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Review

Plastic pollution in terrestrial ecosystems: Current knowledge on impacts of micro and nano fragments on invertebrates

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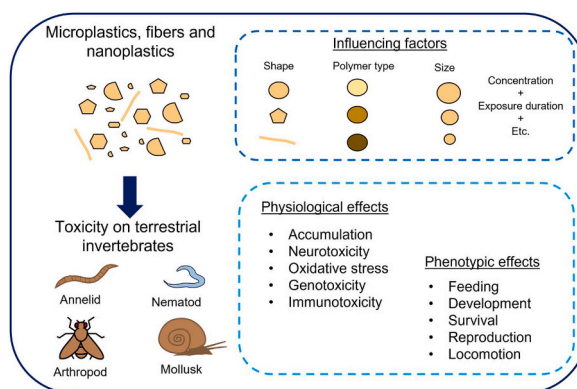
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HIGHLIGHTS

- Micro- and nanoplastics (MNP) induce several responses on terrestrial invertebrates.
- Phenotypic and physiological disorders were observed after MNP exposure.
- Studies on nanoplastics used mostly polystyrene beads.
- A standardize protocol should be used to characterize MNP and their toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing accumulation of small plastic particles, in particular microplastics (>1 μm to 5 mm) and nanoplastics (< 1 μm), in the environment is a hot topic in our rapidly changing world. Recently, studies were initiated to better understand the behavior of micro- and nanoplastics (MNP) within complex matrices like soil, as well as their characterization, incorporation and potential toxicity to terrestrial biota. However, there remains significant knowledge gaps in our understanding of the wide-extent impacts of MNP on terrestrial invertebrates.

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We first summarized facts on global plastic pollution and the generation of MNP. Then, we focused on compiling the existing literature examining the consequences of MNP exposure in terrestrial invertebrates. The diversity of investigated biological endpoints (from molecular to individual levels) were compiled to get a better comprehension of the effects of MNP according to different factors such as the shape, the polymer type, the organism, the concentration and the exposure duration. The sublethal effects of MNP are acknowledged in the literature, yet no general conclusion was drawn as their impacts are highly dependent on their characteristic and experimental design. Finally, the synthesis highlighted some research gaps and remediation strategies, as well as a protocol to standardize ecotoxicological studies.

1. Plastic-production and environmental pollution

1.1. Plastic manufacturing: facts and figures

Industrialization, demographic growth, and globalisation have increased the diversity and amount of environmental contaminants [1-3]. Therefore, concerns about pollution - defined as the presence or introduction of agent in the environment, their extent and impacts on the health of organisms and biodiversity in general - have increased over the years [4-9]. Biodiversity is now threatened by multiple sources of anthropogenic pollution, including light [10] and noise pollution [11], chemical pollution of air, water and soil. For this reason, alarming consequences of humankind resource intensive development on biodiversity health repeatedly alert scientists, the public, and authorities [12-16]. Yet, despite increasing numbers of investigations, research efforts, and regulation tools (e.g. Registration, Evaluation, Authorisation and Restriction of Chemicals, REACH; EU regulation 2006), the growing diversity of anthropogenic emissions of environmental contaminants makes it difficult to assess the potential toxicity of all of them [17].

Plastic-made products, and plastics from personal care products and cosmetics industry, have rapidly emerged as important sources of environmental contamination and their degradation into microplastics seriously impacts biodiversity [18,19]. Due to their increasing use and their persistence, the prevalence of plastic pollution is growing relentlessly and represents a hallmark of the Anthropocene era [20,21]. Since plastics were discovered in the 1900s, plastic production has increased over the years, in particular since the second part of the 1950s [22]. In 2022, the global plastic production was estimated at 400.3 Mt (million metric tons) [23], and it is predicted that production could be doubled by 2050 [24,25].

Plastics are produced by associating several monomers (ethylene, propylene, styrene) reacting with a catalyst to form polymers with the

desired physico-chemical features (hydrophobic behaviour, flexibility, resistance to chemicals, temperature or light / UV, and low weight). Chemical additives (e.g. plasticisers, flame retardants, metals) are added to plastics as softeners, colorants, etc. [26]. The physical and chemical properties of plastics, and the low production cost [27], have made this material an indispensable element of our every day's life [24]. Plastics are highly used by the food industry (Figure A.1, Supplementary materials) which packages many products with polyethylene (PE) (low-density polyethylene, LDPE, and high-density polyethylene, HDPE) and polypropylene (PP). These two polymers alone represent 40 to 45 % of the global plastic production [22,28]. The rest of the production comprises seven other polymers: polyvinyl chloride (PVC, 12 % of the plastic production), polyethylene terephthalate (PET, 6 %), polystyrene (PS, 5 %), polyurethane (PUR, 5 %), other thermoplastics (3 %) and polycarbonate (PC) and polyamide (PA) for 1 % of the plastic production [28,29](Fig. 1).

1.2. Low-recycling rates and waste mismanagement of plastic-made products

The massive plastic production combined with low-recycling rates and waste mismanagement inevitably result in millions of tons of plastic being released into the environment. Worldwide, Ryberg et al. [28] reported that 6.2 Mt of macroplastics (>5 mm) were disseminated without regulation in the environment in the year 2015. This estimation is in line with Jambeck et al. [30] who suggested that 4.8 to 12.7 Mt of land-based plastic sources entered annually in the oceans, additionally highlighting that more than 80 % of marine plastic pollution has a terrestrial origin [31]. The global amount of plastic waste produced in the period 1950–2015 was estimated at 6300 Mt, with only 9 % of this total amount being recycled or reprocessed into a secondary material, and 12 % destroyed thermally by combustion or pyrolysis (extraction of

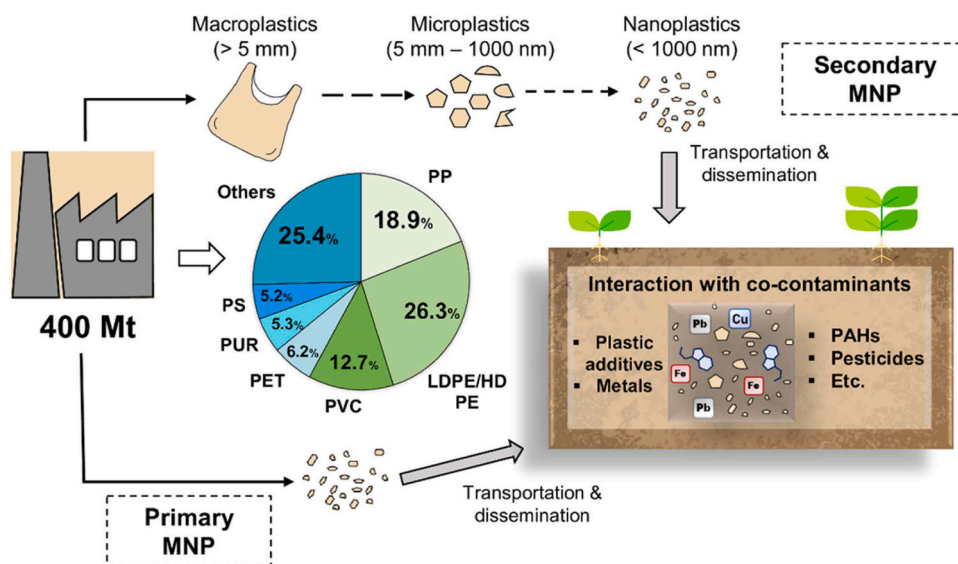


Fig. 1. The fate of plastics in the environment. Data were obtained from Plastics Europe [23]. HDPE: High density polyethylene, LDPE: Low density polyethylene, PAHs: Polycyclic aromatic hydrocarbon, PET: Polyethylene terephthalate, PP: polypropylene, PS: Polystyrene, PUR: Polyurethane, PVC: Polyvinyl chloride.

fuel from plastics waste) [24]. The remaining 79 % of plastic waste was discarded in managed systems (sanitary landfills), or left uncontained in open dumps, or in natural environments [24,32]. The low proportion of recycled plastics and their long degradation time greatly increase the likelihood of their disposal in landfills, ultimately leading to contamination of adjacent habitats and biospheres [33], impairing organisms' health and ecosystems' functioning [34,35]. If the legislation about plastic production, use, and management remains unchanged, environmental plastic pollution will rise continuously in the next decades [27]. In a business-as-usual scenario, plastic pollution transfer to the environment will double by 2040 [32], mirroring the expected plastic production at the horizon 2050 [25]. Plastic quantities may even increase by 2.8 and 2.6-fold in terrestrial and aquatic environments, respectively [32], further increasing toxicity hazards for biodiversity.

1.3. Aims and objectives of the review

To date, the necessary data on the effects of plastic debris on terrestrial invertebrates remains insufficient in terms of taxonomic and geographic coverage [35]. To get a better view of the existing information on the topic, we evaluate the effects of micro- and nanoplastics (MNP) and identify knowledge gaps by compiling and synthesising the available literature. By providing a broad, overarching perspective that spans developmental, behavioural and trophic ecology, life traits, physiology in an integrative way across multiple invertebrate taxa with particular emphasis on terrestrial arthropods, this review amalgamates information on the wide range of MNP-induced effects on biological functions and organism fitness, and the underlying physiological and molecular disorders. First, we briefly present the types and sources of plastics which are accumulating in the environment. Second, we examine the underlying physiological and molecular impacts of MNP. Third, we explore the consequences of MNP on feeding and reproduction activities, and mobility in terrestrial invertebrates, anticipating impacts on behavioural patterns, vital processes and health of these organisms. In every section, scientific observations are compared regarding plastic size (microplastics (MP), nanoplastics (NP) and fibers), polymer type and conditions of exposure, with regard to the characteristics of the studied organism. This approach enables a deeper understanding of plastic hazards by facilitating more effective comparisons, thus highlighting similarities and differences of the impacts of MNP for organisms. To structure the sections, examples are grouped by taxa (whenever possible), starting with below-ground invertebrates and ending with above-ground invertebrates. The compiled information offers an overview of our current knowledge of plastic impacts on the physiological reactions and biological processes in terrestrial arthropods, and identifies knowledge gaps. In the final section, we offer recommendations for forthcoming efforts, emphasising crucial aspects that require immediate attention. Inspired by the studies of Jemec Kokalj et al. [36,37], we advocate for the crucial need of having a standardized set of criteria to harmonize protocols across studies, enabling meaningful comparisons.

2. The journey of plastic-made products in the environment: types, sources and fate of plastics

Two categories of micro (MP, 1000 nm - 5 mm [38]) and nanoplastics (NP, 1–1000 nm [38,39]) can be found in the environment: primary and secondary MP and NP. Primary MNP are synthesised for industrial purposes, and used for industrial air blasting, cosmetics or pharmaceutical products [40,41]. They are generally monodispersed, round and made from the same polymer. Secondary MNP are particles resulting from the fragmentation and degradation of macroplastics (> 5 mm) [42]; they can be oxidised, hydrolysed, fragmented and biodegraded in the environment [43,44] (Fig. 1). Secondary MP include fibers deriving from the degradation of synthetic textiles and having diameters and lengths ranging from 6 to 175 µm and 250 µm to 5 mm, respectively [45,46]. Once in the environment, the surface of plastic

debris is weakened by photo-oxidation with ultraviolet (UV) radiations before plastic debris are fragmented into smaller particles by the action of physical forces, including movements of water, wind, pressure or mechanical work of the soil, and leading to the surface erosion of the debris [47,48]. Biological processes (e.g. bio-fragmentation) by microorganisms or animals may also lead to the partial degradation and fragmentation of plastic debris. Bacteria, such as *Brevibacillus* sp. or *Pseudomonas* sp., and fungi have long been documented and characterised as plastic biodegraders by forming a biofilm on their surface [43, 49–51]. Then, the fragmented small-sized plastic debris can be ingested by aquatic and terrestrial organisms, and, by a combination of friction through digestive gut movement and enzymes, debris are further fragmented into smaller particles [52,53,48]. All these processes contribute to formation of MNP being highly polydisperse in shape, size, and characteristics originating from different polymers.

There are several reasons contributing to environmental MNP pollution, including deficiencies in their collection and processing, or final discarding of plastic waste [28,30]. Domestic products in the form of shopping bags, food wrappers, bottles and packaging represent a significant part of plastic wastes which may be improperly treated and pollute the environment [24]. In addition, agricultural mulch film, sewage sludge and certain organic soil conditioners are contributing directly to the release of MNP in terrestrial environments [54]. For instance, a study conducted in China estimated that 10–30 % of the total amount of MP found in soil came from mulch films liable for specific soil pollution by PE and PVC materials [55]. Sewage sludges, which are residues of wastewater treatment, are also commonly added to agricultural soils to provide plant nutrients. This procedure significantly contributes to contaminating soil with a large variety of MNP [56]: Nizzetto et al. [57] estimated that 63,000–430,000 tons and 44, 000–300,000 tons of microplastics could be present in European and North American farmlands, respectively, through the application of urban sewage sludge. Worst, the quantity of MNP originating from wastewater sludges might be underestimated due to limitations in identification methods, which would drastically increase the actual measured quantity of particles introduced in the environment [58]. Finally, the atmosphere serves as an important source of MNP transport and dissemination [54,59,60]. Dris et al. [61] reported that 29–280 MP items/m²/day in Paris result from atmospheric deposition. Altogether, these sources of transfer of plastic debris to the environment progressively enrich soils with MNP, in turn increasing the likelihood of their ingestion or inhalation through atmospheric exchanges by terrestrial organisms [62].

Substantial quantities of microplastics have already been measured from soil samples [63] all around the world: in China, 40 mg/kg of MP with a size between 0.9 and 2 mm were found in agricultural soils continuously covered by mulch films over several years [64]. In Germany, 0.34 microplastics particles per kg of dry soil were quantified in agricultural soils [65], and amounts up to 67,500 mg/kg of MP were reported from soils near an industrial area in Australia [66].

The detection and quantification of MP in complex matrices such as soil or living organisms was recently improved [67]. In particular, scanning or transmission electron microscopy can be used to visualize MNP and characterize their shape in the studied matrices [68]. Quantification of MP, assessment of chemical and polymer nature can be achieved by vibrational spectroscopy (e.g. Fourier-transformed infrared spectroscopy (FTIR), Raman spectroscopy, hyperspectral imaging), or by Pyrolysis gas chromatography-mass spectrometry (Py-GCMS) [54, 59]. While these techniques are reliable for the identification of man-made MP, we are still missing suitable techniques for characterisation of NP in environmental matrices. Dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) can be performed for assessing NP size and particle concentration [69]. Moreover, Py-GCMS was recently developed to detect NP and determine polymer natures in complex matrices [69,70]. Despite these advancements, there is still no reliable and harmonized procedure to quantify NP from environmental

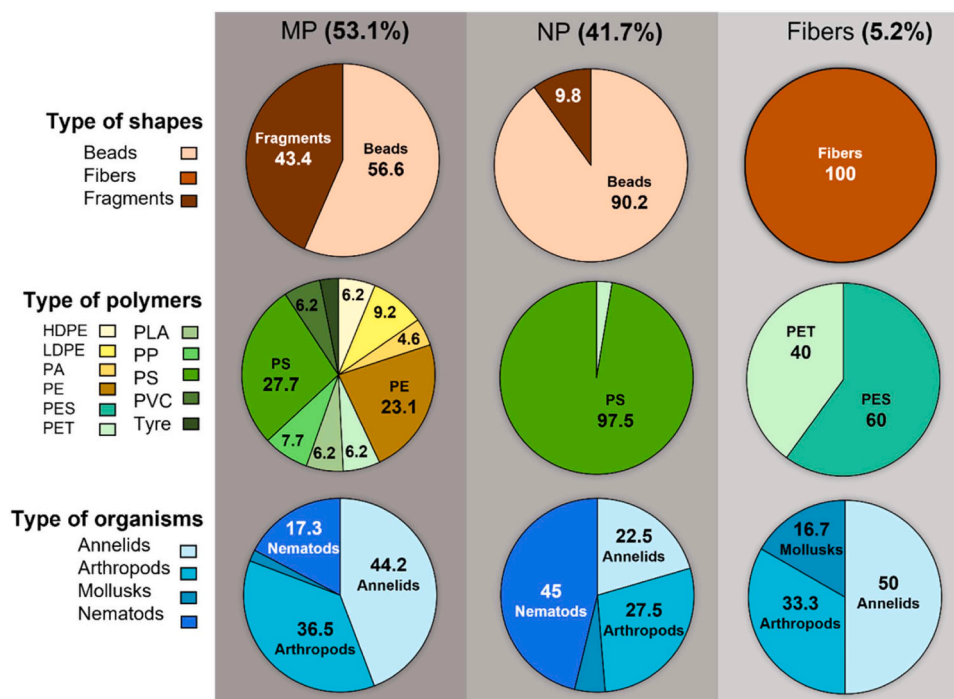


Fig. 2. Visual representation of the information gathered from the literature search. Investigations on the effects of plastic debris on terrestrial invertebrates represented 53.1 %, 41.7 % and 5.2 % for microplastics, nanoplastics, and fibers, respectively. The first line of pie charts shows the proportion of studies using plastic fragments, beads or fibers. The second and third lines of pie charts summarize the type of polymers and the type of organisms considered in the studies for each category of plastic debris (MP, NP and fibers). HDPE: High density polyethylene; LDPE: Low density polyethylene; PA: Polyamide; PE: Polyethylene; PES: Polyester; PET: Polyethylene terephthalate; PLA: Polylactic acid; PP: polypropylene; PS: Polystyrene; PVC: Polyvinyl chloride.

samples. Once transferred in the environment, MNP are easily transported by wind, atmospheric streams, water run-off, or animals [71], allowing them to be dispersed to other compartments such as oceans, groundwaters or deep soil layers [72]. In the soil, MNP act as active substances interfering with the physicochemical and hydrological properties of the ecosystems, including soil aggregation forming, pH, water retention and availability of nutrients, thus affecting biodiversity [35].

3. Plastic chemical additives increase toxicity hazards for biodiversity

The toxicity of MNP can be increased by plastic additives [73], including flame retardants, biocides, metals, or the plasticizers, such as Bisphenol A (BPA) and Di(2-ethylhexyl) phthalate (DEHP), which have non-covalent bonds that facilitate their subsequent release into the environment or directly into the organisms [74]. BPA and DEHP are widely recognised for their endocrine disrupting effects [75-77], and for their carcinogenic effects [78]. Additionally, plastic debris can aggregate other contaminants (co-contaminants), such as organic pollutants (pesticides and pharmaceuticals), (heavy)metals or metalloids. Indeed, MNP have a large specific surface area, a small size, are merely lipophilic and frequently negatively charged [79]. These features increase their capacity to adsorb and carry pollutants, in particular pesticides such as flubendiamide and diflufenzuron [80,81], as well as metals, such as Pb [82]. Thus, MNP contribute to increase the bioavailability and transfer of pollutants in soils and in many terrestrial organisms [79]. The affinity between MNP and contaminants increases with decreasing particle size as smaller particles have a larger specific surface area which enhances their reactivity with both organic and inorganic pollutants [83]. Toxicity of highly hazardous compounds, such as atrazine or cadmium, increased in mixture with MNP [84,85]. Such pollutant mixtures greatly reduce survival, feeding, immunity and antioxidant capacity of invertebrate species like annelids [86,87].

Knowing that most plastic waste ends up in terrestrial environments - and in the oceans for a significant fraction of the debris - we need information of their effects on terrestrial species, and their transfer within the trophic chain including humans [88]. Actually, the research efforts have mainly focused on plastic pollution in the oceans [89,90]. For instance, the meta-analysis of [91] on polystyrene MNP reported 110 articles on aquatic invertebrates, and almost three times less (40) in terrestrial invertebrates. Yet, as ubiquitous components of the terrestrial environments, MP and NP, their additives and potential co-contaminants, may represent a non-negligible part of terrestrial invertebrates' diet [92,93]. A large number of invertebrates ingest plastic debris when feeding, as MNP have been identified in half of the studied macroinvertebrate species [94].

4. Compilation of the literature and visualisation of the current knowledge

To compile the existing information on the effects of MNP on terrestrial invertebrates, we employed the following keywords for conducting the literature search: "microplastics", "nanoplastics", "micro (nano)plastics", "terrestrial", "insect", "invertebrate", "arthropod", "organisms", "effects", "impacts", "ecotoxicology", "phenotypic", "physiology", "metabolism", "reactive oxygen species", "oxidative stress", "hormone", "excretion", "digestion", "reproduction", "fecundity", "lifespan", "development", which allowed compiling review and research articles, book chapters and Ph.D. dissertations from several databases, including SCOPUS, Web of Science and Google Scholar. As the existing literature on above-ground insects remains limited to few taxa, we have incorporated knowledge on below-ground invertebrates (nematodes, earthworms, collembolans, enchytraeids, isopods) so that we could provide a more comprehensive overview of the results obtained so far, and the grand challenges and research avenues that should be primarily explored.

The available information was extracted according to the type (MP,

NP or fibers), shape (beads, fragments or fibers), polymer, particle size, concentration, studied organism, and exposure duration. Table A.1 compiles this information and the associated major findings. Fig. 2 synthesises the distribution of particle characteristics (shape and polymer) and the studied taxa according to plastic types (MP, NP and fibers). Some disparities and research gaps were further emphasized in Fig. 2, evidencing a scarcity of information for the effects of fibers (only 5.2 % of the available literature on the topic). For NP-based investigations, knowledge disparities were also striking, as a significant proportion of research articles focused on the study of the effects of polystyrene beads on nematodes (Fig. 2, Table A.1 (Supplementary material)).

The literature search additionally allowed the identification of the major endpoints which are currently used in investigations assessing the toxicity of MNP on terrestrial invertebrates. Endpoints assessing physiological effects included accumulation (and tissue presence/damage), microbiota, oxidative stress, genotoxicity (DNA damages and gene expression), immunotoxicity and neurotoxicity. Those assessing biological effects of MNP included measurements of feeding (and food intake and excretion), development (and body mass and morphometry), survival (and mortality), reproduction, locomotion (and behaviour). Then, for each article, the following scores were used: (a) 0 when the endpoint was not studied, (b) 1 when it was explored but no effect was observed, (c) 2 when the endpoint was explored and was significantly affected by MNP. In some investigations, different plastic types were studied (for example, MP, NP or fibers) and were treated as independent entities in our exploration of the literature. When several exposure conditions were explored in the study, as for instance examination of the effects of exposure to several polymer types, sizes or concentrations, these conditions were considered dependent, and thus counted as a single article for the endpoints (See Supplementary materials Table B). This scoring code allowed us to visually represent the existing information on the effects of MNP on terrestrial invertebrates by representing for each major endpoint the percentage of articles where an effect (or absence of effect) was observed.

5. Plastic particles ingestion, internalization and diffusion in terrestrial invertebrates

Once in the environment, some of the plastic fragments may be ingested unintentionally by the terrestrial fauna. Plastic consumption may have wide-reaching consequences for the organisms, from abrasion of intestinal tissue to diffusion through the intestinal wall [95,96].

5.1. The routes of MNP entry in terrestrial invertebrates

Ingestion and inhalation are the two main routes for MNP entry in the body of terrestrial invertebrates. The presence of plastic particles in atmospheric air has been reported in several recent studies [97-99] making inhalation a prominent source for respiratory contamination in animals. Inhaled MNP are recognized to be harmful for human health [100], and adverse effects were also reported in mice [101,102]. Surprisingly, contamination by inhalation, and subsequent toxicity from air-breathing to terrestrial invertebrates has remained understudied.

So far, ingestion represents the main studied mechanism of plastic uptake by terrestrial invertebrates, and the journey of MNP through different digestive compartments (digestive tube and intestine) has been studied by fluorescence or confocal microscopy [103-105]. These techniques revealed the presence of PS MP (1.3 μm) in the intestine of *Eisenia fetida* at concentrations of 0.10 $\mu\text{g}/\text{mg}$ [106]. Uptake and elimination of PS MNP from soil in *E. fetida* was size-dependent: the smallest particles (70 nm) were resorbed in the head region, bigger debris (1 μm) were excreted, and the biggest ones (10 μm) remained in the gut lumen [107]. Moreover, quantities of PS NP ranged from 2.09 to 3.8 ng/larva in the gut of MP-fed *Tenebrio molitor*, declined to 0.11 - 0.63 ng in glands, but were not detected in the excrements [108]. In the terrestrial snail *Achatina fulica*, the transfer of PET fibers was detected from the crop to

the stomach [105], and similar findings were made in the giant snail *Achatina reticulata* [104]. Finally, polystyrene MP of 2 μm were also found in different gut portions of the fruit fly *Drosophila melanogaster* [109] (Table A.1), and PE MP were found in gut portions of the black soldier fly (*Hermetia illucens*) larvae [110].

In many instances, terrestrial invertebrates ingest MNP when feeding, as illustrated in the previous examples, especially for soil-dwelling invertebrates which ingest contaminated litter by plastics [111]. Many plastic fragments contain pigments, and the size and colors of MP can make them resemble potential prey. As a result, invertebrates may also confuse MP with prey, thus increasing the likelihood of plastic ingestion by predatory insects. To our knowledge, there is no study investigating this hypothesis for terrestrial species, but it was observed for aquatic invertebrates which fed more on MP than natural prey in plastic-contaminated environments [112]. Carnivorous species may be further exposed to MNP when ingesting plastic-contaminated preys, as observed for spiders eating plastic-contaminated mosquitoes [113]. These ingestions could hint on a possible accumulation and transfer within food webs. Such transfer was also alerted in a 'backyard' study, where uptake of MNP by earthworms and transfer to chicken was evidenced [114].

Finally, ingestion of MNP by invertebrate can occur when consuming on plants and vegetables. MNP uptake by terrestrial plants has recently gained attention with the discovery of accumulation and translocation of plastic particles in different plants structures [115-117], placing herbivorous and omnivorous invertebrates at exposure risk when feeding on contaminated plants and fruits. Under controlled conditions, polystyrene NP transfer from plants to snails was evidenced, with plastic debris having ecotoxicological effects in both organisms [118,119].

5.2. Cellular and tissular impacts, diffusion of MNP in the body

First, plastic particles may damage organism through the abrasion of invertebrates' skin epidermis. This injury, caused by mechanical friction of MNP against the epidermis, and was reported in earthworms whose epithelium was exfoliated in presence of MP. Microscopic observations of earthworm skin further revealed the existence of epidermis lacerations, tissue loss and abrasion at high LDPE MP concentrations (1.5 g/kg dry soil), while no physical damages were noted at lower concentrations (0.1 and 1.0 g/kg dry soil) [120]. Interestingly, the physical injuries were only mentioned for microplastics. It is likely that the smallest-sized plastic debris (NP) caused less surface damage, while the likelihood of their internalization into the animal's body should be way higher [93].

After ingestion, the intestinal epithelium represents the first physical barrier that may limit the diffusion of MNP within the individual's body. In earthworms, the size of intestinal cells and their nuclei were altered and became irregular in individuals that consumed polystyrene MP [106]. The histopathological damages were dose-dependent, with higher changes at higher MP concentrations [106,121]; alterations were also size-dependent with largest beads (1300 nm) inducing more damages in comparison to smaller ones (100 nm) [106]. With larger PS or PE MP, investigations evidenced detachment of gut epithelium, inflammation, fibrosis and congestion [106,121]. Plastic particles have the potential to alter the epithelium barrier: terrestrial snails exposed to high concentrations (0.14 and 0.71 g/kg dry soil) of MP suffered from stomach and intestinal damages, in particular shortening or breakage of intestinal villi [105].

The intestinal epithelium is not impermeable to MNP intrusion. Polystyrene MP and NP may even increase the permeability of intestinal epithelial tissues, as demonstrated in *Caenorhabditis elegans* [122,123]. The internalization of MNP through intestinal cells has been evidenced in a range of terrestrial invertebrates such as earthworms, spiders, flies and silk moths [124,113,109,125,126]. Tang et al. [126] observed the adsorption of polystyrene NP (90-110 nm) in the intestinal cells and in the chloragogenous tissue of *E. fetida*. Similarly, polystyrene and PET NP were internalised by *D. melanogaster* into enterocytes, after they were

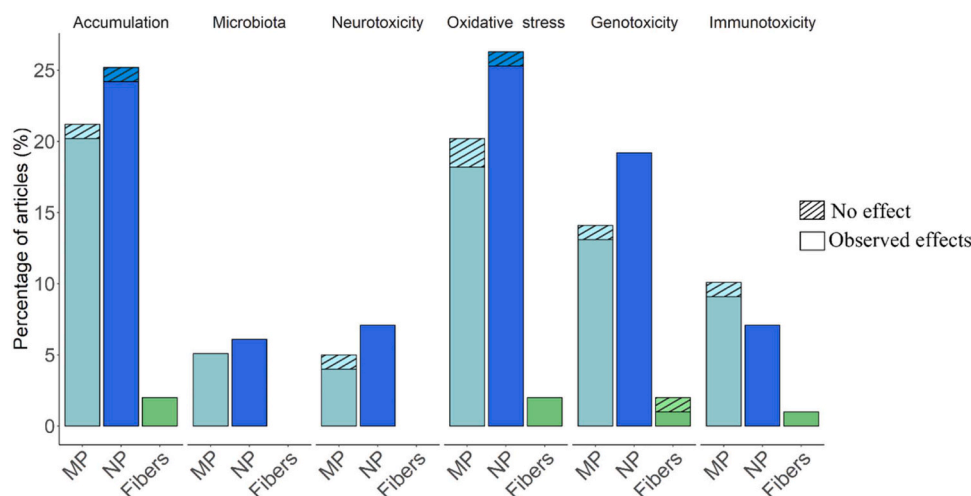


Fig. 3. Physiological effects of fibers, micro- and nanoplastics reported in the available literature. Bioaccumulation, microbiota, neurotoxicity, oxidative stress, genotoxicity, or immunotoxicity incurred by microplastics, nanoplastics and fibers exposures to terrestrial invertebrates were evaluated as physiological endpoints.

transported by extracellular membranes. NP were also observed in cytoplasmic cells (near mitochondria) and in the haemolymph of *D. melanogaster* [103,124]. Importantly, most research focused on NP as their smaller size facilitates their translocation among body compartments [127]; however, microplastics of 2 μm were also detected in flies' bodies [109].

Plastic fragments can subsequently accumulate in different tissues and organs: MNP were detected in Malpighian tubules of flies and moths, potentially impacting animal's excretion [109,125]. Sexual organs and gametogenesis processes were also affected. Polystyrene NP were observed in flies' ovaries exposed to 50 and 100 mg/L PS NP, increasing apoptosis and cell deaths in the oocytes of the F5 generation [128]. Similarly, polyethylene microplastics (1000 mg/kg dry soil) impaired spermatogenesis in *Eisenia andrei*, especially the arrangement of sperm bundles in the seminal vesicles [129]. More precisely, Yu et al. [130] observed apoptotic germ cells in *C. elegans*, and even more surprisingly, germline apoptosis was also detected in offspring from F1-F4 generations after a single maternal exposure.

6. Plastic-induced physiological disorders in terrestrial invertebrates

MNP diffusion among body compartments, tissues and cells may lead to physiological damages and disorders. The major physiological endpoints measured for examining the presence (accumulation), effects - or the absence of effects - of MNP in terrestrial invertebrates are presented in Fig. 3.

6.1. Gut microbiota

The gut microbiota has essential roles in energy metabolism, development and immunity [131-133]. As diet quality can affect gut microbiota composition and functionality, ingestion of MNP-contaminated food and its passage through the digestive tract can influence the microbiome [134]. Microbial community diversity and richness can be approximated with the Chao and the Shannon index, respectively [135]. Both indices were decreased in *E. fetida* exposed to PE and PLA MP [136]. Similar conclusions were drawn by Tang et al. [126] after PS NP ingestion. At the microbial phylum level, the abundance of Proteobacteria was decreased while Firmicutes were increased in comparison to the control group [126]. Similar decreases in microbial community diversity and richness were also measured in *E. crypticus* exposed to PS NP [137], in *F. candida* exposed to PE MP [138,139] and in *A. mellifera* exposed to PS MP [140]. These studies indicate that MNP ingestion led

to a shift in microbiome structure, resulting in changes in predominant phylum [141,138,126,140,136,137]. In sum, MNP ingestion can cause dysbiosis, potentially leading to physiological disorders and impacts at individual scales.

6.2. Neurotoxicity

MNP were reported to provoke neurodegeneration damages when invertebrates are exposed to plastic contamination. For instance, transgenic GFP *C. elegans* strains exposed to polystyrene MP (1 μm) revealed a downregulation of gene expression, involved in cholinergic synaptic transmission, resulting in damage in cholinergic and GABAergic neurons [142].

Neurotoxicity effects can also be assessed through measurements of the activity of acetylcholinesterase (AChE), the enzyme responsible for the cleavage of the neurotransmitter acetylcholine from its postsynaptic receptor in invertebrates and vertebrates. PE and PLA microplastics (100–200 μm) caused AChE stimulation at high concentrations in earthworms [136]. LDPE exposure, however, either inhibited or stimulated AChE activity in earthworms depending on the concentration and time of exposure [120]. These results clearly highlighted neurotoxicity through MNP ingestion and may partially explain the changes in locomotion behaviour at individual scale. Importantly, neurotoxicity induced by MNP is mainly studied on aquatic organisms. Thus, more research is needed to explain the neurodegeneration of terrestrial invertebrates to clarify the underlying mechanisms.

6.3. Oxidative stress

Under normal conditions, production and degradation of ROS are balanced. Exposure to MNP, as with almost all xenobiotics, can strongly alter the redox balance, thus leading to oxidative stress, loss of redox homeostasis and eliciting antioxidant defences [143,144]. The effects of MNP on the oxidative system were frequently reported in the literature, with almost half of the articles studying the effects of MP, NP or fibers on terrestrial invertebrates focusing on antioxidant responses (Fig. 3).

ROS imbalance measured from terrestrial invertebrates which ingested MNP may originate from three different pathways: (i) when ageing into the environment, photooxidation and ultraviolet light radiation lead to weathering of MNP, accompanied with the formation of free radicals at the surface of the plastic debris which react with oxygen [145]. When ingested by organisms, these plastic debris increase extracellular ROS production [143], (ii) less or non-altered MNP induce intracellular increase of ROS production [142], and (iii) ingested MNP

are recognised as foreign compounds by the organisms' immune systems and internalised through cell membranes [146]; the neutralisation process is associated with the production of high quantities of ROS. The membrane permeability is also increased through the transfer of MNP which may facilitate the transfer of ROS into cellular compartments [122,123].

The amount of oxidative stress induced by MNP can be measured by considering intracellular ROS amounts, ROS-induced damages (protein carbonylation, lipid peroxidation, DNA damages), and/or by assessing the antioxidant system which depends on the amount of antioxidant molecules and on the activity of several antioxidative enzymes [147]. The oxidative system includes enzymes as the superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx) and peptides as metallothionein (MT), glutathione (GSH) or vitamins E and C, as well as xanthophylls [144,148]. MNP were effectively reported as oxidative stress inducers in terrestrial invertebrates [124,120,106,149,142,150,105,136,122]. For instance, the exposure to polystyrene MNP (100, 500 nm and 1, 2, 3 μm , at 1 mg/L for 3 days) induced the expression of a GST enzyme - a key enzyme involved in cellular detoxification - in *C. elegans* [142]. ROS production was also increased after ingestion of PS MP (from 1 to 100 $\mu\text{g/L}$) by the nematode [122]. Interestingly, when *E. fetida* was exposed to PE and PLA MP (from 0.5 to 14 % w/w), GST levels were first decreased after 14 days for both polymers, before being increased after 28 days of exposure [136]. The levels of CAT and SOD enzymes, which are key enzymes for scavenging ROS, were also affected after MNP ingestion by *E. fetida*. These responses were modulated by MNP concentration, with higher activities being measured at higher MNP concentrations [136], and was also increased with MNP weathering [84]. In earthworms exposed to 550–1000 μm LDPE MP, antioxidant responses were greater with aged-MP compared to virgin-MP [84]. However, MP did not provoke oxidative stress in the digestive gland of the snails [104]. Similarly, no changes in ROS production was measured in the silkworm moth *Bombyx mori* while SOD activity was slightly reduced (the activity of GST and CAT remained unchanged) [125]. So far, the existing knowledge suggests that antioxidant responses highly depend on the concentration and duration of exposure, size of the particles (smaller-sized particles generate more ROS), and their weathering state [84,143,142].

6.4. ROS-induced damages: lipid peroxidation and DNA damages

When the capacity of the antioxidant system is no longer sufficient for scavenging ROS, the resulting excess of oxidative stress can alter macromolecules, i.e. DNA, lipids, and proteins. Lipid peroxidation results from the free radical-mediated alteration of polyunsaturated fatty acids, thus damaging cell membrane and lipid-containing structures [151]. The oxidation of lipid-membranes changes their structures [152]. This increases membrane permeability and alters homeostasis of the cell, then increasing the possibility for ROS and other contaminants to enter the cells [152]. Once lipids are peroxidised, aldehydes, in particular malondialdehyde (MDA), are formed as secondary peroxidation products, and can be used as reliable biomarkers of oxidative stress [153] (Table A.1).

In *E. fetida* exposed to PE and PLA MP for 14 days, MDA levels were significantly higher compared with controls [136]. Interestingly, the amounts of MDA decreased, and reached values below those measured in the controls when the duration of exposure was prolonged to 28 days. The overproduction and then inhibition of the amount of oxidative stress caused by MP highly correlated with the activation of the antioxidant system [136]. Similarly, MDA content in snails exposed for 4 weeks to PET fibers increased at the highest concentrations (0.71 g/kg dry soil), as a consequence of a depleted antioxidant system [105]. This increase reflected a free radical excess which directly impacted tissues and molecules, moreover MDA is also an important DNA mutagen [154]. Contrastingly, in a similar study using this annelid exposed for 28 days to LDPE MP, the MDA levels remained higher, hence the antioxidant

system did not succeed in reducing oxidative stress [84,120].

MNP may enhance DNA damage both directly and indirectly through excessive ROS production [143]. To assess the potential genotoxicity of MNP, the comet assay can be used which detects double strand DNA breakages [155]. In earthworms (*E. fetida*), larger plastic particles further accentuate the DNA damage (PS MP, 1300 nm versus PS NP, 100 nm), moreover, genotoxicity was dose-dependent with greater DNA impacts at higher concentrations (1000 $\mu\text{g/kg}$ dry soil versus 100 $\mu\text{g/kg}$ dry soil) [106]. In addition, 8-hydroxydeoxyguanosine (8-OHdG) levels, a marker of DNA oxidation, were increased in earthworms exposed to aged-LDPE MP as compared with controls [84]. DNA damages were evidenced after individuals of *D. melanogaster* ingested PET and PS NP at high concentrations (>100 $\mu\text{g/g}$ food) [103,124]. Conversely, smaller particles (50 nm compared to 200 nm) induced more DNA breaks [124].

MNP-induced genotoxicity was reported to accumulate from one generation to another [79]. For instance, using comet assay, Sobhani et al. [156] observed higher DNA damage in the F1 generation of *E. fetida* compared to F0 generation exposed to polystyrene MP at three concentrations (0.01 %, 0.1 % and 0.5 % w/w in soil). Here again, damages were increased when concentrations of MP were augmented [156]. Epigenetic modifications are additionally thought to occur after NP ingestion, with subsequent effects over several generations. Indeed, Yu et al. [130] found a DNA hypomethylation of *ced-3* promoter, increasing the expression of its associated gene and leading to cell apoptosis. In this study, hypomethylation was even found to occur from F0 to F4 generations, causing gene expression perturbations [130], and partially explaining the transgenerational effects described in the above section.

The current body of knowledge suggests that oxidative stress and damages induced by MNP ingestion can impact key life history traits such as longevity as cumulative damages from oxidative stress can accelerate the ageing process by affecting various physiological functions (oxidative theory of ageing, [157]). Likewise, oxidative damage in the brain may alter neuronal function and signalling pathways involved in behaviour regulation.

6.5. Immune system

MNP are exogenous compounds and ingestion of these xenobiotics by terrestrial invertebrates elicits immune responses [143]. The innate immune system was repressed in the nematode species *C. elegans* exposed to NP [158]. Kwak and An [129] studied the viability of coelomocytes, which have a key role in innate immune responses in earthworms, through measurements of esterase activity. The activity of the enzyme decreased significantly in earthworms exposed to polyethylene NP, leading to an attenuation of the immune system. In general, the harmful effects on the immune system are stronger when plastic particles are smaller: the esterase activity was lower when plastic sizes were in the range 180–212 μm compared to plastics with sizes in the range 250–300 μm [129]. Similarly, coelomocyte viability was decreased in *E. fetida* after PS NP exposure inducing oxidative damage in immune cells [159]. More precisely, PS NP interacted directly with immunity protein lysozyme (LZM), a lysosome protein with a key role in the immune system, and inhibited their activity, structure and conformation [159]. Conversely, the innate immune system of *B. mori* was enhanced after ingestion of polystyrene MNP, as evidenced by the over-expression of genes encoding antimicrobial peptides (Cecropin A and Lysozyme) [150].

In invertebrates, immune cells circulate in the haemolymph, and total haemocyte count (THC) is a valuable indicator of responses to a range of environmental stressors [160,161]. No changes in THC were reported from specimens of *Porcellio scaber* exposed to PET and tyre wear MP while the proportion of haemocyte types was altered: the number of semi-granulocytes was decreased and the number of granulocytes was increased, potentially triggering humoral molecules [162]. MNP also disturbed the ability of organisms to defend themselves from microbial

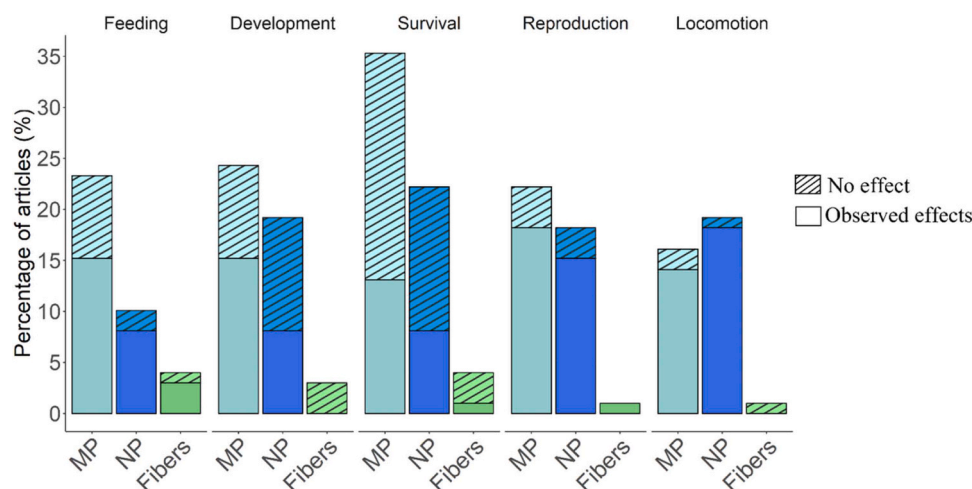


Fig. 4. Biological effects of fibers, micro- and nanoplastics reported in the available literature. Feeding, development, survival, reproduction, or locomotion incurred by micro- and nanoplastic, and fibers exposures to terrestrial invertebrates were evaluated as biological endpoints.

infection, with nanoplastics at concentrations of 10 and 100 $\mu\text{g/L}$ inhibiting the induction of antimicrobial gene expression in nematodes infected by the fungi *Candida albicans* [163]. The authors reported higher mortalities of the nematodes at the highest NP concentration [163]. Similarly, the silkworms were more vulnerable to bacterial infection (*Serratia marcescens* Bm1) when they were exposed to polystyrene NP at 10 $\mu\text{g/ml}$ [150].

In silkworms exposed to NP, no inhibition of the defence system was observed for MP which, conversely, induced a stronger immune response [150]. This finding demonstrates that it is necessary to take into account the size of plastic particles as important drivers of the impacts and physiological and molecular responses of organisms. The smallest plastic fragments might be undetectable, and due to their sizes and easier diffusion among body compartments and tissues, cause dyspepsia and physical injuries that alter the quality of the physiological responses in general. The inflammatory and immune responses on organisms exposed to MNP are more studied on aquatic species [164,165]. More research is needed on terrestrial organisms to explore the underlying mechanisms following MNP ingestion on the immune system and any consequence on pathogens resistance.

To sum up, the available literature revealed that (i) the duration of exposure and the type of plastic debris are important factors determining the magnitude of the physiological impacts, (ii) oxidative stress is triggered by MNP ingestion in several organisms, and the effects depends on the size, concentration and weathering of the plastic fragments, (iii) MNP can be internalized after ingestion and cause oxidative stress with subsequent tissue and macromolecule damages and inducing cascading effects at different scales (Table A.1, Supplementary materials).

7. The effects of micro and nanoplastics on organisms

Once internalised and inducing physiological disorders through various mechanisms, MNP may affect life history traits of invertebrates. The most frequent impacts examined at the organism scale in the available literature are presented in Fig. 4.

7.1. Plastic consequences on feeding

The ingestion of MNP subsequently affects the feeding behaviour of terrestrial invertebrates, particularly altering the amount of food consumed and subsequent excretion. Both, false saturation, or damages of internal organs (mechanical abrasion or physiological interactions) could be underlying mechanisms. For instance, a reduction of faeces

production was observed in earthworms (*Lumbricus terrestris*) which had ingested fibers [93]. Food intake was decreased by 25 to 35 % after snails (*A. fulica*) were fed with fiber-contaminated diet for four weeks, and excretion was even decreased by 60 % at high fiber concentrations [105]. Similarly, food intake and defecation rates of *D. melanogaster* reared on media contaminated with polystyrene MP were significantly reduced [109].

The alteration of food intake inevitably impacts the quality and quantity of body reserves and the body mass of terrestrial invertebrates. A significant body mass decrease (30 %) was measured in the earthworm *E. fetida* exposed to high concentrations of polystyrene MP (1 % and 2 % (w/w)) [166]. This finding supports the results collected on the worm *Enchytraeus crypticus*, where no mortality was induced by ingestion of polystyrene NP while a significant body mass reduction was measured at the highest PS NP concentration (10 % PS NP, w/v) [137]. Furthermore, Fudlosid et al. [167] found that the body size and mass of the cricket *Gryllobates sigillatus* were reduced after MP exposure, and the effect depended on the nature of the polymer: a decrease was observed for PET fibers, while ingestion of PE MP had no impact. Differences may partially result from differential impacts of MNP and fibers on the diversity and richness of gut and intestine microbiota. However, the paucity of data on the topic makes it difficult to appreciate the contribution of microorganisms in the observed differences.

The effects of MNP on body mass can then have cascading consequences on starvation resistance. For instance, the resistance to starvation was decreased in flies (*D. melanogaster*) after MP exposure in a sex-specific manner: only males were affected [109,168]. Yet, these observations are not generalizable as feeding (food consumption, efficiency and energy storage) and defecation rates of a terrestrial isopod species (*P. scaber*) exposed to MP from facial cleanser remained unchanged [169].

7.2. The effects of plastic pollution on survival and reproduction

No mortality was reported when the earthworm *E. fetida* had ingested PS or PE MNP [84,120] (Table A.1). In this species, a 28-day exposure to LDPE MP at concentrations ranging from 62 to 1000 mg/kg of dry soil did not induce mortality [170]. Yet, some investigations evidenced MP-induced lethality when earthworms ingested PP MP at concentrations ranging from 300 to 6000 mg/kg dry soil [171]; the longer duration of exposure (42 days) and the higher concentrations may have increased the likelihood of MP-induced lethality. The survival of flying insects, i.e. *Drosophila* spp. exposed to NP [124,103,172], *B. mori* exposed to PS MNP [150], and *Apis mellifera* exposed to PS MP

[140], was not altered by plastic ingestion. The conclusions on the effects of MNP on survival vary with the study model, the exposure duration and MNP concentration, with extended and high concentrations impacting survival [173]. These results underscore the significance of prolonging exposure duration and examining exposure across multiple generations.

The ingestion of MNP may affect reproduction and fecundity of terrestrial invertebrates. The number of offspring of the nematode *C. elegans* was significantly decreased when the concentration of polystyrene NP (42 and 530 nm) was higher than 10 mg/kg soil [174]. A reduction in offspring was observed when the individuals were exposed to PS NP of 50 nm [149]. The number of *E. crypticus* juveniles was reduced by almost 50 % in comparison to controls when exposed to nylon MP at 120 g/kg dry soil [175]. Similarly, oviposition was reduced by 50 % when *D. melanogaster* females were exposed to PET MP (2 µm at 20 g/L) [168]. After 56 days of exposure to polyethylene MP, the number of *E. andrei* offspring was not affected by the treatment (62.5, 125, 250, 500 and 1000 mg MP/kg dry soil) [121]. Interestingly, polystyrene NP (0.5 %) even stimulated the reproduction of the worm *E. crypticus* whose number of cocoons was increased after 7 days of exposure [137]. As for survival, there is no general rule for the effects of MNP on the reproduction of terrestrial invertebrates, which depend on MNP characteristics and investigated taxa.

Several hypotheses were proposed for the impacts of MNP on reproduction [176]. MNP could decrease energy metabolism, by decreasing food intake and/or nutrient assimilation, as reported in *C. elegans* and *D. melanogaster* whose lipid storage and amino acids production were disturbed [149,168]. Moreover, MNP may directly impact reproductive organs as observed for *D. melanogaster*, where polystyrene NP were found in ovaries, increasing the number of apoptotic and dead cells [128]. These mechanisms are probably inter-related, and combined with DNA damage and ROS production, may explain the effects of MNP on reproduction [177].

7.3. Effects on mobility and behaviour

Locomotion, mobility or behaviour of terrestrial invertebrates can be perturbed by MNP owing to the neurotoxicity and alteration in energy metabolism these plastic particles can cause to the individuals [178]. By quantifying the number of head thrashes and body bends in a given time [179], Lei et al. [142] found that individuals of *C. elegans* exposed to polystyrene MNP at 1 mg/L exhibited a hyperactive behaviour. Conversely, locomotion of *C. elegans* was decreased after the individuals were exposed to polystyrene NP (exposed at 17.3 mg/L and 86.6 mg/L) [149] (Table A.1). Yet again, variations in the concentration and size of plastic particles between the two studies (highest NP concentration and smaller particles in Kim et al. [149] in comparison to Lei et al. [142]), prevent the drawing of reproducible patterns.

Similarly, hyperactive behaviour was measured in the silkworm *B. mori* exposed to PS NP, mainly leading to erratic movements, potentially impacting their fitness [125]. In *D. melanogaster*, the spontaneous activity of the flies over 24 h increased by 20 % to 40 % for females and males after an exposure to PET MP [168]. Using the same experimental set up, Matthews et al. [172] also found an increase in daily activity in males exposed to polystyrene MNP at a concentration of 50 ppm.

7.4. Long-term and multi-generational exposures

Once ingested by organisms, MNP can also be transferred to the next generations and induce ecotoxicological effects. In terrestrial environment, transgenerational effects have been mainly studied on two organisms, *C. elegans* and *D. melanogaster*, after a continuous or a maternal exposure [180,181,128,130]. Fitness was impaired through generations with a decreased fecundity at F4 and F5 (50 and 100 mg/L of PS NP) for the flies [128], and a brood size reduction for worms, until the F4 generation during continuous exposure to 100 nm PS-NP at 50 and

100 mg/L [130]. Brood size was reduced until F6 when the parental generation only was exposed to PS NP (exposure at 100 µg/L with 20 nm NP) [181]. These results suggest that toxicity of MNP through transgenerational effects is dose and size dependent, with smaller particles having more effects. The toxicity of MNP over generations may be explained by their accumulation in ovaries as observed for flies [128], but also by the increase of oxidative stress and DNA damages and epigenetic modification [130,181]. Meanwhile, the mechanisms leading to the transgenerational effects of MNP exposure and ingestion are still unclear.

8. Research gaps

For many years, the impacts of MNP have been predominantly explored in aquatic species [91,182]. More recently, terrestrial plastic contamination has gained attention, but investigations on the effects of MNP on terrestrial invertebrates have mainly focused on a few model species. This prevents our ability to get a full understanding of MNP toxicity for the wildlife. Moreover, we still have little to no information about the toxicity of a large number of environmental MNP as the current knowledge is mainly derived from commercial PS MNP at given sizes and shapes. Consequently, significant considerations should be given to the following points in future studies:

1. Characteristics of plastic particles and fibers: disruption of biological and physiological traits was reported for aquatic invertebrates and to a lesser extent terrestrial invertebrates. In those studies, it has also been frequently concluded that the effects of MNP are polymer, size, concentration, weathering state, and organism dependent. More investigations with polymers having different chemical composition, origin (commercial supply, laboratory production), size (diameter of the particle), shape (morphology of the plastic particle) must be performed. Although there has been a growing number of articles on the effects of NP compared to MP, there is an important imbalance in the polymer type on NP studies with more than 90 % of the articles using polystyrene, as underlined in Fig. 2. It is essential to investigate nanoplastic toxicity by including other polymers as PE, PP and PVC, which are prevalent in the environment.
2. Commercial versus environmental MNP: most of the articles used commercially available MNP (Fig. 2; [182]). Commercial particles are perfectly round and monodisperse, easily accessible for purchase. However, they do not reflect the environmental reality where aged MNP usually display irregular shapes and different sizes. Additional research is required to explore the effects of mixtures of environmental MNP in size and polymers to better understand their environmental impact.
3. Presence of chemical additives: the effects of plastic additives and chemicals/compounds adsorbed onto plastic surfaces in the environment, such as metal, plasticizers, pesticides and others, are rarely represented in the studies of MNP. These compounds are recognized for their hazardous effects, as explained in the introduction, with some of them being biocides and endocrine disruptors. More research testing the effects of MNP in combination with their additives are needed, as this aspect has remained scarcely taken into account in the studies.
4. Tested biological organisms: while the effects of MNP are frequently studied in aquatic species (invertebrates and fishes), there is a noticeable scarcity of research on terrestrial invertebrates. Moreover, the range of considered terrestrial taxa is unequally studied. The main organisms represented are selected (model) species of nematodes, annelids, arthropods and molluscs (Fig. 2) with a largely predominance of investigations on *C. elegans* [183].
5. Studying the effects of plastic pollution is crucial not only for understanding immediate impacts but also for comprehending potential long-term consequences, including transgenerational effects, as acute effects may be subtle or even imperceptible, while chronic

Table 1

Information to be collected in future research for improved hazard characterization of micro and nanoplastics and fibers on terrestrial invertebrates.

Improved hazard characterization	Methodology	List of candidate metrics	Description and relevance
Mortality	High-quality dose-response curves and Benchmark dose	Effect concentration (EC _x), lowest observed effect concentration, no observed effect concentration	Obtaining robust dose-response data is crucial as it reveals the concentrations at which plastic particles and fibers trigger adverse effects
Growth and reproduction	Laboratory, and if possible, in-field measurements of development, lifespan, growth, morphometrics and reproduction	Developmental rate, age at maturity, lifespan, number of offspring, reproductive output, fertility (vitellogenin, energy resources to eggs)	Reproductive abilities and developmental rates of the species exposed to different concentrations and types of MNP Characterization of the impacts on population dynamics and declines
Behavioral effects	Movement patterns, feeding behavior, excretion, seasonal movement, circadian rhythm, aggression	Dispersal distance, straightness/sinuosity of dispersal/movement paths, propensity to disperse, foraging behavior, food intake	Feeding and movement behavior changes after MNP ingestion can influence energy intake at individual scale, and intra- and interspecific interactions at larger scales
Accumulation and internalization	Accumulation of MNP in digestive tract and internalization in targeted organs	Observation of fluorescent MNP with microscopy and quantification of metal-doped MNP	Validation of the ingestion of MNP by the organism Follow the pathway of MNP in the digestive tract and the accumulation in organs, with a quantification
Physiological and molecular effects	Enzyme activity, oxidative stress, immunity, gene expression, genomics, transcriptomics and proteomics, microbiome studies	Reactive oxygen species (H ₂ O ₂), anti-oxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase), quantitative real-time PCR of candidate gene (anti-oxidant, immunity, temperature), mRNA expression and transcriptome analysis	Quantification and understanding of stress responses under ecologically relevant MNP exposure Understand and identify physiological pathways impacted by MNP exposure

exposure could have significant repercussions on invertebrates within ecosystems.

Finally, the crucial priority remains the standardization of experimental protocols, including MNP concentration units to facilitate comparisons between studies. Starting from the information provided by

Jemec Kokalj et al. [36], and completed by Jemec Kokalj et al. [37], we adapted the quality criteria developed by these authors for studying the effects of MNP on aquatic invertebrates (*Daphnia* sp.) so that they can be extended to terrestrial invertebrates (Table A.2, Supplementary materials). In doing so, we aim at further encouraging the standardization of the experimental assays among a large diversity of animal models thriving in different habitats. Standardized approaches and guidance in assessing terrestrial invertebrate health risks will improve our understanding of impacts and potential genericity of the toxicity of MNP across animal species. We believe that the lack of consensus on the potential toxicity of MNP on terrestrial invertebrates partially results in part from some disparity among experimental assays. Thus, guidelines collecting relevant information on specific biological and physiological endpoints are presented in Table 1.

CRediT authorship contribution statement

Paride BALZANI: Writing – review & editing. **David SIAUSSAT:** Writing – review & editing, Supervision, Funding acquisition. **Gwenola GOUESBET:** Writing – review & editing. **Hervé COLINET:** Writing – review & editing, Visualization. **Elsa DEJOIE:** Writing – review & editing, Writing – original draft, Conceptualization. **Claudia WIEGAND:** Writing – review & editing, Supervision. **Chloé M. C. RICHARD:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **David RENAULT:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David RENAULT reports financial support was provided by National Agency for Food Environmental and Occupational Health and Safety. David RENAULT reports financial support was provided by The French Agency for Ecological Transition. David RENAULT reports financial support was provided by National Centre for Scientific Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2024.135299](https://doi.org/10.1016/j.jhazmat.2024.135299).

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Glossary

BPA: Bisphenol A
DEHP: Di(2-ethylhexyl) phthalate
HDPE: H density polyethylene
LDPE: Low density polyethylene
MNP: Micro- and nanoplastic
MP: Microplastic
NP: Nanoplastic
PA: Polyamide
PAHs: Polycyclic aromatic hydrocarbons
PLA: Polylactic acid
PC: Polycarbonate
PES: Polyester
PE: Polyethylene
PET: Polyethylene terephthalate
PP: Polypropylene
PS: Polystyrene
PUR: Polyurethane
PVC: Polyvinyl chloride
THC: Total haemocyte count