Impacts of Micro- and Nano-Sized Plastic Particles on Benthic Invertebrates: A Literature Review and Gap Analysis

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As documented by the numerous publications that have appeared in recent years, plastic pollution of the environment and the effects on the respective ecosystems are currently one of the most intensely discussed issues in environmental science and in society at large. Of special concern are the effects of micro- and nano-sized plastics. A key issue in understanding the fate and potential effects of micro- and nano-sized plastics is their dynamic nature, as the size, shape, and charge of the particles change over time. Moreover, due to various biological processes, such as the aggregation of organic material and/or bacteria ("biofouling"), the density of plastic particles that settle in the sediments of aquatic ecosystems may be several orders of magnitudes higher than that in the surrounding waters. Consequently, the risk posed by plastic pollution to benthic fauna is considerably high. Nonetheless, the vast majority of studies examining the effects of microplastics have focused on pelagic organisms so far. We therefore conducted a comprehensive literature review to examine the impact of micro- and nano-sized plastics on benthic invertebrates, including the physical and chemical effects of leaching and the interactions of plastic particles with contaminants. Overall, 330 papers were reviewed for their fulfillment of different criteria (e.g., test species, plastic material, particle shape, particle size, exposure concentration, exposure route, assay type, assay duration), with 49 publications finally included in our survey. A comprehensive gap-analysis on the effects of plastic particles on benthic invertebrates revealed a wide variety of effects triggered by micro- and/or nano-sized plastics but also distinct differences regarding the plastic materials tested, the size fractions applied, the shape of the respective particles, and the exposure routes tested. Our review concludes with a discussion of the important research gaps concerning freshwater ecosystems and recommendations for future areas of research.

Keywords: microplastic, nanoplastic, sediment, marine, freshwater, invertebrates, toxicity

INTRODUCTION

The pollution of aquatic ecosystems with plastic debris is regarded as one of the most serious environmental issues worldwide. Among this debris, small-sized particles have received increasing attention and are recently of particular concern (e.g., Thompson et al., 2004; Eerkes-Medrano et al., 2015; Rochman et al., 2016). These particles, termed nano- and microplastics, are generally defined by their largest dimensions of 0.001–0.1 μm and 0.1 μm−5 mm, respectively (e.g., Thompson et al., 2004; Moore, 2008) and are both major contributors to plastic pollution in marine as well as freshwater ecosystems (e.g., Thompson et al., 2004; Cole and Galloway, 2015; Chae and An, 2017). Generally, tiny particles could either be manufactured directly for various consumer and industrial applications, serving as primary sources of these particles, or could be derived from the fragmentation of larger plastic particles (e.g., Andrady, 2011; Browne et al., 2011).

An understanding of the environmental fate of small-scale plastic particles is fundamental for the assessment of their potential risks, but this is complicated by the fact that the size, shape, density, and charge of the particles constantly change over time (Galloway et al., 2017). Generally, several plastics, such as Polystyrene (PS), Polyvinylchloride (PVC), or Polyethylene terephthalate (PET), have a specific gravity higher than water, resulting in increased settling rates of these plastic classes in sediments, while plastics with lower densities, such as Low-density polyethylene (LDPE), High-density polyethylene (HDPE), or Polypropylene (PP), are suspected to mainly float in the water column (e.g., Duis and Coors, 2016; Auta et al., 2017). However, due to various biological processes, such as the aggregation of organic material and/or bacteria ("biofouling"), the gravity of plastic particles might become greater, by which their potential to settle in the sediments of aquatic ecosystems is increasing (e.g., Andrady, 2011; Galloway et al., 2017). Subsequently, densities of plastic particles in sediments can become magnitudes higher than in the surrounding waters (Lattin et al., 2004). These processes increase the bioavailability of nano- and microplastics for sediment-inhabiting organisms, especially via ingestion, since the particles are of roughly the same size (or even smaller) as sediment grains (Moore, 2008; Wright et al., 2013a). The ingestion of plastic debris by sediment-dwelling organisms has already been frequently reported and is reviewed elsewhere (e.g., Ivar do Sul and Costa, 2014; Li W. C. et al., 2016; Scherer et al., 2017). Benthic invertebrates are of particular concern, either in marine or freshwater habitats, since they contribute up to 90% of fish prey biomass (e.g., Schindler and Scheuerell, 2002; Weber and Traunspurger, 2015). Hence, for benthic fauna, small-scale plastics may impact trophic energy transfer and/or trophic interactions. However, the vast majority of studies examining the ecotoxicological effects of nano- and microplastics have focused on pelagic rather than benthic organisms so far.

Direct harmful effects of nano- and microplastics may be of physical (mechanical) and/or chemical (toxicological) nature (Barnes et al., 2009; Wright et al., 2013b). The latter include the leaching from plastics of e.g., carcinogenic and endocrine-disrupting contaminants, such as monomers, plastic additives (e.g., Oehlmann et al., 2009; Talsness et al., 2009), and polymer-associated chemicals. In addition, due to their large surface area to volume ratio, small-scale plastic particles can become heavily contaminated, with particle-associated concentrations of the contaminants being several orders of magnitude greater than those in the ambient medium (Mato et al., 2001; Hirai et al., 2011). Among the pollutants with the highest affinity for the hydrophobic surface of plastics are hydrophobic persistent organic pollutants (POPs). After the contaminated particles are ingested by benthic organisms, the possible leaching of associated POPs could result in the bioaccumulation and biomagnification of these chemicals followed by their entry into aquatic food webs (vom Saal et al., 2008).

In this review we assess current knowledge on the effects of nano- and microplastics on benthic invertebrates in aquatic ecosystems. Our assessment is based on a literature analysis of: (i) the impacts on organisms in freshwater and marine environments, (ii) the harmful effects induced by the physical or chemical impacts of plastic particles, (iii) the various particle materials, shapes, and sizes examined, (iv) the exposure matrix and parameters assessed in the respective assays and (v) the interaction of contaminants with nano- and microplastic particles. Subsequently, a gap analysis based on the obtained findings was conducted and areas in need of further research were identified.

METHODS

Using the databases Web of Science and Google Scholar, a comprehensive literature review of the physical and chemical effects of leaching processes as well as the interaction of plastic particles with contaminants in terms of their impacts on benthic invertebrates was conducted. The search was based on a query of the key word terms: microplastic* OR nanoplastic* AND benthic* OR benthos* AND invertebrate* AND effect* OR impact* or a combination thereof.

Overall, 330 papers were reviewed, with 49 publications finally included in this survey based upon their relevance to the topic, in agreement with general criteria for peer-reviewed articles and as judged by the authors of this review. Although a comprehensive literature search was carried out, the retrieved studies may not be fully representative of all studies conducted, since the probability that a given study will be published generally increases with the increased statistical significance of its results. This "file drawer problem" was described by Arnqvist and Wooster (1995). However, for consistency, unpublished results were excluded, with only primary literature reports included in the final review process. Additional validity criteria fulfilled by the included publications were distinct characterizations of the respective plastic material as well as the provision of quality criteria in terms of positive and negative controls. Within the selected publications, investigations of two or more different organisms or particles with various characteristics in terms of, e.g., particle material or shape were considered as separate experiments.

FIGURE 1 | Schematic representation of the review process, publication selection and data analysis.
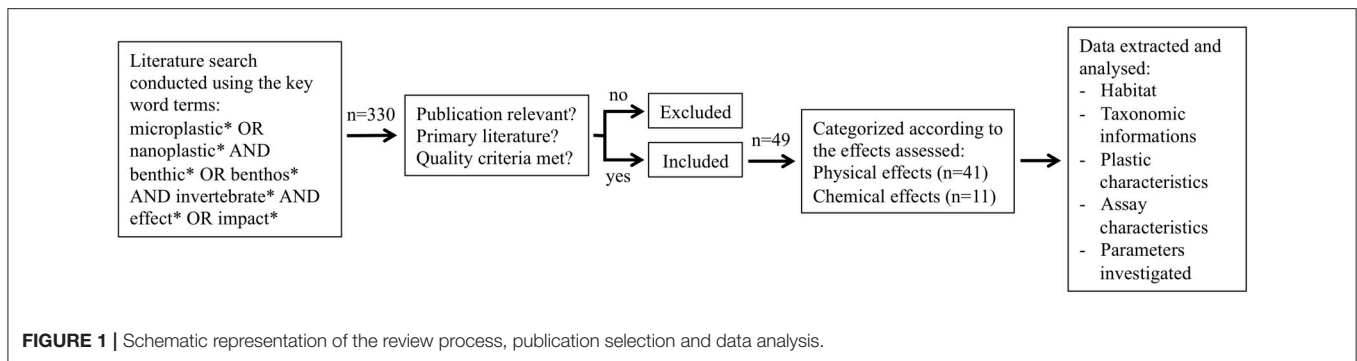
The publications were categorized according to the investigated habitat (freshwater or marine) and the impact (physical or chemical) on the benthic invertebrates assessed. For each publication, the following criteria were analyzed: taxon, species, plastic material, particle shape, particle size, exposure concentration, and matrix endpoints investigated. The general review procedure and the effects identified in those studies are summarized in **Figure 1**.

RESULTS

Physical Impacts
Freshwater Benthic Fauna
The mechanical hazards posed by the ingestion of micro- and nano-sized plastic particles by organisms in freshwater ecosystems were evaluated in 26 experiments reported in 10 publications (**Table 1**). The most frequently used organisms (38%, $n = 10$) were arthropods, mainly crustaceans (**Figure 2A**). The gammarids *Gammarus fossarum, Gammarus pulex,* and *Hyalella azteca* were the target in 8 out of the 26 experiments (**Table 1**), and molluscs and nematodes in 7 (27%) and 6 (23%) of the experiments (**Table 1**; **Figure 2A**). By contrast, very little research focused on the physical effects of plastics on annelids ($n = 2$, 8%) and rotifers ($n = 1$, 4%; **Figure 2A**). The former were limited to the effects on *Lumbriculus variegatus* and *Tubifex* spp., and the latter to those on the rotifer species *Brachionus koreanus* (**Table 1**).

Among the 26 experiments examining the mechanical hazards posed by micro- and nano-sized plastics on benthic organisms in freshwater, 46% ($n = 12$) focused on polystyrene (PS) particles, 23% ($n = 3$) on polyamide (PA), 23% ($n = 3$) on polyethylene (PE), 15% ($n = 2$) on polyvinyl chloride (PVC), and 15% ($n = 2$) on polypropylene particles (PP; **Figure 2B**). The effects of polymethyl methacrylate (PMMA), polyhydroxybutyrate (PHB), polycarbonate (PC), and polyethylene terephthalate (PET) were investigated in single studies (4% each). The vast majority (69%, $n = 18$) of the freshwater investigations examined the physical effects of microplastic fragments, i.e., non-uniform, irregularly shaped particles, and powders (**Figure 2C**). In 23% ($n = 6$; **Figure 2C**) the effects of spheres, i.e., micro- and/or nano-sized beads, were determined. Two studies (8%) assessed the physical effects of polymer fibers (**Figure 2C**). Generally, the effects of micro-sized plastic particles (0.1–5,000 μm) were investigated,

whereas the toxicity of nano-sized particles (<0.1 μm) was rarely tested (**Table 1**).

As shown in **Figure 2D**, in most of the studies on the mechanical hazards of microplastics (54%, $n = 14$) aqueous medium (AM) was the matrix used to apply the investigated plastic particles, followed by plastic spiked sediments (27%, $n = 7$) and of food (8%, $n = 5$).

Mortality
Lethal effects of nano- and micro-sized plastics were investigated in 81% ($n = 21$) of the studies as displayed in **Table 1**. Redondo-Hasselerharm et al. (2018) investigated the lethal effects of various PS-fragments in sediments on the annelids *L. variegatus* and *Tubifex* spp., the arthropods *Asellus aquaticus, G. pulex,* and *H. azteca* and the mollusc *Sphaerium corneum*, without observing lethal effects on any test organism (**Table 1**). Similar results were reported by Blarer and Burkhardt-Holm (2016) and Imhof and Laforsch (2016), who neither found significant lethal effects on *G. fossarum* nor on the mud snail *Potamopyrgus antipodarum* exposed to a variety of polymer fragments (PA, PC, PET, PS, PVC) offered in different shapes, concentrations, and sizes (**Table 1**). However, Ziajahromi et al. (2018) and Lei et al. (2018) reported significantly increased mortality rates for *Chironumus tepperi* and *Caenorhabditis elegans* when exposed to PE- and PS-spheres varying in size and concentration respectively, with effects being distinctly size-dependent (**Table 1**). In terms of investigated effects of plastic fragments and fibers, impacts were reported to be rather dose-dependent as observed for PE-fragments and PP-fibers on *H. azteca* (Au et al., 2015; **Table 1**) and for PA-, PP-, PE-, and PVC-fragments on *C. elegans* (Lei et al., 2018; **Table 1**).

Development
The effects of small-scale plastics on the development of organisms were investigated most frequently, by being of concern in 88% ($n = 23$) of the respective studies. As reported by Redondo-Hasselerharm et al. (2018), effects of PS-fragments on the development were species-specific, with no significant effects reported for *L. variegatus, Tubifex* spp., *A. aquaticus, H. azteca,* and *S. corneum*, while the growth of *G. pulex* was significantly affected (**Table 1**). In the study of Blarer and Burkhardt-Holm (2016), neither PS-spheres nor PA-fibers had significant effects on the development of *G. fossarum*, while exposure to PMMA- and

TABLE 1 | Effects on micro- and nano-sized plastic particles on benthic freshwater invertebrates.

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	References
Annelida	Lumbriculus variegatus	PS	Fragments	20–500 μm	0.1–40% sed. dw	S	Mortality	No effects on mortality	Redondo-Hasselerharm et al., 2018
							Development	No effects on growth	
							Behavior	No effects on feeding rate	
	Tubifex spp.	PS	Fragments	20–500 μm	0.1–40% sed. dw	S	Mortality	No effects on mortality	Redondo-Hasselerharm et al., 2018
							Development	No effects on growth	
							Behavior	No effects on feeding rate	
Arthropoda	Asellus aquaticus	PS	Fragments	20–500 μm	0.1–40% sed. dw	S	Mortality	No effects on mortality	Redondo-Hasselerharm et al., 2018
							Development	No effects on growth	
							Behavior	No effects on feeding rate	
	Chironomus tepperi	PE	Spheres	1–4 μm	500 part. kg^{-1}	S	Mortality	Significantly increased mortality at 1–4, 10–27, and 43–54 μm	Ziajahromi et al., 2018
				10–27 μm			Development	Significantly decreased body length at 1–4, 10–27, and 43–54 μm;	
				43–54 μm				Significantly decreased length of head capsule at 10–27 μm;	
				100–126 μm				Reduced development of head capsule, mouth parts, and antenna at 10–27 μm; Development delay of organisms at 10–27 μm	
							Emergence	Significantly lowered emerging rate for all size ranges	
	Gammarus fossarum	PS	Spheres	≤5 μm	12,500 part. ml^{-1}	AM	Mortality	No effects on mortality	Blarer and Burkhardt-Holm, 2016
							Development	No effects on wet weight	
							Behavior	No effects on feeding rate	
							Assimilation	No effects on assimilation efficiency	
		PA	Fibers	20 × 500 μm	2,680 cm^{-2}	AM	Mortality	No effects on mortality	Blarer and Burkhardt-Holm, 2016
							Development	No effects on wet weight	
							Behavior	No effects on feeding rate	
							Assimilation	Significantly decreased assimilation efficiency	
		PMMA	Fragments	32–64 μm	100,000 part. ind.$^{-1}$	AM	Development	Significantly decreased wet weight	Straub et al., 2017
				64–125 μm			Behavior	No effects on feeding rate	
				125–250 μm			Assimilation	Significantly decreased assimilation efficiency	
		PHB	Fragments	32–64 μm		AM	Development	Significantly decreased wet weight	Straub et al., 2017

(Continued)

TABLE 1 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	References
				64–125 μm			Behavior	No effects on feeding rate	Redondo-Hasselerharm et al., 2018
				125–250 μm			Assimilation	No effects on assimilation efficiency	
	Gammarus pulex	PS	Fragments	20–500 μm	0.1–40% sed. dw	S	Mortality	No effects on mortality	
							Development	Significantly reduced growth (EC$_{10}$: 1.07% sed. dw, EC$_{50}$: 3.57% sed. dw)	
							Behavior	No effects on feeding rate	
	Hyalella azteca	PS	Fragments	20–500 μm	0.1–40% sed. dw	S	Mortality	No effects on mortality	Redondo-Hasselerharm et al., 2018
							Development	No effects on growth	
							Behavior	No effects on feeding rate	
		PE	Fragments	10–27 μm	10–100,000 part. ml^{-1}	AM	Mortality	Significant, dose-dependent increase in mortality (LOEC: 10,000 part. ml^{-1}, LC$_{50}$: 4.6 × 10^4 part. ml^{-1})	Au et al., 2015
							Development	No effects on dw	
					5,000–20,000 part. ml^{-1}		Reproduction	Reproduction significantly decreased at 5,000 part. ml^{-1} (day 28) and at 10,000 part. ml^{-1} (day 28 and 42)	
		PP	Fibers	20–75 × 20 μm	22.5–90 part. ml^{-1}	AM	Mortality	Significant, dose-dependent increase in mortality (LOEC: 45 part. ml^{-1}, LC$_{50}$: 71 part. ml^{-1})	Au et al., 2015
							Development	Significant, dose-dependent decrease in dw (LOEC: 45 part. ml^{-1})	
Mollusca	Potamopyrgus antipodarum	PA	Fragments	4.64–602 μm	30 and 70%	Food	Mortality	No effects on mortality	Imhof and Laforsch, 2016
							Development	No effects on adult development	
							Reproduction	No effects on reproduction	
							Mortality	No effects on mortality	
							Development	No effects on juvenile development	
		PC	Fragments	4.64–602 μm	30 and 70%	Food	Mortality	No effects on mortality	Imhof and Laforsch, 2016
							Development	No effects on adult development	
							Reproduction	No effects on reproduction	
							Mortality	No effects on mortality	
							Development	No effects on juvenile development	
		PET	Fragments	4.64–602 μm	30 and 70%	Food	Mortality	No effects on mortality	Imhof and Laforsch, 2016

(Continued)

TABLE 1 | Continued

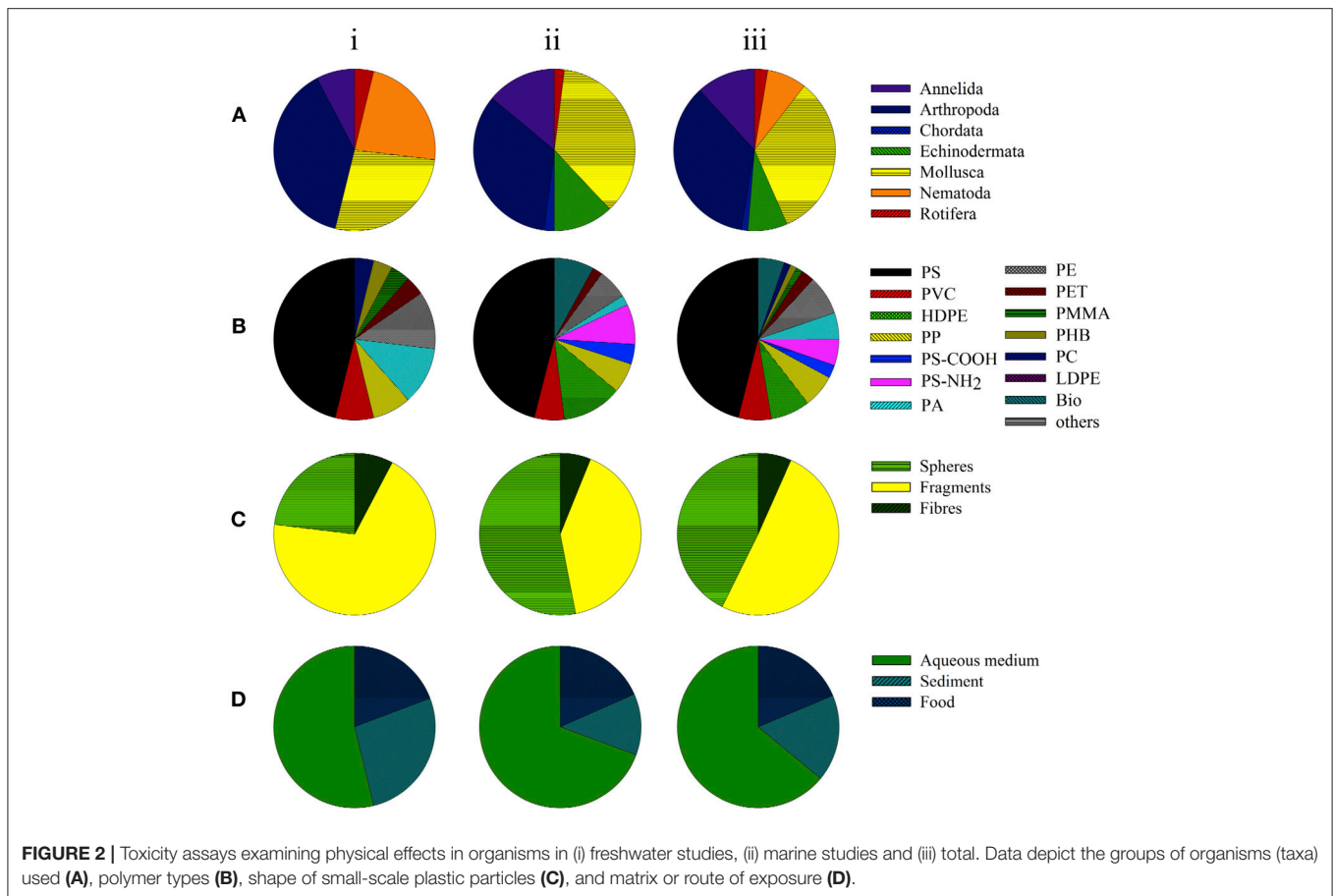| Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	References
							Development	No effects on adult development	
							Reproduction	No effects on reproduction	
							Mortality	No effects on mortality	
		PS	Fragments	4.64–602 μm	30 and 70%	Food	Development	No effects on juvenile development	Imhof and Laforsch, 2016
							Mortality	No effects on mortality	
							Development	No effects on adult development	
							Reproduction	No effects on reproduction	
		PVC	Fragments	4.64–602 μm	30 and 70%	Food	Mortality	No effects on mortality	Imhof and Laforsch, 2016
							Development	No effects on juvenile development	
							Mortality	No effects on mortality	
							Development	No effects on adult development	
							Reproduction	No effects on reproduction	
							Mortality	No effects on mortality	
							Development	No effects on juvenile development	
	Sphaerium corneum	PS	Fragments	20–500 μm	0.1–40% sed. dw	S	Mortality	No effects on mortality	Redondo-Hasselerharm et al., 2018
							Development	No effects on growth	
							Behavior	No effects on feeding rate	
	Dreissena polymorpha	PS	Spheres	1 and 10 μm	$5 \times 10^5 + 5 \times 10^5$ part. l^{-1}, $2 \times 10^6 + 2 \times 10^6$ part. l^{-1}	AM	Cellular response	CAT and gpx activity significantly affected at $5 \times 10^5 + 5 \times 10^5$ part. l^{-1}; No effects on levels of LPO and PCC; DOP significantly increased at $5 \times 10^5 + 5 \times 10^5$ part. l^{-1} (day 3) and $2 \times 10^6 + 2 \times 10^6$ part. L^{-1} (day 6); No effects on SER and GLU; No effects on the activity of ache, MAO and micronuclei frequency	Magni et al., 2018
Nematoda	*Caenorhabditis elegans*	PA	Fragments	~70 μm	0.5–10 mg m^{-2},	AM	Mortality	Significantly increased mortality (LOEC: 0.5 mg m^{-2})	Lei et al., 2018
					5 mg m^{-2}		Development	Significantly decreased body length	
							Reproduction	Number of embryos significantly lowered; No effect on brood size	
							Cellular Response	Calcium level in intestines significantly lowered; No effect on expression of *gst-4*	
		PP	Fragments	~70 μm	0.5–10 mg m^{-2},	AM	Mortality	Significantly increased mortality (LOEC: 0.5 mg m^{-2})	Lei et al., 2018

(Continued)

TABLE 1 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	References
					$5\,mg\,m^{-2}$		Development	Significantly decreased body length	
							Reproduction	Number of embryos significantly lowered; No effect on brood size	
							Cellular Response	No effect on calcium level in intestines; Expression of *gst-4* significantly increased	
		PE	Fragments	$\sim$70 μm	0.5–$10\,mg\,m^{-2}$,	AM	Mortality	Significantly increased mortality (LOEC: $0.5\,mg\,m^{-2}$)	Lei et al., 2018
					$5\,mg\,m^{-2}$		Development	Significantly decreased body length	
							Reproduction	Number of embryos significantly lowered; Brood size significantly decreased	
							Cellular Response	Calcium level in intestines significantly lowered; Expression of *gst-4* significantly increased	
		PVC	Fragments	$\sim$70 μm	0.5–$10\,mg\,m^{-2}$	AM	Mortality	Significantly increased mortality (LOEC: $1\,mg\,m^{-2}$)	Lei et al., 2018
							Reproduction	Number of embryos significantly lowered; Brood size significantly decreased	
							Cellular Response	Calcium level in intestines significantly lowered; Expression of *gst-4* significantly increased	
Nematoda	Caenorhabditis elegans	PS	Spheres	0.1 μm	1–$10{,}000\,μg\,l^{-1}$	AM	Cellular Response	Significant, dose-dependent induction of intestinal ROS production from up 10 μg l^{-1}; Significant, dose-dependent increase in defecation cycle length from up 10 μg l^{-1}	Zhao et al., 2017
							Behavior	Significant, dose-dependent decrease of locomotion (head trash, body bend) from up 10 μg l^{-1}	
							Reproduction	Significant, dose-dependent decrease of reproduction (brood size) from up 10 μg l^{-1}	
							Development	Significant, transgenerational effects on F1-generation in terms of intestinal ROS production, locomotion behavior and reproduction form up 100 μg l^{-1}	

(Continued)

TABLE 1 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	References
			Spheres	0.1 μm	0.5–10 mg m^{-2},	AM	Mortality	Significantly increased mortality (LOEC: 0.5 mg m^{-2}),	Lei et al., 2018
				1 μm	5 mg m^{-2}			Effects significantly size-depending, with slight effects induced by 0.1 μm part., strongest effects induced by 1 μm part.	
				5 μm			Development	Significantly decreased body length	
							Reproduction	Number of embryos significantly lowered for 0.1 and 1 μm part.; Brood size significantly lowered for 0.1 and 1 μm part.	
							Cellular Response	Calcium level in intestines significantly lowered for 1 μm; Expression of gst-4 significantly increased	
Rotifera	Brachionus koreanus	PS	Spheres	0.05 μm	0.1–20 μg ml^{-1},	AM	Reproduction	Reproduction time significantly increased for 0.05 μm at 10 μg ml^{-1};	Jeong et al., 2016
				0.5 μm	10 μg ml^{-1}			Fecundity significantly affected for 0.05 and 0.5 μm (LOEC 0.05 μm: 1 μg ml^{-1}, LOEC 0.5 μm: 20 μg ml^{-1});	
				6 μm				Population growth significantly retarded for 0.05 and 0.5 μm	
							Life-Span	Life-span significantly affected for 0.05 and 0.5 μm (LOEC 0.05 μm: 0.1 μg ml^{-1}, LOEC 0.5 μm: 20 μg ml^{-1})	
							Cellular Response	ROS level significantly increased under microplastic treatments; Significantly affected exposure of antioxidant-related enzymes; Phosphorylation status of p-JNK and p-p38 increased for 0.05 μm	

FIGURE 2 | Toxicity assays examining physical effects in organisms in (i) freshwater studies, (ii) marine studies and (iii) total. Data depict the groups of organisms (taxa) used **(A)**, polymer types **(B)**, shape of small-scale plastic particles **(C)**, and matrix or route of exposure **(D)**.

PHB-fragments in various size-ranges significantly decreased the wet weight of this species (Straub et al., 2017; **Table 1**). Among the studies focusing on freshwater arthropods, size-dependent effects were reported for PE-spheres again (Ziajahromi et al., 2018), with significantly reduced and delayed development of *C. tepperi* induced by small particles (**Table 1**). Effects in a dose-dependent manner were observed by Au et al. (2015), reporting impacts on dry weight of *H. azteca* when exposed to PE-fragments and PP-fibers in various concentrations (**Table 1**). However, PA-, PC-, PET-, PS-, and PVC-fragments included in food did not induce any significant effects on the development of *P. antipodarum* (Imhof and Laforsch, 2016; **Table 1**), while PA-, PP-, PE-, and PVC-fragments significantly reduced the body length of *C. elegans* (Lei et al., 2018). The latter study also showed equivalent reductions in the growth of this nematode species by the application of different PS-spheres (Lei et al., 2018; **Table 1**).

Reproduction

The effects on reproduction were investigated in 50% ($n = 13$; **Table 1**) of the analyzed experiments. Significant dose-dependent effects of plastic particles were reported by Au et al. (2015), investigating the impact of PE-particles on the reproduction of *H. azteca,* and by Zhao et al. (2017), observing dose-depending reductions in brood sizes of *C. elegans* treated with PS-spheres (**Table 1**). Additionally, effects were also reported

to be size-dependent on *C. elegans*, as shown by Lei et al. (2018), who reported significant inhibitory effects of smallest PS-spheres applied (**Table 1**). Comparable size- and dose-dependent effects were also reported for *B. koreanus*, with a significant prolongation of its reproduction time and a reduced fecundity after exposure to PS nanoparticles (Jeong et al., 2016; **Table 1**). Additionally, plastic fragments of various origins (PA, PP, PE, and PVC) significantly reduced reproductive success of *C. elegans*, while only PE- and PVC-fragments affected brood sizes significantly (Lei et al., 2018; **Table 1**). However, fragments of PA, PC, PET, PS, and PVC had no apparent impact on the reproduction of *P. antipodarum* (Imhof and Laforsch, 2016; **Table 1**).

Behavior

Behavioral alterations induced by small-scale plastic particles were investigated in 11 assays, being tantamount with 42% of the experiments described in the included studies (**Table 1**). No alterations in feeding behavior were observed following the exposure of *L. variegatus*, *Tubifex* spp., *A. aquaticus*, *G. pulex*, *H. azteca,* or *S. corneum* to PS-fragments (Redondo-Hasselerharm et al., 2018), or *G. fossarum* to PS-spheres (Blarer and Burkhardt-Holm, 2016). Additionally, neither effects of PA-fibers nor of PMMA- or PHB-fragments on *G. fossarum* could be observed (Blarer and Burkhardt-Holm, 2016; Straub et al., 2017). By contrast, nano-sized PS-spheres significantly reduced

the locomotion of *C. elegans* in a dose-dependent manner (Zhao et al., 2017; **Table 1**).

Cellular response

Cellular responses, including alterations in gene expression, reactive oxygen species (ROS) production, and enzyme activity, were the assessed end points of 31% ($n = 8$) of the studies (**Table 1**). Effects of different PS-spheres at various concentrations on the mussel *D. polymorpha* were investigated by Magni et al. (2018), analyzing various biomarkers (**Table 1**). While neither biomarkers of oxidative damage, neuro-genotoxicity, the activities of neuro-enzymes nor the measured frequency of micronuclei were affected, enzyme activity of catalase (CAT), glutathione peroxidase (GPx), as well as measured dopamine levels were significantly altered in treated organisms (Magni et al., 2018; **Table 1**). The assessed biomarkers of *C. elegans* showed to be material-dependent with a significant up-regulation of glutathione-S-transferase (GST) after exposure to PE-, PP-, and PVC-fragments, while PA-fragments had no effect, and significantly altered intestinal calcium levels observed in *C. elegans* exposed to PA-, PE-, and PVC-fragments exclusively (Lei et al., 2018; **Table 1**). The presence of PS-spheres in various sizes could be shown to induce a general significant up-regulation of GST additionally, whereas measured calcium levels were affected in a size-dependent manner (Lei et al., 2018; **Table 1**). This size-dependent toxicity of PS-spheres on several cellular biomarkers was also reported by Jeong et al. (2016) for *B. koreanus*. While the levels of intracellular ROS were generally higher in treated rotifers, size-dependencies were reported for glutathione reductase (GR), superoxide dismutase (SOD), GPx, GST, and the phosphorylation status of mitogen-activated protein kinase (MAPK) signaling proteins with significant effects after the exposure to smallest spheres applied (Jeong et al., 2016; **Table 1**).

Others

The parameters assimilation efficiency ($n = 4$), life-span ($n = 1$), and emergence ($n = 1$) were assessed in 23% of the respective experiments together. Studies by Blarer and Burkhardt-Holm (2016) and Straub et al. (2017) on the assimilation efficiency of *G. fossarum* revealed significant effects induced by PA-fibers and PMMA-fragments, while PS-spheres and PHB-fragments did not show any effects (**Table 1**). Regarding the parameter life-span, *B. koreanus* revealed size- and dose-dependent impacts of PS-spheres at various concentrations, with life-span being significantly shortened by the smallest spheres applied (Jeong et al., 2016; **Table 1**). However, no dependencies in terms of effects could be shown regarding the emergence of *C. riparius*, being equally lowered by the presence of PE-spheres of various sizes (Ziajahromi et al., 2018).

Marine Benthic Fauna

Mechanically induced effects have also been investigated frequently in marine settings, with 50 studies being included in the review process. Organisms belonging to Mollusca were most commonly used (36%, $n = 18$; **Figure 2A**), especially those of the *Mytilus* complex (mostly *M. edulis* and *M. galloprovincialis*),

followed by the flat oyster *Ostrea edulis* and the Pacific oyster *Crassostrea gigas*, and, in only one study, *Atactodea striata* (**Table 2**). Arthropods (34%, $n = 17$; **Figure 2A**) were another common target organism, with crustaceans investigated exclusively. These consisted mostly of several decapods, including *Carcinus maenas* and *Palaemonetes pugio*, the calanoid species *Calanus finmarchicus* and *C. helgolandicus*, as well as *Centropages typicus* and *Parvocalanus crassirostris* (13%, $n = 3$), but also the isopod *Idotea emarginata* (6%, $n = 1$) and the cyclopoid species *Paracyclopina nana* and *Tigriopus japonicas* (6%, $n = 1$), as shown in **Table 2**. Other targets were annelids (14%, $n = 7$), mostly *Arenicola marina,* but also *Perinereis aibuhitensis* and the ragworm *Hediste diversicolor*. Beside, Echinodermata were investigated frequently (12%, $n = 6$), mostly *Paracentrotus lividus* and the sea urchin *Tripneustes gratilla* (83%, $n = 5$; **Table 2**, **Figure 2A**). Single studies were conducted on the ascidian *Ciona robusta* and the marine rotifer *Brachionus manjavacas* (**Table 2**).

Most studies of marine benthic organisms investigated PS exposure, including 10 experiments assessing the mechanical impact of functionalized PS-particles PS-COOH (4%, $n = 2$) and PS-NH$_2$H (8%, $n = 4$; **Figure 2B**; **Table 2**). PE, specifically, High-density polyethylene (HDPE) particles, was the seconds most frequently tested plastic (12%, $n = 6$), followed by PVC, PP, and PE (6%, $n = 3$ each), biodegradable polyactic acid (PLA, 8%), and PA and PET (4% each; **Figure 2B**; **Table 2**). In 56% ($n = 28$) of the marine studies, the studied plastic particles were applied as spheres (**Figure 2C** and **Table 2**), with fragments contributing 38% ($n = 19$; **Figure 2C**) and polymer fibers 6% ($n = 3$; **Figure 2C**).

The main application route was via aqueous medium (70%, $n = 35$; **Figure 2D**), while effects of plastic particles applied in sediment and in spiked food were analyzed in only 12% ($n = 6$) and 18% ($n = 9$) of the studies, respectively (**Figure 2D**).

Mortality

Lethal effects were examined in 56% ($n = 28$; **Table 2**) of the relevant experiments, by this being the most frequently assessed parameter among the included marine studies. With regard to the model organism *A. marina*, no effects on mortality could be observed when treated with a variety of microplastics in sediments, including PVC-, PLA-, and HDPE-fragments (Green et al., 2016b; **Table 2**) as well as PS-spheres (Besseling et al., 2012; **Table 2**). Even if no effects on mortality could be determined for *H. diversicolor* exposed to PVC-fragments (Gomiero et al., 2018), Leung and Chan (2018) reported significantly increased mortality of another marine annelid, *P. aibuhitensis*, by small PS-spheres in a size-depending manner (**Table 2**). This size-dependency was supported by Lee et al. (2013), reporting significantly increased mortality rates of two generations of *T. japonicus* when treated with PS-spheres in various sizes (**Table 2**). However, the mortality rates of other copepod species (*C. finmarchicus* and *C. helgolandicus*) were not affected when PS-spheres where applied (Cole et al., 2015; Vroom et al., 2017; **Table 2**). The same results were obtained by Watts et al. (2016), reporting no effects of PS-spheres on the decapod *C. maenas*, neither of functionalized nor of non-functionalized particles

TABLE 2 | Effects of micro- and nano-sized plastic particles on benthic marine invertebrates.

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
Annelida	Arenicola marina	PS	Fragments	400–1,300 µm	0.074–7.4% sed. dw	S	Mortality	No effects on mortality	Besseling et al., 2012
							Behavior	Significant, dose-dependent decrease of feeding activity	
							Development	Significant, dose-dependent decrease of dw, no effects on ww, and AFDW	
		PVC	Fragments	63–500 µm	0.5–5% sed. dw	S	Egestion	Significantly prolonged gut residence time at 5% sed.	Wright et al., 2013a
							Behavior	Significantly reduced feeding activity at 5% sed.	
							Cellular response	Significantly increased phagocytic activity at 0.5 and 5% sed.	
							Energy reserves	Significantly reduced energy reserves (J g^{-1} ww.) at 1 and 5% sed.; Significantly lowered lipid reserves for UPVC-exposure	
			Fragments	2.5–316 µm	0.02–2% sed. ww	S	Mortality	No effects on mortality	Green et al., 2016a
							Development	No effects on dw biomass	
							Behavior	Significantly less casts produced at 2% sed. ww	
							O$_2$ consumption	Significantly increased O$_2$ consumption at 2% sed. ww	
		PLA	Fragments	1.4–707 µm	0.02–2% sed. ww	S	Mortality	No effects on mortality	Green et al., 2016a
							Development	No effects on dw biomass	
							Behavior	No effects on bioturbation	
							O$_2$ consumption	Significantly increased O$_2$ consumption at 2% sed. ww	
		HDPE	Fragments	8.7–478 µm	0.02–2% sed. ww	S	Mortality	No effects on mortality	Green et al., 2016a
							Development	No effects on dw biomass	
							Behavior	No effects on bioturbation	
							O$_2$ consumption	Significantly increased O$_2$ consumption at 2% sed. ww	
	Perinereis aibuhitensis	PS	Spheres	8–12 µm	100 and 1,000 part. ml^{-1}	AM	Mortality	Smaller spheres increased mortality significantly	Leung and Chan, 2018
				32–38 µm			Regeneration	Significantly reduced regeneration rate at 1000 part. ml^{-1}, smaller particles being significantly more detrimental	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
	Hediste diversicolor	PVC	Fragments	250 μm	200 and 2,000 part. kg⁻¹	S	Mortality	No effects on mortality	Gomiero et al., 2018
							Cellular response	Significantly increased phagocytic activity at 200 part. kg⁻¹ (10 d) and 2,000 part. kg⁻¹ (10 and 28 d); No effects on extracellular lysosome release, lysosomal membrane stability (LMS) and oxyradical production; No effects micronuclei frequency and on DNA strand breaks; No effects on Lipofuscin content and Catalase activity	
Arthropoda	Calanus finmarchicus	PS	Spheres	15 μm	50 and 500 part. ml⁻¹	AM	Mortality	No effects on mortality	Vroom et al., 2017
	Calanus helgolandicus	PS	Spheres	20 μm	75 part. ml⁻¹	AM	Behavior	Feeding rate significantly reduced; Significant shift to small sized prey	Cole et al., 2015
							Mortality	No significant effects on mortality	
							Reproduction	No significant effects on egg production rate; Hatching success significantly decreased from up day 6	
							Development	Significantly smaller eggs produced from up day 7	
							O₂ consumption	No effects on O₂ consumption	
							Assimilation	2-fold greater energetic losses expected	
	Carcinus maenas	PP	Fibers	1,000–5,000 μm	0.3–1.0% by weight	AM	Behavior	Feeding rate significantly decreased	Watts et al., 2015
							Assimilation	Significantly reduced scope for growth	
	Carcinus maenas	PS	Spheres	8 μm	10⁶ and 10⁷ part. l⁻¹	AM	Mortality	No effects on mortality	Watts et al., 2016
							O₂ consumption	O₂ consumption significantly reduced (1 h) at 10⁷ PS part. l⁻¹	
							Cellular response	Significant effects on Na⁺-Ions and Ca²⁺-Ions (24 h) at 10⁷ PS part. l⁻¹,	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
		PS-COOH	Spheres	8 μm	10^6 and 10^7 part. l^{-1}	AM	Mortality	no effects on K^+-Ion concentration; Significant, dose-dependent effects on hemocyanin concentration; No effect on hemolymph protein concentration; No effects on mortality	Watts et al., 2016
		PS-NH$_2$	Spheres	8 μm	10^6 and 10^7 part. l^{-1}	AM	O$_2$ consumption; Cellular response; Mortality	No effects on O$_2$ consumption; No cellular responses induced; No effects on mortality	Watts et al., 2016
	Centropages typicus	PS	Spheres	7.3 μm	4,000–25,000 part. ml^{-1}	AM	O$_2$ consumption; Cellular response; Behavior	No effects on O$_2$ consumption; No cellular responses induced; Significantly, dose-dependently reduced total algae feeding rate	Cole et al., 2013
	Idotea emarginata	PS	Spheres	10 μm	12 part. mg food^{-1}	Food	Mortality; Behavior; Development	No effects on survival; No effects on feeding rate; No effects on the duration of intermolt periods and growth	Hämer et al., 2014
Arthropoda	Idotea emarginata	PS	Fragments	1–100 μm	20 part. mg food^{-1}	Food	Mortality; Behavior; Development	No effects on mortality; No effects on feeding rate; No effects on the duration of intermolt periods and growth	Hämer et al., 2014
		PA	Fibers	20–2,500 μm	0.3 mg part. mg food^{-1}	Food	Mortality; Behavior; Development	No effects on mortality; No effects on feeding rate; No effects on the duration of intermolt periods and growth	Hämer et al., 2014
	Palaemonetes pugio	PE	Spheres	35–165 μm	50,000 part. l^{-1}	AM	Mortality	Significantly increased mortality by larger particles (>75 μm)	Gray and Weinstein, 2017
		PS	Spheres	30 and 75 μm	50,000 part. l^{-1}	AM	Mortality	Significantly increased mortality by larger particles (75 μm)	Gray and Weinstein, 2017
		PP	Fragments	34 and 93 μm	50,000 part. l^{-1}	AM	Mortality	Significantly increased mortality by larger particles (93 μm)	Gray and Weinstein, 2017

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
			Fibers	34 and 93 μm	50,000 part. l^{-1}	AM	Mortality	Significantly increased mortality	Gray and Weinstein, 2017
	Paracyclopina nana	PS	Spheres	0.05 μm	0.1–20 μg ml^{-1}	AM	Development	Development significantly delayed for 0.05 μm (LOEC: 10 μg ml^{-1})	Jeong et al., 2017
				0.5 μm			Reproduction	Fecundity significantly reduced for 0.05 μm (LOEC: 10 μg ml^{-1}) and 0.5 μm (LOEC: 20 μg ml^{-1})	
				6 μm	20 μg ml^{-1}		Cellular response	Significant increase in intracellular ROS level by 0.05 μm spheres; Significantly increased phosphorylation of p-ERK, p-p38 and Nrf2; Significantly increased activity of GPx (0.05 μm), GR, GST, and SOD	
	Parvocalanus crassirostris	PET	Fragments	5–10 μm	10,000–80,000 part. ml^{-1}	AM	Reproduction	Significantly reduced egg production (LOEC 80,000 part. ml^{-1})	Heindler et al., 2017
					20,000 part. ml^{-1}			Relative population size significantly reduced after 6 and 24 d	
					20,000 part. ml^{-1}		Cellular response	Significantly down regulated *H3* gene expression	
	Tigriopus japonicus	PS	Spheres	0.05 μm	6–313 μg ml^{-1}	AM	Mortality	No effects on mortality	Lee et al., 2013
				0.5 μm	0.125–25 μg ml^{-1}		Mortality	Survival of F_0 generation significantly effected for 0.05 μm (LOEC: 1.25 μg ml^{-1}, LC_{50}: 2.15 μg ml^{-1}),	
				6 μm				Survival of F_1 generation significantly effected for 0.05 μm (LOEC: 1.25 μg ml^{-1}, LC_{50}: 0.16 μg ml^{-1}) and 0.5 μm (LOEC: 25 μg ml^{-1}, LC_{50}: 23.5 μg ml^{-1})	
							Reproduction	Fecundity of F_0 generation significantly decreased for 0.5 μm (LOEC: 0.125 μg ml^{-1}, EC_{50}: 0.07 μg ml^{-1}) and 6 μm (LOEC: 0.125 μg ml^{-1}, EC_{50}: 0.10 μg ml^{-1}),	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
								Fecundity of F_1 generation significantly decreased for 0.5 μm (LOEC: 0.125 μg ml^{-1}, EC$_{50}$: 0.04 μg ml^{-1}) and 6 μm (LOEC: 0.125 μg ml^{-1}, EC$_{50}$: 0.04 μg ml^{-1})	
							Development	Nauplius phase of F_0 generation significantly delayed for 0.05 μm at 1.25 μg ml^{-1}, Generation time of F_1 generation significantly delayed for 0.05 μm at 1.25 μg ml^{-1} and for 0.5 μm at 25 μg ml^{-1} (EC$_{50}$ 21.2–21.4 μg ml^{-1})	Messinetti et al., 2018
Chordata	Ciona robusta	PS	Spheres	10 μm	0.125–25 μg ml^{-1}	AM	Development	Significantly delayed juvenile development, no effects on larvae	Messinetti et al., 2018
							Mortality	No effects on larval and juvenile mortality	
Echinodermata	Paracentrotus lividus	PS	Spheres	10 μm	0.125–25 μg ml^{-1}	AM	Development	Significantly effected body length from up 12.5 μg ml^{-1}, Significantly reduced body weight at 0.125 μg ml^{-1}, Significantly effected arm length at 0.125, 12.5 and 25 μg ml^{-1}	Martínez-Gómez et al., 2017
							Mortality	No effects on larval mortality	
			Spheres	6 μm	10^3–10^5 part. ml^{-1}	AM	Reproduction	Significantly reduced fertilization rate (LOEC 10^3 part. ml^{-1})	
							Development	Significantly affected larval development of pre-exposed zygotes, Significantly reduced larval growth of pre-exposed gametes, Significantly affected embryo development at 10^3 and 10^4 part. ml^{-1} of virgin PS and at each concentration for aged particles	
		PS-COOH	Spheres	0.04 μm	25 μg ml^{-1}	AM	Cellular response	Significantly up regulated Abcb1-genes at 25 μg ml^{-1} 48 hpf	Della Torre et al., 2014
					2.5–50 μg ml^{-1}		Development	No significant effects on development	
		PS-NH$_2$	Spheres	0.05 μm	3 μg ml^{-1}	AM	Cellular response	Significantly up regulated cas-8-genes at 3 μg ml^{-1} 24 hpf	
					1–50 μg ml^{-1}		Development	Significantly increased malformations (EC$_{50}$ 24 hpf 3.82 μg ml^{-1}, 48 hpf 2.61 μg ml^{-1})	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
		HDPE	Fragments	>0–80 μm	0.005–5 g l^{-1}	AM	Development	Significantly affected larval development of pre-exposed zygotes, Significantly reduced larval growth of pre-exposed gametes, Significantly affected embryo development at 10^3 and 10^5 part. ml^{-1} of virgin HDPE and at each concentration for aged particles	Martínez-Gómez et al., 2017
	Tripneustes gratilla	PE	Spheres	10–45 μm	1–300 part. ml^{-1}	AM	Development	Significantly reduced body width at 300 part. ml^{-1}	Kaposi et al., 2014
							Mortality	No effects on larval mortality	
	Atactodea striata	PS	Fragments	63–250 μm	10 and 1,000 part. l^{-1}	AM	Behavior	Feeding rate significantly reduced at 1,000 part. l^{-1}	Xu et al., 2017
							Assimilation	No effects on absorption efficiency	
							O$_2$ consumption	No effects on respiration rate	
Mollusca	*Crassostrea gigas*	PS	Spheres	1 and 10 μm	1–1,000 part. ml^{-1}	AM	Behavior	No significant effects on feeding rate	Cole and Galloway, 2015
					100 part. ml^{-1}		Development	No effects on growth	Sussarellu et al., 2016
				2 and 6 μm	0.023 mg l^{-1}		Behavior	Significantly increased feeding rate	
							Assimilation	Significantly increased absorption efficiency	
							Reproduction	Significantly reduced oocyte number	
							Development	Significantly reduced oocyte diameter and quality (D-larval yield); Significantly reduced sperm velocity; Significantly reduced larval growth	
							Cellular response	Significantly increased sizes of hyalinocytes and granulocytes; Significant effects on transcript expression in digestive glands, gonads and oocytes; Significant effects on the proteome of oocytes	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
	Mytilus edulis	PLA	Fragments	0.6–363 μm	2.5 and 25 μg l⁻¹	Food	Behavior	Significantly decreased feeding rate at 25 μg l⁻¹	Green et al., 2016b
		HDPE	Fragments	0.48–316 μm	2.5 and 25 μg l⁻¹	Food	Behavior	Significantly decreased feeding rate at 25 μg l⁻¹	Green et al., 2016b
			Fragments	>0–80 μm	2.5 g l⁻¹	AM	Mortality	No effects on mortality	von Moos et al., 2012
							Cellular response	Significantly increased granulocytoma formation,	
								Significantly decreased lysosomal membran stability (LMS)	
		PS	Spheres	3 and 9.6 μm		AM	Mortality	No effects on mortality	Browne et al., 2008
							Behavior	No effects on feeding rate	
							Cellular response	No effects on oxidative status of hemolymph, viability and phagocytic activity of hemocytes	
			Spheres	2 and 6 μm	32 μg l⁻¹	AM	Cellular response	Significantly increased number of dead hemocytes;	Paul-Pont et al., 2016
								Significantly increased ROS production;	
								Significantly reduced catalase (CAT) activity,	
								Significantly reduced lipid peroxidation (LPO),	
								Significantly reduced glutathione reductase (GR);	
								Significantly effected anti-oxidant gene expression	
							Development	Significantly increased histopathological observations	
			Spheres	0.03 μm	0.1–0.3 g l⁻¹	AM	Egestion	Significantly increased production of pseudofeces	Wegner et al., 2012
							Behavior	Significantly decreased feeding rates	
	Mytilus galloprovincialis	PS	Spheres	2 and 6 μm	32 μg l⁻¹	AM	Cellular response	Significantly increased number of dead hemocytes;	Paul-Pont et al., 2016
								Significantly increased ROS production;	
								Significantly reduced catalase (CAT) activity,	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
			Fragments	<100 μm	1.5 g l^{-1}	AM	Development	Significantly reduced lipid peroxidation (LPO),	Avio et al., 2015
								Significantly reduced glutathione reductase (GR);	
								Significantly effected anti-oxidant gene expression	
								Significantly increased histopathological observations	
							Mortality	No effects on mortality	
							Cellular response	Significantly decreased LMS	
								No effects on hemocytes and phagocytosis activity	
								Significantly decreased AChE levels in gills	
		PS-NH$_2$	Spheres	0.05 μm	1–50 μg ml^{-1}	AM	Cellular response	Significantly inhibited Se-dependent glutathione peroxidase	Canesi et al., 2015
								Significantly decreased lysosomal membrane stability (LMS; LOEC 5 μg ml^{-1})	
								Significant increase in lysozyme release (LOEC: 1 μg ml^{-1})	
								Significantly increased ROS production (LOEC: 1 μg ml^{-1})	
								Significantly increased NO production	
								Significantly decreased phagocytosis (LOEC: 1 μg ml^{-1})	
								Significantly effected apoptotic parameters (ANX+, ANX+/PI+, TMRE, NAO; LOEC: 50 μg ml^{-1})	
			Spheres	0.05 μm	0.001–20 mg l^{-1}	AM	Development	Significantly decreased larval development (LOEC: 0.01 mg l^{-1}, EC$_{50}$: 0.142 mg l^{-1})	Balbi et al., 2017
					0.15 mg l^{-1}		Cellular response	Significantly effected gene expression (CS, CA, EP, ABCB and LYSO)	
Mollusca	*Mytilus galloprovincialis*	PE	Fragments	<100 μm	1.5 g l^{-1}	AM	Mortality	No effects on mortality	Avio et al., 2015
							Cellular response	Significantly reduced Granulocytes/Hyalinocytes ratio	
								No effects on hemocytes and phagocytosis activity	
								Significantly decreased *AChE* levels in gills	

(Continued)

TABLE 2 | Continued

Taxa	Species	Material	Shape	Size	Conc.	Matrix	Parameter	Effects	Reference
	Ostrea edulis	HDPE	Fragments	0.48–316 μm	2.5 and 25 μg l^{-1}	Food	Behavior	Significantly increased feeding rates at 25 μg l^{-1}	Green et al., 2016b
							Mortality	No effects on mortality	
			Fragments	0.48–316 μm	80 μg l^{-1}	Food	Behavior	No effects on feeding rate	Green, 2016
							Development	No effects on shell growth	
							O$_2$ consumption	No effects on respiration rates	
		PLA	Fragments	0.6–363 μm	2.5 and 25 μg l^{-1}	Food	Behavior	Significantly increased feeding rates at 2.5 and 25 μg l^{-1}	Green et al., 2016b
							Mortality	No effects on mortality	
			Fragments	0.6–363 μm	80 μg l^{-1}	Food	Behavior	No effects on feeding rate	Green, 2016
							Development	No effects on shell growth	
							O$_2$ consumption	No effects on respiration rates	
Rotifera	*Brachionus manjavacas*	PS	Spheres	0.05–3 μm	0.72–5.74 μg ml^{-1}	AM	Behavior	Feeding rate dose-dependently reduced by plastic particles, effects size-dependent up to particles of 0.5 μm	Snell and Hicks, 2011
							Reproduction	Significantly reduced population growth rate at 1.14 μg ml^{-1} for 0.05 μm particles (NOEC 0.57 μg ml^{-1}), no effects for larger particles; No effects on population growth rate of F1 generation	

(**Table 2**). Additionally, mortality of *I. emarginata* was also unaffected by PS-spheres, PS-fragments and PA-fibers (Hämer et al., 2014; **Table 2**). By contrast, Gray and Weinstein (2017) reported increased mortality rates of *P. pugio* treated with PE- and PS-spheres, PP-fragments, and PP-fibers (**Table 2**). PP-fibers generally induced significantly increased mortality, while the PE-spheres, PS-spheres, and the PP-fragments affected mortality rates in a size-dependent manner with larger particles being responsible for significant effects exclusively (**Table 2**). Lethal effects of PS- and PE-spheres were also assessed in *Ciona robusta* (Chordata; Messinetti et al., 2018), *Paracentrotus lividus* (Echinodermata; Messinetti et al., 2018) and *Tripneustus gratile* (Echinodermata; Kaposi et al., 2014; **Table 2**), with no effects being observed. Among the investigated marine molluscs, no lethal effects induced by a variety of plastic particles could be found (**Table 2**). Investigations on the lethality of HDPE-fragments in the blue mussel *M. edulis* and the flat oyster *O. edulis* failed to show any effects (von Moos et al., 2012; Green et al., 2016a; **Table 2**), which was also the case regarding *O. edulis* treated with biodegradable PLA-fragments (Green et al., 2016a). For *M. galloprovincialis*, neither PS- nor PE-fragments induced mortality as reported by Avio et al. (2015; **Table 2**).

Development

Effects on the development of benthic organisms in marine environments were examined in 46% of the studies (*n* = 23), as summarized in **Table 2**. While PVC-, PLA-, and HDPE-fragments did not induce any negative effects on the development of *A. marina* (Green et al., 2016b; **Table 2**), Besseling et al. (2012) reported significant effects on *A. marina* in terms of dry weight (dw) loss in a dose-dependent manner when exposed to PS-spheres (**Table 2**). These findings are in line with negative effects induced by PS-spheres on *C. helgolandicus* (Cole et al., 2015; **Table 2**). However, neither PS- nor PA-particles of various sized and shapes affected *I. emarginata* as reported by Hämer et al. (2014; **Table 2**). Additionally, effects of PS-spheres have further been reported to be size-dependent as indicated for the intergenerational developmental responses of *T. japonicas* (Lee et al., 2013) and development time of *P. nana* (Jeong et al., 2017), with both parameters being affected by nano-sized spheres exclusively (**Table 2**). However, studies examining the impact of small-scale plastics on echinoderms reported significant dose-dependent effects for a variety of applied particles, such as PS-spheres on *T. gratilla* (Kaposi et al., 2014), and significantly affected development of *P. lividus* exposed to plain PS-spheres (Martínez-Gómez et al., 2017; Messinetti et al., 2018), nanospheres of functionalized PS particles (PS-NH$_2$; Della Torre et al., 2014) and HDPE-fragments (Martínez-Gómez et al., 2017). In terms of PS-spheres, comparable results were reported for the ascidian *C. robusta*, with a significant delay in juvenile development but no effects on larvae (Messinetti et al., 2018; **Table 2**). The impacts of micro- and nanoplastics on molluscan development were examined in *C. gigas*, *M. galloprovincialis,* and *O. edulis* (**Table 2**). While no effects were reported for *C. gigas* in a short-term experiment (Cole and Galloway, 2015), enhanced exposure time led to various significantly development parameters (Sussarellu et al., 2016). In terms of *M. edulis*

and *M. galloprovincialis,* significant effects in a dose-dependent manner were reported for functionalized and non-functionalized PS-spheres (Paul-Pont et al., 2016; Balbi et al., 2017; **Table 2**), whereas shell growth of *O. edulis* was neither affected by HDPE- nor PLA-fragments (Green, 2016; **Table 2**).

Behavior

Behavioral alterations of benthic marine organisms due to nano- and micro-sized plastics were assessed in 46% of the studies (*n* = 23; **Table 2**). Studies of potential behavioral alterations induced by plastic particles included an examination of the effects on the bioturbation activity of *A. marina* exposed to PVC-, PLA- and HDPE-fragments (Green et al., 2016b). However, after 1 month, only the PVC-fragments had induced a reduction in behavior of *A. marina*, with no effects were observed for any of the other tested polymers (**Table 2**; Green, 2016). Most of the studies concerning behavioral alterations induced by small-scale plastic particles measured the feeding rate of the test organisms (**Table 2**). This was the case in studies conducted by Besseling et al. (2012) and Wright et al. (2013a) on the effects of PS-spheres and PVC-fragments in *A. marina* respectively, with feeding activity being reported to be affected dose-dependently (**Table 2**). Comparable results were measured for the arthropods *C. helgolandicus* (Cole et al., 2015) and *C. typicus* (Cole et al., 2013) respectively, with reduced feeding activities in a dose-dependent manner after exposure to PS-spheres (**Table 2**). Likewise, dose-dependent effects were also reported regarding the feeding rates of *A. striata* when treated with PS-spheres (Xu et al., 2017), as well as for PLA- and HDPE-fragments on *M. edulis* respectively (Green et al., 2016b; **Table 2**). However, neither PS-fragments or PA-fibers did impact the feeding rates of *I. emarginata* (Hämer et al., 2014; **Table 2**), nor did PS-spheres induce behavioral effects on *M. edulis* (Browne et al., 2008; **Table 2**). Similarly, Cole and Galloway (2015) did not find any significant effects of PS-spheres on the feeding activity of *C. gigas* in a short-term experiment, whereas Sussarellu et al. (2016) reported significantly higher algal consumption by oysters exposed for extended periods of time (**Table 2**). Dose-dependent variations in the impacts of plastic particles on the feeding behavior of *O. edulis* were reported by Green et al. (2016a,b), with HDPE- and PLA-fragments significantly enhancing feeding rates of mussels at particular concentrations exclusively (**Table 2**). Exposed to PS-spheres, size dependency was reported for the feeding activity of the rotifer *B. manjavacas* (Snell and Hicks, 2011), with effects being observed for the smallest particles only (**Table 2**).

Egestion

Closely linked to changes in feeding behavior are changes in egestion, being assessed in two experiments (2%; **Table 2**). Dose-dependent effects of PVC-fragments on the gut residence time of food in *A. marina* were reported by Wright et al. (2013a; **Table 2**), while the presence of nano-sized PS-spheres generally increased the production of pseudofeces in *M. edulis* (Wegner et al., 2012; **Table 2**).

Cellular response

The cellular responses of marine organisms to small-scale plastics were assessed in 36% ($n = 18$) of the studies (**Table 2**). PVC-fragments induced significantly increased phagocytic activity of immune cells in *A. marina* after chronic exposure, which was also the case for *H. diversicolor* as the proportions of phagocytic cells among coelomic fluid cells increased significantly in worms exposed to PVC particles (Gomiero et al., 2018). Heindler et al. (2017) examined the cellular responses of the arthropod *P. crassirostris* to PET-fragments with Histone 3 (*H3*) gene expression was significantly down regulated after 6 days but significant alterations were no longer detected after 18 days of recovery (**Table 2**). Additionally, *Hsp7p0-like* gene expression was not affected neither after 6 days of exposure nor at the end of the recovery phase (Heindler et al., 2017). Jeong et al. (2017) examined the molecular responses of the marine arthropod *P. nana* in terms of the activation of mitogen-activated protein kinase (MAPK), p38 and nuclear factor erythroid 2-related factor 2 (Nrf2), ROS levels, and the activities of the antioxidant enzymes GPx, glutathione reductase (GR), GST, and SOD. For ROS levels, phosphorylation of the MAPK protein extracellular signal-regulated kinase (p-ERK) as well as p38 and Nrf2, significant size-depending alterations were reported with smallest spheres inducing significant increases (**Table 2**). Additionally, size-dependencies were analyzed in antioxidant enzymes, with GPx activity was significantly enhanced in *P. nana* exposed to smallest PS-spheres exclusively, while the activities of GPx, GR, GST, and SOD were generally up regulated after plastic exposure of the copepod (**Table 2**). By contrast, there was no difference in the phosphorylation status of phosphorylated c-Jun N-terminal kinase (Jeong et al., 2017). Watts et al. (2016) also examined the impact of PS, exposing the crab *C. maenas* to non-functionalized and functionalized (PS, PS-COOH, and PS-NH$_2$) spheres. Exposure to neutrally charged spheres resulted in significant effects on several hemolymph constituents and significantly reduced hemocyanin concentrations in a dose-dependent manner, while protein concentrations in the hemolymph of the crabs remained unchanged (**Table 2**). Overall, the exposure of *C. maenas* to carboxyl- or amino-coated plastics had no significant effect on any assessed parameters (**Table 2**; Watts et al., 2016). The impact of functionalized spheres was also assessed by Della Torre et al. (2014), who treated *P. lividus* with nanoparticles of PS-COOH and PS-NH$_2$ (**Table 2**). PS-COOH spheres significantly up-regulated *Abcb1* gene expression, whereas exposure to the amino-coated spheres induced an up-regulation of cas-8. No other effects on further analyzed stress genes (14-3-3ε, Abcc5, cas8, and p-38 MAPK) were observed (**Table 2**). Sussarellu et al. (2016) investigated the effects of PS-spheres on the mollusc *C. gigas* by analyzing its hyalinocyte and granulocyte sizes. Both hemocyte types were significantly larger in exposed organisms and the oxidative activity of these cells was altered (**Table 2**). Additionally, transcriptomic and proteomic analyses revealed further plastic-induced effects on *C. gigas*: within digestive glands, two clusters of transcripts exhibited similar expression patterns (up and down regulated), with glucocorticoid stimulus, fatty acid catabolic processes, respiratory burst, and cellular response to

mechanical stimulus as the main significantly enriched Gene Ontology (GO) biological processes (Sussarellu et al., 2016). In gonads, the expression of transcripts related to glutamine biosynthetic processes, the positive regulation of insulin secretion and of epithelial cell proliferation, and ovarian follicle cell–cell adhesion were among the significantly enriched GO biological processes (Sussarellu et al., 2016). In the transcript of oocytes, the significantly enriched GO biological processes were proteolysis, embryo development, and ion binding. In addition, two abundant protein spots, identified as arginine kinase, were detected in the proteome. The expression of this enzyme was significantly lower in exposed oysters, while the expression of the protein severin was higher in their oocytes (Sussarellu et al., 2016). Regarding effects of irregularly shaped HDPE-fragments on the blue mussel *M. edulis*, von Moos et al. (2012) reported a significant increase in granulocytoma formation and a significantly decreased destabilization time of lysosomes, while no effects on biomarkers of oxyradical damage (lipofuscin accumulation) or on neutral lipid content could be found (**Table 2**). No effects on the oxidative status of the hemolymph or in hemocyte viability and phagocytic activity were measured by Browne et al. (2008), analyzing *M. edulis* treated with PS-spheres. However, Paul-Pont et al. (2016) exposed organisms from the Mytilus-complex (*M. edulis* and *M. galloprovincialis*) to PS-spheres and reported the percentage of dead hemocytes, ROS production and the activity of anti-oxidant enzyme, CAT, and lipid peroxidation (LPO), being significantly affected, while phagocytosis as well as hemocyte and granulocyte concentrations have been unaffected (**Table 2**). In terms of gene expression, only gill mRNA levels changed significantly in response to the PS-spheres, specifically, *lys* levels were enhanced and *cat* levels significantly reduced (**Table 2**). After depuration, both the percentage of dead hemocytes and phagocytosis capacity were significantly higher; granulocyte concentrations and hemocyte counts were significantly lower, GST, and SOD activities were significantly increased, and LPO activity significantly decreased in exposed vs. control mussels. By contrast, after depuration there were no effects on phagocytosis activity, ROS production, and hyalinocyte concentration (**Table 2**). Expression of the genes *sod* and *pk* in the gills of the mussels was increased, whereas that of the genes *cat* and *pgp* was significantly decreased in the gills and digestive glands, respectively (**Table 2**). Using *M. galloprovincialis* as the target organism, impacts of amino-coated (PS-NH$_2$) nano-sized particles were assessed by Canesi et al. (2015) and Balbi et al. (2017). Hemocyte functional parameters (lysosomal membrane stability, lysosomal enzyme release, phagocytosis, ROS, and nitric oxide (NO) production) and apoptotic parameters (mitochondrial membrane potential and cardiolipin peroxidation) were evaluated by Canesi et al. (2015), with significant dose-dependent decreases measured in lysosomal membrane stability (LMS) at highest concentrations tested (**Table 2**). However, lysosomal enzyme release, phagocytosis, and ROS production reacted more sensitive with significant alterations measured even at lowest particle concentrations tested (Canesi et al., 2015; **Table 2**). Additionally, NO production was also increased, with the amount varying depending on the incubation time (Canesi et al.,

2015). In terms of apoptotic parameters measured, significant effects could only be measured at highest concentrations applied (Canesi et al., 2015; **Table 2**). Balbi et al. (2017) evaluated the effects induced on the transcription of genes related to neuroendocrine signaling (serotonin receptor, 5-HTR), antioxidant defense (CAT, SOD), biotransformation (GST, ABC transporter p-glycoprotein [ABCB]), biomineralization (extrapallial protein [EP], carbonic anhydrase [CA]), autophagy, growth and metabolism (mammalian target of rapamycin [mTor]), apoptosis (p53), the immune response (Toll-like receptor I isoform [TLR-i]), shell formation (chitin synthetase [CS]), and the immune response/intracellular digestion lysozyme (LYSO). A time-dependent response of genes were reported, with significant up-regulation of CS and a significant decrease in LYSO after 24 h and a general down-regulation of all genes after 48 h, with significant alterations in the transcription of CS, CA, EP, ABCB, and LYSO (Balbi et al., 2017; **Table 2**). Cellular responses of *M. galloprovincialis* induced by PS- and PE-fragments were investigated by Avio et al. (2015) by assessing the levels of tissue biomarkers of immunological alterations (granolocytes/hyalinocytes ratio, phagocytosis activity, and LMS in hemocytes), neurotoxic responses (acetylcholinesterase [AChE] in hemocytes and gills), cellular, and oxidative stress biomarkers in digestive tissues (acyl-CoA oxidase [AOX], anti-oxidant defenses [CAT, GST, glutathione reductase, total glutathione], total oxyradical scavenging capacity [TOSC], lysosomal latency period [LP], malondialdehyde [MDA], lipofuscin, and neutral lipids), as well as genotoxic effects in hemolymph (DNA strand breaks, micronuclei frequency (MN), and nuclear alterations (NA). Additionally, transcriptional analyses were conducted for organisms treated with the PS-particles exclusively (Avio et al., 2015). Among the immunological responses of hemocytes, there was no significant change in phagocytosis whereas the granulocytes/hyalinocytes ratio decreased significantly in mussels exposed to PE-fragments and LMS decreased significantly in those treated with PS-fragments (**Table 2**). Although there were no obvious genotoxic effects in hemolymph, both the PE- and the PS-fragments induced neurotoxic effects in gills, including a significant decrease in AChE (**Table 2**). Alterations in biomarkers of cellular and oxidative stress included the significant inhibition of Se-dependent glutathione peroxidases in mussels exposed to the PS-fragments, with a similar trend determined for CAT (**Table 2**). Overall, the analysis of transcriptional responses identified a total of 2,143 genes differentially expressed in response to PS exposure, with 1,062 of those genes being down-regulated and 1,081 being up-regulated (Avio et al., 2015).

O_2 consumption

The effects induced by the mechanical hazards of micro- and nano-sized plastics on oxygen (O_2) consumption by marine organisms were examined in 20% ($n = 10$) of the included experiments (**Table 2**). Alterations in the O_2 consumption of the annelid *A. marina* treated with PVC-, PLA-, and HDPE-fragments were analyzed by Green et al. (2016a), reporting dose-dependent effects with significantly increased respiration rates at highest concentrations tested (**Table 2**). As reported by Watts et al. (2016), O_2 consumption rates of *C. maenas* were significantly reduced by the highest applied concentration of plain PS-spheres, whereas there were no significant changes following exposure to functionalized PS-COOH and PS-NH$_2$ spheres (Watts et al., 2016; **Table 2**). However, no effects of plain PS-spheres on O_2 consumption were reported for *C. helgolandicus* (Cole et al., 2015; **Table 2**). Additionally, no significant changes in O_2 consumption induced by fragments of PS, HDPE, and PLA were measured for *A. striata* and *O. edulis* (Green, 2016; Xu et al., 2017; **Table 2**).

Reproduction

The mechanical hazards affecting reproduction (egg production, fecundity, fertilization rates, oocyte number, and population size and growth rate) were assessed in 7 (14%) of the marine studies (**Table 2**). Heindler et al. (2017) reported significant dose-dependent effects of PET-fragments on the reproduction of *P. crassirostris*, with population size being more sensitive than egg production (**Table 2**). Population size was also investigated by Snell and Hicks (2011) assessing two generations of the rotifer *B. manjavacas* treated with PS-spheres. For these particles, distinct size-depending effects were observed with significantly reduced population growth rates for the smallest particles tested (**Table 2**). Size-dependent effects of PS-spheres were also found in *T. japonicus*, with significant effects on the fecundity of organisms exposed to larger spheres at lowest concentrations tested (Lee et al., 2013; **Table 2**). However, significant effects of PS-spheres have additionally been reported in terms of fertilization rates of *P. lividus* (Martínez-Gómez et al., 2017) and the decline in numbers of oocytes of *C. gigas* (Sussarellu et al., 2016; **Table 2**).

Assimilation

Sussarellu et al. (2016) examined the effects of PS-spheres on energy budgets of *C. gigas*, showing absorption efficiency being significantly higher in exposed than in control oysters (**Table 2**). However, PS-fragments did not alter absorption efficiency of *A. striata* (Xu et al., 2017). By contrast, the energy budget of the marine arthropod *C. maenas* showed to be rather sensitive toward exposure with PP-fibers, with a reduction in growth capacity noted at each of the applied concentrations (Watts et al., 2015). Wright et al. (2013a) showed a dose-dependent decrease in total available energy reserves of *A. marina* following exposure to PVC-fragments, with significant reductions at highest concentrations tested, as well as significantly lowered lipid reserves and comparable trends for proteins and sugar reserves (**Table 2**). Based on a conceptual carbon budget model, significant energy losses were also reported in the copepod *C. helgolandicus* treated with PS-spheres (Cole et al., 2015; **Table 2**).

Regeneration

Finally, the regeneration potential of *P. aibuhitensis* after PS-spheres exposure was investigated by Leung and Chan (2018), reporting significant decreases in regeneration rates, especially in worms exposed to the smallest particles (**Table 2**).

TABLE 3 | Effects of plastic leachates on benthic invertebrates.

Habitat	Taxa	Species	Material	Shape	Conc.	Matrix	Leaching	Parameter	Effects	Reference
Marine	Arthropoda	*Amphibalanus amphitrite*	HDPE	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.1 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	Li H. X. et al., 2016
			LDPE	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.1 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	
			PC	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.1 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	
			PET	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.1 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	
			PP	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.1 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	
			PS	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.5 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	
			PVC	Fragments	0.004–0.5 m²m l⁻¹	AM	24 h	Mortality	Significantly increased mortality (LOEC: 0.1 m²m l⁻¹)	Li H. X. et al., 2016
								Development	Significantly inhibited settlement (LOEC: 0.004 m²m l⁻¹)	
		Nitocra spinipes	Bio-bag	Fragments	100 g l⁻¹	AM	72 h	Mortality	Significantly induced mortality (LC₁₀ Bio: 24 g l⁻¹ (0h), 12 g l⁻¹ (96h), 29 g l⁻¹ (192h), 18 g l⁻¹ (288 h); LC₅₀ Bio: >100 g l⁻¹ (0h), 24 g l⁻¹ (96h), 36 g l⁻¹ (192 h), 26 g l⁻¹ (288 h))	Bejgarn et al., 2015
			Bio-PET	Fragments	100 g l⁻¹	AM	72 h	Mortality	No effects on mortality	Bejgarn et al., 2015
			HDPE	Fragments	100 g l⁻¹	AM	72 h	Mortality	No effects on mortality	Bejgarn et al., 2015
			LDPE	Fragments	100 g l⁻¹	AM	72 h	Mortality	No effects on mortality	Bejgarn et al., 2015

TABLE 3 | Continued

Habitat	Taxa	Species	Material	Shape	Conc.	Matrix	Leaching	Parameter	Effects	Reference
			PET	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	No effects on mortality	Bejgarn et al., 2015
			PLA	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	No effects on mortality	Bejgarn et al., 2015
			PP	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	LC_{10} PP: $37\,g\,l^{-1}$ (192 h), $16\,g\,l^{-1}$ (288 h), LC_{50} PP: $71\,g\,l^{-1}$ (192 h), $21\,g\,l^{-1}$ (288 h)	Bejgarn et al., 2015
			PS	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	No effects on mortality	Bejgarn et al., 2015
			PUR	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	LC_{10} PUR: $26\,g\,l^{-1}$ (0h), $40\,g\,l^{-1}$ (96h), $>100\,g\,l^{-1}$ (192h), LC_5C_0 PUR: $85\,g\,l^{-1}$ (0h), $>100\,g\,l^{-1}$ (96h), $>100\,g\,l^{-1}$ (192h)	Bejgarn et al., 2015
			PVC	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	LC_{10} PVC: $16{-}43\,g\,l^{-1}$ (0h), $23{-}35\,g\,l^{-1}$ (96h), $12{-}87\,g\,l^{-1}$ (192 h), $11\,g\,l^{-1}$ (288 h), LC_{50} PVC: $35{-}{>}100\,g\,l^{-1}$ (0h), $35{-}60\,g\,l^{-1}$ (96h), $24{-}52\,g\,l^{-1}$ (192 h), $21\,g\,l^{-1}$ (288 h)	Bejgarn et al., 2015
			Rubber	Fragments	$100\,g\,l^{-1}$	AM	72 h	Mortality	LC_1C_0 Rubber: $6\,g\,l^{-1}$ (0h), $6\,g\,l^{-1}$ (96h), LC_{50} PUR: $8\,g\,l^{-1}$ (0h), $7\,g\,l^{-1}$ (96h)	Bejgarn et al., 2015
	Echinodermata	Lytechinus variegatus	PE	Fragments	$250\,ml\,l^{-1}$	AM	24 h	Development	Significantly affected embryo development in the pellet-water interface assay, Significantly affected embryo development in elutriate assay for virgin pellets exclusively	Nobre et al., 2015
		Paracentrotus lividus	PS	Spheres	$10^3\text{-}10^5$ part. ml^{-1}	AM	30 d	Development	Significantly affected embryo development	Martínez-Gómez et al., 2017
			HDPE	Fragments	$0.005\text{-}5\,g\,l^{-1}$	AM	30 d	Development	Significantly affected embryo development in leachate assays of 0.005 and $5\,g\,l^{-1}$	Martínez-Gómez et al., 2017
	Mollusca	Perna perna	PP	Fragments	$50\text{-}200\,ml\,l^{-1}$	AM	24 h	Development	Significantly affected embryo development in leachate assays with PP (LOEC $50\,ml\,l^{-1}$), Effects significantly higher in tube assays compared to beaker assays	Gandara E Silva et al., 2016
			PP	Fragments	$200\,ml\,l^{-1}$	AM	24 h	Development	Significantly affected embryo development in leachate assays, Effects of beach-collected fragments significantly higher compared to PP fragments	Gandara E Silva et al., 2016
			unknown							
Freshwater	Arthropoda	Ceriodaphnia dubia	PS	Fragments	4 EPS cups l^{-1}	AM	30 min	Mortality / Reproduction	Significantly increased mortality, Significantly decreased total number of offspring; Delay in time to first brood	Thaysen et al., 2018
	Nematoda	Caenorhabditis elegans	PS	Spheres	$10\text{-}10{,}000\,\mu g\,l^{-1}$	AM	1 w	Cellular response / Behavior / Reproduction	Significantly reduced intestinal ROS production at $10{,}000\,\mu g\,l^{-1}$, Significantly decreased locomotion (head trash, body bend) at $10{,}000\,\mu g\,l^{-1}$, Significantly decreased reproduction (brood size) at $10{,}000\,\mu g\,l^{-1}$	Zhao et al., 2017

Chemical Impacts

Leachates

Freshwater benthic fauna

Among the 24 experiments focusing on leachates of micro- and nanoplastics, a single one (4%) targeted a freshwater organism (**Table 3**). Zhao et al. (2017) exposed *C. elegans* to PS-spheres in various concentrations, examining the effects on cellular responses, behavior, and reproduction. However, dose-dependent effects on each assessed parameter were reported with significant alterations at the highest concentration applied (**Table 3**).

Marine benthic fauna

All of the remaining studies (96%, *n* = 23) on chemical hazards posed by leachates of plastics examined marine organisms (**Table 3**), including arthropods (75%, *n* = 18), echinoderms (13%, *n* = 3), and molluscs (8%, *n* = 1; **Figure 3A**). In 13% (*n* = 3) of those studies, effects were examined on leachates of PS, HDPE, PP, and biodegradable plastics (each 12%, *n* = 3; **Figure 3B**). Leachates of LDPE, PVC, and PET were assessed in two studies each (8% of the investigations; **Figure 3B**). PE, PC, PUR, rubber, and an unknown plastic polymer were investigated in single studies (4%, *n* = 1; **Figure 3B**). The leachates derived from plastic fragments were assessed in 22 of the 23 studies, while a single study used spheres (4%; **Figure 3C**). All of the studies were conducted in aqueous medium and mainly assessed mortality (60%, *n* = 18) and/or development (40%, *n* = 12; **Table 3**).
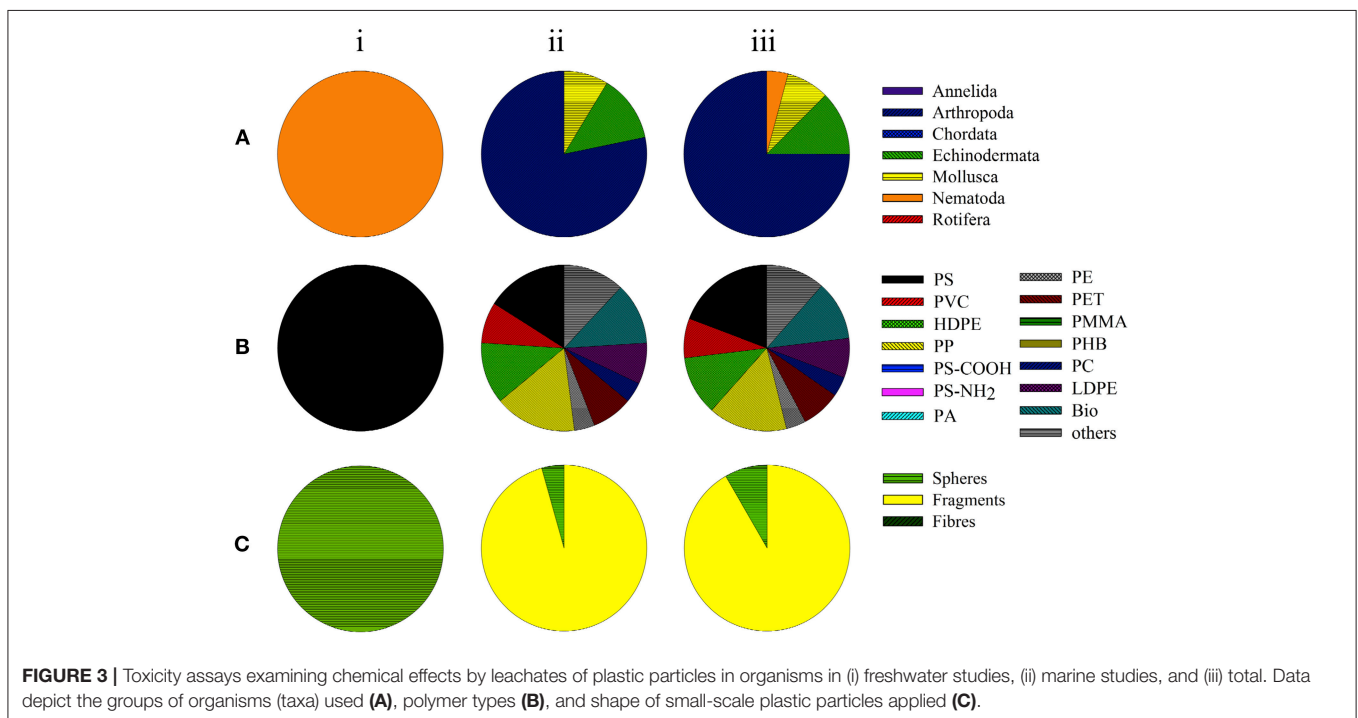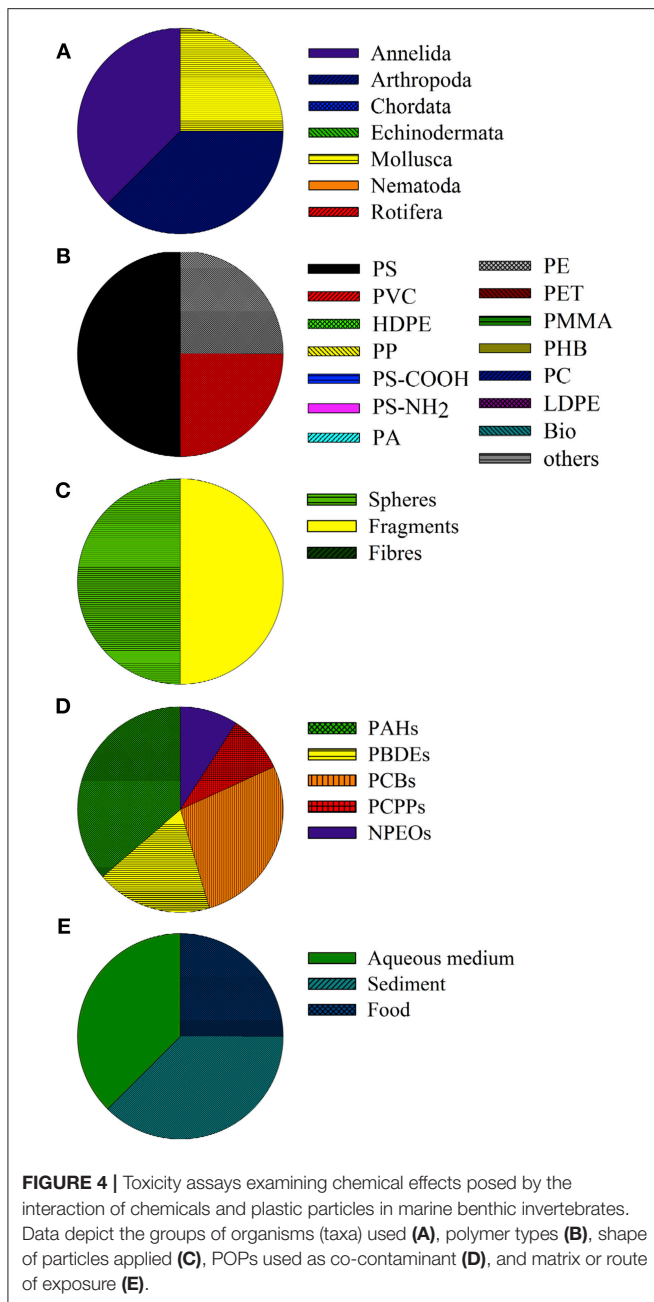
Mortality

Li H. X. et al. (2016) investigated the impact of various plastic polymers (HDPE, LDPE, PC, PET, PP, PS, and PVC), applied as fragments, on the mortality of juvenile *A. amphitrite* (**Table 3**). However, mortality increased in a polymer- and dose-dependent manner, with distinct effects in barnacles at low concentrations of HDPE, LDPE, PC, PET, PP, and PVC, while PS induced significant effects at the highest concentration tested exclusively (**Table 3**). Bejgarn et al. (2015) also found polymer-dependent differences in their examination on the mortality of *N. spinipes* induced by leachates of HDPE, LDPE, PET, PP, PS, PUR, PVC, rubber, biodegradable, and unknown polymers (**Table 3**). Additionally, the same authors compared the effects of selected plastics that had been artificially aged via irradiance with a xenon lamp (wavelength 300–800 nm, 765 W m^2) to simulate natural sunlight (Bejgarn et al., 2015; **Table 3**). However, acute toxicity toward *N. spinipes* was not induced by leachates of 62% of the plastic materials used (*n* = 13), while effects were measured in the remaining 38% (*n* = 8), before and/or after their irradiation (Bejgarn et al., 2015; **Table 3**). Generally, no common trend in toxicity, as a function of irradiation time, could be observed for the various polymers (**Table 3**).

Development

The development of juvenile *A. amphitrite*, measured as settlement success, was investigated by Li H. X. et al. (2016) exposing the organisms to various fragments of HDPE, LDPE, PC, PET, PP, PS, and PVC. Settlement was dose-dependently affected for all polymers investigated, being significantly reduced at the lowest concentrations applied (**Table 3**). Nobre et al. (2015) exposed embryos of *L. variegatus* to leachates of PE-fragments in a pellet-water interface assay and an elutriate assay. Anomalous larval development was observed in sea urchins assayed by either method, together with significant reductions in the proportions showing normal development

FIGURE 3 | Toxicity assays examining chemical effects by leachates of plastic particles in organisms in (i) freshwater studies, (ii) marine studies, and (iii) total. Data depict the groups of organisms (taxa) used **(A)**, polymer types **(B)**, and shape of small-scale plastic particles applied **(C)**.

FIGURE 4 | Toxicity assays examining chemical effects posed by the interaction of chemicals and plastic particles in marine benthic invertebrates. Data depict the groups of organisms (taxa) used **(A)**, polymer types **(B)**, shape of particles applied **(C)**, POPs used as co-contaminant **(D)**, and matrix or route of exposure **(E)**.

Interaction With Chemicals

Studies on effects induced by the interaction of micro- and nanoplastics with pollutants on benthic organisms are scarce. The eight experiments included in the present review were conducted with marine organisms exclusively, three (38%) using annelids and arthropods and two (25%) using molluscs (**Figure 4A**). Plastic particles mainly consisted of PS (50%, $n = 4$; **Figure 4B**) but PVC and PE were examined as well (25% each, $n = 2$ each). These particles were applied as fragments and spheres in four studies each (**Figure 4C**). Among the chemicals investigated, polycyclic aromatic hydrocarbons (PAHs) were most frequently tested, followed by polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), pharmaceuticals and personal care products (PCPPs) as well as nonylphenol ethoxylates (NPEOs; **Figure 4D**). The reviewed assays were conducted in aqueous medium or sediment in 70% of the studies, while exposure via the diet applied was assessed in two studies only (**Figure 4E**).

Development

Devriese et al. (2017) examined the developmental alterations in *N. norvegicus* after exposure to PE- and PS-spheres cross-contaminated with PCBs (applied as congeners 28, 52, 101, 118, 138, 153, 180, CB29, CB112, and CB140). After 3 weeks of exposure, no significant effects on the wet weight (ww), carapace length, and condition of exposed lobsters could be found (**Table 4**). Species of the *Mytilus* complex were treated with PS and the PAH fluoranthene (FLU) over 7 days, followed by 7 days of depuration (Paul-Pont et al., 2016). At day 7, a significant increase in developmental effects, seen as histopathological lesions/abnormalities, was demonstrated in mussels exposed to microplastic particles and FLU, alone or in combination (Paul-Pont et al., 2016; **Table 4**). After 7 days of depuration, the observed effects were still significant for the combined exposure treatments (Paul-Pont et al., 2016; **Table 4**).

Cellular response

The cellular responses of the lugworm *A. marina* to sediment co-contamination with nonylphenol (NPEO), Triclosan (PCPP), phenanthrene (PAH), and PBDE-47 (PBDE) combined with PVC-fragments were studied by Browne et al. (2013; **Table 4**). The phagocytic activity of *A. marina* decreased significantly in treatments consisting of sediment, PVC and NPEO, but also in those in which the sediments were spiked with NPEO alone (Browne et al., 2013). None of the additionally examined POPs induced significant effects on phagocytic activity (**Table 4**). An assay of the coelomic fluid of the lugworm showed that the ingestion of PVC-contaminated sediment resulted in a >30% lower oxidative stress response, while exposure to pollutants and additives through desorption from PVC had no effect (**Table 4**). Gomiero et al. (2018) investigated cellular responses of *H. diversicolor* exposed to PVC-fragments in combination with the PAH benzo(a)pyrene (B(a)P) in sediments over 10 and 28 days. Phagocytic activity was significantly reduced in organisms exposed for 28 days to B(a)P and both PVC concentrations, and for 10 days to B(a)P and highest concentrations of fragments (**Table 4**). Although B(a)P-exposure

patterns (**Table 3**). In comparable assays conducted by Gandara E Silva et al. (2016), *P. perna* was exposed to beach-collected pellets and to commercially available virgin PP-fragments. Exposure to the PP-fragments resulted in malformations or dead embryos in a dose-dependent manner, with significant effects at the lowest concentration tested (**Table 3**). Moreover, the beach-collected pellets were significantly more toxic than the PP-fragments in a comparative assay (**Table 3**). Embryonic development was also assessed by Martínez-Gómez et al. (2017), by treating zygotes of *P. lividus* with leachates of PS-spheres and HDPE-fragments, with abnormalities in the embryonic development being recorded for both polymers applied (**Table 3**).

TABLE 4 | Effects of contaminated plastic particles on benthic (marine) invertebrates.

Habitat	Phylum	Species	Material	shape	PM conc.	Chem.	Chem. conc.	Matrix	Parameter	Effects	Reference
Marine	Annelida	Arenicola marina	PS	Fragments	0.074–7.4% sed. dw	PCBs	1.84 µg kg⁻¹	S	Accumulation	Significantly increased accumulation of PCBs	Besseling et al., 2012
									Behavior	No effects on feeding activity	
									Mortality	No effects on mortality	
			PVC	Fragments	50 mg l⁻¹	Nonylphenol	0.69 µg g⁻¹	S	Accumulation	Significantly less chemicals accumulated under PVC exposure	Browne et al., 2013
						Phenanthrene	0.11 µg g⁻¹		Mortality	Significantly increased mortality by Triclosan, no effects by Nonylphenol, Phenanthrene, PBDE-47	
						Triclosan	57.3 µg g⁻¹				
						PBDE-47	9.49 µg g⁻¹		Cellular response	Significantly reduced phagocytic activity by Nonylphenol, no effects by Phenanthrene, PBDE-47 and Triclosan;	
										Significantly reduced oxidative status by Phenanthrene, no effects by Nonylphenol, Phenanthrene PBDE-47	
									Behavior	Significantly reduced feeding activity by Triclosan, no effects by Nonylphenol, Phenanthrene PBDE-47	
		Hediste diversicolor	PVC	Fragments	200 and 2,000 part. kg⁻¹	B(a)P	4.58 µg kg⁻¹	S	Accumulation	Significantly increased accumulation of B(a)P	Gomiero et al., 2018
									Mortality	No effects on mortality	
									Cellular response	Significantly decreased phagocytic activity at 200 part. kg⁻¹ (28 d), and at 2,000 part. kg⁻¹ (10 and 28 d);	
										No effects on extracellular lysosome release;	
										Significantly decreased mitochondrial activity at 200 part. kg⁻¹ (28 d) and at 2,000 part. kg⁻¹ (10 and 28 d);	
										Significantly decreased lysosomal membrane stability (LMS);	
										Significantly increased oxyradical production	
										Significantly increased micronuclei frequency and DNA strand breaks;	
										Significantly increased Lipofuscin content (28 d) and Catalase activity (10 d)	
Arthropoda		Allorchestes compressa	PE	Fragments	0.1 g l⁻¹	PBDEs	50 and 500 ng g⁻¹ MP	AM	Accumulation	Significantly lowered accumulation of PBDEs, Higher-brominated PBDEs accumulated in greater rates than lower-brominated congeners	Chua et al., 2014
		Nephrops norvegicus	PE	Spheres	3.1 mg l⁻¹	PCBs	8.7 µg g⁻¹ MP	Food	Accumulation	Limited effects of PE on accumulation of PCBs	Devriese et al., 2017
									Development	No effects on condition factor and nutritional state	
			PS	Spheres	3.1 mg l⁻¹	PCBs	8.7 µg g⁻¹ MP	Food	Accumulation	No effects of PS on accumulation of PCBs	Devriese et al., 2017
									Development	No effects on condition factor and nutritional state	

(Continued)

TABLE 4 | Continued

Habitat	Phylum	Species	Material	shape	PM conc.	Chem.	Chem. conc.	Matrix	Parameter	Effects	Reference
	Mollusca	*Mytilus edulis*	PS	Spheres	32 µg l⁻¹	Fluoranthene	30 µg l⁻¹	AM	Accumulation	No effects on accumulation after 7 d, significantly higher concentrations after depuration (7 d)	Paul-Pont et al., 2016
									Development	Significantly higher histopathological lesions after depuration	
									Cellular response	Lowered no. of dead hemocytes; Significantly decreased phagocytosis activity and ROS production; No effects on hemocyte and granulocyte concentration; Significantly reduced CAT activity and LPO, Significantly increased SOD, GST and GR activity after depuration; Significantly increased *cat* levels and increased in *pk* and *sod*, Significantly increased *cat* expression after depuration and lower levels of *pk, gpx, idp, sod,* and *amylase*	
		Mytilus galloprovincialis	PS	Spheres	32 µg l⁻¹	Fluoranthene	30 µg l⁻¹	AM	Accumulation	No effects on accumulation after 7 d, significantly higher concentrations after depuration (7 d)	Paul-Pont et al., 2016
									Development	Significantly higher histopathological lesions after depuration	
									Cellular response	Lowered no. of dead hemocytes; Significantly decreased phagocytosis activity and ROS production; No effects on hemocyte and granulocyte concentration; Significantly reduced CAT activity and LPO, Significantly increased SOD, GST and GR activity after depuration; Significantly increased *cat* levels and increased in *pk* and *sod*, Significantly increased *cat* expression after depuration and lower levels of *pk, gpx, idp, sod,* and *amylase*	

alone reduced phagocytic activity, the effects were significant only in combination with the plastics (Gomiero et al., 2018). In addition, mitochondrial activity and LMS were significantly decreased and oxyradical production significantly increased in *H. diversicolor* after combined exposure (**Table 4**). Again, the effect patterns were similar in organisms exposed to B(a)P alone, but combined contamination greatly intensified the observed alterations (Gomiero et al., 2018). In that same study, the ability of plastic particles to increase dispersion and the effects of genotoxic pollutants, such as B(a)P, were investigated by scoring the increments of micronuclei formation and DNA strand breaks in the coelomocytes of *H. diversicolor*. The results supported those obtained for mitochondrial activity, LMS, and oxyradical production, with co-contamination enhancing the effects of POP alone (Gomiero et al., 2018). An investigation of the effects on the lipofuscin content and CAT activity of *H. diversicolor* likewise showed enhancement by co-contamination, with significantly higher values in organisms treated with B(a)P and PVC than in controls and in worms exposed to sediments spiked only with B(a)P (**Table 4**).

Paul-Pont et al. (2016) focused on hemocyte parameters, enzyme activities and gene expression in *M. edulis* and *M. galloprovincialis* exposed to PS-spheres and FLU. Compared to organisms treated with FLU alone, significant decreases in phagocytic activity and ROS production after combined exposure were observed, together with decreased numbers of dead hemocytes. By contrast, hemocyte and granulocyte concentrations did not differ between treatments (**Table 4**). CAT activity and LPO were also significantly reduced under combined exposure vs. FLU alone (**Table 4**). Gene expression was also altered after combined vs. single exposure, evidenced by significantly higher *cat* levels in gills and the very high levels of *pk* and *sod* mRNA (**Table 4**). After depuration, single exposure to FLU induced a reduction in *cat* mRNA, and combined exposure a significant increase (**Table 4**). The expression of *sod*, *gpx*, *idp*, *pk,* and *amylase* increased significantly under FLU exposure, but not in organisms treated with both PS and FLU (**Table 4**).

Mortality
An effect of combined exposure on mortality was determined in three studies (**Table 4**). Browne et al. (2013) reported the increased mortality of *A. marina* exposed to a combination of PVC-fragments and PCPP, but not to co-contamination with NPEO, PAH, or PBDE-47 (**Table 4**). Besseling et al. (2012) and Gomiero et al. (2018) investigated the effects on the mortality of *A. marina* or *H. diversicolor* in treatments consisting of PS-spheres combined with PCBs and PVC-fragments with B(a)P, respectively, with no alterations being observed under any of the conditions tested (**Table 4**).

Behavior
Behavioral alterations, measured as effects on feeding activity, in the lugworm *A. marina* were assessed by Besseling et al. (2012) and by Browne et al. (2013) with contrary results obtained: while there were no alterations in feeding activity following combined exposure to PS-fragments and PCBs or to PVC-fragments and NPEO, PAH, and PBDE-47, treatments consisting

of PVC-particles combined with PCPP reduced feeding activity significantly (**Table 4**).

Accumulation
POP accumulation and the consequences of POP co-contamination with plastic particles were examined in every study included in this part of the review (**Table 4**). Browne et al. (2013) reported less NPEO, PAH, PCPP, and PBDE-47 accumulation in *A. marina* exposed to the chemicals together with PVC-fragments. These results were supported by those of Chua et al. (2014) in their study of *A. compressa*. The authors found less accumulation of PBDEs when in combination with PE-fragments. However, higher-brominated PBDEs accumulated at higher rates than did lower-brominated congeners (**Table 4**). A polymer-dependent effect on POP accumulation was shown by Devriese et al. (2017), who reported significantly increased body concentrations of PCBs in the tail tissues of *N. norvegicus* exposed to PE-spheres. Co-exposure with PS-spheres, however, did not have any effects on PCB accumulation, independent of the particle size tested (**Table 4**). By contrast, Besseling et al. (2012) reported a significant, but non-dose-dependent increase in PCB accumulation in *A. marina* exposed to sediments additionally spiked with PS-fragments (**Table 4**). Effects of co-contamination on accumulation were also reported for PAH and PVC-fragments, as Gomiero et al. (2018) showed a significant increase in accumulation of B(a)P in the body of *H. diversicolor* under combined exposure (**Table 4**). In addition, Paul-Pont et al. (2016) reported time-dependent effects on the accumulation of FLU induced by PS-spheres for *M. edulis* and *M. galloprovincialis* (**Table 4**). While no effects on accumulation were found after 7 days of exposure, body concentrations in both mussels were significantly higher after depuration (**Table 4**).

CONCLUSION AND GAP-ANALYSIS

The present review is a comprehensive analysis of studies investigating the (eco) toxicological effects of micro- and nanoplastics on benthic invertebrates in marine and in freshwater ecosystems. However, 80% ($n = 39$) of the respective publications analyzed referred to marine organisms and only 20% ($n = 10$) to freshwater organisms. Moreover, the toxic effects induced by leachates of plastic debris on freshwater organisms were analyzed in a single study (Zhao et al., 2017; **Table 3**), whereas the interactive hazards posed by micro- and nanoplastics and POPs in freshwaters have yet to be addressed at all (**Table 4**). Most of the benthic organisms assessed belonged to the macrofauna, investigated in 71% of freshwater studies and 99% of marine studies, with arthropods and molluscs as the most widely used organisms (72% of all studies). By contrast, despite their indisputable ecological relevance, organisms belonging to the micro- or meiofauna have been widely neglected. However, these groups of organisms should be of particular concern regarding nano- and micro-sized plastics, especially due to their key functions in aquatic ecosystems, and necessarily be included in further research.

Among the polymers used in the various assays, PS was the most commonly used: 42 vs. <10% each for other polymers,

including PP, HDPE, and PVC (**Figures 2B**, **3B**, **4B**). Overall, more polymer types were used in studies investigating the hazards posed by plastics on marine than on freshwater organisms, and only PS was used in the leachate study in freshwater (**Figure 3B**). Even though PS is one of the most commonly found polymers in nature (e.g., Barnes et al., 2009; Browne et al., 2010; Klein et al., 2015), other types of plastics present in the environment should also be comprehensively assessed, especially regarding the specific gravity of different plastic classes (e.g., Duis and Coors, 2016; Auta et al., 2017) and the associated individual risk to pose hazards on benthic organisms. Additionally, different polymers vary in their POP-sorption capacity, with consistently higher concentrations of PCBs and PAHs absorbing to HDPE, LDPE, and PP than to PET and PVC, while PAH sorbs more to PE than to PP or PVC (Rochman et al., 2013; Bakir et al., 2014). Hence, the effects of both the polymer and the associated contaminants have to be considered when assessing the potential risks of small-scale plastics in aquatic ecosystems. Effects of weathering or aging have been shown to affect the superficial characteristics of plastic particles and should also be taken into account for assessing potential risks of plastics under environmental relevant conditions. In studies assessing effects posed by non-functionalized vs. functionalized particles comparatively, with coated particles serving as surrogates for naturally altered plastics (Della Torre et al., 2014; Watts et al., 2016), differences in effects were reported. However, these studies investigated physical hazards posed by small-sized plastic particles exclusively, while chemical effects induced might be even more pronounced due to altered surface characteristics.

Regarding the various shapes of plastic particles used to investigate the environmental burden posed, fragments were the most frequent shape (60% of overall studies), whereas spheres still accounted for around 36% of the analyzed studies, and fibers for 5% only. However, particles of primary sources, mainly manufactured as spheres, are of minor relevance in nature and rather irregularly-shaped particles, resulting from the fragmentation of larger plastic items and of materials containing synthetic polymers, are much more prevalent (e.g., Duis and Coors, 2016). By this review, most obvious imbalances could be revealed regarding chemical effects posed by small-scale plastic particles on benthic freshwater organisms again, with spheres being investigated exclusively in terms of potential impacts induced by leachates. Regarding the interaction of chemicals with micro- and/or nanoplastics, the number of studies examining effects of co-contaminations is not representing environmental relevant circumstances, by using fragments and spheres equally (**Figure 4C**).

Furthermore, the exposure of benthic organisms to plastics in nature is doubtless dominated by particles in the sediments, both in marine and in freshwater ecosystems (Moore, 2008; Wright et al., 2013b; Galloway et al., 2017); however, in the vast majority of the reviewed studies exposure was via the aqueous phase (**Figures 2D**, **4E**; **Table 3**). Studies investigating the effects of leachates on benthic organisms were conducted in aqueous medium exclusively (**Table 3**), while particles were applied in more relevant matrices, sediment or food, in terms of studies

on physical hazards and the interaction with POPs (**Figures 2D**, **4E**). However, the scope of further scientific research should be broadened to include investigations under realistic exposure scenarios, mainly by using the ecologically most relevant exposure matrix for the particular organism group tested.

Even though the reviewed studies generally investigated effects of particles in a huge size range, the vast majority of studies applied microplastics, defined as particles ranging between $0.1\,\mu m$ and $5\,mm$ respectively (e.g., Thompson et al., 2004; Moore, 2008; **Tables 1–4**). However, in those studies assessing effects of nano- and micro-sized particles comparatively, distinct size-dependent effects were reported with increased impacts of nanoplastics, both in marine studies (Snell and Hicks, 2011; Lee et al., 2013; Jeong et al., 2017) as well as in experiments concerning freshwater organisms (Jeong et al., 2016; Lei et al., 2018). Irrespectively of the recent technical obstacles concerning the detection of nano-sized particles and the resulting uncertainties about natural concentrations, a subsequent degradation of plastic debris into nano-sized particles is widely accepted (e.g., Andrady, 2011; Lambert et al., 2013; Lambert and Wagner, 2016). By this, distinct research gaps concerning effects of nanoplastics on benthic organisms could be revealed.

The current lack of methodological standardization and harmonization greatly hampers inter-study comparisons, as already noted by Van Cauwenberghe et al. (2015). Generally, small-scale plastics have been tested in concentrations several orders of magnitude higher than current known environmental concentrations. While such approaches may be useful in identifying the general hazards posed by plastic particles, little information is provided on the actual impacts on benthic organisms. However, due to current detection limits for small-scale plastic particles, concrete measures of realistic concentrations are rarely available for particles $>10\,\mu m$ and not available for smaller particles ($<10\,\mu m$).

Even though a general comparison between the various assessed parameters in terms of their susceptibility toward nano- and microplastic exposure is difficult due to varying experimental conditions, sub-lethal parameters indicated to be more sensitive than mortality (**Tables 1–4**). This should be taken into account for upcoming research on this highly relevant topic. Furthermore, single-species tests were the preferred investigation design and the exposures were mostly short or of intermediate duration. However, long-term exposure and interspecific interactions characterize natural conditions; accordingly, the possible effects on the food chain and on the reproductive system of exposed organisms should also be investigated. Such studies are crucial due to the potential impact of small-scale plastics on community structure and population dynamics but also, indirectly, on higher trophic levels. More realistic model ecosystems and controlled experimental conditions would enable explorations of the effects of plastics on whole benthic communities. Extended plastic exposures, even at the low concentrations currently found in nature, may also lead to multi-generational effects on populations and communities.

In conclusion, the present review identified several shortcomings that have limited a comprehensive risk assessment

of the impact of micro- and nanoplastics, as well as future areas of research:

- Few studies have focused on the organisms in freshwater ecosystems, especially chemical effects are widely neglected so far.
- In both marine and freshwater systems, micro- and meiobenthic organisms must be more extensively assessed.
- Greater attention should be devoted to micro- and nano-sized plastics whose polymer composition, shape, surface properties, and exposure routes are those characterizing plastic particles contaminating the natural environment.
- Nano-sized particles should be of concern when assessing potential effects of plastics.
- Long-term assays of multiple species (e.g., model ecosystems) should be conducted to examine effects with higher ecological relevance.
- Standardization of concentrations and exposure conditions are needed together with quality assessments to obtain more reliable and comparable data.

AUTHOR CONTRIBUTIONS

AH contributed conception and design of the review. AH, M-TM, HF, and WT organized and contributed to the database. AH wrote the first draft of the manuscript. M-TM, HF, and WT contributed to manuscript revision, read, and approved the submitted version.

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