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

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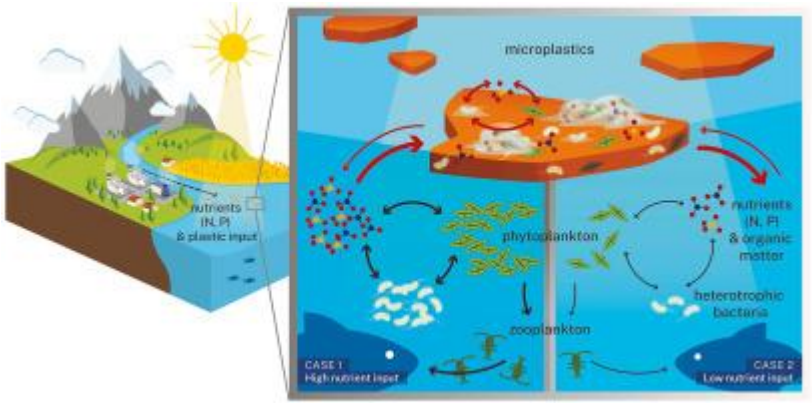
Abstract :

We measured phytoplankton primary production and heterotrophic bacterial activities on microplastics and seawater in the Northwestern Mediterranean Sea during two 3-month spring periods over 2 consecutive years. Microorganisms growing on a 5 mm diameter low density polyethylene films (LDPE; 200 µm thick) faced two contrasting conditions depending on the year. Spring 2018 was characterized by consistent nutrient inputs and bloom development. In spring 2019, nutrient inputs and bloom were low. For the first time, we observed a clear coupling between primary production and heterotrophic prokaryote production on microplastics during both years, but with different intensity between years that reflected the crucial role of the trophic environmental conditions (nutrient supply) in shaping microbial activities on plastics. We found that high primary production on plastics could support the whole (net autotrophy) or the majority of the bacterial carbon demand needed for heterotrophic activities, supplemented by other carbon sources if surrounding waters are highly productive. We propose that microbial activity on plastics influences the microbial community in the surrounding seawater, especially when the environmental conditions are less favorable. An illustrative image of the role of plastics in the environment could be that of an inverter in an electrical circuit that mitigates both positive and negative variations. Our results highlight the potential role of the plastisphere in shaping biogeochemical cycles in the context of increasing amounts of plastic particles in the marine environment.

Graphical abstract



Highlights

► Microplastics are the seat of strong coupled autotrophic and heterotrophic activities. ► Microplastics can be either a sink or a source of organic matter for the environment. ► Microplastic biofilms are relatively unaffected by environmental nutrient depletion. ► Marine primary production is enhanced by material released from the plastic biofilms.

Keywords : Microplastic, Plastisphere, Biogeochemical cycles, Primary production, Bacterial production

Introduction

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

Once in the marine environment, plastics are rapidly colonized by microorganisms, forming the so-called plastisphere (Zettler et al., 2013), and interact with the overall ecosystem depending on their size, from macro- (>2cm) and meso-plastic (between 2cm to 5mm) (Catão et al., 2019) to micro- (between 5mm to 1μm) and nano-plastics (<1mm) (Ter Halle and Ghiglione, 2021). Most of the studies so far revealed a large diversity and a clear niche partitioning among marine bacteria living on plastics compared to free-living (Crisafi et al., 2022; Debroas et al., 2017; Zettler et al., 2013) and organic particle-attached fractions (Dussud et al., 2018b; Oberbeckmann et al., 2018). Photosynthetic microorganisms such as cyanobacteria and diatoms are particularly over-represented on microplastics compared to seawater and other substrates, suggesting the potentially high impact on the global pelagic most of the research dealing with the surface oceans (Amaral-Zettler et al., 2020; Crisafi et al., 2022;

Dussud et al., 2018b; Nava and Leoni, 2021). Because plastic debris offers an abundant growth matrix for microalgae while limiting predation pressure by zooplankton (Kong and Koelmans, 2019), it has been proposed that plastic pollution can promote significant proliferation of microalgae, with consequent harmful effects for aquatic ecosystems already disturbed by eutrophication processes (Durrieu de Madron et al., 2011; Zhang et al., 2020). Moreover, evidence of higher abundance of genes or higher activities involved in nitrogen (nitrogen fixation, ammonia and nitrite oxidation, denitrification) and phosphorus (P accumulation, alkaline phosphatase activity) biogeochemical cycles were found in the plastisphere as compared to the seawater (Bryant et al. 2016, Odobel et al. 2021; Seeley et al. 2020). Because nitrogen and phosphorus exert overall control on the oceanic primary production (Tyrell, 1999), it is assumed that nitrogen and phosphorus release from the plastisphere can indirectly affect primary producers in the surrounding seawater (Mincer et al. 2016). For instance, plastics may represent net autotrophic hot spots in the oligotrophic ocean, with high density of chlorophyll *a* and high oxygen production (Bryant et al., 2016). Despite growing interest on the plastisphere influence on ecosystem functions, direct measurements on microbial activities remain scarce. Some studies found high heterotrophic prokaryotic production (Dussud et al., 2018b; Odobel et al., 2021) and ectoenzyme activities (Odobel et al., 2021) on plastics. It is surprising that primary production in the plastisphere was never recorded in marine ecosystems. Both primary production and heterotrophic activities play a key role in the carbon cycle. The former participate in about half of global primary production (Behrenfeld and Falkowski, 1997), and the latter channel half of the oceanic primary production through the microbial loop (Del Giorgio and Cole, 2000). A recent review underlined the need of coupling primary production and heterotrophic activities to evaluate the potential role of the plastisphere on carbon cycling in the oceans (Jacquin et al., 2019). In oceanic systems, the supply of organic matter from autochthonous (autotrophic) or allochthonous (heterotrophic) primary production is mainly

dependent on nitrogen and phosphorus availability and will define the trophic status of these ecosystems, and in turn, the potential coupling between phytoplankton and bacteria (Dodds and Cole, 2007). Finally, characterization of the trophic state is necessary to understand, food web linkages as well as biogeochemical features and subsequently water quality, including human influence.

To our knowledge, our study provides the first measurements of the marine plastisphere primary production together with bacterial heterotrophic activities over time. We hypothesize that natural environmental factors (such as temperature, salinity, nutrient availability…) play a major role in plastisphere activities, thus influencing the net autotrophy *vs.* heterotrophy budget over time. More precisely, trophic links within the niche created by the plastisphere would be strengthened when external conditions become less favorable (i.e. meso vs oligotrophic). Because a large majority (more than 36%) of microplastic items found at sea is made of polyethylene (PE) (Auta et al., 2017; Geyer et al., 2017), we used PE-based microplastics incubated in natural seawater for two 3-month periods, including a classical spring phytoplanktonic bloom event that occurs every year at the long-term SOLA marine observatory station (NW Mediterranean Sea, France). The experiments revealed a clear link between autotrophic and heterotrophic production of the plastisphere over time, as well as the possible influence of plastisphere microbial activity on the surrounding seawater.

2. Material and methods

2.1. Experimental conditions during 3 months in two consecutive years

We incubated 200 circular pieces of polyethylene of 5 mm diameter and 200 µm thickness (LD-PE, Borealis, ref. FA6224, Austria) in a 50 L aquarium with direct circulation to the sea.

The incubation was repeated twice with a one year interval: the first incubation was performed from 12 February to 7 May 2018 (44^{th} to 127^{th} day of the year in the calendar day, here after D44-127[1]; period of 85 days) and the second incubation was performed from 19 February to 8 May 2019 (D50-133; period of 91 days). Each plastic piece was first sterilized with 70% ethanol and rinsed 3-times with sterile seawater before incubation. The aquarium was placed outside with natural light conditions and covered by a transparent screen. Seawater circulating through the aquarium was pumped from 4 m depth and 30 m from the coast in the Banyuls Bay (NW Mediterranean Sea, France) and the seawater flow rate in the aquarium was ~5 L per hour.

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

2.2 Heterotrophic bacterial production

Heterotrophic bacterial production was measured in triplicate on each plastic and seawater sample at each sampling time by the ^{3}H-leucine incorporation into proteins method, as previously described in Dussud et al. (2018b). Briefly, the plastic pieces were transferred into 1.5 mL of sterilized seawater and a soft cell detachment pre-treatment was applied for each sample consisting in 3 cycles of 1 minute vortexing followed by 3 minutes ultrasonic bath. This pre-treatment greatly improves the signal, especially in the case of mature biofilms (Dussud et al., 2018b). Immediately after cell-detachment, ^{3}H-leucine (specific activity 4.2 x 10^{12}

[1] D1 correspond to the 1st January with this time scale

Bq.mmol^{-1}; Perkin Elmer) was added at a final concentration of 0.9975 x 10^{-9} mol.L^{-1} (completed with cold leucine to 1.49 x 10^{-7} mol.L^{-1}). The same ^{3}H-leucine concentration of 3.97 x 10^{-9} mol.L^{-1} (completed with cold leucine to 3.6 x 10^{-8} mol.L^{-1}) was used for seawater from the aquarium and from the SOLA marine station. No cell detachment pretreatment was used for seawater samples, since Dussud et al. (2018b) showed that it did not influence the signal for free-living bacteria in seawater. All samples were incubated in the dark at 18°C for 2-3 h. The theoretical conversion factor of 1.55 ngC.pmol^{-1} of incorporated leucine was used to calculate heterotrophic bacterial production (Simon and Azam, 1989). Data were normalized for microplastics with blank values according to Dussud et al. (2018b).

2.3 Primary production

Primary production (PP) was measured using a modified protocol of the radioactive ^{14}C tracer technique (Fitzwater et al., 1982). Measurements were carried out in triplicate. One plastic piece was transferred in 10 mL of sterilized seawater. The same volume was taken for seawater from the aquarium and from the SOLA marine station. Each sample was inoculated with $Na_2H^{14}CO_3$ (final activity of 18.5 kBq.mL^{-1}). The introduced quantity was measured by mixing 100 µL ethanolamine to 100 µL of inoculated sample and 10 mL of scintillation cocktail Ultima Gold uLLT. The samples were placed for 5 to 8 h in a thermic and light regulated incubator (14 to 18°C – 500 to 2000 µE.m^{-2}.s^{-1} according to the season). Plastics were isolated and acidified with 6 N HCl and dried during 8 h at 50 °C. Seawater samples were acidified with 6N HCl (final pH = 2) and agitated, lid open, for at least 12 h at 130 rpm and then mixed with scintillation cocktail during 4 hours before measuring radioactivity with the scintillation counter 300 SL Hidex.

The percentage of carbon originating from the extracellular release (ER) that is theoretically available for bacterial heterotrophic activity was calculated as previously described in Van

Wambeke et al. (2002). Briefly, the theoretical ER associated with autotrophic carbon fixation was calculated for a range of 5 and 20% of the PP to cover at least 90% of the wide range of all values found in the literature (Conan et al., 1999). It has been compared to the minimal and maximal theoretical bacterial carbon demand (BCD) ranging from 1 to 25% of the ER (review in Van Wambeke et al., 2002). Comparison between microbial activities on microplastic and in the seawater was calculated by integrating their respective dynamics over the 3 month sampling periods in 2018 and 2019.

2.4 Environmental parameters

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

2.5 Statistical analysis

All statistical and graphical analyses as well as application condition checks were performed using StatEL software v3 for excel (www.adscience.fr).

209 **3. Results**

210 **3.1 Representivity of aquarium with circulating seawater compared to *in situ* conditions**

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

218 In particular, we compared the primary production (PP) and heterotrophic bacterial
219 production (BP) in the aquarium with continuous seawater circulation to the measurements
220 carried out on water sampled at SOLA station (Fig. 1). Regression analysis showed that the data
221 are fit by a linear model with slope of 0.98 ± 0.17 ($p<0.00001$; n=24, the slope is not
222 significantly different from 1 and the origin is not significantly different from 0; $p<0.05$). The
223 same patterns were obtained with temperature, salinity, chlorophyll *a* and nutrients thus
224 indicating the absence of an "aquarium effect" during our 3 month-experiments.

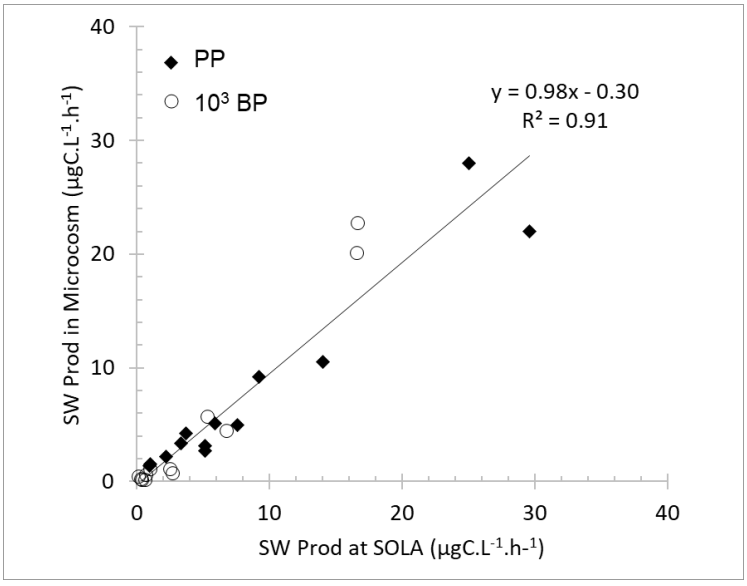


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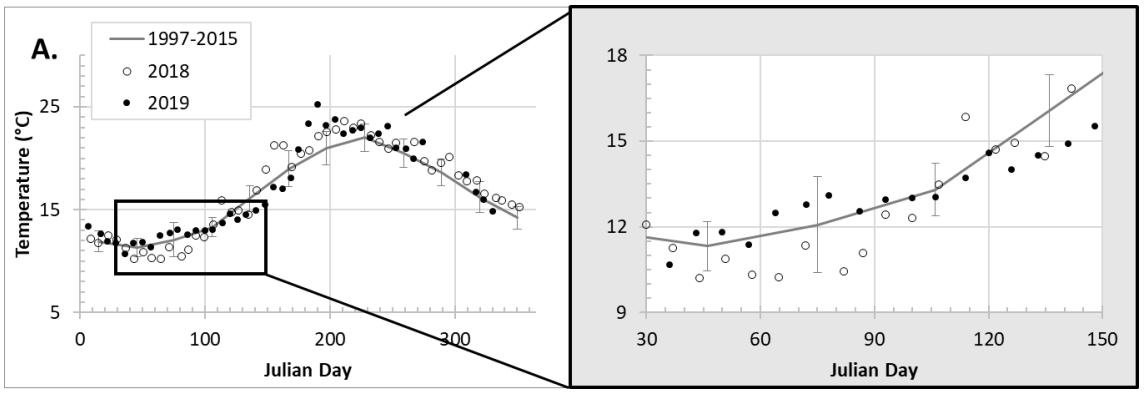
Figure 1: Comparison of phytoplankton primary production (PP = ◆) and heterotrophic bacterial production (BP = ○) in the aquarium with continuous seawater circulation and at the long-term observatory SOLA marine station (Banyuls Bay, NW Mediterranean sea). Note that the heterotrophic bacterial production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

3.2 Biogeochemical conditions during 2 year experiments in a local temporal context

Our two experiments from 2018 and 2019 were done using the same 50-L aquarium with circulating seawater pumped in the Banyuls Bay, in the vicinity of the SOLA observatory station. Weekly measurements are made at SOLA since 1997 as part of the National Observation Service (SNO-SOMLIT). We compared the biogeochemical conditions of our experiments, *i.e.* from 13 February 2018 (D44) to 14 May 2019 (D133), to the nearly 24 years of observation at the SOLA station (see www.somlit.fr/mysomlit/). Very contrasted environmental conditions were observed between 2018 and 2019, with higher variations of temperature, salinity, nutrients and chlorophyll a concentrations during the first year (Fig. 2). Indeed, when comparing the data of the 2 years with respect to the long-term average values represented by the continuous line (Fig. 2A and 2B), the 2018 measurements are variable and distributed rather below the average from the 1976-2005 climatology, while the 2019 measurements are more stable, close to the average in temperature (Fig. 2A) but largely above for salinity (Fig 2B). This leads to important differences in terms of nutrient concentration, particularly visible for nitrate (Fig. 2E), with 2018 being substantially richer than 2019 which was characterized by minimal nutrient concentrations.

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

In 2019, the temperatures remained close to the climatologic curve during the experimental period, although the summer period was warmer (Fig. 2A). Throughout the experiment, salinity was high, with values varying around 38 but the time series was marked by 2 weak decreases for D72 (37.7) and D120 (37.5) (Fig. 2B). As a result, all nutrient concentrations were rather low. Phosphate concentrations were relatively stable at about 0.03 µM and marked by 2 maxima (0.05 µM for D86 and 0.11 µM for D114; Fig. 2C). Ammonium concentrations were close to the detection limit, but again, we observed 2 peaks (0.2 µM for D93 and 0.23 µM for D114; Fig. 2D). A similar pattern was observed for nitrate, with very low concentrations throughout the period, and 2 minor peaks at D64 and D100 (Fig. 2E). The low abundance of nutrients in 2019 resulted in a rather weak spring bloom with chlorophyll *a* maximal concentrations of 1.5 $mgCHL.m^{-3}$ recorded between D115 and D125 (Fig. 2F).



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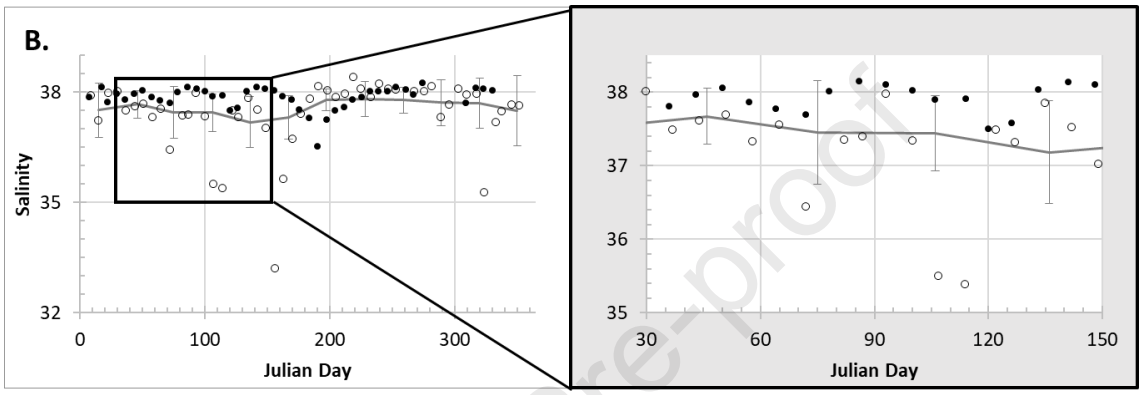

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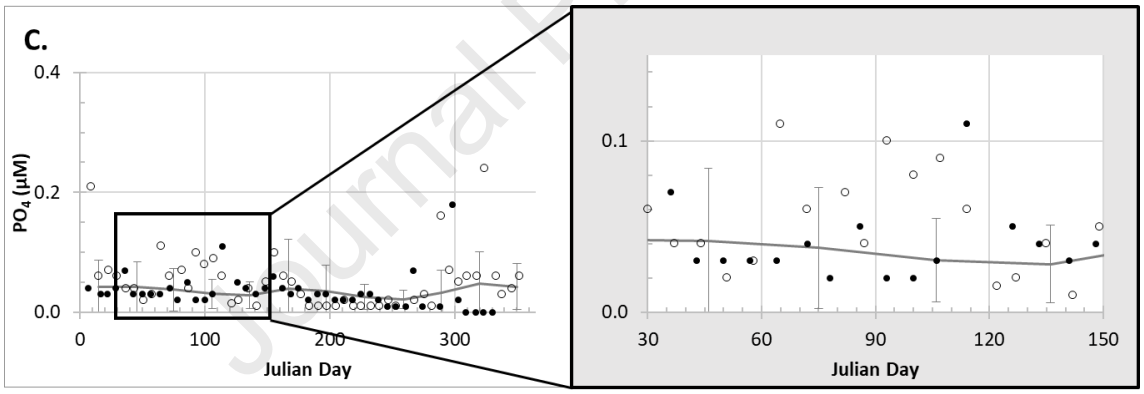

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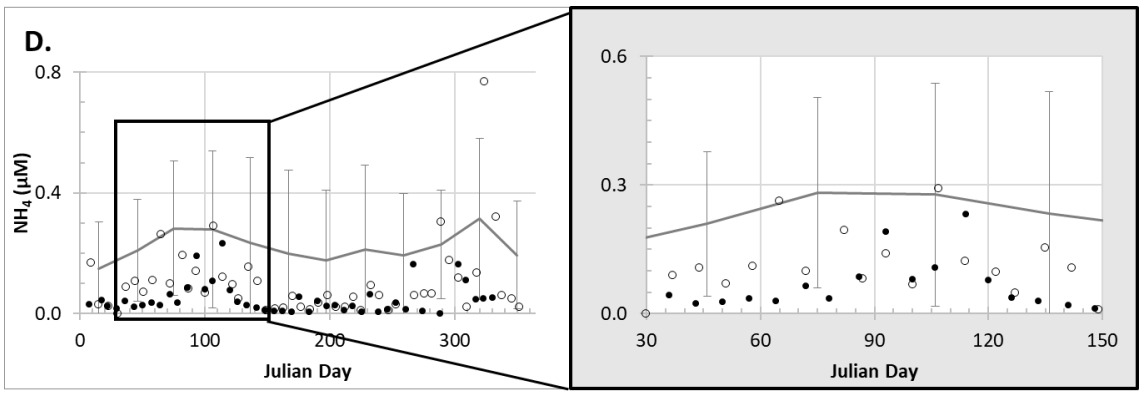

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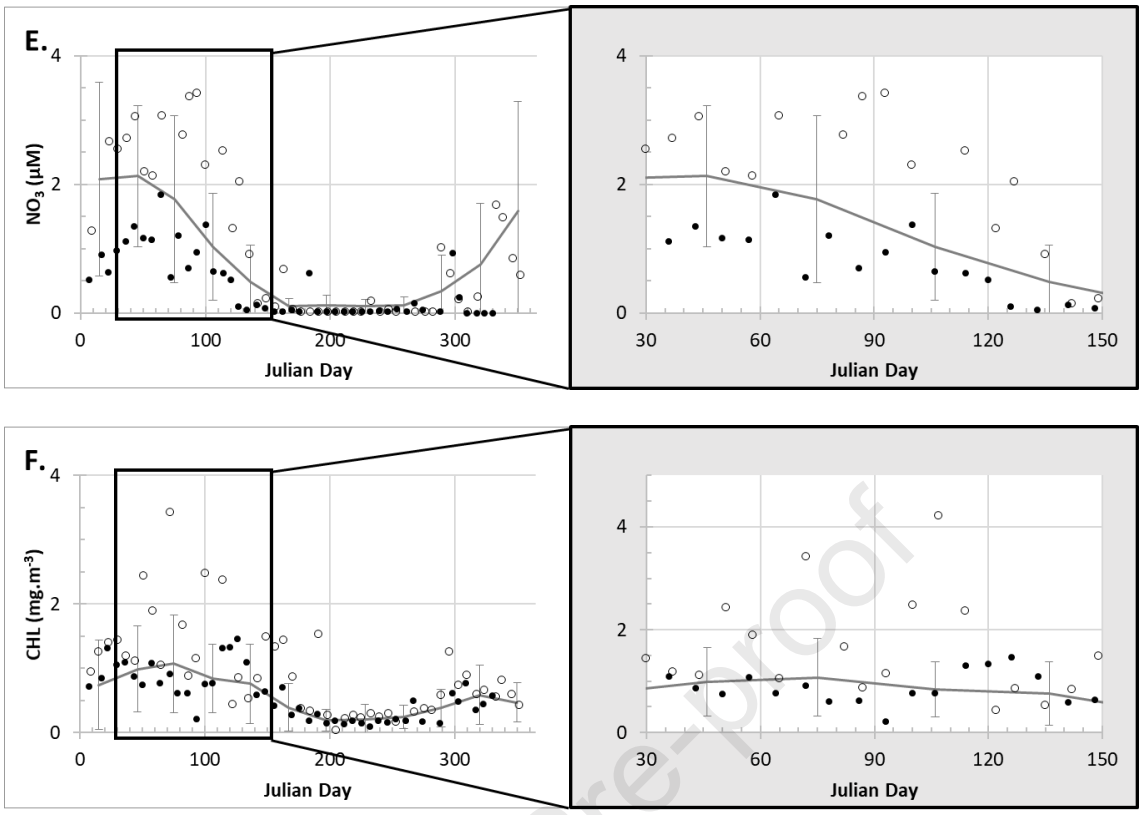


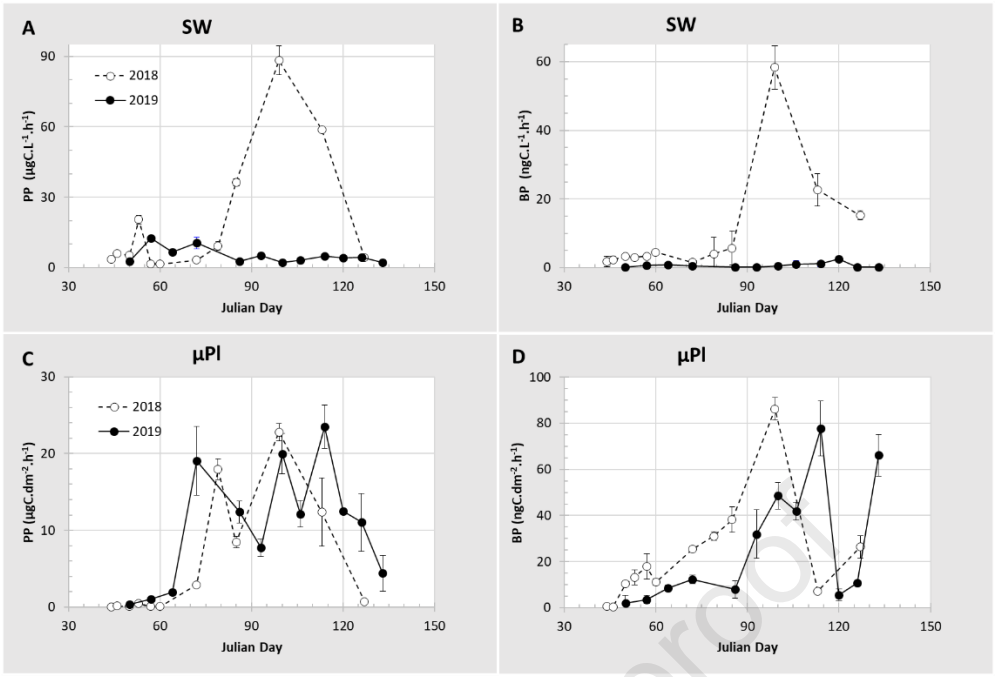
Figure 2: Seasonal evolution at SOLA observatory station for 2018 (D44-127, open dots) and 2019 (D50-133, full dots) compared to the 1997-2015 climatology (black solid line and confidence interval) of A. temperature in °C, B. salinity, C. phosphate in µM, D. ammonium in µM, E. nitrate in µM, and F. chlorophyll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

3.3. Primary production and heterotrophic bacterial production in seawater and on microplastics

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

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

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



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313 **Figure 3**: Temporal evolution during the incubation time in 2018 (D44-127, open dots and
314 dashed line) and 2019 (D50-133, full dots and black line) of **A.** Primary production, PP (in
315 µgC.L^{-1}.h^{-1}), **B.** Heterotrophic bacterial production, BP (ngC.L^{-1}.h^{-1}) in aquarium seawater
316 (SW) and of **C.** Primary production (in µgC.dm^{-2}.h^{-1}), **D.** Heterotrophic bacterial production
317 (ngC.dm^{-2}.h^{-1}) on microplastics (µPl). Whiskers represent standard deviations.

318

319

320 **3.4. Comparison of microbial activities on microplastics and in seawater**

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

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

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

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

		SEAWATER				µPLASTIC			
Year	Nb day	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
		Potential Carbon PP-source consumed for BP							
2018	88	MIN - MAX (%)		2	25	MIN - MAX (%)		14	167
2019	88	MIN - MAX (%)		0.5	6	MIN - MAX (%)		8	101

3.5. Influence of microplastic microbial activities on the surrounding seawater

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

To clarify the origin of this significant increase, we separated the data into 2 subsets (Fig. 4): the developing plastisphere (D44-D60) and the “mature” plastisphere (D65 - D100). There was no significant difference between the PP of seawater incubated with or without plastic during the first 26 days ($PP_{seawater + Plastic} = 0.97 \times PP_{seawater} - 0.08$; $R^2 = 0.99$; $p<0.00001$). The slope of the regression model was not different from 1 (Anova after a Mann-Whitney test, $p<0.05$) and the intercept was not different from 0 ($p<0.05$). The situation was quite different

for the second period (D65 - D100), when the platisphere was well developed (Fig. 4). In this case, the PP in seawater incubated with plastic increased by 2.5 times compared to PP in seawater that was not in contact with plastic ($PP_{seawater + Plastic}$ = 2.49 x $PP_{seawater}$ + 12.36 ; R^2 = 0.95 ; p<0.00085). The slope of this regression model is significantly different from 1 (Ancova after a Mann-Whitney test, p<0.00067) and the intercept is significantly different from 0 (p<0.00085).

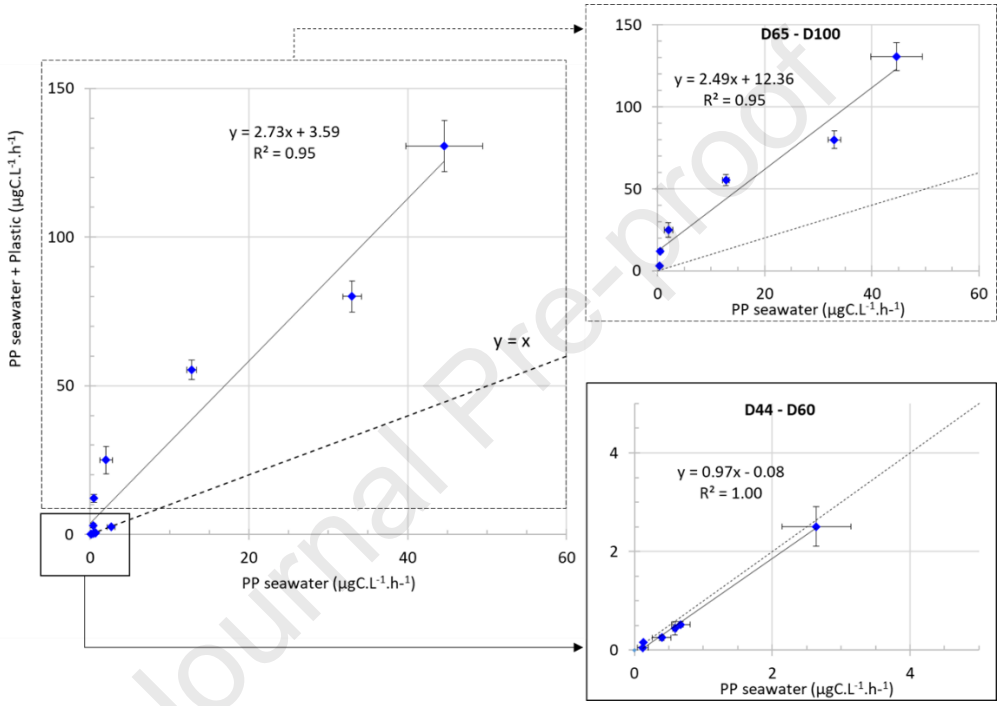

Figure 4: Comparison of primary production (PP) in seawater without (SW) or with microplastic (SW+MP) during the 2018 experiment. Linear regression is represented by a full line and significant equations are indicated (p<0.001; n = 12). Dashed line represents y = x. Pooled data are presented on the left, whereas on the right, data are separated into 2 periods as symbolized by the squares (the developing plastisphere D44 – D60 on the bottom, and the "mature" plastisphere D65 - D100 on the top)

4. DISCUSSION

The impact of plastic pollution on biogeochemical cycles can no longer be considered limited today, since the amount of microplastics found at sea could soon cover more than 4.2 million km^2 of the sea surface (Eriksen et al., 2014; Hidalgo-Ruz et al., 2012). To date, most of the research dealing with the plastisphere has focused on its biodiversity that give very few indications on the microbial functions and do not answer the crucial question of "how the plastisphere may influence the biogeochemical cycles" (Jacquin et al., 2019). Here, we present the first data of primary production (PP) coupled with heterotrophic bacterial production (BP) on microplastics. After a month of colonization of polyethylene microplastics in seawater, we observed that the mature biofilm presented high PP levels, up to 20 ± 3 $\mu gC.dm^{-2}.h^{-1}$ compared to the levels at the beginning of the experiment. These results are in accordance with the classical observation of three successive phases of biofilm development in marine seawater, i.e. the primo-colonization (during few hours to a couple of days after immersion in seawater), the growing phase (during few days) and the maturation phase (generally reached after few weeks) (De Tender et al., 2017; Lorite et al., 2011). Each phase was shown to consistently differ from another in term of abundance, diversity and activity of the biofilm (Cheng et al., 2021), which could explain the changes of photosynthetic activity during the biofilm formation in our conditions.

Consistent to our results, phototrophs such as diatoms and cyanobacteria have been commonly described in the plastisphere, at least on plastics that are exposed to sunlight (Amaral-Zettler et al., 2020; Decelle et al., 2015; Cheng et al., 2021), confirming that microplastics floating at the ocean surface provide good substrates for the development of autotrophic microorganisms. For example, Crisafi et al. (2022) recently studied the growth of microbial biofilms on medical masks in a coastal area affected by different anthropogenic inputs. The authors observed that after one month in the field, the masks were colonized by a

bacterial community significantly different from that detected in natural matrices in the same areas (seawater and sediments), characterized by the growth and enrichment of phototrophic microorganisms such as Oxyphotobacteria, Ruminococcaceae, Gracilibacteria, and Holophageae in particular.

Plastics in the environment are composed of a large variety of organic and inorganic constituents according to their surface properties mediated by physical forces common to all molecular systems (*i.e.* van der Waals, electrostatic and others), the so-called eco-corona (Monopoli et al., 2012; Ter Halle and Ghiglione, 2021). Together with light and CO_2 availability, nutrients such as NO_3^{2-}, NH_4^+ and PO_4^{3-} are abundant in the biomolecular corona of microplastics (Galloway et al., 2017), thus potentially fueling the intense PP observed in our experimental conditions. Our results showed a direct link between PP and BP within the microplastic biofilms. BP reached 86 ± 5 $ngC.dm^{-2}.h^{-1}$ of the same order of magnitude as reported for the few previous studies recording this parameter on microplastics in marine waters (Dussud et al., 2018b; Odobel et al., 2021).

Heterotrophic bacterial activities play a key role in different biogeochemical cycles such as carbon, since it is known that about half of the oceanic primary production is re-mineralized by bacteria into the microbial loop (Azam et al., 1983; Cole et al., 1988), thus transforming the particulate organic matter to dissolved organic matter (Grossart et al., 2007). Indeed in marine waters, the proportion of carbon that is taken up by bacteria determines its final fate in the water column (respired back as CO_2 vs. stored in the ocean as particulate/ dissolved organic matter, Cole et. al., 1988). Addressing phytoplankton-bacteria coupling in the plastic environment will then affect the response of marine ecosystems to anthropogenic pressures. Bacteria can be decoupled from phytoplankton when they are submitted to environmental stressors like nutrient limitation (case of oligotrophic systems, Conan et al., 1999; Turley et al., 2000) or temperature (case of high latitude ecosystems, Kirchman et al,. 2009) or when additional organic matter

428 sources exist (Morán et al., 2002). Allochthonous organic matter sources could lead to
429 ecosystem heterotrophy, when bacterial carbon demand exceeds carbon fixation. The
430 dominance of BP over PP is one of the hypotheses put forward to explain the low fisheries
431 production, the low vertical flux of material transfer and the low biomass of benthic organisms
432 in the eastern Mediterranean regions (Turley et al. 2000).

433 Here, we propose for the first time that heterotrophic microbes have the capacity to
434 process considerable amounts of organic matter produced on microplastics by phytoplankton
435 activities. We tested two possible scenarios with our experimental design: *In case 1*, BP
436 dominates the plastisphere, exceeding PP release via direct incorporation of carbon from the
437 plastic or surrounding water into attached bacterial cells. The plastisphere would then be net
438 heterotrophic. This material consumed by the biofilm would locally induce a potential loss for
439 the pelagic ecosystem, thus limiting transfers to higher trophic levels. *In case 2*, plastics mostly
440 exert a surface for the attachment of photosynthetic microbes. From the carbon that is fixed by
441 photosynthesis in a plastic surface, only a fraction is taken up by attached bacteria; therefore
442 the plastisphere would be net autotrophic. The excess material produced on the microplastics
443 could be then potentially release towards the pelagic ecosystem, thus locally limiting the effects
444 of stress depletion in seawater.

445 Indeed, photosynthates are known to be particularly labile and rapidly consumed by
446 bacteria (Conan et al., 1999; Van Wambeke et al., 2002). The authors showed that the
447 lability/quality of this material increased with its freshness (age) and with trophic conditions
448 (i.e. material produced in oligotrophic environments is generally less labile than that produced
449 in mesotrophic and eutrophic environments). The spatial proximity of the protagonists and their
450 greater number within the plastisphere would be a factor in strengthening the trophic link, both
451 in the autotrophic to heterotrophic direction and in the other direction. Interestingly, our results
452 also indicate that PP can theoretically support most of the heterotrophic activities within the

453 microplastic biofilm. If we assume that taking the extremes, the orders of magnitude cover most
454 values encountered in natural environments and in biofilms, and considering the ranges of ER
455 between 5 and 20% and BGE on labile material between 10 and 30% (Baines and Pace, 1991;
456 Conan et al., 1999; Del Giorgio and Cole, 1998; Hubas et al., 2007; Lemee et al., 2002; López-
457 Sandoval et al., 2010; Moran et al., 2002; Van Wambeke et al., 2002), we observed that the
458 carbon requirement of free-living heterotrophic bacteria could be largely covered by PP for
459 both years.

460 Similarly, ER might have theoretically covered most of the BCD in 2018 (min. 14% and
461 max. 167%, with <100% corresponding to the BCD totally covered by ER) and in 2019 (from
462 8-101%). Thus, while in 2019 the BCD appeared to be fulfilled by autotrophic production on
463 microplastics (*i.e.* when PP in surrounding seawater was low), it is possible that in 2018 an
464 additional organic carbon source was required to supplement the needs of attached
465 heterotrophic bacteria. This observation is consistent with the fact that in 2018, PP in the
466 surrounding water was particularly strong and could logically have been a supplemental source.
467 However, our results suggest that the use of such a complementary carbon source by the
468 plastisphere and the depletion of carbon and nitrogen in the surrounding seawater would rather
469 be an exception than a rule. Additional sources of labile carbon from seawater could be use by
470 heterotrophic bacteria in the plastisphere, but rather as an opportunity because this
471 allochthonous source is not essential to the functioning of the biofilm. This confirms that
472 microplastic itself constitutes an ecological niche (Dussud et al., 2018b), where strong
473 phytoplankton-bacteria interactions exist that control nutrient cycling and biomass production
474 at the scale of microplastic pieces. Roughly, plastics appear to be comparatively less autotrophic
475 than surrounding seawater during our experiments.

476 Another originality of our study was to take into account two contrasting environmental
477 conditions, during each of the 2 years. It is indeed very likely that biofilm composition and

478 microbial interactions within plastic surfaces are in turn influenced by environmental
479 perturbations (Crisafi et al., 2022). For example, the study of Allgaier et al. (2008) in natural
480 mesocosms was designed to test whether the predicted change in pCO_2 would affect the
481 communities of heterotrophic bacteria during a phytoplankton bloom development. The authors
482 concluded that bacterial abundance and activities were similar among the various treatments
483 but the community structure of free-living bacteria changed with pCO_2 unlike that of attached
484 bacteria. The latter were tightly coupled to phytoplankton bloom development. During spring
485 2018, we observed conditions favoring a classical phytoplankton bloom in seawater triggered
486 by nutrient input from coastal water intrusions (Olita et al., 2014). Less favorable conditions
487 were observed in spring 2019, where low nutrient concentrations limited the phytoplankton
488 bloom development. Such irregular nutrient supply from year to year is a classical observation
489 in the North Western Mediterranean Sea (Sánchez-Pérez et al., 2020), that strongly structures
490 microbial communities by promoting planktonic blooms and by stimulating the growth of
491 certain microbes (Céa et al., 2015; Conan et al., 2007; Ghiglione et al., 2005; Lambert et al.,
492 2019). In contrast, we observed that the variability of the nutrient supply in the seawater does
493 not drastically influence the activities of microplastic biofilms. Indeed, primary and secondary
494 productions were similar on microplastics over the two years, whereas a clear decrease in these
495 activities was observed in spring 2019 in seawater, when the environmental conditions were
496 less favorable as compared to spring 2018. It is classically admitted that sessile microorganisms
497 forming a biofilm on natural or engineered materials have greater access to nutrients and any
498 resources that accumulate and concentrate on the biofilm surface (Salgar-Chaparro et al., 2020),
499 which are classically diluted in the open ocean and less accessible for planktonic
500 microorganisms (Dang and Lovell, 2016). Again, these results strengthen the vision of
501 microplastic biofilm as a niche in itself, with the presence of an eco-corona together with strong
502 phytoplankton-bacteria interactions that will control the exchange and interaction with

materials, minerals and other components of the surrounding seawater. Several studies already underlined significant difference between microplastics biofilms and glass, rocks or wood biofilms (Jacquin et al. 2019, Cheng et al. 2021, Zhang et al. 2022). We propose that biofilm development on microplastics is one of the possible strategy for microbial survival in the marine environment, especially in oligotrophic seawaters such as the Mediterranean Sea, which is one of the most microplastic-polluted areas in the world ocean (Cózar et al., 2015; Dussud et al., 2018a).

Finally, interactions between microplastic biofilms and surrounding seawater could be bi-directional. Nutrient and organic matter input on microplastics supports phytoplankton development together with heterotrophic activities (see above discussion), and *vice versa* with the release of materials from the microplastic biofilm to the seawater. In our study, we also incubated seawater with and without microplastics in spring 2018 and we observed a 2-3 fold increase of PP when microplastics were present together with their biofilm. This effect is significant in our samples as soon as the mature biofilm is established (after about 20 days in our experiment) and at least until the end of our time-series (for more than 1 month). As mentioned earlier, a more thorough assessment of the spatial scale and origin of the different processes reported is necessary. ER and the release of other molecules produced by the microplastic biofilm or even the death and breakdown of cells may explain the positive effect on PP in the surrounding waters (example with DOC; Romera-Castillo et al., 2018). Comparing oxygen measurements with and without microplastics, a previous study in the North Pacific gyre measured a net autotrophic production of about 30-60 $\mu gC.L^{-1}.h^{-1}$ for communities attached to microplastics harvested by manta net, while they found zero or even net BP in the surrounding waters (Bryant et al., 2016). These authors proposed that microplastic particles create net autotrophic 'hot spots' in the oligotrophic ocean. However, such a comparison is difficult because of the method used: oxygen measurements in this experiment integrated the

activities of the microplastic biofilms and also of planktonic organisms surrounding the microplastics during incubation. The use of radioactive carbon incorporation removes this ambiguity and confirms the clear influence of the microplastic biofilm on the increased productivity in the surrounding seawater, despite the contrasting environmental conditions encountered in spring 2018 and 2019. Moreover, it is actually the presence of the plastisphere that is at the origin of the increase in production since it is necessary to wait for the installation of the biofilm to observe the 2 to 3-fold stimulation of the PP in the surrounding sea water. Our results are nevertheless limited in time and by the size of the microplastic pieces incubated in 10 mL of seawater. Further studies are needed to evaluate the period and the volume of seawater impacted around the microplastic particles under more realistic conditions.

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

5. CONCLUSION

Our study provides an essential step in understanding the importance of the coupling between phytoplankton autotrophy and bacterial heterotrophic production on microplastics and its relationship with surrounding seawater. The plastic waste quantity entering the oceans is

553 predicted to increase by up to one order of magnitude by 2025 (Gewert et al., 2015), with
554 potential major consequences for marine microbes and the biogeochemical cycles in the ocean.
555 On the basis of the 2 scenarios that we considered, we demonstrate that scenario 2 associated
556 with a net autotrophy of the plastisphere was certainly the most widespread because
557 characteristic of oligotrophic/mesotrophic waters. Scenario 1 where the plastisphere is net
558 heterotroph is dependent upon an important PP as during phytoplankton bloom. Further studies
559 considering the influence of microplastic features and water environmental characteristics are
560 needed. This will enable the development of predictive models for the impact of biofilm
561 activities on the carbon cycle in marine ecosystems.

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Highlights

- Microplastics are the seat of strong coupled autotrophic and heterotrophic activities
- Microplastics can be either a sink or a source of organic matter for the environment
- Microplastic biofilms are relatively unaffected by environmental nutrient depletion
- Marine primary production is enhanced by material released from the plastic biofilms

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☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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