Coursework Declaration and Feedback Form

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Course Code : ENG4110P	Course Name : Individual Project 4
1st Supervisor: Dr Umer Ijaz	2nd Supervisor: Dr Ciara Keating
Title of Assignment : Identifying Key Bacteria in Microbiomes of Microplastics Found in Marine Environments for Possible Degradation.	

Declaration of Originality and Submission Information

I affirm that this submission is all my own work in accordance with the University of Glasgow Regulations and the School of Engineering requirements Signed (Student) :	
Date of Submission : 16/04/2023	

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Identifying Key Bacteria in Microbiomes of Microplastics Found in Marine Environments for Possible Degradation.

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Abstract

The abundance of microplastic pollution in aquatic environments are a growing issue in todays world as they pose threats to marine life. To remove some of these microplastics that cannot be collected with usual methods of gathering pollution (nets, barges, etc.) other procedures must take place. Plastic degradation suing bacteria is a potential method of removing these plastics. By identifying key bacterial members in microbiomes of microplastics that have polymer degrading qualities we can investigate ways to accelerate this and reduce the amount of microplastics in the earths water systems.

Using multiple methods of statistical analysis such as alpha diversity, beta diversity, Taxonomy abundance plots, Core microbiome analysis and CODA LASSO comparisons on meta data derived form a number of studies looking at microplastic pollution in water if some from or another we can identify promising microorganisms to promote plastic degradation.

The taxonomy found in fibres and pellets were diverse and plentiful, this indicates a healthy microbiome allowing for microbes to thrive on the materials. Flavobacterium, Erythrobacter, Lentibacter were some bacteria that indicate a possibility for plastic degradation. This information can be used for further development of degradation of plastic, to eventually lead to a significant reduction of plastics in the marine environment.

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1. Introduction

Microplastic pollution is a global problem affecting the environment which is gaining more and more interest, worry, and investigation each year. The building public concern has garnered this rise in investigation of microplastics in the earth's waterways, seas, ocean beds, rivers, etc. It is estimated that there is approximately 269,000 tons of plastic particles in oceans as of 2020 with 80% of that coming from land and it is unsure on how much this number increases each year (Andrady, 2011). Plastic pollution has an adverse effect on the marine environment that they pollute.

Microplastics are defined as plastic items less than 5 mm in size. They vary from visible plastic fragments to plastic fibres not visible to the naked eye, these plastic particulates originate from a variety of sources such as fishing debris, washing textiles, manufacturing of plastic products and farming. The initial reports of plastics in oceans began in the early 1970's (Carpenter et al., 1972) found concentrations of microplastics in the Sargasso Sea (region of Atlantic Ocean) possibly a result of the carcinogenic compound Polychlorinated Biphenyls, an industrial product now banned, which was present in ocean organisms at the time. Due to its general resistance, ease in manufacturing, good thermal and shock insulation, cost and many other benefits, the plastic industry continued to boom. from the initial study discovering plastic pollution which resulted in an unsettling amount of Microplastic litter in marine environments.

Any amount of plastic can have a serious impact on the environment, just how large of an impact needs further research. It was discovered by many sources that plastics not only have been found to choke and suffocate marine animals but marine life such as phytoplankton and much larger animals like whales scan ingest microplastics. Turtles and birds have been found to eat plastic litter (Mallory, 2008), (Mascarenhas, Santos and Zeppelini, 2004) . The ingestion of larger plastic debris can cause obvious physical harm such as cuts down the throat or suffocation from swallowing but microplastics don't carry these issues, they carry much different ones. On a microscopic level. Evidence of microplastics in the human food chain has now been discovered.

Although marine life has no capacity to digest and breakdown these plastics in their internal systems, the problem lies in the bacteria that inhabit the microplastics. Bacteria resides on outer films of microbes commonly known as a Plastisphere or microbiome, which when consumed it can pass onto organisms and has potential for harmful effects. Bacteria found in the microbiomes can range from dormant microbes to pathogenic bacterium, even some bacteria have been found to degrade the plastics the reside on. An in-depth analysis on these bacteria is warranted, to raise awareness of the serious issues that microplastic pollution has on marine life, but also some possible solutions to clean the Earths waters and make it safer for marine life as well as humans.

1.1 Plastics studied

This study will sample data from multiple papers researching microplastics in marine environments. These studies covered a range of polluting materials including, wood, metal, rubber, sediments, stone, glass, and many forms of plastic. The plastic materials are arranged from type of plastic, such as Polystyrene, Polyethylene, PE, PS,PET,NYLON, LDPE, HDPE, PVC, PP, PU, PMMA, PAH, PCB, PHBV. And plastic “shape”, Microplastic fibres, Pellets, Plastic Marine debris, macroplastics. This study will focus on how bacteria interact with shape. The shapes under investigation are:

1.1.1 Fibres

Microplastic Fibres are a thin “string” of plastic material, most commonly originating from the washing of textiles of materials, polyesters, and polyamides (nylon). These enter waterways from washing wastewater. This contaminated water is treated at Waste Water Treatment Plants (WWTP) which can fail to filter out fibres, causing them to end up in rivers and oceans.

1.1.2 Pellets

Microplastic pellets are small and roughly spherical in size, they can be made from several plastics, not relegated to one or two types. Pellets can originate from the plastic manufacturing process, the beauty industry (Small plastic pellets intended for skin exfoliation) or the degradation of larger plastic debris.

1.1.3 PMD

Plastic Marine Debris are a varied source of plastic pollution. PMD is not always considered microplastic as they can be over 5mm in size. They come from numerous sources, the main one being simple pollution. The term Plastic Marine Debris comes from 1 study that includes multiple different plastics but cannot denote them as microplastics so have used an umbrella term for the samples.

1.1.4 MACROPLASTICS

Macroplastics are plastics above 5 mm in size. Like PMD they are a result of general pollution. Due to sample strength not being strong enough they cannot be included in this thesis.

1.1.5 Reason for study

Investigating the bacteria present on the microbiome of microplastics is an important area of study. This report will outline the steps taken to analyse all samples pertaining to the study, bacteria discovered and advise on further steps to be taken to help the problem of microplastic pollution.

2. Materials and Methods

2.1 Literary Review

A literary review was the first step to begin understanding and identifying the problem and ways of beginning my individual review of plastics. Using the meta data table, provided by the plastisphere team I was working with, 8 Studies were identified as studies relating to the area of study.

Study (out of 43)	Author	Title	Year	Journal	DOI
Study 2	Amanda R. McCormick, Timothy J. Hoellein, Maxwell G. London, Joshua Hittie, John W. Scott, John J. Kelly	Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages	2016	Ecosphere	**10.1002/ecs2.1556**
Study 3	Sonja Oberbeckmann, and Matthias Labrenz and Matthias Labrenz	Environmental Factors Support the Formation of Specific Bacterial Assemblages on Microplastics	2018	Frontiers in Microbiology	10.3389/fmicb.2017.02709
Study 9	Alice Delacuvelleriea, Valentine Cyriaquea, Sylvie Gobertb, Samira Benalic, Ruddy Wattieza,	The plastisphere in marine ecosystem hosts potential specific microbial degraders including Alcanivorax borkumensisas a key player for the low-density polyethylene degradation	2019	Journal of Hazardous Materials	10.1016/j.jhazmat.2019.120899
Study 11	C. Dussud, A.L.Meistertzheim, P.Conan, M.Pujo-Pay, M. George, P. Fabre, J. Coudane, P. Higgs, A. Elineau, M.L. Pedrotti, G. Gorsky, J.F. Ghiglione	Evidence of niche partitioning among bacteria living on plastics, organic particles, and surrounding seawater	2018	Environmental Pollution	10.1016/j.envpol.2017.12.027
Study 27	Peilin Jiang [1], Shiye Zhao [1], Lixin Zhu, Daoji Li	Microplastic-associated bacterial assemblages in the intertidal zone of the Yangtze Estuary	2018	Science of the Total Environment	10.1016/j.scitotenv.2017.12.105

Study 29	Timothy Hoellein , Miguel Rojas, Adam Pink, Joseph Gasior, John Kelly	Anthropogenic Litter in Urban Freshwater Ecosystems: Distribution and Microbial Interactions	2014	PLOS ONE	10.1371/journal.pone.0098485
Study 30	Martin Ogonowski, Asa Motiei,Karolina Ininbergs, Eva Hell, Zandra Gerdes, Klas I. Udekwu, Zoltan Bacsik4andElena Gorokhova1	Evidence for selective bacterial community structuring on microplastics	2018	Environmental Biology	10.1111/1462-2920.14120
Study 31	Lucy C. Woodall , Anna D. Jungblut, Kevin Hopkins, Andie Hall, Laura F. Robinson, Claire Gwinnett, Gordon L. J. Paterson	Deep-sea anthropogenic macro debris harbours rich and diverse communities of bacteria and archaea	2018	PLOS ONE	10.1371/journal.pone.0206220

Table 1: Summary of Relevant Studies

Study 2

A paper from 2016, analysing microplastics in urban rivers of USA and the bacteria assemblages on them, with the key goal of understanding how great of an impact WWTP have on the microplastic concentration on upstream and downstream rivers. The study indicated high microplastic concentrations due to the lesser water volume of rivers in comparison to oceans. The initial observations suggested that fibres, pellets, foam, film, and fragments were the key pollutants, some of these were made up of the polymers polypropylene, polyethylene and polystyrene.

The samples were taken from WWTP across three main areas, 9 streams in total, (Chicago metropolitan area of north-eastern Illinois and Northwest Indiana and central Illinois), using micromesh netting. Samples were stored and processed to extract DNA, gene sequencing occurred and bunched into operational taxonomic units (OTUs) and this data was analysed using two-way ANOVA to compare microplastic concentrations and one-way ANOVA for finding differences across the streams. Bray-Curtis index was calculated to compare the contents of bacterial assemblages of the microbiomes.

The study found that there was significantly different bacterial assemblages between upstream water column, downstream water column and, downstream organic material. Upstream found Flavobacteriaceae, unclassified Actinomycetales, and Cytophagaceae to be most common families and in downstream Flavobacteriaceae, unclassified Actinomycetales, and Cytophagaceae were identified.

The paper concluded that WWTP discharge is a large contributor to microplastic abundance in urban waterways which flow into the larger marine communities. Bacterial assemblages on organic matter was also collected, this comparison showed that there was an taxonomic abundance on the plastic microbiome which is likely due to the "hard surface and organic carbon source". It stated "that pseudomonas produce enzymes such as serine hydrolases, esterases, and lipases, which assist in plastic biodegradation", and that it is shown to be a "rapid" process. This is of great importance as plastic degradation is key to the removal of microplastics that have been proven to sometimes avoid the filtration systems of WWTP.

The paper concluded that microplastics are rife in urban rivers and the large concentration provides easy flow of microplastics to downstream locations. It has stated that further studies should be undertaken to understand how much and to also understand how microplastics affect other types of waterways and its inhabitants. (McCormick *et al.,* 2016c)

Study 3

This intends to develop a greater understanding of microplastics acting as a host for microplastic assemblages. This paper takes place as a controlled experiment, rather than collecting already existing microplastics currently in the water, polystyrene, polyethylene and wooden pellets were subjected to natural water sources in order to develop a microbiome to analyse. The wooden pellets were used as a control to compare as a non-plastic material.

This paper was included in by the meta table as a study to be included in the data analysis of the pellets samples, however this is an oversight as wooden pellets shouldn't have been classified under the pellet category. Therefore will not be analysed in this study or used in its data interpretation. (Oberbeckmann, Kreikemeyer and Labrenz, 2018d)

Study 9

The intention of study 9 was to identify key bacterial degraders, something that is very important to my investigation. This paper identifies only 4 samples of microplastic fibres. this is expected as the paper focuses on the degradation of LDPE, but the samples collected will still be included in data analysis.

Bacterial degradation of polymers is very slow and there is a lack of understanding of how to develop these communities. Pervious studies have indicated that *Alphaproteobacteria* and *Gammaproteobacteria*, are main colonizers of plastics but their ability to degrade plastics is relatively unknown. This study intends to investigate the biospheres of plastics to see their compositions from a wild setting and discover more about the degrading capabilities. (Delacuvellerie *et al.,* 2019b)

Study 11

This study is the largest sample size of the 8 studies it provides us with 148 PMD samples which is the only PMD related study obtained. This study analyses Plastic Marine Debris from the Mediterranean sea and will look at how the plastic properties and environment will affect the ecosphere of the plastics. PMD samples were analysed and found that Cyanobacteria was the most common type of bacteria found, but also that eukaryotic organisms like fungi were also found, some dangerous pathogens like species of Vibrio were for on a small amount of plastics. Pathogens related to marine life, *Tenacibaculum, Phormidium* sp. and *Leptolyngbya* sp were present in 27.7% of PMD microbiomes. Some bacteria were able to "hitchhike" from plastics. (Dussud *et al.,* 2018b)

Study 27

The microbial community attached to plastic marine debris in the sediment environment is still limited. 16s rRNA sequencing was used to identify diverse bacterial communities that colonize plastic marine debris of various types in three different sampling sites in China. The plastisphere communities that were analysed is heavily populated mainly by Proteobacteria, Bacteroidetes, Cyanobacteria, and Actinobacteria. The taxonomy of the bacterial families detected on microplastics are like those in natural microbial communities of the surrounding environments. The study also found no significant differences in microbial composition among polyethylene, polypropylene, and polystyrene types. Alphaproteobacteria and Bacteroidetes were found to be keystone species, with Rhodobacterales being a dominant and ubiquitous primary surface colonizer in temperate coastal waters of the world. Essentially this study highlights that although microbial colonization of polymers is well-known, the detailed investigations of the plastisphere community using culture-independent methods are still uncommon. The influx of freshwater from rivers can influence the microbial assemblages colonizing the microplastics, and the origin of plastic colonizers suggests a close association with the natural microbial communities of the surrounding environments. (Jiang *et al.,* 2018b)

Study 29

This passage describes a study that aims to explore the accumulation and ecosystem effects of anthropogenic litter (AL) in freshwater ecosystems, focusing on urban freshwaters. The study's objectives were to measure the AL density in freshwater, compare it to terrestrial and marine ecosystems, and characterize the activity and composition of AL biofilms in freshwater habitats. The researchers found that the main driver of biofilm community composition was the location that incubation took place. For example, biofilms on organic subsets had lower initial productivity than synthetic substrates, and bacterial communities on organic substrates were distinct in composition from those on hard substrates. The study also revealed that Lake Michigan beaches had significantly less AL than the Chicago River riparian and benthic zones. The dominant types of AL across all ecosystems were plastic, paper, and glass. The study's findings could inform future research on AL sources, ecosystem effects, and fate across multiple ecosystem types, benefitting the management and reduction of global AL accumulations. The passage also discusses the ecological effects of marine debris and the role of microbial biofilms in nutrient cycling and serving as an important food source for higher trophic levels in aquatic habitats. Finally, the passage

highlights the lack of previous research on community composition and activity of biofilms colonising AL in freshwaters. (Hoellein *et al.,* 2014b)

Study 30

This study begins with the understanding that microplastics have recently been recognized as a substrate for various aquatic microorganisms that propagate on the surfaces of certain plastic materials, which possibly contribute to their deterioration/degradation and help with burial in the sediments. Synthetic polymers are not water soluble, and biofilm-forming bacteria degrade such materials more efficiently than planktonic strains, where UV-driven degradation cannot be effective, whereas bacteria-mediated degradation could be more important.

A recent study exposed ambient Baltic bacterioplankton to different polymers, including PS, PE, PP, glass, and cellulose, under controlled conditions to investigate the variability of biofilms on different plastic materials. The highest biofilm density was found on cellulose, followed by PE, glass, PS, and PP. The biofilm density was 56% higher on plastic compared with cellulose, and the biofilm density on PP was 22% and 29% lower compared with glass and PE, respectively.

The composition of taxa displayed a high level of consistency within a given marine region, suggesting particular bacterial taxa are able to successfully colonize plastic materials. The most dominant classes found on plastics recovered from different areas were Alpha- and Gammaproteobacteria, whereas Flavobacteria and Gammaproteobacteria dominated plastic litter communities collected in the North Sea. (Ogonowski *et al.,* 2018b)

Study 31

This study investigates bacterial assemblages on marine debris, including plastic, fabric, rubber, metal, and glass, collected from the deep sea. Using 16S rRNA gene high-throughput sequencing, the authors found that the microbial assemblages varied across the different materials. The study showed that Proteobacteria accounted for more than half of total OTU's followed by Bacteroidetes at 10 % and Crenarchaeota at 9%. The study also found that the physical makeup, surface texture, and previous environments can affect the biofilm communities, with diverse microbial assemblages found in biofilms from plastic debris collected from ocean surface water, shallow coastal seabed, and intertidal zones. The authors suggest that the accumulation of marine debris, including plastic, rubber, metal, and glass, in the deep sea is a growing challenge, and the colonisation of debris by microbes poses a new risk to the environment. The study also found that the bacteria in biofilms from litter samples were different from those found in sediment samples. The microbial analysis of microplastics removed from sediment samples was not successful due to insufficient DNA extraction. The study highlights the need for further research to establish the environmental risk posed by the colonisation of debris by microbes. (Woodall *et al.,* 2018b)

Study	Material(s) Pertaining to thesis	Location	Type of Water	No. of Samples
Study 2	Microplastic Fibres	North America, USA (Chicago metropolitan area of north-eastern Illinois and Northwest Indiana and central Illinois)	Fresh Water Urban Rivers	61
Study 3	N/A	Europe, Germany	Freshwater	-
Study 9	Microplastic Fibres	Europe, France	Seawater (Mediterranean Sea)	4
Study 11	Plastic Marine Debris	Europe, France	Seawater	148
Study 27	Microplastic Fibres	China, Asia, Yangtze Estuary	Freshwater	9
Study 29	Pellets	North America, USA	Freshwater (Chicago River)	144
Study 30	Microplastic Fibres	Europe, Sweden	Sea Water (Baltic Sea)	18
Study 31	Microplastic Fibres	Europe, Germany	Saline water	8

Table 2: Study summary of marterial and Souce of materical

2.2 Method

In regard to steps taken in this study the majority of the base work was already completed. Sample collection, DNA extraction and gene sequencing was already carried out by the researchers of the studies collated. Studies pertaining to microplastics found in water were collated by a member of the plastisphere team before beginning my research. Meaning this is a very statistical and analytical paper rather than an applied paper.

Meta / Abundance Table

A meta-analysis was conducted by a fellow student and member of the plastisphere team, using studies that were found by the staff advisors of the team. Overall 43 different studies with many plastic samples were collated and using microbial analysis software the raw data of the samples was analysed. The meta table was populated with all the necessary information found from the study data to provide a thorough table for in depth analysis further down the line.

2.3 Data Analysis / R Studio

R Studio is a coding package that uses the R coding language. R Studio is great for statistical analysis and visualisation of graphics, the software provides a number of packages that were utilised in the code.

This study revolves around the understanding of bacteria, what they reside on, the abundancy of them, what types of bacteria are present, and that is something that needs to be shown and analysed. Code was written and provided by Dr Umer Ijaz, code was accessible via a shared folder on Outlook. The scripts provided allowed for a graphical output of both alpha and beta diversity, Taxonomy (Taxa) plot, core microbiomes of materials and CODA LASSO comparisons between materials.

2.3.1 Alpha Diversity

Alpha Diversity is the diversity of microbial families present on the samples of one sample. It is calculated by finding the different types of microbes and how much of each is present in the sample and gathers the relative abundance of microorganisms. This alpha diversity script will use the individual sample diversity and mean the results and allow us to compare the relative diversity of one material with others. Using the Shannon Index which is to measure the to diversity of communities with regard to the amount of species found and the abundance of each species. It ranges from 0 to a number dependant on the number of species found, and the higher the number the greater the diversity of the community (Pielou, 1966)

2.3.2 Beta Diversity

Beta diversity plots the comparison of microbial diversity between two or more material sets. The ability to have a direct comparison is advantageous as it'll gives a simple visual representation of how similar the average bacterial composition of different material types are.

Bray Curtis distance it indication of how dissimilar samples are in terms of spices inhabitants. The further away from 0 the more dissimilar the bacteria present are.

2.3.2 Taxa Plot

Taxa plots are a graphical method of allocating microbes based on the taxonomy of the bacteria present (Domain, Kingdom, phylum, Class, Order, family, Genus, Species). It recognises the most abundant microbes present on the samples looked at and allows for identification of key bacterial genus that families that develop on the microbiomes. Taxa plot will label the abundant microbes which helps us identify differences in the taxa between all sample sets.

2.3.3 Core Microbiome

The core microbiome refers to the group of organisms that are consistent to the samples groups. This is presented as a heat map of the most commonly found species found on the biofilms, this helps, with the help of other data sets, to identify and discern between basic bacteria expected to be found and outliers which are highlighted for further research.

2.3.4 CODA LASSO

CODA LASSO is an instrument which compares two material types is used to identify species that are key components of the microbial community's growth and development. By using CODA LASSO, we can focus on the important characters in the ecosystems and see what species are driving factors in the microbiome and isolate for further research.

3. Results

3.1 Alpha Diversity

An alpha diversity table was compiled using the provided scripts which shows the diversity of Fibres (orange), Pellets (green) and PMD (pink) and is shown in figure 1. As shown fibres generally contain a relatively larger bacterial diversity amongst samples, the diversity using the Shannon index was found to be just above 4.0. Pellets were also shown to have a large diversity but not as great as fibres with its Shannon index at just below 4.0. PMD was shown to have the lowest diversity from the three groups with a Shannon index at between 3.0 and 3.5.

From this result it is visible that fibres have the greatest diversity thus being able to adapt and change over time due to its multitude of different microorganisms. This is only a simple rendition of bacterial diversity and more analysis will have to be done to fully understand the makeup of bacterial assemblages.

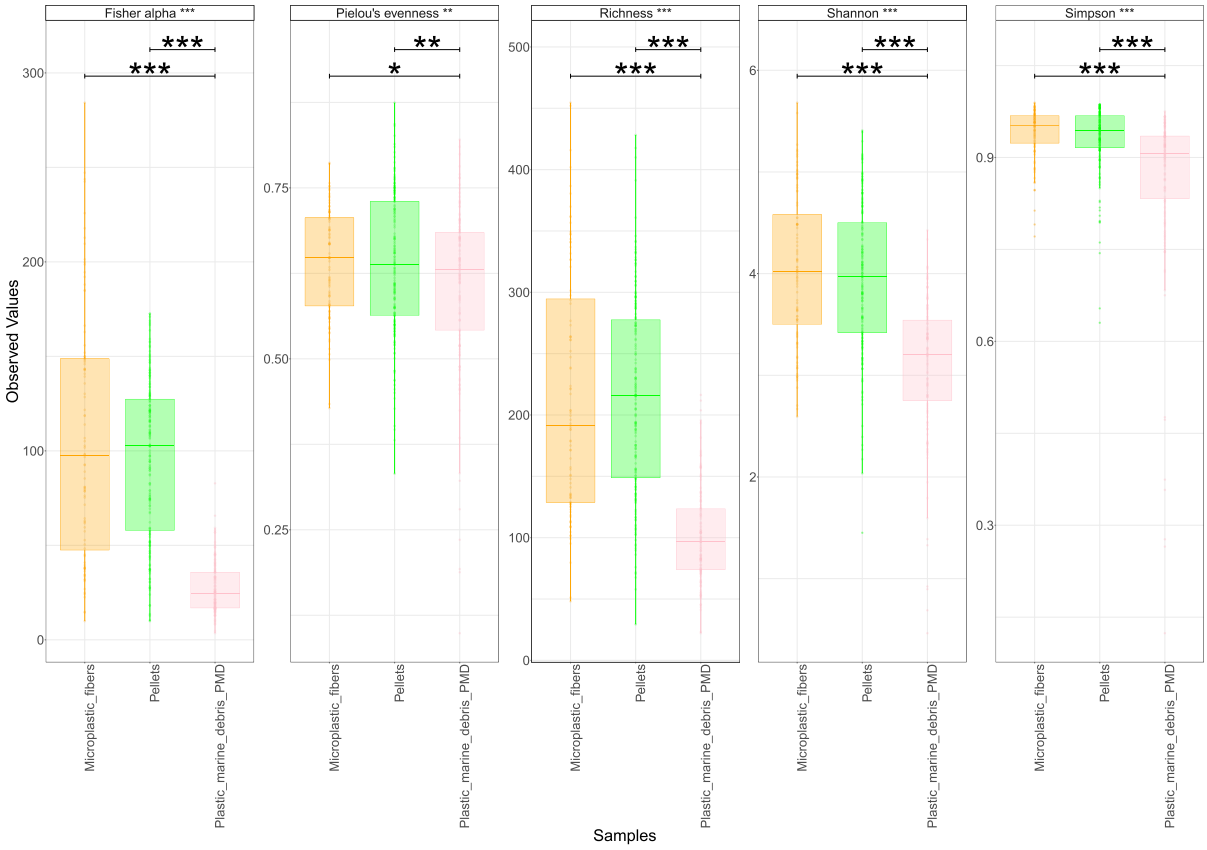

Figure 1 : Alpha diversity of fibres, pellets and PMD using the Fisher alpha, Pielou's evenness, Richness, Shannon index and simpson index.

3.2 Beta Diversity

Figure 2 is a beta diversity graph showing the diversity 3 material sets: Fibres (orange), Pellets (green) and PMD (pink). The dots represent the individual sample microbe result. These results are derived from the material samples as they are, they are not divided by salinity, water type or temperature as this is a material shape study.

Fibres have a tight cluster at 0.0, 0.0 indicating it shares some similar species, which should be expected as there are only a few types of plastics that fibres arise from; this is evidence of bacterial assemblages favouring individual plastic types. Pellets have a wider cluster compared to fibres; this is most likely due to more polymer types being under the pellet umbrella. PMD has the widest range of results, this is due the lack of consistency in the PMD definition, as it is a catch all term for one study it is difficult to denote whether plastic types have a key impact on this result.

There is a cluster of all three which indicate a definite overlap of bacteria and a possible overlap of similar polymer types.

Figure 2 : Beta diversity for fibres, pellets and PMD using the Bray-Curtis Distance

3.3 Taxa Plot

This taxa plot, figure 3 ,shows the 25 most abundant taxa for each material type and their prevalence on it. Figure 3 confirms the diversity of pellets which was indicated by figure 2.

The plot shows bacteria Caldilineaceae to be present in all three material sets this is of the Chloroflexi phylum. The abundance of this family would indicate that this is a naturally occurring microorganism in aquatic regions, rather than one that hooks itself to certain plastics.

In Pellets the genus Flavobacterium, is very common along with many other members of the phylum bacteriodidota. These include the genus' Pseudarcicella, lewinella, Sediminibacterium, aquibacter and Fluviicola.

Hgcl_clade shared was found to be relatively abundant in both fibres and pellets.

This taxa plot helps to affirm and label what was graphically displayed in the beta diversity graph, It identifies the bacteria that would overlap clusters and those that would show no overlap.

It is important to state that approximately half of the other taxa are unlisted and would benefit from further analysis as these could be potential beneficial organisms which are not abundant enough to study, these become prevalent in the core microbiome graph.

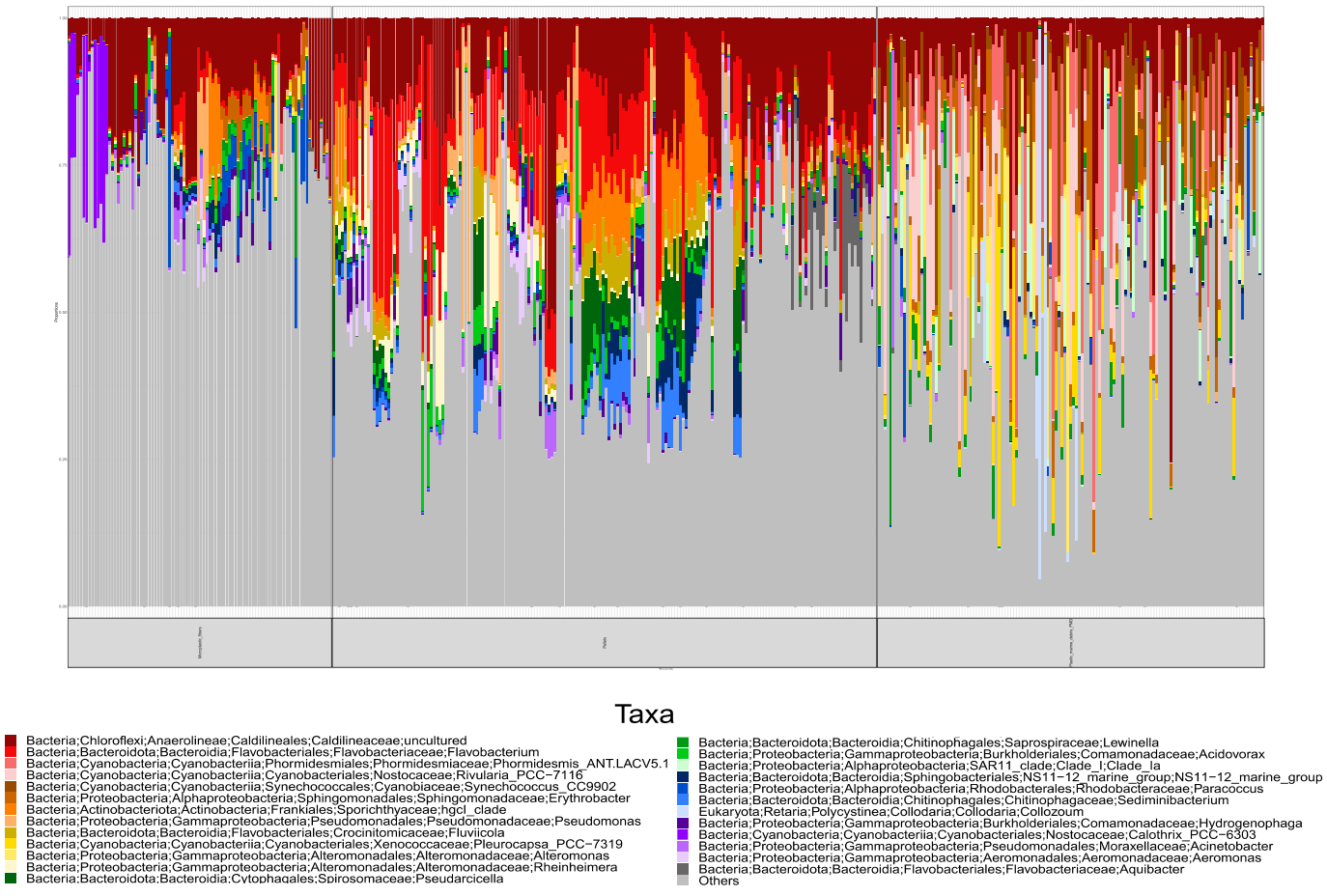

Figure 3 : Taxonomy abundance plot for the 25 most abundant taxa on fibres, pellets and PMD

3.4 Core Microbiome

The core microbiomes indicate a number of prominent taxa, for our purposes the top 4 most prevalent will suffice. 66 types of microorganisms were found on fibres, 60 on pellets and 16 on PMD.

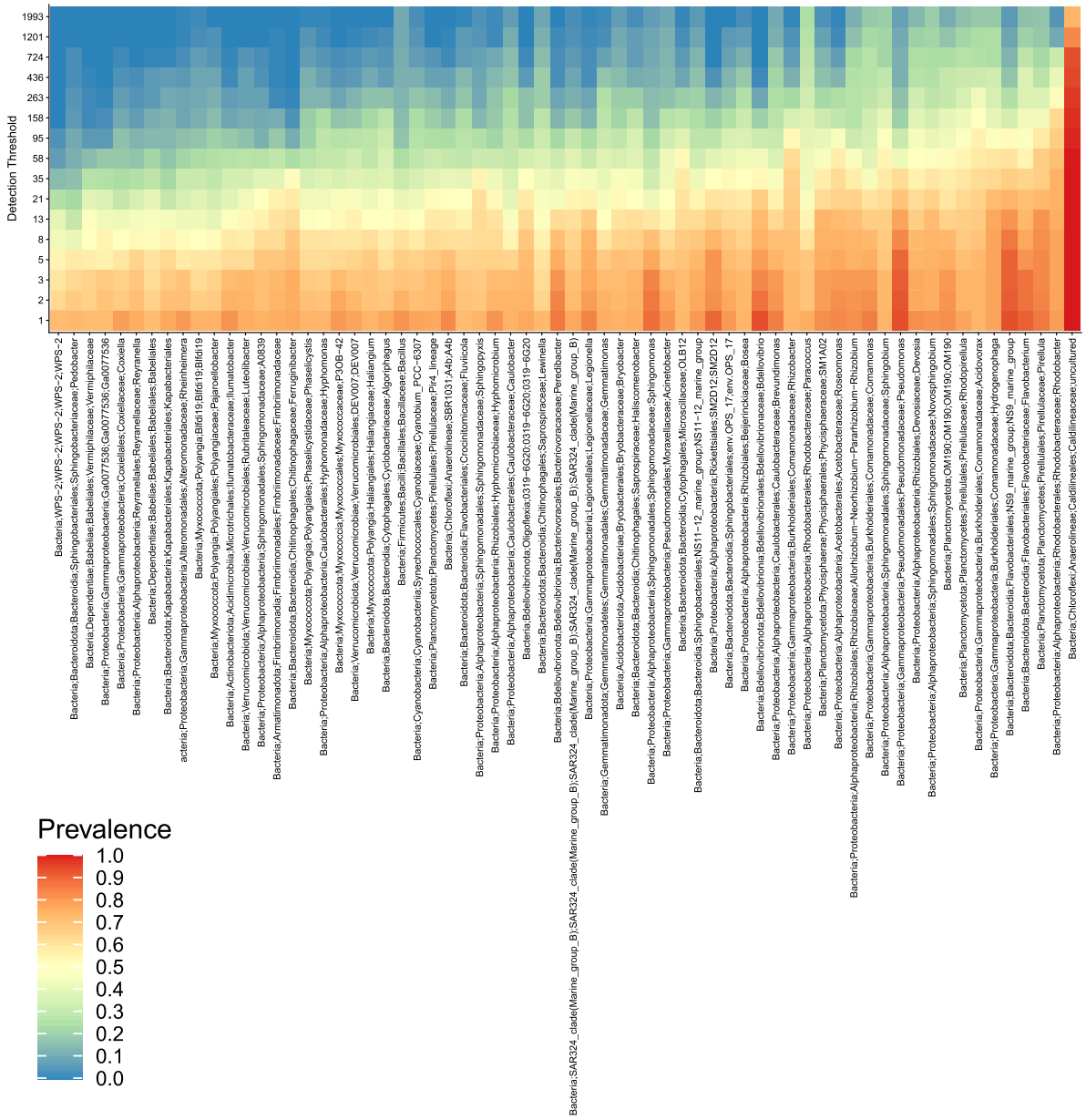

Figure 4 : Core Microbiome for Microplastic fibres

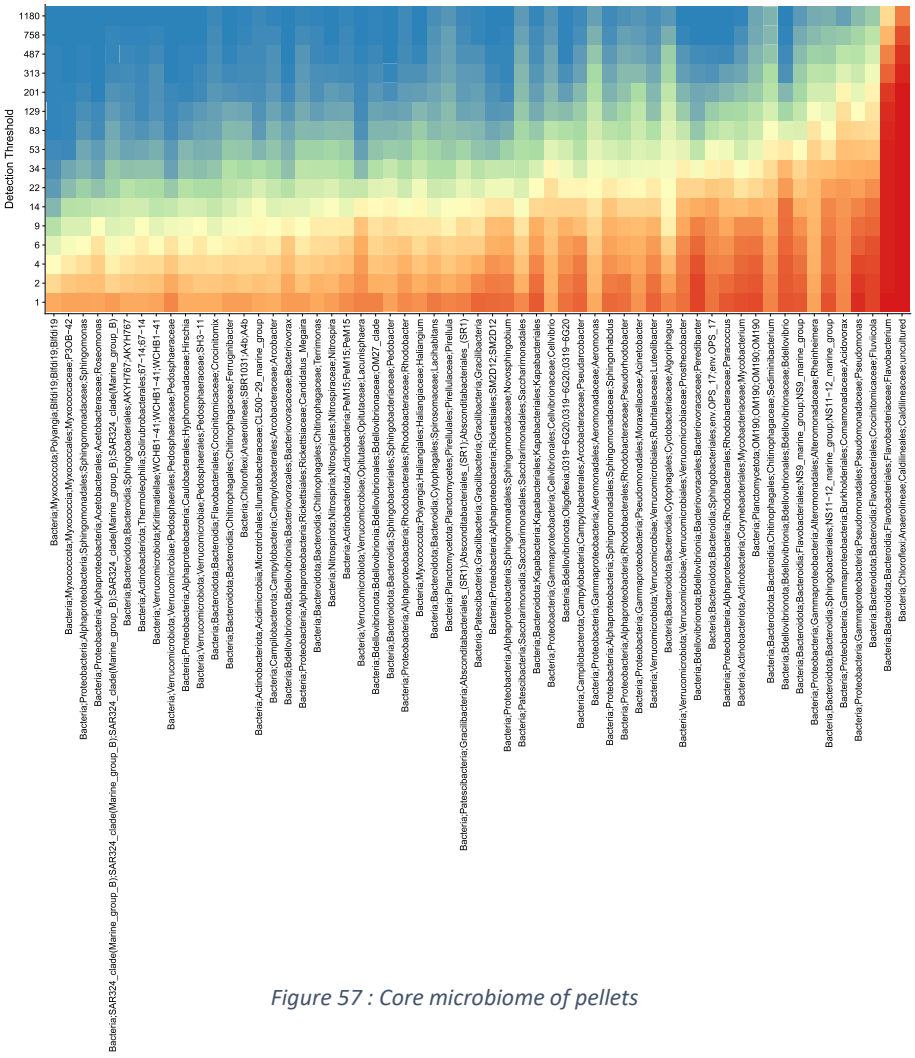

Figure 57 : Core microbiome of pellets

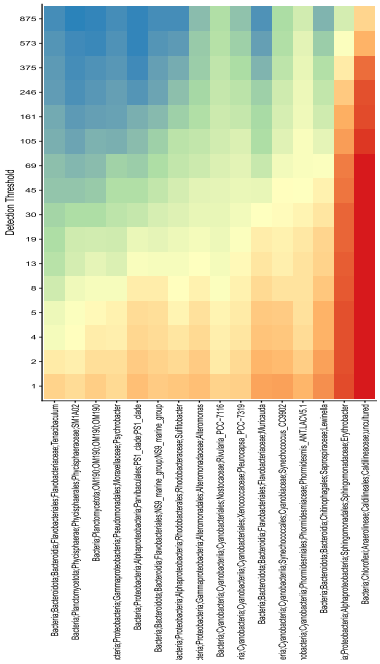

Figure 6 : Core Microbiome for PMD

Material	Bacteria
Microplastic Fibres	Bacteria Chloroflexi Anaerolineae Caldilineales Caldilineaceae uncultured
	Bacteria Planctomycetota Planctomycetes Pirellulales Pirellulaceae Pirellula
	Bacteria Proteobacteria Alphaproteobacteria Rhodobacterales Rhodobacteraceae Rhodobacter
	Bacteria Bacteroidota Bacteroidia Flavobacteriales Flavobacteriaceae Flavobacterium
	Bacteria Proteobacteria Gammaproteobacteria Burkholderiales Comamonadaceae Hydrogenophaga
Pellets	Bacteria Chloroflexi Anaerolineae Caldilineales Caldilineaceae uncultured
	Bacteria Bacteroidota Bacteroidia Flavobacteriales Flavobacteriaceae Flavobacterium
	Bacteria Bacteroidota Bacteroidia Flavobacteriales Crocinitomicaceae Fluviicola
	Bacteria Proteobacteria Gammaproteobacteria Pseudomonadales Pseudomonadaceae Pseudomonas
Plastic Marine Debris	Bacteria Chloroflexi Anaerolineae Caldilineales Caldilineaceae uncultured
	Bacteria Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae Erythrobacter
	Bacteria Bacteroidota Bacteroidia Chitinophagales Saprospiraceae Lewinella
	Bacteria Cyanobacteria Cyanobacteriia Phormidesmiales Phormidesmiaceae Phormidesmis_ANT.LACV5.1
	Bacteria Cyanobacteria Cyanobacteriia Synechococcales Cyanobiaceae Synechococcus_CC9902

Table 3 : Table of 5 most prominant bacteria of each core microbiomes

3.5 CODA LASSO

We use CODA LASSO to identify abundant dissimilar taxa between material sets, several taxa were identified as significantly associated with the only certain material sets. Pairwise comparisons were preformed between: Fibres vs Pellets, Fibres vs PMD, and Pellets vs PMD

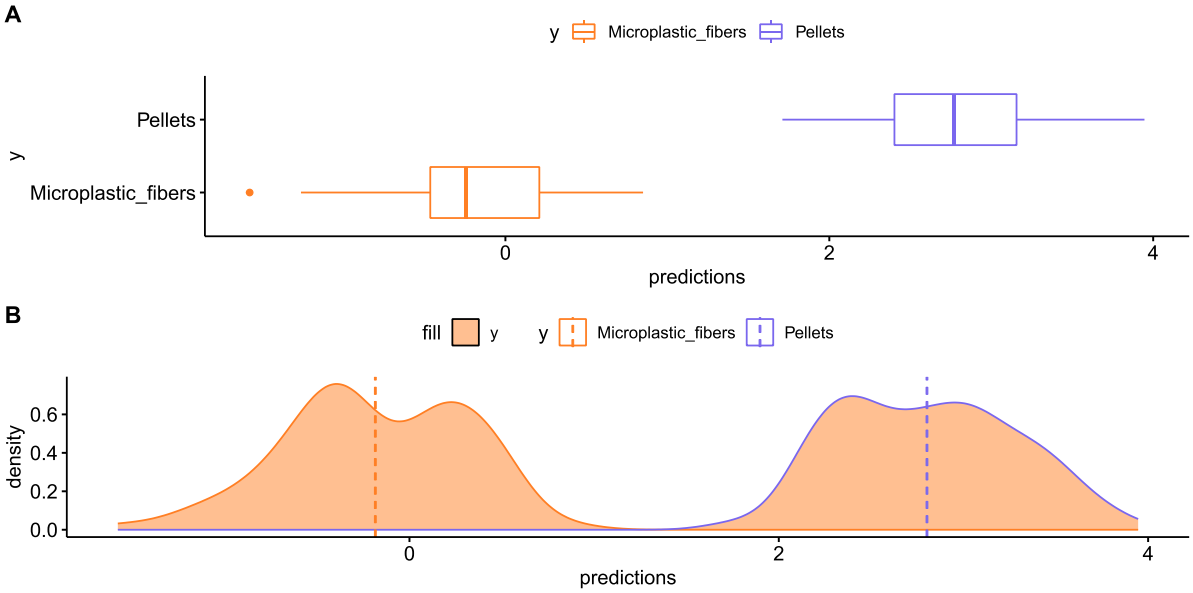

Figure 78 : CODA LASSO comparison for Fibres vs Pellets

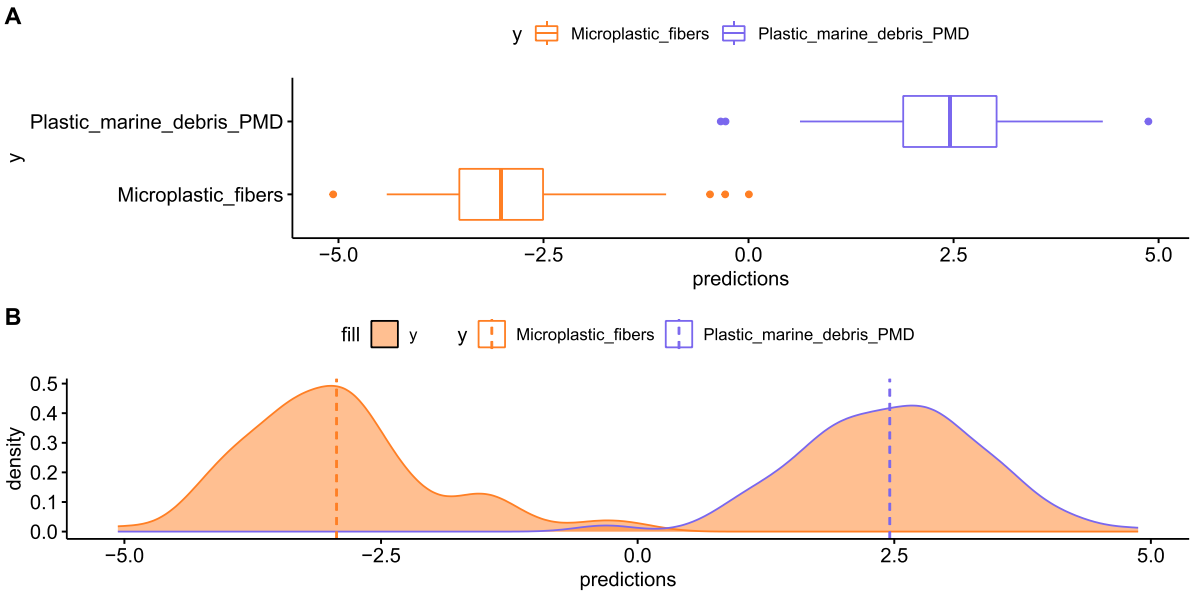

Figure 8: CODA LASSO comparison for Fibres vs PMD

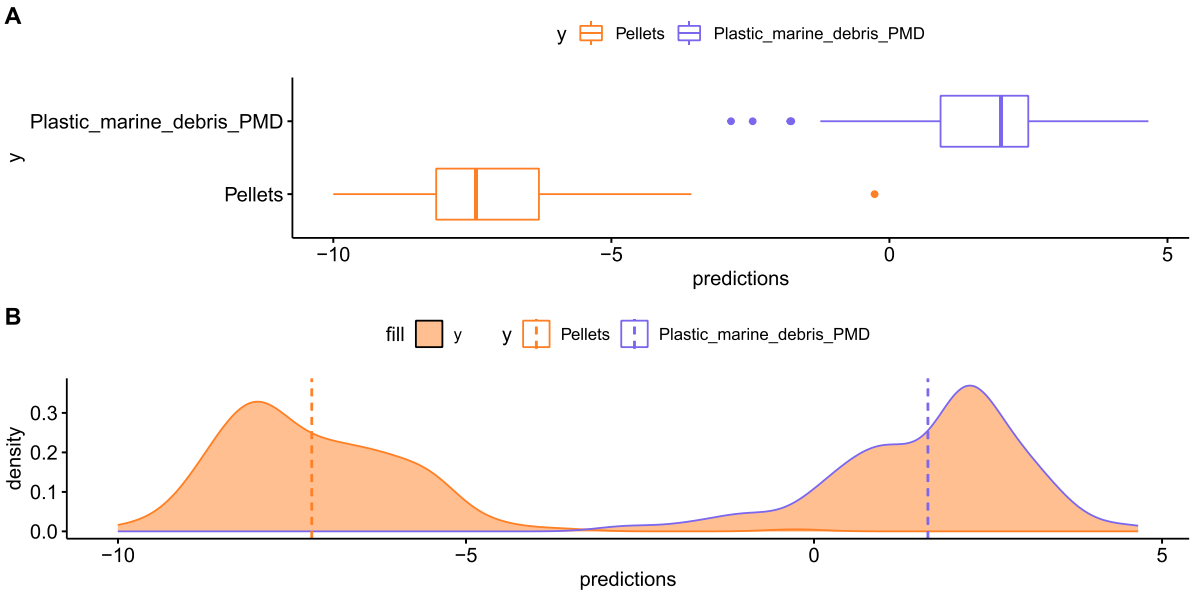

Figure 9 : CODA LASSO comparison for Pellets vs PMD

Figures (10, 11, 12) show a significant difference in bacterial assemblages as there are distinct bacterial congregations for each material set. We can now identify individual members of the microbiome at a genus level.

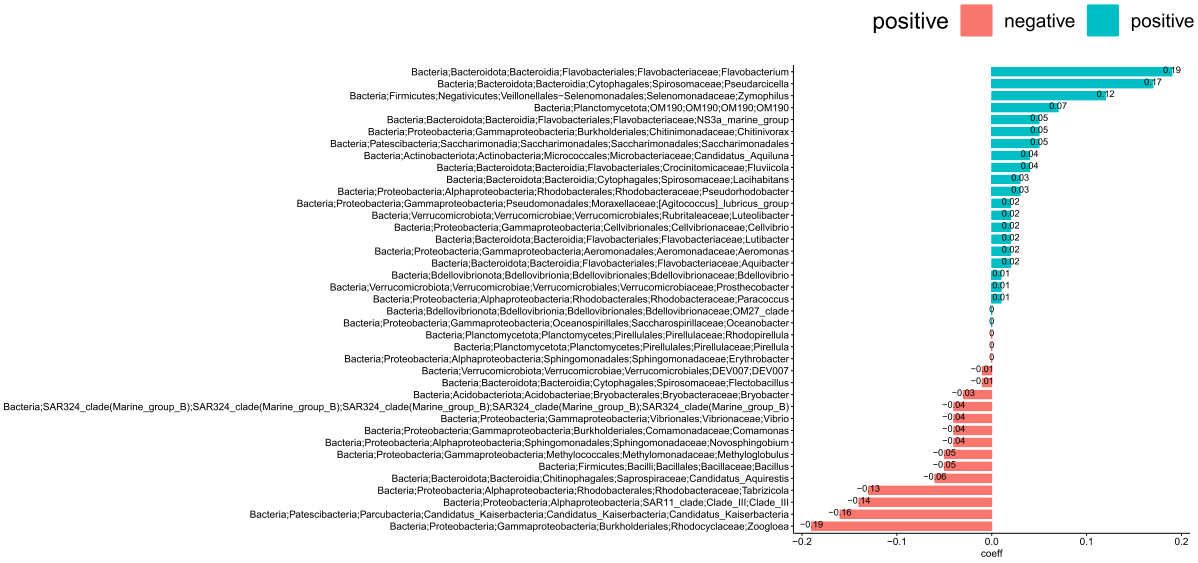

Figure 10 : Bacterial comparisons for Fibres vs Pellets

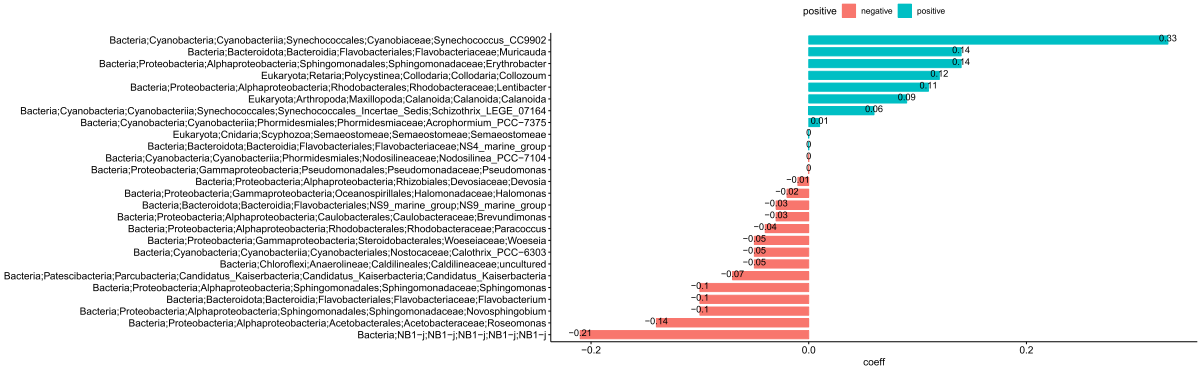

Figure 11 : Bacterial comparisons for Fibres vs PMD

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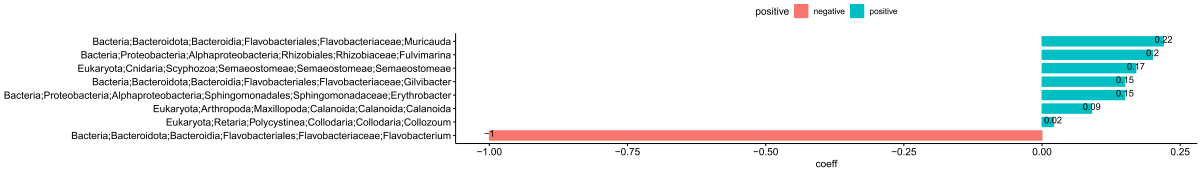

Figure 12 : Bacterial comparisons for Pellets vs PMD

The negative and positive values correspond to the likelihood of what material set the microorganisms will be inhabiting.

OTU - Family, Genus	Description	Negative	Positive
Fibres vs Pellets			
Rhodocyclaceae, Zoogloea	The genus Zoogloea can occur not anchored to any material in organically polluted fresh waters and found in wastewaters.		
Candidatus Kaiserbacteria, Candidatus Kaiserbacteria	-		
Clade III, Clade III	Often associated with ear infections. Have drug resisting capabilities and can reside well on plastics even after cleaning.		
Rhodobacteraceae, Tabrizicola	Found in a number of varied water sources, needs little nutrient, evidence has shown that it utilises photosynthesis.		
Flavobacteriaceae, Flavobacterium	Can be found in multiple types of water (fresh, waste, sea) and genus can adapt to survive in cold habitats like Antarctic lake. Has been found to degrade Nylon and other complex polymers.		
Spirosomaceae, Pseudarcicella	-		
Selenomonadaceae, Zymophilus	Minor evidence of glycerol fermenting properties		
Fibres vs PMD			
Cyanobiaceae, Synechococcus_CC9902	Can adapt to different saline environments and light intensity although salinity can split up Synechococcus strains.		
Flavobacteriaceae, Muricauda	OTU capable of degrading plastics		
Sphingomonadaceae, Erythrobacter	Heavily associated with plastic bacterial assemblages with some supporting evidence of plastic degradation (PE) when present in biofilms.		
Collodaria, Collozoum	Important contributor to the marine food chain. In warm waters it has exhibited host cell degradation and possible infection of other cells.		

Rhodobacteraceae, Lentibacter	Associated with algae blooms. Members of family are known as initial colonizers. Some members of family are known plastic degrading taxa, unsure if this genus shares the same properties.		
NB1−j;NB1−	-		
Acetobacteraceae, Roseomonas	-		
Sphingomonadaceae, Novosphingobium	Hydrocarbon degrading properties, found to be able to degrade a range of plastic compounds.		
Pellets vs PMD			
Flavobacteriaceae, Muricauda	OTU capable of degrading plastics		
Rhizobiaceae, Fulvimarina	Can live on a wide variety of bases. No evidence of plastic degradation		
Flavobacteriaceae, Gilvibacter	-		
Sphingomonadaceae, Erythrobacter	Heavily associated with plastic bacterial assemblages with some supporting evidence of plastic degradation (PE) when present in biofilms.		
Flavobacteriaceae, Flavobacterium	Can be found in multiple types of water (fresh, waste, sea) and genus can adapt to survive in cold habitats like Antarctic lake. Has been found to degrade Nylon and other complex polymers.		

Table 4 : Bacterial comparisons derived from CODA LASSO

4. Discussion

In its research this project has collated and highlighted the vast amount of plastic pollution in marine environments. Steps have already been taken to minimise the amount of plastic pollution in the environment, legislation has been passed to remove microplastics from some consumer material, filters are installed in WWTP and heavy fines are in place for pollution. These are only small steps to fix the already existing problem and greater solutions need to happen. Macroplastic litter is relatively easy as collection with nets is only inhibited by the sheer scale of pollution and funding. Microplastic litter need more specific methods as they pass through these nets. This is where plastic degradation would help.

Throughout a series of data analysis procedures, diversity of samples, abundance taxa, core microbiomes and pairwise comparisons of material sets, the main taxa in the plastispheres of material shapes have been identified. Focusing solely on material shape has led to some stretched conclusions which would need more support to confidently state. The analysis showed important microorganisms that should be investigated further, as some are pathogenic and need to be minimised or avoided in entering the food chain or have degrading properties which can be utilised by biotechnologists.

Plastic Degradation

The ability to degrade plastics is a beneficial one however a lot of microplastic litter comes from the degradation of macroplastics over time, this is why in order to clear up the Earths waters both must be removed concurrently.

The genus, Novosphingobium, is which is found in fibres has been shown to have the ability to degrade certain hydrocarbons and also Polyvinyl Alcohol (PVA).

Flavobacterium is found in abundantly in Pellets, this is another known plastic degrader which is able to degrade complex polymers.

PMD exhibited the most types of possible plastic degradation bacteria, Erythrobacter, Lentibacter, Muricauda all were promising organisms for degradation as there is evidence that the genus exhibits plastic degradation or the family it comes from does.

Deciding which bacteria is the best for plastic degradation is difficult to state without making some vast conclusions. As there is no direct relation to a specific type of polymer the bacteria inhabits it would be difficult to introduce the bacteria to specific subsets of plastics for the intention of degradation.

I do believe that the five bacteria identified (Novosphingobium, Flavobacterium, Erythrobacter, Lentibacter, Muricauda) would be great candidates for further investigation by biotechnicians. Bioengineering these organisms in order to boost their degradation properties and inputting them back into the environments where they thrive would speed up plastic degradation, in attempts to remove microplastics from the marine environments.

PMD Stands to be a difficult material set to analysis as it is not fully quantified, study 11 which PMD is sourced from uses PMD as a term for all plastic collected. PMD is difficult to look at with fibres and pellets as some samples could fall into both categories. Ideally the PMD samples would be classified under multiple terms such as polymer types, macroplastics, fibres and any other type of material collected.

This analysis covers only the microbial assemblages relative to a material shape, this is beneficial to developing a greater understanding of the microorganisms present on the plastispheres. However, would greatly benefit from further analysis on a smaller spectrum. As speculated here material shape plays a small role in the taxonomic makeup of biofilms and the abundance of certain taxa are a result of many factors, those being, the environments are sourced or incubated, polymer type temperature, salinity, and history. Some evidence has been displayed that surface texture may play a part in certain taxa present however this study does not account for that. A further in-depth analysis of the samples would provide a much greater understanding of the plastisphere and steps that can be taken to minimise microplastic pollution. Creating subsets of these material sets in terms of shape and polymer type and repeating this study would give a much better analysis of how shape affects the biofilms of polymer types.

Another way to improve this study is to include salinity and temperature in the data analysis, certain bacteria thrive in high salinities and vice versa, same goes for temperature. This would have a correlation with location but would indicate common bacteria present in common marine environments, as opposed to ones based on polymer types.

5. Conclusion

In conclusion the vastly growing interest and analysis of microplastic pollution is promising and more and more research articles are being published which is thrusting the problem into the public eye but sadly the problem is also growing at an alarming rate. More advanced research will take place and further the development of eradicating the plastic pollution worldwide.

Plastics can serve as a haven for microbial growth in marine environments, the survival of these organisms depend on the resilience of plastics which serve as a host to bacteria and a scourge to our environment.

The research done in this paper could also have benefits for design engineers to use the current manufacturing plastics in a more sustainable fashion. Further research could indicate potential ideal plastics to use as packaging, or one use plastic items and especially ones to avoid, if some bacteria do not inhabit certain plastics that makes them much less susceptible to degradation. Some form of implementation in plastic products could be researched further however designing a product to fail in time can be unethical, as it encourages more of the product to be bought and disposed of. It also comes with a lot of hesitation, as the question of why not build it to last is asked. Introducing bacteria in consumer products also has some serious negative social implications especially in food related products and childrens products.

This paper has set out to identify key bacteria in Microplastic biofilms in order to highlight them for degradation. Bacteria have been isolated as plastic degraders however it is difficult to say how effective they would be as a form of degradation for each material set as the shapes are made of different polymers. Some bacteria are known for degrading different types of materials like polyethylene for example, so it's hard to state which bacteria would be ideal for further research and bioengineered to make an ideal degrader. In this sense the paper hasn't achieved its initial goal.

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