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Differentiation of bacterial communities on five common plastics after six days of exposure to Caribbean coastal waters

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ABSTRACT

Plastic pollution in coastal areas, particularly in subtropical and tropical regions, remains a pervasive environmental issue. Marine plastic debris provides an artificial surface that rapidly accumulates a dynamic microbial biofilm upon entering the marine ecosystem. Especially the early stages of colonization are critical in shaping the microbial community. This study investigates the early microbial colonization, in less than a week, on five different plastic polymers in Caribbean coastal waters through 16S rRNA gene amplicon sequencing. We discovered shared bacterial taxa among the various plastic polymers and sampling timepoints, with dominant orders being Flavobacteriales, Rhodobacterales, Rhizobiales, and Pseudomonadales. Statistical analysis confirmed significant differences in community composition between the two sampling points, with polystyrene exhibiting a distinct microbial community on day 6 compared to polyethylene, polypropylene, and nylon. We found the same for polyethylene compared to nylon and polyethylene-terephthalate. Further examination identified 47 genera responsible for these differences, primarily belonging to the phyla Proteobacteria and Bacteroidota. Our data indicate an influence of both environmentally related stochastic processes and plastic-related specific factors during early colonization. Interestingly, we noticed an increase in the relative abundance of hydrocarbon and potentially plastic-degrading bacteria (PDB) from 12.4 to 34.5 % between the first and sixth day, suggesting their vital role in shaping the epiplastic community. Notably, some identified PDB have been reported to degrade the specific polymers studied, thus the monitored increase in relative abundance supports their role in plastic degradation. However, more research is required to fully understand their functioning and potential role in the epiplastic community. Our study provides insights into the prokaryotic colonization of marine plastics in the Caribbean basin, where to date studies have been limited despite high pollution rates.

1. Introduction

Unmanaged plastic waste presents a global environmental crisis, exerting detrimental effects on both terrestrial and aquatic ecosystems (Joos and De Tender, 2022; de Souza Machado et al., 2018; Wayman and Niemann, 2021; Xanthos and Walker, 2018). Among all plastic waste generated worldwide, marine plastic debris (MPD) accounts for a substantial portion, with estimates suggesting that 0.1–4.1 % of plastic waste ends up in the oceans (Cózar et al., 2014; Jambeck et al., 2015). Since the 1950s, MPD accumulation in the oceans has surpassed 350 million metric tons (Mt) (Wayman and Niemann, 2021) of which 0.093–3.4 Mt can be accounted for in global budget estimates (Kaandorp et al., 2023; Lebreton et al., 2019; Van Sebille et al., 2015) and MPD represents 42–96 % of the solid waste in marine and coastal environments (Garcés-Ordóñez et al., 2020). The most prevalent polymers in global plastic production are polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyurethane (PU), and polyamide (PA, e.g. nylon) (Geyer et al., 2017), representing 77.5 % (Plastics Europe, 2020) to up to 83.3 % (Geyer et al., 2017) of total production worldwide. Notably, these polymers also account for 70–92 % of total plastic debris in different types of marine habitats (Erni-Cassola et al., 2019). Plastic waste enters the marine environment

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through various pathways, including marine vessels, rivers, coastal littering, and atmospheric deposition (Allen et al., 2022; Lebreton et al., 2022, 2019; Onink et al., 2021). MPD accumulates in well-known hotspots such as subtropical gyres but is also ubiquitous in the deep sea, enclosed basins like the Mediterranean, in coastal sediments and on beaches (Hidalgo-Ruz and Thiel, 2013; Kaandorp et al., 2020; Lebreton et al., 2018; McDermid and McMullen, 2004; Onink et al., 2021; Onink and Laufkötter, 2020; Van Cauwenberghe et al., 2013; Van Sebille et al., 2015). Geographically, coastal pollution with MPD, though globally ubiquitous, is particularly severe in tropical regions (Onink et al., 2019; Silvestrova and Stepanova, 2021).

The Caribbean Large Marine Ecosystem (CLME) is recognized as the most biodiverse region in the Western Tropical Atlantic Ocean, featuring crucial habitats like beaches, mangroves, seagrass beds, and coral reefs, which host a diverse array of resident and migratory organisms (Kanhai et al., 2022). Given its coastal nature and the presence of major rivers, the CLME is susceptible to plastic accumulation (Kanhai et al., 2022) and widespread microplastic pollution has been monitored in the Caribbean basin (Aranda et al., 2022). Furthermore, prior research identified MPD inputs originating from the Atlantic Ocean into the Caribbean basin, uncovering plastic debris exchange facilitated by ocean currents (Courtene-Jones et al., 2021). This exchange combined with the proximity to the North Atlantic Gyre, a known plastic accumulation zone (Law et al., 2010), adds to the basin's susceptivity to plastic pollution (Kanhai et al., 2022). Indeed, compared with other Atlantic islands, Caribbean (island) beaches reveal higher densities of macroplastics, with the items found primarily land-based and from riverine sources (Courtene-Jones et al., 2021; Kanhai et al., 2022; Monteiro et al., 2018). Thus far, research on plastic pollution in the CLME has mainly focused on beach debris accumulation (Bosker et al., 2018; Garcés-Ordóñez et al., 2020; Ivar do Sul and Costa, 2007; Mesquita et al., 2022; Monteiro et al., 2018). In the meantime, little is known about plastic pollution's impact on other marine habitats like sediments and the water column in the CLME (Courtene-Jones et al., 2021) and about biotic interactions with said plastics, such as microbial colonization of PMD, potentially including plastic degraders (Dudek et al., 2020; Kanhai et al., 2022; Vaksmaa et al., 2021b, 2021a).

Since plastic in aquatic environments, including the Caribbean Sea, quickly becomes colonized by a variety of organisms, including bacteria, it is relevant to study the colonization of plastics in the CLME, a process that rarely has been studied to date (Dudek et al., 2020). On surfaces such as plastic, the microbial community forms biofilms, which are complex 3-dimensional structures where microbial cells form aggregates and adhere to interfaces (Amaral-Zettler et al., 2020; Harrison et al., 2014; Vaksmaa et al., 2021b; Zettler et al., 2013). The community of bacteria, archaea, fungi, and larger organisms, forms a unique ecosystem associated with plastic waste in marine environments. Their lifestyle, metabolism, and biogeochemical activity are distinctive from planktonic microbes in the water column (Bryant et al., 2016) and the community is sometimes referred to as the "plastisphere" (Zettler et al., 2013). Biofilms represent a dominant habitat for bacteria and archaea and thus play a vital role in biogeochemical processes. (Bosker et al., 2018; Flemming and Wuertz, 2019). Living in a biofilm community provides bacteria with several advantages over the free-living state, including higher tolerance to stressors, protection against dehydration and predation, and enhanced access to nutrients, e.g. through nutrient recycling or emulsification of substrates (Caruso et al., 2018; Gharibzahedi et al., 2014). Several studies working on plastic-associated biofilms examined collected "wild plastics", which have been present in the marine environment for an unknown amount of time, without taking colonization dynamics into account. On the other hand, the available *in vivo* incubation studies typically focused on longer timeframes, ranging from weeks to months (Wright et al., 2020; Yu et al., 2023), rarely taking changing environmental conditions into account. Environmental factors are believed to significantly shape the microbial communities on plastics, e.g. seasonality, salinity, temperature, and biogeographical properties (Bos et al., 2023; Coons et al., 2021; Oberbeckmann et al., 2018, 2016; Pinnell et al., 2020; Wright et al., 2020; Zhang et al., 2022). In the meantime, much remains unclear about biofilm dynamics soon after plastic enters the marine environment (Wright et al., 2021), even though the first seven days of biofilm formation are likely critical in shaping the subsequent microbial community (Datta et al., 2016). In addition to environmental factors, physiochemical properties of plastics e.g. chemical structure, hydrophobicity, and surface roughness influence early community dynamics (Dussud et al., 2018; Kettner et al., 2017; Oberbeckmann et al., 2018; Rummel et al., 2021, p. 20), while in a later stage, the biofilms are primarily structured by more general biofilm properties (e.g. stochastic settling, nutrient exchange, environmental factors and advantages of biofilm life) (Datta et al., 2016; Oberbeckmann et al., 2014). It remains unresolved whether different polymers support differential microbial communities, including potential plastic degraders, especially during the early stages of colonization. Plastics are hydrocarbon-like compounds and contain substantial chemical energy that might support microbial communities (Jacquin et al., 2019a; Oberbeckmann and Labrenz, 2020). Polymers exhibit chemical diversity, ranging from relatively simple polyolefins like PE and PP to more complex structures with aromatic rings such as polystyrene (PS), as well as backbones containing additional heteroatoms like oxygen and nitrogen (e.g., PET and Nylon) (Fig. 1). Consequently, bacterial degradation of distinct polymers would require a diverse array of specific enzymes, although more unspecific enzymes utilizing reactive oxygen species were recently discussed, too (Bos et al., 2023; Yoshida et al., 2016; Zeghal et al., 2021). Polymers with a heteroatom backbone are likely easier to degrade biologically, due to the extra elements in the backbone (Lear et al., 2022; Wayman and Niemann, 2021). Furthermore, UV exposure and subsequent photooxidation leads to chain scission and introduces oxygen into the polymer backbone, which likely increases the bioavailability of (partially) photodegraded plastics (Delre et al., 2023; Gewert et al., 2018; Wayman and Niemann, 2021).

The role of biofilms in plastic degradation is unclear (Amaral-Zettler et al., 2020; Vaksmaa et al., 2021a). The presence of potential plastic degrading bacteria (PDB) has been reported during early stages of colonization, but also long-term incubations of polymers in the marine environment, as well as on collected wild plastics- i.e., free-floating MPD (Latva et al., 2022; Lemonnier et al., 2022; Pinto et al., 2020; Vaksmaa et al., 2021b; Wright et al., 2021). PDB were recently listed in a curated database (PlasticDB) (Gambarini et al., 2022). However, the identity of

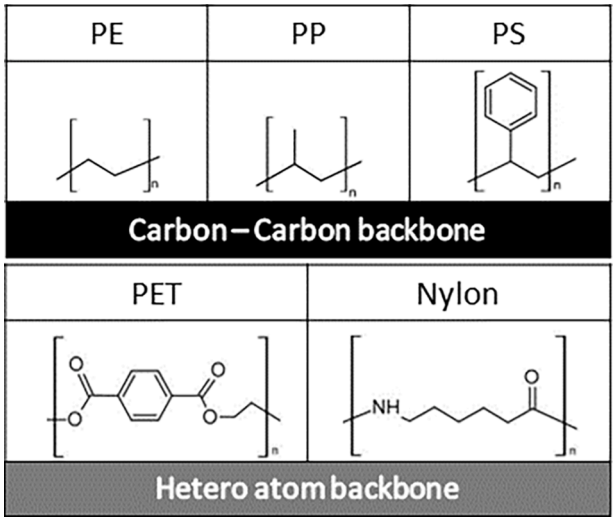

Fig. 1. Backbone structure of the 5 tested polymers comprising a carbon-carbon backbone (PE, PP, and PS) or a backbone with hetero-atoms (Nylon, PET).

potential plastic degraders is most often not constrained (Lear et al., 2022; Wayman and Niemann, 2021). Only a few microbes were identified to degrade specific plastic types, e.g. *Ideonella sakaiensis* to degrade PET (Yoshida et al., 2016), *Rhodococcus* sp. to degrade PE, PP and PS (Auta et al., 2018; Goudriaan et al., 2023; Gravouil et al., 2017; Mor and Sivan, 2008; Rose et al., 2020), and *Alcanivorax* sp. to degrade PE (Rose et al., 2020; Zadjelovic et al., 2022). Furthermore, high abundances of hydrocarbon-degrading bacteria (HCB) were discussed as potential plastic degraders, but unambiguous proof is often missing (Delacuvellerie et al., 2019; Dussud et al., 2018; Erni-Cassola et al., 2020; Koutny et al., 2009; Vaksmaa et al., 2021a).

In this research, we investigate the early stage, i.e. the first six days of microbial colonization of different plastic types (with and without exposure to UV light before incubation) in tropical coastal waters. For this, five different polymer types were immersed close to the shore of the Caribbean island of Saint Eustatius, and the microbial community of the biofilms covering the plastics was analyzed using 16S rRNA gene amplicon sequencing. We investigated changes of the microbial assemblages as a function of time and polymer type and we aimed at identifying taxa associated with plastic and hydrocarbon degradation.

2. Materials and methods

2.1. Test materials and sample preparation

We exposed five different polymers in situ to tropical coastal waters for 6 days: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene-terephthalate (PET), and Nylon-6,6 (NY) (Table 1). The plastic films were cut into strips (2 cm × 15 cm, 6 strips per polymer). Before the experiment, one subset was aged with UV-light by exposure to the sun for 24h, 12h per side facing upwards, and the second subset was not pre-treated. The strips were bound together in triplicates, for each polymer with and without UV-pre-treatment (30 film strips in total). The bundles were then attached to a small mooring for in situ incubation. The mooring comprised a rope with the samples, a weight, and a small floater to keep tension on the rope. The mooring was anchored at a water depth of about 6 m, i.e. the sample strips were suspended at ~5 m depth.

2.2. Field site and incubations sampling

The exposure experiments were carried out in March 2019 in the Caribbean marine sublittoral waters in Oranjestad Bay, Saint Eustatius (17°48'26" N, 62°98'81" W). Samples from plastic strips were taken twice, on days 1 and 6 of the incubation by successively cutting a piece of ~1.5 cm from each strip. The sampled pieces were kept in ambient water in small stand-up bags (118 ml WhirlPak, Madison, WI, USA). To avoid heating of the samples during transport from the sampling side to the field laboratory, all bags were placed in a larger bag filled with ambient seawater. In the laboratory, the sampled strips were placed in individual wells of a 6-well plate, filled with filter-sterilized local seawater (0.2 µm), and the plate was gently swayed to remove loosely attached biofilm and particles (e.g. sand and attached algae). Thereafter the samples were transferred into 2 ml cryovials prefilled with 1 ml RNA-later (Sigma-Aldrich, Germany) for preservation of DNA and stored at -20 °C until further treatment. Samples were transported to our home laboratory (Texel, NL) with a surplus of dry-ice. Geographical information for maps/figures was obtained from Natural Earth database (Massicotte and South, 2023) and Dutch Caribbean Biodiversity database (Verweij et al., 2013).

Table 1
Polymer types tested in this study. Film thickness (FT) and product number of the manufacturer/distributor (Sigma-Aldrich, Germany) are indicated. Abbreviations: Low density polyethylene (LDPE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET), Nylon-6,6 (NY-6,6).

Polymer	FT (mm)	Product #
LDPE	0.23	GF50898910
PP	0.5	GF24105484
PS	0.19	GF91132703
PET	0.25	GF54371695
NY-6,6	0.5	GF45294745

2.3. DNA extraction and 16S rRNA gene amplicon library preparation

Extractions and library preparation were carried out as described before (Vaksmaa et al., 2021b). In short, individual triplicated film pieces were trimmed to 0.5 × 0.5 cm and DNA was extracted from each sample individually with the Powersoil DNA Isolation kit (MoBio Laboratories, Inc, Carlsbad USA). For this, the film pieces were added to the PowerBead tubes directly and a bead beating step (4.00 m s^{-1} for 30 s, 30 s dwell time, repeated 4 times) was added to the protocol, replacing the original cell lysis step. The final elution volume was 25 µl. For library preparation, in short, the V4-V5 regions of 16S rRNA genes from extracted DNA were amplified in technical triplicate using the universal primers 515F-Y and 926R (Parada et al., 2016). The PCR reactions were performed with Phusion High-Fidelity DNA Polymerase (Thermo Scientific). Reagent mixtures, thermocycler settings and post-PCR-treatment of DNA were as described before (Vaksmaa et al., 2021b). Briefly, amplification of individual reactions was confirmed by gel electrophoresis, followed by pooling of triplicated PCR reactions. DNA was then purified, equimolarly pooled, and purified again. Sequencing was carried out at the Useq facility (Utrecht, Netherlands) on an Illumina MiSeq platform (Illumina, USA).

2.4. Sequencing data analysis

Raw sequencing data was processed as described before (Vaksmaa et al., 2022), using the pipeline "Cascabel" v4.6.1 (Abdala Asbun et al., 2020) set to the Amplicon Sequence Variants (ASV) workflow. Filtering and trimming resulted in truncation of forward and reverse reads after 260 and 210 bp respectively, with a maximum allowed error of 5 bp's. Chimeras were detected and removed and paired reads with lengths 402-422 bp were retained. Taxonomy assignment for the ASVs was performed against the Silva database (v138.1 Ref NR 99) (Quast et al., 2013), using the DADA2 RDP classifier algorithm (Wang et al., 2007), with minimum bootstrapping support of 45. The created ASV table was converted to biom format (McDonald et al., 2012), and further analyzed using the R software package (v4.2.1; (R Core Team, 2021). Data visualization was done with ggplot 2 v3.3.6 (Wickham, 2016) unless specified otherwise. Data curation was carried out using phyloseq v1.40.0 (McMurdie and Holmes, 2013). Samples with no reads (one sample) and negative control reactions were removed from the data set. ASVs identified as Eukaryotes, chloroplast, and mitochondria as well as ASVs not assigned on phylum and kingdom level, and singletons were removed. Comparisons of data were based on reads' relative abundance (RA) calculated per sample at the respective taxonomic level. Venn diagrams to represent presence/absence of genera were created with the R-package eulerr v7.0.0 (Larsson et al., 2022). The core microbiome of our dataset was calculated with R-package microbiome v1.20.0 (Lahti and Shetty, 2012), defined by a detection >1 % and prevalence >33.33 %.

Genera were compared against those listed in PlasticDB (version of 19-April-2023)(Gambarini et al., 2022), to identify potential plastic-degrading genera in our datasets. It is the most complete database of plastic-degrading microbes to date. Polymers degraded by microorganisms in PlasticDB were categorized into 6 main categories, corresponding to our tested polymers: PE, PP, PS, PET, Nylon, and Other. Each group contained multiple related compounds, e.g. the PE-group contained polymers consisting of multimeric PE units (i.e. HDPE, LDPE, LDPE Blend, etc.). The categories are described in the Supplemental Materials (Table S1). Furthermore, we checked for HCBs

in our dataset (Gutierrez, 2019; Prince et al., 2019; Yakimov et al., 2022, 2007).

2.5. Statistical analyses

Alpha diversity indices Chao1, Gini-Simpson index, and Shannon index were calculated with the R-package phyloseq, and community beta diversity was analyzed with the R-package vegan v2.6-2 (Oksanen et al., 2020), using square root transformed RA data. We performed non-Metric Multidimensional Scaling (NMDS) ordination on ASV level based on Bray-Curtis distances and used the function stat_ellipse() to calculate the mean and the standard deviation of the NMDS scores of the samples per grouping factor to construct ellipses. Pairwise Adonis tests, from the metagMisc package v0.5.0 (Mikryukov, 2023), were performed to determine significant differences between individual samples (classified by Time × Polymer × UV treatment). Similarity Percentage (SIMPER) analysis was conducted on genus level to determine the role of the individual genera in contributing to the separation of the groups or to the formation of samples within a group. We investigated if and which genera were differentially abundant between different conditions (timepoint, UV treatment, polymer backbone, polymer type, or a combination thereof) with the ALDEx2 package (Fernandes et al., 2013, 2014; Gloor et al., 2016). A complete list of performed tests can be found in the Supplemental Information (Table S8). Additional statistical tests were performed using the PRIMER-e V7 software package (Clarke and Gorley, 2015). Before the permutational tests, the reads per ASV per sample were normalized based on the total number of reads per sample, and a mild square root transformation was performed. Permutational multivariate analysis of variance (PERMANOVA) was applied on the same Bray-Curtis dissimilarity matrix used to produce the NMDS plots. The first PERMANOVA tested all factors and their interactions, and non-significant interactions were removed (UV × Pl and Ti × UV × Pl) for the second PERMANOVA. To assess the significance of UV treatment as a factor, and test which polymers supported a significantly different microbial community on a given timepoint, pair-wise PERMANOVA tests were performed within the timepoint subsets. *Post hoc* Permutational Multivariate Analysis of Dispersion (PERMDISP) was applied to test for homogeneity of dispersion.

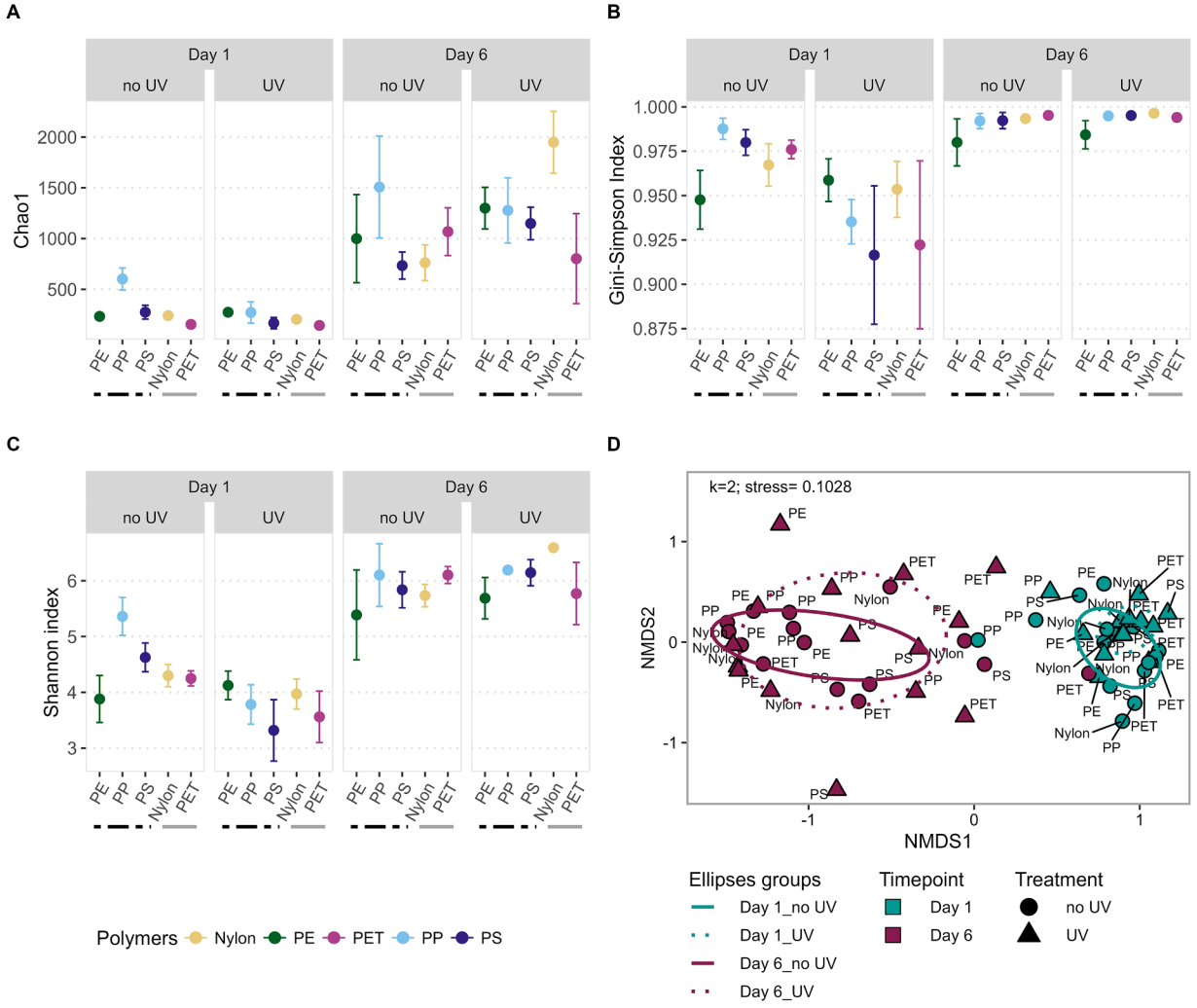

Fig. 2. Alpha and beta diversity of communities on the different polymers, UV treatments, and time points (average with standard deviation of triplicates for alpha diversity measures) **A:** Chao1 index (expected richness). **B:** Gini-Simpson index (evenness) **C:** Shannon index (diversity – also known as Shannon entropy) **D:** Nonmetric multidimensional scaling (NMDS) ordination based on Bray-Curtis distances between square root transformed RA data on ASV level. Symbols represent single sample replicates of Time – Polymer - UV treatment combinations. The centroid and radius of the ellipses represent the mean and the standard deviation of the ordination coordinates of the samples per Timepoint - UV treatment combination. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Alpha and beta diversity of communities on foils

After data treatment, we obtained 9846 unique ASVs from 59 samples (2506 ASVs on day 1 and 8557 ASVs on day 6, 1217 shared). On day 6, the difference in richness (Chao1 index) between polymer types was more pronounced than on day 1 (Fig. 2A), while the patterns of evenness (Gini-Simpson index) (Fig. 2B) and diversity (Shannon index) (Fig. 2C) appeared to be more similar. On day 6, the average richness of communities in UV-treated samples surpassed that of non-treated samples; however, no stark difference in richness was observed between the UV treatments on day 1 (Supplemental Figure S1A). This pattern was particularly notable for Nylon and PS, with a lesser extent observed for PE. Moreover, while UV treatment initially was associated with lower evenness scores and consequently diversity during the first day of colonization (Fig. 2B, C; Supplemental Figure S1B, C) it appeared to enhance evenness and richness over time, as evidenced for PP, PS, and Nylon. Concurrently, the relatively large divergence of average evenness values also diminished with time. Conversely, PE samples exhibited higher evenness and diversity on UV-treated samples compared to non-treated samples on both days, despite similar community richness. On PET samples, UV treatment consistently impacted community richness, evenness, and diversity negatively, regardless of the time point. No definitive correlation between polymer backbone structure and alpha diversity measures was observed. Overall, PP samples exhibited higher community richness, evenness, and diversity compared to most other polymers, while PE samples displayed relatively low richness, moderate evenness, and low community diversity, particularly on day 6. In contrast, both PS and PET supported highly diverse communities on day 6 despite low richness, attributed to their high evenness.

No compositional dissimilarity of microbial communities based on UV treatment or polymer type was observed between samples (Fig. 2D). However, the NMDS plot showed a clear separation between the two time points, indicating that the community changed on all polymer - UV treatment combinations over time. Within timepoints, NMDS showed a separation between UV-treated and non-treated samples on day 1 (Supplemental Figure S2A), but not on day 6 (Supplemental Figure S2B), while no separation based on polymers was observed for both days (Supplemental Figure S2 C, D).

3.1.2. Statistical analysis by PERMANOVA

Statistical analysis using PERMANOVA was conducted leaving out the non-significant and the non-informative interaction terms (Supplemental Materials S3; Supplemental Table S2). The PERMANOVA test showed that there is a significant day effect ($p = 0.0001$), polymer type has a slight effect ($p = 0.014$) and UV has no significant effect, though the interaction term Time × UV treatment is significant ($p = 0.0076$) on the microbial community. These findings are consistent with the NMDS plot (Fig. 2). Considering the different polymers as main factor, there were significant differences in microbial communities among the five Polymer Types (Supplemental Table S2; S3). Pairwise PERMANOVA comparisons between Polymer Types revealed differences between PS & PE, PS & PP, Nylon & PE, PE & PET, and PP & PET, while no significant differences were observed between other polymer pairs (Supplemental Table S4). PERMDISP analysis (Supplemental Materials S5) showed differences in dispersion between day 1 and day 6 (Supplemental Materials S4.1), supporting the findings from the NMDS plot (Fig. 2) and suggesting that besides difference in cluster location dispersion might contribute to the observed differences between the two time points. However, no differences in dispersion were found when comparing different polymer types or UV-treated samples against non-treated samples (Supplemental Materials S4.2, S4.3; Supplemental Figure S2). Hence, dispersion was unlikely to influence differences related to polymer or UV treatment. Notably, a significant difference in dispersion was observed for the interaction term Time × UV (Supplemental Materials S4.4; Supplemental Figure S2).

To further examine the impact of UV treatment or polymer type, pairwise PERMANOVA analyses were performed for each sampling day, revealing that UV-treated and non-treated samples differed on day 6 but not on day 1 (Supplemental Table S6). Additionally, pairwise PERMANOVA analysis considering different polymer types at a given time point (excluding UV treatment) revealed significant differences between five pairs of polymers on day 6, while no significant differences were found on day 1 (Table 2; Supplemental Table S5). The PERMDISP analysis indicated that the dispersion of UV-treated samples significantly differed from non-treated samples on both time points (Supplemental Materials S4.4), whereas the dispersion of polymers did not differ significantly on either time point (Supplemental Materials S4.5). Pairwise Adonis analyses showed that UV treatment did not cause significant differences within individual polymers at each timepoint (Data not shown). For example, on day 6, the two different treatments of PE did not differ significantly from each other.

3.2. Microbial community profiling: 16S rRNA gene sequence analysis

Bacterial reads constituted the overwhelming majority of the samples (>99 %, data not shown), irrespective of treatment and timepoint. Archaea were detected in three samples on day 1 and seven samples on day 6, with a higher RA of archaeal reads observed on day 6 (0.32 ± 0.26 %) compared to day 1 (0.24 ± 0.16 %). A total of 35 unique bacterial phyla were identified, with 26 detected on day 1 and 33 on day 6. Among these, 11 phyla exhibited an RA higher than 1.0 % in at least one sample (Supplemental Figure S4). The dominant phyla, Proteobacteria, Bacteroidota, Actinobacteria, Firmicutes, Verrucomicrobiota, and Cyanobacteria, accounted for 95.7 ± 16.0 % of reads per sample. We identified 184 unique orders, with 115 detected on day 1 and 169 on day 6. Among them, 36 orders had an RA greater than 1.0 % in at least one sample (Supplemental Figure S5). The 10 most abundant orders were Pseudomonadales, Flavobacteriales, Propionibacteriales, Rhodobacterales, Rhizobiales, Chitinophagales, Caulobacterales, Burkholderiales, Chytophagales, and Arenicellales, comprising 77.0 ± 24.4 % of all reads. At the genus level, a total of 624 unique genera were assigned. On day 1, 327 genera were found (189 in UV-treated samples, 270 in untreated samples, with 132 overlapping), while on day 6, 517 genera were detected (383 in UV-treated samples, 415 in untreated samples, with 281 overlapping) (Supplemental Figure S3). A total of 241 genera were common to both day 1 and day 6 (Supplemental Figure S3), and 60 genera exhibited an RA higher than 1.0 % in at least one sample. From the 60 genera with RA>1.0 %, the top 5 abundant genera per individual sample type were selected, which resulted in a total of 26 unique genera for all samples (Fig. 3). The 10 most abundant genera, irrespective of polymer type, timepoint, or UV treatment, accounted for 51.9 ± 30.8 % of all reads; these were *Enhydrobacter, Cutibacterium, Oleiphilus, Bradyrhizobium, Delftia, Tenacibaculum, Staphylococcus, Erythrobacter, Stenotrophomonas*, and *Aureicoccus*. Notably, the genera *Oleiphilus, Delftia, Tenacibaculum,* and *Erythrobacter* contain known HCB and PDB taxa (see section 3.6). This overlaps with the outcome of the core community analysis yielding 9 genera with a detection >1 % and a prevalence >33.33 %, namely *Enhydrobacter, Cutibacterium, Bradyrhizobium, Oleiphilus, Corynebacterium, Staphylococcus, Tenacibaculum, Stenotrophomonas,* and *Acinetobacter*. For a more comprehensive description of the microbial community at the phylum, order, and genus levels, see Supplemental Materials S6.

3.3. SIMPER analysis: key contributors to (dis)similarities

With SIMPER analyses, we determined the 10 main genera contributing the most to the Bray-Curtis distances between samples (Table 3). These genera were identified as *Staphylococcus, Oleiphilus, Cutibacterium, Enhydrobacter, Dietzia, Arenicella, Urania-1B-19 marine sediment group, Marinoscillum, Aquibacter*, and *Roseimarinus*. The average dissimilarity

Table 2
Results of pairwise PERMANOVA between polymers within each timepoints (UV not used as a factor). The results depicted in the top-right show significant differences between some polymers on day 6; the bottom left shows no significant (NS) difference between the polymers on day 1 (all p-values >0.28). *significant ** highly significant

			Day 6				
			Carbon-Carbon			Hetero	
			PE	PP	PS	PET	Nylon
Day 1	Carbon-Carbon	PE		0.1721	0.0314**	0.0813*	0.0761*
		PP	NS		0.0177**	0.2021	0.4434
		PS	NS	NS		0.1721	0.0297**
	Hetero	PET	NS	NS	NS		0.2547
		Nylon	NS	NS	NS	NS	

Fig. 3. Bacterial community profile on genus level with relative abundance (RA) per sample. Represented is the intersection of the top 5 most abundant genera per sample with a RA > 1.0 %. Genera are grouped per order and phylum along the right y-axis. Interaction lines at the bottom indicate whether the polymers have a carbon-carbon backbone (black interrupted line) or backbone with hetero atoms (grey solid line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

between timepoints ranged from 73 % to 80 %, and the 10 most influential genera accounted for up to 27 % of this dissimilarity between timepoints (Table 3). UV and non-UV treated samples exhibited dissimilarity ranging from 53 % to 59 %, with the top 10 genera explaining 15–25 % of this variation. Dissimilarities between polymers with carbon-carbon and hetero atom backbones, as well as between polymer types, ranged from 53 % to 68 %, and the top 10 genera contributed 15 % up to 23 % towards these differences (Table 3). Notably, the average dissimilarity between polymers on day 6 was lower compared to the time-independent tests. We propose that this is caused by the separation between the two time points, leading to an increase in average distance and consequently dissimilarity between polymers overall. Among the top 10 influential genera, *Staphylococcus, Urania-1B-19 marine sediment group*, and *Oleiphilus* typically contributed the most to the dissimilarities.

3.4. Differential abundance analysis: Genera with significantly different RA between sample groups

Differential abundance analysis revealed significant changes in the abundance of genera on day 6 versus day 1 in the complete dataset, independent of treatment and polymer type (Fig. 4). Temporal variations in genera abundances were also observed on subgroups, based on polymer backbone, treatment, or a combination thereof. However, UV treatment, polymer type, and polymer backbone did not independently influence the abundances of genera when either time was excluded, or factors were tested per sampling timepoint. Time emerged as the primary factor responsible for differences in genera abundance. Notably, more genera exhibited changes in abundance over time on polymers with a carbon-carbon backbone compared to those with a hetero-atom backbone (Fig. 4). Moreover, UV-treated polymers displayed a higher number of differentially abundant genera over time, along with a greater

Table 3
Summary of SIMPER results, identifying the top 10 genera explaining 70 % of the between-group Bray-Curtis dissimilarity. The two top rows, given per pairwise SIMPER test (x-axis), show the average between-group dissimilarity on genus level in % and the cumulative difference explained by the top 10 of genera in %. The individual contribution per genera is given in % per test in the lower part of the table. We highlighted potential HCB (+) and PDB (#) genera.

Average dissimilarity	**76.4**	**79.9**	**73**	**74.6**	**77.7**	**67.3**	**66**	**67.8**	**68.1**	**66.2**	**67**	**55.5**	**52.5**	**59**	**56.7**	**52.6**	**59.8**	**57.9**	**56.1**	**56.5**
Cumulative difference caused by 10 genera	**24**	**27**	**22**	**24**	**25**	**22**	**22**	**22**	**21**	**23**	**21**	**15**	**25**	**17**	**15**	**17**	**17**	**18**	**19**	**16**
Roseimarinus	1.1 %	1.2 %	1.0 %	1.1 %	1.1 %	1.0 %	1.0 %	1.0 %	1.1 %	1.1 %	1.1 %	1.1 %	1.0 %	1.1 %	1.1 %	1.0 %	1.1 %	1.0 %	1.1 %	0.9 %
Aquibacter	1.1 %	1.2 %	1.0 %	1.2 %	1.1 %	1.1 %	1.0 %	1.1 %	1.1 %	1.2 %	1.2 %	1.1 %	1.0 %	1.1 %	1.1 %	1.1 %	1.2 %	1.1 %	1.2 %	1.0 %
Marinoscillum	1.1 %	1.2 %	1.0 %	1.2 %	1.1 %	1.1 %	1.1 %	1.1 %	1.2 %	1.2 %	1.3 %	1.1 %	1.1 %	1.2 %	1.1 %	1.1 %	1.2 %	1.2 %	1.3 %	1.1 %
Arenicella	1.3 %	1.4 %	1.4 %	1.4 %	1.6 %	1.4 %	1.2 %	1.4 %	1.5 %	1.5 %	1.6 %	1.4 %	1.2 %	1.3 %	1.2 %	1.1 %	1.4 %	1.6 %	1.8 %	1.4 %
+_Dietzia'	1.6 %	1.6 %	1.6 %	1.6 %	1.6 %	1.5 %	1.6 %	1.6 %	1.5 %	1.6 %	1.6 %	1.8 %	1.3 %	1.4 %	1.2 %	1.3 %	1.4 %	1.7 %	2.0 %	1.5 %
Enhydrobacter	2.3 %	2.4 %	2.2 %	2.2 %	2.4 %	2.3 %	1.7 %	2.5 %	1.9 %	1.9 %	2.1 %	2.3 %	1.5 %	1.5 %	1.5 %	2.0 %	1.6 %	1.8 %	2.1 %	1.7 %
Urania-1B-19 marine sediment group	2.5 %	2.7 %	2.3 %	2.6 %	2.5 %	2.5 %	2.4 %	2.3 %	2.6 %	2.5 %	2.7 %	2.6 %	2.2 %	2.5 %	2.3 %	2.2 %	2.6 %	2.6 %	2.9 %	2.3 %
Cutibacterium	2.7 %	2.9 %	2.5 %	2.4 %	3.1 %	2.5 %	1.8 %	2.8 %	2.2 %	2.3 %	2.5 %	2.3 %	1.6 %	1.6 %	1.6 %	2.0 %	1.6 %	1.8 %	2.1 %	1.7 %
+_Oleiphilus'	3.6 %	4.6 %	2.8 %	3.6 %	3.6 %	2.9 %	1.8 %	3.0 %	3.2 %	2.9 %	3.1 %	2.8 %	1.8 %	1.9 %	1.7 %	2.1 %	1.9 %	2.2 %	2.2 %	2.1 %
#+_Staphylococcus	7.0 %	7.8 %	6.3 %	7.2 %	6.8 %	5.1 %	1.9 %	5.5 %	5.5 %	4.7 %	5.7 %	4.8 %	2.4 %	3.1 %	2.5 %	2.7 %	2.4 %	3.4 %	2.3 %	2.3 %
	Day1 vs Day6	UV Day1 vs Day6	noUV Day1 vs Day6	C-C backbone Day 1 vs Day6	H-A backbone Day 1 vs Day6	C-C vs H-A backbone	C-C backbone Day 6 UV vs noUV	PE vs Nylon	PE vs PET	PP vs PET	PS vs PE	PS vs PP	Day6 UV vs NoUV	H-A backbone Day 6 UV vs noUV	Day 6 C-C vs H-A backbone	Day 6 PE vs Nylon	Day 6 PE vs PET	Day 6 PS vs Nylon	Day 6 PS vs PE	Day 6 PS vs PP

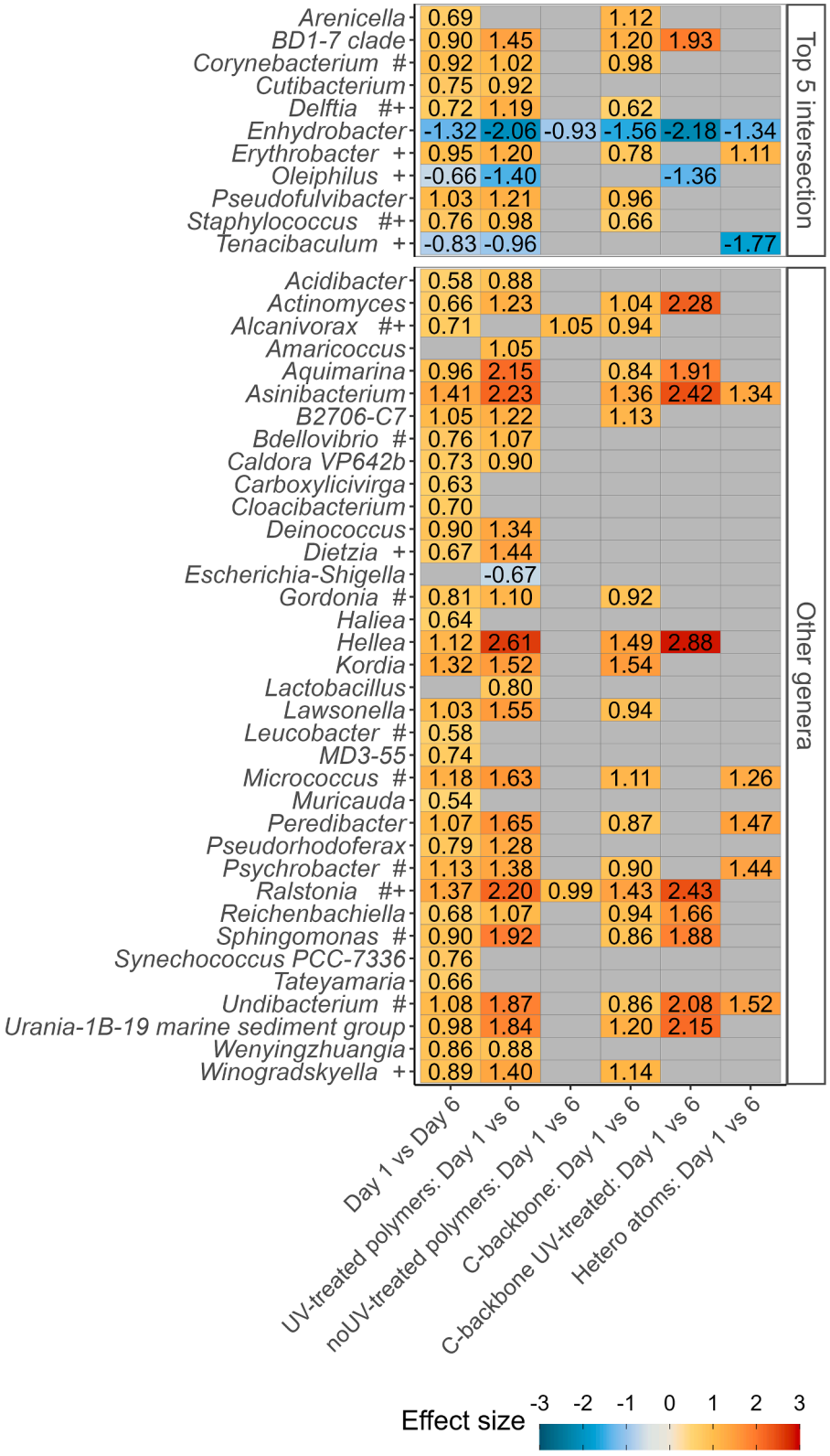

Fig. 4. Differentially abundant genera detected by ALDEx2 tests, with the top panel focusing on the differentially abundant genera among the 26 genera of the top 5 intersection of most abundant genera per sample (as depicted in Fig. 3). Negative/positive effect size indicates that the genus decreases/increases over time in the tested group. We highlighted HCB (+) and PDB (#) genera. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effect size, compared to untreated samples. This suggests that changes in abundance over time (excluding other factors) primarily occurred in UV-treated samples. A similar trend was observed in polymers with a carbon-carbon backbone, where genera exhibited a higher effect size when UV treatment was considered. Consequently, UV treatment appears to accelerate the temporal changes in genera abundance.

The top part of Fig. 4 displays the 11 differentially abundant genera that overlap with the top 5 genera per sample (Fig. 3). A total of 47 differentially abundant genera were identified (Fig. 4). Notably, only *Enhydrobacter, Oleiphilus*, and *Tenacibaculum* exhibited a decrease in abundance over time. Most of the genera exhibiting temporal abundance changes were observed in UV-treated samples, except for *Ralstonia* and *Enhydrobacter* (found in both treatment groups) and *Alcanivorax* (exclusive to non-UV-treated samples). Regarding polymer backbones, temporal changes were predominantly observed in polymers with a carbon-carbon backbone, with only a few genera, including *Erythrobacter* and *Enhydrobacter*, changing in abundance across both backbone structures. Interestingly, *Tenacibaculum* was the sole genus that exclusively exhibited abundance changes over time on the hetero atom backbones.

3.5. Genera comprising potential hydrocarbon and plastic degraders

Amongst all sequences recovered, 50 genera were identified as PDB based on their presence in PlasticDB (Supplemental Figure S7) (Gambarini et al., 2022). Additionally, 21 genera were identified as potential HCB. Together, these 71 genera accounted for an average relative read abundance of 18.8 ± 8.7 % per sample. A subset of 46 genera showed an RA ≥ 0.25 % in at least one sample (Fig. 5), comprising 17.3 ± 9.0 % of the total RA (32 PDB and 14 HCB). Furthermore, 2 HCB genera and 1 PDB genera were found to be relevant according to SIMPER (Table 3), and 8 PDB genera and 5 HCB genera were found to be differentially abundant (Fig. 4). The number of genera detected on UV-treated and untreated polymers was slightly higher on day 6 (49 and 54, respectively) compared to day 1 (43 and 50, respectively). The combined RA of HCB and PDB increased from 9.8 ± 3.2 % to 25.5 ± 7.0 % in UV-treated samples and from 15.0 ± 4.3 % to 25.0 ± 12.3 % in untreated samples. However, the relative read abundance of PDB only showed a modest

Fig. 5. Bubbleplot depicting all genera (with a RA of >0.25 %) detected in this study and which were reported in PlasticDB and/or identified as hydrocarbon degraders in other literature. Polymer categories with which genera are associated in PlasticDB are indicated by Roman numbers after the genus name. Categories are specified in Table S1. I:PE; II:PP; III:PS; IV:Nylon; V:PET. No number means the genus is found on another plastic type in PlasticDB. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

increase on UV-treated samples, from 7.4 ± 2.1 % to 10.4 ± 5.1 %, whereas HCB exhibited an increase ranging from 2 to 10-fold on almost all polymer – UV treatment combinations. Consequently, the overall increase in RA was primarily driven by HCB (Fig. 5). Notably, we observed a wide variety of PDB genera on both sampling days, highlighting their early and persistent presence throughout the initial six-day incubation period.

4. Discussion

Plastic pollution in coastal areas, particularly in subtropical and tropical environments, raises substantial concerns due to its detrimental effects on marine ecosystems and human health (Wayman and Niemann, 2021). Plastic items can undergo partial photodegradation both before and after entering the sea, leading to altered physical and chemical properties and the release of microplastics (Andrady, 2011; Delre et al., 2023; Gewert et al., 2018). Disintegration of MPD is further caused by mechanical processes such as wave action, shear forces, and abrasion with sediments. This ongoing fragmentation of plastics will increase the surface area available for colonization over time (Flemming and Wuertz, 2019), generating non-fouled surfaces for young biofilms to develop. Both virgin and partially photodegraded plastic particles are commonly colonized by diverse microbial communities comprising prokaryotes and eukaryotes (Bryant et al., 2016; Vaksmaa et al., 2021a; Zeghal et al., 2021; Zettler et al., 2013), typically within hours upon entering the marine environment. This work is one of the few reports on of biofilm formation and compositional community change on different plastics *in situ* during the first days of exposure to sea water (Wright et al., 2021; Yu et al., 2023), and informs on microbial colonization dynamics of plastic litter just after entering the marine realm. Previous studies primarily focused on investigating microbial communities on collected "wild plastic" consisting of plastic fragments that likely have been in the sea for extended periods. Research that examines *in situ* incubations mainly explored plastic surfaces exposed to seawater for extended periods, ranging from weeks to months (Dudek et al., 2020; Kirstein et al., 2018; Nauendorf et al., 2016; Oberbeckmann et al., 2016; Pinnell et al., 2020; Wright et al., 2021). More recent studies investigated the colonization of plastic surfaces during the initial hours, days, or weeks of exposure in the marine environment (Harrison et al., 2014; Latva et al., 2022; Lobelle and Cunliffe, 2011; Michels et al., 2018; Pinnell et al., 2020; Wright et al., 2021). Plastic surfaces are sometimes photo- or thermally oxidized, either naturally or artificially (Chiellini et al., 2007; Dodhia et al., 2023; Erni-Cassola et al., 2020; Koutny et al., 2009; Lear et al., 2022; Rummel et al., 2021; Singh and Sharma, 2008). However, the impact of (solar) UV light pre-treatment on the initial colonization patterns of plastic surfaces compared to virgin polymers remains largely unexplored. Although there has been an increasing interest in colonization of plastic surfaces by PDB and HCB (Bryant et al., 2016; Dooley and Bergelson, 2023; Erni-Cassola et al., 2020; Latva et al., 2022; Ogonowski et al., 2018; Roager and Sonnenschein, 2019; Vaksmaa et al., 2021b; Wright et al., 2021), the presence of potential plastic-degrading microbes during the early stage of microbial biofilm formation has thus far received relatively little attention. Our study investigated early-stage biofilm formation, here defined as the first 7 days, and the influence of incubation time, plastic type, and UV treatment. Furthermore, we assessed the presence of potential plastic-degrading (PDB) and/or hydrocarbon-degrading bacteria (HCB) during this early stage of colonization.

4.1. Succession and increasing alpha diversity in early plastic-associated microbial communities

On the first day of the experiment, we already observed biofilm formation on all polymers. Bacteria dominated the biofilm community, while archaeal abundances were low, constituting less than 0.5 % of the total RA. This is consistent with previous studies investigating plastic-associated biofilms (Dussud et al., 2018; Oberbeckmann et al., 2016; Vaksmaa et al., 2022, 2021b; Yu et al., 2023). Other surfaces in oxic oceanic environments are also mainly colonized by bacteria as has been shown for e.g. sand grains (Probandt et al., 2018), shells (Zhang et al., 2022), ceramics (Pinnell and Turner, 2019), wood (Oberbeckmann et al., 2018), and glass (Ogonowski et al., 2018). On day 1, analysis did not reveal significant differences in the microbial community composition between the different polymers and pre-treatments. We argue that the plastic surfaces were colonized by limited numbers of first-day settlers with a high RA per sample, as indicated by low values for species evenness and diversity. In fact, the microbial community on day 1 appeared to consist of generalists such as *Arenicella* (Pollet et al., 2018) and *Tenacibaculum* (Levipan et al., 2019), rather than polymer-specific bacteria. This suggests that stochastic processes (e.g. ecological drift and dispersion of microbes) play a significant role in the initial colonization process (Brislawn et al., 2019; Zhang et al., 2022). As this would lead to random and patchy colonization, this might also explain the relatively large variety in evenness scores in the young biofilm of day 1.

In contrast to day 1, the polymers had attracted a more diverse biofilm community and a greater variety in the number of unique genera per polymer by day 6 (Supplemental Table S6) with more evenly distributed RA values, as is indicated by higher metrics for richness and evenness (Fig. 2 A-C). Furthermore, the differences in diversity and evenness between samples diminished, suggesting convergence in diversity index values over time. Increasing alpha diversity is commonly observed in maturing biofilms (Penesyan et al., 2021) and is attributed to recruitment and coaggregation of new species by the pioneering community (Dang and Lovell, 2016). For incubations with thermo-oxidized and non-weathered PE in Mediterranean water, Erni-Cassola et al. (2020) also reported higher evenness and diversity on day 9 compared to day 2. However, contrasting findings have been reported from other incubations of polymers in Mediterranean waters, either finding no changes (Dussud et al., 2018) or a decrease in diversity (Latva et al., 2022) with time. Among the five polymer types in this study, PE consistently supported the least diverse and even microbial community (Fig. 2B-C), while PET exhibited the lowest richness and number of unique genera (Table S6). Earlier studies, investigating alpha diversity in the first 1-3 weeks of incubation, observed significant differences in terms of diversity indices related to polymer type (Dussud et al., 2018) and found that functional diversity and richness of plastic-associated microbes are polymer-dependent (Miao et al., 2019). In contrast, another study investigating microbes on cellulose, PE, PP, and PS after two weeks of exposure in the Baltic Sea, found hardly any differences in evenness and diversity, while evenness was substantially lower on glass (Ogonowski et al., 2018). Similarly, a study aiming to identify "specific", tightly attached plastic colonizers, found that variation in richness between glass and several (bio)polymers was low after six weeks of exposure in the North Sea (Kirstein et al., 2019). Together, our and the previously published results show that a consensus does not exist (yet) regarding the succession/diversity of biofilms forming on plastics relating to factors such as time, habitat, and material and surface properties. However, the richness of epiplastic communities is generally higher than in the water column (Bryant et al., 2016; Debroas et al., 2017; Dudek et al., 2020). To date, most studies on plastic-associated communities focus on phylogenetic diversity, while information on functional diversity might yield more substrate-specific information including the presence of potential plastic-degrading organisms.

4.2. Common genera in the epiplastic community shared among polymers and time points

Whether microbial taxa are common to every MPD-associated microbiome is an important rationale of ongoing research (Bryant et al., 2016; De Tender et al., 2017; Wright et al., 2021; Zettler et al., 2013). The timespan during which this core of potential common elements is formed is also of interest (Amaral-Zettler et al., 2015) since

several studies have found overlap between so-called early colonizers (i.e. the first 1-4 weeks of colonization, varying per habitat), and the mature community (Bos et al., 2023; Lemonnier et al., 2022; Pinto et al., 2019; Wright et al., 2021). The residence time of a particle in the marine environment influences the potential for biofilm formation, e.g. because of competition for space and nutrients. Along with significant differences in community composition between different treatment and polymer types on day 6, we also found that ~25–40 % of all genera were detected on both sampling days, indicating that many first-day colonizers remained with a decreasing RA over time as they are joined by other settlers. Additionally, the 10 most abundant genera were found in both sampling days, treatment groups, and all five polymers, however, not necessary on all samples (Section 3.2; Fig. 3). Within our dataset, we considered 9 genera to be the core community, defined by the detection limit and prevalence of the genera. Some of these 9 genera have been found on (bio)plastics in tropical marine areas before, and these genera likely play a role in the development of (tropical) epiplastic microbial communities. *Enhydrobacter* was detected on acrylate exposed to the Gulf of Oman for five days (Abed et al., 2019), and on PE found in a subtropical gyre (Vaksmaa et al., 2022). In a meta-analysis of HCB, *Oleiphilus* was detected at several locations worldwide (Yakimov et al., 2022). *Tenacibaculum* has been detected on plastics in the North Sea (Oberbeckmann et al., 2016, 2014), *Cutibacterium* (Wang et al., 2023) and *Bradyrhizobium* (Luo et al., 2022) have been found in association with plastics in soils. *Corynebacterium, Staphylococcus, Stenotrophomonas,* and *Acinetobacter* are mentioned in PlasticDB and were isolated from soils in tropical areas (Gambarini et al., 2022). Another incubation study in the Caribbean also identified Bacteriodota e.g. the orders Cythophagales and Flavobacteria including *Tenacibaculum,* Alphaproteobacteria e.g. Rhodobacteraceae, Gammaproteobacteria, e.g. the order Pseudomonadales including Oleiphilaceae, Planctomycetaceae e.g. Pirellulaceae and *Synechococcus* from the phylum Cyanobacteria as dominant taxa in biofilms on six common polymers, sampled after one, three and six weeks of exposure to the water column (Supplemental Figure S4; S5) (Dudek et al., 2020). Alphaproteobacteria (e.g. Rhodobacterales and Rhizobiales) and Gammaproteobacteria (e.g. Pseudomonadales) have been recognized as early colonizers in the past (Quero and Luna, 2017; Wright et al., 2021) and during incubation studies in the Bay of Brest, ASVs classified as belonging to the orders of Flavobacteriales, Rhodobacterales, Rhizobiales and Pseudomonadales (e.g. *Oleiphilus)* were identified as early colonizers, attaching to PE, PP and PVC within seven days (Lemonnier et al., 2022). Based on this and earlier studies, we can conclude that some genera are common across different polymer types, environments, and geographical locations, during the first days or weeks in the marine environment.

4.3. Beta diversity: driving factors for differences between samples and sample groups

Closely related to research on microbial communities commonly encountered across plastic types and geographical regions, is the question of what drives differential colonization of plastics. Past studies have shown that different factors can influence the microbial community on plastic, including geographical location and climate zone (Amaral-Zettler et al., 2015; Bos et al., 2023; Coons et al., 2021; Oberbeckmann et al., 2016), habitat characteristics such as salinity (Kesy et al., 2019; Kirstein et al., 2019; Li et al., 2020; Oberbeckmann et al., 2018), or sea surface versus submerged conditions (Vaksmaa et al., 2022), and also, residence time in the seawater (Latva et al., 2022; Lemonnier et al., 2022; Wright et al., 2020). We found that microbial communities on the plastics quickly change over time with significant differences in community composition comparing days 1 and 6 (see above). Residence time in the water consequently appears to be a key driver for microbial community development on our plastic films. Several studies uncovered community changes on polymers during the first 1-4 weeks of incubation in natural environments, independent of habitat conditions (Harrison et al., 2014; Oberbeckmann et al., 2014; Pinto et al., 2019; Zhang et al., 2022). Furthermore, other studies have identified a shift in community composition, albeit after varying incubation length (Bos et al., 2023; Brislawn et al., 2019; De Tender et al., 2017; Lemonnier et al., 2022; Oberbeckmann et al., 2014; Pinnell et al., 2020). There is, however, still no consensus on defining early and late developmental stages of plastic associated biofilms. This mainly because the speed of the community development is seemingly dependent on the environment. Nevertheless, based on our data and previous studies, we suggest that incubation duration is a significant cause for community changes and differentiation on plastic surfaces, especially during the first days of biofilm development.

Another important question is whether microbial communities are polymer-specific, since this could point to recruitment of potential plastic degraders. Indeed, we found significant differences in community composition in relation to certain polymers taking both timepoints together; especially, we found highly significant differences between polymer types for day 6. Differences in community structure between different polymer types were found in other studies, too, where plastic was incubated in seawater *in situ* or in microcosms (Hansen et al., 2021; Kirstein et al., 2019, 2018; Lobelle and Cunliffe, 2011; Pinto et al., 2019) or where wild plastic was analyzed (Amaral-Zettler et al., 2015; Vaksmaa et al., 2021b). However, there is also a vast body of studies where no statistical differences were found in biofilm community composition as a function of polymer type during incubations within a certain environment or micro/mesocosm, including studies focusing on the first 1-3 weeks of incubation (Coons et al., 2021; Dudek et al., 2020; Lear et al., 2022; Lemonnier et al., 2022; Miao et al., 2019; Ogonowski et al., 2018). Additionally, several meta-analysis studies of the “plastisphere”, concluded that plastic type has only a minor influence on epiplastic microbiome composition (Oberbeckmann and Labrenz, 2020; Wright et al., 2021, 2020). However, this can be attributed to the fact that plastics are collected or incubated at different geographical locations and habitats, which strongly influences the microbial community on plastic. Also, the variety of analytical protocols used has likely added to differential results. Hence, we suggest that plastic-specific communities can be detected at one habitat or location, but that there is no microbial plastic specificity along environmental gradients.

It is not clear why different polymer types do seemingly not always recruit different microbial communities. Possibly, an explanation might be found from the fact that microbes in the plastic biofilm originate from the particle-associated fraction (e.g. attached to particulate organic matter or phytoplankton) and are thus not representative of the free-living fraction in seawater (Hansen et al., 2021). Attachment, rather than polymer type may thus be a dominant force for selection. Alternatively, this can be explained by the age of the biofilm, as it seems evident that during the first weeks of colonization, the communities on plastics are more different (Bos et al., 2023; Harrison et al., 2014; Pinto et al., 2019), while differences between microbial communities on different plastics seemingly diminish during a longer time frame of incubation (Wright et al., 2021; Zhang et al., 2022). The very first (e.g. within the first day) colonizers could be related to observed differences among polymer types. The identity of these is apparently related to stochastic processes (see above), but they may prime (surface) conditions for secondary colonizers and shape the subsequent microbial community (Datta et al., 2016). For example, initial colonizers of PE, PP, and PVC were significantly influenced by the season of incubation and sampling, which then also determined the later composition of the epiplastic community (Lemonnier et al., 2022). Hence, based on our data and other studies, we suggest that polymer types regularly play an important role in recruiting the first-week colonizers of the surface. However, additional data on the functioning of microbes and their interactions with plastic surfaces at different stages of succession are needed to determine consensus on the roles of the polymer types in recruiting differential microbial communities.

Photo-oxidation changes the surface chemistry of polymers by

introducing oxygen to the carbon backbone, resulting in carbonyl and hydroxyl moieties (Gewert et al., 2018), which are more bio-available and might lead to differences in microbial communities that become attracted (Cai et al., 2019; Huang et al., 2022; Rummel et al., 2021; Wayman and Niemann, 2021; Zeghal et al., 2021). Earlier studies described differences due to surface oxidation/polymer type during the early colonization stage of plastic surfaces (Erdmann et al., 2020; Erni-Cassola et al., 2020; Rummel et al., 2021). Our data indicated that plastics that were irradiated by sunlight before incubation featured a slightly different microbial community when compared to non-treated polymers on day 6 (Supplemental Table S5), however, we cannot rule out that this was caused by dispersion (Supplemental Materials S4.4). We suggest that the exposure to sunlight and thus UV dose applied to our samples might have been too low to cause substantial photo-oxidation and that the selection for specific microbes might thus have been too weak (Erdmann et al., 2020).

4.4. Key contributing genera towards dissimilarity and change

Genera that are identified as main contributors to dissimilarities by SIMPER on different plastic types, are potentially polymer-specific and might be involved in plastic degradation. We identified the same 10 genera as the primary contributors to Bray-Curtis dissimilarities in all performed tests. The SIMPER-derived dissimilarity values furthermore align well with the groupings observed in the NMDS, despite not all observed groups being significantly different according to PERMANOVA. Therefore, we argue that both the presence/absence and RA of genera in our dataset contribute to the Bray-Curtis dissimilarities. Dissimilarity values were highest between the two timepoints, especially on the UV-treated samples.

With ALDEx2 analysis, genera that exhibit a significantly different RA between the tested groups, and hence cause the observed differences between the communities, were identified. Of the genera identified as top-10 contributors by SIMPER analysis, five exhibited differential abundance over time according to ALDEx2 analysis (*Enhydrobacter, Oleiphilus, Staphylococcus, Urania-1B-19 marine sediment group,* and *Dietzia)*. In fact, the majority of the 10 most abundant genera in our dataset (section 3.2) exhibit differential abundance over time, except for *Bradyrhizobium, Stenotrophomonas,* and *Aureicoccus* (Figs. 3 and 4), which are important members of the community at both time points, but with a relatively stable RA over time. This also fits our observations of genera being dominant at day 1 and then decreasing in RA, while by day 6 other genera are introduced or feature an increase in RA. Differential abundance analysis only indicated significant RA changes over time, again suggesting that time was the most influential factor in shaping the microbial communities, and specific polymer-associated genera could not be identified with ALDeX2. This disparity can be attributed to the central log-ratio transformed RA data used by ALDEx2, as opposed to SIMPER/PERMANOVA which are based on Bray-Curtis dissimilarities; ALDeX2 can hence detect other features not captured by PERMANOVA. Additionally, the low significance level of some differences detected by PERMANOVA within timepoints, particularly between treatment groups, coupled with a substantial overlap in the presence/absence of genera in the Venn diagrams, may explain the absence of differentially abundant genera.

Of the genera identified to be influential by SIMPER and found to be differentially abundant, twelve are PDB and five are exclusively HCB. For more information regarding these bacteria, see the following section (Subsection 4.5). Furthermore, a large proportion of the genera resulting from SIMPER and ALDEx2 analysis, although not identified by PDB or as HCB, have been found associated with marine plastics in past research, e.g. *Marinoscillum* (Coons et al., 2021; Zettler et al., 2013) and *Arenicella* (Davidov et al., 2020; Odobel et al., 2021). *Enhydrobacter* species, belonging to the *Vibrionaceae* family, have been detected on plastic particles (Vaksmaa et al., 2022). *Aquibacter* has also been found in plastic-associated biofilms in the North or Baltic Sea (Kirstein et al., 2019; Oberbeckmann et al., 2018) and it has also been influential in differentiating communities on plastic items from the abyssal seafloor (Krause et al., 2020). *Roseimarinus* has exhibited a preference for polyvinyl chloride (PVC) during an incubation study with microplastic in the South China Sea (Zhang et al., 2022). *Pseudofulvibacter* is a marine bacterium belonging to the *Flavobacteriaceae*, a family that is regularly detected on plastic surfaces (Dussud et al., 2018; Roager and Sonnenschein, 2019; Wright et al., 2021). Of the five HCB, including *Tenacibaculum, Winogradskyella* (Wang et al., 2014), and *Erythrobacter* (Wang et al., 2020), *Oleiphilus* is a known oil degrader (Golyshin et al., 2002; Gutierrez, 2019; Yakimov et al., 2022), while *Dietzia* species are also known to stimulate and increase enzyme activity of other HCB (Gharibzahedi et al., 2014). Some of the genera highlighted by SIMPER and/or ALDEx2 have not been described in association with plastics, but have been found in association with petroleum compounds. *BD1-7* clade, a common marine genus, has been found in both Gorgonian-associated microbiomes (Van De Water et al., 2017) and at petroleum-polluted sites (Hamdan et al., 2019). Furthermore, of the genera found influential according to SIMPER, most exhibit an RA >1 % (see also Fig. 3), except for *Urania-1B-19 marine sediment group, Aquibacter,* and *Roseimarinus. Urania-1B-19 marine sediment group* has been found in sediments polluted with petrol off the coast of Lebanon (Hamdan et al., 2019). However, there is limited knowledge about the functioning of the apparently inconspicuous genera *Aquibacter* and *Roseimarinus*, which are also not commonly known to have the potential to degrade plastics or hydrocarbons (PlasticDB; (Gambarini et al., 2022). Similarly, *Cutibacterium* is known as a skin-associated bacterium (Scholz and Kilian, 2016) and is sometimes detected in marine environmental samples (Papale et al., 2020), but is not known as an HCB or PDB. Hence, the association of the majority of genera with plastics in previous research and their overall high abundances, suggest that these might play an important functional role in the plastic-associated biofilms of our samples.

4.5. Hydrocarbon and potentially plastic degrading genera

Studying the early colonizers that settle on plastic during the first week of exposure to the marine environment is of importance, since they influence further biofilm development. As they are attached and in close contact to the surface, they also have, in principal, the opportunity to degrade polymers (Datta et al., 2016; Kirstein et al., 2019). Our data confirms that genera comprising PDB and HCB were common in our samples. The fact that twelve PDB and five HCB were found to influence changes over time and difference between samples among the early-stage colonizers (Fig. 4), suggests that individual PDB and HCB genera also play a crucial role in succession, distinction and diversification of the microbial communities on the plastics.

The combination of higher polymer-specificity of communities and higher RA of HCB on day 6 (Fig. 5) suggests that already within days, plastics select for a community that might use the polymer itself and/or leached hydrocarbons. HCB and PDB, similar to the ones we described, have been identified on plastics before (Debroas et al., 2017; Lacerda et al., 2022; Pinto et al., 2020). Also previous studies found that HCB were either enriched, had a high relative abundance, or were specific for the early stages (1 - 3 weeks) of plastic-associated biofilm formation in the marine environment (Bos et al., 2023; Erni-Cassola et al., 2020; Latva et al., 2022; Lemonnier et al., 2022; Ogonowski et al., 2018; Pinto et al., 2019). In our study, the total RA of PDB roughly remained constant, though some PDB showed an increase in differential abundance over time. We observed diversification of both HCB and PDB genera over time. Diversification of HCB and/or PDB was also detected on PP strips exposed to the Mediterranean Sea for three days (Latva et al., 2022) and on strips of different PE formulations immersed in Mediterranean seawater that were sampled weekly over the course of 6 weeks (Erni-Cassola et al., 2020). Our data combined with published data showing that early colonizers and mature communities on plastic surfaces have a

relatively high amount of HCB and PDB (Dussud et al., 2018; Latva et al., 2022; Vaksmaa et al., 2021b), indicate that these colonizers likely play a role in the potential degradation of the polymer and/or shorter chain degradation products leaching from the plastic (Jacquin et al., 2019b).

It has to be noted that PDB and HCB listed in PlasticDB are associated with a plastic type that sometimes did not match the plastic type on which we found said genus. For example, in PlasticDB, *Staphylococcus* is described as a PE and PU degrader. In our study, in contrast, it was detected on four out of five polymers, and is only differentially abundant on polymers with a carbon-carbon backbone. Likewise, *Delftia* was only found to be differentially abundant over time on carbon-carbon backbone polymers, and was found on all five polymer types in this study, while in PlasticDB it is described as a degrader of PE, PET, and biopolymers, e.g. PHA. *Streptococcus* was found to degrade PE, but is in this study mainly found in PET. Possibly, some PDB can metabolize several plastic types. Alternatively, this could also be related to the several definitions for (potential) plastic degraders used in PlasticDB. For some species/strains, the ability to degrade a polymer is extrapolated from the presence of genes encoding certain enzymes or pathways, while no degradation tests have been performed. Also, the ability to degrade certain polymers was in some cases evidenced by inaccurate/insensitive testing methods that do not trace degradation unambiguously (e.g. SEM, weight loss, CO_2 production, FTIR (Goudriaan et al., 2023; Wright et al., 2020)), or the specific pathway of degradation remains unknown. Hence, the information in PlasticDB can be inconclusive. In addition, the genus might not have been tested (yet) for the polymer we found it on, and is hence not be recorded in the database as such. Furthermore, a substantial fraction of the microbes curated in the database only has the ability to degrade biodegradable polymers (e.g. PHA and PLA). Additionally, the complete composition of the polymer, e.g. presence of additives or plasticizers, is not always provided (Lear et al., 2022). In most studies reported in the database, polymers have been pretreated (e.g. by photo- or thermo- oxidation), and the database offers no proof of degradation of virgin plastics with carbon-carbon backbone thus far (Lear et al., 2022). Hence, it is unclear whether PDB genera can process the virgin plastic itself, or rather additives or shorter chain products originating from the polymer, e.g. photo-oxidation products and leachates. The latter seems a likely scenario for many of the PDB genera where the pathway/enzymes for plastic degradation have not yet been identified, since many PDB genera are also HCB. For example, aforementioned *Staphylococcus,* defined as plastic degrader in PlasticDB, is also a known oil-degrader (Prince et al., 2019). *Acinetobacter* has the proven ability to degrade LDPE, PET, and PS, but is also a known HCB (Gutierrez, 2019). Likewise, *Corynebacterium*, which can be found in PlasticDB, was recently found to have a PETase (Helleckes et al., 2023), but can also degrade hydrocarbons (McGowan et al., 2004). According to PlasticDB, *Alcanivorax* and *Ralstonia* species were found to degrade a wide range of (bio)polymers and LDPE, but they are also HCB (Prince et al., 2019; Yakimov et al., 2022). Moreover, here we only studied bacteria, but other studies show that eukaryotes, especially fungi and diatoms, are important plastic colonizers, too, and can be polymer-dependent (Dudek et al., 2020; Eich et al., 2015). Fungi have also been identified as early colonizers (Kettner et al., 2019; Oberbeckmann et al., 2014), and certain fungal species also can degrade polymers (Vaksmaa et al., 2023; Zeghal et al., 2021) and can be found in PlasticDB. Finally, sequencing data only allow to infer hydrocarbon or plastic degrading potential, but cannot pinpoint if and how plastics are actually degraded by the detected genera.

5. Summary and concluding remarks

In this study, we have investigated the initial stages of colonization of common plastic types in tropical coastal water of the Caribbean Sea, where plastic pollution is a severe problem. After just one day of incubation, our investigation revealed microbial attachment on all plastic polymers. The epiplastic microbial community transformed substantially on a time scale of days. While the initial colonization appeared to be driven by stochastic processes without an apparent selection of distinct communities, subsequent colonization showed signs of influence from specific factors. This led to the differentiation of microbial communities on different polymer types after six days of incubation. However, the pretreatment of plastic films with sunlight had a marginal effect on the development of microbial communities. Notably, we identified genera with the potential to degrade plastics and hydrocarbons among the early settlers, some of which were previously reported to degrade the specific polymers studied suggesting an active role of plastic colonizers in plastic degradation. In addition to timepoint and polymer-specific features, we also identified common ASVs shared across all samples. These shared ASVs were frequently detected in plastic-associated communities at various developmental stages, indicating at least some commonality among global "plastispheres" despite strong habitat influences. It's important to note that amplicon sequencing only allows to deduce the roles and functions of microbes based on their phylogenetic relations. We thus recommend future studies to consider metagenomic and metatranscriptomic analyses and/or direct methods to measure degradation and assimilation, in order to gain a deeper understanding of functionality, including plastic degradation capabilities of the epiplastic microbiome.

Further information

Data availability statement

Raw sequence data have been submitted to Sequence Read Archive (SRA), bioproject PRJNA1005086.

R scripts can be found on Github: https://github.com/MkGoldfish/Caribbean_foils_1week

Supplemental Materials

S1. PlasticDB polymer categories
S2. Sample alpha diversity: richness, diversity, evenness
S3. Sample beta diversity
S4. PERMANOVA results
S5. PERMDISP tests and results
S6. Overlap in community on genus level as shown by Venn diagrams
S7. Microbial community profiling
S8. PlasticDB genera
S9. ALDEx2analysis

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CRediT authorship contribution statement

Maaike Goudriaan: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Emna Zeghal:** Methodology, Data curation, Formal analysis, Validation, Writing – review & editing. **Harry Witte:** Methodology, Formal analysis, Writing – review & editing. **Annika Vaksmaa:** Methodology, Supervision, Validation, Writing – review & editing. **Helge Niemann:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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.

Data availability

Links to sequencing data and Github provided at the Attach File step. GC-data is available on request.

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Supplementary materials

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