SOIL HEALTH RISKS CAUSED BY INTERACTIONS OF MICROPLASTICS AND PESTICIDES

HUI JU

Propositions

1. The coexistence of microplastics and pesticides in soil ecosystems exacerbates ecological impacts and food safety concerns.
   (this thesis)

2. Aging alters the ecological risks of microplastics.
   (this thesis)

3. The use of biodegradable plastics poses a potential obstacle to achieving net-zero greenhouse gas emissions.

4. Mastering the use of artificial intelligence is going to be an essential part of an individual's learning ability.

5. It is better to start preparing thesis propositions at the beginning of the PhD program.

6. Although deadlines are the primary driving force for productivity, overcoming procrastination ensures that you face less pressure before these deadlines.

7. The highly interdisciplinary nature of environmental science makes it unsuitable as a major for undergraduate students.

Soil health risks caused by interactions of microplastics and pesticides

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This research was conducted under the auspices of the Research School for Socio-Economic and Natural Sciences of the Environment (SENSE)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University,
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 1 May 2024
at 1:30 p.m. in the Omnia Auditorium.

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PhD thesis, Wageningen University, Wageningen, the Netherlands (2024)
With references, with summary in English

DOI: 10.18174/653005

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Chapter 1 General introduction

1.1 Plastics and Pesticides in Agriculture: Boon and Bane

Providing sustenance for an ever-growing population, the agricultural sector is critical to human survival and development. 9.3 billion tonnes of primary crops were produced in 2020, 52% more than in 2000 (FAO, 2022). The world population reached 8.0 billion in 2023 and is expected to increase further to 9.1 billion by 2050 (United Nations Department of Economic and Social Affairs, 2022). To meet the surging demand for food, agriculture has evolved significantly over the last few decades, driven by technological advancements. Two of the most transformative technologies that have been developed and extended are plastics and pesticides.

1.1.1 Agro-plastics

Plastics used in agriculture

The versatility, durability, and cost-effectiveness of plastics have revolutionized agricultural practices, bringing about a new era of enhanced efficiency and convenience (Andrady, 2003). From the vast greenhouses covering hectares of land to the simple drip irrigation tubes snaking through fields, plastic has woven itself into the fabric of modern agriculture (Figure 1.1). Plastic mulch films have become a widely used agricultural tool to enhance crop productivity (Kasirajan and Ngouajio, 2012). The use of plastic mulch films has brought about a significant transformation in contemporary agriculture, offering a multitude of benefits. By covering the soil surface, the films help control weed growth by blocking sunlight and creating physical barriers, thereby reducing competition for nutrients and water between crops and weeds. Moreover, plastic mulch films contribute to soil moisture conservation by minimizing evaporation and reducing the frequency of irrigation. They also enhance soil temperature, particularly in cooler climates, by absorbing and retaining solar heat, promoting early crop growth and extending the growing season. Additionally, plastic mulch films create a physical barrier, preventing direct contact between the crops and the soil, reducing the risk of soil-borne diseases (Kader et al., 2017).

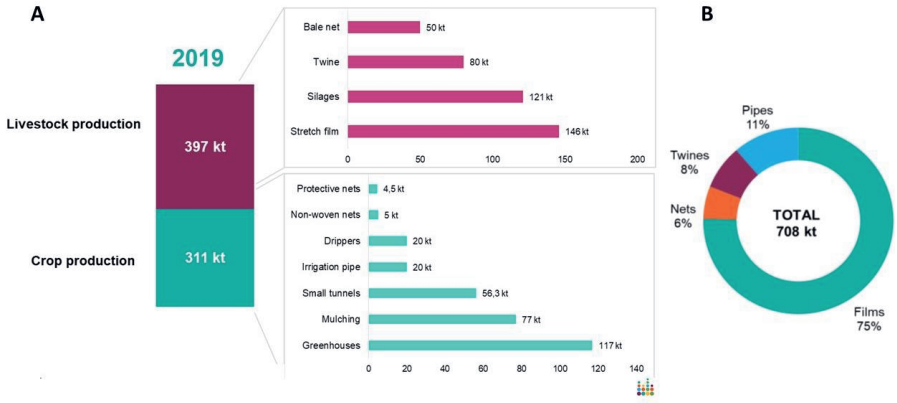

Figure 1.1 *Estimation of the different uses of plastic in agriculture in the EU in 2019 for livestock and crop production (A) and the distribution of different plastic uses (B). Source: EIP-Agri (2021)*

However, the use of nondegradable plastic mulch films has raised environmental concerns due to their persistent nature and potential for accumulation in the environment (Kyrikou and Briassoulis, 2007; Liu et al., 2014). Traditional plastic mulch films are typically made from non-biodegradable materials, such as polyethylene (PE), which can take hundreds of years to degrade, contributing to plastic pollution and ecosystem degradation (Kyrikou and Briassoulis, 2007). The challenge of low recovery rates of plastic film mulch is further compounded due to mechanized cultivation practices and the employment of ultra-thin film technologies, specifically those with thicknesses less than 0.008 mm (Salama and Geyer, 2023). The accumulation of plastic waste in agricultural fields, water bodies, and surrounding ecosystems has prompted the search for more sustainable alternatives. Biodegradable and biobased plastics have emerged as a promising solution to mitigate the environmental impacts of plastic waste in agriculture (Kasirajan and Ngouajio, 2012). Biodegradable or biobased plastics are not a singular substance; rather, they encompass a diverse range of materials with distinct properties and applications. The term 'biobased' indicates that the material or product is either wholly or partially derived from biomass (plants). Biomass utilized for biobased plastic production primarily includes sources such as corn, sugarcane, and cellulose. The process of biodegradation involves the breakdown of materials by microorganisms in the environment, converting the biodegradable plastics into natural substances such as water, carbon dioxide, and biomass. Keep in mind that the term ‘biobased’ does not always mean ‘biodegradable’ (Figure 1.2).

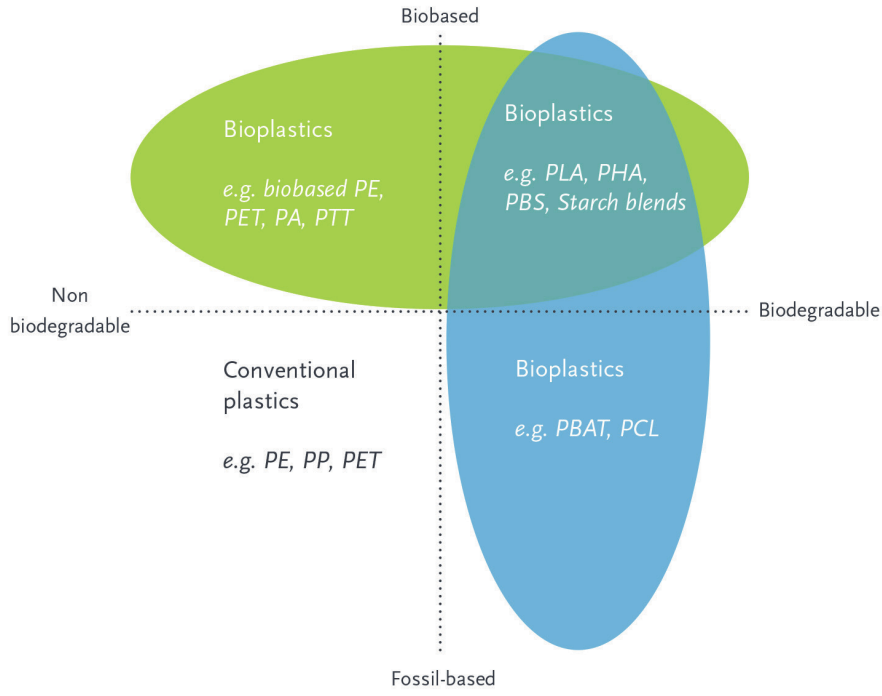

Figure 1.2 *The coordinate system of plastic materials. Source: Fact Sheet of European Bioplastics (2016).*

Biodegradable plastic mulch films are usually made from biopolymers derived from renewable resources, such as polylactic acid (PLA), or from petroleum, like polybutylene adipate terephthalate (PBAT) (Figure 1.3). These materials offer comparable functional properties to traditional plastics, ensuring effective weed control, moisture conservation, and soil warming. Biodegradable plastic mulch films are designed to break down into natural compounds through the action of microorganisms present in the environment. The degradation of biodegradable plastics involves three stages: biodeterioration, biofragmentation, and bioassimilation (Lucas et al., 2008). First, microorganisms inhabit and form a biofilm on or within the plastic, altering its chemical and physical properties. Subsequently, depolymerase produced by microorganisms gradually converts the plastics into oligomers and monomers. Finally, these substances are assimilated by microorganisms for carbon and energy before being transformed into CO_2, H_2O, and biomass (Fan et al., 2022). Unfortunately, the degradation process, especially in soil environments, is not as straightforward as it might seem due to the constraints imposed by limiting environmental conditions (e.g. temperature and moisture), which can lead to the accumulation of biodegradable plastics and unintended environmental consequences in agricultural soil (Han et al., 2021; Liao and

Chen, 2021).

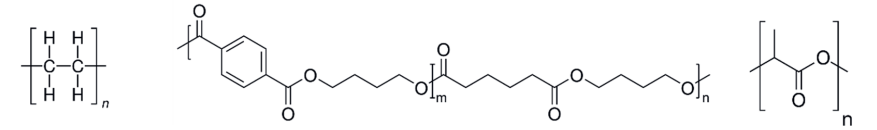

Figure 1.3 *Chemical structure of commonly used plastic polymers in agriculture: fossil-based polyethylene (PE), fossil-based polybutylene adipate terephthalate (PBAT) and biobased polylactic acid (PLA)*

Contamination of plastics in agricultural soils

The fragmentation of larger plastic residues in agriculture has led to a notable increase in the presence of tiny plastic particles. These particles have the capacity to endure in the environment for extended periods of time, exhibiting resistance to natural decomposition processes and accumulating in substantial quantities. Microplastics (MPs), which are defined as plastic particles less than 5 millimetres in diameter, have emerged as a significant environmental concern in recent years, capturing the attention of scientists, policymakers, and the general public alike. MPs can enter agricultural soil in various ways such as the fragmentation of larger plastic particles or the application of sewage sludge, compost, and irrigation (Figure 1.4). A high abundance of MPs has been found in several sites in China, such as the agricultural soils in Xinjiang (40.35 mg kg^{-1}), vegetable farmland soils in Shanghai (78.00 ± 12.91 items kg^{-1}) and Wuhan (320−12560 items kg^{-1}), and agricultural field on the Loess Plateau (<0.54 mg kg^{-1}) (Liu et al., 2018; Zhang et al., 2018; Chen et al., 2020; Li et al., 2020). The majority of the microplastics that originate from mulching films are primarily made up of polyethylene (PE) and the concentration of MPs found in the soil is positively correlated with the application of mulching films (Huang et al., 2020). Sludge is used as a fertilizer on agricultural fields and is another source of MP pollution in soil. In Europe, van den Berg et al. (2020) discovered that agricultural soils in the vicinity of Valencia, Spain exhibited an average light density plastic load of 930 ± 740 MPs kg^{-1} and a heavy density plastic load of 1100 ± 570 MPs kg^{-1} when no sewage sludge was added. Conversely, soils treated with sewage sludge displayed an average light density plastic load of 2130 ± 950 microplastics kg^{-1} and a heavy density plastic load of 3060 ± 1680 MPs kg^{-1}. Moreover, depending on the application dose, compost application can also contribute to MP pollution with an annual plastic input of visible plastics that may reach 0.016 to 1.2 kg ha^{-1} (yearly application rate: 7 t ha^{-1}) up to 0.08 to 6.3 kg ha^{-1} (yearly application rate: 35 t ha^{-1}) (Bläsing and Amelung, 2018). However, the occurrence of biodegradable MPs in various

ecosystems, particularly terrestrial ecosystems, remains unknown. This knowledge gap primarily stems from the lack of viable technical protocols for extracting and identifying biodegradable MPs. For instance, separating biodegradable MPs from natural organic matter during flotation poses a challenge due to their similar densities.

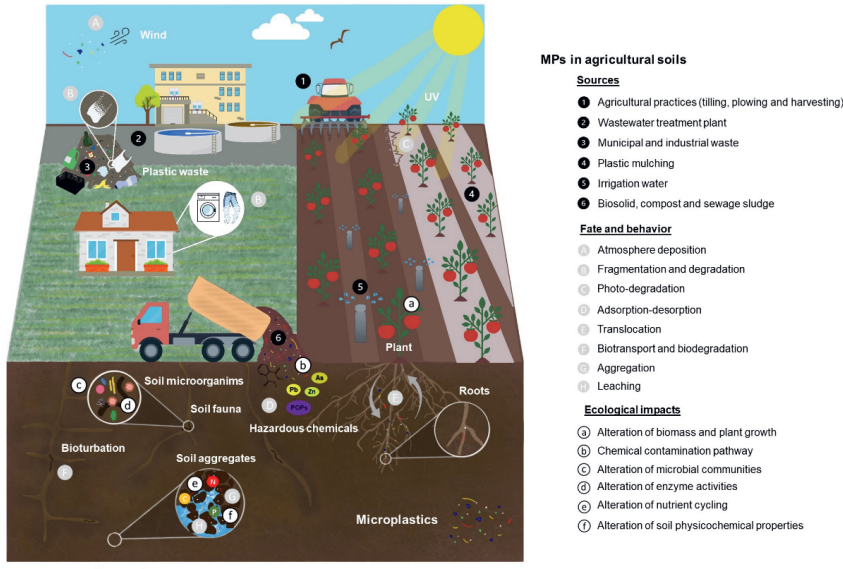

Figure 1.4 *Sources and potential impacts of microplastics in agricultural soil. Source: Pérez-Reverón et al. (2022)*

Risks of plastics for terrestrial ecosystem health

The proliferation of MPs presents multifaceted threats to terrestrial environments. Firstly, their small size and ubiquity enable them to be ingested by soil-dwelling organisms such as earthworms (Huerta Lwanga et al., 2016). Once ingested, MPs can adversely affect the growth, behaviour, oxidative response, gene expression, and gut microbiota of earthworms, with potential repercussions for soil fertility and ecosystem dynamics (Cui et al., 2022). MPs have the potential to induce alterations in soil physical properties, such as soil aggregation, bulk density, and water holding capacity. They can also influence soil chemical characteristics including pH, ion exchange capacity, and nutrient content. Furthermore, MPs may impact microbiological aspects like microbial community composition and enzyme activity (Figure 1.4), albeit with variations dependent on factors such as polymer type, shape, dose, and size (Wang et al., 2022). These changes in soil properties can subsequently exert influences on plant growth and crop productivity (Zhou et al., 2021). These impacts may also vary among plant species, likely resulting in different responses to plant

community composition and potential primary production (Rillig et al., 2019). Moreover, research indicates that certain plants can uptake submicron-plastics in their roots and transport them upward into the shoot through transpiration (Li et al., 2020). This raises concerns regarding the potential transfer of microplastics into the food chain, although the extent and health implications of this phenomenon are areas of ongoing study.

In summary, the use of plastics in agriculture, such as in greenhouses and mulch films, has brought significant benefits by enhancing crop productivity, controlling weeds, conserving soil moisture, and improving soil temperature. This has resulted in higher efficiency and extended growing seasons. However, these advantages are offset by serious environmental concerns. Nondegradable plastics can accumulate in the environment, leading to soil pollution and ecosystem degradation. The challenge is compounded by the prevalence of MPs, which result from the breakdown of larger plastic residues and pose risks to soil health, plant growth, and potentially infiltrate the food chain. While biobased and biodegradable plastics offer a more sustainable alternative, their degradation in soil is complex and can also lead to environmental issues. Thus, while plastics have revolutionized agricultural productivity, their environmental impact highlights the urgent need for more sustainable practices and materials in agriculture.

1.1.2 Agro-pesticides

Pesticides used in agriculture

Pesticides also play a crucial role in modern agriculture, safeguarding crops from destructive pests and diseases, and ensuring food security. Annual crop production suffers a loss of approximately 35% due to pest infestation (Oerke, 2005). Hence, it is imperative to implement effective pest management strategies involving a wide range of pesticides to combat pests and enhance crop yield (Abhilash and Singh, 2009). Pesticides, in the broadest sense, are substances or mixtures of substances intended for preventing, destroying, repelling, or mitigating any pest (FAO, 2002). Their role in safeguarding crops from various biotic stressors is essential in modern agriculture. The term "pesticide" is a comprehensive term that encompasses a variety of products, including insecticides, fungicides, herbicides, rodenticides, nematicides, etc. The worldwide production of pesticides has been steadily rising, with a few countries, notably China, the United States, and certain European nations, emerging as dominant producers (Figure 1.5). This increase in production is driven by several factors, including the expansion of arable land, intensification of farming practices, and the persistent challenge of pesticide resistance. Total pesticide trade reached approximately 7.2 million tonnes of formulated products in 2020, with a value of more than USD 40 billion (FAO,

2022). Global pesticide use reached 2.67 million tons in 2020, led by the United States (with 408 kt of agricultural pesticides), Brazil (377 kt) and China (273 kt) (Figure 1.6) (FAO, 2022).

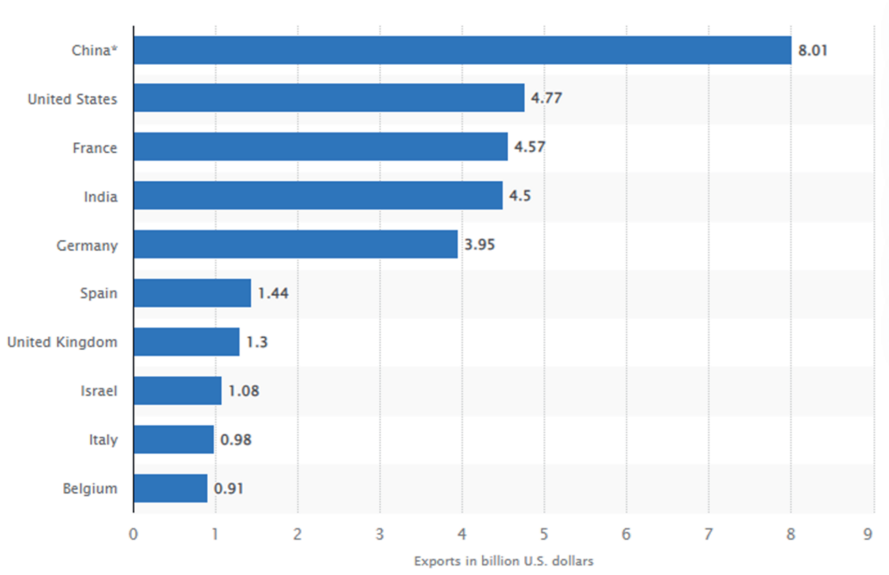

Figure 1.5 *Leading pesticide exporting countries worldwide in 2021, based on value (in billion U.S. dollars). Source: Food and Agriculture Organization of the United Nations, 2021*

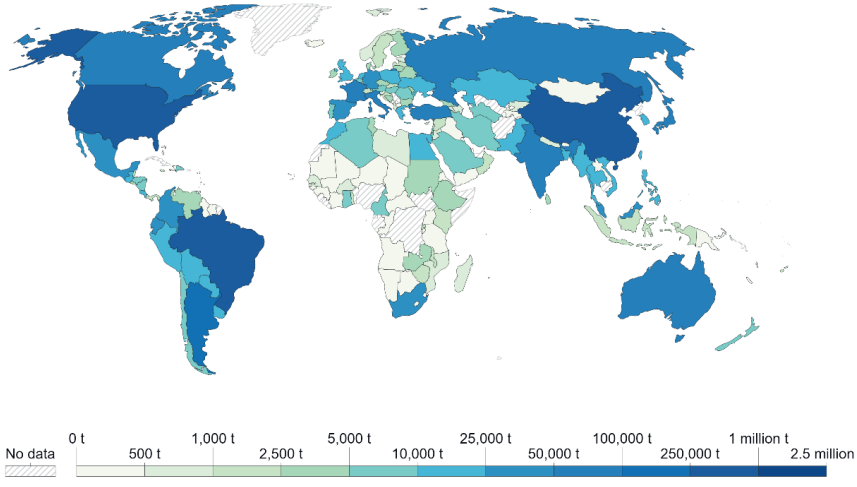

Figure 1.6 *Pesticide use in the world. Source: Food and Agriculture Organization of the United Nations, 2020*

Contamination of pesticides in agricultural soils

However, the widespread use of pesticides can lead to pesticide contamination in agricultural soil. Tang et al. (2021) found that 74.8% of the global agricultural land (approximately 28.8 million km^2) is at some risk of pesticide pollution (risk score (RS) > 0, Figure 1.7); remarkably, 31.4% (approximately 12.1 million km^2) falls within the high-risk class (RS > 3). Regional analysis showed that 61.7% (2.3 million km^2) of European agricultural land is at a high risk of pesticide pollution. Asia has the largest land area at high risk (4.9 million km^2), with 2.9 million km^2 in China and 0.35 million km^2 in Kazakhstan. An increasing amount of evidence has suggested that agricultural soils contain more than one pesticide (Silva et al., 2019; Tang et al., 2021). Globally, 63.7% of the agricultural land is at risk of pollution by more than one active ingredient (AI) and 20.9% by more than ten AIs (Figure 1.8). At the regional level, 93.7%, 73.4% and 69.4% of the agricultural land in Europe, North America and South America, respectively, is contaminated by more than one AI. China is at risk of pollution by the greatest number of AIs, with 8.4% of the agricultural land (0.34 million km^2) contaminated by more than 20 AIs.

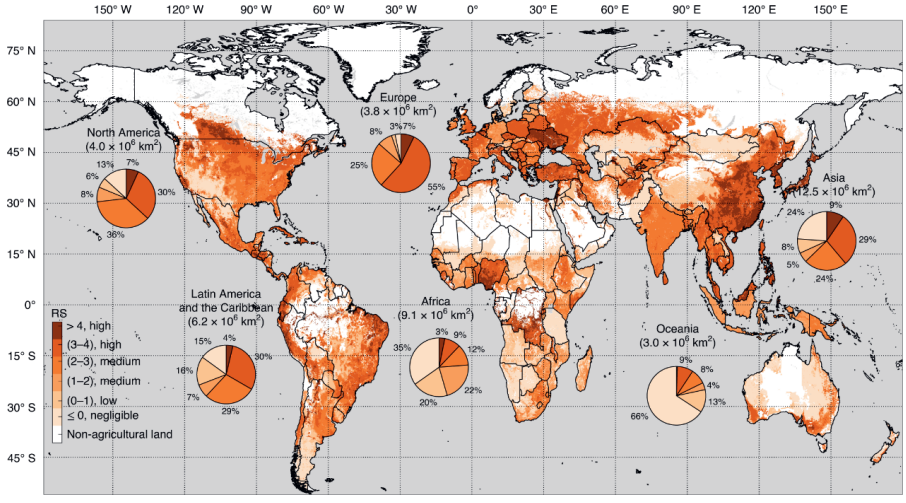

Figure 1.7 *Global map of pesticide risk score (RS). Source: Tang et al. (2021)*

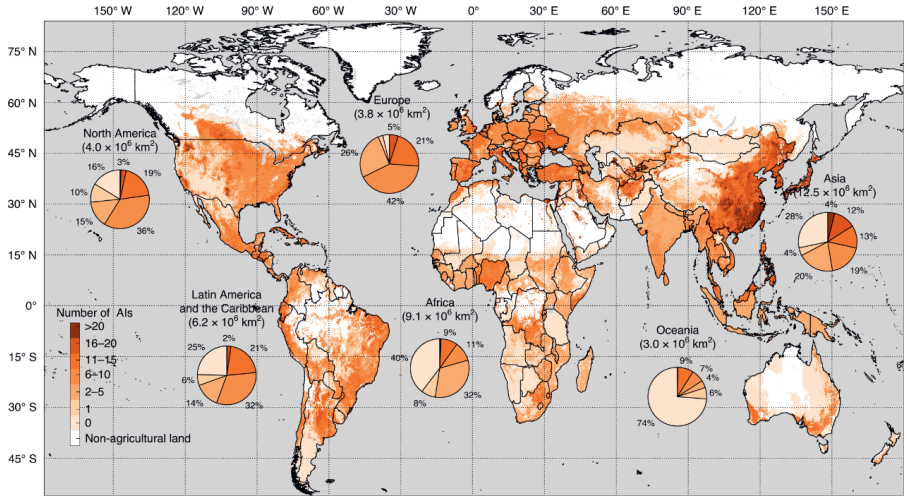

Figure 1.8 *Global map of the number of active ingredients (AIs) posing risks to agricultural soils. Tang et al. (2021)*

In the agricultural soil, pesticides with diverse physicochemical properties can undergo various physical, chemical, and biological transformations, encompassing processes like degradation, adsorption, leaching, and volatilization (Gavrilescu, 2005), ultimately affecting the persistence of pesticides in the soil. Hydrophobic pesticides with high n-octanol/water partition coefficient (K_{ow}) values often exhibit robust sorption to soil organic matter, which may further decrease the bioavailability and biodegradation of pesticides in the soil (Aktar et al., 2009).

Risks of pesticides for terrestrial ecosystem health

Unfortunately, the persistence of pesticides in soil has been reported to have adverse effects in the soil. Firstly, pesticides can pose threats to beneficial microorganisms and associated biochemical reactions in the soil (Hussain et al., 2009). Microorganisms play a pivotal role in nutrient cycling, organic matter decomposition, and overall soil fertility. However, certain pesticides can suppress beneficial microbial activities, such as nitrogen-fixing and phosphorus-solubilizing (Shahid and Khan, 2022). Additionally, it has been reported that pesticides can dimmish the activities of soil enzymes which serve as crucial indicators of soil health (Shahid and Khan, 2022). Beyond the microbial scale, soil pesticide contamination can negatively impact larger soil-dwelling organisms, such as earthworms and beneficial insects, leading to reduced biodiversity in agricultural soils (Beaumelle et al., 2023). Pesticides that persist in the environment can accumulate in the tissues of living organisms over time. This is known as bioaccumulation, and it can lead to increasingly higher concentrations of pesticides in organisms higher up the

food chain, posing risks to top predators (Chormare and Kumar, 2022). Pesticide residues can also be taken up by plants growing in contaminated soil, potentially leading to food products with elevated pesticide levels.

Risks of pesticides for human health

The potential human health risks range from acute toxic effects, such as skin irritation, respiratory distress, and neurotoxicity, to chronic impacts like endocrine disruption, reproductive harm, and carcinogenicity (Xu et al., 2022). The route of exposure—whether inhalation, dermal contact, or ingestion—can influence the severity and type of health outcomes (Damalas and Koutroubas, 2016). Vulnerable populations, including children, pregnant women, and farmworkers, may face heightened risks due to physiological sensitivities or elevated exposure levels. Additionally, the combined exposure to multiple pesticides can result in additive or synergistic effects, further complicating risk assessments (Shahid et al., 2019). Efforts to mitigate soil contamination and environmental persistence of pesticides include improving application techniques to minimize drift, developing and adopting less persistent but environmentally friendly pesticides, and making policies to limit the use of highly persistent and risk pesticides. Additionally, sustainable agricultural practices, such as organic farming and integrated pest management (IPM), prioritize the reduction of pesticide use and promote soil health and biodiversity (Cook et al., 2007).

In summary, pesticides play a critical role in safeguarding crops and securing food production in modern agriculture. However, the persistence of these chemicals in the environment leads to widespread contamination of agricultural land, adversely affecting soil microbiomes and biochemical reactions. The persistence of pesticides can also pose ecotoxicological risks to soil-dwelling organisms and lead to bioaccumulation in crops, thereby increasing health risks in humans. To mitigate these adverse impacts, it is essential to adopt sustainable agricultural practices that focus on minimizing the use of pesticides and promoting the overall health of the soil ecosystem.

1.2 Co-existence of microplastics and pesticides in terrestrial environments

Combined toxicity of microplastics with pesticides

The increasing prevalence of MPs and pesticides in terrestrial ecosystems has become a major environmental concern. Research findings have unveiled that the presence of MPs and pesticides can lead to multifaceted impacts (Figure 1.9). However, the combined toxicity of MPs and pesticides on terrestrial organisms has been studied to a lesser extent than organisms in

aquatic ecosystems. Earthworms, as an important model organism, have been relatively more employed in research investigating the combined effects of MPs and pesticides. The combined effects of MPs and pesticides on earthworms can be either synergistic or antagonistic depending on the types and doses of pollutants. Synergistic effects were observed in most studies. For example, the joint application of the insecticide monocrotophos and PVC and PP MPs increased the oxidative stress on the earthworm *Eudrillus eugeniae* in soil microcosms by increasing lipid peroxidation level and enzyme activities and reducing in protein levels (Mishra et al., 2022). The presence of MPs and dufulin worsened the metabolic profiles of the earthworm *Eisenia fetida* compared to the application of dufulin alone (Sun et al., 2021). In addition, the joint application of environmental MPs (composed of PE, PP, PET, polyethylene vinyl acetate (PEVA) and PA) in different proportions and the herbicide 2,4-D resulted in higher toxicity for the earthworm *Eisenia andrei* by oxidative alterations such as increased glutathione S-transferase and catalase levels, as well as accumulation of malondialdehydes (Boughattas et al., 2022). The antagonistic effects of MPs and pesticides were also observed, which were generally caused by decreased bioavailability of one pollutant caused by the presence of the other (Holzinger et al., 2022; Ju et al., 2022; Peña et al., 2023). Due to inconsistencies in research findings regarding the impacts of MP-pesticide combinations, it is necessary to conduct specific toxicological studies focused on particular mixtures of these pollutants.

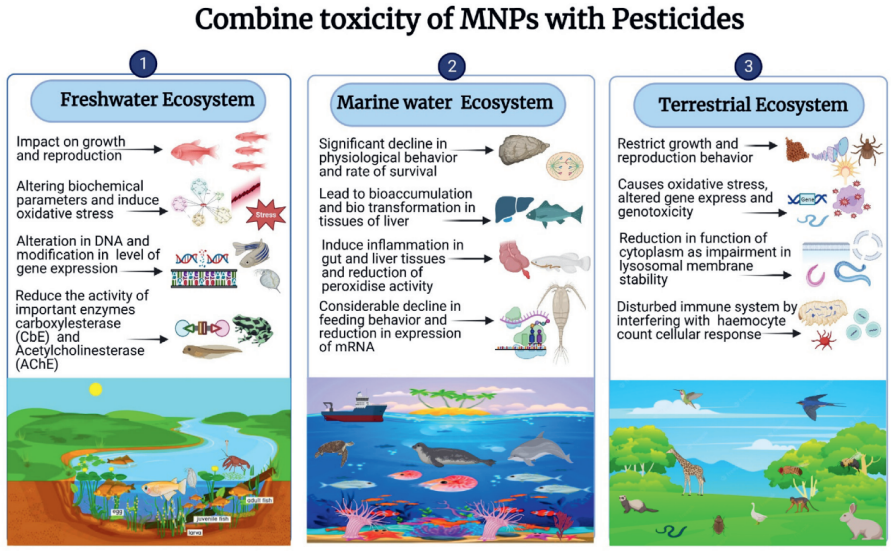

Figure 1.9 *Combined toxicological impacts of MNPs and pesticides in the freshwater, marine water and terrestrial ecosystem. Source: Junaid et al. (2023).*

Interactions of microplastics with pesticides

MPs can adsorb and act as a carrier for other hydrophobic organic contaminants (HOCs) due to their large surface area and hydrophobicity (Jiménez-Skrzypek et al., 2021). The research on the adsorption of MPs to HOCs has advanced more in aquatic environments and adsorption experiments conducted in solutions (Kinigopoulou et al., 2022). The adsorption of HOCs onto MPs is influenced by various factors, including MP properties (particle size, surface area, shape, dose), characteristics of HOCs (molecular weight, hydrophobicity), and environmental factors (temperature, pH, ionic strength) (Prajapati et al., 2022). The adsorption behaviours of pesticides on MPs have been investigated in recent studies, utilizing kinetic and isotherm data to assess the underlying mechanisms (Fu et al., 2021). Generally, the adsorption kinetics of pesticides onto MPs conform to the pseudo-first-order, pseudo-second-order, and intraparticle diffusion models, indicating that the predominant process involves surface adsorption followed by pore filling through diffusion (Xia et al., 2023). The adsorption of pesticides onto MP surfaces is generally rapid, followed by subsequent pore diffusion at varying rates depending on the types (physical and chemical properties, i.e.) of pesticide and MP. Some studies have reported that PE exhibits a higher ability for pesticide adsorption than other nondegradable polymers (Allen et al., 2018; Gomes de Aragão Belé et al., 2021). A proposed explanation is that PE exhibits a rubbery state at room temperature, characterized by an expanded and flexible polymer structure with increased free volume, thereby enhancing the accessibility for organic contaminants. In contrast, other nondegradable MPs studied, such as PS or polyvinyl chloride (PVC), exist in a glassy state with more condensed and cross-linked chains that restrict pesticide diffusion (Pascall et al., 2004; Gong et al., 2019). Due to the prevalence of PE MPs in agricultural soil, they have the potential to act as carriers for pesticides and significantly influence their fate in soils (Tian et al., 2022). In contrast to nondegradable MPs, biodegradable MPs usually have a higher specific surface area, a higher number of pores, and/or different chemical properties (functional groups i.e.) that favour the formation of bonds with pesticides (Gong et al., 2019). Hence, they have shown higher adsorption capacity for different pesticides such as triadimefon, difenoconazole, or fipronil (Gong et al., 2019; Jiang et al., 2020). Another point worth noting is that the particle size and surface properties of biodegradable MPs may change noticeably in a short period of time, which can make their interactions with other contaminants unpredictable.

As previously mentioned, hydrophobic pesticides have the capability to adsorb onto soil organic matter, thereby impacting their bioavailability and

biodegradation. MPs, being exogenous organic matter, also possess the capacity to adsorb pesticides in the soil. Guo et al. (2019) revealed that the concentrations of pesticides in plastic residues (86.4–22213.2 ng g^{-1}) were 20 times higher than those found in soil (9.3–535.3 ng g^{-1}). As a result, the adsorption of pesticides on MPs can affect the environmental fate of pesticides such as their degradation due to reduced bioavailability (Sunta et al., 2020; Wu et al., 2022). Hu et al. (2021) reported that the presence of PP MPs resulted in increased half-lives of imazamox, imazapic, and imazethapyr in sediment. In a separate study conducted in soil, the presence of both PE and PVC in sandy clay loam soil resulted in slower dissipation of the herbicide simazine, leading to higher residues in soil, particularly at high MP doses (20%) that are not environmentally significant (Zhou et al., 2022). The presence of MPs can also inhibit pesticide degradation by changing the soil microbiome. Zhou et al. (2022) found that the addition of PE and PVC MPs caused a shift in microbial composition towards fungi (increased ratio of fungi-to-bacteria), which could potentially impede pesticide degradation primarily driven by bacteria. Studies have also shown that MPs can affect the bioaccumulation of environmental pollutants, including pesticides, by soil biota such as earthworms and plants (Tourinho et al., 2023). In earthworms, some studies have observed an increase in the bioaccumulation of pesticides in the presence of MPs (Sun et al., 2021). This increase has been attributed to the interaction between MPs and pesticides, leading to altered bioavailability and uptake of pesticides by earthworms. In plants, the influence of MPs on the bioaccumulation of pesticides has been less studied compared to other contaminants. However, it is important to note that the presence of MPs has been associated with a decrease in the bioaccumulation of contaminants in plants in general (Tourinho et al., 2023). Further research is required to gain a comprehensive understanding of the specific impacts of MPs on the bioaccumulation of pesticides in plants, particularly in relation to concerns regarding food safety.

Knowledge gaps

In soil ecosystems, even though numerous environmental risk assessments have been conducted for MPs and pesticides individually, there are still significant research gaps in understanding the interaction between MPs and pesticides, the specific mechanisms, and ecological consequences of these interactions. In addition, as previously mentioned, pesticide contamination typically arises from more than one pesticide, which may render existing studies less applicable in predicting realistic contamination scenarios involving different pesticides. For instance, when two pesticides coexist, competition for adsorption sites on MPs may occur. The adsorption of one pesticide onto MPs could potentially diminish the availability of adsorption sites for the other

pesticide. Furthermore, mounting evidence suggests that aged MPs exhibit altered adsorption capacities (often enhanced) compared to pristine MPs due to changes in their physicochemical properties. The majority of studies, however, have utilized pristine MPs as surrogates in investigating the interactions between MPs and other contaminants, thereby compromising the predictability of results with respect to actual contaminant scenarios. Building on this understanding, enhancing our fundamental knowledge of the mechanisms involved in the interactions between contaminants and MPs becomes crucial. This advancement is not only essential for developing more accurate models to evaluate how MPs function as vectors for contaminants but also pivotal for informing effective environmental policy and management strategies. It enables us to better assess the risks and impacts of MPs on ecosystems and human health, thereby guiding targeted mitigation and remediation efforts.

1.3 Thesis outline

1.3.1 Research objectives

This PhD study aims to enhance our understanding of the environmental behaviours and potential risks of MP-pesticide combinations in soil. The MPs utilized throughout the study consisted of LDPE (fossil-based and nondegradable) and PBAT (fossil-based and degradable) blended with PLA (biobased and degradable) (Bio). The MPs were prepared from additive-free raw material pellets which are used to produce plastic mulch films. The LDPE and PBAT/PLA plastic mulch films are widely used in agricultural application (Espí et al., 2006; Serrano-Ruiz et al., 2021). The insecticide chlorpyrifos (CPF) was applied individually or in combination with the fungicide difenoconazole (DIF). These two pesticides were chosen as representatives of a category of hydrophobic pesticides, which theoretically can be strongly adsorbed onto MPs. The two pesticides were widely used and have resulted in adverse effects of pesticides on the agricultural ecosystem (Jiangsheng et al., 2019; Sabzevari and Hofman, 2022). The research objectives are as follows:

1. Investigate the effects of MP ageing on CPF adsorption-desorption on MPs and MP bioconcentration in the casts of the earthworm *Lumbricus terrestris*;
2. Study the combined toxicity of LDPE-MPs and Bio-MPs with CPF on the earthworm *Lumbricus terrestris* and their biogenic transport in soil;
3. Examine the dissipation of a pesticide mixture containing CPF and DIF in soil contaminated with aged LDPE-MPs and Bio-MPs;
4. Explore the effects of aged MPs on the growth and the bioaccumulation of pesticide mixtures containing CPF and DIF of radish *Raphanus sativus*.

1.3.2 Outline

The PhD thesis consists of 4 research chapters (2, 3, 4, 5). The research chapters are introduced and discussed in chapter 1 and 6, respectively. The outline of this PhD thesis is summarized in Figure 1.10.

Chapter 1 provides an overview of the research context and identifies gaps in knowledge regarding MPs and pesticides within soil ecosystems.

Chapter 2 investigates the effects of microplastic ageing on chlorpyrifos adsorption-desorption and microplastic bioconcentration in the casts of the earthworm *Lumbricus terrestris*. To study the adsorption mechanisms, batch adsorption-desorption kinetic and isotherm experiments were conducted using ultraviolet light (UV) aged LDPE-MPs and Bio-MPs, along with chlorpyrifos. The experimental data was fitted with adsorption dynamic and isotherm models to elucidate the distinct adsorption mechanisms between pristine and aged MPs. Additionally, 4-day petri dish experiments were performed with earthworms using LDPE-MPs aged under both UV light and soil conditions, as well as Bio-MPs aged under UV light. The quantities of MPs in earthworm casts were measured to investigate the influence of MP ageing on MP bioconcentration via earthworms.

Chapter 3 studies the combined toxicity of LDPE-MPs and Bio-MPs with chlorpyrifos on the earthworm *Lumbricus terrestris* and their biogenic transport in soil. The mesocosm experiments were conducted for a duration of 60 days, utilizing LDPE-MPs and Bio-MPs (0%, 7%, and 28% w w^{-1} in litter serving as the food source for earthworms) along with chlorpyrifos (0 g ha^{-1}, 250 g ha^{-1}, 1250 g ha^{-1}). Earthworm weight change, mortality, and reproduction were recorded to assess the combined toxicity of MP-chlorpyrifos on earthworms. Measurements of MPs and chlorpyrifos in earthworm burrows were taken to calculate the rate at which they were transported into the soil. Furthermore, the chlorpyrifos and its metabolites in earthworm burrows and bodies were analysed to study the effects of both MPs on the degradation of chlorpyrifos.

Chapter 4 explores dissipation of a pesticide mixture containing chlorpyrifos and difenoconazole in soil contaminated with aged MPs. A 35-day pot experiment was conducted using aged LDPE-MPs and Bio-MPs (0.2% w w^{-1} in soil) along with chlorpyrifos and difenoconazole pesticides (15 mg kg^{-1} in soil). The MPs were aged in the soil for one month prior to the experiment. The pesticides, chlorpyrifos and difenoconazole, were applied both individually and in combination. Soil samples were collected on days 0, 1, 3, 7,

and 14 to measure pesticide residues and study the dynamic dissipation and half-life of these pesticides in the soil.

Chapter 5 further investigates the effects of aged LDPE-MPs and Bio-MPs on radish growth and bioaccumulation of a pesticide mixture containing chlorpyrifos and difenoconazole. The LDPE-MPs and Bio-MPs were aged in soil for 1 month prior to the cultivation of the radishes. The concentrations of MPs and pesticides in the soil were consistent with those applied in Chapter 4. At harvest, the biomass of the radish roots and leaves were measured to assess the effects of aged MPs on the growth of radishes. The contents of pesticides in radish leaves and roots were measured to study the effects of aged MPs on the bioaccumulation of pesticides.

Chapter 6 summarizes and discusses the main findings and implications of this thesis, which also provides the recommendations for further research.

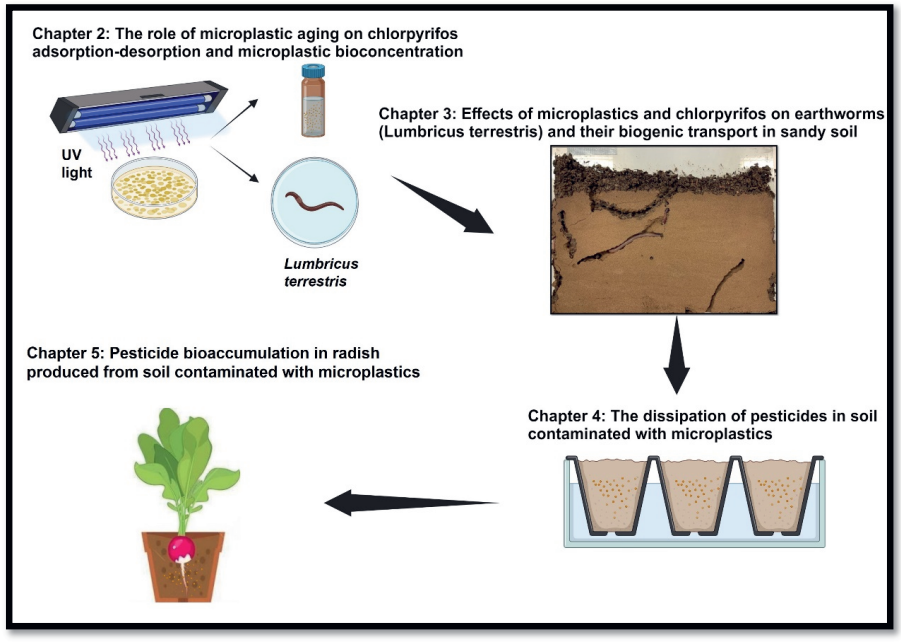

Figure 1.10 *Outline of PhD thesis*

Chapter 2 The role of microplastic aging on chlorpyrifos adsorption-desorption and microplastic bioconcentration

Based on:
Ju, H., Yang, X., Osman, R., Geissen, V., 2023. The role of microplastic aging on chlorpyrifos adsorption-desorption and microplastic bioconcentration. Environ Pollut 331, 121910.10.1016/j.envpol.2023.121910

Abstract

Microplastics (MPs) in soil undergo different aging processes such as photoaging, mechanical abrasion and biodegradation, leading to alterations in the surface properties of MPs. In this study, we investigated the adsorption-desorption of chlorpyrifos (CPF) on pristine and UV light-aged low-density polyethylene (LDPE) and biodegradable (Bio) MPs that were derived from plastic mulch films. We also tested the bioconcentration of pristine and aged MPs (LDPE-MPs and Bio-MPs aged under UV light and LDPE-MPs aged in three different soils) associated with CPF by earthworms (*Lumbricus terrestris*). The results showed that UV-aged MPs showed higher CPF adsorption capacities than pristine MPs, with the adsorption capacities at 184.9±5.3, 200.5±1.8, 193.0±8.7, and 215.9±1.1 μg g^{-1} for pristine LDPE-, UV-aged LDPE-, pristine Bio- and UV-aged Bio-MPs, respectively. The desorption rate of CPF from UV-aged LDPE-MPs within 48 h was lower than the desorption from pristine ones (28.8 ± 7.7% vs. 40.0 ± 3.9%), while both pristine and UV-aged Bio-MPs showed very low CPF desorption rates. A 4-day petri dish experiment showed that UV-aged MPs were significantly less concentrated in earthworm casts than pristine counterparts (52% and 36% lower for UV-aged LDPE-MPs and Bio-MPs), while UV-aged MPs with adsorbed CPF were concentrated significantly more than UV-aged MPs without CPF. Interestingly, LDPE-MPs aged in soil with a high carbon, nitrogen, and carbon-to-nitrogen ratio were significantly more concentrated in earthworm casts than pristine LDPE-MPs. In conclusion, UV-aged MPs acted as stronger vectors for CPF than pristine MPs. The bioconcentration of MPs differed significantly due to microplastic aging, as well as the combined effect with CPF. Moreover, LDPE-MPs aged in soil with enriched carbon and nitrogen were significantly concentrated in earthworm casts. Further studies on the environmental behaviours of aged MPs associated with other pollutants in soil, especially soils high in carbon and nitrogen, are needed.

Keywords: Aging, Biodegradable microplastics, Pesticide, Adsorption/desorption, Earthworm ingestion

2.1 Introduction

Global demand for plastic mulch films increased from 4.4 to 7.4 million tons between 2012 and 2019 (Sintim and Flury, 2017). Low density polyethylene (LDPE) plastic mulch, representative of nondegradable plastic mulch films, has been widely used due to its high puncture resistance, water impermeability, and mechanical stretch properties (Kasirajan and Ngouajio, 2012; Serrano-Ruiz et al., 2021). Unfortunately, these plastic films are rarely if ever recycled or disposed of properly (Sintim and Flury, 2017) resulting in the plastic residues remaining in the soil. As the plastic residues age, they breakdown into microplastics (MPs) which can have adverse effects on the soil ecosystem (Guo et al., 2020). To mitigate the adverse impacts of LDPE plastic mulch, biodegradable plastic mulch (BPM) was developed as a substitute. The BPM was designed to be tilled into the soil after use where it would be degraded by soil microorganisms and eventually broken down into biomass, CO_2, and water (Zumstein et al., 2018). Polybutylene adipate-co-terephthalate (PBAT) and polylactic acid (PLA) are the two polymers most commonly used in the manufacture of BPMs, and often blended together to improve the performance of mulch films (Han et al., 2020). However, some studies have shown that the biodegradation of BPMs in soil does not occur as expected (Weng et al., 2013; Han et al., 2021). Han et al. (2021) found that around 0.9-16% of PBAT was degraded after a 120-day microcosm experiment in 4 different types of soil (lou soil, fluvo-aquic soil, black soil, and red soil). Although PLA has been shown to degrade under compost conditions, it hardly degrades in soil because of the limitations of temperature, moisture, and microbial activity (Radu et al., 2021). In soil, microplastic debris can adsorb hydrophobic organic contaminants (HOCs) like pesticides due to the hydrophobicity and large specific surface areas of MPs (Guo et al., 2020).

Chlorpyrifos (CPF) is an organophosphorus insecticide used broadly for agricultural and residential pest control applications (Lewis et al., 2016). However, studies have proposed that CPF threatens human and animal health. As a result of these findings, more restrictions and bans on the use of CPF have been implemented in the European Union and the United States over the last few years (Jia et al., 2021). Unfortunately, CPF use is still prevalent in developing countries like India and China (Foong et al., 2020). The annual usage of CPF in China has reached more than 2000 tons $year^{-1}$ since 2014 (Su et al., 2016). Studies have found that CPF can cause adverse effects on soil animals like earthworms both alone and when combined with MPs (Ju et al., 2022). Knowledge concerning the adsorption and desorption behaviours of CPF by MPs, especially biodegradable/biobased MPs, is still lacking. Beriot et al. (2020) found that the sorption rates of CPF by PE and

biodegradable mulch were higher than 80% after 15 days, however, the sorption mechanisms of CPF by LDPE and biodegradable MPs remain unclear.

MPs in soil undergo various aging processes including photoaging, mechanical abrasion, and biodegradation (Zha et al., 2022). As a result, aged MPs have different surface morphology, hydrophobicity, and functional groups compared to pristine MPs (Zha et al., 2022). This can impact their behaviours in soil such as their capacities to adsorb and desorb other soil pollutants and their ingestion by organisms (Vroom et al., 2017; Huffer et al., 2018; Lan et al., 2021). Lan et al. (2021) found that the aged agricultural PE MPs had higher adsorption capacities for four pesticides (carbendazim, diflubenzuron, malathion, and difenoconazole) than pristine PE MPs. Compared to PE MPs, biodegradable PBAT and PLA MPs can adsorb more organic pollutants than PE because of various functional groups (carbonyl, alkoxy, aromatic ring, etc.), which can form chemical bonds like hydrogen bonds with adsorbates. In addition, the rubbery domains of these biodegradable MPs are flexible and highly accessible to hydrophobic organic contaminants (HOCs) (Gong et al., 2019; Zuo et al., 2019; Beriot et al., 2020). However, studies related to the adsorption of pesticides on aged biodegradable MPs are still missing. Furthermore, the ingestion of aged MPs by organisms may also differ from pristine MPs. Vroom et al. (2017) observed that the aging of polystyrene MPs in seawater increased the ingestion of MPs by zooplankton due to the formation of biofilms which increased the attractiveness of the polystyrene MPs while polyamide MPs aged with light irradiation and hydrogen peroxide were less ingested by zebrafish compared to pristine MPs (Zou et al., 2020). Although previous studies have reported that earthworms could ingest and transport MPs in soil (Huerta Lwanga et al., 2017; Rillig et al., 2017; Ju et al., 2022), how aged MPs and those with adsorbed pesticides affect the ingestion behaviour of earthworms is still not well-studied.

MP aging in soil may influence the adsorption/desorption of pesticides and affect the ingestion of MPs by soil organisms. Therefore, we studied the effects of UV aging on the adsorption-desorption of CPF by MPs derived from two plastic mulch polymers (LDPE and PBAT/PLA (Bio)). Furthermore, we investigated how aging (under UV light and in soils) and MP-adsorbed CPF influenced the ingestion of MPs by earthworms. The findings and discussion may provide knowledge concerning environmental behaviours of aged MPs interacting with an insecticide and especially aged biodegradable MPs which are poorly understood.

2.2 Materials and methods

2.2.1 Microplastic preparation

Biodegradable plastic pellets consisted of 85% poly (butylene adipate co-terephthalate) (PBAT) blended with 10% polylactic acid (PLA) and 5% calcium carbonate. Low-density polyethylene (LDPE) and biodegradable (Bio) plastic pellets used for blowing mulch films were frozen using liquid nitrogen and then ground and sieved through a 106 μm mesh screen to produce microplastics.

In the UV aging treatment, LDPE and Bio MPs were placed under UVA light (6W, wavelength at 365 nm) at 20 ℃ for 6 months. The UV-aged MPs were sieved through a 106 μm mesh screen before use. The aging treatments for LDPE MPs in 3 different types of soil were conducted in 500 mL glass beakers using a method explained in ASTM D 5988-03 (ASTM, 2003). The textures of the 3 types of soil were loamy sand, silty clay loam, and silt loam which will be hereafter referred to as S1, S2 and S3 , respectively. The properties of each type of soil are shown in Table S2.1. In each beaker, 250 g of soil was mixed with 10 g of cow manure to adjust the carbon-to-nitrogen (C:N) ratio, and soil moisture was kept at 60% of the water holding capacity. To activate microorganisms, the beakers were incubated at 20 ℃ in the dark for 14 days under aerobic conditions and water was replenished every two days. After incubation, 400 mg of LDPE MPs were added to the beakers and thoroughly mixed with the soil. The beakers were then incubated for 4 months under the same conditions as mentioned above and distilled water was replenished weekly to maintain soil moisture. To recover LDPE MPs, the soil was transferred to a bottle and 500 mL of distilled water was added. The bottle was then agitated in a rotary shaker for 1 hour. Afterwards, the bottle was centrifuged at 3500 rpm, and the supernatant was filtered through a 5 μm polycarbonate filter. Large pieces of impurities were removed by hand, small pieces of impurities were removed by sieving through a 106 μm mesh screen. The size distribution of microplastics was characterized using the Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system (Figure S2.1). The changes in the surface functional groups were characterized using Shimadzu Prestige-21 attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic imaging. In order to distinguish whether these functional groups were derived from the LDPE MPs, we cleaned the MPs with 2% sodium dodecyl sulphate (SDS), 70% ethanol, and used an ultrasonic bath to remove any attached organic matter and microorganisms following a widely used protocol (Peixoto et al., 2017). The FTIR spectra showed that these functional groups were from aged LDPE MPs (Figure S2).

2.2.2 Adsorption-desorption experiment

The CPF adsorption kinetic experiment was conducted with pristine LDPE, UV-aged LDPE, pristine Bio, and UV-aged Bio MPs in duplicates. The initial concentration of the CPF solution was 0.2 mg L^{-1} with the background solution consisting of 0.01 mol L^{-1} NaCl and 200 mg L^{-1} NaN_3. The initial pH value of the CPF solution was 6.7. In each treatment, 20 mL of CPF solution and 20 mg of MPs were added to an amber glass vial with a Teflon-lined screw cap. The vials were shaken at 150 rpm for 2 days at 20 ℃. At different time intervals (0, 0.5, 1, 2, 4, 8, 24, 48h), 1 mL of the aliquots were sampled and filtered through a 0.22 μm filter before CPF analysis. The desorption experiment was conducted after the adsorption kinetic experiment. At the end of adsorption kinetic experiment, the CPF solution and the MPs were filtered through a 0.45 μm polycarbonate filter, then the MPs on the filter were washed into the amber glass vial using 20 mL of background solution. The vials were then shaken for two days under the same conditions as in the adsorption experiment. One millilitre of the aliquot was sampled each day. The adsorption isotherm experiment included 7 initial concentrations of CPF at 1, 5, 10, 15, 20, 25, 30 mg L^{-1} in duplicates. In each concentration, 20 mL of CPF solution and 20 mg of pristine or UV-aged MPs were added to the vial. The vials were then shaken at 150 rpm for 2 days at 20 ℃. One millilitre of the aliquot was sampled after the experiment.

The concentration of CPF was determined by HPLC-MS/MS and detailed information about the measurement was explained in our previous study (Ju et al., 2022). The adsorption capacities (q_e) of CPF on MPs were calculated as follows:

Adsorption capacity: $q_e = \frac{C_0 - C_e}{m} V$ (1)

Where C_0 and C_e represent initial and equilibrium concentrations of CPF in the solution (mg L^{-1}), q_e refers to the amount of CPF adsorbed per gram of MPs (μg g^{-1}), V is the volume of CPF solution (L), m is the mass of MPs (g). The adsorption kinetics of CPF on MPs were fitted using the Pseudo-first-order model (PFOM), the Pseudo-second-order model (PSOM) and the Intraparticle diffusion model. The equations are as follows:

Pseudo-first-order model: $log(q_t - q_e) = logQ_e - \frac{k_1 t}{2..03}$ (2)

Pseudo-second-order model: $\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$ (3)

Intraparticle diffusion model: $q_t = k_{id} t^{1/2} + C$ (4)

Where q_e is the adsorption amount of CPF per unit mass of MPs at equilibrium (μg g^{-1}); q_t is the adsorption amount at time t (μg g^{-1}); k_1 is the first-order

sorption rate constant, min^{-1}; k_2 is the second-order sorption rate constant (kg mg^{-1} min^{-1}); k_{id} is the intraparticle diffusion rate constant (μg g^{-1} $h^{1/2}$); C is the intercept.

Linear, Freundlich, and Langmuir adsorption models were used to fit the adsorption isotherms of CPF. The equations are as follows:

Linear model: $q_e = k_d C_e$ (5)

Freundlich model: $q_e = k_f C_e^n$ (6)

Langmuir model: $q_e = \frac{q_m k_l C_e}{1+k_l C_e}$ (7)

Where q_e is the equilibrium adsorption capacity of CPF per unit mass of MPs (μg g^{-1}); Ce is the equilibrium concentration of CPF in solution (mg L^{-1}); k_d is the linear partition coefficient of CPF in the solid phase and the liquid phase (L g^{-1}). k_f is the Freundlich partition coefficient (L g^{-1}); n is the surface heterogeneity factor; q_m is Langmuir adsorption capacity at equilibrium (mg g^{-1}); k_l is Langmuir adsorption constant (L mg^{-1}).

2.2.3 Earthworm ingestion experiment

A four-day petri dish experiment was conducted to study the ingestion of MPs by adult earthworms (*Lumbricus terrestris)*, and the bioconcentration of MPs in earthworm casts. The average weight of earthworms used in this experiment was 3.6 ± 0.5 g. Pristine LDPE and Bio MPs without CPF, pristine LDPE and Bio MPs with adsorbed CPF, UV-aged LDPE and Bio MPs without CPF, UV-aged LDPE and Bio MPs with adsorbed CPF, and LDPE MPs aged in soils S1, S2 and S3 were tested. MPs with adsorbed CPF were prepared as described in the adsorption kinetic experiment. The exposure concentrations of CPF in soil, ranging from 0.9 to 1.1 mg kg^{-1} (Table S2.2), were environmentally relevant (Zhang et al., 2012). In total, 12 treatments were conducted (Table 2.1), and each treatment had 4 replicates. 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. In each glass petri dish, 8 g of dry sandy soil and 40 mg of microplastics were mixed thoroughly to create an exposure concentration of 0.5% (w w^{-1}) which is considered as environmentally relevant (Fuller and Gautam, 2016). Distilled water was added to achieve 20% soil moisture. The earthworms were starved for 2 days prior to the experiment in order to empty their guts. One earthworm was added to each petri dish which was then covered with a glass lid to prevent the earthworm from escaping. The petri dishes were kept in the dark at 15 ℃ for 4 days. After the experiment finished, earthworms were collected, cleaned with distilled water, and put into a new petri dish for 2 days to produce casts. The collected casts were then dried at 40 ℃ and weighed. The MPs in the

earthworm casts were extracted using density flotation. Detailed information concerning the extraction methods has been described in a previous study (Ju et al., 2022). The ingestion rate of MPs was calculated as follows:

$$\text{MP ingestion rate} = \frac{m_1}{m_2 t} \quad (8)$$

Where m_1 is the mass of MPs in the earthworm cast (mg); m_2 is the biomass of the earthworm (g); t is the duration of the experiment (d).

Table 2.1 *Microplastic condition for treatments in the petri dish experiment*

Treatment	MP	Aging in UV light	Chlorpyrifos adsorption	Aging in soil
Control blank (CK)	No MP	X	X	X
Pristine LDPE	LDPE	X	X	X
S1 LDPE	LDPE	X	X	√
S2 LDPE	LDPE	X	X	√
S3 LDPE	LDPE	X	X	√
UV LDPE	LDPE	√	X	X
UV LDPE+CPF	LDPE	√	√	X
Pristine LDPE+CPF	LDPE	X	√	X
Pristine Bio	Bio	X	X	X
UV Bio	Bio	√	X	X
UV Bio+CPF	Bio	√	√	X
Pristine Bio+CPF	Bio	X	√	X

2.2.4 Statistical analysis

Data are presented as mean ± standard deviation (SD). The normality and equality of variances were determined using the Shapiro-Wilk test and Levene's test, respectively. One-way ANOVA with Duncan's post-hoc comparisons were conducted to test the differences among treatments. All the tests were performed using IBM SPSS Statistics 26 and the critical p value of significance was set at 0.05. All the graphs were drawn, and the adsorption models were fitted using originpro 2016.

2.3 Results

2.3.1 Characterization of MPs

After exposure to UV light for 6 months, carbonyl groups were found in the UV-aged LDPE MPs using the ATR-FTIR spectrum and showed peaks at 1717 cm^{-1} (Figure 2.1). The carbonyl index (CI) was 0.028 for UV-aged LDPE MPs and 0.005 for pristine LDPE MPs (Table S2.3), which was calculated according to the method described by La Mantia et al. (2020). UV-aged Bio MPs showed decreased carbonyl group absorbance (1800-1550 cm^{-1}) and decreased CI as compared to pristine Bio MPs. After the aging of MPs in 3 types of soil for 4 months, double bonds (1654 cm^{-1}; -C=C-), nitro groups (1560 cm^{-1}, N-O), alkoxy groups (1032 cm^{-1}; C-O) and terminal double bonds (908 cm^{-1}; H_2C=C-) were determined in LDPE MPs (Figure S2.3).

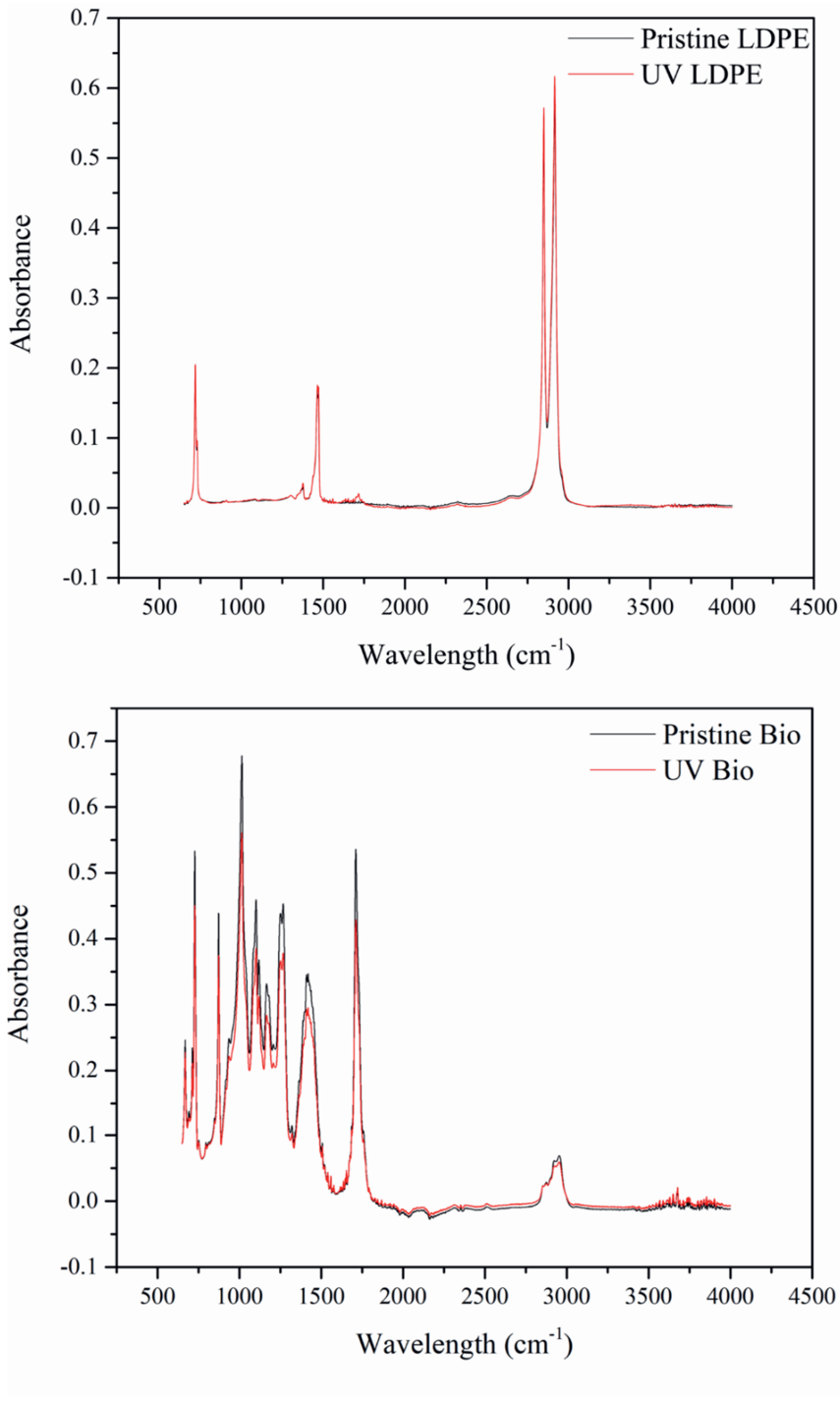

Figure 2.1 *ATR-FTIR spectra for UV-aged LDPE and Bio MPs.*

2.3.2 Adsorption of CPF on MPs

2.3.2.1 Adsorption kinetics

Results of adsorption kinetics showed that the adsorption of CPF by 4 types of MPs reached equilibrium after 4 h, and more than 90% of the sorption was accomplished within the first 2 h (Figure 2.2). The adsorption capacities of CPF for pristine LDPE, UV LDPE, pristine Bio and UV Bio MPs were 184.9±5.3, 200.5±1.8, 193.0±8.7, and 215.9±1.1 μg g^{-1}, respectively. The parameters obtained from the two adsorption kinetic models are summarized in Table 2.2. The adsorption of CPF on pristine LDPE, UV LDPE and pristine Bio MPs was better described by PFOM, while the adsorption of CPF on UV Bio MPs were better fitted by PSOM.

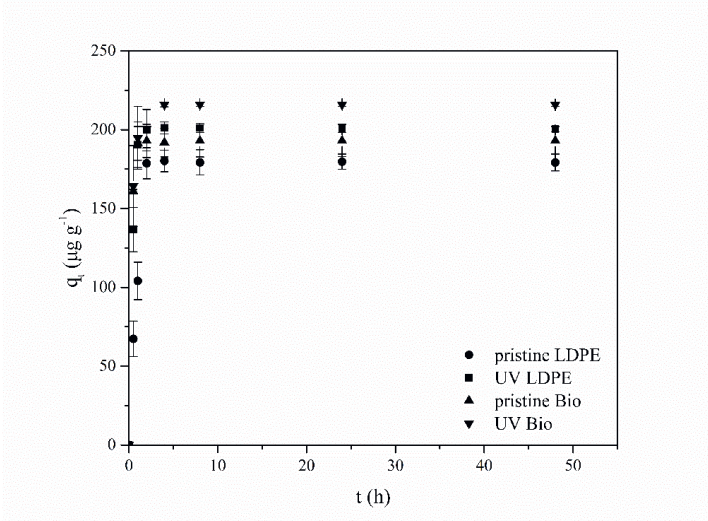

Figure 2.2 *Sorption kinetics of chlorpyrifos on pristine LDPE, UV-aged LDPE, pristine Bio, and UV-aged Bio microplastics.*

Table 2.2 *Adsorption kinetic models of microplastics*

MP	Pseudo-first-order			Pseudo-second-order		
	q_e (μg g^{-1})	k_1	R^2	q_e (μg g^{-1})	k_2	R^2
Pristine LDPE	182.6±5.2	1.0±0.1	0.978	193.9±11.2	0.008±0.003	0.933
UV LDPE	201.7±1.5	2.4±0.1	0.998	208.0±5.5	0.025±0.006	0.982
Pristine Bio	193.2±0.6	3.6±0.1	1.000	196.5±2.9	0.060±0.016	0.993
UV Bio	212.5±2.6	2.8±0.2	0.994	219.3±1.9	0.029±0.003	0.998

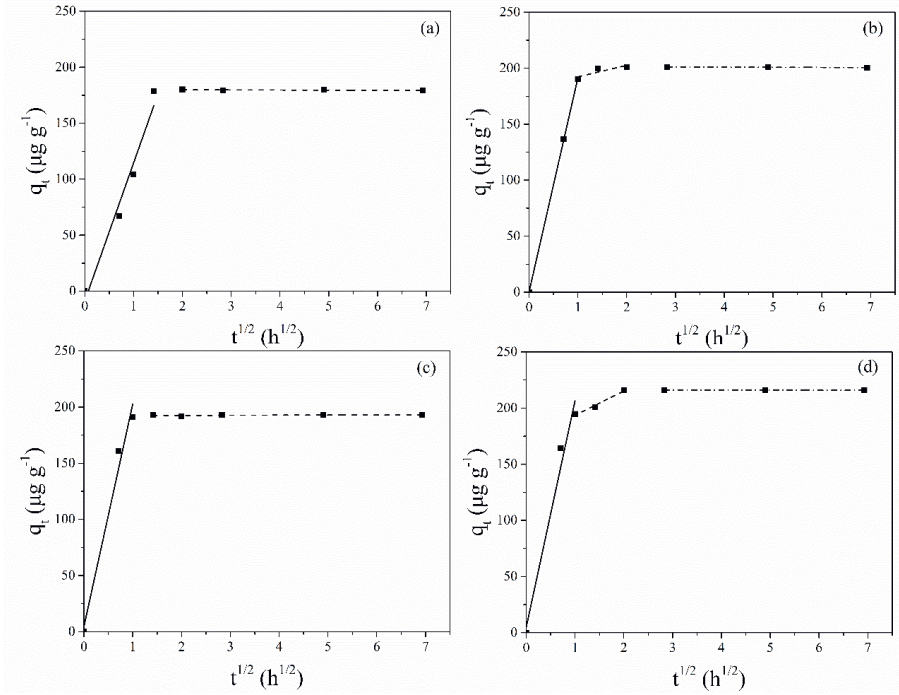

Figure 2.3 *Intra-particle diffusion model plots for chlorpyrifos adsorption by (a) pristine LDPE, (b) UV-aged LDPE, (c) pristine Bio, (d) UV-aged Bio microplastics.*

2.3.2.2 Adsorption isotherms

Linear, Freundlich and Langmuir models were fitted with isotherm data to explore adsorption behaviours of CPF on different MPs (Table 2.3). The adsorption of CPF on MPs was better fitted with a non-linear model than a linear model. The Langmuir model was better to simulate the CPF adsorption isotherms of pristine LDPE and Bio MPs with higher R^2, while the Freundlich model was better to fit the isotherms of UV-aged LDPE and Bio MPs (Table 2.3).

Table 2.3 *Adsorption isotherm models of microplastics*

MP	Linear		Freundlich			Langmuir		
	k_d (L g^{-1})	R^2	k_f (L g^{-1})	n	R^2	K_l (L mg^{-1})	q_m (mg g^{-1})	R^2
Pristine LDPE	0.073	0.917	0.201	0.668	0.957	0.033	4.891	0.961
UV LDPE	0.135	0.880	0.232	1.861	0.960	na	na	na
Pristine Bio	0.171	0.856	0.323	0.713	0.897	0.031	10.795	0.921
UV Bio	0.198	0.981	0.376	1.232	0.990	na	na	na

"na" means the model failed to fit the adsorption isotherm.

2.3.3 Desorption of CPF by MPs

Pristine LDPE MPs exhibited the highest CPF desorption rate. Within 48 h of desorption, 40.0 ± 3.9% of adsorbed CPF desorbed from pristine LDPE MPs (Table 2.4), and most of the CPF was desorbed within the first 24 h of the desorption process. The CPF desorption rate of Bio MPs was much lower than that of LDPE MPs, with only 7.3 ± 5.4 % of adsorbed CPF desorbed from MPs within 48 h. The CPF desorption rate of UV LDPE MPs was lower than that of pristine LDPE MPs with 28.8 ± 7.7% of adsorbed CPF desorbed from MPs within 48 h, and this gap was mainly shown on the first day of desorption. The CPF desorption rate of UV Bio MPs was slightly lower than that of pristine Bio MPs.

Table 2.4 *Chlorpyrifos desorption from microplastics*

MP	Adsorption (%)	Desorption-24 h (%)	Desorption-48 h (%)
LDPE	91.4 ± 0.7%	26.7 ± 5.2%	40.0 ± 3.9%
UV LDPE	93.7 ± 0.1%	13.8 ± 2.7%	28.8 ± 7.7%
Bio	96.8 ± 0.1%	3.1 ± 1.7%	7.3 ± 5.4%
UV Bio	98.6 ± 0.2%	1.8 ± 0.4%	4.2 ± 1.4%

2.3.4 MP concentration in earthworm casts

In the 4-day petri dish experiment, the content of pristine Bio MPs in earthworm casts was 2.41 and 2.16 times higher than the content of pristine LDPE MPs and exposure concentrations of pristine Bio MPs (Figure 2.4).

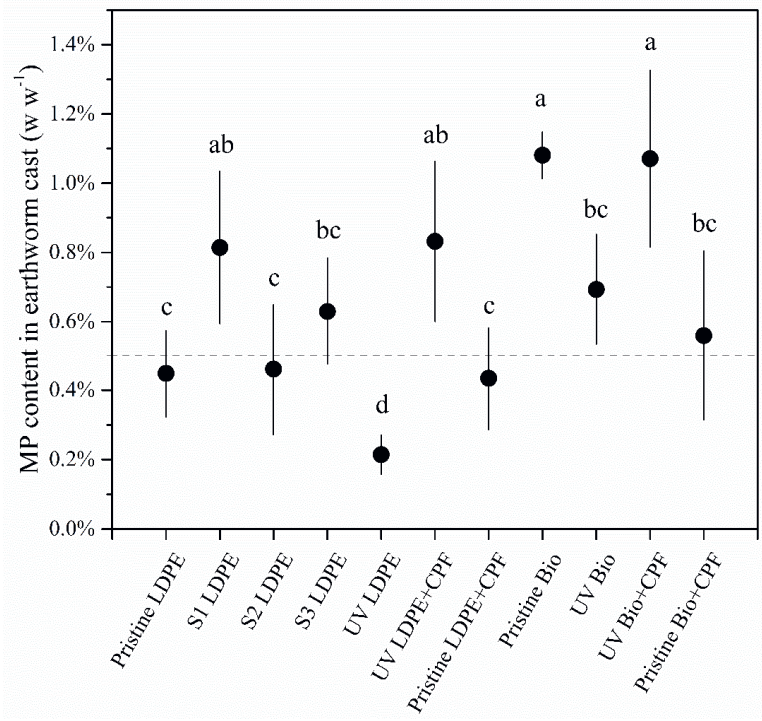

Figure 2.4 *Content of microplastics in earthworm casts among treatments. Different letters indicate significant differences among treatments. The dotted line indicates the exposure concentration of microplastics (0.5%, w* w^{-1}*), the scatter above the dotted line indicates the bioconcentration of microplastics in earthworm casts.*

The contents of UV LDPE and UV Bio MPs in earthworm casts were significantly lower than the contents of their pristine counterparts, with 52% and 36% lower MP contents in earthworm casts for UV LDPE and UV Bio MPs, respectively. The content of pristine LDPE MPs with adsorbed CPF in earthworm casts showed no significant difference compared to that of pristine LDPE MPs. However, the content of pristine Bio MPs with adsorbed CPF in earthworm casts was significantly lower than that of pristine Bio MPs. In addition, the contents of UV-aged MPs with adsorbed CPF in earthworm casts were higher than those of UV-aged MPs without CPF. LDPE MPs aged in soil S1, which was higher in soil carbon and nitrogen, and had a higher carbon-to-nitrogen ratio (C:N), resulted in higher MP content in earthworm casts than pristine LDPE MPs and LDPE MPs aged in soil with lower carbon, nitrogen, and C:N.

However, MP ingestion rates for earthworms showed no significant differences in most of the LDPE-MP treatments compared to that for the

pristine LDPE-MP treatment except in the treatment with LDPE MPs aged in soil S1 (Figure 2.5), where the MP ingestion rate was significantly higher than that in the pristine LDPE-MP treatment. Pristine Bio MPs had a significantly higher ingestion rate than pristine LDPE MPs. Moreover, the ingestion rate of UV Bio MPs, UV Bio MPs with adsorbed CPF and pristine Bio MPs with adsorbed CPF in earthworm casts were all lower than that in the treatments with pristine Bio MPs.

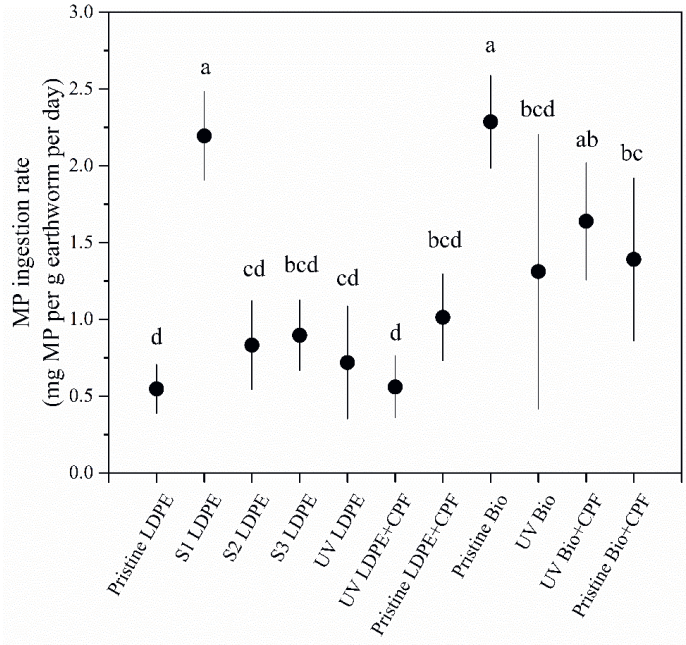

Figure 2.5 *Microplastic ingestion rates among treatments. Different letters indicate significant differences among treatments.*

2.4. Discussion

2.4.1 Effects of MP aging on CPF adsorption-desorption

In this study, we observed that UV-aged MPs (both LDPE and Bio) showed higher adsorption capacities than pristine ones (Figure 2.2). The difference in adsorption behaviours between pristine MPs and aged MPs could be explained by the change in specific surface area and morphology as well as the functional groups and hydrophilicity (Fan et al., 2021). The generation of carbonyl groups in UV-aged LDPE MPs may have facilitated the formation of hydrogen bonds between UV-aged LDPE MPs and CPF. Furthermore, UV aging can also increase the Brunauer–Emmett–Teller (BET) surface area by inducing the formation of pores and cracks in PE-MPs, thereby elevating their adsorption capacities (Wang et al., 2022). A previous study also reported that pores and cracks were observed in aged PBAT MPs after exposure to UV

radiation, indicating increased adsorption sites for adsorbates (Guo et al., 2022). Thus, aged MPs may act as a stronger vector for CPF in the soil ecosystem.

According to the fitted parameters of adsorption kinetic models, PFOM better described the adsorption of CPF on pristine LDPE, UV LDPE and pristine Bio MPs. While the adsorption of CPF on UV Bio MPs was better fitted by PSOM. Previous studies have suggested that the adsorption of hydrophobic organic contaminants on aged MPs was better described by PSOM which is due to the modification of the physicochemical properties of MPs (Fan et al., 2021; Kong et al., 2021). Intraparticle diffusion models showed that the fitted curves could be separated into several linear segments (Figure 2.3). This indicates that CPF adsorption could undergo different stages. In the first stage, the adsorption capacity increased rapidly due to more available adsorption sites on external surfaces. In this stage, the higher intraparticle diffusion rate constant k_{id1} of aged MPs indicated a higher CPF adsorption rate (Table S2.4). It is shown that before reaching the equilibrium stage, aged MPs exhibited second adsorption stages which had lower adsorption rates than those in the first stage. This is because the adsorption sites on the external surface were saturated, and the adsorption was controlled by intra-particle diffusion. This phenomenon might be due to the formation of pores and cracks in aged MPs which led to the adsorption of CPF on the interior of MPs (Fan et al., 2021).

Adsorption isotherm data were fitted using the linear isotherm model, Langmuir model and Freundlich model to further analyse adsorption behaviour of CPF on MPs (Table 2.3). Non-linear models showed better goodness-of-fit for isotherm data which means the adsorption of CPF on MPs was nonlinear. The adsorption process of CPF on pristine LDPE and Bio MPs was more suitable for the Langmuir model while that of aged MPs was more consistent with the Freundlich model. This indicates that the adsorption of CPF on pristine MPs was monolayer while the adsorption on aged MPs was multilayer. Previous studies also found that the adsorption of hydrophobic organic chemicals on aged MPs was well fit to the Freundlich isotherm model, which was due to the pores and cracks in the heterogeneous surfaces of MPs (Liu et al., 2019; Fan et al., 2021). The value of Freundlich constants (k_f) for aged MPs were higher than those for pristine MPs, which was also found in a previous study (Fan et al., 2021). This indicates that aged MPs had a higher adsorption affinity to CPF. Furthermore, the n values for aged MPs were greater than 1, indicating the preferential adsorption of CPF on aged MPs (Liu et al., 2019).

After 48 h of desorption, pristine LDPE MPs desorbed much more CPF than pristine Bio MPs which means that Bio MPs had a stronger affinity to CPF than LDPE MPs. A previous study revealed that the desorption of triclosan

from PE MPs reached equilibrium within 48 h, and more adsorbed triclosan was desorbed from PE MPs than from polystyrene MPs (Chen et al., 2021). This might be because PE has a flexible and rubbery structure while PS has a dense and glassy structure, and the pore-filling of sorbates in the glassy fraction can lead to desorption hysteresis (Chen et al., 2021). Zuo et al. (2019) found that PBAT MPs had a higher desorption capacity for phenanthrene than PE and glassy PS MPs, which was due to the higher proportion of rubbery subfractions in PBAT MPs. However, in the present study, we observed the opposite, which might be attributed to the different chemical structures of both the adsorbate and adsorbent. In this study, UV-aged LDPE MPs showed lower CPF desorption rates than pristine ones. This may be due to the formation of oxygen-containing functional groups in aged MPs which can enhance the affinity of aged MPs and associated contaminants. Similarly, Fan et al. (2021) also observed lower desorption rates of antibiotics from aged PLA and PVC MPs than from pristine ones. The desorption rates of CPF from pristine and UV-aged Bio MPs were both low. Therefore, UV-aged LDPE and Bio MPs can serve as better vectors for CPF than pristine MPs because of the higher adsorption capacities and lower desorption rates of CPF. Previous studies have found that the desorption of organic pollutants from MPs was higher in simulated intestinal fluid than in water (Liu et al., 2020; Fan et al., 2021). Once MPs are ingested by organisms, more adsorbed organic pollutants could be desorbed from MPs in the gut. Therefore, the potential toxicity of MPs, especially aged MPs, associated with organic pollutants for soil organisms like earthworms needs to be considered.

2.4.2 Effects of MP aging and CPF on the bioconcentration of MPs

The content of pristine LDPE MPs in earthworm casts was close to the exposure concentration (0.5%, w w^{-1}), indicating that the pristine LDPE MPs had a low risk of being concentrated in earthworm casts. However, Huerta Lwanga et al. (2016) found that earthworms (*L. terrestris*) can enrich smaller sized PE MPs in the casts, which means that the PE MPs transported into soil by earthworms may pose potential risks to smaller soil biota. We observed that the pristine Bio MPs resulted in significantly higher earthworm ingestion rates and MP content in earthworm casts than the pristine LDPE MPs, suggesting a Bio-MP ingestion preference in earthworms. A previous study found that *p*-phthalic acid and lactic acid, which were the monomers of the Bio MPs used in this study, were attractive to earthworms *Eisenia fetida* (Wang et al., 2022). This is probably due to the sour odour of these polymers which can act as a cue for earthworms when detecting food. However, in our previous mesocosm experiments, we found that the bioconcentration of LDPE and Bio MPs in the earthworm casts were not significantly different when MPs were added to the litter (Ju et al., 2022). This indicates that earthworms are prone

to ingesting Bio MPs in food-deficient situations. Furthermore, Bio MPs were proven to be significantly toxic to earthworms (Ju et al., 2022), thus the adverse effects from Bio MPs may be exacerbated in food-deficient situations. Compared to the pristine LDPE MPs, UV-aged LDPE MPs were significantly less prevalent in earthworm casts. In this study, earthworms produced more casts in the UV LDPE-MP treatments than the pristine LDPE-MP treatments (Figure S2.4), indicating that UV-aged LDPE MPs stimulated the ingestion and egestion of soil by earthworms which may affect the content of UV-aged LDPE MPs in earthworm casts. Previous studies have also reported that the surface chemistry of MPs affected the ingestion and egestion of MPs by organisms (Zou et al., 2020; Liu et al., 2022). For example, Zou et al. (2020) found that the higher depuration rate of polyamide (PA) MPs aged using light irradiation and hydrogen peroxide led to a lower body burden of aged PA MPs in zebrafish compared to pristine PA MPs. CPF had no effect on the bioconcentration of pristine LDPE MPs in earthworm casts, which is probably due to the adsorbed CPF that can be desorbed rapidly from LDPE MPs (Table 2.4). Interestingly, we found that the ingestion and bioconcentration of Bio MPs treated with UV light or CPF adsorption by earthworms were significantly lower compared to that of pristine Bio MPs. This means that after UV aging or the adsorption of CPF, the flavour of Bio MPs changed and made them less appetizing to earthworms. UV-aged MPs (both UV LDPE and UV Bio) with adsorbed CPF showed significantly higher content in earthworm casts than UV-aged MPs without CPF. In treatments with UV-aged MPs with adsorbed CPF, the cast production of earthworms was lower than that in treatments with UV-aged MPs without CPF (Figure S2.4), indicating that UV-aged MPs with adsorbed CPF have a higher risk of being concentrated through earthworm casts than UV-aged MPs without CPF.

In contrast to UV-aged LDPE MPs, we have detected not only carbonyl groups (1654 cm^{-1}), but nitro groups (1560 cm^{-1}), alkoxy groups (1032 cm^{-1}) and terminal double bonds (908 cm^{-1}) in LDPE MPs aged in 3 types of soil for 4 months. These functional groups suggested that LDPE-MPs probably underwent oxidation, vinylene formation and chain scission in the soil (Peixoto et al., 2017). LDPE MPs aged in C and N enriched soil (S1) were significantly ingested and concentrated by earthworms in their casts compared to pristine LDPE MPs and LDPE MPs aged in low C and N soil like S2. This is probably due to the higher C, N and C:N ratios which are conducive to the formation of biofilm on the surface of MPs. Hence, in soil enriched with C and N, earthworm-mediated transport of LDPE MPs through burrowing and excretion may pose greater environmental risks, such as resulting in more profound impacts on deeper soil or groundwater (Yu et al., 2019; Ju et al., 2022). Former studies have reported that biofilms on the surface of MPs can make MPs more attractive to aquatic organisms, leading to increased ingestion of MPs (Rummel et al., 2017; Vroom et al., 2017; Sucharitakul et al., 2021).

However, the ingestion of MPs aged in soil by organisms like earthworms remains unclear. Liu et al. (2022) found that the ingestion of soil-aged PS MPs (24.65 ± 5.20 μm) by earthworms *Eisenia fetida* was lower, which was because soil particles wrapped around the MPs and protected earthworms from direct contact with PS MPs. Whether larger sizes of MPs or MP fibres can also be protected by soil particles in this way and thus reduce soil organism exposure is still unclear. The opposite result from this study is probably due to the different exposure scenarios where the MPs were not well-aggregated with soil particles. Thus, further studies are needed to examine the biological effects (both single effects and combined effects with other pesticides) of MPs that were aged in C and N enriched soil.

2.5 Conclusion

The conclusions of this study can be summarized as follows: (1) UV aging could increase the carrier effect of LDPE and Bio MPs for CPF; (2) the bioconcentration of LDPE and Bio MPs in earthworm casts was negatively affected by UV aging. However, the MP-adsorbed CPF positively affected the bioconcentration of UV-aged MPs in earthworm casts, which is probably due to the change in earthworm cast production. (3) LDPE MPs aged in soil with high levels of soil carbon and nitrogen, as well as a high C:N ratio, were found to be significantly more concentrated in earthworm casts compared to both pristine LDPE MPs and those aged in soils with lower levels of carbon and nitrogen and a lower C:N ratio. Concerning MP aging and its morphological changes, further study is needed to explore the interactions between aged MPs and soil pollutants, especially in soil enriched with carbon and nitrogen.

Acknowledgement

We are grateful for the funding provided by the National Natural Science Foundation of China (41877072), the EU Horizon 2020 project (MINAGRIS) and the China Scholarship Council (CSC: 201904910501).

Supplementary materials

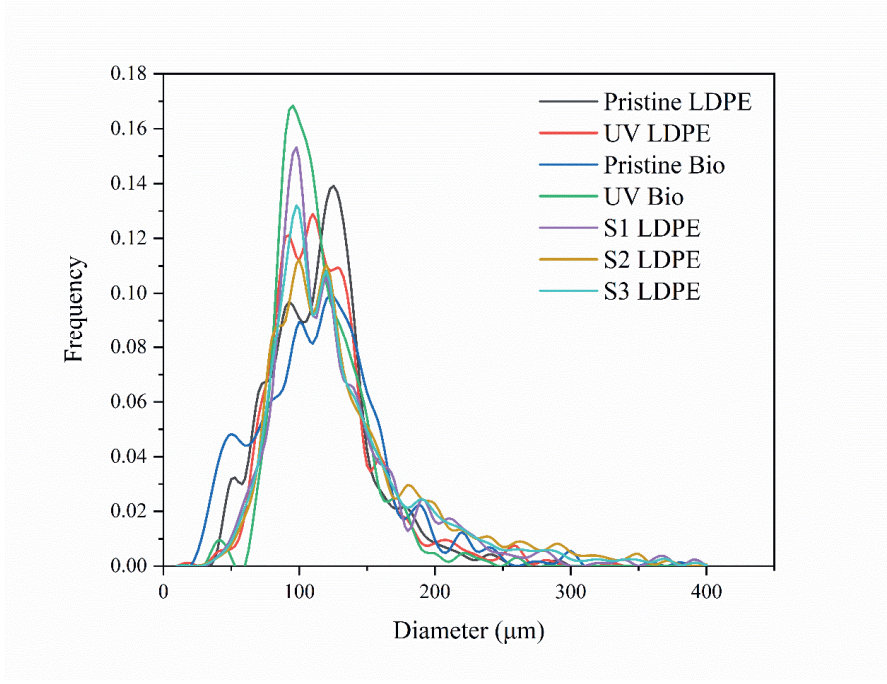

Figure S2.1 *Size distribution of different MPs. Pristine LDPE, UV LDPE, pristine Bio, UV Bio, S1 LDPE, S2 LDPE and S3 LDPE indicate pristine LDPE, UV-aged LDPE, pristine Bio, UV-aged Bio MPs and LDPE MPs aged in soil S1, S2, S3.*

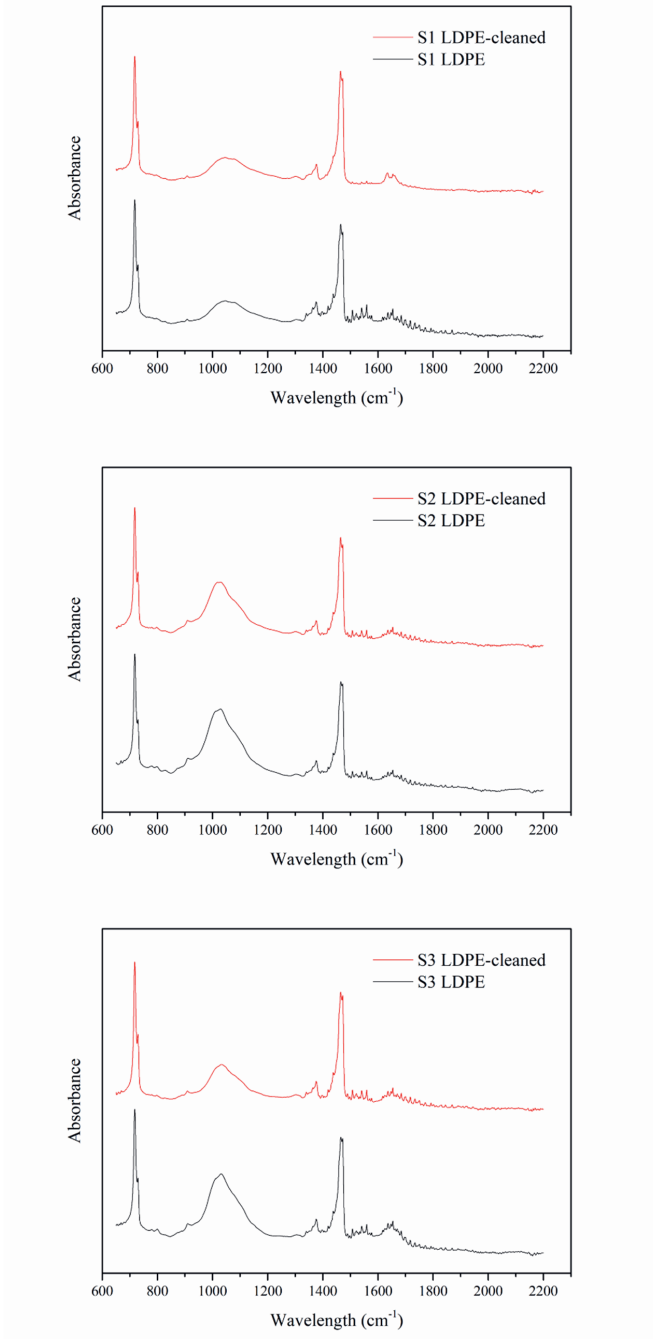

Figure S2.2 *FTIR spectrum for sodium dodecyl sulfate cleaned and uncleaned LDPE MPs aged in different soils.*

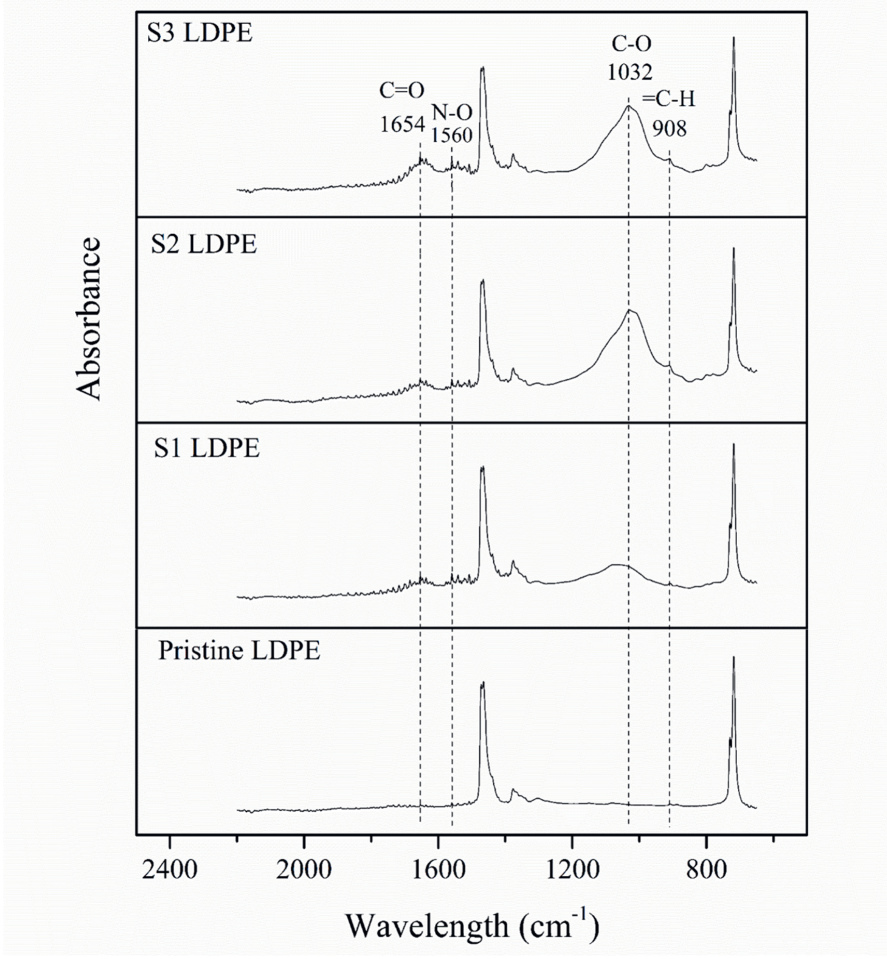

Figure S2.3 *ATR-FTIR spectra for LDPE MPs aged in different soils. Wavelengths at around 908, 1032, 1560 and 1654-1717 cm^{-1} indicate functional groups =C-H, C-O, N-O and C=O, respectively.*

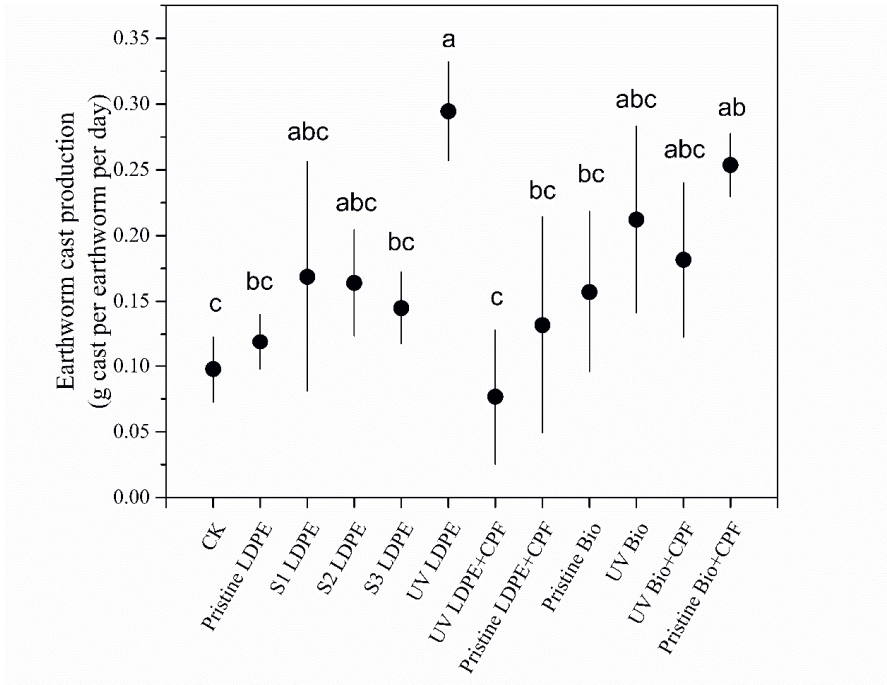

Figure S2.4 *Earthworm cast production in different treatments. S1 LDPE, S2 LDPE, S3 LDPE, UV LDPE and UV Bio indicate LDPE MPs aged in soil S1, S2, S3 and UV light, and Bio MPs aged in UV light. UV LDPE+CPF, pristine LDPE+CPF, UV Bio+CPF, and pristine Bio+CPF indicate UV-aged LDPE and pristine LDPE MPs with adsorbed CPF, and UV-aged Bio and pristine Bio MPs with adsorbed CPF. Different letters indicate significant differences among treatments.*

Table S2.1 *Properties of used soils and cow manure.*

	Soil texture	C(g kg^{-1})	N(g kg^{-1})	C/N after manure addition	pH	WHC
S1	Loamy sand	92.8	5.2	17	7.54	36.0%
S2	Silty clay loam	13.6	1.1	12	7.93	43.3%
S3	Silt loam	9.0	1.2	10	8	38.6%
Manure	-	311.0	27.0	-	-	-

Table S2.2 *Exposure concentration of chlorpyrifos in soil.*

Treatment	Concentration (mg kg^{-1})
Pristine LDPE+CPF	0.9
UV LDPE+CPF	1.0
Pristine Bio+CPF	1.0
UV Bio+CPF	1.1

Table S2.3 *Carbonyl indexes (CI) of pristine and aged microplastics.*

	LDPE					Bio	
	Pristine LDPE	UV LDPE	S1 LDPE	S2 LDPE	S3 LDPE	Pristine Bio	UV Bio
CI	0.005	0.028	0.029	0.032	0.039	3.804	2.592

Table S2.4 *Parameters of intraparticle diffusion model for adsorption of CPF by microplastics.*

MP	Stage Ⅰ			Stage Ⅱ			Stage Ⅲ		
	k_{id1} (μg g^{-1} $h^{1/2}$)	C_1 (μg g^{-1})	R^2	k_{id2} (μg g^{-1} $h^{1/2}$)	C_2 (μg g^{-1})	R^2	k_{id3} (μg g^{-1} $h^{1/2}$)	C_3 (μg g^{-1})	R^2
LDPE	123.27	-8.67	0.957	0.29	178.33	0.952			
UV LDPE	190.95	0.37	1.000	10.17	182.14	0.539	-0.13	201.47	0.954
Bio	198.00	4.73	0.961	0.03	192.86	0.698			
UV Bio	201.65	4.90	0.96	21.48	172.16	0.957	-0.03	216.07	0.875

K_{id} and C mean the intraparticle diffusion rate constant and the value of the intercept.

Chapter 3 Effects of microplastics and chlorpyrifos on earthworms (*Lumbricus terrestris*) and their biogenic transport in sandy soil

Based on:
Ju, H., Yang, X., Osman, R., Geissen, V., 2022. Effects of microplastics and chlorpyrifos on earthworms (Lumbricus terrestris) and their biogenic transport in sandy soil. Environ Pollut, 120483.10.1016/j.envpol.2022.120483

Abstract:

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.

Keywords: Chlorpyrifos; Microplastics; Biodegradable plastic; Earthworms; Biogenic transport

3.1 Introduction

Plastic mulch films are widely used in agriculture to regulate soil temperature, conserve moisture and control weeds (Kasirajan and Ngouajio, 2012). Nondegradable 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 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, 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). 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., 2020; Wang et al., 2020). 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., 2020). 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.

3.2 Materials and methods

3.2.1 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 (Figure S3.1) 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 ℃ 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 ℃ before use.

3.2.2 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 S3.1. 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 ℃ 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 3.1.

Table 3.1 *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 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 ℃ in the dark and then weighed before the experiment was carried out. The average weight of the earthworms in each treatment is shown in Table S3.2 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 ℃ 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.

3.2.3 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 ℃ 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.

3.2.4 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.

3.3 Results

3.3.1 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 S3.4).

Mortality was not significantly different between all treatments with LDPE-MPs as compared to the control (Table 3.2). 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 3.2).

Higher application rates (5C) of CPF alone caused higher earthworm weight losses in both LDPE-MP and Bio-MP experiments (Table 3.2). 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.

Table 3.2 *Earthworm weight change, reproduction and mortality in different treatments*

Indicators	MP	Treatments								
		Control	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±8.5ab	-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±2.6ab
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.

3.3.2 Microplastics and chlorpyrifos in earthworm casts and bodies

3.3.2.1 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 (Figure 3.1a and 3.1b). 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.

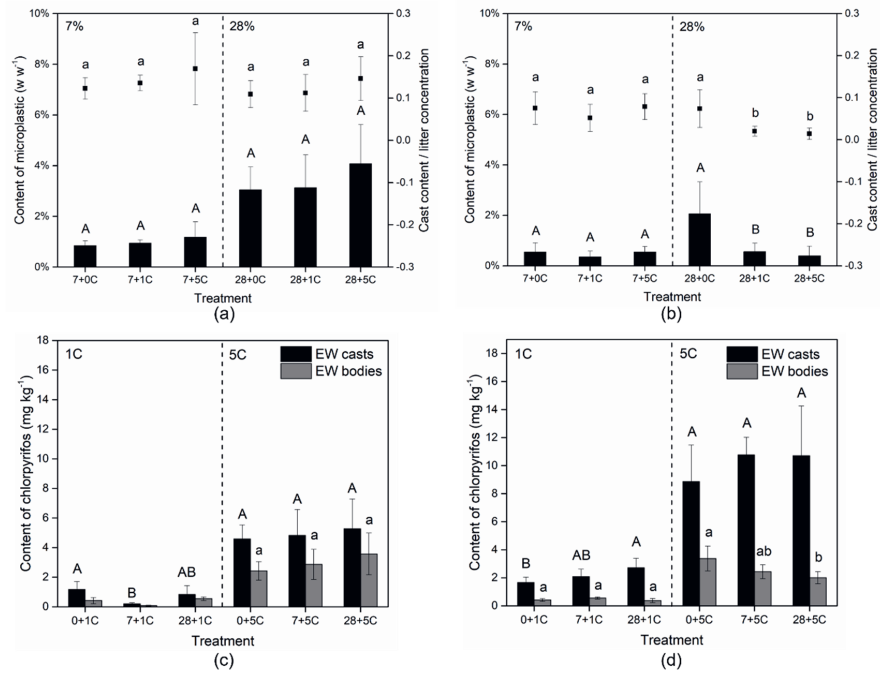

Figure 3.1 *Cast contents of MPs (bars) and ratios of cast contents versus litter concentrations of MPs (scatters) in earthworm casts (a: LDPE-MP experiment; b: Bio-MP experiment), and contents of chlorpyrifos in earthworm*

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.
** 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.*

3.3.2.2 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 (Figure 3.1c). 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 (Figure 3.1d).

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) (Figure 3.1c). Bio-MPs also significantly decreased earthworm body contents of CPF in the 28+5C treatment compared to the 0+5C treatment (Figure 3.1d).

3.3.3 Contents of microplastics and chlorpyrifos in burrow soil

3.3.3.1 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 (Figure 3.2a). 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 (Figure 3.2b). 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.

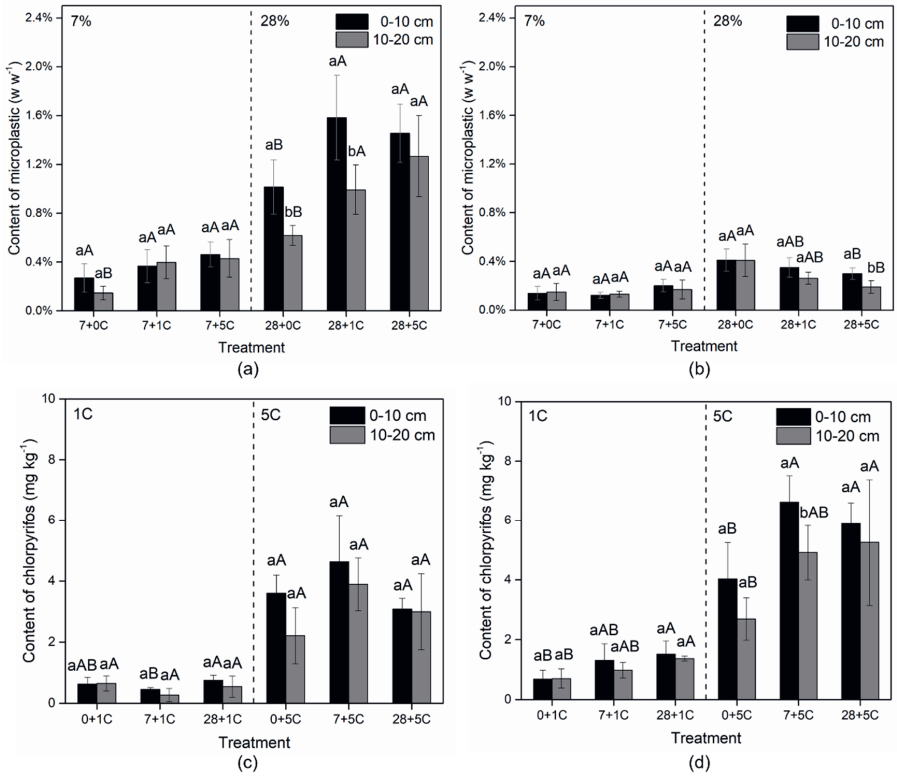

Figure 3.2 *Contents of microplastics (a: LDPE-MP experiment; b: Bio-MP experiment) and chlorpyrifos (c: LDPE-MP experiment; d: Bio-MP experiment) in the 0-10 cm and 10-20 cm depths of burrow soil. Different uppercase letters represent significant differences among treatments in the 1C or 5C chlorpyrifos treatment group, different lowercase letters represent significant differences between the 0-10 cm and 10-20 cm depths of soil.*

3.3.3.2 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 (Figure 3.2c). 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 (Figure 3.2d). 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.

3.3.4 Biogenic transport ratios of microplastics and chlorpyrifos

3.3.4.1 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 (Figure 3.3a). 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%) (Figure 3.3b). However, the presence of CPF did not significantly change the total 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.

3.3.4.2 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 (Figure 3.3c and 3.3d). 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.

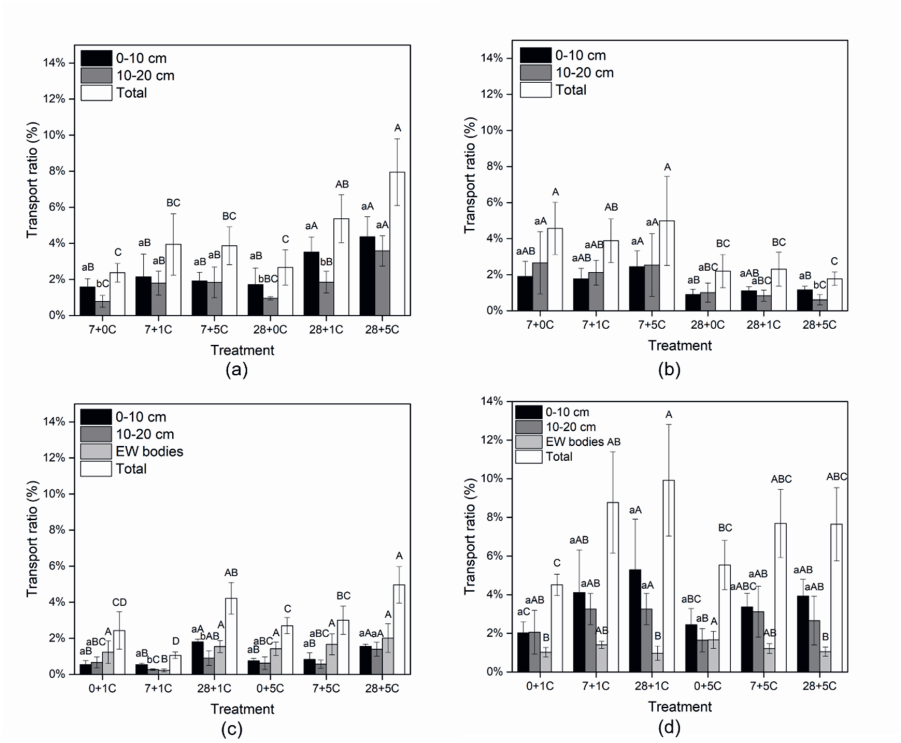

Figure 3.3 *Transport ratios of (a) LDPE-MP and (b) Bio-MP in soil, (c) chlorpyrifos in soil and earthworm bodies in the LDPE-MP experiment and (d) chlorpyrifos in soil and earthworm bodies in the Bio-MP experiment. Different uppercase letters indicate the significant differences among treatments, different lowercase letters indicate the significant differences between the 0-10 cm and 10-20 cm soil depths.*

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

3.3.5.1 Earthworm casts and bodies

Two metabolites of CPF (chlorpyrifos-oxon and 3,5,6-trichloropyridinol (TCP)) were measured in this study (Table S3.5 and S3.6). 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.

3.3.5.2 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 S3.6). 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±0.01 mg kg^{-1}) was significantly lower than that in the 0+5C treatment (0.11±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.

3.4. Discussion

3.4.1 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., 2020). 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 S3.1), 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, 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, 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* (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 S3.7). 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.

3.4.2 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 (1mm 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.

3.4.3 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.

3.5 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.

Acknowledgement

We are grateful for the funding provided by the National Natural Science Foundation of China (41877072), the EU Horizon 2020 project (MINAGRIS) and the China Scholarship Council (CSC: 201904910501).

Supplementary materials

Analysis of microplastics

Both LDPE-MPs and Bio-MPs from burrow soil and earthworm casts were extracted using density flotation. To extract LDPE-MPs, 0.5 g of dried soil or 0.1 g of dried casts were weighed and added to a glass tube. Then, 10 mL of 15% hydrogen peroxide was added to pre-digest organic matter. The digestion process was conducted at 70 ℃ in an oven for 18 h. Then the tube was vortexed for 30 seconds and centrifuged at 3000 rpm for 30 min. After centrifugation, the supernatant was filtered through a 0.4 μm polycarbonate filter and rinsed with distilled water and 70% ethanol. The extraction processes were repeated 2-3 times until no MP was observed in the supernatant. The MPs collected on the filter were then transferred to a pre-weighed tube and dried in the oven at 50℃. To extract Bio-MPs, 0.5 g of dried soil or 0.1 g of dried casts were weighed and put into a glass tube. Then 10 mL of sodium dihydrogen phosphate (NaH_2PO_4) solution at a density of 1.39 g cm^{-3} was added to the tube. To separate the soil from the MPs, the tubes were agitated end-over-end for 18 h and then exposed to an ultrasonic bath for 1 h. The tubes were then vortexed for 30 seconds and centrifuged at 3000 rpm for 30 min. After centrifugation, the supernatant was filtered through a 0.4 μm polycarbonate filter and rinsed with distilled water and 70% ethanol. The extraction processes were repeated 2-3 times until no MPs were observed in the supernatant. The MPs on the filter were then washed down in a pre-weighed tube, and 10 mL of 15% hydrogen peroxide was added to digest organic matter at 50 ℃ for 18h. After digestion, MPs were filtered, rinsed, then transferred to the pre-weighed tube before drying. The mass of the extracted MPs was calculated using the weight difference between the tube before and after the extraction. The MP recovery tests were conducted for both LDPE-MP and Bio-MP with 8 replicates. MPs were added to the clean soil and the extraction processes were the same as described above. The recovery rates for LDPE-MPs and Bio-MPs were $90.4 \pm 4.9\%$ and $107.9 \pm 11.7\%$ (w w^{-1}) respectively.

Analysis of chlorpyrifos and its metabolites

Chemicals and solvents

The reference standards of chlorpyrifos (99%), TCP (99.6%) and ^{13}C-caffeine (99%) were obtained from Sigma-Aldrich (USA). Chlorpyrifos-oxon (98%) was purchased from LGC Standards. Magnesium sulfate ($MgSO_4$; 99.5%) was purchased from Alfa Aesar GmbH (Germany) and sodium acetate (CH_3COONa; ⩾99%) was purchased from Acros Organics (Geel, Belgium). Acetic acid (CH_3COOH; ⩾ 99.8%) was obtained from Biosolve BV (The Netherlands). Acetonitrile (C_2H_3N; 99.95% LC grade) and methanol (MeOH;

99.98%) were purchased from Actuall Chemicals (The Netherlands). Formic acid (≥98% p.a.) from Gevaar (The Netherlands) and ammonium formate (HCO_2NH_4; > 99%) from Sigma (USA) were used for the mobile phases of liquid chromatography-tandem mass spectrometry (LC-MS/MS). Standard stock solutions of chlorpyrifos, TCP and chlorpyrifos-oxon at concentrations of 2000 and 1000 μg mL^{-1} were prepared and all stock solutions and dilutions were stored at 4 °C.

Extraction Methodology of chlorpyrifos and its metabolites

Earthworm casts, earthworm bodies, and burrow soil (stored at -20 ℃) were used for the extraction of CPF and its metabolites. Earthworm bodies were crushed using liquid nitrogen before extraction. The extraction procedure used was based on a QuEChERS approach. One gram of earthworm body was added to 50 mL tubes and then spiked with 25 μL ^{13}C-caffeine (10 μg mL^{-1}). One gram of soil and 0.1 g of earthworm cast were added to seperate 12 mL tubes and then the tubes were spiked with 15 and 5 μL ^{13}C-caffeine, respectively. Five milliliter, 1mL and 0.5 mL Millipore water and 10 mL, 2 mL and 1 mL acetonitrile (ACN) containing 1% acetic acid (HAC) (extraction solvent) were added to earthworm body, soil and earthworm cast samples, respectively. Earthworm bodies were stirred using a stirrer mixer immediately after the addition of the extraction solvent. The tubes of soil and earthworm casts were shaken for 30 min in an end-over-end shaker. Then, all tubes were vortexed and centrifuged at 3500 rpm for 5 min after the addition of CH_3COONa (1, 0.2 and 0.1 g for earthworm body, soil and earthworm cast samples, respectively) and $MgSO_4$ (4, 0.8 and 0.4 g for earthworm body, soil and earthworm cast samples, respectively). After that, 250 μL of supernatant and 250 μL of Millipore water were added to a LC filter vial and analyzed using LC-MS/MS.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The contents of chlorpyrifos, and its two metabolites TCP and chlorpyrifos-oxon were determined by LC–MS/MS using an Acquit UPLC HSST3 RP C18 column (1.8-μm particle size, 100 mm in length, 2.1-mm i.d.) (Waters, The Netherlands). The mobile phases consisted of 5 mM ammonium formate and 0.1 % formic acid in Millipore water (solvent A) and a 95:5 MeOH: H_2O (Millipore) solution (solvent B). The LC gradient times for the separation were: isocratic from 0 to 1 min (100% A: 0% B); from 1 to 2.5 min, a linear increase of B from 0 to 45%; isocratic from 2.5 to 8.5 min (0% A:100% B); from 8.5 to 11.5 min, a linear decrease of B from 100 to 0%; and isocratic from 11.5 to 12 min (100% A:0% B). Initial conditions were re-established every 2 minutes for a total running time of 14 minutes. The column temperature was 45 °C, and

the flow rate was 0.4 mL min^{-1}. Optimization of the ionization and fragmentation conditions for the analytes was obtained by the infusion of solutions of the individual analytes. Optimum responses were obtained by electrospray ionization in the positive and negative-ion mode using the following source parameters: capillary voltage of 3.0 kV, cone voltage of 4 V, source temperature of 150 °C, desolvation gas temperature of 450 °C, and gas flows of 150–148 L hr^{-1} (cone) and 800–794 L hr^{-1} (desolvation). The transitions acquired for chlorpyrifos, TCP and chlorpyrifos-oxon are given in Table S3.3.

Quality control

Analyses were done according to the guidance document (EC, 2019). The quantification of chlorpyrifos, TCP and chlorpyrifos-oxon in the samples was based on multi-level calibrations using solvent standards. Standard calibration curves at concentrations of 0, 0.1, 0.5, 1, 5, 10, 50 ,100 and 250 ng mL^{-1} were prepared from a mix solution that combined the reference standards of all compounds in solvent (ACN with 1 % HAc and Millipore water) and the standard series (0, 0.1, 0.5, 1, 5, 10, 50, 100, 150 and 250 ng mL^{-1}) were prepared in a matrix extract of earthworm and Millipore water. The level of the standard calibration curve is different for each compound and the calibration curve indicated satisfactory linearity, with correlation coefficients > 0.99 and residuals lower than ± 20% response. The concentration exceeding the highest standard of the calibration curve was diluted with a solvent mix of ACN with 1% HAc and Milipore water. The earthworm cast samples were spiked at concentrations of 0.5 µg g^{-1} and 5 µg g^{-1}, the soil samples at 0.005 µg g^{-1} and 0.05 µg g^{-1}, and the earthworm body samples at 0.5 µg g^{-1}, respectively. Unspiked samples were also analyzed to confirm the absence of chlorpyrifos, TCP and chlorpyrifos-oxon residues in the blank samples. The recoveries of the spiked samples in the different matrices were between 70 and 120%. The limit of quantification (LOQ) of each compound corresponds to the lowest calibration standard where a clear identification of the compound was possible. A LOQ of 0.04 µg g^{-1} was achieved for soil samples and 0.1 µg g^{-1} for earthworm cast samples for chlorpyrifos, TCP and chlorpyrifos-oxon while for earthworm body samples, the LOQ was 0.3 µg g^{-1} for chlorpyrifos, 0.03 µg g^{-1} for TCP and 0.005 µg g^{-1} for chlorpyrifos-oxon.

Results

Interaction effects of MPs and CPF

Two-way ANOVA tests were conducted to determine to the interaction effects of two factors, MP and CPF, on their biogenic transport in soil and earthworm health (Table S3.7). Results indicate that Bio-MPs and CPF had a significant interaction effect on earthworm weight change ($F(4,27)=3.023$, $p = 0.033$).

Bio-MPs showed significant interaction effects on the MP contents in earthworm casts ($F(2,18)=5.521$, $p = 0.014$), 0-10 cm soil ($F(2,18)=4.155$, $p = 0.033$) and 10-20 cm soil ($F(2,18)=4.078$, $p = 0.035$). LDPE-MPs and CPF showed significant interaction effects on the CPF contents in earthworm casts ($F(2,18)=4.579$, $p = 0.026$), 0-10 cm soil ($F(2,18)=6.135$, $p = 0.009$) and 10-20 cm soil ($F(2,18)=6.295$, $p = 0.010$). LDPE-MPs and CPF showed a significant interaction effect on the transport rate of MPs in the 10-20 cm soil ($F(2,18)=4.350$, $p = 0.030$). Bio-MPs and CPF had a significant interaction effect on the transport rate of CPF in earthworm bodies ($F(2,18)=4.0.13$, $p = 0.036$).

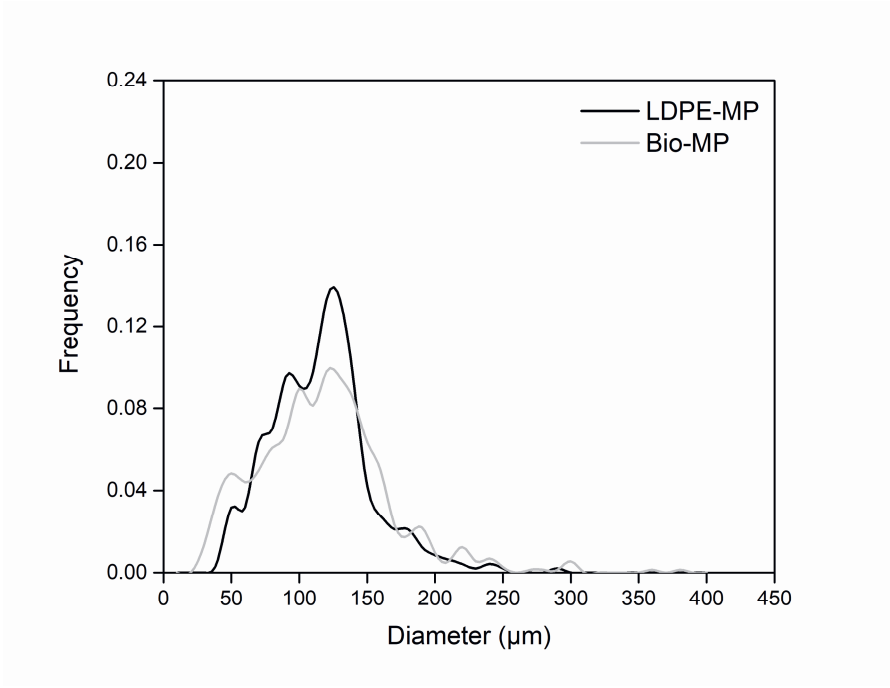

Figure S3.1 *Size distributions of LDPE-MPs and Bio-MPs*

Table S3.1 *Adsorption efficiency and loading capacity of chlorpyrifos on LDPE-MPs and Bio-MPs in different treatments*

MP	Treatment	Adsorption efficiency (%)
LDPE-MP	7+1C	46.7%
	7+5C	10.6%
	28+1C	47.3%
	28+5C	16.7%
Bio-MP	7+1C	66.5%
	7+5C	46.4%
	28+1C	68.4%
	28+5C	53.6%

Table S3.2 *Average earthworm weight ± standard deviation (gram) in different treatments in LDPE-MP and Bio-MP experiment*

Treatment	LDPE-MP experiment	Bio-MP experiment
Control	4.63±0.04	4.06±0.09
Solvent control	4.63±0.09	3.97±0.05
0+1C	4.66±0.08	3.92±0.02
0+5C	4.69±0.10	3.95±0.05
7+0C	4.62±0.06	3.95±0.04
7+1C	4.61±0.10	4.02±0.03
7+5C	4.62±0.05	3.98±0.03
28+0C	4.60±0.01	4.15±0.10
28+1C	4.51±0.04	4.15±0.10
28+5C	4.50±0.04	4.19±0.11

Table S3.3 *The transitions acquired for chlorpyrifos, TCP chlorpyrifos-oxon and 13C-caffeine*

Analyte	Parent ion ($m\ z^{-1}$)	Daughter ion ($m\ z^{-1}$)	Dwell (sec)	Cone Voltage (V)	Collision Energy (eV)
Chlorpyrifos	350	Q:198	0.05	20	17
Chlorpyrifos	352	q:200	0.05	20	17
Chlorpyrifos-oxon	334	Q:197.9	0.05	25	30
Chlorpyrifos-oxon	334	q:277.8	0.05	25	17
TCP	196	Q: 35	0.05	30	15
TCP	198	q :37	0.05	30	15
^{13}C- caffeine	198	140	0.2	20	20

Q: Transition used for quantification; q: transition used for confirmation.

Table S3.4 *Earthworm weight change and reproduction in control and solvent control*

	Weight change (%)			Reproduction (cocoons produced per worm)		
	Control	Solvent control	p value	Control	Solvent control	p value
LDPE-MP	-10.7±7.6	-7.5±8.5	0.594	1.0±0.2	1.1±0.2	0.914
Bio-MP	4.4±5.0	5.8±6.1	0.672	0.6±0.4	0.8±0.2	0.414

Table S3.5 *Contents of two chlorpyrifos metabolites (chlorpyrifos-oxon and TCP) in earthworm casts and bodies in LDPE-MP and Bio-MP experiment.*

			Treatments								
			CK	0+1C	0+5C	7+0C	7+1C	7+5C	28+0C	28+1C	28+5C
EW casts ($mg\ kg^{-1}$)	Chlorpyrifos-oxon	LDPE-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		Bio-MP	<LOQ	<LOQ	0.003±0.005a	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.005±0.006 a
	TCP	LDPE-MP	<LOQ	0.26±0.06b	1.09±0.52a	<LOQ	<LOQ	1.29±0.50a	<LOQ	0.29±0.03ab	1.33±0.41a
		Bio-MP	<LOQ	0.24±0.07d	1.38±0.41b	<LOQ	0.57±0.16c	2.88±0.29a	<LOQ	0.80±0.24c	2.88±0.70a
EW bodies ($mg\ kg^{-1}$)	Chlorpyrifos-oxon	LDPE-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		Bio-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	TCP	LDPE-MP	<LOQ	0.07±0.04b	0.50±0.35a	<LOQ	0.04±0.01b	0.51±0.07a	<LOQ	0.09±0.04b	0.34±0.25a
		Bio-MP	<LOQ	0.04±0.03c d	0.28±0.16a	<LOQ	0.07±0.02b c	0.24±0.13a b	<LOQ	0.03±0.02d	0.11±0.04ab

Different letters indicate significant differences among treatments (one-way ANOVA, $p<0.05$). Treatment abbreviations: 0, 7, 28 represent 0%, 7%, 28% ($w\ w^{-1}$) litter concentrations of microplastics; 1C, 5C represent 250 g ha^{-1}, 1250 g ha^{-1} application rates of chlorpyrifos. LOQ: limit of quantification.

Table S3.6 *Contents of two chlorpyrifos metabolites (chlorpyrifos-oxon and TCP) in 0-10 cm and 10-20 cm soil depths in LDPE-MP and Bio-MP experiment*

			Treatments								
			CK	0+1C	0+5C	7+0C	7+1C	7+5C	28+0C	28+1C	28+5C
0-10 cm (mg kg^{-1})	Chlorpyrifos-oxon	LDPE-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		Bio-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	TCP	LDPE-MP	<LOQ	<LOQ	0.07±0.04a	<LOQ	<LOQ	0.06±0.02a	<LOQ	<LOQ	<LOQ
		Bio-MP	<LOQ	<LOQ	0.11±0.04 a	<LOQ	<LOQ	0.1±0.02a	<LOQ	<LOQ	0.04±0.01b
10-20 cm (mg kg^{-1})	Chlorpyrifos-oxon	LDPE-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		Bio-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	TCP	LDPE-MP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
		Bio-MP	<LOQ	<LOQ	0.08±0.04a	<LOQ	<LOQ	0.07±0.01a	<LOQ	<LOQ	<LOQ

Different letters indicate significant differences among treatments (one-way ANOVA, $p<0.05$). Treatment abbreviations: 0, 7, 28 represent 0%, 7%, 28% (w w^{-1}) litter concentrations of microplastics; 1C, 5C represent 250 g ha^{-1}, 1250 g ha^{-1} application rates of chlorpyrifos. LOQ: limit of quantification.

Table S3.7 *Two-way ANOVA test for the interaction effects of two factors (microplastic and chlorpyrifos) on earthworms (weight change and reproduction), microplastic and chlorpyrifos residues, and their transport rates*

Factors		F (*p*)													
		Weight change	Repro-duction	MP content			CPF content				MP transport rate		CPF transport rate		
				EW casts	0-10 cm	10-20 cm	EW casts	0-10 cm	10-20 cm	EW bodies	0-10 cm	10-20 cm	0-10 cm	10-20 cm	EW bodies
LDPE-MP CPF	*	0.537 (*p*=0.710)	0.633 (*p*=0.643)	0.332 (*p*=0.722)	2.469 (*p*=0.113)	2.031 (*p*=0.160)	4.579 (***p*=0.026**)	6.135 (***p*=0.009**)	6.295 (***p*=0.010**)	-	3.366 (*p*=0.057)	4.350 (***p*=0.030**)	3.593 (*p*=0.051)	1.307 (*p*=0.300)	-
Bio-MP * CPF		3.023 (***p*=0.033**)	0.473 (*p*=0.755)	5.521 (***p*=0.014**)	4.155 (***p*=0.033**)	4.078 (***p*=0.035**)	1.021 (*p*=0.383)	0.975 (*p*=0.396)	0.621 (*p*=0.549)	2.424 (*p*=0.117)	1.053 (*p*=0.369)	0.527 (*p*=0.599)	0.741 (*p*=0.491)	0.357 (*p*=0.705)	4.013 (***p*=0.036**)

"-" means the data was not used due to the results being lower than the limit of quantification (LOQ). For weight change and reproduction, the df_1 and df_2 is 4 and 27, respectively. For MP and CPF content and transport rates, the df_1 and df_2 is 2 and 18, respectively. Df_1 and df_2 mean the degree of freedom of the interaction of factors and degree of freedom of residuals.

Chapter 4 Effects of aged microplastics on the dissipation of mixture pesticides in sandy soil

Based on:
Ju, H., Yang, X., Osman, R., Geissen, V., Effects of aged microplastics on the dissipation of mixture pesticides in sandy soil. To be submitted to Sci Total Environ.

Abstract

The aging of microplastics (MPs) can alter their interactions with other contaminants such as pesticides in soil. However, the effects of aged MPs on the persistence of pesticides, especially pesticide mixtures, remain unclear. In the present study, we examined the effects of aged MPs (low-density polyethylene (LDPE), and biodegradable (polybutylene adipate terephthalate blended with polylactic acid, Bio), 0.2% w w^{-1}) on the dissipation of pesticides (chlorpyrifos (CPF), difenoconazole (DIF), their mixture) in sandy soil. Our results showed that aged MPs increased the degradation half-life (DT50) of CPF when it was applied both individually and in a mixture. In contrast, the DT50 of DIF was not affected by aged MPs. However, the DT50 of DIF was higher when DIF was applied in a mixture than when applied individually. In conclusion, the dissipation of pesticides can be affected by both MPs and pesticide application methods. Further studies should consider the inclusion of aged MPs and mixtures of contaminants as realistic pollution scenarios, to enhance our understanding of the interactions of MPs with other contaminants in soil.

4.1 Introduction

The exponential increase in plastic production and consumption has led to significant environmental challenges, with the emergence of microplastics as a major concern (Thompson et al., 2004). Microplastics have permeated various ecosystems, from the deepest oceans to the highest mountains, posing potential risks to both the environment and human health (Koelmans et al., 2022). Microplastics can originate from two primary sources: primary microplastics and secondary microplastics. Primary microplastics are intentionally manufactured small plastic particles, such as microbeads used in personal care products or microfibers released during the washing of synthetic textiles. Secondary microplastics result from the fragmentation and degradation of larger plastic items, such as plastic mulch films used in agriculture application (An et al., 2020). Plastic mulch film has become a widely used agricultural practice for temperature maintenance, moisture retention, and weed suppression, ultimately enhancing crop productivity. However, traditional plastic mulch films, such as low-density polyethylene (LDPE) film, pose significant environmental concerns due to their non-biodegradability and prolonged persistence in the environment for decades (Khalid et al., 2023). To mitigate the plastic pollution in agriculture soil, biodegradable plastic mulch films have emerged as a promising solution. Biodegradable plastic mulch films are designed to be tilled into soil after use and break down into natural compounds, such as carbon dioxide, water, and biomass, through the action of microorganisms present in the soil (Kasirajan and Ngouajio, 2012). Biodegradable polymers are derived from two resources: synthetic and natural. Polylactic acid (PLA), or polyhydroxyalkanoates (PHAs) are derived from bio-based resources, while polybutylene adipate co-terephthalate (PBAT) is an oil-based resource from fossil (Akhir and Mustapha, 2022). However, the degradation of biodegradable plastics in soil often does not occur as expected. For example, Han et al. (2021) have reported that the degradation rates of PBAT film in four types of soil only ranged from 0.3% to 16% after 120 days. Increasing evidence has reported adverse effects of biodegradable MPs on soi-dwelling organisms and plants (Fan et al., 2022). Therefore, the environmental risks associated with biodegradable plastic debris, especially MPs, need to be aware of.

Pesticides also play a critical role in agriculture application by protecting crops from pests, diseases, and weeds, ensuring higher yields. However, the use of pesticides has raised concerns about their potential impacts on human health and the environment (Rani et al., 2021). Understanding the dissipation process of pesticides in soil is essential for assessing their environmental behaviour and designing sustainable pesticide management strategies.

Pesticide dissipation refers to the gradual decline or disappearance of pesticide residues in soil over time. The dissipation of pesticides in soil can occur through several mechanisms, including physical processes such as evaporation and leaching, chemical degradation, and biological transformations driven by soil microorganisms (Müller et al., 2007). This process involves complex interactions between the pesticide and various environmental factors such as soil properties, climate conditions, microbial activity, and management practices (Farha et al., 2016). Studies have revealed that the introduction of organic residues generated from livestock, urban, agricultural and industrial activities can act as sorbents for pesticides, thereby influencing the adsorption-desorption processes and dissipation of pesticide in soil (Carpio et al., 2021). Microplastics, as an emerging organic contaminant in soil ecosystems, have been proven to be a sorbent for various organic contaminants including pesticides due to their high hydrophobicity and low specific surface area (Fu et al., 2021). The sorption of pesticides onto MPs may change their fate and persistence, potentially prolonging their presence in the environment (Ju et al., 2022). Furthermore, MPs in the environment may undergo aging processes including UV radiation, hydrolysis, mechanical abrasion, and microbial degradation etc. Studies have indicated that aged MPs showed higher adsorption capacities of pesticides, which is due to the changes in the physicochemical properties of MPs (Lan et al., 2021; Ju et al., 2023). Even though studies have disclosed that the presence of pristine MPs can inhibit the dissipation of pesticides in soil (Ju et al., 2022), how aged MPs affect the fate of pesticides in the soil is still unclear.

Pesticide pollution in agriculture soil is often caused by various types of pesticides (Silva et al., 2019; Yang et al., 2022). In most agronomic scenarios, crop protection programs often involve a sequence of herbicide, fungicide, and insecticide applications. Farmers often employ multiple pesticides to target different pests or diseases, which can result in the co-application of different chemical compounds in the same field. Additionally, pesticide residues from previous applications can persist in soil and accumulate over time. Furthermore, pesticide mixtures can occur due to the drift of pesticide sprays from neighboring fields or the use of pesticide-contaminated water sources for irrigation (Guo et al., 2019). Silva et al. (2019) has revealed that the 58% of 317 agricultural topsoil samples from across the European Union contained two or more pesticide residues. Previous studies have indicated that the dissipation of pesticide mixtures differed with the dissipation of individual pesticides (Fogg et al., 2003; Swarcewicz and Gregorczyk, 2012). Swarcewicz and Gregorczyk (2012) have reported that a mixture of pendimethalin, thiamethoxam, and mancozeb significantly inhibited the rate of degradation of pendimethalin. The prolonged persistence of pesticide

mixtures may cause combined effects in soil ecosystems. For example, Van Hoesel et al. (2017) have found that the simultaneous exposure to pesticide mixture including insecticides, fungicides, and herbicides can lead to a synergistic toxicity to earthworms. In addition, multiple pesticides were also found in plastic mulch film residues (Guo et al., 2019), which may affect the persistence of multiple pesticides. Liu et al. (2022) have suggested that the competitive adsorption of pesticides on MPs could occur in the binary pesticide system. This may also happen in soil matrices which indicates that the impact of MPs on the fate of combined pesticides could differ from that of single pesticides. However, the knowledge on the impacts of MPs on the persistence of multiple pesticides in soil is still missing.

In this study, we used LDPE and biodegradable (PBAT blended with PLA) MPs that were aged in soil for 1 month, pesticides chlorpyrifos and difenoconazole to study the effects of aged MPs on the dissipation of pesticides in soil. The pesticides were applied both individually and in a mixture. We hypothesized that: (1) aged MPs may inhibit the dissipation of pesticides in soil; (2) aged MPs may exert different effects on the dissipation of pesticide mixture than single pesticide. Our results and discussion may provide the missing knowledge on the aged MPs on the fate of pesticides, especially pesticide mixtures, in soil.

4.2 Materials & Methods

4.2.1 Materials

We obtained solid chlorpyrifos and difenoconazole (99.6% purity) from HPC Standards GmbH (Cunnersdorf, Germany) and dissolved them in acetone to create a stock solution with a concentration of 2.5 mg mL^{-1}. Low-density polyethylene (LDPE) pellets and biodegradable (Bio) plastic pellets, which consisted of 85% poly(butylene adipate co-terephthalate) (PBAT), 10% polylactic acid (PLA), and 5% calcium carbonate, were used for the preparation of microplastics (MPs). The plastic pellets were frozen with liquid nitrogen, grounded, and sieved to obtain MPs ranging from 200 to 500 µm. Aged LDPE-MPs and Bio-MPs were prepared by introducing pristine LDPE-MPs and Bio-MPs into the experimental soil one month prior to the experiment. The sandy soil used in this study comprised 83% sand, 11% silt, and less than 1% clay, with an organic matter content of 4% and a soil pH of 6.0. Ceramic pots and radish seeds were procured from the horticultural market in Wageningen.

4.2.2 Experiment set-up and sampling

The experiment comprised of three factors: microplastics (MPs) (no MP, 1-month aged LDPE-MP, and 1-month aged Bio-MP) × pesticides (CPF, DIF, a mixture of CPF and DIF) × sampling time (day 0, 1, 3, 7, 14, and 35). In total, the experiment included nine treatments with 6 sampling time points and 3 replications. Fifty grams of dry sandy soil was added in the pot and the soil moisture was set at 20% (w w^{-1}). The exposure concentration of MPs and pesticides was set at 0.2% (w w^{-1}) and 15 mg kg^{-1}, respectively, which were considered as environmentally relevant (references). Prior to the start of the experiment, the soil was incubated in the Unifarm at Wageningen university for one month. The soil moisture was maintained by replenishing water to compensate for any weight loss. The experiment lasted for five weeks, from September 8th, 2022, to October 15th, 2022. On September 8th, 2022, four radish (*Raphanus sativus*) seeds were sown in each pot to simulate the scenario with crop growing. The experiment was conducted indoors at 15 ℃ with natural light resource. After one week, the seedlings were thinned to one per pot. At each time point of sampling, soil samples were collected for the measurement of pesticide residues. The collected soil samples were stored at -20 ℃ before the extraction was implemented.

4.2.3 Pesticide extraction and analysis

The pesticide extraction procedure was conducted following a QuEChERS approach. Five grams of soil sample were placed into 50 mL tubes and then fortified with 25 µL of 13C-caffeine (10 µg mL^{-1}). Then 5 mL of Millipore water and 10 mL of acetonitrile containing 1% acetic acid (serving as the extraction solvent) were separately added to the soil samples. After that, 1 g of CH_3COONa and 4 g of $MgSO_4$ were added in the tubes. Then the tubes were shaken for 30 minutes in an end-over-end shaker, followed with vortex, and centrifuging at 3500 rpm for 5 minutes. After centrifugation, 250 µL of supernatant and 250 µL of Millipore water were added in an LC filter vial for analysis using LC-MS/MS.

4.2.4 Statistics

The dissipation rates are presented as the percentage of dissipated pesticides in the soil. The dissipation data of pesticides was fitted with Zero-order kinetic model, First-order kinetic model and Second-order kinetic model for the calculation of half-life (DT50). The equations are as follows:

Zero-order kinetic model: $C_t = C_0 - kt$ (1)

DT50 = $\frac{C_0}{2k}$ (2)

First-order kinetic model: $C_t = C_0 e^{-kt}$ (3)

DT50 = $\frac{ln2}{k}$ (4)

Second-order kinetic: $C_t = \frac{C_0}{1+C_0kt}$ (5)

DT50 = $\frac{1}{kC_0}$ (6)

where C_t and C_0 indicate the contents (mg kg^{-1}) of pesticides in soil at time t (day) and at t=0. k indicates the dissipation rate constant (day^{-1}).

4.3 Results

4.3.1 Dissipation rate of chlorpyrifos in soil

In CPF only treatment, the CPF showed a more rapid dissipation compared to other treatments throughout the experiment, with the highest dissipation rate at 40.8±5.0% after the experiment (Figure 4.1). In treatment with mixed pesticides, 1-month aged LDPE with CPF and 1-month aged LDPE with mixed pesticides, the dissipation rates of CPF were slightly lower than that in CPF only treatment. However, in treatment with 1-month aged Bio-MPs with CPF and 1-month aged Bio-MPs with mixed pesticides, the dissipation rates of CPF reached 31.6±3.5% and 31.3±4.9% after the experiment, respectively.

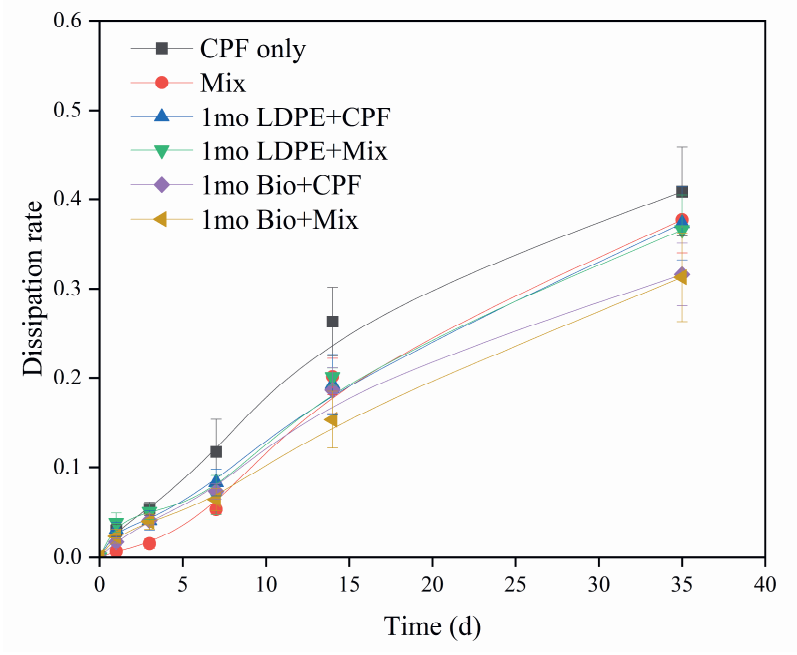

Figure 4.1 *Dissipation rate of chlorpyrifos in soil over time.*

The Zero-order kinetic model, First-order kinetic model and Second-order

kinetic model were fitted with pesticide dissipation data for the calculation of DT50 (Table 4.1). The results showed that the dissipation of CPF was better described by the Second-order kinetic model. The DT50 was 47.7 days in CPF only treatment, which was similar to those in Mix, 1mo LDPE+CPF and 1mo LDPE+Mix treatments. However, the DT50 values were higher in 1mo Bio+CPF and 1mo Bio+Mix treatments at 73.0 and 78.5 days, respectively.

Table 4.1 *Kinetic parameters for the dissipation of chlorpyrifos in soil*

Treatment			First-order kinetic		Second-order kinetic	
	Correlation coefficient (r^2)	DT50 (days)	Correlation coefficient (r^2)	DT50 (days)	Correlation coefficient (r^2)	DT50 (days)
CPF	0.94	41.63	0.97	43.6	0.99	47.7
Mix	0.97	44.57	0.98	48.8	0.98	56.3
1mo LDPE+CPF	0.99	46.69	0.99	51.8	0.99	60.0
1mo LDPE+Mix	0.97	47.77	0.98	53.2	0.98	61.9
1mo Bio+CPF	0.97	54.27	0.98	61.7	0.99	73.0
1mo Bio+Mix	0.99	56.10	0.99	65.0	0.99	78.5

4.3.2 Dissipation rate of difenoconazole in soil

The dissipation of DIF in soil was slower than CPF, with the dissipation rate at 22.0±2.2% after the experiment (Figure 4.2). Similarly, the dissipation of DIF was also more rapid in DIF only treatment compared to other treatments. Compared to single pesticide application treatments (DIF only, 1mo LDPE+DIF, 1mo Bio+DIF), the dissipation of DIF was slower in mixed pesticide treatment (Mix, 1mo LDPE+Mix, 1mo Bio+Mix).

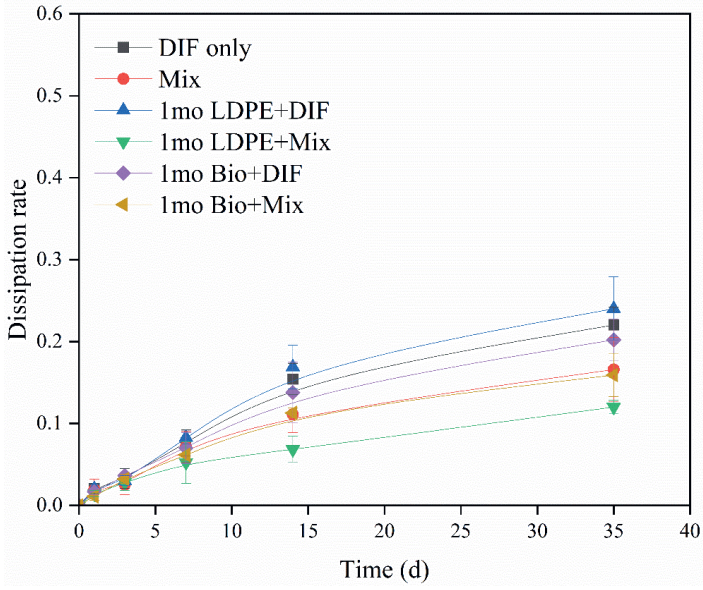

Figure 4.2 *Dissipation rate of difenoconazole in soil over time.*

The dissipation of DIF was also better fitted with the Second-order kinetic model. The DT50 values were 117.9, 113.4 and 118.1 days in DIF only, 1mo LDPE+DIF, 1mo Bio+DIF treatments, which were lower than those in Mix, 1mo LDPE+Mix, 1mo Bio+Mix where the DT50 values were 174.2, 271.5 and 179.9 days, respectively (Table 4.2).

Table 4.2 *Kinetic parameters for the dissipation of difenoconazole in soil*

Treatment	Zero-order kinetic		First-order kinetic		Second-order kinetic	
	Correlation coefficient (r^2)	DT50 (days)	Correlation coefficient (r^2)	DT50 (days)	Correlation coefficient (r^2)	DT50 (days)
DIF	0.92	78.6	0.93	94.7	0.95	117.9
Mix	0.91	107.0	0.92	134.1	0.93	174.2
1mo LDPE+DIF	0.91	73.4	0.93	84.4	0.95	113.4
1mo LDPE+Mix	0.94	155.0	0.94	200.9	0.95	271.5
1mo Bio+DIF	0.94	86.3	0.94	105.7	0.95	118.1
1mo Bio+Mix	0.9	110.2	0.92	138.4	0.93	179.9

4.4 Discussion

Pesticide usage has increased significantly over the years due to expanding agricultural practices, growing global population, and the need to protect crops from pests and diseases, leading to concerns about their potential environmental and health impacts. Understanding the dissipation of pesticides in soil is crucial for managing their environmental impact. The introduction of

MPs into soil can lead to redistribution of pesticides in the soil matrix due to the adsorption of pesticides onto MPs, which might change the availability of pesticides for transport via runoff to surface waters, leaching to groundwater, volatilization into air, degradation/ transformation by microbial activity, and uptake by plants. Increasing evidence has shown that the presence of MPs can increase the persistence of pesticides in soil (Peña et al., 2023). For example, our previous study has shown that both pristine LDPE-MPs and Bio-MPs inhibited the degradation of CPF in earthworm burrow soil (Ju et al., 2022). However, MPs in soil can suffer aging processes (including ultraviolet light radiation, hydrolysis, microbial degradation etc.) and be covered by biofilm, thereby changing the adsorption-desorption behaviours of pesticides on MPs. On the one hand, the change of physicochemical properties after aging might increase the adsorption capacities of MPs, which can facilitate the adsorption of pesticides on the MPs. On the other hand, the MPs may be colonized by biofilm and covered by soil particles which may reduce the opportunity for them to contact pesticides. However, the effects of aged MPs on the behaviours of pesticides in soil are still unclear. In the present study, the order of DT50 of CPF in single pesticide treatments was CPF-only < aged LDPE+CPF < aged Bio+CPF. Hu et al. (2023) have found that LDPE-MPs (0.2%, w w^{-1}) had no effect on the degradation of thiamethoxam in soil while biodegradable MPs (polycaprolactone and polybutylene succinate, 0.2%, w w^{-1}) led to higher DT50 of thiamethoxam compared to LDPE-MPs and soil without MPs. They believed that the addition of biodegradable MPs could affect the degradation of thiamethoxam through changing of soil microbial community. Our previous study has shown that Bio-MPs had higher adsorption capacity of CPF than LDPE-MPs. Moreover, the desorption rate of CPF from Bio-MPs was much lower than that from LDPE-MPs (Ju et al., 2023). This can lead to lower dissipation of CPF in soil. The DT50 of DIF in single pesticide treatments was not affected by aged LDPE-MPs and Bio-MPs. A previous study has indicated that the adsorption capacity of DIF on aged PE-MPs was higher than that on pristine PE-MPs (Lan et al., 2021). However, the dissipation of DIF in soil was not affected by the presence of aged LDPE-MPs. Therefore, the adsorption behaviours of organic contaminants on microplastics in aqueous systems may not always fully explain their dissipation in soil matrices.

Interestingly, the DT50 values of CPF in treatments with pesticide mixtures showed no significant difference compared to those in treatments with single pesticide. However, the DT50 values of DIF were much higher in treatments with pesticide mixtures than those in treatments with single pesticide. Understanding the dissipation of pesticide mixtures in soil is crucial for assessing their environmental fate and potential risks. Despite pesticide

pollution in agricultural soil arising from the presence of multiple pesticides (Silva et al., 2019), the knowledge on the dissipation of pesticide mixtures in the soil is still ambiguous. Fu et al. (2020) found that the half-life of pesticides (cyromazine and acetamiprid) in cowpea and soil in the mixed-pesticide group was comparable to or even shorter than that in the single-pesticide group. Balkan and Kara (2023) found that the degradation of pesticides could be both higher and lower when applied in a mixture compared to single pesticide application. Swarcewicz and Gregorczyk (2012) found that the pendimethalin mixed with mancozeb or with mancozeb and thiamethoxam inhibited the dissipation of pendimethalin in soil. Pesticides within a mixture may compete for the same degradation pathways or be subject to similar metabolic processes within the soil. As a result, the presence of multiple pesticides can create competition among them for degradation resources, leading to a slower degradation rate overall. Pesticides can affect soil microorganisms responsible for the degradation of these compounds. In some cases, one pesticide within a mixture may inhibit the activity of microorganisms involved in the breakdown of another pesticide, resulting in a reduced degradation rate for both compounds (Kaufman, 1966). Even though the evidence has indicated the competitive adsorption of multiple pesticides on MPs, which might increase the bioavailability of pesticide in soil. The dissipation of pesticide mixture was not facilitated in the present study. The increased persistence of pesticide mixture might increase the risk of pesticide being bioaccumulated by crops. Therefore, further research should pay more attention to the effects of MPs on the dissipation of pesticide mixtures in soil.

4.5 Conclusion

The results of this study showed that the dissipation of CPF was affected by aged MPs, resulting in higher DT50 values in treatments with MPs and both single CPF and pesticide mixtures. Differently, the dissipation of DIF was not affected by MPs but was affected by pesticide application method, leading to higher DT50 values in treatments with pesticide mixtures compared to DIF alone. Our study highlights the effects of MPs on the behaviours of other contaminants in soil could be complex in realistic pollution scenarios. Therefore, further studies investigating the interactions between MPs and other contaminants in soil should consider using aged MPs and mixtures of other contaminants.

Acknowledgement

We are grateful for the funding provided by the National Natural Science Foundation of China (41877072), the EU Horizon 2020 project (MINAGRIS) and the China Scholarship Council (CSC: 201904910501)

Chapter 5 Pesticide bioaccumulation in radish produced from soil contaminated with microplastics

Based on:
Ju, H., Yang, X., Tang, D., Osman, R., Geissen, V., 2024. Pesticide bioaccumulation in radish produced from soil contaminated with microplastics. Sci Total Environ 910, 168395.10.1016/j.scitotenv.2023.168395

Abstract

The aging of microplastics (MPs) in soils may affect crop bioaccumulation of coexisting contaminants. We examined the bioaccumulation of pesticides (chlorpyrifos (CPF), difenoconazole (DIF) and their mixture) in radish (Raphanus sativus) planted in soils contaminated with MPs (low-density polyethylene or biodegradable MPs). The experiment was conducted with different contamination scenarios taking into account the use of aged MPs and pesticide mixtures. Radish root biomass was negatively affected in the scenarios with aged MPs. CPF bioaccumulation in radishes appears to be enhanced by the presence of MPs, especially aged MPs, and the pesticide mixture. The results show that food safety risks associated with the bioaccumulation of individual pesticides and their mixtures are increased in soils polluted by MPs, particularly MP after aging.

Keywords: Aged microplastics, Crop biomass, Biodegradable microplastics, Pesticide residues, Food safety

5.1 Introduction

Microplastics (MPs) are receiving increasing attention because of their adverse effects in both aquatic and terrestrial ecosystems. Soil is considered to be the largest reservoir for MPs, and it is estimated that annual plastic mass release to land may be 4-23 times higher than to oceans (Horton et al., 2017). It is recognized that MP pollution in agroecosystems is worse than in other terrestrial ecosystems because of intensive agricultural practices that introduce plastics into the soil, such as mulching and sewage sludge application (Ng et al., 2018). Plastic mulch film has become a widely used agricultural practice for temperature regulation, moisture retention, and weed suppression, to enhance crop productivity. Low-density polyethylene (LDPE) plastic film is the most commonly used plastic mulch, because of its low cost and good performance (Kasirajan and Ngouajio, 2012). However, the debris of LDPE films, including MPs, are non-degradable and can lead to adverse effects on soil ecosystems (Guo et al., 2020). Biodegradable plastic mulch (BDM) was designed as a substitute for non-degradable plastic film, to reduce plastic pollution in the environment. BDMs can be divided into bio-based and fossil-based mulches depending on the origin of polymers. Poly(butylene adipate-co-terephthalate) (PBAT) is representative of fossil-based plastic mulch, and is usually blended with polylactic acid (PLA) to improve its performance (Han et al., 2020). However, the degradability of PBAT/PLA plastic film in soil is not always sufficient, which may result in MP pollution in the soil (Touchaleaume et al., 2016; Han et al., 2021).

Pesticides also play a critical role in modern agriculture, in protecting crops against weeds, insect infestation and diseases. However, pesticides present in the soil can pose a threat to food safety as they can persist in the environment, contaminate crops, and contribute to the presence of pesticide residues in food, which may lead to adverse health effects. Once introduced in the soil, pesticides can be adsorbed by soil organic matter, which may influence the mobility and persistence of pesticides (Kodesova et al., 2011). The adsorption-desorption behaviours of pesticides on MPs have been widely studied because of their high hydrophobicity and specific surface area (Peña et al., 2023). However, as most of these studies were conducted in aqueous systems, how MPs influence the behaviours of pesticides in soils is still unclear (Peña et al., 2023). Limited studies have reported that MPs could affect the transport of pesticides in soil (Ramos et al., 2015; Huffer et al., 2019). In addition, depending on the properties of MPs and pesticides, the presence of MPs can either enhance, decrease, or leave unaffected the degradation rates of pesticides (Yang et al., 2018; Wu et al., 2022; Zhou et al., 2022). This variability can lead to altered distribution patterns and persistence

of pesticides in soil, influencing their potential uptake by crops. Currently, there is a lack of comprehensive understanding regarding the influence of MPs on the bioaccumulation of pesticides in crops which is directly related to food safety. Additionally, MPs present in the soil undergo diverse aging processes that can alter their physiochemical properties, such as morphology (e.g., cracks and pores), particle sizes, and functional groups. These modifications result in different interaction effects with pesticides compared to pristine MPs (Chang et al., 2022; Ju et al., 2023). However, there is still a significant gap in research relating to the influence of aged MPs on the fate of pesticides in soil, specifically regarding their potential implications for food safety and human health.

In agriculture, different pesticides are used depending on the specific pest or problem being addressed. Moreover, mixtures of various pesticides are also used to enhance crop protection performance, which makes the issue of pesticide pollution in soils rather complicated (Tang and Maggi, 2021). Silva et al. (2019) showed that out of 317 agricultural topsoil samples collected from the European Union, 58% were contaminated with two or more types of pesticides. However, the environmental risks posed by pesticide mixtures may not be accurately predicted based only on the findings of individual pesticide exposure studies. The existing studies on the combined effects of pesticides have shown both synergistic/additive and antagonistic toxicity on non-target organisms (Ruiz de Arcaute et al., 2018; Niedobova et al., 2019). For example, Van Hoesel et al. (2017) found that the simultaneous exposure to pesticide mixtures that include insecticides, fungicides, and herbicides can lead to a synergistic toxicity to earthworms. A field investigation has also discovered the presence of multiple pesticides in plastic debris within the soil. Guo et al. (2019) reported that 30 pesticides were detected on plastic mulching films, and that most of the pesticides originated from historical and recent application. Studies on the environmental and ecotoxicological risks of pesticide mixtures, especially associated with MPs in soils, remain lacking. Liu et al. (2022) have suggested that competitive adsorption of pesticides on MPs could occur in when two pesticides are simultaneously present. This suggests that the impact of MPs on the fate of each pesticide in a mixture may differ from that of individual pesticides. Therefore, in this study, we used a broad-spectrum organophosphate insecticide, chlorpyrifos, and a systemic fungicide, difenoconazole, individually as well as in a mixture, to compare the effects of pristine and aged MPs on pesticide bioaccumulation in crop. Radish (*Raphanus sativus*) was selected as a model plant for its relative susceptibility to pesticide residue contamination (Paustenbach, 2000). MPs derived from low-density polyethylene (LDPE-MP) and biodegradable poly butylene adipate co-terephthalate blended with polylactic acid (Bio-MP) plastic mulch films

were used in this study. We aim to study the effects of pristine and aged MPs on the bioaccumulation of pesticides within the edible parts of radishes, considering two different pesticide application treatments: individual and combined pesticide applications. Our hypotheses are as follows: (1) MPs that have undergone aging in soil may exert distinct effects on the bioaccumulation of pesticides in radish compared to pristine MPs. (2) Regardless of the presence of MPs, the bioaccumulation of pesticide mixtures may differ from that of single pesticides. (3) The bioaccumulation of pesticide mixtures may interact with the age of the MPs in the soil. The findings of this study will provide valuable insight into the potential consequences of soil MP contamination on food safety, particularly regarding the bioaccumulation of pesticides, within a realistic agricultural context in which pesticide mixtures and aged MPs are present.

5.2 Materials and methods

5.2.1 Materials

Solid chlorpyrifos and difenoconazole (99.6% purity) were purchased from HPC Standards GmbH (Cunnersdorf, Germany) and were dissolved in acetone to make a 2.5 mg mL^{-1} stock solution. 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 the MPs. To obtain the MPs, the plastic pellets were frozen with liquid nitrogen and then ground and sieved. The size of the MPs ranged from 200 to 500 µm. The scenarios with aged LDPE-MPs and Bio-MPs were prepared by introducing pristine LDPE-MPs and Bio-MPs in the experimental soil 1 month before the experiment, which were named as 1-month LDPE-MPs or 1-month Bio-MPs hereafter. The sandy soil used in this study consisted of 83% sand, 11% silt and <1% clay with an organic matter content of 4%, and the pH of soil was 6.0. The nutrient solution used was purchased from Plagron (Weert, the Netherlands), which has an NPK of 3-2-5. Ceramic pots were purchased at the horticultural market in Wageningen, the Netherlands.

5.2.2 Pot experiment

The pot experiment consisted of 2 factors: MPs (no MP, pristine LDPE-MP, pristine Bio-MP, 1-month LDPE-MP and 1-month Bio-MP) and pesticide application (no pesticide, CPF, DIF, mixture of CPF and DIF). Overall, the experiment included 20 treatments with 4 replicates (Table 5.1). There are 4 scenarios of exposure that were included in this study: (1) pristine MPs with single pesticide, (2) 1-month MPs with single pesticide, (3) pristine MPs with pesticide mixtures, (4) 1-month MPs with pesticide mixtures. The scenario 1 was less realistic but widely applied in previous studies (Khan et al., 2022). The scenarios 2 to 4 were designed to simulate realistic pollution in agricultural soil, including the presence of multiple pesticides and MPs that have undergone aging processes. In each pot, 600 g of sandy soil with moisture at 20% (w w^{-1}) was added and the exposure concentration of MPs and pesticides were set as 0.2% (w w^{-1}) and 15 mg kg^{-1} (for each pesticide in the treatments with pesticide mixtures). The pots incubated in a tunnel greenhouse at Unifarm, Wageningen University & Research for one month before the start of the experiment. The soil moisture was maintained everyday by replenishing the weight loss of water. The experiment lasted for 5 weeks, from the 8th September 2022 to 15th October 2022. On 8th September 2022, 4 radish (*Raphanus sativus*) seeds were sown in each pot. After 1 week, the germination rate of the radish seeds was recorded, and only two seedlings

were retained in each pot through thinning. The germination of radish seeds remained unaffected across all treatments. The soil was fertilized twice (in weeks 3 and 4) with the nutrient solution. At harvest, the radish samples were collected and cleaned with distilled water. Soil samples were collected for the analysis of soil properties and pesticides. The radish and soil samples were stored at -20°C until pesticide extraction. The aboveground part (leaf) and below ground part (root) of radishes were harvested. The fresh biomass, diameter and length of roots, and the fresh biomass and length of leaves were measured. The soil pH and EC were measured using a pH and EC meter at a soil-to-water ratio of 1:5. The soil bulk density was computed by measuring the soil volume. The total soil carbon and nitrogen were measured using a LECO instrument. The NH_4^+-N and NO_x^--N were measured using Segmented Flow Analysis. The data for soil properties is shown in Table S5.1. The pesticide extraction procedure used was based on a QuEChERS approach (Anastassiades et al., 2003), the detailed information is shown in Supporting materials.

Table 5.1 *Summary of treatments*

Microplastic (MP)	Pesticide application	Treatment	Scenario
No MP	No pesticide	CK	-
	Chlorpyrifos	CPF only	-
	Difenoconazole	DIF only	-
	Pesticide mixture	Mix	-
Pristine LDPE	No pesticide	LDPE only	-
	Chlorpyrifos	LDPE+CPF	Scenario 1
	Difenoconazole	LDPE+DIF	Scenario 1
	Pesticide mixture	LDPE+Mix	Scenario 3
Pristine Bio	No pesticide	Bio only	-
	Chlorpyrifos	Bio +CPF	Scenario 1
	Difenoconazole	Bio +DIF	Scenario 1
	Pesticide mixture	Bio +Mix	Scenario 3
1-month LDPE	No pesticide	1-mo LDPE only	-
	Chlorpyrifos	1-mo LDPE+CPF	Scenario 2
	Difenoconazole	1-mo LDPE+DIF	Scenario 2
	Pesticide mixture	1-mo LDPE +Mix	Scenario 4
1-month Bio	No pesticide	1-mo Bio only	-
	Chlorpyrifos	1-mo Bio+CPF	Scenario 2
	Difenoconazole	1-mo Bio+DIF	Scenario 2
	Pesticide mixture	1-mo Bio+Mix	Scenario 4

CK indicates control blank.

5.2.3 Statistics

Data are presented as mean ± standard deviation. The normality and equality of variances were determined using Shapiro-Wilk test and Levene's test, respectively. One-way ANOVA with Duncan's post-hoc comparisons were conducted to test the differences among treatments. Two-way ANOVA tests were conducted to test the effects of two factors: MP (including no MP, pristine LDPE-MP, pristine Bio-MP, 1-month LDPE-MP and 1-month Bio-MP) and pesticide application (including single pesticide application and pesticide mixture application) on radish biomass and pesticide distribution. All the tests were performed using IBM SPSS Statistics 26 and the critical *p* value of significance was set at 0.05. All graphs were drawn using origin 2023.

5.3 Results

5.3.1 Radish growth performance

The fresh root biomass of the radish in treatments with pristine LDPE-MPs and 1-month LDPE-MPS were 100% higher and 44% lower than that in the control (Figure 5.1a). In the treatments with pristine Bio-MPs and 1-month Bio-MPs, the fresh root biomass of the radish was 53% and 59% lower than control. The effects of MPs on leaf fresh biomass and root-to-shoot ratio followed the same trend as fresh root biomass but were not significant (Figure 5.1b and 5.1c). The root diameter and root length of the radish were significantly higher in treatments with pristine LDPE-MPs (Figure 5.1d and 5.1e). However, leaf length was not affected by MPs (Figure 5.1f). The data from treatments with the addition of both MPs and pesticides were combined and shown in the violin plots in Figure 5.1. The results indicate that the effects from MPs and pesticides followed the same trend as when only MPs were present, but without statistical significance, which means the effects on the growth of radish were mainly from MPs.

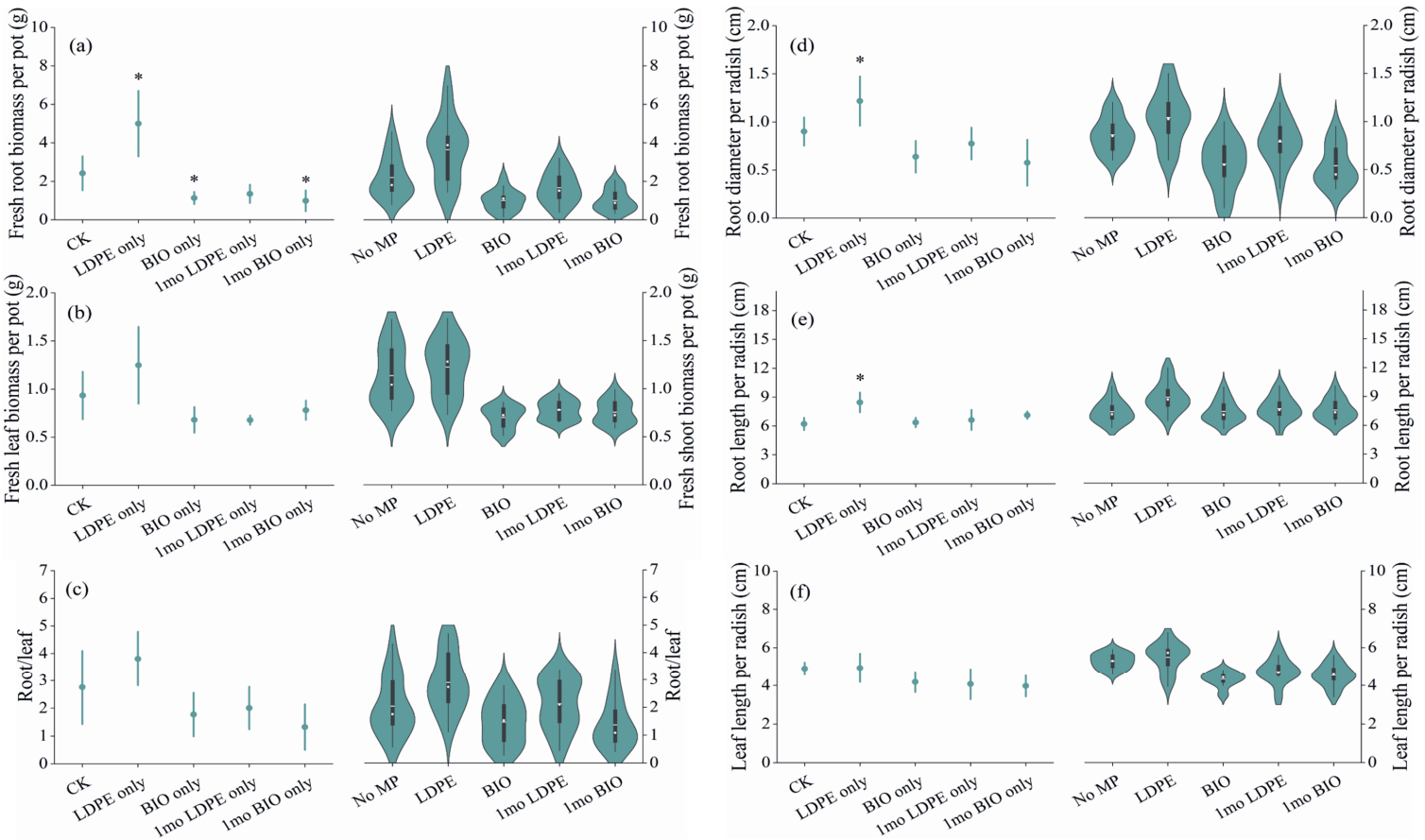
(a)
Fresh root biomass per pot (g)
(b)
Fresh leaf biomass per pot (g)
Fresh shoot biomass per pot (g)
(c)
Root/leaf
(d)
Root diameter per radish (cm)
(e)
Root length per radish (cm)
(f)
Leaf length per radish (cm)
CK
LDPE only
BIO only
1mo LDPE only
1mo BIO only
No MP
LDPE
BIO
1mo LDPE
1mo BIO

Figure 5.1 *The effects of microplastics on radish fresh root biomass (a), fresh shoot biomass (b), root-to-shoot ratio(c), root diameter (d), root length (e) and leaf length (f). The scatter plots (left) indicate the effects of microplastics on radish growth parameters in control and treatments with different microplastic but without pesticide addition. The violin plots (right) indicate the summary of the effect of microplastics on radish growth parameters combining the treatments with pesticides. The line and square in the violin plots represent the mean and the median. The "*" in the scatter plots represents significant difference compared to the control.*

5.3.2 Pesticide residues in soil

The contents of CPF in the soil were significantly higher in most treatments compared to that in the CPF only treatment, except in treatment with pristine LDPE-MPs (Figure 5.2). However, the contents of DIF were only significantly higher in the Bio+DIF and 1-month LDPE+Mix treatments compared to the DIF only treatment (Figure 5.2). Moreover, the contents of CPF in soil were significantly higher in Mix and LDPE+Mix treatments than in CPF-only and LDPE+CPF treaments. The contents of DIF in soil were significantly higher in 1-month LDPE+Mix and 1-month Bio+Mix treatments than l-month aged LDPE+DIF and 1-month Bio+DIF treatments.

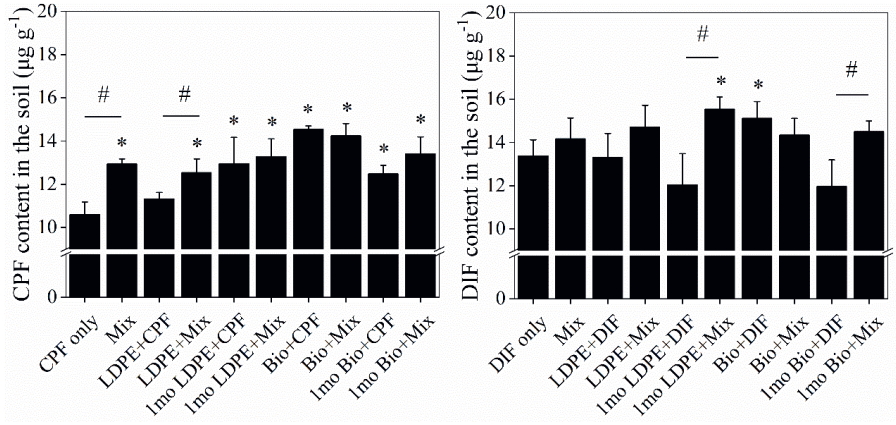

Figure 5.2 *The contents of chlorpyrifos and difenoconazole in the soil at the end of experiment. The "*" represents significant differences compared to pesticide-only treatment (CPF only or DIF only). The "#" represents significant differences between treatments with single pesticides and combined pesticides.*

5.3.3 Pesticide residues in radish

Compared to the CPF-only treatment, the contents of CPF in radish roots were significantly higher in the Mix, LDPE+Mix, 1-month LDPE+Mix, and 1-month Bio+CPF treatments (Figure 5.3a). In addition, the contents of CPF in radish leaves were significantly higher in the Mix, LDPE+Mix, 1-month LDPE+CPF treatment and Bio+CPF, treatment. In treatments with single CPF application, the contents of CPF in radish roots in LDPE+CPF and Bio+CPF treatments were 77.8±34.8 and 157.0±47.4 ng g^{-1}, respectively, which were lower than those in 1-month LDPE+CPF and 1-month Bio+CPF treatments (159.5±12.5 and 344.5±100.2 ng g^{-1}, respectively). In treatments with pesticide mixture, the contents of CPF in radish roots and leaves were higher in Mix treatment (364.8±81.8 ng g^{-1} in roots and 253.5 ±74.9 ng g^{-1} in leaves)

than in CPF-only treatment (109.3±53.2 ng g^{-1} in roots and 130.4±36.1 ng g^{-1} in leaves). In addition, the CPF content in radish roots in the LDPE+Mix and 1-month LDPE+Mix treatment (281.8±70.7 ng g^{-1} and 247.9±44.6 ng g^{-1}, respectively) were higher than those in LDPE+CPF and 1-month LDPE+CPF treatments (77.8±34.8 ng g^{-1} and 159.5±12.5 ng g^{-1}, respectively). The contents of DIF in radish roots and leaves showed no significant difference in any treatment compared to that in the DIF-only treatment (Figure 5.3b). The content of CPF in the roots was significantly lower than the content of CPF in the leaves in the LDPE+CPF treatment. However, in the 1-month Bio+CPF treatment, the content of CPF in the root was significantly higher than the content of CPF in the leaves. The contents of DIF in the roots were significantly higher than that in the leaves in the Bio+DIF and 1-month Bio+DIF treatments.

The bioaccumulation factors (BAFs) of pesticides were determined by calculating the ratio of the contents of pesticides in radish samples to the contents of pesticides in soil at the end of experiment (Table 5.2 and 5.3). The BAFs of CPF in radish roots in Mix and LDPE+Mix treatments were 0.030±0.005 and 0.023±0.007, respectively, which were higher than those in CPF-only treatment (0.010±0.005) and LDPE+CPF treatment (0.007±0.003). The BAFs of CPF in radish leaves, and BAFs of DIF in radish roots and leaves were not significantly different among treatments.

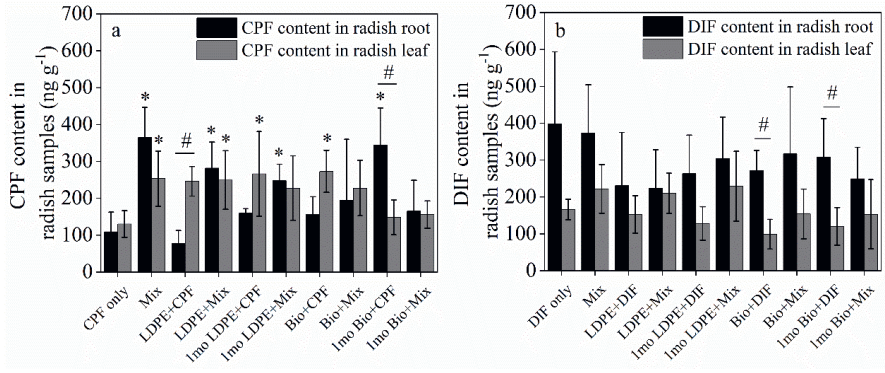

Figure 5.3 *The content of chlorpyrifos (a) and difenoconazole (b) in radish roots and leaves. The "*" represents significant differences compared to pesticide-only treatment (CPF only or DIF only). The "#" represents significant differences between the contents of pesticide in radish roots and leaves.*

Table 5.2 *Bioaccumulation factors (BAFs) of chlorpyrifos (CPF) in radish samples*

Treatment	BAF_{root}	BAF_{leaf}
CPF only	0.010±0.005	0.012±0.003
Mix	0.030±0.005 *	0.020±0.005
LDPE+CPF	0.007±0.003	0.020±0.005
LDPE+Mix	0.023±0.007 *	0.020±0.007
1mo LDPE+CPF	0.012±0.001	0.021±0.011
1mo LDPE+Mix	0.019±0.003	0.017±0.008
Bio+CPF	0.010±0.002	0.017±0.002
Bio+Mix	0.014±0.012	0.016±0.006
1mo Bio+CPF	0.026±0.009 *	0.011±0.004
1mo Bio+Mix	0.013±0.007	0.012±0.003

"*" indicates a significant difference compared to CPF only treatment.

Table 5.3 *Bioaccumulation factors (BAFs) of difenoconazole (DIF) in radish samples*

Treatment	BAF_{root}	BAF_{leaf}
DIF only	0.030±0.014	0.012±0.001
Mix	0.027±0.011	0.016±0.005
LDPE+DIF	0.017±0.010	0.011±0.003
LDPE+Mix	0.015±0.007	0.014±0.004
1mo LDPE+DIF	0.024±0.011	0.012±0.005
1mo LDPE+Mix	0.019±0.007	0.015±0.006
Bio+DIF	0.018±0.004	0.007±0.003
Bio+Mix	0.022±0.013	0.009±0.003
1mo Bio+DIF	0.025±0.007	0.010±0.005
1mo Bio+Mix	0.020±0.010	0.012±0.007

5.3.4 Pesticide distribution

In this study, the fate of pesticide residues can be categorized into four areas: soil, dissipation, radish roots, and leaves (Figure 5.4a and 5.4b). The dissipated pesticides were determined by subtracting the sum of pesticide quantities in soil, radish roots, and leaves from the quantity of added pesticide. The majority of pesticides were either present in soil or dissipated, with a small proportion being absorbed by radishes. The proportions of dissipated CPF were significantly lower in most treatments (except LDPE+CPF) compared to CPF-only treatment. The proportions of dissipated DIF were significantly lower in Bio+DIF and 1-month LDPE+Mix treatments. The distribution of CPF in radish roots was significantly higher in Mix, LDPE+CPF, LDPE+Mix and 1mo LDPE+CPF treatments than CPF-only treatment. The distribution of CPF in radish leaves was significantly higher in Mix and

LDPE+CPF treatments than CPF-only treatment. However, the distribution of DIF in radish roots was significantly higher in DIF-only treatment than those in all other treatments. The distribution of DIF in radish leaves was significantly higher in Mix treatment but significantly lower in Bio+DIF treatment than that in DIF-only treatment.

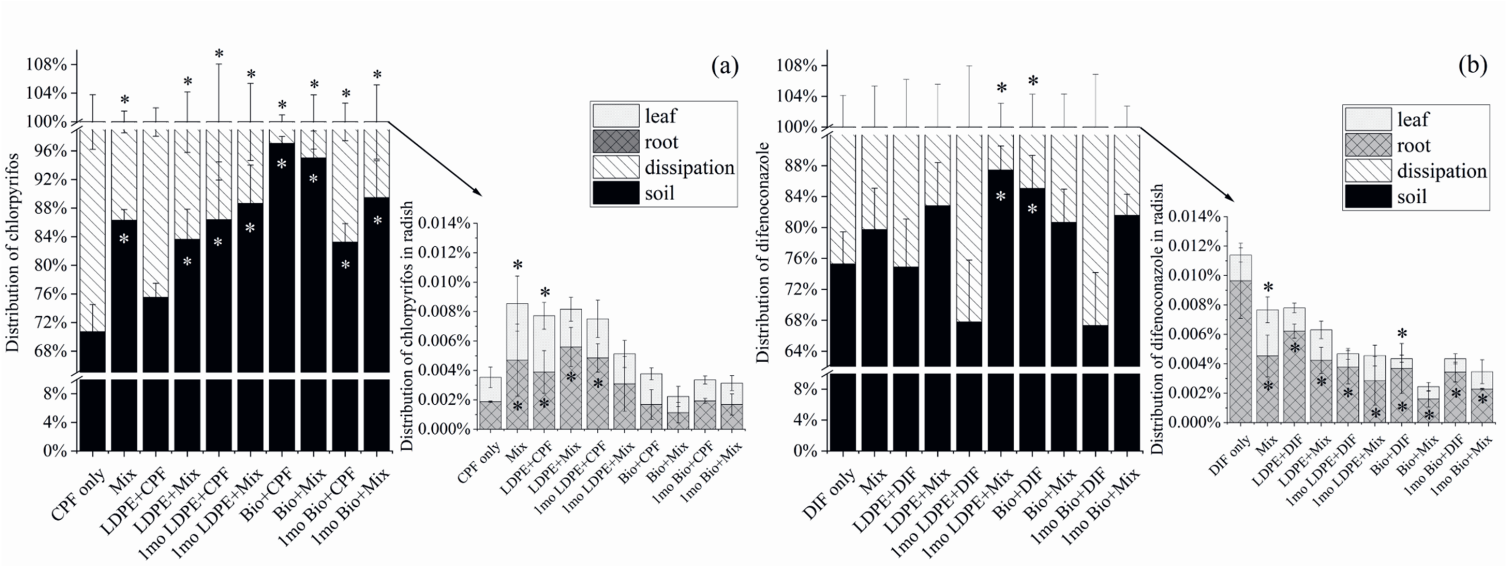

Figure 5.4 *The distribution rates of chlorpyrifos (a) and difenoconazole (b) in soil, dissipation part, radish roots and leaves. The distribution rates of pesticides in radish roots and leaves were presented in small panels due to their low values.*

5.3.5 Factorial analysis

Two-way ANOVA tests were conducted to evaluate the effects of two factors: MP and pesticide application (single or mixture) on the growth of radishes and distribution of pesticides (Table 5.4). MP has significant effects on both root and leaf growth, while pesticide application only has a significant effect on root growth. Both MP and pesticide application have significant effects on the CPF fate towards the soil and dissipation. In contrast, the DIF fate toward soil and dissipation is only affected by pesticide application. MP has significant effects on the proportional distribution of CPF and DIF in both radish roots and leaves. Pesticide application has significant effects on the proportional distribution of CPF and DIF to radish leaves. Furthermore, the interaction effects between MP and pesticide application on radish leaf biomass, pesticide (both CPF and DIF) fate towards the soil and dissipation, and proportional distribution of DIF to radish roots were significant, and the interaction effect on proportional distribution of CPF to radish roots was close to significant.

Table 5.4 *Results of two-way ANOVA tests for two factors (microplastic and pesticide application) on the distribution rates of pesticides.*

Factors	F(*p*)									
	Root biomass	Leaf biomass	CPF distribution				DIF distribution			
			Soil	Dissipation	Root	Leaf	Soil	Dissipation	Root	Leaf
MP	15.096 (***p*=0.000**)	28.165 (***p*=0.000**)	16.336 (***p*=0.000**)	16.881 (***p*=0.000**)	7.299 (***p*=0.001**)	15.899 (***p*=0.000**)	2.537 (*p*=0.062)	2.536 (*p*=0.062)	3.909 (***p*=0.013**)	10.661 (***p*=0.000**)
Pesticide	3.943 (***p*=0.012**)	1.747 (*p*=0.167)	17.990 (***p*=0.000**)	15.827 (***p*=0.000**)	0.645 (*p*=0.431)	21.308 (***p*=0.000**)	23.942 (***p*=0.000**)	23.932 (***p*=0.000**)	0.147 (*p*=0.705)	11.834 (***p*=0.002**)
MP*Pesticide	1.011 (*p*=0.451)	2.727 (***p*=0.005**)	4.098 (***p*=0.011**)	3.751 (***p*=0.011**)	2.721 (*p*=0.059)	2.107 (*p*=0.111)	5.767 (***p*=0.002**)	5.768 (***p*=0.002**)	3.718 (***p*=0.016**)	1.393 (*p*=0.262)

The factor MP includes no MP, pristine LDPE-MP, pristine Bio-MP, 1-month LDPE-MP, and 1-month Bio-MP, and the factor pesticide includes single pesticide and mixed pesticide. The degrees of freedom for the tests on root and leaf biomass were 4, 3, 12, and 60 for the factors MP, pesticide, their interaction, and residuals. Similarly, the degrees of freedom for the tests on pesticide distribution were 4, 1, 4, and 30 for the same factors.

5.4 Discussion

5.4.1 The effects of MPs on radish growth

In this study, we have observed that the presence of pristine LDPE-MPs at a concentration of 0.2% (w w^{-1}) in soil, which is considered to be environmentally realistic (Fuller and Gautam, 2016), exerted a positive influence on the growth of radish roots (edible parts) by increasing their biomass and size. (Figure 5.1). This effect can be attributed to the significant reduction in soil bulk density resulting from the presence of pristine LDPE-MPs (Table S5.1). It has been reported that lowered soil bulk density is favorable for root growth because of its effects on root penetration resistance and soil aeration (Rillig et al., 2019). It should be noted that despite a significant decrease in soil bulk density with 1-month Bio-MPs treatment, there was a significant reduction in radish root biomass, suggesting the presence of other potential mechanisms that may impact radish growth. Even though Botyanszká et al. (2022) also observed significant decreases in soil bulk density after the addition of 5% (w w^{-1}) MPs (PS, PVC and HDPE) to soil, the biomass of radish roots did not significantly change in their study. The different outcomes may be attributed to differences in polymer type, soil characteristics, or the effects on the soil microbiome and enzyme activities. Several studies have observed stimulation effects of low concentration of MPs on aquatic organisms (Sun et al., 2018; Chen et al., 2021), whether the stimulation effects of MPs also apply to higher plants still lack sufficient evidence and a clear mechanism. However, after being aged in the soil for 1 month, the presence of aged LDPE-MPs led to an insignificant decrease in the fresh root biomass of radish compared to both the control. Yet, the majority of studies have concentrated on unaged MPs rather than aged ones, which exhibit distinct physicochemical properties. Very limited studies involving aged MPs have also observed adverse effects of aged MPs on plants *Lolium multiflorum* and *Lepidium sativum* (Pflugmacher et al., 2020; Pflugmacher et al., 2021; Esterhuizen et al., 2022). Therefore, to better understand the environmental risks of MPs, future studies should employ aged MPs instead of pristine MPs.

In contrast to pristine LDPE-MPs, pristine Bio-MPs significantly inhibited the root growth of radish. We observed a significant increase in soil C:N ratio following the addition of Bio-MPs, as compared to the control. This is attributed to the introduction of bioavailable carbon resources into the soil. The elevated C:N ratio may lead to microbial immobilization of nutrients and subsequently negatively impact radish growth (Rillig et al., 2019). Even though pristine LDPE-MPs also led to similar effects on soil C:N as pristine

Bio-MPs, the resulting outcomes were divergent. This is probably due to the inert nature of LDPE-MPs which means the carbon resource from LDPE-MPs is not bioavailable to soil microorganisms (at least within the timeframe of this study). Under the 1-month Bio-MP treatment, NH_4^+-N increased while NO_x^--N decreased in soil 30 days after adding the Bio-MPs, indicating that Bio-MPs affected N transformation in soil. Shi et al. (2022) reported that MPs facilitated the activity of urease and nitrate reductase in soil. Urease catalyzes the hydrolysis of organic nitrogen into NH_4^+, while nitrate reductase facilitates the conversion of NO_3^- to NO_2^-, which is subsequently transformed into NH_4^+. Therefore, the introduction of MPs in soil could result in an elevation of NH_4^+ levels. Shi et al. (2022) also suggested that changes to N transformation by MPs could impact nitrogen bioavailability, as plastic-sphere microorganisms are capable of utilizing mineral N (NH_4^+-N and NO_x^--N). Microorganisms could take up the majority of N released from the soil organic matter and litter (Kuzyakov and Xu, 2013). Therefore, competition for nutrients between microorganisms and crops may affect crop growth performance. There is mounting evidence that biodegradable microplastics (MPs) such as PBAT and PLA have a detrimental impact on plant growth. For example, Qi et al. (2018) found that biodegradable MPs could cause stronger adverse effects on wheat *Triticum aestivum* growth than PE MPs. Similarly, Meng et al. (2021) observed negative impacts on the growth of common bean *Phaseolus vulgaris L.* In our study, we found that both pristine Bio-MPs and aged Bio-MPs inhibited the growth of radish, likely due to alterations to soil properties and nutrient bioavailability. Therefore, given the growing demand for biodegradable plastics in agriculture (Ding et al., 2021), further studies should place greater emphasis on investigating the impacts of biodegradable MPs, including those that have undergone aging processes, on plant growth.

5.4.2 Effects of MPs contamination on pesticide fate

Previous studies have reported that the presence of MPs in soil could negatively affect the dissipation of pesticides in soil, either by decreasing the bioavailability and inhibiting the degradation of pesticide or by changing the soil microbial community and enzyme activities (Chang et al., 2022). In the present study, the pristine LDPE-MPs exhibited negligible effects on the dissipation of both CPF and DIF. The high desorption rate of CPF from pristine LDPE-MPs may account for their minimal effects on CPF dissipation in soil (Ju et al., 2023). Moreover, the pristine LDPE-MPs also exhibited minimal adsorption capacity for DIF (Figure S5.1), which may explain the negligible effects of LDPE-MPs on DIF dissipation. Lan et al. (2021) also observed a lower adsorption capacity of DIF on PE, approximately 50 μg g^{-1}, compared to CPF, despite the similarity in properties such as the log P values of both pesticides. However, the treatment with 1-month LDPE-MPs

showed significantly inhibited dissipation of CPF in soil. This may be attributed to the increased adsorption capacity of LDPE-MPs after aging. Lan et al. (2021) reported that MP aging in soil can increase their adsorption capacity by increasing their specific surface area and generating functional groups such as carbonyl groups that can form hydrogen bonds with other organic pollutants. Moreover, the formation of biofilm on MPs can also enhance the adsorption of pollutants by MPs (Wang et al., 2020). The other reason could be the change in microbial community structure and reduction in enzyme activities after the introduction of MPs in soil (Zhou et al., 2022). In contrast to the pristine LDPE-MPs, the pristine Bio-MPs significantly inhibited the dissipation of both CPF and DIF, which is due to the pristine Bio-MPs having higher CPF and DIF adsorption capacities (Figure S5.1). Our previous study also showed that the desorption of CPF from pristine Bio-MPs was slow, with only 7.3% of adsorbed-CPF being released after 48 h (Ju et al., 2023). The desorption experiment, however, was conducted in the aqueous system within 48 hours. To gain a better understanding of the mechanism, further experiments with soil and for an extended duration will be necessary. Increasing evidence has shown that biodegradable MPs may act as a stronger vector for other contaminants than nondegradable MPs (Gong et al., 2019; Fan et al., 2021). Therefore, it is important to be aware of the potential for Bio-MPs to prolong the persistence of pesticides in soil. However, the dissipation of pesticides was found to be less affected by 1-month Bio-MPs compared to pristine Bio-MPs. The Bio-MPs may undergo degradation in the soil during the aging process, thereby impeding their interaction with pesticides and facilitating the release of pesticides (Lin et al., 2022).

Research on the effects of MPs on the accumulation of hydrophobic organic contaminants (HOCs) by plants is limited (Tourinho et al., 2023). Liu et al. (2021) observed that the co-exposure of PE-MPs and phenanthrene decreased the accumulation of phenanthrene in wheat roots and leaves compared to a treatment with phenanthrene only. Huang et al. (2022) reported that the internalization of 200 nm nanoplastics in ryegrass roots increased the uptake of heavy metals, and this could also be a potential pathway for the bioaccumulation of HOCs in plant. However, these studies only used pristine polymers with a single co-contaminant. In the present study, we investigated the bioaccumulation of pesticides in radishes in four different scenarios, taking into account the aging of MPs and the presence of more than one pesticide in soils. Specifically, the 4 scenarios of exposure include: (1) pristine MPs with single pesticide, (2) 1-month MPs with single pesticide, (3) pristine MPs with pesticide mixtures, (4) 1-month MPs with pesticide mixtures. Generally, the increased bioaccumulation of CPF in radishes could be attributed to the decreased dissipation, which can lead to more

opportunities for root uptake (Hwang et al., 2017). The bioaccumulation of CPF in radish roots was higher in scenario 2 than in scenario 1 (Table S5.2), indicating MPs may pose higher risks on pesticide bioaccumulation by radishes after aging in soil. Studies on the effects of aged MPs on the bioaccumulation of other contaminants are still lacking. Wang et al. (2023) found that aged MPs decreased the bioaccumulation of cadmium in *Brassica juncea* compared to pristine MPs. These inconsistent findings can be attributed to various factors, including the utilization of different types of MPs and contaminants, as well as the selection of different model plants that may exhibit varying uptake patterns for contaminants (Sandanayake et al., 2022). It is worth noting that the presence of 1-month Bio-MPs can not only increase the bioaccumulation of CPF in radishes, but also lead to a reduction in the fresh root biomass of the plants. Increasing evidence have shown that biodegradable MPs can exert adverse effects on crops (Qi et al., 2018; Meng et al., 2021; Yang et al., 2021; Brown et al., 2023; Yu et al., 2023). Hence, soil contamination with biodegradable MPs poses risks regarding food production and safety, necessitating oversight and regulations on the utilization of biodegradable plastic materials in agriculture. Additionally, radish roots exhibited significantly greater contents of CPF in scenarios 3 and 4 compared to scenarios 1 and 2 when LDPE-MPs were present (Table S5.2), indicating pesticide application practices may also affect the pesticide bioaccumulation. Although agricultural soils are typically polluted by multiple pesticides, there is currently a lack of comprehensive environmental risk assessments for pesticide mixtures (Silva et al., 2019). Hence, to prevent the underestimation of pesticide risks, it is important to include MPs (if they are present in the soil) and pesticide mixtures in future studies on the uptake and trophic transfer of pesticides by crops. In contrast, due to the relatively higher standard deviations, the bioaccumulation of DIF remained unaffected by either MPs or the pesticide application method. Given the similar properties between CPF and DIF, further research is needed to explain their differing behaviours. The significant differences in pesticide fate observed among various treatments highlight the impact of MPs and pesticide application on pesticide behavior in the soil-plant system. For CPF, the proportions of dissipated pesticides were lower in most treatments, except LDPE+CPF, suggesting that MPs and pesticide application method may influence the persistence of CPF in the environment. The proportion of applied CPF that ended up in the radish was higher in treatments with LDPE-MPs but not Bio-MPs even though the CPF in soil was clearly higher, due to the low radish biomass in treatments with Bio-MPs. On the other hand, for DIF, the distribution in radish roots was significantly higher in the DIF-only treatment, suggesting that MPs may not increase the potential risks associated with DIF uptake by radishes.

5.4.3 Risk assessment of MPs and pesticides on food safety

MPs and pesticides are environmental pollutants that have come to prominence in recent decades, for their individual ecotoxicological effects and also for their combined effects, which may exacerbate environmental issues. For example, studies have shown that MPs and pesticides can lead to synergistic toxicity in organisms like earthworms (Peña et al., 2023). Even though increasing amounts of evidence has shown that MP pollution in soil can lead to adverse effects on the plant performance (Li et al., 2022), the potential risks for crops grown in soil co-contaminated with MPs and pesticides, especially multiple pesticides, are still unknown. We have observed that radish, a root vegetable, is susceptible to reduced yield and quality in sandy soil polluted by the combination of MPs and multiple pesticides. The results of the two-way ANOVA demonstrate significant interaction effects between MP and pesticide application on radish biomass and pesticide distribution. The significant interaction effects of MP and pesticide application on radish biomass highlight the interactive nature of their impact on plant development. Regarding pesticide distribution, the significant interaction effects between MP and pesticide application on the fate of pesticides imply that the effects of MPs on the uptake or translocation of these pesticides within the radish plant are not consistent across different pesticide applications, and vice versa. Our study implies that the combined effects of MPs and pesticides, including pesticide mixtures, should be an emerging area of concern for food safety. The interactions between MPs and pesticides can exacerbate ecotoxicological effects and lead to unforeseen consequences for human health. Furthermore, we urge for more attention towards risk assessments on biodegradable plastics, which are being found to be harmful to plants in an increasing number of recent studies (Qi et al., 2018; Meng et al., 2021; Yang et al., 2021; Brown et al., 2023; Yu et al., 2023). Therefore, soil pollution by the combination of biodegradable MPs and pesticides is an especially pressing concern for both environmental and human health.

In addition to their effects on the crop bioavailability of pesticides, as investigated in this study, MPs may also act as direct carriers of pesticides into crop tissue. MPs subject to aging processes can undergo fragmentation into smaller particles such as submicron- and nano-plastics, increasing their adsorption capacity and potential for interaction with crops. Li et al. (2020) reported a crack-entry pathway for plant uptake of sub-micrometer plastics, which indicates that plastic debris of this size may carry pollutants such as pesticides directly into crops and into the food chain. Last but not the least, our findings were based on the experimental observation in which the concentration of MPs is elevated compared to real environmental

concentration in soil (Büks and Kaupenjohann, 2020). However, the nondegradable nature of LDPE-MPs may result in their accumulation and subsequent increase in concentration over time. Furthermore, the lack of viable technical protocols for extracting and identifying biodegradable MPs has led to a significant knowledge gap regarding their presence in soil. Biodegradable plastic mulch films are specifically designed to degrade within soil environments. This suggests that biodegradable MPs in soil could be prevalent before they completely decompose. In the future, more comprehensive research is needed to understand the full extent of the combined risks posed by MPs and pesticides.

5.5 Conclusion

The potential combined risks of MPs, particularly aged MPs, and other contaminants such as pesticides on the crops remain unclear. The results from the present study indicated that the aged MPs in the soil, either with or without pesticides, negatively affected the root biomass of radishes. In addition, when the soil was contaminated with single CPF, aged MPs resulted in higher bioaccumulation of CPF in radish roots than pristine MPs. However, when the soil was contaminated with pesticide mixtures and LDPE-MPs, the bioaccumulation of CPF in radish roots was greater than that in soil contaminated with single pesticides and LDPE-MPs. Therefore, further research should be conducted in more realistic scenarios using aged MPs and multiple contaminants to gain a more comprehensive understanding of the environmental risks of MPs.

Acknowledgement

We are grateful for the funding provided by the National Natural Science Foundation of China (41877072), the EU Horizon 2020 project (MINAGRIS) and the China Scholarship Council (CSC: 201904910501).

Supplementary materials

Materials and methods

Adsorption kinetic experiment

The pesticide adsorption kinetic experiments were conducted with pristine LDPE and pristine Bio MPs in duplicates. The initial concentration of each pesticide was 0.2 mg L^{-1} in both single- and mixed-pesticide treatments, with the background solution consisting of 0.01 mol L^{-1} NaCl and 200 mg L^{-1} NaN_3. In each treatment, 20 mL of pesticide solution and 20 mg of MPs were added to an amber glass vial with a Teflon-lined screw cap. The vials were shaken at 150 rpm for 2 days at 20 ℃. At different time intervals (0, 0.5, 1, 2, 4, 8, 24h), 1 mL of the aliquots were sampled and filtered through a 0.22 μm filter before pesticide analysis. The pesticide concentrations were measured using LC-MS/MS.

Pesticide analysis

Chemicals and solvents

The reference standards of chlorpyrifos (99%), difenoconazole (99%) and ^{13}C-caffeine (99%) were obtained from Sigma-Aldrich (USA). Magnesium sulfate ($MgSO_4$; 99.5%) was purchased from Alfa Aesar GmbH (Germany) and sodium acetate (CH_3COONa; ⩾ 99%) was purchased from Acros Organics (Geel, Belgium). Acetic acid (CH_3COOH; ⩾99.8%) was obtained from Biosolve BV (The Netherlands). Acetonitrile (C_2H_3N; 99.95% LC grade) and methanol (MeOH; 99.98%) were purchased from Actuall Chemicals (The Netherlands). Formic acid (⩾98% p.a.) from Gevaar (The Netherlands) and ammonium formate (HCO_2NH_4; > 99%) from Sigma (USA) were used for the mobile phases of liquid chromatography-tandem mass spectrometry (LC-MS/MS). Standard stock solutions of chlorpyrifos and difenoconazole at concentrations of 2000 μg mL^{-1} were prepared and all stock solutions and dilutions were stored at 4 °C.

Extraction methodology of pesticides

The extraction procedure used was based on a QuEChERS approach (Anastassiades et al., 2003). Five grams, 2g, and 0.5 g of soil, radish roots and radish leaves were added to separate 50 mL tubes, respectively. Then the tubes were spiked with 25 and 10 μL ^{13}C-caffeine as a surrogate for soil and radish samples (roots and leaves), respectively. Five milliliters, 2 mL of Millipore water and 10 mL and 4 mL acetonitrile (ACN) containing 1% acetic acid (HAC) (extraction solvent) were added to soil and radish samples, respectively. We used the same amount of water and ACN for the extraction of radish roots and leaves to get better homogenization. The tubes were shaken for 30 min in an end-over-end shaker. Then, all tubes were vortexed

and centrifuged at 3500 rpm for 5 min after the addition of CH_3COONa (1 g and 0.4 g for soil and radish samples, respectively) and $MgSO_4$ (4 g and 1.6 g for soil and radish samples, respectively). After that, 250 µL of supernatant and 250 µL of Millipore water were added to a LC filter vial and analyzed using LC-MS/MS.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The contents of pesticides were determined by LC–MS/MS using an Acquit UPLC HSST3 RP C18 column (1.8-µm particle size, 100 mm in length, 2.1-mm i.d.) (Shimadzu, Japan). The mobile phases consisted of 5 mM ammonium formate and 0.1 % formic acid in Millipore water (solvent A) and a 95:5 MeOH: H_2O (Millipore) solution (solvent B). The LC gradient times for the separation were: isocratic from 0 to 1 min (100% A: 0% B); from 1 to 2.5 min, a linear increase of B from 0 to 45%; isocratic from 2.5 to 8.5 min (0% A:100% B); from 8.5 to 11.5 min, a linear decrease of B from 100 to 0%; and isocratic from 11.5 to 12 min (100% A:0% B). Initial conditions were re-established every 2 minutes for a total running time of 14 minutes. The column temperature was 45 °C, and the flow rate was 0.4 mL min^{-1}. Optimization of the ionization and fragmentation conditions for the analytes was obtained by the infusion of solutions of the individual analytes. Optimum responses were obtained by electrospray ionization in the positive-ion mode using the following source parameters: ion spray voltage at 450 and source temperature of 450 °C. The transitions acquired for chlorpyrifos and difenoconazole are given in Table S5.4.

Quality control

Analyses were done according to the guidance document (EC, 2019). The quantification of pesticides in the samples was based on multi-level calibrations using solvent standards. Standard calibration curves at concentrations of 0, 0.1, 0.5, 1, 5, 10, 50 ,100 and 250 ng mL^{-1} were prepared from a mix solution that combined the reference standards of all compounds in solvent (ACN with 1 % HAc and Millipore water) and the standard series (0, 0.1, 0.5, 1, 5, 10, 50, 100, 150 and 250 ng mL^{-1}) were prepared in a matrix extract of samples (soil and radish) and Millipore water. The level of the standard calibration curve is different for each compound and the calibration curve indicated satisfactory linearity, with correlation coefficients > 0.99 and residuals lower than ± 20% response. The concentration exceeding the highest standard of the calibration curve was diluted with a solvent mix of ACN with 1% HAc and Milipore water. The observed matrix effects were below 20% when comparing the measured concentrations of the standard spiked at 5 ng mL^{-1} in solvent with those spiked in soil or radish matrix. The soil and radish samples were spiked at concentrations of 1 ng g^{-1} for quality control. Unspiked samples were also analyzed to confirm the absence of pesticide residues in the blank samples. The recoveries, limit of quantitation

(LOQ) and limit of detection (LOD) were shown in Table S5.5.

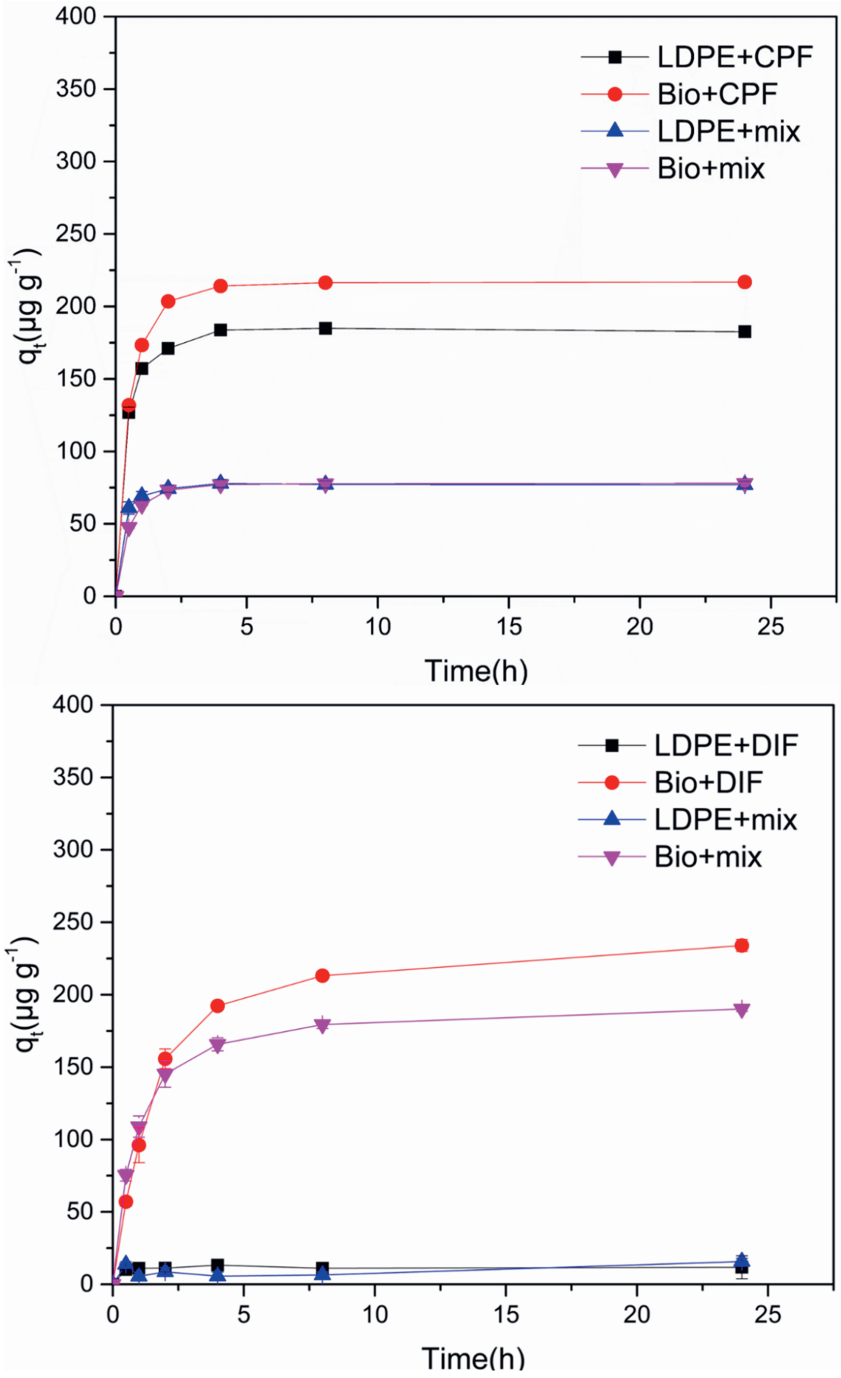

Figure S5.1 *Adsorption kinetics of chlorpyrifos (above) and difenoconazole (below), both alone and in combination, on pristine LDPE-MPs and Bio-MPs*

Table S5.1 *Soil properties for control, LDPE-, Bio-, 1-month LDPE-MP and 1-month Bio-MP treatments.*

		CK	LDPE	Bio	1mo LDPE	1mo Bio
pH	Day 65	7.45±0.03b	7.47±0.03ab	7.48±0.06ab	7.43±0.05b	**7.59±0.09a**
EC (uS cm^{-1})	Day 65	45.33±1.05ab	47.13±0.12a	44.13±1.30b	43.83±2.25b	46.67±1.38a
Bulk density (g cm^{-3})	Day 65	1.49±0.01a	**1.46±0.01b**	1.49±0.02ab	1.48±0.03ab	**1.46±0.02b**
C (g kg^{-1})	Day 0	17.45±4.17	17.45±4.17	17.45±4.17	17.45±4.17	17.45±4.17
	Day 30	15.15±0.21d	**17.50±0.00c**	**17.00±0.00c**	**22.15±0.64a**	**18.20±0.28b**
	Day 65	20.50±6.86a	24.58±6.80a	22.90±4.04a	22.80±5.54a	28.63±4.97a
N (g kg^{-1})	Day 0	1.30±0.28	1.30±0.28	1.30±0.28	1.30±0.28	1.30±0.28
	Day 30	1.15±0.07c	**1.00±0.00d**	**1.00±0.00d**	**1.70±0.00a**	**1.40±0.00b**
	Day 65	1.53±0.46a	1.80±0.49a	1.73±0.26a	1.70±0.40a	2.10±0.36a
C:N	Day 0	13.39±0.30	13.39±0.30	13.39±0.30	13.39±0.30	13.39±0.30
	Day 30	13.20±1.00b	**17.05±0.07a**	**17.45±0.07a**	13.03±0.37b	13.00±0.20b
	Day 65	13.00±0.44b	13.41±0.27ab	13.43±0.41ab	13.39±0.17ab	**13.63±0.20a**
NH_4^+-N (mg kg^{-1})	Day 0	3.55±0.07	3.55±0.07	3.55±0.07	3.55±0.07	3.55±0.07
	Day 30	1.00±0.00c	**0.90±0.00d**	**0.90±0.00d**	**3.35±0.07b**	**5.70±0.00a**
	Day 65	0.50±0.14a	0.38±0.10a	0.48±0.10a	0.35±0.10a	0.43±0.05a
NO_x^--N (mg kg^{-1})	Day 0	14.05±0.07	14.05±0.07	14.05±0.07	14.05±0.07	14.05±0.07
	Day 30	1.75±0.07a	1.65±0.07a	1.65±0.07a	**1.25±0.21b**	**0.20±0.00c**
	Day 65	0.08±0.05a	0.05±0.06a	0.13±0.05a	0.13±0.05a	0.08±0.05a

Day 0 indicates the initial soil properties, day 30 and day 65 indicate the soil properties on the day of sowing and harvesting. Different letters indicate significant difference among treatments.

Table S5.2 *The results of the t-test comparing chlorpyrifos content and bioaccumulation factor (BAF) in radish roots across different scenarios.*

Treatment	Scenario	*p* value	
		Content	BAF
LDPE+CPF 1mo LDPE+CPF	S1 S2	0.029	0.031
Bio+CPF 1mo Bio+CPF	S1 S2	0.047	0.039
LDPE+CPF LDPE+mix	S1 S3	0.004	0.008
Bio+CPF Bio+mix	S1 S3	0.514	0.602
1mo LDPE+CPF 1mo LDPE+mix	S2 S4	0.009	0.01
1mo Bio+CPF 1mo Bio+mix	S2 S4	0.049	0.082

Table S5.3 *The results of the t-test comparing difenoconazole content and bioaccumulation factor (BAF) in radish roots across different scenarios.*

Treatment	Scenario	*p* value	
		Content	BAF
LDPE+DIF 1mo LDPE+DIF	S1 S2	0.731	0.397
Bio+DIF 1mo Bio+DIF	S1 S2	0.549	0.11
LDPE+DIF LDPE+mix	S1 S3	0.942	0.77
Bio+DIF Bio+mix	S1 S3	0.647	0.567
1mo LDPE+DIF 1mo LDPE+mix	S2 S4	0.606	0.534
1mo Bio+DIF 1mo Bio+mix	S2 S4	0.411	0.359

Table S5.4 *LC-MS/MS conditions for detection and quantification of the pesticide.*

Pesticide	Retention time (min)	Transitions		DP	EP	CE	CXP
Chlorpyrifos	11.26	349.900	96.900	41.000	10.000	41.000	20.000
Difenoconazole	10.52	406.000	251.000	136.000	10.000	39.000	26.000

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential.

Table S5.5 *Quality control, Limit of Quantitation (LOQ) and Limit of Detection (LOD) of LC-MS/MS.*

Matrix	Pesticide	QC (1 ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})
Soil	Chlorpyrifos	86%	1	0.35
	Difenoconazole	83%	1	0.10
Leaf	Chlorpyrifos	107%	1	0.36
	Difenoconazole	88%	1	0.12
Root	Chlorpyrifos	103%	1	0.10
	Difenoconazole	86%	1	0.10

Chapter 6 Synthesis

Plastics are extensively utilized in agricultural practices, such as the use of plastic mulching, to enhance crop productivity. However, inadequate management of plastic waste has resulted in soil contamination on a global scale. The fragmentation of larger plastic pieces has led to an explosive increase in the amount of smaller plastic debris present within the soil. From this plastic debris, MPs have emerged as a prominent contaminant which has attracted considerable attention from scientists, governments and other stakeholders in recent years. Due to their high specific surface area and hydrophobic properties, MPs can serve as carriers for various organic contaminants including pesticides which are also prominent contaminants in agricultural soils. Despite the extensive research conducted on the environmental risk assessments of MPs, only a limited number of these studies have been carried out on MPs risks coupled with other contaminants in soil. Furthermore, in order to address the problem of plastic pollution, mainly stemming from nondegradable plastic film use, biodegradable plastics are being introduced into agricultural practices. However, studies show that the degradation of biodegradable plastics in soil does not meet expectations due to environmental limitations (Han et al., 2021). There are still significant research gaps regarding the interactions between biodegradable MPs with pesticides in soil. To address these research gaps, we conducted laboratory experiments to: 1) Investigate the adsorption-desorption behaviours of CPF by pristine and aged LDPE-MPs and Bio-MPs, and the bioconcentration of CPF-adsorbed pristine and aged LDPE-MPs and Bio-MPs in earthworm casts (Chapter 2); 2) Study the combined effects of LDPE-MPs and Bio-MPs and CPF on earthworms, and in turn, examine how earthworms transport MPs and CPF into their burrows. The degradation and bioaccumulation of CPF were also studied in this chapter (Chapter 3); 3) Examine the effects of aged LDPE-MPs and Bio-MPs on the dissipation of a pesticide mixture containing CPF and DIF in soil (Chapter 4); and 4) Explore the effects of aged LDPE-MPs and Bio-MPs on the growth of radishes and the bioaccumulation of a pesticide mixture (Chapter 5). In this chapter, we synthesize our major findings, discuss their implications, acknowledge limitations encountered during this study, and provide recommendations for future research.

6.1 Major findings

The major findings for each research chapter are summarized in Figure 6.1, addressing the research questions proposed in Chapter 1.

In Chapter 2, we found that the adsorption capacities of the CPF were higher, and the desorption rates were lower for aged MPs as compared to pristine MPs. This indicates that aged MPs can act as stronger vectors for CPF. In

addition, a 4-day petri dish experiment was conducted with pristine and aged MPs, including LDPE-MPs aged in both UV light and soil and Bio-MPs aged in UV light, to study the bioconcentration of these MPs in earthworm casts. The results showed that both UV-aged MPs were significantly less concentrated in earthworm casts than pristine MPs. However, UV-aged MPs adsorbed with CPF were significantly more concentrated than UV-aged MPs without CPF. In contrast, LDPE-MPs aged in soil with relatively higher carbon and nitrogen contents were significantly more concentrated in earthworm casts than pristine MPs. Therefore, in the next chapter, we conducted mesocosm experiments to investigate the combined ecotoxicological effects of MPs and CPF on earthworms. We also examined the effects of earthworms on the behaviours of MPs and CPF in soil, specifically focusing on their transport within earthworm tunnels, bioaccumulation potential, and degradation rates.

In Chapter 3, we observed both synergistic and antagonistic toxicity from the MP-pesticide mixture on earthworm growth, which was dependent on the type and dose of the MPs. Moreover, with the addition of CPF, 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 the soil in the treatments with LDPE-MPs and Bio-MPs, with 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 MP additions than without MP additions, indicating that the presence of MPs inhibited CPF degradation.

In Chapter 4, a 35-day pot experiment was conducted to examine the effects of aged LDPE-MPs and Bio-MPs on the dissipation of a pesticide mixture of CPF and DIF in sandy soil. The LDPE-MPs and Bio-MPs were aged in the soil for 1 month before the addition of pesticides. The pesticide residues in soil were measured on days 0, 1, 3, 7, 14, and 35. Our findings indicate that the presence of both aged MPs in soil led to an increase in the half-life (DT50) of CPF, regardless of whether it was applied alone or in combination with DIF. In contrast, the DT50 of DIF was not affected by either aged MP. However, the DT50 of DIF was significantly higher when applied in combination with CPF compared to when it was applied alone. Therefore, in the next chapter, another pot experiment was conducted to study the effects of aged MPs on the bioaccumulation of pesticide mixtures in radish *Raphanus sativus*.

In Chapter 5, we found that the presence of pristine LDPE-MPs resulted in twice as much radish root biomass as the control. However, the radish root biomass decreased by 44% in the treatment with aged LDPE-MPs. In contrast,

pristine Bio-MPs and aged Bio-MPs significantly decreased the radish root biomass by 53% and 59%, respectively, as compared to the control. The addition of pristine LDPE-MPs had no effect on the dissipation of CPF in soil and CPF content in radish roots and leaves. However, aged LDPE-MPs and Bio-MPs (both pristine and aged Bio-MPs) significantly inhibited the dissipation of CPF in the soil, thereby increasing its contents in radish roots and leaves. The dissipation of DIF in soil was significantly inhibited in the treatment with pristine Bio-MPs while the content of DIF in radishes was not changed by any type of MP. Interestingly, the treatments with pesticide mixtures combined with MPs showed a lower dissipation of pesticides and a higher bioaccumulation of pesticides than treatments with a single pesticide and MPs.

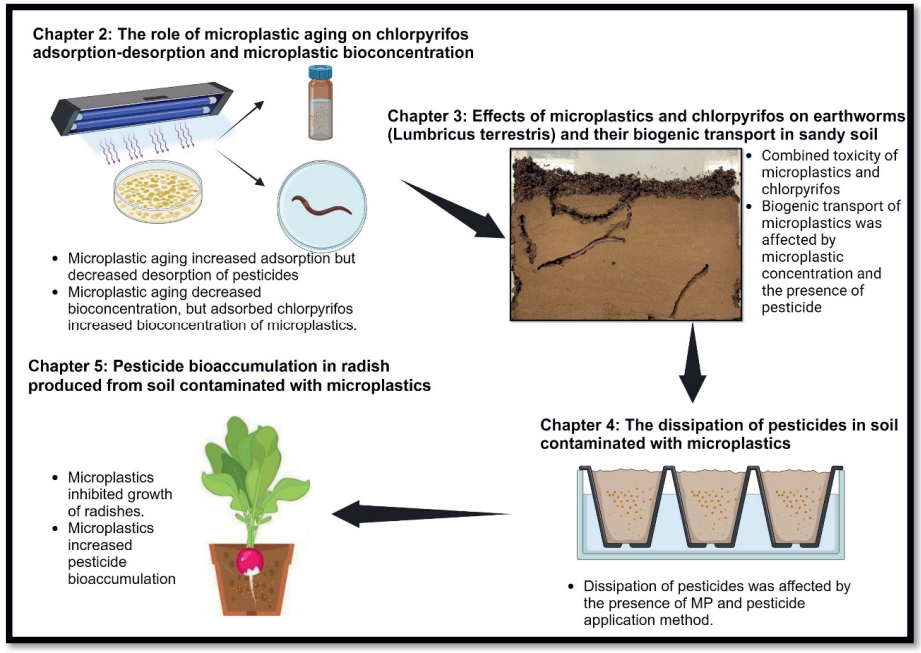

Figure 6.1 *Outline of the PhD thesis with the major finding for each chapter*

6.2 General discussion

6.2.1 Effects of MP ageing on pesticide adsorption-desorption

One of the primary interactions observed between MPs and pesticides is the sorption of pesticides onto the surface of MPs. Some hydrophobic pesticides may preferentially adsorb to MPs due to their lipophilic nature. The degree of sorption is influenced by various factors, including the type and size of MPs, as well as the chemical properties of both MPs and pesticides. The MPs in soil, however, undergo a range of ageing processes such as exposure to

sunlight radiation, physical abrasion, hydrolysis, and biodegradation (Xu et al., 2019). The ageing process accelerates chain scission and crosslinking, and oxidation, which may change the physicochemical properties of MPs (e.g. morphology change including generation of pores and cracks, and the formation of oxygen-containing functional groups such as hydroxyl, carbonyl and carboxyl groups etc.) and lead to enhanced adsorption capacity for pesticides compared to pristine MPs in most cases (Wang et al., 2020; Lan et al., 2021; Ju et al., 2023).

In Chapter 2, it was observed that both aged LDPE-MPs and Bio-MPs exhibited enhanced adsorption and reduced desorption rates of the CPF. Furthermore, alterations in functional groups were detected in aged MPs, which could potentially account for the finding above. For instance, UV-aged LDPE-MPs displayed the generation of carbonyl groups (C=O), capable of forming hydrogen bonds with CPF and consequently increasing its affinity towards MPs. Moreover, ageing processes also augmented the number of adsorption sites in MPs through the formation of cores and poles. Although morphology of MPs was not characterized in this study, numerous previous studies have supported the notion that aged MPs may serve as a stronger carrier for hydrophobic pesticides (Wang et al., 2023). Increased adsorption capacities of aged MPs were also observed in other studies. For example, atrazine adsorption was found to increase on aged PE, PS, and PP (Wang et al., 2022), and carbendazim, diflubenzuron, malathion, difenoconazole showed enhanced adsorption on aged PE surfaces, particularly for the more hydrophobic diflubenzuron and difenoconazole (Lan et al., 2021). Likewise, increased adsorption capacity was observed for endosulfan and endrin on aged PE, polyamide (PA), polystyrene (PS), and PVC, with the highest being that of the more hydrophobic endrin (Yurtsever et al., 2020). In pristine MPs, partitioning and electrostatic attraction were proposed as the dominant mechanisms governing atrazine adsorption; however, in aged MPs, adsorption mainly occurred through surface diffusion and hydrogen bonding. The findings of Lan et al. (2021) support the significance of hydrophobic partitioning in the adsorption process of pesticides to PE. However, it is also important to consider H-bonding in aged PE due to the presence of polar O-containing functionalities on the oxidized MP surface. Gomes de Aragão Belé et al. (2021) studied the adsorption of insecticides chlorpyrifos and dichlorvos on PE, PP and PS before and after treatment with O_3/H_2O_2 oxidation process commonly employed in advanced wastewater treatment plants. They discovered that hydrophobic chlorpyrifos exhibited higher adsorption on virgin MPs due to π-π interactions, while the more polar dichlorvos demonstrated greater affinity for treated MPs where weak electrostatic interactions or hydrogen bonding were proposed mechanisms leading to enhanced polarity

resulting in increased adsorption, particularly for polar pesticides. However, inconsistent findings were also noted. Miranda et al. (2022) reported enhanced adsorption of four pesticides including alachlor, clofibric acid, diuron, and pentachlorophenol following various ageing treatments of low-density polyethylene (PE) and unplasticized polyvinyl chloride (PVC), while the sorption capacity of polyethylene terephthalate (PET) remained unaltered after ageing. Concha-Grana et al. (2022) also found no differences in the adsorption of chlorpyrifos and α-endosulfan on pristine and aged PA and polyhydroxybutyrate (PHB). Despite the presence of evidence indicating that aged MPs exhibit comparable adsorption capacities to pristine MPs, it remains imperative to employ aged MPs during adsorption and desorption experiments in order to prevent underestimation.

In comparison to nondegradable MPs, biodegradable MPs generally possess a higher specific surface area, greater number of pores and/or distinct chemical properties that facilitate bonding with pesticides, potentially leading to enhanced adsorption capacities compared to nondegradable MPs (Gong et al., 2019). However, there remains a lack of research on pesticide adsorption onto biodegradable MPs, particularly those that are aged. In Chapter 2, it was observed that Bio-MPs exhibited a higher adsorption capacity for CPF compared to LDPE-MPs. Furthermore, aged Bio-MPs demonstrated an even higher adsorption capacity than pristine Bio-MPs. Additionally, both aged and pristine biodegradable MPs displayed significantly lower CPF desorption rates within 48 hours in comparison to LDPE MPs. The findings suggest that biodegradable MPs, particularly those subjected to ageing processes, have the potential to serve as effective carriers for pesticides in soil, a notion supported by several studies (Fan et al., 2021; Sun et al., 2021). This aspect should be addressed in future studies considering the increasing utilization of biodegradable plastics in agriculture.

6.2.2 Combined effects of MP-pesticide on earthworms

Studies have shown that MPs can have adverse effects on earthworms, including impacts on growth, behaviour, oxidative response, gene expression, and gut microbiota. The toxicity of MPs on earthworms is influenced by factors such as concentration, size, and polymer type. For example, smaller MPs, especially those smaller than 50 μm and biodegradable, are more likely to be ingested by earthworms and can cause more severe toxicities (Cui et al., 2022). In Chapter 3, we observed that different polymers exhibited varying levels of toxicity towards earthworms. Specifically, LDPE-MPs alone did not have any significant adverse effects on the health of earthworms. However, when Bio-MPs were present at higher concentrations, they resulted in a substantial increase in weight loss and mortality among the earthworm

population. The interaction of MPs and pesticides in soil has emerged as a significant concern in environmental science. However, their combined presence and interaction in soil ecosystems add a layer of complexity that requires comprehensive exploration. Research on the combined impacts of MPs and pesticides on soil organisms is limited, especially when compared to aquatic studies. In Chapter 3, we observed that CPF can increase the toxicity of higher concentration of LDPE-MPs and lower concentration of Bio-MPs on earthworm growth. Based on the results from existing studies which have shown that the co-exposure of earthworms to MPs and pesticides can result in higher mortality rates, reduced growth, altered behaviour, and changes in biochemical and molecular responses compared to exposure to either contaminant alone. For example, the joint exposure of earthworms to MPs and CPF led to increased oxidative stress and alterations in metabolic profiles, indicating a higher level of toxicity compared to exposure to chlorpyrifos alone. Additionally, the presence of MPs can enhance the uptake and bioaccumulation of pesticides in earthworms, potentially leading to prolonged exposure and increased toxicity (Peña et al., 2023). However, we also observed a contrasting result whereby the presence of CPF resulted in a mitigation of Bio-MPs toxicity at higher concentrations, which can be attributed to its ability to reduce earthworm ingestion of Bio-MPs and consequently prevent their toxic effects. There is scant evidence demonstrating that the combination of MPs and other contaminants induces reduced toxicity. Wang et al. (2019) found that PVC MPs alleviated Arsenic (As) toxicity in earthworm *Metaphire californica* by reducing the accumulation of As. The inconsistent findings regarding the combined effects of MPs and pesticides on earthworms could be due to the different types and exposure concentrations of both MPs and pesticides. These findings indicated that the combined toxicity of MPs and pesticides may not always exceed their individual toxicities, indicating a need for further research to enhance our understanding of the combined risks posed by MPs and pesticides in soil ecosystems.

6.2.3 The translocation and transformation of MP-pesticide by earthworms

MPs and pesticides both undergo translocation and transformation processes in soil, influenced by physical, chemical, and biological factors. For instance, they can be physically transported through water (leaching and runoff) or biologically carried by earthworms. Additionally, microorganisms contribute to their degradation while earthworms and plants can bioaccumulate them, among others. All these aspects play a crucial role in determining the fate of MPs and pesticides within soil ecosystems. The translocation and transformation of MPs and pesticides as a combination in soil ecosystems,

however, still lack comprehensive understanding.

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). In Chapter 2 and 3, we studied the potential of earthworms to ingest and transport MPs and CPF in soil using petri-dish experiments and mesocosms experiments, respectively. In Chapter 2, we observed that the pristine LDPE-MPs and Bio-MPs with adsorbed-CPF in soil were not bioconcentrated in earthworm cast compared to pristine MPs without CPF; however, aged LDPE-MPs and Bio-MPs with adsorbed-CPF were significantly bioconcentrated compared to aged MPs without CPF. The findings suggest that MPs undergo ageing processes and become adsorbed with other contaminants in the natural environment, potentially leading to their bioconcentration and translocation by earthworms. Even though increasing studies have focused on the effect of MPs on the uptake of other contaminants by organisms (Tourinho et al., 2023), how the contaminants could ,in turn, affect the ingestion of MPs by organisms has been largely overlooked. In Chapter 3, with the adsorption of CPF, more LDPE MPs were transported into earthworm burrows with higher distribution rates of LDPE-MPs in deeper soil. In turn, the presence of MPs (both LDPE and Bio) also led to more CPF being transported by earthworms into soil. So far, there hasn't been much research examining the biogenic transport of MPs in combination with other contaminants in the soil. 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 MPs alone, probably because of the sizes of the MPs (1 mm and 5 mm) which were too big to be ingested and transported by *E. fetida*. Our results have revealed that the interaction of MPs and pesticides can affect their transport in soil mediated by earthworms. This process could potentially amplify the extent and magnitude of environmental risks associated with MPs and other contaminants in soil.

In Chapter 3, we observed decreased bioaccumulation of CPF in earthworm bodies with the presence of both LDPE-MPs and Bio-MPs, while this is dependent on the exposure concentrations of both MPs and CPF. This result varied from the mainstream findings which conclude that MPs can increase the bioaccumulation of contaminants in earthworm bodies (Tourinho et al., 2023). The MP size, earthworm ecotype (which can determine whether specific size of MPs can be ingested by specific species of earthworms), and exposure concentration of MPs can play important roles in contaminant bioaccumulation by earthworms (Tourinho et al., 2023). Apart from these factors, the exposure scenario can also affect the result. Wang et al. (2020)

revealed that the contribution of MP ingestion to polychlorinated biphenyls (PCBs) bioaccumulation in earthworms was higher under the scenario where PCB-contaminated MPs were added to clean soil than the scenario where soil was contaminated with PCBs and clean MPs. The difference is mainly caused by different diffusion gradients of PCBs between MPs and soils, which can affect the bioavailability of PCBs. In Chapter 3, we used CPF pre-adsorbed to MPs and plant litter which served as a food resource for earthworms in order to simulate realistic exposure scenario. The decreased bioaccumulation of CPF in the presence of MPs indicates that compared to the plant litter, MPs can reduce the bioavailability of CPF to earthworms, thereby reducing the bioaccumulation of CPF in earthworm bodies. The findings in Chapter 5 also revealed that the presence of MPs in soil, particularly aged MPs, significantly enhanced the bioaccumulation of CPF in radishes. This will be further discussed in the subsequent section.

Several studies have suggested that MPs can inhibit the dissipation of pesticides in soil by shielding them from microbial degradation and/or changing the soil microbiome, thereby increasing the persistence of pesticides in the soil (Peña et al., 2023). In Chapter 3, both LDPE and Bio MPs exhibited inhibitory effects on the degradation of CPF in soil, resulting in reduced formation of the metabolite 3,5,6-trichloropyridinol (TCP). Furthermore, in Chapter 4, we observed that aged MPs prolonged the half-life time (DT50) of CPF when applied individually or in combination with the pesticide DIF. However, the DT50 of DIF was not affected by MPs. Similar results were also observed in Chapter 5. Given the similar properties between CPF and DIF ($\log K_{ow}$, solubility, and volatility), further research is needed to explain their differing behaviours. Compared to hydrophobic pesticides such as CPF, the degradation of hydrophilic pesticides such as thiacloprid and glyphosate in soil was negligibly affected by MPs (Yang et al., 2018; Xu et al., 2020). Interestingly, several studies have found that the presence of pesticides can facilitate the degradation of MPs in soil. Even though this is not included in the present thesis, it provides a new perspective regarding the interaction of MPs and pesticides in the soil.

Ultimately, the consequences arising from the interactions between MPs and other contaminants on their translocation and transformation may render their fate and subsequent environmental impacts in soil ecosystems more unpredictable, particularly when relying solely on studies focusing on individual MPs or other contaminants. This underscores the imperative for further investigation to comprehensively comprehend their environmental implications.

6.2.4 Effects of MP-pesticide on plant growth and food safety

MPs can pose a threat to food safety through two mechanisms: (1) direct contamination of food, and (2) indirect interference with the uptake of other contaminants. Regarding the first mechanism, there is a growing body of evidence indicating that MPs, typically in submicron and nano sizes, can pose a threat to food safety by accumulating in the food chain, particularly in fish and crustaceans (Zhang et al., 2019; Usman et al., 2020). Unfortunately, contamination with MPs has become evident in many categories of food products, including some that are consumed frequently and in large quantities, namely, drinking water and kitchen salt (Peixoto et al., 2019; Danopoulos et al., 2020).The contamination of MPs in agricultural products has also been found (Silva et al., 2021). For example, Li et al. (2020) revealed that submicrometre- and micrometre-sized plastic particles can contaminate wheat (*Triticum aestivum*) and lettuce (*Lactuca sativa*), by penetrating through the roots and then being transported to the shoots. Additionally, Oliveri Conti et al. (2020) have reported that a mean concentration of 132,740 particles per gram of MPs and nanoplastics was detected in five commonly consumed fruits and vegetables (apples, pears, broccoli, lettuce, and carrots). Taking these findings as representative for this food group and following the World Health Organization's recommendation to consume a minimum of 400 grams of fruits and vegetables daily (WHO, 2019), it is estimated that humans would ingest approximately 53.1×10^6 particles per day (Domenech and Marcos, 2021). Apart from the direct contamination of agricultural products, the indirect effects from MPs affecting the bioaccumulation of other soil contaminants on food safety are also worth noting. The effects of MPs on the bioavailability of other contaminants have been investigated in several studies, and most of them have shown that the presence of MPs can decrease the bioaccumulation of contaminants in plants (Tourinho et al., 2023). This decrease in bioaccumulation is attributed to the interaction between MPs and contaminants, leading to altered bioavailability and uptake of the contaminants by plants. However, in Chapter 5, the presence of MPs in soil, especially aged MPs, increased the bioaccumulation of CPF in radish roots which is due to inhibited dissipation of CPF with the presence of MPs leading to higher CPF bioavailability. Previous studies attributed the increased bioaccumulation to the increased bioavailability of contaminants due to the presence of MPs decreasing the adsorption of contaminants in soil and/or the uptake of contaminants-sorbed MPs (Li et al., 2021; Wang et al., 2021; Jia et al., 2022). In Chapter 5, we proposed an alternative explanation suggesting that the presence of MPs could potentially enhance the bioaccumulation of hydrophobic organic contaminants by extending their persistence in the environment.

The effects of MPs on plants are an emerging area of research, and the available evidence suggests that MPs can have both positive and negative effects on plant growth and development. Some studies have shown that MPs can act as a physical barrier, reducing water loss and increasing soil moisture, which can benefit plant growth and survival under drought conditions (Lozano and Rillig, 2020). Additionally, MPs can provide a source of nutrients and organic matter to the soil, potentially enhancing soil fertility and plant growth (de Souza Machado et al., 2019). However, other studies have reported negative effects of MPs on plant growth and development. For example, MPs can reduce seed germination, root growth, and biomass production in various plant species (Qi et al., 2018; van Kleunen et al., 2019; Meng et al., 2021; Pflugmacher et al., 2021). However, limited studies have focused on the combined effects of MPs and pesticides on higher plants, resulting in inconsistent outcomes. The combined exposure can cause growth inhibition, activate antioxidant systems, or induce overall toxicity and physical damage. Some circumstances resulted in a reduction in the adverse effects since the adsorption on MPs decreased the toxicity of the pesticides (Xu et al., 2021; Fajardo et al., 2022; Yan et al., 2022; Lu et al., 2023). In Chapter 5, the impacts of MPs and pesticides on radish biomass at harvest varied depending on the type of MP, with the primary effects stemming from MPs rather than pesticides. Specifically, pristine LDPE-MPs with pesticides facilitated the growth of radish roots while aged LDPE-MPs with pesticides decreased the radish root biomass. In contrast, both pristine and aged Bio-MPs decreased the radish root biomass. Therefore, grasping these combined influences of MPs and pesticides on plants is vital for shaping strategies in environmental conservation and sustainable farming. Such understanding demands an integrated perspective, accounting for the intricate interactions and aggregate effects of these contaminants within the soil-plant ecosystem.

6.3 Implications

Our findings revealed that the combination of MPs and pesticides may pose more multifaced and concerning implications in soil ecosystem, especially regarding food safety which is currently under research. Our study has demonstrated that aged MPs can serve as a stronger carrier for hydrophobic contaminants compared to pristine MPs, exhibiting significantly higher adsorption capacities and lower desorption capacities. This suggests that previous studies conducted solely with pristine MPs may have underestimated the carrier effects of MPs. Consequently, the evolving dynamics between MPs and pesticides potentially challenge established norms regarding pesticide behaviour in the environment, emphasizing the necessity for continuous and adaptive research in this field. The study also emphasizes the intricate

correlation between MPs, pesticides, and earthworms. Earthworms play a vital role in maintaining soil health, facilitating nutrient cycling, and enhancing soil structure. The research highlights the combined impact of MPs and pesticides on earthworm health and behaviour, subsequently affecting soil health and ecosystem functionality. Additionally, we present a novel perspective that adsorbed CPF can alter the toxicity resulting from biodegradable MPs by influencing earthworm ingestion of such particles. This contributes to our understanding of the interactions between MPs and other contaminants within soil ecosystems. The investigation into the translocation and transformation of MPs and pesticides within soil provides valuable insights into their environmental behaviours. By elucidating the movement and alteration of MPs and pesticides in soil environments, this study contributes to our comprehension of their fate in the soil. This knowledge is essential for accurately predicting the long-term behaviour of these contaminants and their implications for soil health and broader ecosystem impacts. Moreover, one of the most crucial aspects of this research lies in its implications for food safety and security. The negative effects of MPs and pesticides on crops pose potential risks to the safety of food products. Therefore, the related research is particularly vital in the context of growing global needs for ensuring food safety and security, as well as the increasing use of plastic products and pesticide application. The findings underscore the necessity for rigorous monitoring and management strategies in agricultural practices to prevent contamination of food products by MPs and pesticides. Last but not least, our study reveals that biodegradable MPs, especially aged MPs, not only have adverse effects on plant growth but also significantly influence pesticide bioaccumulation in plants which is observed for the first time. These findings provide us with an additional perspective to comprehend the impacts of biodegradable MPs on soil.

6.4 Limitations and recommendations

The present study provides insights into the behaviours and implications of MPs in the environment, particularly with regard to their interaction with pesticides. However, it is important to acknowledge certain inherent limitations that should be taken into consideration. Consequently, we also offer recommendations for further research in this field.

The adsorption-desorption experiments conducted in solution may not fully capture the complexities of real-world soil environments. The presence of soil and organic matter in natural settings can significantly influence the adsorption behaviours of contaminants, a factor not accounted for in solution-based experiments. Additionally, considering the relatively small quantity of MPs compared to soil mass, current studies might overestimate the

adsorption capacity of MPs. To address this gap, future research should include adsorption-desorption experiments incorporating soil matrices. This approach will provide a more realistic assessment of microplastics' role in the fate of contaminants in natural soil environments.

The use of two types of MPs including LDPE-MPs and Bio-MPs (PBAT blended with PLA), highlights a limitation of our study: the vast diversity of polymers. Different compositions and aging processes among various MP types could lead to diverse environmental interactions. Particularly, MPs which are biodegradable in the soil, due to their dynamic nature, may significantly affect soil ecosystems, altering physicochemical properties, nutrient cycling, and microbiomes. Additionally, biodegradable MPs may possess an increased propensity to release adsorbed contaminants or additives, particularly during their degradation process within soil environments or within biological systems such as the gastrointestinal tracts of soil-dwelling organisms. Alternatively, future research employing environmental MPs as proxies may yield a more comprehensive understanding of the ecological implications associated with MPs. Moreover, our use of two hydrophobic pesticides, particularly CPF which is banned in some regions, suggests the need for research incorporating a broader spectrum of contaminants, including but not limited to various pesticides, to better reflect real-world scenarios.

The MPs we used throughout the study were made from raw materials used to produce commercial plastic mulch films. Therefore, the potential effects of additives were not considered in this study. In the real environment, the additives released from aged MPs could be another source of contamination. This aspect is particularly crucial as it may exacerbate the environmental effects of MPs. Consequently, investigating the environmental behaviours and toxicological impacts of MPs with additives will provide a more comprehensive understanding of their ecological risks.

Compared to the controlled experiments, the complexity of real-world ecosystems is significantly heightened by an array of diverse factors. This intricacy encompasses not just environmental elements but also varied soil compositions and interactions with a broader spectrum of organisms. For instance, the movement and impact of MPs and other contaminants in soil are influenced by a multitude of soil-dwelling organisms, such as earthworms and springtails, and abiotic factors like rainfall. These factors intermingle in dynamic and often unpredictable ways, profoundly impacting ecological interactions and processes. This complexity presents challenges in extrapolating laboratory findings to natural settings, underscoring the need for

future research to consider a wider range of mechanisms and variables. This holistic approach is essential for a more comprehensive and accurate understanding of environmental phenomena.

In acknowledging these limitations, we highlight areas where future research can delve deeper. It's essential to understand that while our study provides a fundamental understanding of the interactions between MPs, pesticides, and soil biota, the intricate web of ecological interactions in natural settings necessitates more comprehensive studies.

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English summary

The thesis begins with an exploration of the widespread use of plastics and pesticides in modern agriculture. The benefits of agro-plastics, particularly plastic mulch films, in enhancing crop productivity are highlighted. However, these practices have led to significant environmental concerns, notably the accumulation of non-biodegradable plastics and microplastics (MPs) in soil. The thesis also addresses the critical role of pesticides in agriculture, outlining their adverse effects on soil health and food safety. This chapter sets the stage for the thesis by discussing the coexistence of MPs and pesticides in the terrestrial environment, their individual and combined impacts, and the need for comprehensive research in this area. This PhD study aims to enhance our understanding of the environmental behaviours and potential risks of MP-pesticide combination in soil.

Chapter 2 delves into the effects of microplastic aging on the adsorption-desorption of the insecticide chlorpyrifos (CPF) and its bioconcentration in earthworm casts. The study finds that aged MPs, both nondegradable (LDPE) and biodegradable (PBAT blended with PLA, Bio), exhibit higher CPF adsorption capacities than their pristine counterparts. This suggests that the aging process enhances MPs' role as vectors for CPF. Additionally, the chapter examines the impact of MP aging and CPF adsorption on the bioconcentration of MPs in earthworm casts, revealing significant differences based on MP aging and combined effects with CPF. By understanding the adsorption-desorption behaviours of CPF on these MPs and their ingestion by earthworms, we further explored their combined toxicity on earthworms, and biogenic transport, bioaccumulation, and degradation.

Chapter 3 explores how the presence of MPs and CPF influences earthworm health, the biogenic transport of MPs and CPF, and the bioaccumulation and degradation of CPF. The results indicate that the combination of MPs and CPF leads to either increased or decreased toxicity compared to MPs alone, which is dependent on the type and concentration of the MPs. Moreover, with CPF addition, more LDPE-MPs (8%) were transported into earthworm burrows and the distribution rates 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.

Chapter 4 investigates the dissipation of a pesticide mixture (CPF and DIF) in soil contaminated with aged LDPE-MPs and Bio-MPs. The results show that aged MPs increased the degradation half-life (DT50) of CPF when it was applied both individually and in a mixture. In contrast, the DT50 of DIF was not affected by aged MPs. However, the DT50 of DIF was higher when DIF was applied in a mixture than when applied individually. By understanding the effects of aged MPs on the dissipation of pesticide mixtures in the soil, we further explore the bioaccumulation of pesticide mixtures by radish cultivated in the soil contaminated with aged MPs.

Chapter 5 studies the growth parameters and pesticide bioaccumulation of radish cultivated in soil contaminated with aged MPs and pesticides. Results show that pristine LDPE-MPs were found to enhance radish root biomass, whereas aged LDPE and both pristine and aged Bio-MPs significantly reduced it. The presence of these MPs also affected the dissipation of CPF in soil, with aged MPs notably inhibiting it, leading to higher CPF content in radish roots and leaves. These results highlight the potential risk of MPs in agricultural soils affecting plant growth and contaminant bioaccumulation.

Chapter 6 delves into a detailed discussion of the findings from previous chapters. This chapter focuses on the nuanced behaviours of MPs, particularly aged types, in soil environments and their interaction with pesticides. The discussion primarily revolves around the implications of these findings for environmental health and food safety. It reviews the observed effects on earthworm toxicity and plant growth, noting the significant role of MPs in altering pesticide bioavailability and persistence in the soil. The chapter further acknowledges the novel insights provided by the study into the potential threat to food safety and food security. This comprehensive analysis emphasizes the need for ongoing research to unravel the full extent of the environmental impact of MPs and other contaminants, particularly in agricultural settings.

Acknowledgements

As I look back on the past four years of my PhD journey, starting from the moment I first stepped into Europe, filled with uncertainty about my future, I feel a deep sense of gratitude towards so many people who have helped me along the way.

A huge thank you to Prof. Dr Violette Geissen, my promotor. It was her who opened the door for me to join SLM and start this incredible journey. I still remember how welcoming she was when I first reached out to her, inviting me not just to the group but also to join her basketball team. Her energy and love for life, from playing sports like table tennis and basketball to running on the streets in the early mornings, have inspired me to find a better balance between work and life. Prof. Geissen always respected my research ideas and gave me the freedom to explore, which was key to my successful journey.

I also want to thank my co-promotor, Dr. Xiaomei Yang, for her invaluable advice and insights throughout my research. She was always there, loudly cheering on my ideas and guiding me when I felt stuck. Her guidance was a big help, especially when things got tough.

A special thanks to the dedicated technicians, Rima, Hennie, Piet, and Harm, whose skill and diligence have been the backbone of my experimental work.

To all my friends at SLM and Wageningen, your support and companionship mean a lot to me. You've all been a part of this incredible chapter in my life.

I would like to express my gratitude to the China Scholarship Council for the funding during my PhD.

Thank you, everyone, for being with me on this journey.

About the author

Hui Ju was born on October 6, 1993, in a lovely village in Anqing, Anhui Province, where he grew up until he was 18. In 2016, after studying for four years at Central South University of Forestry and Technology, he earned his Bachelor's degree in Engineering. Then he started his master in Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. That's where he first got into studying microplastics.

In 2019, Hui Ju decided to take his studies further and started his PhD at Wageningen University, in the Soil Physics and Land Management group. There, he kept on exploring more about microplastics, especially how they interact with pesticides, diving deeper into environmental research.

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Publications

1. **Ju, H.**, Zhu, D., Qiao, M., 2019. Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail, Folsomia candida. Environ Pollut 247, 890-897.10.1016/j.envpol.2019.01.097
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3. **Ju, H.**, Yang, X., Osman, R., Geissen, V., 2023. The role of microplastic aging on chlorpyrifos adsorption-desorption and microplastic bioconcentration. Environ Pollut 331, 121910.10.1016/j.envpol.2023.121910
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Wageningen, 01 May 2024

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The SENSE Research School declares that **Hui Ju** has successfully fulfilled all requirements of the educational PhD programme of SENSE with a work load of 31.7 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2022)
- Research in context activity: 'Organising the WSC Master class – Assessing plastic fate in soil' (2023)

Other PhD and Advanced MSc Courses

- Basic Statistics, PE&RC, & WIMEK (2019)
- Root Ecology, PE&RC, & Copenhagen University (2020)
- Soil Laboratory Skills Course, Wageningen university (2022)
- R and Big Data, PE&RC (2023)
- Generalized Linear Models, PE&RC (2023)
- Introduction to R and R Studio, PE&RC (2023)
- Big data exploration and object-oriented programming with Python, WIMEK (2023)
- Big Data in the Life Sciences, VLAG graduate school (2023)

Oral Presentations

- *The Effects of MPS on the radish production and bioaccumulation of pesticides*. International Conference on Chemistry and the Environment –ICCE 2023, 11-15 June 2023, Venice, Italy

Poster Presentations

- *Combined effects of chlorpyrifos and microplastics derived from conventional and biodegradable mulch film on earthworm (Lumbricus terrestris) and their Fate in Soil.* SETAC EUROPE 32nd annual meeting. 15-19 May 2022, Copenhagen, Denmark
- *Combined effects of microplastics and chlorpyrifos on the anecic earthworm Lumbricus Terrestris.* International Conference on Chemistry and the Environment –ICCE 2023, 11-15 June 2023, Venice , Italy

Colophon

The research described in this thesis was conducted at Soil Physics and Land Management Group, Wageningen University. This research described in this thesis was financially supported by the EU Horizon 2020 project (MINAGRIS) and the China Scholarship Council (CSC: 201904910501)

Cover design: Hui Ju and Stefanie van den Herik (Proefschriftmaken)

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