Electronic supplementary material

Macrofouling communities and the degradation of plastic bags in the sea – an *in situ* experiment

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1. Introduction

There is a variety of sometimes misleading terms for different types of plastics, therefore we focused on three terms in our study: biodegradable, biobased and conventional plastic. Biodegradable plastics are degradable to full remineralisation (CO₂ or CH₄, water and biomass), and can be fossil- as well as biobased. Biobased plastic products consist of biogenic base materials such as starch or vegetable oils. They can be, but are not necessarily, biodegradable. We refer to all synthetic products which are degradable only at very low rates as conventional polymers.

2. Material and Methods

Plastic bags made of two different polymer types, conventional polyethylene (PE) and a biodegradable starch-based polymer blend, were exposed to a benthic and a pelagic coastal habitat. The composition of the two polymers was analysed by Fourier-transform infrared spectroscopy (FTIR) and confirmed to be PE and MaterBi® respectively (Fig. S1).

There were five replicate experimental constructions, each consisted of a pelagic and a benthic system (Fig. S2), which were deployed at 25 m depth in the water column and at 36 m depth on a sediment floor, respectively (Fig. S3).

4. Discussion

4.4 Disintegration and tensile properties

The determination of degradation was conducted by following the loss of sample surface over time photogrammetry. This method, however, intrinsically leads to an underestimation of disintegration at the beginning of the study because a reduction of thickness of the foil is not accounted for as the
images are only two-dimensional. Only when the thinning of the foil leads to the formation of holes, a “loss of surface” will be recorded (Fig. 3). Thus, three-dimensional disintegration is likely to have started before the second half of the experiment. At a very late stage in the study the method tends to overestimate the degradation because fragments are likely to be lost from the mesh bags.

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Figure S1: Results of the Fourier-transform infrared spectroscopy (FTIR) used to analyse the polymer composition. A: FTIR analysis of the biodegradable carrier bag assumed to be MaterBi®. B: FTIR analysis of the conventional fruit and vegetable carrier bag assumed to be Polyethylene (PE). The samples are shown in red, and compared to a polymer of known composition, shown in black. Wavenumber [cm\(^{-1}\)] is depicted on the x-axis. Transmittance of the sample is given in percent and is depicted on the y-axis.
Figure S2. Schematic sketch of the experimental construction of one replicate. There were 5 replicate constructions in total. Plastic films of carrier bags made of Polyethylene (PE) and a biodegradable starch-based plastic blend were secured in mesh bags to avoid the eventual loss of disintegrated fragments. The mesh bags were attached to a benthic and a pelagic experimental system, both connected by ropes to an anchor stone on the seafloor. The benthic system was deployed on the sediment sea floor at 36 m depth. The pelagic system was suspended at 25 m depth in the water column and held upright by floats. On each side of the benthic and pelagic system four samples of the two polymer types were mounted. The numbers represent the pattern in which the samples were retrieved (1=2 months, 2=5.5 months, 3=9.5 months, 4=12.5 months).
Figure S3. Pictures of the pelagic (A) and benthic (B) replicate construction *in situ*. Benthic samples were connected to an anchor stone at a depth of 36 m. Pelagic samples were floating in the water column at 25 m, kept upright by floats.
Figure S4. Abundances (number of specimens per cm$^2$) of all phyla of the fouling communities in the benthic (dark grey) and pelagic (light grey) habitats. Averages across all samples and all exposure times with standard errors are shown.

Figure S5. Selected fouling organisms identified from the polymer samples. A Trochamminidae, Foraminifera, B Spiroloculinidae, Foraminifera, C Planorbulinidae, Foraminifera, D Campanulariidae, Cnidaria, E Mytilidae, Mollusca, F Candidae, Bryozoa. The white bars represent 0.5 mm for A-F, and 1 mm for F.
Figure S6: Average abundances per cm² per family in the benthic (left) and in the pelagic habitat (right). The average abundance of each of the 21 families identified in the fouling community is depicted with the respective standard error.
Figure S7: Results of the analysis of homogeneity of multivariate dispersions. The communities in the two habitats (benthic vs. pelagic) were compared. The average distance of each group to the spatial median (=centroid) in a multivariate space was used as a measure for the dispersion (variance) of the groups. Euclidean distances were calculated based on the principal coordinate axes (PCoA). Eigenvalues are given in percent. The two habitats are depicted in red (pelagic, “P”) and black (benthic, “B”).
Table S1. Means and standard errors (se) for all response variables and all habitat - polymer type combinations. The two polymer types (conventional PE vs. biodegradable plastic) were exposed to a benthic and a pelagic coastal marine habitat. The fouling biomass (measured as optical density (OD) of the stained samples per m²), abundances (specimens per cm²), family richness, Shannon diversity on family level, net oxygen production [µmol L⁻¹ h⁻¹ cm⁻²] of the fouling community and tensile strength of the polymers at break [N] were measured.

| Response variable | Habitat    | Polymer type             | Mean   | se   |
|-------------------|------------|--------------------------|--------|------|
| Fouling biomass   | Benthic    | PE                       | 136.20 | 29.23|
|                   |            | biodegradable plastic    | 215.20 | 38.88|
|                   | Pelagic    | PE                       | 266.60 | 42.06|
|                   |            | biodegradable plastic    | 327.20 | 54.45|
| Abundance         | Benthic    | PE                       | 1.17   | 0.33 |
|                   |            | biodegradable plastic    | 1.45   | 0.59 |
|                   | Pelagic    | PE                       | 11.55  | 2.10 |
|                   |            | biodegradable plastic    | 11.84  | 2.38 |
| Family richness   | Benthic    | PE                       | 1.40   | 0.28 |
|                   |            | biodegradable plastic    | 1.00   | 0.24 |
|                   | Pelagic    | PE                       | 3.40   | 0.56 |
|                   |            | biodegradable plastic    | 3.10   | 0.65 |
| Shannon diversity | Benthic    | PE                       | 0.35   | 0.10 |
|                   |            | biodegradable plastic    | 0.23   | 0.08 |
|                   | Pelagic    | PE                       | 0.68   | 0.11 |
|                   |            | biodegradable plastic    | 0.49   | 0.10 |
| Net oxygen production | Benthic | PE                       | 0.03   | 0.02 |
|                     |            | biodegradable plastic    | -0.01  | 0.02 |
|                     | Pelagic    | PE                       | 0.21   | 0.05 |
|                     |            | biodegradable plastic    | 0.12   | 0.06 |
| Tensile strength  | Benthic    | PE                       | 3.64   | 0.17 |
|                   |            | biodegradable plastic    | 3.29   | 0.52 |
|                   | Pelagic    | PE                       | 3.81   | 0.28 |
|                   |            | biodegradable plastic    | 3.28   | 0.57 |
Table S2: Results of mixed model analysis of the effect of habitat, polymer type, time, and their interactions on family richness. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (combined benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic) per habitat.

|                | numDF | denDF | F-value | p-value |
|----------------|-------|-------|---------|---------|
| (Intercept)    | 1     | 56    | 92.88   | <0.0001 |
| Habitat        | 1     | 4     | 19.71   | 0.0113  |
| Type           | 1     | 8     | 0.58    | 0.4702  |
| Time           | 1     | 56    | 5.44    | 0.0233  |
| Habitat : Type | 1     | 8     | 0.01    | 0.9164  |
| Habitat : Time | 1     | 56    | 0.33    | 0.5701  |
| Type : Time    | 1     | 56    | 0.33    | 0.5685  |
| Habitat : Type : Time | 1 | 56 | 0.09 | 0.7707 |

Table S3: Results of mixed model analysis of the effect of habitat, polymer type, time, and their interactions on abundance for all specimens which were identified to family level. The abundance data were square root transformed to adjust for a non-normal distribution. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (combined benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic per habitat).

|                | numDF | denDF | F-value | p-value |
|----------------|-------|-------|---------|---------|
| (Intercept)    | 1     | 56    | 94.28   | <0.0001 |
| Habitat        | 1     | 4     | 28.20   | 0.006   |
| Type           | 1     | 8     | 0.06    | 0.010   |
| Time           | 1     | 56    | 2.36    | 0.130   |
| Habitat : Type | 1     | 8     | 0.03    | 0.872   |
| Habitat : Time | 1     | 56    | 0.16    | 0.692   |
| Type : Time    | 1     | 56    | 1.12    | 0.295   |
| Habitat : Type : Time | 1 | 56 | 0.02 | 0.882 |
Table S4: Results of mixed model analysis of the effect of habitat, polymer type, time, and their interactions on the Shannon diversity on family level. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (combined benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic) per habitat.

| Shannon diversity      | numDF | denDF | F-value | p-value   |
|------------------------|-------|-------|---------|-----------|
| (Intercept)            | 1     | 56    | 88.78   | <0.0001   |
| Habitat                | 1     | 4     | 8.19    | 0.0459    |
| Type                   | 1     | 8     | 3.43    | 0.1011    |
| Time                   | 1     | 56    | 8.73    | 0.0046    |
| Habitat : Type         | 1     | 8     | 0.01    | 0.9285    |
| Habitat : Time         | 1     | 56    | 0.43    | 0.5146    |
| Type : Time            | 1     | 56    | 1.54    | 0.2203    |
| Habitat : Type : Time  | 1     | 56    | 0.00    | 0.9947    |
Table S5: Results of the PERMANOVA analysis (999 permutations) of the effect of habitat, polymer type, time, and their interactions on the fouling community identified to family level (21 families, taxa that could not be identified to family level were excluded from this analysis). Pairwise Bray-Curtis distances were used.

| Fouling community (families) | Df | Sums of Sq | Mean Sq | F-value | R² | p-value |
|------------------------------|----|------------|---------|---------|----|---------|
| Habitat                      | 1  | 5.44       | 5.44    | 32.94   | 0.4| 0.001   |
| Type                         | 1  | 0.14       | 0.14    | 0.87    | 0.01| 0.465   |
| Time                         | 1  | 0.27       | 0.27    | 1.63    | 0.02| 0.159   |
| Habitat : Type               | 1  | 0.11       | 0.11    | 0.64    | 0.01| 0.669   |
| Habitat : Time               | 1  | 0.23       | 0.23    | 1.39    | 0.02| 0.179   |
| Type : Time                  | 1  | 0.22       | 0.22    | 1.31    | 0.02| 0.227   |
| Habitat : Type : Time        | 1  | 0.32       | 0.32    | 1.92    | 0.02| 0.096   |
| Residuals                    | 42 | 6.93       | 0.17    |         | 0.51|         |
| Total                        | 43 | 13.65      |         |         | 1   |         |

*ESM. Fouling and degradation of plastic bags*
Table S6: Results of mixed model analysis of the effect of habitat, polymer type, time and their interactions on optical density (OD) as a proxy for the biomass of the fouling layer. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (combined benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic) per habitat.

| OD          | numDF | denDF | F-value | p-value |
|-------------|-------|-------|---------|---------|
| (Intercept) | 1     | 55    | 509.99  | <0.0001 |
| Habitat     | 1     | 4     | 33.44   | 0.004   |
| Type        | 1     | 8     | 11.28   | 0.010   |
| Time        | 1     | 55    | 219.37  | <0.0001 |
| Habitat : Type | 1   | 8     | 0.01    | 0.918   |
| Habitat : Time | 1   | 55    | 4.93    | 0.031   |
| Type : Time | 1     | 55    | 6.72    | 0.012   |
| Habitat : Type : Time | 1 | 55    | 0.00    | 0.980   |
Table S7: Results of mixed effect model analysis of the effect of habitat, polymer type, time, and their interactions on optical density (OD). In contrast to Table S1, abundance on family level was tested as a covariable here in order to assess whether the effects of the explanatory variables on OD operated via abundance. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (combined benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic) per habitat.

| OD + Abundance | numDF | denDF | F-value | p-value |
|----------------|-------|-------|---------|---------|
| (Intercept)    | 1     | 54    | 542.52  | <0.0001 |
| Abundance      | 1     | 54    | 56.23   | <0.0001 |
| Habitat        | 1     | 4     | 3.16    | 0.1503  |
| Type           | 1     | 8     | 10.63   | 0.0115  |
| Time           | 1     | 54    | 217.66  | <0.0001 |
| Habitat : Type | 1     | 8     | 0.03    | 0.8636  |
| Habitat : Time | 1     | 54    | 4.04    | 0.0494  |
| Type : Time    | 1     | 54    | 7.12    | 0.0100  |
| Habitat : Type : Time | 1 | 54 | 0.01    | 0.9198  |
Table S8. Results of mixed effect model analysis of the effect of habitat, polymer time and type, and their interactions with net oxygen production. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (combined benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic) per habitat.

|                      | numDF | denDF | F-value | p-value |
|----------------------|-------|-------|---------|---------|
| (Intercept)          | 1     | 29    | 281.38  | <0.0001 |
| Habitat              | 1     | 4     | 11.57   | 0.0272  |
| Type                 | 1     | 8     | 0.12    | 0.7366  |
| Time                 | 1     | 29    | 32.06   | <0.0001 |
| Habitat:Type         | 1     | 8     | 0.28    | 0.6256  |
| Habitat:Time         | 1     | 29    | 0.68    | 0.4165  |
| Type:Time            | 1     | 29    | 0.05    | 0.8163  |
| Habitat:Type:Time    | 1     | 29    | 0.82    | 0.3718  |

Table S9: Results of the mixed effect model analysis of the effect of habitat, polymer time, type, and their interactions on tensile strength. Three nested random effects were included to incorporate the structure of the nested design: ‘Replicate’ for the replicate construction (benthic and pelagic experimental systems), ‘habnest’ to account for the two samples per habitat (benthic vs. pelagic) and ‘typenest’ to account for the two samples of the two polymer types (PE vs. biodegradable plastic) per habitat.

|                      | numDF | denDF | F-value | p-value |
|----------------------|-------|-------|---------|---------|
| (Intercept)          | 1     | 55    | 1103.94 | <0.0001 |
| Habitat              | 1     | 4     | 0.1754  | 0.6969  |
| Type                 | 1     | 8     | 4.2521  | 0.0731  |
| Time                 | 1     | 55    | 118.09  | <0.0001 |
| Habitat:Type         | 1     | 8     | 0.4619  | 0.5159  |
| Habitat:Time         | 1     | 55    | 1.7915  | 0.1863  |
| Type:Time            | 1     | 55    | 96.94   | <0.0001 |
| Habitat:Type:Time    | 1     | 55    | 0.1759  | 0.6765  |