Author(s): Forsell, Venla; Saartama, Vili; Turja, Raisa; Haimi, Jari; Selonen, Salla

Title: Reproduction, growth and oxidative stress in earthworm Eisenia andrei exposed to conventional and biodegradable mulching film microplastics

Year: 2024

Version: Published version

Please cite the original version:

Forsell, V., Saartama, V., Turja, R., Haimi, J., & Selonen, S. (2024). Reproduction, growth and oxidative stress in earthworm Eisenia andrei exposed to conventional and biodegradable mulching film microplastics. Science of the Total Environment, 948, Article 174667. https://doi.org/10.1016/j.scitotenv.2024.174667

Science of the Total Environment 948 (2024) 174667

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Reproduction, growth and oxidative stress in earthworm *Eisenia andrei* exposed to conventional and biodegradable mulching film microplastics

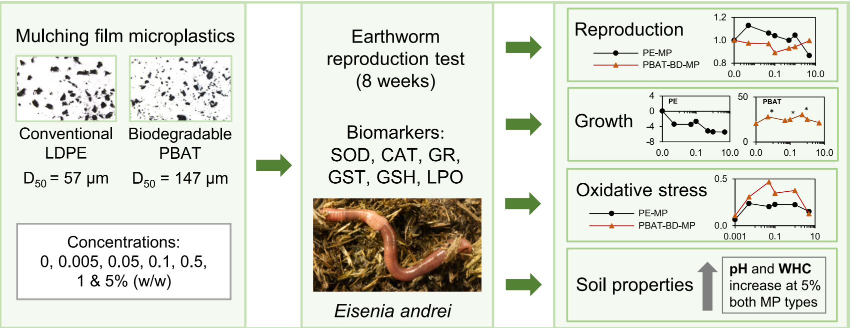
Venla Forsell[a,b], Vili Saartama[a,c], Raisa Turja[a], Jari Haimi[c], Salla Selonen[a,*]

[a] Finnish Environmental Institute Syke, Latokartanonkaari 11, 00790 Helsinki, Finland
[b] University of Helsinki, Faculty of Biological and Environmental Sciences, P.O. Box 4, 00014 University of Helsinki, Helsinki, Finland
[c] University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, 40014 University of Jyväskylä, Jyväskylä, Finland

HIGHLIGHTS

- Both conventional and biodegradable mulching film MPs affected earthworm *E. andrei*.
- PE and PBAT microplastics (MPs) increased soil pH and WHC in high MP concentration.
- Biodegradable PBAT-MPs increased the earthworm growth in low concentrations.
- PE and PBAT microplastics had no clear effect on the number of produced juveniles.
- PE and PBAT-MPs induced oxidative stress in environmentally relevant concentrations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Original content: Reproduction, growth and oxidative stress in the earthworm Eisenia andrei exposed to conventional and biodegradable mulching film microplastics (Original data)

Keywords:
Agricultural soil
Biodegradable plastic
LDPE
PBAT
Environmental stress
Soil ecotoxicology

ABSTRACT

Plastic contamination in agricultural soils has become increasingly evident. Plastic mulching films are widely used in agricultural practices. However, the increased use of biodegradable plastics has, to some extent, replaced their non-degradable counterparts. The fragmentation of plastics generates microplastics (MPs), posing risk to soil functions and organisms. In this study the effects of low-density polyethylene microplastics (PE-MP) and polybutylene adipate terephthalate biodegradable microplastics (PBAT-BD-MP) originating from mulching films on the earthworm *Eisenia andrei* were studied. The earthworms were exposed to seven concentrations (0, 0.005, 0.05, 0.1, 0.5, 1, and 5 % *w*/w) based on environmentally relevant levels and worst-case scenarios on soil contamination. Survival, growth, reproduction, and biomarkers for oxidative stress [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), glutathione (GSH), and lipid peroxidation (LPO)] were analysed. Additionally, the Integrated Biomarker Response Index (IBR) was calculated to assess the overall oxidative stress status of the earthworms. Results showed that PE-MP exposure slightly decreased the biomass of the earthworms towards higher concentrations, whereas PBAT-BD-MPs induced growth at lower concentrations. MPs did not have a significant effect on *Eisenia andrei* reproduction; however, a slight negative trend was observed in juvenile production with increasing PE-MP concentrations. Both PE-MP and PBAT-BD-MP affected antioxidant system, PE-MPs with changes in CAT and GR levels and PBAT-BD-MPs inducing effects on SOD and LPO levels. Additionally, both MPs exhibited effects on soil parameters, resulting in increased soil pH and water-holding capacity at 5 % concentration. Changes in soil parameters can further

---

* Corresponding author.
E-mail address: salla.selonen@syke.fi (S. Selonen).

https://doi.org/10.1016/j.scitotenv.2024.174667
Received 24 February 2024; Received in revised form 2 July 2024; Accepted 8 July 2024
Available online 10 July 2024

affect soil organisms such as earthworms. This study provides understanding of the ecotoxicological effects of conventional and biodegradable microplastics on the earthworm *Eisenia andrei.* It also shows that MP particles of both conventional and biodegradable mulching films induce oxidative stress, considered as an early-warning indicator for adverse ecological effects, in environmentally relevant concentrations.

1. Introduction

Global plastic production starting in the early 1950s has led ubiquitous plastic contamination across various environmental compartments. Plastics are extensively used in agriculture aiming to higher productivity and improved food safety (FAO, 2020). In 2022, worldwide plastic production reached 400 Mt, of which Europe contributed 59 Mt, 4 % founding its application in the agricultural sector (PlasticsEurope, 2023). Predictions suggest a 50 % increase in global agricultural plastic demand by 2030 compared to 2018 levels (FAO, 2021).

Plastic mulching films are extensively used in agricultural practices offering various advantages such as increased crop yields, improvement water use efficiency and weed control (Abouziena et al., 2008; Gao et al., 2019). Non-biodegradable low-density polyethylene (LDPE) is widely used in mulching films. However, challenges arise particularly with thinner mulching films, which are difficult and time consuming to collect from soil after application and use (Steinmetz et al., 2016). This makes them prone to leaving behind plastic fragments in the soil. In a broader context, the low recovery rates of mulching films can lead to significant accumulation of plastic in agricultural land through years (FAO, 2021; Lau et al., 2020). Plastic particles can also breakdown into microplastics (<5 mm, MPs) that are easily ingested by soil organisms and potentially causing diversity of impacts on the soil ecosystem (Li et al., 2022a; Rillig, 2012; Selonen et al., 2020; Yu et al., 2022).

As an alternative to non-degradable plastics, biodegradable mulching films have started to be utilized in agriculture. Polybutylene adipate terephthalate (PBAT) is one of the biodegradable polymers commonly used in biodegradable mulching films. PBAT is fossil-based and it shares similarities in mechanical properties with LDPE (Burford et al., 2023; Jian et al., 2020). However, as opposite to conventional LDPE films, PBAT films are not intended to be collected from the fields after use but are left to decompose in the soil. Nevertheless, the degradation rate of biodegradable plastic polymers often varies from the expected degradation rates due to environmental conditions (Campanale et al., 2024). Thus, concerns have risen that the repeated use of biodegradable mulching films may lead to accumulation of the plastics in the soil (Miles et al., 2017). Furthermore, there is an increasing need for research on the effects of biodegradable MPs on soil systems, given that studies in this area are even scarcer compared to those on conventional plastics.

Microplastics in soil have demonstrated the potential to alter the physiochemical properties of soil, thereby impacting its structure, water-holding capacity, bulk density, soil aggregation and soil-water distribution (de Souza Machado et al., 2018; Jiang et al., 2017). These soil properties are vital for soil health and may determine the suitability of the habitat for various soil organisms (Bünemann et al., 2018). Furthermore, MPs have the capacity to induce adverse effects on soil organisms and ultimately move to aboveground food chains e.g., via consumption of earthworms that have ingested MPs (Huerta Lwanga et al., 2017). Earthworms contribute significantly to soil-based ecosystem services, and are often termed as 'ecosystem engineers', due to their impacts on soil structure, nutrient cycling and microbial interactions. Furthermore, earthworms are widely utilized in ecotoxicological research as bioindicators for soil contamination (Calisi et al., 2013). Moreover, in the field of MP pollution research, earthworms are employed due to their ability to ingest MPs, burrowing activity, and their important role in soil decomposition process (Boughattas et al., 2021; Jiang et al., 2020; Rillig et al., 2017).

Previous studies have shown impacts of various MPs on earthworm growth rate, reproduction, and survival (Cao et al., 2017; Ding et al., 2021; Huerta Lwanga et al., 2016, 2017), with polyethylene and polystyrene being most common plastic materials used in earthworm ecotoxicological studies (Guo et al., 2023). Despite the limited number of conducted studies on biodegradable MPs, Ferreira-Filipe et al. (2022) employed *Eisenia andrei* as the test organism with pristine biodegradable mulching film and observed adverse effects on reproduction rates at concentration range from 0.0125 to 0.05 %.

Moreover, to understand the effects of MPs on earthworms, further information is required on their biological impacts, specifically cellular level responses, known as biomarkers. These biomarkers serve as early warning indicators for adverse effects in the higher levels of the biological hierarchy (Lackmann et al., 2022). MPs have been implicated in triggering oxidative stress in earthworms (Cui et al., 2022). Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the ability of an organism's antioxidant defence mechanisms to neutralize them. ROS, such as superoxide anion ($O_2{}^{\bullet -}$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), are highly reactive molecules containing oxygen, which can cause damage to various cellular components including lipids, proteins, and DNA (Halliwell and Gutteridge, 2007). When the production of ROS exceeds the capacity of antioxidants to remove them, oxidative stress occurs. Antioxidant enzymes help maintain cellular homeostasis by eliminating excess ROS to prevent the harmful effects of oxidative stress. Superoxide dismutase (SOD) serves as the first line of defence by converting superoxide radicals into hydrogen peroxide, which then becomes a substrate for catalase (CAT). CAT, along with glutathione-S-transferase (GST) and glutathione peroxidase (GPx), subsequently detoxifies these harmful ROS metabolites, transforming them into less toxic compounds like water and oxygen. Glutathione reductase (GR), in turn, facilitates the reduction of oxidized glutathione (GSSG) into its reduced form, glutathione (GSH). Glutathione, a critical antioxidant, plays a key role in this process by acting as a substrate for GPx and GST, thereby reducing oxidative stress. Additionally, these enzymes help prevent lipid peroxidation (LPO), a damaging process where free radicals attack lipids in cell membranes, leading to cell damage and dysfunction.

Studies addressing the effects of microplastic exposure have shown significant responses on oxidative stress, histopathological damage, and neurotoxicity in earthworms (Jiang et al., 2020; Rodríguez-Seijo et al., 2018). Chen et al. (2020) found that MPs from LDPE mulching film induced significant oxidative damage with elevating CAT activity and LPO levels. In comparison, another conventional MP type, polystyrene (PS) microspheres, significantly changed GSH level and SOD activity (Jiang et al., 2020).

Currently, there is a need for broader understanding on the ecological consequences of the microplastics that originate from conventional and biodegradable mulching films. This study aims to explore the responses in reproduction, growth, and oxidative stress of earthworm *Eisenia andrei* when exposed to seven different concentrations of conventional polyethylene microplastics (PE-MPs) and biodegradable PBAT microplastics (PBAT-BD-MPs), ranging from environmentally relevant concentrations to worst-case scenario of soil contamination. To assess oxidative stress status, biomarkers of the antioxidant defence system were evaluated, including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), glutathione (GSH) and lipid peroxidation (LPO). Additionally, Integrated Biomarker Response Index was derived with the aim of quantitively evaluate the combined biochemical response.

2. Materials and methods

2.1. Test materials and test soil

Microplastics originating from conventional mulching films made of low-density polyethylene (PE-MP; PAPILLONS code: M-PEDE-45-black-A0, P6) and microplastics originating from biodegradable mulching films consisting of a blend of starch and polybutylene adipate terephthalate (PBAT-BD-MP; PAPILLONS code: M-BIO1EL-15-black-A0, P5) were chosen as test materials due to the extensive utilization of these mulching film types in agriculture. Both materials were produced by cryomilling, resulting in PE-MPs with size below 3600 μm and PBAT-BD-MP with size below 500 μm. However, despite the wider size range, PE-MP material had higher proportion of small particles, 50 % of the particles being smaller than 57 μm, while 50 % of PBAT-BD-MPs were smaller than 147 μm (Fig. S1). Detailed description of the production and characterization of the materials are presented in Hurley et al. (2024) and in Fig. S1 in the Supplementary material.

Lufa 2.2 standard soil (Lufa Speyer, Germany) was used as test soil. The soil was dried for 48 h at 40 °C and stored in room temperature. The detailed properties of the soil are presented in the Table S1.

2.2. Test organism

Earthworms of the species *Eisenia andrei* were obtained from the laboratory cultures of the Finnish Environmental Institute Syke. Earthworms are recognized as a group of key species in soil due to their important role in the decomposition process. *Eisenia andrei*, along with the species *E. fetida*, are commonly used as model organisms in ecotoxicological studies and are also the species used in the standard test guidelines of OECD (OECD, 2016) and ISO/CEN (ISO, 2023). Earthworms were cultured in controlled laboratory conditions with a temperature of 20 °C, photoperiod of 16 h^L:8 h^D, and room moisture set at 40 %. The earthworms were fed with horse manure which had defaunated by three freeze-thaw cycles at − 20 °C, followed by 48 h at 20 °C. Additionally, it was ensured that the horses had not received any medications or treatments with parasite control products for at least two months before collecting the manure, as these substances may adversely affect the earthworms. For the experiment, age-synchronized adult earthworms with a mass between 400 and 600 mg, and with a fully developed clitellum, were selected and placed in Lufa 2.2 soil for 48 h to adapt to the experimental conditions.

2.3. Earthworm reproduction test

Earthworm reproduction test was performed according to the standard protocol ISO 11268-2:2023 (ISO, 2023). Lufa 2.2 soil was spiked with MP test materials in concentrations of 0 % (control), 0.005 %, 0.05 %, 0.1 %, 0.5 %, 1 %, and 5 % (*w*/w of dry soil weight) in five replicates. The exposure concentrations represented environmentally relevant levels and worst-case scenarios of microplastic contamination in soil. High concentrations are needed to assess and compare the effective concentration levels of the two MP types. MPs were mixed in Lufa 2.2. soil in metal bowls using metal spoons to ensure a homogeneous mixture. Moisture was adjusted to 50 % of maximum water-holding capacity of Lufa 2.2 soil. Each concentration of the spiked soil was divided into five 1.5 L glass jars, the mass of soil in each jar equalling to 500 g dry soil. Subsequently, 10 adult earthworms with fully developed clitellum were washed, gently dried, weighed, and transferred into each test jar.

The test jars were incubated for four weeks under laboratory conditions at 20 °C, photoperiod of 16 h^L:8 h^D, and room moisture set at 40 %. Throughout the exposure period, soil moisture was maintained weekly by adding the same amount of MilliQ-H_2O that had evaporated. The earthworms were also fed in the beginning of the test and once a week with moistened horse manure until week 4, equalling to 5 g of dry mass, and spiked with MP at the same concentration as the test soil.

After four weeks of exposure, all adults were removed from glass jars, counted, washed, gently dried, and weighed. From each replicate jar, three earthworm individuals were randomly taken into Eppendorf tubes, snap frozen in liquid nitrogen, and stored at −80 °C for biochemical biomarker analyses. The test was continued for an additional four weeks, with moisture levels checked weekly. At the end of the test, the jars were immersed into 50 °C water bath gradually. After 30 min, the juvenile earthworms emerged to the soil surface where they were removed to Petri dishes, counted, gently dried, and weighed.

2.4. Water-holding capacity and pH measurements

Water-holding capacity (WHC) was initially measured in Lufa 2.2 from freshly spiked soils to ensure the correct moisture content of the test soils ($n = 5$). Additionally, WHC was measured from all concentrations after spiking microplastics to evaluate the effects of MPs on WHC ($n = 3$). WHC measurement followed the Annex E in ISO 1268-2:2023 (ISO, 2023) protocol with a few adjustments. More detailed description of performing WHC measurements can be found in Method S1.

Soil pH was measured from all MP concentrations from the initial spiked test soils (n = 3) and at the end of the test (n = 5). The measurement followed the protocol of OECD (2016). 5 g of soil and 25 mL of 0.01 M $CaCl_2$ solution was added into 50 mL Falcon tubes. Samples were placed on a shaker for 2 h at 200 rpm and pH was measured next day.

2.5. Biomarker assays

The levels of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), glutathione (GSH) and lipid peroxidation (LPO) were measured as biomarkers for oxidative stress in earthworms after a four-week exposure to PE-MPs and PBAT-BD-MPs in six concentrations: 0 % (control), 0.005 %, 0.05 %, 0.1 %, 1 %, and 5 % (*w*/w). Three earthworms from every test replicate jar were collected, resulting in total 15 earthworms per concentration that were analysed for biomarkers individually. Concentration 0.5 % was left out from the analysis due to time limitations.

To prepare earthworm tissues for homogenization, the earthworm's posterior part behind the clitellum was homogenized using the OmniTip Homogenizator in a solution containing 0.1 M potassium phosphate buffer with 0.15 M potassium chloride (pH 7.4), with a sample:buffer ratio of 1:4. The resulting mixture was centrifuged at 10,000 ×*g* for 15 min at 4 °C. Before centrifugation, subsamples were taken for LPO measurements, diluted 1:2 with the homogenization buffer and 4 % butylhydroxytoluene (BHT) was added to inhibit peroxidation. Supernatants were aliquoted and stored at −80 °C until analysis.

The activity of CAT was assessed based on its capability to break down hydrogen peroxide (H_2O_2) (Claiborne, 1985). The assay was performed using UV-transparent microplates with absorption at 240 nm. The reaction mixture consisted of diluted H_2O_2 and 100 mM potassium phosphate buffer (pH 7). SOD assay was performed with Superoxide Dismutase Activity Assay Kit (Sigma-Aldrich) and measured at 450 nm. The SOD activity was expressed as the percentage of SOD inhibition. The activity of GR was determined from reduction of oxidized glutathione (GSSG) to GSH, where GSH undergoes a spontaneous reaction with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) leading to the formation of 5-thio (2-nitrobenzoic acid) (TNB). Additionally, the GR assay included 100 mM potassium phosphate buffer with 2 mM ethylene diamine tetraacetic acid (EDTA) (pH 7.5), 2 mM GSSG, 2 mM nicotinamide adenine dinucleotide phosphate (NADPH), and 3 mM DTNB, and reaction was measured spectrophotometrically at 412 nm (Smith and Johnson, 1988). GST activity was defined using a reaction mixture composed of Dulbecco's phosphate buffered saline, 100 mM 1-chloro-2,4-dinitrobenzene (CDNB), and 100 mM GSH, with absorbance measured at 340 nm (Habig et al., 1974). Analysis of GSH was performed with Glutathione

Colorimetric Detection Kit (Invitrogen, ThermoFisher Scientific) and the GSH activity was measured at 510 nm with excitation at 380 nm. Levels of LPO were determined by quantifying malondialdehyde (MDA) in the samples as described in Ohkawa et al. (1979). The reaction mixture consisted of 12 % trichloroacetic acid (TCA), 0.73 % thiobarbituric acid (TBA), 60 mM Tris-HCl, and 0.1 mM diethylenetriaminepentaacetic acid (DTPA) at pH 7.4. Results were expressed as TBA reactive substances (TBARS), and absorbance was measured at 535 nm. The protein concentrations were determined using the Bradford (1976) method, employing a bovine serum albumin standard, and measuring absorbance at 595 nm. The assays were conducted utilizing a microplate reader (TECAN Spark®) and Magellan software (TECAN).

The Integrated Biomarker Response Index (IBR) was determined following the original protocol of Beliaeff and Burgeot (2002), with adjustments based on Broeg and Lehtonen (2006). A higher IBR value indicates a stronger response in the treatment, reflecting activation or inhibition of a biomarker. The IBR value was normalized by dividing it by the number of biomarkers used (SOD, CAT, GR, GST, GSH, and LPO, $n = 6$).

2.6. Data analysis

R software (R Core Team, 2023) was used to perform statistical analyses. Normality and homogeneity of variance were tested, and when the assumptions of normality and homoscedasticity were met, the data were submitted to one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test to compare the MP treatments with the control. When data were not normally and homogenously distributed, a non-parametric Kruskal-Wallis Test followed with Wilcoxon Rank Sum test was employed. p-Values below 0.05 were considered statistically significant. All data are graphically expressed as mean ± standard error.

3. Results

3.1. Soil properties

After spiking the MPs in soil, the soil pH increased in the highest 5 % concentration compared to control groups in both PE-MPs and PBAT-BD-MP treatments (PE-MP: $F_{6,14} = 3.76$, $p = 0.019$, Dunnett's $p = 0.07$; PBAT-BD-MP: $F_{6,14} = 24.52$, $p < 0.001$, Dunnett's $p < 0.001$; Fig. 1). However, pH increased during the test in all MP concentrations, and no differences between the highest concentrations and control were detected when the test was ended. Instead, in the end of the test the soil pH was higher at 0.005 % PE-MP concentration ($F_{6,14} = 6.89$, $p < 0.001$, Dunnett's $p < 0.001$) and at 0.1 % PBAT-BD-MP concentration ($F_{6,14} = 12.65$, $p < 0.001$, Dunnett's $p < 0.001$) when compared to control (Fig. 1).

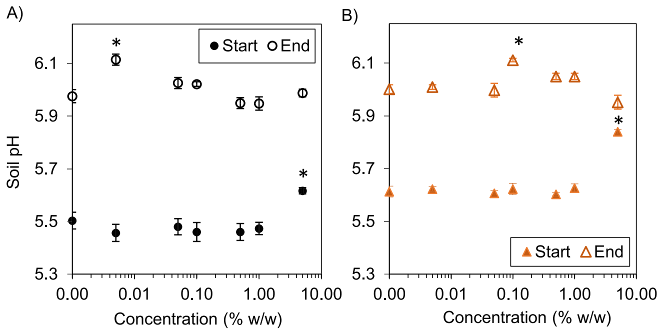


Fig. 1. pH levels of A) PE-MP and B) PBAT-BD-MP in Lufa 2.2 soil spiked with test concentrations (0, 0.005, 0.05, 0.1, 0.5, 1, and 5 % w/w). pH was measured at the start and at the end of the experiment. Microplastic concentrations are presented in logarithmic scale and the results are expressed as mean ± SE. Asterisk (*) indicates a significant difference ($p < 0.05$) compared to the control (0 %).

Increased soil water-holding capacity was observed in PE-MP and PBAT-BD-MP at the highest 5 % concentration when compared to the control groups (PE-MP: $F_{6,14} = 30.0$, $p < 0.001$, Dunnett's $p < 0.001$; PBAT-BD-MP: $F_{6,14} = 159.4$, $p < 0.001$, Dunnett's $p < 0.001$). PBAT-BD-MP exhibited a greater increase between the control and 5 % than PE-MP (Fig. 2).

3.2. Earthworm survival, biomass and reproduction

Based on the requirements of ISO 11268-2:2023 test guideline (ISO, 2023), the results obtained from the present study are considered valid, as the production of juveniles in the control treatment exceeded 30, the coefficient of variation did not exceed 30 %, and mortality in the controls was <10 %.

The four-week exposure of *E. andrei* to PE-MPs and PBAT-BD-MPs treatments showed no effect on survival (Table 1.). In the PE-MP exposure, there were no statistically significant differences observed between treatments regarding the change in earthworm growth, although a declining trend was noted ($F_{6,28} = 0.66$, $p = 0.681$; Fig. 3). In the PBAT-BD-MP exposure, an increase in earthworm growth was observed ($F_{6,28} = 6.23$, $p < 0.001$) at concentrations of 0.005 % ($p < 0.01$), 0.1 % ($p = 0.046$) and 0.5 % ($p < 0.001$) relative to the control group (Fig. 3).

No significant differences in earthworm juvenile production were observed across all concentrations for both MP types (PE-MP: KW, $p = 0.087$, and PBAT-BD-MP: $F_{6,23} = 0.39$, $p = 0.88$; Table 1). However, there was a declining trend in the number of juveniles as PE-MP concentrations increased, beginning from the lowest exposure concentration 0.005 % (Fig. 4). Decrease in the biomass of an individual juvenile was observed in the 5 % concentration of PBAT-BD-MP ($F_{6,28} = 4.40$, $p < 0.01$, Dunnett's $p < 0.001$). In PE-MP treatments no differences between the control were detected, although overall the differences among the treatments were found ($F_{6,28} = 4.09$, $p < 0.01$; Fig. 4).

3.3. Biomarker responses

In PE-MP exposure, CAT exhibited significantly higher activity at a concentration of 0.1 % compared to control group ($F_{5,84} = 3.43$, $p < 0.01$, Dunnett's $p < 0.01$), while a significant increase in GR activity was also noted at the same 0.1 % concentration compared to the control group ($F_{5,84} = 6.24$, $p < 0.001$, Dunnett's $p < 0.001$; Table 2). Furthermore, activities of GSH showed a slight increase, however not being statistically significant. Conversely, no apparent trends were observed in the activities of SOD, GST, and LPO.

Exposure to PBAT-BD-MP resulted in decreased SOD activities, showing significant differences at concentrations of 0.05 %, 1 %, and 5

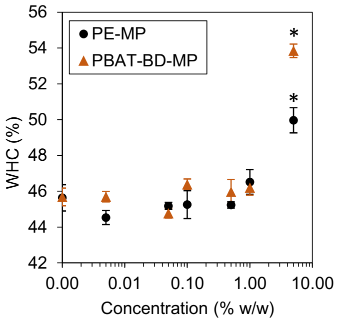


Fig. 2. Water-holding capacity of Lufa 2.2 soil spiked with PE-MPs and PBAT-BD-MPs in concentrations of 0, 0.005, 0.05, 0.1, 0.5, 1, and 5 % (w/w). Microplastic concentrations are presented in a logarithmic scale, and the results are expressed as mean ± SE. Asterisk (*) indicates a significant difference ($p < 0.05$) compared to the control (0 %).

Table 1
Survival (%), growth per adult earthworm (mg), number of juveniles, biomass of juveniles (g) and biomass per juvenile (mg) of earthworms exposed to PE-MPs and PBAT-BD-MPs at concentrations 0, 0.005, 0.05, 0.1, 0.5, 1, and 5 % (*w*/w). Results are expressed as mean ± SE, and statistically significant differences relative to the control are indicated as follows: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

	Concentration of microplastics (% *w*/w)						
	0	0.005	0.05	0.1	0.5	1	5
PE-MP							
Survival (%)	100	100	100	98	100	100	100
Growth per adult earthworm (mg)	−1.0 ± 19.8	−28.2 ± 22.9	−27.0 ± 12.5	−21.0 ± 16.2	−39.9 ± 11.3	−41.4 ± 21.2	−39.3 ± 15.8
Number of juveniles	158.2 ± 13.8	178.6 ± 5.6	168.2 ± 13.2	164.4 ± 4.1	158.2 ± 8.3	165.2 ± 7.7	137 ± 8.1
Biomass of juveniles (g)	1.95 ± 0.07	2.12 ± 0.04	1.98 ± 0.06	2.01 ± 0.03	2.03 ± 0.06	1.88 ± 0.05	1.79 ± 0.04
Biomass per juvenile (mg)	12.6 ± 0.8	11.9 ± 0.3	12.0 ± 0.7	12.2 ± 0.1	12.9 ± 0.4	11.5 ± 0.7	13.3 ± 1.0
PBAT-BD-MP							
Survival (%)	100	100	100	98	100	100	100
Growth per adult earthworm (mg)	124.6 ± 3.9	173.5 ± 10.7**	148.7 ± 8.5	158.3 ± 6.6*	178.2 ± 11.2***	153.1 ± 6.3	123.24 ± 10.5
Number of juveniles	120.6 ± 5.3	117.6 ± 13.4	117 ± 1.6	107.6 ± 5.9	112.2 ± 8.3	113.8 ± 7.5	120.2 ± 4.9
Biomass of juveniles (g)	1.61 ± 0.07	1.46 ± 0.08	1.54 ± 0.06	1.42 ± 0.06	1.45 ± 0.08	1.46 ± 0.06	1.14 ± 0.07***
Biomass per juvenile (mg)	13.3 ± 0.1	12.8 ± 1.0	13.2 ± 0.5	13.3 ± 0.7	13.1 ± 0.9	12.9 ± 0.4	9.5 ± 0.3*

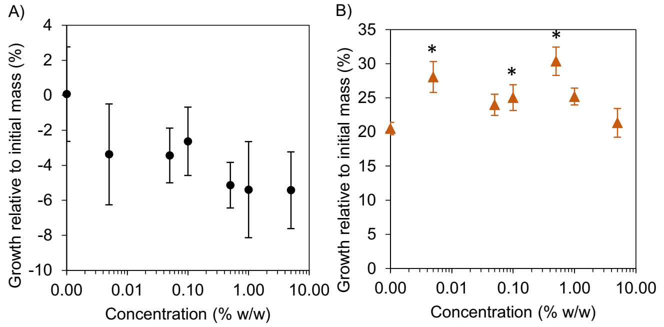


Fig. 3. Adult earthworm growth relative to initial biomass, expressed as percentages (%) in exposure to A) conventional PE-MPs and B) biodegradable PBAT-MPs in concentrations of 0, 0.005, 0.05, 0.1, 0.5, 1, and 5 % (*w*/w). Microplastic concentrations are presented in a logarithmic scale, and the results are expressed as mean ± SE. Asterisk (*) indicates a significant difference ($p < 0.05$) compared to the control (0 %).

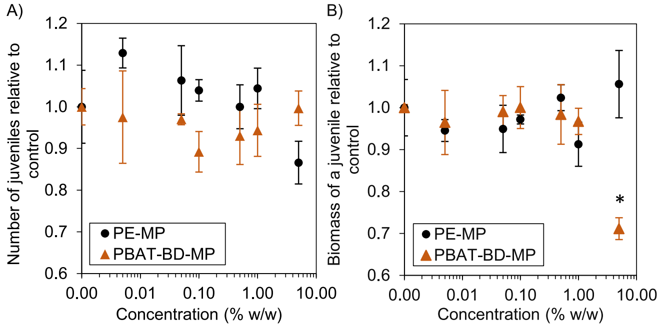


Fig. 4. A) Number of juveniles and B) biomass of a juvenile relative to the control after the exposure of earthworm *Eisenia andrei* to conventional PE-MPs and biodegradable PBAT-MPs in concentrations of 0, 0.005, 0.05, 0.1, 0.5, 1, and 5 % (*w*/w). Microplastic concentrations are presented in a logarithmic scale, and results are expressed as mean ± SE. Asterisk (*) indicates a significant difference ($p < 0.05$) compared to the control (0 %).

Table 2
Biomarker responses measured in earthworm *E. andrei* exposed to PE-MP and PBAT-BD-MPs at concentrations of 0, 0.005, 0.5, 0.1, 1, and 5 % (*w*/w). the measured biomarkers included catalase (CAT, μmol min^{-1} prot^{-1}), superoxide dismutase (SOD, inhibition rate %), glutathione reductase (GR, nmol mg^{-1} prot min^{-1}), glutathione S-transferase (GST, nmol min^{-1} prot^{-1}), glutathione (GSH, μmol/g), and lipid peroxidation (LPO, nmol TBARS/g). Values are presented as mean ± SE, with $n = 15$ for each biomarker except for GSH, where $n = 8$. Statistically significant differences compared to the control are indicated as $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

	Concentration (% *w*/w)					
	0	0.005	0.05	0.1	1	5
PE-MP						
CAT	53.2 ± 4.2	62.8 ± 3.8	64.2 ± 3.2	68.5 ± 3.5**	61.5 ± 4.6	50.6 ± 2.4
SOD	76.1 ± 1.4	79.2 ± 1.2	70.7 ± 2.2	76.5 ± 1.9	76.6 ± 1.3	80.8 ± 0.6
GR	0.99 ± 0.1	1.08 ± 0.1	1.23 ± 0.1	1.38 ± 0.1***	1.28 ± 0.1	0.90 ± 0.0
GST	64.5 ± 3.4	74.3 ± 4.4	72.5 ± 2.7	76.8 ± 4.7	73.6 ± 2.4	61.6 ± 3.2
GSH	16.7 ± 0.09	16.9 ± 0.08	18.1 ± 0.09	17.0 ± 0.13	18.6 ± 0.08	20.1 ± 0.07
LPO	10.8 ± 0.9	10.7 ± 0.8	12.3 ± 0.8	10.0 ± 0.8	9.8 ± 0.6	10.9 ± 0.9
PBAT-BD-MP						
CAT	46.1 ± 4.6	50.0 ± 2.6	57.4 ± 4.7	49.0 ± 3.2	48.9 ± 3.1	48.5 ± 2.7
SOD	74.5 ± 1.4	77.0 ± 1.0	62.4 ± 2.5*	71.6 ± 1.2	58.0 ± 2.2***	64.4. ± 1.6**
GR	0.95 ± 0.0	0.95 ± 0.1	1.04 ± 0.1	0.87 ± 0.1	1.18 ± 0.2	1.00 ± 0.1
GST	66.3 ± 3.2	68.6 ± 2.3	67.9 ± 4.4	61.7 ± 3.0	66.5 ± 1.9	64.0 ± 2.2
GSH	23.1 ± 0.2	21.6 ± 0.1	22.7 ± 0.1	22.0 ± 0.1	21.9 ± 0.1	19.2 ± 0.1
LPO	8.42 ± 0.3	9.04 ± 0.4***	15.4 ± 1.1***	12.6 ± 0.5***	10.7 ± 0.3**	11.6 ± 0.7***

% (KW = 45.13, $p < 0.001$; Table 2). Notably, an increasing trend was observed in LPO levels, with significant differences recorded at concentrations of 0.005 %, 0.05 %, 0.1 %, 1 %, and 5 % compared to control group (KW = 54.90, $p < 0.001$; Table 2). Meanwhile, activities of CAT, GR, GST, and GSH were similar within each treatment group, showing no statistical differences.

Integrated biomarker response index results of PE-MP exposure showed responses across all concentrations relative to the control group (Fig. 5). The responses remained relatively similar level at concentrations of 0.005, 0.05, 0.1, and 1 %, with a subsequent decrease observed at 5 %. In contrast, earthworms exposed to PBAT-BD-MP exhibited a bell-shaped response, where the highest response level was observed at 0.05 %, followed by a decrease in activity levels thereafter (Fig. 5).

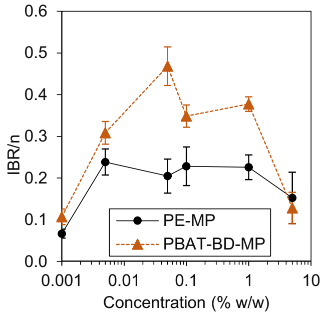


Fig. 5. The Integrated Biomarker Response Index for earthworm *Eisenia andrei* exposed to PE-MP and PBAT-BD-MP at concentrations of 0, 0.005, 0.05, 0.1, 1, and 5 % (*w*/w). Biomarkers used for IBR calculation included SOD, CAT, GR, GST, GSH, and LPO (n = 6). Microplastic concentrations are presented in a logarithmic scale, and all values are expressed as mean ± SE.

4. Discussion

In this study, we investigated the effects of conventional and biodegradable mulching film microplastics on the earthworm *Eisenia andrei* to better understand the potential ecological impacts of soil contamination with these two types of plastics. Both plastic types induced effects on *E. andrei*, albeit in different ways. This study encompassed a wide concentration range, including environmentally relevant concentrations, but also providing the advantage of observing results in more detail with dose-response approach, facilitating the comparison of the two plastic types.

In the present study, no mortality related to the MP treatments was observed, which is consistent with findings from studies by Angmo et al. (2023) and Rodríguez-Seijo et al. (2017). In contrast, Huerta Lwanga et al. (2016) reported earthworm mortality in PE-MP exposure, observing a 25 % mortality rate in earthworm *Lumbricus terrestris* when exposed to 1.2 % PE-MPs (<150 μm) for 60 days. The different outcome of the studies may arise e.g. from different earthworm species, longer exposure time, or differences in microplastic characteristics. The diversity of plastic types, sizes, shapes, as well as their chemical and physical properties, complicates direct comparison of the results of different studies. Recognizing these aspects is crucial when interpreting the results. For instance, the size of MP particles influences toxicity, as highlighted by Cheng et al. (2024), who found that 13 μm sized particles were more toxic to the earthworm *E. fetida* compared to 130 μm PE-MPs, highlighting the increased risk of ingestion associated with smaller particle sizes.

In this study, an increase in PE-MP concentration led to a slight negative trend in earthworm biomass. Previous studies have not demonstrated effects on earthworm growth in respect to lower MP concentrations (<0.1 %) (Angmo et al., 2023; Ding et al., 2021; Holzinger et al., 2023). However, higher concentrations have been shown to impact earthworm biomass. For instance, exposure to PS-MPs at concentrations of 1 % and 2 % (with a size of 58 μm) inhibited earthworm growth (Cao et al., 2017), as did exposure to PE-MP at concentrations of 25 % and 50 % (with an average size of 120 μm; Ding et al., 2021). Nevertheless, it is important to emphasize that the concentrations reported by Ding et al. (2021) do not reflect environmentally relevant levels in agricultural soils.

In our study, exposure to biodegradable PBAT-BD-MP demonstrated a positive impact on earthworm growth at concentrations 0.005 %, 0.1 %, and 0.5 % compared to the control group. This result may be attributed to the consumption of PBAT-BD-MP or the increased microbial biomass by the earthworms. Microbial biomass may have increased when micro-organisms have utilized the biopolymers as an additional carbon source (Morro et al., 2019). However, at the highest concentration of PBAT-BD-MPs, growth remained at the same level as in the control treatment, suggesting that 5 % concentration of biodegradable MPs did not further promote earthworm growth. This, along with the negative trend in biomass change observed in PE-MP exposure, could result from the ingestion of microplastics by the organism and their subsequent accumulation in the gut. Accumulation may lead to damage to the intestines, disruption of antioxidant regulatory systems, and alterations in microbial composition in the digestive tract. These effects were observed from exposure of PE-MP pellets (250–1000 μm; Rodríguez-Seijo et al., 2017) and polystyrene microbeads with sizes 5 μm and 20 μm (Li et al., 2022b). However, Holzinger et al. (2023) did not observe changes in biomass of earthworm *E. fetida* exposed to biodegradable poly(ʟ-lactide) (PLLA) and polycaprolactone (PCL) MPs (size range 45–200 μm).

Earthworm growth is a widely used parameter in ecotoxicology experiments. However, it does not consider the amount of soil and microplastics in the digestive tract of the earthworms when they are weighed. It is possible that a fuller gut due to intestinal obstruction caused by MPs may result in an increased earthworm mass in microplastic exposure studies. However, in the present study, this was likely not the case since the biomass did not increase at the highest MP concentrations.

In the present study, it is also possible that the potential increase in microbial activity at the highest PBAT-BD-MP concentration has resulted in poorer oxygen conditions for the earthworms, although the lids of the jars were allowing the aeration, and the jars were opened every week. This could explain the findings that in the highest PBAT-BD-MP concentration the juveniles were smaller, and the growth of the earthworms was not promoted as in the lower PBAT-BD-MP concentration. Oxygen conditions should be carefully considered in further studies when testing biodegradable plastics.

Earthworm reproduction is an ecologically relevant parameter, making it a useful indicator for observing impacts of environmental stress (Jiang et al., 2020). The results of this study are consistent with some previous findings indicating that conventional MPs did not significantly affect earthworm reproduction in variety of concentrations (Holzinger et al., 2023; Huerta Lwanga et al., 2016; Judy et al., 2019; Rodríguez-Seijo et al., 2017). In contrast, Ding et al. (2021) reported a 10 % reduction in juvenile production in *E. fetida* exposed to PE-MP concentrations at 0.053 % and 0.097 %, along with a 50 % decrease at concentrations at 34.7 % and 50 %.

In the case of biodegradable microplastics, both positive and negative responses in the reproduction of earthworms have been observed. Holzinger et al. (2023) demonstrated a positive correlation between the number of cocoons produced and an increase in the number of juveniles when exposed to PLLA and PCL MPs at concentrations of 1 % and 2.5 % (*w*/w). In contrast, Liwarska-Bizukojc et al. (2023) observed a decline in *E. andrei* reproduction when exposed to concentration at 12.5 % of biodegradable polylactic acid (PLA) MPs. Moreover, biodegradable PLA exhibited a more detrimental impact on reproduction compared to conventional PE-MPs on *E. fetida* (Ding et al., 2021). Ding et al. (2021) further concluded that the concentration of microplastics was a more significant factor than the type of microplastic itself. Additionally, differences in responses can be attributed to variations in energy investment strategies on organism exposed to pollutants (Calow, 1991).

In the present study, despite the lack of responses in the number of juveniles, the biomass of the juveniles was significantly lower in the highest (5 %) PBAT-BD-MP concentration compared to the control. The results exhibited considerable variation, as is customary due to the distinctive nature of each experimental arrangement in earthworm testing. Although, the juvenile biomass parameter is not included in the standard test protocol, it may be a suitable indicator of energy investment allocation under environmental stress.

Oxidative stress responses indicated by biomarkers were observed in both MP exposures, but in different ways. Previous studies have also documented responses in the earthworm antioxidant system, and investigating this aspect offers valuable insights into the interactions between MPs and earthworm physiology and cellular processes. The antioxidant system operates as a balanced system, where both activation and inhibition play a role, ultimately indicating a response to oxidative stress. Antioxidant enzymes protect the cells against oxidative damage and are widely acknowledged as sensitive indicators of environmental contamination (Liang et al., 2017).

In this study, a clear dose-dependent pattern of LPO was observed on earthworms exposed to PBAT-BD-MPs, indicating an excess production of ROS, which has led to oxidative damage. This was also supported by the significant inhibition of SOD observed with increasing concentrations of PBAT-BD-MPs. Furthermore, elevated levels of lipid peroxidation have been previously reported in relation to PE-MPs, underscoring the importance of exposure duration. Chen et al. (2020) observed increased lipid peroxidation levels in *E. fetida* exposed to LDPE-MP at concentration 0.15 % on day 7 and then on day 28 of exposure.

Decreasing trend in SOD activity was observed when *E. fetida* were exposed to 20 % concentrations of PE and PS-MPs (Wang et al., 2019). However, this concentration reported by Wang et al. (2019) is considerably high and not present naturally in the environment. In contrary, significant SOD activation have been discovered on *E. fetida* at 0.25 % concentration of high-density polyethylene (HDPE) and polypropylene (PP; Li et al., 2021). The results of the current study indicates significant effects to the antioxidant defence system already in low, environmentally relevant MP concentrations and the accumulation of superoxide radicals and other ROS in earthworm tissues at high PBAT-BD-MP levels resulting in increased LPO levels. Regarding changes observed in CAT activity, no clear trend was noted. However, Liwarska-Bizukojc et al. (2023) reported an increase in CAT activity *E. andrei* when exposed to biodegradable MPs at a concentration of 12.5 %. Thus, this concentration is remarkably higher than those utilized in the present study, which can impact the results shown.

Earthworms exposed to 0.1 % (*w*/w) PE-MPs showed a significant increase in CAT and GR activities. A significant increase of CAT activity in this study, might indicate the excess production of H_2O_2, to which the enzyme responds by upregulating its activity to defend the organism from oxidative stress. In the higher exposure concentrations, PE-MPs exhibited decreased CAT activities, when *E. fetida* was exposed to1% and 10 % concentrations for 14 days (Zhang et al., 2022).

Increased GR activity suggests an imbalance in GSH levels caused by PE-MPs. However, in the case of exposure to 5 % (*w*/w) PE-MPs, the GR level was even lower than that of the control group. This might indicate an over-production of ROS, leading to the antioxidant capacity being exceeded and reduction in GR activity, thus creating a “bell-shaped response” as a function of exposure levels (Dagnino et al., 2007). In contrast, a slight increase in GSH levels were observed, although it was not statistically significant. Consistent with our findings, comparable responses of increased GR activities were recorded by Angmo et al. (2023) when *E. fetida* was exposed to increasing concentrations of LDPE-MPs. Conversely, the freshwater oligochaete *Tubifex Tubifex* did not show changes in GR activities under PE exposure (Scopetani et al., 2020), nor did *E. andrei* when exposed to car tyre abrasion and polystyrene particles (Lackmann et al., 2022).

Oxidative stress responses can provide valuable insights into an organism's physiological condition, yet antioxidant enzymes and molecules work together in defence system with overlapping functions (Ledford and Niyogi, 2005). Therefore, it is not unusual that significant changes were not observed in the levels of SOD, GSH, LPO, and GST following exposure to PE-MPs, and similarly for CAT, GSH, GR, and GST levels in PBAT-BD-MPs. Therefore, the utilization of the Integrated Biomarker Response Index provides an additional perspective for visualizing biomarker responses in dose-response concentrations. PBAT-BD-MP showed a bell-shaped response where the presence of MPs can upregulate the amount of ROS produced in organism, therefore disturb the antioxidant system functions (Dagnino et al., 2007). The bell-shaped response is most evident when a wide range of concentrations is included, as clearly demonstrated in this study.

Both types of microplastics induced effects on the earthworm antioxidant system, however different biomarkers were affected. The increase in lipid peroxidation observed in PBAT-BD-MP exposure indicates more extensive damage in the earthworm defence system, suggesting that oxidative damage may already be occurring. However, drawing direct conclusions from biomarker responses combined with earthworm growth and reproduction results proves challenging. This limitation may be attributed to the exposure duration, highlighting the importance of focusing on long-term exposure tests in future research.

Especially concerning plastic mulching films, the quantity of plastic present in agricultural soils is influenced by the agricultural practices, duration of application and prevailing climatic conditions (Sa'adu and Farsang, 2023). Sa'adu and Farsang (2022) observed 225 ± 62 pieces kg^{-1} of microplastics within both soil layers (0–20 cm and 20–40 cm) of greenhouse farmland in southern Hungary, and similarly, Zhang and Liu (2018) documented 18,760 particles kg^{-1} in the Chai Valley of China. These findings underscore the location-specific nature of microplastic concentrations, highlighting considerable variations in environmentally relevant levels (Sa'adu and Farsang, 2023), in addition to the influence of the analytical methods on the detected MP concentrations (Jemec Kokalj et al., 2024).

Microplastics may induce effects on soil organisms, not only through ingestion of plastic particles, but also due to changes in the properties of the surrounding soil. Soil parameters such as water-holding capacity, pH, aggregate formation, hydraulic conductivity, and bulk density can be affected by the presence of microplastics (de Souza Machado et al., 2018; Wang et al., 2023). In the present study, a significant increase in soil pH was observed at a 5 % for both microplastic types. Likewise, Qi et al. (2020) observed an elevation in pH after exposure to LDPE-MPs at concentration 1 % over two months, and Zhang et al. (2022) reported similar findings with the same test material at concentrations of 1 % and 10 % after 28 days. In contrary, previous studies have also reported decline in soil pH resulted from exposure to HDPE, polyamide (PA), and PS MPs (Boots et al., 2019; Wen et al., 2022). Varying effects of MPs on soil pH in different studies may reflect the differences in the surface charge and functional groups on the surface of the MPs, which can affect the ion exchange dynamics in the soil (Ma et al., 2023; Song et al., 2024; Zhang et al., 2024). However, in the present study soil pH increased during the eight-week incubation in all treatments, likely due to natural biological activity of the earthworms. Thus, it is unlikely that the increased pH in the highest test concentrations at the beginning of the tests would have posed any major effect on the earthworms, considering that the pH is still below the pH detected at the end of the test and within the optimal pH range for *E. andrei* (ISO, 2023).

The other important soil parameter, water-holding capacity (WHC), was significantly increased at concentration of 5 % (*w*/w) for both plastics in the current study. Wang et al. (2023) also reported an increase in WHC at high concentrations of PE-MPs. Both positive and negative changes in soil water-holding properties have been reported, depending the size and shape of the MPs as well as on the soil structure (de Souza Machado et al., 2018, 2019; Jazaei et al., 2022; Mbachu et al., 2021; Wang et al., 2023). The change in WHC was found to relate to the MP-derived alterations in the pore-size distribution in the soil, but the hydrophobicity of the MP surfaces have also been suggested to play a role (Chia et al., 2022; de Souza Machado et al., 2018, 2019; Jazaei et al., 2022; Mbachu et al., 2021). In any case, it is possible that the increased WHC in the highest test concentrations of the present study have affected the responses seen in *E. andrei* in the highest concentrations of the MPs. The moisture of the soils was adjusted to the 50 % of the maximum WHC of Lufa 2.2 soil, meaning that in the highest concentrations with higher WHC, the soil was relatively drier compared to other concentrations, i.e. requiring more water to reach the 50 % of the WHC of the soil. This illustrates a mechanism of indirect effects of MPs, which may at least partly explain the lower growth and lower biomass of the juveniles in the 5 % PBAT-BD-MP exposure. In any case, further research is needed to explore the relationship between microplastics, soil parameters, and earthworms.

Another crucial consideration is that mulching films contain a variety of chemicals such as plasticizers, colour pigments, and UV stabilizers, which may include harmful substances (Scopetani et al., 2023). These plastic additives may potentially contribute to the observed effects (Sridharan et al., 2022). Further studies should focus on gaining a deeper understanding of the direct and indirect effects of both conventional and biodegradable plastics, as this study has provided valuable insights in this regard.

5. Conclusion

In this study, we evaluated the effects of two types of microplastics, conventional LDPE and biodegradable PBAT derived from commonly used mulching films, on earthworm *Eisenia andrei*. In addition to growth and reproduction, oxidative stress response was analysed across a wide range of concentrations to reveal possible dose-dependent relationships between the MP concentrations and the measured endpoints.

Both types of MPs induced effects on *E. andrei*. Earthworms exposed to PE-MPs exhibited a decreasing trend in biomass towards higher concentrations, whereas PBAT-BD-MP increased earthworm growth in lower concentrations. The MP exposure did not influence the number of juveniles produced. However, a slight negative trend was observed in juvenile production with increasing PE-MP concentration, and the biomass of the juveniles exposed to 5 % PBAT-BD-MP was lower than in control. Significant biomarker responses were observed in earthworms exposed to both plastic types; CAT and GR being the most responsive parameters of PE-MP exposure, while SOD and LPO were affected by PBAT-MP exposure. Additionally, both PE and PBAT increased soil pH and water-holding capacity at the highest MP concentrations, which may contribute to the observed responses in earthworms exposed to these concentrations.

This study highlights the complexity of MP interactions in soil systems, influenced by factors such as plastic type, concentration, environmental conditions as well as microbial biomass and activity. However, to better understand the potential ecological risks of microplastic pollution in the environment, further research is needed to explore the diverse range of environmentally relevant microplastic types with varying properties and their interactions with soil organisms.

CRediT authorship contribution statement

Venla Forsell: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Vili Saartama:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Raisa Turja:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Jari Haimi:** Writing – review & editing, Supervision, Conceptualization. **Salla Selonen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Reproduction, growth and oxidative stress in the earthworm Eisenia andrei exposed to conventional and biodegradable mulching film microplastics (Original data) (Zenodo)

Acknowledgements

We thank Luca Nizzetto, Rachel Hurley and Sarmite Kernchen for organising the production and delivery of the MP test materials, and Rachel Hurley also for providing the information for the characterization of the test materials. We also thank Minna Sepponen and Rami El Dairi for the help in laboratory, Kees van Gestel for his advices and Luca Nizzetto for the coordination of the PAPILLONS project.

Funding

This work was supported by the European Union's Horizon 2020 research from project PAPILLONS - Plastic in Agricultural 480 Production: Impacts, Life-cycles and LONg-term Sustainability [grant agreement No 101000210], Maj and Tor Nessling Foundation and Maa-ja vesitekniikan tuki ry.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (version 3.5; OpenAI, 2022) in order to improve language. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174667.

References

Abouziena, H.F., Hafez, O.M., El-Metwally, I.M., Sharma, S.D., Singh, M., 2008. Comparison of weed suppression and mandarin fruit yield and quality obtained with organic mulches, synthetic mulches, cultivation, and glyphosate. HortScience 43 (3), 795–799. https://doi.org/10.21273/HORTSCI.43.3.795.

Angmo, D., Dutta, R., Singh, J., Chowdhary, A.B., Quadar, J., Thakur, B., Kaur, H., Sharma, M., Singh, S., Vig, A.P., 2023. Biochemical responses, growth and reproduction of earthworm in low density polyethylene (LDPE). Environ. Qual. Manag. 33 (1), 223–237. https://doi.org/10.1002/TQEM.22041.

Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21 (6), 1316–1322. https://doi.org/10.1002/ETC.5620210629.

Boots, B., Russell, C.W., Green, D.S., 2019. Effects of microplastics in soil ecosystems: above and below ground. Environ. Sci. Tech. 53 (19), 11496–11506. https://doi.org/10.1021/acs.est.9b03304.

Boughattas, I., Hattab, S., Zitouni, N., Mkhinini, M., Missawi, O., Bousserrhine, N., Banni, M., 2021. Assessing the presence of microplastic particles in Tunisian agriculture soils and their potential toxicity effects using Eisenia andrei as bioindicator. Sci. Total Environ. 796, 148959 https://doi.org/10.1016/J.SCITOTENV.2021.148959.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Broeg, K., Lehtonen, K.K., 2006. Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. Mar. Pollut. Bull. 53 (8–9), 508–522. https://doi.org/10.1016/J.MARPOLBUL.2006.02.004.

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., de Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – a critical review. Soil Biol. Biochem. 120, 105–125. https://doi.org/10.1016/J.SOILBIO.2018.01.030.

Burford, T., Rieg, W., Madbouly, S., 2023. Biodegradable poly(butylene adipate-co-terephthalate) (PBAT). Phys. Sci. Rev. 8 (8), 1127–1156. https://doi.org/10.1515/psr-2020-0078.

Calisi, A., Zaccarelli, N., Lionetto, M.G., Schettino, T., 2013. Integrated biomarker analysis in the earthworm Lumbricus terrestris: application to the monitoring of soil heavy metal pollution. Chemosphere 90 (11), 2637–2644. https://doi.org/10.1016/J.CHEMOSPHERE.2012.11.040.

Calow, P., 1991. Physiological costs of combating chemical toxicants: ecological implications. Comp. Biochem. Physiol. Part C: Comp. Pharmacol. 100 (1–2), 3–6. https://doi.org/10.1016/0742-8413(91)90110-F.

Campanale, C., Galafassi, S., di Pippo, F., Pojar, I., Massarelli, C., Uricchio, V.F., 2024. A critical review of biodegradable plastic mulch films in agriculture: definitions, scientific background and potential impacts. TrAC Trends Anal. Chem. 170, 117391 https://doi.org/10.1016/J.TRAC.2023.117391.

Cao, D., Wang, X., Luo, X., Liu, G., Zheng, H., 2017. Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. IOP Conf. Ser. Earth Environ. Sci. 61 (1), 012148 https://doi.org/10.1088/1755-1315/61/1/012148.

Chen, Y., Liu, X., Leng, Y., Wang, J., 2020. Defense responses in earthworms (Eisenia fetida) exposed to low-density polyethylene microplastics in soils. Ecotoxicol. Environ. Saf. 187, 109788 https://doi.org/10.1016/J.ECOENV.2019.109788.

Cheng, Y., Guo, Y., Wang, F., Zhang, L., 2024. Effects of polyethylene microplastics stress on soil physicochemical properties mediated by earthworm Eisenia fetida. Environ. Sci. Pollut. Res. 2024, 1–12. https://doi.org/10.1007/S11356-024-32007-8.

Chia, R.W., Lee, J.Y., Jang, J., Kim, H., Kwon, K.D., 2022. Soil health and microplastics: a review of the impacts of microplastic contamination on soil properties. J. Soil. Sediment. 22, 2690–2705. https://doi.org/10.1007/s11368-022-03254-4.

Claiborne, A., 1985. In: Greenwald, R.A. (Ed.), Catalase Activity. Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, pp. 283–284.

Cui, W., Gao, P., Zhang, M., Wang, L., Sun, H., Liu, C., 2022. Adverse effects of microplastics on earthworms: a critical review. Sci. Total Environ. 850, 158041 https://doi.org/10.1016/J.SCITOTENV.2022.158041.

Dagnino, A., Allen, J.I., Moore, M.N., Broeg, K., Canesi, L., Viarengo, A., 2007. Development of an expert system for the integration of biomarker responses in mussels into an animal health index. Biomarkers 12 (2), 155–172. https://doi.org/10.1080/13547500601037171.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M. C., 2018. Impacts of microplastics on the soil biophysical environment. Environ. Sci. Tech. 52 (17), 9656–9665. https://doi.org/10.1021/acs.est.8b02212.

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics can change soil properties and affect plant performance. Environ. Sci. Tech. 53 (10), 6044–6052. https://doi.org/10.1021/acs.est.9b01339.

Ding, W., Li, Z., Qi, R., Jones, D.L., Liu, Q., Liu, Q., Yan, C., 2021. Effect thresholds for the earthworm Eisenia fetida: toxicity comparison between conventional and biodegradable microplastics. Sci. Total Environ. 781 https://doi.org/10.1016/J.SCITOTENV.2021.146884.

FAO, 2020. The State of Food Security and Nutrition in the World 2020. https://doi.org/10.4060/CA9692EN.

FAO, 2021. Assessment of Agricultural Plastics and their Sustainability: A Call for Action. https://doi.org/10.4060/cb7856en.

Ferreira-Filipe, D.A., Paço, A., Natal-da-Luz, T., Sousa, J.P., Saraiva, J.A., Duarte, A.C., Rocha-Santos, T., Patrício Silva, A.L., 2022. Are mulch biofilms used in agriculture an environmentally friendly solution? - an insight into their biodegradability and ecotoxicity using key organisms in soil ecosystems. Sci. Total Environ. 828, 154269 https://doi.org/10.1016/j.scitotenv.2022.154269.

Gao, H., Yan, C., Liu, Q., Ding, W., Chen, B., Li, Z., 2019. Effects of plastic mulching and plastic residue on agricultural production: a meta-analysis. Sci. Total Environ. 651, 484–492. https://doi.org/10.1016/J.SCITOTENV.2018.09.105.

Guo, S., Wang, Q., Li, Z., Chen, Y., Li, H., Zhang, J., Wang, X., Liu, J., Cao, B., Zou, G., Zhang, B., Zhao, M., 2023. Ecological risk of microplastic toxicity to earthworms in soil: a bibliometric analysis. Front. Environ. Sci. 11, 1126847 https://doi.org/10.3389/fenvs.2023.1126847.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

Halliwell, B., Gutteridge, J.M.C., 2007. Free Radicals in Biology and Medicine, 4th edition. Oxford University Press, New York.

Holzinger, A., Hink, L., Sehl, E., Rüppel, N., Lehndorff, E., Weig, A.R., Agarwal, S., Horn, M.A., Feldhaar, H., 2023. Biodegradable polymers boost reproduction in the earthworm Eisenia fetida. Sci. Total Environ. 892, 164670 https://doi.org/10.1016/J.SCITOTENV.2023.164670.

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

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J. de los A., Sanchez del Cid, L., Chi, C., Escalona Segura, G., Gertsen, H., Salánki, T., van der Ploeg, M., Koelmans, A. A., Geissen, V., 2017. Field evidence for transfer of plastic debris along a terrestrial food chain. Sci. Rep. 7 (1), 1–7. https://doi.org/10.1038/s41598-017-14588-2.

Hurley, R., Binda, G., Briassoulis, D., Carroccio, S.C., Cerruti, P., Convertino, F., Dvorakova, D., Kernchen, S., Laforsch, C., Löder, M.G.L., Pulkrabova, J., Schettini, E., Spanu, D., Tsagkaris, A.S., Vox, G., Nizzetto, L., 2024. Production and characterisation of environmentally relevant microplastic test materials derived from agricultural plastics. Sci. Total Environ., 174325 https://doi.org/10.1016/j.scitotenv.2024.174325.

ISO, 2023. European Standard EN ISO 11268-2:2023 "Soil Quality. Effects of Pollutants on Earthworms. Part 2: Determination of Effects on Reproduction of *Eisenia fetida/Eisenia andrei* and Other Earthworm Species".

Jazaei, F., Chy, T.J., Salehi, M., 2022. Can microplastic pollution change soil-water dynamics? Results from controlled laboratory experiments. Water 14 (21), 3430. https://doi.org/10.3390/w14213430.

Jemec Kokalj, A., Kalčíková, G., Selonen, S., Bosker, T., Drobne, D., Dvořáková, D., Hofman, J., Hurley, R., Kernchen, S., Laforsch, C., Löder, M.G.J., van Loon, S., Redondo-Hasselerharm, P.E., Saartama, V., Šmídová, K., Tsagkaris, A.S., Zantis, L.J., Nizzetto, L., van Gestel, C.A.M., 2024. Strategy towards producing relevant and reliable data for the hazard assessment of micro- and nanoplastics in agricultural soils. Trends Anal. Chem. 172, 117567 https://doi.org/10.1016/j.trac.2024.117567.

Jian, J., Xiangbin, Z., Xianbo, H., 2020. An overview on synthesis, properties and applications of poly(butylene-adipate-co-terephthalate)–PBAT. Adv. Ind. Eng. Polym. Res. 3 (1), 19–26. https://doi.org/10.1016/J.AIEPR.2020.01.001.

Jiang, X.J., Liu, W., Wang, E., Zhou, T., Xin, P., 2017. Residual plastic mulch fragments effects on soil physical properties and water flow behavior in the Minqin Oasis, northwestern China. Soil Tillage Res. 166, 100–107. https://doi.org/10.1016/J.STILL.2016.10.011.

Jiang, X., Chang, Y., Zhang, T., Qiao, Y., Klobučar, G., Li, M., 2020. Toxicological effects of polystyrene microplastics on earthworm (Eisenia fetida). Environ. Pollut. 259, 113896 https://doi.org/10.1016/J.ENVPOL.2019.113896.

Judy, J.D., Williams, M., Gregg, A., Oliver, D., Kumar, A., Kookana, R., Kirby, J.K., 2019. Microplastics in municipal mixed-waste organic outputs induce minimal short to long-term toxicity in key terrestrial biota. Environ. Pollut. 252, 522–531. https://doi.org/10.1016/J.ENVPOL.2019.05.027.

Lackmann, C., Velki, M., Šimić, A., Müller, A., Braun, U., Ečimović, S., Hollert, H., 2022. Two types of microplastics (polystyrene-HBCD and car tire abrasion) affect oxidative stress-related biomarkers in earthworm Eisenia andrei in a time-dependent manner. Environ. Int. 163, 107190 https://doi.org/10.1016/J.ENVINT.2022.107190.

Lau, W.W.Y., Shiran, Y., Bailey, R.M., Cook, E., Stuchtey, M.R., Koskella, J., Velis, C.A., Godfrey, L., Boucher, J., Murphy, M.B., Thompson, R.C., Jankowska, E., Castillo, A. C., Pilditch, T.D., Dixon, B., Koerselman, L., Kosior, E., Favoino, E., Gutberlet, J., Palardy, J.E., 2020. Evaluating scenarios toward zero plastic pollution. Science 369 (6509). https://doi.org/10.1126/science.aba9475.

Ledford, H.K., Niyogi, K.K., 2005. Singlet oxygen and photo-oxidative stress management in plants and algae. Plant, Cell Environ. 28, 1037–1045. https://doi.org/10.1111/j.1365-3040.2005.01374.x.

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

Li, H., Liu, L., Xu, Y., Zhang, J., 2022a. Microplastic effects on soil system parameters: a meta-analysis study. Environ. Sci. Pollut. Res. 29 (8) https://doi.org/10.1007/s11356-021-18034-9.

Li, T., Lu, M., Xu, B., Chen, H., Li, J., Zhu, Z., Yu, M., Zheng, J., Peng, P., Wu, S., 2022b. Multiple perspectives reveal the gut toxicity of polystyrene microplastics on Eisenia fetida: insights into community signatures of gut bacteria and their translocation. Sci. Total Environ. 838, 156352 https://doi.org/10.1016/J.SCITOTENV.2022.156352.

Liang, J., Xia, X., Zhang, W., Zaman, W.Q., Lin, K., Hu, S., Lin, Z., 2017. The biochemical and toxicological responses of earthworm (Eisenia fetida) following exposure to nanoscale zerovalent iron in a soil system. Environ. Sci. Pollut. Res. 24 (3), 2507–2514. https://doi.org/10.1007/s11356-016-8001-6.

Liwarska-Bizukojc, E., Bernat, P., Jasińska, A., 2023. Effect of bio-based microplastics on earthworms Eisenia andrei. Sci. Total Environ. 898, 165423 https://doi.org/10.1016/J.SCITOTENV.2023.165423.

Ma, J., Wu, Y., Xu, C., Luo, Z., Yu, R., Hu, G., Yan, Y., 2023. Inhibitory effect of polyethylene microplastics on roxarsone degradation in soils. J. Hazard. Mater. 454, 131483 https://doi.org/10.1016/j.jhazmat.2023.131483.

Mbachu, O., Jenkins, G., Kaparaju, P., Pratt, C., 2021. The rise of artificial soil carbon inputs: reviewing microplastic pollution effects in the soil environment. Sci. Total Environ. 780, 146569 https://doi.org/10.1016/j.scitotenv.2021.146569.

Miles, C., DeVetter, L., Ghimire, S., Hayes, D.G., 2017. Suitability of biodegradable plastic mulches for organic and sustainable agricultural production systems. HortScience 52 (1), 10–15. https://doi.org/10.21273/HORTSCI11249-16.

Morro, A., Catalina, F., Sanchez-León, E., Abrusci, C., 2019. Photodegradation and biodegradation under thermophile conditions of mulching films based on poly (butylene adipate-co-terephthalate) and its blend with poly(lactic acid). J. Polym. Environ. 27 (2), 352–363. https://doi.org/10.1007/s10924-018-1350-0.

OECD, 2016. Test No. 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*). OECD Guidelines for the Testing of Chemicals. Section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264264496-en.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

PlasticsEurope, 2023. Plastics - the facts 2023. In: Plastics Europe 2024. Retrieved January 31, 2024, from. https://plasticseurope.org/knowledge-hub/plastics-the-facts-2023/.

Qi, Y., Ossowicki, A., Yang, X., Huerta Lwanga, E., Dini-Andreote, F., Geissen, V., Garbeva, P., 2020. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J. Hazard. Mater. 387, 121711 https://doi.org/10.1016/J.JHAZMAT.2019.121711.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/ (Version 4.3.2, released on 2023-10-31).

Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Tech. 46 (12), 6453–6454. https://doi.org/10.1021/es302011r.

Rillig, M.C., Ziersch, L., Hempel, S., 2017. Microplastic transport in soil by earthworms. Sci. Reports 7 (1), 1–6. https://doi.org/10.1038/s41598-017-01594-7.

Rodríguez-Seijo, A., Lourenço, J., Rocha-Santos, T.A.P., da Costa, J., Duarte, A.C., Vala, H., Pereira, R., 2017. Histopathological and molecular effects of microplastics in Eisenia andrei Bouché. Environ. Pollut. 220, 495–503. https://doi.org/10.1016/J.ENVPOL.2016.09.092.

Rodríguez-Seijo, A., da Costa, J.P., Rocha-Santos, T., Duarte, A.C., Pereira, R., 2018. Oxidative stress, energy metabolism and molecular responses of earthworms (Eisenia fetida) exposed to low-density polyethylene microplastics. Environ. Sci. Pollut. Res. 25 (33), 33599–33610. https://doi.org/10.1007/s11356-018-3317-z.

Sa'adu, I., Farsang, A., 2022. Greenhouse farming as a source of macroplastic and microplastics contamination in agricultural soils: a case study from Southeast-Hungary. Agrokém. Talajt. 71 (1), 43–57. https://doi.org/10.1556/0088.2022.00120.

Sa`adu, I., Farsang, A., 2023. Plastic contamination in agricultural soils: a review. Environ. Sci. Eur. 35 (1), 1–11. https://doi.org/10.1186/s12302-023-00720-9.

Scopetani, C., Esterhuizen, M., Cincinelli, A., Pflugmacher, S., 2020. Microplastics exposure causes negligible effects on the oxidative response enzymes glutathione reductase and peroxidase in the oligochaete tubifex tubifex. Toxics 8 (1). https://doi.org/10.3390/toxics8010014.

Scopetani, C., Selonen, S., Cincinelli, A., Pellinen, J., 2023. Chemical leaching from polyethylene mulching films to soil in strawberry farming. Front. Environ. Sci. 11 https://doi.org/10.3389/fenvs.2023.1129336.

Selonen, S., Dolar, A., Jemec Kokalj, A., Skalar, T., Parramon Dolcet, L., Hurley, R., Van Gestel, C.A.M., 2020. Exploring the impacts of plastics in soil – the effects of polyester textile fibers on soil invertebrates. Sci. Total Environ. 700, 134451 https://doi.org/10.1016/j.scitotenv.2019.134451.

Smith, D.B., Johnson, K.S., 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene 67 (1), 31–40. https://doi.org/10.1016/0378-1119(88)90005-4.

Song, X., Li, C., Qiu, Z., Wang, C., Zeng, Q., 2024. Ecotoxicological effects of polyethylene microplastics and lead (Pb) on the biomass, activity, and community diversity of soil microbes. Environ. Res. 252, 119012 https://doi.org/10.1016/j.envres.2024.119012.

Sridharan, S., Kumar, M., Saha, M., Kirkham, M.B., Singh, L., Bolan, N.S., 2022. The polymers and their additives in particulate plastics: what makes them hazardous to the fauna? Sci. Total Environ. 824, 153828 https://doi.org/10.1016/J.SCITOTENV.2022.153828.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., Schaumann, G.E., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. 550, 690–705. https://doi.org/10.1016/J.SCITOTENV.2016.01.153.

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

Wang, Z., Li, W., Li, W., Yang, W., Jing, S., 2023. Effects of microplastics on the water characteristic curve of soils with different textures. Chemosphere 317, 137762. https://doi.org/10.1016/J.CHEMOSPHERE.2023.137762.

Wen, X., Yin, L., Zhou, Z., Kang, Z., Sun, Q., Zhang, Y., Long, Y., Nie, X., Wu, Z., Jiang, C., 2022. Microplastics can affect soil properties and chemical speciation of metals in yellow-brown soil. Ecotoxicol. Environ. Saf. 243, 113958 https://doi.org/10.1016/J.ECOENV.2022.113958.

Yu, H., Shi, L., Fan, P., Xi, B., Tan, W., 2022. Effects of conventional versus biodegradable microplastic exposure on oxidative stress and gut microorganisms in earthworms: a comparison with two different soils. Chemosphere 307, 135940. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135940.

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

Zhang, S., Ren, S., Pei, L., Sun, Y., Wang, F., 2022. Ecotoxicological effects of polyethylene microplastics and ZnO nanoparticles on earthworm Eisenia fetida. Appl. Soil Ecol. 176, 104469 https://doi.org/10.1016/J.APSOIL.2022.104469.

Zhang, H., Huang, Y., Shen, J., Xu, F., Hou, H., Xie, C., Wang, B., An, S., 2024. Mechanism of polyethylene and biodegradable microplastic aging effects on soil organic carbon fractions in different land-use types. Sci. Total Environ. 912, 168961 https://doi.org/10.1016/j.scitotenv.2023.168961.