M_w molecular weight change degree of PU degraded by mealworms was slightly higher than that by superworms. This shows that in terms of plastic degradation degree, mealworms are slightly superior to superworms in degrading plastics.

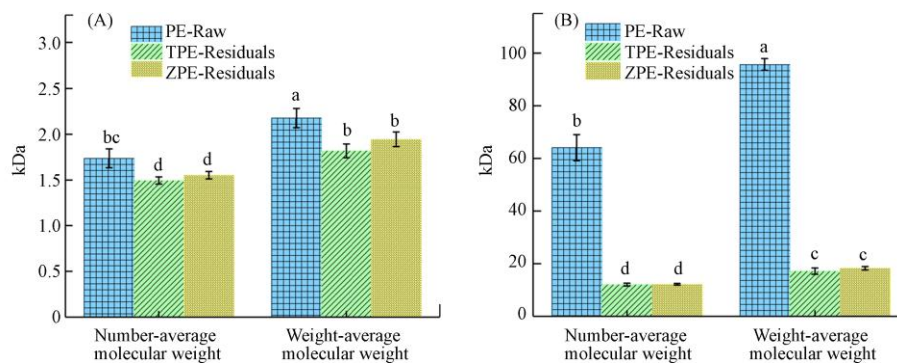


Figure 2 Molecular weight changes of PE (A), PU (B) foam plastics following consumption by larvae of *T. molitor* and *Z. atratus*. Different letters (a, b, c, d) indicated significant differences between groups (ANOVA, $P < 0.05$)

3.2.2 Differences in Structural Changes of PE and PU Foam Plastics After Feeding by Mealworms and Superworms

Comparing the infrared spectra of PE plastic before and after feeding by mealworms and superworms, it was found that the basic characteristic absorption peaks of original PE at 2918, 2853, 1469, 730 cm^{-1} , etc., weakened, and new peaks appeared at 1014 (C-O stretching vibration) and 1640 (C=O stretching vibration), indicating that oxidation occurred in the PE plastic, and the PE plastic was degraded (Fig. 3A) [28]. Comparing the FTIR results of original PU and the residual microplastics after insect feeding, it can be found that the basic characteristic bands of original PU at 3289, 2273, 1715 cm^{-1} (N-H stretching vibration, asymmetric stretching vibration of NCO, C=O stretching vibration) weakened or disappeared, and new bands appeared at 3426, 1386 cm^{-1} (O-H stretching vibration, C-O bending vibration), etc. It can be seen that the plastic components changed significantly, and PU was obviously degraded (Fig. 3B). The infrared spectroscopy results show that during the insect feeding and degradation of polyolefin and polyester plastics, oxidation effects were produced on the plastics. The addition of oxygen-containing functional groups to the polymer chains, reducing their hydrophobicity and molecular weight, is a key and essential step in the plastic biodegradation process [15]. It can be inferred that after feeding by mealworms and superworms, microorganisms in the insect gut colonized the plastic surface, secreted substances such as extracellular enzymes, causing oxidation on the surface of the residual PE and PU plastics, roughening the plastic surface, and damaging the plastic structure, thereby promoting further degradation of the plastics [27, 29]. The infrared spectral differences of residual plastics after degradation of the polyolefin plastic PE by the two insects were small, indicating that the degradation extent of PE plastic by mealworms and superworms was similar. From the infrared spectra of the degraded polyester plastic PU, it can be seen that the intensity of the C-O peak in PU plastic after mealworm feeding was higher than that after superworm feeding. It is speculated that the oxidation degree of PU plastic by mealworms is higher, so the molecular weight change of PU before and after feeding was greater (Fig. 2), and the degradation degree was higher.

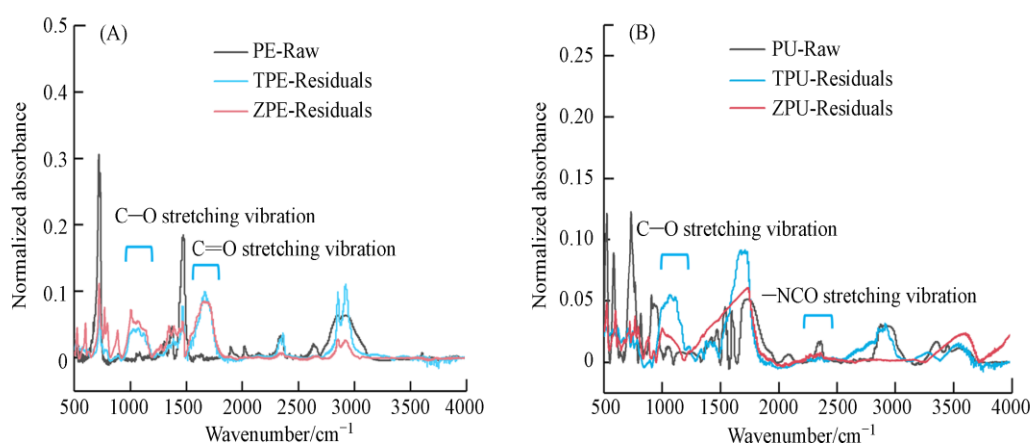


Figure 3 Differences in FTIR results of PE (A) and PU (B) foam plastics following consumption by *T. molitor* larvae and *Z. atratus* larvae

3.2.3 Differences in Thermal Stability Changes of PE and PU Foam Plastics After Feeding by Mealworms and Superworms

Differences in the changes of plastic thermal stability properties before and after insect feeding were compared by thermogravimetric analysis. The original PE plastic showed no significant weight loss under temperature conditions below 400°C, lost 5% of its mass at 446.4°C, and only exhibited one endothermic peak throughout the process (Fig. 4). The residual PE plastic after digestion in the mealworm gut had a 5% mass loss temperature of 183.5°C, significantly lower than the original PE plastic, and the entire weight loss process showed 4

endothermic peaks, indicating the presence of new components in the residual microplastics and reduced thermal stability. Similarly, the thermal stability of the residual PE plastic after superworm feeding was also lower than that of the original plastic, with a 5% mass loss temperature of 327.4°C. The appearance temperature of the endothermic peak was close to that of the original plastic, indicating that it had the same components as the original PE plastic, but the material composition was not exactly the same, and new substances were formed after superworm digestion. From the degree of change in thermal stability of the polyolefin plastic PE before and after feeding and degradation by mealworms and superworms, it can be seen that the thermal stability change of PE after mealworm feeding was greater. This shows that the degradation degree of PE plastic by mealworms is higher than that by superworms. The original PU plastic underwent two weight loss processes, showing two endothermic peaks throughout the process, at 297.8°C and 395.2°C, speculated to be due to molecular chain breakage (Fig. 4). The thermal stability of the residual microplastics after mealworm feeding and degradation of PU decreased, and the weight loss rate accelerated. The thermal stability of the residual microplastics after superworm feeding on PU plastic also decreased to some extent. By comparison, it was found that there were obvious differences from the original PU thermogravimetric curve, indicating that the components of the residual microplastics after insect digestion of PU changed, showing that mealworms and superworms have obvious degradation effects on PU. The 5% mass loss temperature of the residual PU plastic after digestion in the mealworm gut was 213.0°C, lower than that of the residual PU plastic after digestion in the superworm gut (266.7°C). It can be seen that the thermal stability change of PU after mealworm feeding was greater, and the degradation degree of the polyester plastic PU by mealworms is higher than that by superworms.

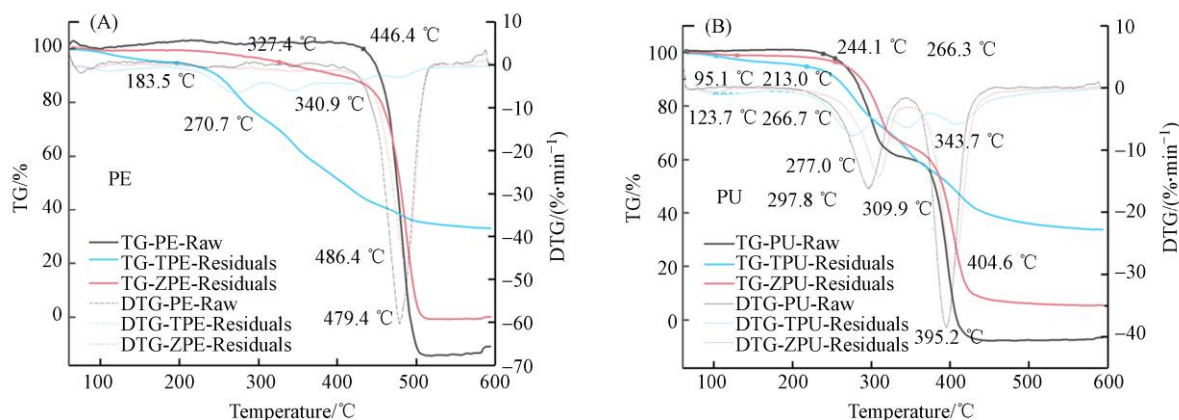


Figure 4 Differences in thermal stability of PE (A) and PU (B) foam plastics following consumption by *T. molitor* and *Z. atratus* larvae

From the changes in physicochemical characteristics of the polyolefin plastic PE and polyester plastic PU before and after feeding by mealworms and superworms, it can be seen that both mealworms and superworms can feed on and degrade polyolefin and polyester foam plastics, with a relatively complete degradation degree. Under the same conditions, the degradation degree of insects on the polyester plastic PU is higher than that on the polyolefin plastic PE, and the degradation degree of mealworms on polyolefin and polyester foam plastics is higher than that of superworms.

3.2.4 Differences in Degradation Intermediate Products of PE and PU Foam Plastics Fed on and Degraded by Mealworms and Superworms

The differences in plastic degradation intermediate products after mealworms and superworms fed on and degraded PE and PU foam plastics were further analyzed by High-Performance Liquid Chromatography-Mass Spectrometry (Fig. 5). After subtracting the chromatographic peaks of the original PE plastic, new peaks appeared in the residual PE plastic after mealworm feeding at retention times of 3.36, 4.75, 9.82, 10.92, and 11.60. By analyzing the structure of fragment ions in the secondary mass spectrometry of the products, the possible degradation products were speculated to be C₆H₁₀O₄, C₁₅H₂₇N₅O₆, C₁₆H₂₉N₃O₆, etc. The degradation products present in the residual PE plastic after superworm feeding were speculated to be C₂₂H₄₀O₂, C₃₁H₅₀O₆, C₄₃H₇₆O₆, etc. Similarly, the possible intermediate small molecule metabolites produced after mealworm feeding on PU

plastic were $C_{20}H_{40}N_2O_3$, $C_{22}H_{40}O_2$, $C_{21}H_{34}N_4O_4$, etc., while the metabolites produced after superworm feeding on PU were possibly $C_{28}H_{49}NO_4$, $C_{24}H_{39}N_3O_3$, $C_{24}H_{43}NO_3$, etc. It can be seen that the main plastic degradation products are substances such as unsaturated fatty acids containing C, H, O, N, and other elements. This shows that depolymerization, oxidation, hydrolysis, and nitrogen fixation play important roles in the plastic degradation process [16, 19, 30]. The small molecules produced by the degradation of the two types of plastics by mealworms and superworms are mainly substances like unsaturated fatty acids, indicating the key role of fatty acid degradation, metabolism, and synthesis in the plastic degradation process. During plastic degradation, plastic monomers and oligomers produced after plastic depolymerization and chain scission enter the fatty acid synthesis and metabolism pathways, and finally enter the β -oxidation mechanism to generate acetyl-CoA, which enters the TCA cycle (Tricarboxylic acid cycle) to oxidize organic matter and produce ATP (Adenosine triphosphate), providing energy for cell metabolism and growth [27, 31]. At the same time, by comparing the developmental toxicity of degradation products after insects fed on plastics, it can be seen that the toxicity of degradation products after mealworm feeding on PE and PU was lower than that of degradation products produced after superworm feeding, although the difference was not significant (ANOVA, $P > 0.05$). Generally, degradation products with larger molecular weights may be less easily biodegraded in the environment, leading to their accumulation in the ecosystem and thus exhibiting higher toxicity [32]. Although it is difficult to determine a complete list of degradation products due to the low mass or complex structure of other degradation products and insufficient fragment ion information in the secondary mass spectrometry, it can be seen that the molecular weight of degradation products produced after mealworm feeding on the two plastics is smaller than that produced after superworm feeding on plastics, and the toxicity of its degradation products is also lower, indicating that the degradation degree of the two plastics by mealworms is relatively higher [24]. Therefore, although the plastic feeding efficiency of superworms is higher, the degradation degree is lower than that of mealworms, and the developmental toxicity of the degradation products is relatively higher. Hence, in practical insect plastic degradation processes, while pursuing degradation efficiency, the green control and reduction of plastic degradation products also need to be considered.

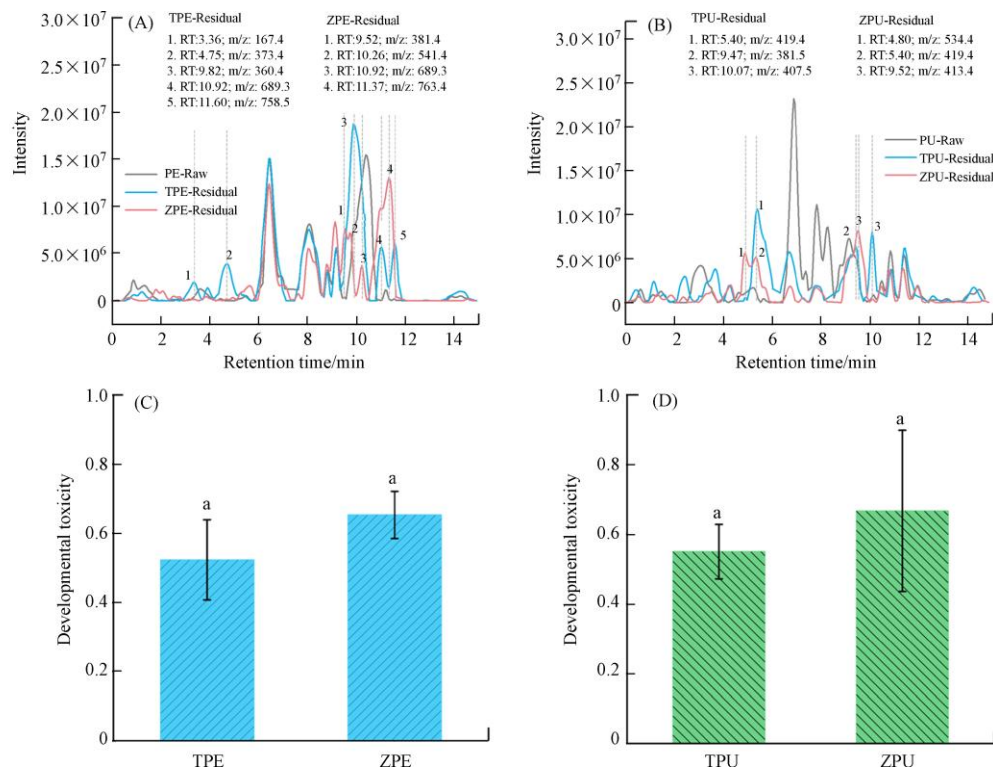


Figure 5 Variations in metabolites of PE (A), PU (B) foam plastics following consumption by the insects and differences in developmental toxicity of metabolites of PE (C), PU (D) foam plastics following consumption by the insects

3.3 Differences in Gut Microbial Response During the Degradation of PE and PU Foam Plastics by Mealworms and Superworms

3.3.1 Differences in Gut Microbes of Mealworms and Superworms After Degrading PE and PU Foam Plastics

After 16S rRNA high-throughput sequencing, a total of 1,015,971 optimized sequences were obtained, totaling 256,709,445 bp, with an average sequence length of 252.68 bp. Species annotation results showed that a total of 9 phyla, 12 classes, 32 orders, 50 families, and 71 genera of bacteria were detected from the gut samples of mealworm and superworm larvae. From the alpha diversity index difference graph, it can be seen that the gut microbial diversity of superworms and mealworms decreased after feeding on the two types of plastics, but there was no significant difference (Fig. 6A, ANOVA, $P > 0.05$). The results of beta diversity index differences between samples based on Bray-Curtis distance showed that the gut microbial community structures of mealworm and superworm larvae were significantly different (Fig. 6B, $P < 0.01$, ADONIS, 999 permutations). The community structures after mealworms and superworms fed on and degraded PE and PU foam plastics are shown in Fig. 6C. Based on Kruskal-Wallis rank-sum test, multiple test correction FDR, and Turkey-Kramer test and analysis of the microbial community, it was revealed that the guts of mealworm and superworm larvae were enriched with corresponding potential plastic-degrading functional bacterial groups after feeding on the polyolefin plastic PE and polyester plastic PU. The dominant bacterial group in the gut of mealworms feeding on PE plastic was mainly un_Enterobacteriaceasp. The gut of mealworms feeding on PU plastic was enriched with Morganellasp. and un_Lachnospiraceasp. bacteria. The gut of superworm larvae feeding on PE plastic was enriched with un_Enterobacteriaceasp., Hafnia-Obesumbacteriumsp., and Acinetobactersp. bacteria. At the same time, the gut of superworm larvae feeding on PU plastic was mainly enriched with Providenciasp. and Morganellasp. bacteria. Among them, bacteria from genera such as Spiroplasmasp., Hafnia-Obesumbacteriumsp., and Acinetobactersp. have been reported to be able to degrade various polymers [13, 16, 33]. This shows that there are obvious differences in the composition of gut functional microorganisms during the feeding and degradation of PE and PU plastics by mealworms and superworms. The inconsistent composition of enriched potential plastic-degrading functional bacteria may be a key factor leading to differences in degradation efficiency and degree of the two types of plastics by the two insects.

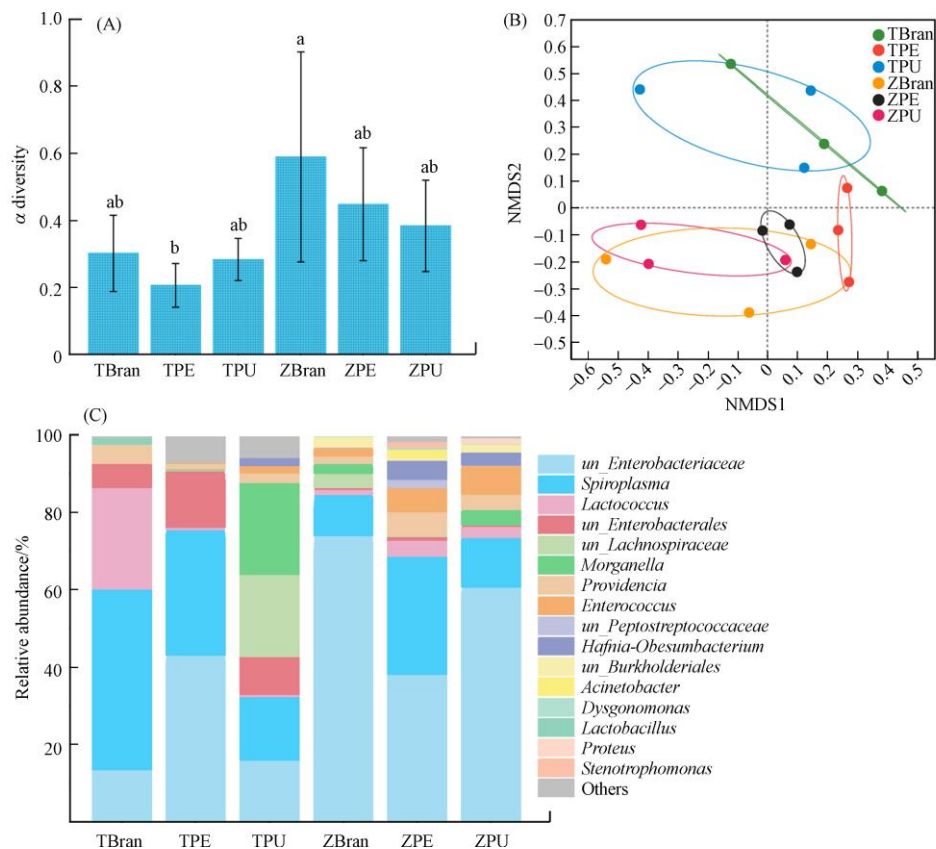


Figure 6 Variations in gut microbial alpha diversity (A) and beta diversity (B), and community composition (C) at the genus level between *T. molitor* and *Z. atratus* larvae after feeding on PE and PU foam plastics

3.3.2 Differences in Metabolic Pathways and Functional Genes During the Degradation of PE and PU Foam Plastics by Mealworms and Superworms

KEGG metabolic pathway function prediction based on PICRUSt2 found that the gene abundance of related metabolic pathways such as benzoate degradation, fatty acid degradation, fatty acid biosynthesis, biosynthesis of unsaturated fatty acids, carbon metabolism, and nitrogen metabolism increased during the feeding and degradation of various plastics by mealworms and superworms (Fig. 7A). This indicates that after insects feed on and digest plastics, they depolymerize the molecular chains in the high-polymer structure of the plastics, generate intermediate products such as long-chain fatty acids, and metabolize them to produce energy to maintain their own growth.

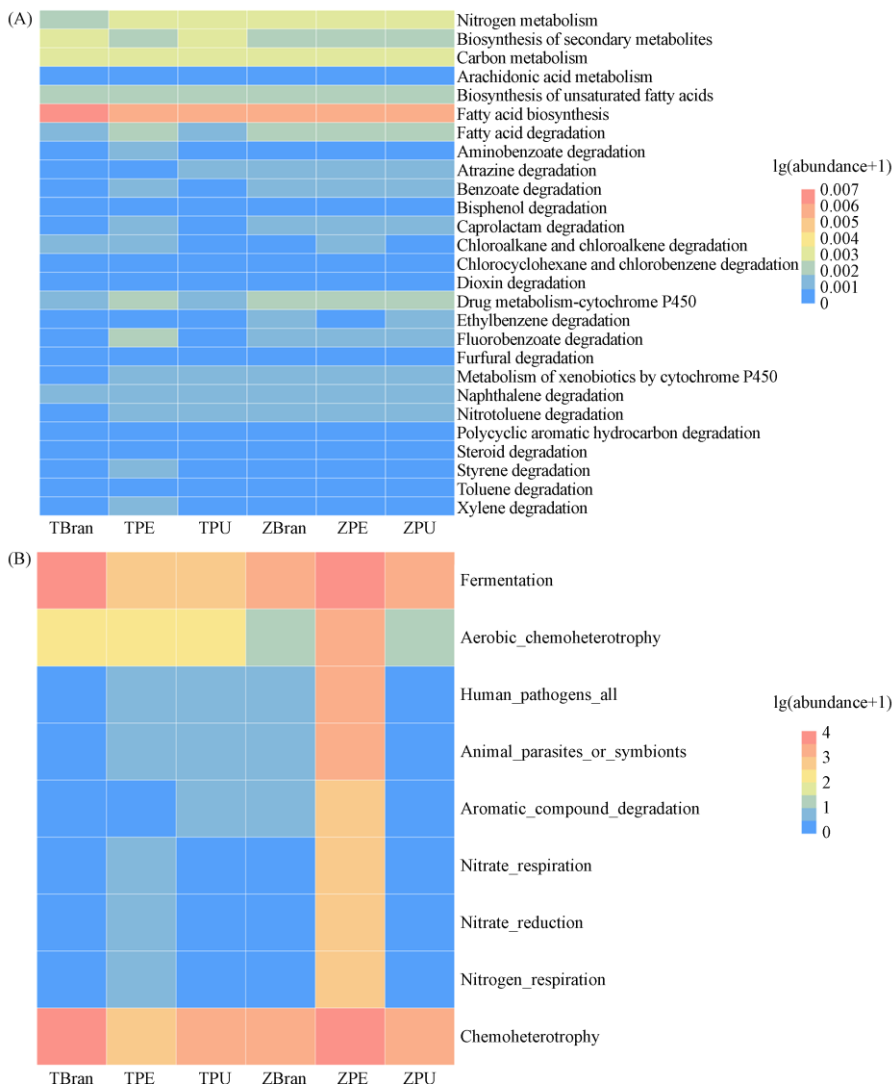


Figure 7 Differences in metabolic pathways based on KEGG (A) and FAPROTAX (B)

C, N, S, P element cycling function prediction based on FAPROTAX shows that metabolic pathways such as aerobic chemoheterotrophy, fermentation, and aromatic compound degradation were significantly enriched in insect groups feeding on various types of plastics (Fig. 7B). Aerobic chemoheterotrophy is a metabolic process in which organisms use organic compounds as a source of energy and carbon and require oxygen in the process [34]. This

type of metabolism is common in many bacteria, fungi, and animals, including the breakdown of organic molecules through aerobic respiration to produce energy in the form of ATP. In this process, organic molecules such as sugars, fats, and proteins are oxidized to produce carbon dioxide, water, and adenosine triphosphate. This illustrates that after insects feed on plastics, they utilize the products of plastic chemoheterotrophy to produce the energy needed to maintain their growth, development, and metabolism. At the same time, it can be noted that nitrogen cycle processes such as nitrate respiration, nitrate reduction, and nitrogen respiration are very important for mealworms and superworms feeding on PE foam plastic [30]. Related reports have also proven that the nitrogen element cycle plays a synergistic and promoting key role in insect feeding and degradation of plastics [19, 30].

COG functional gene analysis based on PICRUSt2 revealed differences in potential plastic-degrading functional enzymes (Fig. 8). It can be found that the expression of dehydrogenases, esterases, hydroxylases, monooxygenases, oxidases, and peroxidases in the gut microorganisms of superworms and mealworms increased compared to the wheat bran control group after feeding on and degrading polyester and polyolefin plastics. From Fig. 8, it can be inferred that genes and enzymes such as dehydrogenase betB, esterase fes, hydroxylase ycfD, monooxygenase ssuD, and peroxidase EfeBplay major roles in PE degradation by mealworms. Dehydrogenase dalD, esterase fes, and hydroxylase ycfD have promoting effects on PU degradation by mealworms. Similarly, genes and enzymes such as dehydrogenases betA, betB, esterase fes, hydroxylases ycfD, ubiH, ubiF, monooxygenases ssuD, pdxH, and peroxidase EfeBplay important roles in the degradation of PE and PU plastics by superworms. Oxidases such as oxygenases, monooxygenases, and peroxidases promote the oxidation and decomposition of complex plastic molecules into smaller molecules or produce soluble compounds through oxidation, promoting further degradation of microplastics [8, 16]. Hydroxylases can catalyze hydroxylation reactions, increasing the hydrophilicity of microplastics, making the substrate more soluble in water, and more easily further degraded and metabolized by microorganisms [35]. Dehydrogenases are also essential for the microbial metabolism of decomposition products produced by microplastic degradation. When microplastics are decomposed into smaller organic molecules by other enzymes, dehydrogenases are responsible for catalyzing the oxidation of these molecules, producing reduced coenzymes (NADH or NADPH), which can then be used in the electron transport chain to produce ATP [36]. This process allows microorganisms to obtain energy from the decomposition products of microplastics, promoting their growth and the microplastic degradation process. Esterases can break ester bonds on polymer chains, thereby forming alcohol and carboxylic acid parts. These decomposition products can be used as nutrients by the microbial community, promoting the biodegradation of plastics in the environment [37].

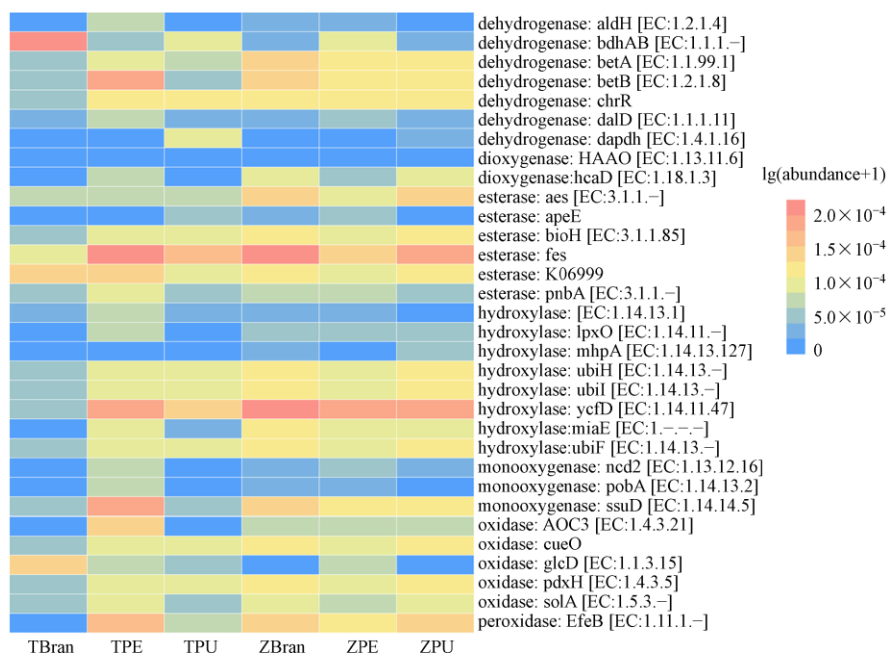


Figure 8 Differences in the abundance of potential plastic degrading enzymes

Overall, it can be seen that the relative abundance of metabolic pathways and functional genes during the feeding of mealworms on PE and PU foam plastics, such as biosynthesis of secondary metabolites, esterase fes, hydroxylases EC:1.14.13.1 and ycfD, oxidase gclD, etc., is higher than that in superworms. This may also be the reason why the degradation degree of the two types of plastics by mealworms is higher than that by superworms. These results indicate that after insects feed on plastics, plastic degradation-related metabolic pathways are greatly stimulated, and a large number of plastic degradation genes are enriched, promoting the biodegradation of plastics by insects.

4 Conclusion

(1) The degradation efficiency and degree of insects on the polyester plastic PU are higher than those on the polyolefin plastic PE. The plastic feeding efficiency of superworms is higher than that of mealworms, but the degradation degree of plastics by mealworms is higher than that by superworms, and the generated degradation products have relatively lower toxicity;

(2) Significant changes occurred in the gut microbial community structure during the degradation of PE and PU plastics by insects, enriching potential plastic-degrading functional bacteria. Un_Enterobacteriaceasp., Hafnia-Obesumbacteriumsp., and Acinetobactersp. bacteria play key roles in PE degradation, while Morganellasp., un_Lachnospiraceasp., and Providenciasp. bacteria are involved in the biodegradation of PU. These potential functional bacteria are expected to be used to design synthetic microbial consortia for synergistic degradation of various plastics, improving plastic biodegradation effects;

(3) During the process of insects feeding on and degrading various plastics, the gene abundance related to the biodegradation and metabolism of xenobiotics, such as fatty acid degradation, carbon synthesis, and nitrogen metabolism, increased. Genes and enzymes such as dehydrogenases, esterases, hydroxylases, monooxygenases, oxidases, and peroxidases play major roles in the plastic degradation process. In particular, dehydrogenase betB, monooxygenase ssuD, and peroxidase EfeBplay important roles in PE degradation, while dehydrogenase dapdh, laccase aes, and hydroxylase mhpAplay key roles in PU degradation. Differential plastic-degrading functional bacteria and enzymes are key factors causing differences in the degradation efficiency and degree of the two insects feeding on the polyolefin plastic PE and the polyester plastic PU.

This study provides an important reference for utilizing insects for plastic biodegradation. Future research can delve deeper into the synergistic effects between microbial metabolisms to better understand the enzyme systems involved in the biodegradation of different plastics. This will help develop green microbial remediation methods for waste plastics based on synthetic microbial consortia.

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