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The combination of microplastics and glyphosate affects the microbiome of soil inhabitant *Enchytraeus crypticus*

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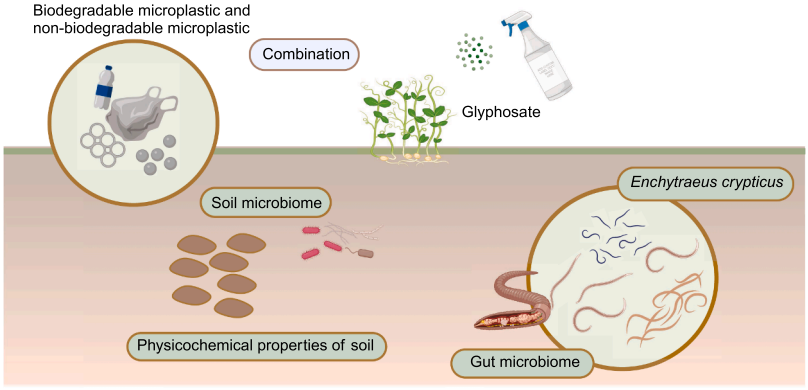
HIGHLIGHTS

- The soil microbiome exhibited more sensitivity to biodegradable PLA than non-biodegradable PET.
- Glyphosate disordered the gut microbiome of soil fauna *Enchytraeus crypticus*.
- Co-exposure of microplastics and pesticides to *E. crypticus* exacerbated oxidative stress.
- The microbial ecological risks of PLA degradation process needs to be concerned.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics and pesticides are emerging contaminants that threaten soil ecosystems, yet their combined effects on soil health and soil fauna remain poorly understood. In this study, we constructed a microcosm to assess the individual and combined effects of microplastics and glyphosate on soil physicochemical properties, microbial communities, and the gut microbiome of soil invertebrates (*Enchytraeus crypticus*). Biodegradable polylactic acid (PLA) and conventional polyethylene terephthalate (PET) were introduced at environmentally relevant concentrations. Our results revealed that PLA had a stronger disruptive effect on soil microbial communities than PET, altering microbial diversity and functional composition. Glyphosate, in contrast, primarily influenced the gut microbiome of *E. crypticus*, reducing microbial diversity and inducing oxidative stress. Combined exposure to

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microplastics and glyphosate significantly intensified oxidative stress but did not amplify microbial dysbiosis beyond the effects of microplastics alone. Compare to PET, PLA combined with glyphosate had the most pronounced effects on both soil and gut microbiomes, suggesting that biodegradable microplastics may pose greater ecological risks than conventional microplastics when used alongside pesticides. These findings underscore the need for a reassessment of biodegradable plastic use in agriculture and highlight the complex interactions between microplastics and pesticides in shaping soil ecosystem health.

1. Introduction

Synthetic plastics have been scrutinized globally due to their widespread use and persistence [31,40,61]. Since the 1950s, global plastic production has surged from 2 million tons to 460 million tons in 2019 [9], contributing to severe environmental pollution. A significant concern is the formation of microplastics through fragmentation processes, which allow them to accumulate in ecosystems over long periods [14,37]. Microplastics enter the soil through multiple pathways, including sewage irrigation, plastic mulch degradation, atmospheric deposition, and direct littering [25,51,65]. Alarmingly, farmlands in China have been found to contain approximately 4537 microplastic particles per kilogram of soil [49], underscoring the ubiquity of microplastic contamination in agricultural environments.

Microplastics alter soil properties and then influence microbial communities [60]. Studies have shown that residual microplastics can modify soil physicochemical characteristics [63], disrupt soil aggregation [66], and impact water retention [64]. Additionally, microplastics selectively enrich certain microbial taxa, leading to shifts in soil microbial diversity and composition [13,45]. Beyond microbial communities, microplastics can affect soil fauna by inducing histopathological damage, oxidative stress, and DNA damage [59], thereby impairing their ecological functions [8,21,73]. Although biodegradable plastics have been proposed as an eco-friendly alternative, recent studies suggest they can degrade into secondary microplastics more rapidly than conventional plastics, potentially exacerbating pollution in soil ecosystems [27,52,75].

Alongside microplastic contamination, pesticide application is another major anthropogenic pressure on soil ecosystems [7,41,56]. Modern agriculture heavily relies on pesticides, particularly herbicides, which constitute 47.5 % of global pesticide use [18]. However, these chemicals can disrupt soil biochemical processes and pose risks to both microbial communities and soil fauna [4,73]. Glyphosate (N-(phosphonomethyl) glycine) is the most widely used herbicide worldwide, with applications reaching 825,804 tons in 2014 and projected to increase to nearly 1 million tons by 2025 [28,73]. Glyphosate residues can persist in soil at concentrations as high as 5 mg/kg [16], disrupting microbial enzymatic functions and altering soil microbiome composition via inhibition of the 5-enolpyruvyl shikimic acid-3-phosphate synthase pathway [26,72]. Since being classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer in 2015 [11], concerns over glyphosate's ecological and human health risks have intensified.

Despite growing awareness of the individual effects of microplastics and pesticides, their combined effects on soil health remain insufficiently studied. Research indicates that interactions between microplastics and pesticides can alter pollutant bioavailability, enhance toxicity, and induce unexpected ecological consequences [30,39]. Soil fauna, including earthworms and arthropods, play essential roles in maintaining ecosystem stability, and their gut microbiome is crucial for mediating their response to environmental stressors [10,36]. *Enchytraeus crypticus*, a standard ecotoxicological model organism, is particularly sensitive to soil contaminants, making it an ideal bioindicator for evaluating soil pollution [1,73].

To systematically assess the impacts of microplastics (both biodegradable and conventional), glyphosate, and their combined exposure on soil and soil fauna, a microcosm experiment was conducted. Polylactic acid (PLA) was selected as a representative biodegradable plastic, while non-biodegradable polyethylene terephthalate (PET) was chosen due to its widespread use in packaging and textiles [47,70]. By comparing microbial diversity and interactions under different treatment conditions, this study aims to: (1) evaluate the individual and combined effects of microplastics and glyphosate on soil and the gut microbiome of *E. crypticus*, (2) differentiate the toxic effects of PLA and PET on soil ecosystems, and (3) explore potential synergistic interactions between glyphosate and different types of microplastics. This research provides novel insights into the combined effects of microplastics and pesticides on soil microecology, highlighting potential risks associated with their concurrent presence in agricultural environments.

2. Materials and methods

2.1. Test soil and test invertebrates

Soil samples were collected from long abandoned agricultural areas in Ningbo City (30.16°N, 121.25°E), which is in the northern region of Zhejiang Province, China. The soil samples were carefully collected from the uppermost 0.5–10 cm layer, after which the samples were transported to the laboratory and preserved in a temperature-controlled foam box at 4 °C. The debris and weeds were carefully expelled, and the samples were homogenized and air-dried in a ventilated area for one week before being sieved to a particle size of 2 mm. The soil was adjusted to 50–60 % of the maximum field water-holding capacity and incubated in an artificial climate chamber for 2 weeks to restore the microbial balance.

E. crypticus, which were originally cultivated at Aarhus University in Denmark, were cultured on disposable Petri dishes adhering to the guidelines set forth by the Organization for Economic Co-operation and Development [44]. The Petri dishes were placed in an artificial climate chamber under stationary conditions (light 800 Lux, light-to-darkness ratio 8 h:16 h, 19 ± 2 °C, 75 % humidity). The test organisms were fed sterilized oat powder twice a week.

2.2. Test microplastics and pesticides

PLA and PET particles (Zhangmutou Plastics, China) had a diameter of 30 μm. These microplastics were added to the soil at a content of 2 % (w/dry w) to simulate plastic residues in agricultural soil. The glyphosate used in this study was purchased from Sigma—Aldrich Company (Shanghai, China) and dissolved in deionized water to obtain a 0.60 g/L stock solution. The stock solution was subsequently added to the dry soil to achieve a maximum concentration of 5 mg glyphosate kg^{-1} dry soil.

2.3. Experimental design

Synchronized *E. crypticus* and eggs were cultivated following OECD guidelines (OECD, 2004). The *E. crypticus* were moved to a clean Petri dish and labelled according to the laying date. Then, we obtained fresh eggs (< 24 h) and placed them in a new sterile Petri dish. We defined these eggs and hatched larvae (< 24 h) as synchronized eggs and synchronized *E. crypticus*, respectively. Sterile water was used to adjust the moisture content of the soil. The soil was then incubated in an artificial climate chamber (800 Lux light, 8 h:16 h light—dark cycle ratio, 18 ± 2 °C, 75 % humidity) for 2 weeks. Several adult individuals of *E. crypticus*

with clearly visible gonads were randomly chosen and transferred to uncontaminated soil to acclimatize to the soil environment and stabilize their gut environment.

Our experiments included 6 treatments with three replicates: (1) CK: only soil; (2) Gly: 5 mg/kg glyphosate; (3) PLA: 2 % (w/w) microplastic PLA; (4) PET: 2 % (w/w) microplastic PET; (5) PLA + glyphosate: 5 mg/kg glyphosate and 2 % (w/w) microplastic PLA; and (6) PET + glyphosate: 5 mg/kg glyphosate and 2 % (w/w) microplastic PET. We simulated the soil environment (sterilized glass beakers) with 240 g of soil and 20 synchronized *E. crypticus* samples as a control group or with microplastics (PLA or PET) and glyphosate uniformly mixed with the soil as treatment groups. The entire experiment was conducted in a stationary climate chamber (800 Lux light, 8 h:16 h light-dark cycle ratio, 18 ± 2 °C, 75 % humidity) for 21 d. At the end of the incubation period, both the soil and *E. crypticus* samples were collected from different treatments, and the number of surviving *E. crypticus* individuals was recorded. Seven living *E. crypticus* samples were preserved in liquid nitrogen for subsequent determination of enzyme activity and extraction of DNA. The gut and soil samples from different treatments were stored at − 80 °C and designated Control, PLA, PET, Glyphosate, PLA-glyphosate, or PET-glyphosate.

2.4. Determination of the physicochemical properties of the soil

The determination of soil organic matter (SOM), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), and available phosphorus (A-P) across various treatments provides a comprehensive understanding of the individual and combined effects of microplastics and glyphosate on the physicochemical properties of soil. The determination of SOM was conducted via a potassium dichromate oxidation spectrophotometric method [48]. The NH_4^+-N in the soil extraction mixture was quantified via the indophenol blue colorimetry method, and NO_3^--N was measured via ultraviolet spectrophotometric detection. The molybdenum blue method was employed for the measurement of A-P levels in the soil [43]. The SOM, NO_3^--N, NH_4^+-N and A-P kits were purchased from Comin Biotechnology (Jiangsu, China).

2.5. Determination of the antioxidants, detoxifying enzymes, survival rates and reproductive quantities of E. crypticus

Four *E. crypticus* specimens previously frozen in liquid nitrogen were homogenized by using a tissue homogenizer (Coyote, China). Saline solution (pH = 7.3–7.5) was subsequently added at a ratio of 1:10, and the suspensions were thoroughly mixed by vortexing for 10 min at 3000 rpm. Then, 10 μL of the supernatant was extracted from the mixture and analyzed using a double-antibody sandwich enzyme-linked immunosorbent assay (Jiangsu, China). Finally, the reactive oxygen species (ROS) and cytochrome P450 (CYP450) levels were measured by determining the absorbance at 450 nm via a microplate reader (Thermo Fisher Scientific Inc, Germany).

To ensure accurate enumeration of juvenile *E. crypticus* in the soil, a standardized protocol was followed. Initially, 10 mL of anhydrous ethanol was dispensed into each beaker to eliminate and immobilize the *E. crypticus* effectively for subsequent processing. The beakers were then gently agitated via a stirring rod. After a 1-min incubation period, 500 mL of 1 % rose bengal mixture was added to each beaker with careful stirring to ensure comprehensive staining of the larvae. After 2 min, 50 mL of distilled water was added, and the solution was thoroughly mixed. The solution was then kept at 4 °C for 6 h and filtered through a 150-mesh sieve. Finally, high-resolution photographs were taken via ImageJ (2023) to obtain an accurate count of *E. crypticus*. We obtained the survival rate of *E. crypticus* by dividing the number of surviving individuals by the initial number of individuals.

2.6. DNA extraction and sequencing

The DNA of the soil (500 mg) was extracted with an MP FastDNA® Spin Kit (Mpbio InC, America). Seven gut samples were randomly chosen from each group, finely grounded, and stored in 1.5 mL centrifuge tubes. A FastPure® Cell & Tissue DNA Isolation Mini Kit (Vazyme International LLC, China) was used to extract DNA from the gut of *E. crypticus*.

We specifically targeted the highly variable V4 region and designed the universal primer 515F-806R (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT) for selective amplification of the 16S rRNA gene [74]. The primers ITS5-1737F (GGAAGTAAAAGTCGTAACAAGG) and ITS2-2043R (GCTGCGTTCTTCATCGATGC) were used to amplify the fungal ITS gene. We constructed the Illumina library on the basis of previous studies [73]. The DNA fragments were quantified and purified for sequencing on the Illumina HiSeq 2500 platform at Novogene (Beijing, China).

We removed primer sequences, ambiguous nucleotides, and low-quality reads to obtain clean data. Microbiome bioinformatics data were processed via QIIME2 v2.33. Dada2 v1.10.1 was used to join paired-end reads and denoise sequences. The Silva v132 database was used to annotate the feature sequences and categorize the microbial amplicon sequence variants (ASVs) for subsequent analysis. During data processing, we removed samples with fewer than 10,000 sequences and removed ASVs with mitochondria and chloroplasts.

2.7. Statistical analysis and visualization

The data were expressed as the means ± standard errors of the means. In this study, we utilized the vegan package in R v3.6.1 to compute the alpha diversity, beta diversity, and Bray—Curtis distance of the microbial community. Additionally, one-way analysis of variance (ANOVA) was performed using the SPSS v27.0 software, followed by post hoc multiple comparisons with the Duncan test to assess differences among multiple groups. Spearman correlation analysis was performed using the R package to analyze correlations between genera ($P < 0.05$, $|r| \geq 0.8$). Correlation matrices were generated via the R package on the basis of the abundance characteristics of species, and correlation network maps were plotted accordingly. We constructed a bipartite association network via R v3.6.1 and Cytoscape v3.8.2. The mean clustering coefficient is used to measure the closeness of nodes, and the average degree serves as an indicator of connectivity density in microbial networks. Co-occurrence network maps were generated via the ggClusterNet package in R v3.6.1. Structural equation modelling (SEM) was performed piecewise utilizing the R package. Histograms and boxplots were generated using GraphPad Prism v9.5.0

3. Results

3.1. Microplastics altered the physicochemical properties of soil

After 21 days of exposure, microplastics significantly altered soil physicochemical properties. PLA and PET increased NH_4^+-N and SOM levels but decreased NO_3^--N levels ($p < 0.05$, Table S1 and Fig. 1a). PET also elevated A-P levels ($p < 0.05$). Glyphosate did not significantly affect the soil parameters. The synergistic effect of microplastics and glyphosate on soil physicochemical properties was consistent with the effects of PLA or PET alone ($p > 0.05$).

3.2. Combination of microplastics with pesticide intensified oxidative stress in E. crypticus

E. crypticus survival rates showed no significant differences between treatments and controls ($p > 0.05$, Table S2 and Fig. 1b). However, reproduction declined significantly with microplastics, glyphosate, and their combination, especially in the PLA treatment ($p < 0.05$, Fig. 1b).

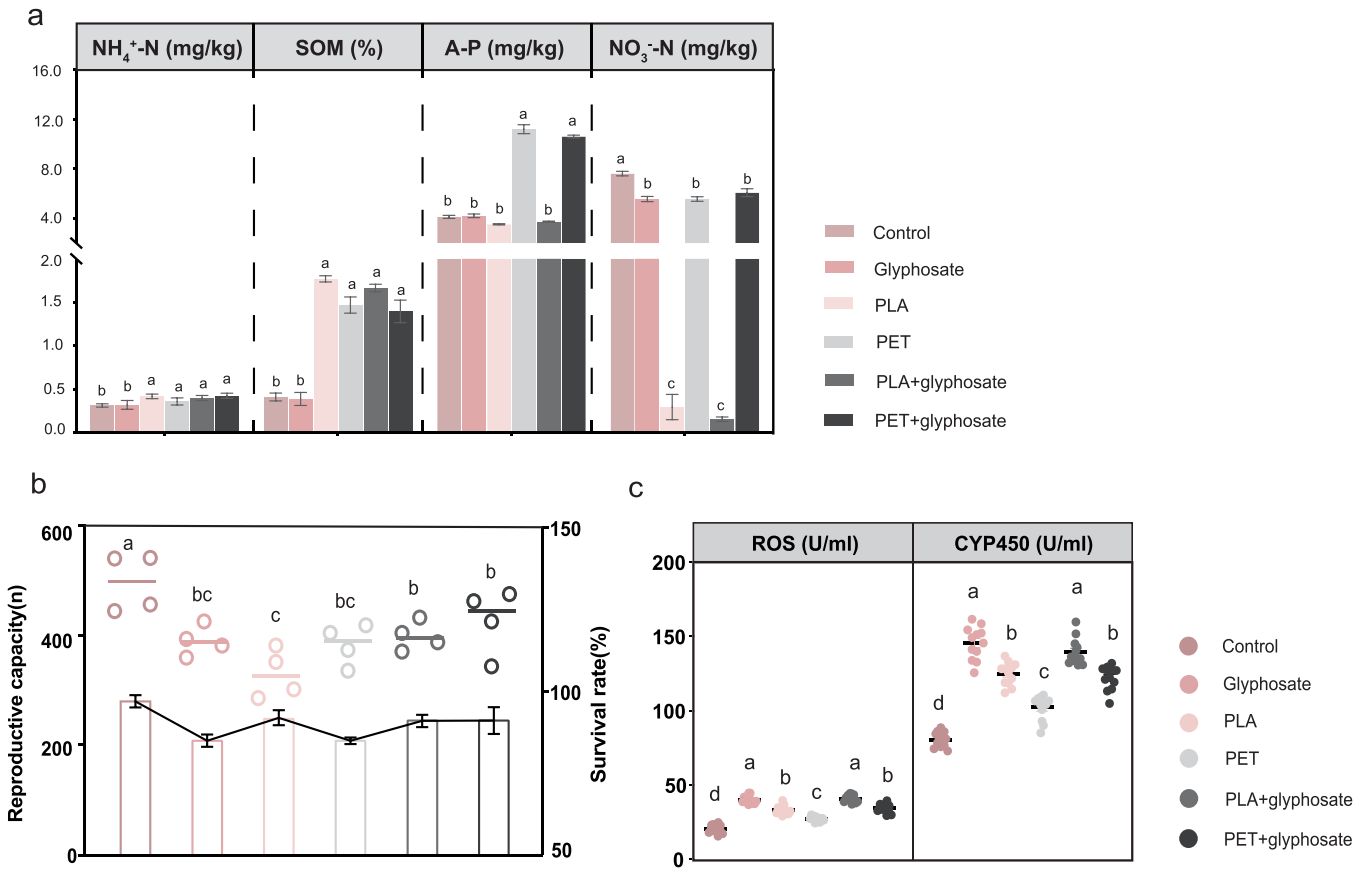

Fig. 1. The impact of microplastics and glyphosate on the physicochemical properties of the soil and the physiology of *E. crypticus* over time. (a) Effects of glyphosate, PLA, PET, PLA + glyphosate and PET + glyphosate on NH_4^+-N, SOM, A-P and NO_3-N levels in soil. (b) Glyphosate, PLA, PET, PLA + glyphosate and PET + glyphosate obviously changed the reproductive capacity (circle), while no significant alteration was observed in the survival rate across all treatment groups (histogram). Specifically, n represent the number of offspring produced per treatment. And the survival rate is represented as a percentage. (c) Effects of different treatments on the antioxidant (ROS) and antidote (CYP450) levels. Different letters indicate statistically significant differences between groups determined by a one-way ANOVA ($p < 0.05$).

CYP450 and ROS levels significantly increased across all treatments ($p < 0.05$, Table S2 and Fig. 1c). Glyphosate increased the most, by 1.8 times. Compared with individual microplastics, the combination of microplastics and glyphosate significantly increased the ROS and CYP450 levels ($p < 0.05$).

3.3. PLA had a stronger impact on soil microbial community than PET and pesticides

In soil, PLA significantly reduced bacterial and fungal Chao1 and Shannon indices ($p < 0.05$, Table S3, S5, Fig. S1 and Fig. 2a), while PET and glyphosate had no significant effect ($p > 0.05$). PCoA showed distinct microbial communities in all treatments compared to controls ($p < 0.001$, Fig. 2b and Fig. S1). Bray-Curtis dissimilarity increased across treatments, with PLA and PLA + glyphosate showing the highest rise (Fig. S2 and S3). The combined effect of microplastics and glyphosate on microbial diversity aligned with PLA or PET alone.

The dominant soil bacteria (> 85 %) included *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, and *Planctomycetota* ($p < 0.05$, Fig. 2c). *Actinobacteria* decreased by 2.35–10.9 % across treatments, while *Proteobacteria* increased (~ 37 %) and both *Firmicutes* and *Bacteroidetes* decreased (98 %) in PLA and PLA + glyphosate treatments. At the family level, *Bacillaceae* declined in all treatments, *Oxalobacteraceae* in PLA and PLA + glyphosate, while *Stenotrophomonas* was enriched in PET and PET + glyphosate ($p < 0.05$, Fig. S4). Fungal communities (> 95 %) were mainly *Ascomycota*, *Mortierellomycota*, and *Basidiomycota* ($p < 0.05$, Fig. S1). *Ascomycota* increased by 20 % and *Mortierellomycota* decreased by 10 % in PLA and PLA + glyphosate treatments, with *Aspergillus* enriched in PET and PET + glyphosate ($p < 0.05$). Volcano plots confirmed significant microbial shifts ($p < 0.05$, Fig. 2d), with PLA and PLA + glyphosate treatments showing the most variation. Overall, PLA had a stronger regulatory effect on soil microbiota than PET, and the synergistic effect of microplastics and glyphosate on soil bacteria was in line with the effects of PLA or PET alone.

3.4. Glyphosate affected the gut microbial community to a greater extent than microplastics

In the gut, PLA and PET had no significant effect on Chao1 or Shannon indices ($p > 0.05$, Table S4, S6, Fig. S1 and Fig. 2a), while glyphosate significantly reduced them ($p < 0.05$). PCoA revealed distinct gut microbial patterns ($p < 0.001$, Fig. 2b and Fig. S1), with Bray-Curtis dissimilarity increasing across treatments (Fig. S2 and S3), especially with glyphosate. The synergistic effect of microplastics and glyphosate on the alpha and beta diversity of gut microbiome was in line with that of PLA or PET alone.

The dominant gut bacteria (> 80 %) included *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Planctomycetota*, *Bacteroidetes*, and *Acidobacteria* ($p < 0.05$, Fig. 2c). *Proteobacteria* increased by 11–33 %, while *Firmicutes*, *Actinobacteria*, and *Chloroflexi* decreased by less than 10 %. At the family level, *Enterobacteriaceae* and *Bacillaceae* declined, whereas *Xanthomonadaceae* and *Lysobacter* increased across all treatments ($p < 0.05$, Fig. S5). Fungal communities (>95 %) were mainly *Ascomycota*,

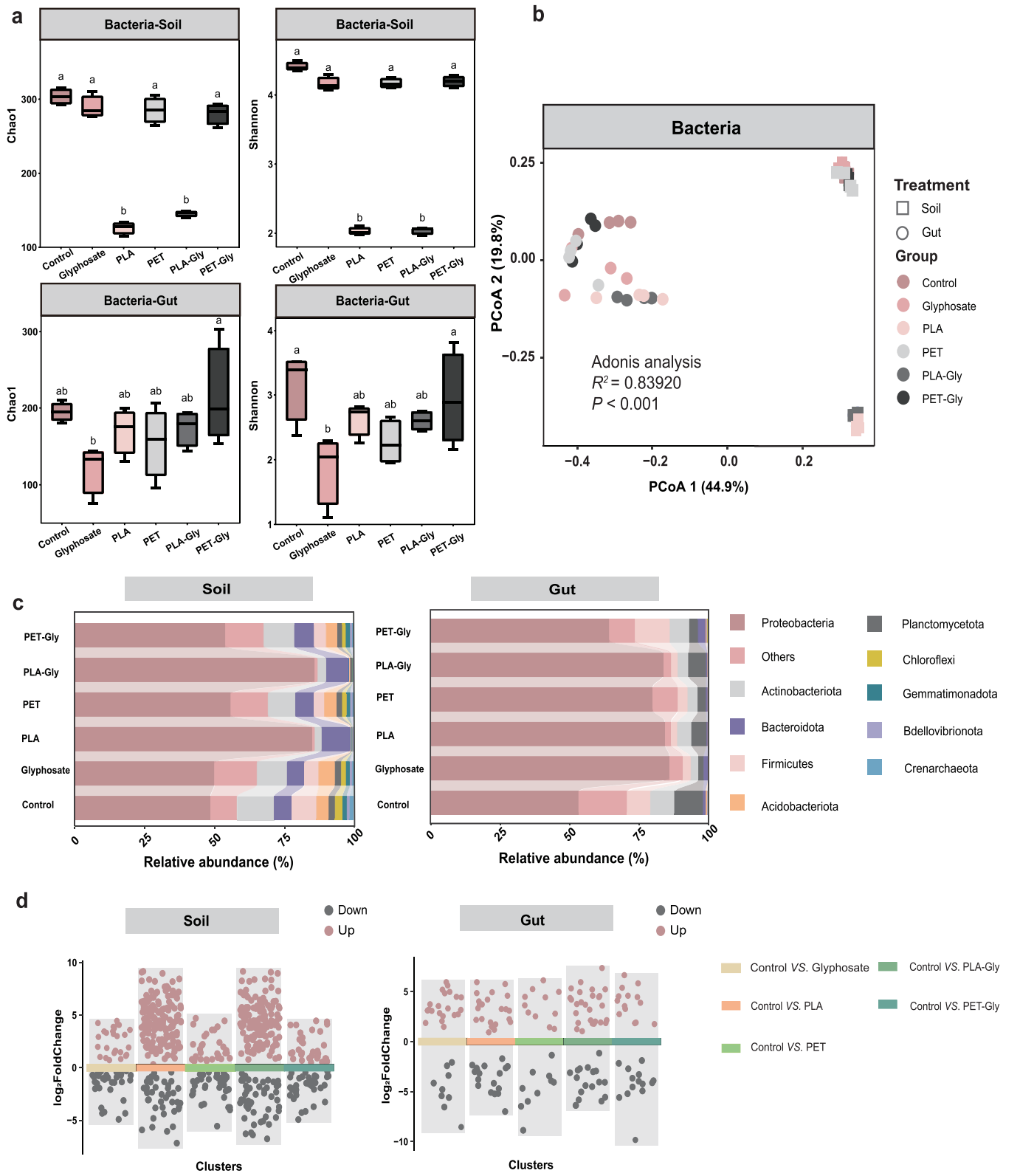

Fig. 2. Effects of microplastics (PLA and PET), glyphosate and their combination on the bacterial communities of the soil and gut over time. (a) Alpha diversity (Chao1 and Shannon) of the bacteria in the soil and the gut. (b) Principal coordinate analysis showed that glyphosate, microplastics (PLA and PET) and their co-addition disturbed the soil and gut bacterial communities. (c) The relative abundance of bacteria at the phylum level in the different treatment groups. (d) Volcano plots of the inter-group differences in the bacterial community. Different letters indicate statistically significant differences between groups determined by a one-way ANOVA ($p < 0.05$).

Mortierellomycota, and *Basidiomycota* ($p < 0.05$, Fig. S1), with *Ascomycota* increasing by 5 % in the PLA, PET, and glyphosate treatments. Volcano plots showed 2.7 % of bacterial taxa were differentially regulated, while fungal genera remained largely unchanged ($p < 0.05$, Fig. 2d and Fig. S1). Overall, glyphosate had a stronger impact on gut microbiota than microplastics, with their combined effect resembling that of PLA or PET alone.

3.5. PLA more strongly impacted the gut bacterial interactions than PET

We constructed co-occurrence networks to compare microbiome interactions in PLA and PET treatments. PLA reduced gut bacterial nodes, edges, average clustering coefficient (Average CC) and the average degree (Average K), weakening bacterial interactions (Fig. 3). PET had a stronger impact on soil microbial networks, lowering Average CC and Average K more than PLA did. High-degree, low-betweenness genera were mainly found in PLA-treated gut bacteria (Fig. S6). Overall, PLA affected gut bacterial interactions more, while PET had a greater impact on soil microbial interactions.

3.6. Microplastics disturbed the soil microecology to a greater extent than glyphosate

The bipartite network showed that while many bacterial species overlapped between environments, soil contained more unique fungal species than the gut (Fig. S7 and S8). Structural equation modeling (SEM) revealed that microplastics directly influenced soil microbial communities or indirectly affected them via changes in NO_3^--N, NH_4^+-N, A-P and SOM (Fig. 4, GFI = 0.6260). Glyphosate significantly impacted gut microbiome and the growth of *E. crypticus*, while combined exposure exacerbated microplastics' effects on ROS and CYP450. These synergistic effects altered soil and gut microbiomes, further influencing

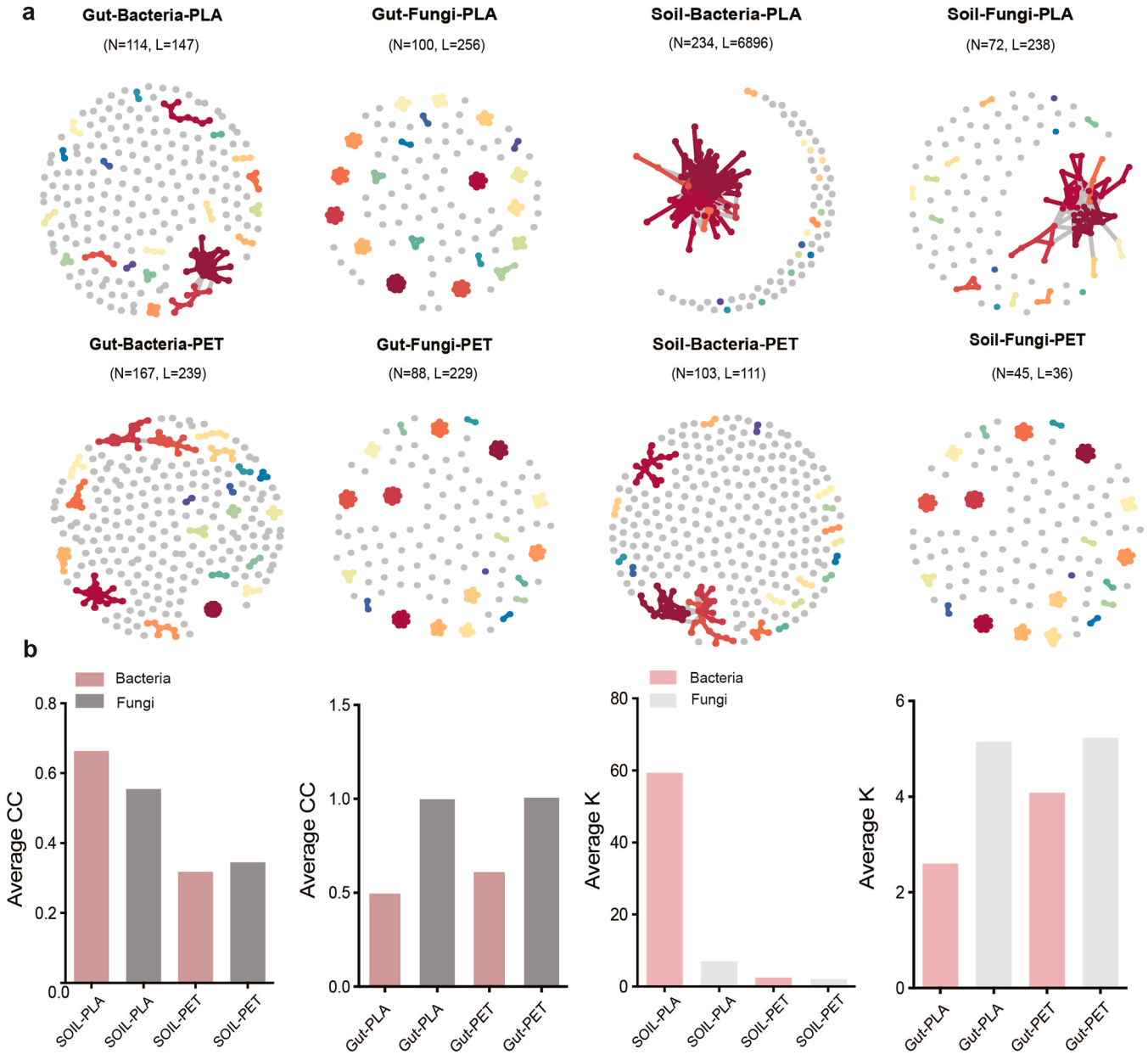

Fig. 3. (a).Visualization of constructed microbial networks. We constructed separate microbial co-occurrence networks for groups containing the glyphosate and the same kind of microplastic and determined their properties. For example, Soil-PLA included the Con, Gly, PLA and PLA + glyphosate treatments of soil. N represents the number of nodes and L represents the number of edges. Nodes represent individual amplicon sequence variants (ASVs) and are filled with color by module attributes (b). The topological parameters of the soil microbial community.

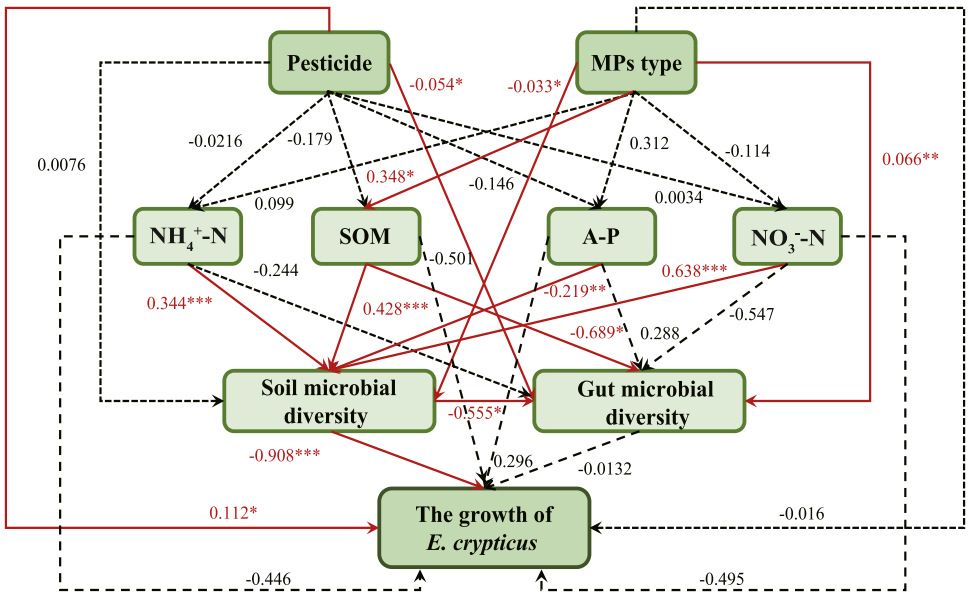

Fig. 4. Driving factors of the alterations in the soil and gut microbiomes. (a) Structural equation models were established considering various factors, such as the presence of pesticides (glyphosate), the types of microplastics (PLA, PET, PLA + glyphosate and PET + glyphosate), the physicochemical properties of the soil (NH_4^+-N, SOM, A-P and NO_3-N), the soil microbial communities (Chao1, Shannon, and PCoA for both bacteria and fungi), the gut microbial communities (Chao1, Shannon, and PCoA for both bacteria and fungi), and the physiological properties (ROS, CYP450, reproduction capacity and survival rate) of *E. crypticus*. The goodness-of-fit for the model was 0.6260. The red and black lines represent significant and insignificant correlations, respectively. (b) "*", "**" and "***" represent significant differences ($p < 0.05$, $p < 0.01$, and $p < 0.001$).

E. crypticus growth. Overall, soil microbiome and physicochemical properties were more sensitive to microplastics, whereas *E. crypticus* physiology and gut microbiome were more affected by glyphosate.

4. Discussion

Microplastics possess substantial specific surface area and hydrophobicity, rendering them excellent adsorbents for pesticides [75]. A comprehensive understanding of the combined ecotoxicity of microplastics and pesticides in terrestrial ecosystems is of paramount importance [42]. Therefore, we established a microcosm system to investigate the individual and combined effects of microplastics and glyphosate on soil and soil fauna.

4.1. Effects of microplastics and combined exposure on soil physicochemical properties

Microplastics and their combination with glyphosate significantly increased soil SOM and NH_4^+-N levels but decreased the NO_3-N level, aligning with previous findings [59]. PLA, being biodegradable, can be decomposed by functional microorganisms [15,69], whereas PET causes the death of soil faunas, and their remains are converted into soil organic matter, contributing to SOM accumulation [24]. Microplastics degradation by functional microorganism creates an oxygen concentration gradient, facilitating nitrogen conversion [20,54]. Both PET and PLA particles obstruct soil voids and alter oxygen content, thereby affecting nitrogen transformation [53]. The PET and PET + glyphosate treatments increased available phosphorus in soil, potentially due to the enrichment of phosphorus-solubilizing microorganisms *Stenotrophomonas* and *Aspergillus* [2,58].

4.2. Microplastics and combined exposure alter soil microbial communities

Microplastics and their combination with glyphosate significantly altered the diversity and composition of soil bacterial and fungal communities, with PLA exerting a greater effect than PET. Different microplastics induced distinct selective pressures on microbial communities [50]. PLA, despite being biodegradable, releases by-products such as lactic acid [12], which may be toxic to specific microbial groups [32]. The accumulation of these by-products can lead to microbial diversity reductions while promoting the proliferation of PLA-degrading taxa [19].

Key bacterial families, including *Bacillaceae*, known for their roles in organic matter cycling and plant pathogen suppression, decreased across all treatment groups [38], suggesting reduced soil resistance to adverse conditions. Similarly, *Oxalobacteraceae*, which play a role in nitrogen acquisition and maize growth [71], were significantly depleted in the PLA and PLA + glyphosate treatments. This indicates that PLA reshapes soil physicochemical properties by altering microbial abundance.

Our study revealed that microplastics and their combination with pesticide altered the soil parameters, thereby potentially affecting soil faunal biodiversity and may threatening the intensified agriculture [5,6,17].

4.3. Microplastics disrupt microbial network stability

Plenty of studies have demonstrated the disruptive effects of PLA or PET on the microbial community of soil and soil fauna [35,68]. In this study, we differentiated their effects by analyzing the co-occurrence networks for microbial groups treated with glyphosate and the same type of microplastics. The PLA treatment decreased the mean clustering coefficient and average degree of gut bacterial interaction networks, signifying reduced complexity of interaction networks. Similarly, in the PET treatment group, reduced clustering coefficients and average degrees suggested disrupted soil microbial symbiosis. PLA contamination significantly altered key bacterial genera essential for microbial homeostasis. Our findings suggest that microplastics disrupt microbial network complexity and symbiotic relationships, destabilizing both soil and gut microbial communities. Notably, biodegradable PLA exerted stronger disruptive effects on gut bacteria, whereas conventional PET had a more pronounced impact on soil microbiome interactions.

4.4. Physiological responses of soil fauna to microplastics and pesticide exposure

Through a comprehensive investigation of the physiological responses of *E. crypticus*, significant increases in ROS and CYP450 were observed across all treatments. Microplastics and glyphosate increased oxidative stress by disrupting cellular processes and causing physical damage [23,73], resulting in an elevation of ROS levels. Meanwhile, the soil fauna activated their metabolic pathways to detoxify the harmful substances, particularly through the increased activity of CYP450 enzymes, thus leading to the elevated CYP450 levels [33]. Combined exposure to microplastics and glyphosate increased their toxicity to soil fauna compared with the case of individual exposure, resulting in more significant oxidative stress reactions. This may be attributed to the toxic effects of glyphosate adsorbed on the surface of the microplastics and ingested by *E. crypticus* [46]. Besides, we found that the combined effects of biodegradable microplastics and pesticides is greater than that of non-biodegradable microplastics. This could be attributed to a more significant effect exerted by biodegradable microplastics on soil and soil fauna during the experimental period than non-biodegradable microplastics. Microplastics can significantly decreased the survival and reproduction of *E. crypticus* through the physical damage. Ingested MPs may cause inflammation, impair nutrient absorption, and lead to malnutrition [29].

Our findings demonstrated that the gut of *E. crypticus* activated its antioxidant system to respond to microplastics and glyphosate exposure, which creates oxidative damage, inflammation and disrupts their physiological balance [62].

4.5. Pesticide alters gut microbial homeostasis

Glyphosate functions as an inhibitor of the shikimate pathway, which is absent in animals but present in many microorganisms [16]. Consequently, it can selectively affect microbial communities by altering the composition and diversity of gut microbiota in soil fauna [73]. Previous studies have reported that glyphosate exposure can lead to dysbiosis in the gut microbiome of earthworms and other soil invertebrates [22], potentially impacting their physiological functions and health. In our study, glyphosate exposure significantly altered the diversity and composition of bacterial and fungal communities in the gut of *E. crypticus*, confirming microbial dysbiosis. This disruption was attributed to pesticide-induced damage to mucosal and epithelial cells [55].

Enterobacteriaceae and *Bacillaceae*, crucial for organic matter degradation and gut health [34,67], decreased across all treatments, suggesting a compromised symbiotic environment [3]. Conversely, *Xanthomonadaceae,* known plant pathogens, increased across all treatment groups, especially under glyphosate exposure [57]. This enrichment suggests potential adverse effects on soil plant populations and biodiversity, leading to shifts in soil structure and microbial communities.

Such alterations in abundance have profound influences on microbiome homeostasis, potentially affecting metabolism and elemental cycling. Microplastics, glyphosate, and their combination therefore have detrimental effects on the health of soil invertebrates. Our findings are crucial for investigating the physiological adaptation mechanisms employed by soil fauna under environmental pollution and ecological stress.

4.6. Differential effects of microplastics and pesticide on soil and gut microbiome

The responses to microplastics and glyphosate exposure differed between soil and gut microbiomes. Soil microbial communities were more affected by microplastics, whereas gut microbiota in soil fauna were more susceptible to glyphosate. Microplastics significantly altered soil physicochemical properties, leading to shifts in microbial community composition. However, glyphosate had relatively minor effects on soil physicochemical properties but exerted a greater impact on gut microbial diversity, ultimately reducing soil fauna reproduction.

5. Conclusion

The microbiome of soil and gut presented distinct responses to microplastics, glyphosate, and their combination. Microplastics were found to alter the physicochemical properties and microbial communities of the soil, whereas glyphosate induced oxidative stress and had a more pronounced impact on the gut of soil fauna. Compared with conventional PET, biodegradable PLA had more disruptive effects on the diversity and composition of bacterial and fungal communities in the soil. Combined exposure to microplastics and glyphosate exacerbated oxidative stress in *E. crypticus*, but did not intensify the individual impact of microplastics on the microbial communities. Meanwhile, the combination of biodegradable polylactic acid and glyphosate exerted more significant effects than the combination of non-biodegradable polyethylene terephthalate and glyphosate, which is in line with the application of microplastics alone. Negative responses on the microbiome of soil and *E. crypticus* warn us that the combined application of biodegradable plastic mulch and pesticides may affect soil functions and raise more ecological threats.

Environmental Implication

Pollutants in terrestrial ecosystems, e.g., microplastics and pesticides, have been increasing. As microplastics possess the ability to adsorb pesticides, the effect of their simultaneous exposure in soil warrants our concerns. In the present study, we systematically reported how microplastics and their combination with pesticides influence the soil and soil fauna gut microbiomes. It is worth noting that biodegradable plastics (like polylactic acid, PLA) have a large impact on the soil microbial ecosystem. Although it will eventually degrade, until then, its impact on the soil microecology may be greater than that of conventional plastics like polyethylene terephthalate (PET). Our study emphasizes that the combination of biodegradable microplastics and pesticide exert significant ecological impacts, highlighting the urgent need for further investigation into the use of biodegradable plastic mulch and pesticides in sustainable agriculture.

Ethics declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Lu Tao: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Zheng Guogang:** Supervision. **Tang Tao:** Writing – review & editing, Supervision, Conceptualization. **Qin Guoyan:** Investigation, Formal analysis. **Xia Shengjie:** Data curation. **Peijnenburg W.J.G.M.:** Writing – review & editing. **Yang Huihui:** Writing – original draft, Visualization. **Cui Rui:** Methodology. **Sun Liwei:** Funding acquisition. **Lei Chaotang:** Visualization. **Liu Meng:** Investigation. **Chen Bingfeng:** Visualization. **Qian Haifeng:** Writing – review & editing, Funding acquisition. **Zhang Qi:** Methodology, Funding acquisition. **Zhang Ziyao:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.137676.

Data availability

Data will be made available on request.

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