Response to the comments / suggestions of reviewer (Dr. Patrick Neale). We thank the detailed review made by Dr. Neale that enhanced the quality of our manuscript and helped to clarify various points from the original version of our Ms.

General Comments

Dr. Neale: *Although there have been several studies of how vertical mixing in lakes and the ocean interacts with UVR inhibition of phytoplankton photosynthesis, this is the first study that has included a third factor, nutrient enrichment, into experiments examining the interaction. All three factors, UV/light climate, vertical mixing and nutrient inputs are expected to vary with climate change. The authors' study systems are three Spanish lakes that can receive nutrient pulses, for example, through Saharan dust events. They use an elegant experimental design that has a full factorial set of treatments, +/-UVR, mixed vs static, with or without nutrient enrichment. The settings of the three studied systems were considerably different, so not too surprisingly, somewhat different patterns of results were obtained for each. The authors describe a synthesis of these results into an overall interaction scheme, including data from other studies, but the support for this scheme was not so clear (so to speak). This is a novel and interesting study that is generally well presented (apart from some technical issues detailed below) but attention is needed to the interpretation of results addressing the issues that are described in the following Specific Comments section.*

Author's response: Below please find our responses to each specific comment / technical issue addressed by Dr Neale.

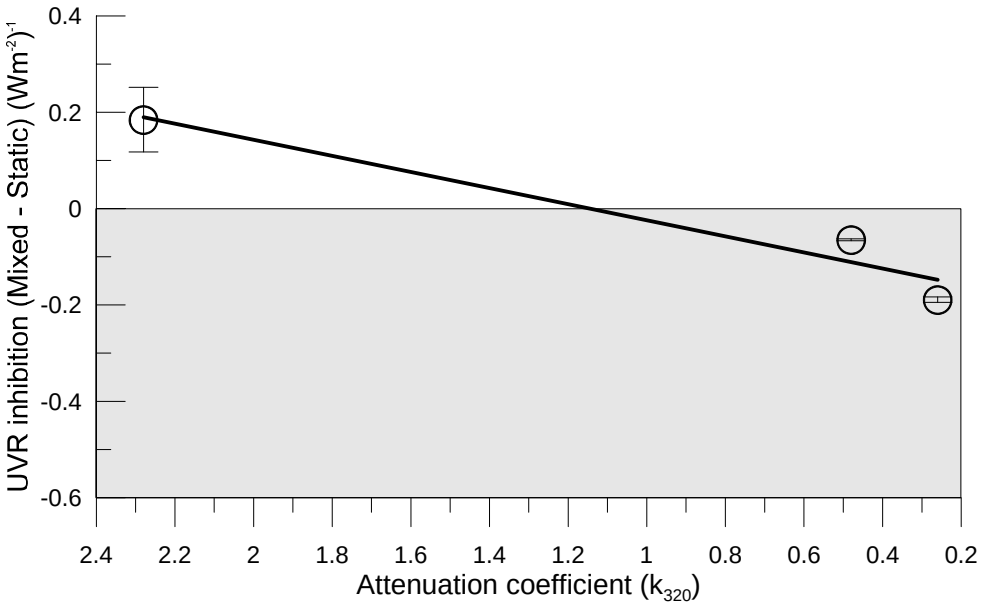
Specific Comments

Dr. Neale: *The authors propose that lake transparency has a dominating effect on how vertical mixing modulates the impact of UVR on primary productivity. This proposition is based partly on the data presented in the paper, but also using data from other studies which actually make the most important contribution to defining a statistically significant trend in the summary figure (Fig. 7). The authors are not very specific about how these extra data points were calculated, however, the position of some points on this graph seem contrary to the conclusions made in the text of these other papers. The graph shows the UVR inhibition results from three previous studies which sampled lakes with Kd320 > 1.0 m-1 and in each case the point on the graph indicates that UVR inhibition was higher in the mixed than in the static samples, supporting the statement in the text that "In opaque lakes (i.e. with high Kd320) the inhibition was greater under mixed than under static conditions" (Pg-Lines 9805-24-25). Point #1 has a citation to Köhler et al. (2001). While Kohler et al present results for a range of different incubations, the results most relevant to this analysis appear to be model calculations for integrated production during a 4 h midday incubation of bottles that were (hypothetically) circulated (0 – 3.9 m) versus static at 1.2 m both having the same average UV exposure. The model estimated less inhibition for the mixed sample (26%) than the static sample (32%) (Köhler et al. pg. 304), so the position of this particular result in Fig. 7 (mixedstatic) would be on the negative side of the y-axis in contrast to the positive point that is shown. Point # 2 is from Hiriart-Baer and Smith, they also present model calculations which compare mixed vs static production, but in this case the opposite conclusion was reached, inhibition was greater in the mixed than in the static, mainly because of slow recovery rates. This does seem to be consistent with the positive position of point # 2 on Figure 7. Point # 3 is from Villafañe et al. (2007), they measured photosynthetic efficiency*

(from PAM fluorescence) in fixed depth incubations vs water column samples. This study only had +/- UV treatments at the surface so it is not clear how UVR inhibition over the water column was estimated. Apparently, they interpreted differences between fixed depth and water column samples in terms of UVR exposure (though it seems PAR exposure could have been important, too). Nevertheless, they conclude that "..., vertical mixing not only counteracted the impact of UVR but also resulted inhigher photosynthetic efficiency at all irradiances (Fig. 6)." The implication seems to be that inhibition was less under mixing than under static conditions, which would result in a negative point on Figure 7 in contrast to the positive point that is shown. These three points account for most of the trend in relationship between UVR inhibition (mixed-static) and Kd in Fig. 7, so it is critical that their calculation be fully documented and any inconsistency with interpretation in the original publication be explained. Was the data specified above used to make the graph? If not please explain how the points were derived. From the way these previous results were discussed in the original papers, at least, there is not general support for the authors' contention that UV inhibition is expected to be enhanced by mixing in low nutrient, opaque lakes. Instead, mixing sometimes enhanced (study 2) and sometimes ameliorated (studies 1 and 3) inhibition in these systems. If this is the case, then neither is there a general contrast based on presence/absence of a nutrient pulse. My own sense is that multiple factors are at work here, in addition to transparency, type and extent of nutrient limitation, taxonomy, depth of mixing and temperature, all of those influencing the rates of damage and recovery which are the ultimate determinants of how UVR inhibition interacts with verticalmixing.

Author's response: We fully agree with Dr. Neale in that multiple factors are at work (as in any aquatic body) but it is almost impossible to address / determine the impact of all of them. As Dr. Neale said, we considered in our experimentation the "manipulation" of three variables – solar radiation, mixing and nutrients addition. We also agree that differences in species composition, temperature, etc., would also have an important share in the variability observed when comparing the different systems / aquatic bodies. However, one thing in common that influences the response of phytoplankton to UVR is the previous light history. Many studies (including also Dr. Neale's various papers) have shown that acclimation of phytoplankton and "activation" of various mechanisms to cope with UVR depends on the light levels previously received by the cells. This was the idea behind the analysis of our data i.e., that phytoplankton from "opaque lakes" received less solar radiation and thus they were less acclimated to cope with solar UVR. In addition, we hypothesized that vertical mixing also conditioned the response of phytoplankton by exposing cells acclimated to different radiation conditions (e.g., opaque and transparent lakes) to high irradiances when they were close to the surface. We are aware that this impact also depends on other factors including species composition, temperature, etc. (as mentioned before) but we established the penetration of solar radiation as a common "driving force" based on the vast literature on this topic.

For doing our analysis, we used the three lakes in which we conducted *in situ* experimentation, and based on the results from these three lakes we determined a trend of higher inhibition under mixing conditions in the opaque lake (see figure below – data shown are those without nutrient addition as in the original Ms).



Of course we wanted to extend these relationship between mixing/static effects and lakes transparency to more opaque lakes that those we sampled, in order to have more data points in the relationship. However, it was impossible to conduct *in situ* experimentation under similar conditions in other opaque lakes, so we decided that one approach was to use data from the literature with experiments using a rather similar mixing approach. This also proved to be a hard task, as there were a lot of variations among the published experiments, different environments, measurements done, timing, etc. Still, this approximation (i.e., considering the three data points obtained from the literature) was important - not to "drive" the relationship, but rather to extend the already determined trend of our data from the three Spanish lakes to others.

Calculations of the three data points added to the Figure: As we mentioned before, we realize now that this was not clearly explained in our original text. The calculations were derived as follows:

Point 1 (Point 3 in Fig. 7): (Köhler et al., 2001). This specific work did not use a static treatment. However, the authors (Dr. Neale was one of the authors of this article) made a comparison between rotating and static treatments (this latter based on a previous work). The authors stated in the abstract "*Compared to the glass bottles, particulate C assimilation in the quartz bottles was reduced by 20-30% at mixing depths between 2 and 10 m*" so we interpreted that these values were due to mixing and therefore we used a mean value of 25% inhibition due to mixing**.** According to Dr. Neale's comment, the value that should be used is the one that appear in the discussion of Köhler's paper. Thus, we recalculated the data presented in Figure 7 as suggested using a -6% inhibition value (i.e., 26% (mixed)-32% (static)), and normalizing it by a mean PAR of 692 μE m^{-2} s^{-1} (148.5 W m^{-2}) as stated in their Table 1 for the 0-3.9 m depth mixing. As a result of this calculation, the data point moved to a slightly negative value (-0.04) but without changing the main trend (see figure below).

Point 2 (Point 2 in Fig. 7): (Hiriart-Baer and Smith, 2005). We used their predictions (as they appear in their Fig 4) based (as the authors mentioned) in data previously collected. From these data we calculated an inhibition difference between mixing and static conditions (i.e., 8%) and we normalized the value by the PAR irradiance. In the text it was not stated the absolute PAR value, but the authors mentioned values of 6.9 and 9.6 % of mean PAR for the West Basin (their Table 1). The authors also quoted Hiriart-Baer and Smith, 2004 (Limnol. Oceanog. 49 (1): 202-214) where maximum values of incident PAR are given for various days (their Table 3, mean of ca. 160 W m^{-2}) so the mean irradiance we used for our calculation was 13 W m^{-2}. Thus we used these values (i.e., 8/13) to estimate the point in Fig 7, obtaining a mean inhibition value of 0.61. There is no change for this point in our Fig. 7.

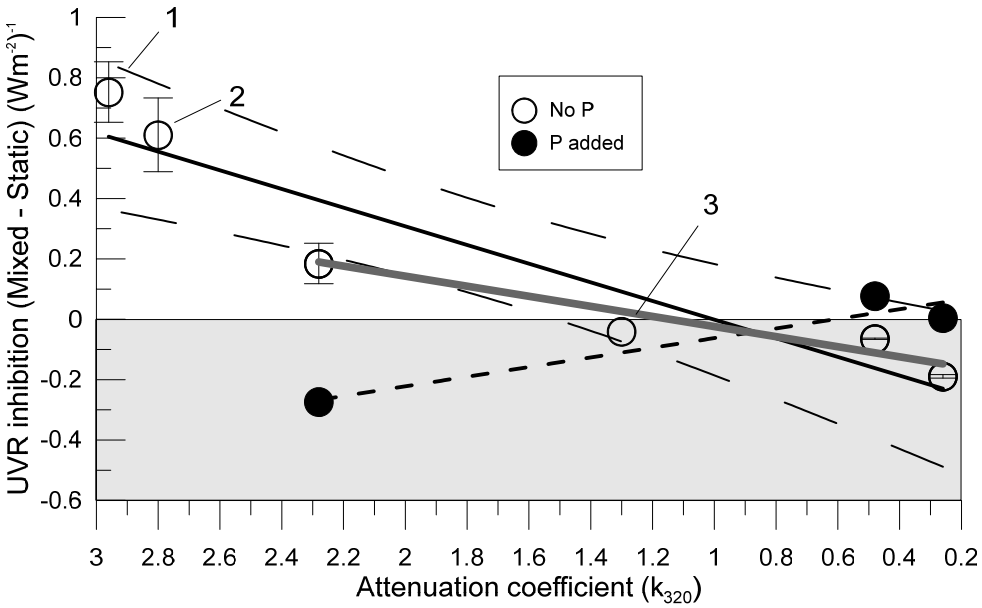
Point 3 (Point 1 in Fi. 7): (Villafañe et al., 2007). For the calculation we used the mixed condition (Fig. 5 in the Ms. of Villafañe et al.) which was established as having the water column well mixed down to 3 m depth (i.e., the mean depth of the lake, as stated in the Ms., pg. 1261). Under this condition, the UVR inhibition in the static sample was approximately 50% (Fig. 5b). The estimation of the inhibition in the mixing condition (i.e., lakes samples) was obtained from Fig. 5c as follows: There was a decrease in yield (during the period of intense mixing) from the initial value in the morning to the one at noon of ~72%. This decrease was caused by both, UVR and PAR acting together. Since in this study we sampled lake water without separating the effects of UVR and PAR, we used a conservative value of 40% inhibition due to PAR, based on the results of the static incubation; the resulting inhibition due to UVR under mixing was ca. 43 % (i.e. 72% * 0.6). It is clear that the overall inhibition was greater in the static samples than in the mixed ones. However, the important difference was the irradiance received in both conditions: Static samples, incubated at the surface of the lake received a mean value of ca. 150 W m^{-2} (stated in page 1261 of the Ms.) whereas the mixed samples received much less (mean of ca 40 W m^{-2}) as they were mixing down to 3 m depth. In order to account for these differences in irradiance we plotted in our Fig. 7 the values normalized by their respective irradiance received, this is why the value in Fig 7 is positive (0.7), reflecting the higher inhibition per unit energy in mixed samples. There was no change of this point in Fig 7.

We explained these points and calculations in the revised version of the text, and we included in separate ways the trend of our three studied lakes and all the points together to show the reader that either way the data support our conclusions. The revised Figure 7 is shown below the next comment.

A second conclusion taken from Fig 7 is that "increased EOC values were also observed in opaque lakes under mixing conditions, and they decreased towards clear lakes" (9805-27), but this interpretation also does not seem consistent with the data presented, indeed the data seem to suggest the opposite conclusion – EOC was less under mixing. It is not stated specifically, but the implied calculation seems to be EOC(Mixed, UVR) – EOC(static, UVR), also unstated whether EOC amount or percentage was used (the latter seems more appropriate). Either way, results in Fig. 6 would not suggest a strongly positive point for LE (Kd320=2.28) shown in Fig. 7. By either measure, EOC(Mixed, UVR) is less than EOC (Static, UVR) in LE, thus the point would be expected to be negative. For LY, text (9805-2) also states "samples under mixing conditions had lower amounts of EOC than in the static ones" consistent with Fig. 6 b but Fig 7

shows a positive value for EOC(Mixed –Static). For LE, EOC was (slightly) greater in mixed than static for amount but no significant difference for percent, but a negative value is shown. Finally, a EOC (Mixed-Static) value is plotted citing Kohler et al. 2001, but they measured EOC only in mixed incubations so I don't see any way to calculate a difference in EOC between mixed vs static.

Author's response: We agree with Dr. Neale's comments as a mistake slipped out in the original Figure. In addition, in our original calculation we determined the percentage difference at mixed and static samples between UVR and PAR exposed samples based on EOC amounts taking the PAR treatment as control. We realize now that this was confusing and we agree that the use of percentage EOC is more appropriate. For each nutrient condition we determined the difference of the EOC percentage in the UVR minus the PAR treatments (i.e., $\Delta EOC_{UVR} = EOC\%_{UVR} - EOC\%_{PAR}$). So we have four values for each lake corresponding to the two nutrient conditions and the mixed and static condition. Then we determined for each nutrient condition the differences of ΔEOC_{UVR} of mixed minus static conditions normalized by PAR (i.e., $EOC_{(m-s)}$ / PAR = $(\Delta EOC_{UVRmixed}-\Delta EOC_{UVRstatic})$ / PAR). There was a slight or no trend in our EOC% data, so we decided to remove the EOC data from Fig 7, softened our statements in the text and placed a question mark in Fig 8, in the left panel for EOC as we originally did in the right panel. Regarding the EOC of Köhler et al., 2001, as mentioned above we interpreted from their Ms. that EOC was due to mixing. So the new Fig 7 looks like this:



Based on these issues, it seems premature to consider the conceptual associations shown in Fig. 8 as something that applies generally to lakes, particularly the indicated directional changes in inhibition and EOC release with increased (no P) or decreased (+P) transparency.

Author's response: We responded to each point raised by Dr. Neale, and we explained how the calculations were made (see above). In addition we show that the relationships and directional

changes for inhibition are correct and we changed and justified the matter of EOC release. Provided these responses / considerations, we trust that the conceptual model (Fig. 8) is valid.

Technical Comments:
Abstract: line 1: Wrong tense: change "had" to "has" Line 11: Correct preposition: change "associated to global change" to "associated with global change" Line 22-23 & 25: Use singular: change "Nutrients" to "Nutrient" Text (pg-line): 9793-5: Wrong tense: change "had" to "has" 9793-9: Use singular: "others" to "other" 9793-17: Redundant phrase: change " nutrients would be used-up and depleted" to "nutrients would be depleted" 9794-16: Change "the raise of temperature" to "periods of elevated temperature" 9794-17: Awkward: instead of "traits" how about "events"? 9794-18: Missing word or typo: "from of terrigenous material"

Author's response: All changed as suggested.

9795-2: Verb case: change "deserve" to "deserves" 9795-3: change "pulsed" to "pulsing"?

Author's response: Changed as suggested.

9797-9: Use singular: change "Nutrients" to "Nutrient"

Author's response: Changed as suggested.

9798-25: "The mean PAR irradiance within the epilimnion was calculated as: " Two of the lakes (LC and LY) did not have a well-defined epilimnion at the time of sampling. Clarify that what is actually being calculated is the mean PAR irradiance in the upper 3 m, the depth of operation of the circulation device. However, note that the average irradiance experienced by samples in the mixed (rotated) incubation may be somewhat different than that calculated with this formula because with a sinusoidal transport rate residence time is not equal at all depths (see discussion in Köhler et al. 2001).

Author's response: We clarified this point about the depth in the revised version. Regarding the use of the equation for the calculation of mean irradiance, and, as Dr. Neale said, Köhler et al. (2001) mentioned that they had a circular path and thus they calculated a sinusoidal transport ("lower vertical speeds at the upper and lower ends"). In our case, the transport was linear and vertical (not circular) via a rope and we did measure similar speeds at various depth intervals, moving 1 m in 4 minutes. It was confusing as by mistake we did state that we performed a "sinusoidal transport", as we did in a different set of experiments already published (van de Poll et al, 2010, Phycologia, 49: 249-259). In this revised version we clarified this point and we mentioned that the rate of mixing was constant.

9799-22: " 3 µmWhatman GF/D filters (25 mm diameter) and then through 0.7 µmWhatman GF/F filters (25 mm diameter)" It should be kept in mind that the minimum size of retained particles on glass fiber filters (in contrast with Nucleopore) is only nominal and depends on such factors as particle shape and filter loading.

Author's response: We agree in this point and we compared our work with previous studies done by the group of Dr. Carrillo (using Nucleopore filters) and we found no differences among both types of filters.

9800-10-11: " Because the time between sampling and the saturating light pulse was in the order of a few seconds, the photochemical effective quantum yield of PSII (Y) in the light was determined (Maxwell and Johnson, 2000)." Based on the description given in 9798-13-15, a 4m, 5 mm ID, tube connecting the PAM fluorometer and the sample bag would contain a volume of 78 mL, resulting in about 19 s dark time before yield measurement at 250 mL min-1. Even a few seconds of dark are sufficient to completely reverse photochemical quenching, so it is incorrect to describe the measurement as effective photochemical quantum yield. A better description is the maximum or intrinsic photochemical efficiency of PSII which primarily reflects the effect ofnon-photochemical quenching.

Author's response: Agreed. We changed to intrinsic photochemical efficiency.

9804-26-27: It appears that %EOC has been calculated as the ratio EOC production to POC incorporation. The calculation of %EOC is different from other reports in the literature (c.f. Obernosterer&Herndl, 1995), it is more usual to calculate %EOC release as 100 x EOC/(EOC+POC).

Author's response: We originally calculated the % EOC as EOC / (EOC+POC) * 100, and we added in M&M how %EOC was calculated in the revised version.

9807-5-8: The authors suggest that the static incubation in LE may have been lightlimited based on the carbon fixation results. But based on the stated light transmissiondata, a static incubation at 1.3 m in LE would get about 64% of surface irradiancesimilar to the average irradiance in the circulated incubation (63%). For the latter,Fig. 3 shows that mean irradiance was around 850 umol photons m-2 s-1 on thecloudy day and a similar mean irradiance would be expected to occur at 1.3 m. Do theauthors really think that photosynthesis can be light limited at such a high irradiance?Moreover, based on the Kd320 of 2.28 m-1 (and figuring a Kd360 about half of that?),an incubation at 1.3 m is only getting around 5-10% of surface UV – a very minorsupplement in photon terms.

Author's response: We agree with the reviewer and, in fact, we were referring to a comparison between lakes mostly based on UVR attenuation. However, the wording in our original text was not correct, so we rephrased this part to accommodate the comment.

9807-27 "however, the opposite would occur in clear lakes, provided that the irradianceconditions at the water surface are similar."
Not clear what is the contrasting result in clear lakes that leads the authors to use theword "opposite".

Author's response: We were comparing clear and opaque epilimnion of equal depth and equal surface irradiance and then making the case that in opaque ones irradiance would be low as

compared to the clear ones, so phytoplankton would be acclimated to different conditions. We rephrased this part to clarify this point.

9809-3 typo: change "addtion" to "addition"

Author's response: Changed as suggested.

Fig. 4 vs Fig. 5. Considering that the values in Fig. 5 are calculated by difference fromthose in Fig. 4, the size of the error bars between the two figures seem inconsistent. Ingeneral, the variance of a difference (Inhibition) combines the variance of values beingsubtracted (UVR and PAR production), but errors bars in Fig. 5 are very small relativeto those in Fig. 4 (they should be larger). I recommend using propagation of errors tocalculate the variance of % Inhibition (see supplemented pdf).

Author's response: We agree with Dr. Neale and we used propagation errors to calculate the variance as suggested.