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Influence of Protein and Cellulose Supplementation on Expanded Polystyrene Biodegradation and Gut Microbial Dynamics in *Tenebrio molitor* and *Zophobas atratus*

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Abstract

Due to its limited environmentally friendly disposal options and resistance to biodegradation, expanded polystyrene (EPS) is a ubiquitous plastic pollutant. Recent research has highlighted that certain insect larvae, most notably mealworms (*Tenebrio molitor*) and superworms (*Zophobas atratus*), can ingest and break down EPS, a process facilitated by their symbiotic gut microbiota. Nevertheless, little is known about how the gut microbial composition, EPS degradation efficiency, and larval diet interact. This study systematically investigated how dietary supplementation—protein, cellulose, and their combination affect EPS degradation rates, larval survival, and gut microbiome dynamics in *T. molitor* and *Z. atratus* over a 14-day period (December 30th, 2024 - January 13th, 2025). The larvae were grouped of 80 (*T. molitor*) and 75 (*Z. atratus*) in each experimental condition. Survival rates and EPS consumption were tracked under four feeding regimes: EPS-only (+bran as baseline), EPS + protein, EPS + cellulose, and EPS + protein + cellulose. Using 16S rRNA gene amplicon sequencing, gut microbial communities were characterized. In all dietary conditions, *Z. atratus* showed higher survival and EPS consumption than *T. molitor*. In *Z. atratus*, larvae fed only EPS exhibited the highest degradation, whereas *T. molitor* consumed the least EPS under EPS-only conditions. There were clear species-specific nutritional trade-offs because adding protein and/or cellulose to the diet increased EPS consumption in *T. molitor* while decreasing EPS intake in *Z. atratus*. Microbial analysis showed that in both species, higher EPS degradation was consistently linked to higher Enterobacteriaceae abundance. Moreover, different microbial community profiles were influenced by diet and species: *T. molitor* carried *Spiroplasma sp.*, *Weissella sp.*, *Enterococcus sp.*, and *Enterobacteriaceae*, whereas *Z. atratus* showed an enrichment of *Enterobacteriaceae* and *Lactococcus sp*. Temporal analysis showed that efficient EPS metabolism was linked to either stable or selectively enriched bacterial consortia. These findings emphasize the critical role of diet-microbiome interactions in optimizing insect-mediated EPS biodegradation and support the development of biologically driven waste management strategies utilizing insect–microbe systems.

Keywords: Tenebrio molitor; Zophobas atratus; Expanded polystyrene; Protein; Cellulose; Gut Microbiota; Unclassified Enterobacteriaceae

1. Introduction

Expanded polystyrene (EPS), also commonly known as Styrofoam, is an exceptionally lightweight and hydrophobic polymer made of stable styrene monomer chains. Due to its properties, it is one of the most widely used materials in the packaging industry, accounting for ~33 million tons of material, equating to around 7% of the annual global plastic production (Wünsch et al., 2000). However, these same properties also make EPS a serious environmental pollutant. Its bulky and lightweight nature hinders efficient collection and transportation, while current disposal practices such as shredding into pellets are geographically limited and can still generate microplastics.

Chemical depolymerization to recover styrene is possible but costly and hazardous, requiring toxic chemicals (Thakur et al., 2018). Consequently, landfilling remains the most common method, even though EPS degrades extremely slowly, occupies large space, and continues to release microplastics and leachates (Febriansya et al., 2023).

Recently, researchers have been looking to utilize the biological alternatives. Certain insect larvae, including *Tenebrio molitor*, *Galleria mellonella*, *Zophobas atratu*s, *Tenebrio obscurus*, *Plodia interpunctella*, *Tribolium castaneum*, *Lasioderma serricorne*, *Rhyzopertha dominica*, and *Sitophilus oryzae*, can ingest and fragment EPS with the aid of their gut microbiota (Jiang et al., 2021; Brandon et al., 2018; Yang et al., 2015; Yang et al., 2018; Cassone et al., 2020; Lou et al., 2020; Machona et al., 2022; Ndotono et al., 2024; Wang et al., 2020). The larvae mechanically break down EPS through chewing, while gut microbes secrete enzymes that further degrade it (Jiang et al., 2021). These findings suggest the potential for developing plastic waste treatment technologies that leverage insect–microbe symbiotic systems. However, while these worms can metabolize EPS, it is not a favorable food source, resulting in frequent complications, such as high rates of dieoff or premature pupation (Jiang et al., 2021). Therefore, the efficiency and practicality of utilizing these insects for large-scale EPS waste management requires further investigation.

Recent studies have demonstrated that diet composition, including supplemental nutrients and co-feeding strategies, can affect EPS degradation rates and larval microbial communities (Brandon et al., 2021; Tay et al., 2023; Wang et al., 2022; Mamtimin et al., 2023). Brandon et al., 2021 studied the adaptability of the gut microbiota of *T. molitor* by feeding them combinations of EPS, polyethylene (PE), and wheat bran (Brandon et al., 2021), finding that the degradation rates of PE and EPS were similar for *T. molitor* despite their distinct chemical structures, indicating that *T. molitor* has gut bacteria that can adapt to accommodate a wide variety of diets. Furthermore, they found that co-feeding either EPS or PE with wheat bran significantly increased plastic consumption rates of the larvae, with microbiota such as *Kosakonia* and *Citrobacter* being associated with the PE and PS degradation. Similarly, Tay et al., 2023 evaluated the effects of co-feeding *Z. atratus* with EPS along with various nutrient-rich supplements, including oatmeal, wheat bran, and cornmeal (Tay et al., 2023). Co-feeding with such supplements improved larval survival and EPS consumption rates compared to EPS-only communities, while solely EPS-fed microbial communities had significantly different microbiomes, notably being composed of genera such as *Klebsiella* and *Enterobacter*. In addition, Mamtimin et al., 2023 showed that polystyrene was not a favored choice by the larvae, but the taxa composing the gut microbiota between EPS and corn straw were extremely similar, potentially indicating that the ability to digest EPS originally came from the microbiome's need to metabolize the large lignocellulose chains found in corn straw. Wang et al., 2022 and Jiang et al., 2021 revealed that *Z. atratus* exhibits faster EPS degradation and maintains a more stable gut microbial structure than *T. molitor* (Jiang et al., 2021; Wang et al., 2022). While these findings underscore the importance of insect gut microbiota in plastic degradation, most existing studies have relied on binary feeding protocols (e.g., EPS vs. bran) or the usage of EPS with a single supplemental diet, largely focusing on endpoint microbial compositions. As a result, key questions remain regarding more complex dietary strategies, particularly around combinations of supplemental nutrients such as protein and cellulose (He et al., 2021; Przemieniecki et al., 2020; van Broekhoven et al., 2015), affect larval survival rates, EPS degradation efficiency, and microbial community dynamics.

This study investigates the effects of dietary combinations involving cellulose and protein alongside EPS on EPS degradation performance and gut microbial dynamics in both *T. molitor* and *Z. atratus*. By comparing the two species, species-specific differences in microbial responses and degradation efficiencies were explored. Key microbial taxa associated with EPS and nutrient metabolism were identified, and their temporal microbial community shifts were monitored across feeding conditions. This approach provided a more comprehensive understanding of insect–microbe interactions in the context of plastic biodegradation and offered practical insights for optimizing feeding strategies in scalable waste management applications.

2. Materials and Methods

2.1 Larva species

All individuals of *T. molitor* and *Z. atratus* used in the experiment were obtained from Timberline (Marion, USA).

The larvae were grouped by weight into cohorts of 80 (*T. molitor*) and 75 (*Z. atratus*) in each experimental condition. Prior to the experiment, the larvae were given a 7-day fasting period to allow them to pass any stomach contents before the experiment began.

2.2 Experimental Setup

4.5 liter plastic containers were utilized as the habitats for each experimental condition. Each container was filled with 140 grams of Red Mill wheat bran, which acts as a substrate to equally provide the larvae with minimal nutrients and cellulose. No water was given to larvae outside, as water is contained within the given feeding sources. The larvae were kept consistently at room temperature (21±1˚C) during the experimental period. Duplicates of each experimental condition were kept to minimize the possibility of statistical outliers. The experimental conditions for evaluating EPS consumption and microbial behavior are summarized in Table 1. There were eight conditions, and those conditions applied to each T. molitor and Z. atratus. The conditions containing EPS, including EPS-only (+bran as baseline) (EPS), EPS with Protein (EPS-P), EPS with cellulose (EPS-C), and EPS with both protein and cellulose (EPS-P-C), were designated as the 120 primary experimental groups used to monitor the survival and EPS consumption rates, while protein (P), cellulose (C), both protein and cellulose (P-C), and no protein and cellulose (Bran-only), which lacked EPS, were designated as controls.

In all experimental groups, EPS in the form of styrofoam boards was used as the common substrate. The EPS boards weigere cut into 100 mm x 140 mm plates, with each being approximately 7 mm thick. Cellulose consists solely of romaine lettuce leaves, and protein source in the form of gelatinous fish feed with a crude protein content of over 40% (Repashy, Oceanside, CA) were also fed according to the feeding strategies. The protein source has been confirmed during the preliminary experiment that worms can utilize it as a substrate. The protein powder was mixed with hot water (97 ± 1˚C) in a 1:4 ratio by weight to maintain uniform consistency and then cut into 9 mm x 100 mm x 140 mm sections, averaging 4.3 ± 0.2g, which were then fed to larvae. The protein source and the lettuce were given as it was consumed by the experimental groups to allow larvae to consistently have access to the food sources over the course of the experiment. Protein and cellulose were consistently provided by replacing them every 3 days to minimize the chances of spoilage as well as replenishing any water content that may have evaporated during that time.

Table 1. Nutrient Composition of Experimental and Control Groups. Each condition applies to *T. molitor* and *Z. atratus*. All conditions include bran as a minimal diet. EPS = expanded polystyrene; P = protein; C = cellulose; + = present; - = absent; M = mealworm *T. molitor*; S = superworm *Z. atratus*.

Species	Group	Name	Bran	EPS	Protein	Cellulose
Z. atratus	Experimental group	S-EPS	+	+	-	-
		S-EPS-P S-EPS-C	+	+	+	-
		S-EPS-P-C	+	+	-	+
	Control group	S-Bran only	+	+	+	+
		S-P	+	-	-	-
		S-C	+	-	+	-
		S-P-C	+	-	-	+
			+	-	+	+
T. molitor	Experimental group	M-EPS	+	+	-	-
		M-EPS-P	+	+	+	-
		M-EPS-C	+	+	-	+
		M-EPS-P-C	+	+	+	+
	Control group	M-Bran only	+	-	-	-
		M-P	+	-	+	-
		M-C	+	-	-	+
		M-P-C	+	-	+	+

2.3 Survival rates and EPS consumption monitoring

The experiment is set for 14 days, with measurements regarding survival rates and EPS consumption of larvae being taken on day 0, 3, 6, 9 and 14. The individuals from each experimental condition were counted and returned to the container. The pupated or dead individuals were also counted and then removed immediately to track the survival rates and prevent cannibalism. The survival rate for each condition was calculated using equation (1). The pupae are treated as non-survivors for the purpose of calculating larval survival rate. They are explicitly subtracted from the initial number of larvae from the numerator.

$$Survival\ rate\ (\%) = \frac{N_0 - N_{pupae} - N_{dead}}{N_0} \times 100 \tag{1}$$

N_0: Initial number of larvae at the start of the experiment
N_{pupae}: Number of larvae that pupated during the observation period
N_{dead}: Number of larvae that died during the observation period

2.4 Worm sample collection, Microbial sequencing and microbial community analysis

These differences in survival and EPS consumption suggest that microbial community composition may play a key role. To explore this relationship, 16S rRNA sequencing of gut microbiota was conducted. A sample of 10 individuals was sampled before the experiment began to characterize the initial microbial community. 10 individuals from each experimental group were also removed on days 9 and 14 to characterize their gut microbiomes. Removed individuals were euthanized via freezing at -20˚C, and their intestinal contents were stored in 5 ml bead-beating tubes.

Genomic DNA was isolated from each sample using a Genomic DNA extraction kit (Qiagen, Hilden, Germany). To target the V4 region of the 16S rRNA gene, PCR amplification was carried out using the 515F-Y and 806R primers. These primers were designed to include unique six-nucleotide barcode sequences for multiplexing, as well as Illumina adapter sequences required for binding and sequencing (Bartram et al., 2011). PCR reactions were conducted in 25 µl volumes containing 1× ThermoPol buffer, 0.2 µM of each primer, 200 µM dNTPs, 15µg of bovine serum albumin, 0.625 U of Taq DNA polymerase (New England Biolabs, USA), and 10 ng of template DNA. Triplicate PCRs were performed with the following thermal profile: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 68°C for 1 minute, with a final elongation step at 68°C for 7 minutes. Amplified products were pooled in equimolar amounts, gel-purified, and cleaned using the Wizard SV Gel and PCR Clean-Up System (Promega, WI, USA). The resulting library, diluted to 3 pM and spiked with 10% PhiX Control v3 (Illumina, CA, USA), was sequenced on the Illumina MiSeq platform using a 2 × 250 bp MiSeq Reagent Kit v2. Paired-end reads were assembled and processed using the Mothur software package (Schloss et al., 2009). Sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence similarity, and representative sequences for each OTU were taxonomically classified using the Silva database (Quast et al., 2013). Hierarchical clustering analysis and principal coordinate analysis (PCoA) were performed by R packages (Ramette et al., 2007).

3. Results and Discussion

3.1 Survival Rates

Z. atratus consistently showed higher survival than *T. molitor*, averaging 98.8 ± 2.8% compared to 56.4 ± 9.7%, indicating stronger physiological adaptation to EPS consumption. This trend has been reported by previous studies (Jiang et al., 2021; Wang et al., 2022). Pupation rates, indicating the transition of larvae to an inactive stage, exhibited distinct trends between *Z. atratus* and *T. molitor*, which corresponded with differences in their mortality rates (Figure 1A). There were no individuals of *Z. atratus* pupated during the experiment, while 22.2 ± 12.5 % of *T. molitor* underwent pupation under certain conditions. Larvae of *Z. atratus* only pupate when isolated from food sources and/or conspecifics; therefore, the experimental conditions, which involved constant contact between larvae and continuous food availability, may have prevented pupation. (Quennedey et al., 1995). However, *T. molitor* does not exhibit biological restrictions on pupation; consequently, older or nutritionally deprived larvae enter the prepupal phase prematurely, which limits their feeding activity (Kim et al., 2015).

Similar to their lower pupation rates, *Z. atratus* larvae exhibited relatively low mortality (1.3 ± 2.9%). In comparison, *T. molitor* showed higher pupation rates accompanied by increased mortality rates of 17.6 ± 8.9%. *Z. atratus* may better tolerate the experimental diets or conditions, maintaining larval health for longer periods, whereas

T. molitor is more sensitive to nutritional stress, which can lead to earlier pupation and increased mortality (Connat et al., 1991).

As mentioned previously, Bran was supplied in all experimental groups as a baseline. Among *Z. atratus* groups, bran-only feeding produced the lowest survival (91.3 ± 2.8%), consistent with studies showing poor outcomes on nutrient-poor diets (Machona et al., 2022; Yang et al., 2020) (Figure 1B). The low survival rate can possibly be attributed to the limited nutritional value of bran, eliminating weaker individuals or increased susceptibility to cannibalism by cohabitated larvae (Tschinkel et al., 1981). Other diets with protein and/or cellulose yielded similar survival rates.

For *T. molitor*, all groups showed substantial pupation and mortality, but EPS-fed groups (M-EPS, M-EPS-P, M-EPS-C, M-EPS-P-C) had higher survival (64.5 ± 5.9%) than controls without EPS (48.3 ± 4.5%) (Figure 1C). It suggests that EPS was utilized by *T. molitor* for its growth. Previous studies also proved that *T. molitor* is capable of uptaking EPS as a viable source of nutrients (Tsochatzis et al., 2021). In addition, M-EPS group did not show a distinct difference from other EPS-feeding groups with added protein or cellulose. This suggests that varying nutrient levels may have a negligible effect on the survival rates of *T. molitor* (Liu et al., 2020).

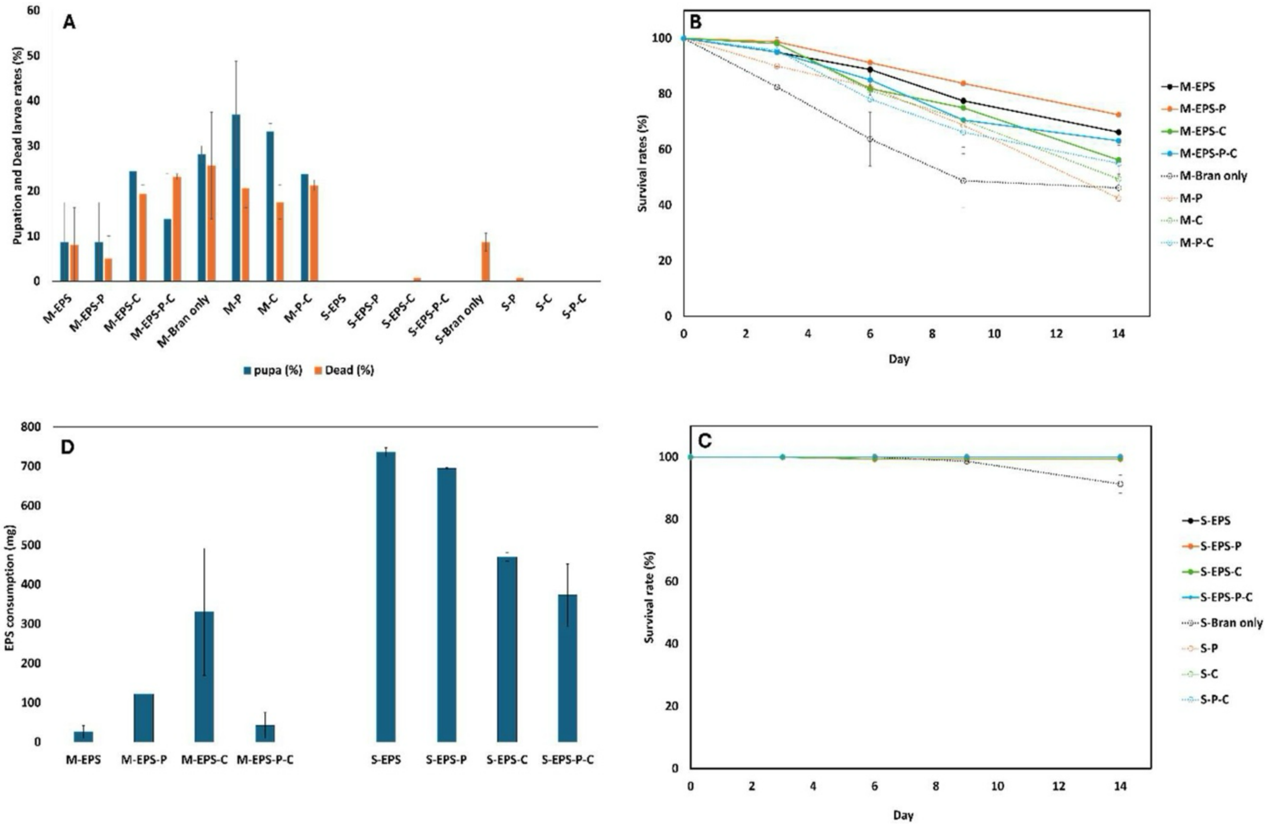

Figure 1. (A) Pupation and death of *Z. atratus* and *T. molitor* with different diets; (B) Survival rates of *Z. atratus*; (C) Survival rates of *T. molitor* (D) Average EPS consumption by *Z. atratus* and *T. molitor* with different diets for 14 days. EPS = expanded polystyrene; P = protein; C = cellulose; M = mealworm *T. molitor*; S = superworm *Z. atratus*

3.2 EPS degradation

Z. atratus consumed substantially more EPS than *T. molitor* (568.7 ± 156.9 mg vs. 170.9 ± 149.2 mg; Figure 1D), consistent with the survival trends. EPS-only groups (S-EPS) had the highest daily intake (52.6 ± 0.8 mg/day), exceeding supplemented groups: S-EPS-P (49.6 ± 0.2), S-EPS-C (33.6 ± 0.8), and S-EPS-P-C (25.6 ± 5.7). This heightened consumption in the EPS-only (+bran as baseline) group is likely due to its restricted feed variety, which limits individuals to concentrate their feeding activity on EPS. In contrast, other groups with supplemental feeds had access to alternative options, such as protein or cellulose, leading to reduced EPS consumption. This trend is also supported by the fact that S-EPS-P-C, which had more types of non-EPS feed to consume, had lower EPS consumption (372.93 ± 78.9 mg) compared to both S-EPS-C (470.75 ± 11.15 mg) and S-EPS-P (694.62 ± 2.98 mg). This pattern is contrasted by some studies where consumption of EPS in the presence of diet supplementation was greater, compared to groups fed solely EPS (Tay et al., 2023; Liang et al., 2025). In addition, Yang et al. (2020) demonstrated that there was a greater chance of cannibalism amongst cohabitated larvae in nutritionally stressed conditions, which could have been derived from restricted food diversity or nutritionally deficient food (Yang et al., 2020). EPS-only (+bran as baseline) feeding, a relatively slower degradable source compared to protein or cellulose, could induce nutritional stress, resulting in cannibalism followed by lower EPS consumption. Despite those tendencies, the reason why this study showed greater EPS consumption might be because of the smaller number of individuals compared to other studies, which might lead to no cannibalism (Brandon et al., 2018; Tay et al., 2023).

T. molitor showed the opposite trend. EPS-only (+bran as baseline) groups consumed the least (1.88 ± 1.2 mg/day), unlike *Z. atratus*. However, both the M-EPS-P (8.78 ± 0.7 mg/day) and M-EPS-C (23.63 ± 11.5 mg/day) groups had larger amounts of EPS consumed compared to M-EPS-P-C (3.1 ± 2.3 mg/day), following a similar feeding trend to *Z. atratus*. These findings further lend support to the notion that the presence of a greater variety of supplements directly inhibits EPS consumption. Notably, while protein promoted EPS consumption in *Z. atratus*, cellulose had a stronger effect in *T. molitor*.

3.3 Microbial communities

Enterobacteriaceae (> 40%) was the dominant taxa within the larval gut microbiome of *Z. atratus* when only EPS (+bran as baseline) was fed (Figure 2A). Given that S-Bran only showed less abundance of *Enterobacteriaceae* (22.6%) compared to S-EPS, *Enterobacteriaceae* may have a significant role in the metabolic breakdown of EPS (Jiang et al., 2021; Li et al., 2024; Miravalle et al., 2024). Compared to S-P, in which *Weissella sp.* was the primary dominant taxa (60.1%), a more diverse array of genera (*Pediococcus sp.*, *Enterococcus sp.*, *Enterobacteriaceae*, and *Hafnia-Obesumbacterium sp.*) were found in groups fed with both EPS and protein. This suggests that feeding a combination of protein and EPS induces a more diverse gut microbiome, leading to relatively higher EPS degradation, potentially due to nutrient dynamics through microbial interactions between microbial taxa. *Lactococcus sp.* were present with the largest population in S-EPS-C as well as S-EPS-P-C. Given this large population mainly restricted to groups containing both EPS and cellulose, and its lower abundance in S-C and S-P-C, it is reasonable to assume that *Lactococcus sp.* has a key role in breaking down EPS as well as cellulose. (Ndotono et al., 2024; Sun et al., 2022; Urbanek et al., 2024)**.** Like S-P, when not in the presence of EPS, S-C also had dominant populations of *Weissella sp.* (80.1%) within their gut microbiomes. Due to their relative absence in groups breaking down EPS, it is possible that *Weissella sp*. may have a reduced role in the EPS metabolic processes in *Z. atratus*.

Unlike the groups of *Z. atratus*, different populations of microbial communities in *T. molitor* were observed despite the same feeding conditions (Figure 2B). *Enterococcus sp.* (28.1%) and *Spiroplasma sp.* (36.6%) were identified as dominant taxa in EPS-only (+bran as baseline) fed conditions (M-EPS), while *Enterobacteriaceae* was dominant (40.4%) when only fed with Bran. *Enterobacteriaceae* (> 50%) was also the dominant taxon within gut microbiomes that were exposed to large quantities of cellulose, suggesting that *Enterobacteriaceae*. In *T. molitor* may have a significant role in the metabolic breakdown of cellulose as well. Given that populations of Enterobacteriaceae were dominant in M-EPS-C and S-EPS conditions, which showed the highest EPS consumption from *T. molitor* and *Z. atratus*, respectively, this family might be highly involved in EPS degradation in both *T. molitor* and *Z. atratus* (Jiang et al., 2021). While it was not a dominant taxon in EPS-fed groups of *Z. atratus*, *Weissella sp.* (>60%) was prominent in groups of *T. molitor* with both EPS and protein (M-EPS-P, M-EPS-P-C), suggesting its key role in the breakdown of both substances in *T. molitor* (Wang et al., 2015). *Erwiniaceae sp*. (>80%), the primary taxa found in M-P, consistently had lower population counts in groups with EPS compared to groups without EPS, suggesting that they are not responsible for EPS breakdown and are outcompeted by bacterial strains that can metabolize EPS.

To better understand how microbial taxonomic relations from different diet conditions are distinct within the gut microbiomes, hierarchical clustering was performed using heatmap visualization based on the relative abundance of the 18 most dominant bacterial genera (Figure 2C). The taxa were organized according to phylogenetic relatedness in the microbial structures. As a result, the bacterial communities were grouped into three major distinct clusters: Cluster I (M–EPS-C and S-EPS), Cluster II (S-EPS-C and S-EPS-P-C), and Cluster III (M-EPS-P and M-EPS-P-C). Cluster 1 was dominated by *Enterococcus sp.* as well as unclassified *Enterobacteriaceae*, and both communities were associated with high EPS consumption. Notably, these communities are shown to be similar in taxonomic relations despite being present in different larvae, indicating a diet-driven rather than host-specific microbial response. Cluster 2 was related to cellulose-rich diets, which had primarily *Lactococcus sp.* as its dominant taxa. Cluster 3 had dominant taxa, most notably *Weissella sp.*, that were associated with a protein-rich diet. These clusters indicate that closely related microbial taxa are responsible for metabolizing similar substrates, even across different host species such as *T. molitor* and *Z. atratus*. This strongly suggests that feeding conditions play a key role in shaping the microbial community structure and, in turn, influence EPS degradation (Mamtimin et al., 2023).

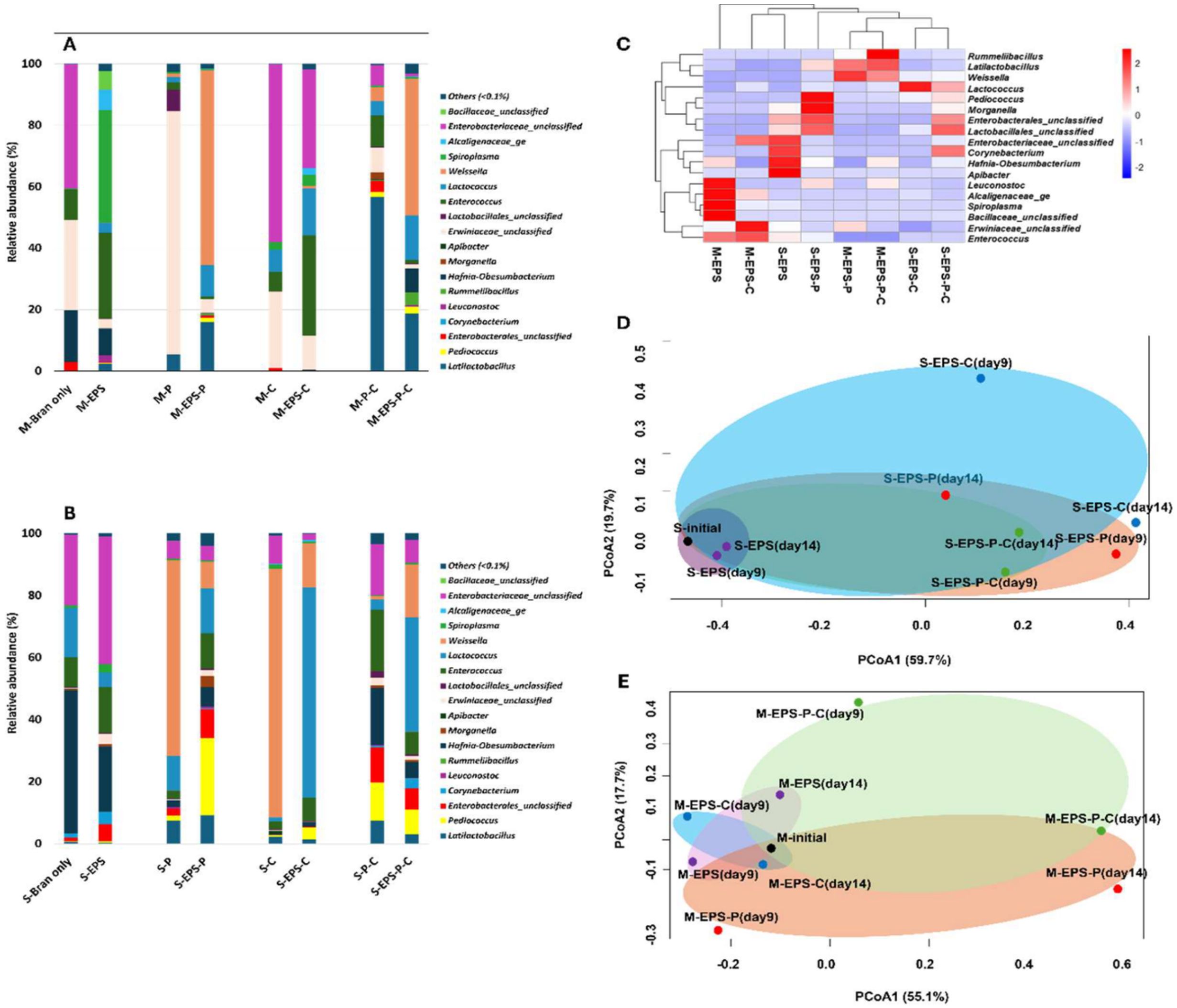

Figure 2. Changes in gut microbiomes on day 14 of *Z. atratus* and *T. molitor* with different diets. (A) Microbial communities of *Z. atratus*; (B) Microbial communities of *T. molitor*. (C) Heatmap and hierarchical cluster analysis of key genera (> 0.1% relative abundance). (D) Principal coordinate analysis (PCoA) of microbial communities of *Z. atratus.* (E) Principal coordinate analysis (PCoA) of microbial communities of *T. molitor*. For Fig. 2D and Fig. 2E, taxa with relative abundance less than 0.1% were excluded. Each ellipse includes the initial community (black circle) as day 0. EPS = expanded polystyrene; P = protein; C = cellulose; M = mealworm *T. molitor*; S = superworm *Z. atratus.*

PCoA was performed to compare the temporal changes in the gut microbiomes of the experimental groups of *T. molitor* and *Z. atratus* (Figure 2D and 2E). Results showed that groups of *T. molitor* that contained only EPS and cellulose (M-EPS-C) had the least microbial changes in the communities, while groups containing protein (M-EPS-P) and both protein and cellulose (M-EPS-P-C) had the most microbial changes. Regarding their EPS consumption rates, it suggests that less shifts in the microbial communities may, over time, induce the rapid enrichment of specific EPS-degrading populations. Interestingly, M-EPS was also shown to have less microbial shift. The EPS consumption rate of this condition was lower than other conditions; thus, this condition might not affect a change in microbial communities, resulting in less microbial shift. This trend also showed for the groups of *Z. atratus*. Groups of *Z. atratus* only containing EPS (S-EPS) had the least microbial changes with a high EPS consumption rate, while S-EPS-C, S-EPS-P-C and S-EPS-P similarly had more microbiome shifts.

4. Conclusion

This study showed that the composition of protein and cellulose in the diet is important for regulating how well mealworms (*Tenebrio molitor*) and superworms (*Zophobas atratus*) break down EPS and for influencing the structure of their gut microbiota. This study has certain limitations. The use of duplicate technical replicates precluded rigorous statistical analysis, and the relatively short experimental duration (14 days) may not fully reflect longer-term biological responses. Further studies with increased biological replication and extended observation periods will be necessary to substantiate and broaden these findings. Despite these limitations, these results emphasize how crucial it is to use co-feeding strategies that are optimized for both plastic degradation and larval survival. Additionally, they imply that the addition of different diets can drastically change preexisting bacterial communities and that gut bacteria are specialized in metabolic processes associated with particular diets. Altogether, our findings point to the potential use of insect–microbe systems in practical waste-management strategies, highlighting their promise for future large- scale applications in plastic biodegradation.

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