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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.

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4 Author names: Loik Sababadichetty^{1,2a}, Guillaume Miltgen^{2,3a}, Bryan Vincent^{4a}, François
5 Guilhaumon^{5a}, Veronique Lenoble⁶, Margot Thibault^{1,7,8}, Sophie Bureau¹, Pablo Tortosa³, Thierry
6 Bouvier⁹ and Philippe Jourand⁵,*.

7

8 Authors affiliations :

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

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

13 ³Université 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 ⁴CIRAD, UMR040 LSTM, Campus Agro Environnemental Caraïbe, BP 214-97285, Cedex 2 le
17 Lamentin, Martinique, Antilles Françaises, France

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

20 ⁶Université de Toulon, Aix Marseille Université, CNRS, IRD, UMR MIO, 83 Toulon, France

21 ⁷The Ocean Cleanup, Rotterdam, The Netherlands

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

24 ⁹UMR 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

28 ^aThese 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/km² 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 10⁷ 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/m³ in water and 8766 particles/m³ 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 1st site is heavily impacted by local anthropogenic activities (Tourrand et al., 2013; Guigue et al.,
139 2015; Lemahieu et al., 2017) while the 2nd 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 μm ; mouth area: 1.125 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/ 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 μm membrane filter resuspension using
229 the reconstituted sterile seawater. The total bacterial flora was counted by inoculating 100 μl 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 cm^{-1}
255 (resolution of 1 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 Chi² 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 / km² on the west coast and from 3,561 to 23,692 items / km² 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 ± 0.31 and 0.022 ± 0.008 item/m² 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 Firmicutes (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 χ^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 (4.82×10^2 CFU/ml of
380 water) or on sand (3.89×10^4 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 β -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/km² and $4,025 \pm 4,760$ items/km² 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/m² on the west coast and 0.0223 items/m² on the east coast) are lower than reported on other
414 Indian Ocean islands such as the Chagos Archipelago (6 items/m², Hoare et al., 2022) or the Maldives
415 (35.8 items/m², 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 3×10^7 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 60×10^{10} CFU/cm² 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 65×10^3 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 pathogens 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 β -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/km² 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 10⁹ CFU/g of microplastics, with a dominance of bacteria from genera
537 *Exiguobacterium* (10⁵ CFU/g of plastic) and *Pseudomonas* (10³ CFU/g of plastic). This plastic debris
538 also carries β -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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608

609 8. References

610 Almeida et al., 2019. M.D. Almeida, S. Sousa, J.S. Rebelo, L. Vales, K. Schifferegger, C.M.M.
611 Almeida. Sand treatment procedures for chemical analysis and beach sand quality. *Environ. Eng.*
612 *Manag. J.*, 18 (1) (2019), pp. 171-183, 10.30638/eemj.2019.017

613

614 Arias-Andres et al., 2018. M. Arias-Andres, U. Klümper, K. Rojas-Jimenez, P.-H. Grossart.
615 Microplastic pollution increases gene exchange in aquatic ecosystems. *Environ. Pollut.*, 237 (2018),
616 pp. 253-261, 10.1016/j.envpol.2018.02.058

617

618 Benard & Malet-Damour 2022. F. Benard, B. Malet-Damour. Assessing potential of plastic waste
619 management policies for territories sustainability: case study of Reunion Island. *World Development*
620 *Sustainability*, 1 (2022), 100030, 10.1016/j.wds.2022.100030

621

622 Besley et al., 2016. A. Besley, M.G. Vivjer, P. Behrens, T. Bosker. A standardized method for
623 sampling and extraction methods for quantifying microplastics in beach sand. *Mar. Pollut. Bull.*, 114
624 (1) (2016), pp. 77-83, 10.1016/j.marpolbul.2016.08.055

625

626 Bhuyan et al., 2023. S. Bhuyan, M. Yadav, S.J. Giri, S. Begum, S. Das, A. Phukan, P. Priyadarshani,
627 S. Sarkar, A. Jayswal, K. Kabyashree, A. Kumar, M. Mandal, S.K. Ray. Microliter spotting and
628 micro-colony observation: A rapid and simple approach for counting bacterial colony forming units. *J.*
629 *Microbiol. Met.*, 207 (2023), 106707, 10.1016/j.mimet.2023.106707
630
631 Bowley et al., 2021. J. Bowley, C. Baker-Austin, A. Porter, R. Hartnell, C. Lewis. Oceanic
632 hitchhikers—assessing pathogen risks from marine microplastic. *Trends Microbiol.*, 29 (2021), pp. 107-
633 116, 10/1016/j.tim.2020.06.011
634
635 Bukin et al., 2019. Y.S. Bukin, Y.P. Galachyants, I.V. Morozov, S.V. Bukin, A.S. Zakharenko, T.I.
636 Zemskaya. The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Sci.*
637 *Data*, 6 (1) (2019), p. 190007, 10.1038/sdata.2019.7
638
639 Caporaso et al., 2010. J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K.
640 Costello, N. Fierer, A.G. Pena, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D.Knights, J.E.
641 Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky,
642 P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight. QIIME allows
643 analysis of high-throughput community sequencing data. *Nat. Methods*, 7 (5) (2010), pp. 335-336,
644 10.1038/nmeth.f.303
645
646 Carbery et al., 2018. M. Carbery, W. O'Connor, T. Palanisami. Trophic transfer of microplastics and
647 mixed contaminants in the marine food web and implications for human health. *Environ Int*, 115
648 (2018), pp. 400-409, 10.1016/j.envint.2018.03.007
649
650 Chao et al., 2016. A. Chao, K.H. Ma, T.C. Hsieh. iNEXT Online: Software for Interpolation and
651 Extrapolation of Species Diversity. Program and User's Guide published at
652 http://chao.stat.nthu.edu.tw/wordpress/software_download/inextonline/
653

654 Chen et al., 2018. S. Chen, Y. Zhou, Y. Chen, J. Gu. Fastp: an ultra-fast all-in-one FASTQ
655 preprocessor. *Bioinformatics*, 34 (17) (2018), pp. i884–i890, 10.1093/bioinformatics/bty560
656

657 Chouchene et al., 2023. K. Chouchene, J. Pinto da Costa, M. Chamkha, M. Ksibi, S. Sayadi. Effects of
658 microplastics' physical and chemical properties on aquatic organisms: state-of-the-art and future
659 research trends. *TrAC Trends Anal. Chem.*, 166 (2023), p. 117192, 10.1016/j.trac.2023.117192
660

661 Cole et al., 2013. M. Cole, P. Lindeque, E. Fileman, C. Halsband, R. Goodhead, J. Moger, T.S.
662 Galloway Microplastic ingestion by zooplankton. *Environ. Sci. Technol.*, 47 (2013), pp. 6646-6655,
663 10.1021/es400663f
664

665 Dar et al., 2020. D. Dar, L.S. Thomashow, D.M. Weller, D.K. Newman. Global landscape of
666 phenazine biosynthesis and biodegradation reveals species-specific colonization patterns in
667 agricultural soils and crop microbiomes. *eLife*, 9 (2020), p. e59726, 10.7554/eLife.59726
668

669 Debeljak et al., 2017. P. Debeljak, M. Pinto, M. Proietti, J. Reisser, F.F. Ferrari, B.Abbas, M.C. van
670 Loosdrecht, B. Slat, G.J. Herndl. Extracting DNA from ocean microplastics: a method comparison
671 study. *Anal. Methods*, 9 (9) (2017), pp. 1521-1526, 10.1039/C6AY03119F
672

673 De Tender et al., 2017. C. De Tender, L.I. Devriese, A. Haegeman, S. Maes, J. Vangeyte, A.
674 Cattrijsse, P. Dawyndt, T. Ruttink. Temporal Dynamics of Bacterial and Fungal Colonization on
675 Plastic Debris in the North Sea. *Environ. Sci. Technol.*, 51 (13) (2017), pp. 7350-7360,
676 10.1021/acs.est.7b00697
677

678 Debroas et al., 2017. D. Debroas, A. Mone, A. Ter Halle. Plastics in the North Atlantic garbage patch:
679 A boat-microbe for hitchhikers and plastic degraders. *Sci. Total Environ.*, 599-600 (2017), pp. 1222-
680 1232, 10.1016/j.scitotenv.2017.05.059
681

682 Derraik, 2002. J.G.B. Derraik. The pollution of the marine environment by plastic debris: a review.
683 Mar. Pollut. Bull., 44 (9) (2002), pp. 842-852, 10.1016/s0025-326x(02)00220-5
684
685 Dixon, 2003. Dixon P. VEGAN, a package of R functions for community ecology. J. Veg. Sci., 14
686 (2003), pp. 927-930, 10.1111/j.1654-1103.2003.tb02228.x
687
688 Djaoudi et al., 2022. K. Djaoudi, J.A. Tesán Onrubia, A. Boukra, L. Guesnay, A. Portas, R. Barry-
689 Martinet, B. Angeletti, S. Mounier, V. Lenoble, J.-F. Briand. Seawater copper content controls biofilm
690 bioaccumulation and microbial community on microplastics. Sci. Total. Env., 814 (2022), p. 152278,
691 10.1016/j.scitotenv.2021.152278
692
693 Erni-Cassola et al., 2019. G. Erni-Cassola, V. Zadjelovic, M.I. Gibson, J.A. Christie-Oleza.
694 Distribution of plastic polymer types in the marine environment; A meta-analysis. J. Hazard. Mater.,
695 369 (2019), pp. 691-698, 10.1016/j.hazmat.2019.02.067
696
697 Fernández-Bravo & Figueras, 2020. A. Fernández-Bravo, M.J. Figueras. An Update on the Genus
698 *Aeromonas*: Taxonomy, Epidemiology and Pathogenicity. Microorganisms, 8 (1) (2020), p. 129,
699 10.3390/microorganisms8010129
700
701 Galloway et al., 2017. T.S. Galloway, M. Cole, C. Lewis. Interactions of microplastic debris
702 throughout the marine ecosystem. Nat. Ecol. Evol., 1 (5) (2017), p. 116, 10.1038/s41559-017-0116
703
704 GESAMP (2019). Guidelines on the monitoring and assessment of plastic litter and microplastics in
705 the ocean (Kershaw P.J., Turra A. and Galgani F. editors),
706 (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on
707 the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 99, 130p.
708

709 Guigue et al., 2015. C. Guigue, L. Bigot, J. Turquet, M. Tedetti, N. Ferretto, M. Goutx, P. Cuet.
710 Hydrocarbons in a coral reef ecosystem subjected to anthropogenic pressures (La Réunion Island,
711 Indian Ocean). *Environ. Chem.*, 12 (3) (2015), pp. 350-365, 10.1071/EN14194.
712
713 Hahnke et al., 2016. R.L. Hahnke, J.P. Meier-Kolthoff, M. García-López, S. Mukherjee, M.
714 Huntemann, N.N. Ivanova, T. Woyke, K.C. Kyrpides, H.-P. Klenk, M. Göker. Genome-Based
715 Taxonomic Classification of Bacteroidetes. *Front. Microbiol.*, 7 (2016), p. 2003,
716 10.3389/fmicb.2016.02003
717
718 Hinlo et al., 2017. R. Hinlo, D. Gleeson, M. Lintermans, E. Furlan. Methods to maximise recovery of
719 environmental DNA from water samples. *PLoS One*, 12 (6) (2017), pp. e0179251,
720 10.1371/journal.pone.0179251
721
722 Hoare et al., 2022. V. Hoare, N. Atchison Balmond, G.C. Hays, R. Jones, H. Koldewey, J.O. Laloë, E.
723 Levy, F. Llewellyn, H. Morrall, N. Esteban. Spatial variation of plastic debris on important turtle
724 nesting beaches of the remote Chagos Archipelago, Indian Ocean. *Mar. Pollut. Bull.*, 181 (2022), p.
725 113868, 10.1016/j.marpolbul.2022.113868
726
727 Huang et al., 2021. T. Huang, J.-C. Fu, S.-H. Dong, Q.-M. Zhang, T.T. Wu, L.-H. Qiu. *Dyella telluris*
728 sp. nov. and *Dyella acidiphila* sp. nov., isolated from forest soil of Dinghushan Biosphere Reserve,
729 China. *Int. J. Syst. Evol. Microbiol.*, 71 (9) (2021), p. 004985, 10.1099/ijsem.0.00498
730
731 Imhof et al., 2017. H.K. Imhof, R. Sigl, E. Brauer, S. Feyl, P. Giesemann, S. Klink, K. Leupolz,
732 M.G.J. Löder, L.A. Löscher, J. Missun, S. Muszynski, A.F.R.M. Ramsperger, I. Schrank, S. Speck, S.
733 Steibl, B. Trotter, I. Winter, C. Laforsch. Spatial and temporal variation of macro-, meso- and
734 microplastic abundance on a remote coral island of the Maldives, Indian Ocean. *Mar. Pollut. Bull.*,
735 116 (1) (2017), pp. 340-347, 10.1016/j.marpolbul.2017.01.010
736

737 Imran et al., 2019. M. Imran, R.K. Das, M.M. Naik. Co-selection of multi-antibiotic resistance in
738 bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat.
739 Chemosphere, 215 (2019), pp. 846-857, 10.1016/j.chemosphere.2018.10.114
740

741 Jacquin et al., 2019. J. Jacquin, J. Cheng, C. Odobel, C. Pandin, P. Conan, M. Pujo-Pay, V. Barbe, A.-
742 L. Meistertzheim, J.-F. Ghiglione. Microbial Ecotoxicology of Marine Plastic Debris: A Review on
743 Colonization and Biodegradation by the “Plastisphere”. Front. Microbiol., 10 (2019), p. 865,
744 10.3389/fmicb.2019.00865
745

746 Junaid et al., 2022. M. Junaid, J.A. Siddiqui, M. Sadaf, S. Liu, J. Wang. Enrichment and dissemination
747 of bacterial pathogens by microplastics in the aquatic environment. Sci Total Environ, 830 (2022),
748 p154720, 10.1016/j.scitotenv.2022.154720
749

750 Kamus et al., 2022. L. Kamus, G. Auger, K. Gambarotto, J. Houivet, M. Ramiandrisoa, S. Picot, N.
751 Lugagne-Delpon, A. Zouari, A. Birer, S. Nogues, A. Collet, M. Lecourt, O. Belmonte, V. Cattoir, G.
752 Miltgen. Investigation of a vanA linezolid- and vancomycin-resistant *Enterococcus faecium* outbreak
753 in the Southwest Indian Ocean (Reunion Island). Int. J. Antimicrob. Agents, 60 (5) (2022), p. 106686,
754 10.1016/j.ijantimicag.2022.106686
755

756 Kertster et al., 2006. K. Kersters, P. De Vos, M. Gillies, J. Swings, P. Vandamme, E. Stackebrandt
757 (Eds.), The Prokaryotes: Volume 5: Proteobacteria: Alpha and Beta Subclasses. Springer, New York,
758 NY (2006), pp. 3-37, 10.1007/0-387-30745-1_1
759

760 Klindworth et al., 2013. A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, F.O.
761 Glöckner. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-
762 generation sequencing-based diversity studies. Nucleic Acids Res., 41 (1) (2013), p. e1,
763 10.1093/nar/gks808
764

765 Kukulka et al., 2012. T. Kukulka, G. Proskurowski, S. Morét-Ferguson, D.W. Meyer, K.L. Law. The
766 effect of wind mixing on the vertical distribution of buoyant plastic debris. *Geophys. Res. Lett.*, 39 (7)
767 (2012), pp. 1–6, 10.1029/2012GL051116

768

769 Kumar et al., 2021. R. Kumar, A. Verma, A. Shome, R. Sinha, S. Sinha, P.K. Jha, R. Kumar, P.
770 Kumar, Shubham, S. Das, P. Sharma, P.V. Vara Prasad. Impacts of Plastic Pollution on Ecosystem
771 Services, Sustainable Development Goals, and Need to Focus on Circular Economy and Policy
772 Interventions. *Sustainability*. 13 (17) (2021), p. 9963, 10.3390/su13179963

773

774 Lambert et al., 2017. S. Lambert, C. Scherer, M. Wagner. Ecotoxicity Testing of Microplastics:
775 Considering the Heterogeneity of Physicochemical Properties. *Integr. Environ. Assess. Manag.*, 13
776 (2017), pp. 470-475, 10.1002/ieam.1901

777

778 Lemahieu et al., 2017. A. Lemahieu, A. Blaison, E. Crochelet, G. Bertrand, G. Pennober, M. Soria.
779 Human-shark interactions: The case study of Reunion Island in the south-west Indian Ocean. *Ocean*
780 *Coast. Manag.*, 136 (2017), pp. 73-82, 10.1016/j.ocecoaman.2016.11.020

781

782 Lenat et al., 2001. J.-F. Lenat, B. Gilbert-Malengreau, A. Galdéano. A new model for the evolution of
783 the volcanic island of Réunion (Indian Ocean). *J. Geophys. Res.*, 106 (B5) (2001), pp. 8645-8663,
784 10.1029/20000JB900448

785

786 Li et al., 2012. W. Li, L. Fu, B. Niu, S. Wu, J. Wooley. Ultrafast Clustering Algorithms for
787 Metagenomic Sequence Analysis. *Brief. Bioinform.*, 13 (6) (2012), pp. 656-668, 10.1093/bib/bbs035

788

789 Li et al., 2021. B. Li, W. Liang, Q.X. Liu, S. Fu, C. Ma, Q. Chen, L. Su, N.J. Craig, H. Shi. Fish Ingest
790 Microplastics Unintentionally. *Environ. Sci. Technol.* 55 (2021), pp. 10471-10479.
791 10.1021/acs.est.1c01753

792

793 Liu et al., 2021. Y. Liu, W. Liu, X. Yang, J. Wang, H. Ling, Y. Yang. Microplastics are a hotspot for
794 antibiotic resistance genes: progress and perspective. *Sci. Total Environ.*, 773 (2021), p. 145643,
795 10.1016/j.scitotenv.2021.145643
796
797 Lincol et al., 2022. S. Lincoln, B. Andrews, S.N.R. Birchenough, P. Chowdhury, G.H. Engelhard, O.
798 Harrod, J.K. Pinnegar, B.L. Townhill Marine litter and climate change: inextricably connected threats
799 to the world’s oceans. *Sci. Total Environ.*, 837 (2022), p. 155709, 10.1016/j.scitotenv.2022.15570
800
801 Löder & Gerds, 2015. M.G.J. Löder, G. Gerds. Methodology Used for the Detection and
802 Identification of Microplastics-A Critical Appraisal. M. Bergmann, L. Gutow, M. Klages (Eds.),
803 Marine Anthropogenic Litter. Springer, Cham (2015), p. 114721, 10.1007/978-3-319-16510-3_8
804
805 Maes et al., 2018. C. Maes, N. Grima, B. Blanke, E. Martinez, T. Paviet-Salomon, T. Huck. A Surface
806 “Superconvergence” Pathway Connecting the South Indian Ocean to the Subtropical South Pacific
807 Gyre. *Geophys. Res. Lett.*, 45 (4) (2018), pp. 1915–1922, 10.1002/2017GL076366
808
809 Magoc & Salzberg, 2011. T. Magoc, S. Salzberg. FLASH: Fast length adjustment of short reads to
810 improve genome assemblies. *Bioinformatics*, 27 (21) (2011), pp. 2957-2963,
811 10.1093/bioinformatics/btr507
812
813 Marathe & Bank, 2022. N.P. Marathe, M.S. Bank. The microplastic-Antibiotic Resistance Connection.
814 Environmental Contamination Remediation and Management. M.S. Bank (Eds.), Microplastic in the
815 Environment: Pattern and Process. Springer, Cham (2022), pp. 311-322, 10.1007/978-3-030-78627-
816 4_9
817
818 Masry et al., 2021. M. Masry, S. Rossignol, J. Gardette, S. Therias, P. Bussière, P. Wong-Wah-Chung.
819 Characteristics, fate, and impact of marine plastic debris exposed to sunlight: a review. *Mar. Pollut.*
820 *Bull.*, 171 (2021), p. 112701, 10.1016/j.marpolbul.2021.112701

821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848

Miltgen et al., 2020. G. Miltgen, P. Cholley, D. Martak, M. Thouverez, P. Seraphin, A. Leclaire, N. Traversier, B. Roquebert, M.-C. Jaffar-Bandjee, M. Ramiandrisoa, S. Picot, A. Lignereux, G. Masson, J. Allyn, N. Allou, P. Mavingui, O. Belmonte, X. Bertrand, D. Hocquet. Carbapenemase-producing Enterobacteriaceae circulating in the Reunion Island, a French territory in the Southwest Indian Ocean. *Antimicrob. Resist. Infect. Control*, 9 (1) (2020), p. 36, 10.1186/s13756-020-0703-3

Miltgen et al., 2021. G. Miltgen, M. Bour, J. Allyn, N. Allou, T. Vedani, J.-B. Vuilleminot, P. Triponney, O. Martinet, N. Lugagne, T. Benoit-Cattin, L. Dortet, A. Birer, M.-C. Jaffar-Bandjee, O. Belmonte, P. Plésiat, A. Potron. Molecular and epidemiological investigation of a colistin-resistant OXA-23-/NDM-1-producing *Acinetobacter baumannii* outbreak in the Southwest Indian Ocean Area. *Int. J. Antimicrob. Agents*, 58 (4) (2021), p. 106402, 10.1016/j.ijantimicag.2021.106402

Miltgen et al., 2022. G. Miltgen, D. Martak, B. Valot, L. Kamus, T. Garrigos, G. Verchere, H. Gbaguidi-Haore, C. Ben Cimon, M. Ramiandrisoa, S. Picot, A. Lignereux, G. Masson, M.-C. Jaffar-Bandjee, O. Belmonte, E. Cardinale, D. Hocquet, P. Mavingui, X. Bertrand. One Health compartmental analysis of ESBL-producing *Escherichia coli* on Reunion Island reveals partitioning between humans and livestock. *J. Antimicrob. Chemother.*, 77 (5) (2022), pp. 1254-1262, 10.1093/jac/dkac054

Muriel-Millán et al., 2021. L.F. Muriel-Millán, S. Millán-López, L. Pardo-López. Biotechnological applications of marine bacteria in bioremediation of environments polluted with hydrocarbons and plastics. *Appl. Microbiol. Biotechnol.*, 105 (19) (2021), pp. 7171–7185, 10.1007/s00253-021-11569-4

Napper & Thompson, 2020. I.E. Napper, R.C. Thompson. Plastic Debris in the Marine Environment: History and Future Challenges. *Global Challenges*, 4 (6) (2020), p. 1900081, 10.1002/gch2.201900081

849 Oberbeckmann et al., 2014. S. Oberbeckmann, M.G.J. Loeder, G. Gerdts, A.M. Osborn. Spatial and
850 seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern
851 European waters. *FEMS Microbiol.*, 90 (2) (2014), pp. 478–492, 10.1111/1574-6941.12409
852

853 Oberbeckmann & Labrenz, 2020. S. Oberbeckmann, M. Labrenz. Marine Microbial Assemblages on
854 Microplastics: Diversity, Adaptation, and Role in Degradation. *Ann. Rev. Mar. Sci.*, 12 (2020), pp.
855 209-232, 10.1146/annurev-marine-010419-010633
856

857 Oliveiros, 2007. Oliveros, J.C. (2007) VENNY. An interactive tool for comparing lists with Venn
858 Diagrams. <https://bioinfogp.cnb.csic.es/tools/venny/index.html>.
859

860 Panti et al., 2019. C. Panti, M. Bainsi, A., Lusher, G. Hernandez-Milan, E.L. Bravo Rebolledo, B.
861 Unger, K. Syberg, M.P. Simmonds, M.C. Fossi. Marine litter: One of the major threats for marine
862 mammals, outcomes from the European Cetacean Society workshop *Environ. Pollut.*, 247 (2019), pp.
863 72-79 10.1016/j.envpol.2019.01.029
864

865 Pattiaratchi et al., 2022. C. Pattiaratchi, M. van der Mheen, C. Schlundt, B. Narayanaswamy, A. Sura,
866 S. Hajbane, R. White, N. Kumar, M. Fernandes, S. Wijeratne. Plastics in the Indian Ocean – sources,
867 transport, distribution, and impacts. *Ocean Sci.*, 18 (1) (2022), pp. 1–28, 10.5194/os-18-1-2022
868

869 Pous et al., 2014. S. Pous, P. Lazure, G. André, F. Dumas, I. Halo, P. Penven. Circulation around La
870 Réunion and Mauritius islands in the south-western Indian Ocean: A modeling perspective. *J.*
871 *Geophys. Res.*, 119 (3) (2014), pp. 1957-1976, 10.1002/2013JC009704
872

873 R Core Team 2021 v4.0.4 R: A Language and Environment for Statistical Computing. R Foundation
874 for Statistical Computing, Vienna. <https://www.R-project.org/>.
875

876 Raghul et al., 2014. S.S. Raghul, S.G. Bhat, M. Chandrasekaran, V. Francis, E.T. Thachil.
877 Biodegradation of polyvinyl alcohol-low linear density polyethylene-blended plastic film by
878 consortium of marine benthic vibrios. *Int. J. Environ. Sci. Technol.*, 11 (7) (2014), pp. 1827–1834,
879 10.1007/s13762-013-0335-8
880
881 Roager & Sonnenschein, 2019. L. Roager, E.C. Sonnenschein. Bacterial Candidates for Colonization
882 and Degradation of Marine Plastic Debris. *Environ. Sci. Technol.*, 53 (2019), pp. 11636-11643,
883 10.1021/acs.est.9b02212
884
885 Rochman, 2015. C.M. Rochman. The Complex Mixture, Fate and Toxicity of Chemicals Associated
886 with Plastic Debris in the Marine Environment. M. Bergmann, L. Gutow, M. Klages (Eds.), *Marine*
887 *Anthropogenic Litter*. Springer, Cham (2015), pp. 117-140, 10.1007/978-3-319-16510-3_5
888
889 Rummel et al., 2017. C.D. Rummel, A. Jahnke, E. Gorokhova, D. Kühnel, M. Schmitt-Jansen. Impacts
890 of Biofilm Formation on the Fate and Potential Effects of Microplastic in the Aquatic Environment.
891 *Environ. Sci. Technol. Lett.*, 4 (7) (2017), pp. 258-267, 10.1021/acs.estlett.7b00164
892
893 Schott et al., 2009. F.A. Schott, S.-P. Xie, J.P. McCreary. Indian Ocean circulation and climate
894 variability, *Rev. Geophys.*, 47, (2009), RG1002, 10.1029/2007RG000245.
895
896 Seeley et al., 2020. M.E. Seeley, B. Song, R. Passie, R.C. Hale. Microplastics affect sedimentary
897 microbial communities and nitrogen cycling. *Nat. Commun*, 11 (1) (2020), p. 2372, 10.1038/s41467-
898 020-16235-3
899
900 Silva et al., 2023. I. Silva., E.T. Rodrigues., M. Tacão, I. Henriques. Microplastics accumulate priority
901 antibiotic-resistant pathogens: Evidence from the riverine plastisphere. *Environ. Pollut.*, (2023), p.
902 121995, 10.1016/j.envpol.2023.121995
903

904 Shan et al., 2022. E. Shan, X. Zhang, J. Li, C. Sun, J. Teng, X. Yang, L. Chen, Y. Liu, X. Sun, J.
905 Zhao, Q. Wang. Incubation habitats and aging treatments affect the formation of biofilms on
906 polypropylene microplastics. *Sci. Total Environ.*, 831 (2022), p. 154769,
907 10.1016/j.scitotenv.2022.154769
908
909 Smith et al., 2018. M. Smith, D.C. Love, C.M. Rochman, R.A. Neff. Microplastics in Seafood and the
910 Implications for Human Health. *Curr. Environ. Health Rep.* 5 (3) (2018), pp. 375-386,
911 10.1007/s40572-018-0206-z
912
913 Sobecky & Hazen, 2009. P.A. Sobecky, T.H. Hazen. Horizontal gene transfer and mobile genetic
914 elements in marine systems. *Methods Mol. Biol.* 532 (2009), pp. 435-453, 10.1007/978-1-60327-853-
915 9_25
916
917 Sooriyakumar et al., 2022. P. Sooriyakumar, N. Bolan, M. Kumar, L. Singh, Y. Yu, Y. Li, C.
918 Weralupitiya, M. Vithanage, S. Ramanayaka, B. Sarkar, F. Wang, D.B. Gleeson, D. Zhang, M.B.
919 Kirkham, J. Rinklebe, K.H. M Siddique. Biofilm formation and its implications on the properties and
920 fate of microplastics in aquatic environments: A review. *J. Hazard. Mater. Adv.*, 6 (2022), p. 100077,
921 10.1016/j.hazadv.2022.100077
922
923 Stabnikova et al., 2021. O. Stabnikova, V. Stabnikov, A. Marinin, M. Klavins, L. Klavins, A.
924 Vaseashta. Microbial Life on the Surface of Microplastics in Natural Waters. *Appl. Sci.*, 11 (24)
925 (2021), p. 11692, 10.3390/app112411692
926
927 Stal & Cretoiu, 2022. L. Stal, M.S. Cretoiu. The Marine Microbiome. 3, The Microbiomes of Humans,
928 Animals, Plants, and the Environment (2022), 10.1007/978-3-030-90383-1
929
930 Staudacher et al., 2009. T. Staudacher, V. Ferrazzini, A. Peltier, P. Kowalski, P. Catherine, F. Lauret,
931 F. Massin. The April 2007 eruption and the Dolomieu crater collapse, two major events at Piton de la

932 Fournaise (La Réunion Island, Indian Ocean). *J. Volcanol. Geotherm. Res.*, 184 (1) (2009), pp. 126-
933 137, 10.1016/j.volgeores.2008.11.005

934

935 Thibault et al., 2023. M. Thibault, L. Hoarau, L. Lebreton, M. Le Corre, M. Barret, E. Cordier, S.
936 Royer, A. Ter Halle, C. Jean, M. Dalleau. Do loggerhead sea turtle (*Caretta caretta*) gut contents
937 reflect types, colors and sources of plastic pollution in the Southwest Indian Ocean ? *Mar. Pollut. Bull.*
938 194 (2023). 10.1016/j.marpolbul.2023.115343

939

940 Thushari & Senevirathna, 2020. G.G.N. Thushari, J.D.M. Senevirathna. Plastic pollution in the marine
941 environment. *Heliyon*, 6 (8) (2020), p. e04709, 10.1016/j.heliyon.2020.e04709

942

943 Trachoo, 2004. N. Trachoo. Biofilm removal technique using stands as a research tool for accessing
944 microbial attachment on surface. *J. Sci. Technol.*, 26 (1) (2004), pp. 109-115

945

946 Türetken et al., 2020. P.S.C. Türetken, G. Altuğ, T. Öksüzoğlu. The levels of plastic-associated
947 heterotrophic bacteria on three different types of plastics. *Aquat. Sci. Eng.*, 35 (2) (2020), pp. 31–35,
948 10.26650/ASE2020679538

949

950 Tuuri & Leterme, 2023. E.M. Tuuri & S.C. Leterme. How plastic debris and associated chemicals
951 impact the marine food web: a review. *Environ. Pollut.*, 321 (2023), p. 121156,
952 10.1016/J.ENVPOL.2023.121156

953

954 Vaksmaa et al., 2021. A. Vaksmaa, K. Knittel, A. Abdala Asbun, M. Goudriaan, A. Ellrott, H.J. Witte,
955 I. Vollmer, F. Meirer, C. Lott, M. Weber, J.C. Engelmann, H. Niemann. Microbial Communities on
956 Plastic Polymers in the Mediterranean Sea. *Front. Microbiol.*, 12 (2021), p. 673553,
957 10.3389/fmicb.2021.673553

958

959 Van Cauwenberghe & Janssen 2014., L. Van Cauwenberghe, C.R. Janssen. Microplastics in bivalves
960 cultured for human consumption. *Environ. Pollut.* 193 (2014), pp. 65-70.
961 10.1016/j.envpol.2014.06.010
962
963 Virsek et al., 2016. M.K. Virsek, A. Palatinus, S. Koren, M. Peterlin, P. Horvat, A. Krzan. Protocol for
964 Microplastics Sampling on the Sea Surface and Sample Analysis. *JoVE*, 118 (2016), p. 55161,
965 10.3791/55161
966
967 Walkinshaw et al., 2020. C. Walkinshaw, P.K. Lindeque, R. Thompson, T. Tolhurst, M. Cole
968 Microplastics and seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicol.*
969 *Environ. Saf.*, 190 (2020), 10.1016/j.ecoenv.2019.110066
970
971 Wang et al., 2021. Z. Wang, J. Gao, Y. Zhao, H. Dai, J. Jia, D. Zhang. Plasticsphere enrich antibiotic
972 resistance genes and potential pathogenic bacteria in sewage with pharmaceuticals. *Sci. Total*
973 *Environ.*, 768 (2021), p 144663, 10.1016/j.scitotenv.2020.144663
974
975 Waring et al., 2018. R.H. Waring, R.M. Harris, S.C. Mitchell. Plastic contamination of the food chain:
976 A threat to human health? *Maturitas* 115 (2018), pp. 64-68, 10.1016/j.maturitas.2018.06.010
977
978 Wu et al., 2019. X. Wu, J. Pan, M. Li, Y. Li, M. Bartlam, Y. Wang. Selective enrichment of bacterial
979 pathogens by microplastic biofilm. *Water Res.*, 165 (2019), p. 114979, 10.1016/j.waters.2019.114979
980
981 Yang et al., 2019. Y. Yang, G. Liu, W. Song, C. Ye, H. Lin, Z. Li, W. Liu. Plastics in the marine
982 environment are reservoirs for antibiotic and metal resistance genes. *Environ. Int.*, 123 (2019), pp. 79-
983 86, 10.1016/j.envint.2018.11.061
984

985 Zettler et al., 2013. E.R. Zettler, T.J. Mincer, L.A. Amaral-Zettler, Y.M. Piceno. Life in the
986 “Plastisphere”: Microbial Communities on Plastic Marine Debris. *Environ. Sci. Technol.*, 47 (13)
987 (2013), pp. 7137–7146, 10.1021/es401288f
988

989 Zhang et al., 2022. G. Zhang, J. Chen J, W. Li. Conjugative antibiotic-resistant plasmids promote
990 bacterial colonization of microplastics in water environments, *J. Haz. Mat.*, 430, (2022), p. 128443,
991 10.1016/j.jhazmat.2022.128443
992

993 Zhurina et al., 2022. M.V. Zhurina, K.I. Bogdanov, A.V. Gannesen, S.V. Mart’yanov, V.K. Plakunov.
994 Microplastics as a New Ecological Niche For Multispecies Microbial Biofilms within the Plastisphere.
995 *Microbiology*. 91 (2) (2022), pp. 107–123, 10.1134/S0026261722020126
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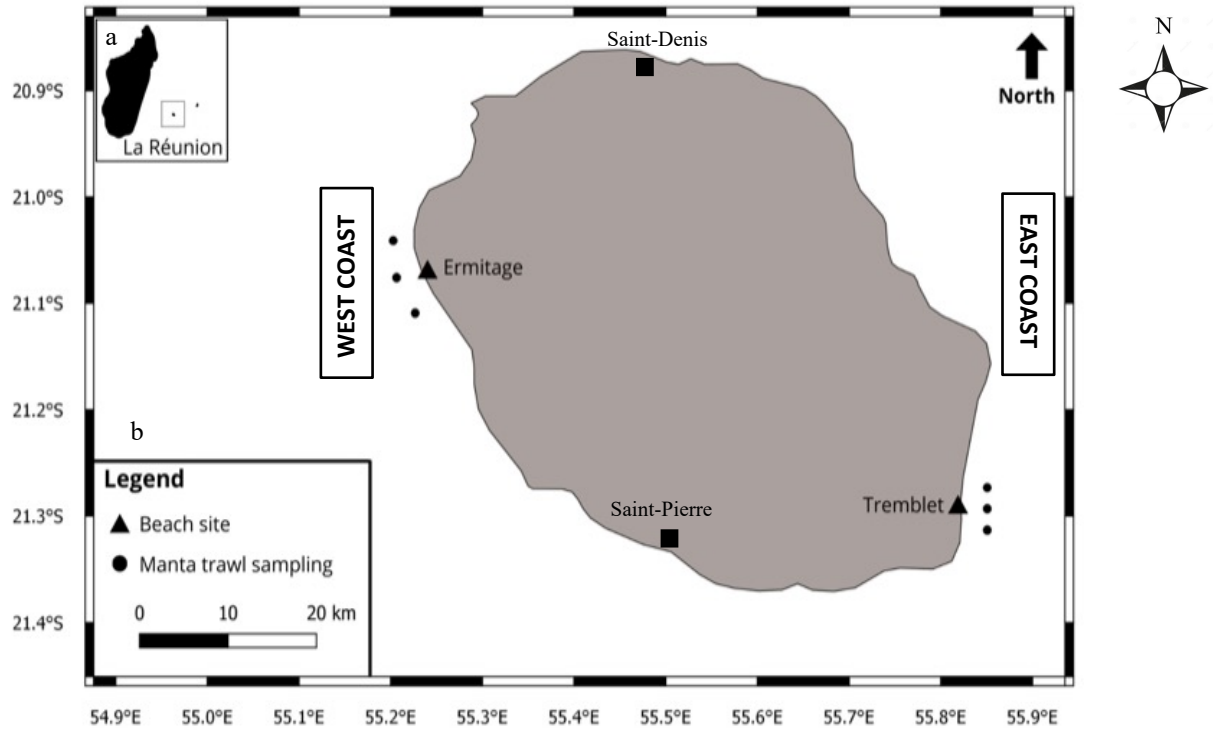
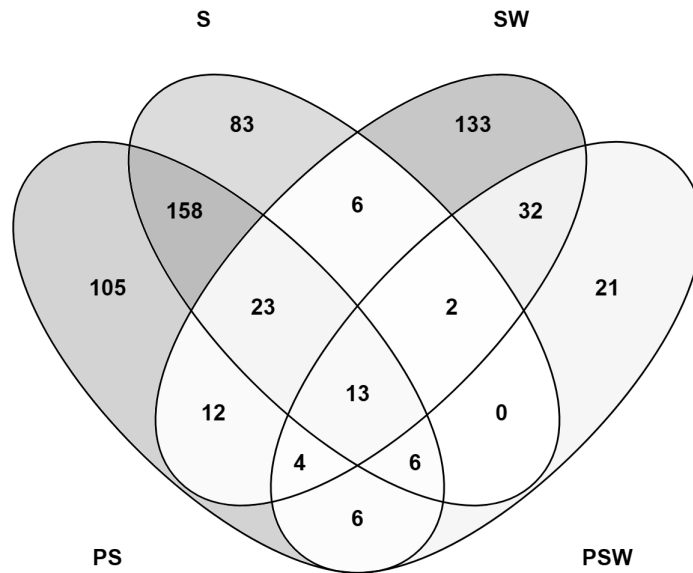


Fig. 1.(a) Location map of Reunion island in the southwest Indian Ocean close to Madagascar and Mauritius island; (b) Map of the Reunion island including the two major cities and the study site locations.

a East Coast : (604 OTUs)



b West Coast : (835 OTUs)

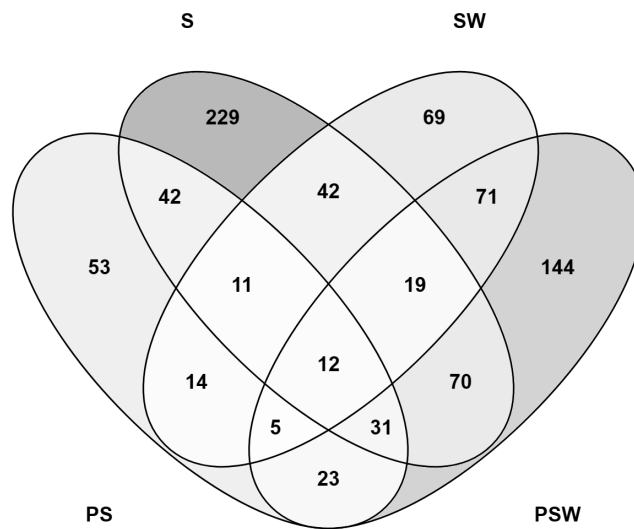
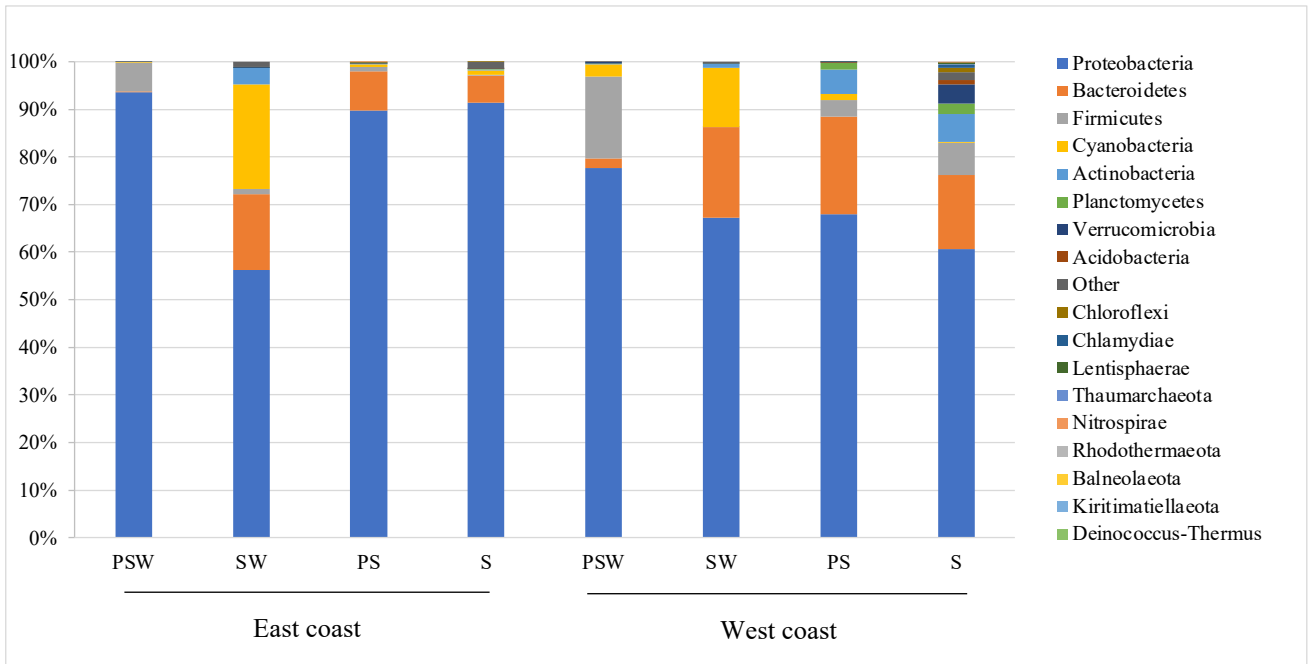


Fig. 2. Venn diagrams of the Operational Taxonomic Unit (OTUs) distribution showing shared and specific OTUs according to site *i.e.* East coast (a) or West coast (b) in all sample types *i.e.* PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand.

a

Bacterial phylum OTUs relative abundance



b

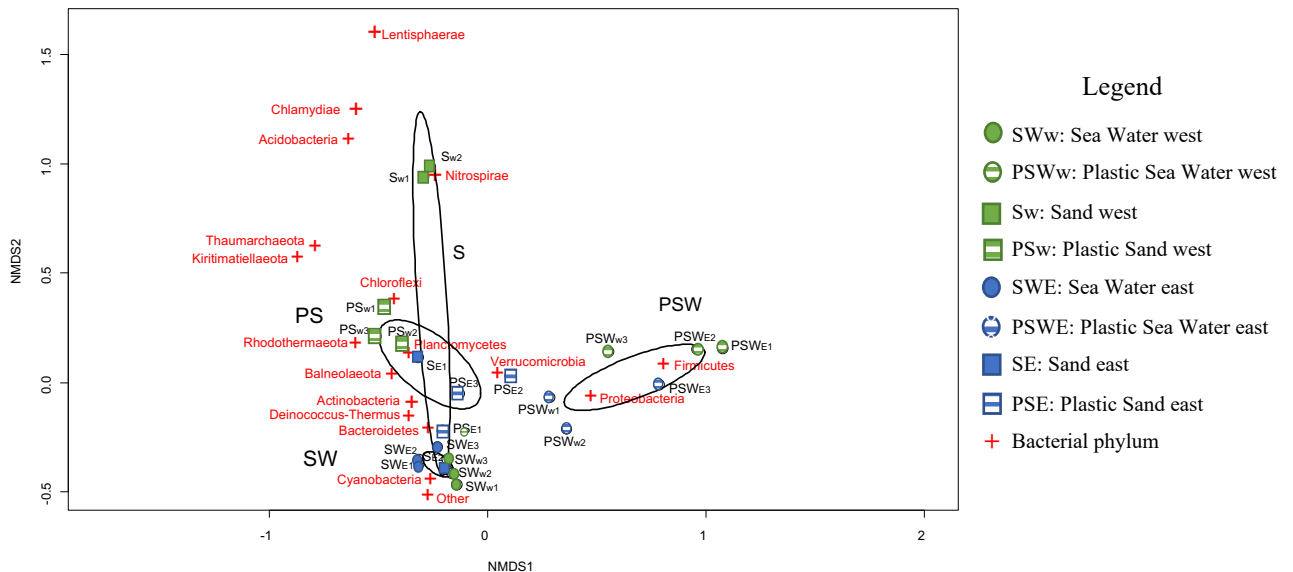
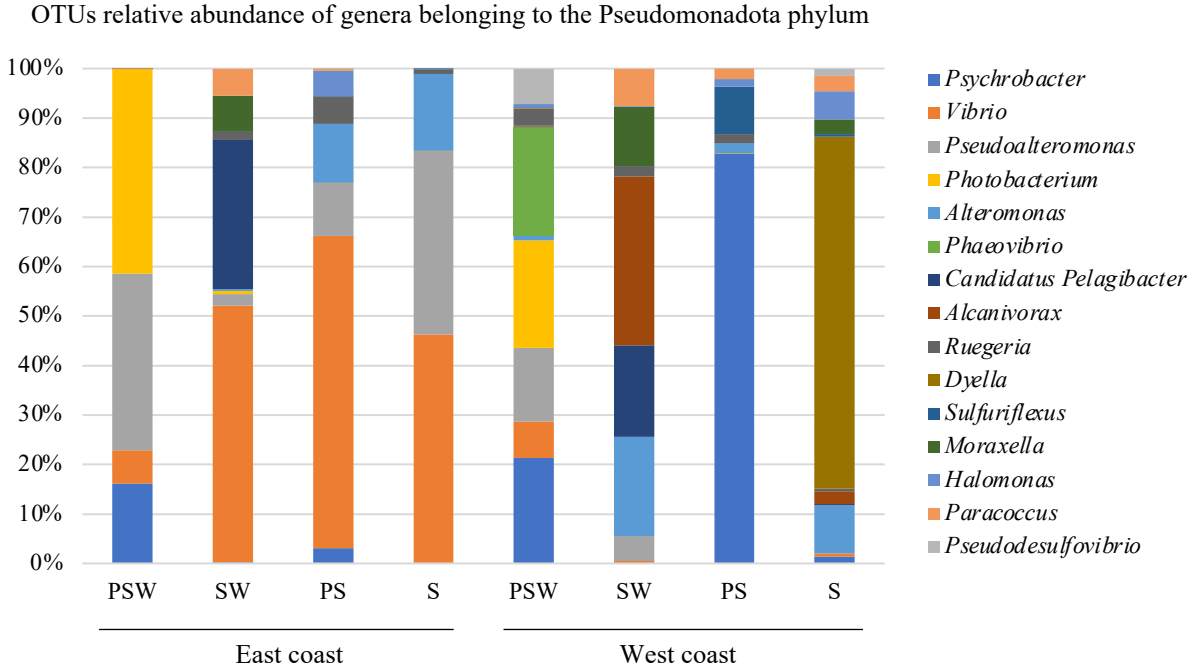


Figure 3 : OTUs distribution of the bacterial community according to the analysis at phylum level for the different substrates: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of bacterial phyla identified in all samples. (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

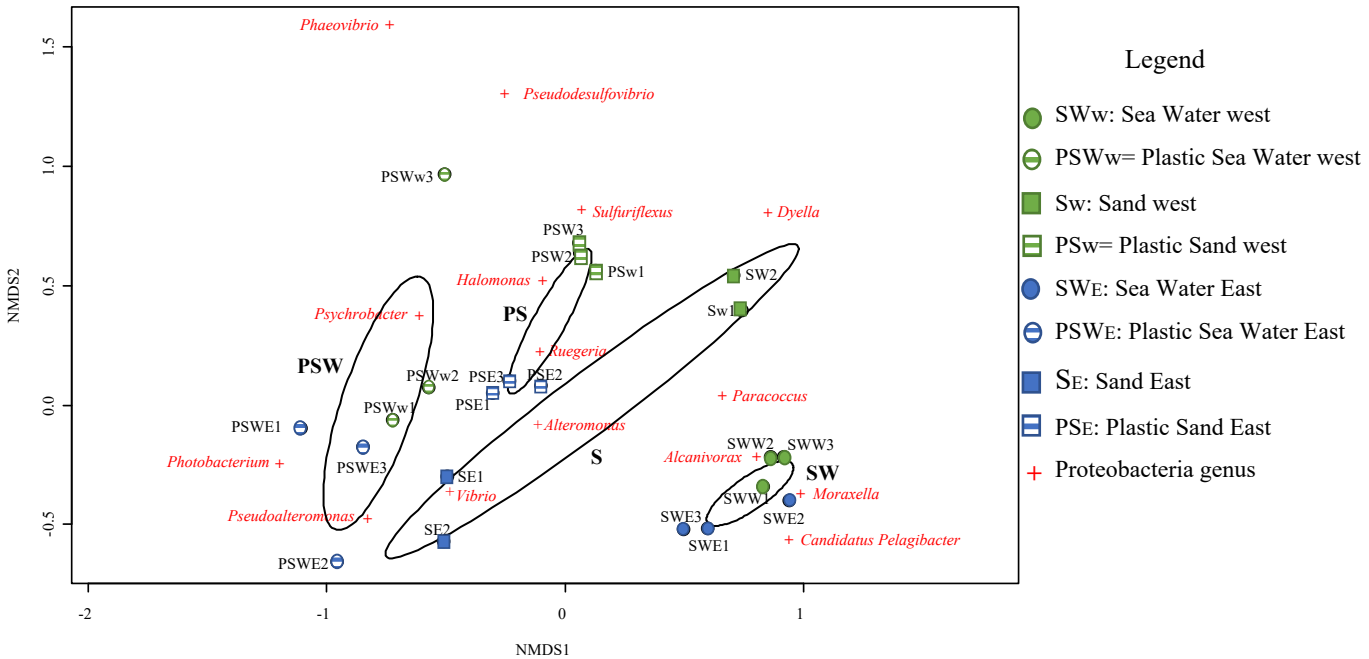


Figure 4 : OTUs distribution of the phylum of the Proteobacteria according to the analysis at genera level for the different substrates: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. (a) Relative abundance of Proteobacteria genus identified 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.

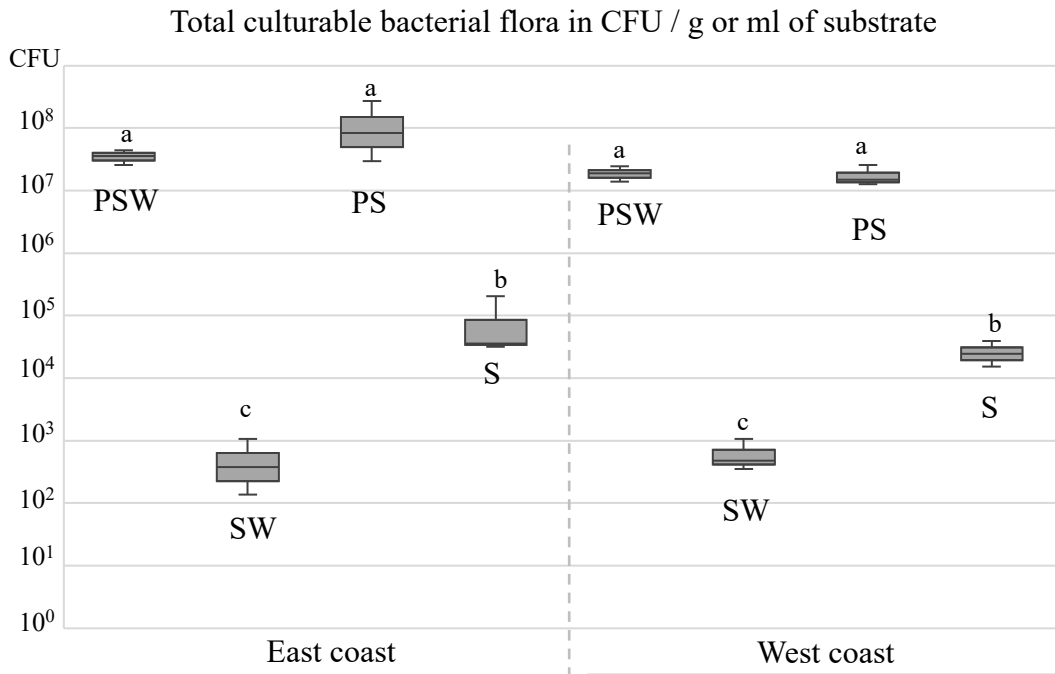


Figure 5 : Total culturable bacterial flora in CFU / g or ml of substrate. Abbreviations: PSW: plastics from coastal sea-water; SW: coastal sea-water; PS: plastics from beach sand; S: beach sand. Plots represent means, and error bars represent standard deviation of means (n = 3) of three independent 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 ²	0.022 \pm 0.008 item/m ²	10,693 \pm 11,275 item/km ²	0.34 \pm 0.31 item/m ²
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	Ticarcillin	
		T3-42	<i>Pseudomonas</i>	TS			
	SW	T5-26	<i>Staphylococcus</i>	Erythromycin	Penicillin		
		T4-54	<i>Bacillus</i>	Amoxicillin	Imipenem	Penicillin G	Vancomycin
		T4-52	<i>Vibrio</i>	Ampicillin			
	PS	A1-2	<i>Pseudomonas</i>	Ticarcillin	TS		
		A2-15	<i>Bacillus</i>	Amoxicillin	Imipenem	Penicillin G	
		A1-13	<i>Vibrio</i>	Ampicillin			
	S	A4-9	<i>Bacillus</i>	Amoxicillin	Penicillin G		
		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	Clindamycin		
	PS	B1-3	<i>Enterococcus</i>	Rifampicin	Vancomycin		
	S	B6-14	<i>Aeromonas</i>	Piperacilin	Ticarcilin	Ticarcilin / Clavulanic acid	PT
		B5-17	<i>Staphylococcus</i>	Clindamycin	Fusidic acid	Penicillin	