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Review

Effects of microplastics on the functional traits of aquatic benthic organisms: A global-scale meta-analysis[☆]M. Berlino^{a, b}, M.C. Mangano^{c, *}, C. De Vittor^b, G. Sarà^a^a Dipartimento di Scienze della Terra e del Mare, DiSTeM, Università degli Studi di Palermo Ed. 16, 90128, Palermo, Italy^b National Institute of Oceanography and Applied Geophysics - OGS, via A. Piccard 54, 34151, Trieste, Italy^c Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology (EMI), Sicily Marine Centre, Lungomare Cristoforo Colombo (complesso Roosevelt), 90149, Palermo, Italy

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ABSTRACT

Microplastics are widespread in the aquatic environment and thus available for many organisms at different trophic levels. Many scientific papers focus their attention on the study of the effects of microplastics on different species at individual level. Here we performed a global scale meta-analysis focusing our work on the study of the effect of microplastics on the functional traits of aquatic benthic organisms. Overall, microplastics showed a moderate negative effect on the examined functional traits of benthic organisms. Our results show that some crucial functional traits, such as those linked to behaviour and feeding, appear to be unaffected by microplastics. In contrast, traits related to the capacity of organisms to assimilate energy are affected. Moreover, traits with possible effects at population level appear to be negatively affected by microplastics. We discuss how the direct impact of organismal performance may have indirect repercussions at higher levels in the ecological hierarchy and represent a risk for the stability and functioning of the ecosystem.

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1. Introduction

Plastic is a worldwide-recognized issue that affects terrestrial and aquatic ecosystems (Duis and Coors, 2016). Despite growing attention on plastic pollution (Thompson et al., 2009), the presence of plastic in the environment is likely to keep increasing in the future (Moore, 2008; Jambeck et al., 2015; Kane et al., 2020). The amount of plastic produced globally has rapidly increased, reaching 360 million tons in 2018 (PlasticEurope, 2019) while, at global level, plastic waste (Barnes et al., 2009) represents 10% of global waste. Plastic debris can be introduced in rivers by intentional or involuntary dumping and runoff from terrestrial environments (Gasperi et al., 2014; Maynard, 2006; Rech, 2015). With half of the global population living in the first 80 km from the sea and the large amount of wastes produced from land and rivers, plastic has a high probability of finding its way into the sea, making plastic items highly diffused in the oceans (Derraik, 2002; Barnes et al., 2009;

Cózar et al., 2014; Eriksen et al., 2014; Moore, 2008). Moreover, in recent years, both scientific and public concern are being directed toward microplastics (i.e. polymers sized less than 5 mm), which can derive from the fragmentation of macroplastics or be produced *ex novo* to be used in different sectors (e.g. industry, personal care, medical applications; Moore et al., 2001; Browne et al., 2008).

Lentic (e.g. lakes etc.) and marine habitats are the most probable ultimate sinks receiving plastics. Organisms linked to the bottom, i.e. vertebrate and invertebrate benthic organisms with different feeding modes (Farrell and Nelson, 2013; Van Cauwenberghe and Janssen, 2014; Schmid et al., 2018; Schessl et al., 2019) are expected to experience new potential negative effects. Nevertheless, although a large amount of research reports on the negative ecotoxicological effects caused by additives coating microplastics (Rios et al., 2007; Teuten et al., 2009; Engler, 2012; Wu and Seebacher, 2020), there is still no full scientific understanding on "if" and "how" microplastics are able to affect individual functional traits and whether this generates repercussions at population level. Functional traits are behavioural, physiological and morphological characteristics (Schoener, 1986). Most concurs to optimizing individual growth and reproduction, the so-called performance traits (Arnold, 1983), in response to changing environmental conditions

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(Violle et al., 2007). According to the current ecological theory, functional traits are considered crucial as they reflect changes of the environment and can thus be used to predict variations in organismal trait performances (Arnold, 1983; Lavorel and Garnier, 2002; Suding, 2008). Reflecting and accommodating effects of anthropogenic-driven environmental change into metabolic and growth rates, changes of functional traits have repercussions on individual fitness (Violle et al., 2007; Enquist et al., 2015). Furthermore, when most individuals of a local population are involved in a functional trait change - and the consequent effects in terms of performance traits due to environmental solicitations - the expected result is an alteration of ecological equilibriums through population demographic traits, thereby undermining ecosystem functioning (Violle et al., 2007; Connell et al., 2017, 2018, 2018, Harley et al., 2017). Benthic organisms living close to the bottom do not evade such a suite of ecological rules and thanks to their spatial position in the habitat they receive the rain of all particles of different sizes falling through the water columns, including plastic materials. They represent the memory of most aquatic system, both freshwater and marine, as a result of their tight link with the substrates. They play a crucial role - often as foundation species or ecosystem engineers - in the increase of biodiversity and ecosystem functioning (Lathlean and McQuaid, 2017). Thus, to elucidate whether microplastics elicit alteration of ecological hierarchy equilibrium through the functional traits of benthic organisms may be effective to increase our understanding of possible repercussions at population level by affecting functional and (i.e. "morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance" *lit.* Violle et al., 2007) and performance traits (i.e. growth rate, reproductive output and survival, Arnold, 1983). Such a kind of information is crucial for increasing the effectiveness of future management measures. To do it, a quantitative synthesis of the scientific information available in the current literature is the first step in creating a robust scientific baseline that could be used to inform stakeholders and address the decision-making process.

Here, we performed a meta-analysis on a global literature dataset to highlight the effect of microplastics on the functional and performance traits of freshwater and marine benthic organisms. We estimated the overall effects size and the effects for most traits reported across the literature in the last decades with the main aim to investigate possible variations in the performance of benthic organisms.

2. Materials and methods

Literature search and data collection. The scientific articles included in our meta-analysis were retrieved by performing a systematic literature review (Pullin and Stewart, 2006; Moher et al., 2009), without applying nor temporal neither spatial geographic scale restriction (to ensure a full temporal and global scale coverage). Only studies focusing on measured effect/impact of microplastics on the functional traits of aquatic benthic organisms were selected (selection criteria). We searched for relevant scientific papers in ISI Web of Knowledge (Web of Science Core Collection package, Clarivate Analytics, 2019) and Scopus databases, using a complex search string involving specific keywords (search performed and ended on March 29, 2019; Table S1 Appendix). Details on the selected search string and the related search string creation strategy are reported in Appendix S1 (Table S1, S2). The search string was created to include four main elements of our primary question (linked by the Boolean operator "AND"; Pullin and Stewart, 2006, Moher et al., 2009; Mangano et al., 2015), respectively: the exposure (e.g. microplastic), the target population (or

subject of the search, e.g. benthos taxa), the measured outcomes (e.g. functioning) and the observation type (e.g. experimental, this latter was added to avoid the inclusion of plastic presence occurrence in the field studies with not associated effect reported). All the synonymous of each element were linked by the Boolean operator "OR"; the final selected search string was:

("microplastic*" OR "micro plastic*" OR "micro-plastic*") AND ("function*" OR "response*" OR "measure*" OR "rate*") AND ("laborator*" OR "mesocosm*" OR "experiment*" OR "trait*" OR "treatment*" OR "manipulat*") AND ("*benth*" OR "shellfish*" OR "shrimp*" OR "clam*" OR "mussel*" OR "fan mussel*" OR "crab*" OR "fiddler crab*" OR "hermit crab*" OR "spider crab*" OR "mud crab*" OR "gost crab*" OR "starfish*" OR "sea cucumber*" OR "*urchin*" OR "tunicate*" OR "sea squirt*" OR "anemon*" OR "*worm*" OR "sponge*" OR "sea slug*" OR "sea urchin*" OR "oyster*" OR "nudibranch*" OR "cockle*" OR "barnacle*" OR "shell*" OR "scallop*" OR "piddock*" OR "arks" OR "crustacea*" OR "isopod*" OR "chiton*" OR "mollusc*" OR "limpet*" OR "echinoderm*" OR "abalon*" OR "welk*" OR "conch*" OR "auger*" OR "wenteltrap*" OR "sea snail*" OR "murex" OR "periwinkle*" OR "sand dollar*" OR "sea star*" OR "sea spider*" OR "brittle star*" OR "basket star*" OR "lobster*" OR "isopod*" OR "amphipod*" OR "beach flea*" OR "scud*" OR "squilla*" OR "polychaet*" OR "gammariid") AND ("insect*").

A total of 272 scientific papers was retrieved (Figure S1 Appendix S1). We removed all spurious results, e.g. those studies specifically dealing with the distribution of microplastics in the environment (i.e. simple monitoring not reporting any measured effects on functional traits), descriptions of microplastics extraction methodologies, records of plastic finding from biotic and abiotic matrix (e.g. presence/absence, abundance, percentage of occurrence) and effects on terrestrial species (see list of excluded studies in Figure S1, Table S3 Appendix S1). We were interested in testing the effects of microplastics on benthic aquatic organisms and included only those studies dealing with the functional traits (measured responses) of both freshwater and marine benthic species. We screened the remaining studies (see list of studies after the first screening, Figure S1, Table S4 Appendix S1) and selected only those with a clear description of experimental design, such as comparisons of experimental treatment groups with one or more control groups (i.e. a group of organisms exposed - "treated" - to microplastics tested against "untreated" organisms, not exposed; see list of studies selected for evidence extraction, Table S4 Appendix S1). Given that our main aim was to focus on the effects of microplastics on the functional traits of benthic aquatic organisms measured at individual level, we included in the meta-analysis all those experimental studies reporting on the mean values of the measured functional trait variables, the number of replicates and a measure of the variability around the mean. Since we wanted to test whether the effects of microplastics translate on a larger scale, we extracted data from individual functional traits (i.e. behaviour, energy & metabolism, feeding) and performance traits (i.e. growth, mortality and reproduction), which have a direct effect on individual performance and ultimately on individual fitness leading to possible effects at higher level on the ecological hierarchy (Violle et al., 2007). Studies using pollutants or other compounds (e.g. antibiotics) added to microplastics, as well as studies focusing on the effect of altered microplastic (e.g. aged microplastic) and those focusing on the effect at sub-organismal level, i.e. studying cellular and subcellular variables such as oxidative stress, gene expression, immunological responses etc. were excluded from our analysis. However, the selection criteria led to the selection of 41 scientific papers (Figure S1 Appendix S1), which were considered suitable for the analysis that we performed in order to answer our primary question and satisfy the logical principles of the experimental design (Underwood, 1997). Details of

the studies (i.e. the variables associated with the studies and the number of case of studies) are reported in Table S5 (Appendix S1). We use a meta-analysis, a more robust than standard approaches to quantitative literature reviews (Gurevitch and Hedges, 2001; Gurevitch et al., 2018), to quantitatively summarise the collated experimental evidence, using an effect size estimator to quantify the direction (positive or negative) and magnitude (small or large) of a particular response variable.

Effects size calculation and analysis. Due to the different approaches used to measure and gather functional trait data, we used the Hedges' *g* as a measure of effect size to estimate the differences in the effects of microplastics between an experimental treatment (i.e. organisms directly exposed to microplastics through food, water or sediment) against a control group.

Hedges' *g* effect size and variance were calculated for each observation within our global dataset (*k* = 831 observations in total) to estimate the differences in the response variable between control and experimental treatment. Hedges' *g*, which is the bias-corrected standardized mean difference between the treatment and control groups (Hedges, 1981; Gurevitch and Hedges, 2001; Sarà, 2007a), weighs cases by their sample size and the inverse of their variance (Sarà, 2007b). The value of Hedges' *g* ranges from $-\infty$ to $+\infty$ and can be interpreted as follows (Borenstein et al., 2011): $|g| \leq 0.2$ considered a small effect; $0.2 \leq |g| \leq 0.5$ a medium effect; $0.5 \leq |g| \leq 0.8$ a large effect; and $|g| \geq 0.8$ a very large effect. The effect size Hedges' *g* was calculated as follows (Sarà, 2007b):

$$\text{Hedges}'g = \frac{(\bar{Y}_c - \bar{Y}_t)}{\text{standard deviation pooled}} \times J$$

where *Y_c* and *Y_t* are the mean of the control and experimental treatment groups, respectively.

The correction for bias attributed to different sample sizes, represented by *J*, was estimated through a differentially weighting studies as follows:

$$J = 1 - \frac{3}{4(N_c + N_t - 2) - 1}$$

While the following formula was used to calculate the pooled standard deviation (standard deviation pooled; Borenstein et al., 2011; Koricheva et al., 2013):

$$\text{standard deviation pooled} = \sqrt{\frac{(N_c - 1) \times s.d._c^2 + (N_t - 1) \times s.d._t^2}{N_c + N_t - 2}}$$

where *N* represented the sample size and s.d. was the standard deviation of the treated or control group. In order to account for inequality in study variance, effect sizes have been weighted using the inverse of the sampling variance, therefore calculating variance for each effect size (*V_g*) as follow (Koricheva et al., 2013):

$$V_g = \frac{N_c + N_t}{n_c n_t} + \frac{g^2}{2(n_c n_t)}$$

As the sign of Hedges' *g* tells the direction of the effect, in order to highlight possible negative effect of microplastics, we changed the sign of the effect sizes. Consequently, a negative value of Hedges' *g* indicates that microplastics have a higher effect on impairing that specific functional trait.

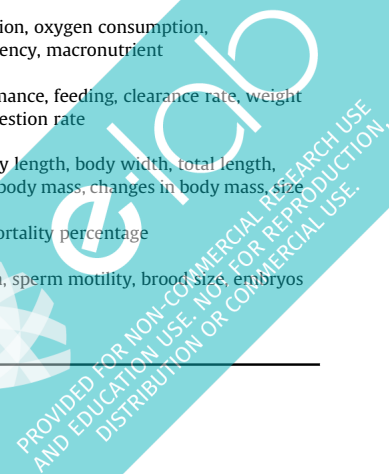
To answer our question, we divided the global dataset into three datasets; one dealing with benthic "adults", the second with benthic "juvenile" organisms and the third with "larvae". We decided to analyse them separately and we obtained an overall pooled effect size and 95% Confidence Interval (CI) for each sub-dataset. This allowed us to reduce the potential biases deriving from the analysis of three ontogenetic stages. In order to measure the effect size on single functional traits, we grouped the identified functional traits into six categories, as follows: behaviour, energy & metabolism, feeding, somatic growth, mortality and reproduction (Table 1 includes the definition of each functional trait used in the present meta-analysis) and ran a model estimating an overall effect size and 95% CI per functional trait.

Finally, we performed subgroup analysis including the following categorical fixed factors as moderators of the mixed-effects model: habitat (freshwater and marine), response level, differences among individual functional traits (e.g. behaviour, feeding etc.) and performance functional traits (e.g. somatic growth, reproduction etc.) with possible repercussions at upper ecological level (community or population); taxa; feeding mode and species (difference of the effects at organismal level). Moreover, we ran a mixed-effect model including microplastic size (1–100, 100–200, 200–500, >500 μm), microplastic shape (fibers, fragments and spheres) and microplastic type (different micro polymers used in the experiment) to investigate possible differences related to experimental condition.

Table 1

List of the six response categories examined in our meta-analysis. The associated description/quantification and the measured variables used (keywords) in the selected studies are reported. Specifically, the three functional traits - behaviour, energy & metabolism, feeding - the three performance traits - somatic growth, mortality, reproduction - as from Arnold's (1983) framework revisited by Violle et al. (2007).

	Trait	Description/Quantification	Measured variables (keywords)
Functional traits	Behaviour (activity)	Organisms behavioural changes reported after the ingestion or exposure to microplastics in comparison to an unexposed control group	Locomotor activity, mobility, interaction with substrate, spontaneous movement
	Energy & Metabolism	Metabolic energy expenditure or available as reserves for the individual after the ingestion of microplastics in comparison to an unexposed control group	Energy consumption, oxygen consumption, assimilation efficiency, macronutrient
	Feeding	Feeding activity of the organisms in presence of microplastics and in comparison to an unexposed control group	Predatory performance, feeding, clearance rate, weight of food eaten, ingestion rate
Performance traits	Somatic growth	Variation in organismal size in the unit of time measured after exposure to microplastics and in comparison to an unexposed control group	Body weight, body length, body width, total length, weight gain rate, body mass, changes in body mass, size increment
	Mortality	Mortality rate of organisms exposed to microplastics and in comparison to an unexposed control group	Mortality rate, mortality percentage
	Reproduction	Variation in gametes or embryos production or impairment in asexual reproduction after exposure to microplastics and in comparison to an unexposed control group	Oocyte production, sperm motility, brood size, embryos production



All statistical models were performed using the 'rma.mv' function of metafor package for R, which use a Wald-type test to determines statistical significance (Viechtbauer, 2010). We run mixed effects models that included study identification number (i.e. the ID of the study as reported in our dataset) and the functional traits as random effects to account for heterogeneity (Viechtbauer, 2007) and non-independence of result from the same study (Auta et al., 2017; Anton et al., 2019; Salerno et al., 2021). Effect sizes for the

models including categorical fixed factor were considered to be significant if their 95% confidence interval (CI) did not overlap with zero and if their $p \leq 0.05$.

3. Results

Estimation of effects on functional traits. The dataset analysed in this study was composed by 831 case studies ($k = 831$),

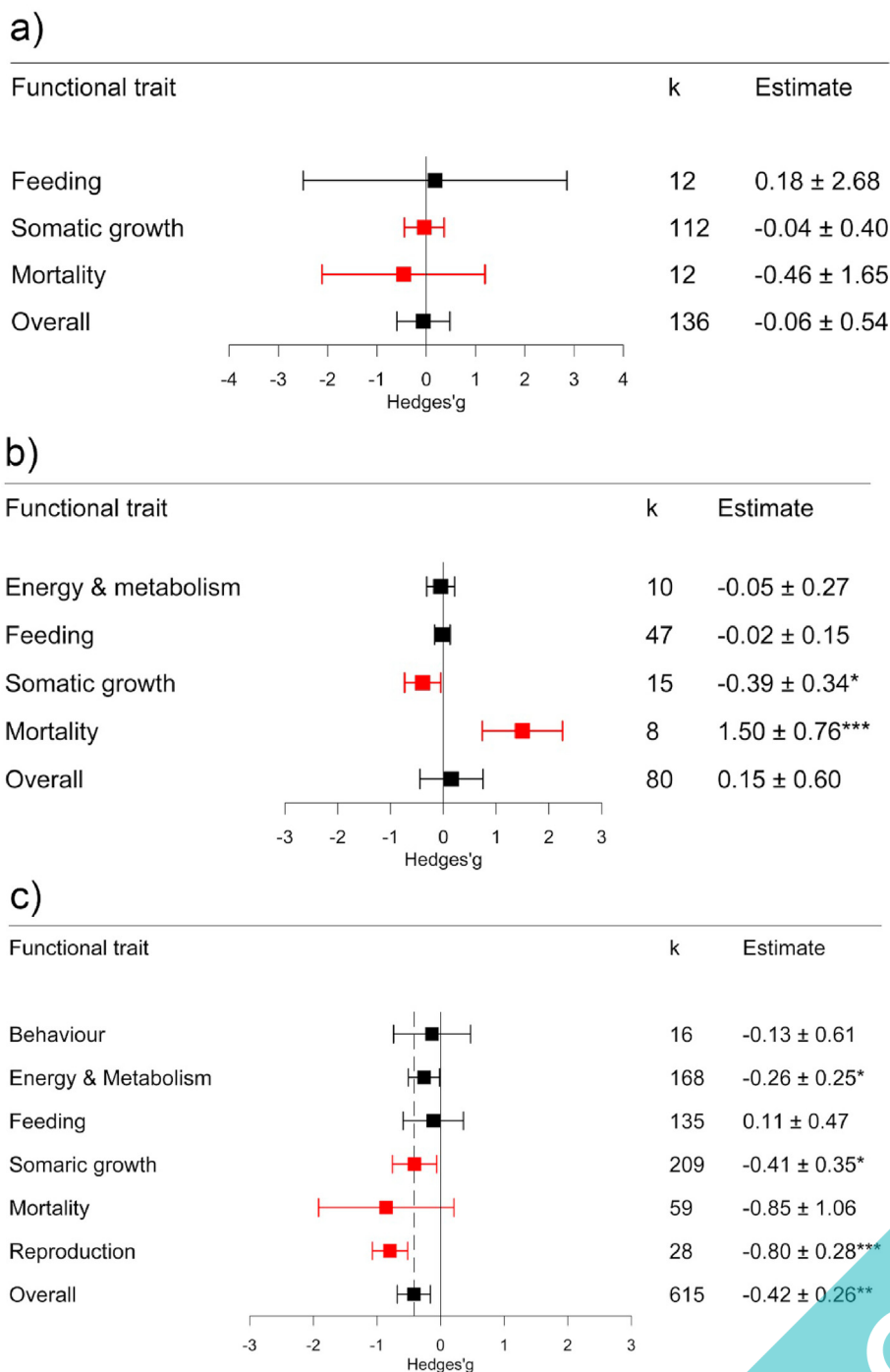


Fig. 1. Effect of microplastics on the functional traits of benthic organism a) larvae, b) juvenile and c) adult. Boxes represent Hedges' g value and the horizontal lines represent the 95% CI for each g value; red boxes indicate performance functional trait; the broken line indicates the overall effects size; asterisks indicate significance level (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). Analysis conducted with mixed-effects model, using the rma.mv function of the metaphor package in R, including study Id and functional trait as random factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



which were extracted from 41 scientific articles meeting our selection criteria and included adults ($k = 615$), juvenile ($k = 80$) and larvae ($k = 136$) of benthic organisms, from both freshwater ($k = 351$) and marine ($k = 480$) systems.

The overall effect size, for both the juvenile ($g = 0.15 \pm 0.60$, $p = 0.609$, ns) and larval ($g = -0.06 \pm 0.54$, $p = 0.832$, ns) dataset was not significant. Moreover, the effect size was not significant looking at the three functional traits analysed within the larvae dataset (i.e. feeding, growth, mortality (Fig. 1a)). Juvenile dataset included only four over the six selected functional traits (i.e. energy & metabolism, feeding, somatic growth and mortality); results showed a significant negative effect only for growth ($g = -0.39 \pm 0.34$, $p = 0.025$, *) and a positive effect on mortality ($g = 1.50 \pm 0.76$, $p < 0.001$, ***) (Fig. 1b). Subgroup analysis was not performed for these two datasets due to the low heterogeneity (i.e. selected subgroup not sufficiently represented).

The analysis of the overall effects for the adult dataset showed a moderately negative effect of microplastics on adult functional traits ($g = -0.33 \pm 0.17$, $p < 0.001$, ***). The large and significant heterogeneity of the dataset allowed us to perform subgroup analysis. Accordingly, three out of the six functional traits (Fig. 2) were significantly affected by microplastics and, specifically, energy & metabolism ($g = -0.26 \pm 0.25$, $p = 0.039$, *), somatic growth ($g = -0.41 \pm 0.35$, $p = 0.020$, *) and reproduction ($g = -0.80 \pm 0.28$, $p < 0.001$, ***) (k values were reported into Fig. 1c).

Habitat and response level. The results obtained from our subgroup analysis performed on adults revealed that microplastics appeared to have a more significant negative effect on the performance functional traits (i.e. somatic growth and reproduction; $g = -0.47 \pm 0.24$, $p < 0.001$, ***) then on individual functional traits (i.e. energy, $g = -0.22 \pm 0.22$, $p = 0.053$, *) (Fig. 2a).

Taking into account habitat as a moderator, both freshwater and marine species showed to be significantly negative affected showing medium effect size value ($g = -0.44 \pm 0.27$, $p = 0.001$, ** and $g = -0.27 \pm 0.21$, $p = 0.018$, ** respectively) (Fig. 2b).

Taxa and individual level. The taxon-specific analysis showed that microplastics have a negative effect on the functional traits of all the examined taxa (i.e. bivalves $g = -0.32 \pm 0.23$, $p = 0.005$, **, crustaceans $g = -0.33 \pm 0.19$, $p < 0.001$ and nematodes $g = -1.75 \pm 0.66$, $p < 0.001$) except for annelids ($g = 0.31 \pm 0.27$, $p = 0.023$, *), while results for gasteropods ($g = -0.17 \pm 1.03$, $p = 0.7114$, ns) were not significant (Table 2). However, it should be kept in mind that the taxa gasteropods and nematodes are actually represented by a single species (i.e. *Potamopyrgus antipodarum* and *Caenorhabditis elegans* respectively) and consequently, the results obtained for these two groups are comparable to the results at the individual level.

Furthermore, the effect of microplastics was significant at species-specific level and correlated with the feeding mode of the benthic organisms (Fig. 3). Our dataset covered 26 species and 7 different feeding modes, as reported in Table 2. A significant negative effect was detected for over 34% of the species, ranging from a medium effect, as expressed by $g = -0.49$ (± 0.49), to a very large effect, as expressed by $g = -1.75$ (± 0.51). The confidence interval and p -value of the effect size for the remaining species did not reveal significant effects (Table 2).

Among feeding modes, bacterivores (-1.75 ± 0.64 , $p < 0.001$, ***), filter feeders (-0.29 ± 0.23 , $p = 0.013$, **) and shredders (-0.57 ± 0.24 , $p < 0.001$, ***) appeared to be significantly affected by microplastics. No significant effect was detected among grazers, predators and omnivores (Fig. 3). Again, as the feeding mode groups "bacterivores" and "grazers" are represented by only one species each, as for the taxa nematodes and gasteropods, results obtained are similar to those for the single species.

Subgroup analysis for experimental condition show a medium

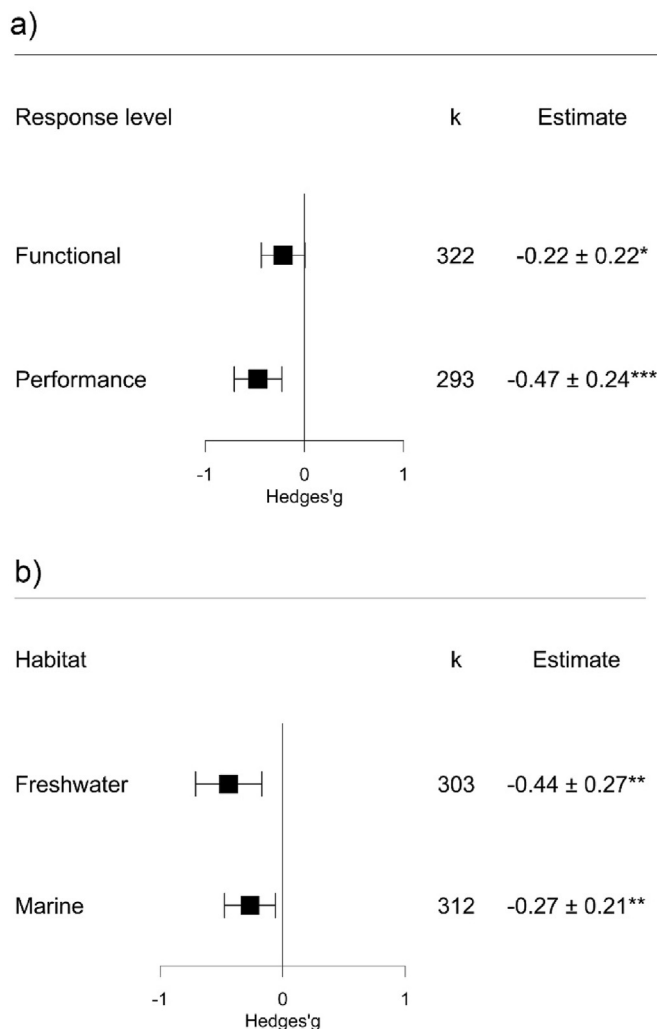


Fig. 2. Result of subgroup analysis with mixed effect model for ecological level a) and habitat b). Boxes represent Hedges' g value and the horizontal lines represent the 95% CI for each g value; asterisks indicate significance level (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). Analysis conducted with mixed-effects model, using the `rma.mv` function of the `metafor` package in R, including study Id and functional trait as random factor.

significant effect for size classes: 1–100 μm ($g = -0.35 \pm 0.19$, $p < 0.001$, ***), 100–200 μm ($g = -0.38 \pm 0.31$, $p = 0.018$, **) and 200–500 μm ($g = -0.29 \pm 0.24$, $p = 0.016$, **), while size class $> 500 \mu\text{m}$ showed a not significant effect ($g = -0.04 \pm 0.65$, $p = 0.898$, ns) (Figure S1). Regarding microplastics shape, spheres and fragment revealed to have a medium negative effect ($g = -0.50 \pm 0.25$, $p < 0.001$, ***; $g = -0.30 \pm 0.23$, $p = 0.013$, ** respectively). Fibers did not show significant effects ($g = -0.30 \pm 0.32$, $p = 0.079$, ns) (Figure S2). Lastly, taking into account microplastic type as a moderator did not show differences between the different polymers except for polystyrene (PS) which show a significant negative effect ($g = -0.42 \pm 0.61$, $p < 0.001$, ***) (Figure S3).

4. Discussion

Our meta-analysis revealed that microplastics exert an overall negative effect on the functional traits of aquatic benthic organisms. Such general results confirm many certainties about the role of microplastics as potential detrimental drivers of ecological

Table 2

Summary of the effects sizes for each species included in the meta-analysis. For each species we reported the belonging taxa (A = annelid; B = bivalve; C = crustacean; N = nematode), the main feeding modes as from the literature (BA= Bacterivore; DP = deposit feeder; FF = filter feeder; GR = grazer; OM = omnivore; PR = predator; SH = shredder), the k = number of case studies, Hedges'g values and the associated p -values (asterisks refer to the level of significance taken into account * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; species showing a significant effect are reported in bold).

Species	Taxa	Feeding mode	k	Hedges'g	p-value
<i>Abra nitida</i>	B	DP	45	-0.24 ± 0.56	0.412
<i>Arenicola marina</i>	A	DP	32	-0.11 ± 0.40	0.593
<i>Asellus aquaticus</i>	C	SH	21	-0.09 ± 0.45	0.362
<i>Atactodea striata</i>	B	FF	18	-0.20 ± 0.62	0.532
<i>Caenorhabditis elegans</i>	N	BA	42	-1.75 ± 0.51***	<0.001
<i>Carcinus maenas</i>	C	OM	39	0.06 ± 0.44	0.776
<i>Cassostrea gigas</i>	B	FF	9	-1.13 ± 0.65***	<0.001
<i>Corbicula fluminea</i>	B	FF	8	-0.16 ± 0.72	0.663
<i>Echinogammarus marinus</i>	C	PR	22	0.59 ± 0.56*	0.039
<i>Einnucula tenuis</i>	B	DP	45	-0.26 ± 0.56	0.370
<i>Eriocheir sinensis</i>	C	OM	15	-0.63 ± 0.55*	0.025
<i>Gammarus fossarum</i>	C	SH	72	-0.26 ± 0.31	0.091
<i>Gammarus pulex</i>	C	SH	41	-0.63 ± 0.55***	<0.001
<i>Hyalella Azteca</i>	C	SH	16	0.09 ± 0.58	0.766
<i>Lumbriculus variegatus</i>	A	DP	24	0.31 ± 0.43	0.163
<i>Mytilus edulis</i>	B	FF	19	-0.85 ± 0.45***	<0.001
<i>Mytilus galloprovincialis</i>	B	FF	3	-1.67 ± 0.95***	<0.001
<i>Nephtys norvegicus</i>	C	PR	12	-0.22 ± 0.49	0.369
<i>Ostrea edulis</i>	B	FF	14	0.52 ± 0.49*	0.039
<i>Perinereis aibuhitensis</i>	A	DP	14	-1.07 ± 0.82**	0.011
<i>Perna viridis</i>	B	FF	3	-2.68 ± 3.20	0.101
<i>Pinctada margaritifera</i>	B	FF	15	-0.49 ± 0.49*	0.050
<i>Platorchestia smithi</i>	C	DP	6	0.09 ± 0.51	0.739
<i>Potamopyrgus antipodarum</i>	G	GR	45	-0.29 ± 0.55	0.302
<i>Sphaerium corneum</i>	B	FF	10	0.26 ± 0.58	0.389
<i>Tubifex spp.</i>	A	DP	24	0.64 ± 0.43**	0.004

performances and contextually open to many questions about the magnitude and extent of the effects that indirectly scale up the ecological levels. Our study supports the scientific evidence that impairment of species traits due to microplastics may have consequences at ecosystem level given that they enter the lower level of the ecological hierarchy and may activate a chain of events that can undermine ecosystem equilibria and functioning (Enquist et al., 2015; Sarà et al., 2021). Our study brings novel information about the role of microplastics in aquatic habitats. Most studies published to date were limited to “monitoring” the presence of plastics and their potential effects at sub-organismal levels.

Here instead, we specifically addressed the question focusing on the effects on the ecological performance of organisms through functional traits. We highlighted that functional traits are threatened by microplastics that can impair ecosystem functioning. What seems more impressive is that the effects on functional traits are ubiquitous among organisms, taxa, feeding modes and habitats. Indeed, our analysis shows that both fresh- and marine water species were affected and this may represent that kind of indirect confirmation that microplastics are widespread through the global aquatic sphere (Auta et al., 2017; Windsor et al., 2019). Microplastics appear to negatively affect all benthic organisms that manipulate sedimentary materials to obtain food or create a refuge (i.e. shredders) and sessile organisms who actively strain out particle from the environment (i.e. filter feeders). Such a finding corroborates the idea that worldwide benthic organisms may be directly or indirectly affected, but also shows that, through ecological interactions such as for example prey-predator relationships, microplastics may move along the food webs and impair trophic interactions (Setälä et al., 2018; Wang et al., 2019). Trophic interactions are the core functions of any ecological system (Duffy et al., 2007), worldwide. Traits related to energy consumption, energy allocation and food assimilation were significantly affected by microplastics, in accordance with the general idea of transversal ecological effects, i.e. that from individuals move to

upper levels of the ecological hierarchy (Sarà et al., 2021). Instead, some behavioural traits (that allow organisms to interact with co-specifics and to drive inter-specific biotic interactions) or feeding-related functional traits (e.g. clearance rate which is a typical feeding mode adopted by most filter feeders clearing water from particles) were unaffected by microplastics (Fig. 2c), which could appear contradictory. Nevertheless, we know that behavioural and feeding traits are the most flexible traits in that they are “evolutionarily designed” to maximize the acquisition of energy under variable condition of food availability and environmental variability (Krebs and West, 2012). Thus, we suggest that this discrepancy can be easily explained by theory, given that the feeding behaviour and most correlated traits involve the most adaptive trait's suite driven by the necessity of organisms to gather energy under any possible condition in order to sustain growth and reproduction and finally individual performance and fitness.

A reduction of the availability of resources such as food can increase the energy spent on food search or acquisition (Charnov, 1967). Microplastics generated an effect on the functional traits involved in the energy budget of the organism (i.e. energy-related functional traits). The occurrence of microplastics in the diet of organisms can in fact produce a chain of events, which is ultimately measurable in terms of rebounds on energy allocation. As a main consequence, growth and reproduction were significantly affected; these two traits are the most important performance traits. They allow organisms to realize the fitness and to compete - through body size - for time, space and trophic resources and are involved in the mating system. Thus, microplastics may generate a sort of food dilution that can impact on total available energy for the organism with repercussions at different levels of organismal traits and performances. Food dilution due to artificial microplastics mixed in the sedimentary material can affect other behavioural traits such as searching for food thus altering the magnitude of the half-saturation constant for food in the Holling functional responses (Holling, 1959) and this could reflect on other loops of

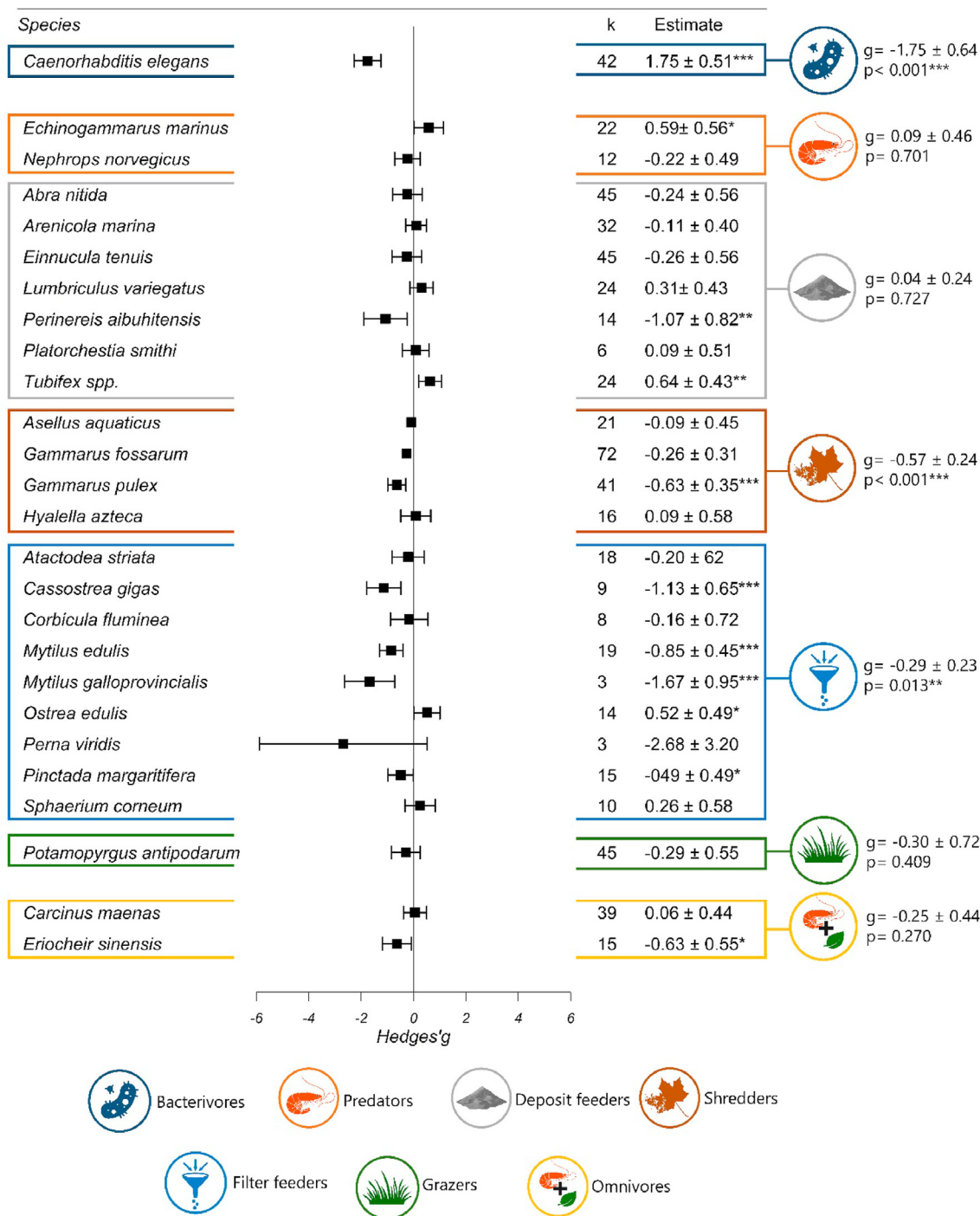


Fig. 3. Result of subgroup analysis with mixed effect model for species subgroups. Boxes represent Hedges' g value and the horizontal lines represent the 95% CI for each g value; asterisks indicate significance level (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). The boxes of different colours group species into their feeding mode (as in the legend). Result for the effects of microplastics for feeding mode subgroups (Hedges'g \pm 95% CI and p -value) are reported on the right side of the feeding mode symbols. Analysis conducted with mixed-effects model, using the rma.mv function of the metaphor package in R, including study Id and functional trait as random factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the energy budget (Kooijman, 2010). Indeed, in the presence of debris in the environment such as microplastics, there is a sort of interplay between food and debris density thus bringing the animal to a sort of misperception of food abundance with repercussions on the amount of energy ingested and energy used for food acquisition. Thus, although some traits specifically involved in the mechanics of feeding such as clearance rates were not significant, we

built on the idea that microplastics work akin the silt particles used as an artefact in the eighties by scientists (Bayne, 1987) to mimic the effect of living and feeding in turbid waters (e.g. estuaries or shallow waters in lagoons). In those seminal experiments carried out with suspension feeders, the effect of silt resulted in an increment of energy requested to obtain food: under these conditions filter feeders need to filter more water in order to obtain the same

amount of organic particles required to sustain somatic maintenance, growth and reproduction. Silt played the role as a sort of dilution factor (McCauley and Bjorndal, 1999) and microplastics may play the same role in that they indirectly and negatively affect the energy budget of organisms with ultimate repercussions on growth and reproduction. Similarly, but at the other side of the phylogenetic tree, the effects of plastics on the energy budget in marine turtles has been modelled (Marn et al., 2020) and it has been hypothesized that plastic dilutes the food and then, as with bivalves and silt, they need to spend more energy to obtain the same amount of food. Most results of our meta-analysis go towards the same direction, supporting that microplastics, affecting the amount of energy necessary to drive the metabolic machinery, have impacts on organismal performances. How the possible energetic bottlenecks created at different levels of the energy budget of organisms by microplastic particles affect the ultimate allocation of growth and reproduction deserves further investigation with both mesocosm and field experiments. However, ultimately, microplastics have the same effect as any other anthropogenic stressful factor; they impact the amount of energy available to individuals, as demonstrated by several studies (Tomlinson et al., 2014).

Overall, the current results reveal a heterogeneous response when assessing the effect of microplastics at taxa and species levels. Indeed, responses to microparticles appear to be species-specific and may depend on the ability of any single species to tolerate stressful habitat conditions: a certain level of an environmental factor may negatively affect the response of a species while not affecting another (Connell et al., 2018). The presence of a source of disturbance may shift environmental variables to a sub-optimal (negative or limiting) level for a species but maintain an optimal level for others (Connell et al., 2017, 2018, 2018; Harley et al., 2017). This is important for the stability of the ecosystem because it can influence the compositional and functional dimensionality of ecological communities (Micheli et al., 1999). Each species has its own biological performance linked to the variation of any environmental factor both of natural and anthropogenic origin, because each functional trait is maximized under optimal conditions with consequences on growth and fitness. Thus, when a community is exposed to disturbance, some species within their optimal window are able to achieve their optimal growth rates and may play a dominant role inside the community while, other species that are out their optimum, may become subordinate species (Enquist et al., 2015). Thus, in this context, given that microplastics affect functional traits and indirectly impair the ability of benthic species to maintain optimal functional performances, they may induce a functional displacement of dominant species and facilitate the expression of subordinates thus moving the barycentre of community equilibrium (Sarà et al., 2021).

Our study showed that microplastic manipulative experiments were characterized by high heterogeneity of methods and experimental designs. Present results show a larger significant effect of plastic spheres than irregular shaped microplastic fragments. This difference could be explained by the fact that regular shape of microspheres could enhance the possible transport and translocation of the particles through the digestive apparatus, after ingestion (Watts et al., 2016). However, such a results can have low impact in terms of realism as regular shaped microspheres are usually adopted in manipulative experiments (Burns et al., 2018) while irregular shaped fragments are more common in natural environments. Our meta-analysis revealed that microplastics size could represent another important characteristic to be considered. We found indeed that the negative effect of microplastic was lower when the size of the polymers increased. Microplastics of smaller dimensions could indeed be ingested by a larger number of

organisms belong to many different species, enhancing the likelihood that particles are canalised through the food webs (Lehtiniemi et al., 2018). Finally, when we tested possible effects exerted by types of polymers on functional traits, we discovered that through the current literature there is no evidence apart from that dealing with polystyrene which was the most used microplastic through analysed studies. As the density of the microplastic depends on the specific composition of the polymer, microplastic type could play an important role in the distribution of plastics in the environment enhancing the encounter likelihood by different benthic organisms (Kane et al., 2019, 2020).

5. Conclusions

By revealing the potential negative effects of microplastics on the functional traits of benthic organisms, our meta-analysis supports the importance to promote more “*sensu strictu*” experimental studies rather than simple monitoring efforts (merely based on the presence of plastics in abiotic and biotic matrices and potential toxicological implications). From here, we suggest to promote more investigations on the cause-effect ecological relationships affected by microplastics (and not only, also dealing with other anthropogenic stressors), and powering studies on the potential effects on biodiversity moving from functional trait responses and how the effects propagate toward upper levels of the ecological hierarchy. As pinpointed, the impairment of functional traits may have direct and proximate consequences on organismal performances and individual fitness, with ultimate potential repercussions on the density of local populations and on the expression of ecological functions on which goods and services depend. Hence, a major experimental research effort is required to measure the causal effects of microplastics, to assess the realistic magnitude and the extent of the associated disturbance, especially now under the globally increasing concern about plastics in nature. An increased awareness among citizens supported by a robust evidence-based knowledge becomes crucial to effectively address the perception of this pervasive environmental global issue. This will allow reinforcing the long-term trust relationship between scientists and society which is essential for joint planning of effective and successful mitigation measures.

Declaration of competing interest

The authors have no competing financial interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

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Authors statement

All persons who met authorship criteria are listed as author of the MS EFFECTS OF MICROPLASTICS ON THE FUNCTIONAL TRAITS OF AQUATIC BENTHIC ORGANISMS: A GLOBAL-SCALE META-ANALYSIS ENVPOL-D-21-00071, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

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