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Effects of microplastics and chlorpyrifos on earthworms (*Lumbricus terrestris*) and their biogenic transport in sandy soil☆

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A R T I C L E I N F O

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A B S T R A C T

Although microplastics (MPs) are ubiquitous in agricultural soil, little is known about the effects of MPs combined with pesticides on soil organisms and their biogenic transport through the soil profile. In this study, we conducted mesocosm experiments to observe the effects of microplastics (polyethylene (LDPE-MPs) and biodegradable microplastics (Bio-MPs)) and chlorpyrifos (CPF) on earthworm (*Lumbricus terrestris*) mortality, growth and reproduction, as well as the biogenic transport of these contaminants through earthworm burrows. The results showed that earthworm reproduction was not affected by any treatment, but earthworm weight was reduced by 17.6% and the mortality increased by 62.5% in treatments with 28% Bio-MPs. Treatments with 28% LDPE-MPs and 7% Bio-MPs combined with CPF showed greater toxicity while the treatment with 28% Bio-MPs combined with CPF showed less toxicity on earthworm growth as compared to treatments with only MPs. The treatments with 1250 g ha^{-1} CPF and 28% Bio-MPs significantly decreased the bioaccumulation of CPF in earthworm bodies (1.1 ± 0.2%, w w^{-1}), compared to the treatment with CPF alone (1.7 ± 0.4%). With CPF addition, more LDPE-MPs (8%) were transported into earthworm burrows and the distribution rate of LDPE-MPs in deeper soil was increased. No effect was observed on the transport of Bio-MPs. More CPF was transported into soil in the treatments with LDPE-MPs and Bio-MPs, 5% and 10% of added CPF, respectively. In addition, a lower level of the CPF metabolite 3,5,6-trichloropyridinol was detected in soil samples from the treatments with MPs additions than without MP additions, indicating that the presence of MPs inhibited CPF degradation. In conclusion, Bio-MPs caused significant toxicity effects on earthworms and the different types of MPs combined with CPF affected earthworms differently, and their transport along the soil profile. Thus, further research is urgently needed to understand the environmental risks of MPs and MP-associated compounds in the soil ecosystem.

1. Introduction

Plastic mulch films are widely used in agriculture to regulate soil temperature, conserve moisture and control weeds (Kasirajan and Ngouajio, 2012). Conventional plastic film made with low-density polyethylene (LDPE) is durable and highly resistant to degradation, which unfortunately means that it often remains in the soil for years (Mierzwa-Hersztek et al., 2019), potentially having adverse effects on soil biota and ecosystems (Kasirajan and Ngouajio, 2012; Huerta Lwanga et al., 2016; Qi et al., 2018). As an alternative, biodegradable plastic mulch films made with poly(butylene adipate-*co*-terephthalate) (PBAT) blended with polylactic acid (PLA) have been developed and are supposedly broken down completely by microorganisms in the soil (Touchaleaume et al., 2016). However, the degradation of PBAT/PLA plastic does not occur as expected in the soil resulting in macro-, micro- and nano-plastic accumulation and pollution in soils (Touchaleaume et al., 2016; Han et al., 2021). For example, Han et al. (2021) revealed that the degradation rate of PBAT in 4 different soils varied from 0.3% to 16% after 120 days. Although there are studies that have shown that PBAT/PLA MPs can affect soil properties such as soil carbon and nitrogen content, possibly due to the degradation of PBAT/PLA MPs (Qi et al., 2019; Meng et al., 2022), there is very little research examining how these compounds affect soil organisms.

Chlorpyrifos (CPF), a broad-spectrum organophosphorus insecticide

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and acaricide, has been widely used worldwide to control pests in agriculture (Lewis et al., 2016). In 2018, the annual yield of CPF was 28, 600 tons and mainly concentrated on rice, corn, wheat, and cotton (Yang et al., 2019). Increasing evidence has shown that CPF can pose risks to human and animal health which has led to more restrictions and bans on the use of CPF in the European Union and United States (Jia et al., 2021). Although, CPF use is still prevalent in developing countries like India and China (Foong et al., 2020). CPF, either alone or in combination with other contaminants, is toxic to many organisms (Zhou et al., 2011; John and Shaike, 2015). S. Zhou et al. (2007) revealed that CPF caused acute toxicity (LC_{50} at 91.87–118.5 mg kg^{-1}) and adverse effects on the growth and reproduction of the earthworm *Eisenia fetida*, depending on the concentration and period of exposure. Karbalaei et al. (2021) found that CPF in combination with polystyrene MPs caused stronger toxicity in rainbow trout, especially in gill tissue, than CPF alone. Garrido et al. (2019) observed that PE-MPs decreased the bioavailability of CPF, thereby decreasing the inhibition of growth in microalgae (*Isochrysis galbana*). These inconsistent findings indicate that the combined effects of CPF and MPs may vary for different organisms. Existing studies have proven that the ingestion of MPs or CPF alone affected the health of the earthworm *Lumbricus terrestris* (Martinez Morcillo et al., 2013; Huerta Lwanga et al., 2016). However, the ingestion of combinations of different MPs (especially biodegradable MPs) and CPF and the combined effects of these compounds on earthworms have rarely been studied.

Anecic earthworms, *Lumbricus terrestris,* are important soil biota which can improve soil structure and hydraulic properties by burrowing (Edwards et al., 1990; Lee and Foster, 1991). Earthworms can carry and transport MPs from the soil surface into deeper soil layers via casts, burrows, egestion and adherence to the earthworm skin (Huerta Lwanga et al., 2017; Rillig et al., 2017). Li et al. (2022) revealed that MPs were detected at a 80–100 cm soil depth, in a range between 2268 and 3529 particles kg^{-1}. Rillig et al. (2017) reported that the earthworm *Lumbricus terrestris* transported more PE-MPs (710–850 μm) into the 7.0–10.5 cm soil depth than into the 0–3.5 cm and 3.5–7.0 cm soil depths. Although the translocation of MPs is affected by tillage and leaching (Blasing and Amelung, 2018), biogenic transport caused by the activities of soil animals, like earthworm burrowing, may also contribute to the translocation of MPs into deep soil layers (Huerta Lwanga et al., 2016, 2017; Yang et al., 2018). However, very few studies have focused on the biogenic transport of MPs into deeper soil layers, or examined the risks of these MPs cause when combined with other contaminants.

MPs in soil can also adsorb hydrophobic organic contaminants (HOCs), such as pesticides, which could potentially affect the bioavailability of these compounds and their durability in the soil environment. This could pose unpredictable risks to soil properties, especially when pesticides are transported together with MPs through different environmental systems (Huffer et al., 2019; Rodríguez-Seijo et al., 2019; Yang et al., 2019; Wang et al., 2020a, Wang et al., 2020b). However, compared to natural sources, the importance of MPs as a source of HOCs is a controversial issue due to the abundance of MPs in the environmental system. Koelmans et al., 2016 said that the bioaccumulation of HOCs derived from MPs was minor compared to the uptake of HOCs via natural pathways in most aquatic habitats. However, Teuten et al., 2007 found that the addition of 1 μg of phenanthrene-contaminated PE-MPs to a gram of sediment could significantly increase the accumulation of phenanthrene by lugworms. Guo et al. 2019 also found that the total concentration of pesticide residues in plastic mulch film residues were about 20 times higher than that found in the soil, indicating the potential risks of plastic particles associated with these compounds, and their unknown behaviours in soil profile need to be aware. Yang et al. (2019) reported that the biogenic transport of glyphosate from the soil surface to deeper layers by earthworms was significantly increased in the presence of LDPE-MPs. Furthermore, the sorption of HOCs onto MPs may also influence the degradation rate of HOCs in the soil (Wang et al., 2020b). Yang et al. (2018) found that LDPE-MPs showed no effects on glyphosate degradation. However, Zhou et al. (2022) reported that PE and polyvinyl chloride MPs could reduce the degradation of simazine in soil. This divergence might be due to the differences in the properties of MPs, pesticides, and soil. Thus, studies looking at the effect of MPs on the degradation of HOC-like pesticides in soil are also needed.

In this study, we hypothesized that different MPs (LDPE-MP and Bio-MP) may cause different effects on earthworms under single exposure or co-exposure with CPF. We also hypothesized that not only could MPs affect earthworm ingestion and biogenic transport of CPF but also that the opposite is true. In addition, MPs may influence the degradation of CPF in soil. Thus, we aim to examine: (1) the single and combined ecotoxicological effects of MPs and CPF on earthworm mortality, growth and reproduction; (2) the effects of MPs and CPF on the biogenic transport of each other at 0–10 cm and 10–20 cm depths of burrow soil; and (3) the differences in CPF degradation in treatments with- and without-MP additions to the soil.

2. Materials and methods

2.1. Materials

Low density polyethylene (LDPE) pellets, and biodegradable plastic (Bio) pellets consisting of 85% poly (butylene adipate co-terephthalate) (PBAT), 10% polylactic acid (PLA) and 5% calcium carbonate were used to prepare MPs. To obtain MPs, the plastic pellets were frozen with liquid nitrogen and then ground and sieved. The size distribution of MPs was characterized using the 8700 Laser Direct Infrared (LDIR) chemical imaging system (Fig. S1) and the size of MPs ranged from 50 to 150 μm, which is considered to be environmentally realistic based on field investigations (Zhang and Liu, 2018). The density was 0.91 g cm^{-3} for LDPE-MPs and 1.35 g cm^{-3} for Bio-MPs. Solid chlorpyrifos (99.6% purity, HPC Standards GmbH, Germany) was dissolved in acetone to make a stock solution of 2.5 mg mL^{-1}. The stock solution was stored at 4 °C in the dark before use. Anecic earthworms (*Lumbricus terrestris*) were purchased from the Star Food Company (Barneveld, The Netherlands). The artificial sandy soil used in this study consisted of 26% sand with brown colour, 24% sand with silver colour, and 50% loamy silt (sand 3.1%, silt 77.3%, clay 19.6%) with 0.2% organic matter and the soil pH was 6.4. The litter from *Populus nigra* was collected from an uncontaminated area in Wageningen, The Netherlands, cut into 1–4 cm pieces, washed with distilled water and dried at 50 °C before use.

2.2. Experiment design and setup

Mesocosm experiments for LDPE-MPs and Bio-MPs were conducted under laboratory conditions from September 2020 and March 2021, respectively. The concentrations of MPs (0%, 7% and 28% in feeding litter, w w^{-1}) were in line with a previous study (Huerta Lwanga et al., 2016). Three CPF concentrations (0 g ha^{-1}, 250 g ha^{-1}, 1250 g ha^{-1}) were selected at 0-, 1-, and 5-times the recommended application rates for pesticide control under good agricultural practices (EFSA, 2014). CPF was pre-adsorbed on both MPs and plant litter to stimulate realistic environmental exposure scenarios and the adsorption efficiencies of CPF on MPs are shown in Table S1. A CPF stock solution was added to 50 mL of organic solvent (acetone for the LDPE-MP experiment and hexane for the Bio-MP experiment) in glass bottles and the MPs were added. The bottles were then shaken at 150 rpm at 20 °C in the dark for 12 h. The mixture of solution and MPs was well-mixed with plant litter. In the end, the treated litter was laid out in a fume hood to evaporate the organic solvent, and distilled water was added to bring the water content to 30%. In addition, a control was added to test the potential toxicity of the added solvent. In total, there were 10 treatments with 4 replicates for each experiment shown in Table 1.

The mesocosm (300 × 405 × 30 mm) was filled with 2 kg of sandy soil. The moisture content was measured using a mobile soil-moisture sensor (TRIME PICO 64, IMKO) and maintained at 20% throughout the experiment. Earthworms were starved for 48 h and kept at 15 °C in

Table 1
Summary of treatments.

MP level (%, w w^{-1})	CPF level (g ha^{-1})	Treatment
0	0 (without solvent)	Control
	0 (with solvent)	Solvent control
	250	0+1C
	1250	0+5C
7	0	7+0C
	250	7+1C
	1250	7+5C
28	0	28+0C
	250	28+1C
	1250	28+5C

the dark and then weighed before the experiment was carried out. The average weight of the earthworms in each treatment is shown in Table S2 and no significant difference was seen among treatments. Four adult earthworms with clitellum were placed in each mesocosm and then 6.5 g of fully treated plant litter was added to the surface of the soil. All the mesocosm boxes were incubated at 15 °C in the dark for 60 days and fully treated plant litters were replenished twice on the 20th day and the 40th day of the experiment.

2.3. Earthworm mortality, growth and reproduction

At the end of the experiment, all the mesocosm boxes were opened and the surviving earthworms were collected and cleaned with distilled water. Cleaned earthworms were transferred to petri dishes to empty their guts for 48 h and then the casts were collected. Earthworms were then cleaned again and weighed. Earthworms were frozen in liquid nitrogen and stored at −20 °C until pesticide extraction could be carried out.

Earthworm mortality was calculated using the percentage of dead individuals after 60 days. The growth of the earthworms was calculated using the change in weight by comparing the weight difference after the experiment with initial earthworm weight. Dead earthworms collected at the end of the experiment were excluded from the calculation. Cocoons produced in each mesocosm box were recorded to evaluate the potential impacts on the reproduction of *L. terrestris* in contaminated situations. All burrow soil (0–2 mm away from the wall of earthworm burrows) from two layers (0–10 cm and 10–20 cm) in each mesocosm box was scraped using a spatula. The extraction of MPs was conducted based on density floatation, and the extraction of CPF and its metabolites was followed using the QuEChERS approach and determined by LC-MS/MS. Detailed methods are described in the Supplementary materials. After all burrow samples were taken, earthworm cocoons were collected from the remaining soil using wet sieving through a 1 mm sieve.

2.4. Statistics

The transport ratios (%) of MPs or CPF in each treatment was calculated as follows:

$$TR = \frac{C * M_1}{M_2} * 100 \quad (2)$$

where C is the mass content of MPs in soils or the content of CPF in soils or earthworm bodies, M_1 is the total mass of the burrow soil, or the earthworms collected after the experiment. The mass of earthworms used for the calculation was their weight before the experiment. M_2 is the mass of MPs or CPF added during the experiment.

All Data except mortality are shown as mean ± standard deviation (SD), and mortality data are shown as median. The normality and equality of variance were determined using the Shapiro-Wilk test and Levene's test. A one-way ANOVA with Duncan's post-hoc comparisons was performed to test the significant differences in the initial weight of the earthworms, the change in weight and the cocoon production as well as the residue contents and transport ratios of CPF and MPs between different treatments. Two-way ANOVA was conducted to test the interaction effects of two factors (MP and CPF exposure concentrations) on earthworm weight changes, reproduction, and content and transport ratios of MPs and CPF in samples. The MP controls (control, 0+1C and 0+5C treatments) and CPF controls (control, 7+0C, 28+0C treatments) were excluded when performing two-way ANOVA analysis on the content and transport ratios of MP or CPF, respectively. A *t*-Test was performed to test the significant differences in the weight change, reproduction and mortality between the blank control and solvent control, and the significant differences of the contents of MPs and CPF within the two soil layers of each treatment. The differences in earthworm mortality between treatments were tested using the non-parametric Kruskal-Wallis test. Critical p values of significance were set at the 0.05 level. All these tests were performed using IBM SPSS Statistics 25 software and graphs were drawn with originpro 2016.

3. Results

3.1. Earthworm mortality, growth and reproduction in different treatments

No statistically significant differences in weight change, cocoon production or mortality of earthworms were observed between the control and solvent control treatments, thus the effect from solvents was excluded (Table S4).

Mortality was not significantly different between all treatments with LDPE-MPs as compared to the control (Table 2). However, in the treatments with Bio-MPs, only the 28+0C treatment caused significantly higher earthworm mortality as compared to other treatments. No significant effect on earthworm reproduction was seen in any treatment (Table 2).

Higher application rates (5C) of CPF alone caused higher earthworm weight losses in both LDPE-MP and Bio-MP experiments (Table 2). The weight changes in the 7% and 28% concentrations of LDPE-MP only treatments (7+0C, 28+0C) and the 7% concentration of Bio-MP only treatment were not significantly different than the controls, while Bio-MP only at 28% caused a significantly higher amount of earthworm weight loss than the control. LDPE-MP at 28% concentration and Bio-MPs at 7% concentration caused significantly higher combined toxicity with 5C CPF on earthworm growth as compared with MP only treatments. However, 28% Bio-MP caused significantly lower combined toxicity with CPF in the 28+1C treatment compared to the 28+0C treatment.

3.2. Microplastics and chlorpyrifos in earthworm casts and bodies

3.2.1. Microplastics

The mean MP contents in earthworm casts in the 28+0C treatment were 3.6 and 3.8 times higher than those in the 7+0C treatment for LDPE-MPs and Bio-MPs, respectively. These ratios were close to the ratios between exposure concentrations (Fig. 1a and b). The ratios of cast contents versus litter concentrations of MPs showed no significant differences between the 7+0C treatment and 28+0C treatment for both LDPE-MPs and Bio-MPs. With the increasing levels of CPF in 7% and 28% LDPE-MP concentration treatments, the cast contents and ratios of cast contents versus litter concentrations of LDPE-MPs were slightly increased as compared to LDPE-MP only treatments but the differences were not significant. However, the cast contents and ratios of cast contents versus litter concentrations of Bio-MPs in 28% concentration treatments significantly decreased with the addition of CPF as compared to 28% concentration of Bio-MP only treatments.

* Statistical analysis was not conducted between contents of CPF in earthworm bodies in the 1C treatments of the LDPE-MP experiment because CPF content in the 7+1C treatment was lower than the limit of quantification.

Table 2
Earthworm weight change, reproduction and mortality in different treatments.

Indicators	MP	Control	Treatments							
			0+1C	0+5C	7+0C	7+1C	7+5C	28+0C	28+1C	28+5C
Weight change (%)	LDPE-MP	−10.7 ± 7.6c	−16.5 ± 12.9abc	−23.6 ± 8.5 ab	−16.9 ± 2.9abc	−15.8 ± 1.9bc	−21.5 ± 4.0abc	−15.2 ± 6.3bc	−21.5 ± 11.5abc	−27.8 ± 5.1a
	Bio-MP	4.4 ± 5.0d	−1.2 ± 7.4cd	−6.3 ± 4.3bc	2.8 ± 3.7d	−0.2 ± 7.1cd	−6.5 ± 1.1bc	−17.6 ± 9.8a	−6.6 ± 7.9bc	−12.3 ± 2.6 ab
Reproduction (cocoons produced per surviving worm)	LDPE-MP	1.0 ± 0.2a	1.1 ± 0.7a	1.1 ± 0.7a	1.1 ± 1.2a	0.8 ± 0.9a	0.6 ± 0.7a	0.6 ± 0.2a	0.4 ± 0.4a	1.1 ± 0.9a
	Bio-MP	0.6 ± 0.4a	0.4 ± 0.1a	0.3 ± 0.3a	0.6 ± 0.3a	0.5 ± 0.4a	0.4 ± 0.2a	0.3 ± 0.4a	0.5 ± 0.5a	0.4 ± 0.1a
Mortality	LDPE-MP	0%a	0%a	12.5%a	0%a	0%a	0%a	0%a	12.5%a	0%a
	Bio-MP	0%a	0%a	0%a	0%a	0%a	0%a	62.5%b	0%a	0%a

Different letters represent significant differences in the indicator among treatments in LDPE-MP or Bio-MP experiments.

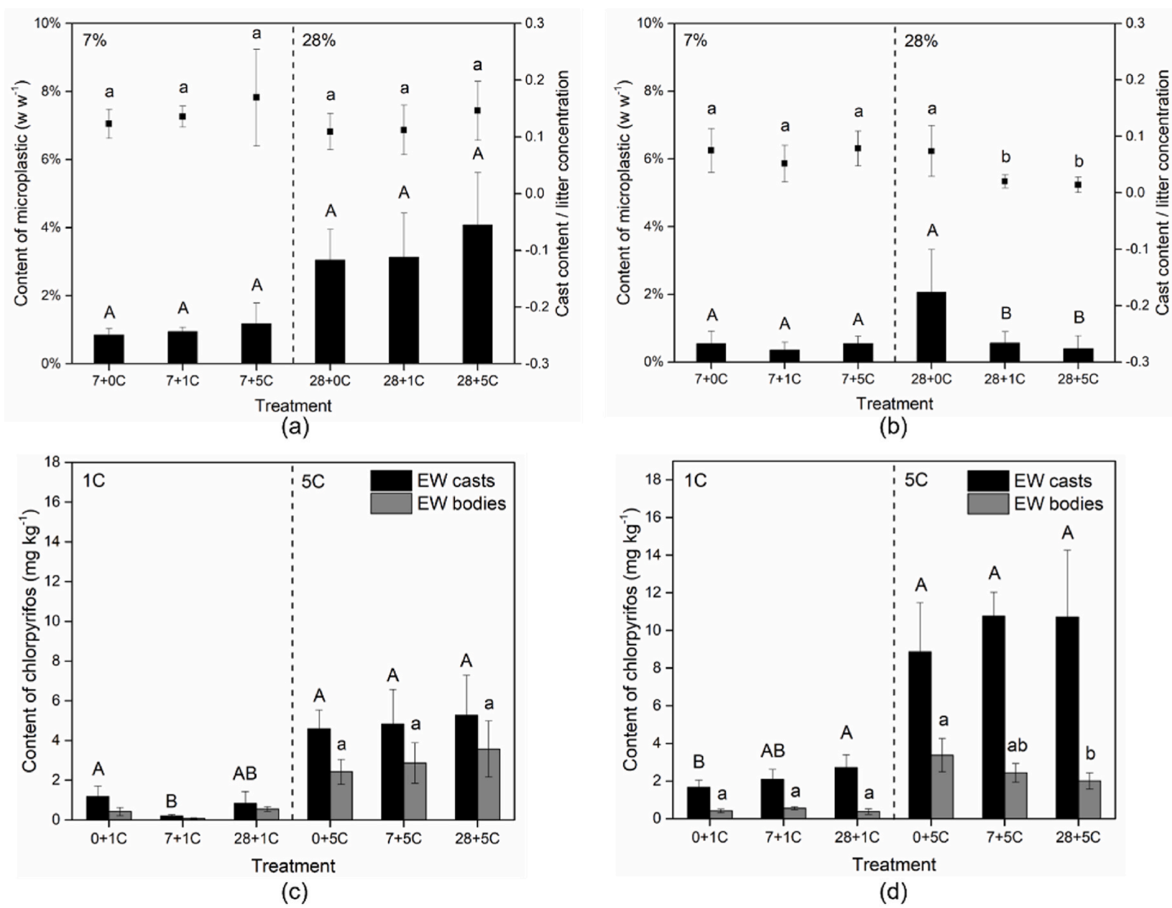

Fig. 1. Cast contents of MPs (bars) and ratios of cast contents versus litter concentrations of MPs (scatters) in earthworm casts (a: LDPE-MP experiment; b: Bio-MP experiment), and contents of chlorpyrifos in earthworm casts and bodies (c: LDPE-MP experiment; d: Bio-MP experiment). In figure a and b, different uppercase letters represent significant differences in microplastic contents among treatments in the 7% or 28% concentration of the microplastic treatment group, different lowercase letters represent significant differences in the ratios of cast contents v litter concentrations of MPs among treatments in the 7% or 28% microplastic treatment group. In figure c and d, different uppercase letters represent significant differences in chlorpyrifos contents in earthworm casts among treatments in the 1C or 5C chlorpyrifos treatment group, different lowercase letters represent significant differences in the chlorpyrifos contents in earthworm bodies among treatments in the 1C or 5C chlorpyrifos treatment group.

3.2.2. *Chlorpyrifos*

In the presence of LDPE-MPs, we only observed a significant decrease of CPF content in the earthworm casts in the 7+1C treatment as compared to the 0+1C treatment (Fig. 1c). However, in the presence of Bio-MPs, the content of CPF in earthworm casts was significantly higher in the 28+1C treatment than 0+1C treatment (Fig. 1d).

In the presence of LDPE-MPs, the content of CPF in earthworm bodies was lower in the 7+1C treatment as compared to the 0+1C treatment, and the content was lower than the limit of quantification (LOQ) (Fig. 1c). Bio-MPs also significantly decreased earthworm body contents of CPF in the 28+5C treatment compared to the 0+5C treatment (Fig. 1d).

3.3. Contents of microplastics and chlorpyrifos in burrow soil

3.3.1. Microplastics

In the 7% LDPE-MP treatments, the content of MPs only significantly increased in the 10–20 cm soil depth in the 7+5C treatment as opposed to the 7+0C treatment (Fig. 2a). However, in the 28% LDPE-MP treatments, the contents of MPs were significantly higher in both 0–10 cm and 10–20 cm soil depths in the 28+1C and 28+5C treatments than 28+0C treatment. LDPE-MP contents in the 0–10 cm depth of burrow soil were significantly higher than those in the 10–20 cm soil depth in the 28+0C and 28+1C treatments. The contents of Bio-MPs showed no significant differences in the 7% MP concentration treatments but in the 28% MP concentration treatments, the contents in both the 0–10 cm and 10–20 cm soil depths were significantly lower in the 28+5C treatment as compared to the 28+0C treatment (Fig. 2b). The content of Bio-MPs in the 0–10 cm burrow soil depth was significantly higher than that in the 10–20 cm depth soil in the 28+5C treatment.

3.3.2. Chlorpyrifos

In both 1C and 5C CPF treatments, the presence of LDPE-MPs did not significantly affect the contents of CPF in the 0–10 cm and 10–20 cm burrow soil depths as compared to the treatments where CPF was added alone (Fig. 2c). However, in the presence of Bio-MPs in both the 1C and 5C CPF treatments, higher CPF contents were found in the 0–10 cm and 10–20 cm soil depths as compared to the treatments without Bio-MPs (Fig. 2d). Only in the 7+5C treatment in the Bio-MP experiment, the content of CPF was significantly higher in the 0–10 cm burrow soil depth than the 10–20 cm soil depth.

3.4. Biogenic transport ratios of microplastics and chlorpyrifos

3.4.1. Microplastics

Earthworms in total transported 2.4 ± 0.5% and 2.7 ± 1.0% of added LDPE-MPs in the 7+0C and 28+0C treatments, respectively (Fig. 3a). In the presence of CPF, earthworms transported significantly more LDPE-MPs into the soil in the 28+1C and 28+5C treatments than the 28+0C treatment. The highest transport ratio reached 8.0 ± 1.9% in the 28+5C treatments. Earthworms transported significantly more LDPE-MPs into the 0–10 cm soil depths than into the 10–20 cm soil depths in the 7+0C and 28+0C treatments. However, in the presence of CPF, higher distribution rates of LDPE-MPs were found in the 10–20 cm soil depths, and the results of statistical analyses showed that the transport ratios for the 0–10 cm soil depth were no longer significantly higher than those for the 10–20 cm soil depths in treatments with CPF additions except in the 28+1C treatment. In the Bio-MP experiment, earthworms transported significantly more MPs in the 7+0C treatment (4.6 ± 1.5%) than in the 28+0C treatment (2.2 ± 0.9%) (Fig. 3b). However, the presence of CPF did not significantly change the total

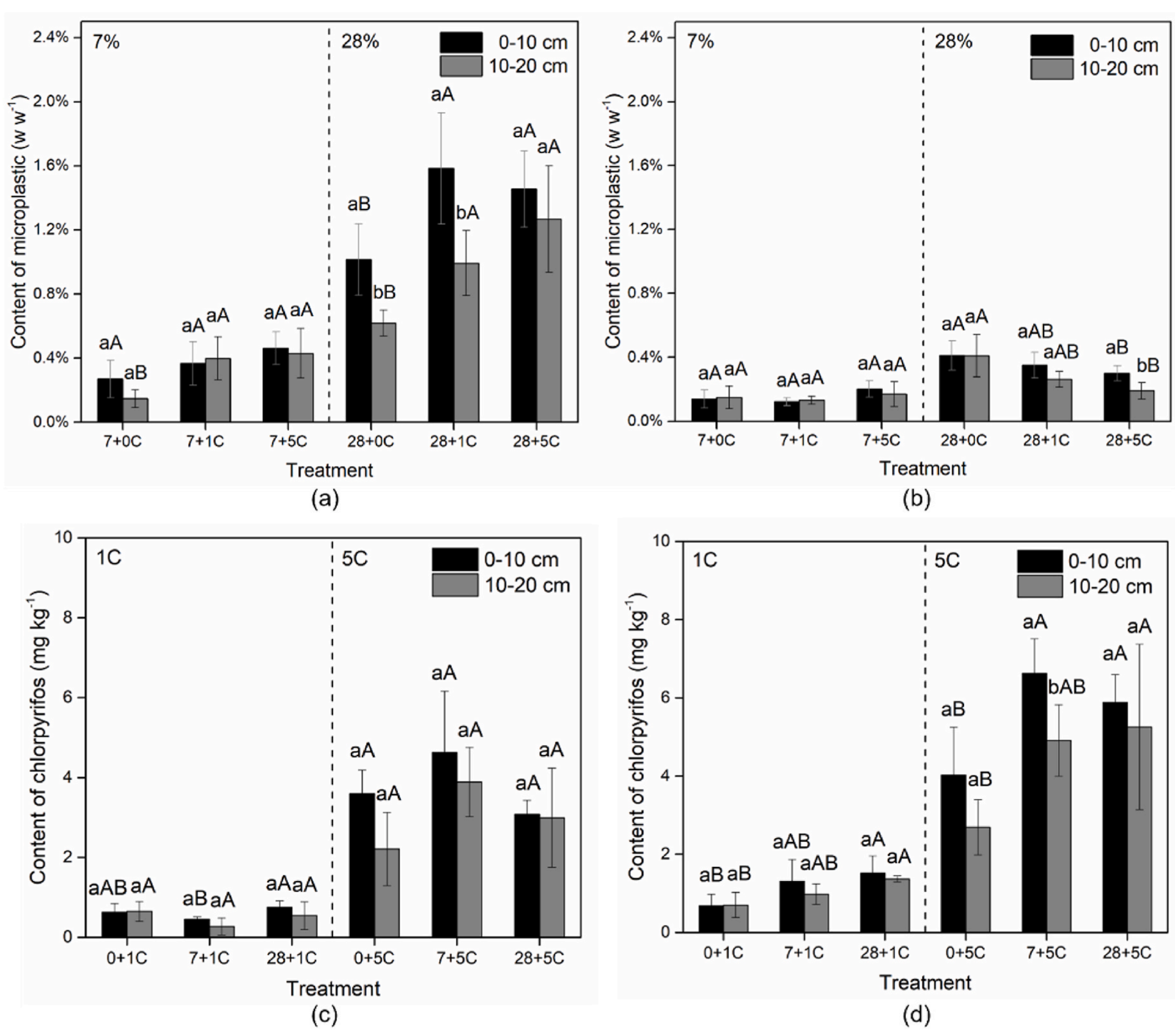

Fig. 2. Contents of microplastics (a: LDPE-MP experiment; b: Bio-MP experiment) and chlorpyrifos (c: LDPE-MP experiment; d: Bio-MP experiment) in the 0–10 cm and 10–20 cm depths of burrow soil. Different uppercase letters represent significant differences among treatments in the 1C or 5C chlorpyrifos treatment group, different lowercase letters represent significant differences between the 0–10 cm and 10–20 cm depths of soil.

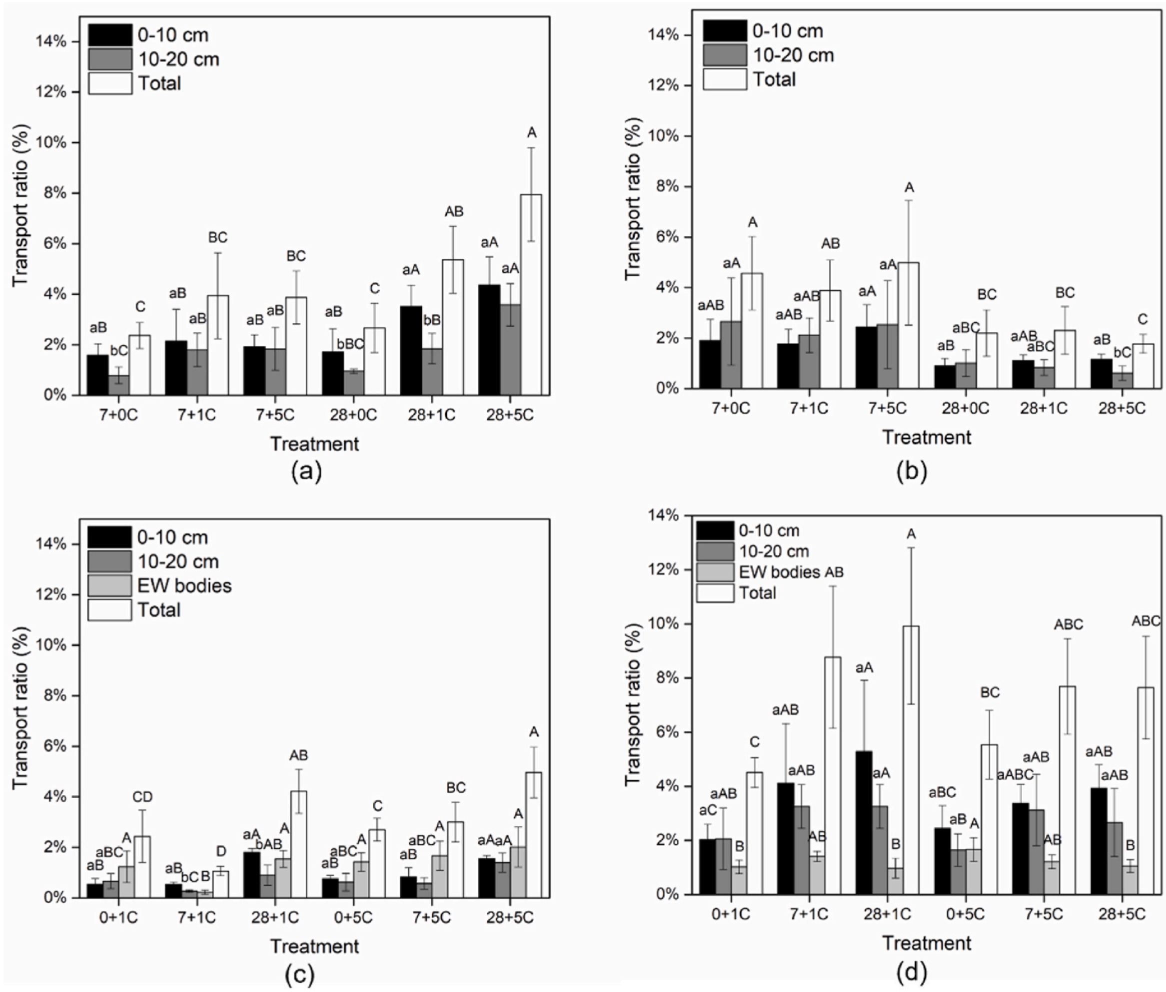

Fig. 3. Transport ratios of (a) LDPE-MP and (b) Bio-MP in soil, (c) chlorpyrifos in soil and earthworm bodies in the LDPE-MP experiment and (d) chlorpyrifos in soil and earthworm bodies in the Bio-MP experiment. Different uppercase letters indicate the significant differences among treatments, different lowercase letters indicate the significant differences between the 0–10 cm and 10–20 cm soil depths.

transport ratios of Bio-MPs. The transport ratio of Bio-MPs in the 0–10 cm soil depth was significantly higher than that in the 10–20 cm soil depth only in 28+5C treatments.

3.4.2. Chlorpyrifos

The total transport ratios of CPF showed no significant differences between the 0+1C and 0 + 5C treatments in both the LDPE-MP and Bio-MP experiments (Fig. 3c and d). Both LDPE-MPs and Bio-MPs increased total transport ratios of CPF and transport ratios of CPF in the 0–10 and 10 – 20 cm soil depths, except in the 7 + 1C treatment in the LDPE-MP experiment. The transport ratios for the 0 – 10 cm soil depth were significantly higher than that for the 10 – 20 cm soil depth only in 7 + 1C and 28 + 1C treatments in the LDPE-MP experiment. Furthermore, in the presence of LDPE-MPs, the amount of CPF in earthworm bodies was significantly lower only in the 7 + 1C treatment as compared to the 0 + 1C treatment. Bio-MPs significantly decreased the amount of CPF in earthworm bodies in the 28 + 5C treatment as compared to the 0 + 5C treatment.

3.5. Contents of chlorpyrifos metabolites in earthworm casts, bodies, and burrow soil

3.5.1. Earthworm casts and bodies

Two metabolites of CPF (chlorpyrifos-oxon and 3,5,6-trichloropyridinol (TCP)) were measured in this study (Tables S5 and S6). We did not find chlorpyrifos-oxon in earthworm bodies, casts or the two layers of soil in the LDPE-MP experiment and only low contents of chlorpyrifos-oxon were found in earthworm casts in the 0 + 5C and 28 + 5C treatments in the Bio-MP experiment. TCP was the main metabolite of CPF found in this study. LDPE-MPs did not significantly affect the contents of TCP in earthworm casts except in the 7 + 1C treatment where the content of TCP was lower than the LOQ. In the Bio-MP experiment, however, both 7% and 28% Bio-MPs significantly decreased the contents of TCP in earthworm casts in the 1C and 5C treatments as compared to the treatments where CPF was added alone. Additionally, the presence of LDPE-MPs and Bio-MPs had no significant effects on the TCP contents in earthworm bodies.

3.5.2. Burrow soil

TCP was not detected in the burrow soil in the 1C treatments in either the LDPE-MP or Bio-MP experiment. In the 0–10 cm soil depth for the LDPE-MP experiment, the content of TCP in the 0 + 5C treatment was 0.07 ± 0.04 mg kg^{-1}, while the presence of 28% LDPE-MP decreased the content of TCP which was lower than the LOQ. While in the 10–20 cm soil depth, the contents of TCP were all lower than the LOQ (Table S6). Similarly, in the 0 – 10 cm soil depths, the contents of TCP decreased with exposure to increasing Bio-MP concentrations as compared to the CPF only treatment. The content of TCP in the 28 + 5C treatment (0.04 $\pm$ 0.01 mg kg^{-1}) was significantly lower than that in the 0 + 5C treatment (0.11 $\pm$ 0.04 mg kg^{-1}). In the 10 – 20 cm soil depth, the content of

TCP was lower in the 28 + 5C treatment which was below the LOQ.

4. Discussion

4.1. Earthworm ingestion of microplastics and chlorpyrifos and their toxicity

MPs can carry organic pollutants to various organisms due to the hydrophobicity and high specific surface area of MPs (Wang et al., 2020c). So far, the combined effects of MPs and organic pollutants have been focused on aquatic organisms. There have been limited studies focusing on soil organisms like earthworms, and very inconsistent results have been achieved. For example, Wang et al. (2019) found that PE and PS MP at a concentration >1% (w w^{-1}) in soil decreased the bioaccumulation of hydrophobic organic contaminants (HOCs) in earthworms, but a MP concentration of 0.1% had no effect on the bioaccumulation in earthworms. However, Sun et al. (2021) found that MPs at a 0.3% (w w^{-1}) concentration significantly increased the bioaccumulation of dufulin in earthworms after 14 days of exposure. Polymer type, size and concentration of MPs might be the main factors affecting the bioaccumulation of pollutants (Zhang et al., 2022). In this study, through dietary exposure of MPs and CPF, we found that different exposure concentrations of LDPE-MP and Bio-MP had different effects on the ingestion and bioaccumulation of CPF by earthworms. For LDPE-MPs, we only observed reduced CPF contents in earthworm casts and bodies in the 7+1C treatment as compared to the 0+1C treatment. This reduction may be due to the adsorption of CPF on LDPE-MPs decreasing the accessibility of CPF to earthworms from plant litter. For comparison, in the 7+5C treatment, the CPF contents in earthworm casts and bodies did not decrease as compared to the 0+5C treatment. This may be due to the fact that the adsorption efficiency of CPF by LDPE-MPs in the 7+5C treatment was lower than that in the 7+1C treatment (Table S1), thus earthworms could uptake more CPF from plant litter in the 7+5C treatment. In addition, in the 28+1C treatment, the contents of CPF in earthworm casts and bodies were higher than those in the 7+1C treatment. This may be because the ingestion of LDPE-MPs in the 28+1C treatment was 3.3 times higher than that in the 7+1C treatment, thus the uptake of MP-adsorbed CPF by earthworms increased. In the Bio-MP experiment, we found that the earthworm bioaccumulation of CPF did not follow the ingestion of CPF. Even though the presence of Bio-MPs increased the ingestion of CPF by earthworms, it still decreased the bioaccumulation of CPF.

In this study, we also examined the effects of CPF on the bioaccumulation of MPs in earthworm casts. We found that the presence of CPF influenced the earthworm cast contents of MPs, but this effect varied with different MPs. The presence of CPF significantly decreased the earthworm cast content of Bio-MP at the 28% exposure concentration. However, the effect of CPF on the bioaccumulation of LDPE-MP was minimal, only slightly increasing earthworm cast contents of LDPE-MP. A previous study suggested that *p*-Phthalic acid and lactic acid, which were the monomers of the Bio-MPs used in this study, were attractive to earthworms (Wang et al., 2022). The author speculated that this may be due to the sour odour of polymers and earthworms use odour cues to forage. If it is true, CPF may disguise the odour from Bio-MPs with its mercaptan odour (Manish, 2017), leading to the reduction of Bio-MPs ingestion by earthworms. However, in our study, this was not our objective. Due to the increasing demand of biodegradable plastic mulches in agriculture, the distribution of Bio-MPs in soil and the risks combined with agrichemicals like pesticides needs to be examined.

In this study, LDPE-MPs alone did not significantly affect the mortality, growth, or reproduction of *Lumbricus terrestris*. However, Huerta Lwanga et al. (2016) found that a LDPE-MPs exposure level of 28% in litter (w w^{-1}) significantly decreased the survival and growth rates of *Lumbricus terrestris*. This may be attributed to the smaller size of the LDPE-MPs used in the study which are prone to be more toxic to earthworms (Li et al., 2021). PE-MPs are harmful for earthworms because they cause intestinal disruption or obstruction and oxidative stress, as well as affect food accessibility and availability (Rodriguez-Seijo et al., 2017; Boots et al., 2019; Chen et al., 2020). However, studies related to the toxicity of biodegradable/biobased MPs are still lacking. Ding et al. (2021) suggested that the toxicity of biodegradable polylactic acid and polypropylene carbonate MPs for earthworms was no less than PE. Several studies have shown that the intermediates of biodegradable MPs caused no adverse effects on earthworms (Siegenthaler et al., 2011; Sforzini et al., 2016). For example, a study to assess the ecotoxicity of composting of Ecoflex® (PBAT) was carried out following the OECD guideline 207 and the earthworms *Eisenia fetida* were exposed to reference soil with a 25% compost addition. No adverse effects on earthworm survival or biomass were observed after 7 and 14 days of exposure (Siegenthaler et al., 2011). However, in our 60-day mesocosm experiment, we revealed significant adverse effects from PBAT/PLA MPs on *Lumbricus terrestris* survival and growth at a 28% exposure concentration in litter. Thus, in the future, studies concerning the risk assessments of biobased/biodegradable MPs should be more often undertaken considering their increasing usage worldwide.

CPF was found to affect the growth of some species of earthworms including *Eisenia fetita*, *Eisenia andre*, and *Aporrectodea caliginosa* (S.-p. Zhou et al., 2007; De Silva et al., 2009; Dasgupta et al., 2012), but little is known about its effects on the growth of the adult *Lumbricus terrestris* (Giménez et al., 2004). In this study, we observed a concentration-dependent effect of CPF on *Lumbricus terrestris* growth but no significant effects on survival or reproduction. Combining CPF with different types of MPs resulted in different combined ecotoxic effects on the growth of *Lumbricus terrestris*. The effect of the combination of LDPE-MPs and CPF on earthworm growth was stronger than LDPE-MPs alone. Shi et al. (2022) found that PE-MPs spiked in soil with fluoranthene also caused greater toxic effects on the growth of the earthworm *Eisenia fetida* than MPs alone. However, the combined effects of biodegradable MPs and CPF on earthworm growth and survival were lower than 28% Bio-MPs alone. It seems that the presence of CPF decreased the ingestion of Bio-MPs by earthworms thus reducing the adverse effects from Bio-MPs. Additionally, a significant interaction effect between Bio-MPs and CPF indicated that the effects of CPF on earthworm growth differed with the exposure level of Bio-MPs (Table S7). In treatments with a 7% concentration of Bio-MPs, the toxicity of the combination of Bio-MPs and CPF on earthworm growth was stronger than Bio-MP alone, this may because the low level of Bio-MPs was not toxic to earthworms and the reduction in earthworm weight was mainly influenced by CPF.

4.2. Biogenic transport of microplastics and chlorpyrifos by earthworms

In this study, we revealed that the biogenic transport of MPs varied with different MPs and their combination with CPF. Different exposure levels of LDPE-MPs alone showed similar transport ratios while Bio-MPs showed a significantly lower transport ratio at higher MP exposure levels. This is probably attributed to the high level of Bio-MPs inhibiting the earthworms' ability to transport Bio-MPs into the soil. So far, there hasn't been much research examining the biogenic transport of MPs in combination with other contaminants in the soil. In this study, we found that the presence of CPF increased the biogenic transport of LDPE-MPs by the earthworm *Lumbricus terrestris*. Rodríguez-Seijo et al. (2019) suggested that the transport of LDPE-MPs sprayed with CPF by the earthworm *Eisenia fetida* did not significantly differ from that of LDPE-MP alone, probably because of the sizes of the MPs (1 mm and 5 mm) which were too big to be ingested and transported by *Eisenia fetida*. We also found that CPF had no significant effect on the biogenic transport of Bio-MPs even though CPF decreased the ingestion of Bio-MPs by earthworms in treatments with high levels of Bio-MPs. These findings revealed that LDPE-MPs may have a higher potential risk of being transported into soil by earthworms, while high concentration of Bio-MPs are less likely to be transported by earthworms due to a greater toxicity.

More LDPE-MPs were transported into the 0–10 cm soil depth as compared to the 10–20 cm soil depth. This may because MPs attached to the skin of earthworms were trapped in this layer when earthworms moved toward deeper soil. Rillig et al. (2017) observed adhesion of LDPE-MPs on the skin of *Lumbricus terrestris* in their study. Therefore, in the 0–10 cm soil depth, MPs may have come from not only earthworm faeces but also adhesion to earthworm skin. While in the 10–20 cm soil depth, earthworm faeces may have been the dominant source of MPs, this was not measured in the current study. Moreover, in the presence of CPF, the distribution rates of LDPE-MPs in deeper soil were higher than those in the LDPE-MP only treatments. This could be because dermal adhesion played a smaller role in the biogenic transport of MPs when earthworms ingested more LDPE-MPs. However, there is little difference in the biogenic transport of Bio-MPs into different soil layers.

In the presence of LDPE-MPs, the transport ratios of CPF increased in both the 0–10 cm and 10–20 cm soil depths except in the 7+1C treatment. Similarly, Yang et al. (2019) also found that even though the presence of LDPE-MPs did not significantly change the contents of glyphosate in earthworm burrow soil, it significantly increased the total amount of glyphosate transported by earthworms. The presence of Bio-MPs increased both the contents and transport ratios of CPF in the 0–10 cm and 10–20 cm soil depths. Bio-MPs increased cast contents of CPF and decreased CPF bioaccumulation in earthworms, thus more CPF was transported into the soil. Furthermore, the increased transport of CPF may also be due to the fact that the presence of MPs decreased the degradation of CPF, this is proven by the lower contents of TCP detected in the MP and CPF combination treatments. Future studies should consider the role of MPs in the transport of other contaminants when they co-exist in soil.

4.3. Effects of MPs on CPF degradation

TCP was the major metabolite detected in this study. However, no significant effects of the bioaccumulation of TCP by earthworms were observed in the presence of MPs. Furthermore, both LDPE-MPs and Bio-MPs decreased the contents of TCP in soil which might be due to the adsorption of CPF on MPs which inhibits the degradation of CPF in the soil. The biochar-adsorbed pesticides showed reduced rates of biodegradation in soil due to the reduced bioavailability to microorganisms. For example, the loss of two pesticides, chlorpyrifos and fipronil, significantly decreased with the increasing levels of biochars in soil (Yang et al., 2010). However, whether microplastics could affect the degradation of pesticides have not been well studied, especially in the soil ecosystem. One recent study found that MPs could decrease the degradation rate of the herbicide simazine in soil, which was attributed to MP-induced change in the microbial community structure and enzyme activity (Zhou et al., 2022). Thus, prolonged effects of microplastics and pesticides in soil ecosystems are expected. In the future, researchers should focus on studying the effects of microplastics on the biodegredation of pesticides as well as the effects of multi-contaminant combinations in soil.

5. Conclusion

In this study, we conducted mesocosm experiments to characterize the combined effects of two types of MPs (LDPE-MPs and Bio-MPs) and one insecticide, CPF, on the earthworm *Lumbricus terrestris* as well as the combined biogenic transport of these compounds in soil. Our findings indicated that Bio-MPs have significant ecotoxicity on earthworm growth and survival but no adverse effect was observed in the treatments containing LDPE-MPs. However, the combination of LDPE-MPs and CPF showed stronger ecotoxicity on earthworm growth than Bio-MPs and CPF at the 28% MP exposure concentration. Interestingly, CPF application increased the biogenic transport of LDPE-MPs but had no significant effect on the transport of Bio-MPs. The differences in the ecotoxicity and biogenic transport between LDPE-MPs and Bio-MPs might be due to the different chemical compositions of MPs which may affect their toxicities and adsorption capacities. LDPE-MPs and Bio-MPs increased biogenic transport and inhibited the degradation of CPF in soil, indicating that the potential risks of the combination of MPs and CPF need to be explored. Further studies should be done to consider MP accumulation in soil ecosystems exposed to multiple pesticides, especially the behaviour of MP-associated compounds in soil.

Conflict of author statement

Hui Ju: Conceptualization, Investigation, Formal analysis, Writing – Original Draft. Writing - review & editing, Visualization. **Xiaomei Yang**: Conceptualization, Writing - Review & Editing, Supervision. **Rima Osman**: Methodology, Validation. **Violette Geissen**: Conceptualization, Resources, Writing - Review & Editing, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Blasing, M., Amelung, W., 2018. Plastics in soil: analytical methods and possible sources. Sci. Total Environ. 612, 422–435. https://doi.org/10.1016/j.scitotenv.2017.08.086.
Boots, B., Russell, C.W., Green, D.S., 2019. Effects of microplastics in soil ecosystems: above and below ground. Environ. Sci. Technol. 53, 11496–11506. https://doi.org/10.1021/acs.est.9b03304.
Chen, Y., Liu, X., Leng, Y., Wang, J., 2020. Defense responses in earthworms (Eisenia fetida) exposed to low-density polyethylene microplastics in soils. Ecotoxicol. Environ. Saf. 187, 109788 https://doi.org/10.1016/j.ecoenv.2019.109788.
Dasgupta, R., Chakravorty, P.P., Kaviraj, A., 2012. Effects of carbaryl, chlorpyrifos and endosulfan on growth, reproduction and respiration of tropical epigeic earthworm, Perionyx excavatus (Perrier). J Environ Sci Health B 47, 99–103. https://doi.org/10.1080/03601234.2012.616771.
De Silva, P.M., Pathiratne, A., van Gestel, C.A., 2009. Influence of temperature and soil type on the toxicity of three pesticides to Eisenia andrei. Chemosphere 76, 1410–1415. https://doi.org/10.1016/j.chemosphere.2009.06.006.
Ding, W., Li, Z., Qi, R., Jones, D.L., Liu, Q., Liu, Q., Yan, C., 2021. Effect thresholds for the earthworm Eisenia fetida: toxicity comparison between conventional and biodegradable microplastics. Sci. Total Environ. 781 https://doi.org/10.1016/j.scitotenv.2021.146884.
Edwards, W.M., Shipitalo, M.J., Owens, L.B., Norton, L.D., 1990. Effect of Lumbricus terrestris L. burrows on hydrology of continuous no-till corn fields. Geoderma 46, 73–84. https://doi.org/10.1016/0016-7061(90)90008-w.
EFSA, 2014. Conclusion on the peer review of the pesticide human health risk assessment of the active substance chlorpyrifos. EFSA J. 12 https://doi.org/10.2903/j.efsa.2014.3640.
Foong, S.Y., Ma, N.L., Lam, S.S., Peng, W., Low, F., Lee, B.H.K., Alstrup, A.K.O., Sonne, C., 2020. A recent global review of hazardous chlorpyrifos pesticide in fruit and vegetables: prevalence, remediation and actions needed. J. Hazard Mater. 400, 123006 https://doi.org/10.1016/j.jhazmat.2020.123006.
Garrido, S., Linares, M., Campillo, J.A., Albentosa, M., 2019. Effect of microplastics on the toxicity of chlorpyrifos to the microalgae Isochrysis galbana, clone t-ISO. Ecotoxicol. Environ. Saf. 173, 103–109. https://doi.org/10.1016/j.ecoenv.2019.02.020.

Gimenez, R., Della Penna, A., Odello, E., 2004. Efectos toxicos de los insecticidas clorpirifos y teflutrina sobre la lombriz de tierra (Lumbricus terrestris L.). Agric. Tec. (Santiago) 64, 10.4067/s0365-28072004000400003.

Guo, B., Meng, J., Wang, X., Yin, C., Hao, W., Ma, B., Tao, Z., 2019. Quantification of pesticide residues on plastic mulching films in typical farmlands of the North China. Front. Environ. Sci. Eng. 14 https://doi.org/10.1007/s11783-019-1181-9.

Han, Y., Teng, Y., Wang, X., Ren, W., Wang, X., Luo, Y., Zhang, H., Christie, P., 2021. Soil type driven change in microbial community affects poly(butylene adipate-co-terephthalate) degradation potential. Environ. Sci. Technol. 55, 4648–4657. https://doi.org/10.1021/acs.est.0c04850.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the terrestrial ecosystem: implications for Lumbricus terrestris (Oligochaeta, lumbricidae). Environ. Sci. Technol. 50, 2685–2691. https://doi.org/10.1021/acs.est.5b05478.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2017. Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environ. Pollut. (Amsterdam, Neth.) 220, 523–531. https://doi.org/10.1016/j.envpol.2016.09.096.

Huffer, T., Metzelder, F., Sigmund, G., Slawek, S., Schmidt, T.C., Hofmann, T., 2019. Polyethylene microplastics influence the transport of organic contaminants in soil. Sci. Total Environ. 657, 242–247. https://doi.org/10.1016/j.scitotenv.2018.12.047.

Jia, W., Shen, D., Yu, K., Zhong, J., Li, Z., Ye, Q., Jiang, J., Wang, W., 2021. Reducing the environmental risk of chlorpyrifos application through appropriate agricultural management: evidence from carbon-14 tracking. J. Agric. Food Chem. 69, 7324–7333. https://doi.org/10.1021/acs.jafc.1c02753.

John, E.M., Shaike, J.M., 2015. Chlorpyrifos: pollution and remediation. Environ. Chem. Lett. 13, 269–291. https://doi.org/10.1007/s10311-015-0513-7.

Karbalaei, S., Hanachi, P., Rafiee, G., Seifori, P., Walker, T.R., 2021. Toxicity of polystyrene microplastics on juvenile Oncorhynchus mykiss (rainbow trout) after individual and combined exposure with chlorpyrifos. J. Hazard Mater. 403 https://doi.org/10.1016/j.jhazmat.2020.123980.

Kasirajan, S., Ngouajio, M., 2012. Polyethylene and Biodegradable Mulches for Agricultural Applications: a Review, vol. 32. Agronomy for Sustainable Development, pp. 501–529. https://doi.org/10.1007/s13593-011-0068-3.

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. Environ. Sci. Technol. 50, 3315–3326. https://doi.org/10.1021/acs.est.5b06069.

Lee, K.E., Foster, R.C., 1991. Soil fauna and soil structure. Soil Res. 29 https://doi.org/10.1071/sr9910745.

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. 22, 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.

Li, B., Song, W., Cheng, Y., Zhang, K., Tian, H., Du, Z., Wang, J., Wang, J., Zhang, W., Zhu, L., 2021. Ecotoxicological effects of different size ranges of industrial-grade polyethylene and polypropylene microplastics on earthworms Eisenia fetida. Sci. Total Environ. 783, 147007 https://doi.org/10.1016/j.scitotenv.2021.147007.

Li, S., Ding, F., Flury, M., Wang, Z., Xu, L., Li, S., Jones, D.L., Wang, J., 2022. Macro- and microplastic accumulation in soil after 32 years of plastic film mulching. Environ. Pollut. (Amsterdam, Neth.) 300, 118945. https://doi.org/10.1016/j.envpol.2022.118945.

Manish, D., 2017. Preparation, characterization and evaluation of human guarded chlorpyrifos urea Co-inclusion complexes. Indian J. Pharmaceut. Sci. 79 https://doi.org/10.4172/pharmaceutical-sciences.1000205.

Martinez Morcillo, S., Yela, J.L., Capowiez, Y., Mazzia, C., Rault, M., Sanchez-Hernandez, J.C., 2013. Avoidance behaviour response and esterase inhibition in the earthworm, Lumbricus terrestris, after exposure to chlorpyrifos. Ecotoxicology 22, 597–607. https://doi.org/10.1007/s10646-013-1051-3.

Meng, F., Yang, X., Riksen, M., Geissen, V., 2022. Effect of different polymers of microplastics on soil organic carbon and nitrogen - a mesocosm experiment. Environ. Res. 204, 111938 https://doi.org/10.1016/j.envres.2021.111938.

Mierzwa-Hersztek, M., Gondek, K., Kopec, M., 2019. Degradation of polyethylene and biocomponent-derived polymer materials: an overview. J. Polym. Environ. 27, 600–611. https://doi.org/10.1007/s10924-019-01368-4.

Qi, Y., Ossowicki, A., Yang, X., Lwanga, E.H., Dini-Andreote, F., Geissen, V., Garbeva, P., 2019. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J. Hazard Mater. https://doi.org/10.1016/j.jhazmat.2019.121711.

Qi, Y., Yang, X., Pelaez, A.M., Huerta Lwanga, E., Beriot, N., Gertsen, H., Garbeva, P., Geissen, V., 2018. Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Sci. Total Environ. 645, 1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229.

Rillig, M.C., Ziersch, L., Hempel, S., 2017. Microplastic transport in soil by earthworms. Sci. Rep. 7, 1362. https://doi.org/10.1038/s41598-017-01594-7.

Rodriguez-Seijo, A., Lourenco, J., Rocha-Santos, T.A.P., da Costa, J., Duarte, A.C., Vala, H., Pereira, R., 2017. Histopathological and molecular effects of microplastics in Eisenia andrei Bouche. Environ. Pollut. (Amsterdam, Neth.) 220, 495–503. https://doi.org/10.1016/j.envpol.2016.09.092.

Rodríguez-Seijo, A., Santos, B., Ferreira da Silva, E., Cachada, A., Pereira, R., 2019. Low-density polyethylene microplastics as a source and carriers of agrochemicals to soil and earthworms. Environ. Chem. 16 https://doi.org/10.1071/en18162.

Sforzini, S., Oliveri, L., Chinaglia, S., Viarengo, A., 2016. Application of biotests for the determination of soil ecotoxicity after exposure to biodegradable plastics. Front. Environ. Sci. 4 https://doi.org/10.3389/fenvs.2016.00068.

Shi, Z., Wen, M., Ma, Z., 2022. Effects of polyethylene, polyvinyl chloride, and polystyrene microplastics on the vermitoxicity of fluoranthene in soil. Chemosphere 298, 134278. https://doi.org/10.1016/j.chemosphere.2022.134278.

Siegenthaler, K.O., Künkel, A., Skupin, G., Yamamoto, M., 2011. Ecoflex® and Ecovio®: biodegradable, performance-enabling plastics. In: Rieger, B., Künkel, A., Coates, G., Reichardt, R., Dinjus, E., Zevaco, T. (Eds.), Synthetic Biodegradable Polymers, Advances in Polymer Science, vol. 245. https://doi.org/10.1007/12_2010_106.

Sun, W., Meng, Z., Li, R., Zhang, R., Jia, M., Yan, S., Tian, S., Zhou, Z., Zhu, W., 2021. Joint effects of microplastic and dufulin on bioaccumulation, oxidative stress and metabolic profile of the earthworm (Eisenia fetida). Chemosphere 263, 128171. https://doi.org/10.1016/j.chemosphere.2020.128171.

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s.

Touchaleaume, F., Martin-Closas, L., Angellier-Coussy, H., Chevillard, A., Cesar, G., Gontard, N., Gastaldi, E., 2016. Performance and environmental impact of biodegradable polymers as agricultural mulching films. Chemosphere 144, 433–439. https://doi.org/10.1016/j.chemosphere.2015.09.006.

Wang, F., Gao, J., Zhai, W., Liu, D., Zhou, Z., Wang, P., 2020a. The influence of polyethylene microplastics on pesticide residue and degradation in the aquatic environment. J. Hazard Mater. 394, 122517 https://doi.org/10.1016/j.jhazmat.2020.122517.

Wang, J., Coffin, S., Sun, C., Schlenk, D., Gan, J., 2019. Negligible effects of microplastics on animal fitness and HOC bioaccumulation in earthworm Eisenia fetida in soil. Environ. Pollut. (Amsterdam, Neth.) 249, 776–784. https://doi.org/10.1016/j.envpol.2019.03.102.

Wang, J., Li, J., Wang, Q., Sun, Y., 2020b. Microplastics as a vector for HOC bioaccumulation in earthworm Eisenia fetida in soil: importance of chemical diffusion and particle size. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.0c03712.

Wang, L., Peng, Y., Xu, Y., Zhang, J., Liu, C., Tang, X., Lu, Y., Sun, H., 2022. Earthworms' degradable bioplastic diet of polylactic acid: easy to break down and slow to excrete. Environ. Sci. Technol. 56, 5020–5028. https://doi.org/10.1021/acs.est.1c08066.

Wang, T., Wang, L., Chen, Q., Kalogerakis, N., Ji, R., Ma, Y., 2020c. Interactions between microplastics and organic pollutants: effects on toxicity, bioaccumulation, degradation, and transport. Sci. Total Environ. 748, 142427 https://doi.org/10.1016/j.scitotenv.2020.142427.

Yang, X., Bento, C.P.M., Chen, H., Zhang, H., Xue, S., Lwanga, E.H., Zomer, P., Ritsema, C.J., Geissen, V., 2018. Influence of microplastic addition on glyphosate decay and soil microbial activities in Chinese loess soil. Environ. Pollut. (Amsterdam, Neth.) 242, 338–347. https://doi.org/10.1016/j.envpol.2018.07.006.

Yang, X., Lwanga, E.H., Bemani, A., Gertsen, H., Salanki, T., Guo, X., Fu, H., Xue, S., Ritsema, C., Geissen, V., 2019. Biogenic transport of glyphosate in the presence of LDPE microplastics: a mesocosm experiment. Environ. Pollut. (Amsterdam, Neth.) 245, 829–835. https://doi.org/10.1016/j.envpol.2018.11.044.

Yang, X.B., Ying, G.G., Peng, P.A., Wang, L., Zhao, J.L., Zhang, L.J., Yuan, P., He, H.P., 2010. Influence of biochars on plant uptake and dissipation of two pesticides in an agricultural soil. J. Agric. Food Chem. 58, 7915–7921. https://doi.org/10.1021/jf1011352.

Yang, Y., 2019. China's (global) chlorpyrifos market status and forecast in 2018. Pesticide Sci. Adm. 18–24.

Zhang, G.S., Liu, Y.F., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. Sci. Total Environ. 642, 12–20. https://doi.org/10.1016/j.scitotenv.2018.06.004.

Zhang, M., Zhang, Y., Wang, W., Cui, W., Wang, L., Sun, H., Liu, C., 2022. Combined effects of microplastics and other contaminants on earthworms: a critical review. Appl. Soil Ecol. 180 https://doi.org/10.1016/j.apsoil.2022.104626.

Zhou, J., Wen, Y., Cheng, H., Zang, H., Jones, D.L., 2022. Simazine degradation in agroecosystems: will it be affected by the type and amount of microplastic pollution? Land Degrad. Dev. https://doi.org/10.1002/ldr.4243.

Zhou, S.-p., Duan, C.-q., Fu, H., Chen, Y.-h., Wang, X.-h., Yu, Z.-f., 2007. Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods. J. Environ. Sci. 19, 854–858. https://doi.org/10.1016/s1001-0742(07)60142-9.

Zhou, S., Duan, C., Fu, H., Chen, Y., Wang, X., Yu, Z., 2007. Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods. J. Environ. Sci. 19, 854–858. https://doi.org/10.1016/s1001-0742(07)60142-9.

Zhou, S., Duan, C., Michelle, W., Yang, F., Wang, X., 2011. Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. J. Environ. Sci. 23, 676–680. https://doi.org/10.1016/s1001-0742(10)60462-7.