

## ***Interactive comment on “Synergistic effects of UVR and simulated stratification on commensalistic algal-bacterial relationship in two optically contrasting oligotrophic Mediterranean lakes” by P. Carrillo et al.***

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We greatly appreciate all the constructive suggestions provided by the reviewer, which have allowed us to improve and clarify the paper. Below, we provide a point-by-point response to the reviewer's comments and specify the changes made in the manuscript.

Reviewer's comment: Carrillo and colleagues present here results on the effect of UV radiation on phytoplankton primary production and extracellular release and bacterial heterotrophic activity in two oligotrophic lakes that differ with respect to water trans-

C6648

parency. The authors apply an exhaustive experimental setup, including different types of radiation regimes and intensities. The originality of the present work lies in the combined investigation of phytoplankton and bacterial parameters in contrasting lake ecosystems, and these data merit publication.

Author's response: We thank Reviewer 2 for her/his constructive general comments as well as for the specific comments that helped us to improve and clarify various points in our manuscript.

Reviewer's comment: I do, however, have one concern that I recommend to be considered in a revised version of the manuscript. Photochemistry, in particular the photochemical transformation of DOM, is more or less neglected in this manuscript, both in the Introduction and the Discussion of the results. I fully understand that it was not possible to include yet another process to the already dense program. But I think the authors should consider these abiotic processes, such as the photochemical consumption of dissolved oxygen or the transformation of DOM to more or less biologically labile forms, in the interpretation of their results. There is extensive literature on this topic that can be used as a basis for discussion. The paper is well written and the results of this overall complex experimental setup are clearly described and illustrated in figures and tables.

Author's response: We agree with the reviewer in that the abiotic processes such as photochemical consumption of dissolved oxygen or the transformation of DOM under UVR are key processes since they could modulate the response of the organisms to UVR. In order to focus the Introduction on the biological processes to be measured, we did not include any information on these photochemical processes. However, it was partially considered in the interpretation of our results in the old Ms. as:

P 12610 ,L.- 6-11“Because this negative effect was greater in opaque ecosystem to UVR due to their DOC content, we propose that the “ideal” photoprotective DOM may become harmful in a scenario of greater stratification and high UVR irradiance induced

C6649

by global warming. Furthermore, UV-B may have harmful effects due to the free radicals ( $O_2$ ,  $H_2O_2$ ,  $OH^-$ ) generated by photo-oxidation of the DOC (Banaszak, 2003; Pullin et al., 2004), exacerbating the negative UVR effect in UVR-opaque lakes". Action taken: We have included the effect of UVR on DOM in the revised version of the manuscript (see below).

#### Specific Comments

##### Abstract:

Reviewer 2.- L. 13-14 This sentence is not easy to follow, because the type of relationship between algae and bacteria is not defined. I suggest the authors explain more explicitly their understanding of strong or weak relationships between bacteria and algal exudates. In a general manner, I prefer the term "phytoplankton" to "algae", because this latter could also make reference to macroalgae. Author's response: Coupling between phytoplankton and bacterioplankton has been defined as the capacity of the carbon (C) released by phytoplankton to support the bacterial carbon requirement. This commensalistic phytoplankton-bacteria relationship is defined as strong when rates of excretion of organic carbon (EOC) exceeded the bacterial carbon demand (BCD), i.e. %BCD:EOC ratio <100, and weak when %BCD:EOC ratio >100 (Morán et al., 2002).

Action taken: "Commensalistic" has been added to the mention of phytoplankton-bacteria relationship. The term algae has been changed to phytoplankton throughout the Ms., as suggested. In the abstract, we have included more details about the meaning of strength of the commensalistic phytoplankton-bacteria relationship: The paragraph now reads: "Under UVR and high mean irradiance, the commensalistic phytoplankton-bacteria relationship was strengthened in the high-UVR lake where excreted organic carbon (EOC) rates exceeded the bacterial carbon demand (BCD)(i.e., %BCD:EOC ratio <100). This did not occur in the low-UVR lake (i.e., %BCD:EOC ratio >100)."

Introduction. The Introduction focuses on the direct effects of UV radiation on phy-

C6650

toplankton and bacterial activity. I was missing a short description of the effects of UV-induced photochemical processes of DOM that will certainly play an important role in the context of the present study.

Author's response: Because we did not measure photochemical processes in our experiments, we focused the Introduction in those variables that were directly measured. However, as suggested by the reviewer, it is interesting to include the effect of UVR on DOM, since photodegradation and photo-oxidation of DOM could play key role in the net response of the organisms to UVR.

Action taken: We have included the following sentence: "Concomitantly, the photochemical reactions mediated by UVR lead to (i) the photodegradation of DOM, altering the composition and absorbance of CDOM and; (ii) the photo-oxidation of DOM producing oxygen free-radicals (Kitidis et al., 2014)."

Reviewer 2.- p. 12592, line 25: The authors expect the readers to be familiar with terms like "B1 and A1 FI scenarios", which is probably not the case. I suggest reformulating this sentence.

Author's response: Both reviewers coincide on the specificity of these terms for the readers of BG.

Action taken: These terms have been eliminated, and the paragraph now reads: "Model predictions indicate greater temperature increases, ranging from 1.5°C to 6.4°C by the end of the century".

Reviewer 2.- p. 12595, line 25-28: This sentence is vague. If the authors want to point out this issue, I suggest they explain in a little more detail the arguments of the paper in question.

Author's response: As also suggested by Reviewer 1, we have included some background information on the debate on bacterial dependence / independence on phytoplankton production, and, accordingly, we have added the references of papers by

C6651

Fouilland and Mostajir, 2010, 2011.

Action taken: We included a paragraph that now reads: "Although the bacterial dependence on C released by phytoplankton is a well-established paradigm in aquatic microbiology (Cole et al., 1988), it is currently under renewed debate. Thus, Fouilland and Mostajir (2010, 2011) proposed that C dependency of bacteria on phytoplankton is uncertain because C sources other than those from algal origin might support the bacterial growth more significantly. However, Morán et al. (2011) rebutted this idea due to uncertainty found in the application of different conversion factors to raw data and modeled rates in the Fouilland and Mostajir's calculations."

Reviewer 2.- Material and Methods. p. 12597, line 3-5. Can you consider the food web as "simple", just because autotrophic picoplankton are missing? Further, this sentence is not clear: What do you mean by size overlap?

Author's response :The structure of the microbial community is simple due to the absence of autotrophic picoplankton, heterotrophic nanoflagellates and the scarcity of ciliates. The meaning of the size overlap is related to the size-abundance distribution. In La Caldera lake the distribution is characterized by a discontinuity in the size range between 2000 and 32000  $\mu\text{m}^3$  (cell volume). Therefore, in this lake did not exist cell size-overlap of the planktonic community (Echevarria et al.,1990).

Action taken: We have added some information about the structure of the microbial community in this lake and we have included "no" before size overlap, because this word was mistakenly omitted in the previous version of the Ms. We have included this point in the text (Model ecosystems subsection) and the sentence now reads: "The pelagic community is relatively simple (Carrillo et al., 2006) and it is characterized by the scarcity of ciliates, absence of heterotrophic nanoflagellates and autotrophic picoplankton, and no size overlap exist between phytoplankton and heterotrophic bacteria (Medina-Sánchez et al., 2002)".

Reviewer 2.- p. 12597 and 12598: I find it very difficult to follow so many different

C6652

abbreviations: HBP, TPR, BR, PAB, PA, P, MIR. I suggest the authors change at least some of them, e.g. PAB to UVB+UVA+PAR, PA to UVA+PAR, P PAR to facilitate the reading of the manuscript.

Author's response:- It is true that we had to use many abbreviations, because we measured several processes in our experiments. However, the reviewer's suggestion perhaps will lengthen the acronym of radiation treatments and this is why we chose to maintain PAB, PA, and PAR. However, we have changed the abbreviations of the stratification treatments throughout the Ms.

Action taken.- We have replaced "high MIR" and "low MIR" by "subsurface" and "mixed" throughout the Ms., Figures and Tables, and in this way we have reduced the number of abbreviations.

Reviewer 2: p. 12602. Respiration rates. How do the authors deal with the photochemical oxygen demand that occurs concomitantly with bacterial or plankton respiration? Do the authors have any previous estimates on this process in their lakes? Neglecting the photochemical oxygen consumption could lead to an overestimation of the respiration rates in the light bottle incubations. This might affect some conclusions as that stated on p. 12608, line 16-17. See for example the recent paper by Kitidis et al. (2014) in *Limnol. Oceanogr.*

Author's response: We did not measure photochemical oxygen demand during the experiments. However, previous experiments using water filtered onto 0.2  $\mu\text{m}$  filters from oligotrophic freshwater systems with different DOM-content did not show significant changes in oxygen content using the optode method during 24 h incubation (Herrera, personal communication). We do know that photochemical processes (photodegradation of DOM or photo-oxidation) are important processes which may contribute to photoinhibition of photosynthesis as well as of production of oxygen free radicals during the light incubation period, with damaging effects on phyto- and bacterioplankton. However, our respiration measurements were made under dark conditions after the ex-

C6653

posure of samples to the light treatments. Therefore, we consider that photochemical oxygen demand did not significantly affect the bacterial or plankton respiration measured.

Action taken: We have specified in the M&M section (Respiration rates) that this measurement was made in dark. The sentence now reads: "TPR and BR rates were measured in darkness using optode sensor-spots. . ."

Figures.

Reviewer 2.- Fig. 2. Can the authors use different symbols for the yield and chl a in figure a and b?

Author's response: Because the Yield data have been eliminated from the figure, the Chl a symbols should be now clear.

Reviewer 2.- Fig. 3. It is not explained in the legend what the different letters stand for.

Author's response: The meaning of the letters was omitted in the original version. They represent the results of a post hoc Bonferroni test used to determine significant differences among treatments.

Action taken: We have modified the captions of Fig. 2 (old Fig. 3), 3 y 4, and now read: "The lines on top of the bars are the standard deviation whereas the letters indicate differences among treatments."

References

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C6654

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C6655