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Title of Assignment : Microbial Communities analysis on Low and High density Polyethylene at different temperatures and salinities	

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Plasticsphere

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Microbial Communities analysis on Low and High density Polyethylene at different temperatures and salinities

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Abstract

At present, plastic pollution is considered as one of the foremost environmental challenges. The high productivity, low recovery of plastics and the poor management of their waste disposal pose a serious threat to the quality of aquatic ecosystems. The degradation of meso- and macro-plastics in the aquatic system to microplastics (MP), which is provides a new ecological niche for the microbiome and forms the 'Plastisphere'. Microplastics pose a potential threat to the aquatic ecosystem food chain as they have the ability to adsorb contaminants onto their surfaces, in addition to the risk of ingestion by aquatic organisms. Both high density polyethylene (HDPE) and low-density polyethylene (LDPE) are utilized in diverse applications including medical, construction, and packaging. At present, there has been a lack of research conducted on the effect of temperature and salinity on the microbiome present on the surface of HDPE and LDPE. In order to enhance comprehension of the microbiome present on LDPE and HDPE, a high-throughput sequencing technique was used through Illumina MiSeq. The samples were already available on online repositories, and were related to aquatic environments, covering both freshwater and marine systems. These were processed using 16S rRNA short read amplicons to given an account of microbial communities that reside on microplastics. Following bioinformatics processing of these samples, we obtained abundance tables of microbial species, which were then processed in R Studio for multivariate statistical analyses that include traditional diversity analyses, as well as differential and core microbiome analyses highlighting which microbes change and which microbes persist on these microplastics.

In general, this research presents novel findings that indicate the effect of temperature, salinity, and attachment substrates on the structure and diversity of microbiomes specific to microplastics. It is discussed that material properties (transparency) may influence the composition of microbiomes by affecting photorespiration. Our findings provide routes to develop intervention strategies for microplastic degradation.

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1. INTRODUCTION

Plastics are a type of organic polymer that originates from fossil fuels [1] [2]. The characteristics of plastics include light weight, low cost, good oxygen/moisture barrier properties and biological inertness. The versatility of this material has resulted in its utilization across diverse domains including medical applications (medical equipment), industrial production (automobiles, aircraft), construction industry, and packaging [3] [4] [3]. The exponential increase in plastic production has exceeded the capacity for proper end-of-life management, resulting to significant contamination of the environment [3] [6]. Microplastics (MPs) are defined as plastic debris which are less than 5 mm in dimension [7]. The small size, large surface area, high adsorption capacity, and resistance to degradation of microplastics make them one of the most important environmental pollutants in the ecosystem presently [6] [3].

1.1 Sources of microplastics

The origins of microplastics contamination are multifaceted, including a) the direct introduction of manufactured microplastics and nanoscale plastics through runoff, b) microplastic particles derived from abrasives present in household products, and c) debris fragments of meso- and macro-plastics that undergo fragmentation and decomposition through in situ weathering, physical, biological, and photochemical degradation processes [5] [6] [8]. The prevalent types of microplastics include high-density and low-density polyethylene (HDPE/LDPE), polyvinyl chloride (PVC), polyethylene (PE), polystyrene (PS), and polypropylene (PP) [2] [6]. Polyethylene (PE) is a polymer which is extensively utilized in materials manufacturing due to its high inertness and resistance to degradation.

1.2 Microplastics in the aquatic environment

Due to wind, tide, and river movement processes, microplastics eventually find their way from the land into the ocean [9] [6]. Plastic enters the sea in amounts 4.8 to 12.7 million metric tons annually [7] [9]. With changes in place, time, environmental conditions, physical pressures, and biological processes, microplastics in the ocean are broken down into smaller fragments or persist in the aquatic. These particles accumulate and dispersal in the marine environmental or shorelines [9]. According to studies, rivers are the primary conduit for the movement of most of the plastic waste from land to the marine environment [10] [11] [12]. However, most of plastic debris is collected by collectors, which causes it to become stuck on riverbanks or overflow into rivers, damaging freshwater ecosystems [13]. The impact of microplastics on aquatic organisms is primarily dependent on the dimensions of the particles. The entanglement caused by large plastics such as fishing lines and nets results in immediate damage to different species including invertebrates, birds, mammals, and turtles. Microplastics have been found to contain diverse toxins, and smaller plastic particles have the potential to be ingested by a range of aquatic

organisms, which could result in gastrointestinal harm as well as modifications in the metabolism [14]. Toxins tend to accumulate in organisms that move up the food chain, resulting into potential changes in behaviour, physiological alterations, reproductive issues, and DNA damage [6]. In addition, microplastics found in aquatic ecosystems are the potential to serve as carriers for deleterious contaminants and pathogenic microorganisms [15] [16].

1.4 Plastisphere

In aquatic systems, these are microbial communities such as fungi, diatoms and bacteria that colonise the surface of Microplastics (MP) and are collectively known as '*Plastisphere*' [17] [18]. The Plastisphere explores the interactions within the microbiome, its metabolic capacity, and its impact on the surrounding environment. The structure and composition of microbial communities can be influenced by various factors involving substrate characteristics, environmental conditions, and timing of colonization of plastics [19]. Substrate properties generally include the type of material, dimensions, density, shape, color, and surface topography. The variability exists between different microplastics. The microbiome composition in aquatic ecosystems is influenced by various environmental factors such as light, pH, temperature, hydrodynamics, nutrient concentrations, and salinity [20]. In addition, pollutants (toxic metals, organic pollutants) also affect the composition and establishment of microbial communities on the MP surface in aquatic systems [21].

1.5 Aims and Objectives

The current research on plastic pollution is primarily focused on the degradation of microplastics by microorganisms. Low density polyethylene (LDPE) and High-density polyethylene (HDPE) account for 20% and 15% of global plastic waste respectively [21] [22]. HDPE is opaque and has a linear structure, LDPE are dendritic and usually transparent. They are widely utilized for construction supplies, daily necessities, medical equipment, and packaging materials. It is crucial to consider the composition of the microbiome on the surfaces of LDPE and HDPE to comprehend the microbial degradation of microplastics. Salinity and temperature have been found to be important environmental parameters that influence the development of microorganisms on the surface of microplastic [23] [242] [25]. However, Insufficient consideration has been given to the physical properties of microplastics how to affect the microbiome composition. The first objective of this research was to characterize the microbial communities on the surfaces of two plastic polymers "LDPE" and "HDPE", as influenced by changes in environmental parameters (temperature, salinity). The second objective was to discuss whether the differences in microbial communities on LDPE/HDPE surfaces are mainly due to the differences in plastic properties or are environmentally driven.

2. LITERATURE SURVEY

Study 1 (PRJNA678316): n = 27 samples, Germany, Europe, Illumina MiSeq - 16S rRNA-gene amplicon sequencing
Study 2 (PRJNA515271): n = 23 samples, Croatia, Europe, Illumina MiSeq - 16S rRNA-gene amplicon sequencing
Study 3 (PRJNA495136): n = 7 samples, France, Europe, Illumina MiSeq - 16S rRNA-gene amplicon sequencing
Study 4 (PRJNA612500): n = 145 samples, France, Europe, Illumina MiSeq - 16S rRNA-gene amplicon sequencing
Study 5 (PRJNA670618): n = 45 samples, China, Asia, Illumina HiSeq 2000 - 16S rRNA-gene amplicon sequencing
Study 6 (PRJNA528407): n = 87 samples, Spain, Europe, Illumina MiSeq - 16S rRNA-gene amplicon sequencing
Study 7 (PRJNA724000): n = 11 samples, Chile, South America, Illumina MiSeq - 16S rRNA-gene amplicon sequencing

The Plastisphere team has collected a total of seven studies on polyethylene (PE). These studies involve the analysis of various types of microplastics, including polystyrene (PS), marine pellets, virgin polyvinyl chloride (PVC), polypropylene (PP), glass, polyethylene terephthalate (PET), and untested samples (ND), in addition to polyethylene. All seven studies provided specific locations and dates of sample collection, which were obtained from publicly available records，,including freshwater and marine environments (Studies1, 2, 6, 7), soils (Study5), sediment and floating material (Study3), and biogenic gut (Study4). Certain studies offered detailed regional information and precise geographic coordinates. The identification of the geographic provenance of individual samples facilitates comprehension of the effect of variations in environmental factors on the microbial community composition of microplastics [26][27]. Environmental variations commonly considered in research encompass different pH [Study 3], salinity concentrations (Study 3), temperature fluctuations (Study 3, Study 5), exposure to light (Study 5), and levels of humidity (Study 5).

Study 2 focused on the effects of distinct light conditions (full exposure to solar radiation or dim light conditions) on the bacterial communities on different sizes of high density polyethylene (HDPE) and low density polyethylene (LDPE) as well as several other materials in the marine. The study findings indicate that there were no significant variations in the composition of microbial communities among glass, HDPE, LDPE, and PP materials, except for PVC. Nevertheless, autotrophs which live and reproduce on inorganic nutrients were in high abundance in the ambient light treatment, due to the energy they obtain through photochemical reactions [28]. Low density

polyethylene (LDPE) and two additional microplastics from maritime sediments and floating objects were the focus of Study 3. The research revealed that an organism known as Alcanivorax borkumensis was able to degrade LDPE and that the shape of the biofilm on the polymer surface was mainly reliant on the material substrate, which has significance for the investigation of microbial degradation of microplastics [29]. High density polyethylene (HDPE) has been shown in study 4 to alter the intestinal flora of blue mussels [30]. Study 5 additionally looked at how LDPE affected soil microbial communities and nitrogen cycling. The findings show that LDPE powder has an effect on the structure of some communities in the soil that are involved in the nitrogen cycle [31]. LDPE and HDPE are of major concern in the field of microplastic pollution due to their extensive utilisation. This study will be focused on the microbiome on the exteriors of these two microplastics, which maintains significance for the investigation of plastic contamination.

According to the findings of Study 2 and Study 3, the properties of materials are a significant factor in the growth of biofilm on microplastic surfaces. The properties of plastic include dimensions, translucency, shape, colour, and density. According to the research, the colour of microplastic surfaces could potentially influence microbial communities [32]. In two additional studies, microbiome remains unaffected by the shape and size of microplastics, regardless of the microplastic type, spatial gradient, and collection date[33][34]. At present, a lack of study exists regarding the effect of transparency on the biofilms in the surface of microplastics. LDPE and HDPE are two distinct varieties of microplastics that exhibit varying degrees of transparency. This study focuses on investigating the impact of transparency on microbial communities residing on microplastics.

Currently, there are research focus on the prevalence of environmental factors in influencing biofilms on microplastics (MP). According to the research, plastics are prone to the effects of light, ultraviolet (UV) radiation, and heat [35]. Andrady (2011) demonstrated that solar infrared absorption causes an increase in temperature of darker coloured plastic fragments, which in consequently accelerates photo-induced oxidative degradation at elevated temperatures [36]. Furthermore, the concentrations of salinity, temperature, and dissolved oxygen also are important factors that impact both the composition and abundance of microbial communities in aquatic environments [37] [38]. Mark et.al (2004) carried out an investigation on the correlation between the abundance of Vibrio traumaticus, a pathogenic bacterium belonging to the genus Vibrio, temperature, and salinity in coastal environments [25]. Nevertheless, it is noteworthy that none of the 7 studies dedicated consideration to the possible effect of environmental factors, especially salinity and temperature, on the microbiome present on microplastics. Low density polyethylene (LDPE), polyethylene terephthalate (PET), and polystyrene (PS) were collected in ocean at 20 °C and a salinity of 38 g/L in Study 3 [39]. A dark environment, 50% relative humidity, and a temperature of 25 °C were applied in Study 5 to examine the effect of powdered LDPE MPs on

the composition of soil microbial communities and the microorganisms engaged in the nitrogen cycle [31]. It has not yet been adequately studied how temperature and salinity specifically affect the microbiome on microplastic surfaces. Therefore, the focus of this study will be on how the microbial communities of LDPE and HDPE change under three salinities (High salinity, Low salinity, freshwater), and three temperatures (Psychrophylic physcrophiles, Mesophylic mesophiles, Thermophylic psychrophiles).

All seven studies were sequenced using 16S rRNA amplicon sequencing based on the Illumina MiSeq platform for the highly variable V3-V4 region. 16S rRNA gene amplicon Sequencing is used extensively in the taxonomy of microbiome [40] [41]. Given the established importance of 16S rRNA gene amplicon sequencing and its extensive citation in studies, this study will employ this method for the purpose of analysing the diversity, taxonomy, and phylogeny of microbial communities present on LDPE and HDPE.

Most studies used the Shannon entropy and the Simpson index for the Alpha diversity analysis. The Shannon index combines the richness and evenness of the community, and the Simpson index estimates the microbial diversity of the samples. Study 4 discussed the Equitability Index in addition to these two indices. Study 5 used the ACE and Chao1 indices, both of which were used to evaluate the numbers of ASVSs in the samples. Study 6 also used the Chao1 index to perform Alpha diversity analysis. According to the different purposes of the different indices, this study will focus on the analysis of microbial diversity, richness, and evenness within the samples by Shannon, Simpson, Richness, Pielou's evenness and Fisher' alpha. The main statistical methods used for Beta diversity analysis included Bray-Curtis distance, UniFrac distance and Jaccard index. Except for Study 2, all other studies utilised the Bray-Curtis distance for the beta diversity analysis. Study 6 also applied the UniFrac distance. Principal Coordinates Analysis (PCoA) was the method for visual statistical analysis of beta diversity in most of studies. Apart from the evaluation of Alpha diversity and Beta diversity, the investigation of the microbiome composition in the samples constitutes a crucial aspect of the analysis process. The microbial community composition and relative abundance of species have been identified through taxa analysis in Study 3 and Study 4. Furthermore, Study 1and Study 5 investigated the intricacy of microbiome interactions within the samples through the utilisation of a Co-occurrence network. The majority of the seven studies analysed only alpha and beta diversity, indicating a limited analysis of the microbiome. In this study, taxa analysis will be performed to determine the microbial composition of the samples and the most abundant microbiome of different groups. The most relevant microbial taxa and differences in microbial taxa within groups will be identified using CODA-LASSO, followed by the calculation of Core microbiome the core microbiome in each group of samples will be identified.

3. MATERIALS AND METHODS

Data collection methodology

Meta-analysis (Meta study) is a statistical technique that utilizes empirical evidence to compare and consolidate the findings of numerous independent studies or amalgamate individual independent studies pertaining to a particular topic [42] [43]. The utilization of meta-analysis enables the combination and statistical assessment of voluminous and complicated literature, resulting to more precise deductions regarding the subject matter under investigation. Additionally, it facilitates the examination of the coherence of evidence across multiple studies and differences between them. The limitations of Meta-analysis that it requires a considerable amount of time to identify appropriate studies, and the reality that not all studies can provide adequate data for inclusion and analysis [44][45]. Applying meta-analysis to the domain of microbiology helps to determine how microplastic properties or environmental factors affect the composition and relative abundance of microbial communities in the ecosystem, as well as their ability to degrade polymers and whether they are commensal or pathogenic bacteria. The primary stage of the meta-analysis centring on the identification, inspection, and compilation of published papers that significance or relevance to the subject -the microbial communities that colonized in HDPE and LDPE. In this study, the literature survey was initiated by the Plastisphere team (research group of Dr Ijaz) two years ago and collected a total of seven studies on polyethylene and 345 publicly accessible individual samples from these studies, which is collected form six countries.

The 16S rRNA gene is a genetic sequence that corresponds to the ribosomal RNA that is encoded in bacteria. This gene has gained significant popularity in the areas of phylogenetics, taxonomy, and microbial diversity studies, and is considered one of the most frequently utilized biomarkers. 16S rRNA amplicon sequencing is a technique for identifying the phylogeny and taxonomy of microorganisms on the surface of LDPE and HDPE， through the analysis of the relative abundance of the variable V3-V4 region of microorganisms utilising high-throughput sequencing techniques [46]. The utilisation of the Illumina MiSeq high-throughput DNA sequencing platform for 16S rRNA sequencing enables the simultaneous sequencing of multiple samples, which facilitates the acquisition of information regarding microbial classification, abundance of species, community composition, phylogeny, and differences in communities. The Illumina Miseq platform has emerged as the leading technology in microbial ecology research due to its exceptional adaptability, capacity for processing large volumes of data, and minimal margin of error [47].

Sequences from each study were processed individually using Qiime2 [48]. Since a meta-analysis combines samples using multiple V regions of the 16 S rRNA gene, two algorithms are typically used for constructing amplicon sequence variants (ASVs): DEBLUR algorithm is typically used for longer amplicon regions (e.g., V3-V4), where it is sometimes difficult to overlap paired-end reads; and b) DADA2 algorithm in all other cases. Further, taxonomy was assigned using a

Bayesian Least Common Ancestor (BLCA) approach (using the SILVA138 database) as recommended by Keating et al. 2020 [49], to obtain more sequence-level resolution. Given the utilisation of multiple V-regions, with some exhibiting distinct features, the goal is to combine a maximal number of ASVs to expedite the production of a phylogenetic tree based on full 16 S rRNA sequences. Our collation strategy is already evaluated to cause minimal loss in beta diversity and is mentioned in detail in Thom et al [50], with the code already released as part of Keating et al 2020. The workflow for a single sample as processed in QIIME2 is given in Figure 2 (From Thom et al. 2022 and is used as it is for plastisphere work).

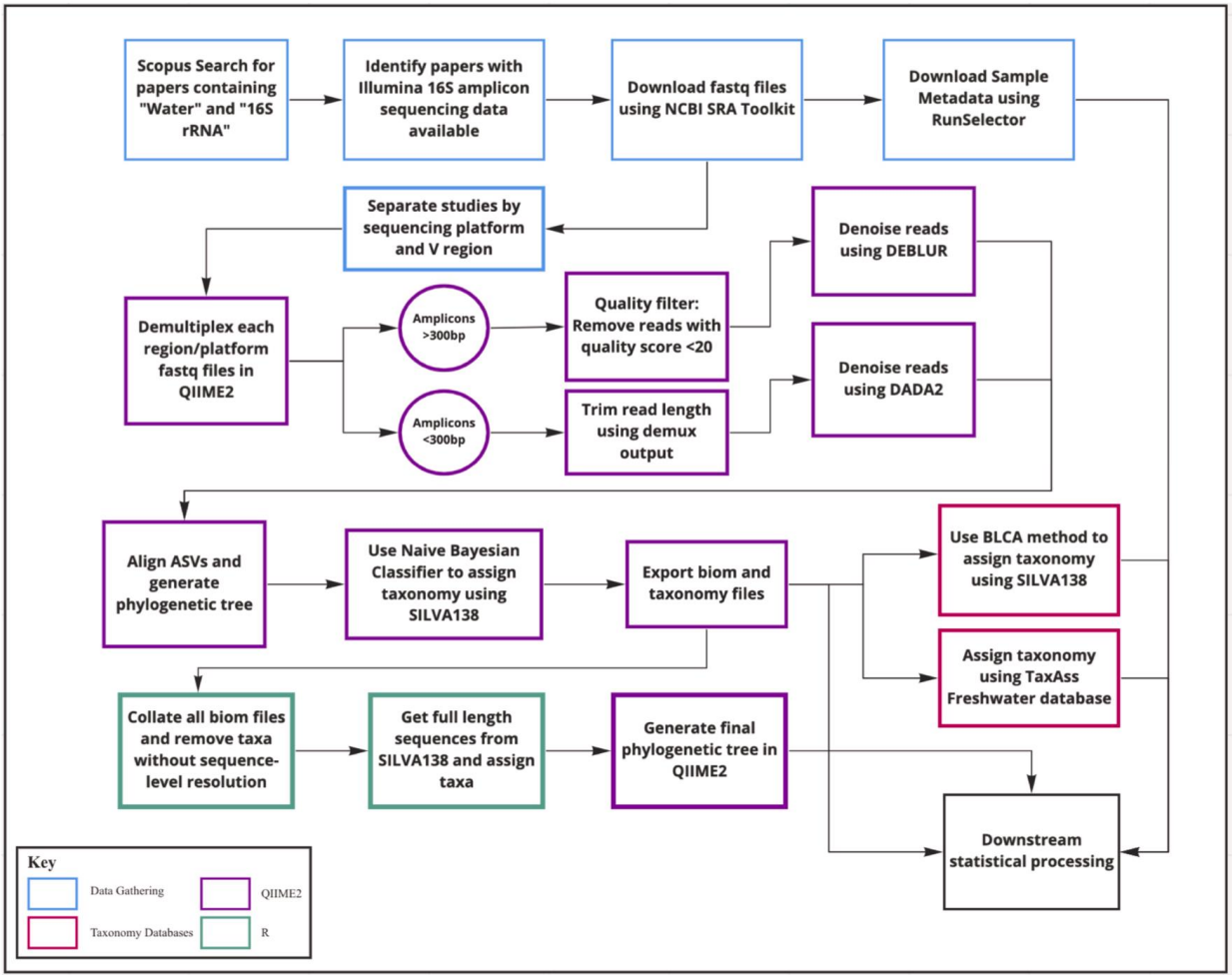

Figure 3.1. Meta-analyses workflow from Thom et al (2022) and utilised in this study to generate ASVs for plastisphere project [50].

Meta table column composition

The meta table was developed based on further research and analysis of the literature survey。 The table includes sample columns and 32 characteristic categories. During the literature survey, the members of the Plastisphere group put forth the suggestion that the present study should focus on the variables of temperature, salinity, and material disparities, specifically regarding transparency. The characteristic categories implemented in this study are as follows:

1) Four Temperature columns：Temperature, Temperature range，Temperature_range_2 and Temperature_range_material

The temperature values of the samples are expressed in degrees Celsius and are further categorised based on their respective temperature intervals. Microorganisms that are detected in samples with temperatures < 20°C are categorised as "Psychrophile" [51], while the range of 15-20°C are classified as "Thermophile". The temperature range of 20-45°C is classified as "Mesophile," while the range of 40-85°C is categorised as "Thermophile" [52]. The microorganisms in the samples were classified as Psychrophylic physcrophiles (7.2 °C < t< 9.1 °C), Mesophylic mesophiles (11.3 °C < t< 27.2 °C) and Thermophylic psychrophiles (16.9 °C < t< 19 °C).

2) Three Salinity columns：Salinity, Salinity_range, Salinity_range_Material

Salinity refers to the concentration of salt that is dissolved in water, measured in grammes of salt per kilogramme of water (g/kg). The column denoted as "Salinity_range" categorises the specimens into three groups based on their salinity levels: freshwater with 0 g/kg salinity, low salinity water with 7.1 g/kg, and high salinity water with a range of 32.2 g/kg to 38.8 g/kg.

3) Material column：

This study will concentrate on the investigation and discussion of high density polyethylene (HDPE) and low density polyethylene (LDPE) in the material column of the meta table, as recommended the plastisphere team.

4) Other features

The meta table encompasses additional feature columns such as pH, location of collection, time of collection, metagenome source, and sequencing platform. Among the sampled individuals, it is noteworthy that not all of them possess complete feature columns. These features will not be considered and discussed further in this study. However, they may be referred to in other sections to help with a more comprehensive interpretation and observation of trends in variance.

Statistical methods

The R software platform is a programming language that prioritises statistical analysis, data science, and visualisation. It is extensively employed for the statistical examination of biome data [**]. The integration of the R with amplicon sequencing analysis enables visualisation of results, thereby providing a clearer representation of community structure and abundance distribution. The necessary data for comprise evolutionary trees, sample metadata, the ASVS abundance table, and the ASVS taxonomy file. The main packages available in RStudio for statistical analysis and visualisation are: '***phyloseq'*** (for analysis and visualisation of high-throughput phylogenetic sequencing data for ASVS clustering) [53], '***vegan'*** (for calculating alpha and beta diversity and taxa analysis), '***ggplot2***'(for visual analysis), '***ape'*** (for phylogenetic and evolutionary analysis) [54], '***coda4microbiome'*** (for taxon characterisation of the microbiome) [55] and '***microbiome'***

package (for core microbial analysis). The scripts pertaining to all implemented methods in executing R can be accessed via the following hyperlink: http://userweb.eng.gla.ac.uk/umer.ijaz/bioinformatics/ecological.html. The microbiome Seq packages can be accessed via the following link: http://www.github.com/umerijaz/microbiomeSeq.

3.3.1 Alpha Diversity

The analysis of alpha diversity is an important component in the exploration of microbial diversity within community ecology, modelling the species as indices of diversity parameters distributed in a logarithmic series. It focuses on analysis the extent of species diversity in a specific geographic area or ecological system. To assess this, various statistical measures are employed to approximate the number of species present (Richness), evenness of distribution of individuals in the community (Evenness) and species diversity in a sample. The Community richness index reflects the abundance of species within the sample, with common indices include Chao's Index and Richness. The evenness of a community is indicative of the manner in which species are distributed within the sample. This distribution is determined by the relative abundance and evenness of each individual species. Additionally, the richness index of a sample, which is commonly referred to as Shannon's evenness or Pielou-e index, is positively correlated with community evenness. The diversity of a community can be assessed by considering both the number of species richness and evenness, that can commonly be measured by Fisher's alpha, Shannon's index, or Simpson's index. During this stage, a total of five different approaches were employed to perform alpha diversity analysis on all samples. These methods included Fisher alpha, Pielou's evenness, Richness, Shannon entropy, and Simpson diversity index.

a) Fisher's alpha (Fisher's logarithmic series model) is a diversity index that attempts to describe the relation between the number of species and the number of individuals in a Random Sample [***]. According to a thorough examination of its properties by Taylor (Taylor, 1978) [**], the log series takes the form:

$$x, \frac{x^2}{2}, \frac{x^3}{3}, \ldots\ x^n/n \qquad 3.1$$

Equation 3.1 – where X^n/n is the number of species predicted to have n individual, S is the total number of species[56].

b) The measurement of the relative abundance of species in a sample is accomplished through the utilisation of evenness. Shannon's evenness（Pielou's evenness） was first proposed by Pielou（Pielou，1966）[57]. It is defined as the ratio of the observed Shannon index in a given sample to the maximum possible Shannon index that can be achieved in a sample with the same number of species. The Pielou index (J) is defined as:

$$J = H/ln(S) \qquad 3.2$$

Equation 3.2 – where H is the Shannon index; S is the sample species richness index; and ln(S) is the maximum Shannon index that can be achieved with equal species richness (when all species in the sample have the same abundance). The value of J is equal to 1 if all species in the sample have the same relative abundance. Whereas the value of J will be infinitesimally near to zero if one species heavily dominates.

c) The Richness index is the sum of the number of species with a community abundance greater than zero, with higher values reflecting a greater variety of species in the sample. However, this approach just considers species is present in the sample and ignores its relative abundance. Additionally, variations in sampling depth have an impact on the richness index.

d) The Shannon index (Shannon entropy index), which considers species richness and evenness within the sample, is used to measure the variety of microorganisms in a sample [58]. Conversely, as the number of species in the community increases, it becomes impossible to determine what species the randomly selected individual is, and increased uncertainty results in increased species diversity in the community. If the community only contains one species, the randomly selected individual can only be this one species in the community, and uncertainty is zero. Therefore, a higher Shannon index denotes more uncertainty. The number of unknowns in the community and the diversity of species increase with increasing degree of uncertainty. The Shannon index has an advantage over other indices in that variations in library sizes have little of an impact on it [59]. The following formula is used to compute the Shannon entropy (H):

$$H(X) := -\sum_{x \in X} p(x) logbp(x) = E[-logbp(X)] \qquad 3.3$$

Equation 3.3 – Σ is a Greek symbol that means "sum"; p(x) is probability; log () is a typical log function. The Shannon index attains its highest value in a state of complete homogeneity within a community, wherein the abundance of all species is uniform.

e) The Simpson index considers both species richness and evenness, but its sensitivity to evenness is greater than that of the Shannon index [60]. The Simpson diversity index exhibits a numerical range that spans from 0 to 1. At the point of minimum species diversity within a community, characterised by the presence of only one species, the Simpson index attains a value of 0. When there are an infinite number of species (highest richness) and the number of each species is consistent (perfectly homogeneous species), the Simpson value is a maximum of 1. The Simpson index exhibits a positive correlation with an increase in richness. The mathematical expression for the Simpson index, denoted by D, is provided below:

$$D = 1 - \sum_{i=1}^{N} (p_i)^2 \qquad 3.4$$

Equation 3.4 – N is the community species richness index (the total number of species types); pi is the relative abundance of species i.

f) In order to accurately represent diversity, it is necessary to convert the Shannon and Simpson indices into an effective number of species [61]. During the early 1970s, Hill (Hill, 1973) integrated three diversity indices, namely Richness, Shannon's index, and Simpson's index, which belong to the same family, into a cohesive concept of diversity. This led to the development of a novel and justifiable definition of evenness [62]. The formula for Hill numbers (D) is as follows:

$$qD = (\sum_{i=1}^{R} p_i^q)^{1/(1-q)} \quad 3.5$$

Equation 3.5 – q is a parameter. Some popular diversity indices correspond to the basic sum as calculated with different values of q. q = 0 is the species richness index; q = 1 is the effective number of species for the Shannon entropy; q = 2 is the effective number of species for the Simpson index. D is specific Hill numbers, which depends on the value of q; R is the species richness index in the community; and pi is the proportion of the community represented by species.

3.1.2 Beta diversity

Following the completion of the Alpha diversity analysis, a Beta diversity analysis was conducted. In contrast to Alpha diversity analysis, Beta diversity is a comparative analysis of the composition and diversity of microbial communities in different samples, measuring the differences between samples and mainly considering the number and abundance of species [63]. The fewer species of organisms shared between different samples and the lower similarity between groups, the higher the Beta biodiversity. Beta diversity, in conjunction with alpha diversity, comprises the complete diversity or biological heterogeneity among samples. Beta diversity is providing:

$$Total\ number\ of\ pairwise\ beta\ diversity\ values = \frac{n(n-1)}{2} \quad 3.6$$

Equation 3.6 – Number of beta diversity results for a sample space of size n.

Principal coordinate axis analysis (PCoA) is used for the visualization of Beta diversity, which presenting similarities or differences in community composition between different samples. This method is used extensively in the area of microbiological analysis.

In order to investigate the potential impact of 'substrate type', 'salinity', and 'temperature' on the microbial community composition, this study primarily employed species distances (Bray-Curtis) and species evolution-based distances (Unifrac distance) to assess the significance of the aforementioned factors. Distance indices have become increasingly prevalent in microbiology for the purpose of analysing beta diversity in recent times. The Bray-Curtis distance, which relies on independent operational taxonomic unit (ASVs) tables, is a mathematical approach utilised to evaluate the level of resemblance among various samples [64]. When the Bray-Curtis distance between two samples is 0, it indicates that the species composition of both samples is identical. Conversely, a distance index of 1 implies that the two communities being compared do not share

any species. It can therefore be directly converted to a similarity index by "1 - distance index = similarity index". A permutation multivariate analysis of variance (PERMANOVA) was conducted utilising the Bray-Curtis distance matrix. The PERMANOVA method is a non-parametric version of multivariate ANOVA that is commonly employed to evaluate the similarities and dissimilarities in the composition of microbial communities [65]. The '***vegan***' package in Rstudio was utilised for conducting the analysis. The permutation test is used for significance analysis, with the purpose of detecting whether there are significant differences in the composition of different groups of colonies. It should be calculated as shown in Equation 2.6:

$$BC(s1, s2) = 1 - \frac{\sum_{s1,s2} \min(OTU)}{\sum_{i=1,2} si} \quad 3.7$$

Equation 3.7 – Expression for calculating the Bray-curtis distance between two samples S1 and S2.

The Bray-Curtis distance metric solely considers the relative abundance and occurrence of species, without incorporating any evolutionary associations among them. The UniFrac distance considers the evolutionary relationships among operational taxonomic units (ASVs) and provides a more objective assessment of the similarity between two community samples [66]. The calculation of the UniFrac distance is predicated on the utilisation of a phylogenetic tree [***]. UniFrac distance value of zero primarily signifies that the evolutionary categorization of the two communities is congruent. The UniFrac distance metric comprises two variants, namely Unweighted and Weighted. The unweighted approach solely considers the existence or non-existence of microbial constituents within the community, without factoring in their relative proportions. The weighted approach considers the phylogenetic interrelationships among members of a community and their relative abundance within their respective communities.

3.1.3 Taxa analysis

To categorise microbial species, present in each sample based on their taxonomic traits, a comparative analysis is conducted between the sequences and a reference database, and the relative abundance of species is counted and plotted for each sample. Within the field of taxonomy, a taxonomic unit refers to a classification unit that denotes a distinct assemblage of organisms situated within diverse taxonomic hierarchies. The table of relative abundance of dominant species is utilised to document the relative prevalence of distinct species in a particular sample or ecosystem, along with the count of individuals represented by each species or as a proportion of the entire community. The assessment of the relative abundance of the most abundant taxa was conducted in R programming language utilising the ***'microbiomeSeq'*** package. The plotting of the relative abundance of taxa was accomplished through the utilisation of the ***'plot_taxa'*** function [67].

3.1.4 CODA LaSSO

The challenge of identifying microbial features holds significant importance in the analysis of microbiomes [68]. The CODA-LASSO model is a regression method. This model has broad applicability and is known for its computational efficiency [68]. This study utilised CODA-LASSO as a statistical tool to examine the taxonomic features of the microbiome in the specimens. The aim was to ascertain the most pertinent microbial species within the cohort and to distinguish between the microbial species in the two samples. In this study, the LDPE and HDPE specimens were segregated into several sub-samples categories according to distinct environmental factors (three temperature classifications and three salinity classifications). The sub-sample groups were subjected to pairwise comparisons, such as compare High salinity HDPE with Low salinity LDPE, in order to elucidate the distinctions in microbial community structure and microbial species among the samples.

3.1.5 CORE MICROBIOME

The practise of conducting core microbiome analysis holds significant prominence in the field of microbial ecology. It is essentially a statistic of the frequency of occurrence of each species in the sample in which it is present and is used to identify the core microbiome in each group of samples. The taxonomic resolution of the core microbiome must be determined prior to sequencing, as the composition of the microbiome is influenced by the choice of 16S rRNA gene amplification regions and associated PCR primers [69]. The quantification of the core microbiome is achieved through a taxonomic method that relies on 16S rRNA sequences to accurately identify taxonomic units that constitute the core microbes. This approach enables the subsequent analysis of the ecological properties and functions of the core microbiome [69]. The process of core microbiome analysis involves clustering the data based on similarity in species abundance, followed by visualisation through the use of a Heatmap. A heatmap is a visual representation of data that utilises colour changes to present information in a two-dimensional matrix or table. The varying shades of colour used in the heatmap correspond to the magnitude of the data values being represented. Similarities and differences in community composition of multiple samples at each taxonomic level are reflected according to the colour gradient and degree of similarity.

4. RESULT

Alpha diversity analysis results

The investigation of alpha diversity was conducted after the compilation of pertinent metadata, with the goal to verify the microbial community diversity present in the samples. This study centred on the impact of temperature and salinity on the bacterial communities that colonized in the surface of LDPE and HDPE. The Alpha diversity of the microbiome within the samples was assessed using five distinct indices, namely Fisher's alpha, Pielou's evenness, Richness, Shannon, and Simpson.

Alpha diversity was assessed to determine the impact of salinity on the microbial community, as depicted in Figure 4.1.1. In this study, the samples were categorised into four cohorts based on the gradient of salinity. In terms of averages for the five indices, LDPE from freshwater environments (blue) and HDPE from low salinity environments (brown) show similar averages and show relatively high alpha diversity, especially when compared to HDPE from high salinity environments (yellow). The study found that the values of Fisher's alpha and Richness indices were marginally lower for LDPE in freshwater environments (Blue) and HDPE samples in low salinity environments (brown) compared to LDPE in high salinity environments (red). However, the Shannon and Simpson diversity calculations yielded more statistically significant outcomes. It is noteworthy that the differences within the groups of LDPE and HDPE samples obtained from the high-salt environment were substantial. In general, based on the alpha diversity calculations, it was observed that the microbial communities on both media exhibited greater diversity in the lower salinity environments. A higher alpha diversity means that there are more species in samples, and a more diverse species means more functionality and better degradation of plastics.

As shown in Figure 4.1.2, samples were divided into five groups based on temperature. The impact of temperature on the microbial community was subsequently evaluated by means of Alpha diversity analysis. However, the constraints of the sampling process resulted in HDPE not being collected in the psychrophilic physcrophiles environment. Under the temperature conditions of Thermophylic, it was observed that HDPE (brown) exhibited a greater degree of alpha diversity in comparison to LDPE (blue). This observation suggests that the microbial community present on HDPE was more diverse and stronger in the thermophilic environment. The results of the study indicate that LDPE (red) obtained from mesophilic environments exhibited a notably greater alpha diversity in comparison to HDPE (yellow). This suggests that the mesophilic environment is more conducive to the growth of microbiome on LDPE. In particular, HDPE from Thermophylic psychrophiles and LDPE from Mesophilic environments (red) show similar indices of mean values. The plot shows that HDPE collected from psychrophilic physcrophiles exhibits the least alpha diversity, implying that psychrophilic temperature conditions are highly unfavourable for the growth of microbial communities on HDPE surfaces. And there was a strong association with the

growth of rare or pathogenic species [70]. The initial analysis into alpha diversity serve to establish a credible conjecture that the diversity of microbial communities on LDPE and HDPE surfaces is influenced by temperature and salinity. The analysis of alpha diversity is a significant metric for assessing the intricacy of microbial communities and their capacity for degradation. However, it is unsuitable for conducting phylogenetic diversity analysis. This study will complement the ASVS diversity information with other analyses of algorithms.

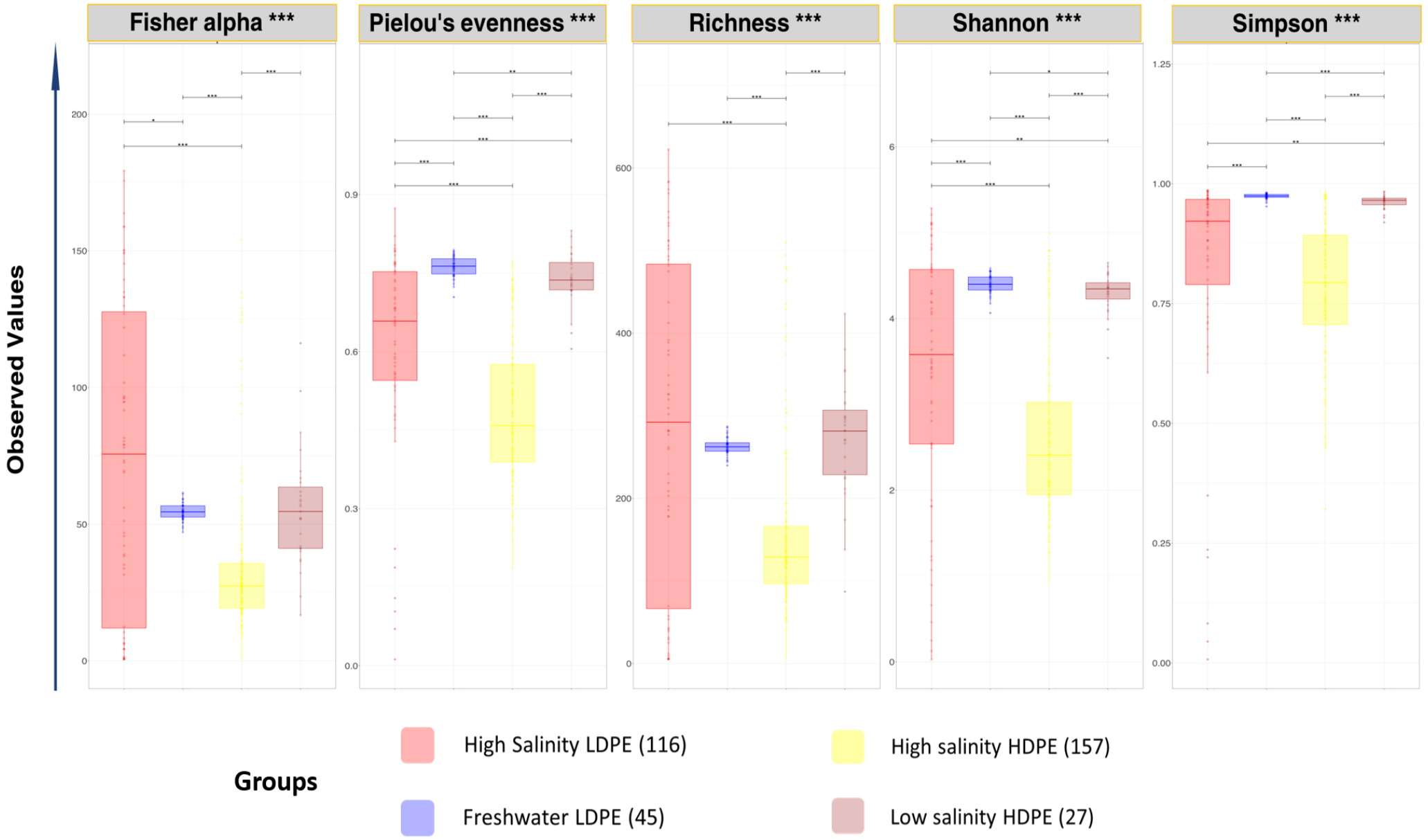

Figure 4.1.1 Alpha diversity of all samples across the LDPE and HDPE in different salinity. Number of samples in brackets. The significance are shown with $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$.

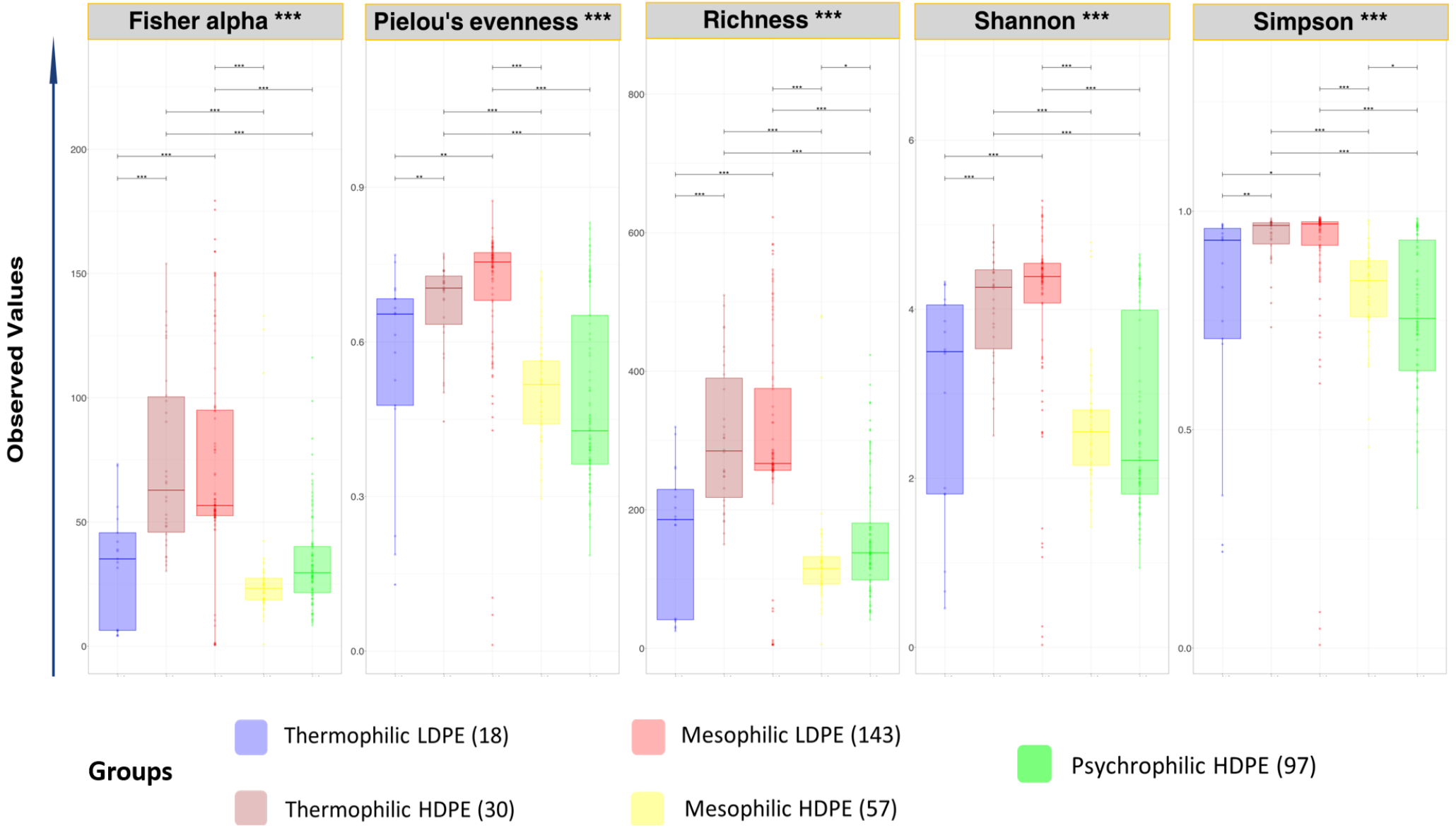

Figure 4.1.2 Alpha diversity of all samples across the LDPE and HDPE in different temperature. Number of samples in brackets. The significance are shown with $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$.

Beta diversity analysis results

As with the Alpha diversity analysis, the LDPE and HDPE samples from the seven studies were divided into four and five groups based on salinity and temperature respectively. The beta diversity analysis of the LDPE and HDPE samples in various salinities was conducted using the Bray-Curtis distance and the Unifrac algorithm. PCoA is used for visualisation of beta diversity analysis. Principal Coordinate Analysis (PCoA) is a statistical method that converts distance matrix data into a visual representation of coordinate axes. The horizontal and vertical axes are scaled according to relative distances, while the percentage value in brackets represents the proportion of variables that can be accounted for by the principal component, without any substantive interpretation. Dots of different colors represent different ASVSs. The plot reveals that the closeness of samples is indicative of their similarity, while greater distance between samples suggests more differences. The plotting of an ellipse utilizing a confidence interval of 95%. The calculation of R^2 values for regression index is performed using PERMANOVA.

The analysis of β-diversity of microbial communities on LDPE and HDPE surfaces at different temperatures was conducted using the Bray-Curtis distance, shown in Figures 4.2.1 and 4.2.2. It is imperative to consider the clustering within the sample while analysing the PCoA plots. As shown in Figure 3.2.1, the yellow points are highly scattered, implying high differences within the HDPE (yellow) group in high salinity conditions. The even distribution of scattered points within this group suggests a reduced number of identical points and a possible increase in species diversity. The results indicate that there is minimal compositional variance within the sample groups, as evidenced by the intra-group clustering of LDPE (blue) in freshwater conditions. Notably, the distance between high salinity HDPE (yellow) and Freshwater LDPE (blue) was extremely far, demonstrating significant differences between the two groups of samples, implying that the composition of the microbial community within the two samples was distinct. In contrast, high salinity LDPE (red) and low salinity HDPE (brown) were almost clustered together, indicating significant similarity.

As shown in Figure 3.2.2, the Mesophylic mesophiles LDPE (red) had extremely within-group variability. The HDPE samples from Mesophylic mesophiles (yellow) and Psychrophylic physcrophiles (green) also showed a high level of dispersion, which means that the samples had significant within-group differences. But the distance between the two groups was minimal, indicating a resemblance in the microbial community structure of both groups. However, there exists a considerable disparity in the microbial community composition between Mesophylic mesophiles LDPE (red) and the preceding groups (yellow, green), as evidenced by the substantial distance separating them. Otherwise, Thermophylic psychrophiles HDPE (brown) and LDPE (blue) have a similar microbiome construction.

The clustering between samples in the beta diversity analysis based on Bray-Curtis distances was more significant than the clustering present in the UniFrac analysis using the identical dataset. The results of the UniFrac analysis are shown in Appendix. This was due to the Bray-Curtis measure of variability between two samples based on independent ASVS tables, whereas UniFrac calculates β-diversity based on evolutionary relationships in the lineages considered ASVSs [71][72]. The findings derived from the Bray-Curtis distance analyses (Figure 3.2.1) and UniFrac demonstrate that high salinity LDPE (red) and low salinity HDPE (brown) exhibit greater similarity in microenvironments and consistent evolutionary patterns. The results of the analysis Bray-Curtis distances (Figure 3.2.1) and UniFrac distances indicate that high salinity LDPE (red) and low salinity HDPE (brown) demonstrate greater similarities in their microenvironments and show consistent evolutionary patterns. Also, there were significant differences between the microbial composition of high salinity HDPE (yellow) and Freshwater LDPE (blue). The utilisation of these approaches can serve as a robust determinant of the microbial composition similarity between Thermophilic Psychrophiles HDPE (brown) and Thermophilic Psychrophiles LDPE (blue).

The observations indicate that HDPE have a higher degree of relative diversity in compared to LDPE. The utilisation of beta diversity in meta-analysis can provide elucidation of attachment materials (LDPE and HDPE) and differences in salinity and temperature are all important factors influencing the microbial community composition.

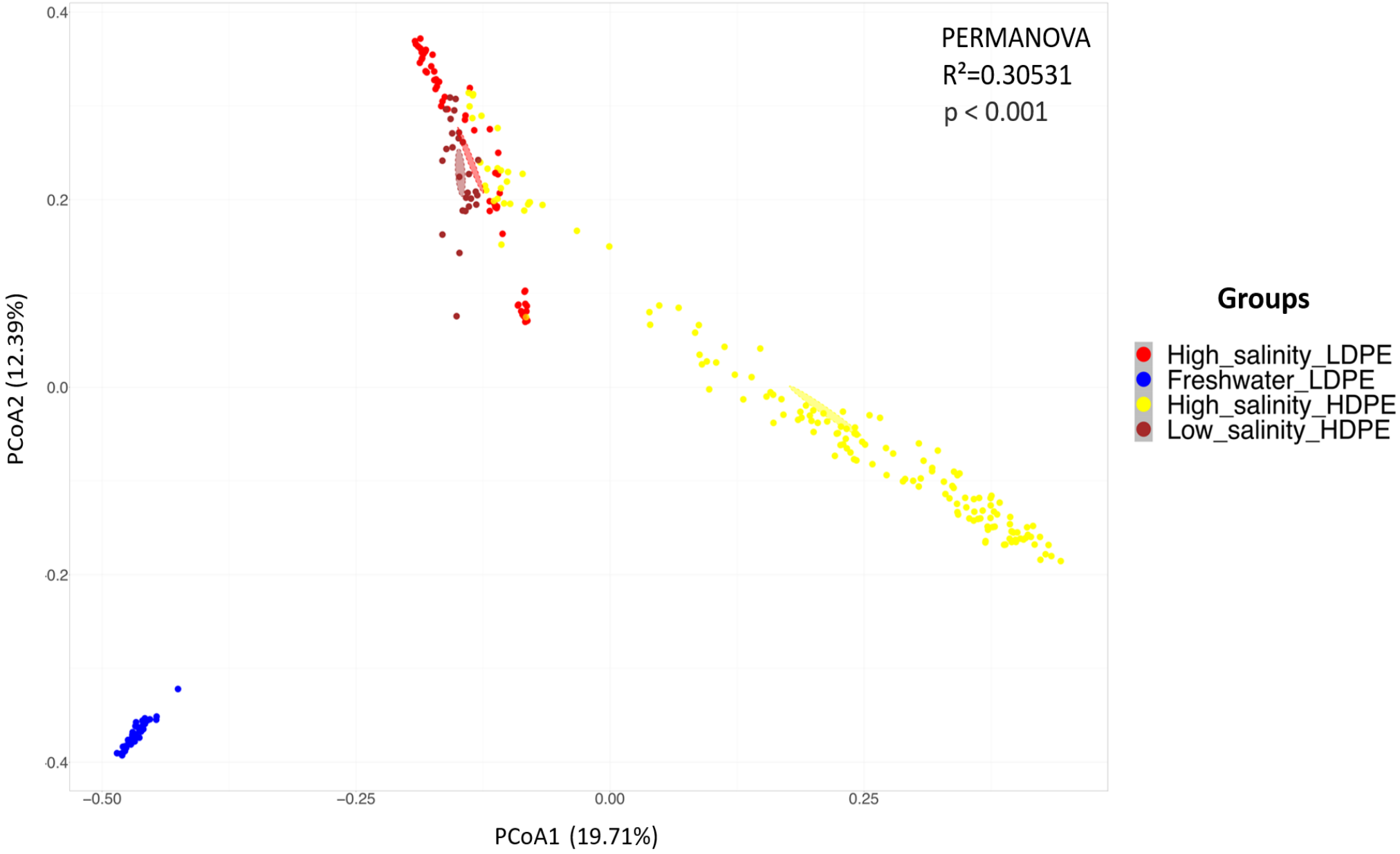

Figure 4.2.1 Beta diversity（Bray-Curtis）of all samples across the LDPE and HDPE in different salinity. R^2 is 0.30531. The significance is shown with $p < 0.001$.

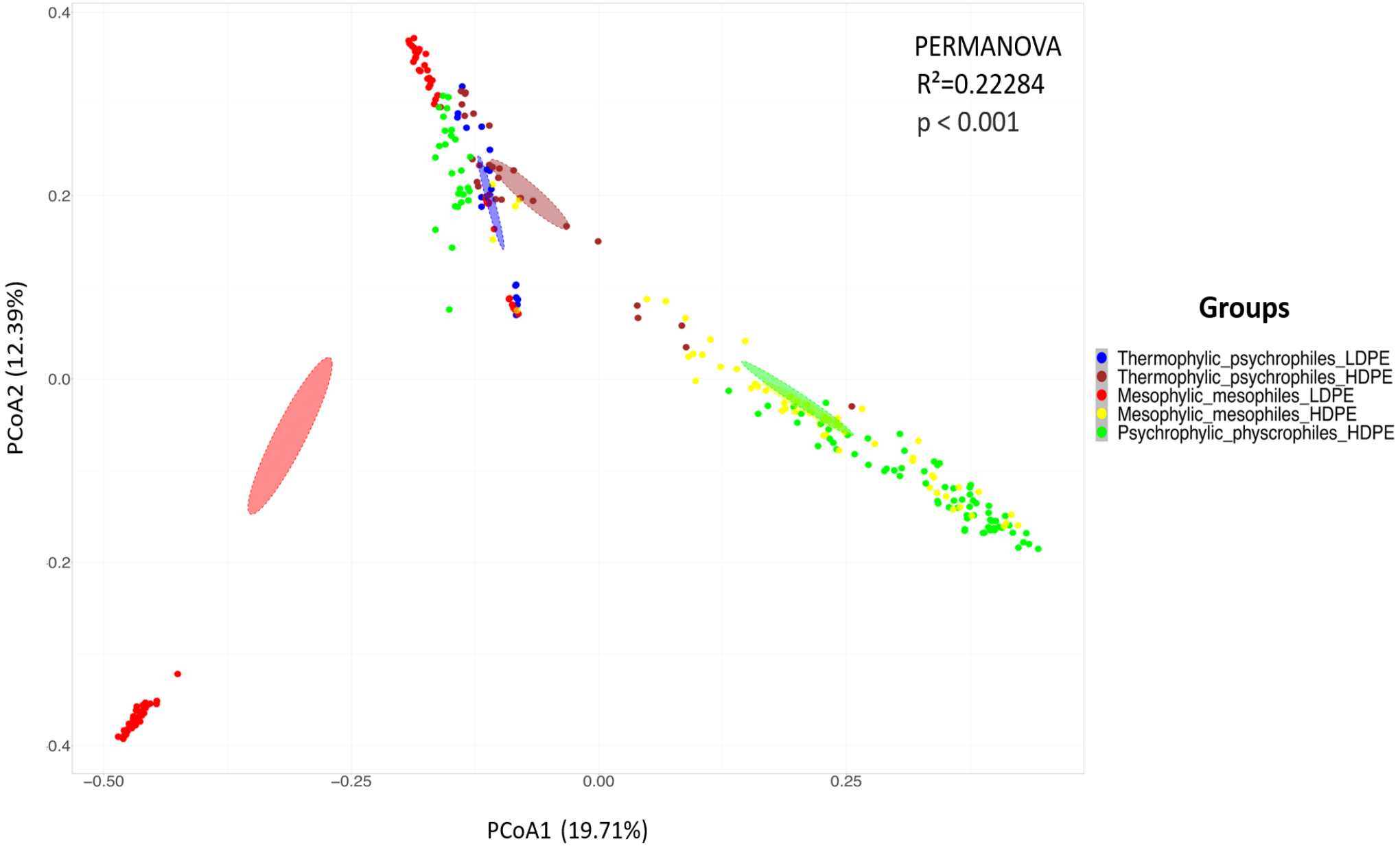

Figure 3.2.1 Beta diversity（UniFrac）of all samples across the LDPE and HDPE in different temperature. R^2 is 0.22284。The significance is shown with $p < 0.001$.

Taxa analysis results

The 25 most abundant microbiomes of the different samples were analyzed by taxa analysis. The LDPE and HDPE specimens were categorised based on three distinct levels of salinity and temperature. This analysis provided the microbial composition of samples and the relative abundance of microbiomes in samples.

As shown in Figure 4.3.1, Caldilineaceae was found as a high abundance genus in all four groups. Most members of the Caldilineaceae family are known to degrade complex organic compounds or to be involved in bioremediation and biofuel production [70]. The most abundant in the Low salinity HDPE are Caldilineaceae and Flavobacterium. The most abundant in the Low salinity HDPE are Caldilineaceae and Flavobacterium. Flavobacterium are Gram-negative bacteria, pathogens prevalent in aquatic environments [71] that can degrade complex organic compounds and while also contributing to the marine carbon cycle [72] . Polaribacter is classified as a Gram-negative aerobic bacterium, representing the most abundant genus of bacteria in the High salinity HDPE. This genus has been found to possess the capacity for fatty acid synthesis and play a crucial role in the carbon and nitrogen cycling processes within the marine ecosystem. Neptuniibacter also has been observed to exhibit high abundance in High salinity HDPE. The ability of Alcanivorax to degrade hydrocarbons was found to be present only in High salinity LDPE. This particular strain of microorganism can be employed for the purpose of purifying oil-polluted marine habitats or for bioremediation. The results indicate that Caldilineaceae, Nocardioides, and Vicinamibacteraceae were found to be the most abundant on LDPE samples in freshwater environments. Nocardioides is classified as a Gram-positive bacterium and contributes to degradation and metabolism of polymers [73]. The Vicinamibacteraceae family holds significant ecological importance in soil and carbon cycling due to their participation in the decomposition of different organic substances. Additionally, it was observed that two species of Eukaryota, namely Mrakiaceae and Cladosporium, exhibited a comparatively high abundant in in High salinity LDPE.

According to the Figure 4.3.2, Caldilineaceae was consistently present in all samples across different temperatures. All identified members of the Caldilineaceae family are all thermophilic that have been observed to be more abundant in both thermophilic and mesophilic conditions. Polaribacter was found to be most abundant in HDPE samples of Mesophylic mesophiles and Psychrophylic physcrophiles. A small amount was present in the HDPE of Thermophylic psychrophiles. Polaribacter was discovered to be most abundant in HDPE samples of Mesophylic mesophiles and Psychrophylic physcrophiles. However, they were not found on any of theother LDPE samples. In the same way, Neptuniibacter was only found in higher abundance in HDPE samples of Mesophylic mesophiles and Psychrophylic physcrophiles. Neptuniibacter exhibited a significant aboundent only in the HDPE samples of Mesophylic mesophiles and Psychrophylic

physcrophiles. Neptuniibacter has been found to have an essential part in nutrient degradation, biogeochemical cycling, and antibiotic production. Nevertheless, there currently exists insufficient evidence to indicate that this microbiome possesses the capability to degrade polymers. Otherwise, Flavobacterium was only detected in high abundant Psychrophylic physcrophiles HDPE, and Alcanivorax was only detected on Thermophylic psychrophiles LDPE. Notably, Mrakiaceae and Cladosporium were discovered to be present exclusively in Mesophylic mesophiles LDPE. Of these, the species in Cladosporium are mainly classified as endophytes or phytopathogens.

Based on the observations, it can be inferred that various species exhibit distinct growth patterns under varying conditions including temperature, salinity, and the utilization of LDPE or HDPE as a growth substrate. The presence of Polaribacter and Neptuniibacter only in HDPE samples indicates a strong preference for HDPE. The high abundance of them is significantly associated with elevated salt concentrations, whereas a thermophilic temperature may impede their proliferation. Alcanivorax exhibits a preference for high temperature and high salinity environments, and its growth is intimately associated with the attachment material LDPE. The absence of Psychrophylic and low salinity environments, which are conducive to the growth of Flavobacterium. The absence of the Alcanivorax and Flavobacterium in LDPE samples suggests that they are not tolerant to the temperature and salinity that are commonly associated with LDPE. It is notable to mention that the presence of two types of fungi, namely Mrakiaceae and Cladosporium, was exclusively observed in LDPE samples. This observation could be attributed to the sequencing method employed, where Eukaryota was primarily utilized for diversity analysis through 18s RNA sequencing [74].

The abundance tables of TAXA demonstrate a distinct impact of attachment medium on both microbial composition and abundance. Additionally, the composition and growth of microbial communities are significantly influenced by temperatures and salinities.

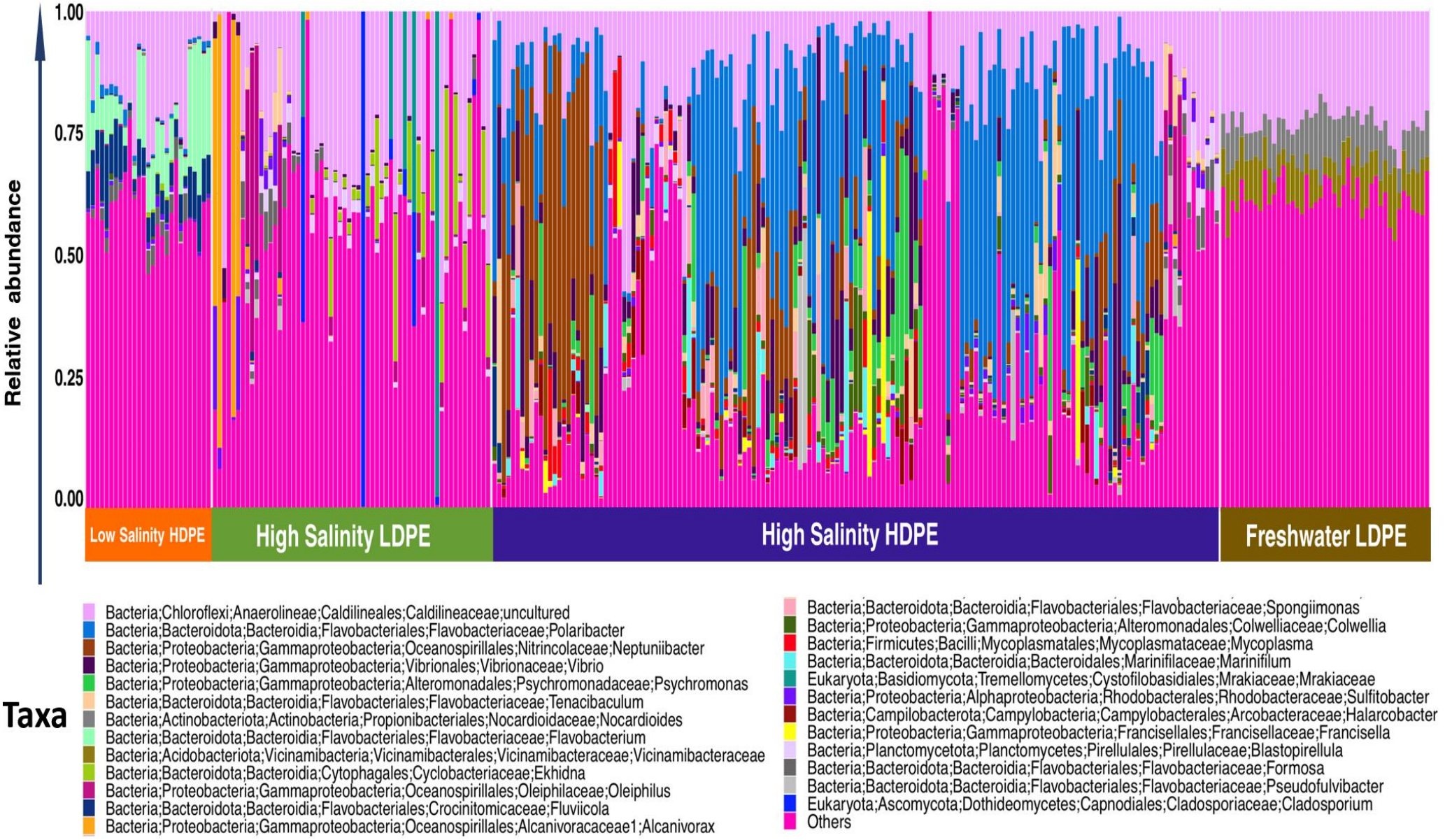

Figure 4.3.1 Taxa analysis of all samples across the LDPE and HDPE in different salinity.

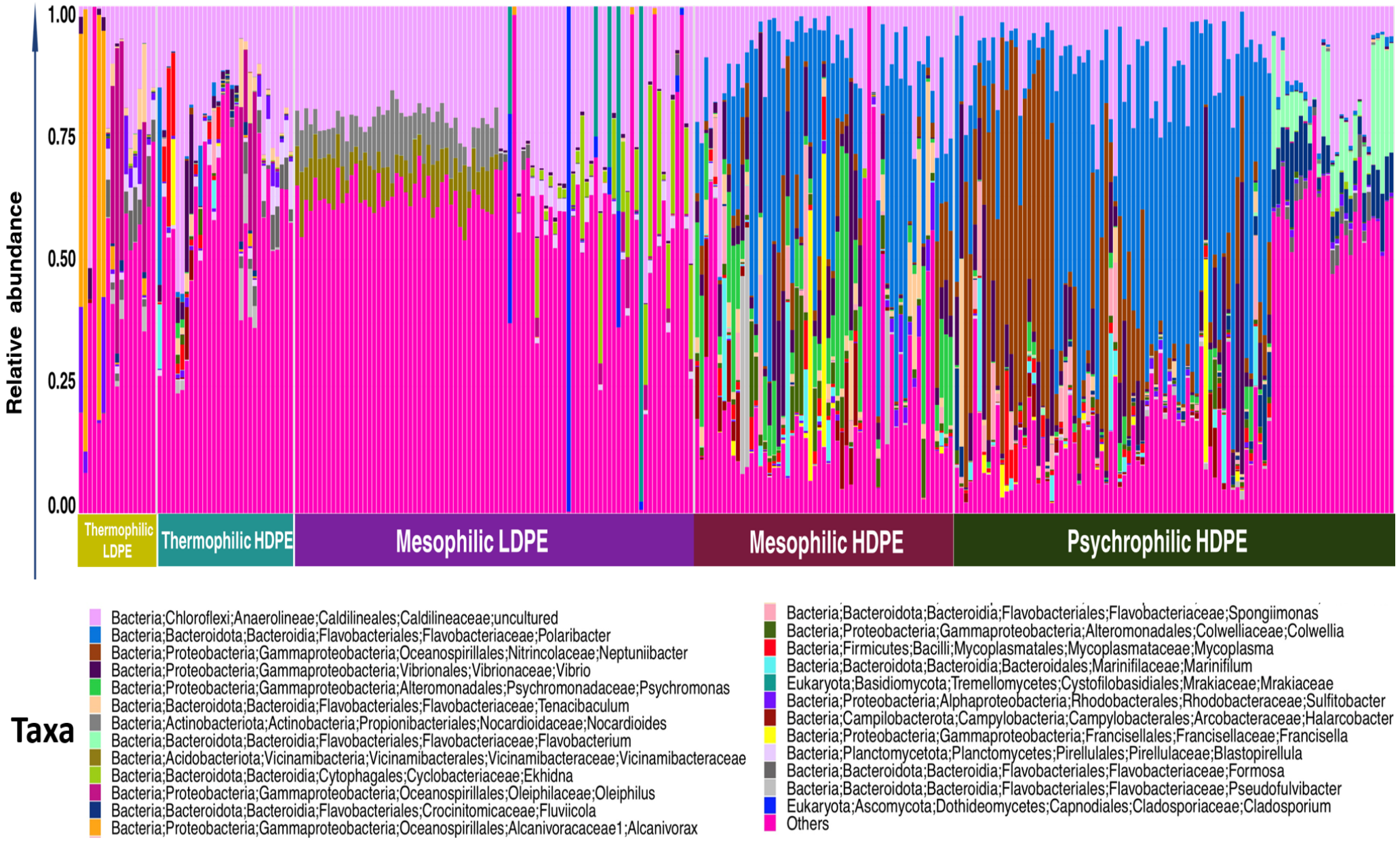

Figure 4.3.2 Taxa analysis of all samples across the LDPE and HDPE in different temperature.

4.4 CODA LaSSO results

At this stage of the study, CODA-LASSO analysis was applied to identify the most relevant microbial taxa within the group and to differentiate between the microbial taxa of the two groups. The samples were divided into groups based on salinity and temperature. The taxon features of the microbiomes in the samples are provided in Figures 4.4.1 and 4.4.2. The left graph indicates the score distribution of the microbial characteristics of two groups. The graph depicted on the right-hand side indicates the relevant microbial species in the two groups. The distribution of the results of the analysis of variance for each species was utilized to ascertain the specifics within a single sample. The size of the bars means how importantly.

It is clear from the distribution in the graphs that the behaviour and composition of the microbial communities differed significantly with the two materials (HDPE and LDPE). As shown in Figure 4.4.1, CODA-LASSO analysis of high salinity HDPE compared to high salinity LDPE revealed a significant difference in the microbial community. Polaribacte and Defluviicoccus were the most relevant bacteria within High salinity HDPE and High salinity LDPE respectively. Clade-la was found to be the most relevant bacteria in the high salinity HDPE, whereas the low salinity HDPE were dominated by Actinomarina. These results provide additional evidence to reinforce prior observations that alterations in microbial species show a relationship not only with modifications in attachment material, but also with the significant effects of salinity on the taxonomic features of the microbiome in the samples. It is notable that microbial features within the same sample can vary considerably depending on the comparison group.

Following the identification of a distinct correlation between the characteristics of microbial communities and both attachment material and salinity, an analysis was conducted to ascertain the effects of temperature on microbial community features. In Figure 4.4.2, we can observe that the differences in microbial communities under Mesophylic mesophiles conditions. They also show differences in the results compared to Psychrophylic physcrophiles HDPE respectively. Based on the above analysis, it was found that temperature was also a key factor influencing the biofilm composition of the microplastic surface.

The results obtained from the CODA-LASSO analysis provide additional confirmation that attachment material, temperature, and salinity have discernible impacts on the microbiome composition of microplastic surfaces. The CODA-LASSO provides an abundance score for each of the identified species within every comparison sample. These results are presented solely for the purpose of reference in Appendix.

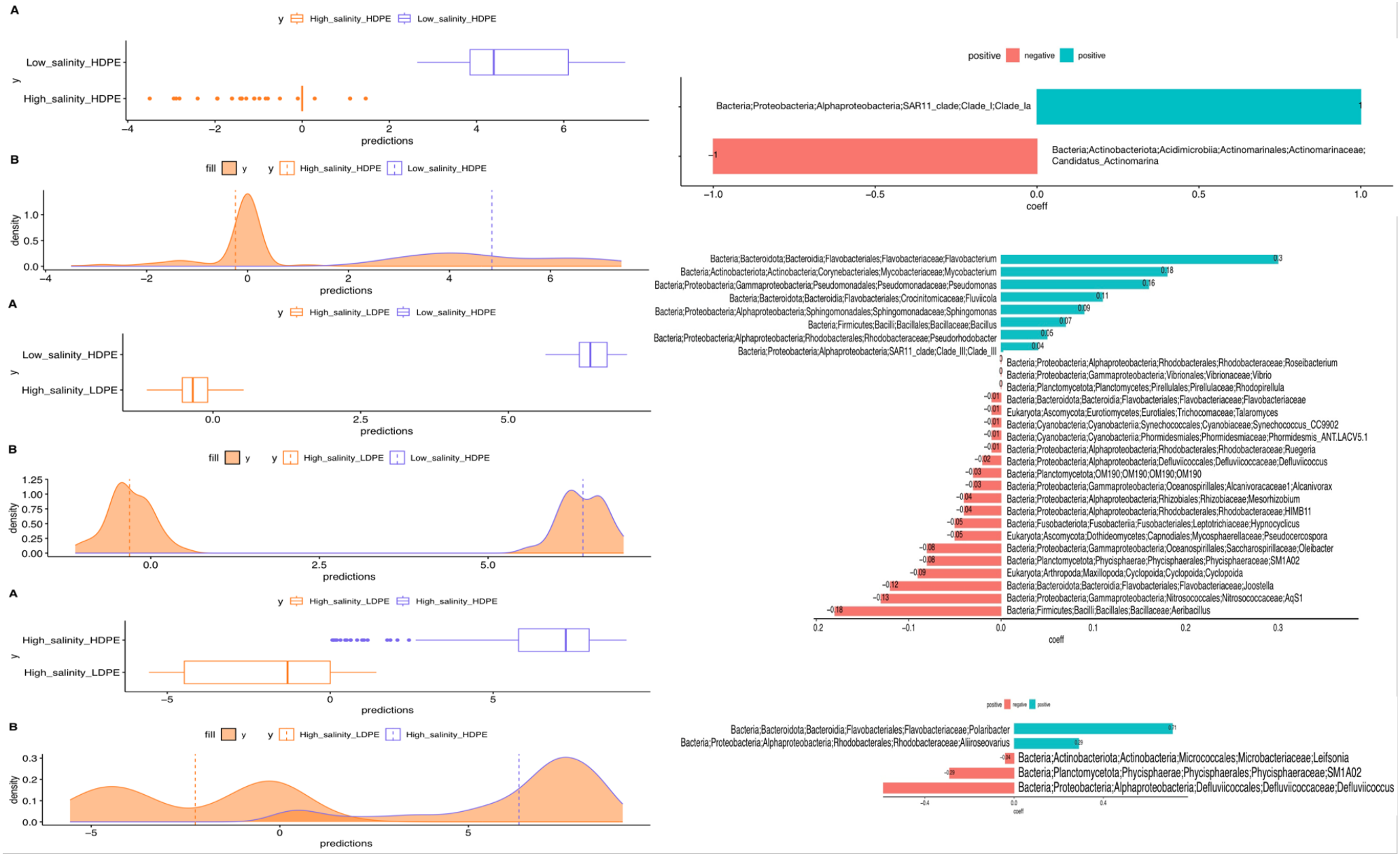

Figure 4.4.1 Coda – Lasso algorithm results (salinity).

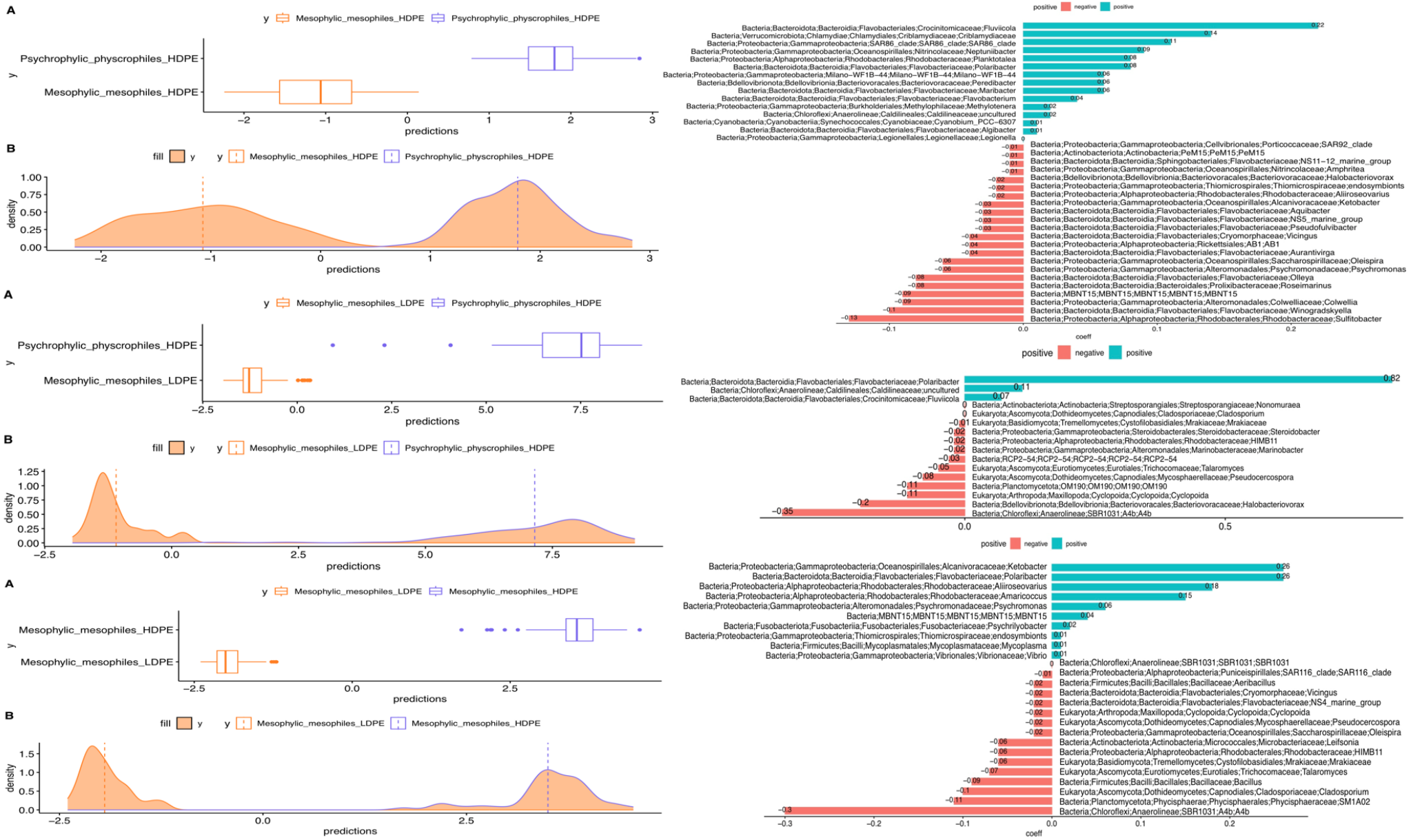

Figure 4.4.2 Coda – Lasso algorithm results (temperature).

The graphs on the right-hand side of Figures 3.4.1 and 3.4.2 present information on the relative abundance and expected representativeness of bacteria in each sample group. The table presented below provides a summary of the comparative results produced by the CODA-LASSO analysis. The table presents distinct descriptions of taxonomic levels and species-specific characteristics. It is directed towards the pathogenicity, symbiotic relationship, and environmental impact of the species.

Table 3.1 CODA-LASSO positive that there is a high degree of correlation between the ASVS genera present in the sample, while a negative value indicates that the correlation is insufficiently low. It is notable that only those genera with an important absolute value exceeding 0.1 were considered in this study in order produce more significant results for the CODA-LASSO analysis.

Material	Posi-tive	Nega-tive	ASVs Genera	Description
High salinity LDPE			AKYG1722	Insufficient information
			TK10	Insufficient information
			Myxococcus	Myxococcus Xanthus-type rod-shaped soil-dwelling bacteria cause the degradation of hydrocarbons. The bacteria produce ammonia through enzymatic hydrolysis. Piston rings of a high-performance thermoplastic composite material, polyether ether ketone (PEEK), are degraded by the enzymatic hydrolysis process of Myxococcus Xanthus.
			Gaiella	Gaiella can use a variety of carbon sources (fructose, glucose, mannose, ribose, xylose, and inositol) as a source of growth carbon. And it is resistant to certain antibiotics and has applications in biotechnology.
Freshwater LDPE			Streptomyces	Streptomyces is a Gram-positive aerobic bacterium, the largest genus in the phylum Actinomycetes. The complex matrix mycelium contributes to the degradation of organic compounds. Streptomyces' have a complex secondary metabolism and they can be used to produce bioactive compounds such as clinical natural antibiotics and immunosuppressants. Streptomyces hawaiiensis can biodegrade a variety of synthetic plastics such as polyethylene polymers, and Streptomyces hawaiiensis can produce several clinically valuable antibiotics such as cyclic acylated peptide (ADEP) antibiotics.
			Nocardioides	Nocardia belongs to a genus of Gram-positive, mild aerobic bacteria in the family Nocardiaceae. Nocardia can break down and metabolise polymers such as PET plastic and polyethylene.
Low salinity HDPE			Fluviicola	Fluviicola lacks oxidase activity and is unable to reduce nitrate and utilise glucose [6]. The class Flavobacteria contains species that may have a role in the flow of carbon and energy in the marine environment. Fluviicola can be used as an indicator index of tolerance in response to grazing parameters. It is used as an indicator for judging water quality due to its association with the diversity of freshwater organisms.

Freshwater LDPE			Caldilineaceae	Caldilineaceae are all chemoheterotrophs and the Caldilinea species is a thermophilic bacterium. There is no known link between Caldilineaceae and LDPE (low density polyethylene). caldilinea enzymes have been shown to catalyse a variety of chemical reactions for use in industrial processes to produce chemicals and pharmaceuticals.
			Mycobacterium	Mycobacterium is a possible pathogen (Quernheim et al., 2008). With the ability to degrade various environmental pollutants, it is being considered for future bioremediation applications.
			Pseudomonas	Pseudomonas aeruginosa is a bacterium (pathogen) that metabolizes a variety of naturally occurring compounds as well as xenobiotic compounds. Pseudomonas aeruginosa, Pseudomonas nidulans and Pseudomonas spp. are susceptible to human infections.
			Rokubacteriales	Rokubacteria utilise different electron donors and acceptors under aerobic and anaerobic conditions [84]. the relative abundance of Rokubacteriales is positively correlated with pH, total nitrogen content and Ca and Mg availability, implying that they derive energy from organic compounds. Rokubacteriales are efficient transformers of organic matter, breaking down complex organic compounds into simpler ones. Rokubacteriales are effective transformers of organic matter, breaking down complex organic compounds into simpler ones.
			RCP2-54	Insufficient information
High salinity HDPE			MBNT15	Insufficient information
			B2M28	B2M28 is an unknown genus of Gammaproteobacteria. The Gammaproteobacteria class is involved in a number of important biogeochemical processes such as nitrogen fixation, sulphur oxidation and aerobic methane oxidation. Some genera of this class are pathogens that cause disease in animals and humans.
			Caldilineaceae	Caldilineaceae are all chemoheterotrophs and the Caldilinea species is a thermophilic bacterium. There is no known link between Caldilineaceae and LDPE (low density polyethylene). caldilinea enzymes have been shown to catalyse a variety of chemical reactions for use in industrial processes to produce chemicals and pharmaceuticals.
			Vicinamibacteraceae	Vicinamibacteraceae plays an important role in soil ecology and the carbon cycle. Involved in the degradation of a wide range of organic compounds including chitin, lignin and cellulose. They are also capable of producing extracellular enzymes involved in the degradation of these compounds and may be involved in nitrogen and phosphorus removal and iron reduction.
			Nocardioides	Nocardia belongs to a genus of Gram-positive, mild aerobic bacteria in the family Nocardiaceae. Nocardia can break down and metabolise polymers such as PET plastic and polyethylene.
			RB41	Insufficient information
Low Salinity HDPE			Clade_Ia	Clade_Ia is an unknown species in the Alphaproteobacteria class. Many bacteria in this class live inside the cells of other organisms or act as parasites or beneficial symbionts within cells. Using CO2 and H2 as

				energy fuel, it produces energy ATP via methanogenesis and glucose via the acetyl coenzyme A pathway.
High salinity_ HDPE			Actinomarina	Actinomarina has an important role in the marine nitrogen cycle . This species produces secondary metabolites with potentially bioactive properties that are used in pharmaceutical and biotechnological applications
Low Salinity HDPE			Flavobacterium	Flavobacterium is a widespread pathogen in the aquatic environment [Astrid Buran Holan,2020]. It can serve as a degrader to degrade organic compounds and is involved in the cycle of marine carbon.
			Mycobacterium	Mycobacterium is a possible pathogen. With the ability to degrade various environmental pollutants, it is being considered for future bioremediation applications .
			Pseudomonas	Pseudomonas aeruginosa is a bacterium (pathogen) that metabolizes a variety of naturally occurring compounds as well as xenobiotic compounds . Pseudomonas aeruginosa, Pseudomonas nidulans and Pseudomonas spp. are susceptible to human infections.
			Fluviicola	Fluviicola lacks oxidase activity and is unable to reduce nitrate and utilise glucose. The class Flavobacteria contains species that may have a role in the flow of carbon and energy in the marine environment. Fluviicola can be used as an indicator index of tolerance in response to grazing parameters. It is used as an indicator for judging water quality due to its association with the diversity of freshwater organisms.
			Aeribacillus	Certain species in the genus Aeribacillus produce enzymes with potential applications in biotechnology（ amylases and proteases.
			AqS1	Insufficient information
			Joostella	Some species of the genus Joostella are potential probiotics, suggesting that they might have a beneficial effect in preventing certain gastrointestinal diseases and improving immune function.
High Salinity HDPE			Polaribacter	Polaribacter is utilised in the production of fatty acids.It facilitates the enhancement of the protein retinal in nutrient-poor seawater. It is an important participant in the carbon and nitrogen cycle in the marine.
			Aliiroseovarius	Aliiroseovarius are biopathogenicp and associated with marine phytoplankton blooms.
High salinity LDPE			Defluviicoccus	Defluviicoccus is able to accumulate large amounts of polyhydroxyalkanoates (PHA). a biodegradable polymer. This ability of Defluviicoccus allows them to be used in the production of bioplastics.
			SM1A02	SM1A02 is proposed as a novel anaerobic ammonia oxidising bacterium and it can oxidise ammonium to nitrite, participating in this nitrogen cycle.
Psychrophylic physcrophiles HDPE			Fluviicola	Fluviicola lacks oxidase activity and is unable to reduce nitrate and utilize glucose [6]. The class Flavobacteria contains species that may have a role in the flow of carbon and energy in the marine environment. Fluviicola can be used as an indicator index of tolerance in response to grazing parameters. It is used as an indicator for judging water quality due to its association with the diversity of freshwater organisms.

Mesophylic mesophiles HDPE			Criblamydiaceae	The genus Criblamydiaceae includes human and animal pathogens that cause a wide range of diseases in humans and animals, including pneumonia, sexually transmitted infections, and eye infections. It has a complex developmental cycle and a biphasic lifestyle with an intracellular replication phase of the reticulum (RB) .
			SAR86_clade	SAR86_clade has the potential to produce phototrophic ATP via the protein retinoid. It is normally found in the surface waters of the ocean and is an important contributor to the marine carbon cycle. It can metabolize dissolved organic matter and recycle it back into the ecosystem..
			Sulfitobacter	The genus Sulfitobacter is an essential member of the marine microbiome and has a role in the marine nutrient cycle and biogeochemical cycles through the degradation of algae. Some species in this genus are toxic to some marine organisms.
			Winogradskyella	The genus Winogradskyella has bioremediation capabilities and is able to degrade a number of organic compounds and produce bioenzymes to remove pollutants from the environment. Some species have potential biotechnological uses.
Psychrophylic physcrophiles HDPE			Polaribacter	Polaribacter is utilised in the production of fatty acids. It is an important participant in the carbon and nitrogen cycle in the marine.
			Caldilineaceae	Caldilineaceae are all chemoheterotrophs and the Caldilinea species is a thermophilic bacterium. There is no known link between Caldilineaceae and LDPE (low density polyethylene). caldilinea enzymes have been shown to catalyse a variety of chemical reactions for use in industrial processes to produce chemicals and pharmaceuticals.
Mesophylic mesophiles LDPE			A4b	Insufficient information
			Halobacteriovorax	Halobacteriovorax can prey on other bacteria by entering the host cell and using their own cellular machinery to replicate and multiply, potentially affecting microbial community structure. They are potential biocontrol agents and alternatives to antibiotics.
			Cyclopoida	Cyclopoida is the main copepod family. Copepods and bacteria are fundamental components of pelagic food webs and play an important part in biogeochemical cycles
			OM190	Insufficient information
Mesophylic mesophiles HDPE			Ketobacter	Ketobacter is capable of degrading hydrocarbons, by degrading petroleum-derived compounds as its main source of carbon and energy
			Polaribacter	Polaribacter is utilised in the production of fatty acids). It is an important participant in the carbon and nitrogen cycle in the marine.
			Aliiroseovarius	Aliiroseovarius are biopathogenicp and associated with marine phytoplankton blooms.
			Amaricoccus	Amaricoccus is a genus of the Rhodobacteraceae. It is involved in biogeochemical cycles, such as the carbon and sulphur cycles, and in the decomposition of complex organic matter.
			A4b	Insufficient information

Mesophylic mesophiles LDPE			SM1A02	SM1A02 is proposed as a novel anaerobic ammonia oxidising bacterium and it can oxidise ammonium to nitrite, participating in this nitrogen cycle.
			Cladosporium	The genus Cladosporium is found in soil, decaying wood and decaying vegetation. Some species of this genus are opportunistic pathogens that result in plant and human diseases, including individual infections, respiratory diseases and and central nervous system infections.
Mesophylic mesophiles LDPE			A4b	Insufficient information.
Thermophylic psychrophiles HDPE			Halioglobus	The genus Halioglobus contains only one species, Halioglobus japonicus. Its ability to reduce nitrate to nitrogen makes it an important player in the sulphur cycle of the marine environment.
			Mesorhizobium	Mesorhizobium is ecologically and agriculturally important as it establishes nitrogen-fixing symbioses on the roots of legumes and the stems of some aquatic legumes. Some species of this genus degrade pollutants such as polycyclic aromatic hydrocarbons (PAHs) and have applications in bioremediation and biotechnology.
Psychrophylic physcrophiles HDPE			Polaribacter	Polaribacter is utilised in the production of fatty acids. It is an important participant in the carbon and nitrogen cycle in the marine.
Thermophylic psychrophiles LDPE			Mesorhizobium	Mesorhizobium is ecologically and agriculturally important as it establishes nitrogen-fixing symbioses on the roots of legumes and the stems of some aquatic legumes. Some species of this genus degrade pollutants such as polycyclic aromatic hydrocarbons (PAHs) and have applications in bioremediation and biotechnology.
			Alcanivorax	Alcanivorax degrades aromatic compounds of alkanes and petroleum. Its ability to degrade hydrocarbons has been used in biotechnological research.
Psychrophylic physcrophiles HDPE			Polaribacter	Polaribacter is utilised in the production of fatty acids It is an important participant in the carbon and nitrogen cycle in the marine.
Thermophylic psychrophiles HDPE			Lutimonas	Lutimonas degrade starch and gelatin, but not elastin, CM cellulose or chitin.
Mesophylic mesophiles LDPE			A4b	Insufficient information
Thermophylic psychrophiles LDPE			Sulfitobacter	The genus Sulfitobacter is an essential member of the marine microbiome and has a role in the marine nutrient cycle and biogeochemical cycles through the degradation of algae. Some species in this genus are toxic to some marine organisms.
Mesophylic mesophiles HDPE			Polaribacter	Polaribacter is utilized in the production of fatty acids. It is an important participant in the carbon and nitrogen cycle in the marine.
			Woeseia	Woeseia use monosaccharides, amino acids, and fatty acids as the sole source of carbon and energy and can hydrolyse proteins to release amino acids and fatty acids. Participates in the cycling of detrital proteins in the marine benthic environment.

			OM60(NOR5)_clade	OM60/NOR5 is metabolised based on anaerobic phototrophy and chemoheterotrophy of bacterial chlorophyll [**]. The aerobic γ-amastigotes in the evolutionary branch of OM60/NOR5 are widespread in saline environments and are of importance to marine ecosystems.
Thermophylic psychrophiles LDPE			Formosa	Certain species in the genus Formosa are involved in the degradation of biopolymers, such as proteins, polysaccharides, and glycoproteins, and are associated with the degradation of marine high molecular weight particulate organic matter. Some species of this genus are opportunistic pathogens.
			Orientia	Species in the genus Orientia are opportunistic pathogens. They cause acute febrile illnesses (typhus) that are transmitted to humans through the bite of infected chiggers, fleas, mites, or ticks.
Mesophyli mesophiles HDPE			Legionella	Legionella is a Gram-negative pathogenic bacterium that includes Legionella pneumophila. Legionella pneumophila replicates in alveolar macrophages and causes Legionnaires, resulting in severe pneumonia.
			MBNT15	Most members of MBNT15 are capable of aerobic respiration and heterotrophic iron reduction. Glycoside hydrolases involved in starch and similar polysaccharide degradation and can mineralise low molecular organic matter formed by microbial degradation of complex polymeric substrates.
			Marinobacter	Some species in the Marinobacter genus degrade hydrocarbons, including polycyclic aromatic hydrocarbons (PHAs), hexanes, heptanes, and petroleum ethers. The ability of this genus to reduce nitrates is used in oilfield development and maintenance. It is enzymatically resistant to arsenic and heavy metals and participates in the biogeochemical cycle of organic matter and metals.
			Psychrilyobacter	Psychrilyobacter preferentially inhabits the guts of marine invertebrates and can act as a probiotic to protect marine animals. Psychrilyobacter atlanticus in this genus is an important primary protein degrader.
Thermophylic psychrophiles HDPE			Alkalimarinus	Alkalimarinus produces extracellular enzymes, such as proteases and lipases. It is associated with the degradation of antitoxins and xenobiotics and has biotechnological potential.
			Lutimonas	Lutimonas degrade starch and gelatin, but not elastin, CM cellulose or chitin.
			Woeseia	Woeseia use monosaccharides, amino acids, and fatty acids as the sole source of carbon and energy and can hydrolyse proteins to release amino acids and fatty acids. Participates in the cycling of detrital proteins in the marine benthic environment.
			KI89A_clade	KI89A_clade performs iron reduction, acting in relation to the carbon cycle, nutrient cycling organisms and geochemical cycles in the aquatic environment.
Thermophylic psychrophiles HDPE			Aliiroseovarius	Aliiroseovarius are biopathogenicp (Kessner et al., 2016) and associated with marine phytoplankton blooms
			Halioglobus	The genus Halioglobus contains only one species, Halioglobus japonicus.Its ability to reduce nitrate to

				nitrogen makes it an important player in the sulphur cycle of the marine environment.
Thermophylic psychrophiles LDPE			Croceitalea	Croceitalea can degrade complex organic compounds such as carbohydrates, proteins, and lipids. The production of active enzymes at low temperatures gives it the ability to consume biopolymers in the marine environment.
			SM1A02	SM1A02 is proposed as a novel anaerobic ammonia oxidising bacterium and it can oxidise ammonium to nitrite, participating in this nitrogen cycle.
			Sulfitobacter	The genus Sulfitobacter is an essential member of the marine microbiome and has a role in the marine nutrient cycle and biogeochemical cycles through the degradation of algae. Some species in this genus are toxic to some marine organisms.
			Rhodopirellula	Members of the genus Rhodopirellulula are characterised by germination and reproduction, species-specific intracellular membrane compartmentalisation and are involved in the carbon and nitrogen cycles. It could degrade complex polysaccharide molecules and is naturally resistant to many antibiotics.
			Orientia	Species in the genus Orientia are opportunistic pathogens. They cause acute febrile illnesses (typhus) that are transmitted to humans through the bite of infected chiggers, fleas, mites, or ticks.

4.5 Core microbiome results

In this study, the abundance and distribution of the core microbiome in samples and explores the extension of the core microbiome concept above taxonomically defined microbial communities to include community function and behavior. Genera are sorted by their abundance in the heat map figures, where those on left of the heat map are low abundant prevalent genera, and those at the right are highly abundant prevalent genera. >0.5 prevalence implies a minimum of 50% prevalence of each genus in the core microbiome.

The core microbiome of the High Salinity HDPE and High Salinity LDPE are shown in Figure 4.5.1. The five core genera in the High salinity HDPE are Caldilineaceae, Polaribacter, Vibro, Neptuniibacter and Tenacibaculum. Members of the Caldilineaceae family can degrade complex organic compounds [75]. Polaribacter, vibro and Tenacibaculum are all gram-negative bacteria. members of the genera Polaribacter and Neptuniibacter contribute to the degradation of algal cells and nutrients，they are important contributors to biogeochemical cycles [76]. Some species of the Vibro genus have the potential to cause illnesses and are unable to survive in freshwater [77]. The genus Tenacibaculum comprises numerous opportunistic pathogens that affect marine fish[78]. Caldilineaceae, Blastopirellula, OM190, Ekhidna and Rhodopirellula are the first five most core genera in the High salinity LDPE. Blastopirellula, OM190, and Rhodopirellula are classified in the Planctomycetes phylum. Their distinctiveness is due to their possession of cell walls with a similarity to those of eukaryotic cells, and their involvement in the carbon and nitrogen cycles of aquatic ecosystems. All these five bacteria are known for their ability to degrade complex organic compounds.

As shown in Figure 4.5.2, the first five core genera identified in the Thermophilic Psychrophiles HDPE and Thermophilic Psychrophiles LDPE included Caldilineaceae, Blastopirellula, and Sulfitobacter. Blastopirellula is a member of the phylum Planctomycetota and involved in the carbon and nitrogen cycle within marine [79]. Sulfitobacter participates in the marine nutrient cycle and biogeochemical cycles through the degradation of algae [80]. Some species within this genus possess toxicity towards select marine organisms [81].

Core microbiome analysis provided core genus for each sample. Other results are shown in the appendix. These results show that different attachment materials (LDPE and HDPE) and temperature and salinity will affect the composition and abundance of the core microbiome. Caldilineacea was the first core microbiome in all samples. we also found Caldilineaceae, Polaribacter and Vibro to be common for the core microbiome of LDPE and HDPE. They are known for their ability to break down complex organic compounds and are of relevance to the field of microplastic degradation. We also found Caldilineaceae, Polaribacter and Vibro to be common for the core microbiome of LDPE and HDPE.

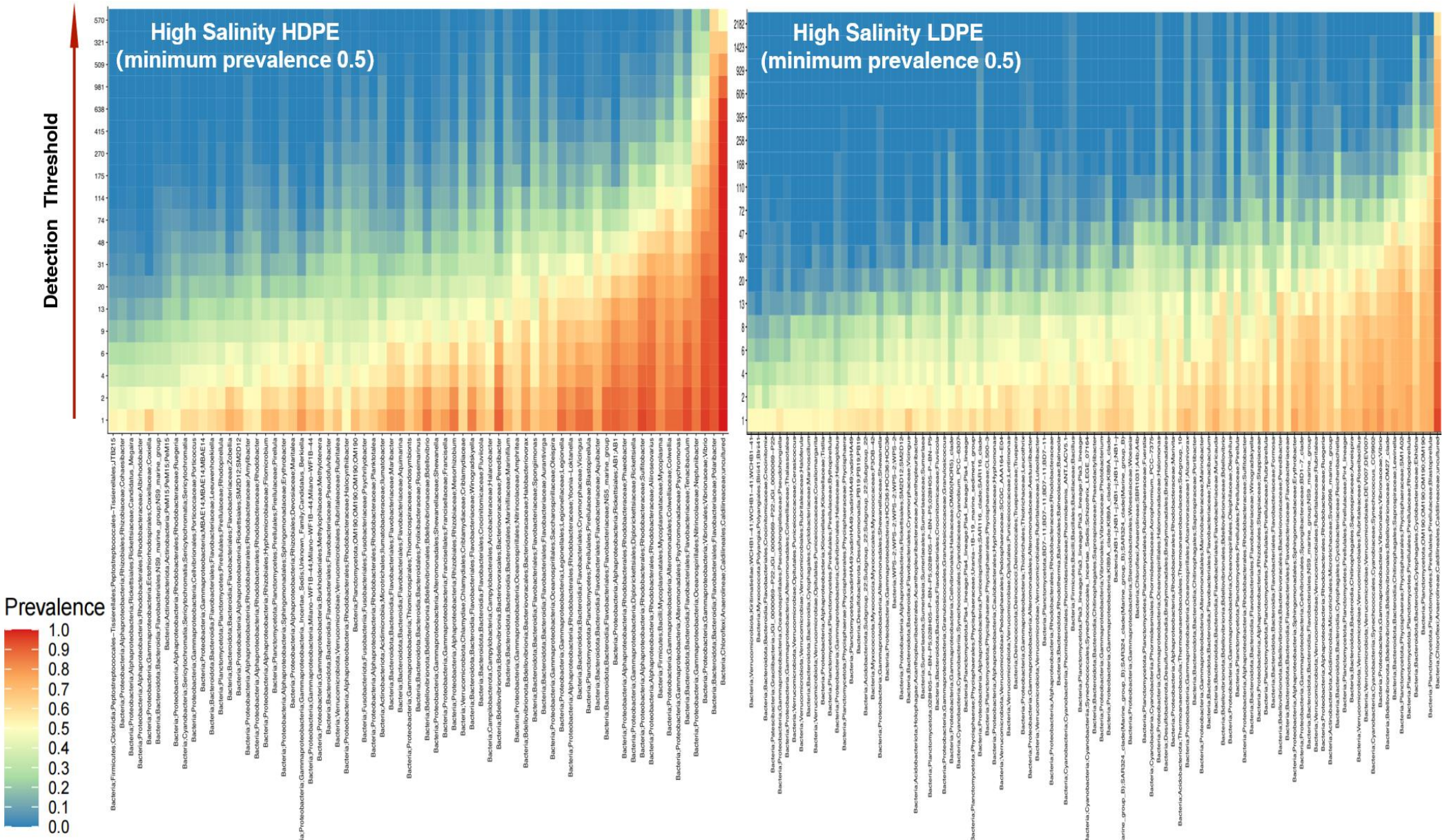

Figure 4.5.1 Core microbiome of High Salinity HDPE and LDPE.

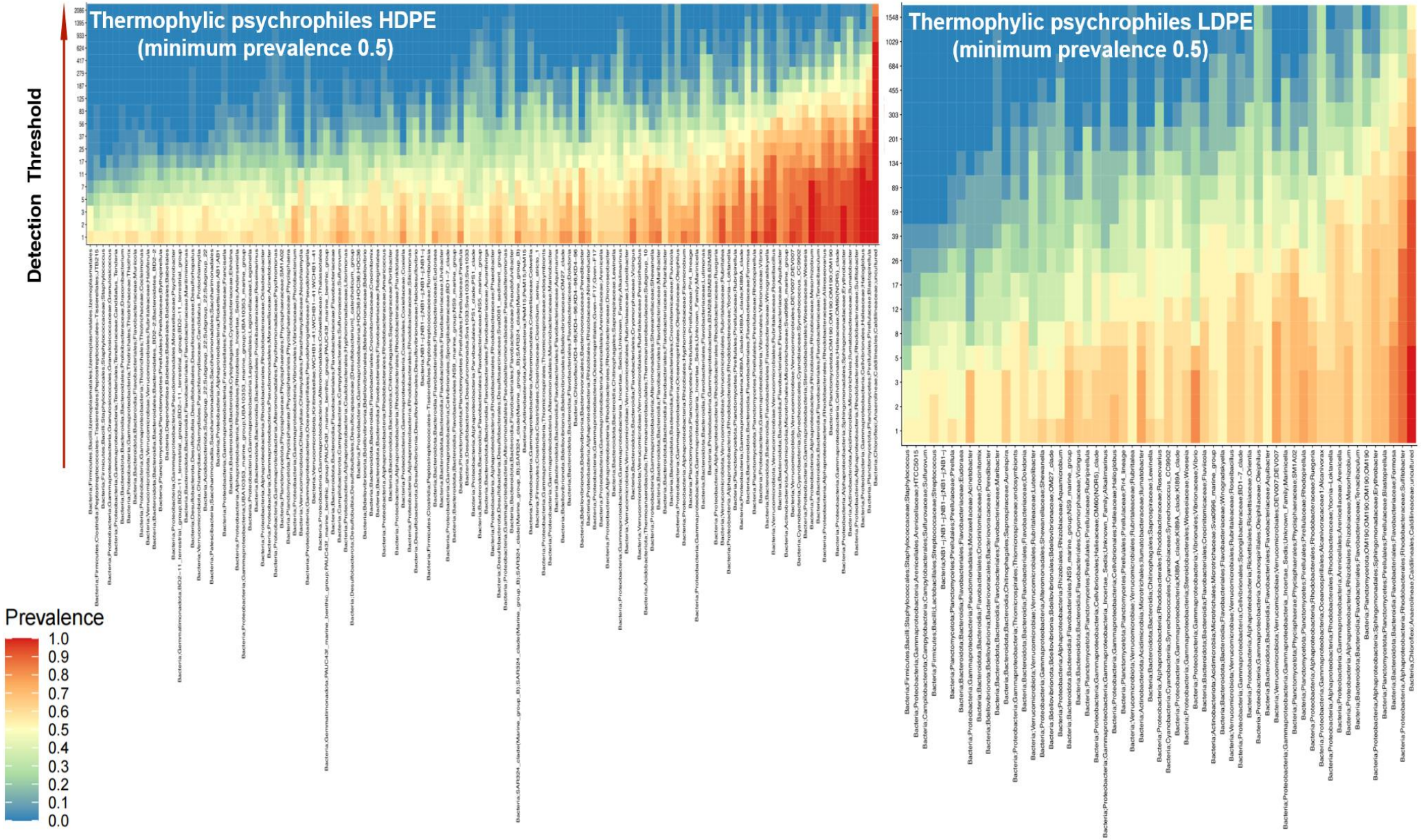

Figure 4.5.2 Core microbiome of Thermophylic psychrophiles HDPE and LDPE.

5. DISCUSSION

In this work, we have used DNA based approach to identify which microbes exist on which plastic type and we can see that the microbiome is different for variation of temperature and salinity.The result is the same as that of Wenjie Li et,al. (2019) who showed that the major factor that affects the diversity of microbiome of microplastic is salinity [82]. In our study, it was observed that microbial communities on the surface of microplastics showed high diversity in freshwater or low salinity environments, and high salinity inhibit the growth of microorganisms. In a study by Sylwia Lew et al. (2022), the total bacterial numbers significantly decreased with increasing salinity[83] . Significant differences in composition of microbiome were observed for all sample along the temperature gradient. Currently, there are no published papers that demonstrate the reasons why temperature affects community composition and diversity. It is notable that the effect of salinity on communities was more significant compared to temperature.

Although the composition of biofilm significantly depends on environmental factors (temperature and salinity), this study demonstrated that the physicochemical properties of the materials are one of the influencing factors. In our study, the diversity of microbial communities on HDPE was significantly higher than on LDPE. these findings are consistent with the results reported by Jessica Song et al. (2022) for plastic samples collected from coastal seawater [84]. The most important species based on CORE microbiome and differential analysis gives us clues on how to degrade these microplastics. This study focuses on their ability to degrade hydrocarbons and their involvement in the marine carbon and nitrogen cycle, including Alcanivorax, Myxococcus, Ketobacter. Caldilineacea was also found to be the most core microbiome in all samples and is known for its ability to degrade complex organic compounds.

A culture-based approach has been used in the majority of investigations on how environmental factors affect microbial communities. In our study, we used the meta-analysis method to combine the results of seven individual studies into a global view of the dataset. The limitation of meta-analysis is that it is influenced by the number of studies. The findings were tentative since there were only 7 studies included in this study due to time constraints. Although our study discovered differences in the microbial communities on LDPE and HDPE surfaces, it was difficult to identify whether these differences were caused by variations in transparency or other physical properties of the materials due to the limitation of data and absence of control variables. Optics defines transparency as the property of an object that permits light to pass through it without being scattering. Different light conditions have been found to influence the composition of different bacterial communities [85].

6. CONCLUSION AND FUTURE WORK

This study provides an initial comprehension of the effects of temperature and salinity on the composition and diversity of LDPE and HDPE communities. This was discovered in the aforementioned demonstrations：the microbial communities on HDPE and LDPE showed high diversity in freshwater or low-salt environments; there was a significant amount of variability observed in the microbial communities on LDPE and HDPE at different temperatures. Furthermore, the presence of specific differences in material properties performed a major factor influencing community composition and abundance. The results of this study indicate that microbial communities on LDPE and HDPE differed significantly in composition and abundance under similar environmental conditions; specific bacteria grew on LDPE and HDPE; and material differences resulted in differences in core microbiome of bacteria. The results of this study indicate that microbial communities on LDPE and HDPE in different temperature and salinity, which provides a new perspective for the investigation on microbial degradation of microplastics. Due to the utilization of meta-analysis and limited dataset collection, the most important limitations in the fact that how transparency affects microbial communities on LDPE and HDPE surfaces remain unanswered.

In the future, datasets will be combined and expanded from the extensive literature and the wider field. Meta-analysis is used to analyses similarities in diversity and phylogenetic abundance, to further provide a theoretical rationale for the way in which transparency will affect the composition, diversity, and relative abundance of microbial communities on LDPE and HDPE. The study confirmed the existence of bacteria able to degrade organic compounds on the surfaces of LDPE and HDPE. Further investigation into processes for exploration microbial degradation and identifying efficient microbial processes for degradation would be an intriguing field for future studies.

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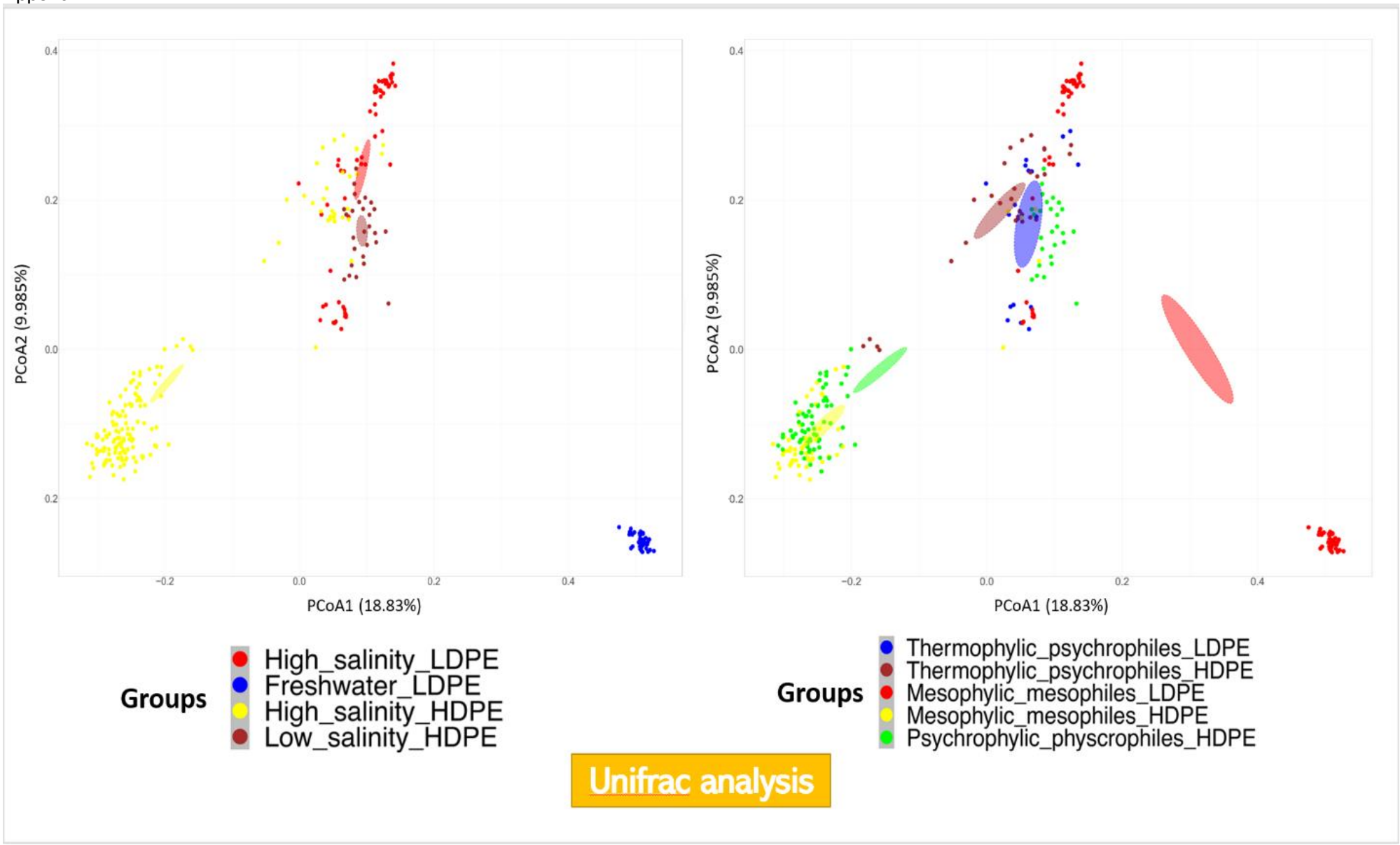
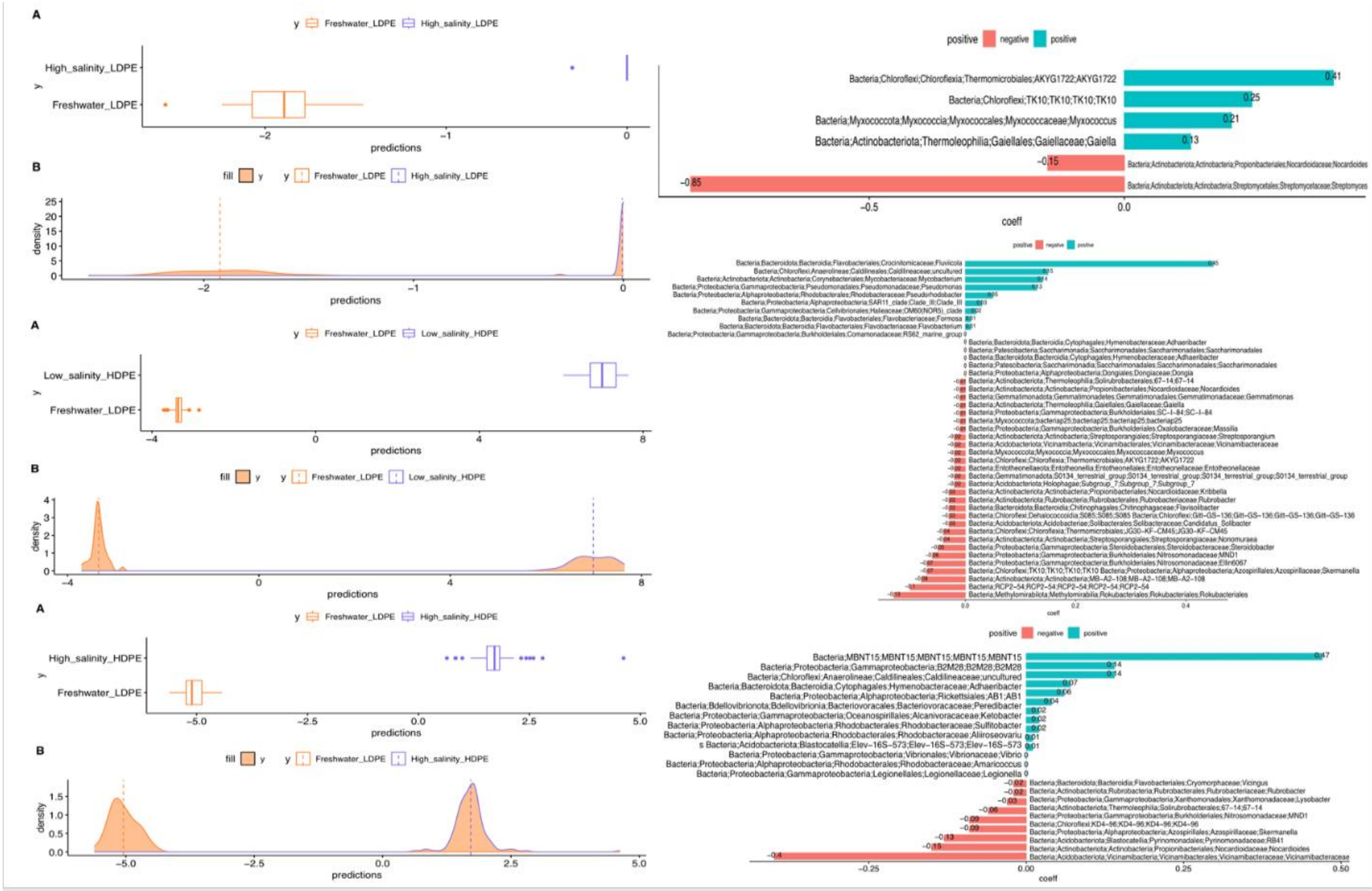
8. APPENDIX

Appendix A:



Appendix B:


A
y Freshwater_LDPE High_salinity_LDPE
High_salinity_LDPE
Freshwater_LDPE
y
predictions
B
fill y
y Freshwater_LDPE High_salinity_LDPE
density
predictions
A
y Freshwater_LDPE Low_salinity_HDPE
Low_salinity_HDPE
Freshwater_LDPE
y
predictions
B
fill y
y Freshwater_LDPE Low_salinity_HDPE
density
predictions
A
y Freshwater_LDPE High_salinity_HDPE
High_salinity_HDPE
Freshwater_LDPE
y
predictions
B
fill y
y Freshwater_LDPE High_salinity_HDPE
density
predictions
positive negative positive
Bacteria;Chloroflexi;Chloroflexia;Thermomicrobiales;AKYG1722;AKYG1722
Bacteria;Chloroflexi;TK10;TK10;TK10;TK10
Bacteria;Myxococcota;Myxococcia;Myxococcales;Myxococcaceae;Myxococcus
Bacteria;Actinobacteriota;Thermoleophilia;Gaiellales;Gaiellaceae;Gaiella
Bacteria;Actinobacteriota;Actinobacteria;Propionibacteriales;Nocardioidaceae;Nocardioides
Bacteria;Actinobacteriota;Actinobacteria;Streptomycetales;Streptomycetaceae;Streptomyces
coeff
positive negative positive
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Crocinitomicaceae;Fluviicola
Bacteria;Chloroflexi;Anaerolineae;Caldilineales;Caldilineaceae;uncultured
Bacteria;Actinobacteriota;Actinobacteria;Corynebacteriales;Mycobacteriaceae;Mycobacterium
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudorhodobacter
Bacteria;Proteobacteria;Alphaproteobacteria;SAR11_clade;Clade_III;Clade_III
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;OM60(NOR5)_clade
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Formosa
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Flavobacterium
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Comamonadaceae;RS62_marine_group
Bacteria;Bacteroidota;Bacteroidia;Cytophagales;Hymenobacteraceae;Adhaeribacter
Bacteria;Patescibacteria;Saccharimonadia;Saccharimonadales;Saccharimonadales;Saccharimonadales
Bacteria;Proteobacteria;Alphaproteobacteria;Dongiales;Dongiaceae;Dongia
Bacteria;Actinobacteriota;Thermoleophilia;Solirubrobacterales;67-14;67-14
Bacteria;Actinobacteriota;Actinobacteria;Propionibacteriales;Nocardioidaceae;Nocardioides
Bacteria;Gemmatimonadota;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;Gemmatimonas
Bacteria;Actinobacteriota;Thermoleophilia;Gaiellales;Gaiellaceae;Gaiella
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;SC-I-84;SC-I-84
Bacteria;Myxococcota;bacteriap25;bacteriap25;bacteriap25;bacteriap25
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Oxalobacteraceae;Massilia
Bacteria;Actinobacteriota;Actinobacteria;Streptosporangiales;Streptosporangiaceae;Streptosporangium
Bacteria;Acidobacteriota;Vicinamibacteria;Vicinamibacterales;Vicinamibacteraceae;Vicinamibacteraceae
Bacteria;Myxococcota;Myxococcia;Myxococcales;Myxococcaceae;Myxococcus
Bacteria;Chloroflexi;Chloroflexia;Thermomicrobiales;AKYG1722;AKYG1722
Bacteria;Entotheonellaeota;Entotheonellia;Entotheonellales;Entotheonellaceae;Entotheonellaceae
Bacteria;Gemmatimonadota;S0134_terrestrial_group;S0134_terrestrial_group;S0134_terrestrial_group;S0134_terrestrial_group
Bacteria;Acidobacteriota;Holophagae;Subgroup_7;Subgroup_7;Subgroup_7
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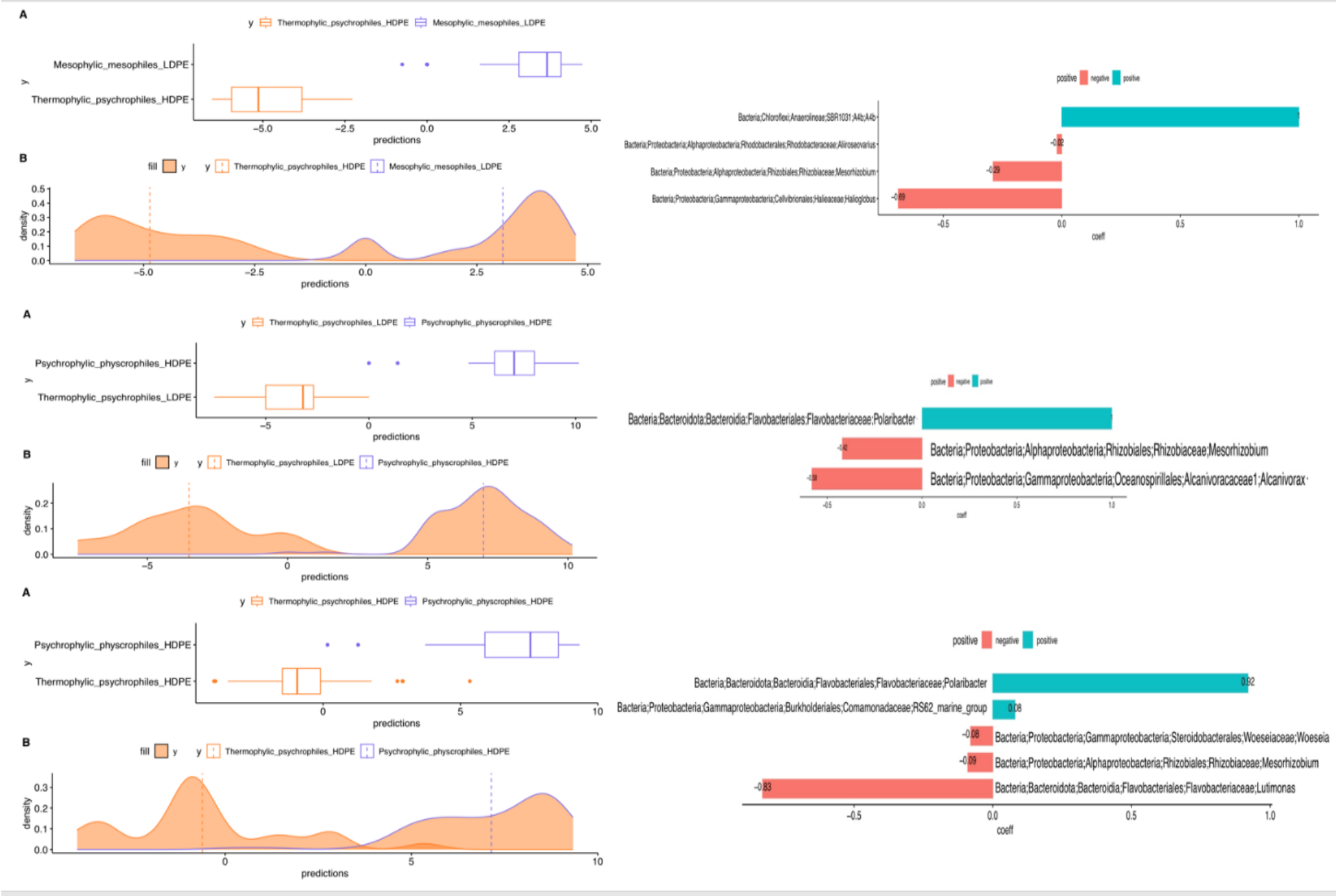
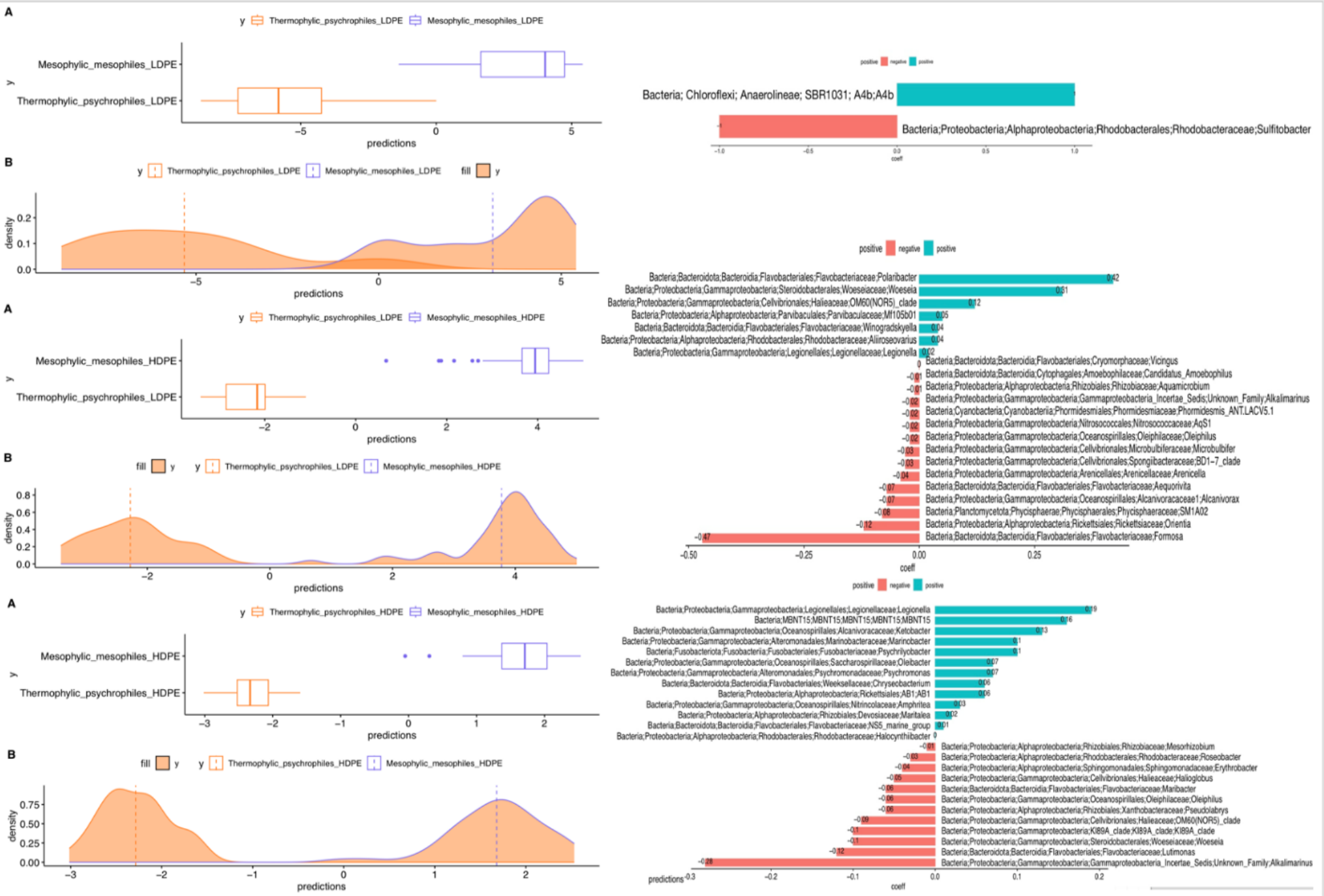
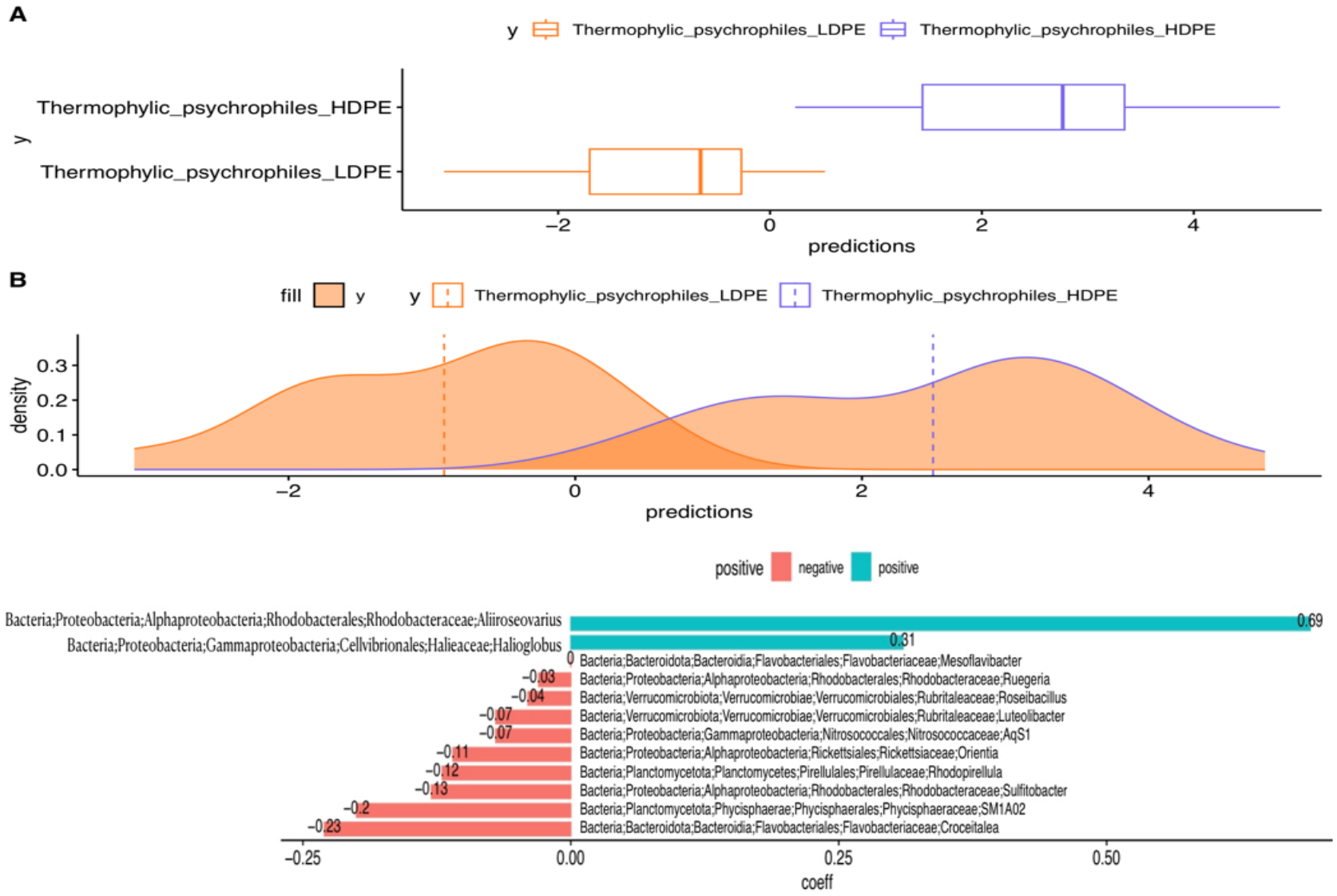
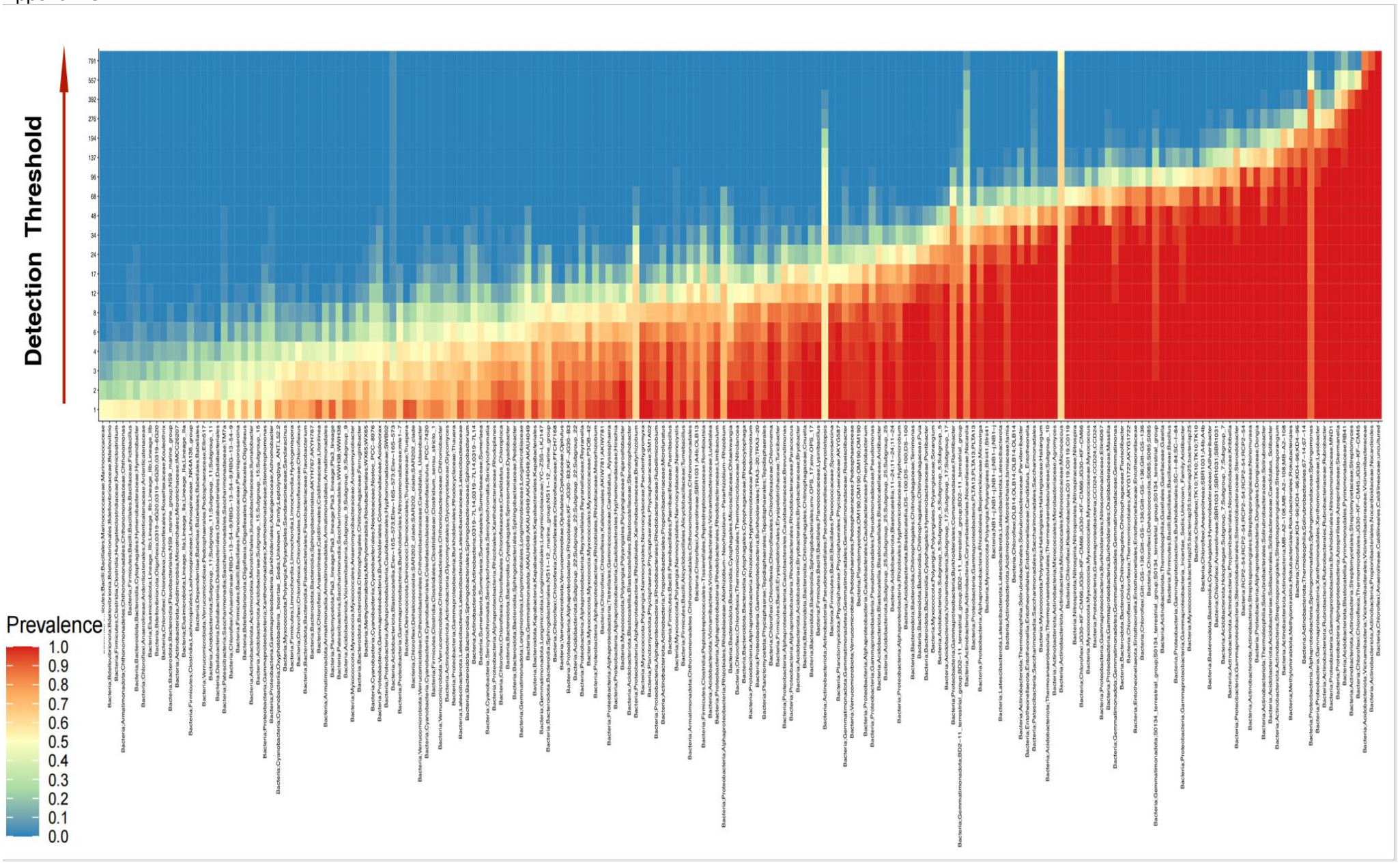
Bacteria;Actinobacteriota;Rubrobacteria;Rubrobacterales;Rubrobacteriaceae;Rubrobacter
Bacteria;Bacteroidota;Bacteroidia;Chitinophagales;Chitinophagaceae;Flavisolibacter
Bacteria;Chloroflexi;Dehalococcoidia;S085;S085;S085 Bacteria;Chloroflexi;Gitl-GS-136;Gitl-GS-136;Gitl-GS-136;Gitl-GS-136
Bacteria;Acidobacteriota;Acidobacteriae;Solibacterales;Solibacteraceae;Candidatus_Solibacter
Bacteria;Chloroflexi;Chloroflexia;Thermomicrobiales;JG30-KF-CM45;JG30-KF-CM45
Bacteria;Actinobacteriota;Actinobacteria;Streptosporangiales;Streptosporangiaceae;Nonomuraea
Bacteria;Proteobacteria;Gammaproteobacteria;Steroidobacterales;Steroidobacteraceae;Steroidobacter
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Nitrosomonadaceae;MND1
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Nitrosomonadaceae;Ellin6067
Bacteria;Chloroflexi;TK10;TK10;TK10;TK10 Bacteria;Proteobacteria;Alphaproteobacteria;Azospirillales;Azospirillaceae;Skermanella
Bacteria;Actinobacteriota;Actinobacteria;MB-A2-108;MB-A2-108;MB-A2-108
Bacteria;RCP2-54;RCP2-54;RCP2-54;RCP2-54;RCP2-54
Bacteria;Methylomirabilota;Methylomirabilia;Rokubacteriales;Rokubacteriales;Rokubacteriales
coeff
positive negative positive
Bacteria;MBNT15;MBNT15;MBNT15;MBNT15;MBNT15
Bacteria;Proteobacteria;Gammaproteobacteria;B2M28;B2M28;B2M28
Bacteria;Chloroflexi;Anaerolineae;Caldilineales;Caldilineaceae;uncultured
Bacteria;Bacteroidota;Bacteroidia;Cytophagales;Hymenobacteraceae;Adhaeribacter
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;AB1;AB1
Bacteria;Bdellovibrionota;Bdellovibrionia;Bacteriovoracales;Bacteriovoracaceae;Peredibacter
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Ketobacter
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Aliiroseovariu
s Bacteria;Acidobacteriota;Blastocatellia;Elev-16S-573;Elev-16S-573;Elev-16S-573
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Amaricoccus
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;Legionella
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Cryomorphaceae;Vicingus
Bacteria;Actinobacteriota;Rubrobacteria;Rubrobacterales;Rubrobacteriaceae;Rubrobacter
Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Lysobacter
Bacteria;Actinobacteriota;Thermoleophilia;Solirubrobacterales;67-14;67-14
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Nitrosomonadaceae;MND1
Bacteria;Chloroflexi;KD4-96;KD4-96;KD4-96;KD4-96
Bacteria;Proteobacteria;Alphaproteobacteria;Azospirillales;Azospirillaceae;Skermanella
Bacteria;Acidobacteriota;Blastocatellia;Pyrinomonadales;Pyrinomonadaceae;RB41
Bacteria;Actinobacteriota;Actinobacteria;Propionibacteriales;Nocardioidaceae;Nocardioides
Bacteria;Acidobacteriota;Vicinamibacteria;Vicinamibacterales;Vicinamibacteraceae;Vicinamibacteraceae
coeff

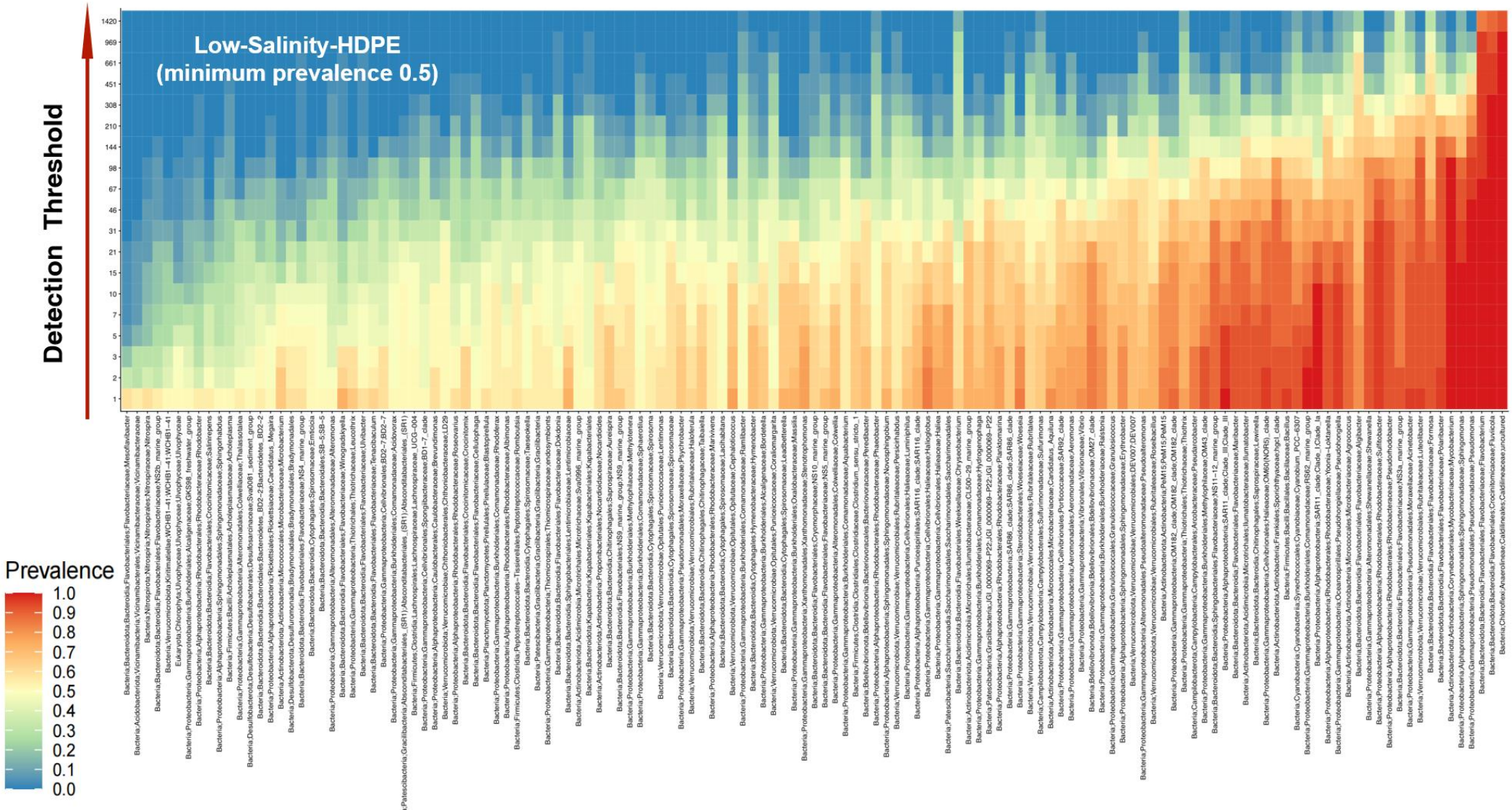

A
y Thermophylic_psychrophiles_HDPE Mesophylic_mesophiles_LDPE
Mesophylic_mesophiles_LDPE
Thermophylic_psychrophiles_HDPE
y
predictions
B
fill y
y Thermophylic_psychrophiles_HDPE Mesophylic_mesophiles_LDPE
density
predictions
positive negative positive
Bacteria;Chloroflexi;Anaerolineae;SBR1031;A4b;A4b
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Aliiroseovarius
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Mesorhizobium
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;Haliogloba
-0.02
-0.29
-0.69
coeff
A
y Thermophylic_psychrophiles_LDPE Psychrophylic_physcrophiles_HDPE
Psychrophylic_physcrophiles_HDPE
Thermophylic_psychrophiles_LDPE
y
predictions
B
fill y
y Thermophylic_psychrophiles_LDPE Psychrophylic_physcrophiles_HDPE
density
predictions
positive negative positive
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Polaribacter
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Mesorhizobium
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae1;Alcanivorax
coeff
A
y Thermophylic_psychrophiles_HDPE Psychrophylic_physcrophiles_HDPE
Psychrophylic_physcrophiles_HDPE
Thermophylic_psychrophiles_HDPE
y
predictions
B
fill y
y Thermophylic_psychrophiles_HDPE Psychrophylic_physcrophiles_HDPE
density
predictions
positive negative positive
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Polaribacter
Bacteria;Proteobacteria;Gammaproteobacteria;Burkholderiales;Comamonadaceae;RS62_marine_group
Bacteria;Proteobacteria;Gammaproteobacteria;Steroidobacterales;Woeseiaceae;Woeseia
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Mesorhizobium
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Lutimonas
0.08
-0.08
-0.09
-0.83
coeff

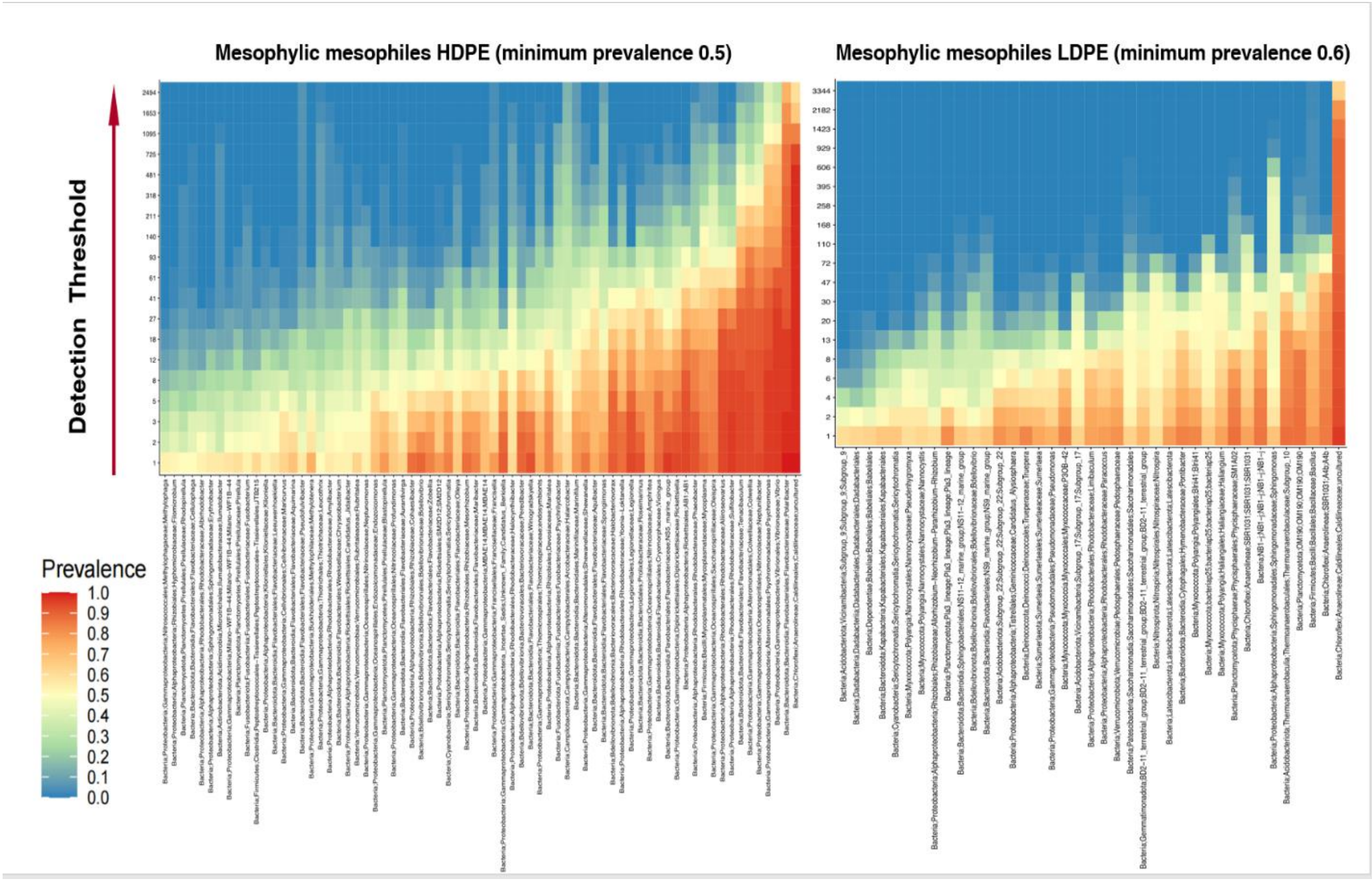

A
y Thermophylic_psychrophiles_LDPE Mesophylic_mesophiles_LDPE
Mesophylic_mesophiles_LDPE
Thermophylic_psychrophiles_LDPE
y
predictions
B
y Thermophylic_psychrophiles_LDPE Mesophylic_mesophiles_LDPE fill y
density
predictions
A
y Thermophylic_psychrophiles_LDPE Mesophylic_mesophiles_HDPE
Mesophylic_mesophiles_HDPE
Thermophylic_psychrophiles_LDPE
y
predictions
B
fill y y Thermophylic_psychrophiles_LDPE Mesophylic_mesophiles_HDPE
density
predictions
A
y Thermophylic_psychrophiles_HDPE Mesophylic_mesophiles_HDPE
Mesophylic_mesophiles_HDPE
Thermophylic_psychrophiles_HDPE
y
predictions
B
fill y y Thermophylic_psychrophiles_HDPE Mesophylic_mesophiles_HDPE
density
predictions
positive negative positive
Bacteria; Chloroflexi; Anaerolineae; SBR1031; A4b;A4b
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
coeff
positive negative positive
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Polaribacter 0.42
Bacteria;Proteobacteria;Gammaproteobacteria;Steroidobacterales;Woeseiaceae;Woeseia 0.31
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;OM60(NOR5)_clade 0.12
Bacteria;Proteobacteria;Alphaproteobacteria;Parvibaculales;Parvibaculaceae;Mf105b01 0.05
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Winogradskyella 0.04
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Aliiroseovarius 0.04
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;Legionella 0.02
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Cryomorphaceae;Vicingus 0
Bacteria;Bacteroidota;Bacteroidia;Cytophagales;Amoebophilaceae;Candidatus_Amoebophilus -0.01
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Aquamicrobium -0.01
Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Alkalimarinus -0.02
Bacteria;Cyanobacteria;Cyanobacteriia;Phormidesmiales;Phormidesmiaceae;Phormidesmis_ANT.LACV5.1 -0.02
Bacteria;Proteobacteria;Gammaproteobacteria;Nitrosococcales;Nitrosococcaceae;AqS1 -0.02
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus -0.02
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Microbulbiferaceae;Microbulbifer -0.03
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Spongiibacteraceae;BD1-7_clade -0.03
Bacteria;Proteobacteria;Gammaproteobacteria;Arenicellales;Arenicellaceae;Arenicella -0.04
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Aequorivita -0.07
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae1;Alcanivorax -0.07
Bacteria;Planctomycetota;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;SM1A02 -0.08
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Rickettsiaceae;Orientia -0.12
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Formosa -0.47
-0.50 -0.25 0.00 0.25
coeff
positive negative positive
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;Legionella 0.19
Bacteria;MBNT15;MBNT15;MBNT15;MBNT15;MBNT15 0.16
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Ketobacter 0.13
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Marinobacteraceae;Marinobacter 0.1
Bacteria;Fusobacteriota;Fusobacteriia;Fusobacteriales;Fusobacteriaceae;Psychrilyobacter 0.1
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Saccharospirillaceae;Oleibacter 0.07
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas 0.07
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Weeksellaceae;Chryseobacterium 0.06
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;AB1;AB1 0.06
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Nitrincolaceae;Amphritea 0.03
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Devosiaceae;Maritalea 0.02
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;NS5_marine_group 0.01
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Halocynthiibacter 0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhizobiaceae;Mesorhizobium -0.01
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter -0.03
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Erythrobacter -0.04
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;Haliolobus -0.05
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Maribacter -0.06
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus -0.06
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Xanthobacteraceae;Pseudolabrys -0.06
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;OM60(NOR5)_clade -0.09
Bacteria;Proteobacteria;Gammaproteobacteria;KI89A_clade;KI89A_clade;KI89A_clade -0.1
Bacteria;Proteobacteria;Gammaproteobacteria;Steroidobacterales;Woeseiaceae;Woeseia -0.1
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Lutimonas -0.12
Bacteria;Proteobacteria;Gammaproteobacteria;Gammaproteobacteria_Incertae_Sedis;Unknown_Family;Alkalimarinus -0.28
predictions -0.3 -0.2 -0.1 0.0 0.1 0.2
coeff

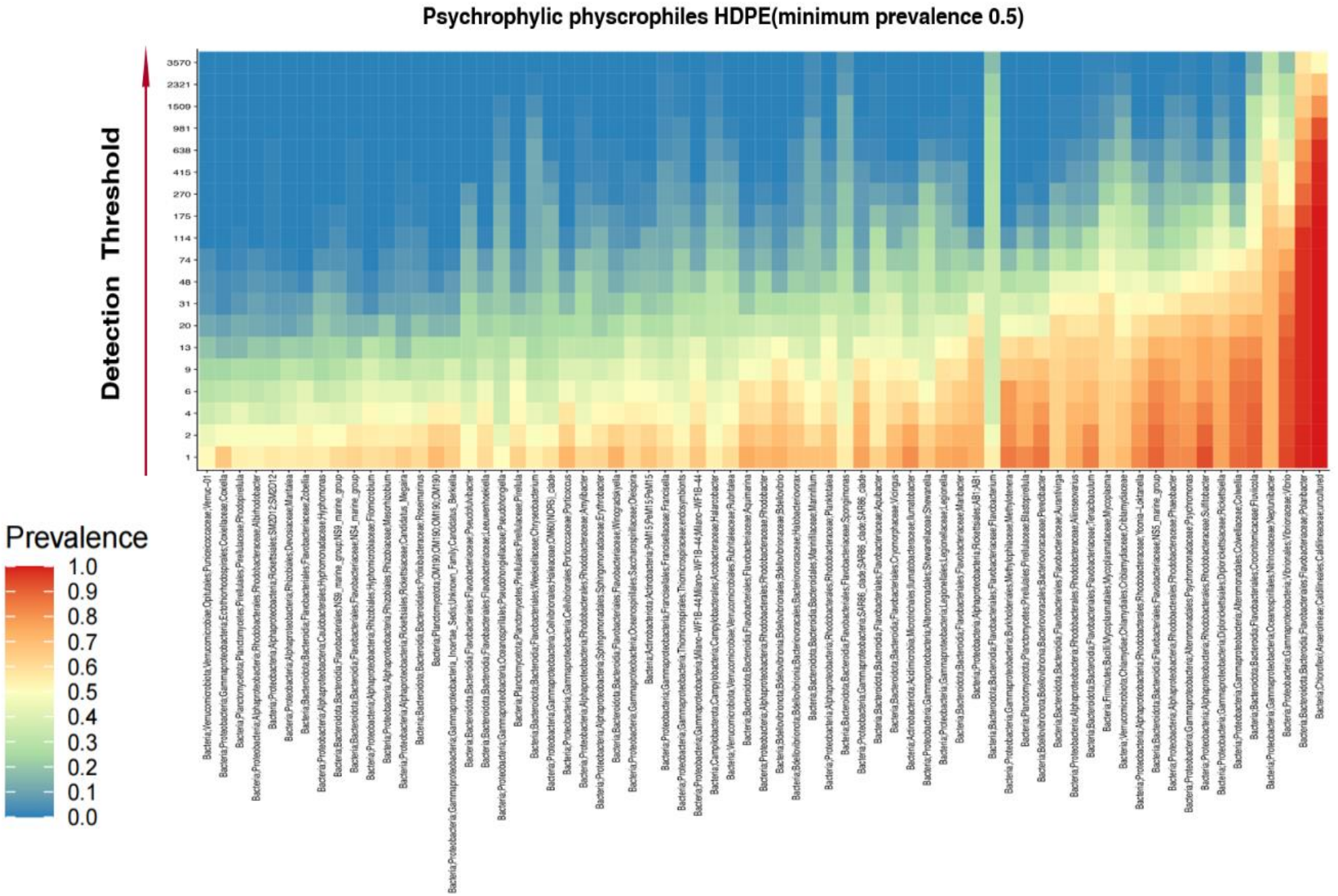

A
y
Thermophylic_psychrophiles_LDPE
Thermophylic_psychrophiles_HDPE
Thermophylic_psychrophiles_HDPE
Thermophylic_psychrophiles_LDPE
y
−2
0
2
4
predictions
B
fill
y
y
Thermophylic_psychrophiles_LDPE
Thermophylic_psychrophiles_HDPE
density
0.3
0.2
0.1
0.0
−2
0
2
4
predictions
positive
negative
positive
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Aliiroseovarius
Bacteria;Proteobacteria;Gammaproteobacteria;Cellvibrionales;Halieaceae;Halioglobus
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Mesoflavibacter
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Ruegeria
Bacteria;Verrucomicrobiota;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Roseibacillus
Bacteria;Verrucomicrobiota;Verrucomicrobiae;Verrucomicrobiales;Rubritaleaceae;Luteolibacter
Bacteria;Proteobacteria;Gammaproteobacteria;Nitrosococcales;Nitrosococcaceae;AqS1
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Rickettsiaceae;Orientia
Bacteria;Planctomycetota;Planctomycetes;Pirellulales;Pirellulaceae;Rhodopirellula
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
Bacteria;Planctomycetota;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;SM1A02
Bacteria;Bacteroidota;Bacteroidia;Flavobacteriales;Flavobacteriaceae;Croceitalea
0.69
0.31
0
−0.03
−0.04
−0.07
−0.07
−0.11
−0.12
−0.13
−0.2
−0.23
−0.25
0.00
0.25
0.50
coeff

Appendix C:


Detection Threshold
791
557
392
276
194
137
96
68
48
34
24
17
12
8
6
4
3
2
1
Prevalence
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0


Low-Salinity-HDPE
(minimum prevalence 0.5)
Detection Threshold
1420
969
661
451
308
210
144
98
67
46
31
21
15
10
7
5
3
2
1
Prevalence
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0


Mesophylic mesophiles HDPE (minimum prevalence 0.5)
Mesophylic mesophiles LDPE (minimum prevalence 0.6)
Detection Threshold
Prevalence
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
2494
1653
1095
725
481
318
211
140
93
61
41
27
18
12
8
5
3
2
1
3344
2182
1423
929
606
395
258
168
110
72
47
30
20
13
8
6
4
2
1


Psychrophylic physcrophiles HDPE(minimum prevalence 0.5)
Detection Threshold
3570
2321
1509
981
638
415
270
175
114
74
48
31
20
13
9
6
4
2
1
Prevalence
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0