

## ***Interactive comment on “Experimental evidence of the potential bioavailability for marine heterotrophic bacteria of aerosols organic matter”***

***by Kahina Djaoudi et al.***

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**Response to the 2nd referee** We would like to thank the referee for his/her relevant comments and suggestions on our submitted manuscript. Here below, we address our response (in bold and in italic) and we highlighted the text modifications in the revised manuscript (in italic between inverted commas). Our response (pdf) is downloaded below as a supplement. Referee 2 1) Inorganic nutrients were added to the microbial cultures to avoid inorganic nutrient limitation. Final concentrations were 1  $\mu$ M-N and 0.3  $\mu$ M-P, which are well above the expected concentration in the surface layer of an oligotrophic region. Given that inorganic nutrients have been added in the same con-

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centrations to all treatments, comparison among treatments is valid but: what about extrapolation of your results to oligotrophic conditions in the field? 2) The WSF of Sahara dust and anthropogenic aerosols do not contain only DON and DOP but also inorganic N (ammonium, nitrite and nitrate) and phosphate. Therefore, even if you have not added inorganic nutrients to the microbial cultures (see point 1), inorganic N and P would be added in the WSF. In this regard, it is relevant that the concentrations of inorganic N and P in the WSF are presented and discussed; We agree with the reviewer on the relevance of inorganic nutrient data. In the revised manuscript, initial inorganic nutrient concentrations were added in the method section and discussed (line: 325-330). Modified text in the method (Lines: 137-142) ‘To avoid nutrient limitation, artificial seawater was enriched with nitrogen ( $\text{NH}_4\text{Cl} + \text{NaNO}_3$ ) and phosphate ( $\text{KH}_2\text{PO}_4$ ), to final concentrations of 1  $\mu\text{M}$  and 0.3  $\mu\text{M}$  in the incubation bottles, respectively. Therefore, initial nutrient concentrations in the control and glucose treatments were  $1.02 \pm 0.02 \mu\text{M}$  and  $1.02 \pm 0.05 \mu\text{M}$  for nitrogen, respectively and,  $0.29 \pm 0.01 \mu\text{M}$  and  $0.27 \pm 0.02$  for phosphorus. However, in anthropogenic and dust treatments, aerosols amendments increased nutrient concentrations in the incubation bottles, reaching  $6.02 \pm 0.34$  in Saharan dust treatment and  $7.03 \pm 0.09$  in anthropogenic treatment for nitrogen concentration and, similarly reaching  $0.40 \pm 0.02$  in Saharan dust treatment and  $0.34 \pm 0.01$  in anthropogenic treatment for phosphorus concentration.’ Modified text in the discussion (Lines: 325-330) ‘Alongside to those nutrient enrichments, aerosol amendments resulted in initial N concentrations up to 7  $\mu\text{M}$  (6  $\mu\text{M}$  and 7  $\mu\text{M}$  in D and A, respectively) and initial P concentrations up to 0.4  $\mu\text{M}$  (0.40  $\mu\text{M}$  and 0.34 in D and A, respectively). Different BGE values were observed between D- and A-treatments despite similar initial nutrient concentration. Moreover, BGE in D was similar to that of the G treatment despite a higher nutrient concentration, suggesting that inorganic nutrients were weakly involved in the control of BGE.’

We agree with the reviewer that the concentrations of nitrogen and phosphorus in the Mediterranean Sea are low in the upper water, especially during the stratification period during which nutrient concentrations fall into nanomolar levels. However, the added

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concentrations were chosen to avoid nutrient limitation over the incubation period, particularly in the glucose treatment. Furthermore, in this study, the seawater used for the inoculum of heterotrophic bacteria was sampled during October. During that period, nitrogen and phosphorus concentrations increase within the surface waters as the mixed layer deepens. During the mixing period, nitrogen and phosphorus concentrations could reach up to 2 and 0.25  $\mu\text{M}$  (Pasqueron de Fommervault et al., 2015), respectively in the upper water of the Mediterranean Sea. Those reported values are in the same order of magnitude as N and P concentrations added in the framework of this study. Of course, we agree that any extrapolation of experimental results to the field can be debated as the encountered variables in natural environments cannot all be reproduced in the context of experimental work. If low inorganic N and P concentrations are encountered in the upper waters, their bioavailability may constrain/ limit the utilization of atmospheric DOC. However, as expected by the reviewer, aerosol amendments delivered inorganic nitrogen and phosphorus, resulting in initial concentrations of  $6.02 \pm 0.34$  in Saharan dust treatment and  $7.03 \pm 0.09$  in anthropogenic treatment for nitrogen concentration and, similarly reaching  $0.40 \pm 0.02$  in Saharan dust treatment and  $0.34 \pm 0.01$  in anthropogenic treatment for phosphorus concentration. In the upper water of oligotrophic regions, N and P supply from atmospheric deposition could alleviate nutrient limitation, thus allowing the utilization of atmospheric DOC. In addition, although inorganic N and P could limit bacterial activity in such oligotrophic regions, DOP and DON concentrations are much higher in the upper waters than inorganic N and P and sustain microbial activity as shown in several nutrient depleted areas (i.e. Van Wambeke et al., 2002; 2020; Mather et al., 2008).

3) It is reported that the DOC concentration in artificial seawater was 6  $\mu\text{M}$  and in the WSF  $<0.3$   $\mu\text{M}$  (once diluted in the ASW). However, the average DOC concentration in the control treatments was 19  $\mu\text{M}$  (calculated from Table 2). What caused this difference? Should we assume that it also occurs in treatments G, A and D?; The concentration of DOC in the control treatment at T0 (T0=6 hours) was indeed higher than the one expected (6.3  $\mu\text{M}$ ). We don't have a straightforward explanation for this

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observed difference. However, a potential release of carbon from heterotrophic bacteria could have occurred, following their senescence as organic matter was not provided in that treatment. This suggestion could be supported by the extremely low bacterial abundance and production observed for that treatment along the incubation experiment. It is unlikely that this also occurred in G, A and D-treatments since initial DOC concentration ( $40 \pm 3$   $\mu\text{M}$ ,  $36 \pm 2$   $\mu\text{M}$  and  $34 \pm 3$   $\mu\text{M}$ ) agrees with the amount of DOC added through the amendments (see Methods section, lines 130-135).

4) The estimates of LDOC(%) are obtained for the 4 treatments (C, G, A and D) comparing the initial DOC and the DOC decrease of each treatment. However, a considerable part of the initial DOC is already present in the control treatment (see point 3). Therefore, how should we interpret the LDOC(%) numbers in Table 2? For example, if the DOC decrease in the control treatment is 5  $\mu\text{M}$  and in the G treatment is 22  $\mu\text{M}$ , the DOC decrease exclusively due to the glucose addition should be 17  $\mu\text{M}$ . Given that the DOC of the glucose addition is 40  $\mu\text{M}$  and in the control is 19  $\mu\text{M}$ , the LDOC(%) of the glucose addition would be 81% ( $= (22 - 5) / (40 - 19)$ ). This is very different from the 55% in Table 2. For treatments A and D the difference is not so large but it is conceptually important; 5) The same reasoning is applicable to the BGE(%) calculations (LDOC is in the denominator of the formulae). For example, for the G treatment BGE(%) should be 9% ( $= (1.7 - 0.7) / (22 - 5)$ ) and for the D treatment 26% ( $= (1.19 - 0.17) / (9 - 5)$ ). It really makes a difference; We understand the Reviewer's reasoning. However, as mentioned previously, if the 'extra' DOC concentration observed in the control treatment had occurred in the glucose treatment (as well as in anthropogenic and Saharan dust treatment) we would have observed an additional 19  $\mu\text{M}$  at T0 for each treatment compared to the actual DOC added, which was not the case. Therefore, we do not think that taking into account the decrease of DOC observed in the control for the calculation of the LDOC and BGE in glucose, Saharan dust and anthropogenic treatments is the appropriate approach. Indeed, if 5  $\mu\text{M}$  decrease of DOC in the control treatment resulted in an initial concentration of 19  $\mu\text{M}$  in, it is unlikely that the background of labile DOC, if there is, was the same in A, D and G treatments as

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any 'extra' DOC was observed in those treatments.

6) Also concerning BGE (%), it should be better to use the bacterial biomass, calculated from BA with a conversion factor, rather than BP; and We recognize that the use of BP in the calculation of BGE could be biased as BP is determined using a leucine to carbon conversion factor. Nevertheless, the use of BA for BGE calculation could be biased as well as: i) it requires the utilization of abundance/biomass conversion factor and biovolume varies substantially during growth. ii) The variation of bacterial abundance is a net variation, reflecting a balance between growth and mortality. This is visible through observations of apparent growth rates calculated from the exponential growth phases of BA and BP, which are lower for abundances than for BP. It is also visible when we look to the lag phase, which was shorter for BP than for BA (Table 3), as BA is the sum of active and inactive cells. This is a classical problem occurring during dilution experiments, used to determine BGEs but also leucine-conversion factors (Kirchman et al., 1982; Amermann et al., 1984). For these reasons we prefer to use BP data for BGE calculations.

Ammermann, J. W., Furhman, J. A., Hagström, A., and Azam, F.: Bacterioplankton growth in sea water : I. Growth kinetics and cellular characteristics in sea water cultures, Mar. Ecol. Prog. Ser., 18, 31-39, 1984. Kirchman, D. L., Ducklow, H. W., and Mitchell, R.: Estimates of bacterial growth from changes in uptake rates and biomass., Appl. Environ. Microbiol., 44, 1296-1307, 1982.

7) Extrapolation of your results to the entire Mediterranean Sea is a bit risky. The fluxes of organic nutrients to the surface layer of the Mediterranean Sea are (probably) an overestimate. Your daily and average annual atmospheric fluxes are obtained from just to two points, off Marseille and in Lampedusa. Do you think that they are representative for the entire Mediterranean Sea? I do not believe that. Section 4.3 is very useful but you must prevent to give the impression that your calculations can be extrapolated to the entire Mediterranean. We fully agree with the reviewer that section 4.3 is very useful but risky. It was not our intention to extrapolate our results to the whole

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Mediterranean basin. The main assumption under the proposed possible influence of our results in marine C cycle in the Mediterranean Sea was that the labile/refractory partitioning obtained in our study is valid for reported atmospheric fluxes of DOC. That is why we keep the estimated fluxes in this section per square meter, in order not to extrapolate to the entire basin. In the revised version of the manuscript, we have rewritten the first part of the section in order to qualify the ideas. Modified text (lines: 403-408): 'Atmospheric fluxes of DOC in the Mediterranean Sea have been reported to be 6 times higher than river fluxes (Djaoudi et al., 2018; Galleti et al., 2020 this issue), highlighting atmospheric deposition as a major allochthonous source of carbon to this marine region. Hereafter, we apply the partitioning between labile (LDOC) and refractory DOC (RDOC) in aerosols obtained in this study to these previously reported atmospheric DOC fluxes in the Mediterranean Sea to propose a tentative estimation of LDOC and RDOC fluxes from both Saharan dust and anthropogenic deposition and to which extent they could potentially contribute to marine carbon cycle'.

MINOR DETAILS Lines 224 and 226. You are referring to Figure 2, not Figure 1. Please, correct. Thank you for this comment. This mistake was corrected in the revised manuscript.

Modified text (Lines: 234-236) 'The initial DON and DOP concentrations in the C-treatment were  $1.6 \pm 0.1 \mu\text{M}$  and below the detection limit, respectively. The G-treatment exhibited the same initial DON concentration as the C-treatment, with values of  $1.8 \pm 0.2 \mu\text{M}$  while DOP concentrations reached  $0.03 \pm 0.01$  (Fig. 2B, C). In A and D-treatments, the concentration of both DON and DOP increased immediately after aerosol addition (T0). The DON concentration reached  $4.3 \pm 0.7 \mu\text{M}$  and  $3.7 \pm 0.2 \mu\text{M}$  in the A and D-treatments, respectively (Fig. 2A), resulting in lower C:N and higher N:P elemental ratios in A and D treatments than in G-treatment (Table S2).'.

Line 422. These numbers should be divided by 1000 or expressed in mol C. Yes, we agree, and we apologize for this mistake. We corrected this paragraph as follow:

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Modified text (Lines: 336-441): 'By applying the percentages of LDOC observed in this to previously daily reported atmospheric fluxes (0.03-1.78 mmol C m<sup>-2</sup> day<sup>-1</sup>) (Djaoui et al., 2018; Galleti et al., 2020 this special issue), daily atmospheric fluxes of LDOC would range between 0.007-0.534 mmol C m<sup>-2</sup> day<sup>-1</sup> for Saharan dust deposition and between 0.006-0.480 mmol C m<sup>-2</sup> day<sup>-1</sup> for anthropogenic deposition. These fluxes would thus represent up to 13% of photosynthetic DOC production, supporting the hypothesis of a potential role of atmospheric deposition of DOC in sustaining secondary production.'

Line 680, Table 2. Please, add a column with the initial concentrations of DOC even if it is redundant. We added this column in the revised manuscript. Please see table. 2 attached below as a figure (please see figure 1 in the caption below).

Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2020-187/bg-2020-187-AC2-supplement.pdf>

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-187>, 2020.

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Table 2. Initial dissolved organic carbon concentration and its decrease and contribution to the initial DOC stocks (expressed as a percentage of labile dissolved organic carbon, LDOC).

	Initial DOC; $\mu\text{mol C L}^{-1}$	DOC decrease; $\mu\text{mol C L}^{-1}$	LDOC [%]
C1	22	5	23
C2	22	6	27
C3	18	4	22
<b>Mean (<math>\pm</math> SD)</b>	<b>20 <math>\pm</math> 2</b>	<b>5 <math>\pm</math> 1</b>	<b>24 <math>\pm</math> 3</b>
G1	36	19	53
G2	42	24	57
G3	41	22.5	54
<b>Mean (<math>\pm</math> SD)</b>	<b>40 <math>\pm</math> 3</b>	<b>22 <math>\pm</math> 3</b>	<b>55 <math>\pm</math> 2</b>
A1	34	6	17
A2	37	11	29
<b>Mean (<math>\pm</math> SD)</b>	<b>36</b>	<b>9 <math>\pm</math> 4</b>	<b>24 <math>\pm</math> 8</b>
D1	31	7	22
D2	36	8.5	24
D3	36	11	30
<b>Mean (<math>\pm</math> SD)</b>	<b>34 <math>\pm</math> 3</b>	<b>9 <math>\pm</math> 2</b>	<b>25 <math>\pm</math> 4</b>

**Fig. 1.**

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