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Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris

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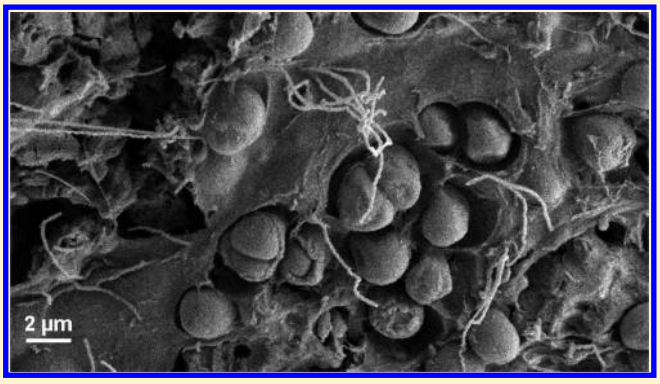
Supporting Information

ABSTRACT: Plastics are the most abundant form of marine debris, with global production rising and documented impacts in some marine environments, but the influence of plastic on open ocean ecosystems is poorly understood, particularly for microbial communities. Plastic marine debris (PMD) collected at multiple locations in the North Atlantic was analyzed with scanning electron microscopy (SEM) and next-generation sequencing to characterize the attached microbial communities. We unveiled a diverse microbial community of heterotrophs, autotrophs, predators, and symbionts, a community we refer to as the "Plastisphere". Pits visualized in the PMD surface conformed to bacterial shapes suggesting active hydrolysis of the hydrocarbon polymer. Small-subunit rRNA gene surveys identified several hydrocarbon-degrading bacteria, supporting the possibility that microbes play a role in degrading PMD. Some Plastisphere members may be opportunistic pathogens (the authors, unpublished data) such as specific members of the genus *Vibrio* that dominated one of our plastic samples. Plastisphere communities are distinct from surrounding surface water, implying that plastic serves as a novel ecological habitat in the open ocean. Plastic has a longer half-life than most natural floating marine substrates, and a hydrophobic surface that promotes microbial colonization and biofilm formation, differing from autochthonous substrates in the upper layers of the ocean.



INTRODUCTION

Plastic has become the most common form of marine debris since it entered the consumer arena less than 60 years ago, and presents a major and growing global pollution problem.[1−3] The current global annual production, estimated at 245 million tonnes[1] represents 35 kg of plastic produced annually for each of the 7 billion humans on the planet, approximating the total human biomass. Some fraction of the increasing amount of postconsumer plastic trash inevitably escapes the recycling and waste streams and makes its way to the global oceans. Additionally, tsunamis and storms can result in large pulses of plastic entering the ocean from coastal areas. Plastic accumulates not only on beaches worldwide, but also in "remote" open ocean ecosystems.[1] Drifter buoys and physical oceanographic models have shown that surface particles such as PMD can passively migrate from Eastern Seaboard locations all the way to the interior of the North Atlantic Subtropical Gyre in less than 60 days,[4] illustrating how quickly human-generated debris can impact the gyre interior that is more than 1000 km from land. Plastic debris in the North Atlantic Subtropical Gyre[4] and North Pacific Subtropical Gyre is well-documented[5−9] and models and limited sampling confirm that accumulations of PMD have formed in all five of the world's subtropical gyres.[10,11]

The effects of plastic debris on animals such as fish, birds, sea turtles, and marine mammals as a result of ingestion,[12−15] and marine entanglement[3,16−18] are well documented, but studies of plastic-associated microbial communities are lacking, and we know little about the impact of this anthropogenic substrate and its attached community on the oligotrophic open ocean. As a relatively new introduction into the marine ecosystem, plastic debris provides a substrate for microbes that lasts much longer than most natural floating substrates and has been implicated as a vector for transportation of harmful algal species[19] and persistent organic pollutants (POPs).[20,21] With a hydrophobic surface rapidly stimulating biofilm formation in the water column, PMD can function as an artificial "microbial reef". PMD at concentrations of up to 5×10^5 pieces/km^2 in the North Atlantic Subtropical Gyre[4] represents a new floating

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substrate for microbial colonization and transportation, and the presence of particles in aquatic systems is known to stimulate microbial productivity and respiration.[22−25] Once trapped in central ocean gyres, there are very few avenues for export, and buoyant plastic particles accumulate and may persist for decades. Increases in PMD have been documented in the North Pacific Gyre,[6] but despite increases in plastic production, use, and presumably input into the ocean, other studies show no significant trend in plastic accumulation in the North Atlantic Subtropical Gyre[4] or in the waters from the British Isles to Iceland since the 1980s.[26] Physical shearing and photodegradation are proposed mechanisms of plastic degradation.[27,28] These physical mechanisms may result in fragmentation into pieces small enough to pass through standard sampling nets.[29] In addition, biofilm formation and colonization by invertebrates can decrease plastic buoyancy allowing some of the plastic debris to sink to deeper waters, with eventual seafloor deposition.[1,30] However, plastic debris is absent from sediment traps[4] suggesting that density-mediated transport of small pieces is relatively low.

PMD has been reported by a number of studies starting in the 1970s[31,32] where authors mention diatoms and other microbes on the debris. However, our study presents the first comprehensive characterization of microbial communities living on PMD in the open ocean with an emphasis on bacteria. We hypothesized that this new man-made substrate is physically and chemically distinct from surrounding seawater and naturally occurring substrates such as macroalgae, feathers, and wood, with the potential to select for and support distinct microbial communities. Using pyrotag sequencing and SEM, we investigated representative microbial communities on pieces of polyethylene (PE) and polypropylene (PP) plastic from geographically distinct areas from the North Atlantic Subtropical Gyre and compared them to the microbial communities in the surrounding seawater. Our analyses unveiled for the first time the breadth of PMD microbes that make up what we call the "Plastisphere".

EXPERIMENTAL SECTION

Sample Collection. Plastics were collected in a 1 × 0.5 m rectangular neuston net with 333-μm mesh towed at the surface from the Sea Education Association (SEA) vessel SSV Corwith Cramer as part of SEA Semester research cruises C-230 and C-241 (Supporting Information (SI) Table S1). Individual pieces of plastic were sorted with sterile forceps and rinsed with sterile seawater prior to subdivision using a sterile razor blade and preservation for DNA extraction and SEM. While the net was in the water, we filtered 4 L from a clean seawater system (periodically freshwater-flushed nonmetallic line drawing water from 3 m below the surface) through a 0.2 μm Sterivex cartridge filter (Millipore, Billerica, MA) to collect microorganisms suspended in the ambient surface water.

Sample Preservation. Plastic and seawater filters for downstream DNA analysis were immediately flooded with Puregene lysis buffer (Qiagen, Valencia, CA) and frozen at −20 °C. PMD samples for SEM were fixed in 4% paraformaldehyde for 2−23 h, then transferred to 50% ethanol in Phosphate Buffered Saline (PBS) and kept at −20 °C.

SEM. Preserved plastic samples for SEM were dehydrated on ice through an ethanol series: 10 min each in 50%, 70%, 85%, 95%, followed by 3 × 15 min in 100% ethanol. Samples were immediately critical point dried using a Samdri 780A (Tousimis, Rockville, MD), sputter coated with 5 nm of platinum using a Leica EM MED020 (Leica Microsystems, Inc. Buffalo Grove, IL), then visualized and imaged on a Zeiss Supra 40VP SEM (Carl Zeiss Microscopy, Thornwood, NY). Cell measurements were made from digital images using ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, MD http://imagej.nih.gov/ij/, 1997-2012).

Raman Spectroscopy. The resin composition of plastic pieces was identified using a PeakSeeker Pro Raman spectrometry system (Agiltron, Woburn, MA) with a microscope attachment that enabled measurement of spectra from very small pieces of plastic. Each sample was compared with reference scans from plastics of known composition. We selected three pieces of polypropylene and three pieces of polyethylene that were large enough to subdivide for SEM and DNA extraction.

Amplicon Pyrotag Sequencing. DNA was extracted using a modified bead-beating approach[33] in combination with the Puregene Tissue DNA extraction kit (Qiagen, Valencia, CA). We amplified bacterial V6−V4 hypervariable regions of the small-subunit rRNA (SSU rRNA) gene using primers targeting *Escherichia coli* positions 518 and 1046.[34] We multiplex-sequenced the resulting amplicons with a barcoded primer strategy[35] on a 454 Genome Sequencer FLX (Roche, Basel, Switzerland) using the manufacturer's suggested protocol for the GS-FLX-Titanium platform. We trimmed sequences of adapter and primer sequences and removed low-quality reads as described previously.[36] We further filtered low quality base calls by applying "anchor trimming" to search for conserved V5 priming regions and trimmed to these conserved regions. We assigned Operational Taxonomic Units (OTUs) to clusters using the UCLUST v3.0.617 de novo clustering algorithm[37] at four percent cluster widths (96% similarity) to further minimize OTU inflation associated with pyrosequencing errors of longer V6−V4 amplicons. Eukaryotic amplicon sequencing targeted the V9 hypervariable region and followed protocols established in Amaral-Zettler et al. (2009).[33] Sequence data are deposited in NCBI's Sequence Read Archive (SRP026054) and conform to the Minimum Information about a MARKer gene Sequence (MIMARKS) standard (SI Table S2).[38]

Data Analyses and Statistical Methods. We used the R package limma[39] to calculate Venn diagrams and plotted the resulting figures using the Venn Diagram Plotter (http://ncrr.pnnl.gov/). Data for subsequent analyses were resampled down to the lowest number of reads recovered (6,102 reads) to standardize for sampling effort. R package routines gplots and heatmap.2 helped to generate the heatmap summary of all OTUs that were encountered with a frequency of greater than 2% in a given sample. OTU bar graphs were generated using Global Alignment Sequence Taxonomy (GAST) algorithms[40] and graphical output in QIIME v1.3.0.[41] We used linear discriminant analysis (LDA) effect size (LEfSe)[42] to identify biomarkers for plastics versus seawater and substrate specific (PP vs PE vs seawater) analyses. To ascertain the closest relative of our dominant *Vibrio* OTU sequence found on the C-230 polypropylene substrate (SI Figure S2), we used the quick-add-sequence-to-tree parsimony feature in the SILVA- 111 reference tree.[43] We then retained only the named type species to summarize the result. We examined co-occurrence patterns using network analysis and significant linear Pearson correlations. For the input matrices we only considered OTUs that occurred in at least 30% of the samples. We used Cytoscape (http://www.cytoscape.org) to visualize the result-

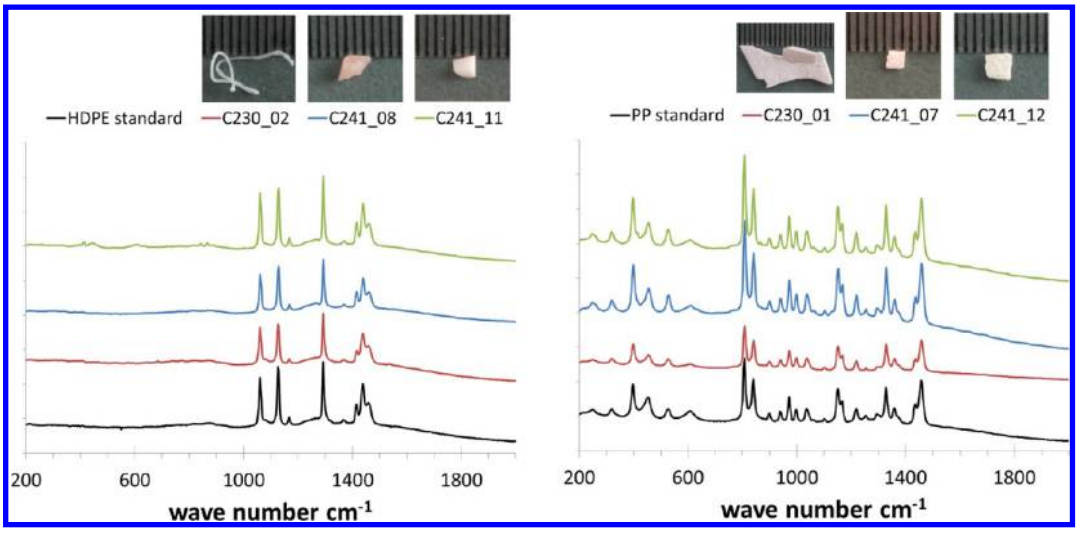

Figure 1. Raman spectroscopy spectra of the plastics collected from the Sargasso Sea that were imaged and sequenced. The bottom scan on each panel is of a known standard. Images along the top are light-micrographs of the plastic samples extracted for DNA analyses ((1-mm gradations; note different magnification on sample C230_01).

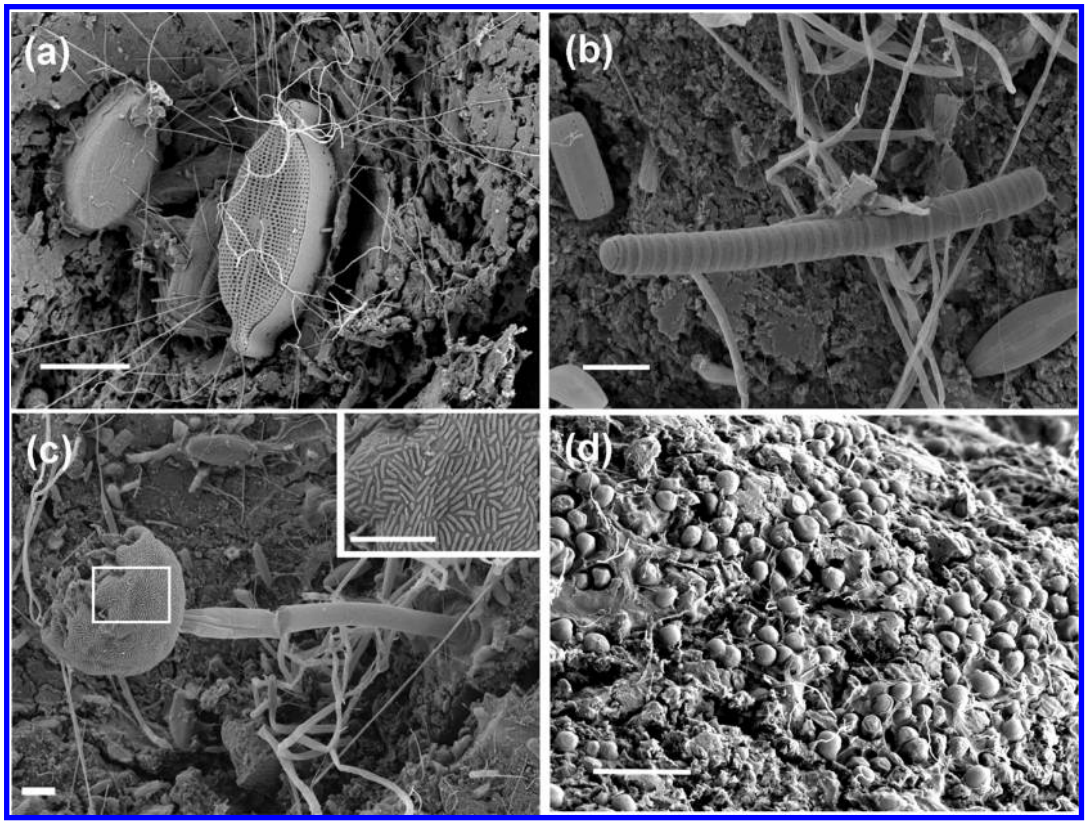

Figure 2. SEM images showing examples of the rich microbial community on PMD: (a) pennate diatom on sample C241_07 with possible prosthecate filaments produced by *Hyphomonas*-like bacteria; (b) filamentous cyanobacteria on sample C230_01; (c) stalked predatory suctorian ciliate in foreground covered with ectosymbiotic bacteria (inset) along with diatoms, bacteria, and filamentous cells on sample C230_01; (d) microbial cells pitting the surface of sample C241_12. All scale bars are 10 μm.

ing network and only considered significant correlations with an *R*-value >0.9.

RESULTS AND DISCUSSION

Identification and Selection of PMD. The majority of plastic pieces recovered in all net tows were fragments of less than 5 mm as has been reported in other studies.[29,44] Even the centimeter-sized pieces chosen to extract DNA and image the same piece with SEM were fragments without identifiable markings of undetermined origin. Pieces we examined with SEM ranged in size from sub-mm diameter monofilament (Figure 1, piece C230_02) to flat fragments that were several cm long before subdivision (Figure 1, piece C230_01). All showed signs of degradation including cracks and pitting as shown in Figure 2. With the microscope-based Raman spectrometer, most fragments collected were positively

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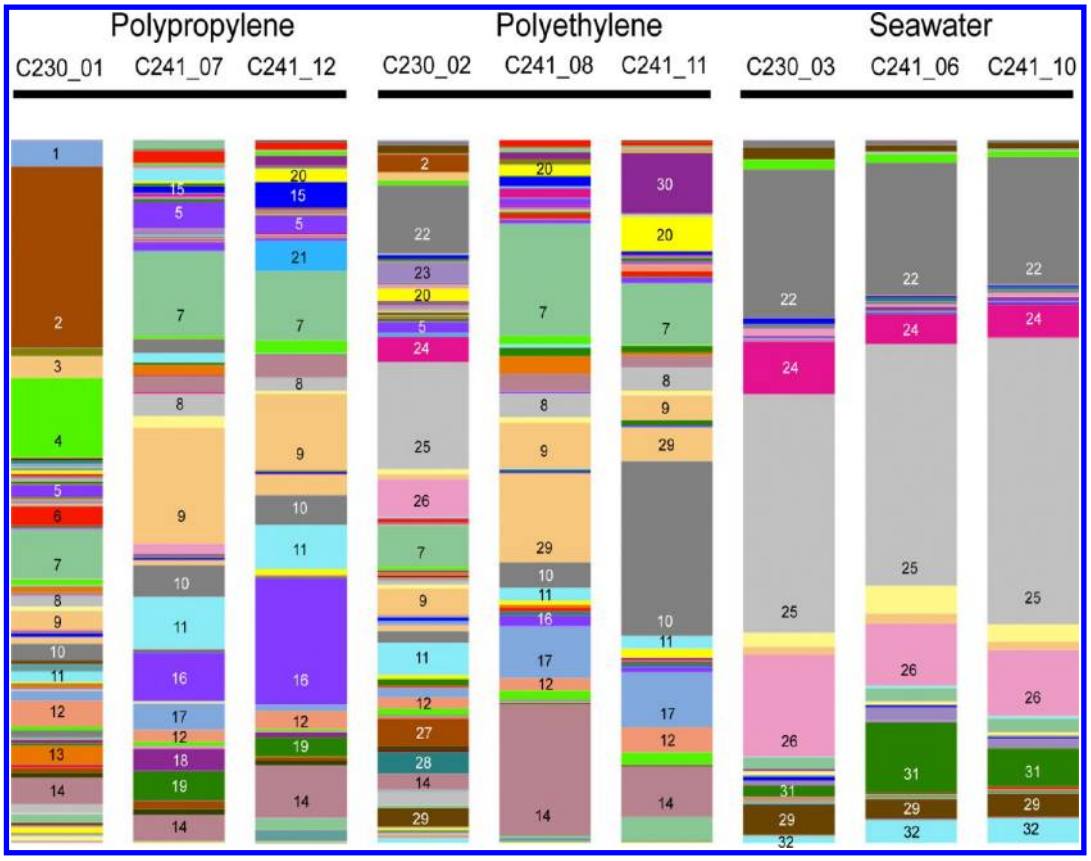

Figure 3. Bar chart showing similarity between all three seawater samples and dominance of a relatively small number of abundant OTUs, versus plastic samples with greater variability between samples and greater evenness indicated by more groups representing smaller proportions of the total population. The most abundant OTUs are listed as follows: (1) Bacteria, Verrucomicrobia, Verrucomicrobiae, Verrucomicrobiales, Rubritaleaceae, Rubritalea; (2) Bacteria, Proteobacteria, Gammaproteobacteria, Vibrionales, Vibrionaceae, *Vibrio*; (3) Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, *Psychrobacter*; (4) Bacteria, Proteobacteria, Gammaproteobacteria; (5) Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Erythrobacteraceae, *Erythrobacter*; (6) Bacteria, Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae, *Thalassobius*; (7) Bacteria, Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae; (8) Bacteria, Proteobacteria, Alphaproteobacteria, Parvularculales, Parvularculaceae, *Parvularcula*; (9) Bacteria, Proteobacteria, Alphaproteobacteria, Caulobacterales, Hyphomonadaceae, *Hyphomonas*; (10) Bacteria, Cyanobacteria, Cyanobacteria, Subsection III, Unassigned, *Phormidium*; (11) Bacteria, Cyanobacteria, Cyanobacteria, Subsection III; (12) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Saprospiraceae, *Lewinella*; (13) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Flammeovirgaceae, *Fulvivirga*; (14) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Chitinophagaceae; (15) Bacteria, Proteobacteria, Deltaproteobacteria, Myxococcales; (16) Bacteria, Chloroflexi, Anaerolineae, Anaerolineales, Anaerolinaceae; (17) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Saprospiraceae, *Saprospira*; (18) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Flammeovirgaceae; (19) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Flammeovirgaceae, *Marinoscillum*; (20) Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, *Alteromonas*; (21) Bacteria, Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae, *Rhodovulum*; (22) Bacteria, Proteobacteria, Gammaproteobacteria, Oceanospirillales, SAR86; (23) Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Pseudoalteromonadaceae, *Pseudoalteromonas*; (24) Bacteria, Proteobacteria, Alphaproteobacteria, Rickettsiales, SAR116; (25) Bacteria, Proteobacteria, Alphaproteobacteria, Rickettsiales, SAR11, *Pelagibacter*; (26) Bacteria, Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae; (27) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Flammeovirgaceae, *Amoebophilus*; (28) Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Chitinophagaceae, *Sediminibacterium*; (29) Bacteria, Bacteroidetes, Flavobacteria, Flavobacteriales, Flavobacteriaceae; (30) Bacteria, Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Oceaniserpentilla*; (31) Bacteria, Cyanobacteria, Cyanobacteria, Subsection I, Unassigned, *Prochlorococcus*; (32) Bacteria, Actinobacteria, Actinobacteria, Acidimicrobiales.

identified as polyethylene and polypropylene based on spectra compared to known standards (Figure 1). This was expected since these two resins are commonly used in packaging and other single-use plastic applications. They are also less dense than seawater so consequently float and accumulate in surface waters.

The Plastisphere Community. Microscopic (phenotypic) and molecular sequence (genotypic) data provided complementary evidence for microbial phototrophy, symbiosis, heterotrophy (including phagotrophy), and predation in our analyses of PP and PE PMD samples. SEM photomicrographs revealed the presence of a rich eukaryotic and bacterial microbiota living on both PP and PE samples (Figure 2, a–d). Cell counts of random images identified over 50 distinct morphotypes covering between 0 and 8% of the surface area of the plastic. Especially intriguing were round cells about 2 μm in diameter embedded in pits in the surface of the PMD (abstract image and Figure 2d). Often occurring in rows or patches, the pits conform closely to the shape of the contained cells, and included dividing cells that suggest active growth. We have not identified these cells but they were the third most common type of morphotype seen, after diatoms and filaments. DNA

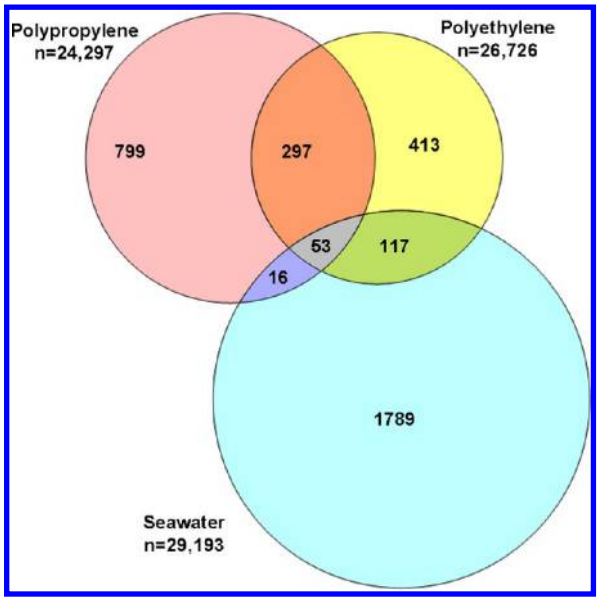

Figure 4. Venn diagram showing bacterial OTU overlap for pooled PP, PE, and seawater samples; *n* = number of sequenced reads per group. Numbers inside the circles represent the number of shared or unique OTUs for a given substrate.

sequence analyses confirmed that these communities were consistently distinct between plastics and the surrounding seawater. For example, photosynthetic filamentous cyanobacteria including *Phormidium* and *Rivularia* OTUs occurred on plastics but were absent from seawater samples where unicellular *Prochlorococcus* dominated the bacterial phototroph community in the seawater samples (Figure 3, SI Figure S1). The presence of cyanobacteria representing *Plectonema*-like genera was also evident in our SEM photomicrographs (Figure 2b).[45] Other conspicuous phototrophs included diatoms (Figure 2a and background of 2b and 2c) that were assigned to a number of bacillariophyte genera including *Navicula*, *Nitzschia*, *Sellaphora*, *Stauroneis*, and *Chaetoceros* based on DNA sequence data. The genera *Navicula*, *Nitzschia*, and *Sellaphora* are commonly substrate-associated and known biofilm formers in aquatic environments.[46] In addition to diatoms, representative OTUs from other protists with known photosynthetic representatives included prasinophytes, rhodophytes, cryptophytes, haptophytes, dinoflagellates, chlorarachniophytes, chrysophytes, pelagophytes, and phaeophytes.

While we only considered eukaryotic diversity for two of our samples, there was corroboration between SEM images and DNA sequence data including the stalked suctorian ciliates (Figure 2c) covered with bacteria (Figure 2c, inset), and sequence data confirming that one of the major ciliates was the genus *Ephelota*, a marine suctorian known to colonize marine surfaces and to harbor ectosymbiotic rod-shaped bacteria.[47] The relationship between the ectosymbiotic bacteria and the *Ephelota* is unknown, but ectosymbiontic bacteria on the surface of other stalked ciliates have been identified as sulfide-oxidizing Gammaproteobacteria[48] of the genus *Thiobios*, and we identified sequences corresponding to members of the same genus in our samples.

Surprisingly, DNA sequences derived from polycystine colonial radiolaria were present on both plastic types and dominated one polypropylene sample but were not identified in our SEM imaging. The discovery of radiolarian OTUs associated with PMD is somewhat unusual in that they are planktonic protists that are not understood to be substrate associated. However, there is precedence for other free-living taxa such as planktonic foraminifera becoming associated with PMD.[49] Furthermore, it is highly likely that radiolarians become passively associated with PMD both in the water column and in our nets given that colonial forms can reach meters in length and have a somewhat gelatinous nature. This may also explain the presence of other traditionally free-living taxa appearing to be associated, typically at low abundance, with our PMD substrates (i.e., *Pelagibacter*, *Prochlorococcus*).

Heterotrophic bacteria in seawater samples were dominated by *Pelagibacter* and other free-living picoplanktonic bacterial groups[50] but showed very different abundance patterns in the plastics samples. A striking example was the dominance of a member of the genus *Vibrio* that constituted nearly 24% of the C230_01 polypropylene sample. This is noteworthy because members of this genus are rarely found in concentrations greater than 1% of the community[51] although members of the species *V. harveyi* are a notable exception.[52,53] To the best of our knowledge, blooms of vibrios have not been associated with particles per se although they can dominate phytoplankton and zooplankton surfaces.[54] Vibrios are also known to have extremely fast growth rates[55] so this may explain their ability to dominate members of the Plastisphere on occasion.

Based on its taxonomic placement (SI Figure S2), the *Vibrio* sequence we recovered in high abundance on the polypropylene plastic sample C230_01 was related to the type species of *V. natriegens*, a known nitrogen fixer.[56] However, this sequence also shared 100% identity with other nontype strain vibrios assigned to the species *V. harveyi*, *V. alginolyticus*, *V. owensii*, *V. azureus*, *V. parahemolyticus*, *V. campbellii*, *V. diabolicus*, *V. communis*, and *V. rotiferianus*, all recent additions to GenBank.

We are unable to assign our dominant *Vibrio* OTU to a specific species based on rRNA sequence data alone so we cannot rule out the possibility that Plastisphere microbes such as vibrios could be animal or human pathogens. Plastic could serve as a vector of infectious diseases since both birds and fishes ingest PMD and a recent study found fishes contain human pathogenic *Vibrio* strains.[57] Because PMD persists longer than natural substrates (e.g., feathers, wood, and macroalgae), it can traverse significant distances, and it has been shown to transport invasive species.[58] Harmful dinoflagellate species belonging to the genus *Alexandrium* were reported from PMD in the Mediterranean,[19] and we also detected several dinoflagellate species including members of this genus on our Atlantic PMD. One property that these groups share is their propensity to adhere to surfaces. In the case of former reports of HAB-associated PMD, the authors specifically hypothesize that it was the "sticky" nature of the vegetative cysts that allowed them to adhere to plastic and may facilitate their dispersal beyond their typical range.[19] This final point reiterates the properties of PMD that set it apart from other types of marine debris: PMD is a selective environment with hydrophobicity that stimulates early colonizers, rapidly driving biofilm formation and succession of other microbes.[25] Additionally, the stimulation of microbial respiration and growth by inert surfaces is a well-characterized phenomenon in which dilute nutrients are concentrated creating a favorable environment for microbial colonization.[59] Termed the "ZoBell effect" (after Claude ZoBell who first thoroughly described the phenomenon[59]), this concentration of micronutrients by abundant PMD in oligotrophic areas of the ocean could play

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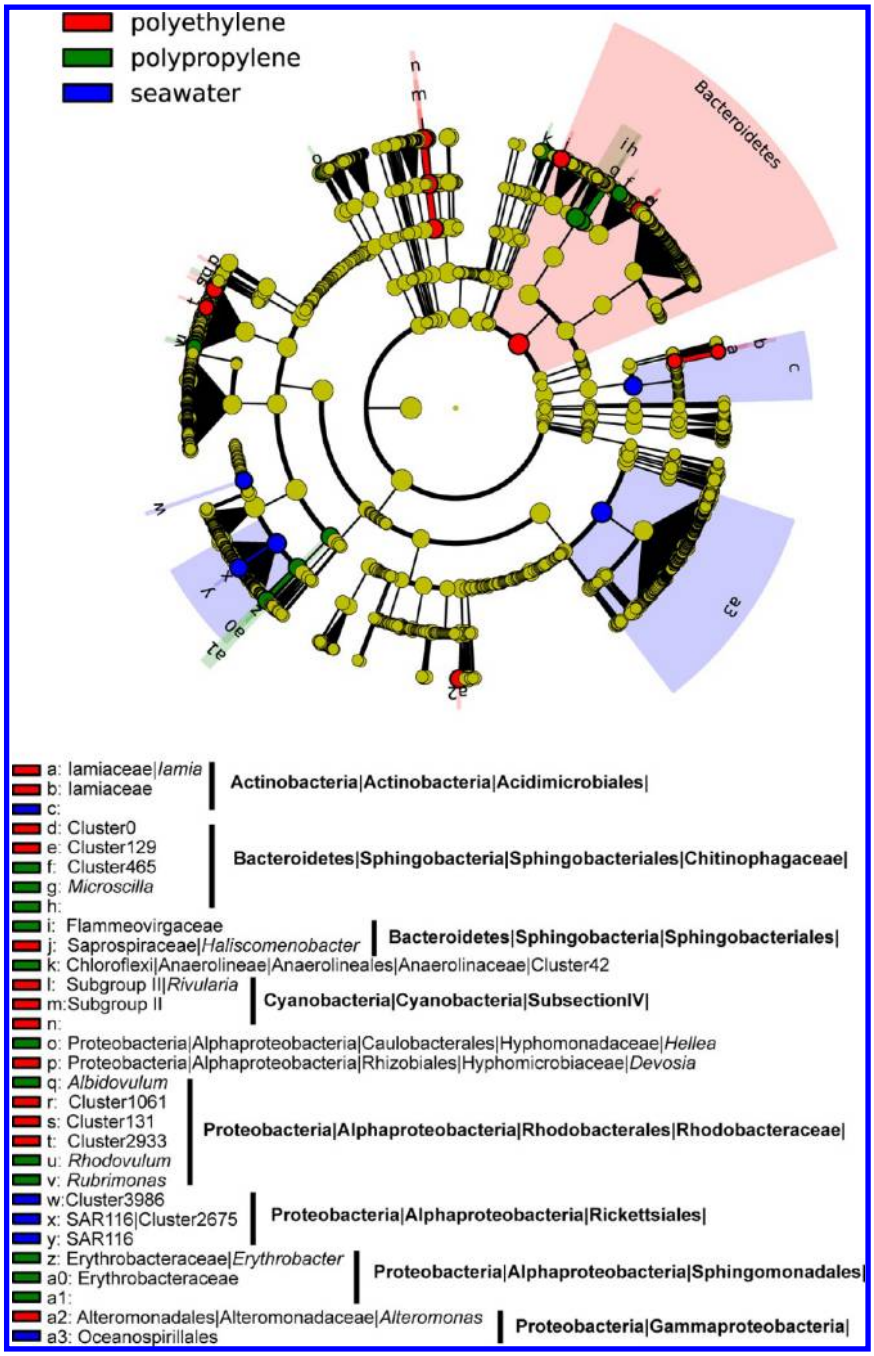

Figure 5. Taxonomic tree generated using the LEfSe online software highlighting the biomarkers that statistically differentiate PP, PE, and seawater samples. Circle diameter is proportional to taxon abundance. The tree highlights both high-level (Class) and genera-specific taxonomic trends. Refer to the legend for substrate color-coding.

a significant role in increasing microbial activity in the upper layer of ocean gyres.

Alpha Diversity. Over a thousand species equivalents, or OTUs were observed from single fragments of PE and PP (SI Table S1). Overall, there were two notable differences between diversity patterns between the plastics samples and the surrounding seawater: (1) average observed richness was much higher in surrounding seawater; but, (2) plastic substrates showed greater evenness than seawater, that is the community was not dominated by a small number of abundant organisms. It is difficult to directly compare richness between seawater and plastics because of sample size considerations. Seawater samples had the highest average richness and polyethylene the lowest, but when normalized with respect to sampling effort (number of reads recovered), the greatest richness in a single sample was associated with polypropylene. Although the wide range of richness values obtained from plastic pieces cautions against drawing general conclusions about richness and plastics, we expect richness to be related to substrate area and observed the highest richness on the largest piece of plastic analyzed (see image of C230_01 in Figure 1). Evenness, on the other hand, was consistently higher on plastics (mean Simpson evenness 0.95) compared to seawater (mean Simpson 0.89) and the brown alga *Sargassum* (mean Simpson 0.90) (data not shown). In other words, seawater was characterized by many more rare taxa that contributed to the richness in these samples.[60,61] This

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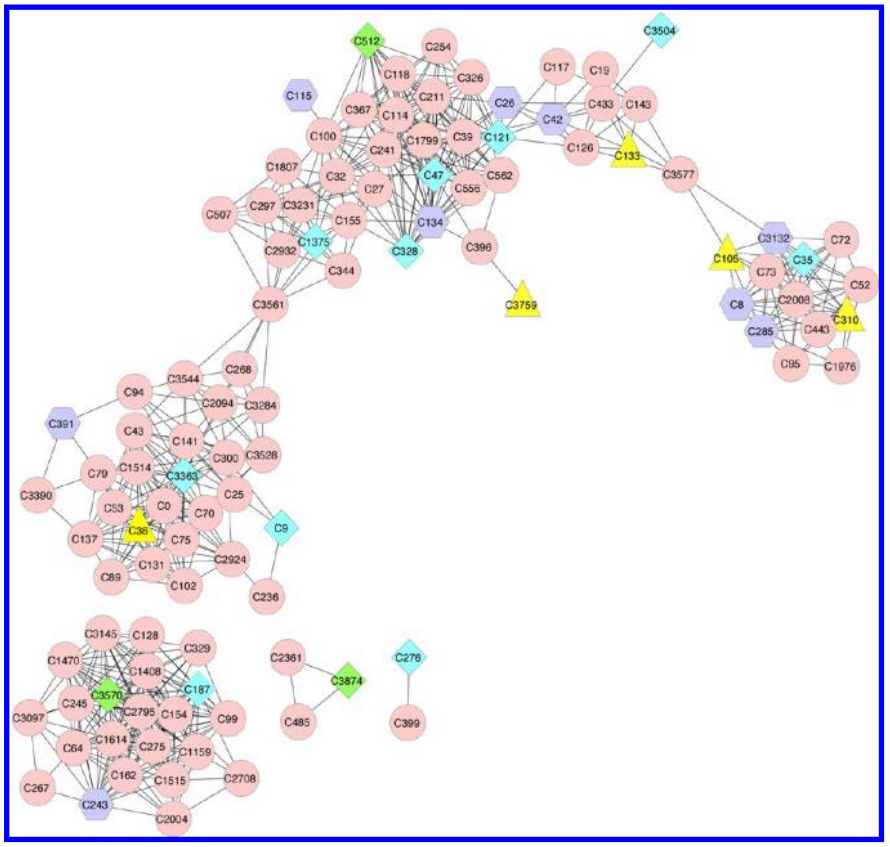

Figure 6. Network analysis diagram of putative hydrocarbon degrading bacterial OTUs. The cyanobacterium *Phormidium* is represented in green diamonds, *Hyphomonas* is depicted in blue diamonds, members of the Chloroflexi are shown in purple hexagons and members of the Myxococcales are represented as yellow triangles. SI Table S3 includes the full taxonomy for all the OTUs in the network.

could be due to a greater abundance of standing stock bacteria in oligotrophic seawater, known to support a high degree of rare taxa, partly due to lower grazing and viral pressures.[60] Additionally, a lower richness is expected in the more selective and metabolically active population of bacteria on the plastic surfaces supported by a relatively higher nutrient microenvironment. The distinctness of microbial communities from PMD was also reflected in the percentage of shared OTUs across the different plastic substrates (Figure 4) and *Sargassum* (data not shown). Collectively we found 350 bacterial OTUs shared between the PE and PP samples. Seawater had the largest number of unique OTUs (n = 1789), but these were mostly rare. Seawater shared a minor proportion of its OTUs with PE (8.6%) and PP (3.5%), respectively. In contrast, OTUs in common between PP and PE represented a substantial proportion of their overall OTU assemblage with 40% of the OTUs shared between PE and PP and 30% of the OTUs shared between PP and PE. Therefore, Plastisphere communities, despite being quite variable, do appear to have a "core" of taxa that characterize them.

Community Membership. To further determine the membership of the "core" Plastisphere community we performed biomarker analyses.[42] Linear discriminant analysis (LDA) effect size (LEfSe) revealed Plastisphere OTUs that characterized PP and PE samples indicating plastic resins may select for particular microbial community members. Of particular interest were OTUs found on both plastics but not in seawater. These included bacteria documented as capable of degrading hydrocarbons including the filamentous cyanobacterium *Phormidium* sp. known to settle on benthic substrates[62] and *Pseudoalteromonas*, a genus frequently associated with marine algae[63] (Figure 3, SI Figure S1). Additionally, the alphaproteobacterial family Hyphomonadaceae, known for forming long holdfast filaments termed prosthecae (which were common in our SEM micrographs) were unique to PMD and comprised almost 8% of the OTUs on PP (SI Figure S1). Members of the Hyphomonadaceae can be methylotrophic, known to degrade hydrocarbons and present in hydrocarbon enrichments.[64,65] Figure 5 summarizes the biomarker results and highlights the differences between each plastic substrate and seawater. LDA scores are shown in SI Figure S3.

Network Analyses. We can make inferences about organism associations from SEM observations of physical location and community architecture on the plastic surface. Although many bacteria cannot be identified visually, it is possible to infer interactions between members of the Plastisphere indirectly via association networks based on sequence data.[66−68] We conducted network analyses to further explore co-occurrence patterns between members of the Plastisphere. Reporting all existing networks is beyond the scope of this paper, so we present only networks associated with putative hydrocarbon-degrading bacteria within our overall network. Figure 6 depicts these networks with the cyanobacterium *Phormidium* highlighted as green diamonds, *Hyphomonas* as blue diamonds, members of the Chloroflexi as purple hexagons and members of the Myxococcales as yellow triangles. The figure only depicts first nearest neighbors in the network with positive correlations having $R > 0.9$. Noteworthy were the co-occurrences of several members of putative hydrocarbon degrading taxa in close proximity to each other in our network.

In addition to the four aforementioned main groups, we further detected other potential hydrocarbon-degrading OTUs that were their nearest neighbors. These included *Hyphomonas*-associated OTUs such as *Haliscomenobacter* (OTU C507, C300, C2094), associated with hydrocarbon contaminated soils,[69] *Devosia* (OTU C137) associated with diesel-contaminated soils[70] and *Oceaniserpentilla* (OTU 1470), one the of major taxa related to OTUs from the Deepwater Horizon oil spill.[71] While the presence of these taxa alone does not implicate them in plastic degradation, our network analyses suggests that consortia of OTUs may be acting in concert to utilize this recalcitrant carbon source and provides a testable hypothesis for future investigation.

PMD age and fate are poorly characterized; despite dramatic increases in plastic production, a 22-year study in the North Atlantic Subtropical Gyre showed no evidence of increasing quantities of PMD collected with a neuston net (333 μm mesh),[4] implying there must be unrecognized sinks to balance the sources. A recent review by Hidalgo-Ruz et al.[29] summarizes much of what is known about PMD and emphasizes the need for further study of how abiotic and biotic factors break it down. Our SEM images show microbial cells embedded in pits in the plastic surface, suggesting that microbes may be taking part in the degradation of plastic via physical or metabolic means (Figure 2d and abstract). These types of cells were found on both PE and PP, and include dividing cells (see image in abstract). Bacteria and fungi are known to degrade refractile compounds including plastic[62,63] but this has not been demonstrated in the open ocean. The pits visualized in PMD surfaces that conform to the shape of cells growing in the pits, and sequences of known hydrocarbon degraders support the possibility that some members of the Plastisphere community are hydrolyzing PMD and could accelerate physical degradation. Future research directions include understanding the genetic mechanisms of how microbes attach to PMD and elucidating the microbes and genes involved in microbially mediated plastic degradation through assaying our extensive culture collection, as well as exploring how these processes influence interactions with larger organisms.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1 shows a heatmap with the relative abundances of the most abundant taxonomic groups from PP, PE and seawater. Figure S2 shows an ARB tree with the placement of our most abundant *Vibrio* OTU. Figure S3 shows the LDA scores for the OTUs that explain the greatest differences between seawater, PP and PE communities. Table S1 provides contextual data for the samples used in this study including observed richness and evenness. Table S2 provides a MIMARKS table. Table S3 provides the taxonomy for the network analysis shown in Figure 6.This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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