Microbial colonization of microplastics in the Caribbean Sea

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Scientific Significance Statement

Microplastics, plastic particles smaller than 5 mm that vary in their chemical makeup depending on the plastic type, are emerging marine pollutants that serve as a novel matrix for microbial colonization in the ocean. The communities growing on the microplastics, also termed the “plastisphere”, may ultimately determine their degradation, deposition, and utilization by the food web, but the factors that shape their composition and development, such as environment, plastic type, and exposure time, are still insufficiently understood. In controlled incubation experiments carried out in a tropical bay in Panama, we show that the composition of a microplastics’ bacterial biofilm is not shaped by plastic type or time exposed the environment. On the other hand, we found that the eukaryotic plastisphere was shaped by both plastic type and exposure time and found that specifically diatom communities exhibited preference for some plastic types.

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, shows that neither plastic polymer type nor time exposed to the environment plays 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.

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Five trillion pieces of plastics are estimated in the global ocean, with 4.8–12.7 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), and so on 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 (PP) particle collected in the Caribbean Sea 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 (PETE) (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 6-week time period, 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 and 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.

**Methods**

**Experimental setup 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 PETE (#1), HDPE (#2), polyvinyl chloride (PVC, #3), low-density polyethylene (LDPE, #4), PP (#5), and PS (#6). Each plastic type was cut into rectangular pieces of 1.5–5 mm in size and washed in 10% hydrochloric acid. The 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 to 995 μm 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 1 g 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 6 weeks (Fig. 1). Microplastics were gently vortexed in filtered (0.2 μm) 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, 250 mL 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).

**Chlorophyll a concentration**

Chlorophyll a (Chl a) concentration in 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 was measured with a Turner Designs TD-700 Fluorometer (Supporting Information Table S1).

**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 DNAeasy 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 (Debeljak et al. 2017). For all samples, Ready-Lyse™lysozyme (10 mL of 1000 units mL−1 stock; Lucigen, Madison, WI) was added and incubated for 30 min in 37°C. The filter samples of ambient seawater were extracted with modified proportions of ATL and Proteinase K, 900 μL and 20 μL, 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 was determined by the Illumina MiSeq 2x300 amplicon sequencing platform of 16S and 18S rRNA genes. Polymerase chain reaction (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. 2019). 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. 2012) databases using trained classifiers. Eukaryotic ASVs classified as Metazoa were removed from the analyses.

**Scanning electron microscopy**

Microplastics were preserved in glutaraldehyde (Sigma-Aldrich, 5% [v/v]), cooled at 4°C for 2–8 h, then transferred into 50% (v/v) ethanol in phosphate-buffered 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 VEGA3 SEM operated at 15 kV.

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 cells). Taxonomic identification of the groups was carried out using reference literature (see Supporting Information Table S8). 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 1760-fold with working distances between 13.36 and 14.02 mm, making each field approximately 200 μm² in size. Diatom density on the plastic surfaces was determined in duplicates for each plastic type and expressed in cells mm⁻².

**Data analyses**

Alpha-diversity indices were calculated using PRIMER v7 (Clarke and 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.
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(\(f\)); and diversity, which considers both richness and evenness, and is represented by the Shannon-Weiner index (\(H^f\)). 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 was 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):

\[
\text{SES} = \frac{\text{BC}_{\text{obs}} - \text{BC}_{\text{exp}}}{\text{SD}_{\text{exp}}}
\]

where \(\text{BC}_{\text{obs}}\) is the BC dissimilarity index calculated between two communities (the original matrix), \(\text{BC}_{\text{exp}}\) is the mean expected BC dissimilarity index calculated from the randomized distribution of the two communities, and \(\text{SD}_{\text{exp}}\) 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” (Kemel 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 https://datadryad.org/stash/dataset/doi:10.5061/dryad.z8w9ghx7m?

Results

Environmental parameters measured on the days microplastics were sampled are shown in Supporting Information 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 0.82 \(\mu\)g L\(^{-1}\) and 0.88 \(\mu\)g L\(^{-1}\) at weeks 1 and 3, respectively, to 0.68 \(\mu\)g L\(^{-1}\) at week 6 (Supporting Information Table S1).

The bacterial plastisphere

Three 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 Bacteroidetes (14–48%). Bacteroidetes consisted mainly of the classes Flavobacteria (30–77%), Saprospirae (6–67%), and Cytophaga (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), \(t\)\(t\) = 12.95, \(p\) << 0.001 (Supporting Information Table S2). Evenness of water column communities was also significantly smaller (mean \(f\) = 0.534) than plastisphere communities (mean \(f\) = 0.7412, \(t\)\(t\) = 14.51, \(p\) << 0.001), that is, the total water column community was dominated by a small number of highly abundant organisms (Supporting Information Table S2). The lower richness and evenness of the total water column contributed to its significantly lower diversity (mean \(H\) = 2.52) compared to the plastospheres (mean \(H\) = 4.00, \(t\)\(t\) = 15.14, \(p\) << 0.001). Plastospheres did not significantly differ in richness, evenness, or diversity as a result of polymer type or time of incubation.

The visualization of bacterial rDNA 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 sequencings data to the null model analysis, those results were not confirmed. Observed BC dissimilarity indices were always low for pairwise comparisons (Supporting Information Tables S4, S5), indicating no significant differences in community composition between plastic types (\(BC \leq 0.51, \text{SES} \leq -8.07, p \leq 0.000\)) 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.

**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

![Fig. 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.”](image-url)
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 and 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 (Supporting Information 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.

Fig. 3. Composition of 15 most relatively abundant taxa based on 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”.
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) (Supporting Information Table S3). 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 values (Supporting Information 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 (Supporting Information 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

![Fig. 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, C) and for each time point (B, D).](image-url)
6 (BC = 0.91–0.94, \( p = 0.2682–0.2881 \)). When BC indices of plastic polymer types were analyzed between time points (Supporting Information Table S7), only PETE exhibited dissimilar communities between all time points (BC \( \geq 0.81, p \geq 0.058 \)), 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 \( \geq 0.87, p \geq 0.207 \)), and weeks 3 and 6 (BC \( \geq 0.89, p \geq 0.247 \)), but not weeks 1 and 3. PS only harbored dissimilar communities between weeks 1 and 6 (BC = 0.83, \( p = 0.063 \)).

**Microscopy reveals polymer preference for diatoms**

Because diatoms only reached sufficient density to be quantitatively analyzed by the end of the incubation (Supporting Information Fig. S1), all diatom analyses are shown for week 6. Distinct diatom communities could be distinguished on the microplastics (Fig. 5, Supporting Information Tables S8, 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 fimбриата*, and *Cocconeis* sp. appeared only on 2–4 plastic types. *Nitzschia sicula* and *Stratiella* sp. were exclusively present on PETE, whereas *Amphora* sp. was observed only on LDPE, *Mastogloia sicula* on PP, and *Pseudo-nitzschia* sp. on PS (Supporting Information 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 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 Supporting Information Fig. S1C).

**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, and the first carried out 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 (Supporting Information 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 (Dussud et al. 2018; Oberbeckmann 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 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 6 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., Supporting Information 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 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, Supporting Information Table S1).

Dominant prokaryotic taxa found in our study included Proteobacteria (Rhodobacteraceae) and Bacteroidetes, most notably Flavobacteriaceae, 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 a significantly different diatom community composition on HDPE and a biopolymer-PETE (biodegradable) plastic in the pelagic zone of the Mediterranean Sea after 33 d of exposure, but not at 15 d of exposure, indicating that both plastic polymer type in addition to time exposed to the environment 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 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 known 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 1 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 PS biofilms by Zettler et al. (2013). These bacteria are known to form prosthecae, or long extensions of the cytosolic cellular membrane (see Fig. 5A in picture of *Mastogloia corticina*, 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; Delacuverelle 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,b), 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 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, possibly making the plastisphere a hotspot for predatory 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 parasitize 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 (Garcías-Bonet et al. 2011). 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 that due to their 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 and Caroppo 2012), and Proorocentrum 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 investigated if biotically derived chemicals, such as toxins associated with HAB species, associate with microplastics in the environment, and what impacts this may have on marine life.

**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 6-week incubation period, such as Mastogloia corsicana on PP or Striatella sp. on PETE, and we hypothesize that the phyocosphere 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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