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1 **Bacterial pathogens associated with the plastisphere of surgical face masks and their dispersion**  
2 **potential in the coastal marine environment**

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17 **Abstract**

18 Huge numbers of face masks (FMs) were discharged into the ocean during the coronavirus  
19 pandemic. These polymer-based artificial surfaces can support the growth of specific  
20 bacterial assemblages, pathogens being of particular concern. However, the potential risks  
21 from FM-associated pathogens in the marine environment remain poorly understood. Here,  
22 FMs were deployed in coastal seawater for two months. PacBio circular consensus  
23 sequencing of the full-length 16S rRNA was used for pathogen identification, providing  
24 enhanced taxonomic resolution. Selective enrichment of putative pathogens (e.g., *Ralstonia*  
25 *pickettii*) was found on FMs, which provided a new niche for these pathogens rarely detected  
26 in the surrounding seawater or the stone controls. The total relative abundance of the putative  
27 pathogens in FMs was higher than in seawater but lower than in the stone controls. FM  
28 exposure during the two months resulted in 3% weight loss and the release of considerable  
29 amounts of microfibers. The ecological assembly process of the putative FM-associated  
30 pathogens was less impacted by the dispersal limitation, indicating that FM-derived  
31 microplastics can serve as vectors of most pathogens for their regional transport. Our results  
32 indicate a possible ecological risk of FMs for marine organisms or humans in the coastal and  
33 potentially in the open ocean.

34

35 **Keywords**

36 Plastic debris; microplastic; Pathogen identification; Biofilm; Bacterial colonization

37

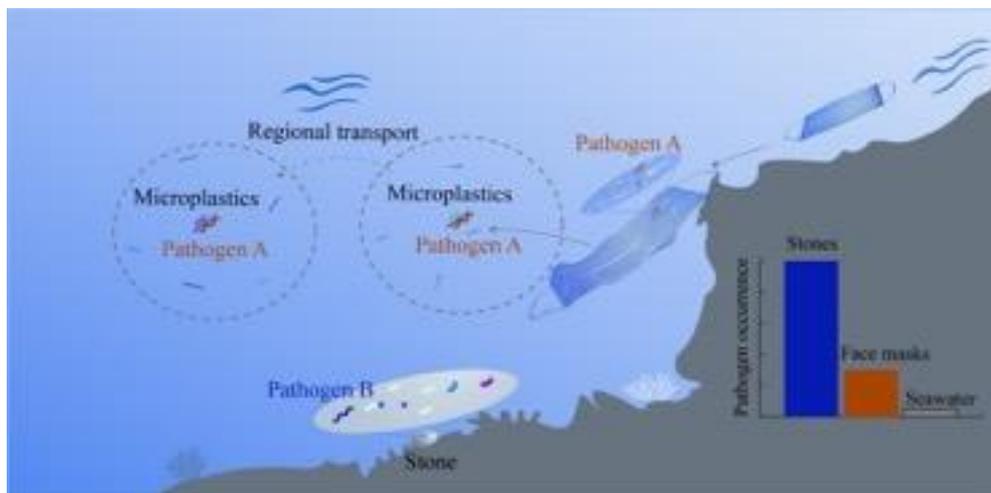
38 **Environmental Implication**

39 The majority of surgical face masks were made of polypropylene plastics in the form of  
40 fibers, which were frequently found in the tissue of marine organisms. They were considered  
41 persistent organic pollutants because of extremely low environmental degradation rates and  
42 toxicity to marine organisms. We showed that FMs could release considerable microfibers *in*  
43 *situ*, exacerbating the plastic pollution in the marine environment. The enrichment of specific  
44 pathogens on FMs and the elevated pathogens' dispersion potential indicates that the FMs or  
45 released microfibers can cause disease to marine organisms in the coastal and even a broader  
46 environment.

47

48 **Graphical abstract**

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51

52

## 53 **1. Introduction**

54 The abrupt outbreak of coronavirus disease in 2019 (COVID-19) led to a global health crisis.  
55 Countermeasures were implemented to restrict the spread of the disease, such as wearing face  
56 masks (FMs), travel restrictions, and lockdowns [1], severely affecting life activities.  
57 Monthly FM consumption was estimated to reach 129 billion in 2020 [2]. However,  
58 extensive use and mismanagement of FMs caused a blooming threat to the fluvial and marine  
59 environment [3]. For example, FMs accounted for 9% of total floating debris from a river  
60 during the pandemic [4]. Considerable FMs can originate from the coast, where up to 100  
61 pieces of FMs can be observed in a beach [5]. It is estimated that 0.39 million tons of FMs  
62 ended up in the ocean within a year during the pandemic [6].

63 Disposable FMs mainly consist of polypropylene microfibers [3]. Recent research  
64 demonstrated that an FM can release thousands of microplastics in seawater *versus* millions  
65 after a UV aging treatment [7–9]. The aging process of FMs was primarily performed within  
66 controlled laboratory environments. Indeed, once a piece of plastic enters the ocean, it can be  
67 rapidly colonized by marine microorganisms, such as bacteria and phytoplankton (e.g.,  
68 diatoms), the so-called biofilm or plastisphere [10–12]. The biofilm formation can reduce the  
69 UV access to FMs, potentially retarding the aging process in the marine environment.  
70 Conversely, the water shear stress [13] and bioerosion [14] can promote the release of  
71 microplastics/nanoplastics *via* fragmentation. Therefore, the aging process of FMs in the  
72 marine environment can be distinct from that in laboratory environments, which cannot be  
73 accurately evaluated without field exposure.

74 Microplastics in the fiber form (i.e., microfibers) in the marine environment can be  
75 particularly detrimental to marine animals. Microfiber was the main shape found in the  
76 marine fish, suggesting a blockage of their digestive tracts [15]. Other studies showed that

77 some additives (such as Mn, Zn, Ni, Pb, Cd, and Cr) and microplastics were toxic to marine  
78 life [16–18]. Over the past decade, intensive research was conducted to characterize the  
79 bacterial structure, assembly mechanisms, and environmental drivers of the plastisphere  
80 bacterial communities, for which the pathogens were of great concern [10]. In 2013, Zettler et  
81 al. investigated the plastisphere from the North Atlantic and described that the potentially  
82 pathogenic *Vibrio* dominated the bacterial community in a polypropylene sample [10]. A  
83 recent meta-analysis also showed that the putative pathogen Vibrionales and *Tenacibaculum*  
84 spp. were more abundant in the plastic samples than the control biofilm or the planktonic  
85 samples in the marine environment [19]. However, some other studies found that the putative  
86 pathogenic species were actually more abundant on natural substrates for members of genera  
87 *Arcobacter*, *Pseudomonas*, *Shewanella*, and *Vibrio* [20]. These methods are generally derived  
88 from second-generation sequencing of the 16S rRNA gene (less than 500 base pairs in  
89 general for the sequence length) [19–22], the higher taxonomic ranks, i.e., at the family level  
90 or the genus level, can contain many non-pathogenic species. For instance, the genus *Vibrio*  
91 comprises over one hundred species, only twelve of which are regarded as human pathogens  
92 [23]. Consequently, low resolution was the main drawback of pathogenic bacteria profiling  
93 for the second-generation sequencing, which makes it hard to assign the pathogens to the  
94 species level and, in turn, makes the pathogen results less relevant to the real condition. Even  
95 through decades of research, limited progress was achieved in this field. Moreover, to  
96 evaluate the pathogen threats to marine ecosystems, the host range at multiple trophic levels  
97 is needed to assess the pathogen risks to the marine environment instead of mainly focusing  
98 on the pathogens of human beings from previous studies.

99 In contrast to bacterial colonization behavior, bacteria can detach from the plastisphere, the  
100 so-called bacterial dispersion, i.e., bacteria escape from the biofilm structure, and both  
101 favorable (e.g., nutritional sufficiency) and unfavorable (e.g., oxygen depletion) conditions

102 can contribute to bacterial dispersion [24]. Previous studies showed that the bacterial  
103 community in the plastsphere was driven by the surrounding environmental conditions,  
104 indicating that during the microplastic transport, there could be bacterial recruitment from the  
105 surrounding seawater to the plastsphere and bacterial dispersal from the plastsphere to its  
106 surrounding seawater [25,26]. This raises the question as to whether pathogens from FM-  
107 derived microplastics will increase pathogen dispersal during their transport in the marine  
108 environment.

109 While fine microfibers of FMs make them susceptible to the ocean current, frictions can  
110 result in the FM breakdowns into microplastics. We hypothesized that the aging process of  
111 FMs will be retarded due to biofilm formation, FMs can selectively enrich pathogens, and the  
112 released microplastics can further promote pathogen dispersion in the marine environment.  
113 Here, surgical face masks were incubated in coastal seawater for two months to allow the  
114 development of a mature biofilm. Three stations were chosen to represent a gradient of  
115 human impacts, which also permitted the assessment of the pathogen dispersal potential.  
116 After the FM exposure, the change in the functional groups was tested using the Fourier  
117 transform infrared (FTIR) spectroscopy. In addition, the weight loss of the FMs was tested  
118 after a biofilm removal process. Scanning electron microscopy was employed to evaluate the  
119 change in surface roughness. To unveil the taxonomies of pathogens in a higher resolution,  
120 i.e., at the species level, the PacBio circular sequencing was adopted to characterize the full-  
121 length 16S rRNA sequences (around 1470 base pairs). Moreover, a custom-made pathogenic  
122 bacteria database was constructed, comprising the pathogen target at multiple trophic levels.  
123 This study aims to 1) test the FM deterioration in the marine environment, 2) characterize the  
124 pathogen profiles in the plastsphere, and 3) assess the dispersion potential of  
125 bacteria/pathogens in the plastsphere from a null model analysis. We aimed to understand

126 the pathogenic behavior and environmental risks during the aging process of masks and  
127 provide services for coastal ecological health/management.

128

## 129 **2 Materials and Methods**

### 130 2.1 Experimental setup

131 Commercially available surgical FMs were fixed inside polyethylene cages, which were  
132 fastened under water quality monitoring buoys at a water depth of around 0.5 m. Ordinary  
133 black agate stones were used as controls. The exposures were performed from Oct. to Dec. in  
134 2021. Three locations along the coast of Shenzhen, China, were chosen to represent different  
135 anthropogenic influences, i.e., an open-water station situated 200 meters from the coast  
136 (114.567°E, 22.465°N), a bay with intensive aquaculture (114.515°E, 22.565°N), and a semi-  
137 enclosed bay of the metropolis (113.946°E, 22.477°N) (Figure 1). Pristine FMs were used in  
138 this study to avoid any heterogeneous absorbent materials after wearing.

139 After 2-month incubation, FMs, stones, and seawater were collected from the three stations.  
140 Before biofilm sampling, FMs and stones were rinsed with 0.2 µm filtered seawater using a  
141 spray bottle to remove loosely attached materials. FMs were aliquoted using a sterile scissor.  
142 Biofilm from the stones was scraped using sterile throat swabs. Besides, one liter of seawater  
143 was filtrated using 0.2 µm polycarbonate membrane filters (47 mm diameter, Nucleopore).  
144 Samples were performed in triplicates and stored at -80 °C after the treatments.

### 145 2.2 *Weight loss and microplastic counting*

146 For the weight loss assay, biofilm on FMs was removed with a nitric acid digestion process at  
147 80 °C for 24 hours using a temperature-controlled hot plate (SINEO, China) [27]. After

148 digestion, FMs were washed with ten cycles of the ultrasonic bath with preheated 0.2  $\mu\text{m}$   
149 filtered milli-Q water for 5 mins at 80 °C. The weight of FM samples was weighted using a  
150 balance with the precision of 0.1 mg after drying (Mettler Toledo, China). Pristine FMs were  
151 performed as controls.

152 In parallel to the FM washing, the eluent was filtered through a 0.2  $\mu\text{m}$  glass fiber membrane,  
153 the microplastics on the glass membrane were resuspended in saturated sodium chloride  
154 overnight to allow the microplastics floating on the liquid surface, and the supernatant was  
155 filtered again through a 0.2  $\mu\text{m}$  CN/CA composite film membrane before the observation  
156 under a stereomicroscope. Triplicate pristine FMs and blanks were performed as controls.

### 157 *2.3 Environmental parameters*

158 Physiochemical parameters such as pH, temperature, and dissolved oxygen were measured *in*  
159 *situ* using a portable Water Quality Meter (SMAT, China). Salinity was measured using a  
160 hand-held practical salinity refractometer. Total organic matter (TOC) was measured using  
161 an Apollo 9000 Total Organic Carbon Analyzer (Teledyne Instruments Tekmar, USA) [28].  
162 Additional environmental factors, such as nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), and inorganic  
163 phosphate ( $\text{PO}_4^{3-}$ ), were measured using a Discrete Chemistry Analyzer (CleverChem Anna,  
164 Germany) according to our previous methods [28]. To test the density of aged FMs, FM  
165 aliquots were placed into seawater with varying levels of salinities. When the FMs were  
166 suspended, their density matched that of the surrounding seawater.

### 167 *2.4 Confocal microscopy and scanning electron microscopy*

168 FM aliquots were fixed in a 2% glutaraldehyde solution overnight and rinsed with phosphate-  
169 buffered saline three times. For confocal microscopy, samples were stained with a 4,6-  
170 diamidino-2-phenylindole (DAPI) solution (final concentration: 50  $\mu\text{M}$ , ZETA<sup>TM</sup>) for 15

171 mins in the dark at ambient temperature before confocal microscopy observations (Nikon,  
172 Japan). The 405 nm laser was used for the excitation and visualization of DAPI signals. The  
173 Z-Step size was set to 1  $\mu\text{m}$  to get regularly spaced cross-sections.

174 For the scanning electron microscopy, the fixed and rinsed aliquots were further dehydrated  
175 with a series of 75% ethanol, 90% ethanol, 100% ethanol, and 100% acetone for 15 minutes  
176 in respective solutions, followed by an air-drying process in a fume hood [25]. Samples were  
177 coated with a sputter coater and further observed under a scanning electron microscope  
178 (Hitachi, Japan).

### 179 *2.5 Fourier transform infrared (FTIR) spectroscopy*

180 FM aliquots were incubated in hydrogen peroxide (30%) to remove the biofilm [29]. Samples  
181 were washed with milli-Q water three times and dried in the fume hood. FM spectra were  
182 recorded using an FTIR spectrometer (PerkinElmer), using 16 scans from 4000  $\text{cm}^{-1}$  to 400  
183  $\text{cm}^{-1}$ . The absorption band strength and polymer type identification were realized *via* Omnic  
184 Spectra software (Thermo Fisher Scientific). To determine the aging process, the carbonyl  
185 index (CI) was calculated from the spectrum of the FMs. The CI value is usually used as a  
186 proxy of oxidation level of polymers, and can be obtained by calculating the adsorption of the  
187 ketone peak at 1715  $\text{cm}^{-1}$  to the methylene peak at 1455  $\text{cm}^{-1}$  [30].

### 188 *2.6 DNA extraction and quantitative PCR (qPCR)*

189 The microbial genomic DNA was extracted using the classical phenol-chloroform protocol  
190 from the outer layer of FMs ( $\sim 5 \text{ cm}^2$ ), the swabs (stone controls), and the membrane filters  
191 (seawater controls) [31]. DNA quality and quantity were verified using NanoDrop One  
192 (Thermo Fisher), and the DNA integrity was checked on 1% agarose gel. The DNA  
193 concentration was quantified using a Qubit 3.0 fluorometer (Invitrogen).

194 To determine the total bacterial abundance in FMs, stones and seawater, bacterial 16S rRNA  
195 gene was quantified by qPCR using QuantStudio™ 5 Real-Time PCR System (Applied  
196 Biosystems) with ChamQ SYBR qPCR Master Mix and the bacterial specific primers (16S  
197 338F, 5'-ACTCCTACGGGAGGCAGCA-3'; 806R 5'-GGACTACHVGGGTWTCTAAT-  
198 3'). The qPCR was conducted in 384-well plates with a 30 µL reaction mixture containing 15  
199 µL qPCR Master Mix, 1 µL of each primer, and 1 µL template DNA. Standard curves were  
200 generated by 10-fold gradient diluted plasmid solutions. All samples were conducted in  
201 triplicates. The amplification efficiencies range from 85% to 105% in different PCR reactions  
202 ( $R^2 > 0.995$ ) for the standard curve.

### 203 *2.7 DNA sequencing, and data processing*

204 Polymerase chain reaction (PCR) amplification was done to target the full-length 16S rRNA  
205 gene using universal small subunit ribosomal RNA (SSU rRNA) primers (27F, 5'-  
206 AGRGTTYGATYMTGGCTCAG-3'; 1492R, 5'-RGYTACCTTGTTACGACTT-3'). PacBio  
207 circular consensus sequencing (CCS) was performed for the 27 samples (Magigene  
208 Biotechnology, China), including triplicate samples from the three stations for FM, stone, and  
209 seawater samples. The PCR amplification profile included an initial denaturation of 15 min at  
210 94 °C, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s,  
211 and amplification at 72 °C for 45 s, in addition to a final elongation step of 10 min at 72 °C.  
212 All the SSU rRNA data are available in the NCBI SRA repository (accession number  
213 PRJNA971390).

214 Processing of SSU rRNA sequences was performed using the QIIME2 microbiome  
215 bioinformatics platform [32]. The plugin UCLUST was used for clustering sequences at a  
216 threshold of 99% to define the operational taxonomic unit (OTU) [33]. OTUs were assigned  
217 against the Greengenes 13.8 database [34], and the OTUs affiliated to eukaryotes, archaea,

218 chloroplast, and mitochondria were removed. Alpha-diversity calculation and histogram  
219 visualization were realized with the MicrobiomeAnalyst server [35].

220 To quantify the degree of connectivity of microbial communities, a community metric  
221 ‘cohesion’ was calculated by integrating the relative bacterial abundance and the co-  
222 occurrence profile. For a given sample type (e.g., FMs), the cohesion is the summation of  
223 each OTU’s negative coefficient to the rest OTUs, divided by that of the positive coefficient,  
224 and weight by bacterial abundance [36].

## 225 *2.8 Pathogen identification and visualization*

226 The bacterial pathogen list was obtained from the Enhanced Infectious Disease Database  
227 (EID2), which used an automated data mining process to extract information on pathogens  
228 and their hosts, and the pathogen-host interactions were verified by literature review [37].  
229 Even though the bacterial pathogenicity was not strictly evidenced, the database was  
230 informative and widely used in recent studies [38,39]. The quality-controlled full-length 16S  
231 rRNA gene sequences in the pathogen list were retrieved from the EzBioCloud database [40].  
232 To identify the pathogens in our samples, the PacBio sequencing data were blasted with the  
233 pathogen database with the criteria of sequence identity > 99%, E-value <  $1 \times 10^{-10}$ , and  
234 coverage > 1300 bp [41]. To compare and assess the performance of the Illumina next-  
235 generation sequencing in identifying pathogens, the variable regions V4-V5 were extracted  
236 from the full-length 16S rRNA gene from the PacBio sequencing, and the used primers were  
237 specified from the previous study [11].

238 Pathogen sequences were aligned using the MAFFT [42], and the phylogenetic tree was  
239 constructed using the FastTree [43] and visualized using the iTOL [44].

## 240 *2.9 Bacterial assembly process*

241 In microbial ecology, both the stochastic (e.g., dispersal and drift) and the deterministic (e.g.,  
242 environment selection) processes were important for their contributions to the bacterial  
243 community assembly [45]. To determine the importance of the stochastic process to  
244 microbial community assembly, the Sloan neutral model was constructed by predicting the  
245 occurrence of the OTUs in a local sample and the metacommunity (e.g., all FM samples) [46].  
246 In the model, death, growth, and dispersal rates of OTUs were assumed to be equivalent.  
247 Thus, the model considers that the stochastic process drives the microbial assembly of a  
248 sample. A single free parameter,  $m$ , is a random loss of an OTU in a local community that  
249 would be replaced by the dispersal from the metacommunity and can be interpreted as a  
250 proxy of dispersal potential. The calculations were performed with the MicEco R package.

251 The relative importance of the stochastic and deterministic processes was quantified using the  
252 null model analysis. In brief, the observed taxa were divided into different groups (so-called  
253 ‘bins’) based on their phylogenetic relationships. The process governing each bin was  
254 quantified based on the phylogenetic diversity using the beta Net Relatedness Index ( $\beta$ NRI)  
255 and taxonomic  $\beta$ -diversities using the Bray-Curtis-based Raup-Crick metric (RC). In terms of  
256 the deterministic process, the homogenous selection usually resulted in communities more  
257 similar, which was in contrast to the heterogeneous selection. Therefore, for each bin, the  
258 pairwise comparison of  $\beta$ NRI value of  $> 1.96$  or  $< -1.96$  suggested the phylogenetic turnover  
259 is greater than the null expectation and was regarded as the heterogenous and homogeneous  
260 selection, respectively. Subsequently, taxonomic  $\beta$ -diversities (i.e., RC) further partition the  
261 stochastic process when the absolute value of  $\beta$ NRI was less than 1.96. The pairwise  
262 comparisons with  $RC > 0.95$  are regarded as dispersal limitation, while those  $< 0.95$  as  
263 homogenizing dispersal. The remaining process (i.e.,  $|\beta \text{ NRI}| \leq 1.96$  and  $|RC| \leq 0.95$ )  
264 represented the percentage of drift, weak selection, diversification, and/or weak dispersal and  
265 was designated as “drift” hereafter. The fraction of the ecological processes in each bin was

266 weighted by their relative abundance and summarized at the community level. The triplicates  
267 of each sample type were averaged, before the quantification of the ecological processes  
268 using the iCAMP R package [47]. In addition, to decipher the assembly process of the  
269 pathogenic and non-pathogenic bacteria from FMs, stones, and seawater, those were  
270 extracted from the total bacterial community.

## 271 *2.10 Statistical analyses*

272 An unweighted-pair group method with arithmetic (UPGMA) dendrogram based on the Bray-  
273 Curtis similarities was used to visualize beta diversity. A similarity profile test (SIMPROF,  
274 PRIMER 6) was performed on the null hypothesis that a specific sub-cluster can be recreated  
275 by permuting the entry species and samples. The significant branch was used as a prerequisite  
276 for defining a bacterial cluster. Bacterial community difference was tested using  
277 permutational multivariate analysis of variance (PERMANOVA) [48], and the homogeneity  
278 of variances was respected using the *betadisper* test of the vegan R package. The student's t-  
279 test was performed using GraphPad Prism 9, and further adjusted with the Benjamini-  
280 Hochberg method to reduce the false discovery rate (FDR) at 5%. The redundancy analysis  
281 was performed using the vegan R package.

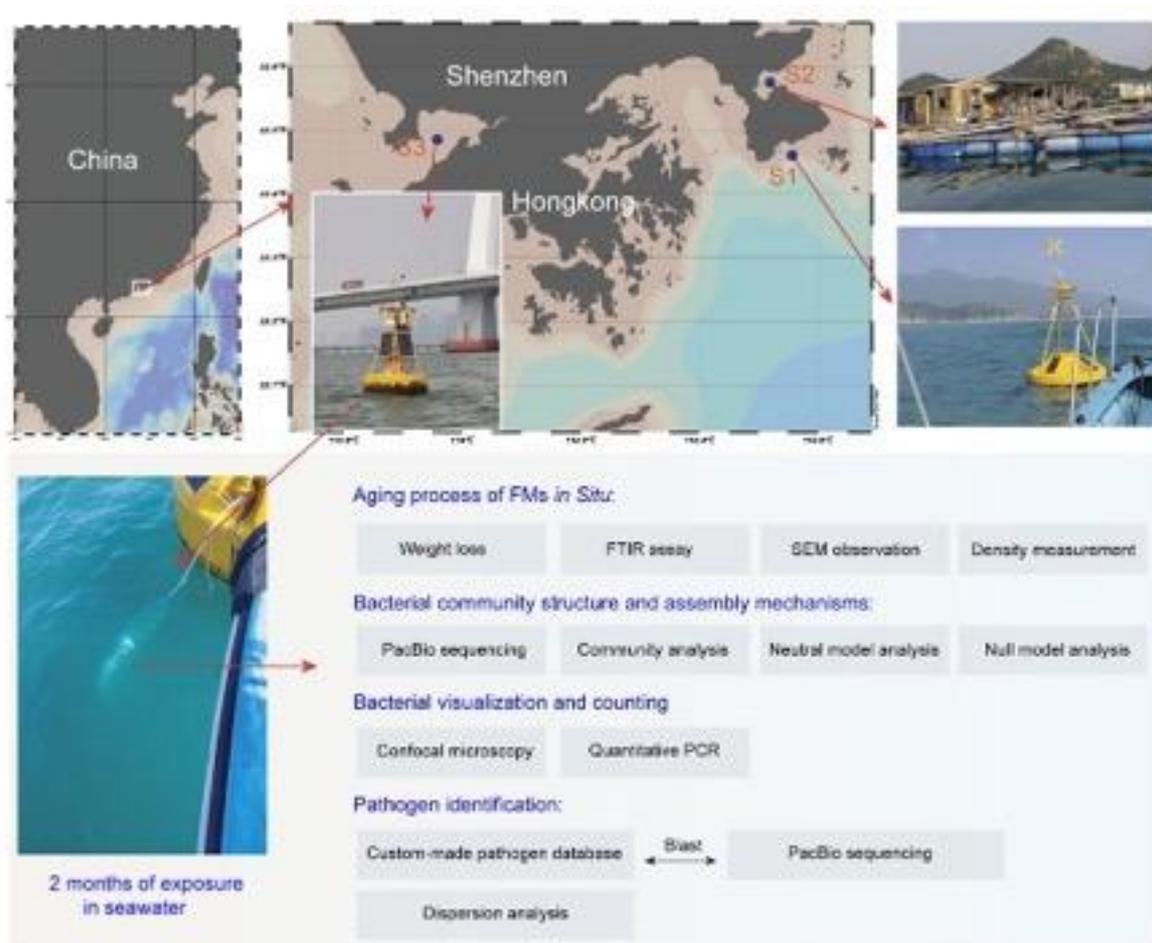
## 282 **3 Results**

### 283 *3.1 Biofouling and physiochemical properties of FMs after exposure*

284 FMs were exposed to the coastal water for two months from three stations *in situ*,  
285 representing different anthropogenic influences, including an open-water station at a distance  
286 of 200 m distance from the coast, a bay with intensive aquaculture (around 4 km<sup>2</sup>), and the  
287 Shenzhen Bay (around 70 km<sup>2</sup>) that is semi-enclosed by the metropolis in China (Shenzhen),  
288 with 17 million inhabitants (designated as S1, S2, and S3 hereafter) (Figure 1). After 2

289 months of exposure, the aging process of FMs was assessed, and the microbial community  
290 profiles and pathogen identification were characterized mainly based on the PacBio  
291 sequencing results.

292 Station S3 had higher concentrations of total organic matter (TOC), nitrite ( $\text{NO}_2^-$ ), nitrate  
293 ( $\text{NO}_3^-$ ), and inorganic phosphate ( $\text{PO}_4^{3-}$ ) compared to S2, followed by S1 (Table S1). These  
294 results indicated that station S3 was more impacted by human activity. The three stations'  
295 temperature, pH, salinity, and dissolved oxygen were around  $20.1 \pm 1.2$  °C,  $8.2 \pm 0.1$ ,  $33 \pm 1.9$   
296 PSU, and  $7.8 \pm 1.1$  mg/L (Table S1).



297

298 Figure 1. Experimental design. Schematic representation of the FM exposure and research  
299 methods, consisting of the assessment of the FM aging process *in situ*, bacterial community  
300 profiling, and pathogen identification.

301 After 2 months of incubation, the FMs' colors changed from blue (pristine, Figure 2a) to grey  
302 because of biofouling (Figure 2b-d). Additionally, high biofouling was visually observed in  
303 S2 compared to S1 and S3 (Figure 2b-d). A wide array of marine organisms was observed on  
304 FMs after the exposure. Amphipods (*Gammarus* sp.) and barnacles (*Balanus amphitrite*)  
305 colonized FMs from all stations (Figure 2 e-h). Mussels (*Perna viridis*) were found in the S1,  
306 with five individuals in maximum observed in one FM's surface (Figure 2i). FMs in S2 had  
307 severe biofouling by macroorganisms consisting of seaweeds (*Gelidium amansii*) and tube-  
308 forming serpulid worms (*Hydroides elegans*) (Figure 2c).

309 FM deterioration was observed from the S1, S3, and to a lesser extent, S2. For example,  
310 damages in FMs were visible after the exposure in S1 (Figure 2j). Besides, the broken  
311 microfibers were observed at the edges of the exterior shells of barnacles (Figure 2f). It  
312 seemed that microplastics and barnacle soft tissue were cross-linked (Figure 2g&h). To  
313 accurately measure the weight loss of the FMs after exposure, the biofilm of FMs was  
314 removed with a rigorous nitric acid digestion process. Triplicate samples from S1 and pristine  
315 controls were used for the analyses. The decoloring effect was observed for the pristine  
316 (Figure 2k) and the aged FMs (Figure 2l). The colors changed from blue to white. In addition,  
317 elastic ear bands were dissolved after the acid digestion. After washing and drying, 3% of  
318 weight loss was detected for the aged compared to the pristine one (t-test,  $p < 0.05$ ), i.e., 70  
319 mg of microplastics released into the ocean from a single FM. The surface topography of  
320 FMs has switched from flat (pristine one, Figure 2m) to rough (aged one, Figure 2n).  
321 Microplastics were detected for the aged FMs after the acid digestion (Figure 2o). The  
322 colonization of microorganisms on the FM surface was observed using the confocal  
323 microscope. As expected, bacteria-like structures were appeared in all FMs from the three  
324 stations and mainly patchily distributed on the FM surface (Figure 2p-r). In specific areas, the  
325 dense bacterial morphotypes formed aggregates, developing biofilm-like structures. Hence,

326 the distribution of the bacteria on the FM surface was heterogeneous. The bacterial  
327 abundances in FMs were further counted, which reached  $1.9 \times 10^6$  (Standard deviation, i.e.,  
328  $SD = 1.9 \times 10^6$ ),  $1.4 \times 10^6$  ( $SD = 4.9 \times 10^5$ ), and  $2.9 \times 10^6$  ( $SD = 4.3 \times 10^5$ ) cells/cm<sup>2</sup> for the  
329 FMs in the station S1, S2, and S3, respectively. By contrast, the bacterial abundance was  
330 determined using quantitative PCR with specific primers for the FMs, stones, and seawater  
331 samples. The bacterial abundances of FM samples were  $7.6 \times 10^3$  ( $SD = 9.2 \times 10^3$ ),  $1.3 \times 10^4$   
332 ( $SD = 2.2 \times 10^4$ ), and  $7.8 \times 10^4$  ( $SD = 4.2 \times 10^4$ ) cells/cm<sup>2</sup> in the station S1, S2, and S3,  
333 respectively. Therefore, the bacterial abundance of FMs measured by the confocal  
334 microscopy was higher than that of qPCR.

335 For the qPCR results of the stones and seawater samples, the bacterial abundances of stone  
336 samples were  $1.4 \times 10^4$  ( $SD = 1.6 \times 10^4$ ),  $3.9 \times 10^3$  ( $SD = 2.2 \times 10^3$ ), and  $3.3 \times 10^4$  ( $SD = 4.8$   
337  $\times 10^4$ ) cells/cm<sup>2</sup> in the station S1, S2, and S3, respectively. No significant difference was  
338 found between the FMs and stone samples ( $p > 0.05$ ). The bacterial abundances of seawater  
339 samples were  $9.5 \times 10^4$  ( $SD = 6.0 \times 10^4$ ),  $4.0 \times 10^5$  ( $SD = 9.5 \times 10^4$ ), and  $8.1 \times 10^4$  ( $SD = 4.4$   
340  $\times 10^4$ ) cells/mL for the seawater in the station S1, S2, and S3, respectively.

341 Due to the formation of the biofilm, the density of the FMs has been increased after the 2-  
342 month exposure. The density of FMs was determined for their outer layers from station S1.  
343 We found that FM aliquots can be suspended in seawater at a salinity of 17 (Figure 2s), for  
344 which the density was around 1.02 g/cm<sup>3</sup>. The salinity of the three stations was between 31  
345 and 35 (Table S1). Therefore, the released microplastics can be floated in the marine  
346 environment.

347 The results from scanning electron microscopy (SEM) showed that the diameter of the outer  
348 layer of fibers was around 20  $\mu$ m. A smooth surface was observed for the pristine FMs  
349 (Figure 3 a-b). By contrast, cracks and rough surfaces appeared for the aged ones (Figure 3 c-

350 h). Dense bacteria-like structures were found in the aged FMs after exposure. Besides, a flake  
351 structure was found in S2 and could be a thick layer of a microbial mat.

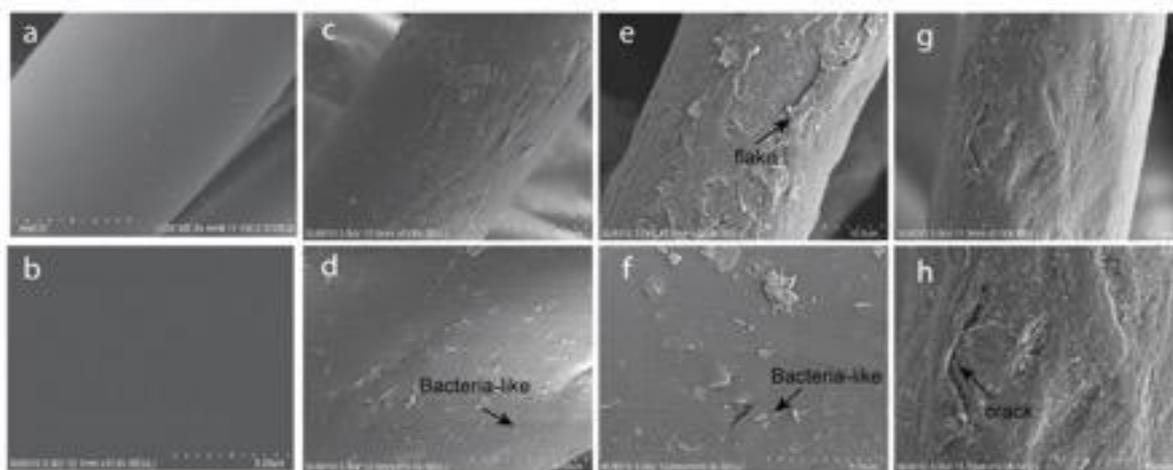
352



353

354 Figure 2. Biofouling and surface deterioration of FMs. Panel a represented the pristine FMs. Panels b-  
355 d represented FMs after 2 months of exposure from station S1, station S2, and station S3, respectively.  
356 Panels e-f represented amphipod (*Gammarus* sp.) and barnacles (*Balanus amphitrite*) that were found  
357 at all stations. Panels g-h represented the soft tissue of barnacles. Panel i represented mussels (*Perna*  
358 *viridis*) that were found in the S1. Panel j represented damages in the three layers of FMs after the

359 exposure. Panel k and l represented the pristine and weathered FMs after the treatment of the acid  
360 digestion process. Panel m and n represented the observation of pristine and weathered FMs using the  
361 stereomicroscope. Panel p represented the microplastic released from FMs. Arrows in panels g-h  
362 represented microplastics. Panels p-r represented confocal microscopy observation of FMs after the  
363 DAPI staining. The scale bars were 50  $\mu\text{m}$ . Panel s represented the density measurement of FM  
364 aliquots.



365

366 Figure 3. Scanning electron microscopy observation of FMs. Panels a and b represented pristine FMs.  
367 Panels c and d represented aged FMs from S1. Panels e and f represented aged FMs from S2. Panels g  
368 and h represented aged FMs from S3.

369 FTIR was used to identify the chemical composition of the FMs. For the pristine FMs, the  
370 three layers were made of polypropylene and the elastic ear bands were made of polyamide 6  
371 (Figure S1 a-b), which was susceptible to acid digestion, as mentioned above. The outer layer  
372 of pristine FMs was subjected to a hydrogen dioxide process, exhibiting good resistance  
373 (Figure S1 b-c). No novel functional groups were observed for the aged FMs after the  
374 hydrogen dioxide process (Figure S1 d-f). The carbonyl groups around  $1715\text{ cm}^{-1}$  can be used  
375 as the proxy of oxidation levels, which were not visually observed from FTIR spectra of  
376 pristine and aged FMs. The values of carbonyl index (CI) of the pristine and aged FMs were  
377  $0.38 \pm 0.03$  (pristine FMs),  $0.37 \pm 0.01$  (aged FMs from S1),  $0.39 \pm 0.01$  (aged FMs from S2),

378 and  $0.33 \pm 0.03$  (aged FMs from S3), respectively. No differences were found for the  
379 pairwise comparisons of the CI value between the pristine FMs and the aged FMs (t-test,  $p >$   
380  $0.05$ ), indicating that the FMs did not undergo photodegradation.

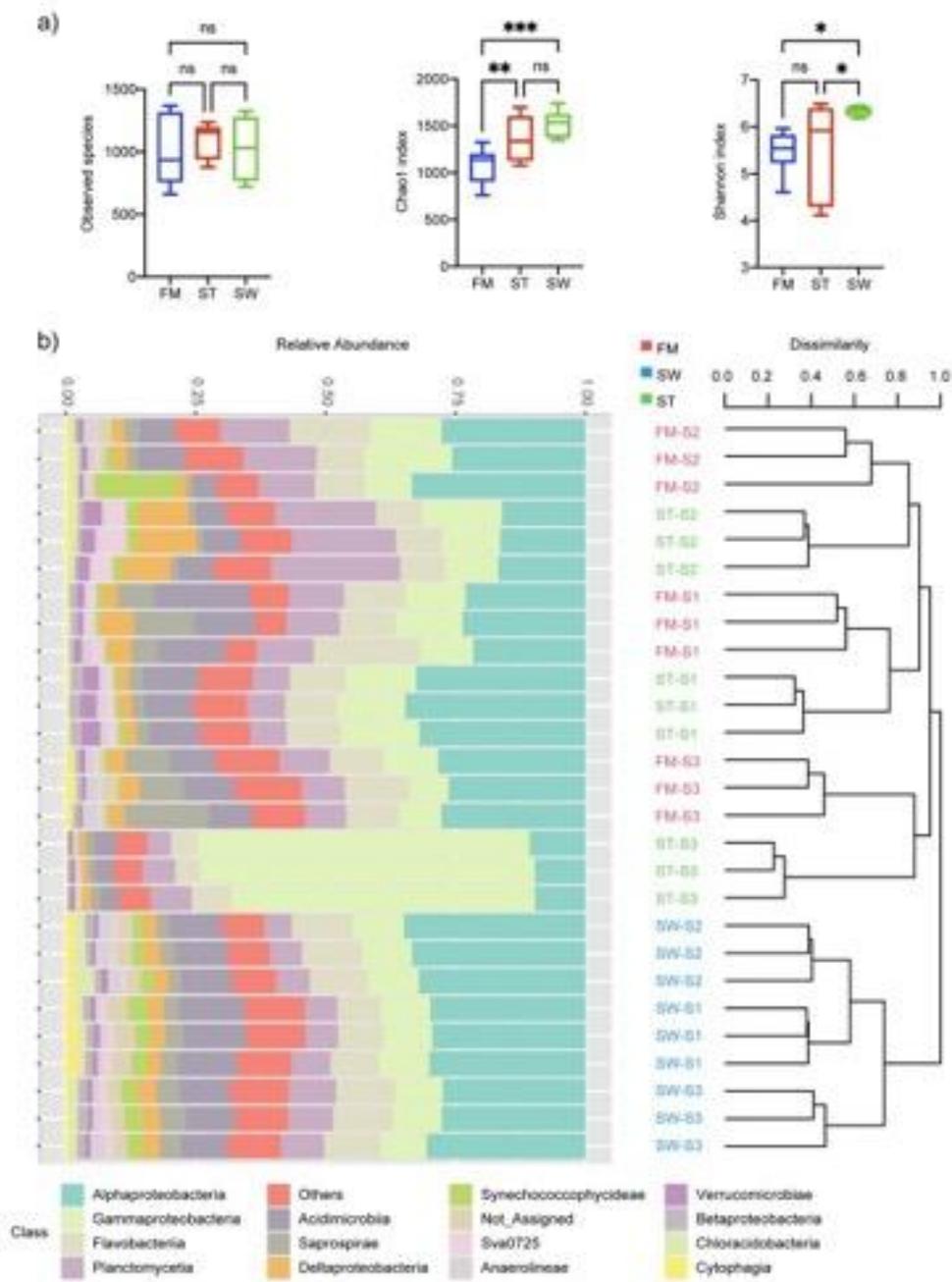
381 Loosely attached microplastics were extracted from the FMs after ten rounds of ultrasonic  
382 baths. It turned out that the aged FMs (Figure 2o) released more microplastics than the  
383 pristine ones (64 fibers *versus* 18 fibers) (t-test,  $p < 0.05$ ). Assuming each layer of FMs  
384 contributed equally to the weight loss, which was 70 mg, and with a diameter of 20  $\mu\text{m}$  (as  
385 indicated in Figure 3), the average length of the released microplastics was calculated to be  
386 889  $\mu\text{m}$  (measured after acid digestion). With a density of polypropylene at  $0.9 \text{ g/cm}^3$ , it was  
387 estimated that one FM could potentially release up to  $9 \times 10^4$  microplastic items during  
388 exposure in the marine environment.

### 389 *3.2 Bacterial community dynamics*

390 PacBio sequencing generated 542,589 sequence tags, falling into 6090 OTUs after randomly  
391 resampling to the lowest number of sequencing tags of 7041 at a 99% similarity threshold.  
392 No difference was found in the observed species between them (t-test,  $p > 0.05$ ). The  
393 bacterial community differences were revealed in other alpha diversity indices. The indices of  
394 the Chao1 richness and the Shannon diversity were higher in the seawater compared to FMs  
395 (t-test,  $p < 0.05$ ) (Figure 4a).

396 UPGMA dendrogram showed that biofilms formed on FMs and black agate stones differed  
397 from seawater samples (SW), with strong dissimilarity observed between these two clades ( $>$   
398 99%). For all the FMs and stone samples, the driving force of bacterial community structure  
399 was shaped by the geographical origins (Dissimilarity of 94% between S1 and (S2 and S3)),  
400 and to a lesser extent, by the material types (Dissimilarity of 74-86% between FMs and the  
401 stones) (Figure 4b). The PERMANOVA results confirmed significant differences among

402 sample types (plastisphere and seawater,  $p < 0.01$ ) or sample stations (S1, S2, and S3,  $p <$   
 403 0.01). The environment parameters were used to detect the variance of the bacterial  
 404 community, and the redundancy analysis showed that less than 3% of the variance could be  
 405 explained for FMs, stones, or seawater.



406

407 Figure 4. Illustration of bacterial alpha diversity (a) and comparison of taxonomic relative abundances  
 408 and community structure of bacteria on the biofilm of FMs, stones (ST), and seawater (SW) by

409 cumulative bar charts comparing relative abundances (left) and by UPGMA dendrogram based on  
410 Bray–Curtis dissimilarities between 16S rRNA-based sequencing profiles (right) (b). Samples were  
411 collected from three stations: an open water station (S1), an aquafarm (S2), and a semi-enclosed bay  
412 of a metropolis (S3). Each sample type was performed in triplicates. P values less than 0.05, 0.01, and  
413 0.001 were designated with one, two, and three asterisks, respectively.

414 Taxonomical analyses confirmed the specificity of the community structure in terms of  
415 sample types. The microbial community was dominated by Alphaproteobacteria and  
416 Flavobacteriia for FM samples ( $25.8 \pm 3.9\%$  and  $12.8 \pm 4.6\%$ , respectively),  
417 Alphaproteobacteria and Gammaproteobacteria for stone samples ( $19.6 \pm 10.8\%$  and  $30.5 \pm$   
418  $20.6\%$ , respectively), and Alphaproteobacteria and Acidimicrobiia for seawater samples ( $30.9$   
419  $\pm 3.6\%$  and  $11.2 \pm 1.4\%$ , respectively). The difference in the class level was significant for  
420 specific taxa. For example, Alphaproteobacteria had a higher proportion in seawater samples  
421 than in FMs or stone samples (FDR < 0.05) (Figure 4b). We further investigated the top 3  
422 OTUs in each sample type (Fig. S2). *Pseudomonas azotoformans* and an unclassified OTU of  
423 Bacteroidetes were particularly dominant for the FM samples. *Aliiroseovarius halocynthiae*,  
424 *Rhodopirellula* sp., and *Psychrobacter nivimaris* were more abundant in stone samples. By  
425 contrast, two unclassified OTUs of Rhodobacteraceae were more abundant in seawater  
426 samples.

### 427 3.3 Bacterial pathogen compositions

428 A bacterial pathogen list was constructed from the Enhanced Infectious Disease Database  
429 (EID2), containing 2252 bacterial species, which have been shown to infect a broad spectrum  
430 of hosts, such as humans, fish, plants, and some invertebrates (Table S2-11). The pathogen  
431 sequences were extracted from the EzBioCloud database, which provided standardized 16S  
432 rRNA sequences between the two most popular PCR primers (27F-1492R), which are the

433 same as the ones used in the PacBio sequencing. The PacBio sequencing results were blasted  
434 to the curated pathogen database, and a total of 191 sequences had significant alignments,  
435 assigned to 70 pathogen species. FMs and stones had a higher putative pathogen prevalence  
436 compared to seawater samples, with 21, 67, and 4 pathogen species found in the three sample  
437 types, respectively (5%, 27%, and 0.01% of the average relative abundance) (Figure 5).  
438 Besides, a phylogenetic tree was constructed for the pathogen species with a relative  
439 abundance higher than 0.1% in a sample type (Figure 5). Three pathogen species were  
440 specifically detected in FM samples. *Ralstonia pickettii* were more abundant in the  
441 plastisphere of FM samples (0.15%) than that in stone samples or seawater ( $p < 0.05$ ). It is  
442 noteworthy that *Ralstonia pickettii* was not found in stone or seawater samples and could  
443 infect a wide range of species, such as humans and arthropods. Besides, *Bacillus*  
444 *amyloliquefaciens* and *Pseudomonas poae* were specifically detected in the FM samples  
445 (0.018% and 0.007%, respectively). For the two pathogen species, *Bacillus amyloliquefaciens*  
446 can infect plants and arthropods, and *Pseudomonas poae* can infect plants and birds.  
447 Moreover, sixteen pathogen species were detected in FM and controls, such as *Acinetobacter*  
448 *johnsonii* (0.01% in FM samples, pathogen of arthropods, plants, humans, fish, and green  
449 algae), *Brevundimonas diminuta* (0.01% in FM samples, pathogen of arthropods, plants, and  
450 humans), *Delftia lacustris* (0.01% in FM samples, pathogen of arthropods), *Enterobacter*  
451 *hormaechei* (0.01% in FM samples, pathogen of arthropods, plants, humans, and fish),  
452 *Escherichia fergusonii* (0.01% in FM samples, pathogen of arthropods and plants),  
453 *Exiguobacterium indicum* (0.004% in FM samples, pathogen of arthropods and fish),  
454 *Exiguobacterium profundum* (0.004% in FM samples, pathogen of arthropods and plants),  
455 *Limnobacter thiooxidans* (0.05% in FM samples, pathogen of plants), *Pseudomonas*  
456 *azotoformans* (0.06% in FM samples, pathogen of plants), *Pseudomonas costantinii* (3.5% in  
457 FM samples, pathogen of fungi), *Psychrobacter cibarius* (0.01% in FM samples, pathogen of

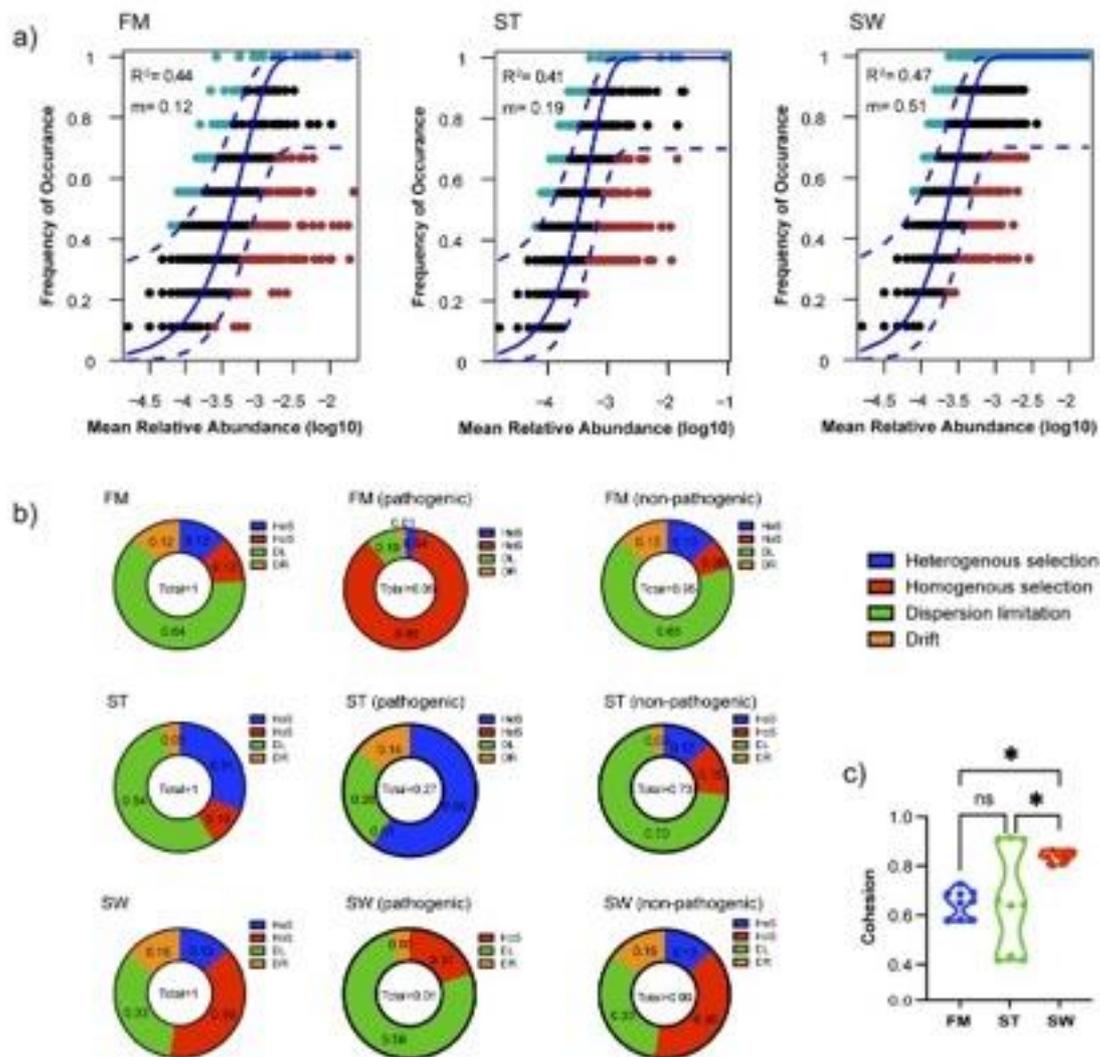


475 Figure 5. Pathogens identified by the PacBio full-length 16S rRNA sequencing. The phylogenetic tree  
476 was constructed for pathogens with relative abundances higher than 0.1% in a sample type. The  
477 branch colors represented taxonomies at the phylum level. Bubble size represented the relative  
478 abundance. The Venn plot showed the number of specific and shared OTUs within different sample  
479 types. The right panel showed the pathogen target spectra.

### 480 *3.4 Community assembly processes*

481 The Sloan neutral model was used to better explore the importance of stochastic processes in  
482 determining bacterial community assembly (Figure 6a). Based on the  $R^2$  value (0.44, 0.41,  
483 and 0.47 for FMs, stones, and seawater, respectively) and the occurrences of the OTUs within  
484 the model prediction (89%, 88%, and 86% for FMs, stones, and seawater, respectively), the  
485 results indicated that the microbial communities in FMs, stones, and seawater were well  
486 described by the neutral model, and the stochastic processes are very important in shaping the  
487 bacterial community assembly. The  $m$  value was higher in seawater samples than in the  
488 stones or FM samples, indicating a higher dispersion potential for the seawater samples. The  
489 relative importance of different assembly processes was further classified, and it turned out  
490 that dispersion limitation was the main driver for the FMs and stone samples, whereas  
491 homogenous selection and dispersion limitation for the seawater samples (Figure 6b). In  
492 detail, the assembly processes of non-pathogenic and pathogenic bacteria in the platisphere  
493 of FMs were further determined. As a subpopulation, it showed that the assembly processes  
494 of the non-pathogenic bacterial assembly process are similar to that of the total bacterial  
495 community from the platisphere. However, the assembly process of pathogens from the  
496 platisphere is dominated by homogeneous selection and less impacted by the dispersion  
497 limitation (Figure 6b). Variations were also found between the non-pathogenic and  
498 pathogenic bacteria in stones and seawater samples (Figure 6b).

499 The cohesion index was calculated to determine the stability of the microbial community. No  
 500 significant difference was found between FMs and stone samples (0.64 *versus* 0.66).  
 501 However, the cohesion value of FMs is significantly lower than that of seawater (0.64 *versus*  
 502 0.84) ( $p < 0.05$ ), indicating that the microbial community in FMs is less stable than that in  
 503 seawater (Figure 6c).



504

505 Figure 6. Bacterial community parameters depicted by the neutral community model (a), the null  
 506 model analysis (b), and the cohesion results (c). In panel a, dots represented OTUs that occurred more  
 507 or less frequently predicted by the neutral model. The predicted occurrence frequency was shown as  
 508 the solid blue line, and the dashed blue line indicated the 95% confidence interval. The R<sup>2</sup> indicated

509 the fit to the model, and the  $m$  value indicated the migration potential of the metacommunity. The  
510 values at the center of the circles of panel b represented the relative bacterial abundance.

511

## 512 **4 Discussion**

### 513 *4.1 A methodology developed for pathogen identification in the plastisphere of FMs*

514 A growing number of studies have been conducted to assess the influence of the plastisphere  
515 on marine ecosystems, and the pathogens were of increasing concern. We summarized 25  
516 studies illustrated in two recent reviews concerning marine plastisphere-associated pathogens  
517 [21,49], and it showed that about 70% of studies adopted the technique of second-generation  
518 sequencing, which is characterized by short reads (less than 500 base pairs in general),  
519 usually targeted at viable regions of V3-V4 or V4-V5 of the 16S rRNA gene (Table S13)  
520 [50,51]. However, the resolution of pathogen classification of this technique was low and  
521 generally at the genus level, making it difficult to know the exact pathogen species. Instead,  
522 some studies adopted other techniques, such as bacterial isolation [52], selective medium [53],  
523 and fluorescence in situ hybridization (FISH). These techniques improved the accuracy.  
524 However, information was limited to specific pathogen types (Table S13) [54]. Previously, it  
525 has been shown that PacBio circular consensus sequencing could provide a near-zero error  
526 rate in the measurement of full-length 16S rRNA [55], which, in turn, can provide the  
527 taxonomic resolution of bacterial communities at the species level [56]. Therefore, it is  
528 plausible to use PacBio circular sequencing for putative pathogen identification. Herein, we  
529 constructed the pathogen database containing a wide range of hosts (e.g., humans, fish, plants)  
530 [37], and pathogen sequences were retrieved from the EzBioCloud database [40]. After  
531 alignments between the PacBio circular sequencing data and the pathogen database, we  
532 detected 70 putative pathogen species. *Vibrio* spp., as serious conditional pathogens from  
533 marine environments, attracted great attention in the plastisphere (Table S13) [57]. In our  
534 study, two *Vibrio* species, i.e., *Vibrio brasiliensis* (0.01%) and *Vibrio owensii* (0.004%) in the  
535 plastisphere were identified, served as pathogens for arthropods [58] and corals [59],

536 respectively, and may have an effect on marine creatures. The results also showed selective  
537 enrichments of pathogens in the plastisphere, such as *Ralstonia pickettii*. It is noteworthy that  
538 *Ralstonia pickettii* is a hydrocarbonoclastic bacterium [60,61]. Therefore, the labile organic  
539 matter from FMs may promote pathogen growth, and FMs can provide a new niche for those  
540 pathogens, as suggested from previous study [62].

541 Additionally, previous studies widely performed pathogen identification *via* alignments (i.e.,  
542 BLAST [41]) between the Illumina sequencing data and their pathogen database at a 99%  
543 similarity threshold [63–65]. To evaluate its performance, we extracted the V4-V5 regions of  
544 16S rRNA genes from our PacBio data, representing the Illumina sequencing result. We  
545 found that 34% of taxa (determined using V4-V5 regions of 16S rRNA gene) were not  
546 detected in our pathogen results (PacBio full-length of 16S rRNA), indicating that the  
547 Illumina sequencing will overestimate the pathogen results.

548 The PacBio sequencing can provide an advantage in pathogen detection compared to the  
549 traditional isolation method in the case of bacteria with unusual phenotypic profiles, rare  
550 bacteria, slow-growing bacteria, uncultivable bacteria, and the pathogen complex [66]. While  
551 there were some limitations to this technique in discriminating bacterial species with close  
552 phylogenetic relationships, which shared high similarity for their 16S rRNA genes (e.g.,  
553 some species of *Bacillus* spp. and *Streptococcus* spp. elaborated from previous study) [67].  
554 Nevertheless, there is great promise for PacBio sequencing to be applied to pathogen  
555 identification, regardless of environmental surveillance or clinical microbiology laboratories.

#### 556 *4.2 Deterioration of FMs and its impact on the bacterial communities of the plastisphere*

557 A significant number of microplastics can be released to the environment, aggravating  
558 current environment plastic pollution [68–70]. Many broken microfibrils were located at the  
559 edges of the exterior shells of barnacles (*Balanus amphitrite*), indicative of biotic

560 deterioration processes, probably resulting from mechanical abrasion (Figure 2). In parallel,  
561 abiotic deterioration played an important role in FM deterioration, and much roughness was  
562 observed on the FM surface after exposure (Figure 2 and Figure 3). The technique of FTIR  
563 can be used to identify polymer types and assess the relative levels of polymer surface  
564 oxidation [71]. FMs showed the appearance of a carbonyl band of the FTIR spectra after  
565 simulating the sunlight aging [72]. On the contrary, this study showed the absence of a  
566 characteristic carbonyl band, suggesting that the photo degradation of plastic is relatively  
567 slow in marine environments. In the laboratory, pristine FMs can release hundreds to millions  
568 of microplastics after shaking or stirring [73]. In our condition, we found a 3% weight loss  
569 for FMs, which could be similar to the real condition because the pristine FMs were  
570 performed as the controls in parallel. By examining the hydrologic condition Field [75], we  
571 found that the water flow can reach 0.3 m/s for station S1 during the winter, indicating that  
572 such a water flow can lead to FM deterioration. During this study, it is estimated that up to  $9$   
573  $\times 10^4$  can be released into the marine environment. The results could be overestimated, and  
574 the mechanical abrasion can lead to the formation of the pills and decrease the number of  
575 microplastics released from the fabrics [74].

576 The formation of biofilm can increase the plastic density [75]. The bulk FMs with the  
577 macroorganisms (e.g., barnacles) were found to be sinking in seawater (data not shown).  
578 However, a salinity of 17 can make the FM aliquots (without macroorganisms) suspended in  
579 this work, suggesting that the released microplastics from this study were floated in the  
580 surface ocean. Indeed, a recent study underlines that the deterioration of sinking plastics can  
581 result in smaller pieces regaining buoyancy and returning to the surface [76]. Therefore, FMs  
582 can have impacts on pelagic and benthic environments.

583 From this study, it is inferred that the bacterial communities in the plastisphere will change as  
584 microplastic transport through different oceanographic areas. Initially, the detached  
585 microplastics from FMs can have similar bacterial and pathogen compositions to those on  
586 FMs. Our previous study showed that polymer size or shape had no significant impact on the  
587 bacterial composition in the plastisphere [11]. Subsequently, along with the microplastic  
588 transport, the alterations of the bacterial community were expected. First, the richness and  
589 cohesion indices of bacterial communities were calculated and used as a proxy for bacterial  
590 stability [77,78]. The Chao1 richness and cohesion results were lower in FMs than in  
591 seawater, indicating bacteria in the plastisphere were less stable under environmental change  
592 (i.e., microplastic transport). Second, our study showed that geography could significantly  
593 impact the bacterial community in the plastisphere, which was supported by previous studies  
594 [79,80]. Therefore, changes in bacterial communities of the plastisphere can occur during  
595 microplastic transport. On the other hand, microplastic transport in the marine environment  
596 can result in bacterial dispersion because of the changes in the environmental conditions [24].

#### 597 *4.3 Rational basis of microplastics serving as vectors for pathogen transport*

598 Reports on whether pathogens preferentially colonize plastics over other materials in the  
599 environment remain inconsistent, which is likely due to the variable environmental  
600 conditions of each study, with the environmental factors often having a stronger influence on  
601 plastisphere diversity than the polymer type [19,20,81]. The *Vibrio* spp. and *Pseudomonas*  
602 spp. were frequently reported from the previous studies in the marine environment [49].  
603 Except for human pathogens, many pathogens can infect other marine organisms. Therefore,  
604 a comprehensive pathogen database, such as the one constructed in this study, will promote  
605 risk assessment for human beings and ecosystems. For this study, pathogens were  
606 investigated on FMs in comparison to stones and seawater controls. We found a high  
607 prevalence of putative pathogens in FMs compared to seawater (21 taxa in FMs *versus* 4 taxa

608 in seawater). In addition, the putative pathogens in the natural biofilm were characterized in  
609 coastal stones. Selective enrichments were found in both FMs and stone samples, and there  
610 was a low prevalence of the pathogens in FMs compared to stone samples (21 taxa in FMs  
611 *versus* 67 taxa in stones). These results suggested that the pathogen's lifestyle preferred  
612 living in the biofilm of FMs or stones during the winter season. However, the role of FMs  
613 serving as pathogen vectors cannot be neglected. First, the marine environment was  
614 considered the final reservoir of the plastics [82]. A higher prevalence of putative pathogens  
615 on FMs than in seawater indicated that FMs and other plastic debris could be hotspots for the  
616 pathogens in the coastal water and, in a broader context, in the open ocean. Second, selective  
617 enrichments can be found in FMs as compared to stones or seawater samples. Moreover,  
618 microplastics released from the FMs can be vectors for pathogen transport in marine  
619 environments, which is not the situation for pathogens in coastal natural biofilm formed on  
620 stones.

621 There is growing concern about the possibility that pathogens can be transported in the  
622 plastisphere in the marine environment [21]. The assembly mechanism was clarified to infer  
623 the pathogenic bacterial response during microplastic transport. The assembly process of the  
624 pathogens from the plastisphere showed that homogeneous selection explained 85% of the  
625 total ecological processes. Indeed, the homogenous selection is an indicator that the  
626 pathogens were mainly influenced by the same environment selection, resulting in similar  
627 compositions among different stations [45]. A relatively low dispersion limitation (10%) also  
628 indicated that the majority of the putative pathogen could be transported along the coast (100  
629 km in this study) without the occurrence of pathogen dispersion (bacteria escape from the  
630 plastisphere).

631 The released microplastics can serve as vectors for transferring pathogens from the  
632 plastisphere to the organisms [83]. Previous studies showed that microbes living within the  
633 biofilm are highly beneficial and can become more infectious than when free-living [21].  
634 Moreover, microplastics in the form of microfibers can be released from FMs. Fish species  
635 were found to ingest and retain the microfibers in their tissues (*Boops boops*, *Cathorops*  
636 *agassizii*) [84,85]. In fact, oyster *Saccostrea cucullata* at station S3 had an average of 8  
637 microplastic items per individual, mainly in the form of microfiber [86]. This ingestion of  
638 microplastics by marine organisms may lead to diseases in those organisms and impact  
639 human health from the trophic transfer [87]. Besides, since many beaches were located near  
640 station S1, the microplastics released from FMs may create a new route for pathogen  
641 infection in humans.

#### 642 **Concluding Remarks**

643 The chemical composition of the face masks was mainly made of polypropylene. After two  
644 months of exposure to the marine environment, a wide array of microorganisms and  
645 macroorganisms colonized the surface of face masks. Pathogens in the face masks were  
646 investigated using the PacBio sequencing of the 16S rRNA marker gene, showing its  
647 applicability to identify the pathogens at the species level. PacBio sequencing can be used as  
648 a main or auxiliary method in detecting pathogen species in many contexts, such as  
649 aquaculture and wastewater treatment. This study found the enrichment of the putative  
650 pathogens in the plastisphere of FMs compared to seawater in a subtropical coastal  
651 environment with intensive human activity. However, the relative abundance of putative  
652 pathogens in the plastisphere was less than in the coastal stones. Therefore, to conclude  
653 whether the FMs or the plastic litter is a reservoir of pathogens, its profiles in the open ocean  
654 are needed and warrant further studies. Even though the classification of pathogens at the

655 species level has greatly improved the accuracy, the pathogenicity should be revealed in  
656 future.

657 In accordance with other laboratory studies, we found severe deterioration of FMs after  
658 exposure, which could be an important source of microfibers in the marine environment. The  
659 FM aliquots can be suspended at a density of approximately 17 PSU, making the released  
660 microfibers transport potentially in the surface ocean. Our results also showed that assembly  
661 processes were distinct for the pathogenic and non-pathogenic bacteria, and the microfibers  
662 were favorable for transporting specific pathogens in the marine environment, which can  
663 increase the possibility of microbial invasion. In general, the occurrence of pathogens in  
664 nature had spatial and temporal patterns, long-term observations of the pathogen profile  
665 together with the host fitness may significantly advance the environmental epidemiology in  
666 this field.

## 667 **Supporting Information**

668 Figure S1 shows the spectra of the Fourier transform infrared spectroscopy. Figure S2. shows  
669 the relative abundance of the top 3 OTUs in each sample type using the bubble plot. Table S1  
670 shows the physiochemical parameters of the seawater. Table S2-11 shows the putative  
671 pathogens for humans, fish, plants, arthropods, algae, fungi, Mollusca, Cnidaria, rodents, and  
672 others. Table S12 shows the relative abundance of the putative pathogens and their target  
673 spectra. Table S13 shows methods used for pathogen identifications from previous studies.

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