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**MICROBIAL COLONIZATION OF DIFFERENT MICROPLASTIC TYPES AND  
BIOTRANSFORMATION OF SORBED PCBs BY A MARINE ANAEROBIC BACTERIAL  
COMMUNITY**

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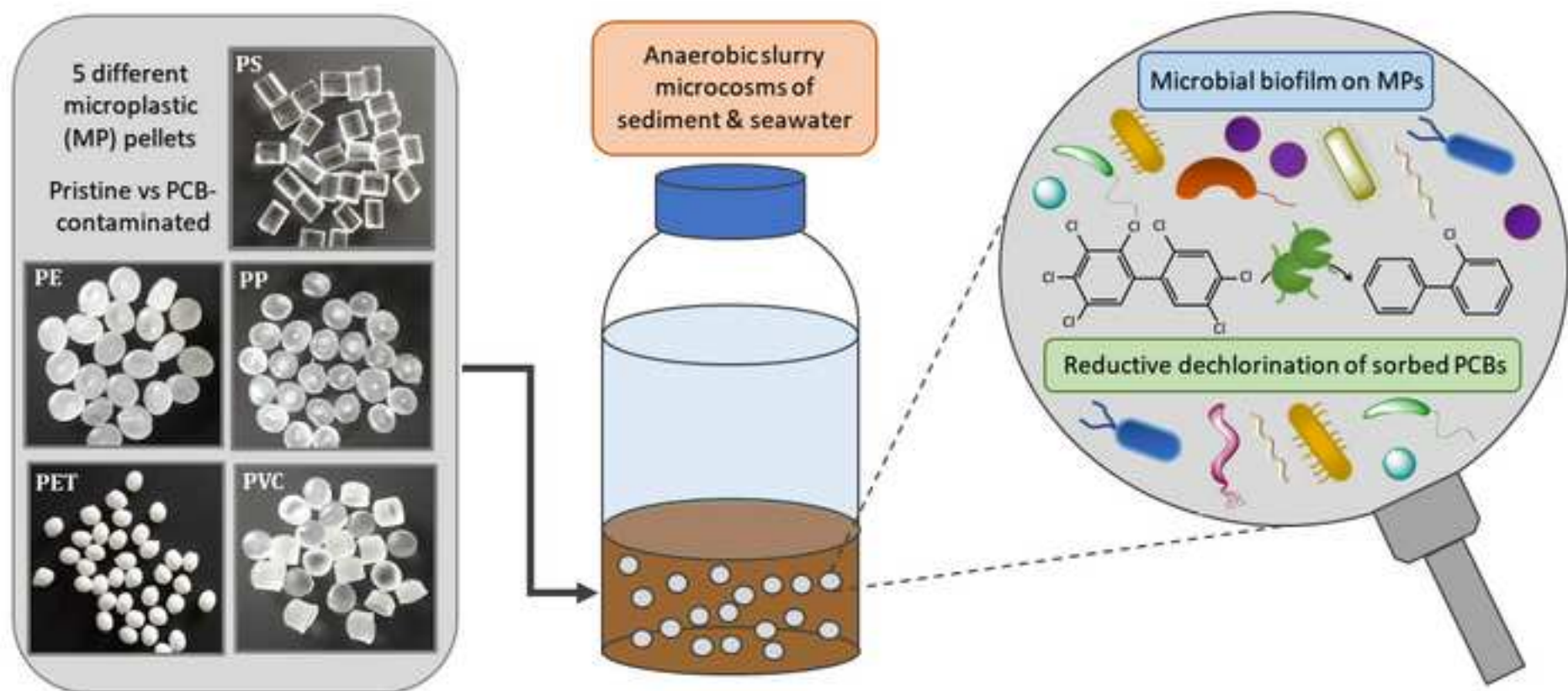
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**Keywords**

Microplastics; biofilm; microbial community; marine sediment; polychlorinated biphenyls;  
reductive dehalogenation



## **Highlights**

- Marine bacterial biofilm composition changes on different MP types.
- MP-associated bacterial communities differ from the surrounding sediment ones.
- MP-sorbed PCBs do not significantly affect the MP-biofilm composition.
- PCBs sorbed on MPs can be rapidly dehalogenated by anaerobic marine biofilms.

# Abstract

We investigated the colonization dynamics of different microplastic (MP) pellets, namely, polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP) and polyvinyl chloride (PVC), either pristine or contaminated with polychlorinated biphenyls (PCBs), by an organohalide respiring marine microbial community and its biotransformation activity towards PCBs sorbed on MPs, in anaerobic laboratory microcosms of a marine sediment.

All MPs were rapidly colonized by the microbial community within 2 weeks of incubation, when approximately  $10^{10}$  16S rRNA gene copies  $\text{cm}^{-2}$  were detected on PVC,  $10^9$  copies  $\text{cm}^{-2}$  on PE, and  $10^8$  copies  $\text{cm}^{-2}$  on PET, PP and PS. A greater biofilm growth on PVC pellets than other MPs was confirmed by quantification of the reducing sugars of the EPS and biofilm staining with crystal violet. Illumina sequencing of the 16S rRNA genes and Principal Coordinate Analysis (PCoA) revealed that the biofilm community on MPs significantly differed from the sediment community, being enriched of chemoorganotrophic fermenting species, and was significantly affected by the type of polymer. The presence of sorbed PCBs did not significantly affect the overall community composition, and mainly resulted in the enrichment of Dehalococcoidia, i.e., of the organohalide respiring members of the community.

Reductive dechlorination of PCBs sorbed to MPs was observed after two weeks of incubation, when the average number of chlorines per biphenyl molecule was reduced from 5.2 to 4.8 - 4.3, and was faster ( $35.2 \pm 1.9$  to  $61.2 \pm 5.8$   $\mu\text{moles of Cl removed kg}_{\text{MP}}^{-1} \text{ week}^{-1}$ ) than that of sediment-sorbed ones ( $33.9 \pm 9.1$   $\mu\text{moles of Cl removed kg}_{\text{sediment}}^{-1} \text{ week}^{-1}$ ), which started only after 10 weeks of incubation. These data suggest that microbial colonization of contaminated MPs might change the composition of sorbed PCB mixtures and therefore the toxicity associated to PCB-polluted MPs.

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28

## 29    **1    Introduction**

30    Plastics use and production has enormously increased since 1950, reaching around 335  
31    million tonnes in 2016, with 60 million tonnes generated in Europe alone (PlasticsEurope,  
32    2018). The most common polymers are high-density and low-density polyethylene (PE),  
33    polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP) and polyvinyl  
34    chloride (PVC), which together represent approximately 90% of the global plastic production  
35    (Andrady and Neal, 2009). A huge amount of plastic materials ends up in the marine  
36    environment, becoming an ever-increasing problem due to the toxicity, persistence and  
37    universal presence of such debris. The estimation of plastic released in the oceans varies from  
38    4.8 to 12.7 million tons annually (Haward, 2018), with size ranging from meters to  
39    micrometers (Ryan et al., 2009). The term microplastics (MPs) was introduced within the last  
40    decade to describe small particles of plastic, commonly defined as < 5mm in diameter (Frias  
41    and Nash, 2019). Once discharged into the marine environment, plastic litter undergoes  
42    different processes, such as weathering, fragmentation and fouling (Cole et al., 2011). In  
43    particular, biomass accumulation on MPs due to bio(fouling) can lead to an increase of their  
44    density and thus their sinking (Morét-Ferguson et al., 2010; Chubarenko et al, 2016; Miao et  
45    al., 2019a). The anaerobic sediment has been indicated as a possible long-term sink for MPs  
46    (Andrady, 2011; Van Cauwenberghe et al., 2015). Microplastic contamination in marine  
47    sediments has been reported by several authors (Thompson et al., 2004; McDermid and  
48    McMullen, 2004; Claessens et al., 2011), with some of the highest concentrations of MPs found  
49    in sediments located in Nova Scotia and Arctic Ocean (up to 8000 and 6595    MPs/kg of  
50    sediment, respectively) (Mathalon and Hill, 2014; Bergmann et al., 2017).

51    The first evidence of microbial colonization of plastic fragments dates back to the early 70s  
52    (Carpenter et al., 1972). Recent studies reported that MPs are readily colonized by  
53    environmental microbial communities in few hours or days of incubation in seawater

(Ogonowski et al., 2018) and coastal marine sediments (Harrison et al., 2014). The bacterial assemblages colonizing MPs in freshwater and marine environments have been reported to significantly differ in taxonomic composition and structure from those present in the surrounding water and/or sediment (Miao et al., 2019b; Frère et al., 2018; Ogonowski et al., 2018; Rummel et al., 2017; De Tender et al., 2015). Microplastic-associated microbial communities were also reported to have a lower alpha diversity (richness, evenness, and diversity) than those associated to natural substrates, indicating a remarkable differentiation between microbial communities and a substrate-type-coupled species sorting (Miao et al., 2019b; Ogonowski et al., 2018). MPs can thus be considered a distinct ecological habitat for diverse microbial communities, the “plastisphere” (Zettler et al., 2013), potentially characterized by distinct microbial and ecological functions (Miao et al., 2019b; Arias-Andres et al., 2018). Less conclusive information has been reported on the selective colonization of MPs of different materials, possibly as the consequence of the potential confounding temporal and environmental variability associated to the dynamic exposure conditions typically occurring in situ (Ogonowski et al., 2018). Recently, Li et al. (2019) reported that environmental factors, such as the salinity and the nutrients (total nitrogen and total phosphorus), affected the growth rate of biofilms on five types of plastics debris (polyvinyl chloride, polypropylene, polyethylene, polystyrene, and polyurethane) in the Haihe Estuary. The same authors also showed that some genera in the bacterial communities exhibit selectivity for the different polymer types (Li et al., 2019).

Given their small size, MPs may be ingested and accumulated by a wide range of marine organisms, causing direct effects such as physical damage in their intestinal tract, or in other tissues or organs (Van Cauwenberghe et al., 2015). However, the uptake of MPs might also have indirect impacts, since they can absorb and concentrate persistent organic pollutant (POPs) by partition and then transfer such contaminants to the marine food web through



79 ingestion (Andrady, 2011; Wang et al., 2018). Polychlorinated biphenyls (PCBs), polycyclic  
80 aromatic hydrocarbons (PAHs), dichloro-diphenyl-trichloroethane (DDTs), polybrominated  
81 diphenyl ethers (PBDEs), alkylphenols and bisphenol A (BPA), are the main contaminants that  
82 have been found on plastics debris in the marine environment, at concentrations ranging from  
83 0.001 to 10 mg/kg (Hirai et al., 2011).

84 Despite their production ban in the 1980s, PCBs are still common contaminants in marine  
85 sediments and, as reported recently, on marine plastic pellets, where they have been detected  
86 at concentrations up to 7.5 mg/kg (Taniguchi et al., 2016). Due to their high lipophilicity, PCBs  
87 are accumulated through the food chain up to humans, where they act as endocrine disrupters  
88 and possible carcinogens. Besseling et al. (2013) showed that an exposure of lugworm  
89 (*Arenicola marina*) to low concentrations of polystyrene MPs mixed with PCB-contaminated  
90 sediment enhanced the bioaccumulation of PCBs. Similar results were obtained by Koelmans  
91 et al. (2013) in their model analysis with *A. marina*, although their experiments showed that  
92 the role of plastic in the bioaccumulation of POPs may not be considered a significant hazard.  
93 Therefore, the release of MPs in the marine environment can have contrasting effects on the  
94 PCBs bioaccumulation in marine organisms, which can depend on different causes, such as the  
95 experimental conditions or the type of PCB congeners, and further investigations in this  
96 regard are necessary (Ziccardi et al., 2016). The toxicity of PCBs varies with the position and  
97 number of chlorine atoms on the biphenyl ring, being the coplanar congeners more toxic  
98 (Tanabe et al., 1987; Hashmi et al., 2017). Under anoxic conditions, PCBs can undergo  
99 reductive dechlorination by organohalide respiring microorganisms occurring in marine  
100 sediments, typically belonging to the Dehalococcoidia class of the Phylum Chloroflexi  
101 (Zanaroli et al., 2015; Nuzzo et al., 2017). This process replaces chlorine atoms mainly in the  
102 *meta* and *para* positions with hydrogen, thus converting highly chlorinated congeners into  
103 less chlorinated products that are often less toxic, less prone to bioaccumulation (less

hydrophobic) and more amenable to degradation by indigenous aerobic bacteria (Fava and Agathos, 2006). If taking place on MP-sorbed PCBs, this microbial process might change the composition of PCBs, and thus the toxicity and bioavailability of the sorbed PCB mixture for consumers ingesting MPs. The effect of microbial colonization of plastics debris on the environmental fate and the biodegradation/biotransformation of the pollutants absorbed on the MPs surface has not been adequately evaluated yet. Microbial colonization of low-density PE pellets incubated in marine and river sediments under aerobic conditions has been shown to increase the biotransformation of MP-sorbed DDTs and PAHs, but not of PCBs (Wu et al., 2017), suggesting that it might affect the toxicity associated with polluted microplastics. However, to the best of our knowledge, no studies investigated the influence of microbial colonization of MPs on MP-sorbed pollutants under anoxic conditions typically present in sediments.

The aim of this study was to investigate the colonization dynamics on different types of MPs (low-density PE, PET, PS, PP and PVC) by an anaerobic marine bacterial community containing organohalide respiring bacteria, and the effect of the microbial biofilm on the biotransformation of sorbed PCBs. The process was studied in anaerobic slurry microcosms consisting of marine sediment suspended in seawater, i.e. under biogeochemical conditions mimicking those occurring in situ and controlled exposure conditions.

122

## 2 Materials and Methods

### 2.1 Microplastics and their contamination

Five types of MPs were selected for this study: low density polyethylene pristine pellets (PE), crystalline poly(ethylene terephthalate) pristine pellets (PET), general purpose polystyrene pristine pellets (PS), homo-polypropylene pristine pellets (PP), and poly(vinyl chloride) soft pristine pellets (PVC). The size of the plastic particles ranged from approximately 2.5 to 3 mm.

129 All MPs were sterilized in 70% ethanol for 15 min under shaking, followed by rinsing 3 times  
130 with sterilized deionized water, before their use. MPs were contaminated in the laboratory  
131 with a commercial mixture of PCBs (Aroclor 1254, UltraScientific, Bologna, Italy) at the final  
132 concentration of 30 mg<sub>PCBs</sub>/kg<sub>MPs</sub>. This concentration, which is approximately the same order  
133 of magnitude of the highest PCBs concentration reported on marine plastic debris (7.5 mg/kg;  
134 Taniguchi et al., 2016), was selected in order to obtain final concentrations of single PCB  
135 congeners (or co-eluting congeners) of the mixture in the range 20 µg/kg<sub>MPs</sub> - 4.8 mg/kg<sub>MPs</sub>,  
136 and thus to better assess the microbial dechlorination processes of the spiked PCB mixture.  
137 The contamination protocol was adapted from Beckingham and Ghosh (2017); synthetic  
138 marine water (1.5 L) was spiked with 0.6 mL of Aroclor 1254 stock solution (20000 mg/L in  
139 acetone) under shaking (acetone:water 0.04%), 400 g of MPs were added immediately after  
140 and incubated under shaking (180 rpm) for 10 days. MPs were recovered by sieving and dried  
141 at room temperature under sterile conditions. The actual amount of PCBs sorbed on MPs was  
142 indirectly estimated by measuring the residual mass of PCBs in the water phase via solid  
143 phase extraction with polydimethylsiloxane (PDMS) fibers. Five-cm PDMS fibers (outer  
144 diameter 558.8 µm, inner diameter 486 µm, thickness annulus 35.4 µm, fiber volume 0.597  
145 µL/cm) were incubated in the water phase under mixing (150 rpm) at room temperature for  
146 40 days. PCBs were then eluted for 16 h in 0.1 mL hexane and analysed as described in section  
147 2.3. The water concentration of PCBs was calculated from their concentration in the PDMS  
148 fiber using the fiber-water coefficient ( $K_{PDMS-W}$ ). The latter was calculated, for each PCB  
149 congener, from the octanol–water partition coefficient ( $K_{ow}$ ) using the equation  $\log K_{PDMS-W} =$   
150  $0.725 \log K_{ow} + 0.479$  (Thomas et al., 2014). Octanol–water partition coefficients for all PCB  
151 congeners were obtained from Hawker and Connell (1988). An almost negligible mass of PCBs  
152 was detected in the water phase, corresponding to 0.71%, 0.15%, 0.16%, 0.01% and 0.01%

153 (in case of PET, PP, PS, PE and PVC, respectively) of the total mass of PCBs initially added for  
154 MPs contamination. The residual mass of PCBs was assumed to be sorbed on MPs.

155

## 156 2.2 *Microcosms set up, incubation and sampling*

157 Sacrificial anaerobic slurry microcosms (200 mL total volume) consisting of sediment (20%  
158 w/v) and seawater collected from Piailassa Baiona (Ravenna, Italy) were set up according the  
159 procedure described in Nuzzo et al. (2017). Microcosms were autoclave-sterilized for 1 h on  
160 three consecutive days, inoculated at 5% (v/v) with a PCB-dechlorinating microbial culture  
161 enriched previously from marine sediments (Nuzzo et al., 2017) and supplemented with 5 g of  
162 MPs, corresponding to a number of MPs ranging from approximately 175 to approximately  
163 300, depending on the density and size of the MP type. This resulted in a final concentration  
164 ranging from approximately 4375 to approximately 7500 MPs/kg of sediment, which is  
165 comparable with the concentrations of microplastics reported in the most MP-impacted  
166 marine sediments (Bergmann et al., 2017; Mathalon and Hill, 2014). For each type of MPs, the  
167 following microcosms sets were prepared: i) supplemented with MPs contaminated by PCBs;  
168 ii) supplemented with pristine MPs (not contaminated). A sterile control set was set up for  
169 each MP type by supplementing sterile, not inoculated slurry microcosms with MPs  
170 contaminated by PCBs. In addition, a MP-free biologically active control was set up by spiking  
171 Aroclor 1254 PCBs in the sediment and inoculating the same marine culture.

172 The microcosms were incubated at 20°C in the dark under static conditions for 28 weeks.  
173 After 2, 5, 10, 14, 19 and 28 weeks of incubation, 3 microcosms for each MP type were  
174 sacrificed and MPs were recovered through sieving on sterilized 0.5 mm sieves for the  
175 analysis of biofilm microbial communities (except from sterile controls) and of the sorbed  
176 PCBs (where spiked).

177

### 178 2.3 *PCB extraction and analysis*

179 PCBs were batch extracted from 1 g of MPs (i.e., approximately 35 to 60 MPs, depending on  
180 the MP type) with 4 mL of hexane overnight (30°C, mixing at 150 rpm) and sonication (Hong  
181 et al., 2017). PCBs in the organic extracts were analyzed with a 6890N gas-chromatograph  
182 equipped with a 63Ni electron capture detector ( $\mu$ ECD) and a 6890 series-automatic sampler  
183 (Agilent Technologies) using a 30m $\times$ 0.25mm HP-5 capillary column (Agilent Technologies)  
184 under the conditions described elsewhere (Fava et al., 2003). Calibration curves were  
185 obtained and verified monthly using standard mixtures of Aroclor 1254 and Aroclor 1242 (0.5  
186 to 30 mg/L concentration range).

187

### 188 2.4 *Quantification of the bacterial biofilm*

189 Prior to each analysis, the MPs were separated from the sediment by sieving (mesh 0.5 mm),  
190 rinsed three times with sterile water, in order to remove not-attached cells, and air dried  
191 under sterile conditions. Three different approaches were used for the quantification of the  
192 biofilm growth on MPs: i) metagenomic DNA extraction followed by qPCR of bacterial 16S  
193 rRNA genes; ii) cell/biofilm staining with crystal violet; iii) quantification of the reducing  
194 sugars after alkaline hydrolysis of the EPS polysaccharides (Costerton et al., 1995).

195 Metagenomic DNA was extracted from a mixture of replicate samples (0.5 g of MPs, i.e.,  
196 approximately 18-30 MPs, depending on the MP type) with the UltraClean Soil DNA kit  
197 (MoBio Laboratories, Carlsbad, CA, USA) following the procedure described by the provider,  
198 with an additional enzymatic cell lysis step before mechanical cell lysis. In particular, MPs  
199 were incubated in the bead solution (provided with the kit) in the presence of Lysozyme (11.9  
200  $\mu$ L of a 100 mg mL<sup>-1</sup> solution) at 37 °C on a rotary shaker for 30 min, and then of Proteinase K  
201 (3  $\mu$ L of a 20 mg mL<sup>-1</sup> solution) at 37 °C on a rotary shaker for 45 min, prior to addition of  
202 SDS and mechanical cell lysis (bead beating on vortex at maximum speed for 10 min). Total

203 DNA was quantified using Qubit® dsDNA HS Assay Kit with a Qubit 3.0 fluorimeter, following  
204 the manufacturer's specifications. The 16S rRNA genes were quantified via qPCR with primers  
205 905f (5'-AAACTCAAAGGAATTGACGG-3') and 1044r (5'-GACARCCATGCASCACCTG-3') using  
206 the reactions conditions described in Nuzzo et al. (2017). 7-point standard curves were  
207 included in each plate using *E. coli* 16S rRNA gene. Samples and standards were analyzed in  
208 triplicate reactions and 16S rRNA gene copy numbers per cm<sup>2</sup> of MPs were finally calculated.  
209 A colorimetric biofilm quantification protocol using crystal violet was adapted from Burton et  
210 al. (2007) and Zanaroli et al. (2011). MPs (0.5 g, i.e., approximately 18-30 MPs, depending on  
211 the MP type) were stained 20 minutes with 2 mL of crystal violet solution (0.05% w/v). MPs  
212 were then rinsed with sterile distilled water and air-dried for 15 min. The crystal violet on  
213 MP-associated biofilm was solubilized by adding 2 mL of 96% (v/v) ethanol and the  
214 absorbance of the destaining solution was spectrophotometrically measured at 570 nm  
215 versus a blank solution obtained with the same procedure using ethanol-sterilized MPs (see  
216 above).

217 Reducing sugars occurring in the EPS polysaccharides of the biofilm were measured via the  
218 colorimetric method described by Bailey (1988), using 3,5 dinitrosalicylic acid, potassium  
219 tartrate and NaOH. MPs (0.5 g, i.e., approximately 18-30 MPs, depending on the MP type) were  
220 incubated in a boiling water bath for 15 minutes. After cooling in ice, the absorbance of the  
221 supernatant was measured at 540 nm versus a blank solution obtained with the same  
222 procedure using ethanol-sterilized MPs (see above).

223 To identify statistically significant differences between MP types, multiple pair-wise  
224 comparisons were performed with the Tukey test ( $P < 0.05$ ).

225

## 226 2.5 Analysis of the biofilm community via PCR-DGGE of the 16S rRNA genes

227 PCR-DGGE analysis of 16S bacterial rRNA genes was used to investigate changes in richness  
228 (Rr) and community structure (Co) of the bacterial biofilm on different MPs over time. The  
229 V3-V5 variable regions of the bacterial 16S rRNA genes were PCR amplified with primers GC-  
230 357f, containing a 40 bp GC-clamp (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG  
231 CCC C CC TAC GGG AGG CAG CAG-3') and 907r (5'-CCG TCA ATT CCT TTG AGT TT-3') (Sass et  
232 al., 2001). The PCR program consisted of an initial denaturation at 95 °C for 5 min, 30 cycles of  
233 repeated denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for  
234 1 min, followed by a final elongation at 72 °C for 10 min. The PCR reaction (50 µL) consisted  
235 of mixtures containing 1× colorless GoTaq® Flexi buffer (Promega Corporation, Italy), 1.5  
236 mM MgCl<sub>2</sub>, 0.2 mM each dNTP, 0.4 µM each primer, 1 U of GoTaq® G2 Flexi DNA Polymerase  
237 (Promega Corporation, Italy), and of 4 µL template DNA.

238 The amplicons were resolved with a D-Code Universal Mutation Detection System (Bio-Rad,  
239 Milan, Italy) on a 7% (w/v) polyacrylamide gel (acrylamide-N,N'-methylenebisacrylamide,  
240 37:1) containing a denaturing gradient from 40% (top) to 60% (bottom) denaturant (100%  
241 denaturant: 7M urea, 40% v/v formamide) as described in Nuzzo et al. (2017). DGGE image  
242 analysis was used to calculate the community richness (Rr) and community organization (Co)  
243 indexes (Nuzzo et al., 2017).

244

## 245 2.6 Analysis of the biofilm community via Illumina sequencing of 16S rRNA genes and 246 statistical analysis

247 DNA extracted from biofilms of selected samples was subjected to sequencing in order to  
248 investigate the bacterial community composition. The selection of samples was based on the  
249 observed colonization dynamics in terms of i) amount of bacterial biofilm formed and, ii)  
250 changes of the community richness and organization over time, detected via PCR-DGGE

analysis (section 3.1), as well as on the observed reductive dechlorination of MP-sorbed PCBs (section 3.3). V1-V3 hypervariable regions of 16S rRNA were PCR amplified and sequenced on Illumina Miseq with 300 bp paired-end approach (BMR genomics, Padova, Italy). The 16S rRNA gene raw sequences were processed using the open source software pipeline Quantitative Insights Into Microbial Ecology 2 (QIIME 2) version 2017.12 (<http://qiime2.org>). SILVA was used as reference database to classify the representative sequences from our dataset, and Jaccard distance matrices as beta-diversity measures. The Principal Coordinate Analysis (PCoA) plot was created in R (version 3.5.1) through community ecology vegan package. The significance of separation among study groups was determined by permutation test with pseudo-*F* ratios using the function *adonis* of vegan package. Significant differences in the relative abundance of Dehalococcoidia between MPs groups – pristine and PCB-contaminated – were evaluated with the Wilcoxon test.

263

### 264 **3 Results**

#### 265 *3.1 Colonization dynamics of different MPs*

The colonization dynamics was evaluated during a 28-weeks experiment using different approaches: i) quantification of the Bacterial 16S rRNA genes via qPCR, as an approximation of cell density on the MPs surface (16S rRNA gene copies per cm<sup>2</sup> of MPs), ii) cell/biofilm staining based on crystal violet, which targets both the bacterial cells and the extracellular polymeric substances (EPS) they produce when growing attached to surfaces, and iii) quantification of reducing sugars occurring in the polysaccharide fraction of EPS. The qPCR analysis revealed that all MPs were rapidly colonized by the bacterial community within the first 2 weeks of incubation, without any remarkable difference between the PCB-contaminated MPs and the pristine (not-contaminated) MPs (Figure 1A,D). The highest cell density was found on PVC pellets, which reached approximately 10<sup>10</sup> 16S rRNA gene copies



276 per cm<sup>2</sup> of MPs, followed by PE (approximately 10<sup>9</sup> 16S rRNA copies cm<sup>-2</sup>), and then PET, PP  
277 and PS (approximately 10<sup>8</sup> 16S rRNA copies cm<sup>-2</sup>). Since the surface area of MPs was  
278 estimated using the geometry of each plastic pellet, i.e., not considering the irregularities of  
279 the shapes and the porosities, the calculated superficial cell density may be an  
280 underestimation of the actual microbial colonization. No remarkable cell density changes  
281 were observed along the rest of the incubation, indicating that cells attachment and growth  
282 were not taking place and/or that were balanced by cells death and detachment from the  
283 surface (McDougald et al., 2012).

284 Staining with crystal violet (Figure 1B,E) and quantification of the reducing sugars (Figure  
285 1C,F) revealed a biofilm increase up to weeks 10-15 on PE, PS, PP and PET, and up to the end  
286 of incubation (week 28) on PVC, without remarkable differences in the presence and absence  
287 of the sorbed pollutants. The biofilm increase over time may indicate the occurrence of a  
288 biofilm maturation phase, taking place after a rapid cells adhesion and growth. The biofilm  
289 amount then changed less markedly, showing slight decreases followed by small increases  
290 that may indicate the succession of detachment and regrowth events involving portions of the  
291 biofilm, which are typical of these dynamic structures (McDougald et al., 2012). The greatest  
292 biomass concentration, approximately one order of magnitude higher than on the other MP  
293 types, was detected on PVC MPs also according to crystal violet and quantification of reducing  
294 sugars and was significantly higher than that on other MP types ( $P < 0.05$ , Tukey test)  
295 according to all measured parameters.

296 A preliminary investigation of the evolution of the biofilm community established on the  
297 different MPs was carried out through PCR-DGGE analysis of the 16S rRNA genes. PCR-DGGE  
298 profiles (Figure S1) were mainly used to evaluate if major changes were taking place over  
299 time in the community developed on each MP type, rather than for an in-depth assessment of  
300 the community diversity and structure or to compare the biofilm communities on the

different MP types. The following parameters were considered: i) community richness (Rr), i.e., the number of species, and ii) community organization (Co), which indicates if the individuals of the population are homogeneously distributed between the species (even community) or if individuals belonging to few species are predominant over those belonging to all others species (uneven community).

The Rr and Co varied markedly between the different type of MPs, but not between the MPs contaminated by PCBs and those not contaminated (Figure S2). In particular, the biofilm communities on PET, PS and PP did not change remarkably in Rr and Co over time, and were characterized by an average Rr of approximately 25 and 18, for the pristine and PCB-contaminated MPs, respectively. The Co resulted to be approximately 25 or lower for all this plastic samples (PET, PS and PP, contaminated and pristine), which indicates a high degree of community evenness. In contrast, a remarkable change of the biofilm community appeared to occur on PE during the first 10 weeks of incubation, where a noteworthy reduction of Rr and a substantial increase of Co were observed. This indicates that a fraction of the biofilm microbial community on the PE surface was able to become dominant over time, causing a loss of biodiversity. Conversely, an increase of Rr and limited fluctuations of the Co were observed on PVC over time. This indicates that an increase of biodiversity took place without affecting the functional organization of the community.

### 3.2 Composition of the biofilm community on different MPs

The composition of the biofilm communities was then investigated more in detail through Illumina sequencing of the 16S rRNA genes. Samples were selected based on the observed colonization dynamics in terms of i) amount of bacterial biofilm formed and, ii) changes of the community richness and organization over time (section 3.1), as well as on the observed reductive dechlorination of MP-sorbed PCBs (section 3.3). In particular, the biofilm

community composition was investigated for all samples after 2 weeks of incubation, since at this sampling point all MP types were colonized by the maximum cells concentration, which then remained almost constant (Figure 1A,D), and a remarkable reductive dechlorination of sorbed PCBs was observed on all MP types (section 3.3, Figure 5); in addition, the final sampling point (28 weeks of incubation) was analysed only for PE and PVC pellets, since substantial changes in Rr and Co were observed between 2 and 28 weeks of incubation only on these materials (Figure S2). The PCB-dechlorinating marine culture inoculated in the microcosms was also analysed to investigate if adhesion and biofilm formation on MPs involves specific members of the community or the whole population. In addition, the sediment present in the microcosms supplemented with PCB-contaminated MPs was analysed to evaluate any differences between the microbial communities adhering to the MPs and those associated to the sediment particles.

At the phylum level (Figure 2), the microbial inoculum mainly consisted of Proteobacteria (58%), Chloroflexi (30%) and, at lower extent, Firmicutes (6%). After 2 weeks of incubation, Firmicutes became dominant in the biofilm communities on all MP types, having a relative abundance that ranged from 88% (pristine PS) to 49% (PCB-contaminated PVC). Conversely, Proteobacteria represented a minor fraction of the biofilm communities, ranging from 1 to 27%, and the fraction of Chloroflexi decreased on all plastic pellets (0.3-13%), except for PVC contaminated by PCBs (45%). Plastic biofilm communities also included, at quite high relative abundances, the phyla Lentisphaerae (0-26%), Deinococcus-Thermus (0-7%), Actinobacteria (0-13%) and Acidobacteria (0-9%), which were not detected in the inoculum or represented less than 2% of the original microbial community.

Relevant changes in composition were detected after 28 weeks of incubation on PE and PVC, when Chloroflexi became more abundant and Firmicutes decreased.

350 The bacterial communities associated to the sediment surrounding the different PCB-  
 351 contaminated MPs were very similar to each other and mainly consisted of bacteria belonging  
 352 to the phyla Firmicutes (30-43%), Chloroflexi (20-28%), Proteobacteria (12-22%), and to less  
 353 extent, Acidobacteria (6-9%), Synergistetes (4-8%), Lentisphaerae (3-7%), and  
 354 Actinobacteria (2-3%). These bacterial communities therefore remarkably differed from the  
 355 microbial inoculum and those colonizing the MPs.

356 At lower taxonomic levels (Table S1), the original marine microbial community was  
 357 dominated by bacteria of the genera *Sulfurovum* (20.4%) and *Sulfurimonas* (15.5%), which are  
 358 both typically sulphur oxidizing, nitrate-reducing bacteria, *Dethiosulfatibacter* (5.3%), a  
 359 sulphur and thiosulfate-reducing genus, *Magnetovibrio* (3.6%), which includes members  
 360 capable of anaerobic thiosulfate oxidation using nitrous oxide, *Celeribacter* and another  
 361 member (unidentified genus) of the *Rhodobacteraceae* family (12.0%) and *Dehalobium*  
 362 (17.7%), an organohalide respiring Dehalococcoidia, along with other non-dehalorespiring  
 363 members of the Chloroflexi phylum (unidentified genus, *Anaerolineaceae* family, 12.8%). Most  
 364 of these genera were not detected in the biofilm communities grown on pristine MPs, or they  
 365 were at much lower abundances. After 2 weeks of incubation, the biofilm present on PS and  
 366 PVC was dominated by a Firmicutes bacterium of the *Peptostreptococcaceae* family  
 367 (unidentified genus), which represented 42.4% and 37.6% of the community, respectively.  
 368 This bacterium was also present at high relative abundance on PE (19.4%) and, at lower  
 369 abundances, on PET (9.4%) and PP (8.5%) after 2 weeks of incubation. The microbial  
 370 community on PE and PP after 2 weeks of incubation was mainly dominated by a different  
 371 Firmicutes bacterium belonging to the SRB2 family of *Thermoanaerobacterales* (unidentified  
 372 genus), which represented approximately 29% of the biofilm community on both MPs types.  
 373 The same bacterium was also detected at high abundance on PET (17.6%) and at much lower  
 374 abundance on PS (5.6%), while was not detected on PVC. A member of the *Clostridiaceae* 1

375 family (unidentified genus) was also dominant or present at relatively high abundances on  
 376 PET (20.5%), PP (21.0%) and PS (16.4%), whereas the community on PVC included at high  
 377 abundances a member of Clostridiales (22.5%), *Celeribacter* (15.0%) and a member of  
 378 Dehalococcoidia (10.0%). Other community members that enriched on pristine MPs after 2  
 379 weeks of incubation were an uncultured *Coriobacteriaceae* (unidentified genus) in PVC  
 380 (12.5%), PE (6.6%), PET (5.3%) and PP (4.3%), a member of Deinococci in PP (6.8%), PE  
 381 (5.9%) and PS (5.3%), *Sedimentibacter* in PE (10.2%), PET (7.3%), PP (6.3%) and PS (3.1%),  
 382 and *Clostridium sensu strictu 1* only in PET (7.9%) and PS (5.4%).

383 After 28 weeks of incubation, the SRB2 family of *Thermoanaerobacterales* and  
 384 *Sedimentibacter* were lost by the biofilm community on PE, where *Peptostreptococcaceae*  
 385 further enriched (40.3%) along with *Anaeroplasma* (6.5%) and *Anarolinaceae* (17.9%). A  
 386 remarkable change was also observed in the biofilm composition of PVC after 28 weeks of  
 387 incubation, when the uncultured *Coriobacteriaceae*, Dehalococcoidia, Clostridiales and  
 388 *Celeribacter* drastically reduced, and uncultured *Anaerolinaceae* remarkably enriched  
 389 (35.4%).

390 The presence of sorbed PCBs had very limited effects on the overall composition of the biofilm  
 391 on MPs, resulting mainly in the enrichment of the class Dehalococcoidia, i.e., the organohalide  
 392 respiring Chloroflexi members of the community (Figure 3). This behavior appears even  
 393 more marked in the case of PVC, where the increase of Dehalococcoidia in the biofilm  
 394 community in the presence of PCBs reaches the statistics significance (P-value = 0.05,  
 395 Wilcoxon test).

396 The PCoA analysis based on Jaccard similarity index was then used to assess the overall OTU-  
 397 level compositional differences in the microbial communities adhering to the different plastic  
 398 types (PE, PET, PS, PP, PVC) and sediment. The data show a clear separation of microbial  
 399 community profiles by MPs type and between MPs and the surrounding sediment (P-value <

0.03, permutation test with pseudo-*F* ratio) (Figure 4A). Permutation test with pseudo-*F* ratio of the significance of pairwise separation between each MP type and MPs and the sediment is reported in Table S2. The PCoA analysis also demonstrated that the communities on PCB-contaminated and pristine MPs did not significantly differ between each other (*P*-value = 0.4) (Figure 4B). The sorption of PCBs on MPs therefore did not significantly change the taxonomic composition of the overall microbial community adhering to them.

### 3.3 PCB dechlorination

No dechlorination of MP-sorbed PCBs was observed in sterile controls during the 28 weeks of incubation. On the contrary, a significant reductive dechlorination of PCBs associated to all different types of MPs was observed in the microcosms inoculated with the PCB-dechlorinating culture after only two weeks of incubation, when the average number of chlorines per biphenyl molecule was reduced from 5.2 to 4.8 on PP, 4.7 on PE, 4.6 on PET, 4.4 on PS and 4.3 on PVC (Figure 5), corresponding to dechlorination rates of  $35.2 \pm 1.9$  (PP),  $44.6 \pm 2.6$  (PE),  $56.8 \pm 6.2$  (PET),  $57.8 \pm 5.4$  (PS) and  $61.2 \pm 5.8$  (PVC)  $\mu\text{moles of Cl removed kg}_{\text{MP}}^{-1} \text{ week}^{-1}$ . The reductive dechlorination of sorbed PCBs then proceeded more slowly, leading to an average number of chlorines per biphenyl molecules after 28 weeks of incubation in the range 4.4-3.9. Remarkably, the biotransformation of PCBs sorbed on MPs was faster than that of PCBs sorbed on sediment, which started only after 10 weeks of incubation and proceeded with a dechlorination rate of  $33.9 \pm 9.1$   $\mu\text{moles of Cl removed kg}_{\text{sediment}}^{-1} \text{ week}^{-1}$  until week 28, when the average number of chlorines per biphenyl molecule was reduced to 4.0 (Figure 5). In general, highly similar dechlorination patterns were observed for PCBs sorbed on different MP types, as indicated by the depletion of the same highly-chlorinated congeners and the accumulation of the same low-chlorinated ones (Figure 6). The lower reduction of the highly-chlorinated congeners and the lower accumulation of the low-chlorinated ones detected on

PE and PP pellets at the end of incubation was in accordance with the less extensive dechlorination process occurred on these MPs. The dechlorination patterns of PCBs sorbed on MPs was similar to that observed on PCBs sorbed on sediment (without MPs), with a main difference: the accumulation in the sediment of the co-eluting hexa-/penta-chlorinated congeners 234-35, 235-34 and 2356-24, which did not accumulate on the MPs, and the concomitant accumulation of the tri-chlorinated congeners 25-3 and 24-3 on MPs and not in the sediment. This indicates that a *meta* and *para* dechlorination of 235-34 and 234-35 to 25-3 and 24-3, respectively, occurred only on MPs.

433

#### 4 Discussion

Microbial colonization of MPs in the marine environment may have several implications on their fate and sedimentation behavior, the fate of MP-sorbed pollutants and their toxicity and bioavailability to marine organisms (Cole et al., 2011; Wang et al., 2018; Mohamed Nor and Koelmans, 2019). While the colonization of plastic debris by marine microorganisms have been recently reported in seawater (Dussud et al., 2018; Frère et al., 2018; Xu et al., 2019), very limited information is available on plastic colonization in marine sediments (Harrison et al., 2014), which are the ultimate sink, as well as an entry point in the food chain through benthic organisms, of plastic debris and hydrophobic pollutants in the marine environment (Kaiser et al., 2017). Very limited information is available also on the fate of pollutants sorbed on plastics and, in particular, on the potential role of microbial biofilms in the biodegradation/biotransformation of plastic-sorbed pollutants (Wu et al., 2017). In this study, the colonization dynamics of microplastic (MP) pellets of different materials, namely, PE, PET, PS, PP and PVC, either pristine or contaminated with polychlorinated biphenyls (PCBs), was investigated in laboratory microcosms of an anoxic marine sediment and seawater collected from the same site, i.e., under laboratory biogeochemical conditions mimicking those

450 occurring in situ and controlled exposure conditions. The sediment was inoculated with a  
451 marine anaerobic microbial community previously selected for its ability to dehalogenate  
452 PCBs (Nuzzo et al., 2017), in order to better assess the potential role of microbial reductive  
453 dehalogenation processes on the fate of MP-sorbed PCBs. The use of a well-defined source  
454 community was also made to better evaluate the possible effects of the MP material and of  
455 MP-sorbed pollutants on the surface colonization dynamics and on structure and composition  
456 of the biofilm (Ogonowski et al. 2018).

457 All MPs were rapidly colonized during the first 2 weeks of incubation, up to a cell density  
458 range of  $1.0 \times 10^8$  to  $1.0 \times 10^{10}$  cells/cm<sup>2</sup>. Harrison et al. (2014) reported a comparable, rapid  
459 colonization of low-density PE by bacteria of coastal marine sediments incubated in Petri  
460 dishes, that reached a density of  $1.0 \times 10^6$  to  $1.0 \times 10^9$  16S rRNA genes DNA per mm<sup>2</sup> of PE  
461 within 7 days. Our work confirms that MPs are a good anthropogenic substrate for  
462 colonization by marine sediment microbial communities also under strictly anoxic conditions,  
463 which are typically present in situ few millimeters below the sediment surface.

464 Remarkable differences were observed in terms of colonization of different MP types. In  
465 particular, biofilm formation was remarkably higher on PVC pellets than on other MP types,  
466 both in terms of cell density reached and amount of EPS produced. Such a higher biofilm  
467 growth on PVC is likely the consequence of a higher availability of energy and carbon sources  
468 that might have been released from the MPs. Many organic additives are commonly  
469 supplemented to plastic materials in order to improve their proprieties, such as tensile  
470 strength, flexibility and durability (Teuten et al., 2009). In particular, PVC is among the  
471 plastics containing the highest amounts of additives, mainly plasticizers and stabilizers, that  
472 may reach up to 50% w/w of the plastic material. Phthalates (alkyl/aryl esters of 1,2-  
473 benzenedicarboxylic acid), as well as low molecular weight, easily biodegradable citrate,  
474 adipate and hexanoate esters, are very commonly used plasticizers in PVC (Markarian, 2007;



475 Babinsky, 2006). The microbial degradation of phthalates to methane and carbon dioxide has  
476 been also reported under anaerobic conditions (Chang et al., 2005; Liang et al., 2008). All  
477 these additives can thus sustain the growth of anaerobic microbes/microbial communities  
478 and could have therefore favored a more extensive biofilm growth on PVC pellets.

479 All MP types selected specific fractions of the inoculated microbial community. The dominant  
480 members of the latter were mainly associated with sulphur cycling, a common process taking  
481 place in marine sediments (Wasmund et al., 2017), as well as with organohalide respiration,  
482 which was a specific feature of the marine microbial community inoculated in the microcosms  
483 (Nuzzo et al., 2017). Most of these members were not detected in any of the biofilm  
484 communities grown on MPs, which were dominated by taxa mainly including  
485 chemoorganotrophic, fermenting bacteria.

486 Remarkable differences were also observed in terms of composition between the microbial  
487 communities associated to the different MP types and the surrounding sediment. Recent  
488 studies have demonstrated that the bacterial assemblages that develop on the surface of  
489 plastic material in seawater or freshwater systems can significantly differ from those  
490 developing on non-plastic substrates, such as wood, cobblestone, cellulose and glass, as well  
491 as from the sediment and water bacterial communities (Miao et al., 2019b, Ogonowski et al.,  
492 2018; Frère et al., 2018; De Tender et al., 2015). Some of these studies have also reported that  
493 the microbial communities developed on PE and PP were quite similar to each other, and  
494 more distinct to those associated with PS pellets (Ogonowski et al., 2018; Frère et al., 2018).

495 The differentiation of biofilm communities on different MP types occurred in this study may  
496 be attributed to many factors. For example, Ogonowski et al. (2018) recently reported a  
497 significant correlation between the substrate hydrophobicity and bacterial composition on  
498 different plastic and non-plastic (glass, cellulose) materials. Since the hydrophobicity of the  
499 materials tested in this study typically changes in a relatively narrow range (contact angle 80-

100; Ogonowski et al., 2018; Gotoh et al., 2011; McGinty and Brittain, 2008), the difference in biofilm composition could be more probably related to other features, such as the presence of different organic and inorganic additives and the different rates they are released from the plastic material. Indeed, the additives released from different MPs may either promote the growth of some microbial species able to use them as carbon and energy source, or limit the growth and survival of some other species that are sensitive to these chemicals. However, the very high variety of additives used in different plastics, as well as the use of different additives for the same plastic type by different producers, make impossible to identify a typical additives composition for each plastic and thus to relate the specific compositions of the biofilm communities observed in this study to the release of specific chemicals. Interestingly, Klaeger et al. (2019) recently reported that the release of biodegradable residual monomers and oligomers from polymers can lead to a remarkable overestimation of the plastic materials degradation, in particular during the first 19 days of incubation. Indeed, styrene and vinyl chloride monomers have been widely reported to leach from PS and PVC pellets, respectively (Pilevar et al., 2019; Fayad et al., 1997) and are known to be susceptible, as well as other hydrocarbons, to anaerobic biodegradation (Varjani, 2017). This suggests that the leaching of residual monomers and/or oligomers from the different plastic types may have also contributed to the differentiation of biofilm compositions on the different MPs we observed especially during the first 2 weeks of incubation. This is in agreement also with the selective enrichment of Dehalococcoidia (i.e., of the organohalide respiring Chloroflexi members of the inoculated community) in the biofilm promoted by the sorbed PCBs on all MPs, without any significant change of the overall community.

The PCBs sorbed on the different MP types were rapidly converted into less chlorinated congeners by the anaerobic marine biofilms. Indeed, the rate of PCB dechlorination we observed may be much higher than that potentially taking place in situ, given the extremely

low concentration of organohalide respiring bacteria typically present in environmental samples and the specialized enriched community with marked organohalide-respiring activity we used in our study under controlled conditions. However, to our knowledge, this is the first report describing the potential occurrence of reductive dechlorination processes towards PCBs sorbed on MPs. Very few studies investigated the biotransformation/biodegradation of pollutants sorbed on MPs; Wu et al. (2017) reported for example that colonization of low-density PE pellets increased the biotransformation of dichloro-diphenyl-trichloroethanes (DDTs) and polycyclic aromatic hydrocarbons (PAHs) in marine and river sediments incubated aerobically, although had not significant effect on PCBs. This claims the need of further studies on the biotransformation of pollutants sorbed on MPs under different environmental conditions and by different microbial communities.

Moreover, our study shows that PCBs sorbed on MPs underwent reductive dechlorination much more rapidly than those sorbed on the sediment. While Mato et al. (2001) showed that sorption of organic compounds, such as PCBs and DDE, is approximately two orders of magnitude higher in PP pellets than in marine sediments, probably due to the plastic hydrophobic surfaces, our results suggest that PCBs are more bioavailable to the dechlorinating marine microbes when sorbed on MPs, or that the colonization of MPs might increase the bioavailability of MP-sorbed PCBs, e.g., through biosurfactants production, better than of the sediment-sorbed ones. PCB dechlorination was more extensive on PVC, PS and PET, followed by PE and PP, which might indicate a slight different degree of PCB bioavailability on different MP materials. The most abundant highly-chlorinated PCB congeners were extensively converted into less chlorinated products, that are commonly less toxic and less hydrophobic, thus potentially more bioavailable and less prone to bioaccumulation. The toxicity of contaminated MPs is potentially due to the combination of several factors, e.g., the intrinsic toxicity of the sorbed pollutants, their bioavailability and

550 capability to bioaccumulate; the observed change in congener composition of the PCB mixture  
551 associated to MPs might thus have remarkable effects on the overall toxicity associated to  
552 polluted MPs, that deserves more investigations.

553

## 554 **5 Conclusions**

555 The colonization dynamics of different MP pellet types (PE, PET, PS, PP, and PVC) by an  
556 anaerobic marine microbial community was investigated for the first time in anoxic marine  
557 sediments, which represent the main sink for MPs in the marine environment. This study  
558 showed that microbial colonization took place very rapidly on all MPs and that the biofilm  
559 composition differed significantly between the five plastic types and from the surrounding  
560 sediment community. The colonization of MPs is thus a selective process that may depend on  
561 several factors, such as the different properties of polymer surface and the presence of  
562 different additives and/or different residual monomers/oligomers that may be released from  
563 the plastics and promote or limit selectively the growth of distinct bacterial species. However,  
564 the factors driving the enrichment of different biofilm communities on the MPs may be  
565 multiple and diverse and need further investigation. This work also showed that PCBs sorbed  
566 on different MP types can be dehalogenated much faster than those sorbed on sediments,  
567 suggesting that they are highly bioavailable. In addition, their susceptibility to be  
568 bioconverted to even more bioavailable, although less toxic, low-chlorinated PCBs indicate  
569 that microbial biofilms growing on contaminated MPs, by changing the congener composition  
570 of the sorbed PCB mixture, might remarkably affect the toxicity of contaminated MPs in the  
571 marine environment, as well as the uptake and bioaccumulation of MP-associated PCBs by  
572 marine organisms. Further investigation should be carried out in order to assess ecotoxicity  
573 changes associated to the biotransformation of PCBs sorbed to MPs particles.

574

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583

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## 775 **Figures**

776 **Figure 1.** Colonization dynamics of PCB-contaminated (A-C) and pristine (D-F) MPs  
777 determined via qPCR of 16S rRNA genes (A, D), reducing sugars in the EPS (B, E), and biofilm  
778 staining with crystal violet (C, F). Values are the mean of triplicate cultures  $\pm$  standard  
779 deviation.

780 **Figure 2.** Composition at the Phylum level of the inoculated microbial community, the biofilm  
781 grown on all PCB-contaminated and pristine MPs after 2 weeks of incubation, the biofilm  
782 grown on PCB-contaminated and pristine PE and PVC after 28 weeks of incubation and the  
783 microbial community associated to the sediment particles surrounding the PCB-contaminated  
784 MPs after 2 weeks of incubation.

785 **Figure 3.** Boxplots showing the relative abundance (%) of Dehalococcoidia in pristine and  
786 PCB-contaminated MPs. \*: P-value = 0.05; Wilcoxon test.

787 **Figure 4.** PCoA plots of bacterial communities in the inoculum, in the sediment and associated  
788 to MPs (A) and of bacterial communities associated to PCB-contaminated and to pristine MPs  
789 (B).

790 **Figure 5.** Reductive dechlorination of PCBs sorbed on the sediment (biotic control  
791 microcosms) and on the different MP types (biologically active microcosms and sterile  
792 controls). Values (average number of chlorines/biphenyl molecule) are the mean of triplicate  
793 cultures  $\pm$  standard deviation.

794 **Figure 6.** PCB congeners and their concentrations on MPs (A) and in the sediment (B) at the  
795 beginning and the end of incubation (28 weeks). Value are the mean of triplicate cultures  $\pm$   
796 standard deviation.

797 **Supplementary information**

798 **Table S1.** Composition at the genus level of the inoculated microbial community, of the  
799 biofilm grown on all MP types after 2 weeks of incubation and after 28 weeks of incubation on  
800 PE and PVC MPs, and of the microbial community associated to the sediment particles.

801 **Table S2.** Results of permutation test with pseudo-F ratio statistics applied to ordination  
802 analysis based on Jaccard similarity index (related to Figure 4A).

803 **Figure S1.** PCR-DGGE gels of the 16S rRNA genes of the inoculated marine microbial culture  
804 (A) and the biofilm microbial community grown on pristine MPs (A) and on PCB-  
805 contaminated-MPs (B).

806 **Figure S2.** Evolution of the biofilm community richness (Rr) and organization (Co) over time  
807 on PCB-contaminated (A) and pristine (B) MPs, based on PCR-DGGE analysis of 16S rRNA  
808 genes (Figure S1).

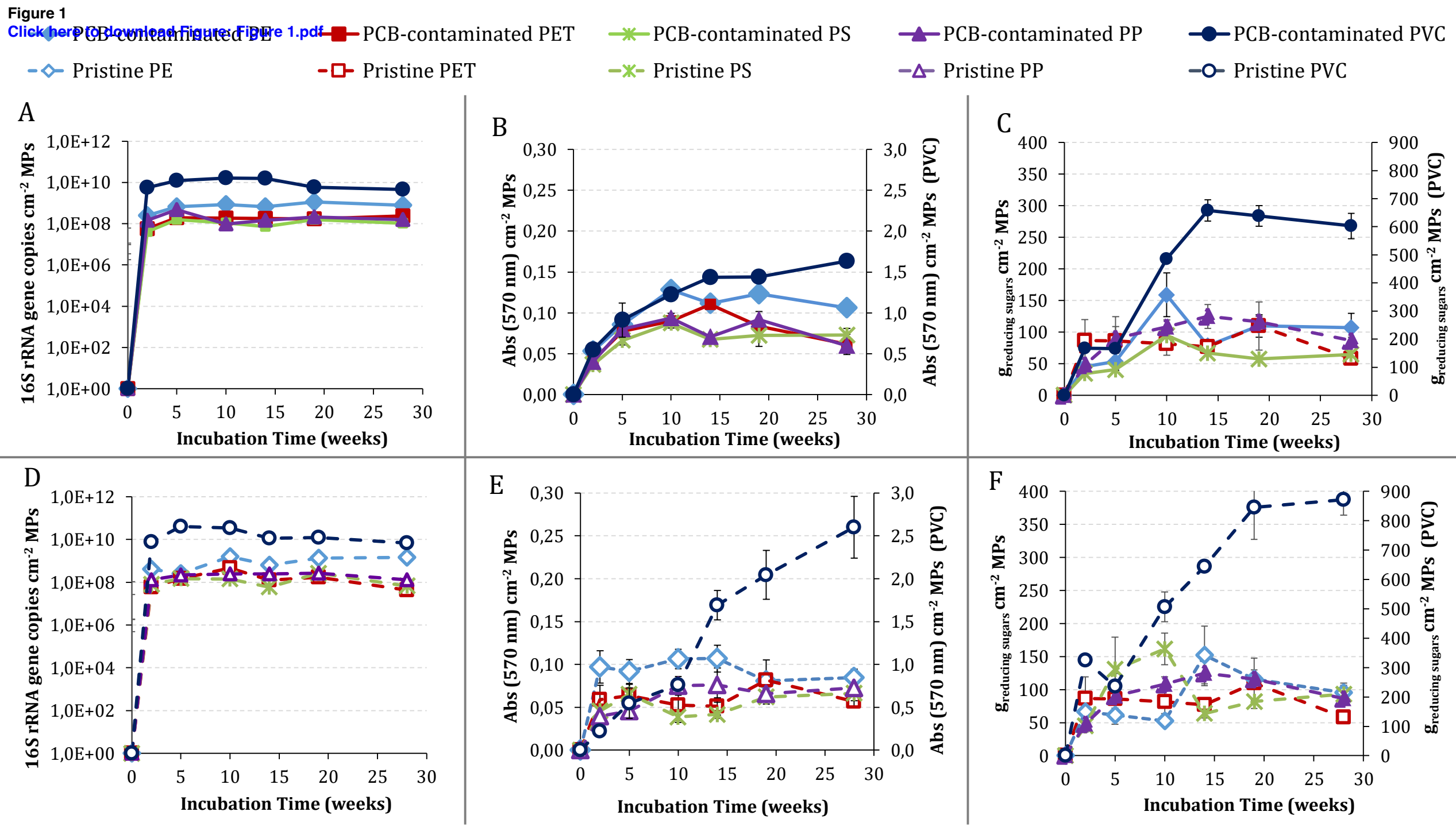


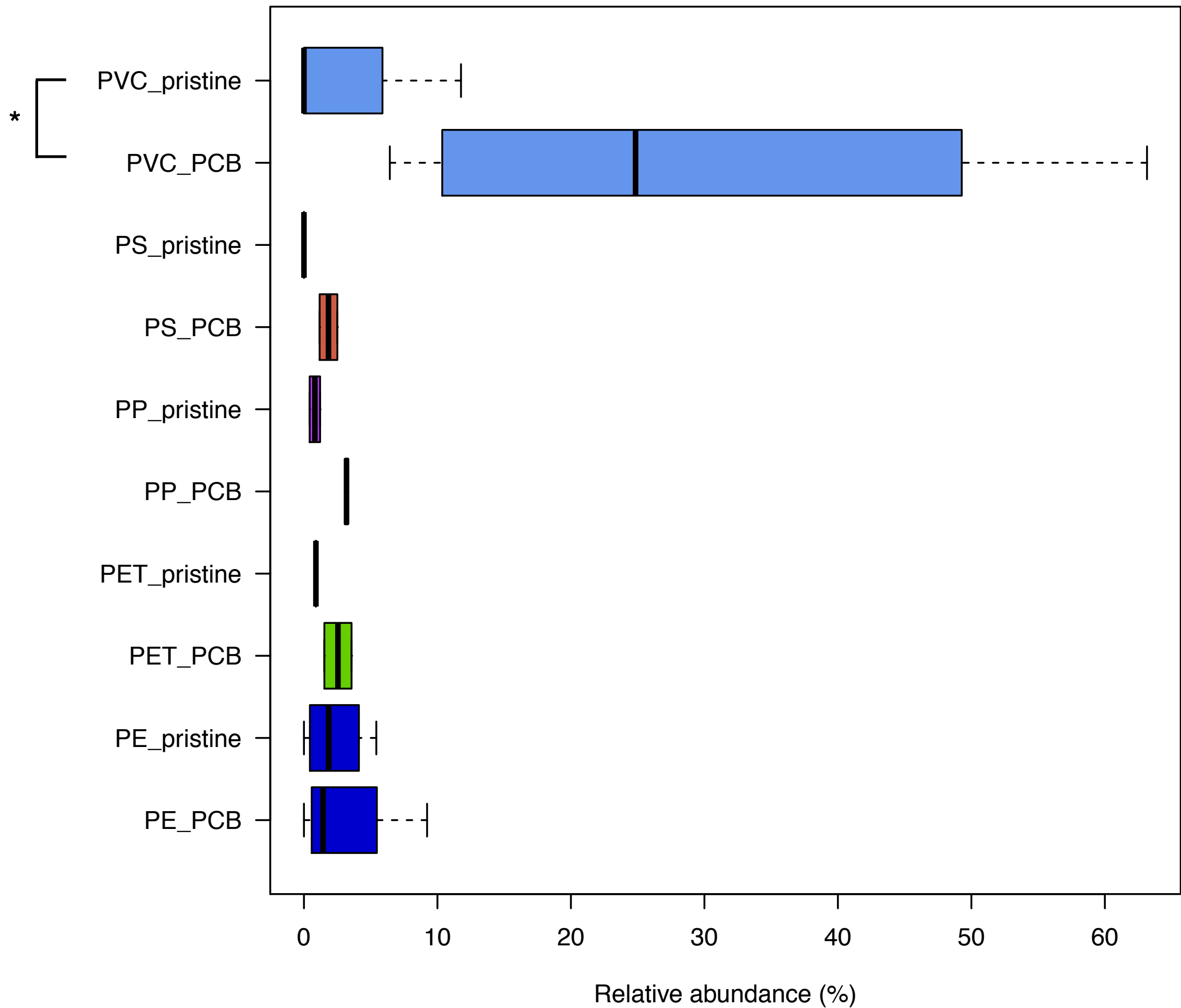




Figure 3

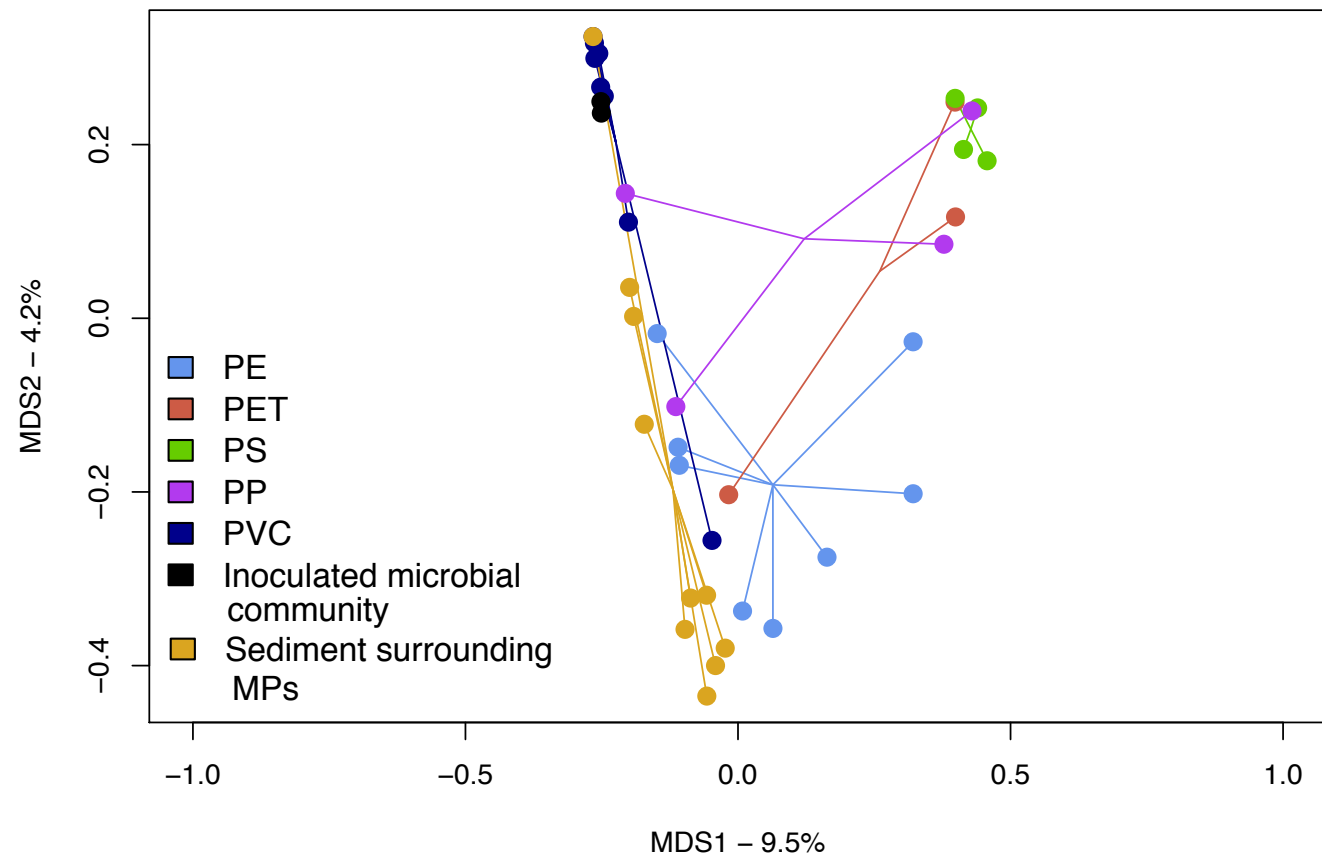
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# Dehalococcoidia



**Figure 4**  
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**A**



**B**

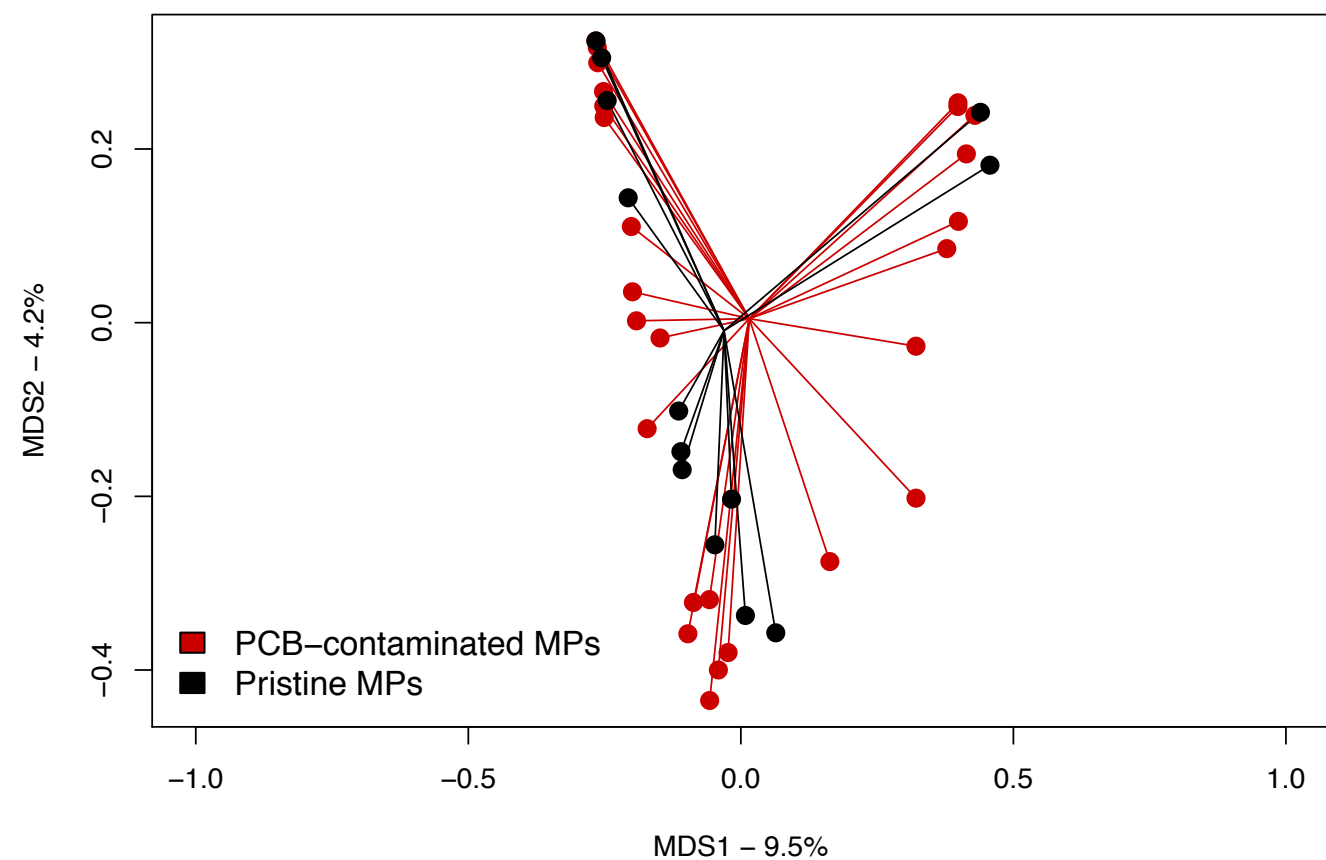


Figure 5  
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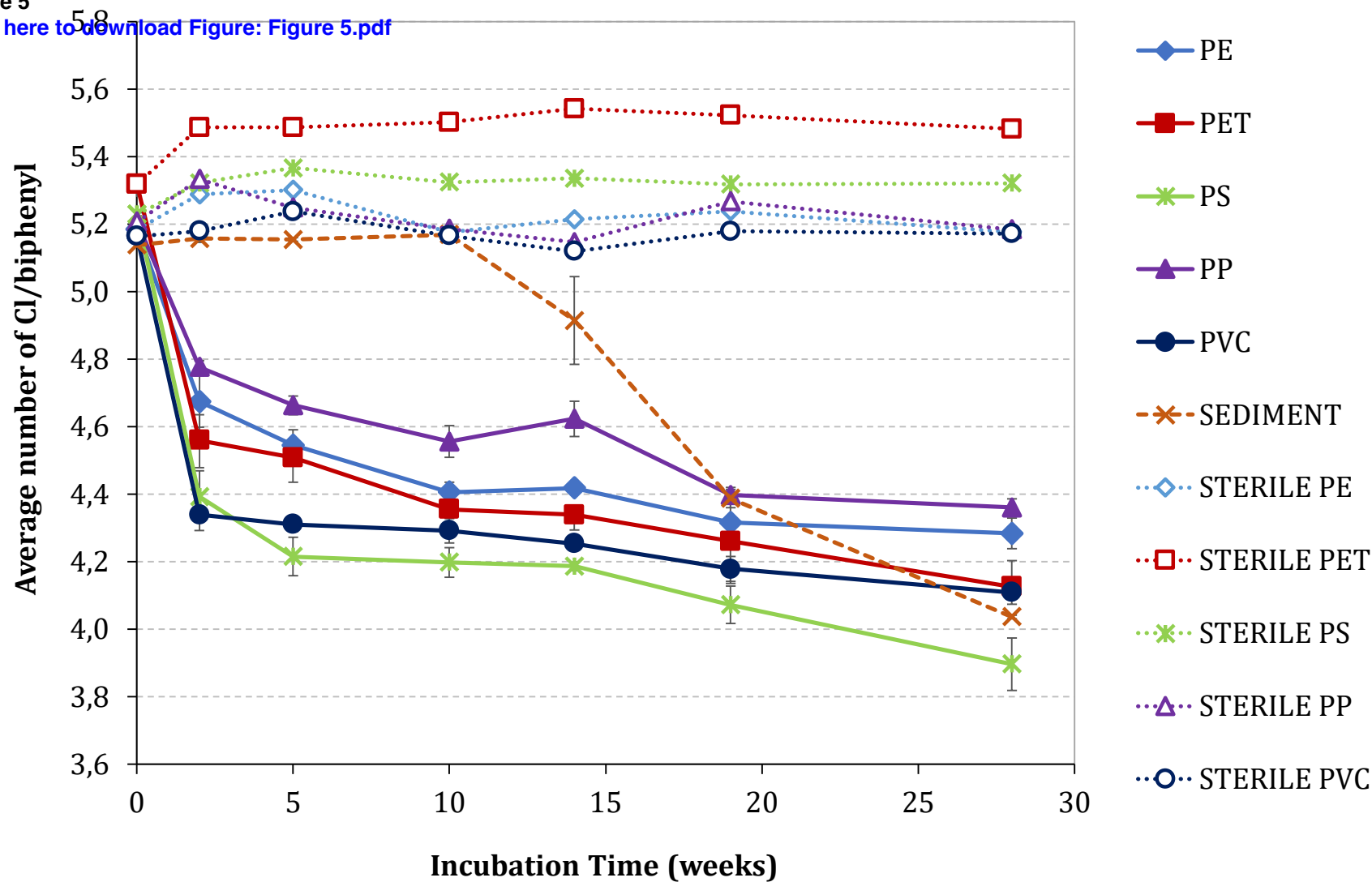
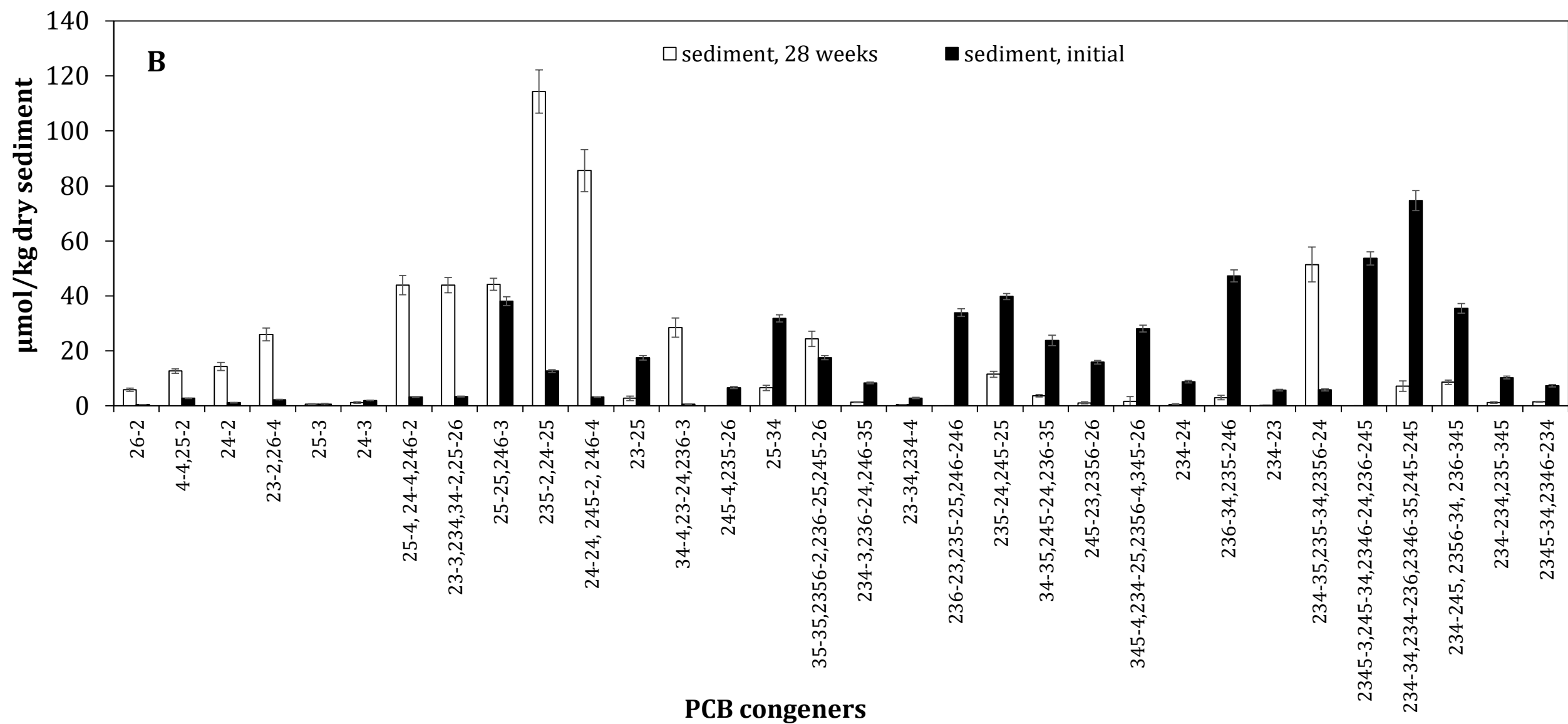
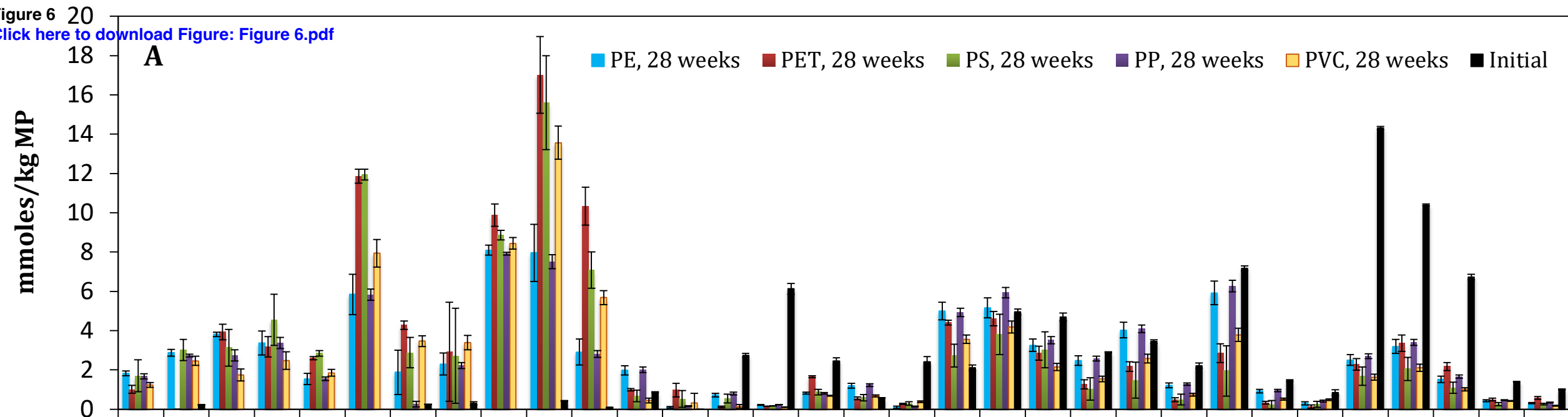


Figure 6  
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## Figure S1

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## Figure S2

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