What do we know about how the terrestrial multicellular soil fauna reacts to microplastic?

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Abstract. The ubiquitous accumulation of microplastic (MP) particles across all global ecosystems comes along with the uptake into soil food webs. In this review, we analyzed studies on passive translocation, active ingestion, bioaccumulation and adverse effects within the phylogenetic tree of multicellular soil faunal life. The representativity of these studies for natural soil ecosystems was assessed using data on the type of plastic, shape, composition, concentration and time of exposure.

Available studies cover a wide range of soil organisms, with emphasis on earthworms, nematodes, springtails, beetles and lugworms, each focused on well-known model organisms. About 58 % of the studies thereby used inappropriate concentrations or units, but 42 % applied MP concentrations similar to amounts in slightly to very heavily polluted soils. In many cases, however, polystyrene microspheres have been used, a combination of plastic type and shape, that is easily available, but does not represent the main plastic input into soil ecosystems. In turn, MP fibers are strongly underrepresented compared to their high abundance within contaminated soils. A few studies also examined the comminution of macroplastic by the soil fauna. Further properties of plastic such as aging, coating and additives were insufficiently documented. Despite these limitations, there is a recurring pattern of active intake followed by a population shift within the gut microbiome and adverse effects on motility, growth, metabolism, reproduction and mortality in various combinations, especially at high concentrations and small particle sizes.

For the improvement of future studies, we identified problems of past experiments and recommend that coming studies take into account the type, shape, grade of aging, specific concentrations of MP fractions and long-term incubation, in natural and contaminated soils.

1 Introduction

Imagine a compact plastic cube of nearly 2 km side length and a weight of 7300000000 tons, with major percentages by weight of 36 % polyethylene (PE), 21 % polypropylene (PP), 12 % polyvinyl chloride (PVC) and 10 % of each polyethylene terephthalate (PET), polyurethane (PU) and polystyrene (PS). That is the accumulated global non-fiber production of the six main plastic types until 2015. It accounts to 87 % of the all-time plastic production, which evolved exponentially, since the early 1950s, from some megatons (Mt) to 8300 Mt in 2015, with only 260 Mt annual output in 2009 increased to 380 Mt in 2015 (Thompson et al., 2009; Geyer et al., 2017). Of this ever produced plastic, 6300 Mt became waste until 2015, of which only 21 % were recycled or incinerated, whereas 5000 Mt ended up in landfills and nature (Geyer et al., 2017). As a corollary of production, use and disposal, a certain part of plastic waste is constantly released into the environment through various pathways, but our knowledge about rates of mass flow into global ecosystems is very limited. Based on waste generation in coastal countries, Jambeck et al. (2015) calculated the global plastic input to marine ecosystems to be roughly 4.8 to 12.7 Mt in 2010. Such data on soils are lacking, but Nizzetto et al. (2016) estimated that the load of microplastic (MP) to agricultural sites in Europe is in the same order of magnitude as that in marine environments.

By littering, plastic mulching, the application of sewage sludge, digestates and composts as well as windblown dispersal (Bertling et al., 2018; Weithmann et al., 2018; Zhang et al., 2019; Wang et al., 2019a), plastic from our technosphere arrives in soil ecosystems in various forms as large and small fragments, fibers and particles. Exposed to UV radiation, mechanical stress and processing by soil organisms, plastic items become weathered and prone to a successive comminution towards the size range of MP with increased surface, charge and biofilm cover (Kale et al., 2015; Andrady, 2017). However, the resistance of plastic to metabolization causes a constant accumulation in soils as long as the release rate from human processes is above the very slow rate of degradation.

Due to a lack of monitoring programs, data on MP concentrations in terrestrial soils are rare, and those using w/w concentrations represent only a small part compared to item concentrations. In soils with only slightly contaminated conditions, amounts seem to average about 1 mg kg^{-1} soil dry weight (and approx. 200 items kg^{-1} dry soil) (Rezaei et al., 2019). On sites with industrial activity or intensive use of plastic mulching and sewage sludge in agriculture, concentrations can be increased by 2 to 4 orders of magnitude (Fuller and Gautam, 2016; Zhang and Liu, 2018; Huang et al., 2020). Semisubhydric soils such as beaches, mudflats, mangroves or lagoons, that are additionally contaminated from the aquatic side, contain MP of the order of 10 to 100 items kg^{-1} dry soil and single extreme samplings contained several thousand items (Nor and Obbard, 2014; Naji et al., 2017; Garcés-Ordóñez et al., 2019; Li et al., 2018a). More informative data using mg kg^{-1} are only available for beaches and coastal deconstruction yards in municipal neighborhoods and amount to 0.5 and

70 mg kg^{-1} dry soil, 0.00005 and 0.007 % w/w, respectively (Reddy et al., 2006; Claessens et al., 2011). All these concentration data represent a wide range of particle sizes between 0 and 5000 µm with different materials, shapes and degrees of aging.

Plastic particles can possibly enter and accumulate in the food web by either direct uptake from soil or by consumption of other soil biota contaminated by adhesion or ingestion (Huerta Lwanga et al., 2017a). There is evidence, that MP is incorporated even by plants and unicellular organisms at the base of the food web. **Bacteria**, for example, that are reasonably assumed to avoid MP uptake due to their minor size and the prevalent lack of phagocytosis, were shown to take up inorganic nanoparticles of a few nanometers (Kumar et al., 2011). Although the physiochemical properties of weathered nanoparticulate plastics might differ from these, also their uptake seems likely.

A similar argument can be made for **fungi** and soil **algae**, but studies on incorporation are lacking, whereas the transfer into a freshwater food web by adhesion of nanoplastic on algae has been shown by Chae et al. (2018). The uptake of MP into **plant roots** is also inhibited (Rillig et al., 2019), but occurred for nanoplastics that permeate into the plant tissue (Li et al., 2019). Also the integration into root tissue after adsorption to the rhizodermis has yet to be studied.

In contrast, **protozoa** feature phagocytosis for the active ingestion of particles. Diverse soil, freshwater and marine ciliates ingest PS/latex beads of 0.1 to 14.4 µm in laboratory experiments, with preferences to their natural prey size (Fenchel, 1980; Jonsson, 1986; Lavin et al., 1990). Soil amoebas act similarly, but additionally select according to food quality (Weisman and Korn, 1967; Vogel et al., 1980; Bowers and Olszewski, 1983; Avery et al., 1995; Elloway et al., 2006).

Finally, many soil microbiota live protected within biofilms. Plastic particles were shown to be a potential surface for the formation of those biofilms (Lobelle and Cunliffe, 2011), which are a food source for grazing primary consumers. Inadvertent ingestion might also transfer occluded or abrased MP to higher trophic levels.

But what about the larger organisms that feed on all these, free plastic particles, contaminated microorganisms, biofilms and one another? Recent work discussed the effects of MP on soil biota (Chae and An, 2018) or called for intensified research on certain taxonomic groups (Rillig and Bonkowski, 2018). Thus, we were motivated to give on our part a review with focus on the most-produced plastics and their passive translocation, ingestion, bioaccumulation and adverse effects on the multicellular soil fauna. The types, sizes and shapes of plastic used in former laboratory studies were compared with the available knowledge on plastic in the environment, and recommendations are given for future research. This analysis aims to support the assessment of the influence of MP on the ecosystem services provided by diverse soil organisms.

2 Search pattern

Within the tree of life, edaphic branches were identified comprising taxa that permanently inhabit the soil, are both-sided part of the soil food web and/or the burrowing macro- and megafauna or have active subterranean larval stages. The resulting tree of soil life based on the NCBI taxonomy database (Fig. 1) was drawn using the software phyloT and shows the leading taxonomic rank, which is mainly the family, but in exceptions – e.g. if one species represents the only soil-born between many aquatic – a lower rank.

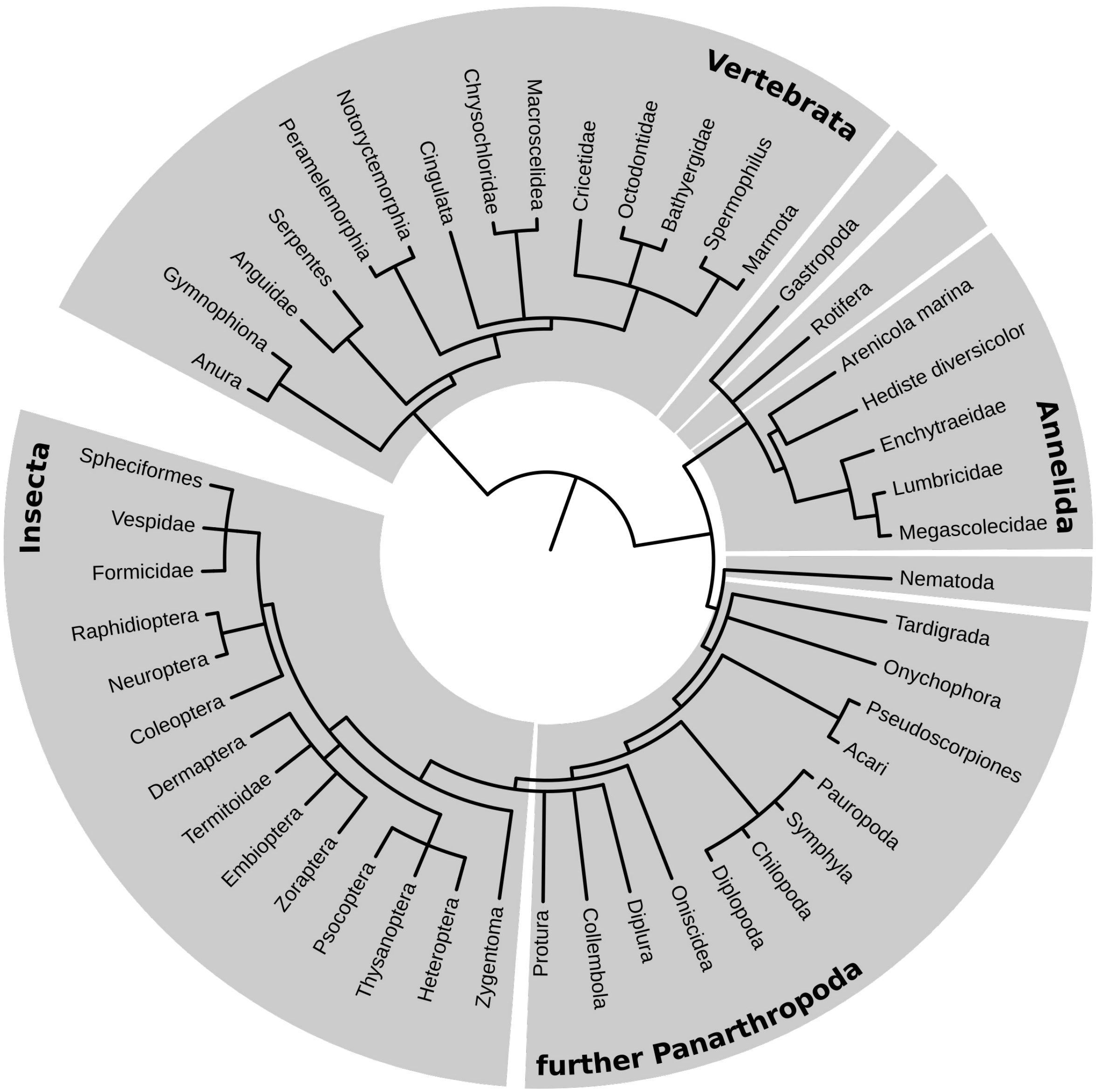

Figure 1: Tree of edaphic fauna. Taxonomic ranks, that were examined in this qualitative study, are placed at the outer rim of the diagram. The length of the connecting line between two taxa represents the grade of phylogenetic relationship.

A pattern of search terms was established (*Table 1*), consisting of „taxon“ (Linné‘s binominal nomenclature, common name, plural-sensitive search), „plastic type“ (plastic, microplastic, nanoplastic, PE or polyethylene, PP or polypropylene, PVC or polyvinyl chloride, PS or polystyrene, PU or polyurethane, PET or polyethylene terephthalate and latex) and „common shapes“ (fragments, particles, fibers, microfibers, beads, microbeads, microspheres). Some type- shape combinations caused problems, as they led to a very large amount of unuseful, off-topic papers – e.g. using any taxon combined with PET, papers with the use of PET bottles in experimental set-ups were selected or also studies on pets. Those combinations of search terms were excluded from this pattern. Further plastic types and shapes occuring within the found studies were also included in the review. Data on microspheres and microbeads were pooled, as both names describe one and the same.

Table 1: Types and shapes of microplastic particles in edaphon studies within this review. (X) symbolizes combinations excluded from the search pattern. The number counts for how often type-shape combinations were used in all reviewed experimental setups independently of organism. Empty fields stand for zero results. Microbeads and microspheres are often mixed up terms and, thus, counted together.

Organism: Linné‘s systematic names OR common name	-	fragments	particles	fibers	microfibers	beads	microbeads	microspheres	other, diverse, N/A
plastic	X								
microplastic									
nanoplastic									
PE *OR* polyethylene	X	4	10	1	1	1		4	7
PP *OR* polypropylene	X	1							
PVC *OR* polyvinyl chloride	X	4	6	1					
PS *OR* polystyrene	X	6	3					24	4
PU *OR* polyurethane	X								
PET *OR* polyethylene terephthalate	X	3		2			X		
latex	X				X			6	
other		6	3		1				1
N/A		1		1		2			3

The search was applied between June 2019 and January 2020 within the Web of Science Core Collection Database, repeated in the first week of January 2020 and covers publications until January 2020. The search strings result from combinations of taxon, plastic type and particle shape shown in *Table 1*. Based on the search pattern, data on passive transport, ingestion, bioaccumulation and adverse effects were collected for each edaphic group. Studies that only use uncommon, local, outdated, weird or nicknames are excluded by the search pattern. Studies testing injection to tissues, lymph or blood were excluded, as they do not represent natural ways to incorporate MPs. Data on inhalation by the megafauna in fact represent a natural way of uptake, but were also excluded as they are exclusively related to above-ground organisms, that only occur on the outer edge of the food-web. Also running debates on phylogenetic classifications are not part of this work and the taxonomists will be able to adjust the branches accordingly to their purpose.

The data of related taxonomic groups were pooled and evaluated for their environmental representativity based on exposure time, plastic concentrations and properties used. From this synthesis recommendations for a structured experimental design were derived for application in future studies.

3 Data collection

3.1 Insects

Within the Panarthropoda, the insects comprise the highest taxonomic diversity. And, regarding MPs, they represent an unevenly studied taxonomic group.

Within the Insecta, the **Coleoptera** (beetles) are an extraordinarily diverse and abundant taxon. Studies on plastic uptake into adult individuals mainly focus on the subfamily of Scarabaeinae (dung beetles). Comprehensive experiments with latex microbeads showed, that many species only ingest smaller particles with maximum diameters of about 10 to 83 µm and retain them within the gut – with a slightly positive dependency on body size. Larger particles were rejected by a filtering mechanism within the mouth region and not ground with the mandibles (Holter, 2000; Holter et al., 2002; Holter and Scholtz, 2005). Beside those on Nematods, these data comprise by far the most detailed information about size-dependent uptake of MP particles compared to other edaphic taxa. This gives a good foundation for future studies on adverse concentrations. In addition, several studies with plastic as predominant food source could show chewing, ingestion and intestinal degradation of different PS and PE foams in feeding experiments with *Tenebrio sp.* larvae (mealworms). These experiments also pointed out an alteration of the gut microbiome, but no adverse effects on reproduction and survival, with only one case of a non-significant tendency to higher mortality after 1 month of exposure (Yang et al., 2015; Brandon et al., 2018; Yang et al., 2018; Peng et al., 2019).

The **Isoptera** (termites), recently categorized as part of the order Blattodea, are the oldest social insects with a tribal history of about 130 million years (Korb, 2008). Especially in arid ecosystems with a lack of earthworms they play an important role in homogenization of soils, but also in sorting of soil mineral particles for building mounds as well as decomposition and distribution of organic matter (De Bruyn and Conacher, 1990). Tsunoda et al. (2010) and Lenz et al. (2012) could show, that different termite species are picky feeders and erode PE, but avoid other plastic cable sheathings. This suggests the excretion of ground MP particles by termites, but metabolic impacts are unknown. In contrast to termites, data on **other Blattodea** (e.g. cockroaches) were not found.

The suborder **Apocrita** comprises some flying insects, that inhabit burrows within the soil, such as ground-dwelling wasps within the **Vespidae** superfamily, mining bees within the **Apoidea** superfamily and the **Spheciformes**. They mostly do not prey and feed on subterrestrial organisms, but may move MP particles into the ground, as implied by a report of Allasino et al. (2019) on solitary bees, which built nests fully made of plastic fragments. The Apocrita also contain the **Formicidae** (ants). Some ant species are considered an important factor for seed dispersal, a behavior, that could also be shown for artificial plastic seeds with ~2 mm diameter (Hughes and Westoby, 1992; Angotti et al., 2018). Robins and Robins (2011) found that this also includes differently shaped cultural objects: *Rhytidoponera metallica*, a representative of ground-nesting, omnivore ants, is capable not only of a remarkable bioturbation, but also of an active, apparently random burying of anthropogenic plastic artifacts >1 mm. Seeds are used as a food source, thus, the ingestion of plastic bites is conceivable, but not documented. The uptake of latex microspheres ≥0.88 µm with liquids by larvae of *Solenopsis invicta* seems to be prevented by filtration within the mouth and the particles are released as larger aggregates, whereas other species ingest by far larger particles up to 150 µm (Glancey et al., 1981). However, also here data on adverse effects are missing.

Further insects with edaphic adult stages, e.g. **Dermaptera** (earwigs), **Heteroptera** (true bugs) and **Zygentoma** (silverfish, fishmoth, firebrat) or soil- or litter-dwelling larvae such as **Embioptera** (webspinners, footspinners), **Thysanoptera** (thrips), **Psocoptera** (booklice, barklice, barkflies), **Neuroptera** (lacewings)**, Raphidioptera** (snakeflies) or **Zoraptera** (angel insects) were not yet researched with focus on soil MP.

Regarding insects, mainly studies on translocation and uptake of MP were carried out. In contrast, work on bioaccumulation is completely lacking and adverse effects are sparsely tested using *Tenebrio sp.* larvae. Such studies could provide information whether or not the input of MP in soil ecosystems is one of many factors causing the global decline of the entomofauna (Oliveira et al., 2019; Sánchez-Bayo and Wyckhuys, 2019).

Table 2: Microplastic studies on Coleoptera, Blattodea (Blattod.), Apoidea (A.) and Formicidae (mb=microbeads, fr=fragments, ms=microspheres, b=beads, N/A=information not available). Concentrations refer to mg kg^{-1} dry soil, if not specially marked.

	organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
Coleoptera	*Aphodius erraticus* *Aphodius rufipes* *Aphodius ater* *Aphodius fimetarius* *Aphodius contaminatus* *Aphodius fossor*	Petri dish	latex	N/A	N/A	N/A	mb	5 2..39 2..39 2..39 2..39 2..39	N/A	45 min	N/A	no ≤14 µm ≤14 µm ≤18 µm ≤18 µm ≤18 µm	N/A	N/A	Holter (2000)
	diverse dung beetles	vial	latex	N/A	N/A	N/A	mb	2..83	N/A	45 min	N/A	≤10..≤60 µm	N/A	N/A	Holter et al. (2002)
	diverse dung beetles	N/A	latex	N/A	N/A	N/A	mb	2..83	N/A	45 min	N/A	≤4..≤95 µm	N/A	N/A	Holter and Scholtz (2005)
	Tenebrio molitor larvae	container	PS	N/A	N/A	no	foam	N/A	100% w/w (food)	31 d	N/A	yes	biodegrad.	N/A	Yang et al. (2015)
	Tenebrio molitor larvae	container	LD-PE PS	N/A	N/A	no flame retardant	foam	*8..27 cm³*	50..100% w/w (food)	32 d	N/A	yes	biodegrad.	microbiome	Brandon et al. (2018)
	Tenebrio molitor larvae	container	PS	N/A	N/A	N/A	foam	N/A	4..100% w/w (food)	32 d	N/A	yes	biodegrad.	no	Yang et al. (2018)
	Tenebrio molitor larvae *Tenebrio obscurus* larvae	N/A	PS	N/A	N/A	no	foam	N/A	86..100% w/w (food)	31 d	N/A	yes	biodegrad.	microbiome	Peng et al. (2019)
Blattod.	*Coptotermes formosanus*	mesocosm	LD-PE others	yes/no	N/A	N/A	cable sheets	*4 cm,* *∅ 0.8 cm*	N/A	42 d	N/A	yes no	N/A	N/A	Tsunoda et al. (2010)
	diverse termites	in situ	MD-PE PA	no	N/A	anti-oxidant stabilizer	cable sheets	*30 cm,* *∅ 1.4 cm*	N/A	6 yr.	N/A	yes no	N/A	N/A	Lenz et al. (2012)
A.	*Megachile sp.*	in situ	N/A	N/A	N/A	N/A	fr	N/A	N/A	N/A	yes	N/A	N/A	N/A	Allasino et al. (2019)
Formicidae	*Solenopsis invicta*	Petri dish	latex	N/A	N/A	fluorescence	ms	0.9..4.5	2.5% w/w (food)	direct	N/A	filtration	N/A	N/A	Glancey et al. (1982)
	Rhytidoponera metallica *Aphaenogaster longiceps* *Pheidole sp.*	in situ	N/A	N/A	N/A	N/A	b	N/A	50 items per nest	3 d	yes	N/A	N/A	N/A	Hughes and Westoby (1992)
	Rhytidoponera metallica	mesocosm	N/A	N/A	N/A	N/A	diverse	*<75.5 cm*	N/A	26 mos.	yes	N/A	N/A	N/A	Robins and Robins (2011)
	diverse ants	in situ	N/A	N/A	attractant	N/A	b	*1.8 cm*	N/A	1 d	yes	N/A	N/A	N/A	Angotti et al. (2018)

3.2 Other panarthropods

Apart from the insects, **Acari** (mites) comprise many abundant soil-living taxa, that feed on litter, fungi and fauna as predators and parasites and are bioindicators, as they are sensitive to changes in the soil physiochemical environment (Gulvik, 2007). Experiments indicated, that mites passively transport MP due to pushing and dragging after attachment to their cuticle, as shown with 80 to 250 µm sized PVC particles in a Petri dish experiment without soil (Zhu et al., 2018a). The population within manure pats slightly declines when exposed to mm-sized unweathered PE and PS particles at concentrations of 5 % v/v and declines strongly at ≥60 % v/v (Stamatiadis and Dindal, 1990). This could probably be an effect of moisture deficiency due to a reduced water holding capacity in an unnaturally enriched substrate, but not necessarily through plastic intake. In contrast, no data was found on their arachnoid, preying relatives, the order of **Pseudoscorpiones** (false scorpions).

Just as many other highly abundant and diverse representatives of the soil mesofauna, the **Oniscideae** (woodlice) contribute to the decomposition of litter by chewing and passage through their digestive system (Warburg, 1987) and react strongly to environmental pollution, thus, they are potentially used as bioindicators (van Gestel et al., 2018). They practice a strict selection of natural food sources (Hassall and Rushton, 1984). This is also demonstrated for starch and cellulose based plastic films (4 cm^2), which were consumed and digested in experiments with the model organism *Porcellio scaber*, in contrast to PHB (polyhydroxybutyrate) films, that reduces the feeding rate (Wood and Zimmer, 2014). Smaller PE particles (137±51 µm and 183±93 µm) embedded into food pellets (0.4 % w/w) were taken up easily by *Porcellio scaber*, and the smaller fraction caused a slight and non-significant reduction of body mass after 14 days of exposure, but not of feeding, defecation or energy reserves (Kokalj et al., 2018).

Other panarthropodean groups are even less studied in terms of MP. We did not find literature on the subphylum of Myriapoda containing the classes of **Diplopoda** (millipedes), **Chilopoda** (centipedes), **Pauropoda** and **Symphyla** (pseudocentipedes or symphilids), which are important litter-feeders and predators within various soil ecosystems.

The situation is nearly similar with the phylum of **Tardigrada** (water-bears or tardigrades), that has many ecologically relevant and well studied species feeding on microorganisms and detritus particles. Sparse field research in semisubhydric environments showed no uptake of MP fibers by tardigrada (Gusmão et al., 2016), but comprehensive data on terrestrial soils are lacking. Similarly, the related phylum of **Onychophora** (velvet worms), primordial invertebrates that are mainly native in litter and soils with high water holding capacity under pleistocene-like forest vegetation within tropical and moderate regions (Monge-Nájera, 1994).

The phylum of **Collembola** (springtails), together with the **Diplura** and **Protura** (Westheide and Rieger, 1996; Pass et al., 2011), is an intensively studied morphological group, that exhibits similar ecological functions, such as distribution and decomposition of organic matter as well as the control of fungal abundance (Hopkin, 1997). Springtails provide up to 27 % of the soil biomass and up to 33 % of the total soil respiration (with higher shares in colder ecosystems) (Petersen, 1994) with up to 100000 individuals per square meter (Hopkin, 1997). Thus, their well-being plays an important role for ecosystem functioning.

In a Petri dish experiment without soil, Maaß et al. (2017) showed the passive transport of urea-formaldehyde particles <400 µm and undefined PET fragments by two Collembola species (*Folsomia candida* and *Proisotoma minuta*) due to attachment, but found no ingestion. Within a soil matrix, trials of Kim and An (2019) indicated hindrance of collembolan migration by larger PS particles (44±39, 282±131 and 676±479 µm) at concentrations of 1000 mg kg^{-1} corresponding to highly contaminated soils. In addition, they found suppressed mobility due to the attachment of even smaller PS microbeads (0.47 to 0.53 µm) at concentrations of 8 mg kg^{-1} dry soil, which is equivalent to values found in nature. Small particles <50 µm were moved, while larger particles were most likely cast off. When *F. candida* encounters two of its predators, the mites *Damaeus exspinosus* and *Hypoaspis aculeifer*, the dispersal of 80 to 250 µm PVC particles is enhanced as shown by Zhu et al. (2018a) in a Petri dish experiment. Without proving the ingestion or the minimal effective MP concentration, Zhu et al. (2018b) published an alteration of the gut microbiome and adverse effects on growth and reproduction of *F. candida* by 80 to 250 µm PVC particles mixed in soil at concentrations of 1000 mg kg^{-1} dry soil. These data were not considered robust (van Gestel and Selonen, 2018), but match with a later study that found inhibited reproduction at ≥1000 mg kg^{-1} and avoidance behavior as well as microbiome alteration at ≥5000 mg kg^{-1} (Ju et al., 2019). Such concentrations can occur in highly contaminated soils (Fuller and Gautam, 2016). However, documentations on the active uptake, gnawing and grinding of MP by springtails proposed by Rillig (2012) is still lacking and also studies on Diplura and Protura were not found.

Table 3: Microplastic studies on Acari, Oniscidea (Onisc.), Tardigrada (T.) and Collembola (fr=fragments, p=particles, mf=microfibers, mb=microbeads, ms=microspheres, [s]=semisubhydric, N/A=information not available). Concentrations refer to mg kg^{-1} dry soil, if not specially marked.

	organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
Acari	diverse mites	microcosm	PE PS	no	N/A	N/A	fr	<4800 >2000	0..90% v/v (manure)	16 d	N/A	N/A	N/A	≥5% v/v: abundance ↓	Stamatiadis and Dindal (1990)
Acari	*Hypoaspis aculeifer* *Damaeus exspinosus*	Petri dish	PVC	N/A	no	N/A	p	80..250	5000 items per dish	N/A	yes	N/A	N/A	N/A	Zhu et al. (2018a)
Onisc.	*Porcellio scaber*	mesocosm	PHB	no	N/A	N/A	fr	*4 cm²*	1 item per cosm	14 d	N/A	yes	N/A	feeding ↓	Wood and Zimmer (2014)
Onisc.	*Porcellio scaber*	Petri dish	PE	N/A	N/A	N/A	fr	183±93 137±51	0.4% w/w (food)	14 d	N/A	yes	N/A	no	Kokalj et al. (2018)
T.	diverse tardigrades [s]	in situ	N/A	N/A	N/A	N/A	mf	N/A	N/A	N/A	N/A	no	N/A	N/A	Gusmão et al. (2016)
Collembola	*Folsomia candida* *Proisotoma minuta*	cup	UF, PET	N/A	no	N/A	p,fr	<400	2.5..5 mg per cup	N/A	yes	N/A	N/A	N/A	Maaß et al. (2017)
Collembola	*Folsomia candida*	Petri dish	PVC	N/A	no	N/A	p	80..250	5000 items per dish	N/A	yes	N/A	N/A	N/A	Zhu et al. (2018a)
Collembola	*Folsomia candida*	microcosm	PVC	N/A	no	N/A	p	80..250	1000	56 d	N/A	N/A	N/A	microbiome, growth ↓, reproduction ↓	Zhu et al. (2018b)
Collembola	*Folsomia candida*	microcosm	PE	N/A	no	N/A	mb	<500	0..10000 0..10000 0..5000	7 d 28 d 28 d	N/A	N/A	N/A	≥5000: avoidance ≥1000: reproduction ↓ ≥5000: microbiome	Ju et al. (2019)
Collembola	*Lobella sokamensis*	soil sample	PS	N/A	carboxyl	fluorescence	mb	0.5	4..8	≤3 min	yes	N/A	N/A	avoidance, motivity ↓	Kim and An (2019)
			PE	no	N/A	fluorescence	ms	27..32	1000		yes				
			PE	no	N/A	fluorescence	ms	250..300	1000		N/A				
			PS	no	N/A	no	fr	44±39	1000		yes				
			PS	no	N/A	no	fr	282±131	1000		N/A				
			PS	no	N/A	no	fr	676±479	1000		N/A				

3.3 Annelida

Land-based Annelida comprise another large group of invertebrates. The **Lumbricidae** (earthworms) are a well-studied family (Darwin, 1881; Lavelle et al., 2006), represented in high abundance and diversity in many ecosystems all around the world (Phillips et al., 2019). Earthworms are often used as indicators for soil health (Fründ et al., 2011; Pulleman et al., 2012), as they are ecosystem engineers which through their burrowing activity influence various soil physical, chemical and biological processes (Jouquet et al., 2006; Lavelle et al., 2006).

By far the most of the studies on the influence of MP on earthworms are performed with PE and the species *Lumbricus terrestris* or *Eisenia fetida*, but there are also single studies with *Aporrectodea rosea* (Boots et al., 2019) and *Eisenia andrei* (Rodriguez-Seijo et al., 2017) and with the less common species *Metaphire californica* (Wang et al., 2019b). We found one field study of earthworms and MPs (Huerta Lwanga et al., 2017a) among many laboratory experiments with MPs mixed into soil volumes (concentrations ranging up to 20000 mg kg^{-1} dry soil) or applied with litter on top of the soil surface (≤60% w/w). The particles sizes were usually <1 mm in diameter, but some were even up to 2x2 cm^2, and the duration of experiments was generally 14 to 28 days, few lasted up to 60 days.

The uptake of MPs of a broad size range by earthworms was shown in studies based on particles in earthworm casts of *Lumbricus terrestris* (Huerta Lwanga et al., 2016; Cao et al., 2017; Hodson et al., 2017; Rillig et al., 2017; Prendergast-Miller et al., 2019; Yu et al., 2019; Huerta Lwanga et al., 2017a), *Eisenia fetida* (Rodríguez-Seijo et al., 2018; Chen et al., 2020; Wang et al., 2019c), *Eisenia andrei* (Rodriguez-Seijo et al., 2017) and *Metaphire californica* (Wang et al., 2019b). Zhang et al. (2018) showed that relatively large PE particles of 1.5 x1.5 cm^2 are not ingested by *Lumbricus terrestris*, but partial ingestion of such large particles of biodegradable MPs does take place after initial weathering in soil or in compost has occurred. In some laboratory experiments, MPs were found in the gut of dissected earthworms (Huerta Lwanga et al., 2016; Hodson et al., 2017; Rodriguez-Seijo et al., 2017), but the concentration of MPs in the gut was not significantly different between treatments nor significantly different from the bulk soil concentration, so there was no evidence of accumulation of MPs in the earthworm bodies (Hodson et al., 2017). Chen et al. (2020) assume an accumulation of MP takes place in *Eisenia fetida*, based on an observed increase of MP concentrations in the casts in the course of 4 weeks. Huerta Lwanga et al. (2017a) supposed an accumulation of MPs in the food chain as the concentration of MPs in chicken gizzards is strongly increased compared to that in the earthworm casts in the same experiments. However, mainly the amount of large particles, i.e. macroplastics, in the gizzards was very large, thus it seems likely that the chicken directly fed on plastics and an accumulation through the food chain cannot be proven with the current knowledge and should be further investigated.

Several studies did not find significant negative effects of MPs on earthworms' avoidance behaviour (Judy et al., 2019), nor on growth (Hodson et al., 2017; Rodriguez-Seijo et al., 2017; Judy et al., 2019; Wang et al., 2019c), mortality Hodson et al. (2017); Rillig et al. (2017); Rodriguez-Seijo et al. (2017); Judy et al. (2019); Prendergast-Miller et al. (2019) or reproduction (Huerta Lwanga et al., 2016; Rodriguez-Seijo et al., 2017). However, other studies do show adverse effects of the uptake of MP in different degrees and on different aspects of earthworms' fitness: A reduced growth was shown by Cao et al. (2017) for *Eisenia Fetida* and the mortality increased at an exposure of concentrations ≥10000 mg kg^{-1} dry soil. At lower concentrations no significant effects were found. The growth of *Aporrectodea rosea* was also inhibited when exposed to biodegradable polylactic acid, conventional high-density polyethylene (at 1000 mg kg^{-1} dry soil), and MP clothing fibers (at 10 mg kg^{-1} dry soil) (Boots et al., 2019). Huerta Lwanga et al. (2016) showed a decrease in growth and increased mortality at concentrations ≥28% w/w in litter and after 60 days, though after just 14 days no mortality occurred in these experiments.

In some studies, additional effects such as histopathological changes or stress biomarkers were measured. For *Eisenia fetida* Chen et al. (2020) observed skin damage at 1500 mg MP kg^{-1} in soil, measured an increase in catalase activity and malondialdehyde content at 1000 mg kg^{-1} and at ≥1000 mg kg^{-1} acetylcholine esterase was significantly stimulated. Wang et al. (2019c) tested *Eisenia fetida* and found that MPs only increased the catalase and peroxidase levels as well as the level of lipid peroxidation and decreased the activity of superoxide dismutase and glutathione S-transferase at an exposure of 200000 mg kg^{-1} dry soil for 14 days. No discernible influence was found at 100000 mg kg^{-1}. However, Rodríguez-Seijo et al. (2018) also found for *Eisenia fetida* a significant positive correlation of MP concentration with different biomarker responses: catalase, glutathione S-transferase, lactate dehydrogenase and thiobarbituric acid reactive substances. In addition, Rodriguez-Seijo et al. (2017) observed histological damage of the gut and occurrence of inflammatory processes as well as an increase of stress response indicators associated with MP exposure of *Eisenia andrei*. For *Lumbricus terrestris* Prendergast-Miller et al. (2019) showed an increase in metallothionein expression at an exposure with ≥1000 mg kg^{-1} dry soil and a decrease in heat shock protein 70 at a concentration of ≥10000 mg kg^{-1}.

Due to the large differences in experimental conditions – e.g. size of the MPs, addition of MPs to soil or to litter, duration of experiments, earthworm species – the current knowledge is not sufficient to detect whether there is a threshold in MP size and concentration at which the MP become harmful for earthworms and how this threshold differs for different earthworms species and MP shapes. The results of Huerta Lwanga et al. (2016), who found no effects of MPs on earthworms at 14 days, but significant influence on growth and mortality after 60 days, indicate the importance of longer measurements. This is consistent with Pelosi et al.

(2015), who concluded that the influence of pesticides on earthworm communities should be tested in long term field experiments.

Earthworms activity also increased the transport of MP in soil columns to deeper soil layers (Rillig et al., 2017; Yu et al., 2019; Huerta Lwanga et al., 2017b). The smaller the MP the stronger the transport. Particles are transported both actively – ingested and later cast out – and passively after attachment to the earthworm's body or by water flow through the biopores. As Huerta Lwanga et al. (2018) showed that the bacteria in the gut of *Lumbricus terrestris* can decompose MPs, it seems likely that particles taken up at the surface are egested as smaller particles in deeper layers.

Microplastics might well serve as a vector for contaminant transport to soil organisms. Though adsorption on plastics was seen to be lower than on the soil matrix, the desorption of Zn was seen to be higher in synthetic earthworm guts. However, there was no measurable negative effect of Zn or the PE on *Lumbricus terrestris* (Hodson et al., 2017). Wang et al. (2019b) studied the influence of MP on arsenic uptake and negative effects on *Metaphire californica* and concluded that MPs decreased the uptake of arsenic and that MPs reduced the influence of arsenic on the gut bacterial communities. Rodríguez-Seijo et al. (2019) showed altered enzyme activities and enhanced avoidence behavior in face of LD-PE pellets spiked with the insecticide chlorpyriphos. Yang et al. (2019a) studied the influence of MPs on the transport of glyphosate, however they mainly showed that the glyphosate transport was increased by earthworm activity, the role of MPs in this transport could not be determined with this study. These studies show that MP might have very different influences on the uptake and the adverse effects of different pollutants on earthworms and further investigation is needed in order to understand the influence of MPs on pollutant transport.

In contrast to the recently well-researched Lumbricidae, a near relative, the family of **Megascolecidae** (giant earthworms), is not yet mentioned in literature. Another branch within the Annelida, the small **Enchytraeidae** (potworms), were shown to suffer adverse effects on body weight and microbiome with PS microspheres (0.05 to 0.1 µm) at concentrations of ≥10 % w/w within their food source, but an unexpected increase of reproduction at 0.5 % w/w (Zhu et al., 2018b). The reproduction was reduced at abnormal concentrations of 90 g kg^{-1} dry soil of polyamid particles (13 to 150 µm), but not with PVC (Lahive et al., 2019).

The edaphon of semisubhydric soils is often treated as a marginal group between the area of interest of soil and aquatic scientists. As a highly diverse soil biocenosis outside the focus of this paper, the benthos along seashores and fresh waters is also affected by MPs and should therefore be shortly mentioned by reviewing the lugworm **Arenicola marina**, a well examined deposit-feeder of the tidal flats. In situ, MP accumulates within its tissue and feces (Van Cauwenberghe et al., 2015). In laboratory experiments, PS particles ≥500 µm were avoided as food-source and passively translocated within the sediment at concentrations of ~2 g kg^{-1}

(Gebhardt and Forster, 2018), but were measured within the feces at ~74 g kg^{-1} causing effects on feeding activity and body weight with no influence on the survival rate (Besseling et al., 2012). PS microspheres ≤30 µm remained within the animal without any adverse effects regardless of particle size (Van Cauwenberghe et al., 2015). Other studies found adverse effects on respiration, energy reserves, feeding, egestion and casting after uptake of PVC particles ≤478 µm at different sediment concentrations of >2 g kg^{-1}, but neither on biomass and survival nor due to HD-PE (Wright et al., 2013; Green et al., 2016). There is further a difficulty in distinguishing between the adverse effects of MPs and substances adsorbed on or leached from MPs (Besseling et al., 2012). When adding PCB-spiked PE to mud flat sediment with concentrations up to 5000 mg kg^{-1} dry mass, there was no significant change of survival rate or body weight. The decreased feeding activity and heap mass could be attributed to increasing plastic concentrations, but not to enhanced PCB bioaccumulation via PE uptake (Besseling et al., 2017). However, all these studies found adverse effects at MP concentrations orders of magnitude above natural values.

Table 4: Microplastic studies on Lumbricidae (p=particles, ms=microspheres, b=beads, f=fibers, ms=microfibers, N/A=information not available). Concentrations refer to mg kg^{-1} dry soil, if not specially marked.

Lumbricidae

organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
Lumbricus terrestris	mesocosm	PE	washed (C_5H_{12}, C_8H_{18})	N/A	N/A	p	<150	0..60% w/w (litter)	14 d / 60 d	yes	yes	N/A	at 60 d, ≥28% w/w: survival ↓, growth ↓	Huerta Lwanga et al. (2016)
Eisenia fetida	glass beaker	PS	N/A	N/A	N/A	ms	50..80	0..20000	30 d	N/A	yes	N/A	≥5000: survival ↓ ≥10000: weight ↓	Cao et al. (2017)
Lumbricus terrestris	bag	PE	N/A	N/A	N/A	p	*0.92±1.09 mm²*	3500	28 d	N/A	yes	no	no	Hodson et al. (2017)
Lumbricus terrestris	home yard	diverse	yes	N/A	N/A	N/A	N/A	0.87±1.9 items g^{-1}	N/A	N/A	yes	conc. in chickens > in earthworms	N/A	Huerta Lwanga et al. (2017a)
Lumbricus terrestris	mesocosm	PE	washed (C_5H_{12}, C_8H_{18})	N/A	N/A	p	<150	0..60% w/w (litter)	14 d	yes	yes	N/A	N/A	Huerta Lwanga et al. (2017b)
Lumbricus terrestris	mesocosm	PE	N/A	no	no	b	710..2800	750 µg on 2.5 kg soil	21 d	yes	yes	N/A	no	Rillig et al. (2017)
Eisenia andrei	mesocosm	LD-PE	N/A	N/A	N/A	pellets	250..1000	0..1000	28 d	N/A	yes	N/A	≥62.5: intestinal damage	Rodriguez-Seijo et al (2017)
Lumbricus terrestris (gut bacteria)	mesocosm glass bottle	PE	washed (C_5H_{12}, C_8H_{18})	N/A	N/A	p	150	7% w/w (litter) 10000	60 d (earthworms) 21 d (bacteria)	N/A	yes	N/A	N/A	Huerta Lwanga et al. (2018)
Eisenia fetida	mesocosm	LD-PE	washed (EtOH)	N/A	N/A	pellets	250..1000	0..1000	28 d	N/A	yes	N/A	≥125: altered enzyme activity	Rodriguez-Seijo et al (2018)
Aporrectodea rosea	mesocosm	PLA, PE N/A	N/A	N/A	N/A	p f	N/A	1000 10	30 d	N/A	yes	N/A	growth ↓	Boots et al. (2019)
Eisenia fetida	mesocosm	HD-PE, PET, PVC	no	N/A	no	f	<2000	soil extract	48 h / 56 d	N/A	N/A	N/A	no	Judy et al. (2019)
Lumbricus terrestris	bag	PE	N/A	N/A	N/A	mf	∅40.7±3.8 x 361.6±387.0	0..10000	35 d	N/A	yes	N/A	≥1000: metallothionein expression ↑ ≥10000: heat shock protein 70 ↓	Prendergast-Miller et al. (2019)
Eisenia fetida	mesocosm	LD-PE	washed (EtOH)	N/A	chlorpyrifos (CPF)	pellets	5000 250..1000	40 items on 0.5 kg soil 180..200 items on 0.5 kg soil	14 d	N/A	N/A	N/A	with CPF: altered enzyme activity, avoidance of MPs	Rodriguez-Seijo et al (2019)
Metaphire californica	mesocosm	PVC	N/A	N/A	sodium arsenate	p	N/A	2000	28 d	yes	yes	N/A	microbiome	Wang et al. (2019b)
Eisenia fetida	glass beaker	PE PS	washed (MetOH)	N/A	PAHs, PCBs, Nile Red (NR)	p	<300 <250	0..200000 0..100	14 d 28 d	N/A	yes	N/A	≥200000: altered enzyme activity	Wang et al. (2019c)
Lumbricus terrestris	mesocosm	PE	washed (C_5H_{12}, C_8H_{18})	N/A	glyphosate	p	<150	0..7% w/w (litter)	14 d	N/A	N/A	N/A	N/A	Yang et al. (2019a)
Lumbricus terrestris	mesocosm	PE	N/A	N/A	N/A	N/A	<1000	7% w/w (litter)	14 d	yes	yes	N/A	N/A	Yu et al. (2019)
Lumbricus terrestris	Petri dish mesocosm	PE and div. biode-gradables	unweathered, field or compost	N/A	N/A	p	*1.5x1.5 cm²* *2x2 cm²*	4 items per dish 10 items per dish	14 d 50 d	yes	no yes	N/A	N/A	Zhang et al. (2018)
Eisenia fetida	bag	PE	washed (EtOH)	N/A	N/A	p	<400	0..1500	28 d	N/A	yes	yes	skin damage, ≥250 mg/kg: oxidative stress ≥1000 mg/kg: neurotoxicity ↑	Chen et al. (2020)

Table 5: Microplastic studies on Enchytraeidae and *Arenicola marina* (mb=microbeads, p=particles, ms=microspheres, sed.=sediment, [s]=semisubhydric, N/A=information not available). Concentrations refer to mg kg^{-1} dry soil in terrestrialsoils and mg kg^{-1} dry sediment in semisubhydric soils, if not specially marked.

	organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
Enchyt.	*Enchytraeus crypticus*	Petri dish	PS	N/A	N/A	N/A	mb	0.05..0.1	0..10% w/w (food)	7	N/A	yes	N/A	at 0.5% w/w: reproduction ↑ ≥10% w/w: microbiome, weight ↓	Zhu et al. (2018c)
Enchyt.	*Enchytraeus crypticus*	microcosm	PA PVC	N/A	N/A	fluorescence N/A	p	13..150 106..150	20000..120000 90000	20 h / 21 d	N/A	yes	N/A	≥90000: reproduction ↓ no	Lahive et al. (2018)
Arenicola	*Arenicola marina* [s]	in situ	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	yes	1.2±2.8 items g^{-1}	N/A	Cauwenberghe et al. (2015)
Arenicola		liquid culture	PS	no	N/A	N/A	ms	10..90	10000..50000 items kg^{-1}	14 d	N/A	yes	10 µm: 9600±1800 items kg^{-1} 30 µm: 800±700 items kg^{-1}	no	
Arenicola	*Arenicola marina* [s]	mesocosm	PS PA	yes	biofilm	N/A	p	500..1000	~2000 ~1000	106..240 d	yes	no	N/A	N/A	Gebhardt and Forster (2018)
Arenicola	*Arenicola marina* [s]	mesocosm	PS	N/A	N/A	N/A	p	400..1300	0..74000	28 d	N/A	≥400 µm	no	≥74000: feeding ↓, weight ↓	Besseling et al., (2012)
Arenicola	*Arenicola marina* [s]	mesocosm	PVC HD-PE	N/A	N/A	N/A	p	9..478 3..316	0..20000 mg kg^{-1} wet sed.	31 d	N/A	N/A	N/A	>2000: respiration ↓, casting ↓ no	Green et al. (2016)
Arenicola	*Arenicola marina* [s]	mesocosm	PE	N/A	PCBs	fluorescence	mb	10..180	0..5000	28 d	N/A	yes	no	feeding activity ↓, heap mass ↓	Besseling et al. (2017)
Arenicola	*Arenicola marina* [s]	mesocosm	PVC	N/A	N/A	not leaching	p	~130	0..50000	28 d	N/A	N/A	N/A	≥10000: energy reserves ↓ ≥50000: feeding ↓, egestion ↓, casting ↓	Wright et al. (2013)

3.4 Further invertebrates

As part of the microfauna, the phylum **Nematoda** (nematodes or roundworms) is an ecologically important branch containing >25000 species (Zhang, 2013) in freshwater, marine, endobiotic and soil habitats. Due to their diverse trophic interactions nematodes hold a central position in both bottom-up and top-down controlled food webs (Yeates, 2001; Ferris, 2010) and thus most likely the uptake and transfer of MP.

Active feeding of adults and larvae of different species on 0.5 to 6 µm PS/latex microspheres (the size of their bacterial prey) was proven by Nika et al. (2016) and Fueser et al. (2019). However, most MP experiments on Nematodes are based on the bacterial-feeding model organism *Caenorhabditis elegans*. Kiyama et al. (2012) showed the favored uptake of PS microspheres with sizes of 0.5 to 3 µm by adult and 0.5 µm by larval *C. elegans*. The ingestion of MP decreased in the presence of bacteria as the natural food source.

When larval stages and adults ingested PS between 0.05 and 5 µm within an aqueous suspension or on agar plates, adverse effects such as oxidative stress, neurodegeneration, intestinal and DNA damage or dysfunction in motility, growth, life span, defecation, reproduction or energy metabolism appeared from a wide spectrum of concentrations from ≥1 µg l^{-1} up to ≥86.3 mg l^{-1} (Zhao et al., 2017; Dong et al., 2018; Kim et al., 2019; Lei et al., 2018a; Lei et al., 2018b; Qu et al., 2019a). These effects are not seen below 1 µg l^{-1} (Qu et al., 2019b), and are enhanced due to amino modifications on micropshere surfaces (Qu et al., 2019c). The incubation on agar plates with PE, PP and PVC particles <70 µm caused similar influences on survival, fertility, brood size and intestinal function (Lei et al., 2018b). Leachates from soils amended with 5 mg kg^{-1} dry soil of HD-PE and PVC decreased reproduction in laboratory cultures, but there was no effect shown on survival and after application of PET (Judy et al., 2019). Furthermore, silica nanoparticles (0.05 µm) are not only taken up orally but also via the vulva and spermathecae and migrate into gonad cells (Scharf et al., 2013), This process was confirmed for PS nanoparticles with the potential of a transfer to the progenity (Zhao et al., 2017).

The clear adverse effects of these studies are limited in their representativity by a narrow restriction to liquid cultures and a single model organism. Broader studies like on prominent soil-born nematodes such as *Acrobeloides buetschlii* (Frey, 1971) are still lacking. When assuming in first proximity mg l^{-1} solution = mg kg^{-1} dry soil, the applied concentrations between 0.001 and 86.8 mg l^{-1} match lower levels of soil contamination.

Feeding studies on the phylum **Rotifera** with MPs are fully based on PS microbeads and model organisms of the planktonic genus *Brachionus*. However, this data can carefully be transferred to soil environments as also soil rotifers are aquatic organisms living in water-filled pores and waterfilms. Different *Brachionus sp.* ingest microbeads <10 µm with strong preference for particles the size of their natural food source, namely bacteria and algae with

2 to 5 µm in diameter (Vadstein et al., 1993; Heerkloß and Hlawa, 1995; Baer et al., 2008; Jeong et al., 2016). The uptake appears to be selective as microbeads are fewer incorporated than bacteria and algae (Vadstein et al., 1993). The egestion of particles ≤0.5 µm is hindered compared to 6 µm (Jeong et al., 2016). In suspension, microbeads ≤0.5 µm cause adverse effects on fertility and life span at ≥0.1 mg l^{-1} as well as oxidative stress and less growth at ≥10 mg l^{-1} (Jeong et al., 2016; Sun et al., 2019).

Terrestrial mollusks comprise snails and slugs within the class of **Gastropoda**. These grazers feed on bacterial biofilms, fungi and plant tissue (Parkyn and Newell, 2013). Studies on terrestrial species are sparse, but data on the benthic *Littorina sp.* imply passive transport and non-selective MP uptake by feeding on surfaces with contaminated feces and mucus trails of other snails (Gutow et al., 2019). With focus on benthic snails, Imhof and Laforsch (2016) found no significant influence on growth parameters and fertility of juveniles and adult *Potampoyrgus antipodarum* even when a food source with 70 % w/w of 5 to 600 µm sized fragments was given (a mixture of PA, PC, PET, PS, PVC). In contrast, adverse effects were found in recent work on the terrestrial snail *Achatina fulica*, that showed uptake and complete gastrointestinal passage within 48 h with partial degradation of PET fibers (appr. 1258x76 µm), but reduced excretion and food intake as well as increased oxidative stress at concentrations of ≥0.01 g kg^{-1}, ≥0.14 g kg^{-1} and ≥0.71 g kg^{-1} dry soil, respectively (Song et al., 2019).

Table 6: Microplastic studies on nematods (ms=microspheres, fr=fragments, np=nanoparticles, mb=microbeads, ms=microspheres, ox.=oxidative, N/A=information not available). Concentrations refer to mg kg^{-1} dry soil, if not specially marked.

Microplastic studies on nematods (ms=microspheres, fr=fragments, np=nanoparticles, mb=microbeads, ms=microspheres, ox.=oxidative). Concentrations refer to mg kg^{-1} dry soil, if not specially marked.

	organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
Nematoda	*Caenorhabditis elegans*	agar plate	PS	N/A	carboxyl sulfate amino	fluorescence	ms	0.1..6.6	N/A	0.5..2 h	N/A	yes	0.5..3 µm	N/A	Kiyama et al. (2012)
	Caenorhabditis elegans	liquid culture	PS	N/A	carboxyl	fluorescence	ms	0.1	0.001..10 mg l^{-1}	4.5 d	N/A	Yes	N/A	≥0.01 mg l^{-1}: motivity ↓, growth ↓, defecation ↓, within gonads	Zhao et al. (2017)
	Caenorhabditis elegans	liquid culture	PS	N/A	ζ=-10mV	fluorescence	ms	0.1	0.00001..0.001 mg l^{-1}	N/A	N/A	Yes	N/A	≥0.001 mg l^{-1}: motivity ↓, ox. stress ↑	Dong et al. (2018)
	Caenorhabditis elegans	liquid culture	PS	N/A	N/A	preservatives, fluorescence	ms	0.05..0.2	0.001..86.8 mg l^{-1} 17.3..86.8 mg l^{-1}	24 h	N/A	Yes	N/A	≥17.3 mg l^{-1}l: motivity ↓, reproduction ↓ ≥86.3 mg l^{-1}: ox. stress ↑ ≥17.3 mg l^{-1}: metabolic dysf.	Kim et al. (2019)
	Caenorhabditis elegans	liquid culture	PS	N/A	ζ=-10mV	fluorescence	ms	0.1	0.001..1 mg l^{-1}	N/A	N/A	Yes	N/A	≥1 mg l^{-1}: neurodegeneration ≥0.01 mg l^{-1}l: motivity ↓	Qu et al. (2019a)
	Caenorhabditis elegans	liquid culture	PS	N/A	N/A	N/A	ms	0.1..5	1 mg l^{-1}	3 d	N/A	Yes	N/A	motivity ↓, survival ↓, growth ↓, ox. stress ↑, neurotoxicity	Lei et al. (2018a)
	Caenorhabditis elegans	agar plate	PE, PP, PVC, PS	no	N/A	N/A	fr, ms	0.1..200	0.5..10.0 mg m^{-2}	2 d	N/A	Yes	N/A	≥0.5 mg m^{-2}: survival ↓ at 5 mg m^{-2}: growth ↓, reproduction ↓, ox. stress ↑, intestinal damage	Lei et al. (2018b)
			PS	N/A		fluorescence	ms	0.1..5						mainly 1µm: intestinal damage	
	Caenorhabditis elegans	agar plate	silica gel	N/A	N/A	N/A	np	0.05	2500 mg l^{-1}	7 d	N/A	Yes	N/A	within tissue and gonades	Scharf et al. (2013)
	Caenorhabditis elegans	liquid culture	HD-PE, PET, PVC	no	N/A	no	fr	<2000	soil extract	72 h	N/A	N/A	N/A	reproduction ↓	Judy et al. (2019)
	Caenorhabditis elegans	agar plates	latex	N/A	N/A	fluorescence	mb	0.5	N/A	30 min	N/A	yes	N/A	N/A	Nika et al. (2016)
	Caenorhabditis elegans *Panagrolaimus thienemanni* *Plectus acuminatus* *Poikilolaimus regenfussi* *Acrobeloides nanus* *Pristionchus pacificus* *Aphelenchoides parietinus*	liquid culture	PS	N/A	N/A	fluorescence	ms	0.5..6	$3 \cdot 10^{9}..10^{10}$ items l^{-1} *(~0.2..1200 mg l^{-1})*	4..73 h	N/A	≤3µm ≤0.5µm ≤1µm ≤1µm ≤1µm ≤6µm no	N/A	N/A	Fueser et al. (2019)
	Caenorhabditis elegans	liquid culture	PS	N/A	N/A	N/A	ms	0.1	0.0001..0.001 mg l^{-1}	N/A	N/A	N/A	N/A	no	Qu et al. (2019b)
	Caenorhabditis elegans	liquid culture	PS	N/A	no amino	N/A	ms	0.1	0.001..1 mg l^{-1}	N/A	N/A	yes	N/A	≥0.01 mg l^{-1}: reproduction ↓, DNA damage ≥0.001 mg l^{-1}: reproduction ↓, DNA damage	Qu et al. (2019c)

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Table 7: Microplastic studies on Rotifera and Gastropoda (ms=microspheres, mb=microbeads, fr=fragments, f=fibers, ox.=oxidative, pref.=preferential, [p]=planctic , [b]=benthic, N/A=information not available). Concentrations refer to mg kg^{-1} dry soil, if not specially marked.

	organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
Rotifera	*Brachionus plicatilis* [p]	liquid culture	PS	N/A	carboxyl	fluorescence	ms	1.6..20	$5 \cdot 10^9$ µm³ l^{-1} *(~5.25 mg l^{-1})*	35 min	N/A	≤10 µm	pref. 4.5 µm	N/A	Bear et al. (2008)
Rotifera	*Brachionus plicatilis* [p]	liquid culture	latex	N/A	N/A	fluorescence	mb	0.3..3.1	$3 \cdot 10^7..7 \cdot 10^8$ items l^{-1} *(~0.0004..11 mg l^{-1})*	20 min	N/A	yes	pref. ≥2 µm	N/A	Vadstein et al. (1993)
Rotifera	*Brachionus koreanus* [p]	liquid culture	PS	no	N/A	fluorescence	mb	0.05..6	0...20 mg l^{-1}	1 d	N/A	yes	egestion rate 0.05 µm < 0.5 µm < 6 µm	≤0.5 µm, ≥0.1 mg l^{-1}: reproduction ↓, survival ↓ ≤0.5 µm, 10 mg l^{-1}: oxidative stress ↑	Jeong et al. (2016)
Rotifera	*Brachionus plicatilis* [p]	liquid culture	PS	N/A	N/A	N/A	mb	0.07..7	0..20 mg l^{-1}	N/A	N/A	yes	N/A	≤0.07 µm, ≥10 mg l^{-1}: reproduction ↓, growth ↓ ≤0.07 µm and ≥0.1 mg l^{-1}: survival ↓	Sun et al. (2019)
Rotifera	*Brachionus quadridentatus* [p] *Brachionus plicatilis* [p]	liquid culture	PS	N/A	N/A	N/A	ms	2..10	N/A	8..10 d	N/A	pref. 3..5 µm pref. 2 µm	N/A	N/A	Heerkloß and Hlawa (1993)
Gastropoda	*Littorina littorea* [b]	microcosm	PMMA	N/A	N/A	fluorescence	fr	10..100	increasing	16 h	N/A	yes	N/A	N/A	Gutow et al. (2019)
Gastropoda	*Potampoyrgus antipodarum* [b]	aquarium	PET, PS, PVC, PA, PC	N/A	N/A	no	fr	5..600	0..70% w/w (food)	≤141 d	N/A	yes	N/A	no	Imhof and Laforsch (2016)
Gastropoda	*Achatina fulica*	mesocosm	PET	N/A	N/A	no / stained	f	approx. 1258x76 µm	10..710	28 d	N/A	yes	excretion after 48 hours	≥140: food intake ↓ ≥10: excretion ↓ ≥710: ox. stress ↑, gastrointestinal damage	Song et al. (2019)

3.5 Vertebrates

Different taxa of the class of Amphibia have a predator function within the edaphic food web (e.g. preying on invertebrates) (Hebrard et al., 1992). While no data on the reaction to soil MPs are available neither for the legless **Gymnophiona** nor for adults of the order **Anura**, sparse data on tadpoles of aquatic frogs suggest uptake followed by regular excretion of PS microspheres as shown with *Xenopus tropicalis* (Hu et al., 2016). Further, there exist no data on the families **Serpentes** (snakes) and **Anguidae** within the class of Reptilia, residing at the outer rim of the food web.

Within the broad field of Mammalia, studies on MP ingestion are sparse and focus on **mice** as a rodent model organism. Feeding of mice with PS microspheres of 1 to 14 µm in concentrations of 1.49×10^6 to 4.55×10^7 particles at a volume of 10 ml kg^{-1} body weight for 4 weeks showed no adverse effects (Stock et al., 2019). In contrast, longer exposition (6 weeks) with lower concentrations of particles with the same shape and size range changed the mouse microbiome and caused metabolic and intestinal dysfunction (Lu et al., 2018; Jin et al., 2019), which comes along with bioaccumulation within organs (Yang et al., 2019b). These studies are regularly conducted with passive feeding and exclude active foraging on perceptible plastic particles. However, the uptake via prey or feeding on contaminated roots and litter is highly probable. Further Rodentia – **Cricetidae** (hamsters, lemmings, voles)**,** **Bathyergidae** (blesmols, mole-rats), **Octodontidae** as well as **Spermophilus** (ground squirrels) and **Marmota** (marmots) within the family of **Sciuridae** – were not yet studied, just as other mammalian (sub)orders like **Chrysochloridae** (golden moles), **Cingulata** (armadillos), **Macroscelidea** (elephant shrews), **Notoryctemorphia** and **Peramelemorphia**.

Table 8: Microplastic studies on Anura (An.) and Rodentia (ms=microspheres, [a]=aquatic, N/A=information not available).

	organism	experimental environment	plastic type	aging	coating	additives	shape	size span [µm]	concentrations	exposure time	passive transport	active uptake	bioaccum. dynamics	measured adverse effects	reference
An.	*Xenopus tropicalis* [a]	Petri dish	PS	N/A	N/A	fluorescence	ms	1..10	$100..10^8$ items l^{-1} *($55{\cdot}10^{-9}..55$ mg l^{-1})*	48 h	N/A	yes	egestion within days	N/A	Hu et al. (2016)
Rodentia					carboxyl			1	$4.55{\cdot}10^7$ items per mouse *(0.025 mg per mouse)*						
	transgenic mice	in vivo	PS	N/A	sulfate	fluorescence	ms	4	$4.55{\cdot}10^7$ items per mouse *(1.6 mg per mouse)*	28 d	N/A	yes	N/A	no	Stock et al. (2019)
					sulfate			10	$1.49{\cdot}10^6$ items per mouse *(0.8 mg per mouse)*						
	mice	in vivo	PS	N/A	N/A	fluorescence	ms	5	0.1..1 mg l^{-1} (food)	42 d	N/A	yes	N/A	≥0.1 mg l^{-1}: microbiome, metabolic dysfunction	Jin et al. (2019)
	mice	in vivo	PS	N/A	N/A	N/A	ms	0.5..50	0.1..1 mg l^{-1} (food)	35 d	N/A	N/A	N/A	≥0.1 mg l^{-1}: microbiome, metabolic dysfunction ≥1 mg l^{-1}: body weight ↓	Lu et al. (2018)
	Mus musculus	in vivo	PS	N/A	N/A	fluorescence	ms	5..20	200 mg l^{-1} (food)	28 d	N/A	yes	8x, 8±5 and 0.71±0.14 mg kg^{-1} body weight	N/A	Yang et al. (2019b)

4 Synthesis

4.1 Summarized observations

Our systematic search comprised recent research on the interaction of soil organisms with MP, but also studies with focus on feeding experiments, that are published much earlier than the awareness on plastic in the environment appeared. The numerous studies found with focus on the ingestion of MPs consistently showed the active uptake by diverse soil organisms with few exceptions spread over the whole branch of invertebrates. In addition, also studies on adverse effects caused by the intake of MP contaminated food (e.g. of food pallets by dung beetles) imply the ingestion into the test organism. Distinct size preferences are observed in dung beetles, nematodes, rotifers and ants showing that mainly particles are ingested, that are small enough to enter the gastrointestinal tract. In contrast, active comminution by gnawing on larger particles was tested only for a few taxa and confirmed for woodlice, termites and mealworms, and in the case of earthworms only after initial weathering.

After the ingestion, MP is translocated actively until excretion or death of the transporting organism, which was only directly shown in experiments with earthworms. The passive transport by attachment, dragging and pushing was investigated in a few experiments with earthworms, mites and springtails that partly worked without soil substrate and consistently showed positive results.

After exposition to MP, a pattern of adverse effects can be seen: Across various taxa, altered microbiomes, reduced motility, body mass, fertility and life span as well as increased oxidative stress and metabolic malfunctioning occur in different combinations mainly due to µm-sized MP in and above the whole known natural range of concentrations. For some taxa such as Nematodes, Gastropoda and Rotifera these effects appear at natural and increased MP concentrations (<100 mg kg^{-1} dry soil), for Collembola and Lumbricidae at concentrations like in highly contaminated sites (≥1000 mg kg^{-1} dry soil) and for Enchytraeidae, *Arenicola marina* and in further experiments with earthworms at implausibly high values. The data show a tendency, that the effects occur at lower concentrations, when the added particles are smaller. Small sized particles also provide the highest surface/volume ratio and thus the highest reactive surface per weight.

Most studies work with defined increasing MP concentrations and particle sizes in soil substrates and food sources, which can be used to determine relationships between environmental concentrations and adverse effects. However, the lack of information about intake rates, grades of accumulation and effective prey-predator transfer leads to a gap within the chain of explanation for toxic effects on the soil organisms. In some experiments, the intestinal passage of MP and sizes preferably retained within the gut were shown, but there are no experiments that could demonstrate quantitative bioaccumulation. In contrast, quantification of the retained and egested MP particle size fractions might be biased due to gnawing and intestinal comminution as shown for woodlice, termites, mealworms, snails and earthworms.

In order to improve our understanding of processes underlying adverse effects of MP on soil organisms, data on ingestion rates, dwell times, biodegradation and egestion rates are important bricks e.g. to reveal bioaccumulation dynamics. However, there are only a few data on biodegradation (mealworms, snails, earthworms), egestion (rotifers, frogs, snails, earthworms) and remaining concentrations in the body (lugworm, mice, earthworms).

4.2 Limitations of previous studies

The available studies worked with items within the full size span of micro- and nanoplastics (≤5000 µm). Approximately 72 % of the experiments used microplastic (0.1 to 5000 µm), only 6 % nanoplastic (<0.1 µm), 10 % included macroplastic (>5000 µm) and 12 % used microplastic of undefined size. When MP ≥50 µm was applied, mainly particles and fragments made of PE and PVC were used, whereas PS/latex microspheres were mainly applied for sizes ≤10 µm (*Table 1*). The latter are readily available, highly standardized and are mostly used with fluorescent dyes and either without additional functional groups, carboxylated or, more rarely, with amino or sulfate groups. However, there are indications that the spectrum of particle type and shape used in experiments does not correspond to the properties of particles in soils. In different natural as well as agriculturally and industrially contaminated terrestrial and semisubhydric sites, fibers and fragments of PE and PP, mostly ≤100 µm, were much more abundant than PVC, PET and PS items (Claessens et al., 2011; Vianello et al., 2013; Nor and Obbard, 2014; Naji et al., 2017; Zhang and Liu, 2018; Li et al., 2018a). This is probably caused by high loads of MP fibers in discharged waste water and sewage sludge, which is used in agricultural sites worldwide (Mahon et al., 2016; Li et al., 2018b). It is likely that shape plays an important role for the ingestion of MP items. Unfortunately, we did not find studies that have carried out a complete classification of sampling sites according to plastic origin, size and type, that could help to evaluate differences between former experimental and natural plastic composition to achieve the most realistic experimental conditions. Little knowledge about the size distribution of MP in soils furthermore complicates the determination of realistic concentrations for the addition of a certain particle size spectrum. All reviewed studies either arbitrarily set their applied concentrations or had to base them on measurements of total specific MP masses, regardless of how much of this mass is in the tested size range. This may lead to a false estimation of total adverse MP concentrations.

In contrast to particle type and shape, the documentation of chemical properties of MP samples in most of these studies is fragmentary. Some experiments explicitly mentioned that the added plastic was unweathered, whereas most studies lack information about the degree of aging implying that unweathered items were used. Only a few experiments involved aging of MP, but without comparison to results of natural weathering (Tsunoda et al., 2010; Gebhardt and Forster, 2018). That is in conflict with natural conditions, as plastic that remains within the soil after littering, sewage sludge application or plastic mulching shows signs of weathering, e.g. modified carbonyl indices (Andrady, 2017), while unweathered soil MP might be rare. In addition, Zhang et al. (2018) showed that earthworms actively comminute only weathered bioplastics. In experiments using PS microspheres, carboxylation is often used to imitate a reduced hydrophobicity due to weathering. However, according to manufacturer information microplastics only have little influence on hydrophobicity.

Weathering of MP surfaces within soils comes along with biofilm growth and adsorption of organic molecules, which could potentially affect the attractiveness or toxicity for grazers and other organisms. Such coatings were applied only in a few cases (Besseling et al., 2017; Angotti et al., 2018; Gebhardt and Forster, 2018), but were not documented in most studies. Similarly, the type and concentration of additives such as flame retardants, anti-oxidants or stabilizers often remained undocumented, with exception of fluorescent dyes, that are well mentioned. The release of additives can have a harmful effect on the test organism, as shown for aquatic environments (e Silva et al., 2016). Some studies on the ingestion of MP by the soil mesofauna indicate that the diameter of the gastrointestinal tract is a useful upper size limit for added particles, as far as the organism is unable to crush them (Heerkloß and Hlawa, 1995; Holter, 2000; Holter et al., 2002; Holter and Scholtz, 2005; Baer et al., 2008; Fueser et al., 2019). However, using only ingestible particle sizes in their natural concentrations neglect the adverse effects of plastic leachates, which can also get into the soil solution and onto the mineral phase from larger particles and affect soil life.

The conditions of incubation differ considerably in terms of habitats and duration of exposure. In most studies, the exposure ranges from a few minutes to a few days in experiments with micro- and small mesofauna and hours to several weeks in experiments with large meso- and macrofauna and is mainly based on excretion or reproductive cycles. Long-term studies, which are indeed difficult to carry out in mesocosms, practically do not exist. However, certain adverse effects might only establish themselves after long term trials, as was shown for the influence of pesticides (Pelosi et al., 2015).

Some experiments were carried out in soil-free test environments such as liquid cultures or Petri dishes with nutrient solutions or a specific food source (nematods, rotifers, mice). Therefore, motivity is less restricted and feeding behavior can be altered compared to cultivation within soil environments. For example, the ingestion of MP by nematodes decreases in the presence of an alternative and more natural food source like bacteria, which can significantly reduce the bioaccumulation and thus the effective toxicity (Kiyama et al., 2012). This can lead to less consumption of MP in soil environments and an overestimation of the toxicity in liquid culture experiments. Also, all laboratory feeding experiments were carried out by use of only one species. The complexity of the food web in soils is thereby excluded and the potential accumulation from prey to predators still unexplored.

4.3 Pinpoints for future research

Most studies reviewed in this work have a pioneering role in MP research and, thus, are subject to some experimental limitations caused by an early state of knowledge. The adverse effects recently found are alarming, but must be considered under the restrictions named above. We propose the following points as part of a *modus operandi* for future MP research.

In past studies, particular adverse effects of MP were measured only for certain sizes, shapes, coatings, leachates or adsorbed substances (*Tables 2 to 8*). Experimental concentrations were assumed randomly or derived from cumulative concentrations of one or more MP types measured in natural soils (approx. 1 to some 1000 mg kg^{-1} dry soil), regardless of size. For those specific experiments coming, the spectrum of concentrations used should be adapted to the quantities of the size spectrum, that occurs within the soil. For future studies on mixed contaminations, we recommend to evaluate the overall adverse effects of PE, PP, PVC, PET, PU and PS to certain test organisms by use of typical MP-specific spans of concentration, size and shape distribution in natural soils or food samples. This previously requires well-structured data of appropriate MP type, shape and size for different soils in differently contaminated areas.

Experiments on adverse effects should be applied within soil matrices to allow the interplay of plastic, natural organic and mineral matter. The MP should be weathered, as plastic in soils underlie broad environmental aging. Pre-weathering of MP should therefor not only be performed in climate chambers (e.g. following DIN EN ISO 4892-2/3), but also include subsequent leaching and equilibration of additives or coatings within the soil matrix before the main experiment. Furthermore, the experimental design may consider coatings with biofilms or attractants and even particle color to regulate the preference of the test organisms.

Most detailed information about ingestion are available for dung beetles, nematods and earthworms, data on adverse effects on nematods, earthworms, lugworms and collembola. Future experiments should focus on a larger variety of ecologically relevant taxa like Coleoptera, Formicidae, Acari, Oniscidea, Collembola, Lumbricidae, Enchytraeidae, Nematoda and Gastropoda. The studies are recommended to conduct with emphasis on uptake, accumulation and key adverse effects like on survival rate, motility, growth and fertility as well as on the stability of the intestinal microbiome. Further studies with more than one test organism are important to foster our understanding of MP within certain food chains. Also long-term experiments might reveal adverse effects, which evolve slowly within populations. This may enable the assessment of the distribution and effects of MP within the food web and the resulting long-term impact on soil ecosystems.

5 Conclusion

Our review of 77 studies on the impact of microplastic on the soil fauna shows a considerable diversity and distribution of adverse effects within the soil tree of life. However, these effects have to be considered carefully, as many experiments did not use plastic matching properties within natural soils and found adverse effects only at concentrations like in highly contaminated soils or above. To elucidate effective concentrations and properties for short and long-term effects on soil faunal health, the most exact reproduction of plastic properties within the soil matrix and natural living conditions of the test organisms is necessary together with a better knowledge on common concentrations and size distributions of soil microplastic. For future experiments we therefore recommend to choose compositions of type, shape, size, concentration, grade of weathering, leachability and coating with biofilms and other organic matter as expected in the habitat to be examined. Furthermore, coming studies should include long-term exposure and food chain experiments to get a better look at the effect of even smaller MP concentrations and their enrichment within the food web. This may give us a better way of assessing the impact of global microplastic contamination on e.g. soil biodiversity, soil carbon cycles and soil quality.

Author contribution

Frederick Büks developed the review concept, collected data and prepared the manuscript except for earthworms. Nicolette Loes van Schaik did all the work on earthworms. Martin Kaupenjohann supervised the study by participating in structural discussions on the idea and concept of the paper as well as the final corrections.

The authors declare that they have no conflict of interest.

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Competing interests

The authors declare that they have no conflict of interest.

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