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Brief Report

Biodegradation of textile waste by marine bacterial communities enhanced by light

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Summary

Knowledge of biofilm formation on pollutants in the marine realm is expanding, but how communities respond to substrates during colonization remains poorly understood. Here, we assess community assembly and respiration in response to two different micropollutants, virgin high-density polyethylene (HDPE) microbeads and textile fibres under different light settings. Raman characterization, high-throughput DNA sequencing data, quantitative PCR, and respiration measurements reveal how a stimulation of aerobic respiration by micropollutants is translated into selection for significantly different communities colonizing the substrates. Despite the lack of evidence for biodegradation of HDPE, an increased abundance and respiration of bacterial taxa closely related to hydrocarbonoclastic *Kordiimonas* spp. and *Alteromonas* spp. in the presence of textile waste highlights their biodegradation potential. Incubations with textile fibres exhibited significantly higher respiration rates in the presence of light, which could be partially explained by photochemical dissolution of the textile waste into smaller bioavailable compounds. Our results suggest that the development and increased respiration of these unique microbial communities may potentially play a role in the bioremediation of the relatively long-lived textile pollutants in marine habitats, and that the respiration of heterotrophic hydrocarbon-degrading bacteria colonizing marine pollutants can be stimulated by light.

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Introduction

Plastics are synthetic organic polymers that are composed of long chains of monomers primarily made from petrochemical sources (Scott, 1999). The mismanagement of waste in regions with high coastal population density has been linked to high plastic input into the ocean, resulting in an annual flow of 4.8–12.7 million tons per year since 2010 (Jambeck *et al*., 2015). Once released in the environment, debris are readily colonized by complex microbial communities (Frère *et al*., 2018). Consequently, macrolitter and microlitter may facilitate microbial dispersal throughout the marine realm. However, knowledge gaps regarding the mechanisms of microbial biodegradation of plastic and textile waste.

Plastic-degrading microorganisms have been known for decades, and studied since the 1960s (Summer, 1964). More recent studies have shed some light on the diversity of microbial communities colonizing synthetic polymers. For example, Zettler and colleagues (2013) identified a highly diverse microbial community settled on plastic debris, the 'plastisphere' (Zettler *et al*., 2013), whose species richness appears to be higher compared with the surrounding seawater (Zettler *et al*., 2013; Frère *et al*., 2018). The colonization of plastic debris by bacteria is hypothesized as a two-step settlement: primary colonization by α- and γ-proteobacteria, and subsequent secondary colonization by Bacteroidetes (Oberbeckmann *et al*., 2015; Quero and Luna, 2017).

Bioremediation of plastic pollution can be aided by heterotrophic bacteria (Krueger *et al*., 2015) by extracting the carbon from plastic particles, via hydrolysis of the hydrocarbon polymer (Krueger *et al*., 2015; Quero and Luna, 2017). One example is the bacterium, *Ideonella sakainesis*, which can degrade polyethylene (Yoshida

et al., 2016). The plastisphere also harbours a variety of potential pathogens, such as *Vibrio* spp. (Zettler *et al*., 2013; Oberbeckmann *et al*., 2015; Frère *et al*., 2018) and bacterial pathogens can be transferred from plastic litter to reef-building corals, causing diseases leading to coral mortality (Lamb *et al*., 2018). In addition to microplastics, microparticles of textile waste (i.e. synthetic and natural fibres) enter the ocean due to atmospheric deposition and poor wastewater incubation plant filtration systems allowing the leakage of fibres to the aquatic environment, making it one of the most abundant and recorded micropollutants at sea (Browne *et al*., 2011; Dris *et al*., 2016). Thus, this study aims to assess the potential for bioremediation of the microplastic high-density polyethylene (HDPE) and textile waste by marine microbial communities in controlled microcosm experiments.

The main questions addressed here are whether specific micropollutant-associated microbial communities develop in the presence of HDPE microbeads and textile fibres as sole source of carbon, and how these substrates influence their aerobic respiration rates. Furthermore, we investigated whether light has an impact on the development and respiration of these communities. The results contribute to our understanding of how light enhances the formation and development of plastics- and textile-waste-associated microbial communities, the utilization of these pollutants as a growth substrate, and the associated respiration rates. Our findings demonstrate that light can increase respiration of marine microbial communities growing on textile waste as a sole carbon source, stimulating the development of a unique community that is responsible for degrading this widespread micropollutant.

Results and discussion

O_2 consumption rates and community respiration

During the first 30 h of the experiment (Fig 1), a noticeable decrease in oxygen concentration was measured in all incubations containing micropollutants and tween that was not observed in the control (Fig. 2A,C). This increased oxygen consumption in the presence of micropollutants and tween indicates that microbial metabolism was stimulated by these substrates, and increased copies of 16S rRNA genes in those same incubations compared with the seawater control suggests their utilization as a carbon source for growth. Moreover, it is consistent with the theory that artificial surfaces, such as plastics, are colonized within 24 h in the natural environment (Oberbeckmann *et al*., 2015). HDPE and tween incubations reached equilibrium between respiration and oxygen diffusion through the parafilm after 30 h. However, microbial communities in the textile fibre incubations continued to consume oxygen past 40 h, down to suboxic levels of 25 μM in the presence of light (Fig. 2). Furthermore, in the textile fibre incubations, there were higher oxygen consumption rates in the presence of light

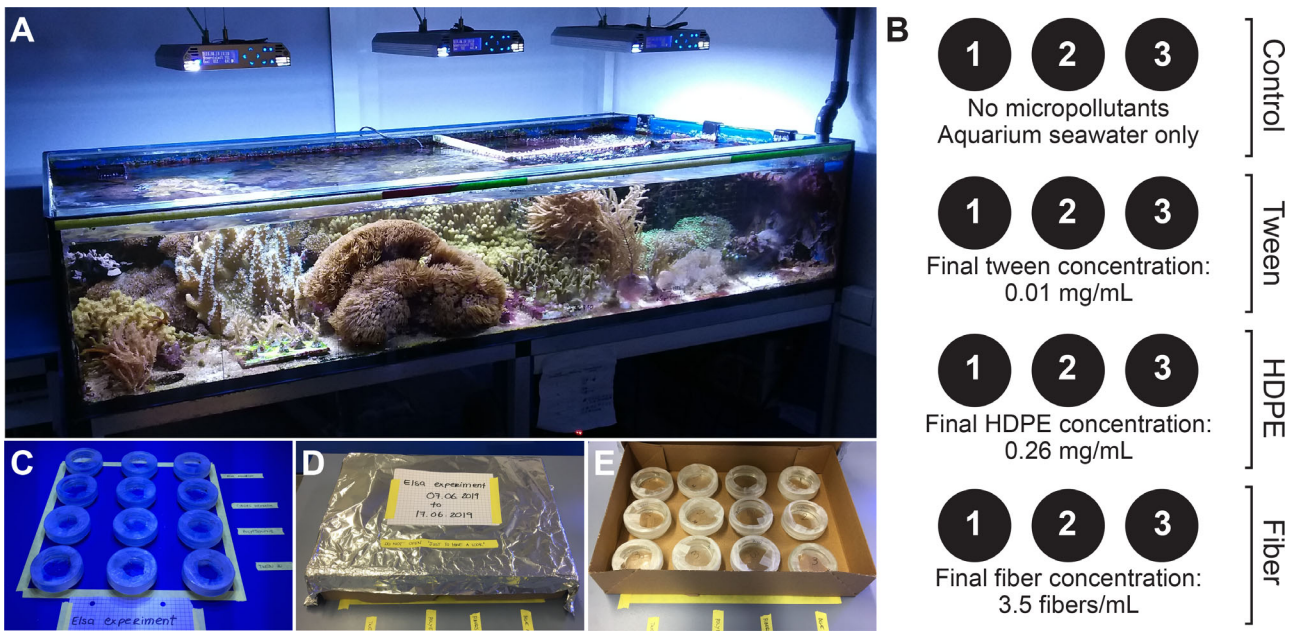

Fig 1. Experimental setup. A. Aquarium hosting a small reef ecosystem from which 15 ml was transferred into each incubation petri dish. B. Incubation set of 12 glass petri dishes and associated incubations. C. Experimental display under artificial sunlight. D, E. Experimental display with limited access to light.

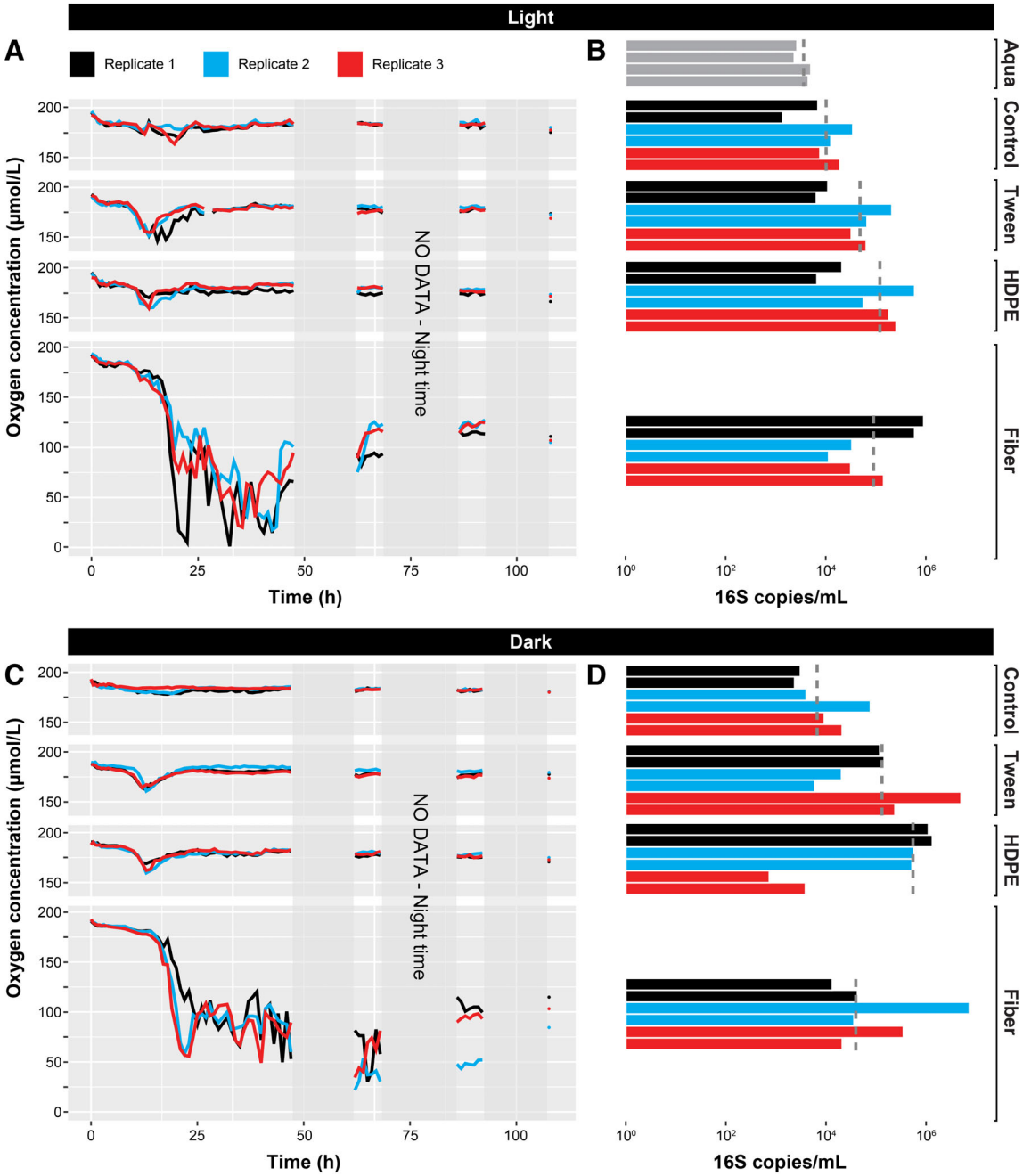

Fig 2. (A, C) Oxygen concentration curve measured over the course of the experiment for each incubation, highlighting the oxygen consumption by microorganisms. Dark bands display night times when no oxygen measurements were taken. (B, D) Associated 16S copies values measured with qPCR at 108 h (the end of the experiment) depicting the microbial growth in the incubations. Incubation replicates are indicated with a different colour (black, blue and red). Each replicate was divided into two for DNA analysis, hence two 16S copies values per replicate. Dashed grey lines represent the median value of the 16S copies per incubation type. (A, B) Data from incubations under light and (C, D) under dark settings.

[5.5 (SD: 0.016) µmol l^{-1} h^{-1}] compared with dark conditions [4.1 (SD: 0.24) µmol l^{-1} h^{-1}] after 30 h of incubation, which was consistent between replicates (Fig. 2) and was statistically significant (Welch two sample *t*-test: $P = 0.036$). A higher respiration rate indicates a higher microbial metabolic activity was induced by the textile fibres, compared with the other tested substrates. The metabolic activity must be higher, because the increased reduction of O_2 must be coupled to an increased oxidation of the added substrate, in this case the textile fibres. Thus, the increased O_2 reduction rate is connected to oxidation of the textile fibres.

It is worth noting that remnant organic matter on the fibres, despite thorough washing steps, might have contributed to the increased oxygen consumption observed in the fibre incubations. The significantly higher oxygen consumption rates in the textile fibre incubations in the presence of light, compared with the dark incubations (Fig. 2), could be explained by ultraviolet (UV) photolysis of polymers resulting in more bioavailable smaller compounds fuelling the metabolism of aerobic bacterial heterotrophs.

These fibres were mainly pigmented with a black dye (CI reactive black 5 or 8) and a blue dye [phthalocyanine 15 (PB15)] according to the Raman analysis (Bouchard *et al*., 2009; Buzzini and Massonnet, 2013). Indeed, as much as 42% of the fibre spectra expressed only the fibre pigment, covering the fabric signal and preventing the identification of the polymer composition (Fig. 3). Here, only cotton expressed a signal strong enough to be recognized in the Raman spectra, with characteristic Raman peaks at positions 379, 435, 953, 1091 and 1116 cm^{-1} (Fig. 3). Hence, at least 44% of all fibres collected in the intertidal zone were identified as cotton, which has been reported to be one of the most abundant fibre materials found in the environment (Dris *et al*., 2016, 2017). The unidentified pigmented fibres were most likely made of either plastic polymers or a mixture between natural and petrochemical fabrics (both referred to as microplastic; Dris *et al*., 2016), as approximately a third of fibres found in the environment are of synthetic material, such as polypropylene (Dris *et al*., 2017).

As textile fibres were sampled directly from an intertidal sandy beach in Indonesia (Table S1), they were already

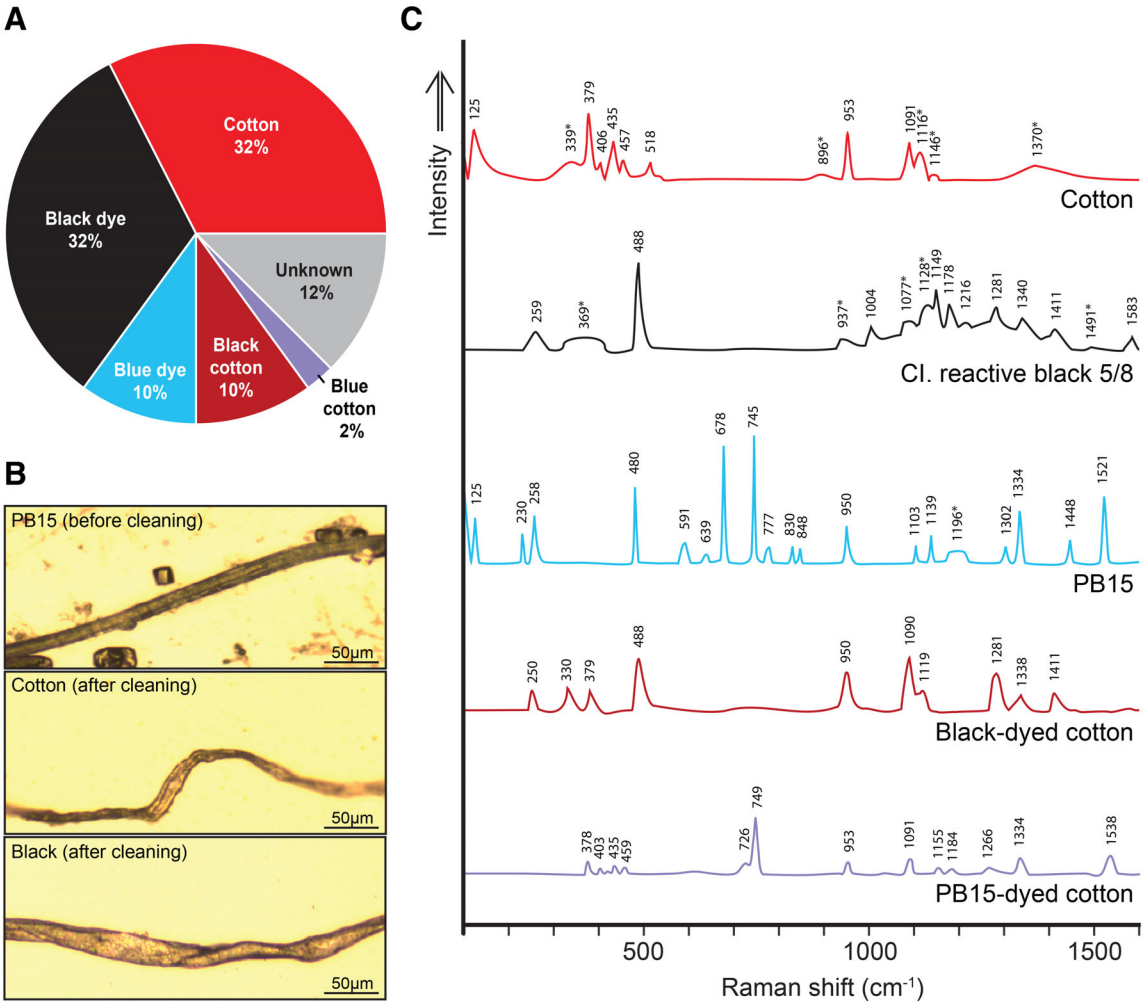

Fig 3. Analysis of the textile fibre sample extracted from the intertidal sediment at Coral Eye Resort. A. Fibre type ratio based on signals obtained with Raman spectroscopy. B. Photograms of different fibres measured with Raman spectroscopy, illustrating examples of fibres before and after cleaning. C. Raman spectra of five fibre types identified. Numbers indicate the position of the peaks (cm^{-}) 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 fibres. Only cotton seems to have a signal strong enough to overcome the pigment signature.

exposed to high UV radiation and temperature, which are the main factors participating in polymer degradation and fragmentation on beaches (Barnes *et al*., 2009; Corcoran *et al*., 2009; Andrady, 2017). Indeed, UV radiation causes photooxidative degradation, which results in breaking of the polymer chains, produces free radicals and reduces the molecular weight, causing deterioration of the material, after an unpredictable time (Singh and Sharma, 2008). It stands to reason that these processes rendered the textile fibres more subject to microbial colonization in comparison to virgin HDPE microbeads, due to their advanced deteriorated state, and likely facilitated the hydrolysis of carbon by hydrocarbon-degrading bacteria (Wilkes and Aristilde, 2017). Another study, by Romera-Castillo *et al*., demonstrated that irradiated plastic debris stimulated microbial activity in a mesocosm experiment in comparison to virgin plastics, supporting the results obtained in our study (Romera-Castillo *et al*., 2018).

Microbial community assembly

After sequencing of the V4 hypervariable region of the 16S rRNA genes a total of 1 463 028 sequences were obtained, from 50 samples. After the quality control on the data, all sequences were clustered in 3884 OTUs, of which 3321 (85%) could be taxonomically classified. In addition to the higher respiration rates in the presence of the added substrates, the higher average concentrations of 16S rRNA gene copies in tween, HDPE and textile fibre incubations compared with the controls indicates that the added substrates were used as a carbon source for growth (Fig. 2B,D). However, differences between 16S rRNA gene copy concentrations in the treatments and the controls were not statistically significant (Welch two sample *t*-test: $P > 0.05$); only the HDPE incubation had significantly higher concentrations of 16S copies in comparison to the starting values in the aquarium (Welch two sample *t*-test: $P = 0.009$). This may be possibly due to a high variability in the number of 16S rRNA gene copies between replicates (Fig. 2). This high variability between replicates was likely due, for example, to an accumulation of textile fibres, clogged together, during the process of DNA extraction, which accumulated at the bottom of the Amicon filters causing some replicates to have higher copy numbers than others. The higher concentration of 16S rRNA gene copies in those replicates however highlights that microbes indeed had colonized the fibres.

Moreover, microbial communities between the seawater control and those on the fibres were significantly different (analysis of similarity test: $R^2 = 0.53$, $P = 0.001$) (Fig. 4), and the textile fibre incubations were enriched with bacteria from the Kordiimonadaceae and Cellvibrionaceae (Fig. 5). The significant difference between microbial community structures from the control and textile incubations (Fig. 4) might also be explained by an accumulation of dissolved organic carbon (DOC) in the microcosms (Romera-Castillo *et al*., 2018). The different microbial communities in the textile fibres incubations indicates the development of polymer-dependent taxa, which is also supported by the findings of Frère and

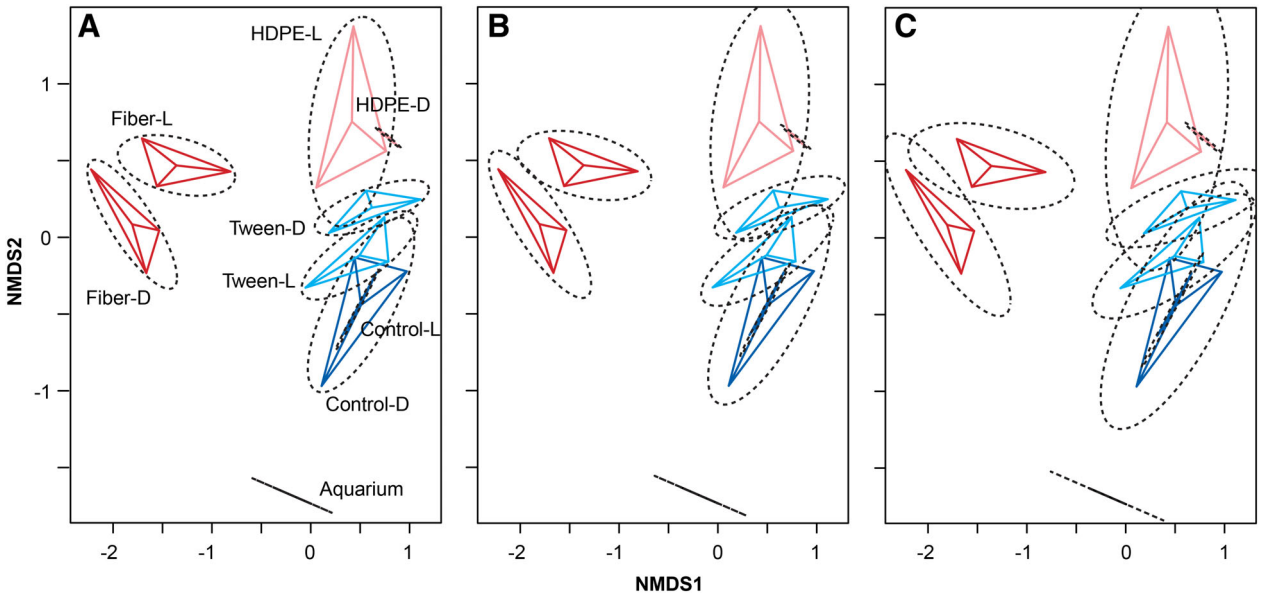

Fig 4. Non-metric multidimensional scaling (NMDS) analysis highlighting the difference in arbitrary distances between incubation-specific microbial communities (ANOSIM: $P = 0.003$). Black dotted lines illustrate (A) 90%, (B) 95% and (C) 99% confidence intervals (CI).

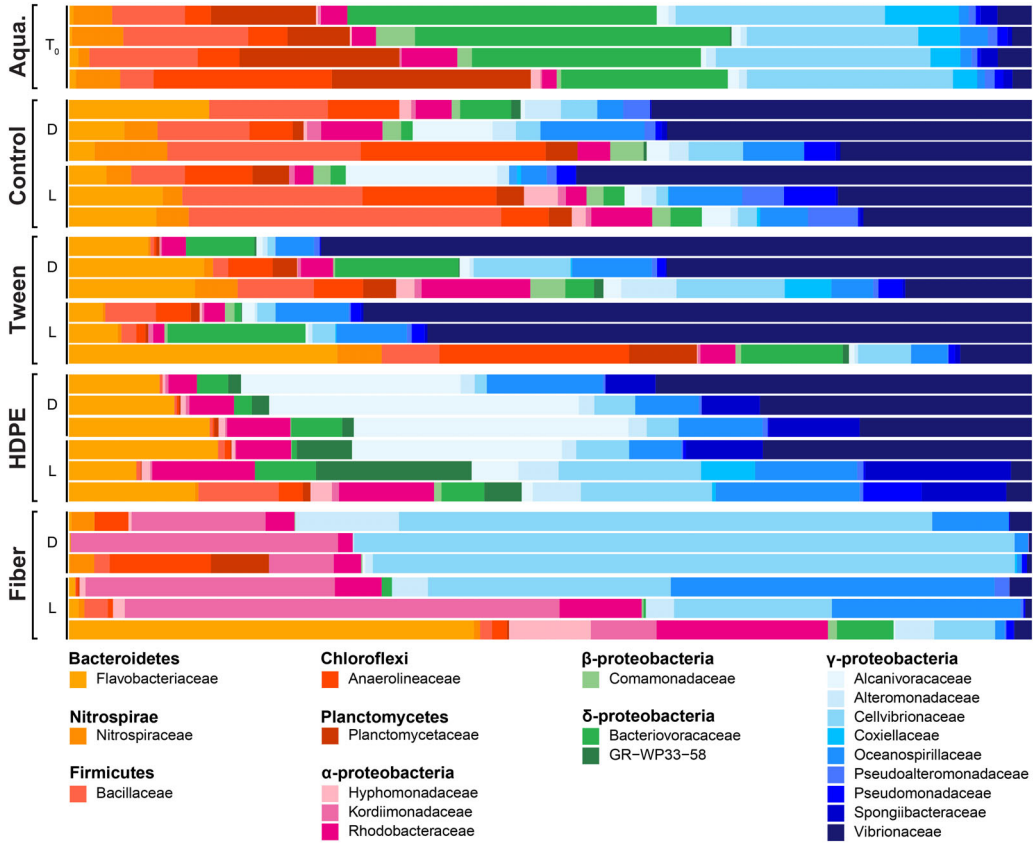

Fig 5. Bar charts illustrating the relative proportion of the 20 most important families across all incubations, separated into incubation types and light conditions (T_0: initial community; D: dark; L: light).

colleagues (2018). Indeed, textile fibres were especially colonized by taxa affiliated with the α- and γ-proteobacteria, groups previously identified as first colonizers (Oberbeckmann *et al*., 2015; Quero and Luna, 2017).

The HDPE and tween (control for HDPE) incubations had significantly different microbial communities (analysis of similarity test: $R^2 = 0.51$, $P = 0.001$), and the differing community structures could be observed both in the ordination analysis (Fig. 4) and the heatmap analysis (Fig. 5). Only one taxon was enriched in the tween incubation and shared some OTUs with HDPE and control incubations, which were largely affiliated with the γ-proteobacteria (Fig. 6). However, the respiration rates between HDPE and tween control incubations were not significantly different (Fig. 1). Thus, the HDPE stimulated the development of a unique microbial community, but this was not translated into an increased respiration rate (as observed in the textile fibre incubations). HDPE incubations were mainly characterized by the development of Bacteroidetes (i.e. Family Flavobacteriaceae), in addition to taxa affiliated with the α- and γ-proteobacteria, hypothesized to colonize plastics at a later stage (Oberbeckmann *et al*., 2015; Quero and Luna, 2017). Furthermore, the families Oceanospirillaceae, Vibrionaceae, Flavobacteriaceae and Rhodobacteraceae are putative members of 'the plastisphere' with common representatives in HDPE and textile fibre incubations, however less diverse than previously observed in other studies (Zettler *et al*., 2013; Frère *et al*., 2018). This might be related to the short experimental time; micropollutants and debris are otherwise accumulating over months and years in the ocean (Lebreton *et al*., 2018).

Impact of light on microbial development

Ordination analysis of the 16S rRNA genes data clearly shows that the added substrate (textile fibres, HDPE, tween) had the strongest effect on the community assembly (Fig. 4, analysis of similarity test: $P = 0.003$). However, within the individual substrate treatments there appears to be a possible additional effect of light on the assembly of the microbial community because communities from light and dark treatments occupy different regions of the ordination space (Fig. 4). Specifically, the difference in microbial communities incubated with textile fibres with, and without light, was found to have weak

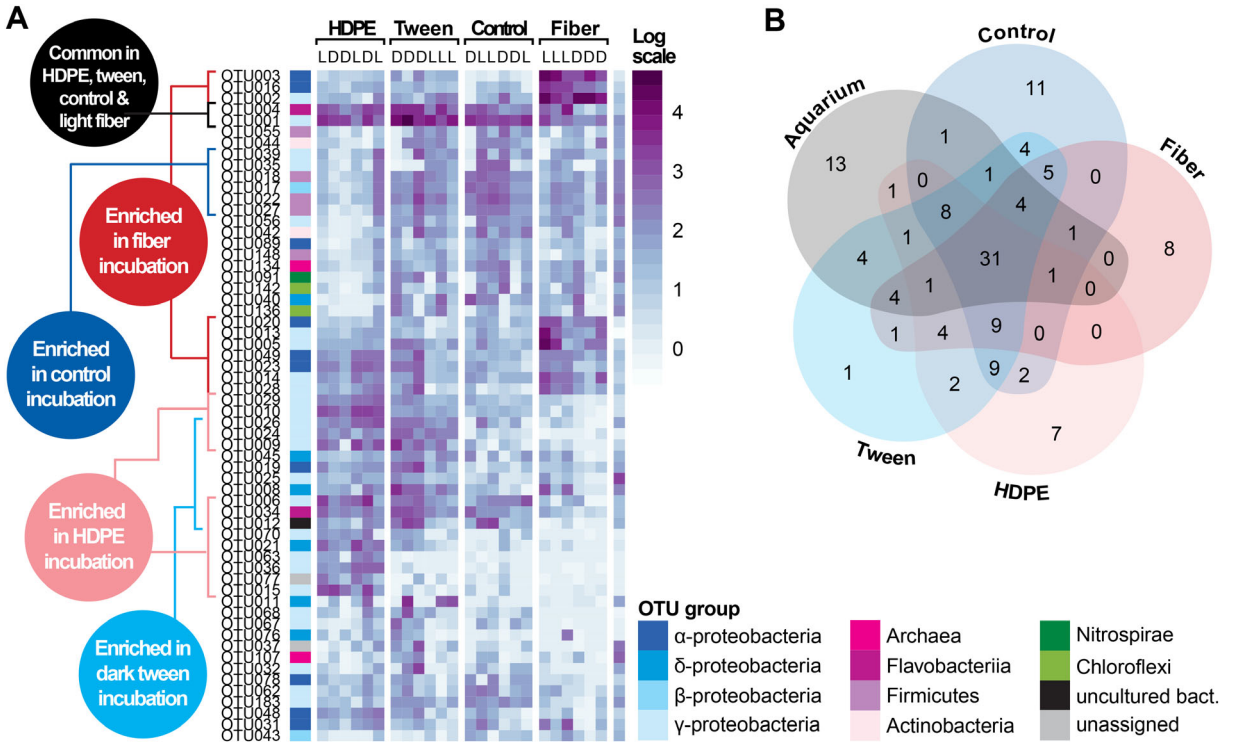

Fig 6. Representation of the 10 most abundant OTUs per sample, clustered per incubation. A. Log-scaled heat map highlighting the abundance of incubation-enriched OTUs (n = 60), including the aquarium community. Light conditions are indicated by the letters 'L' (light) and 'D' (dark). B. Venn diagram (n = 134) associated to the heatmap showing an important core community, nonetheless with incubation-enriched OTUs. Note: OTUs accounting for less than 10 reads were interpreted as absent in the community.

statistically significance (analysis of similarity test: R^2 = 0.36, P = 0.02) (Fig. 4). Since the communities in the fibre incubations were enriched in aerobic heterotrophic hydrocarbonoclastic bacteria, the increased respiration in presence of light (with an O_2 drawdown to suboxic levels of 25 μM, Fig. 2) cannot be explained by increased oxygen availability via photosynthesis since cyanobacteria were in the minority of taxa in all incubations, which was not higher in the fibre incubations. Rather, it appears that light actually stimulated the aerobic respiration of the heterotrophic hydrocarbonoclastic bacteria in the presence of the fibres, and resulted in a significantly different community composition. This is known to occur in photoheterotrophic marine bacteria that contain proteorhodopsins (Fuhrman *et al*., 2008; Sánchez *et al*., 2017).

Differences in the microbial communities between light and dark incubations were also observed in the microbial communities incubated with HDPE (analysis of similarity test: R^2 = 0.31, P = 0.01), which was evident in the ordination analysis (Fig. 4). Thus, similar to the textile fibres, light also resulted in the development of a unique community of heterotrophic hydrocarbon degrading bacteria colonizing the HDPE substrates. HDPE incubations in the dark were dominated by the three families Vibrionaceae, Alcanivoracaceae and Flavobacteriaceae, whereas the light-incubated HDPE microbial communities were more diverse with a shared dominance between eight families (Rhodobacteraceae, GR-WP33-58, Spongiibacteraceae, Vibrionaceae, Cellvibrionaceae, Oceanospirillaceae, Alcanivoracaceae and Flavobacteriaceae) (Figs. 4 and 5).

Many taxa had higher relative abundance across all substrate incubations, and the higher average abundance as measured with quantitative PCR (qPCR) indicates the ability of many taxa to utilize carbon from various sources (HDPE microbeads, Tween 20, textile fibres) and may indicate an opportunistic behaviour (Fredricks, 1966). It is also a possibility that a higher level of available DOC in light-exposed textile fibre incubations caused the higher respiration rates and microbial growth, caused by the polymer exposure to artificial sunlight (Romera-Castillo *et al*., 2018; Zhu *et al*., 2020). These findings may help us better understand the plastisphere dynamic in situations similar to, for example, microorganisms settled on plastic debris initially floating in the photic zone and later buried in the sediment or sinking in regions with limited light availability.

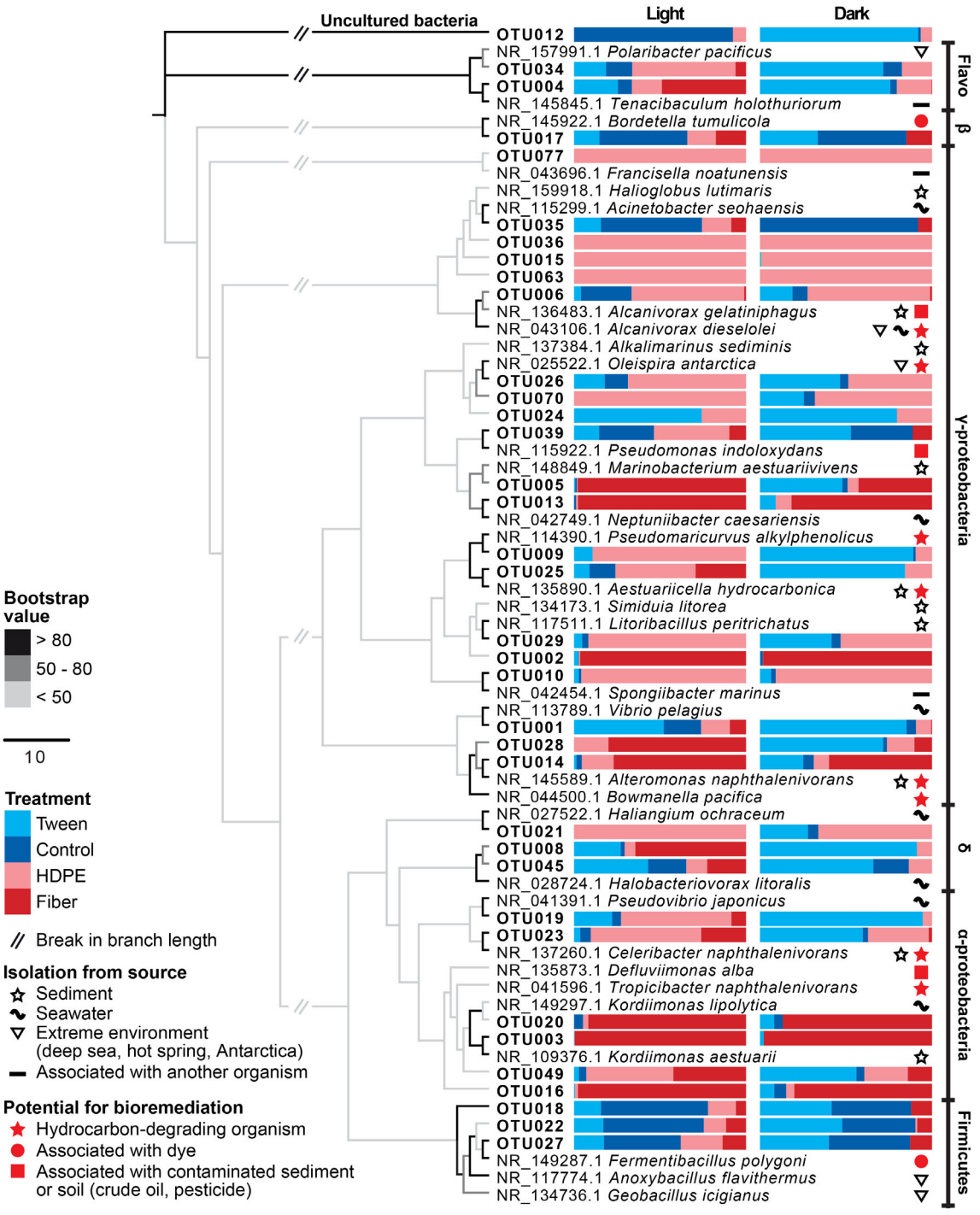

Fig 7. Phylogenetic reconstruction of 36 OTUs identified as incubation-enriched in Fig. 6 and their closest associated named species from NCBI database. The relative abundance of OTUs in each incubation (pale blue: tween; dark blue: control; pale red: HDPE; dark red: fibre) is represented by bar charts, 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).

Hydrocarbon-degrading bacteria

Several OTUs enriched in the different incubation types revealed to be closely related to known hydrocarbon-degrading microorganisms (Fig. 7). This, together with the increased rates of oxygen consumption in those incubations, and the higher average microbial abundance in those incubations relative to the controls (Fig. 2),

highlights their potential for utilization of organic carbon from micropollutants. Utilization of carbon from those micropollutants would lead to biotransformation, and potentially bioremediation, of these compounds. The six most abundant OTUs of the textile fibre incubation (OTU002, -003, -005, -013, -016 and -020) are closely related to the genera *Kordiimonas* and *Defluviimonas* (α-proteobacteria), and *Simiduila*, *Marinobacterium* and *Neptuniibacter* (γ-proteobacteria) according to the inferred phylogenetic tree. OTU003 and -020 were also closely related to *K. gwangyangensis* (NR_043103.1), which can hydrolyze six different polycyclic aromatic hydrocarbons (PAHs) (Kim and Kwon, 2010), giving this taxon a potential role in microplastic bioremediation. The genus *Alteromonas*, hosting hydrocarbon-degrading microorganisms, has been previously identified as part of the plastisphere from North Adriatic Sea and Atlantic Ocean (Zettler *et al*., 2013; Viršek *et al*., 2017). More specifically, *Alteromonas naphthalenivorans* was identified as a naphthalene consumer (Jin *et al*., 2015). *Defluviimonas alba* was isolated from an oilfield water sample suggesting that it has a potential for degradation of hydrocarbons, similar to *Defluviimonas pyrenivorans* (Zhang *et al*., 2018); however, to the best of our knowledge, it has not yet been tested for hydrocarbon hydrolysis (Pan *et al*., 2015). *Bowmanella pacifica* was identified during a search for pyrene-degrading bacteria (Lai *et al*., 2009), which is a PAH highly concentrated in certain plastics (Chen *et al*., 2018). *Bowmanella* spp. were also identified on polyethylene terephthalate (PET) specific assemblages from Northern European waters (Oberbeckmann *et al*., 2014). Because these taxa were enriched in the textile fibre incubations which had higher average microbial abundance, and respiration rates compared with the controls, we speculate that they were utilizing the polymer as both a carbon source (the increased abundance) and energy source (the increased respiration rates), owing to their established capability to grow on hydrocarbons as a primary substrate determined in prior experiments with pure cultures. Although approximately half of the fibres used in the experiment were of natural fabric (i.e. cellulose), it has been observed that cotton is a powerful sorbent used to treat oil spill (Choi *et al*., 1993). Thus, cotton fibres may absorb traces of oil present in the environment (Singh *et al*., 2013) that are accessed by hydrocarbonoclastic bacteria forming biofilms on the cottom fibres.

Whilst our results do not directly support that HDPE was biotransformed by the microbial community, the HDPE incubation stimulated the enrichment of six OTUs (OTU001, -004, -006, -010, -015 and -026) closely related to hydrocarbon-degrading bacteria, different from those enriched in textile fibre incubations. For instance, OTU006 was affiliated with the genus *Alcanivorax*, which are specialized in degrading alkanes, especially in contaminated marine environments (Barbato *et al*., 2016; Zadjelovic *et al*., 2020). This genus was also identified as being potentially important for PET degradation in the natural marine environment (Oberbeckmann *et al*., 2014). All other five OTUs were abundant mainly in light-grown HDPE communities, which were affiliated with the taxa *Pseudomaricurvus alkylphenolicus*, *Celeribacter naphthalenivorans*, *Oleispira antarctica*, *Tropicibacter naphthalenivorans* and *Aestuariicella hydrocarbonica*. The increased microbial growth in the HPDE incubation and different community assembly compared with the Tween 20 treatment (an emulsifier for the HDPE microbeads) demonstrate a unique effect on microbial community formation due to the HDPE itself (as opposed to microbes that may just be eating the emulsifier Tween that is coated onto the HDPE).

Extrapolating from our experiment to the natural environment

Although this study did not use 'natural' microbial communities from the intertidal habitats from which the textile fibres derive, it tests for the potential response of microbial communities to widespread micropollutants. Since the identified hydrocarbonoclastic bacteria in our incubations are also common to both natural marine sediments and water column (Lai *et al*., 2009; Kim and Kwon, 2010; Zettler *et al*., 2013; Oberbeckmann *et al*., 2014; Barbato *et al*., 2016; Zadjelovic *et al*., 2020), this study contributes to the developing understanding of how light effects respiration of hydrocarbon-degrading bacteria colonizing textile waste, and their utilization of these micropollutants as a carbon source.

Three main lines of evidence suggest that textile fibres were utilized as a carbon and energy source by heterotrophic hydrocarbonoclastic aerobic bacteria during the experiment: (i) higher rates of oxygen consumption relative to all other treatments, (ii) increased abundances as indicated by qPCR compared with the controls and (iii) the development of unique microbial communities dominated by heterotrophic hydrocarbonoclastic aerobic bacteria specific to this micropollutant. Moreover, the same groups of hydrocarbonoclastic bacteria found in our study, such as *Alteromonas* spp. and *Kordiimonas* spp., have been commonly observed in the marine environment. Moreover, the significantly higher respiration rates in the presence of light in the textile fibre incubations indicate that light availability can stimulate the aerobic respiration of hydrocarbon-degrading bacterial communities in the presence of this micropollutant. We speculate that this may be due to photochemical dissolution of the textile substrates, which potentially allow for more readily bioavailable substrates (smaller molecules)

to fuel microbial metabolism. Deeper study into the effects of light on *in situ* aerobic hydrocarbon-degrading bacterial communities should help to better understand microbial mechanisms of plastic and textile waste in the marine environment.

Experimental procedures

Experimental setup and sampling

A total of 15 ml of aquarium seawater containing microbial communities were incubated in 20 ml glass petri dishes for 108 h at room temperature, which received the following four treatments: (i) no micropollutants (control), (ii) polysorbate-20 (Tween 20) (0.01 mg ml^{-1}), (iii) HDPE microbeads added at a concentration of 0.26 mg ml^{-1} (which have a Tween 20 emulsifier at a concentration of 0.01 mg ml^{-1}) and (iv) textile fibres at a concentration of 3.5 fibre ml^{-1} (Fig. 1, Supporting Information Table S1). Tween 20 at a concentration of 0.01 mg ml^{-1} was used as a control in treatment (Jambeck *et al*., 2015) since it is used as an emulsifier at this same concentration for the HDPE microbeads in treatment (Frère *et al*., 2018) and thus serves to test whether the microbes respond only to the Tween coating on the beads, or are effected by the HDPE itself. The artificial seawater microbial community comes from an aquarium (642 l) built of imported live rocks, which hosts many reefs organisms, such as hexacorals, octocorals, gorgonians, sea anemones (*Aiptasia* sp.), marine sponges (*Lendenfeldia chondrodes*, *Tethya wilhelma*), marco-algae (*Chaetomorpha linum*) and cyanobacteria, mussels (*Mytilus edulis*) and benthic foraminifera (*Elphidium crispum*) (Fig. 1A). For each of these incubations, one set was placed under LEDs (Mitras LX6200 HV; light spectrum of 380 nm to 700 nm) with a 12 h light/12 h dark cycle (referred to as 'light') and the other one placed inside a cardboard box covered with aluminium foil to block incoming light (referred to as 'dark') (Fig. 1). Each incubation set consists of 12 glass petri dishes sealed with parafilm containing a submerged fibre optic oxygen sensor spot (PreSens Precision Sensing): three controls and nine incubations (Fig. 1B). The oxygen sensor spot was positioned at the bottom of the petri dish to measure the minimal concentration of O_2 that could be dissolved into the bottom water of the petri dish after diffusion from the overlying headspace. Because the tops of the petri dishes were covered with parafilm, atmospheric oxygen could continuously diffuse into the seawater.

The oxygen concentration in each incubation was closely monitored over the first 48 h of the experiment using a Stand-alone Fibre Optic Oxygen Meter (PreSens Fibox 4). At the beginning of the experiment, four samples of 15 ml were collected from the aquarium to assess the initial microbial community (T0; referred to as 'aquarium'). The entire 15 ml volume of the seawater incubations, and the aquarium samples, were concentrated down to 100 μl using 50 KDa Amicon Ultra Centrifugal Filters (4000 r.p.m., RCF 3399 × *g*, 10 min. at 20°C). This 50 kDa size selection concentrated all particles (free living microbes, particle associated particles, and particles) and high molecular weight dissolved organic matter (including any extracellular DNA from dead or lysed cells). From each incubation, the 100 μl concentrate from the Amicon filters was split into equal volumes (50 μl) and added to two Lysing matrix E tubes to serve as technical replicates to capture variability in the DNA extraction.

DNA extraction and qPCR

The DNA was extracted from the concentrate as described by Pichler and colleagues (2018), using 1 ml of a C1 extraction buffer mixed from 38 ml saturated $NaPO_4$ 1 mol l^{-1} buffer, 7.5 ml 100% ethanol, 4 ml MoBio's lysis buffer solution C1 (MoBio, Carlsbad, CA) and 0.5 ml 10% SDS (Pichler *et al*., 2018). To lyse cells, the samples were subsequently heated at 99°C for 2 min, frozen at −20°C for 1 h, thawed at room temperature, and heated again at 99°C for 2 min. After homogenization and centrifugation, high molecular weight DNA in the supernatant was concentrated using 50 kDa Amicon filters, down to a final volume of *c*. 100 μl. The 100 μl concentrate was further purified using the DNeasy PowerClean Pro Cleanup Kit (Qiagen, Hilden, Germany). To assess the concentration of 16S rRNA gene copies per millilitre at the end of the experiment, the V4 hypervariable region of the 16S rRNA gene was amplified in all samples using qPCR (Bio-Rad CFX connect Real-Time System). Every reaction contained 4 μl of DNA template, 10 μl of Supermix, 5.2 μl H_2O, and 0.4 μl of forward (515F 5′-TATGGTAATTGTGTGCCAGCMG CCGCGGTAA-3′) and reverse (806R 5′-AGTCAGTCAG CCGGACTACHVGGGTWTCTAAT-3′) primer, and were subject to the following PCR program: denaturation at 95°C for 3 min, and 40 amplification cycles (denaturation at 95°C for 10 s, annealing 55°C for 30 s). All qPCR reactions were set up using an Eppendorf EpMotion pipetting robot that has < 5% technical variation and results in qPCR reaction efficiencies (standard curves) having > 90% (Coskun *et al*., 2018).

16S amplicon Illumina library preparation

To assess the diversity of the microbial community in the experimental samples, the V4 hypervariable region 16S rRNA gene (*c*. 250 base pairs) was amplified with the primers 515F and 806R, respectively, combined to a forward (P5 5′-AATGATACGGCGACCACCGAGATCTAC

AC-3′) and reverse (P7 5′-CAAGCAGAAGACGGC ATACGAGAT-3′) adaptor, and unique dual indices for every sample (Pichler *et al*., 2018). The preparation of the samples for the polymerase chain reaction (PCR) was done according to Pichler and colleagues (2018). In short, 4 μl of extracted DNA was mixed to 5 μl 5× PCR buffer, 1 μl 50 mM dNTP, 1 μl forward 515F and 1 μl reverse 806R primers, 9.9 μl H_2O, 3 μl $MgCl_2$ and 0.1 μl Taq DNA polymerase, for a total volume of 25 μl for each sample. The amplification took place under specific PCR settings: denaturation at 95°C for 3 min, 35 amplification cycles (denaturation at 95°C for 10 s, annealing 55°C for 30 s, elongation 72°C for 1 min), and elongation at 72°C for 5 min to ensure polymerization of all amplified DNA strands. PCR products were run through a 1.5% (w/v) agarose gel, and DNA strands were subsequently extracted using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The DNA concentration was quantified using the fluorometer QuBit 2.0 (Life Technologies, Grand Island, NE) and its associated dsDNA high-sensitivity assay kit. As preparation for 16S amplicon sequencing, all samples were pooled together by adding 5 μl of every sample at a DNA concentration of 1 nM.

Illumina sequencing

A metagenome, used as high diversity library, was added to the 16S amplicon pool, which is a typical practice to enhance the recognition of the 16S sequences by the Illumina MiniSeq (Coskun *et al*., 2018). The DNA was denatured by adding of 0.1 nM NaOH for a short period of 5 min, which was then directly neutralized with a Tris–HCl buffer (pH 7) to avoid hydrolyzation of the DNA. To not overload the flow cell, a two-step dilution was performed on the 16S pool for a final DNA concentration of 1.8 pM, resulting in a final volume of 500 μl MiniSeq solution. Four additional sequencing primers after (Pichler *et al*., 2018) were added to successfully undergo the dual-index barcoding with the MiniSeq. Finally, the prepared 1.8 pM solution of 16S, transcriptomes, and the four sequencing primers was loaded into the reagent cartridge.

Data analysis

The Illumina MiniSeq raw data were quality controlled, curated for contaminants (which were removed), and analysed using USEARCH v11.0 (https://drive5.com/usearch) (Edgar, 2018) following the method described by Pichler and colleagues (2018). Most similar sequences sharing at least 97% of bases were grouped, and associated to an operational taxonomic unit (OTU). Each OTU was classified within the Taxonomic Classification System using MacQiime v1.9.1 (http://qiime.org/), against the SILVA database version 132 (Quast *et al*., 2013). Low abundance OTUs in 16S rRNA gene Illumina datasets are a result of contamination, sequencing errors, and real rare species and thus cannot be reliably trusted (Edgar, 2018; Pichler *et al*., 2018). Therefore, any OTUs having than 10 reads across samples were discarded prior to further analysis. The statistical analysis ANOSIM (analysis of similarity) was performed using 999 permutations in the Vegan package (Oksanen *et al*., 2017) to evaluate the similarity in community composition of 16S rRNA gene OTUs between incubation types. Welch two sample *t*-tests (using the t.test() command in R) were used in order to assess the significance of respiration rates and 16S rRNA gene copies between incubations (as determined via qPCR), using R v3.3.3 (R Core Team, 2017). For phylogenetic reconstruction, the closest cultured and uncultured relatives of OTUs in our datasets were identified using BLASTn (BLAST, 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 v4.7 (Gouy *et al*., 2010) under PhyML optimized settings (General Time Rerversable model), including 100 bootstrap replicates (Guindon *et al*., 2010). All related primary data and R scripts are stored on GitHub (https://github.com/PalMuc/PlasticsBacteria), and 16S rRNA sequences resulting from the sequencing run are available in the European Nucleotide Archive (Project PRJEB37536).

Raman spectroscopy

Forty textile fibres were randomly subsampled and their associated spectrum obtained with a HORIBA JOBIN YVON XploRa ONE micro Raman spectrometer belonging to the Mineralogical State Collection Munich (SNSB). The used Raman spectrometer is equipped with edge filters, a Peltier cooled charged coupled device (CCD) detector and three different lasers working at 532 nm (green), 638 nm (red) and 785 nm (near IR). To perform the measurements the near infrared (IR) Laser (785 nm) was used, with a long working distance objective, magnification 100× (Olympus, series LMPlanFL N), resulting in a 0.9 μm laser spot size on the sample surface. The wavelength calibration of the IR laser was performed by manual calibration with a pure Si wafer chip, the main peak intensity had values in the interval 520 cm^{-1} ± 1 cm^{-1}. The wave number reproducibility was checked several times a day providing deviation of less than < 0.2 cm^{-1}. Monthly deviation was in the range of 1 cm^{-1} before calibration. The necessary power to obtain a good-quality spectrum varied between 10% and 50% (i.e. respectively, 2.98 mW and 18 mW ± 0.1 mW on the sample surface) depending on the type and

degraded stage of the measured textile fibre. The pinhole and the slit were, respectively, set at 300 and 100. Each acquisition included two accumulations with a grading of 1200 T and an integration time of 5 s over a spectral range of 100–1600 cm^{-1}. The precision of determining Raman peak positions by this method is estimated to be ±1 to ±1.5 cm^{-1}. Resulting Raman spectra were analysed using LabSpec Spectroscopy Suite software v5.93.20, treated in R v3.3.3, manually sorted in Adobe Illustrator CS3, and compared with available spectra from published work. All related Raman spectra and R scripts are stored on GitHub (https://github.com/PalMuc/PlasticsBacteria).

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Description of each incubation type, equally exposed to light and dark conditions (ASW: artificial seawater).