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LIFE ON PLASTIC
The microbial ecology of the plastisphere

Diversity and discovery of phages
Gotta catch ‘em all

Ecology of the plastisphere

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Abstract
The plastisphere, which comprises the microbial community on plastic debris, rivals that of the built environment in spanning multiple biomes on Earth. Although human-derived debris has been entering the ocean for thousands of years, microplastics now numerically dominate marine debris and are primarily colonized by microbial and other microscopic life. The realization that this novel substrate in the marine environment can facilitate microbial dispersal and affect all aquatic ecosystems has intensified interest in the microbial ecology and evolution of this biotope. Whether a 'core' plastisphere community exists that is specific to plastic is currently a topic of intense investigation. This Review provides an overview of the microbial ecology of the plastisphere in the context of its diversity and function, as well as suggesting areas of further research.

Introduction

As global plastics production approached 350 million tonnes in 2017[1] and continues to rise (FIG. 1[2]), public awareness of plastic pollution in our environment has increased. With the United States and Canada recently placing restrictions on the use of microbeads in cosmetics, and the European Union Commission's decision to ban some single-use plastics (which will come into effect in 2021) there is an increase in public pressure and legislation to dampen the input of plastic debris into our ocean and the environment overall. In 2010, approximately 5-13 million tonnes of plastic entered the ocean[3], contributing to 15-51 trillion floating plastic particles circulating in the marine environment[4]. This increasing stream of contamination threatens to double by 2030 if the present rate of release continues[5].

Research efforts have focused on exploring the influence of plastic litter on aquatic ecosystems, such as the coastal and open ocean. Plastic debris provides a durable substrate that can be colonized by microorganisms, transported long distances, and supports the growth of microbial biofilms that include potential pathogens[6] and harmful algal bloom species[7]. This new human-made ecosystem is referred to as the plastisphere[6]; this term originally referred to life on microplastics (plastic litter < 5 mm) collected from the North Atlantic Subtropical Gyre, where the accumulations of floating debris were alluded to as 'garbage patches', but has since been used to describe life associated with plastic debris in many aquatic environments[8, 9]. A large percentage of plastic debris (about 80%) originates from land-based sources, including rivers and

wastewater treatment facilities that unintentionally release microplastics (FIG. 2). Biomass of the known plastisphere has previously been approximated to 0.01–0.2% of total microbial biomass in open ocean surface waters[10]. Given that recent studies confirm that we can only account for about 1% of the plastic litter that is released into the marine environment,[4] the plastisphere biomass in the global ocean is likely to be substantial. Where is the missing plastic, and what are the ecological effects of plastic debris in the marine environment? Fragmentation results in many particles that are too small to quantify due to limitations in sampling and analysis techniques, and even much of floating plastics (polyethylene, polypropylene and expanded polystyrene Supplementary Table S1) is hypothesized to ultimately end up below the ocean surface in different oceanic compartments such as the sediments via biofouling and the marine food web via ingestion[11, 12].

Elucidating the role of plastic debris in the microbial loop, especially in the oceanic gyres or accumulation zones, is key to helping us understand the ecological impact of plastic pollution on open ocean environments. Although carbon is not a limiting nutrient in the ocean, nitrogen, iron or phosphorous are, and plastic debris offers a surface where limiting nutrients are more available in these nutrient-depleted oceanic deserts[10]. Furthermore, microorganisms have the ability to biotransform plastic debris into compounds that could pose a risk to human health and food security[13].

Early studies of the plastisphere relied primarily on microscopy, which identified morphologically distinct organisms such as diatoms and filamentous bacteria[14], but the application of modern molecular methods and especially high-throughput DNA sequencing is increasing our understanding of the diverse microorganisms that inhabit the plastisphere[15]. Recent studies investigate the microorganisms that thrive on plastic debris, revealing that many of those microorganisms are natural biofilm formers and prefer an attached lifestyle over a free-living one[8], and other studies examine community assembly[16] and succession, or explore the interactions within the communities[17], their metabolic capacities and how those communities affect their surrounding ecosystem[18]. Despite the current interest in the topic, there are still limited studies on the plastisphere using high-throughput DNA sequencing. Most samples that are analyzed are from Europe, with only a few studies targeting samples from Asia and America, and none from Africa. Data are lacking from polar regions and the Southern Hemisphere, where three of the five major ocean plastic accumulation zones are located (Supplementary FIG. S2). Only one metagenomic survey is available[18], studies in freshwater systems are still rare[9] and only very few studies have examined microorganisms on plastic below the water surface[19]. Most studies are focused on incubation experiments with known polymer types and only a few have examined communities on environmentally collected plastic debris and in the open ocean.

There has been increasing interest in the role of bacteria and other microorganisms in the degradation of plastics polluting the environment, in the hope that they might provide ‘solutions’ to the plastic pollution problem by guiding the design of more efficient enzymes for recycling facilities, landfills and plastics polluting the sea (BOX 1). Although plastics are a fairly new habitat for microorganisms, microbial hydrocarbon degradation activities have been known for some time with natural and anthropogenic hydrocarbon sources in the ocean ranging from 0.4-4 Gt/yr[20]. Thus, the 10^{29} microbial inhabitants of the ocean[21] are metabolically diverse and not naïve to natural hydrocarbons present in aquatic environments.

Despite noteworthy advances in the field, several key questions remain. What is the species composition (microbiome) of the plastisphere and to what extent is it unique to plastic? Does the plastisphere shift or increase the carrying capacity of the open ocean microbial community with the potential to perturb open ocean ecosystems? What is the fate of plastic debris? What harm, if any, does plastic debris pose to the flora and fauna of the environment and ultimately to humans? In this Review we provide an overview of the microbial ecology of the microbial community of the plastisphere in the context of their diversity, function and fate in the environment, and suggest areas of future research. We explore plastic as a metabolic substrate but do not exhaustively summarize the microorganisms that can degrade plastics to varying extents, which is reviewed elsewhere[22-24].

Community membership

Community richness and diversity. Some studies have shown that the microbial community on plastic is different compared to that on other particles in the same environment[8, 25] and certain microbial groups are consistently found on plastic, but to our knowledge no microorganisms occur only on plastic. Given the novelty of this substrate in the environment, it is likely that microorganisms are still adapting to colonizing and utilizing plastic. Currently, the carrying capacity of the community attached to the surface of plastic debris remains unknown. This raises the question of how many species can co-exist on the surface of a small piece of plastic, particularly in the open ocean, where most communities have a distinct dominance structure. Standardization in molecular approaches to answering this interesting question are lacking, but it is still possible to compare trends between studies[15]. In addition, studies have focused on colonization experiments whereby different substrates are placed in the environment, subsampled at various time points and compared in terms of their overall community composition, species richness and changes over time. In this section we focus on some broad observations from studies in which plastics were collected from the natural environment and then assessed for species richness and evenness, and also compared across different locations.

Reports of diversity (species richness and beta diversity) found on microplastics in the environment are still rare, which is likely to be due to the expense of obtaining such samples at sea, as well as the downstream processing costs. Another challenge working with natural' microplastic samples is low biomass (at least those in the < 5 mm size fraction) available for DNA extractions and subsequent microbial profiling, which affects the success in producing amplifiable DNA. The method of choice for comparative molecular ecology studies has been amplicon sequencing - initially via 454 pyrosequencing and later via Illumina MiSeq or HiSeq sequencing. This approach is still quite viable and probably the best way to obtain richness and evenness estimates from different types of environments.

Existing studies point to some interesting similarities and differences in aquatic environments. Both freshwater and marine aquatic environments show differences in bacterial community composition between plastic debris and the surrounding water[6, 26-31], with most studies indicating lower richness on plastic[6, 9, 16, 26, 30] but greater evenness[6, 25, 26, 28, 30] when measured. However, exceptions exist; in samples collected from coastal waters of France[28] richness on plastic debris was higher than in surrounding seawater, but a caveat of this study is that plastic samples were pooled. Studies contrasting species compositions and richness on

plastic found in sediments are even less common. Again, this is likely to be due to the labor-intensive nature of the sample collection and processing effort. Richness levels of bacteria and archaea in sediments versus macroplastic samples from the seafloor in the equatorial Atlantic were similar[19], and in the North Sea plastic debris, sediment and water had distinct communities, but similar levels of richness between seawater and plastic debris, with sediments possessing the highest richness levels[27].

To date, most plastisphere studies target bacterial and archaeal diversity (although not using archaeal-specific primers), and very few focus on eukaryotes. However, studies that do target natural plastic debris samples clearly indicate that eukaryotic taxa, especially microbial eukaryotes, are common members of the plastisphere[6, 18]. Difficulties in estimating the diversity of microbial eukaryotes stem from varying copy numbers of their rRNA marker genes that are typically targeted in biodiversity surveys, in which they can dominate the DNA content. Unlike water column studies that can size fractionate samples and exclude eukaryotic DNA to a large extent, plastic samples do not offer this option. However, eukaryotes represent a natural part of the community on plastic in the environment, and to understand the plastisphere community holobiome, eukaryotic taxa need to be included in future studies in addition to bacteria and archaea.

Microbial guilds in the plastisphere. Early scanning electron micrographs of biofilms on plastic substrates[14] hinted at the diversity within the microbial community in the plastisphere. Biofilms are aggregates of cells either attached or unattached to a substrate that grow within matrix composed of extracellular polymeric substances (EPS)[32]. Biofilm formation typically constitutes a considerable change in the lifestyle of a microorganism from a planktonic or motile state, whereby specific gene sets involved in chemotaxis, communication, adhesion and substrate transport are expressed to enable individual cells to form a matrix analogous to tissues[33] as well as fluid channels that help distribute nutrients between cells. More recent studies combining scanning electron microscopy (SEM) with molecular data[6, 18, 25, 34] confirmed that the plastisphere can be a crowded, surface-based micro-ecosystem in the oligotrophic open ocean that includes primary producers (for example, phototrophs), predators symbionts and decomposers (FIG. 3).

Phototrophs. Most studies show that phototrophs such as diatoms are common and omnipresent residents of the plastisphere, at least on plastics that are exposed to sunlight (SupplementaryTable S3). It is unclear whether their occurrence indicates recent colonization of the plastic surface, as they are often reported as early and sometimes dominant colonizers on plastic debris[17, 35-39]. Diatoms described morphologically in the very first paper reporting plastic in the ocean from the Sargasso Sea[40] included *Mastogloia angulata*, *Mastogloia pusilla*, *Mastogloia hulburti*, *Cyclotella meneghiniana* and *Pleurosigma* sp. whereas amplicon reads reported from the Sargasso Sea 40 years later[6] included identities best assigned to the genera *Sellaphora*, *Amphora* and *Nitzschia*. Moreover, *Mastogloia*, *Nitzschia* and *Amphora* genera were also reported from the Arabian Gulf based on morphological characteristics alone[41]. These findings suggest that diatom species tend to inhabit the sunlit plastisphere. New chloroplast databases[42] make it possible to assign eukaryotic phototroph data from bacterial amplicon surveys, thereby uncovering additional phototrophs. These data confirm the presence of diatoms on open ocean microplastics collected from both the Atlantic and Pacific gyres, as well as from

experimental marine incubation studies off Woods Hole, Massachusetts, United States and the island of Grenada[15, 26]. The findings also point to the abundance of other protistan phototrophs such as chlorarachniophytes (for example, *Chlorarachnion reptans*), which are less identifiable based on morphology alone compared to diatoms.

Although microscopy-based studies as well as amplicon sequencing studies have indicated that diatoms are common members of the plastisphere, metagenomic surveys[18] placed diatom clades at less than 1% of the eukaryotic community, which suggests that they are replaced over time as the community matures (see below; FIG. 4). Low diatom recovery was also a hallmark of recent work in estuarine and freshwaters, where diatoms were not well represented in amplicon comparative surveys, and instead the heterotrophic dinoflagellate *Pfiesteria* (in this study the most abundant eukaryotic genus on polyethylene and third most abundant on polystyrene) dominated polyethylene substrates[17]. The most abundant phototroph in this same study was the genus *Ulva* - with a Holozoan being the most abundant taxon on polystyrene. However, a limitation of using amplicon sequencing to infer eukaryotic abundances is that even a few metazoans such as macroalgae or bryozoans on a small piece of plastic can easily dominate amplicon read data owing to the high copy number of rRNA genes per cell.

Cyanobacteria typically join diatoms among the photosynthetic representatives that contribute to making net primary production positive on plastic substrates in contrast to the surrounding water column[18], as evidenced from chlorophyll *a* measurements combined with oxygen production and respiration measurements. Filamentous genera, including *Phormidium*, *Rivularia* and *Leptolyngbya*, are consistently found across different ocean basins on microplastics. The light-harvesting adaptation strategies (termed complementary chromatic adaptation) that many of these filamentous genera of cyanobacteria possess, help them overcome high-light and low-light challenges typical of the oligotrophic waters of the open ocean[18]. The report that the cyanobacterium *Microcystis* constitutes up to 4% of the bacterial community in a polyethylene terephthalate (PET) bottle incubation study in marine waters off of Oman[41] raises the question of whether this freshwater harmful algal bloomer may have been transported to a marine system via a propagule attached to the substrate. As microcystin-producing cyanobacteria like *Microcystis* do not typically occur in marine settings, the finding suggests that it may have arisen from an allochthonous source.

Photoheterotrophs and heterotrophs. In addition to phototrophs that derive energy from light, common residents of the plastisphere in sunlit portions of the ocean include potential photoheterotrophic bacteria of the genera *Erythrobacter* and *Roseobacter*. Some members of these genera possess genes that are affiliated with aerobic anoxygenic photoheterotrophy, and they contain bacteriochlorophyll, photosynthesize without producing oxygen, can fix CO_2 and engage in heterotrophy[43]. Organisms like some roseobacters could be classified as 'mixotrophic', a common microbial eukaryotic term but less frequently encountered when referring to bacteria[44, 45]. This and earlier studies reinforce the notion that even traditional phototrophs such as Cyanobacteria are able to use organic substrates heterotrophically[46, 47]. Some plastisphere studies have inferred function by comparing the taxonomy of genera represented on plastic debris[25, 48], but genes related to functions such as mixotrophy highlight that caution is warranted when drawing conclusions based on taxonomy alone.

Various bacterial cell morphologies are commonly seen in SEM images of plastic debris,

and molecular data confirm the presence of many heterotrophic bacteria. Although heterotrophic roseobacters have been identified on plastic debris, the classic 'heterotrophic' bacteria such as *Candidatus* Pelagibacter that dominate marine open waters tend to be scarce. Culturing efforts to grow bacteria with plastics as a sole carbon source (such as polypropylene or PET) have produced an assortment of isolates, including members of the Gammaproteobacteria (for example, *Pseudomonas* spp.[49], *Azotobacter* spp.[50]), and Firmicutes (for example *Bacillus* spp.[51]), as well as Actinobacteria (for example, *Rhodococcus* spp.)[52].

Fungal sequences have been reported in studies that targeted overall eukaryotes[6, 17, 48] on plastic debris, but only one study[53] specifically addressed fungi growing on plastic debris. The potential trophic role of saprotrophs such as fungi in the plastisphere includes decomposition, parasitism, predation, symbiosis and pathogenesis. Fungal diversity in the plastisphere remains relatively underexplored, but recent field experiments reveal that members of the Chytridiomycota, Cryptomycota and Ascomycota dominate the fungal assemblages on polyethylene and polystyrene substrates in brackish and freshwaters[17] where fungal reads contributed up to 4% of the total eukaryotic reads. A study of ocean-collected polyethylene microplastic[6], which is accessible through the public plastisphere portal called Visual Analysis of Microbial Population Structures (VAMPS), [vamps2.mbl.edu] also revealed the presence of fungal groups, in very low abundance but present above singleton copies, that included, among others, members of the genus *Malassezia*, which was recently shown to be abundant in the marine environment[54].

Predators. A combination of SEM and molecular data uncovered a striking example of symbiosis in the plastisphere[6, 17] between the predatory ciliate *Ephelota* and its sulfite-oxidizing ectosymbiotic bacteria (FIG. 3). Common epibionts on copepods, these suctorian ciliates require surface attachment, and plastic debris aptly provides such a surface. In addition, more correlative evidence is available from co-occurrence networks[17] that pointed to positive associations of *Amoebophrya* with Suessiaceae on polyethylene. This result is interesting as *Amoebophrya* are typically parasitic on other dinoflagellates so one might expect this association to be negative; however host and parasites must co-occur at some point in time and the association probably depends on the timing of sampling.

Despite differences in dominant taxa inhabiting the plastisphere in fresh versus marine environments (see below), the attached predatory ciliate *Ephelota* seems to be common on microplastics from marine and freshwater and/or brackish samples, which provides an important insight into the ability of this ciliate to potentially survive long distances from the coast. Furthermore, at least one study in Japan describes the prevalence of this genus on plastic associated with mariculture of seaweed[55]. Other species of *Ephelota* cause infestations in Antarctic krill[56], which makes them ectoparasites of animals in addition to microbial predators that eat ciliates and other small eukaryotes. Apart from ciliates that make up the predatory guild in the plastisphere, SEM and molecular data provide evidence that choanoflagellates, radiolaria and small flagellates such as *Micromonas* are present, which are all known to consume bacteria and other organisms. In some cases, radiolaria can represent a large fraction of the plastisphere community recovered from molecular surveys, although it is not clear whether they are living on the plastic or passively stick to it during sampling via manta trawls [6, 18] due to the gelatinous make-up of their cells.

Pathogens. In addition to reported threats from invasive species and toxic chemicals associated with plastic[57], the colonization of plastic by microbial groups that include pathogens has recently been recognized as another risk factor since the initial report showing that members of the genus *Vibrio* and other potentially pathogenic microorganisms are attached to plastic debris[6]. Since then the presence of various potential pathogens (for example, members of the Campylobacteraceae, *Aeromonas salmonicida, Arcobacter* spp.) has been reported from environmental plastic samples around the world in both temperate[27, 28, 58-60] and tropical[61] marine environments, as well as in freshwater[9, 30]. In particular, members of the genus *Vibrio* are endemic to marine environments, and although many are harmless, some can cause disease in wildlife and humans. Microbial diseases in fish, crustaceans and mollusks[62] are a major source of loss in aquaculture settings. *Vibrio* spp. are the most common pathogen of fish and shellfish in aquaculture systems, and these facilities may function as reservoirs of pathogenic *Vibrio* species and contribute to the emergence and spread of antibiotic resistance[63]. Aquaculture facilities also use plastic for floats, pens, nets lines, among others, which might increase the chance that potentially harmful bacteria colonize plastic surfaces. Phototrophic species responsible for harmful algal blooms have also been reported on plastic debris[7, 37, 64]. It is important to note that most evidence stems from molecular sequence data and does not prove pathogenicity or toxicity, and most studies have reported relatively low abundances of potential pathogens. Plastic, a long-lived floating substrate that can travel long distances and is frequently consumed by marine fauna, represents an ideal fomite. Exposure is increasing as the amount of plastic in the environment increases, and rising seawater temperatures in the North Sea over the past 45 years have been correlated with higher numbers of *Vibrio* species and infections from bathing in the ocean[65]. *Vibrio* bacteria can dominate bacterial plastisphere communities, particularly during the summer when they are known to bloom in response to higher water temperatures (FIG. 5). In addition to the risk from ingestion[57], plastic has been shown to transport potential protistan coral pathogens[66], to increase disease in corals[67] and to carry a known fish pathogen[68]. Passage of plastic through wastewater treatment plants and into waterways could be another potential source of human pathogens attached to plastic. A recent study[30] found a higher abundance of the family Campylobacteraceae, which is known to cause human gastrointestinal infections, attached to microplastics downstream from a sewage treatment plant. Despite all the attention, the role of plastic debris as a fomite for pathogenic microorganisms is unknown, and thus this topic requires additional research, particularly as plastic continues to accumulate in marine environments.

Community assembly

Experiments documenting the colonization of plastic in marine and freshwater systems have used many types of conventional and biobased plastic polymers, in different systems, at different times of year and under different conditions (see Supplementary Table S3). Some studies use post-consumer plastic such as PET bottles or plastic bags[41, 60], whereas others use 'raw' plastic from known manufacturing sources[53, 69]. Incubation conditions have included suspension *in situ* within the natural water column, laboratory aquaria of various sizes with flow-through seawater systems exposed to light[70] or in the dark[8, 34], static laboratory systems in containers of various

sizes with water collected once from the aquatic system of interest[61, 71, 72], and sediments[69]. The variation in experimental designs make it difficult to compare studies directly, but some specific examples provide evidence of common core members of the plastisphere, such as Rhodobacteraceae[15], but also geographic, temporal-, substrate- and environmental-dependent differences. Geographic differences have been reported at various scales from global surveys of bacterial communities on plastic collected in nets in the Atlantic and Pacific oceans[26], to regional differences on PET submerged at locations in the North Sea off the coast of England less than 200 km apart[60]. The North Sea study harvested samples after 5-6 weeks exposure during winter, spring and summer[60] and showed difference in microbial communities between the three seasons, but no significant difference between the communities on PET and their glass controls. This is in contrast to another study that described differences between communities on plastic and glass[34]. Other studies have reported different microbial communities on different types of plastic in both field-collected[6, 48] and experimental systems[8, 72]. Environmental conditions, including salinity gradients, clearly influence microbial communities, including the plastisphere[73].

Microbial settlement and biofilm formation is a complex process that has been intensely studied in systems ranging from biofouling of ship hulls to colonization of medical implants, and we refer the reader to some previously published informative and thorough reviews ranging from active (for example, chemical settling deterrent) types of biofilm inhibition[74-76] to passive biofilm inhibition (for example, physical modification of surfaces to inhibit settlement)[75]. Settlement on plastic polymers presents advantages such as increased access to limited nutrients[77] but also challenges, such as increased susceptibility to grazing pressure. The majority of published plastisphere studies have examined community composition via colonization experiments. Figure 4 illustrates community changes (based on operational taxonomic groupings assigned in Global Assignment of Sequence Taxonomy (GAST)) using a combined approach of microscopy and small subunit (SSU) rRNA marker gene profiling from a published study[26]. Microorganisms can colonize plastic substrates within hours after immersion in seawater[69, 78], where microbial abundances are greater than one million per milliliter. SEM images show various individual and filamentous pennate diatoms dominating the polyethylene biofilm after 1 week (FIG. 4). By week 2 many of the diatoms have been killed or grazed from the surface as other organisms such as filamentous cyanobacteria and associated heterotrophic bacteria attach and the community becomes more diverse. After 4 weeks the community continues to diversify and more biogenic debris is accumulating, including filaments, broken diatom frustules and 'slime', which is the EPS matrix that helps contain exuded enzymes and nutrients and forms the foundation of the biofilm structure. After 8 weeks, a more three-dimensional structure develops as mounds and clumps of cells build up, and after 16 weeks crowded, complex 3D structure of cells and debris is visible. The average coverage increased rapidly for the first couple weeks, then stabilized at around 15%-25% of the plastic surface for live cells. Molecular data from the same samples provided additional information about proportions of different organisms and community change over time. Diatoms (using chloroplast genetic data as a phylogenetic proxy) dominate early then decrease in relative abundance, although they remain a consistent member of the plastisphere. Rhodobacteriacea are abundant at all time points and they are important for biofilm formation as they are able to colonize new surfaces and the produced EPS promotes the settlement of other microorganisms. The abundance of distinct microbial groups that increased on plastic over time include Rhodospirillaceae, which includes purple sulfur bacteria, many that can fix N_2 and thus

can provide 'fertilizer' to the community members and increase carrying capacity. Other groups include Flavobacteriaceae, which are often associated with diatoms and members of this family have been described as keystone taxa in biofilms that feed off exudates (some have the ability to prey on diatoms via lysis)[79], and Xanthomonadales, which include species associated with dinoflagellates and diatoms, as well as a number of hydrocarbon degraders. The number of bacterial taxonomic groups observed on plastic substrates in the colonization experiment[26] increased quickly to over 800 in the first week, and then slowly almost doubled by week 16.

More data are required to provide a more complete picture, but in general, location (biogeography and anthropogenic influences) and time of year (season) seem to influence the microbial community that develops on plastic surfaces in aquatic environments. Within a given system, the polymer type and surface characteristics of the plastic influence what microorganisms attach, but communities on different substrates converge over time as the biofilms mature[10]. Besides those initial findings, important questions remain. What organisms colonize the plastic surface in the first hours and days after plastic is immersed in seawater, and how does this influence the community composition over time? Is the progression of species presence and dominance on a polymer within a specific environment predictable, and can this help us determine how long a piece of plastic has been immersed? Is there a stable 'climax' community in a given environment, or is the plastisphere community constantly changing? Do some members of the plastisphere community persist via resistant resting stages or sheltered in refuges within the biofilm or fouling community as plastic is transported through different environments?

Function and metabolism

Currently, the functional diversity and metabolic capacity of microorganisms found in the plastisphere are not well understood. The first metagenomic study to explore the metabolic potential of microorganisms on plastic debris[18] hypothesized that the microorganisms found on plastic would be more metabolically active and have distinct sets of genes compared to those in the surrounding water column. Between 40%-99% of the rRNA gene reads from the metagenomes recovered from plastic debris mapped to eukaryotic rRNAs; however it is not clear whether this represents 'true' abundances because microbial eukaryotes can have greatly disparate copy numbers of their rRNA genes[80]. Although our awareness of the role of microbial eukaryotic occupancy of the plastisphere is not new, this finding reiterates the importance of their inclusion in future culture-independent studies such as metagenomics and metatranscriptomics to increase our understanding of the ecology of this microbial habitat.

Chemotaxis and adhesion. Polyethylene and polypropylene have been shown to 'off-gas' and release dissolved organic matter (DOM) primarily in the form of the hydrocarbons, methane, and to a lesser extent ethane and ethylene, predominantly in warmer waters in the photic zone[81]. Microorganisms can rapidly take up DOM according to bacterial abundance measurements and ^{3}H-leucine uptake assays as a proxy for bacterial biomass production[82], and DOM may influence the composition of microorganisms settling on these polymers, possible owing to microbial chemotaxis. Chemotaxis settlement cues in plastic are underexplored, but microorganisms are known to migrate towards hydrocarbons[83]. Moreover, chemotaxis-like gene sets are well

represented in metagenomic datasets derived from floating plastic in the Pacific[18]. Several studies suggest the microbial community varies between different plastics in pelagic and benthic environments[6, 19, 48], which could be due to different cues emitted by different polymers. Some polymers such as polyamide contain nitrogen and provide a near Redfield ratio of reduced nitrogen to carbon. However, it is unclear to what extent cue-specific recruitment of microorganisms influences microbial community assembly of the plastisphere. As discussed, diatoms are consistent members of the plastisphere, and diatom biofilm formation is an active area of research in bio-inspired adhesive materials, on which they are known to secrete EPS to form an adherence pad that, in the case of the diatom *Seminavis* sp*.,* varies in adhesive strength with surface type. For example, a hydrophilic surface enables the diatom to swarm over the surface and attach and release, but a hydrophobic surface like virgin plastic causes the diatom to produce a stronger adhesive pad to which the diatom sticks firmly[84].

In general, biofilm settlement and formation of complex microbial communities on plastic debris are poorly understood, but many factors have been implicated in those processes, such as timing of initial colonizers, available nutrients and the presence of predators. Biofilms have also been shown to have a greater potential for gene transfer via conjugation, and type IV secretion systems[18, 85]. Fimbriae and pili, bacterial appendages involved in attachment, have a well-known role in biofilm formation. In the short-term, bacterial cells tend to maximize the contact area to surface ratio, and physical deformations in the micro-to-nanoscale that disrupt this ratio hinder biofilm formation[86], but may be less important long-term. It could be expected that specific microorganisms colonizing a virgin surface could have a distinct advantage over other microbial lineages for a time, and this dominance could influence the carrying capacity of a plastic surface until succession events equilibrate on the surface over time. These time periods have not been quantified as yet but are likely to be on the scale of weeks to months.

Metals. Microbial interactions with metals on plastic is an active research area. Metals found in plastic can be derived from the polymerization process or from additives or be adsorbed from the surrounding environment[87]. With the exception of oxo-degradable plastics (now banned in the EU) that contain metals (for example, Co, Mn, Fe or Ti) that are designed to induce radical formation and polymer chain scission upon exposure to UV light or high temperature, most additives confer some desirable properties to the plastic such as color, flexibility, antimicrobial properties or UV light resistance. A recent study examining plastic debris in the North Atlantic Subtropical Gyre (NASG)[87] found higher concentrations of certain heavy metals (As, Ti, Ni, and Cd) in environmentally weathered plastic debris than in new plastic items, and the authors attributed the finding to increased levels of oxidation in the environmental plastic debris. The same study indicated that ocean-derived samples contained higher concentrations of metals than aged collected pellets from the beach. Given that many microorganisms could derive energy directly from metal metabolism or use metals as micronutrients (for example, Fe, Co and other trace metals), they may be contributing to metal transformations of plastic debris.

Plastic degradation. Microorganisms have been implicated as a possible solution to the problem of plastic pollution in aquatic environments (BOX 1). A caveat to interpreting published research on degradation of conventional polymers is that most studies to date use pretreated polyethylene (either containing additives, having been exposed to UV light or other forms of

thermal treatment)[88-92] prior to measuring degradation, so it is difficult to know whether the microbial activity described is in response to high-molecular weight polymers or smaller breakdown products generated by abiotic factors. As already mentioned, leaching of small molecules (low-molecular weight DOM) from plastics that have been incubated in seawater may stimulate microbial settlement and contribute to microbial metabolism. Microorganisms must take up polymers to oxidize them intracellularly, so the molecular weight of the polymer must be low enough (<500 Mw)[49] to pass through cell membranes (FIG. 6). To this end, plastics that are hydrolysable (that is, with backbones consisting of components other than just C-C or C-H; for example, PET, polyurethane (PUR) and polycarbonate) are more likely to be substrates for microbial degradation in the environment than non-hydrolysable polymers most commonly encountered in the pelagic marine environment (polyethylene, polypropylene and expanded polystyrene)[23]. A class of enzymes that may be effective at degrading recalcitrant polymers such as polyethylene are those that degrade alkanes such as hexadecane[49]. A mesophilic marine beach soil-derived *Pseudomonas* strain incubated with low-molecular-weight polyethylene (LMWPE) as a sole carbon source is often cited as the best example of the potential of 'biodegradation of polyethylene [49]. Although this and other papers hint at the possibilities for microbial solutions to plastic pollution, LMWPE does not commonly occur in the marine environment and conditions in the ocean result in very slow rates of degradation.

Plastics that are hydrolysable (for example, polyamides, PET or PUR) may also be susceptible to preexisting degradation pathways present in microorganisms (such as extracellular hydrolases that are involved in the degradation of cellulose and proteins) but the environmental conditions often limit complete biodegradation. The discovery of PETase, an enzyme that hydrolyzes plastic polymers such as PET, in the bacterium *Ideonella sakaiensis*[93] and the subsequent recovery of related enzymes from marine and terrestrial metagenomes in public databases[94], indicates that PET-degrading capacity may be ubiquitous in those environments. However, incomplete microbial hydrolysis of plastic polymers or extreme oxidation through microbial biotransformation can lead to the generation of nanoplastics. Although understudied, nanoplastics may have the ability to be ingested by humans via the food chain. Once ingested by humans, microplastics of less than 150 µm have been shown to translocate across the gut epithelium into the lymphatic system, causing systemic exposure and eventually affecting human health[95]. However, it is important to note that the effect of nanoplastics on human health is underexplored.

Terrestrial fungal representatives that degrade plastic polymers include the Ascomycete *Engyodontium album*, the Basiomycote fungi *Phanerochaete chrysosporium* and *Trametes versicolor*, which is better known by its common name white-rot fungus, and the soft-rot fungus *Humicola insolens*[23]. However, most microbial degradation studies are conducted in vitro, and field degradation rates are exceedingly low in both soil (0.1-0.4% in 800 days) and seawater (1.6-1.9% weight loss in 1 year)[96, 97]. Many factors account for the observed differences in lab versus field studies including absence of UV-light pre-treatment to accelerate degradation, sub-optimal temperatures for degradation, microbial preference for more palatable carbon options, and polymer form and extent of crystallinity. A recent workshop report considered whether fungi have a role in plastic degradation in the marine environment given their ability to degrade other recalcitrant polymers (like lignins)[98], but few studies have been performed in the ocean, especially below the ocean surface where higher concentrations of fungi might be expected.

Homobasidiomycetes or 'higher fungi' possess esterases and other polymer-degrading enzymes and have been found to degrade various types of plastics in a laboratory setting, including nylon and polyethylene [99, 100]. Although homobasidiomycetes are rare in the marine environment they do exist, particularly in mangrove habitats; however, these particular fungi remain underexplored regarding their metabolic potential.

Outlook

As human activities continue to modify our planet, it is important to assess the impact of our actions. Plastic materials have extended our lives with many life-saving technologies, provided numerous benefits to society and simplified our lives with convenience. Perhaps it is time to reflect on the value we place on such modern convenience. Planet Earth, with myriad habitats and more than 10^{30} microbial inhabitants,[21] has found a way to biotransform plastic materials and even degrade them to some extent. However, the ocean is not a continuous bioreactor of uniform temperature, but a dynamic system of varying densities, nutrient limitations and biota that are continually interacting with plastic fragments at all scales. Future studies aimed at understanding the roles of the plastisphere in chemical biotransformation and physical modification of plastic debris that alter the size, density and oxidation state of polymers is an important area to explore. No doubt, multi-omics approaches will have an important role in deciphering microbial-mediated biochemical transformations and answer many outstanding questions remain. Furthermore, future studies are required to answer the many open questions in plastisphere research. For example, do the compounds that plastic emit in aquatic environments provide chemo-attractant plumes? If so, how does this plume influence the initial plastisphere composition? Microbial biotransformations of plastic debris may have a substantial role in the generation of micron and sub-micron scale polymer particles. These nanoplastics could have implications for human health and food security as they could be incorporated into tissues. Although this phenomenon is known to occur, it is still unclear to what extent nanoplastics pose a threat, if at all. It is clear that taking hundreds of millions of metric tonnes of hydrocarbons out of the Earth's interior and producing refractory materials which are allowed to escape into aquifers and marine systems has set an 'experiment' in play for which we are only beginning to interpret the results.

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L.A.-Z, E.Z. and T.J.M. researched data for the article, substantially contributed to the discussion of the content, wrote the article and reviewed and edited the manuscript before submissions.

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Supplementary information

Supplementary information is available for this paper at https://doi.org/10.1038/s41579-019-0308-0.

Related links:

Visual analysis of microbial population structures (VAMPS): vamps2.mbl.edu

Fig. 1 | **A timeline of plastic innovation, discovery and pollution**. Semi-synthetic materials have been around since the 1850s when cellulose nitrate was invented to replace limited natural materials such as tortoiseshell, horn, baleen and ivory. In 1907, the first plastic material Bakelite, and five years later, polyvinyl chloride (PVC) and polyvinyl acetate were discovered. In the 1930's polyamide polymer (known commercially as Nylon) was introduced, the first synthetic fiber which was immensely popular and of great utility during World War II, as well as polymethyl methacrylate (Plexiglas®) (not shown) used in the windows of aircraft. Immense innovation and development continued in the 1930s and 1940s during which time most of the common polymers were discovered. After World War II many of these polymers found their way to the general public in the form of low-cost, disposable, single-use items, inspiring the term 'throwaway living' which remains part of the public mindset today, although more individuals recognize the need to limit single-use plastics in the future. Synthetic polymers have a life beyond Earth since a plastic (polyamide) flag was first planted on the moon in 1969. By the 1970's plastics became the most widely used materials in the world[103]. Polymer materials have played key roles in economic expansion, and the production of low- priced goods in the emerging world market, particularly in the 1990s, and continue to be a growth industry[104–115]. Adapted with permission from REF.[2]. MARPOL , International Convention for the Prevention of Pollution from Ships; PET, polyethylene terephthalate.

Fig. 2 | **The lifecycle of plastic litter.** Diagram illustrating many of the possible pathways during the lifecycle of plastic litter on its journey from land to sea. Plastic debris enters the ocean through both aquatic (rivers, accidental escape at sea) and land-based sources (littering, escape from municipal waste management, such as waste water treatment plants (WWTPs). Depending on the density of the plastic material, plastic items will remain afloat for a given part of their life cycle, or as they become weighted down by biofouling will begin to sink into the water column ultimately to the ocean bottom. Mechanical, photochemical and biological forces break down plastic debris into micro- and nanoplastics that subsequently become incorporated into the marine food web. Organisms such as filter feeders may further concentrate these smaller particles given their capacity to filter large volumes of water. Microorganisms begin to attach, colonizing plastic in the water within hours and can include potentially harmful microorganisms such as disease-causing pathogens. The 99% 'missing plastic' refers to the fact that estimates of surface plastic account for only 1% of what has been released into the ocean[4]. PET, polyethylene terephthalate; PVC, polyvinyl chloride.

Fig. 3 |**The plastisphere community**. Conceptual model of the diverse, three-dimensional plastisphere community showing a microbial ecosystem of bacteria, protists and animals in the oligotrophic open ocean. Members include cyanobacteria and diatom primary producers,

predatory ciliates and hydroids, grazers including ciliates and bryozoans, symbiotic relationships and heterotrophs.

Fig. 4 | **Assessing community assembly**.

Shown is an example of microbial biofilms developing on strips of high-density polyethylene (HDPE) from a 1 gallon bottle of water during an *in situ* colonization experiment performed off a dock in Woods Hole, Massachusetts, USA (GPS coordinates: 41.525, -70.673) from July 2013 until November 2013[26]. Scanning electron micrographs from 0-16 weeks show the increasing diversity and structural complexity of the community that develops over time. Bar charts at the bottom represent taxonomic groupings at the family level based on amplicon sequencing of the V6 hypervariable region of the 16S rRNA gene for corresponding samples (shown are groupings represented at greater than 4% of the overall community, rendered through the visualization and analysis of microbial population structures (VAMPS) plastisphere portal [vamps2.mbl.edu][15]). Diatoms (based on chloroplast sequences) and Rhodobacteriaceae are the most abundant groups. Groups that increased over time included Rhodospirillaceae, Flavobacteriaceae, and Xanthomonadales. Shown are taxonomic groups down to the family level. Data based on REF[26].

Fig. 5 | ***Vibrio* blooms in the plastisphere**. a, b) A comparison is shown of bacterial communities on a polyethylene sample from sediment (the North Sea coastal sediment) and a polypropylene sample from the water column (North Atlantic open ocean water), which are both dominated by *Vibrio* species. Pie charts (part a) reveal the relative abundances for each taxonomic grouping (genus level or higher), and the bar graph (part b) shows the comparative contribution of each taxon in the corresponding sample; for example, there were 0 *Phormidium* in the North Sea sample and 413 in the North Atlantic sample, so within *Phormidium*, the North Atlantic contributed 100% of the total. The taxonomic groupings shown represent greater than 1% of the overall community and are normalized by percentage, rendered through the Visualization and Analysis of Microbial Population Structures (VAMPS) plastisphere portal[15]. Despite the dominance of *Vibrio* species in both samples, the *Vibrio* communities are otherwise very different. Based on data from REFS[6,27].

Fig. 6 | **Degradation of plastic materials**
(a) Degradation of conventional plastics is a combination of physical, chemical and biological interactions. Our knowledge of the biological process is largely informed from cultured strains and consortia that are reared in the laboratory, and many of those strains are found in terrestrial environments. Thus, the schematic shown is a hypothetical model of the processes leading to plastic degradation in aquatic settings like the open ocean. Floating plastic debris is subjected to different types of degradation induced by sunlight. The visible spectrum leads to heating and thermal degradation, whereas UV light leads to photodegradation of the polymers into monomers through bond scission, and infrared radiation can result in thermal oxidation of polymer chains. (b) Biological pathways of polymer degradation include the mechanical action of organisms that grow in cracks and crevices of the polymer surface (not shown), but also enzymatic processes that can hydrolyze the polymer into oligomers and ultimately monomers.

Polyethylene, polypropylene and expanded polystyrene contain very stable backbones and are difficult to degrade, whereas polyethylene terephthalate (PET), polyurethane (PUR) and polycarbonate are more susceptible to hydrolysis and the enzymes that catalyze these reactions[23,50,93,116-118]. Enzymes that can hydrolyze polypropylene and polycarbonate have not yet been reported to the best of our knowledge (indicated by the question marks).

BOX 1

Degradation versus biodegradation

Much attention has recently turned to the members of the plastisphere as a possible solution to plastic pollution in the marine environment through microbially mediated biodegradation of environmental plastic debris. The scientific literature abounds with different definitions creating confusion both within the scientific community and among the public. We endorse a definition of biodegradation that is adopted by the international standards communities wherein biodegradation refers to a complete breakdown of plastic into CO_2, H_2O and biomass in aerobic settings and CO_2, CH_4 and biomass in anaerobic settings, in a reasonable timeframe (that is, >90% carbon converted to CO_2 within 180 days in an industrial composting facility; at least 30% carbon converted to CO_2 within 180 days at 30°C in marine environments). It cannot be overstated that partial breakdown is not the intended goal of products designed to be biodegradable and compostable, and therefore describing microorganisms that partially biodegrade materials as biodegradation is misleading. There is also some confusion over the use of standards to determine the 'inherent biodegradability' of a product. For the marine standards that are presently under revision and improvement, only test methods that include respirometry measurements are considered tests of biodegradability. The D7473-12 test of the ASTM International (formerly known as the American Society for Testing and Materials) is a weight loss method and thus does not measure biodegradability and is not intended to be a replacement for ASTM test method D6691: 'Test method for determining aerobic biodegradation of plastic materials in the marine environment by a defined microbial consortium or natural sea water inoculum'. ASTM D6691 measures CO_2 evolution, and D7473-12 is a weight loss test method to be applied after D6691, in Phase II testing to determine the behavior of a product or plastic material under conditions intended to be more similar to the natural environment. There is consensus in the scientific community that present standards need to be updated and are inherently imperfect[101], but they do provide the means to compare the fate of different forms of plastic materials. Criticisms of respirometric methods include the fact that plastics can attract organic molecules that can be subsequently consumed by members of the plastisphere (overestimating biodegradation) or that CO_2 originating from photosynthetic members of plastisphere communities (underestimating biodegradation)[22]. The latter issue is typically addressed by running experiments in the dark. In addition to these caveats, recent work demonstrating that plastic debris can release dissolved organic matter (for example, methane, ethane and ethylene[81]) that is available for use by members of the plastisphere raises the issue of what carbon substrates are being consumed and the mechanisms behind the production of these low molecular weight compounds (abiotic versus biotic forces).

Combined approaches that take advantage of isotope labeling methods to ascertain the extent of microbial assimilation of the plastic material might address some of these issues. For example a recent study[102] used ^{13}C-labeled poly(butylene adipate-co-terephthalate) (PBAT) and nanoscale secondary ion mass spectrometry (NanoSIMS) to show microbial assimilation of the labeled carbon from the polymer. These authors argue that both CO_2 evolution and incorporation of polymer-derived carbon into microbial biomass should be taken into account while determining biodegradability. Although this example examined plastic used for mulch applications in the terrestrial environment, similar experiments could be performed for aquatic settings. In fact, an earlier ASTM test method D6692-01 used radioactive ^{14}C materials in carbon dioxide respirometry measurements to confirm the origins of the CO_2 evolution in marine aquaria.

Glossary

Microplastic - Generally refers to plastic particles smaller than 5 mm in size.
Nanoplastic - Generally refers to plastic pieces smaller than 1 μm.
Phototrophs - Organisms that harness light energy and convert it into chemical energy.
Heterotrophs - Organisms that use organic compounds as a carbon and energy source for biosynthesis.
Symbionts - An organism that lives with another organism where both derive benefits from the arrangement.
Predators - Organisms that kill and ingest other organisms for nutrition.
Saprotrophs - Organisms that feed on the organic matter of decaying organisms.
Grazers - Organisms that ingest organisms or parts of other organisms for nutrition.
Epibiont - a symbiont living attached to the outside of another organism.
Parasites - Organisms that derive nutrients and energy from larger organisms while causing harm to their 'host'.
Mariculture – Refers to marine agriculture.
Manta Trawl - a net resembling the shape of a manta ray used for sampling plankton and plastic at the surface of the ocean.
Degradation - The physical, chemical or biological break down of a substrate (synthetic polymers, biomass) into smaller units.
Fragmentation - Physically breaking an item into smaller pieces.
Biodegradation - The biological break-down of a carbon-based product into water and carbon dioxide or methane.
Carrying capacity - The number of organisms that can be sustained in a given environment.
Species richness - The total number of different species in a community.
Beta diversity - Compares the variation in species composition between two different environments.
Evenness - A diversity index that refers to how equally abundant different members of a given community are represented.
Redfield ratio – The consistent stoichiometric ratio of carbon:nitrogen:phosphorous in marine phytoplankton, typically 106:16:1.

Figure 1

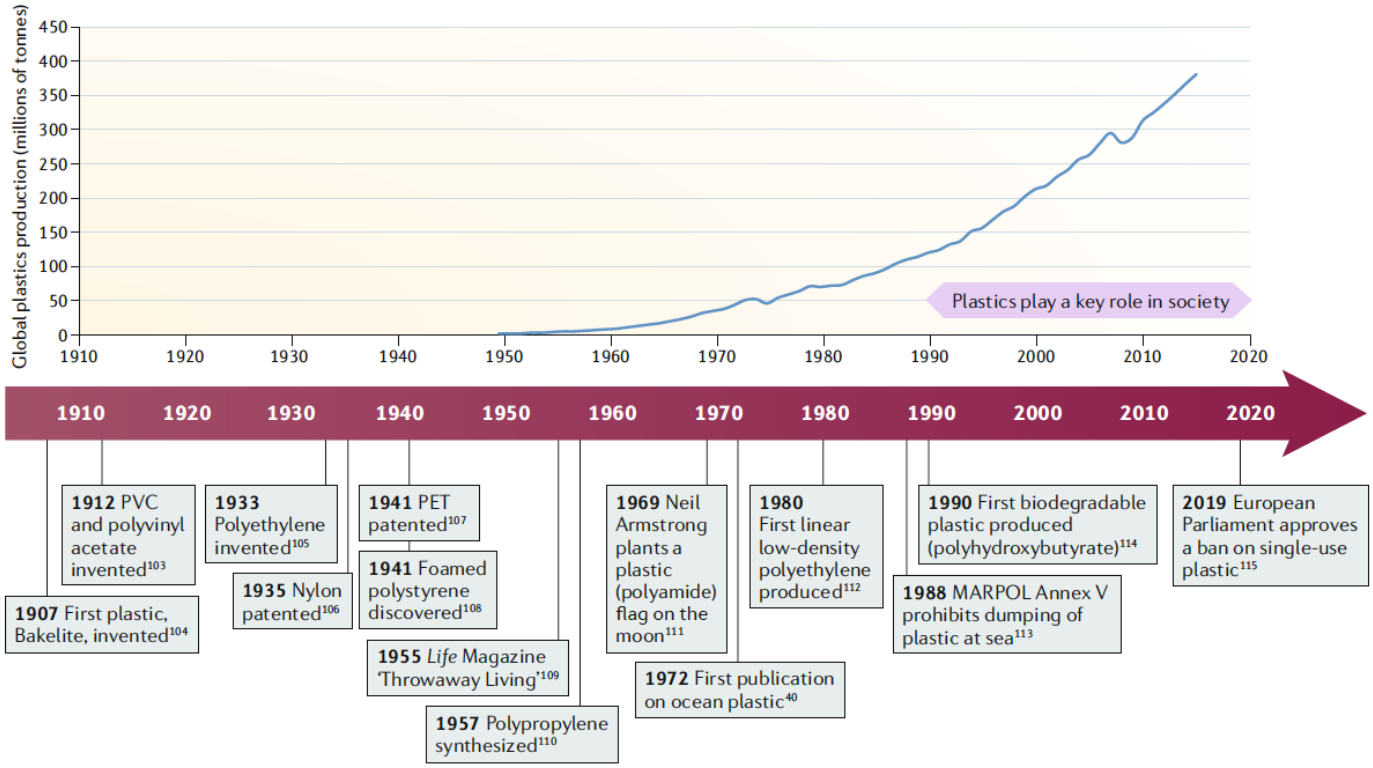

Global plastics production (millions of tonnes)
450
400
350
300
250
200
150
100
50
0
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
2010
2020
Plastics play a key role in society
1907 First plastic, Bakelite, invented[104]
1912 PVC and polyvinyl acetate invented[103]
1933 Polyethylene invented[105]
1935 Nylon patented[106]
1941 PET patented[107]
1941 Foamed polystyrene discovered[108]
1955 Life Magazine 'Throwaway Living'[109]
1957 Polypropylene synthesized[110]
1969 Neil Armstrong plants a plastic (polyamide) flag on the moon[111]
1972 First publication on ocean plastic[40]
1980 First linear low-density polyethylene produced[112]
1988 MARPOL Annex V prohibits dumping of plastic at sea[113]
1990 First biodegradable plastic produced (polyhydroxybutyrate)[114]
2019 European Parliament approves a ban on single-use plastic[115]

Figure 2

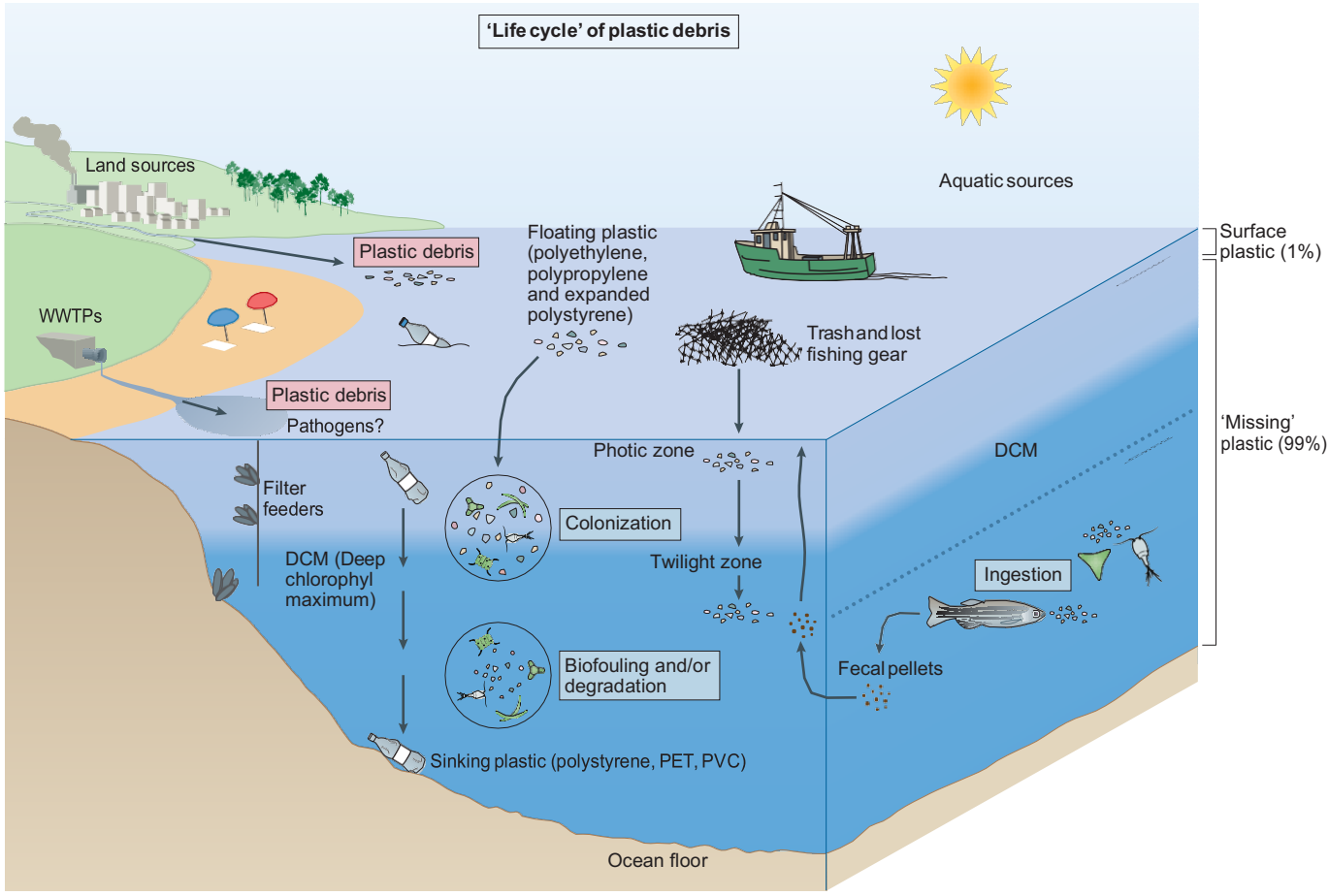

'Life cycle' of plastic debris
Land sources
Aquatic sources
Plastic debris
Floating plastic (polyethylene, polypropylene and expanded polystyrene)
WWTPs
Trash and lost fishing gear
Plastic debris
Pathogens?
Photic zone
DCM
Filter feeders
Colonization
DCM (Deep chlorophyl maximum)
Twilight zone
Ingestion
Biofouling and/or degradation
Fecal pellets
Sinking plastic (polystyrene, PET, PVC)
Ocean floor
Surface plastic (1%)
'Missing' plastic (99%)

Figure 3

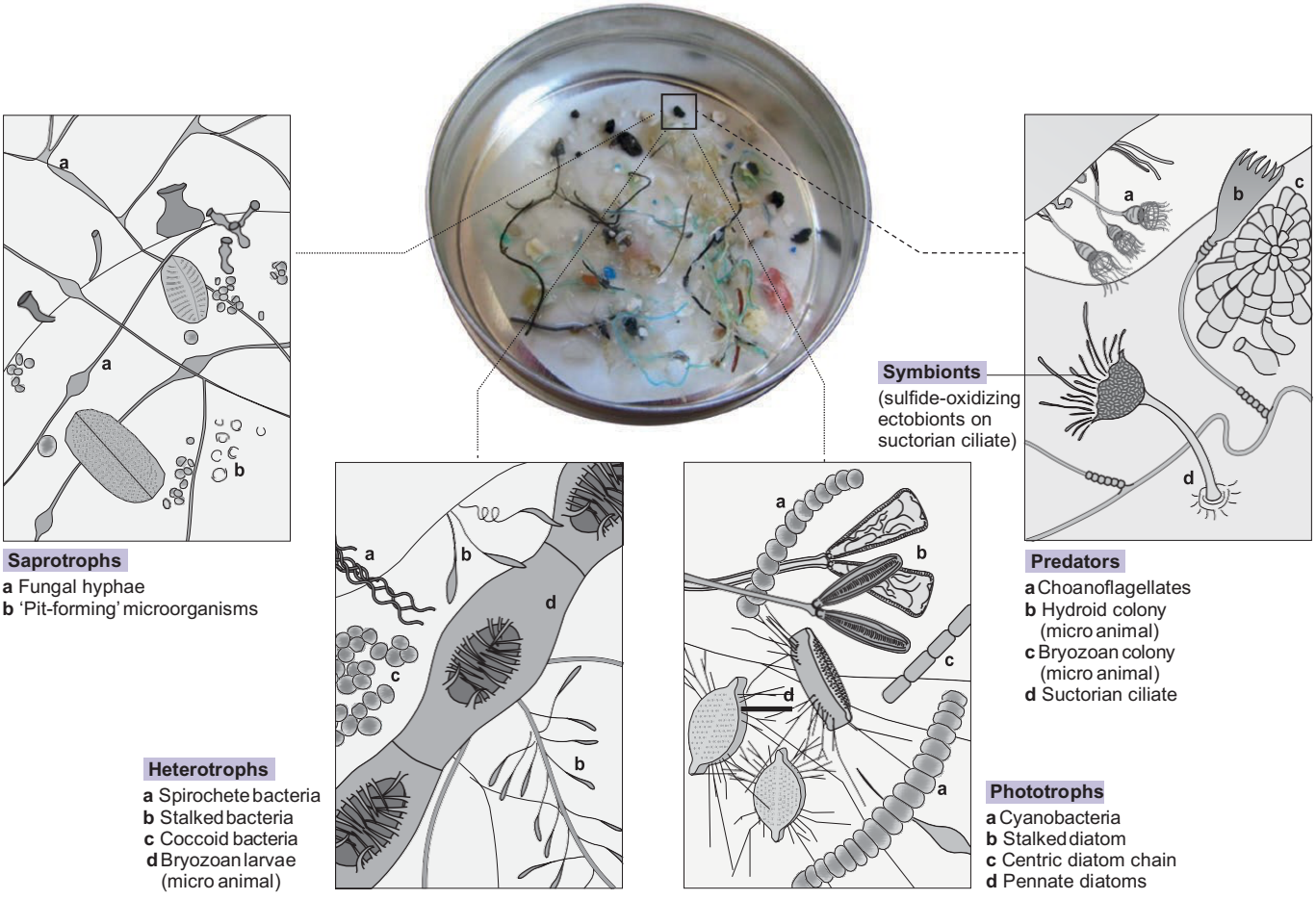

a
a
b
Saprotrophs
a Fungal hyphae
b 'Pit-forming' microorganisms
a
b
c
d
Heterotrophs
a Spirochete bacteria
b Stalked bacteria
c Coccoid bacteria
d Bryozoan larvae
(micro animal)
a
b
c
d
a
Phototrophs
a Cyanobacteria
b Stalked diatom
c Centric diatom chain
d Pennate diatoms
Symbionts
(sulfide-oxidizing
ectobionts on
suctorian ciliate)
a
b
c
d
Predators
a Choanoflagellates
b Hydroid colony
(micro animal)
c Bryozoan colony
(micro animal)
d Suctorian ciliate

Figure 4

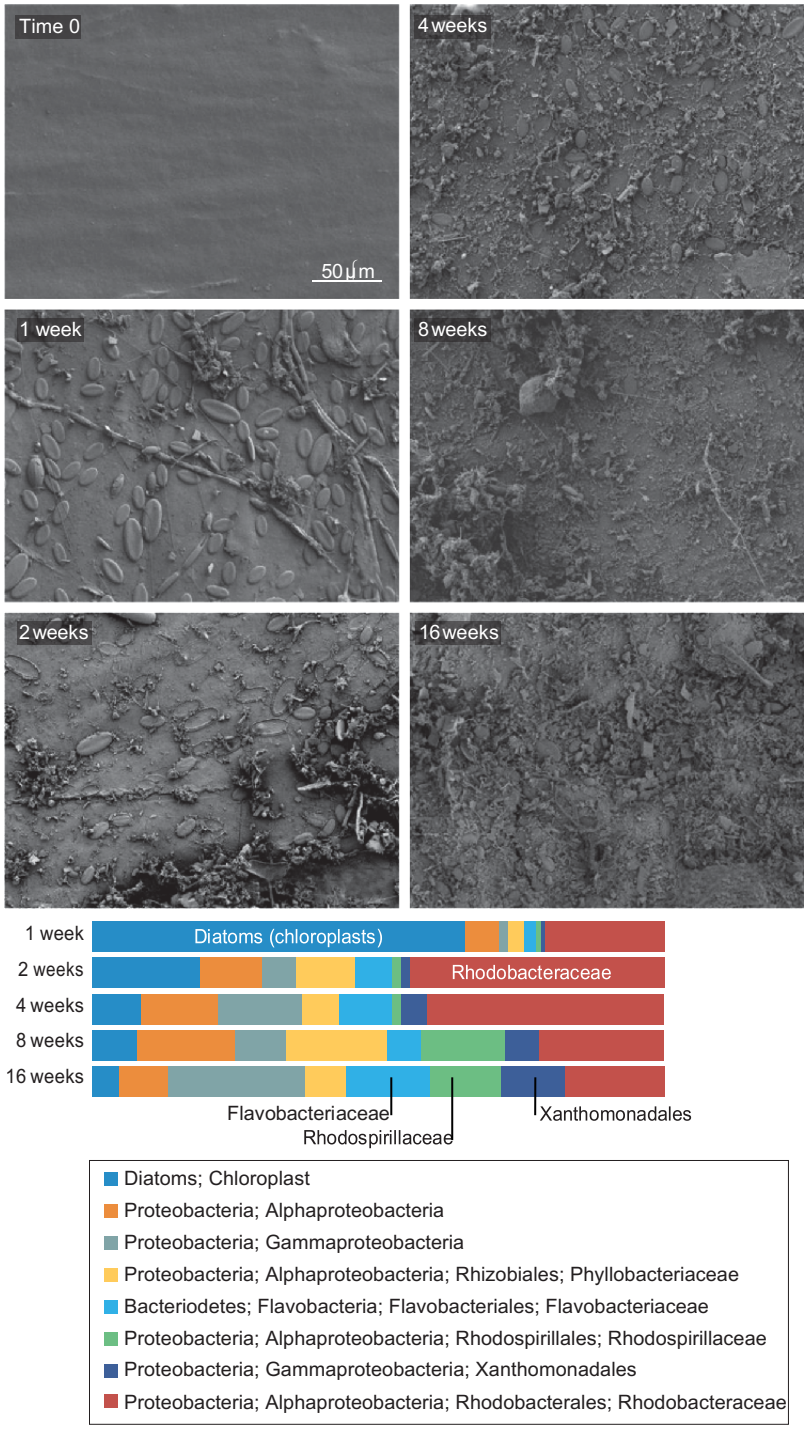

Time 0
50 µm
4 weeks
1 week
8 weeks
2 weeks
16 weeks
1 week
2 weeks
4 weeks
8 weeks
16 weeks
Diatoms (chloroplasts)
Rhodobacteraceae
Flavobacteriaceae
Rhodospirillaceae
Xanthomonadales
Diatoms; Chloroplast
Proteobacteria; Alphaproteobacteria
Proteobacteria; Gammaproteobacteria
Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae
Bacteriodetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae
Proteobacteria; Gammaproteobacteria; Xanthomonadales
Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae

Figure 5

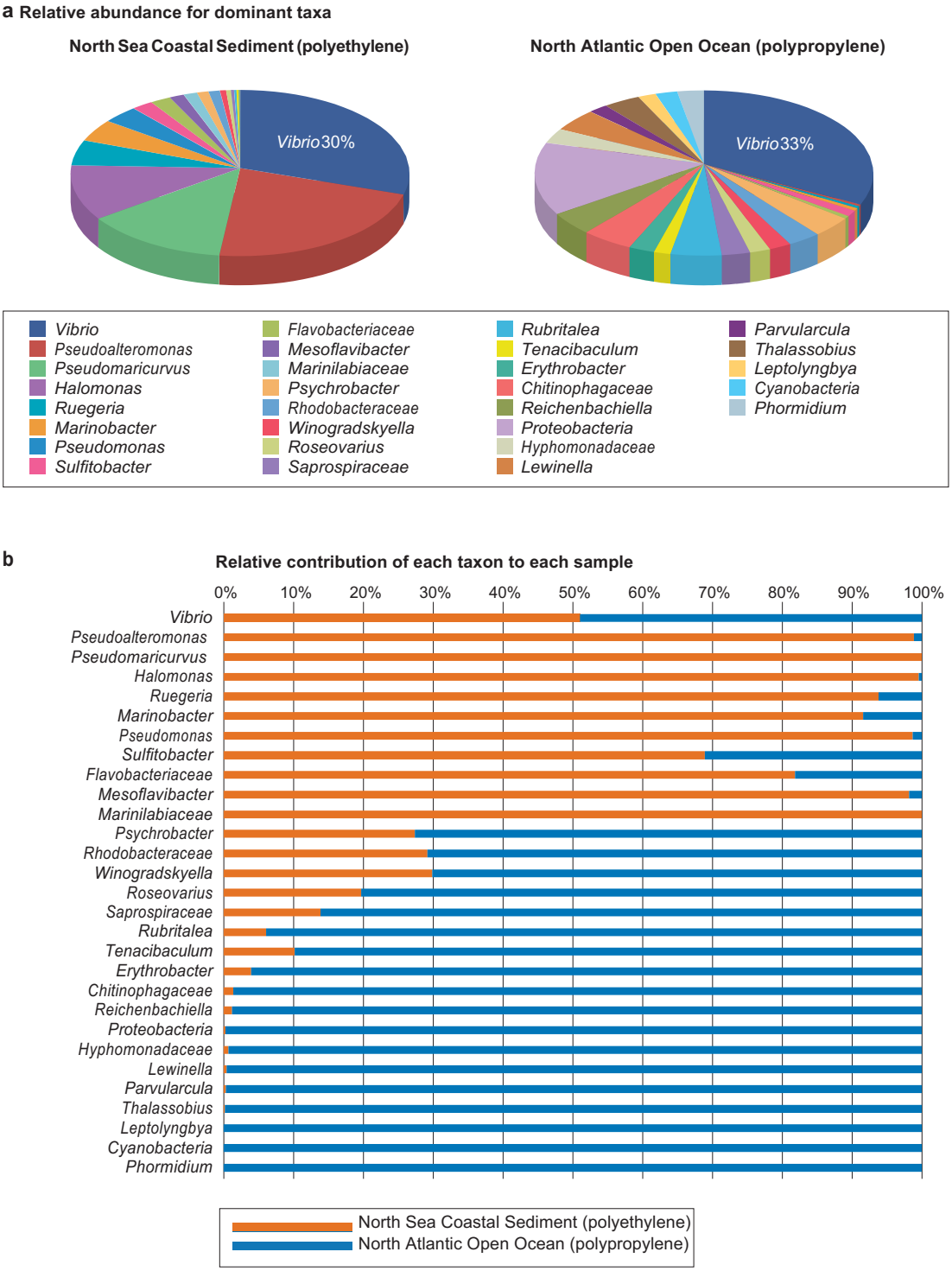

a Relative abundance for dominant taxa
North Sea Coastal Sediment (polyethylene)
North Atlantic Open Ocean (polypropylene)
Vibrio 30%
Vibrio 33%
Vibrio
Pseudoalteromonas
Pseudomaricurvus
Halomonas
Ruegeria
Marinobacter
Pseudomonas
Sulfitobacter
Flavobacteriaceae
Mesoflavibacter
Marinilabiaceae
Psychrobacter
Rhodobacteraceae
Winogradskyella
Roseovarius
Saprospiraceae
Rubritalea
Tenacibaculum
Erythrobacter
Chitinophagaceae
Reichenbachiella
Proteobacteria
Hyphomonadaceae
Lewinella
Parvularcula
Thalassobius
Leptolyngbya
Cyanobacteria
Phormidium
b
Relative contribution of each taxon to each sample
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Vibrio
Pseudoalteromonas
Pseudomaricurvus
Halomonas
Ruegeria
Marinobacter
Pseudomonas
Sulfitobacter
Flavobacteriaceae
Mesoflavibacter
Marinilabiaceae
Psychrobacter
Rhodobacteraceae
Winogradskyella
Roseovarius
Saprospiraceae
Rubritalea
Tenacibaculum
Erythrobacter
Chitinophagaceae
Reichenbachiella
Proteobacteria
Hyphomonadaceae
Lewinella
Parvularcula
Thalassobius
Leptolyngbya
Cyanobacteria
Phormidium
North Sea Coastal Sediment (polyethylene)
North Atlantic Open Ocean (polypropylene)

Figure 6

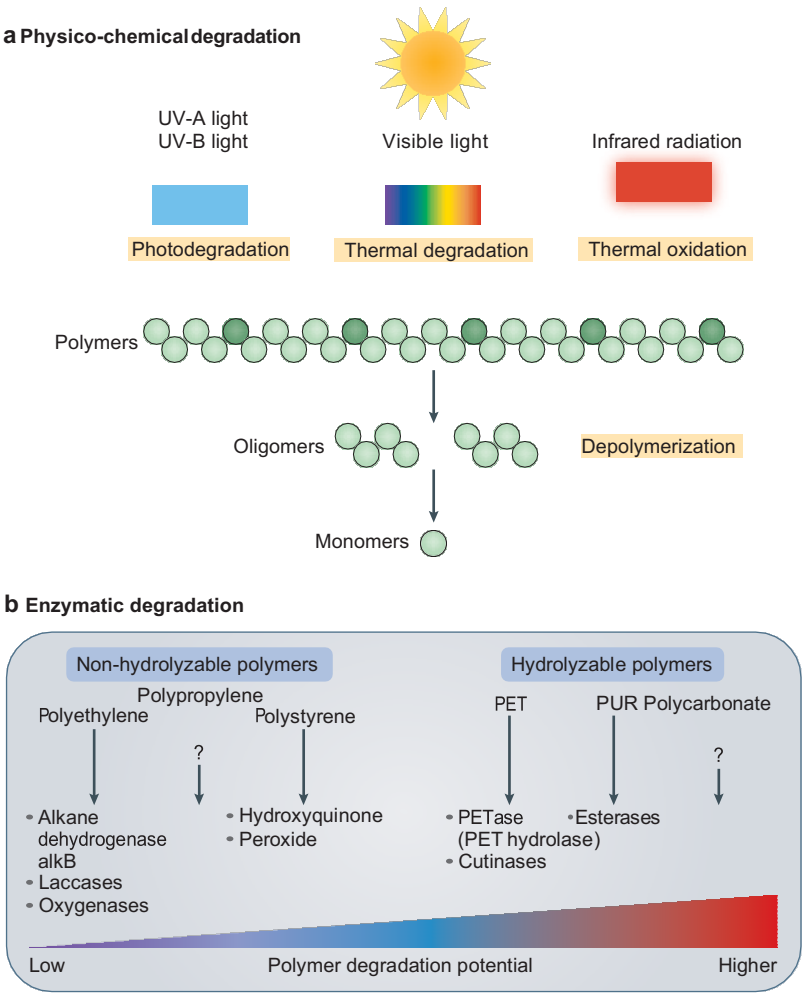

a Physico-chemical degradation
UV-A light
UV-B light
Visible light
Infrared radiation
Photodegradation
Thermal degradation
Thermal oxidation
Polymers
Oligomers
Depolymerization
Monomers
b Enzymatic degradation
Non-hydrolyzable polymers
Hydrolyzable polymers
Polypropylene
Polyethylene
Polystyrene
PET
PUR Polycarbonate
?
?
• Alkane dehydrogenase alkB
• Laccases
• Oxygenases
• Hydroxyquinone
• Peroxide
• PETase (PET hydrolase)
• Cutinases
• Esterases
Low
Polymer degradation potential
Higher

Table S1. Petroleum and bio-based plastics and their characteristics.

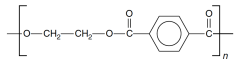
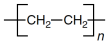
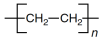
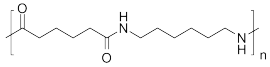
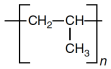
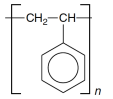
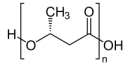
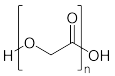
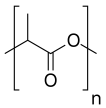
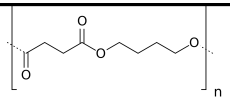
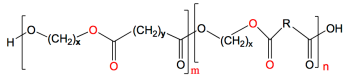
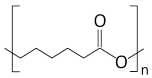
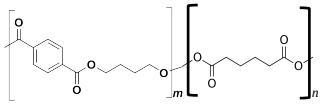
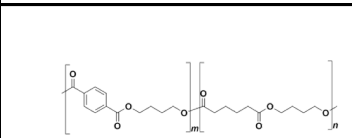
Plastic	Abbr.	Resin Code	Production	Origin(s)	Behavior in Marine Water Column	World Production[1] (millions of tons)	Biodegradability	Polymer building blocks
Polyethylene terephthalate	PET	1	synthetic	petroleum or bio-based	sinks	33	no	
High-Density Polyethylene	HDPE	2	synthetic	petroleum or bio-based	floats	52	no	
Polyvinyl chloride	PVC	3	synthetic	petroleum	sinks	38	no	
Low-Density Polyethylene	LDPE	4	synthetic	petroleum or bio-based	floats	64	no	
Nylon	Other	7	synthetic	Petroleum	sinks	-	some	
Polypropylene	PP	5	synthetic	petroleum	floats	68	no	
Polystyrene	PS	6	synthetic	petroleum	sinks	25	no	
Expanded Polystyrene	PS	6	synthetic	petroleum	floats	-	no	
Poly-3-hydroxybutyrate	PHB	7	biological	bio-based	sinks	-	yes	
Poly-hydroxyvalerate	PHV	7	biological	bio-based	sinks	-	yes	Same as PHB except -CH_3 replaced with CH_2-CH_3
Polyglycolic acid	PGA	7	synthetic	petroleum or bio-based	sinks	-	yes	

Table S1 (cont). Petroleum and bio-based plastics and their characteristics.

1Plastic	Abbr.	Resin Code	Production	Origin(s)	Behavior in Marine Water Column Environment	World Production[1] (millions of tons)	Biodegradability	Polymer building blocks
Polylactic acid	PLA	7	synthetic	petroleum or bio-based	sinks	-	yes	
Polybutylene succinate	PBS	7	synthetic	petroleum	sinks	-	yes	
Polybutylene succinate adipate*	PBSA	7	synthetic	petroleum	sinks	-	yes	
Polycaprolactone	PCL	7	synthetic	petroleum	sinks	-	yes	
Polybutylene succinate terephthalate	PBST	7	synthetic	petroleum	sinks	-	yes	
Polybutylene adipate terephthalate	PBAT	7	synthetic	petroleum	sinks	-	yes	
Polytetramethylene adipate terephthalate	PTMAT	7	synthetic	petroleum	sinks	-	yes	

All structures obtained from Wikipedia and are part of the creative commons license agreement https://creativecommons.org/

*x=4; y=2; and R= -$(CH_2)_4$

Figure S2. A global map showing locations of studies where Plastisphere research employing high-throughput DNA sequencing has been conducted. Studies are concentrated in Europe, with only a handful from Asia and America, and none from Africa. There is a complete lack of data from polar regions and the Southern Hemisphere, where three of the five major ocean accumulation zones are located. There is only one metagenomic survey, studies in freshwater systems are still rare, and there are very few studies that looked at microbes on plastic below the water surface. SW natural refers to studies that looked at communities on pieces of plastic collected from the pelagic marine environment; SW expt refers to pieces of plastic from experiments in marine systems; SWB natural are studies using plastic collected from the benthic marine environment; FW natural are samples collected from freshwater environments; FW expt are from experiments in freshwater systems; SWB expt are from experiments in marine benthic systems.

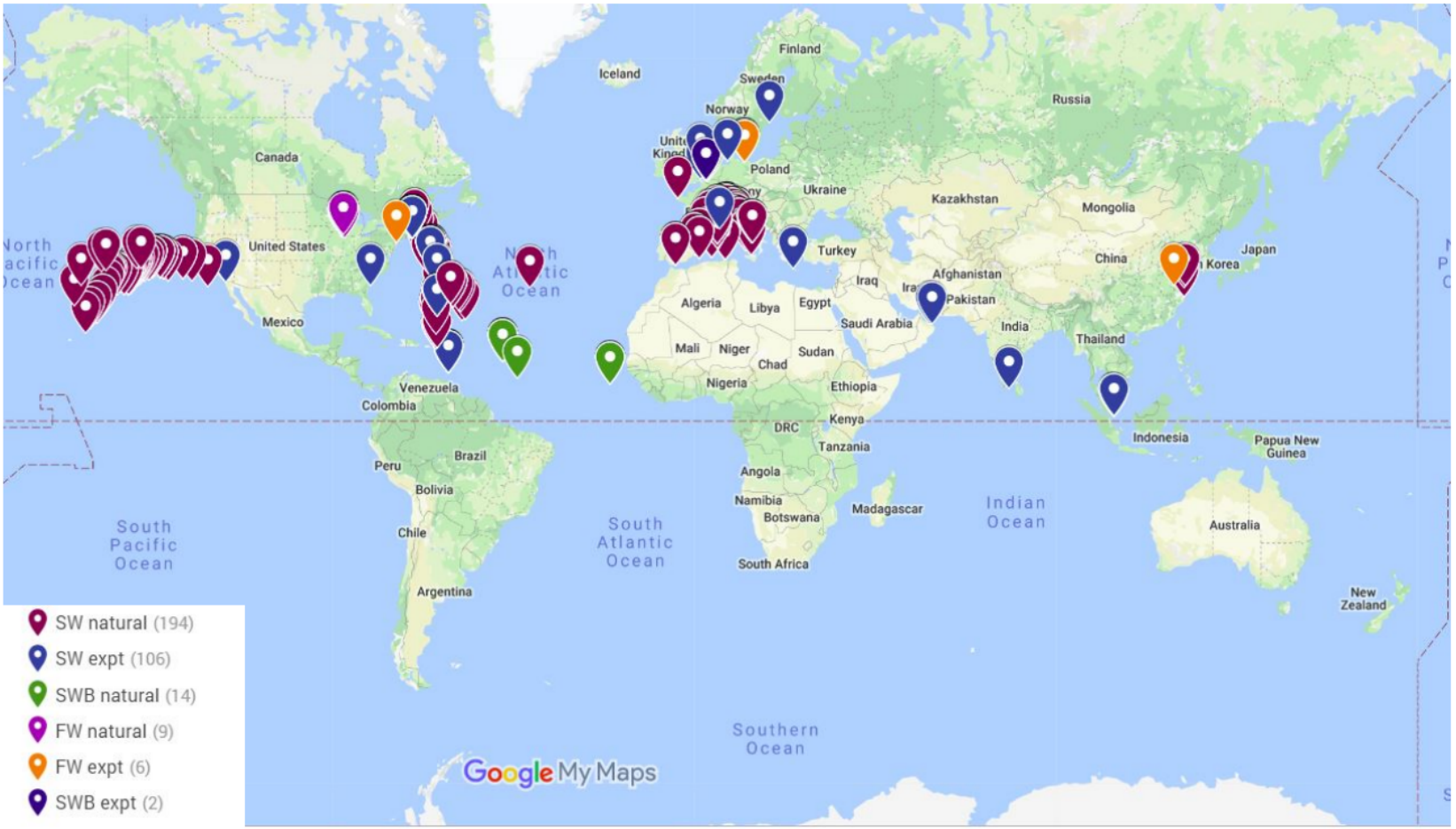


Table S3. Substrates and sampling conditions for “colonization experiments”

Study	Substrates	Sample type	Environment	Conditions	Duration
2013; Zettler, Mincer, Amaral-Zettler[2]	PE, PP	field-collected	open Atlantic	1m in-situ	unknown/variable
2014; Harrison *et al.*[3]	LDPE	defined	sediments, UK estuary	petri dish; 4C, dark	2min-14 days
2015; Amaral-Zettler *et al.*[4]	PE, PETE, PP, PS	field, post-consumer, pellets	open and coastal ocean	1m *in situ*	unknown/variable
2016; Oberbeckmann *et al.*[5]	PET, glass	water bottles, glass slides	North Sea in three different seasons	*in situ*	5-6 weeks
2017; Debroas *et al.*[6]	PE, PETE, PP, PS	field-collected	open Atlantic	1m *in situ*	unknown/variable
2017; De Tender *et al.*[7]	PE, PETE, PP, PS	field, post-consumer, pellets	open and coastal ocean	1m *in situ*	unknown/variable
2017: Kettner *et al.*[8]	PE, PS, wood pellets	defined	Baltic Sea, River, WWT plant	1-3m *in situ*	14 days
2018; Dussud *et al.*[9]	OXO-PE, PHBV	defined	Mediterranean	dark in 1.5Lflow-through aquaria	7-45 days
2018; Muthukrishnan *et al.*[10]	PE, PET, wood, steel	post consumer	Arabian Gulf	2m *in situ*	30 days
2018; Oberbeckmann *et al.*[11]	PE, PS, wood pellets	defined	Baltic Sea, River, WWT plant	1-3m *in situ*	14 days
2018; Kirstein *et al.*[12]	HDPE, LDPE, PP, PS, PET, PLA, SAN, PESTUR, PVC, glass	defined	North Sea water	dark flow through system	15 months
2019; Kirstein *et al.*[13]	HDPE, LDPE, PP, PS, PET, PLA, SAN, PESTUR, PVC, glass	defined	North Sea water	dark flow through system	21 months
2019; Parrish *et al.*[14]	PE, PS spheres	defined	freshwater/WW	Stirred 100 mL batch reactors	48 hours
2019; Curren and Leong[15]	not specified	field collected on beach	Singapore beaches	tropical beaches	unknown/variable
2019; Miao *et al.*[16]	PE, PP	defined	China lake water	aquaria in greenhouse	21 days

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