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Biogeosciences Discussions

Interactive comment on "Interaction between elevated CO_2 and phytoplankton-derived organic matter under solar radiation on bacterial metabolism from coastal waters" *by* Antonio Fuentes-Lema et al.

Anonymous Referee #2

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General comments The paper by Fuentes-Lema and co-workers addresses a topic of interest in marine biogeochemistry. The experimental design is appropriate although the results are far from concluding and I feel the authors have overexploited a bit their findings. Some of the conclusions do not hold or do so only for one of the sampling points, which diminishes the overall relevance of their contribution. I think they should be much more cautious in some statements. Another problem that complicates the interpretation of the dataset is that not all variables were sampled at the same time (e.g. bacterial abundance data are lacking on days 2, 4 and 6 and respiration is lacking on

days 1, 3, 5 and 6). This makes the assessment of the effect of high CO2 concentrations on DOM-heterotrophic bacteria interactions very difficult. I suggest an alternative approach. Rather than focusing on the analysis of specific sampling times I would like to see the analysis of integrated values of bacterial biomass, production and respiration over the course of the 7 days of the incubation of the second phase. Maybe the conclusions will change but they will be more reliable than in the current version. In general, the paper is well-written although there are a number of instances in which English usage and grammar needs to be improved.

Specific comments An important concern is related to the authors' point about the lability of DOC. By examining their Figure 7 one cannot really say anything about DOC lability since only in the HC treatments there was a net, although very slight, decrease in DOC concentration, presumably due to bacterial uptake. How can the authors explain the general pattern of increase rather than decrease in DOC for most of the experiment?

The manuscript implicitly assumes that UV played a distinct role in the amount and quality of DOC produced by phytoplankton (DOCp) but there is no way of distinguishing the effect of UV from the effect of PAR in their experimental design.

By incubating the samples in the dark the authors are introducing a potential source of error in their results. I fully concur with them that solar radiation plays an important role in DOM-microbial plankton interactions but then, why stop the normal diel cycle of light and darkness during 8 days? The authors should be aware of the possible role of photoheterotrophy in bacterioplankton communities (e.g. Ruiz-González et al. 2013 Frontiers in Microbiology) and their response to the two types of DOM. Moroever, the DOM enriched seawater could also be subject to further transformations caused purely by sunlight that are not accounted for in their setup.

Since no attempt was made to estimate empirical leucine-to-carbon conversion factors for calculating bacterial production, known to change dramatically in different en-

vironmental conditions (see for instance Teira et al. 2015 Applied and Environmental Microbiology, and references therein), presumably met during their second phase incubations, the uncertainties of this variable (BP) and that of bacterial carbon demand (BCD) are very high.

Information about phytoplankton cell counts of two idly defined groups (Region 1 and Region 2 in Table 1 for which we do not even know their sizes) in flow cytometry analysis, assuming that the huge initial (Day 0-1) increase in chlorophyll a concentration was mostly due to large cells not detected by flow cytometry make this section virtually irrelevant. Also, I guess that Synechococcus cyanobacteria were surely present at least in Day 2, with abundances much higher than 1000-10000 cells mL-1. The authors should ellaborate more on these results or simply delete them.

Technical The title is very confusing. The interaction is established between DOM and bacteria, not between elevated CO2 and phytoplankton-derived DOM. Also, what does it mean "Interaction... on bacterial metabolism"? The expression "Under solar radiation" is not necessary to be included in the title. "bacterial metabolism from coastal waters" also reads awkwardly. Please change to a more informative, correct title.

L. 54-55. "Phytoplankton" and "heterotrophic counterparts" are not logical choices. Please refer to autotrophic and heterotrophic microbes or something similar.

L. 60. What do the authors refer by "The other way round"? Please explain.

L. 64-65. Provide more detail about "the abiotic AND biotic factors".

L. 67. "Adaptation towards a fast acclimation" sounds odd. The underlying mechanisms are different, please rephrase.

L. 103. "subjected... concentrations" can be safely eliminated here.

L. 142. Surely there were other phytoplanktonic taxa/groups present along with "maily diatoms".

L. 194. The R2 value does not inform about the significance of this difference. Did the aothors performed a t-test/one-way ANOVA to support their statement?

L. 206. Duplicate Winkler bottles seem too few for oxygen changes measurements. Usually a minimum of 4-5 bottles are used.

L. 249. "to compare non-parametric paired samples" is an odd phrasing.

L. 293. It does not seem so obvious to me.

L. 302. Why using RMANOVA for comparing differences at one single sampling point?

L. 310. I do not follow the rationale for using the two statistical tests here. There is some confusion about statistics throughout the manuscript. The authors should clearly state which tests they used and why or try a different analysis (see my general comment) with changes integrated over the course of the incubation of phase 2.

L. 328. "biased" is probably not the best word here.

L. 329. Replace "on" by "of".

L. 341. "there have been...simulate" reads awkward. Please rephrase.

L. 345. Are the authors sure of this statement?

L. 370-371. This is not true in view of the different sampling points and the data shown in the corresponding figures.

L. 396-397. "having... production rates" is not correct English usage.

L. 398-400. Pleaase see my previous comment about lability.

L. 432. Do the authors imply that their water samples collected on June 27th were "cold"? Maybe there was a strong upwelling on that day but this information is not provided and ca. 15°C is not exactly cold.

L. 454. Respiration rate and organic matter are not independent.

L. 455. These results are far from “demonstrating” that claim.

I am not conviced that “acidified organic matter” and “non acidified organic matter” are the best terms for their treatments, did they check that the resultant DOM was of lower pH in the former treatment? Fig. 5B simply shows that the sample water had a lower pH but not that the DOM was indeed of lower pH.

μM is not the appropriate SI unit, please correct it to μmol L-1.

Fig. 4. Please replace the “P” in the Y-axes by TOC, POC and DOC. This is not a very relevant figure and can be eliminated or moved to supplementary information.

The authors use total abundance of bacteria but probably data about the contribution of low and high nucleic acid content (LNA and HNA) cells are available, as well as some indication of changes in cell size that would provide a good estimate of bacterial biomass that could be compared with changes in BP, even if they were assuming data from the literature to convert from leucine incorporation rates to carbon units.

Dubbing cells able to reduce CTC as “viable” is not the best term. Most authors, including the cited references, refer to them as cells actively respiring or showing active respiration but the number of viable cells in their incubations was likely much higher, just by comparing the cell abundance numbers of Figs. 6 and 8. It is uncommon to show CTC positive cells before total bacterial abundance. Also, why not showing the dynamics of CTC positive bacteria for the entire experiment rather than only at day 7?

Fig. 9B. BGE is given either as a percentage or as a ratio, what does “r.u.” mean? Also, given the use of a very high and constant leucine-to-carbon conversion factor of 3.1 kg C mol Leu-1, if the values are close to 80% BGE, they are extremely high, very difficult to reconcile with almost no net uptake of DOC (Fig. 7).

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