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1 **Deep-sea plastisphere: long-term colonization by plastic-associated bacterial and archaeal**
2 **communities in the Southwest Atlantic Ocean**

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13 **Abstract**

14 Marine plastic pollution is a global concern because of continuous release into the oceans over the
15 last several decades. Although recent studies have made efforts to characterize the so-called
16 plastisphere, or microbial community inhabiting plastic substrates, it is not clear whether the
17 plastisphere is defined as a core community or as a random attachment of microbial cells. Likewise,
18 little is known about the influence of the deep-sea environment on the plastisphere. In our
19 experimental study, we evaluated the microbial colonization on polypropylene pellets and two
20 types of plastic bags: regular high density polyethylene (HDPE) and HDPE with the oxo-
21 biodegradable additive BDA. Gravel was used as control. Samples were deployed at three sites at
22 3,300 m depth in the Southwest Atlantic Ocean and left for microbial colonization for 719 days.
23 For microbial communities analysis, DNA was extracted from the biofilm on plastic and gravel
24 substrates, and then the 16S rRNA was sequenced through the Illumina Miseq platform.
25 Cultivation was performed to isolate strains from the plastic and gravel substrates. Substrate type
26 strongly influenced the microbial composition and structure, while no difference between sites was
27 detected. Although several taxa were shared among plastics, we observed some groups specific for
28 each plastic substrate. These communities comprised taxa previously reported from both epipelagic
29 zones and deep-sea benthic ecosystems. The core microbiome (microbial taxa shared by all plastic
30 substrates) was exclusively composed by low abundance taxa, with some members well-described
31 in the plastisphere and with known plastic-degradation capabilities. Additionally, we obtained
32 bacterial strains that have been previously reported inhabiting plastic substrates and/or degrading
33 hydrocarbon compounds, which corroborates our metabarcoding data and suggests the presence of
34 microbial members potentially active and involved with degradation of these plastics in the deep
35 sea.

36 **Keywords:** core microbiome, microbial colonization, plastics, 16S rRNA

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37 ## 1. Introduction

38 Plastic waste has become a global challenge. Although many countries are involved in public
39 policies to mitigate this problem, tons of plastic waste continue to enter the oceans annually
40 (GESAMP, 2019; Jambeck et al., 2015; PlasticEurope, 2019; Schmidt et al., 2017). As a
41 consequence, marine plastic contamination is ubiquitous, including in remote marine environments
42 such as the deep ocean (Woodal et. al., 2014). In the marine environment, plastic waste is exposed
43 to physical and chemical factors that contribute to the degradation and fragmentation of this
44 material into microplastics (i.e. plastic objects < 5 mm in diameter) (Roager and Sonnenschein,
45 2019). Microplastic chemical composition, density and shape determine whether it is more likely
46 to float or sink, which will influence the distribution in pelagic or benthic ecosystems (Cole et al.,
47 2011; Pierdomenico et al., 2019; Pinnell and Turner, 2019; Van Cauwenberghe et al., 2013;
48 Woodall et al., 2014). Although both macro- and microplastics have already been reported in deep-
49 ocean regions, knowledge regarding plastic colonization by deep-sea prokaryotic communities in
50 both pelagic and benthic ecosystems is incipient (Bergmann and Klages, 2012; Chiba et al., 2018;
51 Galgani et al., 1996; Krause et al., 2020; Pierdomenico et al., 2019; Schlining et al., 2013; Woodall
52 et al., 2018).

53 Plastics can be used as substrata and be rapidly colonized by microorganisms, which form biofilms
54 on the plastic surface, a community we refer to as the “plastisphere” (Zettler et al., 2013). The
55 ability to colonize and metabolize substrate surfaces is a mechanism that promotes advantages for
56 microorganisms in situations with nutritional limitation (Dang and Lovell, 2016), as can be the
57 case in deep-sea regions. Plastic substrates in the deep ocean may offer a new hotspot of
58 colonization, as well as a relevant source of carbon to support the microbial community,
59 proportionally magnifying the abundance of potentially plastic degrading microorganisms within

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60 the plastisphere. A growing number of studies have reported microorganisms capable of degrading
61 hydrocarbons (Didier et al., 2017; Oberbeckmann et al., 2017), raising the hypothesis that they
62 would be consuming this material in nutritionally limited conditions, such as those found in deep-
63 sea ecosystems.

64 In addition to the influence of the environment (which can be prone to microbial colonization or
65 not), polymer composition is suggested to modulate the structure and composition of the
66 plastisphere (Dussud et al., 2018a; Dussud et al., 2018b; Kirstein et al., 2018, 2019; McCormick
67 et al., 2014; Pinto et al., 2019; Zettler et al., 2013). Moreover, geographic locations have also been
68 indicated to exert influence on the microbial community of the plastisphere (Oberbeckmann et al.,
69 2016). In contrast, independent of environmental factors or plastic substrate type, some microbial
70 taxa have been reported in multiple plastispheres (Tu et al., 2020; Zettler et al., 2013) and they are
71 now referred to as "core microbiome". The core microbiome members comprise taxa with high
72 occupancy across a dataset that are hypothesized to reflect functional relationships with the host
73 (or substrate) (Shade and Handelsman, 2012). In the plastisphere, these members are thought to be
74 involved in biofilm formation and/or metabolizing compounds from the plastic substrate (Didier
75 et al., 2017; Tu et al., 2020). Bacterial families such as Oleiphilaceae and Hyphomonadaceae have
76 been found colonizing multiple substrates (Bryant et al., 2016; De Tender et al., 2017;
77 Oberbeckmann et al., 2018; Pinto et al., 2019; Zettler et al., 2013). The known capacity of these
78 families to metabolize hydrocarbon compounds may be the key functional trait enabling them to
79 colonize different plastics (Golyshin et al., 2002; Ozaki et al., 2007).

80 In the last decade, the number of studies on the plastisphere has increased (Amaral-Zettler et al.,
81 2020). However, efforts have been concentrated mainly on epipelagic (e.g. Bryant et al., 2016;
82 Carson et al., 2013; Zettler et al., 2013) or shallow benthic systems (Pinnel and Turner, 2019).

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83 Only a few studies, however, analyzed the plastisphere in deep-sea habitats (Krause et. al., 2020;
84 Woodal et. al, 2018). Studies that assessed marine plastisphere microbial communities can be
85 classified into three basic groups: those that randomly collected plastic marine debris (PMD) or
86 microplastics (Bryant et al., 2016; De Tender et al., 2015; Zettler et al., 2013); studies that deployed
87 plastic substrates in the ocean (Oberbeckmann et al., 2016; Tu et al., 2020) and those conducted in
88 laboratory conditions (Kirstein et al., 2018; Ogonowski et al., 2018). The microbial community
89 structure inhabiting deployed plastic substrates for long periods is as yet poorly studied (Kirstein
90 et al., 2018; Oberbeckmann et al., 2016, 2018; Tu et al., 2020). Based on these studies, the marine
91 plastic bacterial community is mainly represented by the Gammaproteobacteria,
92 Alphaproteobacteria and Bacteroidetes classes, while the archaeal community is represented by
93 the Euryarchaeota and Crenarchaeota phyla (Eriksen et al., 2014; Oberbeckmann et al., 2018;
94 Oberbeckmann and Labrenz, 2020; Quero and Luna, 2017; Urbanek et al., 2018; Woodall et al.,
95 2018).

96 Here, we conducted the first *in situ* experimental study that characterized the structure and
97 composition of the microbial community (the bacterial and archaeal) associated with different
98 types of plastic substrates deployed for 719 days at three sites in deep waters in the Southwest
99 (SW) Atlantic Ocean (3,300 m). Our objectives in this study were to understand (i) if there are
100 differences in microbial communities inhabiting plastics polymers as opposed to control samples
101 and adjacent seawater, and differences among multiple sites, (ii) if there is a core microbiome
102 among plastics that can contribute to the description of the plastisphere in the deep SW Atlantic
103 Ocean (different from shallow waters), and (iii) if it is possible to isolate viable bacteria through
104 cultivation of plastic substrates that are potentially related to plastic degradation. For this, we
105 performed a high-throughput sequencing of the 16S rRNA gene to access bacterial and archaeal

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106 communities and used traditional culturing methods to assess the plastics as substrates for
107 microbial growth.

108

109 **2. Materials and Methods**

110 2.1. Studied area and deployment methods

111 Experimental sites are located in a region that encompasses the transition between the North
112 Atlantic Deep Water (NADW) and the Lower Circumpolar Deep Water (LCDW). This region is
113 characterized by water temperature between 3 °C and 4 °C, salinity between 34.6 and 35.0, oxygen
114 concentration above 5 mL $L^{-1}$ and low nutrient levels (i.e. oligotrophic) (Gonzalez-Silvera et al.,
115 2004; Krueger et al., 2015).

116 Autonomous aluminum structures that housed multiple experiments, called landers, were deployed
117 at three sites along the southeastern Brazilian continental margin, and between 21°S and 28°S. The
118 sites were named ES (22º50'27.24"S; 38º24'58.68"W), RJ (25º20'17.88"S; 39º38'28.32"W) and SP
119 (28º1'42.24"S; 43º31'43.32"W), with distances of 304 km between ES and RJ, 786 km between ES
120 and SP, and 496 km between RJ and SP (Figure 1). Landers were deployed in June 2-6, 2013 at
121 3,300 m depth, using the R/V Alpha Crucis from the Oceanographic Institute at Universidade de
122 São Paulo (IO-USP). On each lander, single use plastic bags of two different polymers, 60g of
123 pristine PP pellets (Braskem) and 60 g of inorganic material as a control were deployed. Plastic
124 bags included: (i) regular high density polyethylene (HDPE) grocery bag material (Valbags,
125 ValGroup Brasil), (ii) biodegradable grocery bag material made from HDPE with the oxo-
126 biodegradable additive BDA (Willow Ridge Plastics, Inc.). All types of substrates were placed

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127 inside fiberglass mesh bags (~8 x 15 cm; mesh size, 1 mm), attached to the metal lander frame and
128 secured with nylon fishing line and rope before deployment.

129 After 719 days in all cases, 23.6 months (on May 22-25, 2015), samples were collected with the
130 support of NPo Almirante Maximiano (H-41, Brazilian Navy). For DNA extraction, 30 pellets of
131 gravel, plastic and cut strips of plastic bag material were rinsed lightly with autoclaved distilled
132 water. These were then placed into vials (10 per vial for pellets or sufficient to occupy
133 approximately the same vial volume as pellets for bags, n = 3 replicate vials), filled with RNAlater
134 buffer solution (Thermo Fisher Scientific, Waltham, MA, USA), and stored at -20 ºC until analysis.
135 For live culturing, an additional 5 pellets or cut strips of plastic bag material were placed directly
136 into Eppendorf tubes and filled with sterilized seawater without rinsing. Eppendorf tubes were
137 shaken gently, and stored at 4 ºC until analysis. All handling materials were sterilized between
138 every step.

139 Before retrieving each lander, water samples from the same depth and current as the plastic samples
140 were collected using a Rosette water sampler equipped with Niskin bottles. Water samples
141 (adjacent water - AW) were collected to analyze the structure of the microbial communities in the
142 environment where plastics were deployed. Each water sample collected (~ 10 L) was immediately
143 filtered through a 0.22 μm polycarbonate membrane (diameter 45 mm; Millipore, Bedford, MA,
144 USA) using a peristaltic pump, and stored at -80 °C.

145

146 2.2. DNA extraction, 16S rRNA gene amplification and sequencing

147 Samples were processed at LECOM, the Microbial Ecology Laboratory at the Oceanographic
148 Institute (IO-USP) of University of São Paulo. Extraction of the total DNA from the plastics was

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149 performed in triplicate using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA).
150 Extraction of DNA from the water was performed using a PowerWater DNA Isolation Kit (MoBio,
151 Carlsbad, CA, USA) according to the manufacturer's specifications.

152 Six PCR reactions from each sample were pooled and purified with the DNA Clean &
153 Concentrator$^{TM}$ kit (Zymo Research, Irvine, CA, USA), and quantified with Qubit 1.0 fluorometer
154 (Thermo Fisher Scientific, Waltham, MA, USA) and the Qubit® dsDNA HS Assay Kit (Thermo
155 Fisher Scientific, Waltham, MA, USA). PCR was conducted following the Earth Microbiome 16S
156 Illumina Amplicon Protocol. Amplicons were sent to the Molecular Research - MR. DNA
157 company (Texas, USA) for sequencing on the Illumina Miseq platform in a 2x300 bp pair-end
158 system. The V4 hypervariable region of the 16S rRNA gene from Bacteria and Archaea was
159 amplified with the universal primers 515F and 806R (Caporaso et al., 2010) with specific adapters
160 for the Illumina Miseq platform. Sequence data (raw .fastq files) have been submitted to the
161 GenBank under accession number PRJNA692207.

162

163 2.3. Bioinformatics and statistical analyses

164 Processing and quality control of reads was performed using QIIME2 version 2019.10 (Bolyen et
165 al., 2019). After graphic inspection of quality profiles, raw reads were subjected to trimming and
166 filtering, then clustered into Amplicon Sequence Variants (ASV) with DADA2 denoising
167 (Callahan et al., 2016) using the QIIME 2 package (Bolyen et al., 2019). Sequence counts were
168 rarefied to 45,020 reads per sample across all samples to mitigate uneven sequencing depth.

169 The ASV richness, Shannon and InvSimpson diversity indexes were calculated using *phyloseq* and
170 *vegan* packages. Normality and Homogeneity of variances was assessed by Shapiro-Wilk

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171 normality and Levene's test, respectively. If the data showed a normal distribution and the
172 variances were homogeneous, differences between groups were assessed by one-way analysis of
173 variance (ANOVA) and subsequent post-hoc Tukey's tests, which were performed using *stats* and
174 *agricolae* packages in R (v.3.6.1) to assess differences in diversity indexes among substrates and
175 sites. To compare the structure of the bacterial communities among substrates and sites, non-metric
176 multidimensional scaling (NMDS) ordination was performed, based on weighted unifrac
177 dissimilarities among all samples. Differences in the microbial community structure among
178 substrates and sites were tested by performing a permutational multivariate analysis of variance
179 (PERMANOVA) on the community matrix (Anderson, 2001).

180 To identify an ASV that was significantly more abundant among substrate types, we performed
181 DESeq2, Differential Expression analysis for Sequence count data (Love et al., 2014). The DESeq2
182 input was a rarefied microbial dataset previously treated using the Prevalence Interval for
183 Microbiome Evaluation (PIME) package (Roesch et al., 2020). PIME uses machine-learning to
184 generate ASV prevalence among samples, and validate it by comparison with control Monte Carlo
185 simulations with randomized variations of sequences (Roesch et al., 2020). The full rarefied dataset
186 consisting of 5,199 ASVs was filtered using the PIME R package (Roesch et al., 2020). PIME
187 removes the within-group variations and captures only biologically significant differences which
188 have high sample prevalence levels. PIME employs a supervised machine-learning algorithm to
189 predict random forests and estimates out-of-bag (OOB) errors for each ASV prevalence dataset at
190 5% intervals. High OOB errors indicate that a given prevalence dataset interval is noisy, while the
191 minimal OOB errors (OBB error = zero) represent the absence of noise. Here, the minimal OOB
192 errors occurred with a 70% prevalence interval, which represented 471,078 sequences distributed
193 among 535 ASVs. This 70% prevalence dataset was used for DESeq2 subsequent analyses.

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194 To observe the occurrence of ASVs among substrate types, the samples were grouped by substrate
195 type and the taxa abundance table transformed to presence/absence. The unique and shared ASVs
196 were then visualized using an UpSet plot, *UpSetR* package (Conway et al., 2017). The ASVs shared
197 by all plastic types were considered the core microbiome.

198

199 2.4. Cultivable Plastic-associated Bacteria

200 In sterile Petri dishes, samples of plastic substrates were inoculated directly into the mineral culture
201 medium described by Sekiguchi et al., (2010). The medium was prepared to contain per liter of
202 distilled water: 1.87% of Marine Broth (Difco), 1.5% NaCl, 0.35% KCl, 5.4% $MgCl_2$. $6H_2O$, 2.7%
203 $MgSO_4$. $7H_2O$, 0.5% $CaCl_2$. $2H_2O$, 1.2% agar with 0.25% poly-β-hydroxybutyrate (PHB) granules
204 added. The samples were incubated for 15 days at 13 °C, or until the growth of colonies around
205 the plastic samples was observed. All morphologically different macroscopic colonies were
206 selected using the depletion technique two to three times until pure colonies for sequencing were
207 obtained. The isolates were preserved in 20% glycerol in an ultra-freezer at -80 °C.

208 The genomic DNA of 22 isolates was extracted using the Purelink Genomic DNA kit (Invitrogen
209 by Thermo Fisher Scientific, Carlsbad, USA), according to the manufacturer's specifications.
210 Amplification of the RNAr 16S gene was conducted using primers 515F (5 '-
211 GTGYCAGCMGCCGCGGTAA - 3') and 1401R (5 '- CGGTGTGTACAAGGCCCGGGA - 3').
212 The polymerase chain reaction (25 µL reaction) was performed using Gotaq Mix Hot Start, 0.25
213 µL of each primer and 2 µL of DNA template. The PCR conditions were: initial denaturation
214 temperature of 95 °C, 3 min; followed by 30 cycles of 94 °C, 1 min; 53 °C, 30 seconds; 72 °C, 1
215 min; and a final extension at 72 °C for 10 min. The PCR product was purified using the DNA

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216 Clean & Concentrator kit (Zymo Research, Irvine, USA) according to the manufacturer's
217 specifications and sent for sequencing at Genomic Engenharia Molecular, where they were
218 sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Palo
219 Alto, USA) with the 515F primer.

220 Sequence analysis was initially performed using CodonCodeAligner Software (CodonCode
221 Corporation, Dedham, MA, USA). Through this software, the sequences were checked for quality
222 and treated. After obtaining the treated sequences, the SILVA v138 database (High-Quality
223 Ribosomal RNA Databases) was used to align the sequences, to identify the isolates and to
224 construct the phylogenetic trees through MEGA X software (Kumar et al., 2018), using the
225 maximum-likelihood method (999 bootstraps). All sequencing data was deposited in GenBank
226 (National Center for Biotechnology Information Sequence Read Archives) under accession
227 numbers between MW216888 and MW216902.

228

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229 **3. Results**

230 3.1. ASV richness and Alpha diversity among substrata and sites

231 From the 15 samples sequenced, a total of 1,086,988 valid sequences (i.e. reads) were obtained,
232 representing an average of 72,465±12,068 (SD) reads per sample. The obtained reads were
233 clustered into 5,310 ASVs, representing an average of 702±149 ASVs per sample. Rarefaction
234 curves indicated a stationary phase, suggesting sufficient depth of sequencing to account for the
235 diversity of the microbial community on the plastic substrates, gravel, and seawater samples
236 (Figure S1).

237 Overall, among substrate type the ASV richness measured by the Chao1 estimator was
238 significantly higher on gravel samples (898.33±84.77) (ANOVA; $F = 4.85$; $df = 4$; $p = 0.027$),
239 while the microbial richness from AW samples and plastic substrates (HDPE and PP) was lower
240 (631±106, 616±80 and 583±141, respectively) (Figure S2A). Although the Shannon diversity was
241 not affected by substrate types (ANOVA; $F = 2.77$; $df = 4$; $p = 0.102$), the diversity observed on
242 gravel samples was 1.05, 1.07, and 1.2-fold higher than PP, HDPE-OXO, and HDPE samples,
243 respectively (Figure S2B). The evenness was also not affected significantly by substrate type
244 (ANOVA; $F = 1.65$; $df = 4$; $p = 0.111$), but a lower mean evenness was observed on HDPE (0.66),
245 AW (0.68), and HDPE-OXO (0.71), while the PP samples showed the higher evenness (0.76)
246 (Figure S2C).

247 The site did not exhibit a significant effect on any diversity indexes measured. However, the ASV
248 richness measured by the Chao1 estimator was higher from RJ samples (741.09±159.06)
249 (ANOVA; $F = 0.61$; $df = 2$; $p = 0.564$), while SP and ES showed similar richness (675.54±81.16;
250 681.38±203.29, respectively) (Figure S2D). Shannon index averages were high in RJ samples

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251 (4.78±0.55) and SP (4.71±0.20), while ES showed the lowest mean values (4.51±0.55) (ANOVA;
252 F = 0.38; df = 2; p = 0.517) (Figure S2E). Finally, the lowest evenness was observed in the ES
253 samples (0.69±0.07) and similar between RJ and SP (0.72±0.06 and 0.72±0.02, respectively)
254 (ANOVA; F = 0.61; df = 2; p = 0.565) (Figure S2F).

255

256 3.2. Microbial community structure among substrata and sites

257 Non-metric multidimensional scaling (NMDS) based on weighted Unifrac dissimilarities revealed
258 that the global pattern of microbial diversity was significantly explained by the substrate type
259 (Figure 2). Based on Permutational Multivariate Analysis of Variance (PERMANOVA) the
260 microbial community structure was highly dependent on the substrate type ($R^2$ = 0.78, P = 0.001),
261 while the site effect was lower and not significant ($R^2$ = 0.11, P = 0.054). Adjacent water (AW)
262 samples were particularly distinct, while gravel samples showed more similarities to plastic
263 samples.

264

265 3.3. Microbial composition and taxa differential abundance between plastic and control samples

266 The microbial community of all substrates were composed in majority by the phylum
267 Proteobacteria (40 to 77%), while other phyla showed different colonization patterns amongst
268 plastic substrates (HDPE, HDPE-OXO and PP), gravel and AW, including both presence/absence
269 and differences in relative proportions of the phyla (Figure 3A). For example, Firmicutes was
270 abundant in all PP samples (average 20±6%), while found in smaller proportions in HDPE
271 (6.6±6.2%), HDPE-OXO (6.1±5.6%) and gravel samples (3.8±1.9%). Among plastics, NB1-j was
272 more abundant in HDPE (4.6±5.9%) and archaeal phyla (such as Crenarchaeota and

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273 Nanoarchaeota) were prevalent in HDPE and HDPE-OXO (5.1±4 and 7.3±4.7%, respectively).
274 The lowest proportions of those archaeal phyla were observed in PP (0.7±0.4%). Chloroflexi,
275 Marinimicrobia (SAR406 clade) and SAR324 (Marine group B) were mainly present in AW
276 samples (7.1±1.6, 5.6±0.28 and 6.9±0.1%, respectively) but not in the plastic substrates used in
277 this study.

278 Differences between plastics and gravel samples were strongly evident when we examined deeper
279 taxonomic levels. We observed ASVs from a total of 37 families that were significantly more
280 abundant in plastic substrates when compared with gravel samples; these include families, such as
281 Methylologellaceae, Colwelliaceae, Pseudomonadaceae, Haliangiaceae, Micrococcaceae,
282 Halieceaea, Oleiphilaceae, Flavobacteriaceae, Rhizobiaceae, Microtrichaeae, Rhodobacteraceae,
283 among others (Figure 2B).Seven families showed ASVs with differential abundances for both
284 plastic and gravel samples, such as Cyclobacteriaceae, OM190, Spongiibacteraceae, NB1-j,
285 Cryomorphaceae, Nitrosopumilaceae and unclassified Bacteroidia. Gravel samples exhibited 16
286 families significantly more abundant in comparison with plastics (e.g. Kangiellaceae, OM182
287 clade, Crocinitomicaceae, Sphingomonadaceae and Bacteriovoracaceae ) (Figure 2B).

288

289 3.4. Microbial composition and taxa differential abundance among plastic types

290 Among each substrate type, the largest number of unique ASVs was found in gravel samples (1096
291 ASVs), followed by AW, PP, HDPE-OXO and HDPE, with respective values of 954, 736, 732 and
292 475 ASVs. Grouping those ASVs into families to examine abundance, we identified a
293 predominance of low abundance families on all substrates deployed (relative abundance < 2% of

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294 total community) on PP (71.9%), HDPE-OXO (63.9%), gravel (56.8%) and HDPE (40.2%). The
295 percentage of low abundance families in AW samples was lower (21.4%) (Figure 4).

296 Overall, the number of common ASVs amongst substrates deployed (pairwise combinations
297 among HDPE, HDPE-OXO, PP and gravel) was higher than common ASVs between AW and
298 substrates deployed (Figure 4). These results may confirm taxa effectively colonizing the
299 substrates over long periods. Another piece of evidence to support this idea is the high number of
300 ASVs (201) shared among all substrates deployed, while only 91 ASVs were observed shared
301 among the substrates deployed and the adjacent water (AW) (Figure 4).

302 Similar polymer composition was also suggested as exerting influence on the microbial taxa
303 composition. We identified 163 ASVs shared between HDPE and HDPE-OXO, plastic substrates
304 composed basically by HDPE. In contrast, a lower number of ASVs were shared between PP and
305 HDPE-OXO (68) and PP and HDPE (46), substrates composed of different polymers (Figure 4).

306 Based on the 44 families with ASVs significantly more abundant in plastic substrates than in gravel
307 samples (Figure 2B), we checked the distributions of these families amongst plastic substrates
308 types (Figure 5). Results showed us three major family groups: (i) the generalists, found with
309 significant abundance in all plastic types (HDPE, HDPE-OXO, and PP), (ii) the plastic HDPE
310 generalists, found with significant abundance in plastic HDPE substrates (HDPE and HDPE-
311 OXO), and finally, (iii) the specialists group, composed by families found in differential
312 abundances in specific plastic substrates.

313 Fourteen families were classified into a generalist group, which included Bdellovibrionaceae,
314 Halieaceae, Microtrichaceae, Pseudomonadaceae, and uncultured families. The HDPE plastic
315 generalists were composed of 13 families, including CCM11a, Cryomorphaceae, Oleiphilaceae,

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316 Rhizobiaceae and Flavobacteriaceae, Nitrosopumilaceae. Among specialist families, the
317 Beijerinckiaceae and Staphylococcaceae were significantly more abundant in PP samples, whereas
318 NB1-j, OM190, Saccharospirillaceae, Spongiibacteraceae, Micavibrionaceae and unclassified
319 class of Alphaproteobacteria were more abundant in HDPE-OXO samples. We did not identify
320 families specifically associated with HDPE samples.
321

322 3.5. Core microbiome of the plastisphere community

323 To identify the core microbiome of the plastisphere from the deep Southwest Atlantic Ocean, the
324 shared ASVs among plastic substrate types were examined (Figure 4). A total of 28 ASVs were
325 shared among plastic types (Table S1), comprising the core microbiome of the plastisphere from
326 the deep Southwest Atlantic Ocean. These ASVs were classified within 23 bacterial families, while
327 17 families included 97% of total core microbiome (Figure 4). This families were composed by
328 Oleiphilaceae (30% of core microbiome), Rhizobiaceae (22%), unclassified Bacteria (13%), NB1-
329 j (7%), Halieaceae (5%), Hyphomonadaceae (4%), Sneathiellaceae (3%), unclassified Bacteroidia
330 class (3%), Micrococcaceae (2%), AB1 (1%), OM190 (1%), Thermaceae (1%), Paenibacillaceae
331 (1%), Haliangiaceae (1%), Microtrichaceae (1%), Nannocystaceae (1%) and Eel-36e1D6 (1%).
332 Six other families represented approximately 3% of the core microbiome (Figure 4).

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333 3.6. Cultivable plastic-associated Bacteria

334 Bacterial strains were cultivated from all substrates, including all plastic types and gravel (Table
335 S2). Overall, 15 strains were affiliated according to their 16S rRNA gene sequences to a bacterial
336 genus. The most dominant family identified among the strains was Halomonadaceae, recovered
337 from all substrates. This family was represented by two genera, *Salinicola* and *Halomonas*. Four
338 families were isolated only from plastic substrates, Flavobacteriaceae (HDPE and HDPE-OXO),
339 Pseudoalteromonadaceae (HDPE), Marinobacteraceae (HDPE-OXO) and Rhodobacteraceae (PP).
340 In contrast, Micrococcaceae and Bacillaceae were isolated only from gravel. The comparison of
341 the sequences of all isolates with the SILVA rRNA database confirmed the identities of the isolates.
342 A phylogenetic tree was constructed with the bacterial sequences deposited in the database most
343 closely related to our isolates (Figure S3). All the bacterial genera isolated in this study have been
344 reported associated with or degrading hydrocarbon compounds (Table S2).

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345 **4. Discussion**

346 Deep-sea environments are characterized by low temperatures, high pressure, absence of light and
347 the consequent absence of photosynthetic primary production (Corinaldesi, 2015). Together with
348 a general reduction of organic matter input, these extreme conditions promote high selective
349 pressures on the microbial community. The input of anthropogenic substrates (i.e., sources of
350 carbon) into the deep sea, such as plastic substrates, creates new habitats (or food sources) to be
351 colonized by microorganisms. However, the composition of these substrata may select pelagic
352 microorganisms with specific features that allow them to colonize and metabolize this carbon
353 source (Dussud et al., 2018a; Dussud et al., 2018b).

354 Although the input of organic matter is suggested to be low, the pool of organic matter in deep
355 waters could be enough to support high microbial richness (Luna et al., 2012), as indicated by our
356 results. The highest richness on the gravel samples indicates that more microbial taxa were capable
357 of attaching to the gravel in comparison with the plastic substrates, suggesting that plastics likely
358 offer a strong selective pressure to colonization by the surrounding microbial community
359 (McCormick et al., 2014). This is also supported by the lower microbial evenness observed in the
360 plastic substrates (Figure S1C). However, statistical differences in alpha-diversity indices between
361 plastic substrates and AW were not observed. Despite the high variability among samples and a
362 limited number of replicates, these results indicate that the substrate effect on microbial
363 communities is reflected by taxonomic composition rather than by diversity indexes. To the best
364 of our knowledge, there is no previous study that has experimentally addressed the microbial
365 colonization on plastic substrates in deep waters. Several previous studies have reported on surface
366 waters (either from laboratory or field experiments) a lower richness and diversity of plastic
367 substrates in comparison with the surrounding waters, suggesting that plastic substrates select for

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368 a specific and less diverse microbial community (McCormick et al., 2014; Ogonowski et al., 2018;
369 Zettler et al., 2013). Nevertheless, deep water is generally oligotrophic, while surface waters have
370 a constant input of organic matter from primary production from the phytoplanktonic community
371 and could support high microbial diversity. These general oligotrophic conditions could also be
372 attributed to the lack of significant differences in microbial richness and diversity among sites,
373 which are exposed to the same depths and water mass. Abiotic parameters associated with water
374 mass are suggested to be the main driver of the microbial community in the pelagic system. The
375 similarity of these drivers observed among the sites could thus explain the lack of significant
376 differences found in our study, as well as the high similarity among AW samples from different
377 sites (Figure 2). Although at different sites, all samples were deployed at the same depth (3300 m),
378 under similar temperatures (3-4 °C), salinities (34.6-35 psu), oxygen concentrations (above 5 mL
379 $L^{-1}$) and with similar low nutrient levels (oligotrophic) (Gonzalez-Silvera et al., 2004; Krueger et
380 al., 2015).

381 Our results showing the strong effect of substrate type on microbial community structure
382 (PERMANOVA, $R^2$=0.78, $P < 0.001$) indicates niche partitioning of microbial communities
383 among substrates (Dussud et al., 2018a). The substrate-dependence has been reported by studies
384 regarding environmental and controlled conditions (Dussud et al., 2018a; Dussud et al., 2018b;
385 Kirstein et al., 2019, 2018; McCormick et al., 2014; Zettler et al., 2013), showing evidence of the
386 selective effect of plastic substrates. Under natural environmental conditions, substrate-
387 dependence has also been reported in studies that randomly collected PMD (Plastic marine debris)
388 (Didier et al., 2017; Dussud et al., 2018a; Ogonowski et al., 2018; Zettler et al., 2013) or that
389 deployed plastic substrates (Kirstein et al., 2019), showing that the microbial community attached
390 to a plastic substrate are distinct from free-living seawater communities or those attached to other

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391 hard substrata. Besides the substrate composition, the shape of the plastic particle could also act as
392 a driver of the microbial diversity. For example, it is possible that the differences in the microbial
393 structure and composition found between HDPE and PP substrates could also be attributed to shape
394 differences in the plastics, not only to differences in composition. The HDPE substrate used in our
395 study was in the form of a film, while the PP was a pellet. Because we didn't have the same plastic
396 type with multiple shapes available among our samples, we could not evaluate the isolated effect
397 of plastic shapes on microbial diversity. Some recent studies have indicated that particle shape
398 affects the microbial biofilm thickness (Wright et al., 2020), but if this reflects in changes in the
399 microbial community composition is as yet unknown.

400 The microbial communities from gravel samples were more similar to the plastic substrates, while
401 adjacent water samples were particularly distinct (Figure 2). These results were expected, as the
402 plastic samples and gravel were deployed for 719 days; water samples represented only a single
403 moment in time, while the plastic and gravel samples are the results of cell deposition and a
404 dynamic succession over a long period. The AW samples are therefore a type of control, providing
405 information on the microbial taxa present in the water column before retrieving the substrates.
406 Corroborating our results, Oberbeckmann et al., (2016) demonstrated significant differences of
407 multiple taxonomic groups when comparing plastic biofilm communities and the surrounding
408 seawater communities; although the bacterial communities attached to PET bottles were distinct
409 from the free-living seawater communities, the authors also found that PET-associated
410 communities were similar to other types of particle-associated or glass-bound communities
411 collected in the surrounding seawater. Those results confirm the ability of pelagic microorganisms
412 to colonize a range of substrates without specificity (Dussud et al., 2018a).

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413 At deeper taxonomic levels, the differences between the plastic and gravel samples were strongly
414 evident. We identified 37 families significantly more abundant in plastic substrates than in gravel
415 samples (Figure 2B), such as such as Methylologellaceae, Colwelliaceae, Pseudomonadaceae,
416 Haliangiaceae, Micrococcaceae, Halieceaea, Oleiphilaceae, Rhizobiaceae, Microtrichaeaed,
417 Flavobacteriaceae, Rhodobacteraceae and unclassified families of Alpha- and
418 Gammaproteobacteria. Corroborating our results, previous studies have shown that microplastics
419 were mainly colonized by Alpha- and Gammaproteobacteria, which were shown to act as primary
420 colonizers, and Flavobacteria (Bacteroidetes), which appeared to act as secondary colonizers (Lee
421 et al., 2008). Additionally, bacterial families classified as Flavobacteriaceae, Pirellulaceae,
422 Rhodobacteraceae (Alphaproteobacteria) and Microtrichaceae (Acidimicrobiia) were identified as
423 the most dominant families on microplastic (PE) biofilms exposed for 135 days to the marine
424 environment at 12 m depth (Tu et al., 2020).

425 When comparing our microbial taxa from plastic substrates with previous studies related to the
426 plastisphere in epipelagic ecosystems we found several families in common, such as
427 Microtrichaceae, Rhizobiaceae, Halieaceae, Spongiibacteraceae, Rhodobacteraceae,
428 Micavibrionaceae, Flavobacteriaceae, Halomonadaceae, Kangiellaceae, Hyphomonadaceae,
429 Comamonadaceae, Oleiphilaceae and Bacillaceae (Amaral-Zettler et al., 2020; Feng et al., 2020;
430 Pinto et al., 2019; Rogers et al., 2020). The family Oleiphilaceae comprises members that
431 obligately utilize hydrocarbons through the alkane hydroxylase (*AlkB*) pathway (Golyshin et al.,
432 2002); its detection in our plastic samples likely indicates their potential role in degrading plastic
433 substrates in deep-sea ecosystems. Further, among the families described by these authors,
434 Rhodospirillaceae members were not detected in our samples, which is in agreement with their
435 photosynthetic capacity and thus their prevalence in epipelagic ecosystems. We detected a few

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436 families in our plastic samples which were not identified in these previous studies, such as
437 Nitrosopumilaceae. Nitrosopumilaceae members are widely distributed in several deep-sea
438 environments and have an important role as primary producers through ammonia oxidation (Zhong
439 et al., 2020). Their presence in our plastic substrates likely reflects the high abundance of this
440 family in these ecosystems, which might favor their attachment to a variety of substrates available
441 for colonization.

442 Remarkable differences were observed when we grouped the microbial families by their
443 distributions amongst plastic types as generalists (significantly abundant on all plastic types,
444 HDPE, HDPE-OXO and PP), HDPE plastic generalists (significantly abundant in HDPE and
445 HDPE-OXO), and specialists (significantly abundant in specific plastic substrates). Members of
446 Bdellovibrionaceae, Halieaceae, Microtrichaceae and Pseudomonadaceae families were identified
447 as generalists. The ability to colonize and potentially metabolize the carbon from plastic polymers
448 of different substrates in deep environments, under oligotrophic conditions, confer advantages on
449 these microbes in comparison to the entire microbial community. Some of these families have been
450 previously described in association with different types of microplastics from several locations
451 (e.g. Dussud et al., 2018a; Jiang et al., 2018; Tu et al., 2020). Tu et al., (2020) found a high
452 abundance of Microtrichaceae members within biofilms of polyethylene microplastics from coastal
453 seawater in the Yellow Sea, China, with increasing abundance according to longer exposure
454 periods (135 days). Halieaceae members were detected in polyethylene, polypropylene and
455 polystyrene microplastics from the Yangtze estuary (Jiang et al., 2018) and polyethylene
456 microplastics from the Yellow Sea (Tu et al., 2020), both in China. This family is composed by
457 marine bacteria that are capable of assimilating propylene through alkene monooxygenase genes
458 (Suzuki et al., 2019). In addition, we observed microbial families associated specifically to HDPE

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459 samples (with and without biodegradable additives). Those microbes, in contrast to the generalist
460 group, are suggested to be more adaptive to colonizing HDPE polymers, with weak or no influence
461 from biodegradable additives. The influence of biodegradable additives was observed in the
462 specialist taxa group (i.e. those microbes more adapted to a specific polymer type). The presence
463 of additives in the polymer compositions may support microbial dynamics over time (Dussud et
464 al., 2018b). Additionally, those additives could be an extra source of nutrients that may reflect in
465 the multiplication of the different microorganisms. Similar results were reported by Dussud et al.,
466 (2018b) that suggested a strong effect of the polymer type on the bacterial community, because the
467 composition of microbial biofilm on LDPE and LDPE-OXO (PE with pro-oxidant additives) was
468 completely distinct, while AA-LDPE-OXO (artificially aged LDPE-OXO) and PHBV (poly(3-
469 hydroxybutyrate- co-3-hydroxyvalerate) showed higher similarity, all under controlled conditions.
470 As observed by these authors for shallow waters, we also observed an influence of the plastic types
471 HDPE and HDPE-OXO on selecting specific microbial taxa in deep waters.

472 A total of 28 ASVs were identified as core microbiome members in the plastisphere. Defining a
473 common core microbiome in the plastisphere across different studies may be difficult, because
474 variations between experimental designs make it difficult to compare studies directly, as do the
475 variety of study-specific approaches used to define the core (Didier et al., 2017; Tu et al., 2020;
476 Zettler et al., 2013). However, some taxa observed in our study were widely described by previous
477 studies, which provides evidence of common core members of the plastisphere from both surface
478 and deep waters. ASVs from the bacterial families Oleiphilaceae and Hyphomonadaceae were
479 found as members of the core microbiome in our plastic samples. These families have members
480 known to degrade hydrocarbons (Golyshin et al., 2002; Ozaki et al., 2007) or are frequently

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481 associated to plastic substrates in the marine environment (Bryant et al., 2016; De Tender et al.,
482 2017; Oberbeckmann et al., 2018; Pinto et al., 2019; Zettler et al., 2013).

483 In addition, taxa reported from plastic substrates, but not in the core microbiome were also
484 identified. For instance, Microtrichaceae were reported as a dominant taxon on a PE surface during
485 the early phase of biofilm formation (Tu et al., 2020) and Sneathiellaceae colonized plastic debris
486 along a transect through the North Pacific Subtropical Gyre (Bryant et al., 2016). Notably, taxa not
487 previously described from plastic substrates were also identified. Micrococcaceae is a well-
488 documented bacterial family inhabiting deep-sea sediments (Chen et al., 2005, 2016; Sass et al.,
489 2001); their members were already identified in sediments from the Southwest Indian Ridge at
490 depths ranging from 1,662 to 4,000 m (Chen et al., 2016), in a hypersaline 3,500 m depth site in
491 the Mediterranean Sea (Sass et al., 2001), and were isolated from an Antarctic lake and deep-sea
492 sediments from the tropical West Pacific (Chen et al., 2005). *Allorhizobium-Neorhizobium-*
493 *Pararhizobium-Rhizobium* spp. (Rhizobiaceae), a taxon reported as nitrogen-fixing (Franche et al.,
494 2009), was an abundant member of the core microbiome. The family Rhizobiaceae is commonly
495 involved in plant-microbe interactions and was reported recently in marine environments (Kimes
496 et al., 2015). In deep-sea environments, the species of Rhizobiaceae *Georhizobium profundi* was
497 isolated from sediment collected at 4,524 m depth (Cao et al., 2020), but its association with plastic
498 substrata were only described in freshwater environments (Wang et al., 2020; Wen et al., 2020).
499 Moreover, another taxa that comprised our core microbiome was NB1-j, an uncultivated bacterial
500 family that was previously found in Japan Trench sediment at 6,292 m depth (Yanagibayashi et
501 al., 1999), and in 800 to 1,450 m depth sediments heavily impacted by an oil spill in the northern
502 Gulf of Mexico (Handam et al., 2018). Finally, taxa from AB1 family (previously assigned as
503 unclassified Alphaproteobacteria) and Eel-36e1D6 (previously assigned as unclassified

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504 environmental clone groups), which also comprised the core microbiome, were reported in deep-
505 sea hydrothermal fields, as well as in ferromanganese crusts (Nitahara et al., 2011). Overall, these
506 results highlight a significant number of deep-sea taxonomic groups that were not described by
507 previous studies inhabiting the plastic substrates but were found inhabiting our plastic substrates
508 in the deep SW Atlantic Ocean.

509 We identified some taxa in the core microbiome that might be potentially related to plastic
510 degradation, according to previous studies. For example, *Arthrobacter* spp. (Micrococcaceae)
511 isolated from plastic waste in the Gulf of Mannar, India, was reported degrading high-density
512 polyethylene (HDPE); after 30 days incubation, they had reduced the weight of the substrate by
513 12% (Balasubramanian et al., 2010). In addition, members of the Halieaceae family that have
514 known capabilities of assimilating propylene through alkene monooxygenase genes (Suzuki et al.,
515 2019), were described in plastic substrates from the Yangtze estuary (Jiang et al., 2018) and Yellow
516 Sea (Tu et al., 2020), both in China. Members of the Paenibacillaceae family, such as *Paenibacillus*
517 spp. have shown high potential to degrade LPDE and HDPE when in consortia with *Pseudomonas*
518 spp., *Stenotrophomonas* spp. and *Bacillus* spp. (Skariyachan et al., 2017). Furthermore, we were
519 able to isolate bacteria from our plastic substrates that comprise families and genera previously
520 described as colonizing or degrading hydrocarbon substrates (Table S2). The isolation of these
521 bacteria indicates that they were at least viable in deep-sea conditions and are important members
522 to be explored in future studies to reveal their plastic-degradation capacity.

523 Information about microbial communities associated with the plastic substrata in the deep-ocean
524 is scarce in published research studies (Krause et al., 2020; Woodall et al., 2018). Additionally,
525 results from other experiments deploying samples for a long period in deep sea environments have
526 not yet been published to date. Our pioneer study showed that several taxonomic groups previously

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527 described as plastic colonizers in surface waters seem to also colonize the plastic substrates in the
528 deep sea. However, we also identified some groups in the plastisphere that are typically found
529 inhabiting deep-sea sediments, such as NB1-j, Rhizobiaceae and Eel-36e1D6 members, most of
530 them still poorly characterized and not yet cultivated. In addition, 13% of taxa in the core
531 microbiome were not classified to any microorganism previously deposited in the reference
532 databases, which might indicate sequencing artefacts or that we identified potential novel groups
533 not yet described. Our study addresses the gap in the knowledge of microbial colonization in
534 plastics deployed for a long period in the deep sea, highlighting the taxa potentially involved with
535 plastic degradation processes. However, further studies are needed to better understand their role
536 in plastic colonization and degradation in deep-sea ecosystems.

537 In summary, this study reported for the first time that deep-sea microbial communities of the
538 Southwest Atlantic Ocean are involved in the colonization of plastic substrates. Furthermore, the
539 microbial community colonizing the plastic surfaces were distinct and dependent on polymer type,
540 but the site where the samples were deployed had no effect on the microbial community. Our
541 results demonstrated a core microbiome exclusively composed of low abundance taxa; some
542 members were not previously described as associated with plastic substrates, while other bacterial
543 families had previously been described degrading plastics, but not in deep-sea environments.
544 Additionally, we were able to cultivate and isolate some of these bacterial families from our plastic
545 substrates. Our results indicate that a specific microbial community from the deep-sea can attach,
546 colonize and potentially degrade plastic substrates. It is important to note that some of those
547 members were reported degrading plastics in controlled conditions, and their ability to degrade the
548 plastic compounds under deep-water conditions remains unknown without further investigation.
549 We provide the first evidence of an unexplored microbial community inhabiting the deep-sea

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550 plastisphere, which may be used as a baseline to future studies about the functionality and the
551 potential of degradation of these microbial communities living in oligotrophic conditions, in the
552 absence of sunlight, under high pressure and low temperature.

553

554 **CRediT authorship contribution statement**

555 **Luana Agostini:** Conceptualization, Methodology, Investigation, Data curation, Writing - original
556 draft, review & editing. **Julio Cezar Fornazier Moreira:** Formal analysis, Data curation, Writing
557 - original draft, review & editing, Visualization. **Amanda Gonçalves Bendia:** Formal analysis,
558 Writing - original draft, review & editing, Visualization. **Maria Carolina Pezzo Kmit:** Writing -
559 original draft, review & editing. **Linda Gwen Waters:** Conceptualization, Methodology,
560 Investigation, Writing - review & editing. **Marina Ferreira Mourão Santana:** Conceptualization,
561 Methodology, Investigation, Writing - review & editing. **Paulo Yukio Gomes Sumida:**
562 Conceptualization, Methodology, Writing - review & editing, Project administration, Funding
563 acquisition. **Alexander Turra:** Conceptualization, Methodology, Writing - review & editing,
564 Project administration, Funding acquisition. **Vivian Helena Pellizari:** Conceptualization,
565 Methodology, Writing - review & editing, Supervision.

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576 **References**

577 Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J., 2020. Ecology of the plastisphere. Nat. Rev.
578 Microbiol. 18, 139–151. https://doi.org/10.1038/s41579-019-0308-0

579 Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral
580 Ecol. 32–46.

581 Bergmann, M., Klages, M., 2012. Increase of litter at the Arctic deep-sea observatory
582 HAUSGARTEN. Mar. Pollut. Bull. 64, 2734–2741.
583 https://doi.org/10.1016/j.marpolbul.2012.09.018

584 Bolyen, E., Rideout, J.R., Dillon, M.R., et al., 2019. Reproducible, interactive, scalable and
585 extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.
586 https://doi.org/10.1038/s41587-019-0209-9

587 Bryant, J. A., Clemente, T.M., Viviani, D.A., Fong, A.A., Thomas, K.A., Kemp, P., Karl, D.M.,
588 White, A.E., DeLong, E.F., 2016. Diversity and Activity of Communities Inhabiting Plastic Debris
589 in the North Pacific Gyre. mSystems 1, 1–19. https://doi.org/10.1128/msystems.00024-16

590 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016.
591 DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–
592 583. https://doi.org/10.1038/nmeth.3869

593 Cao, J., Wei, Y., Lai, Q., Wu, Y., Deng, J., Li, J., Liu, R., Wang, L., Fang, J., 2020. Georhizobium
594 profundi gen. Nov., sp. nov., a piezotolerant bacterium isolated from a deep-sea sediment sample
595 of the New Britain Trench. Int. J. Syst. Evol. Microbiol. 70, 373–379.
596 https://doi.org/10.1099/ijsem.0.003766

597 Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-lyons, D., Lozupone, C.A., Turnbaugh, P.J.,
598 Fierer, N., Knight, R., 2010. Global patterns of 16S rRNA diversity at a depth of millions of
599 sequences per sample. Proceedings of the national academy of sciences, 108(Supplement 1), 4516-
600 4522. https://doi.org/10.1073/pnas.1000080107

601 Carson, H.S., Nerheim, M.S., Carroll, K.A., Eriksen, M., 2013. The plastic-associated
602 microorganisms of the North Pacific Gyre. Mar. Pollut. Bull. 75, 126–132.
603 https://doi.org/10.1016/j.marpolbul.2013.07.054

604 Chen, M., Xiao, X., Wang, P., Zeng, X., Wang, F., 2005. Arthrobacter ardleyensis sp. nov., isolated
605 from Antarctic lake sediment and deep-sea sediment. Arch. Microbiol. 183, 301–305.
606 https://doi.org/10.1007/s00203-005-0772-y

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.26.428295; this version posted January 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

607 Chen, P., Zhang, L., Guo, X., Dai, X., Liu, L., Xi, L., Wang, J., Song, L., Wang, Y., Zhu, Y.,
608 Huang, L., Huang, Y., 2016. Diversity, biogeography, and biodegradation potential of
609 actinobacteria in the deep-sea sediments along the southwest Indian ridge. Front. Microbiol. 7, 1–
610 17. https://doi.org/10.3389/fmicb.2016.01340

611 Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., Ogido, M., Fujikura, K., 2018.
612 Human footprint in the abyss: 30 year records of deep-sea plastic debris. Mar. Policy 0–1.
613 https://doi.org/10.1016/j.marpol.2018.03.022

614 Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the
615 marine environment: A review. Mar. Pollut. Bull. 62, 2588–2597.
616 https://doi.org/10.1016/j.marpolbul.2011.09.025

617 Conway, J.R., Lex, A., Gehlenborg, N., 2017. UpSetR: An R package for the visualization of
618 intersecting sets and their properties. Bioinformatics 33, 2938–2940.
619 https://doi.org/10.1093/bioinformatics/btx364

620 Corinaldesi, C., 2015. New perspectives in benthic deep-sea microbial ecology. Front. Mar. Sci. 2,
621 1–12. https://doi.org/10.3389/fmars.2015.00017

622 Dang, H., and Lovell, C. R. (2016). Microbial surface colonization and biofilm development in
623 marine environments. Microbiology and Molecular Biology Reviews, 80(1), 91-138.

624 De Tender, C.A., Devriese, L.I., Haegeman, A., Maes, S., Ruttink, T., Dawyndt, P., 2015. Bacterial
625 Community Profiling of Plastic Litter in the Belgian Part of the North Sea. Environ. Sci. Technol.
626 49, 9629–9638. https://doi.org/10.1021/acs.est.5b01093

627 De Tender, C., Schlundt, C., Devriese, L.I., Mincer, T.J., Zettler, E.R., Amaral-Zettler, L.A., 2017.
628 A review of microscopy and comparative molecular-based methods to characterize "Plastisphere"
629 communities. Anal. Methods 9, 2132–2143. https://doi.org/10.1039/C7AY00260B

630 Didier, D., Anne, M., Alexandra, T.H., 2017. Plastics in the North Atlantic garbage patch: A boat-
631 microbe for hitchhikers and plastic degraders. Sci. Total Environ. 599–600, 1222–1232.
632 https://doi.org/10.1016/j.scitotenv.2017.05.059

633 Dussud, C., Meistertzheim, A.L., Conan, P., Pujo-Pay, M., George, M., Fabre, P., Coudane, J.,
634 Higgs, P., Elineau, A., Pedrotti, M.L., Gorsky, G., Ghiglione, J.F., 2018a. Evidence of niche
635 partitioning among bacteria living on plastics, organic particles and surrounding seawaters.
636 Environ. Pollut. 236, 807–816. https://doi.org/10.1016/j.envpol.2017.12.027

637 Dussud, Claire, Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., Delort, A.M.,
638 Eyheraguibel, B., Meistertzheim, A.L., Jacquin, J., Cheng, J., Callac, N., Odobel, C., Rabouille,

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.26.428295; this version posted January 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

639 S., Ghiglione, J.F., 2018b. Colonization of non-biodegradable and biodegradable plastics by
640 marine microorganisms. Front. Microbiol. 9, 1–13. https://doi.org/10.3389/fmicb.2018.01571

641 Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F.,
642 Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic
643 Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS One 9, 1–15.
644 https://doi.org/10.1371/journal.pone.0111913

645 Feng, L., He, L., Jiang, S., Chen, J., Zhou, C., Qian, Z.J., Hong, P., Sun, S., Li, C., 2020.
646 Investigating the composition and distribution of microplastics surface biofilms in coral areas.
647 Chemosphere 252, 126565. https://doi.org/10.1016/j.chemosphere.2020.126565

648 Franche, C., Lindström, K., Elmerich, C., 2009. Nitrogen-fixing bacteria associated with
649 leguminous and non-leguminous plants. Plant Soil 321, 35–59. https://doi.org/10.1007/s11104-
650 008-9833-8

651 Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep sea floor off the
652 French Mediterranean coast. Mar. Ecol. Prog. Ser. 142, 225–234.

653 GESAMP, 2019. Guidelines or the monitoring and assessment of plastic litter and microplastics in
654 the ocean (Kershaw P.J., Turra A. and Galgani F. editors). Rep. Stud. GESAMP 99, 130.

655 Golyshin, P.N., Chernikova, Ta.N., Abraham, W.R., Lünsdorf, H., Timmis, K.N., Yakimov, M.M.,
656 2002. Oleiphilaceae fam. nov., to include Oleiphilus messinensis gen. nov., sp. nov., a novel
657 marine bacterium that obligately utilizes hydrocarbons. Int. J. Syst. Evol. Microbiol. 52, 901–911.
658 https://doi.org/10.1099/ijs.0.01890-0

659 Gonzalez-Silvera, A., Santamaria-Del-Angel, E., Garcia, V.M.T., Garcia, C.A.E., Millán-Nuñez,
660 R., Muller-Karger, F., 2004. Biogeographical regions of the tropical and subtropical Atlantic
661 Ocean off South America: Classification based on pigment (CZCS) and chlorophyll-a (SeaWiFS)
662 variability. Cont. Shelf Res. 24, 983–1000. https://doi.org/10.1016/j.csr.2004.03.002

663 Hamdan, L.J., Salerno, J.L., Reed, A., Joye, S. B., Damour, M. The impact of the Deepwater
664 Horizon blowout on historic shipwreck-associated sediment microbiomes in the northern Gulf of
665 Mexico. Sci Rep 8, 9057 (2018). https://doi.org/10.1038/s41598-018-27350-z

666 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law,
667 K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-. ). 347, 768–771.
668 https://doi.org/10.1126/science.1260352

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.26.428295; this version posted January 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

669 Jiang, P., Zhao, S., Zhu, L., Li, D., 2018. Microplastic-associated bacterial assemblages in the
670 intertidal zone of the Yangtze Estuary. Sci. Total Environ. 624, 48–54.
671 https://doi.org/10.1016/j.scitotenv.2017.12.105

672 Kimes, N.E., López-Pérez, M., Flores-Félix, J.D., Ramírez-Bahena, M.H., Igual, J.M., Peix, A.,
673 Rodriguez-Valera, F., Velázquez, E., 2015. Pseudorhizobium pelagicum gen. nov., sp. nov.
674 isolated from a pelagic Mediterranean zone. Syst. Appl. Microbiol. 38, 293–299.
675 https://doi.org/10.1016/j.syapm.2015.05.003

676 Kirstein, I.V., Wichels, A., Gullans, E., Krohne, G., Gerdts, G., 2019. The plastisphere –
677 Uncovering tightly attached plastic “specific” microorganisms. PLoS One 14, 1–17.
678 https://doi.org/10.1371/journal.pone.0215859

679 Kirstein, I. V., Wichels, A., Krohne, G., Gerdts, G., 2018. Mature biofilm communities on
680 synthetic polymers in seawater - Specific or general? Mar. Environ. Res. 147–154.
681 https://doi.org/10.1016/j.marenvres.2018.09.028

682 Krause, S., Molari, M., Gorb, E. V., Gorb, S.N., Kossel, E., Haeckel, M., 2020. Persistence of
683 plastic debris and its colonization by bacterial communities after two decades on the abyssal
684 seafloor. Sci. Rep. 10, 1–15. https://doi.org/10.1038/s41598-020-66361-7

685 Krueger, M.C., Harms, H., Schlosser, D., 2015. Prospects for microbiological solutions to
686 environmental pollution with plastics. Appl. Microbiol. Biotechnol. 99, 8857–8874.
687 https://doi.org/10.1007/s00253-015-6879-4

688 Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary
689 genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
690 https://doi.org/10.1093/molbev/msy096

691 Lee, J.W., Nam, J.H., Kim, Y.H., Lee, K.H., Lee, D.H., 2008. Bacterial communities in the initial
692 stage of marine biofilm formation on artificial surfaces. J. Microbiol. 46, 174–182.
693 https://doi.org/10.1007/s12275-008-0032-3

694 Love, M.I., Anders, S., Huber, W., 2014. Differential analysis of count data - the DESeq2 package,
695 Genome Biology. https://doi.org/110.1186/s13059-014-0550-8

696 Luna, G.M., Bianchelli, S., Decembrini, F., De Domenico, E., Danovaro, R., Dell’Anno, A., 2012.
697 The dark portion of the Mediterranean Sea is a bioreactor of organic matter cycling. Global
698 Biogeochem. Cycles 26, 1–14. https://doi.org/10.1029/2011GB004168

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699 McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an
700 abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48, 11863–11871.
701 https://doi.org/10.1021/es503610r

702 Nitahara, S., Kato, S., Urabe, T., Usui, A., Yamagishi, A., 2011. Molecular characterization of the
703 microbial community in hydrogenetic ferromanganese crusts of the Takuyo-Daigo Seamount,
704 northwest Pacific. FEMS Microbiol. Lett. 321, 121–129. https://doi.org/10.1111/j.1574-
705 6968.2011.02323.x

706 Oberbeckmann, S., Labrenz, M., 2020. Marine Microbial Assemblages on Microplastics:
707 Diversity, Adaptation, and Role in Degradation. Ann. Rev. Mar. Sci. 12, 209–232.
708 https://doi.org/10.1146/annurev-marine-010419-010633

709 Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., et al., 2018. Interactions of microplastic debris
710 throughout the marine ecosystem. Front. Microbiol. 1, 1–8. https://doi.org/10.1038/s41559-017-
711 0116

712 Oberbeckmann, S., Kreikemeyer, B., Labrenz, M., 2017. Environmental factors support the
713 formation of specific bacterial assemblages on microplastics. Front. Microbiol. 8, 2709.
714 https://doi.org/10.3389/FMICB.2017.02709

715 Oberbeckmann, S., Osborn, A.M., Duhaime, M.B., 2016. Microbes on a Bottle : Substrate , Season
716 and Geography Influence Community Composition of Microbes Colonizing Marine Plastic Debris.
717 PLoS One 1–24. https://doi.org/10.1371/journal.pone.0159289

718 Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I., Bacsik, Z.,
719 Gorokhova, E., 2018. Evidence for selective bacterial community structuring on microplastics.
720 Environ. Microbiol. https://doi.org/10.1111/1462-2920.14120

721 Ozaki, S., Kishimoto, N., Fujita, T., 2007. Change in the Predominant Bacteria in a Microbial
722 Consortium Cultured on Media Containing Aromatic and Saturated Hydrocarbons as the Sole
723 Carbon Source. Microbes Environ. 22, 128–135. https://doi.org/10.1264/jsme2.22.128

724 Pierdomenico, M., Casalbore, D., Chiocci, F.L., 2019. Massive benthic litter funnelled to deep sea
725 by flash-flood generated hyperpycnal flows. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-
726 019-41816-8

727 Pinnell, L.J., Turner, J.W., 2019. Shotgun metagenomics reveals the benthic microbial community
728 response to plastic and bioplastic in a coastal marine environment. Front. Microbiol. 10.
729 https://doi.org/10.3389/fmicb.2019.01252

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.26.428295; this version posted January 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

730 Pinto, M., Langer, T.M., Hüffer, T., Hofmann, T., Herndl, G.J., 2019. The composition of bacterial
731 communities associated with plastic biofilms differs between different polymers and stages of
732 biofilm succession. PLoS One 14, 1–20. https://doi.org/10.1371/journal.pone.0217165

733 PlasticEurope, 2019. Plastics-the Facts 2019 – An analysis of European plastics production,
734 demand and waste data.

735 Quero, G.M., Luna, G.M., 2017. Surfing and dining on the "plastisphere": Microbial life on plastic
736 marine debris. Adv. Oceanogr. Limnol. 8, 199–207. https://doi.org/10.4081/AIOL.2017.7211

737 Roager, L., Sonnenschein, E.C., 2019. Bacterial Candidates for Colonization and Degradation of
738 Marine Plastic Debris. Environ. Sci. Technol. 53, 11636–11643.
739 https://doi.org/10.1021/acs.est.9b02212

740 Roesch, L.F.W., Dobbler, P.T., Pylro, V.S., Kolaczkowski, B., Drew, J.C., Triplett, E.W., 2020.
741 pime: A package for discovery of novel differences among microbial communities. Mol. Ecol.
742 Resour. 20, 415–428. https://doi.org/10.1111/1755-0998.13116

743 Rogers, K.L., Carreres-Calabuig, J.A., Gorokhova, E., Posth, N.R., 2020. Micro-by-micro
744 interactions: How microorganisms influence the fate of marine microplastics. Limnol. Oceanogr.
745 Lett. 5, 18–36. https://doi.org/10.1002/lol2.10136

746 Sass, A. M., Sass, H., Coolen, M. J. L., Cypionka, H., Overmann, J. (2001). Microbial
747 Communities in the Chemocline of a Hypersaline Deep-Sea Basin (Urania Basin, Mediterranean
748 Sea). Applied and Environmental Microbiology, 67(12), 5392–5402.
749 https://doi.org/10.1128/AEM.67.12.5392-5402.2001

750 Skariyachan, S., Setlur, A.S., Naik, S.Y., Naik, A. A., Usharani, M, Vasist, K. S. Enhanced
751 biodegradation of low and high-density polyethylene by novel bacterial consortia formulated from
752 plastic-contaminated cow dung under thermophilic conditions. Environ Sci Pollut Res 24, 8443–
753 8457 (2017). https://doi.org/10.1007/s11356-017-8537-0

754 Schlining, K., von Thun, S., Kuhnz, L., Schlining, B., Lundsten, L., Jacobsen Stout, N., Chaney,
755 L., Connor, J., 2013. Debris in the deep: Using a 22-year video annotation database to survey
756 marine litter in Monterey Canyon, central California, USA. Deep. Res. Part I Oceanogr. Res. Pap.
757 79, 96–105. https://doi.org/10.1016/j.dsr.2013.05.006

758 Schmidt, C., Krauth, T., Wagner, S., 2017. Export of Plastic Debris by Rivers into the Sea. Environ.
759 Sci. Technol. 51, 12246–12253. https://doi.org/10.1021/acs.est.7b02368

760 Sekiguchi, T., Sato, T., Enoki, M., Kanehiro, H., Kato, C., 2010. Procedure for isolation of the
761 plastic degrading piezophilic bacteria from deep-sea environments. J. Japanese Soc. Extrem. 9,
762 25–30.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.26.428295; this version posted January 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

763 Shade, A., Handelsman, J., 2012. Beyond the Venn diagram: The hunt for a core microbiome.
764 Environ. Microbiol. 14, 4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x

765 Suzuki, T., Yazawa, T., Morishita, N., Maruyama, A., Fuse, H., 2019. Genetic and physiological
766 characteristics of a novel marine propylene-assimilating halieaceae bacterium isolated from
767 seawater and the diversity of its alkene and epoxide metabolism genes. Microbes Environ. 34, 33–
768 42. https://doi.org/10.1264/jsme2.ME18053

769 Tu, C., Chen, T., Zhou, Q., Liu, Y., Wei, J., Waniek, J.J., Luo, Y., 2020. Biofilm formation and its
770 influences on the properties of microplastics as affected by exposure time and depth in the
771 seawater. Sci. Total Environ. 734. https://doi.org/10.1016/j.scitotenv.2020.139237

772 Urbanek, A.K., Rymowicz, W., Mironczuk, A.M., 2018. Degradation of plastics and plastic-
773 degrading bacteria in cold marine habitats. Appl. Microbiol. Biotechnol. 102, 7669–7678.
774 https://doi.org/doi.org/10.1007/s00253-018-9195-y

775 Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in
776 deep-sea sediments. Environ. Pollut. 182, 495–499. https://doi.org/10.1016/j.envpol.2013.08.013

777 Wang, L., Luo, Z., Zhen, Z., Yan, Y., Yan, C., Ma, X., Sun, L., Wang, M., Zhou, X., Hu, A., 2020.
778 Bacterial community colonization on tire microplastics in typical urban water environments and
779 associated impacting factors. Environ. Pollut. 265, 114922.
780 https://doi.org/10.1016/j.envpol.2020.114922

781 Wen, B., Liu, J.H., Zhang, Y., Zhang, H.R., Gao, J.Z., Chen, Z.Z., 2020. Community structure and
782 functional diversity of the plastisphere in aquaculture waters: Does plastic color matter? Sci. Total
783 Environ. 740, 140082. https://doi.org/10.1016/j.scitotenv.2020.140082

784 Woodall, L.C., Jungblut, A.D., Hopkins, K., Hall, A., Robinson, F., Gwinnett, C., Paterson, G.L.J.,
785 2018. Deep-sea anthropogenic macrodebris harbours rich and diverse communities of bacteria and
786 archaea. PLoS One 1–11. https://doi.org/10.1371/journal.pone.0206220

787 Woodall, L.C., Sanchez-vidal, A., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers,
788 A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic
789 debris. R. Soc. Open Sci. 1. https://doi.org/http://dx.doi.org/10.1098/rsos.140317

790 Wright, R. J., Erni-Cassola, G., Zadjelovic, V., Latva, M., & Christie-Oleza, J. A. (2020). Marine
791 Plastic Debris: A New Surface for Microbial Colonization. Environmental Science & Technology,
792 54(19), 11657-11672. https://doi.org/10.1021/acs.est.0c02305

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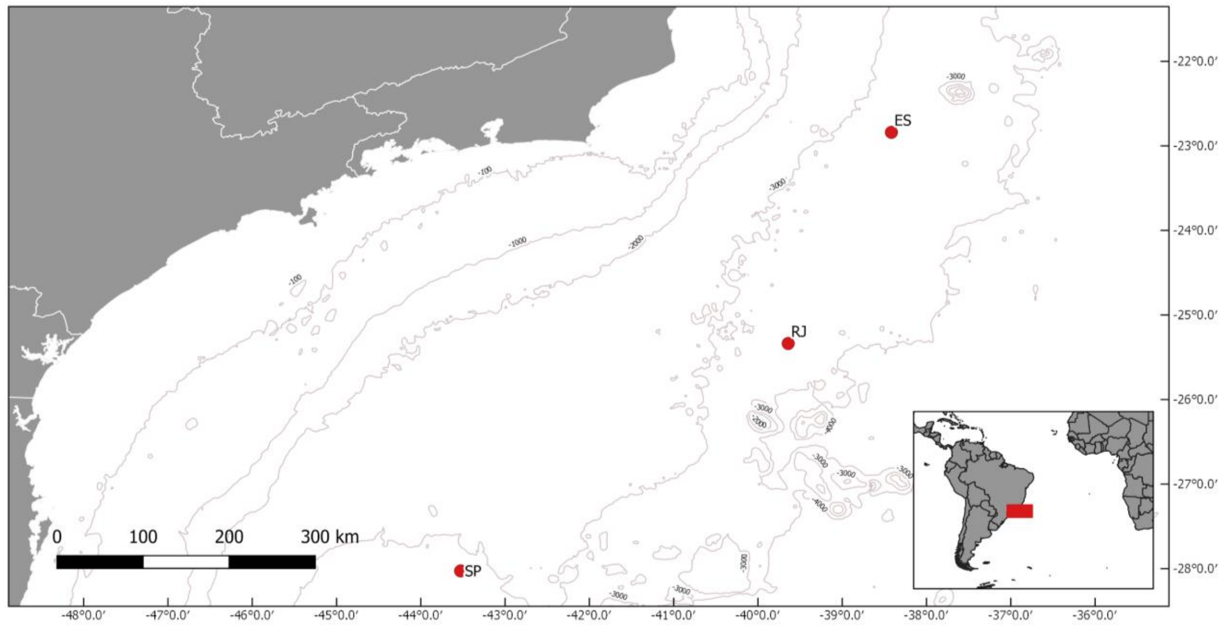
793 Yanagibayashi, M., Nogi, Y., Li, L., Kato, C., 1999. Changes in the microbial community in Japan
794 Trench sediment from a depth of 6292 m during cultivation without decompression. FEMS
795 Microbiol. Lett. 170, 271–279. https://doi.org/10.1016/S0378-1097(98)00556-4

796 Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the " Plastisphere ": Microbial
797 Communities on Plastic Marine Debris. Environ. Sci. Technol. 47, 7137–7146.
798 https://doi.org/dx.doi.org/10.1021/es401288x

799 Zhong, H., Lehtovirta-Morley, L., Liu, J., Zheng, Y., Lin, H., Song, D., Todd, J., Tian, J., Zhang,
800 X.-H., 2020. Novel insights into the Thaumarchaeota in the deepest oceans: their metabolism and
801 potential adaptation mechanisms 1–16. https://doi.org/10.21203/rs.3.rs-16227/v2

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.26.428295; this version posted January 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

802 **Figures**




803

804 **Figure 1**: Map of the study region (Southwestern Atlantic) indicating the three experimental sites
805 where the landers were deployed (red dots), all placed along the 3,300 meter bathymetric line.
806 Sites: ES, Espírito Santo; RJ, Rio de Janeiro; SP, São Paulo. Source: GeoMapApp®.

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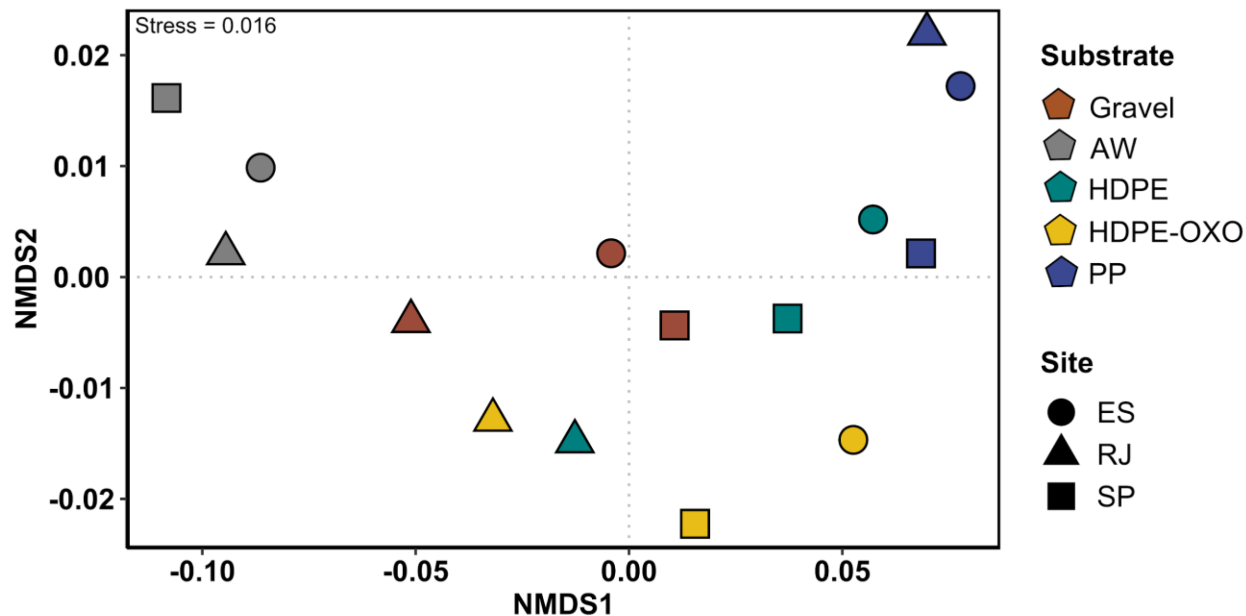


809

810 **Figure 2.** Non-metric multidimensional scaling (NMDS) ordination of weighted unifrac
811 dissimilarities in microbial community structures. Substrate: Gravel; AW, adjacent water; HDPE,
812 High density polyethylene bag (film); HDPE-OXO, High density polyethylene bag (film) with
813 oxo-biodegradable additive BDA; PP, Polypropylene pellets. Sites: ES, Espírito Santo; RJ, Rio de
814 Janeiro; SP, São Paulo.

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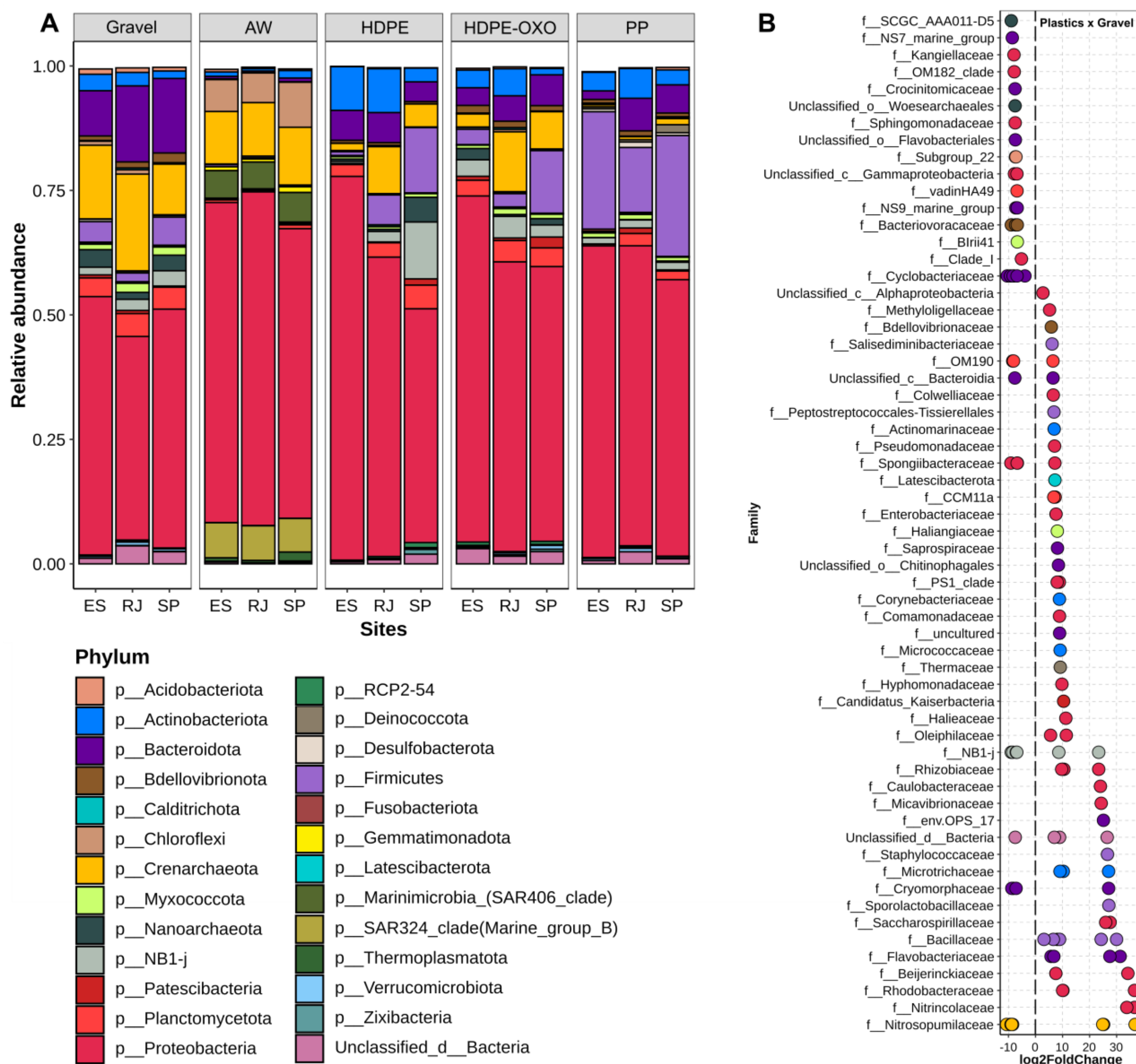


815

816 **Figure 3.** (A) Relative abundance of the microbial community at the Phylum level (Bacteria and
817 Archaea) among sites. (B) Differentially abundant Amplicon Sequence Variant (ASV) comparing
818 plastic substrates (HDPE, HDPE-OXO and PP merged) and gravel. Substrate: Gravel; AW,
819 adjacent water; HDPE, High density polyethylene; HDPE-OXO, High density polyethylene with
820 oxo-biodegradable additive BDA; PP, Polypropylene. Significant ASVs (padj $< 0.05$) are
821 represented by single data points, grouped by family on the y-axis and by color according to the
822 taxonomic phylum from which the ASV originates. Positive values (log2FoldChange) indicate
823 ASVs significantly more abundant in plastic substrates; Negative values indicate the opposite.
824 Unclassified taxons were represented by the prefix Unassigned_.

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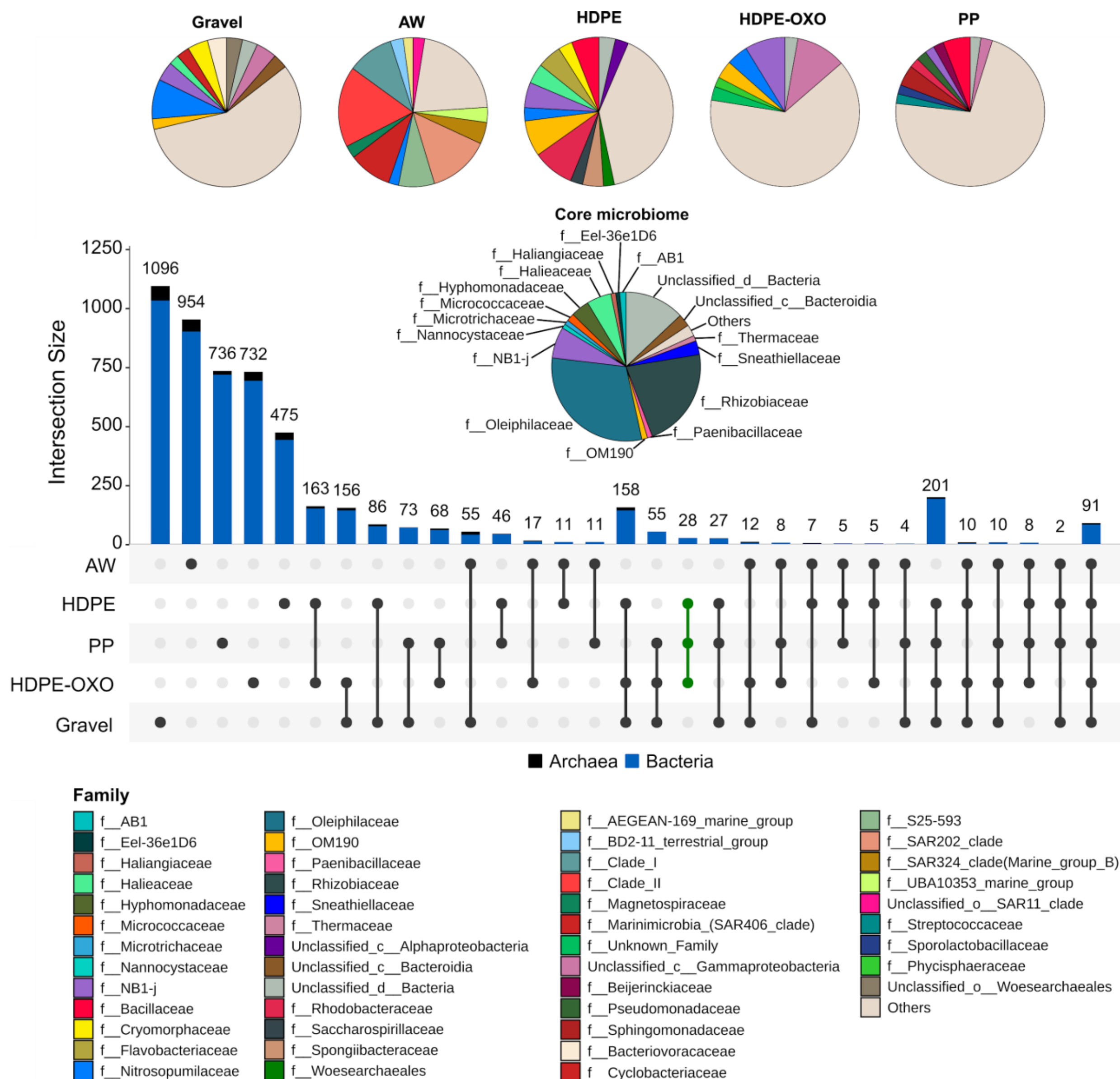


825

826 **Figure 4.** Upset plot composed by ASVs identified among substrates. Circles indicate substrates.
827 Black lines connecting circles indicate shared ASVs. Vertical bars indicate intersection size
828 (number of ASVs) on each set. Blue and black bars represent Bacteria and Archaea ASVs,
829 respectively. The green line represents ASVs shared by all plastic samples. Substrates: AW,
830 adjacent water; HDPE, High density polyethylene; HDPE-OXO, High density polyethylene with
831 biodegradable additive BDA; PP, Polypropylene. Pie charts show microbial composition specific
832 to each substrate (families with abundance > 2%) and those shared among plastics types (core
833 microbiome). Substrate: Gravel; AW, adjacent water; HDPE, High density polyethylene; HDPE-
834 OXO, High density polyethylene with oxo-biodegradable additive BDA; PP, Polypropylene.

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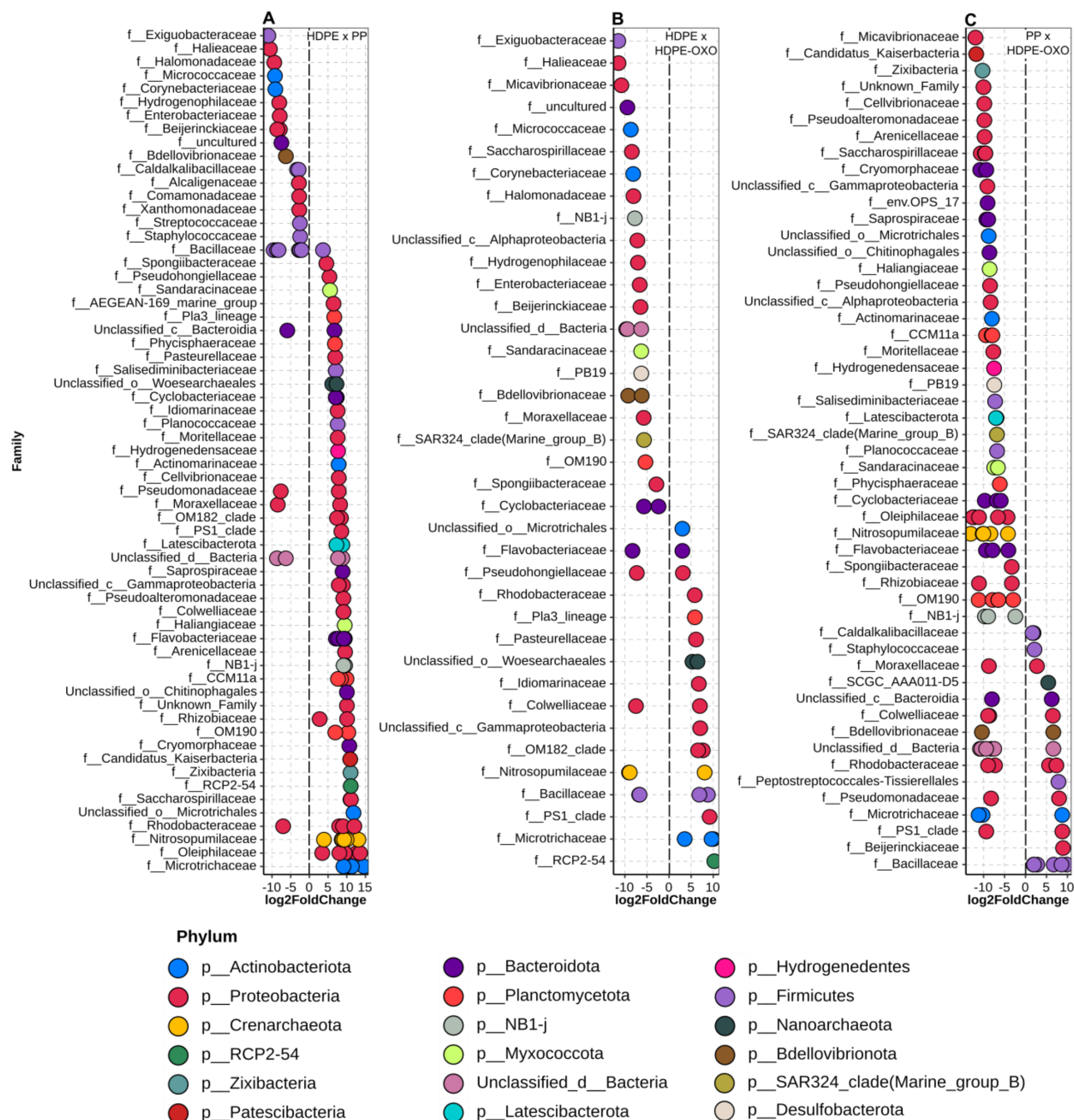


835

836 **Figure 5.** Differentially abundant Amplicon Sequence Variant (ASV) comparing HDPE and PP
837 (A), HDPE and HDPE-OXO (B); PP and HDPE-OXO (C). Significant ASVs (padj $< 0.05$) are
838 represented by single data points, grouped by family on the y-axis and by color according to the
839 taxonomic phylum from which the ASV originates. Positive values (log2FoldChange) indicate
840 ASVs that were significantly more abundant in HDPE (A and B) and PP (C) plastic substrates;
841 Negative values indicate the opposite. Substrate: Gravel; AW, adjacent water; HDPE, High density
842 polyethylene; HDPE-OXO, High density polyethylene with oxo-biodegradable additive BDA; PP,
843 Polypropylene.