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Mapping the Diversity of Beach Fungal Plastisphere: Insights from Metagenomic Approaches

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Abstract

Exploration of fungal communities within the plastisphere reveals a complex interaction between microbial life and plastic pollution. This study investigates the prevalence and distribution of fungal plastisphere from the beach. Plastic samples were obtained at Marunda beach-Jakarta, Cipta beach-Semarang, and Baros beach-Yogyakarta, Java Island, Indonesia. Three type of plastic were high density polyethylene (HDPE), low density polyethylene (LDPE), and polypropylene (PP), which were subjected to scanning electron microscope with energy dispersive X-ray (SEM-EDX) analyzes to determine their surface topography, chemical composition, and adherent biofilms. Internal Transcribed Spacers (ITS) of the ribosomal RNA genes were used in fungal metagenome study. The existence of topographical structures and degrading effects, which promote microbial attachment on the plastic surface, as well as Na, Mg, Cl, Si, Al, S, Ca, Mn, and K element were discovered by SEM-EDX analysis. The results showed the presence and diversity of fungi in plastispheres found on the beach of Java, Indonesia. LDPE is especially suitable for plastisphere than HDPE, and PP. The observed species fungal plastisphere from Baros beach is higher than from Marunda and Cipta beaches. Ascomycota and *Saccharomyces* were dominant at the phylum and genus taxa, respectively. Moreover, these research results provide for fungal plastisphere ecological importance and also their potential role in bioremediation efforts aimed at addressing the global plastic waste challenge.

Keywords: Ascomycota, HDPE, ITS, LDPE, PP, Plastic pollution, *Saccharomyces*

Introduction

In recent decades, the presence of plastic in our environment has become a significant global problem, affecting both terrestrial and marine ecosystems. Plastics, especially in the form of microplastics, have spread to almost all habitats on earth [1]. Plastic waste on beaches is a serious problem caused by a variety of factors related to human activities. Plastic pollution in the ocean mainly comes from mismanaged plastic waste originating from land, which is carried by rivers [2]. It can also come from marine-based activities. The percentage of plastic waste in solid waste generated in Indonesia ranged between 11 and 17 % in the last 4 years (2018 - 2021) [3]. Polyethylene (PE) and polypropylene (PP) are the main types of plastic that are predominantly found wasted in the environment [4]. The main types of "nonbiodegradable" plastics include PE and PP [5]. Polymer types of low density polyethylene (LDPE), high density polyethylene (HDPE), PP are the main macroplastic and microplastic pollutants in Indonesian beaches [6]. Research is needed to better understand the

distribution of plastic particles made of different polymers in the water column as well as the influence of abiotic environmental factors on distribution, behavior, and bioavailability [7].

Plastic pollution phenomenon has created a new environment known as the "plastisphere", which is an ecosystem that develops on the surface of plastic materials that accumulate in the natural environment [8]. Microorganisms within the plastisphere can use plastic as a physical substrate and possible supply of carbon [9]. Compared to community analysis that focus on prokaryotes, eukaryotic communities in the plastisphere are still much less studied [10]. Meanwhile eukaryotic, including fungi, play an important role in forming and maintaining the plastisphere [11]. Fungi, with their ability to degrade various organic compounds, including some plastic components, are key players in the plastic degradation process [12]. Fungi employ a combination of physical attachment strategies, invasive growth forms, enzymatic degradation capabilities, and nutrient exploitation mechanisms to effectively colonize plastic debris. These mechanisms enhance their ability to adhere and thrive on these synthetic surfaces. The plastics can be adhered to and broken down by fungi, and the mycelium that they create changes the plastic's physical structure, which promotes plastic biodegradation [13]. Several fungal enzymes, i.e. laccases, peroxidases, cutinases, and lipases, also contribute to the breakdown of the main plastic's polymers [14]. Research on plastisphere fungi that colonise and multiply on plastic surfaces is important. It can provides new insights into how microbial life adapts to and survives the extreme conditions caused by plastic accumulation.

Application of genomics reveals the full biodiversity of the environment, including the placement of new specimens in their evolutionary context [15]. Metagenomics, an approach that utilizes environmental DNA analysis to identify and understand the genetic diversity of microorganisms, including fungi, offers a very useful tool in exploring the plastisphere [16]. This technique allows us to explore complex and diverse microbial communities without the need for isolation of individual species, providing a comprehensive picture of the structure and function of plastisphere ecosystems [17].

Although the microbiome is involved in plastic degradation, not many analyses have been conducted to compare the composition of plastisphere fungi on HDPE, LDPE, and PP plastics discarded at different beach locations. Using culture-independent and internal transcribed spacer (ITS) methods, the main objective of this study was to identify plastisphere fungi from plastics on beaches in the northern, central, and southern regions of Java Island, Indonesia. Although plastic degradation by fungi has been widely studied, little is known about the metagenome of beach plastisphere fungi. In the future, the use of plastic-degrading fungi may offer an excellent and environmentally friendly plastic degradation process. Therefore, through an in-depth understanding of plastisphere fungi, we can identify their biological potential to degrade plastics, develop new biotechnological solutions, and better understand the ecological impacts of plastic pollution in the marine environment. This research not only expands our knowledge of microbial adaptations and interactions in ecosystems affected by plastic pollution, but also paves the way for more sustainable plastic waste management strategies. In this study, 3 beaches of Marunda-Jakarta, Cipta-Semarang, and Baros-Yogyakarta, Java, Indonesia, were studied. Three locations were chosen because they include plastic-contaminated beaches as hotspots to find plastisphere fungi communities.

Materials and methods

Sample collection

The research was conducted during June-July 2024, including the study of plastisphere fungi metagenome from plastic waste. We selected with visible plastic waste sampling locations at 3 beaches, Marunda Beach, Jakarta (6°05'32"S 106°57'48"E); Cipta Beach, Semarang (6°56'31"S 110°24'42"E); and Baros Beach, Yogyakarta (8°00'31"S 110°16'57"E). This beach was chosen based on its proximity to tourist destinations in the south and north of Java Island and polluted with various types of plastic waste. Physicochemical measurements include air, water, and sand. Sampling was carried out at the same time range in the morning between 07:00 - 10:00 am. A total of 9 samples consist of 3 plastic waste HDPE (High Density Polyethylene), 3 LDPE (Low Density Polyethylene), and 3 PP (Polypropylene), respectively, for each beach.

Nine sample codes are JH: Marunda-Jakarta-HDPE; JL: Marunda-Jakarta-LDPE; JP: Marunda-Jakarta-PP;SH: Cipta-Semarang-HDPE; SL: Cipta-Semarang-LDPE; SP: Cipta-Semarang-PP; YH: Baros-Yogyakarta-HDPE; YL: Baros-Yogyakarta-LDPE; YP: Baros-Yogyakarta-PP. Plastic waste and the top soil are gathered, packed in sterile plastic bags, and brought to the lab on ice. The plastic fragments extracted from the samples are shaken to eliminate bulk dirt. A plastisphere (P) sample is made by physically scraping the layer on plastic with a sterile spoon after it has been allowed to stick firmly to the plastic surface. An acceptable sample of the surrounding soil is thought to be the topsoil that was obtained 10 cm from the spot where the plastic pieces were found. The environment's condition includes various parameters i.e, wind velocity, light intensity, humidity, temperature, pH, turbidity, total suspended solid (TSS), salinity, dissolved oxygen (DO), biological oxygen demand$_5$/BOD$_5$, total ammonia, ortofosfat, total phenolic, and lipid. The water and soil physicochemical analysis were examined at PT Greenlab Indo Global, Yogyakarta, Indonesia.

SEM-EDX analysis

Scanning electron microscope with energy dispersive X-ray (SEM-EDX) analysis to get information about the morphology, structure, and composition of the plastic sample. Plastic strips of around 0.5 cm^2 and containing plastisphere were fixed at 4 °C in sterile seawater with 50 mM sodium cacodylate (pH 7.2) and 2.5 % glutaraldehyde. Before being processed for scanning electron microscopy, the samples were kept in the fixative at 4 °C for 5 days. The samples were gradually dehydrated using a series of 10-minute ethanol baths at 30, 50, 70 and 90 % concentrations. This was followed by 3 10-minute baths in 100 % ethanol [18]. After that, the samples were placed on pellets on a carbon-covered copper grid for SEM-EDX analysis (JSM-6510LA, Japan).

Extraction, amplification, and sequencing of total DNA

About 10 g of plastic for each sample were sent for DNA extraction to PT Genetika Science Indonesia. Following the manufacturer's instructions. Total DNA was extracted from each sample using a ZymoBIOMICS^{TM} DNA Miniprep Kit (Qiagen, Hilden, Germany) [19]. Initial quantification and purity observation with the Nanodrop ND2000 spectrophotometer (Thermo Scientific, Waltham, USA). PCR amplification with Phusion™ Plus PCR Master Mix (F631S). The PCR cycle used was 94 °C initial denaturation for 2 min, 40 cycles including denaturation at 94°C for 15s, annealing at 56°C for 30 s, extension at 68°C, and final extension at 68°C for 10 min 40 s. PCR and product PCR visualization with Agarose Gel Electrophoresis. Accurate quantification with Qubit dsDNA HS Assay Kits (Thermo Scientific, Waltham, USA).

The principle of metagenomic diversity analysis is based on DNA analysis taken directly from the plastisphere. The internal transcribed spacer (ITS) rRNA is part of the non-transcriptional region of the fungal rRNA gene and is used for fungal identification. The ITS region shows higher variability compared to the better-preserved rRNA coding sequences, making it valuable for phylogenetic studies and species identification. The replicated metagenomic DNA was pooled, and the ITS 1 gene was amplified using ITS5-1737F primer (GGAAGTAAAAGTCGTAACAAGG) and the ITS2-2043R primer (GCTGCGTTCTTCATCGATGC) pair to generate an amplicon library. The fungal community was analyzed using Illumina Miseq PE300 platform (Illumina, San Diego, USA).

Bioinformatic analysis

Paired-end raw reads generated from the final library were sequenced on the Illumina platform. FastQC checks raw readings for quality assurance. Cutadapt (v1.17) and fastp (v0.20.0) were used to remove less than 50 bp of fake, low-quality sequences (sliding window 5, quality score <20) from raw sequence readings [20]. QIIME 2 (Quantitative Insights into Microbial Ecology) (v2022.2) with the DADA2 plugin was used to perform quality control and feature table construction on raw sequences. DADA2 was used to correct sequencing errors, remove low-quality sequences, and remove chimera errors [21]. The Amplicon Sequence Variants (ASV) data was used for taxonomic classification against the UNITE (ITS) database. By comparing the fungal gene sequences to

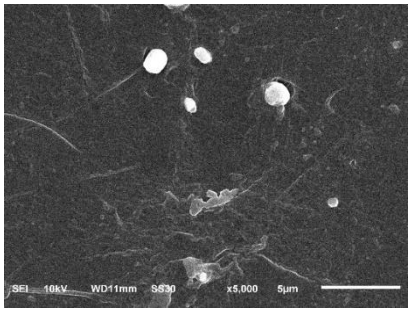
the UNITE (v7) database, they were grouped into operational taxonomic units (OTUs). Additionally, a 97 % similarity level served as the basis for the OTU identification. Downstream analysis and visualizations were performed using packages in RStudio (R version 4.2.3) (https://www.R-project.org/), Krona Tools (https://github.com/marbl/Krona), PICRUSt2 (https://github.com/picrust/picrust2). Group 1, 2, and 3 for PP, HDPE, and LDPE, respectively.

Results and discussion

Measuring environmental factors during microbial state analysis is critical to understanding how microbes interact with their surroundings and how these conditions affect microbial growth, activity, and characteristics. Environmental factors measured during analysis can provide insight into the conditions that favor or inhibit microbial life, as well as identify changes that occur in microbial responses to environmental changes. **Table 1** presents the results of physicochemical factor measurements at the 3 beaches where plastic samples were collected. Numerous environmental factors, including pH, water temperature, dissolved oxygen, illumination, nutrient concentration, salinity, and hydrodynamics, have been found to have an impact on the plastisphere's composition and functionality. Even though earlier research has highlighted various specific environmental aspects, including turbidity, suspended particles, and hydrodynamics, little is known about how each of these elements affects the water plastisphere on its own [22]. Moreover, topography, wind and water currents, and proximity to pollution sources control the amount and type of plastic along the coastline, while degradation processes determine how long plastic debris remains on the beach. Environmental condition with pH 7 and temperature 30°C are suitable for the growth of plastic degrading fungi [23].

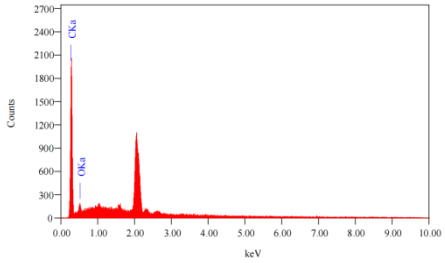
Table 1 Physicochemical measurements of the sampling site environment.

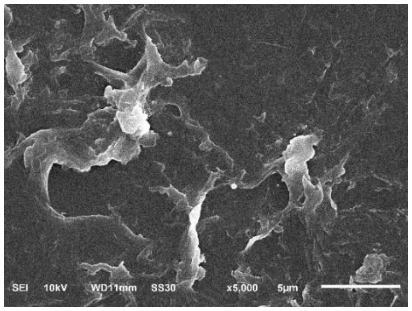
Analysis	Beach location		
	Marunda	**Cipta**	**Baros**
Wind velocity (m/s)	1.1	3.0	4.15
Light intensity (lux)	3.731×10	9.615×10	8.897×10
Air humidity (%)	72	35	75
Air temperature (°C)	29	30	30
Water temperature (°C)	27.5	29.6	26.6
pH of water	6.99	7.39	7.17
Sand humidity (%)	3.0	5.85	4.5
Water turbidity (NTU)	29.6	24.4	38.6
Total suspended solid/TSS (mg/L)	15.8	16.7	34.5
Salinity (‰)	33.0	33.0	33.0
Dissolved oxygen/DO (mg/L)	7.95	6.61	7.55
Biological oxygen demand$_5$/BOD$_5$(mg/L)	0.587	0.940	1.80
Total ammonia (mg/L)	0.575	0.027	0.267
Orthophosphate (mg/L)	0.012	0.014	1.20
Total phenolic (mg/L)	0.063	0.0013	< 0.018
Lipid (mg/L)	0.500	0.600	0.800



JL

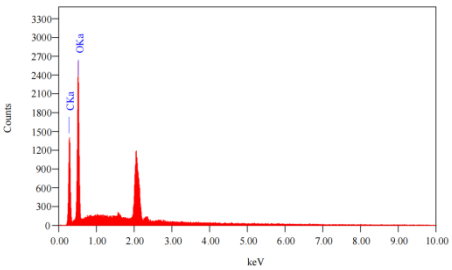
Element	Weight (%)	Atomic (%)
C	84.02	87.51
O	15.98	12.49

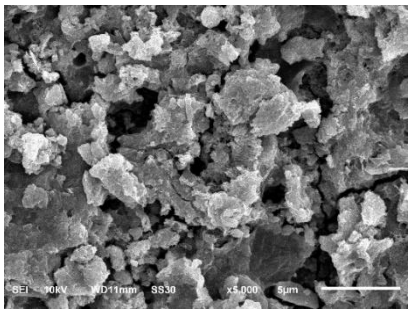




JP

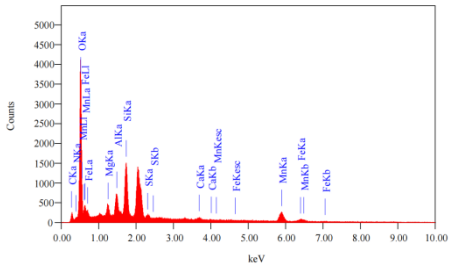
Element	Weight (%)	Atomic (%)
C	30.57	36.97
O	69.43	63.03

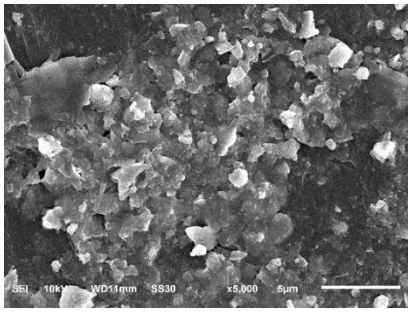




SH

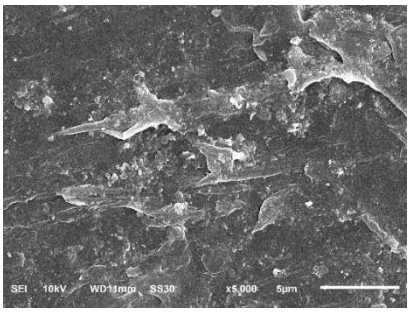
Element	Weight (%)	Atomic (%)
C	3.00	5.91
N	2.38	4.03
O	39.56	58.60
Mg	2.20	2.14
Al	3.73	3.28
Si	10.26	8.65
S	1.53	1.13
Ca	1.27	0.75
Mn	28.92	12.48
Fe	7.15	3.03



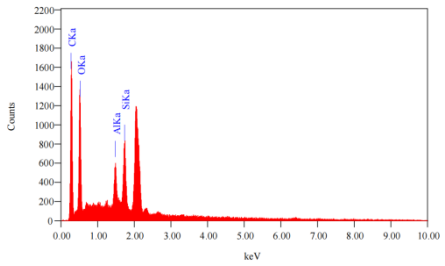


SL

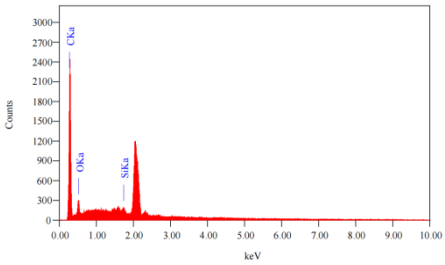
Element	Weight (%)	Atomic (%)
C	42.12	52.46
O	41.21	38.54
Al	5.90	3.27
Si	10.77	5.74



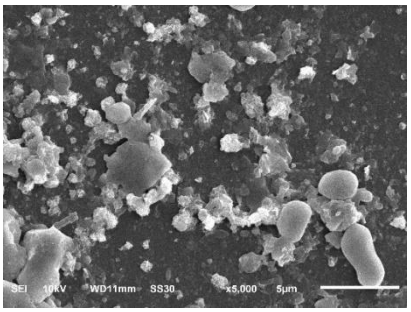
SP



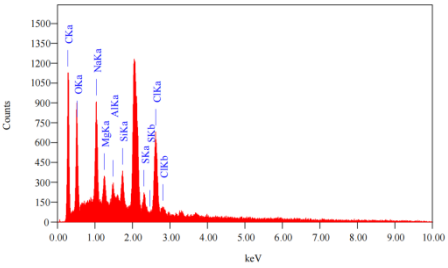
Element	Weight (%)	Atomic (%)
C	76.45	81.97
O	20.88	16.80
Si	2.67	1.22



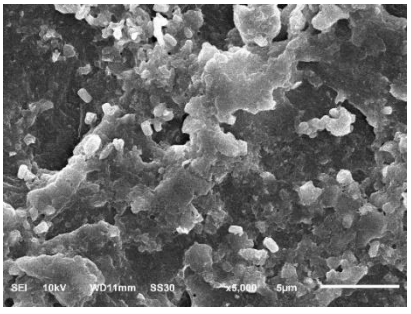
Element	Weight (%)	Atomic (%)
C	43.07	57.55
O	25.54	24.62
Na	7.74	5.40
Mg	2.32	1.53
Al	1.57	0.94
Si	3.35	1.92
S	3.48	1.74
Cl	13.92	6.30



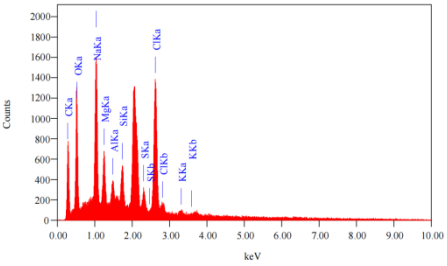
YH

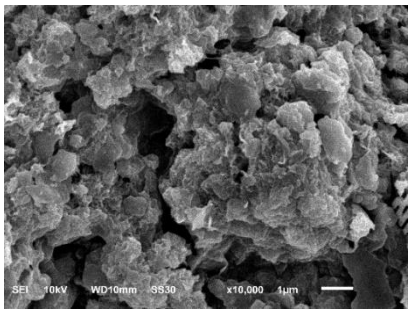


Element	Weight (%)	Atomic (%)
C	28.20	42.64
O	25.21	28.62
Na	10.68	8.44
Mg	4.07	3.04
Al	1.84	1.24
Si	3.85	2.49
S	3.64	2.06
Cl	21.35	10.94
K	1.15	0.53



YL





YP

Element	Weight (%)	Atomic (%)
C	2.18	4.58
O	38.77	61.07
Na	0.79	0.87
Mg	2.46	2.55
Al	2.36	2.20
Si	7.49	6.72
S	1.83	1.44
Ca	1.96	1.23
Mn	42.16	19.34

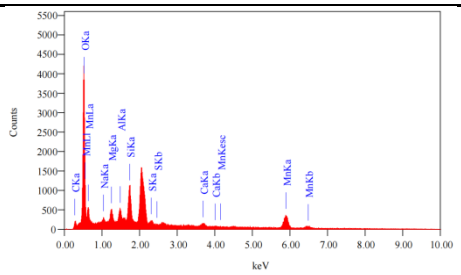


Figure 1 SEM-EDX microscopy of plastic waste. JH: Marunda-Jakarta-HDPE; JL: Marunda-Jakarta-LDPE; JP: Marunda-Jakarta-PP; SH: Cipta-Semarang-HDPE; SL: Cipta-Semarang-LDPE; SP: Cipta-Semarang-PP; YH: Baros-Yogyakarta-HDPE; YL: Baros-Yogyakarta-LDPE; YP: Baros-Yogyakarta-PP.

The use of SEM-EDX in fungal plastisphere studies provides important insights into the interactions between plastic and fungal in aquatic environments. The fungus's composition can also be linked to polymer characteristics like roughness. By analyzing surface morphology and chemical composition, this study helps to understand how plastics affect aquatic ecosystems and their potential impact on environmental health. According to the SEM analysis at **Figure 1**, the plastic surface exhibits delamination at different depths and textures. The deeper layers have a more gritty texture, while the surface layer appears smoother. SEM observations showed the presence of a layer on the surface of the plastic, which is an indicator of the presence of a plastisphere. The presence of plastic spheres on the surface of the plastic will affect the physicochemical properties of the polymer, including an increase in surface area and pore volume [24]. The physical characteristics, biota, and environmental interactions of plastics are greatly influenced by their surface shape and content. Adsorption, transit, sedimentation, and the fate of specific plastic are all determined by the accessible surface and its structure, even if it is smaller than the biofilm size [25]. Different layer thicknesses show different elements, when the thin layer on JL and JP samples, the detected elements are only C and O. When the observed layer is thicker, more elements are detected. Examination of surface roughness provides fungal colonization on plastic. Rougher surface provide more area for fungal attachment, facilitating plastisphere formation [26]. Surface irregularities can trap moisture and organic matter, creating microenvironment conducive to fungal growth [27]. Weight percent (%) is the percentage of an element's mass relative to the total mass of the sample. Atomic percent (%) is the percentage of an element's atom relative to the total number of atoms in the sample. When atoms have substantially varying atomic weights, atomic percent can give more accurate approximations. For instance, lighter elements might have a higher fraction by atom count but be present in smaller amounts by weight. Based on the EDX analysis, the elements C and O were found in all samples. Carbon and oxygen are 2 vital elements that influence microbial growth. Carbon provides an energy source and structural components, while oxygen supports efficient aerobic respiration. Understanding this relationship is important for managing microbial ecosystems in various applications, including plastisphere formation. For the purpose of distinguishing carbon-dominant polymers from other interfering materials, it is highly helpful to know the elemental composition of a particle. Other elements

found are sodium (Na), magnesium (Mg), chloride (Cl), silica (Si), alumunium (Al), sulphur (S), calsium (Ca), manganese (Mn) and kalium (K). This is an indicator of the presence of organic, inorganic, microplastic, and metallic materials. EDX analysis is particularly useful for the discrimination of organic (rich in Ca/Mg/Sr) and inorganic (minerals, salts) and microplastics (rich in C/Cl/S/Ti) [28]. C, Ti, Ba, Si, Al, Fe, and O atoms are distributed on microplastic debris found in the coastal area [29]. Arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), tin (Sn), titanium (Ti) and vanadium (V) are trace elements that can be accumulated in the plastisphere [30].

The results of metagenome analysis of the diversity of plastisphere fungi found in plastic waste from all locations showed differences. As **Table 2** shows, the comparison curve of fungal species diversity in 3 beach locations. Alpha-diversity (α-diversity) is the average species diversity in a location at a local scale, mainly focusing on the number of species in a homogeneous local habitat. In the field of microbial ecology, analyzing alpha diversity from amplicon sequence data is a common 1st step to assess differences in microbial environments. The Shannon index is a measure used to estimate the diversity of microbial species in a given sample. Higher Shannon values signify higher levels of community diversity. The highest shannon index is JL sample followed by YL, and YP. Simpson's index is used as a measure of concentration when identifying individuals, and, as such, is used to estimate one index of microbial diversity in a sample. A lower Simpson's index value indicates a more diverse community, while a higher value indicates that 1 or few species dominate. The lowest Simpson's index is SP. The total number of species observed from Marunda, Cipta, and Baros beaches were 587, 455, and 1,225, respectively. Abiotic factors affect the formation of the plastisphere, Total suspended solid (TSS) in Baros beach is higher than Marunda and Cipta beach. Overall, TSS is the amount of solid matter suspended in water and provides information about water pollution. Suspended compounds in the water column have a tendency to create a conditioning coating on the plastic surface, altering its hydrophobicity and facilitating the attachment of microorganisms [31]. The elevated TSS levels in aquatic environments promote fast biofilm development and colonization on plastic fragments [32]. The highest of orthophosphate content in Baros beach also support to fungal growth. Orthophosphate is a crucial macronutrient for fungi, influencing of fungal growth, metabolism, and interaction with other organisms [33].

Table 2 Result of alpha diversity.

Sampel	*Observed species*	Shannon	Simpson
JH	204	3.305	0.899
JL	229	4.657	0.981
JP	154	3.071	0.921
SH	199	3.267	0.919
SL	167	3.197	0.899
SP	89	2.622	0.751
YH	328	3.424	0.827
YL	461	4.323	0.950
YP	436	3.912	0.935

JH: Marunda-Jakarta-HDPE; JL: Marunda-Jakarta-LDPE; JP: Marunda-Jakarta-PP; SH: Cipta-Semarang-HDPE; SL: Cipta-Semarang-LDPE; SP: Cipta-Semarang-PP; YH: Baros-Yogyakarta-HDPE; YL: Baros-Yogyakarta-LDPE; YP: Baros-Yogyakarta-PP.

Table 2 displays the alpha diversity index of plastisphere fungi from 9 plastic samples of different plastic types and sampling locations. The current study shows species observed were highest in the Baros Beach

Yogyakarta for both LDPE, HDPE, and PP plastic types. While the highest diversity in samples from Marunda Beach is LDPE plastic, followed by Baros Beach Yogyakarta LDPE plastic. The lowest diversity was in PP plastic from Cipta Beach. Prior research has demonstrated that the organization and makeup of microbial communities on plastic and natural surfaces exhibit distinct patterns. Other elements that affect the formation of biofilms include the surface functional groups of plastics, their diverse morphologies, and their biochemical interactions with microbial communities [34]. According to fungal species detected, LDPE (857) is greater than HDPE (731), and PP (679). LDPE particularly suitable for plastisphere compared to HDPE and PP due to several characteristics than enhance its interaction with microbial community and environmental factors. Recent studies have indicated that LDPE is more readily degraded by certain microbial communities than HDPE and PP [35].

The plastisphere is distinctive due to the wide range of abiotic environmental influences. Even for plastic particles in the same area, the variety and quantity of microbial species can vary, despite the plastisphere's microbial phylum being relatively identical [36]. The plastisphere's particular microbiological makeup varies based on a number of variables, including the season, location, polymer types [37], physical and chemical properties, and time of exposure [38]. One of the influencing factors is environmental conditions. The structure of the fungal community is influenced by pH and BOD_5 [39]. pH plays a crucial role in assessing the degree of toxicity of some contaminants in water as well as whether a chemical or biological reaction can occur [40]. BOD_5 is used as an indicator to assess the level of organic pollution in water. The higher the BOD_5 value, the more organic matter is present, which indicates the potential for pollution and its impact on water quality and aquatic life.

Metagenomic data analysis, in addition to each sample, was also grouped by plastic type. Groups 1, 2, and 3 are HDPE, LDPE, and PP plastics. **Figure 2** represents the top 10 phylum and genus of the 3 groups. For phylum level, relative abundance of group 1 are Ascomycota (83.01 %), Basidiomycota (5.90 %), and Chytridomycota (4.68 %). Relative abundance for group 2 are Ascomycota (48.74 %), Chlorophyta (22.13 %), and Basidiomycota (19.82 %). Moreover, Ascomycota (80.92 %), Basidiomycota (16.80 %), and Cercozoa (1.90 %) are dominant for group 3. This result is in line with previous studies that find Ascomycota, Basidiomycota, Chytridiomycota, and Mucoromycota were dominant in the terrestrial-marine ecotone [41]. The Ascomycota, Basidiomycota, and Chytridiomycota phyla on biodegradable plastics and foamed polystyrene substitutes submerged in a marina [42]. Ascomycota dominant in the plastisphere even in extreme weather conditions [43]. Chytridiomycota and Cryptomycota species were found in freshwater plastisphere [44]. Both for the 3 group, Ascomycota was superior, as was the same with other studies that Ascomycota was the majority in the Alpine and Arctic terrestrial plastisphere [45] Ascomycota, Mortierellomycota, and Basidiomycota dominated in polyethylene (PE) and polybutylene adipate terephthalate/polylactide (PBAT/PLA) microplastics in farmland [46].

Furthermore, *Saccharomyces* (60.03 %), *Cladosporium* (22.39 %), and *Nigrospora* (7.33 %) are dominant genera in group 1. The 3 superior genera of group 2 are *Saccharomyces* (24.53 %), *Trichosporon* (24.36 %), and *Cercozoa* (10.38 %). Additionally, *Hortaea* (34.02 %), *Saccharomyces* (22.65 %), and *Neodevriesia* (14.44 %) were superior genera in group 3 in this study. This result not in line with previous studies examine *Fusarium* mainly on plastic's surface [47]. This is different from previous research, which shows *Gibberella* (12 %), *Trichosporon* (3.1 %), and *Paraphoma* (2.8 %) were dominant [48]. *Alternaria* being specifically enriched in the soil plastisphere [49]. Moreover, other research showed that fungal genera from the plastisphere i.e. *Aspergillus* [50], *Penicillium*, *Peacilomyces*, *Absidia*, and *Cochliobolus* [51], *Trichoderma*, *Monascus*, *Clitocybe*, and *Phanerochaete* [52] demonstrated plastic-degrading ability.

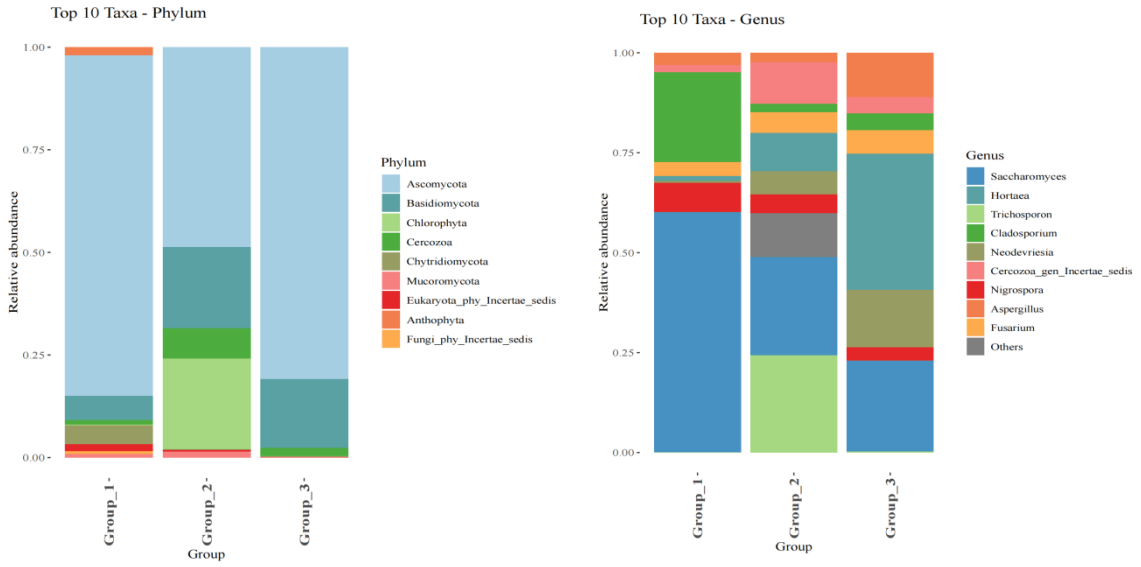


Figure 2 The top 10 relative abundance of phylum and genus taxa based on group 1 (HDPE), 2 (LDPE), 3 (PP).

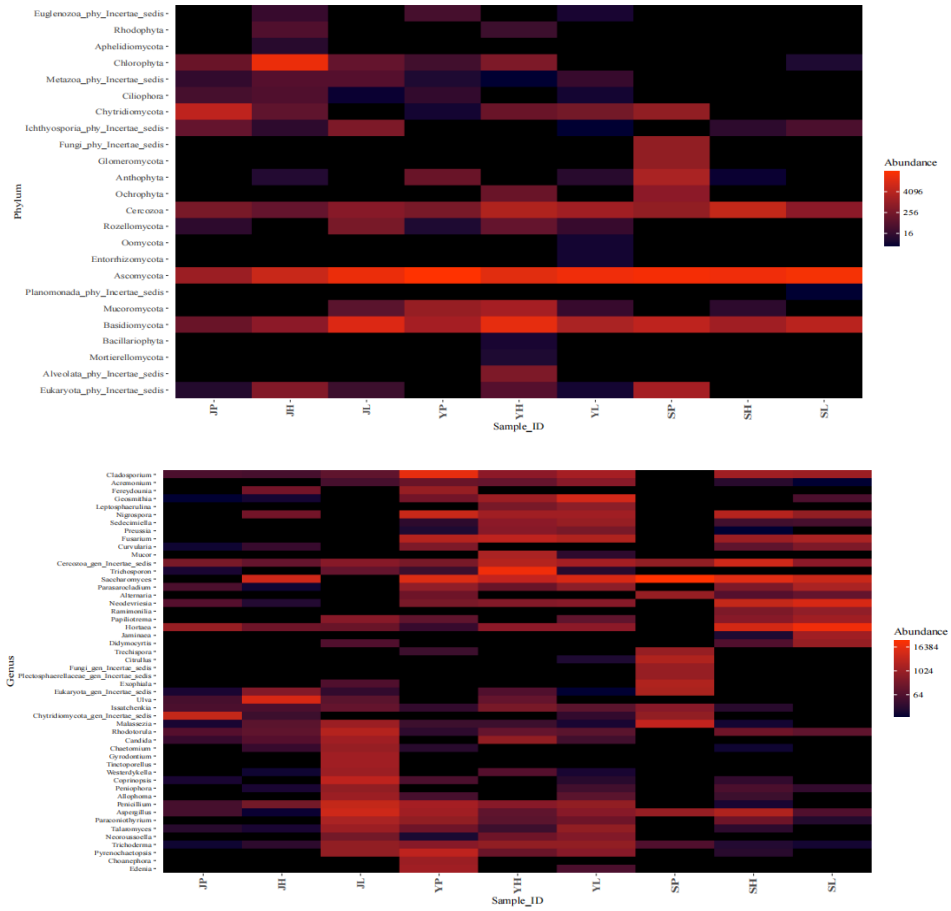


Figure 3 Heatmap analysis of the fungal community (a. phylum; b.genus taxa) of fungal plastisphere and plastic type.

The percentage of each taxon can be used to illustrate the organization of fungal communities from the plastisphere. Results of the heatmap analyzis of the samples are presented in **Figure 3**. At the phylum taxa level, it appears that Ascomycota, followed by Basidiomycota were dominant in all samples. These results match those observed in earlier studies that Ascomycota and Basidiomycota were predominant at

the plastisphere [53]. The Ascomycota phylum includes a wide range of species with unique characteristics, which allow them to fill various ecological niches in the plastisphere. Interestingly, Ascomycota and Basidiomycota have survival capability with increasing temperature and CO_2 levels [54]. This diversity and growth strategy helps them compete with other microorganisms and dominate the microbial community in the plastisphere. However, at the genera level, there is almost no equal dominance in all samples. It is apparent from this heatmap that *Saccharomyces* is dominant in 7 of the 9 samples. *Saccharomyces*'s ability to quickly adapt [55], and withstand environmental stress [56] makes it a dominant force in the plastisphere ecosystem. The presence of *Saccharomyces* in the plastisphere underscores adaptability this fungi to diverse environment. While its primary roles may not only for plastic degradation.

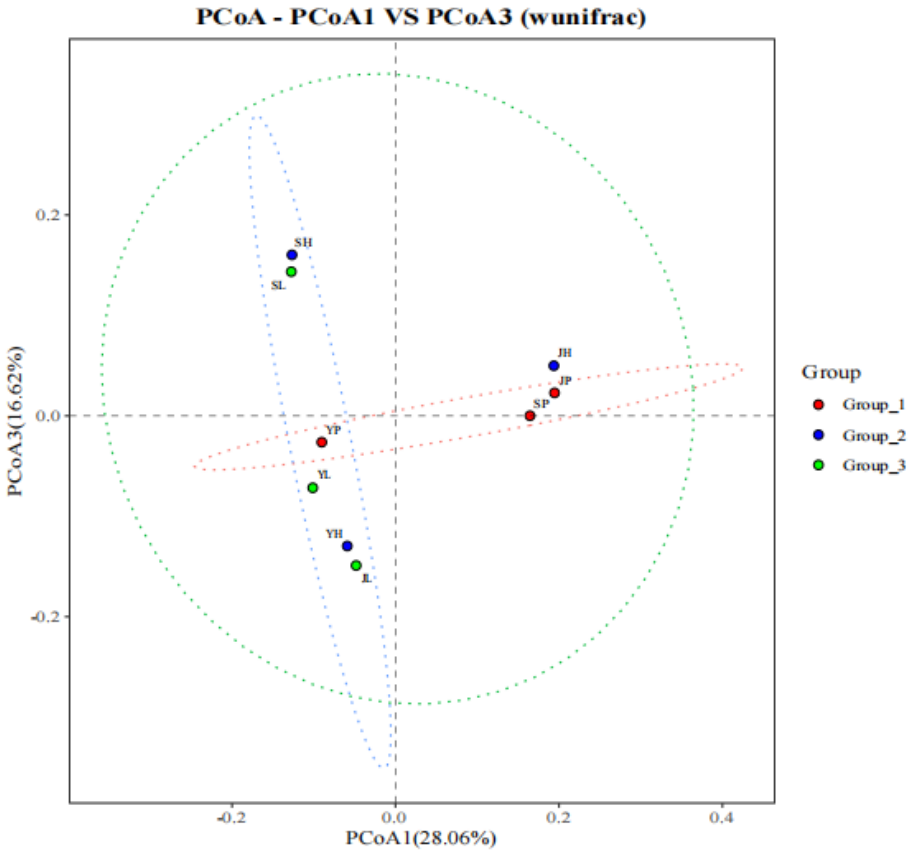


Figure 4 Principal coordinates analysis (PCoA) of the fungal plastisphere.

A multivariate statistical method called principal component analysis, or PCA, aids in visualizing the distribution patterns and genetic relationships of the recovered sequences. The fungal communities in the plastisphere, clustered along the 1st (28.06 %) and 2nd principal coordinates (16.62 %). As shown in **Figure 4**, according to the PCoA scatter plot there are 3 distinct groups. The same locations tend to cluster in 1 group, i.e SH-SL, JH-JP, YP-YL. This shows that the same environmental conditions tend to affect the diversity of plastisphere fungi more than the type of plastic. Plastic types can provide a substrate for fungi to thrive, but their presence is outweighed by the influence of wider environmental factors. For example, although some fungi can utilize plastics as a carbon source, their existence remains dependent on favorable environmental conditions. The results of this study are in line with previous research that shows the type of plastic does not affect the composition of plastisphere fungi [58]. SP, JL, YH samples which do not cluster according to location can be related several ecological, methodological, and environmental factors. The

ecological variability i.e. species composition and microhabitat differences. SP, JL, and YH samples may composed different species compositions that are not influenced by their geographical proximity. Even within the same geographical area, microhabitat can be vary significantly.

Conclusions

In this study, fungal communities of different plastic types (HDPE, LDPE, PP) from 3 beaches (Marunda-Jakarta, Cipta-Semarang, Baros-Yogyakarta), on Java island, Indonesia, were analyzed. Morphological observation and elemental composition by SEM-EDX showed various elemental compositions and also morphological and degrading effects of the samples, which promote microbial attachment on the plastic surface. ITS-based metagenome analysis showed abundant OTUs identified at different taxonomic levels. The number of species observed was highest in Baros beach, followed by Marunda and Cipta. Morever, at the phylum level, it was dominated by Ascomycota and Basidiomycota, while at the genera level was *Saccharomyces*.

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References

[1] H Hamdhani, G Ghitarina, R Eryati and DE Eppehimer. Occurrence of microplastic ingestion by commercial fish species from the pangempang estuary in Indonesia. *Trends in Sciences* 2024; **21(7)**, 7762.

[2] MM Sari, P Andarani, S Notodarmojo, RK Harryes, MN Nguyen, K Yokota and T Inoue. Plastic pollution in the surface water in Jakarta, Indonesia. *Marine Pollution Bulletin* 2022; **182**, 114023.

[3] Z Arifin, D Falahudin, H Saito, T Hendrawati, M Hafizt and Y Suteja. Indonesian policy and researches toward 70 % reduction of marine plastic pollution by 2025. *Marine Policy* 2025; **155**, 105692.

[4] P Li, X Wang, M Su, X Zou, L Duan and H Zhang. Characteristics of plastic pollution in the environment: A review. *Bulletin of Environmental Contamination and Toxicology* 2021; **107(4)**, 577-584.

[5] X Zhai, XH Zhang and M Yu. Microbial colonization and degradation of marine microplastics in the plastisphere: A review. *Frontiers in Microbiology* 2023; **14**, 1-15.

[6] P Vriend, H Hidayat, JV Leeuwen, MR Cordova, NP Purba and AJ Löhr. Plastic pollution research in Indonesia: state of science and future research directions to reduce impacts. *Frontiers in Environmental Science* 2021; **9**, 692907.

[7] M Oliveira and M Almeida. The why and how of micro(nano)plastic research. *TrAC Trends in Analytical Chemistry* 2019; **114**, 196-201.

[8] ALDF Lacerda, JD Taylor, LDS Rodrigues, F Kessler, E Secchi and MC Proietti. Floating plastics and their associated biota in the Western South Atlantic. *The Science of the Total Environment* 2022; **805**, 150186.

[9] A Delacuvellerie, V Cyriaque, S Gobert, S Benali and R Wattiez. The plastisphere in marine ecosystem hosts potential specific microbial degraders including *Alcanivorax borkumensis* as a key player for the low-density polyethylene degradation. *Journal of Hazardous Materials* 2019; **380**, 120899.

[10] M Latva, CJ Dedman, RJ Wright, M Polin and JA Christie-Oleza. Microbial pioneers of plastic colonisation in coastal seawaters. *Marine Pollution Bulletin* 2022; **179**, 113701.

[11] M González-Pleiter, D Velázquez, MC Casero, B Tytgat, E Verleyen, F Leganés, R Rosal, A Quesada and F Fernández-Piñas. Microbial colonizers of microplastics in an Arctic freshwater lake. *Science of The Total Environment* 2021; **795**, 148640.

[12] A Safdar, F Ismail and M Imran. Biodegradation of synthetic plastics by the extracellular lipase of *Aspergillus niger*. Environ. Adv., 2024; **17**, 100563.

[13] Y Du, X Liu, X Dong and Z Yin. A review on marine plastisphere: Biodiversity, formation, and

role in degradation. *Computational and Structural Biotechnology Journal* 2022; **20(3)**, 975-988.

[14] MEE Temporiti, L Nicola, E Nielsen and S Tosi. Fungal enzymes involved in plastics biodegradation. *Microorganisms* 2022; **10(6)**, 1-27.

[15] ÂM Ribeiro, AD Foote, A Kupczok, B Frazão, MT Limborg, R Piñeiro, S Abalde, S Rocha and RRD Fonseca. Marine genomics: News and views. *Marine genomics* 2017, **31**, 1-8.

[16] I Mani. *Metagenomics approach for bioremediation: Challenges and perspectives*. Elsevier Inc, New York, 2020, p. 275-285.

[17] R Kirubakaran, KN ArulJothi, S Revathi, N Shameem and JA Parray. Emerging priorities for microbial metagenome research. *Bioresource Technology Reports* 2020; **11**, 100485.

[18] IV Kirstein, A Wichels, E Gullans, G Krohne and G Gerdts. The plastisphere - Uncovering tightly attached plastic 'specific' microorganisms. *PLoS One* 2019, **14(4)**, e0215859.

[19] ND Takarina, AM Sukma, A Adiwibowo and S Sitaresmi. Water quality and microbial community assessment in artisanal mining- affected sediments of Cikidang River, Banten, Java, Indonesia. *Sains Malaysiana* 2022, **51(5)**, 1339-1352.

[20] Y Zhang, J Tao, Y Bai, F Wang and B Xie. Incomplete degradation of aromatic-aliphatic copolymer leads to proliferation of microplastics and antibiotic resistance genes. *Environment International* 2023; **181(10)**, 108291.

[21] Y Li, R Yang, L Guo, W Gao, P Su, Z Xu, H Xiao, Z Ma, X Liu, P Gao, B Li, X Sun, G Yan and W Sun. The composition, biotic network, and assembly of plastisphere protistan taxonomic and functional communities in plastic-mulching croplands. *Journal of Hazardous Materials* 2022; **430**, 128390.

[22] Y Yu, L Miao, TM Adyel, K Waldschläger, J Wu and J Hou. Aquatic plastisphere: Interactions between plastics and biofilms. *Environmental Pollution* 2023; **322**, 121196.

[23] MF Fachrul, A Rinanti, S Salmiati and T Sunaryo. Degradation of polyethylene plastic waste by indigenous microbial consortium and fungi. *Indonesian Journal of Urban and Environmental Technology* 2021; **5(1)**, 86-103.

[24] J Kapelewska, J Karpińska, U Klekotka and A Piotrowska-Niczyporuk. Effect of polyethylene microplastic biodegradation by algae on their sorption properties and toxicity. *Chemosphere* 2025; **370**, 143993.

[25] A Dąbrowska, M Gniadek and P Machowski. The proposal and necessity of the numerical description of nano- and microplastics' surfaces (Plastisphere). *Polymers* 2021; **13(14)**, 2255.

[26] C Maddison, CI Sathish, D Lakshmi, OC Wayne and T Palanisami. An advanced analytical approach to assess the long-term degradation of microplastics in the marine environment. *npj Materials Degradation* 2023, **7(1)**, 1-11.

[27] B Küppers, S Schloegl, G Oreski, R Pomberger and D Vollprecht. Influence of surface roughness and surface moisture of plastics on sensor-based sorting in the near infrared range. *Waste Management & Research* 2019; **37(8)**, 843-850.

[28] M Gniadek and A Dąbrowska. The marine nano- and microplastics characterisation by SEM-EDX: The potential of the method in comparison with various physical and chemical approaches. *Marine Pollution Bulletin* 2019; **148(51)**, 210-216.

[29] AD Forero-López, A Poza, CV Colombo, NGM Pontet, GN Rimondino, A Toniolo, FE Malanca and SE Bott. Chemical analysis of marine microdebris pollution in macroalgae from the coastal areas of Argentina. *Science of the Total Environment* 2024; **923(1)**, 171390.

[30] V Lenoble, AM Cindri, JF Briand, ML Pedrotti, AL Lacerda, S Muniategui-Lorenzo, V Fernandez-Gonzalez, CM Moscoso-Perez, JM Andrade-Garda, R Casotti, C Murano, V Donnarumma, S Frizzi, C Hannon, H Joyce, R Nash and J Frias. Bioaccumulation of trace metals in the plastisphere: Awareness of environmental risk from a European perspective. *Environmental Pollution* 2024; **348(13)**, 123808.

[31] ADF López, GE De-la-Torre, MD Fernandez SeveriA, G Prieto, LI Brugnoni, CV Colombo, DC Dioses-Salinas, GN Rimondino and CV Spetter. Chemical-analytical characterization and leaching of heavy metals associated with nanoparticles and microplastics from commercial face masks and the abundance of personal protective equipment (PPE) waste in three metropolitan cities of South

America. *Marine Pollution Bulletin* 2023; **191**, 114997.

[32] TŽ Pfeiffer, DS Maroni, F Stevi, AG Balki, N Bek, A Martinovi, T Mandir, R Nikolaˇsevi and D Janji. Plastisphere development in relation to the surrounding biotic communities. *Environmental Pollution* 2022; **306**, 119380.

[33] K Bhalla, X Qu, M Kretschmer and JW Kronstad. The phosphate language of fungi. *Trends in Microbiology* 2022, **30(4)**, 338-349.

[34] P Sooriyakumar, N Bolan, M Kumar, L Singh, Y Yu, Y Li, C Weralupitiya, M Vithanage, S Ramanayaka, B Sarkar, F Wang, DB Gleeson, D Zhang, MB Kirkham, J Rinklebe and KHM Siddique. Biofilm formation and its implications on the properties and fate of microplastics in aquatic environments: A review. *Journal of Hazardous Materials Advances* 2021; **6**, 100077.

[35] JA Wallbank, G Lear, JM Kingsbury, L Weaver, F Doake, DA Smith, F Audre´Zet, SDM Maday, V Gambarini, L Donaldson, B Theobald, M Barbier and O Pantos. Into the Plastisphere, where only the generalists thrive: Early insights in plastisphere microbial community succession. *Frontiers in Marine Science* 2022, **9**, 841142.

[36] KHD Tang. Terrestrial and aquatic plastisphere: formation, characteristics, and influencing factors. *Sustainability* 2024; **16(5)**, 2163.

[37] R Tavelli, M Callens, C Grootaert, MF Abdallah and A Rajkovic. Foodborne pathogens in the plastisphere: Can microplastics in the food chain threaten microbial food safety? *Trends in Food Science & Technology* 2022; **129**, 1-10.

[38] F Audrézet, A Zaiko, G Lear, S Wood, LA Trembla and Pochon. Biosecurity implications of drifting marine plastic debris: Current knowledge and future research. *Marine Pollution Bulletin* 2021; **162**, 111835.

[39] S Yang, L Li, X Peng and L Song. Leachate microbiome profile reveals bacteria, archaea and eukaryote dynamics and methanogenic function during solid waste decomposition. *Bioresource Technology* 2021; **320(A)**, 124359.

[40] NS Harun, MM Hanafiah, NUM Nizam and A Rasool. Water and soil physicochemical characteristics of different rice cultivation areas. *Applied Ecology and Environmental Research* 2020; **18(5)**, 6775-6791.

[41] G Pang, X Li, M Ding, S Jiang, P Chen, Z Zhao, R Gao, B Song, X Xu, Q Shen, FM Cai and IS Druzhinina. The distinct plastisphere microbiome in the terrestrial-marine ecotone is a reservoir for putative degraders of petroleum-based polymers. *Journal of Hazardous Materials* 2023; **453**, 131399.

[42] A Philippe, M Salaun, M Quemener, C Noël, K Tallec, C Lacroix, E Coton and G Burgaud. Colonization and biodegradation potential of fungal communities on immersed polystyrene vs. biodegradable plastics: A time series study in a marina environment. *Journal of Fungi* 2024; **10(6)**, 428.

[43] K Karkanorachaki, E Syranidou and N Kalogerakis. Extreme weather events as an important factor for the evolution of plastisphere but not for the degradation process. *Water Research* 2023; **246**, 120687.

[44] V Bocci, S Galafassi, C Levantesi, S Crognale, S Amalfitano, R Congestri, B Matturro, S Rossetti and FD Pippo. Freshwater plastisphere: A review on biodiversity, risks, and biodegradation potential with implications for the aquatic ecosystem health. *Frontiers in Microbiology* 2024; **15**, 1395401.

[45] J Rüthi, M Cerri, I Brunner, B Stierli, M Sander and B Frey. Discovery of plastic-degrading microbial strains isolated from the Alpine and Arctic terrestrial plastisphere. *Frontiers in Microbiology* 2023; **14**, 1178474.

[46] K Li, L Xu, X Bai, G Zhang, M Zhang and Y Huang. Differential fungal assemblages and functions between the plastisphere of biodegradable and conventional microplastics in farmland. *Science of the Total Environment* 2024; **906**, 167478.

[47] H Tian, L Wang, X Zhu, M Zhang, L Li, Z Liu and S Abolfathi. Biodegradation of microplastics derived from controlled release fertilizer coating: Selective microbial colonization and metabolism in plastisphere. *Science of the Total Environment* 2024; **920**, 170978.

[48] LY Yang, XR Huang, R Neilson, SYD Zhou, ZL Li, XR Yang and XX Su. Characterization of microbial community, ecological functions and

antibiotic resistance in estuarine plastisphere. *Science of the Total Environment* 2023; **866(3)**, 161322.

[49] Y Sun, S Xie,J Zang, M Wu, J Tao, S Li, X Du and J Wang. Terrestrial plastisphere as unique niches for fungal communities. *Communications Earth & Environment* 2024; **5(1)**, 483.

[50] X Li, X Qi, X Liu, M Khalid, J Ye, M Romantschuk, F Chen, Y Hua and N Hui. PET addition delays the composting mature process and promotes microbiota associated with plastic degradation in plastisphere. *Journal of Cleaner Production* 2023; **389**, 136066.

[51] N Chaimusik, N Sombuttra, Y Nakaramontri, P Sompongchaiyakul, C Charoenpong, B Intra and J Euanorasetr. The comparative plastisphere microbial community profile at Kung Wiman beach unveils potential plastic-specific degrading microorganisms. *PeerJ* 2024; **12(4)**, e17165.

[52] RT Kapoor and M Rafatullah. Leveraging biotechnological approaches for the degradation of plastics: A sustainable tool for plastic waste management and environment protection. *Journal of Water Process Engineering* 2025; **69(1)**, 106736.

[53] N Xue, Q Fang, X Pan and D Zhang. Distinct fungal plastisphere across different river functional zones: A watershed scale study. *Science of the Total Environment* 2021; **752(10)**, 141879.

[54] B Song, Y Li, Z Yu, J Jin, Z Liu, R Yang, JM Adams and BS Razavi. Changes in enzyme activity, structure and growth strategies of the rhizosphere microbiome influenced by elevated temperature and CO_2. *Science of The Total Environment* 2024; **954(1)**, 176522.

[55] SM Mirsalami and M Mirsalami. Evolutionary adaptation in the laboratory and the process of retrograde engineering augment autotrophic proliferation in Saccharomyces cerevisiae. *Biochemical Engineering Journal* 2024; **205(1)**, 109278.

[56] J Sica, B Bovo, C Nadai, M Carlot, A Giacomin and V Corich. Effect of CUP1 copy number and pH on copper resistance of *Saccharomyces cerevisiae* enological strains. *Heliyon* 2024; **10(15)**, e34885.

[57] T Sérvulo, JD Taylor, MC Proietti, LDS Rodrigues, IP Puertas, RA Barutot and ALDF Lacerda. Plastisphere composition in a subtropical estuary: Influence of season, incubation time and polymer type on plastic biofouling. *Environmental Pollution* 2023; **332**, 121873.