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# Microplastics in the insular marine environment of the Southwest Indian Ocean carry a microbiome including antimicrobial resistant (AMR) bacteria: A case study from Reunion Island

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1 Title: Microplastics in the insular marine environment of the Southwest Indian Ocean carry a  
2 microbiome including antimicrobial resistant (AMR) bacteria: a case study from Reunion Island.

3

4 Author names: Loik Sababadichetty<sup>1,2a</sup>, Guillaume Miltgen<sup>2,3a</sup>, Bryan Vincent<sup>4a</sup>, François  
5 Guilhaumon<sup>5a</sup>, Veronique Lenoble<sup>6</sup>, Margot Thibault<sup>1,7,8</sup>, Sophie Bureau<sup>1</sup>, Pablo Tortosa<sup>3</sup>, Thierry  
6 Bouvier<sup>9</sup> and Philippe Jourand<sup>5</sup>,\*.

7

8 Authors affiliations :

9 <sup>1</sup>Université de La Réunion, UMR ENTROPIE, 15 Avenue René Cassin, CS 92003, 97744, Saint Denis  
10 Cedex 9, La Réunion, France.

11 <sup>2</sup>CHU, Laboratoire de Bactériologie, CHU Félix Guyon, Allée des Topazes, 97400, Saint-Denis, La  
12 Réunion, France.

13 <sup>3</sup>Université de La Réunion, UMR PIMIT Processus Infectieux en Milieu Insulaire Tropical, CNRS  
14 9192, INSERM 1187, IRD 249. Plateforme de recherche CYROI, 2 rue Maxime Rivière, 97490 Ste  
15 Clotilde, La Réunion, France

16 <sup>4</sup>CIRAD, UMR040 LSTM, Campus Agro Environnemental Caraïbe, BP 214-97285, Cedex 2 le  
17 Lamentin, Martinique, Antilles Françaises, France

18 <sup>5</sup>IRD, UMR ENTROPIE, 15 Avenue René Cassin, CS 92003, 97744, Saint Denis Cedex 9, La  
19 Réunion, France.

20 <sup>6</sup>Université de Toulon, Aix Marseille Université, CNRS, IRD, UMR MIO, 83 Toulon, France

21 <sup>7</sup>The Ocean Cleanup, Rotterdam, The Netherlands

22 <sup>8</sup>CNRS, Université Toulouse III, Laboratoire des Interactions Moléculaires et Réactivité Chimique et  
23 Photochimique (IMRCP), UMR 5623, Toulouse, France

24 <sup>9</sup>UMR MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Montpellier, France

25 \*Corresponding author : Philippe Jourand, IRD, UMR ENTROPIE, 15 Avenue René Cassin, CS  
26 92003, 97744, Saint Denis Cedex 9, La Réunion, France. Tel: +262 0262 52 89 19; E-mail:  
27 [philippe.jourand@ird.fr](mailto:philippe.jourand@ird.fr)

28 <sup>a</sup>These authors contributed equally to this work.

29 **Highlights**

- 30 • Severe marine plastic pollution impacts Southwest Indian Ocean insular ecosystems
- 31 • Plastic debris from Southwest Indian Ocean host rich microbiomes
- 32 • Proteobacteria dominate such marine plastic microbiomes
- 33 • These debris carry a consequent culturable bacterial flora including potential pathogens
- 34 • AMR bacteria hitchhike on these plastics

35

36 **Abstract**

37 The increasing threats to ecosystems and humans from marine plastic pollution require a  
38 comprehensive assessment. We present a plastisphere case study from Reunion Island, a remote  
39 oceanic island located in the Southwest Indian Ocean, polluted by plastics. We characterized the  
40 plastic pollution on the island's coastal waters, described the associated microbiome, explored viable  
41 bacterial flora and the presence of antimicrobial resistant (AMR) bacteria. Reunion Island faces plastic  
42 pollution with up to 10,000 items/km<sup>2</sup> in coastal water. These plastics host microbiomes dominated by  
43 Proteobacteria (80%), including dominant genera such as *Psychrobacter*, *Photobacterium*,  
44 *Pseudoalteromonas* and *Vibrio*. Culturable microbiomes reach 10<sup>7</sup> CFU/g of microplastics, with  
45 dominance of *Exiguobacterium* and *Pseudomonas*. Plastics also carry AMR bacteria including β-  
46 lactam resistance. Thus, Southwest Indian Ocean islands are facing serious plastic pollution. This  
47 pollution requires vigilant monitoring as it harbors a plastisphere including AMR, that threatens  
48 pristine ecosystems and potentially human health through the marine food chain.

49

50 Keywords: Reunion Island, plastic marine pollution, microbiome, Proteobacteria, AMR bacteria

51 1. Introduction

52 Marine pollution by plastic wastes and debris is an important source of anthropogenic  
53 contamination in the oceans (Thushari & Senevirathna, 2020). This pollution is increasingly seen as a  
54 major concern not only for the environment, *i.e.*, contamination of pristine ecosystems with loss of  
55 biodiversity, but also for human health through contamination of marine trophic networks (Smith et  
56 al., 2018; Wright et al. 2020; Kumar et al., 2021). Plastics, when they enter the marine ecosystem,  
57 influence the marine food chain differently in different species due to factors such as size, shape,  
58 polymer composition and chemical additives (Tuuri and Laterme, 2023). Plastics contaminate marine  
59 life at many levels, from zooplankton (Cole et al., 2013) to bivalves (Van Cauwenberghe and Janssen,  
60 2014), fishes (Li et al.2021), reptiles such as turtles (Thibault et al., 2023) to marine mammals (Panti  
61 et al., 2019) and, at the end of the marine food chain, humans (Waring et al., 2018). For example,  
62 microplastics have been found in most marine organisms along trophic levels in various organs such  
63 as intestinal contents, gills or even flesh (Walkinshaw et al., 2020). In addition, the physical and  
64 chemical properties of plastic debris allow them to absorb numerous contaminants such as chemicals,  
65 metals and bacteria (Imran et al., 2019), acting as vectors for these contaminants when ingested by  
66 organisms. Therefore, bioaccumulation of plastics for higher trophic organisms has wider impacts on  
67 marine food webs with potential impacts on human health (Carbery et al., 2018).

68 Plastics slowly degrade over time into smaller and smaller particles including those called  
69 "microplastics" (particle size between 0.1 - 5 mm) through weathering and physical processes as well  
70 as microbial activities (Galloway et al., 2017; Rummel et al., 2017; Jacquin et al., 2019). As a result,  
71 plastics remain present in the marine environment over very large time scales and accumulate, with an  
72 estimation of microplastic particles in 2014 reaching up to 50 trillion particles and weighing over  
73 200,000 metric tons (van Sebille et al., 2015). Microplastic contamination of coastal and marine  
74 ecosystems reaches up to 140 particles/m<sup>3</sup> in water and 8766 particles/m<sup>3</sup> in sediments (Thushari &  
75 Senevirathna, 2020).

76 These microplastics are durable, often floating substrates with physical and chemical  
77 properties that can have negative impacts on entire marine ecosystems over all bathymetric zones  
78 (Rochman, 2015; Masry et al., 2021). The physical traits of microplastics negatively impacting the

79 ecosystems and marine organisms are the plastic size, their shape, the polymer composition and the  
80 surface chemistry (Lambert et al., 2017). Moreover, the potential harm of microplastics in marine  
81 environments is linked to their ability to accumulate contaminants and transport toxic substances and  
82 pathogens (Imran et al., 2019; Chouchene et al., 2023). Microplastic substrates offer new habitats and  
83 very effective dispersal ways to microbial communities that can attach through the formation of  
84 biofilms and drift along oceanic currents (Oberbeckmann & Labrenz 2020). In addition,  
85 microorganisms' communities associated with plastic, the so-called "plastisphere" (Zettler et al.,  
86 2013), have been shown to be enriched with pathogenic bacteria, including members of the genus  
87 *Vibrio* (Oberbeckmann & Labrenz 2020). Thus, pathogen-enriched floating microplastics have the  
88 potential to disperse over long distances and spread pathogenic bacteria to new marine areas and  
89 ecosystems and should therefore be considered as a threat to marine ecosystems as well as to animal  
90 and human health (Bowley et al., 2021; Stabnikova et al., 2021).

91         Among the pathogens underscored on microplastics, many bacterial strains have been found to  
92 harbor antimicrobial resistance (AMR) as well as resistance to heavy metals (MRG) (Bowley et al.,  
93 2021). These multidrug resistances are correlated with the presence of heavy metals, organic  
94 pollutants, and traces of antibiotics in the marine environment, which can adsorb onto plastic biofilms  
95 (Imran et al., 2019). These substances are known to promote horizontal gene transfer (HGT) of  
96 virulence and resistance *via* mobile genetic elements (MGE) within bacterial communities (Sobecky &  
97 Hazen, 2009). Therefore, microplastics and their associated adsorbed chemicals, by promoting  
98 horizontal gene transfer in bacteria, contribute to the selection and dispersal of antimicrobial and metal  
99 resistance (Arias-Andres et al., 2018; Marathe & Bank, 2022). Finally, microplastics can have a  
100 significant impact on the spread of multidrug-resistant (MDR) pathogens, which may represent an  
101 additional threat (in terms of dangerousness compared to simple bacterial contamination) to the entire  
102 marine-related trophic network, including humans (Wright et al. 2020).

103         In the Indian Ocean (IO), recent reports highlight plastic accumulation along the coasts from  
104 Australia to India, in the Arabian Peninsula, along the coasts of East Africa and of the IO islands  
105 (Pattiaratchi et al., 2022). In the open ocean, the authors reported the absence of any rubbish patch in  
106 the northern IO, while a significant patch was identified in the southern IO in relation to the South

107 Atlantic Ocean (Pattiaratchi et al., 2022) and the South Pacific Ocean (Maes et al., 2018). According  
108 to Pattiaratchi et al. (2022), Reunion Island, an oceanic island located in the southwest part of the IO,  
109 is also impacted by this marine plastic pollution. This island, located at the crossroads of southern  
110 Africa and the Indian subcontinent, is also strongly affected by the phenomenon of AMR. In the  
111 human sphere, Reunion Island is subject to a very high pressure of importation of MDR and  
112 extensively drug-resistant (XDR) pathogens, linked to the population flow in the area (Miltgen et al.,  
113 2020; Miltgen et al., 2021; Kamus et al., 2022). However, there is very little data on the environmental  
114 spread of these pathogens from human excreta after the discharge of these effluents from the  
115 wastewater treatment plants into the ocean (Miltgen et al., 2022). The same is true for microplastics  
116 that are subject to human pollution in other territories and that can drift *via* the oceans to Reunion  
117 Island.

118 Thus, the microbial communities existing on the plastic marine pollution that reaches the  
119 coasts of Reunion Island could be affected by this AMR phenomenon. Therefore, it is of utmost  
120 importance to determine whether the microbial communities colonizing the marine microplastic debris  
121 drifting off the coast of Reunion Island host pathogenic bacteria, potentially resistant to several  
122 antimicrobials, which should then be considered as a threat to public health.

123 The present study aims at *(i)* characterizing the microbiome hosted by marine microplastics  
124 drifting in the coastal waters of Reunion Island and *(ii)* addressing the presence of AMR potential  
125 pathogens carried by these microplastics. To our best knowledge, this study is the first in the  
126 southwest Indian Ocean islands, including physico-chemical, genomic, and microbiological  
127 approaches. It integrates public health concerns and local environmental issues with the aim of  
128 shedding light on the role of microplastics and the consequences that this new human-induced niche  
129 may have, not only on the marine environment and island ecosystems, but also potentially on the  
130 entire marine food chain, up to humans, in a One Health approach (Wright et al., 2020).

## 131 2. Materials and methods

### 132 2.1 Site description and sampling methods

133 Reunion Island is located at 55° East 21° South, 700 km east of Madagascar (Fig. 1). The two  
134 selected sites (see map in Fig. 1) are distinct in terms of anthropogenic disturbance and oceanic

135 influence: the first site, Livingstone (21°05'02.5"S 55°13'33.6"E), is located on the leeward west coast,  
136 at the level of the Ermitage lagoon, in Saint-Gilles municipality, while the second site *i.e.* the Tremblet  
137 beach in Saint-Philippe municipality (21°17'38"S 55°48'19"E) is located on the windward east coast.  
138 The 1<sup>st</sup> site is heavily impacted by local anthropogenic activities (Tourrand et al., 2013; Guigue et al.,  
139 2015; Lemahieu et al., 2017) while the 2<sup>nd</sup> site is a newly formed beach, almost untouched by human  
140 activity, resulting from a volcanic eruption that occurred in 2007 (Staudacher et al., 2009). The  
141 collected samples were, on one hand, the plastics from the coastal seawater (PSW) and the sand beach  
142 (PS), and on the other hand, the substrates *i.e.* the coastal sea-water (SW) and the beach sand (S).  
143 beach sand.

144 Plastic debris were collected from the seawater surface at 200 m from the shoreline using a  
145 manta net (mesh size: 500  $\mu\text{m}$ ; mouth area: 1.125  $\text{m}^2$ ) provided by the non-governmental organization  
146 (NGO) "The Ocean Clean Up" (Rotterdam, The Netherlands; <https://theoceancleanup.com>) (Virsek et  
147 al., 2016)). The speedboat was sailing at 2 knots and the sampling time was 20 minutes, with sampling  
148 days chosen in fair weather. Three transects were set at each site to generate replicates per area  
149 (GESAMP, 2019). Between each replicate, the manta net was rinsed externally with a jet of seawater  
150 and all plastic particles were collected. Plastic concentration was calculated following Kukulka et al.  
151 (2012). Plastic debris collection numbered by sample according to site and substrate are reported in  
152 Supplemental Table 1. At the same time, seawater samples collected from the coastal area (3 replicates  
153 of 2 L samples per collection) were processed according to the protocol of Hinlo et al. (2017). In  
154 parallel to seawater collection, plastic debris were collected from the beach at each site following the  
155 protocols of Besley et al. (2016) and using a 1 m  $\times$  1 m sampling quadrat. Three 50 x 10 m corridor  
156 transects running parallel to the sea were conducted for each site. We report the density of collected  
157 plastic wastes in items/ $\text{km}^2$ . At the same time, beach sand samples were collected in triplicates  
158 according to the protocol reported by Almeida et al. (2019). From these plastic sample sorting, sub-  
159 samples of 50 microplastics particles (size < 5 mm) were randomized (Löder & Gerts, 2015) to carry  
160 out optimal DNA extraction as suggested by Debeljack et al. (2017). To avoid air contamination, the  
161 separation of the microplastics (< 5 mm) was carried out in a clean room and under a binocular

162 magnifier under sterile conditions. These randomized subsamples were assembled in triplicates for  
163 both DNA and living microbe extractions (see further for the pre-treatment detailed protocol).

## 164 2.2. Sub-samples pre-treatments

165 Each microplastic sub-sample of 50 particles was treated according to the protocol of Trachoo  
166 (2004) by gentle abrasion to extract DNA and cultivable bacteria while keeping the polymer structure  
167 of the plastic intact. For this, 10 g of washed and sterile (see below) silica sand (Sigma-Aldrich,  
168 Darmstadt, Germany) was added to a sterile 50 ml Falcon tube (Fischer, Illkirch, France) containing  
169 50 particles of microplastic. Silica sand was treated before use as follows: sand was first washed for  
170 10 min with 2% hydrochloric acid (Merck, Darmstadt, Germany), then rinsed 3 times with MilliQ  
171 water. Silica sand was then washed for 10 minutes with sodium hypochlorite solution Emplura at final  
172 concentration of 2% (Merck, Darmstadt, Merck, Germany) and rinsed 3 times with MilliQ water.  
173 Finally, sand was rinsed once for 10 minutes with MilliQ water before being autoclaved (120°C, 20  
174 min, 1 bar). An artificial seawater solution was reconstituted by dissolving 35 g of NaCl (Sigma-  
175 Aldrich, Darmstadt, Germany) in 1 L of MilliQ water and then autoclaved (120°C, 20 min, 1 bar). A  
176 volume of 20 mL of reconstituted sterile seawater and 20 g of sterile and washed silica sand were  
177 added to each microplastic sample. Similarly, the beach sand samples (20 g in a 50 ml Falcon tube)  
178 were supplemented with 20 ml of reconstituted sterile seawater. All samples (microplastics and beach  
179 sand) were vortexed for 60 seconds. The supernatant was collected and divided into two aliquots of 10  
180 ml for DNA extraction and microbiological analysis. Seawater samples collected from the coastal area  
181 (3 replicates of 2 L samples per collection) were processed according to the protocol of Hinlo et al.  
182 (2017). For each 2 L sample of sea water, there were two separate filtrations of 1 L on a sterile  
183 nitrocellulose membrane (0.22 µm): one filter was stored at -20°C for DNA extraction and the other  
184 one underwent resuspension of bacteria by vortexing in 5 ml of reconstituted sterile seawater for  
185 microbiological analyses.

## 186 2.3 DNA extractions, 16S PCR and library preparation



187           The 10 ml of supernatants from the plastic abrasion or sediment extractions were filtered and  
188 sterilized through 0.22 µm nitrocellulose membranes (Merck Millipore, Cork, Ireland). DNA was  
189 extracted from all nitrocellulose membranes resulting from the plastic, sediment supernatant and water  
190 filtrations, following the protocols of Debeljack et al. (2017). Briefly, the membranes were placed in  
191 Qiagen DNeasy Blood and Tissue kit columns (Qiagen GmbH, Hilden, Germany) and DNA was  
192 extracted according to the Qiagen manufacturer's instructions. After extraction, DNA was quantified  
193 using a Nanodrop spectrophotometer (Thermo Scientific France, Illkirch-Graffenstaden, France). The  
194 DNA samples were then sent to Macrogen's Next Generation Sequencing (NGS) platform (Macrogen,  
195 Seoul, Korea) for mass DNA sequencing. Library construction and sequencing were performed  
196 according to Illumina 16S metagenomic sequencing library protocols to amplify the V3 and V4 region  
197 of 16S DNA (Bukin et al., 2019). Two nanograms of genomic DNA were amplified by PCR with 5x  
198 reaction buffer, 1 mM dNTP mix, 500 nM of each of the universal F/R PCR primers and Herculase II  
199 fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycling condition for the 1st  
200 PCR was 3 min at 95°C, and 25 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, followed  
201 by a final extension of 5 min at 72°C. The V3-V4 domain of the 16S rDNA was amplified by PCR  
202 using the following primers V3-341F: 5'-CCTACGGGNGGCWGCAG-3', V4-805R: 5'-  
203 GACTACHVGGGTATTAATCC-3' (Klindworth et al., 2013) with Illumina adaptor overlays. The  
204 PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA) and 2 µl of the  
205 purified product was PCR amplified for construction of the final library containing the index using the  
206 Nextera XT index primer. The cycling condition for the 2nd PCR was the same as the 1st PCR  
207 conditions. The PCR product was purified with AMPure beads. The final purified product was then  
208 quantified using qPCR according to the qPCR quantification protocol guide (KAPA library  
209 quantification kits for Illumina sequencing platforms) and qualified using the TapeStation D1000  
210 ScreenTape (Agilent Technologies, Waldbronn, Germany).

211           2.4 NGS analyses.

212           Paired sequencing (2×300 bp) was performed using the MiSeq™ platform (Illumina, San  
213 Diego, USA). Adapter pruning was performed using the fastp program, adapter sequences were

214 removed and error correction was performed in overlapping sequences (Chen et al., 2018). The read  
215 assembly was performed by assembling pair-end sequences created by sequencing both directions of  
216 the library. The program used in this process is \*FLASH (v1.2.11) (Magoc & Salzberg, 2011).  
217 Assembled reads shorter than 400 bp or longer than 500 bp were removed. Next, the preprocessing  
218 and clustering process was performed according to the protocols of Li et al. (2012): data with  
219 sequence errors were removed in order to obtain accurate OTUs. Reads containing ambiguous bases  
220 and chimeric sequences were also removed. After this process, clustering was performed based on  
221 sequence similarity with a cut-off value of 97% using CD-HIT-OTU, a comprehensive program based  
222 on cd-hit-est. Community diversity and taxonomy were analyzed according to Caporaso et al. (2010)  
223 using QIIME (v1.9.0), which is used for OTU analysis and taxonomy information. The main sequence  
224 of each OTU was referenced in the NCBI 16S database, and taxonomic information was obtained with  
225 BLASTN (v2.4.0).

#### 226 2.4. Microbiological analysis

227 Successive serial dilutions from 1 to  $10^{-2}$  were prepared from the bacterial supernatant  
228 resulting from the plastic / sand abrasion or the seawater 0.22  $\mu\text{m}$  membrane filter resuspension using  
229 the reconstituted sterile seawater. The total bacterial flora was counted by inoculating 100  $\mu\text{l}$  of these  
230 dilutions onto plate agar of Mueller Hinton + PolyViteX (PVX, BioMérieux, Marcy l'Etoile, France)  
231 and Mueller Hinton E (MHE, BioMérieux, Marcy l'Etoile, France) media as previously described  
232 (Miltgen et al., 2020; Bhuyan et al., 2023). Once inoculated, the media were incubated at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$   
233 for 24 to 72 h, until the microbial colonies appeared visible. The total bacterial flora was quantified  
234 and expressed as colony forming units (CFU) per g of plastic or sand or ml of seawater. The following  
235 selective media were also used: Columbia NaladixicAcid Agar (CNA, bioMérieux, Marcy l'Etoile,  
236 France) for the identification of the Gram-positive bacteria, Drigalski (DRIG, bioMérieux, Marcy  
237 l'Etoile, France) for Gram negative bacteria and chromID CPS ELITE (CPSE, bioMérieux, Marcy  
238 l'Etoile, France) for a control. For each sample, subcultures were analyzed, and each phenotypically  
239 distinct colony was re-isolated on Mueller Hinton agar (MHE) and incubated at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . After 24  
240 to 72 hours, the individual colonies were identified using MALDI-TOF (Matrix Assisted Laser

241 Desorption Ionization Time-Of-Flight) mass spectrometry (Bizini & Greub, 2010). After  
242 identification, pathogens were counted on selective media to estimate the relative abundance of each  
243 bacterial genus. The antimicrobial susceptibility of each pathogen was assessed by the disk diffusion  
244 or gradient strips methods (Miltgen et al., 2020). After 18-24 hours, the inhibition diameter around  
245 each antibiotic disc or the MIC (minimal inhibitory concentration) were measured and the  
246 bacterial/antibiotic pair was categorized susceptible, intermediate or resistant (S/I/R) following the  
247 recommendations of The European Committee on Antimicrobial Susceptibility Testing (2020  
248 EUCAST, <https://www.eucast.org>), while the resistance for isolates belonging to *Vibrio* spp. was  
249 categorized using the Clinical Laboratory Standards Institute (CLSI <https://clsi.org/>)  
250 recommendations.

## 251 2.5. Microplastic polymer identification

252 Microplastic particles were retrieved after the sand abrasion as described above. They were  
253 characterized by using Fourier Transform InfraRed spectroscopy (FTIR Nicolet i550, Thermo fisher)  
254 in ATR (Attenuated Total Reflection) mode, with wavelengths ranging from 400 to 4000  $\text{cm}^{-1}$   
255 (resolution of 1  $\text{cm}^{-1}$ ). The pieces were one by one pictured then pressed between diamond and base  
256 (Djaoudi et al., 2022). The diamond was cleaned between each particle analysis. Final infrared  
257 spectrums (average of 40 scans) were analyzed using SpectraGryph software and its database. Only  
258 correspondences higher than 85% were validated. The polymer FTIR identification and frequency per  
259 sample are reported in Supplemental Table 1.

## 260 2.6 Microbial community analyses based on OTUs abundance, taxonomy and statistical 261 methods

262 A full overview of this analytical approach is presented in Supplemental Fig. 1. We used  
263 OTUs abundance data resulting from NGS analyses to compare microbial communities between: (i)  
264 sampling coasts (East or West coast), (ii) the matrices from which plastics were sampled (sand beach  
265 or seawater) and (iii) the sampled materials (plastic, seawater, or sand beach). The sampling DNA  
266 sequencing quality was firstly checked with rarefaction curves (Supplemental Fig. 2a, b, c, d)

267 computed with iNEXT online (Chao et al., 2016). We used a top-down taxonomic approach to explore  
268 the differences between microbial communities by first analyzing the full data at the phylum level then  
269 focusing only on the most frequent genera belonging to the Proteobacteria phylum (genera  
270 representing more than 1% OTUs total abundance: 15 out of the 405 genera identified in the full  
271 database, Supplemental Fig. 3). OTU abundances raw data are available as Supplemental table 2. The  
272 composition of microbial communities is presented using (i) barplots of the relative abundance of  
273 organisms (cumulated abundances are given in Supplemental tables 2a at phylum level and  
274 Supplemental table 3a for genera belonging to the Proteobacteria phylum level) and (ii) Non-Metric  
275 Multidimensional Scaling (NMDS) (based on Bray-Curtis dissimilarities) plots using the "vegan"  
276 package for the R software (R Core Team 2021, v4.0.4). In both graphical representations, data were  
277 grouped according to environmental parameters (combination of matrix and material), with NMDS  
278 plots showing ellipses for 95% confidence intervals. Overall differences in bacterial communities'  
279 composition were statistically assessed between sample sites, substrates and ecosystems using analysis  
280 of similarities (ANOSIM) on the same Bray-Curtis distance matrix (Dixon, 2003). Pairwise  
281 differences between groups were statistically assessed using Chi<sup>2</sup> tests and corrected according to the  
282 Bonferroni method. Ecological diversity was measured using several indices: specific richness and the  
283 Shannon and Simpson diversity indices. Overall differences in ecological diversity between groups  
284 were tested using non-parametric Kruskal Wallis test, followed by Duncan's post-hoc tests when more  
285 than two groups were compared and non-parametric Wilcoxon Mann-Whitney test if two groups were  
286 compared. Venn diagrams were used to illustrate the dissimilarity in composition between groups  
287 (Oliveiros, 2007).

288 Linear models were used to compare (i) the abundance of plastic debris collected at the sea  
289 surface and on beaches between sites (East vs West) and polymer type and (ii) the abundance of  
290 culturable bacterial populations isolated from plastic, water and sand samples from different sites or  
291 substrates. Tukey signed-rank tests were further used to evaluate pairwise differences.

## 292 3. Results

### 293 3.1. Plastic data

294 At the sea surface, concentrations of particles were highly variable across samples, ranging  
295 from 0 to 7,391 items / km<sup>2</sup> on the west coast and from 3,561 to 23,692 items / km<sup>2</sup> on the east coast  
296 (Table 1 and Supplemental Table 1). There was no significant difference between the west and east  
297 coasts in terms of item concentrations (p.value > 0.05) (Table 1). Most of the debris (85%) have been  
298 successfully classified by FTIR analysis. Both on the west and east coasts, the most abundant plastic  
299 polymers identified were polyethylene (PE), representing 75% and 84% of particles respectively; and  
300 polypropylene (PP) representing the remaining 25% and 16% (Table 1). There was no significant  
301 difference between the west and east coast in terms of polymer type (p.value > 0.05). For the beaches,  
302 two significant different concentrations of  $0.34 \pm 0.31$  and  $0.022 \pm 0.008$  item/m<sup>2</sup> were estimated for  
303 the west and east coast, respectively. Significant differences between the diversity of polymers on the  
304 strandings were found on the west coast, there was 50% PE, 38% PP, 9% polystyrene (PS) and 3%  
305 polyvinyl chloride (PVC) while on the east coast, there was 70% PE, 29% PP and 1% PS (Table 1).

### 306 3.2. Bacterial microbiome analyses based on the 16S rDNA sequencing.

#### 307 3.2.1 Total OTUs diversity and abundance

308 A total of 4,052,436 reads were retained ( $184,201 \pm 22,611$  reads per sample on average) after  
309 quality filtering and chimera checking, reads abundances ranged from 138,698 to 213,256 for the  
310 plastics sampled from seawater of the West Coast (PSWw) and seawater samples from East Coast  
311 (SWe), respectively (Supplemental Tables 2a, b, c). Read numbers did not differ significantly between  
312 plastics and substrates and between coasts (Supplemental Tables 2a, b) and no clear grouping was  
313 detected when samples were compared pair wisely (Supplemental Table 2c). All rarefaction curves  
314 showed an early stationary phase indicating sufficient sequencing depth of the taxa amplified in the  
315 microplastic, sand and seawater matrices (Supplemental Fig. 2a, b, c, d). Overall, high-quality  
316 sequences were grouped into 1,084 OTUs, 877 OTUs were identified from microplastic samples, 747  
317 from the sand samples and 468 from seawater samples. These 1,084 OTUs (Supplemental Table 3)  
318 were tallied at an overall mean of  $21,299 \pm 5,127$  OTUs / sample, with the difference between the East  
319 coast and West coast sites being not significant (Supplemental Table 2a,  $p > 0.05$ ), as the difference  
320 between plastics sampled in sea water and on plastics sampled in sand (Supplemental Table 2b,  $p >$   
321  $0.05$ ). However, OTUS were significantly more abundant for plastics than for both of their substrates

322 (seawater or sand), with OTUS being 1.46 times more abundant in plastic DNA samples than in  
323 seawater DNA samples, and 1.32 times more abundant for plastics sampled in sand (Supplemental  
324 Table 2b,  $p < 0.05$ ). Microplastic debris from seawater shared 28.5% and 60.7% of OTUs with  
325 seawater in the western and eastern sites, respectively, while microplastics from the sandy beach  
326 shared 50.2% and 61.2% of OTUs with sand beach in the western and eastern sites, respectively (Fig.  
327 2).

### 328 3.2.2 Diversity of OTUs at the phylum level

329 Phylum richness was significantly higher on sand than on water (Table 2). However, there  
330 was no significant difference between the study sites (East versus West) nor between plastic versus  
331 non-plastic substrates (seawater or sand beach). Shannon and Simpson diversities differed  
332 significantly between study sites and between plastic and non-plastic samples (Table 2) but not  
333 between sand and water. Shannon and Simpson diversities were higher on the east coast than on the  
334 west coast and in non-plastic samples compared to plastic samples (Table 2). The full diversity  
335 indexes of the bacterial phylum distribution according to the site and the substrate are presented in  
336 Supplemental Table 4a.

337 An overall number of 19 phyla was identified in both plastics and their substrates samples  
338 (Fig. 3a, Supplemental Table 4b). All samples were dominated by Proteobacteria (75%), Bacteroidetes  
339 (11%), Cyanobacteria (5%) and Fimicutes (4%). Actinobacteria, Planctomycetes and Verrucomicrobia  
340 were also present across all the samples but at lower levels (<2%). At the phylum level, bacterial  
341 communities associated with plastics harvested in sea water (PSW) stands out from other groups  
342 (ANOSIM and  $\chi^2$  tests, Fig. 3b and Supplemental Tables 4c and d). Height phyla were detected in  
343 seawater and sand but not on plastics: Acidobacteria, Chlamydiae, Gemmatimonadetes,  
344 Ignavibacteriae, Kiritimatiellaeota, Lentisphaerae, Spirochaetes and Thaumarchaeota. Phylum  
345 communities did not differ between sites (West vs. East) and matrices (samples harvested from  
346 seawater vs. samples harvested from the sand).

### 347 3.2.3 Proteobacteria

348 Proteobacteria were the dominant phylum (75.6% of the total of OTUs) and were further  
349 analyzed. To better analyze data at the genus level, we filtered out the least frequent Proteobacteria

350 OTUs (frequency <1%). 15 out of 405 genera, representing 79.4 % of the total Proteobacteria OTUs  
351 were kept (see Supplemental Fig. 3a and Supplemental Table 3 for full results). Genera richness and  
352 Shannon diversity did not differ between sites (East vs. West) and environmental parameters (sand or  
353 seawater), nor between plastic and non-plastic samples (Table 2 and Supplemental Table 5a, b and c).  
354 Simpson diversity did not differ between sites (East versus West) nor between plastic versus non-  
355 plastic samples but differed significantly between substrates (sea water versus sand) ( $p < 0.05$ ). Across  
356 all samples four genera accumulated more than 70% of the overall OTU abundance: *Psychrobacter*  
357 (21.9% in all samples and 28.8% in plastics), *Vibrio* (20.2% in all samples and 17.1% in plastics),  
358 *Pseudoalteromonas* (17.6% in all samples and 18.1% in plastics) and *Photobacterium* (14.8% in all  
359 samples and 19.4 % in plastics) were among the most found abundant genera belonging to the phylum  
360 of the Proteobacteria. Nonetheless, the composition of proteobacterial communities differed strongly  
361 between the west and the east coasts (Fig. 4a, Supplemental Table 5c). Considering plastic, seawater,  
362 and sand samples, on east coast *Vibrio* (33.1%), *Pseudoalteromonas* (25.6%) and *Photobacterium*  
363 (19.3%) were dominant whereas the genus *Psychrobacter* (39.7%) was the most abundant on the West  
364 coast. Plastic samples showed genera compositions different from seawater or sand samples (Fig. 4a,  
365 Supplemental Table 5c) with a dominance of *Psychrobacter* (28.8%), *Photobacterium* (19.5%),  
366 *Pseudoalteromonas* (18.1%) and *Vibrio* (17.1%) on plastics, and *Vibrio* (30.1%), *Pseudoalteromonas*  
367 (15.8%), *Candidatus Pelagibacter* (12.7%), *Alcanivorax* (12.6%) and *Alteromonas* (10%) in seawater  
368 and sand. Proteobacterial communities associated with microplastics or found in the water column or  
369 on the beach sand were further differentiated using NMDS ordinations and ANOSIM tests (Fig. 4b).  
370 The distribution of proteobacterial communities differed significantly (Supplemental Table 5d)  
371 between sites (green symbols vs blue symbols on Fig 4b), material collected (S + SW filled symbols vs  
372 PS + PSW empty symbols on Fig 4b), and the type of sample (combination of both site and material,  
373 represented with four ellipses on Fig 4b). However, the proteobacterial communities found on sand  
374 beaches (PS + S) vs coastal waters (PSW + SW) were similar (Fig 4b; Supplemental Table 5d).

375

376 3.3. Cultivable bacterial flora analysis and antimicrobial resistance

377 A dense cultivable bacterial flora was found on the microplastics from Reunion Island ( $3.13 \times$   
378  $10^7$  colony forming units (CFU) /g of microplastics on average, Fig. 5, Supplemental Table 6). The  
379 total cultivable flora was significantly denser on microplastics than in seawater ( $4.82 \times 10^2$  CFU/ml of  
380 water) or on sand ( $3.89 \times 10^4$  CFU/g of sand) whatever the studied site. On the contrary, no  
381 differences were found between the density of this culturable flora on microplastics collected on the  
382 east or the west coast, or between the seawater and the sand. The fraction of potentially pathogenic  
383 bacterial microflora was isolated on selective media: a total of 105 bacterial strains could be identified  
384 at the genus level using MALDI-TOF analysis: summarized data are presented in Supplemental Table  
385 7 while numeration data of these strains per genus are presented in Supplemental Table 8. Various  
386 genera belonging to the phylum Proteobacteria, Firmicutes and Bacillota were found on plastics,  
387 seawater, or sand. On plastics these culturable bacteria reach significantly higher abundances ( $10^3$  to  
388  $10^5$  CFU/g of plastic) than in seawater (1 CFU/ml of seawater) and on sand ( $10^2$  CFU/g of sand).  
389 Noteworthy, on plastics from both sites (East or West) and both matrices (seawater or sand), the most  
390 dominant bacterial genera were *Exiguobacterium* and *Pseudomonas* with several culturable bacteria  
391 scaling from  $10^3$  CFU/g for *Pseudomonas* to  $10^5$  CFU/g for *Exiguobacterium*. Acquired resistance  
392 profiles could be sorted for 16 of the 105 strains identified by MALDI-TOF (Table 3 and  
393 Supplemental Table 9a, b). Microplastics carried bacterial strains with AMR, including strains with  
394 non-intrinsic resistances to antibiotics. The main antibiotic resistances detected concerned  $\beta$ -lactams  
395 such as penicillin, ampicillin and ticarcillin. On plastics, the AMR potential pathogens detected were  
396 strains belonging to *Bacillus*, *Enterococcus*, *Pseudomonas* and *Pantoea* genera.

#### 397 4. Discussion

398 This study contributes to the knowledge of the health risk associated with the plastisphere in  
399 an oceanic region still very little documented.

##### 400 4.1 Reunion Island suffers from plastic pollution

401 Our observation shows that surface coastal waters around Reunion Island are polluted by plastic.  
402 Concentrations of  $10,693 \pm 1,275$  items/km<sup>2</sup> and  $4,025 \pm 4,760$  items/km<sup>2</sup> were measured on the West  
403 and East coasts respectively. Plastics pollution at Reunion Island may come from local plastic waste  
404 sources (Benard et al., 2022) but also from plastic marine debris from the Indian ocean subtropical



405 plastic gyre (Pattiaratchi et al., 2022), with these plastic debris mainly coming from Southeast Asia  
406 and carried by the south equatorial current (Thibault et al., 2023). These results are consistent with  
407 observations in most other seas and oceans (Thushari & Senevirathna, 2020). In addition, despite of  
408 the geographical isolation of Reunion Island in the Indian ocean, the data of the present study argue in  
409 favor the hypothesis that (i) marine plastic pollution is a global connected threat to all the world's  
410 oceans (Lincoln et al., 2022), and (ii) most scattered oceanic islands are threatened by such a pollution  
411 (Derraik et al., 2002).

412 However, the concentrations of these stranded plastics on the beaches of Reunion Island (0,35  
413 items/m<sup>2</sup> on the west coast and 0.0223 items/m<sup>2</sup> on the east coast) are lower than reported on other  
414 Indian Ocean islands such as the Chagos Archipelago (6 items/m<sup>2</sup>, Hoare et al., 2022) or the Maldives  
415 (35.8 items/m<sup>2</sup>, Imhof et al., 2017). Plastic abundance varies with environmental settings such as wind  
416 speed, swell intensity, marine currents velocity, seasonality, and the morphology of the island (Imhof  
417 et al., 2017). Reunion Island is a young volcanic island (Lenat et al., 2001) harboring few coral reefs  
418 and subject to oceanic swell and strong marine currents impacting the few beaches located on the east  
419 coast (Pous et al., 2014). Alternatively, the low plastic abundance may result from distinct oceanic  
420 influences as well as the remoteness of Reunion Island. The island is mostly under the influence of  
421 south Easterly trade winds and currents, and waters circulate from Western Australia to Reunion  
422 Island without colliding any important land mass (Schott et al., 2009). Concerning the nature of the  
423 plastic polymers found in Reunion Island, whether on the sea surface or in the sand of the beaches,  
424 polyethylene and polypropylene are the most abundant polymers. The dominance of these two  
425 polymers is hegemonic across the world's seas and oceans as reported in the meta-analysis by Erni-  
426 Cassola et al. (2019).

427

#### 428 4.2 Marine microplastic debris reaching Reunion Island host a specific microbiome

429 In the present study we found that a substantial proportion of up to 60% of the operational  
430 taxonomic units (OTUs) was shared between microplastics and their environment (seawater or sand  
431 beach). Seawater microplastic debris are colonized by planktonic microorganisms forming a biofilm  
432 whose composition presents expected high similarities with seawater microbiome (Zettler et al., 2013;

433 De Tender et al., 2017). Our observation highlights the colonizing role of bacterioplankton but also  
434 suggests a relatively new colonization (40% of OTUs are specific to plastics). Such renewal of  
435 microbiome composition is known as resulting from the oceanic environmental factors as temperature,  
436 salinity, light, and UV radiations: these factors affect the microbial biofilm composition and its  
437 renewal with an enrichment in pathogens (Sooriyakumar et al., 2022; Shan et al., 2022).

438 Similarly, plastic debris reaching beaches, after being introduced into the marine environment,  
439 undergo physical and biological processes that break them down into smaller fragments, which can  
440 eventually be incorporated as part of the beach sand (Napper & Thompson, 2020). Through this  
441 process, the plastic fragments and the sandy beach substrate can contribute to each other's bacterial  
442 enrichment, which the high proportion of shared OTUs may reflect. A recent study has shown that a  
443 high proportion of OTUs shared between plastic debris and sand samples suggests that plastic debris  
444 have a significant impact on microbial communities in marine sediment (Seeley et al., 2020).  
445 Nevertheless, there is still an important proportion of OTUs (up to 38%) that are specific to the plastic  
446 debris indicating that plastics are also a microbial ecological niche for specific communities compared  
447 to the communities from the environment. A recent review suggests that such specific communities  
448 result from biofilm formation and evolution processes, with an enrichment in bacteria able to use the  
449 plastic polymer as carbon source (Sooriyakumar, et al., 2022; Zhurina et al., 2022).

450

#### 451 4.3. Proteobacteria and Bacteroidota phyla dominate marine microplastic microbiome

452 The microbial community structure on Reunion Island's plastic pollution exhibits a notable  
453 dominance of Proteobacteria and Bacteroidota phyla across various sample types, including seawater-  
454 plastic, sand-plastic, seawater, and beach sand. These phyla, particularly Proteobacteria (75% average  
455 relative abundance) and Bacteroidota (11%), remain consistent irrespective of the substrate or site  
456 studied, reflecting their adaptability to diverse environmental conditions. Proteobacteria and  
457 Bacteroidota are diverse groups of bacteria known for their ability to adapt to a wide range of  
458 environmental conditions (Kertsters et al., 2006; Hahnke et al., 2016). They are commonly found in  
459 the oceans and their high abundance is well documented in seawater and marine sediments (Stal &

460 Cretiou, 2022). Our observation is consistent with other reports that also observed dominance of  
461 Proteobacteria and Bacteroidota phyla associated to plastic biofilm, e.g. Northern Europe seas,  
462 Mediterranean Sea and the Atlantic Ocean (Oberbeckmann et al., 2014; Vaksmaa et al., 2021, Debroas  
463 et al., 2017).

464 Within the dominant Proteobacteria phylum, specific dominant genera stand out. On seawater  
465 plastics, *Photobacterium* (33%), *Pseudoalteromonas* (27%), and *Psychrobacter* (18%) are highly  
466 abundant, comprising up to 78% of the Proteobacteria OTUs found on plastics. In surrounding  
467 seawater, dominant *Proteobacteria* genera, including *Candidatus* (24%), *Vibrio* (24%), *Alcanivorax*  
468 (18%), and *Alteromonas* (11%), collectively represent over 77% of the OTUs. These significant  
469 differences between Proteobacteria found on plastics and in surrounding waters suggest that marine  
470 microorganisms have adapted to plastics as a colonization surface (Roager & Sonnenschein, 2019).  
471 Moreover, several of these dominant Proteobacteria genera (*Photobacterium*, *Pseudoalteromonas*, and  
472 *Psychrobacter*) are known for their ability to biodegrade and utilize plastics as a carbon source and  
473 nutrient (Raghul et al., 2014; Muriel-Millán et al. 2021; Atanasova et al., 2021). This presence on  
474 seawater plastics indicates that the plastics reaching Reunion Island have likely undergone degradation  
475 during prolonged floating, in line with the concept of plastic debris transporting microorganisms as  
476 suggested by Pattiaratchi et al. (2022).

477 The diversity and abundance of Proteobacterial OTUs on plastics and sand from the East coast  
478 resemble each other, with dominant genera such as *Vibrio*, *Pseudoalteromonas*, and *Alteromonas*,  
479 suggesting a role for plastics in transporting and enriching the sand bacterial community. In contrast,  
480 on the West coast, the Proteobacteria community structure significantly differs between sand plastics,  
481 dominated by *Psychrobacter* (>80%), and sandy beach sand, where the Proteobacteria community is  
482 led by the genus *Dyella* (>70%). This variation may be linked to the specific types of polymers present  
483 on the plastics reaching each coast, with *Psychrobacter* known for its biodegradation of polyurethane  
484 and polycaprolactone polyester (PCL) (Atanasova et al., 2021a). The dominance of *Dyella* in beach  
485 sand may indicate residual wastewater contamination from forest or cultivated soils, where *Dyella* is  
486 primarily isolated (Dar et al., 2020; Huang et al., 2021).

487  
488

#### 4.4 Cultivable bacteria isolated from marine microplastic debris

489           The study of cultivable bacteria isolated from marine microplastic debris reveals significant  
490 microbial activity, with an average concentration of  $3 \times 10^7$  colony forming units (CFU)/g of plastic.  
491 Notably, this demonstrates that microplastic serve as an ecological niche where bacteria remain  
492 metabolically active and cultivable. Comparatively, recent research found that plastic microbeads  
493 could harbour up to  $60 \times 10^{10}$  CFU/cm<sup>2</sup> of plastic (Türetken et al., 2020). In the present study at  
494 Reunion Island, the number of cultivable bacteria on plastics exceeded those in beach sediments by  
495 800 times and coastal waters by up to  $65 \times 10^3$  times.

496           Similarly, potential pathogens cultivable bacteria were significantly more abundant on plastic  
497 debris (ranging from  $10^3$  to  $10^5$  CFU/g of plastic) than in surrounding environments like seawater or  
498 sediment (1 to 1000 CFU/g of sand or ml of seawater). This is consistent with Wu et al. (2019) work  
499 that showed higher abundance of pathogenic bacterial families on microplastics, compared to  
500 substrates. The most encountered potentially pathogenic cultivable bacterial strains belong to the  
501 *Bacillus*, *Exiguobacterium*, and *Pseudomonas* genera, raising concerns about the potential  
502 transmission of infectious pathogens to humans or animals (Bergan, 1981; Mandic-Mulec et al., 2015;  
503 Chen et al., 2017). Remarkably, many of these potentially pathogenic bacteria also possess the  
504 capacity to degrade plastics, exemplified by the genus *Pseudomonas*, which is both abundant (up to  
505  $10^5$  CFU/g of plastic) and proficient in plastic degradation (Wilkes & Aristilde, 2017).

506

#### 507 4.5 AMRs bacteria

508 Marine plastics have been shown to enrich the plastisphere with pathogens, including genera like  
509 *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Aeromonas*, and the Enterobacterales family, many of which  
510 have acquired resistance to antibiotics (Junaid et al., 2022). This poses a significant threat to public  
511 health and the environment (Marathe & Bank, 2022). In the Western Indian Ocean, this study  
512 represents one of the first reports of isolating cultivable pathogens from plastics for potential  
513 antimicrobial susceptibility testing (AST). Resistance to antibiotics was observed in strains of  
514 *Bacillus*, *Enterococcus*, *Pseudomonas*, and *Vibrio* isolated from Reunion Island's seawater or sandy

515 beaches. The majority of observed resistance was directed against molecules in the  $\beta$ -lactam family,  
516 such as ampicillin, penicillin, and ticarcillin. This is consistent with recent findings that residual beta-  
517 lactams, including ampicillin, are common emerging pollutants that chemically adsorb onto plastic,  
518 promoting resistance among the plastisphere's bacterial community (Imran et al., 2019; Wang et al.,  
519 2021). Furthermore, beta-lactam resistance genes (*bla* genes family) are often associated with mobile  
520 genetic elements (MGEs), facilitating horizontal gene transfer within the plastisphere (Wang et al.,  
521 2021; Zhang et al., 2022; Silva et al., 2023). This data, collected in a high-income country in the  
522 Southwest Indian Ocean, underscores that microplastic debris serves as a reservoir of potentially  
523 antibiotic-resistant pathogens (AMR), aligning with similar reports from around the world (Yang et  
524 al., 2019; Bowley et al., 2021; Liu et al., 2021). Importantly, this should be viewed from a One Health  
525 perspective, acknowledging that highly anthropized coastal environments can be contaminated by  
526 enteric bacteria from human or animal sources, potentially including MDR bacteria, based on regional  
527 epidemiology (Fernandes et al., 2020; Miltgen et al., 2022).

528

## 529 5. Conclusions

530 In this study, we presented a first case of the plastisphere assessment from Reunion Island, a  
531 remote oceanic island located in the Southwest Indian Ocean, polluted by plastic debris from various  
532 geographical origins. The characterization of plastic pollution in the island's coastal waters and  
533 beaches indicates that Reunion Island is facing plastic pollution with up to 10,000 objects/km<sup>2</sup> in the  
534 coastal waters, mainly consisting of polyethylene (up to 75%) and polypropylene (up to 25%). Plastic  
535 debris host dense microbiomes, dominated by Proteobacteria (80%). In addition, the cultivable  
536 microbiotes reached 10<sup>9</sup> CFU/g of microplastics, with a dominance of bacteria from genera  
537 *Exiguobacterium* (10<sup>5</sup> CFU/g of plastic) and *Pseudomonas* (10<sup>3</sup> CFU/g of plastic). This plastic debris  
538 also carries  $\beta$ -lactam resistant AMR bacteria such as certain strains of the genera *Bacillus*,  
539 *Enterococcus* and *Pantoea* resistant to ampicillin, penicillin and ticarcillin. Overall, our results  
540 confirm, as it has already been described for other islands in other oceans and seas, that the islands of  
541 the Indian Ocean are facing severe marine plastic pollution, the debris of which host a dense

542 plastisphere including AMR bacteria. Our data also suggests potential risks associated with a plastic-  
543 specific microbiome for Southwest Indian Ocean socio-ecosystems.

544

545 6. The following list is the Supplemental data related to this article.

546 Supplemental Fig. 1: Diagram of NGS data analysis process.

547 Supplemental Fig. 2a, b, c, d: Alpha rarefaction (Chao1) curves showing the observed sampling  
548 effort. (a) East coast Plastic Sea water (PSW) + Sea water (SW); (b) East coast Plastic Sand (PS) +  
549 Sand (S)

550 Supplemental Fig 3: Proteobacteria OTUs abundances according to genera. The full list of OTUs  
551 repartition according to the Proteobacteria genera including the rare Proteobacteria genera (<1%) is  
552 reported as an Excel file in Supplemental Table 3.

553 Supplemental Table 1: Plastic debris collection number by sample according to site and environmental  
554 parameters (seawater or sand beach) and polymer IFTR identification frequency per sample.

555 Supplemental Table 2a, b, c: NGS full data: number of total reads and OTUs per sample including  
556 means and standard deviation calculations. Two-way Anovas were carried out to compare OTUs  
557 abundances data by site (a) or substrate (b) or samples (c) and letters indicate significantly different  
558 means according to a test of Duncan (at  $p < 0.05$ ). Abbreviations: S: sand; SW: sea water; PSW:  
559 plastic from sea water; PS: plastic from sand.

560 Supplemental Table 3: Excel file presenting the full taxonomic list of the 1084 identified OTUs and  
561 their sample distribution at Phylum level and Proteobacteria genera level.

562 Supplemental Table 4a: Diversity indexes of the bacterial phylum distribution according to the site  
563 and the substrate. Data are mean  $\pm$  standard errors. Letters indicate significantly different means,  
564 according to a post-hoc Dunn's multiple comparison test (at  $p < 0.05$ ).

565 Supplemental Table 4b: Total sample OTUs number and distribution per Phylum. Abbreviations: S:  
566 sand; SW: sea water; PSW: plastic from sea water; PS: plastic from sand.

567 Supplemental Table 4c: Phylum OTUS distribution Chi2 test results and associated p.values.

568 Supplemental Table 4d: Analysis of similarity tests (ANOSIM) results for phylum. The groups tested  
569 are i) the site (East or West), ii) the matrix (sand beach or sea water), iii) the material harvested (sand,

570 water, or plastic); and iv) the sample type (combination of site and material). The results are given as  
571 ANOSIM R values and its significance.

572 Supplemental Table 5a: Diversity indexes of the Proteobacteria genus distribution according to the site  
573 and the substrate. Data are means  $\pm$  standard errors. Letters indicate significantly different means,  
574 according to a post-hoc Dunn's multiple comparison test (at  $p < 0.05$ ).

575 Supplemental Table 5b: Total sample OTUs number and distribution per Proteobacteria genus of  
576 which frequency was  $>1\%$ . Abbreviations: S: sand; SW: sea water; PSW: plastic from sea water; PS:  
577 plastic from sand.

578 Supplemental Table 5c: Proteobacteria genera (15 genera  $> 1\%$ ) distribution Chi2 test results and p.  
579 values.

580 Supplemental Table 5d: Analysis of similarity tests (ANOSIM) results for Proteobacteria genera  $>1\%$ .  
581 The groups tested are (i) the site (East or West), (ii) the matrix (sand beach or sea water), (iii) the  
582 material harvested (sand, water or plastic) and (iv) the sample type (combination of site and material).  
583 The results are given as ANOSIM R values and its significance.

584 Supplemental Table 6: Total culturable bacterial flora in CFU / g or ml of substrate, Abbreviations:  
585 PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach  
586 sand, Values are means  $\pm$  standard deviation of means ( $n = 3$ ) of three independent samplings, A two-  
587 anova was carried out and the different letters indicate significant differences as determined by Tukey  
588 HSD test ( $p \leq 0.05$ ).

589 Supplemental Table 7: Excel file presenting the list of the 105 cultivable bacterial strains isolated  
590 from selective media and identified at genus level by MaldiTof.

591 Supplemental Table 8a: Numbering of culturable bacterial strains according to the genus identified by  
592 Maldi Tof and expressed as CFU per g or ml of substrate *i.e.* plastic from sea water (PSW), plastic  
593 from sand beach (PS), sea water (SW) or sand beach (S) and associated literature indicating the  
594 bacterial genus potential pathogenicity and plastic degradation capacities.

595 Supplemental Table 8b: List of the bibliographic references of the Supplemental Table 8a.

596 Supplemental Table 9a and b: Full antibiograms data. Antibiograms were carried out according to  
597 antibiotic specific spectrum related to the bacterial genus tested. From 8 to 16 ATB were tested

598 according to the specific ATBiograms carried out to test the bacterial strains genus. In bold: ATB  
599 resistance, in non-bold: ATB sensitivity.

600

601 Data accessibility: NGS raw data 16SrDNA sequences are deposited in zenodo data bank:  
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603

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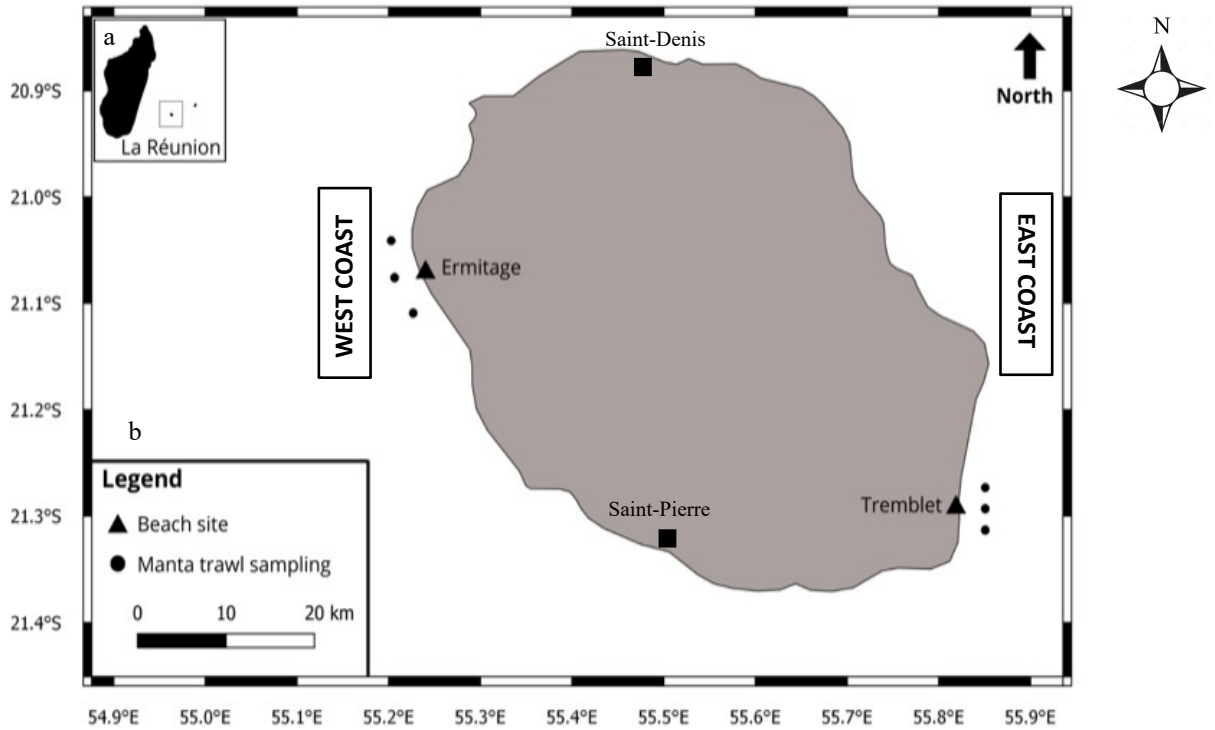
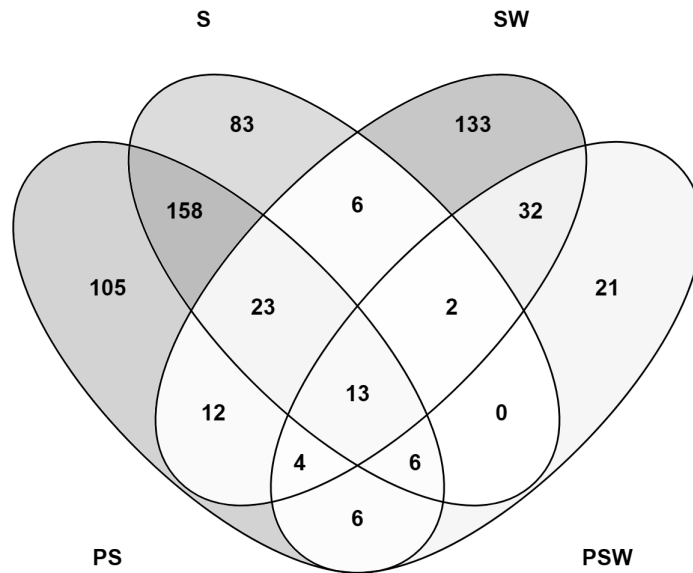


Fig. 1.(a) Location map of Reunion island in the southwest Indian Ocean close to Madagascar and Mauritius island; (b) Map of the Reunion island including the two major cities and the study site locations.

a East Coast : (604 OTUs)



b West Coast : (835 OTUs)

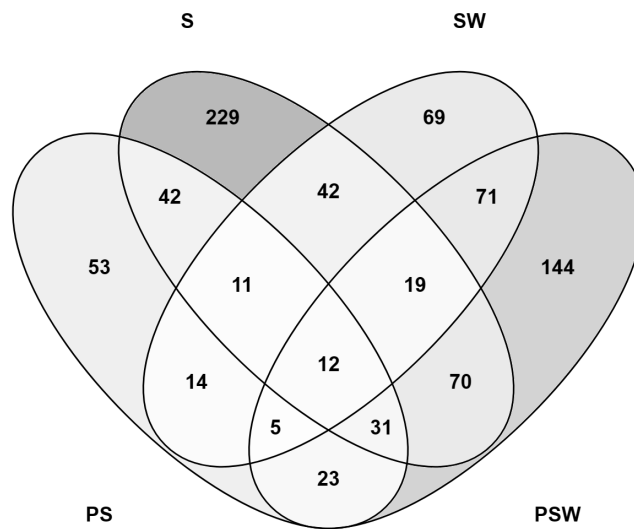
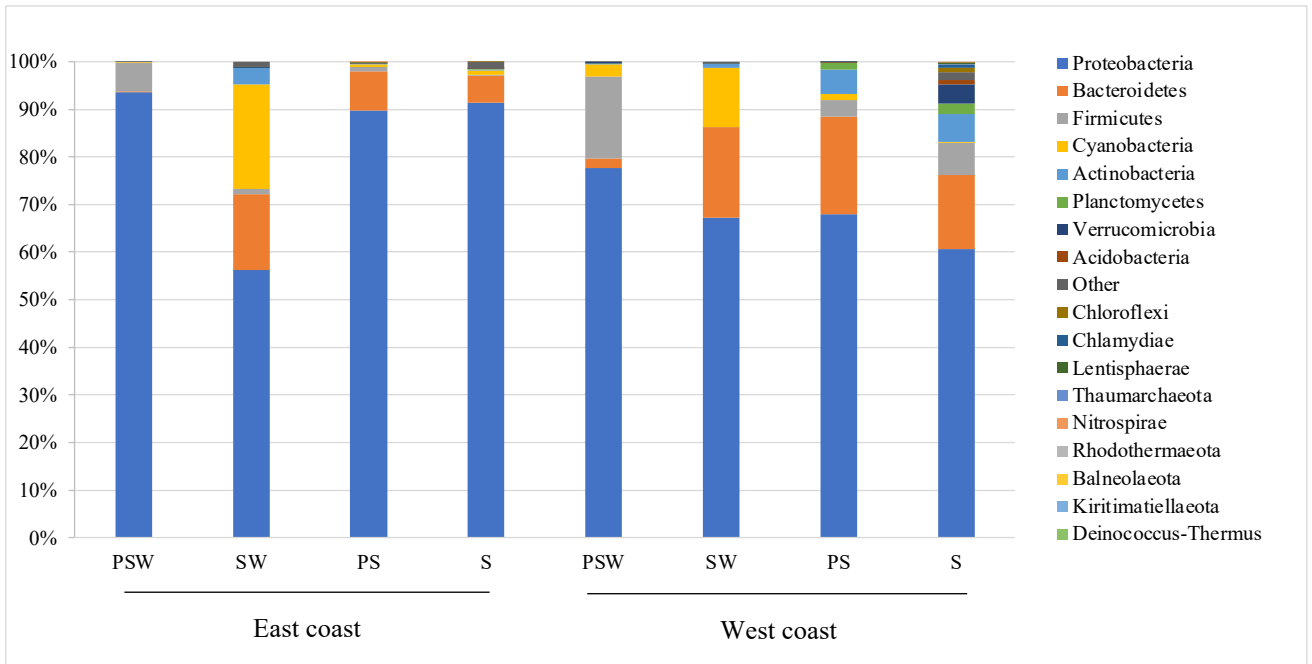


Fig. 2. Venn diagrams of the Operational Taxonomic Unit (OTUs) distribution showing shared and specific OTUs according to site *i.e.* East coast (a) or West coast (b) in all sample types *i.e.* PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand.



a

Bacterial phylum OTUs relative abundance



b

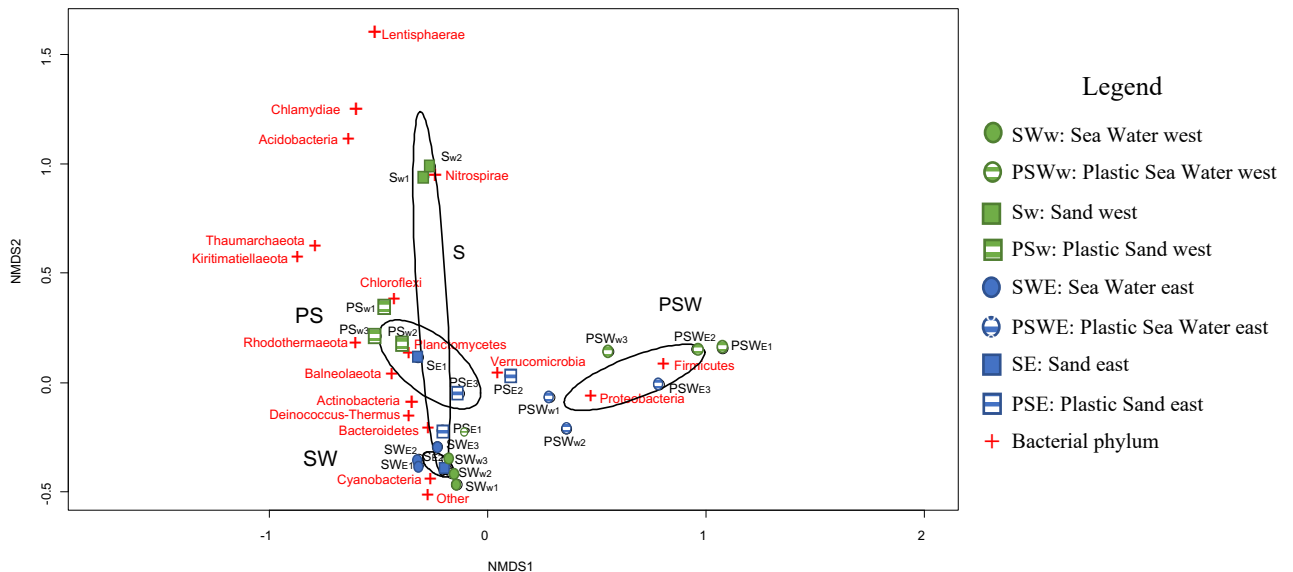
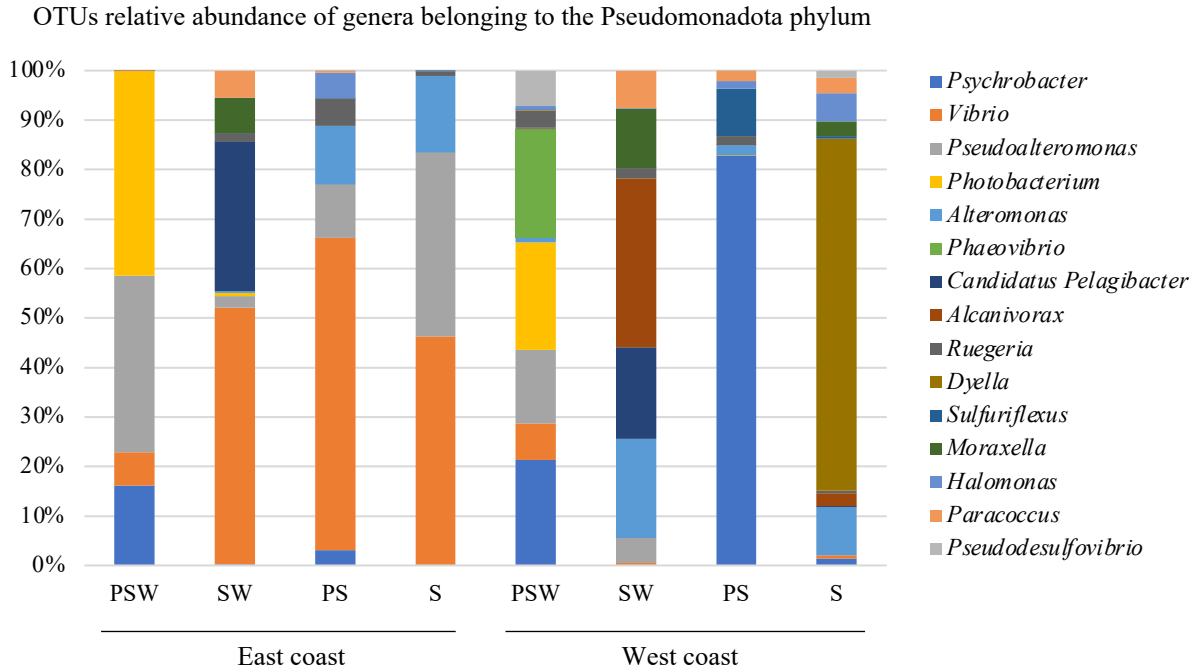


Figure 3 : OTUs distribution of the bacterial community according to the analysis at phylum level for the different substrates: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of bacterial phyla identified in all samples. (b) Two-dimensional NMDS ordination of bacterial community structure. Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95%) per substrate.

a



b

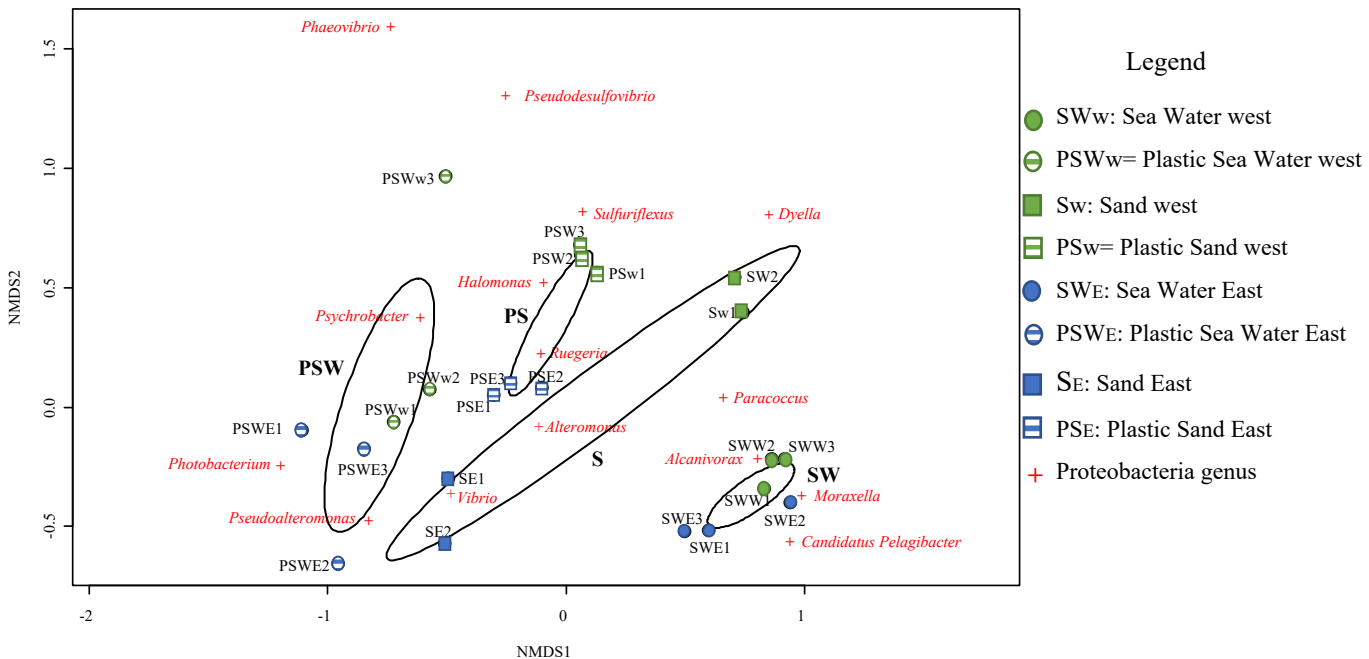


Figure 4 : OTUs distribution of the phylum of the Proteobacteria according to the analysis at genera level for the different substrates: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of Proteobacteria genus identified in all samples. (b) Two-dimensional NMDS ordination of Proteobacterial community structure. Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95%) per substrate.

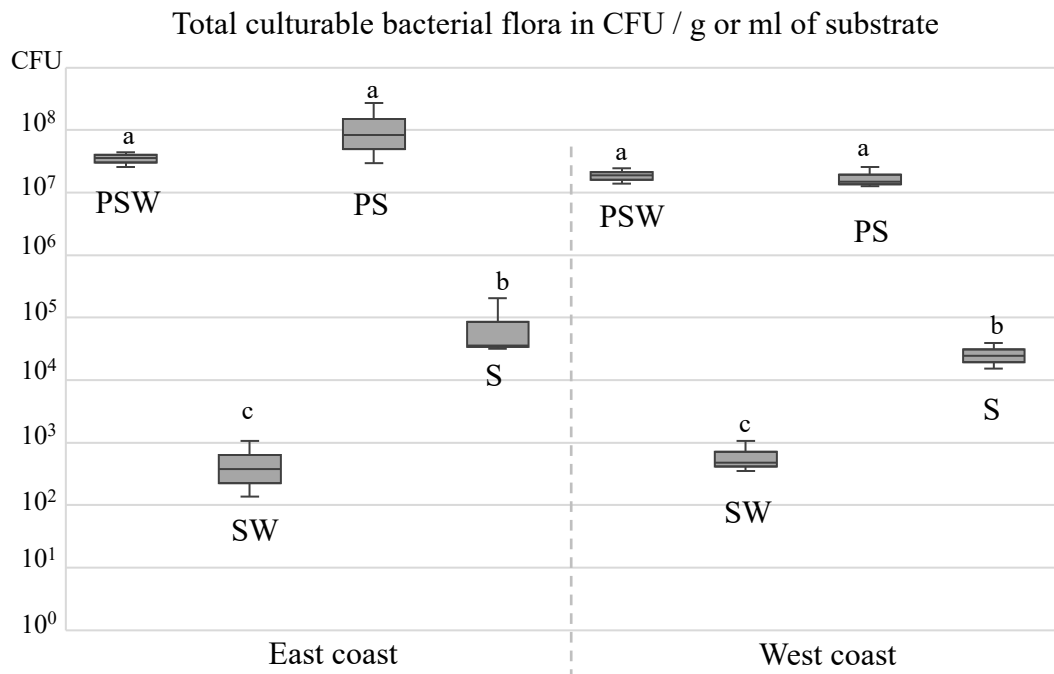


Figure 5 : Total culturable bacterial flora in CFU / g or ml of substrate. Abbreviations: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. Plots represent means, and error bars represent standard deviation of means (n = 3) of three independent samplings. The different letters above plots indicate significant differences as determined by Tukey HSD test ( $P \leq 0.05$ ).

Table 1: Quantification and characterization of plastic debris collected on East coast and West coast, in seawater or on sand beach. Except for plastic polymer nature, all data are means  $\pm$  stdv of samples (n = 3) / site and substrate collection.

Site		East		West	
Substrate		Seawater	Sand beach	Seawater	Sand beach
Plastic item concentration (mean $\pm$ stdv)		4,025 $\pm$ 4,760 item/km <sup>2</sup>	0.022 $\pm$ 0.008 item/m <sup>2</sup>	10,693 $\pm$ 11,275 item/km <sup>2</sup>	0.34 $\pm$ 0.31 item/m <sup>2</sup>
Weight of plastic item in mg / item (mean $\pm$ stdv)		1.4 $\pm$ 1.3	31.4 $\pm$ 51.2	2.6 $\pm$ 7.4	56.5 $\pm$ 43.2
Weight of microplastic in mg / sample (mean $\pm$ stdv)		24.7 $\pm$ 0.1	202 $\pm$ 90	46.7 $\pm$ 0.7	1,798 $\pm$ 110
Plastic polymer (%)	Polyethylene	75	70	84	50
	Polypropylene	25	29	16	38
	Polystyrene	0	1	0	9
	Polyvinyl Chloride	0	0	0	3

Table 2: Diversity indexes of the marine bacterial phyla and Proteobacteria genera found according to the combination of the site, the substrate, and the presence of plastic. Data are pooled according to three parameters: the study site (East or West), the type of substrate (Sea Water or Sand) and the presence of plastic (Plastic or non-plastic substrate i.e., Seawater or Sand). Data are reported as means  $\pm$  standard errors. Letters indicate significantly different means according to a test of Wilcoxon–Mann–Whitney (at  $P < 0.05$ ). Abbreviations: S: sand; SW: sea water; PSW: plastic from sea water; PS: plastic from sand. Full data of the diversity indexes per site and per substrate are presented in supplemental table 3.

Parameters	Groups	Richness		Shannon diversity		Simpson diversity	
		Phylum	Proteobacteria	Phylum	Proteobacteria	Phylum	Proteobacteria
Site	East (S + SW + PSW)	9.8 $\pm$ 3.1 a	8.9 $\pm$ 0.6 a	2.7 $\pm$ 0.8 a	1.05 $\pm$ 0.06 a	2.0 $\pm$ 0.5 a	0.53 $\pm$ 0.03 a
	West (S + SW + PSW)	8.4 $\pm$ 2.7 a	10.3 $\pm$ 0.4 a	1.9 $\pm$ 0.9 b	1.21 $\pm$ 0.11 a	1.6 $\pm$ 0.7 b	0.56 $\pm$ 0.06 a
Substrate	Sand (S + PS)	11.8 $\pm$ 1.8 a	10 $\pm$ 0.6 a	2.4 $\pm$ 1.1 a	1.00 $\pm$ 0.08 a	1.7 $\pm$ 0.6 a	0.48 $\pm$ 0.04 a
	Water (W + PS)	6.9 $\pm$ 1.3 b	9.3 $\pm$ 0.5 a	2.2 $\pm$ 0.8 a	1.24 $\pm$ 0.09 a	1.8 $\pm$ 0.6 a	0.61 $\pm$ 0.04 b
Plastic	Plastic (PSW + PS)	8.6 $\pm$ 2.8 a	9.7 $\pm$ 0.6 a	1.9 $\pm$ 0.6 b	1.03 $\pm$ 0.09 a	1.5 $\pm$ 0.4 b	0.51 $\pm$ 0.05 a
	Non-Plastic (SW or S)	9.8 $\pm$ 3.0 a	9.5 $\pm$ 0.6 a	2.8 $\pm$ 1.0 a	1.24 $\pm$ 0.09 a	2.1 $\pm$ 0.7 a	0.60 $\pm$ 0.04 a

Table 3: Antibiotic multiresistances detected among the culturable bacterial strains. In bold are noticed non-natural antibiotic resistances. Abbreviations: PSW : plastic from sea water; PS : plastic from sand beach; SW : sea water; S : sand beach; TS: Trimethoprim/ sulfamethoxazole; PT: Piperacilin/ Tazobactam; AC: Amoxicillin / Clavulanic acid.

Site	Substrate	Bacterial strain MT code	Bacterial genus	Antibiotic Resistances			
				ATB1	ATB2	ATB3	ATB4
East	PSW	T2-14	<i>Pantoea</i>	Ampicillin	Cefadroxil	<b>Ticarcillin</b>	
		T3-42	<i>Pseudomonas</i>	TS			
	SW	T5-26	<i>Staphylococcus</i>	<b>Erythromycin</b>	<b>Penicillin</b>		
		T4-54	<i>Bacillus</i>	Amoxicillin	<b>Imipenem</b>	Penicillin G	<b>Vancomycin</b>
		T4-52	<i>Vibrio</i>	<b>Ampicillin</b>			
	PS	A1-2	<i>Pseudomonas</i>	<b>Ticarcillin</b>	TS		
		A2-15	<i>Bacillus</i>	Amoxicillin	<b>Imipenem</b>	<b>Penicillin G</b>	
		A1-13	<i>Vibrio</i>	<b>Ampicillin</b>			
	S	A4-9	<i>Bacillus</i>	Amoxicillin	<b>Penicillin G</b>		
		A6-6	<i>Enterobacter</i>	Ampicillin	AC	Cefalexin	
West	PSW	MEEP141	<i>Pseudomonas</i>	TS			
	SW	E5-28	<i>Pseudomonas</i>	TS			
		E4-13	<i>Bacillus</i>	Amoxicillin	<b>Clindamycin</b>		
	PS	B1-3	<i>Enterococcus</i>	<b>Rifampicin</b>	Vancomycin		
	S	B6-14	<i>Aeromonas</i>	<b>Piperacilin</b>	<b>Ticarcilin</b>	<b>Ticarcilin / Clavulanic acid</b>	<b>PT</b>
		B5-17	<i>Staphylococcus</i>	<b>Clindamycin</b>	<b>Fusidic acid</b>	<b>Penicillin</b>	