Response to the comments / suggestions of reviewer (Dr. Emmanuel Boss). We thank Dr. Emanuel Boss for his general comments as well as for those inserted in the text (supplementary material) that helped us to clarify various points and improved the quality of our manuscript.

Dr. Boss: This paper present the result of an experiment carried in three lakes where the effects of changing mixing regime, spectral light and nutrients on phytoplankton productivity and exudation were conducted. The lakes and their flora are different in both their properties as well as the response to the manipulation experiments. The topic is of interest to BGC readers and within its scope. I have several major comments on the paper that I feel, if addressed, could significantly improve it:

Author’s response: We thank Dr. Boos for his input and below each of his major comments we clarified and responded to them.

Dr. Boss: 1. The link to climate is weak at best. The duration of the experiments was short and represents a very specific state in the evolution of the lake through the growth season. Phytoplankton populations continuously adapt to changing conditions from passing storms to longer term changes in nutrient availability, predators etc’. Assuming we could draw conclusions on changes that are likely to occur due seems a stretch to me. I would not be surprised if two weeks earlier or later from the occurrence of the experiments described here the phytoplankton species composition were different.

Author’s response: We agree with Dr. Boss in that phytoplankton continuously acclimate to changing environmental conditions, and to be able to relate this acclimation to climate it would require long-term monitoring of lake variables as well as of weather conditions. However, in our experimentation (and Ms.) we are not attempting to do this: On the contrary, we are focusing on the short-term pulse of nutrients input which occur within a time frame of 1-2 days. This pulse of nutrients, caused by wind transportation and deposition of Saharan dust is known to affect the short-term response of phytoplankton as demonstrated in previous studies (see references in our original text). In our particular case, we are not addressing the long-term changes in species composition or acclimation that might occur (in fact these were the objectives of a separate set of microcosms experiments that we performed in these lakes) but rather we evaluated the short-term responses such as PSII fluorescence mechanisms and carbon incorporation that can reveal generalized underlying physiological algal responses to stressors. However, we do agree in that if one could perform experiments in other seasons the results might be different due to a combination of factors (i.e., different incident solar radiation, temperature, mixing conditions due to wind stress as well as species composition). In our particular case, it is important to note that for these in situ experiments we had to accommodate the sampling and time frame experimentation to many variables: Researchers of the University of Granada (co-authors of this Ms.) have been following the dynamics of at least two of the studied lakes (Lakes La Caldera and Las Yeguas) for some years, and it is seen that they are “open” (i.e., not frozen) for only about three months a year (when ice melts away during Summer). Therefore, the timing of our experimentation in these two lakes was conditioned by this situation but, on the other hand, it allowed us to sample at the peak of development of communities. In addition, the environmental conditions during that period are such that the variability in species composition is rather small, thus we can consider that it is representative of these lakes. In the case of Lake Enol, it does not
freeze during the year; however, our experiments in this lake were done during Summer to allow for a comparison (in terms of maximum incident solar radiation) with the results obtained in Lakes La Caldera and Las Yeguas. Finally, and regarding acclimation, we cannot expect that it would be the same throughout the year, but in our Ms we intended to address the worst-case scenario in terms of solar radiation (i.e., high irradiance conditions characteristic of Summer), in combination with an expected increased thermal stratification and mixing during Summer. Thus, our experiments, that were designed and performed in situ during mid Summer, mimicked the expected future scenario of summer stratification and mixing regime (under high solar radiation fluxes) in the upper layers of these mountain lakes. We added some sentences in the revised version to stress this point.

*Dr. Boss:* 2. I am not at all surprised to see that phytoplankton in the lake with the most UV attenuation be the one that are the most stressed when exposed to a larger UV dose than that which they are used to. Phytoplankton, for example, produce MAAs as sun screen to UVR when needed. Imagine taking an Alaskan inuit (or me from Maine) to the equator in the middle of the winter. He will feel a shock too and stress out. However, if the cells and ecosystem could have time to acclimate to new conditions (from individual cells producing MAAs to another phytoplankton specie dominating by being better adapted), the ecosystem response may be very different from a short-term un-natural perturbation as performed in the experiments presented here.

*Author’s response:* We partially agree with Dr. Boss in this comment. We do not consider that our experiments had “short-term un-natural perturbations” as in our experiments we are manipulating three variables, solar radiation (quality and quantity), nutrients (in situ versus enhanced) and mixing (statics versus mixing) in conditions rather similar to those encountered in Nature as follows: In the case of irradiance, we did in situ incubations, using solar radiation: Quantity was changed by changing the depth in the water column (as normally occurs in any aquatic body) and quality was changed by both, changing the depth in the water column (via mixing) and using specific filters to absorb UVR. Therefore, and with the exception of the use of a filter, our conditions were natural. In the case of nutrients, we used the in situ conditions (i.e., the nutrient concentration at the sampling time) and for the enhanced concentration we added enough amount to simulate the nutrient input due to Saharan dust as previously published (Morales-Baquero et al., 2006). Finally, and for vertical mixing, we estimated the mixing speed previous to our experiments by conducting in situ measurements of phytoplankton PSII fluorescence based on a previous study of our group (Villafañe et al., 2007). This estimated speed was then applied to our mixing device to simulate the changing irradiance conditions (quality and quantity) in each lake. Perhaps the most “artificial” condition was the static condition, with phytoplankton staying at the same depth for a period of time, but this situation might also be encountered in calm days when vertical mixing is very slow and swimming (if motile phytoplankton are encountered) still cannot oppose turbulence. We further clarified all these points (including dust deposition vs. nutrient addition) in the revised version of the Ms., so the reader should not have the impression that our experimental conditions are un-natural. As mentioned above, and of course there are some manipulations that are necessary for the experiments, such as for example those related with the enclosure of phytoplankton in a container to measure carbon incorporation, etc. Because we considered that “bottle effects” would increase with increasing time of experimentation, we performed short-term incubations to
assess carbon incorporation and fluorescence measurements. These variables changed very fast (seconds) as in the case of PSII fluorescence and hours to measure carbon incorporation, therefore we performed incubations lasting only 4hs. Regarding the first point addressed by Dr. Boss, we fully agree (and this was discussed in our original Ms.) with the possibility of acclimation mechanisms acting under different radiation conditions. However, and in spite of the obviousness of higher sensitivity towards UVR of organisms living in opaque environments, it has to be also considered (and as we also stated in our Discussion) that increasing attenuation in lakes due to increasing DOM is thought to protect the organisms, being considered the “ozone of the underwater world” (as so defined in Williamson & Rose, 2010, Science); therefore, while these organisms could be especially vulnerable to solar UVR, the impact of these wavelengths could be lower than expected, precisely due to this natural protection (i.e., DOM). In our Ms. we further determined that mixing needs to be included in models and system evaluation as it affects the exposure and responses of organisms to solar radiation, as also demonstrated in previous studies (Villafañe et al., 2004, Arch. Hydrobiol.).

Dr. Boss: 3. Some phytoplankton swim and hence have the potential avoid stressors such as UVR (when not incubated) and access nutrients even under increased stratification.

Author’s response: We fully agree with Dr. Boss’ statement, but this is so provided that phytoplankton are in a water column under calm conditions and thus swimming can counteract the turbulent mixing; however, for doing this, phytoplankton will also need to spend energy. In addition, phytoplankton can access to high nutrients when swimming down if a nutricline (or at least increasing concentration of nutrients with depth) is observed. In our studied lakes none of these characteristics (i.e., presence of nutricline nor increased nutrients concentration) was observed as phytoplankton were well mixed within the epilimnion and, most important and what we simulated, is that nutrients input was from above, either as cattle activity (Lake Enol) or Saharan dust (in the three lakes). We further stressed this point in the revised version.

4. In many shallow marine environments benthic production can dominate that of the water column (E.g. works by Nelson and others by Gatusso). Do you know whether this is important in the shallow lakes investigated here? I am mentioning it as you specifically avoided deep lakes with DCMs.

Author’s response: Previous studies evaluating the dynamics of these lakes have already established that there were no DCMs. In addition, we were interested in what happened to primary production in the water column under a simulated perturbation (e.g., pulse of nutrients due to input of Saharan dust). Moreover, we were particularly interested in phytoplankton and not in the benthos, as phytoplankton responses in the water column would be the first indication on any impact from UVR, surface nutrient inputs and or temperature increases. Under a scenario of increasing temperature, a stronger stratification would occur, thus exposing cells to higher UVR and nutrients conditions. We further stressed this point in the revised version.

Dr. Boss: 5. Kd PAR is not constant even in a layer of constant optical properties (e.g. Morel, 1988, JGR) due to the rapid change in light spectra as well as changes of direction and degree of ‘diffusion’ of surface light by absorption (which tends to focus it) and scattering (which tends
to diffuse it). You measure Kd so can directly integrate it w/o the need to make this assumption (e.g. in Equ. 1).

Author’s response: We measured the irradiance at different depths in the water column and we used these depth profiles, that considered those variations in light that Dr. Boss mentioned, for the calculation of Kd. Kd was calculated by fitting a profile of solar irradiance versus depth with and exponential curve, so the exponent was the Kd, so we are using this value as the mean Kd for that specific profile. For the calculation of the mean irradiance in the 3 m upper water column we used eq. 1. This equation comes from solving the integral of the penetration of solar radiation as a function of the depth considered (in our case: 3 m) together with the mean Kd calculated as mentioned before using all the data points in the profile, and not just 2 points (e.g. surface and 3 m).

Dr. Boss: 6. The fluctuation of light near the surface is consistent with waves (e.g. Zaneveld et al., 2001, Applied optics) than with clouds passage (which can produce negative Kd). In addition the differences in surface illumination for the days of the experiments seem very similar, such that clouds are not likely to have played a major role.

Author’s response: We agree in that the fluctuations seen in Fig. 1 near the surface are due to waves and not to clouds, as the profile took a very short time to be done and to be influenced by clouds. We did mention the clouds’ influence as we had a radiometer continuously measuring incident solar radiation (every minute) and this was clearly seen, especially during experiments carried out in Lake Enol (Fig 3b).

Dr. Boss: 7. It is not clear how the measurement of Carbon with a CHN analyzer provided you with phytoplankton carbon (in the ocean most of POC is not phytoplankton, e.g. works by Durand and Olson). Can you please elaborate?

Author’s response: We estimated phytoplankton carbon from microscopic observations and calculations of biovolumes and applying equations that correlate them with carbon; we did not use a CHN analyzer for this calculation, as we were aware of the problems that Dr. Boss mentioned.

Dr. Boss: Dear authors: I am not a limnologist (though I worked in lakes) and have limited knowledge of your field. If you feel my comments are wrong feel free to contact me directly and I will be happy to change my comments.

Author’s response: No answer is needed.

Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/9/C4690/2012/bgd-9-C4690-2012

Page: 9792
Number: 1 Author: emmanuelboss : Sticky Note Date: 10/4/2012 7:12:35 AM
I assume you mean ‘under in-situ nutrient addition’
Author’s response: We did not add nutrients to this treatment and we are referring to the nutrient concentration that each lake had at the moment of sampling.

Number: 2 Author: emmanuelboss : Sticky Note Date: 10/4/2012 7:13:01 AM
define EOC

Author’s response: Defined as suggested.

Number: 3 Author: emmanuelboss : Sticky Note Date: 10/4/2012 7:14:09 AM
These climate changed related scenarios of

Author’s response: Changed as suggested.

Page: 9793
Number: 1 Author: emmanuelboss : Sticky Note Date: 10/4/2012 6:39:54 AM
Increasing

Author’s response: Changed as suggested.

Page: 9795
Number: 1 Author: emmanuelboss : Sticky Note Date: 10/13/2012 4:32:52 PM
the description of the ecosystem is for the time of the experiments. It does not cover the whole growth season where some conditions could be significantly different.

Author’s response: The descriptions of the ecosystems are based on previous studies conducted at different times (as mentioned in the original text) so they represent the general conditions of these lakes. Since at the time of our sampling the conditions were within the range presented in this previous literature we treated them as representative for the systems. We clarified this point in the revised version.

Number: 2 Author: emmanuelboss : Sticky Note Date: 10/4/2012 7:29:52 AM
You provide different descriptors to each lake (e.g. DOC levels for some etc'). Can you provide a table summarizing the salient features of each lake. Also, please define oligotrophy - is it based on levels of [chl] and provide the trophic status of LC.

Author’s response: As suggested by Dr. Boss we added the main features of each lake to Table 1, clearly indicating what is from the literature and what are our own measurements. In the case of “oligotrophy” we were quoting the work of Velasco et al., 1999 that based this on the low chl-a content of Lake Enol. We also added the trophic status of LC as suggested.

Number: 3 Author: emmanuelboss : Sticky Note Date: 10/4/2012 7:32:40 AM
not clear how this lake is oligotrophic given low light penetration (is CDOM taking up all the light) and influence by nutrient coming in from cattle activity. Are the descriptions year-arround characteristics or those of summer/late-summer.

Author’s response: As mentioned in the previous comment this was based on the chl-a content.
Have you considered benthic productivity? In shallow coastal areas of the ocean, the bulk of productivity can occur in the benthos (e.g. works by Nelson or Gatusso).

Author’s response: In our work we did not consider the benthic production and, as mentioned above, we evaluated in our experiments the response of phytoplankton in the water column as a first and rapid indication of the impact of some climate change variables on these lakes.

If that is the case, is mixing to 3m a realistic representation of phytoplankton that are likely cycling the full depth of the water column?

Author’s response: This is a good point. By doing this, we were able to compare the three lakes and we also mimicked stratification as would occur due to an increase in temperature. In addition, our profiles in Fig. 2 show stratification in the upper meters of the water column. Also, previous studies have shown that this type of stratification in the upper section of the lakes occurs during Summer. We added this information to the Discussion and we clarified this point.

What other inputs can they receive at the shore?

Author’s response: These lakes, and especially Lake Enol, receive a lot of tourists and sometimes the shore is not very clean (there is also important cattle activity around). Also, a small stream runs into Lake Enol, so we wanted to avoid any potential change in the optical conditions due to this. The mid-section of the lake was clean and with minimal influence of any trash or particles that might influence the turbidity of the water.

since you have these measurements you can compute from the profiles the position of 50% light level.

Author’s response: Yes, we could compute directly the 50% light levels, but since we are not using this value in the discussion of our data we did not compute it.

this assume kdPAR to be constant. It is well known that kdPAR varies faster near the surface than at depth for a homogeneous column of water (e.g. Morel, 1988). Since you have the profile you can compute I_m directly.

Author’s response: We fully agree with this comment. We used this equation as we did various profiles for each lake and computed a mean kdPAR, and we used this mean value in the equation.
The variations between profiles within each lake were minimal and this is why we showed in Fig. 1 representative profiles. Nevertheless, we computed the $I_{\text{m}(\text{PAR})}$ using both approaches and the differences were minimal and did not change our overall conclusions. The advantages of using eq. 1, is that it is much easier to take into account the variations in surface irradiance as in the equation $I_{0\text{PAR}}$ is the mean incident PAR during the incubation period of 4 hours as measured by a terrestrial radiometer as stated in the text. We further clarified this point in the text.

Author’s response: We agree - Lakes Las Yeguas and La Caldera are similar in terms of PAR penetration but they are different in terms of UVR penetration as shown in our Fig. 1.

Author’s response: We did not show the surface irradiance and also we are not referring to the vertical profiles but rather to incident radiation. We realize that this was confusing in the text, so we clarified this point in the revised version.

Author’s response: The mean irradiances, as stated in our text, referred to the 4 hours experimental period, and we agree with the reviewer in that there were no differences in these mean values. However, clouds were important during some days as seen in the graph below that show the incident solar radiation of one experimental day at Lakes La Caldera and Enol. However, we do not consider it necessary to show this type of detail in our original Ms. but we mentioned the influence of clouds in the text.
Author’s response: As mentioned above phytoplankton carbon was estimated from microscopic measurements i.e., by calculating the biovolume of cells and converting this to carbon content. Therefore the only source of C was from phytoplankton organisms. This was stated in the Material and Method section.

Author’s response: We agree on this and this was stated in the text. However (see above) there were differences in UVR penetration among the three lakes.

Author’s response: See our response above referred to clouds and surface irradiance (i.e., important influence of clouds during some days, especially at Lake Enol).

Author’s response: In this part we are referring to Fig. 4a, for Lake Enol, and in the Figure the white bars refer to the fraction > 3 µm, while the black ones are the 3-0.7 µm size fraction. This was indicated in the Figure caption, but as Dr. Boss indicated in the figure, the text was hard to read. We enlarged the text so it should be clear now the differences in size in each graph.

Author’s response: We agree in that this statement holds for static samples, while the ones under mixing regime had a significantly greater inhibition. This, in fact, was one of the key points of our Ms. i.e., that in spite that CDOM may confer some protection to cells, mixing needs to be considered at the time to evaluate the impact of global change variables in high CDOM environments.

Author’s response: Is this significant given the uncertainties in your method?
**Author’s response:** Yes, they were significant and we stated in the Figure what was statistically significant and what was not. We further clarified this in the text.

**Number: 2** Author: emmanuelboss : Sticky Note Date: 10/13/2012 5:03:46 PM
*is this surprising given that in lakes with lots of CDOM cells will not invest in photoprotective pigments?*

**Author’s response:** As mentioned above, and although this is not entirely surprising, we are not aware of any other study manipulating multiple variables as we did to prove this. In fact, many papers report CDOM as a “protecting factor” as cells receive less solar UVR however, the importance of mixing is generally disregarded. It is then of key importance the results obtained in our study, in which we particularly addressed the importance of mixing in regulating inhibitory effects.

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**Number: 1** Author: emmanuelboss : Sticky Note Date: 10/13/2012 5:04:41 PM
*your simulation do not take into account the placticity of phytoplankton taxa and species to adapt to their environment. What you have looked at is how cells existing today will fare if suddenly moved to a different regime. You can say that you varied their growth conditions using forcing by variable that are affected by climate change.*

**Author’s response:** As previously mentioned, we did not intend to evaluate the long-term acclimation of phytoplankton, which includes the change in taxa (among other changes), but we rather looked at the short-term responses (photosynthesis) and how this was affected by our simulation of natural conditions of nutrients and mixing. We are aware that this cannot be extrapolated to growth or long-term periods, but neither a long-term simulation using mesocosms would allow us to evaluate the interplay of these variables, as mixing is not constant in time and, moreover, it is attenuated after a storm or strong wind passes. We rephrased this part of the Discussion so the reader has a clear understanding that we are evaluation short-term effects on photosynthesis.

**Number: 2** Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:30:59 PM
*you are assuming that phytoplankton species are the same throughout the growth season and all affected similarly. In actuality it is most likely that there is a continuous selection to different species with time scales of weeks in addition to inner species adaptation.*

**Author’s response:** We are not assuming that phytoplankton species are the same throughout the season. In fact, we are aware of these changes, but we are emphasizing the short-term response of a particular phytoplankton population that we consider representative of the Summer conditions in the lakes. In order to avoid confusions, we rephrased this section to accommodate this comment, clearly stating that doing our experimentation in Summer, we not only have open water in two of the lakes (as mentioned above) but also we are addressing the effects under a maximum conditions of solar radiation and nutrient inputs (Saharan dust).

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**Number: 1** Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:33:07 PM
this is all pure speculation

Author’s response: We rephrased this part to accommodate what we were stating i.e., that vertical mixing and nutrient input complicates the simple view that CDOM protects organisms from solar radiation.

Number: 2 Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:33:36 PM
yes and their acclimation state
N
Author’s response: We agree, and this is why we stated “resulted in different acclimation to solar radiation”

umber: 3 Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:34:28 PM
that make sense as CDOM was very high in this lake.

Author’s response: We agree in this point.

Number: 4 Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:35:18 PM
there is no significant evidence for clouds.

Author’s response: See our responses above.

Number: 5 Author: emmanuelboss : Sticky Note Date: 10/13/2012 5:05:13 PM
what about photooxidation increase due to increase in T? There are so many effects and counter effects that it seems to me premature to speculate about climate effects.

Author’s response: We agree with this, and this is why we quoted the work of Chris Osburn on photooxidation of CDOM. However, we are not speculating with climate effects but rather stating that a new balance will be created.

Number: 6 Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:40:04 PM
they do it all the time or species that are more adapted replace them.

Author’s response: We agree, and this is what we are saying, but with different wording.

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Number: 1 Author: emmanuelboss : Sticky Note Date: 10/13/2012 12:41:28 PM
in a time scale of ~1 hour or less.

Author’s response: The time scale will depend on the depth of the upper mixed layer (or epilimnion), the strength of the pycnocline and the forcing and turbulence within the UML. Calculations go from minutes to weeks as previously published (Denman and Gargett, 1983; Neale et al 2003).

Number: 2 Author: emmanuelboss : Sticky Note Date: 10/13/2012 2:57:28 PM
It shouldn’t be if you let the ecosystem acclimate to its environment.
Author’s response: We consider that the ecosystem is already “acclimated” to their conditions at the time of sampling, and our experimental conditions just mimicked the natural environment as mentioned above. Of course, the acclimation is in continuous change, due to variations in clouds, wind, etc., and this is why we stated under what conditions our experiments were done and how realistic they were. We further clarified this point in the revised version as the input of nutrients does not occur “continuously” but rather in pulses.

Number: 3  Author: emmanuelboss  Sticky Note Date: 10/13/2012 2:58:12 PM
such mixing could occur regularly during passages of storms

Author’s response: We agree; we are just saying that increasing temperature would decrease the depth of the UML and it would increase the strength of the pycnocline thus making the consideration of mixing more important.

Page: 9809  Number: 1  Author: emmanuelboss  Sticky Note Date: 10/13/2012 2:59:38 PM
these effect are all incorporated into modern sophisticated model. I think that you mean that they were not been experimented with simultaneously.

Author’s response: We agree and this is what we meant. We rephrased this statement.

Page: 9820  Number: 1  Author: emmanuelboss  Sticky Note Date: 10/13/2012 5:07:16 PM
I suggest using continuous gray lines rather than dashed ones.

Author’s response: Changed as suggested.

Page: 9823  Number: 1  Author: emmanuelboss  Sticky Note Date: 10/13/2012 5:07:56 PM
Hard to read texts on graphs.

Author’s response: We enlarged the text.

Page: 9824  Number: 1  Author: emmanuelboss  Sticky Note Date: 10/13/2012 5:08:11 PM
hard to read text on graphs.

Author’s response: We enlarged the text.

Page: 9826  Number: 1  Author: emmanuelboss  Sticky Note Date: 10/13/2012 5:08:50 PM
hard to make a meaningful 95% confidence interval from a line with 5 points.

Author’s response: We do not understand this point and we are not sure if Dr. Boss is suggesting that we take out the 95% lines? In any case, with the 6 points that we have, the 95% confident
limit is statistically significant and very close to the regression line (that was also significant). The 95% confidence limits just tell us how good is our fit, in contrast to the prediction band that indicate how scattered our points are.

I find this figure rather confusing.

Author’s response: We enlarged the text and modified slightly the figure.