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Discussions

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Interactive comment on "Biologically labile photoproducts from riverine non-labile dissolved organic carbon in the coastal waters" by V. Kasurinen et al.

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Interactive comment on "Biologically labile photoproducts from riverine non-labile dissolved organic carbon in the coastal waters" by V. Kasurinen et al.

A: We are grateful to the anonymous referees for their comments on our manuscript. Below we provide a detailed response to each comment and clarify both major and minor concerns of the reviewers. In our opinion, the comments of reviewers are not able to show any major weakness in our study. The comments of the reviewer, however, allow us to improve the original manuscript into a much clearer and focused revised version.

The major concern of the first reviewer was the method used for the determination of spectral apparent quantum yields (AQYs). The methodological description in the original manuscript was not detailed enough to express all technical details involved in the determination of AQYs. For the revised manuscript we shall be more specific and can provide these methodological details e.g., as a separate supplementary material.

The second reviewer was primarily concerned about the integrity of water samples during the transport and storage prior to the experiments performed. In the revised version of manuscript, we wish to emphasize that the photochemical transformation of terrestrial DOM (tDOM) takes place primarily offshore after a considerable transportation and modification of tDOM (Medeiros et al. 2015). During this transport the biological communities as well as the properties of tDOM change. The transport and storage of water samples was a part of our study design to change the properties of the original samples into those, which are representative for the majority of tDOM experiencing photochemical transformation in the coastal waters.

Both reviewers were concerned that the Congo River sample is driving the coefficient of determination (R^2) and the values of slope in the statistical analysis., We used additional statistical methods with and without the Conge River To show that our results are valid even if the Congo River sample is deleted from the data. In the following we reply to each question pointed out by referees.

Anonymous Referee 1 Received and published: 21 July 2015

Response to Anonymous Referee 1

R1: In order to produce accurate AQY estimates, we need correct information for both the numerator and the denominator of the AQY (Equation 1). Until molecular level information becomes available that identify all the specific absorbing compounds responsible for specific photoproducts, we remain limited by using overall solution absorption coefficients to determine the rate of photon absorption, the denominator, for all products. However, this still means that accurate quantification of absorbed photon

doses within sample containers is critical for any AQY experiment. The authors here have irradiated their samples in quartz flasks in a water bath, with dark control flasks wrapped in tin foil in the same water bath.

First, I am not sure the best method for quantifying absorbed photon doses in a sphere. The authors only briefly mention that this was done following the methods of Aarnos et al. (2012). In the Aarnos et al. paper, the authors only describe how they determined CDOM absorption coefficients (which is standard) and say that absorbed photons was quantified following equations in supplementary material (text S1 in Aarnos et al.). However, when I tried to look at the supplementary material for Aarnos et al. (2012), I could not find this information. The text S1 referenced in the Aarnos et al. paper gives a table with measured and modeled photomineralization rates of DOC.

A: We apologize that the supplementary material is not available in the web page of Journal of Geophysical Research (JGR). The supplementary material submitted to JGR is provided in a separate attachment of the present reply. If needed, we are willing to modify the supplementary material of Aarnos et al. 2012 to match the details of present study.

R1: This tells me nothing about how they calculated photon absorption rates. Even if the math the authors use to calculate absorb photons is correct, I am still worried that they are missing elements critical to this calculation. Since this is not discussed, I can only guess at things that were/were not considered in this study.

A: The Aarnos et al. 2012 supplement will tell the details requested by the reviewer #1.

R1: For example, having their darks in the water bath during irradiations means they are adding a hard reflector (tin foil) to the system, which will certainly change photon doses when compared with their absence. Were darks placed in the same spot every time? Were darks present during the light measurements that were used to calculate absorbed photon doses?

A: The reflection by the dark controls was accounted for in our irradiation measurements. The measurements of irradiation were done upon those conditions used for the irradiation of samples. The dark controls were always placed in the same spot.

R1: Were light measurements performed above or in the water bath and at different locations?

A: Light measurements quantified the photon flux density incident to samples from above and below. These measurements were done in water by taking the cosine collector through an opening in the wall of water bath. The measurements were done at one location.

R1: Having worked with CPS solar simulators, the light is not entirely uniform throughout and this needs to be accounted for.

A: Lam et al. (2003) reported that the light field of Suntest CPS+ varied <1% among different sites of irradiation chamber. In our opinion, this variation is so small that it does not need to be accounted for. However, we avoided irradiating our samples close to the walls to avoid possibly biased irradiation at these sites.

R1: Absorbed photon calculations must include accurate path length information, but when light hits a curved surface it will bend and focus the light, so what is the pathl ength inside a quartz sphere? I am hoping the authors can tell me in the next iteration so I can learn something. And, thiswill certainly be affected by the differences in refractive indices between fresh water (the water bath) and their salty samples (salinity in this study 15). It might be small but may also artificially increase the pathlength and I am not sure how the authors account for this. And finally, maybe because I am not familiar with these calculations, I would love to see something to verify these photon dose calculations are correct (i.e. actinometry).

A: The method of path length calculation is given in the Aarnos et al. 2012 Supplement. The light focusing should not be an issue as the samples were kept in the water bath

like in our study (Jankowski et al. 2000). Aarnos et al. 2012 supplements will tell the details of photon dose measurements and calculations. Actinometry is not viable option in the present study, where the spectral information of absorbed photons is essential. Instead of actinometry, we have validated our results with the experiments done in situ and with monochromatic irradiations (Vähätalo et al. 2000, Vähätalo and Wetzel 2004; Aarnos et al. 2012).

R1: My second major issue is with the AQY calculation itself. To me, apparent quantum yields as reported here should reflect spectral information. This can either be done with monochromatic (irradiate at single wavelengths throughout the solar spectrum) or polychromatic (irradiate with a variety of polychromatic light fields with various long bandpass cutoff filters throughout the solar spectrum) methods. Here, the authors have chosen polychromatic light, but used only ONE light field. In this way ALL spectral information is lost. It is fine to divide their BP or BR rates by absorbed photons (assuming it is correct), but I am opposed to them calling this a spectral AQY. The authors should call it a broadband (e.g. Fichot and Benner 2014) AQY or a pseudo-AQY or anything but a spectral AQY. I have such a strong objection to this because it is very misleading to the community; especially to research groups that are not familiar with AQY determinations and blindly follow a published method that includes only one light treatment (and likely less time required to do the experiment). Furthermore, since there is only one light treatment, this means there is only one point the authors can use to model the AQY spectrum (see common methods for polychromatic determinations, e.g. Johannessen and Miller, 2001; Koehler et al., 2014; Zhang et al., 2006). Mathematically you can't define an exponential line using one data point. Or even two data points. Here the authors model one point with exponential Equation 2 and solve for TWO variables! How is this at all constrained? It doesn't matter that these model equations give BP/BR rates that are in agreement with what has been measured in previous studies, it is just plain wrong. The authors should therefore either do more light treatments (giving multiple points for an exponential equation) or drop this equation entirely. Since I am assuming the authors are not going to be able to repeat these

experiments, it will probably have to be the latter option.

A: The Aarnos et al. 2012 supplementary material explains in detail how the AQYs were calculated. Our method for the calculation of AQYs was originally published in Vähätalo et al. 2000. This method is different from any of the options suggested by the reviewer #1. Our method uses polychromatic irradiation. In the present study, the number of absorbed photons is quantified at 461 different wavelengths. Our method like e.g., that used by Johannessen and Miller, 2001; Koehler et al., 2014; Zhang et al., 2006 assumes that spectral dependence of AQY follows exponential equation specified by two variables. Mathematically our method iterates the values of two unknown variables based on spectral information from 461 different wavelengths. For this method one irradiation with polychromatic light is enough. Our method has validated with monochromatic irradiations (Vähätalo et al. 2000) and several in situ experiments (Vähätalo et al. 2000, 2002, Vähätalo Wetzel 2004; Aarnos et al. 2012). The method has been used additionally by Vähätalo and Zepp 2005; and Vähätalo et al. 2011. All these studies and the present study report spectral AQYs.

DETAILED COMMENTS:

Abstract

R1: Lines 11&15: please drop 'spectral' and call it 'broadband' or something else (as stated above), and change numbers to reflect this.

A: Our method determines spectral AQYs and therefore the expression used in the manuscript is correct.

R1: Intro Line 22: "BLPs are linearly related to CDOM photobleaching." Is this true everywhere, all the time?

A: The linear relationship between the amount of BLPs and photobleaching has been found in the five studies cited in this sentence. When we checked the cited studies again, we noticed that Brinkmann et al., 2003 actually reports the linear relationship

Table 1. The relationship between the production of BLPs and the photobleaching of CDOM

DOM	BLP	CDOM*****	n	fit	r2	slope	r
various sources (***)	Formaldehyde	300	18	linear	0.99	1400	1
various sources (***)	Acetaldehyde	300	18	linear	0.99	1100	1
various sources (***)	Glyoxylate	300	18	linear	0.96	2000	1
humic substances (****)	Carbon monoxide	350	8	linear	>0.9	562	2
coastal seawater	Carbon monoxide	350	7	linear	> 0.9**	665	2
from 38 lakes	sum of four carboxyl acids	250	38	linear	0.83	nr	3
from 38 lakes	sum of four carboxyl acids	365	38	linear	0.67	nr	3
Adriatic Sea	bacterial growth on BLPs*	365	7	linear	>0.85**	nr	4

*enhancement factor. **visual estimate from a plot as the values were not reported, ***wetland, river, coastal and offshore waters, ****isolated from a saltmarsh in coastal seawater, *****wavelength of CDOM photobleaching, 1# Kieber et al. 1990, 2# Miller and Moran 1997, 3# Bertilsson and Tranvik 2000, 4# Obernosterer and Herndl 2000 nr = not reported

between the production of BLPs and the amount of absorbed energy. In a related study, the absorbed energy is linearly related to photobleaching (Bertilsson and Tranvik 2000). Thus, the claim in our discussion paper seems to hold also for Brinkmann et al. 2003. However, we plan to drop out Brinkmann et al., 2003 from the revised manuscript as it does directly support our claim as the authors did not measure photobleaching. We cannot claim this is true everywhere all the time, but this relationship has been found in all earlier publications, which have measured simultaneously the photoproduction of BLPs and the photobleaching of CDOM in surface waters (e.g., Kieber et al. 1990; Miller and Moran, 1997; Bertilsson and Tranvik, 2000; Obernosterer and Herndl, 2000). These samples examined in earlier studies include DOM from 38 lakes, a wetland, a river, salt march, humic isolates, coastal water, and offshore water.

A: We intentionally left out the wavelength, because photobleaching has been and can be measured at several different wavelengths. There is not any standardized wavelength for the quantification of photobleaching. Nevertheless, the photobleaching at

one wavelength will correlate with the photobleaching at another wavelength.

R1: Modeling fading isn't trivial either.

A: We agree with the reviewer. It is easy to measure photobleaching in a laboratory experiments like in the present study, but also in experiments carried out in situ (Nelson et al. 1998; Vähätalo et al. 2002, Vähätalo and Wetzel 2004). Photobleaching can be also modelled (e.g., Whitehead et al. 2000, Vähätalo and Wetzel 2004; Miller and Moran 2002), but this modelling is not trivial.

R1: Page 8202 lines 3-5: How is the light-dark difference to determine BLPs different from other studies? I thought this was common practice. Or has no one determined BGE for BLPs before?

A: Several earlier studies have determined BGEs separately in the irradiated samples or in the non-irradiated dark control samples (reviewed by Abboudi et al. 2008). However, according to our knowledge, BGE for BLPs (the difference between irradiated and dark control) has not been previously reported

R1: 8202 line 20: How do you get a unit of Mt C for CDOM? CDOM is an optical property with units of m-1; it does not mean carbon. Is there some sort of relationship used to get to [DOC]? If so, say this.

A: Thank you for pointing out a unit error. The correct unit is $m^{-2}\,yr^{-1}$ as explained in the methodology section (page 8202 lines 25-27). We will correct this to next version.

R1: Materials/methods 2.1 Materials a) The use of detergent (a carbon source surely) for bottle cleaning worries me. How did you verify that all detergent was removed? Could this change or alter DOC/CDOM concentrations? Maybe I'm just being paranoid but, any left over could be a carbon source for these microbial communities and would seriously alter the results of this study.

A: We share the same paranoia of contamination with the reviewer. Therefore, our cleaning protocol of vessels was meticulous. In the early part of protocol, the vessels

were washed with our laboratory dishwasher. The washing program of dishwasher includes a use of detergent (deconex 21 lig, a mildly alkaline special cleaning concentrate, Borer Chemie AG, Switzerland) to remove potential organic and inorganic residues, which might had not dissolved to water alone. Later, the washing program of dishwasher includes several rinses with water purified with reverse osmosis (RIOS). After the vessels were washed with the laboratory dishwasher, the washed vessels were soaked in 7% HCl for > 1 day. This procedure exchanged any cations (or anions) attached to the surface of glass or quartz. The HCl was removed from the vessels by rinsing them copious (> 6) times with the cleanest waver available (ion-exchanged water produced by MilliQ water purification system). The rinsed glass and quartz vessels were combusted (> 2 h) in +450 C. This step removes remaining organic compounds. We conclude that after our cleaning protocol a possibility for contamination was negligible, the concentrations of DOC/CDOM were unaltered and bacterial communities grew on DOM originating from our river water samples.

R1: b) Artificial seawater preparation. Although the formula from Kester et al. (1967) was followed to make artificial seawater, there are no details on how (or if) these salts were cleaned. Commercially available salts can be very dirty (contaminated with organic carbon and trace metals) and should at least be baked in a muffle furnace (e.g. Nelson et al., 2007) to lower carbon contamination here. If this was not done it should at least be noted since it could affect both their light and dark results.

A: We considered baking the salts in a muffle furnace but declined this step, because the high temperature of muffle furnace may potentially transform salts or cause selective losses in mass. In order to avoid contamination, we purchased the purest form of salts available (pro analysis grade) for the preparation of artificial seawater. We carried out an experiment to test possible organic contamination in the salts. In this experiment, the St. Lawrence water sample was filtered and used as a carbon source as such or diluted 1:1 to artificial seawater. After the irradiation experiment, bacterial biomass based on BLPs (measured as described in the Methods) in the diluted sam-

ple was about half of that in the undiluted sample. This result suggests that artificial seawater did not contain any additional bioavailable carbon to bacteria.

R1: 2.2 Experimental 2.2.2 Please see general comment above for major issues with this section R1: 8204 line 21: How was this irradiation time selected?

A: The irradiation time was selected to photobleach ca. 50% of CDOM. The selected irradiation time thus represents the median of photochemical transformation for riverine DOM in the coastal waters. In our opinion, the selected irradiation time is the best to describe the majority of photoreactive tDOM. Additionally, the selected irradiation time produced BLPs in such amounts, which we were able to quantify reliably with the methods used.

R1: I'd like to see a comparison to a 'real world' photon flux.

A: The 'real world' comparison was omitted from the Methods of this study, because the samples were collected from different latitudes having seasonally variable photon fluxes. It is hard to give a single simple comparison applicable to all sampling locations. However, ca. 4 h irradiation with our solar simulator corresponded to a day dose of ultraviolet radiation during summer in the Baltic Sea as explained in our earlier publication (Vähätalo and Zepp 2004). If needed we can also convert the used photo flux into an average planetary value (independent of season and latitudes) as we have done in our earlier publication (Vaalgamaa et al. 2011).

R1: And does this group have any information on how BR/BP changes with different photon doses?

A: In this study, we used only one irradiation time as explained in the Methods. In earlier studies, e.g., BP has increased nearly linearly along with the irradiation time (Wetzel et al. 1995, Farjalla et al 2001).

R1: 44-46 hours in a Suntest (causing 50% CDOM fading at 300 nm here), must be a rather large photon flux.

A: The method section of present study expresses precisely how large the photon flux was. Our irradiation power was 765 Wm^{-2}, which is 765 $Js^{-1}m^{-2}$. The irradiation time of 44 h equals 33660 seconds. The dose is power times the irradiation time, which is 25 MJm^{-2}. We wish to point out that tDOM exposes to extensive solar radiation when it arrives to coastal waters. These doses are higher than used in this study and eventually cause complete fading of tCDOM if it stays in the mixing layer of ocean (see a recent review by Nelson and Siegel 2013).

R1: In another study with similar irradiation conditions (round quartz flasks, sealed with no head space, irradiated with a 1000 W Oriel xenon lamp; Xie et al., 2004), CDOM absorption also decreased by 50-55% in two river samples from the Southeastern USA after 46 h of irradiation. On the other hand, oxygen concentrations also decreased by 86% in both rivers, but exponentially. This was reflected in the rates of DIC photoproduction in this study, which were faster during the early (e.g. $2 - 4$ h) stages of the experiment.

A: We are well aware about the excellent study by Xie et al. (2004). In that study, the 86% photochemical oxygen consumption was involved in the photochemical transformation of DOM from Altahama and Satilla rivers high in DOC and iron. In these rivers, iron causes photochemical consumption of oxygen (see Gao and Zepp 1998). Our samples were mixed (1:1) with artificial seawater and filtered to mimic precipitation of iron in estuaries. Therefore, the photochemical consumption of O_2 by iron was minimal in our samples. The 1:1 dilution of our samples with artificial seawater decreased also the concentration of DOC to a half of that in the original river water samples. Thus, the study by Xie et al. (2004) suggests that photochemical oxygen consumption in our samples was <43%. Thus, at least more than half of the initial oxygen was present in the sample in the end of irradiation.

R1: For a "true" AQY calculation, initial rates are a requirement, but after 46 h, the rate of photoproduction can be significantly slower than at the beginning of the experiment, even when correcting for CDOM photobleaching (Xie et al., 2004), rates of DIC pro-

duction normalized to average ag(320) were 0.29 M m h-1 for the first 4 h and 0.14 M m h-1 after 46 h). This point is at least worthy of discussion so other researchers consider these points in their experiments because BLP production rates may not be the same over time.

A: We fully agree with the reviewer: photochemical reactivity of DOM decreases along with irradiation time. The decrease is most rapid in the early phase of irradiation in particular for "fresh" samples without previous exposure to solar radiation (discussed in detail in Vähätalo and Wetzel 2004). For DOM with previous exposure to solar radiation (like our samples from large rivers), the photochemical reactivity decreases only slowly along with irradiation time (Vähätalo and Wetzel 2004). Much of the photochemical transformation of riverine DOM in coastal waters takes place after DOM is transported across estuaries and turbidity maximum to optically transparent coastal waters (Medeiros et al. 2015). Our aim is this study is to provide AQYs applicable for the bulk riverine DOM not only for the small most reactive part of DOM. Therefore short irradiations suggested by the reviewer would provide too high AQY to be representative for bulk riverine DOM. We selected to photobleach ca. 50% of photoreactive DOM (i.e., CDOM) in our irradiation to get AQY representative for bulk riverine DOM. Our AQYs are as "true" as those obtained with shorter irradiation times, because AQYs can be calculated to any time point along the photochemical transformation of DOM. In this case, however, the photobleaching of CDOM needs to be accounted for as done in the present study (Aarnos et al. 2012 Supplement).

R1: Reader and Miller (2014) do a nice job showing and discussing the effects of different irradiation times on the photoproduction of BLPs, and it is pretty clear that some BLPs are both biolabile and photolabile, and selection of photon dose is critical to interpreting the results.

A: We agree with the reviewer: photochemistry can degrade BLPs, which are both biolabile and photolabile (Bertilsson and Tranvik 1998). However, we wish to point out that photochemical reactions can also transform bioavailable DOM into biologically recalci-

trant form (Kieber et al. 1997), and this process can explain at least a part of observations done by Reader and Miller (2014). The observations by Reader and Miller (2014) may not be applicable to our DOM without bioavailable DOM. In Reader and Miller (2014), some irradiations of DOM had negative effect on bacterioplankton, while our irradiations had always positive effects on bacterioplankton. The difference can be explained by the age of DOM. Reader and Miller (2014) irradiated freshly collected DOM including the most bioavailable fraction of DOM. If the bioavailability of DOM is already high in the beginning of irradiation, experimental irradiations reduce the bioavailability of DOM (Obernosterer et al. 1999„ Mopper and Kieber 2002). In this case, photochemical reactions can transform bioavailable DOM into less bioavailable humic-like substances (Kieber et al. 1997). Irradiation of these humic-like substances will produce BLPs later. Thus, the irradiation of bioavailable DOM includes two sequential events with contrasting effects on bioavailability: #1) photochemical transformation of bioavailable DOM into biologically recalcitrant but photo-labile humic-like DOM, and #2) photochemical transformation of biologically recalcitrant photo-labile humic-like DOM into BLPs. Upon irradiation of bioavailable DOM, low dose of photos may first decrease the bioavailability of DOM, while high doses of photons may increase the bioavailability of DOM. Although experimental irradiations can transform bioavailable DOM into biologically recalcitrant forms (sequential event #1 above), we argue that environmental importance of this process (event #1) is relatively low. In surface waters, microbes assimilate effectively bioavailable DOM, reduce its life time and possibility for photochemical transformation. In contrast, the lifetime of biologically recalcitrant DOM is long and its photochemical transformation into BLPs (event #2) makes an environmentally important sink for riverine DOM in coastal waters. Our study focuses on the event 2 alone. Our experimental design included a pre-treatment for the removal of bioavailable DOM, so that irradiations concerned biologically recalcitrant but photochemically labile forms of riverine DOM. When this type of DOM is irradiated, photochemical transformations produce BLPs and have positive effect on bacterioplankton (as explained in many reviews and observed in this study). We cannot exclude a possibility that some

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of our BLPs would have photochemically transformed further into other forms. However, the majority of photochemical transformations must have concerned biologically recalcitrant but photolabile DOM, because only ca. 50% of initial CDOM was photochemical transformed. In our study, bioavailable DOM was absent and the production of BLPs was expected to be more or less linearly dependent on the amount of absorbed photons.

R1: Section 2.2.6

Why stop at 12 d if max biomass has not been reached?

A: The aim of bioassays was to determine the response of bacteria to BLPs AND determine the BGE on BLPs at the same time. A few studies have determined BGE on irradiated DOM (Farjalla et al. 2001; Anesio et al. 2000; Amado et al. 2006; McCallister et al. 2005; Pullin et al. 2004; Reche et al. 1998; Smith Benner 2005; Anesio et al. 2005; Abboudi et al. 2008; Vähätalo et al. 2003). In all previous studies, the responses of bacteria and BGE have been determined at fixed times, which have been ≤ 8 days (see Table below). We designed the length of our bioassays according to the previous studies and extended the length of bioassays up to 12 days assuming that the peak biomass will be reached earlier like have been observed or assumed in earlier studies (Aarnos et al. 2012; Farjalla et al. 2001; Anesio et al. 2000; Amado et al. 2006; McCallister et al. 2005; Pullin et al. 2004; Reche et al. 1998; Smith and Benner 2005; Anesio et al. 2005; Abboudi et al. 2008; Vähätalo et al. 2003). In contrast to earlier studies using fixed times, we made a large effort to find out the maximum biomass of each bacterial community growing on irradiated DOM. In our bioassays, the bacterial communities typically followed a growth dynamics of a single bacterial population in a batch culture including a lag-phase and a clear maximum biomass prior to the end of bioassays (≤ 12days). In two exceptions, the maximum biomass was found in the last day of bioassay on 12 d. We cannot exclude a possibility that the maximum biomass would have been reached after 12 d or that the biomass maximum was on day 12. Nevertheless in these cases, a fixed time (= 12 d) makes our study comparable to an

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Table 2. Comparison of times (days) selected for the determinations of BGE

BGE determination*		Reference
Fixed time	Following maximum biomass of bacteria	
4		Farjalla et al. 2001
3		Anesio et al. 2000
4		Amado et al. 2006
7		McCallister et al. 2005
7		Pullin et al. 2004
7.1 or 7.8		Reche et al. 1998
4		Smith and Benner 2005
1.8		Anesio et al. 2005
1.5		Abboudi et al. 2008
4		Vähätalo et al. 2004
-	From 5 to 12	This study

approach used in all previous studies.

R1: After 46 h of irradiation, I can imagine that there is a significant buildup of H2O2 in all irradiated solutions. Microbes will have to devote some of their metabolic energy at the beginning of the bioassays to deal with external H2O2 stress (perhaps reflected in the low respiration at the beginning of the BR curves, fig. 3), enzymes which coincidently give O2 back. Could you be underestimating BR if the bioassay was stopped early in solutions with significant oxidative stress (i.e. the St. Lawrence and the Mekong)?

A: Irradiation indeed will produce H_2O_2, which have been interpreted as the most important factor responsible for negative responses of bacteria in early part of bioassays in this and many earlier studies. In the present study, the lag-phase was more prominent in the irradiated than non-irradiated (dark control) samples. The prolonged lag-phase in the irradiated samples may be explained at least in a part by photochemically produced H_2O_2. Microbes use enzymes such as catalase to remove H_2O_2. It is hard to evaluate

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in which extent a potential oxidative stress by H_2O_2 changes BGE. However, it is likely that microbes removed H_2O_2 in the early part of bioassay when their biomass was low, and the major increase of biomass up to two orders took place after the lag-phase. As the bacteria grew primarily after the lag-phase and after the expected depletion of H_2O_2, the impact of H_2O_2 on BR was likely low. Although our irradiation times were long, our irradiated samples had low concentration of iron, because the river waters received artificial seawater and possible precipitates of Fe were removed by filtration. Therefore, the photochemical formation of H_2O_2 in this study may have been smaller in earlier determinations done in freshwaters including relatively high concentrations of iron, an important sensitizer for photochemical production of H_2O_2. To best of our knowledge, all earlier determinations of BGEs on irradiated DOM have involved a similar potential oxidative stress by H_2O_2 like our study, because no attempts for removal H_2O_2 have done in the present or earlier studies (Farjalla et al. 2001; Anesio et al. 2000; Amado et al. 2006; McCallister et al. 2005; Pullin et al. 2004; Reche et al. 1998; Smith and Benner 2005; Anesio et al. 2005; Abboudi et al. 2008; Vähätalo et al. 2003).

R1: Section 2.4

Again please call "broadband" or something similar and lose the model, as stated in the general comment above.

A: Spectral AQY is the correct expression in this study.

R1: Section 2.5 Please add an equation to describe the Q calculation. At first it sounds like a product of solar radiation and CDOM in the water column but then (starting line 6 pg. 8208) is sounds like you're multiplying solar ration by global radiation? Please clarify.

A: The method explains "Q_λ was calculated as the product of a standard solar radiation spectrum (ASTM G173-03; Chu and Liu, 2009) normalized with global radiation and the annual mean global radiation determined for the area of each river plume examined."

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Q_λ for each river (hereafter $Q_{\lambda,river}$) can be also expressed as a product of two parameters:

$$Q_{\lambda,river} = Q_\lambda(normalized\ to\ GR)GR_{river}\ (1)$$

where $Q_{\lambda,river}$ is the spectral photon flux density absorbed to the water column in the front of each studied river (mol photons $m^{-2}\,nm^{-1}\,s^{-1}$ at spectral range from 290 nm to 750 nm); Q_λ (normalized to GR) is a standard solar radiation spectrum ASTM G173-03 normalized with global radiation (mol photons $m^{-2}\,s^{-1}\,nm^{-1}$/ $W\,m^{-2}$ GR); and GR_{river} is the annual mean global radiation in the coastal ocean in the front of each studied river ($W\,m^{-2}$).

GR_{river} refers to the annual mean downward shortwave radiation ($W\,m^{-2}$; reported in Hatzianastassiou et al. 2005) (Fig. 1 shown in the end of reply). The annual mean downward shortwave radiation was derived from monthly values over a 16 year period (Hatzianastassiou et al. 2005). Although the values can be derived from a publication (Hatzianastassiou et al. 2005), Nikos Hatzianastassiou kindly provided the values (through a personal communication) for the estimated plume areas of each river examined in this study.

Figure 1 caption: Mean monthly shortwave radiation (n = 16; years 1984-2000) for the plume areas of examined rivers provided by Dr. Nikos Hatzianastassiou (Laboratory of Meteorology, Department of Physics, University of Ioannina, Greece).

GR_{river} values express the total energy of shortwave solar radiation incident to the plume area of each river. The shortwave radiation is also called global radiation and it refers a solar spectrum integrated over the wavelengths from 290 nm to ca. 4000 nm. The calculations of photochemical rates require spectrally resolved solar radiation, and therefore we assumed that the spectral shape of solar radiation is the same as in the

ASTM G173-03 standard spectrally resolved shortwave solar radiation spectrum (Chu and Liu, 2009). The spectrally resolved ASTM G173-03 ($W\,m^{-2}\,nm^{-1}$) was converted to photon flux densities (mol $m^{-2}\,s^{-1}\,nm^{-1}$) and divided by the global radiation calculated as the integral over the wavelengths from 290 nm to 4000 nm resulting in Q_λ (normalized to GR).

Figure 2 caption. Q_λ (normalized to GR) i.e., a standard solar radiation spectrum ASTM G173-03 normalized with global radiation (mol photons $m^{-2}\,s-1\,nm^{-1}$ / $W\,m^{-2}$ GR) (Fig. 2 shown in the end of reply).

$Q_{\lambda,river}$ refers to the annual mean values of the spectral photon flux density absorbed to the water column in the front of each studied river (mol photons $m^{-2}\,nm^{-1}\,yr^{-1}$ at spectral range from 290 nm to 750 nm). For calculation of daily rates, $Q\lambda,river$ was multiplied 86400 $s\,d^{-1}$. Annual rates were calculated by multiplying the daily rates by 365 $d\,yr^{-1}$.

R1: 8208 line 6: integrating out to 750 nm is not appropriate because the majority of photochemistry (for all products I know of) comes from wavelengths < 400 nm. Otherwise we'd all get a sunburn indoors. Some groups (e.g. Simon Belanger/ Huixiang Xie) integrate out to 600 nm, but I don't think there's any measureable photochemistry beyond 500 nm. Changing this may lower the results of their BLP photoproduction model, maybe not significantly but it is worth doing.

A: We agree with the reviewer, ultraviolet radiation is primarily responsible for the photochemical production of BLPs (see e.g., the action spectrum shown in Fig. 6F of Aarnos et al. 2012). However, it is hard to define the longest wavelength responsible for a photochemical reaction. The wavelength is likely somewhere between 400 nm and 500 nm like suggested by the reviewer, but no-one can point out a specific wavelength where AQY drops to zero. In our approach, AQY decreases exponentially with increasing wavelength and becomes negligible (but not zero) at the visible part of spectrum. Our spectral fitting determines AQYs primarily based on the absorbed

photons at the ultraviolet region of spectrum, which is responsible for photochemical reactions. The spectral fitting is insensitive to the wavelengths >500 nm, which do not promote photochemistry. For our determination of AQY, the upper end of integration range does not matter. The results of AQYs are practically the same, if the integration is done up to 500 nm, 600 nm or 750 nm (unpublished calculations with different spectral regions). The insensitivity of method to visible part of spectrum is one proof that our fitting method and modeling based on spectral AQYs is correct.

The comment related to sunburn is not relevant here. We do not get sunburn indoors, because the window glass will cut off the ultraviolet part of solar radiation.

Results R1: Section 3.5: Again, I think these results need to be reworked to reflected broadband calculations.

A: This study provides spectral AQYs.

Section 4.4.1 R1: 8213 line 23: Have you also considered that the high r2 value is due to the fact that you have one point with a large delta and a large BR? How does this change without this point?

A: The largest values come from the Congo River sample, where the photobleaching of CDOM and consequently bacterial responses were largest among our samples. We evaluated the effect of Congo River sample on statistics by excluding it from the statistical analysis. For all samples shown in Figure 2, r^2 is 0.88 and p-value < 0.001. Without Congo River sample, r^2 is 0.69 and p-value 0.002 being still statistically highly significant. Our discussion manuscript states that our r^2 is larger than the corresponding one in an earlier study (Bertilsson and Tranvik 2000). This statement holds even without the Congo River sample. Although the largest value in any linear regression has a high leverage, in our regression the Congo River sample was not driving the regression, which remained highly significant even without the Congo sample.

R1: 8214 lines 1-4: This is a dangerous statement. I like that this study uses rivers

from all over the world, but to say that their correlation between BLPs and CDOM loss is a useful BLP proxy is very misleading.

A: The text on page 8214 at lines 1-4 is "this and many other studies (Kieber et al., 1990; Miller and Moran, 1997; Bertilsson and Tranvik, 2000; Obernosterer and Herndl, 2000; Brinkmann et al., 2003) is that the production of BLPs is quantitatively linked the photobleaching of CDOM, which makes it as a useful proxy for the production of BLPs." We believe that the reviewer is worried about the statement: "the production of BLPs is quantitatively linked the photobleaching of CDOM". Thus statement draws a conclusion from the results of present study (e.g, Figure 2) and many earlier published studies (Kieber et al., 1990; Miller and Moran, 1997; Bertilsson and Tranvik, 2000; Obernosterer and Herndl, 2000). All these studies show a relationship between the amount of BLPs and the amount of photobleached CDOM with r^2 up to 0.99. We cannot understand why it is misleading or dangerous to point out the relationship between BLPs and photobleaching found in this and many earlier studies?

R1: This relationship is from 7 samples, and includes one rather high point in figure 2, driving the whole correlation.

A: As described above, the sample from Congo River is not driving the regression alone. If this data point will be left out, the effect regarding the slope based on CDOM photobleaching and BP is minor.

R1: How does this change for other times of year?

The correlation between BLPs and photobleaching of CDOM is a ubiquitous phenomenon independent on the time of year (Kieber et al., 1990; Miller and Moran, 1997; Bertilsson and Tranvik, 2000; Obernosterer and Herndl, 2000; this study).

R1: With more samples?

A: The correlation between BLPs and photobleaching of CDOM holds in this study and also in other studies, where more samples have been analyzed (Kieber et al., 1990;

Miller and Moran, 1997; Bertilsson and Tranvik, 2000; Obernosterer and Herndl, 2000).

R1: With a different irradiation time?

A: The general correlation between BLPs and photobleaching of CDOM should be applicable at all irradiation times, which cause photobleaching (e.g., Kieber et al., 1990).

R1: I think the claim made here needs to be softened quite a bit to reflect the way this relationship could (and certainly does) change.

A: We still feel confident to claim that "the production of BLPs is quantitatively linked the photobleaching of CDOM". However, we do not want to claim that the relationship (i.e., the slope of regression analysis) is a constant ubiquitous value. Our relationship (i.e„ the slope of regression) quantifies the relatively fast (≤ 12day) response of bacterioplankton upon our experimental conditions to the observed photobleaching of CDOM at 300 nm upon irradiation with simulated solar radiation at +20°C. The slope is of course different, for example, if another photochemical reaction (e.g., photoproduction of individual BLP-compound) is examined. For example, the photoproduction of formaldehyde and acetaldehyde are highly ($r2 = 0.99$) correlated to the photobleaching of CDOM at 300 nm (Kieber et al. 1990). The slopes of this regression were different for these BLP-compounds (1.4E3 for formaldehyde, but 1.1E3 for acetaldehyde; Table 2 in Kieber et al. 1990), but independent on sampling site or irradiation time (Kieber et al. 1990).

R1: Same goes for the conclusions (8217 lines 7-9) of this paper.

A: "Similar estimates can be made by multiplying photobleaching of CDOM (Whitehead et al., 2000; Osburn et al., 2001; Vähätalo and Wetzel, 2004) with 0.335 mmol C m-2 (Fig. 2)."

The conclusion aims to highlight that the relationship between BLP and photobleaching of CDOM can be used to estimate the fate of non-labile photoreactive tDOM in the coastal ocean. To best of our knowledge, this kind of approach is applied for the

first time in this study. The relationship is related to CDOM, an important component in environmental photochemistry, which seems to have an exponentially increasing importance in science (based on scientific citations on topic "CDOM" found in the Web of Science;se Fig. 3 in the end of reply).

The sentence pointed out by the reviewer specifies the quantitative relationship (i.e., the slope) observed in this study. In the revised version, we plan to soften the claim by dropping out the actual value of slope. This type of value is provided for the first time in the present study. The value can be dependent on the experimental design used for its determination. Further research is needed to evaluate the variability of slope and its applicability to different environments (e.g., in freshwaters). We designed our study to evaluate the photochemical transformation of non-labile tDOM in global coastal ocean. For example, irradiations with simulated solar radiation targeted tDOM alone, were carried out in seawater matrix and it the absence of metals, which precipitate out in estuaries. Our experimental irradiation was not able to fully trace the complex environmental variability in global coastal ocean. For example, the selected temperature, salinity and irradiation time we selected to represent the average conditions in the global coastal ocean. We believe these conditions are representative for bulk tDOM, but it is possible that the slope would be different for extreme (low vs. high) conditions in salinity, temperature or the irradiation doses.

R1: 8214 lines 7-10: Because you used reference fluxes for the Mississippi, Lena and St. Lawrence and calculated the flux for the rest, I'd like to know what kind of numbers this calculation gives for these three rivers. Is it even close?

A: We used reported CDOM flux estimate for Lena River (Stedmon et al. 2011), which was 27.8 $\times 10^{12}$ m^2 yr^{-1} at 300 nm. The calculated CDOM flux based on reported discharge and CDOM absorption of our sample was 11.0 $\times 10^{12}$ m^2 yr^{-1} at 300 nm. For Lena River, the estimate of Stedmon et al. 2011 is 2.5 times higher than the one based on our samples. The estimated CDOM flux for Missisippi River in Spencer et al. 2011 was 8.8 $\times 10^{12}$ m^2 yr^{-1} and for St. Lawrence 0.61 $\times 10^{12}$ m^2 yr^{-1} at 300 nm. The

calculated flux for the same rivers based on reported discharge and our samples were 5.6 and $4.08 \times 10^{12} \, \text{m}^2 \, \text{yr}^{-1}$ at 300 nm. As a conclusion, previously reported CDOM flux for these three rivers was $36.4 \times 10^{12} \, \text{m}^2 \, \text{yr}^{-1}$ at 300 nm and the estimate calculated by using the absorbance of our samples was $20.7 \, \text{times} \, 10^{12} \, \text{m}^2 \, \text{yr}^{-1}$ at 300 nm. This type of differences are expected to be explained e.g., by inter-annual natural fluctuation in the fluxes. We decided to use the CDOM flux estimates reported in literature, because they base on rigorous determinations of seasonal discharges and CDOM.

R1: Section 4.1.2. Please note when you are comparing to spectral AQY and broad-band AQY experiments. Table 5 (as per general comment), should not be included.

A: Our results describe spectral AQYs and all data reported in Table 5 is essential regarding the method we have used for AQY determination.

R1: Technical corrections: 8204 line 13: what is the 2.2.1 in reference to?

A: This is an error. Instead of 2.2.1 there should be two references Minor et al. (2006), White et al. (2010). We will correct this to next version of the manuscript.

R1: 8213 line 17: BLPs and again on pg.8215

A: We thank Anonymous Referee 1 for pointing out these spelling errors, which will be corrected in the next version.

R1: Please check all references, I found these but there may be others: 8213 Line 27: Wagner et al. (2015) ref. not found in references section White et al. (2010) is in reference list but not in text.

A: We are grateful that Anonymous Referee #1 checked the manuscript in such detail. We found out that some error has occurred in manuscript preparation because in page 8204 line 12 should be two references: Minor et al. (2006), White et al. (2010), but in the current version these are replaced with the number 2.2.1, which is same as the number of the chapter sample preparation. We will revise this error in the next version. At the time of the submission, Wagner et al. (2015) was submitted to Environmental

Science Technology and was not citable. Now, this study is published and correct reference will be included to the next version and this citation is also included to reference list here.

References:

All references are provided in the response to Anonymous Referee # 2

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C5355/2015/bgd-12-C5355-2015-supplement.pdf

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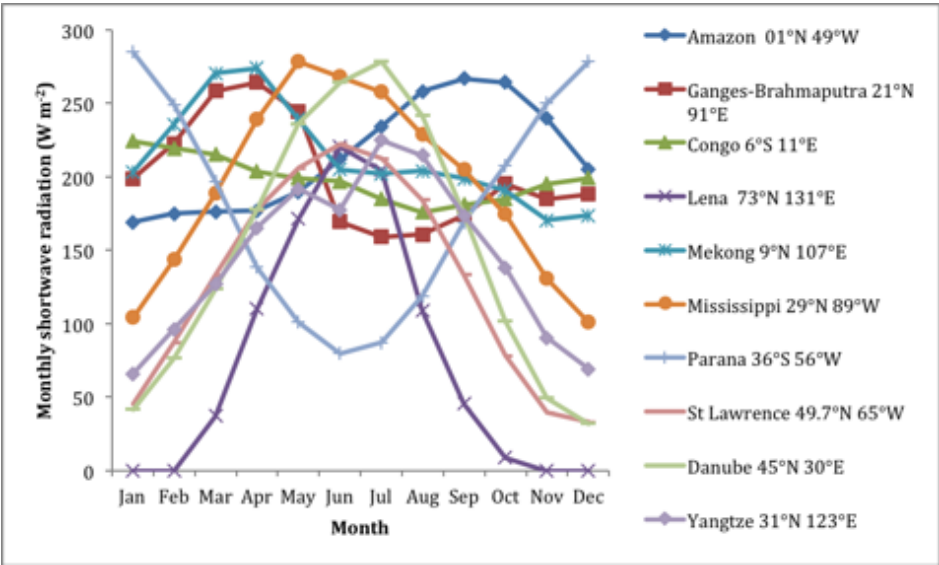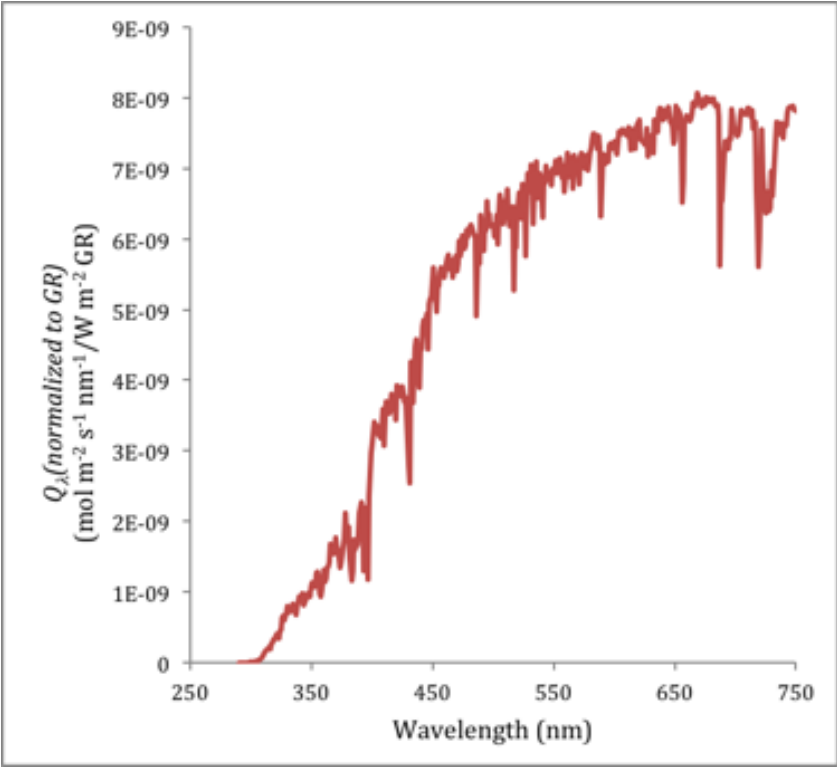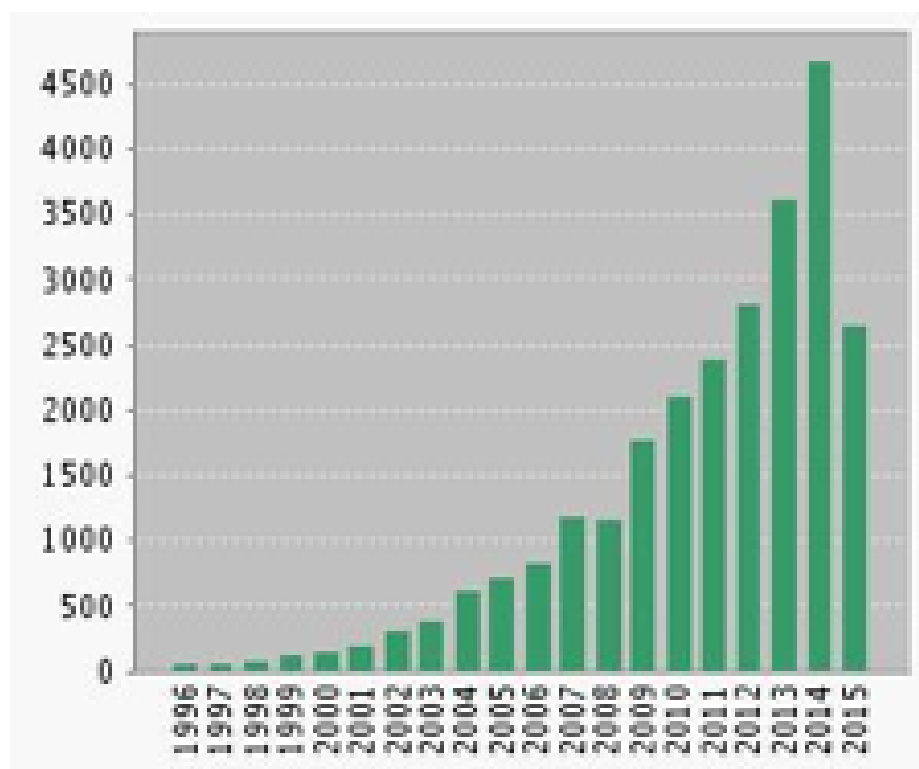Interactive comment on Biogeosciences Discuss., 12, 8199, 2015.

Fig. 1.

Fig. 2.

Fig. 3.

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