Authors' response for revision of the manuscript:

Changes in optical characteristics of surface microlayers hint to photochemically and microbially-mediated DOM turnover in the upwelling region off Peru

We thank the three referees for their thorough evaluation of our manuscript and the valuable suggestions for its improvement. Below, we list the point-by-point responses to the referees' comments.

Response to referee #1:

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

1. Referee:

Page 19374, lines 10-14. Because the data of DOC, amino acids, marine gels, and bacterial abundance were cited from Engel and Galgani (2015), it is more appropriate to describe these biochemicals in the discussion section. I suggest that the authors omit the sentences "In order to understand.. . .microbial alteration processes" from the abstract.

Authors: we have rephrased that part of the abstract.

2. Referee:

Page 19376, line 7. "biological liability" should be biological lability

Authors: We have corrected it.

3. Referee:

Page 19378, lines 6-8. Please clarify and supplement the purpose of this study. It can be emphasized that the meaning of the CDOM differs from those of other biochemicals (DOC, amino acids, etc) and is more specific about what scientific questions will be addressed in this study.

Authors: in the revised version at the end of the introduction, we have discussed more extensively about the purpose of this study and the importance of CDOM characterization in the Peruvian upwelling.

4. Referee:

Page 19380, the section "2.2 Chemical and biological analyses" need some reorganization for conciseness. I found that the analyses procedure of DOC, amino acids, phytoplankton, gel particles and heterotrophic bacteria were mostly copied from the paper of Engel and Galgani (2015). I think that there was no need to make a detailed description of the analytical methods for these compounds.

Authors: we have shortened the methods description for DOC, DHAA, marine gels, phytoplankton and heterotrophic bacteria and combined DOC and DHAA into DOM, and phytoplankton and heterotrophic bacteria together making reference to Engel and Galgani (2016).

5. Referee: Page19381, lines 11-13. In this study, using 2% (THAA%-DOC) as the threshold for DOM lability may be inappropriate, because the THAA yields in different sea areas are not comparable. I think that a direct comparison for their values is more reasonable. If possible, I suggest that the authors could calculate the "degradation index" (Dauwe and Middelburg, 1998; Dauwe et al., 1999; Kaiser and Benner, 2009;Peter et al., 2012) based on the amino acids mole percentages, which can help to evaluate the degradation states of organic matter between the SML and ULW. Dauwe, B., Middelburg, J.J., 1998. Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnology and Oceanography 43, 782–798. Dauwe, B., Middelburg, J.J., Herman, P.M.J., Heip, C.H.R., 1999. Linking diagenetic alteration of amino acids and bulk organic matter reactivity. Limnology and Oceanography 44, 1809–1814. Kaiser, K., Benner, R., 2009. Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. Marine Chemistry 113, 63-77. Peter, S., Y. Shen, K. Kaiser, R. Benner, and E. Durisch- Kaiser, 2012. Bioavailability and diagenetic state of dissolved organic matter in riparian groundwater, J. Geophys. Res., 117, G04006, doi:10.1029/2012JG002072.

Authors: The study by Peter and colleagues (2012) refers to ground water, presenting three indicators of DOM diagenesis: amino acids concentrations, carbon-normalized yields

of amino acids, and degradation index. We have compared our results with the study by Kaiser and Benner (2009) and other references already cited in the text (e.g. Davis and Benner, 2007). We are aware of the studies suggested by the referee and the calculation of the degradation index for amino acids. However, the degradation index calculated by Dauwe and colleagues refers to POM in sediments and could at best also be only an indication for DOM diagenesis. In the revised version, we have added the following sentence: “Amino acids generally comprise a large fraction of bioavailable organic matter and are preferentially consumed by microbial activity quite rapidly. In surface waters they may be easily photodegraded too. Therefore, the amount of carbon included in amino acids is considered as a good indicator of DOM diagenesis and a value of ~ 2% of DHAA%-DOC may indicate the threshold between labile and semi-labile and refractory DOM (Davis and Benner, 2007).”

6. Referee: I suggest the authors avoid discussing data in the results section. For example, sentences on lines 4-7 (page 19386), lines 21-23 (page 19386) and lines 1-4, (page 19391) belong to the discussion section.

Authors: We have thoroughly revised the results section avoiding discussing data in that context.

7. Referee: P19392, in the section 4.1. Lots of data including temperature, salinity, wind speed, radiation and different DOC type refer to Engel and Galgani (2015) in the SML and ULW. If the authors can combine these environmental parameters to discuss the enrichment of CDOM, that will help to increase our understanding of CDOM enrichment.

Authors: we have inserted table 1, recalling hydrographic data, and table 5, described in results 3.3, which analyses correlations between CDOM optical properties and salinity, water temperature, wind speed and particulate organic carbon (POC). The discussion has been revised accordingly.

8. Referee: P19393, line 26. The component F1 showed a protein-like fluorescence of autochthonous material, and those (F2, F3 and F5) had the characteristics of terrestrially derived fulvic-acid like or humic-acid like DOM. But as showed in Table 3, the autochthonous component F1 negatively correlated to salinity, and no correlations were found between the terrigenous components and salinity. It is in contradiction that terrigenous material usually negatively correlated with salinity.

Authors: It is true that terrigenous material may have negative correlations to salinity as their concentration is higher in freshwater bodies. However, in the SML of the Peruvian EBUS, a combination of processes due to the complexity of the system may be responsible for DOM accumulation and alteration, such as upwelling of colder waters (as also indicated by the negative relationship of F1 to temperature). Therefore a straightforward relationship between CDOM and salinity cannot always be established in this case. In our study the negative correlation coefficient of F1 to salinity, although significant, was low (-0.24). In the revised version, we have modified paragraph 4.1. with the following text, as also commented to Referee 3, comment nr. 3 […] "In the Peruvian EBUS, we observed a general enrichment of CDOM in the SML with respect to the ULW, based on values of the specific absorption coefficient $a(\lambda)$ measured at 325 nm. Higher values for CDOM absorbance were observed in the coastal upwelling stations characterized by lowest salinity, temperature and highest enrichment of organic components, both in the particulate and dissolved fraction (Engel and Galgani, 2016). These high values may be associated to an input of terrestrial material from urban and agricultural activities at the coast and from inland, as it is commonly observed that the spectral loadings of allochtonous/terrestrial DOM decrease with increasing salinity (Murphy et al., 2008). However, we did not observe such trend in our samples. Instead, we found a negative correlation of protein-like fluorophore F1 to salinity and temperature, and no clear enrichment of humic-acid like fluorophores F2, F3 and F5 in the SML. Therefore, we think 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 enriched in the SML such as the amino-acidic compounds F1 and F4 found at the upwelling stations may therefore reflect other processes rather than input of allochtonous DOM from land." […]

9. Referee: P19394, line 22. Table 2 should be Table 3.

Authors: We corrected it. However, we have added two tables: table 1 and 5. Thus, tables have been renumbered.

10. Referee: P19395, lines 23-25. The authors present a good example of the conceptual model of CDOM production and removal between the SML and ULW. I suggest that the author could emphasize this model in the abstract section to attract readers.

Authors: we appreciate the suggestion and we think it is a good idea to have the conceptual model as graphic abstract. In the revised version, we emphasized the model and used the figure as graphical abstract. Therefore, figure 11 does not exist anymore, the abstract has been changed, and the discussion text has been corrected accordingly (4.2).

Response to referee #2:

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

1. Referee: Figure 1. S7 and S12_3 are not shown on the map.
Authors: The referee is right. The location of station S7 and S7_2 coincides but S7 is not indicated. We have adjusted the figure. S12_3 is shown as S12_1/3 (as S12_1 and S12_3 are in the same location, just different times). We have clarified that in the figure's legend.

2. Referee: Figure 2. It would be helpful to label the stations shown in the figure so that the reader does not need to go between the text, Figure 1 and Figure 2. Even just putting S10 for all of the S10 sites would help.
Authors: We have labelled principal stations of figure 2 a(325).

3. Referee: Figure 3 caption: The box and whisker plots should be explained in the caption. What are the percentile values for horizontal lines? What do the black circles represent? Are they the individual samples? It would be helpful if these were colored by station number.
Authors: We have addressed the Referee's comment by replacing figure 3 with a new box and whisker plots, where outliers are identified by labels and colors. Percentiles are explained in the figure caption.

4. Referee: Page 19386, lines 7 – 8: It is hard to see that "generally CDOM was enriched in the SML" based on the results shown in Figures 2 and 3. Figure 3 shows a few EF values > than 2 and a few < 1. Based on the text and Figure 2, the higher EFs appear to be associated with regions of terrestrial input or regions of coastal upwelling. The discussion on p. 19386 should reflect this.
Authors: CDOM concentration is expressed as absorption coefficient at 325 nm, a(325). Median EF for a(325) is 1.2 (figure 2). This means that at least 50% of our observations (and more) account for CDOM enrichment in the SML, with a median EF >1 as figure 2 shows. This is also visible in figure 4, where lower EFs (and EFs < 1) are found at higher distance from the coast. We modified the text to: "CDOM was enriched in the SML at most stations (Figure 3), with median EF for a(325) = 1.2 in a range varying between 0.4 and

2.8. A median EF = 1.2 means that at least 50% of our observations accounted for a CDOM-enriched SML. Besides the southern transect, higher EF values were observed at the northern stations S2 and S2_2, and in the southern coastal upwelling stations S15_1 to S15_3. Lower EFs and EFs < 1, indicating a depletion of CDOM in the SML, were observed at higher distance from the coast (Figure 4)." We have addressed these points as suggested in paragraph 4.1 of the discussion as well.

5. Referee: Page 19386, lines 19 – 21: Figure 3 indicates two populations of S(275-295) values – one with EFs <1 and the other with EFs >1. Are these not statistically different? If they are, reporting a median EF of 1 is misleading.

Authors: We don't understand the point raised by the Referee. In lines 19-21 (page 19386) we describe that values for spectral slope parameter S(275-295) were similar between sea-surface microlayer and underlying water, two distinct compartments. This means, that no real enrichment in one compartment or another was observed. This is clearly visible in figure 3, where median EF for S(275-295) is = 1. No statistically significant differences were found between SML and underlying water for S(275-295). This has been added to the results section 3.1.

6. Referee: Figure 4: Again – it would be helpful if the stations were labeled in the figure.

Authors: We have labelled principal stations of figure 4, EF for a(325). Also, we have labelled the principal stations of figure 6 (EF for F1).

7. Referee: Page 19388, lines 21 – 24: On average, F2 did not show a clear enrichment in the SML but it did regionally – especially at S2, S10, and the southernmost stations. It is not clear why average EFs are emphasized rather than the regional values – especially since no data points coincide with the median values (as shown in Figure 7).

Authors: The referee raised an important point and we have addressed the regional enrichment in as suggested (Results) and added explanation on percentiles in the caption of figure 7. Apart for a few stations, F2 was not particularly enriched in the SML as figures 6, and 7 in particular, show, with a median value of EF = 1, that is, similar concentration in SML and underlying water for component F2. Box and Whiskers plots of figure 7 have been replaced and outliers identified by labels and colours.

8. Referee: Page 19393, lines 2-4: Not sure what is meant by this sentence.

Authors: We believe the Referee refers to the sentence […] "**Moreover, it helps tracking changes in DOM "quality" deriving from the exposure of SML to solar radiation more than any other marine environment**. […]. In this context we meant that optical analysis of CDOM helps in understanding sources and fate of DOM in the SML, in particular photochemical processes that alter DOM composition (such as compounds and diagenetic state), that, in an environment so exposed to solar radiation such as the SML, might be of extreme importance. We have rephrased the sentence to "Moreover, it helps tracking changes in DOM "quality" deriving from higher DOM exposure to solar radiation at the sea-surface than deeper in the water column. ".

9. Referee: Page 19393, lines 7 – 9: The highest a(325) EFs were observed at S10. Earlier in the paper it is said this may be due to the input of terrestrial material or upwelling. Can anything more be said based on the other reported measurements (F factors, etc.) about the relative importance of terrestrial inputs?

Authors: At S10, we saw an accumulation of CDOM in the SML with respect to the underlying water (measured as *a*(325)). However, F components of terrestrial origin (F2, F3, F5) were not enriched in the SML of those stations, while F1 and F4 preferentially accumulated in the SML. We may argue that these compounds (F1, F4) containing more protein-like DOM and enriched in the SML as an important component of CDOM, may directly derive from upwelling or from a microbial response to solar radiation, thereby implying an autochthonous microbial source in the euphotic zone or in the SML itself. Terrestrial material, containing more refractory DOM, showed similar concentrations in SML and underlying water (reminding that underlying water is still considered surface ocean, as in this study goes up to ~20 cm). Since amino acids tend to accumulate in the SML quite ubiquitously (e.g. Cunliffe et al. 2013, Progress in Oceanography 109, 104–116), in this setting the contribution of terrestrially-derived material is, in our opinion, overwhelmed by the high productivity of the Peruvian EBUS (reflected in F1 and F4 and amino-acidic compounds). In other words, in highly productive oceanic regions DOM components prevailing in the SML may have different origin than terrestrial ones, even if close to the coast. Characterizing these features in the SML is important when addressing the issue of gas exchange between the ocean and the atmosphere, in particular within the context of worldwide expanding oxygen minim zones. In the revised version we have

modified the text (4.1, Discussion) to the following: ” Higher values for CDOM absorbance were observed in the coastal upwelling stations characterized by lowest salinity, temperature and highest enrichment of organic components, both in the particulate and dissolved fraction (Engel and Galgani, 2016). It is commonly observed that spectral loadings of allochtonous/terrestrial-like CDOM decrease with increasing salinity (Murphy et al., 2008). However, we did not observe such trend in our samples. Instead, we found a negative correlation of amino-acid like fluorophore F1 to salinity and temperature, and no clear enrichment of humic-acid like fluorophores F2, F3 and F5 in the SML. Therefore, we think that in the SML of the study region the contribution of terrestrially derived CDOM, if any, is overwhelmed by the high productivity of the upwelling system. Organics enriched in the SML such as the amino-acidic compounds F1 and F4 found at the upwelling stations may therefore reflect other processes rather than input of allochtonous CDOM from land.”

Response to referee #3:

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, 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. We have revised the manuscript as we agree with the Referee: we think the main process responsible for CDOM accumulation in the SML relates more to the upwelling rather than to coastal inputs of terrestrial material. As also evidenced by Referee#1, comment n. 7, we have included table 1, which recalls average data on salinity, temperature, wind speed and global radiation and table 5, described in results 3.3, which analyses correlations between CDOM optical properties and salinity, water temperature, wind speed and particulate organic carbon (POC). The discussion has been revised accordingly.

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: We have added the following text: “After normalization to R.U. units, data were smoothed to remove remaining 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 dominates the dataset, components are strongly correlated (Murphy et al., 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 “$S_4C_6T_3$” (see Murphy et al. 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. The maximum fluorescence intensities of the five fluorophores at specific Ex-Em wavelengths ranges are described in table 2. Figures with the model comparison for both excitation and emission for the 5-components model are included in the supplementary material (Figures S1 and S2).”

We have added the following figures to the supplementary information:

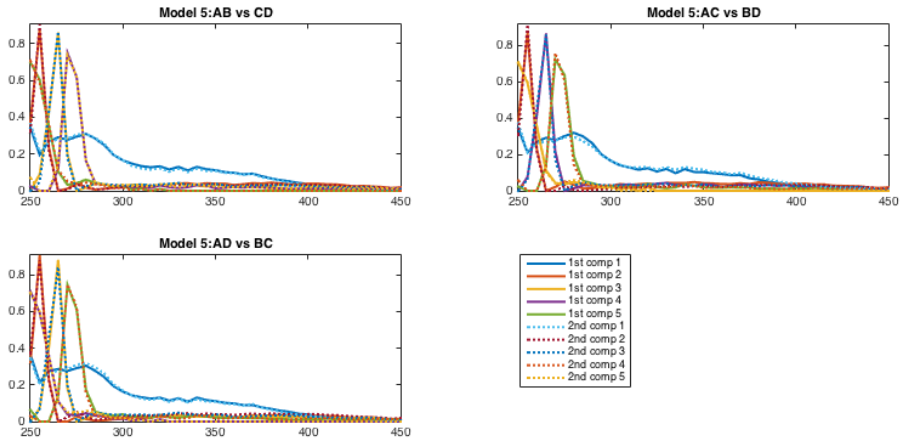


Figure S1. 5-components model validation for multiple comparisons – excitation.

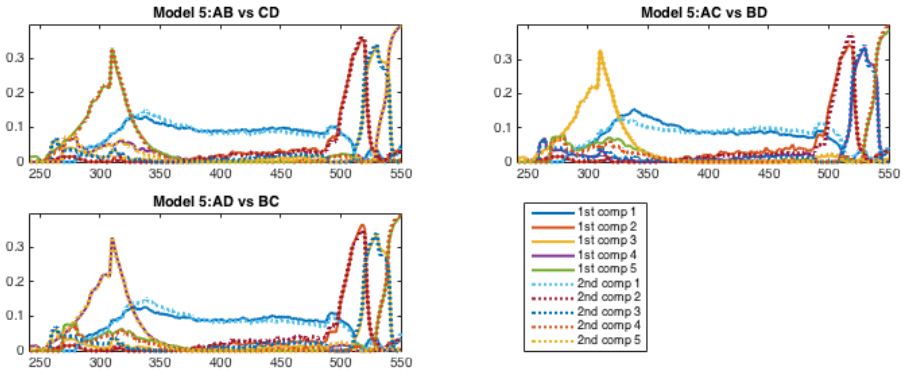
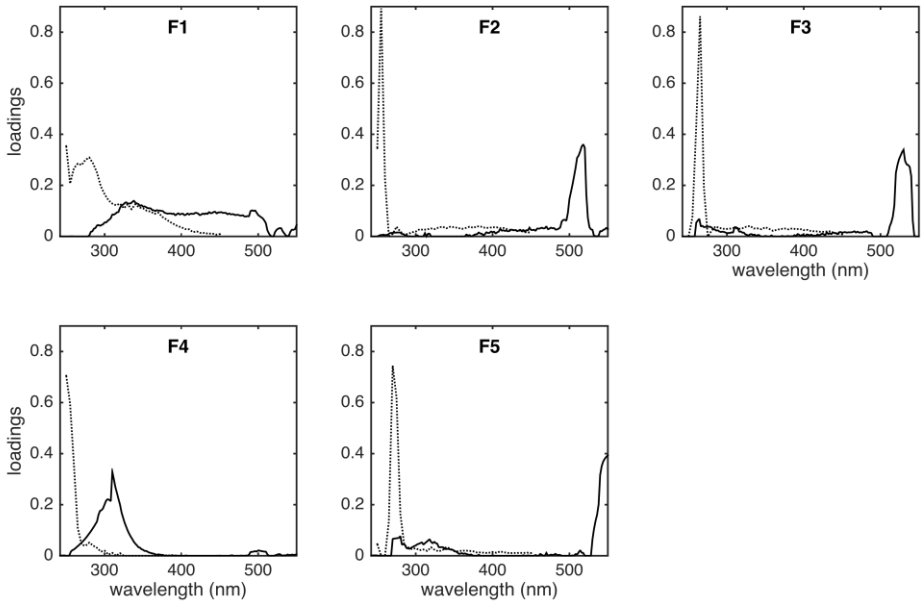


Figure S2. 5-components model validation for multiple comparisons – emission.

The figure of overlaid spectra for the 5-components model validated with 3 split comparisons is already presented in the text (Figure 5).



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

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 are 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 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, so we cannot detect it. Organics in the SML and their negative correlation to salinity (as for F1) may therefore reflect local upwelling and DOM production/turnover within the SML itself rather than allochtonous inputs from land. In the revised version, we have modified paragraph 4.1. with the following text […] "In the Peruvian EBUS, we observed a general enrichment of CDOM in the SML with respect to the ULW, based on values of the specific absorption coefficient $a(\lambda)$ measured at 325 nm. Higher values for CDOM absorbance were observed in the coastal upwelling stations characterized by lowest salinity, temperature and highest enrichment of organic components, both in the particulate and dissolved fraction (Engel and Galgani, 2016). It is commonly observed that spectral loadings of allochtonous/terrestrial-like CDOM decrease with increasing salinity (Murphy et al., 2008). However, we did not observe such trend in our samples. Instead, we found a negative correlation of amino-acid like fluorophore F1 to salinity and temperature, and no clear enrichment of humic-acid like fluorophores F2, F3 and F5 in the SML. Therefore, we think that in the SML of the study region the contribution of terrestrially derived CDOM, if any, is overwhelmed by the high productivity of the upwelling system. Organics enriched in the SML such as the amino-acidic compounds F1 and F4 found at the upwelling stations may therefore reflect other processes rather than input of allochtonous CDOM from land." […]

4. RC: Finally, I am somewhat concerned about the authors' conclusions from Fig. 8, which depicts correlations of PARAFAC component F1 (tryptophane-like) and socalled 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

383 controlled irradiations [Dainard et al., 2015; C.A. Stedmon et al., 2007], but not with field
384 observations alone.
385 Secondly, comparing indicators of CDOM abundance to instantaneous irradiance neglects
386 the effects of photodegradation kinetics. For example, let's assume that any processes
387 other than photodegradation may be neglected over the diel cycle (sampling occurred
388 between sun rise and sun set). Then, CDOM bleaching would be expected to continue
389 throughout the entire photoperiod, leading to monotonically decreasing F1 and SUVA
390 during the day. That is, the highest CDOM levels should be present at sunrise (i.e. at
391 lowest global irradiance) and the lowest CDOM levels should be present at sunset (i.e.
392 also at lowest global irradiance). If anything, it would have made more sense to plot
393 CDOM indicators against time-integrated global irradiance, starting from the point of first
394 sampling on the day. There are of course cases where tightly coupled production &
395 consumption kinetics result in diurnal cycles (e.g. CO photoproduction and microbial
396 consumption [Doney et al., 1995], but these also show the maximum impact of
397 photochemistry in the late afternoon. Regarding CDOM, however, reported photobleaching
398 half lives in the order of days to weeks [Dainard et al., 2015; Moran et al., 2000] argue
399 against a pronounced diel cycle. I suggest that Fig. 8 should be removed, and that results
400 and discussion sections are amended accordingly.
401
402 AC: In the SML of stations with multiple measurements we observed a significant
403 decrease in F1 concentration due to radiation intensity ($R^2 = 0.56$, $p = 0.013$, $n = 10$, linear
404 regression), while such decrease was not observed for other fluorophores. We agree with
405 the referee's comment, as we cannot provide any indication of CDOM photodegradation
406 kinetics. It is also difficult to unravel photodegradation from other processes responsible
407 for decrease in fluorophores' concentration. However, we believe this strong correlation of
408 F1 with global radiation indicated by the regression analysis could be a clear indication of
409 F1 removal by photodegradation in the SML. Thus, we have removed the section and both
410 figures 8 and 9. In the main text we have discussed effects of photodegradation of F1,
411 referring also to processes mentioned by the referee, as we think it supports our
412 hypothesis. All figures after figure 9 have been renumbered.
413
414 Specific comments:
415
416 Abstract:

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

AC: we have modified the abstract including Figure 11 as graphical abstract, which summarizes our major findings. Figure 11 does not exist anymore.

References:

6. RC: A number of references are missing from the bibliography, starting with GESAMP, 1995 (Page19376, 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 have edited the manuscript and included all missing references. 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 changed the sentence to: “Sampling was performed on a rubber boat; in order to obtain a well standardized procedure and to minimize biases introduced by sampling, the same person always took the samples with a repeatable withdrawal speed of the SML.”

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 have included this information in the methods section. The new sentence now reads “Different devices can be applied to sample the SML. The glass plate approach we choose collects a thinner SML (~60 - 150 µm) when compared to i.e. the Garrett Screen (150 - 300 µm), one of the mainly recognized practices introduced by Garrett in 1965 (Cunliffe et al., 2011; Garrett, 1965). “

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.

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. We rephrased the sentence to "Absorbance is an optical characteristic of CDOM, which allows to quantify the amount of CDOM in the samples. Therefore, the absorption coefficient *a* is considered as a proxy for CDOM concentration. To estimate CDOM concentration, we calculated the absorption coefficient at 325 nm as often used for the open ocean (Swan et al., 2009;Nelson and Siegel, 2013)." We also rephrased the sentences related to CDOM and FDOM throughout the text, referring to high and low absorption and fluorescence (and not concentration).

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 have rephrased the sentence to:"Both *S*(275-295) and SR increase a) with irradiation (photobleaching), b) with decreasing DOM molecular weight, and c) at higher salinity reflecting mixing of water masses along a salinity gradient."

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-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

485 SUVA254 enrichment factors and SUVA254 correlations with fluorophores F1 and F3 do
486 not change dividing by 2.303 to reverse from absorption coefficient to Absorbance.
487 Corrected SUVA254 values are lower than we previously reported and comparable to
488 oceanic waters as indicated by Weishaar and colleagues (2013). We have corrected it in
489 the revised version throughout the text and in the supplementary tables accordingly.
490
491 12. RC: Page 19384, lines 14 ff. HIX. Similarly, please use a distinct acronym for your
492 modified HIX, as your wavelength ranges significantly depart from those in the original
493 paper by Zsolnay et al 1999.
494 AC: We have introduced the acronym “SMHIX”, where SM stands for Surface Microlayer
495 and thoroughly revised the text and the figures for the same acronym.
496
497 13. RC: Page 19385, lines 5 ff, Enrichment factors. Perhaps this is a matter of taste:
498 Enrichment factors (EFs) usually refer to a difference in abundance between SML and
499 ULW. However, HIX, spectral slopes and SR etc. are not measures of abundance, and
500 therefore I personally would prefer using something other than 'EF' to denote differences in
501 DOM properties between SML and ULW.
502 AC: We agree with the referee comment, that the term EF might not be appropriate for all.
503 However, to avoid confusion and introducing more parameters, we kept the same wording
504 for the same calculation and we have specified: “EFs are normally used for quantitative
505 parameters, i.e., that are measured in abundance and concentration such as DOC, DHAA,
506 CDOM, marine gels and cell abundances. Here, we applied the EF calculation for
507 qualitative ratios and indexes too, like S(275-295), SR, SMHIX, $SUVA_{254}$, DHAA-%DOC.
508 We kept the same wording, which is useful to describe differences between SML and ULW
509 for both quantitative and qualitative parameters.”
510
511 Results:
512
513 14. RC: Page 19387, line 1 ff. Socalled SUVA values: 0.6 mg C L-1 m-1 is
514 NOT“*comparable to riverine waters*”. This value was obtained with Pacific Ocean fulvi
515 acids [*Weishaar et al.*, 2003]. Please correct your statement.
516 AC: We have rephrased the sentence accordingly, also in light of the correction applied to
517 our previous calculation of SUVA254 (see comment 11).
518

519 15. RC: Page 19388, lines 24 ff: Origin of PARAFAC component F2: Please clarify why
520 you state that a positive correlation of F2 with SST and bacterial abundance might suggest
521 "*a refractory DOM component of terrestrial origin*" ??? My hunch would be that bacterial
522 abundance could well be related to primary production, which in your study area is likely to
523 be fuelled mainly by coastal upwelling? I also do not agree with your description of F2 as a
524 "refractory DOM component" resulting from either photochemical or microbial DOM
525 degradation. Please revise.
526 AC: We have rephrased the sentences, as in this setting terrestrial origin might not be the
527 appropriate description for F2 (see comment #3). We have rephrased the discussion (4.2).
528 However, we think that F2, being probably not anymore bioavailable, reflects the
529 fluorescence of highly degraded organic matter originated in the underlying
530 production/degradation processes brought up by upwelled waters. It may represent the
531 ultimate product of microbial or photochemical degradation happening in the sunlit ocean.
532
533
534 16. RC: Furthermore, positive correlations between F2 on the one hand and F3 and F5 on
535 the other does not necessarily support your statement that F2 is derived from these other
536 two FDOM fractions. Either all 3 fractions are formed by the same underlying process
537 (then I'd expect positive correlations between them), or F2 is formed from F3 & F5, that is
538 an F2 increase must cause decreases in F3 & F5. Please revise.
539 AC: we agree with the interpretation given by the referee that the components, showing
540 positive correlation, may derive from the same underlying processes, in this case microbial
541 reworking of larger components which can be still subject to photochemical degradation.
542 We have added this concept in the discussion, suggesting the concept of a progressive
543 photochemical and microbial alteration of DOM jointly with a local HMW-DOM microbial
544 release.
545 However, due to the size-continuum of DOM, and to the microbial life comprised in the
546 surface film and below, it may be not necessarily true either that an increase in F2 implies
547 a decrease in the other two fractions (F3 and F5); these compounds may be constantly
548 replenished by the complex biogeochemical processes of the upwelled waters.
549
550 17. RC: Page 19391, lines 19 ff, Mycosporine like amino acids: The authors try to link
551 mycosporine like amino acids (MAAs) to their CDOM characteristics. This is rather
552 speculative. Besides, MAAs are not of high molecular weight as stated here. This section

553 does not add value to the results section and should be deleted.
554 AC: The referee is right that MAAs are LMW and not HMW. The point here, is to find
555 supporting evidence for our hypothesis of a local microbial release of protein-like DOM as
556 a response to high solar radiation. We do that by referring to available studies on SML
557 optical properties, which are really scarce, and on microbial response to UV radiation. We
558 agree that this paragraph is not well suited for the results section and has been moved to
559 the discussion, paragraph 4.2.
560
561 Discussion:
562
563 18. RC: Page 19393, lines 20 ff: SR, DHAA%, and "lability": Please spell out how SR and
564 DHAA% support the notion of a labile DOC fraction in the SML. Are you referring to lability
565 with respect to microbial consumption or with respect to photochemical degradation?
566 AC: We have added a correlation table (table 5, described in the results section) also
567 retrieving data on POC from Engel and Galgani (2016). Plus, we have added the following
568 text (Discussion, 4.1): "According to Helms et al. (2008), an increase in *S*(275-295) and
569 SR suggests DOM photodegradation and decreasing molecular weight. DHAA%-DOC is
570 used here as an indicator for DOM diagenesis, thus, the extent of microbially altered DOM.
571 The higher DHAA%-DOC, the more labile, bioavailable, recent and less altered DOM in
572 the sample. We observed a negative correlation when comparing DHAAA%-DOC and
573 POC to *S*(275-295) and to SR. The higher DHAA%-DOC, the lower *S*(275-295) and SR.
574 Microorganisms adopt several strategies against tough environments; the correlation
575 between DHAA%-DOC to *S*(275-295) and SR was stronger in the SML than in the ULW,
576 suggesting an accumulation of HMW-DOM related to the contribution of microorganisms
577 directly in the SML or in the proximity due to cell lysis or exudation, which has been
578 previously proposed (Tilstone et al., 2010). Thus, the close correlations of optical
579 parameters to POC and marine gels lead to hypothesize that autochtonous CDOM
580 produced in the very surface ocean can actually be incorporated in the gelatinous organic
581 carbon pool."
582
583 19. RC: Page 19394, lines 16 ff, nitrous oxide binding to aromatic groups. The authors
584 refer to Cao et al. (2015) who found evidence for the formation of complexes between
585 nitrous oxide and some mono-aromatics. However, Cao's study was conducted in a Ne
586 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. We rephrased the sentence (Discussion, section 4.2.): "A recent laboratory study reported π non-covalent interactions between N_2O and phenolic groups in phenylalanine and tyrosine (Cao et al., 2014). Although performed in a setting non-comparable to our study area, these findings may suggest an interaction of N_2O with biological macromolecules enriched in the SML of the Peruvian EBUS, and thus with the exchange of N_2O between the ocean and the atmosphere (Engel and Galgani, 2016)."

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 have rephrased this part: "In this study we did not find a correlation of F2 to global radiation but a positive correlation to temperature and to bacterial abundance (Table 4)."

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 which may occur because of the high radiation received by the SML. We have revised the last part of the discussion (4.2).

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. As we have previously 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 have addressed the referee's

655 comment by revisiting the section and avoiding repetitions.
656
657
658 The following figures were changed:
659
660 Fig. 2, 3, 4, 6, 7: Station labels were added
661
662 Original figures 8 and 9 were omitted.
663

Changes in optical characteristics of surface microlayers hint to photochemically and microbially-mediated DOM turnover in the upwelling region off the coast off Peru

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Abstract

The coastal upwelling system off Peru is characterized by high biological activity and a pronounced subsurface oxygen minimum zone, as well as associated emissions of atmospheric trace gases such as N_2O, CH_4 and CO_2. During the METEOR (M91) cruise to the Peruvian upwelling system in 2012, we investigated the composition of the sea-surface microlayer (SML), the oceanic uppermost boundary directly subject to high solar radiation, often enriched in specific organic compounds of biological origin like Chromophoric Dissolved Organic Matter (CDOM) and marine gels. In the SML, the continuous photochemical and microbial recycling of organic matter may strongly influence gas exchange between marine systems and the atmosphere. We analyzed SML and underlying water samples at 38 stations focusing on CDOM spectral characteristics as indicator of photochemical and microbial alteration processes. CDOM composition was characterized by spectral slope (*S*) values and Excitation-Emission Matrix fluorescence (EEMs), which allow to track changes in molecular weight (MW) of DOM, and to determine potential DOM sources and sinks. We identified five fluorescent components (F1-5) of the CDOM pool, of which two had excitation/emission characteristics of amino-acid like fluorophores (F1, F4) and were highly enriched in the SML. CDOM composition and changes in spectral slope properties suggested a local microbial release of DOM directly in the SML as a response to light exposure in this extreme environment. In the conceptual model **of the sources and modifications of optically active DOM in the** SML and underlying seawater (ULW), we describe processes we think may take place (see graphical abstract). The production of C**DOM** of probably higher MW **by microbial release through growth, exudation and lysis in the euphotic zone,** includes the identified **fluorophores (F1, F2, F3, F4, F5). Specific amino-acid like fluorophores (F1, F4) accumulated in the SML with respect to the ULW,** as p**hotochemistry may enhance microbial CDOM release by a) photoprotection mechanisms and b) cell-lysis processes. Microbial and photochemical degradation are potential sinks of the amino-acid like fluorophores (F1, F4), and potential**

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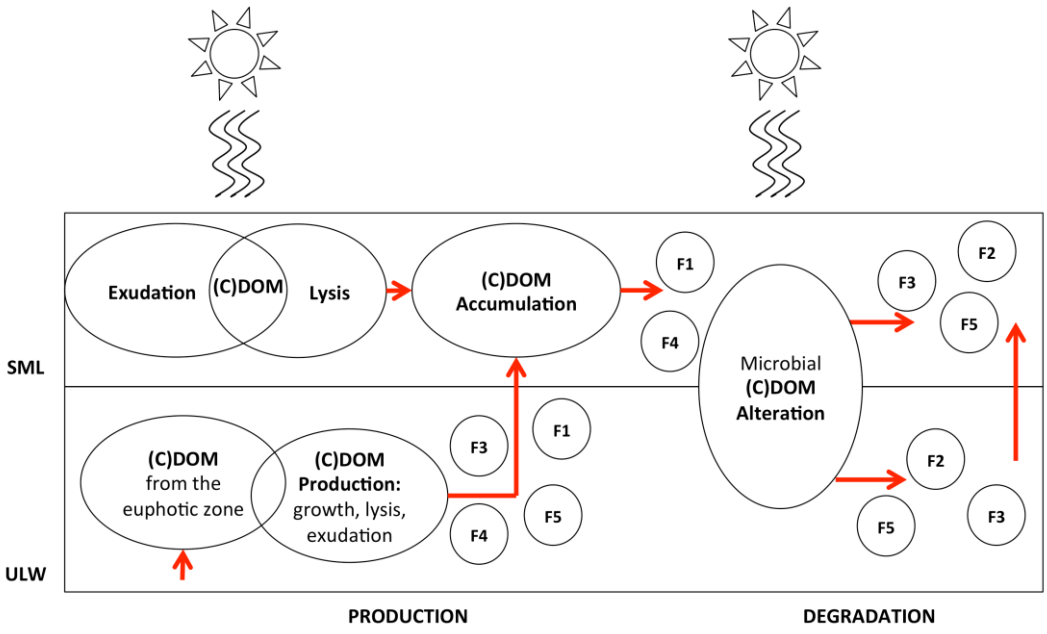
sources of reworked and more refractory humic-like components (F2, F3, F5). In the highly productive upwelling region along the Peruvian coast, the interplay of microbial and photochemical processes controls the enrichment of amino-acid like CDOM in the SML. We discuss potential implications for air-sea gas exchange in this area.



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1. Introduction

The Peruvian Eastern Boundary Upwelling System (EBUS), extending along the coast off Peru between 4° and about 40° South, is among the most productive marine ecosystems worldwide (Capone and Hutchins, 2013;Chavez and Messié, 2009;Rosenberg et al., 1983) and it is characterized by high biological activity, involving high export rates of organic carbon both vertically and laterally (Arístegui et al., 2004;Muller-Karger et al., 2005). The high productivity is sustained by winds year-round that promote the upwelling of nutrient-rich deep waters into the euphotic zone, thus favoring phytoplankton photosynthesis and organic matter production (Chavez and Messié, 2009). High rates of organic matter production are counterbalanced by heterotrophic

737 respiration, which provides sinks for the oxygen produced by autotrophs and leads to subsurface
738 Oxygen Minimum Zones (OMZs) (Lachkar and Gruber, 2011). OMZs are expanding worldwide
739 due to reduced solubility at increasing temperatures, as well as a consequence of reduced oceanic
740 ventilation and enhanced stratification (Keeling et al., 2010;Stramma et al., 2008). OMZ become
741 increasingly important as key marine regions for the emission of climate-relevant gases like carbon
742 dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and hydrogen sulfide (H_2S) (Paulmier et al.,
743 2008;Paulmier et al., 2011). N_2O is a strong greenhouse gas and ozone-reactive: 30% of its
744 atmospheric concentration has an oceanic source (Solomon et al., 2007), of which, up to 75% is
745 supported by OMZs (Bange et al., 2001). Therefore, OMZs are key environments to assess the
746 oceanic contribution to the concentration of atmospheric gases. Defining the processes that regulate
747 gas fluxes across the water-air interface is a central objective to better understand the reciprocal
748 relationship between changes in our climate and marine environments.
749 The uppermost oceanic layer in contact with the atmosphere is the sea-surface microlayer (SML),
750 which mediates major climate-relevant processes including air-sea gas exchange and sea-spray
751 aerosol emission (Liss and Duce, 2005). This interface between a liquid (hydrosphere) and a gas
752 phase (atmosphere) accumulates organic matter of biological origin, creating a sort of "skin" of
753 surface-active compounds able to damp capillary waves and "capping the flux" of gases across the
754 water-air interface (GESAMP, 1995). Natural organic compounds in the SML include a vast array
755 of photosynthesis products including carbohydrates, amino acids and lipids, as well as other carbon-
756 rich compounds like dissolved organic matter (DOM) and marine gels (e.g. Cunliffe et al., 2013).
757 The DOM pool represents a continuum of molecular weights and biological lability ranging from
758 refractory to labile DOM being utilized rapidly by microorganisms (Benner, 2002;Carlson, 2002),
759 or photochemically degraded (Kieber, 2000). These compounds, produced in the oceanic photic
760 zone and brought to the SML through rising bubbles (Hardy, 1982), contribute to the enrichment of
761 a natural surface biofilm and favor specific SML heterotrophic communities that are very active in
762 recycling this organic material (Hardy, 1982;Cunliffe et al., 2011). While bulk dissolved organic

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765 carbon is not generally enriched in the SML, specific DOM fractions are present occasionally at
766 much higher concentrations than in the underlying water (Cunliffe et al., 2013). These enriched
767 pools of organic matter include marine gel particles (Wurl and Holmes, 2008), chromophoric
768 dissolved organic matter (CDOM) (Zhang and Yang, 2013;Tilstone et al., 2010) and phenolic
769 material (**Carlson, 1982;**Carlson and Mayer, 1980).
770 CDOM is the principal light-absorbing constituent of DOM, strongly absorbing UV (100 - 400 nm)
771 and visible radiation (400 - 700 nm), and it can comprise 20%-70% of the DOM in oceanic waters
772 (Coble, 2007). CDOM plays a major role in the attenuation of UV wavelengths and can reduce the
773 availability of underwater photosynthetically active radiation for primary production (Bracchini et
774 al., 2011). Photolysis of CDOM promotes the formation of low molecular weight (LMW)
775 compounds from the breakdown of high molecular weight DOM (HMW-DOM), facilitating the
776 bioavailability of carbon uptake for microbial growth from biologically refractory material, and
777 representing an important loss pathway for CDOM in the oceans (Kieber et al., 1989). Other major
778 by-products of CDOM photolysis are carbon monoxide (CO), which often exists at supersaturated
779 concentrations in the oceans' surface (Blough, 2005, and references therein), CO_2 (Miller and Zepp,
780 1995) and reactive chemical species (Loiselle et al., 2012). To initiate a photochemical reaction,
781 light must first be absorbed and in this respect the SML is very well exposed to elevated solar
782 radiation (Liss and Duce, 2005). CDOM photolysis may affect biological processes within the SML
783 as well as the structure of accumulated organic matter. Optical properties and photochemical
784 cycling of DOM have been widely investigated in the oceans: CDOM alters light spectra in the
785 surface ocean and its spatial and temporal distribution have been used in characterizing water
786 masses exchange (Nelson and Siegel, 2013). However, processes within the SML remain poorly
787 understood. Possible effects of photochemistry on SML chemical composition have been discussed
788 in the past (Blough, 2005), but still little is known on CDOM fluorophores, sources and sinks
789 (Tilstone et al., 2010;Zhang and Yang, 2013). To discern sources, sinks and modification of DOM
790 in surface waters, whether microbially or photochemically-induced, we investigated optical

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properties of organic sea-surface microlayers and underlying water samples in the highly productive EBUS off Peru. We applied optical spectroscopy measurements combined with chemical and biological analysis to identify different compounds within the CDOM pool and their partitioning between the SML and the underlying water. The use of excitation-emission matrix fluorescence spectroscopy (EEMs) allowed us to discriminate different compound classes in the SML and underlying water based on their excitation and emission maxima (Coble, 1996).

At present, the oceans are subject to many changes in physical and chemical properties like pH, temperature, and dissolved oxygen concentration, which potentially will affect the biological cycling of carbon (Riebesell et al., 2009;Keeling et al., 2010;Bopp et al., 2002). Whether the oceans are sources or sinks of carbon depends on the production rate of organic matter with respect to its biological degradation (Del Giorgio and Duarte, 2002), and high DOM degradation in the SML might represent a net source of CO_2 to the atmosphere (Garabétian, 1990). It is well known that the composition of the SML reflects biological processes of the euphotic zone (Galgani et al., 2014;Gao et al., 2012;Matrai et al., 2008;Bigg et al., 2004), and that elevated concentrations of organic matter may accumulate in the SML in highly productive regions like the Peruvian EBUS (Engel and Galgani, 2016). The enrichment of light-absorbing DOM in the SML may increase the photochemical formation and fluxes of reactive chemical species at the surface, with potentially important consequences for the composition of the SML itself and for the fate of compounds passing through this interface (Blough, 2005). Last but not least, the photochemical DOM breakdown may increase the biological availability of carbon, thus increasing heterotrophic respiration and CO_2 flux to the atmosphere.

CDOM contributes to the dissolved organic carbon (DOC) pool, but while DOC is a bulk measure, CDOM is a characteristic of DOM rather than a discrete class of compounds (Nelson and Siegel, 2013). Positive correlations have been observed between CDOM and DOC in coastal systems and plankton enclosures (Loginova et al., 2015), but the strenght of these correlations varies much across regional and seasonal differences (Blough and Del Vecchio, 2002). CDOM is a precursor for

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photochemical reactions that may drive the emission of trace gases from photochemically-altered DOM (e.g. Ciuraru et al., 2015). Therefore, in upwelling areas associated with OMZs, CDOM characteristics in the SML are worth to be investigated as they may impact the exchange of gases between the ocean and the atmosphere.

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2. Material and methods

2.1. Study area

The R/V METEOR cruise M91 was an integrated biogeochemical study in the upwelling region off Peru, with the aim to assess the importance of oxygen minimum zones (OMZs) for the air-sea exchange of gases relevant for climate and tropospheric chemistry (Bange 2013). A total of 39 samples for SML and underlying water were collected in December 2012 between 5°S and 16°S off the Peruvian coast. Data that we report here additionally from what previously described by Engel and Galgani (2016) refer to 38 stations. For easiness of comparison, table 1 recalls salinity, water temperature, radiation and wind speed, as already described in the companion manuscript (Engel and Galgani, 2016).

Some stations were revisited for multiple sampling (Table 2): S7 and S7_2; S12_1, S12_2, and S12_3; S16_1, S16_2, S16_3; S20 and S20_2. These stations were sampled within a time frame of 24 hours for SML and ULW, as we were interested in monitoring the evolution of CDOM optical properties in the SML and ULW at different times of the day depending on the solar irradiation. Whenever possible, we sampled at sunrise, midday and sunset. For security reasons, it was not possible to sample later than sunset, as the zodiac operations were not allowed out at dark. Exact latitude and longitude were not always possible to retrieve after a certain time, but were similar for the stations sampled in a few hours time lag.

The sampling approach for the SML was chosen as a silicate glass plate of 500 mm (length) x 250 mm (width) x 5 mm (thickness) with an effective sampling area of 2000 cm^2 as indicated in Engel and Galgani (2016). Briefly, the glass plate was inserted into the water perpendicular to the surface

and withdrawn at a controlled rate of ~ 20 cm s^{-1} as first suggested by Harvey and Burzell (1972). Different devices can be applied to sample the SML. The glass plate approach we choose collects a thinner SML (~60 - 150 µm) when compared to i.e. the Garrett Screen (150 - 300 µm), one of the mainly recognized practices introduced by Garrett in 1965 (Cunliffe et al., 2011; Garrett, 1965). The glass plate was chosen because it allows the sampling of enough volume required for analysis while keeping a minimal dilution with underlying water. Sampling was performed on a rubber boat; in order to obtain a well-standardized procedure and to minimize biases by sampling, the same person always took the samples with a repeatable withdrawal speed of the SML. The rubber boat was positioned as far upwind of the ship as possible and away from the path taken by the ship in order to avoid any potential surface contamination. The outboard motor of the rubber boat was switched off and samples were collected in upwind clean waters.

Before collecting the sample into the bottle, we let the plate drain for 20 s approximately. Then, the sample retained on both sides of the plate was removed with a Teflon wiper, and the procedure repeated about 20 times to obtain the necessary volume for analysis. The exact amount of dips per sample has been tracked. The first sample was discarded and used to rinse the collecting bottle (HCl 10% cleaned and Milli-Q rinsed). Glass plate and wiper were acid cleaned (HCl 10%) and Milli-Q rinsed prior use, and at sampling site they were copiously rinsed with in situ seawater to minimize any contamination with alien material during transport and handling. Underlying seawater (ULW) was collected right after SML at about ~ 20 cm depth by opening an acid cleaned (HCl 10%) and Milli-Q rinsed glass bottle and closing it underwater. The thickness (d, m) of our reference SML that we were able to collect was estimated as follows:

$$d = V/(A \times n) \quad (1)$$

Where V is the SML volume collected, i.e. 60-140 mL, A is the sampling area of the glass plate (A = 2000 cm^2) and n is the number of dips. During this cruise, the apparent sampling thickness of the SML ranged between 45 and 60 µm, with an overall mean of 49 ± 8.9 µm (Engel and Galgani, 2016). Many factors may influence the thickness of the SML such as withdrawal rate, dipping time,

and plate dimensions. With a withdrawal speed of ~ 20 cm s^{-1}, the apparent SML thickness was in accordance with previous findings at similar withdrawal rate reporting 60 – 100 µm (Harvey and Burzell, 1972) and 50 – 60 µm (Zhang et al., 1998). The sampling thickness was very well comparable among all stations, indicating that no major biases due to sampling procedure may have occurred.

After sampling, bottles were stored in the dark and the samples immediately processes in the laboratory onboard, within maximum 30 minutes from sampling.

2.2. Chemical and biological analyses

Dissolved organic matter (DOM): Sampling, calibration and analysis procedure for dissolved organic carbon (DOC) and for dissolved hydrolysable amino acids (DHAA), have been described in details in Engel and Galgani (2016). Additionally, to track DOM diagenetic state and bioavailability, we used the carbon-normalized yields of dissolved amino acids to DOC, expressed as DHAA%-DOC (Amon and Fitznar, 2001;Benner, 2002;Kaiser and Benner, 2009;Davis and Benner, 2007). Amino acids generally comprise a large fraction of bioavailable organic matter and are preferentially consumed by microbial activity quite rapidly. In surface waters they may be easily photodegraded too. Therefore, the amount of carbon included in amino acids is considered as a good indicator of DOM diagenesis and a value of ~ 2% of DHAA%-DOC may indicate the threshold between labile and semi-labile and refractory DOM (Davis and Benner, 2007).

Samples for chromophoric and fluorescent DOM (CDOM and FDOM) were filtered through 0.45 µm PES syringe filters and collected into 40 mL pre-combusted (8 h, 500° C) amber glass vials. Samples were stored in the dark at 4° C with no other treatment than pre-filtering. Since storage procedures may affect the absorbance and fluorescence properties of DOM, absorbance and fluorescence readings were performed directly on-board within a few hours from sampling or the next day according to Schneider-Zapp and colleagues (2013). Prior to measurements, samples were stored in the dark and acclimatized at room temperature. For CDOM, triplicate absorbance

measurements were made on a Shimadzu 1800 UV-Visible Spectrophotometer in the range 220 to 700 nm with 0.5 nm increments, in a 10 cm path-length quartz cuvette against Milli-Q water as a reference. For FDOM, 3-D fluorescence spectroscopy was performed with a Varian Cary Eclipse Fluorescence Spectrophotometer equipped with a xenon flash lamp and data assembled into Excitation/Emission matrices (EEMs) which enable to individuate single DOM fluorophores (Coble, 1996) and to perform parallel factor analysis PARAFAC (Stedmon and Bro, 2008). Samples have been acclimatized and scanned at a fixed 20°C temperature (Cary Single Cell Peltier Accessory, VARIAN) in 1 cm path length quartz cuvette. Scans were performed at 600 nm/min using an excitation range (Ex) of 240-450 nm with 5 nm increments and recorded emission (Em) in the range 242-600 nm with 2 nm increments. Samples were run in a mode of 5 nm slit for both excitation and emission and 0.1 s integration time.

Particulate Organic Carbon (POC) and gel particles: Total numbers of gel particles were determined by microscopy after Engel (2009). A detailed description of the method used during M91 cruise can be found in Engel and Galgani (2016). POC data were retrieved after Engel and Galgani (2016). We refer to this companion publication for further analytical details.

Phytoplankton and heterotrophic bacteria: Samples, calibration and analysis for phytoplankton and heterotrophic bacteria counts for M91 are described in details in Engel and Galgani (2016).

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2.3. Data analysis

CDOM: The measured absorbance at every wavelength λ was converted to absorption coefficient $a(\lambda)$, (m^{-1}), according to the following equation (Bricaud et al., 1981):

(2) $a(\lambda) = 2.303A_\lambda/L$

where A_λ is the absorbance and L is the path-length of the cuvette (here 0.10 m). Absorbance is an optical characteristic of CDOM, which allows quantifying the amount of CDOM in the samples. Therefore, the absorption coefficient $a(\lambda)$ is considered as a proxy for CDOM concentration. To estimate CDOM concentration, we calculated the absorption coefficient at 325 nm as often used for

934 the open ocean (Swan et al., 2009;Nelson and Siegel, 2013). The dependence of *a* on the
935 wavelength was determined by analyzing the spectral slope parameter *S* (nm^{-1}) in the discrete
936 wavelength ranges of 275-295 nm and 350-400 nm, determined by linear regression of log-
937 transformed absorption spectra against the wavelength (Bricaud et al., 1981;Helms et al., 2008):

938 (3) $a(\lambda) = a(\lambda_0)e^{-S(\lambda - \lambda_0)}$

939 where $a(\lambda_0)$ is the absorption coefficient at a reference wavelength λ_0. *S* measured in the wavelength
940 range 275-295 nm (*S*(275-295), nm^{-1}) and 350-400 nm (*S*(350-400), nm^{-1}) as well as slope ratio
941 (SR) defined as *S*(275-295): *S*(350-400) are good indicators to characterize CDOM (Helms et al.,
942 2008). SR is characterized by lower values for terrestrial CDOM compared to CDOM produced by
943 autochthonous marine sources and instead of *S* alone, could be a more sensitive indicator of
944 photochemically induced changes in the molecular weight of the CDOM pool as an increase in SR
945 suggests photodegradation processes, while a decrease is often related to microbially altered CDOM
946 (Helms et al., 2008). Both *S*(275-295) and SR increase with a) irradiation (photobleaching), b) with
947 decreasing DOM molecular weight, and c) at higher salinity reflecting mixing of water masses
948 along a salinity gradient. As such they are useful as tracers to determine mixing and coastal inputs.
949 We also determined the $SUVA_{254}$ index, that is, the specific ultraviolet absorbance (*A*) at 254 nm
950 normalized to DOC concentration. This index was shown to correlate significantly with increasing
951 aromaticity of DOM (Weishaar et al., 2003):

952 (4) $SUVA_{254}$ ($mg\ C\ L^{-1}\ m^{-1}$) = *A*(254) (m^{-1})/ DOC ($mg\ L^{-1}$)

953 **FDOM:** The 3-D recorded spectra were corrected for the instrumental biases both for excitation
954 and emission using correction curves provided by the manufacturer (Stedmon and Bro, 2008).
955 Additionally, spectra were corrected against a Milli-Q water blank run every day before the samples
956 to remove water Raman peaks. No correction for inner filter effects was applied to our data as for
957 each sample the relative $a(\lambda)$ value was below 10 m^{-1} (Lawaetz and Stedmon, 2009;Stedmon and
958 Bro, 2008). As an example, *a*(254) was on average 2±2 m^{-1} for SML and 1.6±1.3 m^{-1} for underlying
959 water (ULW) samples. Fluorescence spectra were normalized to Raman Units (R.U.) by integrating

960 the Raman peak of 350 nm Ex and 382 to 407 nm Ex extracted by the daily Milli-Q water blank.
961 Calibration to R.U. was done with the FDOMcorrect toolbox for Matlab (The MathWorks Inc.)
962 incorporated in DrEEM toolbox (Murphy et al., 2013). We choose to normalize to R.U. as these
963 units are widely used in open ocean measurements and we could compare our results.
964 PARAFAC analysis was applied to EEMs in order to identify and quantify independent underlying
965 components of the CDOM pool, and was performed by the N-way toolbox for Matlab in DrEEM
966 (Murphy et al., 2013). After normalization to R.U. units, data were smoothed to remove remaining
967 scatter peaks, Raman and Rayleigh signals by creating a sub-dataset. We then performed a
968 preliminary outlier analysis generating models with 3 to 7 factors with non-negativity constraints,
969 comparing the spectra to unconstrained models. When dilution dominates the dataset, components
970 are strongly correlated. To investigate biases due to dilution, we performed a test for correlations
971 between the components, as suggested by the DrEEM tutorial by Murphy and colleagues (2013).
972 We then normalised the dataset by the DrEEm function *normeem* to reduce the co-linearity related
973 to the concentration, thus giving low-concentrated samples a possibility to enter the model,
974 followed by the outlier test again on the normalised data. After visually comparing the spectra and
975 looking at the error residuals for models with 4 to 7 components, we then compared the models by
976 the sum of squared errors (SSE) expressed as a function of wavelength, choosing the models with
977 lower SSE. At this stage, we choose models with 5, 6 and 7 components and reversed the
978 normalization to obtain the unscaled scores before validation. Models with 5, 6 and 7 components
979 were validated by split half analysis "$S_4C_6T_3$" (see Murphy et al. 2013) where it was ensured that in
980 each test the dataset halves being compared had no samples in common. The validation was
981 successful for 5-components model, for all comparison. The maximum fluorescence intensities of
982 the five fluorophores at specific Ex-Em wavelengths ranges are described in table 3. Figures with
983 the model comparison for both excitation and emission for the 5-components model are included in
984 the supplementary material (Figures S1 and S2).

In fluorescence spectroscopy, the humification index (HIX), first introduced by Zsolnay et al. (1999), is a powerful tool to study CDOM dynamics in soils, as humification is associated with a shift to longer emission wavelengths (Senesi, 1990). It has been first applied to aquatic CDOM in estuarine waters by Huguet and colleagues (2009), and is calculated as the ratio H/L of two spectral region areas of the emission spectrum scanned at 254 nm excitation. Area L is calculated between the emission wavelengths 300 nm and 345 nm, and area H between 435 nm and 480 nm. When the degree of aromaticity of CDOM increases, the emission spectrum at excitation 254 nm is shifted towards the red (longer wavelengths), implying an increase in H/L ratio and in HIX. High HIX implies maximum fluorescence intensity at long wavelengths and therefore the presence of complex molecules like HMW aromatic CDOM (Senesi et al., 1991). We applied a slight modification to the HIX index for our samples, introducing the "SMHIX" index, where SM stands for Surface Microlayer. As we did neither have the scanned excitation wavelength of 254 nm, nor the scanned spectrum at excitation 345 nm and 435 nm, we calculated SMHIX index as follows:

(5) SMHIX = $(\sum I_{434\rightarrow480})/\sum I_{300\rightarrow346})$

Where $\sum I_{434\rightarrow480}$ is the sum of all fluorescence intensities at every emission wavelength between 434 nm and 480 nm, and $\sum I_{300\rightarrow346}$ is the sum of all fluorescence intensities at every emission wavelength between 300 nm and 346 nm, both scanned with excitation = 255 nm.

Enrichment Factors: Enrichment Factors (EF), allow tracking of accumulation patterns of any compound in the SML with respect to the underlying water (ULW) and comparison among different compounds. EF are calculated according to the following:

(6) $EF = [X]_{SML} / [X]_{ULW}$

Where [X] is the concentration of a given parameter in the SML or ULW, respectively (GESAMP, 1995). EF > 1 indicates an enrichment, EF < 1 indicates a depletion in the SML. EFs are normally used for quantitative parameters, i.e., measured in abundance and concentration such as DOC, DHAA, CDOM, marine gels and cell abundances. Here, we applied the EF calculation for qualitative ratios and indexes too, like *S*(275-295), SR, SMHIX, $SUVA_{254}$, DHAA%-DOC. We

kept the same wording, which is useful to describe differences between SML and ULW for both quantitative and qualitative parameters.

Statistical tests in data analysis have been accepted as significant for $p < 0.05$. Calculations, statistical tests and illustration were performed with Microsoft Office Excel 2010, Sigma Plot 12.0 (Systat), Prism (GraphPad), Ocean Data View and Matlab R2009b (The MathWorks Inc.).

3. Results

Results on dissolved organic carbon and amino acids, gel particles (TEP and CSP), phytoplankton and bacterial abundance and the relative enrichment of these components in the SML of our sampling sites have been described elsewhere (Engel and Galgani, 2016). Here, we focus on the optical properties of DOM to identify possible sources, sinks and dynamics in the SML and underlying water of the Peruvian upwelling region.

3.1. CDOM optical absorption properties

In the upwelling region off Peru, values for CDOM absorption coefficient $a(325)$ ranged from 0.09 to 1.47 m^{-1} in the SML and from 0.07 to 1.47 m^{-1} in ULW. Highest values were observed at stations S10_1 to S10_4 along the coast for both SML and ULW. CDOM was enriched in the SML at most stations (Figure 3), with median EF for $a(325)$ = 1.2 in a range varying between 0.4 and 2.8. A median EF = 1.2 means that at least 50% of our observations accounted for a CDOM-enriched SML. Besides the southern transect, higher EF values were observed at the northern stations S2 and S2_2, and in the southern coastal upwelling stations S15_1 to S15_3. Lower EFs and EFs < 1, indicating a depletion of CDOM in the SML, were observed at higher distance from the coast (Figure 4).

The spectral slope parameter between 275 and 295 nm ($S(275\text{-}295)$, nm^{-1}) is a good indicator for CDOM molecular weight as an increase of this parameter indicates decreasing molecular weight, thus revealing accumulation or degradation processes of bioavailable CDOM (Helms et al., 2008).

In our samples, *S*(275-295) ranged from 0.012 to 0.038 nm^{-1} in the SML and from 0.017 to 0.043 nm^{-1} in ULW. In general, *S*(275-295) was quite similar between SML and ULW, and no statistically significant differences were found between SML and ULW for *S*(275-295). Higher spectral slopes were observed in the ULW of the southern stations below 15°S (S19, S19_2, S20, S20_2, S1778). In the coastal stations S10_1 to S10_4 and S14_1 to S15_3 lower *S*(275-295) values were determined for both SML and ULW. Median enrichment factor (EF) for *S*(275-295) was 1 (Figure 3), thus indicating similar molecular weight of CDOM compounds in the SML and ULW. Lower EFs were observed in the northernmost and southernmost stations and along the coast.

The $SUVA_{254}$ and SMHIX indexes are related to the degree of CDOM aromaticity and to its humic content, respectively. In our study, SUVA254 ranged from 0.49 to 1.74 mg C L^{-1} m^{-1} in the SML, with highest values at coastal southern stations S10_1 to S10_4 and S14_1 to S17_2. Similar values were recorded for ULW, ranging from 0.49 to 1.21 mg C L^{-1} m^{-1}. Generally, $SUVA_{254}$ values in our samples were comparable to the Pacific Ocean with a typical $SUVA_{254}$ of 0.6 mg C L^{-1} m^{-1} (Helms et al., 2008;Weishaar et al., 2003). Median EF for $SUVA_{254}$ was 1.1, with higher values in correspondence of northern stations and coastal southern stations (S2, S2_2, S15_1 to S15_3 and S19 to S1778) where the higher EF for *a*(325) were also observed (Figures 3 and 4). SMHIX ranged from -1.33 to 2.05 for SML and from -0.1 to 3.03 for ULW, with highest values in ULW. Enrichment factors showed an overall depletion of high-humic acid containing CDOM in the SML (Figure 3), with median EF = 0.8. Higher humic acid enrichment in the SML was observed on the southern transect S19 to S1778 (Figure 4), where we recorded the highest enrichment of CDOM (as *a*(325)) as well.

The carbon-normalized yields of dissolved amino acids (DHAA%-DOC) as indicator of DOM diagenetic state, ranged from 1.4% to 8.1% in SML samples and from 0.9% to 3.6% in ULW samples, indicating relatively more labile DOM in the SML. This observation was supported by the enrichment factors (EF), which showed a general enrichment of more labile DOM in the SML

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(Figure 3), with median EF values for DHAA%-DOC of 1.5. Highest EFs were recorded in the northernmost stations S1 to S3, and on the southernmost transect S19 to S1778.

3.2. PARAFAC analysis for CDOM fluorophores

Five optically active components were identified by PARAFAC analysis with the DrEEM toolbox in Matlab (Murphy et al., 2013), hereafter named F1, F2, F3, F4 and F5 (Figure 5). The spectral characteristics of the five identified components were compared to previous studies as described in table 3. F1 had an excitation range of 250-290 nm with emission peaks between 320 and 350 nm, which corresponds to peak T of the amino-acid like fluorescence of tryptophan, derived by *in-situ* primary production (Coble, 1996). This component (F1) was generally enriched in the SML (Figures 6, 7) with a median EF = 1.5, between a minimum EF of 0.5 and a maximum EF of 3.3. Potential loss processes of this compound are its destruction by UV light and microbial degradation (Stedmon and Markager, 2005b). F1 has also been related to protein-like fluorescence of extracellular polymeric substances (Liu and Fang, 2002). Fluorescence intensities of F1 were the lowest compared to the other fluorophores, but significantly higher in the SML compared to the ULW (Mann-Whitney Rank Sum Test, $p < 0.001$, $n = 38$). Both in SML and ULW, fluorescence intensities of F1 were positively correlated to components F3, F4 and F5 (Spearman Rank Order Correlation coefficient $C = 0.37$, $p < 0.001$, $n = 76$ with F3; $C = 0.41$, $p = 0.001$, $n = 57$ with F4; $C = 0.38$, $p < 0.001$, $n = 76$ with F5).

Component F2 had a short wavelength excitation range (250-260 nm) with emission at longer wavelengths (500-520 nm), corresponding to peak A of fulvic acids and humic acids (Stedmon and Markager, 2005a;Singh et al., 2010;Yamashita and Jaffé, 2008;Coble, 2007;Santín et al., 2009). F2 showed a regional enrichment in the SML, with highest values at the northernmost stations S2 to S3 and at stations S10_1 to S10_4 (Figure 6). F2 enrichment was not ubiquitous (Figure 7), with median EF = 1, ranging from a minimum EF = 0.5 and a maximum EF = 3.6. F2 positively correlated with bacterial abundance and temperature (Table 4) and to F3 and F5 components

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Gelöscht: protein-like

Gelöscht: Based on its excitation/emission maxima and fluorescence intensities, and its relationship to other parameters in this study (table 3), we can assume F1 as a tryptophan-like fluorophore, originating by *in situ* primary production, relatively labile and included in the formation of gel particles. Its negative correlation to SR may hint to a loss of F1 during microbial processing, as a decrease in SR suggests microbially reworked CDOM (Helms et al., 2008).

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Gelöscht: Component F2 has been characterized as of terrestrial origin, allochtonous in marine environments, and was found in bays, rivers and coastal waters. It is assumed to reflect small-sized molecules, being resistant to photodegradation, biologically not available, and mainly derived from photobleached terrestrial humic acids in marine waters with highest concentrations near the surface (Ishii and Boyer, 2012).

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Gelöscht: Highest SML concentrations of F2 were observed at the northernmost stations S2 to S3 (Figure 7).

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(Spearman Rank Order Correlation coefficient C = 0.74, p < 0.001, n = 76 with F3, and C = 0.71, p < 0.001, n = 76 with F5).

Component F3 was characterized by a clear excitation peak at 265 nm, with emission maxima in the longer wavelength range 520-540 nm. Component F3 showed a median EF = 1.1 (minimum EF = 0.3, maximum EF = 4.7), indicating a slight enrichment in the SML (Figure 7), with higher accumulations close to the coast at stations S19_2 to S1778 and at the edge of the continental shelf at stations S4 and S8 (Figure 6), in correspondence with the highest enrichment of gel particles in the SML (Engel and Galgani, 2016). In our study F3 was positively correlated with the abundance of bacteria, proteinaceous particles and increasing $SUVA_{254}$ (Table 4). It showed an inverse correlation to salinity (Table 4). Besides F1 and F2, F3 was significantly correlated to F5 (Spearman Rank Order Correlation coefficient C = 0.87, p < 0.001, n = 76).

Component F4 was not detectable at all stations, but showed high enrichment in the SML close to the coast and along the continental shelf at stations S10_1 to S10_4, S13_1 to S13_3, S14_1 to S15_2 (Figure 6). F4 was generally enriched in the SML (Figure 7) with median EF = 1.5 (in a minimum-maximum EF range of 0.4 – 14.9) and with significant differences in fluorescence intensity compared to the ULW (Mann-Whitney Rank Sum Test, p < 0.001, n = 38). F4 featured characteristics of an amino-acid like fluorophore with excitation/emission maxima in the range 250-265/284-320 in the fluorescence peak T region of tyrosine (Coble, 1996;Murphy et al., 2008;Aoki et al., 2008;Yamashita and Tanoue, 2003) and phenylalanine (Yamashita and Tanoue, 2003;Jørgensen et al., 2011) (Table 3). F4 was negatively correlated to bacterial abundance (Table 4), and to slope ratio SR with SR = (*S*(275-295):*S*(350:400)). F4 was also negatively correlated to SMHIX, indicating a low humic-acid content of this fluorophore. As for F1, it positively correlated with $SUVA_{254}$ and DHAA%-DOC (Table 4). Interestingly, F4 showed the highest fluorescence intensities among all samples.

Component F5 was quite difficult to identify, as we found no comparable spectra in the literature. It showed typical characteristics of allochtonous humic-like material with excitation/emission ranges

Gelöscht: , potentially hinting to a refractory DOM component of terrestrial origin, characterized by small molecules completely degraded either photochemically or microbially and therefore of low bioavailability. F2 strongly correlated to F3 and F5 components, further suggesting its origin from larger organic molecules (Spearman Rank Order Correlation coefficient C = 0.74, p < 0.001, n = 76 with F3, and C = 0.71, p < 0.001, n = 76 with F5). ¶

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[5] nach unten: Earlier studies attributed this optical behavior to fulvic acid C-like components showing a peak in region A. According to Ishii and Boyer (2012), F3 like components may comprise larger hydrophobic molecules that are photodegradable by UV light, of terrestrial or microbial origin, biologically degraded and produced.

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[3] nach unten: This may point to a fluorophore characterized by large and hydrophobic compounds, containing aromatic groups, and probably produced *in-situ* by marine bacteria.

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Gelöscht: which could represent a sink for this autochtonous compound, also confirmed by the negative correlation to slope ratio SR,

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in the peak A and C regions, which have been observed in bay and offshore waters (Mostofa et al., 2013). F5 had the highest fluorescence intensities both in the SML and ULW but was not clearly enriched in one or the other compartment (Figure 7). EF ranged from a minimum of 0.5 and a maximum of 3, with median value = 1.1. Highest enrichments in the SML were observed at northern stations S4 and S4_2, at stations S10_1 to S10_4, and in the southern stations S20 to S1778 (Figure 6). F5 was similar in characteristics to component F3, and positively correlated to bacterial abundance and proteinaceous CSP particles (Table 4). Component F5 was also positively correlated to all other fluorophores F1, F2, F3 as described, and to F4 (Spearman Rank Order Correlation coefficient C = 0.34, p = 0.009, n = 57).

On the revisited stations with multiple measurements, only component F1 showed a direct dependency on light exposure, significantly decreasing in fluorescence – thus concentration – with increasing global radiation intensity (R^2 = 0.56, ***p*** = 0.013, ***n*** = 10). Components F2 to F5 showed no significant change with increased irradiation (Spearmank Rank Order Correlation analysis).

3.3. Changes in CDOM properties related to the biological and physical environment

Both in the SML and ULW, CDOM optical properties as absorption coefficient ***a***(325), ***S***(275-295), and $SUVA_{254}$ were compared to salinity, temperature, wind speed and particulate organic carbon (POC) (Table 5). Data on POC have been described in details in Engel and Galgani (2016). CDOM absorption coefficient ***a***(325) decreased at higher salinity, temperature and wind speed in the SML and ULW, with stronger dependency on these physical parameters in the SML (Table 5). In both compartments, there was a positive correlation of *a*(325) to POC. The spectral slope parameter *S*(275-295), indicator for DOM molecular weight, source, and degradation processes (Helms et al., 2008), increased at higher salinity and temperature (Figure 8d) in the SML and ULW. It did not show any correlation to wind speed, but a significant negative correlation to POC in both compartments (Table 5). Moreover, an increase of bacterial and phytoplankton cells led to a lower *S*(275-295) both in the SML and ULW (Figures 8a, b). The dependency of *S*(275-295) on bacteria

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<#>**"Revisited stations"**¶
During R/V Meteor cruise leg M91, we sampled four stations within a time frame of 24 hours for SML and ULW, as described in table 1. Exact latitude and longitude were not always possible to retrieve after a certain time, but were similar for the stations sampled in a few hours time lag. We were interested in monitoring the spectral characteristics of CDOM in the SML and ULW at different times of the day depending on the solar irradiation and therefore in the evolution of the optical properties. Whenever possible, we sampled at sunrise, midday and sunset. For security reasons, it was not possible to sample later than sunset, as the zodiac operations were not allowed out at dark.

Gelöscht: levels of solar radiation

Gelöscht: Figure 8

Gelöscht: As previously observed, F1 was characterized by higher aromaticity (measured as SUVA254), which also decreased at higher solar radiation (Figure 8), implying DOM photobleaching. On the revisited stations, c

Gelöscht: F4

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Gelöscht: showed a significant decrease (p = 0.032, n = 16) in concentration with increasing abundance of bacteria both in the SML and underlying water (Figure 9), suggesting heterotrophic activity as a major sink for this class of fluorophores. Components F2, F3 and F5 instead

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Gelöscht: Both in the SML and ULW, an increase of bacterial and phytoplankton cells led to a lower *S*(275-295), **suggesting an accumulation of HMW-DOM related to the contribution of microorganisms.**

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in the SML (Spearman Rank Order Correlation Coefficient C = - 0.59, p < 0.001, n = 35) was stronger than in the ULW (C = - 0.38, p = 0.02, n = 36), potentially indicating a higher bacterial CDOM contribution. S(275-295) was also negatively correlated to phytoplankton abundance with a stronger relationship in the ULW (C = - 0.64, p = 0.001, n = 22) than in the SML (C = - 0.47, p = 0.004, n = 35). In the SML, we observed a significant decrease in S(275-295) with increasing abundance of gelatinous proteinaceous particles (CSP) (Figure 8c), while in the underlying water a lower S(275-295) was highly related to increasing concentration of polysaccharidic gels (TEP). In both SML and ULW, higher salinity, temperature and wind speed were related to lower $SUVA_{254}$ indexes, as indicators of DOM aromaticity. A positive correlation was observed instead between $SUVA_{254}$ and POC (Table 5). An increment in temperature was inversely correlated to DOM lability, and therefore bioavailability, expressed as DHAA%-DOC, indicating a higher degree of DOM degradation (Spearman Rank Order Correlation coefficient C = -0.68, p < 0.001, n = 29 in the SML and C = -0.66, p < 0.001, n = 29 in the ULW). DHAA%-DOC was also lower at higher salinity (Spearman Rank Order Correlation coefficient C = -0.42, p = 0.02, n = 29 in the SML and C = -0.63, p < 0.001, n = 29 in the ULW). As for S(275-295), we observed similar trends in SR (data not shown): SR was negatively correlated to DHAA%-DOC (Spearman Rank Order correlation coefficient C = -0.50, p < 0.001, n = 75) and to both gel particles abundance (Spearman Rank Order correlation coefficient C = -0.37, p < 0.001, n = 75 for TEP and C = -0.33, p = 0.004, n = 75 for CSP). SR did not show any significant correlation to total bacteria or phytoplankton abundance, but was significantly lower in the SML, with a median EF = 0.9 (Mann-Whitney Rank Sum Test, p = 0.013, n = 38). Furthermore, DHAA%-DOC was significantly higher in the SML (Mann-Whitney Rank Sum Test, p = 0.036, n = 38).

4. Discussion

4.1. CDOM enrichment and production in the top surface layer of the ocean

[1] nach unten: An enhanced release of HMW DOM may derive from cell disintegration, or from a microbial protection strategy due to the exposure to UVB light in a demanding environment such as the SML (Ortega-Retuerta et al., 2009). Mycosporine-like amino acids (MAAs), for example, serve as a natural microbial UV sunscreen against photodamage (Garcia-Pichel et al., 1993) and have been observed in enriched concentrations in the SML (Tilstone et al., 2010).

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S(275-295) increased with increasing temperature (Figure 10d) both in the SML and in the ULW. Moreover, temperature increment was i

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The enrichment of organic material in the SML has been mainly related to biological processes in the euphotic zone below the surface (Hardy, 1982;Liss and Duce, 2005). EBUS are among the most productive regions in the ocean and therefore interesting systems to investigate the relationship between organic matter accumulation and SML biogeochemical properties. The Peruvian EBUS is associated with an extensive OMZ and a key region for the study of gas fluxes from the ocean (Paulmier et al., 2008;Paulmier and Ruiz-Pino, 2009;Keeling et al., 2010). The presence of an organics-enriched surface layer may strongly affect gas exchange between the marine and the atmospheric systems (Engel and Galgani, 2016). The characterization of CDOM via its optical properties adds relevant information to the organic matter composition in the SML, as it allows discriminating between terrestrial and marine sources of DOM that may be equally enriched at the surface. Moreover, it helps tracking changes in DOM "quality" deriving from higher DOM exposure to solar radiation at the sea-surface than deeper in the water column. As such, microbial and photochemical DOM turnover in the SML may contribute to the atmospheric emission of gases and chemical reactive species, and interfere with the microbial carbon loop in the ocean.

In the Peruvian EBUS, we observed a general enrichment of CDOM in the SML with respect to the ULW, based on values of the specific absorption coefficient $a(\lambda)$ measured at 325 nm. Higher values for CDOM absorbance were observed in the coastal upwelling stations characterized by lowest salinity, temperature and highest enrichment of organic components, both in the particulate and dissolved fraction (Engel and Galgani, 2016). **It is commonly observed that spectral loadings of allochtonous/terrestrial-like CDOM decrease with increasing salinity (Murphy et al., 2008). However, we did not observe such trend in our samples. Instead, we found a negative correlation of amino-acid like fluorophore F1 to salinity and temperature, and no clear enrichment of humic-acid like fluorophores F2, F3 and F5 in the SML. Therefore, we think that in the SML of the study region the contribution of terrestrially derived CDOM, if any, is overwhelmed by the high productivity of the upwelling system. Organics enriched in the SML such as the amino-acidic compounds F1 and F4 found at the upwelling stations** may therefore

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reflect other processes rather than input of allochtonous CDOM from land. DOC concentrations in the SML were related to DOC concentrations in the ULW (Engel and Galgani, 2016), and the same was true for CDOM absorption coefficient *a*(325) (Spearman Rank Order Correlation coefficient *C* = 0.82, $p < 0.001$, $n = 38$), implying a direct dependency of SML CDOM on the organic matter in the ULW (Zhang and Yang, 2013). CDOM absorption coefficient *a*(325) as well as its spectral slope *S*(275-295) did not show any correlation to changes in DOC concentrations neither in the SML, nor in the ULW, but were significantly related to DOM diagenesis (DHAA%-DOC), POC, and abundance of autotrophic and heterotrophic microorganisms suggesting a recent production of labile or semi-labile substrates driven by *in-situ* microbial or photochemical processes in the underlying euphotic zone or at the immediate sea surface. A closer look on CDOM spectral properties revealed significant differences between SML and ULW. According to Helms et al. (2008), an increase in *S*(275-295) and SR suggests CDOM photodegradation and decreasing 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, recent and less altered DOM in the sample. We observed a negative correlation when comparing DHAA%-DOC and POC to *S*(275-295) and to SR. The higher DHAA%-DOC, the lower *S*(275-295) and SR. Microorganisms adopt several strategies against tough environments; the correlation between DHAA%-DOC to *S*(275-295) and SR was stronger in the SML than in the ULW, suggesting **an accumulation of HMW-DOM related to the contribution of microorganisms** directly in the SML or in the proximity due to cell lysis or exudation, which has been previously proposed (Tilstone et al., 2010). Thus, the close correlations of optical parameters to POC and marine gels lead to hypothesize that autochtonous CDOM produced in the very surface ocean can actually be incorporated in the gelatinous organic carbon pool.

4.2. CDOM composition

Gelöscht: 5

Gelöscht: concentrations

Gelöscht: concentration

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Gelöscht: ,

Gelöscht:

Gelöscht: nutrient-rich

Gelöscht: However, a closer look on CDOM spectral properties revealed significant differences between SML and ULW in CDOM source and lability as evidenced by spectral slope ratio SR and DHAA%-DOC.

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[2] verschoben

Gelöscht: . This observation suggested a microbial production of relatively recent and labile DOM directly in the SML due to lysis or exudation, which has been previously proposed (Tilstone et al., 2010). ¶

[2] nach oben: due to lysis or exudation, which has been previously proposed (Tilstone et al., 2010).

The analysis of EEMs allowed the identification of five fluorescent components both in the SML and ULW, of which two (F1 and F4) showed an amino-acid like fluorescence of autochtonous material, and three (F2, F3 and F5) had the characteristics of fulvic-acid like or humic-acid like CDOM (Table 3). These classes of fluorophores are commonly found in marine environments (Coble, 2007;Mostofa et al., 2013), but EEMs analyses of SML samples are scarce and up to now revealed the enrichment in humic-acid like fluorophores only (Zhang and Yang, 2013). Phenolic materials deriving from humic and fulvic acids transported by river drainage, and from macroalgae polyphenols, are often enriched in the SML, and indicate the presence of surface slicks (Carlson, 1982;Carlson and Mayer, 1980). Here, we observed a significant enrichment of amino-acid like fluorophores F1 and F4 with respect to ULW, in good accordance with previous reports on amino acids enrichment in the SML (Kuznetsova et al., 2004;Cunliffe et al., 2013;Tilstone et al., 2010), and with our own observations for the Peruvian EBUS (Engel and Galgani, 2016). F1 has shown the greatest production rates during algal blooms whereas its major sinks are UV light and microbial degradation (Stedmon and Markager, 2005b). Moreover, it is assumed that F1 relates to the fluorescence of amino acids still bound in the proteinaceous matrix (Stedmon and Markager, 2005b). Based on these previous findings and on our results (Table 4), we suggest that F1 is a tryptophan-like fluorophore, originating by *in-situ* primary production, relatively labile as it features an increase in fluorescence intensity correlated to increasing DHAA%-DOC, and possibly included in the gel particles surface matrix. F4 showed very high fluorescence intensities compared to F1, F2 and F3. In the literature, F4 has been associated to the fluorescence of amino acids in peptides (Stedmon and Markager, 2005b). Similarly to F1, F4 showed a positive correlation to DHAA%-DOC, as to indicate its labile nature. The aromatic content of DOM is highly responsible for its photoreactivity (e.g. Mopper et al., 2014); F4 correlation to DOM lability (DHAA%-DOC) and aromatic content ($SUVA_{254}$) was weaker than for F1. In our study, this may indicate F4 as an intermediate product of photochemically-driven aggregation or microbial degradation of labile CDOM. F4 has been linked to the fluorescence of tyrosine and phenylalanine (e.g. Coble,

Gelöscht: protein-like

Gelöscht: terrestrially derived

Gelöscht: 2

Gelöscht:

Gelöscht: protein-like

Gelöscht: 5

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Gelöscht: it

Gelöscht: Component F4 was characterized

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1996;Murphy et al., 2008;Jørgensen et al., 2011) and both amino acids were enriched in the SML of the Peruvian EBUS (Engel and Galgani, 2016). A recent laboratory study reported **π non-covalent interactions between N_2O and phenolic groups** in phenylalanine and tyrosine (Cao et al., 2014). Although performed in a setting non-comparable to our study area, these findings may suggest an interaction of N_2O with biological macromolecules enriched in the SML of the Peruvian EBUS, and thus with the exchange of N_2O between the ocean and the atmosphere (Engel and Galgani, 2016). The enrichment of fluorophores F1 and F4 in the SML could be partly due to the upwelling of colder nutrient-rich waters that boost primary production in the euphotic zone. Salinity and temperature gradients may thus explain the variation of F1 in the SML (Table 4), reflecting local upwelling and DOM production. The observed accumulation of amino-acid like CDOM may additionally derive from a local microbial release within the SML itself due to **cell disintegration, or as protection strategy for the exposure to UVB light in a demanding environment (Ortega-Retuerta et al., 2009). Mycosporine-like amino acids (MAAs), for example, serve as a natural microbial UV sunscreen against photodamage (Garcia-Pichel et al., 1993) and have been observed in enriched concentrations in the SML (Tilstone et al., 2010).** Major losses of autochtonous protein-like fluorophores in the SML may be related to photochemical and microbial degradation: **negative correlations of F1 and F4 to SR may hint to photochemical degradation**, recalling that an increase in SR is usually related to photobleached material (Helms et al., 2008). The negative correlation of F4 to bacterial abundance may be instead an indication of a microbial sink of this fluorophore.

The fulvic acid or humic acid-like components F2, F3 and F5 were ubiquitous in SML and ULW, with no significant differences in fluorescence intensities between the two compartments. F2 and F3 have been previously observed in coastal marine environments (e.g. Jørgensen et al., 2011;Ishii and Boyer, 2012). In the literature, component F2 has been characterized as of terrestrial origin, allochtonous in marine environments, found in bays, rivers and coastal waters. It is assumed to reflect small-sized molecules, being resistant to photodegradation, biologically not available, and

Gelöscht: -like fluorophore,

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Gelöscht: that

Gelöscht: the greenhouse gas N_2O can bind to aromatic groups in phenylalanine and tyrosine

Gelöscht: , thus suggesting

Gelöscht: processes

Gelöscht: in the Peruvian EBUS

Gelöscht: 5

Gelöscht: protein-like

Gelöscht: be related

Gelöscht: (as indicated by an inverse correlation of F1 to salinity and temperature, table 2). However, a

Gelöscht: may be another source for protein-like fluorophores in the SML

Gelöscht: .**An enhanced release of HMW DOM may derive from**

[1] verschoben

Gelöscht: production

Gelöscht: as response to high solar radiation

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Gelöscht: **from a microbial**

Gelöscht: **due to**

Gelöscht: **such as the SML**

Gelöscht: were

Gelöscht:

Formatiert: Nicht Hervorheben

Gelöscht: , as evidenced during the revisited stations.

mainly derived from photobleached terrestrial-like humic acids in marine waters with highest concentrations near the surface (Ishii and Boyer, 2012). In this study we did not find a correlation of F2 to global radiation but a positive correlation to temperature and to bacterial abundance (Table 4). We also observed an increase of bacterial abundance with increasing sea-surface temperature, which is well supported by existing literature (e.g. Morán et al., 2015). Higher temperature also stimulates the activity of marine bacteria (e.g. Piontek et al., 2009). Thus, as F2 probably reflects the fluorescence of highly degraded small molecules, we may characterize F2 as the ultimate product of microbial CDOM degradation in the surface ocean, not bioavailable anymore. F3-like fluorophores have been identified as an intermediate product of terrestrially derived DOM, still subject to further photochemical degradation (Stedmon et al., 2007). Earlier studies attributed this optical behavior to fulvic acid C-like components showing a peak in region A. According to Ishii and Boyer (2012), F3 like components may comprise larger hydrophobic molecules that are photodegradable by UV light, of terrestrial or microbial origin, biologically degraded and produced. Moreover, F3 appearance has been related to apparent oxygen utilization (Yamashita et al., 2010), further suggesting a microbial source of this material (Jørgensen et al., 2011). In this study, F3 showed a slight enrichment in the SML and was related to heterotrophic bacteria as well as to CSP particles, possibly indicating its origin in microbial reworking of larger organic compounds. F5 showed characteristics of humic acid fluorophores, with fluorescence maximum ranges to the lower end of F3 emission indicating a more pronounced CDOM alteration with respect to F3. Showing similar correlations to heterotrophic bacteria and CSP, F5 may as well derive from a microbial *in-situ* reworking of larger organic molecules both in the SML and ULW contributing to the size continuum and reactivity of the gel particles pool in surface waters. In fact, a net production and accumulation of humic-like CDOM in surface waters may occur in upwelling regions (Nieto-Cid et al., 2005;Jørgensen et al., 2011), whereas photochemical loss is thought to be the major removal mechanism of this material (e.g. Mopper and Schultz, 1993). In this study, fulvic acid/humic acid-like fluorophores well correlated among each other, suggesting a common underlying origin.

Gelöscht: F2 fluorescence appears to be related to DOM exposure to sunlight,

Gelöscht: although

Gelöscht: significant

Gelöscht: in this study,

Gelöscht: instead

Gelöscht: 2

Gelöscht: This kind of fluorophores has been shown having highest intensities near the surface, decreasing with depth (Yamashita et al., 2008). The positive correlation to bacterial abundance might suggest F2 as the ultimate product of microbial degradation in the surface ocean.

Gelöscht: -

[5] verschoben

Gelöscht: an

Gelöscht: related to

Gelöscht: material

Gelöscht: allochtonous

[3] verschoben

Gelöscht: This may point to a fluorophore characterized by large and hydrophobic compounds, containing aromatic groups, and probably produced *in-situ* by marine bacteria.

Gelöscht: n

Gelöscht: indirect

Gelöscht: starting terrestrial

Gelöscht: material

Gelöscht: that contribute

Gelöscht: in surface waters.

Gelöscht: (

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Based on CDOM absorption and fluorescence characteristics, we propose a conceptual model for the control of CDOM production and loss in the SML and ULW by microbial and photochemical processes (see graphical abstract). In this model, the accumulation of CDOM in the SML is the result of a) the biological production of CDOM in the ULW and deeper water column, stimulated by the upwelling of nutrient-rich waters to the sunlit surface and b) the local microbial release of CDOM as a response to elevated solar radiation. Previous and our own observations on amino-acid fluorophores (F1, F4), as well as on the enrichment of CSP and amino acids in the SML described elsewhere (Engel and Galgani, 2016), suggest a rapid turnover of fresh DOM in the sea-surface itself. On one hand, microbes release fresh DOM directly within the SML or in the upper first centimetres, as a consequence of high light exposure. On the other hand, and both in the SML and ULW, microbial and photochemical degradation would lead to the loss of amino-acid like fluorophores (F1, F4) and to the accumulation of less labile and humic-like components completely degraded (F2) or still subject to further photochemical degradation (F3, F5).

Gelöscht: ¶
Gelöscht: , in figure 11 we summarized potential
Gelöscht: es
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4.3. Implications for surface ocean dynamics and future perspectives

Optical properties of DOM in the Peruvian EBUS revealed a SML characterized by amino-acid like CDOM fluorophores. CDOM enrichment in the SML has been observed in different marine regions associated with enrichment in phenolic compounds, MAAs and humic acids (Carlson, 1982;Carlson and Mayer, 1980;Tilstone et al., 2010;Zhang and Yang, 2013). MAAs for example (LMW-DOM) are well known as microbial sunscreen in aquatic environments (Bhatia et al., 2011;Shick and Dunlap, 2002), and were observed in higher concentrations in the SML during surface slicks development (Tilstone et al., 2010). Here, the accumulation of amino-acid like CDOM may have a major microbial source directly in the SML or the immediate subsurface water, whereas fulvic acid/humic acid-like CDOM likely originated in the sunlit zone below by microbial and photochemical processing of upwelled organic material. Accumulation of amino acids in the SML has been related to a reduced bacterial activity, being the SML an extreme environment where the

Gelöscht: protein-like
Gelöscht: protein-like
Gelöscht: derived from high primary production in the euphotic zone, but likely had
Gelöscht: a
Gelöscht: microlayer.

consumption of amino acids may be lower (Santos et al., 2012). A reduced bacterial activity may thus also explain the amino acids enrichment in the SML of the Peruvian EBUS (Engel and Galgani, 2016). We may assume that in the top layer of the ocean, and at higher extent in the SML, exposure to light may have determined three main processes: 1) microbial release amino-acid like CDOM as a sunscreen function, 2) increased availability of biological substrate by CDOM photolysis and 3) further photochemical degradation of microbially-altered CDOM. Photochemistry is able to alter the HMW fraction making it more available for microbial attack (Kieber et al., 1989), but at the same time it may lead to a net loss of bioavailable substrates (Kieber, 2000). Therefore, the interplay of photochemistry and microbial activity controls the accumulation and loss of organic compounds at the sea-surface, implying consequences on gas fluxes worth deeper investigations in climate-relevant marine regions such as the OMZ off Peru. As an example, high microbial DOM respiration can lead to higher production of CO_2 in the SML (Garabétian, 1990), whereas high concentrations of isoprene may be released from photosensitized DOM reactions in the SML, proving an abiotic source of this gas uncoupled from biological production (Ciuraru et al., 2015).

It remains unclear whether in the Peruvian EBUS an increase in bioavailable carbon may have implied a higher heterotrophic respiration and CO_2 production in the SML, and this is an attractive hypothesis for future studies in this direction. It may be suggested however, that a net DOM production in the SML may take place independently of the biological productivity of the underlying waters as a sole microbial response to light exposure. We assessed the enrichment of light-absorbing proteinaceous organic material in the SML of a highly productive oceanic system, which may interfere with correct estimates of primary production from remote measurements. To conclude, we suggest that further primary production estimates may take into account the CDOM enrichment in the first centimeters of the ocean.

Gelöscht: Nevertheless, the dynamics of a surface-active biofilm enriched in organic matter depend both on microbial and photochemical DOM alteration processes that need deeper investigations in climate-relevant marine regions such as the OMZ off Peru.

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This manuscript is accompanied by supplementary material.

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Tables

Table 1. Data on average, maximum and minimum salinity, water temperature, global radiation and wind speed during M91. Data were retrieved from Dship data server of R/V Meteor.

	Salinity [PSU]	Temperature [°C]	Global Radiation [W m^{-2}]	Wind Speed [m s^{-1}]
Average	34.9	19.2	539	5.5
SD	0.2	1.7	352	2.1
Min	34.4	15.9	10	0.6
Max	35.3	21.9	1088	9.0

Table 2. Stations with multiple measurements. Metadata with date, local and UTC time of sampling, coordinates, and average global radiation retrieved from Dship data server of R/V Meteor.

Station Ship ID	Nr.	Station nr.	Samples	Date	Time [UTC]	Time [Local]	Lat, S [°]	Long, W [°]	Average Global Radiation [W m^{-2}]
1733-5	1	S7	sml/ulw	08-12-12	11:30	6:30	9°31.258'	79°17.886'	10
1733-9		S7_2	sml/ulw	08-12-12	19:45	14:45	9°32.75'	79°18.43	837
1752-2	2	S12_1	sml/ulw	13-12-12	12:00	7:00	12°55.20'	78°.42.00'	380.5
1752-7		S12_2	sml/ulw	13-12-12	20:30	15:30	12°59.79'	78°.41.00'	704.5
1752-9		S12_3	sml/ulw	13-12-12	23:10	18:10	12°55.20'	78°.42.03'	47
1764-4	3	S16_1	sml/ulw	17-12-12	12:40	7:40	14°7.708'	76°52.759'	381
1764-6		S16_2	sml/ulw	17-12-12	17:40	12:40	14°11.11'	76°55.95'	1043
1764-9		S16_3	sml/ulw	17-12-12	22:00	17:00	14°11.10'	76°55.99'	161.5
1777-2	4	S20	sml/ulw	22-12-12	18:00	13:00	15°31.174'	75°36.015'	1088
1777-10		S20_2	sml/ulw	23-12-12	15:00	10:00	15°36.42'	75°38.60'	1046

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Table 3. Fluorescent components identified in this study in both SML and ULW samples, according to their Ex/Em maxima ranges (nm), maximum fluorescence intensity range Fmax (R.U.), corresponding peaks individuated in previous studies (peak name, region, Ex/Em ranges) and properties.

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Components of this study	Ex/Em maxima [nm]	Fmax range [R.U.]	Literature peak name (region, Ex/Em)	Reference	Properties
F1	250-290/ 320-350	0.001-0.228	(T) (275/340)	A	Protein-like fluorescence of tryptophan Autochtonous material. Source: in situ primary production.
			6(B) (280/338)	B	Protein-like fluorescence of tryptophan, autochtonous material. Source: algal growth. Sink: microbial reworking, UVB.
			T (280-285/340-350)	C	Protein-like, extracted from EPS.
F2	250-260/ 500-520	0.048-1.709	2(A) (250/504)	D	Fulvic acid C-like allochtononus material present in all environments. Terrestrial/autochthonous fulvic acid fluorophore group.
			1(A) (250/520)	E	Fulvic acid C-like. Bay waters, allochtonous.
			2(A) (<260/>500)	F	Humic Acid C-like, river and coastal waters, allochtonous. Terrestrial humic.
			1(A) (<230-260/400-500)	G	Small sized molecules, photoresistant and biologically not available. Source: photochemistry, terrestrially derived humic acids in marine waters, highest concentrations near the water surface.
			2(A?) (250/504)	H	UVA humic-like, fulvic acid, terrestrial, autochtonous.
			C2(-) (256/>500)	I	Humic acid C-like, estuaries of the Iberian peninsula, allochtonous.
F3	265/520-540	0.019-1.640	2(A+C) (<240-275/434-520)	G	Larger molecules, hydrophobic compounds, photodegradable by UVA light. Source: terrestrial or microbial, intermediate inputs of minimal exposure to sunlight, biologically degraded and produced.
			C1 (~275/400-550)	L	Humic-like CDOM microbially produced.
			1(A/C) (<260/466)	O	Humic-like CDOM oxidized *in situ* by microbial processes.
F4	250-265/ 284-320	0.002-6.507	(T) (275/300)	J	Protein-like fluorescence of tyrosine. Autochtonous material. Source: in situ primary production, North Pacific and Atlantic Ocean.
			4(T) (275/306(338))	B	Fluorescence of tryptophan and tyrosine in peptides. Greatest production rates during establishment of algal bloom. Source: algae in exponential growth phase. Sinks: not identified (microbial uptake or aggregation?)
			(B) (275/310)	A	Tyrosine-like, marine waters, autochtonous.
			C(T) (270-290/250-365)	K	Autochtonous protein-like hydrophobic acid fraction from phytoplankton cultures.
			C3(T)	L	Protein-like fluorescence of phenylanine.
			Standard (255-265/284-285)	M	Protein-like fluorescence of phenylanine. Source: standard.
			(B) (265-280/293-313)	M	Protein-like fluorescence of tyrosine. Source: autochtonous.
F5	270-275/	0.023-	(A,C)	N	Humic acid C-like or A-like, allochtonous

	540-550	1.714	(<260-270/>508)	material in bay and marine waters.

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Table 4. Spearman Rank Order Correlation coefficients (*C*) between fluorescent components (F1-5) and total bacterial and phytoplankton cells, TEP and CSP particles, $SUVA_{254}$, *S*(275-295), *SR*, *a*(325), DHAA%-DOC, SMHIX, salinity and temperature measured in our study, both in the SML and ULW. Statistical significance was accepted for $p < 0.05$. *n* = number of samples. Only statistically significant correlations are shown. Bold characters indicate negative correlations.

Component [R.U.]	Statistics	Bacteria [cells mL^{-1}]	Phytoplankton [cells mL^{-1}]	TEP [L^{-1}]	CSP [L^{-1}]	$SUVA_{254}$ [mg C L^{-1} m^{-1}]	*S*(275-295) [nm^{-1}]	*SR*	*a*(325) [m^{-1}]	DHAA%-DOC [%]	SMHIX	Salinity [psu]	Temperature [° C]
F1	*C* *p* *n*	-	*0.285* *0.031* *57*	*0.281* *0.014* *76*		*0.620* *< 0.001* *76*	***- 0.257*** ***0.025*** ***76***	***- 0.387*** ***< 0.001*** ***75***	*0.406* *< 0.001* *76*	*0.696* *< 0.001* *76*	***- 0.342*** ***0.003*** ***76***	***- 0.261*** ***0.023*** ***76***	***- 0.323*** ***0.004*** ***76***
F2	*C* *p* *n*	*0.393* *< 0.001* *71*	-	-	-	-	-	-	-	-	*0.225* *0.050* *76*	-	*0.238* *0.038* *76*
F3	*C* *p* *n*	*0.355* *0.002* *71*	-	-	*0.411* *< 0.001* *76*	*0.305* *0.007* *76*	***- 0.221*** ***0.055*** ***76***	***- 0.226*** ***0.051*** ***76***	-	-	-	***- 0.273*** ***0.017*** ***76***	-
F4	*C* *p* *n*	***- 0.409*** ***0.003*** ***52***	-	-	-	*0.346* *0.008* *56*	-	***- 0.410*** ***0.002*** ***56***	-	*0.392* *0.008* *56*	***- 0.536*** ***< 0.001*** ***57***	-	-
F5	*C* *p* *n*	*0.270* *0.023* *71*	-	-	*0.402* *< 0.001* *76*	-	-	-	-	-	-	-	-

Table 5. Spearman Rank Order Correlation (C) between CDOM optical properties both in the SML and ULW with salinity (C_{PSU}), water temperature (C_T), wind speed (C_U) and particulate organic carbon (C_{POC}). Significant correlations ($p < 0.01$) are marked in bold (except [a] = $p < 0.05$). n is the number of samples, except * = 36 samples.

SML	C_{PSU}	C_T	C_U	C_{POC}	n
CDOM a(325)	**-0.420**	**-0.728**	**-0.535**	**0.579**	38
S(275-295)	**0.640**	**0.616**	0.318	**-0.597**	38
$SUVA_{254}$	**-0.380**[a]	**-0.634**	**-0.460**	**0.537**	38
ULW	C_{PSU}	C_T	C_U	C_{POC}	n
CDOM a(325)	**-0.329**[a]	**-0.637**	**-0.386**[a]	**0.656***	38
S(275-295)	**0.493**	**0.613**	0.24	**-0.622***	38
$SUVA_{254}$	**-0.326**[a]	**-0.458**	**-0.324**[a]	**0.495***	38

Figures' legend

Figure 3, Maps showing all sampled stations. Stations with multiple measurements are: (1) S7/7_2, (2) S12_1/3 and S12_2, (3) S16_1, S16_2/3, (4) S20 and S20_2. The locations of S7 and S7_2; S12_1 and S12_3; S16_2 and S16_3 coincide, as sampling was performed at different times.

Gelöscht: 1

Figure 4, CDOM absorption coefficient a(325), [m^{-1}], in SML and underlying water (ULW) and spectral slope parameter between 275 and 295 nm, S(275-295), [nm^{-1}].

Gelöscht: 2

Figure 5, Box and Whiskers plot of enrichment factors for CDOM absorption coefficient a(325), aromaticity (SUVA254), DOM diagenetic state (DHAA%-DOC), spectral slope S(275-295), and modified surface microlayer humification index (SMHIX). The horizontal lines of the boxes represent 25%, 50% (median) and 75% percentiles (from bottom to top). In the boxes, crosses represent the mean. Whiskers represent minimum and maximum values, and circles are outliers. Outliers are staggered to better visualize them. To identify the station, see outliers' labels and color legend. For a(325), SUVA254 and S(275-295) n = 38. For SMHIX, n = 37 and for DHAA%-DOC n = 29.

Gelöscht: 3

Figure 6, Enrichment factors (EF) in the Peruvian upwelling region. From the top left, EF for absorption coefficient measured at 325 nm both in SML and ULW, spectral slope parameter S(275-295) as indicator for changes in DOM molecular weight, SUVA254 as indicator for DOM aromatic content, DHAA%-DOC as indicator of DOM lability, and SMHIX as indicator of humic content of DOM.

Gelöscht: 4

Figure 7, (Above) Contour plots of five fluorescent components as identified by PARAFAC analysis and (below) relative spectral loadings of overlaid spectra for the 5-components model validated with 3 split comparisons. The axes of contour plots have been scaled to better visualize the fluorescence intensities (R.U.). A figure with the complete spectrum is included in the supplementary material (Figure S3). The dashed black line in the spectral loadings indicates excitation maxima for each component, the solid black line indicates emission peaks.

Gelöscht: 5

Figure 8. Distribution of enrichment factors (EF) for fluorescent components F1, F2, F3, F4, F5 identified in this study. Maximum EF for F4 has been recorded at station S10_2, with a value of 14.9. For visualization purposes, this data point is not included in the figure and fluorescence intensities have been scaled down to a maximum EF = 6.

Figure 9. Box and Whiskers plot of enrichment factors for fluorescent components F1, F2, F3, F4 and F5. The horizontal lines of the boxes represent 25%, 50% (median) and 75% percentiles (from bottom to top). In the boxes, crosses represent the mean. Whiskers represent minimum and maximum values, and circles are outliers. Outliers are staggered to better visualize them. To identify the station, see outliers' labels and color legend. For F4, *n* = 24. For all other components, *n* = 38.

Figure 8a-d. (a) Linear regression between bacterial abundance [10^6 cells mL^{-1}] and spectral slope *S*(275-295) [nm^{-1}] in SML and ULW. (b) Linear regression (ULW) and Spearman Rank Order Correlation (SML) between phytoplankton abundance [10^4 cells mL^{-1}] and spectral slope *S*(275-295) [nm^{-1}]. (c) Linear regression between CSP abundance [10^8 particles L^{-1}] and spectral slope *S*(275-295) [nm^{-1}] in the SML and between TEP abundance [10^8 particles L^{-1}] and spectral slope *S*(275-295) [nm^{-1}] in the ULW. (d) Linear regression between temperature [°C] and *S*(275-295) [nm^{-1}] in SML and ULW. Black triangles: SML, open dots: ULW.

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