

BRIEF REPORT

Microbes and microplastics: Community shifts along an urban coastal contaminant gradient

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Abstract

Plastic substrates introduced to the environment during the Anthropocene have introduced new pathways for microbial selection and dispersal. Some plastic-colonising microorganisms have adapted phenotypes for plastic degradation (selection), while the spatial transport (dispersal) potential of plastic colonisers remains controlled by polymer-specific density, hydrography and currents. Plastic-degrading enzyme abundances have recently been correlated with concentrations of plastic debris in open ocean environments, making it critical to better understand colonisation of hydrocarbon degraders with plastic degradation potential in urbanised watersheds where plastic pollution often originates. We found that microbial colonisation by reputed hydrocarbon degraders on microplastics (MPs) correlated with a spatial contaminant gradient (New York City/Long Island waterways), polymer types, temporal scales, microbial domains and putative cell activity (DNA vs. RNA). Hydrocarbon-degrading taxa enriched on polyethylene and polyvinyl chloride substrates relative to other polymers and were more commonly recovered in samples proximal to New York City. These differences in MP colonisation could indicate phenotypic adaptation processes resulting from increased exposure to urban plastic runoff as well as differences in carbon bioavailability across polymer types. Shifts in MP community potential across urban coastal contaminant gradients and polymer types improve our understanding of environmental plastic discharge impacts toward biogeochemical cycling across the global ocean.

INTRODUCTION

Plastic pollution in the global ocean has provided microorganisms with novel carbon substrates with unknown pathways of removal or sedimentation (Amaral-Zettler et al., 2020; Jambeck et al., 2015). Some taxa have evolved phenotypes for plastic degradation which are often derived from hydrocarbon degradation pathways (Jacquin et al., 2019), but it remains unclear how microbial interactions with these xenobiotic substrates differ among plastic polymer types and aquatic habitats. In marine settings, we postulate that plastic-adapted phenotypes are most common in coastal and estuarine environments due to contaminant loadings. Microbial communities inhabiting

plastic material in the receiving waters of New York City and Long Island, New York are of particular interest because of the watershed's high population density and its attendant plastic discharge rates (Miller et al., 2017). High plastic pollution loadings to aquatic microbial communities are postulated to select for microbial phenotypes capable of plastic degradation. Subsequent transport of coastal MP microbiomes likely leads to significant gene exchange among plastic biofilm communities and gene dispersal across neighbouring marine ecosystems. Improved understanding of urban-sourced plastisphere communities could help predict downstream impacts of plastic pollution on biogeochemical cycling and gene flow. Further, plastic pollution has introduced highly recalcitrant forms of carbon

with extremely low biological accessibility into the global ocean. The fate and transport of marine plastics and their impact on global carbon budgets may thus need adjustment based on loadings of specific polymer types and on proximity to plastic pollution sources.

Extensive research has been conducted on plastisphere communities collected from the marine environment (Bryant et al., 2016; Oberbeckmann et al., 2014; Oberbeckmann & Labrenz, 2020; Zettler et al., 2013), deployed within the environment (Latva et al., 2022; Lobelle & Cunliffe, 2011; Oberbeckmann et al., 2016) and incubated in controlled laboratory settings (Ogonowski et al., 2018; Pinto et al., 2019; Wright et al., 2021). Although results are highly variable among studies, overall patterns indicate that polymer type and geographic location are both significant factors determining plastisphere community composition and functional potential (Frère et al., 2018; Hansen et al., 2021; Oberbeckmann et al., 2014; Ogonowski et al., 2018; Pinto et al., 2019). Geographic location of plastic contaminants has often been shown to be a more significant determinant of plastisphere community composition than plastic polymer type (Amaral-Zettler et al., 2015; Muthukrishnan et al., 2019; Oberbeckmann et al., 2014; Oberbeckmann et al., 2016). However, cross-comparisons of studies of diversely sourced marine plastics often yield inconsistent results due to varying methodologies (i.e., biases from different PCR primer types, DNA extraction kits, sample collection and processing), as well as potential site-specific influences introducing other sources of variation. These uncertainties severely hinder broader understanding of polymer-specific surface community functioning and plastic-adapted phenotypes. A more rigorous assessment of polymer-specific effects on hydrocarbon-degrader colonisation and plastisphere community function would benefit from thorough investigation of colonisation across polymer types within urbanised estuarine systems where plastic debris often originates.

Marine plastic debris is often selectively colonised by hydrocarbon-degrading microbes (Delacuvellerie et al., 2019; Erni-Cassola et al., 2020; Zadjelovic et al., 2020; Zettler et al., 2013), suggesting that hydrocarbon degraders have distinct functional roles on marine plastic surfaces. With the exponential increase of plastic polymer discharge into the ocean, it is critical to understand its overall biotic degradation potential. Xenobiotic-degrading phenotypes, like many other bacterial traits, likely evolved in response to repeated environmental exposures to contaminants (e.g., chemical waste, biocides, etc.) (Gillings, 2017; Wright et al., 2008). Prolonged exposure of microbial cells to plastic polymers in the natural environment could have potentially produced a similar outcome. Understanding hydrocarbon degrader colonisation selectivity across many common plastic polymer types is essential for determining the outcome of marine plastic-biotic

interactions and their influence on the global ocean. Microbial plastic degradation potential has been correlated with concentrations of plastic debris across broad spatial scales within open ocean environments (Zimec et al., 2021). Large scale metagenomic data mining has revealed that genes for plastic-degrading enzymes were more abundant in Mediterranean Sea and South Pacific Ocean contaminant hotspots. However, it is unclear how plastic degrading potentials relate to contaminant loadings in highly urbanised coastal environments, and assessment of hydrocarbon degrader colonisation in these urban systems is a critical step toward addressing plastic degradation potentials. The Hudson and East Rivers have been identified repeatedly as significant sources of urban runoff and contamination to Long Island Sound and the Atlantic Ocean, yet remain uninvestigated for plastic-microbial colonisation selectivity (Ayres & Rod, 1986; McCrone, 1966; Miller et al., 2017; Polanco et al., 2020). Plastisphere investigations within this urbanised marine ecosystem could provide a benchmark for understanding microbial interactions with plastic debris across other highly urbanised coastal ecosystems.

This study aims to answer the question ‘do urban coastal contaminant gradients influence plastisphere community structure and do effects vary among polymer types?’ To that end, we investigated microplastic (MP) microbiomes associated with six common polymer types along a spatial contaminant gradient. We hypothesize that coastal sampling sites proximal to densely populated New York City will yield more hydrocarbon-degrading colonisers due to their relatively high plastic loadings. Enhanced exposures to plastic debris potentially lead to increased selection of hydrocarbon-degrading taxa. Differences in sampling site characteristics (e.g., salinity, nutrient load histories) may also modify colonisation selection rules. We hypothesize that plastisphere microbiomes also vary according to polymer type along the contaminant gradient. Our results will ultimately improve understanding of polymer-specific bioavailability of recalcitrant carbon sources and changes in biogeochemical cycling caused by plastic pollution. We also analyse both DNA and RNA recovered from naturally colonised plastic polymers as a means to assess activity levels of specific phylogenetic groups and their putative functional roles within plastic microbiomes. To our knowledge, RNA-based microbial profiling has not been previously used in marine plastisphere colonisation studies. This approach could serve as a standard for relating marine plastic DNA surveys to active members of plastic microbiomes. Furthermore, identifying relative abundances of active hydrocarbon-degrading taxa is a preliminary step toward predicting microbial transformation effectiveness for plastic-based carbon from individual cells to whole ecosystems.

EXPERIMENTAL PROCEDURES

Sample collection

Test substrates included MP beads of six different polymer types, high density polyethylene (HDPE), polystyrene (PS), polypropylene (PP), polycarbonate (PC), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) and distributed by LNS Technologies®, Domino Plastics Company Inc., Total Petrochemicals and Refining USA Inc. Plastic distributors could not provide any information on potential additives and/or monomers within our test substrates. Microplastic and glass control beads were between 3 and 5 mm in diameter. For

field deployments, they were packaged in individual nylon mesh bags (1.5 mm mesh size) and secured to Pex-A pipe frame (Figure 1A–D). A vinyl-coated steel chain was attached to the Pex-A frame's bottom member as a counterweight to submerge these buoyant samplers at ~0.5 m depth. These passive MP samplers were secured to floating docks with nylon rope for 8 weeks at SUNY Maritime College (denoted as MAR site; The Bronx, NY), Flax Pond salt marsh (FP site; Old Field, NY), Southampton (SH site; Shinnecock Bay, NY) and Eastern Sound Station (ESS site; Avery Point, CT) (Figure 1E). Temporal colonisation dynamics were investigated at the MAR site only by deploying replicate mesh bags with PE or glass substrate

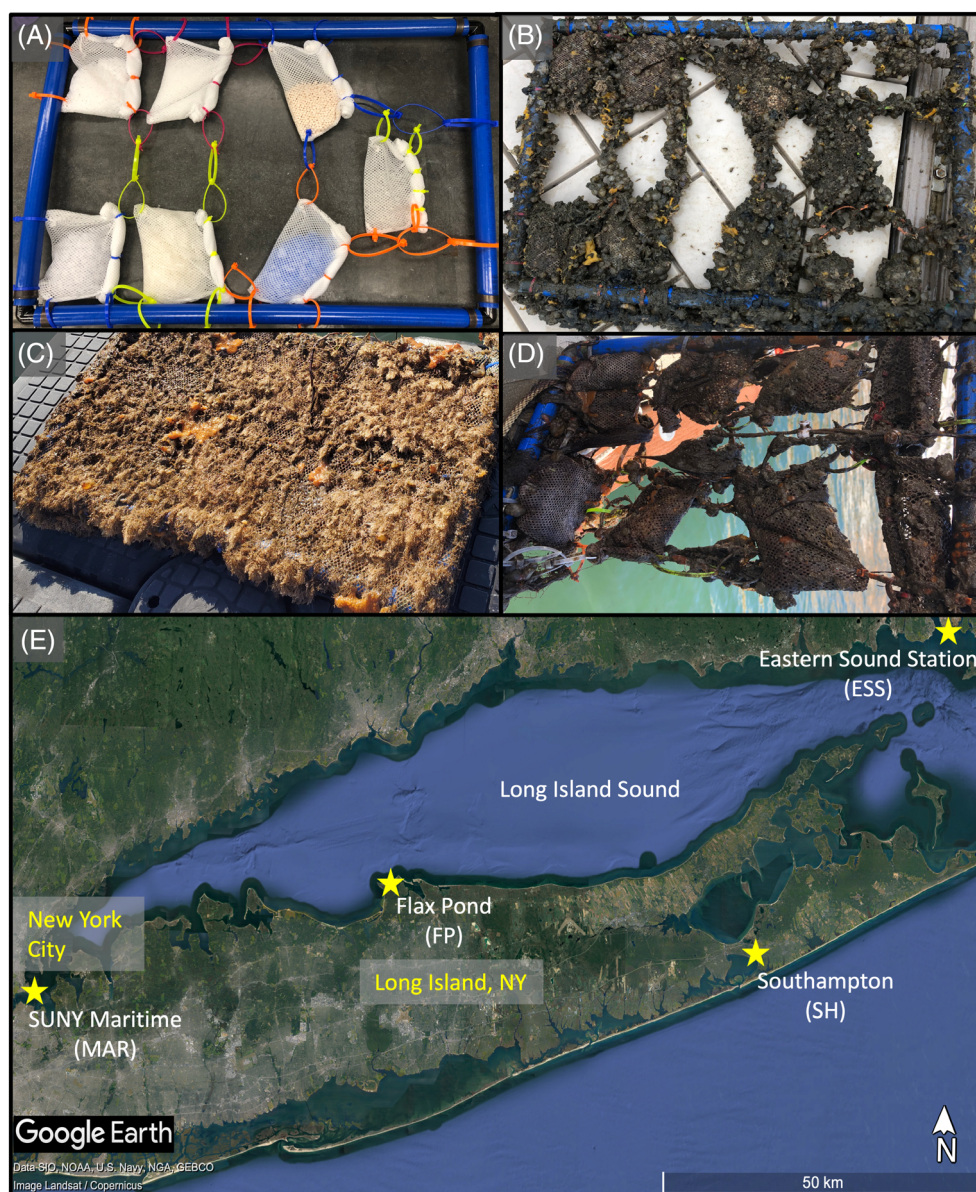


FIGURE 1 (A) Microplastic deployment sampler design. Frames were constructed of Pex-a pipe, and each microplastic polymer type was packaged in an individual nylon mesh bags. In situ biofilm community accumulations are shown after 8 weeks of incubation at (B) Southampton, (C) Eastern Sound Station and (D) SUNY Maritime sites. (E) Map of all deployment sites surrounding Long Island, NY.

(including beads and flat rectangular samples $25 \times 75 \text{ mm}^2$ for each substrate type) and recovering after 1, 2, 3, 4 and 8 weeks. Sampling site salinity and water temperature were recorded upon deployment and retrieval using a refractometer and laboratory thermometer.

Salinity and temperature varied over a relatively narrow range (26–29 PSU, 25°C – 26°C) throughout deployments among all sites, consistent with historical salinity data (Lee & Lwiza, 2005; Swanson et al., 2021; Weiss et al., 2007). Upon sample retrieval, nitrile gloves and scissors were sterilised with 70% ethanol to ensure aseptic handling of samples. Samplers were retrieved slowly and carefully to avoid disturbance of loosely attached biofilms. Each sampler was photographed to document site-specific visual differences in biofilm development. Individual mesh bags were placed in separate presterilised Ziploc bags. Finally, 3 L of seawater were collected at each site in autoclaved glass bottles for subsequent analysis of planktonic communities to serve as references for source seawater. Samples were transported back to the laboratory on ice for processing.

Sample processing

In the lab, each mesh bag was aseptically opened and colonised MP and glass beads were transferred using flame-sterilised forceps to 15 mL Falcon tubes and stored at -80°C prior to nucleic acid extraction. Colonised substrates were not rinsed in order to limit removal of loosely attached microbes and maximise capture of MP microbiome members. Mesh bags themselves were also processed alongside substrates for ESS site samples only as another surface substrate comparison. Seawater subsamples (1 L each) were immediately filtered through three separate $0.2 \mu\text{m}$ polyethersulfone filters (Sterlitech® Corp.) and stored at -80°C prior to nucleic acid extraction. Colonisation time course samples (planar substrates) were subjected to image analysis to document biofilm maturation. Images were captured with an AMscope MU130 camera attached to an Olympus SZ 60-PT binocular microscope. Images were processed using ImageJ software (Abràmoff et al., 2004). Briefly, threshold and bandpass filters were adjusted to display only grey-scale images. Then, the ‘analyse particles’ feature was used to measure area and percent coverage of biofilm material for each sample.

Enrichment cultures

Naturally colonised MPs of each polymer type and inert glass controls from the ESS site were used to inoculate enrichment cultures. These cultures were maintained

for ~5 months to compare average surface cell abundance over time in a controlled environment depleted of labile organic substrates. Roughly 5 g (~100 beads) of environmentally colonised MPs were used as enrichment culture inocula. Prior to inoculation, colonised samples to be used for enrichment cultures were gently rinsed with site water to remove loose organic material and limit labile organics introduction to cultures. Cell counts and culture media changes were performed every 2 weeks for the first 2 months, and monthly thereafter. For each media change, all beads were aseptically transferred to freshly autoclaved media to continually decrease labile substrate concentrations and promote growth of plastic-specific microorganisms. Artificial seawater (MBL formula; Cavanaugh, 1975) and aged natural seawater (from open Atlantic Bight waters stored at room temperature in the dark for ~3 months to promote nutrient depletion) were filtered ($0.2 \mu\text{m}$ polycarbonate Millipore®), autoclaved, diluted to site salinity and used for culture media. These two medias were used in tandem to compare natural and artificial media impacts on the growth of plastic-specific microbiomes. Guillard’s (F/2) marine water enrichment solution ($1\times$; Sigma-Aldrich®) provided all essential mineral nutrients and vitamins commonly found in seawater with no significant source of organic carbon. Cultures were continuously shaken on an orbital shaker at 100 RPM and stored at 18°C in the dark to select for growth of aerobic marine heterotrophic microbes. For cell counts, ~75 mg MP subsamples were aseptically transferred into 1 mL of artificial seawater with 20 mM EDTA to promote disassociation of cells from MP surfaces, and then cells were preserved in 2% formaldehyde. To further remove cells from MP surfaces, suspensions were placed in a sonication bath for 15 min. Suspension subsamples were filtered onto $0.2 \mu\text{m}$ polycarbonate Millipore® filters, rinsed with $1\times$ PBS, and stained with DAPI in Vectashield® Plus for cell enumeration under epifluorescence microscopy. Roughly 20 fields of view were randomly selected and enumerated to determine abundances of prokaryotes and eukaryotes present in each subsample.

Nucleic acid extraction and sequencing

One hundred eighty-one DNA and RNA samples were used to characterise plastic-associated microbial communities. Colonised MP samples (~250 mg) were transferred to bead tubes with sterilised forceps and replicates were either extracted for DNA (Qiagen DNeasy PowerWater kit) or for RNA (Qiagen RNeasy PowerWater kit) to identify ‘total’ and ‘active’ portions of the microbial community, respectively (Campbell et al., 2009). RNA analysis was included because DNA profiles alone often include extracellular DNA and inactive or dead microbial cells that are not representative

of ecosystem function (Cardoso et al., 2017; Dell'Anno et al., 1998; Freedman et al., 2015). Extracted RNA was reverse transcribed into cDNA using the Qiagen QuantiTect® Reverse Transcription kit prior to sequencing to allow for 16S rRNA gene amplicon sequencing and subsequent inference of 'active' microbial community members. Filtered seawater samples were also extracted for DNA (and RNA at ESS site only) using half filters. Amplicon fragments were PCR-amplified from DNA and cDNA using a high-fidelity phusion plus polymerase (Comeau et al., 2017) and sequenced on an Illumina MiSeq platform by Integrated Microbiome Resource (IMR, Dalhousie University, Halifax, Canada). The universal V4-V5 region (515FB/926R) was targeted to capture the majority of microbial taxa from both freshwater and marine origins due to the estuarine characteristics of the study sites (Parada et al., 2016; Walters et al., 2016; Yang et al., 2016). This primer set recovers a higher diversity of marine plastic biofilm inhabitants than other primer sets often used in marine plastic studies (Pollet et al., 2018). An additional fungi-specific rRNA target (ITS2, Op De et al., 2014) was used for ESS site samples to investigate fungal diversity in surface biofilms developing on different plastic polymer types. Negative sequencing controls were provided by us, while mock community positive controls are performed routinely at the IMR facility (Comeau et al., 2017).

Data processing and analysis

Amplicon sequence data were processed using the DADA2 v.3.15 analysis pipeline (Callahan et al., 2016). Amplicon sequence variants (ASVs) from bacteria, archaea and fungi were determined using the Silva reference database v.132 (Quast et al., 2012) and the UNITE ITS reference database v.8.3 (Nilsson et al., 2019). ASV-specific ratios of RNA-based 16S rRNA genes to DNA-based 16S rRNA gene were calculated according to percent of RNA and DNA reads for each ASV in a sample library following methods by Jones and Lennon (2010) and Bowsher et al. (2019). 'Phantom taxa', that is, ASVs detected by RNA reads but absent in DNA profiles (Klein et al., 2016), were assigned a ratio of '100'. Sequence data were normalised using a scaling with ranked subsampling method, an alternative to rarefying, which improves reproducibility of results and reduces distortion of community structure compared to traditional rarefaction techniques (Beule & Karlovsky, 2020). Non-metric multidimensional scaling (NMDS) plots were created in R using vegan and ggplot2 packages with the metaMDS function and Bray–Curtis similarity matrices (R Core Team, 2016). Significance of differences between sample groups were evaluated using analysis of similarity (ANOSIM; Clarke, 1993; Warton et al., 2012) in R. Community diversity indices (richness, evenness and Shannon diversity; Shannon, 1948) were calculated

using the vegan package in R. Boxplots depict sample group medians, the interquartile range (>25% and <75% of all observations) and whiskers representing the feasible data range. Welch's *t*-test (t.test package in R) was used to compare significance of diversity indices between sample groups (Moser & Stevens, 1992).

An indicator species analysis was performed using the indic.species package (De Cáceres & Legendre, 2009) in R to determine ASVs among sample groups that were deemed indicators for certain sample types or deployment sites. Probability percentages and *p*-values were determined for indicator taxa based on exclusivity (whether the ASV was found in only one particular substrate type) and fidelity (whether the ASV was found at all sites on that particular substrate type). Levins' niche breadth analysis was used to investigate whether certain substrate types or collection sites contained differing proportions of ASVs that could be categorised as either 'generalist' or 'specialist' taxa (i.e., whether they were commonly shared among all samples within the dataset). Niche breadth was calculated using the formula $B = 1/\sum p_i^2$, where *B* represents niche breadth and *p_i* represents proportion of a specific ASV within sample *i* (Levins, 1968). ASVs were assigned niche breadth values based on the number of samples within which that taxon appeared. High niche breadth values suggest a more 'generalist' ASV that is common among all samples analysed, whereas low niche breadth values suggest a more 'specialist' ASV that is rare among samples. The plotly package in R created three-dimensional surface plots for visualising distributions of generalists and specialists across samples. The presence of potential 'plastic-degrading' taxa were investigated by quantifying sequence reads classified as taxa with known functions as hydrocarbon degraders. These taxa have also been previously shown to possess the ability for plastic degradation (Zadjelovic et al., 2022) or have high metabolic potential for plastic degradation (Dussud et al., 2018; Girard et al., 2020; Yakimov et al., 2022).

RESULTS

Plastisphere microbiomes differed among sampling sites and polymer types

DNA-based plastisphere community profiles differed significantly across substrate types (Figure 2A) (ANOSIM test: $R = 0.36$, $p < 0.001$) and sampling sites ($R = 0.46$, $p < 0.001$). Plastic polymer type was also a significant determinant for community assembly on a single-site basis (Figure S1) (ESS: $R = 0.35$, $p < 0.001$; FP: $R = 0.35$, $p < 0.05$; MAR: $R = 0.56$, $p < 0.001$; SH: $R = 0.41$, $p < 0.01$). Furthermore, all biofilm microbial communities were significantly different from surrounding seawater communities (ESS: $R = 0.99$, $p < 0.01$; FP: $R = 0.81$, $p < 0.01$; MAR:

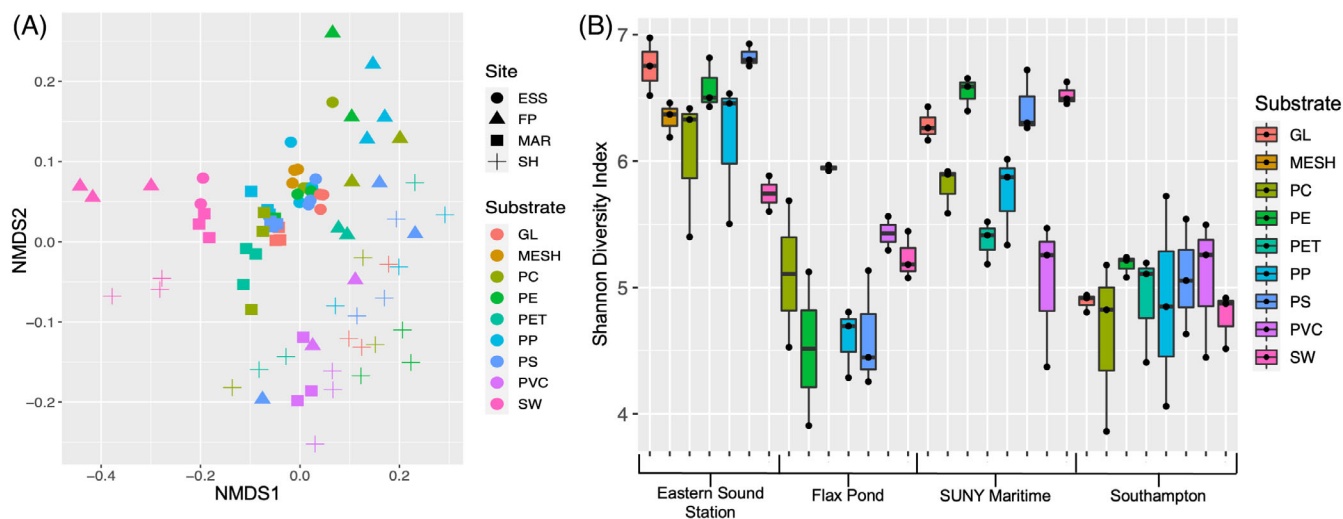


FIGURE 2 (A) Non-metric multidimensional scaling (NMDS) plot of DNA-based microbial community profiles from 16S rRNA gene sequencing among all recovered microplastic samples. Community profiles were significantly different among polymer types ($R = 0.36$, $p < 0.001$) and deployment locations ($R = 0.46$, $p < 0.001$). (B) Shannon diversity boxplots of DNA profiles across all samples. Shannon diversity of plastisphere communities was higher on average at Eastern Sound and Maritime sites and lower at Flax Pond and Southampton sites.

$R = 0.67$, $p < 0.01$; SH: $R = 0.50$, $p < 0.001$). Shannon diversity indices of DNA-based plastisphere microbiome profiles were higher on average at the Eastern Sound Station and Maritime sites compared to Flax Pond and Southampton sites (Welch's t -test: $t = 10.17$, $df = 83$, $p < 0.001$) (Figure 2B). Taxon richness and evenness showed similar trends (Figure S2). Taxon plots at the class level reveal more pronounced community differences between sampling sites than between substrate types (Figure S3). The most notable differences include a higher proportion of phototrophic microbes (i.e., *Oxyphotobacteria*) in surrounding seawater samples compared to biofilm samples, and higher proportions of nitrogen-cycling microbes (e.g., *Nitrososphaeria*, *Nitrospina* and *Nitrospira*) on MPs at SUNY Maritime College site compared to glass bead controls and MPs at other sites.

DNA-based fungal profiles (for ESS site only) were also distinct among substrate types ($R = 0.616$, $p < 0.001$), with PC and seawater samples being the most distinct from other sample types (Figure S4). Shannon diversity indices of fungal DNA were highest on PE samples. Taxon plots at the class level reveal more fungal community diversity in biofilms than in seawater samples (Figure S5). *Agaricomycetes* and *Sordariomycetes* were more abundant in biofilms, including glass substrates, whereas some of the less abundant colonisers (e.g., *Exobasidiomycetes*, *Walleriomycetes*, *Mortierellomycetes* and *Pucciniomycetes*) were specific to plastic biofilms.

Indicator species analysis revealed which taxa among sample groups were indicators for specific plastic polymer types based on extracted DNA (Table 1). At the genus level, indicator taxa for PE were *Alcanivorax* sp., *Desulfotalea* and *Maritimibacter*. The only indicator

taxon for PP was *Sedimenticola*. Indicator taxa for PVC were *Marinagarivorans*, *Aestuariicella*, *Oleiphilus*, *Porticoccaceae* gen. *C1-B045* and *Hyphomonas*. A multi-group indicator taxon for PE and PC was *Fusibacter*. A multi-group indicator taxon for PS, PET and PVC was *Lacinutrix*. A multi-group indicator taxon for PC, PET and PVC was *Methylothera*.

Niche breadth analysis determined the proportions of 'generalist' and 'specialist' taxa found at each sampling location and from each sample type based on extracted DNA (Figure 3). High niche breadth values suggest a more 'generalist' taxon that were commonly found among all samples, whereas low niche breadth values imply a more 'specialised' taxon restricted to a certain location or sample type. SUNY Maritime samples as a whole had the highest proportion of 'specialists' compared to other sites (30% more specialists than ESS, Flax and SH), indicated by the broad peak across all MAR samples. The most 'specialists' among MAR samples were observed within PE biofilm communities. Specialists were also common in PVC samples from the FP, MAR and SH sites. In addition, all sites except ESS had higher proportions of 'specialists' on plastic polymers than on glass. At the ESS site, proportions of 'specialists' on plastic and glass were indistinguishable.

Strong temporal succession of plastisphere community members at the most urbanised sampling site

DNA-based community composition of biofilms on PE and glass beads at the SUNY Maritime site varied significantly over time (ANOSIM test: $R = 0.66$, $p < 0.001$) and between PE and glass substrates ($R = 0.16$, $p < 0.01$)

TABLE 1 Indicator species analysis (indic.species test) based on extracted DNA shows taxa that are indicators for certain plastic polymer types.

Polymer	Taxa	R	p	% Abund ^a	Sites
PE	<i>Alcanivorax</i>	0.63	<0.05	0.3–10.2	MAR, SH
	<i>Desulfotalea</i>	0.57	<0.05	0.3	MAR
	<i>Maritimibacter</i>	0.52	<0.05	0.5	MAR
PP	<i>Sedimenticola</i>	0.47	<0.05	0.4–1.3	ESS, SH
PVC	<i>Marinagarivorans</i>	0.94	<0.001	4.5–16	FP, MAR, SH
	<i>Aestuariicella</i>	0.77	<0.001	0.1–1.8	FP, MAR, SH
	<i>Oleiphilus</i>	0.77	<0.001	0.2–5.5	FP, MAR
	<i>C1-B045</i>	0.66	<0.01	1–5.5	FP, MAR
	<i>Hyphomonas</i>	0.61	<0.01	0.2–0.9	FP, MAR, SH
PE, PC	<i>Fusibacter</i>	0.56	<0.05	0.1–2.7	MAR, SH
PS, PET, PVC	<i>Lacinutrix</i>	0.55	<0.05	0.2–2.9	ESS, FP, SH
PC, PET, PVC	<i>Methylothera</i>	0.76	<0.001	0.2–3.6	ESS, FP, MAR, SH

Abbreviations: ESS, Eastern sound station; FP, Flax pond; MAR, Maritime College; PC, polycarbonate; PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene; PVC, polyvinyl chloride; SH, Southampton.

^a% Abundance of a specific ASV DNA reads within a sample library.

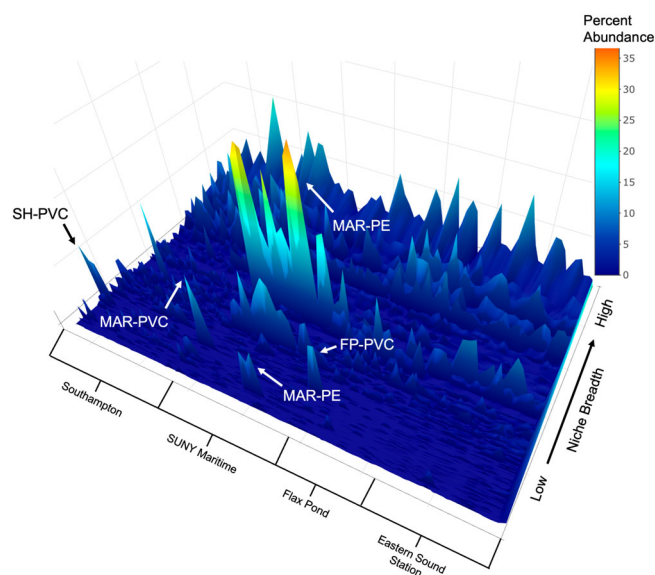


FIGURE 3 Niche breadth analysis of microbial community profiles from DNA-based 16S rRNA gene abundance among all microplastic samples and sites. High niche breadth values suggest more ‘generalist’ taxa commonly found among all samples. Whereas, low niche breadth values suggest more ‘specialised’ taxa that is rare among other samples. SUNY Maritime plastic polymer samples contained the highest proportion of ‘specialists’ among all biofilms and all sites. No ‘specialists’ peaks were found on any glass biofilms across all sites. The highest ‘specialists’ peaks were found on polyethylene samples at the MAR site and on polyvinyl chloride samples at FP, MAR and SH sites.

(Figure 4A and Figure S6). In contrast, planktonic communities at this site did not change significantly over our time course observations. The largest difference in community composition between PE and glass beads was observed in weeks 1 and 3. Shannon diversity values of glass and PE were indistinguishable from surrounding seawater in week 1 but diverged over time (Figure 4B and Figure S7). Comparisons of plastic community

composition at weeks 1 and 8 revealed that proportions of *Bacteroidia* and *Alphaproteobacteria* in PE biofilms decreased, while proportions of *Gammaproteobacteria*, *Nitrososphaeria* and *Nitrospina* increased (Figure S8). A closer look at genus-level relative abundances in polyethylene and glass biofilm communities reveals plastic selectivity across successional timescales (Figure S9). After 1 week, *Oxyphotobacteria* and *Saprospiraceae* reads had higher proportional abundances on PE than on glass, 4.2% and 2.9% higher, respectively. At 2 weeks, *Flavirhabdus* (+3.2%) and *Saprospiraceae* (+2.5%) reads exhibited the highest enrichment and selectivity toward PE. At 3 weeks, PE selectivity was dominated by *Alcanivorax* (+5.9%), *Saprospiraceae* (+5.8%) and *Flavirhabdus* (+2.7%) reads. At 4 weeks, PE selectivity was most evident in *Alcanivorax* (+6.3%) reads. At 8 weeks, PE selectivity was dominated by *Alcanivorax* (+7.5%) and *Nitrosopumilus Archaea* (+12.3%) reads.

Image analysis of successional biofilm formation was also conducted on glass and PE ‘slides’ that were deployed alongside the PE and glass bead samples at SUNY Maritime site (Figure S10). Biofilm coverage on both surfaces was not visible to the eye after 1 week, followed by more extensive coverage on glass (~50%) than on PE (~7%) after 2 weeks (Figure S11). PE biofilm coverage increased after 3 weeks (~30%) and eventually became greater than glass after 8 weeks (PE: 85%, GL: ~75%).

Hydrocarbon degraders selectively colonise polyethylene and polyvinyl chloride, particularly at urbanised sites

High read abundances for hydrocarbonoclastic bacteria (obligate hydrocarbon degraders: *Alcanivorax*, *Oleiphilus*, *C1-B045* *Porticoccaceae* gen. and *Aestuariicella*)

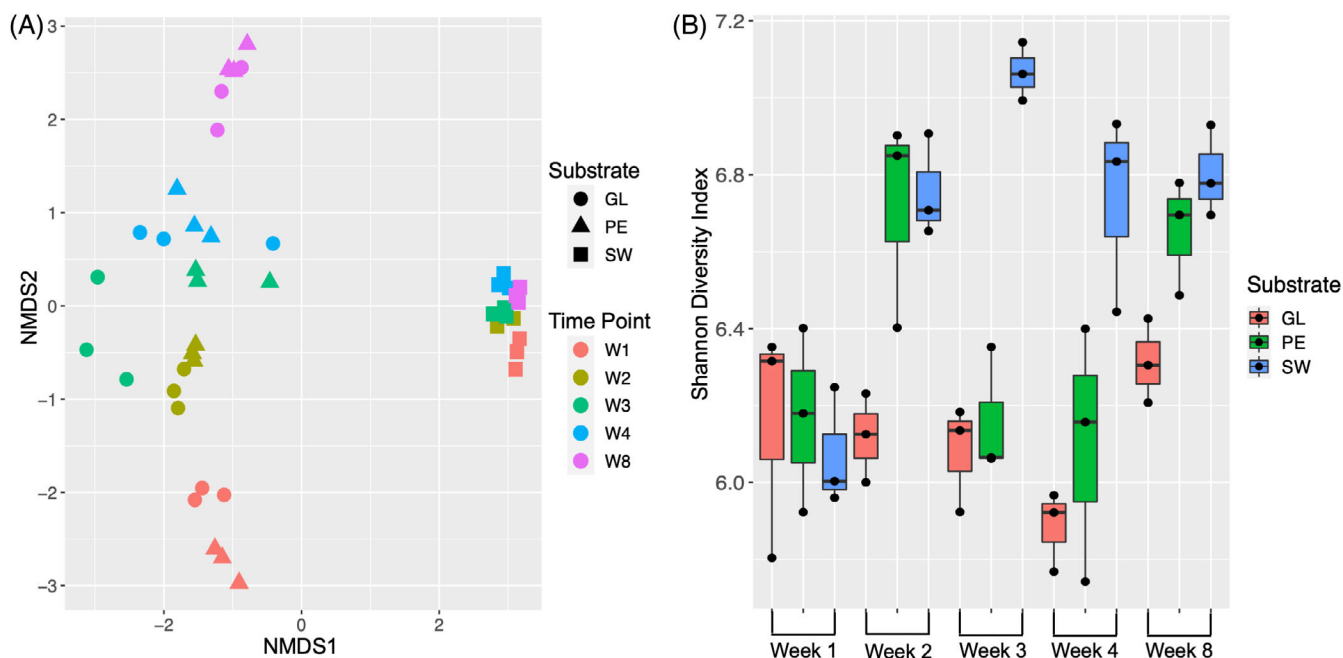


FIGURE 4 (A) Non-metric multidimensional scaling (NMDS) plot of DNA-based microbial community succession from 16S rRNA gene abundances recovered from polyethylene, glass and seawater at SUNY Maritime site over 8 weeks. Polyethylene and glass biofilm communities varied significantly through time (ANOSIM test: $R = 0.66$, $p < 0.001$) and between substrates ($R = 0.16$, $p < 0.01$), while seawater communities remained relatively constant over time. (B) DNA-based Shannon diversity of glass, polyethylene and seawater samples over 8 weeks at SUNY Maritime site.



FIGURE 5 Relative abundances of hydrocarbonoclastic bacterial taxa (obligate hydrocarbon degraders) found on each sample type from extracted DNA superimposed on a sampling site map. Highest abundance of hydrocarbon degrader reads was found at SUNY Maritime site and second highest abundance was found at Flax pond site.

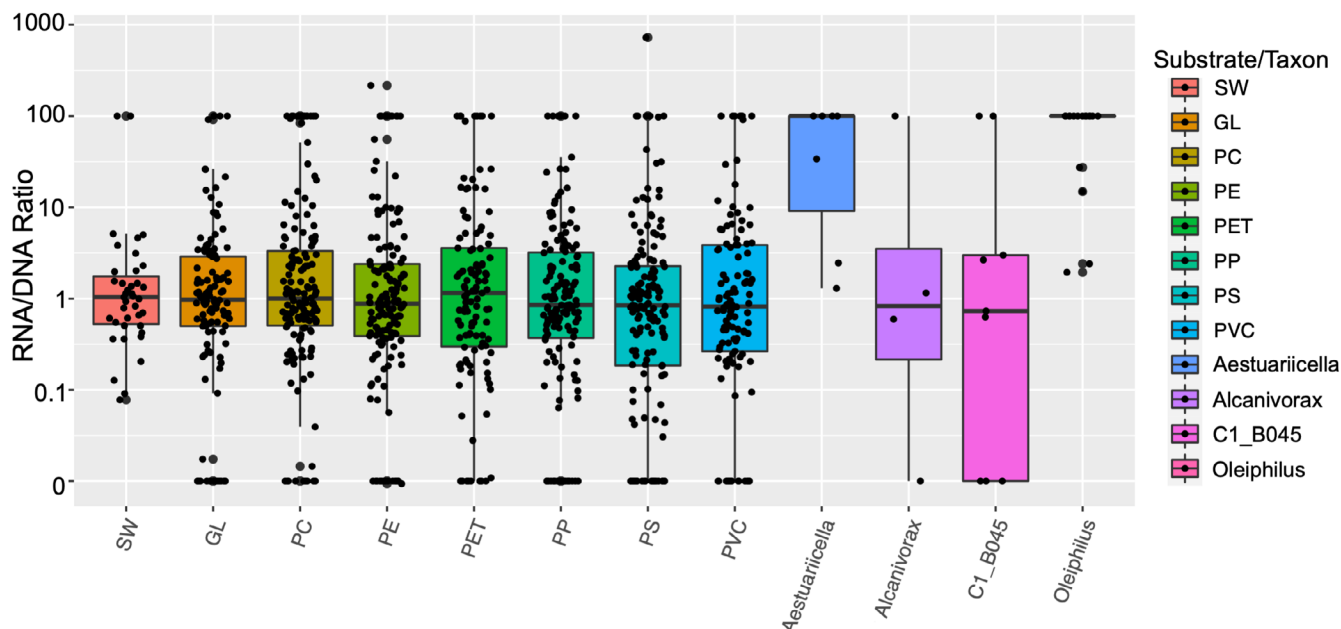


FIGURE 6 RNA:DNA ratios illustrating community activity potentials across all substrate types and suspected plastic-degrading taxa. Median RNA:DNA ratios were high (≥ 1) in all substrate types with many ‘phantom taxa’ present. *Aestuariicella* and *Oleiphilus* had the highest RNA:DNA ratios among putative plastic-degrading taxa.

were repeatedly and exclusively recovered from extracted DNA for PE and PVC polymers (Figure 5). Their relative read abundances were highest at the SUNY Maritime site and next highest at Flax Pond site (60 km east). *Alcanivorax* reads were most highly represented on PE at the MAR site (9.1% of total reads), followed by *Oleiphilus* (4.3%), *C1-B045 Porticoccaceae gen.* (3.1%) and *Aestuariicella* (1.5%) on PVC at MAR site. At Flax Pond, relative abundances of *Oleiphilus* and *C1-B045 Porticoccaceae gen* on PVC were 1.6% and 1.5% of total sample reads, respectively. No reads for hydrocarbonoclastic taxa were recovered from PE at FP site. Hydrocarbon-degrading taxa recovered from SH and ESS sites were $\leq 0.5\%$ relative abundance.

Plastisphere communities have high proportions of active cells across all polymer types

Plastisphere community profiles generated from extracted RNA were used to calculate ratios of RNA-based 16S rRNA gene abundance to DNA-based 16S rRNA gene abundance and compare putative community activity among plastic polymer types (Figure 6). Average RNA:DNA ratios were high (≥ 1) among all substrate types. Hydrocarbon-degrading taxa exhibited high RNA:DNA ratios and many ‘phantom taxa’ (i.e., ASVs detected by RNA reads but absent in DNA profiles), with *Aestuariicella* and *Oleiphilus* having the highest ratios across all samples. Overall, ASV

abundance profiles based on RNA were distinct from those based on DNA (ANOSIM test: $R = 0.16$, $p < 0.001$) (Figure S12). RNA community profiles also differed significantly among substrates ($R = 0.14$, $p < 0.001$) and sites ($R = 0.56$, $p < 0.001$) (Figure S13).

Enrichment cultures can sustain natural plastisphere communities

Enrichment cultures inoculated with naturally colonised MPs revealed that all plastic polymers sustained significantly higher prokaryote and eukaryote cell abundances over time compared to glass (up to 20 $\times$ higher; t -test: $t = 10.6$, $df = 65$, $p < 0.05$) (Figure 7). Prokaryote cell abundances on average were highest in PE cultures across both natural and artificial media types, while eukaryote abundances were highest in PC and PP cultures. Prokaryote and eukaryote cell abundances across all cultures further exhibited cyclical patterns of increasing and decreasing abundance.

DISCUSSION

A total of 181 environmentally deployed MP and glass bead samples were examined to determine plastisphere community variations along an urban coastal contaminant gradient within receiving waters of New York City and Long Island, New York, USA. Community composition of biofilms from sampling sites near New

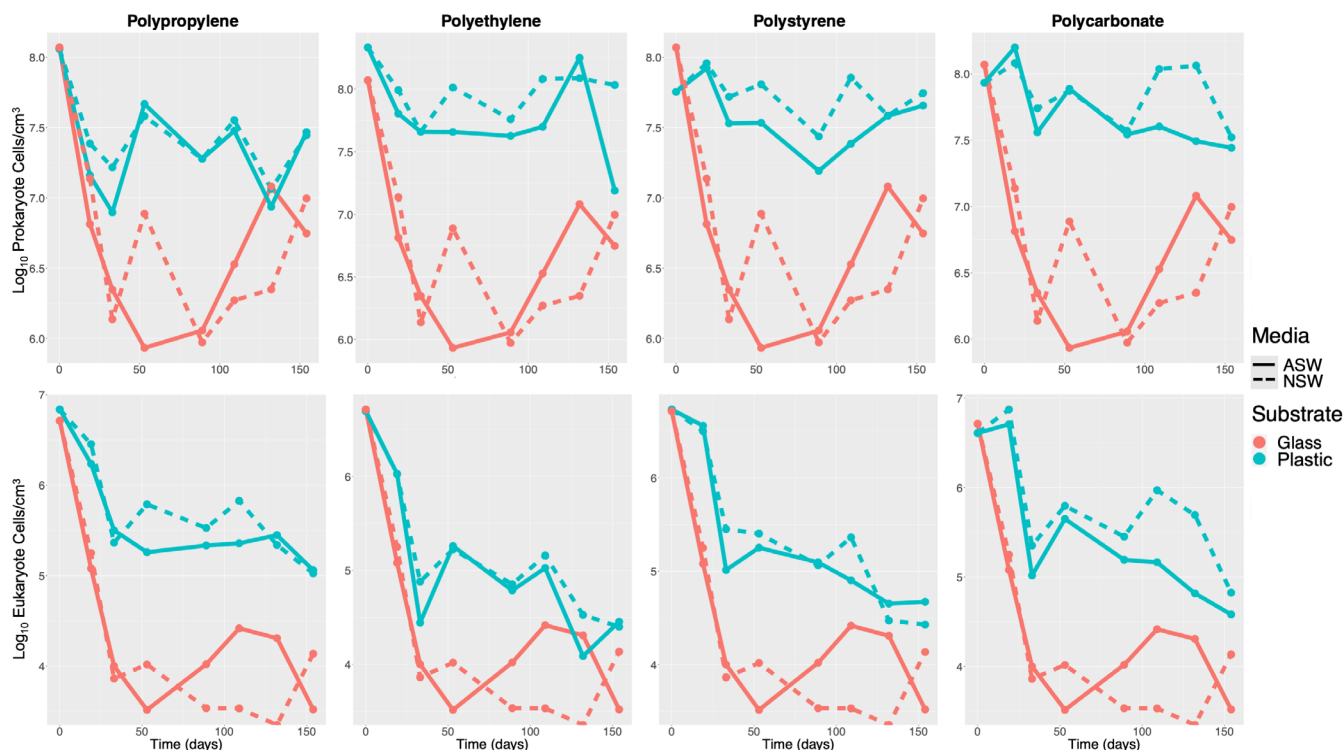


FIGURE 7 Prokaryote and eukaryote cell abundance dynamics in enrichment cultures of microplastics and glass bead controls colonised at the ESS site. Labile organic substrate concentrations in culture media were continually reduced over time to promote growth of plastic-specific microorganisms. Cells were counted using epifluorescence microscopy. Cell abundance reported as cells/cm³ due to greater feasibility of measuring weight of colonised substrates and utilising known density rather than measuring surface area. All plastic polymers enrichments sustained a significantly higher cell abundances over time than glass beads.

York City were distinct from those from less urbanised sites, populated by higher relative abundances of hydrocarbon-degrading taxa. These urbanised non-point sources of high plastic discharge to aquatic microbial communities could lead to increased selection for microbial phenotypes capable of plastic degradation and likely derived from hydrocarbon degradation genotypes. Subsequent dispersal of urban-sourced MP communities may influence genetic selection pathways within receiving marine ecosystems and influence regional biogeochemical cycling. For example, MP particles may provide novel surface environments for nutrient cycling and gene exchange. Dispersal of these MP microbiomes can inevitably lead to altered community compositions reflecting changes in surrounding abiotic conditions (Song et al., 2022). Nonetheless, transport of novel lineages and genotypes remain important considerations for understanding microbial adaptations to these xenobiotic substrates. Dispersal potential of these MPs is likely controlled by differences in polymer-specific density, with PE and PP being most buoyant (density = 0.95 and 0.9 g cm⁻³, respectively) and therefore most prone to transport via surface currents. However, density of buoyant plastics inevitably increases temporally as microbiofouling matures (Amaral-Zettler et al., 2021; Kaiser et al., 2017), which

can lead to sedimentation offshore and subsequent disruption of deep-sea ecosystems. PS, PC, PET and PVC all have higher densities (1.05, 1.2, 1.38 and 1.4 g cm⁻³, respectively) than seawater and thus lower surface water dispersal potentials and higher sedimentation potentials near their initial source. The differences in polymer-specific biofilm assemblages reported herein could therefore lead to significantly different ecosystem impacts following downstream dispersal and sedimentation. Further understanding of the advection and vertical transport of these plastisphere communities and the accompanying adaptive phenotypes could help to predict the impact of hydrocarbon-degrading organisms on global ocean plastic carbon budgets.

The urban contaminant gradient characterising our study system resulted in high representation of obligate hydrocarbon degraders at the most urbanised study site. *Alcanivorax* reads were highly represented in both DNA-based and RNA-based community profiles for PE beads at the MAR site, supporting the hypothesis that more urbanised sites harbour plastisphere communities with high phylogenetic selection. Interestingly, *Alcanivorax* relative read abundance in seawater controls at the MAR site was not the highest among sites (0.83% and 1.42% relative abundances in MAR and SH

seawater, respectively). In contrast, *Alcanivorax* read abundance on PE at the MAR site was 100x higher than that of the SH site. This suggests that taxon abundances in source water does not limit hydrocarbon degrader colonisation rates. Other obligate hydrocarbon degraders, *Oleiphilus* (Yakimov et al., 2022) and *C1-B045 Porticoccaceae* (Zhou et al., 2022), were moderately abundant indicators for PVC beads at both the MAR and FP sites, indicating that plastisphere community ‘specialisation’ can also occur at locations downstream from pollution epicentres. Away from urban receiving waters, the relative read abundance of both taxa decreased (4.3% and 3.1% relative abundances of *Oleiphilus* and *C1-B045 Porticoccaceae*, respectively, at MAR site; 1.6% and 1.5% relative abundances of *Oleiphilus* and *C1-B045 Porticoccaceae*, respectively, at FP site). A fourth obligate hydrocarbon degrader, *Aestuariicella* (Muthukrishnan et al., 2019), was found at moderate relative read abundances only at the MAR site and may be specific to chronically contaminated sites. These four hydrocarbon-degrading taxa also had high average ratios of RNA-based 16S rRNA gene abundance to DNA-based 16S rRNA gene abundance, indicating that they are active and important members of plastisphere communities. Our results show that proximity to urbanised coastal systems and polymer type both likely exert an important selective pressure on biofilm community structure, favouring colonisation of hydrocarbon-degrading taxa.

Temporal changes in plastisphere community composition throughout biofilm maturation further revealed that *Alcanivorax* reads increased in relative abundance on PE substrates throughout the 8-week time course, reinforcing the role of hydrocarbon degrader colonisation throughout plastic biofilm development. The hydrocarbon degrader *Oleiphilus* may be a ‘pioneer’ coloniser for plastic surfaces, as this taxon was observed only in week 1 samples. Additional investigations into the functional roles of *Oleiphilus* and other early pioneer hydrocarbonoclastic colonisers across different polymer types could provide insight into its early demise within the community. Other highly represented PE-specific community members throughout the time course include members of the *Saprospiraceae* family, known for their ability to degrade complex organic carbon sources (McIlroy & Nielsen, 2014), and *Nitrosopumilus*, an ammonia-oxidising archaea (Schouten et al., 2008). The significant prevalence of ammonia oxidizers on PE suggests that plastisphere communities could have a role in overall nitrogen cycling at this study site. Interestingly, more community similarity between PE and glass substrates was observed at early and late successional time points, and less similarity at intermediate time points. This suggests that early colonisers are less selective of substrate composition but may become more substrate-specific with each passing week. This could also be

influenced by progressive abiotic weathering of the plastic surface (i.e., UV degradation) over time, which is known to release leachates such as alkanes with higher bioavailability than long-chain polymers (Wu et al., 2023; Zadjelovic et al., 2022). The rebound in community similarity observed between PE and glass at later time points could be due to generalist colonisers dominating the biofilm’s outer layer over time. Visual observations of temporal succession further illustrate that PE biofilm formation may be slower than glass, but may also exhibit higher coverage over time. These temporal data provide a spectrum of successional characteristics for plastisphere microbiomes that could serve as phylogenetic references for plastispheres in other similar ecosystems.

The overall polymer-specific variations in community composition show that substrate composition is an important factor for plastisphere community assembly in urbanised coastal systems. Interestingly, polymer type had the least significant effect on community composition at the SH site, which had the lowest salinity (26 PSU) and is historically characterised by high nutrient loadings (Gobler et al., 2008). Meanwhile, MAR site samples exhibited the most significant effect of polymer type on community composition despite having high nutrient loads and high salinity (Li et al., 2018; Taillie et al., 2020). In general, polymer-specific influence on plastisphere community composition across previous studies is inconsistent. These inconsistencies highlight the importance of experimental design and ecosystem context in interpreting impacts of marine plastisphere communities on regional ecosystem function. For example, Ogonowski et al. (2018) found lower community evenness and diversity among biofilms forming on common polymer types compared to seawater controls. In contrast, our analyses revealed higher community evenness and Shannon diversity on MPs than in surrounding planktonic communities, suggesting diverse molecular compounds of interest on MP surfaces. It is important to note that Ogonowski et al. (2018) used 16S rRNA primers targeting the V3-V4 region (341F/805R), which likely underestimates community diversity (Yang et al., 2016; Pollet et al., 2018). Nonetheless, higher plastisphere community diversity and evenness as reported here are often positively associated with high stability and productivity of microbial communities (Haig et al., 2015; Schmidt et al., 2020; Wittebolle et al., 2009). These community characteristics are not unique to plastic surfaces, as surface biofilms in aquatic environments have repeatedly shown higher community diversity and productivity than their planktonic neighbouring communities (Dang & Lovell, 2016; Pepe-Ranney & Hall, 2015). However, the comparable diversity indices on glass and plastic substrates observed in this study suggests that surface biofilms in urbanised coastal systems are not significantly hindered by plastic polymer substrates and are

likely to be stable and biogeochemically productive environments.

Plastisphere communities were generally taxonomically distinct from surrounding planktonic communities, reinforcing the postulate that marine plastics offer distinct niches (Dussud et al., 2018; Yang et al., 2020). For instance, the proportions of bacterial phototrophs were lower on plastic surfaces compared to surrounding seawater suggesting increased prevalence of net heterotrophy on marine plastic surface environments or less adaptive ability for phototrophs to live in plastic biofilms. Although the reduced sunlight penetration caused by the presence of outer mesh bags may have contributed to this difference in phototroph abundances. Seawater communities also consistently held higher proportions of *Alphaproteobacteria* and lower proportions of *Gammaproteobacteria* compared to plastisphere communities. Meanwhile, fungi exhibited distinct representation in plastisphere communities, being enriched in *Agaricomycetes* and *Sordariomycetes* compared to surrounding seawater. Fungal colonisers were most diverse on PE, from which we postulate that this polymer has higher fungal community productivity than other tested polymers. Functional potential varied among sites as well, with plastisphere communities at the MAR site comprised of ~25% nitrogen cyclers (i.e., *Nitrososphaeria*—ammonia-oxidising archaea, *Nitrospina* and *Nitrospira*—nitrite-oxidising bacteria). This observation is consistent with high nitrogen loadings at this site which lies within urban sewage receiving waters where exceptional NH_4^+ and NO_x concentrations have long been reported (Anderson & Taylor, 2001; Suter et al., 2014). Levin's niche breadth analysis revealed that MAR site community members as a group were generally more 'specialised' among all MP polymer types and unique from other sampling sites. This suggests that the MAR site supports a unique source community of microbes that may favour colonising MP substrates more than microplankton from the other three sites, presumably due to contaminant loadings. Polymer-specific 'specialists' were also found exclusively within PE and PVC communities, suggesting that biofilms on these polymers may exert stronger selective pressure than other polymer types tested in this study. The observed spatial and polymer selectivity could have important implications for localised biogeochemical cycling in this urbanised estuarine system.

The location- and polymer-specific effects on plastisphere community composition reported herein are strengthened by RNA and culture-based data. RNA results support marine plastic surfaces as productive micro-environments, as all polymer types on average had high ratios (≥ 1) of RNA-based 16S rRNA gene abundance to DNA-based 16S rRNA gene abundance. Similar to DNA profiles, RNA-derived profiles were significantly different among polymer types and sampling

locations, which further supports the hypothesis that plastisphere assemblies are controlled by location- and polymer-specific factors. However, RNA-derived community profiles as a whole diverged from DNA-derived profiles across all samples, suggesting that biogeochemical cycling within plastispheres may not reflect DNA-based taxon representation. This is highlighted by the common occurrence of 'phantom taxa' (where $\text{RNA:DNA} = \infty$) among all samples. Future DNA-based plastisphere studies should therefore take this into account when predicting functional potential. The naturally colonised plastisphere communities were additionally maintained in controlled laboratory culture environments across a wide range of polymer types. Results show that even in a laboratory setting, all plastic polymer types supported higher prokaryote and eukaryote cell abundances in their biofilms than glass surfaces. In addition, oscillations in prokaryote and eukaryote cell abundance are probably indicative of grazing by bacterivorous protists within cultures and warrant future investigation. Our data suggest that plastisphere microbiomes can be sustained for long periods (>5 months) in a culture setting that provides no organic carbon supplement and potentially selects for plastic-specialised microorganisms.

Previous studies have suggested plastic-specific community members to be relatively rare (<0.1% relative abundance within the community; Kirstein et al., 2019). Results from this study present new evidence for plastisphere 'specialists' comprising up to 10% of the biofilm community in highly urbanised ecosystems. PE and PVC polymers clearly select for hydrocarbon-degrading microbes in urban receiving waters, from which we infer that those polymers are more bioavailable than other polymers tested in this study. This study shows that plastics introduced to the environment are colonised by distinct microbial communities that vary phylogenetically across contaminant levels, and are strongly influenced by polymer type and exposure times. These significant differences in microbiome composition were observed across inferred cell activities and all domains of microbial life. Microorganisms have thus shown adaptive abilities to utilise novel xenobiotic substrates in the environment and fill vacant ecological niches within a short geologic time period (the Anthropocene). Differences observed between DNA-based 16S rRNA gene abundance and RNA-based 16S rRNA gene abundance profiles among all samples highlight the utility of including RNA surveys in future marine plastic studies as a benchmark for relating phylogeny and actively growing plastic biofilm community members. The clear prevalence of hydrocarbon-degrading taxa at the most urbanised site supports the hypothesis that geographic regions with high plastic contaminant loadings select for hydrocarbon-degrading phenotypes that may also be capable of plastic degradation. Differences in

plastisphere microbiomes among polymer types suggest differential ecosystem impacts that are influenced by polymer density and dispersal potential. Observed phylogenetic differences among locations and polymer types indicate that upstream plastic discharge source, dispersal potential and carbon bioavailability must all be carefully integrated into forecasts of plastic pollutants' fate and transport, as well as their subsequent impacts on global ocean plastic carbon budgets and ecosystem function.

AUTHOR CONTRIBUTIONS

Cody E. Garrison: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; project administration; data curation; supervision; resources. **Maria G. Pachiadaki:** Conceptualization; supervision; resources; project administration; writing – review and editing; funding acquisition. **Sammer Soliman:** Investigation; writing – review and editing; methodology. **Anthony Helfrich:** Investigation; writing – review and editing. **Gordon T. Taylor:** Conceptualization; funding acquisition; writing – review and editing; methodology; project administration; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequence data can be found under the NCBI Sequence Read Archive BioProject accession PRJNA1002351: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1002351>. All data processing and analysis pipelines are available at https://github.com/cgarr017/Microbes_Microplastics.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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