



UNIVERSITY OF
PLYMOUTH

PEARL

Microplastics Affect the Ecological Functioning of an Important Biogenic Habitat.

Green, DS; Boots, B; O'Connor, NE; Thompson, R

Published in:

Environmental Science & Technology

Publication date:

2017

Link:

[Link to publication in PEARL](#)

Citation for published version (APA):

Green, DS., Boots, B., O'Connor, NE., & Thompson, R. (2017). Microplastics Affect the Ecological Functioning of an Important Biogenic Habitat. *Environmental Science & Technology*, 0(0).

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Wherever possible please cite the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Download date: 15. Sep. 2024

1 'This is a copy of the accepted paper as submitted for final publication. The final published version
2 can be found at <http://pubs.acs.org/doi/abs/10.1021/acs.est.6b04496>

3

4

5

6

7 **Microplastics affect the ecological functioning of an important biogenic habitat**

8

9 Dannielle Senga Green^{1,2,3*}, Bas Boots², Nessa E. O'Connor^{4,5} and Richard Thompson⁶

10

11 *¹Department of Life Sciences, Anglia Ruskin University, Cambridge, United Kingdom,*
12 *CB11PT.*

13 *²School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry, United*
14 *Kingdom, CV47AL.*

15 *³Biogeochemistry Research Group, Geography Department, School of Natural Sciences,*
16 *Trinity College Dublin, Dublin 2, Ireland.*

17 *⁴Queen's University Marine Laboratory, Portaferry, Northern Ireland, United Kingdom.*

18 *⁵School of Biological Sciences, Queen's University Belfast, Northern Ireland, United*
19 *Kingdom, BT71NN.*

20 *⁶School of Marine Science and Engineering, Plymouth University, Plymouth, Devon, United*
21 *Kingdom, PL48AA.*

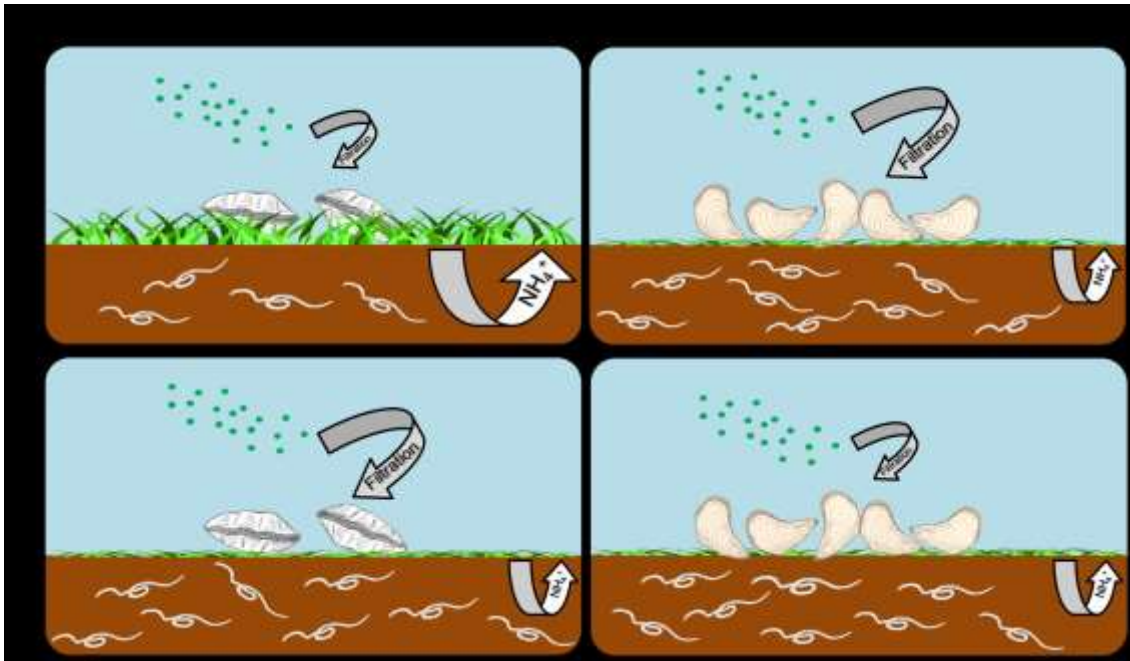
22

23 **Keywords:** plastic pollution, *Ostrea edulis*, *Mytilus edulis*, polyethylene, polylactic acid,
24 biogeochemistry, nutrient cycling

25 **Abstract**

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

43



44

45 **Introduction**

46 Microplastics contaminate marine habitats across the globe¹ and are recognised as a
47 significant environmental challenge requiring urgent management². It has recently been
48 suggested that they are the most abundant form of solid waste on Earth¹ and their abundance
49 is increasing³. Although there is much uncertainty regarding the concentrations of
50 microplastics in the environment, high concentrations in seawater of $\sim 3\text{-}23 \mu\text{g L}^{-1}$ and even
51 up to $\sim 4500 \mu\text{g L}^{-1}$ have been reported^{4,5,6} in some heavily contaminated areas. Despite this
52 prevalence, their effects on marine ecosystems are not well understood. Research to date has
53 mostly focused on effects of microplastics on individual species, but effects on assemblages
54 and ecosystem functioning within coastal habitats remain largely unknown^{7,8}.

55 Previous research has concentrated on organisms that ingest microplastics directly, such as
56 filter-feeders, including marine mussels^{9,10,11} and oysters^{12,13}. These organisms are typically
57 chosen for exposure experiments due to their great filtration capacity. For example,
58 individual mussels and oysters can filter $\sim 0.5\text{-}2.5^{14}$ and $\sim 5\text{-}25^{15}$ L of seawater h^{-1}
59 respectively. As such, they are very likely to ingest microplastics¹⁶, and indeed, specimens
60 from the field have been found to contain microplastics^{11,17,18}. Exposure to relatively high
61 densities of microplastics has been found to alter the respiration rates¹³, immunology¹⁰,
62 reproductive capacity and filtration rates¹² of bivalves. Owing to their role as ecosystem
63 engineers, such effects are likely to permeate beyond the individual organism. For example,
64 reefs created by mussels and oysters provide refugia and nursery grounds for other,
65 commercially-important species and can support diverse communities^{19,20}. In addition, filter
66 feeding leads to benthic-pelagic coupling; channelling nutrients from the water column and
67 locally concentrating them via biodeposition (i.e. deposition of faeces and pseudo-faeces).
68 Mussels and oysters can, therefore, enhance the release of limiting inorganic nutrients, such
69 as ammonium, from sediments, fuelling primary productivity of the microphytobenthos (such

70 as diatoms and cyanobacteria) in the sediment, which in turn supports benthic and pelagic
71 food webs²¹. If microplastics alter the ability of these organisms to filter feed, there may be
72 wider impacts on their associated communities and on the functioning of coastal ecosystems.
73 In addition, biodeposition is a likely mechanism by which suspended microplastics are
74 transported from the pelagic zone onto sediments²². Mussels and oysters may, therefore,
75 locally concentrate microplastics potentially altering biogeochemical processes, the biomass
76 of primary producers and macrofaunal assemblages within the sediment.

77 In response to concerns of globally increasing plastic pollution, demand for biodegradable
78 plastics has risen, with annual global production predicted to quadruple over the next five
79 years²³. It is thought that the replacement of conventional plastics, such as high density
80 polyethylene (HDPE), with biodegradable alternatives, such as polylactic acid (PLA) will
81 reduce the persistence, and therefore the impacts, of plastic pollution²⁴. However, methods
82 developed to assess the rate and extent of biodegradability of plastics in marine environments
83 (e.g. ASTM International D7991-15)²⁵ are still limited in their ability to predict degradation
84 in natural habitats²⁶. The potential for PLA and other bioplastics, or biodegradable plastics to
85 persist as microscopic particles, or to affect assemblages of organisms in the marine
86 environment before they degrade, remains largely unknown. Recently, however, Green,
87 (2016)¹³ showed that PLA microplastics can lead to alterations in assemblage structure of
88 macrofauna in sandy sediment with oysters.

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

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

99

100 **2. Methodology**

101 *2.1. Experimental design and set-up*

102 Two separate mesocosm experiments, one focusing on *M. edulis* and one on *O. edulis*
103 habitats, were set up simultaneously at the outdoor flow-through mesocosm facility at
104 Queen's University Marine Laboratory, Portaferry, Northern Ireland. Both experiments had
105 the same asymmetric design, with two fixed, orthogonal factors: "Plastic", with two levels:
106 polylactic acid (PLA) and high density polyethylene (HDPE) and "Dose", with two levels:
107 $2.5 \mu\text{g L}^{-1}$ and $25 \mu\text{g L}^{-1}$ seawater. A single treatment, without any added microplastics, was
108 used as a control. To estimate the densities of microplastics in each treatment, water samples
109 were taken from each "Plastic x Dose" treatment on days 1, 26 and 48 and microplastic
110 particles were counted using a haemocytometer (Table S1). For each experiment, all
111 treatments were replicated five times ($n = 5$, $N = 25$ per species) for a total of 50 mesocosms.
112 Although the applied doses were relatively high compared with average densities observed
113 and reported in the literature from $\sim 330 \mu\text{m}$ plankton-net tow samples²⁷, at smaller mesh
114 sizes ($50 \mu\text{m}$) densities of up to $7800 \text{ particles L}^{-1}$ (equating to $\sim 4500 \mu\text{g L}^{-1}$) have been
115 found in heavily contaminated coastal waters⁶. Furthermore, the densities used in the current
116 study are among the lowest used experimentally to date²⁸ and were chosen to approximately
117 reflect high values currently ($2.5 \mu\text{g L}^{-1}$) and in the future ($25 \mu\text{g L}^{-1}$) based on the prediction
118 that global plastic waste input will increase 10-fold by 2025²⁷.

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 2016¹³). Each
121 mesocosm had an overflow pipe, allowing drainage directly into the basin. Waste water did
122 not come into contact with other mesocosms 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 (\pm S.E.M.) wet biomass of 20.1 \pm
136 1.7 g, maximal length of 47.9 \pm 0.6 mm, width of 21.5 \pm 0.4 mm and height of 23.5 \pm 0.3 mm
137 (n = 175). The collected *O. edulis* had an initial average (\pm S.E.M.) wet biomass of 36.0 \pm 5.2
138 g, maximal length of 63.0 \pm 1.6 mm, width of 60.1 \pm 1.1 mm and height of 14.9 \pm 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%

144 cover and 5 individuals m⁻² for *M. edulis* and *O. edulis* respectively, as defined by OSPAR²⁹).
145 The bivalves were placed on the surface of the sediment to mirror how they occurred locally
146 in the field. There were no significant differences between the biomasses of individuals
147 allocated to the different treatments at the start of the experiment (one-way ANOVA based on
148 averaged dimensions in each mesocosm: *M. edulis*: $F_{4,20} = 0.32$, $P = 0.861$, *O. edulis*: $F_{4,20} =$
149 0.26 , $P = 0.902$). The microplastic particles used in the experiment were of a similar colour
150 (white) and size range, although their volume-weighted mean diameters differed: 65.6 μm
151 (range = 0.6–363 μm) for PLA and 102.6 μm (range = 0.48–316 μm) for HDPE. In order to
152 introduce microplastics into the mesocosms in a realistic manner, a dietary exposure method
153 was used. In brief, microplastics were added to separate cultures (10 L) of the microalgae,
154 *Isochrysis galbana* and left for 3 days with constant aeration. This was long enough for the
155 microplastics to become more neutrally buoyant; i.e. move more freely within the culture
156 containers rather than clinging to the sides or floating on top of the water. Fresh batches of
157 control and microplastic dosed algae cultures were made up weekly. In order to ensure that
158 the concentrations of *I. galbana* did not differ between treatments, algal cells were counted
159 from each culture using a haemocytometer (on days 1, 26 and 48, Table S2). There were no
160 significant differences in the density of *I. galbana* cells between treatments (one-way
161 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: $F_{4,20} =$
162 0.28 , $P = 0.891$) and no aggregations of microalgae and microplastics were observed during
163 the experiment. Cultures of *I. galbana* were prepared using seawater (35 psu), which was
164 filtered with 0.45 μm aperture membranes and sterilised with UV light. Every day, each
165 mesocosm received 250 mL of $\sim 2 \times 10^6$ cells mL⁻¹ of microalgae containing either 0
166 (control), 80 or 800 $\mu\text{g L}^{-1}$ of PLA or HDPE microplastics, equating to final densities in the
167 mesocosms of 2.5 $\mu\text{g L}^{-1}$ or 25 $\mu\text{g L}^{-1}$ (i.e. 250 mL diluted by the 8 L mesocosm
168 volume). During feeding the flow of water was stopped for two hours and air bubblers were

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

176

177 2.2. Filtration rates of *M. edulis* and *O. edulis*

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

186

187 2.3. Porewater nutrients; ammonium, nitrate and nitrite

188 Porewater samples were collected using RhizonTM membranes (Rhizosphere Research
189 Products B.V., The Netherlands) inserted into the sampling ports of the mesocosms. This
190 allowed water to be sampled at the surface (1 cm above the sediment), sediment-water
191 interface (0 cm) and at 1 and 4 cm depths in the sediment. The flow of seawater into
192 mesocosms was stopped and porewater was drawn by attaching a needle to each RhizonTM
193 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 $\sim 0.01 \text{ mg L}^{-1}$) 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 (BenthosTorch, bbe-Moldaenke GmbH, Schwentinental, Germany³²)
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 activities. The BenthosTorch 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 BenthosTorch on similar sediment mesocosms found it to
212 mirror the patterns of chlorophyll-a extraction using solvents³³.

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

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

221

222 2.6. Statistical data analyses

223 Statistical analysis was done using the R environment (R v3.2.3; R core team 2015). The data
224 were screened for normality (q-q plots, and Shapiro-Wilk tests) and homogeneity of variance
225 (Levene's test, using the *car* (v2.1-2) package³⁵) to ascertain assumptions for ANOVA.
226 Transformation of some data was necessary to enable them to conform to these assumptions
227 (specific transformations are stated in the results). Data were analysed separately for each of
228 the two experiments (i.e. *M. edulis* and *O. edulis* were not compared in the statistical
229 analyses). Since the design was asymmetrical (i.e. having a single control group for the two
230 factors "Plastic" and "Dose"), the data were analysed by using the mean squares from two
231 independent ANOVAs³⁶ (see Green et al., 2016³³ for more details on calculations). Briefly,
232 this included partitioning of the variance by calculating: (1) one-way ANOVA with all
233 treatments as separate levels ($a=5$, $n=5$, $N=25$); and (2) a full-factorial two-way ANOVA of
234 "Plastic" by "Dose" without the control ($a=2$, $b=2$, $n=5$, $N=20$). The residuals of the 1st
235 ANOVA were used to assess differences between the levels within the 2nd ANOVA, allowing
236 the variation associated with controls and that of the other treatments to be distinguished ("C
237 vs. O"), which is contrasted with one degree of freedom³⁶. When a significant effect in the "C
238 vs. O" contrast was found Dunnett's test was used to contrast the control versus each level of
239 the significant term using the *multcomp* (v1.4-6) package³⁷. Pairwise comparisons for the
240 factors in ANOVA (2) were computed using Tukey HSD tests when the main terms were
241 significant. Statistical significance was assumed at $\alpha = 0.05$.

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

244 transformed data with 9999 permutations under the reduced model using Type I sum of
245 squares (SS) using PERMANOVA+ add-on (PRIMER-E Ltd. Plymouth, UK). The
246 asymmetrical analyses were achieved by fitting each main effect (“Plastic” and “Dose”) in
247 turn with a Type I (sequential) SS model, then swapping the order of the terms and
248 combining the results of the two analyses³⁸. When a factor was significant, contrasts were
249 used to determine the specific differences. Results of the PERMANOVA were visualised
250 with 2-dimensional ordination using canonical analysis of principal coordinates (CAP)³⁹.
251 Where assemblage structures differed, SIMPER analysis was used to quantify the
252 contribution of different taxa to dissimilarities between treatments.

253

254 **3. Results**

255 *3.1. Effects of microplastics on the filtration rates of bivalves*

256 Two mussels died during the experiment and were removed. There were no oyster
257 mortalities. When exposed to 25 $\mu\text{g L}^{-1}$ of PLA or HDPE microplastics, *M. edulis* filtered
258 ~2.4 times less microalgae (*I. galbana*) per hour than when exposed to none of the
259 experimental microplastics (Figure 1a, Table S3, Dunnett's *Control vs 25 $\mu\text{g L}^{-1}$* : $t=2.42$, $P=0.045$).
260 There was no effect of 2.5 $\mu\text{g L}^{-1}$ of either type of microplastic on the filtration of *M. edulis*.
261 On the contrary, *O. edulis* in the control mesocosms filtered ~7.5 times less microalgae than
262 those in mesocosms with any type or density of microplastic (Figure 1b, Table S3, Dunnett's
263 *Control vs 25 $\mu\text{g L}^{-1}$* : $t=-3.09$, $P=0.011$, *Control vs 2.5 $\mu\text{g L}^{-1}$* : $t=-2.74$, $P=0.024$, *Control vs PLA*: $t=-2.51$,
264 $P=0.038$, *Control vs HDPE*: $t=-2.74$, $P=0.024$) compared to when not exposed to microplastics.

265

266 *3.2. Effects of microplastics on ammonium in sediment porewater*

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

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

277

278 3.3. Effects of microplastics on the microphytobenthos

279 The biomass of diatoms was not significantly different between the microplastic treatments
280 for sediments with *M. edulis* or *O. edulis* (Table S4). The biomass of cyanobacteria, however,
281 was significantly less in sediments which contained microplastics with *O. edulis* (Table S4)
282 (but not those with *M. edulis*, Figure 3a), and was ~2 times greater in the controls than in
283 mesocosms dosed with either type or density of microplastics (Figure 3b, Dunnett's *Control vs 25*
284 $\mu\text{g L}^{-1}$ *PLA*: $t=4.77$, $P<0.001$; *Control vs 2.5* $\mu\text{g L}^{-1}$ *PLA*: $t=3.91$, $P=0.003$; *Control vs 25* $\mu\text{g L}^{-1}$ *HDPE*: $t=4.31$,
285 $P=0.001$; *Control vs 2.5* $\mu\text{g L}^{-1}$ *HDPE*: $t=3.05$, $P=0.022$).

286

287 3.4. Effects of microplastics on infaunal assemblages

288 There were no significant differences between the structure of infaunal invertebrate
289 assemblages (Figure 4a, Table S5), the diversity indices (Figure 5a, Table S6) nor the
290 abundance of individual taxa (Table S6) in sediments with *M. edulis*. Sediments with *O.*
291 *edulis*, however, had significantly different assemblage structures in treatments dosed with
292 microplastics, at any density or type of plastic compared to controls (Figure 4b, Table S5)
293 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 $25 \mu\text{g L}^{-1}$ of HDPE microplastics
296 (Figure 5b, Table S6, Dunnett's *Control vs 25 $\mu\text{g L}^{-1}$ 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 $\mu\text{g L}^{-1}$ PLA*: $t=3.53$, $P=0.008$; *Control vs 2.5 $\mu\text{g L}^{-1}$ PLA*:
300 $t=3.99$, $P=0.002$; *Control vs 25 $\mu\text{g L}^{-1}$ HDPE*: $t=4.27$, $P=0.001$; *Control vs 2.5 $\mu\text{g L}^{-1}$ 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 $25 \mu\text{g L}^{-1}$ of either type of microplastic (Figure 6b, Table
303 S6, Dunnett's *Control vs 25 $\mu\text{g L}^{-1}$* : $t=-3.27$, $P=0.007$). There were also ~2.6 times more *Lineus*
304 *longissimus* nemerteans in sediments when exposed to $25 \mu\text{g L}^{-1}$ of PLA than in those
305 exposed to $2.5 \mu\text{g L}^{-1}$ of PLA or no microplastics (Figure 6c, Table S6, Tukey's HSD *2.5 $\mu\text{g L}^{-1}$*
306 *PLA vs 25 $\mu\text{g L}^{-1}$ PLA*: $P=0.026$, Dunnett's *Control vs 25 $\mu\text{g L}^{-1}$ PLA*: $t=-2.66$, $P=0.049$).

307

308 4. Discussion

309 *Mytilus edulis* and *Ostrea edulis* responded differently to contamination with microplastics.
310 The blue mussels filtered fewer algal cells h^{-1} when exposed to $25 \mu\text{g L}^{-1}$ 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 $25 \mu\text{g L}^{-1}$ 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 $23 \mu\text{g L}^{-1}$ of $6 \mu\text{m}$ polystyrene microplastics¹². 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 $4000 \text{ cells mL}^{-1}$ and *M. edulis* reaches optimum filtration
329 rates at 2000 and $6000 \text{ cells mL}^{-1}$, whilst *O. edulis* requires concentrations an order of
330 magnitude greater than this for optimum filtration rates to be reached⁴⁴. 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 pseudofaeces⁴⁵. 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,

344 HDPE or PVC microplastics (2% of wet sediment weight) directly added to sandy sediment
345 led to reductions in the biomass of benthic diatoms, but not of cyanobacteria³³. This
346 difference could be due to the grain size of the sediment. Cyanobacteria are less able to build
347 stable microbial mats on fine (muddy) sediment than they are on coarse (sandy) sediment,
348 whilst diatoms are stable on muddy sediment⁴⁶. Nano- or micro- plastics have also been
349 found to reduce the productivity of other primary producers. For example, Besseling et al.
350 (2014)⁴⁷ found that nanoplastics reduced growth of green algae and overall chlorophyll
351 concentrations in laboratory microcosm experiments, and in another study Bhattacharya et al.
352 (2010)⁴⁸ reported a reduction of photosynthesis by microalgae. Cyanobacteria are key
353 primary producers in sedimentary systems⁴⁹, vital in food-web dynamics^{50,51}. Together with
354 euglenids and diatoms, they can supply up to 45% of the organic budget of an estuary¹⁴ and
355 are important for stabilising sediments⁵². Decreases in the biomass of primary producers
356 (including cyanobacteria) could, therefore, induce cascading impacts on biodiversity and
357 ecosystem services⁵³.

358 In the oyster-dominated mesocosms, perhaps in response to the decrease in cyanobacteria,
359 invertebrate assemblage structure was different in all treatments exposed to microplastics
360 compared with controls. Although species richness and the total abundance of infauna were
361 not affected by microplastics, assemblages in *O. edulis* treatments exposed to 25 $\mu\text{g L}^{-1}$ of
362 HDPE had lower Shannon-Weiner diversity indices, indicating that assemblages were more
363 homogeneous compared with controls. These differences were mostly caused by a greater
364 dominance of oligochaetes, *Tubificoides benedii*, in all treatments with microplastics (which
365 contributed ~30% of the difference between controls and each microplastic treatment, Table
366 S7). Oligochaetes typically respond opportunistically to stressors and have long been
367 considered as indicators of pollution in marine⁵⁴ and freshwater⁵⁵ systems. Mesocosms dosed
368 with 25 $\mu\text{g L}^{-1}$ 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 systems⁵⁶.
371 Additionally, the abundance of *Lineus longissimus* (bootlace worms) was greater in
372 treatments with 25 $\mu\text{g L}^{-1}$ PLA microplastics compared to those with 2.5 $\mu\text{g L}^{-1}$ or no
373 microplastics. These worms are also a potential indicator species of pollution⁵⁷. 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 sink⁵⁸, 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 species⁵⁹. 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 pollutants⁶⁰, 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 recruitment⁶¹ and other complex processes
399 involving larger organisms (such as fish), are excluded from the cores⁶². 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 $\mu\text{g L}^{-1}$ of HDPE or PLA microplastics¹³.
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 $\mu\text{g L}^{-1}$. Globally,
409 oyster populations are under threat, and due to overfishing, parasites and disease⁶⁸, 85% of
410 oyster reefs have been lost world-wide⁶⁹. 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 zone⁷⁰. 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*

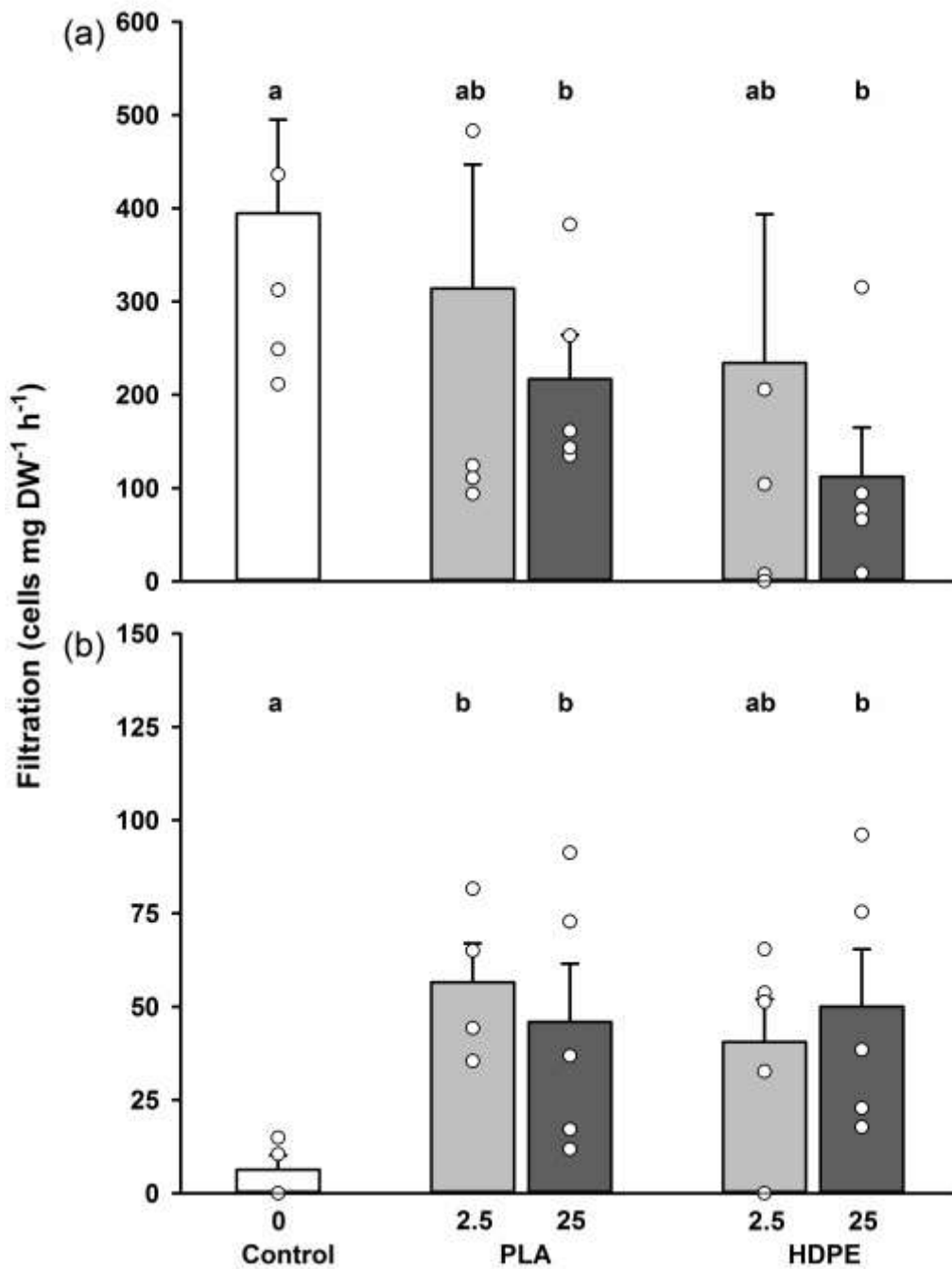
417 Since microplastics composed of PLA did not rapidly decompose, they caused many of the
418 same impacts as HDPE to *M. edulis* and *O. edulis* and the sediment they inhabit. This,

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 microbeads⁷² or enter the environment as larger litter.

423 Alterations to invertebrate assemblages in oyster-dominated sediment was detected at just 2.5
424 $\mu\text{g L}^{-1}$, although this dose is high, it is conservative compared to other recent experimental
425 studies e.g. 1250 - 25000⁷³ $\mu\text{g L}^{-1}$ and 200 - 4800²² $\mu\text{g L}^{-1}$. It is also much lower than current
426 levels found in some heavily contaminated coasts, for example, $\sim 4500 \mu\text{g L}^{-1}$ in Korea⁶.

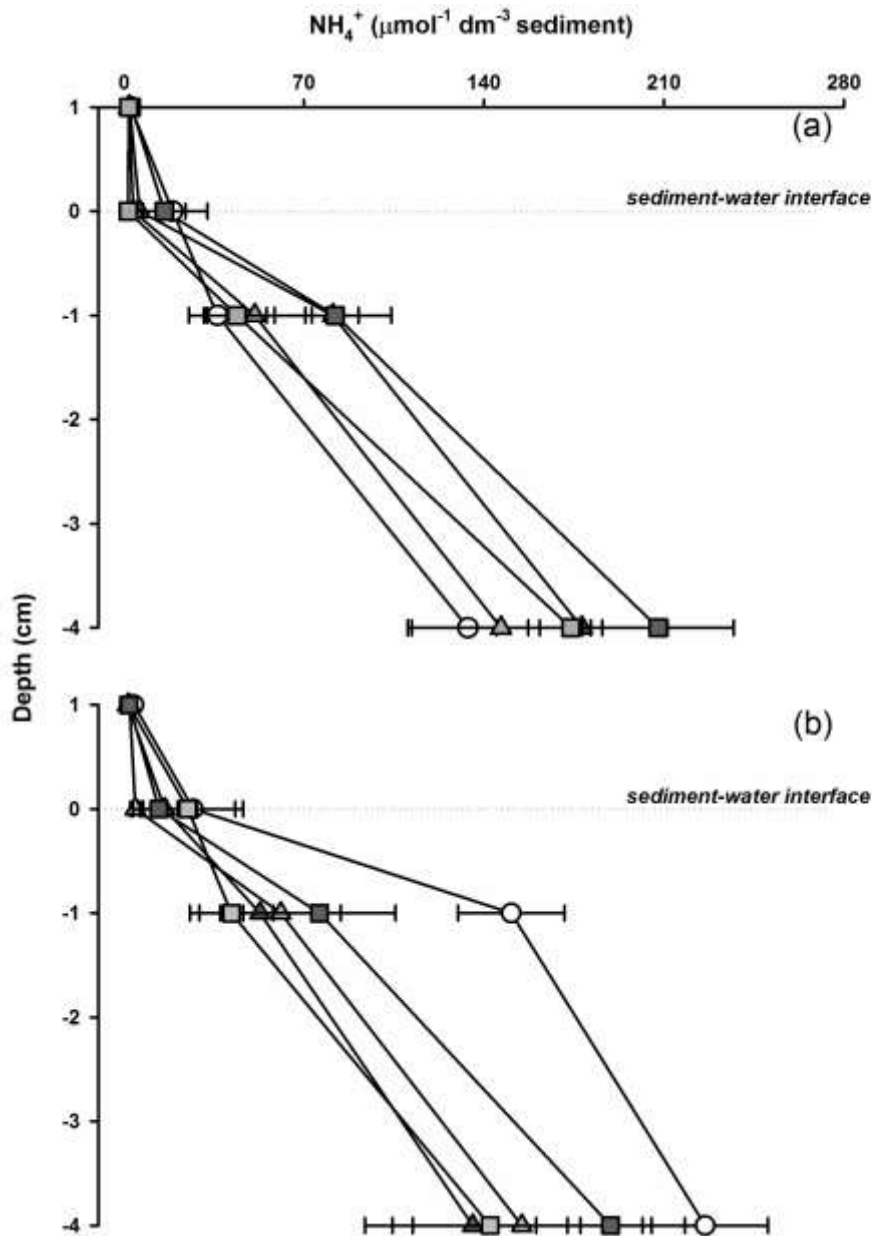
427 Current plankton net sampling techniques, however, typically have a lower size limit of ~ 300
428 μm , therefore underestimating current densities of microplastics, possibly by between 3 and 6
429 orders of magnitude when compared to a 10 μm mesh⁷⁴. Also, given that the cumulative input
430 of plastic waste is expected to increase in the coming decades²⁷ and that the fragmentation of
431 macroplastic litter already present in the environment will continue, concentrations of
432 microplastics are expected to increase⁷⁵. In the current study, effects on ammonium
433 concentrations and biomass of cyanobacteria occurred at just 2.5 $\mu\text{g L}^{-1}$. 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 ecosystems⁷. 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).



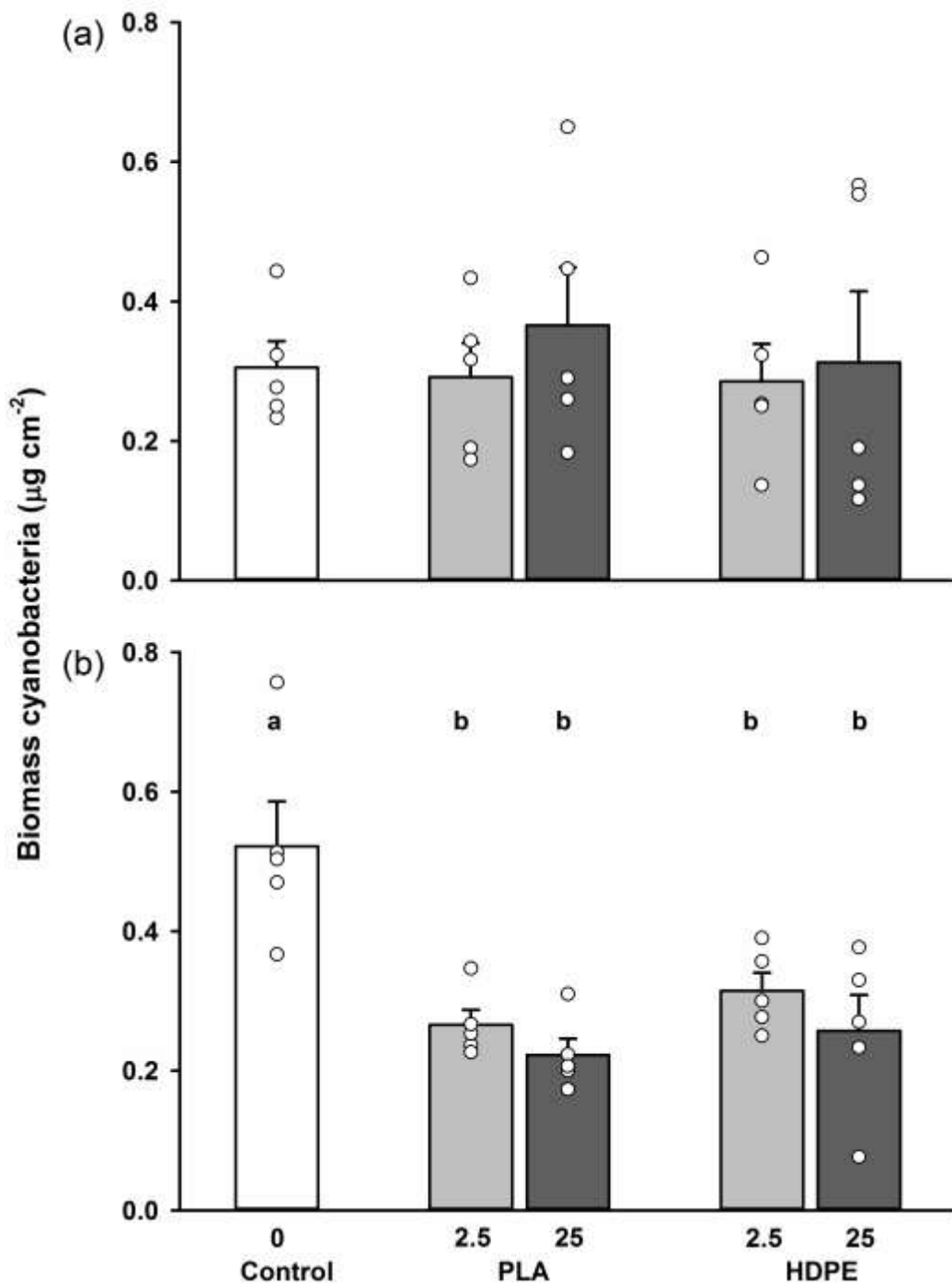
444

445 **Figure 1.** Filtration rates of (a) *M. edulis* and (b) *O. edulis* in mesocosms with 2.5 µg L⁻¹ or
 446 25 µg L⁻¹ of PLA or HDPE or with no microplastics (Control) after 50 days. Different letters
 447 indicate significant differences among treatments as determined by *post-hoc* comparisons or
 448 Dunnett's tests. Circles represent raw data, bars are means (± S.E.M.) with n = 5.



449

450 **Figure 2.** Concentrations of NH_4^+ in mesocosms with (a) *M. edulis* or (b) *O. edulis* of surface
 451 water (1 cm), the sediment-water interface (0 cm), and 1 & 4 cm into the sediment in
 452 mesocosms with 2.5 $\mu\text{g L}^{-1}$ (△) or 25 $\mu\text{g L}^{-1}$ (▲) of PLA or 2.5 $\mu\text{g L}^{-1}$ (◻) or 25 $\mu\text{g L}^{-1}$ (◼)
 453 of HDPE microplastics or no microplastics (Control = ○) after 50 days. Data are means (\pm
 454 S.E.M.) with n = 5.



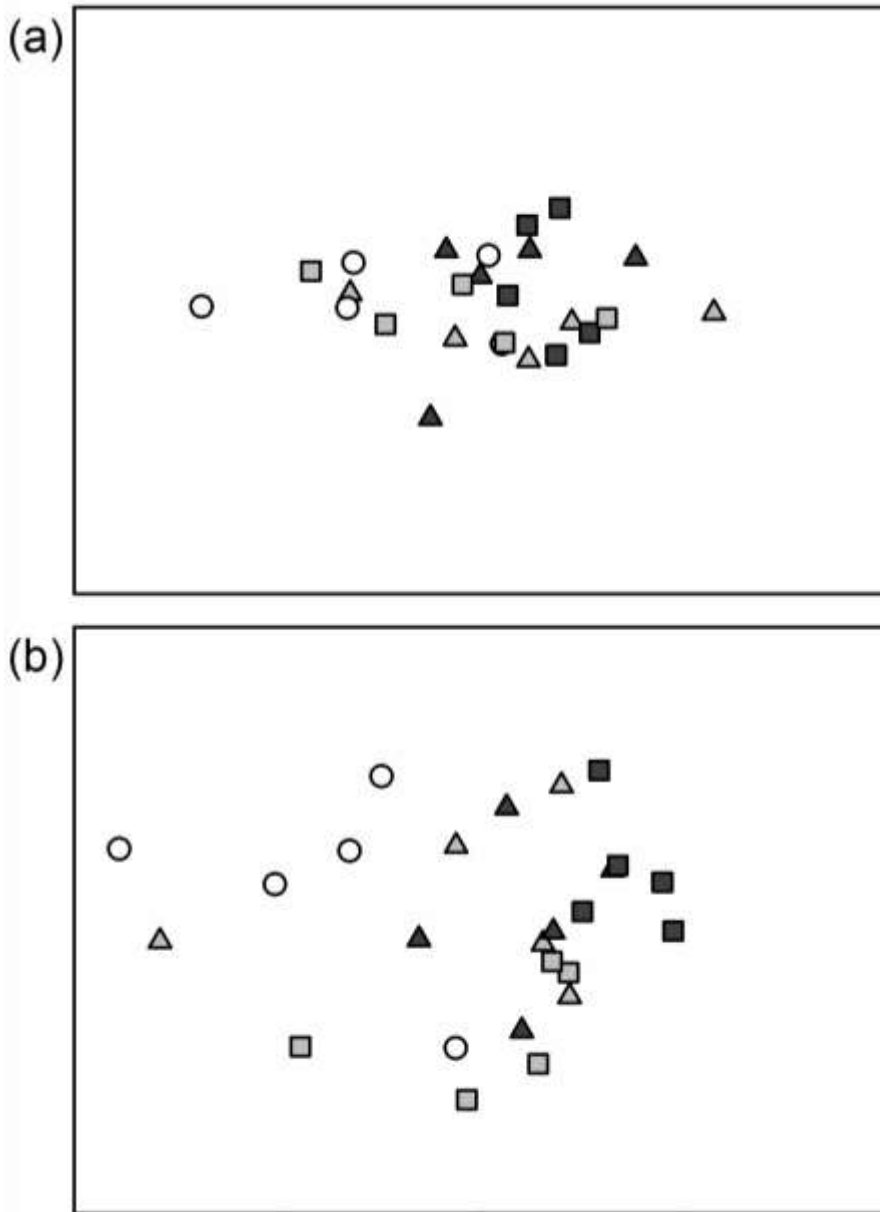
455

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*

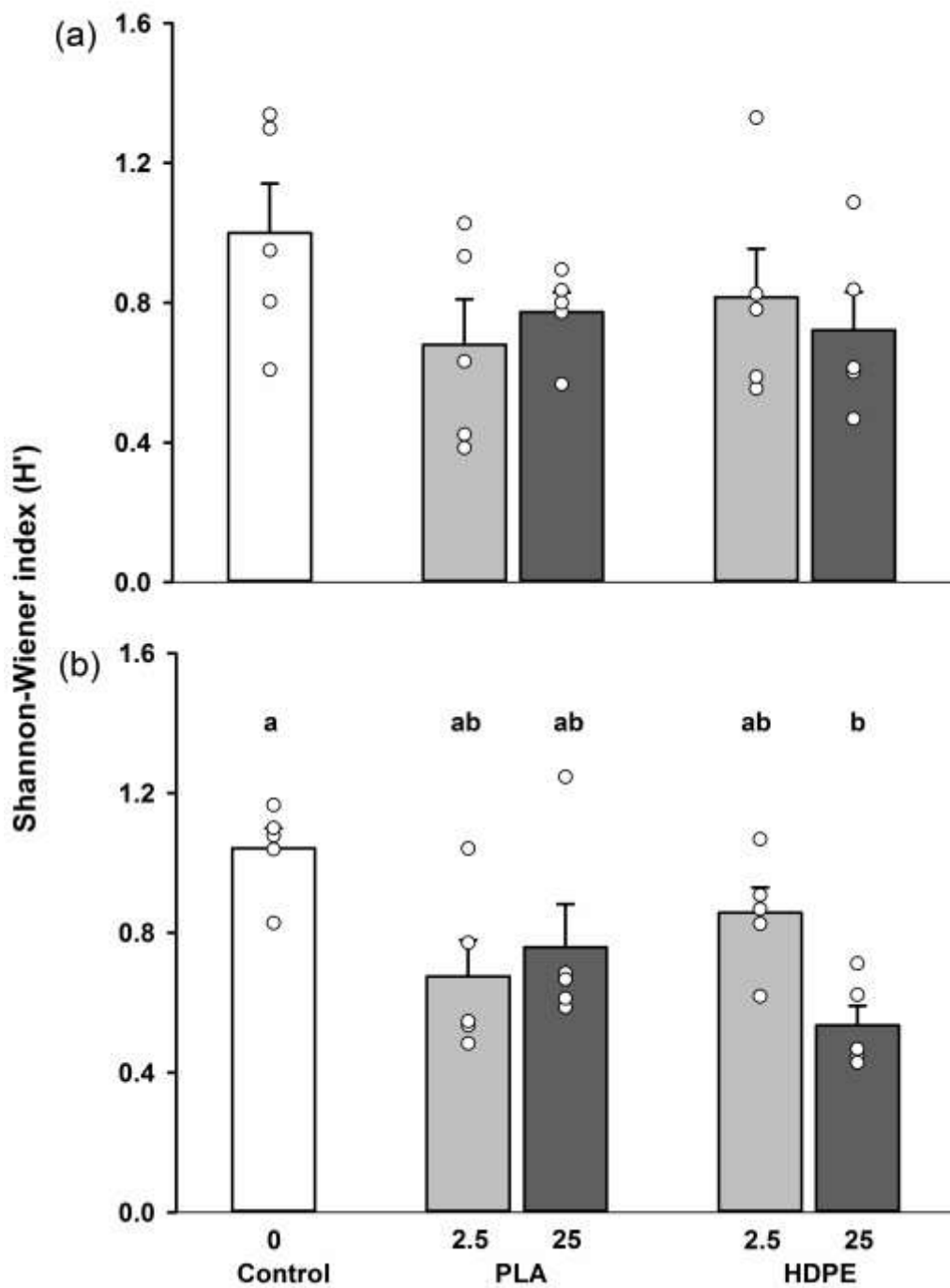
459 comparisons or Dunnett's tests. Circles represent raw data and bars are means (± S.E.M.) with

460 n = 5.



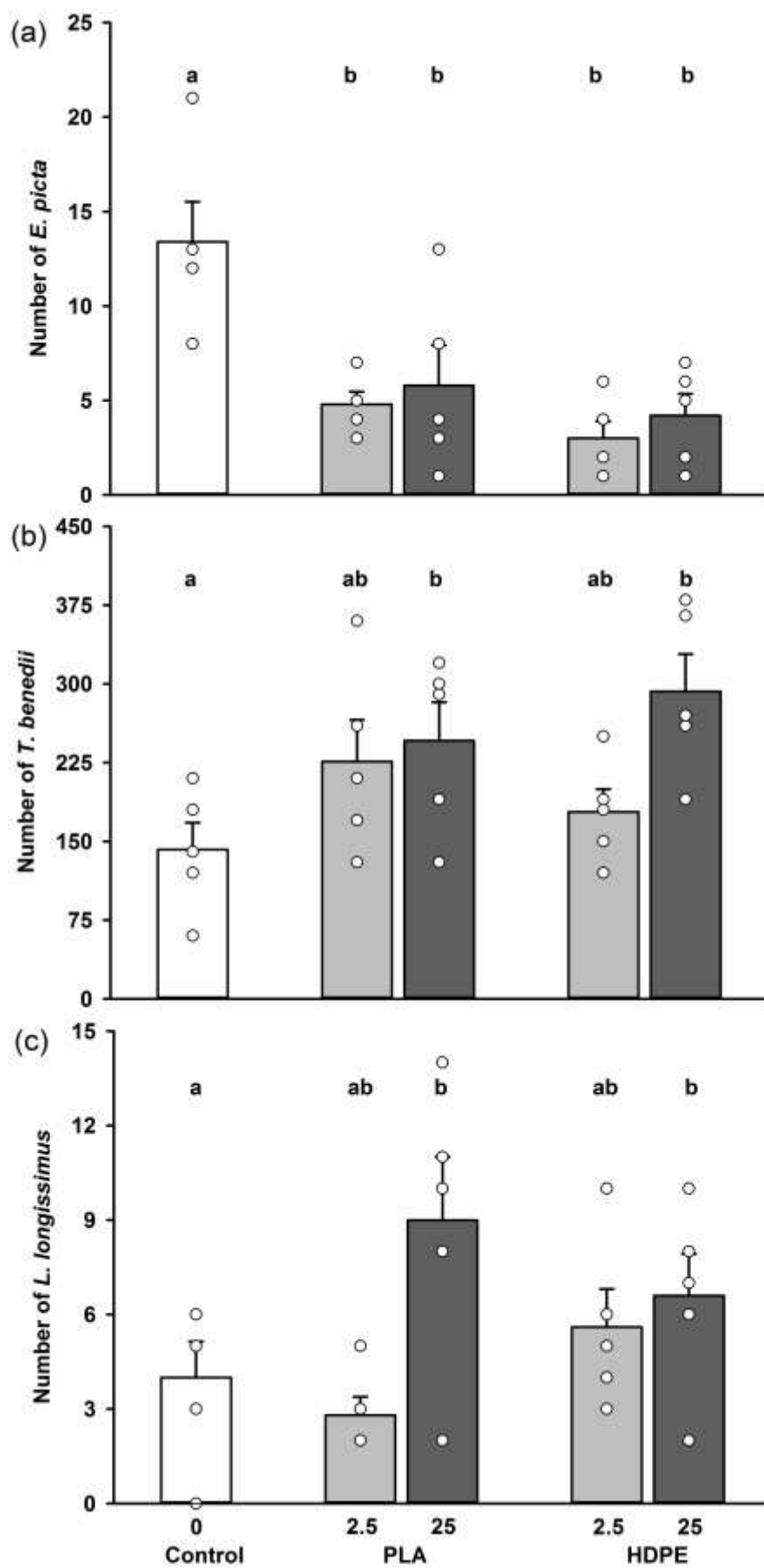
461

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. edilis* and 2.5 $\mu\text{g L}^{-1}$ (\blacktriangle) or 25 μg
 464 L^{-1} (\blacktriangle) of PLA or 2.5 $\mu\text{g L}^{-1}$ (\square) or 25 $\mu\text{g L}^{-1}$ (\blacksquare) of HDPE microplastics or with no
 465 microplastics (control = \circ) after 50 days, n = 5.



466

467 **Figure 5.** Shannon Wiener diversity index in mesocosms with (a) mussels or (b) oysters and
 468 2.5 $\mu\text{g L}^{-1}$ or 25 $\mu\text{g L}^{-1}$ of PLA or HDPE or with no microplastics (control) after 50 days
 469 Different letters indicate significant differences among treatments as determined by *post-hoc*
 470 comparisons or Dunnett's tests. Circles represent raw data, and bars are mean (\pm S.E.M.) with
 471 $n = 5$.



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

478 **Table 1.** Porewater ammonium pool ($\mu\text{mol dm}^{-3}$) and flux ($\mu\text{mol h}^{-1}$) 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 (\pm S.E.M.) with $n = 5$.

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

482

ASSOCIATED CONTENT

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

AUTHOR INFORMATION

*Corresponding Author

Department of Life Sciences,

Anglia Ruskin University,

Cambridge,

United Kingdom

Email: danniellegreen@gmail.com

Phone: +44 (0) 8452712234

Author Contributions

D.S.G. conceived the idea and designed the experiment. D.S.G. and B.B. carried out the field and laboratory work and analysed the data. All authors contributed to writing the manuscript and all authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interests.

Funding Sources

This research was funded the Irish Research Council with a Postdoctoral Research Project Grant (GOIPD/2013/306) and the G.M. Williams Fund and Royal Society Research Grant (RG120432).

Acknowledgements

The authors are very grateful to F. Glynn, B. McNamara and C. Guillaumot for assistance in the field, to E. Gorman for microalgal culturing and to S. Jiang for nutrient analysis. Thanks to G. Chapman and M. Anderson for advice on asymmetrical analyses. This research was funded by the Irish Research Council with a Postdoctoral Research Project Grant (GOIPD/2013/306) awarded to D.S.G., the G.M. Williams Fund and Royal Society Research Grant (RG120432) to N.E.O'C.

References

1. Eriksen, M.; Lebreton, L. C. M.; Carson, H. S.; Thiel, M.; Moore, C. J.; Borerro, J. C.; Galgani, F.; Ryan, P. G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* **2014**, *9* (12).
2. UNEP, GRID-Arendal. Marine Litter Vital Graphics. United Nations Environment Programme and GRID-Arendal. Nairobi and Arendal. *www.unep.org* 2016.
3. Law, K. L.; Thompson, R. C. Microplastics in the seas. *Science* **2014**, *345* (6193), 144-145.
4. Reisser, J.; Slat, B.; Noble, K.; du Plessis, K.; Epp, M.; Proietti, M.; de Sonnevile, J.; Becker, T.; Pattiaratchi, C. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. *Biogeosciences* **2015**, *12* (4), 1249-1256.
5. Collignon, A.; Hecq, J. H.; Galgani, F.; Voisin, P.; Collard, F.; Goffart, A. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. *Mar. pollut. bull.* **2012**, *64* (4), 861-864.

6. Kang, J. H.; Kwon, O. Y.; Shim, W. J. Potential Threat of Microplastics to Zooplanktivores in the Surface Waters of the Southern Sea of Korea. *Arch. Environ. Con. Tox.* **2015**, *69* (3), 340-351.
7. Rochman, C. M. Ecologically relevant data are policy-relevant data. *Science* **2016**, *352* (6290), 1172-1172.
8. Browne, M. A.; Underwood, A. J.; Chapman, M. G.; Williams, R.; Thompson, R. C.; van Franeker, J. A. Linking effects of anthropogenic debris to ecological impacts. *P. Roy. Soc. Lond. B Bio.* **2015**, *282* (1807).
9. Browne, M. A.; Dissanayake, A.; Galloway, T. S.; Lowe, D. M.; Thompson, R. C. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* **2008**, *42* (13), 5026-5031.
10. von Moos, N.; Burkhardt-Holm, P.; Koehler, A. Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. *Environ. Sci. Technol.* **2012**, *46* (20), 11327-11335.
11. Van Cauwenberghe, L.; Claessens, M.; Vandegehuchte, M. B.; Janssen, C. R. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ. Pollut.* **2015**, *199*, 10-17.
12. Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M. E. J.; Le Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; Corporeau, C.; Guyomarch, J.; Robbens, J.; Paul-Pont, I.; Soudant, P.; Huvet, A. Oyster reproduction is affected by exposure to polystyrene microplastics. *P. Natl. Acad. Sci. USA* **2016**, *113* (9), 2430-2435.
13. Green, D. S. Effects of microplastics on European flat oysters, *Ostrea edulis* and their associated benthic communities. *Environ. Pollut.* **2016**, *216*, 95-103.

14. Asmus, H.; Asmus, R. M.; Prins, T. C.; Dankers, N.; Frances, G.; Maass, B.; Reise, K. Benthic-pelagic flux rates on mussel beds - tunnel and tidal flume methodology compared. *Helgolander Meeresun.* **1992**, *46* (3), 341-361.
15. Korringa, P. Recent Advances in Oyster Biology. *Q. Rev. Bio.* **1952**, *27* (3), 266-308.
16. Setälä, O.; Norkko, J.; Lehtiniemi, M. Feeding type affects microplastic ingestion in a coastal invertebrate community. *Mar. pollut. bull.* **2016**, *102* (1), 95-101.
17. Li, J.; Yang, D.; Li, L.; Jabeen, K.; Shi, H. Microplastics in commercial bivalves from China. *Environ. Pollut.* **2015**, *207*, 190-195.
18. Rochman, C. M.; Tahir, A.; Williams, S. L.; Baxa, D. V.; Lam, R.; Miller, J. T.; Teh, F. C.; Werorilangi, S.; Teh, S. J. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci. Rep.* **2015**, *5*, 14340.
19. O'Connor, N. E.; Crowe, T. P. Biodiversity among mussels: separating the influence of sizes of mussels from the ages of patches. *J. Mar. Biol. Assoc. UK* **2007**, *87* (2), 551-557.
20. Grabowski, J. H.; Brumbaugh, R. D.; Conrad, R. F.; Keeler, A. G.; Opaluch, J. J.; Peterson, C. H.; Piehler, M. F.; Powers, S. P.; Smyth, A. R. Economic Valuation of Ecosystem Services Provided by Oyster Reefs. *Bioscience* **2012**, *62* (10), 900-909.
21. Barbier, E. B.; Hacker, S. D.; Kennedy, C.; Koch, E. W.; Stier, A. C.; Silliman, B. R. The value of estuarine and coastal ecosystem services. *Ecol. Monogr.* **2011**, *81* (2), 169-193.
22. Cole, M.; Lindeque, P. K.; Fileman, E.; Clark, J.; Lewis, C.; Halsband, C.; Galloway, T. S. Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. *Environ. Sci. Technol.* **2016**, *50* (6), 3239-3246.
23. World Economic Forum; The New Plastics Economy - Rethinking the future of plastics. <http://www.ellenmacarthurfoundation.org/publications> 2016.

24. Song, J. H.; Murphy, R. J.; Narayan, R.; Davies, G. B. H. Biodegradable and compostable alternatives to conventional plastics. *P. Roy. Soc. Lond. B Bio.* **2009**, *364* (1526), 2127-2139.
25. ASTM D7991-15, Standard Test Method for Determining Aerobic Biodegradation of Plastics Buried in Sandy Marine Sediment under Controlled Laboratory Conditions, ASTM International, West Conshohocken, PA, **2015**, www.astm.org
26. Nauendorf, A.; Krause, S.; Bigalke, N. K.; Gorb, E. V.; Gorb, S. N.; Haeckel, M.; Wahl, M.; Treude, T. Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments. *Mar. pollut. bull.* **2016**, *103* (1-2), 168-78.
27. Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768-771.
28. Lenz, R.; Enders, K.; Nielsen, T. G. Microplastic exposure studies should be environmentally realistic. *P. Natl. Acad. Sci. USA* **2016**, *113* (29), E4121-E4122.
29. OSPAR Commission website. <http://www.ospar.org/work-areas/bdc/species-habitats/mapping-habitats-on-the-ospar-list-of-threatened-or-declining-species-and-habitats>.
30. McCahon, C. P.; Pascoe, D. Episodic pollution: causes, toxicological effects and ecological significance. *Funct. Ecol.* **1990**, *4*, 375-383.
31. Handy, R. D. Intermittent exposure to aquatic pollutants: assessment, toxicity and sublethal responses in fish and invertebrate. *Comp. Biochem. Physiol. C.* **1994**, *107*, 171-184.
32. Aberle, N.; Moldaenke, M. B. C.; Wiltshire, K. H. 'Spectral fingerprinting' for specific algal groups on sediments in situ: A new sensor. *Archiv fur Hydrobiologie* **2006**, *167* (1-4), 575-592.

33. Green, D. S.; Boots, B.; Sigwart, J.; Jiang, S.; Rocha, C. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. *Environ. Pollut.* **2016**, *208*, 426-434.
34. Hayward, P. J.; Ryland, J. S. *Handbook of the Marine Fauna of North-West Europe*. Oxford University Press: Oxford, U.K., 1995.
35. Fox, J.; Weisberg, S. *An Companion to Applied Regression, Second Edition*. Sage, 2011; <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.
36. Underwood, A. *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, U.K., 1997.
37. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous Inference in General Parametric Models. *Biometrical J.* **2008**, *50* (3), 346-363.
38. Anderson, M. J.; Clarke, R. K.; Permanova+ for Primer: Guide to Software and Statistical Methods PRIMER-E. *Plymouth* 2008.
39. Anderson, M. J.; Willis, T. J., Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **2003**, *84* (2), 511-525.
40. Wegner, A.; Besseling, E.; Foekema, E. M.; Kamermans, P.; Koelmans, A. A. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). *Environ. Toxicol. Chem.* **2012**, *31* (11), 2490-2497.
41. Pernet, F.; Tremblay, R.; Comeau, L.; Guderley, H. Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids. *J. Exp. Biol.* **2007**, *210* (17), 2999-3014.
42. Mafra Jr, L. L.; Bricelj, V. M.; Fennel, K. Domoic acid uptake and elimination kinetics in oysters and mussels in relation to body size and anatomical distribution of toxin. *Aquat. Toxicol.* **2010**, *100* (1), 17-29.

43. Riisgard, H. U.; Saavedra, I. B.; Feeding Behaviour of the Mussel, *Mytilus edulis*: New Observations, with a Minireview of Current Knowledge. *J.Mar. Biol.* **2011**, *doi:10.1155/2011/312459*.
44. Wilson, J. Retention efficiency and pumping rate of *Ostrea edulis* in suspensions of *Isochrysis galbana*. *Mar. Ecol. Prog. Ser.* **1983**, *12*, 51-58.
45. Barnes, P. *Shellfish Culture and Particulate Matter Production and Cycling: A Literature Review*. BC Aquaculture Research & Development Committee AE 02.03-02.01, 2006; <https://www2.viu.ca/csr/documents/BarnesLiteratureReviewCSR.pdf>.
46. Watermann, F.; Hillebrand, H.; Gerdes, G.; Krumbein, W. E.; Sommer, U. Competition between benthic cyanobacteria and diatoms as influenced by different grain sizes and temperatures. *Mar. Ecol. Prog. Ser.* **1999**, *187*, 77-87.
47. Besseling, E.; Wang, B.; Lürling, M.; Koelmans, A. A. Nanoplastic Affects Growth of *S. obliquus* and Reproduction of *D. magna*. *Environ. Sci. Technol.* **2014**, *48* (20), 12336-12343.
48. Bhattacharya, P.; Lin, S.; Turner, J. P.; Ke, P. C., Physical Adsorption of Charged Plastic Nanoparticles Affects Algal Photosynthesis. *J. Phys. Chem. C.* **2010**, *114* (39), 16556-16561.
49. Currin, C. A.; Levin, L. A.; Talley, T. S.; Michener, R.; Talley, D. The role of cyanobacteria in Southern California salt marsh food webs. *Mar. Ecol.* **2011**, *32* (3), 346-363.
50. MacIntyre, H. L.; Geider, R. J.; Miller, D. C. Microphytobenthos: The ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries* **1996**, *19* (2), 186-201.
51. Yamamuro, M. Importance of epiphytic cyanobacteria as food sources for heterotrophs in a tropical seagrass bed. *Coral Reefs* **1999**, *18* (3), 263-271.

52. Stal, L. J. Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecol. Eng.* **2010**, *36* (2), 236-245.
53. Díez, B.; Ininbergs, K. Ecological importance of cyanobacteria, in Cyanobacteria: An Economic Perspective. In *Cyanobacteria*; Sharma, N. K.; Rai, A. K.; Stal, L. J. Eds.; John Wiley & Sons: Chichester, U.K. 2014.
54. Barnett, B. E. Oligochaetes as indicators of pollution in the Humber estuary, with special reference to *Tubificoides benedeni*. *Environ. Pollut. A.* **1983**, *30* (4), 277-291.
55. Brinkhurst, R. O.; Kennedy, C. R. Studies on the biology of the Tubificidae (annelida, oligochaeta) in a polluted stream. *J. Anim. Ecol.* **1965**, *34* (2), 429-443.
56. O'Gorman, E. J.; Fitch, J. E.; Crowe, T. P. Multiple anthropogenic stressors and the structural properties of food webs. *Ecology* **2012**, *93* (3), 441-448.
57. McEvoy, E. G.; Sundberg, P. Patterns of trace metal accumulation in Swedish marine nemerteans. *Hydrobiologia* **1993**, *266* (1), 273-280.
58. Kiorboe, T.; Mohlenberg, F. Particle selection in suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* **1981**, *5* (3), 291-296.
59. Goodsell, P. J.; Connell, S. D. Complexity in the relationship between matrix composition and inter-patch distance in fragmented habitats. *Mar. Biol.* **2008**, *154* (1), 117-125.
60. Mayer-Pinto, M.; Matias, M. G.; Coleman, R. A. The interplay between habitat structure and chemical contaminants on biotic responses of benthic organisms. *PeerJ* **2016**, *4*, e1985.
61. Cowie, R. P.; Widdicombe, S.; Austen, C. M. Effects of physical disturbance on an estuarine intertidal community: field and mesocosm results compared. *Mar. Biol.* **2000**, *136* (3), 485-495.

62. Underwood, A. J., Peterson, C. H. Towards an ecological framework for investigating pollution. *Mar. Ecol. Prog. Ser.* **1988**, *46*, 227-234.
63. Fletcher, R.; Reynoldson, T. B.; Taylor, W. D. The use of benthic mesocosms for the assessment of sediment contamination. *Environ. Pollut.* **2001**, *115* (2), 173-182.
64. Fletcher, R.; Reynoldson, T. B.; Taylor, W. D. Maintenance of intact sediment box cores as laboratory mesocosms. *Environ. Pollut.* **2001**, *115* (2), 183-189.
65. Srivastava, D. S.; Kolasa, J.; Bengtsson, J.; Gonzalez, A.; Lawler, S. P.; Miller, T. E.; Munguia, P.; Romanuk, T.; Schneider, D. C.; Trzcinski, M. K. Are natural microcosms useful model systems for ecology? *Trends Ecol. Evol.* **2004**, *19* (7), 379-384.
66. Bayne, B. L.; Addison, R. F.; Capuzzo, J. M.; Clarke, K. R.; Gray, J. S.; Moore, M. N.; Warwick, R. M. An overview of the GEEP workshop. *Mar. Ecol. Prog. Ser.* **1988**, *46* (1-3), 235-243.
67. Karle, I. M.; Agrenius, S.; Molander, S.; Magnusson, K.; Blanck, H.; Dahl, B.; Hall, P.; Dahllöf, I. Verification of a benthic boxcosm system with potential for extrapolating experimental results to the field. *J. Exp. Mar. Biol. Ecol.* **2007**, *353*(2), 265-278.
68. Kamphausen, L.; Jensen, A.; Hawkins, L. Unusually High Proportion of Males in a Collapsing Population of Commercially Fished Oysters (*Ostrea edulis*) in the Solent, United Kingdom. *J. Shellfish Res.* **2011**, *30* (2), 217-222.
69. Beck, M. W.; Brumbaugh, R. D.; Airoidi, L.; Carranza, A.; Coen, L. D.; Crawford, C.; Defeo, O.; Edgar, G. J.; Hancock, B.; Kay, M. C.; Lenihan, H. S.; Luckenbach, M. W.; Toropova, C. L.; Zhang, G. F.; Guo, X. M. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *Bioscience* **2011**, *61* (2), 107-116.
70. Loren, D. C.; Robert, D. B.; David, B.; Ray, G.; Mark, W. L.; Martin, H. P.; Sean, P. P.; Tolley, S. G., Ecosystem services related to oyster restoration. *Mar. Ecol. Prog. Ser.* **2007**, *341*, 303-307.

71. Green, D. S.; Boots, B.; Blockley, D. J.; Rocha, C.; Thompson, R. Impacts of Discarded Plastic Bags on Marine Assemblages and Ecosystem Functioning. *Environ. Sci. Technol.* **2015**, *49* (9), 5380-5389.
72. Rochman, C. M.; Kross, S. M.; Armstrong, J. B.; Bogan, M. T.; Darling, E. S.; Green, S. J.; Smyth, A. R.; Verissimo, D. Scientific Evidence Supports a Ban on Microbeads. *Environ. Sci. Technol.* **2015**, *49* (18), 10759-10761.
73. Lee, K. W.; Shim, W. J.; Kwon, O. Y.; Kang, J. H. Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod *Tigriopus japonicus*. *Environ. Sci. Technol.* **2013**, *47* (19), 11278-11283.
74. Norén, F.; Norén, K.; Magnusson, K. 2014. Marint mikroplastiskt skräp: undersökning längs svenska västkusten 2013 & 2014. Länsstyrelsen i Västra Götalands län, vattenvårdsenheten.
75. Duis, K.; Coors, A. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Env. Sci. Eur.* **2016**, *28* (1), 1-25.

SUPPORTING INFORMATION

Microplastics affect the ecological functioning of an important biogenic habitat

Dannielle Senga Green^{1,2,3*}, Bas Boots², Nessa E. O'Connor^{4,5} and Richard Thompson⁶

¹*Department of Life Sciences, Anglia Ruskin University, Cambridge, United Kingdom.*

²*School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry, United Kingdom.*

³*Biogeochemistry Research Group, Geography Department, School of Natural Sciences, Trinity College Dublin, Dublin, Ireland.*

⁴*Queen's University Marine Laboratory, Portaferry, Northern Ireland, United Kingdom.*

⁵*School of Biological Sciences, Queen's University Belfast, Northern Ireland, UK.*

⁶*School of Marine Science and Engineering, Plymouth University, Plymouth, Devon, United Kingdom.*

*corresponding author at:

Department of Life Sciences, Anglia Ruskin University, Cambridge, United Kingdom.

Email: danniellesgreen@gmail.com

This file has 10 pages (cover page included), containing one figure and 7 tables as referred to in the main manuscript:

Figure S1 (page S3): Schematic diagram of bucket as mesocosm.

Table S1 (page S4): Approximate density of microplastic particles per treatment estimated using a haemocytometer.

Table S2 (page S5): Microalgae cell counts estimated a haemocytometer.

Table S3 (page S6): Asymmetric ANOVA results for filtration.

Table S4 (page S7): Asymmetric ANOVA results for nutrients and microalgal biomass.

Table S5 (page S8): Asymmetric permutational multivariate ANOVA results for assemblage structures

Table S6 (page S9): 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*.

Table S7 (page S10): SIMPER results of benthic assemblages.

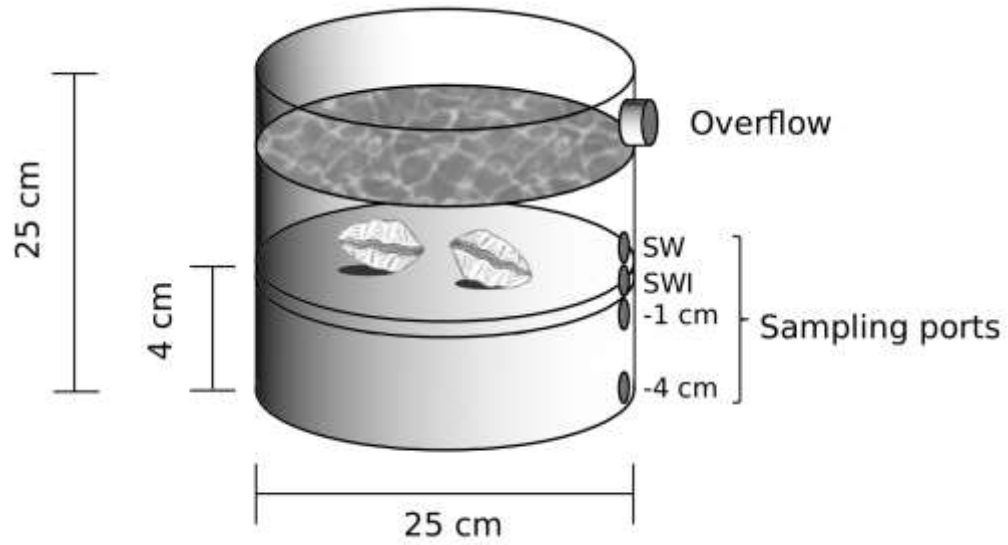


Figure S1. Diagram showing the design of the mesocosms with sampling ports for surface water (SW), the sediment-water interface (SWI) and for porewater at 1 and 4 cm into the sediment.

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 \pm 125.43	156.25 \pm 45.54	138.89 \pm 45.35
	25	1406.25 \pm 193.48	1163.19 \pm 165.29	1319 \pm 189.99
HDPE	2.5	104.17 \pm 65.88	86.81 \pm 42.31	86.81 \pm 33.95
	25	937.50 \pm 213.48	815.97 \pm 80.44	763.89 \pm 126.88

Table S2. Mean (\pm 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 $\mu\text{g L}^{-1}$ of PLA or HDPE microplastic treatments or in Controls (with no microplastics) on days 1, 26 and 48 of the experiment.

Plastic	Dose ($\mu\text{g L}^{-1}$)	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. edulis* and *O. edulis* 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**.

<i>M. edulis</i>		
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
<i>O. edulis</i>		
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 ($\mu\text{mol dm}^{-3}$) and flux ($\mu\text{mol h}^{-1}$) of NH_4^+ , and biomass ($\mu\text{g cm}^{-2}$) 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*.

<i>M. edulis</i>								
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
<i>O. edulis</i>								
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. edulis* or *O. edulis* and 2.5 or 25 $\mu\text{g L}^{-1}$ of PLA or HDPE microplastics, or controls (C) with no microplastics after 50 days. When the factors "Plastic" or "Dose" were significant (at $\alpha = 0.05$, indicated in **bold**), contrasts were used to determine any differences among treatments between levels.

Source	Contrasts	d.f.*	<i>M. edulis</i>		<i>O. edulis</i>	
			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 $\alpha=0.05$ 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.

<i>M. edulis</i>												
Source	R		N		H'		<i>E. picta</i>		<i>T. benedii</i>		<i>L. longissimus</i>	
	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
<i>O. edulis</i>												
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

1 **Table S7.** SIMPER analyses based on square-root transformed abundance data within the
 2 sediment from the *O. edulis* mesocosms with no microplastics (control = C) versus 2.5 µg L⁻¹
 3 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
 4 of HDPE (25 HDPE).

Taxon	Average abundance		Av.Diss*	Diss/SD**	Contrib %***	Cum.%****
	C vs. 2.5 PLA					
<i>T. benedii</i>	12.74	16.71	8.43	1.44	29.93	29.93
<i>Corophium</i> 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
<i>Glycera</i> sp.	2.87	2.82	2.34	1.38	8.32	66.05
<i>E. picta</i>	3.08	2.27	1.92	1.62	6.82	72.86
<i>L. longissimus</i>	2.2	1.48	1.89	1.23	6.7	79.57
<i>Hydrobia</i> sp.	1.2	0.51	1.63	1.2	5.79	85.36
C vs. 25 PLA						
<i>T. benedii</i>	12.74	15.56	7.04	1.43	26.92	26.92
<i>Corophium</i> sp.	2.89	2.99	4.03	1.4	15.4	42.32
<i>Glycera</i> 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
<i>E. picta</i>	3.08	2.53	1.95	1.4	7.46	71.89
<i>L. longissimus</i>	2.2	2.52	1.78	1.29	6.78	78.68
<i>Hydrobia</i> sp.	1.2	0.6	1.73	1.24	6.6	85.28
C vs. 2.5 HDPE						
<i>T. benedii</i>	12.74	14.63	7.04	1.27	28.2	28.2
<i>Corophium</i> 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
<i>Glycera</i> sp.	2.87	2.4	2.38	1.44	9.52	62.04
<i>E. picta</i>	3.08	2.11	2.16	1.5	8.64	70.67
<i>Hydrobia</i> sp.	1.2	0.62	1.59	1.19	6.37	77.05
<i>L. longissimus</i>	2.2	2.47	1.54	1.25	6.18	83.23
C vs. 25 HDPE						
<i>T. benedii</i>	12.74	16.81	8.87	1.38	32.39	32.39
<i>Corophium</i> 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
<i>Glycera</i> sp.	2.87	3.4	2.71	1.34	9.91	67.55
<i>E. picta</i>	3.08	2.29	1.94	1.41	7.09	74.65
<i>Hydrobia</i> sp.	1.2	0.5	1.82	1.21	6.65	81.3
<i>L. longissimus</i>	2.2	2.23	1.49	1.22	5.43	86.74

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

6 dissimilarities

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

8 across all pairs of samples

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

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

