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

Response to Anonymous Referee for minor revision of the manuscript.

We thank the referee for the evaluation of our revised manuscript and for the constructive additional comments provided. All issues are addressed below.

RC = Referee Comment
AC = Author Comment

RC1: The abstract still does not contain cruise dates, nor does it carry any quantitative information. I did request this in my initial comments, and I still believe that cruise / sampling dates should always be reported, and that publications reporting field observations should carry at least some quantitative info, not simply some qualitative description of the findings.

AC: The referee is right; we did not include the required information in the previous version. We have now modified the abstract including the dates in which the cruise took place, the exact coordinates, as well as some quantitative results on enrichment factors, spectral slope ranges, CDOM absorption ranges and enrichment factors for components F1 and F4.

RC2; N2O interactions with 'phenolic groups' (lines 1449 ff in authors' response): I still believe that the statement on possible implications of N2O-FDOM interactions (F1, F4 = proteinaceous fluorescence) for N2O cycling is rather too speculative, given that it is based on experiments in a neon matrix carried out at or below 8 K using millimolar levels of both aromatic monomeres and N2O (Cao et al. 2014). I appreciate the authors' opinion on speculative discussion. However, I recommend that they clarify the conditions of the experimental work used in Cao et al. (2014) (rather than simply state “non-comparable to our study area ”) if they want to retain this statement.

AC: We appreciate the suggestion given by the referee and we adopt it. In the revised version of the manuscript (lines 560 - on) we have added the following clarification on why we mention the work carried out by Cao and colleagues:

“Recently, Cao and colleagues (2014) performed a laboratory study aimed at understanding intermolecular interactions between N2O and phenol (C6H5OH), cresol (CH3C6H4(OH)), and toluene (CH3C6H5), which are representative aromatic compounds and useful models of various biomolecules such as the aromatic amino acids tyrosine and phenylalanine containing a benzene ring. Their experiment was carried out in a Ne matrix at about 8 K (-265.15 °C) with millimolar concentrations of the aromatic compounds (Cao et al., 2014), therefore in a setting not at all comparable to our experimental setup. Cao and colleagues found interesting π non-covalent interactions between N2O and the aromatic compounds, suggesting an interaction of N2O with tyrosine and phenylalanine of great interest for biological processes. We mention it here, as the presence of these specific amino acids in the SML of the Peruvian EBUS may interfere with the exchange of N2O between the ocean and the atmosphere, as suggested previously (Engel and Galgani, 2016).”

RC: Additional minor comment: phenylalanine is an aromatic but not a phenolic amino acid. Please correct your wording.
AC: We apologize for the mistake, and we thank the referee to pointing it out. We have corrected the wording.

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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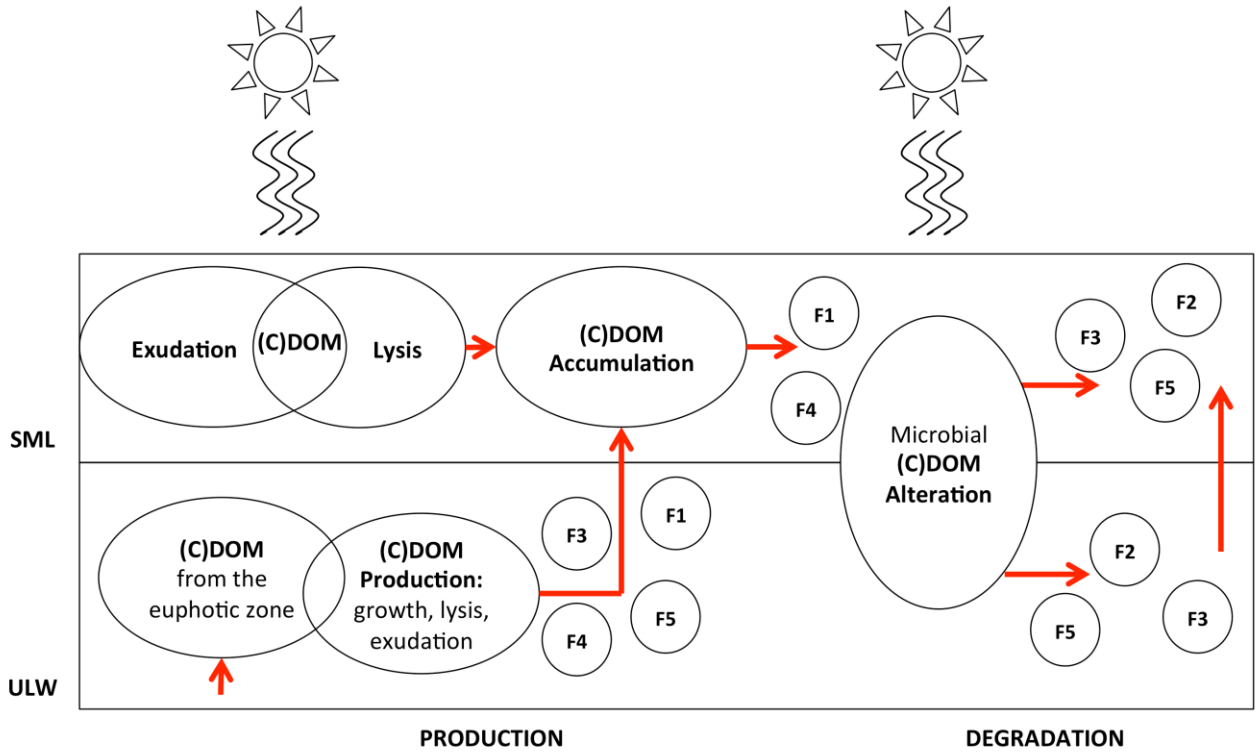
Abstract

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. From December 3rd to 23rd 2012, METEOR (M91) cruise took place in the Peruvian upwelling system between 4.59° S and 15.4° S, and 82.0° W to 77.5° W. During M91 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 (ULW) 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. Spectral slope *S* varied between 0.012 to 0.043 nm^{-1} and was quite similar between SML and ULW, with no significant differences between the two compartments. Higher *S* values were observed in the ULW of the southern stations below 15°S. By EEMs, 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, with a median ratio SML:ULW of 1.5 for both fluorophores. In the study region, values for CDOM absorption ranged from 0.07 to 1.47 m^{-1}. CDOM was generally highly concentrated in the SML, with a median enrichment with respect to the ULW of 1.2. 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 a 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 CDOM of 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) accumulate in the SML with respect to the ULW, as photochemistry 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 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.



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 respiration, which provides sinks for the oxygen produced by autotrophs and leads to subsurface Oxygen Minimum Zones (OMZs) (Lachkar and Gruber, 2011). OMZs are expanding worldwide due to reduced solubility at increasing temperatures, as well as a consequence of reduced oceanic ventilation and enhanced stratification (Keeling et al., 2010;Stramma et al., 2008). OMZ become increasingly important as key marine regions for the emission of climate-relevant gases like carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and hydrogen sulfide (H_2S) (Paulmier et al., 2008;Paulmier et al., 2011). N_2O is a strong greenhouse gas and ozone-reactive: 30% of its atmospheric concentration has an oceanic source (Solomon et al., 2007), of which, up to 75% is supported by OMZs (Bange et al., 2001). Therefore, OMZs are key environments to assess the oceanic contribution to the concentration of atmospheric gases. Defining the processes that regulate gas fluxes across the water-air interface is a central objective to better understand the reciprocal relationship between changes in our climate and marine environments.

The uppermost oceanic layer in contact with the atmosphere is the sea-surface microlayer (SML), which mediates major climate-relevant processes including air-sea gas exchange and sea-spray aerosol emission (Liss and Duce, 2005). This interface between a liquid (hydrosphere) and a gas phase (atmosphere) accumulates organic matter of biological origin, creating a sort of “skin” of surface-active compounds able to damp capillary waves and “capping the flux” of gases across the water-air interface (GESAMP, 1995). Natural organic compounds in the SML include a vast array of photosynthesis products including carbohydrates, amino acids and lipids, as well as other carbon-rich compounds like dissolved organic matter (DOM) and marine gels (e.g. Cunliffe et al., 2013). The DOM pool represents a continuum of molecular weights and biological lability ranging from

refractory to labile DOM being utilized rapidly by microorganisms (Benner, 2002;Carlson, 2002), or photochemically degraded (Kieber, 2000). These compounds, produced in the oceanic photic zone and brought to the SML through rising bubbles (Hardy, 1982), contribute to the enrichment of a natural surface biofilm and favor specific SML heterotrophic communities that are very active in recycling this organic material (Hardy, 1982;Cunliffe et al., 2011). While bulk dissolved organic carbon is not generally enriched in the SML, specific DOM fractions are present occasionally at much higher concentrations than in the underlying water (Cunliffe et al., 2013). These enriched pools of organic matter include marine gel particles (Wurl and Holmes, 2008), chromophoric dissolved organic matter (CDOM) (Zhang and Yang, 2013;Tilstone et al., 2010) and phenolic material (Carlson, 1982;Carlson and Mayer, 1980).

CDOM is the principal light-absorbing constituent of DOM, strongly absorbing UV (100 - 400 nm) and visible radiation (400 - 700 nm), and it can comprise 20%-70% of the DOM in oceanic waters (Coble, 2007). CDOM plays a major role in the attenuation of UV wavelengths and can reduce the availability of underwater photosynthetically active radiation for primary production (Bracchini et al., 2011). Photolysis of CDOM promotes the formation of low molecular weight (LMW) compounds from the breakdown of high molecular weight DOM (HMW-DOM), facilitating the bioavailability of carbon uptake for microbial growth from biologically refractory material, and representing an important loss pathway for CDOM in the oceans (Kieber et al., 1989). Other major by-products of CDOM photolysis are carbon monoxide (CO), which often exists at supersaturated concentrations in the oceans' surface (Blough, 2005, and references therein), CO_2 (Miller and Zepp, 1995) and reactive chemical species (Loiselle et al., 2012). To initiate a photochemical reaction, light must first be absorbed and in this respect the SML is very well exposed to elevated solar radiation (Liss and Duce, 2005). CDOM photolysis may affect biological processes within the SML as well as the structure of accumulated organic matter. Optical properties and photochemical cycling of DOM have been widely investigated in the ocean: CDOM alters light spectra in the surface ocean and its spatial and temporal distribution have been used in characterizing water

masses exchange (Nelson and Siegel, 2013). However, processes within the SML remain poorly understood. Possible effects of photochemistry on SML chemical composition have been discussed in the past (Blough, 2005), but still little is known on CDOM fluorophores, sources and sinks (Tilstone et al., 2010;Zhang and Yang, 2013). To discern sources, sinks and modification of DOM in surface waters, whether microbially or photochemically-induced, we investigated optical 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 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.

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

218 latitude and longitude were not always possible to retrieve after a certain time, but were similar for
219 the stations sampled in a few hours time lag.
220 The sampling approach for the SML was chosen as a silicate glass plate of 500 mm (length) x 250
221 mm (width) x 5 mm (thickness) with an effective sampling area of 2000 cm^2 as indicated in Engel
222 and Galgani (2016). Briefly, the glass plate was inserted into the water perpendicular to the surface
223 and withdrawn at a controlled rate of ~ 20 cm s^{-1} as first suggested by Harvey and Burzell (1972).
224 Different devices can be applied to sample the SML. The glass plate approach we choose collects a
225 thinner SML (~60 - 150 µm) when compared to i.e. the Garrett Screen (150 - 300 µm), one of the
226 mainly recognized practices introduced by Garrett in 1965 (Cunliffe et al., 2011; Garrett, 1965).
227 The glass plate was chosen because it allows the sampling of enough volume required for analysis
228 while keeping a minimal dilution with underlying water. Sampling was performed on a rubber
229 boat; in order to obtain a well-standardized procedure and to minimize biases by sampling, the same
230 person always took the samples with a repeatable withdrawal speed of the SML. The rubber boat
231 was positioned as far upwind of the ship as possible and away from the path taken by the ship in
232 order to avoid any potential surface contamination. The outboard motor of the rubber boat was
233 switched off and samples were collected in upwind clean waters.
234 Before collecting the sample into the bottle, we let the plate drain for 20 s approximately. Then, the
235 sample retained on both sides of the plate was removed with a Teflon wiper, and the procedure
236 repeated about 20 times to obtain the necessary volume for analysis. The exact amount of dips per
237 sample has been tracked. The first sample was discarded and used to rinse the collecting bottle (HCl
238 10% cleaned and Milli-Q rinsed). Glass plate and wiper were acid cleaned (HCl 10%) and Milli-Q
239 rinsed prior use, and at sampling site they were copiously rinsed with in situ seawater to minimize
240 any contamination with alien material during transport and handling. Underlying seawater (ULW)
241 was collected right after SML at about ~ 20 cm depth by opening an acid cleaned (HCl 10%) and
242 Milli-Q rinsed glass bottle and closing it underwater. The thickness (*d, m*) of our reference SML
243 that we were able to collect was estimated as follows:

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

Where V is the SML volume collected, i.e. 60-140 mL, A is the sampling area of the glass plate (A = 2000 cm²) 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).

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

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

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

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

FDOM: The 3-D recorded spectra were corrected for the instrumental biases both for excitation and emission using correction curves provided by the manufacturer (Stedmon and Bro, 2008).

Additionally, spectra were corrected against a Milli-Q water blank run every day before the samples to remove water Raman peaks. No correction for inner filter effects was applied to our data as for each sample the relative $a(\lambda)$ value was below 10 m^{-1} (Lawaetz and Stedmon, 2009;Stedmon and 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 water (ULW) samples. Fluorescence spectra were normalized to Raman Units (R.U.) by integrating the Raman peak of 350 nm Ex and 382 to 407 nm Ex extracted by the daily Milli-Q water blank. Calibration to R.U. was done with the FDOMcorrect toolbox for Matlab (The MathWorks Inc.) incorporated in DrEEM toolbox (Murphy et al., 2013). We choose to normalize to R.U. as these units are widely used in open ocean measurements and we could compare our results.

PARAFAC analysis was applied to EEMs in order to identify and quantify independent underlying components of the CDOM pool, and was performed by the N-way toolbox for Matlab in DrEEM (Murphy et al., 2013). 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. 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

348 successful for 5-components model, for all comparison. The maximum fluorescence intensities of
349 the five fluorophores at specific Ex-Em wavelengths ranges are described in table 3. Figures with
350 the model comparison for both excitation and emission for the 5-components model are included in
351 the supplementary material (Figures S1 and S2).
352 In fluorescence spectroscopy, the humification index (HIX), first introduced by Zsolnay et al.
353 (1999), is a powerful tool to study CDOM dynamics in soils, as humification is associated with a
354 shift to longer emission wavelengths (Senesi, 1990). It has been first applied to aquatic CDOM in
355 estuarine waters by Huguet and colleagues (2009), and is calculated as the ratio H/L of two spectral
356 region areas of the emission spectrum scanned at 254 nm excitation. Area L is calculated between
357 the emission wavelengths 300 nm and 345 nm, and area H between 435 nm and 480 nm. When the
358 degree of aromaticity of CDOM increases, the emission spectrum at excitation 254 nm is shifted
359 towards the red (longer wavelengths), implying an increase in H/L ratio and in HIX. High HIX
360 implies maximum fluorescence intensity at long wavelengths and therefore the presence of complex
361 molecules like HMW aromatic CDOM (Senesi et al., 1991). We applied a slight modification to the
362 HIX index for our samples, introducing the "SMHIX" index, where SM stands for Surface
363 Microlayer. As we did neither have the scanned excitation wavelength of 254 nm, nor the scanned
364 spectrum at excitation 345 nm and 435 nm, we calculated SMHIX index as follows:
365 (5) $SMHIX = (\sum I_{434\rightarrow480}) / \sum I_{300\rightarrow346})$
366 Where $\sum I_{434\rightarrow480}$ is the sum of all fluorescence intensities at every emission wavelength between
367 434 nm and 480 nm, and $\sum I_{300\rightarrow346}$ is the sum of all fluorescence intensities at every emission
368 wavelength between 300 nm and 346 nm, both scanned with excitation = 255 nm.
369 **Enrichment Factors:** Enrichment Factors (EF), allow tracking of accumulation patterns of any
370 compound in the SML with respect to the underlying water (ULW) and comparison among
371 different compounds. EF are calculated according to the following:
372 (6) $EF = [X]_{SML} / [X]_{ULW}$

373 Where [X] is the concentration of a given parameter in the SML or ULW, respectively (GESAMP,
374 1995). EF > 1 indicates an enrichment, EF < 1 indicates a depletion in the SML. EFs are normally
375 used for quantitative parameters, i.e., measured in abundance and concentration such as DOC,
376 DHAA, CDOM, marine gels and cell abundances. Here, we applied the EF calculation for
377 qualitative ratios and indexes too, like *S*(275-295), SR, SMHIX, $SUVA_{254}$, DHAA%-DOC. We
378 kept the same wording, which is useful to describe differences between SML and ULW for both
379 quantitative and qualitative parameters.
380 Statistical tests in data analysis have been accepted as significant for $p < 0.05$. Calculations,
381 statistical tests and illustration were performed with Microsoft Office Excel 2010, Sigma Plot 12.0
382 (Systat), Prism (GraphPad), Ocean Data View and Matlab R2009b (The MathWorks Inc.).
383

384 ## 3. Results

385 Results on dissolved organic carbon and amino acids, gel particles (TEP and CSP), phytoplankton
386 and bacterial abundance and the relative enrichment of these components in the SML of our
387 sampling sites have been described elsewhere (Engel and Galgani, 2016). Here, we focus on the
388 optical properties of DOM to identify possible sources, sinks and dynamics in the SML and
389 underlying water of the Peruvian upwelling region.
390

391 ### 3.1. CDOM optical absorption properties

392 In the upwelling region off Peru, values for CDOM absorption coefficient *a*(325) ranged from 0.09
393 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
394 S10_1 to S10_4 along the coast for both SML and ULW. CDOM was enriched in the SML at most
395 stations (Figure 3), with median EF for *a*(325) = 1.2 in a range varying between 0.4 and 2.8. A
396 median EF = 1.2 means that at least 50% of our observations accounted for a CDOM-enriched
397 SML. Besides the southern transect, higher EF values were observed at the northern stations S2 and
398 S2_2, and in the southern coastal upwelling stations S15_1 to S15_3. Lower EFs and EFs < 1,

399 indicating a depletion of CDOM in the SML, were observed at higher distance from the coast
400 (Figure 4).
401 The spectral slope parameter between 275 and 295 nm (S(275-295), nm^{-1}) is a good indicator for
402 CDOM molecular weight as an increase of this parameter indicates decreasing molecular weight,
403 thus revealing accumulation or degradation processes of bioavailable CDOM (Helms et al., 2008).
404 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
405 nm^{-1} in ULW. In general, S(275-295) was quite similar between SML and ULW, and no statistically
406 significant differences were found between SML and ULW for S(275-295). Higher spectral slopes
407 were observed in the ULW of the southern stations below 15°S (S19, S19_2, S20, S20_2, S1778).
408 In the coastal stations S10_1 to S10_4 and S14_1 to S15_3 lower S(275-295) values were
409 determined for both SML and ULW. Median enrichment factor (EF) for S(275-295) was 1 (Figure
410 3), thus indicating similar molecular weight of CDOM compounds in the SML and ULW. Lower
411 EFs were observed in the northernmost and southernmost stations and along the coast.
412 The $SUVA_{254}$ and SMHIX indexes are related to the degree of CDOM aromaticity and to its humic
413 content, respectively. In our study, SUVA254 ranged from 0.49 to 1.74 mg C L^{-1} m^{-1} in the SML,
414 with highest values at coastal southern stations S10_1 to S10_4 and S14_1 to S17_2. Similar values
415 were recorded for ULW, ranging from 0.49 to 1.21 mg C L^{-1} m^{-1}. Generally, $SUVA_{254}$ values in our
416 samples were comparable to the Pacific Ocean with a typical $SUVA_{254}$ of 0.6 mg C L^{-1} m^{-1} (Helms
417 et al., 2008;Weishaar et al., 2003). Median EF for $SUVA_{254}$ was 1.1, with higher values in
418 correspondence of northern stations and coastal southern stations (S2, S2_2, S15_1 to S15_3 and
419 S19 to S1778) where the higher EF for a(325) were also observed (Figures 3 and 4). SMHIX
420 ranged from -1.33 to 2.05 for SML and from -0.1 to 3.03 for ULW, with highest values in ULW.
421 Enrichment factors showed an overall depletion of high-humic acid containing CDOM in the SML
422 (Figure 3), with median EF = 0.8. Higher humic acid enrichment in the SML was observed on the
423 southern transect S19 to S1778 (Figure 4), where we recorded the highest enrichment of CDOM (as
424 a(325)) as well.

425 The carbon-normalized yields of dissolved amino acids (DHAA%-DOC) as indicator of DOM
426 diagenetic state, ranged from 1.4% to 8.1% in SML samples and from 0.9% to 3.6% in ULW
427 samples, indicating relatively more labile DOM in the SML. This observation was supported by the
428 enrichment factors (EF), which showed a general enrichment of more labile DOM in the SML
429 (Figure 3), with median EF values for DHAA%-DOC of 1.5. Highest EFs were recorded in the
430 northernmost stations S1 to S3, and on the southernmost transect S19 to S1778.
431
432 **3.2. PARAFAC analysis for CDOM fluorophores**
433 Five optically active components were identified by PARAFAC analysis with the DrEEM toolbox
434 in Matlab (Murphy et al., 2013), hereafter named F1, F2, F3, F4 and F5 (Figure 5). The spectral
435 characteristics of the five identified components were compared to previous studies as described in
436 table 3. F1 had an excitation range of 250-290 nm with emission peaks between 320 and 350 nm,
437 which corresponds to peak T of the amino-acid like fluorescence of tryptophan, derived by *in-situ*
438 primary production (Coble, 1996). This component (F1) was generally enriched in the SML
439 (Figures 6, 7) with a median EF = 1.5, between a minimum EF of 0.5 and a maximum EF of 3.3.
440 Potential loss processes of this compound are its destruction by UV light and microbial degradation
441 (Stedmon and Markager, 2005b). F1 has also been related to protein-like fluorescence of
442 extracellular polymeric substances (Liu and Fang, 2002). Fluorescence intensities of F1 were the
443 lowest compared to the other fluorophores, but significantly higher in the SML compared to the
444 ULW (Mann-Whitney Rank Sum Test, $p < 0.001$, $n = 38$). Both in SML and ULW, fluorescence
445 intensities of F1 were positively correlated to components F3, F4 and F5 (Spearman Rank Order
446 Correlation coefficient $C = 0.37$, $p < 0.001$, $n = 76$ with F3; $C = 0.41$, $p = 0.001$, $n = 57$ with F4; C
447 $= 0.38$, $p < 0.001$, $n = 76$ with F5).
448 Component F2 had a short wavelength excitation range (250-260 nm) with emission at longer
449 wavelengths (500-520 nm), corresponding to peak A of fulvic acids and humic acids (Stedmon and
450 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 (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 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, 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 detail 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 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

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

555 region the contribution of terrestrially derived CDOM, if any, is overwhelmed by the high
556 productivity of the upwelling system. Organics enriched in the SML such as the amino-acidic
557 compounds F1 and F4 found at the upwelling stations may therefore reflect other processes rather
558 than input of allochtonous CDOM from land. DOC concentrations in the SML were related to DOC
559 concentrations in the ULW (Engel and Galgani, 2016), and the same was true for CDOM
560 absorption coefficient $a(325)$ (Spearman Rank Order Correlation coefficient $C = 0.82$, $p < 0.001$, n
561 = 38), implying a direct dependency of SML CDOM on the organic matter in the ULW (Zhang and
562 Yang, 2013). CDOM absorption coefficient $a(325)$ as well as its spectral slope $S(275\text{-}295)$ did not
563 show any correlation to changes in DOC concentrations neither in the SML, nor in the ULW, but
564 were significantly related to DOM diagenesis (DHAA%-DOC) POC, and abundance of autotrophic
565 and heterotrophic microorganisms suggesting a recent production of labile or semi-labile substrates
566 driven by *in-situ* microbial or photochemical processes in the underlying euphotic zone or at the
567 immediate sea surface. A closer look on CDOM spectral properties revealed significant differences
568 between SML and ULW. According to Helms et al. (2008), an increase in $S(275\text{-}295)$ and SR
569 suggests CDOM photodegradation and decreasing molecular weight. DHAA%-DOC is used here
570 as an indicator for DOM diagenesis, thus, the extent of microbially altered DOM. The higher
571 DHAA%-DOC, the more labile, bioavailable, recent and less altered DOM in the sample. We
572 observed a negative correlation when comparing DHAA%-DOC and POC to $S(275\text{-}295)$ and to SR.
573 The higher DHAA%-DOC, the lower $S(275\text{-}295)$ and SR. Microorganisms adopt several strategies
574 against tough environments; the correlation between DHAA%-DOC to $S(275\text{-}295)$ and SR was
575 stronger in the SML than in the ULW, suggesting an accumulation of HMW-DOM related to the
576 contribution of microorganisms directly in the SML or in the proximity due to cell lysis or
577 exudation, which has been previously proposed (Tilstone et al., 2010). Thus, the close correlations
578 of optical parameters to POC and marine gels lead to hypothesize that autochtonous CDOM
579 produced in the very surface ocean can actually be incorporated in the gelatinous organic carbon
580 pool.

581

582 **4.2. CDOM composition**

583 The analysis of EEMs allowed the identification of five fluorescent components both in the SML
584 and ULW, of which two (F1 and F4) showed an amino-acid like fluorescence of autochtonous
585 material, and three (F2, F3 and F5) had the characteristics of fulvic-acid like or humic-acid like
586 CDOM (Table 3). These classes of fluorophores are commonly found in marine environments
587 (Coble, 2007;Mostofa et al., 2013), but EEMs analyses of SML samples are scarce and up to now
588 revealed the enrichment in humic-acid like fluorophores only (Zhang and Yang, 2013). Phenolic
589 materials deriving from humic and fulvic acids transported by river drainage, and from macroalgae
590 polyphenols, are often enriched in the SML, and indicate the presence of surface slicks (Carlson,
591 1982;Carlson and Mayer, 1980). Here, we observed a significant enrichment of amino-acid like
592 fluorophores F1 and F4 with respect to ULW, in good accordance with previous reports on amino
593 acids enrichment in the SML (Kuznetsova et al., 2004;Cunliffe et al., 2013;Tilstone et al., 2010),
594 and with our own observations for the Peruvian EBUS (Engel and Galgani, 2016). F1 has shown
595 the greatest production rates during algal blooms, whereas its major sinks are UV light and
596 microbial degradation (Stedmon and Markager, 2005b). Moreover, it is assumed that F1 relates to
597 the fluorescence of amino acids still bound in the proteinaceous matrix (Stedmon and Markager,
598 2005b). Based on these previous findings and on our results (Table 4), we suggest that F1 is a
599 tryptophan-like fluorophore, originating by *in-situ* primary production, relatively labile as it features
600 an increase in fluorescence intensity correlated to increasing DHAA%-DOC, and possibly included
601 in the gel particles surface matrix. F4 showed very high fluorescence intensities compared to F1,
602 F2 and F3. In the literature, F4 has been associated to the fluorescence of amino acids in peptides
603 (Stedmon and Markager, 2005b). Similarly to F1, F4 showed a positive correlation to DHAA%-
604 DOC, as to indicate its labile nature. The aromatic content of DOM is highly responsible for its
605 photoreactivity (e.g. Mopper et al., 2014); F4 correlation to DOM lability (DHAA%-DOC) and
606 aromatic content ($SUVA_{254}$) was weaker than for F1. In our study, this may indicate F4 as an

607 intermediate product of photochemically-driven aggregation or microbial degradation of labile
608 CDOM. F4 has been linked to the fluorescence of tyrosine and phenylalanine (e.g. Coble,
609 1996;Murphy et al., 2008;Jørgensen et al., 2011) and both amino acids were enriched in the SML of
610 the Peruvian EBUS (Engel and Galgani, 2016). Recently, Cao and colleagues (2014) performed a
611 laboratory study aimed at understanding intermolecular interactions between N_2O and phenol
612 (C_6H_5OH), cresol ($CH_3C_6H_4OH$), and toluene ($CH_3C_6H_5$), which are representative aromatic
613 compounds and useful models of various biomolecules such as the aromatic amino acids tyrosine
614 and phenylalanine containing a benzene ring. Their experiment was carried out in a Ne matrix at
615 about 8 K (-265.15 °C) with millimolar concentrations of the aromatic compounds (Cao et al.,
616 2014), therefore in a setting not at all comparable to our experimental setup. Cao and colleagues
617 found interesting π non-covalent interactions between N_2O and the aromatic compounds,
618 suggesting an interaction of N_2O with tyrosine and phenylalanine of great interest for biological
619 processes. We mention it here, as the presence of these specific amino acids in the SML of the
620 Peruvian EBUS may interfere with the exchange of N_2O between the ocean and the atmosphere, as
621 suggested previously (Engel and Galgani, 2016). The enrichment of fluorophores F1 and F4 in the
622 SML could be partly due to the upwelling of colder nutrient-rich waters that boost primary
623 production in the euphotic zone. Salinity and temperature gradients may thus explain the variation
624 of F1 in the SML (Table 4), reflecting local upwelling and DOM production. The observed
625 accumulation of amino-acid like CDOM may additionally derive from a local microbial release
626 within the SML itself due to cell disintegration, or as protection strategy for the exposure to UVB
627 light in a demanding environment (Ortega-Retuerta et al., 2009). Mycosporine-like amino acids
628 (MAAs), for example, serve as a natural microbial UV sunscreen against photodamage (Garcia-
629 Pichel et al., 1993) and have been observed in enriched concentrations in the SML (Tilstone et al.,
630 2010). Major losses of autochtonous protein-like fluorophores in the SML may be related to
631 photochemical and microbial degradation: negative correlations of F1 and F4 to SR may hint to
632 photochemical degradation, recalling that an increase in SR is usually related to photobleached

633 material (Helms et al., 2008). The negative correlation of F4 to bacterial abundance may be instead
634 an indication of a microbial sink of this fluorophore.
635 The fulvic acid or humic acid-like components F2, F3 and F5 were ubiquitous in SML and ULW,
636 with no significant differences in fluorescence intensities between the two compartments. F2 and F3
637 have been previously observed in coastal marine environments (e.g. Jørgensen et al., 2011;Ishii and
638 Boyer, 2012). In the literature, component F2 has been characterized as of terrestrial origin,
639 allochtonous in marine environments, found in bays, rivers and coastal waters. It is assumed to
640 reflect small-sized molecules, being resistant to photodegradation, biologically not available, and
641 mainly derived from photobleached terrestrial-like humic acids in marine waters with highest
642 concentrations near the surface (Ishii and Boyer, 2012). In this study we did not find a correlation
643 of F2 to global radiation but a positive correlation to temperature and to bacterial abundance (Table
644 4). We also observed an increase of bacterial abundance with increasing sea-surface temperature,
645 which is well supported by existing literature (e.g. Morán et al., 2015). Higher temperature also
646 stimulates the activity of marine bacteria (e.g. Piontek et al., 2009). Thus, as F2 probably reflects
647 the fluorescence of highly degraded small molecules, we may characterize F2 as the ultimate
648 product of microbial CDOM degradation in the surface ocean, not bioavailable anymore. F3-like
649 fluorophores have been identified as an intermediate product of terrestrially derived DOM, still
650 subject to further photochemical degradation (Stedmon et al., 2007). Earlier studies attributed this
651 optical behavior to fulvic acid C-like components showing a peak in region A. According to Ishii
652 and Boyer (2012), F3 like components may comprise larger hydrophobic molecules that are
653 photodegradable by UV light, of terrestrial or microbial origin, biologically degraded and produced.
654 Moreover, F3 appearance has been related to apparent oxygen utilization (Yamashita et al., 2010),
655 further suggesting a microbial source of this material (Jørgensen et al., 2011). In this study, F3
656 showed a slight enrichment in the SML and was related to heterotrophic bacteria as well as to CSP
657 particles, possibly indicating its origin in microbial reworking of larger organic compounds. F5
658 showed characteristics of humic acid fluorophores, with fluorescence maximum ranges to the lower

659 end of F3 emission indicating a more pronounced CDOM alteration with respect to F3. Showing
660 similar correlations to heterotrophic bacteria and CSP, F5 may as well derive from a microbial *in-*
661 *situ* reworking of larger organic molecules both in the SML and ULW contributing to the size
662 continuum and reactivity of the gel particles pool in surface waters. In fact, a net production and
663 accumulation of humic-like CDOM in surface waters may occur in upwelling regions (Nieto-Cid et
664 al., 2005;Jørgensen et al., 2011), whereas photochemical loss is thought to be the major removal
665 mechanism of this material (e.g. Mopper and Schultz, 1993). In this study, fulvic acid/humic acid-
666 like fluorophores well correlated among each other, suggesting a common underlying origin.
667 Based on CDOM absorption and fluorescence characteristics, we propose a conceptual model for
668 the control of CDOM production and loss in the SML and ULW by microbial and photochemical
669 processes (see graphical abstract). In this model, the accumulation of CDOM in the SML is the
670 result of a) the biological production of CDOM in the ULW and deeper water column, stimulated
671 by the upwelling of nutrient-rich waters to the sunlit surface and b) the local microbial release of
672 CDOM as a response to elevated solar radiation. Previous and our own observations on amino-acid
673 fluorophores (F1, F4), as well as on the enrichment of CSP and amino acids in the SML described
674 elsewhere (Engel and Galgani, 2016), suggest a rapid turnover of fresh DOM in the sea-surface
675 itself. On one hand, microbes release fresh DOM directly within the SML or in the upper first
676 centimetres, as a consequence of high light exposure. On the other hand, and both in the SML and
677 ULW, microbial and photochemical degradation would lead to the loss of amino-acid like
678 fluorophores (F1, F4) and to the accumulation of less labile and humic-like components completely
679 degraded (F2) or still subject to further photochemical degradation (F3, F5).
680

681 **4.3. Implications for surface ocean dynamics and future perspectives**

682 Optical properties of DOM in the Peruvian EBUS revealed a SML characterized by amino-acid like
683 CDOM fluorophores. CDOM enrichment in the SML has been observed in different marine regions
684 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 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.

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730

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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

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.

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 1. 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.

Figure 2. CDOM absorption coefficient $a(325)$, [m^{-1}], in SML and underlying water (ULW) and spectral slope parameter between 275 and 295 nm, $S(275\text{-}295)$, [nm^{-1}].

Figure 3. Box and Whiskers plot of enrichment factors for CDOM absorption coefficient $a(325)$, aromaticity (SUVA254), DOM diagenetic state (DHAA%-DOC), spectral slope $S(275\text{-}295)$, 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 $S(275\text{-}295)$ $n = 38$. For SMHIX, $n = 37$ and for DHAA%-DOC $n = 29$.

Figure 4. 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 $S(275\text{-}295)$ 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.

Figure 5. (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.

Figure 6. 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 7. 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.

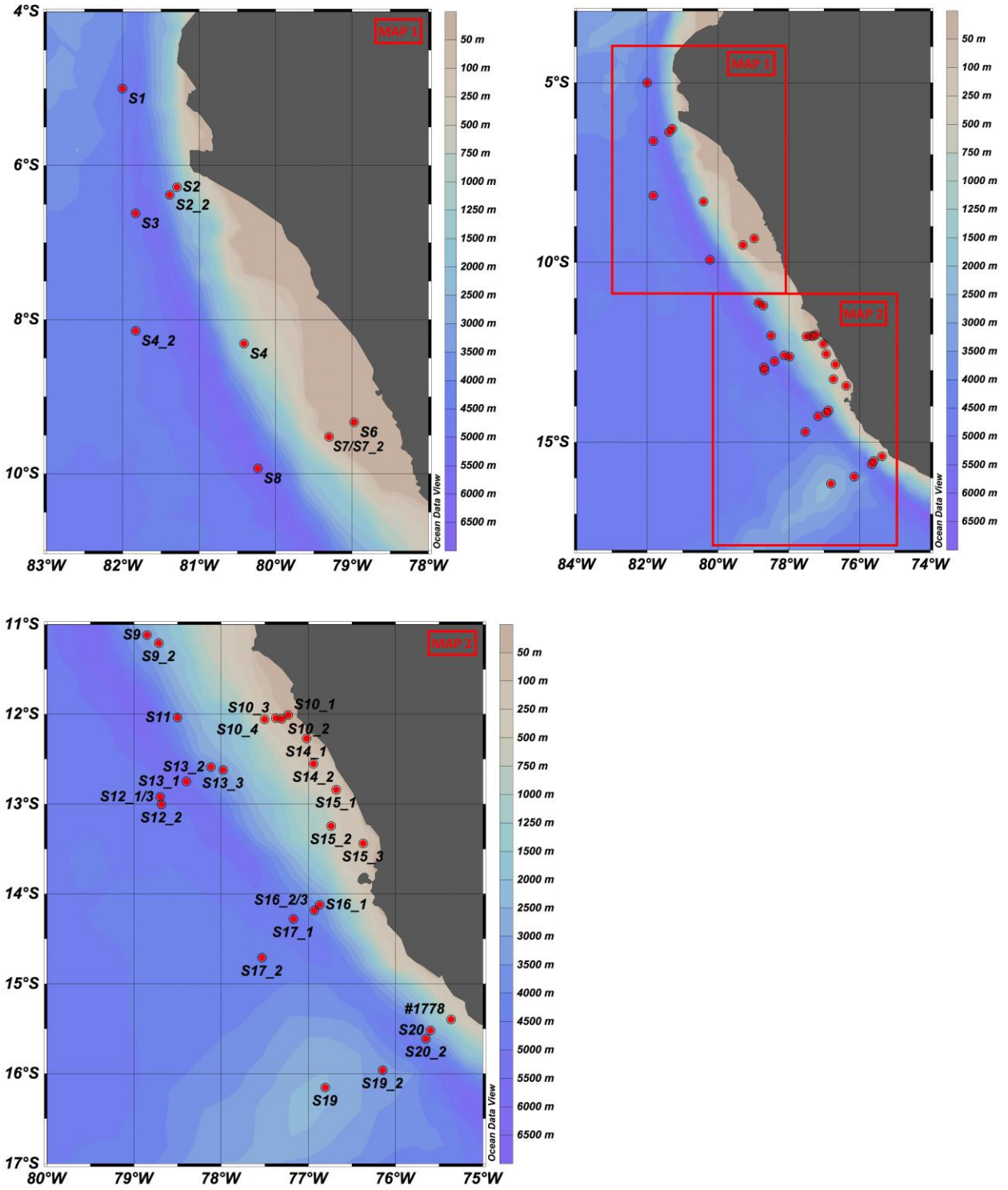
MAP 1
S1
S2
S2_2
S3
S4_2
S4
S6
S7/S7_2
S8
MAP 1
MAP 2
MAP 3
S9
S9_2
S10_3
S10_1
S11
S10_4
S10_2
S14_1
S13_2
S14_2
S13_1
S13_3
S12_1/3
S15_1
S12_2
S15_2
S15_3
S16_2/3
S16_1
S17_1
S17_2
#1778
S20
S20_2
S19_2
S19
Ocean Data View
50 m
100 m
250 m
500 m
750 m
1000 m
1250 m
1500 m
2000 m
2500 m
3000 m
3500 m
4000 m
4500 m
5000 m
5500 m
6000 m
6500 m
4°S
6°S
8°S
10°S
83°W
82°W
81°W
80°W
79°W
78°W
5°S
10°S
15°S
84°W
82°W
80°W
78°W
76°W
74°W
11°S
12°S
13°S
14°S
15°S
16°S
17°S
80°W
79°W
78°W
77°W
76°W
75°W

Figure 8

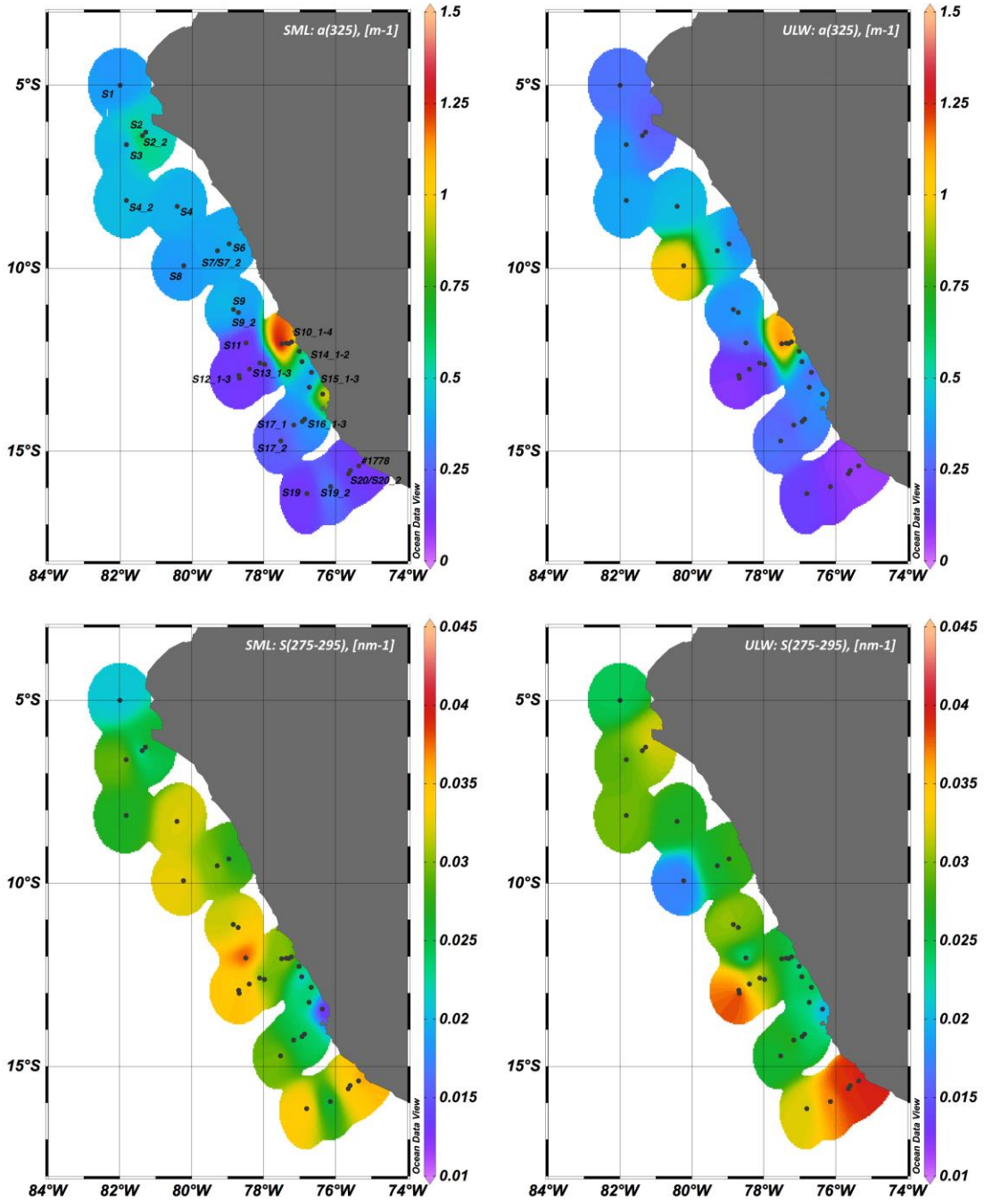
SML: a(325), [m-1]
ULW: a(325), [m-1]
SML: S(275-295), [nm-1]
ULW: S(275-295), [nm-1]
5°S
10°S
15°S
84°W
82°W
80°W
78°W
76°W
74°W
1.5
1.25
1
0.75
0.5
0.25
0
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
Ocean Data View
S1
S2
S2_2
S3
S4_2
S4
S6
S7/S7_2
S8
S9
S9_2
S10_1-4
S11
S14_1-2
S12_1-3
S13_1-3
S15_1-3
S17_1
S16_1-3
S17_2
#1778
S20/S20_2
S19
S19_2

Figure 9

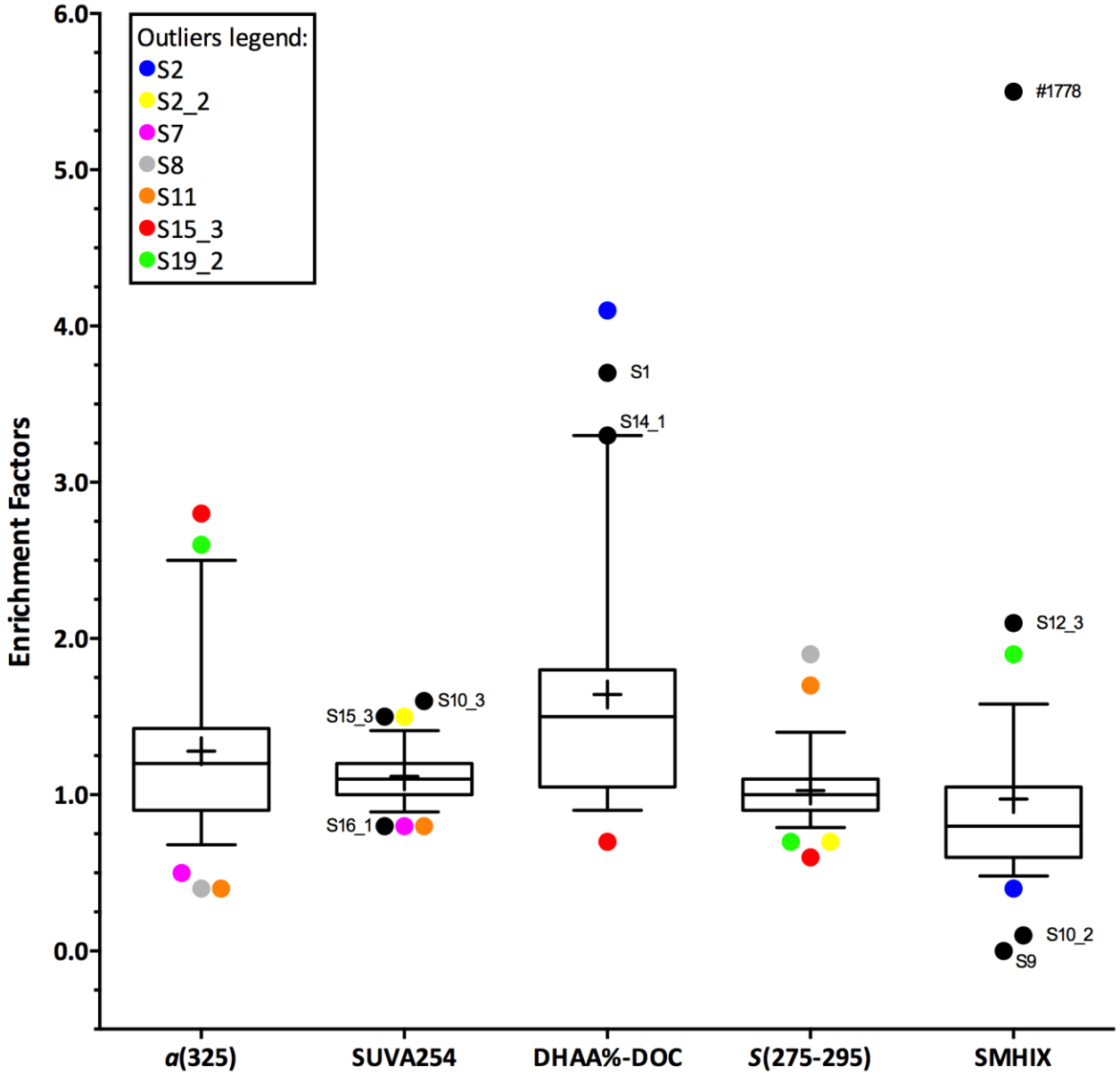
Outliers legend:
S2
S2_2
S7
S8
S11
S15_3
S19_2
Enrichment Factors
6.0
5.0
4.0
3.0
2.0
1.0
0.0
#1778
S1
S14_1
S12_3
S10_3
S15_3
S16_1
S10_2
S9
a(325)
SUVA254
DHAA%-DOC
S(275-295)
SMHIX

Figure 10

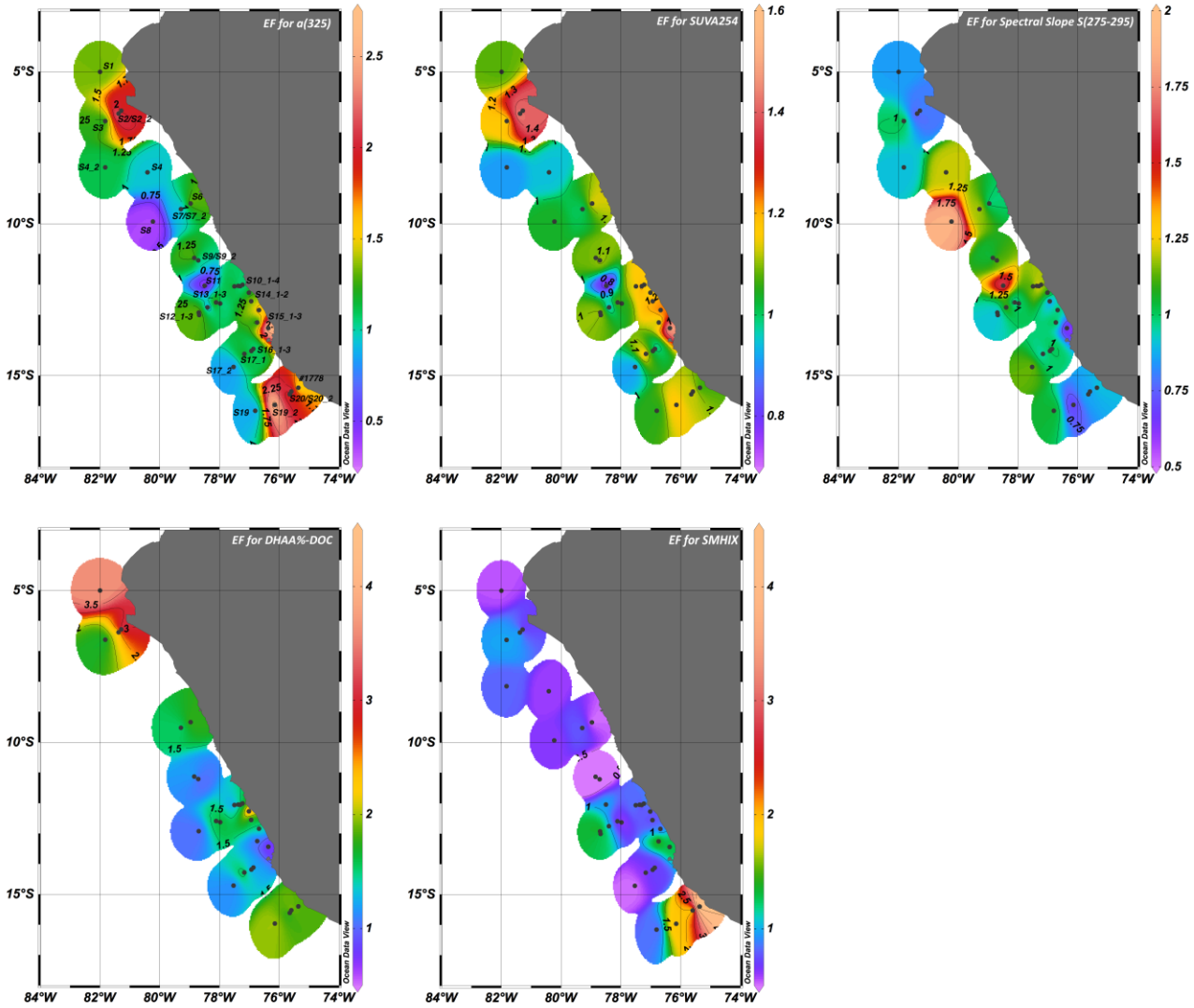
EF for a(325)
EF for SUVA254
EF for Spectral Slope S(275-295)
EF for DHAA%-DOC
EF for SMHIX
5°S
10°S
15°S
84°W
82°W
80°W
78°W
76°W
74°W
Ocean Data View

Figure 11

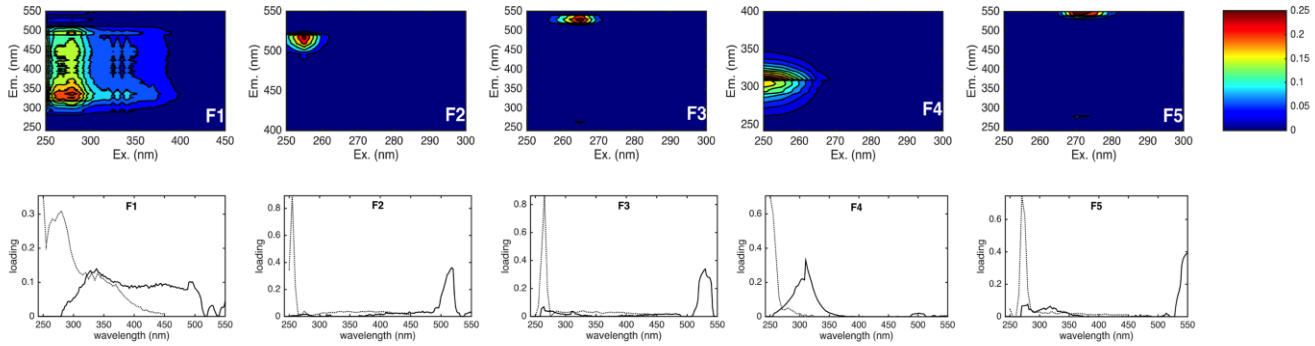

Figure 12

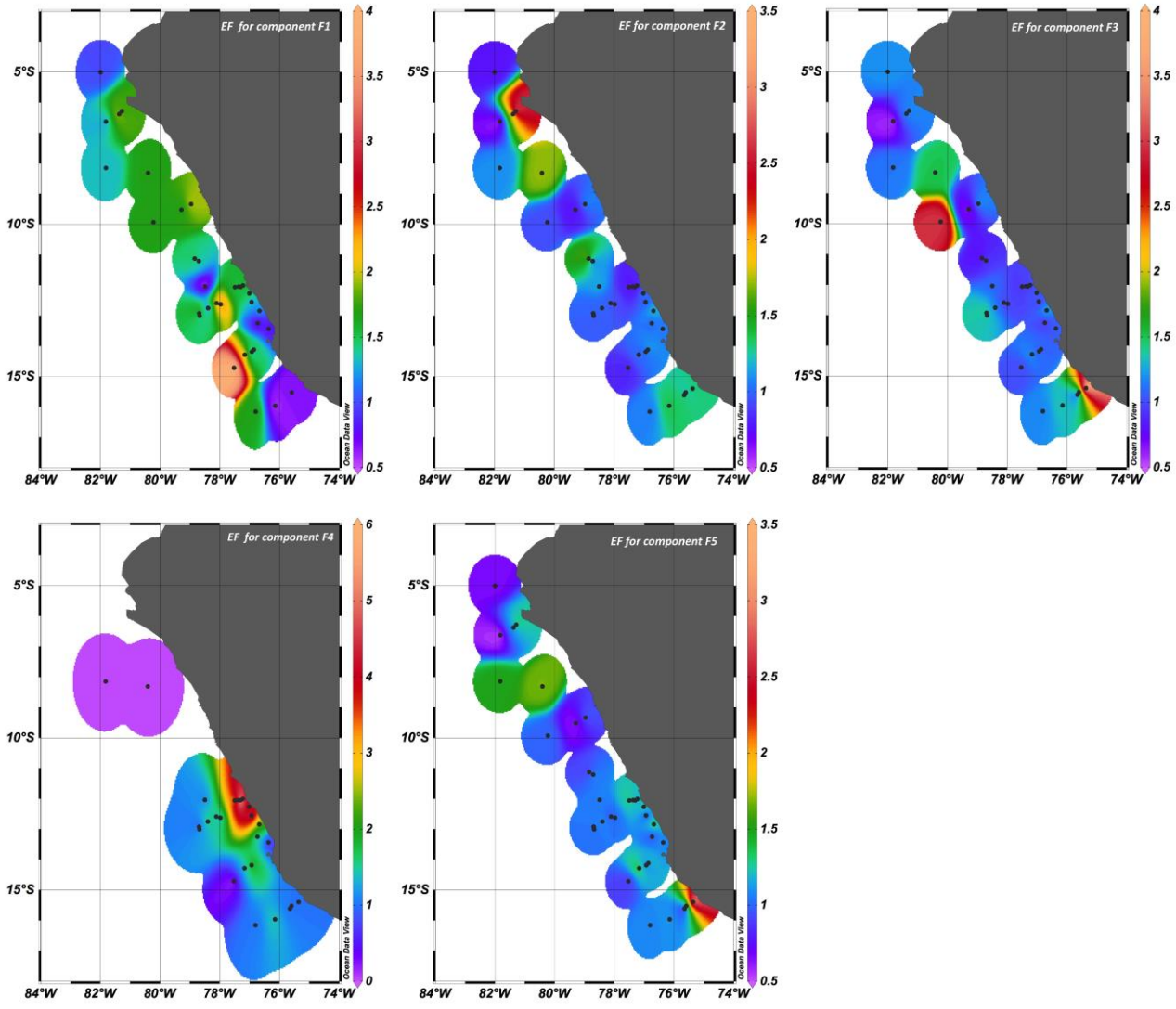

Figure 13

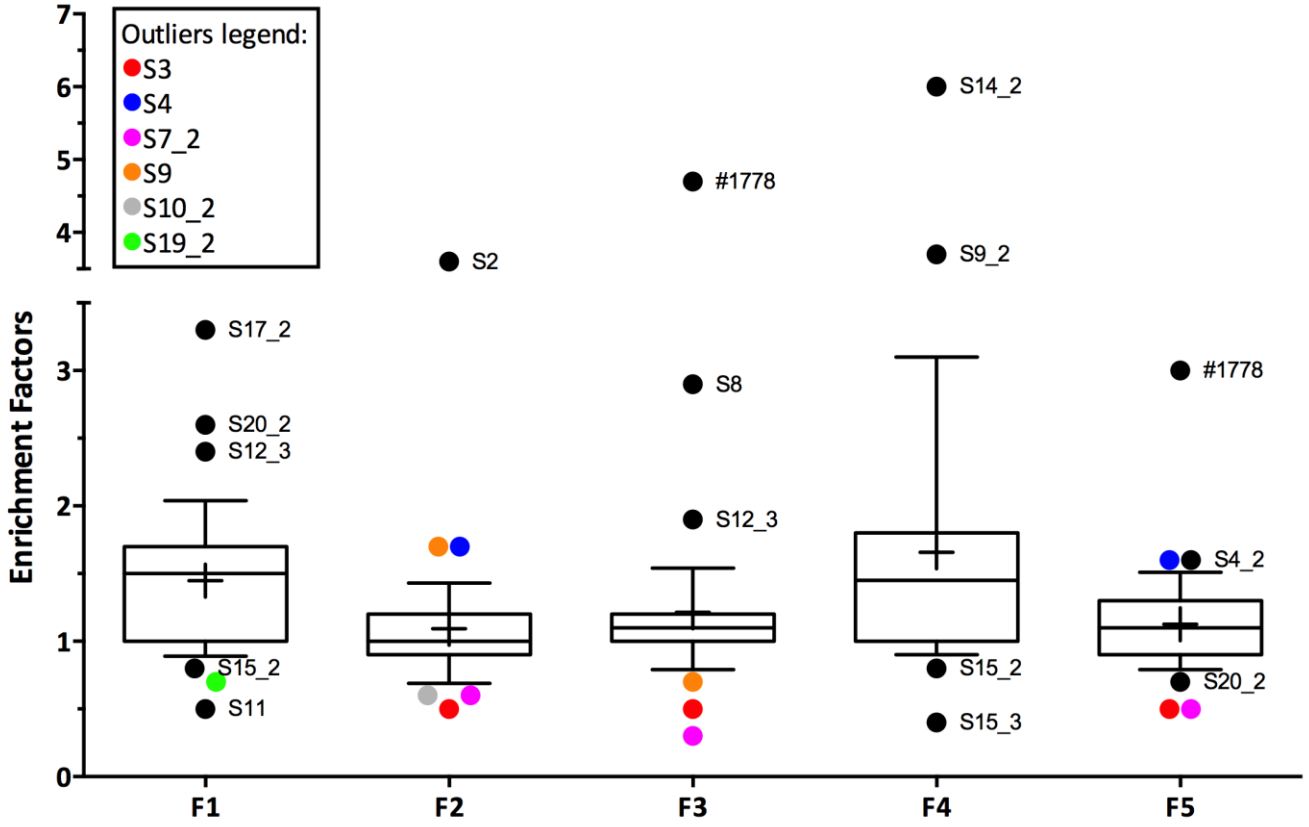
Outliers legend:
S3
S4
S7_2
S9
S10_2
S19_2
Enrichment Factors
7
6
5
4
3
2
1
0
S17_2
S20_2
S12_3
S15_2
S11
S2
#1778
S8
S12_3
S14_2
S9_2
S15_2
S15_3
#1778
S4_2
S20_2
F1
F2
F3
F4
F5

Figure 14

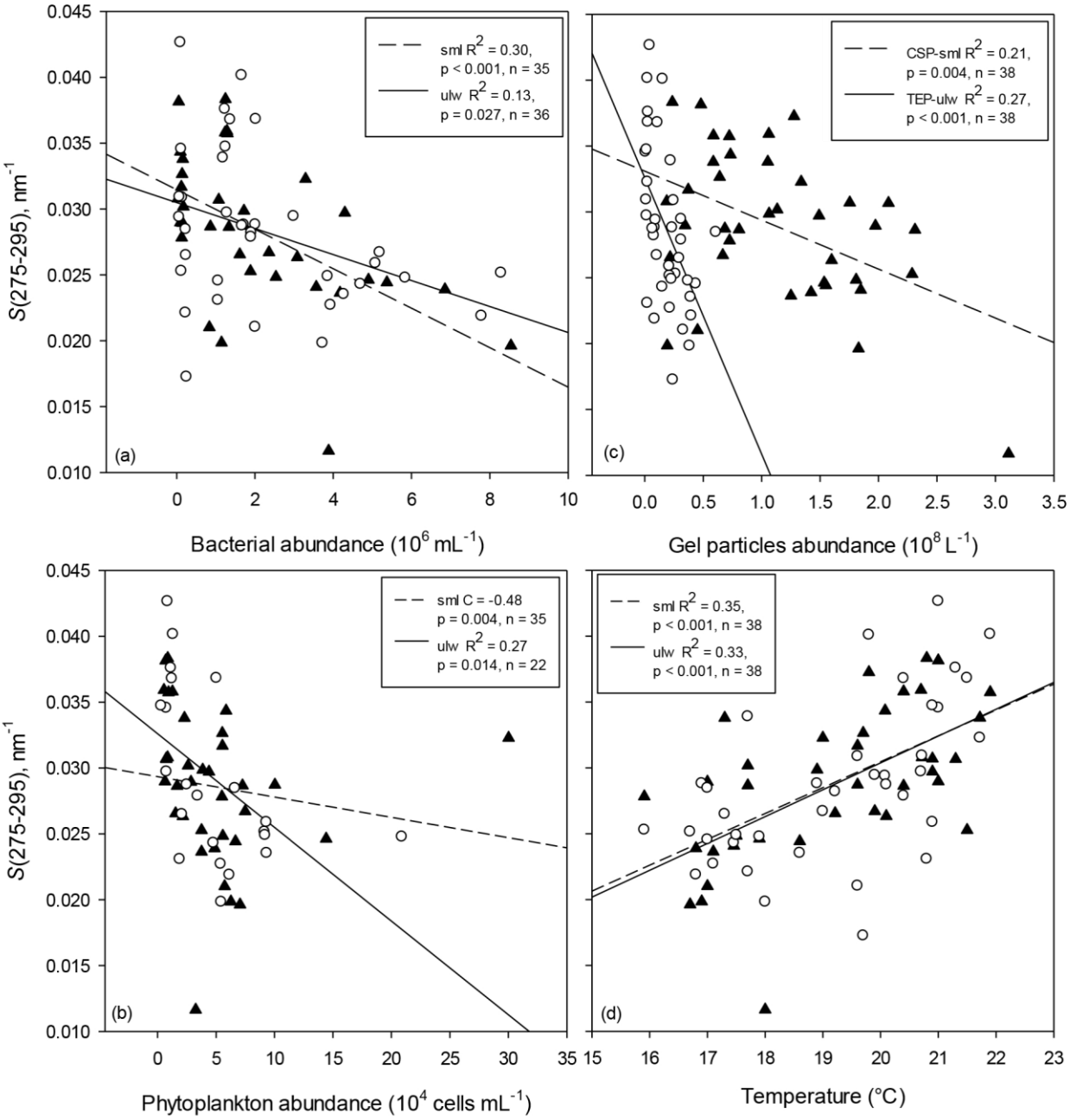
S(275-295), nm-1
sml R2 = 0.30,
p < 0.001, n = 35
ulw R2 = 0.13,
p = 0.027, n = 36
(a)
Bacterial abundance (10^6 mL^-1)
CSP-sml R2 = 0.21,
p = 0.004, n = 38
TEP-ulw R2 = 0.27,
p < 0.001, n = 38
(c)
Gel particles abundance (10^8 L^-1)
sml C = -0.48
p = 0.004, n = 35
ulw R2 = 0.27
p = 0.014, n = 22
(b)
Phytoplankton abundance (10^4 cells mL^-1)
sml R2 = 0.35,
p < 0.001, n = 38
ulw R2 = 0.33,
p < 0.001, n = 38
(d)
Temperature (°C)

Figure 15