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Biofilm development as a factor driving the degradation of plasticised marine microplastics

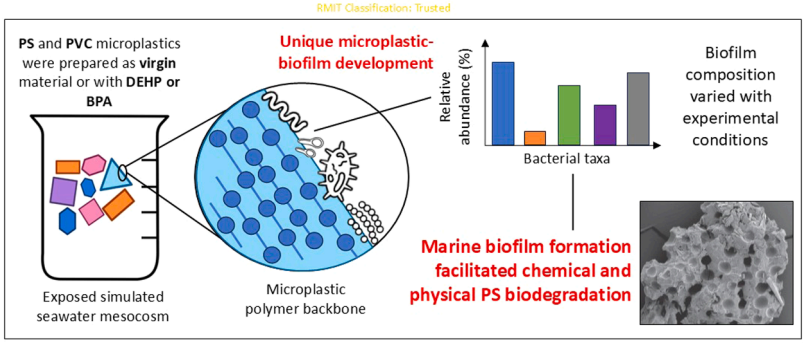
Alexandra M. Gulizia [a,b,c,d,*], Sara C. Bell [b,e], Felicity Kuek [e], Marina M.F. Santana [a,b,e], Richard C. Edmunds [e], Yun Kit Yeoh [b,e], Yui Sato [a,e], Pirjo Haikola [c,e], Lynne van Herwerden [a], Cherie A. Motti [b,e], David G. Bourne [a,b,e], George Vamvounis [a,b]

[a] College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia
[b] AIMS@JCU, Division of Research and Innovation, James Cook University, Townsville, QLD 4811, Australia
[c] School of Design, Royal Melbourne Institute of Technology (RMIT), Melbourne, VIC 3000, Australia
[d] Bioplastics Innovation Hub (BIH), Food Futures Institute, Murdoch University, Perth, WA 6150, Australia
[e] Australian Institute of Marine Science (AIMS), Townsville, QLD 4810, Australia

HIGHLIGHTS

- A developing marine microplastic biofilm is significantly impacted by time, polymer and plasticiser composition.
- Putative plastic degrading bacteria were identified in the incoming seawater and on all examined microplastics substrates.
- Biodegradation initiated during biofouling prompted plastic biodegradation, especially of the PS-BPA substrates.
- The microbiome of Australian tropical reef waters possesses plastic biodegradative capabilities.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradation of microplastics facilitated by natural marine biofouling is a promising approach for ocean bioremediation. However, implementation requires a comprehensive understanding of how interactions between the marine microbiome and dominant microplastic debris types (e.g., polymer and additive combinations) can influence biofilm development and drive biodegradation. To investigate this, polystyrene (PS) and polyvinyl chloride (PVC) microplastics (< 200 μm in diameter) were prepared either without any additives (i.e., virgin) or containing 15 wt% of the plasticisers diethylhexyl phthalate (DEHP) or bisphenol A (BPA). Each polymer-plasticiser microplastic combination was exposed to environmentally relevant conditions in a simulated seawater mesocosm representative of tropical reef waters over a 21-day period to allow for natural biofilm development. Following this, microplastic degradation and the colonising bacterial biofilm was assessed as a function of time, polymer and plasticiser type using infrared, thermal, gel permeation and surface characterisation techniques, as well as 16S ribosomal RNA bacterial gene sequencing, respectively. Together, these analyses revealed time-, polymer- and plasticiser-dependent degradation, particularly of the PS-BPA microplastics.

* Corresponding author at: Bioplastics Innovation Hub (BIH), Food Futures Institute, Murdoch University, Perth, WA 6150, Australia.
E-mail address: alexandra.gulizia@murdoch.edu.au (A.M. Gulizia).

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Degradation of the PS-BPA microplastics also coincided with changes in bacterial community composition and an increased total relative abundance of putative biodegradative bacteria. These findings indicate that the metabolic potential and biodegradative capability of the colonising marine biofilm can be significantly impacted by the chemical properties of the microplastic substrate, even within short timeframes.

1. Introduction

Microplastics (< 5 mm in diameter) and their associated chemical additives (e.g., phthalate and diphenol plasticisers) are hazardous environmental contaminants [1]. Due to the recalcitrant nature of these polymer-additive mixtures [2], marine ecosystems including coral reefs are susceptible to the long-term consequences of microplastic accumulation [3–7]. To mitigate these impacts, current research aims to develop methods that isolate and remove plastics from oceans worldwide [8,9]. Microbial biodegradation of synthetic plastics exploits microorganisms that can break down long-chain polymers into more easily metabolised compounds and offers a natural and cost-effective way to completely remove plastics from marine matrices [10–16]. However, there are still many knowledge gaps limiting the implementation of these strategies [15]. Firstly, the availability of putative biodegradative microorganisms as well as their propensity to colonise a substrate are significant factors that influence the effectiveness of natural plastic biodegradation [13, 15–17], especially in marine environments where microbial diversity and density can fluctuate temporally [18], spatially [19] and with water biogeochemistry [18,20]. Moreover, substrate physicochemistry and morphology can also impact microorganism colonisation and biofilm formation (i.e., biofouling) [21–26]. Given that microplastics can have vastly different properties (i.e., polymer-additive composition [27]), effective biodegradation strategies for these materials relies on a comprehensive understanding of how these chemical factors influence biofouling [28].

Marine plastic-associated biofilms (i.e., the plastisphere [15,29]) are diverse assemblages of microorganisms, mainly bacteria, that colonise the surface of plastics immersed in seawater [28]. The bacterial community composition of the plastisphere and its metabolic potential can be impacted by the physicochemical properties of the plastic (e.g., polymer-additive composition [22,26,30–33] and morphology [34–37]). These physicochemical variables influence theplastic's surface properties, most importantly, topography, hydrophobicity and functionalisation [26,38,39]. Consequently, taxonomically distinct biofilms have been reported on polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyvinyl chloride (PVC) plastics collected from a range of marine environments worldwide [22,26,30,31,33]. However, the combined impact of incorporated functional additives such as plasticisers on microplastic biofouling under environmentally relevant conditions has been understudied to date [40,41]. Plasticisers such as diethylhexyl phthalate (DEHP) and bisphenol A (BPA) are common toxic additives incorporated at high concentrations into plastic polymers (1 – 70 wt% [42,43]) [27]. They have been detected in marine environments at concentrations between 7.4 and 71,700 ng/L [44,45] with reported widespread and detrimental impacts on environmental microbiomes (e.g., inducing gut dysbiosis in some fishes) [46–48]. Controlled preparation and comprehensive characterisation of the polymer-additive properties of microplastics employed for biofouling studies will ensure that differences in the developing biofilm can be reliably identified with time and correlated to the property of the plastic (e.g., polymer or additive type) [49,50]. These insights will help to promote better quality assurance and control protocols (QA/QC) for future research, specifically by elucidating differences in biofilm community composition over time. Consequently, more effective restoration initiatives that promote natural and timely biodegradation will be informed [32,51–53].

The phylogenetic database of microorganisms with metabolic capability to biodegrade synthetic polymers is expanding [13]. In laboratory studies, bacteria with these capabilities have been cultured and grown on plastics, and evidence of their biodegradation potential within reasonable timeframes (i.e., less than 1 year) is promising [13,15]. Using a variety of chemical (e.g., thermal stability, spectral and molecular weight information) and physical (e.g., microscopy and hardness) techniques, polymer degradation initiated during biofouling can be understood and linked to the property of the plastic as well as the presence and abundance of specific microorganism(s). However, direct evidence of microplastic degradation by biofouling microbes under natural marine conditions is limited [15], especially for plasticised-PS and -PVC [10,54,55]. PS and PVC microplastics are ranked among the highest concern polymers in marine environments according to parameters such as organism ingestion rate (exceeding 0.01 – 100 particles/day for some biota), global waste generation (15 – 17 million tonnes/year), toxic chemical association (i.e., leaching/adsorption of additives and monomers) and degradation rate (2 – 5 years) [56,57]. More specifically, these microplastic polymers in tropical marine and coral reef ecosystems can negatively impact coral physiology and alter the behaviour of associated coral species leading to the destruction of these habitats [58,59]. Thus, there is a need for multidisciplinary studies that target these marine recalcitrant polymer-additive mixtures and aim to understand biofouling as a factor driving their biodegradation [60].

In this study, smaller-sized PS and PVC microplastics (< 200 μm in diameter) prepared as a virgin material (i.e., without additives) or with 15 wt% of the plasticisers DEHP or BPA, were deployed for 21 days into a simulated seawater mesocosm representative of tropical reef waters. During early marine biofouling, rapid changes in bacterial colonisation and successive patterns are heavily influenced by the physicochemical and surface properties of the substrate, therefore, this experimental timeframe is predicted to highlight specifically the influence of polymer-plasticiser composition on marine plastic biofouling and biodegradation [26,61]. As such, a combined chemical and genetic approach using spectroscopic analyses (e.g., infrared, thermal, gel permeation and surface characterisation [9]) and high-throughput sequencing of the 16S ribosomal RNA (rRNA) bacterial genes, respectively, was applied to explore polymer degradation and biofilm community structure as a function of microplastic chemical composition (polymer-plasticiser composition) and time (0, 7 and 21 days). Given the differences in chemical properties of the prepared microplastics, it is hypothesised that plastic substrates with different polymer-plasticiser compositions would develop unique and taxonomically distinct biofilms that influence biodegradation. Therefore, revealing the relationship between plasticised microplastics and the marine microbiome. Overall, this work will better inform on the impacts of plasticisers on microbial communities, contribute to the advancement of plastic bioremediation strategies and refinement of QA/QC protocols [62].

2. Methods

2.1. Materials

PS (weight-average molecular weight (*Mw*) = 192 K Daltons; 430102–1 Kg), PVC (*Mw* = 55 K Daltons; 389239–500 g), tetrahydrofuran (THF; HPLC Grade), DEHP, BPA, sodium lauryl sulfate (SDS), Trizma® base (CAS 77–86–1), phenol:chloroform:isoamyl alcohol (IAA) (25:24:1, *v/v*) and chloroform:IAA (24:1, *v/v*) were sourced from Sigma Aldrich. Sodium hydroxide (NaOH), acetone, ethanol (EtOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2; 30 % grade) were sourced from Univar. Proteinase K (20 mg/mL; recombinant; PCR Grade), lysozyme and UltraPure™ distilled water (DNase/RNase-Free)

were sourced from ThermoFisher. Ethylenediaminetetraacetic acid (EDTA) and sucrose were sourced from Astral Scientific. Isopropanol (80 % *v/v;* HPLC grade) was sourced from BDH Chemicals. PCR reagents including AmpliTaq Gold 360 Master Mix (2X concentrate) and GC enhancer were sourced from Applied Biosystems. Forward and reverse PCR primers were supplied by Sigma Aldrich and reconstituted to 100 μM on arrival.

2.2. Preparation and characterisation of plastics

Virgin (i.e., containing no additives) and additive loaded (15 wt% DEHP or 15 wt% BPA) PS and PVC microplastics were prepared as per Gulizia et al. [49,50]. All three preparations of PS (PS-virgin, PS-DEHP and PS-BPA) and PVC (PVC-virgin, PVC-DEHP and PVC-BPA) were then sorted separately over a 200 μm aperture stainless-steel test sieve (Glenammer Sieves), with all microplastics smaller than 200 μm in diameter collected and used for experiments. Gel permeation chromatography (GPC) was used to determine the concentration (wt%) of DEHP and BPA incorporated into the PS and PVC polymer blends, and was calculated using prepared calibration curves of PS, PVC, DEHP and BPA in THF from 0 – 1.05 mg/mL ($R^2 > 0.99$). For these analyses, PS (3 – 5 mg) and PVC (1.5 – 3 mg) microplastics were dissolved in THF (1.5 mL), filtered through a 0.22 μm MS® polytetrafluoroethylene (PTFE) filter (Membrane Solutions), and 50 μL aliquots were injected into a 1260 Infinity II Multi-Detector GPC (Agilent Technologies) equipped with an ultraviolet (UV) absorbance and refractive index detector. Two PLgel 5 μL MIXED-C columns (300 x 7.5 mm; Agilent Technologies) were calibrated using PS narrow standards (Agilent EasiVial PS-M). Thermal gravimetric analysis (TGA; Fig. S1) and Fourier Transform-Infrared Spectroscopy (FT-IR; Fig. S2) were used to characterise signature chemical properties, while optical microscopy (Leica MZ26A; Fig. S3) was used to conduct size analysis of the microplastics, as described in Gulizia et al. [49,50]. Physical observation and morphological characterisation of the microplastics was performed using a Canon 5D Mk 111 with a Canon 100 mm f2.7 Macro Lens and a Joel Superprobe JXA-8200 Standard Electron Microscope (SEM).

These analyses revealed that all microplastics were white-opaque in colour, irregular shaped and sized between 88 – 157 μm, with the untreated PS and PVC microplastics (i.e., 0 days of exposure), having a *Mw* of 171 and 58 kg/mol and a polydispersity index (PDI) of 2.54 and 1.97, respectively. These polymer-plasticiser blends (including polymer and plasticiser type and concentration [42,43]), as well as the morphology of the prepared microplastics were chosen as they are representative of dominant plastic contamination detected in aquatic and biological matrices worldwide [2,63–66]. Furthermore, the chemical behaviours (i.e., leaching and degradation) of these polymer-plasticiser combinations at this timescale were characterised in previous work [49,50], thus ensuring that the impact of biofilm formation on microplastic degradation could be studied in isolation.

2.3. Experimental setup

Biofilm formation and microplastic degradation were investigated as functions of polymer and plasticiser composition over 21 days in a seawater mesocosm at the National Sea Simulator (SeaSim) at the Australian Institute of Marine Science (AIMS), Townsville (Table 1). Prepared PS and PVC microplastics (PS-virgin, PS-DEHP, PS-BPA, PVC-virgin, PVC-DEHP and PVC-BPA; ≈ 0.30 g each) were placed inside empty Biotage® Sfär DVL 10 g cartridges fitted with a 26 μm mesh filter and a poly(methyl methacrylate) frit (to prevent microplastic loss). The cartridges containing microplastics (n = 5 cartridges per polymer-plasticiser combination, with n = 5 removed after 7 and 21 days) were fitted to a manifold system inside a water bath to reduce climate fluctuations and exposed to a constant flow of seawater sourced from the outlet of eight mesocosm tanks (Fig. S4). Incoming seawater was pre-filtered to 1 μm and the whole experiment was covered with a nylon mesh to simulate natural cloud cover and normalise sunlight exposure. The mesocosms were maintained at seasonal average conditions of the coastal, central Great Barrier Reef (GBR) World Heritage Area, Australia (Table S1) and contained representative organisms of these ecosystems (e.g., hard and soft corals, snails, sea cucumbers, urchins, seagrass, coral reef fishes and clams [67–69]). The flow (mL/min) through each cartridge was monitored weekly by measuring the volume of water leaving the cartridge within 10 s (Fig. S5). After 7 and 21 days of exposure to the seawater, the cartridges containing microplastics were collected, drained and microplastic samples stored at −20°C for subsequent DNA extractions and plastic degradation analyses.

2.4. Assessment of microplastic degradation

Chemical and physical analyses of the biofouled microplastics were performed using TGA, FT-IR, GPC and SEM, as described above. These techniques provide information regarding polymer thermal stability (e.g., decline in glass transition temperature and/or changes in plasticiser composition), new bond formation (e.g., carbonyl and hydroxyl bonds formed during polymer oxidation), molecular weight information (e.g., changes to the polymers hydrodynamic radii initiated during bond cleavage) and surface morphology (e.g., pitting), respectively, which can be used to evaluate polymer degradation [9,54,60,70]. For all techniques, the starting profiles of all prepared microplastic particles were characterised prior to experimentation, ensuring their homogeneity and allowing for comparison of microplastic degradation throughout the experiment. For TGA and FT-IR, representative samples (n = 1 replicate sample per polymer-plasticiser combination) were selected at random and tested neat, i.e., no pre-treatment was required. For GPC (n = 3 replicate samples per polymer-plasticiser combination) and SEM (n = 1 replicate sample per polymer-plasticiser combination) analyses, biofouled microplastics were first rinsed thoroughly with water, acetone and H_2O_2 to remove remaining biological material [9], and the solvent evaporated by air drying under ambient conditions (≈ 20 min) then under reduced pressure overnight.

2.5. DNA extraction, sample selection and quality control

Solutions prepared for DNA extraction and quality control assays were prepared as follows: 0.5 M EDTA was prepared by dissolving EDTA

Table 1
Experimental exposure conditions for the polystyrene (PS) and polyvinyl chloride (PVC) microplastics; virgin (i.e., containing no additives) or containing 15 wt% diethylhexyl phthalate (DEHP) or 15 wt% bisphenol A (BPA).

Polymer type	Additive composition	Nomenclature	Sampling time (days)	Replicates per polymer-plasticiser type	Total replicates per polymer type
PS	Virgin	PS-virgin	0[a], 7 and 21	5	45
	DEHP	PS-DEHP	0[a], 7 and 21	5	45
	BPA	PS-BPA	0[a], 7 and 21	5	45
PVC	Virgin	PVC-virgin	0[a], 7 and 21	5	45
	DEHP	PVC-DEHP	0[a], 7 and 21	5	45
	BPA	PVC-BPA	0[a], 7 and 21	5	45

[a] These plastics were not exposed to biofouling conditions and therefore served as a negative control to compare marine microplastic biofouling and subsequent polymer degradation in tropical waters.

(18.6 g) in Milli-Q water (100 mL) at pH 8.0. 1 M Tris-HCl was prepared by dissolving Trizma® base (12.113 g) in Milli-Q water (100 mL) at pH 8.0. Sucrose lysis buffer was prepared by dissolving 1 M Tris-HCl (5 mL), 0.5 M EDTA (8 mL) and sucrose (25.6 g) in MilliQ water (100 mL) before filter sterilisation (0.22 μm; Millipore). All solutions were autoclaved prior to use.

Total genomic DNA (gDNA) from the plastic-associated biofilms after 0 (i.e., no immersion in seawater), 7 and 21 days was extracted from 0.05 to 0.105 g of microplastics using the DNeasy® PowerBiofilm® Kit (Qiagen) following manufacturer protocol, except that samples were incubated at 65°C for 5 min before homogenisation by bead beating (FastPrep-24™ 5 G; MP Biomedicals) for 40 s at 4 m/s. Three kit blanks containing no plastic material were also extracted concurrently to establish environmental and laboratory contamination levels. All DNA was eluted into 50 μL elution buffer and immediately frozen at −20°C.

To profile the microbial community inherent in the seawater introduced into the mesocosm, 2 L of incoming seawater was collected twice throughout the experiment corresponding to sampling days on day 7 and day 21, respectively ($n = 3$ per sampling time). Microbes were collected from seawater using 0.22 μm Millipore Sterivex™ GP filter units connected to a peristaltic pump with a Masterflex easy load pump head (MilliPore XX8020ELO; 50 rpm) with sterilised tubing. Sterivex filters were immediately sealed with parafilm at both ends to prevent contamination and stored at −20°C until DNA extraction. DNA was extracted according to methods described in Botte et al. [20] with the following alterations: sucrose lysis buffer (1.8 mL) and lysozyme (100 mg/mL; 18 μL) was added directly into the filter units and then mixed by gentle inversion for 1 min. Filter units were incubated at 37°C with rotation for 1 h after which proteinase K (20 mg/mL; 20 μL) and SDS (10 %; 18.38 μL) were added, mixed by inversion, and incubated at 55°C with rotation for 1 h. The liquid (≈ 2 mL) was collected into two 2 mL microtubes and extracted using equal volumes of phenol:chloroform:IAA, mixed by inversion and centrifuged (16,000 x *g*; ambient) for 10 min to phase-separate. The aqueous layer was recovered from each sample and equal volumes of chloroform:IAA was added, mixed and centrifuged, as before. The aqueous layer was recovered from each sample again and DNA precipitated by adding isopropanol (≈ 0.7 mL), mixing by gentle inversion, incubation at ambient conditions for 15 min before centrifugation (20,000 x *g*, 4°C) for 30 min to pelletise DNA. The supernatant was then discarded, and the pellet washed by adding EtOH (80 %; 0.5 mL), followed by gentle inversion and centrifugation (20,000 x *g*; ambient) for 10 min. The supernatant was discarded, and the DNA pellets air dried under ambient conditions until no EtOH remained (≈ 20 min). Finally, pellets were resuspended in 25 μL PCR-grade water and stored at −20°C.

DNA quality was determined for all samples using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific). Representative samples ($n = 20$) were selected across all treatment groups spanning a range of concentrations for quantification using the Qubit High Sensitivity DNA kit (Invitrogen) on the Qubit 3.0 Fluorometer (Invitrogen) and to perform PCR amplification checks. PCR amplification was undertaken using the following reaction mix in 25 μL: AmpliTaq 360 PCR Master Mix (X1 concentrate; 12.5 μL), forward primer (1 μL 27 F; 0.4 μM), reverse primer (1 μL 1492R; 0.4 μM), template DNA (< 15 ng), GC enhancer (1 μL) and PCR-grade water (8.5 μL) [71]. Cycling conditions were as follows: 7 min at 95°C followed by 30 cycles of 30 s at 95°C, 30 s at 55°C and 90 s at 72°C followed by a final extension of 7 min at 72°C. To check for amplification, each sample was run on a 1.5 % agarose gel in 1x TAE buffer. Qubit concentrations were used to normalise DNA samples to ≈ 5 ng/μL before dispatch for sequencing.

2.6. 16S rRNA gene sequencing and bioinformatics

16S amplicon samples underwent 2 x 250 bp high-throughput DNA sequencing on the Illumina MiSeq® platform at the Ramaciotti Centre for Genomics (University of New South Wales, Australia) using the primer pair 515F/806R to amplify the V4 region of the 16S gene [72]. After quality control using FastQC (v0.11.9) and MultiQC (v1.14) [73], five raw samples that contained less than 2500 sequence counts were excluded from downstream analysis (< 5 % of dataset). The remaining high-quality sequence files were tested for compliance with tabular bioinformatics file formats using the Keemei add-in [74], imported into QIIME2 v2022.11 [75] and trimmed to 147 bp [76]. The filtered reads were then classified using the Silva 138 99 % full-length 16S sequences database to assign taxonomic information to the recovered amplicon sequence variants (ASVs). Sequence counts were rarefied and a total of 5,456,975 valid sequences (i.e., reads) were obtained, representing an average of 30,317 ± 1137 reads per sample (Fig. S6). The obtained reads were clustered into 11,570 ASVs, representing an average of 471 ± 17 ASVs per sample. Alpha diversity metrics including Chao1 richness and Simpson's evenness indices were calculated to assess global community structure and diversity of the plastisphere. Beta diversity and microbiome divergence were explored using multidimensional scaling (MDS) based on Bray-Curtis dissimilarity and compared using principal coordinate analysis (PCoA) [77,78]. Additionally, bacterial clades with metabolic capability to biodegrade PS and PVC were identified in the literature [79–83], grouped at the genus level and then cross-referenced with the ASVs identified in the incoming seawater and on each marine biofouled microplastic sample.

2.7. Statistical analyses

Data collation, visualisation and statistical analyses were undertaken in Microsoft Excel and R v4.2.2 through RStudio v2022.12.0–353 using the *BiocManager, eulerr, phyloseq* and *vegan* packages [78,84–86]. Further data filtering was performed to restrict statistical analyses to bacterial sequences with assignment beyond the kingdom level only [87]. Statistical differences between *Mw* and PDI indices obtained using GPC were calculated using pairwise *t*-tests and compared over time (i.e., 0 vs 7 and 21 days) within each treatment group, respectively. General linear models (GLMs) were used to identify significant changes in global diversity metrics (Eq. 1 [88]) and the relative abundance of ASVs (Eqs. 2 and 3) among treatment groups (PS-virgin, PS-DEHP, PS-BPA, PVC-virgin, PVC-DEHP and PVC-BPA) with time (0, 7 and 21 days) using the *DESeq2* package in R [89]. Due to the impact of time on biofouling (Fig. S7), all metrics (Chao1, Simpson's evenness and ASV relative abundance) in Eqs. 1 and 3 were examined independently of time to elucidate community differences with polymer and plasticiser composition. Alpha was set to 0.05 for all statistical comparisons.

$$\text{Global diversity metric} \sim \text{polymer type} + \text{plasticiser composition} \quad (1)$$

$$\text{ASVs relative abundance} \sim \text{exposure time} \quad (2)$$

$$\text{ASVs relative abundance} \sim \text{polymer type} * \text{plasticiser composition} \quad (3)$$

where, Global diversity metric = Chao1 and Simpson's evenness; polymer type = PS or PVC; plasticiser composition = virgin (i.e., no additive), 15 wt% DEHP or 15 wt% BPA; and exposure time = 0, 7 and 21 days. For each GLM, exploratory data analysis (not shown) dictated whether factors (i.e., polymer type, plasticiser type or exposure time) were considered nested or independent.

3. Results

3.1. Physicochemical properties of microplastics after immersion in seawater

Signature chemical peaks, thermal stability, Mw, PDI and surface morphology (TGA, FT-IR, GPC and SEM, respectively) as a function of time (i.e., 0, 7 and 21 days) and polymer-plasticiser composition (i.e., PS or PVC, virgin or containing DEHP or BPA) confirmed polymer

degradation was initiated during marine biofouling at this experimental timescale.

The TGA curves and FT-IR spectra of the biofouled microplastics revealed key changes consistent with polymer biodegradation. Though the thermal degradation profiles of all virgin microplastics were within error, a slight displacement of the curves was observed for all PS-DEHP and PS-BPA plastics after 7 and 21 days (Fig. S1). Supporting this, the FT-IR spectra of the biofouled PS microplastics revealed the presence of new peaks at 3380 cm^{-1} (-OH), increased intensity of peaks at 1650 cm^{-1} (-C═O) and broadened peaks between 900 and 1000 cm^{-1} (-C═C, -C-O, v -C-O-C); changes that are commonly associated with carbon bond oxidation during polymer degradation (Fig. S2) [70]. FT-IR of the PVC-microplastics also revealed a decline in the intensity of peaks centred at 827 cm^{-1} (v -C-Cl), which could suggest a breakdown of the PVC polymer backbone [90]. Changes to these signature peaks in both the TGA and FT-IR spectra were especially evident after biofouling for 21 days, indicating prolonged exposure to marine biofouling may enhance degradation.

GPC-derived molecular weights data of biofouled microplastics revealed more notable changes to the chemical properties of the PS microplastics than PVC microplastics (Fig. 1). The mean *Mw* of PS microplastics displayed a significant time- and plasticiser-dependent decline up to 10 % (p-value = $2.57E^{-3}$). Specifically, the mean *Mw* of the PS-virgin, PS-DEHP and PS-BPA microplastics decreased from a common 171 kg/mol (0 days), to 154, 167 and 168 kg/mol after 7 days and 155, 163 and 167 (p-value < 0.05) kg/mol after 21 days, respectively. Moreover, the mean PDI of PS-virgin, PS-DEHP and PS-BPA microplastics decreased from a common 2.54 (0 days) to 2.37, 2.13 (p-value < 0.05) and 2.36 after 7 days and 2.39, 2.23 (p < 0.05) and 2.43 after 21 days, respectively (Table S2). These changes in *Mw* and PDI suggest a substantial change in the hydrodynamic radii of PS, which could indicate oxidative cleavage and breakdown of the polymeric backbone into shorter chain fragments [9,60]. Contrary, no significant variation in molecular weights information was observed for any PVC microplastics.

SEM images also revealed notable changes in the morphology of all biofouled PS microplastics and indicated time- and plasticiser-dependent effects consistent with biodegradation (Fig. 2e, f, h and i). After 7 and 21 days of biofouling, PS-DEHP and PS-BPA microplastics were substantially degraded, evident by the formation of small holes on the particles surface. These holes increased in frequency with time and were most pronounced on PS-BPA microplastics. No evident changes to the surface of PVC microplastics were observed throughout the duration of the biofouling experiment (Fig. S8).

3.2. Bacterial community patterns in a developing microplastic biofilm

Macroscopic observation of PS and PVC microplastics revealed substantial biofouling as depicted by biomass formation (Fig. S9 and S10) [22]. Representative SEM images also depicted colonisation by marine eukaryotes (e.g., diatoms) as well as evidence of some bacterial filaments (Fig. S11) [26]. 16S rRNA gene amplicon sequencing revealed a distinctness between the biofouled microplastics and the incoming seawater, with increased ASV richness (Chao1) observed after 7 and 21 days (Fig. 3). Assessment of the plastisphere community richness and evenness (Simpson's) also revealed time-, polymer- and plasticiser-dependence. Both measured indices changed after immersion in seawater for up to 21 days, with Chao1 values increasing from a common 100 (0 days) to 1650 (21 days) while Simpson's values decreased from a common 0.3 (0 days) to 0.04 (21 days), respectively. This indicated enhanced community complexity over time. Moreover, while Chao1 and Simpson's diversity metrics remained fairly constant from 7 to 21 days (1100 – 1800 and 0.025 – 0.05) and with plasticiser composition (1000 – 2000 and 0.025 – 0.075), respectively, significant differences in community complexity between PS and PVC was observed at this timescale. Evenness was significantly lower for biofouled PVC microplastics relative to the biofouled PS microplastics after both 7 and 21 days of seawater immersion (GLM p-value < 0.05), while the richness of the PS-DEHP biofilms was significantly lower than all other treatments (GLM p-value < 0.0001), irrespective of chemical composition (Fig. 3).

The developing plastisphere was dominated by Alphaproteobacteria and Gammaproteobacteria, which represented between 20 % and 40 % of retrieved 16S sequences, respectively (Fig. 4 and Fig. S12). Bacteroidota represented the second most dominant group (10 – 40 %), followed by Verrucomicrobiota (< 5 – 20 %) and Planctomycetota (< 5 – 10 %). Dominant members of these phyla at the class level included Bacteroidia (10 – 40 %), Kiritimatiellae (< 5 – 20 %), Planctomycetes (< 5 – 10 %) and Bdellovibrionia (< 5 – 10 %).

The largest shifts in community composition were observed after immersion in seawater from 0 to 7 and 21 days. The 16S sequences affiliated with members of Actinobacteria and Bacilli decreased in abundance by up to 40 %, whilst Bacteroidia, Bdellovibrionia, Planctomycetes, Kiritimatiellae, Alphaproteobacteria and Gammaproteobacteria all increased in abundance by up to 20 % (Fig. 4 and Fig. S7) [91]. Time-dependent shifts in community composition, illustrated using

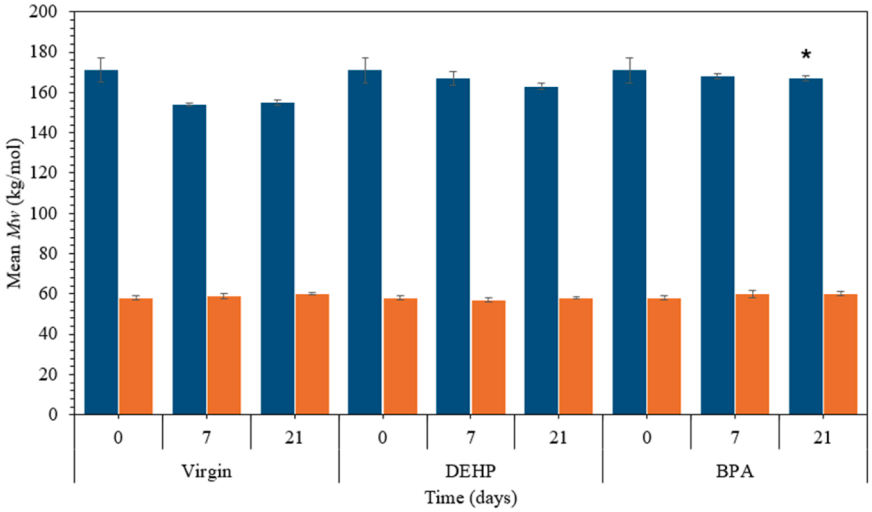

Fig. 1. The mean weight-average molecular weight (*Mw*; kg/mol ± standard deviation) of the polystyrene (blue) and polyvinyl chloride (orange) microplastics; virgin (i.e., containing no additives) or containing 15 wt% diethylhexyl phthalate (DEHP) or 15 wt% bisphenol A (BPA) after 0, 7 and 21 days of marine biofouling. The significant differences between the untreated, control samples (i.e., 0 days) and biofouled microplastics after 7 and 21 days are indicated by * (p-value ≤ 0.05).

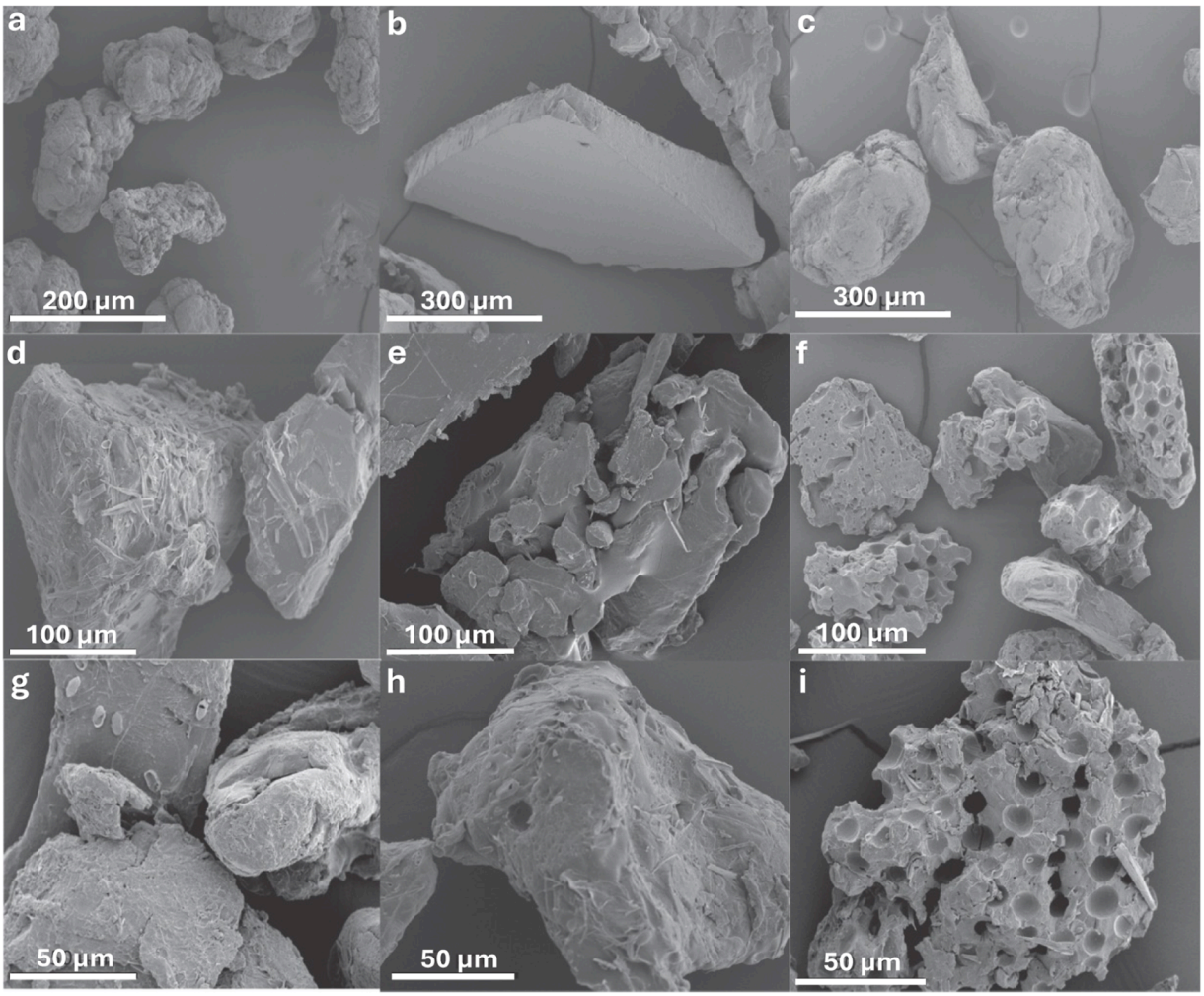

Fig. 2. Representative images of the polystyrene (PS) microplastics after (a – c) 0, (d – f) 7 and (g – i) 21 days of marine biofouling. Microplastics are virgin (i.e., containing no additive; a, d, g), containing 15 wt% diethylhexyl phthalate (DEHP; b, e, h) or 15 wt% bisphenol A (BPA; c, f, i). Scale bars are indicated on each image.

PCoA (Fig. S7) and tested by GLM (Eq. 2), revealed significant microbiome divergence from 0, 7–21 days, which corresponded to a 5.4 – 6.7-fold increase in the mean relative abundance of Gammaproteobacteria (GLM p-value < 0.0001) and Bacteroidia (GLM p-value < 0.0001) (Table S3). Other ASVs detected in relatively high abundance in response to seawater exposure time include species of *Tropicibacter* (63-fold decrease), Rhodobacteraceae (9-fold increase) and Neochlamydia (8-fold decrease). Additionally, community dissimilarity and distinctness in bacterial composition was also associated with polymer and plasticiser type (Fig. 5). The GLM (Eq. 3) identified a total of 30 ASVs that were significantly impacted by polymer-plasticiser composition (Table S4) and mainly corresponded with low abundance members of Enterobacterales, Pseudomonadales, Rhodobacterales and Rhizobiales belonging to the classes Gammaproteobacteria, Cyanobacteriia and Bacteroidia.

Finally, following bacterial community assessment of the alpha diversity and compositional patterns, 16S amplicon sequences affiliated with bacterial clades possessing metabolic capability to degrade PS and/or PVC polymers were identified on the biofouled microplastics after 7 and 21 days [13,54,55,60,92]. Out of 34 genera previously reported with the capability [13], a total of 11 genera were identified in the incoming seawater and on the biofouled microplastics, and included members of *Alcanivorax, Vibrio, Thalassospira, Cohaesibacter, Bacillus, Desulfovibrio, Bravundimonas, Novosphingobium, Alteromonas, Pseudomonas* and *Corynebacterium* (Fig. 6). Unique compositional changes among these bacteria over time were particularly evident when comparing the microbiome of the incoming seawater and that of the developing microplastic-biofilms, with the PS-virgin, PS-DEHP and PS-BPA substrates showing the greatest dissimilarity. For the PS-virgin substrates, a decrease in the total relative abundance of members of *Brevundimonas, Pseudomonas* and *Corynebacterium* was observed between 7 and 21 days of biofouling. For the PS-DEHP substrates, a decrease in *Alcanivorax, Vibrio, Thalassospira, Cohaesibacter* and *Desulfovibrio* was observed between 7 and 21 days, whereas for PS-BPA substrates, an increase in these genera was observed, respectively. Notably, all PVC substrates retrieved the lowest number of affiliated sequences, irrespective of plasticiser composition.

4. Discussion

Comparisons between the chemical (i.e., TGA, FT-IR and GPC) and physical (i.e., SEM) data of the untreated (i.e., prior to seawater exposure; 0 days) and marine biofouled microplastics (i.e., 7 and 21 days) exposed to environmentally relevant tropical marine conditions revealed considerable physicochemical changes indicative of plastic degradation [9]. Likewise, the 16S rRNA gene amplicon sequencing data proved effective in characterising the community patterns of the developing microplastic-biofilms and identified key bacteria, previously reported, that were potentially involved in biodegradation [13,54,55,60,92].

The predominant plastisphere community was comprised of highly abundant and opportunistic marine colonisers, notably including

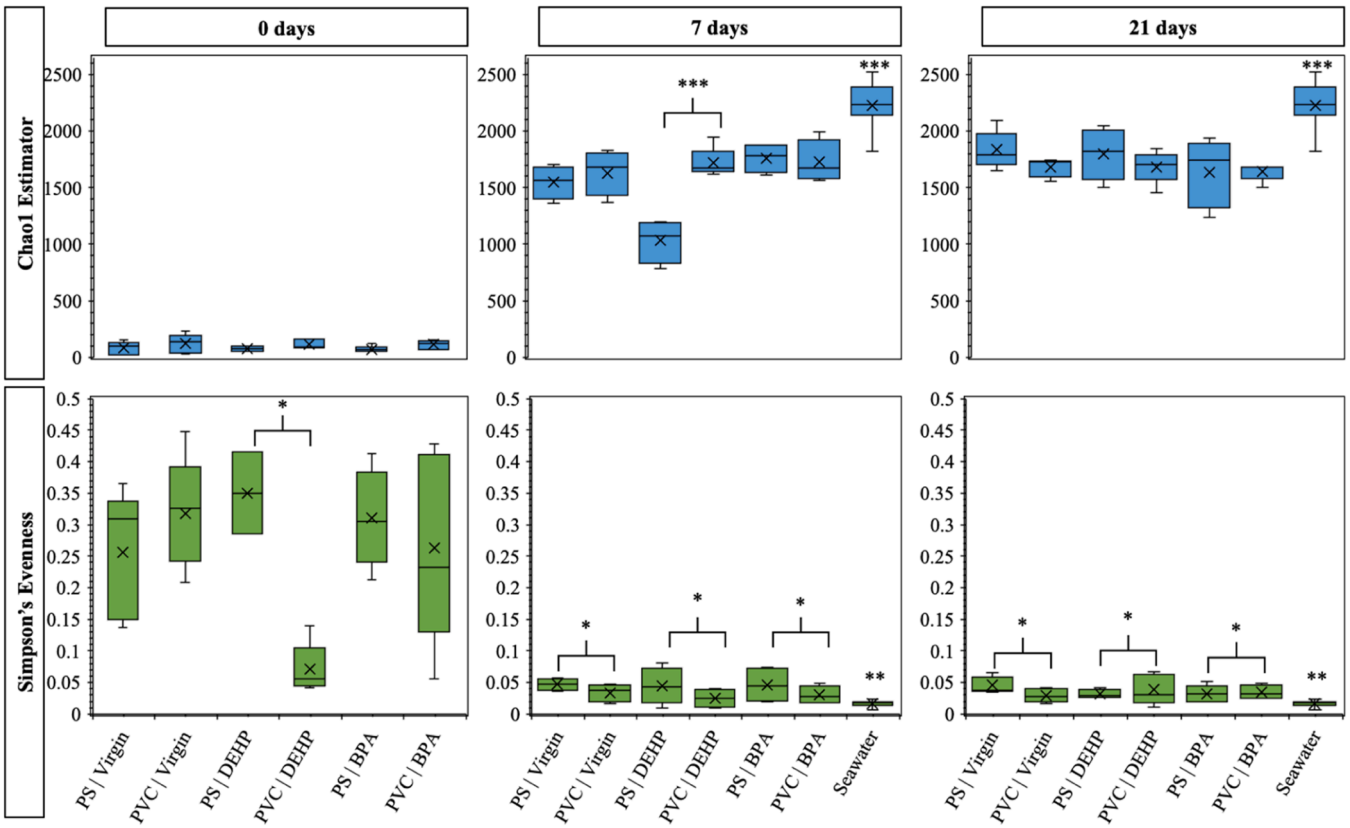

Fig. 3. Alpha diversity metrics: richness (Chao1; blue) and evenness (Simpson's; green) of the background seawater and of the biofouled polystyrene (PS) and polyvinyl chloride (PVC) microplastics; virgin (i.e., no additives) or containing 15 wt% diethylhexyl phthalate (DEHP) or 15 wt% bisphenol A (BPA) after 0, 7 and 21 days. P-values are indicated by * (≤ 0.05), ** (≤ 0.01) and *** (≤ 0.001).

members of Proteobacteria, Bacteroidota and Planctomycetota [91,93,94]. The identified community structure aligns with findings from previous studies on marine plastic biofouling [18,22,24,35,61,95,96], and together highlights some similarities in bacterial composition across different geographic regions and irrespective plastic composition [95]. Bacterial groups that persisted across the 21-day timescale irrespective of polymer and plasticiser type included members of Bacteroidia, Alphaproteobacteria and Gammaproteobacteria such as Flavobacteriales, Rhodobacteriales and Pseudomonadales, respectively (Fig. S12). Members of Flavobacteriales and Rhodobacteriales are recognised as dominant and primary colonisers of marine plastics worldwide and play defining roles in long-term biofilm maturation [29]. Within Gammaproteobacteria, the order Pseudomonadales have also been identified as important marine colonisers of microplastics, with members of this group particularly highlighted for their biodegradative metabolic capabilities [13,29]. Moreover, while similarities in community composition consistent with previous studies were observed here, unique temporal trends were also evident. Most notable was the diversification of bacterial communities upon immersion in seawater and the shifts in community complexity (i.e., richness) and ASV relative abundance between 7 and 21 days (Fig. S7). These community changes suggest a transition from predominately opportunistic primary colonisers at 7 days to more specialised bacteria after 21 days [90,93,94]. These early changes influenced by the polymer-plasticiser composition of the microplastic substrate are likely to affect long-term succession patterns and biofilm maturation, which could ultimately shape the metabolic potential of the plastisphere (e.g., for biodegradation). Based on this information, early intervention strategies that target specific temporal patterns in the plastisphere (i.e., primary colonisers), could be employed to encourage and promote the long-term succession of plastic degrading bacteria for particular polymer-additive microplastic mixtures [92]. Thereby optimising natural plastic removal pathways *in-natura*.

Biofouling at this narrow timescale was also heavily influenced by the physicochemical properties of the plastic [26,61]. The PCoA (Fig. 5) highlighted differences in community structure with respect to the chemical composition of the microplastic at each experimental timepoint. These analyses revealed a polymer- and/or plasticiser-dependency during early-stage marine plastisphere development. Specifically, higher bacterial diversity exhibited selectivity towards PS-DEHP and PS-BPA when compared to PS-virgin, PVC-virgin, PVC-DEHP and PVC-BPA compositions (Fig. 3). While similar polymer-dependent marine biofouling has been previously reported [22,26,30,31,33], this study was the first to also consider the combined impact of incorporated plasticisers under controlled environmentally relevant conditions. Given differences in community composition on the virgin, DEHP and BPA plasticised microplastics, it is likely the contrasting PS (i.e., strongly hydrophobic due to repeating phenyl groups) and PVC (i.e., moderately hydrophobic due to repeating C-Cl bonds) chemical properties in combination with the different leaching behaviours of DEHP (i.e., high polymer retention [49,50,97]) and BPA (i.e., fast and concentrated release [49,50,98]) in seawater uniquely impacted the attachment and succession of primary colonising bacteria [18,38], resulting in differences in biofilm composition. Furthermore, it is likely the presence and concentration of these additives [21,32,99] – both within the polymer and as leachates in the surrounding water – also influenced the biofouling potential of some marine bacteria [100]. With this novel information, QA/QC protocols can be updated to ensure researchers consider how discrete differences in plastic physicochemistry impact colonising bacteria [53]. This is particularly relevant for microplastic exposure studies that employ biofouled microplastics, as differences in biofilm formation can impact the long-term fate of microplastics in different environments, i.e., by encouraging deep sea

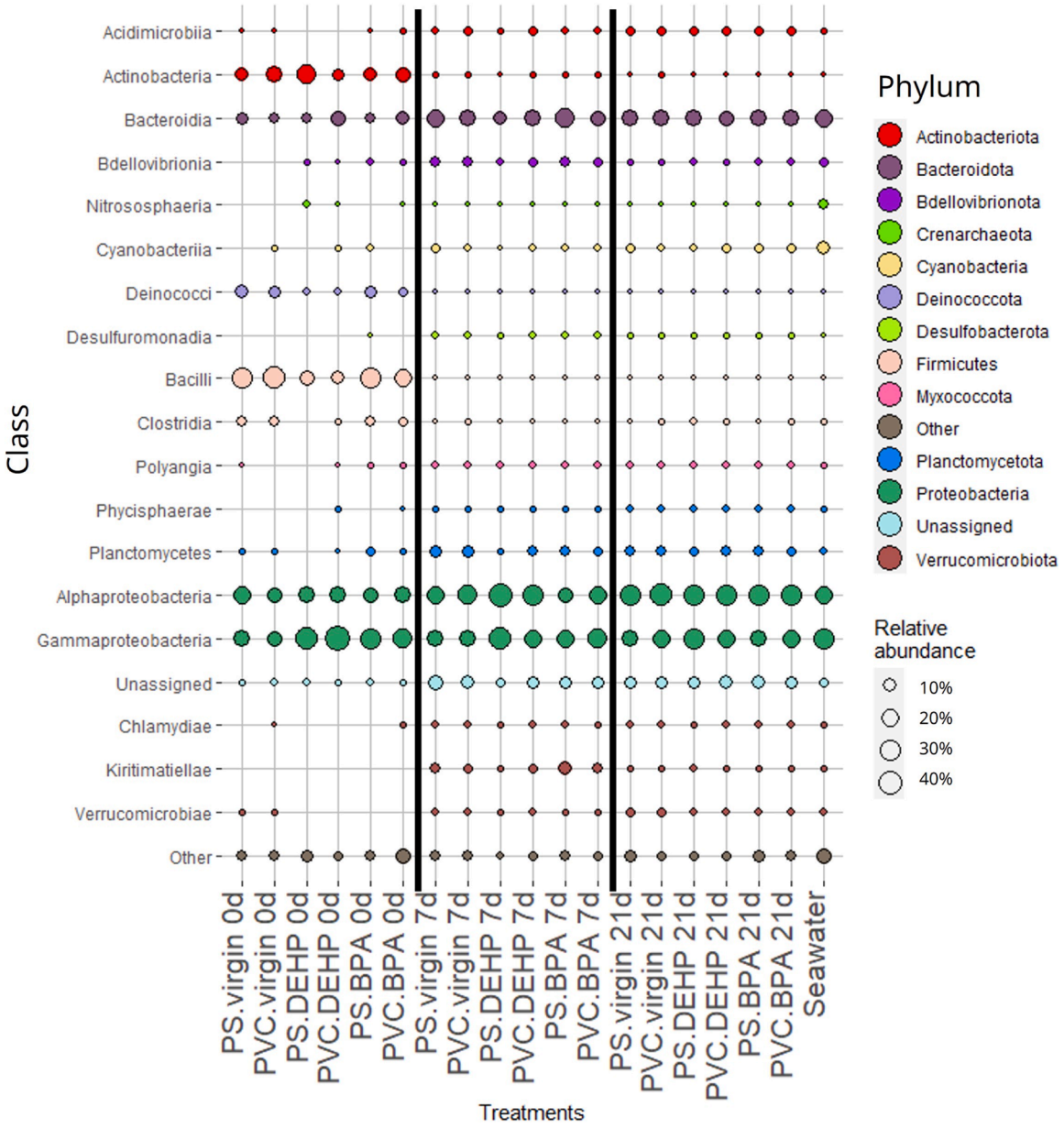

Fig. 4. Mean relative abundance (%) of the most abundant (top 20) bacterial classes present in the background seawater (averaged across both sampling points taken on 7 and 21 days) and on the biofouled polystyrene (PS) and polyvinyl chloride (PVC) microplastics; virgin (i.e., no additive) or containing 15 wt% diethylhexyl phthalate (DEHP) or 15 wt% bisphenol A (BPA) after 0, 7 and 21 days of marine biofouling. "Other" refers to bacterial taxa with less than 1 % affiliated sequences and "unassigned" refers to sequences that could not be taxonomically classified.

settlement [101] and/or by changing biota feeding preferences[53] This underscores the importance of considering both polymer and additive composition when characterising the plastisphere in different marine environments [18,38], especially in sensitive ecosystems with high levels of plastic pollution such as coral reefs[6].

Biofouling and the presence of bacterial clades with metabolic potential to degrade synthetic polymers was considered a significant factor driving microplastic degradation in this study. Degradation of the PS microplastics – especially those incorporated with BPA – was substantial and highlighted a polymer- and plasticiser-dependent susceptibility to marine biodegradation that was enhanced upon extended immersion in seawater from 7 day to 21 days. Biodegradation of these PS-BPA substrates could be due to the presence of bacterial clades identified in the developing biofilm with metabolic capability to degrade the polymer (Fig. 6). Genera of particular interest here include, *Alcanivorax, Novosphingobium, Alteromonas* and *Bacillus*, which when cultured on PS substrates under laboratory conditions have been shown to biodegrade the polymer within similar experimental timeframes [54,102,103]. Biofouling of these bacteria on plastic surfaces have been shown to change the FT-IR spectra (e.g., increased carbonyl bonds [54,104]), comparatively decrease *Mw* values (e.g., < 10 % [10]) and impact surface morphology (e.g., appearance of holes [54,103]), which collectively corroborate the results presented here. Additionally, the time- and plasticiser-dependent data also indicate that progressive exposure to biofouling conditions at this timescale and/or the prolonged settlement of putative biodegradative bacteria can promote marine plastic degradation, with plasticised polymers displaying enhanced susceptibility[52]. Therefore, by understanding these unique trends on different types of dominant microplastic debris (i.e., plasticised-PS and -PVC), bioremediation strategies can be aligned with both the temporal dynamics of microbial colonisation as well as the manufacturing history of the plastic material (i.e., polymer-additive composition) [105]. Finally, these data

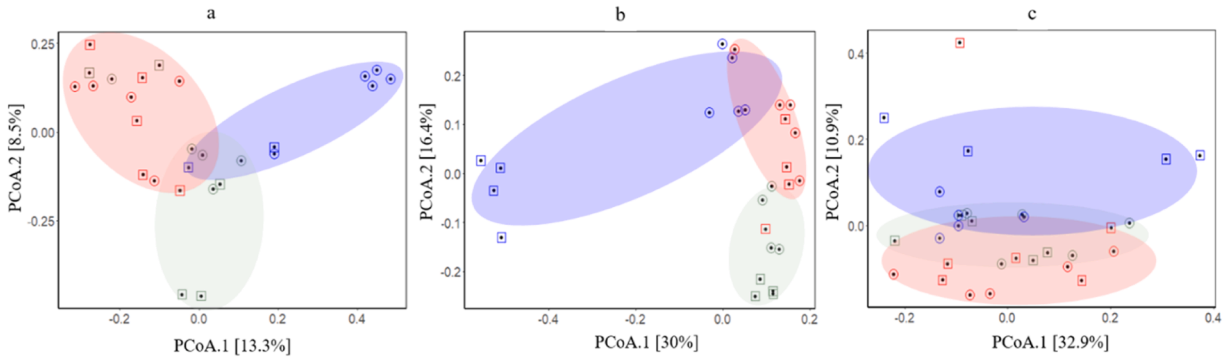

Fig. 5. Principal coordinate analysis (PCoA) plots of 16S amplicon sequences identified on the biofouled polystyrene (PS; square) and polyvinyl chloride (PVC; circle) microplastics; virgin (i.e., no additive; red) or containing 15 wt% diethylhexyl phthalate (DEHP; blue) or 15 wt% bisphenol A (BPA; green) after (a) 0, (b) 7 and (c) 21 days of marine biofouling.

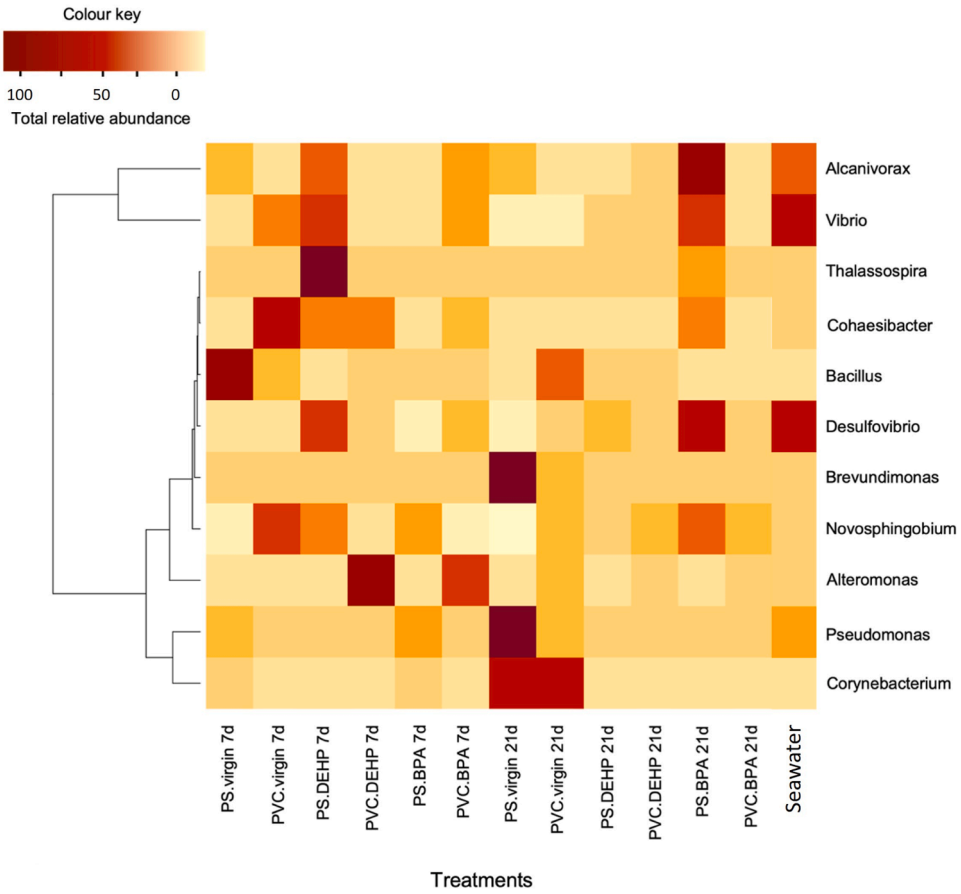

Fig. 6. Heat map showing the total relative abundance (%) of amplicon sequence variants (ASVs) belonging to the 11 genera with biodegradative metabolic potential, which were identified in the background seawater (sum of both sampling points taken day 7 and 21) and on the biofouled polystyrene (PS) and polyvinyl chloride (PVC) microplastics after 7 and 21 days of seawater exposure. Microplastics were prepared virgin (i.e., no additive) or containing 15 wt% diethylhexyl phthalate (DEHP) or 15 wt% bisphenol A (BPA).

also implicate the biofouling and biodegradative potential of the marine microbiome in tropical waters (e.g., along the GBR [19]), which not only offers promising perspectives for effective and low-cost restoration initiatives in these areas, but also in other, similar and threatened coral reef systems worldwide [106]. However, more comprehensive omics analyses and bacterial load data (i.e., concentration of bacteria present in the biofilm) are needed to definitively identify key species and metabolic pathways involved in the observed PS degradation, as well as those that are activated and/or inhibited in response to different plasticiser combinations [39].

5. Conclusion

Microbial colonisation of microplastics holds exciting potential for synthetic polymer degradation [14,16,29]; however, the link between biofouling and plastic degradation remains unclear [107]. Here, time (0, 7 and 21 days), polymer (PS or PVC) and plasticiser composition (-virgin, -DEHP or -BPA) uniquely impacted bacterial biofouling and promoted substantial microplastic degradation, specifically of the PS-BPA substrates. Putative biodegrading bacteria were identified in the incoming seawater, which highlights the biodegradative potential of

marine microbiomes in Australian tropical waters along the GBR and offers promising perspectives for effective and low-cost restoration initiatives in these ecosystems [19]. Furthermore, these novel findings can be used to guide more effective restoration initiatives for tropical waters and coral reefs that integrate both biofilm temporal dynamics (e.g., encouraging early-intervention [92]) and plastic manufacturing history (e.g., incorporating specific polymer-additive mixtures [52]). Further, better QA/QC protocols can be employed in future studies to ensure that potential differences in the developing plastisphere are understood and considered [53,101]. However, given the novelty of the topic and the paucity of literature, more refined and comprehensive analyses such as omics approaches and load data are needed to elucidate the mechanisms underpinning the observed bacterial growth patterns, as this will help to clarify the bacterial-driven biodegradation pathways [39]. Such studies should also assess the impacts of biofouling on other prominent synthetic (e.g., PP or PE) and biopolymer (e.g., polyhydroxyalkanoates (PHAs)) substrates [37]. In conclusion, this study provides valuable insights into the importance of microplastic polymer-plasticiser composition on short-term marine biofouling and resulting polymer biodegradation, which can be driven by coral reef ecosystem bacterial communities [10].

Environmental Implication

This study highlights the potential for natural marine microbiomes to facilitate timely biodegradation of high risk and recalcitrant microplastics. Controlled preparation of the polymer-additive microplastics and exposure to environmentally relevant marine biofouling conditions ensured that the developing biofilm and subsequent biodegradation was representative of natural marine ecosystems. Thus, time-, polymer- and plasticiser-dependent trends in microplastic biofilm formation and biodegradation were highlighted. These results allow for the optimisation of environmental remediation strategies that are aligned with both the temporal dynamics of the microbial community (i.e., effective intervention timing) as well as the manufacturing history of the material (i.e., polymer-additive composition).

CRediT authorship contribution statement

Marina M.F. Santana: Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. **Richard C. Edmunds:** Writing – review & editing, Writing – original draft, Validation, Supervision. **Yun Kit Yeoh:** Writing – review & editing, Validation, Supervision, Formal analysis. **Yui Sato:** Writing – review & editing, Validation, Supervision, Data curation. **Sara C. Bell:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **Felicity Kuek:** Writing – review & editing, Supervision, Resources, Methodology. **Alexandra M. Gulizia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **George Vamvounis:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Pirjo Haikola:** Funding acquisition. **Lynne van Herwerden:** Writing – review & editing, Writing – original draft, Conceptualization. **Cherie A. Motti:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **David G. Bourne:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136975.

Data Availability

Data will be made available on request.

References

[1] Andrady, A.L., 2017. The plastic in microplastics: a review. Mar Pollut Bull 119 (1), 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082.
[2] Yuan, Z., Nag, R., Cummins, E., 2022. Ranking of potential hazards from microplastics polymers in the marine environment. J Hazard Mater 429, 128399. https://doi.org/10.1016/j.jhazmat.2022.128399.
[3] Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. Mar Environ Res 128, 2–11. https://doi.org/10.1016/j.marenvres.2016.05.012.
[4] Santana, M.M.F.Presence, Abundance and Effects of Microplastics on the Great Barrier Reef, 2021.
[5] Jensen, L.H., Motti, C.A., Garm, A.L., Tonin, H., Kroon, F.J., 2019. Sources, distribution and fate of microfibres on the Great Barrier Reef, Australia. Sci Rep 9 (1), 9021. https://doi.org/10.1038/s41598-019-45340-7.
[6] Kroon, F.J., Motti, C.E., Jensen, L.H., Berry, K.L.E., 2018. Classification of marine microdebris: a review and case study on fish from the Great Barrier Reef, Australia. Sci Rep 8 (1), 16422. https://doi.org/10.1038/s41598-018-34590-6.
[7] McCormick, M.I., Chivers, D.P., Ferrari, M.C.O., Blandford, M.I., Nanninga, G.B., Richardson, C., Fakan, E.P., Vamvounis, G., Gulizia, A.M., Allan, B.J.M., 2020. Microplastic exposure interacts with habitat degradation to affect behaviour and survival of juvenile fish in the field. Proc R Soc B: Biol Sci 287 (1937), 20201947. https://doi.org/10.1098/rspb.2020.1947.
[8] Zhou, Y., Kumar, M., Sarsaiya, S., Sirohi, R., Awasthi, S.K., Sindhu, R., Binod, P., Pandey, A., Bolan, N.S., Zhang, Z., Singh, L., Kumar, S., Awasthi, M.K., 2022. Challenges and opportunities in bioremediation of micro-nano plastics: a review. Sci Total Environ 802, 149823. https://doi.org/10.1016/j.scitotenv.2021.149823.
[9] Gulizia, A.M., Brodie, E., Daumuller, R., Bloom, S.B., Corbett, T., Santana, M.M. F., Motti, C.A., Vamvounis, G., 2022. Evaluating the effect of chemical digestion treatments on polystyrene microplastics: recommended updates to chemical digestion protocols. Macromol Chem Phys 223 (13), 2100485. https://doi.org/10.1002/macp.202100485.
[10] Giacomucci, L., Raddadi, N., Soccio, M., Lotti, N., Fava, F., 2020. Biodegradation of polyvinyl chloride plastic films by enriched anaerobic marine consortia. Mar Environ Res 158, 104949. https://doi.org/10.1016/j.marenvres.2020.104949.
[11] Taniguchi, I., Yoshida, S., Hiraga, K., Miyamoto, K., Kimura, Y., Oda, K., 2019. Biodegradation of PET: current status and application aspects. ACS Catal 9 (5), 4089–4105. https://doi.org/10.1021/acscatal.8b05171.
[12] Kim, H.-W., Jo, J.H., Kim, Y.-B., Le, T.-K., Cho, C.-W., Yun, C.-H., Chi, W.S., Yeom, S.-J., 2021. Biodegradation of polystyrene by bacteria from the soil in common environments. J Hazard Mater 416, 126239. https://doi.org/10.1016/j.jhazmat.2021.126239.
[13] Gambarini, V., Pantos, O., Kingsbury, J.M., Weaver, L., Handley, K.M., Lear, G., 2021. Phylogenetic distribution of plastic-degrading microorganisms. mSystems 6 (1). https://doi.org/10.1128/msystems.01112-20.
[14] Mohanan, N., Montazer, Z., Sharma, P.K., Levin, D.B., 2020. Microbial and enzymatic degradation of synthetic Plastics. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.580709.
[15] Zhai, X., Zhang, X.-H., Yu, M., 2023. Microbial colonization and degradation of marine microplastics in the plastisphere: a review. Front Microbiol 14. https://doi.org/10.3389/fmicb.2023.1127308.

[16] Oberbeckmann, S., Labrenz, M., 2020. Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. Ann Rev Mar Sci 12 (1), 209–232. https://doi.org/10.1146/annurev-marine-010419-010633.
[17] Atanasova, N., Stoitsova, S., Paunova-Krasteva, T., Kambourova, M., 2021. Plastic degradation by extremophilic bacteria. Int J Mol Sci 22 (11), 5610. https://doi.org/10.3390/ijms22115610.
[18] Oberbeckmann, S., Loeder, M.G.J., Gerdts, G., Osborn, M.A., 2014. Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. FEMS Microbiol Ecol 90 (2), 478–492. https://doi.org/10.1111/1574-6941.12409.
[19] Frade, P.R., Glasl, B., Matthews, S.A., Mellin, C., Serrão, E.A., Wolfe, K., Mumby, P.J., Webster, N.S., Bourne, D.G., 2020. Spatial patterns of microbial communities across surface waters of the Great Barrier Reef. Commun Biol 3 (1), 442. https://doi.org/10.1038/s42003-020-01166-y.
[20] Botté, E.S., Nielsen, S., Abdul Wahab, M.A., Webster, J., Robbins, S., Thomas, T., Webster, N.S., 2019. Changes in the metabolic potential of the sponge microbiome under ocean acidification. Nat Commun 10 (1), 4134. https://doi.org/10.1038/s41467-019-12156-y.
[21] Peng, G., Pu, Z., Chen, F., Xu, H., Cao, X., Chun Chen, C., Wang, J., Liao, Y., Zhu, X., Pan, K., 2023. Metal leaching from plastics in the marine environment: an ignored role of biofilm. Environ Int 177, 107988. https://doi.org/10.1016/j.envint.2023.107988.
[22] Delacuvellerie, A., Benali, S., Cyriaque, V., Moins, S., Raquez, J.M., Gobert, S., Wattiez, R., 2021. Microbial biofilm composition and polymer degradation of compostable and non-compostable plastics immersed in the marine environment. J Hazard Mater 419, 126526. https://doi.org/10.1016/j.jhazmat.2021.126526.
[23] Ganesan, S., Ruendee, T., Kimura, S.Y., Chawengkijwanich, C., Janjaroen, D., 2022. Effect of biofilm formation on different types of plastic shopping bags: structural and physicochemical properties. Environ Res 206, 112542. https://doi.org/10.1016/j.envres.2021.112542.
[24] Caroppo, C., Azzaro, M., Dell'Acqua, O., Azzaro, F., Maimone, G., Rappazzo, A.C., Raffa, F., Caruso, G., 2022. Microbial biofilms colonizing plastic substrates in the Ross Sea (Antarctica). J Mar Sci Eng 10 (11), 1714. https://doi.org/10.3390/jmse10111714.
[25] Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S., Li, T., 2019. Distinct community structure and microbial functions of biofilms colonizing microplastics. Sci Total Environ 650, 2395–2402. https://doi.org/10.1016/j.scitotenv.2018.09.378.
[26] Ramsperger, A.F.M., Stellwag, A.C., Caspari, A., Fery, A., Lueders, T., Kress, H., Löder, M.G.J., Laforsch, C., 2020. Structural diversity in early-stage biofilm formation on microplastics depends on environmental medium and polymer properties. Water (Basel) 12 (11), 3216. https://doi.org/10.3390/w12113216.
[27] Marturano, V., Cerruti, P., Ambrogi, V., 2019. Polymer additives. Phys Sci Rev 2 (6). https://doi.org/10.1515/psr-2016-0130.
[28] Oberbeckmann, S., Löder, M.G.J., Labrenz, M., 2015. Marine microplastic-associated biofilms – a review. Environ Chem 12 (5), 551. https://doi.org/10.1071/EN15069.
[29] Zhai, X., Zhang, X.-H., Yu, M., 2023. Microbial colonization and degradation of marine microplastics in the plastisphere: a review. Front Microbiol 14. https://doi.org/10.3389/fmicb.2023.1127308.
[30] Hansen, J., Melchiorsen, J., Ciacotich, N., Gram, L., Sonnenschein, E.C., 2021. Effect of polymer type on the colonization of plastic pellets by marine bacteria. FEMS Microbiol Lett 368 (5). https://doi.org/10.1093/femsle/fnab026.
[31] Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., Kerninon, S., Cassone, A.-L., Lambert, C., Reveillaud, J., Paul-Pont, I., 2018. Microplastic bacterial communities in the Bay of Brest: influence of polymer type and size. Environ Pollut 242, 614–625. https://doi.org/10.1016/j.envpol.2018.07.023.
[32] Luo, J., Chen, Z., Sun, Y., 2006. Controlling biofilm formation with AnN-halamine-based polymeric additive. J Biomed Mater Res A 77A (4), 823–831. https://doi.org/10.1002/jbm.a.30689.
[33] Fernández-Juárez, V., López-Alforja, X., Frank-Comas, A., Echeveste, P., Bennasar-Figueras, A., Ramis-Munar, G., Gomila, R.M., Agawin, N.S.R., 2021. The good, the bad and the double-sword" effects of microplastics and their organic additives in marine bacteria. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.581118.
[34] Pedersen, K., 1990. Biofilm development on stainless steel and pvc surfaces in drinking water. Water Res 24 (2), 239–243. https://doi.org/10.1016/0043-1354(90)90109-J.
[35] Dang, H., Lovell, C.R., 2016. Microbial surface colonization and biofilm development in marine environments. Microbiol Mol Biol Rev 80 (1), 91–138. https://doi.org/10.1128/MMBR.00037-15.
[36] Okshevsky, M., Gautier, E., Farner, J.M., Schreiber, L., Tufenkji, N., 2020. Biofilm formation by marine bacteria is impacted by concentration and surface functionalization of polystyrene nanoparticles in a species-specific manner. Environ Microbiol Rep 12 (2), 203–213. https://doi.org/10.1111/1758-2229.12824.
[37] Wu, C., Tanaka, K., Tani, Y., Bi, X., Liu, J., Yu, Q., 2022. Effect of particle size on the colonization of biofilms and the potential of biofilm-covered microplastics as metal carriers. Sci Total Environ 821, 153265. https://doi.org/10.1016/j.scitotenv.2022.153265.
[38] Tu, C., Chen, T., Zhou, Q., Liu, Y., Wei, J., Waniek, J.J., Luo, Y., 2020. Biofilm formation and its influences on the properties of microplastics as affected by exposure time and depth in the seawater. Sci Total Environ 734, 139237. https://doi.org/10.1016/j.scitotenv.2020.139237.
[39] Sooriyakumar, P., Bolan, N., Kumar, M., Singh, L., Yu, Y., Li, Y., Weralupitiya, C., Vithanage, M., Ramanayaka, S., Sarkar, B., Wang, F., Gleeson, D.B., Zhang, D., Kirkham, M.B., Rinklebe, J., M Siddique, K.H., 2022. Biofilm formation and its implications on the properties and fate of microplastics in aquatic environments: a review. J Hazard Mater Adv 6, 100077. https://doi.org/10.1016/j.hazadv.2022.100077.
[40] Wang, H., Yu, P., Schwarz, C., Zhang, B., Huo, L., Shi, B., Alvarez, P.J.J., 2022. Phthalate esters released from plastics promote biofilm formation and chlorine resistance. Environ Sci Technol 56 (2), 1081–1090. https://doi.org/10.1021/acs.est.1c04857.
[41] Zhao, E., Xiong, X., Li, X., Hu, H., Wu, C., 2024. Effect of biofilm forming on the migration of Di(2-Ethylhexyl)Phthalate from PVC plastics. Environ Sci Technol 58 (14), 6326–6334. https://doi.org/10.1021/acs.est.3c09021.
[42] Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-Water. In IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.; International Agency for Research on Cancer: Lyon, 2013; Vol. 101.
[43] Palsania, P., Singhal, K., Dar, M.A., Kaushik, G., 2024. Food grade plastics and Bisphenol A: associated risks, toxicity, and bioremediation approaches. J Hazard Mater 466, 133474. https://doi.org/10.1016/j.jhazmat.2024.133474.
[44] Billings, A., Jones, K.C., Pereira, M.G., Spurgeon, D.J., 2024. Emerging and legacy plasticisers in coastal and estuarine environments: a review. Sci Total Environ 908, 168462. https://doi.org/10.1016/j.scitotenv.2023.168462.
[45] Corrales, J., Kristofco, L.A., Steele, W.B., Yates, B.S., Breed, C.S., Williams, E.S., Brooks, B.W., 2015. Global assessment of Bisphenol A in the environment. Dose-Response 13 (3). https://doi.org/10.1177/1559325815598308.
[46] Zhu, F., Yan, Y., Doyle, E., Zhu, C., Jin, X., Chen, Z., Wang, C., He, H., Zhou, D., Gu, C., 2022. Microplastics altered soil microbiome and nitrogen cycling: the role of phthalate plasticizer. J Hazard Mater 427, 127944. https://doi.org/10.1016/j.jhazmat.2021.127944.
[47] Zaborowska, M., Wyszkowska, J., Borowik, A., 2020. Soil microbiome response to contamination with Bisphenol A, Bisphenol F and Bisphenol S. Int J Mol Sci 21 (10), 3529. https://doi.org/10.3390/ijms21103529.
[48] Adamovsky, O., Bisesi, J.H., Martyniuk, C.J., 2021. Plastics in our water: fish microbiomes at risk? Comp Biochem Physiol Part D Genom Proteom 39, 100834. https://doi.org/10.1016/j.cbd.2021.100834.
[49] Gulizia, A.M., Philippa, B., Zacharuk, J., Motti, C.A., Vamvounis, G., 2023. Plasticiser leaching from polyvinyl chloride microplastics and the implications for environmental risk assessment. Mar Pollut Bull 195, 115392. https://doi.org/10.1016/j.marpolbul.2023.115392.
[50] Gulizia, A.M., Patel, K., Philippa, B., Motti, C.A., van Herwerden, L., Vamvounis, G., 2023. Understanding plasticiser leaching from polystyrene microplastics. Sci Total Environ 857, 159099. https://doi.org/10.1016/j.scitotenv.2022.159099.
[51] Bhagwat, G., O'Connor, W., Grainge, I., Palanisami, T., 2021. Understanding the fundamental basis for biofilm formation on plastic surfaces: role of conditioning films. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.687118.
[52] Selke, S., Auras, R., Nguyen, T.A., Castro Aguirre, E., Cheruvathur, R., Liu, Y., 2015. Evaluation of biodegradation-promoting additives for plastics. Environ Sci Technol 49 (6), 3769–3777. https://doi.org/10.1021/es504258u.
[53] Polhill, L., de Bruijn, R., Amaral-Zettler, L., Praetorius, A., van Wezel, A., 2022. Daphnia magna's favorite snack: biofouled plastics. Environ Toxicol Chem 41 (8), 1977–1981. https://doi.org/10.1002/etc.5393.
[54] Liu, R., Zhao, S., Zhang, B., Li, G., Fu, X., Yan, P., Shao, Z., 2023. Biodegradation of polystyrene (PS) by marine bacteria in mangrove ecosystem. J Hazard Mater 442, 130056. https://doi.org/10.1016/j.jhazmat.2022.130056.
[55] Khandare, S.D., Chaudhary, D.R., Jha, B., 2021. Bioremediation of polyvinyl chloride (PVC) films by marine bacteria. Mar Pollut Bull 169, 112566. https://doi.org/10.1016/j.marpolbul.2021.112566.
[56] Yuan, Z., Nag, R., Cummins, E., 2022. Ranking of potential hazards from microplastics polymers in the marine environment. J Hazard Mater 429, 128399. https://doi.org/10.1016/j.jhazmat.2022.128399.
[57] Marcharla, E., Vinayagam, S., Gnanasekaran, L., Soto-Moscoso, M., Chen, W.-H., Thanigaivel, S., Ganesan, S., 2024. Microplastics in marine ecosystems: a comprehensive review of biological and ecological implications and its mitigation approach using nanotechnology for the sustainable environment. Environ Res 256, 119181. https://doi.org/10.1016/j.envres.2024.119181.
[58] Chapron, L., Peru, E., Engler, A., Ghiglione, J.F., Meistertzheim, A.L., Pruski, A.M., Purser, A., Vétion, G., Galand, P.E., Lartaud, F., 2018. Macro- and microplastics affect cold-water corals growth, feeding and behaviour. Sci Rep 8 (1), 15299. https://doi.org/10.1038/s41598-018-33683-6.
[59] Rahman, Md.N., Shozib, S.H., Akter, Mst Y., Islam, A.R., Md, T., Islam, Md.S., Sohel, Md.S., Kamaraj, C., Rakib, Md.R.J., Idris, A.M., Sarker, A., Malafaia, G., 2023. Microplastic as an invisible threat to the coral reefs: sources, toxicity mechanisms, policy intervention, and the way forward. J Hazard Mater 454, 131522. https://doi.org/10.1016/j.jhazmat.2023.131522.
[60] Saeed, S., Iqbal, A., Deeba, F., 2022. Biodegradation study of polyethylene and PVC using naturally occurring plastic degrading microbes. Arch Microbiol 204 (8), 497. https://doi.org/10.1007/s00203-022-03081-8.
[61] Lobelle, D., Cunliffe, M., 2011. Early microbial biofilm formation on marine plastic debris. Mar Pollut Bull 62 (1), 197–200. https://doi.org/10.1016/j.marpolbul.2010.10.013.
[62] Lear, G., Kingsbury, J.M., Franchini, S., Gambarini, V., Maday, S.D.M., Wallbank, J.A., Weaver, L., Pantos, O., 2021. Plastics and the microbiome: impacts and solutions. Environ Micro 16 (1), 2. https://doi.org/10.1186/s40793-020-00371-w.

[63] Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9 (12), e111913. https://doi.org/10.1371/journal.pone.0111913.
[64] Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debeljak, P., Maral, H., Schoeneich-Argent, R., Brambini, R., Reisser, J., 2018. Evidence that the great pacific garbage patch is rapidly accumulating plastic. Sci Rep 8 (1), 4666. https://doi.org/10.1038/s41598-018-22939-w.
[65] Gigault, J., Halle, A. ter, Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: what is a nanoplastic? Environ Pollut 235, 1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024.
[66] Allen, D., Allen, S., Abbasi, S., Baker, A., Bergmann, M., Brahney, J., Butler, T., Duce, R.A., Eckhardt, S., Evangeliou, N., Jickells, T., Kanakidou, M., Kershaw, P., Laj, P., Levermore, J., Li, D., Liss, P., Liu, K., Mahowald, N., Masque, P., Materić, D., Mayes, A.G., McGinnity, P., Osvath, I., Prather, K.A., Prospero, J.M., Revell, L.E., Sander, S.G., Shim, W.J., Slade, J., Stein, A., Tarasova, O., Wright, S., 2022. Microplastics and nanoplastics in the marine-atmosphere environment. Nat Rev Earth Environ 3 (6), 393–405. https://doi.org/10.1038/s43017-022-00292-x.
[67] Wolfe, K., Byrne, M., 2022. Overview of the Great Barrier Reef sea cucumber fishery with focus on vulnerable and endangered species. Biol Conserv 266, 109451. https://doi.org/10.1016/j.biocon.2022.109451.
[68] Scott, M.E., Tebbett, S.B., Whitman, K.L., Thompson, C.A., Mancini, F.B., Heupel, M.R., Pratchett, M.S., 2022. Variation in abundance, diversity and composition of coral reef fishes with increasing depth at a submerged shoal in the northern Great Barrier Reef. Rev Fish Biol Fish 32 (3), 941–962. https://doi.org/10.1007/s11160-022-09716-9.
[69] Mellin, C., Peterson, E.E., Puotinen, M., Schaffelke, B., 2020. Representation and complementarity of the long-term coral monitoring on the Great Barrier Reef. Ecol Appl 30 (6). https://doi.org/10.1002/eap.2122.
[70] Brandon, J., Goldstein, M., Ohman, M.D., 2016. Long-term aging and degradation of microplastic particles: comparing in situ oceanic and experimental weathering patterns. Mar Pollut Bull 110 (1), 299–308. https://doi.org/10.1016/j.marpolbul.2016.06.048.
[71] Lane, D. 16S/23S RRNA Sequencing; Stackebrandt, E., Goodfellow, M., Eds.; Wiley: New York, 1991.
[72] Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., Prill, R.J., Tripathi, A., Gibbons, S.M., Ackermann, G., Navas-Molina, J.A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J.T., Mirarab, S., Zech Xu, Z., Jiang, L., Haroon, M.F., Kanbar, J., Zhu, Q., Jin Song, S., Kosciolek, T., Bokulich, N.A., Lefler, J., Brislawn, C.J., Humphrey, G., Owens, S. M., Hampton-Marcell, J., Berg-Lyons, D., McKenzie, V., Fierer, N., Fuhrman, J.A., Clauset, A., Stevens, R.L., Shade, A., Pollard, K.S., Goodwin, K.D., Jansson, J.K., Gilbert, J.A., Knight, R., Rivera, J.L.A., Al-Moosawi, L., Alverdy, J., Amato, K.R., Andras, J., Angenent, L.T., Antonopoulos, D.A., Apprill, A., Armitage, D., Ballantine, K., Bárta, J., Baum, J.K., Berry, A., Bhatnagar, A., Bhatnagar, M., Biddle, J.F., Bittner, L., Boldgiv, B., Bottos, E., Boyer, D.M., Braun, J., Brazelton, W., Brearley, F.Q., Campbell, A.H., Caporaso, J.G., Cardona, C., Carroll, J., Cary, S.C., Casper, B.B., Charles, T.C., Chu, H., Claar, D.C., Clark, R.G., Clayton, J.B., Clemente, J.C., Cochran, A., Coleman, M.L., Collins, G., Colwell, R. R., Contreras, M., Crary, B.B., Creer, S., Cristol, D.A., Crump, B.C., Cui, D., Daly, S.E., Davalos, L., Dawson, R.D., Defazio, J., Delsuc, F., Dionisi, H.M., Dominguez-Bello, M.G., Dowell, R., Dubinsky, E.A., Dunn, P.O., Ercolini, D., Espinoza, R.E., Ezenwa, V., Fenner, N., Findlay, H.S., Fleming, I.D., Fogliano, V., Forsman, A., Freeman, C., Friedman, E.S., Galindo, G., Garcia, L., Garcia-Amado, M.A., Garshelis, D., Gasser, R.B., Gerdts, G., Gibson, M.K., Gifford, I., Gill, R.T., Giray, T., Gittel, A., Golyshin, P., Gong, D., Grossart, H.-P., Guyton, K., Haig, S.-J., Hale, V., Hall, R.S., Hallam, S.J., Handley, K.M., Hasan, N.A., Haydon, S.R., Hickman, J.E., Hidalgo, G., Hofmockel, K.S., Hooker, J., Hulth, S., Hultman, J., Hyde, E., Ibáñez-Álamo, J.D., Jastrow, J.D., Jex, A.R., Johnson, L.S., Johnston, E.R., Joseph, S., Jurburg, S.D., Jurelevicius, D., Karlsson, A., Karlsson, R., Kauppinen, S., Kellogg, C.T.E., Kennedy, S.J., Kerkhof, L.J., King, G. M., Kling, G.W., Koehler, A.V., Krezalek, M., Kueneman, J., Lamendella, R., Landon, E.M., Lane-deGraaf, K., LaRoche, J., Larsen, P., Laverock, B., Lax, S., Lentino, M., Levin, I.I., Liancourt, P., Liang, W., Linz, A.M., Lipson, D.A., Liu, Y., Lladser, M.E., Lozada, M., Spirito, C.M., MacCormack, W.P., MacRae-Crerar, A., Magris, M., Martín-Platero, A.M., Martín-Vivaldi, M., Martínez, L.M., Martínez-Bueno, M., Marzinelli, E.M., Mason, O.U., Mayer, G.D., McDevitt-Irwin, J.M., McDonald, J.E., McGuire, K.L., McMahon, K.D., McMinds, R., Medina, M., Mendelson, J.R., Metcalf, J.L., Meyer, F., Michelangeli, F., Miller, K., Mills, D.A., Minich, J., Mocali, S., Moitinho-Silva, L., Moore, A., Morgan-Kiss, R.M., Munroe, P., Myrold, D., Neufeld, J.D., Ni, Y., Nicol, G.W., Nielsen, S., Nissimov, J. I., Niu, K., Nolan, M.J., Noyce, K., O'Brien, S.L., Okamoto, N., Orlando, L., Castellano, Y.O., Osuolale, O., Oswald, W., Parnell, J., Peralta-Sánchez, J.M., Petraitis, P., Pfister, C., Pilon-Smits, E., Piombino, P., Pointing, S.B., Pollock, F.J., Potter, C., Prithiviraj, B., Quince, C., Rani, A., Ranjan, R., Rao, S., Rees, A.P., Richardson, M., Riebesell, U., Robinson, C., Rockne, K.J., Rodriguezl, S.M., Rohwer, F., Roundstone, W., Safran, R.J., Sangwan, N., Sanz, V., Schrenk, M., Schrenzel, M.D., Scott, N.M., Seger, R.L., Seguin-Orlando, A., Seldin, L., Seyler, L. M., Shakhsheer, B., Sheets, G.M., Shen, C., Shi, Y., Shin, H., Shogan, B.D., Shutler, D., Siegel, J., Simmons, S., Sjöling, S., Smith, D.P., Soler, J.J., Sperling, M., Steinberg, P.D., Stephens, B., Stevens, M.A., Taghavi, S., Tai, V., Tait, K., Tan, C.L., Tas¸, N., Taylor, D.L., Thomas, T., Timling, I., Turner, B.L., Urich, T., Ursell, L.K., van der Lelie, D., Van Treuren, W., van Zwieten, L., Vargas-Robles, D., Thurber, R.V., Vitaglione, P., Walker, D.A., Walters, W.A., Wang, S., Wang, T., Weaver, T., Webster, N.S., Wehrle, B., Weisenhorn, P., Weiss, S., Werner, J.J., West, K., Whitehead, A., Whitehead, S.R., Whittingham, L.A., Willerslev, E., Williams, A.E., Wood, S.A., Woodhams, D.C., Yang, Y., Zaneveld, J., Zarraonaindia, I., Zhang, Q., Zhao, H., 2017. A communal catalogue reveals earth's multiscale microbial diversity. Nature 551 (7681), 457–463. https://doi.org/10.1038/nature24621.
[73] Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32 (19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354.
[74] Rideout, J.R., Chase, J.H., Bolyen, E., Ackermann, G., González, A., Knight, R., Caporaso, J.G., 2016. Keemei: cloud-based validation of tabular bioinformatics file formats in google sheets. Gigascience 5 (1), 27. https://doi.org/10.1186/s13742-016-0133-6.
[75] Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G. A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S. C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0209-9.
[76] Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from illumina amplicon sequencing. Nat Methods 10 (1), 57–59. https://doi.org/10.1038/nmeth.2276.
[77] Ramette, A., 2007. Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62 (2), 142–160. https://doi.org/10.1111/j.1574-6941.2007.00375.x.
[78] Lahti, L.; Sudarshan, S. microbiome R package: Tools for microbiome analysis in R. http://microbiome.github.com/microbiome.
[79] Das, G., Bordoloi, N.K., Rai, S.K., Mukherjee, A.K., Karak, N., 2012. Biodegradable and biocompatible epoxidized vegetable oil modified thermostable poly(vinyl chloride): thermal and performance characteristics post biodegradation with pseudomonas Aeruginosa and Achromobacter Sp. J Hazard Mater 434–442. https://doi.org/10.1016/j.jhazmat.2012.01.043.
[80] Gambarini, V., Pantos, O., Kingsbury, J.M., Weaver, L., Handley, K.M., Lear, G., 2021. Phylogenetic distribution of plastic-degrading microorganisms. mSystems 6 (1). https://doi.org/10.1128/msystems.01112-20.
[81] Oberbeckmann, S., Löder, M.G.J., Labrenz, M., 2015. Marine microplastic-associated biofilms - a review. Environ Chem 12 (5), 551–562. https://doi.org/10.1071/EN15069.
[82] Zhang, Z., Peng, H., Yang, D., Zhang, G., Zhang, J., Ju, F., 2022. Polyvinyl chloride degradation by a bacterium isolated from the gut of insect larvae. Nat Commun 13 (1), 5360. https://doi.org/10.1038/s41467-022-32903-y.
[83] Giacomucci, L., Raddadi, N., Soccio, M., Lotti, N., Fava, F., 2020. Biodegradation of polyvinyl chloride plastic films by enriched anaerobic marine consortia. Mar Environ Res 158, 104949. https://doi.org/10.1016/j.marenvres.2020.104949.
[84] Zackular, J.P., Baxter, N.T., Iverson, K.D., Sadler, W.D., Petrosino, J.F., Chen, G. Y., Schloss, P.D., 2013. The gut microbiome modulates colon tumorigenesis. mBio 4 (6). https://doi.org/10.1128/mBio.00692-13.
[85] Micallef, L., Rodgers, P., 2014. EulerAPE: drawing area-proportional 3-venn diagrams using ellipses. PLoS One 9 (7), e101717. https://doi.org/10.1371/journal.pone.0101717.
[86] Wilkinson, L., 2012. Exact and approximate area-proportional circular venn and euler diagrams. IEEE Trans Vis Comput Graph 18 (2), 321–331. https://doi.org/10.1109/TVCG.2011.56.
[87] Latva, M., Dedman, C.J., Wright, R.J., Polin, M., Christie-Oleza, J.A., 2022. Microbial pioneers of plastic colonisation in coastal seawaters. Mar Pollut Bull 179, 113701. https://doi.org/10.1016/j.marpolbul.2022.113701.
[88] Santana, M.M.F.. Presence, Abundance and Effects of Microplastics on the Great Barrier Reef, 2021.
[89] Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15 (12), 550. https://doi.org/10.1186/s13059-014-0550-8.
[90] Yu, J., Sun, L., Ma, C., Qiao, Y., Yao, H., 2016. Thermal degradation of PVC: a review. Waste Manag 48, 300–314. https://doi.org/10.1016/j.wasman.2015.11.041.
[91] Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., Djahanschiri, B., Zeller, G., Mende, D.R., Alberti, A., Cornejo-Castillo, F.M., Costea, P.I., Cruaud, C., d'Ovidio, F., Engelen, S., Ferrera, I., Gasol, J.M., Guidi, L., Hildebrand, F., Kokoszka, F., Lepoivre, C., Lima-Mendez, G., Poulain, J., Poulos, B.T., Royo-Llonch, M., Sarmento, H., Vieira-Silva, S., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Bowler, C., de Vargas, C., Gorsky, G.,

Grimsley, N., Hingamp, P., Iudicone, D., Jaillon, O., Not, F., Ogata, H., Pesant, S., Speich, S., Stemmann, L., Sullivan, M.B., Weissenbach, J., Wincker, P., Karsenti, E., Raes, J., Acinas, S.G., Bork, P., Boss, E., Bowler, C., Follows, M., Karp-Boss, L., Krzic, U., Reynaud, E.G., Sardet, C., Sieracki, M., Velayoudon, D., 1979. Structure and function of the global ocean microbiome. Science 2015 (348), 6237. https://doi.org/10.1126/science.1261359.

[92] Amobonye, A., Bhagwat, P., Singh, S., Pillai, S., 2021. Plastic biodegradation: frontline microbes and their enzymes. Sci Total Environ 759, 143536. https://doi.org/10.1016/j.scitotenv.2020.143536.

[93] Lu, J., Shu, Y., Zhang, H., Zhang, S., Zhu, C., Ding, W., Zhang, W., 2023. The landscape of global ocean microbiome: from bacterioplankton to biofilms. Int J Mol Sci 24 (7), 6491. https://doi.org/10.3390/ijms24076491.

[94] Wallbank, J.A., Lear, G., Kingsbury, J.M., Weaver, L., Doake, F., Smith, D.A., Audrézet, F., Maday, S.D.M., Gambarini, V., Donaldson, L., Theobald, B., Barbier, M., Pantos, O., 2022. Into the plastisphere, where only the generalists thrive: early insights in plastisphere microbial community succession. Front Mar Sci 9. https://doi.org/10.3389/fmars.2022.841142.

[95] Basili, M., Quero, G.M., Giovannelli, D., Manini, E., Vignaroli, C., Avio, C.G., De Marco, R., Luna, G.M., 2020. Major role of surrounding environment in shaping biofilm community composition on marine plastic debris. Front Mar Sci 7. https://doi.org/10.3389/fmars.2020.00262.

[96] Eich, A., Mildenberger, T., Laforsch, C., Weber, M., 2015. Biofilm and diatom succession on polyethylene (PE) and biodegradable plastic bags in two marine habitats: early signs of degradation in the pelagic and benthic zone? PLoS One 10 (9). https://doi.org/10.1371/journal.pone.0137201.

[97] Ekelund, M., Azhdar, B., Gedde, U.W., 2010. Evaporative loss kinetics of Di(2-Ethylhexyl)Phthalate (DEHP) from pristine DEHP and plasticized PVC. Polym Degrad Stab 95 (9), 1789–1793. https://doi.org/10.1016/j.polymdegradstab.2010.05.007.

[98] Sun, P., Liu, X., Zhang, M., Li, Z., Cao, C., Shi, H., Yang, Y., Zhao, Y., 2021. Sorption and leaching behaviors between aged MPs and BPA in water: the role of BPA binding modes within plastic matrix. Water Res 195, 116956. https://doi.org/10.1016/j.watres.2021.116956.

[99] Latorre, I., Hwang, S., Sevillano, M., Montalvo-Rodríguez, R., 2012. PVC biodeterioration and DEHP leaching by DEHP-degrading bacteria. Int Biodeterior Biodegrad 69, 73–81. https://doi.org/10.1016/j.ibiod.2011.12.011.

[100] Skovhus, T.L., Søborg, D.A., Braga, F.S., Højris, B., Kristensen, K.B., Hansen, K.L., 2022. Effects of early biofilm formation on water quality during commissioning of new polyethylene pipes. Environ Sci (Camb 8 (9), 1992–2005. https://doi.org/10.1039/D2EW00200K.

[101] Kaiser, D., Kowalski, N., Waniek, J.J., 2017. Effects of biofouling on the sinking behavior of microplastics. Environ Res Lett 12 (12), 124003. https://doi.org/10.1088/1748-9326/aa8e8b.

[102] Yang, Y., Yang, J., Wu, W.-M., Zhao, J., Song, Y., Gao, L., Yang, R., Jiang, L., 2015. Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 2. Role of Gut Microorganisms. Environ Sci Technol 49 (20), 12087–12093. https://doi.org/10.1021/acs.est.5b02663.

[103] Xie, H., Chen, J., Feng, L., He, L., Zhou, C., Hong, P., Sun, S., Zhao, H., Liang, Y., Ren, L., Zhang, Y., Li, C., 2021. Chemotaxis-selective colonization of mangrove rhizosphere microbes on nine different microplastics. Sci Total Environ 752, 142223. https://doi.org/10.1016/j.scitotenv.2020.142223.

[104] McGivney, E., Cederholm, L., Barth, A., Hakkarainen, M., Hamacher-Barth, E., Ogonowski, M., Gorokhova, E., 2020. Rapid physicochemical changes in microplastic induced by biofilm formation. Front Bioeng Biotechnol 8. https://doi.org/10.3389/fbioe.2020.00205.

[105] Agostini, L., Moreira, J.C.F., Bendia, A.G., Kmit, M.C.P., Waters, L.G., Santana, M. F.M., Sumida, P.Y.G., Turra, A., Pellizari, V.H., 2021. Deep-sea plastisphere: long-term colonization by plastic-associated bacterial and archaeal communities in the Southwest Atlantic Ocean. Sci Total Environ 793, 148335. https://doi.org/10.1016/j.scitotenv.2021.148335.

[106] McCook, L.J., Ayling, T., Cappo, M., Choat, J.H., Evans, R.D., De Freitas, D.M., Heupel, M., Hughes, T.P., Jones, G.P., Mapstone, B., Marsh, H., Mills, M., Molloy, F.J., Pitcher, C.R., Pressey, R.L., Russ, G.R., Sutton, S., Sweatman, H., Tobin, R., Wachenfeld, D.R., Williamson, D.H., 2010. Adaptive management of the great barrier reef: a globally significant demonstration of the benefits of networks of marine reserves. Proc Natl Acad Sci 107 (43), 18278–18285. https://doi.org/10.1073/pnas.0909335107.

[107] Danso, D., Chow, J., Streita, W.R., 2019. Plastics: environmental and biotechnological perspectives on microbial degradation. Appl Environ Microbiol 85 (19). https://doi.org/10.1128/AEM.01095-19.