UNIVERSIDADE TÉCNICA DO ATLÂNTICO
INSTITUTO DE ENGENHARIA E CIÊNCIAS DO MAR

WEST AFRICAN SCIENCE SERVICE CENTRE ON CLIMATE CHANGE AND ADAPTED LAND USE

Master Thesis

16S rRNA GENE SEQUENCE ANALYSIS OF THE MICROBIAL COMMUNITY ON MICROPLASTIC SAMPLES FROM THE NORTH ATLANTIC AND GREAT PACIFIC GARBAGE PATCHES

DKAWLMA TORA

Master Research Program on Climate Change and Marine Sciences

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Dkawlma Tora

Master's thesis presented to obtain the master's degree in Climate Change and Marine Sciences by the Institute of Engineering and Marine Sciences, Atlantic Technical University, in the framework of the West African Science Service Centre on Climate Change and Adapted Land Use.

Supervisor

Prof. Dr Ute Hentschel Humeida
GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany

Co-supervisor

Dr Erik Borchert
GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany

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Dkawlma Tora

Panel defence

President

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Examiner 1

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Examiner 2

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Dedication

In the memory of my younger brother Don de Dieu TORA and my parents, namely Isabelle Wima MAKEOUMA and Mananté W. TORA

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Resumo

O lixo plástico marinho representa um substrato relativamente novo e cada vez mais abundante para a colonização por organismos microbianos, embora o potencial funcional total destes organismos ainda não tenha sido descoberto. O aumento exponencial da produção de plástico levou à sua subsequente acumulação no ambiente, particularmente nos oceanos, uma vez que os actuais tratamentos convencionais de resíduos plásticos são de eficiência mínima. No presente estudo, comparámos comunidades microbianas de plástico, conhecidas como "Plastisfera", dos oceanos Atlântico e Pacífico, para ver se eram significativamente distintas. Além disso, identificámos potenciais degradadores de plástico dentro destes Plastispheres. Por conseguinte, utilizámos 16S rRNA *amplicon* analysis em amostras de microplásticos e de água recolhidas nas aglomerações de lixo no Grande Pacífico e no Atlântico Norte, respectivamente, em Junho e Agosto de 2019 e revisão bibliográfica. Os plásticos recolhidos compreendiam quatro tipos diferentes de polímeros: polietileno de alta densidade (PEAD), polietileno de baixa densidade linear (PEBD), polipropileno (PP) e polietileno (PE). Verificámos que comunidades microbianas diferiam significativamente entre os dois oceanos. Identificámos trinta e dois taxas diferencialmente abundantes ao nível da classe entre os dois oceanos. Verificámos também que as comunidades que vivem em polímeros plásticos no Atlântico e no Pacífico não eram significativamente distintas dentro de cada área, juntamente com as comunidades que vivem na água e no plástico no Pacífico. Proteobacteria, Cianobacteria e Bacteroidota foram as mais proeminentes phyla relativamente abundantes nos três substratos. Finalmente, encontrámos 40 géneros pertencentes aos Filos Actinobacteriota, Bacteroidota, Firmicutes e Proteobacteria dentro da Plastisfera, documentados a litterature como potenciais degradadores de plástico. Terminámos o trabalho com recomendações para estudos futuros, nomeadamente a integração das propriedades de ambos os oceanos, como temperatura, oxigénio dissolvido, salinidade e pH, para determinar os prováveis motores destas diferenças na colonização das comunidades.

Palavras-chave: Plastisphere, Atlântico, Pacífico, comunidades microbianas, microplásticos, degradadores de plástico

Abstract

Marine plastic debris represents a relatively new and increasingly abundant substrate for colonization by microbial organisms, although the total functional potential of these organisms is yet to be uncovered. The exponential increase of plastic production has led to their subsequent accumulation in the environment, particularly in oceans, as current conventional treatments of plastic waste are of minimal efficiency. In the present study, we compared microbial communities on plastic, known as "Plastisphere", from the Atlantic and Pacific oceans to see whether they were significantly distinct. In addition, we identified potential plastic degraders within these Plastispheres. Therefore, we used 16S rRNA amplicon analysis on microplastic and water samples collected in the Great Pacific and North Atlantic Garbage Patches, respectively, in June and August 2019 and literature review. Four polymer types composed the plastics: high-density polyethylene (HDPE), linear low-density polyethylene (LDPE), polypropylene (PP) and polyethylene (PE). We found that microbial communities differed significantly between the two oceans. We identified thirty-two differentially abundant taxa at the class level between the two oceans. We also found that communities living on plastic polymers in the Atlantic and the Pacific are not significantly distinct within each area, along with communities living in water and plastic in the Pacific. Proteobacteria, Cyanobacteria and Bacteroidota were the most prominent relative abundant phyla on the three substrates. Finally, we found 40 genera belonging to the phyla Actinobacteriota, Bacteroidota, Firmicutes and Proteobacteria within the Plastisphere, documented in the literature as potential plastic degraders. We ended the work with recommendations for future studies, notably the integration of the properties of both oceans like temperature, dissolved oxygen, salinity and pH to determine likely drivers of these differences in communities' colonization.

Keywords: Plastisphere, Atlantic, Pacific, microbial communities, microplastics, plastic degraders

Abbreviations and acronyms

A_	Atlantic
ASV	Amplicon Sequence Variant
CCGA	Competence Centre for Genomic Analyses
CoA	Coenzyme A
CSV	Comma Separated Values
DIPLANOAGAP	Distribution of Plastics in the North Atlantic Garbage Patch
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
GEOMAR	Helmholtz Centre for Ocean Research, Kiel
GPGP	Great Pacific Garbage Patch
Biobank	Strain collections of Research Division 3: Marine Ecology, Research Unit: Marine Symbioses (GEOMAR)
HCB	Hydrocarbon Degrading Bacteria
HDPE	High-density Polyethylene
LCA	Least Common Ancestor
LDA	Linear Discriminant Analysis
LDPE	Low-density Polyethylene
LEfSe	Linear Discriminant Analysis Effect Size
MARPOL	International Convention for the Prevention of Pollution from Ships
NAGP	North Atlantic Garbage Patch
MT	Metric Tons
NMDS	Non-metric Multidimensional Scaling
OTU	Operational Taxonomic Unit
P_	Pacific
PA	Polyamide
PCR	Polymerase Chain Reaction
PD	Phylogenetic Diversity
PERMANOVA	Permutational Multivariate Analysis of Variance
PE	Polyethylene
PET	Polyethylene Terephthalate
PP	Polypropylene
PMMA	Polymethyl Methacrylate
PMD	Plastic Marine Debris
PS	Polystyrene
QIIME	Quantitative Insights Into Microbial Ecology
R/V	Research Vessel
rRNA	Ribosomal Ribonucleic Acid
TCA	Tricarboxylic Acid
TSV	Tab-Separated Values

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1. Introduction

1.1 Background

Over the last few decades, the promiscuous and uncontrolled use of plastics led to millions of tons of plastic waste in the terrestrial and marine environment. It elevated the risk of environmental pollution and climate change. For instance, sea surface plastic degradation is estimated to release 76 metric tons of methane (a potent greenhouse gas) yearly (Royer et al., 2018). And microplastic pollution can alter the ocean's climate mitigation potential by reducing its blue carbon storage capacity and resilience to climate change (Stoett and Vince, 2019; Sjollema et al., 2016). The concern arises more due to the reckless and unscientific disposal of plastics containing high molecular weight polymers, notably polystyrene, polyamide, polyvinylchloride, polypropylene, polyurethane, and polyethylene, etc., which seems very difficult to degrade. Plastic debris, notably larger plastic debris, has harmful effects, such as entanglement and ingestion by animals in the marine environment (Laist, 1997).

Throughout the past ten years, research efforts have focused on the ecological impacts of smaller plastic particles known as microplastics (Arthur et al., 2009) on marine animals such as bivalves and fish (Sussarellu et al., 2016; Lusher et al., 2016), and zooplankton (Cole et al., 2013), and the link to humans through food web interactions (Cox et al., 2019). Microplastics are plastic particles less than 5 mm long. They come from resin pellets used in plastic manufacturing and manufactured plastic beads (primary microplastics) used in health and beauty products. They also stem from larger plastic pieces that have broken apart (secondary microplastics) (NOAA, "National Oceanic and Atmospheric Administration", Institute, USA). According to NOAA, they come in many forms, including beads, fragments, pellets, and fibres. They get into the ocean through varied pathways, including littering, landfill run-off and loss at Sea (Browne, 2015). Alarmingly, 4.8 to 12.7 million metric tons are estimated to enter the ocean each year, and this is expected to increase by order of magnitude by 2025 (Jambeck et al., 2015). Microplastics are found globally because of their high mobility and long residence time (Naidoo & Glassom, 2019). In the ocean, plastics in water bodies can be found (Auta et al., 2017) or ingested by marine life (Guzzetti et al., 2018), floating at the surface, buried within the sediments (Robbin et al., 2020) or mixed with marine snow (Porter et al., 2018).

Plastic debris often concentrates in oceanic gyres (Law et al., 2010) but has also been discovered in remote regions, including Arctic Sea ice (Peeken et al., 2018) and at depths greater than 4,000 m in the Pacific Ocean (Krause et al., 2020). The standard prevention techniques to reduce plastics in the ocean come from outreaches such as 'stop throwing the plastic garbage away', 'buy environment-safe cleaning products'; from National and International policies like Conventions (MARPOL, 1973; London Convention and Protocol, respectively 1972 and 1996). Appeals for eliminating single-use plastic products and packaging caps in virgin plastic production and manufacturing biodegradable plastics are encouraged.

Some techniques were also discovered and tested. Physical techniques like coagulation, flocculation, sand filtration, and adsorption on activated carbon have been tried. Likewise, chemical methods like photosensitized oxidation and adsorption have been tested (Li et al., 1999; Plumlee et al., 2008). But they were quickly abandoned (Ferreira et al., 2001; Saquib and Muneer, 2003). Advanced oxidation processes and photocatalytic nanocoating devices were created to convert fragment contaminants into CO_2 and H_2O and to break down microplastics into harmless elements (Tofa et al., 2019; Mohsen et al., 2020). With swift advancements in molecular techniques, researchers recently started to describe the microbial life colonizing marine plastic debris. The aim was to clarify which microorganisms are present (Zettler, Mincer & Amaral-Zettler, 2013) and to explore them for their potential plastic degradation (Coons et al., 2021).

1.2 Relevance and importance of this research

The microbial diversity could have important implications for understanding their interactions with plastic and its management on a global scale. Microbes dominate the diversity and biomass of marine ecosystems and control biogeochemical cycling in these systems. However, very little is known about the communities of microbes that develop on marine plastic debris and how they interact with and transform plastic, one of the most common anthropogenic pollutants in the sea. Recognizing this knowledge gap, Harrison et al. (2011) called for research to determine spatiotemporal patterns of taxonomic composition and the functional potential of plastic-colonizing microbes as critical to informing management decisions. Biotechnology will be crucial in developing strategies for reducing plastic pollution. Besides, the study samples comprised polyethylene (PE) and polypropylene (PP) pieces, for which only scarce information concerning biodegradation is available.

1.3 Problem Statement

Microbial communities on the surfaces of marine plastic debris are known as 'Plastisphere', describing the biofilm-forming communities on plastic debris surfaces (Zettler et al., 2013). These biofilms consist of diverse microorganisms such as bacteria, algae, protozoans, and fungi (Cooksey & Wigglesworth-Cooksey, 1995). Many studies have investigated both hemispheres (Coons, 2020) but much more in the northern hemisphere (Wright et al., 2020). Various experiments in coastal- and open-ocean surface waters have been conducted (Coons et al., 2021), including the Pacific (Tobias-Huenefeldt et al., 2021), Atlantic (Debroas et al., 2017), Indian Ocean (Muthukrishnan et al., 2019), Baltic (Kesy et al., 2019), North (Oberbeckmann et al., 2016), and Mediterranean Sea (Dussud et al., 2018). Biogeography and the substrate type were shown to influence the diversity of the communities (Zettler et al., 2015). Indeed, using water and plastic samples from the North Atlantic and the Great Pacific Garbage patches collected in 2015, Zettler et al. demonstrated that the microbial diversity was distinct between the Atlantic and the Pacific Oceans. Some signs like rising temperatures affect biodiversity while changing rainfall patterns, extreme weather events, and ocean acidification pressure species[1] already threatened by other human activities (Weiskopf et al., 2020). In this context, could the result of Zettler et al., 2015 be observed with microplastic and water samples collected in 2019 in the same areas?

1.4 The objective of the work

The main goal was to compare Atlantic to Pacific bacterial communities using microplastic and water samples collected in 2019 to determine whether their communities differed significantly. Secondly, the work sought potential plastic degraders within the present Plastisphere. Eventually, the work discussed the study of Zettler et al., 2015, which had the same comparison.

This objective posed the following questions:

- What microbial communities live on the Atlantic plastic, Pacific plastic or in Pacific water?
- Do microbial communities on Atlantic, or Pacific plastic differ significantly from the microbial community in Pacific water?
- Do microbial communities on Atlantic and Pacific plastics significantly distinct?

[1] https://www.eea.europa.eu/signals/signals-2015/articles/climate-change-and-the-seas

- What are the main differentially abundant classes of Atlantic and Pacific Plastispheres?
- Are there potential plastic degraders within Atlantic and Pacific plastic communities?

1.5 Structure of the work

The workflow of this thesis is organized as follows from here onward:

- The literature review exhibited in section two went through previous studies related to microplastic and microbial communities on plastic, notably the definition of the term “microplastic”, the inconvenient of plastic in the environment, the input of plastic in the ocean, attempt to fight plastic pollution and different studies related to Plastisphere;
- The study area and the methodology used to get the data and to perform the study are presented in section three;
- The results that characterized the microbial communities on plastic and in water, statistical analysis and discussion of the results are shown in section four;
- The conclusion and future work recommendations are shown in section five.

2. Literature review

Literature related to microplastic pollution is abundant (Bergman et al., 2015; Zeng et al., 2018). The area evolved recently and is getting a lot of attention (Zeng et al., 2018) regarding its harmful effects. According to Frias (2019), authors found a consensus to define microplastic as any synthetic solid particle or polymeric matrix, with regular or irregular shape and a size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water.

The term 'microplastic' appeared in Thompson et al. (2004) for the first time when he was trying to find out where all the millions of metric tons of plastics produced annually end up. The study results showed that microscopic plastic fragments and fibres (microplastics) are widespread in the oceans and have accumulated in the pelagic zone and sediments resulting from the degradation of more oversized items. Ingested by marine organisms, its consequences on the environment were still unknown. Subsequently, studies have focused on the impact of microplastics on different settings, such as the marine environment. Using bioimaging techniques, Cole et al. (2013) employed feeding rate studies to determine the impact of microplastics on algal ingestion rates in copepods. Afterwards, with the aid of fluorescence and coherent anti-Stokes Raman scattering, they found that marine microplastic debris can significantly decrease algal feeding and thus can negatively impact zooplankton function and health.

Before that, using respectively CO_2 depletion and ROS (Reactive Oxygen Species) assays, Bhattacharya et al. (2010) conducted a study showing that the adsorption of plastic beads hinders algal photosynthesis, possibly through the physical blockage of light and airflow by the nanoparticles. Subsequently, plastic adsorption promotes algal ROS production. They linked the climate change issue, and their analysis pointed out that the above impacts may compromise the carbon cycling function of the ocean ecosystem (Stoett & Vince, 2019).

Evidence suggests that plastic ingestion by coral reefs adds to the threat of climate change and ocean acidification (Stoett & Vince, 2019). Additionally, an analysis of samples from sub-surface plankton close to inshore reefs run by Hall et al. (2015) pointed out that ingestion of high concentrations of microplastic debris could potentially affect the health of corals. Microplastics can also absorb toxic compounds such as persistent organic pollutants (e.g., industrial chemicals, dioxins, pesticides) and heavy metals from seawater, leading to extra damage to the biota (Mato et al., 2001; Horn et al., 2019).

Besides that, the potential accumulation of microplastics in the food chain, especially in fish and shellfish, exposes human consumers (GESAMP, 2015). Lusher et al. (2013) reported that 36% of pelagic and demersal fish collected from the English Channel had microplastics in their Gastrointestinal Tract. Nelms et al. (2019) observed that every specimen sampled from stranded marine mammals along the British coast contained microplastics in its gut. Several finfish investigations highlighted that microplastics accumulate in the gills, alimentary tract, liver and muscle (Avio et al., 2015; Choi et al., 2018; Greven et al., 2016; Su et al., 2019).

Microplastics come from different pathways to get in the marine environment. Dris et al. (2016) related an atmospheric fallout between 2 and 355 particles/m^2/day, while most other studies focus on littering, landfill run-off and loss at Sea (Browne, 2015). Jambeck et al. (2015) estimated the mass of land-based plastic waste entering the ocean from 4.8 to 12.7 million metric tons. One year before, Eriksen et al. (2014) estimated a minimum of 5.25 trillion particles weighing 268.940 tons floating at the sea surface. Trawling the water's surface with 333 μm mesh nets, Moore et al. (2001) reported the mass of microplastic in samples from the North Pacific gyre was six times that of coincident plankton.

Investigating plastic content at the surface of the western North Atlantic Ocean and the Caribbean Sea, Law et al. (2010) linked the movement at sea to surface currents predicted by Ekman dynamics. They also observed the highest concentration of plastic debris in subtropical latitudes. Finally, the model from Maximenko and Niiler identified five hot spots of plastic accumulation in the world's oceans. These hot spots are the five ocean gyres: North Pacific, South Pacific, North Atlantic, South Atlantic, and Indian gyre (Ocean Debris, 2008), identified as significant accumulation zones for marine debris.

For plastic pollution reduction, actions are being undertaken, and research and politics focus on processes that can help with plastic removal and against its dump in the environment. Some studies investigated techniques like adsorption on green algae, removal using membrane technology, removal using advanced filtration technologies in wastewater treatment plants, and chemical methods to treat microplastics. Sundbaek et al. (2018) studied the adherence behaviour of fluorescent microplastic particles on the surface of edible marine microalgae, seaweed, named *Fucus vesiculosus*. The results revealed high sorption of microplastics (94.5%), mainly near the cut surfaces of the seaweed, which is explained by the role of released alginate compounds from cell walls in the cut regions. The diameter size of the polystyrene microplastics was almost 20 μm. At the same time, the plant cells of the

sorbent contained very narrow microchannels to restrict the translocation of polystyrene microplastics into the tissues.

Meanwhile, Talvitie et al. (2017) investigated the removal of various microplastics from wastewater treatment plant effluents using advanced final-stage treatment technologies, including membrane bioreactor, disk filter, rapid sand filtration, and dissolved air floating. They concluded that the membrane bioreactor eliminated 99.9%, from 6.9 to 0.005 microplastic particles per litre. Besides that, 11 wastewater treatment plants in Changzhou, China, were studied for their efficiency in removing microplastics by following the abundance, colour, shape and dimensional changes during the removal steps (Ma et al. 2019). All plants that used several treatment steps, such as subsequent tanks for floating and sedimentation, and filtration processes, eliminated more than 90% of microplastics from the influents, with a final removal efficiency reaching 97.15%.

Nowadays, research focuses on biological removal. The preliminary results of a four-month study on reducing high-density polyethylene secondary microplastics in seawater using two marine communities, from Agios and Souda, were published by Cocca et al. (2017). According to the recorded weight reduction results, the Souda community was more efficient. Interestingly, their finding in monitoring the cell content and populations suggested that microplastics acted as a rich carbon source to feed the organisms. Paço et al. (2017), meanwhile, explored the capability of the fungus *Zalerion maritimum*, a naturally occurring fungus in marine ecosystems, for the polyethylene microplastics biodegradation based on mass and size variations of the microplastics in a batch reactor. They measured the resulting microplastic substance's concentration at various time intervals. The evidence from biological content measurements, such as protein reduction and carbohydrate increase with exposure time, revealed that the *Zalerion maritimum* community possibly uses the microplastics as a nutrient source.

Plastic is a high molecular weight synthetic polymer of a long chain of hydrocarbon derived from petrochemicals (Ahmed et al., 2018). The biological deterioration of plastic pollutants depends on many factors: surface area, functional groups, molecular weight, hydrophilic and hydrophobicity, melting temperature, chemical structure, crystallinity, etc. (Okada, 2002). Microbial degradation of plastic involves many steps: biodeterioration, bio-fragmentation, assimilation, and mineralization, as shown in Figure 1**.** Indeed, the physical deterioration and chemical degradation of plastic polymers lead to their fragmentation into simpler oligomeric and monomeric forms: benzoic acid, benzyl alcohol, benzaldehyde, carboxylic acid, ethylene, ethylbenzene, propylene benzene, phenol, Poly-β-hydroxybutyrate,

ketones, styrene, and vinyl chloride, *etc*. that are less recalcitrant and harmful for the environment (Pathak, 2017; Dussud and Ghiglione., 2014). The oligomers and monomers of plastic are assimilated by various microbes inside their cell and catabolized to produce energy. The assimilation helps convert monomeric plastic into secondary metabolites, which are later excreted into the environment (Swapnil et al., 2015). These metabolites are either utilized by other microbes for further degradation or remain deposited in the background with other non-assimilative compounds. These metabolites are completely oxidized through consecutive assimilation and degradation into minerals such as CO_2, N_2, CH_4, and H_2O (Nakajima-Kambe et al., 1995).

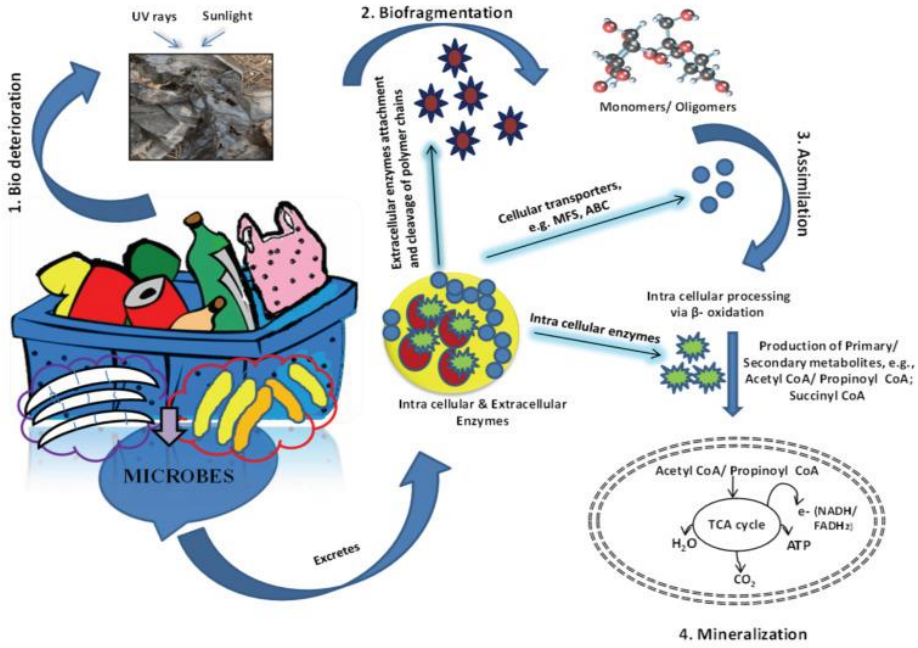

Figure 1. Mechanism of plastic biodegradation (Purohit et al., 2020**)**

Many actions are also undertaken against the plastic pollution issue around the world. In Africa, Rwanda has banned non-biodegradable plastic since 2008, which was complemented later in 2019 by a ban on all single-use plastics. The ban prohibited the manufacturing, use, import and sale of plastic carrier bags and forbade travellers into Rwanda to come with such products. They introduced a community cleanup, 'Umuganda', held on the last Saturday of every month. Senegal tightened its efforts against single-use plastic by banning plastic water sachets and coffee cups in 2020 but is still making some allowance because of the Covid-19 situation. Nigeria announced a ban on plastic bags in 2013, and in 2020, it strengthened its legislation by including a fine of 1072.16 Euro or three years jail terms for any store found giving plastic bags to customers. In the Gambia, a person who manufactures, imports, uses or sells plastic bags commits a criminal offence. In Botswana, a minimum thickness for bags

was established and mandated that retailers apply a minimum levy to thicker bags, which would support government environmental projects. Subsequently, a study of four retail chains 18 months after implementing the charge showed that bag use dropped by 50%. Kenya leads the way with the strictest ban on single-use plastic globally. Importing, manufacturing or selling single-use plastic bags could incur companies a fine of 40000USD; using one, on the other hand, could see individuals facing a penalty of 500USD. In 2019 the President of Kenya announced a ban on single-use plastics in protected areas (Greenpeace, 2021).

Microplastics are subjected to different processes within the ocean, including degradation, burial in sediments, sinking to the bottom, ingestion by marine animals (Cózar et al., 2014) or colonization by different microorganisms known as 'Plastisphere'. Zettler, Mincer & Amaral-Zettler (2013) coined the term “Plastisphere” to describe biofilm-forming communities on marine plastic debris. They collected marine plastic debris at multiple locations in the North Atlantic to analyze the microbial consortia attached to it. They found diverse microbial communities, including heterotrophs, autotrophs, predators, and symbionts, which they called a 'Plastisphere'. Fletcher & Loeb (1979) used a species of marine *Pseudomonas* NCIMB 2021 in laboratory experiments. Their results showed that hydrophobic surfaces were more rapidly colonized than hydrophilic surfaces. Subsequently, Ogonowski et al. (2018) demonstrated that substrate type is crucial for biofilm forming. They exposed ambient Baltic bacterioplankton to polyethylene, polypropylene, polystyrene, cellulose and glass beads substrates. They found that all biofilm communities displayed lower diversity and evenness than the source community, suggesting substrate-driven selection. Moreover, the plastics-associated communities differed from those on the non-plastic substrates.

Many studies regarding bacteria colonization of plastic have been performed and are underway. Many other aspects of substrate-driven selection were also considered in the analysis to decipher and understand different microbial interactions with plastic. In their review ‘Adhesion of bacteria and diatoms to surfaces in the sea’, Cooksey & Wigglesworth-Cooksey (1995) found that, in marine environments, submerged material provides a substratum for the rapid formation of biofilms. These biofilms comprise organic matter, bacteria, and microalgae and develop into mature biofouling communities over time. Since then, many studies have investigated different marine environments for their microbial contents, focusing on plastics. Debroas et al. (2017) used a metabarcoding approach and statistical analysis associated with network building to study the structure of Eukaryotes, Bacteria and Archaea in the Atlantic Ocean. Their system defined a core microbiome at the plastic surface. The results showed that most of the bacteria significantly associated with the

plastic waste originated from marine and non-marine ecosystems, and numerous species can be considered hitchhikers. They also pointed out that others act as keystone species (e.g., Rhodobacterales, Rhizobiales, Streptomycetales and Cyanobacteria) in the biofilm. The chemical analysis provides evidence for specific colonization of the polymers. Alphaproteobacteria and Gammaproteobacteria significantly dominated mesoplastics consisting of polyethylene, polyethylene-terephthalate and polystyrene. Furthermore, the study indicated that the bacteria inhabiting plastics harboured distinct metabolisms from those in the surrounding water.

Meanwhile, Bryant et al., 2016 investigated the Pacific Ocean in its GPGP (Great Pacific Garbage Patch). Scanning electron microscopy and metagenomic sequencing of plastic-attached communities revealed the dominance of a few metazoan taxa. It also showed a diverse assemblage of photoautotrophic and heterotrophic protists and bacteria. Bryozoa, Cyanobacteria, Alphaproteobacteria, and Bacteroidetes, dominated all plastic particles, regardless of particle size. Bacteria on plastic were taxonomically distinct from the surrounding picoplankton. These bacteria appeared well adapted to a surface-associated lifestyle. Their findings suggest that plastic debris forms a habitat for complex microbial assemblages with lifestyles, metabolic pathways, and biogeochemical activities distinct from those of free-living planktonic microbial communities.

For the Atlantic and Pacific oceans together, Coons et al. (2021) investigated plastic-type and incubation location as drivers of marine bacterial community structure development on plastic via 16S rRNA gene amplicon analysis. Four distinct plastic types were incubated at four different biogeographic locations, including HDPE, LDPE, PA, PMMA, and glass-slide controls (Cape Verde, Chile, Japan, and South Africa). They found that the primary driver of the coastal Plastisphere composition was incubation location, while substrate type did not significantly affect bacterial community composition. The bacterial communities were consistently dominated by the classes Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia, irrespective of sampling location or substrate type. Similarly, in 2015, Amaral-Zettler et al. used next-generation DNA sequencing to characterize bacterial communities from the water of the Pacific and Atlantic oceans and plastic samples composed of PP, PE, PS and PET from the North Pacific and North Atlantic subtropical gyres. Their objective was to determine whether the composition of Plastisphere communities reflects their biogeographic origins. They found that these communities differed between ocean basins and, to a lesser extent, between polymer types and displayed latitudinal gradients in species richness.

Four years later (because the samples of this study were retrieved in 2019 from the North Atlantic and the Great Pacific Garbage Patches), as in the previous research, this present master thesis tried to compare the Atlantic to the Pacific to determine whether there was a significant difference between their microbial communities. In addition to the work of Zettler et al. (2015), this work pointed out communities responsible for the discrepancy. 16S rRNA gene amplicon sequence analysis was used on microplastic samples composed of PP, LDPE, PE and HDPE from North Pacific and North Atlantic subtropical gyres and Pacific water samples. Both results were discussed, and differences were pointed out. Additionally, in this work, potential plastic degraders within both Plastisphere were detected.

3. Study area, data collection, materials and methods

This part exhibited the study area, data collection, the methods used to extract the DNA of the microbes, their sequencing and bioinformatic analysis. The materials used are shown throughout the description of the different techniques.

3.1 Study area

The present study used two areas where samples were collected, notably the North Atlantic Garbage Patch and the Great Pacific Garbage Patch, displayed in Figure 2. Both are among the most significant areas of marine debris accumulation. These accumulations are linked to the circulation of the oceanic currents (Ocean Debris, 2008; Law et al., 2010).

The Great Pacific Garbage Patch is the most renowned collection of plastic pollution found in the North Pacific gyre. It is located halfway between Hawaii and California. On average, the patch orbits around 32°N and 145°W. It is estimated to have a total mass of 96,400 MT, made up of 1990 billion pieces (Ritchie and Roser, 2018; Lebreton et al., 2012). Plastics account for 99.9% of the debris within the GPGP (Lebreton et al., 2018), and 94% of the debris pieces are microplastics (Greenly et al., 2021). These plastics are made up of primarily rigid Polyethylene (PE) and Polypropylene (PP) plastics and discarded fishing nets remaining afloat (Lebreton et al., 2018), with fishing gear consisting of 52% of the total mass (Ritchie and Roser, 2018). The size of the GPGP is estimated at 1.6 billion km^2. It is the most severe and considerable accumulation of garbage out of the five patches littered across the world's oceans (Ocean cleanup, 2020).

Besides that, it was reported that 20% of the global inventory of floating plastic debris is accumulated in the North Atlantic, and most of these materials are concentrated in the North Atlantic Garbage Patch (Cózar et al., 2014; Eriksen et al., 2014). The region is between the Azores and Bermuda (Cózar et al., 2014). Plastic inventories averaged 400 g/km^2 within the zone, with maxima as high as 2500 g/km^2 (Cózar et al., 2014). Eriksen et al. (2014) reported plastic particle densities of up to 106 km^2 within the same area. Plastic particles within the region comprise a wide range of compositions and sizes, including nanoplastics (Halle et al., 2017). Small particles may include a significant mass fraction in the region (Poulain et al., 2019). Indeed, a study of tiny microplastics (32–651 μm) in the North Atlantic water column found a combined mass of 11.6 – 21.1 million tons for polyethylene, polypropylene, and polystyrene suspended in the top 200 m (Pabortsava and Lampitt, 2020).

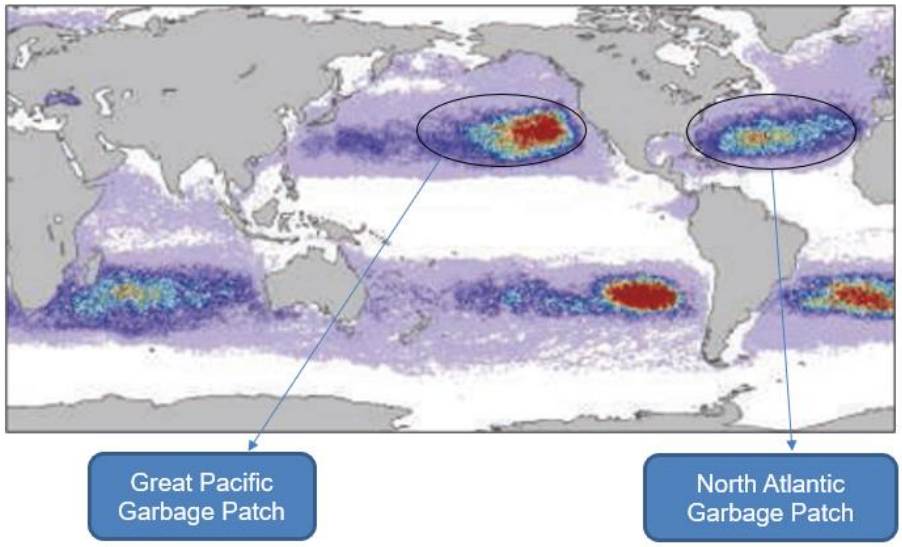

Figure 2. Sampling locations from (Kaiser, 2010)

Besides that, the Pacific Ocean covers 30% of the Earth's surface while the Atlantic covers 20%. The environmental conditions show the North Atlantic Ocean to be warmer and saltier than the North Pacific Ocean (Reid, 1961). Likewise, the pH of the Atlantic was shown to be lower than the Pacific (Voelker, 2018), and the dissolved oxygen between both oceans varies (Boyer et al., 1999).

3.2 Data collection

The samples were collected in the North Atlantic and Great Pacific Garbage Patches. The pieces from the Atlantic were collected between 26-08-2019 and 04-09-2019 during the POS536 cruise project 'Distribution of Plastics in the North Atlantic Garbage Patch' (DIPLANOAGAP) aboard the German research vessel (R/V) Poseidon. A Neuston catamaran onboard R/V Poseidon, equipped with a microplastic trawl net (mesh size 300 µm, mouth opening 70 cm x 40 cm), was used to collect the plastic samples from the sea surface. A flowmeter was deployed along with the net to measure the volume of water filtered during each 20-minute tow.

After each tow, the body was rinsed using a seawater hose, directing all contents to the cod-end. All microplastic fragments were removed from the trawl sample and conserved in a saturated ammonium sulphate solution (700 g/l ammonium sulfate, 20 mM sodium citrate, 25mM EDTA, pH 5.2). This solution precipitated all proteins, preventing DNA and RNA degradation for an extended time, even at room temperature. Verification of plastic-type by Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy analysis was subsequently performed by TUTECH GmbH in Hamburg, Germany.

Another cruise (SO268/3), this time in the Pacific, between 05-06-2019 and 27-06-2019, was used to collect plastic samples at the sea surface. Microplastics were collected using a scoop net sampling method. Microplastic surfaces were scraped with flame-sterilized scalpels, and biofilms were collected directly into microcentrifuge tubes. The sampling was 16 x 16 mm, and tubes were immediately frozen in liquid nitrogen. At each station, 1 litre of pacific water was filtered through a 3 µm filter (3 µm Isopore TSTP 04700 Millipore, Merck KGaA, Frankfurt, Germany) and a 0.22 µm filter (0.22 µm Isopore GTTP04700 membrane filters Millipore, Merck KGaA, Frankfurt, Germany). Also, the filters were transferred to microcentrifuge tubes and immediately frozen in liquid nitrogen.

3.3 Extraction of nucleic acids

Atlantic samples

Sections of the different plastic samples were cut with a sterile scalpel and placed into 2 ml MP Biomedicals™ Lysing Matrix E tubes (MP Biomedicals, Eschwege, Germany). Then physically disrupted using a bead-beating technique, with a single cycle of 30s at a speed of 5.500 rpm in a FastPrep homogenizer (Qiagen, Hilden, Germany). The DNA extraction from the lysis product was then performed using the Qiagen AllPrep DNA/RNA Minikit according to the manufacturer's instructions. The quality and quantity of the DNA extraction were assessed using the A260/280 ratio from a NanoDrop Spectrophotometer (Desjardins & Conklin, 2010).

Segments of the resulting DNA extracts were amplified using the 16S rRNA gene primer pair 27F and 1492R for a quality check via polymerase chain reaction (PCR), with the following PCR conditions: an initial denaturation at 95°C for 3 min, 34 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 90 s, followed by a final extension at 72°C for 5 min and held at 10°C. The resulting PCR products were visually assessed via 1% gel electrophoresis. Sequencing of the V3-V4 region of the 16S rRNA gene was conducted using v3 chemistry on a MiSeq Illumina sequencing platform at the Competence Centre for Genomic Analysis (CCGA) Kiel, Germany. For amplicon sequencing, the V3-V4 hypervariable region of the 16S rRNA gene was amplified using primer pair 341F (50-CCTACGGGAGGCAGCAG-30; Muyzer, De Waal & Uitterlinden, 1993) and 806R (50-GGACTACHVGGGTWTCTAAT-30; Caporaso et al., 2011) with the cycler conditions as follows: initial denaturation at 98°C for 30 s, 30 cycles of 98°C for 9 s, 55°C for 60 s, and 72°C for 90 s, followed by a final extension at 72°C for 10 min and held at 10°C.

Pacific samples

DNA was extracted from the biofilm pellets and water filters using the Macherey Nagel DNA Nucleo spin soil kit (Nucleo Spin TM Soil kit Macherey-Nagel TM, Düren, Germany) according to the manufacturer's instructions. DNA concentration was measured using a nano Qubit (ThermoFisher). Next-generation Illumina Sequencing was performed on an Illumina MiSeq platform using a V3 (300bp paired-end read) kit with a sequencing amount of 20 million reads, using the 341F (CCTACGGGNGGCWGCAG) and 785R primer set (GACTACHVGGGTATCTAAKCC).

3.4 Bioinformatic analysis

The resulting sequenced samples were organized into seven different types of datasets for further bioinformatics analysis. One set was made only by the Atlantic plastic samples for diversity comparisons within the Atlantic based on plastic polymer types, one by only Pacific plastic samples for the diversity comparisons within the Pacific based on plastic polymer types, and another one by only Pacific water samples for the microbial diversity within the Pacific water. Another dataset merged all the pieces for further analysis. The other three types also comprised combined datasets between Atlantic plastic and water, Pacific plastic and water (for diversity measures analysis), and the last dataset merged plastics from both oceans for the Plastisphere exploration and comparison. Each sample was identified with a unique number, and each set was processed within QIIME2, a program dedicated to microbial community analysis. The 'manifest file' and 'metadata file' were used to get the different datasets when reading the samples (all put together) within the QIIME2 pipeline.

3.4.1 Quantitative Insights Into Microbial Ecology (QIIME2) pipeline

Each of the seven datasets underwent the following procedure similar to the one described in Coons et al. (2021). Indeed, raw amplicon sequences were processed using the open-source Quantitative Insights into Microbial Ecology (QIIME2) framework (version 2020.11) following a pipeline developed by Kathrin Busch (GEOMAR). Forward primers and heterogeneity spacers were trimmed from forward-only single-end fastq files using the *cutadapt* plugin (Martin, 2011). The quality of the demultiplexed reads was verified using the *qualityfilter* plugin for PHRED-based filtering and trimming (Bokulich et al., 2013). An interactive plot was used to visualize these results using the *summarise* method of the *demux* plugin to determine an appropriate truncation length. Reads were denoised using the *denoise-*

single method of the DADA2 algorithm (Callahan et al., 2016), which truncated the ends and produced a total length of 270 nucleotides, removed chimeric sequences, and inferred sample composition using a parametric error model. Truncation to 270 nucleotides length increased the quality of the reads significantly but reduced the overlap between forward and reverse reads; therefore, only forward reads were used for the analysis.

Amplicon sequence variant (ASV; Callahan et al., 2017) taxonomy was classified at an 80% confidence level using the most recent SILVA 138 16S rRNA gene reference database (Quast et al., 2013; Yilmaz et al., 2014). That was possible *via* the feature-classifier plugin's pre-fitted classifysklearn taxonomy method (Bokulich et al., 2018; Pedregosa et al., 2012). Common eukaryotic contaminants (chloroplasts, mitochondria) and unassigned sequences were removed using the *filter-features* method of the *featuretable* plugin. The filtered dataset was rarefied to 8,000 sequences due to a good saturation of the alpha rarefaction curves for this number of features. A phylogenetic backbone tree was constructed using FastTree (Price, Dehal & Arkin, 2009; Price, Dehal & Arkin, 2010) and MAFFT (Katoh & Standley, 2013) alignment *via* the *phylogeny* plugin. The resulting tree was used to compute core diversity metrics. QIIME2 artefacts containing phylogenetic and non-phylogenetic diversity metrics were computed for downstream analyses along with an alpha-rarefaction curve *via* the *diversity* plugin.

3.4.2 Diversity measures

We adopted the standard significant measure, p-value = 5%, for the analysis. All the results showing a p-value below this standard describe a significant difference between the compared parameters and vice versa. Before the diversity measures, the study defined some valuable parameters.

Study parameters

Based on the study's objective, three factors were defined and accounted for in the 'metadata' file for QIIME2 processing. The parameters were also adapted for each set.

Ocean: this parameter shows where the sample comes from, the Atlantic or the Pacific Ocean. It helped for microbial diversity comparisons between the Atlantic and the Pacific oceans and whether there was a significant difference between them.

Plastic type: this parameter considered the different polymer types of the plastic samples in their parent ocean and or without water. It helped for microbial diversity comparisons on

plastic polymer types (sometimes including water for merged datasets) of the respective oceans to see if a significant difference could influence the diversity between the Atlantic and Pacific Oceans.

Sample type: considered the plastics under their parent ocean regardless of their polymer types and or without water. So, this parameter informed whether the sample was plastic from the Pacific or plastic from the Atlantic or water. The aim was to compare the biofilms of Atlantic plastic to Pacific plastic, Atlantic plastic to water and Pacific plastic to water to see if there was a significant difference in the diversity.

Alpha diversity measures

Alpha diversity analyses were performed on all the different datasets at the ASV level except the one from water (the dataset of water alone did not have any categorical data column in its metadata file). The alpha diversity was investigated according to unique ASVs per sample (species richness), taking into consideration the number of times each ASV occurs in the sample (Pielou's evenness) and the phylogenetic relatedness of each sample community (Faith's PD). It used *'qiime diversity alpha-group-significance*' plugin in QIIME2 to assess the diversity within each area. The results were displayed through Kruskal-Wallis (all groups) and Kruskal-Wallis (pairwise) results. The separate analysis aimed to see whether there was a significant difference within the different sites before comparing the two regions using the merged plastic datasets (the Plastisphere).

Non-phylogenetic (evenness) and phylogenetic (Faith's PD) diversity indices were visualized using the QIIME2 view in boxplots. Eventually, if the comparison revealed a significant difference in microbial diversity, Kruskal-Wallis pairwise was considered among groups to see where the difference lies.

Beta diversity measures

Beta diversity analyses were performed on all the datasets at the ASV level (except the one from water). Beta diversity measures assessed the differences between groups following the different parameters. It used *'qiime diversity beta-group-significance*' plugin in QIIME2. The analysis was performed using the non-metric multidimensional scaling method (NMDS; Kruskal, 1964) with a sample-wise unweighted UniFraq distance matrix (Lozupone & Knight, 2005). The unweighted UniFraq distance is a qualitative, phylogenetic beta diversity measure, calculated based on the presence or absence of ASVs in a sample community, as opposed to its quantitative counterpart (weighted UniFraq; Lozupone et al., 2007), which accounts for

relative abundances. Each group was assessed based on its distance from the other groups in QIIME2; boxplots were displayed simultaneously with the PERMANOVA results and pairwise PERMANOVA results between groups. PERMANOVA produces a probability related to the null hypothesis that there is no significant difference among group means concerning the diversity measure of choice (unweighted UniFraq). The PERMANOVA group significance and pairwise tests were run simultaneously through the *betagroup-significance* method (non-parametric MANOVA; Anderson, 2001) of the *diversity* plugin with an unweighted UniFraq matrix and 999 permutations as input.

3.4.3 Different taxonomic levels analysis

First, the feature ASVs table was exported in biom format in QIIME2. Subsequently, the taxonomy metadata file was added to the biom file and exported in TSV file format using '*biom convert*' plugin in QIIME2. Further analyses outside the QIIME2 environment were performed using the resulting TSV file table, which could be manipulated in Excel. Besides that, the same feature table was collapsed at level 3 (class level) and level 6 (genus level) of the different taxonomic ranks using the '*qiime taxa collapse*' plugin. Then the resulting tables were exported and converted into a TSV file format for analysis using tools outside the QIIME2 environment. Furthermore, and in many cases, the online converter CSV to TSV or TSV to CSV was used to get an eventual format needed. The header with the sample's identification was replaced by its different appropriate names in Excel.

Barplots: Excel displayed bar plots showing ASVs distribution on plastic samples between the Atlantic and the Pacific oceans. That helped to look at the distribution of microbial diversity between Atlantic Pacific plastics regardless of/considering the polymer types.

A Sunburst plot was used to display microbial shares at different taxonomic levels on plastics up to level 6 for genera greater than 20,000 reads. The plot was performed using 'plotly.express' package. Therefore, water samples were removed from the CSV file and imported into python. Abundances below 20,000 (number of reads) at the genus level were cut to simplify the plot and make it readable. In python, the taxonomic names were split into six columns (representing the different taxonomic levels up to the genus level). This plot aimed to explore the different abundances and their different proportions within each Plastisphere.

A Heatmap plot was used to explore abundances at one taxonomy level. High and low masses were visualized between the Pacific and the Atlantic plastics at the 'class level' (level

3). The data was obtained from the collapsed feature table at level 3, converted into TSV file format in QIIME2 and then imported into python. The taxonomic names were split, and level 3 was kept in python. Unclassified reads were removed with the python command, and the heatmap was performed with the aid of the 'seaborn' package.

Venn diagrams: displayed the numbers of unique and shared ASVs on the three substrates using 'vennDiagram' package. The converted ASVs table file was used, organized in Excel and imported into R. Indeed, the values of the samples were regrouped in Excel according to the different substrates. The null values were removed using python for each variable and then exported. The remaining rows were replaced by their respective ASV identification in Excel and run in R.

Linear discriminant analysis (LDA) effect size (LEfSe): the plot was performed within 'galaxy online'[2]. It displayed the differential abundance classes between the Atlantic and Pacific Plastisphere. The level 3 data was used, arranged within Excel (according to the different oceans) and imported into Galaxy for LEfSe analysis. The graphics were performed on the microbial community relative abundance data in both oceans. Grouped data were first analyzed using the Kruskal-Wallis test with a significance set to 0.05 to determine if the data was differentially distributed between groups. The differentially distributed taxa classes were used for the LDA model analysis to rank the relative abundance difference between groups. The log (10) transformed score demonstrates the effect size.

[2] https://huttenhower.sph.harvard.edu/galaxy/

4. Results

Through this section, the results stemming from the analysis are displayed. It started with the general overview of the samples, the investigation of each Plastisphere, the statistical analysis of some of the merged datasets, and continued with the Principal Coordinates Analysis of all the samples in one dataset, the exploration and statistical analysis of the Plastisphere (combined plastic samples from both oceans). Finally, the results displayed the differentially abundant microbial classes between the two oceans and potential plastic degraders within the Plastisphere.

4.1 General overview of the samples

The samples showed four plastic polymer types: HDPE (27 pieces with two from the Pacific and 25 from the Atlantic), PP (15 pieces with ten from the Pacific and five from the Atlantic), LDPE (2 pieces only from the Pacific) and PE (24 pieces only from the Pacific). These polymers were grouped in 68 microplastic pieces. The Atlantic Ocean accounted for 30 pieces, and the Pacific Ocean, 38 pieces. Fourteen water samples from the Pacific Ocean were added to the previous samples, making up 82 samples. The distribution in percentages based on the defined study parameters is shown in Table 1.

Table 1. Distribution of the data in percentages based on the study parameters.

In %	**Ocean**		**Plastic type**							**Sample type**		
	Pacific	Atlantic	A_HDPE	A_PP	P_HDPE	P_PP	P_LDPE	P_PE	Water	P_plastic	A_plastic	Water
Total samples	63.41	36.59	30.49	6.1	2.44	12.2	2.44	29.27	17.07	46.34	36.59	17.07
Plastic samples	55.88	44.11	36.76	7.35	2.94	14.71	2.94	35.29		55.88	44.11	

After processing all samples in QIIME2, 11,852 demultiplexed ASVs, shared in different taxonomic levels, were recorded and summarized in Table 2. Pacific plastic shows more microbial diversity than Atlantic Plastic, which offers more variety than Pacific water.

Table 2. Samples' distribution at different taxonomic levels (Unclassified percentages were not included within the classified).

Taxonomic level	Atlantic plastic		Pacific plastic		Water	
	Classified	%Unclassified	Classified	%Unclassified	Classified	%Unclassified
Kingdom	3	0	3	0	3	0
Phylum	35	0.51	33	0.12	27	0.13
Class	74	0.57	74	0.26	55	0.21
Order	161	4.19	172	2.30	142	2.91
Family	241	5.75	252	5.35	206	5.67
Genus	369	34.09	400	35.33	323	38.56
ASVs	4,454		7,081		3,623	

4.2 Atlantic Plastisphere

Atlantic samples were composed of two polymer types: HDPE with 25 pieces and PP with five pieces. The number of taxa revealed by 16S rRNA gene sequences at different taxonomic levels is in Table 2. From the analysis, most of the relative abundances were bacteria (99.91%). Three bacterial phyla, notably Proteobacteria, Cyanobacteria and Bacteroidota accounted for more than 90% of the relative abundance, while 29 other phyla (including the phyla of Bacteria, Archaea and Eukaryota) accounted for 4.70% (each of these 29 phyla accounted below 1% of the relative abundances). Verrucomicrobiota, Bdellovibrionota and Firmicutes (from Bacteria) were perceived as median relative abundances accounting for more than 1% each. The distribution of the masses within the Atlantic Plastisphere at different taxonomic ranks can be seen in the Appendix, Table (a).

The taxonomic affiliations, as displayed in Figure 3 for the number of reads (genus level) greater than 20,000, show Alphaproteobacteria and Gammaproteobacteria stemming from Proteobacteria, among the highest relative abundances at the class level. Most of Gammaproteobacteria's highest reads belonged to Pseudomonadales at the order level, Moraxellaceae at the family level, and Acinetobacter genus. The other big classes of these reads were Cyanobacteriia from Cyanobacteria and Bacteroidia from Bacteroidota. The highest percentage of the reads assigned to Alphaproteobacteria belonged to Rhodobacterales and Caulobacterales at the order level; Rhodobacteraceae and Hyphomonadaceae at the family level. At the genus level, Rhodobacteraceae were most represented by unclassified, and the reads of an uncultured species most represented Hyphomonadaceae.

Phormidesmiales and Cyanobacteriales most represented Cyanobacteriia's reads at the order level; Phormidesmiaceae and Xenococcaceae at the family level; *Phormidesmis*_ANT.LACV5.1 and *Acrophormium*_PCC-7375 at the genus level. Meanwhile,

Bacteroidia's reads were most represented by Chitinophagales at the order level, Saprospiraceae at the family level and the genus *Lewinella*.

Among the small percentage reads, Eukaryota (0.09%) were represented by the phyla Amorphea (0.08%) and SAR (0.002%) and the classes of Obazoa and Alveolata. Likewise, the reads of Archaea (0.0002%) were represented by the phylum of Nanoarchaeota and the class of Nanoarchaeia**.**

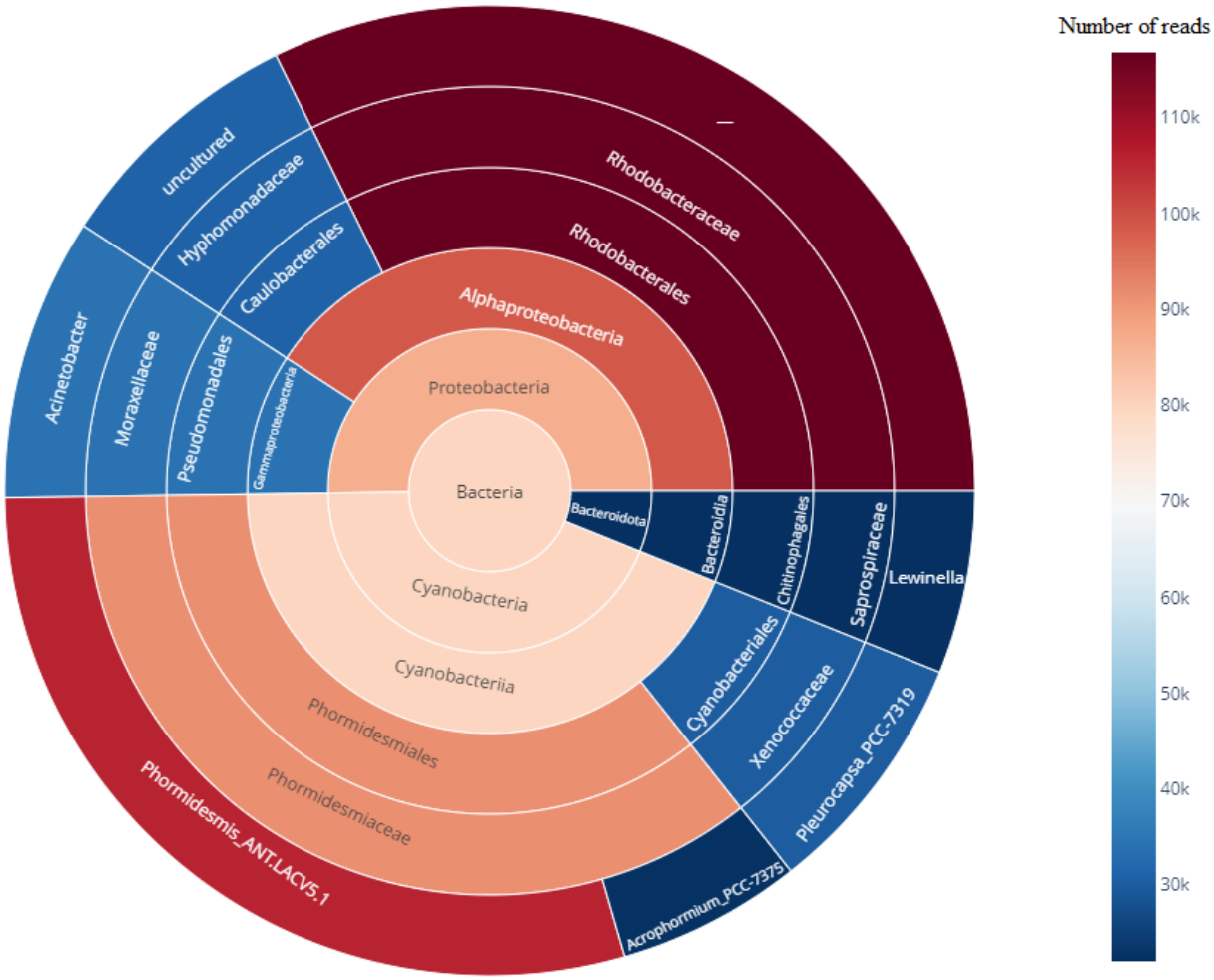

Figure 3. Distribution of recurring communities on the Atlantic Plastisphere. Sunburst chart displaying the affiliations of genera communities that reached values above 20,000 reads at different taxonomic levels. Each plot crown represents one taxonomic level from the Kingdom to the genus.

Diversity measures

Alpha diversity measures within the Atlantic area were performed for both non-phylogenetic (evenness) and phylogenetic (Faith's PD) diversity. These two tests exhibited p-

values > 0.05. They were respectively 0.80 and 0.67. The variety within the area was not significantly different, along with the evenness of the communities in the area.

The microbial communities in the Atlantic did not vary significantly by polymer type. Beta diversity measures between plastic groups showed no significant difference in the microbial community diversity on plastic polymer types. Indeed, PERMANOVA results displayed a p-value = 0.35.

4.3 Pacific samples

The Pacific Ocean dataset comprised 38 microplastic and 14 water samples. This section explored the Pacific Plastisphere, its diversity measures and the investigation of water samples.

4.3.1 Pacific Plastisphere

The plastic samples were composed of 4 polymer types, namely HDPE (2), LDPE (2), PP (10) and PE (24). In summary, Table 2 shows the number of taxa revealed at different taxonomic levels by 16S rRNA gene sequences. After processing, 99.38% of the reads belonged to the Kingdom of Bacteria. Proteobacteria, Cyanobacteria and Bacteroidota were the essential reads with almost 91% of the total. Twenty-seven phyla stemming from Bacteria, Archaea and Eukaryota accounted for 2.76% (each of the 27 recorded below 1% of the reads). The median relative abundance phyla were classified as Planctomycetota, Actinobacteriota and Verrucomicrobiota and were from Bacteria. They accounted for 6.23% of the total reads. The distribution of the abundances within the Pacific Plastisphere at different taxonomic ranks can be seen in the Appendix, Table (a).

The taxonomic affiliations displayed in Figure 4 for genera reads greater than 20,000 show the classes of Alphaproteobacteria, Cyanobacteriia, Bacteroidia and Gammaproteobacteria from the phyla Proteobacteria, Cyanobacteria and Bacteroidota as the most important of these relative abundances. Most of the highest reads assigned to Alphaproteobacteria belonged to Rhodobacterales, SAR11_clade and Caulobacterales at the order level; Rhodobacteraceae, Clade_I and Hyphomonadaceae at the family level. At the genus level, Rhodobacteraceae were most represented by unclassified and *Actibacterium*; Clade_Ia most represented Clade_I's reads and the reads of uncultured species most represented Hyphomonadaceae. Most of the highest reads from Gammaproteobacteria

belonged to the order of SAR86_clade; most unclassified reads represented the family and genus levels.

Besides that, the relative abundances of Cyanobacteriia were most represented by Cyanobacteriales, Synechococcales, and Phormidesmiales at the order level; Nostocaceae, Cyanobiaceae, Phormidesmiaceae at the family level and by the reads of *Rivularia*_PCC-7116, *Prochlorococcus*_MIT9313 and *Phormidesmis*_ANT.LACV5.1 at the genus level. Meanwhile, Bacteroidia's reads were most represented by Flavobacteriales at the order level, Flavobacteriaceae at the family level and by the reads of *Muricauda* at the genus level.

Among the negligible reads, Archaea (0.62%) showed more diversity in the Pacific than within the Atlantic and were represented by the phyla Thermoplasmatota (0.62%), Nanoarchaeota (0.00058%) and Halobacterota (0.00008%). At the class level, Archaea were represented by Thermoplasmata, Nanoarchaeia and Methanosarcinia. Meanwhile, Eukaryota (0.00018%) displayed less diversity than within the Atlantic. They were represented by one phylum, SAR and one class, Stramenopiles**.**

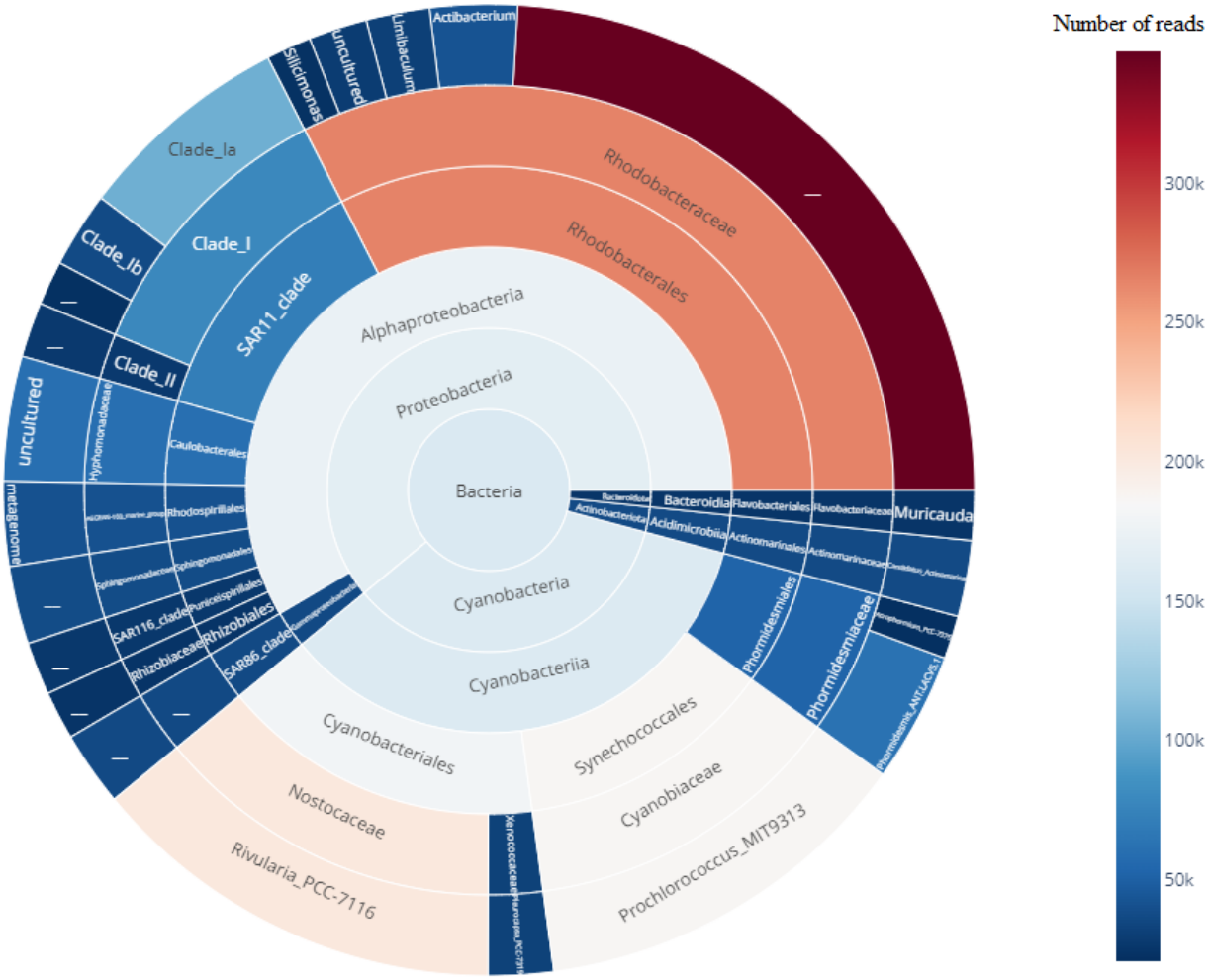

Figure 4. Distribution of recurring communities on the Pacific Plastisphere. Sunburst chart displaying the affiliations of genera communities that reached values above 20,000 reads at different taxonomic levels. Each plot crown represents one taxonomic level from the Kingdom to the genus.

Diversity measures

The alpha diversity measure showed no significant difference in the microbial communities of the different plastics within the Pacific. The assessment showed for Non-phylogenetic (evenness) measure, a p-value = 0.60 and phylogenetic (Faith's PD), a p-value = 0.57 through Kruskal-Wallis (all groups) test; both are greater than 0.05.

For the beta diversity measure, the PERMANOVA results showed a p-value = 0.84. So, the difference in microbial diversity between the different plastic groups showed no significant difference. The microbial colonization did not vary significantly based on the different polymer types in the Pacific.

4.3.2 Pacific water samples

Water samples analysis was performed to compare microbial communities on plastic and water. Many studies showed that water communities differ from communities on plastic. The study wanted to see if there was a significant difference between Pacific plastic communities and Pacific water communities on the one hand and, on the other hand, between Atlantic communities and Pacific water communities.

From the analysis, the number of taxa revealed by 16S rRNA gene sequences at different taxonomic ranks is summarized in Table 2. Bacteria were the most prominent reads with 99.62%. Its phyla Proteobacteria, Cyanobacteria and Bacteroidota accounted for more than 91% of the relative abundances. Actinobacteriota, Verrucomicrobiota, Planctomycetota and Patescibacteria constituted the median relative abundances with 7.11%. The rest (20), stemming from Bacteria, Archaea and Eukaryota, accounted for 1.83% of the reads. The distribution of abundances within the Pacific water at different taxonomic ranks can be seen in the Appendix, Table (a).

Archaea in water (0.37%) were represented by the phylum of Thermoplasmatota and the class of Thermoplasmata. Meanwhile, Eukaryota (0.0019%) were represented by the phylum of Amorphea and the class of Obazoa. Pacific plastic presented more diversity than Pacific water (see Table 2).

4.4 Merged set: Atlantic Plastisphere and Pacific water

This section presented diversity measures results on the dataset formed by the Atlantic Plastisphere and Pacific water samples. Both Alpha and Beta diversity measures were performed.

Alpha diversity measures

Alpha diversity measure within the merged datasets constituted by Atlantic and Pacific water samples showed no significant difference for the Non-phylogenetic (evenness) measure (p-value = 0.76 for plastic communities assessment regardless of the polymer types and p-value = 0.93 for plastic communities assessment taking into account the polymer types). Regarding the phylogenetic (Faith's PD) measure, Kruskal-Wallis (all groups) test showed different p-values as follows:

For plastic communities' assessment taking into account the plastic polymer types, a non-significant p-value = 0.08 was displayed. For plastic communities' evaluation, regardless of

the polymer types, a significant p-value = 0.02 was displayed. For this later one, the repartition of the phylogenetic distances within the given dataset led to two different groups of samples, as shown in Figure 5. Most of the communities are close within each location and far from one another between both locations.

Kruskal-Wallis (pairwise) test, taking into account the plastic polymer types, revealed a significant difference between HDPE and water communities (p-value = 0.03). In contrast, the difference between PP and water communities was not significant (p-value = 0.16). For the assessment, regardless of the polymer types, Kruskal-Wallis (pairwise) test pointed out a significant p-value = 0.02 between Atlantic plastic and Pacific water. Based on the results, regardless of the plastic polymer types, there is a significant difference in microbial colonization between Atlantic plastic and Pacific water.

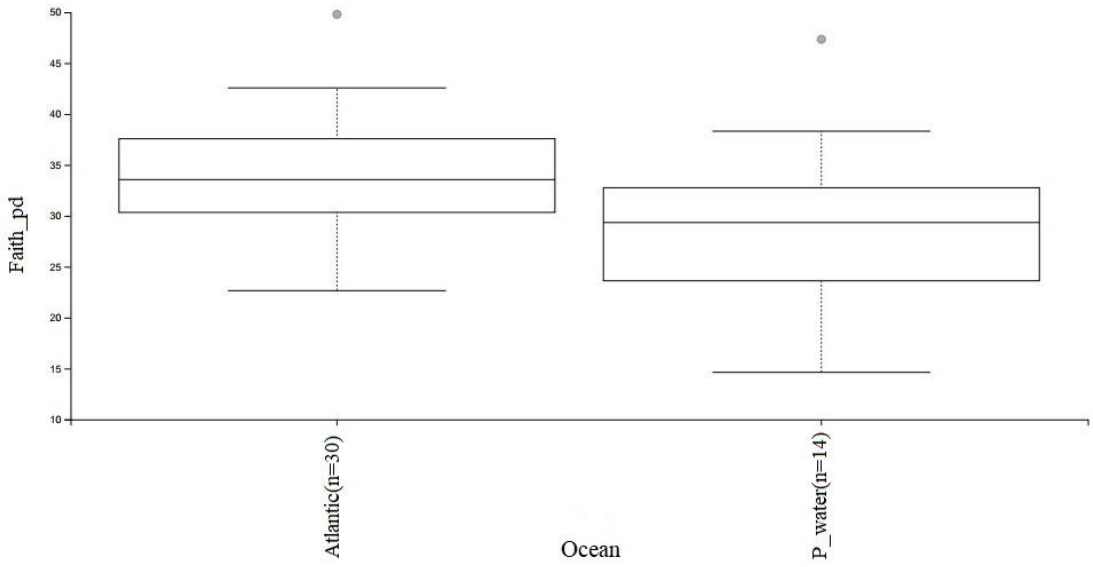

Figure 5. Influence of location (Ocean) on alpha diversity. Boxplots displaying Faith's PD (p-value = 0.02) of phylogenetic distances within the grouped dataset formed by Atlantic plastic samples (n=30) and Pacific water samples (n=14). Atlantic and Pacific water groups are distinct, as shown by the different positions of each boxplot on the Faith (PD) axis. The p-value related to the plot indicates the significance. "n=number of samples."

Beta diversity measures

Beta diversity measures also revealed a significant difference between the different groups shaped by Atlantic plastic and Pacific water (p-value = 0.001) through PERMANOVA. Pairwise PERMANOVA results indicated a p-value = 0.001 between Atlantic HDPE and Pacific water; a p-value = 0.004 between Atlantic PP and Pacific water. The difference was significant between the Atlantic plastic polymer types and Pacific water. These results reveal a substantial difference in the microbial diversity between Atlantic plastic and Pacific water.

4.5 Merged set: Pacific Plastisphere and Pacific water

This section went through the diversity analysis of the merged dataset constituted by the Pacific Plastisphere and the Pacific water samples. Both Alpha and Beta diversity measures were performed.

Alpha diversity measures

The alpha diversity measures within Pacific plastic and water samples showed no significant difference in the microbial community diversity. The p-value = 0.40 for phylogenetic (Faith's PD) and 0.94 for Non-phylogenetic (evenness) measures through Kruskal-Wallis (all groups) tests. These results show plastic and water microbial communities are not significantly different in the Pacific.

Beta diversity measures

Beta diversity showed no significant difference between the microbial community's Pacific plastic and water sample groups. PERMANOVA results exhibited a p-value = 0.59 for assessment regardless of the polymer type and p-value = 0.8 for evaluation considering the polymer types. There is no significant difference in microbial diversity based on polymer types.

4.6 Synthesis and all samples analysis

The analysis of all the samples within QIIME2 pointed out 11,852 ASVs shared in different taxonomic levels, as shown in Table 2. Proteobacteria, Cyanobacteria, and Bacteroidota were present in all the samples and were the most relative abundances phyla on every substrate. No significant difference in the microbial diversity was recorded based on the different plastic polymer types within the areas and between the Pacific Plastisphere and its surrounding water. The Atlantic Plastisphere community was significantly distinct from the Pacific water community.

The following diagram shows the shares of ASVs between Atlantic Plastisphere, Pacific Plastisphere, and Pacific water. 5% of the reads are shared among the three, and 8% are exclusively shared between Atlantic plastic and Pacific plastic. Atlantic plastisphere and Pacific water, with a significant difference in their community diversity, share exclusively 6% of the reads. Meanwhile, with no significant difference in their community diversity, Pacific Plastisphere and Pacific water share exclusively 18% of the reads.

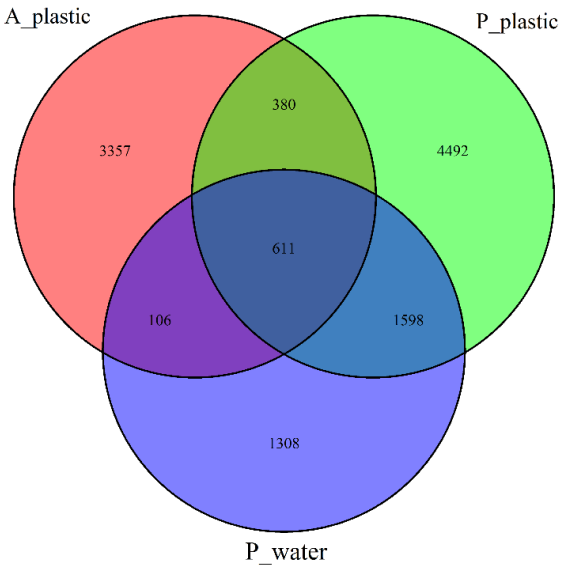

Figure 6. Unique and shared ASVs between Atlantic plastic, Pacific plastic, and Pacific water samples

Furthermore, a PCoA plot was generated from all samples within the QIIME2 pipeline. Three clusters, as shown in Figure 7**,** were formed. It shows that communities in the Atlantic are distinct from communities in the Pacific. Besides that, Atlantic communities are closer than in the Pacific, where two clusters separated far away from one another are recorded. That confirms the difference between the Atlantic and the Pacific regarding their communities and, in addition, informs that Pacific communities are distinct.

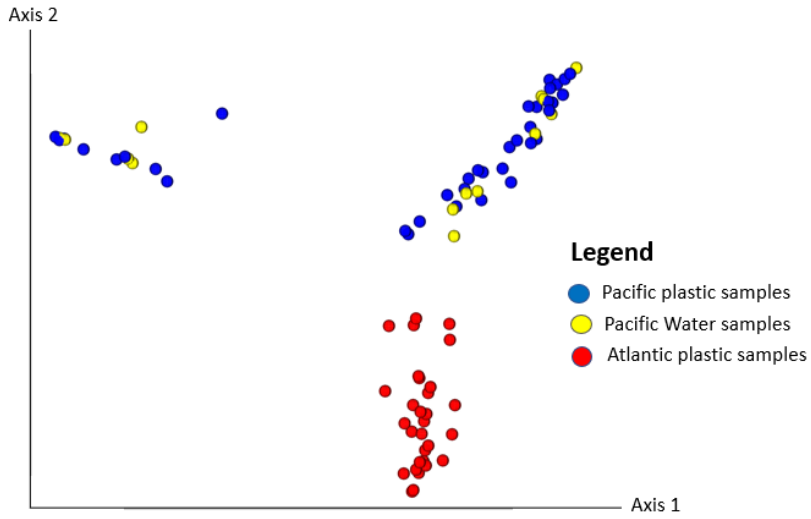

Figure 7. Principal coordinates analysis (PCoA) of all samples showing grouped communities. The phylogenetic distances calculated within the dataset show three clusters indicating how far or close the samples are from each other.

4.7 The Atlantic and Pacific Plastisphere

This part took into account only plastic samples from the two oceans. Pacific plastic samples were 38, and Atlantic plastic samples were 30 making a merged dataset of 68 plastic

samples. The microbial diversity on the samples was represented within the three kingdoms shared in 38 phyla and 0.22% unclassified, 94 classes and 0.35% unclassified, 206 orders and 2.83% unclassified, respectively 303 families and 5.46% unclassified, 527 genera and 34.98% unclassified. While the assigned reads to Bacteria (99.53%) and Archaea (0.45%) were found on all the plastic polymers, Eukaryota (0.025%) were found only on HDPE (0.024%) and PP (0.0001%).

At the ASV level, the total number of ASVs found was 10,544. Their distributions among the two oceans and polymer types under their different oceans are as follows in Figure 8. The Pacific Ocean showed more ASVs (61.39%) than the Atlantic Ocean (38.61%). 36.87% of ASVs in the Pacific were affiliated with PE, 17.02% to PP, 5.21% to LDPE and 3.72% with HDPE. In the Atlantic Ocean, 26.30% of the ASVs were affiliated with HDPE and 10.88% with PP.

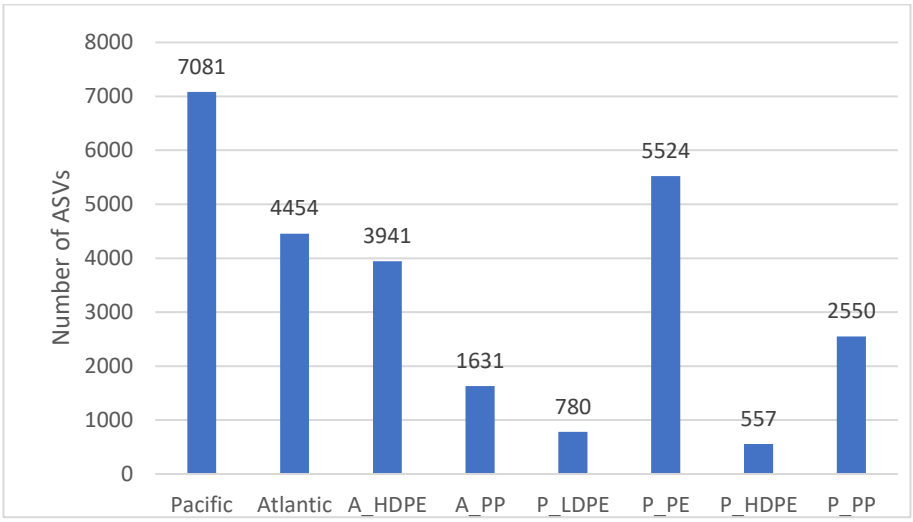

Figure 8. ASV distributions within the Plastisphere. P_PE=Pacific polyethylene, P_PP=Pacific polypropylene, P_HDPE=Pacific high-density polyethylene, P_LDPE=Pacific linear low-density polyethylene, A_HDPE=Atlantic high-density polyethylene, A_PP=Atlantic polypropylene

For a glimpse, the taxonomic class level was extracted, and Figure 9 shows the heatmap displaying the different abundances regarding both oceans and plastic polymer types. Both oceans shared 60% of the 94 classes; 19.35% were found only in the Atlantic and 20,65% in the Pacific. For instance, the following classes, Alphaproteobacteria (45.57%), Acidimicrobiia (1.83%), Verrucomicrobiae (1.36%), Phycisphaerae (1.08%), Bdellovibrionia (0.59%), Parcubacteria (0.35%) and Gracilibacteria (0.10%) were shared between the two oceans. Bdellovibrionia reads were more diverse in the Atlantic (1.61%) than in the Pacific (0.2%), and Acidimicrobiia reads were more diverse in the Pacific (2.28%) than in the Atlantic

(0.68%). Pla4_lineage (0.0014%) and Alveolata (0.00067%, from Eukaryota) were found in the Atlantic but not in the Pacific. The reads of Thermoplasmata (0.44%, from Archaea) were present in the Pacific but not in the Atlantic, whereas Obazoa's reads (0.023%, from Eukaryota) were detected only in the Atlantic.

Regarding the plastic polymer types, 24% of the 94 classes were detected on all the plastic types. It is the cases of Alphaproteobacteria, Cyanobacteriia, Acidimicrobiia, Bdellovibrionia. A_HDPE and P_PE, each one, recorded 22% of the 94 classes; P_PP recorded 17.62%; A_PP recorded 16.35%; P_LDPE recorded 13.53% and P_HDPE recorded 8.50%. For instance, the reads of Bacilli, Anaerolineae, Chlamydiae, Saccharimonadia and Vampirivibrionia were detected on all the plastics except P_HDPE. Thermoplasmata'reads (from Archaea) was not detected on A_HDPE, A_PP and P_LDPE, and Obazoa'reads (from Eukaryota) were detected only on A_HDPE.

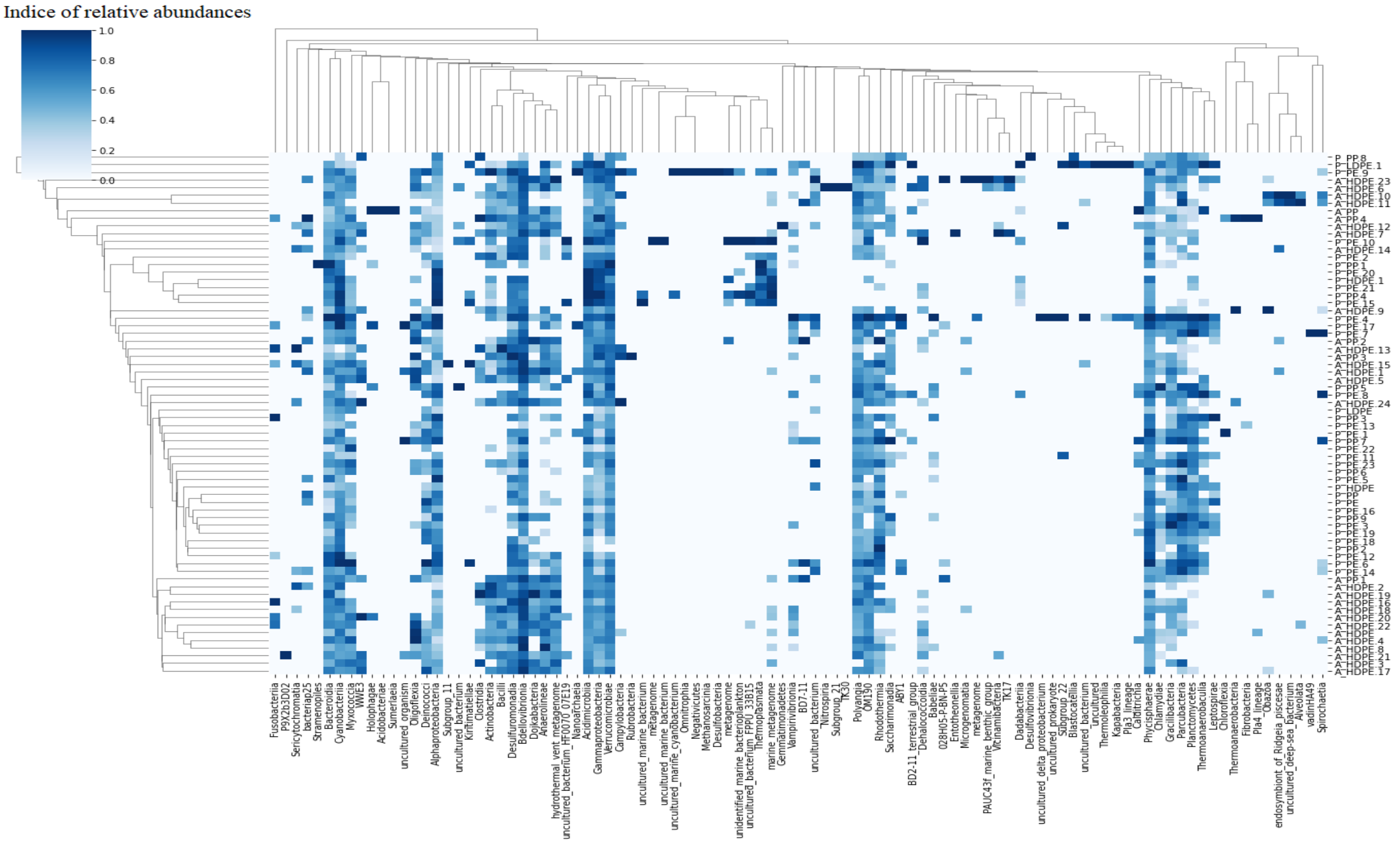

Figure 9. Class taxonomic level abundances in the different oceans and on plastic polymer types

The number of shared and unique ASVs between the Atlantic and Pacific is confined in Figure 10. 9% of ASVs are shared between the Atlantic and Pacific oceans. 33% are unique to the Atlantic and 58% to the Pacific. The number of shared ASVs is meagre compared to the number of ASVs carried by each area, which shows that the Atlantic and Pacific communities are very distinct.

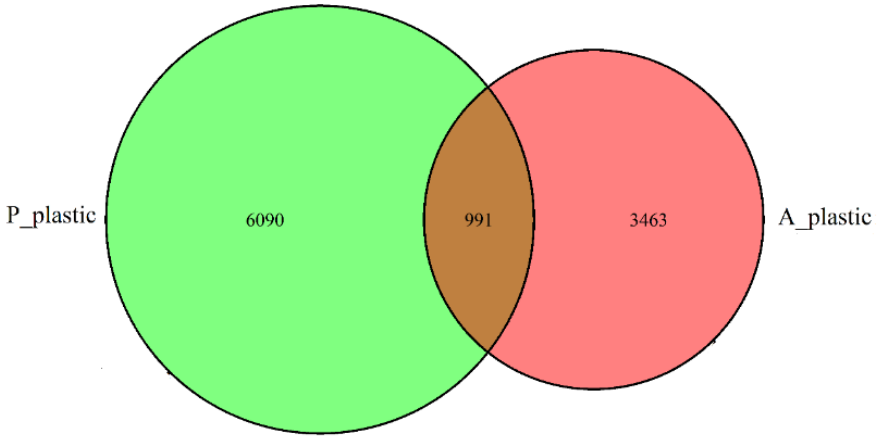

Figure 10. Unique and shared ASVs between Atlantic and Pacific Plastisphere

Alpha diversity measure

Non-phylogenetic (evenness) measure showed no significant difference within the Plastisphere (p-value = 0.38 for plastic communities' assessment regardless of the polymer types and p-value = 0.82 when considering the polymer types). Contrary, the phylogenetic (Faith's PD) measure, exhibited through Kruskal-Wallis (all groups) test, a significant p-value = 0.000063 (for plastic communities' assessment regardless of the polymer type), and also a significant p-value = 0.002 when considering the type of polymer. These results show a significant difference within the Plastisphere based or not on the plastic polymer types.

The Kruskal-Wallis (pairwise) test taking into account the polymer types, shows that the above significance difference within the Plastisphere (p-value=0.002) is mainly induced by five polymer pairs where the p-values are significant and vary from 0.001 to 0.04 as displayed in Table 3. Each couple of the five pairs was made of plastic polymers from both oceans. No pair between the different polymer types from the same ocean showed a significant difference, as seen in Table 3. These results confirm a significant difference between the two oceans in microbial community composition. Likewise, the Kruskal-Wallis (pairwise) test taking into account the plastics regardless of the polymer types, also shows that there is a significant difference between the community on Atlantic plastic (number of samples = 30)

and the community on Pacific plastic (number of samples = 38). That was shown by a p-value = 0.00006 between the two groups.

Table 3. Kruskal-Wallis (pairwise) between plastic polymer types combined with their parent ocean

		p-value
Group 1	Group 2	
A_HDPE (n=25)	A_PP (n=5)	**0.676411**
	P_HDPE (n=2)	**0.033222**
	P_LDPE (n=2)	**0.8531**
	P_PE (n=24)	**0.001935**
	P_PP (n=10)	**0.00244**
A_PP (n=5)	P_HDPE (n=2)	**0.052808**
	P_LDPE (n=2)	**0.698535**
	P_PE (n=24)	**0.043308**
	P_PP (n=10)	**0.014306**
P_HDPE (n=2)	P_LDPE (n=2)	**0.438578**
	P_PE (n=24)	**0.177932**
	P_PP (n=10)	**0.390154**
P_LDPE (n=2)	P_PE (n=24)	**0.77283**
	P_PP (n=10)	**0.829896**
P_PE (n=24)	P_PP (n=10)	**0.256839**

Beta diversity measure

PERMANOVA results showed a significant difference (p-value = 0.001) between the combined plastic polymer types group under their parent ocean. Pairwise PERMANOVA results revealed a difference between plastic polymer types from different oceans, as shown in Table 4**.** The p-values are significant for all the pairs and vary from 0.001 between 3 pairs to 0.046 between two pairs.

Table 4. Pairwise PERMANOVA results from plastic polymer types under their parent ocean

		Sample size	Permutations	**p-value**
Group 1	Group 2			
A_HDPE	A_PP	30	999	**0.357**
	P_HDPE	27	999	**0.008**
	P_LDPE	27	999	**0.003**
	P_PE	49	999	**0.001**
	P_PP	35	999	**0.001**
A_PP	P_HDPE	7	999	**0.046**
	P_LDPE	7	999	**0.046**
	P_PE	29	999	**0.003**
	P_PP	15	999	**0.001**

P_HDPE	P_LDPE	4	999	**1**
	P_PE	26	999	**0.823**
	P_PP	12	999	**0.974**
P_LDPE	P_PE	26	999	**0.214**
	P_PP	12	999	**0.39**
P_PE	P_PP	34	999	**0.785**

Regardless of the polymer type, the Plastisphere's diversity is significantly different between the Atlantic and the Pacific, with a p-value = 0.001 exhibited by PERMANOVA results. Indeed, based on the phylogeny, distances within the Atlantic group were compared to distances within the Pacific group. It shows that most of the communities within the Atlantic Plastisphere are close and differ from most of the communities within the Pacific Plastisphere (also close between them), as characterized in Figure 11.

Pairwise PERMANOVA results showed this difference with the previous p-value between the two groups.

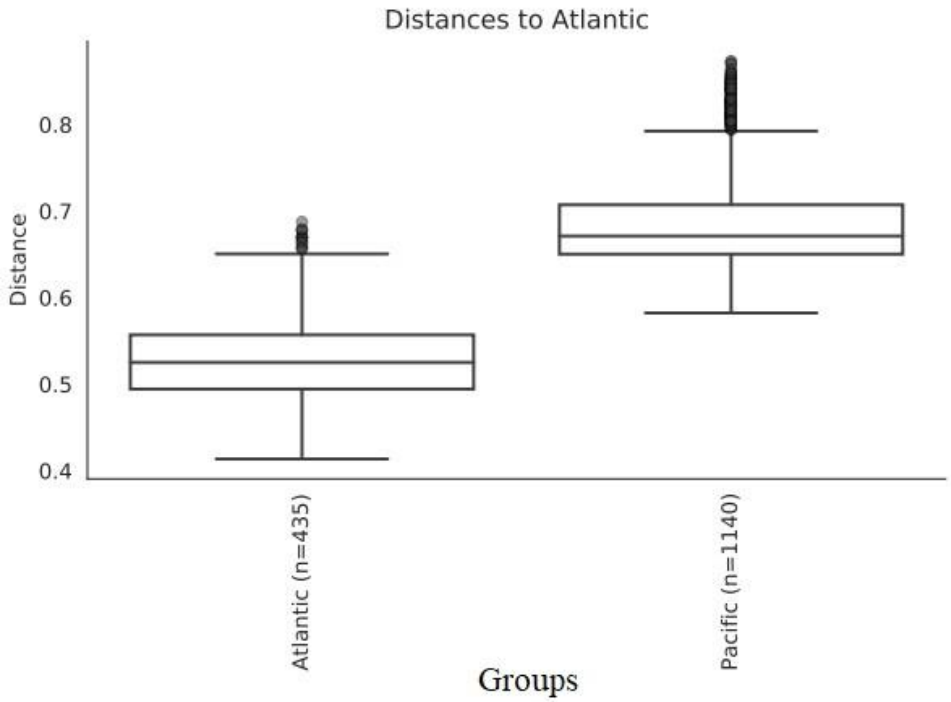

Figure 11. Influence of location (Ocean) on beta diversity. Boxplots displaying PERMANOVA results (p-value = 0.001) of phylogenetic distances between Atlantic and Pacific Plastisphere. Each boxplot's different distances (positions) show that the groups are distinct. The p-value related to the plot indicates the significance. “n = number of distances within the group”.

There is a significant difference in the diversity of microbial communities between the Atlantic and the Pacific Plastisphere, as shown by the previous p-values. Linear discriminant analysis (LDA) effect size (LEfSe) was used to predict the class level abundances between the Atlantic and the Pacific for their different masses. It revealed 32 differentially abundant

classes (LDA log score > ±2) between the Atlantic and the Pacific, as displayed in Figure 12. The Atlantic shows 23 less abundant classes, among which Alveolata and Obazoa are from Eukaryota. In comparison, the Pacific shows nine more abundant classes, among which Thermoplasmata, from Archaea. Among these 32 classes, 12 had an LDA score > ±3, including eight from the Atlantic (in ascending order Desulfuromonadia, TK17, Verrucomicrobiae, Anaerolineae, Bacilli, Bdellovibrionia, Gammaproteobacteria) and four from the Pacific (in ascending order Parcubacteria, Thermoplasmata, Planctomycetes, and Alphaproteobacteria). Alphaproteobacteria, Gammaproteobacteria, Bacteroidia had an LDA score > ±4. Thermoplasmata, ABY1 and Desulfovibrionia were unique to the Pacific, while Obazoa, endosymbiont_of_Ridgeia_piscesae, Vicinamibacteria, Alveolata and TK17 were unique to the Atlantic.

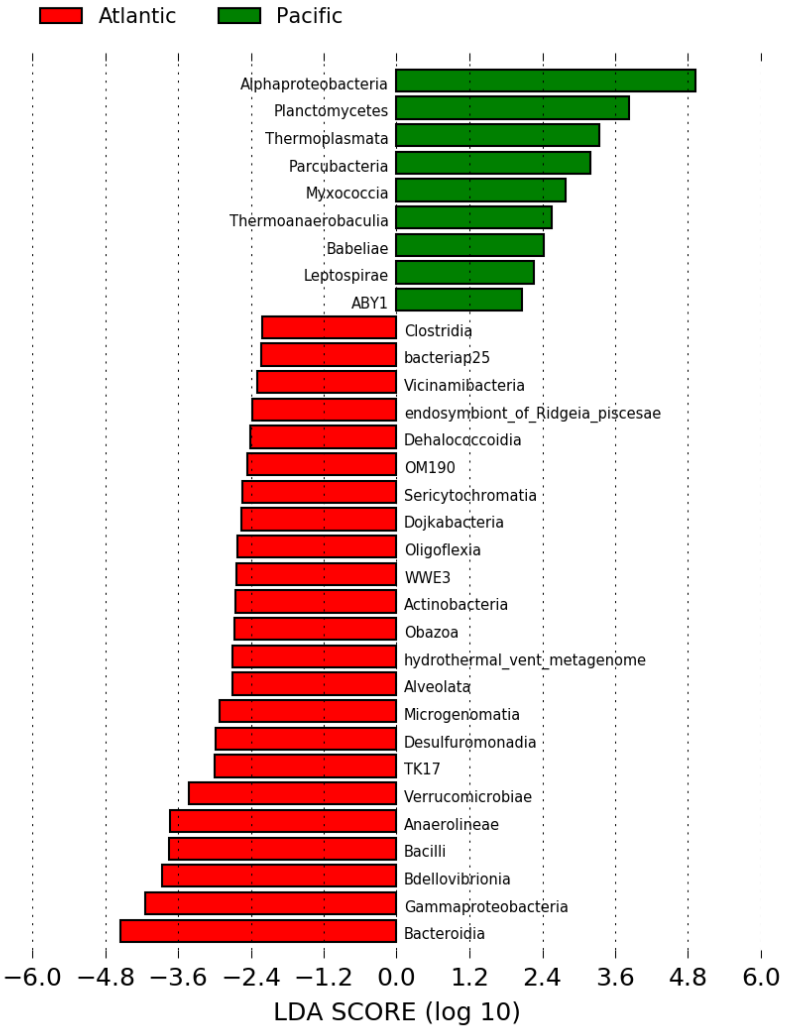

Figure 12. Differentially abundant classes between the Atlantic and the Pacific oceans. Linear discriminant analysis (LDA) effect size (LEfSe) results per ocean. Bar plots depict all classes which had an LDA log score > ±2 between all plastic samples (N = 68) in the Atlantic (n=30) or Pacific (n=38) oceans.

4.8 Potential plastic degraders

Among the microbial community on the present Plastisphere, 40 genera previously described to include hydrocarbon-degrading bacteria (HCB), as shown in Table 5, were deciphered. These genera represent 4.07% of the relative abundances of the whole Plastisphere. They were shared in 4 phyla, five classes, 21 orders and 32 families (see Appendix B, Table (a)). Proteobacteria was the most represented, with 22 genera. Actinobacteria came after that with eight genera, Bacteroidota with seven genera and Firmicutes with three genera. Twelve genera were exclusively detected in the Atlantic and three in the Pacific, while 25 were shared between the two oceans.

At least 90% of the 40 genera were present on all the plastic except on LDPE (below 25%). All plastics shared 15% of the total genera. 18%, 62%, 59%, 18%, 67% and 15% were respectively shared between PE - LDPE, PE - PP, PE - HDPE, PP - LDPE, PP – HDPE and PP - LDPE. Besides that, 2% were unique to PE, 15% to HDPE, 2% to PP and none to LDPE.

Table 5. Genera of potential plastic degraders within the studied Plastisphere

Genus&Reference	Atlantic	Pacific	PP	HDPE	LDPE	PE	Total
Lewinella (Vaksmaa et al., 2021)	0.73	0.42	0.19	0.68	0.006	0.29	**1.16**
Acinetobacter (Chaineau et al., 1999)	1.10	0.01	0.41	0.68	0	0.01	**1.11**
Erythrobacter (Harwati et al. 2007)	0.08	0.29	0.18	0.05	0	0.13	**0.37**
Algimonas (Vaksmaa et al., 2021)	0.12	0.14	0.06	0.11	0	0.09	**0.26**
Vibrio (Hedlund and Staley, 2001)	0.18	0.032	0.03	0.18	0.001	0.008	**0.21**
Winogradskyella (Wang et al. 2014)	0.03	0.16	0.07	0.03	0.0009	0.1	**0.19**
Tenacibaculum (Wang et al. 2014)	0.07	0.08	0.02	0.04	0	0.08	**0.14**
Alteromonas (Iwabuchi et al., 2002)	0.09	0.04	0.02	0.07	0.03	0.009	**0.12**
Brevundimonas (Chaineau et al., 1999)	0.1	0.002	0.05	0.05	0	0.002	**0.1**
Roseovarius (Peeb et al., 2022)	0.007	0.08	0.03	0.007	0.005	0.05	**0.09**
Pseudomonas (Le Petit et al., 1975)	0.06	0.001	0.04	0.03	0	0.001	**0.07**
Hyphomonas (Yakimov et al., 2005)	0.04	0.01	0.006	0.04	0	0.006	**0.05**
Flavobacterium (Stucki and Alexander, 1987)	0.05	0.001	0.01	0.04	0	0.001	**0.05**
Fabibacter (Wang et al. 2014)	0.02	0.007	0.004	0.02	0	0.006	**0.03**
Dokdonia (González et al., 2011)	0.02	0.006	0.006	0.01	0.0007	0.004	**0.02**

Stenotrophomonas (Juhasz et al., 2000)	0.02	0	0.01	0.009	0	0	**0.02**
Marinobacter (Gauthier et al., 1992)	0.0008	0.01	0.0004	0.0008	0	0.01	**0.01**
Halomonas (Wang et al., 2007)	0.006	0.007	0.002	0.005	0	0.006	**0.01**
Oleiphilus (Golyshin et al., 2002)	0.01	0	0	0.01	0	0	**0.01**
Methylobacterium-Methylorubrum (Bodour et al., 2003)	0.003	0.007	0.002	0.001	0	0.007	**0.01**
Staphylococcus (Saadoun et al., 1999)	0.005	0.004	0.001	0.004	0	0.003	**0.009**
Hyphomicrobium (Ozaki et al., 2006)	0	0.008	0.003	0	0.002	0.003	**0.008**
Corynebacterium (Chaineau et al., 1999)	0.004	0.003	0.001	0.003	0	0.003	**0.007**
Pseudoxanthomonas (Yue et al., 2021)	0.004	0	0.0007	0.004	0	0	**0.004**
Chryseobacterium (Szoboszlay et al., 2008)	0.003	0.0001	0.001	0.002	0	0.0001	**0.003**
Thalassospira (Kodama et al., 2008)	0	0.003	0.0001	0	0	0.003	**0.003**
Alkanindiges (Bogan et al., 2003)	0.001	0	0.001	0.0004	0	0	**0.001**
Alcanivorax (Yakimov et al., 1998)	0.0003	0.001	0	0.0003	0	0.001	**0.001**
Micrococcus (Ilori et al., 2000)	0.0002	0.001	0.0002	0	0	0.001	**0.001**
Kocuria (Dashti et al., 2009)	0.0009	0	0	0.0009	0	0	**0.0009**
Rhodococcus (Meyer et al., 1999)	0.0008	0	0.0008	0	0	0	**0.0008**
Methylophaga (Mishamandani et al. 2014)	0	0.0008	0	0	0	0.0008	**0.0008**
Oleispira (Yakimov et al., 2003)	0.0003	0.0005	0	0.0003	0	0.0005	**0.0008**
Mycobacterium (Willumsen et al., 2001)	0.0004	0.0004	0.0002	0.0004	0	0.0001	**0.0007**
Nocardioides (Hamamura and Arp, 2000)	0.0003	0	0.0002	0.0002	0	0	**0.0003**
Arthrobacter (Le Petit et al., 1975)	0.0003	0	0	0.0003	0	0	**0.0003**
Actinomyces (ZoBell, 1946)	0.0002	0	0.0001	0.0001	0	0	**0.0002**
Achromobacter (Le Petit et al., 1975)	0.0002	0	0	0.0002	0	0	**0.0002**
Lactobacillus (Floodgate, 1984)	0.0001	0	0	0.0001	0	0	**0.0001**
Bacillus (Li et al., 2008)	0.00006	0	0	0.00006	0	0	**0.00006**

5. Discussion

Influence of location on bacterial community composition

Our results showed a significant difference between the Atlantic Plastisphere and the Pacific water (PERMANOVA results; p-value = 0.001) and the Atlantic and Pacific Plastispheres (PERMANOVA results; p-value = 0.001) in microbial diversity. It confirms the results obtained by Amaral-Zettler et al. seven years ago on the same topic when assessing the diversity between Atlantic and Pacific communities. They found the same significance level (p-value = 0.001); distinct grouping based on the oceanic biogeographic zone (Atlantic versus Pacific). Biogeography is incontestably a driver of microbial diversity. Similar results were also obtained by Coons et al., 2021 who found that the biogeography influences Plastisphere community structure more than substrate type. Differences in the biofilm community composition are related to different factors. The temperature was recently thought to be the best predictor of bacterial diversity in surface waters (Ibarbalz et al., 2019). Future studies on the same topic should include more environmental parameters to determine the drivers of this difference between Atlantic and Pacific in microbial diversity.

Regarding this study, the plastic particles were collected at the surface of different waters. They could have attracted microbial communities able to evolve at the water surface, such as Flavobacteriaceae (Zheng et al., 2018) and Saprospiraceae (McIlroy & Nielsen, 2014), opportunistic colonisers (Rhodobacteriaceae; Dang & Lovell, 2016), and biofilm formers (Hyphomonodaceae; Abraham & Rohde, 2014). These bacteria comprised 3.77%, 3.48%, 23.13%, and 3.63% of the bacterial community herein. On the other hand, we also found when investigating the lifestyle of one of the Pacific big communities set (see Figure 7), namely Thermoplasmata (from Archaea), that their lifestyle was attached to high acidity (Gupta et al., 2021). That means pH can be a driver of microbial diversity. So, the pH (as it varies between the Atlantic and the Pacific), the dissolved oxygen, the salinity or the surface temperature (as it also varies between both oceans) could be responsible for this difference in microbial diversity between the Atlantic and Pacific oceans.

Most dominant classes that made the difference between the Atlantic and the Pacific (see Figure 12) belong to the phyla Proteobacteria, Bacteroidota, Planctomycetota, Bdellovibrionota, Bacilli, Verrucomicrobiota and Thermoplasmatota (from Archaea). SAR and Amorphea (from Eukaryota) were also part of the differentially abundant microorganisms.

Bacterial diversity of the Plastisphere

We found members of all three domains of life on the plastic particles (Eukarya, Bacteria, and Archaea). The data presented demonstrate that three bacterial phyla, Proteobacteria (classes Alphaproteobacteria and Gammaproteobacteria), Cyanobacteria (class Cyanobacteriia) and Bacteroidota (class Bacteroidia), respectively 45.57%, 29.98% and 11.43% dominated the bacterial communities across the three substrates. Many of the taxa found to be abundant on the plastics were also prevalent in the marine-incubated plastic-coated slides study by Coons et al., 2021 in the North Atlantic. The same results were also obtained from plastics that were retrieved from the North Atlantic Gyre (Zettler, Mincer & Amaral-Zettler, 2013; Coons, 2020), the Mediterranean Sea (Annika et al., 2021) and the Pacific (Tobias-Huenefeldt et al., 2021).

Meanwhile, a recent 16S rRNA gene amplicon data meta-analysis from 35 Plastisphere studies revealed the successive colonization of the Plastisphere (Wright et al., 2020). The authors discovered that, at early time points of succession, Alphaproteobacteria are significantly more abundant. And a significant increase in Bacteroidia usually coincides with the arrival of Gammaproteobacteria at a later stage. That led us to hypothesize that biofilms on our samples were not at an early stage of formation. So, our samples were drifting in the oceans for quite some time. We found Actinobacteria and Cyanobacteria; members of the phylum Actinobacteria, have been reported as abundant components of plastic debris communities (Salta et al., 2013; Pinto et al., 2019), as well as Cyanobacteria, which were highly represented on PP and PE items (Zettler et al., 2013). Many hydrocarbon-degrading genera were also found in our samples (see 4.8 Potential plastic degraders), suggesting a possible role of plastic-inhabiting microbes in the degradation of plastic polymers.

Microplastics are increasingly discussed as potential vectors for microorganisms, especially pathogens, multidrug-resistant strains and as vectors for chemical pollutants (Shen et al., 2019). The ordinary community includes bacteria that prefer a surface-attached lifestyle, such as Flavobacteriaceae (Zheng et al., 2018) and Saprospiraceae (McIlroy & Nielsen, 2014), 3.77% and 3.48% of the bacterial community composition in this study. Some *Vibrio* species have been described as pathogenic, and their dispersion is helped by debris abundance (Zettler et al., 2013; Kirstein et al., 2016; Debroas et al., 2017). Other *Vibrio* species have been seen as promising candidates for the remediation of plastics (Danso et al., 2019). *Vibrio* accounted for 0.21% of the total Plastisphere in this study, and we got three species, notably *Vibrio_azureus*, *Vibrio_caribbeanicus*_ATCC_BAA-2122 and *Vibrio_penaeicida*. Of the many *Vibrio* species, 12 are human pathogens (Kokashvili et al.,

2015). Oberbeckmann & Labrenz (2020) suggested that most *Vibrio* species represent opportunistic biofilm generalists that favour natural substrates, such as wood, over plastic particles.

Influence of plastic polymer types on bacterial diversity

In this study, no significant difference in microbial community composition was revealed between plastic polymer types in the Atlantic (HDPE and PP) and the Pacific (HDPE, PP, LDPE and PE) for both alpha diversity and beta diversity measures. Amaral-Zettler et al. (2015) used four different polymers, notably PE, PS, PP and PET. They only found significant differences between polystyrene and polyethylene or polystyrene and polypropylene with pairwise polymer tests (polystyrene was not part of our study). Likewise, they also found no significant difference between PP and PE as in this study. Coons et al. (2021) performed a study using HDPE, LDPE, PA, PMMA, and glass-slide controls after five weeks of incubation in the Pacific and Atlantic (coastal water column). She also found no significant difference between these polymer types within each area.

The differences in experimental design make it challenging to determine how far differences in microbial community structure depend on the polymer type. However, previous studies have suggested that microbial communities are more polymer-specific during the early stages of colonization (Pinto et al., 2019). Plastic-specific patterns have emerged in Plastisphere composition during short times of incubations: two minutes (Harrison et al., 2014) and two weeks (Ogonowski et al., 2018). They have also emerged after 21 months of incubation; mature biofilms were closely attached and selectively enriched under controlled conditions (Kirstein et al., 2019). The results demonstrated that polymer type has no significant effect in determining Plastisphere community composition in mature biofilm, especially compared to biogeography (Oberbeckmann & Labrenz, 2020). That could confirm the maturity of the biofilm formed on our samples and the immaturity of biofilms formed on samples of Amaral-Zettler et al. (2015).

Some studies showed that the substratum physicochemical properties (hydrophobicity, roughness, vulnerability to weather) and the surface chemodynamics (surface conditioning or nutrient enrichment) play a role in microbial diversity (Dang and Lovell, 2016). Besides physicochemical surface properties, it has been shown that the composition of biofilm communities associated with synthetic polymers differed significantly for different ocean basins (Amaral-Zettler et al., 2015) and underlay both seasonal and spatial effects, e.g., in North Sea waters (Oberbeckmann et al., 2014). That could explain why in this study, we

found that polymer types from different oceans significantly differed. For instance, our study showed a significant difference in beta diversity analysis between A_PP and P_PP (same polymer types; p-value = 0.001 with Pairwise PERMANOVA). In contrast, those in the same ocean did not. It might revive the discussions on the properties of waters from the seas and their impact on the substratum.

Regarding our analysis, P_PE and A_HDPE exhibited the most considerable ASVs diversity (see Figure 8). P_HDPE and P_LDPE showed the most negligible ASVs variety. However, with the same number of samples between P_HDPE and P_LDPE, P_LDPE exhibited more ASVs diversity than P_HDPE. On the other hand, P_PE with fewer samples than A_HDPE showed more variety. That could indicate that the different grade of PE, especially its rate of crystallinity, has a role in microbial diversity development. This hypothesis can be verified by the rate of ASVs diversity on LDPE and HDPE in the Pacific. LDPE shows more diversity than HDPE, although both exhibited the same number of samples. PE is the most hydrophobic polymer, which is also the least vulnerable to enzymatic attack (Min et al., 2020). HDPE shows a higher degree of crystallinity than LDPE, further impeding potential microbial colonization and enzymatic accessibility.

Also, this study observed no significant difference in community composition between the plastics and their surrounding water in the Pacific. Contrary to these results, Amaral-Zettler et al. (2015) found that bacterial communities, those free-living in the water column versus those associated with Plastic Marine Debris (PMD), were significantly different. Based on the successive microbial colonization (Pinto et al., 2019), it could confirm that the plastic particles used by Amaral-Zettler et al. (2015) were at their early time of bacterial colonization. Besides that, we found more diversity on plastic than in water samples within the merged set of Pacific plastic and Pacific water (see Table 2). Bacteria and archaea showed more reads on plastic (respectively 73.52% and 0.46%) than in water (25.92% and 0.09%), while Eukaryota showed more reads in water (0.0005%) than on plastic (0.0001%). From the 34 phyla, Dependentiae (0.005%), PAUC34f (0.002%), Nanoarchaeota (0.0004%, from Archaea), SAR (0.0001%, from Eukaryota), Latescibacterota (0.0001%), Fibrobacterota (0.0001%) and Halobacterota (0.00007%) were found only on Pacific plastic. Amorphea (0.0005%, from Eukaryota) was found only in water. That could probably explain that some microorganisms are specific to a certain type of substrate.

Potential plastic degraders

The plastic-degrading potential of the Plastisphere community is an ongoing topic (Zettler et al., 2013). Exploring our Plastisphere, we detected 40 genera previously reported as potential plastic degraders (see Table 5). They belong to the phyla Actinobacteriota, Bacteroidota, Firmicutes and Proteobacteria, as shown in Appendix, Table (b). Our samples were comprised of polycarbonates (PP and PE) known to have greater resistance to hydrolysis. The distribution of PE degrading microorganisms seems limited, although PP appears to be non-biodegradable. However, it was reported that lower molecular weight PE oligomers were partially degraded by *Acinetobacter* sp. 351 upon dispersion, while high molecular weight PE could not be impaired (Tsuchii et al., 1980). The genus *Acinetobacter* (1.11%) was found within the present Plastisphere.

Environmental degradation of PE proceeds by the synergistic action of photo- & thermo-oxidative degradation and biological activity (i.e., microorganisms). Moreover, the biodegradability of PE could be improved by blending it with biodegradable additives, photoinitiators or copolymerization (Griffin, 2007; Hakkarainen & Albertsson, 2004). A blending of PE with additives generally enhances auto-oxidation, reduces the molecular weight of the polymer, and then makes it easier for microorganisms to degrade the low molecular weight materials.

Meanwhile, the possibility of degrading PP with microorganisms has been investigated (Cacciari et al. 1993). In that study, it was shown that aerobic and anaerobic species with different catabolic capabilities could act in close cooperation to degrade polypropylene films. Some *Pseudomonas* (present in this Plastisphere) species were pointed out in the process of polypropylene degradation. Besides that, many species of *Pseudomonas* were indicated to degrade Polyethylene (Zheng et al., 2005), Polyvinyl chloride (Danko et al., 2004), while *Rhodococcus* was shown to degrade Polyethylene (Sivan et al., 2006).

In 2018, Danso et al. developed a Hidden Markov Model (HMM) to profile marine and terrestrial metagenomes for PET hydrolase candidate genes, finding key candidates to cluster in the bacterial phyla Actinobacteria, Bacteroidota, and Proteobacteria (classes Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria). Additionally, in a recent review of microbial plastic degradation, the same research group compiled a list of known enzymes and microorganisms involved in the degradation of high-molecular-weight artificial polymers (Danso et al., 2019).

In their activities, the synthesis of lipase and cutinase for polyesters degradation was associated with some *Pseudomonas* species. A large number of bacterial genera have been

affiliated with PE degradation. Among those were Gram-negatives affiliated with *Pseudomonas*, *Ralstonia*, *Stenotrophomonas* and many Gram-positives (e.g., *Rhodococcus*, *Staphylococcus*, *Streptomyces*, *Bacillus*, and others) (Kumar Sen & Raut, 2015; Restrepo-Florez et al., 2014). In this study, Gram-negatives and Gram-positives affiliated genera, potentially degrading PE were respectively *Pseudomonas* (0.07%), *Stenotrophomonas* (0.02%) and *Rhodococcus* (0.0008%), *Staphylococcus* (0.009%), *Bacillus* (0.00006%).

Oberbeckmann et al. (2016, 2020) recognized many families of the Bacteroidota phyla, such as Flavobacteriaceae, Cryomorphaceae, and Saprospiraceae, to be known for degrading complex carbon molecules. These families, respectively 3.77%, 0.08%, and 3.48%, were among the families within the present Plastisphere. The detected genera belonging to Cryomorphaceae do not match the ones described as potential plastic degraders. The other families were represented. Flavobacteriaceae was represented by *Dokdonia*, *Flavobacterium*, *Tenacibaculum*, *Winogradskyella* and *Chryseobacterium*, whereas *Lewinella* represented Saprospiraceae.

Microbial communities associated with plastic degradation composition and species richness are influenced by spatiotemporal phenomena like habitats/geographical location, ecosystem, and seasonal variation (Kirstein et al., 2019; Pinto et al., 2019). Further, the physiochemical nature of plastics like polyethylene, polypropylene, polystyrene, also regulates this degradation (Pinnell & Turner, 2019). The composition and specificity of microbial assemblage associated with polyethylene (PE) and polystyrene (PS) in the marine aquatic ecosystem (coastal Baltic Sea) are indicated by an abundance of Flavobacteriaceae (*Flavobacterium*), Rhodobacteraceae (*Rhodobactor*), Methylophilaceae (*Methylotenera*), Plactomycetaceae (Planctomyces, *Pirellula*), Hyphomonadaceae (*Hyphomonas*), Planctomycetaceae (*Blastopirellula*), Erythrobacteraceae (*Erythrobacter*), Sphingomonadaceae (*Sphingopyxis*), etc. (Oberbeckmann et al., 2018). Kirstein et al., 2019 found that the microbial community composition associated with various plastics is significantly varying, and it is also changing with the different phases of the plastic degradation process. In our study, the genera, *Flavobacterium* (0.05%), *Hyphomonas* (0.01%) and *Erythrobacter* (0.29%) were precisely found to be associated with PE (0.27%, all PE types of our study regrouped), but also PP (0.2%).

In a marine ecosystem, some bacterial genera, viz., *Dokdonia* (0.02%), *Erythrobacter* (0.37%), *Roseovarius* (0.09%) (found in this study), *Flexithrix*, *Hirschia*, *Parvularcula*, Phyllobactereacea, *Ulvibacter* have some specific association on the different types of plastics and play significant, but an undefined role in decomposition (Stern & Howard, 2000; Howard

et al., 2007; Akutsu et al., 1998). Many other researchers also reported this specific microbiome association with different plastic types. The members of the family Alcanivocareacea (*Alcanivorax*), Cryomophaceaea, and the genus *Erythrobacter* show higher abundance on the surface of Polyethylene (PE) and Polyethylene terephthalate (PET) (Stern & Howard, 2000; Peng et al., 2014). These associations with PE were also found in the present study. *Alcanivorax* (0.0013%) was found only on PE and HDPE, and Cryomophaceaea (0.07%) was found highly prominent on all PE (0.06%). Meanwhile, *Erythrobacter* (0.37%) was found on all PE (0.18%) and PP (0.18%) at the same rate.

Similarly, members of the family Oleiphilaceae (*Oleiphilus*) and Arenicellaceae are dominating on PE and PP (Peng et al., 2014; Oceguera-Cervantes et al., 2007), whereas the members of Hyphomonadaceae and Erythrobacteraceae form biofilm on PE and Polystyrene (PS) surfaces (Akutsu et al., 1998). The genera *Oleiphilus* (0.01%) was found within our potential plastic degraders along with the families Arenicellaceae (0.05%); Hyphomonadaceae (0.31%), represented by the genera *Algimonas* and *Hyphomonas*; Erythrobacteraceae (0.37%) represented by the genus *Erythrobacter*. *Oleiphilus* was found only on HDPE, and Arenicellaceae (0.05%) was highly found on all PE (0.04%), acknowledging part of the results above. Similarly, Hyphomonadaceae was found highly prominent on all PE (0.25%), and Erythrobacteraceae were found prominent on all PE (0.18%) and PP (0.18%).

More recently, some studies reported a significant weight loss of plastic by using some organisms over three months. Indeed, in their research, *Bacillus cereus*, *Bacillus sphaericus*, *Vibrio furnisii* and *Brevundimonas vesicularis*, herein found at genera level (*Bacillus* (0.00006%), *Vibrio* (0.21%), *Brevundimonas* (0.1%)), were identified as potential nylon degraders (Sudhakar, 2007). However, the genes and enzymes associated with nylon degradation remain to be identified. Besides that, a larger number of bacterial genera are known to be able to metabolize the monomer styrene as a sole source of carbon. Styrene degradation in bacteria is well studied in *Pseudomonas*, *Xanthobacter*, *Rhodococcus*, *Corynebacterium* and others (Tischler et al., 2009). Within these genera, only *Xanthobacter* was not found in this study.

According to Coons, 2020, Bacteria that displayed enzymatic activity on all lipolytic and polyester substrates were largely from the classes Actinobacteria (4), Bacilli (5), Gammaproteobacteria (20; families Heromonadaceae, Pseudoalteromonadaceae, Pseudomonadaceae, Schewanellaceae, Vibrionaceae), along with a single Alphaproteobacteria (family Rhodobacteriaceae). The latter is a bacterial family of high

interest in Plastisphere research (Amaral-Zettler et al., 2020). In our work, genera stemming from Actinobacteria (8), Bacilli (3), Gammaproteobacteria (13), Alphaproteobacteria (9) represented respectively 0.01%, 0.009%, 0.46% and 0.89% of the total Plastisphere. *Bacillus subtilis* sp. are appreciated in metabolic engineering due to their high protein secretion capacity (Zhang & Zhang, 2010). As such, *Bacillus subtilis* sp. was successfully chosen as an expression host by Huang et al. (2018) and efficiently secreted the highly active PETase of *Ideonella sakaiensis*, further optimized by Wang et al. (2020)

6. Conclusion

This present thesis aims to compare microbial communities from the Atlantic and Pacific oceans to see whether they show a significant difference between them and to find potential plastic degraders from communities of both Plastispheres. We used 16S rRNA gene amplicon sequence analysis to achieve those objectives on microplastic samples from the North Atlantic Garbage Patch and the Great Pacific Garbage Patch. These microplastic samples were collected in the summer of 2019 at the water surface of both oceans and were composed of PP, PE, LDPE and HDPE polymer types. Water samples collected simultaneously in the Pacific were also used to perform different comparisons. Secondly, we used a literature review to explore the Plastisphere to decipher potential plastic degraders.

The extensive experiments carried out for this work showed that the microbial community on plastic particles in the Atlantic Ocean is significantly distinct from those in the Pacific Ocean. This result especially confirmed those of Amaral-Zettler et al. (2015), who reached the same conclusion after comparing microbial communities in the same areas. Additionally, we found that the Atlantic and Pacific showed no significant difference in microbial diversity within each site based on plastic polymer types. Amaral-Zettler et al. (2015) got the same results with PP and PE (polymers used in this study) but additionally found a significant difference between PS and PE and between PS and PP (PS was not part of our study). In addition, the present study showed 32 microbial classes to be differentially abundant between the two oceans.

Our other result revealed no significant difference between the Plastisphere and surrounding water in the Pacific. In contrast to these results, Amaral-Zettler et al. (2015) found that bacterial communities those free-living in the water column versus those associated with PMD were significantly different. This contrasted results between the two studies could be due to the different maturities of the different Plastispheres. We also characterized each substrate community and found that Proteobacteria, Cyanobacteria and Bacteroidota were the most prominent relative abundances everywhere. These phyla accounted for more than 90% of the relative abundances of each substrate. Exploring the Plastisphere, our last result showed 40 genera previously documented as potential plastic degraders as being present on our plastic samples. They belonged to the phyla of Actinobacteriota, Bacteroidota, Firmicutes and Proteobacteria.

Uncovering the solution to the man-made plastic problem is an urgent issue, but the work does not end here. Biotechnological approaches are directing scientists toward developing a

more environmentally friendly alternative to mechanical or chemical degradation of plastic waste for a more sustainable, circular end life of plastics. Once a candidate has revealed plastic degrading potential, as in the well-documented case of *Ideonella sakaiensis* (Yoshida et al., 2016), the responsible gene must be uncovered and cloned into a host organism (e.g., *Bacillus subtilis*; Austin et al., 2018). Then the protein secretion must be optimized (Wang et al., 2020). Ultimately, the goal is to uncover organisms able to aid in the remediation of man-made polymers.

We are delighted with this study's outcomes. All our goals are well achieved. We have contributed to reinforcing the current knowledge regarding the microbial community diversity between the Atlantic and the Pacific oceans. Biogeography is undeniably a parameter in the diversity of the microbial community. We also came up with the type of potential plastic degraders that are probably found within the Atlantic and the Pacific oceans. For this work to be complete, we recommend that future studies try integrating environmental parameters like temperature, dissolved oxygen, salinity and pH of both oceans' respective properties in their analysis. It will help to discover the potential drivers of the difference in microbial communities between the Atlantic and Pacific oceans. And also, within the Pacific Ocean, where the PCoA plot showed two different clusters of communities.

7. References

Abraham, W. R., & Rohde, M. (2014). The Family Hyphomonadaceae. *The Prokaryotes*, 283–299. https://doi.org/10.1007/978-3-642-30197-1_260.

Ahmed, W., Zhang, Q., Lobos, A., Senkbeil, J., Sadowsky, M. J., Harwood, V. J., Saeidi, N., Marinoni, O., & Ishii, S. (2018). Precipitation influences pathogenic bacteria and antibiotic resistance gene abundance in storm drain outfalls in coastal sub-tropical waters. *Environment International*, *116*, 308–318. https://doi.org/10.1016/j.envint.2018.04.005.

Akutsu, Y., Nakajima-Kambe, T., Nomura, N., & Nakahara, T. (1998). Purification and Properties of a Polyester Polyurethane-Degrading Enzyme from *Comamonas acidovorans* TB-35. *Applied and Environmental Microbiology*, *64*(1), 62–67. https://doi.org/10.1128/aem.64.1.62-67.1998.

Amaral-Zettler, L. A., Zettler, E. R., & Mincer, T. J. (2020). Ecology of the plastisphere. *Nature Reviews Microbiology*, *18*(3), 139–151. https://doi.org/10.1038/s41579-019-0308-0.

Amaral-Zettler, L. A., Zettler, E. R., Slikas, B., Boyd, G. D., Melvin, D. W., Morrall, C. E., Proskurowski, G., & Mincer, T. J. (2015). The biogeography of the Plastisphere: implications for policy. *Frontiers in Ecology and the Environment*, *13*(10), 541–546. https://doi.org/10.1890/150017.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Austin, H. P., Allen, M. D., Donohoe, B. S., Rorrer, N. A., Kearns, F. L., Silveira, R. L., Pollard, B. C., Dominick, G., Duman, R., el Omari, K., Mykhaylyk, V., Wagner, A., Michener, W. E., Amore, A., Skaf, M. S., Crowley, M. F., Thorne, A. W., Johnson, C. W., Woodcock, H. L., . . . Beckham, G. T. (2018). Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proceedings of the National Academy of Sciences*, *115*(19). https://doi.org/10.1073/pnas.1718804115

Auta, H., Emenike, C., & Fauziah, S. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environment International*, *102*, 165–176. https://doi.org/10.1016/j.envint.2017.02.013.

Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Marine Environmental Research*, *111*, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014.

Bergmann, M., Gutow, L., & Klages, M. (Eds.). (2015). Marine Anthropogenic Litter. *Springer, Berlin*. https://doi.org/10.1007/978-3-319-16510-3.

Bhattacharya, P., Lin, S., Turner, J. P., & Ke, P. C. (2010). Physical Adsorption of Charged Plastic Nanoparticles Affects Algal Photosynthesis. *The Journal of Physical Chemistry C*, *114*(39), 16556–16561. https://doi.org/10.1021/jp1054759.

Bodour, A. A., Wang, J. M., Brusseau, M. L., & Maier, R. M. (2003). Temporal change in culturable phenanthrene degraders in response to long-term exposure to phenanthrene in a soil column system. *Environmental Microbiology*, *5*(10), 888–895. https://doi.org/10.1046/j.1462-2920.2003.00481.x.

Bogan, B. W., Sullivan, W. R., Kayser, K. J., Derr, K., Aldrich, H. C., & Paterek, J. R. (2003). Alkanindiges illinoisensis gen. nov., sp. nov., an obligately hydrocarbonoclastic, aerobic squalane-degrading bacterium isolated from oilfield soils. *International Journal of Systematic and Evolutionary Microbiology*, *53*(5), 1389–1395. https://doi.org/10.1099/ijs.0.02568-0.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., & Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, *6*(1). https://doi.org/10.1186/s40168-018-0470-z.

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., Mills, D. A., & Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods*, *10*(1), 57–59. https://doi.org/10.1038/nmeth.2276.

Browne, M. A. (2015). Sources and Pathways of Microplastics to Habitats. *Marine Anthropogenic Litter*, 229–244. https://doi.org/10.1007/978-3-319-16510-3_9.

Boyer, T., Conkright, M., & Levitus, S. (1999). Seasonal variability of dissolved oxygen, percent oxygen saturation, and apparent oxygen utilization in the Atlantic and Pacific Oceans. *Deep Sea Research Part I: Oceanographic Research Papers*, *46*(9), 1593–1613. https://doi.org/10.1016/s0967-0637(99)00021-7.

Bryant, J. A., Clemente, T. M., Viviani, D. A., Fong, A. A., Thomas, K. A., Kemp, P., Karl, D. M., White, A. E., & DeLong, E. F. (2016). Diversity and Activity of Communities Inhabiting Plastic Debris in the North Pacific Gyre. *mSystems*, *1*(3). https://doi.org/10.1128/msystems.00024-16.

Cacciari, I., Quatrini, P., Zirletta, G., Mincione, E., Vinciguerra, V., Lupattelli, P., & Giovannozzi Sermanni, G. (1993). Isotactic polypropylene biodegradation by a microbial community: physicochemical characterization of metabolites produced. *Applied and Environmental Microbiology*, *59*(11), 3695-3700.

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, *108*(supplement_1), 4516–4522. https://doi.org/10.1073/pnas.1000080107.

ChaîNeau, C., Morel, J., Dupont, J., Bury, E., & Oudot, J. (1999). Comparison of the fuel oil biodegradation potential of hydrocarbon-assimilating microorganisms isolated from a temperate agricultural soil. *Science of The Total Environment*, *227*(2–3), 237–247. https://doi.org/10.1016/s0048-9697(99)00033-9.

Choi, J. S., Jung, Y. J., Hong, N. H., Hong, S. H., & Park, J. W. (2018). Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow

(Cyprinodon variegatus). *Marine Pollution Bulletin*, *129*(1), 231–240. https://doi.org/10.1016/j.marpolbul.2018.02.039.

Climate change and the seas. (2015). European Environment Agency. https://www.eea.europa.eu/signals/signals-2015/articles/climate-change-and-the-seas.

Cocca, M., Di Pace, E., Errico, M. E., Gentile, G., Montarsolo, A., Mossotti, R. (2017). *Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea*. AGRIS: International Information System for the Agricultural Science and Technology. https://agris.fao.org/agris-search/search.do?recordID=XF2018001623.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., & Galloway, T. S. (2013). Microplastic Ingestion by Zooplankton. *Environmental Science & Technology*, *47*(12), 6646–6655. https://doi.org/10.1021/es400663f.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*, *62*(12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Cooksey, K., & Wigglesworth-Cooksey, B. (1995). Adhesion of bacteria and diatoms to surfaces in the sea: a review. *Aquatic Microbial Ecology*, *9*, 87–96. https://doi.org/10.3354/ame009087.

Coons, A. (2020). Variability of the global Plastisphere and identification of potential plastic degraders. Master's Thesis from Biological Oceanography, Christian-Albrechts-University, Kiel; pp. 105.

Coons, A. K., Busch, K., Lenz, M., Hentschel, U., & Borchert, E. (2021). Biogeography rather than substrate type determines bacterial colonization dynamics of marine plastics. *PeerJ*, *9*, e12135. https://doi.org/10.7717/peerj.12135.

Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F., & Dudas, S. E. (2019). Human Consumption of Microplastics. *Environmental Science & Technology*, *53*(12), 7068–7074. https://doi.org/10.1021/acs.est.9b01517.

Cózar, A., Echevarría, F., González-Gordillo, J. I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, L. T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M. L., & Duarte, C. M. (2014). Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences*, *111*(28), 10239–10244. https://doi.org/10.1073/pnas.1314705111.

Dang, H., & Lovell, C. R. (2016). Microbial Surface Colonization and Biofilm Development in Marine Environments. *Microbiology and Molecular Biology Reviews*, *80*(1), 91–138. https://doi.org/10.1128/mmbr.00037-15.

Danko, A. S., Luo, M., Bagwell, C. E., Brigmon, R. L., & Freedman, D. L. (2004). Involvement of linear plasmids in aerobic biodegradation of vinyl chloride. *Applied and environmental microbiology*, *70*(10), 6092–6097. https://doi.org/10.1128/AEM.70.10.6092-6097.2004.

Danso, D., Chow, J., & Streit, W. R. (2019). Plastics: Environmental and Biotechnological Perspectives on Microbial Degradation. *Applied and Environmental Microbiology*, *85*(19). https://doi.org/10.1128/aem.01095-19.

Danso, D., Schmeisser, C., Chow, J., Zimmermann, W., Wei, R., Leggewie, C., Li, X., Hazen, T., & Streit, W. R. (2018). New Insights into the Function and Global Distribution of Polyethylene Terephthalate (PET)-Degrading Bacteria and Enzymes in Marine and Terrestrial

Metagenomes. *Applied and Environmental Microbiology*, *84*(8). https://doi.org/10.1128/aem.02773-17.

Dashti, N., Khanafer, M., El-Nemr, I., Sorkhoh, N., Ali, N., & Radwan, S. (2009). The potential of oil-utilizing bacterial consortia associated with legume root nodules for cleaning oily soils. *Chemosphere*, *74*(10), 1354–1359. https://doi.org/10.1016/j.chemosphere.2008.11.028.

De Tender, C., Devriese, L. I., Haegeman, A., Maes, S., Vangeyte, J., Cattrijsse, A., Dawyndt, P., & Ruttink, T. (2017). Temporal Dynamics of Bacterial and Fungal Colonization on Plastic Debris in the North Sea. *Environmental Science & Technology*, *51*(13), 7350–7360. https://doi.org/10.1021/acs.est.7b00697.

Debroas, D., Mone, A., & ter Halle, A. (2017). Plastics in the North Atlantic garbage patch: A boat-microbe for hitchhikers and plastic degraders. *Science of The Total Environment*, *599–600*, 1222–1232. https://doi.org/10.1016/j.scitotenv.2017.05.059.

Desjardins, P., & Conklin, D. (2010). NanoDrop Microvolume Quantitation of Nucleic Acids. *Journal of Visualized Experiments*, *1*. https://doi.org/10.3791/2565.

Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Marine Pollution Bulletin*, *104*(1–2), 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006.

Dussud, C., Ghiglione, J. F. (2014). Bacterial degradation of synthetic plastics. Proceedings of the CIESM workshop monograph, monaco city. pp. 43-48.

Dussud, C., Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., Delort, A. M., Eyheraguibel, B., Meistertzheim, A. L., Jacquin, J., Cheng, J., Callac, N., Odobel, C., Rabouille, S., & Ghiglione, J. F. (2018). Colonization of Non-biodegradable and Biodegradable Plastics by Marine Microorganisms. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01571.

Dussud, C., Meistertzheim, A., Conan, P., Pujo-Pay, M., George, M., Fabre, P., Coudane, J., Higgs, P., Elineau, A., Pedrotti, M., Gorsky, G., & Ghiglione, J. (2018). Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. *Environmental Pollution*, *236*, 807–816. https://doi.org/10.1016/j.envpol.2017.12.027.

Eriksen, M., Lebreton, L. C. M., Carson, H. S., Thiel, M., Moore, C. J., Borerro, J. C., Galgani, F., Ryan, P. G., & Reisser, J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE*, *9*(12), e111913. https://doi.org/10.1371/journal.pone.0111913.

Ferreira, C., Domenech, S., & Lacaze, P. (2001). Synthesis and characterization of polypyrrole/TiO2 composites on mild steel. *Journal of Applied Electrochemistry*, *31*(1), 49–56. https://doi.org/10.1023/a:1004149421649.

Fletcher, M., & Loeb, G. I. (1979). Influence of Substratum Characteristics on the Attachment of a Marine Pseudomonad to Solid Surfaces. *Applied and Environmental Microbiology*, *37*(1), 67–72. https://doi.org/10.1128/aem.37.1.67-72.1979.

Floodgate, G. D. (1984) The fate of petroleum in marine ecosystems. In Petroleum Microbiology. *RM Atlas (ed.). New York: MacMillan*, pp. 355–397.

Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., Kerninon, S., Cassone, A. L., Lambert, C., Reveillaud, J., & Paul-Pont, I. (2018). Microplastic bacterial

communities in the Bay of Brest: Influence of polymer type and size. *Environmental Pollution*, *242*, 614–625. https://doi.org/10.1016/j.envpol.2018.07.023.

Frias, J., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. *Marine Pollution Bulletin*, *138*, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022.

Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P., & Bertrand, J. C. (1992). Marinobacter hydrocarbonoclasticus gen. nov., sp. nov., a New, Extremely Halotolerant, Hydrocarbon-Degrading Marine Bacterium. *International Journal of Systematic Bacteriology*, *42*(4), 568–576. https://doi.org/10.1099/00207713-42-4-568.

Golyshin, P. N., Chernikova, T. N., Abraham, W. R., Lünsdorf, H., Timmis, K. N., & Yakimov, M. M. (2002). Oleiphilaceae fam. nov., to include Oleiphilus messinensis gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. *International Journal of Systematic and Evolutionary Microbiology*, *52*(3), 901–911. https://doi.org/10.1099/00207713-52-3-901.

González, J. M., Pinhassi, J., Fernández-Gómez, B., Coll-Lladó, M., González-Velázquez, M., Puigbò, P., Jaenicke, S., Gómez-Consarnau, L., Fernàndez-Guerra, A., Goesmann, A., & Pedrós-Alió, C. (2011). Genomics of the Proteorhodopsin-Containing Marine Flavobacterium Dokdonia sp. Strain MED134. *Applied and Environmental Microbiology*, *77*(24), 8676–8686. https://doi.org/10.1128/aem.06152-11.

Greenly, C., Gray, H., Wong, H., Chinn, S., Passmore, J., Johnson, P., and Zaidi, Y. (2021). *Observing and Tracking the Great Pacific Garbage Patch*. DigitalCommons@USU. https://digitalcommons.usu.edu/smallsat/2021/all2021/222/.

Greenpeace, 34 Plastic Bans in Africa | A Reality Check. https://www.greenpeace.org/africa/en/blogs/11156/34-plastic-bans-in-africa/.

Greven, A. C., Merk, T., Karagöz, F., Mohr, K., Klapper, M., Jovanović, B., & Palić, D. (2016). Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, *35*(12), 3093–3100. https://doi.org/10.1002/etc.3501.

Griffin, G. J. L. (2007). Degradation of polyethylene in compost burial. In *Journal of Polymer Science: Polymer Symposia* (Vol. 57, No. 1, pp. 281-286). New York: Wiley Subscription Services, Inc., A Wiley Company.

Gupta, A., Saha, A., & Sar, P. (2021). Thermoplasmata and Nitrososphaeria as dominant archaeal members in acid mine drainage sediment of Malanjkhand Copper Project, India. *Archives of Microbiology*, *203*(4), 1833–1841. https://doi.org/10.1007/s00203-020-02130-4.

Guzzetti, E., Sureda, A., Tejada, S., & Faggio, C. (2018). Microplastic in marine organism: Environmental and toxicological effects. *Environmental Toxicology and Pharmacology*, *64*, 164–171. https://doi.org/10.1016/j.etap.2018.10.009.

Hall, N. M., Berry, K. L. E., Rintoul, L., & Hoogenboom, M. O. (2015). Microplastic ingestion by scleractinian corals. *Marine Biology*, *162*(3), 725–732. https://doi.org/10.1007/s00227-015-2619-7.

Hakkarainen, M., & Albertsson, A. C. (2004). Environmental degradation of polyethylene. *Long term properties of polyolefins*, 177-200.

Hamamura, N., & Arp, D. J. (2000). Isolation and characterization of alkane-utilizing*Nocardioides*sp. strain CF8. *FEMS Microbiology Letters*, *186*(1), 21–26. https://doi.org/10.1111/j.1574-6968.2000.tb09076.x.

Harrison, J. P., Sapp, M., Schratzberger, M., & Osborn, A. M. (2011). Interactions Between Microorganisms and Marine Microplastics: A Call for Research. *Marine Technology Society Journal*, *45*(2), 12–20. https://doi.org/10.4031/mtsj.45.2.2.

Harwati, T. U., Kasai, Y., Kodama, Y., Susilaningsih, D., & Watanabe, K. (2007). Characterization of Diverse Hydrocarbon-Degrading Bacteria Isolated from Indonesian Seawater. *Microbes and Environments*, *22*(4), 412–415. https://doi.org/10.1264/jsme2.22.412.

Hedlund, B. P., & Staley, J. T. (2001). Vibrio cyclotrophicus sp. nov., a polycyclic aromatic hydrocarbon (PAH)-degrading marine bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *51*(1), 61–66. https://doi.org/10.1099/00207713-51-1-61.

Horn, D. A., Granek, E. F., & Steele, C. L. (2019). Effects of environmentally relevant concentrations of microplastic fibers on Pacific mole crab (*Emerita analoga*) mortality and reproduction. *Limnology and Oceanography Letters*, *5*(1), 74–83. https://doi.org/10.1002/lol2.10137.

Howard, G., Mackie, R., Cann, I., Ohene-Adjei, S., Aboudehen, K., Duos, B., & Childers, G. (2007). Effect of insertional mutations in the pueA and pueB genes encoding two polyurethanases in Pseudomonas chlororaphis contained within a gene cluster. *Journal of Applied Microbiology*, *103*(6), 2074–2083. https://doi.org/10.1111/j.1365-2672.2007.03447.x.

Howard, G., Mackie, R., Cann, I., Ohene-Adjei, S., Aboudehen, K., Duos, B., & Childers, G. (2007). Effect of insertional mutations in the pueA and pueB genes encoding two polyurethanases in Pseudomonas chlororaphis contained within a gene cluster. *Journal of Applied Microbiology*, *103*(6), 2074–2083. https://doi.org/10.1111/j.1365-2672.2007.03447.x.

Huang, X., Cao, L., Qin, Z., Li, S., Kong, W., & Liu, Y. (2018). Tat-Independent Secretion of Polyethylene Terephthalate Hydrolase PETase in *Bacillus subtilis* 168 Mediated by Its Native Signal Peptide. *Journal of Agricultural and Food Chemistry*, *66*(50), 13217–13227. https://doi.org/10.1021/acs.jafc.8b05038.

Ibarbalz, F. M., Henry, N., Brandão, M. C., Martini, S., Busseni, G., Byrne, H., Coelho, L. P., Endo, H., Gasol, J. M., Gregory, A. C., Mahé, F., Rigonato, J., Royo-Llonch, M., Salazar, G., Sanz-Sáez, I., Scalco, E., Soviadan, D., Zayed, A. A., Zingone, A., . . . Wincker, P. (2019). Global Trends in Marine Plankton Diversity across Kingdoms of Life. *Cell*, *179*(5), 1084–1097.e21. https://doi.org/10.1016/j.cell.2019.10.008.

Ilori, M. O., Amund, D., & Robinson, G. K. (2000). Ultrastructure of two oil-degrading bacteria isolated from the tropical soil environment. *Folia Microbiologica*, *45*(3), 259–262. https://doi.org/10.1007/bf02908956.

International Pacific Research Center. (2008). *Tracking Ocean Debris*. Vol. 8, No. 2. http://iprc.soest.hawaii.edu/newsletters/newsletter_sections/iprc_climate_vol8_2/tracking_ocean_debris.pdf.

Iwabuchi, N., Sunairi, M., Urai, M., Itoh, C., Anzai, H., Nakajima, M., & Harayama, S. (2002). Extracellular Polysaccharides of *Rhodococcus rhodochrous* S-2 Stimulate the Degradation of Aromatic Components in Crude Oil by Indigenous Marine Bacteria. *Applied*

and Environmental Microbiology, *68*(5), 2337–2343. https://doi.org/10.1128/aem.68.5.2337-2343.2002.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768–771. https://doi.org/10.1126/science.1260352.

Juhasz, A., Stanley, G., & Britz, M. (2000). Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by Stenotrophomonas maltophilia strain VUN 10,003. *Letters in Applied Microbiology*, *30*(5), 396–401. https://doi.org/10.1046/j.1472-765x.2000.00733.x.

Kaiser, J. (2010). The Dirt on Ocean Garbage Patches. *Science*, *328*(5985), 1506. https://doi.org/10.1126/science.328.5985.1506.

Kale, S. K., Deshmukh, A. G., Dudhare, M. S., & Patil, V. B. (2015). Microbial degradation of plastic: a review Swapnil. *Journal of Biochemical Technology*, *6*(2), 952–961.
Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010.

Kesy, K., Oberbeckmann, S., Kreikemeyer, B., & Labrenz, M. (2019). Spatial Environmental Heterogeneity Determines Young Biofilm Assemblages on Microplastics in Baltic Sea Mesocosms. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01665.

Kirstein, I. V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., & Gerdts, G. (2016). Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. *Marine Environmental Research*, *120*, 1–8. https://doi.org/10.1016/j.marenvres.2016.07.004.

Kirstein, I. V., Wichels, A., Gullans, E., Krohne, G., & Gerdts, G. (2019). The Plastisphere – Uncovering tightly attached plastic "specific" microorganisms. *PLOS ONE*, *14*(4), e0215859. https://doi.org/10.1371/journal.pone.0215859.

Kodama, Y., Stiknowati, L. I., Ueki, A., Ueki, K., & Watanabe, K. (2008). Thalassospira tepidiphila sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from seawater. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *58*(3), 711–715. https://doi.org/10.1099/ijs.0.65476-0.

Kokashvili, T., Whitehouse, C. A., Tskhvediani, A., Grim, C. J., Elbakidze, T., Mitaishvili, N., Janelidze, N., Jaiani, E., Haley, B. J., Lashkhi, N., Huq, A., Colwell, R. R., & Tediashvili, M. (2015). Occurrence and Diversity of Clinically Important Vibrio Species in the Aquatic Environment of Georgia. *Frontiers in Public Health*, *3*. https://doi.org/10.3389/fpubh.2015.00232.

Krause, S., Molari, M., Gorb, E. V., Gorb, S. N., Kossel, E., & Haeckel, M. (2020). Persistence of plastic debris and its colonization by bacterial communities after two decades on the abyssal seafloor. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-66361-7.

Kumar Sen, S., & Raut, S. (2015). Microbial degradation of low density polyethylene (LDPE): A review. *Journal of Environmental Chemical Engineering*, *3*(1), 462–473. https://doi.org/10.1016/j.jece.2015.01.003.

Laist, D. W. (1997). Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species with Entanglement and Ingestion Records. *Springer Series on Environmental Management*, 99–139. https://doi.org/10.1007/978-1-4613-8486-1_10.

Law, K. L., Morét-Ferguson, S., Maximenko, N. A., Proskurowski, G., Peacock, E. E., Hafner, J., & Reddy, C. M. (2010). Plastic Accumulation in the North Atlantic Subtropical Gyre. *Science*, *329*(5996), 1185–1188. https://doi.org/10.1126/science.1192321.

Le Petit, J., Bertrand, J. C., N'Guyen, M. H., & Tagger, S. (1975). Etude taxonomique et physiologique de bactéries se développant sur hydrocarbures en milieu marin [On the taxonomy and physiology of bacteria utilizing hydrocarbons in the sea (author's transl)]. *Annales de microbiologie*, *126*(3), 367–380.

Lebreton, L. M., Greer, S., & Borrero, J. (2012). Numerical modelling of floating debris in the world's oceans. *Marine Pollution Bulletin*, *64*(3), 653–661. https://doi.org/10.1016/j.marpolbul.2011.10.027.

Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debeljak, P., Maral, H., Schoeneich-Argent, R., Brambini, R., & Reisser, J. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-22939-w.

Li, G., He, Z., An, T., Zeng, X., Sheng, G., & Fu, J. (2008). Comparative study of the elimination of toluene vapours in twin biotrickling filters using two microorganismsBacillus cereus S1 and S2. *Journal of Chemical Technology & Biotechnology*, *83*(7), 1019–1026. https://doi.org/10.1002/jctb.1908.

Li, X., Liu, G., & Zhao, J. (1999). Two competitive primary processes in the photodegradation of cationic triarylmethane dyes under visible irradiation in TiO2 dispersions. *New Journal of Chemistry*, *23*(12), 1193–1196. https://doi.org/10.1039/a906765e.

Lozupone, C. A., Hamady, M., Kelley, S. T., & Knight, R. (2007). Quantitative and Qualitative β Diversity Measures Lead to Different Insights into Factors That Structure Microbial Communities. *Applied and Environmental Microbiology*, *73*(5), 1576–1585. https://doi.org/10.1128/aem.01996-06.

Lusher, A. L., O'Donnell, C., Officer, R., & O'Connor, I. (2016). Microplastic interactions with North Atlantic mesopelagic fish. *ICES Journal of Marine Science*, *73*(4), 1214–1225. https://doi.org/10.1093/icesjms/fsv241.

Lusher, A., McHugh, M., & Thompson, R. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin*, *67*(1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

Ma, B., Xue, W., Ding, Y., Hu, C., Liu, H., & Qu, J. (2019). Removal characteristics of microplastics by Fe-based coagulants during drinking water treatment. *Journal of Environmental Sciences*, *78*, 267–275. https://doi.org/10.1016/j.jes.2018.10.006.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., & Kaminuma, T. (2001). Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. *Environmental Science & Technology*, *35*(2), 318–324. https://doi.org/10.1021/es0010498.

McIlroy, S. J., & Nielsen, P. H. (2014). The Family Saprospiraceae. *The Prokaryotes*, 863–889. https://doi.org/10.1007/978-3-642-38954-2_138.

Meyer, S., Moser, R., Neef, A., Stahl, U., & Kämpfer, P. (1999). Differential detection of key enzymes of polyaromatic-hydrocarbon-degrading bacteria using PCR and gene probes. *Microbiology*, *145*(7), 1731–1741. https://doi.org/10.1099/13500872-145-7-1731.

Min, K., Cuiffi, J. D., & Mathers, R. T. (2020). Ranking environmental degradation trends of plastic marine debris based on physical properties and molecular structure. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-14538-z.

Mishamandani, S., Gutierrez, T., & Aitken, M. D. (2014). DNA-based stable isotope probing coupled with cultivation methods implicates Methylophaga in hydrocarbon degradation. *Frontiers in Microbiology*, *5*. https://doi.org/10.3389/fmicb.2014.00076.

Muthukrishnan, T., al Khaburi, M., & Abed, R. M. M. (2019). Fouling Microbial Communities on Plastics Compared with Wood and Steel: Are They Substrate- or Location-Specific? *Microbial Ecology*, *78*(2), 361–374. https://doi.org/10.1007/s00248-018-1303-0.

Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, *59*(3), 695–700. https://doi.org/10.1128/aem.59.3.695-700.1993.

Naidoo, T., & Glassom, D. (2019). Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. *Marine Pollution Bulletin*, *145*, 254–259. https://doi.org/10.1016/j.marpolbul.2019.02.037.

Nakajima-Kambe, T., Onuma, F., Kimpara, N., & Nakahara, T. (1995). Isolation and characterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source. *FEMS microbiology letters*, *129*(1), 39–42. https://doi.org/10.1016/0378-1097(95)00131-N.

Nelms, S. E., Barnett, J., Brownlow, A., Davison, N. J., Deaville, R., Galloway, T. S., Lindeque, P. K., Santillo, D., & Godley, B. J. (2019). Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-018-37428-3.

NOAA (National Oceanic and Atmospheric Administration). (2017). What are microplastics? *National Ocean Service Website*. https://oceanservice.noaa.gov/facts/microplastics.html.

Oberbeckmann, S., & Labrenz, M. (2020). Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. *Annual Review of Marine Science*, *12*(1), 209–232. https://doi.org/10.1146/annurev-marine-010419-010633.

Oberbeckmann, S., Kreikemeyer, B., & Labrenz, M. (2018). Environmental Factors Support the Formation of Specific Bacterial Assemblages on Microplastics. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.02709.

Oberbeckmann, S., Loeder, M. G., Gerdts, G., & Osborn, A. M. (2014). Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern

European waters. *FEMS Microbiology Ecology*, *90*(2), 478–492. https://doi.org/10.1111/1574-6941.12409.

Oberbeckmann, S., Osborn, A. M., & Duhaime, M. B. (2016). Microbes on a Bottle: Substrate, Season and Geography Influence Community Composition of Microbes Colonizing Marine Plastic Debris. *PLOS ONE*, *11*(8), e0159289. https://doi.org/10.1371/journal.pone.0159289.

Oceguera-Cervantes, A., Carrillo-García, A., López, N., Bolaños-Nuñez, S., Cruz-Gómez, M. J., Wacher, C., & Loza-Tavera, H. (2007). Characterization of the polyurethanolytic activity of two Alicycliphilus sp. strains able to degrade polyurethane and N-methylpyrrolidone. *Applied and environmental microbiology*, *73*(19), 6214–6223. https://doi.org/10.1128/AEM.01230-07.

Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K. I., Bacsik, Z., & Gorokhova, E. (2018). Evidence for selective bacterial community structuring on microplastics. *Environmental Microbiology*, *20*(8), 2796–2808. https://doi.org/10.1111/1462-2920.14120.

Okada, M. (2002). Chemical syntheses of biodegradable polymers. *Progress in Polymer Science*, *27*(1), 87–133. https://doi.org/10.1016/s0079-6700(01)00039-9.

Ozaki, S., Kishimoto, N., & Fujita, T. (2006). Isolation and Phylogenetic Characterization of Microbial Consortia able to Degrade Aromatic Hydrocarbons at High Rates. *Microbes and Environments*, *21*(1), 44–52. https://doi.org/10.1264/jsme2.21.44.

Pabortsava, K., & Lampitt, R. S. (2020). High concentrations of plastic hidden beneath the surface of the Atlantic Ocean. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-17932-9.

Paço, A., Duarte, K., da Costa, J. P., Santos, P. S., Pereira, R., Pereira, M., Freitas, A. C., Duarte, A. C., & Rocha-Santos, T. A. (2017). Biodegradation of polyethylene microplastics by the marine fungus Zalerion maritimum. *Science of The Total Environment*, *586*, 10–15. https://doi.org/10.1016/j.scitotenv.2017.02.017.

Padervand, M., Lichtfouse, E., Robert, D., & Wang, C. (2020). Removal of microplastics from the environment. A review. *Environmental Chemistry Letters*, *18*(3), 807–828. https://doi.org/10.1007/s10311-020-00983-1.

Pathak, V. M., & Navneet. (2017). Review on the current status of polymer degradation: a microbial approach. *Bioresources and Bioprocessing*, *4*(1). https://doi.org/10.1186/s40643-017-0145-9.

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Müller, A., Nothman, J., Louppe, G., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Cournapeau, D., Brucher, M., & Perrot, M. (2012). Scikit-learn: Machine Learning in Python Pedregosa, Varoquaux, Gramfort et al. In *Journal of Machine Learning Research* (Vol. 12). http://scikit-learn.org.

Peeb, A., Dang, N. P., Truu, M., Nõlvak, H., Petrich, C., & Truu, J. (2022). Assessment of Hydrocarbon Degradation Potential in Microbial Communities in Arctic Sea Ice. *Microorganisms*, *10*(2), 328. https://doi.org/10.3390/microorganisms10020328.

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., & Gerdts, G. (2018). Arctic sea ice is an important temporal sink and means

of transport for microplastic. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-03825-5.

Peng, Y. H., Shih, Y. H., Lai, Y. C., Liu, Y. Z., Liu, Y. T., & Lin, N. C. (2014). Degradation of polyurethane by bacterium isolated from soil and assessment of polyurethanolytic activity of a Pseudomonas putida strain. *Environmental Science and Pollution Research*, *21*(16), 9529–9537. https://doi.org/10.1007/s11356-014-2647-8.

Pinnell, L. J., & Turner, J. W. (2019). Shotgun Metagenomics Reveals the Benthic Microbial Community Response to Plastic and Bioplastic in a Coastal Marine Environment. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01252.

Pinto, M., Langer, T. M., Hüffer, T., Hofmann, T., & Herndl, G. J. (2019). The composition of bacterial communities associated with plastic biofilms differs between different polymers and stages of biofilm succession. *PLOS ONE*, *14*(6), e0217165. https://doi.org/10.1371/journal.pone.0217165.

Plumlee, M. H., Larabee, J., & Reinhard, M. (2008). Perfluorochemicals in water reuse. *Chemosphere*, *72*(10), 1541–1547. https://doi.org/10.1016/j.chemosphere.2008.04.057

Porter, A., Lyons, B. P., Galloway, T. S., & Lewis, C. (2018). Role of Marine Snows in Microplastic Fate and Bioavailability. *Environmental Science & Technology*, *52*(12), 7111–7119. https://doi.org/10.1021/acs.est.8b01000.

Poulain, M., Mercier, M. J., Brach, L., Martignac, M., Routaboul, C., Perez, E., Desjean, M. C., & ter Halle, A. (2018). Small Microplastics As a Main Contributor to Plastic Mass Balance in the North Atlantic Subtropical Gyre. *Environmental Science & Technology*, *53*(3), 1157–1164. https://doi.org/10.1021/acs.est.8b05458.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Molecular Biology and Evolution*, *26*(7), 1641–1650. https://doi.org/10.1093/molbev/msp077.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE*, *5*(3), e9490. https://doi.org/10.1371/journal.pone.0009490.

Purohit, J., Chattopadhyay, A., & Teli, B. (2020). Metagenomic Exploration of Plastic Degrading Microbes for Biotechnological Application. *Current Genomics*, *21*(4), 253–270. https://doi.org/10.2174/1389202921999200525155711.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219.

Reid, J. L. (1961). On the temperature, salinity, and density differences between the Atlantic and Pacific oceans in the upper kilometre. *Deep Sea Research (1953)*, *7*(4), 265–275. https://doi.org/10.1016/0146-6313(61)90044-2.

Restrepo-Flórez, J. M., Bassi, A., & Thompson, M. R. (2014). Microbial degradation and deterioration of polyethylene – A review. *International Biodeterioration & Biodegradation*, *88*, 83–90. https://doi.org/10.1016/j.ibiod.2013.12.014.

Ritchie, H. and Roser, M. (2018) - "Plastic Pollution". *Published online at OurWorldInData.org.* Retrieved from: 'https://ourworldindata.org/plastic-pollution' [Online Resource].

Robin, R., Karthik, R., Purvaja, R., Ganguly, D., Anandavelu, I., Mugilarasan, M., & Ramesh, R. (2019). Holistic assessment of microplastics in various coastal environmental matrices, southwest coast of India. *Science of The Total Environment*, *703*, 134947. https://doi.org/10.1016/j.scitotenv.2019.134947.

Royer, S. J., Ferrón, S., Wilson, S. T., & Karl, D. M. (2018). Production of methane and ethylene from plastic in the environment. *PLOS ONE*, *13*(8), e0200574. https://doi.org/10.1371/journal.pone.0200574.

Saadoun, I., al-Akhras, M. A., & Abu-Ashour, J. (1999). Bacterial degradation of hydrocarbons as evidenced by respirometric analysis. *Microbios*, *100*(395), 19–25.

Salta, M., Wharton, J. A., Blache, Y., Stokes, K. R., & Briand, J. F. (2013). Marine biofilms on artificial surfaces: structure and dynamics. *Environmental Microbiology*, n/a. https://doi.org/10.1111/1462-2920.12186.

Saquib, M. (2003). TiO2-mediated photocatalytic degradation of a triphenylmethane dye (gentian violet), in aqueous suspensions. *Dyes and Pigments*, *56*(1), 37–49. https://doi.org/10.1016/s0143-7208(02)00101-8.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60.

Shen, M., Zhang, Y., Zhu, Y., Song, B., Zeng, G., Hu, D., Wen, X., & Ren, X. (2019). Recent advances in toxicological research of nanoplastics in the environment: A review. *Environmental Pollution*, *252*, 511–521. https://doi.org/10.1016/j.envpol.2019.05.102.

Sivan, A., Szanto, M., & Pavlov, V. (2006). Biofilm development of the polyethylene-degrading bacterium Rhodococcus ruber. *Applied microbiology and biotechnology*, *72*(2), 346–352. https://doi.org/10.1007/s00253-005-0259-4

Sjollema, S. B., Redondo-Hasselerharm, P., Leslie, H. A., Kraak, M. H., & Vethaak, A. D. (2016). Do plastic particles affect microalgal photosynthesis and growth? *Aquatic Toxicology*, *170*, 259–261. https://doi.org/10.1016/j.aquatox.2015.12.002.

Stern, R. V., & Howard, G. T. (2000). The polyester polyurethanase gene (*pueA*) from*Pseudomonas chlororaphis*encodes a lipase. *FEMS Microbiology Letters*, *185*(2), 163–168. https://doi.org/10.1111/j.1574-6968.2000.tb09056.x.

Stoett, P., & Vince, J. (2019). The Plastic–Climate Nexus. *Climate Change and Ocean Governance*, 345–361. https://doi.org/10.1017/9781108502238.021.

Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., & Shi, H. (2019). The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *Journal of Hazardous Materials*, *365*, 716–724. https://doi.org/10.1016/j.jhazmat.2018.11.024.

Sudhakar, M., Priyadarshini, C.H., Doble, M., Murthy, P.S., & Venkatesan, R. (2007). Marine bacteria mediated degradation of nylon 66 and 6. *International Biodeterioration & Biodegradation, 60*, 144-151.

Sundbæk, K. B., Koch, I. D. W., Villaro, C. G., Rasmussen, N. S., Holdt, S. L., & Hartmann, N. B. (2018). Sorption of fluorescent polystyrene microplastic particles to edible seaweed Fucus vesiculosus. *Journal of Applied Phycology*, *30*(5), 2923–2927. https://doi.org/10.1007/s10811-018-1472-8.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M. E. J., le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., & Huvet, A. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences*, *113*(9), 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Szoboszlay, S., Atzel, B., Kukolya, J., Toth, E. M., Marialigeti, K., Schumann, P., & Kriszt, B. (2008). Chryseobacterium hungaricum sp. nov., isolated from hydrocarbon-contaminated soil. *INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY*, *58*(12), 2748–2754. https://doi.org/10.1099/ijs.0.65847-0.

Talvitie, J., Mikola, A., Koistinen, A., & Setälä, O. (2017). Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. *Water Research*, *123*, 401–407. https://doi.org/10.1016/j.watres.2017.07.005.

ter Halle, A., Ladirat, L., Martignac, M., Mingotaud, A. F., Boyron, O., & Perez, E. (2017). To what extent are microplastics from the open ocean weathered? *Environmental Pollution*, *227*, 167–174. https://doi.org/10.1016/j.envpol.2017.04.051.

The Ocean Cleanup. (2020). *"The Great Pacific Garbage Patch."* https://theoceancleanup.com/great-pacific-garbage-patch/.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W., McGonigle, D., & Russell, A. E. (2004). Lost at sea: where is all the plastic?. *Science (New York, N.Y.)*, *304*(5672), 838. https://doi.org/10.1126/science.1094559.

Tischler, D., Eulberg, D., Lakner, S., Kaschabek, S. R., van Berkel, W. J., & Schlömann, M. (2009). Identification of a novel self-sufficient styrene monooxygenase from Rhodococcus opacus 1CP. *Journal of bacteriology*, *191*(15), 4996–5009. https://doi.org/10.1128/JB.00307-09.

Tobias-Hünefeldt, S. P., Wenley, J., Baltar, F., & Morales, S. E. (2021). Ecological drivers switch from bottom–up to top–down during model microbial community successions. *The ISME Journal*, *15*(4), 1085–1097. https://doi.org/10.1038/s41396-020-00833-6.

Tofa, T. S., Kunjali, K. L., Paul, S., & Dutta, J. (2019). Visible light photocatalytic degradation of microplastic residues with zinc oxide nanorods. *Environmental Chemistry Letters*, *17*(3), 1341–1346. https://doi.org/10.1007/s10311-019-00859-z.

Tsuchii, A. (1980). Microbial degradation of polyethylene oligomers. *Report Ferment. Res. Inst*, *55*, 35-40.

Vaksmaa, A., Knittel, K., Abdala Asbun, A., Goudriaan, M., Ellrott, A., Witte, H. J., Vollmer, I., Meirer, F., Lott, C., Weber, M., Engelmann, J. C., & Niemann, H. (2021). Microbial Communities on Plastic Polymers in the Mediterranean Sea. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.673553.

Voelker, C. (2018). *Re: Does the pH of the Atlantic, Pacific and Indian oceans differ?* Researchgate. Retrieved August 8, 2022, from https://www.researchgate.net/post/Does-the-

pH-of-the-Atlantic-Pacific-and-Indian-oceans-differ/5b0adad210569f02850f202f/citation/download

Wang, N., Guan, F., Lv, X., Han, D., Zhang, Y., Wu, N., Xia, X., & Tian, J. (2020). Enhancing secretion of polyethylene terephthalate hydrolase PETase in *Bacillus subtilis* WB600 mediated by the SP_{amy} signal peptide. *Letters in Applied Microbiology*, *71*(3), 235–241. https://doi.org/10.1111/lam.13312.

Wang, W., Zhang, R., Shan, D., & Shao, Z. (2014). Indigenous oil-degrading bacteria in crude oil-contaminated seawater of the Yellow sea, China. *Applied microbiology and biotechnology*, *98*(16), 7253–7269. https://doi.org/10.1007/s00253-014-5817-1.

Wang, Z. M., Wagner, J., Ghosal, S., Bedi, G., & Wall, S. (2017). SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and fish guts. *Science of The Total Environment*, *603–604*, 616–626. https://doi.org/10.1016/j.scitotenv.2017.06.047.

Weiskopf, S. R., Rubenstein, M. A., Crozier, L. G., Gaichas, S., Griffis, R., Halofsky, J. E., Hyde, K. J., Morelli, T. L., Morisette, J. T., Muñoz, R. C., Pershing, A. J., Peterson, D. L., Poudel, R., Staudinger, M. D., Sutton-Grier, A. E., Thompson, L., Vose, J., Weltzin, J. F., & Whyte, K. P. (2020). Climate change effects on biodiversity, ecosystems, ecosystem services, and natural resource management in the United States. *Science of The Total Environment*, *733*, 137782. https://doi.org/10.1016/j.scitotenv.2020.137782.

Willumsen, P., Karlson, U., Stackebrandt, E., & Kroppenstedt, R. M. (2001). Mycobacterium frederiksbergense sp. nov., a novel polycyclic aromatic hydrocarbon-degrading Mycobacterium species. *International journal of systematic and evolutionary microbiology*, *51*(Pt 5), 1715–1722. https://doi.org/10.1099/00207713-51-5-1715.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, *178*, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.

Yakimov, M. M., Denaro, R., Genovese, M., Cappello, S., D'Auria, G., Chernikova, T. N., Timmis, K. N., Golyshin, P. N., & Giluliano, L. (2005). Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. *Environmental Microbiology*, *7*(9), 1426–1441. https://doi.org/10.1111/j.1462-5822.2005.00829.x.

Yakimov, M. M., Giuliano, L., Gentile, G., Crisafi, E., Chernikova, T. N., Abraham, W. R., Lünsdorf, H., Timmis, K. N., & Golyshin, P. N. (2003). Oleispira antarctica gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. *International journal of systematic and evolutionary microbiology*, *53*(Pt 3), 779–785. https://doi.org/10.1099/ijs.0.02366-0.

Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R., Abraham, W. R., Lünsdorf, H., & Timmis, K. N. (1998). Alcanivorax borkumensis gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *International journal of systematic bacteriology*, *48 Pt 2*, 339–348. https://doi.org/10.1099/00207713-48-2-339.

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., & Glöckner, F. O. (2014). The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research*, *42*(D1), D643–D648. https://doi.org/10.1093/nar/gkt1209.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., & Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*, *351*(6278), 1196–1199. https://doi.org/10.1126/science.aad6359

Yue, W., Yin, C. F., Sun, L., Zhang, J., Xu, Y., & Zhou, N. Y. (2021). Biodegradation of bisphenol-A polycarbonate plastic by Pseudoxanthomonas sp. strain NyZ600. *Journal of Hazardous Materials*, *416*, 125775. https://doi.org/10.1016/j.jhazmat.2021.125775.

Zaiko, A., Schimanski, K., Pochon, X., Hopkins, G. A., Goldstien, S., Floerl, O., & Wood, S. A. (2016). Metabarcoding improves detection of eukaryotes from early biofouling communities: implications for pest monitoring and pathway management. *Biofouling*, *32*(6), 671–684. https://doi.org/10.1080/08927014.2016.1186165.

Zeng, E. (2018). Microplastic Contamination in Aquatic Environments – An Emerging Matter of Environmental Urgency. *Elsevier*, *424p*.

Zheng, Y., Yanful, E. K., & Bassi, A. S. (2005). A review of plastic waste biodegradation. *Critical reviews in biotechnology*, *25*(4), 243–250. https://doi.org/10.1080/07388550500346359

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. *Environmental Science & Technology*, *47*(13), 7137–7146. https://doi.org/10.1021/es401288x.

Zhang, Q., Ma, H., Mai, K., Zhang, W., Liufu, Z., & Xu, W. (2010). Interaction of dietary Bacillus subtilis and fructooligosaccharide on the growth performance, non-specific immunity of sea cucumber, Apostichopus japonicus. *Fish & Shellfish Immunology*, *29*(2), 204–211. https://doi.org/10.1016/j.fsi.2010.03.009.

Zobell C. E. (1946). ACTION OF MICROORGANISMS ON HYDROCARBONS. *Bacteriological reviews*, *10*(1-2), 1–49.

Appendix-TABLES OF ABUNDANCES

Table (a): Different taxonomic levels abundances in percentage deciphered within each Plastisphere and water. Taxa displayed from Phylum to Genus levels are those with abundances >1% at least in one of the three substrates.

Abundances	%Atlantic	%Pacific	%Water
Kingdom			
Bacteria	99.91	99.38	99.62
Archaea	0.088	0.618	0.37
Eukaryota	0.00017	0.00017	0.0019
Phylum			
Proteobacteria	45.58	55.66	59.73
Cyanobacteria	27.28	25.5	22.76
Bacteroidota	17.72	9.82	8.55
Actinobacteriota	0.84	2.31	2.09
Verrucomicrobiota	1.74	1.49	1.91
Bdellovibrionota	1.69	0.2	0.28
Firmicutes	1.25	0.02	0.03
Planctomycetota	0.62	2.43	1.91
Patescibacteria	0.37	0.57	1.18
Class			
Alphaproteobacteria	34.6	49.8	53.53
Cyanobacteriia	27.26	25.48	22.75
Bacteroidia	17.04	9.26	8.09
Gammaproteobacteria	10.96	5.84	6.18
Acidimicrobiia	0.67	2.27	2.03
Phycisphaerae	0.46	1.32	1.18
Verrucomicrobiae	1.7	1.23	1.35
Planctomycetes	0.007	1.01	0.64
Bdellovibrionia	1.6	0.19	0.25
Bacilli	1.19	0.02	0.03
Order			
Rhodobacterales	20.01	24.32	25.65
Cyanobacteriales	6.69	11.45	9.34
SAR11_clade	0.02	8.92	9.41
Synechococcales	1.03	8.72	8.54
Flavobacteriales	2.46	5.02	4.77
Phormidesmiales	18.48	4.72	3.58
Caulobacterales	7.39	3.6	2.9
Rhizobiales	3.3	3.08	4.9
SAR86_clade	0.08	3.07	2.93
Chitinophagales	11.51	2.66	2.27
Rhodospirillales	0.05	2.53	2.54
Sphingomonadales	1.55	2.21	1.83
Pseudomonadales	4.35	0.01	0.26
Puniceispirillales	0.13	1.64	2.47
Alteromonadales	2.29	0.54	0.15
Actinomarinales	0.005	1.62	1.66
Cytophagales	2.32	1.28	0.89
Phycisphaerales	0.44	1.08	1.16

Verrucomicrobiales	1.39	0.48	0.25
Bdellovibrionales	1.3	0.17	0.23
Opitutales	0.28	0.67	1.08
Rickettsiales	0.86	0.64	1.01
Unclassified	4.19	2.3	2.9
Family			
Rhodobacteraceae	20.01	24.32	25.65
Nostocaceae	1.66	9.15	5.06
Cyanobiaceae	0.09	8.62	8.46
Clade_I	0.02	7.22	7.86
Flavobacteriaceae	2.01	4.44	3.97
Phormidesmiaceae	17.32	4.18	3.31
Hyphomonadaceae	5.32	3.02	2.48
AEGEAN-169_marine_group	0.02	2.48	2.5
Sphingomonadaceae	1.55	2.21	1.83
Rhizobiaceae	1.57	2.02	3.76
Saprospiraceae	7.74	1.83	1.56
SAR116_clade	0.13	1.63	2.47
Alteromonadaceae	2.15	0.07	0.07
Actinomarinaceae	0.004	1.62	1.66
Xenococcaceae	4.96	1.44	3.08
uncultured	3.93	1.28	1.35
Clade_II	0.004	1.17	0.93
Parvularculaceae	1.71	0.56	0.36
Phycisphaeraceae	0.44	1.08	1.16
Cyclobacteriaceae	0.91	1.03	0.65
Moraxellaceae	4.07	0.01	0.25
Bdellovibrionaceae	1.3	0.17	0.23
Amoebophilaceae	1.14	0.16	0.06
Nodosilineaceae	1.13	0.39	0.25
Puniceicoccaceae	0.28	0.67	1.08
Unclassified	5.74	5.35	5.66
Genus			
*Rivularia*_PCC-7116	1.64	9.01	4.43
*Prochlorococcus*_MIT9313	0.07	8.32	8.13
uncultured	7.82	6.37	6.24
*Clade*_Ia	0.01	4.64	4.92
*Phormidesmis*_ANT.LACV5.1	13.53	2.78	1.8
*Acrophormium*_PCC-7375	2.63	0.9	0.94
Acinetobacter	3.95	0.01	0.24
Actibacterium	0.33	1.87	1.03
metagenome	0.01	1.81	1.8
Candidatus_Actinomarina	0.004	1.62	1.66
Candidatus_Amoebophilus	1.12	0.08	0.04
*Clade*_Ib	0.003	1.62	1.86
*Pleurocapsa*_PCC-7319	4.75	1.39	3.04
Lewinella	2.63	0.58	0.43
Limibaculum	2.2	1.36	0.56
Muricauda	0.29	1.06	1.21
*OM27*_clade	1.1	0.13	0.18
Unclassified	34.08	35.33	38.55

Table (b): Taxonomic affiliation of the 40 genera potentially able to degrade plastic

Phylum	Class	Order	Family	Genus&Reference
Actinobacteriota (1.9%)	Actinobacteria (0.07%)	Actinomycetales (0.0005%)	Actinomycetaceae (0.0005%)	*Actinomyces* (ZoBell, 1946)
		Corynebacteriales (0.009%)	Corynebacteriaceae (0.007%)	*Corynebacterium* (Chaineau et al., 1999)
			Nocardiaceae (0.0008%)	Rhodococcus (Meyer et al., 1999)
			Mycobacteriaceae (0.0007%)	*Mycobacterium* (Willumsen et al., 2001)
		Micrococcales (0.01%)	Micrococcaceae (0.005%)	*Arthrobacter* (Le Petit et al., 1975)
				Kocuria (Dashti et al., 2009)
				Micrococcus (Ilori et al., 2000)
		Propionibacteriales (0.04%)	Nocardioidaceae (0.0003%)	*Nocardioides* (Hamamura and Arp, 2000)
Bacteroidota (12.02%)	Bacteroidia (11.43%)	Chitinophagales (5.13%)	Saprospiraceae (3.48%)	*Lewinella* (Vaksmaa et al., 2021)
		Cytophagales (1.57%)	Cyclobacteriaceae (1%)	*Fabibacter* (Wang et al. 2014)
		Flavobacteriales (4.31%)	Flavobacteriaceae (3.77%)	*Dokdonia* (González et al., 2011)
				Flavobacterium (Stucki and Alexander, 1987)
				Tenacibaculum (Wang et al. 2014)
				Winogradskyella (Wang et al. 2014)
			Weeksellaceae (0.01%)	*Chryseobacterium* (Szoboszlay et al., 2008)
Firmicutes (0.37%)	Bacilli (0.35%)	Bacillales (0.02%)	Bacillaceae (0.01%)	*Bacillus* (Li et al., 2008)
		Lactobacillales (0.02%)	Lactobacillaceae (0.0001%)	*Lactobacillus* (Floodgate, 1984)
		Staphylococcales (0.009%)	Staphylococcaceae (0.009%)	*Staphylococcus* (Saadoun et al., 1999)
Proteobacteria (52.86%)	Alphaproteobacteria (45.57%)	Caulobacterales (4.66%)	Caulobacteraceae (0.1%)	*Brevundimonas* (Chaineau et al., 1999)
			Hyphomonadaceae (3.67%)	*Algimonas* (Vaksmaa et al., 2021)
				Hyphomonas (Yakimov et al., 2005)
		Rhizobiales (3.15%)	Beijerinckiaceae (0.01%)	*Methylobacterium-Methylorubrum* (Bodour et al., 2003)
			Hyphomicrobiaceae (0.56%)	*Hyphomicrobium* (Ozaki et al., 2006)
		Rhodobacterales (23.13%)	Rhodobacteraceae (23.13%)	*Roseovarius* (Peeb et al., 2022)
		Rhodospirillales (1.84%)	Thalassospiraceae (0.003%)	*Thalassospira* (Kodama et al., 2008)
		Sphingomonadales (2.03%)	Erythrobacteraceae (2.02%)	*Erythrobacter* (Harwati et al. 2007)
	Gammaproteobacteria (7.27%)	Alteromonadales (1.03%)	Alteromonadaceae (0.65%)	*Alteromonas* (Iwabuchi et al., 2002)

			Marinobacteraceae (0.01%)	*Marinobacter* (Gauthier et al., 1992)
		Burkholderiales (0.12%)	Alcaligenaceae (0.0004%)	*Achromobacter* (Le Petit et al., 1975)
		Nitrosococcales (0.007%)	Methylophagaceae (0.002%)	*Methylophaga* (Mishamandani et al. 2014)
		Oceanospirillales (0.25%)	Alcanivoracaceae1 (0.001%)	*Alcanivorax* (Yakimov et al., 1998)
			Halomonadaceae (0.03%)	*Halomonas* (Wang et al., 2007)
			Oleiphilaceae (0.01%)	*Oleiphilus* (Golyshin et al., 2002)
			Saccharospirillaceae (0.0008%)	*Oleispira* (Yakimov et al., 2003)
		Pseudomonadales (1.23%)	Moraxellaceae (1.15%)	*Alkanindiges* (Bogan et al., 2003)
				Acinetobacter (Chaineau et al., 1999)
			Pseudomonadaceae (0.08%)	*Pseudomonas* (Le Petit et al., 1975)
		Vibrionales (0.26%)	Vibrionaceae (0.26%)	*Vibrio* (Hedlund and Staley, 2001)
		Xanthomonadales (0.025%)	Xanthomonadaceae (0.03%)	*Pseudoxanthomonas* (Yue et al., 2021)
				Stenotrophomonas (Juhasz et al., 2000)