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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 thank Reviewer 1 for her/his outstanding general comments, as well as for the great efforts done with the specific comments, that helped us to clarify various points and improved the quality of our manuscript. We respond below each of his/her major comments / concerns.

General Comments Reviewer 1.- The authors have chosen to address interesting questions but obtained a somewhat limited dataset to address them. There is some uncer-

tainty when generalizing from a single set of measurements from one clear and one brownish lake to what may generally be observed in nature as climate change progresses. Some replication through time or (more usefully) across multiple lakes of each optical category would have strengthened the study considerably.

Author's response: We agree with the reviewer in that the dataset presented could seem limited, since to know how climate change evolves require long-term monitoring of lake variables as well as of weather conditions. However, in our experimentation (and in our Ms.) we are not attempting to do this. On the contrary, we focused on just one important aspect of climate change, such as stratification, which could trap organisms near surface, increasing their exposure to damaging UVR. We used these lakes as model ecosystems where to test our hypothesis, because they represent two ends of the optical properties within the oligotrophic state in Southern Spain.In our work, we simultaneously quantified a great number of metabolic variables, measured in situ; hence, replication of this kind of experiments in several lakes, maintaining a similar UVR environmental natural regime, was not feasible due to the logistic complexity as well as to the scarcity of lakes with similar optical characteristics in our region. This study is part of a more comprehensive project that focus on the quantification of the effects of multiple stressors on algal and bacterial communities; part of the results has been already published in Helbling et al., 2013 (BG) and Duran et al., 2014 (BGD), and they further evidence the high sensitivity of organisms and processes in low-UVR lakes(opaque lakes.Therefore, this study is strengthened by those previous results. We want stand out that the originality of our study was the evaluation of interactive effects of radiation quality and "static" regime on algal and bacterial metabolic processes, being the first one which measured in situ the bacterial respiration and bacterial growth efficiency(BGE)under these experimental conditions, providing a reliable estimation of BCD. This allowed us to assess algal–bacterial coupling via the ratio between bacterial demand for C and algal C supply. We consider that these investigations have the added value of establishing a range of responses, besides identifying their mechanisms in realistic ambient conditions. Ultimately, they may be crucial to improve the

parameterization of predictive models.

Reviewer 1.- Furthermore, the results from the subsurface incubations are probably not representative of what should be expected under an altered stratification regime in the future (I would not expect a drastic reduction in mixing depth from 3 m to 0.5 m,for instance.

Author's response: We consider that our results indeed represent a realistic approach to the expected changes in the vertical physical structure in the water column as consequence of global warming, since most of the lakes of the Southern Iberian Peninsula are shallow, with mean depth less than 5 m (Medina-Sánchez et al.,2010.Besides, extreme events occurring in the Mediterranean region, such as heat-waves, are frequent and they produce daily cycles of micro-stratification with a consequent shallow mixed layer(Giorgi and Lionello 2008.This effect could be more relevant in high altitude mountain lakes, as La Caldera Lake, where it has been found a frequent occurrence of mixed layers with thicknesses as small as 0.5 m on a short-term scale during two-thirds of the ice-free periods in the early 90's (see Fig. 1 from Rodriguez-Rodriguez et al., 2004).

In addition, we consider that a decrease from 3 m to 0.5 m would not be a drastic reduction in mixing depth,since greater changes, encompassing even tenths of meters, have been observed on daily scale in the ocean(Neale et al., 2003).

Reviewer 1.- Despite these problems, it is an interesting dataset, the analysis of which has highlighted a number of interesting patterns; the results deserve a more comprehensive, thoughtful discussion than that currently presented – some of the points mentioned in the Introduction could be revisited in light of the data obtained (for instance, more discussion (and references) regarding how UVR affects the rate of release of exudates by phytoplankton).

Author's response: We have revisited throughout the text these points mentioned in the Introduction in light of the data obtained, as suggested by the reviewer. Thus, we have included in the M&M, Results (supplementary figure) and Discussion sections

information about %EOC. From this normalized variable we have discussed how UVR affects the C excretion rates by phytoplankton.

Action taken: We have included the following information:

1.- In the M&M section: "The total organic carbon (TOC)produced was measured on 4-mL aliquots before filtration"."The % EOC was estimated as: %EOC=100x(EOC/TOC)"

2.- In the Results section:Subsection 3.3: "Likewise, the % EOC was significantly affected by UV-B, increasing to 22% and 21% in subsurface and in mixed treatments, respectively (Fig.1a in Supplement)". Subsection 3.4:"%EOC did not show differences due to radiation in none of the stratification treatments (Fig.1b in Supplement)".

3.- In the Discussion section: "Interestingly, the UVR effect on %EOC was only significant in the high-UVR lake; the release of C has been described as a protective mechanism to prevent photosystem damage from reducing power excess under high irradiance of PAR (Wood and Van Valen, 1990) and also of UVR (Carrillo et al., 2002, 2008). The lack of this "escape valve", which helps to prevent over-excitation of PSII, might be the final cause of the higher sensitivity of phytoplankton communities in the low-UVR lakes".

Reviewer 1.- The quality of the writing could use considerable improvement. The Introduction was generally well written, but the Discussion needs serious revision. The interpretation of the results was hard to follow at times due to the phrasing. I have noted a number of errors below in the Technical Corrections. Further assistance from a scientist with a high level of English proficiency would help to make the MS more readable.

Author's response: We thank Reviewer 1 for her/his detailed work, which has significantly improved the manuscript.

Action taken: A thorough review of the Discussion has been conducted. Also, the English has been revised and corrected throughout the text.

Reviewer 1.- The data visualization could be improved somewhat. For instance, it is difficult to make comparisons among the lakes/strata with the data contained in separate figures (3,4,5) which have different ranges of values on their Y-axes. However, a good summary of the results is provided in Figure 6.

Author's response: We agree in that the data visualization can be improved.

Action taken: We have homogenized the ranges of values in the Y-axes for each variable response (Figs. 2, 3 and 4).

Reviewer 1.- I find the language around UVR and MIR (especially as interacting factors, e.g. p12595, L10-11 -"the interactive effects of radiation quality and increased MIR") confusing; what is being manipulated is the mean irradiance and its spectral balance– this should be made more clear – it is odd to speak of joint effects of UVR and MIR when MIR is partly composed of UVR. This is also an issue for the statistical analysis, as MIR and UVR are not independent, but are used as the two (independent) factors in 2-way ANOVA. Additionally, the terms low MIR and high MIR are somewhat coarse, as the low MIR is not just a lower level of irradiance, but represents a dynamic irradiance treatment (varying from high (subsurface) to low (5 m depth) irradiance over time) whereas the high MIR treatment is static. Why not call the treatments "subsurface" and "mixed" or something along those lines?

Author's response: We agree with the reviewer in that the language could be somewhat confusing when using UVR and MIR as factors. Therefore, we have followed her/his suggestion and we have named "stratification" instead of mean irradiance (MIR) to the second factor, and each level of this factor as "subsurface" and "mixed". In fact, our experimental design included PAB, PA, P, or Dark as levels of solar radiation quality ("UVR" factor) and "subsurface" and "mixed" as levels of stratification conditions, i.e., a factor implying static vs. dynamic irradiance ("STRAT" factor), as indicated by the reviewer; therefore, we consider justified that there were two independent factors to be used in a 2-way ANOVA.

Action taken: We have modified Figs. 2, 3 and 4 and tables 2, 3 and 4, and the terms throughout the Ms.

Reviewer 1.- The term "excreted organic carbon" is used throughout the MS to mean the rate of organic carbon excretion. These are not the same things: the former is a substance, the latter is a process.

Author's response: In order to make the text more fluent to the reader, and because this term was defined in M&M section, "rate" was (sometimes) omitted in the Ms.

Action taken: To improve the precision of the Ms. language, we have included "EOC rate" throughout the Ms.

Reviewer 1.- The validity of the BCD values for the 'UVR-clear' lake depends on the supposed absence of autotrophic picoplankton, which was not confirmed by the authors. A reference (Medina-Sánchez et al., 2002) is provided, which contains two references (from 1990 and 1999) which (I assume) confirmed this empirically. Is it possible that the size distribution of the phytoplankton community has changed since 1999?

Author's response: We have followed the dynamics of planktonic communities during the last 30 years and we can assure that the biological communities during our experiments are indeed representative of this lake. The lack of picoplankton is an intrinsic trait of this lake, which even led us to propose a food-web structure and a functioning model, based on the dual role of mixotrophic-algae (Medina-Sánchez et al., 2004).

The lack of autotrophic picoplankton has also been corroborated in further experimental studies on the microbial food web structure and function of La Caldera lake (Medina-Sánchez et al., 2013 and during the same periods (i.e. summer) in which the present experiments were performed (Dorado-García et al., 2014).

Reviewer 1.- Instead of using BCD:EOC ratio as a proxy for the strength of algal-bacterial coupling, why was the proportion of EOC actually incorporated by bacteria

not measured as the amount of 14C-labelled seston retained on a 1 or 2-um pore-size filter at the end of the primary production/EOC incubations?

Author's response: We do not agree with the reviewer since 14C-labelled seston retained on a 1 or 2-μm pore-size filter in the high-UVR lake is only constituted by phytoplankton, and in the low-UVR lake this portion corresponded to autotrophic picoplankton, nanoplankton and a proportion of heterotrophic bacteria. Therefore, the procedure suggested by the reviewer would not allow us to discriminate between carbon produced by autotrophs and that incorporated by heterotrophic bacteria.

Reviewer 1.- The variable fluorescence (PSII quantum yield) data, as currently presented and interpreted, have no apparent relevance to the study. These data are presented in the Results section but not interpreted in the Discussion. Nor is the significance of the nutrient data (TN, TDN, NO3, TP, TDP, SRP),beyond providing background information on the lakes, obvious to me.

Author's response: The reviewer's is correct in that the variable fluorescence data (PSII quantum yield) do not have enough relevance for this study. On the other hand, we agree with reviewer that the data on nutrient variables serve as background information of the lakes. However, in our opinion, these data are useful for a non-specialized reader (e.g. non limnologist) since they emphasize the fact that both ecosystems are oligotrophic and highly transparent to PAR.

Action taken: Data on PSII quantum yield have been removed.

Reviewer 1.- Further discussion of the relative importance of EOC in lakes that differ in DOC content is warranted. In a DOC-rich lake, is EOC as important to HBP as it would be in a low-DOC lake?

Author's response: Following the reviewer's suggestion, we have included this aspect in the Discussion section. EOC is preferred by bacteria even in lakes with considerable input of terrestrial carbon to subsidize their growth (Kritzberg et al. 2005, 2006). It

is a matter of DOC-quality rather than DOC-quantity, as it has been widely reported that EOC is composed by small molecules (such as glycolate) readily assimilable by bacteria, whereas the DOC pool in lakes is constituted frequently by refractory carbon non-readily assimilable.

Action taken: We have included this point in the text (Discussion section) and the sentence now reads "As noted above, UVR and stratification exerted an interactive effect on PP and HBP in the epilimnetic layer in both lakes. However, this interactive effect was only exerted on EOC in the low-UVR lake, where the EOC rates values were 3-fold higher (except under PAB-subsurface treatment) than in the high-UVR lake. The carbon released by phytoplankton is composed mainly of low-molecular-weight compounds that are readily assimilable by bacteria (Amon et al., 2001). This source of carbon is preferred by bacteria, even in lakes with considerable input of terrestrial carbon to subsidize their growth (Kritzberg et al., 2005, 2006), because the non-readily assimilable organic matter, mostly composed of high molecular-weight (HMW) compounds, must be hydrolyzed by bacterial ectoenzymes before the assimilation."

SPECIFIC COMMENTS

Reviewer 1.- Abstract – "natural microplankton communities" – what about pico- and nano-plankton?

Author's response: This was a mistake. Indeed, we were referring to pico- and nanoplanktonic communities. These terms are more precise to describe the size structure of the planktonic communities sampled in this study.

Action taken: "Microplanktonic communities" has been changed to "pico- and nanoplanktonic communities".

Reviewer 1.- p. 12595, L13-15 – citing a single reference from 1991 (without specifying that it is a review) does not appear to support the claim that there is a "growing body of literature"!

Author's response: We have included more recent references to emphasize that this is a relevant topic in the literature.

Action taken: We have included the references of works carried out by Norrman et al., 1995, and Morán et al., 2011.

Reviewer 1.- p12595, l25-28 – likewise, more references should be supplied to illustrate that there is in fact a "renewed debate" regarding phytoplankton-bacterial coupling, and to provide the reader with background information on this (putative) debate

Author's response: As suggested, references have been included: Fouilland and Mostajir, 2010, 2011, and Morán et al., 2011.These references include the above mentioned debate, which was published in FEMS Microbial Ecology.

Action taken: We have included this point in the text (Introduction section) and the sentence now reads: "Although the bacterial dependence on C released by phytoplankton is a well-established aquatic microbiological paradigm (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 other C sources 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 modelled rates in the Fouilland and Mostajir′s calculations".

Reviewer 1.- p.12596, l22 – unless it is the journal's policy, why not give DOC and TP in mass units, as is conventional in limnology, rather than uM or mM?

Author's response: We partially agree with the reviewer. It is very frequent that DOC is given as molar units, although it is also true that, among limnologists, it is also accepted as mass units. Therefore, we kept the units of DOC and TP as molar, for homogeneity.

Reviewer 1.- p.12596,l26 – does the max depth really vary between 2 and 14 m? If so, explain, otherwise I assume that this is a typo, and should read 12 m to 14 m.

Author's response: The values presented are correct. It is not a typo; the lake depth

fluctuates inter-annually between 2-14 m, because it is very sensitive to drought, as it relies on precipitation regime due to its small drainage area (Villar-Argaiz et al., 2002; García-Jurado et al., 2012).

Reviewer 1.- p.12597, L3 - Would the terms "high-UVR" and "low-UVR" be more accurate than "UVR-clear" and "UVR-opaque"? My dictionary defines "opaque" as "impenetrable by light" – this is not the case for UVR in Lake La Conceja where UVR of short and longer wavelengths appears to be measureable beyond several meters depth (Fig 1).

Author's response:The reviewer is correct. High- and low-UVR are more accurate terms.

Action taken: We have changed "UVR-clear" and "UVR-opaque" to "high-UVR" and "low-UVR" throughout of Ms.

Reviewer 1.- p.12597 – L3-5 – Medina-Sánchez et al., 2002 did not examine the phytoplankton size distribution of Lake Caldera– cite a primary reference to support this claim regarding the absence of autotrophic picoplankton (Personally, I find it surprising that the lake would be devoid of autotrophic picoplankton, given its oligotrophic nature, but perhaps there are other factors at work here.) The current phrasing is also ambiguous – add the word "no" before "size overlap" to clarify it.

Author's response: It is true that the phytoplankton size distribution was not examined by Medina-Sánchez et al. (2002); however, the authors did evaluate the net segregation between algae and bacteria. Regarding the absence of autotrophic picoplankton, this concern has been discussed above i.e., the lack of picoplankton is an intrinsic trait in La Caldera lake.In fact, we proposed a bypass of C flux towards grazing chain mediated by mixotrophic-algae (Medina-Sánchez et al., 2004) as an alternative strategy to make more efficient the carbon flux in oligotrophic high mountain lakes, explaining the scarce development of the autotrophic picoplanktonic fraction.

Action taken: We have added "no" before “size overlap”.

Reviewer 1.- P.12598, L. 15 – how realistic is immediately-subsurface (0.5 m) irradiance as a scenario for altered mean epilimnetic irradiance due to climate change?

Author’s response: This concern has been discussed above. Briefly, and due to characteristic shallowness of the lakes in the Southern Iberian Peninsula, and the existence of the heat waves, it is frequent the occurrence of mixed layers with thicknesses as small as 0.5 m. The phenomenon of microstratification has also been also observed in the ocean at daily scale (see Neale et al., 2003).

Reviewer 1.- P.12598, L. 15. No justification is given for this light level representing a “worst-case stratification scenario”.

Author’s response: We believe to have justified better this sentence referring to the “worst-case stratification scenario” due to the high irradiance conditions as consequence of the formation of near-surface thermoclines during the Summer in the Mediterranean Region.

Action taken: We have included this point in the text (M&M section) and the sentence now reads: “This treatment represents the worst-case scenario in terms of solar radiation (i.e., high summer irradiance conditions), in combination with a sharp increase of thermal stratification (i.e., simulating the formation of near-surface thermoclines) during the usually warm Mediterranean summer”.

Reviewer 1.- P. 12600, Chl-a fluorescence section - a few comments here: 1. What was measured was not the effective or functional PSII quantum yield (called the ‘intrinsic photochemical quantum yield’ by the authors), because of the time the sample spent in darkness prior to application of a saturation pulse – this would allow re-oxidation of the PQ pool and a decrease in PSII fluorescence below Ft’ to something closer to Fo’ 2. Equation 1 is incorrect and should read: Y = F:F’m = (F’m – F’t) : F’m (but see comment 1) 3. The term “Yield” and symbol “Y” are non-specific – use “PSII quantum

yield” and “PSII” 3. I believe most Water-PAMs use red light not white light 4. Were PAM fluorescence values corrected for dissolved fluorescence? If not, I suspect that this is why the quantum yields appear relatively low in the high DOC lake.

Author’s response: This variable has been removed according to a previous suggestion (see above). Nevertheless, and to clarify the points raised by the reviewer, we trust that the “recovery” of the samples in the darkness was minimum. The size of the tubes (stated in the text) was such that, when pumping from 3 m depth, it was filled with < 60 mL, and thus the samples from that depth took <15 sec before PAM readings. Given the slow recovery of the samples we consider this as almost negligible. We thank the reviewer as there was a “minus” symbol missing in the equation. Also, it is correct that the water PAM use a red light. The samples were not corrected for dissolved fluorescence.

Action taken: This variable has been removed from the M&M and from the Results section.

Reviewer 1.- P. 12604, L. 2-4 – why are two different tests listed for testing normality and two for homoscedasticity? When was each test used and why? Why not just use 1 test for normality and 1 for homoscedasticity for consistency?

Author’s response: We listed two tests for normality and two for homoscedasticity because, while all variables fulfilled the assumptions of parametric analyses by means of the least restrictive tests (Kolmogorov-Smirnov’s test for normality, and Cochran, Hartley & Bartlett’s test for homoscedasticity), some variables did not fulfill these assumptions by means of more restrictive tests (Shapiro-Wilks’ and Levene’s tests for normality and homoscedasticity, respectively).

Action taken: We have maintained one test for normality (Kolgomorov-Smirnov’s test) and one for homoscedasticity (Cochran, Hartley & Bartlett’s test) for simplicity and consistency, as suggested by the reviewer.

Reviewer 1.- P.12604, L. 13-20 – is all this text and Fig 1 necessary? Why not just refer to Table 2 for the irradiance data and add DOC data to Table 1?

Author's response: We partially agree with the reviewer; we have included the values of vertical attenuation coefficients in the Table 1 and, thus, we have removed the Fig. 1; however, DOC and temperature data were included in old Fig. 2 (now Fig.1) because their variation in the vertical profile is relevant for this study as it is the main factor responsible of the UVR attenuation. In the text, we highlighted the main traits which made optically contrasting these ecosystems.

Action taken: We have removed the Fig. 1 and modified the Fig. 2 by including DOC and temperature data, and by removing Yield data.

Reviewer 1.- P. 12605, L.12 – do not use the word "significantly" unless it is meant in the statistical sense, and accompanied by a p value

Author's response: We have revised thoroughly the text for any wrong use of word "significant".

Reviewer 1.- P. 12605, L.17 – phytoplankton abundance did not increase with depth – it was higher at the deepest depth but approximately equal at the two more shallow depths – there is no trend as the current phrasing suggests.

Author's response: We have introduced the correction suggested by the reviewer.

Action taken: We changed the sentence "phytoplankton abundance increased with depth" by "phytoplankton abundance was higher at the deepest depth".

Reviewer 1.- P.12605, L. 21 – did these two species also dominate the phytoplankton biovolume, or just cell counts? Author's response:-The two species were dominant in terms of both abundance and biomass.

Action taken: We have included this information on the text, as well as the values of total algal and bacterial mean biomass in Table 1. We have also included in the

M&M section how phytoplankton and bacteria biomass were estimated: "Phytoplankton biovolumes were estimated from measurements of 20–30 cells of each species using image analysis (Inverted microscope Axio Observer A1, Zeiss – High resolution microscopy camera Axiocam HRc, Zeiss).Cell volume was calculated according to Carrillo et al. (1995), and converted to phytoplankton carbon using the conversion factors reported by Rocha and Duncan (1985)" "Bacterial biomass was estimated from bacterial biovolume, measured from bacterial images obtained by transmission electron microscopy (TEM) as described by Medina-Sánchez, et al.,(1999)".

Reviewer 1.- P. 12609, L. 10 – title – change "Sensitiveness" to "Sensitivity"

Action taken: The title has been changed as suggested by the reviewer.

Reviewer 1.- P. 12609, L.19-22 – Harrison and Smith 2011 (Limnol. Oceanogr. 56: 2115–2126) is relevant here

Author's response: We thank Reviewer 1 for her/his suggestion, since this paper is very appropriate for our discussion.

Action taken: We have included the reference of the work by Harrison and Smith, 2011 (Limnol. Oceanogr. 56: 2115–2126).

Reviewer 1.- P. 12610, L.3-5 – if stratification were substantially altered to resemble the 0.5-m treatment used here (which is extremely unlikely) it should be considered that the DOC would become bleached and therefore more UVR transparent.

Author's response: Previous studies (see Rodríguez and Rodríguez et al., 2004; Neale et al., 2003) have reported that the micro-stratification encompass the reduction in the depth of the upper mixed layer to thicknesses as small as 0.5 m both in freshwater and marine ecosystems. We have included the potential effect of DOC photobleaching to make the lake more UVR-transparent.

Action taken: We have introduced this point in the text (Discussion section) with the following sentence: "In addition, DOC would become bleached and therefore the lake

would be more UVR transparent (Reche et al., 2001), increasing the negative effect of UVR on organisms".

Reviewer 1.- P. 12610, L.7 – I don't follow the reasoning here – why would photoprotective DOM become harmful? It is the shading of the DOM that left the plankton in the less-clear lake more sensitive to UVR – if the UVR in the less-clear lake were to increase due to changes in stratification/mixing, these plankton would likely acclimate or adapt.

Author's response: Our reasoning is that, despite the fact that DOM can avoid the UVR penetration in depth and, therefore, could exert a protective role against direct-damage of UVR, the indirect effect by photo-oxidation of the DOC at short-term could result in a net harmful outcome to the more sensitive cells. In our opinion, the cells may acclimate or adapt to UVR in the less-clear lake, but only at long-term scale. It is reported that photobleaching would reduce CDOM light absorption by 50% over 18-44 days under summer conditions (Reche et al. 2000), and over this temporal scale it is plausible that acclimation or shifts in the taxonomic composition towards UVR-resistant species take place.

Action taken: We have rewritten the text to clarify our ideas and the paragraph now reads: "Taken all together, our results show that stratification, by trapping the cells in a shallower epilimnion, with increased UVR exposure, triggered or exacerbated the inhibitory effect of UVR on phytoplanktonic and bacteria metabolism under mixing conditions. Because this negative effect was greater in brownish waters due to their DOC content, we propose that the "ideal" photoprotective DOM may become harmful on planktonic communities in a scenario of greater stratification. Our proposal is based on the indirect harmful UV-B effects due to the free radicals (O-2, H2O2, OH-) generated by photo-oxidation of the DOC (Banaszak, 2003; Pullin et al., 2004), which can exacerbate the negative UVR effect in low-UVR lakes. In addition, DOC would become bleached and therefore the lake would be more UVR transparent (Reche et al., 2001), thus increasing the negative effect of UVR on organisms. However, cell acclimation

to UVR or a shift in the taxonomic composition towards UVR-resistant species could counteract the net negative UVR effect in a long-term scale".

Reviewer 1.- P 12610, L12 – do the authors not find it surprising that the hypolimnetic community was about as UVR-sensitive as the epilimnetic community? This contrast strongly with previous studies (Harrison and Smith, 2011, Freshwater Biol. 56: 980-992; Xenopoulos and Schindler, 2003, cited by the authors)

Author's response: It is true that both communities had similar % inhibition of UVR at subsurface conditions, indicating the threshold of damage by UVR under our experimental conditions. However, we have noticed that the hypolimnetic algal community was more sensitive to UVR than the epilimnetic, because they underwent strong negative UVB and UVA effects even under mixed conditions, in contrast to the epilimnetic algal communities that were not inhibited by UVR under mixed conditions.

Action taken: In order to clarify this point, we have rewritten the paragraph that now reads: "UVR was the main factor that affected the non-acclimated hypolimnetic community, and thus PP and HBP underwent negative UV-B and UV-A effects in both subsurface and mixed conditions (Table 4). These responses reflect the higher sensitivity of the hypolimnetic than the epilimnetic community to UVR, because only the hypolimnetic community was negatively affected by UVR, under mixed conditions. These results agree with previous reports of higher photosynthetic impairment under UVR exposure of phytoplankton from deep chlorophyll maxima (Harrison and Smith, 2011b) or from the bottom of the mixed layer (Xenopoulos and Schindler, 2003)".

Reviewer P.12610, L20 – "gross negative effect"? the net effect would be the damage remaining after repair, would it not?

Author's response: The reviewer's is correct; we used an incorrect term.

Action taken: We have changed "net negative effect" to "gross negative effect".

Reviewer1.- P.12613, L1 – clarify here that the strength of the "commensalistic algal-

bacterial dependence" is synonymous with the magnitude of the BCD:EOC ratio (or this was my understanding.

Author's response: The strength of the "commensalistic algal-bacterial dependence" is not a synonymous of the magnitude of the %BCD:EOC ratio. This ratio quantifies the bacterial carbon demand related to C supply by algae; a value near 100 means a balanced phytoplankton-bacteria relationship, below 100 means a stronger relationship and above 100 means a weaker one.

Action taken: The meaning of the strength of the "commensalistic algal-bacterial dependence" has been clarified in the text as follows: "These results partially support our hypothesis because the interaction between UVR and stratification strengthened the commensalistic phytoplankton-bacteria relationship (decreasing %BCD:EOC ratio <100) in the high-UVR lake, but weakened (increasing %BCD:EOC ratio >100) this relationship in the low-UVR lake (Fig. 2f and 3f)".

Reviewer 1. -P.12613, L 13 – what is meant by the "interactive effect of UVR and stratification"? Yes, micro-stratification would increase the UVR exposure of the plankton trapped within the micro-layer (and, it should be recognized, decrease the UVR exposure of the plankton below it), but, in this context, increased UVR is a direct effect of a change in the physical structure of the water column; enhanced UVR and micro-stratification are not two independent factors producing interactive effects; one causes the other.

Author's response: In this context an interactive effect of UVR and stratification refers to the increased UVR exposure as a result of the stratification.

Action taken: To avoid any misunderstandings, we have rewritten the sentence. We have changed "interactive effect of UVR and stratification" to "the increased effect of UVR at upper layers on" ...as this is more accurate.

Tables

Reviewer 1.- Tables 2 – I assume that these numbers correspond to the PAB treatment? This should be stated in the caption.

Author's response: These values represent the mean irradiance that reached the subsurface and mixed conditions during the incubation time.

Action taken: We modified the figure caption that now reads: "Mean irradiances in subsurface and mixed layers. . .. . ."

Reviewer 1.- Table 3 – if the degrees of freedom are listed it should be clear what they represent (e.g., the sample vs. residual df) – "df1" is not meaningful. - p values should be shown as "<0.001" not as "0.000"

Author's response: In regard to degrees of freedom, df1 = (k-1) and df2= (n-k), being k numbers of groups (treatments) and n the number observations. Because the response variables underwent different radiation treatments (i.e., PP: three-levels, HPB: four-levels, TPR: two- levels in the low- UVR lake; BR: three-levels in the high-UVR lake), we consider that it is necessary to specify the degrees of freedom to fully understand the significance of the F-values for each variable.

Action taken: The p-values have been changed as suggested by the reviewer.

Reviewer 1.- Table 4 – caption – "heterotrophic bacterial production" not "bacterial heterotrophic production"

Action taken: Changed as suggested.

Figures

Reviewer 1.- Figure 1 – why do the profiles in a) and c) not extend to 10 m? Also, the "(c)" is obscuring a datum in panel c. As stated above, I'm not sure this figure is necessary. I would just include the temperature profile in figure 2 and the DOC and light data in Tables 1 and 2.

Action taken: The Figure 1 has been eliminated. The temperature profile and the DOC

data have been included in the old figure 2 (now Figure 1).

Reviewer 1.-Figure 2 – symbols for Chl-a and yield are hard to distinguish (both are black diamonds)

Author's response: We eliminated yield data because this information is not very relevant in the context of our Ms.

Action taken: The Figure 2 has been changed because yield data were removed; thus, symbols for Chl a should be now clear.

Reviewer 1.- Figure 4 – the caption for panel f is not distinguished with "(f)" as the others are – are the dashed lines in panel f an attempt to interpolate between the P and PAB values, because the PA ones are missing ? This is not explained and looks odd.

Author's response: The dashed lines in panel "f" indicate a min-max range of BCD:EOC ratio, where BCD was calculated assuming a BR as 50% and 75% of Total Planktonic Respiration (TPR). The lines are dashed because the PA treatment was not performed.

Action taken: The caption has been corrected including (f) before "Bacterial Carbon Demand (BCD): Excreted Organic Carbon (EOC) as a percentage". The meaning of the dashed lines has been included.

Technical Corrections

Pg. 12592

Title – should be "relationships" (plural) not "relationship" and "optically contrasting" should be hyphenated ("optically-contrasting")1st sentence of Abstract – "shallowing" is not a word – why not phrase L18 – change "global change" to "global climate change" ?

Action taken: We have changed "relationship" to "relationships"; global change" to "global climate change". We have included a hyphen in "optically-contrasting". The

term "shallowing" has been replaced by "a reduction in the depth of the upper mixed layer".

L25 – readers may not be familiar with the terms "scenario B1" and "scenario A1FI"

Action taken: The terms "scenario B1" and "scenario A1FI" have been removed.

Pg. 12593

L15 - the word "their" is not preceded by a subject in this sentence

Action taken: We have replaced "their" by "nutrient".

L17 – change to "light available for growth"

Action taken: Changed as suggested.

L25 – what is meant by "differential" acclimation capacity? Rephrase this.

Action taken: We have replaced "differential acclimation" by "high acclimation".

L26 – change "UVR-stressed" to "high-UVR" – obviously if the organisms have adapted or acclimated and UVR is not producing negative effects it is not accurate to describe the ecosystems as "UVR-stressed"

Action taken: Changed as suggested.

L24 – change "limitation" to "supply of inorganic nutrients"

Action taken: Changed as suggested.

Pg. 12594L4 – "low values"

Action taken: Changed as suggested.

Pg. 12595 L10-12 – reword

Action taken: The paragraph has been rephrased and now reads: "However, despite the key role of phytoplankton and heterotrophic bacteria production as a link between

the microbial and grazing food webs, no studies on the interactive effects of radiation quality and increased stratification on the commensalistic phytoplankton-bacteria relationship have been done in ecosystems with high- and low-CDOM contents".

L16 – replace "rise" with "increase"

Action taken: Changed as suggested.

Pg. 12596L1-2 – reword

Action taken: The sentence has been eliminated.

L11 – hyphenate "UVR-resistant"

Action taken: Changed as suggested.

L13-16 - reword

Action taken: The paragraph has been rephrased and now reads: "To test our hypothesis, we carried out in situ experiments to assess the combined impact of solar radiation (i.e., quality) and simulated stratification on metabolism of phytoplankton and bacteria, and their commensalistic relationship in two oligotrophic lakes with contrasting transparency to UVR in the Mediterranean Region".

Pg. 12597

L. 10 – should be "composed of" not "composed by" Pg. 12598

Action taken: Changed as suggested.

L. 13 – change "associated to waves" to "associated with waves"

Action taken: Changed as suggested.

Pg. 12599

L. 12-13 – change to "to estimate the strength of stratification and the depth of the epilimnion" and omit "in the water column"

Action taken: Changed as suggested.

L. 20 – TP was already defined on a previous page

Action taken: Total phosphorus has been removed as suggested.

Pg. 12600

L. 3 – "pre-combusted"

Action taken: Changed as suggested.

L. 26 – "until analysis" not "until their analysis"

Action taken: Changed as suggested.

Pg. 12601

L. 11 – "filtered onto a 0.2 um" not "filtered through"

Action taken: Changed as suggested.

L. 20 "0.2 um pore-size Nucleopore filters" ?????

Action taken.-The sentence has been rephrased and now reads: "The samples for PP were filtered onto 0.2-μm filters (25 mm diameter, Nuclepore, Whatman)".

Pg. 12603

L9 – typo: "where picoplankton autotroph and bacteria"

Action taken: "Where" has been replaced by "since", and the sentence now reads: "This, however, was not possible in the low-UVR lake, since picoplankton autotroph and bacteria coexisted in the < 3 μm fraction".

L10 – "BR values lie within" not "lies"

Action taken: Changed as suggested.

Pg. 12604

L. 8-10 – rephrase this last sentence for better clarity

Action taken: The sentence has been rephrased and now reads: “Regression analyses were done to assess the dependence of the BGE on the EOC rates for the experimental data in each lake”.

References

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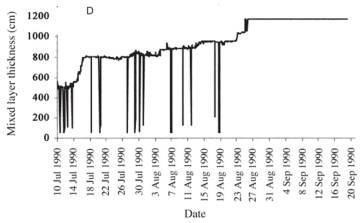

Fig 1.-Time series showing the mixed layer depth (in cm) in La Caldera Lake during the period July 10 to September 20, 1990 (from Rodriguez-Rodriguez et al., 2004)

Fig. 1.