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# Underestimated and ignored? The impacts of microplastic on soil invertebrates—Current scientific knowledge and research needs

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The accumulation of plastics in the environment is a major problem in the Anthropocene. As most plastic is produced, used and discarded on land, ~4–23 times more plastics are deposited in soils than in the oceans. However, there is far too little knowledge on the ecological consequences of plastic pollution, especially for soil ecosystems. Microplastics (<5 mm), whether derived from larger plastic pieces through physical, chemical and biological degradation or produced as primary particles, is of considerable interest, as they can be ingested by organisms at the basis of the trophic net and transferred to higher trophic levels. Nonetheless, although the assessment of microplastic effects on soil invertebrates is of undeniable relevance, most studies have focussed on nano- and microplastics in aquatic environments. This review examines the current state of knowledge regarding the effects of microplastics on soil invertebrates. As part of the soil biota, these organisms are of utmost importance for carbon cycling, respiration and biodiversity. Based on strict quality criteria, the data of 45 papers reporting ecotoxicological effects on soil invertebrates were analyzed, considering various test organisms and types of microplastic (in terms of polymer, shape and size). However, although different impacts were demonstrated, a deduction of general effect tendencies of microplastics in soils was difficult due to the scarcity of data and the use of diverse methodological setups. Moreover, almost all experiments were based on short-term single-species testing involving only a small number of species and single microplastic types. The review concludes with a discussion of the remaining knowledge gap and the needs for a standardized approach allowing an ecologically relevant risk assessment of the impacts of microplastic on invertebrates in terrestrial ecosystems.

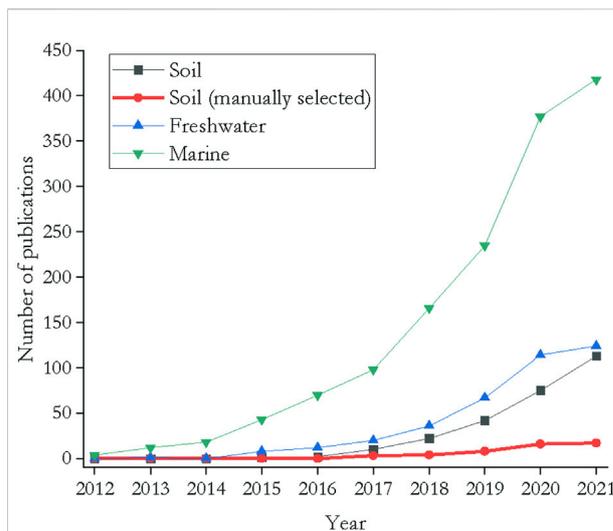
## KEYWORDS

invertebrates, mesofauna, microplastic, nanoplastic, soil, toxicity

## 1 Introduction

Environmental contamination with plastic has rapidly become a major problem of global proportion. The mass production of plastics started in the 1940s and has steadily increased (Thompson et al., 2009a; Cole et al., 2011; Hohn et al., 2020; PlasticsEurope, 2020), given the numerous desirable features of this material, including its low-cost, durability, lightweight and resistance to biodegradation. However, the recalcitrance of plastic has led to its accumulation in the environment and therefore its environmental risk (Thompson et al., 2005; Gautam et al., 2007; Shah et al., 2008; Barnes et al., 2009; Thompson et al., 2009b; Imhof et al., 2013). In 2020, ~367 million tonnes of plastics were produced globally (PlasticsEurope, 2021). About 54% of anthropogenic waste discarded into the environment consists of plastic (Hoellein et al., 2014). In the environment plastics can be found in different particle sizes: macroplastic (>25 mm), mesoplastic (5–25 mm), microplastic (<5 mm) and nanoplastics (<100 nm) (Zalasiewicz et al., 2016; Horton et al., 2017; Alimi et al., 2018). The focus of this study is microplastics, which are highly abundant in the environment, consist of a wide range of polymers (e.g. polyethylene, polystyrene, polypropylene) and forming various shapes (e.g. beads, fragments, fibers or films) (He et al., 2018). Microplastics are also defined according to their source: Primary microplastics are plastic particles produced directly for commercial or industrial use and most commonly consists of polypropylene, polyethylene and polystyrene (Horton et al., 2017; Helmberger et al., 2020). Secondary microplastics emerge through the physical, chemical and biological degradation of macroplastics (Thompson et al., 2005; Ryan et al., 2009; Gewert et al., 2015). Both plastic types can reach the soil via improper waste management, tire abrasion or the application of sewage sludge and waste water on agricultural fields, which is a common practice throughout the world (Blaesing & Amelung, 2018; Li et al., 2018; Corradini et al., 2019).

Plastic production, usage and disposal mainly happen in terrestrial ecosystems rather than in oceans. It was estimated that 80% of the litter found in oceans derives from terrestrial sources (Jambeck et al., 2015). Terrestrial ecosystems have therefore a high risk of being contaminated with plastic debris, and, consequently, have to be considered as long-term sinks or at least intermediate transport pathways (Zubris & Richards, 2005; Rillig, 2012). In fact, soils contain 4–23 times more plastic than oceans (Horton et al., 2017). It is thus surprising that most studies have focussed on marine or freshwater systems rather than soil ecosystems (Andrady, 2011; Browne et al., 2011; Wright et al., 2013; Erkes-Medrano et al., 2015; He et al., 2018; Wang et al., 2019). The first study of microplastics in soil was published in 2012 (Rillig, 2012). The scarcity of studies has continued as a Web of Science query revealed that in 2021 there were 418 publications examining the effects of microplastics in the marine environment, with far fewer studies of freshwater systems



**FIGURE 1**

Number of studies published between 2012 and 2021 that examined the impact of micro- and nanoplastics in different environments. Search terms: for marine ecosystems (microplastic\* OR nanoplastic\*) AND (marine\* OR sea\*) AND (invertebrate\* OR mesofauna\* OR meiobenthos\* OR organism\*); for freshwater systems (microplastic\* OR nanoplastic\*) AND (freshwater\* OR limn\*) AND (invertebrate\* OR mesofauna\* OR meiobenthos\* OR organism\*); for soil (microplastic\* OR nanoplastic\*) AND (soil\* OR terrestr\*) AND (invertebrate\* OR mesofauna\* OR organism\*). A manual selection of the hits was performed by excluding all publications that did not match the search terms (exclusion of false positives). Query performed on 12 October 2021.

(124 publications) and even fewer studies that focussed on soil (113 publications) (Figure 1). If the several false positive hits provided by the Web of science search for microplastic research in soils are excluded, a small number of 17 publications remain for the year 2021, which states a relative count of 0–3% between 2012 and 2021.

Soil is proposed to provide most of the living biomass and to be one of the most biodiverse habitats, harbouring about 25% of all species on Earth. Soil organisms are key drivers for ecosystem functions and processes, such as nutrient cycling and water transfer (Bar-On et al., 2018; Bardgett & van der Putten, 2014; Bowker et al., 2010; Lavelle & Spain, 2001; Porazinska et al., 2003; Roger-Estrade et al., 2010; van den Hoogen et al., 2019). The heterotrophic soil biota are responsible for up to half of the soil respiration and soil animals including the mesofauna considerably determine decomposition rates of organic matter (Cisneros-Dozal et al., 2006; Frouz, 2018).

Arrived in the soil, plastics exert both short- and long-term impacts on soil organisms (Steinmetz et al., 2016). The ingestion of the omnipresent micro- and nanoplastic by soil invertebrates is of particular concern as these organisms form the base of the trophic net and allow the transfer of plastic particles to higher trophic levels (Duis & Coors, 2016; Carbery et al., 2018). The

physical or mechanical effects of microplastics occur outside the body of the organism or after ingestion (Anbumani & Kakkar, 2018; Padervand et al., 2020). The strength of the effect was shown to inversely correlate with the plastic size, with smaller particles more likely to be ingested (da Costa et al., 2016; Horton et al., 2017). However, independent of particle size, the ecotoxicological effects of microplastics could be accurately predicted by their total surface area (shown for PS beads; Mueller et al., 2020). Furthermore, in addition to direct toxicity, indirect effects, via the interaction of microplastics with the food of the tested organisms, have been reported (e.g. Mueller et al., 2020b; Rauchschalbe et al., 2021; Besseling et al., 2013; Wright et al., 2013; Scherer et al., 2018; Oganowski et al., 2018). Finally, the additives used in plastic production, such as plasticizers and stabilizers, can leach into the soil and accumulate in the food chain, leading to chemical effects and biomagnification (Teuten et al., 2009; Haegerbaeumer et al., 2019a; Halle et al., 2020).

The ecotoxicological effects of microplastic on soil biota have been examined in several studies but drawing conclusions from those studies is hindered by differences in experimental methods and the concentration, type, shape and color of the tested microplastics (e.g. Boots et al., 2019). In addition, detailed quantitative analyses of the data are lacking (e.g. He et al., 2018; Wang et al., 2019; Kim Y.-N. et al., 2020). Therefore, in this review we evaluated the current state of knowledge regarding the effects of microplastics on soil invertebrates. To identify future research needs, the reviewed studies were differentiated in terms of (i) the test organism and toxicity endpoints and (ii) the type, shape, size and concentration of the microplastic. By this, we could compare effect threshold concentrations for various types of plastics and organisms and provided insights into possible toxicity mechanisms.

## 2 Methods of the literature research

The literature search was performed to identify all published results regarding ecotoxicological impacts of microplastics on soil invertebrates. The Web of Science and Google Scholar search was executed on the 12 November 2021 using 'Harzing's Publish or Perish' (Windows GUI Edition) 8.1.3625.7987 under usage of the following search term: TOPIC ((microplastic\* OR nanoplastic\*) AND (soil\* OR terrestri\*) AND (invertebrate\* OR mesofauna\* OR organism\*)). As the first study of the effects of microplastics on soil organisms was published in 2012 (Rillig, 2012), the query was performed for the years 2012–2021. The publications in the resulting list were assessed based on several quality criteria. First, the publications within the

raw list (I) were evaluated manually for their title and abstract to remove false positives (II). In the final step, the following quality criteria were applied: the use of soil as the testing medium in at least one experiment; a detailed description of type (shape and/or polymer), size and concentration of the microplastic and, if applied, the concentration of environmental chemicals (e.g. heavy metals); and the use of negative controls and statistical analyses (III). The steps comprising the literature search and the quality criteria are summarized in Figure 2.

Bar charts (OriginPro 2020 64-bit) were created to condense the general characteristics of the reviewed publications: experimental setup/soil, microplastic type/shape, the taxonomic group of the test organisms and the effect categories cellular response, (gut) microbiota, mortality, development, behavior, reproduction and community structure.

As not indicating an impact, any results regarding the ingestion, egestion, bioturbation of microplastic and the microplastic-related accumulation of other environmental chemicals were separately examined (Tables 1–3).

The effect-related results were condensed in lists (Tables 4–6) summarizing the tested species, associated taxa, the microplastic type (polymer, shape), size and concentration, the quantity and quality of the observed effects on the test organism, the corresponding author and the publication date. For clarity the effects were grouped within the above-mentioned parameter categories, whereby community structure was only physically affected, and can therefore be found in Table 4 only.

## 2.1 Chemical vs physical interactions

In this paper, we differentiate between chemical effects and the physical interactions of the studied organisms with microplastics. When considered together, they constitute an observed adverse effect (OAE) (according to ECETOC, 2018):

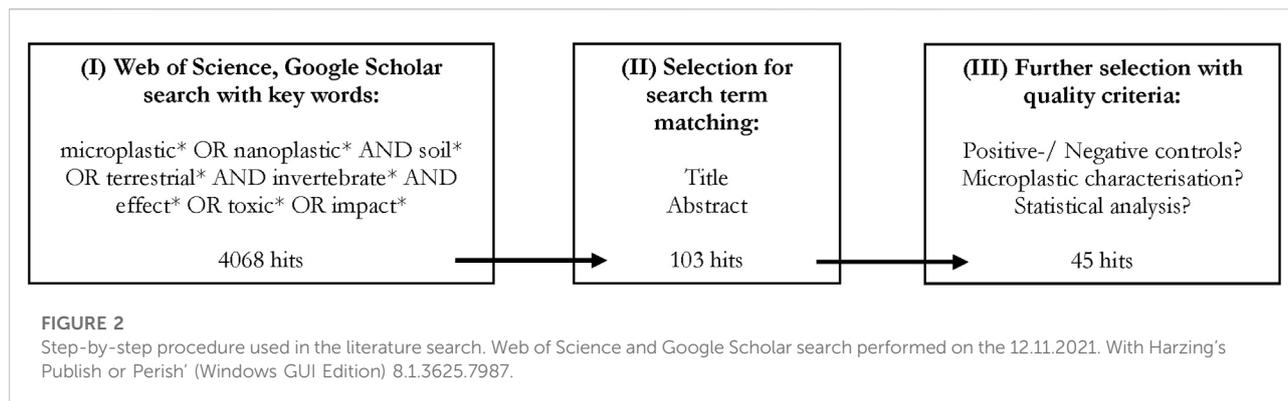
$$OAE = E_{IT} + E_{PInt}$$

where  $E_{IT}$  is the intrinsic toxic effect and  $E_{PInt}$  the physical interaction.

We provide following definitions for these terms:

Physical interactions are related to the physical properties of microplastics, including size, shape, rigidity, specific density, surface area and structure. In contrast to dissolved chemicals, insoluble, particulate microplastics can interact directly with the organism to cause physical injury or congestion. Indirect impacts can also occur, such as when microplastics interact with the soil to cause food dilution (Table 4).

The adverse effects of chemicals are related to the ability of the compounds to enter the cells of organisms, interact with



chemical receptors and thus cause a molecular, sub-organismic or organismic response. For microplastics, chemical effects arise from the leaching of chemicals associated with the microplastics, including additives (e.g. plasticizers or flame retardants), monomers, or compounds absorbed from the environment (e.g. polychlorinated biphenyls (PCBs), phenanthrene) (Tables 5, 6) (Teuten et al., 2009; Carbery et al., 2018).

## 3 Results

### 3.1 General characterization of the reviewed publications

From the initial raw list of 4,068 publications, 45 recently published papers (since 2017) were finally included in this literature review (Figure 2).

The publications describe experiments with exposure times between 3 min and 287 days. Physical effects of microplastics on soil invertebrates were examined in 32 of the 45 publications. Eighteen studies considered the effects of leached chemicals and environmental chemicals associated with microplastics. Overall, seven different effect endpoints were evaluated, with the most frequent being mortality, cellular response and development, analyzed in 29, 25, and 24 publications respectively, followed by reproduction and behavior (16 and 15 papers). The influence of microplastics on the invertebrate's (gut) microbiota was examined in six papers and the effects on the structure of the soil invertebrate communities in two papers (Figure 3).

Microplastics consisting of 13 different polymers were applied, namely polyethylene (PE, including high-density PE: HDPE, low-density PE: LDPE, linear low-density PE: LLDPE), polystyrene (PS), polyvinyl chloride (PVC), synthetic clothing fibers, polyethylene terephthalate (PET), polyester, polyamide (PA; nylon), polyacrylonitrile (PAN), synthetic clothing fibers (SCF), melamine-formaldehyde fragrance encapsulates (MFR), antifouling paint particles (APP) and biodegradable polylactic acid (PLA). PE was the most frequently studied polymer

(22 publications) followed by PS, which was used in 11 publications. PP, tires abrasions, PVC and PET were applied in three to six publications each (Figure 4A).

Four different microplastic shapes were evaluated for their effects on soil invertebrates: spheres, fragments, fibers and films. Fragments were tested in 22 studies, spheres in 10, fibers in 9, and plastic films in one. In eight publications the microplastic shape was not specified (Figure 4B).

In the microplastic exposure experiments, the amounts of dry soil ranged from <1 g (four publications) to >1 kg (five publications). Most approaches used 100–600 g (21 publications), followed by 10–100 g (12 publications). In three papers the amount of soil was not specified. Only publications were considered in which the organisms were exposed to microplastics at least partly via the soil matrix (Figure 4C).

Most studies made use of field soils (28 publications), although the LUF standard soil type 2.2 and OECD artificial soil were also commonly applied in nine and six publications, respectively. Additionally, a further artificial soil or other grounds were used (Figure 4D).

In general, 13 species of soil invertebrates were studied for their response to the addition of microplastics. In 34 publications annelids were tested, with the standard test organisms *Eisenia fetida* and *Folsomia candida* used in 15 and five publications respectively, collembolans in 12, *Porcellio scaber* (isopoda) in four, and the mite *Oppia nitens* in one. As representative of the nematodes and molluscs, *Caenorhabditis elegans* and *Achatina fulica* were used in three and one publication as test organism, respectively. In two studies, a natural occurring soil community was exposed to microplastics (Figure 5).

### 3.2 The uptake and bioturbation of microplastics through soil invertebrates

While ingestion, egestion and bioturbation are not strictly ecotoxicological effects, they contribute to the adverse effects of

TABLE 1 Ingestion, egestion and distribution of microplastics through soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Concentration	Parameter	Comment	References	
Annelida	<i>Lumbricus terrestris</i>	LDPE	Fragments	<150 µm	7–60% in dry surface litter w/w	Distribution	+ Amount of LDPE and organic matter in burrows + smaller LDPE particles in burrows than in surface litter; size-dependent transport	Huerta Lwanga et al. (2017)	
		PE	Spheres	710–2800 µm	75–2625 particles (750 mg PE/2.5 kg fresh soil)	Distribution	+ Transport of PE in deeper soil layers + transport of smallest PE class in deeper soil layers; transport depth- and size-dependent Further transport mechanism: adherence of PE to <i>L. terrestris</i> ' body	Rillig et al. (2017)	
						Egestion	✓ Size-dependent; only 710–1400 µm PE was found in casts		
		PET	Fibers	633.7 ± 282.8 µm × 30 µm	50–5000 µg g <sup>-1</sup>	Ingestion	✓	Lahive et al. (2021)	
						Egestion	✓		
							- 500 µg g <sup>-1</sup> - Fiber concentration in the faeces than in the soil		
		<i>Eisenia fetida</i>	PS	Spheres	187 nm	22–2206 µg g <sup>-1</sup>	Ingestion	✓	
						Egestion	✓		
							- Sphere concentration in the faeces than in the soil		
			LLDPE	Fragments	250–1000 µm	62.5–1000 mg kg <sup>-1</sup>	Ingestion	✓	Rodriguez-Seijo et al. (2017)
			MFR	Spheres	10–25 µm	50 mg kg <sup>-1</sup>	Ingestion	✓	Kuehr et al. (2021)
			PP (of face masks)	Fibers, fragments	<300 µm	1000 mg kg <sup>-1</sup>	Egestion	✓	Kwak & An (2021a)
	<i>Eisenia andrei</i>	HDPE	Fragments	28–1464 µm	0.25% w/w	Ingestion	✓	Zhou et al. (2020)	
		PE	Spheres	180–300 µm	1000 mg kg <sup>-1</sup>	Ingestion	✓	Kwak and An (2021b)	
		PE	Not sp	≤300 µm	0.1–10% w/w	Ingestion	✓ ≥1% PE	Wang et al. (2019)	
		PP	Fragments	8–1660 µm	0.25% w/w	Ingestion	✓	Li et al. (2021b)	
		PP	Spheres	<150 µm	0.03–0.9% w/w	Ingestion	✓ Concentration-dependent Accumulation of PP in tissues; concentration-dependent	Zhou et al. (2020)	
						Egestion	✓ Concentration-dependent		
	<i>Eisenia fetida</i>	PS	Spheres	100 and 1300 nm	100 and 1000 µg kg <sup>-1</sup>	Ingestion	✓ Concentration in intestines higher for 1300 nm than for 100 nm PS	Jiang et al. (2020)	
		PS	Not sp	≤250 µm	0.1–10% w/w	Ingestion	✓ ≥1% PS	Wang et al. (2019)	

(Continued on following page)

TABLE 1 (Continued) Ingestion, egestion and distribution of microplastics through soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Concentration	Parameter	Comment	References
		PS	Not sp	100 nm–100 µm	10 mg kg <sup>-1</sup>	Ingestion	✓ Size-dependent: 10 µm > 100 µm > 1 µm > 100 nm	Xu et al. (2021a)
		Tire	Fragments	<25 µm–2 mm	1–20% w/w	Egestion	✓ Concentration-dependent	Sheng et al. (2021)
	<i>Enchytraeus crypticus</i>	Nylon	Fragments	13–150 µm	2–12% w/w	Ingestion	✓ Size-dependent	Lahive et al. (2019)
		PE	Fibers	12 µm–24 mm	0.02–1.5% w/w; in soil/food	Ingestion	✓ Concentration-dependent	Selonen et al. (2020)
						Egestion	✓ Concentration-dependent	
	<i>Metaphire guillelmi</i>	HDPE	Fragments	25 µm	0.25% w/w	Ingestion	✓	Cheng et al. (2021)
		PP	Fragments	13 µm		Ingestion	✓	
Collembola	<i>Cryptopygus antarcticus</i>	PS	Foam	NA	NA	Ingestion	✓	Bergami et al. (2020)
	<i>Folsomia candida</i>	PP (of face masks)	Fibers, fragments	<300 µm	3 mg yeast per 1 mg PP	Ingestion	✓	Kwak and An (2021a)
						Egestion	✓	
		PVC	Not sp	80–250 µm	1 g kg <sup>-1</sup>	Ingestion	✓	Zhu et al. (2018)
Isopoda	<i>Porcellio scaber</i>	PE	Fibers	12 µm–24 mm	0.02–1.3% w/w	Ingestion	✓ Concentration-dependent	Selonen et al. (2020)
Mollusca	<i>Achatina fulica</i>	PET	Fibers	1257.8 × 76.3 µm	0.01–0.17 g kg <sup>-1</sup>	Ingestion	✓	Song et al. (2019)
						Egestion	✓	
							- Excretion rate; concentration-dependent Deterioration of PET after digestion	

not sp. = not specified. See section 3.1 for polymer abbreviations. Significant results represent comparison with the control treatment unless stated otherwise. The concentrations of the applied microplastic are those in dry soil unless stated otherwise. + = significant higher/more. . ., - = significant lower/fewer. . . ✓ = ingestion/egestion of microplastic particles.

TABLE 2 Accumulation of leached chemicals.

Taxa	Species	Material	Shape	Size	Conc	Leaching	Effect	Chemical accumulation	References
Annelida	<i>Eisenia fetida</i>	EPS	Fragments	<830–2000 µm	0.25% w/w	14, 21 days, 28 days	+	in HBCDD-EPS than in HBCDD treatment; highest values after 28 days	Li et al. (2019)
		Tire particles	Fragments	<25–2000 µm	1, 5, 10, 20% w/w	14, 28 days	+	Zn, Cd, Pb; time-dependent The smaller the tire particles, the higher the metal concentration	Sheng et al. (2021)
	<i>Metaphire guillelmi</i>	EPS	Fragments	<830–2000 µm	0.25% w/w	14, 21 days, 28 days	+	in HBCDD-EPS than in HBCDD treatment; highest values after 28 days	Li et al. (2019)

Abbreviations: dw = dry weight. The concentrations (conc.) of the applied microplastic are stated for dry soil. All effects are reported in comparison with the control treatment unless stated otherwise. + = significantly higher chemical accumulation.

TABLE 3 Microplastic influence on the accumulation of environmental chemicals.

Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Effect	Chemical accumulation	References	
Annelida	<i>Eisenia fetida</i>	PE	Not sp	<300 µm	7–30% w/w	Cd	2, 10 mg kg <sup>-1</sup>	28 days	+	in tissues; correlation with PE/Cd conc. In soil	Huang et al. (2021)	
		PE	Not sp	30, 100 µm	0.01, 0.05, 0.1% w/w	Cu	100 mg kg <sup>-1</sup>		+		Li et al. (2021a)	
						Ni	40 mg kg <sup>-1</sup>		+	in Cu-PE than in Cu treatment; time-, PE conc.-dependent		
			PP	Spheres	<150 µm	0.03–0.9% w/w	Cd	8.4 mg kg <sup>-1</sup>	42 days	+	Ni-PE than in Ni treatment	
			PS	Not sp	100 nm–100 µm	10, 100 mg kg <sup>-1</sup>	As, Cd	18.8, 0.2 mg kg <sup>-1</sup>	3–21 days	+	in Cd-PP than in Cd treatment	Zhou et al. (2020)
									+	in MP than in NP treatments	Xu et al. (2021b)	
			PS	Not sp		10 mg kg <sup>-1</sup>	PHE	5 mg kg <sup>-1</sup>	21 days	+		Xu et al. (2021a)
									+	in PHE-PS than in PHE treatment		
			PVC	Not sp	<125 µm	0.1–1000 mg kg <sup>-1</sup>	PFOA	10 mg kg <sup>-1</sup>	28 days, 56 days	+	≥500 mg kg <sup>-1</sup> PVC (bioconcentration factor)	Sobhani et al. (2021a)
							PFOS			+	≥1 mg kg <sup>-1</sup> PVC (bioconcentration factor)	
						PFOA/PFOS	5/5 mg kg <sup>-1</sup>		+	1 mg kg <sup>-1</sup> PVC (bioconcentration factor)		
	<i>Enchytraeus crypticus</i>	PA	Not sp	30 µm	1000 mg kg <sup>-1</sup> dry	TC	20 mg kg <sup>-1</sup>	21 days	+		Ma et al. (2020)	
									+	no sig. Different between TC and TC-PA treatment		
		PVC	Not sp						+	no sig. Different between TC and TC-PVC treatment		
	<i>Lumbricus terrestris</i>	HDPE	Fragments	600 µm–2.04 mm	0.35% w/w	Zn	236–4,505 mg kg <sup>-1</sup>	28 days	0	in gut, chloragog	Hodson et al. (2017)	
									0	of HDPE (gut)		

Abbreviations: MP = microplastic, NP = nanoplastic, not sp. = not specified, PHE = phenanthrene, TC = tetracycline, d = days. The concentrations (conc.) of the applied microplastic are stated for dry soil. All effects are stated in comparison with the control treatment unless stated otherwise. + = significant higher chemical accumulation. . ., 0 = no chemical accumulation. . .

TABLE 4 Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
Acari	<i>Oppia nitens</i>	PE	Fibers	4–24 mm	0.5% w/w	Mortality	0		Selonen et al. (2020)
						Reproduction	0		
						Mortality	0	0.5% w/w; in food	
						Reproduction	0		
Annelida	<i>Aporrectodea rosea</i>	Biodegradable PLA	Not sp	0.6–363 µm	0.1% w/w	Development	0		Boots et al. (2019)
						Mortality	0		
		HDPE	Not sp	0.48–316 µm	0.1% w/w	Development	–		
						Mortality	0		
		Synthetic clothing fibers	Fibers	<2, 2–7 and >7 mm	0.001% w/w	Development	0		
						Mortality	0		
	<i>Lumbricus terrestris</i>	LDPE	Fragments	<150 µm	7–60% surface litter <sub>dw</sub> w/w	Behavior	+	burrow formation (7% LDPE)	Huerta Lwanga et al. (2017)
							+	bioturbation activity (7% LDPE)	
							0	burrow length/volume	
							+	burrow weight/wall density (7%, 45% LDPE)	
						Development	–	7% LDPE	
						Development	0		
PE	Spheres	710–2800 µm	75–2625 particles (750 mg MP/2.5 kg fresh soil)	Mortality	0		Rillig et al. (2017)		
				Behavior	0	avoidance behavior			
				Development	0				
				Behavior	0	avoidance behavior			
				Cellular response	+	<i>hsp70</i> expression (1% polyester)			
					–	<i>mt-2</i> expression; dose-dependent increase			
PET	Fibers	633.7 ± 282.8 µm × 30 µm	50, 500, 5000 µg g <sup>-1</sup>	Behavior	0	avoidance behavior	Lahive et al. (2021)		
				Development	0				
				Behavior	0	avoidance behavior			
				Cellular response	+	<i>hsp70</i> expression (1% polyester)			
					–	<i>mt-2</i> expression; dose-dependent increase			
					0	<i>sod-1</i> expression			
Polyester	Fibers	361.6 ± 387 µm, × 40.7 ± 3.8 µm	0, 0.1, 1% w/w (0, 0.3 and 3 g)	Behavior	0	avoidance behavior	Prendergast-Miller et al. (2019)		
				Cellular response	+	<i>hsp70</i> expression (1% polyester)			
					–	<i>mt-2</i> expression; dose-dependent increase			
					0	<i>sod-1</i> expression			
				Development	0				
				Mortality	0				
<i>Eisenia andrei</i>	LLDPE	Fragments	250–1000 µm	62.5–1000 mg kg <sup>-1</sup>	Cellular response	+	inflammatory reactions; LOEC 125 mg kg <sup>-1</sup> dry soil	Rodriguez-Sejjo et al. (2017)	
						0	FTIR-ATR spectra		
					Development	0			
					Development	0			

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
						Mortality	0		
						Reproduction	0		
		PP (of face masks)	Fibers, fragments	<300 $\mu\text{m}$	1000 mg kg <sup>-1</sup>	Cellular response	-	intracellular esterase activity	Kwak & An, (2021a)
							0	oxidative stress (coelomocytes)	
							0	lysosomal stability	
							-	mature sperms, spermids (seminal vesicle score reduced to 0.8)	
							0	number of mature oocytes	
							0	tissue damage	
						Mortality	0		
	<i>Eisenia fetida</i>	LDPE	Beads	250 $\mu\text{m}$ –1000 mm	180–200 beads in 500 mg kg <sup>-1</sup>	Cellular response	0	TBARS content	Rodriguez-Seijo et al., (2018b)
						Development	0		
						Mortality	0		
				5 mm	8 beads in 500 mg kg <sup>-1</sup>	Cellular response	0	TBARS content	
						Development	0		
						Mortality	0		
		LDPE (unaged)	Film	550–1000 $\mu\text{m}$	0.25% w/w	Cellular response	+	Hsp70, CRT, TCTP gene expression levels	Cheng et al., (2020)
							0	ANN gene expression level	
							0	8-OHdG level	
							+	ROS level	
							-	CAT, GST activity	
							+	MDA content	
							0	SOD activity	
						Mortality	0		
		LDPE (aged)	Film			Cellular response	+	ANN, CRT, Hsp70, TCTP gene expression levels	
							+	8-OHdG level	
							+	ROS level	
							-	CAT, GST, SOD activity	
							0	MDA content	
						Mortality	0		
		PE	Spheres	180–212, 250–300 $\mu\text{m}$	1000 mg kg <sup>-1</sup>	Cellular response	0	ROS level	Kwak & An, (2021b)
							-	esterase activity (coelomocytes)	
							0	oogenesis	
							-	sperm density, mature sperm bundles	

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
							+	damage to sperm plasma membranes	
							+	disarranged male germ cells	
							+	disorder of tissue structure/intestinal tissue	
						Mortality	0		
		PE	Not sp	100 $\mu\text{m}$	0.05% w/w	Cellular response	+	CAT, POD, SOD activity, MDA content	Li et al., (2021a)
		PE	Not sp	$\leq 300 \mu\text{m}$	1, 5, 10, 20% w/w	Cellular response	+	CAT (20% PE), POD activity	Wang et al. (2019)
							-	GST, SOD activity (both 20% PE)	
							0	MDA conc	
						Development	0	growth	
		PP	Spheres	$< 150 \mu\text{m}$	0.03, 0.3, 0.6, 0.9% w/w	Cellular response	+	LPO level	Zhou et al. (2020)
							+	GSH content; dose-dependent increase	
						Development	-	growth rate after 14 days ( $\geq 0.6\%$ PP)	
						Mortality	+	after 42 days ( $\geq 0.3\%$ PP)	
		PS	Spheres	100 and 1300 nm	100 and 1000 $\mu\text{g kg}^{-1}$	Cellular response	+	DNA damage (1000 $\mu\text{g kg}^{-1}$ PS)	Jiang et al. (2020)
							+	DNA damage (1300 nm-sized PS) enlarged cells with irregular shapes/altered size of cell nuclei, increased intestinal cell lysis; more pronounced with 1300 nm PS (1000 $\mu\text{g kg}^{-1}$ )	
							-	SOD activity	
							+	GSH level (100 nm-, 1300 nm-sized (100 $\mu\text{g kg}^{-1}$ ) PS)	
						Development	+	growth rate	
						Mortality	-	100 $\mu\text{g kg}^{-1}$	
							+	1000 $\mu\text{g kg}^{-1}$ (100 nm-sized PS)	
		PS (com.)	Fragments	65–125 $\mu\text{m}$	0.01–0.5% w/w	Cellular response	+	DNA damage in F0 (0.1, 0.5% PS), F1	Sobhani et al., (2021b)
						Development	0	growth	
						Mortality	0		
						Reproduction	-	F0, F1; dose-dependent decrease	
		PS (pure)	Fragments	65–125 $\mu\text{m}$		Cellular response	+	DNA damage in F0 (0.1, 0.5% PS), F1	
						Development	0	growth	
						Mortality	0		
						Reproduction	-	F0, F1; dose-dependent decrease	
		PS	Not sp	$\leq 250 \mu\text{m}$	1–20% w/w	Cellular response	+	CAT activity ( $\geq 5\%$ PS)	Wang et al. (2019)
							0	GST activity	

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
		PS	Not sp	100 nm–100 µm	10 mg kg <sup>-1</sup>		+	MDA conc. (20% PS); POD activity, dose-dependent increase	
							–	SOD activity (20% PS)	
						Development	0	growth	
						Cellular response	+	DNA damage	Xu et al., (2021a)
							–	CAT, GST gene expression level	
							+	Hsp70, MT, SOD, TCTP gene expressidsupon level	
						Development	–		
		HDPE	Fragments	28–145, 133–415, 400–1464 µm	0.25% w/w	Mortality	0		
						Cellular response	+	8-OHdG content	Li et al., (2021b)
							+	disturbance in metabolic pathways related to oxidative stress, inflammation, neurotoxicity	
							–	CAT activity (28–415 µm HDPE), GST, SOD activity	
								MDA content	
						Mortality	0		
		PP	Fragments	8–125, 71–383, 761–1660 µm	0.25% w/w	Cellular response	+	8-OHdG content	
							+	disturbances in metabolic pathways related to oxidative stress, inflammation, neurotoxicity	
							–	CAT, GST, SOD activity	
							–	MDA content	
						Mortality	0		
		PVC	Not sp	<125 µm	0.1–1000 mg kg <sup>-1</sup>	Development	0		Sobhani et al., (2021a)
						Mortality	0		
						Reproduction	–	number of juveniles (1000 mg kg <sup>-1</sup> PVC)	
		LDPE	Fragments	250–1000 µm	62.5–1000 mg kg <sup>-1</sup>	Cellular response	0	FTIR-ATR spectra	Rodriguez-Seijo et al., (2018a)
								sig. Differences in NMR biochemical profile (62.5 mg kg <sup>-1</sup> LDPE)	
							–	CAT activity (125 mg kg <sup>-1</sup> LDPE); dose-dependent decrease	
							+	GST activity (1000 mg kg <sup>-1</sup> LDPE); TBARS conc. (≥250 mg kg <sup>-1</sup> LDPE); LDH activity (1000 mg kg <sup>-1</sup> LDPE), correlation with GST activity; always dose-dependent increase	
						Development	0		
						Mortality	0		

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References		
	<i>Enchytraeus crypticus</i>	HDPE	Fragments	4 mm	0, 2, 4, 8% w/w	Cellular response	+	CAT, GST activity (4, 8% HDPE); always dose-dependent increase	Pflugmacher et al. (2020)		
Behavior						+	avoidance of the polluted site				
Cellular response					+	CAT (8% HDPE), GST activity (4, 8% HDPE)					
Behavior					+	avoidance of polluted site					
							Cellular response	+		CAT activity (8% HDPE) (2/8% pairing)	
PA					Not sp	30 µm	1000 mg kg <sup>-1</sup>	Cellular response		+	GST activity
		+	abundance of ARGs								
		0	abundance of MGEs								
		Microbiota	–	sig. change of microbial composition							
			–	alpha diversity							
			0	Mortality							
PE		Fibers	12 µm–2.87 mm	0.02–1.3% w/w	Mortality	0	Selonen et al., (2020)				
	+					Reproduction					
	0					Reproduction					
	4–24 mm				0.02–1.5% w/w; in food	0		Mortality			
						0		Reproduction			
						0		Mortality			
	PVC		Not sp	30 µm	1000 mg kg <sup>-1</sup>	0.02–1.3% w/w	0	NOEC 0.06%			
							–		Reproduction		
							0		Mortality		
						0.5% w/w; in food	Reproduction		0	Ma et al. (2020)	
									+		Development
									–		Microbiota
PVC	Fragments	106–150 µm	9% w/w	Cellular response	+	abundance of ARGs	Lahive et al. (2019)				
					0	abundance of MGEs					
					0	Development					
				Nylon	Fragments	13–150 µm		2–12% w/w	Mortality	0	sig. change of microbial composition
										–	alpha diversity
										0	Mortality
PVC	Fragments	106–150 µm	9% w/w	Mortality	0	Lahive et al. (2019)					
					0		Reproduction				
Nylon	Fragments	13–150 µm	2–12% w/w	Mortality	0	Lahive et al. (2019)					
					0		Reproduction				

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References					
Arthropoda	<i>Metaphire guillelmi</i>	HDPE	Fragments	25 $\mu\text{m}$	0.25% w/w	Reproduction	–	13–18 $\mu\text{m}$ ( $\text{EC}_{50}$ 108 $\pm$ 8.5 g $\text{kg}^{-1}$ ) and 90–150 $\mu\text{m}$ ; dose-dependent decrease	Cheng et al. (2021)					
						Gut microbiota	0	alpha diversity (richness, diversity)						
	Arthropod community	PP	Fragments	13 $\mu\text{m}$			Mortality	0	alpha diversity (richness, diversity)					
							Gut microbiota	0						
		LDPE	Fragments	0.3–400 $\mu\text{m}$		5, 10, 15 g $\text{m}^{-2}$		Community structure	–	sig. change in microarthropod community structure	Lin et al. (2020)			
									–	abundance of dipteran larvae (15 g $\text{m}^{-2}$ LDPE: $-30.5 \pm 9.3\%$ ), lepidopteran larvae (15 g $\text{m}^{-2}$ LDPE: $-41.5 \pm 12.2\%$ ), ants (Hymenoptera) (15 g $\text{m}^{-2}$ LDPE: $-62.5 \pm 7.5\%$ ), oribatid mites (15 g $\text{m}^{-2}$ LDPE: $-15.3 \pm 5.7\%$ )				
		PE	Fibers	2–3 mm			0.4% w/w	Behavior	0	non-oribatid mites	Barreto et al. (2020)			
									0	feeding activity of microarthropod community				
								Community structure	+	decomposition rate i.c.w. C, PP treatments				
									0	oribatid mite species richness				
PP	Fibers	5–6 mm				Community structure	0	MP type/length on abundance of oribatid mites (adults, immatures, adults + immatures) and microarthropod community composition						
							0	abundance of Mesostigmata, Prostigmata, Astigmata, Acari, other invertebrates, total microarthropodes						
						Behavior	0	feeding activity of microarthropod community						
							0	decomposition rate						
Collembola	Collembolan community	LDPE	Fragments	0.3–400 $\mu\text{m}$	5, 10, 15 g $\text{m}^{-2}$	Community structure	0	abundance	Lin et al. (2020)					
							PE	Fibers	2–3 mm $\times$ 22.92 $\pm$ 0.17 $\mu\text{m}$	0.4% w/w	Community structure	0	abundance	Barreto et al. (2020)
												0	MP type/length on abundance	
	PP	Fibers					Community structure	0	abundance					

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
				5–6 mm × 33.33 ± 0.07 μm		Community structure	0	MP type/length on abundance	
	<i>Folsomia candida</i>	PE	Fibers	12 μm–24 mm	0.02–1.3% w/w	Mortality	0		Selonen et al. (2020)
					0.02–1.5% w/w; in food	Reproduction	0		
						Mortality	0		
						Reproduction	0		
				4–24 mm	0.02–1.3% w/w	Mortality	0		
						Reproduction	0		
					0.5% w/w; in food	Mortality	0		
						Reproduction	0		
			Spheres	<50, 50–500 μm	0.5, 1% w/w	Behavior	+	avoidance behavior; dose-dependent increase	Ju et al. (2019)
					0.5% w/w	Mortality	0		
					0.005–1% w/w	Gut microbiota	–	sig. change of microbial composition in guts bacterial alpha diversity	
						Reproduction	–	reproduction rate (0.1% PE); dose-dependent decrease; calculated EC <sub>50</sub> = 0.29%	
						Mortality	–	number of adults (1% PE)	
		PP (of face masks)	Fibers, fragments	<300 μm	1000 mg kg <sup>-1</sup>	Cellular response	0	esterase activity	Kwak & An, (2021a)
							0	oxidative stress	
						Development	–	growth (decreased to 92.9%)	
						Mortality	0		
						Reproduction	–	decrease to 48.2%	
		NA	NA	NA	NA	Behavior	0	light avoidance	
		PVC	Not sp	80–250 μm	1000 mg kg <sup>-1</sup>	Cellular response	+	level of δ <sup>15</sup> N and δ <sup>13</sup> C in tissues	Zhu et al. (2018)
							0	C:N ratio in tissue	
						Development	–		
						Gut microbiota	–	sig. change of microbial composition in guts bacterial alpha diversity	
						Mortality	0		
						Reproduction	–		
	<i>Lobella sokamensis</i>	PE	Spheres	25–262 μm	1000 mg kg <sup>-1</sup>	Behavior	–	movement activity	Kim & An, (2019)
		PS	Fragments	5–1155 μm		Behavior	–	movement activity	
			Spheres	0.5 ± 0.01 μm	4, 8 mg kg <sup>-1</sup>	Behavior	–	movement activity	
Isopoda	<i>Porcellio scaber</i>	PE	Fibers	12–2870 × 6 μm	0.05–1.5% w/w		0	total/differential haemocyte count	Dolar et al. (2021)

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
						Cellular response	0	viability of haemocytes	
					0.5% w/w	Cellular response	+	PO-like activity	
		PE	Fibers	12 µm–24 mm	0.02–1.3% w/w	Behavior	0	feeding activity; dose-dependent decrease	Selonen et al. (2020)
						Cellular response	0	carbohydrate, lipid, protein content	
						Development	0		
				4–24 mm		Mortality	0		
						Behavior	0	feeding activity	
						Cellular response	0	carbohydrate, lipid, protein content	
						Development	0		
						Mortality	0		
		Tire particles	Fragments	<180 µm	0.05, 0.5, 1.5% w/w	Cellular response	+	total haemocyte count (0.05% TPs)	Dolar et al. (2021)
							0	differential haemocyte count	
							0	viability of haemocytes	
					0.05, 1.5% w/w	Cellular response	0	PO-like activity	
Mollusca	<i>Achatina fulica</i>	PET	Fibers		0.01–0.71 g kg <sup>-1</sup>	Behavior	–	food intake (0.14, 0.71 g kg <sup>-1</sup> PET)	Song et al. (2019)
						Cellular response		tissue damages in stomach and intestines (0.14, 0.71 g kg <sup>-1</sup> PET)	
							0	liver, kidney histology	
							–	GPx content, T-AOC (0.71 g kg <sup>-1</sup> PET)	
							+	MDA content (0.71 g kg <sup>-1</sup> PET)	
						Egestion	–		
						Development	0	shell diameter or length	
						Mortality	0		
Nematoda	<i>Caenorhabditis elegans</i>	HDPE	Fragments	<250–1000 µm	0.01–1%	Reproduction	–	number of juveniles (1% HDPE)	Kim et al., (2020b)
		LDPE	Films	<630 µm		Reproduction	0	number of juveniles	
		PAN	Fibers		0.001–0.1%	Reproduction	–	number of juveniles (0.1% PAN)	
		PET	Fragments	<250–630 µm	0.01, 0.1, 1%	Reproduction	–	number of juveniles (0.1, 1% PET)	
		PP	Fragments	<250–1000 µm		Reproduction	–	number of juveniles (1% PP, <250 µm)	
		PS	Fragments			Reproduction	–	number of juveniles (1% PS)	
		PS	Spheres	60–76 nm	0.01–100 mg L <sup>-1</sup>	Reproduction	–	100 mg l <sup>-1</sup> PS	Kim et al., (2020a)

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TABLE 4 (Continued) Effects of macro-, micro- and nanoplastics on soil invertebrates.

Taxa	Species	Polymer	Shape	Size	Conc	Parameter	Effect	Endpoint	References
					0.01–100 mg kg <sup>-1</sup>	Reproduction	–	100 mg kg <sup>-1</sup> PS (EC <sub>50</sub> > 100 mg kg <sup>-1</sup> ); toxic effect dependent on soil physicochemical properties	
				482–510 nm	0.01–100 mg L <sup>-1</sup>	Reproduction	–	100 mg l <sup>-1</sup> PS	
					0.01–100 mg kg <sup>-1</sup>	Reproduction	–	10, 100 mg kg <sup>-1</sup> PS (EC <sub>50</sub> 14.23 mg kg <sup>-1</sup> ); toxic effect dependent on soil physicochemical properties	
		TP	Fragments	34–265 μm	1–10000 mg kg <sup>-1</sup>	Development	–	short-term exposure (48 h)	Kim et al. (2021)
							–	growth (≥100 mg kg <sup>-1</sup> TPs)	
						Mortality		short-term exposure (48 h)	
							0	survival rate	
								long-term exposure (10 days)	
							–	survival rate after 8 days (≥10 mg kg <sup>-1</sup> TPs); reduced to 27–45% after 10 d	
						Reproduction		Short-term exposure (48 h)	
							–	brood size (≥1000 mg kg <sup>-1</sup> TPs)	
	Nematode community	LDPE	Fragments	0.3–400 μm	5, 10, 15 g m <sup>-2</sup>	Community structure		sig. change in nematode trophic structure	Lin et al. (2020)
							–	nematodes abundance (5 g m <sup>-2</sup> LDPE: –15.4 ± 5.9%, 10 g m <sup>-2</sup> LDPE: –18.2 ± 4.3%, 15 g m <sup>-2</sup> LDPE: –19.7 ± 3.4%)	
							–	abundance of omnivorous/predatory (15 g m <sup>-2</sup> LDPE)/plant-feeding (10, 15 g m <sup>-2</sup> LDPE) nematodes	
							0	abundance of fungal-/bacterial feeding nematodes	

The parameters are listed in alphabetical order. Abbreviations: conc. = concentration, dw = dry weight, fw = fresh weight, i. c.w. = in comparison with, MP = microplastics, not sp. = not specified, 8-OHdG (indicator for DNA damage) = 8-hydroxy-2'-deoxyguanosine, sig. = significant. The concentrations of the applied microplastic are stated for dry soil unless stated otherwise. 0 = no effect on. . ., + = significant higher/more, - = significant lower/fewer. The parameter development applies to the biomass (fw) unless stated otherwise. The abbreviations of evaluated genes and enzymes are spelled in full in the text above describing the physical effects of microplastics on soil invertebrates. All effects are stated in comparison with the control treatment unless stated otherwise.

TABLE 5 Effects of plastic leachates on soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Leaching	Parameter	Effects	References	
Annelida	<i>Eisenia andrei</i>	APP	Fragments	<63 µm	0.01, 0.14%	3–56 d	Development	– biomass (fw, 0.14% APPs after 56 days)	Soroldoni et al. (2021)	
							Mortality	+ with 1.5% APPs after 3 d		
							Reproduction	– number of juveniles (0.01% APPs after 56 days and overall)		
								– number of cocoons (0.01% APPs after 42 days)		
		<i>Eisenia fetida</i>	TP	Fragments	13–1400 µm	0.0048–3.0%	21 d	Gut microbiota	0 alpha diversity of bacteria	Ding et al. (2020)
								+ community of bacteria, fungi significantly changed		
								+ more sensitive towards TPs than soil microbiota		
	Mortality							– survival rate (≥0.024% TPs)		
	Reproduction							– with ≥0.12% TPs; dose-dependent decrease; reproduction logarithmic correlated with TP conc.		
		TP	Fragments	<25 µm	1–20%	14, 28 d	Cellular response	+ CAT activity, MDA conc.; GST activity after 28days; POD activity (≥10% TPs)	Sheng et al. (2021)	
	– SOD activity (≥10% TPs)									
	25–50 µm			Cellular response			+ CAT, POD activity (≥10% TPs); GST activity (≥5% TPs after 28 days); MDA conc			
	0 SOD activity									
			50–350 µm	Cellular response	+ CAT activity (≥10% TPs after 14 days); GST activity (≥5% TPs after 28 days); MDA conc. (≥10% TPs); POD activity		0 SOD activity			
				Cellular response	+ CAT activity (≥10% TPs after 14 days); GST activity (≥5% TPs after 28 days); MDA conc. (≥10% TPs); POD activity		0 SOD activity			
			350 µm–2 mm	Cellular response	0 CAT, GST, SOD activity		+ MDA conc. (20% TPs after 28 days); POD activity (≥10% TPs after 28 days)			
				Reproduction	– number of juveniles (0.02, 1.5% TPs)					
	<i>Enchytraeus crypticus</i>	TP	Fragments	<180 µm	0.02–1.5%	21 d	Mortality	0	Selonen et al. (2021)	
							Reproduction	0 number of juveniles		
							Mortality	0		
Collembola	<i>Folsomia candida</i>	TP	Fragments			21 d	Reproduction	0 number of juveniles	Selonen et al. (2021)	
								Mortality		0
								Reproduction		0 number of juveniles
								Mortality		0
Isopoda	<i>Porcellio scaber</i>	LDPE (virgin)	Fragments	39.8 ± 8.8 µm	0.02–1.5%	21 d	Cellular response	+ granulocytes (0.05, 1.5% LDPE)	Kokalj et al. (2021)	
				<125 µm			0 haemocyte viability, total haemocyte count			
							– semigranulocytes (0.05, 1.5% LDPE)			

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TABLE 5 (Continued) Effects of plastic leachates on soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Leaching	Parameter	Effects	References
Nematoda	<i>Caenorhabditis elegans</i>	LDPE (recycled)	Fragments	205 ± 144 µm <418 µm			Behavior	– feeding rate (0.05, 1.5% LDPE)	
								0 feeding rate i.c.w. recycled LDPE	
							Mortality	0 i.c.w. C, recycled LDPE	
							Cellular response	0 granulocytes, haemocyte viability, total haemocyte count – semigranulocytes (0.05, 1.5% LDPE)	
			Behavior	0 feeding rate i.c.w. C, virgin LDPE					
			Mortality	0 i.c.w. C, virgin LDPE					
			Behavior	0 feeding activity	Selonen et al. (2021)				
			Cellular response	– AChE activity (EC <sub>50</sub> = 1.2%, NOEC = 0.5%) 0 electron transfer system					
			Reproduction	– number of juveniles (1% HDPE) 0 number of juveniles (HDPE extract)	Kim et al. (2020b)				
				– number of juveniles (HDPE extract bound to glass beads; corresponds to 1% PET)					
			HDPE		24 h (additive extracted HDPE)	Reproduction	– number of juveniles after one extraction cycle (0.01, 0.1% HDPE) 0 number of juveniles after two extraction cycles		
			LDPE	Films	<630 µm		24 h	Reproduction	
	LDPE			1%	24 h (additive extracted PP)	Reproduction	0 number of juveniles after one extraction cycle		
	LDPE			0.01, 0.1, 1%	6, 12, 18 days (one wet-dry cycle every 6 days)	Reproduction	– number of juveniles after 6, 12, 18 days higher toxicity with repeating wet-dry cycles		
	PAN	Fibers	<630 µm	0.001, 0.01, 0.1%	24 h	Reproduction	– number of juveniles (0.1% PAN) 0 number of juveniles (PAN extract) 0 number of juveniles (PAN extract bound to glass beads; corresponds to 0.1% PAN)		
	PAN			0.1%	24 h (additive extracted PAN)	Reproduction	0 number of juveniles after one extraction cycle		
	PAN			0.001, 0.01, 0.1%	6, 12, 18 days (one wet-dry cycle every 6 days)	Reproduction	– number of juveniles after 6 days (0.001, 0.01% PAN), 12days, 18 days higher toxicity with repeating wet-dry cycles		

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TABLE 5 (Continued) Effects of plastic leachates on soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Leaching	Parameter	Effects	References
		PET	Fragments	<250–630 $\mu\text{m}$	0.01, 0.1, 1%	24 h	Reproduction	– number of juveniles (0.1, 1% PET) 0 number of juveniles (PET extract)	
		PET			1%	24 h (additive extracted PET)	Reproduction	– number of juveniles (PET extract bound to glass beads; corresponds to 1% PET) 0 number of juveniles after one extraction cycle 0 number of juveniles after two extraction cycles	
		PP	Fragments	<250–1000 $\mu\text{m}$	0.01, 0.1, 1%	24 h	Reproduction	– number of juveniles (1% PP, <250 $\mu\text{m}$ ); size-dependent effect 0 number of juveniles (PP extract) – number of juveniles (PP extract bound to glass beads; corresponds to 1% PP, <250 $\mu\text{m}$ )	
		PP			1%	24 h (additive extracted PP)	Reproduction	0 number of juveniles after extraction cycles	
		PS	Fragments	<250–1000 $\mu\text{m}$	0.01, 0.1, 1%	24 h	Reproduction	– number of juveniles (1% PS) 0 number of juveniles (PS extract) – number of juveniles (PS extract bound to glass beads; corresponds to 1% PS)	
		PS			1%	24 h (additive extracted PS)	Reproduction	0 number of juveniles after extraction cycles	
		TP	Fragments	34–265 $\mu\text{m}$	1–10000 $\text{mg kg}^{-1}$	0, 30, 75 days preinc. of MP with soil	Development	Short-term exposure (48 h) – growth ( $\geq 100 \text{ mg kg}^{-1}$ TP) – growth ( $\geq 1 \text{ mg kg}^{-1}$ TP); with preinc. of TP in soil for 30 and 75 d	Kim et al. (2021)
							Mortality	Short-term exposure (48 h) 0 survival rate	
							Reproduction	Short-term exposure (48 h) – brood size (reduced by 3–33%) – brood size ( $\geq 1000 \text{ mg kg}^{-1}$ TP) – brood size ( $\geq 1 \text{ mg kg}^{-1}$ TP); with preinc. of TP in soil for 30 and 75 days – pregnant individuals; preinc.-time-dependent decrease	
						0, 75 days preinc. of MP with soil	Mortality	long-term exposure (10 days) – survival rate ( $\geq 10 \text{ mg kg}^{-1}$ TP after 8 days); survival rate reduced to 27–45% after 10 days – survival rate (10000 $\text{mg kg}^{-1}$ TP after 6 days); with preinc. Of TP in soil for 75days; survival rate reduced to 17–50% after 10 d	

The parameters are listed in alphabetical order. Abbreviations: APP = antifouling paint particle, conc. = concentration, d = days, fw = fresh weight, i. c.w. = in comparison with, preinc. = preincubation, TP = tire particle. The concentrations of the applied microplastic are stated as % dry soil w/w or  $\text{mg kg}^{-1}$  dry soil. All effects are based on comparisons with the control treatment unless stated otherwise.

TABLE 6 Influence of microplastics on the bioavailability of environmental chemicals in soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Parameter	Effects	References	
Annelida	<i>Eisenia fetida</i>	LDPE (unaged)	Film	550–1000 $\mu\text{m}$	0.25% w/w	ATZ	0.02, 2.0 $\text{mg kg}^{-1}$	28 d	Cellular response	+	8-OHdG level	<a href="#">Cheng et al. (2020)</a>
										+	ANN, CRT, Hsp70 gene expression levels	
										0	TCTP gene expression level	
		+	ROS level i.c.w. C, LDPE (aged/unaged) or ATZ treatment									
		–	CAT, GST, SOD activity									
		–	MDA content (ATZ 0.02 $\text{mg kg}^{-1}$ -LDPE treatment)									
			IBR values higher									
			Mortality	0								
			Cellular response	sig. change of gene expression levels (ANN, CRT, TCTP, Hsp70)								
		IBR value slightly higher in ATZ 0.02 $\text{mg kg}^{-1}$ -LDPE (aged) treatment										
		+	8-OHdG level									
		+	ROS level; i.c.w. C, LDPE (aged/unaged) or ATZ treatment									
		–	CAT, GST, SOD activity									
		–	MDA content (ATZ 2.0 $\text{mg kg}^{-1}$ -LDPE (aged) treatment)									
			IBR values higher									
			Mortality	0								
		LDPE	Pellets	<1 mm	180–200 beads/500 $\text{mg kg}^{-1}$	CPF	4 L $\text{ha}^{-1}$	14 d	Mortality	0	<a href="#">Rodriguez-Seijo et al. (2018b)</a>	
Behavior	+									earthworms at the bottom of the containers		
	Cellular response									0		AChE activity
			+	TBARS content								
Development	–											
	Mortality		0									
Behavior	+	earthworms at the bottom of the containers										
	Cellular response	–	AChE activity									
		–	TBARS content									
Development	0											
	Mortality	0										
		Pellets	5 mm	8 beads/500 $\text{mg kg}^{-1}$						–	AChE activity	
Cellular response	–									TBARS content		
	Development									0		
Mortality	0											

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TABLE 6 (Continued) Influence of microplastics on the bioavailability of environmental chemicals in soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Parameter	Effects	References
		PE	Not sp	<300 $\mu\text{m}$	7–30% w/w	Cd	2, 10 $\text{mg kg}^{-1}$	48 h	Behavior	+ avoidance rate	Huang et al. (2021)
								28 d	Cellular response	+ GSH, MDA content; always dose (PE)-dependent increase – POD, SOD activity	
					7, 15% w/w		2, 10 $\text{mg kg}^{-1}$	28 d	Development	–	
					7–30% w/w		10 $\text{mg kg}^{-1}$		Reproduction	– cocoon reproduction	
									Cellular response	+ sperm damage; dose (PE)-dependent increase + epidermal necrosis, muscle fibroses; dose (PE)-dependent increase + abnormalities of intestinal epithelial/chlorogenic tissue (vesicle enlargement, layer fibrosis, tissue necrosis/disintegration)	
		PE	Not sp	30 $\mu\text{m}$	0.05% w/w	Cu	100 $\text{mg kg}^{-1}$	7, 14, 21 d	Cellular response	+ CAT, POD, SOD activity; i.c.w. C, PE treatments + MDA content i.c.w. C, PE treatment; time-dependent increase	Li et al. (2021a)
				100 $\mu\text{m}$	0.01, 0.05, 0.1% w/w				Cellular response	+ CAT, POD, SOD activity; i.c.w. C, PE treatments + MDA content (i.c.w. C, PE treatments); time-dependent increase	
				30 $\mu\text{m}$	0.05% w/w	Ni	40 $\text{mg kg}^{-1}$		Cellular response	+ CAT, POD, SOD activity; i.c.w. C, PE treatments + MDA content (i.c.w. C, PE (30 $\mu\text{m}$ ; 100 $\mu\text{m}$ , 0.05%) treatment); time-dependent increase	
				100 $\mu\text{m}$	0.01, 0.05, 0.1% w/w				Cellular response	+ CAT, POD, SOD activity; i.c.w. C, PP treatment + MDA content (i.c.w. C, PE treatment); time-dependent increase – MDA content i.c.w. 30 $\mu\text{m}$ PE (0.05%)	
		PP	Spheres	<150 $\mu\text{m}$	0.03–0.9% w/w	Cd	8.4 $\text{mg kg}^{-1}$	42 d	Cellular response	+ GSH content, LPO level i.c.w. C, PP treatments	Zhou et al. (2020)
								Development	– growth rate i.c.w. C, PP treatment		
									Mortality	+ after 42 d	
		PS	Not sp	10 $\mu\text{m}$	10, 100 $\text{mg kg}^{-1}$	As, Cd	0.2, 18.8 $\text{mg kg}^{-1}$	3–21 d	Cellular response	– CAT, MT mRNA level + GST, SOD mRNA level	Xu et al. (2021b)

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TABLE 6 (Continued) Influence of microplastics on the bioavailability of environmental chemicals in soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Parameter	Effects	References
				100 $\mu\text{m}$						abnormal epithelial cells, incomplete coelom tissue, exfoliation in intestinal epithelium + CAT activity after 3 days - CAT activity (10 mg kg <sup>-1</sup> after 14days, 100 mg kg <sup>-1</sup> after 21 days) + MDA content, SOD activity after $\geq 3$ days - MT activity sig. change in protein expression and metabolic profiles	
									Development	- 100 mg kg <sup>-1</sup>	
									Mortality	0	
									Cellular response	- CAT, MT mRNA level + GST, SOD mRNA level + CAT activity after 3 days - CAT activity after $\geq 14$ days + MDA content, SOD activity after $\geq 3$ days + MT activity sig. change in protein expression and metabolic profiles	
				100 nm						abnormal epithelial cells, incomplete coelom tissue, exfoliation in intestinal epithelium	
									Development	0	
									Mortality	0	
									Cellular response	- CAT, MT mRNA level + GST, SOD mRNA level + CAT activity after 3, 7ays, 14 days (10 mg kg <sup>-1</sup> ) - CAT activity after 21 dayays (10 mg kg <sup>-1</sup> ) + MDA content after $\geq 3$ days + MT activity + SOD activity after $\geq 14$ days sig. change in protein expression and metabolic profiles	

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TABLE 6 (Continued) Influence of microplastics on the bioavailability of environmental chemicals in soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Parameter	Effects	References
										abnormal epithelial cells, incomplete coelom tissue, exfoliation in intestinal epithelium	
									Development	0	
									Mortality	0	
		PS	Not sp	100 $\mu\text{m}$	10 mg kg <sup>-1</sup>	PHE	5 mg kg <sup>-1</sup>	21 d	Cellular response	+ DNA damage i.c.w. C, PS, PHE treatment	Xu et al. (2021a)
										- CAT, GST mRNA level	
										- CAT mRNA level i.c.w. PS treatment	
										+ Hsp70, MT, SOD, TSTP mRNA level i.c.w. C, PS treatments	
										- CAT, POD, SOD activity	
										+ GSH content after 21 days	
										+ MDA content, TPC	
									Development	-	
									Gut microbiota	sig. change of composition	
									Mortality	0	
				10 $\mu\text{m}$					Cellular response	+ DNA damage i.c.w. C, PS, PHE and all other PHE-PS treatments	
										- CAT, GST mRNA level i.c.w. C, PS treatments	
										+ Hsp70, MT, SOD, TSTP mRNA level i.c.w. C, PS treatments	
										- CAT, POD, SOD activity	
										- GSH content	
										+ MDA content, TPC	
									Development	-	
									Gut microbiota	sig. change of composition	
									Mortality	0	
				1 $\mu\text{m}$					Cellular response	+ DNA damage i.c.w. PS, PHE treatments	
										- CAT, GST mRNA level i.c.w. C, PS treatments	
										+ Hsp70, MT, SOD, TSTP mRNA level i.c.w. C, PS treatments	

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TABLE 6 (Continued) Influence of microplastics on the bioavailability of environmental chemicals in soil invertebrates.

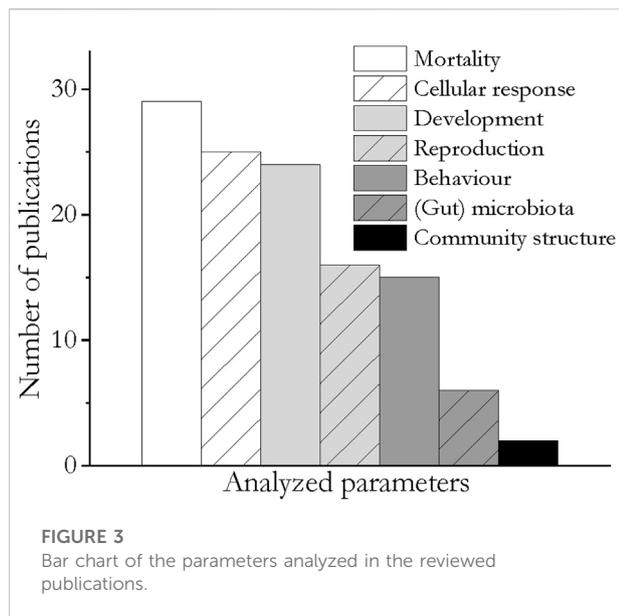
Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Parameter	Effects	References
				100 nm						<ul style="list-style-type: none"> <li>- CAT activity; GSH content after 21 days</li> <li>+ MDA content</li> <li>- POD, SOD activity after 21 days</li> <li>+ TPC after 21 d</li> </ul>	
									Development	-	
									Gut microbiota	sig. change of composition	
									Mortality	0	
									Cellular response	<ul style="list-style-type: none"> <li>+ DNA damage in i.c.w. PS, PHE treatments</li> <li>- CAT, GST mRNA level i.c.w. C, PS treatments</li> <li>+ Hsp70, MT, SOD, TCTP mRNA level i.c.w. C, PS treatments</li> <li>- CAT activity</li> <li>- POD/SOD activity after 21 days</li> <li>- GSH content after 14 days</li> <li>+ MDA content</li> <li>+ TP content after 14 d</li> </ul>	
									Development	-	
									Gut microbiota	sig. change of composition	
									Mortality	0	
		PVC	Not sp	<125 $\mu$ m	0.1–1000 mg kg <sup>-1</sup>	PFOA	10 mg kg <sup>-1</sup>	28days, 56 d	Development	0	Sobhani et al. (2021a)
									Mortality	0	
									Reproduction	- number of juveniles ( $\geq$ 500 mg kg <sup>-1</sup> PVC)	
						PFOS			Development	0	
									Mortality	0	
									Reproduction	- number of juveniles ( $\geq$ 500 mg kg <sup>-1</sup> PVC)	
						PFOA/ PFOS	5/5 mg kg <sup>-1</sup>		Development	0	
									Mortality	0	

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TABLE 6 (Continued) Influence of microplastics on the bioavailability of environmental chemicals in soil invertebrates.

Taxa	Species	Material	Shape	Size	Conc	Chem	Chem. con	Leaching	Parameter	Effects	References							
	<i>Enchytraeus crypticus</i>	PA	Not sp	30 $\mu\text{m}$	1000 $\text{mg kg}^{-1}$	TC	20 $\text{mg kg}^{-1}$	21 d	Reproduction	– number of juveniles ( $\geq 500 \text{ mg kg}^{-1}$ PVC)	Ma et al. (2020)							
Cellular response									+ abundance of ARGs and MGEs									
Microbiota									sig. change of microbial composition – alpha diversity									
Mortality		0																
Reproduction		–																
Cellular response		+ abundance of ARGs and MGEs																
Arthropoda	<i>Lumbricus terrestris</i>	PVC							Development	+								
									Microbiota	sig. change of microbial composition – alpha diversity								
									Mortality	0								
		Reproduction	–															
		Behavior	0															
		Development	0															
	HDPE	Fragments		600 $\mu\text{m}$ –2.04 mm	0.35% w/w	Zn	236–4,505 $\text{mg kg}^{-1}$	28 d	Egestion	0 of Zn	Hodson et al. (2017)							
Mortality									0									
Cellular response									– AChE activity ( $\geq 0.8 \text{ mg kg}^{-1}$ ) + THC (2.0 $\text{mg kg}^{-1}$ ) + proportion of hyalinocytes (0.8 $\text{mg kg}^{-1}$ CP-PE i.c.w. CP treatment) 0 proportion of granulocytes, semigranulocytes, haemocyte viability									
	PE	Fibers		12–2870 $\mu\text{m}$	0.5% w/w	CPF	0.2–2.0 $\text{mg kg}^{-1}$	21 d	Cellular response	– AChE activity ( $\geq 0.8 \text{ mg kg}^{-1}$ ) + THC (2.0 $\text{mg kg}^{-1}$ ) + proportion of hyalinocytes (0.8 $\text{mg kg}^{-1}$ CP-PE i.c.w. CP treatment) 0 proportion of granulocytes, semigranulocytes, haemocyte viability	Dolar et al. (2021)							
									Tire particles	Fragments		<180 $\mu\text{m}$					Cellular response	0 THC + proportion of granulocytes (0.4 $\text{mg kg}^{-1}$ ) – proportion of semigranulocytes (0.2, 0.6 $\text{mg kg}^{-1}$ ) 0 proportion of hyalinocytes, haemocyte viability

The parameters are listed in alphabetical order. Abbreviations: ATZ = atrazine, C = control, CPF = chlorpyrifos, d = days, i. c.w. = in comparison with, not sp. = not specified PHE = phenanthrene. The concentrations of the applied microplastic are stated for dry soil unless stated otherwise. The parameter development applies to biomass (fw) unless stated otherwise. The chemical abbreviations are defined in the text. All effects are based on comparison with the control treatment unless stated otherwise.



microplastics in soil (Table 1) via their incorporation and transport by soil invertebrates (Duis & Coors, 2016; Rillig et al., 2017). These processes were considered in 16 of 45 papers, 14 of which focussed on ingestion and/or egestion and the remaining two on bioturbation (Table 1). Microplastics of several different types, shapes and concentrations and ranging in size from 100 nm to 2800 µm were shown to be ingested and egested by annelids, arthropods and molluscs. Among the Annelida, *L. terrestris*, *E. andrei*, *E. fetida*, *E. crypticus* and *M. guillelmi* ingested and/or egested PE, PS, PP and MFR spheres with sizes between 100 nm and 2800 µm (Rillig et al., 2017; Jiang et al., 2020; Zhou et al., 2020; Kuehr et al., 2021; Lahive et al., 2021). Other studies demonstrated that annelids ingested and/or egested fragments (8–2000 µm) of LLDPE, PP, tires, nylon, HDPE (Rodriguez-Seijo et al., 2017; Lahive et al., 2019; Zhou et al., 2020; Kwak & An, 2021a; Li M. et al., 2021; Cheng et al., 2021; Sheng et al., 2021), fibers (12–2400 µm) of PET, PP, PE (Selonen et al., 2020; Kwak and An, 2021a; Lahive et al., 2021), PE beads (180–300 µm; Kwak & An, 2021b) and particles (100 nm–300 µm) of PE and PS of unspecific shape (Wang et al., 2019; Xu et al., 2021a). The arthropods *F. candida* (Zhu et al., 2018; Kwak and An, 2021a) and *P. scaber* (Selonen et al., 2020) ingested and/or egested PP fiber-fragment mixtures (<300 µm), PVC particles (80–250 µm) and PE fibers (12–2400 µm). The study of Bergami et al. was the only one that analyzed the uptake of PS foam (size not specified) in a natural occurring population of the collembolan *C. antarcticus* (Bergami et al., 2020). The study of Song et al. (2019) was the only one that focussed on a molluscan species *A. fulica*, which was shown to ingest and egest PET fibers (12578.8 µm in length) (Table 1).

In most studies, the ingestion/egestion of microplastics was dose- or size-dependent (e.g. Selonen et al., 2020). Evidence of the retention of micro- and nanoplastics in the tissues and organs

of soil invertebrates was provided in several cases (Jiang et al., 2020; Zhou et al., 2020; Lahive et al., 2021) (Table 1).

Bioturbation by microplastics was analyzed in *L. terrestris*. Exposure to LDPE fragments increased the concentration of microplastic found in the earthworm's burrows. The transport of LDPE and PE particles was size-dependent, as the fragments found in the burrows were smaller than those in the surface litter and in deeper soil layers (Huerta Lwanga et al., 2017; Rillig et al., 2017) (Table 1).

### 3.3 Accumulation of microplastic-associated chemicals

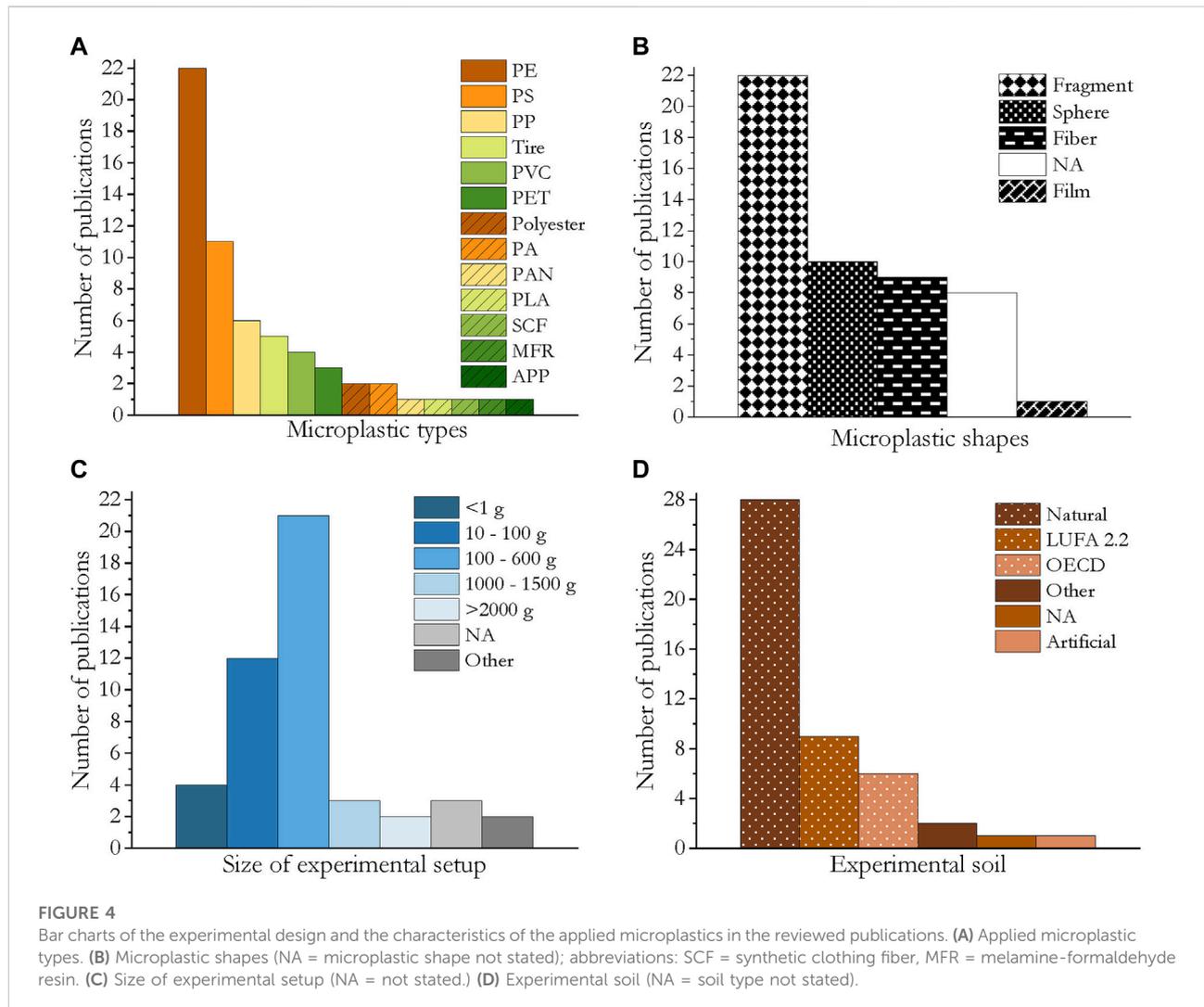
The accumulation of chemicals in tissues of soil invertebrates through microplastic exposure is also not a strictly ecotoxicological effect. Nevertheless, in contrast to microplastic particles, the body burdens of dissolved chemicals in organisms are clearly related to their adverse effects (McCarty et al., 2013) and therefore relevant in risk assessments (Schaefer et al., 2015). Microplastics can act as vectors for environmental chemicals in two ways. (1) by the leaching of their stabilizers, plasticizers and other associated chemical compounds into the environmental medium, allowing their subsequent ingestion by organisms and thus their bioaccumulation (Teuten et al., 2009; Halle et al., 2020) and (2) by absorbing environmental chemicals (Table 3), such as heavy metals, pesticides, or persistent organic pollutants (POPs), which are then ingested with the microplastics by soil organisms (Akdogan and Guven, 2019; Torres et al., 2021). The accumulation of microplastic-associated chemicals has been evaluated only in annelid species. Those studies generally demonstrated that microplastics are a potential source of hazardous leachates (Table 2) and facilitate the accumulation of toxic chemicals (Table 3).

#### 3.3.1 Accumulation of leached chemicals

Two publications analyzed the accumulation of leached chemicals. A significant bioaccumulation of endogenous hexabromocyclododecanes (HBCDDs) from EPS (expanded polystyrene) was found in the digestive fluid of *E. fetida*, and *M. guillelmi* after 28 days. Accumulation increased with the decreasing size of EPS particles and prolonged exposure time (Li et al., 2019). Tire fragments induced an accumulation of metals (Zn, Cd, Pb) in *E. fetida* after 14 and 28 days. In that study, the metal concentrations in the earthworms' tissues were related to microplastic size and exposure time (Sheng et al., 2021) (Table 2).

#### 3.3.2 Accumulation of environmental chemicals

The influence of microplastics on the bioavailability of environmental chemicals was examined in eight studies, using *E. fetida*, *L. terrestris* and *E. crypticus* as the test species (Table 3). After the co-exposure of *E. fetida* to PE particles and Cd and Cu



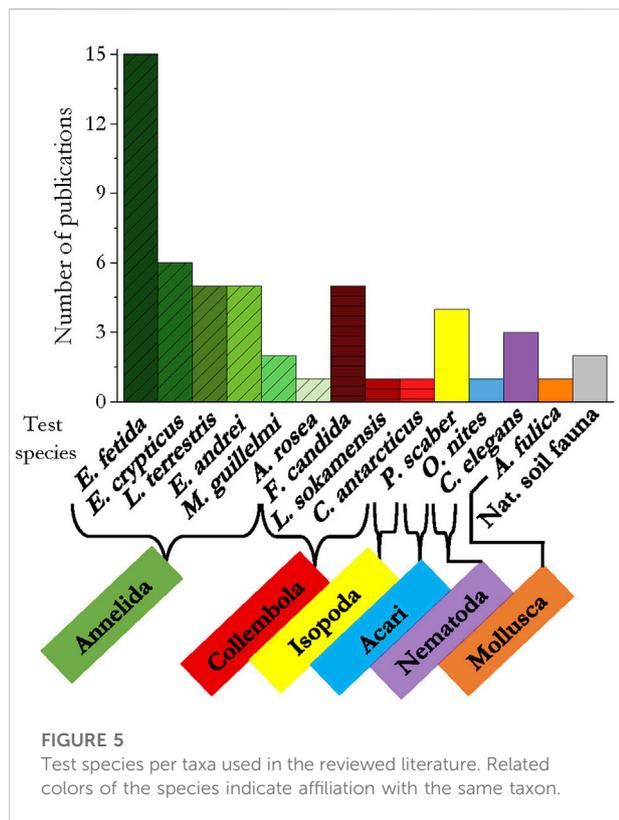
for 28 days, the levels of the metals in the worm's tissues were significantly increased (Li B. et al., 2021; Huang et al., 2021). Exposure to PS particles in Cd- and As-contaminated soil resulted in significantly elevated metal levels in *E. fetida*'s tissues after 21 days. Microplastics induced a higher accumulation than did nanosized plastics (Xu et al., 2021b). Zhou and co-authors reported a significantly higher accumulation of Cd in the tissues of *E. fetida* when PE beads were added (Zhou et al., 2020). The bioaccumulation of phenanthrene in the tissues of *E. fetida* was higher in the presence than the absence of PS particles (Xu et al., 2021a). Perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) (both 10 mg kg<sup>-1</sup>) and a mixture of PFOA/PFOS (5/5 mg kg<sup>-1</sup>) accumulated in the tissues of *E. fetida*. Bioaccumulation was dependent on the concentration of PVC: 1000 mg PVC kg<sup>-1</sup> significantly increased the incorporation of PFOA/PFOS, at least 500 mg PVC kg<sup>-1</sup> was needed for a

significant accumulation of PFOA, but only ≥1 mg PVC kg<sup>-1</sup> facilitated the significant accumulation of PFOS (Sobhani et al., 2021a) (Table 3).

However, in other studies there was no increase in the accumulation of chemicals in the presence of microplastics. The addition of PA or PVC did not enhance the accumulation of tetracycline in *E. crypticus* after 21 days (Ma et al., 2020). In a co-contamination study with HDPE fragments, Zn levels in the gut or chloragoc cells of *L. terrestris* were not elevated after 28 days (Hodson et al., 2017) (Table 3).

### 3.4 Physical effects of microplastics on soil invertebrates

The morphological features of microplastics, including their size, shape or surface structure, might pose mechanical hazards



to organisms (Barnes et al., 2009), in turn affecting their cellular responses, microbiota, mortality, development, behavior and reproduction. Of the 45 reviewed papers, 34 dealt with physical effects (Figure 4). It should be noted that while the respective authors assumed a physical impact, additional effects of possibly leached endogenous chemicals from the experimentally applied microplastics could not be excluded.

In the following, the different effect endpoints considered as indicators of physical impacts of microplastics are considered from a sub-organismic to organismic to community level.

### 3.4.1 Cellular response

Microplastics can induce changes in cellular responses via genetic damages/alterations, gene expression (levels), metabolic pathways, enzyme activity/levels, the production of reactive oxygen species (ROS), miscellaneous inflammatory reactions, cell structure/lysis, or the lipid, carbohydrate and protein content of tissues (Table 4). In most experiments, a negative influence of microplastics on the cellular responses, including oxidative stress reactions and histopathological changes, of several organisms was determined (Table 4). The expression levels of several genes and oxidative indicators were used as indicators, including annetocin (ANN), calreticulin (CRT), catalase (CAT), glutathione (GSH), glutathione S-transferase (GST), glutathione peroxidase (GPx), heat shock protein 70 (Hsp70), metallothionein (MT), translationally controlled

tumor protein (TCTP), thiobarbituric acid reactive substances (TBARS), lactate dehydrogenase (LDH), lipid hydroperoxide (LPO), malondialdehyde (MDA), phenoloxidase (PO) and superoxide dismutase (SOD). Changes in the levels of those biochemical markers indicated an oxidative stress response. For example, the radical scavengers CAT, SOD and the detoxifying enzyme GST were shown to be important for inactivating ROS and obviating cell damages (Liu et al., 2012; Zhang et al., 2014; Hu et al., 2016). High levels of ROS induce detectable changes in the synthesis or modification of enzymes responsible for inactivation (Liu et al., 2011; Wang et al., 2016; Cheng et al., 2020).

The effects of microplastics were analyzed in the annelids *E. crypticus*, *E. andrei* and, most often, *E. fetida*. Exposure to HDPE, LDPE, PP and PS induced DNA damages in the F0 and the F1 generation of *E. fetida* (Cheng et al., 2020; Jiang et al., 2020; Xu et al., 2021a; Li M. et al., 2021; Sobhani et al., 2021b). The extent of the damage was larger at higher particle concentrations and larger bead sizes (Jiang et al., 2020).

The levels of GSH, ROS, LDH, LPO, MDA, and TBARS, the activity of CAT, GST, POS, SOD and the transcript levels of ANN, CRT, Hsp70, MT, SOD and TCTP increased significantly in annelids exposed to several shapes of LDPE, PE, PP and PS (Rodriguez-Seijo et al., 2018a; Wang et al., 2019; Cheng et al., 2020; Jiang et al., 2020; Zhou et al., 2020; Li B. et al., 2021; Xu et al., 2021a). Conversely, exposure to HDPE, LDPE, PE, PP, PS significantly decreased CAT, SOD, and GST activity, MDA levels and CAT and GST transcript levels (Rodriguez-Seijo et al., 2018a; Wang et al., 2019; Cheng et al., 2020; Jiang et al., 2020; Xu et al., 2021a; Li M. et al., 2021). Finally, LDPE and PE had no impact on SOD and GST activity or on TBARS, MDA or ROS levels in *E. fetida* (Rodriguez-Seijo et al., 2018b; Wang et al., 2019; Cheng et al., 2020; Kwak and An, 2021b).

On the tissue level, PS spheres induced the enlargement and deformation of *E. fetida* cells, altered the size of cell nuclei and caused cell lysis after 14 days (Jiang et al., 2020). The cell viability of *E. fetida* was affected by PE beads, as the esterase activity of the coelomocytes was significantly lowered. While female reproductive organs were not affected, male reproductive organs were significantly damaged (Kwak & An, 2021b).

In *E. andrei* a LOEC of 62.5 mg LLDPE fragments kg<sup>-1</sup> dry soil caused histopathological damage, by significantly inducing fibrosis, inflammatory infiltrates and congestion (Rodriguez-Seijo et al., 2017). In *E. andrei* and *E. fetida*, these effects were dose-dependent (Rodriguez-Seijo et al., 2017; Rodriguez-Seijo et al., 2018a). After exposure to a facemask-derived PP fiber-fragment mixture, spermatogenesis in *E. andrei* was significantly damaged. The cell viability of coelomocytes was affected, but there were no signs of oxidative stress nor was tissue damage, including bleeding, swelling and thinning, observed. Neither lysosomal stability nor the number of mature oocytes was impaired (Kwak & An, 2021a). Oxidative reactions were observed in *E. crypticus* as well, as HDPE fragments (4 mm)

provoked a dose-dependent increase in GST and CAT activity (Pflugmacher et al., 2020). In the same organism, PA and PVC triggered an increase in the abundance of ARGs (antibiotic resistance genes), whereas MGEs (mobile genetic elements) were not affected (Ma et al., 2020). In *L. terrestris*, exposure to polyester microfibers caused a dose-dependent increase in the transcript level of *mt-2*. The expression of the *hsp70* was significantly reduced but there was no effect on *sod-1* expression (Prendergast-Miller et al., 2019).

The results for microplastic effects in springtail species are ambiguous. There was no evidence of oxidative stress, as esterase activity in *F. candida* was not affected by exposure to a facemask-derived PP fiber and fragment mixture (Kwak & An, 2021a). In *F. candida*'s tissues, PVC treatment for 28 and 56 days resulted in a significant enrichment of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  while the C:N ratio itself was not altered. The nitrogen and carbon isotopes were used as indicator of the springtail's feeding habits and metabolic turnover rates (Zhu et al., 2018). In the springtail *P. scaber*, neither the viability of haemocytes nor the lipid, carbohydrate and protein content was affected by PE fibers, whereas PO-like activity was increased in the presence of 0.5% PE. Tire fragments had no effect on the differential haemocyte count, haemocyte viability or PO-like activity but the total haemocyte count (THC) significantly increased (Selonen et al., 2020; Dolar et al., 2021).

Oxidative stress was also demonstrated in *A. fulica*, as GPx levels and total antioxidant capacity (T-AOC) significantly decreased and the MDA content was significantly increased after exposure of the mollusc to PET fibers for 28 days. Exposure also resulted in mechanical tissue damage in the stomach, intestines and the villi of the gastric wall of the snail, whereas kidney and liver were unaffected (Song et al., 2019).

### 3.4.2 Microbiota

Only four publications focussed on (gut) microbiota (Table 4). Different types of microplastics (PA, PVC, PE) were reported to significantly change the microbial composition in soil invertebrates (*F. candida*, *E. crypticus*). Bacterial alpha diversity was significantly decreased in microplastic treatments (Zhu et al., 2018; Ju et al., 2019; Ma et al., 2020). In comparison, there was no effect on the bacterial alpha diversity and density of *M. guillemi* by either HDPE or PP fragments (Cheng et al., 2021).

### 3.4.3 Mortality

Compared to all other analyzed parameters, the mortality of soil invertebrates, examined in 22 publications (65%), was the least sensitive towards microplastics exposure. In four studies, microplastics decreased survival at higher particle contents or a prolonged exposure time. The majority of studies revealed no effect of several microplastic types on the mortality of annelids (Boots et al., 2019; Cheng et al., 2020, 2021; Hodson et al., 2017;

Kwak & An, 2021a, b; Lahive et al., 2019; Li M. et al., 2021; Ma et al., 2020; Prendergast-Miller et al., 2019; Rillig et al., 2017; Rodriguez-Seijo et al., 2017, ; Selonen et al., 2020; Sobhani et al., 2021a; Xu et al., 2021a) or *P. scaber*, *O. nitens*, *F. candida* or *A. fulica* (Kwak and An, 2021a; Selonen et al., 2020; Song et al., 2019; Zhu et al., 2018; Table 4). Jiang et al., 2020 found a significantly higher mortality of *E. fetida* exposed to 1000  $\mu\text{g}$  PS  $\text{kg}^{-1}$ . In another study, exposure to PP spheres (<150  $\mu\text{m}$ ) at concentrations >0.3% soil dry weight increased the mortality of *E. fetida* after 42 days (Zhou et al., 2020). A long-term experiment with natural soil demonstrated significantly higher dose- and time dependent mortality in the annelid species *L. terrestris* when exposed to LDPE for 60 days (Huerta Lwanga et al., 2016). In a 1-week experiment, Ju et al., 2019 showed no lethal effects of PE spheres on *F. candida*, whereas in an experiment lasting 28 days mortality was significantly higher. The mortality of *C. elegans* was not affected by exposure to tire microplastics for 2 days whereas survival was significantly reduced after a 10-day exposure (Kim et al., 2021) (Table 4).

### 3.4.4 Development

Developmental alterations caused by microplastics include effects on growth (rate), biomass and body length and were examined in 19 publications. No general impact tendencies of microplastic on soil invertebrates could be derived, as contradictory results were found. In *A. rosea* and *L. terrestris* both negative and no effects on development were reported. Similarly in *E. fetida* and *F. candida* both no impact or a striking elevation/reduction of the growth rate was determined. Tire fragments decreased the growth in *C. elegans* (Kim et al., 2021). LLDPE, PE and PET had no influence on the biomass of *E. andrei*, *P. scaber* and *A. fulica* (Rodriguez-Seijo et al., 2017; Selonen et al., 2020; Song et al., 2019; Table 4).

In *A. rosea* exposed for 30 days to HDPE, biomass was significantly reduced. The growth and weight of *L. terrestris* exposed to LDPE and PS were significantly reduced after 7, 14 and 60 days; the effect on the growth rate was dose-dependent (Huerta Lwanga et al., 2016). By contrast, HDPE, PE, PET, PLA, polyester fibers and synthetic clothing fibers had no effect on the weight of either *L. terrestris* (Hodson et al., 2017; Rillig et al., 2017; Prendergast-Miller et al., 2019; Lahive et al., 2021) or *A. rosea* (Boots et al., 2019).

LDPE pellets, PVC particles, PS fragments (pure and commercial)/particles and PE particles had no significant effects on the weight of *E. fetida* (Rodriguez-Seijo et al., 2018a; Sobhani et al., 2021a, Wang et al., 2019), whereas Jiang et al., 2020 reported a significantly increased growth rate after exposure of the worm to PS spheres for 14 days. Another study showed that exposure to PP spheres (<150  $\mu\text{m}$ ) at a concentration of  $\geq 0.6\%$  soil dry weight and to PS particles (100 nm–100  $\mu\text{m}$ ) reduced the growth and weight of *E. fetida* after 14 and 21 days, respectively (Zhou et al., 2020; Xu et al., 2021a). However, in *E. crypticus* exposed to PVC particles for

21 days, body weight was significantly increased (Ma et al., 2020). The collembolan *F. candida* was not affected by exposure to PVC (Zhu et al., 2018) whereas face-mask-derived PP microplastics significantly reduced its growth, by 92.9% after 28 days (Kwak and An, 2021a).

### 3.4.5 Behavior

Alterations in behavior by microplastics comprise changes in movement, foraging activity and the stimulation of avoidance behavior. Microplastics were shown to have significant impacts on the movement activities of soil invertebrates. Ambiguous results were found focussing on the feeding activity and avoidance behavior (Table 4).

The addition of PE/PS spheres and PE fragments significantly reduced the movement of *L. sokamensis* (Kim & An, 2019). Significantly more burrow formation and bioturbation activity were provoked in *L. terrestris* exposed to 7% LDPE over 2 weeks (Huerta Lwanga et al., 2017).

The food intake of the mollusc *A. fulica* was reduced by the ingestion of PET fibers (Song et al., 2019).

Barreto and co-authors analyzed the impacts of PE and PP fibers on a natural soil community. The feeding activity of microarthropods was not affected after 4 weeks, whereas exposure to PE but not PP increased the decomposition rate (Barreto et al., 2020). In a study of avoidance behavior, *F. candida* significantly evaded PE spheres in a dose-dependent manner (Ju et al., 2019). In a two-sided test arena, *E. crypticus* clearly avoided the side containing a higher HDPE concentration (0/2, 0/4, 0/8, 2/4, 2/8, 4/8% dw) (Pflugmacher et al., 2020). In comparison, PE/PET/polyester fibers, PP fragments and fibers triggered neither avoidance behavior in *L. terrestris* (Prendergast-Miller et al., 2019; Lahive et al., 2021) or *F. candida* (Kwak and An, 2021a) nor a change in the feeding activity of *P. scaber* (Selonen et al., 2020).

### 3.4.6 Reproduction

Reproduction was examined in 12 publications. There was no significant reduction in *L. terrestris*, *P. scaber*, *O. nitens*, *E. andrei*, *E. crypticus*, *F. candida* and *C. elegans* (Huerta Lwanga et al., 2016, Kim et al., 2020b, Rodriguez-Seijo et al., 2017 and Selonen et al., 2020; Table 4). Lahive et al. (2019) observed a polymer-dependent impact on *E. crypticus* reproduction with PVC particles having no effects, while after 21 days of exposure polyamide particles at very high concentrations (>90 g/kg soil) decreased reproduction significantly (Table 4). In *E. crypticus* exposed to PA particles, the reproduction rate was elevated but it decreased significantly in response to PVC (Ma et al., 2020). After 4 weeks the number of *E. fetida* juveniles was significantly reduced by 1000 mg PVC particles kg<sup>-1</sup> (Sobhani et al., 2021a). In a long-term experiment, a significant a dose-dependent decrease in the reproduction of F0 and F1 generations of *E. fetida* was determined when the worm was exposed to pure or commercial PS fragments, thus revealing transgenerational effects of microplastic exposure

(Sobhani et al., 2021b). A significant inhibition by PE and PP on the reproduction rate of *F. candida* after 28 days was reported as well (Ju et al., 2019; Kwak and An, 2021a). PS beads, PET, HDPE, PP, PS, tire fragments and PAN fibers significantly reduced the number of offspring in *C. elegans* (Kim et al., 2020a; Kim et al., 2021).

### 3.4.7 Community structure

The effects of microplastic on the community structure of soil invertebrates was analyzed in two studies. The composition of the trophic structure, species richness, and the abundance of taxa served as indicators.

In a natural soil community exposed to LDPE fragments for 287 days in a field experiment, significant impacts on nematode trophic structure and a reduction in the overall abundance of nematodes were determined (Table 4). The abundances of omnivorous, predatory and plant-feeding nematodes declined, whereas fungal- and bacterial-feeding members were unaffected. In general, microarthropod structure was significantly altered by the addition of LDPE. The abundance of dipteran/lepidopteran larvae, ants and oribatid mites significantly decreased. Only the abundance of non-oribatid mites and collembolans remained unaffected (Lin et al., 2020). However, Barreto and co-authors found no effects of microplastics on either the species richness or the abundance of several taxa in a natural soil community, tested in a climate chamber (Barreto et al., 2020).

## 3.5 Chemical effects of microplastic-associated compounds on soil invertebrates

As discussed above, the leaching of chemical compounds associated with microplastic (e.g. plasticizers or flame retardants) or absorbed from the environment (e.g. polychlorinated biphenyls (PCBs), phenanthrene) can result in harmful impacts on organisms (Teuten et al., 2009; Carbery et al., 2018). While most of the following publications assumed a chemical impact, additional effects of the physical properties of the applied microplastic could not be excluded.

### 3.5.1 Effects of leaching compounds

Seven publications examined the effects of chemical leachates from microplastics (Table 5). Three different annelid species and *P. scaber*, *F. candida* and *C. elegans* were exposed for 3–56 days.

#### 3.5.1.1 Cellular response

The cellular responses of soil organisms towards microplastic leachates were examined in *P. scaber* and *E. fetida* (Table 5). Oxidative stress was induced in *E. fetida* exposed to tire particles. POD, CAT, GST activity and MDA levels were elevated, whereas SOD activity decreased (Sheng et al., 2021).

Tire particles did not affect electron transfer in *P. scaber*, whereas acetylcholinesterase (AChE) activity was significantly decreased, with a NOEC of 0.5% and an EC<sub>50</sub> of 1.2% (Selonen et al., 2021). When *P. scaber* was exposed to virgin LDPE, neither the total haemocyte count nor haemocyte viability were affected. However, the proportion of granulocytes increased and that of semigranulocytes decreased. Recycled LDPE did not influence the total haemocyte count, haemocyte viability or the proportion of granulocytes, but the proportion of semigranulocytes decreased in response to virgin LDPE (Kokalj et al., 2021).

### 3.5.1.2 Gut microbiota

One publication analyzed the effect of leachates on one annelid species (Table 5). Applied tire fragments did not affect the alpha diversity of the gut microbiota in *E. fetida*, whereas both the bacterial and the fungal community were significantly changed after exposure for 21 days. The study showed that the gut microbiota was more sensitive than soil bacteria to tire particles (Ding et al., 2020).

### 3.5.1.3 Mortality

Ambiguous results on the mortality of soil invertebrates were reported in five publications. Neither tire particles, nor virgin/recycled LDPE fragments and their leachates affected the survival of *P. scaber*, *F. candida* or *E. crypticus* (Kokalj et al., 2021; Selonen et al., 2021), whereas the survival rate of *E. fetida* and *E. andrei* exposed to tire fragments and APPs decreased significantly (Ding et al., 2020; Soroldoni et al., 2021). The mortality of *C. elegans* was not affected by exposure of the nematode to tire fragments for 2 days, independent of the preincubation time of the microplastics with the testing soil. However, in a longer experiment survival was significantly reduced after 8 days. Preincubation of the microplastic with the soil for 75 days resulted in a significant inhibition already after 6 days (Kim et al., 2021) (Table 5).

### 3.5.1.4 Development

Two publications found impacts of leachates on the development of invertebrates (Table 5). Antifouling paint particles (0.14%, dry soil w/w) scraped off from a boat's hull reduced the biomass of *E. andrei* after 56 days (Soroldini et al., 2021). In *C. elegans* exposed to  $\geq 100$  mg tire fragments kg<sup>-1</sup> for 2 days, growth was significantly reduced. A significant inhibition of growth at lower concentrations (1 mg kg<sup>-1</sup> and higher) was obtained when the tire particles were preincubated with the testing soil for 30 or 75 days, indicating an effect of leaching compounds (Kim et al., 2021).

### 3.5.1.5 Behavior

The behavior of soil invertebrates exposed to leachates has been analyzed only in *P. scaber* (Table 5). Exposure of the woodlouse to 0.5 and 1.5% virgin LDPE fragments for 21 days significantly reduced its feeding rate whereas recycled LDPE or

tire fragments had no effect on the feeding rate or activity (Kokalj et al., 2021; Selonen et al., 2021).

### 3.5.1.6 Reproduction

Five studies examined the impact of microplastic leachates on three annelid species, the nematode *C. elegans* and the collembolan *F. candida* (Table 5). Annelida were clearly impacted, as the exposure of *E. fetida* to tire fragments ( $\geq 0.12\%$  microplastic content) for 21 days caused a dose-dependent decrease in reproduction. A logarithmic correlation between reproduction and the concentration of tire particles was determined (Ding et al., 2020). The number of juveniles and cocoons produced by *E. andrei* was reduced after 56 and 42 days, respectively, when the worm was exposed to 0.01% APPs (Soroldoni et al., 2021). Reproduction in *E. crypticus* was not affected by microplastic mixed with food, whereas 0.02 and 1.5% tire particles in soil significantly decreased the number of offspring (Selonen et al., 2021).

The exposure of *C. elegans* to extractable additive solutions of PE, PAN, PP for 24 h did not affect reproduction, whereas extracts derived from PET significantly reduced the number of offspring. After the removal of those additives by extraction, the toxic effect was lost. The adverse impact of LDPE films and PAN fibers on *C. elegans* reproduction increased with prolonged exposure and frequent wet-dry cycles of the testing soil (Kim et al., 2020b). When the nematode was exposed to tire fragments for 2 days its brood size was significantly reduced. This decreasing effect was aggravated when the microplastic was preincubated with the testing soil (Kim et al., 2021). Finally, the number of juveniles in *F. candida* was not affected by tire particles added to soil or food (Selonen et al., 2021).

## 3.5.2 Influence of microplastic on the toxicity of environmental chemicals

Eleven of the reviewed papers examined the vector effects of microplastics on soil invertebrates, in three annelid species in ten publications and the arthropod *P. scaber* in one publication (Table 6). The impacts of the microplastics in co-exposure experiments with atrazine, chlorpyrifos, phenanthrene, PFOA, PFOS, tetracycline and the metals As, Cd, Cu, Ni and Zn were analyzed. The exposure time ranged between 7 and 56 days.

### 3.5.2.1 Cellular response

Microplastic-chemical-co-exposures triggered several oxidative stress responses in *E. fetida*, *E. crypticus* and *P. scaber*. *E. fetida* was the most well-studied (Table 6). Its co-exposures to PE-Cd, PS-phenanthrene and LDPE-atrazine induced DNA damage (Cheng et al., 2020; Xu et al., 2021a; Huang et al., 2021). LDPE-atrazine, LDPE-chlorpyrifos, PP-Cd, PE-Cd, -Cu or -Ni, and PS-phenanthrene, -Cd or -As triggered increases in LPO and ROS levels, GSH, MDA and TBARS content, total protein, IBR, the activity of CAT, MT, POD, SOD and GST as well as the mRNA levels of HSP70, MT,

SOD and TCTP (Cheng et al., 2020; Huang et al., 2021; Li B. et al., 2021; Rodriguez-Seijo et al., 2018b; Xu et al., 2021a; Zhou et al., 2020). In general, cellular responses were aggravated by co-exposure compared to the chemical or microplastic treatment alone (Ma et al., 2020; Zhou et al., 2020; Li B. et al., 2021; Xu et al., 2021a).

By contrast, the levels/activities of several indicators of oxidative stress were decreased in *E. fetida* after its co-exposure for 28 days. Thus LDPE-atrazine, LDPE-chlorpyrifos, PE-Cd and PS-phenanthrene, -Cd or -As resulted in significantly lower AChE, CAT, GST, POD, SOD activities, GSH, MDA, TBARS contents and CAT, GST MT mRNA levels (Cheng et al., 2020; Huang et al., 2021; Rodriguez-Seijo et al., 2018b; Xu et al., 2021a). No effect on the AChE activity were observed when *E. fetida* was exposed to 0.25–1 mm LDPE pellets in combination with chlorpyrifos (Rodriguez-Seijo et al., 2018b).

On tissue level, in *E. fetida* exposed for 21 days to PS particles (100 µm, 10 µm, 100 nm) in Cd- and As-contaminated soil cellular damage such as abnormal epithelial cells, incomplete coelom tissues and exfoliated intestinal epithelium were observed (Xu et al., 2021b; Huang et al., 2021). The exposure of *E. crypticus* to PA or PVC particles in combination with tetracycline for 21 days resulted in a significantly higher amount of ARGs and MGEs (Ma et al., 2020).

AChE activity was significantly lower in *P. scaber* exposed to PE fibers (12–2870 µm) and 0.2–2 mg kg<sup>-1</sup> CP for 3 weeks. Both the total haemocyte count (THC) and the proportion of hyalinocytes were elevated whereas the proportion of granulocytes, semigranulocytes and the viability of haemocytes were not affected. The co-exposure of *P. scaber* to tire fragments (<180 µm) and CP for 3 weeks increased the proportion of granulocytes and decreased that of semigranulocytes. Haemocyte viability and the proportion of hyalinocytes were not affected (Dolar et al., 2021).

### 3.5.2.2 Gut microbiota

Only two publications focussed on the impacts of co-exposure on the gut microbiota. Thus, the exposure of *E. fetida* to PS of several sizes and to phenanthrene significantly changed the composition of the gut microbial community (Xu et al., 2021a). Similar results were found in *E. crypticus*, in which PA/PVC-tetracycline treatment induced a significant change in microbial composition and a reduction in microbial alpha diversity (Ma et al., 2020) (Table 6).

### 3.5.2.3 Mortality

Almost all publications with experiments lasting 3–56 days reported no effects on the mortality of the test species (Table 6). Most data are available for *E. fetida*: The combinations LDPE-chlorpyrifos (Rodriguez-Seijo et al., 2018b), aged/unaged LDPE-atrazine (Cheng et al., 2020), PA-Cd or -As (Xu et al., 2021b), PS-phenanthrene (Xu et al., 2021a) and PVC-PFOA and/or PFOS (Sobhani et al., 2021a) did not influence the annelid's mortality.

However, co-exposure to Cd-PS spheres for >42 days significantly reduced survival. In another study the effect of PS-phenanthrene was mostly mediated by the PS component, as phenanthrene addition did not aggravate the effect (Zhou et al., 2020).

### 3.5.2.4 Development

Eight publications examined the effects of environmental chemicals on developmental traits, with *E. fetida* as the test organism in most of those studies. After a maximum of 28 days of co-exposure to PE-Cd or PS-phenanthrene, -As or -Cd, the weight of the worm was significantly reduced (Xu et al., 2021a; Huang et al., 2021) (Table 3). Additionally, in *E. fetida* exposed to PP-Cd for 42 days the growth rate was significantly decreased. The decline was greater in the co-exposure treatment than in the Cd alone treatment (Zhou et al., 2020). However, the exposure of *E. fetida* to LDPE pellets contaminated with chlorpyrifos caused significant weight loss after 28 days (Rodriguez-Seijo et al., 2018b). Similar results were found for *L. terrestris* exposed to PVC-tetracycline for 21 days (Ma et al., 2020) whereas the co-exposure of *E. fetida* or *L. terrestris* to LDPE beads and chlorpyrifos, PS particles and Cd or As, PVC-PFOA and/or PFOS and HDPE-Zn had no impact on the weight of either organism (Hodson et al., 2017; Rodriguez-Seijo et al., 2018b; Sobhani et al., 2021a; Xu et al., 2021b) (Table 6).

### 3.5.2.5 Behavior

The effects of environmental chemicals and microplastic on the behavior of soil invertebrates were investigated in three studies. Significant avoidance behavior was reported for *E. fetida* exposed to PE-Cd or to LDPE pellets contaminated with chlorpyrifos (Rodriguez-Seijo et al., 2018b; Huang et al., 2021) whereas *L. terrestris* did not avoid contaminated HDPE fragments (Hodson et al., 2017) (Table 6).

### 3.5.2.6 Reproduction

Co-exposure treatments significantly reduced the reproduction of soil invertebrates (Table 6). Cocoon production by *E. fetida* exposed to Cd and PE particles decreased (Huang et al., 2021), and PVC combined with PFOA, PFOS or a mixture of both significantly reduced the number of juveniles (Sobhani et al., 2021a). In *E. crypticus* exposed for 21 days to PVC or PA combined with tetracycline, reproduction was significantly reduced (Ma et al., 2020).

## 4 Discussion

Soils can be hotspots of microplastic pollution, as here dumping, decay, fragmentation and accumulation of plastic takes place. Moreover, soils play a key role in the transition to aquatic environments. Consequently, soil invertebrates are likely to be exposed to elevated concentrations of microplastics, potentially leading to adverse effects on soil ecosystems and

thus higher trophic levels. Due to patchy data availability and the heterogenous, non-standardized methodologies used in the different studies, general microplastic-specific effect mechanism in soils could not be deduced. However, our literature review suggests trends that should be the basis of future research.

#### 4.1 Variability in species sensitivity

In general, there was little diversity in the test organisms, as 64% of all studies analyzed only annelid species. As comparisons of species sensitivity with environmental thresholds are the basis of risk assessments, including the risk posed by the specific or unspecific impacts of microplastics (Koelmans et al., 2017), toxicity data must be required for a larger number of soil invertebrate species, to cover at least the most dominant ones.

Nonetheless, the published data revealed differences in the sensitivity of different soil invertebrates to microplastics. Annelida tended to be more sensitive than Collembola. The levels of stress biomarkers were increased in the annelid *E. fetida* exposed to 0.05% PE particles (Li B. et al., 2021) (Table 4). Cellular responses, development, mortality, and reproduction were negatively impacted in *E. andrei*, *E. fetida* and *E. crypticus* exposed to APP and tire fragments and/or its possible leachates (Ding et al., 2020; Selonen et al., 2021; Sheng et al., 2021; Soroldoni et al., 2021) (Table 5). In comparison, a concentration of at least 0.1% was needed to reduce the reproduction rate in the springtail *F. candida* (Ju et al., 2019) (Table 4). In *F. candida*, neither mortality nor reproduction was affected by the leachates of tire fragments (Selonen et al., 2021) (Table 5). However, studies with other chemicals, such as pesticides, showed that the toxicity ranking among soil invertebrates is highly substance specific (Frampton et al., 2006). For example, reproduction in *F. candida* was more sensitive than *E. fetida* to the insecticide toxaphene ( $EC_{50} = 3.6 \text{ mg kg}^{-1}$  soil dry weight vs  $EC_{50} = 54.5 \text{ mg kg}^{-1}$  soil dry weight) (Bezchlebova et al., 2007) and to Ag nanoparticles (Heckmann et al., 2011; Zhang and Filser, 2020) whereas the effect of nickel was comparably (*E. fetida*:  $EC_{50} = 362 \text{ mg Ni kg}^{-1}$  soil dry weight; *F. candida*:  $EC_{50} = 391\text{--}461 \text{ mg Ni kg}^{-1}$  soil dry weight) (Lock and Janssen, 2002).

Considering the large heterogeneity of microplastics as a contaminant group, also here a substantial variability in the sensitivity of soil organisms can be expected. Furthermore, the lack of standardized test protocols (including with respect to concentration: mass, and particle numbers; dry or wet weight) hampers reliable comparisons between studies (Cunningham and Sigwart, 2019; De Ruijter et al., 2020). The development of standardized experimental and analytical methods will enable the comparability and reproducibility of results and in turn allow the identification of valid trends in the sensitivities within soil communities.

#### 4.2 Role of the properties of microplastics (polymer/shape/size)

The toxicity of microplastics differing in their polymer composition, shape and type and of mixtures thereof remains to be reliably determined. None of the reviewed papers except one simultaneously tested the effect of more than one microplastic type or shape (Kim and An, 2021a). While one-third of all publications evaluated the interference of PE, PS and PP with soil invertebrates (Figure 4A; Tables 4–6), there are roughly 30,000 EU-approved types of plastic and several plastic types have already been detected in a diversity of soils around the world, including their spatial temporal dynamics (Geyer et al., 2017; Horton et al., 2017; Huerta Lwanga et al., 2018; Büks and Kaupenjohann, 2020; Wong et al., 2020).

Given the limited data on the impacts of microplastics on soil invertebrates, systematic investigations of the influence of particle properties on the ecotoxicological behavior of microplastic in soil are rare and the determination of toxicity mechanisms related to microplastic properties accordingly difficult. Studies using bacteria (*Aliivibrio fischeri*), plants (*Nelumbo nucifera*) or fish (*Danio rerio*) suggest that PVC is more toxic than PS (Lei et al., 2018; Zimmermann et al., 2019; Esterhuizen and Kim, 2022), but this could not be shown for soil invertebrates. Several studies demonstrated that PS affects the cellular responses of soil organisms (Sobhani et al., 2021b; Jiang et al., 2020; Wang et al., 2019; Xu et al., 2021a; Table 4), but a direct comparison of the effects of PS vs those of other polymers is difficult, as in the respective studies the PS particles were smaller ( $<250 \mu\text{m}$ ) than particles of other microplastic types (e.g. up to 2.4 mm for PE). Particle size is known to play a decisive role in the toxic effects of microplastics on soil invertebrates (e.g. Lei et al., 2018; Mueller et al., 2020), as ingestion is dependent on the size of the organism's mouth opening (Fueser et al., 2019; Koelmans et al., 2020).

Fragments and fibers are the most common shape found in soil environments (Büks and Kaupenjohann, 2020). In several studies, fragments were shown to negatively impact cellular responses (Rodriguez-Seijo et al., 2017; Sobhani et al., 2021b; Li M. et al., 2021; Table 4) which suggests shape-specific effects. Unfortunately, none of the reviewed publications directly compared the effects of the microplastic shapes with a standardized polymer type, size and concentration. Sublethal effects in *Daphnia magna* were found to be related to microplastic size, polymer type and shape, with beads and fragments being more toxic than fibers (Schwarzer et al., 2022). Further studies are needed to evaluate the role of microplastic shape in the toxicity of microplastics to soil invertebrates (Büks and Kaupenjohann, 2020).

#### 4.3 Distinguishing physical from chemical effects

To distinguish between the major effect pathways of microplastics, studies were categorized into those examining

physical and chemical effects of microplastics. The former accounted for 71% of the reviewed publications. In fact, this ascription neglects that in most studies, due to their simultaneous occurrence, the two toxicity pathways are not readily distinguished. For example, some publications reported DNA damages after microplastic exposure, but this could not be unambiguously attributed to strictly chemical vs strictly physical effects (e.g. Cheng et al., 2020; Sobhani et al., 2021b; Jiang et al., 2020; Table 4). Thus, test setups are required that the experimental separation of these two effect pathways, such as by testing extracted leachates separately from the particles to test for strictly chemical effects (Kim et al., 2020b) and by testing (non-plastic) reference particles with characteristics similar to those of microplastics to account for the physical effects of particles (Koelmans et al., 2022).

#### 4.4 Experimental approaches

Most of the studies evaluating the potential adverse effects of microplastics on soil invertebrates consisted of single-species toxicity tests. Only two studies assessed the impacts of microplastics on natural soil communities (Barreto et al., 2020; Lin et al., 2020; Table 4). Although lower-tier testing is a valuable approach for obtaining reliable toxicity thresholds, assuming standardized methods are used, realistic exposure scenarios are best achieved in community-based or long-term population-based approaches, such as in micro- or mesocosm studies. These systems have several advantages over acute, single-species tests:

- 1) Whole-community tests allow the toxicity of microplastics to be synchronously assessed in several species, thus significantly expanding the species database on microplastic toxicity.
- 2) The impacts of microplastics on whole food webs and the transfer of microplastics from one trophic level to another are unclear but can only be elucidated in multispecies systems (Haegerbaeumer et al., 2019b). Several studies have shown that microplastic particles can exert their toxic effects on organisms not only directly, but also indirectly, such as via the dilution of food through or the formation of food-microplastic agglomerates (Hanna et al., 2018; Mueller et al., 2020; Rauchschalbe et al., 2021). Higher-tier studies, especially terrestrial model ecosystems (TMEs), can reveal both the direct and indirect effects of microplastics on several taxa, trophic, population levels or developmental stages of organisms (Gestel et al., 2020; Mueller et al., 2020).
- 3) In publications dealing with chemical effects, it could be reported that the exposure time influenced the magnitude of leaching or vector impact (Xu et al., 2021b; Soroldoni et al., 2021). Moreover, as microplastics are highly persistent in the environment (Thompson et al., 2005; Thompson et al., 2009a; Barnes et al., 2009; Horton et al., 2017; Selonen et al., 2020)

chronic effects on the soil fauna can be expected, such that risk assessments should be conducted in long-term studies, as has been done in freshwater and marine ecosystems (e.g. Santana et al., 2018; Chisada et al., 2019; Redendo-Hasselerharm et al., 2020; Rauchschalbe et al., 2022). As transgenerational effects of microplastics on soil invertebrates have been reported (Sobhani et al., 2021b), these should be further analyzed in model ecosystems to evaluate long-term effects of microplastic in soil.

The experimental approaches in the reviewed studies were highly diverse, ranging from single to multiple species set ups, and therefore so were their findings. Terrestrial and aquatic studies with environmental chemicals often yielded more sensitive responses of soil and sediment communities when in microcosms than as single-species tests (Clements et al., 2013; Haegerbaeumer et al., 2016; Yin et al., 2018; Boots et al., 2019). However, a microcosm study using freshwater nematodes showed that the sensitivity of a native nematode community to microplastics in the sediment of the microcosms (Rauchschalbe et al., 2022) was similar to that determined in a single-species sediment toxicity test (Höss et al., 2022). Analogous to the risk assessment of other environmental chemicals, lower-tier testing with representative species is necessary to determine reliable toxicity thresholds, with higher-tier testing using microcosms or semi-field approaches allowing the validation of these thresholds under more realistic exposure conditions.

#### 4.5 Differences between different soils

Since microplastics can affect the physical properties of soil, including soil structure, pH, size distribution of water-stable soil aggregates and the availability of nutrients, the impact of microplastics on soil invertebrates may depend on the nature of the tested soil (Liu et al., 2017; Machado et al., 2018; Boots et al., 2019; Machado et al., 2019). For example, the toxicity of the fungicide mancozeb on the reproduction of the two soil invertebrates was shown to be highly dependent on the soil type (Carniel et al., 2019). Among the reviewed studies, only one investigated the toxic effect of PS spheres on *C. elegans* in different soil types. The results showed that the physicochemical properties of the soil influenced the response of *C. elegans* to soil microplastics (Kim et al., 2020a). However, a comparable study in freshwater sediments found no influence of physico-chemical properties on the response of this nematode (Höss et al., 2022). Microplastic exposure in OECD soil, but not natural field soil (LUF) significantly increased the mortality of *E. fetida* (Zhou et al., 2020; Xu et al., 2021a). Nonetheless, although these two studies used microplastics of similar concentration and size, the different experimental set ups hamper a direct comparison of the tested soils.

## 4.6 Risk assessment: Comparing effects with exposure

The use of effect assessment data, as presented in this review, in a microplastic risk assessment requires realistic exposure estimates, i.e. predicted environmental concentrations. However, for the smaller size fractions of microplastics (<20  $\mu\text{m}$ ), reliable data on environmental concentrations are still missing, due to the obvious analytical challenges posed by the presence of these particles in complex matrices, such as soils. In agricultural or horticultural sites, microplastic concentrations of up to 4.5  $\text{mg kg}^{-1}$  dry soil have been determined, whereby and concentrations at industrial used sites exceeded such values up to four times (Büks & Kaupenjohann, 2020). Based on the lowest effect concentrations resulting in toxicity, 100  $\mu\text{g kg}^{-1}$  of dry soil, as demonstrated in *E. fetida* (Jiang et al., 2020; Table 4), an environmental risk for soil invertebrates at microplastic hotspots cannot be ruled out. Moreover, including the small fraction, still not reliably covered by analytical studies, an even higher risk might be realistic.

A second important component in a risk assessment is the identification of reliable toxicity thresholds, such as those derived from dose-response curves (effect concentrations: EC<sub>x</sub>; LOEC, NOEC). However, for microplastics, toxicity thresholds comparable to those available for pesticides and other chemicals are scarce (Table 4; Rodriguez-Seijo et al., 2017; Selonen et al., 2021). A study consisting of an alignment of available microplastic effect thresholds for a variety of organisms (Koehlmans et al., 2020) was unable to include findings for soil invertebrates, due to the lack of threshold data. Therefore, studies producing effect thresholds on the basis of dose-response curves are needed for soil ecosystems.

## 5 Recommendations

- A larger number of soil invertebrate species should be included in the routine testing of microplastics in soil.
- Robust toxicity thresholds (i.e. effect concentrations) determined on the basis of dose-response testing remain to be established for microplastics. Chronic toxicity endpoints should be included to account for subtle effects and the time-dependent leaching of chemicals from plastic particles.
- Toxicity thresholds for microplastics, based on single-species testing, should be verified by testing under realistic exposure scenarios, including multiple species (whole communities), trophic interactions (and thus also indirect effects) and long-term exposure.
- Test systems are required that distinguish between chemical and physical effects. This includes the

testing of extracted leachates and the use of non-plastic reference particles as a negative control for physical particle effects.

- As also reported for microplastics research in aquatic environments, harmonized, standardized methods are urgently needed in tests of the effects of microplastics on soil organisms, as these will allow comparisons of reported effects.

## Author contributions

ACFM created the first draft of the manuscript, prepared data organization/collection and contributed to manuscript revision. AH guided manuscript revision and contributed to the organization of the database. SH was responsible for study conceptualization, manuscript writing and editing, and WT for study conceptualization and manuscript revision.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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