

# Impacts of Discarded Plastic Bags on Marine Assemblages and Ecosystem Functioning

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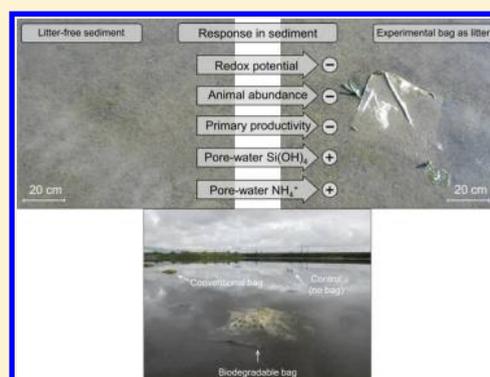
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## S Supporting Information

**ABSTRACT:** The accumulation of plastic debris is a global environmental problem due to its durability, persistence, and abundance. Although effects of plastic debris on individual marine organisms, particularly mammals and birds, have been extensively documented (e.g., entanglement and choking), very little is known about effects on assemblages and consequences for ecosystem functioning. In Europe, around 40% of the plastic items produced are utilized as single-use packaging, which rapidly accumulate in waste management facilities and as litter in the environment. A range of biodegradable plastics have been developed with the aspiration of reducing the persistence of litter; however, their impacts on marine assemblages or ecosystem functioning have never been evaluated. A field experiment was conducted to assess the impact of conventional and biodegradable plastic carrier bags as litter on benthic macro- and meio-faunal assemblages and biogeochemical processes (primary productivity, redox condition, organic matter content, and pore-water nutrients) on an intertidal shore near Dublin, Ireland. After 9 weeks, the presence of either type of bag created anoxic conditions within the sediment along with reduced primary productivity and organic matter and significantly lower abundances of infaunal invertebrates. This indicates that both conventional and biodegradable bags can rapidly alter marine assemblages and the ecosystem services they provide.



## 1. INTRODUCTION

Plastic items have become an integral part of daily life in many societies, and use is increasing, with an estimated annual global production of 299 million tonnes in 2013.<sup>1</sup> Of this, single-use packaging items account for the majority, almost 40%, of total production.<sup>1,2</sup> It is estimated that almost 5% of the plastic produced is transported via wastewater flows, inland waterways, wind, or tides and ends up in the marine environment as litter.<sup>3–7</sup> Indeed, plastic waste accounts for up to 80% of all litter found in marine habitats.<sup>4</sup> Of this litter, plastic bags are one of the most common items<sup>8</sup> especially on intertidal<sup>9,10</sup> and subtidal<sup>11</sup> benthos.

Biodegradable plastics have been proposed as an alternative to conventional plastics such as polyethylene. Biodegradable plastic bags are intended to break down more rapidly than conventional plastic bags and are, therefore, believed to be less persistent as litter. Yet there have been few studies evaluating their degradation in natural habitats, and the extent to which any enhanced degradation might reduce marine litter is not clear.<sup>12,13</sup> Indeed some degradable polyethylene formulations have been shown to persist in the environment for years after their disposal.<sup>14</sup> Given their relative recalcitrance to decom-

position under natural conditions, conventional and biodegradable plastic bags pose a potential threat to organisms in coastal ecosystems when present as litter.

Contamination of marine habitats by plastic litter can be aesthetically detrimental, leading to negative socio-economic consequences.<sup>15</sup> There is also considerable evidence relating to consequences for wildlife. Over 660 species are known to encounter marine debris, and negative consequences including physical damage from entanglement and choking and mortality are reported for individuals from a wide range of species,<sup>4</sup> including birds,<sup>16</sup> mammals,<sup>4</sup> and invertebrates.<sup>17</sup> While information on effects at the individual level is of considerable value, evidence of effects at higher levels of biological organization, i.e., species assemblages, communities, and populations, is often of critical importance to decision makers since it can be used to inform policy measures. Providing this information is necessary, for example, to inform decisions about

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legislation to reduce the quantity of single use, disposable items including plastic bags and/or about the efficacy of alternative materials with enhanced degradability.

It has been estimated that up to 70% of all plastic debris settles onto the benthos<sup>18</sup> with considerable accumulations in intertidal habitats worldwide;<sup>19</sup> yet, to date, there have been few studies assessing the effect of plastic debris on these habitats and no work on the ecosystem services they provide, such as primary productivity and nutrient cycling. Coastal ecosystems are extremely diverse and productive, supplying vital ecosystem services such as the production of food, stabilization of shorelines, removal of pollutants, and nutrient turnover.<sup>20</sup> Within these, sedimentary habitats are of particular importance, recycling nutrients that support economically important benthic and pelagic food-webs through a myriad of biogeochemical processes.<sup>21</sup> It is possible that plastic debris might decrease the biomass of microphytobenthos (important primary producers) in sedimentary habitats by blocking light. Consequently this could alter biogeochemical processes such as decomposition of organic matter, which is essential for the release of inorganic nutrients (such as nitrogen as ammonium). This, in turn, supports primary production and, therefore, food-webs. To date, two studies have examined the effects of plastic debris at ecologically relevant scales; one mensurative study on infauna<sup>22</sup> and one manipulative subtidal experiment on epibiota and infauna.<sup>23,24</sup> It has been suggested that, when on the surface, plastic debris might physically asphyxiate the underlying sediment, potentially impeding nutrient exchange processes at the sediment–water interface, leading to anoxia and decreasing the abundance of infaunal organisms,<sup>6</sup> but neither this nor the effects of plastic debris on infaunal assemblages in intertidal habitats have ever been experimentally evaluated.

To assess the impact of plastic bags as litter within a coastal habitat, a manipulative field experiment was done to test the following hypotheses: the addition of plastic carrier bags locally alters (i) invertebrate assemblage structure and composition and (ii) biogeochemical processes within the sediment and (iii) carrier bags made of different types of material (biodegradable or conventional nonbiodegradable plastic) have different effects on biological assemblages and biogeochemical processes within the sediment.

## 2. MATERIALS AND METHODS

**2.1. Site Description and Experimental Design.** Booterstown Marsh (Booterstown, Co. Dublin, Ireland, 53°18.65' N, 6°11.9' W) is a nature reserve, sheltered from the open sea, managed by An Taisce, the National Trust for Ireland (An Taisce 2013) and has been proposed to be a Natural Heritage Area. Permission to execute the experiment at this nature reserve was granted by An Taisce. It is a designated bird sanctuary with hydrologically low energy depositional areas consisting of very fine clay and silt forming mud-flats overlain with brackish water. As the marsh receives freshwater input from a stream, salinity fluctuates with the tidal cycle. At the time of sampling, the salinity of the surface water was 20 ppt during falling to low tide, and during rising to high tide it was 35 ppt. It is in close vicinity to a highly urbanised area and is prone to accumulate waste litter, including plastic items.

White plastic carrier bags made of either conventional high density polyethylene (HDPE) or biodegradable plastic manufactured from corn starch were used. Both bags were of a single-use disposable nature rather than of a more substantial

“bags for life” thickness. The biodegradable bags were labeled as “biodegradable” and “compostable” and claimed to completely disintegrate into carbon dioxide, water, and biomass within 10–12 weeks in standard composting conditions and are certified “OK COMPOST” by EU standard EN 13432 and International standard ASTM D6400-99. The dimensions of both types of bag were approximately 38 × 46 cm and 65 μm thick. On 13 March 2014, conventional and biodegradable plastic bags and control plots without bags ( $n = 10$  for each treatment) were randomly interspersed on the surface in an area (10 m × 25 m) of pristine mud-flat, i.e., free of observable plastic debris on the surface. Each plot was separated by at least 2 m to avoid potential edge effects crossing over between treatments and was assumed to be similar at the beginning of the experiment. Each bag was securely positioned flat using metal pins inserted into each corner to simulate the manner in which plastic bags had been observed to become trapped at the surface of the sediment elsewhere in the marsh (personal observation). Control plots ( $n = 10$ ) were also marked out with metal pegs at the corners to identify dedicated plots of the same size as the bags. Prior to deployment of the bags, each plot including the controls received a custom-made, in situ profiler to allow for pore water measurements (see section 2.3.1 for details). Compared to the typical persistence of plastic debris in the environment, our experiment was short-term and was destructively sampled to assess impacts on infaunal assemblages (section 2.2) and ecosystem functioning (section 2.3) after 9 weeks (i.e., after 75% of the time stated for complete disintegration of biodegradable plastic bags under composting conditions). Weather conditions (daily temperature and hours of sunshine) during the experimental period are displayed in the Supporting Information, Figure S1.

**2.2. Assessing Infaunal Diversity.** A 10 cm diameter corer was inserted down to 5 cm depth as much to the center of each plot as possible. The core sample (~393 cm<sup>3</sup>) was transferred to sealable bags, stored at 4 °C and upon arrival in the laboratory immediately sieved through a 125 μm mesh sieve pan to retain macrofauna and large meiofauna. All material recovered from the sieves was preserved separately for each sample in 70% methylated ethanol for enumeration and identification to the lowest discernible clade possible using a dissecting microscope.

**2.3. Assessing Ecosystem Functioning.** **2.3.1. Redox and Pore-Water Nutrient Concentrations.** Prior to any other measurements being taken, five plots from each treatment were randomly selected to determine the redox potential of the surface sediment (~0.5 cm) using a redox electrode (Elit 31 C ORP). Next, pore-water was sampled prior to any disturbance created by the other sampling procedures. Pore-water samples were collected using purpose-built in situ profilers based on the design in Seeberg-Elverfeldt et al.<sup>2</sup> and as described in Rocha et al.<sup>26</sup> and Green et al.<sup>27</sup> Briefly, the profilers, made of poly(methyl methacrylate) sheets, had grooves cut at intervals to attach Rhizon membranes (Rhizosphere Research Products B.V., The Netherlands) on a vertical sequence, allowing sampling at 0 (sediment–water interface), 2, and 4 cm depths. The profilers were inserted vertically into the sediment, secured by metal pins, and a single biodegradable or conventional bag was placed centered on top. Control plots had a profiler inserted and secured with pins only. At the end of the experiment, pore-water was sampled by attaching a needle to each Rhizon membrane, and water was collected directly into sterile vacuum tubes (according to Ibanhez and Rocha).<sup>28</sup>

Within 24 h, ammonium ( $\text{NH}_4^+$ ) and biogenic silicate ( $\text{Si}(\text{OH})_4$ ) were measured from the water samples using a Lachat QuickChem 8000 flow injection autoanalyser (Lachat Instruments, USA) following Lachat methods 31-107-06-1-B ( $\text{NH}_4^+$ ) and 31-114-27-1-A ( $\text{Si}(\text{OH})_4$ ). All concentrations of pore-water were corrected for sediment porosity and were standardized to dry bulk density following Eleftheriou and McIntyre.<sup>29</sup>  $\text{NH}_4^+$  and  $\text{Si}(\text{OH})_4$  inventories were calculated within the depth profile by integration of linear pore-water concentration gradients, corrected for porosity, down to 4 cm depth.

**2.3.2. Chlorophyll Content and Light Measurements.** Chlorophyll measurements were done colorimetrically. The oxic surface layer of sediment (approximately the top 0.5 cm) was sampled using clean spatulas, immediately wrapped in tin foil to protect from light, and stored at 4 °C. Within 24 h of collection, 10 mL of 90% acetone was added to ~1 g of field-moist, homogenized sediment, left to extract total chlorophyll for 1 h at room temperature under constant shaking in the dark, and centrifuged at 3000g for 5 min to settle sediment.<sup>30</sup> Chlorophyll-a, -b, and -c concentrations were measured from the supernatant using a spectrophotometer (at  $\lambda = 430$  and 664, 460 and 647, and 630 nm respectively) and concentrations of chlorophyll were calculated according to equations by Jeffrey and Humphrey<sup>31</sup> and expressed as  $\mu\text{g}$  chlorophyll  $\text{g}^{-1}$  dry sediment.

The plastic bags became colonised with algae during the experiment; therefore, chlorophyll-a, -b, and -c were also measured from the surfaces of the bags. Bags were kept in the dark and stored at 4 °C for 24 h before being processed for analysis in order to preserve chlorophyll at levels similar to field conditions. Total chlorophyll contents of microalgae that had colonised the surfaces of the bags were estimated by cutting 4  $\text{cm}^2$  squares from each corner (leaving 5 cm margins at all sides) and one from the center, representing a surface area of 20  $\text{cm}^2$ . From each bag the five squares were placed together into 10 mL of 90% acetone, left to extract for 1 h at room temperature in the dark under constant shaking with intermittent vortexing to thoroughly mix the plastic fragments. After centrifuging at 3000g for 5 min to settle the plastic fragments, 1 mL of supernatant was used for measuring on a spectrophotometer in a similar fashion as was done with the sediment samples.

In addition, the percentage of light blocked by the plastic bags (transparency) was measured using a LI-COR LI-250A light meter (LI-COR Inc., USA). On average, the conventional bags blocked 51.1 ( $\pm 0.6$ ) and biodegradable bags blocked 50.3 ( $\pm 1.6$ ) % of photosynthetically active radiation.

**2.3.3. Organic Matter Content of the Sediment and Grain Size.** Surface (top ~0.5 cm) sediment samples were collected by scraping using a clean spatula, while the underlying sediment (approximately 4 cm depth) was sampled using a mini-corer adapted from a 60 mL syringe (26.7 mm diameter). Two samples were randomly taken from each plot to account for within-plot heterogeneity. Organic matter content was determined by loss on ignition (LoI) following Eleftheriou and McIntyre.<sup>29</sup> Briefly, ~2.5 g of oven-dried (80 °C, 12 h) sediment was placed in a muffle furnace at 450 °C for 24 h. The difference in weight is expressed as a percentage of ash-free dry weight.

Grain size distribution of the sediment was estimated by laser diffraction using a Mastersizer 2000 (Malvern Instruments) from three plots randomly selected from each treatment.

Briefly, 1 g of dry sediment was digested in hydrogen peroxide to remove organics prior to analysis according to Gray et al.<sup>32</sup>

**2.4. Statistical Analyses.** All data were analyzed using R v3.1.1.<sup>33</sup> The data were checked for normality (normal quantile plots and Shapiro-Wilk tests) and homogeneity of variance (Levene's tests), and no transformations were deemed necessary. All response variables from the sediment samples (total amount of individuals ( $N$ ), number of species ( $S$ ), Shannon–Wiener index ( $H'$ ), chlorophyll contents in sediments, loss-on ignition (LoI), nutrient concentrations at each depth, and nutrient pools and fluxes) were individually analyzed using a one-way ANOVA with “Treatment” as a factor having three levels (control, conventional, and biodegradable). Redox potential was measured from only five randomly selected replicates from each treatment in the field due to time restrictions posed by the tide. The redox data were analyzed using the same model as before. When the main test was significant, a posthoc pairwise comparison of the means was computed (Tukey HSD test, at  $\alpha = 0.05$ ) to ascertain the a priori hypotheses. Chlorophyll-a, -b, and -c contents on the bags (two levels: Conventional and Biodegradable) were compared using separate two-tailed Welch's  $t$  tests.

Multivariate data were analyzed using the *vegan* package v2.2-0.<sup>34</sup> Assemblage structures were computed using species count data (4th-root transformed abundance data to account for highly dominant observations), whereas assemblage compositions were computed based on presence/absence of species. Bray–Curtis similarity indices were calculated between samples, and differences are shown using two-dimensional nonmetric multidimensional scaling (nMDS) ordinations. The nMDS were calculated using 250 iterations or until the lowest 2D stress was reached using the *metaMDS* function in *vegan* employing the *monoMDS* engine. Before calculating permutational analyses of variance (PERMANOVA), multivariate homogeneity of variances were checked (*betadisp* function in the *vegan* package). Differences between assemblage structure and composition within the sediment were analyzed with “Treatment” as a single factor with three levels (control, conventional, and biodegradable) by computing a one-way PERMANOVA using the *adonis* function in *vegan*, with probabilities calculated based on 9999 permutations of the raw data. To assess which identified groups contributed most to dissimilarities between treatments, similarity percentages were computed using the *simper* routine in the *vegan* package.

### 3. RESULTS

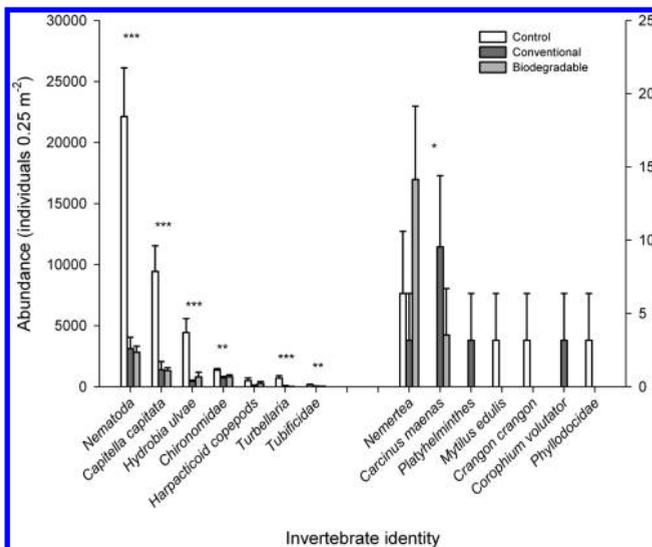
**3.1. Effects of Plastic Bags on Infaunal Diversity, Assemblage Composition, and Structure.** All bags were still in situ when collected, and no immediate signs of degradation nor fragmentation were observed. A total of 14 different groups of infaunal invertebrates were identified within the top 5 cm sediment layer. The total numbers of individuals ( $N$ ) were significantly lower beneath plastic bags compared to in control plots (One-way ANOVA,  $F_{2,27} = 48.97$ ,  $P < 0.001$ ), but similar numbers of individuals were found under the biodegradable and conventional bags (Table 1). Sediment beneath biodegradable and conventional bags had on average 6.2 and 6.4 times fewer individuals compared to that of control plots, but species richness (number of species,  $S$ ) and Shannon–Wiener diversity ( $H'$ ) was not significantly different from that found in control plots (Table 1). The most abundant taxa were Nematoda (potworms), *Capitella capitata* (gallery worm), *Hydrobia ulvae* (mudsnails), and Chironomidae

**Table 1. Total Number of Individuals (*N* per 0.25 m<sup>2</sup>), Number of Different Species (*S*), and Shannon-Wiener Diversity Index (*H'*) of Invertebrates Found within the Top 5 cm Layer in Plain Sediment (control), Underneath Conventional Bags (conv), and Underneath Biodegradable Bags (bio)<sup>a</sup>**

treatment	<i>N</i>	<i>S</i>	<i>H'</i>
control	38.8 × 10 <sup>4</sup> ± 5.4 × 10 <sup>3</sup> <sup>a</sup>	7.4 ± 0.2 <sup>a</sup>	1.105 ± 0.085 <sup>a</sup>
conv	6.0 × 10 <sup>3</sup> ± 1.4 × 10 <sup>3</sup> <sup>b</sup>	6.9 ± 0.4 <sup>a</sup>	1.288 ± 0.087 <sup>a</sup>
bio	6.2 × 10 <sup>3</sup> ± 7.9 × 10 <sup>2</sup> <sup>b</sup>	6.8 ± 0.4 <sup>a</sup>	1.242 ± 0.050 <sup>a</sup>
ANOVA	<i>F</i> = 48.97, <i>P</i> < 0.001	<i>F</i> = 0.85, <i>P</i> = 0.439	<i>F</i> = 1.56, <i>P</i> = 0.229
<i>df</i> <sub>1</sub> = 2, <i>df</i> <sub>2</sub> = 27 <sup>b</sup>	<i>R</i> <sup>2</sup> = 0.71	<i>R</i> <sup>2</sup> = 0.06	<i>R</i> <sup>2</sup> = 0.10

<sup>a</sup>Values are means ± standard error of the mean, *n* = 10. Different superscript letters indicate significant differences between treatments at *P* < 0.05 based on separate Tukey tests. <sup>b</sup>*df*<sub>1</sub> and *df*<sub>2</sub> are degrees of freedom for the numerators and denominators of the *F*-statistic, respectively.

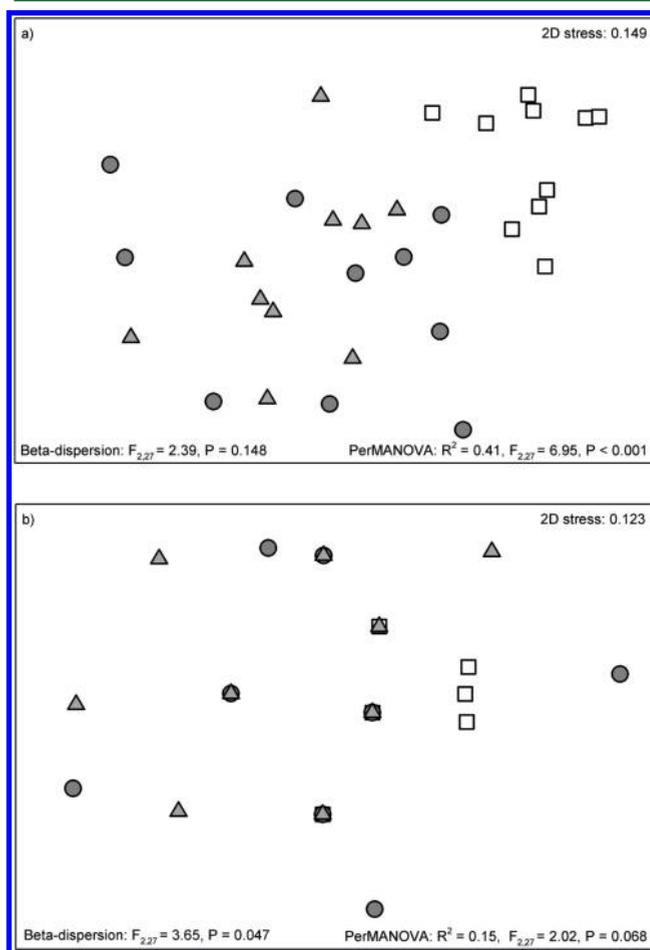
(midge) larvae which, although numerically dominant in all treatments, were less abundant beneath bags compared to control plots (Figure 1). Furthermore, more individuals of *Carcinus maenas* (green shore crab) were found under bags compared to within control plots, but the abundances overall were low (Figure 1).



**Figure 1.** Species abundance in the top 5 cm layer of plain sediment (control), underneath conventional and biodegradable plastic bags. Values are means ± sem, *n* = 10. Asterisks indicate significant difference between treatments, with \*\*\* *P* < 0.001, \*\* *P* < 0.01, and \* *P* < 0.05. N.B.: The right half of the data set is represented by the right *y* axis.

There were significant differences (one-way PERMANOVA, *F*<sub>2,27</sub> = 6.95, *P* < 0.001) between infaunal assemblage structures (based on abundance data of each group identified). Specifically, assemblages beneath the biodegradable and conventional bags were similar to each other (*F*<sub>1,18</sub> = 1.27, *P* = 0.302.) but significantly different from that in the control plots (control vs biodegradable: *F*<sub>1,18</sub> = 15.78, *P* < 0.001; control vs conventional: *F*<sub>1,18</sub> = 13.26, *P* < 0.001). This is

visualized by nMDS ordination (Figure 2a) and supported by similarity percentage (*simper*) analysis. Lesser abundances of

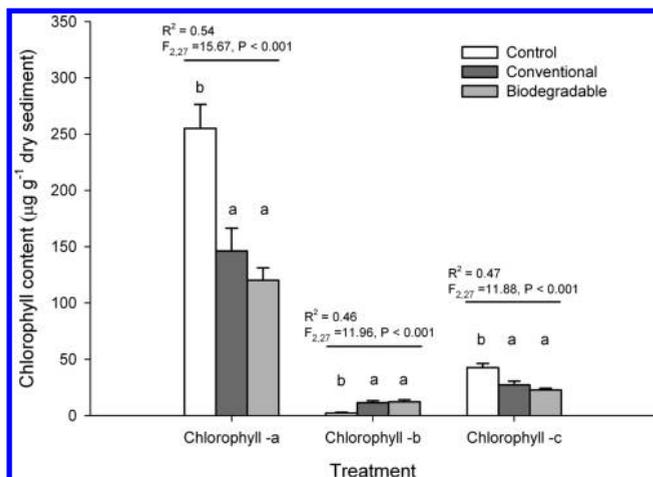


**Figure 2.** Nonmetric multidimensional scaling ordination of assemblage structure (a) and assemblage composition (b) of invertebrate groups in the top 5 cm layer of plain sediment (control: □), underneath conventional (●), and biodegradable (▲) plastic bags. Included are Kruskal 2D stress values, multivariate dispersion, and PerMANOVA, goodness of fit (*R*<sup>2</sup>), pseudo-*F* and *P* values based on 9999 possible permutations.

Nematoda and *C. capitata* contributed the most to differences in assemblage structure of invertebrates under the bags compared to the controls (Supporting Information, Table S1). However, when considering assemblage composition (based on species presence/absence data alone), there were no significant differences between the controls and either type of bag (Figure 2b).

### 3.2. Effects of Plastic Bags on Ecosystem Functioning.

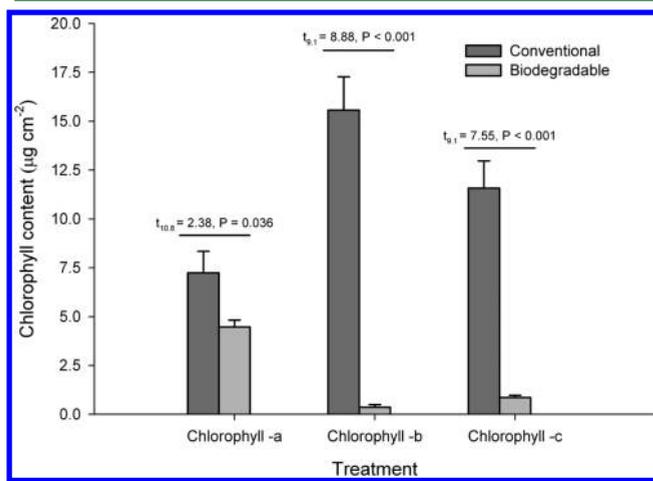
**3.2.1. Chlorophyll Content in Sediment Surface and on Plastic Bags.** Chlorophyll-a, -b, and -c contents in the top ~0.5 cm surface layer of the sediment were measured to approximate potential primary production of the microphytobenthos. The chlorophyll-a content (g<sup>-1</sup> dry sediment) was significantly less (one-way ANOVA, *F*<sub>2,27</sub> = 15.67, *P* < 0.001) beneath plastic bags (Figure 3) compared to control plots, which had on average 2.12 and 1.74 fold more chlorophyll-a than beneath “biodegradable” and conventional bags, respectively. The chlorophyll-b content, however, was significantly greater (one-way ANOVA, *F*<sub>2,27</sub> = 11.69, *P* = <0.001) beneath both



**Figure 3.** Chlorophyll-a, -b, and-c contents in the surface sediment without bags (control), underneath conventional and biodegradable plastic bags. Values are means  $\pm$  sem,  $n = 10$ . Included are ANOVA  $R^2$ ,  $F$ , and  $P$  values with different letters indicating significant differences between treatments at  $P < 0.05$  based on separate Tukey tests.

types of bags compared to within control plots (Figure 3), which had 5.15 and 4.78 fold less chlorophyll-b compared to beneath biodegradable and conventional bags, respectively. The content of chlorophyll-c was also significantly different (one-way ANOVA,  $F_{2,27} = 11.88$ ,  $P = <0.001$ ) between the treatments (Figure 3) but with an average 1.88 and 1.56 fold less chlorophyll-c present beneath biodegradable and conventional bags respectively than in the control plots. Chlorophyll contents of the surface sediment did not significantly differ between the two types of bags; chlorophyll-a (one-way ANOVA,  $P = 0.571$ , TukeyHSD), chlorophyll-b (one-way ANOVA,  $P = 0.926$ , TukeyHSD), and chlorophyll-c (one-way ANOVA,  $P = 0.541$ , TukeyHSD).

The chlorophyll content on the surfaces of the bags did, however, differ. Conventional bags had more algal biomass on the surface than the biodegradable bags (personal observation, Supporting Information, Figure S2), which corresponded to greater concentrations of chlorophyll (Figure 4). The surface of conventional bags contained more (1.62 fold) chlorophyll-a



**Figure 4.** Chlorophyll-a, -b, and-c contents on the surface of the conventional and biodegradable plastic bags. Values are means  $\pm$  sem,  $n = 10$ . Included are separate results of two-tailed Welch's  $t$  tests between the bag types.

than the biodegradable bags ( $t$  test,  $t_{10.84} = 2.37$ ,  $P = 0.036$ ), similarly the chlorophyll-b content was an average 43 fold greater on the surface of the conventional bags compared to the biodegradable bags ( $t$  test,  $t_{9.12} = 8.88$ ,  $P < 0.001$ ), and the chlorophyll-c content was an average of 11 times greater on the conventional than the biodegradable bags ( $t$  test,  $t_{9.11} = 7.65$ ,  $P < 0.001$ ).

**3.2.2. Redox Potential, Organic Matter Content, Grain Size, and Nutrient Pools and Profiles in Sediment.** The redox potential ( $E_h$ ) of the top sediment layer was negative under both the biodegradable and conventional bags, while it was positive in the control plots (Table 2). The amount of organic matter in the top sediment layer as measured by loss on ignition (LoI) was significantly less for sediment under either type of bag compared to the controls (One-way ANOVA,  $F_{2,27} = 12.31$ ,  $P < 0.001$ ), with approximately 13% less organic matter beneath bags. At 4 cm beneath the surface of the sediment the concentrations of organic matter was similar under the two types of bags and the controls (Table 2). The grain size distribution did not significantly differ among treatments (One-way ANOVA,  $F_{2,6} = 1.62$ ,  $P = 0.273$ ), and the average grain size was 44.98 ( $\pm 3.93$ ), 41.02 ( $\pm 2.13$ ), and 34.66 ( $\pm 3.62$ ) for control, biodegradable, and conventional treatments, respectively. Ammonium ( $\text{NH}_4^+$ ) and biogenic silicate ( $\text{Si}(\text{OH})_4$ ) pools within the first 4 cm of sediment were significantly different, with less  $\text{NH}_4^+$  in the control pore-water compared to that under the biodegradable bags. The biodegradable and conventional bags had similar amounts of  $\text{NH}_4^+$  in the underlying pore-water. Similarly, the total amount of  $\text{Si}(\text{OH})_4$  under the biodegradable bags was greater compared to sediment in the control plots and under conventional bags, with the two types of bags having similar amounts of  $\text{Si}(\text{OH})_4$  in the pore-water (Table 2). The concentration of  $\text{NH}_4^+$  was significantly different between the treatments (One-way ANOVA,  $F_{2,27} = 11.96$ ,  $P < 0.001$ ) at 4 cm depth, with  $\text{NH}_4^+$  being less in the control plots compared to beneath biodegradable and conventional bags. Similarly, the concentrations of  $\text{Si}(\text{OH})_4$  associated with the bags were greater in surface waters (One-way ANOVA,  $F_{2,27} = 3.77$ ,  $P = 0.036$ ) and at 4 cm depth (One-way ANOVA,  $F_{2,27} = 6.93$ ,  $P = 0.004$ ) compared to the control, albeit only significant for biodegradable bags (Figure 5).

## 4. DISCUSSION

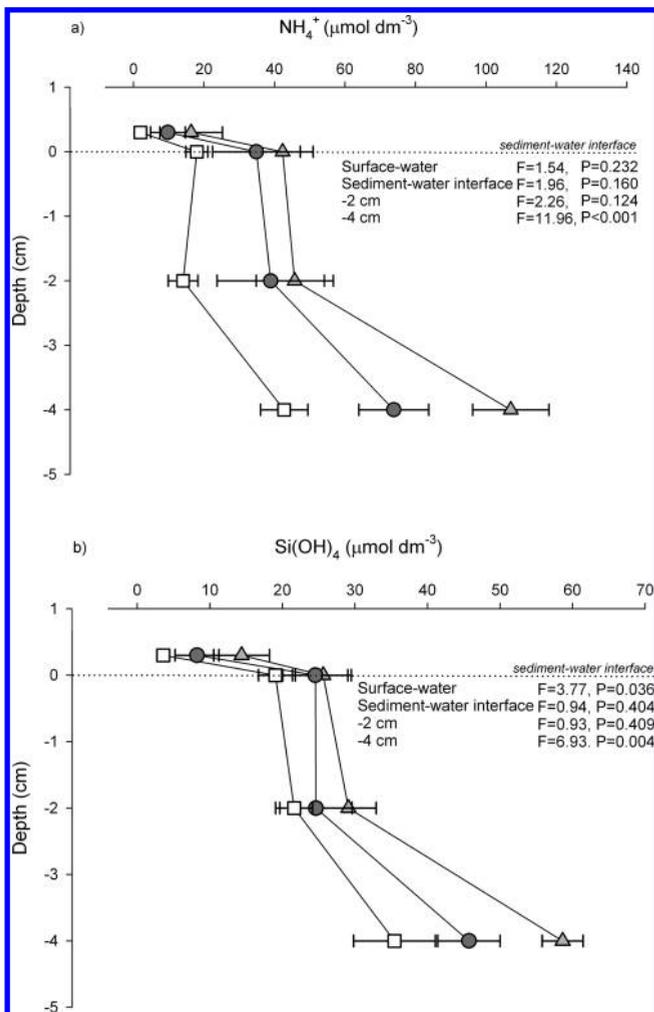
**4.1. Impacts of Plastic Debris on Benthic Infauna and Ecosystem Functioning.** In the current study, within less than 9 weeks, the presence of plastic debris in the intertidal significantly altered the community structure and abundance of sediment infauna, reducing the number of individuals of invertebrates living within the sediment. The presence of both types of bags caused the assemblages to differ from those in control treatments. This was driven by differences in relative abundances, rather than to changes to the types of taxa, the number of species, or change in the relative dominance of individual taxa. The nMDS ordination showing assemblage composition suggests that the presence of plastic bags increased multivariate variance, which was confirmed by the *beta-disp* routine testing for homogeneity of variance. This indicates that variability in the infaunal composition among replicates from the bag treatments was greater than in the control plots.

The addition of plastic bags, whether biodegradable or conventional, created anoxic conditions in the sediment, as indicated by reduced redox potentials and increased ammo-

**Table 2. Loss on Ignition (LoI) in the Top and Bottom Sediment Layer, Redox Potential ( $E_h$ ) in the Surface Sediment Layer and Inventory (Inv.  $\mu\text{mol dm}^{-3}$ ) of Ammonium ( $\text{NH}_4^+$ ) and Silica ( $\text{Si}(\text{OH})_4$ ) in Plain Sediment (Control), Underneath Conventional Bags (Conv) and Underneath Biodegradable Bags (Bio)<sup>a</sup>**

treatment	LoI <sub>top</sub> (%)	LoI <sub>bottom</sub> (%)	$E_h$ (mV)	Inv. $\text{NH}_4^+$	Inv. $\text{Si}(\text{OH})_4$
control	20.3 ± 0.2 <sup>a</sup>	19.5 ± 0.3 <sup>a</sup>	33.6 ± 6.7 <sup>a</sup>	52.6 ± 5.7 <sup>a</sup>	58.3 ± 6.2 <sup>a</sup>
conv	19.1 ± 0.1 <sup>b</sup>	19.9 ± 0.5 <sup>a</sup>	-51.5 ± 21.5 <sup>b</sup>	114.5 ± 30.7 <sup>ab</sup>	75.4 ± 11.0 <sup>ab</sup>
bio	19.1 ± 0.2 <sup>b</sup>	19.1 ± 0.3 <sup>a</sup>	-48.5 ± 10.9 <sup>b</sup>	149.7 ± 20.2 <sup>b</sup>	91.2 ± 6.7 <sup>b</sup>
ANOVA	$F_{2,27} = 12.31$ , $P < 0.001$ $R^2 = 0.48$	$F_{2,27} = 1.33$ , $P = 0.281$ $R^2 = 0.09$	$F_{2,12} = 11.15$ , $P = 0.002$ <sup>b</sup> $R^2 = 0.65$	$F_{2,27} = 5.25$ , $P = 0.012$ $R^2 = 0.28$	$F_{2,27} = 4.01$ , $P = 0.030$ $R^2 = 0.23$

<sup>a</sup>Values are means ± standard error of the means,  $n = 10$ . Different superscript letters indicate significant differences between treatments at  $\alpha = 0.05$  based on separate Tukey tests. <sup>b</sup>Degrees of freedom differ from other tests because five replicates were measured instead of ten.



**Figure 5.** Concentrations of ammonium (a) and silica (b) of pore and surface water in depth of plain sediment (control: □), underneath conventional (●) and biodegradable (▲) plastic bags. Values are means ± sem,  $n = 10$ . Included are ANOVA  $F$  ( $df_1 = 2$ ,  $df_2 = 27$ ) and  $P$  values for each depth.

niium pools. The physical effect of sealing the surface and effectively blocking oxygen diffusion into the sediment probably contributed to the reduction in redox potential, while the reduced oxygen availability at the water–sediment interface likely accounted for the decrease in infaunal abundances. Similar results, including decreases in biomass/abundances of infauna, have been found as a result of hypoxia and anoxia due to eutrophication.<sup>35</sup> Furthermore, decreased amounts of

organic matter in the sediment beneath the bags indicates that the bags acted as a barrier, preventing organic matter deposition into the sediment. It is likely that mineralization (i.e., the decomposition of organic matter resulting in the release of inorganic nutrients) beneath bags depleted the pre-existing stores of organic matter in the surface layer, and this is reflected in the increase of ammonium beneath the bags. Biogenic silicate, however, is not affected by redox reactions (e.g., mineralization). Given that the physical barrier created by the presence of the bags impaired transport across the sediment–water interface, a slight accumulation of silicate within the sediment is not surprising. On the contrary, because ammonium is the byproduct of organic matter mineralization, both this and impaired transport are likely to have led to its accumulation beneath the bags. Besides covering the sediment and directly affecting the benthic assemblages, indirect effects due to asphyxiation such as changes in redox potential may have led to altered benthic assemblages.

Overall, most species that were found inhabiting the sediment are tolerant to fluctuations in environmental conditions, as are many intertidal organisms. For example, *C. capitata* is an opportunistic polychaete often used as an indicator species for other types of pollution such as organic enrichment and nitrogenous runoff.<sup>36</sup> Given that organisms in the intertidal are usually very tolerant of fluctuating environmental conditions,<sup>37</sup> the results of this experiment may give a conservative estimate of the effects of plastic debris when compared to other, less resilient, assemblages. For example, the responses noted here in an intertidal marsh could be more pronounced in subtidal coral reefs and deep sea sediments, which are also known to accumulate litter.<sup>38,39</sup> Despite being resilient against environmental pollution, the abundance of *C. capitata* and other organisms was substantially reduced under both the biodegradable and conventional bags compared to control plots of sediment. It is possible that other, more sensitive groups of invertebrates would be even more profoundly affected by plastic debris. The most reduced taxa were deposit feeders, including *C. capitata*, *H. ulvae*, and individuals belonging to the Nematoda. Deposit feeders, and indeed any organisms which feed or live within the sediment, are more likely to be negatively affected by plastic debris due to the sealing of the sediment surface, reducing light, algal growth, and oxygen penetration.

Evidence in the literature of assemblage level effects of plastic debris is very scarce, and to date, only two studies have considered these impacts in benthic habitats<sup>22–24</sup> and neither evaluated the effect of the debris on the physio-chemical characteristics of the habitat. In Ambon Bay, Indonesia, Unepetty and Evans<sup>22</sup> compared areas where litter had already

accumulated with areas free from litter. They found that, underneath plastic debris, there was an increase in the abundance of meiofauna, decreased diatom abundances but no changes to macrofauna. However, it was not clear how long the litter had been present, nor how similar the areas of seabed were prior to its arrival. In a long-term (one-year), manipulative experiment the addition of plastic and glass debris to a subtidal sediment habitat resulted in an increase in the total abundance and the number of species of megafauna compared to control sediments. However, this was mainly as a consequence of migration of mobile species, such as hermit crabs, or settlement of invertebrates, such as ascidians and sponges on the hard substratum offered by the litter.<sup>23</sup> Subsequent examination of sediments from the same experiment found altered assemblage compositions, driven by an increase in opportunistic macrofaunal species, associated with experimentally added plastic and glass bottles.<sup>24</sup> Contrary to this previous study, the current short-term experiment found no changes in the number of species but did find a dramatic decrease in the abundances of several macrofaunal species beneath plastic debris. The plastic debris used by Katsaneakis et al.<sup>23</sup> and Akoumianaki et al.<sup>24</sup> was mostly bottles and jars, whereas the current study used plastic bags. Perhaps the effect of flat plastic sheeting is more pronounced than three-dimensional debris, in that sheeting smothers larger areas of the sediment, sealing off the sediment–water interface from the water column above. This may have prevented settlement of some infaunal species which depend on a planktonic larval stage before settling and recruiting. In addition, many of the changes described by Katsaneakis et al.<sup>23</sup> related to colonisation of the plastic by epifauna and migration of mobile species. Colonisation by epifauna is a longer term process and was not considered in the present study. *C. maenas* was, however, found under several of the bags and not in the control plots. Similarly, other artificial structures associated with fisheries, such as tiles, have been found to increase crab abundances due to the provision of refugia.<sup>40</sup> Despite this, the majority of organisms found in the present study were less abundant beneath plastic bags than in uncovered control plots.

Apart from the physical sealing effect of the plastic, our study showed that several physicochemical characteristics were altered beneath the plastic bags, and these may have indirectly accounted for the reduction in the abundances of benthic infauna. There was a reduction in organic matter and chlorophyll-a and -c beneath the bags indicating that there had been a decrease in the biomass of primary producers in the sediment, such as microphytobenthos.<sup>30</sup> A reduction in microphytobenthos means that there is less food available to higher trophic level organisms, and this could also account for the decrease in the biomass of invertebrates in the sediment.

Microphytobenthos play an important role in system metabolism within shallow coastal bays, and changes in autotrophic community structure will ultimately affect nutrient turnover in the system and could have cascading effects for the food-web. It is likely, for example, that the reduction of photosynthetic biomass beneath plastic bags led to reduced inputs of organic matter that serve as food sources for the deposit feeding detritivores, including surface and subsurface feeding groups. Indeed, other studies found reductions in macro-invertebrate infaunal abundances as a result of reduced microphytobenthic algae due to shading.<sup>41,42</sup>

In addition, since microphytobenthos strongly influences sediment organic matter quality and quantity,<sup>43</sup> this likely

accounts for the reduction in organic matter content in the surface sediment beneath bags. In fact, the ability of plastic sheeting to smother the underlying sediment has been utilized as a method, called “plasticulture”, to reduce weed growth by blocking out light in terrestrial agricultural practices.<sup>44</sup> Similarly, the reduction in light penetration caused by the bags during our experiment likely accounts for the reduction in chlorophyll-a and -c beneath the bags compared to the control sediment. Light attenuation is one of the principal limiting factors of primary productivity of microphytobenthos in shallow and intertidal estuarine habitats.<sup>45,46</sup> Some microphytobenthic species, however, show evidence of photoacclimation to low-light environments and are not affected by shading.<sup>45</sup> Chlorophyll-b concentrations were greater beneath plastic bags compared to bare sediment. Many green photosynthetic organisms contain chlorophyll-b as an accessory pigment, but there are microphytoplankton which have their light harvesting complex based on chlorophyll-b, and in this way, chlorophyll-b may indicate the presence of algae from the classes Chlorophyceae and/or Euglenophyceae.<sup>47,48</sup> Therefore, the increase in chlorophyll-b may indicate a change in types of microalgae dominating the sediment, but further work would be required to confirm this. Despite a lack of information on specific photosynthetic species, these results suggest that the algal community has been altered or, at least the amount of photosynthetic pigment was altered, due to the presence of the bags.

Interestingly, biodegradable plastic bags had substantially less chlorophyll on their surfaces than the conventional bags did, suggesting that the type of plastic influenced the recruitment and/or persistence of algae on the surface. Over time, this may result in differential effects, but this hypothesis would require further investigation. Aloy et al.<sup>49</sup> found a reduction in grazing by gastropods over conventional plastic bags compared to rock. It is possible that grazing is differentially affected by different types of plastic, thus accounting for the differences in algal biomass on biodegradable and conventional bags found in the current study.

Aside from the differences in chlorophyll concentrations, our short-term study showed that the biodegradable and conventional bags caused almost identical changes to invertebrate abundance and ecosystem functioning and that effects on assemblages occur much more rapidly than any meaningful degradation of the plastic itself; indeed we also demonstrated that, in the intertidal, breakdown of the biodegradable plastic was slower than would be expected according to EN 13432. Collectively, the potential for rapid effects coupled with the persistence of plastic debris, including plastics described as “compostable” or “biodegradable”,<sup>14</sup> emphasizes the need to focus on reducing and reusing plastic packaging and subsequently recycling, rather than designing materials with enhanced degradation which could lead to inappropriate, indiscriminate disposal and further accumulation in marine environments and/or compromise recycling streams.<sup>50</sup> Moreover, it is possible that, when certain biodegradable plastics do break down into smaller pieces, their effects could still be deleterious. For example, in a laboratory study by Doering et al.,<sup>51</sup> using powdered biodegradable plastics, including a bacterially derived polyester and ethylene vinyl alcohol and comparing to cornstarch as a control, it was found that normal nutrient exchange patterns were altered by addition of the plastic, including an increase of ammonia fluxes. They hypothesized that the high biological oxygen demand resulting

from decomposition of such materials could lead to the development of anoxia in the sediment. Similarly, the physical presence of fragments of conventional plastics have been shown to reduce the ability of marine worms to store energy,<sup>52</sup> and there are concerns about the potential for plastic fragments to transport and release contaminants.<sup>53</sup> It is possible that fragments of biodegradable plastic could have similar effects to conventional types, accumulating organic and metal pollutants, leaching additives, and causing physical harm upon ingestion, but this is yet to be examined.

**4.2. Wider Implications of Plastic Litter and Recommendations.** The impacts of disturbance on ecosystems are often context-dependent. Since the current study was set up in a single habitat and measured once after 63 days, it is limited in its ability to predict impacts on assemblages and ecosystem functioning at larger spatial or temporal scales. Furthermore, our model system was subject to very low wave energy, and in other habitats, plastic debris may remain for shorter periods of time in a single location. This study does emphasize, however, that assemblage level effects can occur rapidly, much faster than the time scales of any meaningful degradation, even of materials designed to quickly break down. This either indicates considerable lack of relevance of EN 13432 to degradation (i.e., composting) in the natural environment, or a failure of the biodegradable bag to perform to EN 13432. Over the short duration of this experiment, littering by plastic debris altered important components of the sediment ecosystem, including abundances of benthic infauna and physicochemical characteristics associated with nutrient cycling. The bags posed a physical barrier to mass, and therefore energy, exchange across the sediment–water interface. Since the functioning of the benthic ecosystem is essentially regulated by the extent of exchanges occurring across the sediment–water interface, the plastic bags (either biodegradable or conventional) drove the system to another balance point, leading to a depletion of organic matter, whether living or dead. While biodegradable plastics are promoted to have less negative impacts on the environment because their persistence is shorter, the current study suggests that the rate at which they break down is not sufficiently quick to have any meaningful advantage over conventional bags in terms of consequences when considering benthic habitats.

It is estimated that approximately 70% of the marine litter that enters the sea ends up accumulating on the sea bed;<sup>18</sup> indeed plastic debris accounts for the majority of litter recorded on the benthos in many parts of the world including the Mediterranean Sea,<sup>54</sup> European coastal regions,<sup>55</sup> and even remote Alaskan<sup>56</sup> and deep Arctic seafloors.<sup>59</sup> Therefore, if the amount of plastic in the environment increases as predicted,<sup>57</sup> the potential for wider scale impacts is considerable. Although it had previously been suggested that plastic litter can alter nutrient exchange between sediments and overlying water and therefore affect ecosystem services,<sup>56</sup> this had not been tested until now. Besides negative aesthetic effects, it is apparent from this study that littering of sedimentary habitats by plastic debris can alter key components of ecosystems, including primary productivity, invertebrate biomass, and benthic turnover rates of important limiting nutrients. Plastic debris is increasingly accumulating in coastal habitats worldwide, and the full extent of impacts in different habitats is not clear. While the effects of marine debris on individuals are well documented, this study indicates the potential for rapid effects on assemblages and several important ecosystem services.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Two figures, one showing weather meta-data and one showing images of the plastic bags, and a table with SIMPER analysis results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

D.S.G. and R.C.T. conceived the idea. D.S.G. and B.B. designed the experiment. B.B. did the statistical analysis. D.S.G., B.B., and D.J.B. carried out the field and laboratory work. 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.

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## ■ REFERENCES

- (1) Plastics Europe Web site; <http://www.plasticseurope.org/Document/plastics-the-facts-2012.aspx?FolID=2>.
- (2) Thompson, R. C.; Moore, C. J.; vom Saal, F. S.; Swan, S. H. Plastics, the environment and human health: current consensus and future trends. *Philos. Trans. R. Soc., B* **2009**, *364* (1526), 2153–66 DOI: 10.1098/rstb.2009.0053.
- (3) Jambeck, J. R.; Andrady, A.; Geyer, R.; Narayan, R.; Perryman, M.; Siegler, T.; Wicox, C.; Lavender Law, K. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768–771 DOI: 10.1126/science.1260352.
- (4) Derraik, J. G. The pollution of the marine environment by plastic debris: a review. *Mar. Pollut. Bull.* **2002**, *44* (9), 842–52 DOI: 10.1016/S0025-326X(02)00220-5.
- (5) Goldberg, E. D. Diamonds and plastics are forever? Editorial. *Mar. Pollut. Bull.* **1994**, *28*, (466); DOI 10.1016/0025-326X(94)90511-8.
- (6) Goldberg, E. D. Plasticizing the seafloor: an overview. *Environ. Technol.* **1997**, *18* (2), 195–202 DOI: 10.1080/09593331808616527.
- (7) Thompson, R. C.; Olsen, Y.; Mitchell, R. P.; Davis, A.; Rowland, S. J.; John, A. W. G.; McGonigle, D.; Russell, A. E. Lost at sea: where is all the plastic? *Science*. **2004**, *304* (838); DOI: 10.1126/science.1094559.
- (8) Ocean Conservancy Website; <http://www.oceanconservancy.org/>.
- (9) Thiel, M.; Hinojosa, I.; Vásquez, N.; Macaya, E. Floating marine debris in coastal waters of the SE Pacific (Chile). *Mar. Pollut. Bull.* **2003**, *46* (2), 224–231 DOI: 10.1016/S0025-326X(02)00365-X.
- (10) Willoughby, N. G.; Sangkoyo, H.; Lakaseru, B. O. Beach litter: an increasing and changing problem for Indonesia. *Mar. Pollut. Bull.* **1997**, *34* (6), 469–478 DOI: 10.1016/j.marpollbul.2005.04.044.
- (11) Galgani, F.; Burgeot, T.; Bocquene, G.; Vincent, F.; Leaute, J. P. Distribution and abundance of debris on the continental shelf of the Bay of Biscaye and in the Seine Bay. *Mar. Pollut. Bull.* **1995**, *30* (1), 58–62 DOI: 10.1016/0025-326X(94)00101-E.
- (12) Tosin, M.; Weber, M.; Siotto, M.; Lott, C.; Degli Innocenti, F. Laboratory Test Methods to Determine the Degradation of Plastics in

Marine Environmental Conditions. *Front. Microbiol.* **2012**, *3*, 225 DOI: 10.3389/fmicb.2012.00225.

(13) O'Brine, T.; Thompson, R. C. Degradation of plastic carrier bags in the marine environment. *Mar. Pollut. Bull.* **2010**, *60* (12), 2279–2283 DOI: 10.1016/j.marpolbul.2010.08.005.

(14) Briassoulis, D.; Babou, E.; Hiskakis, M.; Kyriku, I. Analysis of long-term degradation behaviour of polyethylene mulching films with pro-oxidants under real cultivation and soil burial conditions. *Environ. Sci. Pollut. Res.* **2015**, *22* (4), 2584–2598 DOI: 10.1007/s11356-014-3464-9.

(15) Mouat, J.; Lozano, R. L.; Bateson, H. *Economic impacts of marine litter*. KIMO (Kommunenenes Internasjonale Miljøorganisasjon), 2010.

(16) Azzarello, M. Y.; Van Vleet, E. S. Marine birds and plastic pollution. *Mar. Ecol.: Prog. Ser.* **1987**, *37*, 295–303 DOI: 10.3354/meps037295.

(17) Gall, S. C.; Thompson, R. C. The impact of debris on marine life. *Mar. Pollut. Bull.* **2015**, *29* (1–2), 170–179 DOI: 10.1016/j.marpolbul.2014.12.041.

(18) Hammer, J. I.; Kraak, M. H.; Parsons, J. R. Plastics in the marine environment: the dark side of a modern gift. *Rev. Environ. Contam. Toxicol.* **2012**, *220*, 1–44 DOI: 10.1007/978-1-4614-3414-6\_1.

(19) Barnes, D. K. A.; Galgani, F.; Thompson, R. C.; Barlaz, M. Environmental accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc., B* **2009**, *364* (1526), 1985–1998 DOI: 10.1098/rstb.2008.0205.

(20) 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 DOI: 10.1890/10-1510.1.

(21) Martínez, M. L.; Intralawan, A.; Vázquez, G.; Pérez-Maqueo, O.; Sutton, P.; Landgrave, R. The coasts of our world: ecological, economic and social importance. *Ecol. Econ.* **2007**, *63* (2–3), 254–272 DOI: 10.1016/j.ecolecon.2006.10.022.

(22) Unepetty, P.; Evans, S. M. The impact of plastic debris on the biota of tidal flats in Ambon Bay (eastern Indonesia). *Mar. Environ. Res.* **1997**, *44* (3), 233–242 DOI: 10.1016/S0141-1136(97)00002-0.

(23) Katsanevakis, S.; Verriopoulos, G.; Nicolaidou, A.; Thessalou-Legaki, M. Effect of marine litter on the benthic megafauna of coastal soft bottoms: a manipulative field experiment. *Mar. Pollut. Bull.* **2007**, *54* (6), 771–778 DOI: 10.1016/j.marpolbul.2006.12.016.

(24) Akoumianaki, I.; Kontolefas, P.; Katsanevakis, S.; Nicolaidou, A.; Verriopoulos, G. Subtidal littering: Indirect effects on soft substratum macrofauna? *Mediterr. Mar. Sci.* **2008**, *9* (2), 35–52 DOI: 10.12681/mms.131.

(25) Seeberg-Elverfeldt, J.; Schluter, M.; Feseker, T.; Kolling, M. Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. *Limnol. Oceanogr.: Methods* **2005**, *3* (8), 361–71 DOI: 10.4319/lom.2005.3.361.

(26) Rocha, C.; Ibanhez, J.; Leote, C. Benthic nitrate biogeochemistry affected by tidal modulation of Submarine Groundwater Discharge (SGD) through a sandy beach face, Ria Formosa, Southwestern Iberia. *Mar. Chem.* **2009**, *115* (1–2), 43–58 DOI: 10.1016/j.marchem.2009.06.003.

(27) Green, D. S.; Boots, G. B.; Crowe, T. P. Effects of non-indigenous oysters on microbial diversity and ecosystem functioning. *PLoS One* **2012**, *7* (10), e48410 DOI: 10.1371/journal.pone.0004.

(28) Ibanhez, J. S. P.; Rocha, C. Effects of recirculation of seawater enriched in inorganic nitrogen on dissolved organic carbon processing in sandy seepage face sediments. *Mar. Chem.* **2014**, *166*, 48–58 DOI: 10.1016/j.marchem.2014.09.012.

(29) Eleftheriou, A.; McIntyre, A. *Methods for the study of marine benthos*; Blackwell Science: Oxford, U.K., 2007; DOI 10.1002/9780470995129.

(30) Aminot, A.; Rey, F. *Standard procedure for the determination of chlorophyll a by spectroscopic methods*; International Council for the Exploration of the Sea Techniques in Marine Environmental Sciences: Copenhagen, Denmark, 2000.

(31) Jeffrey, S. W.; Humphrey, G. F. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants,

algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **1975**, *167*, 191–194.

(32) Gray, A. B.; Pasternack, G. B.; Watson, E. B. Hydrogen peroxide treatment effects on the particle size distribution of alluvial and marsh sediments. *Holocene* **2010**, *20* (2), 293–301 DOI: 10.1177/0959683609350390.

(33) R Core Team. *R: A language and environment for statistical computing*; R Foundation for Statistical Computing: Vienna, Austria, 2014; URL <http://www.R-project.org/>.

(34) Oksanen, J.; Blanchet, F. G.; Kindt, R.; Legendre, P.; Minchin, P. R.; O'Hara, R. B.; Simpson, G. L.; Solymos, P.; Stevens, M. H. H.; Wagner, H. *vegan: Community Ecology Package*. R package version 2.2–0, 2014; <http://CRAN.R-project.org/package=vegan>.

(35) Levin, L. A.; Ekau, W.; Gooday, A. J.; Jorissen, F.; Middelburg, J. J.; Naqvi, S. W. A.; Neira, C.; Rabalais, N. N.; Zhang, J. Effects of natural and human-induced hypoxia on coastal benthos. *Biogeosciences* **2009**, *6* (10), 2063–2098 DOI: 10.5194/bg-6-2063-2009.

(36) Henriksson, R. Influence of pollution on the bottom fauna of the Sound (Öresund). *Oikos* **1969**, *20* (2), 507–523.

(37) Newell, R. C. *Adaptation to Environment: Essays on the Physiology of Marine Animals*; Butterworths: London, 1976.

(38) Smith, S. D. A.; Edgar, R. J. Documenting the density of dubtidal marine debris across multiple marine and coastal habitats. *PLoS One* **2014**, *9* (4), e94593 DOI: 10.1371/journal.pone.0094593.

(39) Bergmann, M.; Klages, M. Increase of litter at the Arctic deep-sea observatory HAUSGARTEN. *Mar. Pollut. Bull.* **2012**, *64* (12), 2734–2741 DOI: 10.1016/j.marpolbul.2012.09.018.

(40) Sheehan, E. V.; Thompson, R. C.; Coleman, R. A.; Attrill, M. J. Positive feedback fishery: Population consequences of 'crab-tiling' on the green crab *Carcinus maenas*. *J. Sea Res.* **2008**, *60* (4), 255–261 DOI: 10.1016/j.seares.2008.09.002.

(41) Stocks, K. I.; Grassle, J. F. Effects of microalgae and food limitation on the recolonization of benthic macrofauna into *in situ* saltmarsh-pond mesocosms. *Mar. Ecol.: Prog. Ser.* **2001**, *221*, 93–104 DOI: 10.3354/meps221093.

(42) Struck, S. D.; Craft, C. B.; Broome, S. W.; Sanclements, M. D.; Sacco, J. N. Effects of bridge shading on estuarine marsh benthic invertebrate community structure and function. *Environ. Manage.* **2004**, No. 1, 99–111 DOI: 10.1007/s00267-004-0032-y.

(43) Hardison, A. K.; Canuel, E. A.; Anderson, I. C.; Tobias, C. R.; Veuger, B.; Waters, M. N. Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments. *Biogeosciences* **2013**, *10* (8), 5571–5588 DOI: 10.5194/bg-10-5571-2013.

(44) Grundy, A. C.; Bond, B. Use of non-living mulches for weed control. In *Non-chemical weed management: principles, concepts and technology*; Upadhyaya, M. K., Blackshaw, R. E., Eds.; CABI: Wallingford, U.K., 2007; pp 135–154, DOI 10.1079/9781845932909.0135.

(45) Sundbäck, K.; McGlathery, K. J. Interaction between benthic macro- and microalgae in the marine environment. In *Interactions between macro- and microorganisms in marine sediments*; Kristensen, E., Kostka, J. E., Haese, R. H., Eds.; John Wiley & Sons, Inc.; American Geophysical Union: New York, 2005; pp 7–29, DOI 10.1029/CE060p0007.

(46) Valiela, I.; McClelland, J.; Hauxwell, J.; Behr, P. J.; Hersh, D.; Foreman, K. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnol. Oceanogr.* **1997**, *42* (5), 1105–1118 DOI: 10.4319/lo.1997.42.5\_part\_2.1105.

(47) Riaux-Gobin, C.; Llewellyn, C. A.; Klein, B. Microphytobenthos from two subtidal sediments from North Brittany. II. Variations of pigment compositions and concentrations determined by HPLC and conventional techniques. *Mar. Ecol.: Prog. Ser.* **1987**, *40* (3), 275–283 DOI: 10.3354/meps040275.

(48) Hoek van den, C. *Algen, Einführung in die Phykologie*; Georg Thieme Verlag: Stuttgart, Germany, 1978.

(49) Aloy, A. B.; Vallejo, B. M.; Juinio-Meñez, M. A. Increased plastic litter cover affects the foraging activity of the sandy intertidal

gastropod *Nassarius pullus*. *Mar. Pollut. Bull.* **2011**, *62* (8), 1772–1779 DOI: 10.1016/j.marpolbul.2011.05.021.

(50) Environmental Audit Committee. *Plastic bags: Government response to the Committee's Eleventh Report of Session 2013–14. First Special Report of Session 2014–15*. Authority of the House of Commons; The Stationery Office: London, 2014.

(51) Doering, P. H.; Sullivan, B. K.; Jeon, H. Effects of biodegradable plastic components on metabolism of an estuarine benthos. *J. Environ. Polym. Degrad.* **1994**, *2* (4), 271–275 DOI: 10.1007/BF02071975.

(52) Wright, S. L.; Rowe, D.; Thompson, R. C.; Galloway, T. S. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* **2013**, *23* (23), 1031–1033 DOI: 10.1016/j.cub.2013.10.068.

(53) Teuten, E. L.; Saquing, J. M.; Knappe, D. R. U.; Barlaz, M. A.; Jonsson, S.; Björn, A.; Rowland, S. J.; Thompson, R. C.; Galloway, T. S.; Yamashita, R.; Ochi, D.; Watanuki, Y.; Moore, C.; Viet, P. H.; Tana, T. S.; Prudente, M.; Boonyatumanond, R.; Zakaria, M. P.; Akkavong, K.; Ogata, Y.; Hirai, H.; Iwasa, S.; Mizukawa, K.; Hagino, Y.; Imamura, A.; Saha, M.; Takada, H. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc., B* **2009**, *364*, 2027–2045 DOI: 10.1098/rstb.2008.0284.

(54) Galgani, F.; Souplet, A.; Cadiou, Y. Accumulation of debris on the deep sea floor off the French Mediterranean coast. *Mar. Ecol.: Prog. Ser.* **1996**, *142* (1–3), 225–234 DOI: 10.3354/meps142225.

(55) Galgani, F.; Leaute, J. P.; Moguedet, P.; Souplet, A.; Verin, Y.; Carpentier, A.; Goragner, H.; Latrouite, D.; Andral, B.; Cadiou, Y.; Mahe, J. C.; Poulard, J. C.; Nerisson, P. Litter on the sea floor along European coasts. *Mar. Pollut. Bull.* **2000**, *40* (6), 516–527 DOI: 10.1016/S0025-326X(99)00234-9.

(56) Hess, N. A.; Ribic, C. A.; Vining, I. Benthic marine debris, with an emphasis on fishery-related items, surrounding Kodiak Island, Alaska, 1994–1996. *Mar. Pollut. Bull.* **1999**, *38* (10), 885–890 DOI: 10.1016/S0025-326X(99)00087-9.

(57) Rochman, C. M.; Browne, M. A.; Halpern, B. S.; Hentschel, B. T.; Hoh, E.; Karapanagioti, H. K.; Rios-Mendoza, L. M.; Takada, H.; Teh, S.; Thompson, R. C. Classify plastic waste as hazardous. *Nature* **2013**, *494* (7436), 169–171 DOI: 10.1038/494169a.