

1 **Textile waste and microplastic induce activity and**
2 **development of unique hydrocarbon-degrading marine**
3 **bacterial communities**

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18 **KEYWORDS**

19 Microplastic, Fiber, Hydrocarbon-degrading bacteria, Microbial community, Pollution
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22 **ABSTRACT**

23 Biofilm-forming microbial communities on plastics and textile fibers are of growing interest since
24 they have potential to contribute to disease outbreaks and material biodegradability in the
25 environment. Knowledge on microbial colonization of pollutants in the marine realm is expanding,
26 but metabolic responses during substrate colonization remains poorly understood. Here, we assess
27 the metabolic response in marine microbial communities to three different micropollutants, virgin
28 high-density polyethylene (HDPE) microbeads, polysorbate-20 (Tween), and textile fibers.
29 Intertidal textile fibers, mainly cotton, virgin HDPE, and Tween induced variable levels of
30 microbial growth, respiration, and community assembly in controlled microcosm experiments.
31 RAMAN characterization of the chemical composition of the textile waste fibers and high-
32 throughput DNA sequencing data shows how the increased metabolic stimulation and
33 biodegradation is translated into selection processes ultimately manifested in different
34 communities colonizing the different micropollutant substrates. The composition of the bacterial
35 communities colonizing the substrates were significantly altered by micropollutant substrate type
36 and light conditions. Bacterial taxa, closely related to the well-known hydrocarbonoclastic bacteria
37 *Kordiimonas* spp. and *Alcanivorax* spp., were enriched in the presence of textile-waste. The
38 findings demonstrate an increased metabolic response by marine hydrocarbon-degrading bacterial
39 taxa in the presence of microplastics and textile waste, highlighting their biodegradation potential.
40 The metabolic stimulation by the micropollutants was increased in the presence of light, possibly
41 due to photochemical dissolution of the plastic into smaller bioavailable compounds. Our results
42 suggest that the development and increased activity of these unique microbial communities likely
43 play a role in the bioremediation of the relatively long lived textile and microplastic pollutants in
44 marine habitats.

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46 **INTRODUCTION**

47 Plastics are synthetic organic polymers that are composed of long chains of monomers primarily
48 made from petrochemical sources ¹. The mismanagement of waste in regions with high coastal
49 population density has been linked to high plastic input into the ocean, resulting in an annual flow
50 of 4.8 to 12.7 million tons per year since 2010 ². Once released in the environment, debris are

51 readily colonized by complex microbial communities³. Consequently, macro- and micro-litter
52 may facilitate microbial dispersal throughout the marine realm. However, knowledge gaps
53 regarding the mechanisms of microbial biodegradation of plastic and textile waste.

54 Plastic-degrading microorganisms have been studied since the 1960's. Summer studied the
55 inhibition of microorganism growth for lasting polymers, to counter the deterioration of plastics
56 due to mold and bacteria, because some plasticisers (chemical additives used to provide strength
57 and flexibility), e.g., Ester-type plasticisers, are in turn a source of nutrients, which sustains
58 microbial activity leading to natural degradation of the polymer⁴. More recent studies have shed
59 some light on the diversity of microbial communities colonizing synthetic polymers. For example,
60 Zettler *et al.* (2013) identified a highly diverse microbial community settled on plastic debris,
61 termed as the "plastisphere", unraveling polymer-dependent communities⁵. Moreover, its species
62 richness appears to be more important than the microbial community in seawater samples for a
63 given surface^{3,5}. The colonization of plastic debris by bacteria is hypothesized as a two-step
64 settlement: primary colonization by α - and γ -proteobacteria, and subsequent secondary
65 colonization by Bacteroidetes^{6,7}.

66 Bioremediation of plastic pollution can be aided by heterotrophic bacteria⁸. These
67 microorganisms may survive by extracting the carbon from plastic particles, via hydrolysis of the
68 hydrocarbon polymer^{7,8}. Recent studies have identified a few bacterial species able to deteriorate
69 plastics, for example, *Ideonella sakainesis*, which is a betaproteobacterium actively degrading
70 polyethylene (PE)⁹. The plastisphere also harbours a variety of potential pathogens, i.e., harmful
71 microorganisms to animals, such as *Vibrio* spp.^{3,5,6}. Indeed, Lamb *et al.* observed the transfer of
72 harmful bacteria from plastic litter to reef-building corals, causing three diseases (skeletal eroding
73 band disease, white syndromes and black band disease), which led to coral mortality¹⁰. Such
74 observation highlights the need for reducing and taking action on plastic pollution in the
75 environment.

76 Microparticles of textile waste (i.e., synthetic and natural fibers) enter the ocean due to
77 atmospheric deposition and poor wastewater incubation plant filtration systems allowing the
78 leakage of fibers to the aquatic environment, making it one of the most abundant and recorded
79 micropollutants at sea^{11,12}. Thus, this study aims to assess the potential for bioremediation of
80 microplastics and textile waste by marine microbial communities in a controlled microcosm
81 experiments.

82 The main questions addressed here are whether specific micropollutant-associated microbial
83 communities develop in the presence of high-density polyethylene (HDPE) microbeads and textile
84 fibers as sole source of carbon, and how these substrates influence their metabolic state.
85 Furthermore, we investigated whether light has an impact on the development and metabolism of
86 these communities. We hypothesized that hydrocarbon-degrading microbes can use microplastics
87 and textile waste as the sole carbon source, and that different types of microplastics will select for
88 unique communities with different levels of activity. Because light also plays a role in the abiotic
89 degradation of organic matter in aquatic environments¹³, we hypothesized that exposure to light
90 may also improve the ability of the bacteria to utilize carbon from plastic polymers as a growth
91 substrate due to its photochemical dissolution. The results contribute to our understanding of the
92 formation and development of plastics and textile-waste-associated microbes and their potential
93 role in bioremediation of these widespread environmental micropollutants.

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96 MATERIAL AND METHODS

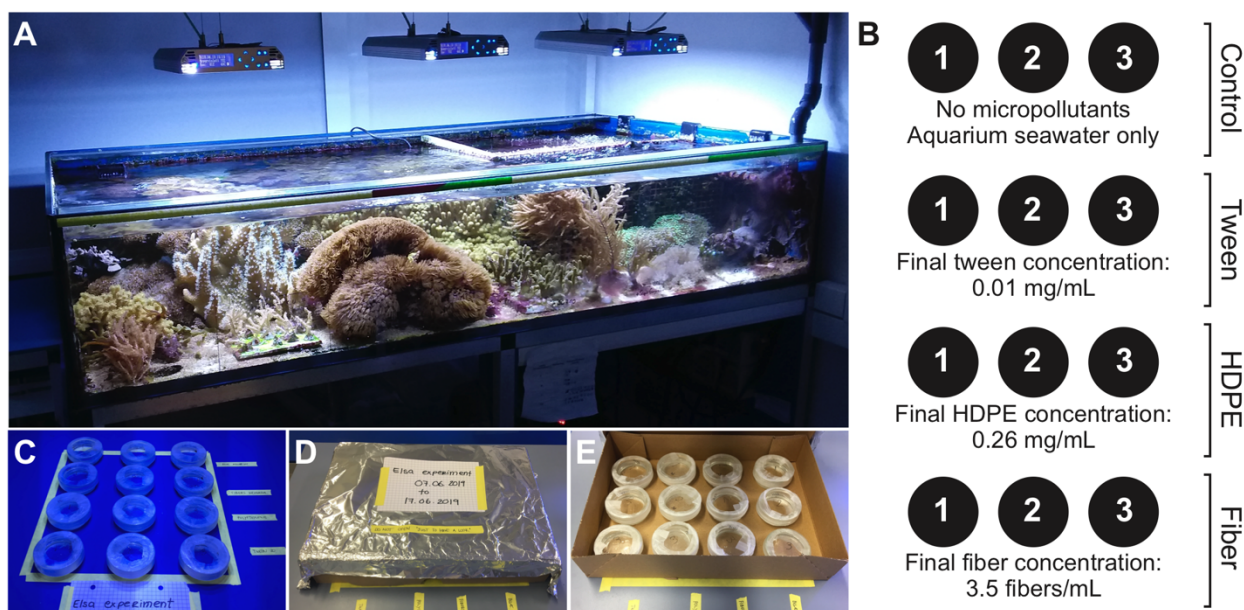
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98 **Experimental setup and sampling.** A total of 15 mL of aquarium seawater containing
99 microbial communities were incubated in 20 mL glass petri dishes for 108 h at room temperature,
100 which received either no micropollutants (control), polysorbate (Tween) 20, Tween 20 and HDPE
101 microbeads, or textile fibers (Tab. 1, Fig. 1). Tween 20 was used as a control since it is used as an
102 emulsifier for the HDPE microbeads and thus serves to test whether the microbes respond only to
103 the Tween or also are effected by the HDPE itself. The artificial seawater microbial community
104 comes from an aquarium (642 L) built of imported live rocks, which hosts many reefs organisms,
105 such as hexacorals, octacorals, gorgonians, sea anemones (*Aiptasia* sp.), marine sponges
106 (*Lendenfeldia chondrodes*, *Tethya wilhelma*), marco-algae (*Chaetomorpha linum*) and
107 cyanobacteria, mussels (*Mytilus edulis*) and benthic foraminifera (*Elphidium crispum*) (Fig. 1A).
108 For each of these incubations, one set was placed under LEDs (Mitras LX6200 HV; light spectrum
109 of 380 nm to 700 nm) with a 12/24 h light cycle (referred to as “light”) and the other one placed
110 inside a cardboard box covered with aluminium foil to block incoming light (referred to as “dark”)
111 (Fig. 1). Each incubation set consists of twelve glass petri dishes sealed with parafilm containing
112 a submerged oxygen sensor spot (PreSens Precision Sensing): three controls and nine incubations

113 (Fig. 1B). The oxygen sensor spot was positioned at the bottom of the petri dish to measure the
114 minimal concentration of O₂, that could be dissolved into the bottom water of the petri dish after
115 diffusion from the overlying headspace.

116 The oxygen concentration in each incubation was closely monitored over the first 48 h of the
117 experiment using a Stand-alone Fiber Optic Oxygen Meter (PreSens Fibox 4) interacting with the
118 oxygen sensor spots. At the beginning of the experiment, four samples of 15 mL were collected
119 from the aquarium to assess the initial microbial community (T₀; referred to as “aquarium”). All
120 incubations and aquarium samples were processed through 4 or 15 mL Amicon® Ultra Centrifugal
121 Filters (4000 rpm, RCF 3399 *g, 10 min. at 20 °C) to concentrate microbes and associated particles
122 depending on the incubation. The concentrated supernatant was equally transferred in two Lysing
123 matrix E tubes for every incubation.

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126 **Figure 1.** Experimental setup. A) Aquarium hosting a small reef ecosystem from which 15 mL was transferred
127 into each incubation petri dish. B) incubation set of twelve glass petri dishes and associated incubations. C)
128 Experimental display under artificial sunlight. D) and E) experimental display with limited access to light.

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Incubation Description

Control	Control petri dishes containing only 15 mL of artificial seawater, with no micropollutants.
Tween	0.1% Tween 20 solution produced following the solubilisation protocol of Cospheric LLC (https://www.cospheric.com/). 150 μ L of tween solution was added to 15 mL of artificial seawater. Final tween concentration: 0.01 mg/mL.
HDPE	Virgin HDPE microbeads (1-4 μ m; 0.96 g/cm ³) solubilized in 0.1% Tween 20 solution (final concentration of 2.6% solid solution of HDPE). 150 μ L of HDPE solution was added to 15 mL of artificial seawater. Final HDPE concentration: 0.26 mg/mL.
Textile fiber	Ca. 500 textile fibers were collected from intertidal sediment at Coral Eye Resort (Bangka Island, North Sulawesi, Indonesia). Fibers were washed twice in Ethanol >99%. 150 μ L of Milli-Q H ₂ O containing 50-60 fibers was added to 15 mL of artificial seawater. Final fiber concentration: 3.5 fibers/mL.

133

134 **Table 1.** Description of each incubation type, equally exposed to light and dark conditions.

135

136 **Quantitative PCR.** The DNA was extracted as in Pichler *et al.* (2018), using 1 mL of a C1
137 extraction buffer¹⁴. To lyse cells, the samples were subsequently heated at 99 °C for 2 min, frozen
138 at -20 °C for 1 h, thawed at room temperature, and heated again at 99 °C for 2 min. After
139 homogenization and centrifugation, the samples were concentrated a second time through Amicon
140 filters, down to a final volume of ca. 100 μ L. The supernatant was purified following the DNeasy®
141 PowerClean® Pro Cleanup Kit (Qiagen, Hilden, Germany). To assess the number of 16S copies
142 at the end of the experiment, all samples were amplified using quantitative PCR (qPCR; Bio-Rad
143 CFX connect™ Real-Time System). Every reaction contained 4 μ L of DNA template, 10 μ L of

144 Supermix, 5.2 μL H₂O, and 0.4 μL of forward and reverse primer, and were subject to the following
145 PCR program: denaturation at 95 °C for 3 min, and 40 amplification cycles (denaturation at 95 °C
146 for 10 s, annealing 55 °C for 30 s). All qPCR reactions were set up using an Eppendorf EpMotion
147 pipetting robot that has <5% technical variation and results in qPCR reaction efficiencies (standard
148 curves) having >90%¹⁵.

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150 **16S amplicon library preparation.** To assess the diversity of the microbial community in the
151 experimental samples, the V4 hypervariable region 16S rRNA gene (ca. 250 base pairs) was
152 amplified with a set of primers (515F 5'-TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'
153 and 806R 5'-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3'), combined to a forward
154 (P5) and reverse (P7) adaptor, and unique dual indices for every sample¹⁴. The preparation of the
155 samples for the polymerase chain reaction (PCR) was done according to Pichler *et al.* (2018). In
156 short, 4 μL of extracted DNA was mixed to 5 μL 5x PCR buffer, 1 μL 50 mM dNTP, 1 μL forward
157 515F and 1 μL reverse 806R primers, 9.9 μL H₂O, 3 μL MgCl₂ and 0.1 μL Taq DNA polymerase,
158 for a total volume of 25 μL for each sample. The amplification took place under specific PCR
159 settings: denaturation at 95 °C for 3 min, 35 amplification cycles (denaturation at 95 °C for 10 s,
160 annealing 55 °C for 30 s, elongation 72 °C for 1 min), and elongation at 72 °C for 5 min to ensure
161 polymerization of all amplified DNA strands. PCR products were run through a 1.5% (w/v)
162 agarose gel, and DNA strands were subsequently extracted using the QIAquick® Gel Extraction
163 Kit (Qiagen, Hilden, Germany). The DNA concentration was quantified using the fluorometer
164 QuBit 2.0 (Life Technologies, Grand Island, USA) and its associated dsDNA high-sensitivity
165 assay kit. As preparation for 16S amplicon sequencing, all samples were pooled together by adding
166 5 μL of every sample at a DNA concentration of 1 nM.

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168 **16S amplicon sequencing.** A high diversity library was added to the 16S amplicon pool to
169 enhance the recognition of the 16S sequences by the Illumina MiniSeq. The DNA was denatured
170 by adding of 0.1 nM NaOH for a short period of 5 min, which was then directly neutralized with
171 a tris-HCl buffer (pH 7) to avoid hydrolyzation of the DNA. To not overload the flow cell, a two-
172 step dilution was performed on the 16S pool for a final DNA concentration of 1.8 pM, resulting in
173 a final volume of 500 μL MiniSeq solution. Four additional sequencing primers after¹⁴ were added
174 to successfully undergo the dual-index barcoding with the MiniSeq. Finally, the prepared 1.8 pM

175 solution of 16S, transcriptomes, and the four sequencing primers was loaded into the reagent
176 cartridge.

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178 **Data analysis.** To transform the demultiplexed sequences from Illumina MiniSeq into an OTU
179 table, the raw data was manipulated using USEARCH v11.0 (<https://drive5.com/usearch>)¹⁶
180 following the method developed by Pichler and colleagues (2018). Most similar sequences sharing
181 at least 97% of bases were grouped, and associated to an operational taxonomic unit (OTU). Each
182 OTU was classified within the Taxonomic Classification System using MacQiime v1.9.1
183 (<http://qiime.org/>). To keep a control on the analyzed data, OTUs of less than 10 reads to all
184 samples were discarded. Statistical analyses were computed in R v3.3.3¹⁷. For phylogenetic
185 reconstruction, most abundant selected OTUs were identified using blastn (BLAST®,
186 <https://blast.ncbi.nlm.nih.gov/>). Sequences were aligned in MAFFT v7.427 (<https://mafft.cbrc.jp/alignment/software/>). The phylogenetic tree was inferred using Seaview v4.7¹⁸ under PhyML
187 optimized settings (GTR model), including 100 bootstrap replicates¹⁹. All related primary data
188 and R scripts are stored on GitHub (<https://github.com/PalMuc/PlasticsBacteria>).

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191 **Raman spectroscopy.** Forty textile fibers were randomly subsampled and their associated
192 spectrum obtained with a HORIBA JOBIN YVON XploRa ONE micro Raman spectrometer
193 belonging to the Mineralogical State Collection Munich (SNSB). The used Raman spectrometer
194 is equipped with edge filters, a Peltier cooled CCD detector and three different lasers working at
195 532 nm (green), 638 nm (red) and 785 nm (near IR). To perform the measurements the near IR
196 Laser (785 nm) was used, with a long working distance objective (LWD), magnification 100x
197 (Olympus, series LMPlanFL N), resulting in a 0.9 μm laser spot size on the sample surface. The
198 wavelength calibration of the IR laser was performed by manual calibration with a pure Si wafer
199 chip, the main peak intensity had values in the interval $520\text{ cm}^{-1} \pm 1\text{ cm}^{-1}$. The wave number
200 reproducibility was checked several times a day providing deviation of less than $< 0.2\text{ cm}^{-1}$.
201 Monthly deviation was in the range of 1 cm^{-1} before calibration. The necessary power to obtain a
202 good-quality spectrum varied between 10% and 50% (i.e., respectively 2.98 mW and 18 mW \pm
203 0.1 mW on the sample surface) depending on the type and degraded stage of the measured textile
204 fiber. The pin-hole and the slit were respectively set at 300 and 100. Each acquisition included two
205 accumulations with a grading of 1200 T and an integration time of 5 s over a spectral range of 100

206 to 1600 cm^{-1} . The precision of determining Raman peak positions by this method is estimated to
207 be ± 1 to ± 1.5 cm^{-1} . Resulting Raman spectra were analyzed using LabSpec Spectroscopy Suite
208 software v5.93.20, treated in R v3.3.3, manually sorted in Adobe Illustrator CS3, and compared
209 with available spectra from published work. All related Raman spectra and R scripts are stored on
210 GitHub (<https://github.com/PalMuc/PlasticsBacteria>).

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213 **RESULTS & DISCUSSION**

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215 For four days, a coral reef aquarium microbial community was incubated with Tween 20, HDPE
216 microbeads and intertidal textile fibers in a microcosm experiment to test its potential to
217 bioremediate widespread micropollutants. After sequencing of the V4 hypervariable region of the
218 16S rDNA genes a total of 1,463,028 sequences were obtained, from a total of 50 samples. After
219 the quality control on the data, all sequences were clustered in 3,884 OTUs, of which 563 (85%)
220 could be taxonomically classified.

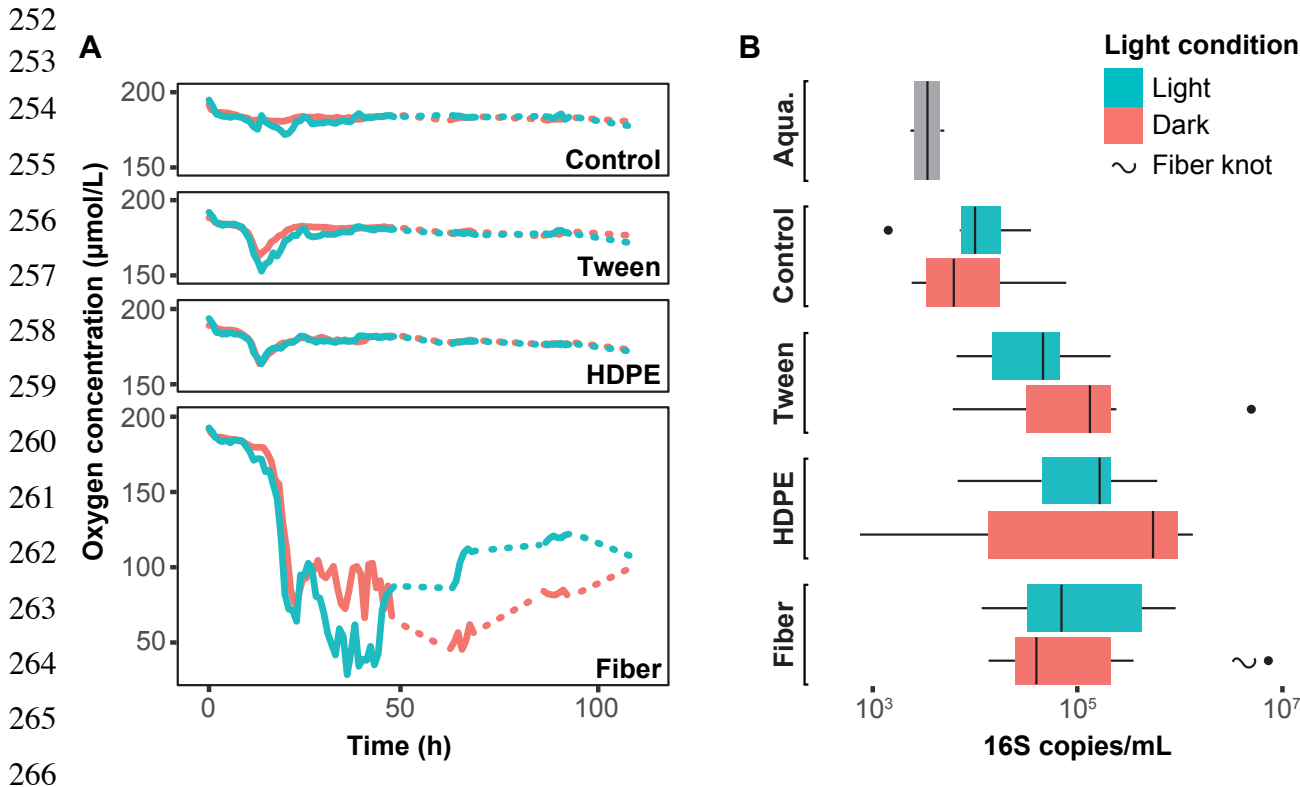
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222 **Respiration and induced microbial activity.** Ten to 12 hours after the beginning of the
223 experiment, a noticeable decrease in oxygen concentration was measured in all incubations
224 containing micropollutants that was not observed in the control (Fig. 2A). This increased oxygen
225 consumption in the presence of micropollutants indicates that microbial metabolism was
226 stimulated by these pollutants, and their utilization as a carbon source for growth. It is likely that
227 the transition between initial and final microbial communities initiated at this time in all
228 incubations, which correlates with the theory that plastic surfaces are colonized within 24 h in the
229 natural environment ⁶.

230 Control, tween and HDPE incubations reached an equilibrium between oxygen consumption and
231 production after this time, whereas microbial communities in the textile fiber incubations
232 continued to consume oxygen at a high rate (Fig. 2A). This indicates a higher microbial activity
233 induced by the intertidal textile fibers. These fibers were mainly pigmented with a black dye (CI.
234 reactive black 5 or 8) and a blue dye (phthalocyanine 15 (PB15)) according to the Raman analysis
235 ^{20,21}. Indeed, as much as 42% of the fiber spectra expressed only the fiber pigment, covering the
236 fabric signal and preventing the identification of the polymer composition (Fig. 3). Here, only

237 cotton expressed a signal strong enough to be recognized in the Raman spectra, with characteristic
238 Raman peaks at positions 379, 435, 953, 1091 and 1116 cm^{-1} (Fig. 3). Hence, at least 44% of all
239 fibers collected in the intertidal zone were identified as cotton, which appears to be one of the most
240 abundant fiber materials found in the environment ^{11,22}.

241 As textile fibers were sampled directly from an intertidal sandy beach in Indonesia (see Table
242 1), they were already exposed to high ultraviolet (UV) radiation and temperature, which are the
243 main factors participating in polymer degradation and fragmentation on beaches ²³⁻²⁵. Indeed, UV
244 radiation causes photooxidative degradation, which results in breaking of the polymer chains,
245 produces free radicals and reduces the molecular weight, causing deterioration of the material,
246 after an unpredictable time ²⁶. This process rendered the textile fibers more subject to colonization
247 in comparison to virgin HDPE microbeads, due to their advanced deteriorated stage, and likely
248 facilitated the hydrolysis of carbon by hydrocarbon-degrading bacteria ²⁷. Another study, by
249 Romera-Castillo et al., demonstrated that irradiated plastic debris stimulated microbial activity in
250 a mesocosm experiment in comparison to virgin plastics, supporting the results obtained in our
251 study ²⁸.



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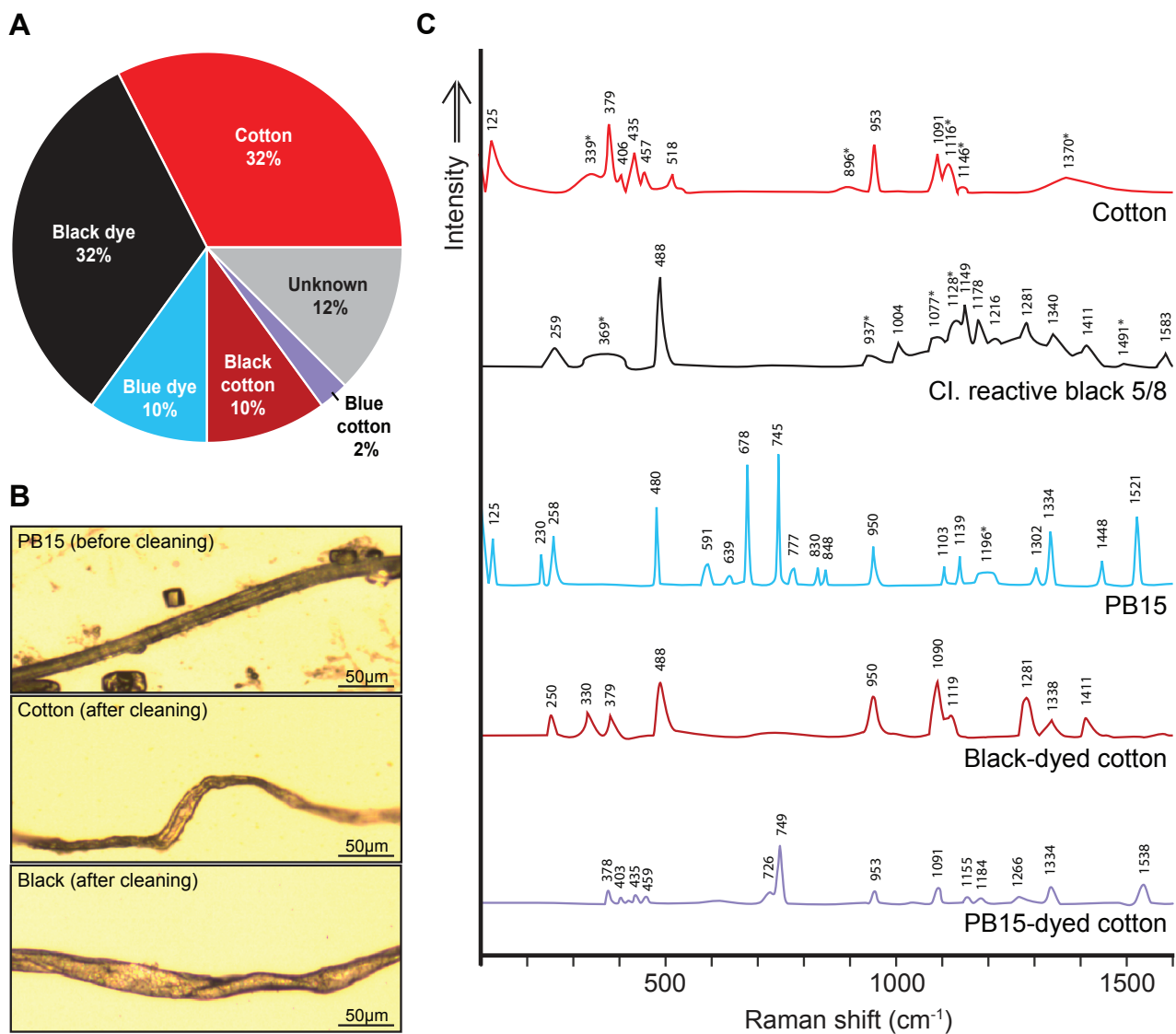


Figure 3. Analysis of the textile fiber sample extracted from the intertidal sediment at Coral Eye Resort. A) fiber type ratio based on signals obtained with Raman spectroscopy. B) photomicrographs of different fibers measured with Raman spectroscopy, illustrating examples of fibers before and after cleaning. C) Raman spectra of five fiber types identified. Numbers indicate the position of the peaks (cm⁻¹) and the associated asterisk (*) indicates a broad peak. Note: in 42% of the measurements, only the pigment signals were expressed, covering the polymer signature and preventing the identification of the fabric of those fibers. Only cotton seems to have a signal strong enough to overcome the pigment signature.

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274 **Microbial community development.** Another indication that microbial communities developed
275 according to the provided carbon source (i.e., tween, HDPE microbeads and intertidal textile
276 fibers) is the higher amount of 16S copies in tween, HDPE and textile fiber incubations measured
277 using qPCR, in comparison to the control incubations (Fig. 2B). Moreover, microbial communities
278 were significantly different (Analysis of Similarity: $P < 0.01$) between incubation types (Fig. 4),
279 in comparison to the initial microbial community measure from the aquarium, which was
280 dominated by the families Bacillaceae, Planctomycetaceae, Bacteriovoraceae and
281 Cellvibrionaceae (Fig. 5).

282 Flavobacteriaceae and Vibrionaceae were particularly ubiquitous in control, tween and HDPE
283 incubations. However, HDPE-incubated communities were enriched with bacteria from the Family
284 Alcanivoraceae, whereas communities from the textile fiber incubations were enriched with

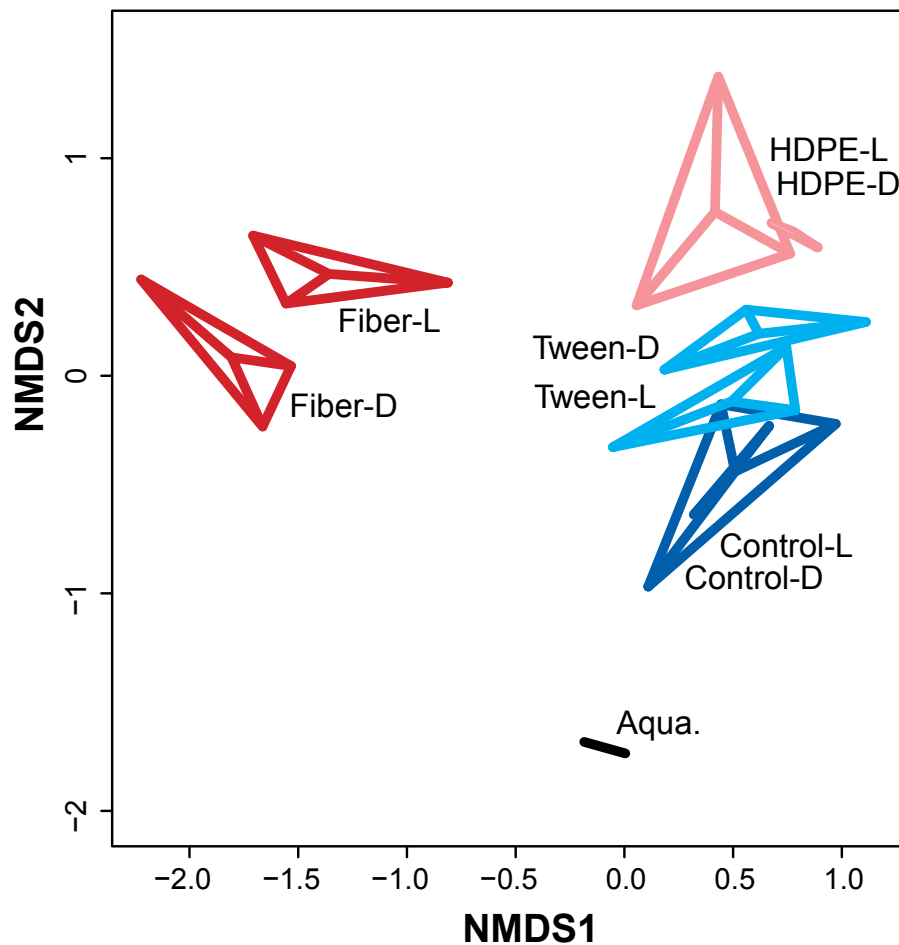


Figure 4. Non-metric multidimensional scaling (NMDS) analysis highlighting the difference in arbitrary distances between incubation-specific microbial communities (ANOSIM $p = 0.001$).

285 bacteria from the Kordiimonadaceae and Cellvibrionaceae (Fig. 5). The observed difference
 286 between microbial communities from micropollutant-bearing and control incubations might also
 287 be explained by an accumulation of plastic-associated dissolved organic carbon (DOC) in the
 288 microcosms²⁸. Romera-Castillo et al. discovered that plastic debris releases a non-negligible
 289 amount of DOC, most of it leaches within the first few days after the initial contact with seawater,
 290 which would be applicable to the virgin HDPE microbeads²⁸.

291 Control and tween incubations had a highly variable microbial communities between replicates,
 292 whereas HDPE and textile fiber incubations showed very similar replicates. Nonetheless, all had
 293 differences in the community composition between light and dark settings (Fig. 4, 5). The disparity
 294 between microbial communities from incubations with HDPE microbeads and textile fibers

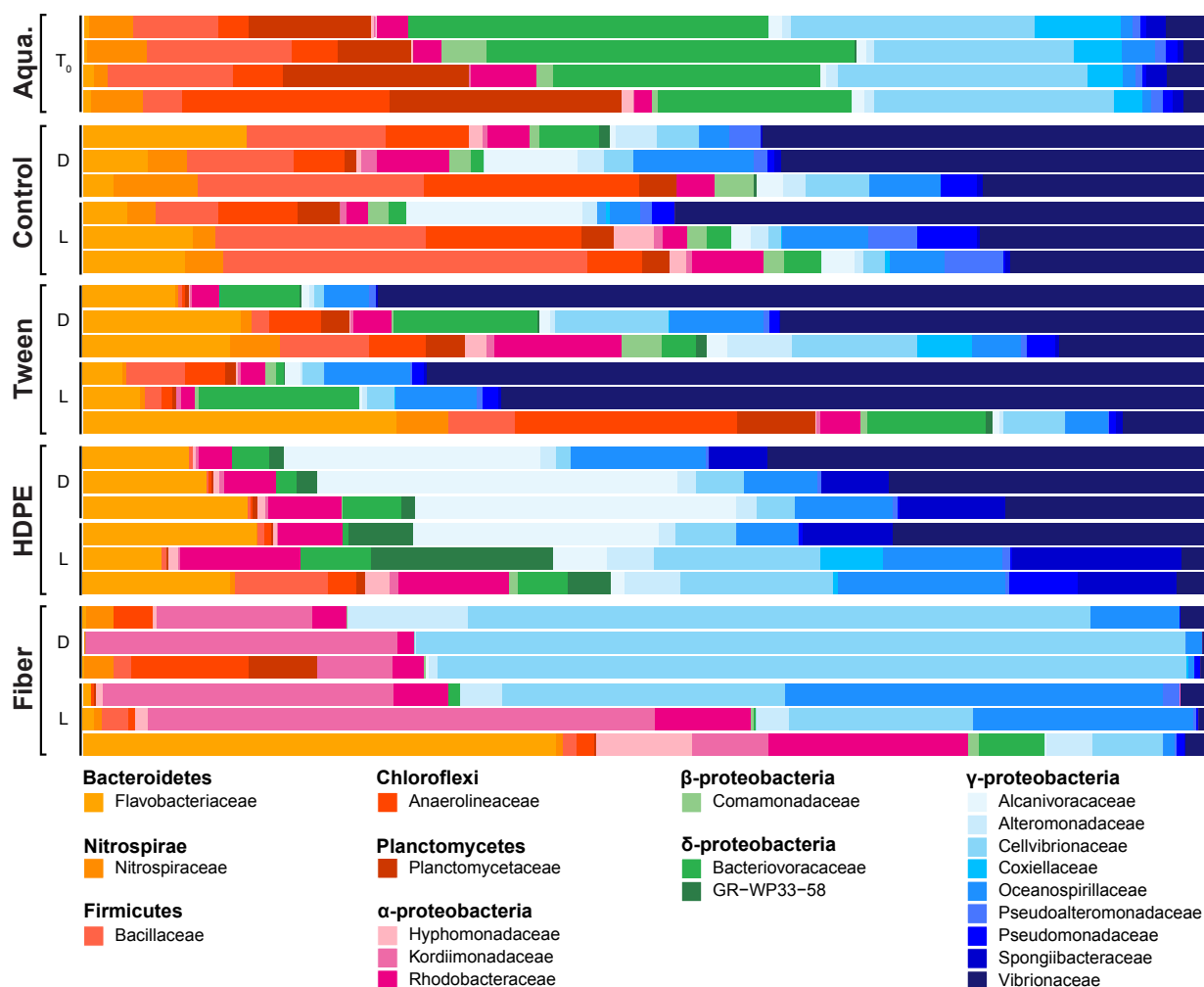


Figure 5. The relative proportion of the 20 most abundant families across all incubations, separated into incubation types and light conditions (T₀: initial community; D: dark; L: light).

295 indicates the development of polymer-dependent taxa, which is also supported by the findings of
 296 Frère et al. (2018). The families Oceanospirillaceae, Vibrionaceae, Flavobacteriaceae and
 297 Rhodobacteraceae are putative to the plastisphere with common representatives in HDPE and
 298 textile fiber incubations, however less diverse than previously observed in other studies^{3,5}. This
 299 might be related to the short experimental time; micropollutants and debris are otherwise
 300 accumulating over months and years in the ocean³⁰.

301 Operational taxonomic units (OTUs) of bacteria that were enriched in particular incubations
 302 were identified (Fig. 6). Thirty-one OTUs are shared between all incubation types and the
 303 aquarium water itself, of which two OTUs, i.e., OTU001 (γ -proteobacteria) and OTU004
 304 (Flavobacteriia), were highly abundant across all incubations (Fig. 6). Only one taxon was
 305 especially enriched in the tween incubation and shared some OTUs with HDPE and control
 306 incubations, which were largely affiliated with the γ -proteobacteria. Textile fibers were especially
 307 colonized by α - and γ -proteobacteria, identified as first colonizers^{6,7}. HDPE incubations were
 308 mainly characterized by the development of Bacteroidetes (i.e., Family Flavobacteriaceae),
 309 additionally to α - and γ -proteobacteria, hypothesized to colonize plastics at a later stage^{6,7}.

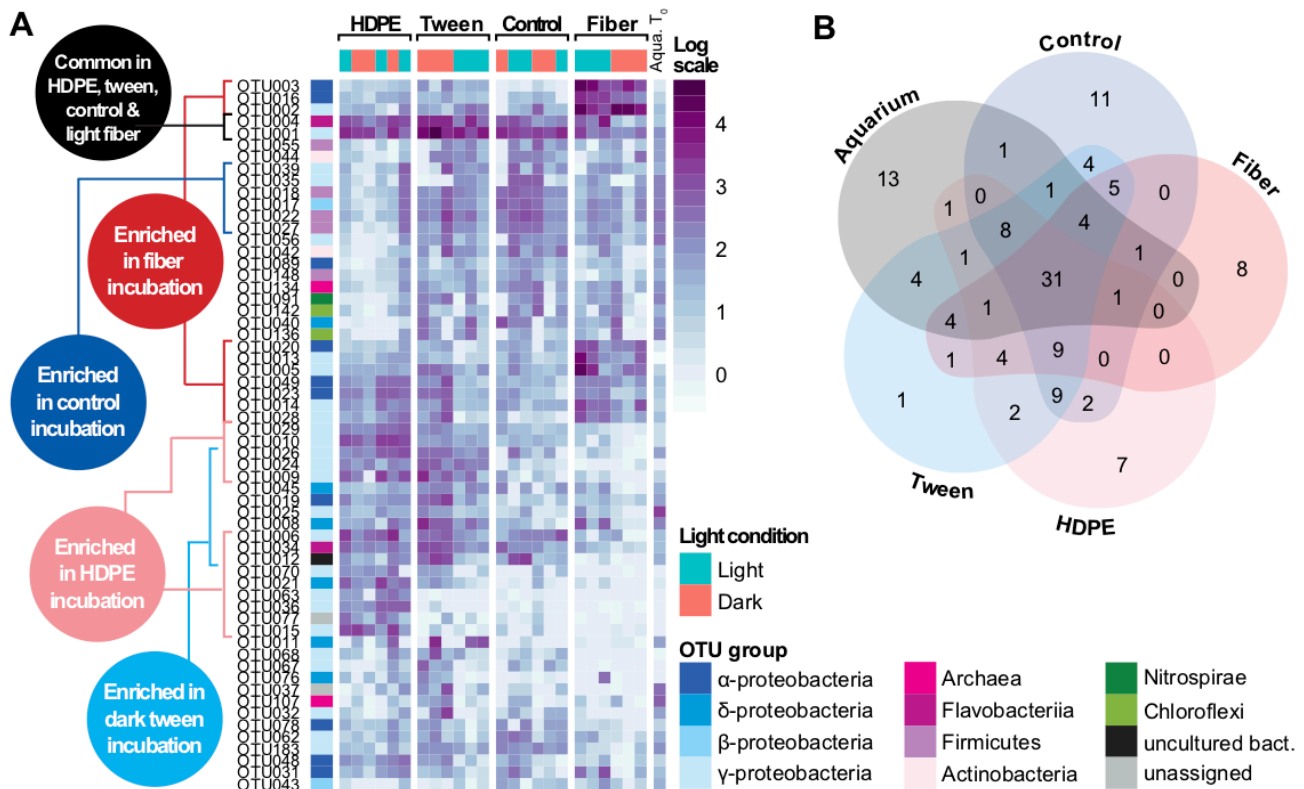


Figure 6. A) Log-scaled heat map highlighting the abundance of the 10 most abundant OTUs in each incubation. B) Venn diagram showing a core community, and incubation-specific OTUs.

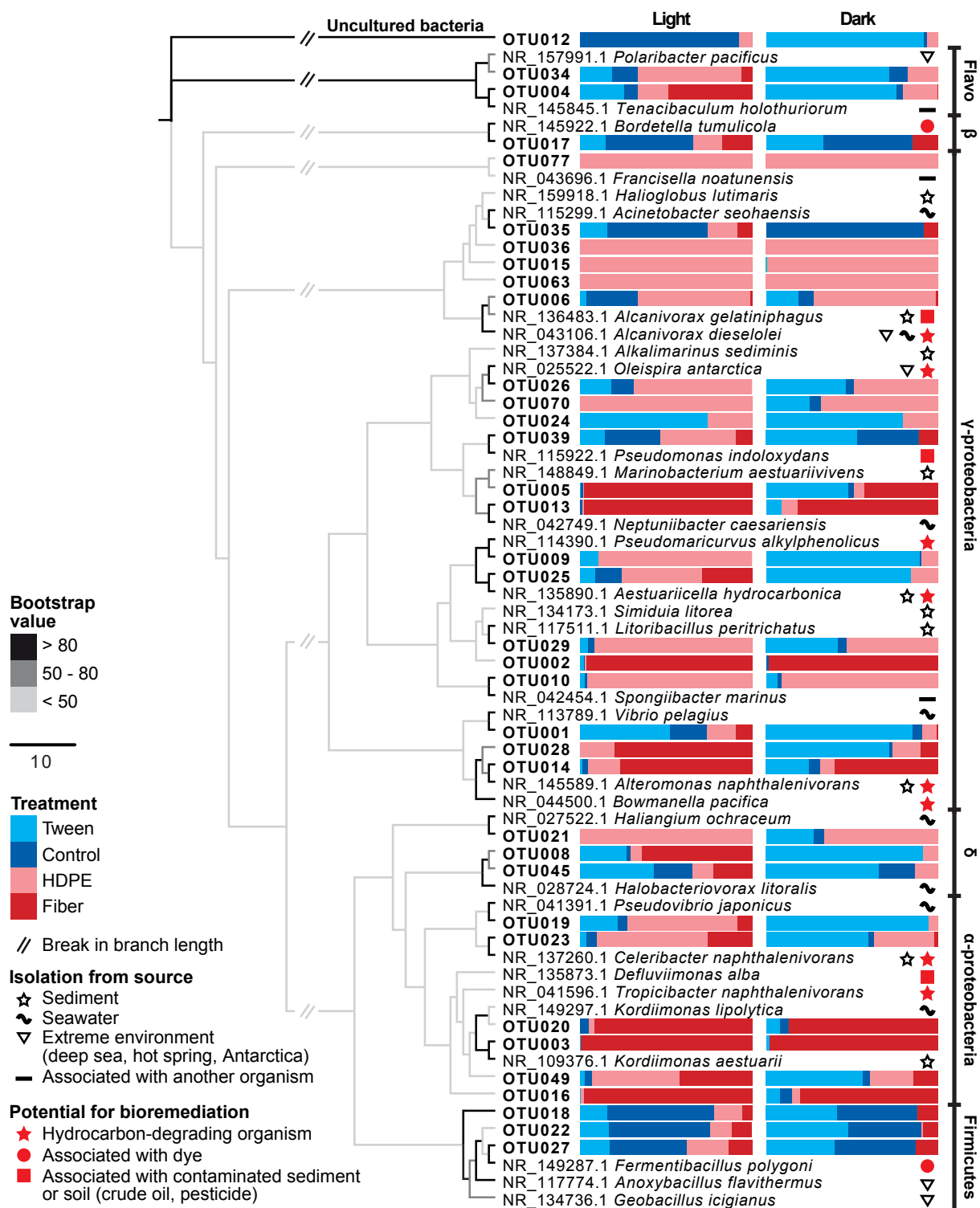


Figure 7. Phylogenetic reconstruction of 36 OTUs identified as incubation-enriched in Fig. 6 and their closest associated named species from NCBI database. The relative abundance of OTUs in each incubation (pale blue: tween; dark blue: control; pale red: HDPE; dark red: fiber) is represented by bar charts, divided according to the light condition. Named- species microorganisms were classified based on their isolation from the source (black star, wave, triangle and line) and marked for their potential to bioremediate microplastics (red star, circle and square).

311 **Impact of light on microbial development.** The presence of light was correlated with the
312 development of unique microbial communities, especially observable in HDPE and textile fiber
313 incubations, similar to the findings of Fuhrman et al. (2008) and Sánchez et al. (2017) regarding
314 the impact of light on the microbial communities^{31,32}. Indeed, the dark-incubated HDPE microbial
315 community was dominated by the three families Vibrionaceae, Alcanivoracaceae and
316 Flavobacteriaceae, whereas the light-grown HDPE microbial community was more diverse with a
317 shared dominance between eight families (Rhodobacteraceae, GR-WP33-58, Spongiibacteraceae,
318 Vibrionaceae, Cellvibrionaceae, Oceanospirillaceae, Alcanivoracaceae, and Flavobacteriaceae)
319 (Fig. 5).

320 Some taxa were enriched under artificial sunlight when incubated with HDPE and textile fibers,
321 but enriched in the presence of Tween 20 under dark conditions. This observation is well
322 represented by OTU028 and OTU049, which were most abundant in textile fiber and HDPE
323 incubations in the presence of light, otherwise dominant in the tween incubation under dark
324 conditions. In general, OTUs enriched in Tween-20 incubations had a higher relative abundance
325 in dark settings, also supported by our qPCR results. The flexibility these taxa show in using carbon
326 from various sources (HDPE microbeads vs Tween 20) depending on the light availability might
327 highlight an opportunistic behaviour³³. Another hypothesis suggests a higher level of available
328 DOC in light-exposed textile fiber and HDPE incubations, caused by the polymer exposure to
329 artificial sunlight^{13,28}. These findings may help us better understand the plastisphere dynamic in
330 situations similar to, for example, microorganisms settled on plastic debris initially floating in the
331 photic zone and later buried in the sediment or sinking in regions with limited light availability.

332
333 **Hydrocarbon-degrading bacteria.** Several OTUs enriched in the different incubation types
334 revealed to be closely related to known hydrocarbon degrading microorganisms (Fig. 7). This,
335 together with the increased rates of oxygen consumption in those incubations, highlights their
336 potential for biodegradation of organic carbon based micropollutants. The six most abundant
337 OTUs of the textile fiber incubation (OTU002, -003, -005, -013, -016 and -020) are closely related
338 to the genera *Kordiimonas* and *Defluviimonas* (α -proteobacteria), and *Simiduila*,
339 *Marinobacterium* and *Neptuniibacter* (γ -proteobacteria) according to the inferred phylogenetic
340 tree. OTU003 and -020 were also closely related to *K. gwangyangensis* (NR_043103.1), which
341 can hydrolyze six different polycyclic aromatic hydrocarbons (PAHs)³⁴, giving this taxon a strong

342 potential for microplastic bioremediation. The genus *Alteromonas*, hosting hydrocarbon-degrading
343 microorganisms, has been previously identified as part of the plastisphere from North Adriatic Sea
344 and Atlantic Ocean ^{5,35}. More specifically, *Alteromonas naphthalenivorans* was identified as a
345 naphthalene consumer ³⁶. *Defluviimonas alba* was isolated from an oilfield water sample
346 suggesting that it has a potential for degradation of hydrocarbon similarly to *Defluviimonas*
347 *pyrenivorans* ³⁷, however not tested for hydrocarbon hydrolysis ³⁸. *Bowmanella pacifica* was
348 identified during a search for pyrene-degrading bacteria ³⁹, which is a PAH highly concentrated in
349 certain plastics ⁴⁰. *Bowmanella* spp. were also identified on polyethylene terephthalate (PET)
350 specific assemblages from Northern European waters ⁴¹. Because these taxa were probably
351 biofilm-forming microorganisms developed on the surface of textile fibers, it is very likely that
352 they partly consumed the polymer, an available source of carbon and energy, owing to their
353 capability to hydrolyze hydrocarbons. Although approximately half of the fibers used in the
354 experiment were of natural fabric, it has been observed that cotton is a powerful sorbent used to
355 treat oil spill ⁴². The latter suggest that cotton fibers may absorb traces of hydrocarbon present in
356 the environment ⁴³ and, therefore, provide a source of hydrocarbon for hydrocarbonoclastic
357 bacteria.

358 The HDPE incubation stimulated the activity and enrichment of six OTUs (OTU001, -004, -006,
359 -010, -015 and -026) closely related to hydrocarbon-degrading bacteria, different from those
360 enriched in textile fiber incubations. For instance, OTU006 was affiliated with the genus
361 *Alcanivorax*, which are specialized in degrading alkanes, especially in contaminated marine
362 environments ⁴⁴. This genus was also identified as being potentially important for PET degradation
363 in the natural marine environment ⁴¹. All other five OTUs were abundant only in light-grown
364 HDPE communities, which were affiliated with the taxa *Pseudomarcus alkylphenolicus*,
365 *Celeribacter naphthalenivorans*, *Oleispira antarctica*, *Tropicibacter naphthalenivorans*, and
366 *Aestuariicella hydrocarbonica*. Given their enrichment in the HDPE incubations in the presence
367 of light, and the increased oxygen consumption relative to the control, it seems likely that these
368 conditions selected for these specific organisms that were uniquely adept to utilize organic carbon
369 from the HDPE as a carbon and energy source. This indicates their potential for the bioremediation
370 of this plastic type. The different respiration rates of HPDE treatment and community assembly
371 compared to the Tween 20 treatment (an emulsifier for the HDPE microbeads) and demonstrates

372 a unique effect on microbial community formation due to the HDPE itself (as opposed to microbes
373 that may just be eating the Tween that is coated on the HDPE).

374
375 **Extrapolating from our experiment to the natural environment.** Although this study did not
376 use “natural” microbial communities from the intertidal habitats from which the textile fibers
377 derive, it tests for the potential response of microbial communities to widespread micropollutants.
378 Since the identified hydrocarbonoclastic bacteria in our incubations are also common to both
379 natural marine sediments and water column^{5,34,39,41,44}, this study contributes to the developing
380 understanding of how hydrocarbon-degrading bacteria utilize various fabric types as a carbon and
381 energy sources during bioremediation processes. Three main lines of evidence indicate that the
382 plastics and textile fibers were consumed by hydrocarbon-degrading microorganisms during the
383 experiment: (1) higher rates of oxygen consumption relative to the control, (2) increased
384 abundance as indicated by qPCR and (3) the development of unique microbial communities that
385 form in incubations containing different types of micropollutants. Moreover, the same groups of
386 hydrocarbonoclastic bacteria found in our study are present in the marine environment. The
387 findings resulting from this study also demonstrated that light availability is an important factor
388 shaping hydrocarbon-degrading bacterial communities. We speculate that this is due to
389 photochemical dissolution of the plastic and textile substrates, which might allow for more readily
390 bioavailable substrates for the developing biofilms. A deeper study into the topic is necessary to
391 enlarge our understanding behind microbial adaptation to changing environmental conditions,
392 including photochemical dissolution of plastic waste.

393

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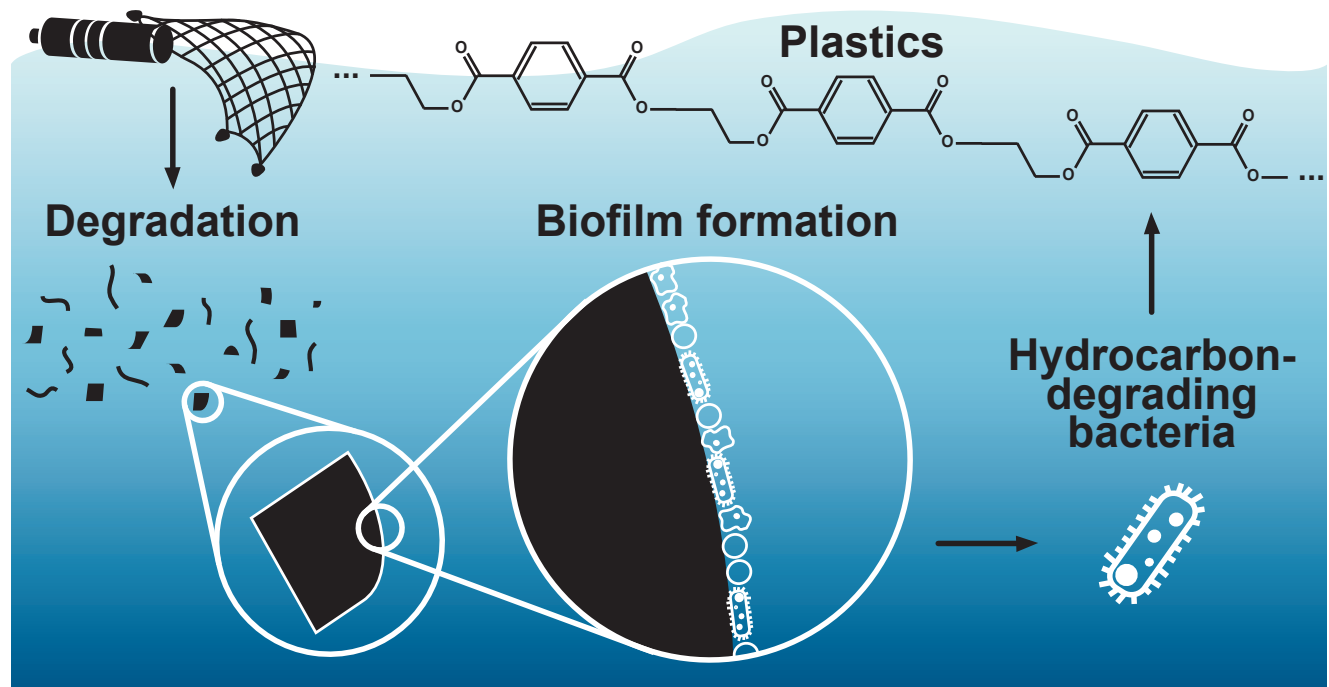
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Graphical Abstract



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