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Microplastics affect the ecological functioning of an important biogenic habitat

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Abstract

Biological effects of microplastics on the health of bivalves have been demonstrated elsewhere, but ecological impacts on the biodiversity and ecosystem functioning of bivalve-dominated habitats are unknown. Thus, we exposed intact sediment cores containing European flat oysters (*Ostrea edulis*) or blue mussels (*Mytilus edulis*) in seawater to two different densities (2.5 or 25 μg L^{-1}) of biodegradable or conventional microplastics in outdoor mesocosms. We hypothesised that filtration rates of the bivalves, inorganic nitrogen cycling, primary productivity of sediment dwelling microphytobenthos, and the structure of invertebrate benthic assemblages would be influenced by microplastics. After 50 days, filtration by *M. edulis* was significantly less when exposed to 25 μg L^{-1} of either type of microplastics, but there were no effects on ecosystem functioning or the associated invertebrate assemblages. Contrastingly, filtration by *O. edulis* significantly increased when exposed to 2.5 or 25 μg L^{-1} of microplastics, and porewater ammonium and biomass of benthic cyanobacteria decreased. Additionally the associated infaunal invertebrate assemblages differed, with significantly less polychaetes and more oligochaetes in treatments exposed to microplastics. These findings highlight the potential of microplastics to impact the functioning and structure of sedimentary habitats and show that such effects may depend on the dominant bivalve present.

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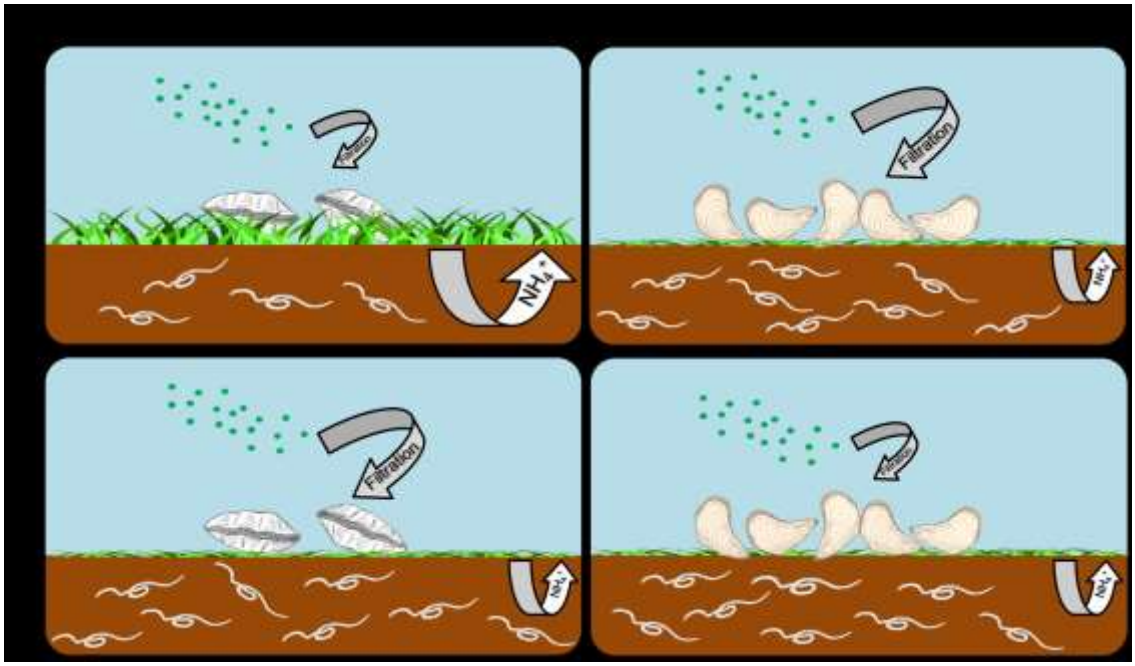
Filtration
NH_4^+
Filtration
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NH_4^+
Filtration
NH_4^+

Introduction

Microplastics contaminate marine habitats across the globe[1] and are recognised as a significant environmental challenge requiring urgent management[2]. It has recently been suggested that they are the most abundant form of solid waste on Earth[1] and their abundance is increasing[3]. Although there is much uncertainty regarding the concentrations of microplastics in the environment, high concentrations in seawater of ~ 3-23 μg L^{-1} and even up to ~ 4500 μg L^{-1} have been reported[4,5,6] in some heavily contaminated areas. Despite this prevalence, their effects on marine ecosystems are not well understood. Research to date has mostly focused on effects of microplastics on individual species, but effects on assemblages and ecosystem functioning within coastal habitats remain largely unknown[7,8].

Previous research has concentrated on organisms that ingest microplastics directly, such as filter-feeders, including marine mussels[9,10,11] and oysters[12,13]. These organisms are typically chosen for exposure experiments due to their great filtration capacity. For example, individual mussels and oysters can filter ~ 0.5-2.5[14] and ~ 5-25[15] L of seawater h^{-1} respectively. As such, they are very likely to ingest microplastics[16], and indeed, specimens from the field have been found to contain microplastics[11,17,18]. Exposure to relatively high densities of microplastics has been found to alter the respiration rates[13], immunology[10], reproductive capacity and filtration rates[12] of bivalves. Owing to their role as ecosystem engineers, such effects are likely to permeate beyond the individual organism. For example, reefs created by mussels and oysters provide refugia and nursery grounds for other, commercially-important species and can support diverse communities[19,20]. In addition, filter feeding leads to benthic-pelagic coupling; channelling nutrients from the water column and locally concentrating them via biodeposition (i.e. deposition of faeces and pseudo-faeces). Mussels and oysters can, therefore, enhance the release of limiting inorganic nutrients, such as ammonium, from sediments, fuelling primary productivity of the microphytobenthos (such

as diatoms and cyanobacteria) in the sediment, which in turn supports benthic and pelagic food webs[21]. If microplastics alter the ability of these organisms to filter feed, there may be wider impacts on their associated communities and on the functioning of coastal ecosystems. In addition, biodeposition is a likely mechanism by which suspended microplastics are transported from the pelagic zone onto sediments[22]. Mussels and oysters may, therefore, locally concentrate microplastics potentially altering biogeochemical processes, the biomass of primary producers and macrofaunal assemblages within the sediment.

In response to concerns of globally increasing plastic pollution, demand for biodegradable plastics has risen, with annual global production predicted to quadruple over the next five years[23]. It is thought that the replacement of conventional plastics, such as high density polyethylene (HDPE), with biodegradable alternatives, such as polylactic acid (PLA) will reduce the persistence, and therefore the impacts, of plastic pollution[24]. However, methods developed to assess the rate and extent of biodegradability of plastics in marine environments (e.g. ASTM International D7991-15)[25] are still limited in their ability to predict degradation in natural habitats[26]. The potential for PLA and other bioplastics, or biodegradable plastics to persist as microscopic particles, or to affect assemblages of organisms in the marine environment before they degrade, remains largely unknown. Recently, however, Green, (2016)[13] showed that PLA microplastics can lead to alterations in assemblage structure of macrofauna in sandy sediment with oysters.

In this study the effects of microplastics composed of HDPE or PLA, at two densities, on the structure and functioning of bivalve-dominated habitats were assessed using intact sediment cores in outdoor mesocosms, providing controlled, semi-natural conditions. Two experiments were conducted using two common, filter-feeding bivalves; blue mussels (*Mytilus edulis*) and European flat oysters (*Ostrea edulis*). The experiments tested the hypotheses that repeated exposure to biodegradable (PLA) and conventional (HDPE) microplastics in the water

column would alter the: (i) filtration rates of the bivalves; (ii) concentration and fluxes of benthic inorganic nitrogen; (iii) biomass of benthic micro-algae; and (iv) diversity and abundance of macrofauna within sedimentary habitats associated with either species of bivalve.

2. Methodology

2.1. Experimental design and set-up

Two separate mesocosm experiments, one focusing on *M. edulis* and one on *O. edulis* habitats, were set up simultaneously at the outdoor flow-through mesocosm facility at Queen's University Marine Laboratory, Portaferry, Northern Ireland. Both experiments had the same asymmetric design, with two fixed, orthogonal factors: "Plastic", with two levels: polylactic acid (PLA) and high density polyethylene (HDPE) and "Dose", with two levels: 2.5 μg L^{-1} and 25 μg L^{-1} seawater. A single treatment, without any added microplastics, was used as a control. To estimate the densities of microplastics in each treatment, water samples were taken from each "Plastic x Dose" treatment on days 1, 26 and 48 and microplastic particles were counted using a haemocytometer (Table S1).For each experiment, all treatments were replicated five times (n = 5, N = 25 per species) for a total of 50 mesocosms. Although the applied doses were relatively high compared with average densities observed and reported in the literature from ~330 μm plankton-net tow samples[27], at smaller mesh sizes (50 μm) densities of up to 7800 particles L^{-1} (equating to ~4500 μg L^{-1}) have been found in heavily contaminated coastal waters[6]. Furthermore, the densities used in the current study are among the lowest used experimentally to date[28] and were chosen to approximately reflect high values currently (2.5 μg L^{-1}) and in the future (25 μg L^{-1}) based on the prediction that global plastic waste input will increase 10-fold by 2025[27].

119 The mesocosms were made using clean, opaque 10 L polypropylene buckets (height x
120 diameter = 25 x 25 cm), placed onto large basins (as shown in Green 2016[13]). Each
121 mesocosm had an overflow pipe, allowing drainage directly into the basin. Waste water did
122 not come into contact with other mescosms and each mesocosm was an independent
123 replicate. In order to minimize disturbance to the sediment water interface, mesocosms were
124 equipped with sampling ports, drilled at 0, 1 and 4 cm into the sediment (Figure S1). These
125 ports were plugged until required for nutrient sampling (see section 2.3).
126 Each mesocosm was filled up to 4 cm depth with an intact core of muddy sediment, collected
127 using a mesocosm with the bottom cut out, from an area (~25 x 25 m) of a nearby shore
128 where *M. edulis* and *O. edulis* were abundant. Sand-filtered seawater, sourced directly from
129 Strangford Lough (54°22'51.1"N; 5°33'04.0"W) was delivered via dedicated, individual
130 hoses to each mesocosm at constant flow rates (~500 mL $minute^{-1}$), giving an overlying water
131 column of ~8 L and a daily turnover rate of 60 L day^{-1}. The mesocosms were left to
132 acclimatise for 48 h before live *M. edulis* or *O. edulis* were added. *M. edulis* and *O. edulis*
133 were collected from the same shore as the mud and were measured, weighed and allocated
134 randomly to treatments in order to ensure that no biases due to size were introduced into the
135 experiments. The collected *M. edulis* had an initial average (± S.E.M.) wet biomass of 20.1 ±
136 1.7 g, maximal length of 47.9 ± 0.6 mm, width of 21.5 ± 0.4 mm and height of 23.5 ± 0.3 mm
137 (n = 175). The collected *O. edulis* had an initial average (± S.E.M.) wet biomass of 36.0 ± 5.2
138 g, maximal length of 63.0 ± 1.6 mm, width of 60.1 ± 1.1 mm and height of 14.9 ± 0.6 mm (n
139 = 50). Dimensions were measured with a calliper. On the 24th of August 2014, seven
140 individuals of *M. edulis* (equivalent to individuals 142.6 m^{-2}) were placed into 25 separate
141 mesocosms and two individuals of *O. edulis* (equivalent to individuals 40.7 m^{-2}) were placed
142 into each of the other 25 mesocosms. These densities were chosen to reflect those high
143 enough to be considered "*M. edulis* dominated" or "*O. edulis* dominated" habitats (i.e. > 30%

cover and 5 individuals m^{-2} for *M. edulis* and *O. edulis* respectively, as defined by OSPAR[29]). The bivalves were placed on the surface of the sediment to mirror how they occurred locally in the field. There were no significant differences between the biomasses of individuals allocated to the different treatments at the start of the experiment (one-way ANOVA based on averaged dimensions in each mesocosm: *M. edulis*: $F_{4,20} = 0.32$, $P = 0.861$, *O. edulis*: $F_{4,20} = 0.26$, $P = 0.902$).The microplastic particles used in the experiment were of a similar colour (white) and size range, although their volume-weighted mean diameters differed: 65.6 µm (range = 0.6–363 µm) for PLA and 102.6 µm (range = 0.48–316 µm) for HDPE. In order to introduce microplastics into the mesocosms in a realistic manner, a dietary exposure method was used. In brief, microplastics were added to separate cultures (10 L) of the microalgae, *Isochrysis galbana* and left for 3 days with constant aeration. This was long enough for the microplastics to become more neutrally buoyant; i.e. move more freely within the culture containers rather than clinging to the sides or floating on top of the water. Fresh batches of control and microplastic dosed algae cultures were made up weekly. In order to ensure that the concentrations of *I. galbana* did not differ between treatments, algal cells were counted from each culture using a haemocytometer (on days 1, 26 and 48, Table S2). There were no significant differences in the density of *I. galbana* cells between treatments (one-way ANOVA for day 1: $F_{4,20} = 0.21$, $P = 0.927$, day 26: $F_{4,20} = 0.08$, $P = 0.986$ and day 48: $F_{4,20} = 0.28$, $P = 0.891$) and no aggregations of microalgae and microplastics were observed during the experiment. Cultures of *I. galbana* were prepared using seawater (35 psu), which was filtered with 0.45 µm aperture membranes and sterilised with UV light. Every day, each mesocosm received 250 mL of $\sim 2 \times 10^6$ cells mL^{-1} of microalgae containing either 0 (control), 80 or 800 µg L^{-1} of PLA or HDPE microplastics, equating to final densities in the mesocosms of 2.5 µg L^{-1} or 25 µg L^{-1} (i.e. 250 mL diluted by the 8 L mesocosm volume).During feeding the flow of water was stopped for two hours and air bubblers were

switched on in order to prevent anoxia and sedimentation of particulates. After this, the water flow in the mesocosms was resumed, replacing each mesocosm with clean seawater. The 2 hour daily exposure was chosen because in aquatic habitats, intermittent (as opposed to constant) exposure of contaminants is more likely to occur and, therefore, may be more environmentally relevant[30,31]. The experiment ran for 50 days, from the 26th of August until the 14th of October 2014. During this period the mean (± S.E.M) temperature of the water in the mesocosms was 15.4 ± 1.2.

2.2. Filtration rates of M. edulis *and* O. edulis

After 50 days, filtration rates were assessed by removing a single, randomly selected individual mussel or oyster from each mesocosm and holding them in separate 500 mL glass beakers with clean seawater each containing $4*10^3$ cells of *I. galabana* mL^{-1}. Samples of 5 mL were taken after 0, 30, and 60 minutes and suspended algal cells were counted using a coulter counter. Tissue from each replicate was frozen at -20°C and later the dry biomass of each individual was determined by drying at 60°C for 24 h and weighing to the nearest µg to account for body mass. Filtration rates are expressed as the number of cells filtered mg^{-1} of dry biomass h^{-1}.

2.3. Porewater nutrients; ammonium, nitrate and nitrite

Porewater samples were collected using Rhizon™ membranes (Rhizosphere Research Products B.V., The Netherlands) inserted into the sampling ports of the mesocosms. This allowed water to be sampled at the surface (1 cm above the sediment), sediment-water interface (0 cm) and at 1 and 4 cm depths in the sediment. The flow of seawater into mesocosms was stopped and porewater was drawn by attaching a needle to each Rhizon™ membrane collecting 10 mL of water directly into sterile vacuum tubes (BD Vacutainer®).

194 Surface water was sampled a second and third time (at 30 minute intervals) to estimate
195 nutrient fluxes. The water samples were stored in the vacuum tubes at 4°C prior to measuring
196 concentrations of ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) using a Lachat Quick-
197 Chem 8000 flow injection autoanalyser with Lachat methods 31-107-06-1-B (NH_4^+) and 31-
198 107-04-1-A (NO_2^- and NO_3^- nitrate and nitrite). Porewater nutrient concentrations were
199 adjusted for sediment porosity and standardised to dry bulk density. Pools of nutrients were
200 calculated within the depth profile by integrating linear porewater concentration gradients,
201 corrected for porosity, down to 4 cm depth. Concentrations of nitrate and nitrite were too
202 minute (i.e. below the detection limit of ~0.01 mg L^{-1}) to be measured with confidence and
203 were omitted from further analysis.
204
205 *2.4. Microalgal biomass on sediment surface*
206 A benthic fluorometer (BenthoTorch, bbe-Moldaenke GmbH, Schwentinental, Germany[32])
207 was used to estimate the biomass of diatoms and cyanobacteria on the sediment surface.
208 Measurements were taken after 48 days, before any disturbance caused by other sampling
209 activties. The BenthoTorch was placed on the surface at three random locations and averaged
210 to serve as a single replicate measurement per mesocosm. Measurements are expressed in µg
211 biomass cm^{-2}. Previous use of the BenthoTorch on similar sediment mesocosms found it to
212 mirror the patterns of chlorophyll-a extraction using solvents[33].
213
214 *2.5. Infaunal assemblages in the sediment*
215 Finally, all sediment was removed from each mesocosm and sieved separately through a 500
216 µm mesh to retain macrofauna, which were placed into containers and topped up with 5%
217 formalin and later enumerated and identified in the laboratory using Hayward and Ryland
218 (1995)[34] as a key. Individuals were identified to species level where possible, and the number

of taxa (R), the total number of individuals (N) and Shannon-Wiener diversity (H') (with *e* as the base) were calculated as alpha-diversity measurements.

2.6. Statistical data analyses

Statistical analysis was done using the R environment (R v3.2.3; R core team 2015). The data were screened for normality (q-q plots, and Shapiro-Wilk tests) and homogeneity of variance (Levene's test, using the *car* (v2.1-2) package[35]) to ascertain assumptions for ANOVA. Transformation of some data was necessary to enable them to conform to these assumptions (specific transformations are stated in the results). Data were analysed separately for each of the two experiments (i.e. *M. edulis* and *O. edulis* were not compared in the statistical analyses). Since the design was asymmetrical (i.e. having a single control group for the two factors "Plastic" and "Dose"), the data were analysed by using the mean squares from two independent ANOVAs[36] (see Green et al., 2016[33] for more details on calculations). Briefly, this included partitioning of the variance by calculating: (1) one-way ANOVA with all treatments as separate levels (a=5, n=5, N=25); and (2) a full-factorial two-way ANOVA of "Plastic" by "Dose" without the control (a=2, b=2, n=5, N=20). The residuals of the 1st ANOVA were used to assess differences between the levels within the 2nd ANOVA, allowing the variation associated with controls and that of the other treatments to be distinguished ("C vs. O"), which is contrasted with one degree of freedom[36]. When a significant effect in the "C vs. O" contrast was found Dunnett's test was used to contrast the control versus each level of the significant term using the *multcomp* (v1.4-6) package[37]. Pairwise comparisons for the factors in ANOVA (2) were computed using Tukey HSD tests when the main terms were significant. Statistical significance was assumed at $\alpha = 0.05$.

Differences in invertebrate assemblage structure among treatments were compared using a two-factor permutational ANOVA based on Bray-Curtis dissimilarities of square root

transformed data with 9999 permutations under the reduced model using Type I sum of squares (SS) using PERMANOVA+ add-on (PRIMER-E Ltd. Plymouth, UK). The asymmetrical analyses were achieved by fitting each main effect ("Plastic" and "Dose") in turn with a Type I (sequential) SS model, then swapping the order of the terms and combining the results of the two analyses[38]. When a factor was significant, contrasts were used to determine the specific differences. Results of the PERMANOVA were visualised with 2-dimensional ordination using canonical analysis of principal coordinates (CAP)[39]. Where assemblage structures differed, SIMPER analysis was used to quantify the contribution of different taxa to dissimilarities between treatments.

3. Results

3.1. Effects of microplastics on the filtration rates of bivalves

Two mussels died during the experiment and were removed. There were no oyster mortalities. When exposed to 25 µg L^{-1} of PLA or HDPE microplastics, *M. edulis* filtered ~2.4 times less microalgae (*I. galbana*) per hour than when exposed to none of the experimental microplastics (Figure 1a, Table S3, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}}$: t=2.42, P=0.045). There was no effect of 2.5 µg L^{-1} of either type of microplastic on the filtration of *M. edulis*. On the contrary, *O. edulis* in the control mesocosms filtered ~7.5 times less microalgae than those in mesocosms with any type or density of microplastic (Figure 1b, Table S3, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}}$: t=-3.09, P=0.011, $_{Control\ vs\ 2.5\ \mu g\ L^{-1}}$: t=-2.74, P=0.024, $_{Control\ vs\ PLA}$: t=-2.51, P=0.038, $_{Control\ vs\ HDPE}$: t=-2.74, P=0.024) compared to when not exposed to microplastics.

3.2. Effects of microplastics on ammonium in sediment porewater

Concentrations of ammonium increased with depth in the sediment in all mesocosms (Figure 2). Sediment with *M. edulis* had no significantly different ammonium pools and ammonium

flux from the surface and was not significantly different between microplastic treatments (Table 1 and S4). Sediment with *O. edulis*, however, contained ~1.8 times more ammonium when no experimental microplastics were present compared with those dosed with either type of microplastic at both densities (Table S4, Dunnett's $_{Control\ vs\ PLA}$: t=2.63, P = 0.030; $_{Control\ vs\ HDPE}$: t=2.94, P=0.015). In addition, ammonium fluxes from the sediment into the water column were significantly different in mesocosms with *O. edulis* dosed with microplastics than in controls (Tables 1 and S4), however, *post-hoc* tests were unable to determine further significant differences.

3.3. Effects of microplastics on the microphytobenthos

The biomass of diatoms was not significantly different between the microplastic treatments for sediments with *M. edulis* or *O. edulis* (Table S4). The biomass of cyanobacteria, however, was significantly less in sediments which contained microplastics with *O. edulis* (Table S4) (but not those with *M. edulis*, Figure 3a), and was ~2 times greater in the controls than in mesocosms dosed with either type or density of microplastics (Figure 3b, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}\ PLA}$: t=4.77, P<0.001; $_{Control\ vs\ 2.5\ \mu g\ L^{-1}\ PLA}$: t=3.91, P=0.003; $_{Control\ vs\ 25\ \mu g\ L^{-1}\ HDPE}$: t=4.31, P=0.001; $_{Control\ vs\ 2.5\ \mu g\ L^{-1}HDPE}$: t=3.05, P=0.022).

3.4. Effects of microplastics on infaunal assemblages

There were no significant differences between the structure of infaunal invertebrate assemblages (Figure 4a, Table S5), the diversity indices (Figure 5a, Table S6) nor the abundance of individual taxa (Table S6) in sediments with *M. edulis*. Sediments with *O. edulis*, however, had significantly different assemblage structures in treatments dosed with microplastics, at any density or type of plastic compared to controls (Figure 4b, Table S5) and there were several differences in dominance (Table S7). Although species richness and

294 total abundance did not differ significantly (Table S6), the Shannon-Wiener index (H') was
295 ~2 times greater in controls than in mesocosms dosed with 25 µg L^{-1} of HDPE microplastics
296 (Figure 5b, Table S6, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}\ HDPE}$: t=0.14, P=0.004). There was a ~3 times
297 greater abundance of *Eteone picta* polychaetes present in sediments not dosed with
298 experimental microplastics than in treatments that received microplastics of either type
299 (Figure 6a, Table S6, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}\ PLA}$: t=3.53, P=0.008; $_{Control\ vs\ 2.5\ \mu g\ L^{-1}\ PLA}$:
300 t=3.99, P=0.002; $_{Control\ vs\ 25\ \mu g\ L^{-1}\ HDPE}$: t=4.27, P=0.001; $_{Control\ vs\ 2.5\ \mu g\ L^{-1}\ HDPE}$: t=4.83, P<0.001).
301 On the contrary, sediments in the controls had ~1.9 times fewer *Tubificoides benedii*
302 oligochaetes than those dosed with 25 µg L^{-1} of either type of microplastic (Figure 6b, Table
303 S6, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}}$: t=-3.27, P=0.007). There were also ~2.6 times more *Lineus*
304 *longissimus* nemerteans in sediments when exposed to 25 µg L^{-1} of PLA than in those
305 exposed to 2.5 µg L^{-1} of PLA or no microplastics (Figure 6c, Table S6, Tukey's HSD $_{2.5\ \mu g\ L^{-1}\ PLA\ vs\ 25\ \mu g\ L^{-1}\ PLA}$:
306 P=0.026, Dunnett's $_{Control\ vs\ 25\ \mu g\ L^{-1}\ PLA}$: t=-2.66, P=0.049).
307
308 **4. Discussion**
309 *Mytulis edulis* and *Ostrea edulis* responded differently to contamination with microplastics.
310 The blue mussels filtered fewer algal cells h^{-1} when exposed to 25 µg L^{-1} of PLA or HDPE
311 microplastics. This supports findings of Wegner et al. (2012)[40] who found decreasing
312 filtration rates with increasing concentrations (constant exposure of 0.1 - 0.3 g) of
313 polystyrene nanoplastics (30 nm), but is in contrast with Browne et al., (2008)[9] which found
314 no effect of constant exposure of 0.51 g of 3.0 or 9.6 µm polystyrene microbeads on the
315 filtration rates of *M. edulis* after 48 days. On the contrary, *O. edulis* exposed for 2 hours per
316 day to 2.5 or 25 µg L^{-1} of PLA or HDPE microplastics filtered more algae h^{-1} than when
317 exposed to no microplastics. This is similar to another recent experiment, which found an
318 increase in filtration rates of another species of oyster, *Crassostrea gigas*, in response to

319 constant exposure of 23 μg L^{-1} of 6 μm polystyrene microplastics[12]. From this selection of
320 studies, albeit small, there is a pattern emerging suggesting a trend that mussels filter less and
321 oysters filter more in response to plastic particles. More research is needed, however, to
322 determine whether responses of filtration rates are generally applicable for bivalves in
323 response to microplastics across different environmental contexts and with different polymer
324 types.
325 Overall, the net filtration rates (corrected for dry weight of animal tissue) were greater for *M.*
326 *edulis* than for *O. edulis*. Others have also found greater filtration rates (corrected for weight)
327 in mussels than in oysters[41,42]. This could be because the microalgal concentrations in the
328 filtration measurements were at 4000 cells mL^{-1} and *M. edulis* reaches optimum filtration
329 rates at 2000 and 6000 cells[43] mL^{-1}, whilst *O. edulis* requires concentrations an order of
330 magnitude greater than this for optimum filtration rates to be reached[44]. Different bivalves
331 may use different strategies when coping with an increase in particles (which would have
332 occurred with the addition of microplastics). For example, under increased microalgae
333 concentrations, mussels often decrease their filtration rates in order to maintain a constant
334 consumption rate, whilst oysters increase theirs, along with their production of
335 pseudofaeces[45]. Due to their importance in benthic-pelagic coupling, such alterations to
336 filtration rates could lead to cascading effects on nutrient cycling and primary productivity in
337 sedimentary habitats. In the current study, this occurred in the oyster-dominated mesocosms.
338 The pool and flux of ammonium was less in the sediment pore-water with *O. edulis* exposed
339 to microplastics. Although more research is required to ascertain the mechanisms to account
340 for this result, it is possible that microbially-mediated processes which control the production
341 (ammonification) and reduction (nitrification and denitrification) of ammonium were altered
342 by the microplastics. Likely in response to there being less ammonium in the porewater, there
343 was also less biomass of cyanobacteria. In a similar outdoor mesocosm experiment, PLA,

HDPE or PVC microplastics (2% of wet sediment weight) directly added to sandy sediment led to reductions in the biomass of benthic diatoms, but not of cyanobacteria[33]. This difference could be due to the grain size of the sediment. Cyanobacteria are less able to build stable microbial mats on fine (muddy) sediment than they are on coarse (sandy) sediment, whilst diatoms are stable on muddy sediment[46]. Nano- or micro- plastics have also been found to reduce the productivity of other primary producers. For example, Besseling et al. (2014)[47] found that nanoplastics reduced growth of green algae and overall chlorophyll concentrations in laboratory microcosm experiments, and in another study Bhattacharya et al. (2010)[48] reported a reduction of photosynthesis by microalgae. Cyanobacteria are key primary producers in sedimentary systems[49], vital in food-web dynamics[50,51]. Together with euglenids and diatoms, they can supply up to 45% of the organic budget of an estuary[14] and are important for stabilising sediments[52]. Decreases in the biomass of primary producers (including cyanobacteria) could, therefore, induce cascading impacts on biodiversity and ecosystem services[53].

In the oyster-dominated mesocosms, perhaps in response to the decrease in cyanobacteria, invertebrate assemblage structure was different in all treatments exposed to microplastics compared with controls. Although species richness and the total abundance of infauna were not affected by microplastics, assemblages in *O. edulis* treatments exposed to 25 μg L^{-1} of HDPE had lower Shannon-Weiner diversity indices, indicating that assemblages were more homogeneous compared with controls. These differences were mostly caused by a greater dominance of oligochaetes, *Tubificoides benedii*, in all treatments with microplastics (which contributed ~30% of the difference between controls and each microplastic treatment, Table S7). Oligochaetes typically respond opportunistically to stressors and have long been considered as indicators of pollution in marine[54] and freshwater[55] systems. Mesocosms dosed with 25 μg L^{-1} of PLA or HDPE microplastics also had less *E. picta* (paddle worms). A

369 reduction in the abundance of paddle worms, which are often specialist predators, has been
370 found in response to other stressors, such as nutrient enrichment, in sedimentary systems[56].
371 Additionally, the abundance of *Lineus longissimus* (bootlace worms) was greater in
372 treatments with 25 μg L^{-1} PLA microplastics compared to those with 2.5 μg L^{-1} or no
373 microplastics. These worms are also a potential indicator species of pollution[57]. The
374 dominance of opportunistic species, suggests a simplification of the food web in response to
375 high levels of microplastic contamination.
376 Interestingly, there were no measurable effects of microplastic exposure on infaunal
377 invertebrate assemblages in the sediments with *M. edulis* mussels. This may be due to the
378 different effects of microplastics on filtration activity of the bivalves. For example, the
379 increase in filtration rates of *O. edulis* were likely accompanied by an increase in the
380 production of pseudofaeces. Particles rejected by bivalves as pseudofaeces are embedded in
381 mucous and sink[58], possibly increasing the availability of microplastics to the *O. edulis*
382 benthic communities. It is also possible that greater habitat complexity, due to the presence of
383 more shells in the mussel experiment, mitigated any effects of microplastics on ecosystem
384 functioning or on assemblages compared to in the oyster experiment. Habitat structure can
385 influence movement and resource utilisation of organisms and can alter the direct and indirect
386 interactions between species[59]. In order to fully understand the role of different ecosystem
387 engineers in mediating or exacerbating the effect of microplastics, experiments comparing
388 community level effects with and without ecosystem engineers present are needed.
389 Regardless of the mechanisms, this study shows that microplastics may affect ecosystem
390 functioning and biodiversity but, as has been found for other pollutants[60], such effects are
391 context-dependent.
392 Manipulation of microplastics in field conditions would be difficult and would pollute,
393 therefore, the use of an outdoor mesocosm system, with natural seawater and weather

394 conditions and recruitment of meio- and micro-organisms, provided an ideal compromise
395 between the highly controlled conditions of a laboratory experiment and the realism of a field
396 experiment. The extrapolation of results from any mesocosm experiment should, however,
397 proceed with caution since the assemblages represented in the mesocosms are simplified,
398 compared to the field. For example, pelagic larval recruitment[61] and other complex processes
399 involving larger organisms (such as fish), are excluded from the cores[62]. Regardless, semi-
400 field experiments, such as those using intact cores are a useful technique for evaluating the
401 effects of stressors on infaunal communities[63,64,65] and they have been found to produce
402 ecologically relevant data[66,67]. A similar mesocosm experiment using intact cores and *Ostrea*
403 *edulis* (in vegetated, sandy rather than muddy sediment) also found alterations to assemblage
404 structure and a reduction in diversity, specifically with less isopods, amphipods and
405 periwinkle snails after 60 days of exposure to 80 μg L^{-1} of HDPE or PLA microplastics[13].
406 Together these two pioneering studies indicate that microplastics could alter benthic
407 assemblages in *O. edulis*-dominated sedimentary habitats if they are repeatedly exposed
408 (even for just 2 hours per day) to concentrations as high as 2.5, 25 or 80 μg L^{-1}. Globally,
409 oyster populations are under threat, and due to overfishing, parasites and disease[68], 85% of
410 oyster reefs have been lost world-wide[69]. Declines in oyster reefs are cause for concern, not
411 only because they provide protein sources and support the fishing industry, but also because
412 of their role in the provision of ecosystem services in the coastal zone[70]. The current study
413 suggests that microplastics may represent an additional pressure to the organisms living in
414 these already threatened habitats.
415
416 *Wider implications and recommendations*
417 Since microplastics composed of PLA did not rapidly decompose, they caused many of the
418 same impacts as HDPE to *M. edulis* and *O. edulis* and the sediment they inhabit. This,

419 combined with evidence from other studies[13,33,71], supports recommendations that the term
420 "biodegradable" should be redefined to ensure that material, such as PLA, that apparently
421 does not rapidly and fully degrade in aquatic habitats does not enter drainage systems as
422 microscale particles such as microbeads[72] or enter the environment as larger litter.
423 Alterations to invertebrate assemblages in oyster-dominated sediment was detected at just 2.5
424 μg L^{-1}, although this dose is high, it is conservative compared to other recent experimental
425 studies e.g. 1250 - 25000[73] μg L^{-1} and 200 - 4800[22] μg L^{-1}. It is also much lower than current
426 levels found in some heavily contaminated coasts, for example, ~4500 μg L^{-1} in Korea[6].
427 Current plankton net sampling techniques, however, typically have a lower size limit of ~300
428 μm, therefore underestimating current densities of microplastics, possibly by between 3 and 6
429 orders of magnitude when compared to a 10 μm mesh[74]. Also, given that the cumulative input
430 of plastic waste is expected to increase in the coming decades[27] and that the fragmentation of
431 macroplastic litter already present in the environment will continue, concentrations of
432 microplastics are expected to increase[75]. In the current study, effects on ammonium
433 concentrations and biomass of cyanobacteria occurred at just 2.5 μg L^{-1}. Wider effects of
434 microplastics on nutrient cycling and invertebrate assemblages could, therefore, already be
435 occurring in heavily contaminated oyster-dominated habitats, however more research,
436 including mensurative studies, are needed to ascertain this.
437 The current study provides ecologically relevant data on the effects of contamination by
438 microplastic of different polymers, focusing on assemblage-level effects and ecosystem
439 functioning. Such data is currently still rare in the literature, but is vital in order to inform
440 policy and prevent damage to ecosystems[7]. In order to fully assess the ecological impacts of
441 microplastics, however, we also need to test their effects at low concentrations and using
442 realistic mixtures of polymer types (as opposed to one type at a time).

Figures

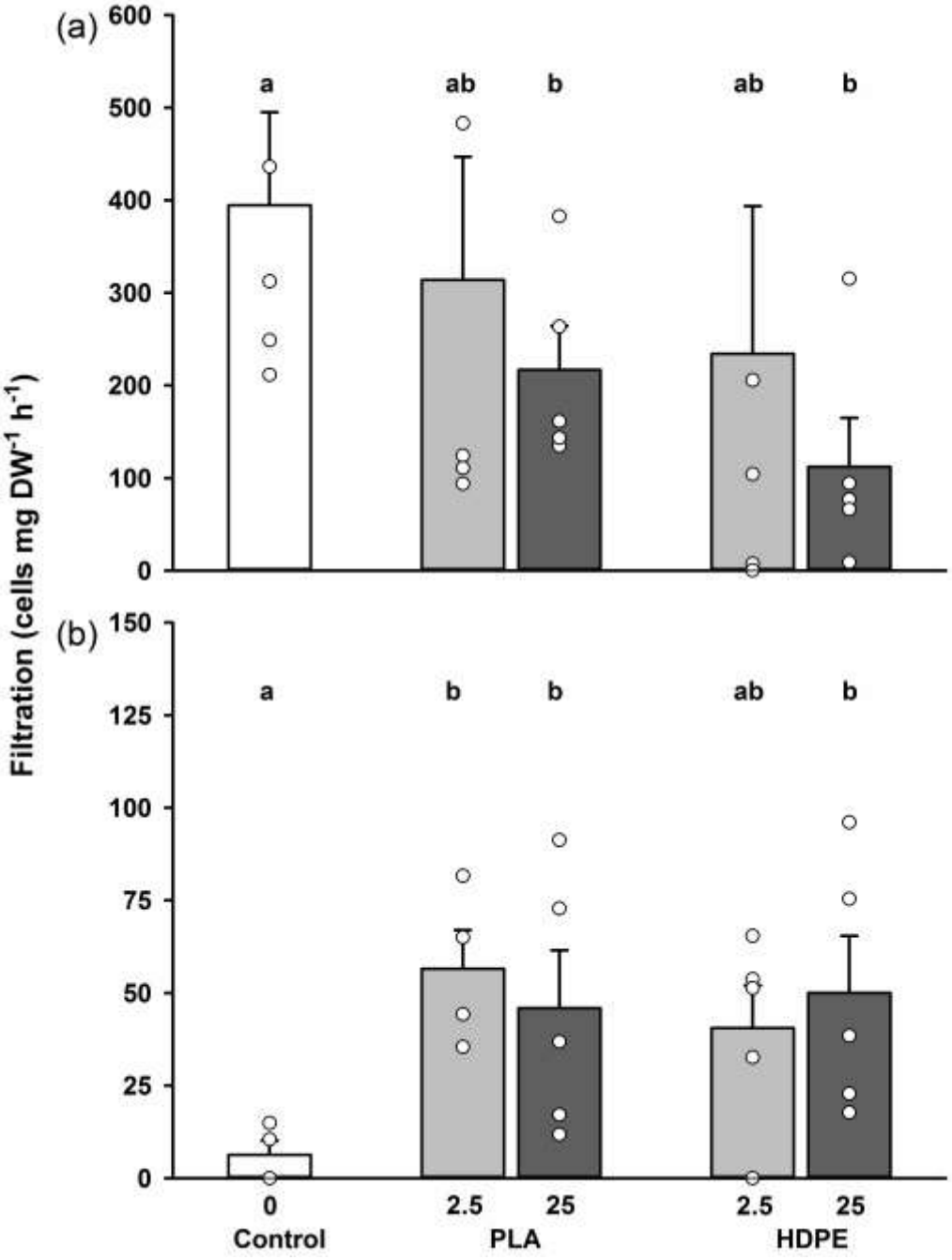


Figure 1. Filtration rates of (a) *M. edulis* and (b) *O. edulis* in mesocosms with 2.5 µg L^{-1} or 25 µg L^{-1} of PLA or HDPE or with no microplastics (Control) after 50 days. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, bars are means (± S.E.M.) with n = 5.

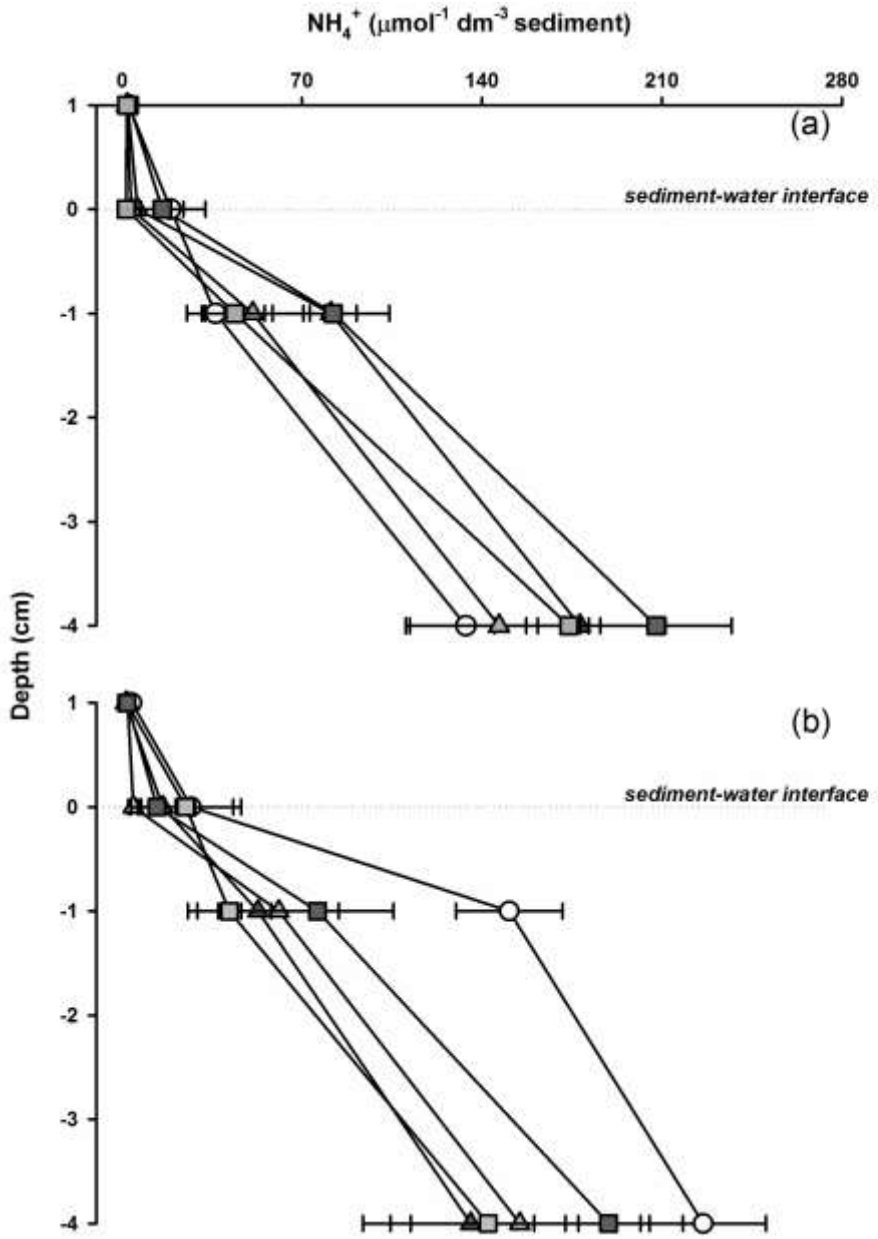
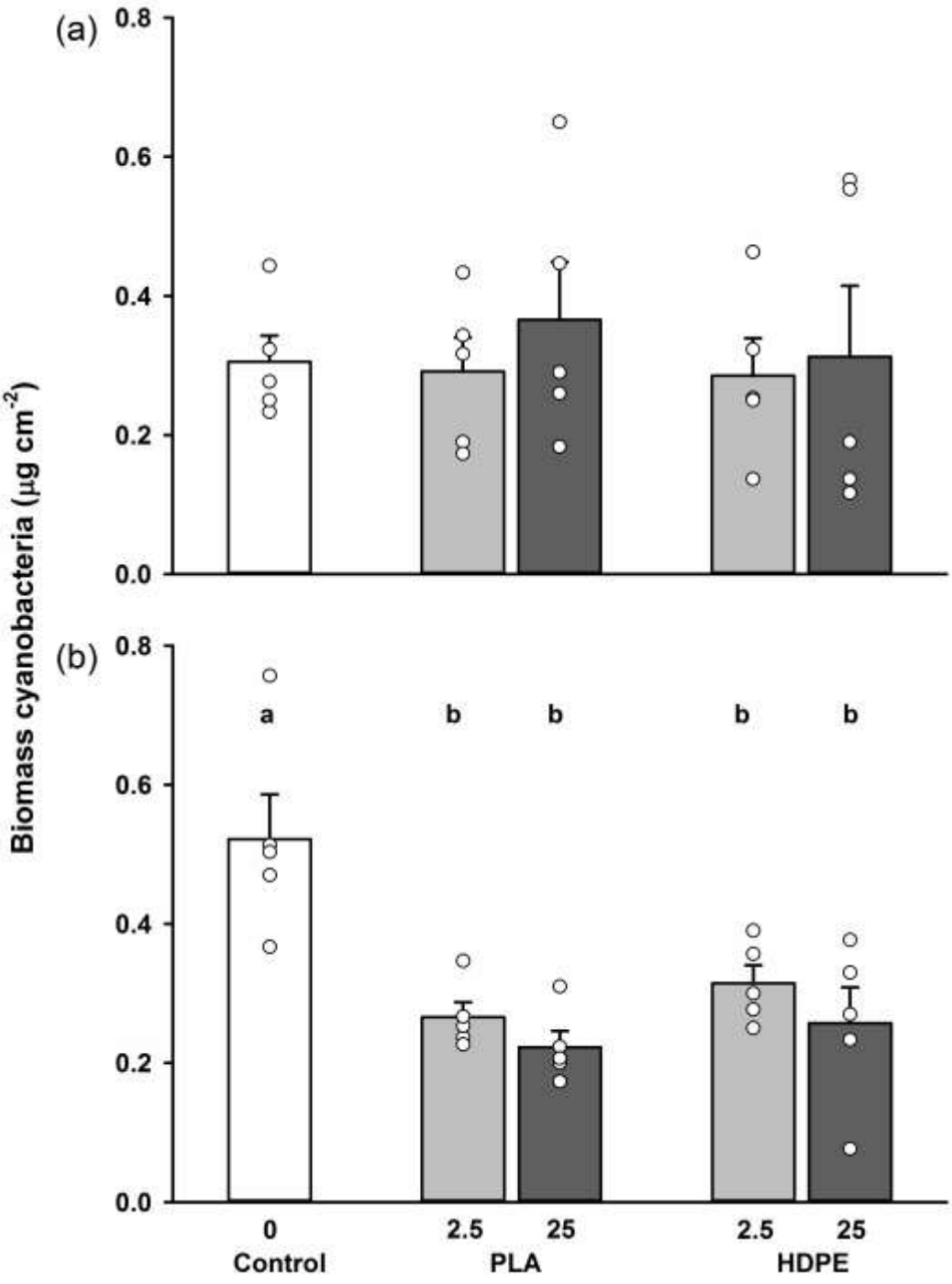
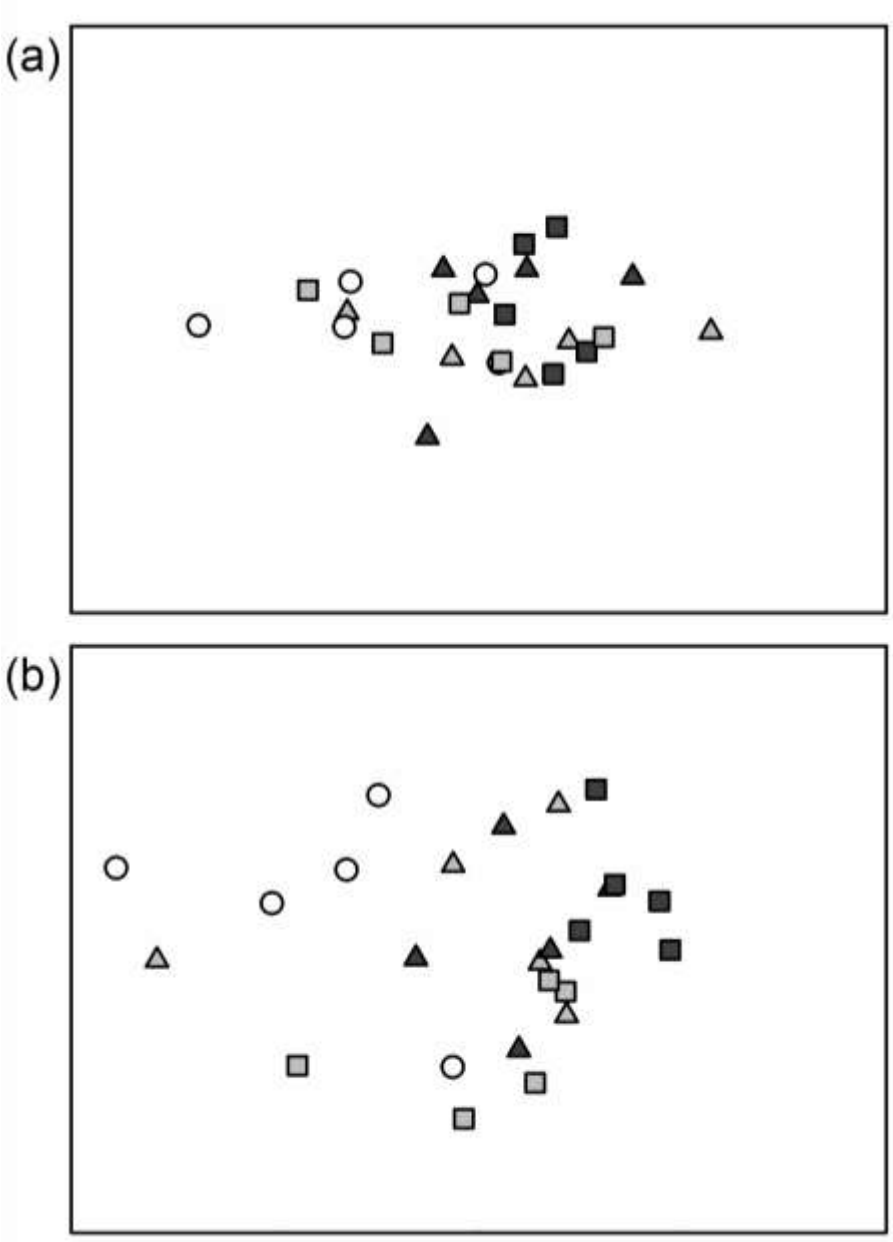


Figure 2. Concentrations of NH_4^+ in mesocosms with (a) *M. edulis* or (b) *O. edulis* of surface water (1 cm), the sediment-water interface (0 cm), and 1 & 4 cm into the sediment in mesocosms with 2.5 µg L^{-1} (△) or 25 µg L^{-1} (▲) of PLA or 2.5 µg L^{-1} (□) or 25 µg L^{-1} (■) of HDPE microplastics or no microplastics (Control = ○) after 50 days. Data are means (± S.E.M.) with n = 5.



455

456 **Figure 3.** Biomass of cyanobacteria in mesocosms with (a) *M. edulis* or (b) *O. edulis* and 2.5
457 µg L^{-1} or 25 µg L^{-1} of PLA or HDPE or with no microplastics (Control) after 48 days.
458 Different letters indicate significant differences among treatments as determined by *post-hoc*
459 comparisons or Dunnett's tests. Circles represent raw data and bars are means (± S.E.M.) with
460 n = 5.



461

462 **Figure 4.** Canonical analysis of principal coordinates of square root transformed community
463 structure data in mesocosms with (a) *M. edulis* or (b) *O. edilus* and 2.5 µg L^{-1} (△) or 25 µg
464 L^{-1} (▲)of PLA or 2.5 µg L^{-1} (□) or 25 µg L^{-1} (■) of HDPE microplastics or with no
465 microplastics (control = ○) after 50 days, n = 5.

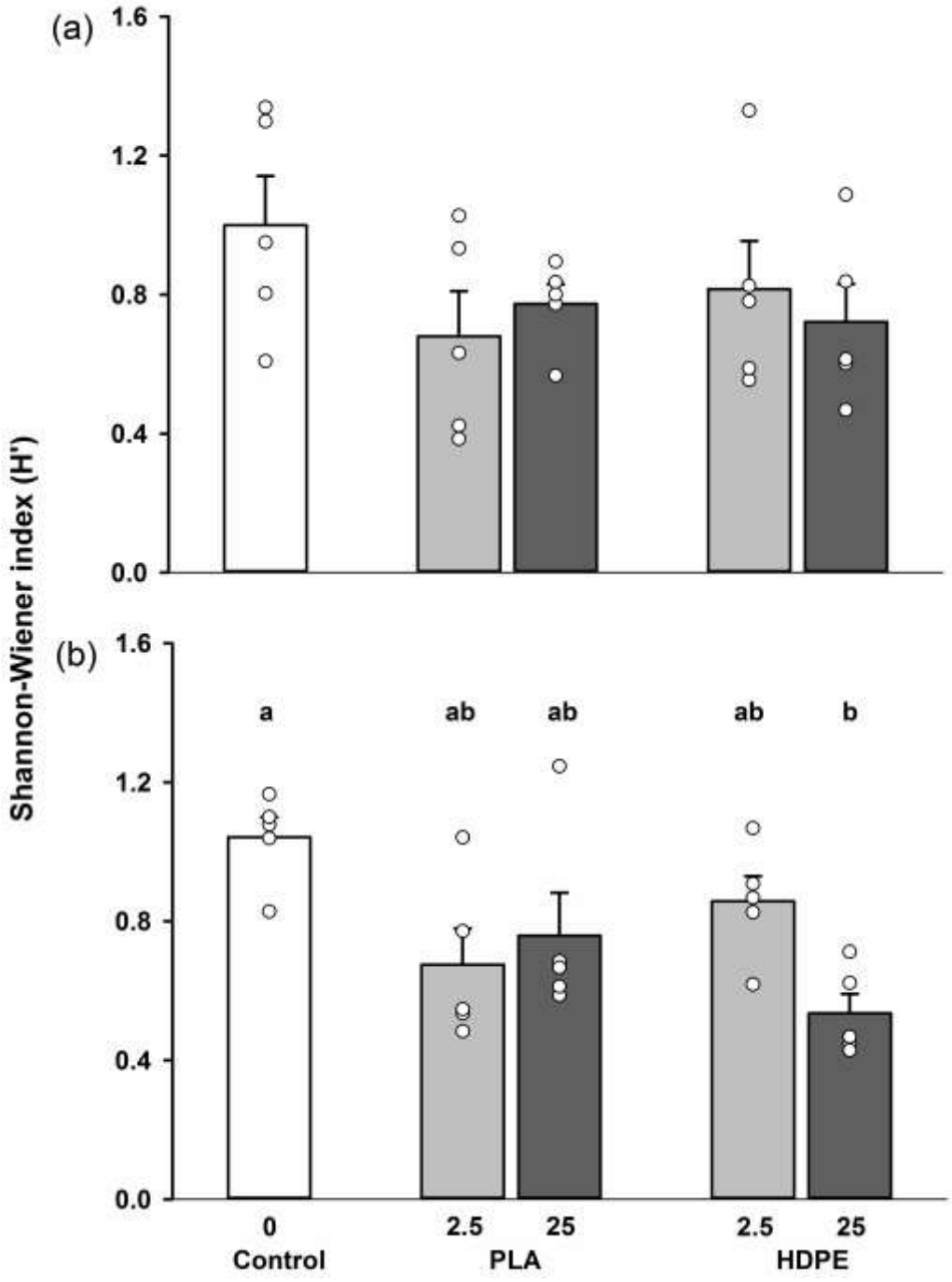
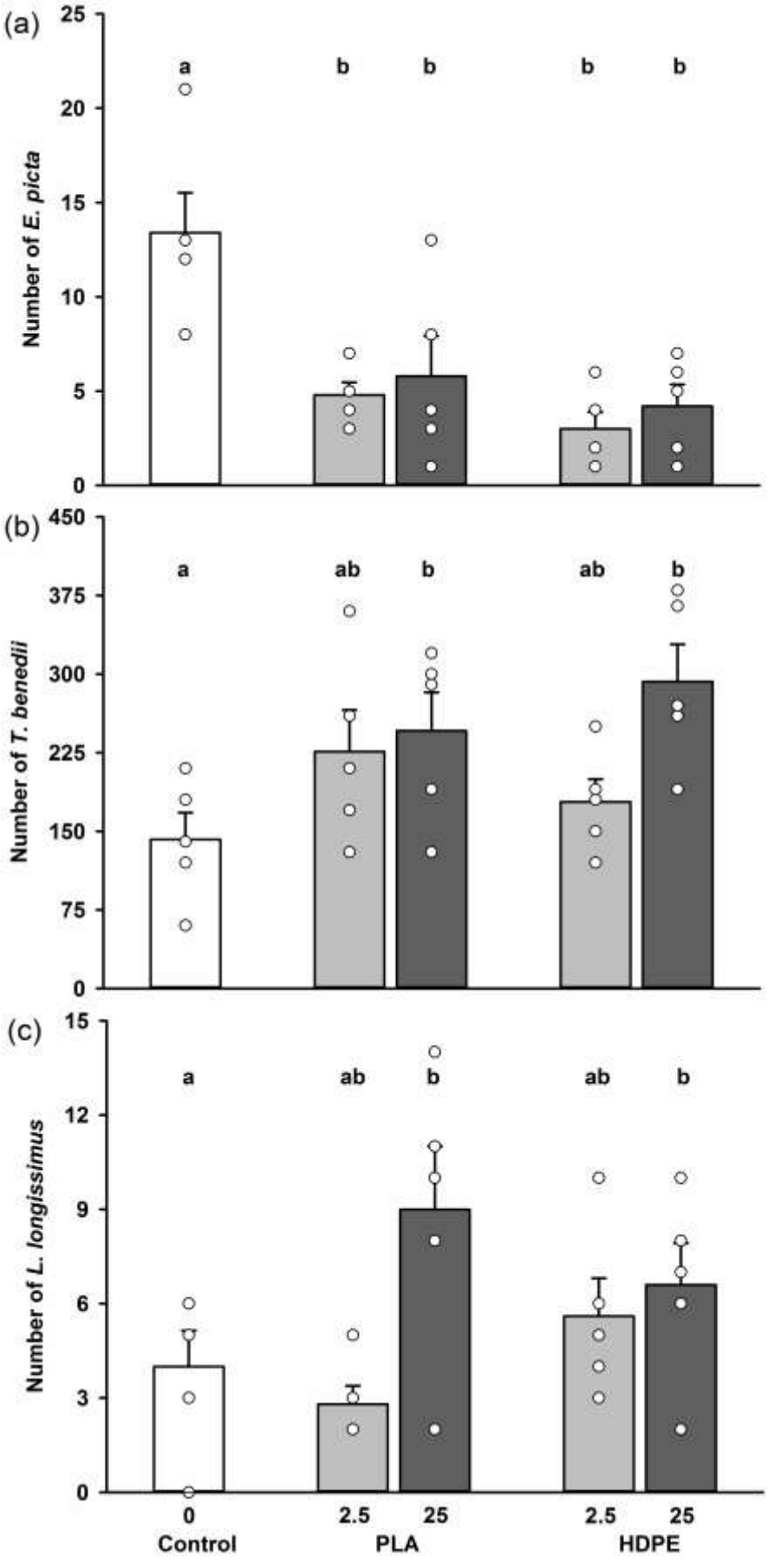


Figure 5. Shannon Wiener diversity index in mesocosms with (a) mussels or (b) oysters and 2.5 µg L^{-1} or 25 µg L^{-1} of PLA or HDPE or with no microplastics (control) after 50 days Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, and bars are mean (± S.E.M.) with n = 5.


(a)
Number of E. picta
25
20
15
10
5
0
a
b
b
b
b
(b)
Number of T. benedii
450
375
300
225
150
75
0
a
ab
b
ab
b
(c)
Number of L. longissimus
15
12
9
6
3
0
a
ab
b
ab
b
0
2.5
25
2.5
25
Control
PLA
HDPE

472

Figure 6. Abundances of (a) *E. picta*, (b) *T. benedii* and (c) *L. longissimus* in oyster treatments with 2.5 µg L^{-1} or 25 µg L^{-1} of PLA or HDPE or with no microplastics (control) after 50 days. Data from *M. edulis* mesocosms are not shown. Different letters indicate significant differences among treatments as determined by *post-hoc* comparisons or Dunnett's tests. Circles represent raw data, and bars are mean (± S.E.M.) with n = 5.

478 **Table 1.** Porewater ammonium pool (μmol dm^{-3}) and flux (μmol h^{-1}) in mesocosm sediment
479 after 50 days with *M. edulis* or *O. edulis* and no microplastics (control), or the two doses of
480 microplastics. Different superscript letters indicate significance between treatments. Data are
481 means (± S.E.M.) with n = 5.

		M. edulis		*O. edulis*	
		NH_4^+ pool	NH_4^+ flux	NH_4^+ pool	NH_4^+ flux
Control	**0 ug L^{-1}**	436.89 ± 77.63[a]	0.14 ± 3.99[a]	669.23 ± 80.57[a]	-57.18 ± 38.61[a]
PLA	**2.5 ug L^{-1}**	293.68 ± 33.29[a]	-19.68 ± 13.98[a]	359.52 ± 108.41[b]	-19.88 ± 4.52[a]
	25 ug L^{-1}	493.00 ± 52.43[a]	-25.58 ± 9.96[a]	325.83 ± 54.25[b]	2.34 ± 2.54[a]
HDPE	**2.5 ug L^{-1}**	326.25 ± 79.16[a]	-3.28 ± 8.25[a]	322.85 ± 69.08[b]	-8.76 ± 2.38[a]
	25 ug L^{-1}	351.19 ± 24.21[a]	-14.34 ± 6.56[a]	450.64 ± 94.07[b]	-10.42 ± 9.08[a]

482

ASSOCIATED CONTENT

File name “Supporting Material.pdf” containing 10 pages (cover page included), containing 1 figure and 7 tables.

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Notes

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SUPPORTING INFORMATION

Microplastics affect the ecological functioning of an important biogenic habitat

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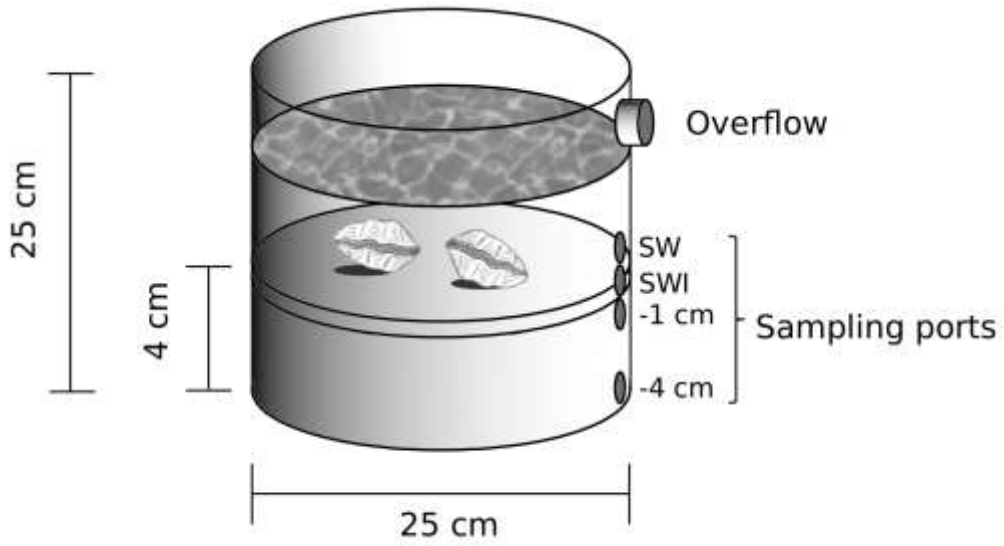

Figure S1. Diagram showing the design of the mesocosms with sampling ports for surface water (SW), the sediment-water interface (SWI) and for porewater at 1 and 4 cm into the sediment.

Table S1. Approximate number of microplastic particles per treatment (L^{-1}), estimated using haemocytometer counts on water samples taken from mesocosms directly after dosing on days 1, 26 and 48 of the experiment.

Plastic	Dose ($\mu g\ L^{-1}$)	Day 1	Day 26	Day 48
Control	0	0	0	0
PLA	2.5	260.42 ± 125.43	156.25 ± 45.54	138.89 ± 45.35
	25	1406.25 ± 193.48	1163.19 ± 165.29	1319 ± 189.99
HDPE	2.5	104.17 ± 65.88	86.81 ± 42.31	86.81 ± 33.95
	25	937.50 ± 213.48	815.97 ± 80.44	763.89 ± 126.88

Table S2. Mean (±S.E.M, n = 5) number of cells of *Isochrysis galbana* (mL^{-1}) estimated using haemocytometer counts in batches algal cultures for use in 2.5 or 25 μg L^{-1} of PLA or HDPE microplastic treatments or in Controls (with no microplastics) on days 1, 26 and 48 of the experiment.

Plastic	Dose (μg L^{-1})	Day 1	Day 26	Day 48
Control	0	$2.16 \times 10^6 \pm 2.37 \times 10^5$	$1.94 \times 10^6 \pm 1.37 \times 10^5$	$1.96 \times 10^6 \pm 2.31 \times 10^5$
PLA	2.5	$2.43 \times 10^6 \pm 6.01 \times 10^5$	$1.97 \times 10^6 \pm 3.30 \times 10^5$	$1.92 \times 10^6 \pm 2.22 \times 10^5$
	25	$2.45 \times 10^6 \pm 3.47 \times 10^5$	$1.90 \times 10^6 \pm 7.07 \times 10^4$	$2.07 \times 10^6 \pm 2.14 \times 10^5$
HDPE	2.5	$2.19 \times 10^6 \pm 2.59 \times 10^5$	$1.91 \times 10^6 \pm 2.82 \times 10^5$	$1.81 \times 10^6 \pm 1.72 \times 10^5$
	25	$2.52 \times 10^6 \pm 1.39 \times 10^5$	$1.80 \times 10^6 \pm 1.72 \times 10^5$	$2.16 \times 10^6 \pm 3.94 \times 10^5$

Table S3. Asymmetric ANOVA results of filtration of *M. edilus* and *O. edilus* after 50 days. The term "One-way" has 4,20 degrees of freedom (numerator and denominator, respectively) and all other terms have 1,20 degrees of freedom. F ratios with P significant at $\alpha = 0.05$ are indicated in **bold**.

M. edulis		
Source	F ratio	P value
One-way	1.62	0.330
C vs. O*	**4.58**	**0.045**
Plastic (P)	1.46	0.241
Dose (D)	0.37	0.548
P x D	0.06	0.805
O. edulis		
One-way	**3.20**	**0.038**
C vs. O	**11.63**	**0.003**
Plastic (P)	0.30	0.593
Dose (D)	0.00	0.958
P x D	0.86	0.366

* C vs. O = contrast comparing the control versus all others

Table S4. Asymmetric ANOVA on pool (µmol dm^{-3}) and flux (µmol h^{-1}) of NH_4^+, and biomass (µg cm^{-2}) of diatoms and cyanobacteria in the sediment after 48 days. The term "One-way" has 4,20 degrees of freedom (numerator and denominator, respectively) and all other terms have 1,20 degrees of freedom. Data are F ratios with P values (those significant at $\alpha = 0.05$ are indicated in **bold)**. In order to conform to the assumptions of normality, data for cyanobacteria were square-root transformed in the experiment with *O. edulis*.

	M. edulis							
Source	NH_4^+ pool		NH_4^+ flux		Diatoms		Cyanobacteria	
	F ratio	P value	F ratio	P value	F ratio	P value	F ratio	P value
One-way	2.03	0.129	1.40	0.271	0.17	0.953	0.21	0.927
C vs. O	1.20	0.286	2.39	0.138	0.00	0.959	0.01	0.914
Plastic (P)	0.89	0.356	2.27	0.148	0.05	0.827	0.18	0.672
Dose (D)	3.76	0.067	0.85	0.367	0.49	0.491	0.54	0.469
P x D	2.27	0.147	0.08	0.782	0.12	0.727	0.12	0.736
	O. edulis							
One-way	**3.05**	**0.041**	1.63	0.206	1.33	0.293	**7.23**	**0.001**
C vs. O	**10.7**	**0.004**	**5.74**	**0.027**	3.30	0.084	**25.71**	**<0.001**
Plastic (P)	0.28	0.603	0.00	0.964	0.01	0.922	0.88	0.360
Dose (D)	0.32	0.579	0.33	0.573	0.94	0.344	2.25	0.149
P x D	0.94	0.345	0.44	0.513	1.07	0.313	0.08	0.780

Table S5. Asymmetric permutational multivariate ANOVA results for assemblage structures in sediments with *M. edilus* or *O. edulis* and 2.5 or 25 μg L^{-1} of PLA or HDPE microplastics, or controls (C) with no microplastics after 50 days. When the factors "Plastic" or "Dose" were significant (at α = 0.05, indicated in **bold**), contrasts were used to determine any differences among treatments between levels.

			M. edulis		*O. edulis*	
Source	Contrasts	d.f.*	F-value	P-value	F-value	P-value
One-way		4	0.93	0.500	1.83	0.088
Plastic (P)		2	1.10	0.350	**2.51**	**0.037**
	PLA vs. HDPE	1	-	-	0.32	0.781
	PLA vs. C	1	-	-	**3.61**	**0.028**
	HDPE vs. C	1	-	-	**3.84**	**0.018**
Dose (D)		2	1.21	0.277	**3.10**	**0.026**
	2.5 vs. 25	1	-	-	1.65	0.167
	2.5 vs. C	1	-	-	**3.00**	**0.043**
	25 vs. C	1	-	-	**4.72**	**0.015**
PxD		1	0.78	0.545	0.82	0.461

*d.f. = degrees of freedom of nominator

Table S6. Asymmetric ANOVA results of number of taxa (R), total abundance (N), Shannon Wiener diversity (H') and abundances of *E. picta, T. benedii* and *L. longissimus* after 50 days. The term "One-way" has 4,20 d.f.'s and all other terms have 1,20 d.f.'s. (numerator and denominator, respectively) F ratios (F) with P significant at α=0.05 are indicated in **bold**. In order to conform to the assumptions of homogeneity of variance, R and N were square-root transformed for data from the *M. edulis* experiment.

	M. edulis											
Source	R		N		H'		*E. picta*		*T. benedii*		*L. longissimus*	
	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
One-way	0.81	0.536	0.55	0.703	1.08	0.393	0.52	0.725	0.61	0.658	1.37	0.279
C vs. O	2.80	0.109	1.06	0.314	3.57	0.073	0.83	0.373	1.27	0.272	1.61	0.219
Plastic (P)	0.24	0.630	0.08	0.777	0.12	0.730	0.12	0.732	0.00	0.990	1.70	0.206
Dose (D)	0.18	0.674	0.20	0.659	0.00	1.000	0.56	0.465	0.11	0.740	0.37	0.548
P x D	0.00	0.950	0.84	0.370	0.62	0.439	0.56	0.465	1.07	0.313	1.79	0.196
	O. edulis											
One-way	2.09	0.119	1.59	0.217	**4.84**	**0.007**	**7.36**	**0.001**	**3.25**	**0.033**	**3.24**	**0.033**
C vs. O	0.01	0.936	3.49	0.076	**11.95**	**0.003**	**27.67**	**<0.001**	**6.61**	**0.018**	1.81	0.194
Plastic (P)	2.70	0.116	0.23	0.638	0.06	0.812	1.25	0.277	0.00	0.989	0.02	0.882
Dose (D)	1.63	0.216	2.02	0.171	1.88	0.185	0.52	0.478	4.28	0.052	3.81	0.065
P x D	4.03	0.058	0.61	0.45	**5.47**	**0.029**	0.00	0.948	2.12	0.161	**7.31**	**0.014**

Table S7. SIMPER analyses based on square-root transformed abundance data within the sediment from the *O. edulis* mesocosms with no microplastics (control = C) versus 2.5 µg L^{-1} of PLA (2.5 PLA), 25 µg L^{-1} of PLA (25 PLA), 2.5 µg L^{-1} of HDPE (2.5 PLA) or 25 µg L^{-1} of HDPE (25 HDPE).

	Average abundance		Av.Diss*	Diss/SD**	Contrib %***	Cum.%****
Taxon			C vs. 2.5 PLA			
T. benedii	12.74	16.71	8.43	1.44	29.93	29.93
Corophium sp.	2.89	4.52	5.22	1.49	18.52	48.45
Spionidae	4.03	3.22	2.61	1.37	9.28	57.73
Glycera sp.	2.87	2.82	2.34	1.38	8.32	66.05
E. picta	3.08	2.27	1.92	1.62	6.82	72.86
L. longissimus	2.2	1.48	1.89	1.23	6.7	79.57
Hydrobia sp.	1.2	0.51	1.63	1.2	5.79	85.36
			C vs. 25 PLA			
T. benedii	12.74	15.56	7.04	1.43	26.92	26.92
Corophium sp.	2.89	2.99	4.03	1.4	15.4	42.32
Glycera sp.	2.87	2.8	2.9	1.33	11.07	53.39
Spionidae	4.03	3.92	2.89	1.27	11.04	64.43
E. picta	3.08	2.53	1.95	1.4	7.46	71.89
L. longissimus	2.2	2.52	1.78	1.29	6.78	78.68
Hydrobia sp.	1.2	0.6	1.73	1.24	6.6	85.28
			C vs. 2.5 HDPE			
T. benedii	12.74	14.63	7.04	1.27	28.2	28.2
Corophium sp.	2.89	3.71	3.66	1.38	14.68	42.87
Spionidae	4.03	4.28	2.41	1.52	9.65	52.52
Glycera sp.	2.87	2.4	2.38	1.44	9.52	62.04
E. picta	3.08	2.11	2.16	1.5	8.64	70.67
Hydrobia sp.	1.2	0.62	1.59	1.19	6.37	77.05
L. longissimus	2.2	2.47	1.54	1.25	6.18	83.23
			C vs. 25 HDPE			
T. benedii	12.74	16.81	8.87	1.38	32.39	32.39
Corophium sp.	2.89	2.53	4.08	1.29	14.91	47.31
Spionidae	4.03	3.04	2.83	1.34	10.34	57.65
Glycera sp.	2.87	3.4	2.71	1.34	9.91	67.55
E. picta	3.08	2.29	1.94	1.41	7.09	74.65
Hydrobia sp.	1.2	0.5	1.82	1.21	6.65	81.3
L. longissimus	2.2	2.23	1.49	1.22	5.43	86.74

* Av. Diss. = average "absolute" contribution of taxon to total dissimilarity between pairs based on Bray-Curtis dissimilarities

** Diss/SD = ratio of average contribution to dissimilarity and the standard deviation among all contribution across all pairs of samples

*** Contrib % = contribution of taxon in % to dissimilarity between the two samples

**** Cum. % = cumulative percentage of contribution of taxon to the dissimilarity between the two sample.

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