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## Evidence of coupled autotrophy and heterotrophy on plastic biofilms and its influence on surrounding seawater

Pascal Conan, Léna Philip, Eva Ortega-Retuerta, Charlène Odobel, Clélia Duran, Caroline Pandin, Carolane Giraud, Anne-Leila Meistertzheim, Valérie Barbe, Alexandra ter Halle, et al.

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1 **Title:**

2 Evidence of coupled autotrophy and heterotrophy on plastic biofilms  
3 and its influence on surrounding seawaters

4

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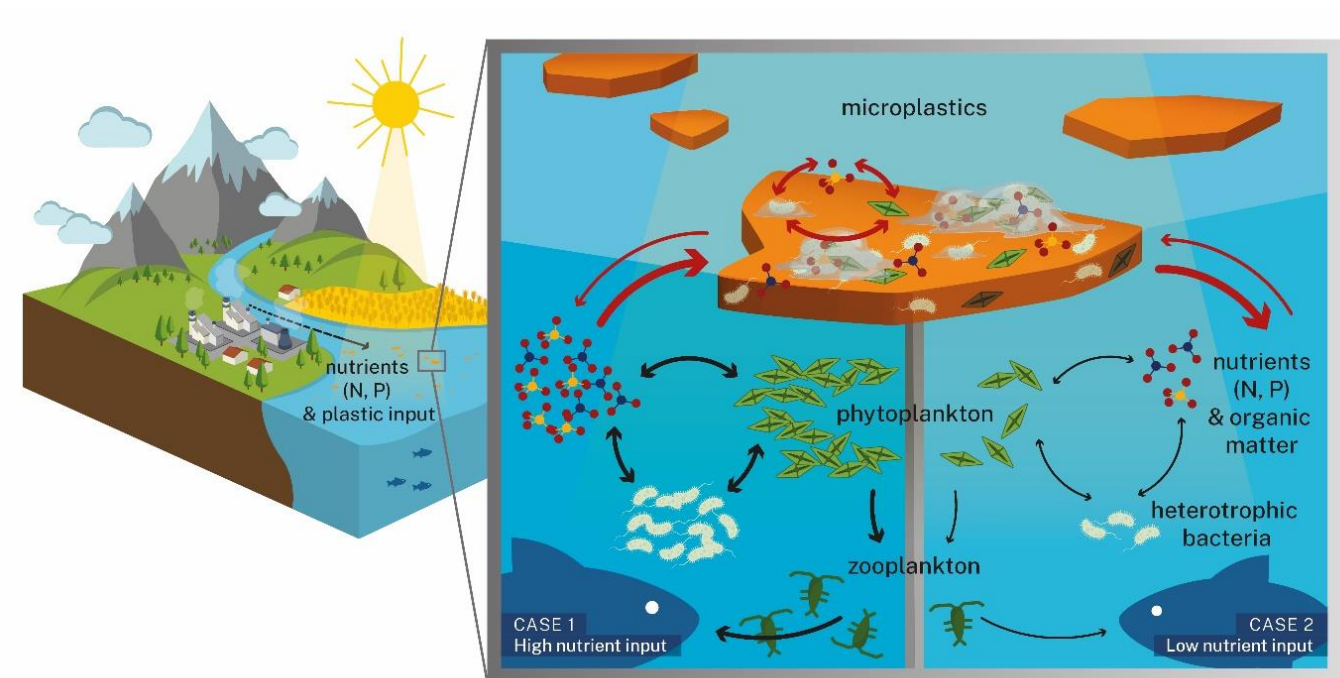
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29

30 **Graphical Abstract**

31



33

34

35 **Abstract**

36 We measured phytoplankton primary production and heterotrophic bacterial activities on  
37 microplastics and seawater in the Northwestern Mediterranean sea during two 3-month spring  
38 periods over consecutive years. Microorganisms growing on a 5 mm diameter low density  
39 polyethylene (LDPE) film (200  $\mu\text{m}$  thick) were faced with two contrasting conditions from one  
40 year to the next. Spring 2018 was characterized by consistent nutrient inputs and bloom  
41 development. In spring 2019, nutrient inputs and bloom were low. For the first time, we  
42 observed a clear coupling between primary production and heterotrophic prokaryote production  
43 on microplastics during both years, but with different intensity between years that reflected the  
44 crucial role of the trophic environmental conditions (nutrient supply) in shaping microbial  
45 activities on plastics. Indeed, we proved that high primary production on plastics could support  
46 the whole (net autotrophy) or the majority of the bacterial carbon demand needed for  
47 heterotrophic activities, supplemented by other carbon sources if surrounding waters are highly  
48 productive. We also propose that microbial activity on plastics influence the microbial  
49 community in the surrounding seawaters, especially when the environmental conditions are less  
50 favorable. An illustrative image of the role of plastics in the environment could be that of an  
51 electrical inverter in a circuit that allows for the attenuation of both positive and negative  
52 variations. Our results highlight the potential role played by the plastisphere shaping  
53 biogeochemical cycles in a context of an increasing amount of plastic particles in the marine  
54 environment.

55

## 56 **Introduction**

57       The growing use of plastic worldwide together with waste mismanagement resulted in an  
58 estimated of 24.4 trillion plastic particles floating in the world's oceans, which majority has a  
59 particle-size <5 mm, the so-called microplastics, discharged mainly from rivers (Isobe et al.,  
60 2021; Weiss et al., 2021). Indeed, the introduction of microplastics into the aquatic ecosystems  
61 is mainly related to domestic runoff that contains microbeads and fragments of microplastics  
62 (used in cosmetics and other consumer products) and also to the fragmentation of large plastic  
63 waste. Plastic manufacturing industries and coastal activities (fishing, aquatic tourism...) are  
64 also sources of microplastic pollution in the marine ecosystems (Subhankar and Shivika, 2019).  
65 The emissions of microplastic are estimated to increase, even under the most optimistic  
66 scenarios (Borrelle et al., 2020). This pollution is considered "poorly reversible" with potential  
67 impacts including changes to the carbon and nutrient cycles, co-occurring biological impacts  
68 on endangered or keystone species, ecotoxicity and other related societal impacts (MacLeod et  
69 al., 2021).

70       Once in the marine environment, plastics are rapidly colonized by microorganisms,  
71 forming the so-called plastisphere (Zettler et al., 2013), and interact with the overall ecosystem  
72 depending on their size (PVC panels of 5 × 5 cm or 15 × 3.5 cm for Catão et al., 2019; Nano-  
73 to Micro-plastics for Ter Halle and Ghiglione, 2021). Most of the studies so far revealed a large  
74 diversity and a clear niche partitioning among marine bacteria living on plastics compared to  
75 the free-living (Crisafi et al., 2022; Debroas et al., 2017; Zettler et al., 2013) and organic  
76 particle-attached fractions (Dussud et al., 2018b; Oberbeckmann et al., 2018). Autotrophic  
77 microorganisms such as cyanobacteria and diatoms have been shown to be particularly over-  
78 represented in microplastics, suggesting the potentially high impact on the global pelagic  
79 primary production and more generally on the carbon and nitrogen biogeochemical cycles in  
80 the surface oceans (Amaral-Zettler et al., 2020; Crisafi et al., 2022; Dussud et al., 2018b; Nava

81 and Leoni, 2021). Because plastic debris offer an abundant growth matrix for microalgae while  
82 limiting predation pressure by zooplankton (Kong and Koelmans, 2019), it has been proposed  
83 that plastic pollution can promote significant proliferations of microalgae, with consequent  
84 harmful effects for aquatic ecosystems already disturbed by eutrophication processes (Durrieu  
85 de Madron et al., 2011; Zhang et al., 2020). Moreover, processes involved in the nitrogen  
86 (nitrogen fixation, ammonia and nitrite oxidation, denitrification) and phosphorus cycling (P  
87 accumulation, alkaline phosphatase activity) are overexpressed in the plastisphere, which can  
88 modify the nutrient concentration in the surrounding seawater and thus indirectly affect primary  
89 producers (Chen et al., 2020). For instance, plastics may represent net autotrophic hot spots in  
90 the oligotrophic ocean, with high density of chlorophyll *a* and high oxygen production (Bryant  
91 et al., 2016). Despite growing interest on the plastisphere influence on ecosystem functions,  
92 direct measurements on microbial activities remain scarce. Some studies found also high  
93 heterotrophic prokaryotic production (Dussud et al., 2018b; Odobel et al., 2021) and  
94 ectoenzyme activities (Odobel et al., 2021) on plastics. It is surprising that primary production  
95 in the plastisphere was never recorded in marine ecosystems. Both primary production and  
96 heterotrophic activities play a key role in the carbon cycle. The former participating in about  
97 half of global primary production (Behrenfeld and Falkowski, 1997), and the latter channeling  
98 half of the oceanic primary production through the microbial loop (Del Giorgio and Cole, 2000).  
99 A recent review underlined the need of coupling primary production and heterotrophic activities  
100 to evaluate the potential role of the plastisphere on carbon cycling in the oceans (Jacquin et al.,  
101 2019). In oceanic systems, the supply of organic matter from autochthonous (autotrophic) or  
102 allochthonous (heterotrophic) primary production is mainly dependent on nitrogen and  
103 phosphorus availability and will defined the trophic status of these ecosystems, and in turn, the  
104 potential coupling between phytoplankton and bacteria (Dodds and Cole, 2007). Finally, the

105 characterization of the trophic state is necessary for understanding food web linkages as well  
106 as biogeochemical features and subsequently water quality, including human influence.

107 Our study provides the first measurements to our knowledge of the marine plastisphere  
108 primary production together with bacterial heterotrophic activities over time. We hypothesize  
109 that natural environmental factors (such as temperature, salinity, nutrient availability...) play a  
110 major role in plastisphere activities, thus influencing the net autotrophy *vs.* heterotrophy  
111 budgets over time. More precisely, trophic links within the niche created by the plastisphere  
112 would be strengthened when external conditions become less favorable (i.e. meso *vs.*  
113 oligotrophic). Polyethylene-based microplastics were incubated in natural seawater for two 3-  
114 month periods, including a classical spring phytoplanktonic bloom event that occurs every year  
115 at the long-term SOLA marine observatory station (NW Mediterranean Sea, France). The  
116 experiments revealed a clear link between autotrophic and heterotrophic productions of the  
117 plastisphere over time, as well as a possible influence of the microbial activities on  
118 microplastics in surrounding seawaters.

119

120

## 121 **2. Material and methods**

### 122 **2.1. Experimental conditions during 3 months in two following years**

123 We incubated 200 circular pieces of polyethylene of 5 mm diameter and 200  $\mu\text{m}$  thickness  
124 (LD-PE, Borealis, ref. FA6224, Austria) in a 50 L aquarium with direct circulation to the sea.  
125 The incubation was repeated twice at one year interval: the first incubation was performed from  
126 12 February to 7 May 2018 (44<sup>th</sup> to 127<sup>th</sup> day of the year, here after D44-127<sup>1</sup>; period of 85  
127 days) and the second incubation was performed from 19 February to 8 May 2019 (D50-133;

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<sup>1</sup> D1 correspond to the 1<sup>st</sup> January with this time scale

128 period of 91 days). Each plastic piece was first sterilized with 70% ethanol and rinsed 3-times  
129 with sterile seawater before incubation. The aquarium was placed outside with natural light  
130 conditions, and covered by a transparent screen. Seawater circulating through the aquarium was  
131 pumped from 4 m depth and 30 m from the coast in the Banyuls bay (NW Mediterranean Sea,  
132 France) and the seawater flow rate in the aquarium was ~5 L per hour.

133 Triplicate plastic pieces were sampled 12 times during each experiment with sterile  
134 forceps at each sampling time (*i.e.* D44, D46, D50, D53, D57, D60, D72, D79, D85, D99, D113,  
135 D127 for 2018 and D50, D57, D64, D72, D86, D93, D100, D106, D114, D120, D126, D133  
136 for 2019). In parallel, 3 L of seawater were sampled in a sterilized glass vial at the same  
137 sampling days from the aquarium or at the SOLA marine observatory located at 0.5 nautical  
138 miles from the coast in the Banyuls Bay (42°29'300 N; 03°08'700 E).

139

## 140 **2.2 Heterotrophic bacterial production**

141 Heterotrophic bacterial production was measured in triplicate on each plastic and  
142 seawater sample at each sampling time by the <sup>3</sup>H-leucine incorporation into proteins method,  
143 as previously described in Dussud et al. (2018b). Briefly, the plastic pieces were transferred  
144 into 1.5 mL of sterilized seawater and a soft cell detachment pre-treatment was applied for each  
145 sample consisting in 3 cycles of 1 minute vortexing followed by 3 minutes in an ultrasound  
146 bath. This pre-treatment greatly improves the signal, especially in the case of mature biofilms  
147 (Dussud et al., 2018b). Immediately after cell-detachment, <sup>3</sup>H-leucine (specific activity 4.2 x  
148 10<sup>12</sup> Bq.mmol<sup>-1</sup>; Perkin Elmer) was added at a final concentration of 0.9975 x 10<sup>-9</sup> mol.L<sup>-1</sup>  
149 (completed with cold leucine to 1.49 x 10<sup>-7</sup> mol.L<sup>-1</sup>). A same <sup>3</sup>H-leucine concentration of 3,97  
150 x 10<sup>-9</sup> mol.L<sup>-1</sup> (completed with cold leucine to 3.6 x 10<sup>-8</sup> mol.L<sup>-1</sup>) was used for seawater from  
151 the aquarium and from the SOLA marine station. No cell detachment pretreatment was used for  
152 seawater samples, since Dussud et al. (2018b) showed that did not influence the signal for free-



153 living bacteria in seawater. All samples were incubated in the dark at 18°C for 2-3 h. The  
154 theoretical conversion factor of 1.55 ngC.pmol<sup>-1</sup> of incorporated leucine was used to calculate  
155 heterotrophic bacterial production (Simon and Azam, 1989). Data were normalized for  
156 microplastics with blank values according to Dussud et al. (2018b).

157

### 158 **2.3 Primary production**

159 Primary production (PP) was measured using a modified protocol of the radioactive <sup>14</sup>C  
160 tracer technique (Fitzwater et al., 1982). Measurements were carried out in triplicate. One  
161 plastic piece was transferred in 10 mL of sterilized seawater. The same volume was taken for  
162 seawater from the aquarium and from the SOLA marine station. Each sample was inoculated  
163 with Na<sub>2</sub>H<sup>14</sup>CO<sub>3</sub> (final activity of 18.5 kBq.mL<sup>-1</sup>). The introduced quantity was measured by  
164 mixing 100 µL ethanolamine to 100 µL of inoculated sample and 10 mL of scintillation cocktail  
165 Ultima Gold uLLT. The samples were placed for 5 to 8 h in a thermic and light regulated  
166 incubator (14 to 18°C – 500 to 2000 µE.m<sup>-2</sup>.s<sup>-1</sup> according to the season). Plastics were isolated  
167 and acidified with 6 N HCl and dried during 8 h at 50 °C. Seawater samples were acidified with  
168 6N HCl (final pH = 2) and agitated, lid open, for at least 12 h at 130 rpm and then mixed with  
169 scintillation cocktail during 4 hours before measuring radioactivity with the scintillation counter  
170 300 SL Hidex.

171 The percentage of carbon originating from the extracellular release (ER) that is theoretically  
172 available for bacterial heterotrophic activity was calculated as previously described in Van  
173 Wambeke et al. (2002). Briefly, the theoretical ER associated to the autotrophic carbon fixation  
174 was calculated for a range of 5 and 20% of the PP to cover at least 90% of the wide range of all  
175 values found in the literature (Conan et al., 1999). It has been compared to the minimal and  
176 maximal theoretical bacterial carbon demand (BCD) ranging from 1 to 25% of the ER (review  
177 in Van Wambeke et al., 2002). Comparison between microbial activities on microplastic and in

178 the seawater was calculated by integrating their respective dynamics over the 3 months  
179 sampling periods in 2018 and 2019.

180

## 181 **2.4 Environmental parameters**

182 Temperature, salinity, nutrients and chlorophyll *a* were measured in the aquarium with  
183 direct circulation to the sea and at the SOLA marine observatory station all along both 3-month  
184 incubations. Temperature, salinity and fluorescence were continuously recorded with a CTD  
185 probe (Seabird SBE16+) equipped with a fluorimeter (ECO FLNTU WETLab). All chemical  
186 measurements were carried out following standard procedures defined by SOMLIT protocols  
187 ([www.somlit.fr/parametres-et-protocoles/](http://www.somlit.fr/parametres-et-protocoles/)). Briefly, ammonium was detected at nanomolar  
188 concentrations by fluorimetric detection according to Holmes et al. (1999) using a Turner  
189 Designs Trilogy fluorimeter. Nitrate, nitrite and phosphate concentrations were simultaneously  
190 measured in 10 mL of sample, on a continuous flow Autoanalyser III Seal-Bran&Luebbe  
191 (Aminot and K erouel, 2007). For Chlorophyll *a*, 250 mL samples were filtered using pre-  
192 combusted 25 mm diameter Whatman® GFF filters (~0.4-0.7 µm porosity). Concentrations  
193 were determined by fluorimetry (Lorenzen, 1966) on a turner design Trilogy fluorimeter.

194

## 195 **2.5 Statistical analysis**

196 All statistical and graphical analyses as well as application condition checks were  
197 performed using StatEL software v3 for excel ([www.adscience.fr](http://www.adscience.fr)).

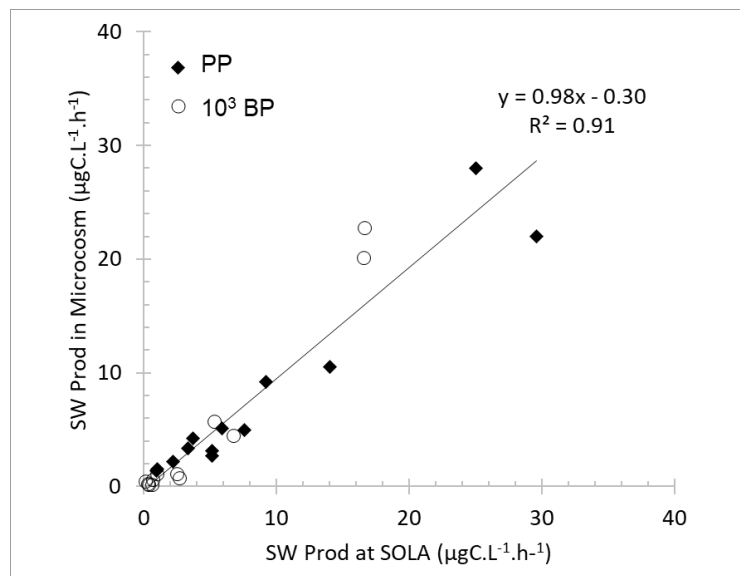
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199 **3. Results**

200 **3.1 Representativeness of aquarium with circulating seawater compared to *in situ***  
201 **conditions**

202 We measured the temporal evolution of several environmental parameters in order to test  
203 that the experimental setup in the 50 L aquarium with continuous circulating seawater  
204 originating from the bay of Banyuls (flow rate of ~5.0 L per hour) was representative of the *in*  
205 *situ* conditions classically observed at the coastal observatory marine station (SOLA located in  
206 the Banyuls bay). We found significant correlations for all parameters ( $R > 0.78$ ,  $p < 0.05$ )  
207 between our experimental aquaria and the natural seawater in both experiments performed in  
208 2018 and 2019.

209 In particular, we compared the primary production (PP) and heterotrophic bacterial  
210 production (BP) in the aquarium with continuous seawater circulation and the measurements  
211 carried out with water sampled at SOLA station (Fig. 1). Analysis of the comparison points  
212 showed a linear regression slope of  $0.98 \pm 0.17$  ( $p < 0.00001$ ;  $n = 24$ , slope is not significantly  
213 different from 1 and the origin is not significantly different from 0;  $p < 0.05$ ). The same patterns  
214 were obtained with temperature, salinity, chlorophyll *a* and nutrients thus confirming the  
215 absence of an “aquarium effect” during our 3 month-experiments.



216

217 **Figure 1:** Comparison of phytoplankton primary production (PP = ◆) and heterotrophic  
 218 bacterial production (BP = ○) in the aquarium with continuous seawater circulation and at  
 219 the long-term observatory SOLA marine station (Banyuls bay, NW Mediterranean sea). Note  
 220 that the heterotrophic bacterial production units are 1000x. The linear regression is  
 221 represented by the black line and the corresponding equation is indicated ( $p < 0.00001$ ;  $n =$   
 222 24).

223

224

### 225 3.2 Biogeochemical conditions during 2 years experiments in a local temporal context

226 Our two experiments from 2018 and 2019 were done using the same 50-L aquarium with  
 227 circulating seawater pumped in the Banyuls Bay, in the vicinity of the SOLA observatory  
 228 station. Weekly measurements are made at SOLA since 1997 as part of the National  
 229 Observation Service (SNO-SOMLIT). We compared the biogeochemical conditions of our  
 230 experiments, *i.e.* from 13 February 2018 (D44) to 14 May 2019 (D133), to the nearly 24 years  
 231 of observation at the SOLA station (see [www.somlit.fr/mysomlit/](http://www.somlit.fr/mysomlit/)). Very contrasted  
 232 environmental conditions were observed between 2018 and 2019, with higher variations of

233 temperature, salinity, nutrients and chlorophyll *a* concentrations during the first year (Fig. 2).  
234 Indeed, when comparing the data of the 2 years with respect to the long-term average values  
235 represented by the continuous line (Fig. 2A and 2B), the 2018 measurements are variable and  
236 distributed rather below the normals, while the 2019 measurements are more stable, close to  
237 the normals in temperature (Fig. 2A) but largely above for salinity (Fig 2B). This leads to  
238 important differences in terms of nutrients, particularly visible on nitrates (Fig. 2E), with 2018  
239 being rich compared to 2019 marked by minimal nutrient concentrations.

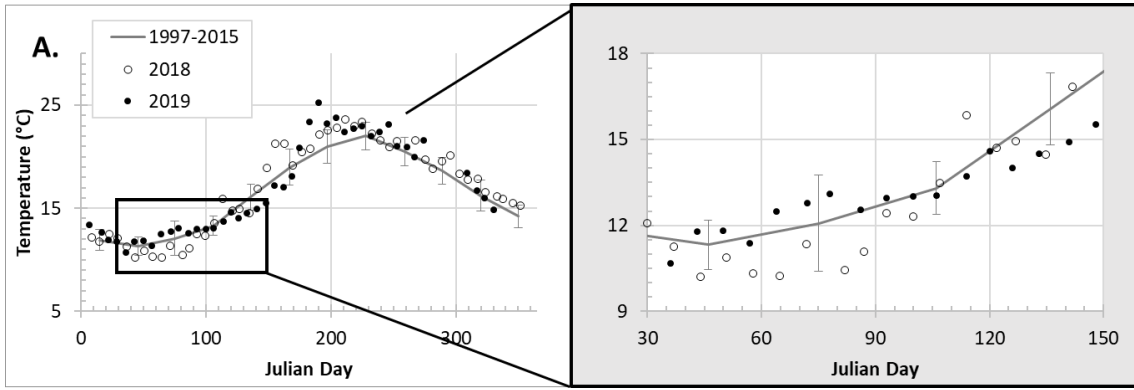
240         Then, the spring conditions in our 2018 experiment were particularly cold between D30  
241 and D90 (Fig. 2A) and marked by two episodes of low salinity water at D72 (36.4) and D105  
242 to D115 (35.3 and even 33 for D156; Fig. 2B). These desalted episodes caused strong variations  
243 in nutrient concentrations during 2018, especially phosphate, marked by 2 peaks with nearly  
244 0.1  $\mu\text{M}$  (Fig. 2C). While ammonium concentrations were rather low ( $\sim 0.1 \mu\text{M}$ ) compared to  
245 “usual values”, we still observed 2 peaks approaching 0.3  $\mu\text{M}$  each (Fig. 2D). Nitrate  
246 concentrations were high throughout the period, with peaks above 3  $\mu\text{M}$  (Fig. 2E). The  
247 abundance of nutrients in 2018 resulted in a particularly pronounced spring bloom with  
248 chlorophyll *a* maximal concentrations close to 3.5 and 4.1  $\text{mgCHL}\cdot\text{m}^{-3}$  for D72 and D107  
249 respectively (Fig. 2F).

250         In 2019, the temperatures remained close to the climatologic curve during the  
251 experimental period, while the summer period was warmer (Fig. 2A). Throughout the  
252 experiment, salinity was high, with values varying around 38 but the time series was marked  
253 by 2 weak decreases for D72 (37.7) and D120 (37.5) (Fig. 2B). As a result, all nutrient  
254 concentrations were rather low. Phosphate concentrations were relatively stable at about 0.03  
255  $\mu\text{M}$  and marked by 2 maxima (0.05  $\mu\text{M}$  for D86 and 0.11  $\mu\text{M}$  for D114; Fig. 2C). Ammonium  
256 concentrations were close to the detection limit, but again, we observed 2 peaks (0.2  $\mu\text{M}$  for  
257 D93 and 0.23  $\mu\text{M}$  for D114; Fig. 2D). A similar pattern was observed for nitrate, with very low

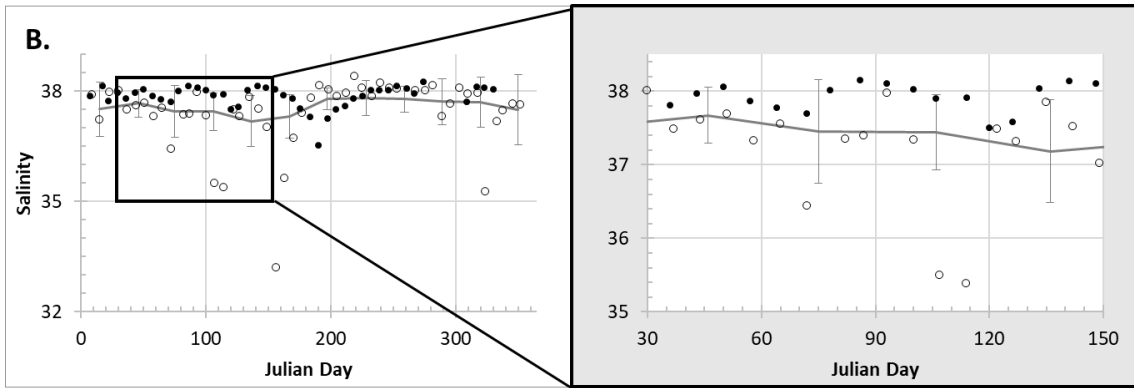
258 concentrations throughout the period, and 2 minor peaks at D64 and D100 (Fig. 2E). The low  
259 abundance of nutrients in 2019 resulted in a rather weak spring bloom with chlorophyll *a*  
260 maximal concentrations of 1.5 mgCHL.m<sup>-3</sup> recorded between D115 and D125 (Fig. 2F).

261

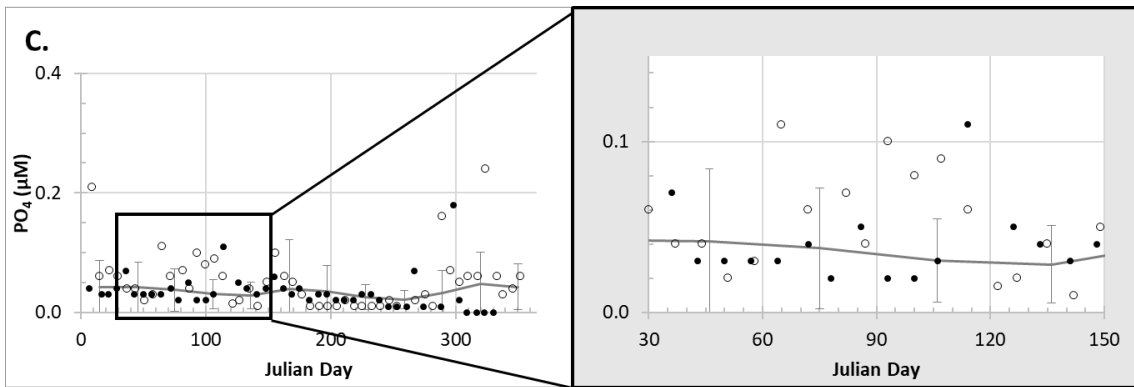
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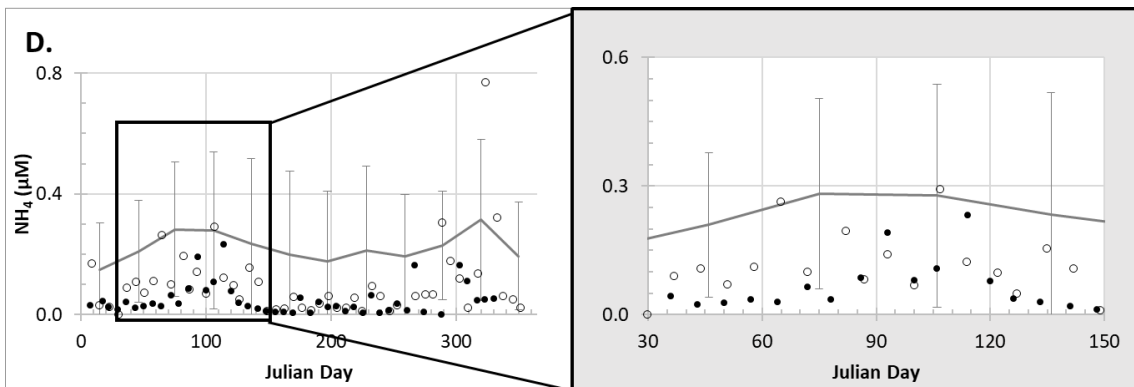
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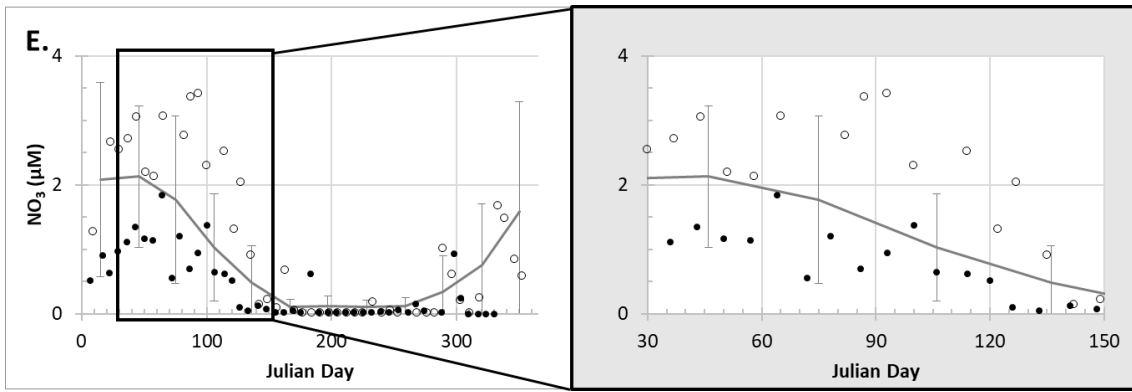
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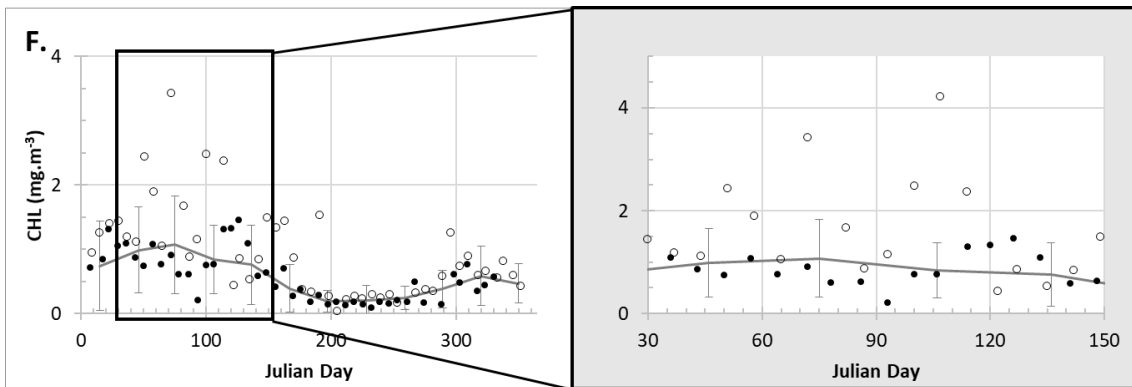
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266



267



268 **Figure 2:** Seasonal evolution at SOLA observatory station for 2018 (D44-127, open dots) and  
269 2019 (D50-133, full dots) compared to the 1997-2015 climatology (black solid line and  
270 confidence interval) of A. temperature in °C, B. salinity, C. phosphate in  $\mu\text{M}$ , D. ammonium  
271 in  $\mu\text{M}$ , E. nitrate in  $\mu\text{M}$ , and F. chlorophyll a in  $\text{mgCHL}\cdot\text{m}^{-3}$ . The grey inset on the right of  
272 each main graph focuses on the experimental period D30 to D150.

273

274

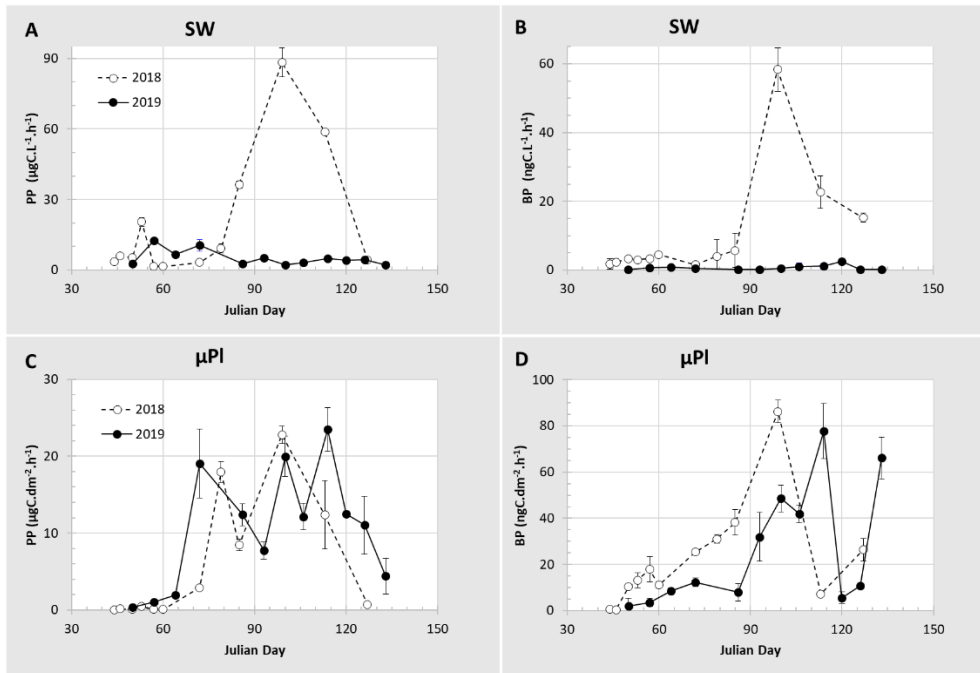
### 275 3.3. Primary production and heterotrophic bacterial production in seawater and on 276 microplastics

277 Significant differences were found when comparing the evolution of both primary and  
278 heterotrophic bacterial productions in seawater between the experiments conducted in 2018 and  
279 2019 (Fig. 3A & 3B). In 2018, primary and heterotrophic bacterial productions presented a  
280 succession pattern over the 3 months spring period. Production rates varied from low activities



281 between D40 and D80 (on average  $5.9 \pm 5.0 \mu\text{gC.L}^{-1}.\text{h}^{-1}$  for PP [Fig. 3A] and  $3.3 \pm 1.3 \text{ngC.L}^{-1}.\text{h}^{-1}$  for heterotrophic bacterial production [Fig. 3B]) to a high peak observed at D100 for both  
282 activities ( $88.2 \pm 6.1 \mu\text{gC.L}^{-1}.\text{h}^{-1}$  and  $58.3 \pm 6.4 \text{ngC.L}^{-1}.\text{h}^{-1}$  for PP and heterotrophic bacterial  
283 production, respectively) followed by a rapid decrease until the end of the incubation (~D130).  
284 In 2019, the evolution of primary and heterotrophic bacterial productions were clearly different  
285 compared to 2018, with no synchronicity or succession observed between the 2 types of  
286 productions. Primary production varied over time with maximal values at the beginning of the  
287 experiment between D50 and D90 (maximum rate at  $12.5 \pm 0.1 \mu\text{gC.L}^{-1}.\text{h}^{-1}$ ) and then remained  
288 low and relatively stable until the end of incubation ( $<5.1 \pm 0.1 \mu\text{gC.L}^{-1}.\text{h}^{-1}$ ; Fig. 3A). In  
289 contrast, heterotrophic bacterial production had a similar evolution to 2018, but with lower  
290 values and a low later peak (maximum for D120 with  $2.5 \pm 0.5 \text{ngC.L}^{-1}.\text{h}^{-1}$ ; Fig. 3B).

292 Years 2018 and 2019 showed sharp contrasted spring conditions, reflected in the distinct  
293 evolution of primary and heterotrophic bacterial productions in seawater. On the contrary, the  
294 productions measured on microplastics showed a surprisingly similar evolution during the two  
295 years (Fig. 3C & 3D). Primary production increased rapidly after D60 to reach maximum  
296 activities of about  $20 \pm 3 \mu\text{gC.dm}^{-2}.\text{h}^{-1}$  (Fig. 3C) and remained variable (from  $8 \pm 1$  to  $23 \pm 2$   
297  $\mu\text{gC.dm}^{-2}.\text{h}^{-1}$ ) until D100 in 2018 and D115 in 2019 to finally drop rapidly until the end of the  
298 experiment. Heterotrophic bacterial production increased during about 80 days to reach a  
299 maximum of  $86 \pm 5 \text{ngC.dm}^{-2}.\text{h}^{-1}$  in 2018 at D99 and  $78 \pm 10 \text{ngC.dm}^{-2}.\text{h}^{-1}$  in 2019 at D114.  
300 During both years, peaks were followed by a rapid decrease and a second phase of increase  
301 until the end of the incubation (Fig 3D).



302

303 **Figure 3:** Temporal evolution during the incubation time in 2018 (D44-127, open dots and  
 304 dashed line) and 2019 (D50-133, full dots and black line) of **A.** Primary production, PP (in  
 305  $\mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ ), **B.** Heterotrophic bacterial production, BP ( $\text{ngC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ ) in aquarium seawater  
 306 (SW) and of **C.** Primary production (in  $\mu\text{gC}\cdot\text{dm}^{-2}\cdot\text{h}^{-1}$ ), **D.** Heterotrophic bacterial production  
 307 ( $\text{ngC}\cdot\text{dm}^{-2}\cdot\text{h}^{-1}$ ) on microplastics ( $\mu\text{PI}$ ). Whiskers represent standard deviations.

308

309

### 310 **3.4. Comparison of microbial activities on microplastics and in seawater**

311 We calculated the percentage of carbon originating from the extracellular release (ER)  
 312 that was available for bacterial heterotrophic activity, based on the theoretical ranges of ER  
 313 (from 5 to 20% of the PP) and the bacterial carbon demand (BCD) considering a Bacterial  
 314 Growth Efficiency (BGE) of 10 to 30%. Indeed, BGE is the amount of new bacterial biomass  
 315 produced per unit of organic C substrate assimilated. The wide ranges used in our calculation  
 316 are sufficient to cover about all natural and mesocosm situations for phytoplanktonic excretion  
 317 (Baines and Pace, 1991; López-Sandoval et al., 2010; Moran et al., 2002), or BGE (Del Giorgio

318 and Cole, 1998; Lemee et al., 2002) and match also with the rates found in benthic communities  
319 (Hubas et al., 2007).

320 During the spring sampling periods, we found that the free-living seawater bacteria might  
321 theoretically consume between 2 and 25% of the carbon excreted by autotrophs in the 2018  
322 conditions and between 0.5 and 6% in the 2019 conditions (Tab. I). The situation was quite  
323 different on microplastics, where we found that between 14 to 167% of the extracellular release  
324 was needed to cover the BCD of the heterotrophic community in 2018 and between 8 to 101%  
325 in 2019.

326

327

328 **Table I. Comparison between microbial activities on microplastics and in the surrounding**  
329 **seawater.** *On top:* integrated values of primary production and bacterial heterotrophic  
330 production (BP) in seawater (in  $\text{mgC.L}^{-1}$ ) and on microplastics ( $\text{mgC.dm}^{-2}$ ) over the 88 days  
331 of incubation. *In the middle:* theoretical extracellular release (range for 5 to 20% excretion,  
332 as mentioned by Conan et al., 1999) and theoretical bacterial carbon demand (with a BGE  
333 of 10 to 30% as mentioned by Van Wambeke et al., 2002). *At the bottom:* theoretical carbon  
334 from extracellular release available for (BP) bacterial heterotrophic activity (see text for  
335 more explanation).

Year	Nb day	SEAWATER				μPLASTIC			
		Integrated PP	sigma	Integrated BP	sigma	Integrated PP	sigma	Integrated BP	sigma
2018	88	26.7	6.0	0.0339	0.0294	7.3	3.1	0.061	0.025
2019	88	4.6	1.1	0.0013	0.0004	9.6	4.5	0.049	0.075
		Excretion 5 % - 20%		BCD 10% - 30%		Excretion 5 % - 20%		BCD 10% - 30%	
2018	88	1.3	5.3	0.339	0.113	0.4	1.5	0.611	0.204
2019	88	0.2	0.9	0.013	0.004	0.5	1.9	0.486	0.162
		<b>Potential Carbon PP-source consumed for BP</b>							
2018	88	MIN - MAX (%)		2	25	MIN - MAX (%)		14	167
2019	88	MIN - MAX (%)		0.5	6	MIN - MAX (%)		8	101

336

337

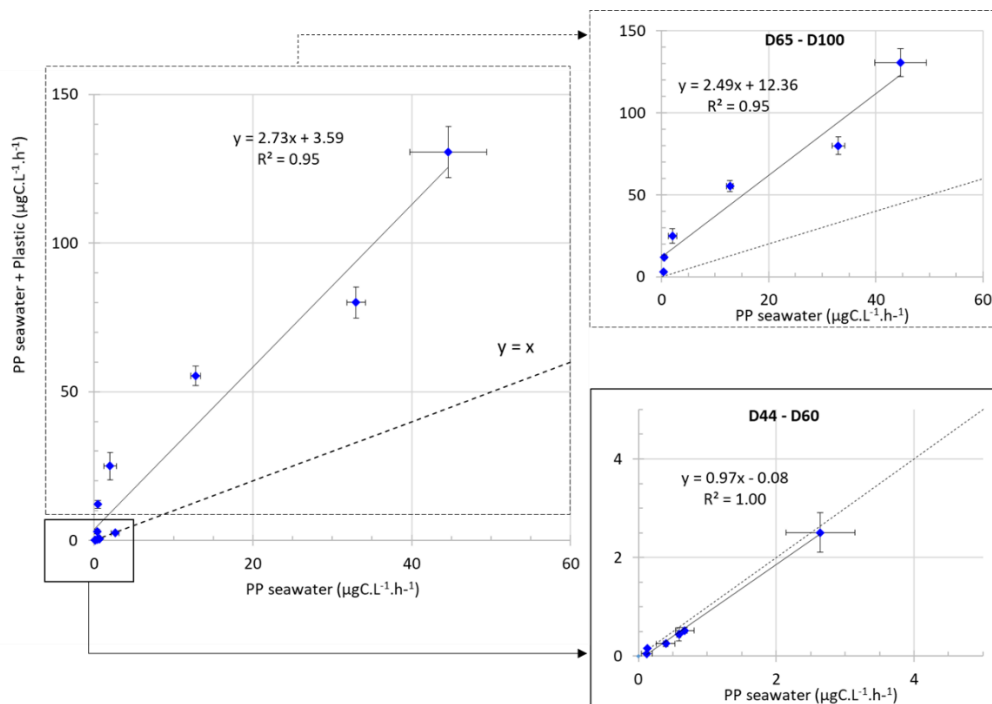
338

### 339 3.5. Influence of microplastic microbial activities on the surrounding seawater

340 In the 2018 experiment, we compared measurements of total primary production in the  
341 seawater samples that were incubated with or without microplastics (Fig. 4). Primary  
342 production in seawater incubated in the presence of plastic is higher than when no plastic is  
343 present. The positive linear correlation between samples is estimated using a Spearman's test  
344 ( $p < 0.005$ ), and then the regression is calculated using Pearson's regression ( $PP_{\text{seawater} + \text{Plastic}} =$   
345  $2.73 \times PP_{\text{seawater}} + 3.59$ ;  $R^2 = 0.95$ ;  $p < 0.00001$ ). The intercept of the regression is not different  
346 from 0 ( $p < 0.05$ ), and so the increase in the rate of primary production of seawater in the  
347 presence of plastic is about 2.8 over the incubation period.

348 In order to clarify the origin of this significant increase, we separate the data into 2 subsets  
349 (Fig. 4), the first grouping the measurements during the development of the platisphere (D44 -  
350 D60), and the second including the data in the presence of the “mature platisphere” (D65 -  
351 D100). Finally, there was no significant difference between the PP of seawater incubated with

352 or without plastic during the first 26 days ( $PP_{\text{seawater} + \text{Plastic}} = 0.97 \times PP_{\text{seawater}} - 0.08$  ;  $R^2 = 0.99$  ;  
 353  $p < 0.00001$ ). The slope of the regression model was not different from 1 (Ancova after a Mann-  
 354 Whitney test,  $p < 0.05$ ) and the intercept was not different from 0 ( $p < 0.05$ ). The situation was  
 355 quite different for the second period (D65 - D100), when the platisphere was well developed  
 356 (Fig. 4). In this case, the PP in seawater incubated with plastic increased by 2.5 times compared  
 357 to PP in seawater that has not been in contact with plastic ( $PP_{\text{seawater} + \text{Plastic}} = 2.49 \times PP_{\text{seawater}} +$   
 358  $12.36$  ;  $R^2 = 0.95$  ;  $p < 0.00085$ ). The slope of this regression model is significantly different  
 359 from 1 (Ancova after a Mann-Whitney test,  $p < 0.00067$ ) and the intercept is significantly  
 360 different from 0 ( $p < 0.00085$ ).



361  
 362 **Figure 4:** Comparison of primary production (PP) in seawater without (SW) or with  
 363 microplastic (SW+MP) during the 2018 experiment. Linear regression are full line and  
 364 significant equations are indicate ( $p < 0.001$ ;  $n = 12$ ). Dashed line represents  $y = x$ . Pooled  
 365 data are presented on the left, whereas on the right, date are separated into 2 periods as  
 366 symbolized by the squares (D44 – D60 on the bottom, D65 - D100 on the top)

367

#### 368 4. DISCUSSION

369 The impact of plastic pollution on biogeochemical cycles can no longer be considered  
370 limited today, since the amount of microplastics found at sea could soon cover more than 4.2  
371 million km<sup>2</sup> of the sea surface (Eriksen et al., 2014; Hidalgo-Ruz et al., 2012). To date, most of  
372 the research dealing with the plastisphere has been focused on its biodiversity (including  
373 putative pathogens or harmful algae), with little documentation on how the plastisphere may  
374 influence the biogeochemical cycles (Jacquin et al., 2019). Here, we present the first data of PP  
375 coupled with heterotrophic bacterial production on microplastics. After a month of colonization  
376 of polyethylene microplastics in seawater, we observed that the mature biofilm presented high  
377 PP levels, up to  $20 \pm 3 \mu\text{gC}\cdot\text{dm}^{-2}\cdot\text{h}^{-1}$  compare to the beginning of the experiment. Consistent to  
378 our results, phototrophs such as diatoms and cyanobacteria have been commonly described in  
379 the plastisphere, at least on plastics that are exposed to sunlight (Amaral-Zettler et al., 2020;  
380 Decelle et al., 2015), confirming that microplastics floating at the ocean surface provide good  
381 substrates for the development of autotrophic microorganisms. For example, Crisafi et al.  
382 (2022) recently studied the growth of microbial biofilms on medical masks in a coastal area  
383 affected by different anthropogenic inputs. The authors observed that after one month in the  
384 field, the masks were colonized by a bacterial community significantly different from that  
385 detected in natural matrices in the same areas (seawater and sediments), and allowed the growth  
386 and enrichment of phototrophic microorganisms such as Oxyphotobacteria, Ruminococcaceae,  
387 Gracilibacteria, and Holophageae in particular.

388 Plastics in the environment are composed by a large variety of organic and inorganic  
389 constituents according to their surface properties and mediated by physical forces common to  
390 all molecular systems (*i.e.* van der Waals, electrostatic and others), the so-called eco-corona  
391 (Monopoli et al., 2012; Ter Halle and Ghiglione, 2021). Together with light and CO<sub>2</sub>  
392 availability, nutrients such as NO<sub>3</sub><sup>2-</sup>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> are abundant in the biomolecular corona

393 of microplastics (Galloway et al., 2017), thus potentially fueling the intense PP observed in our  
394 experimental conditions.

395 Our results also showed a direct link between PP and heterotrophic bacterial production  
396 (BP) within the microplastic biofilms. BP reached  $86 \pm 5 \text{ ngC.dm}^{-2}.\text{h}^{-1}$ , which was in the same  
397 order of magnitude than the few previous studies recording this parameter on microplastics in  
398 marine waters (Dussud et al., 2018b; Odobel et al., 2021). Heterotrophic bacterial activities play  
399 a key role in different biogeochemical cycles such as carbon, since it is known that half of the  
400 oceanic primary production on average is re-mineralized by bacteria into the microbial loop  
401 (Azam et al., 1983; Cole et al., 1988), thus transforming the particulate organic matter to  
402 dissolved organic matter (Grossart et al., 2007). Here, we propose for the first time that  
403 heterotrophic microbes have the ability to process considerable amounts of organic matter  
404 produced on microplastics by phytoplankton activities. Here, we propose for the first time that  
405 heterotrophic microbes have the capacity to process considerable amounts of organic matter  
406 produced on microplastics by phytoplankton activities. Indeed, photosynthates are known to be  
407 particularly labile and rapidly consumed by bacteria (Conan et al., 1999; Van Wambeke et al.,  
408 2002). The authors showed that the lability/quality of this material increased with its freshness  
409 (age) and with trophic conditions (i.e. material produced in oligotrophic environments is  
410 generally less labile than that of mesotrophic and eutrophic environments). The spatial  
411 proximity of the protagonists and their greater number within the plastisphere would be a factor  
412 in strengthening the trophic link, both in the autotrophic to heterotrophic direction and in the  
413 other direction. Interestingly, our results also indicate that PP can theoretically support most of  
414 the heterotrophic activities within the microplastic biofilm. If we assume that taking the  
415 extremes, the orders of magnitude cover most values encountered in natural environments and  
416 in biofilms, and considering the ranges of ER between 5 and 20% and BGE on labile material  
417 between 10 and 30% (Baines and Pace, 1991; Conan et al., 1999; Del Giorgio and Cole, 1998;

418 Hubas et al., 2007; Lemee et al., 2002; López-Sandoval et al., 2010; Moran et al., 2002; Van  
419 Wambeke et al., 2002), we observed that the carbon requirement of free-living heterotrophic  
420 bacteria could be largely covered by PP for both years.

421 Similarly, ER might have theoretically covered most of the BCD in 2018 (min. 14% and  
422 max. 167%, with <100% corresponding to the BCD totally covered by ER) and in 2019 (from  
423 8-101%). Thus, while in 2019 the BCD appeared to be fulfilled by autotrophic production on  
424 microplastics (*i.e.* when PP in surrounding seawater was low), it is possible that in 2018 an  
425 additional organic carbon source was required to supplement the needs of attached  
426 heterotrophic bacteria. This observation is consistent with the fact that in 2018, PP in the  
427 surrounding water was particularly strong and could logically have been a supplemental source.  
428 However, our results suggest that the use of such a complementary carbon source by the  
429 plastisphere and the depletion of carbon and nitrogen in the surrounding seawater would rather  
430 be an exception than a rule. This confirms that microplastic is an ecological niche in itself  
431 (Dussud et al., 2018b), where strong phytoplankton-bacteria interactions exist that control  
432 nutrient cycling and biomass production at the scale of microplastic pieces. Roughly, plastics  
433 appear to be comparatively less autotrophic than surrounding seawater during our experiments.

434 Another originality of our study was to take into account two contrasted environmental  
435 conditions during each of the 2 years. It is indeed very likely that biofilm composition and  
436 microbial interactions within plastic surfaces are in turn influenced by environmental  
437 perturbations (Crisafi et al., 2022). For example, the works of Allgaier et al. (2008) in natural  
438 mesocosms were designed to test whether the predicted change in  $p\text{CO}_2$  will affect the  
439 communities of heterotrophic bacteria during a phytoplankton bloom development. The authors  
440 concluded that bacterial abundance and activities were similar among the various treatments  
441 but the community structure of free-living bacteria changed with  $p\text{CO}_2$  on the contrary of that  
442 of attached bacteria. These latter were tightly coupled to phytoplankton bloom development.



443 During spring 2018, we observed conditions favoring a classical phytoplankton bloom in  
444 seawater triggered by nutrient inputs from coastal water intrusions (Olita et al., 2014). Less  
445 favorable conditions were observed in spring 2019, where low nutrient concentrations limited  
446 the phytoplankton bloom development. Such irregular nutrient supply from year to year is a  
447 classical observation in the North Western Mediterranean sea (Sánchez-Pérez et al., 2020), that  
448 strongly structures microbial communities by promoting planktonic blooms and by stimulating  
449 the growth of certain microbes (Céa et al., 2015; Conan et al., 2007; Ghiglione et al., 2005;  
450 Lambert et al., 2019). In contrast, we observed that microplastic biofilms were not influenced  
451 by the irregularity of the nutrient supply in the seawater. Indeed, primary and secondary  
452 productions were similar on microplastics over the two years, whereas a clear decrease in these  
453 activities was observed in spring 2019 in seawater, when the environmental conditions were  
454 less favorable as compared to spring 2018. It is classically admitted that sessile microorganisms  
455 forming a biofilm on natural or engineered materials have greater access to nutrients and any  
456 resources that accumulate and concentrate on the biofilms surface (Salgar-Chaparro et al.,  
457 2020), which are classically diluted in the open ocean and less accessible for planktonic  
458 microorganisms (Dang and Lovell, 2016). Again, these results strengthen the vision of  
459 microplastic biofilm as a niche in itself, with the presence of an eco-corona together with strong  
460 phytoplankton-bacteria interactions that will control the exchange and interaction with  
461 materials, minerals and other components of the surrounding seawater. We propose that biofilm  
462 development on microplastics is a possible strategy for microbial survival in the marine  
463 environment, especially in oligotrophic seawaters such as the Mediterranean Sea, which is one  
464 of the most microplastic-polluted areas in the world ocean (Cózar et al., 2015; Dussud et al.,  
465 2018a).

466 Interactions between microplastic biofilms and surrounding seawater could be finally bi-  
467 directional, *i.e.* nutrient and organic matter inputs on microplastics support the phytoplankton

468 development together with heterotrophic activities (see above discussion), and *vice versa* with  
469 the release of materials nutrients from the microplastic biofilm to the seawater. In our study, we  
470 also incubated seawater with and without microplastics in spring 2018 and we evidenced a 2-3  
471 fold increase of PP when microplastics were present together with their biofilm. As mentioned  
472 earlier, a more thorough assessment of the spatial scale of the different processes reported is  
473 necessary. ER and the release of other molecules produced by the microplastic biofilm or even  
474 the breakdown of cells may explain the positive effect on PP in the surrounding waters (example  
475 with DOC; Romera-Castillo et al., 2018). Further experiments are obviously needed to  
476 investigate the molecular exchanges related to this positive effect. Comparing oxygen  
477 measurements with and without microplastics, a previous study in the North Pacific gyre  
478 measured a net autotrophic production of about 30-60  $\mu\text{gC}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$  for communities attached to  
479 microplastics harvested by manta net, while they found zero or even net heterotrophic bacterial  
480 production in the surrounding waters (Bryant et al., 2016). These authors proposed that  
481 microplastic particles create net autotrophic 'hot spots' in the oligotrophic ocean. However, such  
482 comparison is difficult because of the method used, as oxygen measurements in their  
483 experiment integrated the activities of the microplastic biofilms and also of planktonic  
484 organisms surrounding the microplastics during incubation. The use of radioactive carbon  
485 incorporation removes this ambiguity and confirms the clear influence of the microplastic  
486 biofilm on the increased productivity in the surrounding seawater, despite the contrasted  
487 environmental conditions encountered in spring 2018 and 2019. Moreover, it is actually the  
488 presence of the platisphere that is at the origin of the increase in production since it is necessary  
489 to wait for the installation of the biofilm to observe the stimulation of 2 to 3 times of the PP in  
490 the surrounding sea water. Our results are nevertheless limited by the size of the microplastic  
491 pieces incubated in 10 mL of seawater. Further studies are needed to evaluate the volume of  
492 seawater that could be impacted around the microplastic particles in more realistic conditions.

493           Interestingly, a previous study estimated that globally up to 23,600 metric tons of  
494 dissolved organic compounds (from truly dissolved substances to any polymeric nanoplastic  
495 that might have been also fragmented from the plastic surface) are released from the estimated  
496 35,000 metric tons of all the microplastics floating in the ocean (Romera-Castillo et al., 2018).  
497 These numbers consider the release of compounds from the microplastic itself, but do not take  
498 into account the biofilm covering its surface. Finally, a funny and illustrative image of the role  
499 of plastics in the environment could be that of an electrical inverter in a circuit that allows the  
500 attenuation of both positive and negative variations. A better estimation of the carbon fluxes  
501 generated by microplastic biofilm exudates may help to refine the balance between direct  
502 microplastics leaching and the contribution of their associated biofilm.

503

## 504 **5. CONCLUSION**

505           Our study provides an essential step in understanding the importance of the coupling  
506 between phytoplankton autotrophy and bacterial heterotrophic production on microplastics and  
507 its relationship with surrounding seawater. The plastic waste quantity entering the oceans is  
508 predicted to increase by up to one order of magnitude by 2025 (Gewert et al., 2015), with  
509 potential major consequences for marine microbes and the biogeochemical cycles in the ocean.  
510 Further studies considering the influence of microplastic features and water environmental  
511 characteristics are needed. This will enable the development of predicting models for the impact  
512 of biofilm activities on the carbon cycle in marine ecosystems.

513

514

515

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522

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