



## Microbial communities on plastic particles in surface waters differ from subsurface waters of the North Pacific Subtropical Gyre

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### ABSTRACT

The long-term fate of plastics in the ocean and their interactions with marine microorganisms remain poorly understood. In particular, the role of sinking plastic particles as a transport vector for surface microbes towards the deep sea has not been investigated. Here, we present the first data on the composition of microbial communities on floating and suspended plastic particles recovered from the surface to the bathypelagic water column (0–2000 m water depth) of the North Pacific Subtropical Gyre. Microbial community composition of suspended plastic particles differed from that of plastic particles afloat at the sea surface. However, in both compartments, a diversity of hydrocarbon-degrading bacteria was identified. These findings indicate that microbial community members initially present on floating plastics are quickly replaced by microorganisms acquired from deeper water layers, thus suggesting a limited efficiency of sinking plastic particles to vertically transport microorganisms in the North Pacific Subtropical Gyre.

### 1. Introduction

Since the onset of production of synthetic polymers in the 1950s, the annual plastic production has undergone exponential growth, currently exceeding 400 million metric tons per year (Geyer et al., 2017; PlasticsEurope, 2019; Wayman and Niemann, 2021). The exceptional growth in plastic use also resulted in a concomitant increase in the amount of mismanaged plastic waste, of which a portion eventually enters the ocean, either directly from coastal environments, via river transport, through atmospheric deposition or direct littering at sea (Jambeck et al., 2015; Lebreton et al., 2017; Zhang et al., 2020b; Meijer et al., 2021). While a probably large fraction of the floating plastic debris is redeposited at the shore (Lebreton et al., 2019; Olivelli et al., 2020; Onink et al., 2021), the remainder is dispersed horizontally with a tendency to accumulate in subtropical gyres and enclosed seas (Lebreton et al., 2012; Maximenko et al., 2012; van Sebille et al., 2012). In addition

to horizontal transport, floating plastics may also sink in the water column (Egger et al., 2020; Kvale et al., 2020; Pabortsava and Lampitt, 2020; Zhao et al., 2022). The highest offshore concentrations of positively buoyant plastic debris have been recorded in the North Pacific Subtropical Gyre, which was hence also termed the North Pacific Garbage Patch (NPGP) (Howell et al., 2012; Eriksen et al., 2014; Law et al., 2014; van Sebille et al., 2015; Lebreton et al., 2018). The total amount of floating plastic in this region has been estimated with ~21,300 tons (Law et al., 2014) to up to 45,000–129,000 tons (Lebreton et al., 2018). The majority of floating plastic in this subtropical gyre are polymers less dense than seawater (Lebreton et al., 2018; Egger et al., 2020), e.g. polyethylene and polypropylene, similar to the polymer distribution recorded in other oceans and seas (Cozar et al., 2014; Suaria et al., 2016; Erni-Cassola et al., 2019; Adamopoulou et al., 2021). The fate of these positively buoyant plastics accumulating at the sea surface remains uncertain. Once in the ocean, such debris is subjected to a wide

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range of physical and biological processes (van Sebille et al., 2020; Wayman and Niemann, 2021). Floating plastic debris fragments into smaller and smaller items from macro (>5 cm) to mesoplastics (5 mm–5 cm), microplastics (1 µm–5 mm) and potentially to nanoplastics (<1 µm) by the action of solar radiation, waves, temperature variations and marine organisms (Andrady, 2011; Gewert et al., 2015; Ter Halle et al., 2016; Zhu et al., 2020). Some of these plastics are subsequently lost to the underlying deep-sea through sinking (Egger et al., 2020; Kane et al., 2020; Karkanorachaki et al., 2021). Several sedimentation mechanisms have been proposed, including incorporation into marine snow (Porter et al., 2018), fecal pellets (Cole et al., 2016; Wieczorek et al., 2019), aggregation with suspended particles (Besseling et al., 2017; Michels et al., 2018; de Haan et al., 2019) as well as changes in buoyancy because of biofouling (i.e., the colonization with bacteria, algae, and other marine organisms - also known as the “Plastisphere” - Zettler et al., 2013; Fazey and Ryan, 2016; Kaiser et al., 2017; Kooi et al., 2017; Amaral-Zettler et al., 2020; Lobelle et al., 2021). For marine microorganisms, plastics primarily serve as an attachment surface and the microbial community on plastic is distinct from the surrounding seawater (Bryant et al., 2016; Dussud et al., 2018a; Dussud et al., 2018b).

Investigations of microbial communities associated with ocean plastic have revealed the presence of recurrent microbial taxa such as *Rhodobacteraceae*, *Flavobacteriaceae*, *Cyclobacteriaceae*, and *Alteromonadaceae* (Dang and Lovell, 2000; Zettler et al., 2013; Oberbeckmann et al., 2016; Miao et al., 2019; Schlundt et al., 2020; Vaksmaa et al., 2021b). These taxa have been found both in open ocean or semi-enclosed seas, and on different types of plastic polymers (Bryant et al., 2016; De Tender et al., 2017; Kettner et al., 2017; Dussud et al., 2018a; Frère et al., 2018; Wright et al., 2020). Microbial taxa on plastic particles are highly similar over geographical regions. However, the specific plastic colonizers may not be the most abundant taxa, but can belong to the rare biosphere (Kirstein et al., 2019), and mostly remain unclassified beyond family level (Scales et al., 2021). Selection mechanisms determining which microorganisms colonize plastics are not well constrained but are likely related to the nature of the plastic item, either polymer composition or the organisms' ability to adhere to surfaces (Cai et al., 2019).

At present, it remains unknown if the microorganisms colonizing plastic debris are involved in plastic degradation or if they are opportunistic settlers (Vaksmaa et al., 2021a). For example, hydrocarbon-degrading bacteria (HCBs) seem to be frequently detected plastic colonizers; these microorganisms express enzymes that could directly or indirectly be involved in plastic degradation (Vaksmaa et al., 2021b). Confirming this function, a marine HCB was recently shown to break down polyethylene terephthalate (PET; Denaro et al., 2020). Furthermore, several marine bacterial and fungal strains have been shown to degrade specific types of plastic. For example, *Pseudomonas aestuvaria* was found to encode for a carboxylic ester hydrolase able to degrade PET (Bollinger et al., 2020) and the marine fungus *Zalerion maritimum* was shown to degrade polyethylene (Paço et al., 2017). This indicates that microbial plastic degradation could be a potentially important yet unquantified sink for plastics in the ocean.

Studies on microbial community composition of ocean plastics have mainly been conducted with floating plastics collected from surface waters. Furthermore, floating plastics are proposed to facilitate horizontal long-range transport for microorganisms attached to these particles (Masó et al., 2003; Barnes and Milner, 2005; Kiessling et al., 2015; Simkanin et al., 2019). Contrastingly, studies on microbial communities on sinking, subsurface or sedimented plastic particles remain scarce (Harrison et al., 2014; Woodall et al., 2018; Pinnell and Turner, 2019; Krause et al., 2020; Tu et al., 2020; Agostini et al., 2021). Moreover, it is unknown to which extent sinking plastic particles may act as a vertical transport vector, carrying along microorganisms from the surface and translocating them to deeper water layers.

In this study, we investigated the composition of microbial communities adhered to plastic particles collected from the surface of the

North Pacific Subtropical Gyre (NPSG), as well as from deeper water layers down to the bathypelagic zone (2000 m water depth). We addressed three research questions to gain insights into the fate of ocean plastic, both in terms of vertical transport and degradation: (i) What are the changes in microbial community composition on plastic particles after they have sunk from surface waters? (ii) Do sinking plastic function as a vertical transport vector for microbes? (iii) Which taxa could account for plastic degradation? We found that plastics from surface and subsurface waters harbor distinct microbial communities, which seemingly follow bathymetric gradients. We therefore hypothesize that the efficiency of sinking plastics to act as a transport vector translocating surface microbial communities to the deep sea is limited. Our results show an abundance of hydrocarbon-degrading bacteria on all plastic particles; thus, these might be involved in plastic degradation.

## 2. Materials and methods

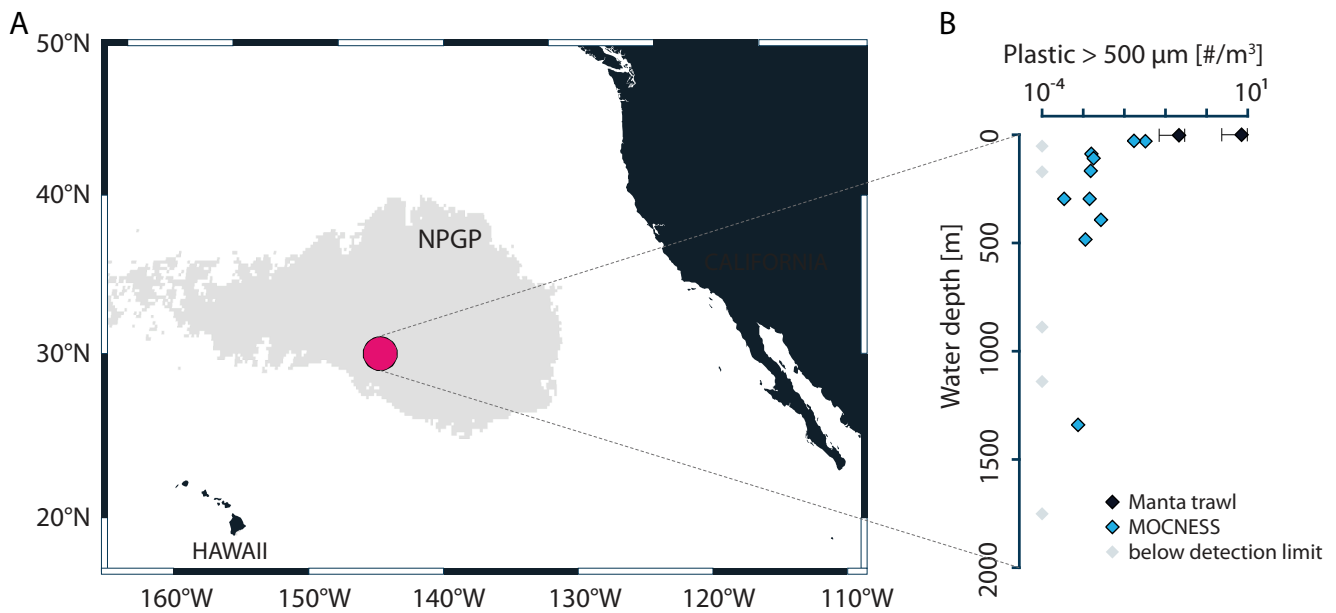
### 2.1. Sampling

Surface and subsurface samples from different depths were collected onboard the ship “MS Maersk Transporter” (IMO: 9388649) from the North Pacific Subtropical Gyre in November 2018 (Fig. 1, Station 3 in Egger et al. (2020)). Plastic particles afloat at the ocean surface were collected using a Manta trawl (Ocean Instruments, Inc.), with a trawl mouth area of 90 cm × 15 cm (width × height) and a net mesh size of 500 µm. All nets used in this study were square mesh netted. Upon recovery, the net was rinsed from the outside with freshwater and the cod-end (333 µm mesh size) was removed. Plastic particles present in the cod-end were manually picked using stainless steel tweezers and transferred into individual 1 ml tubes (Eppendorf) pre-filled with RNA-later solution (Sigma Aldrich). The tubes were immediately closed, and the plastic particles were covered entirely with RNA-later by gently swinging the tubes. Subsequently, the tubes were stored frozen (−20 °C) until further analysis in the shore-based laboratory.

Close to the Manta trawl deployment locations, submerged plastic particles were collected with a Multiple Opening and Closing Net with an Environmental Sensing System (MOCNESS). The MOCNESS consists of a total of 9 individual nets (Biological Environmental Sampling Systems, Inc.) with cod ends (333 µm). Towed, the net opening equaled 1 m<sup>2</sup>. First, the MOCNESS was lowered to 2000 m with the first net open (i.e., net #0). Once the MOCNESS reached the deepest depth, net #0 was closed and net #1 opened. The MOCNESS was then slowly hauled towards the sea surface and eight specific water layers were sampled (nets #1 to #8). In total, the MOCNESS was deployed twice at the same location. Note that any samples towed in net #0 were not used for further analyses due to possible contamination with plastic fragments from the surface waters. Upon retrieval on deck, the contents of the individual MOCNESS cod-ends were immediately transferred into single-use cod-ends (333 µm mesh size). The latter were sealed with staples, placed in individual zip-lock bags, wrapped in aluminum foil, and stored frozen (−20 °C) until further analysis in our home laboratories. Back in the onshore laboratory, the MOCNESS samples were thawed and a random subset of plastic particles hand-picked using stainless steel tweezers and transferred into individual 1 ml tubes (Eppendorf) pre-filled with RNA-later solution. The tubes were immediately closed, and the plastic particles completely covered with RNA-later. Subsequently, the tubes were stored frozen (−20 °C) until further analysis.

### 2.2. DNA extraction and polymer analysis

DNA from individual plastic fragments was extracted using the Powersoil DNA Isolation kit (MoBio laboratories, Inc., Carlsbad USA). For this, single particles were taken out of RNA-later with sterile tweezers and added to the PowerBead tubes directly. As a modification to the manufacturer's protocol, we added a bead beating step of 4.55 m/s for



**Fig. 1.** (A) Study site (30°N/154°W; red point) located in the North Pacific Subtropical Gyre. The grey area indicates the zone where microplastic concentrations exceeded 1 kg/km<sup>2</sup> (recalculated from [Lebreton et al., 2018](#)). This plastic accumulation zone is also known as the North Pacific Garbage Patch. (B) Vertical distribution of plastic particles (0.5 cm to 5 cm in size) at the study site (recreated from [Egger et al., 2020](#)).

30 s 3 times with dwell time of 10 s, replacing the cell lysis step in the original protocol. The remaining DNA extraction was performed according to the protocol provided by the manufacturer. The final elution volume was 30 µl. DNA concentrations were measured on a Qubit fluorometer using the Qubit dsDNA HS Assay Kit (Invitrogen, USA) by adding 2 µl of DNA sample to 198 µl of Qubit working solution. Particles used for DNA extractions were subsequently analyzed individually by Raman spectroscopy (Agiltron, Inc.; PeakSeeker PEK-785) and resulting sample spectra were compared to an in-house Raman plastic reference library ([Egger et al., 2020](#)) to identify the corresponding plastic polymer types. All black colored particles were further analyzed by Fourier Transform Infrared Spectroscopy (Thermo Scientific; Nicolet 6700 FTIR), as no conclusive Raman spectra could be obtained on these particles due to strong absorption of the laser and subsequent burning of the particle surface.

### 2.3. 16S rRNA gene amplicon library preparation and sequencing

DNA from each extraction was subjected to PCR amplification (conducted in triplicate) in 50 µl reaction by using the universal prokaryotic primer pair 515F-Y (5' GTGYCAGCMGCGCGGTAA 3') and 926R (5' CCGYCAATYMTTTRAGTTT 3') ([Parada et al., 2016](#)) for the amplification of V4-V5 regions of the 16S rRNA gene. The forward and reverse primers were both barcoded with a unique 12 nucleotide Golay code. Briefly, the PCR reaction mix contained 25.5 µl PCR grade water, 10 µl 5× Phusion HF buffer, 4 µl dNTPs (2.5 mM), 2 µl BSA (920 mg/ml), 0.5 µl Phusion™ High-Fidelity DNA Polymerase (2 U/µl) (ThermoFisher), 3 µl of each primer (forward and reverse; 10 µM), and 2 µl of the DNA. Control reactions were carried out by replacing the DNA with PCR grade water. The thermal PCR program consisted of the following steps: 98 °C 30 s, 35 cycles of 98 °C 30 s, 50 °C 30 s, 72 °C 30 s, followed by 72 °C 7 min and after that kept at 4 °C. All triplicate PCR reactions were pooled and 5 µl of the PCR was used for quantification against a known standard by 1 % agarose gel electrophoresis stained with ethidium bromide. Thereafter, the PCR products were pooled in equimolar amounts and purified using the QIAquick PCR purification kit (Qiagen, USA) according to manufacturer instructions, followed by a second 1 % gel electrophoresis. The pooled sample was divided into wells and was run for 50 min at a current of 75 V. The bands were visualized on an UV

illuminator, cut from the gel and purified with the Qiaquick gel extraction kit (QIAGEN, USA). Sequencing was carried out at the Useq facility (Utrecht, Netherlands) on an Illumina MiSeq platform (Illumina, USA).

### 2.4. Sequencing data analysis and statistics

Sequencing data were processed using the pipeline Cascabel ([Abdala Asbun et al., 2020](#)) with the amplicon sequence variant (ASV) workflow (all commands and related parameters can be found in Appendix A). The quality of the raw reads was verified using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Library demultiplexing was carried out using the Qiime version 1.9 ([Caporaso et al., 2010](#)) scripts `extract_barcode.py` and `split_libraries_fastq.py`. Next, by using Cutadapt version 1.16 ([Martin, 2011](#)), primers were removed from “single sample” fastq files and processed with R version 4.0.3 ([RCoreTeam, 2021](#)) using the ‘Dada2’ package version 1.9.1 ([Callahan et al., 2016](#)). Reads were truncated to a length of 250 and 230 base pairs from R1 and R2 reads respectively and filtered with a maximum expected errors (maxEE) of 7 and 5 for forward and reverse reads, respectively, for both options. Following this, reads were merged and ASVs were obtained utilizing the ‘Dada()’ function. Chimeras were removed with ‘removeBimeraDenovo()’ and non-chimeric ASVs with a length between 350 and 400 base pairs were retained, i.e. 8089 unique amplicon sequence variants. Taxonomy assignments were performed with the Dada2’s RDP classifier ([Wang et al., 2007](#)) implementation ‘assign-Taxonomy()’ against the Silva database ([Quast et al., 2013](#)) update v138. Finally, an ASV table was created and converted to the biom format ([McDonald et al., 2012](#)). Downstream analysis was performed on the ASV table in R version 4.0.3 ([RCoreTeam, 2021](#)). Alpha- and beta-diversity analysis was carried out using the ‘pyloseq’ package version 1.34.0 and the ‘vegan’ package version 2.5–6 implemented in R ([McMurdie and Holmes, 2013](#); [Oksanen et al., 2020](#)). Graphs were created using the ‘ggplot2’ package version 3.3.2 ([Wickham, 2016](#)). For this, data were filtered based on taxonomy and ASVs assigned to eukaryota (only prokaryotic amplification with the primers 515-Y and 926R was used) or not assigned to any domain were removed from the dataset. Of the remaining data, ASVs not assigned to any phylum, and ASVs assigned to Chloroplast and Mitochondria were removed. Alpha

diversity indices were calculated using the 'estimate\_richness()' function in 'phyloseq', and the Wilcoxon rank-sum test ('wilcox.test()') function) was used to verify statistically significant differences between alpha diversity measures of different groups ( $p < 0.05$ ). Prior to non-metric multidimensional scaling (NMDS) analysis, the data set was further filtered and ASVs only occurring once (singletons) were removed. Abundance values were transformed to median sampling depth by dividing each ASV count value by the sum of count values in the respective samples, and then multiplied by the median of count sums across all samples. NMDS plots were constructed using the 'metaMDS()' function with Bray-Curtis distance and 1000 maximum random starts to find a stable solution. Analysis of similarities ('anosim()') function) was used to test for significant differences between groups. Throughout the manuscript, the term "abundance" refers to relative sequence abundance. The sequences have been submitted to the Sequence Read Archive (SRA), bioproject PRJNA685772.

### 3. Results

#### 3.1. Plastic composition and size class

In total, we analyzed 63 individual plastic particles, 46 of which were retrieved from the ocean surface by Manta trawling (i.e., the upper 0.15 m) and 17 were collected from subsurface waters (2–2000 m water depth). The polymer composition of all analyzed plastic particles was dominated by polyethylene (PE), accounting for 96 % of plastic particles collected from the ocean surface (Supplementary Table 1) and for 88 % of particles recovered from the subsurface water column (Supplementary Table 2). Polypropylene (PP) accounted for 2 % and 12 % of the plastic particles collected from the ocean surface and subsurface water column, respectively. One plastic particle collected from the ocean surface remained unclassified as no conclusive spectrum could be obtained. Large microplastics (i.e., plastics between 0.15 and 0.5 cm in size) accounted for 63 % of the particles collected from the ocean surface, and 32 % of the particles were between 0.5 and 1.5 cm in size (Supplementary Table 1). The remaining surface water particles were between 1.5 and 5 cm (2 %) and 5 and 10 cm (2 %) in size. For plastic particles collected from the water column, 6 % were between 0.05 and 0.15 cm, 29 % between 0.15 and 0.5 cm, 53 % between 0.5 and 1.5 cm, and 12 % between 1.5 and 5 cm in size, respectively. Plastic particles were further divided into different type categories (Lebreton et al., 2018). Most of the surface water particles (83 %) were classified as fragments made of hard plastic, plastic sheet or film (H-type) (Supplementary Table 1). The remaining surface water particles were classified as pre-production plastic pellets (P-type, 13 %) and as fragments of plastic lines, ropes, and fishing nets (N-type, 2 %). Only H-type and N-type plastics were collected in the water column, accounting for 41 % and 59 % each, respectively (Supplementary Table 2).

#### 3.2. Microbial communities on plastic retrieved from surface and subsurface waters

A total of 10,454,282 reads were generated by high-throughput sequencing of the 16S rRNA gene. After trimming and quality filtering, 9,917,431 reads remained. After further removal of low read samples, eukaryotic, mitochondria and chloroplast assigned reads as well as chimeras, the remaining reads comprised of 34 archaeal and 5895 bacterial Amplicon Sequence Variants (ASV). Overall, the microbial communities on different particles were rather heterogeneous. For instance, no ASV could be found that was present and shared by all particles (Figs. S1, S2, S3). Nevertheless, general trends could be observed. Based on NMDS analysis, microbial communities on particles retrieved from surface waters were different from those on particles recovered from subsurface waters (Fig. 2). Analysis of similarities (ANOSIM) supported a significant clustering of the two groups ( $R = 0.623$ ,  $p = 0.001$ ). Neither particle size nor color were found to have an

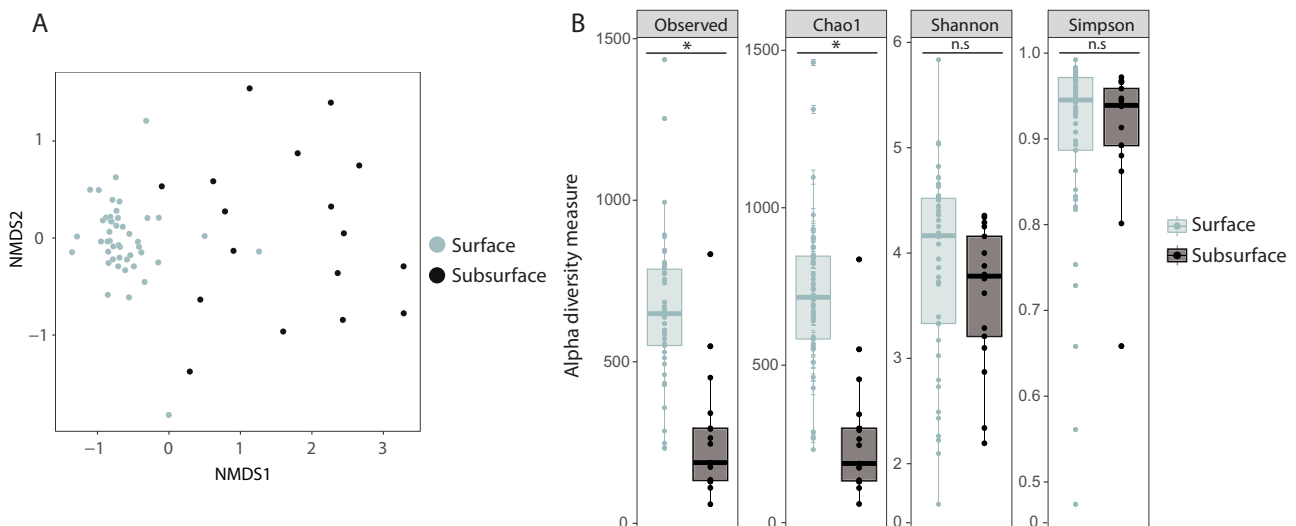
effect on the microbial community composition. Different types of plastic (H-type versus N-type) might have different microbial communities (Fig. S5); however, more replicates are needed to test this hypothesis.

To evaluate microbial richness and diversity on surface and deeper-water plastic particles, we compared a variety of diversity indices (Fig. 2). The surface samples showed a significantly higher ASV richness than subsurface samples (Observed richness:  $W = 708$ ,  $p < 0.05$ ; Chao1:  $W = 723$ ,  $p < 0.05$ ). However, Shannon and Simpson diversity indices were not significantly different ( $p > 0.05$ ) for surface and subsurface samples. The separation of surface and subsurface samples as revealed by NMDS was also reflected by differences in community compositions at different taxonomic levels (Figs. 3, 4, S1, S2, S3, S4).

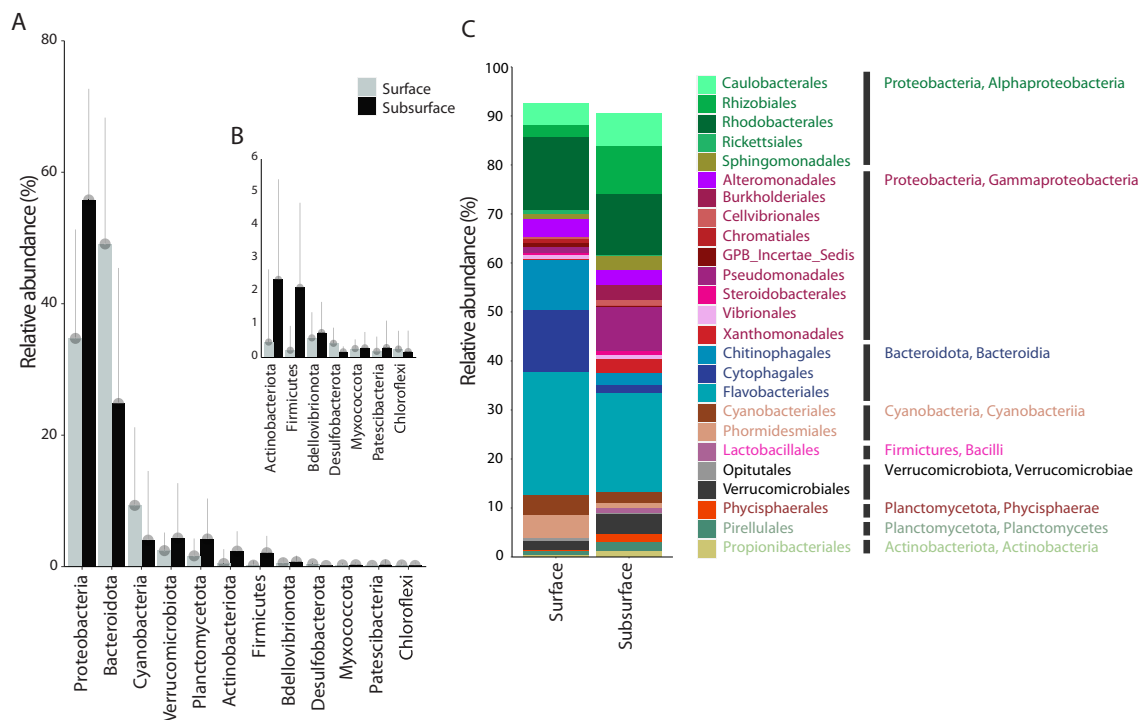
Bacteria comprised the most abundant domain on both, surface and subsurface plastic particles and accounted for the majority with >99 % of 16S rRNA gene read assignments. Archaea were detected at <1 % relative abundance on plastic particles in both surface and subsurface waters. Higher archaeal relative abundance was only found in a single subsurface sample with 1.4 % of reads assigned to the *Nitrosopumilaceae* family. At phylum level, the surface particles were dominated by Bacteroidota ( $49.1 \pm 19.2\%$ ), which accounted for >50 % of reads in 21 out of 46 surface samples, and reached >70 % in 9 samples (most abundant phyla and orders are depicted in Fig. 3 and genera per sample in Figs. S1, S2, S3, S4). The second most abundant phylum was Proteobacteria ( $34.7 \pm 16.6\%$ ) with a relative abundance of >50 % in 9 out of 46 surface samples. The opposite trend was observed for subsurface particles. Bacteroidota showed generally lower relative abundances at greater depths ( $24.8 \pm 20.6\%$ ), while Proteobacteria ( $55.8 \pm 16.9\%$ ) were above 50 % in 10 out of 17 samples. Phyla of lower abundances (1–10 %) included Cyanobacteria, Verrucomicrobia and Planctomycetota. Of these, Cyanobacteria were more abundant at the surface than subsurface while we observed the reverse for the Verrucomicrobia and Planctomycetota. Among Cyanobacteria, the main families were *Phormidemiaceae* ( $4.2 \pm 6.4\%$  on surface particles,  $0.9 \pm 2.1\%$  on subsurface particles) and *Noctaceae* ( $4.1 \pm 9.0\%$  on surface particles,  $2.2 \pm 8.4\%$  on subsurface particles). The most abundant Planctomycete families were *Pirellulaceae* ( $0.9 \pm 2.2\%$  on surface particles,  $1.8 \pm 4.6\%$  on subsurface particles), *Phycisphaeraceae* ( $0.4 \pm 0.8\%$  on surface particles,  $1.8 \pm 3.4\%$  on subsurface particles) and *Rubinisphaeraceae* ( $0.04 \pm 0.09\%$  on surface particles,  $0.2 \pm 0.4\%$  on subsurface particles). Sequences affiliated to the phylum Verrucomicrobia also showed differential distribution across the water column, with DEV007 as the most abundant family on both surface and subsurface ( $1.5 \pm 2.5\%$  and  $3.5 \pm 7.2\%$ , respectively (Fig. 4).

At the genus level, we investigated abundances and composition of known and putative hydrocarbon-degrading bacteria (HCB) and identified 43 taxa with members that are known to degrade complex organic compounds such as oil polycyclic aromatic hydrocarbons (PAH) and other straight chain or branched alkanes. In surface samples, HCBs genera accounted for 28 % of reads, and in subsurface samples for 35 %. However, variations were found in the relative abundance of specific genera. In surface samples, *Tenacibaculum* was the most abundant HCB, with  $12.3 \pm 22.4\%$  relative abundance. It was followed by *Lewinella* ( $3.8 \pm 5.8\%$ ), *Muricauda* ( $3.7 \pm 5.5\%$ ), and *Roseovarius* ( $2.0 \pm 2.5\%$ ). We further identified the genera *Winogradskyella*, *Pseudoalteromonas*, *Acinetobacter*, *Erythrobacter*, *Loktanella* and *Fabibacter* with >1 % of relative read abundance. In the subsurface, the most abundant genus was also *Tenacibaculum*, although at lower relative abundance ( $6.4 \pm 16.3\%$ ). Furthermore, the subsurface was characterized by high abundances of *Ulvibacter* ( $4.7 \pm 11.7\%$ ), *Enhydrobacter* ( $4.6 \pm 6.5\%$ ), *Acinetobacter* ( $4.3 \pm 6.8\%$ ), *Stenotrophomonas* ( $2.6 \pm 3.4\%$ ) and *Winogradskyella* ( $1.7 \pm 5.2\%$ ). Moreover, the genera *Sulfitobacter*, *Pseudoalteromonas*, *Roseovarius*, *Erythrobacter*, *Muricauda*, *Staphylococcus* and *Loktanella* were frequently identified HCBs, with >1 % relative abundance (Fig. 5). At lower abundance ( $0.2 \pm 0.2\%$ ), we also detected sequences assigned to *Ideonella* in subsurface plastic samples, with abundances even lower in





**Fig. 2.** (A) Beta diversity analyzed by non-metric multidimensional scaling (NMDS) of the microbial community present on particles collected from the sea surface and subsurface water column of the North Pacific Subtropical Gyre (NMDS stress = 0.12). (B) Box and whiskers plot of microbial alpha diversity of the same particles as in panel A. Observed ASV richness (Observed), estimated richness (Chao1), and the Shannon and Simpson diversity indices are shown. The asterisk indicates a significant difference of alpha diversity between surface and subsurface communities according to the Wilcoxon rank-sum test ( $p < 0.05$ ). n.s = not significant.



**Fig. 3.** (A) Microbial relative abundances at phylum level for plastic particles collected from the sea surface and subsurface water column of the North Pacific Subtropical Gyre (top 12 phyla). (B) Zoom in to less abundant phyla from panel A. (C) Microbial relative abundances at order level in surface and subsurface samples (top 20 orders). The respective phylum and class are indicated on the right.

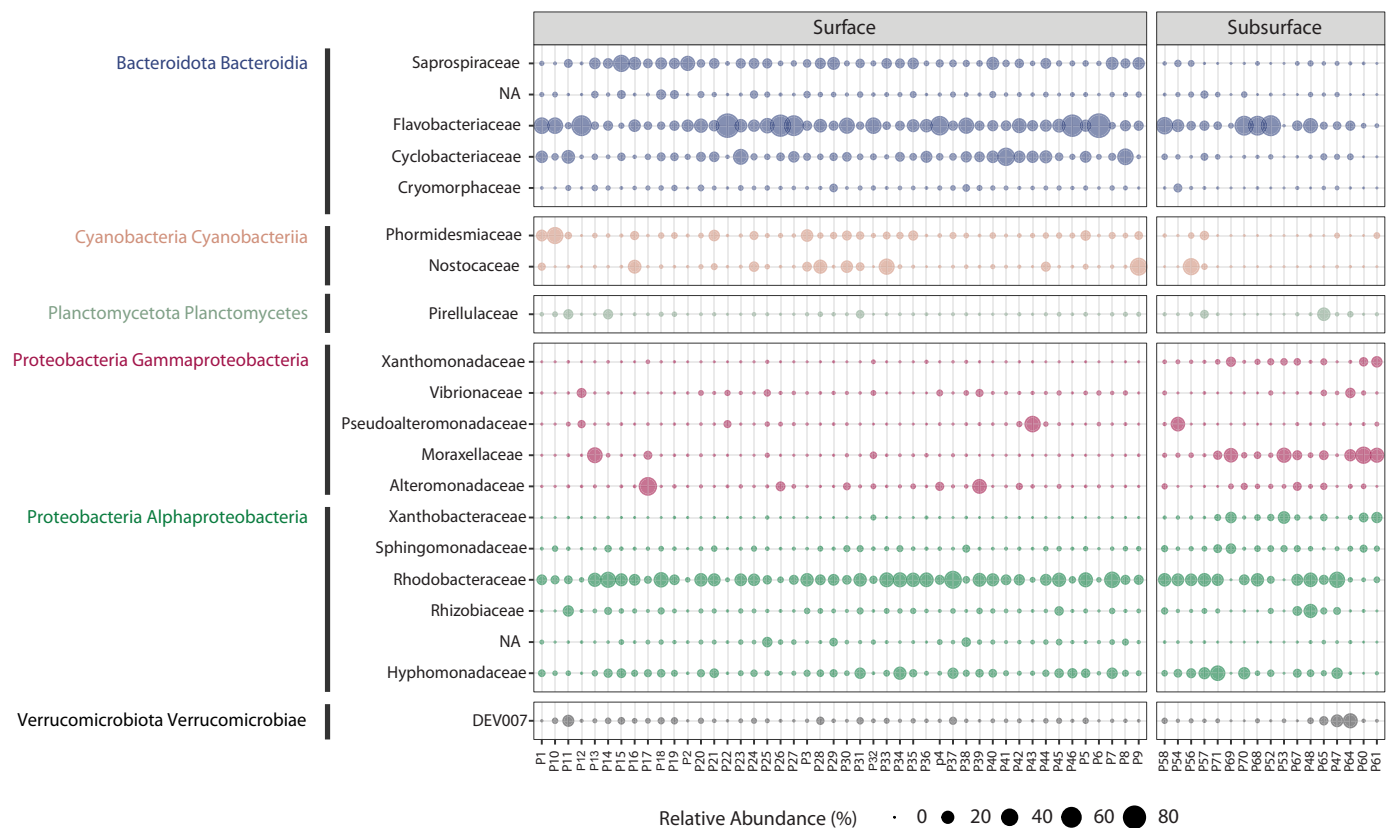
surface plastic samples.

Finally, we also detected sequences assigned to genera containing pathogens, such as *Vibrio* in the surface ( $0.7 \pm 1.6$  %) and subsurface ( $0.2 \pm 0.4$  %) samples. *Escherichia*, *Enterococcus* and *Shewanella* were also found on both sample types with relative abundance of  $<0.5$  %.

#### 4. Discussion

##### 4.1. Plastic debris constitutes a new ecosystem across the water column

Plastic debris in the ocean is an environmental problem of increasing magnitude with unforeseeable consequences for the marine environment (Wayman and Niemann, 2021). Plastic has been detected in all ocean compartments and biofouling has been shown to cause changes in the buoyancy of the plastic – fouling community aggregate (Fazey and Ryan, 2016; Kaiser et al., 2017; Kooi et al., 2017; Lobelle et al., 2021).



**Fig. 4.** Relative abundance of top 20 microbial families found on particles collected from the sea surface and subsurface water column of the North Pacific Subtropical Gyre. NA, non-assigned at family level. Subsurface samples are presented in the order of increasing water depth. ASVs occurring in only one sample have been removed prior creating the dotplot.

The overballasted particles eventually become either neutrally buoyant, drifting and remaining in a given water layer, or they will become negatively buoyant slowly sinking downwards (Kooi et al., 2017; Lobelle et al., 2021). Alternatively, when defouled, the particles may move upwards in the water column and resurface (Ye and Andradý, 1991). Our knowledge on the vertical distribution of plastic particles in the global ocean is rather limited but the fraction of plastic submerged and suspended in the ocean's interior could be substantial (Choy et al., 2019; Pabortsava and Lampitt, 2020). Yet, whether sinking plastics constitutes a vertical transport vector for ocean surface microorganisms similar to the proposed horizontal long-range transport (Gregory, 2009) remains unknown. In this study, for the first time, we show microbial community compositions on plastic particles recovered from the sea surface down to the bathypelagic water column.

## 4.2. Microbial diversity

### 4.2.1. Microbial communities on plastic: distinct but follows general trends of water column microbial communities

Bacteroidota, Proteobacteria (Alpha and Gammaproteobacteria), and Cyanobacteria were the main phyla on the plastic particles analyzed here. These are also abundant bacterial phyla generally found in ocean waters (Brown et al., 2009; Sunagawa et al., 2015). However, other highly abundant free living ocean taxa such as the SAR324 clade (Marine Group B; (Boeuf et al., 2021), the Marinimicrobia (SAR406; (Hawley et al., 2017), SAR86 (Hoarfrost et al., 2020) or SAR11 (West et al., 2016) were nearly absent on our plastic samples. Also abundant archaea in ocean waters such as Thaumarchaeota and marine group II were very rare taxa on plastic, which indicates the plastic surfaces select for specific microorganisms (Sunagawa et al., 2015; Pereira et al., 2019). In general microbial communities in surface waters differ from

microbial communities in the deeper water layers of the ocean. This is thought to derive from the alteration of environmental factors (temperature, oxygen, light and pH), which are substantially different in the photic and aphotic zones of the ocean (Haro-Moreno et al., 2018) and which shape these microbial communities (Brown et al., 2009; Bryant et al., 2012; Sunagawa et al., 2015).

We found similarities of plastic associated communities when compared to the microbial community in the water column. Already at the phylum level, our analysis revealed differences among microbial taxa when comparing plastic particles from surface versus deeper water layers. We found a higher abundance of reads assigned to Cyanobacteria and Bacteroidota on plastic debris retrieved from surface waters when compared to deeper water layers. In contrast, a higher read abundance of Proteobacteria was found on plastic particles retrieved from deeper water layers. Cyanobacteria, Bacteroidota and Proteobacteria on plastic follow the typical water column microbial composition in the Pacific Ocean. There, Cyanobacteria and Bacteroidetes are more abundant in the photic zone, whereas in deeper water layers, Proteobacteria dominate (Walsh et al., 2016; Wang et al., 2020). The same pattern of Cyanobacteria being more abundant in surface waters and Proteobacteria in deeper water layers, has been reported for the Indian Ocean (Wang et al., 2016). Cyanobacteria constitute one of the most abundant phyla in marine environments, with total cell counts ranging between  $4 \times 10^4$  and  $2 \times 10^5$  cells  $\text{ml}^{-1}$  in Pacific waters (Flombaum et al., 2013). Prochlorococcus and Synechococcus have been detected as main free living Cyanobacteria in the North Pacific Ocean Gyre (Brown et al., 2009), but in contrast to this, our data indicate that plastic debris associated communities are enriched in families of filamentous Cyanobacteria *Phormidesmiaceae* and *Nostocaceae*, which were particularly abundant on plastic fragments retrieved from surface waters. Filamentous Cyanobacteria, such as *Trichodesmium* spp., are known to be specialized in

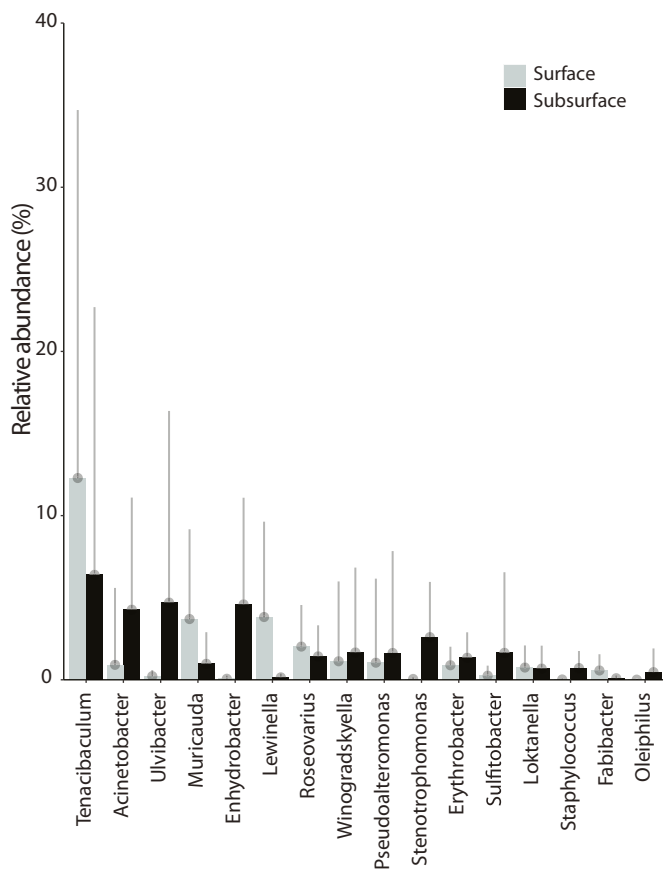


Fig. 5. Relative abundance of bacterial genera containing previously described hydrocarbon-degrading bacteria. The sampling position (sea surface, subsurface water column) is indicated.

trapping particles in suspension and releasing important nutrients involved in biogeochemical cycles (Rubin et al., 2011; Basu et al., 2019). Furthermore, we found more reads assigned to Verrucomicrobia, Planctomycetota and Actinobacteria on plastic particles in subsurface than surface waters. Both, Verrucomicrobia as well as Planctomycetota have been shown to increase in relative read abundance with increasing water depth in the Black Sea (Cabello-Yeves et al., 2021), which may be linked to oxygen availability. However, representatives of Verrucomicrobia have been found in both surface (Cottrell and Kirchman, 2000; Gründger et al., 2021) as well as subsurface waters in other ocean regions (Cardman et al., 2014). Planctomycetota have been shown to increase in relative abundance on PE in incubation experiments over 2 months (Cheng et al., 2021). Planctomycetota, are a relatively novel phylum consisting of 29 genera, including members with the ability to degrade complex hydrocarbons and sugars (Wiegand et al., 2018; Wiegand et al., 2020), thus they may be more important in aphotic zones of the ocean. The most abundant Planctomycetota genus in our samples was *Rhodopirellula*, which was twice more abundant on plastic particles in subsurface compared to surface waters. Species of this genus have previously been isolated from different particle surfaces, both natural as well as plastics (Kallscheuer et al., 2020). In addition, Actinobacteria have been shown to increase in relative abundance over depth in the water column of the Pacific Ocean (Wang et al., 2020). To a certain extent, microbial communities on plastic particles recovered from the sea surface thus resemble the typical surface water microbial community whereas the microbial communities on submerged plastic particles resemble the microbial communities in deeper layers of the water column. In conclusion, this indicates that sinking plastic particles acquire mostly taxa from the surrounding water layers and seem to rather rapidly lose their microbial load from the sea surface. This also suggests

that sinking plastic particles are not an efficient transport vector to vertically translocate microbial communities through the water column.

Interestingly, the microbial richness (number of different taxa observed) on plastic particles does not follow the general trend observed for ocean waters. The taxa richness of all dominant phyla was higher on surface plastics compared to particles retrieved from subsurface water layers (Figs. S1, S2, S3, S4). Even for taxonomic groups that increased in relative abundance on subsurface plastics, such as the Alpha- and Gammaproteobacteria and Planctomycetota, the taxa richness decreased. This is opposite to the global trend that shows increased microbial richness in the mesopelagic zones of oceans compared to the surface waters (Sunagawa et al., 2015). The reduced diversity of taxa on sinking/suspended plastic might indicate a more specialized community with an advantage over opportunistic settlers.

#### 4.2.2. Plastic associated core-microbiome on surface water plastic particles

Microbial communities on floating plastic particles were dominated by *Flavobacteriaceae*, *Rhodobacteraceae*, *Cyclobacteriaceae*, *Saprospiraceae* and *Hyphomonadaceae*. *Flavobacteriaceae* and *Rhodobacteraceae* have been identified as dominant microbial taxa on low-density polyethylene (LDPE), high-density polyethylene (HDPE) and PP in the Mediterranean Sea (Pinto et al., 2019; Vaksmaa et al., 2021b). In six week incubation experiments in the Caribbean Sea, these taxa were also found to colonize all tested polymers (PET, HDPE, polyvinyl chloride (PVC), LDPE, PP and PS (Dudek et al., 2020). *Flavobacteriaceae* and *Rhodobacteraceae* were furthermore found on PE and PP during incubation experiments in a harbor in the NW Atlantic (Zettler et al., 2013). *Flavobacteriaceae* and *Rhodobacteriaceae* have been described as general colonizers, which may explain their presence on plastics in different geographical locations. They have been detected both as early colonizers in incubation experiments as well as on “wild” plastics with unknown residence times in the ocean surface waters. To date, their role in colonizing different polymer types has not been revealed. Whether representatives of the *Flavobacteriaceae* and *Rhodobacteriaceae* are narrow-range (more polymer specific) plastic colonizers or general plastic colonizers remains unknown. *Saprospiraceae* have been detected on different polymers, such as PS and PE (Oberbeckmann et al., 2018; Kirstein et al., 2019) as well as on PET, HDPE, PVC, LDPE, PP and PS (Dudek et al., 2020). *Cyclobacteriaceae* have mainly been found in freshwater systems as plastic-colonizing taxa (Miao et al., 2019). Though several of these families have been detected on floating plastic polymers, their functional roles including their potential to act as direct or indirect plastic degraders still needs to be demonstrated.

#### 4.2.3. Differences in microbial community structure on surface and submerged plastics

Submerged and sinking plastic have gained increasing attention because a substantial fraction of all positively buoyant plastic that has been released to the ocean since the 1950s cannot be accounted for - a phenomenon that was termed the “missing plastic paradox” (Cressey, 2016; Wayman and Niemann, 2021). Plastic from the surface sinks to deeper water layers and sediments, a process that is influenced by biofouling of initially floating plastics (Kaiser et al., 2017; Lobelle et al., 2021). However, relatively little is known about microbial communities on submerged plastic particles, in contrast to floating plastics (Harrison et al., 2014; Woodall et al., 2018; Pinnell and Turner, 2019; Krause et al., 2020; Tu et al., 2020; Agostini et al., 2021). Microbial community structure has been investigated on plastics retrieved from the deep sea (Woodall et al., 2018; Krause et al., 2020; Kelly et al., 2022). Other studies conducted exposure experiments, for example with PE exposed to coastal sediments (Harrison et al., 2014), PE exposed to marine subsurface waters (Tu et al., 2020) and PET, and PHA exposed to water sediment interface in microcosms (Pinnell and Turner, 2019).

Knowledge of microbial community structure on submerged pelagic plastics is crucial for our understanding of microbial community succession during the vertical movement of plastic particles from the sea

surface to deeper water layers and sediments. Additionally, this can shed light on the importance of sinking plastics as a vertical transport vector; i.e., whether surface microorganisms that are “alien” to deeper waters are introduced into the native microbiome of the deep waters. Our results indicate that *Flavobacteriaceae*, *Rhodobacteraceae*, *Moraxellaceae*, *Hyphomonadaceae*, *Xanthobacteraceae* and the poorly resolved verrucomicrobial family DEV007 are the dominant microbial families on plastic particles submerged in the NPSG water column. In previous investigations, *Flavobacteriaceae* and *Rhodobacteraceae* were found on PE sheets and dolly ropes exposed to the seafloor in a harbor and offshore in the North Sea (De Tender et al., 2017). *Flavobacteriaceae*, which have been found recurrent in our analysis, include several species whose genome encodes proteins involved in adhesion and motility processes, indicating potential adaptation to a particle-associated life cycle (Fernández-Gómez et al., 2013; McBride and Zhu, 2013; Gavriilidou et al., 2020). *Moraxellaceae* are known as mesophilic or psychrophilic, and contain genera such as *Psychrobacter*. These are adapted to high salinity and colder temperatures (Welter et al., 2021), which are typical for subsurface waters of the central Pacific. *Moraxellaceae* have been identified on plastics recovered from the North Atlantic Ocean (Zettler et al., 2013). Furthermore *Moraxella* spp. have been shown to seemingly degrade polyethylene bags and plastic cups in mangrove soils (Kathiresan, 2003). *Hyphomonadaceae* have frequently been identified as part of plastic microbial communities (Bryant et al., 2016; Kirstein et al., 2019; Pinto et al., 2019) and as a core plastic-associated taxa in the deep ocean (Agostini et al., 2021). *Hyphomonadaceae* have been shown to degrade variety of hydrocarbons (Kappell et al., 2014), and may thus be directly involved in polymer degradation. The role of the DEV007 (Verrucomicrobia) has not yet been clarified. Nevertheless, this family is widely distributed across different ecosystems (Orsi et al., 2016; Coskun et al., 2019).

#### 4.2.4. Diversity of putative and known hydrocarbon-degrading bacteria (HCBs)

The list of potential plastic degraders (prokaryotic and eukaryotic) is continuously expanding (Yamada-Onodera et al., 2001; Gilan et al., 2004; Matsumiya et al., 2009; Herrero Acero et al., 2011; Tanasupawat et al., 2016; Paço et al., 2017; Palm et al., 2019; Zhang et al., 2020a). Specifically, we suggest that HCBs in our samples are potential plastic degraders because plastics are hydrocarbons or hydrocarbon-like compounds. Several studies have reported HCBs as abundant taxa on plastic surfaces in the marine environment (Debroas et al., 2017; Dussud et al., 2018a; Oberbeckmann et al., 2018; Davidov et al., 2020; Erni-Cassola et al., 2020; Vaksmaa et al., 2021b). We identified 43 genera of putative HCBs, comprising a summed relative abundance of 28 % and 35 % on floating and submerged plastic particles, respectively. Sea surface samples were dominated by the genus *Tenacibaculum* followed by *Muricauda* and *Lewinella*. Similarly, plastic fragments from subsurface waters were also dominantly colonized by *Tenacibaculum*, too. But in contrast to surface particles the 2nd and 3rd most abundant genera were *Ulvibacter* and *Enhydrobacter*, pinpointing the demarcation of communities on surface versus subsurface plastic particles. *Tenacibaculum* has been detected on plastics recovered from surface waters of the North Sea and in colonization experiments with water from the Mediterranean Sea (Oberbeckmann et al., 2016; Dussud et al., 2018a). In our study, most *Tenacibaculum* reads retrieved from sea surface samples belonged to *Tenacibaculum jejuense*. *T. jejuense* is a strictly aerobic bacterium, which has been shown to degrade crude oil (Wang et al., 2014) and is able to hydrolyze carboxymethylcellulose, gelatin, starch and xylan (Oh et al., 2012). *Muricauda* and *Lewinella* have previously been identified on plastic debris in the North Pacific Ocean (Bryant et al., 2016), and *Lewinella* was also detected in biofilms on PET bottles in North Sea waters (Oberbeckmann et al., 2016). Members of the genus *Lewinella* have been described as starch and carboxymethylcellulose degraders (Khan et al., 2007). Hydrocarbonoclastic bacteria, which include species such as *Balneola* sp., *Marinobacter* sp., *Winogradskyella* sp., and *Lewinella*

sp. were dominant bacteria on enrichment experiments with PP (Jacquin et al., 2021). *Ulvibacter* has been identified as part of tightly adhered communities on plastic (Kirstein et al., 2019) and to colonize PET bottles in the North Sea (Oberbeckmann et al., 2016). However, the role of this genus is not well defined (Purohit et al., 2020).

As prime example of HCBs, bacterial species such as *Rhodococcus* spp., able to degrade oil, hexane, other recalcitrant pollutants and PAHs (Sorkhoh et al., 1990; Lee et al., 2010; Bourguignon et al., 2014), have been shown to efficiently degrade several types of plastic polymers such as PE, PP and PS (Gilan et al., 2004; Mor and Sivan, 2008; Gravouil et al., 2017). The ability to break down plastics may be supported by the metabolic capabilities of HCBs. These organisms contain enzymes that degrade straight chain and more complex hydrocarbons including aromatic compounds that are also found in crude oil, organic pollutants, and plastics. Among these, carboxylesterases, hydrolases, lipases and cutinases have been proposed to enable the hydrolysis of plastics (Müller et al., 2005). However, bacteria containing specific enzymes to degrade specific plastic types have only seldomly been characterized. For example, *Ideonella sakaiensis* was shown to degrade PET with a cutinase (termed PETase) and to assimilate the PET-derived carbon (Tanasupawat et al., 2016; Yoshida et al., 2016; Palm et al., 2019; Tournier et al., 2020). HCBs have frequently been detected on plastic particles in the marine environment and some species have been confirmed to degrade plastics. However, HCBs might not necessarily degrade plastics directly, but rather utilize daughter products produced during physicochemical weathering process, most importantly photodegradation (Wayman and Niemann, 2021). These include short chain volatile compounds such as methane (Royer et al., 2018), but also longer chain alkanes with and without carbonyl or hydroxyl moieties as well as plastic oligomers (Gewert et al., 2018) and nanoplastics (Gigault et al., 2016). At least some of these compounds were found to be utilized by marine microorganisms (Romera-Castillo et al., 2018). It thus remains to be revealed in future studies, whether HCBs make use of the virgin polymer or of the plastic degradation products.

#### 4.2.5. Microbial community on surface and subsurface plastic particles - the role of hitchhikers

Previously, it has been postulated that microorganisms and other organisms attached to plastic can disperse over large horizontal distances in the marine system by means of transoceanic rafting (Masó et al., 2003; Barnes and Milner, 2005; Kiessling et al., 2015; Carlton et al., 2017; Simkanin et al., 2019). Thus, plastics allow attached organisms to overcome large distances as ‘hitchhikers’, and to invade new geographical regions. This has implications for local ecosystems, for example if the invading ‘hitchhiker’ community includes non-native organisms, which may outcompete members of the original biome. In addition, floating plastics has been proposed to function as a horizontal dispersal vector for pathogenic microorganisms (Kirstein et al., 2016; Debroas et al., 2017). For example, *Vibrio* spp. have been found to colonize plastics in different geographical locations (Zettler et al., 2013; De Tender et al., 2015; Frère et al., 2018; Keszy et al., 2019). Similar to the previous studies, we found members of the potentially pathogenic bacterial genera *Vibrio*, *Escherichia*, *Enterococcus* and *Shewanella*, with *Vibrio* accounting for ~1 % of total reads on surface plastic. However, we cannot account for the origin of these organisms, i.e., whether they have been transported over long distances to the sampling location or if these have been acquired from the surrounding water.

A fraction of the floating surface plastic sinks (Kaiser et al., 2017; Kooi et al., 2017; Lobelle et al., 2021). Incubation experiments have shown that PE can already sink after 6 weeks in coastal waters (Kaiser et al., 2017) and that sinking may be followed by resurfacing (Ye and Andradý, 1991). Sinking dynamics of plastic particles are influenced by the particle's surface to volume ratio: a larger surface to volume ratio supports a relatively high fouling community mass including multicellular organisms (Amaral-Zettler et al., 2021). To determine the importance of microorganisms on sinking plastic particles, we firstly estimated



bathy- and epipelagic standing stock of water column microorganisms and compared that to an estimate of the total amount of microorganisms attached to floating plastic particles. Prokaryotic cell densities in open ocean waters are  $\sim 5 \times 10^5$  cells  $\text{ml}^{-1}$  close to the ocean's surface (upper 200 m) decreasing logarithmically to  $\sim 5 \times 10^4$  cells  $\text{ml}^{-1}$  at greater depths, below 200 m (Whitman et al., 1998; Aristegui et al., 2009). The average water depth in the NPSG is about 4500 m (Weatherall et al., 2021). Integrating the surface and deep ocean cell numbers over depth and spatially extrapolating, this yields a total of  $\sim 10^{20}$  microbial cells in a 4500 m deep and 1  $\text{km}^2$  wide water column. Incubation experiments have shown that ocean plastic fragments (PE, PP, PS after 8 to 12 weeks incubation) can support biofilms with cell densities of  $1 \times 10^7$  to  $2 \times 10^7$  cell  $\text{cm}^{-2}$  (Zhao et al., 2020). Similarly,  $2 \times 10^7$  cells  $\text{cm}^{-2}$  were found on floating plastic pieces recovered from intertidal zones (Basili et al., 2020). On a global scale, biofilms on floating plastic particles have thus been identified as a potentially important compartment of the surface ocean's ecosystem (Zhao et al., 2020; Amaral-Zettler et al., 2021). To roughly determine the total surface area of floating plastic particles in 1  $\text{km}^2$  of the NPSG, and thus the potential load of microorganisms attached to it, we made the following considerations: most floating plastic pieces in the ocean are microplastics (1  $\mu\text{m}$ –5 mm), of which small microplastics  $\ll 1$  mm are probably predominant (Poulain et al., 2019; Lindeque et al., 2020; Pabortsava and Lampitt, 2020; Wayman and Niemann, 2021). We thus assumed two scenarios: All floating plastic particles are spherical with a diameter of (i) 0.1 mm or (ii) 5 mm. For any particle, the density is 0.97  $\text{g cm}^{-3}$  and the biofilm has a cell density of  $10^7$  cells  $\text{cm}^{-2}$ . Highest concentrations of floating plastic were estimated with up to  $10^5$   $\text{g km}^{-2}$  in the NPSG (van Sebille et al., 2015; Lebreton et al., 2018), this comprises both macro- and microplastics. In accordance with our considerations of small (0.1 mm diameter) and large microplastics (5 mm diameter), the plastic mass of  $10^5$   $\text{g km}^{-2}$  translates to a combined surface area of floating plastic of  $1.2 \times 10^6$  to  $6.2 \times 10^7$   $\text{cm}^2$ . Assuming cell densities of  $\sim 10^7$  cells  $\text{cm}^{-2}$  on floating plastic particles, the combined surface area of plastics per  $\text{km}^2$  in the gyre's center could thus support  $1.2 \times 10^{13}$  to  $6.2 \times 10^{14}$  cells. Compared to the number of pelagic microbial cells per  $\text{km}^2$  ( $\sim 10^{20}$ , see above), the number of microbial cells on floating plastic is hence  $\geq 6$  orders of magnitude lower. It thus appears unlikely that the biomass of microorganisms on sinking plastics adds substantially to the pelagic ecosystem as a whole. However, locally, a plastic particle with a surface area of a few  $\text{mm}^2$  may add a substantial number of microbial cells to the water column in the particles immediate surroundings. Furthermore, microorganisms dissociating from biofilms on sinking plastic could act as invading species with adverse effects for the pelagic microbial community. Future studies should investigate the impacts of transported “hitchhikers” into deep waters on ecosystem function.

For future research, the question thus arises if and over which vertical distances sinking plastic particles are functioning as an “elevator” carrying along microorganisms from the surface to deeper water layers. How quickly are microorganisms on plastics exchanged with adjacent waters? Also, do some microbial taxa stay attached to plastics longer than others and could thus overcome vertical distances more efficiently? Alternatively, microbial communities on sinking plastics might be exchanged with the surrounding water column rather rapidly during downward movement of the plastic particle, which would limit the potential vertical reach of surface microorganisms. Our results show that plastic particles harbor microbial communities that are rather typical for the water layer from where the particle was recovered. This indicates that communities are rather rapidly exchanged once the particle sinks down from the surface. Alternatively, the sinking velocities of particles might be rather slow. This could also explain the observed differences in microbial community structure, even if the exchange rate was low. Both scenarios result in a limited drag of the surface communities towards deeper water layers. Our results thus show that sinking particles act as an elevator, but that the microbial passengers are apparently exchanged at each water layer.

## 5. Summary, conclusions and outlook

Our results indicate that microbial communities on plastic debris afloat at the sea surface differ from communities on plastic particles deeper in the water column. Thus, plastic sinking from surface waters towards the underlying deep sea provides only a limited transport for surface microbial communities to deeper water layers. Instead, sinking plastics appear to exchange microorganisms with deeper water layers on time scales relevant for the particle's sinking velocity. Considering the abundance of free microorganisms in the water column, sinking plastic does not carry along substantial microbial biomass to the epipelagic and bathypelagic subsurface compartments. Future studies are required to elucidate impacts of surface microorganisms transported to deeper waters on microbial and ecosystem function. We found indications for a more specialized microbial community on deep-water plastics and we also found putative and known hydrocarbon-degrading bacteria but their plastic-degrading potential and metabolic pathways remain unknown. Our methodology for DNA extraction from small individual plastic particles resulted in low DNA amounts requiring PCR amplification prior to 16S rRNA gene sequencing, which can introduce biases related to microbial abundances.

Unravelling microbial roles in plastic biofilms will require finer taxonomic resolution, application of non-amplicon based methods to evaluate microbial community structure and quantitative abundances of microbes attached to plastic as well ecophysiology studies. Further efforts should also focus on higher sampling resolution, to assess potentially rapid changes in plastic-associated microbial communities with water depth. Additionally, further research is needed to determine the velocity of community exchange (plastic-seawater, seawater-plastic) during the downward movement of plastic particles, as well as cell abundances and biomass carbon that could be transported vertically on sinking plastic to deep waters and sediments.

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### CRedit authorship contribution statement

Annika Vaksmaa: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Investigation, Writing - original draft.

Matthias Egger: Conceptualization, Sampling, Methodology, Validation, Writing - original draft, review & editing.

Claudia Lücke: Data curation, Formal analysis, Visualization, Writing - review & editing.

Paula Dalcin Martins: Data curation, Formal analysis, Writing - review & editing.

Riccardo Rosselli: Data curation, Formal analysis, Writing - review & editing.

Alejandro Abdala Asbun: Methodology, Validation, Bioinformatics, Writing - review & editing.

Helge Niemann: Conceptualization, Supervision, Validation, Resources, Funding acquisition, Writing - review & editing.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Matthias Egger reports financial support and travel were provided by The Ocean Cleanup.

Matthias Egger works for The Ocean Cleanup, a non-profit organization aimed at advancing scientific understanding and developing solutions to rid the oceans of plastic, headquartered in the Netherlands.

### Data availability

Data has been deposited to public repositories

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