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Comment

## ***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.***

**Anonymous Referee #1**

Received and published: 9 September 2014

I have reviewed the MS entitled “Synergistic effects of UVR and simulated stratification on commensalistic algal-bacterial relationship in two optically contrasting oligotrophic Mediterranean lakes” by P. Carrillo et al. (bg-2014-385) for the journal Biogeosciences.

The authors performed in situ incubations in two lakes of contrasting optical properties (due to different DOC concentrations) and measured phytoplankton and heterotrophic bacterial production, extracellular release of organic carbon by phytoplankton, and respiration rates. The incubations were performed at both a fixed depth (0.5 m) and under artificial mixing (0-5 m) conditions in bottles that produced 3 spectral treatments:

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UVB+UVA+PAR, UVA+PAR, and PAR. Data on Chl-a concentration and variable fluorescence, DOC, nutrients, and algal and bacterial abundances were also collected.

### General Comments

The authors have chosen to address interesting questions but obtained a somewhat limited dataset to address them. There is some uncertainty 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. 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). 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).

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.

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.

I find the language around UVR and MIR (especially as interacting factors, e.g. p12595,

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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?

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.

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-Sanchez 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?

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  $^{14}\text{C}$ -labelled seston retained on a 1 or 2- $\mu\text{m}$  pore-size filter at the end of the primary production/EOC incubations?

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,  $\text{NO}_3$ , TP, TDP, SRP), beyond providing background information on the lakes, obvious to me.

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?

### Specific Comments

Abstract – “natural microplankton communities” – what about pico- and nano-plankton?

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”!

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

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  $\mu\text{M}$  or  $\text{mM}$ ?

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.

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 measurable beyond several meters depth (Fig 1).

p.12597 – L3-5 – Medina-Sanchez 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.

P.12598, L. 15 – how realistic is immediately-subsurface (0.5 m) irradiance as a sce-

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nario for altered mean epilimnetic irradiance due to climate change? No justification is given for this light level representing a “worst-case stratification scenario”.

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  $F_t'$  to something closer to  $F_o'$

2. Equation 1 is incorrect and should read:  $Y = \Delta F : F_m' = (F_m' - F_t') : F_m'$  (but see comment 1)

3. The term “Yield” and symbol “Y” are non-specific – use “PSII quantum yield” and “ $\Phi_{PSII}$ ”

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.

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?

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?

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

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

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P.12605, L. 21 – did these two species also dominate the phytoplankton biovolume, or just cell counts?

P. 12609, L. 10 – title – change “Sensitiveness” to “Sensitivity”

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

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.

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.

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

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

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)

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.

## Tables

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

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”

Table 4 – caption – “heterotrophic bacterial production” not “bacterial heterotrophic production”

## Figures

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.

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

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.

## 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 as in the next sentence, and say “a reduction in the depth of the upper mixed layer”?

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11, C5028–C5036, 2014

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L18 – change “global change” to “global climate change” ?

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

Pg. 12593

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

L17 – change to “light available for growth”

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

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”

L24 – change “limitation” to “supply of inorganic nutrients”

Pg. 12594

L4 – “low values”

Pg. 12595

L10-12 – reword

L16 – replace “rise” with “increase”

Pg. 12596

L1-2 - reword

L11 – hyphenate “UVR-resistant”

L13-16 - reword

Pg. 12597

L. 10 – should be “composed of” not “composed by”

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Pg. 12598

L. 13 – change “associated to waves” to “associated with waves”

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”

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

Pg. 12600

L. 3 – “pre-combusted”

L. 26 – “until analysis” not “until their analysis”

Pg. 12601

L. 11 – “filtered onto a 0.2 um” not “filtered through”

L. 20 “0.2 um pore-size Nucleopore filters”

Pg. 12603

L9 – typo: “where picoplankton autotroph and bacteria”

L10 – “BR values lie within” not “lies”

Pg. 12604

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

...there are a number of errors in the remainder of the paper ...

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Interactive comment on Biogeosciences Discuss., 11, 12591, 2014.

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