

Interactive comment on “Changes in optical characteristics of surface microlayers hint to photochemically and microbially-mediated DOM turnover in the upwelling region off Peru” by L. Galgani et al.

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We thank the referee for the evaluation of our manuscript and for the constructive review provided. Specific issues raised are addressed here below.

RC = Referee Comment AC = Author Comment

General comments:

1. RC: I do understand that this manuscript is part of a special issue in Biogeosciences,

C9725

and that some ancillary data are presented elsewhere [Engel and Galgani, 2015]. This, however, does make it quite difficult to relate observations to hydrography: for example both sea surface temperature (SST) and salinity are invoked in section 3.1 ff. in order to explain CDOM distribution patterns and their relation to coastal upwelling and terrestrial inputs, but the manuscript itself contains neither SST nor salinity. Including an overview of SST and sea surface salinity here would really help. This may also help support the authors statement that high CDOM absorption coefficients at stations S10_1 to S10_4 were “probably due to an input of terrestrial material” (page 19385, top paragraph). These high CDOM absorbance values seem to fall into a patch of upwelled water (see Fig 2, [Engel and Galgani, 2015]), and it remains unclear if SST and salinity characteristics at S10_1 to 4 are consistent with terrestrial inputs.

AC: The referee raised a good point. Even if those data have been presented elsewhere, it is good to include again an overview of SST and sea-surface salinity to make it easier to follow our argumentation.

2. RC: I do believe that the authors’ analytical methodology, including CDOM fluorescence analysis, is sound. Unfortunately, however, neither section 2 (Materials and methods) nor section 3.2 (PARAFAC analysis for CDOM fluorophores) includes any details on the PARAFAC modelling and the verification of the final 5 component model chosen by the authors. It remains unclear, if their PARAFAC analysis tested for different numbers of components, and on what basis they identified the appropriate number of components in their model, e.g. residuals, split half analysis, Tucker congruency, ... ? It is worth noting that such detail is usually provided in publications presenting PARAFAC models, although at times in a concise manner [Dainard et al., 2015; Murphy et al., 2008; C. A. Stedmon and Bro, 2008].

AC: After normalization to R.U. units as described in the text, data were smoothed to remove scatter peaks, Raman and Rayleigh signals by creating a sub-dataset. We then performed a preliminary outlier analysis generating models with 3 to 7 factors with non-negativity constraints, comparing the spectra to unconstrained models. When dilution

C9726

dominates the dataset, components are strongly correlated (Murphy et al., Analytical Methods, ESI, 2013). To investigate biases due to dilution, we performed a test for correlations between the components, as suggested by the DrEEM tutorial by Murphy and colleagues (2013). We then normalised the dataset by the DrEEM function *normeem* to reduce the co-linearity related to the concentration, thus giving low-concentrated samples a possibility to enter the model, followed by the outlier test again on the normalised data. After visually comparing the spectra and looking at the error residuals for models with 4 to 7 components, we then compared the models by the sum of squared errors (SSE) expressed as a function of wavelength, choosing the models with lower SSE. At this stage, we choose models with 5, 6 and 7 components and reversed the normalization to obtain the unscaled scores before validation. Models with 5, 6 and 7 components were validated by split half analysis “S4C6T3” (see Murphy et al. Analytical Methods, ESI, 2013) where it was ensured that in each test the dataset halves being compared had no samples in common. The validation was successful for 5-components model, for all comparison. We will include details on the model validation in the revised version and in the supplementary information we will include the figures with the model comparison for excitation and emission for the 5-components model (here below).

3. RC: The discussion of spectral CDOM absorbance leans heavily on a paper by Helms et al [Helms et al., 2008], which is based on an analysis of a coastal DOM gradient from high DOC swamp waters into the northwestern Atlantic off Georgia. As such, results in Helms et al [Helms et al., 2008] are heavily weighted towards terrestrial (allochthonous) DOM. There is nothing wrong per se with applying CDOM spectral slopes and slope ratios as defined in Helms et al [Helms et al., 2008] to a contrasting study area such as the Peruvian upwelling. However, DOM spectral signatures alone do not unambiguously define terrestrial (allochthonous) origin. For example, low spectral slope coefficients may also result from autochthonous CDOM production in situ [Kitidis et al., 2006]. Furthermore, freshwater DOM inputs into the study area are conceivably small. Therefore, it might be useful to revisit the wording used for describing

C9727

ing CDOM characteristics, in order to clarify that spectral signatures similar to those of allochthonous/terrestrial DOM don't necessarily suggest the presence of terrestrial material in the Peruvian upwelling. This recommendation also extends to the discussion of PARAFAC components of DOM fluorescence, where any attribution of terrestrial sources would need to be underpinned by further supporting evidence, e.g. observations of decreasing load with increasing salinity [Murphy et al., 2008]. However, no such further analysis is presented.

AC: The referee raised a good point also evidenced by Referee #1, comment nr. 8, on the negative correlation between component F1 and salinity, and by Referee #2, comment nr. 9 about terrestrial inputs of DOM into the Peruvian upwelling. As in the responses to previous reviews (#1 and #2), we hypothesize that in the SML of the study region the contribution of terrestrially-derived DOM, if any, is overwhelmed by the high productivity of the upwelling system. Organics in the SML and their negative correlation to salinity (as for F1) may therefore reflect local upwelling and DOM remineralisation within the SML itself rather than allochthonous inputs from land. In the revised version, we will revisit the wording to express these concepts, as suggested by the Referee.

4. RC: Finally, I am somewhat concerned about the authors' conclusions from Fig. 8, which depicts correlations of PARAFAC component F1 (tryptophane-like) and so-called SUVA with the instantaneous global irradiance at the sea surface. Their statement in section 3.3 concludes that Fig. 8 provides evidence for “DOM photobleaching”. I disagree on the following grounds. Firstly, the field data shown in Fig. 8 only show the net, overall change in F1 and SUVA, but do not allow identification of individual processes. Identification of photodegradation as the process responsible would require an experimental setup able to isolate photochemical effects from other factors including microbial production and consumption occurring simultaneously. This can be done in controlled irradiations [Dainard et al., 2015; C.A. Stedmon et al., 2007], but not with field observations alone. Secondly, comparing indicators of CDOM abundance to instantaneous irradiance neglects the effects of photodegradation kinetics. For example, let's assume

C9728

that any processes other than photodegradation may be neglected over the diel cycle (sampling occurred between sun rise and sun set). Then, CDOM bleaching would be expected to continue throughout the entire photoperiod, leading to monotonically decreasing F1 and SUVA during the day. That is, the highest CDOM levels should be present at sunrise (i.e. at lowest global irradiance) and the lowest CDOM levels should be present at sunset (i.e. also at lowest global irradiance). If anything, it would have made more sense to plot CDOM indicators against time-integrated global irradiance, starting from the point of first sampling on the day. There are of course cases where tightly coupled production & consumption kinetics result in diurnal cycles (e.g. CO photoproduction and microbial consumption [Doney et al., 1995], but these also show the maximum impact of photochemistry in the late afternoon. Regarding CDOM, however, reported photobleaching half lives in the order of days to weeks [Dainard et al., 2015; Moran et al., 2000] argue against a pronounced diel cycle. I suggest that Fig. 8 should be removed, and that results and discussion sections are amended accordingly.

AC: In the SML of stations with multiple measurements we observed a significant decrease in F1 concentration due to radiation intensity ($R^2 = 0.56$, $p = 0.013$, $n = 10$, linear regression), while such decrease was not observed for other fluorophores. We agree with the referee's comment, as we cannot provide any indication of CDOM photodegradation kinetics. It is also difficult to unravel photodegradation from other processes responsible for decrease in fluorophores' concentration. However, we believe this strong correlation of F1 with radiation indicated by the regression analysis could be a clear indication of F1 removal by photodegradation in the SML. Thus, we will remove the section and figure 8. In the main text we will discuss effects of photodegradation of F1 referring also to processes mentioned by the referee, as we think it supports our hypothesis.

Specific comments:

Abstract:

C9729

5. RC: Some tangible information should be added to the abstract, for example dates, and some quantitative information.

AC: we will include more detailed information in the abstract of the revised version, as well as we may consider to use Figure 10 as graphical abstract.

References:

6. RC: A number of references are missing from the bibliography, starting with GESAMP, 1995 (Page 19376, line 3). Van Blough 2005 should be Blough 2005. Others: Engel 2015 (p 19380) Zsolnay et al 1999 Bange et al 2013..Please edit carefully throughout.

AC: we will edit the manuscript and include all references missing in the final reference list. We apologize for this mistake.

Materials and Methods:

7. RC: Page 19378, lines 20 ff Please clarify what was meant by "great care was taken that the sampling procedure was well standardized".

AC: We will rephrase the sentence in the revised version. We meant, that in order to obtain a well standardized procedure, the same person took the sample, with a repeatable withdrawal speed of the SML, to minimize any bias introduced by the sampling procedure.

8. RC: Page 19379, lines 14 ff. Not really a criticism: however, it might be worth stating that the SML thickness as sampled by the glass plate is less than that by e.g. the Garrett screen.

AC: we will include this information in the methods section.

9. RC: Page 19382, lines 21 ff. CDOM absorbance is simply an optical characteristics and not a 'concentration'. Please do not use 'concentration' when describing CDOM optical characteristics.

C9730

AC: we agree with the referee that absorbance is an optical characteristic of CDOM. However, since to the best of our knowledge UV-Vis absorbance measurements are the only way of quantifying the amount of CDOM in the samples, the absorption coefficient $a(325)$ (at 325 nm or at other wavelengths, as described in the literature for different environments) is considered as a proxy for CDOM concentration. Therefore, we will rephrase the sentences referring to CDOM absorption (higher, lower) and fluorescence (for individual FDOM components), and mention that absorption is a proxy for concentration in the introduction or method description.

10. RC: Page 19383, lines 14 ff. Effect of salinity on spectra slope. Please clarify that the variation of CDOM spectral characteristics in this context is simply reflecting 2 end member mixing along a salinity gradient, and not salinity effects per se.

AC: we will rephrase the sentence as suggested by the referee, explaining that a salinity gradient indicating mixing of water masses can be reflected in CDOM spectral characteristics. We agree it may be misleading here.

11. RC: Page 19383, lines 15. Definition of SUVA: SUVA is defined in EPA Document #: EPA/600/R-09/122, "DETERMINATION OF TOTAL ORGANIC CARBON AND SPECIFIC UV ABSORBANCE AT 254 nm IN SOURCE WATER AND DRINKING WATER" (2009) $SUVA (L/mg \cdot M) = UVA(cm^{-1}) / DOC (mg/L) * 100 cm/M$ UVA Calculation: $UVA = A / d$ where UVA = The calculated UV absorbance of the sample in absorbance units (cm^{-1}), A = The measured UV absorbance at 254 nm of the sample that is filtered through a 0.45- μm filter media, and d = The quartz cell path length in cm. Your DOC normalised absorption coefficient at 254 nm is NOT SUVA, so please do not call it that.

AC: We thank the referee as we realized the mistake. In our equation we used the absorption coefficient $a(254nm)$ defined as $a(254) = A(254) * 2.303/d$, where A is the Absorbance (in absorbance units) and d is the path length (cm or m). Our findings on SUVA₂₅₄ enrichment factors and SUVA₂₅₄ correlations with fluorophores F1 and F3 do not change dividing by 2.303 to reverse from absorption coefficient to Absorbance.

C9731

Corrected SUVA₂₅₄ values are lower than we previously reported and comparable to oceanic waters as indicated by Weishaar and colleagues (2013). In the revised version we will correct it (page 19386 line 28 and page 19387 line 1).

12. RC: Page 19384, lines 14 ff. HIX. Similarly, please use a distinct acronym for your modified HIX, as your wavelength ranges significantly depart from those in the original paper by Zsolnay et al 1999.

AC: We will call the index differently as suggested by the referee, suggesting the acronym, "SMHIX", where SM stands for Surface Microlayer.

13. RC: Page 19385, lines 5 ff, Enrichment factors. Perhaps this is a matter of taste: Enrichment factors (EFs) usually refer to a difference in abundance between SML and ULW. However, HIX, spectral slopes and SR etc. are not measures of abundance, and therefore I personally would prefer using something other than 'EF' to denote differences in DOM properties between SML and ULW.

AC: We agree with the referee comment, that the term EF might not be appropriate for all. However, to avoid confusion and introducing more parameters, we'd rather keep the same wording for the same calculation. We will specify in the methods that EFs are "quantitative ratios" for some parameters, and for others such as SR, Slope, HIX, EFs are "qualitative ratios".

Results:

14. RC: Page 19387, line 1 ff. Socalled SUVA values: 0.6 mg C L⁻¹ m⁻¹ is NOT "comparable to riverine waters". This value was obtained with Pacific Ocean fulvic acids [Weishaar et al., 2003]. Please correct your statement.

AC: We think there was a misinterpretation here. We meant that the value for Pacific Ocean fulvic acids obtained by Weishaar et al., 2003 of 0.6 mg C L⁻¹ m⁻¹ was lower than the values we observed in SML and underlying water samples, and that OUR values were more comparable, in number, to riverine waters. We will rephrase the

C9732

sentence accordingly.

15. RC: Page 19388, lines 24 ff: Origin of PARAFAC component F2: Please clarify why you state that a positive correlation of F2 with SST and bacterial abundance might suggest “a refractory DOM component of terrestrial origin” ??? My hunch would be that bacterial abundance could well be related to primary production, which in your study area is likely to be fuelled mainly by coastal upwelling? I also do not agree with your description of F2 as a “refractory DOM component” resulting from either photochemical or microbial DOM degradation. Please revise.

AC: We will rephrase the sentences, as in this setting terrestrial origin might not be the appropriate description for F2 (see comment #3). We saw an increase of bacteria with increasing temperature, which is well supported by the literature. As we found that fluorophores with Ex/Em ranges similar to F2 could hint to small molecules of low bioavailability, highly degraded, the fact that higher temperature stimulates bacterial activity could be reflected in F2, thus suggesting a more refractory or, better said, more microbially-altered DOM.

16. RC: Furthermore, positive correlations between F2 on the one hand and F3 and F5 on the other does not necessarily support your statement that F2 is derived from these other two FDOM fractions. Either all 3 fractions are formed by the same underlying process (then I'd expect positive correlations between them), or F2 is formed from F3 & F5, that is an F2 increase must cause decreases in F3 & F5. Please revise.

AC: we agree with the interpretation given by the referee that the components, showing positive correlation, may derive from the same underlying processes, in this case microbial reworking of larger components which can be still subject to photochemical degradation. However, due to the size-continuum of DOM, and to the microbial life comprised in the surface film and below, it may be not necessarily true either that an increase in F2 implies a decrease in the other two fractions (F3 and F5); these compounds may be constantly replenished by the complex biogeochemical processes at

C9733

the air-sea interface. We will revise the wording, suggesting the concept of a progressive photochemical and microbial alteration of DOM jointly with a local HMW-DOM microbial release.

17. RC: Page 19391, lines 19 ff, Mycosporine like amino acids: The authors try to link mycosporine like amino acids (MAAs) to their CDOM characteristics. This is rather speculative. Besides, MAAs are not of high molecular weight as stated here. This section does not add value to the results section and should be deleted.

AC: The referee is right that MAAs are LMW and not HMW. The point here, is to find supporting evidence for our hypothesis of a local microbial release of protein-like DOM as a response to high solar radiation. We do that by referring to available studies on SML optical properties, which are really scarce, and on microbial response to UV radiation. We agree that this paragraph is not well suited for the results section and should be moved to the discussion.

Discussion:

18. RC: Page 19393, lines 20 ff: SR, DHAA%, and “lability”: Please spell out how SR and DHAA% support the notion of a labile DOC fraction in the SML. Are you referring to lability with respect to microbial consumption or with respect to photochemical degradation?

AC: According to Helms et al. (2008), an increase in SR suggests photodegradation processes and DOM of lower molecular weight. DHAA%-DOC is used here as an indicator for DOM diagenesis, thus, the extent of microbially-altered DOM. The higher DHAA%-DOC, the more labile, bioavailable, and recently produced DOM in the sample (and less altered). In this context, we compared DHAA%-DOC and SR (and also S(275-295)) finding a negative correlation between the two. The higher DHAA%-DOC, the lower SR and S(275-295). The correlation was stronger in the SML than in the ULW. In our opinion, this suggests a local microbial release and accumulation of relatively “fresh” DOM of higher molecular weight in the SML which may derive from cell

C9734

disintegration, probably due to photochemical processes, or to a release of exudates as a protection strategy against UV. Microorganisms adopt several strategies against tough environments, and this is what may have happened in the SML. Since we saw a higher correlation to heterotrophic bacteria, we may argue that bacteria rather than phytoplankton are more successful at the immediate sea-air interface.

19. RC: Page 19394, lines 16 ff, nitrous oxide binding to aromatic groups. The authors refer to Cao et al. (2015) who found evidence for the formation of complexes between nitrous oxide and some mono-aromatics. However, Cao's study was conducted in a Ne matrix using millimolar concentration, i.e. rather different from the conditions at the sea surface. In my view, this section is far too speculative and should be removed.

AC: The Referee is right, the section is speculative and the experiment by Cao and colleagues cannot be translated to our setting. However, to the best of our knowledge no previous studies linked SML DOM optical properties and sea-air exchange of climate-relevant gases (such as N₂O) in the highly productive Peruvian EBUS. In our opinion, some speculation based on recent findings on N₂O interaction with biological macromolecules, can provide fertile ground and interesting ideas worth further investigation in the Peruvian EBUS and other key oceanic regions.

20. RC: Page 19395, line 1 ff. Origin of F2. What is your evidence for your statement that "F2 fluorescence appears to be related to DOM exposure to sunlight [...]?"

AC: we did not express our idea properly here. We meant that F2 in our study was comparable to component 1 as found by Ishii and Boyer (2012), as described in table 2 of our manuscript. According to these authors, F2 (or component 1 in their study) reflects "[...] small-sized molecules [...] and mainly derived from photobleached terrestrial humic acids in marine waters with highest concentrations near the surface [...]" (as we wrote on page 19388, lines 18-21). In the literature this fluorophore can be related to DOM exposure to sunlight, but we did not find such correlation. We will rephrase this part.

C9735

21. RC: Page 19395, lines 23 ff. The discussion here speculates on the sunlight induced release of DOM fractions but cannot provide any supporting evidence from the field data shown. Please remove this section.

AC: we don't have measurements on microbial release of DOM induced by UV as this was not the purpose of this study. Such measurements should be made in controlled laboratory experiments with controlled irradiation. Our study was meant to investigate the origin and processes controlling DOM turnover in the SML in the Peruvian EBUS. Based on our observations and on previous findings in the literature, we summarize potential DOM production and loss processes that may happen in the SML. Therefore, we do not agree with the referee's comment on this section, as this is kind of discussion is needed to highlight our findings. The analysis of gel particles, microorganisms, optical DOM properties and indicators of DOM diagenesis well support the hypothesis of a local microbial DOM release (Fig. 10), which may occur because of the high radiation received by the SML.

22. RC: Page 19396, line 1 ff. Section 4.3 Implications: This section is somewhat repetitive in that it repeats statements regarding the possible roles of photodegradation and UV-induced stress. Much of this section is highly speculative and either not well or not at all supported by the authors own observations. Some of the statements on fluorescent organic matter remain unclear and need thorough revision in the light of the observational evidence available here. For example, components F3 and F5 are described as high molecular weight and more humic and refractory, but at the same time related to microbial cycling fuelled by the upwelling system. This seems a little confused, given that the currently accepted size reactivity continuum model implies that HMW material is less complex and more bioavailable (i.e. labile) than low molecular weight DOM [Benner and Amon, 2015]. I recommend a rewrite of this section, focused on the less speculative aspects that can be gained from this interesting data set.

AC: We do not agree completely with the Referee's comment on this section. The section indeed needs some rewriting and better wording, but as we have previously

C9736

stated, no studies up to now have investigated SML optical properties in the Peruvian upwelling, and in general, studies on SML-CDOM are extremely scarce. Therefore, some argumentation or speculation is necessary to support our ideas, which is based on evidences of our results. One study is certainly not enough and we present our hypothesis based on our observations, but it is clear that future investigations are needed, indeed. Based on previous studies reporting comparable Ex/Em ranges, we suggest that component F3 could be characterized by large and hydrophobic compounds and probably produced in situ by microbial reworking of organic material, fuelled by the high productivity of the upwelling. F5 could be at a later stage of microbial reworking, and both components could be included in the pool of the so-called marine gels, which represent a size continuum of organic matter, from dissolved colloids to macromolecules spanning over several millimetres and yet, quite complex molecules. We will address the referee's comment by revisiting the section and avoid

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C9737

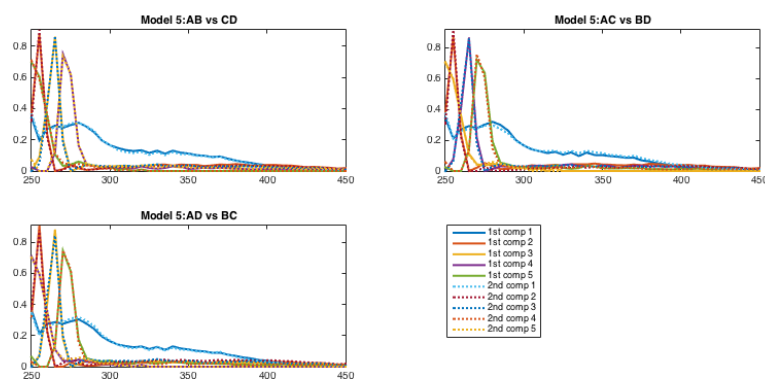


Fig. 1. Figure 1. 5-components model validation for multiple comparisons – excitation

C9738

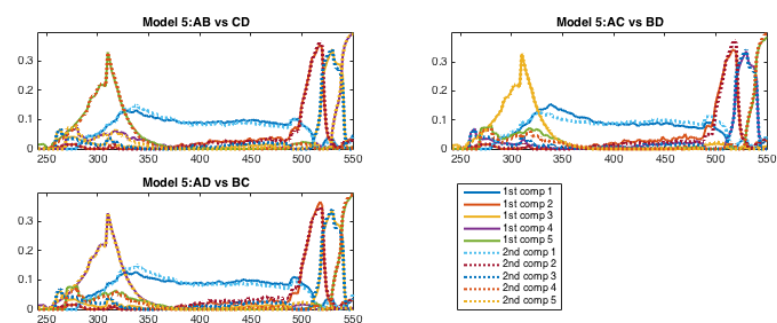


Fig. 2. Figure 2. 5-components model validation for multiple comparisons – emission.

C9739

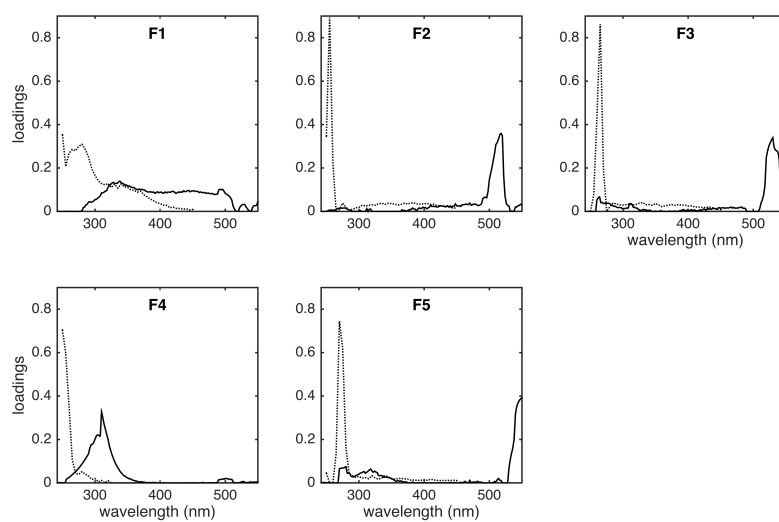


Fig. 3. Figure 3. Overlaid spectra. 5-components model validated with 3 split comparisons.

C9740