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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. Anonymous Referee #2 Received and published: 30 July 2015

R2: General comments This study measured the AQYs of BLPs from non-labile tDOC for 10 large worldwide rivers, established a quantitative relationship between BLPs production and photobleaching, and estimated BLPs production fluxes in global coastal oceans. In particular, the authors simultaneously determined AQYs of BP and BR on BLPs, thereby making it possible to evaluate the relative importance of carbon respira-

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tion and incorporation in bacterial utilization of BLPs. Such data, if obtained with sound methodology (see comments below), will greatly improve our understanding of the fate of tDOC in global oceans and thus the role of coupled photochemistry and biology in the global carbon cycle.

A: We are grateful from the encouraging feedback of Anonymous Referee #2.

R2: My main concern is the potential compromising of the sample integrity associated with the often lengthy sample transport by air cargo (17-155 d, average: 33 d). Were temperatures during transport similar to those in situ at the time of sampling?

A: Before answering to the detailed questions we wish to clarify the aims of this study and the rationale for the selected experimental design. This study focuses on the photochemical transformation of biologically non-labile but photoreactive tDOM into biologically labile forms in the global coastal ocean.

The removal of biologically labile portion of DOM during the transport prior to irradiation experiments is a part of experimental design. In the perspective of present study, the fate of biologically labile DOM is not interesting as this fraction of DOM will be quickly consumed in a predictable way by bacterioplankton as demonstrated by hundreds of earlier experiments (Lønborg and Álvarez Salgado 2012). We additionally argue that photochemistry plays a minor role in the consumption of labile DOM compared to its major role in the transformation of non-labile photoreactive DOM, the topic of present study. Additionally, the involvement of labile DOM during irradiations would complicate the responses of bacterioplankton during the following bioassays (see an detailed answer to referee #1 earlier). Therefore, we selected to remove the biologically labile DOM by allowing biological activity during the transport/storage prior to experimental irradiations to focus on the photochemical transformation of non-labile tDOM. We have used successfully the same strategy even for the freshly collected samples in our earlier studies (Baltic Sea, Vähätalo and Zepp 2004; Vähätalo et al. 2011; Aarnos et al. 2012)

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Our experimental design allowed us to set the focus on the photochemical transformation of terrestrial DOM in the marine recipient. It is extremely hard to separate the photochemical transformation of terrestrial DOM from that of autochthonous marine DOM in any study carried out in situ at the coastal waters. In our experimental design, riverine DOM was mixed 1:1 with artificial seawater and potential precipitates were filtered out prior to irradiation experiments. This simple experiment cannot naturally simulate a continuum of biological and physical events, which take place along the transport of terrestrial DOM towards offshore. In the terms of environmental photochemistry our simple experimental design is enough (Minor et al. 2006, White et al. 2010). Photochemical reactivity of terrestrial DOM changes quickly in low salinity (< 5) because of selective precipitation of photoreactive iron associated with terrestrial matter. After this precipitation event, the photoreactivity of tDOM remains more or less similar at higher salinities (Minor et al. 2006, White et al. 2010). Thus, our simple experimental setup allows us to study photochemical transformation of terrestrial DOM in the way how it takes place in the marine recipient.

Microbial communities are perhaps the most dynamic biological component in coastal waters. They originate partly from riverine freshwater communities and in part from marine environment (Kisand et al. 2005; Chauhan et al. 2009). The composition of these communities is never constant but changes constantly in response to abiotic (temperature, salinity, nutrients, DOM, etc) and biotic factors (e.g., grazing, viruses, etc; Pineiro et al. 2013). A community collected on a certain time at a certain site may not be representative for the same site e.g., a week later or for a different site at the same time. Thus, no single microbial community can fully represent the temporal and spatial variability in coastal microbial communities over the large areas, where the plumes of rivers will spread. Additionally, a microbial community is only representative for a site during the collection of sample, but after that the community starts to change. In the present study, we were forced to accept the adaptations of biological communities to the changing conditions in the collected samples, because the experiments were carried out at Helsinki (Finland) with the samples collected around the world. Fol-

lowing the legacy of Martinus Willem Beijerinck (1851-1931) we argue that despite the changes during transportation our bacterial communities still showed high adaptability to environmental conditions and were relevant for testing microbial responses to BPLs, labile part of DOM. The quality of DOM is one of the major abiotic variables regulating the composition and activity of microbial community (e.g., Judd et al. 2006). In our experiments, the riverine bacteria increased their biomass by two orders of magnitude. This clear growth in all samples represents the natural selection and adaptation of riverine bacteria to coastal waters. This process takes place when bacterial communities from rivers are transported to marine waters, where grazing and an increase in salinity cause selection in riverine communities leading to success of some species, represented of those bacteria which thrived in our experiments. Thus, the bacterial communities used in this study were natural components of diverse microbial communities in our study areas and relevant for testing the production and respiration based on BLPs.

The answer to the specific question is here. The temperatures of samples were not monitored during the sampling or during the transportation.

R2: If not, what were the differences? Would these differences affect bacterial species composition and physiology?

A: We do not have any data on bacterial species composition and physiology during the transportation.

R2: Would DOM and POM properties and their interactions change significantly after transport, including a change of the proportion and properties of tDOC?

A: During the transport and storage, microbes mineralized the labile part of organic matter as well as altered tDOM and tPOM into non-labile forms. For the St Lawrence sample the measured loss of DOC was 19% as reported in the Methods of original manuscript (Lalonde et al. 2014).The biological diagenesis in our samples was meant to simulate similar process along the transport of tDOM or tPOM towards offshore,

where the majority of photochemical transformation of non-labile photoreactive tDOM takes place. We argue that our experiments with diagenetically altered tDOC (instead of fresh tDOC) are well representative for the majority of tDOC, which is photochemically transformed in the coastal waters.

R2: (Note that samples were not filtered and thus contained all living stuff including phytoplankton and zooplankton which should mostly perished during transport).

A: Our non-filtered samples contained also eukaryotic communities. The composition of these communities obviously changed during the transport. These changes were meant to simulate similar changes in riverine communities upon transport to estuaries and further to coastal waters. Although these changes in the eukaryotic communities increased the environmental relevance of present study, their impact on the non-labile photoreactive DOM (the main interest of our study) was likely small or negligible.

R2: I also doubt the validity of use of the mixture of river water and artificial seawater to represent coastal waters.

A: The experimental preparation of "coastal waters" by mixing (1:1) river water with artificial seawater was designed in the perspective of photochemical transformation of tDOC. We did not use natural seawater, because we wanted to exclude marine DOC in our study focusing on tDOC. The photochemical reactivity of tDOC is larger in a freshwater than in a seawater matrix (Minor et al. 2006; White et al. 2010). The main reason for the difference is the precipitation of dissolved photoreactive Fe complexed with tDOM at low salinities (Minor et al. 2006; White et al. 2010). The experimental mixing raised the salinity to 16 and high enough to precipitate photoreactive Fe, which was removed by filtration. The inorganic ions of seawater are not photoreactive at natural salinities; and therefore our experimental conditions are representative to those of coastal waters, where the photochemical transformation of tDOM primarily takes place.

R2: First, bacteria that is indigenous in river waters is often not indigenous anymore in

coastal waters; there must be shifts in species composition. Second, estuarine mixing is a gradual process in terms of changes in salinity and pH, in a marked contrast with the "shock" treatment of adding artificial seawater to river water at a 1:1 ratio.

A: We agree with Anonymous Referee #2 that the species composition of communities in river water will shift upon mixing with seawater. Despite the osmotic shock, our bacteria from river waters were able to increase their biomass during the bioassays typically by two orders of magnitude. In agreement with the referee #2, we believe that the environmental conditions during bioassay resulted in a strong selection in the indigenous microbial community of river water. The species with dramatic increase in their biomass represent those riverine bacteria, which can not only survive but also reproduce and consume non-labile tDOM or BLPs from it in the coastal waters. These bacteria must be natural members of active microbial communities in the coastal regions in the front of major rivers.

R2: Salinity and pH affect DOM concentrations and properties and influence trace metal concentrations and speciation and thus alter the photoreactivity of DOM.

A: We designed our experimental accounting for the major factors affecting photochemical reactivity of tDOC in coastal waters based on earlier detailed studies (Minor et al. 2006; White et al. 2010). Already at low (< 5) salinities, the dramatic increase in the concentration of divalent cations leads to cationic exchange in DOM. Ferric iron complexed with tDOM will break loose and OH^- will replace the organic ligand resulting in insoluble $[Fe(III)(OH^-)_3(H_2O)_3]^0$, which will precipitate out of solution. Our experimental design (1:1 mix with artificial seawater followed by filtration) accounts for the alterations in the photoreactivity of tDOM in coastal waters.

R2: Third, biological activity occurring in coastal waters, which was lacking in the authors' artificial seawater, also affect trace metal concentrations and speciation.

A: Our experiments did not include the biological activity found in the coastal waters. This biological activity for example produces autochthonous DOM, which we wanted to

exclude from our study focusing on tDOM.

R2: I found the correlation between BP on BLPs and photobleaching as shown in figure 2 is not robust enough for estimating BLPs production from photobleaching. The relatively high R2 is largely due to a single point at delta(aCDOM) at ca. 12 m^{-1}. What is the R^2 after this point is removed? To minimize the bias, please try plotting data on a log-to-log scale.

A: We replied to this question also to Anonymous Referee #1. The regression is not driven only by the Congo River sample with large absorbance. If this point is removed, the slope estimate decreases from 0.33 to 0.26 and R^2 is 0.69 and p-value 0.001. Additionally, we analysed the relationship of CDOM photobleaching and BP based on BLPs using generalised least squares for linear model from the R-cran package "nlme" (Pinheiro et al 2014). The used gls-function allows the errors to be correlated/and or have unequal variance. By using this method, a linear model can be evaluated allowing heteroscedastical structure and evaluate Akaike Information Criteria (AIC) as well as Bayesian Information Criteria (BIC). We analysed linear models using all data and excluding Congo River data point, which both referees criticized. In these analyses three different weights were used and they described:

1) exponential of a variance covariate (varExp) 2) power of variance covariate (var-Power) 3) constant variance (varIndent)

The results from these analyses are given in table 1.

Based on the generalized least square estimates, there are no significant differences that would depend on the used weight. For all data points varPower and varIdent provides similar slope estimate compared to linear regression. If Congo River sample is removed from the analysis, varExp weight produces similar slope than estimates for all data points. It should be noticed that differences in AIC and BIC estimates between different methods and data sets are small and highly supports our understanding that the regression is not driven only by the Congo River sample.

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Table 1. Statistical summary from the linear regression describing the relationship of CDOM photobleaching and bacterial production based on BLPs

Generalized least squares	AIC	BIC	logLik	Slope	Std. Error	t-value	p-value
all data (varExp)	28.21	28.81	-11.11	0.33	0.06	5.72	3.00E-04
all data (varPower)	28.31	28.90	-11.16	0.34	0.05	6.46	1.00E-04
all data (varIdent)	27.64	28.04	-11.82	0.34	0.04	8.29	1.00E-03
Congo excluded (varExp)	24.89	25.13	-9.45	0.34	0.08	4.38	2.30E-03
Congo excluded (varPower)	25.13	25.37	-9.56	0.28	0.06	4.36	2.40E-03
Congo excluded (varIndent)	23.28	23.44	-9.64	0.26	0.06	4.61	1.70E-03

Table 2. Statistics for linear regression between bacterial biomass based on BLPs and CDOM photobleaching

Linear regression	Estimate	Std. Error	t-value	p-value
all data	0.33568	0.04048	8.293	1.66E-05
all data (log:log)	0.2638	0.1297	2.034	0.0725

As suggested, we plotted the data in log-to-log scale and found that for all data slope estimate was 0.26 (Table 2).

Although it is possible to carry out different type of transformations (e.g., log or trigono-metric) for the original data, the earlier studies strongly indicate that the relationship between the production of BLPs and the photobleaching of CDOM is linear (See a Table related to reply of R1). Therefore, we wish to report linear relationships in the present study. For this linear relationship, we report 95% confidence intervals. This 95% confidence interval is accounted for as an error in the estimated production of BLPs based on the photobleaching of CDOM.

R2: Detailed comments:

Detailed comments (xxxx.xx stands for page xxxx and line xx)

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8201.19: delete "to" after "yielding" 8202.3: "Despite" not "Despite of"

A: We shall correct these for the revised manuscript.

R2: 8203.3: please provide in situ water temperatures and DOC concentrations in Table 1 at the time of sampling.

A: Water temperatures or DOC concentration were not measured systematically from our samples without few exceptions (e.g., St Lawrences water sample).

8204.2-3:how to prove this?

A: The turn over time of labile DOM is typically defined to be a few days but it accepted to span time scales from hours up to ca. two weeks according to the older as well the latest reviews on the biological reactivity of DOC (Ogura 1975; Søndergaard and Middelboe, 1995; Carlson and Hansell 2015). The transport/storage time of our samples was >14 days. Thus, by the definition of labile DOM, it must have been consumed during the transport/storage of our samples.

R2: 8204.6: Is 19% the mean value for world oceans?

A: The value refers the data presented in two reviews (Søndergaard and Middelboe 1995; Lønborg and Álvarez Salgado 2012). Søndergaard and Middelboe (1995) report that labile DOC consist 19 ± 16 % (mean $\pm$ SD) of the total DOC in river water based on 16 studies they reviewed. Lønborg and Álvarez Salgado (2012) analyzed 491 incubation experiments carried out in ca. 30 coastal regions of global ocean and concluded that labile DOC represents 22 ± 12 % (mean $\pm$ SD) of the total DOC. Thus it seems that $1/5^{th}$ of riverine DOC is labile (Søndergaard and Middelboe 1995) and this fraction is also biologically removed in the global coastal ocean (Lønborg and Álvarez Salgado 2012).

In the revised version of manuscript, we shall drop out the single individual study (Asmala 2014) and clarify the value of labile DOC.

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R2: Section 2.2.2 Irradiations: any concern of self-shading?

A: Any potential self-shading is accounted for in the calculations of AQYs. No concern of self-shading for the relationship between BLPs and the photobleaching of CDOM, because any potential self-shading would affect the both photoreactions similarly.

R2: acdom(330) for Congo is >20 m-1!

Yes, Congo River had high CDOM content. It should be noticed the $a_{CDOM,300}$ of Congo River in Table 2 refers to our Congo River water diluted (1:1) with artificial seawater without any CDOM. Thus, the $a_{CDOM,300}$ in the original sample was two times higher than $a_{CDOM,300}$ in the diluted sample. Our $a_{CDOM,300}$ in Congo River samples agrees well with similarly high values reported e.g., by Spencer et al. (2009).

R2: 8211.23:this CDOM fluxes actually also contain a portion of labile CDOM.

A: In the present study, the CDOM flux estimates are based in a part on our own measurements and in a part values reported in literature. CDOM is an optical property of DOM and its biological lability has not been evaluated and categorized e.g., in the way done for DOC (Carlson and Hansell 2015). However, much of CDOM gets its color from dissolved humic substances (Xiao et al. 2013; 2015), which are typically consider non-labile. Thus, owning the non-lability of CDOM, our CDOM values reported in this study are likely close to those in the original sample and "labile CDOM" does not pose a problem in our calculations.

R2: 8213.22-23: so the actual R^2 could be smaller even without considering the bias resulting from the lack of intermediate values as commented previously.

A: Based on the additional statistical testing, R^2 is slightly lower if Congo River sample is excluded. However, according to our opinion, Congo River sample is representing the real variation in river derived CDOM absorbance and it should not be removed from the analysis. The regression is statistically significant for all generalized least square methods with or without Congo River sample and it did not have a significant

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effect to the slope estimate, which is important for this study and scaling of our results. Therefore, we do not see the lack of intermediate values as a big problem. We think that our experimental approach was able to describe well the bacterial responses based on non-labile DOC (not utilized from few weeks to few months time scale) after irradiation experiments and BP based on BLPs. BP based on BLPs was calculated as a difference of BP in irradiated samples and their dark controls describing the fraction of semi-labile or semi-refractory DOC that cannot be assimilated by bacteria in such time scale that it could be considered labile. We acknowledge that more samples from different rivers and different seasons would provide also intermediate values and help us to improve our estimate.

R2: 8215.9: would the in-situ coastal bacterial species composition be similar or identical to that of the riverine bacteria surviving the salinity shock introduced during your experiment? See general comments as well.

A: We argue that the bacteria used in the experiments are representatives of active microbial communities in the coastal regions in the front of major rivers. Coastal bacterial communities include also species specific to marine environment (e.g., Kisand et al. 2005; Cauhan et al. 2009; Pineiro et al. 2013). Marine species were not involved in our experiments, and therefore the communities used in this study were not identical to the composition of bacterial communities in coastal waters. Bacteria collected in-situ from marine environment also respond positively to BPLs like observed in the present study (e.g., the North Sea, the Adriatic Sea, the Baltic Sea, coastal Georgia; Obernosterer and Herndl 2000; Vähätalo et al. 2011; Reader and Miller 2014).

R2: 8216.20-21: If it can be assimilated by bacteria, then it should not be termed "nonlabile".

A: We apologize using the jargon related to DOM (Ogura 1975; Søndergaard and Middelboe; 1995; Carlson and Hansell 2015). According to this jargon, "nonlabile" refers DOC having turnover time more than few weeks. We shall clarify this for the

revised version.

R2: 8216.24: "in" not "at" estuaries. 8217.6: "river" not "rivers" plumes.

A: We will correct this spelling error in next version of the manuscript.

R2: 8217.10: "a robust estimate" is an overstatement.

A: We agree with the reviewer. In the revised version, we wish to rather emphasize that our study is the first, which determined the production of BLPs from tDOM in the global coastal ocean using the photobleaching of tCDOM as a tool. Being the first estimate of this kind, the estimate is rather rough but is expected to become more accurate with the future work.

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