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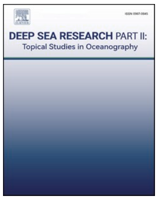

Plastic pollution: A potential vector of both pathogens and probiotics for corals on the Mascarene Ridge, Indian Ocean

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ABSTRACT

In marine environments, plastic debris serves as a vector for pathogenic bacteria that can negatively impact marine fauna, including corals. The Saya de Malha and Nazareth Banks, located in the Indian Ocean, harbor an extensive area of coral reefs that may be susceptible to diseases associated with plastic pollution. In this context, we aimed to determine whether plastic debris in this region can act as a vector for coral-associated pathogenic bacteria. During the "Indian Ocean mission 2022," floating plastic debris (>500 μm) was collected from the Saya de Malha region using manta trawling. The collected debris were quantified, classified by type, size, and weighed. The associated microbiome, along with the viable bacterial communities attached to the plastic, were analyzed using 16S rDNA high-throughput sequencing and traditional molecular and microbiological techniques. A high concentration of plastic debris was recorded both on Saya de Malha and Nazareth Bank, with 10^5 items. km^{-2}. The mean viable bacterial count was 15,519 ± 9340 CFU/g of plastic debris. Nine bacterial phyla were identified, with Firmicutes (58 % of the total) and Proteobacteria (42 %) being the dominant groups. The bacterial diversity on the plastic was measured at 0.92, including coral pathogens, such as *Acinetobacter* (27 %), *Psychrobacter* (2 %), and *Pseudomonas* (2 %). In addition, probiotic bacteria such as *Exiguobacterium* (58 %), *Rheinheimera* (1 %), and *Idiomarina* (1 %) were also detected. This preliminary study states the presence of both pathogenic and probiotic coral-associated fixed to plastic. Further research is needed to confirm their direct effect on coral reef disease. Nevertheless, these findings add to the growing evidence of the widespread presence of plastic debris floating in the region.

1. Introduction

Coral reefs are a crucial ecosystem, covering less than 0.1 % of the ocean's surface (Huang et al., 2021). Despite their small area, they are home to more than 25 % of all marine species (Klein et al., 2024). Marine species rely on coral reefs for reproduction, feeding, and as nurseries (Cumming et al., 2024; Nagelkerken et al., 2002). Primarily located in pantropical latitudes, coral reefs are often found in countries, atolls, and islands that are less economically developed (Birkeland, 2017; Klein et al., 2024). Despite their vulnerability to overharvesting for export, reef fisheries have served as major sources of food for hundreds, and in some cases, thousands of years. The economy of reef islands is heavily dependent on coral reefs, whether it is based on fisheries (Birkeland, 2017), aquaculture (Pomeroy et al., 2006) or tourism (Spalding et al., 2017). For example, in Australia, coral reefs generate $6.4 billion per year from tourism related to the Great Barrier Reef (Deloitte Touche Tohmatsu, 2017) and globally, coral reef ecosystems contribute more than $375 billion annually in goods and services (Pandolfi et al., 2005).

Global warming and anthropic pressures are severely impacting the health of coral reefs (Hoegh-Guldberg et al., 2017). The gradual or exceptional increase in sea surface temperature, such as during El Niño events (Goreau and Hayes, 2024; Hausfather, 2023; Li et al., 2023) has led to significant bleaching episodes and coral mortality. After bleaching, coral resilience is not always effective, especially when additional

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pressures are present, such as the discharge of chemical pollutants (e.g., pesticides; Ross et al., 2015), sedimentation (Erftemeijer et al., 2012), and plastic pollution (Hamman et al., 2024; Lamb et al., 2018). These factors can further impact the coral holobiont, disrupting symbiosis and leading to a decline in coral health by enhancing the growth of both algae and pathogens, which increases the probability of coral diseases (Bruno et al., 2003; Lamb et al., 2018; Weil et al., 2006).

Plastic pollution continues to rise, entering the ocean from various sources including atmospheric deposition (Bianco and Passananti, 2020), maritime activities (Lebreton, 2022), rivers (Meijer et al., 2021), and poorly managed landfills (Jambeck et al., 2015). Once in the ocean, even if plastic debris can float for long distances and are highly resistant, they can be degraded in smaller pieces as microplastics (<5 mm) and nanoplastics (10^{-9} nm; Andrady, 2011; Ter Halle et al., 2017). Plastic debris have already been studied in relation to coral reefs (Hamman et al., 2024; Hoeksema and Hermanto, 2018; Lamb et al., 2018; Singleton et al., 2023; Vered and Shenkar, 2023). According to Lamb et al. (2018), there are an estimated 11.1 billion pieces of plastic debris impacting coral reefs in the Asia-Pacific region, and this number is expected to increase by 40 % by 2025. Indeed, depending on their size, macroplastics and megaplastics, such as plastic bags, fishing lines, and ghost nets, can become entangled with corals, covering polyps and impeding light penetration. This interference disrupts the respiratory processes and photosynthetic activity of the zooxanthellae (Suka et al., 2020). Additionally, a variety of microorganisms colonise the surface of plastics, forming an ecosystem known as the “plastisphere” (Dussud, 2018; Zettler et al., 2013). These microorganisms can include eukaryote from larva macrofauna (such as bryozoans, and barnacles) to microfauna (diatoms, rotifers, algae), as well as prokaryotes within the phyla Proteobacteria and Bacteroidota (Latva et al., 2022; Wright et al., 2021). Some of these bacteria can be pathogenic to corals, such as *Vibrio* bacteria, *Escherichia coli*, *Rhodobacter* and *Flavobacteracea* causing diseases such as black band, white band, bleaching, and tissue damage (Beloe et al., 2022; Feng et al., 2020; Sheffey, 2023). These diseases can lead to significant changes in coral reproduction and growth rates, which in turn affect community structure, species diversity, and the abundance of reef-associated organisms (Loya et al., 2001). This can impact the entire reef ecosystem (Sokolow, 2009). To date, no study has demonstrated a correlation between bacteria colonizing plastic debris and diseases affecting corals.

The Western Indian Ocean contains 16 % of the world’s coral reefs, and the region is now thought to host the second peak of coral biodiversity globally (Chabanet et al., 2016; Naim, 1993; Obura et al., 2017; Spencer et al., 2000; Vogt-Vincent et al., 2024). Research on plastic pollution in this region is relatively recent, and indicates that the majority of plastic debris found on islands such as Seychelles, Saint Brandon, Tromelin, and Sainte Marie (Madagascar) originates from Southeast Asia (Cartraud et al., 2019; Hoarau et al., 2014; Honorato-Zimmer et al., 2022; Mattan-Moorgawa et al., 2021; Thibault et al., 2023; Vogt-Vincent et al., 2022). This origin data highlights the movement and persistence of these wastes. Nevertheless, the dynamics of plastic debris and coral pathogens in the Indian Ocean remain recently documented (Ben-haim et al., 2003; Hamman et al., 2024; Sabadadichetty et al., 2024). Given that plastic debris harbors a new ecosystem called the plastisphere, it has been observed in Australia that they can serve as vectors for coral disease (Lamb et al., 2018). To our knowledge, no study has directly identified coral pathogen bacteria attached to plastic debris in the southwest Indian Ocean. We aim to test the hypothesis that floating plastic debris acts as a vector for coral-specific pathogenic bacteria.

During the Indian Ocean Expedition 2022 along the Mascarene Ridge, coordinated by Monaco Explorations, we had the opportunity to collect plastic debris. This allowed us to (1) quantify the concentration of floating plastic debris in this region, (2) describe the associated microbiome and the viable bacterial communities attached to the plastic, and (3) assess, through a comprehensive review, whether the bacteria found on the plastic debris are coral species’ pathogens.

2. Material and methods

2.1. Survey area

The Indian Ocean Expedition 2022 was conducted on board the vessel *S.A. Agulhas II* in the Western Ocean between the latitudes of Mauritius, and Seychelles, from October to November 2022, precisely on the Mascarene Ridge. The two banks of Saya de Malha and Nazareth are prominent underwater features of the Mascarene Ridge. The Saya de Malha Bank, is a submerged plateau located between latitudes 9°30-12°50′S and longitudes 59.30–62.30°E, with an estimated size of 41,000 km^2 (Coopen et al., 2021; Vortsepneva, 2008). It is located between the Exclusive Economic Zones (EEZ) of Seychelles, Mauritius and France, with a part of the international zone providing significant ecological benefits, particularly related to fisheries. The Nazareth Bank (13°S-18°S), where Saint Brandon Island (16°40′S) is located, is entirely included in the Mauritian EEZ (Fig. 1).

2.2. Pretreatment before the expedition

Prior to the expedition, we prepared a sterile sand following the protocol of Trachoo (2004). This sand is used to mechanically abrade the surface of plastic debris, thereby the detachment of biofilm-associated microorganisms. The sand was initially washed with 2 % hydrochloric acid (Merck, Darmstadt, Germany) for 10 min, followed by three rinses with MilliQ water. The sand then underwent a final 10-min rinse with MilliQ water before being sterilized by autoclaving at 120 °C for 20 min at 1 bar pressure. In parallel, we prepared an artificial seawater solution by dissolving 35 g of NaCl (Sigma-Aldrich, Darmstadt, Germany) in 1 L of MilliQ water. This solution was also sterilized by autoclaving under the same conditions (120 °C, 20 min, 1 bar).

2.3. Plastic debris sampling

During the expedition, plastic debris were collected at four sites on the sea surface (Fig. 1), using a manta net (mouth: rectangular, width: 0.88 m, height: 0.165 m, mesh size: 500 μm, Egger et al., 2020; Lebreton et al., 2018). The manta net and cod-ends were pre-rinsed with 70 % ethanol and sterile seawater before each deployment to remove potential contaminants. Each trawl used a new, sterile cod-end to prevent cross-contamination between samples. Three 30 min replicates were conducted at each site at a speed of 2 knots, with an individual single-use cod-end for each transect. The replicates were conducted one after the other along the same track. For each transect, the manta net was deployed more than 30 m behind the vessel to avoid the vessel’s wake. A flowmeter (©General Oceanic, Inc., model 438,110; Hydro-bios: https://www.hydrobios.de) was attached at the net entrance (Lebreton et al., 2018). Between transects, the net was rinsed with seawater on the outside of the net to move any missed plastic debris towards the cod-end. The cod-end was then detached, placed into an annotated plastic bag (date, mission, cod-end identification number), and stored in a freezer (−20 °C) until analysis in the onboard wet laboratory. For the next deployment, a new cod-end was placed. For each manta trawling, the following environmental parameters were recorded: wave height (m), wind speed ($m.s^{-1}$), surface area of sampling (km^{-2}, flowmeters, GPS points; Supplementary A: Parameters of manta sampling). To avoid errors in plastic concentration in the net and the bioturbation of debris in the water column, debris were collected only when wave height was less than 2 m.

2.4. Molecular processing

For all manipulations, the scientists in charge of sorting had received prior training in the morphological identification (rigid, pellet, fibers,

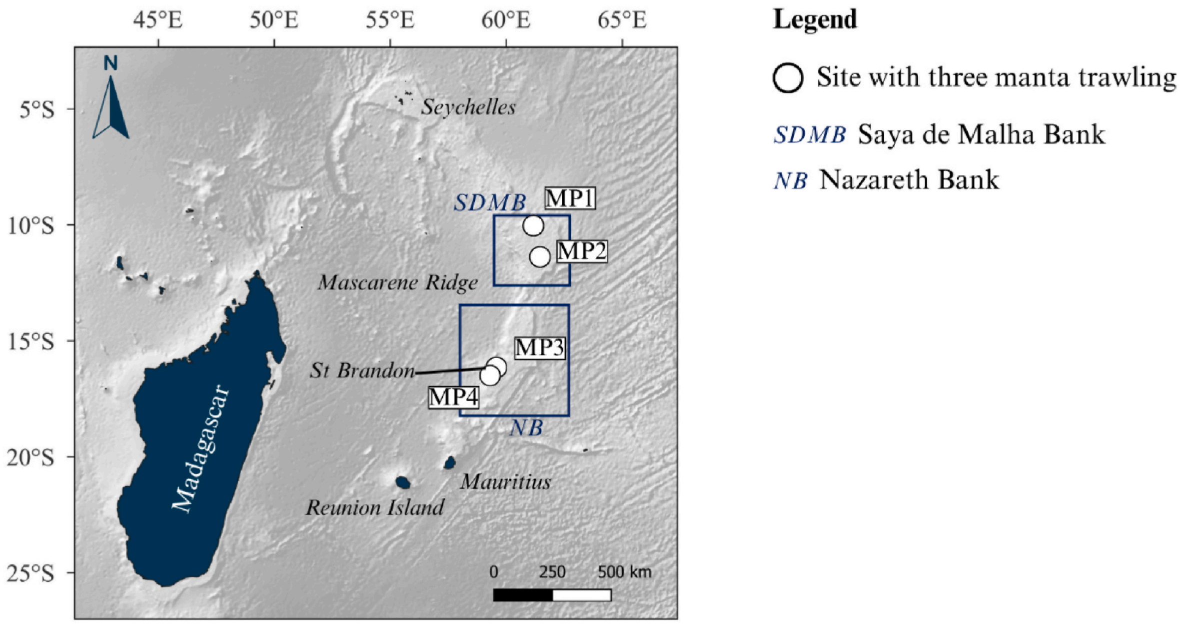

Fig. 1. Study area at the Mascarene Ridge with the four sites surveyed (MP1-MP4), including a total of 12 manta trawling at the sea surface. MP1-MP2 are located on Saya de Malha Bank (SDMB) and MP3-MP4 are located on the Nazareth Bank (NB) close to St Brandon Island.

floated) of plastic debris. This training took place during previous research expedition, such as École Bleue Outre Mer on the *Marion Dufresne* (Thibault et al., 2025). These measures were taken to minimise errors in identifying plastic debris. In addition, scientists wore latex gloves and lab coats to avoid bacterial and textile fiber contamination.

The wet laboratory onboard was cleaned with 70 % ethanol before and after each session. Immediately after the three-manta sampling, the cod-end were taken from freezer, externally rinsed with filtered and sterilized Milli-Q water, and its contents were deposited onto a 500 μm sieve. Under light and magnifying glass, all items visually showing the characteristics of a plastic debris (rigid, pellet, fibers, floated) were collected with ultra-fine tweezers (300 μm point diameter, GESAMP, 2019) and placed in a glass Petri dish. An image was taken of each Petri Dish with a camera (Nikon D7500 - lens: AF-S MICRO NIKKOR 105 mm). A sterile glass Petri dish was left open in the laboratory environment for the duration of the sorting process. Any particles or microbes detected in this blank were considered background contamination and excluded from the analysis.

Then, we pooled all plastic debris collected from each site. Microbial analyses were subsequently normalised per gram of plastic to ensure comparability across sites. This pooled debris was placed into a sterile 50 mL Falcon tube (Fischer, Illkirch, France) along with 10 g of sterile sand (Sigma-Aldrich, Darmstadt, Germany; Trachoo, 2004). To this mixture, we added 20 mL of sterile artificial seawater (NaCl, 35 g/L).

After thorough shaking, the resulting supernatant was collected and divided into two 10 mL portions for further site-specific analyses: (a) microbiological culture and (b) DNA extraction. For the microbiological analysis, the 10 mL sample was transferred to a new 50 mL Falcon tube, mixed with 60 % sterile glycerol, and stored at −20 °C. For DNA extraction, another 10 mL was placed in a 15 mL Falcon tube and stored at −20 °C.

Each sample was carefully labeled with the collection date, sample number, and the corresponding method of analysis. The residual plastic debris in the Eppendorf tubes were subsequently retained for additional analyses described in 2.8.

2.4.1. *Microbiological culture-based analysis*

To quantify the number of micro-organisms present in a sample, we followed a standardised protocol for counting Colony-Forming Units (CFU) as reported in Sababadichetty et al. (2024). At the laboratory ENTROPIE, University of Reunion Island, a series of serial dilutions ranging from 1 to 10^{-5} were prepared from the bacterial supernatant obtained after the abrasion of plastic and sand. The serial dilutions were prepared using sterile artificial seawater (35 g/L NaCl in Milli-Q water, autoclaved at 120 °C for 20 min). This medium was chosen to maintain osmotic conditions compatible with marine bacterial viability during dilution, avoiding osmotic shock that could occur with deionized water or standard saline solutions. The total bacterial load was determined by plating 100 μl of these dilutions onto Mueller Hinton agar plates (Bio-Mérieux, Marcy l'Etoile, France), following the method described in Miltgen et al. (2020). After inoculation, the plates were incubated at 30 °C ± 2 °C for 24–72 h, allowing microbial colonies to become visible. The total bacterial count was then calculated and reported as CFU per gram of plastic. Bacterial strains were isolated from the Mueller Hinton agar, is a non-selective medium recommended for environmental microbial isolation, allowing diverse bacterial growth while minimising bias. Bacterial strains were isolated by morphological differentiation is a standard method for preliminary strain separation before molecular identification (e.g.,Sambrook and Russell, 2001). During microbial culture, uninoculated Mueller Hinton agar plates were incubated alongside the samples to check for airborne or media contamination.

2.4.2. *Molecular identification of the bacterial strain*

Each pure and isolated bacterial strain was cultivated in 5 ml LB broth 24h at 30 °C until dense culture. Then after centrifugation, the bacterial pellet was used to extract DNA (gDNA) using a Qiagen Blood and Tissue ProKit (Qiagen, Hilden, Germany). DNA samples were controlled for quality and quantification using a Nanodrop (Thermo Fischer scientific, Illkirch-Graffenstaden, France).

DNA samples were sent for conventional SANGER genotyping to the ADNid platform (ADNid, Montpellier, France). The bacterial full 16S rRNA gene sequence was assessed using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATC-TAATCC-3'; Klindworth et al., 2013).

For data acquisition and preprocessing, ABI Prism-generated raw sequencing files in ABI format were analyzed (Chen et al., 2014). Chromatogram data from these ABI files were processed to assemble forward and reverse sequences into a consensus sequence using BioEdit software.

To correct errors in chromatogram reads, poor-quality regions at the beginning and end of sequences were trimmed, and base misreads were corrected. The refined consensus sequence was saved in FASTA format for further analysis.

This sequence underwent BLASTN analysis against the GENBANK database using a bash script with the following parameters: '*blastn -db nt -query $FILE -max_target_seqs 3 -outfmt "6 qseqid sseqid pident stitle length mismatch gapopen evalue bitscore" -remote*'. The analysis identified the genus and species information from the top 3 BLASTN results, with the hit showing the highest probability (lowest e-value) and the highest percentage identity being recorded.

2.4.3. *DNA extraction*

Before DNA extraction, one blank sample (sterile Milli-Q water) was included in each batch of extractions to monitor reagent of kit contamination. Then firstly, the 10 mL supernatants obtained from plastic abrasion or sediment extractions were filtered and sterilized using 0.22 μm nitrocellulose membranes (Merck Millipore, Cork, Ireland). DNA was then extracted from all membranes resulting from the filtration of plastic, sediment supernatant, and water samples, following the protocols of Debeljak et al. (2017). Secondly, the membranes were placed in Qiagen DNeasy Blood and Tissue kit columns (Qiagen GmbH, Hilden, Germany), and DNA extraction was carried out according to the manufacturer's instructions. After extraction, DNA concentration was measured using a Nanodrop spectrophotometer (Thermo Scientific, Illkirch-Graffenstaden, France).

2.4.4. *Libraries generation and sequencing*

The DNA samples were then sent to Microsynth platform (Microsynth, Balgach, Switzerland) for mass DNA sequencing. Library construction and sequencing were performed according to Illumina 16S metagenomic sequencing library protocols to amplify the V3 and V4 region of 16S DNA (Bukin et al., 2019). The first PCR amplification for the 16S V3-V4 region was performed with 30 cycles. Quality control included quantification using PicoGreen and qualification on QIAxcel. The PCR mix consisted of 5 μl (or 25 ng) of gDNA, 5X HOT BIOAmp® BlendMaster Mix (12.5 mM MgCl2) from Microsynth, 10X GC-rich Enhancer from Microsynth, and 20 mg/ml BSA, in a final volume of 25 μl. A second indexing PCR was performed with 10 cycles using the product from PCR 1, followed by quality control. The sequencing libraries were then standardized equimolarly and pooled.

The sequencing library pool, supplemented with 5 % phiX, was deposited on a V3 Flow Cell and sequenced on a MiSeq Flow Cell using paired-end 2 x 300 bp reads (301 x 8 x 8 x 301). A total of 193,252 reads were obtained, and the sequences are available under NCBI BioProject PRJNA1083625, spanning from SAMN40254788 to SAMN40254795.

2.5. *Bioinformatics*

2.5.1. *Working environment*

The pipeline was run on the Nouméa Institut de Recherche pour le Développement's cluster, running under CentOS Linux release 8.3.2011, and then downstream analysis proceeded on macOS Mojave 10.14.6 (x86_64-apple-darwin17.0 (64-bit)). All scripts created and used for this pipeline can be found at https://github.com/PLStenger/MADCAPS_NGS.

2.5.2. *Pre-processing*

First, the quality of raw Illumina sequences and datasets was assessed using FastQC (V. 0.11.9), with aggregated reports generated by MultiQC (V. 1.10.1; Ewels et al., 2016). The sequences were then cleaned and adaptors removed using Trimmomatic (V. 0.39; Bolger et al., 2014) with the following parameters: 'illuminaclip 2:30:10′, 'leading 30′, 'trailing 30′, and 'minlen 150'. After cleaning, the data quality was re-evaluated with FastQC and MultiQC.

2.5.3. *Qiime2 framework*

Microbiome analysis was conducted using the QIIME 2 framework (V. 2021.4.0; Bolyen et al., 2018). Dereplicated and trimmed paired-end sequences (Phred33V2) were imported and denoised with the DADA2 plugin (V. 1.8; Callahan et al., 2017)), which removed singletons, chimeras, and sequencing errors, producing a table of exact amplicon sequence variants (ASVs; Callahan et al., 2017). Contaminant sequences identified in negative controls were excluded (Galan et al., 2016). ASVs present in only one sample were filtered out to avoid potential PCR or sequencing errors (Fernandez Nuñez et al., 2021). Samples were then rarefied using the alpha-rarefaction tool in QIIME2, with a maximum depth of 16,694 for Shannon entropy and Faith's PD (Faith, 1992). A rarefaction depth of 11,129 was used. A multiple sequence alignment was generated with MAFFT (V. 7.310; Katoh and Standley, 2013), and a rooted phylogenetic tree was constructed using FastTree (V. 2.1.10; Price et al., 2009). Taxonomy was assigned via Naive Bayes classifiers trained with the *q2-feature-classifier* tool, using the SILVA-138-SSURef-Full-Seqs database. For the V4 region of the 16S rRNA gene, the forward 341F ('CCTACGGGNGGCWGCAG') and reverse 805R ('GACTACHVGGGTATCTAATCC') primers were used to extract reads.

2.6. *Microbial diversity*

Alpha diversity analysis was used to assess species richness and evenness within each sample, aiming to identify the highest or lowest diversity levels. In this study, diversity was quantified using different indices: Chao1 (expected richness; Chao, 1984), Simpson evenness (a measure of diversity that accounts for the number of organisms and the number of species), Fisher alpha (Fisher et al., 1943), and Shannon entropy. Chao1 is specifically designed to estimate the true species richness of a community, including rare and hard-to-detect species, by accounting for those observed only once or twice (singleton and doubleton occurrences). This helps correct sampling biases and estimate the number of undetected species. In contrast, Shannon and Simpson indices incorporate both species richness and relative abundance, placing more weight on common species, which can lead to the underestimation of rare species' importance. An accumulation curve based on the Chao1 index was generated using the *iNEXT* (V. 2.0.20) R package and visualized with the *ggplot2* (V. 3.3.5) R package (Wickham et al., 2019).

Beta diversity was performed with a non-metric multidimensional scaling (NMDS) analysis on the dissimilarity matrix based on the Bray-Curtis index, in order to explore the similarities between samples in terms of community composition with the *vegan* R package.

2.7. *Microbiome effect on coral*

After identifying all bacterial phyla, we conducted a comprehensive review to determine their interactions with coral, categorizing them as mutualistic (beneficial to both partners), commensal (benefiting the bacteria without affecting the coral), symbiotic (a close and long-term association), or pathogenic (harmful to the coral, Bourne et al., 2016; Peixoto et al., 2017).

2.8. *Concentration of plastic debris*

We analyze the images taken by the camera to attribute the following parameters by items: (i) type (hard, foam, pellet, fiber); (ii) size class defined by (Lebreton et al., 2018): 0.05–0.15 cm; 0.15–0.5 cm; 0.5–1.5 cm; 1.5–5 cm (ImageJ software 1.5K, (Thibault, 2024; Thibault et al., 2025). To obtain the dry mass, plastic debris pooled by site in Eppendorf tubes were placed in an oven at 60 °C for 30 min. The total dry weight of the plastic debris was then measured using a precision balance with an accuracy of 10^{-5} g.

Then the concentration of plastic debris was calculated by incorporating the effect of wind mixing into the calculation of the concentration at the sea surface (Kukulka et al., 2012):

$$C_i = \frac{c_s}{1 - e^{-dW_b\left(1.5\sqrt{\frac{\rho_a}{\rho_w}C_dU^2},k,\frac{0.96}{g},\sigma^{\frac{3}{2}},C_d,U^2\right)^{-1}}} \tag{1}$$

Where C*i* is the depth-integrated concentration for the upper 5 m of the water column (item/km^{-2}), C*s* corresponds to the raw concentration of plastic debris type and size class as measured in the laboratory linked with the sampling surface area (km^{-2}), d is the depth of the manta net, W_b is the rising velocity by plastic type and size (m.s^{-1} determined by Lebreton et al., 2018), ρ_a is the air density (kg.m^{-3}), ρ_w is the seawater density (kg.m^{-3}), C_d is the drag coefficient (0.0012), U is the sea surface wind speed during sampling (m.s^{-1}), k is the Karman constant (0.4), g is the gravitational constant (9.81 m s^{-2}) and σ is the wave age equal to the constant 35.

After testing for normality and homoscedasticity, non-parametric tests were performed to compare concentrations across sites, difference between each type and size classes, using the Kruskal-Wallis test with pairwise Wilcoxon and p-value adjust with Benjamini–Hochberg ($p \leq 0.05$).

3. Results

3.1. Plastic debris concentration

We identified a total of N = 157 plastic debris items and N = 0 was found in the blank. After applying the correction of Kukulka et al. (2012), differences in concentrations between sites were non-significant, with 10^4 items.km^{-2} and 10^5 items.km^{-2} recorded at the Saya de Malha Bank, and 10^5 items.km^{-2} at the Nazareth Bank for both sites (Kruskal-Wallis chi-squared = 2.4, df = 1, p-value = 0.1213) Precisely, MP1 has 150,388 ± 92,825 items.km^{-2} (mean of plastic debris corrected concentration ± SE), MP2 has 47,956 ± 9493 items.km^{-2}; MP3 has 238,753 ± items.km^{-2} and MP4 has 167,365 ± 130,210 items.km^{-2} (Fig. 2A).

The Kruskal–Wallis test indicated a significant difference among debris types ($\chi^2 = 11.541$, df = 3, p = 0.009). However, pairwise Wilcoxon comparisons with p-value adjustment did not identify significant differences between individual types (all adjusted $p > 0.05$), although comparisons involving hard plastic showed near-significant trends ($p \approx 0.053$). Only N = 1 foam and N = 1 fiber like fishing line were counted. No pellets were collected. Regarding size classes, the Kruskal–Wallis test indicated a significant difference among debris types ($\chi^2 = 11.916$, df = 3, p = 0.007678), pairwise Wilcoxon tests with Benjamini–Hochberg adjustment revealed significant differences between S1 and S2 (p = 0.046), S1 and S3 (p = 0.046), and S2 and S4 (p = 0.046). Other comparisons were not significant ($p > 0.05$, Fig. 2B; Supplementary; B: Plastic debris concentration).

3.2. Viable microbiota on plastic debris

Contamination controls included field blanks (sterile seawater deployed during trawling), sorting blanks (open Petri dishes exposed during debris sorting), extraction blanks (sterile water), and PCR no-template controls. No microbial growth were detected in the field or sorting blanks. Extraction blanks revealed trace bacterial DNA (<0.1 % of reads), primarily from known laboratory contaminants (e.g., Ralstonia), which were removed using the decontam package (Davis et al., 2018). Precisely, among all sites, the mean (±SE) of viable bacteriais 15, 519 ± 9340 CFU/g of plastic debris, with maximum located at site MP3 with 49,456 CFU/g (Table 1; Supplementary C: CFU_raw_data).

3.3. Microbial diversity

Alpha diversity indices varied among samples (Table 2). MP1 and MP2 showed the highest species richness (Chao1 = 50 and 39,

Table 1
Colony-Forming Unit (CFU) by mass of plastic debris calculated by data from CFU and plastic debris mass (g).

Code	CFU	Plastic debris mass (g)	CFU/Mass (g)
MP1	150	1.461071	103
MP2	86000	7.728476	11128
MP3	103500	2.092759	49456
MP4	3510	2.519454	1393

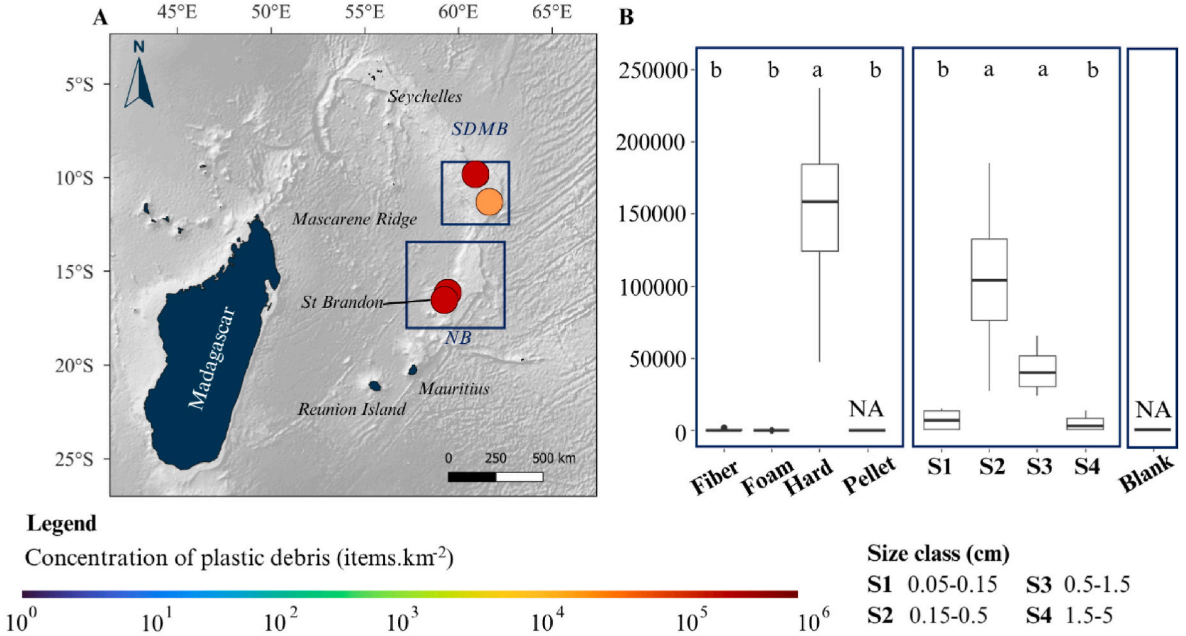

Fig. 2. A) Mean concentration of plastic debris by site (items.km^{-2})and B) by type and size classes. Different letters indicate significant differences between types or size-class concentrations (Pairwise Wilcoxon tests with Benjamini–Hochberg adjustment, $p < 0.05$). NA indicates N = 0.

Table 2
Comparison of richness and diversity indices across samples (MP1–MP4).

Samples	Chao1	Fisher Alpha	Simpson	Shannon
MP1	50	6.75	0.92	2.92
MP2	39	5.07	0.94	3.03
MP3	38,67	4.92	0.92	2.92
MP4	18	2.1	0.9	2.39

respectively) and diversity (Shannon = 2.92 and 3.03; Simpson = 0.92 and 0.94), indicating communities with both high richness and relatively even species distributions. MP3 displayed comparable diversity values to MP1 (Shannon = 2.92; Simpson = 0.92), although with slightly lower estimated richness (Chao1 = 38.7). In contrast, MP4 exhibited the lowest diversity (Chao1 = 18; Shannon = 2.39; Simpson = 0.90), suggesting a less diverse and more uneven community structure. (Table 2).

The accumulation curve of diversity reflects the “species richness” of each sample. For this analysis, we selected the minimum accumulation point of the lowest curve, as we were not focused on extrapolating indices. For MP2 and MP4, their slopes are steep and quickly reach a plateau at 6 and 3, respectively. In contrast to MP3 and MP1, even if their slopes are very similar, the only difference is that no plateau is reached, a steady increase is observed instead, especially for MP3 (Fig. 3A).

The nMDS analysis revealed three distinct clusters in terms of microbiome distribution. MP1 and MP2 are the same, while MP3 and MP4 are distinctly different from the two other sites. (Fig. 3B). The taxonomic analysis of the plastic debris microbiome revealed nine bacterial phyla (Fig. 4). The Firmicute and the Proteobacteria are the most represented with 58 % and 42 % of the total, respectively. Within the Proteobacteria phylum, twenty-two genera were identified, with *Acinetobacter* being the most abundant at all samples, representing over 50 % of Proteobacteria (Fig. 5A). For the Firmicutes phylum, four genera were identified, with *Exiguobacterium* showing 100 % of that phylum relative abundance for MP2, MP3 and MP4. Except for MP1 sample, *Bacillus* accounted for 5 % and 95 % of *Exiguobacterium* of that same phylum (Fig. 5B). The two other genera, *Clostridisalibacter* and *Lactococcus*, were not observed and were recorded as 0 %

3.4. Bacteria interaction with coral

Among the 32 genera identified, 24 were quantified at our sites. We identified coral pathogens, with *Acinetobacter* (27 % of the total), followed by *Psychrobacter* (2 %) and *Pseudomonas* (2 %) from the Proteobacteria phylum, which are associated with diseases, tissue deformation, or bleaching. Other bacteria exhibited mutualistic interactions with corals, such as probiotic activity, facilitating coral adaptation to their environment, and providing stress resistance like *Exiguobacterium* (58 %), *Rheinheimera* (1 %), and *Idiomarina* (1 %). Another exhibited both pathogenic and probiotic properties and were commensal such as *Erythrobacter* with less than 1 %. Finally, we also identified a human pathogen, *Brevundimonas* (7 %). (Table 3; Supplementary D: Raw data by genera).

4. Discussion

This study reveals that plastic debris concentrations are abundant at the sea surface of the Mascarene Ridge, with 10^5 items.km^{-2} recorded on the Saya de Malha and Nazareth banks. This preliminary study describes also the identification of coral pathogens, but also probiotic bacteria beneficial to corals.

4.1. Plastic concentration

In the Western Indian Ocean, plastic debris originates primarily from rivers in Southeast Asia, carried by the south equatorial current (Vogt-Vincent et al., 2022), but also from maritime traffic, including fishing activities in regions such as the Maldives (Imhof et al., 2017; Yadav et al., 2020), Madagascar (White et al., 2022), and the Seychelles (Marsac, 2018). These mismanaged plastics end up on tropical islands like Aldabra (Burt et al., 2020), Sainte Marie Island (Madagascar, Thibault et al., 2023), and Seychelles (Duhec et al., 2015). The two banks of the Mascarene Ridge are crossed by the South Equatorial Current, which transports plastic debris from east to west. (Vogt-Vincent et al., 2024). However, due to the proximity of seagrass beds near the surface, Saya de Malha Bank may also trap sunken plastic debris such as fishing nets and other marine debris with higher density like polystyrene or polyvinyl chloride (GESAMP, 2019).

This study confirms the significant amount of debris previously beached on the Saint Brandon island, in the Nazareth Bank (Bouwman et al., 2016). Hamman et al. (2024) revealed the presence of hard and fiber plastic debris primarily in soft corals, rather than in hard corals, at Saint Brandon and Rodrigues. Our samples collected west of Saint Brandon revealed a concentration of 10^5 items.km^{-2} of floating plastic debris, comparable to the plastic debris accumulated in the South Atlantic patch (Suaria et al., 2023), but less than the North Pacific patch with 10^7 items.km^{-2} (Egger et al., 2020, 2021; Lebreton et al., 2018). The study of the Indian Ocean patch has started only recently (Connan et al., 2021; Honorato-Zimmer et al., 2022). A, a recent research work

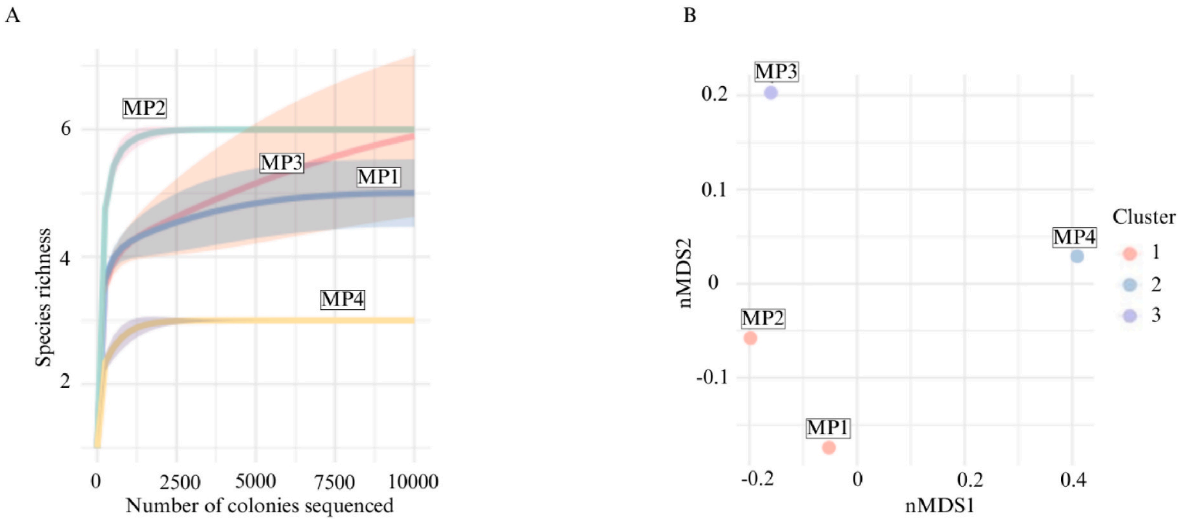

Fig. 3. (A) Accumulation curve of alpha diversity by sample with Chao1 index; (B) nMDS indicated microbiome distribution by sites.

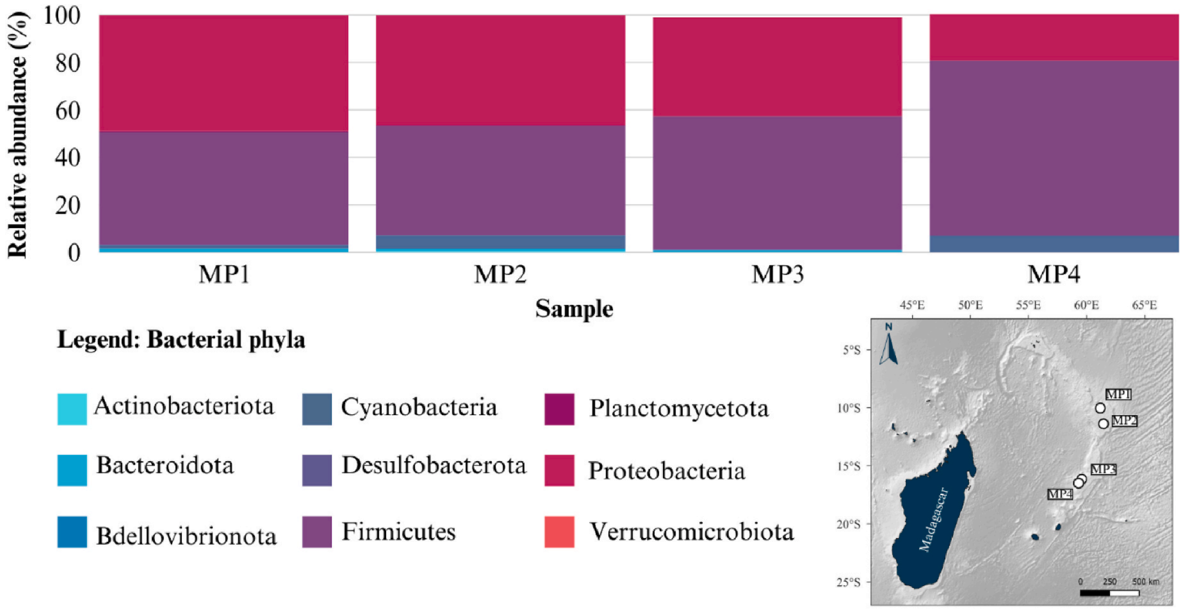

Fig. 4. Bacteria phyla relative abundance by sample.

combining 19 oceanographic campaigns in the Western Indian Ocean, found a gradient concentration of plastic debris along the latitudes 30–33°S, from 40°E with 10^3 items.km^{-2} to 65°E with 10^5 items.km^{-2} (Thibault et al., 2025). The authors also described an important concentration on the Northwest of Reunion Island with 10^6 items.km^{-2}. This accumulation can also be explained by the island mass effect that creates retention on the lee of the islands (Chandelier, 2024; Chandelier et al., 2023). A similar effect could be suggested for Saint Brandon Island, on the Nazareth Bank, in relation to the important concentration noted there. As plastic debris are transported across the ocean and accumulate in certain regions, it serves as a novel substrate for the development of a distinct microbial community, referred to as the "plastisphere," which can colonise its surface and spread over long distances (Rummel et al., 2017; Zettler et al., 2013).

4.2. *Microbiome fixed on plastic debris: density, diversity and richness specific*

Although plastic debris concentrations were similar between sites, microbiome densities were higher at MP3 (49,456 CFU/g) and MP4 (1393 CFU/g) on the Nazareth Bank, where species richness and diversity were the lowest, and communities dominated by Firmicutes rather than Proteobacteria. These patterns may stem from distinct environmental conditions between the two banks. The Nazareth Bank has cooler waters (≈24–26 °C), with possible coastal inputs from St Brandon or Mauritius, enhancing nutrient availability and favouring opportunistic taxa as Firmicute showing higher density (Mattan-Moorgawa et al., 2021; Soondur et al., 2022). By contrast, Saya de Malha Bank is under equatorial influences (≈28–30 °C), pelagic, isolated from island effects, with warm waters which could be benefic for high species diversity and richness like Proteobacteria phyla with lower density (Halo et al., 2017; Vortsepneva, 2008). Important densities of viable bacteria was also described close to Reunion Island with 10^{7-8} CFU/g (Sababadichetty et al., 2024) and lower in Madagascar but mainly formed of human pathogenic bacteria (Raherimino et al., 2025). Another study, demonstrated that Firmicute are mainly found in the nearshore area in contrast to Proteobacteria were found mainly offshore (Naudet et al., 2025; Wang et al., 2024). Differences might also arise from polymer type or surface properties influencing microbial adhesion (Setiyawan et al., 2023), or from strong competition among early colonizers such as *Pseudomonas*, *Vibrio* or *Alteromonas*, which can outcompete others in nutrient-rich, stable or coastal environments (Lemonnier et al., 2022).

4.3. *Pathogenic to corals or not?*

The plastisphere is primarily composed of diverse microbial phyla, including Actinobacteria, Acidobacteria, Bacteroidetes, Cyanobacteria, Euryarchaeota, Firmicutes, Patescibacteria, Planctomycetes, Proteobacteria, and Verrucomicrobia. These groups interact distinctly with coral microbiome communities by different actions (Curren and Leong, 2019; Oberbeckmann et al., 2016; Patel et al., 2024; Roager and Sonnenschein, 2019). In the Indian Ocean, recent studies observed presence of potential human pathogens and antimicrobial resistant bacteria fixed on plastic debris present in coral reef lagoons (Raherimino et al., 2025; Sababadichetty et al., 2024).

4.3.1. *Pathogenic*

Bacteria are widely recognized as crucial pathogens affecting corals, yet understanding their exact pathogenic mechanisms remains challenging (Lamb et al., 2018; Rosales et al., 2019). Studies have shown that bacterial richness tends to increase in diseased corals, suggesting a role for opportunistic bacteria during disease outbreaks (Sussman et al., 2008). Among the Proteobacteria, *Vibrio* species are identified as common pathogenic bacteria, posing a significant threat due to their potential role in increasing the ecological risks associated with plastic debris in coral reefs (Liu et al., 2019). *Arcobacter* spp is known to generate white band disease, and *Serratia marcescens* for white pox (Sheridan et al., 2013; Sussman et al., 2008). Although our study did not detect *Vibrio, Arcobacter* or *Serratia marcescens* species, we identified several other potential coral pathogens belonging to Proteobacteria.

These Proteobacteria have been implicated in various coral diseases and coral bleaching events (Sussman et al., 2008). *Acinetobacter* species have been linked to coral bleaching and black band disease, contributing to the degradation of coral health (Larasati et al., 2023; Li et al., 2014; Pereira et al., 2017). *Pseudomonas* sp. and *Psychrobacter*, which are known to cause white band disease, are often found in bacterial consortia alongside other pathogens (Feng et al., 2020; Junaid et al., 2022; Patel et al., 2024; Sweet et al., 2013; Thinesh et al., 2013).

Environmental factors, particularly rising temperatures, can significantly alter probiotic assemblages, exacerbating coral disease dynamics and complicating efforts to identify specific pathogens (Séré et al.,

Fig. 5. Relative abundance (%) of genera identified within the phyla (A) Proteobacteria and (B) Firmicutes.

2013). High temperatures, can intensify these issues by increasing the presence of both human and coral pathogens associated with plastic debris, such as *Escherichia-Shigella*, *Brevundimonas*, *Streptococcus*, *Acinetobacter*, *Staphylococcus*, *Lactobacillus*, *Enterobacteriaceae*, and *Sphingomonas* (Sun et al., 2022). Under such conditions, potentially pathogenic bacteria may come to dominate the coral microbiome. This interplay of environmental stressors and microbial dynamics underscores the complexity of coral disease and highlights the challenge of pinpointing precise pathogens amidst shifting microbial communities.

4.3.2. Non-pathogenic

Interestingly, our findings revealed also the presence of coral probiotic bacteria, including mainly *Exiguobacterium* followed by *Rheinheimera*, *Idiomarina* and *Bacillus*. *Exiguobacterium* is known for its antimicrobial properties, which are beneficial to coral health (Chen et al., 2010; Krediet et al., 2013; Sabdono and Radjasa, 2006). Similarly, *Bacillus* species are significant in coral environments due to their antimicrobial activity, with certain strains producing secondary metabolites that inhibit harmful bacteria and thereby support coral health (Pereira et al., 2017; Sabdono and Radjasa, 2006). The plastisphere is shaped by selective microbial attachment, interspecific competition, and high species richness, reflecting a deliberate microbial selection process (Feng et al., 2020). Another genus in our results, *Erythrobacter* (Proteobacteria), has been shown to combat *Vibrio* species on corals (Pereira et al., 2017). Corals and their associated microbiomes employ chemical defenses to counter microbial attacks, with these bacteria playing crucial roles in nutrient cycling and pathogen defense, which enhances coral resilience to environmental stress (Peixoto et al., 2017). However, variations in microbial consortia associated with coral diseases depend on factors such as geographic location, seasonal changes, and the physical characteristics of coral habitats (Sokolow, 2009).

4.4. Implication on conservation and research perspective

The Saya de Malha Bank is a rich and valuable ecosystem located at the intersection of three Exclusive Economic Zones (EEZs): those of

Table 3
_ List of bacteria phyla with effect on coral within the samples of plastic debris collected along the Mascarene ridge.

Phyla	Genera	% of the total	% of Firmicute	% of Proteobacteria	Which site	Interaction with coral	Effect	Publication
Firmicutes	*Exiguobacterium*	58	99	–	all sites	Mutualism	Antimicrobial	Krediet et al. (2013)
Firmicutes	*Bacillus* sp	0	1	–	MP1	Mutualism	Antibiotic - anti-pathogenic - pathogenic with pesticide	Negri et al. (2009); Pereira et al. (2017); Pham et al. (2017); Sabdono and Radjasa (2006)
Proteobacteria	*Rheinheimera*	1	–	2	MP1, MP3	Mutualism	Antimicrobial	Chen et al. (2010)
Proteobacteria	*Halomonas*	1	–	2	MP1	Mutualism - pathogen	Composition of coral fat acids - Antimicrobial	Villela et al. (2023)
Proteobacteria	*Acinetobacter*	27	–	63	all site	Pathogen	Coral disease and bleaching	Chen et al. (2014); Sun et al. (2022)
Proteobacteria	*Brevundimonas*	7	–	16	MP1, MP3, MP4	Pathogen	No information but human pathogen	Koh et al. (2023)
Proteobacteria	*Sphingobium*	1	–	3	MP3, MP4	Commensal	Seems good; thermal resistance	Li et al. (2014)
Proteobacteria	*Azospirillaceae*	0	–	0	MP1	NA	NA	NA
Proteobacteria	*Erythrobacter*	0	–	1	MP1, MP2	Mutualism - pathogen	Antimicrobial against Vibrio - disease tissue	Jaafar et al. (2023); Pereira et al. (2017)
Proteobacteria	*Psychrobacter*	2	–	4	MP1, MP2, MP3	Mutualism - pathogen	Produce biosurfactant	Pereira et al., 2017; Pham et al. (2017)
Proteobacteria	*Pseudomonas*	2	–	4	MP1, MP3, MP4	Pathogen	Coral disease - destroy Idiomarina	Chen et al. (2014); Feng et al. (2020); Huang et al. (2021); Jaafar et al. (2023); Sweet et al. (2013);
Proteobacteria	*Idiomarina*	1	–	1	MP1, MP3	Mutualism	Use for for biogeochemical cycling of sulfur	Raina et al. (2009)
Proteobacteria	*Pseudoaleteromonas*	0	–	1	MP1	Mutualism	Antimicrobial	Shnit-Orland et al. (2012)
Proteobacteria	*Comamonas*	0	–	0	MP1	Mutualism	tolerance and adaptation of coral-associated bacteria	Sun et al., 2022
Proteobacteria	*Cobetia*	0	–	0	MP4	Mutualism	Antimicrobial - the oil degradation - resilience	Garzon-Machado et al. (2024); Villela et al., 2023

Seychelles, Mauritius, and France (Bertrand, 1988; Lebeau and Cueff, 1975). These waters are not only a hub for fishing activities but also a busy route for maritime traffic (Vespe et al., 2015). The biodiversity and coral reefs in this area are increasingly vulnerable to these human activities (Bouchet and Bail, 1991; Webber et al., 2023). With the ongoing threats of climate change, plastic pollution, and the emergence of harmful bacteria, coral ecosystems are at greater risk of disease epidemic episodes and may struggle to withstand future heat waves or adapt to changing conditions. Even if we observe coral antimicrobial bacteria attached to plastic, the danger remains. Plastic debris is still a major problem, on land (Chen et al., 2021), in the air (Liu et al., 2019), and in the ocean (Fujieda et al., 2008; Jambeck et al., 2015; Maes et al., 2018; Suaria et al., 2020). Regardless of their size, plastic debris have negative impacts on marine life and humans (entanglement, Panda et al., 2025; bioaccumulation, Neumann et al., 2021; Verma et al., 2016; PFASs, Scott et al., 2021). Today, it is as alarming as climate change, and an international treaty against plastic pollution is currently being developed to limit it (Zhou and Xu, 2025). However, our study is preliminary, and future research should focus on examining the bacteria associated with plastics entangled in coral reefs and benthic material. Additionally, it would be valuable to sample the northern seagrass beds of the Saya de Malha Bank to collect plastic that may have sunk. Beyond studying bacterial communities, it is also important to investigate the toxicity of plastics on corals, especially considering the potential for hydrocarbons to cause endocrine disruption and other health issues in marine life (Syberg et al., 2020; Thompson et al., 2009).

Further research could also explore the broader ecological consequences for the Indian Ocean region. For example, examining how plastic pollution affects migratory species or how localized coral degradation might impact fisheries and food security across neighbouring countries (Apete et al., 2024; Jang et al., 2024). Additionally, investigating potential mitigation strategies, such as reducing plastic waste at its source or restoring degraded coral habitats, could be crucial for the long-term preservation of this unique marine ecosystem.

5. Conclusion

The Mascarene Ridge appears to be a convergence zone for plastic debris at the sea surface. The bacteria found on these plastics include a range of coral potential pathogens such as *Acinetobacter, Psychrobacter* and *Pseudomonas* as well as probiotics bacteria like *Exiguobacterium, Rheinheimera* and *Idiomarina*. As a preliminary study, we recommend continuing precise research on the direct interactions between coral disease, plastic debris, and bacteria. Depending on abiotic and biotic conditions, these microbiomes can evolve and potentially lead to coral diseases. With the ongoing climate change and increasing plastic pollution, it is crucial to continue protecting both banks of the Mascarene Ridge for the years to come.

CRediT authorship contribution statement

Vyctoria Marillac: Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Philippe Jourand:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Conceptualization. **Pierre-Louis Stenger:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Gwennais Fustemberg:** Writing – review & editing, Investigation. **Julie Gindrey:** Writing – review & editing, Investigation. **Margot Thibault:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Margot Thibault reports article publishing charges and travel were provided by Monaco Explorations. Margot Thibault reports equipment, drugs, or supplies was provided by The Ocean Cleanup. Vyctoria Marillac reports equipment, drugs, or supplies and travel were provided by French Biodiversity Office. Vyctoria Marillac reports travel was provided by Directive des Ressources Internationales. Margot Thibault reports a relationship with The Ocean Cleanup that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr2.2025.105556.

Data availability

Data will be made available on request.

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