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Sediment-driven plastisphere community assembly on plastic debris in tropical coastal and marine environments

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ABSTRACT

Coastal habitats have been suggested to serve as a sink for unaccounted plastic debris, *i.e.*, "missing plastic" in the sea, and hence, a hotspot of plastic pollution in the marine and coastal environments. Although the accumulation of plastic debris may pose significant threats to coastal ecosystems, we know little about the fate of these plastic debris and their ecological impacts due to the lack of studies on plastic-microbe interactions in coastal habitats, especially for the tropical marine and coastal environments. In this study, we collected plastic debris from 14 sites consisting of various coastal ecosystems (seagrass meadows, mangrove forests, and beaches), and marine ecosystem (coral reef) around Singapore and characterized the prokaryotic and eukaryotic microbial communities colonized on them. Our results showed that the composition of plastisphere communities in these intertidal ecosystems was predominantly influenced by the sediment than by the plastic materials. Compared with surrounding sediment and seawater, the plastic debris enriched potential plastic degraders, such as *Muricauda*, *Halomonas*, and *Brevundimonas*. The plastic debris was also found to host taxa that play significant roles in biogeochemical cycles (*e.g.*, cyanobacteria, *Erythrobacter*), hygienically relevant bacteria (*e.g.*, *Chryseobacterium*, *Brevundimonas*), and potential pathogens that may negatively impact the health of coastal ecosystems (*e.g.*, *Thraustochytriaceae*, *Labyrinthulaceae*, *Flavobacterium*). Taken together, our study provides valuable insights into the plastic-microbe interactions in tropical coastal and marine ecosystems, highlighting the urgent need for plastisphere studies to understand the fate and ecological impacts of plastic debris accumulated in coastal habitats.

1. Introduction

Plastics are being used and produced extensively, with global production reaching 460 million tonnes in 2019 (OECD, 2022). Due to the low recycling rate and mismanagement of plastic waste, it is estimated that 12 million tonnes of plastic enter the marine environment annually (Boucher et al., 2020). Once in the marine environment, plastic debris is colonised by microorganisms, forming a microbial community distinct from the surrounding environment, known as the plastisphere (Zettler et al., 2013). Research on plastisphere has been booming in recent years, with studies focusing on the mining of microbial resources for plastic waste processing, the potential role of plastics to serve as vectors for harmful microorganisms (*e.g.*, pathogens and toxic algal species), and the impact on the fate and transport of marine plastic debris (Latva et al., 2021). However, plastisphere studies were primarily conducted in the temperate or subtropical regions (Wright et al., 2021), leaving a gap in tropical marine environments, particularly in the Southeast Asian (SEA) region that accounts for a significant portion of plastic waste leakage into marine environments (Koh et al., 2023).

In the SEA region, coastal habitats are one of the most important

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marine environments with around 77% of the region's total population (~2 billion in coastal areas) (PEMSEA Partnerships in Environmental Management for the Seas of East Asia, 2015). In addition, recent studies have suggested that coastal habitats may serve as a sink for unaccounted plastic debris, *i.e.*, "missing plastic", and hence, a hotspot of plastisphere (Bond et al., 2018; Isobe and Iwasaki, 2022; Lebreton et al., 2019). Coastal habitats, including coral reefs, seagrass meadows, and mangrove forests, not only support the biodiversity of marine organisms but also contribute to coastal protection, carbon sequestration, and water purification (Dahl et al., 2021; Laffoley et al., 2014; Sanchez-Vidal et al., 2021). Deposition of marine plastic debris may cause physical damage (*e.g.*, abrasion and shearing) to the habitats and impede sediment oxygenation, resulting in mangrove suffocation and potential leaf loss (van Bijsterveldt et al., 2021), and sedimentation and reduction of seagrass cover (Menicagli et al., 2021). Furthermore, there is an increased incidence rate of coral diseases (*e.g.*, skeletal eroding band disease, white syndrome, and black band disease) when corals are associated with plastic debris (Lamb et al., 2018). Our current understanding of plastispheres, specifically their associated microbial communities, in tropical marine and coastal habitats remains limited. The lack of knowledge impedes our ability to comprehend their ecological impacts. Specifically, key research questions include: (i) What environmental factors shape plastisphere microbial communities in the intertidal tropical marine and coastal ecosystems? (ii) What are the eukaryotic members in the tropical marine and coastal plastisphere, and how are the communities assembled?

To address this knowledge gap, we collected plastic debris from tropical marine and coastal ecosystems and the open waters along the Singapore coastline and characterised both the prokaryotic and eukaryotic microbial communities in the plastispheres. We also identified key environmental factors driving the microbial community assembly. This study offers new insights into the structure and assembly of plastisphere microbial communities in tropical marine and coastal environments.

2. Materials and methods

2.1. Collection of marine plastic debris, seawater, and sediments

Plastic debris was collected from 14 coastal sites around Singapore (1°17′ N, 103°50′ E), consisting of coral reefs, seagrass meadows, mangrove forests, and beaches, as well as from waters off-coast (at least 100 m) of the island, *i.e.,* open waters (Fig. 1). Examples of the collected marine plastic debris are shown in Figures S1 and S2. Singapore is a tropical island city-state in Southeast Asia. It is surrounded by the Strait of Malacca to the west, the Singapore Strait to the south, the South China Sea to the east, and the Straits of Johor to the north. The samples were collected during low tides, except those collected from coral reefs and open waters. The collected samples were cut into approximately 1.5 cm × 5 cm strips and rinsed with autoclaved Milli-Q to remove loosely attached debris before transporting them back to the laboratory in 15-mL centrifuge tubes.

Seawater and sediment samples were also collected. Three 1-L seawater samples (collected from the coastline) and three 5-mL sediment samples (top 5 cm) were collected in 1-L bottles and 15-mL centrifuge tubes, respectively. Samples were transported back to the laboratory on ice. Seawater was pre-filtered through a 150-μm sock (Bubble Magus) before filtering through a 0.2-μm polycarbonate membrane (47 mm diameter, Isopore, Millipore) to harvest microorganisms from the water samples. Plastic debris and sediment samples were stored at −20 °C, and the microorganisms retained on the polycarbonate membrane filters were stored at −80 °C until DNA extraction.

2.2. DNA extraction and amplicon PCR

DNA from the plastic debris, sediment samples (0.5 g each), and seawater (microorganisms retained on the polycarbonate filters) was extracted with the DNeasy PowerBiofilm Kit (Qiagen) according to the manufacturer's instructions. Due to the low DNA yield from sediments collected from coral reefs, DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen). The extracted DNA was purified using the

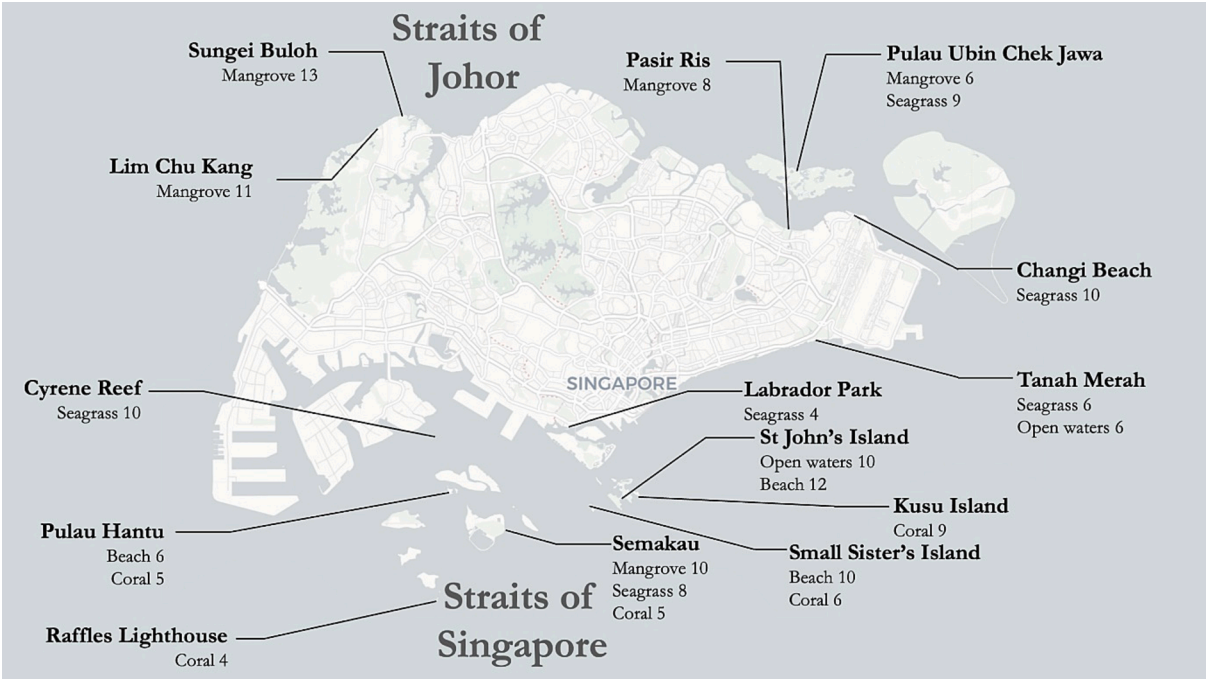

Fig. 1. A total of 168 plastic debris were collected from 14 coastal sites covering different coastal habitats (coral reef: 29, seagrass meadow: 47, mangrove forest: 48, and beach: 28) and the open waters (16) around Singapore. The number following each coastal habitat represents the number of plastic debris collected from that site. The geographic coordinates of the sampling sites are listed in Supplementary Table 1. Map of Singapore was obtained from the R package "leaflet".

Genomic DNA Clean and Concentrator™ −10 Kit (Zymo Research) and diluted to 5 ng/μL before being used for PCR. The V4 region of the 16S rRNA gene was amplified using the 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (with Illumina adapters). The PCR was conducted as follows: an initial denaturation step at 95 °C for 3 min, followed by 25 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The V4 region of the 18S rRNA gene was amplified using the primers Eu565F (5′-CCAGCASCYGCGGTAATTCC-3′) and Eu981R (5′-ACTTTCGTTCTTGATYRA-3′) primers (with Illumina adapters). The PCR reaction volume and components were identical to those of the 16S rRNA gene amplification, with the following cycling condition: an initial denaturation step at 95 °C for 3 min, followed by 28 cycles of 98 °C for 20 s, 52 °C for 15 s, 72 °C for 15 s, followed by a final extension at 72 °C for 1 min. For each sample, three PCR replicates were pooled and purified using the Agencourt Ampure XP beads (Beckman Coulter) with a ratio of 1:1.8. The quality of the eluted DNA was assessed with Nanodrop and quantified with the Qubit dsDNA HS Assay Kit (Invitrogen, ThermoFisher Scientific). The length of the purified PCR products was then evaluated using electrophoresis on a 1.5% agarose gel. The eluted DNA was then diluted to 10 ng/μl, and indexing was performed using the Nextera XT Index Kit v2 (dual barcoded) and sequenced on the Illumina MiSeq V3 300 × 300 bp in the Singapore Centre for Environmental Life Sciences Engineering (SCELSE). The 16S and 18S rRNA amplicon sequences have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under Bioprojects PRJNA988256 and PRJNA989750, respectively.

2.3. Sequencing data processing and analyses

Demultiplexed FASTQ files were processed with the DADA2 pipeline (version 1.22.0) in R (version 4.1.3) to generate amplicon sequencing variants (ASVs). Although both Operational Taxonomic Units (OTUs) and ASVs are often used for clustering and taxonomy assignment, we chose ASVs because they did not require an arbitrary dissimilarity threshold for clustering and provided high sensitivity for taxonomic assignment (Callahan et al., 2017). ASVs generated were used for taxonomy assignment with the SILVA database (Quast et al., 2012) (version 138.1) for the 16S rRNA gene and the PR^2 database (Version 4.14.0) (Guillou et al., 2012) for the 18S rRNA gene. Assigned taxonomy for bacteria and archaea was filtered to remove eukaryotes, mitochondria, chloroplasts, and unassigned ASVs at kingdom and phylum levels. For the eukaryotic community, assigned taxonomy was filtered to remove bacteria, metazoan, nucleus, and unassigned ASVs at kingdom and phylum levels. In addition, ASVs with less than a cumulative number of reads of ten were removed. The α- and β- diversity analyses were conducted on R (version 4.2.0) with the package 'phyloseq' (McMurdie and Holmes, 2013). The reads were rarefied prior to the α-diversity analysis, while raw counts were converted to relative abundance for the β-diversity analysis. The Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) (Lin and Peddada, 2020) was used to determine taxa enriched on plastic debris compared with sediment and seawater. ANCOM-BC was performed after agglomerating taxa by genus with the function phyloseq::tax_glom(), and differential abundance of genera was tested between plastics against sediment and seawater combined. The package 'NetCoMi' (Peschel et al., 2021) was used to construct the prokaryotic and eukaryotic communities network. Detailed data processing statistics can be found in SI (S2).

2.4. Plastic identification

A few strands of fibres or pieces of plastic (~1 cm × 1 cm) from each piece of plastic debris were kept at −20 °C prior to the polymer identification using the attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR). Plastic samples were submerged overnight in a 2% sodium dodecyl sulphate (SDS) solution, then wiped with 70% ethanol and allowed to dry overnight at room temperature. The analysis was performed using Shimadzu IRPrestige-21 with a diamond ATR unit (SPECAC). Spectra were recorded using 45 scans with a resolution of 4 cm^{-1}, measuring between 400 and 4000 cm^{-1}. The atmospheric spectrum was subtracted from the captured spectra and then used to identify the polymer type using OpenSpecy (Cowger et al., 2021).

3. Results

3.1. Community structure and keystone taxa of tropical coastal plastispheres

The α-diversity indices (observed taxa, Chao1, Shannon, and Inverse Simpson) were calculated after rarefying from 62,582 to 62,268 ASVs for the 16S rRNA gene and from 17,363 to 15,757 ASVs for the 18S rRNA gene. The richness and evenness of the prokaryotic communities in plastispheres were found to be significantly lower ($p < 0.05$, Table S2, Fig. S3A) than sediment communities but significantly higher than microbial communities in seawater ($p < 0.05$, Table S2, Fig. S3A). In contrast, the richness and evenness of the eukaryotic communities in plastispheres were lower than those in the sediment and seawater ($p < 0.05$, Table S2, Fig. S3B). The α-diversity for the detected eukaryotic communities was comparable for the sediment and water samples ($p > 0.05$, Table S2, Fig. S3B).

Across all the plastic debris collected from various coastal habitats, the families *Flavobacteriaceae, Rhodobacteraceae,* and *Sphingomonadaceae* dominated the prokaryotic communities in the plastispheres (Fig. 2A). Other abundant prokaryotic families in plastispheres from all the habitats except coral reefs include *Saprospiraceae* and *Hyphomonadaceae.* Interestingly, the dominant prokaryotic taxa in plastispheres from coral reefs and open waters differed from those from seagrass meadows, mangrove forests, and beaches. For example, several families of cyanobacteria (*e.g., Xenococcaceae*, *Synechococcales Incertae Sedis*, *Phormidesmiaceae*) dominated the prokaryotic communities in plastispheres from seagrass meadows, mangrove forests, and beaches. In contrast, *Vibrionaceae*, *Pseudoalteromonadaceae*, *Nitrosopumilaceae*, *Alteromonadaceae*, *Woeseiaceae*, *Cyclobacteriaceae*, and *Nitrososphaeraceae* were most abundant in the prokaryotic communities in plastispheres from coral reefs, while the open-water plastispheres were dominated by *Woeseiaceae*, *Moraxellaceae*, *Puniceicoccaceae*, and *Pirellulaceae*.

As for eukaryotic communities, raphid pennate diatoms were consistently found to dominate the plastispheres across all the coastal habitats and the sediments, contrasting with their markedly lower abundance in seawater (Fig. 2B). In addition, the protist *Acinetidae* was almost exclusively found on plastic debris in all habitats except coral reefs. The protist *Labyrinthulaceae* was also only present on plastics found across all habitats with varying degrees of abundance. The algal families *Ceramiaceae* and *Dasyaceae* were part of the coral reef plastispheres only.

We conducted network analyses for each habitat to identify the keystone taxa of prokaryotic and eukaryotic plastispheres (Figures S4-S7). No keystone eukaryotic taxa was identified for plastispheres from mangrove forests, beaches, and open waters. This was likely due to the limited number of plastic samples when we conducted analyses based on individual habitats. Although at each sampling site, we tried to collect plastic debris from various polymer types, FTIR analyses showed that the collected plastic debris was mostly polyethylene (LDPE and HDPE) and polypropylene. In addition, it was also challenging for us to collect more samples from the open waters because of the limited number of plastic debris floating on the open waters around Singapore. Nevertheless, keystone taxa of plastispheres from each habitat are summarised in Table 1.

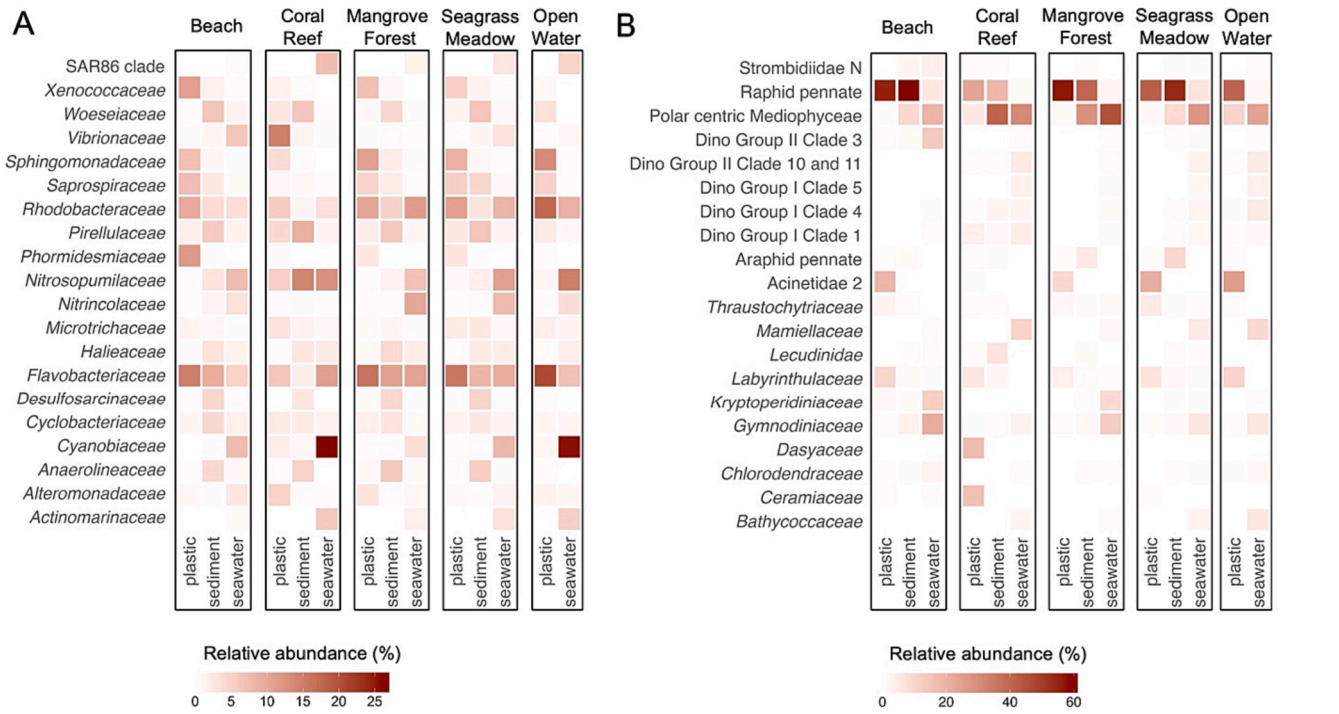

Fig. 2. Relative abundance of the top 20 prokaryotic families (A) and eukaryotic families (B) with samples grouped by habitats (beach, coral reef, mangrove forests, seagrass, and open waters) and sample types (plastic debris, sediment, and seawater). The complete lists are shown in the Supplementary Excel file.

3.2. Enriched prokaryotes and eukaryotes in plastispheres

Differential abundance analysis with ANCOM-BC showed that hydrocarbonoclastic organisms and potential plastic degraders were enriched in the plastispheres (Fig. 3; Excel file in supplementary). The hydrocarbonoclastic families (*Hyphomonadaceae* and *Rhodobacteraceae*) and the genera (*Muricauda*, *Phormidium, Erythrobacter*, *Winogradskyella*, and *Blastocatella*) were present across all the coastal habitats. *Paracoccus* was enriched on plastic debris of all habitats except open waters. *Acinetobacter* was enriched in plastics from coral reefs and seagrass meadows, while *Flavobacterium* was enriched in plastics from seagrass meadows. Furthermore, potential pathogenic genera such as *Acinetobacter*, *Chryseobacterium*, *Flavobacterium*, *Brevundimonas*, and *Tenacibaculum* were also enriched in plastispheres from all the coastal habitats. Several genera of cyanobacteria were also enriched on plastics. In particular, the genera *Pleurocapsa* and *Chroococcidiopsis* were enriched on plastics across all the habitats. *Myxosarcina* was enriched on plastics from coral reefs, seagrass meadows, and mangrove forests, while *Xenococcus* was enriched on plastics from mangrove forests only.

For the eukaryotic plastisphere, apart from plastics collected from coral reefs, several genera belonging to the algal class Ulvophyceae (*e.g.*, *Acrochaete*, *Ruthnielsenia*, and *Ulva*) were enriched on plastics across all the habitats (Fig. 4; the Supplementary Excel file). Curiously, single-celled protists considered seagrass pathogen Labyrinthulomycetes (with special emphasis on the genus *Labyrinthula*) are enriched on plastic surfaces collected from all habitats. In plastispheres from coral reefs and mangrove forests, the top 20 enriched eukaryotic genera primarily comprise organisms belonging to the phyla Rhodophyta and Chlorophyta.

3.3. Coastal plastispheres are predominantly shaped by the sediment of coastal habitat

Out of the 168 plastic debris, the 16S and 18S rRNA genes were successfully amplified from 136 samples. Among them, the polymer types were identified as polypropylene (PP) (52), polyethylene (PE) (37), polyamide (PA) (13), polyethylene terephthalate (PET) (12), polystyrene (PS) (6), polyvinyl chloride (PVC) (5), polyester urethane (2), polyurethane (1), and poly(methyl methacrylate) (PMMA) (1). Seven pieces of plastic debris remained unidentified due to a low percentage match against the database. A detailed breakdown of the occurrence of plastics in the various habitats can be found in the supplementary (Table S3). Out of the nine identified polymers, only PP, PE, PA, PET, PS and PVC were used for downstream analyses, as there are insufficient samples for polyester urethane, polyurethane, and PMMA for statistical analyses.

To compare the α-diversity of plastisphere from different polymer types, rarefaction was done for plastic samples, resulting in 39,837 ASVs for the 16S rRNA gene and 7,787 ASVs for the 18S rRNA gene. The α-diversity indices of both the prokaryotic and eukaryotic communities in the plastispheres were comparable for different polymer types ($p > 0.05$) (Figure S8). We conducted a multivariate analysis using non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarities and clustered the plastisphere communities based on polymer types (Fig. 5A and 5B). Our results suggested that neither the prokaryotic nor eukaryotic community in the plastispheres was polymer-specific. To further confirm our observation, we conducted linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) on the Galaxy framework (https://huttenhower.sph.harvard.edu/galaxy/) and could not identify plastic-specific biomarker taxa. Due to insufficient representative samples in one or more polymer groups in a single habitat, we could not provide statistical insights on whether plastisphere communities at each habitat were plastic-specific. Although not supported by statistical analyses, the plastisphere communities seemed unlikely to be shaped by polymer types as the community structures of plastispheres at individual habitats were closely related (Figure S13).

While we did not obtain distinct clusters of prokaryotic and eukaryotic plastisphere community structures when clustered by polymer type, clustering by habitat showed distinct clusters; coastal plastisphere communities are shaped by the habitat (Fig. 5C and 5D), which is also supported by the pairwise PERMANOVA analysis (Table S5). To determine the factors that resulted in the clustering of plastispheres by

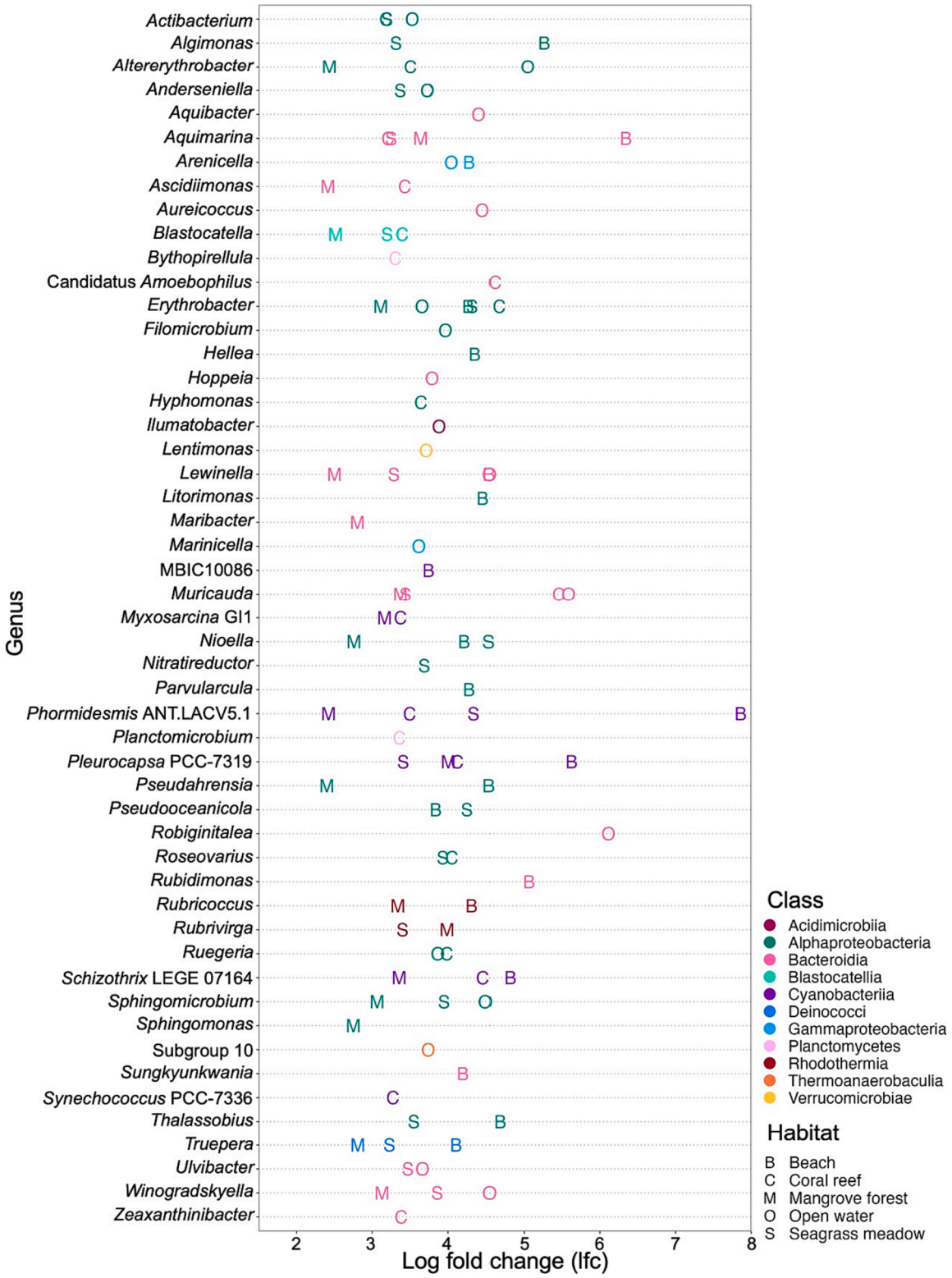

Fig. 3. Prokaryotic genera that are enriched in plastispheres. ANCOM-BC was conducted between plastic and sediment/seawater for each habitat (beach, coral reef, mangrove forest, open water, and seagrass meadows). The top 20 genera with a log-fold change of ≥ 2 from each habitat were shown here. A complete list can be found in the Supplementary Excel file.

habitat, multivariate analyses of the surrounding sediment and seawater communities were performed using nMDS based on Bray-Curtis dissimilarities (Figures S9-10).

PERMANOVA showed that the prokaryotic and eukaryotic communities of the coastal sediments differed between habitats ($p = 0.001$) and locations ($p = 0.001$). The temperature, salinity, pH, and dissolved inorganic nutrients of the surrounding seawater were identified to be key environmental factors contributing to the clustering of sediment by habitat (Figure S9). Similarly, both the prokaryotic and eukaryotic communities of seawater could also be clustered by habitat (F = 48.2

Table 1
Keystone taxa of prokaryotic and eukaryotic plastispheres.

Habitat	Prokaryotic keystone taxa	Eukaryotic keystone taxa
Coral reef	*Psychrobacter* (family: *Moraxellaceae*) *Woeseia* (family: *Woeseiaceae*)	*Labyrinthula* (family: *Labyrinthulaceae*) *Nitzchia* (phylum: Ochrophyta)
Mangrove forest	*TM7a* (family: *Saccharimonadaceae*) *Flavobacterium* (family: *Flavobacteriaceae*) *Chroococcidiopsis* PCC-6712 (family: *Xenococcaceae*) *Pleurocapsa* PCC-7319 (family: *Xenococcaceae*) *Robiginitalea* (family: *Flavobacteriaceae*)	Nil*
Seagrass meadows	*Hellea* (family: *Hyphomonadaceae*) *Altererythrobacter* (family: *Sphingomonadaceae*) *Aquimarina* (family: *Flavobacteriaceae*) *Ketobacter* (family: *Alcanivoracaceae*) *Schizothrix* LEGE 07164 (family: *Synechococcales incertae sedis*)	*Boloceroides* (family: *Boloceroididae*)
Beaches	*Phormidesmis* ANT.LACV5.1 (family: *Phormidesmiaceae*) *Rubidimonas* (family: *Saprospiraceae*) *Algimonas* (family: *Hyphomonadaceae*)	Nil*
Open waters	*Marinicella* (order: Gammaproteobacteria incertae sedis)	Nil*

* Nil: No keystone eukaryotic taxa were identified.

(prokaryotic) or 65.0 (eukaryotic), $p = 0.001$) and location ($F = 18.6$ (prokaryotic) or 32.5 (eukaryotic), $p = 0.001$) (Figure S10). The temperature, salinity, pH and dissolved inorganic nutrients were identified to drive the separation of the microbial communities of seawater (Figure S10). As seawater from mangroves was only collected from the Straits of Johor, where most of the mangrove forests we sampled were located, the seawater communities were more likely to be clustered by location rather than habitat. In fact, the seawater communities could be clustered into three main clusters: coastal water from the Straits of Johor, coastal water from the Straits of Singapore, and open waters from the Straits of Singapore (Figure S10), which is consistent with a previous study reporting that microbial communities of seawater around Singapore were clustered based on the Straits (Chénard et al., 2019).

Further multivariate analyses showed that the plastisphere communities were distinct from the communities in the sediment and seawater ($p < 0.05$) (Figs. 6-7; Tables S6 and S7). In addition, the nMDS plots with a 95% confidence ellipse show that the plastisphere communities, especially for coral reefs (Fig. 6A and 7A), mangrove forests (Fig. 6B and 7B), and seagrass meadows (Fig. 6C and 7C), mostly overlapped or were closely related to the sediment communities, while differed substantially from the seawater communities.

The prokaryotic and eukaryotic plastisphere communities shared 4.5% to 34.2% and 14.4% to 53.7% of their ASVs with the surrounding environment, respectively, at the time of sample collection (Table S8). Intriguingly, the plastisphere and the sediment communities shared 15.9% to 24% for prokaryotes and 28.5% to 34.6% for eukaryotes (Table S8; Figures S11 and S12). In contrast, only 4.5% to 14.6% of the prokaryotic ASVs and 12% to 25.2% of the eukaryotic ASVs were shared between the plastisphere and seawater microbial communities.

4. Discussion

Although coastal ecosystems have been suggested to be major sinks for plastic debris (Bond et al., 2018; Isobe and Iwasaki, 2022; Lebreton et al., 2019), there is a lack of understanding of the plastispheres in these habitats, especially for tropical marine and coastal areas in Southeast Asia. In this study, we characterised the prokaryotic and eukaryotic plastisphere communities from 168 plastic debris collected from seagrass meadows, mangrove forests, coral reefs, beaches, and open waters around Singapore. We found that the prokaryotic and eukaryotic communities on the plastic debris deposited in coastal ecosystems differed from the surrounding seawater and sediment communities. This is in agreement with observations of plastic debris collected from non-coastal areas in the temperate and subtropical waters, where plastispheres were also found to be distinct from the seawater (Amaral-Zettler et al., 2015; Basili et al., 2020; Bryant et al., 2016; De Tender et al., 2015; Debroas et al., 2017; Delacuvellerie et al., 2019; Dussud et al., 2018; Woodall et al., 2018; Zettler et al., 2013) and sediment (Basili et al., 2020; De Tender et al., 2015; Delacuvellerie et al., 2019). In addition, the coastal plastisphere communities were found to be independent of the type of polymers. When the plastispheres were clustered by polymer types, distinct clusters were lacking (Fig. 5A and 5B), which is also supported by the lack of biomarker taxa for different plastics. This finding agrees with a recent *meta*-analysis that concluded environmental variables, *e.g.*, geographical location and temperature, not the types of polymers, play a more prominent role in shaping plastisphere communities (Wright et al., 2021).

For the studied tropical coastal habitats, the plastisphere communities were found to be mainly influenced by the surrounding sediment and seawater. Specifically, the plastispheres in this study were shaped predominantly by the sediment (Fig. 5C and 5D, and S9). Further, the plastisphere and sediment communities shared a higher percentage of prokaryotic and eukaryotic ASVs (Figure S11, S12, and Table S8). As the plastic samples were collected from the intertidal zones of the coastal habitats, they would have experienced frequent physical contact with the sediment communities. In addition, sediment-conditioned plastic surfaces may favour colonisation by marine microorganisms, facilitating the plastisphere community formation. The coastal plastispheres contained a substantial fraction (46.3% for eukaryotes and 65.8% for prokaryotes) of unique ASVs not detected in the surrounding sediment or water. This result suggested that plastic debris deposited at the sampling coastal sites were likely transported from elsewhere, and the plastisphere communities might have recruited microbial community members before their deposition. Interestingly, the community structures of plastispheres on plastic debris collected from open waters are strikingly similar to those from other habitats (Fig. 6A and 6B), suggesting that plastics collected from open waters may be transported from coastal habitats into the open waters or from one coastal habitat to another.

Based on our analyses, neither the prokaryotic nor eukaryotic community in the marine and coastal plastispheres was polymer-specific, which is consistent with several previous studies showing a lack of polymer specificity of plastispheres from plastic debris collected from the environment (Basili et al., 2020; Jiang et al., 2018; Wu et al., 2020). Although pairwise PERMANOVA seemed to result in distinct clustering of the plastisphere communities for polyamide from other plastics, it is likely due to sampling bias as polyamide plastic debris collected from this study were mostly from coral reefs with only two pieces of polyamide debris from seagrass meadows. In addition, the plastisphere communities of the two pieces of polyamide plastic debris from seagrass meadows positioned away from those collected from coral reefs (Figure S13). The lack of polymer-specific microbial assemblage may be attributed to the unknown duration of deposition of plastic debris in the environment, where microbial communities of different polymers have been shown to converge as incubation time increases (Pinto et al., 2019). Prokaryotic plastisphere communities for all the habitats were primarily dominated by the families *Flavobacteriaceae*, *Sphingomonadaceae*, and *Rhodobacteraceae*. Previous studies suggested that *Flavobacteriaceae* and *Rhodobacteraceae* dominated in secondary or mature biofilms on plastics (Du et al., 2022), supporting the idea that the plastic debris collected in this work might not be newly deposited in the coastal and marine environments. In addition, the physicochemical properties (*e.g.*, degree of biotic and abiotic degradation, crystallinity, and additives present) of plastic debris deposited in the coastal and marine environments may

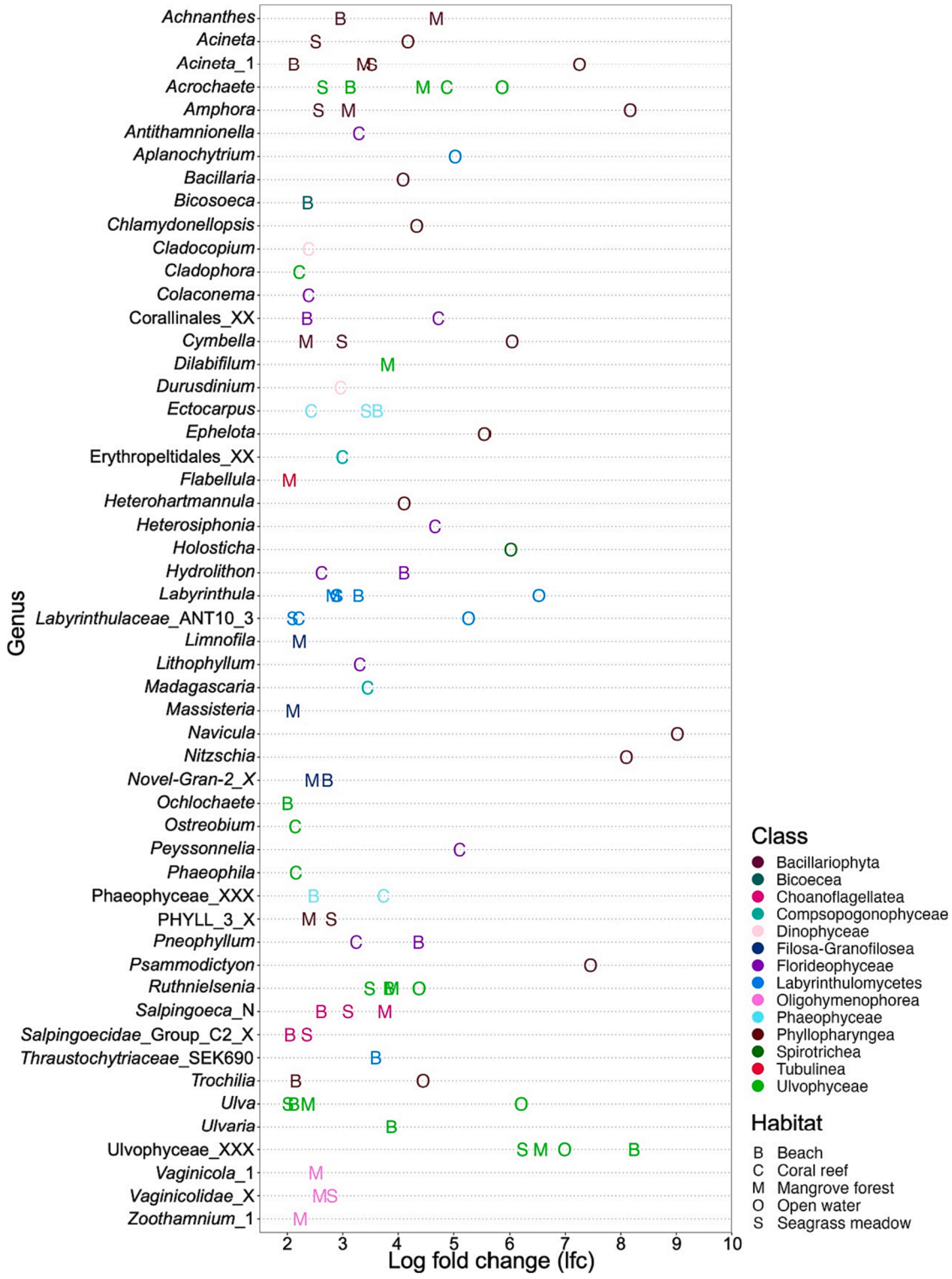

Fig. 4. Eukaryotic genera that are enriched in plastispheres. ANCOM-BC was conducted between plastic and sediment/seawater for each habitat (beach, coral reef, mangrove forest, open water, and seagrass meadows). The top 20 genera with a log-fold change of ≥ 2 from each habitat were shown here. A complete list can be found in the Supplementary excel file.

have been varying over time, which could have also contributed to the lack of polymer-specific plastisphere communities. Furthermore, microbial biofilms are naturally sorptive (Flemming et al., 2016); hence, the enrichment of nutrients in the biofilms might attract surrounding microorganisms or other grazers into the communities, to which the lack of polymer-specific plastisphere communities may also be attributed (Flemming et al., 2016).

Cyanobacteria (*e.g.*, *Trichodesmium, Xenococcaceae*, *Synechococcales Incertae Sedis*, *Phormidesmiaceae*), *Erythrobacter*, *Ruegeria*, and *Nitrosomonas* were enriched and dominated in the prokaryotic plastisphere communities (with several genera to be keystone taxa on plastic debris collected from mangrove forests and beaches (Table 1)). These

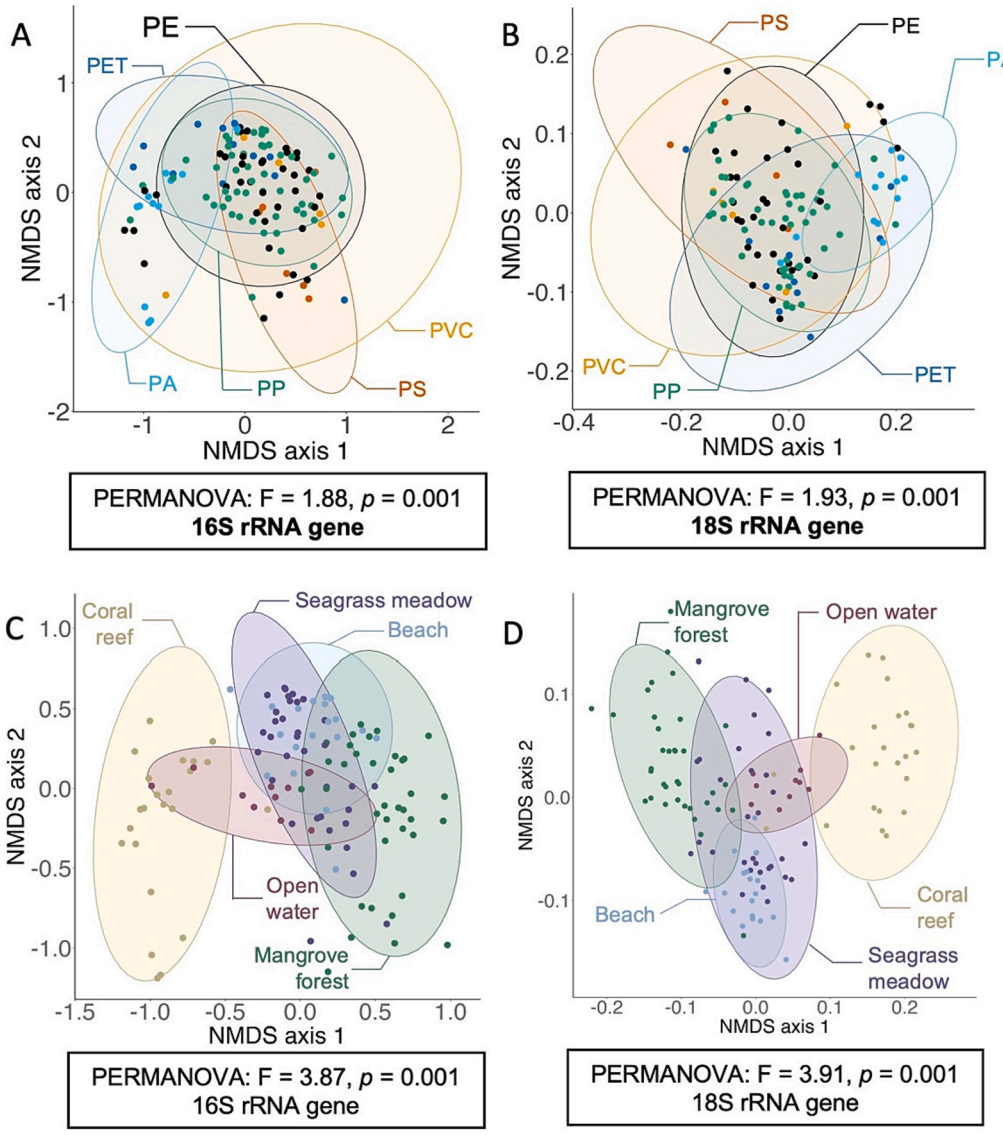

Fig. 5. nMDS ordination of the plastisphere communities for (a) prokaryotes (based on the 16s rRNA gene; n = 3, stress = 0.13) and (B) eukaryotes (based on the 18S rRNA gene; n = 3, stress = 0.15), clustered by polymer types: A, polyethylene (PE); B, polyethylene terephthalate (PET); C, polyamide (PA); D, polypropylene (PP); E, polystyrene (PS); and F, polyvinyl chloride (PVC). nMDS ordination of the plastisphere communities for (C) prokaryotes (based on the 16S rRNA gene; n = 3, stress = 0.13) and (D) eukaryotes (based on the 18S rRNA gene; n = 3, stress = 0.15), clustered by coastal habitats: beach, coral reef, mangrove forest, seagrass meadow, and open water.

organisms play an important role in biogeochemical cycles in the marine and coastal environment. In particular, *Ruegeria* (enriched on plastic surfaces from coral reefs and open waters) and *Nitrosomonas* (enriched on plastic surfaces from coral reefs) may synergistically participate in the carbon, nitrogen, and sulfur cycles (Meng et al., 2022). On the other hand, harmful cyanobacteria in the coastal plastisphere communities may pose health risks. The genera *Lyngbya* (Order: Cyanobacteriales, Family: *Phormidiaceae*), *Trichodesmium* (Order: Cyanobacteriales, family: *Phormidiaceae*), and *Moorea* (order: Cyanobacteriales, family: *Coleofasciculaceae*) were enriched on the plastics collected from the coastal habitats. They have been associated with major human health impacts (*e.g.*, secondary metabolites that are cytotoxic and neurotoxic) (Mazard et al., 2016).

Differential abundance analysis (ANCOM-BC) has also identified the enrichment of several genera associated with plastic degradation. For example, *Muricauda* sp. has been associated with PET degradation (Debroas et al., 2017). Other genera enriched on plastic debris that have also been associated with plastic degradation include *Halomonas* (shown to cause a 1.72% weight loss in LDPE after 90 days incubation (Khandare et al., 2021)) and *Brevundimonas* (shown to potentially degrade plastics like nylon (Sudhakar et al., 2007)). Finally, the order Bacillales with species shown to degrade PP and PS (Gambarini et al., 2021) has also been found on the plastic debris collected from coral reefs and seagrass meadows. Although the extent of biodegradation of the plastic debris could not be examined in the present study, plastisphere communities from tropical coastal habitats serve as a potential bioresource for the bioremediation of plastic waste (Amaral-Zettler et al., 2020).

Enrichment of potential pathogens, especially *Vibrio* (Curren and Leong, 2019; Delacuvellerie et al., 2019; Frere et al., 2018; Silva et al., 2019; Zettler et al., 2013), in plastispheres has been reported to be a potential threat to marine ecosystems. Although *Vibrio* was not enriched on the plastic debris collected in this study, other genera with pathogenic species were detected. For example, *Acinetobacter* sp., a common infectious pathogen highly resistant to antibiotics, were detected on plastic debris collected from coral reefs and seagrass meadows. The genus *Acinetobacter* and family Parvularculaceae were enriched on plastic debris, and they have been highly associated with the Dark Spot Syndrome in Stephanocoenia intersepta, suggesting that plastispheres may have a role in coral diseases (Sweet et al., 2013). The genera *Chryseobacterium* (*e.g.*, *C. meningosepticum* and *C. indologenes* that cause bacteremia, meningitis and other human diseases) (Gong et al., 2019), *Flavobacterium* (*e.g.*, *F. branchiophilum* that causes bacterial gill disease), and *Brevundimonas* (*e.g.*, *B. diminuta* and *B. vesicularis* are considered of species with clinical importance) (Ryan and Pembroke, 2018) are also enriched on plastics in seagrass meadows. *Tenacibaculum* enriched on plastics collected from beaches carries species that can cause ulcerative

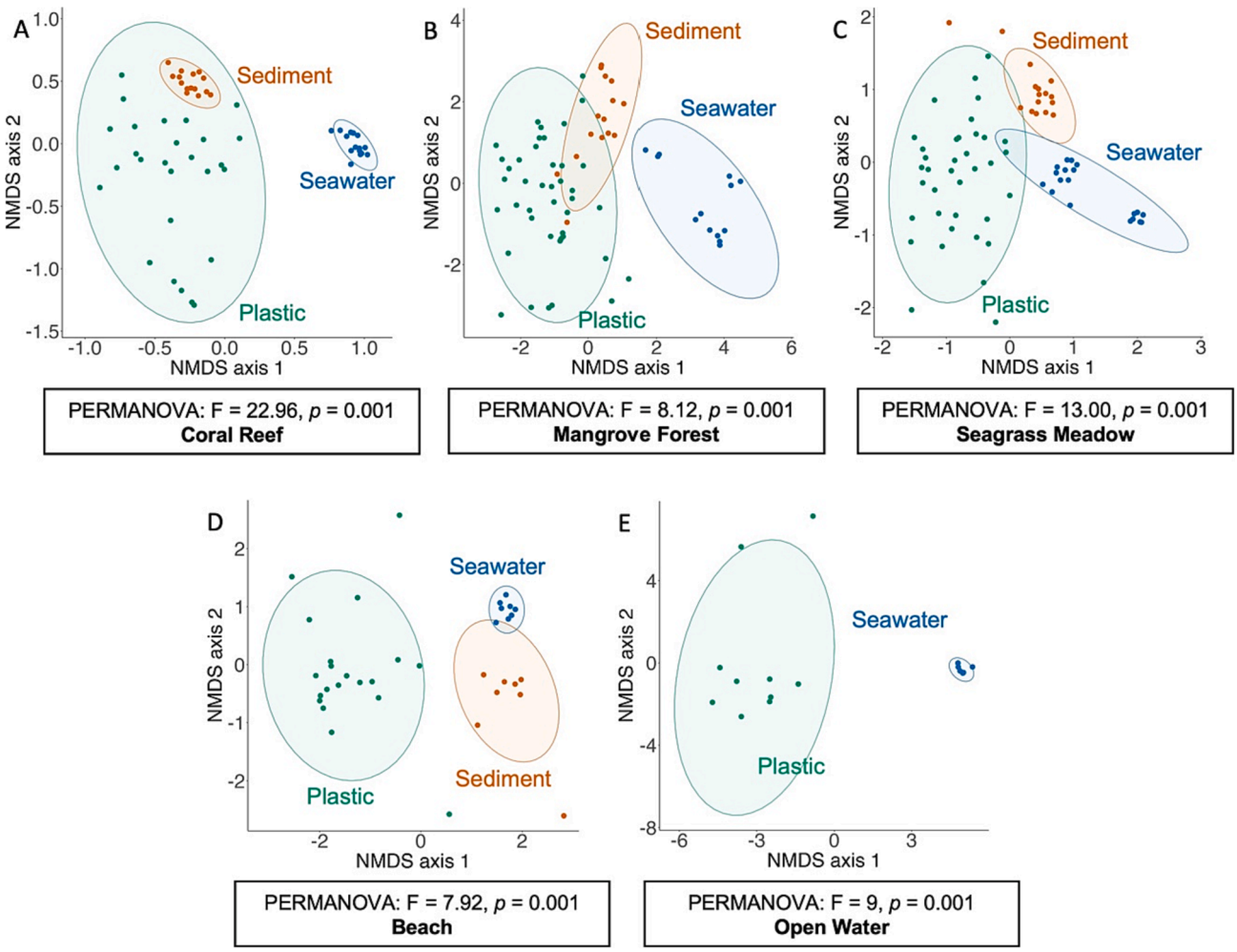

Fig. 6. nMDS ordination for prokaryotic communities grouped by plastic, sediment, and seawater samples from (A) coral reefs (n = 2, stress = 0.12), (B) mangrove forests (n = 3, stress = 0.13), (C) seagrass meadows (n = 2, stress = 0.17), (D) beaches (n = 2, stress = 0.10), and (E) open waters (n = 2, stress = 0.07).

disease (tenacibaculosis) in fishes (Bridel et al., 2020). Intriguingly, *Chryseobacterium* spp. has also been associated with the biodegradation of polyurethane and its additives (Gaytán et al., 2020), and *Brevudimonas* spp. have been identified as potential bioremediators of hydrocarbons (Ryan and Pembroke, 2018).

The prokaryotic plastisphere has received more attention than the eukaryotic plastisphere, and consequently, the available data on the latter is scarce. This study, therefore, contributes to the understanding of eukaryotic plastispheres. In contrast to the prokaryotic plastisphere, the eukaryotic plastisphere seems less diverse, with raphid pennate diatoms and Acinetidae (Order: Suctoria) dominating. Raphid pennate diatoms, known as biofilm-forming sessile taxa and important representatives of the microfouling communities (Salta et al., 2013), are consistently found across all habitats, dominating the plastisphere and the sediments. The protist Acinetidae, on the other hand, was almost exclusively found on plastics in all habitats except the coral reef. The order Suctoria is known sessile epibionts often found on crustacea and algae (Chatterjee et al., 2019), but this is the first time this taxon has been reported on plastics.

Thraustochytriaceae and *Labyrinthulaceae*, belonging to the class of Labyrinthulomycetes, were also only found to be enriched on plastic debris across all habitats, with varying degree of abundance (Fig. 2B; supplementary Excel file). Taxa belonging to this group (*e.g., Labyrinthula zosterae*) are notoriously known pathogens causing the seagrass wasting disease, responsible for decimating up to 90% of eelgrass meadows in North America (Short et al., 1987; Sullivan et al., 2018). They are also known as epibionts on diatom and could therefore be attached to the raphid diatoms found in all samples. Finding *Labyrinthulida* on the plastisphere is a cause for concern, as this implies that plastics serve as pathogen vectors that can move between habitats with the potential of infecting seagrass populations. Differential abundant analysis revealed that *Labyrinthulomycetes* genera were enriched only on plastic samples across all five habitats, further increasing our understanding of these pathogens on plastic surfaces.

It is widely accepted that plastic can act as a potential dispersal vector of marine species since 61–87% of all marine debris is plastic, making it the most common debris material in the marine environment (Eriksen et al., 2014; Serra-Gonçalves et al., 2019). Marine ecosystems are at risk from an invasion of non-native alien species (NNAS) as a direct result of plastic colonisation and subsequent transport of microorganisms, algae, invertebrates, and fish (Carson et al., 2013; Goldstein et al., 2014) as plastics are globally distributed and with a high potential for attachment (García et al., 2021). Algae have been reported as NNAS as reviewed by García et al. (2021). Although algae are not as common as the typical NNAS, their capacity to attach to plastics and invade a new ecosystem is troubling. The current study identified numerous algal species on plastics across all five environments, some reported as part of the plastisphere for the first time. Plastics and their plastisphere can be transported passively via the currents or ballast waters, traditional marine NNAS vectors (Bailey, 2015), potentially transporting harmful algae or pathogens out into open waters.

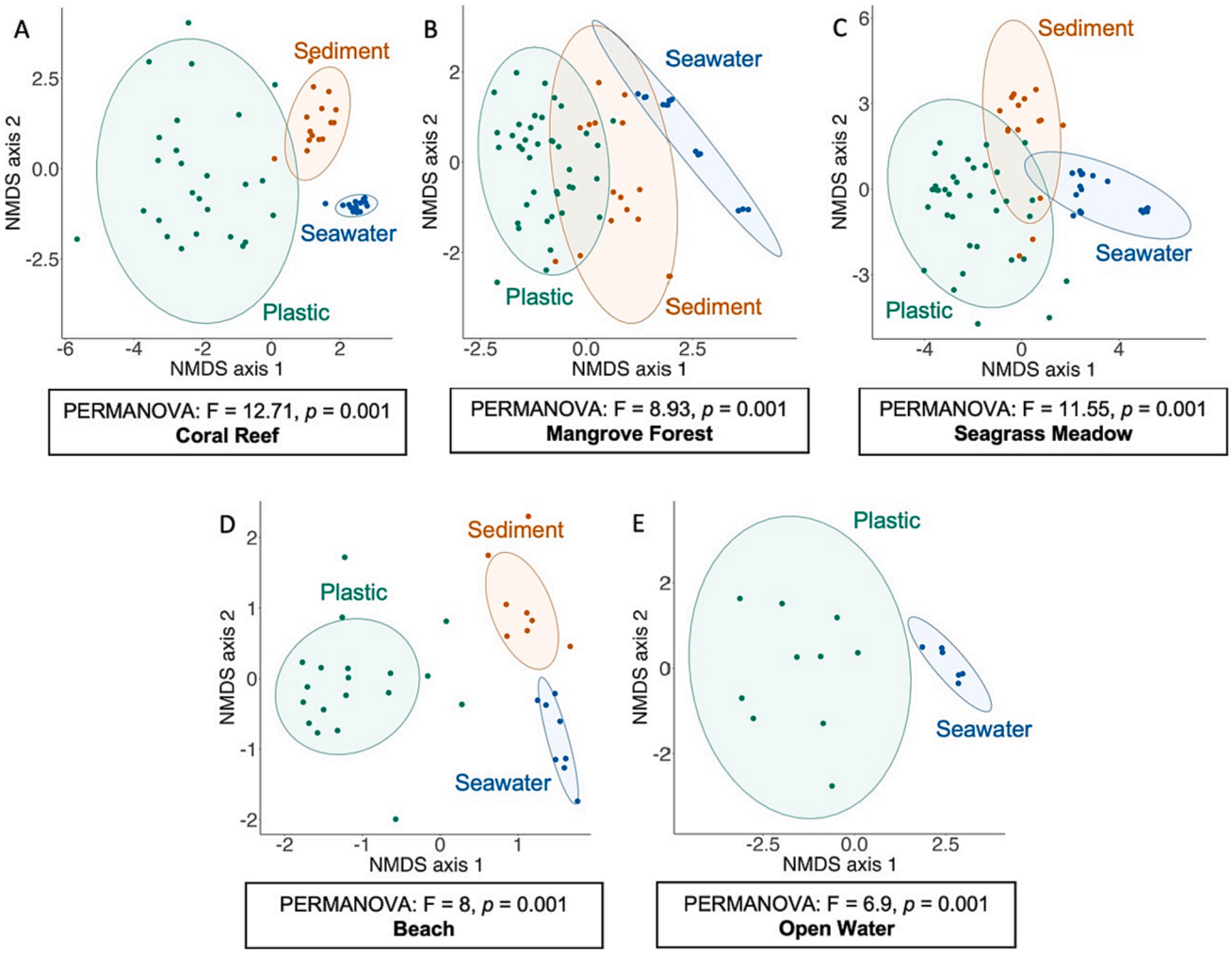

Fig. 7. nMDS ordination for eukaryotic communities grouped by plastic, sediment, and seawater samples from (A) coral reefs (n = 2, stress = 0.12), (B) mangrove forests (n = 3, stress = 0.15), (C) seagrass meadows (n = 3, stress = 0.15), (D) beaches (n = 2, stress = 0.16), and (E) open waters (n = 2, stress = 0.09).

5. Conclusions

As plastics accumulate in coastal habitats, it is evident that the plastisphere may have a role to play in the biogeochemical cycles or may present as a threat to the ecosystems due to potential pathogens. In addition, as plastics are buoyant and persist in the environment for years, they may be transported out of these coastal habitats by tides, potentially transporting harmful algae or pathogens out into the open waters. While we have identified potential pathogens to be enriched on plastics, metabarcoding cannot identify species/strains, and pathogenicity cannot be determined. Nevertheless, we have also identified potentially harmful cyanobacteria enriched on plastic surfaces. The bioaccumulation of cyanotoxins produced by these cyanobacteria (*e.g.*, *Lyngbya*) can cause the poisoning of marine life (particularly shellfish) in these habitats (Mazard et al., 2016). While it is unclear if marine animals consume colonised plastic debris in these habitats, the ecotoxicological effects of the plastisphere cannot be ignored. While we did not investigate the effect of cyanobacteria and other prokaryotes that have been shown to impact biogeochemical cycles potentially, the enrichment of these taxa on plastic debris suggests a role of plastisphere in the biogeochemical cycles. Therefore, our study highlights the enrichment of potential pathogens on plastic debris deposited in coastal environments that can threaten coastal ecosystems or potentially impact the biogeochemical cycles of these critical habitats, especially when these habitats continue to accumulate marine plastic debris. It is prudent that we minimise the use of plastics, and plastic wastes should be appropriately managed to prevent the accumulation of plastics in coastal ecosystems and open waters.

CRediT authorship contribution statement

Jonas Koh: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Sakcham Bairoliya:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Maria Salta:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Zin Thida Cho:** Data curation, Formal analysis, Investigation, Validation, Writing – original draft. **Jenny Fong:** Data curation, Formal analysis, Investigation, Methodology. **Mei Lin Neo:** Investigation, Methodology, Resources, Funding acquisition. **Simon Cragg:** Investigation, Methodology, Resources, Funding acquisition. **Bin Cao:** Conceptualization, Supervision, Formal analysis, Methodology, Resources, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108153.

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