The Marine Plastisphere: Microbial Colonization of Polymer Surfaces and its Role in

Microplastic Degradation and Deposition

by

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ABSTRACT

There is an estimated five trillion pieces of plastic in the global ocean, with 4.8 to

12.7 million metric tons entering the ocean annually. Much of the plastic in the ocean is

in the form of microplastics, or plastic particles <5mm in size. Microplastics enter the

marine environment as primary or secondary microplastics; primary microplastics are

pre-manufactured micro-sized particles, such as microbeads used in cosmetics, while

secondary microplastics form from the degradation of larger plastic objects, such water

bottles. Once in the ocean, plastics are readily colonized by a consortium of prokaryotic

and eukaryotic organisms, which form dense biofilms on the plastic; this biofilm is

termed the "plastisphere". Despite growing concerns about the ecological impact of

microplastics and their respective plastispheres on the marine environment, there is little

consensus about the factors that shape the plastisphere on environmentally relevant

secondary microplastics. The goal of my dissertation is to comprehensively analyze the

role of plastic polymer type, incubation time, and geographic location on shaping

plastisphere communities attached to secondary microplastics. I investigated the

plastisphere of six chemically distinct plastic polymer types obtained from common

household consumer products that were incubated in the coastal Caribbean (Bocas del

Toro, Panama) and coastal Pacific (San Diego, CA) oceans. Genotyping using 16S and

18S rRNA gene amplification and next-generation Illumina sequencing was employed to

identify bacterial and eukaryotic communities on the polymer surfaces. Statistical

analyses show that there were no polymer-specific assemblages for prokaryotes or

eukaryotes, but rather a microbial core community that was shared among plastic types. I

also found that rare hydrocarbon degrading bacteria may be specific to certain chemical properties of the microplastics. Statistical comparisons of the communities across both sites showed that prokaryotic plastispheres were shaped primarily by incubation time and geographic location. Finally, I assessed the impact of biofilms on microplastic degradation and deposition and conclude that biofilms enhance microplastic sinking of negatively buoyant particles and reduce microplastic degradation. The results of my dissertation increases understanding of the factors that shape the plastisphere and how these communities ultimately determine the fate of microplastics in the marine environment.

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INTRODUCTION

1.1 Microplastics in the marine environment and the plastisphere

It is estimated that there are roughly five trillion pieces of plastic in the global ocean, with 4.8 to 12.7 million metric tons entering the ocean annually (Eriksen et al., 2014; Jambeck et al., 2015). The majority of plastics in the ocean are microplastic, or particles < 5 mm in diameter (Hidalgo-Ruz et al., 2012, Goldstein et al., 2013). These microplastics can either be classified as primary microplastics, which are produced to be intentionally micro-sized (i.e., microbeads), or secondary microplastics, which form from the degradation of larger plastic pieces through mechanisms such as mechanical degradation (i.e., wave action), UV degradation, and microbial degradation. Like any substrate in the ocean, plastics act as a novel source for bacterial and protistan colonization, forming what researchers have termed the "plastisphere" (Zettler et al. 2013). A growing body of research has focused on determining the factors that shape plastispheres and have found plastisphere divergence as a result of plastic polymer type (Oberbeckmann et al., 2014, 2018; Amaral-Zettler et al., 2015, Eich et al. 2015; Debroas et al., 2017), geography (Amaral-Zettler et al., 2015), and seasonality (Oberbeckmann et al. 2014). In controlled plastic incubation studies, significant differences were found between the bacterial assemblages associated with polystyrene (PS) and high-density polyethylene (HDPE), but only in low nutrient environments in the North Sea (Oberbeckmann et al., 2018), and not for their respective eukaryotic communities (Kettner et al., 2019). In similar reports of eukaryotic assemblages within the plastisphere

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Kirstein et al., (2018) could not conclude polymer specificity in eukaryotes. Using light

microscopy, however, Eich et al. (2015) found certain diatom taxa to preferentially

colonize HDPE or a biopolymer-polyethylene terephthalate ("biodegradable") in the

Mediterranean Sea after 33 days, but not 15 days. These observations support the

complex interplay of the factors involved in shaping plastisphere communities (reviewed

by Jacquin et al., 2019), and the importance of understanding the colonization of new

plastics in the environment over time.

1.2 Hydrophobic Organic Contaminant Sorption to Microplastics

Other extrinsic factors that interplay with biofilm formation onto microplastics are

the sorption of hydrophobic organic contaminants (HOCs), such as polycyclic aromatic

hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, etc. The partitioning

of HOCs between plastic debris and the water column may be affected by biofilms due to

a biofilm's sorptive properties (independent of the plastic particle) as well as a biofilm's

ability to metabolize HOCs (Wolfaardt et al., 1994; Headley et al., 1998; Writer et al,.

2011; Ding et al., 2015). Chemical sorption onto microplastics depends largely on the

polymer/water partition ratios of any given molecule, which can be approximated by the

octanol/water partition ratios (K_{OW}) (Ziccardi et al., 2016), and may also depend on

plastic polymer type. Rochman et al. (2013) showed that PAHs and PCBs, polyethylene

terephthalate (PETE) and polyvinyl chloride (PVC) reach equilibrium in the marine

environment much faster than HDPE, low-density polyethylene (LDPE), and

polypropylene (PP). Moreover, concentrations of PAHs and PCBs sorbed to HDPE,

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LDPE, and PP were consistently much greater than concentrations sorbed to PETE and PVC. Additionally, chemicals with lighter molecular weight and smaller K_{OW} achieve saturation faster, possibly having a greater influence on the plastisphere communities. A wide range of bacteria, fungi, and algae are capable of utilizing HOCs as a carbon source (Ghosal et al., 2016), which is why they can be used for bioremediation of surface waters *in situ* or in engineered bioreactors (Demeter et al., 2017; Wu et al., 2014). This demonstrates the high relevance of biofilms for the accumulation and/or removal via metabolization of plastic-associated chemicals (Writer et al., 2011), or even the plastic polymer itself (Andrady, 1994), which may affect their bioavailability for consumers ingesting microplastics. Ingestion of microplastic particles, with or without additional HOCs, has been shown to induce immune-toxicological responses, alter gene expression, lower reproductive abilities, and cause cell death (Lithner et al.; 2011). Despite known impacts of plastic on higher organisms, much less is known about the interactions between marine microbiota and microplastics, and how those interactions influence microplastic degradation and sinking, leading to the removal of microplastics from the surface ocean.

1.2.1 Polycyclic Aromatic Hydrocarbons

PAHs are a particularly important class of HOCs that, due to their low water solubility, toxicity, and potential to bioaccumulate up the food web, are recognized both by the Environmental Protection Agency and United Nations Environment Programme as high-priority pollutants that can be carcinogenic to humans (Schoeny & Poirier, 1993).

PAHs enter and persist in the marine environment through a variety of methods that include biological sources (marine and terrestrial plants), anthropogenic sources (fluvial input, atmospheric fallout, surface runoff, and oil spills), and secretion at active tectonic zones (hydrothermal plumes, natural oil seeps, and volcanic eruption). The entry of PAHs into the marine environment immediately subjects them to abiotic and biotic degradation processes and often leads to the enrichment of indigenous populations of marine bacteria that can break down and utilize these compounds. In addition to oil-impacted sites, PAH-degraders have been isolated during enrichment experiments and even cultures of marine phytoplankton (Gutierrez et al., 2012). The organisms described in Gutierrez et al. (2012) represent novel "specialist" hydrocarbon degraders, i.e., they exhibit an almost exclusive requirement for hydrocarbons as an energy source, yet, interestingly, they are not well represented in genomic libraries, even from highly PAH-polluted environments, such as oil-impacted sites. This conundrum could be due to the possibility that they inhabit an explicit and relatively unexplored niche, i.e., the cell surface of eukaryotic phytoplankton.

1.2.2 Eukaryotic phytoplankton and hydrocarbon-degrading bacteria

Eukaryotic phytoplankton, in particular diatoms, may function as an important habitat for hydrocarbon-degrading bacteria (Gutierrez et al., 2013), specifically PAH degrading bacteria (Mishamandani et al., 2016). This association may stem from the capacity of diatoms to accumulate PAHs on their cell surfaces (Binark et al., 2000), which would create a PAH-enriched zone around the phycosphere, a mucosal region around the cell rich in organic matter, and in turn attract PAH-degrading bacteria to

colonize this zone. The rationale for this association is further evidenced in a few studies

that present data correlating the influence of eukaryotic phytoplankton with the removal

of PAHs and other hydrocarbons from the marine water column (Binark et al., 2000;

Witt, 2002). Interestingly, it is being increasingly shown that PAH-degrading bacteria can

utilize the same metabolic pathways to degrade plastic polymers, such as *Arthrobacter*

sp. and *Alcanivorax sp.* (Urbanek et al., 2018, Balasubramanian et al., 2010). So, while

microplastics have an independent capacity to sorb PAHs, it is possible that diatom

colonization recruits hydrocarbon degrading bacteria to microplastics, and that

microplastics and PAHs, when interacting in consortium with a microplastic's biofilm,

may together create a hotspot for their respective degradation.

1.3 Degradation of Microplastics

       The fate of plastic debris in the marine environment is governed by degradation

processes as they influence the condition of the plastic material and its hydrodynamic

behavior (Ter Halle et al., 2016). Global problems with plastic waste are inherent to its

design: plastic is made to endure. The longevity of plastics is estimated to be hundreds or

even thousands of years depending on properties of the plastic polymer as well as the

surrounding environmental conditions (PlasticsEurope 2019). Although at a very slow

rate, environmental weathering still causes the breakdown of plastics through by abiotic

and biotic processes, and are described by their causative agent:

       1. Photodegradation – breakdown initiated by ultraviolet radiation from sunlight.

       2. Thermal degradation – heat-induced breakdown.

3. Mechanical degradation – breakdown due to the action of external forces.

4. Hydrolysis – breakdown in water.

5. Biodegradation – enzymatic breakdown by living organisms.

The focus here will be on the photodegradation and biodegradation as these will be the dominant processes in the marine environment.

1.3.1 Photodegradation of plastics

In any environment, photodegradation, particularly by UV-B (290-315 nm) and UV-A (315-400 nm) irradiation, is considered the most important processes that initiates the degradation of any plastic polymer (Zhang et al., 2021). Photooxidation may be divided into three main steps: (1) initiation [polymer-chain scission induced by ultraviolet (UV) light and formation of free radicals], (2) propagation (auto-oxidation), and (3) termination (formation of inert products, typically olefins, aldehydes, and ketones). The degradation mainly acts on the material surface that is exposed to UV light. As a result, the plastic surfaces may display a modified topography, an increase in surface roughness, and altered chemistry (e.g., becoming more hydrophilic because of the formation of carbonyl groups) (Andrady, 2015; Fotopoulou & Karapanagioti, 2015; Cooper & Corcoran, 2010; Feldman, 2002). These processes may favor the adhesion of microorganisms (Donlan, 2002) and the composition and structure of the microbial communities (Kerr & Cowling, 2003, Cazzaniga et al., 2015). In addition, successive fragmentation into smaller particles accompanied by an increased surface-to-volume ratio is an important prerequisite for biodegradation (Barnes et al., 2009; Lambert et al., 2017) and thus it is assumed that photodegradation must take place prior to biodegradation due

to the lack of bioavailability of most plastic polymers. However, certain plastic polymers are more or less resistant to photodegradation depending on their chemical structure.

Polyethylene terephthalate (PETE) is a polyester compound consisting of alternating ethylene glycolate and terephthalate subunits. This polymer is moderately susceptible to photo-oxidative attack as UV irradiation leads to the cleavage of those ester bonds directly. This leads to the formation of terephthalic acid, anhydrides, carboxylic acids, and other smaller compounds, allowing them to further be photodegraded (Fairbrother et al., 2019). Alternatively, these smaller compounds become more bioavailable for microbial utilization (Zhang et al., 2021).

Polyethylene (HDPE and LDPE) is generally resistant to photodegradation due to the lack of chromophores (pigment molecules), but the presence of impurities, such as carbonyl groups within the PE backbone or structural defects in polymers during manufacture or weathering can act as chromophores (Fairbrother et al., 2019).

Polyvinyl chloride (PVC) undergoes rapid dehydrochlorination under UV irradiation and generates short sequences of conjugated unsaturation in the polymer, causing instability and susceptibility for further photodegradation. Similarly, in HDPE and LDPE, the presence of chromophores in PVC due to impurities can absorb UV radiation and generate free radicals that can break the carbon backbone of PVC (Law 2016; Yang et al., 2018).

Polypropylene (PP) is less stable than HDPE and LDPE due to the presence of tertiary carbon, which is more susceptible to oxygen attack (Weber et al., 2011). The mechanisms in which photodegradation occurs on PP, however, are like those of PE and

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PVC in that the presence of chromophores in PP due to impurities allows the formation of radicals under UV radiation. These radicals can then break the carbon backbone of PP.

Finally, polystyrene (PS) is relatively susceptible to photodegradation due to the presence of phenyl rings, which get excited and form triplet state (an electronic state where electrons in different molecular orbitals have parallel spins) under UV radiation. This triplet energy can be transferred to the nearest C – H or C – C bond, and when the polymer is in the presence of oxygen, different radicals can be produced leading to chain scission and cross-linking to create individual styrene monomers, carbonyl compounds, and olefins (Zhang et al., 2021; Kumar et al., 2020; Dris et al., 2017).

1.3.2 Biodegradation of plastics

The role microbial biofilms play in the degradation process of plastics, and their potential for bioremediation in plastic pollution strategies is under recent investigation (Shah et al., 2008; Sánchez 2019; Pathak and Navneet 2017; Wu et al., 2019, Bahl et al., 2021). General processes for plastic degradation are shown in Fig. 1, though further degradation mechanisms have been detailed elsewhere (Debroas et al., 2017; Shah et al., 2008; Gu 2003). While many hydrocarbon-degrading, or plastic-degrading bacteria have been described (Shah et al., 2008; Bhardwaj et al., 2013; Kale et al., 2015; Pathak 2017, Jacquin et al., 2019), *Arthrobacter*, *Corynebacterium*, *Micrococcus*, *Pseudomonas*, *Rhodococcus*, and *Streptomyces*, were the prominent and consistent microbial taxa shown to utilize plastics as a sole carbon source in laboratory settings. Biodegradation can be

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summarized into 4 main steps, described in further detail in Dussud and Ghiglione (2014):

1. **Bio-deterioration** relates to the biofilm growing on the surface and proliferating inside the cracks and pores caused by photodegradation on plastic, which increases the pore size and provokes cracks to widen. This weakens the physical properties of the plastic. Additionally, biofilms can release acid compounds that modify the pH inside the pores and results in changes in the microstructure of the plastic matrix.

2. **Bio-fragmentation** corresponds to the action of extracellular enzymes, mostly oxygenases, lipases, esterases, and depolymerases, released by bacteria colonizing the polymer surface. These enzymes will reduce the molecular weight of polymers and release oligomers and then monomers that can be assimilated by cells.

3. **Assimilation** allows oligomers of less than 600 Daltons to be utilized by the cells as a carbon source, thus increasing the microbial biomass.

4. **Mineralization** is the final step in the biodegradation of a plastic polymer and results in the excretion of completely oxidized metabolites (CO_2, N_2, CH_4, and H_2O), further leading to an increase in microbial biomass. While this process can occur in anaerobic conditions, it takes a much longer time for complete mineralization (Gu 2003).

1.4 Deposition of Microplastics

As biofilms form on positively buoyant microplastics in the marine environment, they lose their buoyancy and eventually sink below the surface over time. Kaiser et al, (2017) demonstrated this in incubations of HDPE in the coastal Baltic Sea, though the HDPE microplastics only began to sink after mussel colonization. This may partially explain that only ~1% of the total plastic pollution estimated to be in the ocean is found on the surface (Jambeck et al., 2015; Lebreton et al., 2017). Biofilm formation may lead to an increase in the density of the particle and a decrease in its buoyancy, but is largely depended on polymer type (Lagarde et al., 2016), microplastic size, and ambient algal concentrations (Lobelle et al., 2021). Since the sinking rate of a microplastic is a function of particle size (surface area:volume ratio) and density; an increase in density above that of ambient water (1020-1029 kg/m^3) implies deposition and sedimentation. Furthermore, during biofilm formation, microplastic becomes sticky because of the EPS matrix exuded by both prokaryotes and eukaryotes within the plastisphere, which promotes the formation of hetero-aggregates, including microplastics, microbial communities, and detritus (Long et al., 2015), and other inorganic molecules such as iron (Lesier et al., 2020). Moreover, possible preferential ingestion of microplastic with well-developed biofilms may promote downward transport of microplastic particles incorporated into fecal pellets of zooplankton (Cole et al., 2016; Gorokhova et al., 2015; Kvale et al., 2020). Despite all the aforementioned ways for microplastics to potentially sink down the water column, it has been hypothesized that microplastics may actually be decreasing the efficiency of the biological carbon pump. The biological carbon pump is one of the main drivers of CO_2 sequestration in the ocean. Shen et al., (2020) showed that when fecal

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pellets are contaminated with microplastics, their equivalent spherical diameters significantly decrease, with a reduction in sinking rates by 1.35-fold. In addition, the fecal pellets contaminated by microplastics are more likely to be fragmented than uncontaminated pellets (Wieczorek et al., 2019). While I do not focus on zooplankton mediation in my studies, it is important nonetheless to keep these food-web mediated processes in mind when considering the ultimate fate of the microplastics in the ocean.

1.5 Dissertation Objectives

The overall goal of this study is to investigate the factors that contribute to shaping the plastisphere on microplastics of six different common plastic polymer types, and how those plastispheres ultimately control the fate of these microplastics in the marine water column. Specifically, I wanted to further investigate the role of plastic polymer type, incubation time, geographic location, and sorbed PAHs in plastisphere formation on PETE, HDPE, PVC, LDPE, PP, and PS. Finally, I wanted to investigate the role of these plastispheres on their respective plastics' capacity to degrade or sink in two different oceanic regions.

The specific results chapters of my dissertation are the following:

- **Chapter 1: Microbial colonization of microplastics in the Caribbean Sea.** In this study, I investigated the role of plastic polymer type and incubation time in shaping the prokaryotic and eukaryotic members of the plastisphere. My main goal was to determine the extent at which the chemical structure of the plastic substrate influenced its biofilm, or if microplastics followed the patterns of

general marine biofilm formation. I extracted the DNA of these plastispheres incubated over a time series in Bocas del Toro, Panama and used next-generation Illumina sequencing to determine community composition. In addition, I used scanning electron microscopy to visualize the plastics' surfaces and the attached organisms. This chapter is published in Limnology and Oceanography Letters (**https://doi.org/10.1002/lol2.10141**).

- **Chapter 2: Marine microplastic-associated bacterial communities are determined by exposure to the environment and geography, but not plastic type.** In this study, I used next generation Illumina Sequencing to determine the taxonomical composition of the plastisphere attached to the same plastic polymers studied in **Chapter 1**, but incubated in seawater sourced off the coast of San Diego, CA. The goal of this chapter was not only to determine if plastic polymer type, incubation time, or geographic location determines the composition of the plastisphere, but also to identify a "core plastisphere," or a bacterial community that unites the plastispheres. Finally, I determined PAH concentrations sorbed onto the microplastics and made inferences about their impact on members of the plastisphere.

Chapter 3: Biotic and abiotic factors in microplastic degradation and deposition. The goal of this chapter was to investigate the role of biofilms in microplastic degradation and deposition. I carried out incubations in the Caribbean and the Pacific, settings described in Ch. 2 and 3. I incubated the microplastics in both biotic and abiotic conditions and carried out scanning

electron microscopy to determine differences in physical degradation, and

measured phthalate concentrations as a chemical indicator of degradation.

Furthermore, I measured sinking velocities of the microplastics to determine the

ultimate fate of positively and negatively buoyant microplastics incubated in the

different treatments and different ambient conditions.

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CHAPTER 1 – MICROBIAL COLONIZATION OF MICROPLASTICS IN THE

CARIBBEAN SEA

The authors have acknowledged the use of this manuscript in my dissertation.

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2.1 Abstract

Microplastics in the ocean function as an artificial microbial reef, with diverse communities of eukaryotic and bacterial microbiota colonizing its surface. It is not well understood if these communities are specific for the type of microplastic on which they develop. Here, we carried out a 6-week long incubation experiment of six common plastic polymers in Bocas del Toro, Panama. The community composition of prokaryotes based on 16S rRNA gene sequencing data, when judged under a null model analysis, show that neither plastic polymer type nor time exposed to the environment play a significant role in shaping biofilm communities. However, the null model analyses of eukaryotic communities based on 18S rRNA gene sequences reveal that they can be significantly influenced by plastic polymer type and time incubated. This was confirmed by scanning electron microscopy, which allowed us to distinguish plastic-specific diatom communities by the end of the incubation period.

2.2 Introduction

Five trillion pieces of plastics are estimated in the global ocean, with 4.8 to 12.7 million metric tons entering the ocean annually (Eriksen et al., 2014; Jambeck et al., 2015). The vast majority of plastics in the ocean are microplastic, or particles < 5 mm in diameter (Hidalgo-Ruz et al., 2012; Goldstein et al., 2013), which can either form from the degradation of larger plastic pieces through mechanisms such as UV degradation, microbial degradation, or mechanical degradation (i.e., wave action), or be industrially produced as such (i.e., microbeads). These microplastics represent a novel matrix in the marine environment, providing a surface for hydrophobic organic contaminants such as pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), etc. to sorb and leach (Engler 2012; Rochman et al., 2013), and for bacterial and eukaryotic organisms to colonize. This colonization forms a biofilm community termed the "plastisphere" (Zettler et al., 2013).

The structure of the plastisphere has been found to be shaped by plastic polymer type (Oberbeckmann et al., 2014, 2018; Amaral-Zettler et al., 2015, Eich et al., 2015; Debroas et al., 2017), geography (Amaral-Zettler et al., 2015), and seasonality (Oberbeckmann et al., 2014). Zettler et al. (2013) found a high-relative abundance of a potentially pathogenic *Vibrio* spp. on a polypropylene particle collected in the North Atlantic. However, because of sampling constraints, it is difficult to ascertain whether the presence of this taxon was a product of time exposed to the environment, polymer type, or simply a random occurrence. In controlled plastic incubation studies, significant differences were found between the bacterial assemblages associated with polystyrene

(PS) and high-density polyethylene (HDPE), but only in low nutrient environments in the North Sea (Oberbeckmann et al., 2018). However, this was not the case for eukaryotic communities in a companion study (Kettner et al., 2019), and in similar reports of eukaryotic assemblages within the plastisphere (Kirstein et al., 2018) that also did not find polymer specificity in eukaryotes. Using light microscopy, however, Eich et al., (2015) found certain diatom taxa to specifically colonize HDPE or a biopolymer-polyethylene terephthalate ("biodegradable") in the Mediterranean Sea. These observations support the complex interplay of the factors involved in shaping plastisphere communities (reviewed by Jacquin et al., 2019), and the importance of understanding the colonization of new plastics in the environment over time.

Here, we combine microscopy and DNA sequencing analyses to determine if polymer specific communities emerge over a controlled time series by incubating six common plastic polymers over a six-week time series, *in situ,* in a tropical bay in Bocas del Toro, Panama. Since Panama acts as a catch basin for marine debris in the Caribbean (Garrity & Levings, 1993), it is of particular interest in the study of plastic pollution. This is, to our knowledge, the first investigation assessing biofilm formation on microplastics in the Caribbean, and the first that is comprehensive with respect to all six common plastic types.

2.3 Materials and Methods

2.3.1 Experimental set up and sample collection

The incubation experiments were carried out at the Smithsonian Tropical Research Institute's (STRI) Bocas del Toro Research Station in Almirante Bay, Panama (Fig. 1), during June and July 2017. We investigated microplastics of the six most common plastic types, colloquially known as plastics "1-6" on consumer products. These are polyethylene terephthalate (PETE, #1), high-density polyethylene (HDPE, #2), polyvinyl chloride (PVC, #3), low-density polyethylene (LDPE, #4), polypropylene (PP, #5), and polystyrene (PS, #6). Each plastic type was cut into rectangular pieces of 1.5-5mm in size and washed in 10% Hydrochloric Acid. The source of plastics used to produce the microplastics came from common household items, such as disposable cups (PETE and PS), milk and yogurt containers (HDPE and PP, respectively), squirt bottles (LDPE), and unused, blank ID cards (PVC). These microplastics ranged in thickness, from 220-995µm with 0-2.9% variation among replicates, with PS being the thinnest and PVC the thickest (PETE: 0.23 mm, HDPE: 0.57 mm, LDPE: 0.86 mm, and PP: 0.34 mm). Approximately 1g of each microplastic type was secured in nylon sachets (1 sachet per plastic, per week) with a mesh pore size of 1 mm, anchored approximately 1 m below the surface at the base of the station's sensor platform approximately 60 m away from shore, and sampled weekly over six weeks (Fig. 1). Microplastics were gently vortexed in filtered (0.2µm), autoclaved sterile seawater for the removal of any free-living organisms not interacting with the substrate and then picked and sorted with sterile forceps. Microplastics were sampled for amplicon sequencing and scanning electron microscopy

(SEM). Additionally, 250mL of surface seawater was passed through GF/F filters for DNA extraction and amplicon sequencing analyses of the total water column community. Environmental parameters at the time of sampling were obtained as part of the STRI Physical Monitoring Program (https://biogeodb.stri.si.edu).

2.3.2 Chlorophyll a concentration

Chlorophyll *a* (Chl *a*) concentration in the ambient water was analyzed at each sampling time point by filtering 65-250mL in duplicate onto GF/F filters that were kept at -20°C until extraction in 5mL of 90% acetone at 4°C for 24 hours back at the ASU laboratory. Fluorescence was measured with a Turner Designs TD-700 Fluorometer (model# 7000-009) (Table S1).

2.3.3 DNA extraction, amplicon sequencing, and sequence analysis

At each sampling point, 15 microplastic pieces of each plastic polymer type were randomly selected from their respective sachets and stored in ATL buffer at -20°C after proteinase K digestion prior to being transported and extracted for DNA as per the Qiagen Dneasy Blood and Tissue kit manufacturer's protocol (Qiagen, Valencia, CA). DNA was extracted from a composite sample made after pooling the 15 biological replicates with modifications recommended to standardize DNA extractions from microplastics (Debeljack et al., 2017). For all samples, Ready-Lyse™ lysozyme (10 ml of 1000 units per ml stock; Lucigen, Madison, WI) was added and incubated for 30 minutes in 37°C. The filter samples of ambient water were extracted with modified proportions of

ATL and Proteinase K, 900μl and 20μl, respectively, to account for the size of the filter. Blank controls were run throughout the extraction and sequencing process. For all samples, successful DNA isolation was confirmed by agarose gel electrophoresis and quantified with a Qubit system utilizing the High Sensitivity dsDNA reagents (Invitrogen, Carlsbad, CA).

The taxonomic composition of bacterial and eukaryotic communities were determined by the Illumina MiSeq 2x300 amplicon sequencing platform of 16S and 18S rRNA genes. PCR amplification was performed using primers 515F and 926R (Quince et al., 2011; Parada et al., 2016) to amplify the V4-V5 region of bacterial 16S rRNA genes and primers eukv4F and eukv4R (Stoeck et al., 2010) to amplify the V4 region of eukaryotic 18S rRNA genes. Amplicons were sequenced in a paired end format and processed using the QIIME 2 v2018.4 platform (Bolyen et al., 2018). Reads were assembled, demultiplexed, and trimmed to salvage reads that had a median quality score above 25. Any PhiX reads and chimeric sequences were filtered using DADA2 (Callahan et al., 2016). Amplicon sequences variants (ASVs) were classified against the Silva (bacteria) and Protist Ribosomal Reference (eukaryotes) (Guillou et al., 2013) databases using trained classifiers. Eukaryotic ASVs classified as Metazoans were removed from the analyses.

2.3.4 Scanning Electron Microscopy

Microplastics were preserved in glutaraldehyde (Sigma-Aldrich, 5% (v/v)), cooled at 4°C for 2-8 hours, then transferred into 50% (v/v) ethanol in Phosphate Buffer

Solution (PBS) and stored at -20°C until further preparation and imaging at the ASU laboratory. Samples were then dehydrated through a graded ethanol series and critical-point dried. The dried samples were mounted on aluminum stubs and sputter-coated with 10-15 nm of gold-palladium (60/40). Images were generated using a TESCAN VEGA[3] SEM operated at 15kV.

We focused on the taxonomic description of the diatom community, which are a major component of marine biofilms and can be taxonomically distinguished based on their morphology. We classified diatoms into 14 morphologically distinct groups denoted D1 through D14, with D14 comprising all "other" diatoms that were counted in low abundance (< 2) Taxonomic identification of the groups was carried out using reference literature (See Table S1). Five fields under SEM were counted, which amounted to 1-58 cells for a taxonomic group depending on its density on the plastic piece. Each field had a SEM magnification of 1760x with working distances between 13.36-14.02 mm, making each field approximately 200 μm^2 in size. Diatom density on the plastic surfaces was determined in duplicates for each plastic type and expressed in cells/mm^2.

2.3.5 Data Analyses

Alpha-diversity indices were calculated using PRIMER v7 (Clarke & Gorley, 2015) after rarefying abundances based on the lowest recovered reads (21,580 for prokaryotes and 27,581 for eukaryotes). These indices include: observed richness, or total number of species (S); evenness, or the numerical distribution of each species within the community, represented by Pielou's index (J'); and diversity, which considers both

richness and evenness, and is represented by the Shannon-Weiner index (H'). Significant differences in the richness, evenness, and diversity between samples were calculated using a Student's t-test (assuming equal variances, as determined first by Levene's Test) or a Mann-Whitney U-test if normality or equal variances were not met.

We evaluated differences in the microbial community composition of both prokaryotes and eukaryotes (at the genus-level) using the Bray-Curtis (BC) dissimilarity index as an estimator of the taxonomic distance between samples. BC dissimilarities were calculated between samples and visualized using Principal Coordinate Analysis (PCoA).

      To determine if the BC indices were due to chance, we built a null distribution model to which observed BC indices were compared, by generating abundance matrices of random communities (9999 iterations, Gotelli 2008). The use of this algorithmic null distribution model is recommended as it is not susceptible to Type I errors, that is, there is a low probability that the null model produces a statistically significant pattern incorrectly (Gotelli 2008). Statistical significance of observed BC indices were then evaluated by comparing them to the distribution of BC distances calculated after the randomization procedure (Swenson 2014). Communities were considered statistically similar in composition if the observed BC index fell within the lower 5% tail of the BC distribution, which corresponds to a p-value < 0.05. Standardized effect size (SES) was additionally calculated in order to avoid directional bias associated with a decrease in variance in expected BC indices with increasing species richness. SES was calculated with the following formula (Swenson 2014):

$$SES = \frac{BC_{obs} - BC_{exp}}{SD_{exp}}$$

where BC_{obs} is the BC dissimilarity index calculated between two communities (the original matrix), BC_{exp} is the mean expected BC dissimilarity index calculated from the randomized distribution of the two communities, and SD_{exp} is the standard deviation of the expected BC dissimilarity index. Negative SES values denote an observed BC index lower than the average expected value, and typically indicate communities that are similar to one another, whereas a positive SES indicates a segregation between communities (i.e., the random generation of communities had much less co-occurrence of species), denoting dissimilar community composition accompanied by a p-value > 0.05. According to Swenson (2014), dissimilarity between communities is not significant until a p-value > 0.95 is reached.

All statistical analyses and graphs were completed in Rstudio (version 1.2.1335) using the "vegan" (Oksanen et al., 2018) and "picante" (Kembel et al., 2010) packages, except the analyses of the relative abundances of the diatoms identified using SEM, which were visualized using a heatmap made in PRIMER v7. Hierarchical Cluster Analysis using the averages of unweighted pair-groups (UPGMA) was applied to cluster the plastic samples of similar diatom composition, visualized using a dendrogram. The Similarity Profile Routine (SIMPROF) was then used to test for significant differences between the clusters (999 iterations with a significance level of 5%). All data, including accession numbers of sequence data deposited to NCBI's Sequence Read Archive are available on Data Dryad at

2.4 Results

Environmental parameters on the days microplastics were sampled are shown in Table S1. Average water temperature at the site over the time series was 30.1°C (range 29.1-30.9°C) and salinity averaged 32.6‰. The Chl a concentration changed from 0.82µg/L and 0.88µg/L at weeks 1 and 3, respectively, to 0.68µg/L at week 6 (Table S1).

2.4.1 The Bacterial Plastisphere

Time points (week 1, week 3, and week 6) were chosen to represent the biofilm community composition at the initial, mid, and final points of the experiment. 16S rRNA gene sequence analyses of the prokaryotic community (Fig. 2) revealed that all biofilms included a high proportion of Proteobacteria (31-45%) with Alpha- (48-85%) and Gammaproteobacteria (10-41%) as well as Bacteriodetes (14-48%). Bacteriodetes consisted mainly of the classes Flavobacteriia (30-77%), Saprospirae (6-67%), and Cytophagia (1-27%). Total water column communities were dominated by Cyanobacteria (17-34%), most notably *Synechococcus*, which constituted a minor component (0-6%) on the plastisphere.

Over the time series, water column communities consistently had significantly lower richness (mean observed richness: 114) than plastisphere communities (mean observed richness: 222), t(17)=-12.95, p << 0.001 (Table S2). Evenness of water column

communities was also significantly smaller (mean J': 0.534) than plastisphere

communities (mean J': 0.7412, t(17)=-14.51, p << 0.001), that is, the total water column

community was dominated by a small number of highly abundant organisms (Table S2).

The lower richness and evenness of the total water community contributed to its

significantly lower diversity (mean H': 2.52) compared to the plastispheres (mean H':

4.00, t(17) = -15.14, p << 0.001). Plastispheres did not significantly differ in richness,

evenness, or diversity as a result of polymer type or time of incubation.

The visualization of bacterial rRNA gene sequences using PCoA revealed that

prokaryotic biofilms clustered away from water column communities (Fig. 4A), and

clustered as a result of time, not plastic type (Fig. 4B). While the PCoA depicted a

clustering based on BC dissimilarity, when subjecting the sequencing data to the null

model analysis, those results were not confirmed. Observed BC dissimilarity indices were

always low for pairwise comparisons (Table S4, Table S5), indicating no significant

differences in community composition between plastic types (BC ≤ 0.51, SES ≤ -8.07, p

≤ 0.000) or time of incubation (BC ≤ 0.62, SES ≤ -7.99, p ≤ 0.000) at the available

taxonomic resolution. Moreover, observed BC indices between water column and plastic

adhered communities were higher (BC ranging 0.77-0.82), but the indices did not differ

from the random distribution of BC indices calculated from the null model (SES ≤ -7.99,

p < 0.05), which indicates that the water column and plastic adhered communities were

not significantly different from one another.

2.4.2 The Eukaryotic Plastisphere

Eukaryotic rRNA gene sequencing data (Fig. 3) predominately showed diatoms, dinoflagellates, red, green, and brown algae, as well as parasitic ciliates and apicomplexans in all plastispheres. Dinoflagellates initially contributed much more to the relative abundance of the water column community, but over the time series these taxa became much more prevalent within the plastispheres as well. It should be noted, however, that not the same dinoflagellates were associated with both the water column and the plastic adhered communities. For example, *Gyrodinium sp*. Dominated the water column, as well as several unclassified Dinophyceae members (65-84% of total Dinophyceae relative abundances), whereas the plastisphere communities were dominated by *Amphidinium sp.* At weeks 1 and 3, but shifted to *Prorocentrum sp.* And *Alexandrium sp.* At week 6 of incubation. No dinoflagellates, however, were observed under SEM, possibly due to them being washed away during SEM preparation, or alternatively, by being overrepresented in sequencing libraries due to their high nuclear gene copy number (Medinger et al., 2010; Amacher et al., 2011; Keeling & del Campo, 2017). Raphid and Araphid pennate diatoms were much more relatively abundant on all plastics during the initial biofilm forming stages, but decreased over the time series on all plastic types despite their increase in abundance under SEM (Fig. S1). This is likely due to their initial presence on the microplastics and subsequent colonization by other eukaryotes, which would then dilute the relative contribution of diatom sequences.

Alpha indices among the eukaryotes exhibited higher richness within the water column at weeks 1 and 3 of the experiment (observed richness: 146-168) in comparison to all plastisphere communities (average observed richness: 50.1) (Table S6). At week 6,

the richness of the water column pointedly decreased (observed richness = 86), accompanied by a decrease in phytoplankton biomass indicated by lower Chl *a* value (Table S1), however, the water column communities, independent of time, still had significantly higher richness than plastispheres (Mann-Whitney, U = 52, p = 0.006). Evenness between water column (average J': 0.5854) and plastisphere communities (average J': 0.5169) did not deviate from one another significantly over the time series (Mann-Whitney, U = 31, p = 0.740), however, water column communities exhibited significantly higher diversity (mean H': 2.801) than plastispheres (mean H': 2.02) (t(19) = 2.70, p = 0.014).

Eukaryotic plastispheres, in contrast to prokaryotic plastispheres, did not cluster away from total water column communities (Fig. 4C). However, the eukaryotic plastispheres mirrored prokaryotic plastispheres in that clustering occurred as a result of time, not plastic type (Fig. 4D). The analysis of significance of observed BC indices within the null model showed that differences in eukaryotic plastispheres were variable when comparing plastic polymer types per time point, in addition to total water column communities over the time series (Table S6). At week 1 of incubation, no plastic polymer significantly differed from one another, and all plastic polymers (with the exception of PVC) were dissimilar from the total water column community (BC ≥ 0.85, p ≥ 0.06). By week 3, PETE was the only polymer that differed from other polymers, specifically from PVC and LDPE (BC ≥ 0.84, p ≥ 0.054), but not from HDPE, PP, or PS (p < 0.05). At week 6 dissimilar communities developed between PETE and HDPE (BC = 0.92, p = 0.234), PETE and LDPE (BC = 0.9, p = 0.301), HDPE and PVC (BC = 0.91, p = 0.098),

PVC and LDPE (BC = 0.82, p = 0.065), and LDPE and PP (BC = 0.9, p = 0.213). Only LDPE and HDPE harbored eukaryotic communities different from the total water column community at week 6 (BC = 0.91-0.94, p = 0.2682-0.2881). When BC indices of plastic polymer types were analyzed between time points (Table S7), only PETE exhibited dissimilar communities between all time points (BC ≥ 0.81, p ≥ 0.058), whereas PVC and PP did not differ between any time points. LDPE and HDPE both differed in community composition between weeks 1 and 6 (BC ≥ 0.87, p ≥ 0.207), and weeks 3 and 6 (BC ≥ 0.89, p ≥ 0.247), but not weeks 1 and 3. PS only harbored dissimilar communities between weeks 1 and 6 (BC = 0.83, p = 0.063).

2.4.3 Microscopy reveals polymer preference for diatoms

Because diatoms only reached sufficient density to be quantitatively analyzed by the end of the incubation (Fig. S1), all diatom analyses are shown for week 6. Distinct diatom communities could be distinguished on the microplastics (Fig. 5, Table S8, Table S9). While *Cocconeis placentula*, *Fragilara sp.,* and *Navicula sp.* Appeared on all plastic types, albeit in varying abundances, several diatoms exhibited polymer preference. *Mastogloia sp. I, Mastogloia sp. II, Mastogloia fimbriate,* and *Cocconeis sp.* Appeared only on 2-4 plastic types. *Nitzschia sicula* and *Striatella sp.* Were exclusively present on PETE, whereas *Amphora sp.* Was observed only on LDPE, *Mastogloia epiphytic* on PP, and *Pseudo-nitzschia sp.* On PS (Table S9).

We used hierarchical clustering analysis (Fig. 5B) to determine whether diatom community composition was influenced by plastic polymer type. HDPE and LDPE had

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similar diatom community composition, as did PP and PVC, and both groups contrasted with resident communities on PETE and PS. The community on PS was the most distinct (Fig. 5B), with D11 (*Pseudo-nitzschia sp.*) observed exclusively on its surface (as seen also in Fig. S1 C).

2.5 Discussion

This study is the first to compare *in situ* biofilm development of prokaryotic and eukaryotic communities on all six common plastic types, over a controlled time series in the Caribbean Sea. We found through rRNA gene sequencing analyses that bacterial plastispheres formed were not significantly shaped as a result of plastic polymer type, but by incubation time, as shown in the PCoA ordination (Fig. 4B); however, the null model derived BC index comparisons did not confirm temporal differentiation (Table S5). These results agree with those of Pinto et al. (2019), and while certain "core" taxa have been associated with early, intermediate, and late successional stages of biofilm development (De Tender et al., 2017), results of the same study suggest that time does not significantly shape plastic bacterial communities. In our study, the lack of significant differentiation between plastic polymer types indicates that general biofilm processes, rather than plastic-polymer associated characteristics (i.e., hydrophobicity, surface roughness, etc.), shape a core, plastic-associated community.

PCoA ordination showed significant differences between prokaryotic water column communities and the plastisphere (Fig. 4A), however, the null model derived results did not confirm this differentiation. It is notable though that BC indices, SES

values, and p-values were higher when comparing total water column communities and any given plastic type in pairwise comparisons, but not high enough to denote significant dissimilarity. These results are in contrast to other controlled incubation studies assessing bacterial colonization on microplastics that found significant dissimilarity between water column and plastic adhered communities (Oberbeckmann et al., 2018; Dussud et al., 2018).

Eukaryotic plastispheres, which exhibited similar clustering as the prokaryotic communities (Fig. 4D), clustered as a result of time, but not between the total water column communities and plastic polymer type. However, this was not the case in several scenarios when subjected to the null model analysis. With the exception of PVC and PP, all eukaryotic plastispheres differed between the beginning (week 1), and end (week 6) of the experiment, but only PETE differed between the beginning and middle (week 3) of the experiment according to the null model. We can attribute much of this variability to the presence of a high relative abundance of indicator species, such as the coralline algae *Pneophyllum conicum* (~75% of total relative abundances) on HDPE at week 6. However, the fact that we see more differentiation among the eukaryotes after six weeks, and much less so after 3 weeks, could explain why studies arrive at varying conclusions on polymer specificity of plastispheres; that is, they not only vary in methodologies and plastic types, but also in incubation times, which limits generalizations on polymer specific communities. Additionally, despite the overlap of total water column communities and plastisphere communities among eukaryotes (Fig. 4C), those that exhibited dissimilarity between water column and a given plastic type from the null

model analysis (i.e., PETE week 3, LDPE and HDPE week 6, etc., Table S6) also were furthest from one another in the PCoA plot. Our results showing dissimilarity between total eukaryotic water column communities and those associated with the plastisphere support results reported by Kettner et al. (2019) from incubations of HDPE and PS in the Baltic Sea.

We found bacterial richness to be higher in the plastisphere than in the water column, confirming results found by Bryant et al. (2016) in the North Pacific, De Tender et al. (2015) in the North Sea, and Debroas et al. (2017) in the North Atlantic, where plastics were collected directly from the environment. Contrastingly, richness within the eukaryotic communities was higher in the total water column, which confirms results by Kettner et al. (2019) who incubated plastics *in situ* in the Baltic Sea. Furthermore, bacterial plastispheres were higher in evenness, attributing to their overall higher diversity, whereas eukaryotic plastispheres were much less even and when coupled with lower richness, resulted in lower diversity. The total water column eukaryotic communities became less diverse than the plastispheres on PETE, LDPE, and PS at week 6, when the concentration in ambient phytoplankton decreased (as measured by a decrease in Chl *a*, Table S1).

Dominant prokaryotic taxa found in our study included Proteobacteria (Rhodobacteraceae) and Bacteriodetes, most notably Flavobacteraceae, Cryomorphaceae, and Saprospiraceae – all of which are known to degrade complex carbons. These are the same dominant taxa found in microplastics from other controlled incubation studies (Oberbeckmann et al., 2014, 2016, 2018; Bryant et al., 2016; Pinto et al., 2019); thus, we

infer that location may not be particularly relevant in forming the core members of the prokaryotic plastisphere. On the other hand, less relatively abundant taxa may be specific for our location. For example, the bacterial family Pirellulaceae, ammonia-oxidizing bacteria found in sponges and corals, was in high relative abundance across all plastic types in our study and has so far not been observed in the plastisphere in other studies.

Additionally, there are similarities between our eukaryotic rRNA gene sequencing results and results from other studies, such as the occurrence of diatoms as pioneer colonizers (Carpenter and Smith, 1972), as well as a high occurrence of dinoflagellates and different algal species (Zettler et al., 2013; Oberbeckmann et al., 2016; Debroas et al., 2017; Kettner et al., 2019). However, the fact that we could differentiate distinct polymer specific diatom communities via microscopy shows that sequencing analyses alone, with its inherent limitations as a result of available sequences in databases, may not reveal enough taxonomic resolution to distinguish statistically significant differences between the communities. Our results support those of Eich et al. (2015), who, using light microscopy, found significantly different diatom community composition on HDPE and a biopolymer-polyethylene terephthalate ("biodegradable") plastic in the pelagic zone of the Mediterranean Sea after 33 days of exposure, but not at 15 days of exposure, indicating that both plastic polymer type in addition to time exposed to the environment may play integral roles in shaping diatom members of the plastisphere.

Diatoms may function as an important habitat for hydrocarbon-degrading bacteria (Gutierrez et al., 2013), specifically polycyclic aromatic hydrocarbon (PAH) degrading

bacteria (Mishamandani et al., 2016). This association may stem from the capacity of diatoms to accumulate PAHs on their cell surfaces (Binark et al., 2000), which would create a PAH-enriched zone around the phycosphere, a mucosal region around the cell rich in organic matter, and in turn attract PAH-degrading bacteria to colonize this zone. Hydrocarbon degrading bacteria were detected on all plastic types. For instance, *Arthrobacter sp.,* which are shown to be diatom associated (Baker and Kemp 2014) utilize both LDPE and HDPE as a carbon source (Satlewal et al., 2008, Balasubramanian et al. 2010), and can additionally metabolize PAHs (Cerniglia 1993). *Arthrobacter sp.* Was found in high relative abundance (~17%) on LDPE after one week of incubation and on no other polymer at no other time point. It is possible that the presence of PAHs could have selected for these taxa.

Additional diatom associated hydrocarbon degraders present in the plastisphere include members of the Hyphomonadaceae, a family found in polyethylene and polystyrene biofilms by Zettler et al. (2013). These bacteria are known to form prosethecae, or long extensions of the cytosolic cellular membrane (see Fig. 5 A in picture of *Mastogloia Corsicana, D4*). Other PAH-degraders include *Nautella sp.* (37-63% of Rhodobacteraceae among all plastic types at week 1, and 3-18% at week 6), a taxon associated with the Deepwater Horizon oil spill (Severin et al., 2016), as well as *Marinobacter sp., Alcanivorax sp.*, and *Tenacibaculum sp.* (Gauthier et al., 1992, Schneiker et al., 2006; Wang et al., 2014). While the presence of these taxa does not necessarily mean they are capable of plastic degradation, many PAH degraders are also known to degrade plastics, such as *Arthrobacter sp.* And *Alcanivorax sp.* (Urbanek et al.,

2018; Delacuvellerie et al., 2019). Pinto et al. (2019) found in ambient light, but not in dim light, a high relative abundance of hydrocarbon degrading members of Alteromonadaceae (specifically *Marinobacter* and *Alteromonas*) and speculated that their relative resistance to UV radiation coupled with their capacity to degrade hydrocarbons gave them a selective advantage for growth in ambient light conditions. Both *Marinobacter* and *Alteromonas* are also known to be diatom-associated (Amin et al., 2012a, 2012b), thus, it is likely that these bacteria are found in less relative abundance under dim conditions due to the lower diatom abundance observed by Pinto et al. (2019). We hypothesize that diatom colonization on microplastics recruits hydrocarbon degrading bacteria, and that microplastics and PAHs, when interacting in consortium with a biofilm, may together create a hotspot for their respective degradation.

Diatoms were the dominant group of eukaryotes visualized by microscopy, but DNA analyses revealed other protists such as dinoflagellates and amoeba on microplastics, possibly making the plastisphere a hotspot for predatorial activity. The relative abundance of Vampyrellida, a group of predatory Rhizarian amoebae, was higher on all microplastics and nearly absent in the water column. Some species of this group feed on protists and others parasitize fungi and small metazoans (Berney et al., 2013). Additionally, many of the detected dinoflagellates were heterotrophs or mixotrophs that can feed on diatoms, such *Protoceratium reticulatum*. We also found pathogenic protists of the family Labyrinthulaceae. Isolates of members of this family were found to induce lesions on several seagrass species and have been classified as the agent that causes seagrass wasting disease and mass mortality of these plants (Garcias-Bonet et al., 2011).

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Given that Labyrinthulaceae were an initial colonizer on microplastics, most notably PETE and PS, it is possible that the microplastics may act a vector for these pathogens.

Many of the microplastic-associated dinoflagellates we could identify could be associated with harmful algal blooms (HABs), such as *Alexandrium sp.*, where some species of this genus cause paralytic shellfish poisoning in humans. Plastic-associated HAB formers were first described in Masó et al. (2003), who found temporary cysts of *Alexandrium taylori*, due to its sticky nature, adhered to plastic culture bottles. Additionally, Kettner et al., (2019) described a strong enrichment of *Pfiesteria* on microplastics PE and PS, possibly *Pfiesteria piscicida*, which produces neurotoxins and is harmful to fish. Other potentially toxic dinoflagellates in our plastisphere samples included *Amphidinium sp.*, members of which are known to disrupt sea urchin development (Pagliara & Caroppo 2012), and *Prorocentrum sp.,* which contains several toxic species, some of which inhibit diatom growth (Ji et al. 2011). While microplastics have been assessed as a vehicle for organic contaminants into the food web (Ziccardi et al., 2016), no studies to date have assessed if biotically derived chemicals, such as toxins associated with HAB species, associate with microplastics in the environment, and what impacts this may have to marine life.

2.6 Conclusion

This study is the first systematic investigation comparing biofilms developing on microplastics of all six common plastic polymer types, and the first on microplastic biofilm development in a Caribbean coastal site. We did not observe a polymer specific

assemblage of bacteria, nor were the plastisphere communities significantly distinct from the water column. We infer that the bacterial plastisphere in our study was influenced more so by the time the plastics were exposed to the environment than by plastic polymer type, however this difference was not statistically confirmed. Based on our sequence-derived eukaryotic community data, we did find evidence of some polymer specific communities that also changed significantly over time and deviated from water column communities. We observed that some diatoms, specifically, exhibited polymer preference by the end of the six-week incubation period, such as *Mastogloia Corsicana* on PP or *Striatella sp.* On PETE, and we hypothesize that the phycosphere of diatoms may play a role in attracting plastic degrading bacteria. We also find evidence that microplastics could serve as a vehicle for both pathogenic and toxigenic eukaryotes, a notion expressed in earlier studies, which would make those organisms susceptible to transport from the coastal bays into the open ocean via currents or uptake by zooplankton and fish, or possibly affect benthic communities by sinking. Our results show that in investigations of the plastisphere of microplastics, exposure time needs to be taken into consideration in addition to contrasting plastic polymers, and that a complementary approach that includes both DNA-based and microscopy-based investigations is necessary to comprehensively determine the differences between communities in the plastisphere.

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Figures
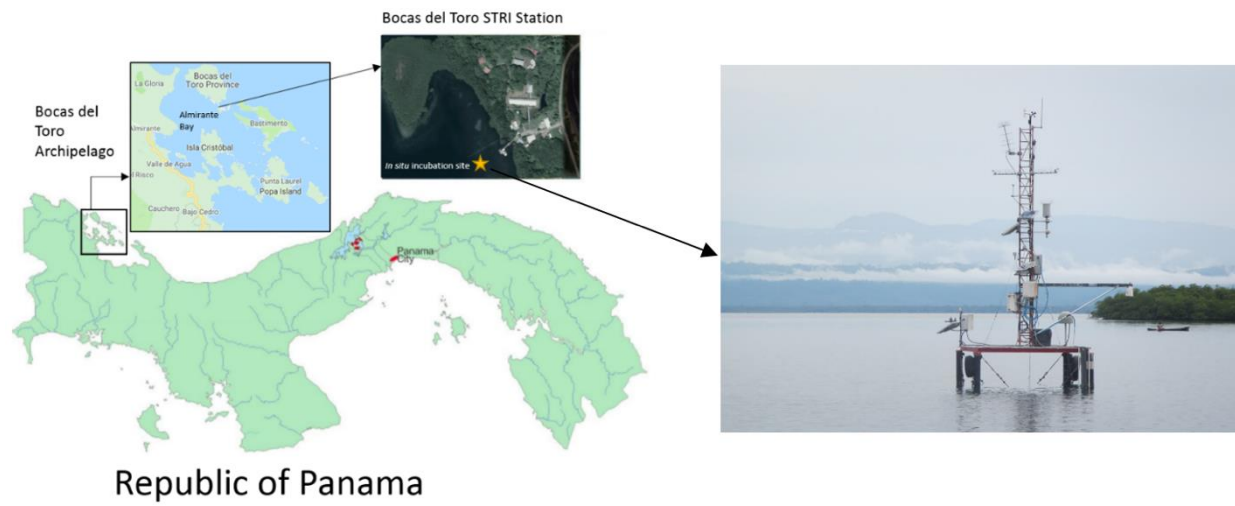
Figure 1. Location of study area and *in situ* incubation site in Bocas del Toro, Panama at the Smithsonian Tropical Research Institute's sensor platform.

Figure 2. Composition of 15 most relatively abundant taxa based on bacterial 16S rDNA amplicon analysis from incubations of microplastics and the ambient water at **(A)** week 1, **(B)** week 3, and **(C)** week 6. Sequences were classified to genus-level when possible, otherwise a higher-level classification is shown. Taxa that are not in the top 15 most relative abundant are shown as "Other".
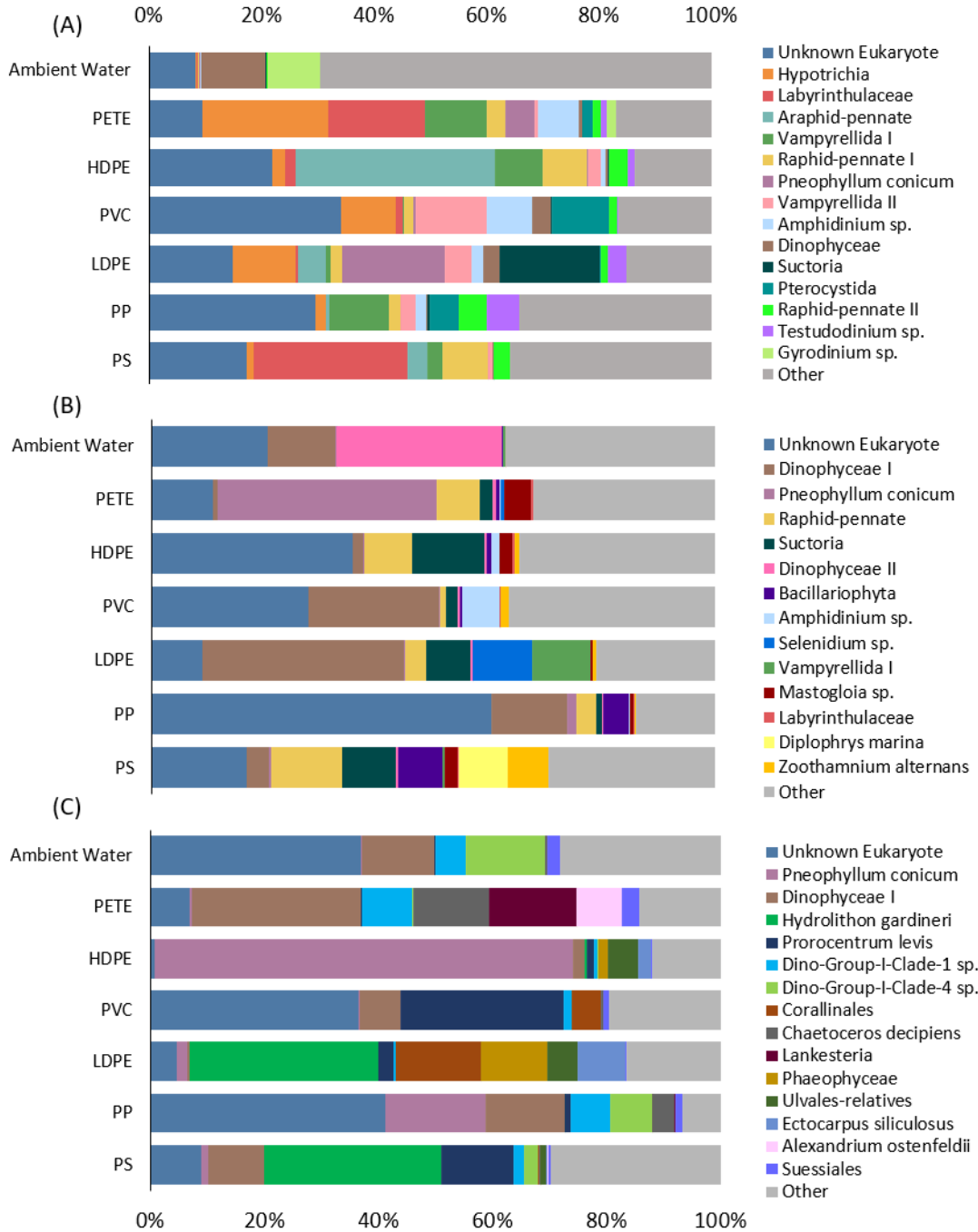
Figure 3. Composition of 15 most relatively abundant taxa based on based eukaryotic 18S rDNA amplicon sequence data from incubations of microplastics and the ambient water at **(A)** week 1, **(B)** week 3, and **(C)** week 6. Sequences were classified to species-level when possible, otherwise a higher-level classification is shown. *Sequences that represent "Unknown Eukaryote" were compared with sequences in the GenBank database, but results from the BLAST search yielded only 80-91% identity for various diatoms, dinoflagellates, and apicomplexans, thus is shown as "Unknown". Taxa that are not in the top 15 most relative abundant are shown as "Other".
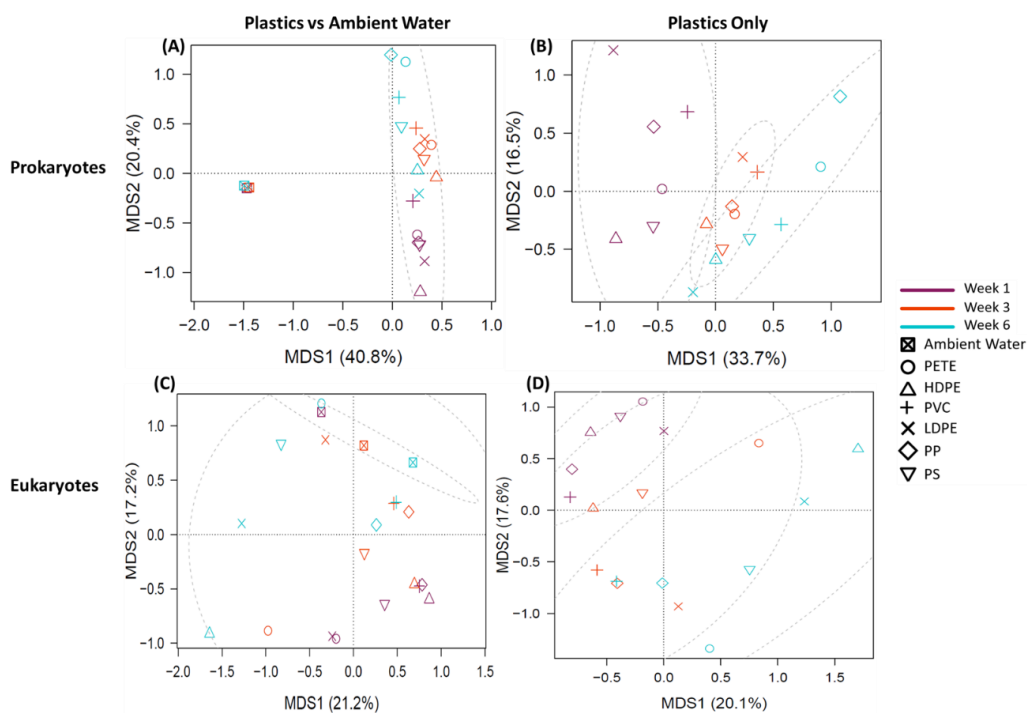
Figure 4. Principal coordinate analysis ordination of the Bray-Curtis dissimilarities computed between prokaryotic plastispheres with **(A)** and without **(B)** total water column communities as well as between eukaryotic plastispheres with **(C)** and without **(D)** total water column communities. Each ellipse indicates the 95% confidence interval of all plastics vs ambient water samples **(A and C)** and for each time point **(B and D)**.

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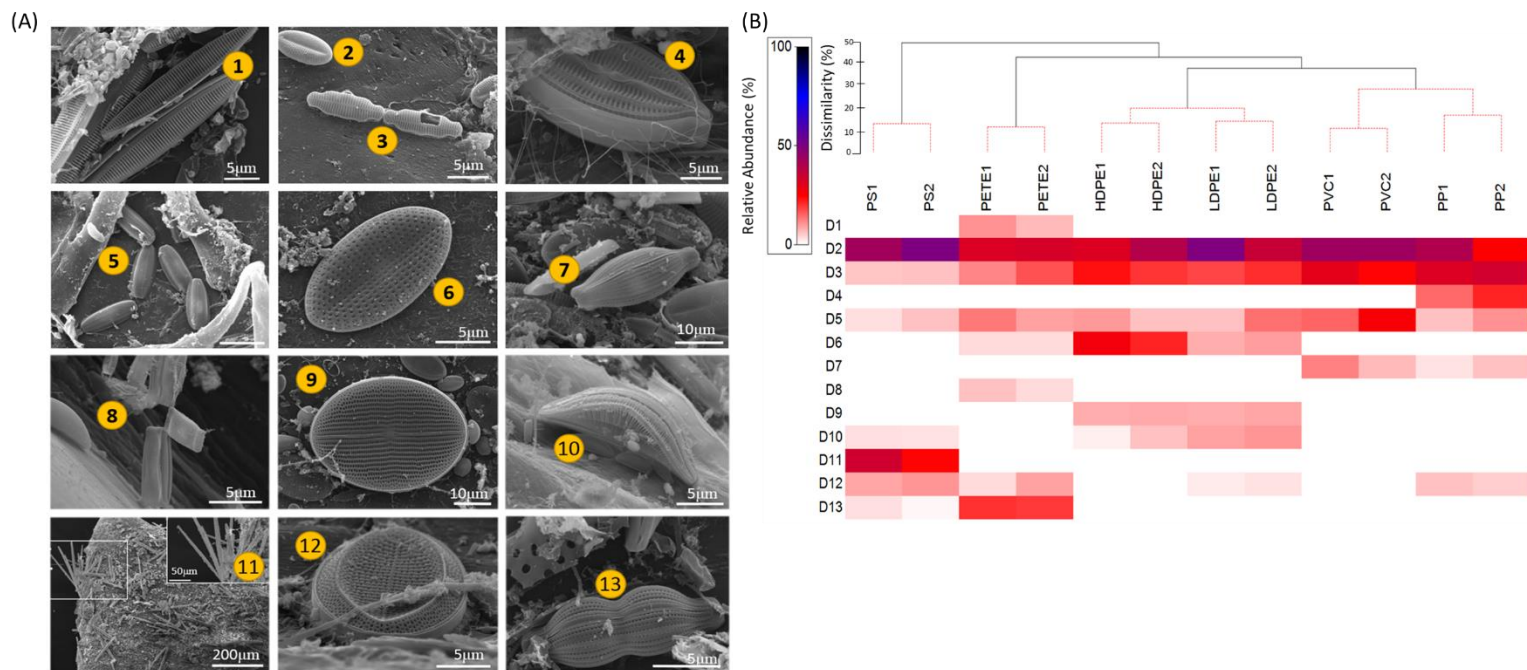
Figure 5. **A.** SEM photomicrographs of diatoms colonizing microplastics at week 6. 1) *Nitzschia sicula* **(D1),** 2) *Coccoeneis plancentula* **(D2)**, 3) *Fragilara sp.* **(D3)**, 4) *Mastogloia corsicana* **(D4)**, 5) *Navicula sp.* **(D5)**, 6) *Mastogloia sp.* I **(D6)**, 7) *Mastogloia sp.* II **(D7)**, 8) *Striatella sp.* **(D8)**, 9) *Mastogloia fimbriate* **(D9)**, 10) *Amphora sp.* **(D10)**, 11) *Pseudo-nitzschia sp.* **(D11)**, 12) *Cocconeis sp.* **(D12)** 13) *Diploneis sp.* **(D13)** See Table S8 for further taxonomic information. **B.** Heatmap depicting the relative abundances of the diatom taxa on each plastic sample, overlaid with a dendrogram that shows clustering of plastics with similar diatom composition. Black lines represent significant differences between plastic samples (p < 0.05, SIMPROF test).

CHAPTER–2 – MARINE BACTERIAL PLASTISPHERE COMMUNINITIES ARE DETERMINED INCUBATION TIME AND AMBIENT COMMUNITIES, BUT NOT PLASTIC TYPE

The co-authors have acknowledged the use of this manuscript in my dissertation.

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3.1 Abstract

Microplastics have arisen as a global threat to marine ecosystems. In this study, we explored the role that plastic polymer type, incubation time, and geographic location have on shaping the microbial community adhered to the microplastics, termed the plastisphere. We performed 6-week long incubations of six common household plastic polymers: polyethylene terephthalate (PETE), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS). Microplastics were incubated in a flow-through tank system with coastal Pacific water at La Jolla, CA. Ribosomal gene-sequencing analyses revealed that prokaryotic community composition did not exhibit significant preference for plastic type, but was instead driven by exposure time. In addition, the presence of polycyclic aromatic hydrocarbons (PAHs) sorbed onto microplastics might have influenced plastisphere communities, favoring PAH degraders, such as *Marinomonas sp.* And *Arthrobacter sp*. An indicator species analysis identified several taxa as pioneer biofilm formers (i.e., *Corynebacterium sp*. And *Halomonas sp*.) as well as late specialist colonizers such as *Dinoroseobacter sp.*. These bacterial communities were compared to the plastisphere communities grown on identical microplastic particles incubated in the coastal Caribbean Sea at Bocas del Toro, Panama. A differential abundance analysis revealed over 400 taxa being significantly different between communities found in the Pacific or Caribbean waters. However, we identified a "core plastisphere" composed of 57 taxa common to all plastic types, incubation times, and both sites. This study further

confirms the role of geography, in addition to exposure time, in the composition of the plastisphere.

3.2 Introduction

Microplastics, universally defined as plastic particles being less than five millimeters in size (Thompson et al., 2004; Frias and Nash, 2019), are ubiquitous emerging marine pollutants that have been widely established as a global environmental concern (Law & Thompson, 2014; Gago et al., 2018, Yang et al., 2018). Microplastics can be divided into primary or secondary categories depending on their source. Primary microplastics are already produced as micro-sized pieces, as in the case of pre-production pellets (Ogata et al., 2009) or microbeads found in cosmetics or hygiene products (Fendall & Sewell 2009; Gregory 2009). These particles typically have rounded shapes, a defined size range (e.g., in the US, 74–420 μm, Beach 1972), and their source locations are typically predictable. Therefore, contamination by primary microplastics is relatively easy to tackle, and effective preventive measures against it have been already undertaken. Secondary microplastics come from the degradation (i.e., by wave action, photolysis, or biodegradation) of larger objects, such as water bottles, milk cartons, or fishing nets (Cooper & Corcoran 2010). Given the large amount of macroplastics entering the environment, an estimated 4.8-12.7 million metric tons per year (Jambeck et al., 2015), it is generally assumed that most microplastics in the environment are secondary microplastics (Andrady, 2011; Hidalgo-Ruz et al., 2012; Duis and Coors, 2016). Because it is known that microplastic shape and size (Cheng et al., 2020), and even color (Wen et al., 2020) impact the plastisphere, it is imperative to study these microbial-microplastic

interactions using microplastic particles that are environmentally relevant and mimic common microplastics found in the ocean.

Microplastics are unique in the marine environment, having a large surface area-to-volume ratio and relatively high chemical stability (Engler 2012). These characteristics allow microorganisms such as bacteria, fungi, and eukaryotes to readily colonize onto microplastics to form a biofilm termed the "plastisphere" (Zettler et al., 2013). Notably, several studies have shown that microplastics adsorb various toxic chemicals (e.g., organic pollutants, heavy metals, and additives) from the surrounding environment (Hirai et al., 2011; Turner and Holmes, 2015; Rochman et al., 2013), among which polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread contaminants that sorb onto microplastics. Rochman et al. (2013) showed that for PAH sorption, polyethylene terephthalate (PETE) and polyvinyl chloride (PVC) reach equilibrium in the marine environment much faster than high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene (PP). Moreover, concentrations of PAHs sorbed to HDPE, LDPE, and PP are consistently much greater than concentrations sorbed to PETE and PVC (Rochman et al., 2013). Since organic contaminants readily partition into biofilms (Headley et al., 1998), and there are different sorption capacities among plastic polymers (Rochman et al., 2013), it is possible that this will influence the composition of their respective plastisphere communities as well. It should be noted that microplastics also leach chemicals added to them during production (i.e., flame retardants, anti-

microbials, phthalates, etc.), and Pinto et al., (2019) showed that, at least in the case of phthalates, these compounds can significantly alter plastisphere community structure.

Research on the plastisphere has led to divergent conclusions as to the variables that shape their communities. Microplastic-attached microbial communities are clearly significantly different from ambient communities (Zettler et al., 2013; Oberbeckmann et al., 2014; Amaral-Zettler et al., 2015; Oberbeckmann et al., 2016; Dudek et al., 2020). Some studies found them to differ in composition from those colonizing other types of particulate surfaces, such as wood or glass (Kirstein et al., 2018; Kesy et al., 2019), but other studies failed to detect such differences (Oberbeckmann et al., 2016). It is clear that environmental conditions such as salinity and nutrient availability (Oberbeckmann et al., 2018), seasonal effects (Amaral-Zettler et al., 2015), or time of exposure to the environment (Xu et al., 2019; Kirstein et al., 2019; Dudek et al., 2020; Zhang et al., 2021) play a role. In addition, some studies found the plastisphere under controlled conditions to be polymer specific (Oberbeckmann et al., 2014; Kirstein et al., 2019; Abed et al., 2021), likely implying that the chemical composition or surface properties, such as hydrophobicity of the substrate, influence community assembly and dynamics of the plastisphere. However, other studies failed to detect a polymer-specific effect (Bryant et al., 2016; Oberbeckmann et al., 2018; Pinto et al., 2019; Xu et al., 2019; Dudek et al., 2020; Zhang et al., 2021). Geographic location, which amalgamates a variety of environmental conditions, has emerged as an important factor influencing microplastic-attached microbial communities (summarized in Wright et al., 2020).

After a decade of research on the plastisphere, investigators continue to study the variables that contribute to the formation and maturation of the plastisphere, but none to date have comprehensively assessed these microplastic-attached communities as a function of plastic type, exposure time, and geographic location using comparable controlled experimental design and identical secondary microplastics. This study aims to (i) describe the prokaryotic plastisphere colonizing common household plastics incubated in water from the coastal Pacific near San Diego, California as a function of plastic polymer type and incubation time; (ii) compare the communities to those found in a study carried out by Dudek et al. (2020) in the coastal Caribbean; (iii) and investigate if there is a core community that is common to different plastic types, exposure times, and geographic locations.

3.3 Materials and Methods

3.3.1 Experimental design and sampling

We investigated microplastic biofilms of the six most common plastic types, commonly known as plastics "#1-6" (#1: polyethylene terephthalate; #2: high-density polyethylene; #3: polyvinyl chloride; #4: low-density polyethylene; #5: polypropylene; #6: polystyrene). The experimental design and analyses followed closely those of Dudek et al. (2020) who carried out *in-situ* incubations of microplastics in the Caribbean at Bocas del Toro, Panama. These plastics were cut into rectangular pieces of 1.5-5 mm in diameter; the thickness ranged between 220 and 995µm with 0.0-2.9% variation among replicates (Table 1). Surface area and thickness of the particles were determined using images captured using scanning electron microscopy (SEM) analyzed using the ImageJ

software (v1.4.3.x). 1g of each microplastic type, determined using an analytical balance (Mettler Toledo Model: XS105), was secured in nylon mesh sachets (1 sachet per plastic type, per sampling period) with a mesh pore size of 1 mm and incubated in a flow-through open-air sea water tank system (20L) with a water exchange rate of 0.48 L/min at Scripps Institute of Oceanography in La Jolla, CA and sampled from March to April 2018, in weekly intervals. Microplastics incubated at the early (week 1), intermediate (week 3) and end (week 6) of the time series were analyzed. After 3 weeks of incubation, algal growth was conspicuous on the wall of the tanks and sachets. The tanks were subsequently emptied and cleaned, and the sachets were cleaned of any algal growth on a weekly basis. Microplastics were processed for 16S rRNA gene amplicon sequencing, SEM, and gas chromatography with mass spectrometry (GC/MS) as detailed below. Additionally, we filtered 250 mL of the inflow seawater, which had passed through a sand filter to remove macrofauna and detritus, onto GF/F filters for amplicon sequencing analyses of the microbial community contained in the source water.

3.3.2 Environmental Parameters

For samples incubated in Pacific waters, salinity and temperature over the incubation period were obtained from the National Oceanic and Atmospheric Administration's (NOAA) Integrated Ocean Observing System in Southern California (https://sccoos.org/data/autoss/). This system consists of a suite of moored sensors attached to piers, and data used were obtained by the Scripps Pier sensors. At each sampling event, salinity and temperature were also measured with a YSI Model 85. Solar

radiation metrics over the incubation period were obtained through the California

Irrigation Management Information System (CIMIS) (https://cimis.water.ca.gov/) and

obtained manually upon sampling both ambient and light loss due to attenuation from

algal growth on the microplastics' receptacles using a Quantum Solar Laboratory

Radiometer (Model# QSL-2101).

Environmental parameters for samples incubated in the Caribbean over the

incubation period and at the time of sampling were obtained as part of the STRI Physical

Monitoring Program (https://biogeodb.stri.si.edu) and is described further in Dudek et al.

(2020).

3.3.3 DNA extraction, amplicon sequencing, and sequence analysis

At each sampling point, 15 microplastic pieces of each plastic polymer type were

randomly selected from their respective sachets and stored in an ATL buffer/proteinase K

mixture at -20°C prior to being transport and subsequent DNA extraction with the Qiagen

DNeasy Blood and Tissue kit, following manufacturer's protocol (Qiagen, Valencia,

CA), with modifications recommended (Debeljack et al., 2017) to standardize DNA

extractions from microplastics. DNA was extracted from a composite sample made after

pooling the 15 biological replicates. For all samples, Ready-Lyse™ lysozyme (10 ml of

1000 units per ml stock; Lucigen, Madison, WI) was added and the plastics incubated for

30 minutes at 37 °C. The ambient water filters were extracted with modified proportions

of ATL and Proteinase K, 900 μl and 20 μl, respectively, to account for the size of the

filter. We also extracted blank filters and zircon silica beads as an inert surface for

comparison controls. However, the zircon silica beads did not have enough DNA to be

successfully amplified for sequencing. For all samples, successful DNA extraction was confirmed by agarose gel electrophoresis and quantified with a Qubit system utilizing the High Sensitivity dsDNA reagents (Invitrogen, Carlsbad, CA).

The taxonomic composition of bacterial communities was determined by sequencing their DNA using a MiSeq Illumina platform (2x300bp paired-end) after PCR amplification of the V4 region of bacterial 16S rRNA genes using primers 515F (Parada et al. 2016) and 806R (Apprill et al., 2015). Bioinformatic processing and analyses were performed using QIIME 2 v2020.2 (Bolyen et al., 2018) where reads were assembled, demultiplexed, and trimmed to salvage reads that had a median quality score above 25. PhiX reads and chimeric sequences were filtered using DADA2 (Callahan et al. 2016). Amplicon sequences variants (ASVs) were classified against the Silva (version 138) database using a trained classifier and collapsed into taxa at the highest resolution possible. ASVs assigned to mitochondria or chloroplast were removed from the analyses a posteriori. Due to insufficient sequence read yield, the 3-week water column sample was not included in analyses. We targeted both the 16S and 18S rRNA genes for amplification, but due to insufficient 18S amplicon sequence reads, this study focuses on the description of the prokaryotic communities.

Microplastic samples used for comparison incubated in the Caribbean (Dudek et al., 2020) used the primer pair 515F and 926R, instead of the 515F and 806R used for the microplastics incubated in the Pacific, and were thus trimmed so that each sample was classified based on identical regions. Reads from both studies were merged before being subject to the bioinformatic pipeline in QIIME2, including the utilization of an updated

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Silva database from that used in Dudek et al. (2020). Due to the updated database, the taxonomic results specific to the Caribbean samples may not exactly reflect those reported by Dudek et al. (2020).

3.3.4 Statistical Analyses

Alpha-diversity indices were calculated using the *vegan* package (Okansan et al., 2018) in R (version 1.3.959-1). Sequence variant richness was calculated by rarefying read counts to the lowest number of reads (5498). Evenness was calculated as J = H/logS, where H is the Shannon diversity index and S is rarefied richness (Pielou, 1977). Significant differences in the richness, evenness, and diversity between samples were calculated using a non-parametric Kruskal-Wallis H Test as normality and equal variances assumptions were not met as determined first by Shapiro-Wilks Test and Levene's Test. Pairwise comparisons were evaluated using a Tukey HSD post-hoc test.

Hierarchical Cluster Analysis using the averages of unweighted pair-groups (UPGMA) was applied to cluster samples of similar prokaryotic community composition, and the Similarity Profile Routine (SIMPROF) was used to test for significant differences between the clusters (999 iterations with a significance level of 5%) and visualized using a dendrogram. Dendrograms were overlaid on heatmaps of Log_{10} (X+1) transformed relative abundances of taxa present on the microplastics. Dendrograms, heatmaps, as well as the SIMPROF routine were performed within PRIMER v7 (Clarke and Gorley, 2015) on the rarefied collapsed taxonomy table classified to the family level. Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson 2005) tests were used to

test for significant differences between the prokaryotic communities associated with sample type (plastic polymer type), time of incubation (week 1, week 3, and week 6), as well as between locations – the Pacific (this study) and the Caribbean (Dudek et al., 2020). Differences in the composition of prokaryotic communities were evaluated by creating a Bray-Curtis (BC) dissimilarity matrix at the highest taxonomic resolution available before being visualized using Principal Coordinate Analyses (PCoA). PERMANOVA and PcoA ordinations were performed within the *vegan* (Okansan et al., 2018) package in RStudio statistical software (version1.3.959-1).

To determine if the BC indices were due to random chance, we built a null distribution model using R packages *vegan* (Okansan et al. 2018) and *picante* (Kemble et al., 2010) to which observed BC indices were compared by generating abundance matrices of random communities (9999 iterations, Gotelli 2008). This algorithmic null distribution model was used in Dudek et al. (2020) to determine dissimilarity, or similarity, between microbial communities attached to different plastic polymer types, as well as the effect of incubation time on the communities attached to the same plastic polymer type. Statistical significance of observed BC indices were assessed by comparing them to the distribution of BC distances calculated after the iterative process (Swenson 2014). If the observed BC index fell with the lower 5% tail of the BC distribution (p-value <0.05), communities were considered statistically similar in composition. Statistically significant dissimilar community composition was observed if the BC index fell within the higher 5% tail of the distribution (p-value > 0.95). We additionally calculated standardized effect size (SES) in order to avoid directional bias

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associated with a decrease in variance in expected BC indices with increasing species

richness. SES was calculated with the following formula (Swenson 2014):

$$SES = \frac{BC_{obs} - BC_{exp}}{SD_{exp}}$$

where BC_{obs} is the BC dissimilarity index calculated between two communities (the

original matrix), BC_{exp} is the mean expected BC dissimilarity index calculated from the

randomized distribution of the two communities, and SD_{exp} is the standard deviation of

the expected BC dissimilarity index. Negative SES values denote an observed BC index

lower than the average expected value, and typically indicate communities that are

similar to one another, whereas a positive SES indicates a differentiation between

communities (i.e., the random generation of communities had much less co-occurrence of

species), denoting dissimilar community composition accompanied by a p-value > 0.05

and significant dissimilarity is signified at a p-value > 0.95.

We also carried out indicator species analyses (Dufrêne and Legendre 1997) to

determine if there were taxa that were specific to time of incubation or plastic polymer

type by using the *indicspecies* R package (function *multipatt*, version 1.7.8) on the

rarefied feature tables classified to highest taxonomic resolution possible. This is a

widely used tool in ecology to identify indicator species, characterized by their exclusive

occurrence (i.e., specificity) and distribution in the sampling sites of a particular habitat

(i.e., fidelity). The following algorithm provides a statistic (Indicator Value) ranging from 0 (not suitable indicator) to 1 (ideal indicator):

$$IndVal_{ij} = \sqrt{100(A_{ij} * B_{ij})}$$

where A_{ij} is the specificity, i.e., abundance of species i in group j and B_{ij} is the fidelity, i.e., frequency by which species i appears in group j. Monte Carlo permutational tests within the *multipatt* function were used to assess the statistical significance of each taxon's indicator value, and only those with $p < 0.05$, and a √IndVal >0.8 were considered true indicator taxa.

We performed a differential abundance analysis using the *DEseq2* R package (Love et al., 2014) to identify taxa that were differently abundant between the Pacific site and Caribbean site (Dudek et al., 2020). This analysis estimates variance-mean dependence in count data from high-throughput sequencing assays and tests for differential expression based on a model using a negative binomial distribution.

3.3.5 Environmental and Microplastic PAH Concentrations

Deployed microplastics and standard length (91.5 cm) semi-permeable membrane devices (SPMDs) were analyzed for PAHs using previously reported methods (Polidoro et al., 2009, Allen et al., 2018). SPMDs were prepared from pre-washed LPDE and analytical grade triolein (Arcos Organics) spiked with ~ 30 µg p-terphenyl before deployment. These devices act as "artificial fish," to sample the equilibrium concentrations of nonpolar organic compounds in the ambient water in order to estimate

the lipophilic chemical profile of the water column. For processing and analyses, microplastics were removed from each sachet, including controls, weighed, and then placed in a borosilicate glass test tube with Teflon-coated lids. Samples were treated with 15 ml of hexane, spiked with the recovery standard p-terphenyl, and then spun on a rotary mixer for 48 hours. Hexane extracts were removed through glassfiber filtration, dried with $NaSO_4$ and concentrated to a final volume of 0.5 ml with nitrogen gas. Final extracts were spiked with the internal standard tetracosane-d50, and then analyzed for PAHs using a Varian 3800 gas chromatograph in tandem with a Saturn 2200 electron ionization mass spectrometer. Because incubation time is not a factor for chemical sorption, as the chemical profile of the water column is constantly changing, chemical analyses were conducted at Day 4, and weekly from weeks 1-6. Samples at the Pacific site for week 1 – PS, week 2 – PVC, and week 6 – HDPE were omitted from the chemical analyses due to the high viscosity of samples and unsuccessful processing. Results are reported in micrograms of PAH per gram of plastic, or parts per million (ppm).

Collected SPMDs remained frozen until processing. In the lab, SPMDs were dialyzed in hexane for 24 hours and then again for 8 hours. The hexane dialysates were concentrated to 5 ml by nitrogen gas, cleaned-up for large compounds using gel permeation chromatography (e.g., Biobeads SX-3, BioRad), and dried with $NaSO_4$. Final extracts were concentrated to a volume of 0.5 ml with nitrogen gas and followed the remainder of the protocol as stated above. Average PAH water concentrations over time were calculated from PAHs quantified in SPMDs based on established compound-

specific uptake rates (CERC 2018). Results are reported in micrograms of PAH per milliliter of water, or ppm.

Method recoveries for detected PAHs were 0% to > 100% depending on the targeted chemical and plastic polymer. A complete list of potential contaminants analyzed, with method recoveries for each plastic polymer type, is shown in Table S1, with the exception of PS where controls could not be processed due to the sample dissolving in the hexane.

3.4 Results

Environmental parameters for both the Pacific and Caribbean sites, which includes average measurements over each time series, are shown in Figure 6. During the incubation period, at the Pacific site, water temperatures increased from 11.3°C to 18.1°C, and averaged 14.9°C whereas salinity averaged 35.1‰. Average solar radiation was 20.3 MJ/m^2/day. The Caribbean site had an average surface water temperature of 29.4°C (range 28.2-30.9°C), an average salinity of 31.1‰, and average solar radiation was 14.7 MJ/m^2/day over its respective time series. The Caribbean site had significantly higher temperatures ($t_{88} = 224.18$, $p \ll 0.001$), significantly lower salinity ($t_7 = -4.07$, $p = 0.002$), and significantly lower solar radiation ($t_{167} = -6.17$, $p \ll 0.001$) than the Pacific site. All averaged measurements include those measured at night. In addition, Manual measurements of solar radiation at the Pacific site showed that up to 78% of light was lost because of algal growth in the tanks and on the sachets at the end of the time series prior to being cleaned. This biofouling did not occur at the Caribbean study site, though some

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light attenuation, even at 1 meter below the surface, naturally would have occurred due to reflection at the water surface, and light attenuation in the water influenced by particulate and colored dissolved organic matter (Bricaud et al., 1998).

3.4.1 Exposure Time Determines Microplastic-attached Community Composition

Diverse bacterial communities on microplastics incubated in Pacific waters had 311, 341, and 386 observed taxa at week 1, week 3, and week 6 of incubation, respectively and cumulatively for all plastic types. Microbial assemblages on microplastics were dominated by Alphaproteobacteria, Bacteriodia, Gammaproteobacteria, and Planctomycetes across all sampling time points and plastic types. They together accounted for 54.3-84.4% of biofilms' relative abundance. At the family level, bacterial communities varied between the initial (week 1), mid (week 3), and end (week 6) of the incubation period. At week 1, the five most common families were Alteromonadaceae, Rhodobacteraceae, Saprospiraceae, Flavobacteriaceae, and Verrucomicrobiaceae with a combined contribution of 26.3-67.1% of sequence reads among the various plastic polymers (Fig. 7). HDPE, PVC, and PS had much lower taxonomic richness (85, 43, and 26, respectively, Table S2, Fig. S1) and total number of sequence reads (5498, 10107, and 6357, respectively, Table S2) at 1 week of incubation compared to PETE, LDPE, and PP (richness: 137, 142, 176; and number of sequence reads: 36527, 73848, 34354, respectively, Table S2, Figure S1), thereby contributing to a potential overrepresentation of certain taxa, such as Verrucomicrobiaceae, Moaxellaceae, or Halomonadaceae, within the plastispheres of HDPE, PVC, and PS (Fig. 7). By week 3,

the communities among all plastic types shifted toward Rickettsiales (AB1),

Marinomonadaceae, and Hyphomonadaceae in addition to the Rhodobacteraceae and

Flavobacteriaceae that were already present in week 1, making up 55.0-80.5% of the

community among all plastic types (Fig. 7). At the end of the incubation period (week 6),

communities continued to be dominated by Rhodobacteraceae, Flavobacteriaceae, and

Hyphomonadaceae, but Saprospiraceae and Sphingomonadaceae became even more

dominant on all plastic types with a combined contribution of 52.3-73.9% of the total

relative community composition (Fig. 7). A dendrogram based on the unweighted pair

group method with arithmetic means (UPGMA) revealed distinct similarity clustering

with time (SIMPROF, $p < 0.05$; Fig. 7). Water column communities, by contrast, were

dominated by several Rhodobacteraceae such as *Amylibacter sp.*, *Planktomarina sp.*, and

unidentified Rhodobacteraceae, with a cumulative relative contribution of 10.3 (week –) -

23.2% (week 6) of the total community based on sequence reads. (Fig. S2). Other highly

abundant taxa found in the water column (>5%) belong to the Flavobacteriaceae,

Actinomarinaceae, Cyanobiaceae, and several SAR clades (Figure S2).

Overall, plastisphere communities generally had lower richness and higher

evenness than their respective water column communities. In other words, seawater

communities were characterized by many more rare taxa compared to the plastisphere,

yet were also dominated by a large number of relatively abundant organisms. Richness in

all plastic types increased over time (mean richness at week 1: 101.5; week 3: 124.8; and

week 6: 176.8, Table S2, Figure S1). A non-parametric Kruskal-Wallis H test was

conducted to examine the difference in richness, evenness, and diversity according to

time of incubation. Significant differences were observed among species richness ($H(2) =$ 6.22, p-value = 0.045) and evenness ($H(2) = 9.63$, p-value = 0.008), specifically when comparing weeks 1 and 6 (richness Tukey HSD; p-value = 0.03, evenness Tukey HSD; p-value = 0.01), however, no significance was observed when comparing Shannon diversity indices of communities between time points ($H(2) = 2.24$, p-value = 0.33).

Principle coordinate analyses were used to visualize the relationships among communities between the plastispheres and the water column (Fig. 8). Generally, the prokaryotic plastisphere clustered apart from total water column communities regardless of incubation time (Fig. 8B). Within the plastispheres, communities again clustered as a result of incubation time, not plastic type (Fig. 8A). Communities appear to coalesce into a climax community by week 6, with very little variability within the ordination. Permutational multivariate analyses of variance (PERMANOVA) further exemplified that incubation time was a strong predictor of community variability ($R^2 = 0.42$ p = 0.001, Table 2), while plastic polymer type was not ($R^2 = 0.23$, p = 0.891, Table 2). This observation confirms the conclusion drawn from comparisons of plastisphere communities in the Caribbean (Dudek et al. 2020) where community composition correlated better with incubation time ($R^2 = 0.36$, p = 0.001, Table 2) and not plastic polymer type ($R^2 = 0.27$, p = 0.698, Table 2).

The shifts in community composition with time are confirmed when communities are subject to a null model analysis. BC and SES indices were generally high amongst pairwise comparisons between week 1 and week 6, most of which were significant, (BC:

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0.92-0.98, SES: 1.41-4.84 p-value = 0.97-1, Table S3). LDPE was the sole exception with communities that were neither significantly similar or dissimilar between week 1 and week 3 (BC = 0.86, SES = -0.30, p-value = 0.31, Table S3). While the plastisphere on all plastic types showed no dissimilarity between week 3 and week 6, communities between week 1 and week 6 exhibited significant dissimilarity on most plastic polymer types (BC: 0.92-99, SES: 1.15-4.16, p-value: 0.99-1, Table S3), with LDPE constituting again the exception (BC: 0.90, SES: 1.08, p-value: 0.89, Table S3). The null model analysis revealed no dissimilarity between plastic types within the same sampling time at weeks 3 and 6 (Table S4). At week 1, however, LDPE showed significant dissimilarity when compared to HDPE and PVC (BC: 0.92-0.93, SES: 1.36-2.14, p-value: 0.95-0.99, Table S4). Biofilms on all plastics were significantly dissimilar from the total water column community at week 1 (BC: 0.94-0.99, SES: 3.83-5.37, p-value: 1, Table S4) and week 6 (BC: 0.95-0.97, SES: 2.71-4.96, p-value: 1, Table S4).

Indicator species analyses further provide support of the notion that the plastisphere was shaped by time of exposure rather than by plastic polymer type. Among the 601 taxa classified within the plastispheres, 80 taxa exhibited a significant association (using a minimum association value of $\sqrt{\text{IndVal}} = 0.8$ as a threshold) with exposure time (after 9999 permutations, $\alpha = 0.05$). Among them, 32 were indicators for a specific time point (10 taxa for week 1, 22 taxa for week 6) and 48 taxa indicated specificity within a combination two of time points (2 taxa for week 1 and week 6; 46 taxa for week 3 and week 6) (Table 3, Table S5). 19 taxa were confirmed to be indicators for all time points, i.e., these taxa represent a core community not influenced by incubation time (Table S6).

Notably, we could not identify any indicator taxa for any specific plastic polymer type. Indicators that were specific for 1 week of incubation had 3 taxa present on all plastic types: *Corynebacterium sp*., *Flavobacterium succinicans*, and *Anaerococcus sp*.. Plastics incubated for 3 weeks did not exhibit any indicator taxa however, by week 6, we see not only more indicators than in week 1, but the indicators were present on all plastics regardless of polymer type (Table 3, Table S5). Additionally, two indicator taxa for week 6, *Plesiocystis sp.* and *Acinetobacter radioresistens*, were exclusive to microplastic microbial communities sampled on week 6 (designated by an √indicator value of 1). Among indicators between two time points, the two taxa *Tenacibaculum sp.* and an unclassified Saprospirales were indicators for weeks 1 and 6. Indicator taxa did not differ significantly in relative abundance between week 1 and week 6 of incubation (Mann-Whitney U, *p-value* > 0.05). Of the 46 taxa that were indicators for weeks 3 and 6, five taxa (*Lewinella cohaerens, Croceitalea dokdonensis, Sulfitobacter litoralis, Congregibacter sp.,* and *Marinomonas sp*.) were exclusive to weeks 3 and 6 (designated by a √Indicator Value of 1), i.e., these taxa were secondary colonizers. The taxa BD7-3, *Nautella sp*., and *Marinomonas sp*., were significantly more abundant at week 3 than at week 6 (Mann-Whitney U, *p-value* < 0.05) of incubation.

3.4.2 Geographic location significantly determines microplastic-associated microbial community composition

We observed significant differences in the microbial community composition of the microplastics exposed to the Pacific Ocean and the Caribbean Sea (Bocas del Toro,

Panama, Dudek et al. 2020). PERMANOVA results showed that while time of incubation is a significant factor in shaping these plastic-adhered microbial communities between locations ($R^2 = 0.13$, p-value = 0.006, Table 2), and plastic type plays little to no role at all ($R^2 = 0.08$, p-value = 1, Table 2), communities better associate with geographic location ($R^2 = 0.35$, p-value = 0.001, Table 2). a PCoA ordination plot showed that communities differed in composition more between locations than among incubation times (Fig. 9) as the distance between locations is greater (along the x-axis where 38.4% of variability in communities is explained) than that of incubation times (y-axis where 21.2% of variability is explained, for Pacific samples). Ambient water column communities also differed significantly in composition (Fig. S2). While both sites shared some taxa, notably unclassified Rhodobacteraceae, Flavobacteriaceae, Altermonadaceae, and Cryomorphaceae, most of the taxa diverge between sites (Fig. S2). The Pacific site was dominated by Actinomarinaceae, *Synechococcus sp.*, and various Rhodobacteraceae (i.e., Amylibacter sp.), Flavobacteriaceae (i.e., Marine group NS5), and various SAR 11 clades. The Panama site had a higher relative abundance of unclassified Cyanobiaceae, as well as several Rhodobacteraceae (i.e., Planktomarina sp.), Flavobacteriaceae (i.e., Marine group NS4) (Fig. S2), as well as various SAR 11 clades. Most of these clades were different between sites (Fig. S2).

A differential abundance analysis revealed that 586 taxa differed significantly (adjusted p < 0.01) in relative abundance between sites. On a broad taxonomical level, several taxa within the Cyanobacteria, Planctomycetes, Chloroflexi, Acidobacteria, and Actinobacteria were overrepresented at the Caribbean site (Fig. 10). At the order level,

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Rhodobacterales, Sphingomonadales, and Rickettsiales were generally overrepresented at the Pacific site. Highly abundant taxa, such as *Marinomonas sp.* (Gammaproteobacteria: Oceanospirillales), Rickettsiales (Alphaproteobacteria), and several Rhodobacterales such as unidentified Rhodobacteraceae, and numerous *Roseobacter*, *Sulfitobacter*, and *Nautella* species, were all overrepresented in the Pacific site (Fig. 10).

3.4.3 A Core Plastisphere

Among the 601 taxa that were identified in the Pacific dataset, and 684 taxa that were identified in the Caribbean dataset, 57 taxa were detected at both locations and at every time point (Fig. 11). Of these, 12 were classified as Bacteroidetes, and 31 were classified as Proteobacteria with the highest mean relative abundances across all samples in the family Rhodobacteraceae (3 core taxa). A mean relative abundance of greater than 1% across all samples was reached by three of the core taxa, which were classified as members of the families Rhodobacteraceae and Saprospiraceae (unclassified at the genus level) and unidentified Alphaproteobacteria. The core community comprised 27.1% (Pacific – Week 1), 38.1% (Pacific – Week 3), 44.7% (Pacific – Week 6), 54.1% (Caribbean – Week 1), 48.8% (Caribbean – Week 3), and 49.6% (Caribbean – Week 6) of reads.

3.4.4 PAH sorption onto microplastics

PAHs were found to be associated with microplastics for all plastic types except PETE and LDPE in the Pacific (Fig. 12). PS has the highest concentrations of ΣPAHs

(2.03 µg·g^{-1} (ppm)) despite water column concentrations ranging between 0.02-0.09 mg·L^{-1} (ppm) over the time series. Detected PAHs were predominantly phenanthrene, anthracene, 4H-Cyclopenta[def]phenanthrene, fluoranthene, and benzo[k]fluoranthene where concentrations were dependent on the time of incubation and plastic type. PVC at week 4 of incubation had additional PAHs (Table S8). PAH partitioning onto HDPE ranged from 0.0005-0.004 µg·g^{-1}, PVC only had detectable ΣPAHs at week 4 at 1.1 µg·g^{-1}, PP ranged from 0.09-0.13 µg·g^{-1}, and PS ranged from 0.005-2.03 µg·g^{-1} (Fig. 12, Fig. S3, Table S8).

At the Caribbean site in Bocas del Toro, Panama, LDPE after week 1 exhibited the highest concentration of ΣPAHs (0.15 µg·g^{-1}, Fig. 12, Table S9). PETE, PP, and HDPE had measurable concentrations of ΣPAH (0.002-0.04) throughout the time series (Fig. 12, Fig. S3, Table S9). PS had two peaks of ΣPAHs at day 4 (0.07µg·g^{-1}) and week 3 (0.08 µg·g^{-1}) (Fig. 12, Fig. S3, Table S9). The water column exhibited the highest ΣPAH concentrations on day 4 (0.09 mg·mL^{-1}). No PAHs could be detected in week 2 whereas samples collected in weeks 4 and 6 had comparable concentrations (between 0.04-0.07 mg·L^{-1}, Fig. S3, Table S9). Dominant PAHs associated with microplastics were phenanthrene, fluoranthene, pyrene, and benzo[ghi]perylene. other PAHs were also present in lower concentration (Table S9). Many of these concentrations at both sites exceed both NOAA's acute or chronic screening thresholds for fresh, marine, or estuarine waters (Buchman, 2008), as well as those set by the EPA (<0.0001-0.0004 mg·L^{-1} for a given PAH compound (USEPA, 2015)).

3.5 Discussion

Our study focuses on the role of plastic polymer type, time exposed to the environment, and geographic location on the composition of the bacterial plastisphere of environmentally relevant secondary microplastics. We investigated the plastisphere developing on six common household consumer plastic type products incubated in ambient seawater of the coastal Pacific (San Diego, CA), and compared those communities with those growing on the same types of microplastics in incubations carried out in the Caribbean (Bocas del Toro, Panama, Dudek et al., 2020). Below we discuss the role of the different factors influencing the plastisphere.

3.5.1 Plastisphere composition in comparison with other studies

Dominant prokaryotic taxa found colonizing microplastics in this study from the Pacific site were Proteobacteria, most notably Rhodobacteraceae, Marinomonadaceae, Sphingomonadaceae, and Hyphomonadaceae, as well as Bacteroidetes, with Saprospiraceae and Flavobacteriaceae being the most dominant families. Several members of the Rhodobacteraceae, Hyphomonadaceae, and Flavobacteriaceae families have consistently been identified within the plastisphere. Zettler et al. (2013) identified OTUs within these families to be associated with, some uniquely, to plastic fragments floating in the Sargasso Sea, though many species of the Rhodobacteraceae, Hyphomonadaceae, and Flavobacteriaceae families were also present in the water column albeit in different relative abundances. Even in other controlled incubation studies, these families were omnipresent members of the plastisphere from HDPE and PS in the Baltic Sea (Oberbeckmann et al., 2018), or PVC, PP, HDPE, and LDPE in the Northern Adriatic

Sea (Pinto et al., 2019), PETE, HDPE, PVC, LDPE, PP, and PS in the Caribbean Sea (Dudek et al., 2020), and even PVC and PP pellets incubated in both the South China Sea and the Yellow Sea (Xu et al., 2019). Table 4 shows a comprehensive comparison between results obtained in our study with similar studies conducted in other ocean regions.

The families Sphingomonadaceae, Hyphomonadaceae, and Marinomonadaceae were significantly more abundant on all microplastics regardless of time compared to the ambient communities in this study. Many members of these families are known biofilms formers (Dang and Lovell, 2002; Stolz, 2009; López-Pérez and Rodriguez-Valera, 2014; Masák et al., 2014). For example, of the Sphingomonadaceae, the genera *Erythrobacter*, *Sphingopyxis,* and *Sphingomonas* have consistently been found in plastisphere samples, both in experimental incubations as well as from microplastics sampled directly from the environment (Zettler et al., 2013; Hoellein et al., 2014; Jiang et al., 2018; Oberbeckmann et al., 2018; Ogonowski et al., 2018). Many Sphingomonadaceae, including members of the genera *Erythrobacter* and *Sphingobium*, as well as members of the family Hyphomonadaceae, which are prosthecate bacteria that produce a polysaccharide holdfast allowing them to both attach firmly to surfaces, were all more abundant on microplastics than in the water column. Organisms within the Hyphomonadaceae family, too, are described as putative hydrocarbon degraders and have repeatedly been isolated from environments contaminated with petroleum-derived hydrocarbons (Kumar et al., 2008)

Consistent with results of previous studies (Zettler et al., 2013; Dussud et al., 2018; Jiang et al., 2018), bacteria of the genus *Vibrio* were observed on microplastics,

albeit at a very low relative abundance (<0.2%) on all samples. *Vibrio spp.* within the plastisphere have garnered attention due to the fact that some species are pathogenic to humans. It has been suggested that *Vibrio spp.* could act as a "hitchhiker" and use microplastics as a vector to travel into non-habitats (Kirstein et al., 2016) distributed by ocean currents (Barnes, 2002; Gregory, 2009), or via trophic utilization (Senderovich et al., 2010; Foekema et al., 2013; Cluzard et al., 2015). *Vibrio spp.* was not found to be abundant in the plastispheres investigated in our study, but was found to be a member of the core plastisphere. That is, *Vibrio spp.* was extant on microplastics at all time points (week 1, week 3, and week 6) and both sites (Caribbean and Pacific) suggesting that *Vibrio spp.* can grow in diverse environmental conditions. It is possible that *Vibrio's* presence relies on zooplankton mediation, as *Vibrio spp.* are known to be tightly associated with particles (i.e. fecal pellets) and copepod guts (Shoemaker and Moisander 2017). This may explain the phenomenon that *Vibrio spp*. dominate the plastisphere community in some studies (Zettler et al., 2013; Kirstein et al., 2016), while it rarely occurs in others (Myers et al., 2007; Bryant et al., 2016; Debroas et al., 2017, Oberbeckmann et al., 2018; Xu et al., 2019; Dudek et al., 2020). Additionally, while this group has been extensively studied for its ability to degrade the natural polymer chitin (Hays et al., 2017; Giubergia et al., 2016; Hunt et al., 2008), strains of this group have also been found to degrade PAHs (Hedlund & Staley 2001) which warrants further study into their bioremediation potential.

An indicator species analysis revealed a pattern of microbial colonization onto the microplastics in the Pacific. Pioneer biofilm formers are typically known to be versatile

in terms of their ability to successfully grow in a variety of temperature and pH conditions. Late biofilm members, on the other hand, have the tendency to be associated with eukaryotes or more specialized in nutrient acquisition. For example, *Halomonas sp.* which exhibited the highest relative abundance on PS at week 1 at 29.26%, are found from Arctic ice to hydrothermal vents (Okamoto et al., 2004). Other pioneer biofilm forming indicators from the Pacific site include the ubiquitous *Corynebacterium sp.,* which has been isolated from nearly every environment from human mucosal membranes (Rudresh et al., 2015) to marine sediments (Du et al., 2010), though our review of the literature shows that they are rarely found in microplastic biofilms. They are prominent microbial agents that are being studied for their degradation potential of plastic polymers and bioremediation potential of plastic pollution (Pathak 2017). Additionally, early biofilm indicators were *Prosthecobacter sp*., which thrive in low nutrient environments, as well as *Flavobacterium succinicans*, which were isolated from and associated with gill disease in Rainbow Trout in aquaculture (Good et al., 2015). While there were no intermediate (week 3) indicator taxa, the indicator taxa for the late-stage biofilms (week 6) contained several taxa associated with algae or phytoplankton, such as *Winogradskyella thalassocola* (Nedashkovskaya et al., 2005)*, Crocinitomix sp.,* (Shi et al., 2017) and members of the family Phycisphaeraceae (Fukunaga et al., 2009). *Dinoroseobacter sp.* was another plankton-associated indicator taxon for week 6; these bacteria are reported to have both mutualistic and pathogenic symbioses with dinoflagellates (Biebl et al., 2005). While low in relative abundance (0.02-0.66%), their presence likely depends on dinoflagellate mediation.

3.5.2 Factors determining plastisphere composition

3.5.2.1 The role of plastic type and incubation time

Hierarchical Cluster Analyses using the averages of unweighted pair-groups (UPGMA) and PCoA ordination analyses both reveal time of incubation to be a strong predictor of community composition, whereas plastic polymer type had an insignificant role (Fig. 7, Fig. 8, Table 2). The results derived from the null model analysis, however, did not always confirm this distinction. The prokaryotic communities on LDPE did not exhibit significant dissimilarity between time points, though the communities also were not significantly similar. The null model, too, revealed the plastisphere on LDPE to be significantly dissimilar to HDPE and PVC at one week of incubation, and dissimilar (albeit not significantly) to the plastisphere on PS (Table S4). The disparity in overall richness and sequence reads demonstrated between HDPE, PVC, PS and PETE, LDPE, PP likely explains this result, as LDPE had the highest number of reads (Table S2), whereas HDPE, PVC, and PS had the lowest (Table S2), and may explain why we do not see comparable patterns with PETE and PP (Table S2, Table S4) during week 1. Beyond the differences in week 1, our results support other studies that concluded that plastic polymer type is not a significant factor in shaping biofilm communities (Oberbeckmann et al., 2018; Kirstein et al., 2018; Ogonowski et al., 2018; Kirstein et al., 2019; Xu et al., 2019; Kesy et al., 2019, Table 4), even when identical microplastic particles and experimental methodologies, and source waters, are utilized (Dudek et al., 2020, Table 4). Furthermore, while this study investigated secondary microplastics, the results are in

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accordance with previous analogous studies that used primary microplastics

(Oberbeckmann et al., 2018; Pinto et al., 2019; Xu et al., 2019) suggesting a small role in

microplastic shape, stage of weathering, or potential chemical additives (i.e., anti-

microbials, flame retardants, phthalates, etc.) in shaping the plastisphere. Additionally,

the results of this study agree with a meta-analysis of 35 existing plastisphere studies by

Wright et al. (2020). These authors highlighted the importance of primer pair and DNA

extraction methodology in characterizing microbial communities, and also showed the

role of experimental design and environmental factors – but not plastic type, in the

composition of the microplastic-attached microbial communities. For example,

significantly different communities were observed on field-collected low-density

polyethylene (LDPE), polyethylene terephthalate (PET) and polypropylene (PP) in the

North Sea using denaturing gradient gel electrophoresis (DDGE) profiles

(Oberbeckmann et al., 2014). The improved resolution offered by next generation

sequencing, however, did not lead to any significant differences between the

communities developing on the same plastic polymers collected in the North Atlantic

(polyethylene and polypropylene; Zettler et al., 2013).

The temporal variability in community development as well as ambient exposure

conditions *in situ*, such as ambient communities, light, oxygen, nutrient availability, etc.,

may lead to a differentiation between plastic-associated communities on field-collected

particles. This would likely hamper comparisons between biofilm assemblages on field-

collected plastic debris with those colonizing plastic debris incubated in controlled

experiments. In field samples, where the history and age of the collected material are

unknown, substrate-specificity in biofilm composition is difficult to establish because species sorting is likely to be detectable only during the early stages of colonization (Zhang et al., 2014; Datta et al., 2016) and is often obscured by environmental conditions (Lee et al., 2015). While field studies are essential to provide the full range of microbial diversity in biofilms growing on plastics, it is necessary to design experiments with controlled exposure and a well-defined source community to be able to evaluate the variables that contribute to shaping the plastisphere.

3.5.2.2 The role of geography

There is a growing body of literature that focuses on the spatiotemporal distribution of microplastics, and the impact geographic location has on marine plastispheres. Based on a 6-week *in situ* exposure experiment of PETE at various locations in the North Sea, Oberbeckmann et al. (2014, 2016), found location-dependent microbial community assemblages. Additionally, Amaral-Zettler et al. (2015) found latitudinal gradients in species richness as well as polymer specific communities between microplastics collected in the North Atlantic and North Pacific subtropical gyres, with more significant differences in prokaryotic community composition between locations than between polymer types. In a metanalysis of studies focusing on the role geographic location on plastisphere composition, Oberbeckmann and Labrenz (2020; n = 5) reported that geographic location (the Baltic Sea, the North Sea, and the Yangtze Estuary), driven by differences in salinity and nutrient concentrations, and not plastic polymer type, significantly shaped community composition on microplastics. In our study, when comparing the microplastic-attached prokaryotic communities from the coastal San

Diego site to those from the coastal Caribbean in Bocas del Toro, Panama (Dudek et al., 2020), we found that incubation time drives both communities significantly. Geographic location, and their respective ambient communities, however, had a more significant impact on the composition of the plastisphere. While both the Pacific and Caribbean sites shared taxa, ambient communities, the most immediate source of microorganisms inoculating the plastic surface, were significantly different (Figure S2). In addition, the indicator taxa at the Pacific site did not correlate with any indicator taxa from the Caribbean site (Table S4, Table S7).

3.5.2.3 The role of PAHs

The differential abundance analysis revealed many taxa associated with either the Pacific or Caribbean sites. Marinomonadaceae, in particular the genus *Marinomonas*, had the second greatest log2-fold change value (behind Hyphomonadaceae). *Marinomonas* was an indicator taxon for weeks 3 and 6 at the Pacific site, signifying that it is not a pioneer colonizer, but rather that these organisms proliferate under more favorable conditions, such as when pioneer biofilm formers create a nutritionally rich conditioning film. Species of this genus are also known PAH degraders (Yuan et al., 2015; Dong et al., 2015). This *Marinomonas* was differentially abundant, and in high relative abundance, at the Pacific site in comparison with the Caribbean site. While the differences in temperature, salinity, light availability, or other environmental parameters (Fig. S1) could have caused the lack of *Marinomonas* in the Caribbean, the average concentration of PAHs sorbed onto the microplastics, in particular PS, was significantly higher in the Pacific site. *Marinomonas* was most relatively abundant at week 3 on LDPE (24.2% with

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18767 reads) but had the highest number of 16S gene copies for week 3 on PS (12.4% relative abundance with 37664 reads). While present on LDPE, which had no detectable PAHs throughout the time series (Fig. 12, Fig. S3), it is possible that the presence of sorbed PAHs, while toxic to some bacteria, provided *Marinomonas* with more nutrients and less competition on PS. *Erythrobacter* and the families Rhodobacteraceae and Hyphomonadaceae, many of which are known to utilize PAHs as a carbon and energy source, also significantly exhibited preference for the microplastics incubated at the Pacific site. Whether these organisms have the capacity to degrade the carbon-backbone of the plastic polymers is not known. A metagenome study of the plastisphere discovered an overrepresentation of genes involved in xenobiotic degradation processes (Bryant et al., 2016), however, whether the bacteria are exclusively taking advantage of the volatile compounds released from the plastics, such as monomers, additives, or of the organic pollutants that sorb to and leach from the surface of the polymers (Mato et al., 2001), or are instead actively degrading the polymer remains to be fully understood. Another known PAHs degrader that was differentially abundant was *Arthrobacter sp.*, which was more abundant at the Caribbean site. As discussed in Dudek et al. (2020), *Arthrobacter sp.* was most markedly abundant on LDPE after 1 week of incubation and on no other polymer at no other time point. LDPE at 1 week of incubation in the Caribbean site also exhibited the highest concentrations of PAHs (Fig 7, Table S9). While these contaminants may be contributing to significant differential abundance of specific taxa between plastic polymers or geographic locations, these same contaminants are not significantly altering the plastisphere as a whole.

3.5.2.4 The core plastisphere

While many studies aim to classify microplastic-attached taxa that are polymer-specific, temporally dependent, or defined for a particular location, this is the first study, to the best of our knowledge, to identify a core community that unites the plastispheres. A "core microbiome" has previously been described for biofilms present on both polyethylene sheet fragments and ropes incubated *in situ* in the North Sea (De Tender et al., 2017). In this study we found 57 core taxa shared between all time points and both sites (Fig. 11A). Many of these core taxa are unclassified beyond the family level, but we do see many ubiquitous taxa commonly associated with microplastics in the marine environment, such as Rhodobacteraceae, Flavobacteriales, Saprospiraceae, Hypomonadaceae, Altermonadaceae, and *Vibrio sp.* (Fig. 11B). It is likely that this core plastisphere is adapted for ideal cohabitation. For example, Rhodobacteraceae, in particular the Roseobacter group have been shown to be versatile in their physiologies (Collins et al., 2015) and account for large portions of bacterioplankton and biofilm communities alike (Wemheuer et al., 2015). Many of these marine Rhodobacteraceae (but not terrestrial taxa) have the gene encoding (S)-2-haloacid dehalogenase (Simon et al., 2017). The vast majority of organohalogens in the marine environment are produced by macroalgae, sponges, corals, tunicates, polychaetes and other marine organisms. These exudates are toxic for several bacteria, such as *Vibrio sp.* and *Acinetobacter sp.* (Simon et al., 2017). These rhodobacters presumably use dehalogenases for detoxification and potential utilization of the compounds as substrates (Novak et al., 2013). Conversely, as previously described, organisms such as *Vibrio sp.*, as well as

several Saprospiraceae species and Hypomonadaceae species, can utilize and break down other potentially toxic chemicals, such as the PAHs.

3.6 Conclusion

This study provides detailed prokaryotic plastisphere community analyses on secondary microplastics of six different polymers incubated in coastal Pacific waters and compares them to those incubated in the coastal Caribbean. We did not observe polymer specific assemblages, but instead determined that plastisphere composition was driven by the incubation time and geographic location. This study builds on a previous study with comparable analyses on bacterial plastispheres incubated in the coastal Caribbean (Dudek et al., 2020) and extends the concept of a "core microbiome" to a "core plastisphere" where possible synergies between taxa are observed. We inferred that while the presence of PAHs may not significantly alter the plastisphere, these compounds could be allowing more specialist bacteria, such as the PAH degraders of *Marinomonas sp.* and *Arthrobacter sp.* to proliferate.

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Tables

Table 1. Characteristics of microplastic particles used in this study.

Particle Type	Surface Area* (mm^2)	Weight* (mg)	Thickness* (mm)	Shape	Color	Plastic source
Polyethylene terephthalate (PETE)	6.44	2.09	0.270	Rectangular	Transparent	Disposable cups
High-density polyethylene (HDPE)	7.13	2.28	0.335	Rectangular	Cloudy/transparent	Milk containers
Polyvinyl chloride (PVC)	6.60	8.15	0.995	Rectangular	White	Unused ID cards
Low-density polyethylene (LDPE)	5.45	4.96	0.981	Rectangular	Cloudy/transparent	Squirt bottles
Polypropylene (PP)	9.49	3.15	0.360	Rectangular	White	Yogurt containers
Polystyrene (PS)	5.80	1.27	0.220	Rectangular	Transparent	Disposable cups

*Measurements are based on the average of ten particles

Table 2. PERMANOVA results testing the predictive strength of location, time of incubation, and plastic polymer type at each sire and between sites. "df": degrees of freedom, "SS": sum of squares, "R^2": variation in distances explained by the factor. P-values in bold indicate significance.

Factor	df	SS	R^2	p-value
Pacific site				
Time of incubation	2	2.08	0.42	**0.001**
Polymer Type	5	1.14	0.23	0.891
Caribbean site				
Time of incubation	2	0.61	0.36	**0.001**
Polymer Type	5	0.45	0.27	0.698
Pacific and Caribbean sites				
Location	1	3.54	0.35	**0.001**
Time of incubation	2	1.35	0.13	**0.006**
Polymer Type	5	0.77	0.08	1

Table 3. Indicator taxa at the highest classified taxonomic resolution that were significant (p-value <0.05, √Indicator value >0.8) for each time point and their range relative abundances across all plastic types. For each category, the top 10 indicator species (based on lowest p-values) are shown. See Supplemental Table S5 for a complete list of indicator taxa and their respective relative abundances.

Indicator Taxa	√Indicator Value	Relative Abundance (%)
Week 1		
Corynebacterium sp.	0.99	0.51-6.41
Flavobacterium succinicans	0.99	0.26-2.10
Chitinophagaceae	0.90	0.00-3.81
Sphingobacteriales	0.86	0.00-1.65
Prosthecobacter sp.	0.81	0.00-0.06
Cytophagaceae	0.81	0.00-1.47
Flavobacterium sp.	0.82	0.00-0.40
Halomonas sp.	0.91	0.00-29.26
Enterobacteriaceae	0.89	0.00-3.68
Week 6		
Amoebophilaceae; SC3-56	0.98	0.18-1.08
GMD14H09 (Order)	0.99	0.23-1.03
Plesiocystis sp.	1	0.02-0.23
Acinetobacter radioresistens	1	0.01-0.14
Winogradskyella thalassocola	0.98	0.02-0.93
Phycisphaeraceae	0.97	0.03-0.26
Myxococcales	0.89	0.16-0.37
Crocinitomix sp.	0.97	0.13-0.69
Dinoroseobacter sp.	0.96	0.02-0.66
Cryomorpha sp.	0.97	0.09-0.70
Week 1 and Week 6		
Tenacibaculum sp.	0.87	**Week 1**: 0.00-0.71; **Week 6**: 0.02-0.22
Saprospirales	0.87	**Week 1**: 0.00-1.19; **Week 6**: 0.01-0.18
Week 3 and Week 6		
Lewinella cohaerens	1	**Week 3**: 0.03-0.28; **Week 6**: 0.13-1.00
Croceitalea dokdonensis	1	**Week 3**: 0.07-0.49; **Week 6**: 0.40-0.86
BD7-3 (Order)	0.99	**Week 3**: 2.40-12.05; **Week 6**: 1.84-4.19
Hyphomonadaceae	0.97	**Week 3**: 6.67-9.19; **Week 6**: 4.83-9.75
Nautella sp.	0.98	**Week 3**: 1.39-9.48; **Week 6**: 1.43-3.43
Octadecabacter antarcticus	0.99	**Week 3**: 0.85-2.23; **Week 6**: 0.35-2.39
Sulfitobacter litoralis	1	**Week 3**: 0.01-0.50; **Week 6**: 0.10-0.45
Bacteriovorax sp.	0.99	**Week 3**: 0.51-1.12; **Week 6**: 0.19-0.41
Congregibacter sp.	1	**Week 3**: 0.11-0.75; **Week 6**: 0.29-0.86
Marinomonas sp.	1	Week 3: 7.18-24.25; **Week 6**: 0.43-1.71

Table 4. List of relevant studies that performed controlled microplastics incubations over an established time utilizing at least two different polymer types.

Location	Experimental Design/Sample Type	Time series	Plastic category	Plastic polymer type	Significant community differences as a result of:			Reference
					Plastic type	Incubation time	Location	
Coastal Baltic and effluent of a WWTP*	*In situ* controlled incubation	2 weeks	Primary	HDPE and PS	No	N/A	Yes	Oberbeckmann et al. 2018
Baltic Sea	Microcosm (glass beakers)	2 weeks	Primary	PE, PP, PS	No	N/A	N/A	Ogonowski et al. 2018
North Sea	Mesocosm controlled incubation	15 months	Macroplastic - films	HDPE, LDPE, PP, PS, PET, PLA, SAN, PESTUR, PVC	No	N/A	N/A	Kirstein et al. 2018
North Sea	Mesocosm controlled incubation	6 weeks and 5 months	Macroplastic - films	HDPE, LDPE, PP, PS, PET, PLA, SAN, PESTUR, PVC	Yes	Yes	N/A	Kirstein et al. 2019
Baltic Sea	Aerated indoor tanks	7 days	Primary	HDPE and PS	No	N/A	Yes	Kesy et al. 2019
Coastal Northern Adriatic	*In situ* controlled incubation	1 week, 1- and 2-months	Primary/ Secondary	LDPE, PP, PVC (Primary) HDPE (Secondary)	Yes	Yes	N/A	Pinto et al. 2019
The Yellow Sea and the South China Sea	*In situ* controlled incubation	1-, 3-, 6-, and 12-months	Primary	PVC and PP	No	Yes	Yes	Xu et al. 2019
Arabian Gulf	*In situ* controlled incubation	20 and 80 days	Secondary (plastic bags)	OXO-PE (oxo-biodegradable polyethylene), PE, PETE	Yes	Yes	N/A	Abed et al., 2020
Daya Bay of Shenzhen (Guangdong Province, China)	*In situ* controlled incubation	1-, 4-, and 8-weeks	Primary	PE, PP, PS, PVC, ABS (acrylonitrile butadiene styrene)	No	Yes	N/A	Zhang et al., 2021
Coastal Caribbean (Panama)	*In situ* controlled incubation	1-, 3-, and 6-weeks	Secondary	PETE, HDPE, PVC, LDPE, PP, PS	No	Yes	N/A	Dudek et al. 2020
Coastal Pacific (San Diego, CA)	Mesocosm controlled incubation	1-, 3-, and 6-weeks	Secondary	PETE, HDPE, PVC, LDPE, PP, PS	No	Yes	Yes	*This study*

PLA: Polylactic Acid; SAN: Styrene-acrylonitrile resin; PESTUR: Polyesterurethane

Figures
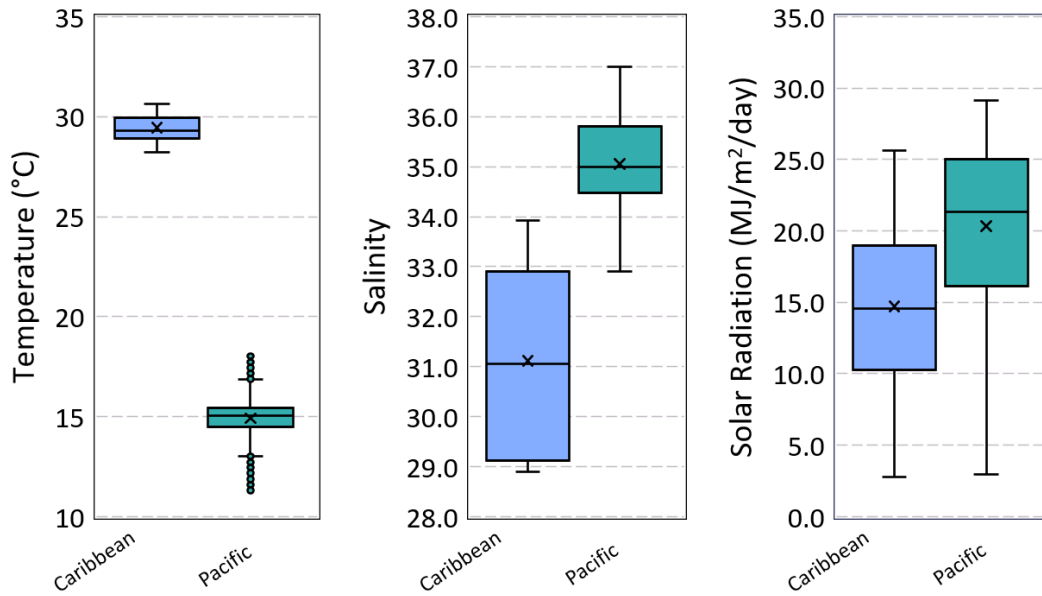
Figure 6. Box and whisker plot of environmental parameters of temperature (°C), salinity (‰), and solar radiation (MJ/m^2/day) for the Caribbean site in Bocas del Toro and the Pacific site in San Diego, California. Circles are outliers, the (x) is the average, the quartiles are divided by the median presented by a horizontal line.
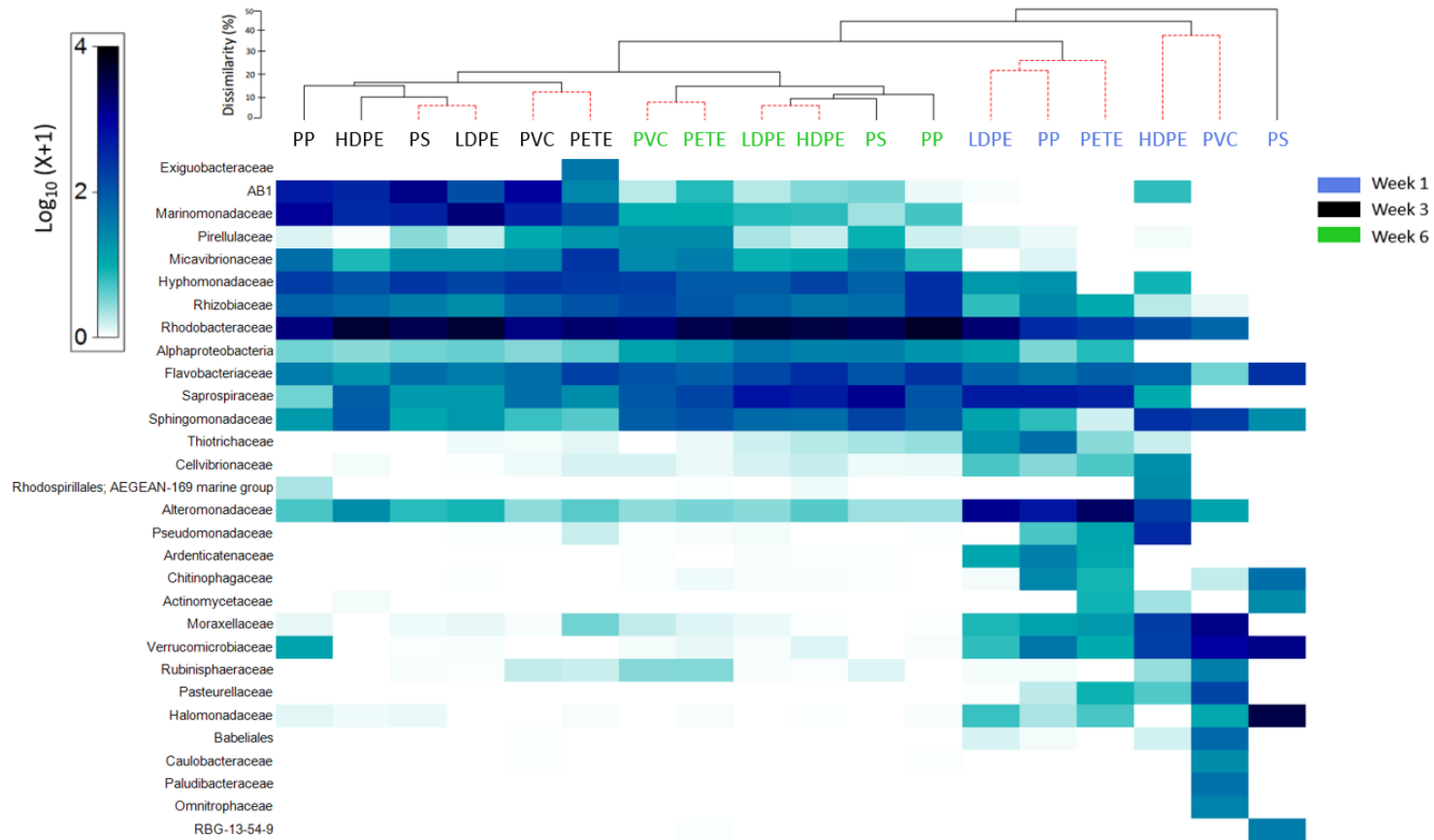
Figure 7. Heatmap depicting the relative abundances of the most abundant 30 prokaryotic taxa across all plastic samples incubated off the Scripps Pier (San Diego, CA) overlaid with a dendrogram that shows clustering of plastics with similar community composition. Black lines represent significant differences between plastic samples (p < 0.05, SIMPROF test) whereas red dotted lines signify similarity.
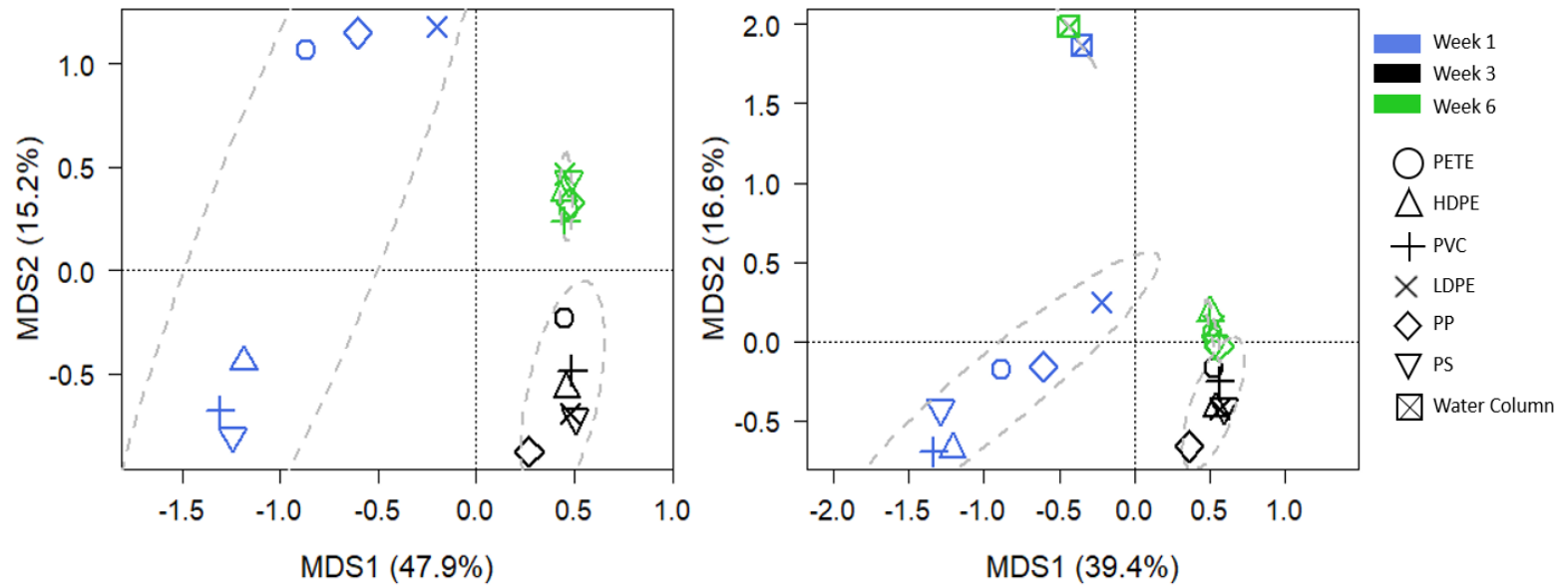
Figure 8. Principal coordinate analysis ordination plot based on Bray-Curtis dissimilarity between prokaryotic communities associated with microplastics at week 1, week 3, and week 6 of incubation without (A) and with (B) total water column communities. Hatched ellipses indicate 95% confidence intervals of all plastics for each time point.
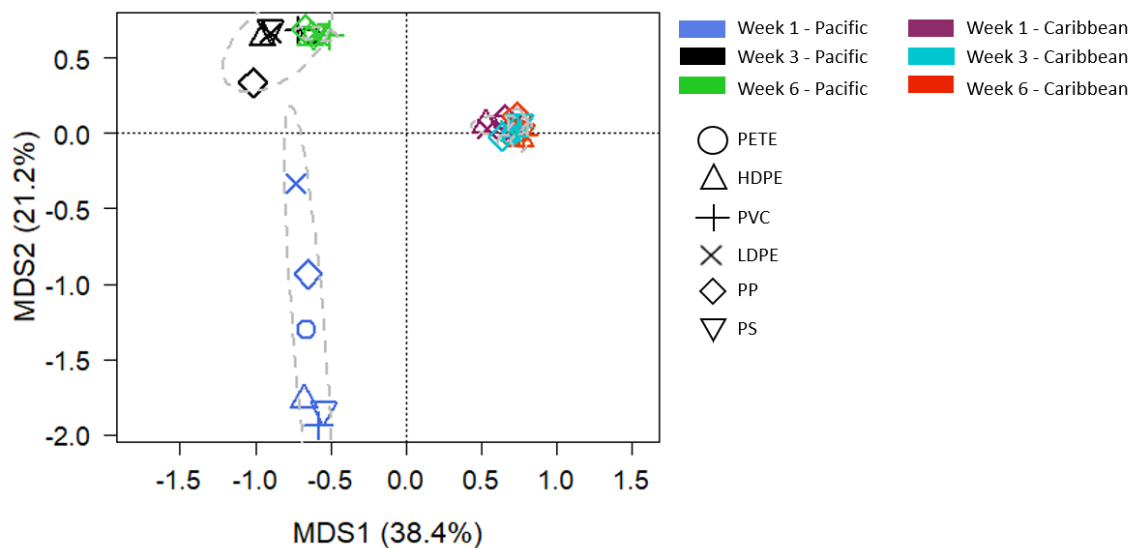
Figure 9. Principal coordinate analysis ordination plot based on Bray-Curtis dissimilarity between prokaryotic communities associated with microplastics at week 1, week 3, and week 6 of incubation at the Pacific site and Caribbean site. Hatched ellipses indicate 95% confidence intervals.
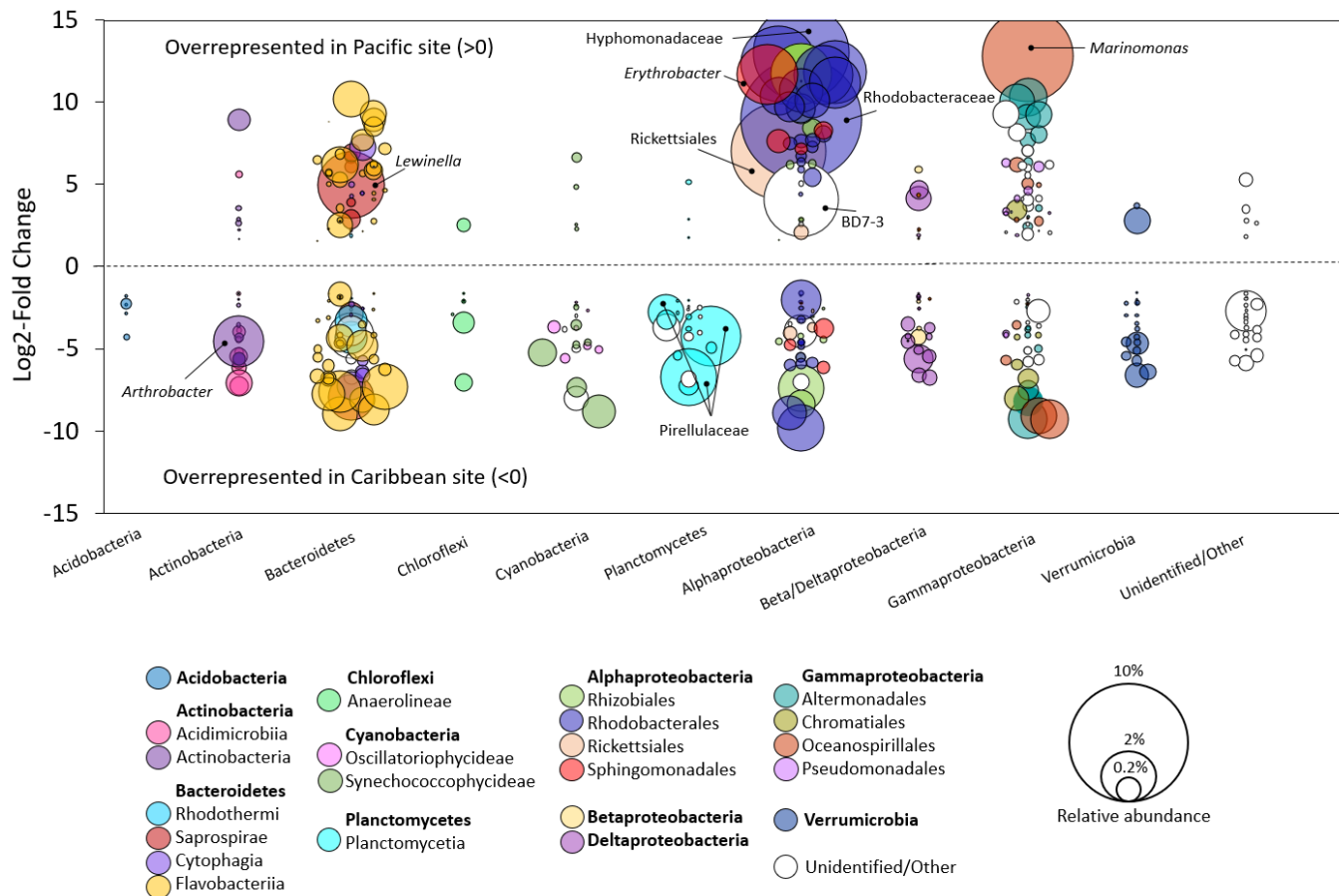
Figure 10. Differential abundance analysis of 16S rRNA gene sequences comparing Pacific (San Diego, CA) and Caribbean (Bocas del Toro, Panama) coastal sites. Positive log2 fold-change values indicate a significantly (adjusted p < 0.01) higher abundance at the Pacific sites, while negative values indicate significantly higher abundances in Caribbean sites. The area of each circle representing an individual taxon is proportional to the average relative abundance of that taxon across all samples within the site in which it is differentially abundant. Taxa that are characterized by either high log2-fold change values, high relative abundance, or both, are identified to a higher taxonomic level.
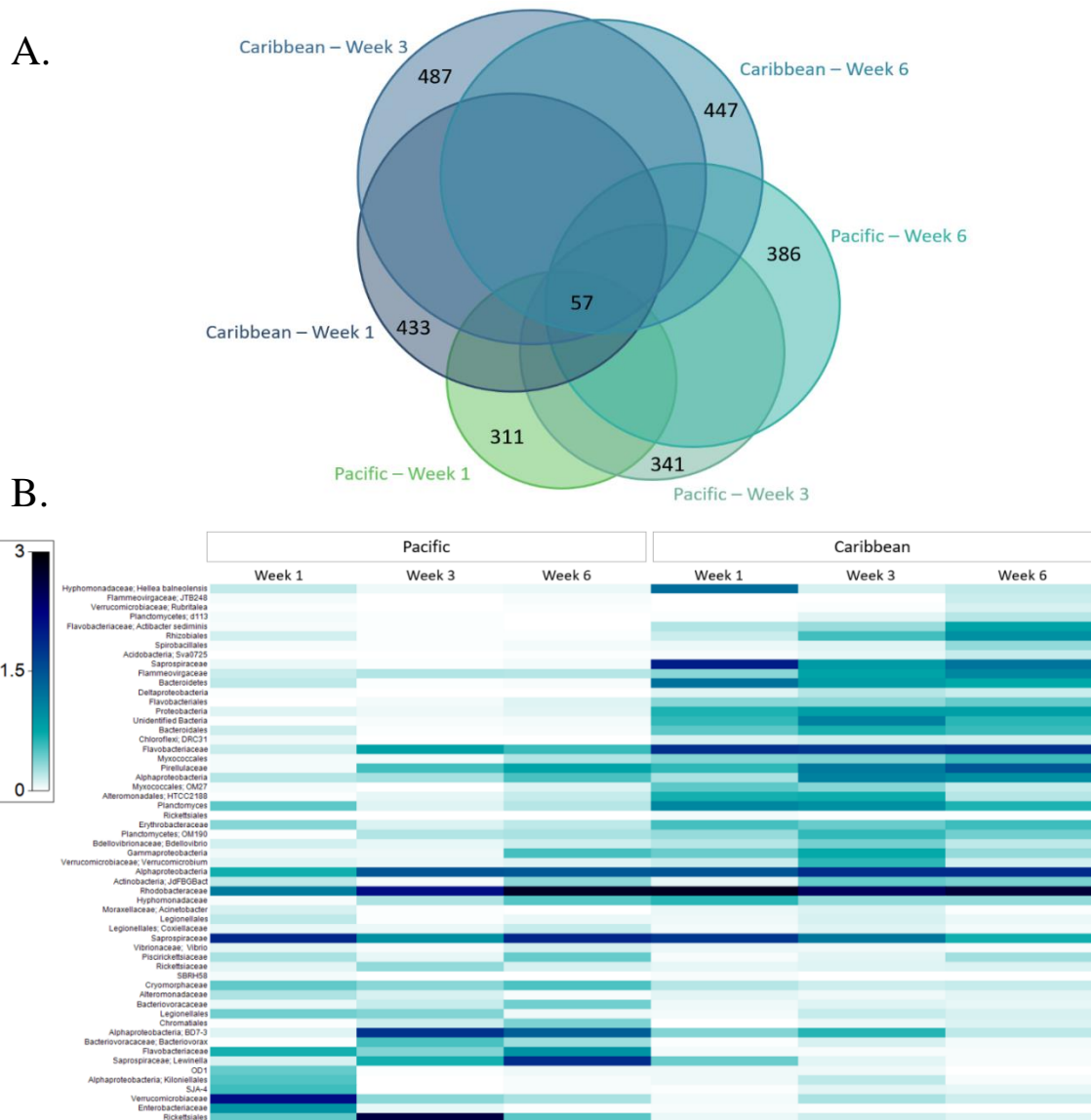
Figure 11. Venn diagram (**A**) displaying number of category specific (i.e. Pacific – Week 1, etc.) and core taxa in microbial communities across all plastic types in the Pacific Ocean and Caribbean Sea (Dudek et al. 2020. The size of the circle is proportional to the number of classified taxa. Heatmap (**B**) with all 57 core taxa and their relative abundance ($\log_{10}$) across time and location within the core community.
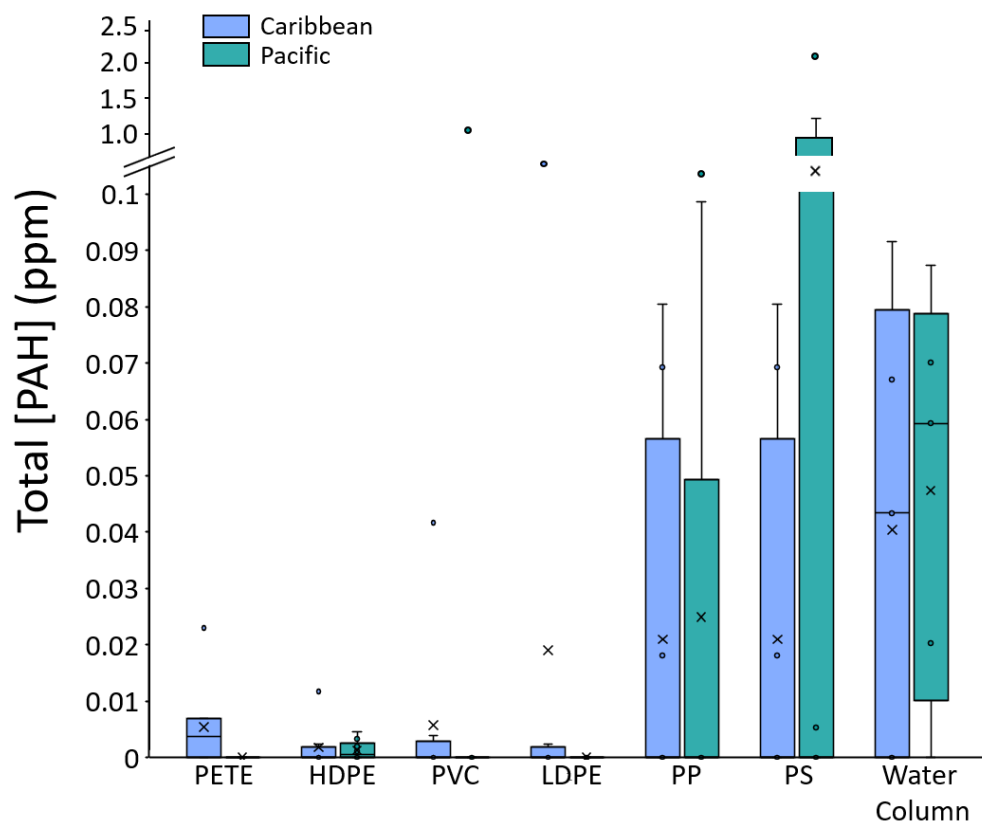
Figure 12. Box and whisker plot of the total PAH concentrations sorbed onto microplastics and in the surrounding water column across the time series' in both the Caribbean and Pacific sites. Circles are outliers, the (x) is the average, the quartiles are divided by the median presented by a horizontal line. In cases where outliers are near averages, the median is near zero.

CHAPTER 3 - BIOTIC AND ABIOTIC FACTORS AFFECTING MICROPLASTIC DEGRADATION AND DEPOSITION

The co-authors have acknowledged the use of this manuscript in my dissertation.

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4.1 Abstract

Although microplastics are ubiquitous in marine systems, our current knowledge on how biofilms that form on them affect their degradation and removal from the surface ocean is limited. Plastics denser than seawater entering the ocean logically sink (i.e., polyvinyl chloride or polyethylene terephthalate), but the fate in the water column becomes unpredictable for polymers like polystyrene (PS), whose density range (960-1040 kg/m^3) overlaps with that of surface seawater (1020-1029 kg/m^3). While plastic biodegradation by bacteria growing on microplastics is well known, biofilms can also slow plastic degradation by shielding them from UV radiation, or by enhancing microplastic sedimentation rates, but this has rarely been studied. We incubated microplastic particles (< 5 mm) of the common household consumer products polyethylene terephthalate (PETE, #1), high-density polyethylene (HDPE, #2), polyvinyl chloride (PVC, #3), low-density polyethylene (LDPE, #4), polypropylene (PP, #5), and polystyrene (PS, #6) in coastal waters of the Pacific (San Diego, CA) and the Caribbean (Bocas del Toro, Panama) under either sterile or non-sterile conditions to determine how biofilm formation affects their degradation and sinking rates. The formation of biofilms slowed degradation processes, as indicated by scanning electron microscopy (SEM) observations, and a reduced loss of phthalates, an indicator of plastic degradation. Additionally, biofilm formation enhanced sinking velocities of PETE, PVC, and PS at both sites, whereas PS required a biofilm in order to sink at the Pacific site. Our result show that biofilm formation impacts the degradation and transport behavior of microplastics in the ocean.

4.2 Introduction

Plastics are man-made long-chain polymeric materials that are extensively used in many aspects of everyday life due to their excellent characteristics such as light weight, flexibility, thermal and electrical insulation, corrosion resistance, and low cost. However, these same convenient characteristics allow plastics to persist in the environment, particularly in the marine realm (Oberbeckmann et al., 2015; van Sebille et al., 2015; Law 2017). Estimates of the plastic debris in the surface ocean range in the tens to hundreds of thousands of metric tons (Cózar et al., 2015; Law 2017; van Sebille et al., 2015), and only account for approximately 1% of the estimated millions of metric tons of floating plastic input from land (Jambeck et al., 2015; Lebreton et al., 2017). Once in the environment, plastic waste can slowly break down and generate numerous smaller plastic debris under the action of physical (i.e., wave action), chemical (i.e., photo-oxidation) and biological (i.e., biodegradation) degradation processes (PlasticsEurope 2019). These particles, typically less than 5 mm in size, are termed as "microplastics," and their persistence in the environment has become a global issue of concern (Thompson et al., 2004, Galloway and Lewis 2016; Eerkes-Medrano et al., 2015; Abel et al., 2018).

The fate of plastic debris in the marine pelagic environment is governed by transport and degradation processes (Halle et al., 2017). Although many studies have examined the aging of plastic polymers polyethylene (PE) and polypropylene (PP) (approximately 46% of all plastics produced worldwide (Rabnawaz et al., 2017)) (Stark and Matuana, 2004; La Mantia and Morreale, 2008), almost all have been conducted

utilizing accelerated weathering devices that utilize much higher temperatures or radiation exposures than those that would occur in the natural environment (Stark and Matuana, 2004). These studies do not consider the microbial biofilms that naturally grow on microplastic particles in the environment (Artham et al., 2009; Abed et al., 2020; Denaro et al., 2020; Dudek et al., 2020). These biofilms may reduce plastic degradation rates by acting as a sunscreen for floating microplastics, as shown by Weinstein et al. (2016) who found that biofilm formation on PP and polystyrene (PS) strips resulted in a decrease in UV transmittance by approximately 95%. Additionally, accreting biofilms may aid in the sedimentation of microplastic particles to greater ocean depths, thereby reducing degradation by removing the particles from the sunlit surface ocean to the dark and much colder deep ocean (Kershaw et al., 2011). The biofilms, alternatively, could aid in degradation, for example, by hosting hydrocarbon or plastic-degrading microbes, which are becoming more widely studied in their bioremediation potential for plastic pollution (Caruso 2015; Roager & Sonnenschein 2019). On the other hand, Emi-Cassola et al. (2020) concluded that while mature biofilms (9 days) on PE particles incubated in coastal Mallorca, Spain, did not utilize the plastic surface as a carbon source, early colonizing organisms (2 days) were perhaps able to metabolize plastic sub-products, such as phthalates (ester plasticizers) that leached from the plastic, thus contributing to the plastics' deterioration. Phthalates are not covalently bound to the plastic polymer and are leached out of the plastic into the environment, animal tissue, or even other plastics during abiotic/biotic aging processes (Andrady 2011; Palluselli et al., 2018).

Furthermore, Krause et al. (2020) used scanning electron microscopy (SEM) and contact

angle measurements to determine that no evidence of physical or chemical degradation on PS and PE consumer products (bags, lids, etc.) could be detected on the Pacific abyssal floor after 19-26 years after sedimentation. However, signs of biodeterioration possibly occurred due to altered wettability compared to reference materials. Because photodegradation, predominantly of wavelength from 0.295 to 0.400 µm, is widely recognized as the most important process leading to plastic degradation (Liu et al., 2019, Zhang et al., 2021), sinking of plastic below the euphotic zone of the ocean would inevitably slow this plastic degradation process.

It has been long suspected that the seafloor is a major sink for much of the plastic introduced to surface waters (Cózar et al., 2017; Van Cauwenberghe et al., 2013; Woodall et al., 2014). However, a large amount of plastic is positively buoyant in the marine environment (Cózar et al., 2015) and should not sink. And yet, both high-density and low-density polyethylene (HDPE and LDPE, respectively) alone, which have a specific density lower than seawater, are found on the sea floor (Holmström 1975), speaking for the existence of mechanisms that facilitate their sinking. Possible explanations for enhanced sinking are either the formation of biofilms that increase the specific density of the floating macro and microplastics they colonize (Morét-Ferguson et al., 2010; Andrady 2011; Woodall et al., 2014; Chubarenko et al., 2016; Fazey & Ryan 2016), or promote their incorporation into marine snow aggregates (Long et al., 2015; Porter et al., 2018) or zooplankton fecal pellets (Cole et al., 2016). Microbial colonization and community composition on microplastics varies in space and time as ambient water conditions differ between geographic locations or seasons (Carson et al., 2013;

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Oberbeckmann et al., 2014, 2015, 2016; Wright et al., 2020; Zhang et al., 2021, Dudek et al., in prep.). Additionally, differences in water temperature or salinity not only impact the ambient microbial communities, but the density of the ambient water, which has implications for certain plastic polymers whose density ranges overlap with typical oceanic water ranges (range: 1020-1029 kg/m^3, average: 1023 kg/m^3). For example, PS (non-expanded) has a density range of 960-1040 kg/m^3. Thus, a given PS microplastic could be positively, negatively, or neutrally buoyant depending on both ambient conditions and the presence and nature of existing biofilms.

The density, and thus fate, of plastic debris in the marine environment is governed by a net effect of bacterial and eukaryotic colonization as well as degradation and fragmentation. In order to establish whether biofilm formation influences the sinking behavior or degradation of microplastics, we incubated microplastics particles in ambient communities in the coastal Pacific (San Diego, CA) and coastal Caribbean (Bocas del Toro, Panama), as well as in sterile controls at both sites. We hypothesize that (1) biofilms decrease microplastic degradation by attenuating UV radiation, (2) biofilms increase the sinking velocity of negatively buoyant microplastic particles and cause the sinking of positively buoyant microplastic particles, and that (3) the effects of biofilm formation on sinking behavior of microplastics varies between oceanic environments.

4.3 Materials and Methods

Microplastic particles (1.5-5 mm in size) of the common household consumer products polyethylene terephthalate (PETE, #1), high-density polyethylene (HDPE, #2),

polyvinyl chloride (PVC, #3), low-density polyethylene (LDPE, #4), polypropylene (PP, #5), and polystyrene (PS, #6) were incubated in coastal waters of the Pacific (San Diego, CA) and the Caribbean (Bocas del Toro, Panama). Microplastic creation followed the processes described in **Chapter 1** and **Chapter 2**.

4.3.1 Microplastic incubations

  Caribbean site, Bocas del Toro, Panama. Two treatments, here identified as the "biotic" and "abiotic" treatments, were set up by incubating samples in natural or sterilized seawater inside of quartz tubes. Quartz tubes will transmit essentially all UV and visible light from the solar spectrum, thus mimicking the natural radiation environment while maintaining sterility and continuity between treatments. Tubes for "biotic" treatments were filled with natural seawater and capped (3 tubes per plastic type), which were exchanged with fresh seawater weekly. Sterile, abiotic treatments followed the same experimental design, but were filled with 0.2 µm pore sized filtered, autoclaved seawater and the water was never exchanged through-out the sampling period. Tubes were sampled at 1, 4, and 12 weeks of incubation from May to July 2019 where a tube was sacrificed for each sampling event. For both biotic and abiotic treatments, a 20g x ½ inch needle was punctured through the plastic cap of each tube and affixed with a 0.1 µm pore size filter to allow gas exchange and prevent pressure build-up. All tubes were incubated in 10L outdoor flow-through tanks (turn-over rate of about 5 minutes). At each sampling period, microplastics from both the biotic and abiotic treatments were placed in a settling cylinder (32.2cm) to calculate sinking speeds. The remaining plastics were

preserved for SEM, dry weight, and gas chromatography with mass spectroscopy (GC/MS) analyses.

   Pacific Site, San Diego, CA. Microplastics were incubated in a flow-through open-air sea water tank system (20L), with a turn-over rate of about 42 minutes, at Scripps Institute of Oceanography in La Jolla, San Diego, CA and sampled from March to July 2018, in weekly to biweekly time points. Only microplastics incubated at 1 week, 4 weeks, and 12 weeks for both the biotic and abiotic treatments were used for analyses. Unlike the samples incubated in the Caribbean, the microplastics of the biotic treatment at the Pacific site were incubated in sachets (see **Chapter 2**), while the microplastics for the abiotic treatment were incubated in quartz tubes and placed into the sachets. Quartz tubes for the abiotic treatment were filled with autoclaved artificial seawater (Instant Ocean®). Upon sampling, microplastics from both the biotic and abiotic treatments were placed in a settling cylinder (32.2cm) to calculate sinking speeds. The remaining plastics were preserved for SEM, dry weight, and gas chromatography with mass spectroscopy (GC/MS) analyses.

4.3.2 Environmental parameters

   At the Pacific coastal location, we obtained salinity, temperature, and chlorophyll *a* (Chl a) concentrations over the incubation period from the National Oceanic and Atmospheric Administration's (NOAA) Integrated Ocean Observing System in Southern California (https://sccoos.org/data/autoss/). This system consists of a suite of moored sensors attached to piers, and data were obtained from the Scripps Pier sensor. At each

sampling event, salinity and temperature were also measured manually with a YSI Model 85. Solar radiation metrics over the incubation period were obtained through the California Irrigation Management Information System (CIMIS) (https://cimis.water.ca.gov/). Additionally, a Quantum Solar Laboratory Radiometer (Model# QSL-2101) was used to measure both ambient radiation and light loss due to attenuation from algal growth on the microplastics' receptacle at each sampling event. In the Caribbean, environmental parameters were obtained as part of the STRI Physical Monitoring Program (https://biogeodb.stri.si.edu). Chl *a* concentration of the ambient water was analyzed at each sampling time point by filtering 65–250 mL in duplicate onto GF/F filters that were kept at −20°C until extraction in 5 mL of 90% acetone at 4°C for 24 h back at the ASU laboratory. Fluorescence of the chlorophyll extract was measured with a Turner Designs TD-700 Fluorometer.

4.3.3 Scanning electron microscopy

Microplastic samples after incubation were preserved in glutaraldehyde (Sigma-Aldrich, 5% (v/v)), cooled at 4°C for 2-8 hours, then transferred into 50% (v/v) ethanol in Phosphate Buffer Solution (PBS) and stored at -20°C until further preparation and imaging at the ASU laboratory. Samples were then dehydrated through a graded ethanol series and critical-point dried. The dried samples were mounted on aluminum stubs and sputter-coated with 10-15 nm of gold-palladium (60/40). Images were generated using a TESCAN VEGA[3] SEM operated at 15kV.

4.3.4 Phthalate measurements (GC/MS)

Microplastics of the biotic and abiotic treatments were preserved for GC/MS by placing 0.5-1g of microplastic particles in aluminum foil packets. These were then frozen in -20°C prior to being transported back to the ASU laboratory. Each microplastic subsample, including controls, was placed in a borosilicate glass test tube with Teflon-coated lids. Samples were treated with 15 ml of hexane, spiked with the recovery standard p-terphenyl, and then spun on a rotary mixer for 48 hours. Hexane extracts were removed, dried with $NaSO_4$ and concentrated to a final volume of 0.5 ml with nitrogen gas. Final extracts were spiked with the internal standard tetracosane-d50, and then analyzed for phthalates using a Varian 3800 gas chromatograph in tandem with a Saturn 2200 electron ionization mass spectrometer. Results are reported in micrograms of phthalates per gram of plastic, or parts per million (ppm).

4.3.5 Dry weights

After sampling, 10 particles of each polymer were placed in pre-weighed tin cups for determination of their weight after drying at 40°C for 24 hours (Analytical balance Mettler Toledo Model: XS105).

4.3.6 Microplastic sinking velocity

Upon sampling, microplastics 1-6 in both biotic and abiotic treatments were removed from their respective receptacles with sterile forceps and placed into a one liter settling column to determine their sinking velocities. Sinking velocities were determined

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in the field outdoors and the settling column was filled with GF/F filtered natural

seawater with temperature and salinity determined for each sinking experiment. The

sinking velocities of 6-9 negatively buoyant microplastics were measured with a

stopwatch through a vertical distance of 32.2 cm in the settling column and converted to

velocities in meters per day. For PS, the control (unincubated microplastics) was not

negatively buoyant at the Pacific site. In this instance, rising velocity was determined

instead by placing PS microplastics at the bottom of a wider 24.6 cm column with sterile

30.4 cm tweezers.

4.3.7 Microplastic excess density

Microplastic excess density ($\Delta\rho$) was determined using the Navier–Stokes drag

equation (Eq. 1) as in Iversen and Ploug (2010):

$$\Delta\rho = \frac{C_D \rho_w v^2}{\frac{4}{3}gESD} \tag{1}$$

where C_D is the dimensionless drag force defined in Eq. 2 for a Reynolds number

(Re, *see* Eq. 3) > 1, ρ_w is the density of seawater calculated at each sampling point using

the sea surface temperature(°C) and salinity, v is the average measured sinking velocity in

cm·s^{-1}, g is the gravitational acceleration of 981 cm·s^{-2}, and *ESD* is the equivalent

spherical diameter in cm (Eq. 4).

$$C_D = \left(\frac{24}{Re}\right) + \left(\frac{6}{1 + Re^{0.5}}\right) + 0.4 \tag{2}$$

Re is defined as:

$$Re = \frac{vESD\rho_w}{\eta} \tag{3}$$

where η is the dynamic viscosity of seawater (g·cm^{-1}·s^{-1}) determined at each sampling

point using the sea surface temperature(°C) and salinity and *ESD* is defined as:

$$ESD = \left(\frac{V}{\pi}\right)^{1/2} \tag{4}$$

where *V* is the measured volume (length x width x height; mean of n=10,) of each

microplastic particle (Pabst and Gregorova 2007).

4.3.8 Statistical Analyses

In order to test if biofilm formation modified microplastic weight, we tested for

the significance of any differences in dry weights between incubated and control (not

incubated) microplastics at the end of the time series (week 12) for both the biotic and

abiotic treatments via paired t-tests (two-tailed). Two-tailed t-tests were additionally

employed to determine if microplastic excess densities or sinking velocities were

significantly different between sites for both biotic and abiotic treatments, or within sites

between biotic and abiotic treatments.

4.4 Results

During the incubation period, at the Pacific site, water temperatures increased

from 11.3°C to 18.1°C and averaged 14.9°C whereas salinity averaged 35.1‰ (Fig. 13,

Table 5). Average solar radiation was 20.3 MJ/m^2/day and average chl *a* concentrations

were 4.21 μg·L^{-1} (Fig. 13). The Caribbean site had an average surface water temperature of 29.4°C (range 28.2-30.9°C), an average salinity of 31.1‰ (Fig. 13, Table 5), average solar radiation was 14.7 MJ/m^2/day over its respective time series, and an average chlorophyll *a* concentration of 0.79 μg·L^{-1} (Fig. 13). All averaged measurements include those measured at night. Water density was higher in the Pacific (1029.0-1030.4 kg/m^3) because of lower water temperatures and higher salinities compared to the Caribbean site (1022.9-1024.5 kg/m^3) (Fig. 13).

Manual measurements of solar radiation inside the sachets at the Pacific site showed that up to 78% of light was lost because of algal growth in the tanks and on the sachets at the end of the time series prior to being cleaned. This biofouling did not occur at the Caribbean study site, though some light attenuation, even at 1 meter below the surface, naturally would have occurred due to reflection at the water surface, and light attenuation in the water influenced by particulate and colored dissolved organic matter (Bricaud et al., 1998).

4.4.1 Degradation of microplastics

Degradation of microplastics was evidenced by the presence of extensive cracks, fractures, flakes, and pits in scanning electron microscopic (SEM) images of microplastics incubated in both the Caribbean (Fig. 14) and the Pacific (Fig. 15). In the Caribbean, microplastics incubated for 1 week in *in situ* (biotic) conditions exhibited several bacterial cells on all plastic types, with few diatoms present. PETE was the only microplastic type at week 1 that had several areas covered by a web of filamentous algae.

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By week 12, similar filamentous organisms could be seen on PETE and HDPE (Fig. 14) in addition to more diatoms and bacteria on most plastic types. Interestingly, LDPE had little biota attached to the microplastic particles (n=4) at 12 weeks of incubation, though it exhibited degradation in the form of small cracks across its entire surface (Fig 14, Fig 16A). PP, and to a lesser extent PVC, also exhibited signs of degradation within the biotic treatment by week 12 with long cracks observed on its surface. PETE, HDPE, and PS did not appear to show visual signs of degradation in the Caribbean in the biotic treatment at week 12. In the abiotic treatment, there were no clear signs of degradation at one week of incubation, but at week 12, pits can be seen in PETE, LDPE, and PS, where cracks can be seen on PVC and PP. LDPE exhibited distinctly different degradation patterns in the abiotic treatment compared to the biotic treatment (Fig. 16). The LDPE particles incubated in the biotic treatment at week 12 shows small cracks on its surface (Fig. 16A), whereas the microplastics incubated in the abiotic treatment exhibited pits (Fig. 16B). There were no visual signs of degradation for HDPE.

The microplastics incubated in the Pacific generally showed little growth at week 1 and week 12 in the biotic treatment. At week 1, few diatoms, in addition to bacteria, could be seen on PETE (n=3), PP (n=4), and PS (n=3), but HDPE, PVC, and LDPE only appeared to have bacterial cells attached to its surface (Fig. 15). At week 12, fewer diatoms were observed compared to week 1 across all plastic types. PVC, LDPE, and PP showed signs of degradation at week 12 via cracks and fissures whereas PETE, HDPE, and PS showed no visual signs of degradation (Fig. 15). In the abiotic treatment incubated in the Pacific, there were no clear signs of degradation at one week of

incubation for most of the plastic types, much like microplastics incubated in abiotic conditions in the Caribbean. PVC and LDPE, however, did show few pits on their respective surfaces at week 1. By week 12, HDPE, PVC, LDPE showed signs of degradation via cracks on the plastics' surfaces, while PP showed signs of degradation via pits, and PETE and PS exhibited no visual signs of degradation (Fig. 15). Unincubated microplastics were used for control comparisons for both the biotic and abiotic treatments at both sites (Fig. 17).

The loss or gain of phthalates associated with microplastics was measured throughout the time series in both the biotic and abiotic treatments at both sites. Identifiable phthalates were Diethyl phthalate, Dibutyl phthalate, 2,4-Bis(a,a-dimethylbenzy), Dicyclohexyl phthalate, Di(2-ethylhexyl)phthalate (DEHP), Di(2-ethylhexyl)-iso Phthalate (DEHP-iso), and Di-n-octyl phthalate. The biotic treatment at the Caribbean site showed a decrease in total phthalate concentrations across most plastic types, the exception being PP, which gradually increased in phthalate concentrations up to week 4 (0.55-4.67 $\mu g \cdot g^{-1}$) before decreasing to 0.05 $\mu g \cdot g^{-1}$ at week 12 (Fig. 18A). Cumulative phthalate concentrations on other microplastics (not including the control) ranged from 0.11-0.32 $\mu g \cdot g^{-1}$ on PETE, 0.03-0.55 $\mu g \cdot g^{-1}$ on HDPE, 0.17-2.0 $\mu g \cdot g^{-1}$ on PVC, 0.03-6.86 $\mu g \cdot g^{-1}$ on LDPE, and 0.05-1.20 $\mu g \cdot g^{-1}$ on PS (Fig. 18A). For the abiotic treatments, PETE ranged from 0.03-0.10 $\mu g \cdot g^{-1}$, HDPE ranged from 0.12-0.21 $\mu g \cdot g^{-1}$, PVC ranged from 0.04-0.09 $\mu g \cdot g^{-1}$, LDPE ranged from 2.49-5.13 $\mu g \cdot g^{-1}$, PP ranged from 0.05-0.24 $\mu g \cdot g^{-1}$, and PS ranged from 0.01-0.42 $\mu g \cdot g^{-1}$ (Fig. 18B).

The biotic treatment at the Pacific site yielded contrasting results to the Caribbean

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site, in that summed phthalate concentrations were highest at the end of the incubation period (week 12) for the plastics HDPE, LDPE, PP, and PS (0.28-11.70 $\mu g \cdot g^{-1}$, Fig. 18C) – concentrations that exceed those measured on the initial controls (0-10.22 $\mu g \cdot g^{-1}$, Fig. 17C). PETE and PVC were similar to the biotic treatment phthalate concentrations from the Caribbean, ranging from 0.05-0.22 $\mu g \cdot g^{-1}$ for PETE and 0.34-0.86 $\mu g \cdot g^{-1}$ for PVC (Fig. 18C). The sample for LDPE for the Pacific site biotic treatment at week 4 was lost in the field. For the abiotic treatments, PETE ranged from 0.03-0.07 $\mu g \cdot g^{-1}$, HDPE ranged from 0.13-1.62 $\mu g \cdot g^{-1}$, PVC ranged from 0.07-0.51 $\mu g \cdot g^{-1}$, LDPE ranged from 2.64-5.02 $\mu g \cdot g^{-1}$, PP ranged from 0.14-0.26 $\mu g \cdot g^{-1}$, and PS ranged from 0.03-2.66 $\mu g \cdot g^{-1}$ (Fig. 18D).

4.4.2 Weight change, excess density and sinking velocity of microplastics

At the Caribbean site, HDPE and PS significantly increased in weight by week 12 of incubation (HDPE: $t(9) = 4.57$, $p = 0.001$; PS: $t(9) = 4.53$, $p = 0.001$, Fig. 19, Table 6). For the abiotic treatments, no significance was observed, though we do see a general pattern of weight loss with each plastic type. At the Pacific site, PETE and PS significantly increased in dry weight in the biotic treatment (PETE: $t(9) = 5.30$, $p < 0.001$; PS: $t(9) = 3.41$, $p = 0.01$, Fig. 19, Table 6), and PETE, PVC, LDPE, and PP significantly decreased in dry weight in the abiotic treatment (PETE: $t(9) = -3.34$, $p = 0.01$; PVC: $t(9) = -2.92$, $p = 0.02$; LDPE: $t(9) = -2.86$, $p < 0.02$; PP: $t(9) = -3.10$, $p = 0.01$, Fig. 19, Table 6). PVC was the only plastic polymer to consistently decrease in weight regardless of incubation conditions. When comparing dry weights between the Caribbean and Pacific sites within the same treatment type and incubation time, PS had

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significantly higher weights in both the biotic and abiotic treatments at the Caribbean site (Table 7), while PVC had significantly higher weight in the abiotic treatment only at the Caribbean site (Table 7). The excess density of microplastics compared to the ambient seawater shows differences between biotic and abiotic treatments within a site (Fig. 20B, D, Table 8) and within the same treatment between sites (Fig. 20B, D, Table 9). In the Caribbean site, PETE had significantly greater excess densities at week 4 and week 12 of incubation in the biotic treatments ($t(6) = 3.25\text{-}6.07$, p = 0.02-<0.001, Table 8). PVC was significantly denser than the surrounding seawater at week 1 only in the abiotic treatment ($t(6) = -2.67$, p = 0.04, Table 8). PS showed significantly higher excess densities in the biotic treatment at all time points ($t(6) = 4.26 – 8.39$, p = <0.001 – 0.01, Table 8). For the Pacific site, PETE and PS microplastics had significantly greater excess densities in the biotic treatments at all time points (PETE: $t(6) = 14.09 – 18.51$, p < 0.001; PS: $t(6) = 5.62 – 17.86$, p = <0.001-0.005, Table 8).

When comparing excess densities within the same treatment, but between sites, PETE was significantly denser than the surrounding seawater in the Pacific at week 4 ($t(6) = -6.80$, p = 0.001. Table 9) but was significantly higher in excess density in the Caribbean at week 12 $t(6) = 3.99$, p = 0.01, Table 9). PVC had significantly higher excess densities in the Pacific at week 4 ($t(6) = -5.45$, p = 0.003, Table 9). PS did not exhibit any significant differences in excess densities in the biotic treatments between sites. In the abiotic treatments, PETE and PS both showed significantly higher excess densities in the Caribbean at all time points (PETE: $t(6) = 17.12 – 19.70$, p = <0.001, Table 5; PS: $t(6) = 7.01 – 15.45$, p < 0.001, Table 9). PVC was significantly higher in

excess density in the Caribbean at week 12 only ($t(6) = 3.44$, p = 0.02, Table 9).

Paired T-tests were used to compare sinking velocities between biotic and abiotic treatments of the same plastic type at the same time point between within the same site. In the Caribbean PETE sank significantly faster in the biotic treatments at week 4 ($t(6) = 2.86$, p = 0.01) and week 12 ($t(6) = 6.07$, p < 0.001, Table 10, Fig. 20). PVC exhibited significantly higher sinking velocities for the abiotic treatment at week 1 of incubation ($t(6) = -2.78$, p < 0.04). PS had significantly higher sinking velocities in the biotic treatment at every time point: week 1 ($t(6) = 5.59$, p = 0.001), week 4 ($t(6) = 3.57$, p = 0.01), and week 12 ($t(6) = 5.53$, p = 0.002, Table 10). In the Pacific site, PETE and PS showed significantly higher sinking velocities in the biotic treatment compared to the abiotic treatment (PETE: $t(6) = 18.06 – 26.13$, p = <0.001; PS: point ($t(6) = 7.11 – 20.32$, p = 0.002 - <0.001, Table 10, Fig. 20). PVC showed significantly higher sinking velocities in the biotic treatment at week 4 only ($t(5) = 3.84$, p = 0.01, Table 10, Fig. 20). When comparing the microplastics incubated in biotic conditions between the Caribbean and Pacific sites, PETE sank significantly faster in the Caribbean site than the Pacific site at week 1 ($t(6) = 3.21$, p = 0.02) and week 12 ($t(6) = 5.47$, p = 0.002, Table 11, Fig. 20) of incubation. PS sank significantly faster at the Caribbean site at week 4 of incubation ($t(6) = 2.61$, p = 0.04, Table 11, Fig. 20), and PVC did not exhibit any significance in sinking velocities between sites at any time point. When comparing the microplastics incubated in abiotic conditions between the Caribbean and Pacific sites, PETE and PS sank significantly faster at all time points (PETE: $t(7) = 21.59\text{-}34.68$, p < 0.001; PS: $t(7) = 13.74\text{-}37.34$, p < 0.001, Table 11, Fig. 20), and PVC significantly differed at week 4

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($t(7) = 5.76$, p = 0.001) and week 12 ($t(9) = 4.12$, p = 0.01, Table 11, Fig. 20) of incubation.

4.5 Discussion

Qualitative and quantitative results support the hypothesis that biofilms reduce microplastic degradation, likely by attenuating UV radiation. At both sites, visual signs of degradation were more numerous and more commonly observed in the abiotic treatments compared to the biotic treatments. In general, phthalate and weight (mass) loss was greater in abiotic treatments in comparison to biotic treatments at both sites, further indicating enhanced plastic degradation in comparison with the biotic treatments, though mass loss in the biotic treatment could have been counteracted by mass gain from biofilm formation, which did not occur in the abiotic treatments.

The hypothesis that biofilms increase the sinking velocity of negatively buoyant microplastic particles was supported, but the hypothesis that biofilms cause the sinking of positively buoyant microplastic particles was not. At both sites, negatively buoyant microplastics PETE and PS significantly increased in sinking velocity and excess density, whereas PVC was negligibly affected by its biofilm. Positively buoyant microplastics HDPE, LDPE, and PP never sank in either the biotic or abiotic treatments at either site. Finally, the hypothesis that biofilm formation affects the sinking behavior of microplastics varies between locations was supported, but was dependent on plastic type and incubation time.

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4.5.1 Role of biofilms on plastic degradation

        While hydrocarbon degrading bacteria exist within the plastisphere on

microplastics incubated in ambient seawater, such as *Arthrobacter sp.* and *Alcanivorax*

sp. in the Caribbean (Dudek et al., 2020) or *Erythrobacter sp.* and *Marinomonas sp.* in

the Pacific (Dudek et al., in prep), our results show that biofilm formation ultimately

decreased microplastic degradation. SEM images show visual signs of degradation at

week 12 in abiotic treatments, and even biotic treatments. For example, the LDPE

particles in the biotic treatment incubated in the Pacific began to crack at week 12, but

the stage of degradation in the abiotic treatment is far greater as evidenced by many more

cracks and fissures (Fig. 15). As the density of LDPE ranges between 910-940 kg/m^3, it is

unlikely to sink in most marine systems. The presence of a biofilm appears to slow the

degradation process of this polymer not by allowing it to sink, but by attenuating UV

radiation. Plastic polymers HDPE and PVC, too, appear to be protected to some extent by

its accreting biofilm in the Pacific location as loss in plastic integrity is clear in the

abiotic treatment in comparison the biotic treatment at 12 weeks (Fig. 15). With PVC,

however, because it will likely always sink in the marine environment (PVC density

~1380 kg/cm^3), photodegradation plays a small role. While UV radiation clearly

enhances the degradation rates of this polymer, its polymer break-down in the natural

environment is likely affected by biodegradation or hydrolysis. Interestingly, these signs

of degradation in the abiotic treatments in the Pacific site are not mirrored in the

Caribbean site. While the Pacific site had significantly higher solar radiation than the

Caribbean site, the effects of the algal growth in the tank and sachets, despite being

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regularly cleaned, reduced light availability in the sachets. Any visual evidence of plastic degradation in the Caribbean was in the form of pits instead of the cracks seen in microplastics at the Pacific site. In the abiotic treatments, the only difference between the sites was that the Caribbean microplastics were incubated in filtered, autoclaved natural seawater, whereas the Pacific samples were incubated in artificial seawater. Because plastics are resistant to chemical corrosion, especially at such small time scales, any additional ions present in the natural seawater should not have had an influence in plastic degradation.

In addition, phthalate loss was on average much higher in the abiotic treatments compared to the biotic treatments. Phthalate loss is a proxy for plastic degradation (King et al., 2020), as phthalate loss leads to decreased flexibility and increased fragmentation of plastics (Richardson et al., 2014). While phthalates readily leach from plastic, they, too, readily sorb back to its surface using a process called 'cross migration,' and is heavily dependent on the polymer pore size, the molecular weight of the phthalate, and water solubility of the phthalate (Teuten et al., 2009). This process likely occurred for microplastics HDPE, LDPE, and PP incubated in the Pacific as phthalate concentrations were higher than the controls at week 12 of incubations for the biotic treatment. DEHP was the dominant phthalate on HDPE, LDPE, and PP at 12 weeks of incubation (70-96% of total phthalates). DEHP are more resistant to migration owing to their hydrophobicity and high molecular weight, which causes less release from the polymer surface, but a higher capacity to sorb onto plastics once in the environment (Teuten et al., 2009).

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The differences in the microscopic biofilm between particles incubated at the Caribbean and Pacific sites is due in part to the different water conditions. While Chl *a* concentrations were much higher at the Pacific site, with an average of 4.21 $\mu g \cdot L^{-1}$ and a maximum of over 25 $\mu g \cdot L^{-1}$, the Caribbean site had a much lower phytoplankton biomass with an average concentration of 0.79 $\mu g \cdot L^{-1}$. Despite this, due to the algal growth on sachets previously described at the Pacific site, less light was available for photosynthetic organisms at the Pacific site. Cleaning of this algal growth on sachets, as well as the incubation tanks, could only be done on a weekly to biweekly basis. This, coupled with slower water exchange rates, led to the stagnant build-up of algae in the tanks as well as the sachets and, to a lesser extent, on the quartz tubes incubated at the Pacific site. These algae, by the end of the incubation period, caused up to 87% of ambient light loss on microplastics incubated in the biotic treatment, and 52% of light loss on microplastics incubated in the abiotic treatment. At the Caribbean site, the tanks could be monitored daily, and the water exchange rates were much higher, so algal over-growth did not occur. The observed differences on microplastic degradation between biotic and abiotic treatments at both sites show that while biofilm formation by photosynthetic organisms might be decreased at the Pacific location, differences between treatments were well captured by this experiment.

4.5.2 The role of biofilms in microplastic sinking rates

Biofilm formation on microplastics enhanced sinking. Biofouling significantly increased the sinking velocities of negatively buoyant microplastic particles of PETE and

PS, though did not have a significant impact on negatively buoyant PVC. Our hypothesis that biofouling would allow positively buoyant microplastics (HDPE, LDPE, and PP) to sink was not supported as these plastics never sank during the time series. However, longer time series, or different ambient conditions, may yield different results. Additionally, as hypothesized by Kaiser et al., (2017), a microscopic biofilm may be insufficient to allow a positively buoyant microplastic to become negatively buoyant, but that the colonization by macro-organisms, such as mussels or barnacles, is necessary to transport positively buoyant microplastics down the water column. This may explain why much of the "lighter" plastic polymers found on the seafloor are in the form of macro-plastics, such as bags, bottles, or lids (Krause et al., 2020). Due to the small size of microplastics, macro-organisms are unlikely to attach to the surface of microplastics. It is possible that low-density microplastics found in sediments may have fragmented after reaching the seafloor, perhaps due to mega-fauna interactions (Angiolillo et al., 2021), anoxic biodegradation (Giacomucci et al., 2020), or mechanical fragmentation upon sampling. Additionally, Amaral-Zettler et al. (2021) concluded that microorganisms alone cause positively buoyant microplastics to sink only with surface-area:volume (SA:V) ratios above 100, and that it is primarily multicellular organisms that cause the sinking of plastics with SA:V ratios below 100. This suggests a shift in importance of invertebrates vs. microbes to cause these floating microplastics (i.e., PE and PP) to sink depending on microplastic shape and size. Additionally, Kaiser et al., (2017) found that cylindrical PE particles (approximated SA:V = 5.99) only began to sink after being colonized by mussels after six weeks of incubation in the coastal Baltic Sea . SA:V ratios

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of all microplastics used in our study were between 3.15 – 7.85. Despite their size or shape, any attached multicellular organisms were not enough to cause positively buoyant HDPE, LDPE, and PP to sink in the Caribbean nor Pacific sites.

For the negatively buoyant microplastics, our hypothesis that biofilm formation affects the sinking behavior of microplastics and is variable between oceanic environments was supported. At the Caribbean site, PETE and PS significantly increased in excess densities and sinking velocities after biofilm formation, though this significance was observed later for PETE (week 4 to week 12) than for PS (week 1 to week 12) (Table 5, Table 7) despite the excess of algal growth observed on PETE and not PS at week 1 (Fig. 14). Algal and bacterial cells were observed in lower densities on PS compared to PETE. It is possible that microorganisms have difficulties attaching to PS, which has a water contact angle of 83-91° (Van Melkebeke et al., 2020), whereas PETE has a water contact angle of 63-83° (Van Melkebeke et al., 2020). Polymers with water contact angles < 90° are hydrophilic, and water contact angles > 90° are hydrophobic (Ko et al., 1981, Van Melkebeke et al., 2020). Microorganisms attach more rapidly, and proliferate quicker, when attached to hydrophobic, nonpolar surfaces (most plastics) compared to hydrophilic surfaces (i.e., glass or metal) (Donlan 2002), which causes low attachment efficiency and leads to cell stress and subsequent extracellular polymeric substance (EPS) production (Donlan 2002; Vosshage et al., 2018). These few, but stressed cells could have produced more EPS on PS than on PETE, increasing the densities and sinking velocities for PS earlier in the time series.

At the Pacific site, biofilms significantly enhanced sinking of PETE and PS at all time points. Biofilms particularly influenced PS, causing the particles to change from positively buoyant to negatively buoyant. The unincubated PETE particles sank much slower in the Pacific than in the Caribbean, indicating the role of ambient water density on the sinking behavior of this polymer. In addition, biofilm formation significantly impacted PETE excess densities and sinking velocities throughout the entire time series at both sites in the biotic treatments (Fig. 20, Tables 8-11). However, PETE at week 1 and week 12 had significantly higher sinking velocities in the biotic treatments in the Caribbean (Fig. 20A, C, Table 11), in that these particles sank faster in the warmer, less saline tropical waters than they did in the colder, more saline coastal Pacific waters. The web of filamentous algae observed on the Caribbean PETE samples (Fig. 14), and near absence eukaryotic of growth on the PETE microplastics incubated in the Pacific (Fig. 15), likely contributed to these differences in sinking velocities. The integral role of biofilms in the deposition of PS is also evident in the Pacific location. Prior to biofilm formation, PS remained positively buoyant and only began to sink once a biofilm had formed. Excess densities and sinking velocities of PS were all significantly higher in the biotic treatment compared to the abiotic treatment at all times points. When comparing the PETE and PS microplastics in the abiotic treatments between sites, excess density and sinking velocities were significantly higher in the Caribbean at all time points. Again, the role in ambient water density is vital in understanding the hydrodynamic behavior of these particles.

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Much of the current research on the role of biofilm formation on microplastic sinking has been carried out in freshwater systems, but studies share conclusions with this study. In three freshwater environments in China (the Niushoushan River, the Qinhuai River, and East Lake), microplastic polymers made from common household consumer products, PETE and PVC significantly increased in sinking velocity and density after 44 days of incubation, whereas PP remained positively buoyant after the incubation period (Miao et al., 2021). In contrast, Chen et al. (2019) reported that PP (in the form of floating sheets) became negatively buoyant in East Lake (Wuhan, China) after 30 days of incubation in three different seasons, and that the sinking rates were season dependent (i.e., faster in warmer seasons, slower in colder seasons), supporting the role ambient water density has on microplastic sinking behavior. In addition, these contrasting results in the same location, utilizing the same plastic polymer type, reinforce the importance of shape in sinking behavior (Van Melkebeke et al., 2020).

4.6 Conclusion

This study provides extensive analyses on the effect of biofilm formation on the degradation and sinking velocities of six different polymers incubated in natural seawater. Biofilms appear to slow degradation processes for positively buoyant particles HDPE and LDPE, though the extent of which was dependent on ambient seawater conditions. Biofilm formation, too, enhanced the excess densities and sinking velocities of negatively buoyant microplastics PETE, PVC, and PS. We also show the importance of ambient water density in the hydrodynamic behavior of negatively buoyant

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microplastics, in particular PETE and PS. PS exhibits a range of densities that closely

overlap with that of seawater, and was particularly sensitive to changes in ambient

conditions, and controls only sank in the warmer, less saline Caribbean. In the colder,

more saline Pacific water, PS needed the growth of a biofilm to become negatively

buoyant. We conclude that these biofilms also decrease degradation rates for negatively

buoyant microplastics by increasing their sinking velocities to deeper water where

photooxidation cannot occur.

4.7 References

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Tables

Table 5. Environmental parameters at the Caribbean and Pacific sites at each time point during sinking experiments.

Location	Time of incubation	Temperature (°C)	Salinity	Water Density (kg/m^3)
Bocas del Toro, Panama	Week 1	29.0	32.6	1024.5
	Week 4	30.3	31.0	1022.9
	Week 12	29.0	31.2	1023.8
La Jolla, San Diego, CA	Week 1	15.0	35.1	1030.4
	Week 4	15.2	34.7	1030.1
	Week 12	21.7	35.5	1029.0

Table 6. T-statistics and p-values of paired T-tests (two-tailed) comparing dry weight gain or dry weight loss between unincubated microplastics (control) and microplastic incubated for 12 weeks among biotic and abiotic treatments in the Caribbean and Pacific sites. Significant results ($\alpha = 0.05$) are shown in bold.

Plastic Type	Caribbean				Pacific			
	Biotic		Abiotic		Biotic		Abiotic	
	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value
PETE	1.73	0.117	-1.76	0.113	**5.30**	**<0.001**	**-3.34**	**0.01**
HDPE	**4.57**	**0.001**	-1.82	0.10	2.17	0.06	-0.92	0.38
PVC	-0.96	0.36	-1.54	0.16	-0.66	0.52	**-2.92**	**0.02**
LDPE	0.43	0.68	-1.04	0.33	-0.40	0.70	**-2.86**	**0.02**
PP	1.08	0.31	-2.02	0.07	1.07	0.31	**-3.10**	**0.01**
PS	**4.53**	**0.001**	0.28	0.79	**3.41**	**0.01**	-1.92	0.09

Table 7. T-statistics and p-values of paired T-tests (two-tailed) comparing dry weight gain or dry weight loss between sites (Caribbean and Pacific) within the biotic or abiotic treatments at week 12 of incubation. Significant results ($\alpha = 0.05$) are shown in bold.

Plastic Type	Caribbean vs Pacific			
	Biotic		Abiotic	
	t-stat	p-value	t-stat	p-value
PETE	-0.93	0.38	0.60	0.56
HDPE	0.34	0.74	-0.94	0.37
PVC	-1.09	0.30	**2.91**	**0.02**
LDPE	1.06	0.32	1.72	0.12
PP	-0.09	0.93	-0.28	0.78
PS	**2.41**	**0.04**	**3.53**	**0.001**

Table 8. T-statistics and p-values of paired T-tests (two-tailed) comparing **excess densities between the biotic and abiotic treatments** at week 1, week 4, and week 12 of incubation **withing the Caribbean or Pacific sites**. A positive t-stat indicates higher excess densities on microplastics compared to the ambient seawater in the Caribbean, whereas a negative t-stat indicates higher excess densities for the Pacific site. Significant results ($\alpha = 0.05$) are shown in bold.

Plastic Type	Biotic vs Abiotic - Caribbean						Biotic vs Abiotic - Pacific					
	Week 1		Week 4		Week 12		Week 1		Week 4		Week 12	
	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value
PETE	1.65	0.15	3.25	**0.02**	6.07	**<0.001**	18.51	**<0.001**	17.65	**<0.001**	14.09	**<0.001**
PVC	-2.67	**0.04**	1.72	0.14	-2.44	>0.05	1.57	0.18	3.24	**0.02**	3.23	**0.02**
PS	5.43	**0.005**	4.26	**0.01**	8.39	**<0.001**	5.62	**0.005**	17.86	**<0.001**	9.39	**0.002**

Table 9. T-statistics and p-values of paired T-tests (two-tailed) comparing **excess densities within the biotic and abiotic treatments** at week 1, week 4, and week 12 of incubation **between the Caribbean and Pacific sites**. A positive t-stat indicates higher excess densities on microplastics compared to the ambient seawater in the Caribbean, whereas a negative t-stat indicates higher excess densities for the Pacific site. Significant results ($\alpha = 0.05$) are shown in bold.

Plastic Type	Biotic Treatment						Abiotic Treatment					
	Week 1		Week 4		Week 12		Week 1		Week 4		Week 12	
	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value
PETE	1.58	0.17	-6.80	**0.001**	3.99	**0.01**	17.83	**<0.001**	17.12	**<0.001**	19.70	**<0.001**
PVC	-1.39	0.21	-5.45	**0.003**	-2.62	0.05	2.06	0.09	0.34	0.67	3.44	**0.02**
PS	-0.53	0.62	-0.27	0.80	-1.53	0.19	15.45	**<0.001**	7.01	**<0.001**	7.82	**<0.001**

Table 10. Paired T-tests (two-tailed) comparing sinking velocities of the biotic and abiotic treatments of the same plastic type and the same time point. A positive t-stat indicates higher weight in the Caribbean site, while a negative t-stat indicates higher weight in the Pacific site. Significant results (α = 0.05) are shown in bold.

Plastic Type	Biotic vs Abiotic - Panama						Biotic vs Abiotic - Pacific					
	Week 1		Week 4		Week 12		Week 1		Week 4		Week 12	
	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value
PETE	1.65	0.08	2.86	**0.01**	6.07	**<0.001**	25.20	**<0.001**	26.13	**<0.001**	18.06	**<0.001**
PVC	-2.78	**0.04**	1.69	0.14	-2.42	>0.05	0.52	0.62	3.84	**0.01**	2.29	0.07
PS	5.59	**0.001**	3.57	**0.01**	5.53	**0.002**	7.11	**0.002**	20.32	**<0.001**	11.62	**<0.001**

Table 11. T-statistics and p-values of paired T-tests (two-tailed) comparing **sinking velocities within the biotic and abiotic treatments** at week 1, week 4, and week 12 of incubation **between the Caribbean and Pacific sites**. A positive t-stat indicates faster sinking velocities, whereas a negative t-stat indicates higher sinking velocities. Significant results ($\alpha = 0.05$) are shown in bold.

Plastic Type	Biotic Treatment						Abiotic Treatment					
	Week 1		Week 4		Week 12		Week 1		Week 4		Week 12	
	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value
PETE	3.21	**0.02**	-0.93	0.39	5.47	**0.002**	21.59	**<0.001**	22.05	**<0.001**	34.68	**<0.001**
PVC	0.79	0.46	2.18	0.08	-1.53	0.19	2.13	0.08	5.76	**0.001**	4.12	**0.01**
PS	0.95	0.40	2.61	**0.04**	-1.23	0.26	13.74	**<0.001**	37.34	**<0.001**	16.54	**<0.001**

Figures
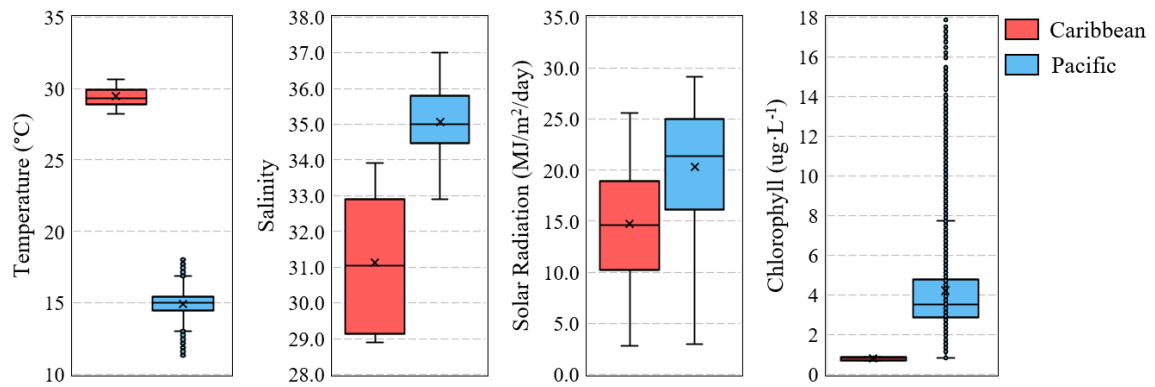
Figure 13. Box and whisker plot of environmental parameters of temperature (°C), salinity (‰), solar radiation (MJ/m^2/day), and chlorophyll *a* concentrations for the Caribbean site in Bocas del Toro, Panama and the Pacific site in San Diego, California. The (x) is the average, and quartiles are divided by the median presented by a horizontal line.
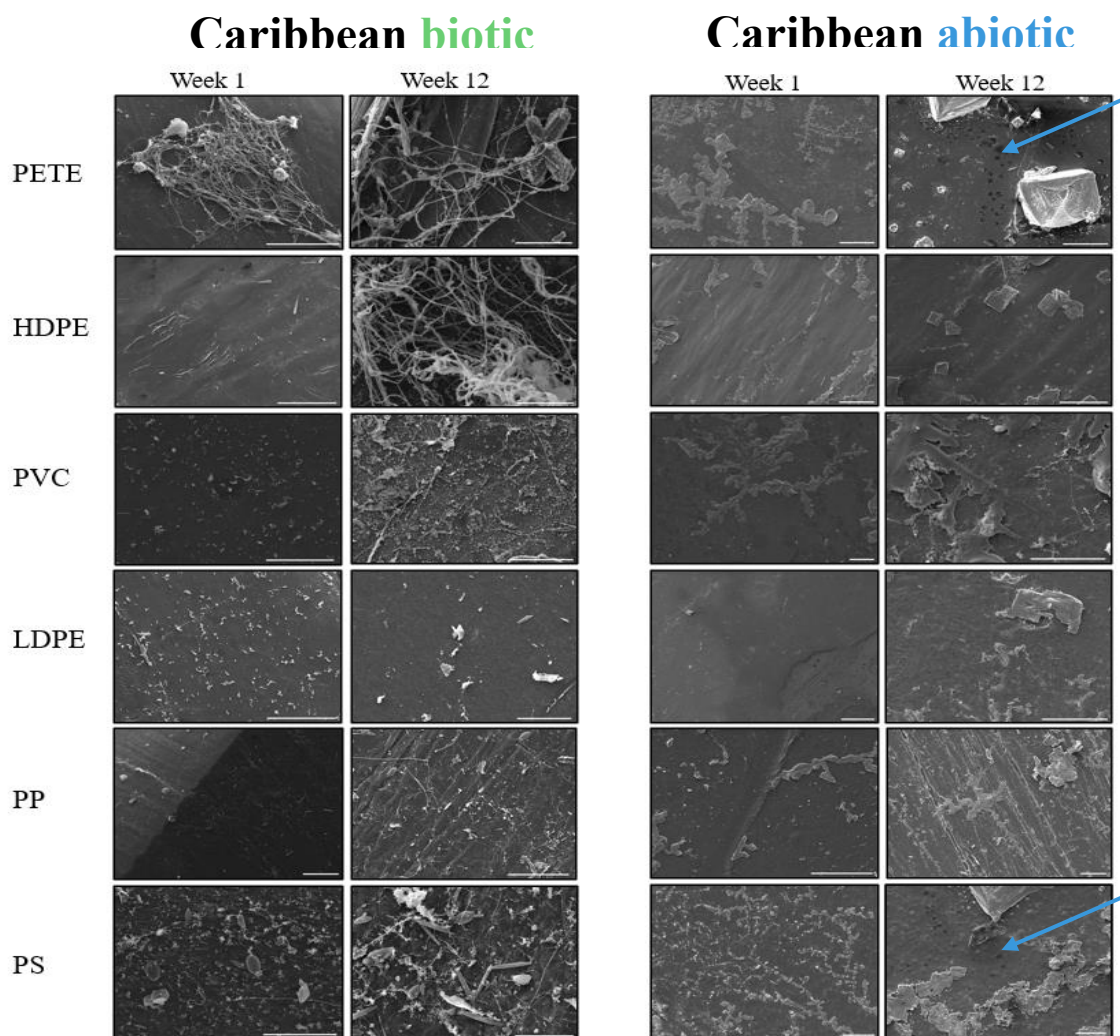
Figure 14. Photomicrographs of plastics 1-6 incubated in biotic (left panel) and abiotic (right panel) conditions in the Caribbean at the initial (week 1) and final (week 12) incubation time points. Scale bars are 50 μm. Arrows indicate pits and cracks observed as signs of degradation.

Pacific biotic   **Pacific abiotic**
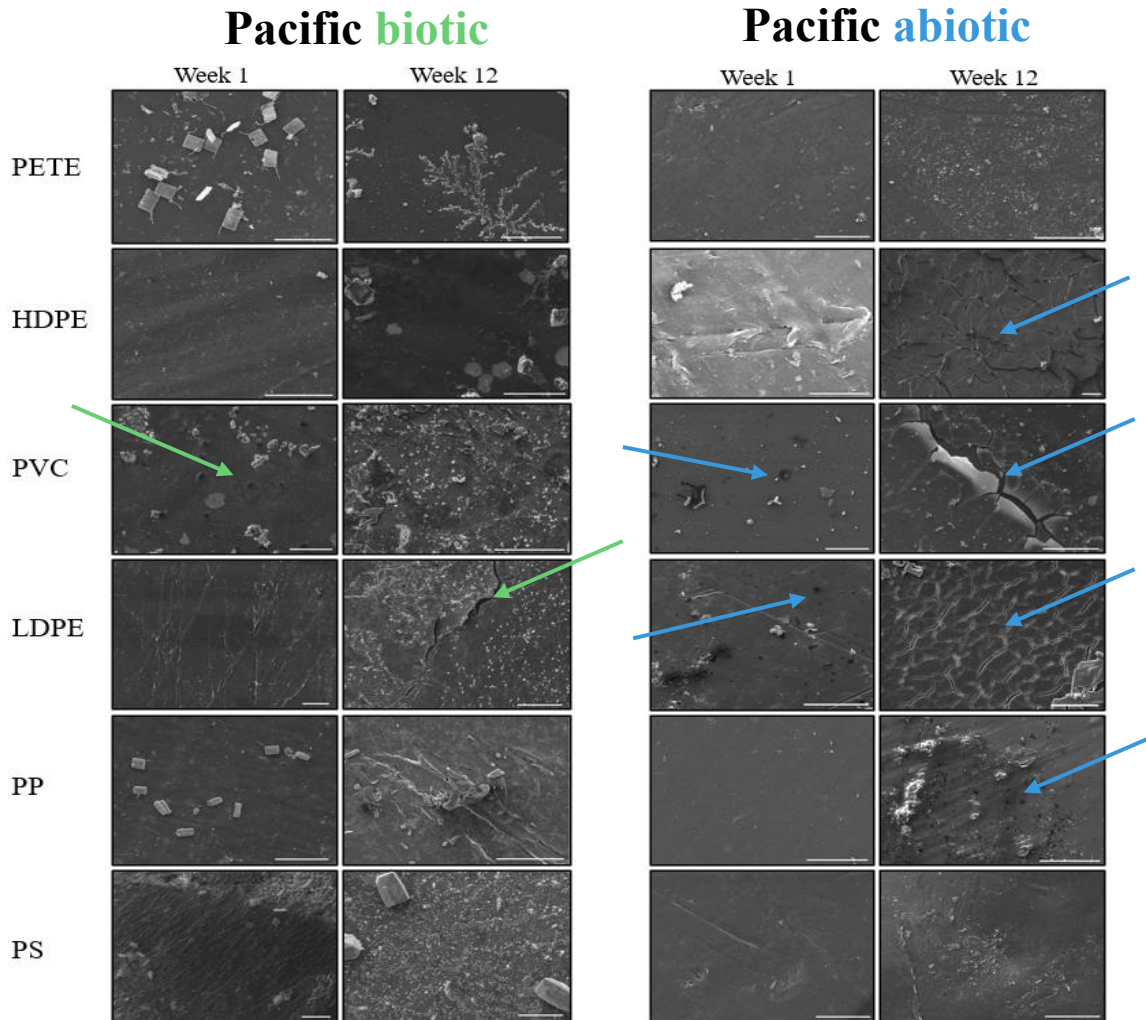
Figure 15. Photomicrographs of plastics 1-6 incubated in biotic (left panel) and abiotic (right panel) conditions in the Pacific at the initial (week 1) and final (week 12) incubation time points. Scale bars are 50 μm. Arrows indicate pits and cracks observed as signs of degradation.
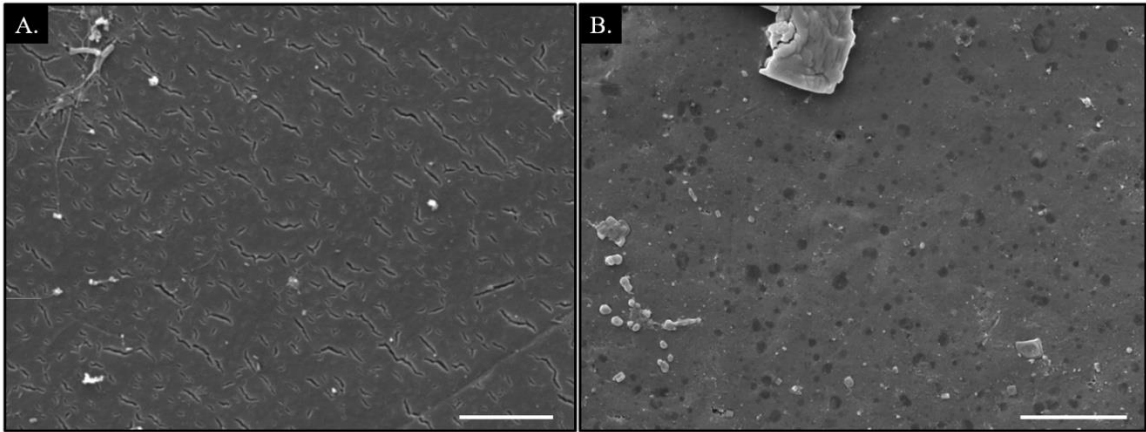
Figure 16. Photomicrographs of LDPE at 12 weeks of incubated in the Caribbean in biotic (A) and abiotic (B) conditions -is this in reverse? Looking at LDPE in Fig. 14, . the patterns in A look like those in the abiotic ones-Scale bars are 10 μm.
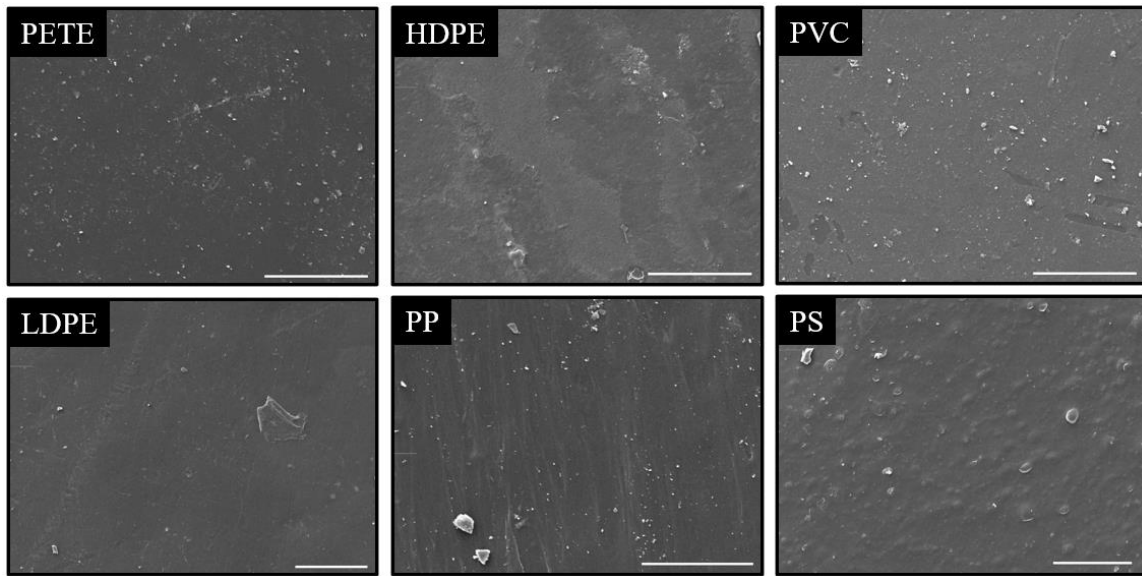
Figure 17. Photomicrographs of unincubated (control) microplastics. Scale bars are 50 µm.
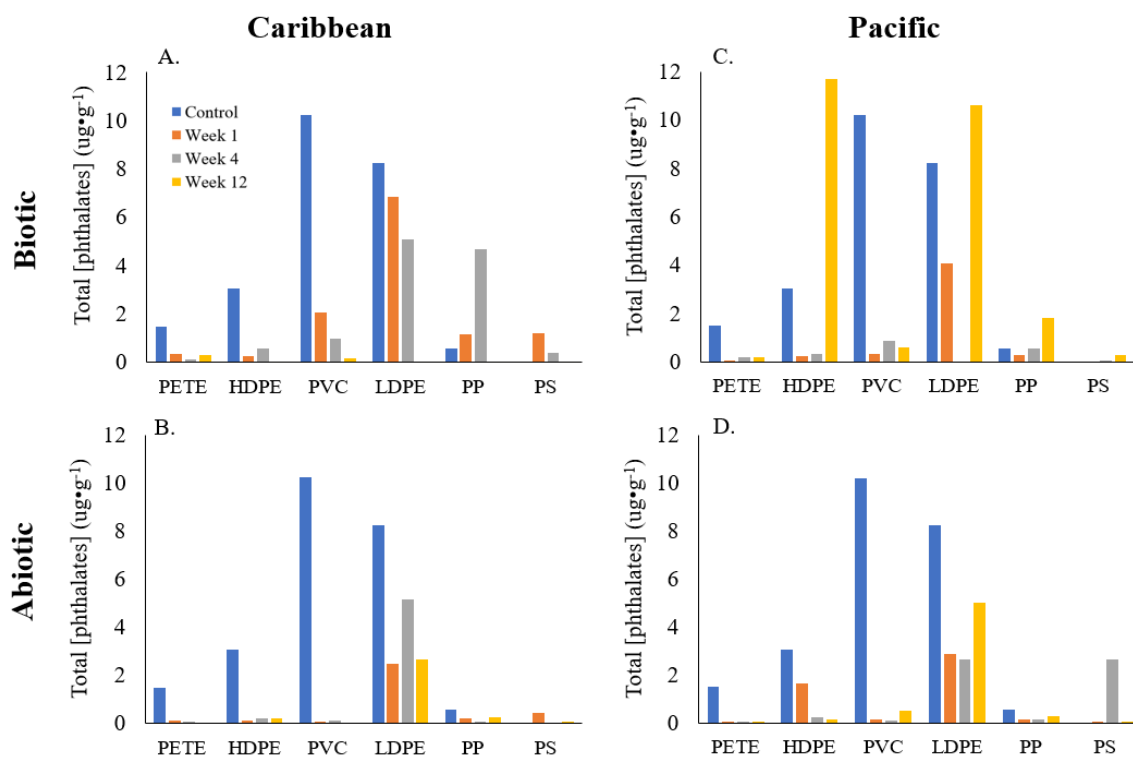
Figure 18. Summed phthalate concentrations (ug·g⁻¹) of unincubated microplastics (control) and microplastics incubated at the initial (week 1), intermediate (week 4) and end (week 12) of the times series in the Caribbean (A-B), and the Pacific (C-D) in biotic (A, C) and abiotic (B, D) conditions.
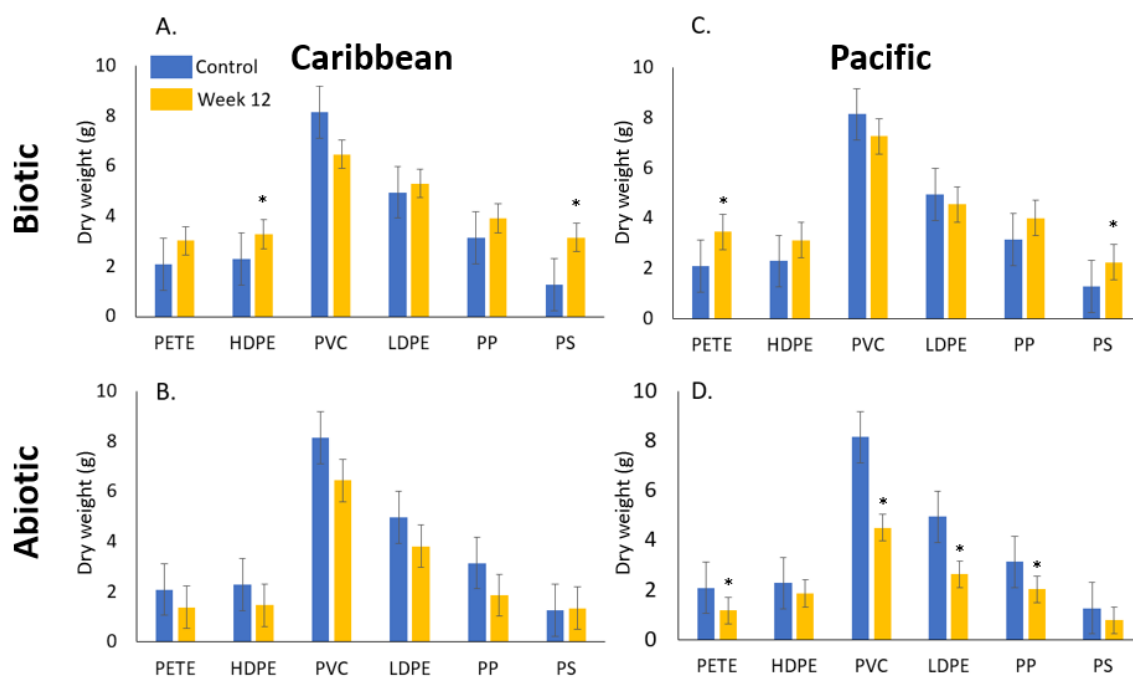
Figure 19. Dry weight (g) of unincubated microplastics (control) and microplastics incubated for 12 weeks in the Caribbean (Bocas del Toro, Panama, A-B), and the Pacific (San Diego, CA, C-D) in biotic (A, C) and abiotic (B, D) conditions.
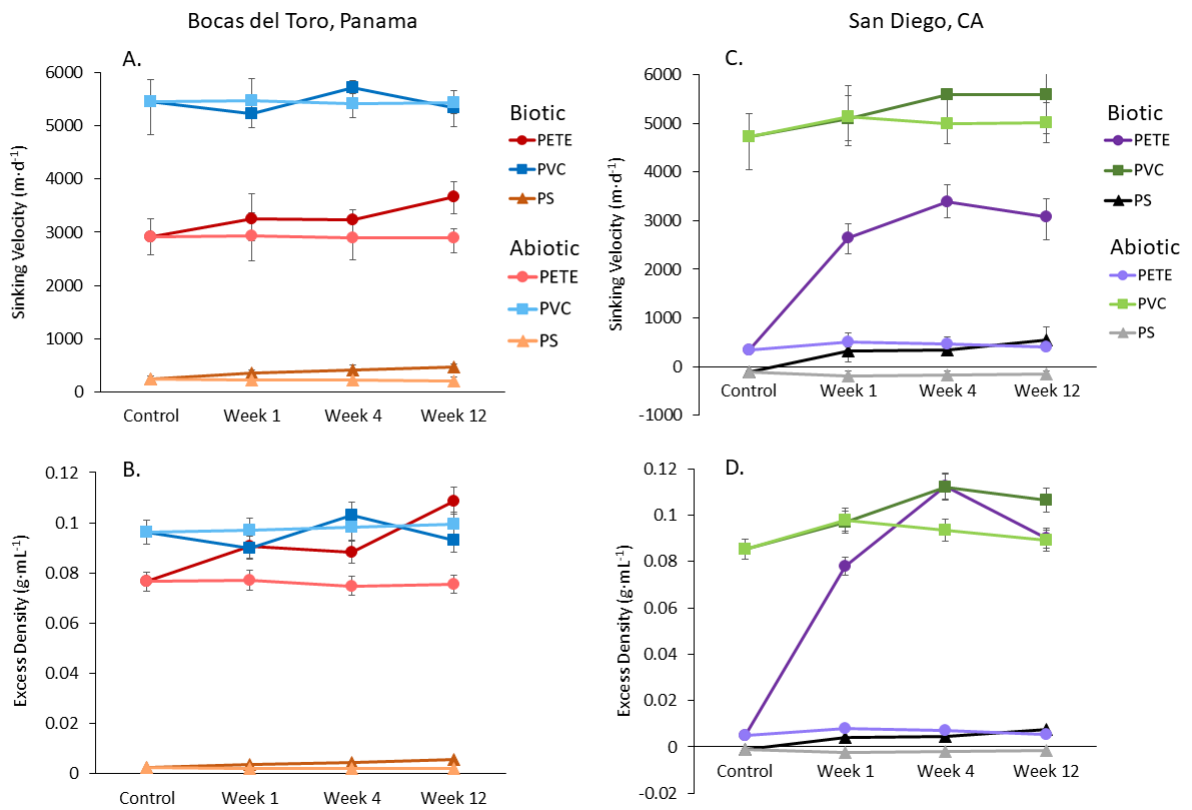
Figure 20. Sinking velocity and excess density of microplastics PETE, PVC, and PS incubated in the Caribbean (A and B respectively) and Pacific (C and D respectively) for both biotic and abiotic treatments. Error bars indicate standard error of the mean.

CONCLUSION

5.1 Dissertation contribution

In this study, I investigated the role of plastic polymer type, time of incubation, and geographic location on plastisphere formation and how these plastispheres ultimately control microplastics' fate in the water column and degradation capacities.

In Chapter 1, I focused on incubations of microplastics in the coastal Caribbean. Using amplicon sequencing data, I found that there were no polymer specific assemblages of bacteria, nor were the plastisphere communities significantly distinct from the water column. Instead, I inferred that the bacterial plastisphere was shaped by exposure to the environment. The eukaryotic members of the plastisphere, however, did exhibit polymer specificity that also changed significantly over time and deviated from water column communities. More specifically, using SEM, I observed that some diatoms, specifically, exhibited polymer preference, such as *Mastogloia corsicana* on PP or *Striatella* sp. on PETE, and I hypothesized that the phycosphere of diatoms may play a role in attracting plastic degrading bacteria. I also found evidence that microplastics could serve as a vector for both pathogenic and toxigenic eukaryotes, such as protists of the family Labyrinthulaceae, or *Alexandrium sp.* which would make those organisms susceptible to transport into non-native environments from the coastal bays into the open ocean via currents or uptake by zooplankton and fish, or possibly affect benthic communities by sinking.

In chapter 2, I focused on incubations of microplastics in water from the coast of the Pacific in San Diego, CA. Again, as in Chapter 1, I found no polymer specificity among the prokaryotic plastispheres. Instead, plastisphere composition was once again driven by incubation time. When comparing the plastispheres incubated in the Pacific with those from the coastal Caribbean (Ch. 1), I found that geographic location had an even more significant role in shaping the plastisphere. In addition, I found that there was a core community common to all plastic types and locations, and extended the concept of a "core microbiome" to a "core plastisphere", or a community that unites the plastispheres regardless of plastic polymer type, incubation time, or geographic location. I discussed in detail the possible synergies between taxa found within the core plastisphere.

In my third chapter I studied the role of the plastisphere on plastic degradation and deposition by comparing microplastics incubated in biotic and abiotic conditions at both the Caribbean (Chapter 1) and Pacific (Chapter 2) sites. I found that biofilms decrease degradation rates in microplastics. For positively buoyant microplastics HDPE and LDPE, I hypothesize that biofilms shaded the particles from ambient UV irradiation. For negatively buoyant microplastics, the plastisphere enhanced their sinking velocities. I inferred that enhanced sinking leads to enhanced sedimentation rates, which would reduce, possibly eliminate, the amount of light the microplastics are exposed to in the open ocean. Given that photooxidation is the primary source of microplastic degradation, only much slower degradation processes, such as hydrolysis or biodegradation, would occur. I also show the importance of ambient water density on the hydrodynamic

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behavior of negatively buoyant microplastics, in particular PETE and PS. In the warmer, less saline site in the Caribbean, PETE and PS sank without a biofilm. In the colder, more saline Pacific site, PETE sank significantly slower, and PS did not sink at all without a biofilm.

This dissertation shows the importance of incubation time and geographic location in shaping the plastisphere, but perhaps more importantly, I showed that plastic polymer type does not select for specific bacterial communities. This is in contrast to other studies that have concluded polymer specificity both within an environment (Oberbeckmann et al., 2014; Kirstein et al., 2019) and between environments (Zettler et al., 2013). Many of these were carried out with microplastics sampled directly from the environment where incubation time is not a measured variable. It is known that microplastics can enter new environments with oceanic currents (Sherman et al., 2016), fish ingestion/egestion (Lusher et al., 2016), or possibly horizontal migration of zooplankton (Ilamner & Hauri, 1981) and other food-web interactions. Thus, without knowing the amount of time a plastic particle has been exposed to the environment, or where the particle even initially came from, it is difficult to make the conclusion of polymer specificity. However, plenty of controlled incubated studies exist now that also found polymer specific assemblages (Table 4). Almost all, however, utilize primary microplastics, which are typically spherical in shape and are not representative of the majority of microplastics found in the ocean, which are not uniform in shape. The results of this dissertation, utilizing environmentally relevant secondary microplastics, show that there is a core community shared among plastic polymer types in two distinct ocean

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regions. This plastisphere consistency among polymer types may ultimately prove to be ideal when assessing the ecological impact these plastispheres may have. For example, instead of determining the potential pathogenicity or toxigenicity of the plastisphere on a polymer by polymer basis, perhaps instead these investigations can be more broadly addressed

While the plastisphere may not be polymer specific, microplastic hydrodynamic behavior, and thus sinking abilities, certainly are. In addition, plastic polymers depend significantly on ambient water density, which is controlled more so by salinity than by temperature (Fofonoff 1985). This has implications for sedimentation rates of microplastics, where microplastic accumulation in the sediments may be more concentrated in lower saline environments, such the Arctic, or tropical regions with heavy rainfall. The fact that I found significant sinking differences of PETE and PS in two ocean regions with different ambient water densities reinforces this concept.

In addition, I showed the importance of combining different methodologies to better understand and visualize plastisphere composition and microplastic degradation. A complementary approach that included both DNA-based and microscopy-based techniques allowed me to find the highest resolution possible for eukaryotic taxa, in particular diatoms. Scanning electron microscopy allowed me to determine that polymer specificity is occurring with these organisms – a conclusion not captured with sequencing technologies alone. In addition, when assessing microplastic degradation, I was able to utilize both qualitative techniques – visualizing the plastics

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surface, as well as quantitative techniques – phthalate concentration as well as weight changes.

5.2 The challenges ahead

Plastic waste entering the ocean is predicted reach up to 90 million metric tons (Mt) per year by 2030 if waste generation trends continue without the intervention of improved waste management – approximately a 300% increase from plastic emissions estimated in 2016 (Borrelle et al., 2020). While mitigation strategies should take precedence, we must deal with the plastic pollution already present in the ocean, and the concept of utilizing hydrocarbon degrading bacteria has gained attention to combat this issue (Jacquin et al., 2019). In addition, while emissions of PAHs are expected decrease up to 46-71% by 2030 (Shen et al., 2013), their anthropogenic presence in the environment will persist if people continue to rely on oil, or practice burning trash as a waste management practice. It is imperative to understand how plastics and PAHs interact with one another, how these interactions influence the plastisphere, and if we can utilize these interactions for pollution control strategies.

Eukaryotic phytoplankton, in particular diatoms, may function as an important habitat for hydrocarbon-degrading bacteria (Gutierrez et al., 2013), specifically PAH degrading bacteria (Mishamandani et al., 2016). This association may stem from the capacity of diatoms to accumulate PAHs on their cell surfaces (Binark et al., 2000), which would create a PAH-enriched zone in the phycosphere, a mucosal region around the cell rich in organic matter, and in turn attract PAH-degrading bacteria to colonize this

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zone. The rationale for this association is further evidenced in a few studies that present

data correlating the influence of eukaryotic phytoplankton with the removal of PAHs and

other hydrocarbons from the marine water column (Binark et al., 2000; Witt, 2002).

Interestingly, studies have shown that PAH-degrading bacteria utilize the same metabolic

pathways to degrade plastic polymers, such as *Arthrobacter sp.* and *Alcanivorax sp.*

(Urbanek et al. 2018, Balasubramanian et al. 2019). Both PAH- and plastic-degrading

bacteria were detected on all plastic types in both incubation experiments from the

Caribbean (Chapter 2) and the Pacific (Chapter 3). Was the presence of these bacteria

independent of eukaryotic phytoplankton colonization, specifically diatoms, or,

alternatively, is diatom colonization necessary for these bacteria to interact with the

plastics' surface? So, while microplastics have an independent capacity to sorb PAHs, it

is possible that diatom colonization recruits hydrocarbon degrading bacteria to

microplastics, and that microplastics and PAHs, when interacting in consortium with a

microplastic's biofilm, may together create a hotspot for their respective degradation.

Alternatively, do biofilms, regardless of hydrocarbon degrader presence, ultimately slow

the degradation of microplastics as I show in Chapter 3? In addition, high hydrostatic

pressure and low temperatures – parameters that would naturally occur in the deep ocean

– adversely affect PAH biodegradation (Louvado et al., 2015). Thus, microplastic

deposition would not only decrease its respective degradation rates, but the degradation

of PAHs as well.

These questions and concerns should motivate us to further investigate the role of

the plastisphere not only in microplastic degradation, but PAH degradation as well, in all

oceanic regions. For example, in highly plastic polluted regions, plastic can cause

shading effects, leading to reduced light intensity and affect microalgal photosynthesis

(Yurtsever et al., 2017, Chia et al., 2020). Does this reduction in phototrophs lead to a

reduction in hydrocarbon bacteria interacting with plastics, PAHs, or both?

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APPENDIX A

CHAPTER 1 SUPPLEMENTAL

Table S1. Environmental parameters measured at the sampling site at each sampling point.

	Rain (mm)	Relative Humidity (%)	Avg Air Temp (°C)	Wind Speed (km/hr)	Solar Radiation (MJ/m^2)	Salinity	Water Temp (°C)	[Chl-a] (μg/L)
Week 1	2	81.5	27.4	6.6	24.7	31	30.4	0.82
Week 3	8.9	84.7	26.9	9.7	10	32.9	29.7	0.88
Week 6	5.6	88.1	26	9.2	5.5	32.3	28.8	0.68

Table S2. Alpha-diversity indices for prokaryotic communities obtained using Pielou index (J') for evenness, and Shannon-Wiener (H') for diversity

S: total number of species (observed richness); N: total number of individuals.

Sample	S	N	Alpha Indices (Prokaryotes)	
			J'	H'($\log_e$)
Week 1				
Ambient Water	106	62302	0.5233	2.437
PETE	212	27581	0.7922	4.243
HDPE	161	40696	0.6052	3.075
PVC	212	39974	0.796	4.264
LDPE	201	57659	0.6698	3.559
PP	197	37120	0.7277	3.844
PS	208	39507	0.7148	3.815
Week 3				
Ambient Water	114	51160	0.5456	2.591
PETE	260	37203	0.8077	4.491
HDPE	260	41230	0.7759	4.318
PVC	238	35496	0.7929	4.339
LDPE	274	46685	0.7708	4.327
PP	241	35651	0.7751	4.251
PS	240	32000	0.7831	4.292
Week 6				
Ambient Water	120	55943	0.5338	2.553
PETE	253	39294	0.7533	4.168
HDPE	192	47193	0.7308	3.842
PVC	244	41244	0.7195	3.958
LDPE	168	35472	0.7348	3.765
PP	231	42457	0.7354	4.006
PS	192	32650	0.6595	3.467

Table S3. Alpha-diversity indices for eukaryotic communities obtained using Pielou index (J') for evenness, and Shannon-Wiener (H') for diversity

S: total number of species (observed richness); N: total number of individuals.

Sample	S	N	Alpha Indices (Eukaryotes)	
			J'	H'($\log_e$)
Week 1				
Ambient Water	168	67408	0.7294	3.737
PETE	49	64234	0.5835	2.271
HDPE	37	90170	0.4081	1.474
PVC	42	68081	0.5669	2.119
LDPE	48	121423	0.5475	2.119
PP	66	143203	0.5625	2.357
PS	42	79902	0.4954	1.852
Week 3				
Ambient Water	146	99365	0.5256	2.619
PETE	44	25842	0.5724	2.166
HDPE	50	34108	0.5826	2.279
PVC	60	79031	0.5804	2.376
LDPE	45	116725	0.4579	1.743
PP	47	120470	0.2995	1.153
PS	53	53449	0.6420	2.549
Week 6				
Ambient Water	62	70554	0.5012	2.069
PETE	51	48955	0.5603	2.203
HDPE	44	85716	0.3470	1.313
PVC	46	47204	0.5017	1.921
LDPE	52	87003	0.5599	2.212
PP	48	114241	0.4659	1.804
PS	78	60825	0.5708	2.487

Table S4. Summary statistics of null models calculated with randomized microbial community data to assess similarity based on BC indices between plastic type per time point. AW, ambient water, or total water column community; BC, Bray-Curtis dissimilarity index, used as an estimate of community composition shift based eukaryotic communities; SES, standardized effect size; (p-values lower than 0.05 denote significantly similar community composition between samples). None of the pairwise comparisons among the plastispheres resulted in dissimilar communities (p > 0.05).

PROKARYOTES

Week 1

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.5	-15.05	0	0.36	-17.89	0	0.51	-15.44	0	0.36	-17.18	0	0.32	-20.82	0	0.81	-5.33	2.00E-04
HDPE				0.49	-12.17	0	0.32	-16.51	0	0.32	-17.01	0	0.36	-15.97	0	0.84	-2.41	0.0262
PVC							0.54	-14.32	0	0.4	-15.68	0	0.36	-15.6	0	0.81	-4.67	2.00E-04
LDPE										0.47	-16.66	0	0.49	-17.3	0	0.86	-1.84	0.045
PP													0.33	-17.13	0	0.83	-3.49	0.0064
PS																0.83	-3.97	0.001

Week 3

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.27	-20	0	0.35	-16.3	0	0.3	-19.2	0	0.34	-17.41	0	0.3	-18.5	0	0.81	-4.72	1E-0.4
HDPE				0.35	-16.5	0	0.35	-17.31	0	0.35	-17.01	0	0.28	-19.43	0	0.82	-3.73	0.005
PVC							0.34	-17.44	0	0.39	-15.04	0	0.31	-17.78	0	0.8	-5.22	1.00E-04
LDPE										0.28	-19.67	0	0.32	-17.41	0	0.79	-5.7	0
PP													0.32	-17.75	0	0.77	-6.14	0
PS																0.8	-5.04	0

Week 6

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.5	-11.94	0	0.31	-19.32	0	0.53	-10.31	0	0.31	-18.24	0	0.51	-14.4	0	0.79	-5.89	0.002
HDPE				0.4	-15.1	0	0.36	-14.58	0	0.58	-8.88	0	0.45	-15.24	0	0.82	-3.45	0.009
PVC							0.43	-13.93	0	0.37	-16.56	0	0.41	-18.28	0	0.75	-7.24	0
LDPE										0.58	-8.07	0	0.49	-13.31	0	0.82	-3.58	0.004
PP													0.53	-12.77	0	0.75	-7.5	0
PS																0.75	-7.39	0

Table S5. Summary statistics of null models calculated with randomized microbial community data of prokaryotic sequences to assess similarity based on BC indices between time exposed to the environment of a given plastic polymer type. AW, ambient water, or total water column community; BC, Bray-Curtis dissimilarity index, used as an estimate of community composition shift based prokaryotic communities; SES, standardized effect size; (p-values lower than 0.05 denote significantly similar community composition between samples). None of the pairwise comparisons resulted in dissimilar communities (p > 0.05).

PROKARYOTES

PETE	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.48	-12.38	0	0.59	-8.33	0
W3				0.45	-13.35	0

HDPE	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.5	-13.37	0	0.52	-10.45	0
W3				0.45	-13.83	0

PVC	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.39	-15.03	0	0.44	-14.63	0
W3				0.38	-16.63	0

LDPE	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.36	-12.24	0	0.21	-14.85	0
W3				0.47	-12.68	0

PP	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.41	-15.56	0	0.62	-7.99	0
W3				0.47	-12.83	0

PS	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.35	-15.78	0	0.52	-12.83	0
W3				0.44	-16.91	0

AW	W3 BC	W3 SES	W3 p	W6 BC	W6 SES	W6 p
W1	0.43	-11.44	0	0.24	-16.07	0
W3				0.34	-12.63	0

Table S6. Summary statistics of null models calculated with randomized microbial community data of eukaryotic sequences to assess similarity based on BC indices between plastic per time point. AW, ambient water, or total water column community; BC, Bray-Curtis dissimilarity index, used as an estimate of community composition shift based eukaryotic communities; SES, standardized effect size; (p-values lower than 0.05 denote significantly similar community composition between samples.) Indices in **bold** indicate dissimilar communities (p > 0.05).

EUKARYOTES

Week 1

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.67	-4.46	0.002	0.61	-4.79	<0.001	0.61	-5.21	<0.001	0.71	-4.31	0.001	0.6	-5.32	<0.001	**0.85**	**-1.83**	**0.06**
HDPE				0.62	-4.78	0.01	0.61	-5.07	<0.001	0.62	-5.97	0.002	0.55	-6.12	<0.001	**0.9**	**-1.01**	**0.12**
PVC							0.56	-5.91	<0.001	0.57	-7.34	<0.001	0.7	-3.16	0.02	0.82	-3.13	0.02
LDPE										0.66	-4.72	<0.001	0.67	-3.83	0.01	**0.88**	**-1.63**	**0.08**
PP													0.73	-3.61	0.01	**0.89**	**-1.11**	**0.13**
PS																**0.88**	**-1.16**	**0.12**

Week 3

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.65	-4.19	0.01	0.84	-1.59	0.05	**0.88**	**-1.36**	**0.08**	0.84	-3.15	0.04	0.74	-2.86	0.02	**0.92**	**-0.94**	**0.09**
HDPE				0.58	-7.48	0	0.69	-5.82	0	0.7	-7.4	0	0.49	-7.89	<0.001	0.78	-5.55	0.01
PVC							0.62	-5.92	<0.001	0.54	-9.34	0	0.7	-4.52	<0.001	0.6	-8.86	<0.001
LDPE										0.68	-4.82	0.01	0.7	-4.63	<0.001	0.76	-4.86	0.007
PP													0.7	-6.3	<0.001	0.68	-8.74	<0.001
PS																0.82	-3.73	0.007

Week 6

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	**0.92**	**-0.3**	**0.23**	0.79	-2.18	0.04	**0.9**	**-0.22**	**0.30**	0.63	-6.14	0	0.74	-3.33	0.01	0.65	-5.46	0.001
HDPE				**0.91**	**-0.68**	**0.10**	0.79	-2.53	0.02	0.74	-3.63	0.01	0.87	-1.78	0.04	**0.94**	**-0.08**	**0.29**
PVC							**0.82**	**-1.8**	**0.07**	0.69	-4.73	0.002	0.61	-6.34	0.002	0.6	-6.68	<0.001
LDPE										**0.9**	**-0.46**	**0.21**	0.54	-8.28	0	**0.91**	**-0.23**	**0.27**
PP													0.78	-3.37	0.03	0.45	-9.75	0
PS																0.74	-3.91	0.01

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Table S7. Summary statistics of null models calculated with randomized microbial community data of eukaryotic sequences to assess similarity based on BC indices between time exposed to the environment of a given plastic polymer type. AW, ambient water, or total water column community; BC, Bray-Curtis dissimilarity index, used as an estimate of community composition shift based eukaryotic communities; SES, standardized effect size; (p-values lower than 0.05 denote significantly similar community composition between samples.) Indices in **bold** indicate dissimilar communities (p > 0.05).

EUKARYOTES

PETE	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.81**	**-1.39**	**0.06**	**0.88**	**-0.5**	**0.23**
W3				**0.86**	**-0.88**	**0.12**

HDPE	W3			W6		
	BC	SES	p	BC	SES	p
W1	0.69	-5.42	0.002	**0.95**	**0.6**	**0.89**
W3				**0.92**	**0.09**	**0.44**

PVC	W3			W6		
	BC	SES	p	BC	SES	p
W1	0.53	-6.79	<0.001	0.63	-5.31	0.002
W3				0.6	-6.38	<0.001

LDPE	W3			W6		
	BC	SES	p	BC	SES	p
W1	0.71	-3.52	0.01	**0.87**	**-0.5**	**0.21**
W3				**0.89**	**-0.46**	**0.25**

PP	W3			W6		
	BC	SES	p	BC	SES	p
W1	0.61	-7.56	0.001	0.63	-5.86	0.01
W3				0.42	-9.28	<0.001

PS	W3			W6		
	BC	SES	p	BC	SES	p
W1	0.73	-3.05	0.01	**0.83**	**-1.59**	**0.06**
W3				0.73	-3.68	0.01

AW	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.81**	**-1.39**	**0.06**	**0.88**	**-0.5**	**0.23**
W3				**0.86**	**-0.88**	**0.12**

Table S8. Diatom taxonomic groups found by SEM observation of plastispheres and reference study. Group numbers refer to diatoms shown in Figure 5A and in the heatmap analysis shown in Figure 5B.

Diatom Group	Name	Reference
D1	Nitzschia sicula	Siqueiros-Beltrones et al. 2016
D2	Cocconeis placentula	Romero and Jahn 2013
D3	Fragilara sp.	Spaulding and Edlund 2008
D4	Mastogloia corsicana	Navarro and Hernández-Becerril 1997
D5	Navicula sp	Piccinetti et al. 2016
D6	Mastogloia sp. I	López-Fuerte 2013
D7	Mastogloia sp. II	López-Fuerte 2013
D8	Striatella sp.	Veselá et al. 2011
D9	Mastogloia fimbriate	Park et al. 2018
D10	Amphora sp.	Cavalcante et al. 2014
D11	Pseudo-nitzschia sp.	Tomas 1997
D12	Cocconeis sp.	Romero and Jahn 2013
D13	Diploneis sp.	Pennesi et al. 2017

Table S9. Diatom abundances (cells/ mm^2, ± standard error of the mean of duplicates) for each microplastic particle at 6 weeks of incubation.

Diatom Group	PETE	HDPE	PVC	LDPE	PP	PS
D1	1 ± 0.2	0	0	0	0	0
D2	3.7 ± 1.0	6.5 ± 0.5	4.1 ± 0.2	6.1 ± 0.5	3.3 ± 0.5	6.5 ± 1.2
D3	1.6 ± 0.3	3.9 ± 1.2	2.5 ± 0.1	1.4 ± 0.5	3.4 ± 0.5	0.8 ± 0.1
D4	0	0	0	0	1.9 ± 0.5	0
D5	1.3 ± 0.2	1.4 ± 0.4	1.9 ± 0.1	1.5 ± 0.7	0.9 ± 0.3	0.7 ± 0.3
D6	0.4 ± 0.0	4.5 ± 0.7	0	1.3 ± 0.2	0	0
D7	0	0	0.9 ± 0.2	0	0.5 ± 0.2	0
D8	0.5 ± 0.1	0	0	0	0	0
D9	0	0.6 ± 0.0	0	1.2 ± 0.1	0	0
D10	0	0	0	1.4 ± 0.2	0	0
D11	0	0	0	0	0	1.2 ± 0.3
D12	0.7 ± 0.3	0	0	0.3 ± 0.1	0.5 ± 0.0	1.3 ± 0.3
D13	2.1 ± 0.0	0	0	0	0	0.3 ± 0.1
D14	0	0	2.9 ± 0.4	0	1.3 ± 0.3	0.3 ± 0.1

Figure S1. Time series of microbial succession on polystyrene at one week (A, D), three weeks (B, E), and six weeks (C, F) of incubation. Scale bars are 500µm (A-C), and 20µm (D-F). We show polystyrene as an example, but all plastic polymer types exhibited this pattern of diatom succession.
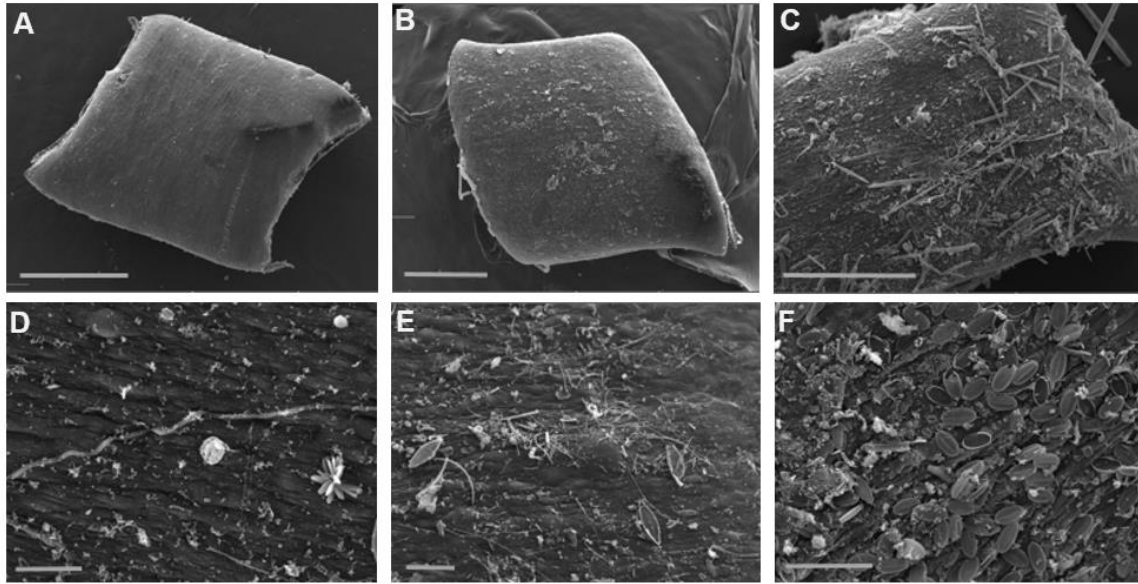
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APPENDIX B

CHAPTER 2 SUPPLEMENTAL

Table S1. Method percent recovery of targeted PAHs for PETE, HDPE, PVC, LDPE, and PP. Method recoveries for PS are unavailable due to samples being too viscous and unable to be processed.

Polycyclic Aromatic Hydrocarbon	PETE	HDPE	PVC	LDPE	PP
1-Methylnaphthalene	9.49	6.95	8.57	4.57	0
Acenaphthylene	40.10	6.62	42.24	17.22	40.76
Acenaphthene	45.33	37.91	41.34	43.54	46.60
Fluorene	46.06	40.35	42.87	46.49	4.86
Phenanthrene	55.47	46.01	47.21	44.98	44.33
Anthracene	55.85	49.41	49.62	0	0
4H-Cyclopenta[def]phenant	0.07	0.06	0.06	0.06	0.06
Fluoranthene	51.55	44.55	51.31	48.49	49.66
Pyrene	59.71	52.97	60.21	62.48	51.32
Benz[a]anthracene	52.96	40.83	31.22	41.95	51.10
Chrysene + Triphenylene	34.51	70.23	77.72	8.80	51.95
Benzo[b]fluoranthene	17.41	8.96	17.28	12.67	1.20
Benzo[k]fluoranthene	145.59	129.79	149.28	52.37	22.38
Benzo[b+j+k]fluoranthene	114.19	93.70	116.37	48.99	15.74
Benzo[a]pyrene	55.53	45.04	55.78	49.51	51.48
Indeno[1,2,3-cd]pyrene	0.85	1.17	0.27	4.33	1.32
Dibenz[a,h]anthracene	10.99	14.43	16.69	14.03	45.29
Benzo[ghi]perylene	22.56	35.40	10.27	5.45	58.33

Table S2. Alpha-diversity indices for prokaryotic communities on microplastics at the Pacific site obtained using Pielou index (J') for evenness, and Shannon-Wiener (H') for diversity.
S: total number of species (observed richness); N: total number of sequence reads.

Sample	S	N	Alpha Indices	
Week 1			J'	H'($\log_e$)
Ambient Water	257	226495	0.71	3.99
PETE	137	36527	0.87	3.87
HDPE	85	5498	0.87	3.87
PVC	43	10107	0.75	3.27
LDPE	142	73848	0.78	3.71
PP	176	34354	0.82	4.05
PS	26	6357	0.79	2.69
Week 3				
Ambient Water	Sample removed from analyses due to insufficient DNA yield			
PETE	171	243197	0.74	3.21
HDPE	80	95796	0.73	3.48
PVC	183	227235	0.67	3.03
LDPE	106	77402	0.65	3.31
PP	68	19262	0.79	3.14
PS	141	303581	0.63	3.21
Week 6				
Ambient Water	180	128412	0.58	3.00
PETE	227	235365	0.75	4.06
HDPE	174	175431	0.67	3.44
PVC	188	123057	0.78	4.09
LDPE	174	141573	0.63	3.24
PP	144	96368	0.69	3.45
PS	154	254285	0.64	4.06

Figure S1. ASV richness, Pielou's evenness, and Shannon Diversity indices of prokaryotic communities attached to microplastics at week 1, week 3, and week 6 of incubation at the Pacific site and Caribbean sites.
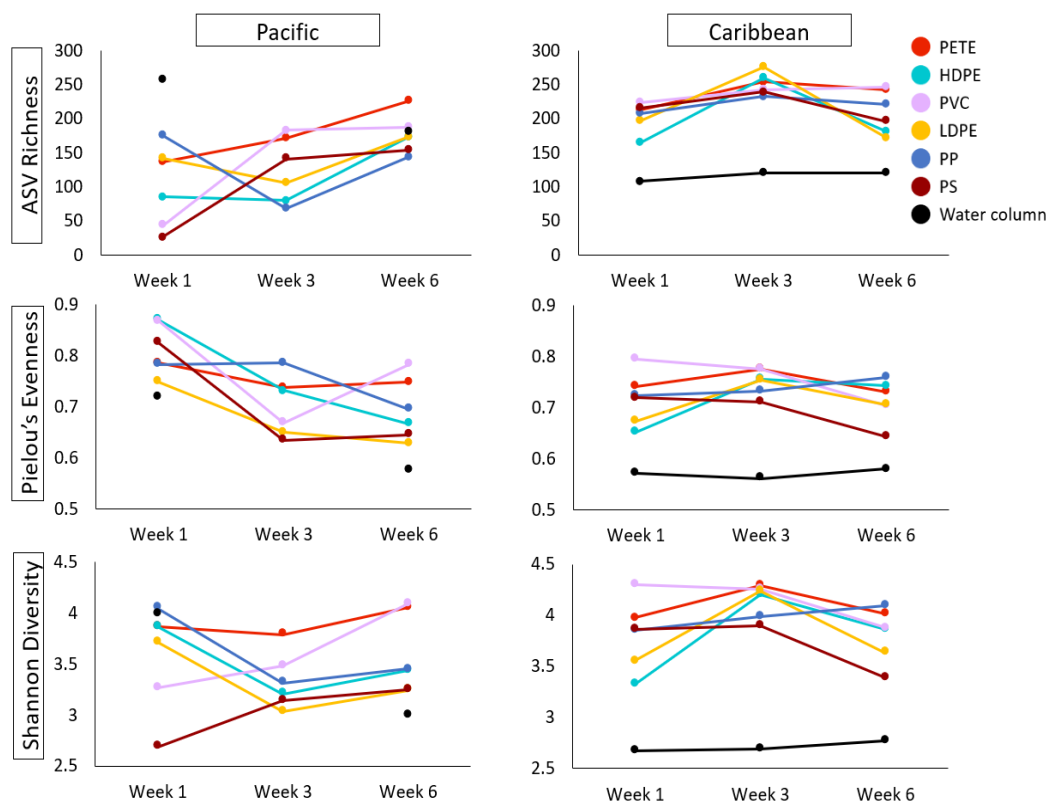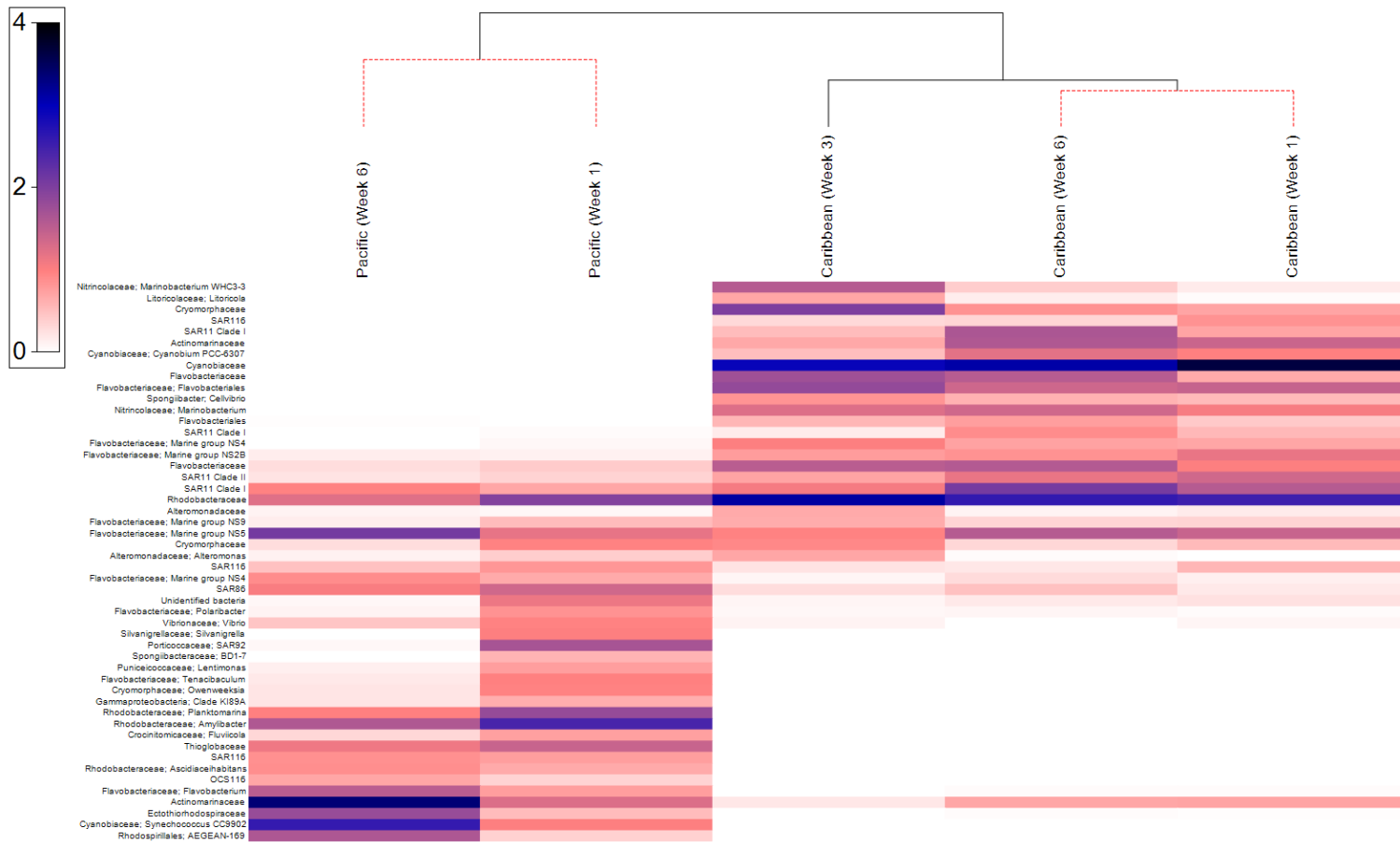
Figure S2. Heatmap depicting the relative abundances of the most abundant 50 prokaryotic taxa across all total water column samples from the Pacific (San Diego, CA) and Caribbean (Bocas del Toro, Panama) sites overlaid with a dendrogram that shows clustering of water column communities with similar community composition. Black lines represent significant differences between plastic samples (p < 0.05, SIMPROF test) whereas red dotted lines signify similarity.

Table S3. Summary statistics of null models calculated with randomized microbial community data of prokaryotic sequences to assess similarity based on BC indices between time exposed to the environment of a given plastic polymer type. AW, ambient water, or total water column community; BC, Bray-Curtis dissimilarity index, used as an estimate of community composition shift based eukaryotic communities; SES, standardized effect size; (p-values lower than 0.05 denote significantly similar community composition between samples.) Indices in **bold** indicate significantly dissimilar communities (p > 0.95).

PROKARYOTES

PETE	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.96**	**4.84**	**1**	**0.94**	**2.36**	**0.99**
W3				0.62	-22.64	0

HDPE	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.97**	**4.02**	**1**	**0.99**	**1.15**	**1**
W3				0.73	-13.14	0

PVC	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.98**	**2.62**	**1**	**0.98**	**3.31**	**1**
W3				0.66	-22.50	0

LDPE	W3			W6		
	BC	SES	p	BC	SES	p
W1	0.86	-0.30	0.31	0.90	1.08	0.89
W3				0.76	-12.26	0

PP	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.92**	**4.12**	**1**	**0.92**	**4.16**	**1**
W3				0.87	-3.87	0.005

PS	W3			W6		
	BC	SES	p	BC	SES	p
W1	**0.98**	**1.41**	**0.97**	**0.99**	**3.63**	**1**
W3				0.73	-14.99	0

AW	W3			W6		
	BC	SES	p	BC	SES	p
W1				0.71	-21.91	0
W3						

Table S4. Summary statistics of null models calculated with randomized microbial community data of prokaryotic sequences to assess similarity based on BC indices between plastic per time point. AW, ambient water, or total water column community; BC, Bray-Curtis dissimilarity index, used as an estimate of community composition shift based eukaryotic communities; SES, standardized effect size; (p-values lower than 0.05 denote significantly similar community composition between samples.) Indices in **bold** indicate significantly dissimilar communities (p > 0.95).

PROKARYOTES

Week 1

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.86	-1.93	0.03	0.84	-1.35	0.05	0.74	-4.77	0.01	0.67	-8.94	0	0.67	8.93	0	**0.97**	**4.96**	**1**
HDPE				0.68	-6.23	5e-04	**0.92**	**1.36**	**0.95**	0.77	-6.01	9e-04	0.55	-6.88	4e-04	**0.99**	**5.10**	**1**
PVC							**0.93**	**2.14**	**0.99**	0.82	-4.19	0.003	0.70	-3.63	0.007	**0.99**	**3.83**	**1**
LDPE										0.58	-12.93	0	0.93	0.67	0.75	**0.94**	**4.14**	**1**
PP													0.81	-4.13	0.01	**0.95**	**5.37**	**1**
PS																**0.99**	**3.94**	**1**

Week 3

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.61	-14.20	0	0.37	-26.98	0	0.58	-17.41	0	0.89	-7.45	0	0.42	-23.55	0			
HDPE				0.57	-21.30	0	0.31	-17.20	0	0.76	-6.90	0	0.55	-19.44	0			
PVC							0.56	-21.81	0	0.88	-10.53	0	0.30	-28.99	0			
LDPE										0.72	-9.89	0	0.60	-18.18	0			
PP													0.90	-8.99	0			
PS																		

Week 6

	HDPE			PVC			LDPE			PP			PS			AW		
	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p	BC	SES	p
PETE	0.37	-20.04	0	0.41	-28.97	0	0.40	-27.45	0	0.52	-22.64	0	0.38	-30.30	0	**0.96**	**3.95**	**1**
HDPE				0.52	-22.32	0	0.19	-32.85	0	0.48	-22.65	0	0.33	-29.67	0	**0.95**	**2.50**	**1**
PVC							0.50	-22.41	0	0.38	-23.86	0	0.59	-21.84	0	**0.96**	**4.96**	**1**
LDPE										0.47	-22.73	0	0.34	-29.27	0	**0.96**	**3.29**	**1**
PP													0.60	-18.96	0	**0.95**	**2.71**	**1**
PS																**0.97**	**3.34**	**1**

Table S5. Indicator taxa at the Pacific site for each time point and groups of time points classified to the highest resolution possible with √Indicator values and p-values. Only significant indicators (√Indicator values > 0.8 and p-value < 0.05) are shown.

Week 1		
k__Bacteria.p__Actinobacteria.c__Actinobacteria.o__Actinomycetales.f__Corynebacteriaceae.g__Corynebacterium.s__	0.99	3.00E-04
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Flavobacterium.s__succinicans	0.99	3.00E-04
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.Tissierellaceae..g__Anaerococcus.s__	0.94	0.001
k__Bacteria.p__Bacteroidetes.c__.Saprospirae..o__.Saprospirales..f__Chitinophagaceae.g__.s__	0.90	0.005
k__Bacteria.p__Bacteroidetes.c__Sphingobacteriia.o__Sphingobacteriales.f__.g__.s__	0.86	0.0073
k__Bacteria.p__Verrucomicrobia.c__Verrucomicrobiae.o__Verrucomicrobiales.f__Verrucomicrobiaceae.g__Prosthecobacter.s__	0.81	0.0131
k__Bacteria.p__Bacteroidetes.c__Cytophagia.o__Cytophagales.f__Cytophagaceae.g__.s__	0.81	0.0145
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Flavobacterium.s__	0.82	0.0162
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Oceanospirillales.f__Halomonadaceae.g__Halomonas.__	0.91	0.0284
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Enterobacteriales.f__Enterobacteriaceae.__.__	0.89	0.0401
Week 6		
k__Bacteria.p__Bacteroidetes.c__Cytophagia.o__Cytophagales.f__.Amoebophilaceae..g__SC3.56.s__	0.98	5.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__GMD14H09.f__.g__.s__	0.99	5.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Myxococcales.f__Nannocystaceae.g__Plesiocystis.s__	1	5.00E-04
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__HTCC2188.f__HTCC2089.g__Acinetobacter.s__radioresistens	1	5.00E-04
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Winogradskyella.s__thalassocola	0.98	6.00E-04
k__Bacteria.p__Planctomycetes.c__Phycisphaerae.o__Phycisphaerales.f__Phycisphaeraceae.g__.s__	0.96	8.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Myxococcales.f__.g__.s__	0.89	9.00E-04
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Cryomorphaceae.g__Crocinitomix.s__	0.96	0.0011
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Dinoroseobacter.s__	0.96	0.0011
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Cryomorphaceae.g__Cryomorpha.s__	0.96	0.0012
k__Bacteria.p__Cyanobacteria.c__Synechococcophycideae.o__Pseudanabaenales.f__Pseudanabaenaceae.g__.s__	0.91	0.0013
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Myxococcales.f__OM27.g__.s__	0.89	0.0014
k__Bacteria.p__Proteobacteria.c__Epsilonproteobacteria.o__Campylobacterales.f__Campylobacteraceae.g__Arcobacter.s__	0.91	0.0025
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Roseivivax.s__	0.90	0.0026
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Oceanospirillales.f__Saccharospirillaceae.g__Saccharospirillum.s__	0.91	0.0026
k__Bacteria.p__Planctomycetes.c__Planctomycetia.o__Pirellulales.f__Pirellulaceae.g__planctomycete.s__MS1399	0.84	0.0085
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__Cohaesibacteraceae.g__.s__	0.82	0.0097
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Erythrobacteraceae.g__.s__	0.90	0.0108
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__.f__.g__.s__	0.94	0.0111
k__Bacteria.p__Bacteroidetes.c__Cytophagia.o__Cytophagales.f__.Amoebophilaceae..g__.s__	0.81	0.0145
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Ruegeria.__	0.81	0.0147
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Xanthomonadales.f__Xanthomonadaceae.g__.s__	0.81	0.0153

Week 3 and Week 6

k__Bacteria.p__Bacteroidetes.c__.Saprospirae..o__.Saprospirales..f__Saprospiraceae.g__Lewinella.s__cohaerens	1	3.00E-04
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.__.__	0.96	3.00E-04
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Croceitalea.s__dokdonensis	1	3.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__BD7.3.f__.g__.s__	0.99	3.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Hyphomonadaceae.g__.s__	0.97	3.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Nautella.s__	0.98	3.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Octadecabacter.s__antarcticus	0.99	3.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Sulfitobacter.s__litoralis	1	3.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Bdellovibrionales.f__Bacteriovoracaceae.g__Bacteriovorax.s__	0.99	3.00E-04
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Alteromonadales.f__OM60.g__Congregibacter.s__	1	3.00E-04
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Oceanospirillales.f__Oceanospirillaceae.g__Marinomonas.s__	1	3.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.__.__	0.95	5.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Sulfitobacter.s__japonica	0.97	6.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__.g__.s__	0.95	9.00E-04
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Gilvibacter.s__sediminis	0.95	0.001
k__Bacteria.p__Chlamydiae.c__Chlamydiia.o__Chlamydiales.f__.g__.s__	0.95	0.001
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__Phyllobacteriaceae.g__.s__	0.97	0.0012
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Roseobacter.s__litoralis	0.95	0.0012
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Roseovarius.s__aestuarii	0.95	0.0012
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Alteromonadales.f__Alteromonadaceae.g__Glaciecola.s__punicea	0.95	0.0012
k__Bacteria.p__Bacteroidetes.c__Cytophagia.o__Cytophagales.f__Flammeovirgaceae.g__Fulvivirga.s__	0.95	0.0016
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Maribacter.s__	0.95	0.0016
k__Bacteria.p__Planctomycetes.c__OM190.o__agg27.f__.g__.s__	0.93	0.0022
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Erythrobacteraceae.g__Erythrobacter.s__	0.96	0.0023
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Hyphomonadaceae.__.__	0.94	0.0028
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Marivita.s__	0.95	0.0034
k__Bacteria.p__Bacteroidetes.c__.Saprospirae..o__.Saprospirales..f__Saprospiraceae.g__Lewinella.s__	0.94	0.004
k__Bacteria.p__Planctomycetes.c__Phycisphaerae.o__Phycisphaerales.f__.g__.s__	0.91	0.0046
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Jannaschia.s__	0.91	0.0046
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Alteromonadales.f__Alteromonadaceae.g__Candidatus.Endobugula.s__	0.91	0.0048
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Marinicellales..f__.Marinicellaceae..g__Marinicella.s__	0.91	0.0055
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rickettsiales.f__Rickettsiaceae.g__.s__	0.94	0.0062
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Oceanospirillales.f__.g__.s__	0.91	0.0084
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Psychroserpens.s__	0.90	0.0091
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Sphingomonadaceae.g__.s__	0.91	0.0109
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rickettsiales.f__.g__.s__	0.97	0.0114
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Winogradskyella.s__	0.92	0.0134
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Ruegeria.s__	0.96	0.0152
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__HTCC2188.f__HTCC2089.g__.s__	0.91	0.0167
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Bdellovibrionales.f__Bacteriovoracaceae.g__.s__	0.86	0.0256
k__Bacteria.p__Planctomycetes.c__Planctomycetia.o__Pirellulales.f__Pirellulaceae.g__.s__	0.90	0.0278
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__.s__	0.90	0.0352
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Erythrobacteraceae.g__Erythrobacter.s__longus	0.81	0.0427
k__Bacteria.p__Planctomycetes.c__Planctomycetia.o__Pirellulales.f__Pirellulaceae.g__planctomycete.__	0.81	0.0467
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Pontirhabdus.s__pectinivorans	0.81	0.0485
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Alteromonadales.f__HTCC2188.g__.s__	0.85	0.0497

Week 1 and Week 6

k__Bacteria.p__Bacteroidetes.c__.Saprospirae..o__.Saprospirales..f__.g__.s__	0.86	0.0175
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Tenacibaculum.s__	0.86	0.0276

Table S6. Core indicator community taxa among Pacific communities only.

Taxonomic classification	√Indicator Value
Core Taxa	
k__Bacteria.p__Bacteroidetes.c__.Saprospirae..o__.Saprospirales..f__Saprospiraceae.g__.s__	0.94
k__Bacteria.p__Bacteroidetes.c__Cytophagia.o__Cytophagales.f__Flammeovirgaceae.g__.s__	0.88
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Cryomorphaceae.g__.s__	0.91
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__.s__	0.97
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Krokinobacter.s__eikastus	0.84
k__Bacteria.p__Bacteroidetes.c__Flavobacteriia.o__Flavobacteriales.f__Flavobacteriaceae.g__Lutimonas.s__	0.81
k__Bacteria.p__Proteobacteria.__.__.__.__	0.81
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.__.__.__	0.94
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__Hyphomicrobiaceae.g__.s__	0.84
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Loktanella.s__	0.94
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Octadecabacter.s__	0.88
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Phaeobacter.s__	0.94
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Pseudoruegeria.s__	0.94
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Sulfitobacter.__	0.94
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Erythrobacteraceae.__.__	0.81
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Bdellovibrionales.f__Bdellovibrionaceae.g__Bdellovibrio.s__	0.88
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Alteromonadales.f__Alteromonadaceae.g__Glaciecola.s__	0.94
k__Bacteria.p__Proteobacteria.c1__Gammaproteobacteria.o__Alteromonadales.f__OM60.g__.s__	0.81
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Thiohalorhabdales.f__.g__.s__	0.91

Table S7. Indicator taxa at the Caribbean site for each time point and groups of time points classified to the highest resolution possible with √Indicator values and p-values. Only significant indicators (√Indicator values > 0.08 and p-value < 0.05) are shown.

Week 1

k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhodobacterales.f__Rhodobacteraceae.g__Loktanella.s__	0.81	0.0142
k__Bacteria.p__TM6.__.__.__.__.__	0.81	0.0145
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Oceanospirillales.f__Oceanospirillaceae.g__Oleibacter.s__	0.81	0.0148
k__Bacteria.p__Planctomycetes.c__028H05.P.BN.P5.o__.f__.g__.s__	0.87	0.0433
k__Bacteria.p__TM6.c__SBRH58.o__.f__.g__.s__	0.87	0.0037

Week 3

k__Bacteria.p__Planctomycetes.c__Phycisphaerae.o__mle1.8.f__.g__.s__	0.99	5.00E-04
k__Archaea.p__.Parvarchaeota..c__.Parvarchaea..o__YLA114.f__.g__.s__	0.96	5.00E-04
k__Bacteria.p__Verrucomicrobia.c__.Methylacidiphilae..o__Methylacidiphilales.f__LD19.g__.s__	0.95	5.00E-04
k__Bacteria.p__Caldithrix.c__Caldithrixae.o__Caldithrixales.f__BA059.g__.s__	0.96	8.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__MIZ46.f__.g__.s__	0.92	0.001
k__Bacteria.p__Actinobacteria.c__Acidimicrobiia.o__Acidimicrobiales.f__TK06.g__.s__	0.84	0.002
k__Bacteria.p__WS3.c__PRR.12.o__Sediment.1.f__.g__.s__	0.86	0.0032
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Kordiimonadales.__.__.__	0.81	0.015
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Legionellales.f__Coxiellaceae.g__Aquicella.s__	0.85	0.005
k__Bacteria.p__Spirochaetes.c__.Brachyspirae..o__.Brachyspirales..f__Brachyspiraceae.g__.s__	0.88	0.0045
k__Bacteria.p__Cyanobacteria.c__Nostocophycideae.o__Nostocales.f__Nostocaceae.g__.s__	0.81	0.0154
k__Bacteria.p__PAUC34f.c__.o__.f__.g__.s__	0.81	0.0167
k__Bacteria.p__Verrucomicrobia.c__Opitutae.o__.Pelagicoccales..f__.Pelagicoccaceae..g__Pelagicoccus.s__	0.84	0.0222
k__Bacteria.p__Cyanobacteria.c__4C0d.2.o__SM1D11.f__.g__.s__	0.85	0.033

Week 6

k__Bacteria.p__Bacteroidetes.c__Cytophagia.o__Cytophagales.f__Flammeovirgaceae.g__JTB248.s__	0.96	8.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Myxococcales.f__Haliangiaceae.g__.s__	0.92	0.001
k__Bacteria.p__Bacteroidetes.c__Sphingobacteriia.o__Sphingobacteriales.f__NS11.12.g__.s__	0.94	0.0012
k__Bacteria.p__Verrucomicrobia.c__Verrucomicrobiae.o__Verrucomicrobiales.f__Verrucomicrobiaceae.g__Persicirhabdus.s__	0.91	0.0024
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Thiohalorhabdales.f__Thiohalorhabdaceae.g__.s__	0.89	0.0025
k__Bacteria.p__Actinobacteria.c__Acidimicrobiia.o__Acidimicrobiales.f__C111.g__.s__	0.82	0.0142

Week 3 and Week 6

k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__NB1.j.__.__.__	1	1.00E-04
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__FAC87.f__.g__.s__	1	1.00E-04
k__Bacteria.p__Acidobacteria.c__.Chloracidobacteria..o__RB41.f__Ellin6075.g__.s__	0.99	1.00E-04
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__Hyphomicrobiaceae.g__Hyphomicrobium.s__	0.99	1.00E-04
k__Bacteria.p__Proteobacteria.c__TA18.o__PHOS.HD29.f__.g__.s__	0.99	3.00E-04
k__Bacteria.p__Acidobacteria.c__RB25.o__.f__.g__.s__	0.98	3.00E-04
k__Bacteria.p__Chloroflexi.c__Anaerolineae.o__Caldilineales.f__Caldilineaceae.g__.s__	0.97	4.00E-04
k__Bacteria.p__SBR1093.c__EC214.o__.f__.g__.s__	0.95	0.001
k__Bacteria.p__Cyanobacteria.__.__.__.__.__	0.95	0.0016
k__Bacteria.p__Proteobacteria.c__Betaproteobacteria.o__.f__.g__.s__	0.94	0.0023
k__Bacteria.p__Proteobacteria.c__TA18.o__CV90.f__.g__.s__	0.95	0.0032
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__NB1.j.f__NB1.i.g__.s__	0.92	0.0043
k__Bacteria.p__Planctomycetes.c__C6.o__d113.f__.g__.s__	0.94	0.005
k__Bacteria.p__WS3.c__PRR.12.o__Sediment.1.f__CV106.g__.s__	0.87	0.0131
k__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.__.__.__	0.85	0.0151
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Legionellales.f__Legionellaceae.g__.s__	0.93	0.0158
k__Bacteria.p__Cyanobacteria.c__4C0d.2.o__MLE1.12.f__.g__.s__	0.86	0.0167
k__Bacteria.p__Spirochaetes.c__.Leptospirae..o__Leptospirales..f__Leptospiraceae.g__Leptonema.s__	0.86	0.0187
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.o__Myxococcales.f__Cystobacterineae.g__.s__	0.91	0.0204
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__HOC36.f__.g__.s__	0.87	0.0215
k__Bacteria.p__Cyanobacteria.c__Gloeobacterophycideae.o__Gloeobacterales.f__Gloeobacteraceae.g__Gloeobacter.s__	0.87	0.0218
k__Bacteria.p__Acidobacteria.c__AT.s2.57.o__.f__.g__.s__	0.81	0.0351
k__Bacteria.p__Cyanobacteria.c__4C0d.2.o__SM2F09.f__.g__.s__	0.84	0.0416
k__Bacteria.p__.c__.o__.f__.g__.s__	0.81	0.042
k__Bacteria.p__Proteobacteria.c__Deltaproteobacteria.__.__.__	0.87	0.0446

Figure S3. Total PAH concentrations for each plastic polymer incubated at the Caribbean site (A), and Pacific site (C) and water column concentrations at the Caribbean site (B) and the Pacific site (D).
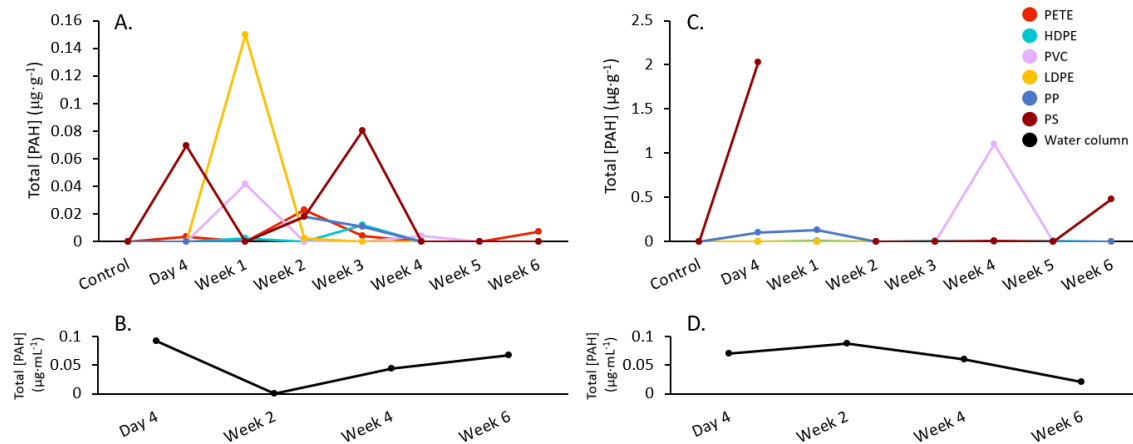
Table S8. List of PAHs recovered from each sample at the Pacific site. P = plastics. D = Day (i.e., D4 P6 is Day 4, Plastic 6, etc.).

	P1 Blank	P2 Blank	P3 Blank	P4 Blank	P5 Blank	P6 Blank	D4 P1	D4 P2	D4 P3	D4 P4	D4 P5	D4 P6	W1 P1	W1 P2	W1 P3	W1 P4	W1 P5	W2 P1	W2 P2	W2 P4	W2 P5	W2 P6	W3 P1	W3 P2	W3 P3	W3 P4	W3 P5	W3 P6	W4 P1	W4 P2	W4 P3	W4 P5	W4 P6	W5 P1	W5 P2	W5 P3	W5 P4	W5 P5	W5 P6	W6 P1	W6 P3	W6 P4	W6 P5	W6 P6
1-Methylnaphthalene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acenaphthylene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acenaphthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluorene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenanthrene	0	0	0	0	0	0	0	0	0	0	0	1.445	0	0.001769	0	0	0	0	0.001181	0	0	0	0	0.001772	0	0	0	0	0	0	0.003309	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthracene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.001067	0	0	0	0	0.001601	0	0	0	0	0	0.000767	0.005705	0	0	0	0.001426	0	0	0	0	0	0	0	0	0
4H-Cyclopenta[def]phenant	0	0	0	0	0	0	0	0	0	0	0.07579	0	0	0	0	0	0.03683	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.006291	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.005551	0	0	0	0	0	0	0	0	0	0	0	0	0
Benz[a]anthracene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1179	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysene + Triphenylene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1651	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[b]fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.008182	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[k]fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02052	0	0.003243	0	0	0	0	0	0	0	0	0	0	0
Benzo[b+j+k]fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02043	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[a]pyrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Indeno[1,2,3-cd]pyrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01617	0	0	0	0	0	0	0	0	0	0	0	0	0
Dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5001	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[ghi]perylene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2651	0	0	0	0	0	0	0	0	0	0	0	0	0
Dibenzo[a,e]pyrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table S9. List of PAHs recovered from each sample at the Caribbean site. P = plastics. D = Day (i.e., D4 P6 is Day 4, Plastic 6, etc.).

	P1 Blank	P2 Blank	P3 Blank	P4 Blank	P5 Blank	P6 Blank	D4 P1	D4 P2	D4 P3	D4 P4	D4 P5	D4 P6	W1 P1	W1 P2	W1 P3	W1 P4	W1 P5	W1 P6	W2 P1	W2 P2	W2 P3	W2 P4	W2 P5	W2 P6	W3 P1	W3 P2	W3 P43	W3 P4	W3 P5	W3 P6	W4 P1	W4 P2	W4 P3	W4 P4	W4 P5	W4 P6	W5 P1	W5 P2	W5 P3	W5 P4	W5 P5	W5 P6	W6 P1	W6 P2	W6 P3	W6 P4	W6 P5	W6 P6
Acenaphthene	0	0	0	0	0	0	0	0	0	0	0.005936	0.005174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fluorene	0	0	0.001622	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.022869	0	0	0.00272	0
Phenanthrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014787	0	0	0	0	0	0.001232	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.015847	0	0	0
Fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.036316	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.034394	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysene + Triphenylene	0	0	0	0	0	0	0	0	0	0.023924	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[b+j+k]fluoranthene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014874	0	0	0	0
Indeno[1,2,3-cd]pyrene	0	0	0	0	0	0	0	0	0	0	0.001449	0	0	0	0.010515	0	0	0	0.005129	0	0	0	0	0	0.003673	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dibenz[a,h]anthracene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.010869	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Benzo[ghi]perylene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.006868	0	0	0	0	0	0	0.004508	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.229812	0	0	0	0
Dibenzo[a,l]pyrene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0