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**Spatial distribution of microplastics in sediments and surface waters of the southern North Sea.**

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1 Spatial distribution of microplastics in  
2 sediments and surface waters of the southern  
3 North Sea

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**18 Abstract:**

19 Microplastic pollution within the marine environment is of pressing concern globally. Accordingly,  
20 spatial monitoring of microplastic concentrations, composition and size distribution may help to  
21 identify sources and entry pathways, and hence allow initiating focused mitigation. Spatial  
22 distribution patterns of microplastics were investigated in two compartments of the southern  
23 North Sea by collecting sublittoral sediment and surface water samples from 24 stations. Large  
24 microplastics (500–5000  $\mu\text{m}$ ) were detected visually and identified using attenuated total  
25 reflection (ATR) Fourier transform infrared (FTIR) spectroscopy. The remaining sample was  
26 digested enzymatically, concentrated onto filters and analyzed for small microplastics  
27 (11–500  $\mu\text{m}$ ) using Focal Plane Array (FPA) FTIR imaging. Microplastics were detected in all  
28 samples with concentrations ranging between 2.8–1188.8 particles  $\text{kg}^{-1}$  for sediments and 0.1–  
29 245.4 particles  $\text{m}^{-3}$  for surface waters. On average 98% of microplastics were  $<100 \mu\text{m}$  in  
30 sediments and 86% in surface waters. The most prevalent polymer types in both compartments  
31 were polypropylene, acrylates/polyurethane/varnish, and polyamide. Polymer composition  
32 differed significantly between sediment and surface water samples as well as between the  
33 Frisian Islands and the English Channel sites. These results show that microplastics are not  
34 evenly distributed, in neither location nor size, which is illuminating regarding the development of  
35 monitoring protocols.

36

**37 Keywords**

38 FTIR imaging, microplastic, enzymatic-oxidative treatment, enzymatic purification,  
39 PERMANOVA, clustering, spatial distribution patterns, horizontal transport

## 40 **Introduction**

41 Due to its ubiquity and longevity, plastic pollution in the marine environment has been  
42 recognized as a threat globally and is one of the “novel entities” referred to in the planetary  
43 boundaries concept<sup>1-3</sup>. Moreover, it has been included as part of descriptor 10 (marine litter) to  
44 be monitored in the European Marine Strategy Framework Directive (2008/56/EC) (MSFD)<sup>4, 5</sup>.  
45 Increasing plastics production and improper disposal have consequently led to an input of  
46 plastics into the marine environment which has been quantified to up to 12 million tons  
47 worldwide in 2010<sup>6</sup>. Once plastics reach the oceans, they are almost impossible to remove and  
48 merely disintegrate by chemical, physical and biological processes over time into smaller and  
49 more numerous microplastics (MP, <5 mm<sup>7</sup>).

50 With decreasing particle size, the unambiguous identification of polymer type becomes more  
51 challenging. As of yet, still no universally accepted standard operating procedure (SOP) exists,  
52 the harmonization of methods is urgently needed to allow for a comparison of data<sup>8-10</sup>.

53 According to Kroon et al.<sup>11</sup> a merely visual identification of the potential MP particles is not  
54 sufficient. More than 60% of the particles might be misassigned<sup>8, 11</sup>, if results are not validated by  
55 chemical identification. According to Rivers et al.<sup>12</sup>, a meaningful inter-study comparison requires  
56 not only data on particle number but also on particle size. As stated by Potthoff et al.<sup>13</sup> it is very  
57 important to gain as much information as possible from MP particles, like number, polymer type,  
58 shape, size distribution and weathering status, to do a qualified risk assessment. This stresses  
59 the need to use spectroscopic methods, which can provide this information.

60 In this study, we aimed to gain a valuable insight into the spatial distribution of MP in the  
61 southern North Sea, in terms of the MP concentrations, polymer types and size classes. To  
62 achieve this, we sampled two marine compartments, sediment and surface water, at 24 stations.  
63 We employed state-of-the-art techniques to extract MP and analyze samples based on  
64 attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectroscopy and focal plane  
65 array (FPA) based FTIR imaging. In the present study we also applied a novel approach based

66 on uni- and multivariate statistics to investigate the questions: (1) Do MP metric parameters  
67 differ significantly between the two sampled compartments?; (2) Which polymer types and size  
68 classes contribute most to these differences?; (3) Do spatial patterns driven by polymer  
69 composition or size distribution exist within each compartment?

70 In this study we applied innovative analytical techniques to expand the field of environmental MP  
71 research and to generate assured and comparable data for the ongoing development of MP  
72 monitoring strategies.

## 73 **Materials and Methods**

### 74 **Sampling**

75 Sampling was conducted during a cruise aboard the RV Heincke (He430) in the southern North  
76 Sea between the 30<sup>th</sup> of July and the 11<sup>th</sup> of August 2014. Sediment and water samples were  
77 taken at 24 stations (Figure 1, Supporting Information (SI) Table S1). Surface water was  
78 sampled using a Neuston Catamaran (HydroBios Apparatebau GmbH) towed alongside the  
79 vessel for up to 20 min. The attached net had an opening of 0.15 m x 0.30 m and a mesh size of  
80 100 µm. The sampled water volume and surface area were determined by a mechanical  
81 flowmeter (HydroBios Apparatebau GmbH) mounted at the opening of the net (SI Table S1). All  
82 materials collected in the cod end were rinsed into a 1-L bottle (polyvinyl chloride, Kautex  
83 Textron GmbH & Co KG). Sediment samples were taken with a Van-Veen grab deployed at  
84 each station, with the upper 5 cm of the sediment transferred into 1-L Kautex bottles with a metal  
85 spoon. All samples were immediately stored at -20 °C until further processing in the lab.

### 86 **MP extraction from sediment and surface water samples**

87 Multiple steps were taken to remove natural organic and inorganic matter to facilitate effective  
88 MP analysis. Measures for contamination prevention during sample processing are described in  
89 the SI Paragraph S1. Sediment samples were defrosted, transferred into glass jars, and then  
90 homogenized. Triplicates of approximately 5 cm<sup>3</sup> each were taken to determine the dry weight of  
91 each sediment samples. Extraction of MP from the remaining sediment was performed using the  
92 MicroPlastics Sediment Separator (MPSS, HydroBios Apparatebau GmbH)<sup>14</sup>. The general  
93 procedure of density separation followed the methodology of Bergmann et al.<sup>15</sup> and Haave et  
94 al.<sup>16</sup> and is shortly described in the SI Paragraph S2.

95 A size fractionation of each sample was conducted, as sample processing and analytical  
96 approaches varied for different MP size categories. The extracted sediment samples and the  
97 defrosted surface water samples were screened over a 500-µm stainless steel mesh (Haver &  
98 Boecker OHG). The material retained on the mesh was thoroughly rinsed with filtered water

99 (Milli-Q, 0.2  $\mu\text{m}$ ), and Ethanol (30%) also to remove any residual zinc chloride ( $\text{ZnCl}_2$ ) in the  
100 case of the extracted sediment samples. This step divided the sample into two size fractions  
101 potentially containing particles of either  $>500 \mu\text{m}$  and  $<500 \mu\text{m}$  respectively.

102 All steps taken in the laboratory are displayed in a flow scheme (SI Figure S1), and are  
103 explained in the subsequent sections.

#### 104 **MP $>500 \mu\text{m}$**

105 The  $>500 \mu\text{m}$  fraction was rinsed into a beaker and manually sorted in a Bogorov chamber  
106 under a stereo microscope (Olympus SZX16, Olympus) at a 100–320x magnification. All  
107 putative MP particles were transferred into glass petri dishes, photographed under the  
108 microscope (Olympus DP26 Digital Camera, Olympus) and measured (length at their longest  
109 dimension) using image analysis software (cellSens, Olympus).

#### 110 **MP $<500 \mu\text{m}$**

111 The general approach to purify and extract MP from the  $<500 \mu\text{m}$  fraction followed the  
112 enzymatic-oxidative treatment published by Löder et al.<sup>17</sup> which had proven effective for the  
113 degradation of a broad range of environmental matrices. This protocol was performed in newly  
114 developed Microplastic Reactors<sup>18</sup> (SI Figure S2). These semi-enclosed filtration units contain  
115 20- $\mu\text{m}$  stainless steel filters (Haver & Boecker OHG) and allow to add and remove solutions via  
116 vacuum and pressure filtration. Samples could be kept in the reactors and be exposed to the  
117 different chemical and enzyme treatment steps without requiring further sample transfers, by that  
118 reducing the risk of particle loss and sample contamination. The  $<500 \mu\text{m}$  sample fraction was  
119 transferred into the reactors before sodium dodecyl sulfate solution (SDS, 10%, Carl Roth) was  
120 added and the reactors were sealed and incubated at 50 °C for 24 hours. Following this, the  
121 technical enzyme purification steps with protease, cellulase and chitinase (ASA Spezialenzyme  
122 GmbH) as well as hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 35%, Carl Roth) were performed sequentially. The  
123 samples were incubated at optimal pH and temperature conditions for each step. Between

124 treatments the samples were flushed and rinsed several times using Milli-Q. A more detailed  
125 description of the full multi-step procedure can be found in the SI Paragraph S3.

126 About two-thirds of the surface water samples (n=16), containing large amounts of biomass,  
127 were additionally treated with a Proteinase-K step after the second H<sub>2</sub>O<sub>2</sub> treatment. The  
128 procedure was adapted from the protocol of Cole et al.<sup>19</sup>, using H<sub>2</sub>O<sub>2</sub> instead of sodium  
129 perchlorate (NaClO<sub>4</sub>).

130 Inorganic material (e.g. sand, calcium carbonate) was removed by a density separation  
131 performed in separation funnels containing ZnCl<sub>2</sub> (minimum density of 1.7 g cm<sup>-3</sup>). Over a period  
132 of usually one to three days (n=30), but in some cases extended to seven days (n=16), the  
133 denser materials settled to the bottom and were removed.

134 The upper phase of the density separation treatment, containing the lighter material, was passed  
135 through 20-µm stainless steel filters to remove the ZnCl<sub>2</sub>. The retained material was transferred  
136 into 100-mL glass bottles by rinsing the filter with Milli-Q and stored for analysis. For FTIR  
137 measurement the processed sample needed to be transferred onto aluminum oxide filters  
138 (Anodisc, 0.2 µm, Whatman GmbH)<sup>20</sup>. Each sample was concentrated onto a 13 mm diameter  
139 filter area, using in-house fabricated glass filter funnels and a vacuum filtration unit. Prior to this  
140 a FlowCam (Fluid Imaging Technologies) was used to determine a suitable subsample volume  
141 that could be applied onto the filter area without overloading it (SI Paragraph S4)<sup>15</sup>. Based on the  
142 FlowCam assessments, aliquots ranging from 1.2–73.2% (surface water samples) and 10.8–  
143 75.4% (sediment samples) of the total sample volumes were used on each aluminum oxide filter  
144 (SI Table S2). The loaded filters were transferred into covered glass petri dishes and dried for 48  
145 hours at 30 °C.

#### 146 **Identification and quantification of MP >500 µm using ATR-FTIR**

147 All putative plastic particles were identified individually using an ATR-FTIR unit (Bruker Optik  
148 GmbH) with the exception of 30 particles which were analyzed within an accompanying study by  
149 Cabernard et al.<sup>21</sup> using Raman spectroscopy. The IR spectra were collected in the spectral

150 range of 400–4000  $\text{cm}^{-1}$  and compared against our reference library<sup>22</sup>. Particles with a match of  
151 at least 700 (out of 1000) were counted as safely identified. If the match ranged between 600  
152 and 700 the spectra were manually compared to database spectra and evaluated based on  
153 expert knowledge, as suggested by other studies<sup>11, 23, 24</sup>.

#### 154 **Spectral analysis of MP <500 $\mu\text{m}$ using FTIR imaging**

155 Particles of the smaller size fraction were analyzed using a FTIR-microscope (Hyperion 3000  
156 coupled to a Tensor 27 spectrometer, Bruker Optik GmbH) equipped with a 15x objective and a  
157 focal plane array (FPA) detector. Settings for the measurements were similar to previous  
158 studies<sup>15, 20, 22, 25-28</sup> defining the lower detection limit of 11  $\mu\text{m}$ . By measuring a grid of 77 x 77  
159 (surface waters) or 78 x 78 (sediments) FPA fields, corresponding to a filter area of 184  $\text{mm}^2$   
160 and 188  $\text{mm}^2$ , respectively, every particle was analyzed.

161 The FTIR imaging data were automatically analyzed following Primpke et al.<sup>28</sup> with the adaptable  
162 database design<sup>22</sup>. Based on the identified spectra a subsequent image analysis provided  
163 particle numbers, polymer types and size classes of all identified particles. The threshold for this  
164 image analysis was set based on a manual spectra evaluation of a data-subset (SI Paragraph  
165 S5 and Table S3).

#### 166 **Data handling and statistical analysis**

167 The MP counts of each analyzed filter were extrapolated to the respective sample based on the  
168 analyzed proportion (SI Table S2) and corrected for contamination recorded in procedural blank  
169 samples ( $n=6$ ). The total amount of MP in both size fractions was combined and presented as  
170 MP per kg dry weight sediment (particles  $\text{kg}^{-1}$  (DW)) or water volume sampled (particles  $\text{m}^{-3}$ ).

171 Percentages of polymer types and size classes were arcsine square root (univariate statistics) or  
172 square root transformed (multivariate statistics)<sup>29</sup>. The multivariate analyses were carried out  
173 based on Hellinger distance transformation, a recommended measure for ordination and  
174 clustering of (polymer) species abundance data, which does not put high weights on rare  
175 (polymer) species<sup>30, 31</sup>. To assess the polymer diversity, species richness and Shannon-Wiener-

176 index  $H'$  (log base e) was calculated. To test if samples from the two compartments (sediment,  
177 surface water) differed significantly in their polymer composition or size class distribution,  
178 permutational multivariate analysis (PERMANOVA)<sup>32</sup> with 999 permutations at a significance  
179 level of  $p < 0.001$  was applied. To visualize these differences cluster analysis and canonical  
180 analysis on the principal coordinates (CAP)<sup>33</sup> were performed with PRIMER plus the add-on  
181 PERMANOVA+ (PRIMER-E version 7.013)<sup>34</sup>.

182 Analyses of variances (ANOVA) was performed with Statistica 13 (Statsoft) to show which  
183 polymer types or size classes had the greatest influence on observed differences. To identify  
184 which stations of both compartments individually group in terms of polymer composition and size  
185 distribution a non-hierarchical clustering based on k-means and coupled to similarity profile test  
186 (SIMPROF, henceforth referred to as kR-clustering) was performed using PRIMER-7 on the  
187 basis of the Hellinger distance matrix with square root transformed data. The significance level  
188 for SIMPROF was set to 5% and performed with 999 permutations to define the optimal number  
189 of k-groups (between 2 and 10) to describe the clustering of the samples, which is based on  
190 maximizing  $R$ <sup>34</sup>. An ANOVA followed by a Tukey HSD test was used to test the influence of each  
191 polymer type on the respective group.

192 Maps showing the geographical position of the samples along with the MP concentration,  
193 polymer composition and diversity as well as assigned groups were prepared using QGIS  
194 (version 3.2 'Bonn'). The displayed shoreline data was taken from the Global Self-consistent,  
195 Hierarchical, High-resolution Geography Database (GSHHS)  
196 <https://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html>, hosted by the National Oceanic And  
197 Atmospheric Administration (NOAA) by Wessel and Smith<sup>35</sup>.

## 198 **RESULTS**

### 199 **Comparison of two size fractions of MP in sediment and surface water samples**

200 Microplastics from 23 sediment and 23 surface water samples, out of 24 sampled stations, could  
201 successfully be extracted and analyzed. For each sample two size fractions (MP >500  $\mu\text{m}$  and  
202 MP <500  $\mu\text{m}$ ) were analyzed separately and their results later combined for statistical analyses.

203 The two size fractions differed considerably concerning polymer composition and size  
204 distribution. Overall, only one polymer type (polyester) of MP >500  $\mu\text{m}$  was identified in sediment  
205 samples and eight different polymer types of MP >500  $\mu\text{m}$  were found in surface water samples.  
206 Concerning MP <500  $\mu\text{m}$ , 21 different polymer types were found in the sediment samples and  
207 19 different polymer types in surface water samples.

208 In sediment samples only 0.04% of all detected particles were >500  $\mu\text{m}$  while 99.96% were MP  
209 <500  $\mu\text{m}$ . For surface water samples 6.02% of all the detected particles were >500  $\mu\text{m}$  while  
210 93.98% accounted for MP <500  $\mu\text{m}$ . A selection of MP >500  $\mu\text{m}$  detected in five surface water  
211 samples can be found in the SI Figure S3. Two examples of filters with MP <500  $\mu\text{m}$  and their  
212 respective false color plot of one station for sediment and surface water each are shown in the  
213 SI Figure S4.

### 214 **Comparison of MP occurrence in sediments and surface waters of the southern North** 215 **Sea**

216 Combining both size fractions, MP concentrations in sediments ranged from 2.8 (station 11) to  
217 1188.8 particles  $\text{kg}^{-1}$  (DW) (station 23) and for surface waters from 0.1 (station 22) to  
218 245.4 particles  $\text{m}^{-3}$  (station 20) (SI Table S4, Figure 1).

219 The two investigated compartments, sediment and surface water, differed greatly regarding MP-  
220 concentration (Figure 1) and polymer composition (PERMANOVA,  $p$ -value=0.001, SI Table S5).  
221 This was also confirmed in the cluster analysis where samples from the same environmental  
222 compartment were grouped together (SI Figure S5). The separation of most of the samples into  
223 the two a priori defined groups, sediment and surface water, was also supported by CAP,

224 depicted in SI Figure S6, with a reasonably high correlation value of 0.89 and revealed six  
225 samples to be mismatched with a mis-classification error of 13.04%. Sediment samples from  
226 three stations (11, 18, and 21) were falsely allocated to surface waters and three surface water  
227 samples (stations 6, 22, and 23) were allocated to sediments. ANOVA was significant when  
228 comparing polymer richness and Shannon-Wiener index  $H'$  based on polymer types for both  
229 compartments ( $p$ -value=0.001) (SI Table S6). The spatial distribution of this polymer diversity is  
230 depicted in SI Figure S7. Figure 2 shows the relative polymer composition for sediments (Figure  
231 2a) and surface waters (Figure 2b). The number of different polymer types in sediment samples  
232 was on average 11 and ranged between 3 (station 11) and 15 (station 1) with Shannon-Wiener  
233 index  $H'$  ranging between 0.89 (station 11) and 2.29 (station 5). For surface water samples on  
234 average 7 different polymer types were detected ranging from 1 (station 15) to 14 (stations 2, 6  
235 and 17) with Shannon-Wiener index  $H'$  ranging from 0 (station 15) to 2.07 (station 17).

236 Polyethylene (PE), polypropylene (PP), and polyester/polyethylene terephthalate (PEST) were  
237 present in more than 75% of all samples from both compartments. In sediment samples  
238 acrylates/polyurethane/varnish (acrylates/PUR/varnish) were omnipresent. Additionally to the  
239 aforementioned polymers, PE chlorinated, polystyrene (PS), polyamide (PA), polyvinyl chloride  
240 (PVC), and rubber type 3 were frequent. Figure 3 shows that in sediment samples 13 different  
241 polymer types contributed on average between 1.3% (nitrile rubber) and 21.0%  
242 (acrylates/PUR/varnish) to the polymer composition while eight polymer types contributed less  
243 than 1% (in descending order): ethylene vinyl acetate (EVA), polycaprolactone, rubber type 1,  
244 PE oxidized, polyisoprene chlorinated, acrylonitrile butadiene, polycarbonate (PC), and  
245 polysulfone (PSU). In surface water samples nine different polymer types contributed on  
246 average between 1.3% (PS) and 26.7% (PP) to the polymer composition while ten polymer  
247 types contributed less than 1% (in descending order): cellulose chemically modified (CA),  
248 polycaprolactone, EVA, rubber type 1, polyoxymethylene (POM), PE oxidized, PC, nitrile rubber,  
249 rubber type 2, and polychloroprene (Figure 3). Furthermore, an ANOVA revealed the polymer

250 types contributing most to the dissimilarity of surface water and sediment samples: PE (ANOVA,  
251  $p$ -value=0.010), PP (ANOVA,  $p$ -value=0.042), PVC (ANOVA,  $p$ -value=0.007), nitrile rubber  
252 (ANOVA,  $p$ -value=0.001), polychloroprene (ANOVA,  $p$ -value=0.000), and POM (ANOVA,  $p$ -  
253 value=0.000) (SI Table S7).

254 When comparing the size distribution (11–5000  $\mu$ m) of polymers in sediment and surface water  
255 samples, they differed significantly (PERMANOVA,  $p$ -value=0.001) (SI Table S8). Cluster  
256 analysis revealed a higher variance in surface water than in sediment samples (SI Figure S8).  
257 This was highlighted by CAP which had a moderately large correlation value of 0.61 and  
258 revealed only surface water samples ( $n=8$ ) to be mis-classified with a relatively low mis-  
259 classification error of 17.39% (SI Figure S9). Furthermore, in both compartments the size  
260 distribution of MP is clearly skewed towards the smallest size classes (Figure 4). The ANOVA for  
261 the individual size classes revealed that the average abundance of polymers in the size classes  
262 25–50  $\mu$ m ( $p$ -value=0.008) and 50–75  $\mu$ m ( $p$ -value=0.007) was significantly higher for sediment  
263 than surface water samples. This was the opposite for size classes 500–1000  $\mu$ m ( $p$ -  
264 value=0.031), 2000–2500  $\mu$ m ( $p$ -value=0.038), and 2500–3000  $\mu$ m ( $p$ -value=0.004) (SI Table  
265 S9).

### 266 **Spatial distribution of MP occurrence in the southern North Sea**

267 After confirming significant differences between the sediment and surface water compartments,  
268 intra-compartmental variations between sampling stations were investigated.

269 A kR-clustering with SIMPROF was applied to highlight stations that were more similar  
270 concerning their polymer composition. Figure 5 shows the spatial distribution of these identified  
271 kR groups. Five groups (A–E) were found to best represent the clustering of sediment samples  
272 ( $R=0.921$ ) (SI Figure S10) with most samples belonging to group D ( $n=12$ ) (Figure 5a).  
273 Furthermore, the result of CAP reinforces the significant differences between the samples of the  
274 five groups with high correlation values (0.931–0.996) with only two samples being mis-classified  
275 (SI Figure S11). One sample from group C was mis-classified as belonging to group B (station

276 11) and one sample from group B was mis-classified as being part of group E (station 15) with a  
277 low mis-classification error of 8.70%. To discover which polymer types drive this clustering in  
278 sediment samples an ANOVA with a subsequent Tukey HSD was performed. This analysis  
279 revealed six polymer types that contributed significantly to the dissimilarity between the grouped  
280 stations (SI Table S10), which are PE chlorinated, PA, CA, acrylates/PUR/varnish,  
281 polychloroprene and rubber type 3. Polychloroprene dominated samples belonging to group D  
282 (n=12, stations 1–3, 5–10, and 22–24). Polyamide was preeminent for group E (n=5, stations 4,  
283 12, and 19–21). Sediment samples from the Rhine-Meuse-delta (station 14, 15) belonged to  
284 group B (n=2) and were dominated by rubber type 3. Group C (n=2) was characterized by a low  
285 polymer diversity (station 11, 16) and the prevalence of acrylates/PUR/varnish and CA was  
286 distinct for group A (n=2, station 13, 18).

287 For surface water samples kR-clustering revealed four groups (A–D) that represented the best  
288 possible clustering (R=0.868), where most samples are clustering closely and belong to group C  
289 (n=13) (Figure 5b, SI Figure S12). The clustering was confirmed by CAP with relatively high  
290 correlation values (0.811–0.970) (Figure S13). Only two samples (stations 15 and 18) were mis-  
291 classified to group C instead of group D resulting in an overall low mis-classification error of  
292 8.7%. Six polymers, (PE, PE chlorinated, PVC, PEST, acrylates/PUR/varnish and  
293 rubber type 3), were revealed by ANOVA, with a subsequent Tukey HSD, to show significant  
294 differences between the four identified groups (SI Table S11). The presence of rubber type 3  
295 characterized group C (n=13, station 1–2, 4–8, 11, 17, 20–21, and 23–24). Group B had  
296 significantly relatively more abundant ( $p$ -value=0.000) PEST (station 4, 13, 16, and 22). The  
297 absence of PE, PVC, and rubber type 3, as well as the relatively high presence of PE  
298 chlorinated, characterized group A (n=3, station 9, 10, and 19). Group D (n=3) was defined by  
299 an absence of PE chlorinated, PEST, and rubber type 3, as well as a relatively high presence of  
300 PVC and acrylates/PUR/varnish (station 14, 15, and 18).

## 301 Discussion

302 Through the integration of effective sample preparation together with state-of-the-art FTIR  
303 imaging and an automated analysis technique, MP could be detected in all analyzed sediment  
304 and surface water samples in the southern North Sea. The investigated compartments differed  
305 significantly in their polymer composition and particle size distribution. Average MP  
306 concentrations were considerably higher in sediments ( $234.5 \pm 254.3$  particles  $\text{kg}^{-1}$  (DW)) than in  
307 surface waters ( $27.2 \pm 52.5$  particles  $\text{m}^{-3}$ ). However, a direct comparison is difficult because of  
308 different units of reference. When comparing the abundance of MP in a certain surface area the  
309 difference is even more striking with numbers ranging from 9.5 to 4041.9 particles  $\text{m}^{-2}$  in  
310 sediments and 0.01 to 24.5 particles  $\text{m}^{-2}$  in surface waters (SI Table S4). Furthermore, both  
311 compartments were clearly dominated by MP  $< 500 \mu\text{m}$ . Although the numbers of MP  $> 500 \mu\text{m}$  in  
312 surface water samples were noticeable, they had hardly any influence on the sample  
313 comparison between stations.

314 Along the West and East Frisian Islands (station 1–9) MP concentrations were of average extent  
315 in surface waters ( $3.5$ – $58.6$  particles  $\text{m}^{-3}$ ) as well as sediments ( $38.7$ – $318.4$  particles  $\text{kg}^{-1}$  (DW)).  
316 Furthermore, sediment samples from this region showed high polymer diversities and similar  
317 polymer compositions, and were therefore grouped together by cluster analysis. Both  
318 compartments in this region showed the lowest MP concentration at station 9, situated north of  
319 Texel, the most western Frisian Island, and the highest concentration at station 6, north of  
320 Ameland. It is very striking, if expected, that samples from the mouth of the Scheldt and Rhine-  
321 Meuse-delta (station 14 and 15) differed greatly from all the others concerning their polymer  
322 composition, with surface water samples being dominated by acrylates/PUR/varnish and  
323 sediments additionally by rubber type 3, both indicating the influence of high marine traffic. In the  
324 English Channel region (station 16–19) polymer diversity and composition showed high  
325 variability, which was also true for the west coast of the Netherlands (station 10–13).

326 There is no clear gradient visible for surface waters, but those in the proximity to the English  
327 Channel, particularly station 20, which are influenced by riverine input from the Thames and the  
328 Rhine as well as channel water, exhibited the highest MP concentration (245.4 particles  $m^{-3}$ ) with  
329 a decrease in the northern direction. These results agree with results from two studies where  
330 frequency of MP in fish and fulmars were highest in the Channel area<sup>36, 37</sup>. For sediments MP  
331 concentration was highest at station 23 (1188.8 particles  $kg^{-1}$  (DW)), situated at the Dogger  
332 Bank and characterized by fine sediments. Further spatial patterns could be revealed by  
333 applying multivariate statistics, which has been attempted in previous studies as well<sup>38, 39</sup>.  
334 Hajbane and Pattiaratchi<sup>38</sup> used multi-dimensional scaling (nMDS) to describe the differences in  
335 MP concentration and size classes between three distinct stations over time. Suaria et al.<sup>39</sup> used  
336 principal component analysis (PCA) to compare a large number of surface water samples ( $n=74$ )  
337 regarding the relative frequencies of the seven most common polymer types. We used kR-  
338 clustering to reveal a spatial pattern regarding polymer composition followed by ANOVA to  
339 uncover the polymer types which drive this structure. The differences in polymer composition  
340 show a clear pattern with an imaginary line that can be drawn along  $53^{\circ}$  N. Stations below  $53^{\circ}$  N  
341 are more diverse in their polymer composition, which might be related to the influence coming  
342 from the English Channel as well as the high riverine input by the Thames, the Scheldt, the  
343 Rhine and the Meuse. On the contrary, stations located above  $53^{\circ}$  N exhibit a more similar  
344 polymer composition. ANOVA revealed that the sediment samples of these stations were  
345 characterized by the presence of polychloroprene and surface water samples by rubber type 3,  
346 represented by ethylene-propylene-diene monomer rubber (EPDM).

#### 347 **Interstudy comparison**

348 In sediments sampled along the Belgian coast, Claessens et al.<sup>40</sup> detected MP concentrations of  
349 up to 269 particles  $kg^{-1}$  (DW) on the shelf sea samples and 390 particles  $kg^{-1}$  (DW) in harbor  
350 areas, both in the size range of 38–1000  $\mu m$ . They extracted MP by applying a density  
351 separation approach with sodium chloride (NaCl) and MP were visually preselected and

352 identified using FTIR spectroscopy. In sediments of the Dutch North Sea coast Leslie et al.<sup>41</sup>  
353 detected between 100–3600 particles kg<sup>-1</sup> (DW). In their extensive study, they analyzed several  
354 compartments, among others sublittoral sediments close to the stations of this study. They  
355 analyzed only small aliquots of 20 g each which infers a large degree of extrapolation when  
356 referring to particles per kg and used NaCl for density separation which might have resulted in  
357 an underestimation of some denser polymer types. Furthermore, they mostly detected fibers of  
358 which only a small proportion (6%) was analyzed spectroscopically. Regardless of these  
359 potential issues, concentrations were of the same order of magnitude as found in this study.  
360 Another study by Maes et al.<sup>42</sup> reported similar numbers for MP in sediments in the same area  
361 (0–3146 particles kg<sup>-1</sup> (DW)). It is noteworthy to highlight that no MP fragments were counted in  
362 this study but mostly spheres and fibers contrary to our study. Maes et al.<sup>42</sup> also used small  
363 aliquots of 25 g for density separation with NaCl and identified plastics based on visual criteria  
364 only. When comparing results for surface water samples from the same study, MP  
365 concentrations were considerably higher in our study (0–1.5 vs. 0.1–245.4 particles m<sup>-3</sup>). The  
366 most considerable differences between the two studies were in the mesh size of the sample net  
367 (333 µm vs. 100 µm) and the method for identification of MP (visual vs. ATR-FTIR and FTIR  
368 imaging). Relatively small aliquots of the samples were processed in these studies, especially  
369 regarding sediment samples. Our protocol enabled us to process the whole sample consisting of  
370 1309–1770 g dry sediment and 15500–51300 L surface water concentrated by the net,  
371 respectively. It was however inevitable that we would only be able to analyze aliquots of the  
372 processed samples on the measuring filters, for practical reasons, i.e. to improve spectra quality  
373 and reduce time demand for analysis. However, for sediments the aliquots comprised on  
374 average 48.8% of the samples, being equivalent to a range of 141 to 1253 g sediment (DW),  
375 and for surface water samples the aliquots comprised on average 15.6% of the sample resulting  
376 in an equivalent volume of sea water ranging from 190 to 18236 L. To overcome the need for  
377 any extrapolation, multiple filters per sample could have been analyzed, but this would have

378 increased the time demand by at least two days per filter. With new advances in reducing the  
379 analysis time, more filters could be analyzed more rapidly than has been possible until now.  
380 These developments in methodology are discussed further in the SI Paragraph S6.

381 We acknowledge that the lower limit of the filtration during sample processing was theoretically  
382 20  $\mu\text{m}$ . However, this limit can be considered lower when filters become clogged. For this  
383 reason, the number of particles between 11–20  $\mu\text{m}$  should be considered as semi-quantitative,  
384 and therefore the total detected number is most likely an underestimation. Same applies to some  
385 extent also to the fraction 10-100  $\mu\text{m}$  of the surface water samples due to the mesh size of the  
386 net. However, this emphasizes even more that very small MP are numerous and should be  
387 included in further research.

388 As in agreement with other studies<sup>8, 43, 44</sup>, difficulties in interstudy comparisons due to variable  
389 sampling, sample preparation and analytical methods are well illustrated. Concerning possible  
390 analytical methods, the range includes simple visual identification only<sup>42</sup>, visual presorting  
391 followed by chemical identification of (a subset of) putative plastics<sup>41, 45, 46</sup>, spectroscopic imaging  
392 of whole sample filters using FTIR imaging<sup>15, 25-27, 47, 48</sup>,  $\mu\text{Raman}$  spectroscopy<sup>21</sup>, and  
393 thermoanalytical methods like Pyrolysis gas chromatography coupled to mass spectrometry (Py-  
394 GC-MS)<sup>49</sup> or thermal extraction-desorption (TED) GC-MS<sup>50, 51</sup>.

395 It has been shown that visual identification alone is insufficient<sup>11, 52</sup> and the same can be said for  
396 methods relying on a visual preselection stage from which only a subset are verified by  
397 spectroscopic or thermoanalytical methods. When aiming for particle related data, the  
398 complementary spectroscopic methods of Raman microscopy and FTIR imaging are the most  
399 commonly used<sup>23, 53, 54</sup>. Generally, there is a requirement within the research field to establish the  
400 usage of polymer characterization methods that omit the need for pre-sorting. If a pre-selection  
401 is unavoidable, it should follow certain criteria<sup>8, 11, 55</sup> or be aided by dyeing with Nile red<sup>56, 57</sup>.

## 402 **Implications**

403 Beyond the harmonization of methods for reliable comparisons of MP data, another important  
404 issue in the field of marine environmental MP research is the need for clear identification of  
405 pathways, especially entry pathways. Previous research has shown that rivers are to be  
406 considered as one of the major sources<sup>58, 59</sup>. Studies focusing on rivers entering into the North  
407 Sea report relatively high numbers of MP<sup>41, 60, 61</sup>. Noteworthy, it has been shown in a recent  
408 study by Hurley et al.<sup>62</sup> that MP from the river beds (approx. 70%) can be resuspended, flushed  
409 by flooding events and hence be introduced into the oceans. This implies that MP from riverine  
410 surface waters alongside those locked in bed sediments should both be considered as having  
411 the potential of entering the North Sea. In this regard, it is even more notable, considering that  
412 most of the polymer types reported in these riverine studies would be buoyant in seawater  
413 ( $\rho \leq 1.025 \text{ g cm}^{-3}$ ), that the concentrations detected in North Sea surface waters are surprisingly  
414 low. One explanation might be the very conservative approach of our study. The other, more  
415 significant, factor might be that the hydrodynamics of the North Sea have a generally anti-  
416 clockwise circulation in the center and a northeasterly export along the coast<sup>63, 64</sup>. This makes it  
417 less likely for MP to accumulate in North Sea surface waters. Another aspect to acknowledge  
418 when considering distribution pathways is the vertical transport of MP. Möhlenkamp et al.<sup>65</sup>  
419 showed that buoyant MP that become incorporated into aggregates can sink and eventually  
420 settle into the benthic boundary layer, providing an explanation to the presence of positively  
421 buoyant plastics in sublittoral sediments. This evidence was backed by another study by Porter  
422 et al.<sup>66</sup> in which they showed the incorporation of different plastic types (PE, PP, PA, PS, and  
423 PVC), of different shapes (fibers, spheres, and fragments), and sizes (7–3000  $\mu\text{m}$ ) into marine  
424 snow. The incorporation of normally buoyant polymers in organic-rich aggregates increased the  
425 sinking velocity of all tested polymer types<sup>66</sup>. The authors of both studies recorded that in case of  
426 a polymer buoyant in seawater, such as PE, as well as with a mixture of plastic microbeads  
427 (extracted from facial cleansers), sinking velocities increased to up to 818 and 831  $\text{m d}^{-1}$

428 respectively<sup>65, 66</sup>. This exceeds general sinking velocities of phytoplankton aggregates  
429 ( $53 \pm 22 \text{ m d}^{-1}$ )<sup>65</sup> and fecal pellets, which range, depending on the composition of the  
430 phytoplankton bloom, between 70–100  $\text{m d}^{-1}$ <sup>67</sup>.

431 A final consideration on the topic of vertical transport would be that the bioavailability of MP to  
432 filter and suspension feeders has been shown to be increased by their incorporation in  
433 aggregates<sup>66, 68</sup>. Recently, Katija et al.<sup>69</sup> showed that MP are captured by larvaceans and that  
434 these can be contained in their fecal pellets or discarded houses, and may thus sink to the  
435 seafloor as part of these structures where they are available for benthic organisms.

436 Robust monitoring protocols are needed which should not only focus on large MP ( $>500 \mu\text{m}$ ). In  
437 this study we demonstrated that it is not possible to extrapolate conclusions from analyzing MP  
438  $>500 \mu\text{m}$  to ascertain data on MP  $<500 \mu\text{m}$ . For the development of risk management and  
439 assessment protocols, and to monitor trends in changes of polymer concentrations and  
440 composition, it is of importance that the focus should shift to be on the small or even very small  
441 size fraction ( $<100 \mu\text{m}$ ). In agreement with previous studies<sup>41, 42</sup> we found North Sea sediments  
442 to be considerably more contaminated than surface waters and that these may act as potential  
443 sinks. This clearly supports the statement of Leslie et al.<sup>41</sup> to track changes in MP pollution in  
444 sediments in future monitoring approaches.

445 **Supporting Information.** Paragraphs, figures and tables describing the sampling, sample  
446 processing and analysis in greater detail as well as presenting result for the statistical analysis

447

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455

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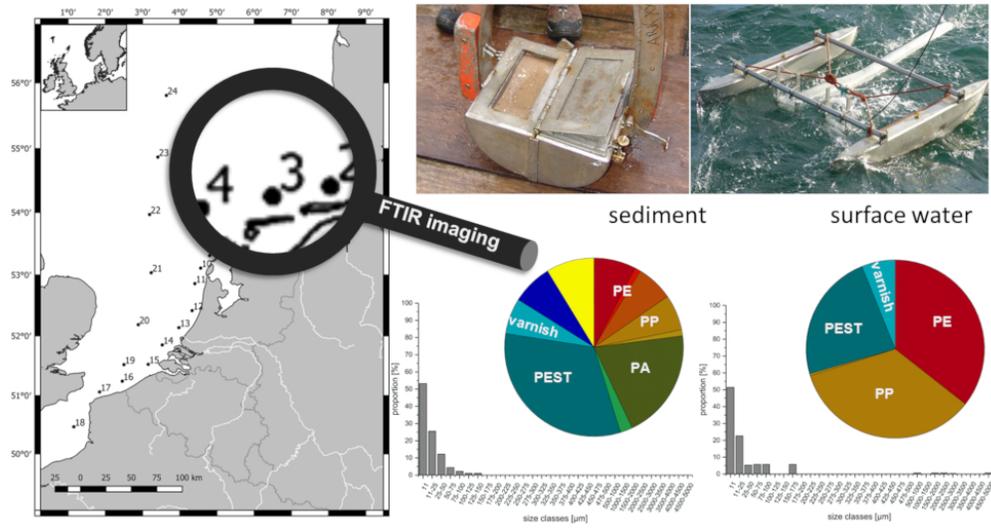
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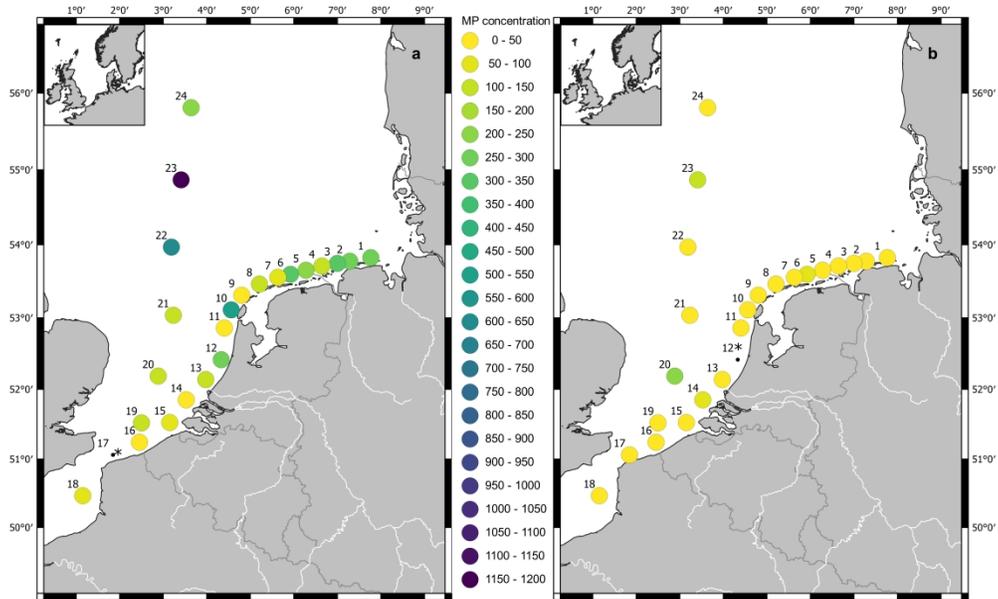


Figure 1. Geographic distribution of microplastic concentration in the southern North Sea. Microplastic concentration for sediment samples (a) reported in particles per kg and surface water samples (b) reported in particles per m<sup>3</sup>. Stations where no sample was analyzed are marked \*.

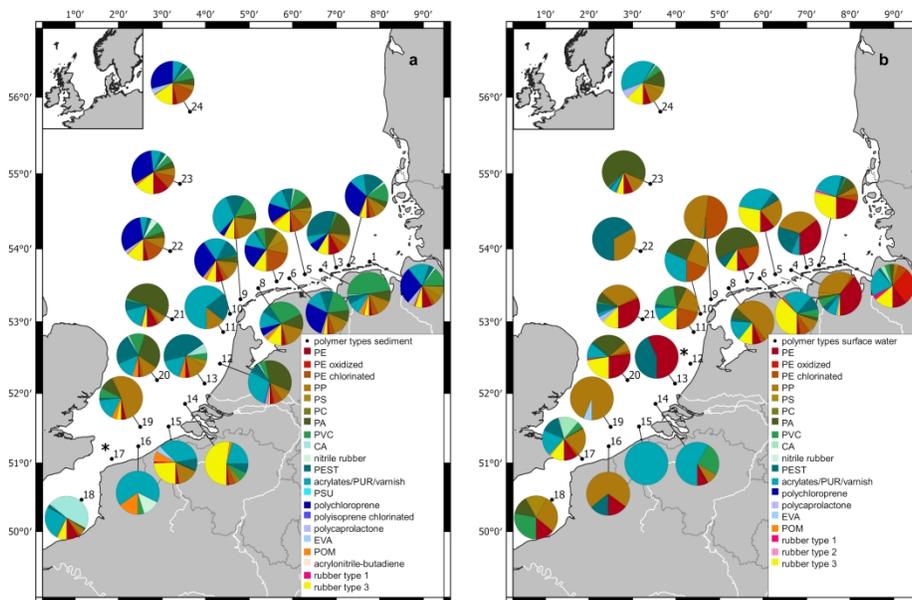


Figure 2. Spatial distribution of the relative polymer composition for sediment (a) and surface water samples (b). PE: polyethylene, PP: polypropylene, PS: polystyrene, PC: polycarbonate, PA: polyamide, PVC: polyvinyl chloride, CA: cellulose chemically modified, PEST: polyester/polyethylene terephthalate, PUR: polyurethane, PSU: polysulfone, EVA: ethylene vinyl acetate, POM: polyoxymethylene. Stations where no sample was analyzed are marked with \*.

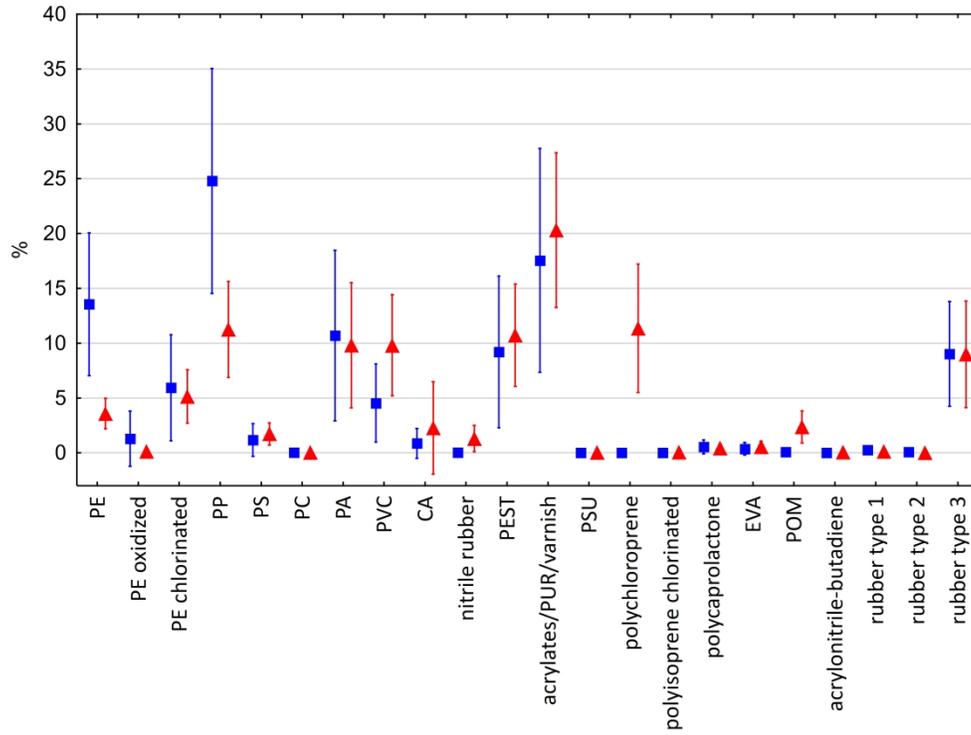


Figure 3. Mean percentage of each polymer type for sediment (red triangle) and surface water samples (blue square). PE: polyethylene, PP: polypropylene, PS: polystyrene, PC: polycarbonate, PA: polyamide, PVC: polyvinyl chloride, CA: cellulose chemically modified, PEST: polyester/polyethylene terephthalate, PUR: polyurethane, PSU: polysulfone, EVA: ethylene vinyl acetate, POM: polyoxymethylene. Whiskers show the 95% confidence interval.

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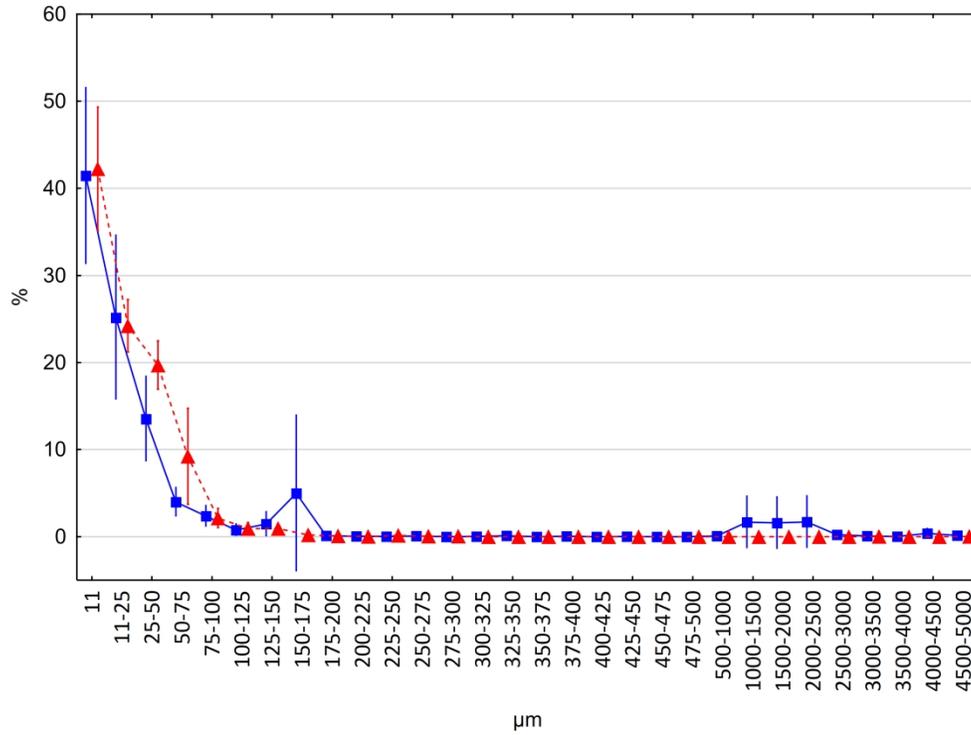


Figure 4. Mean percentage of each size class in µm for sediment (red triangle) and surface water samples (blue square). Whiskers show the 95% confidence interval.

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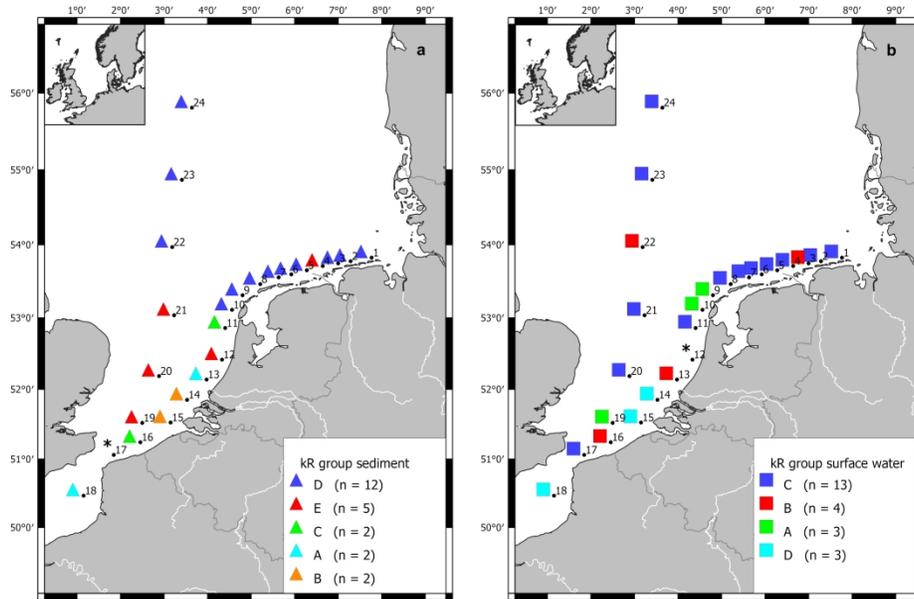


Figure 5. Map of the southern North Sea with the 24 stations of sediment (a) and surface water sampling (b) and their assigned groups (different colors) respectively based on kR-clustering. Stations where no sample was analyzed are marked with \*.