Optical Characterization of Dissolved Organic Matter in the Amazon River Plume and the
Adjacent Ocean: Examining the Relative Role of Mixing, Photochemistry, and Microbial
Alterations

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Abstract

The Amazon River is a major source of terrestrially-derived dissolved organic matter (DOM) to the Atlantic Ocean. Measurements of dissolved organic carbon (DOC) and its optical properties (absorbance and fluorescence) were made in the water within and adjacent to the Amazon River plume during two cruises conducted at periods of low (September-October/2011) and high (July/2012) river discharges. Four fluorescent components were resolved by excitation emission matrix fluorescence combined with parallel factor analysis (EEM-PARAFAC) as two terrestrial humic-like components, one marine humic-like component, and one autochthonous protein-like component. The distribution and dynamics of optically active fraction of DOM were largely controlled by physical mixing along the Amazon River-ocean continuum, with the exception of the protein-like component whose distribution was driven by factors other than dilution. Principal component analysis (PCA) enabled differentiation of water samples following gradient of terrestrial signatures and distinction of source materials associated with different fluorescent components. On board water incubation experiments with photochemical and microbial alterations revealed that photochemistry was primarily responsible for the remineralization of optically active fraction of DOM whereas the contribution of microbial transformation to this fraction was minor. Results from this work will provide a better understanding of DOM compositions and transformations in a globally significant river plume-ocean continuum.

Keywords: Amazon River plume; Chromophoric dissolved organic matter (CDOM); Fluorescence; Excitation emission matrix (EEM); PARAFAC model; Principal component analysis; Photochemical degradation; Microbial degradation.

1. Introduction

The Amazon River is the world's largest river by far in terms of water flow, numbers of tributaries, and catchment area. It drains nearly 7,000 km² across the South American continent and accounts for ~15% of global river discharge (Coles et al., 2013). The river delivers large amounts (approximately 22 Tg carbon/yr) of dissolved organic carbon (DOC) (Richey et al., 1990) derived predominantly from tropical rainforests and savannah and thus has a terrestrial origin, to the Western Tropical Atlantic Ocean. A significant portion of these DOC inputs are generally considered biologically refractory in nature owing largely to the high abundance (60%) of terrestrial humic substances, which is also capable of absorbing light (Ertel et al., 1986). These light-absorbing materials, known as chromophoric dissolved organic matter (CDOM), become dominant in the total constituent absorption in the UV in areas closest to shore (Salisbury et al., 2011). Via absorption of sunlight, CDOM greatly influences the underwater light field and subsequently affects photoecological processes, including primary production and microbial activities (Moran and Zepp, 2000). Despite its significance on these processes, optical signatures of riverine CDOM in the Amazon plume as well as changes during transport to the ocean remain poorly characterized. A fraction of CDOM fluoresces after absorbing light and this allows additional methods for characterizing different CDOM components. These optical features, with their derivative optical indices including CDOM spectral slope (S) and specific ultraviolet absorbance (e.g., SUVA₂₅₄), have been widely used to study DOM sources and composition in aquatic environments (e.g., Coble, 1996; Fichot and Benner, 2012; Helms et al., 2008; Yamashita et al., 2010). Although temporal evolution and spatial variability of CDOM and its fluorescence within and adjacent to the Amazon River plume have been previously described *in situ* (Del Vecchio and Subramaniam, 2004) and synoptically from satellite observations

(Salisbury et al., 2011), characterization of CDOM fluorescence properties and their dynamics during mixing of riverine and oceanic waters is however still lacking.

Global oceanic DOC inputs from rivers alone are sufficient to account for the entire DOC pool in the ocean, yet only small quantities of DOC found in marine systems appear to be land-derived (Hedges et al., 1997). The timing and mechanisms by which these substantial terrestrial inputs are transformed remain largely unknown. Photochemistry is a commonly recognized process that both directly removes dissolved organic matter (DOM) as CO₂ and CO by photooxidation (Powers and Miller, 2015), and alters its availability for microbial consumption. The combined and relative magnitudes of these photochemical processes, and exactly how they control the DOC cycle is debatable, however, largely due to great uncertainty in quantifying the stimulatory effect on bacterial metabolism (Mopper and Kieber, 2002 and references therein). Many studies have shown that exposure to sunlight structurally alters intrinsic bio-resistant DOM, producing photoproducts that are readily used for bacterial metabolism, thereby enhancing the productivity of microorganisms and fostering the general belief that photochemistry promotes microbial biomass production and increased respiration (e.g., Kieber et al., 1990; Moran and Zepp, 1997). However, other studies have found that photochemical and biological processes compete in the mineralization of terrigenous DOC with photooxidation potentially removing bioreactive DOC directly. This could account for some studies that report reduced bacterial activity following irradiation (Obernosterer and Benner, 2004). Moreover, most previous work of DOM decomposition has been conducted by isolating these two processes, using microbe-free, natural/stimulated solar irradiations to determine the kinetics of photodegradation (e.g., Miller and Zepp, 1995) and dark incubations alone to quantify the extent of microbial process (e.g., Moran et al., 1999). Recently, a limited number of studies have begun

to address the combined effects of photo- and bio-degradation with a few focusing on the changes in optical properties when these two processes co-occur (Benner and Kaiser, 2011). The Amazon River plume represents an area that both supports diverse microorganisms (Goes et al., 2014) and receives intense solar radiation, thus creating an ideal reactor for significant interactions between photo- and microbial processes. To date, the partitioning of photochemical and biological processes in this area is largely unstudied.

Thus the primary focus of this study is to trace the dynamics of DOM optical properties within the Amazon River-ocean continuum and to evaluate the relative connection between photochemical and microbial processes with observed DOM optical changes. Here we characterize the absorbance and fluorescent properties of waters taken within the Amazon River plume and the adjacent ocean. Excitation and emission matrix spectroscopy, coupled with parallel factor analysis (EEM-PARAFAC) and principal component analysis (PCA) on fluorescent DOM, were used as proxies to investigate the distribution and dynamics of CDOM in this region. Photochemical, microbial, and combined photo-/microbial degradation experiments were conducted to quantify relative contributions to optical changes and removal of DOM. Findings from this study contribute to the understanding of the fate of riverine DOM in the tropical Atlantic Ocean by providing insight on the sources and mechanisms of that transform DOM in the Amazon River plume.

2. Materials and methods

2.1. Chemicals and labware

All chemicals were American Chemical Society (ACS) grade and used without further purification. All reagents, standards, and spectrophotometric blanks were prepared using 18.2

MΩ ultrapure water (Milli-Q, Millipore). All labware (borosilicate, quartz, and filtration components) was acid cleaned (0.01 M HCl), rinsed with Milli-Q water before either drying or flushing with sample. As required, some non-volumetric borosilicate, quartz glassware, and GF/F filters were also pre-combusted at 450°C for at least 5 h as indicated below.

2.2. Sample collection

Samples were collected along the Amazon River plume and the adjacent ocean (Fig. 1) during two river flow conditions: low flow discharge in September/October 2011 ($\sim 110,000 \text{ m}^3 \text{ s}^{-1}$) and high flow discharge in July 2012 ($\sim 267,000 \text{ m}^3 \text{ s}^{-1}$). The collection included both surface ($\sim 2 \text{ m}$) and below plume depths (at $\sim 20 \text{ m}$, $\sim 1000 \text{ m}$, and $\sim 2000 \text{ m}$) as described by Medeiros et al. (2015). Immediately after collection, plume/oceanic samples were filtered using $0.2 \mu\text{m}$ Pall Supor membranes, and aliquots were collected into 60 mL pre-combusted amber bottles and kept frozen (-20°C) until total DOC analysis within one month of the end of each cruise. Filtrate aliquots were also collected into 100 mL pre-combusted amber glass bottles and kept in the refrigerator (4°C) until optical analyses within six months after sampling. Samples of similar coastal (Powers and Miller, 2015) and open ocean (Swan et al., 2012) waters have shown no measurable changes in CDOM absorption spectra over 12 months of storage.

2.3. Photo-irradiation and microbial incubation experiments

Water incubations were performed at sea in a flow-through incubator with constantly flowing surface seawater keeping the flasks at ambient surface water temperature. For dark microbial incubations, water samples were first filtered through $0.7 \mu\text{m}$ pre-combusted Whatman GF/F filters, and collected into 1 L pre-combusted amber glass bottles in triplicate, wrapped in aluminum foil, and kept submerged for 5 days enclosed by a black plastic bag. Although we did

not perform any microbial activity measurements on these dark-incubation samples, analysis of the DOM composition using 0.7 μm filtered water from the Amazon mouth and plume have revealed evident changes in composition at the molecular level before and after dark incubations (Medeiros et al., 2015; Seidel et al., 2015). Therefore, samples filtered through 0.7 μm filters were considered microbially active. For photochemical incubations, samples were a) filtered using 0.2 μm membranes (photochemical only) and b) filtered using 0.7 μm combusted GF/F filters (photochemical and microbial combined), respectively. These two filtrate sets were collected in triplicate into 1.5 L pre-combusted quartz flasks with a 500 mL headspace. Due to cruise length constraints, flasks were exposed continuously on deck to natural sunlight for 5 days only, with the lower part of the flask submerged in a flowing surface seawater bath to regulate temperature. Since no irradiance measurements were available, we estimated an average total solar irradiance (5.88 kWh/m²/d; 0.78 kW/m² solar noon) for the general conditions of the exposure (5° N, 50° W; July) using climatological data obtained from the NASA Langley Research Center Atmospheric Science Data Center and the Surface Meteorology and Solar Energy calculator (Version 6.0). Noon UV irradiance (280-400nm) for mid July was also estimated to be 6.2 W/m² using the UCAR/NCAR Quick TUV Calculator (http://cprm.acom.ucar.edu/Models/TUV/Interactive_TUV/). After incubation, all samples were 0.2 μm filtered and collected for total DOC and optical analyses as described before.

2.4. DOC analysis

Total DOC concentrations for water samples were measured using a Shimadzu TOC-V_{CPH} with daily potassium hydrogen phthalate standard curves. Standard solutions were prepared either on the same day or the day before DOC sample analysis. Analytical accuracy and precision were tested against the Consensus Reference Material for DOC in seawater (42-45 μM)

obtained from the University of Miami (Hansell, 2005; Sharp, 2002) and were better than 5%.

2.5. Optical measurements

2.5.1. Absorbance measurement

Water samples were adjusted to room temperature prior to optical measurements. UV-visible absorbance scans for CDOM were made over the wavelength range 250 to 800 nm at 1 nm intervals on a single-beam spectrophotometer (Agilent 8453 UV-Vis) using a 5 cm quartz cuvette. Daily-measured Milli-Q water was used as the reference. Baseline corrections were made by subtracting an offset value from the absorbance spectra, according to Green and Blough (1994). The offset-corrected absorbance was used to calculate absorption coefficients following Eq. (1):

$$a_g(\lambda) = 2.303 \times A(\lambda)/L \quad (1)$$

where $a_g(\lambda)$ (m^{-1}) is the Napierian absorption coefficient of CDOM at wavelength λ , $A(\lambda)$ (unitless) is the offset-corrected measured CDOM absorbance at λ , and L (m) is the path length of the spectrophotometric cell and $L = 0.05$ m.

To determine CDOM spectral slope coefficient (S) over the spectral ranges 275–295 nm and 350–400 nm, denoted hereafter as $S_{275-295}$ and $S_{350-400}$, respectively, $a_g(\lambda)$ spectra were determined using a non-linear least square regression fitting routine (*nlinfit*) in the MATLAB® Statistics Toolbox (MathWorks, MA) applied over the spectral ranges of 275–295 nm and 350–400 nm for best fit to the following equation:

$$a_g(\lambda) = B \times \exp(-S \times \lambda) \quad (2)$$

where a_g is the absorption coefficient (m^{-1}) of CDOM at wavelength λ , B is a fitting parameter, and S is the desired spectral slope coefficient (nm^{-1}). CDOM spectral slope ratio (S_R) was subsequently calculated as: $S_R = S_{275-295}/S_{350-400}$ (Helms et al., 2008). Specific ultraviolet absorbance (SUVA_{254}) has been used to estimate DOM aromaticity (Weishaar et al., 2003), with fresh terrestrial DOM generally associated with higher SUVA_{254} . SUVA_{254} ($\text{mg C L}^{-1} \text{ m}^{-1}$) was obtained by dividing the absorption coefficient $a_g(254)$ (m^{-1}) by the sample DOC concentration (mg C L^{-1}).

2.5.2. Fluorescence measurement

Excitation-emission matrix (EEM) fluorescence spectra were measured on an Aqualog spectrofluorometer (HORIBA Jobin Yvon Inc., NJ, USA), using a 1-cm quartz cell with Milli-Q as the blank. Fluorescence intensities (FI) of EEM spectra were recorded by scanning the excitation range between 240 and 450 nm (5 nm intervals) and capturing emission spectra over wavelengths from 280 to 500 nm (3.2 nm intervals), using a range of optimal integration times. Optimal integration times for each sample (ranged from 0.5s to 10s) were determined by maximizing emission intensity at low excitation wavelength without saturating the detector at high excitation wavelength (Gentry-Shields et al., 2013). Post-processing steps to correct and calibrate the fluorescence spectra followed Murphy et al. (2010) and included: (1) EEM spectra were corrected for instrument configuration using manufacturer-provided excitation and emission correction parameters; (2) samples with high absorption coefficients that could alter excitation and emission intensities were corrected for inner filter effects according to McKnight et al. (2001); (3) fluorescence spectra were normalized to the Raman peak area at 350 nm excitation in Milli-Q water scanned daily and blanks were subtracted from the sample fluorescence spectra; (4) all spectrally corrected EEM data were calibrated against quinine

sulfate standard solutions and reported in quinine sulfate units (QSU), where 1 QSU is equivalent to the maximum fluorescence intensity of 1 ppb quinine sulfate in a 0.1 N H₂SO₄ solution measured at $\lambda_{\text{excitation}}/\lambda_{\text{emission}} = 350/450$ nm; (5) Rayleigh and water Raman scatter signals were removed using an interpolation protocol developed by Bahram et al. (2006).

2.6. PARAFAC modeling

A total of 64 samples from two cruises, including samples for photo- and microbial degradation experiments, were subjected to PARAFAC modeling, using DOMFluor toolbox in MATLAB (Stedmon and Bro, 2008). Due to the dominance of a high fluorescence signal in plume waters, EEM data were scaled to unit intensity by normalizing to individual fluorescence intensity maxima (Murphy et al., 2008) prior to the modeling process. PARAFAC analysis was applied to these scaled EEM spectra. No samples were identified as outliers and the model was constructed and further validated by split-half analysis. The modeled fluorescence intensities of each component were then multiplied by their maximum intensity to reconstruct actual fluorescence intensities of components in a given sample.

2.7. Statistical analyses

Because of the small number of samples, we performed non-parametric Mann-Whitney *U* tests and Kruskal-Wallis tests to assess the differences in water properties from different regions under two distinct hydrological events. All the statistical analyses used to investigate the relationship between the variables were conducted using the open source statistical computing software “R” (<http://www.r-project.org/>). Statistical significance levels are reported as non-significant ($p > 0.05$), significant ($0.001 < p < 0.05$), or highly significant ($p < 0.001$). All the values of “*r*” are Pearson correlation coefficients unless otherwise noted. Principal component

analysis (PCA) was conducted using the relative abundance of fluorescent PARAFAC components. Relative abundance was calculated by log-linearizing the absolute abundance, subtracting the individual means, and dividing by their standard deviations. Values for the mean and standard deviation of each PARAFAC component were also calculated from the log-linearized abundance.

For the photo- and microbial degradation experiments, percent losses of absorptivity and fluorescent intensities as a function of incubation treatments were quantified according to Eq. (3) and (4):

$$\Delta a_g(\lambda)\% = [a_g(\lambda)^{t=0} - a_g(\lambda)^{t=final}] / a_g(\lambda)^{t=0} \times 100 \quad (3)$$

$$\Delta F(i)\% = [F_{max}(i)^{t=0} - F_{max}(i)^{t=final}] / F_{max}(i)^{t=0} \times 100 \quad (4)$$

where $\Delta a_g(\lambda)\%$ is the percent loss of absorptivity at wavelength λ and $\Delta F(i)\%$ is the percent loss of fluorescence intensity of the i th component; $a_g(\lambda)^{t=0}$ and $F_{max}(i)^{t=0}$ are the CDOM absorption coefficient at wavelength λ and the fluorescence intensity of the i th component, respectively, both at the initial time point ($t = 0$) of the experiment. Likewise, $a_g(\lambda)^{t=final}$ and $F_{max}(i)^{t=final}$ represent the corresponding optical properties at the end ($t = \text{final}$) of the experiment. Negative values indicate the percent increase of $a_g(\lambda)$ or fluorescence intensity of the i th component (i.e., $\Delta F(4)\%$ for component 4 in this study).

3. Results and discussion

3.1. Spectral properties of fluorescent PARAFAC components

Four fluorescent components of DOM, hereafter referred to as components C1 to C4, were identified and validated by PARAFAC modeling (Table 1; Fig. 2). A comparison between the four components found in this study and those that have been previously reported by other studies are also listed in Table 1. There is an excitation maxima at 250 nm with its emission peak at 460 nm for component 1, a feature close to that of the humic-like fluorescent peak A proposed by Coble (1996) as originating from terrestrial sources. This component is now recognized as being ubiquitous, existing in freshwater (e.g., component 1 in Yamashita et al. (2010)), coastal (e.g., component 1 in Kowalczyk et al. (2009)) as well as open oceanic (e.g., component 1 in Walker et al. (2009)) environments. EEM component 2 possesses double excitation peaks, occurring at < 260 and 305 nm, and an intensive emission maximum at 400 nm. This component resembles Coble's peak M and could possibly be associated with microbial metabolism. Component 3 is representative of fluorophores with the longest excitation wavelengths and broadest emission range (peak at 460 nm), considered to contain wide-spread, terrestrial humic-like organic matter. Component 4 shows features that are typical of autochthonous protein-like fluorophores, with an excitation peak around 270 nm. However, with an emission maxima at 320 nm, it is not possible to completely resolve this component to either a tyrosine (peak emission = 304 nm) or tryptophan-like component (peak emission = 340 nm).

3.2. Variations of DOM and its optical characteristics

Using the distinct signature of the Amazon River plume (salinity < 35) (Goes et al., 2014), we divided our study region into three sub-areas based on geographic locations and salinity: (1) the low-salinity plume core between 1° and 4 °N during high discharge (2012, salinity < 30) confined near the Amazon River mouth (0°42'28"N, 50°5'22"W), including the southern patch and water northward to French Guiana; (2) the plume extending northwestward

alongshore and eastward offshore from French Guiana, including surface and subsurface waters (salinity < 35 during low discharge in 2011 and $30 < \text{salinity} < 35$ when the flow was high in 2012); and (3) oceanic surface water that was not influenced by the plume (salinity > 35 for both low and high flow conditions, water depth >20 m). Fig. 3 presents the distribution of salinity and DOM quantitative parameters. Salinity ranged from ~0.05 at the river mouth to ~36.3 in oceanic waters below the plume (e.g., waters at 20 m depth). DOC concentrations varied strongly, ranging from 61 to 277 μM . Maximum DOC levels were observed from the core of the plume in the vicinity of the river mouth under high flow condition ($\text{DOC} = 149.3 \pm 68 \mu\text{M}$, $n = 7$), progressively decreasing northward along the plume pathway ($\text{DOC} = 87.8 \pm 25 \mu\text{M}$, $n = 5$), and then offshore into the western Atlantic Ocean ($\text{DOC} = 63 \pm 2.5 \mu\text{M}$, $n = 3$). DOC concentrations around the river mouth were significantly higher than those in the plume and oceanic waters (Kruskal-Wallis test, $p < 0.001$), indicating that a considerable amount of riverine-derived DOC was carried from the Amazon mainstream into the plume and adjacent ocean. However, different flow conditions did not result in significant DOC changes to the north and east of French Guiana, in either the plume (mean $\text{DOC} = 91 \mu\text{M}$ at low flow, $n = 17$; compared to $87.8 \mu\text{M}$ during high flow event, $n = 5$; Mann-Whitney U test, $p > 0.05$) or in surface oceanic water (mean $\text{DOC} = 82 \mu\text{M}$ during low flow, $n = 1$; compared to $63 \mu\text{M}$ under high flow discharge, $n = 3$; $p > 0.05$), suggesting that the influence of water discharge on DOC in the plume and oceanic waters was minor, and only limited to a certain area (i.e., the plume core from river mouth alongshore to French Guiana).

Most of the optical properties of DOM, except fluorescent C4, generally displayed similar distribution patterns to that of DOC, with highest values occurring near the river mouth during high-flow conditions (e.g., $a_g(300) = 20.8 \text{ m}^{-1}$, FI = 64.6, 24.6, and 29 QSU of C1, C2,

and C3, respectively, at salinity ~ 0.05) and lowest values occurring in more saline oceanic surface waters (e.g., $a_g(300) = 0.3 \text{ m}^{-1}$, FI = 0.75, 0.3, and 0.2 QSU for C1, C2, and C3, respectively, at salinity ~ 36.1). Moreover, strong linear relationships of DOC to CDOM and to humic-like FDOM (i.e., C1, C2, and C3) were found ($r \sim 0.9$, $p < 0.05$), indicating the dynamics of optically active compounds, including the humic-like fluorescent fraction in the DOM pool, were tightly coupled to that of DOC. This is not surprising given that a large fraction of organic matter drained from the Amazon River system are terrestrial macromolecules (Ward et al., 2013), many of which contain a variety of light-absorbing and fluorescent moieties, including aromatic and unsaturated aliphatic compounds (Ertel et al., 1986).

Fluorescent component C4 did not exhibit substantial variability, instead showing an overall less varied distribution when compared between the two flow conditions (mean FI = 1.3 and 0.9 QSU for plume and surface seawater in low discharge, compared to mean FI = 1.6, 1.9, and 1.4 QSU for waters at the river mouth, in the plume and oceanic surface waters during high flow, respectively). Similar to other fluorescent components, enhanced outflow resulted in significantly higher C4 fluorescence in waters near the river mouth, relative to that in the alongshore plume and seawater (Kruskal-Wallis test, $p < 0.05$). However, no significant differences for C4 in the plume water were found between different discharge events ($p > 0.05$), indicating that C4 in the plume was likely regulated by factors other than river discharge. Furthermore, no significant correlation between DOC and C4 across the continuum was observed ($r = 0.03$, $p = 0.3$), suggesting that C4 was most regulated by processes different from those governing DOC variability. One possible explanation is that the protein-like C4, as compared to humic-like compounds, was more labile and susceptible to bacterial cycling and rapid consumption during phytoplankton blooms that occurred throughout the sampling period

(Fellman et al., 2010a; Medeiros et al., 2015) resulting in low, rather than high concentrations in the plume. Additionally, bacterial carbon consumption rates (i.e., production and respiration) are closely related to the bio-available fraction of dissolved organic carbon (Middelboe et al., 2012). Thus, although more DOC was found near the river mouth, it likely contains an abundance of humic compounds that are resistant to bacterial utilization (Bauer and Bianchi, 2011; Benner and Biddanda, 1998; Wiegner and Seitzinger, 2001). As a consequence, bacterial uptake capacity, as investigated by optical analyses, could be limited due to the depletion of bioavailable DOC in the vicinity of river mouth, producing only low levels of microbially derived protein-like components in the plume.

3.3. Relationships of salinity with DOM and its optical properties

To investigate DOM processes in the Amazon River-ocean continuum, we examined the relationship between salinity and measured properties in surface waters (water depth < 20 m) regardless of river discharge. Changes of DOC and its optical properties with salinity are given in Fig. 4. Both DOC and $a_g(300)$ were generally lower at higher salinity and there was a strong correlation between salinity and DOC ($\text{DOC} = -5.3 \times \text{salinity} + 257$, $r = 0.92$, $p < 0.001$, $n = 32$). This behavior of DOC, however, cannot simply be interpreted as conservative mixing of the plume with oceanic water alone. A noticeable nonlinear trend ($r = 0.04$, $p > 0.05$) was found at higher DOC levels (Fig. 4(a)) in the outer plume (salinity > 33) and oceanic waters below the plume, suggesting more than two distinct water masses were mixing. This is similar to the nonlinear distribution of DOC within the salinity gradient in the Baltic-North Sea transition zone where three different water masses are reported to mix (Osburn and Stedmon, 2011). In contrast, a tight correlation between $a_g(300)$ and salinity was found in the entire study area ($a_g(300) = -0.50 \times \text{salinity} + 17.7$, $r = 0.92$, $p < 0.001$, $n = 32$), even in the high salinity region where no

correlation between DOC and salinity was observed ($a_g(300) = -0.11 \times \text{salinity} + 4.6$, $r = 0.4$, $p < 0.05$, salinity > 33). Note that there was a small deviation of $a_g(300)$ from the conservative mixing line with lower values in the mesohaline water ($30 < \text{salinity} < 35$) (inset plot in Fig. 4(b)), suggesting a removal of CDOM within the Amazon plume. This could represent photobleaching, a recognized dominant sink for CDOM (Del Vecchio and Blough, 2006). Considering the extraordinarily dynamic nature of the continuum, however, other factors such as particle adsorption (Guo et al., 2007; Uher et al., 2001), vertical mixing with subplume flow (Del Vecchio and Subramaniam, 2004), and photoflocculation (Helms et al., 2013) may also contribute to the loss of color from the DOM pool. Additionally, two samples with moderate salinity (~ 25) deviated slightly from the mixing line (Fig. 4(b)) and were associated with waters with observed *Trichodesmium* and coastal diatom blooms (Medeiros et al., 2015; Seidel et al., 2015). Rather than CDOM increases from primary production as reported by Del Vecchio and Subramaniam (2004), reduced CDOM could be due to *in situ* removal processes involving microorganisms (Zhang et al., 2013) in these highly-productive regions.

The humic-like fluorescent components (C1, C2, and C3) generally exhibited a linear correlation with salinity ($C1 = -1.84 \times \text{salinity} + 64.6$, $C2 = -0.68 \times \text{salinity} + 24.9$, and $C3 = -0.78 \times \text{salinity} + 27.0$, with $r = 0.96$, 0.97 , and 0.95 , respectively, $p < 0.001$, $n = 32$, Fig. 4(c-e)) over the entire mixing regime, suggesting that the substantial terrestrial input of these components behaved conservatively in mixing with marine end-members. A similar trend for terrestrial humic-like components (C1 and C3) has been observed in various other mixing regimes including the South Atlantic Bight (Kowalczyk et al., 2009), Hudson Bay (Guéguen et al., 2011), and Danish coastal waters (Osburn and Stedmon, 2011). Humic-like component C1 dominates the total fluorescence signal within the entire study regime (range = 24%–56%; avg. =

~40%) and best correlates with DOC ($r = 0.90$, $p < 0.001$), indicating persistence throughout the mixing process. Interestingly, while marine humic-like component C2 is thought to be produced by bacterial reworking of humic-like components, the magnitude of any microbial transformation here was not sufficient to influence the conservative behavior of C2 during mixing. Overall, the strong dilution of river water with seawater was the dominant process in shaping the conservative behavior of humic-like components along the salinity gradient in agreement with Medeiros et al. (2015).

In contrast, the protein-like component C4 does not correlate with salinity ($r = 0.04$, $p > 0.05$, Fig. 4(f)). This independence of C4 from salinity has been observed elsewhere, including Japan's Ise Bay (Yamashita et al., 2008), the subtropical Jiulong Estuary in China (Guo et al., 2011), and in near-shore marine ecosystems that experiences glacial runoff (Fellman et al., 2010b). Absence of correlation between C4 and salinity may result from (1) *in situ* addition along the salinity gradient (Kowalczyk et al., 2009), as further supported by the lack of correlation between C4 and C1 (shown later in Fig. 5(c)), and/or (2) C4 present as tyrosine residuals in biorefractory low molecular weight DOM, regarded as a remnant of autochthonous DOM biodegradation (Yamashita et al., 2008, and references therein).

3.4. Behavior of fluorescent components in the plume and seawater

To further explore the variations of fluorescent components across the continuum, we performed regression analysis between the four EEM components in surface waters (water depth < 20 m) based on the model proposed by Murphy et al. (2008). Because C1 is considered to be almost exclusively terrestrial, comparison of C1 with the other three components should highlight the importance of non-terrestrial or autochthonous protein sources relative to terrestrial

input, revealing possible sinks and/or sources for organic matter during transport. Concentrations of $\log(C2)$ and $\log(C1)$ were generally highly correlated ($r = 0.94$, $p < 0.001$, Fig. 5(a)) in the entire study area, suggesting C2 was largely terrestrial in origin and conservative mixing played a dominant role in controlling C2 distribution. On closer inspection, however, the slope coefficient (0.85) in the low salinity plume area ($30 < \text{salinity} < 35$) suggests a decoupling of C2 and C1 and that other non-conservative factors alter C1 and C2 at different rates (Guéguen et al., 2011). Deviation of $\log(C2):\log(C1)$ from the 1:1 line toward a lower ratio (Fig. 5(a)) indicates that the removal rate of C1 outpaced that of C2. This could occur even if they were both governed by the same process, for example photochemistry, since C1 has been reported to be more susceptible to photo-alteration (Murphy et al., 2008) than C2.

A stronger linear correlation existed between $\log(C3):\log(C1)$ (Fig. 5(b)), with a slope of 1.1 and an intercept (-1.28) that nearly equals the C1/C3 ratio found in the terrestrial endmember. This strikingly high degree of covariance of the terrestrial humic-like components agrees well with previous findings made in open ocean (Murphy et al., 2008), estuarine (Guo et al., 2011), and fresh waters (Stedmon and Markager, 2005). This result suggests a common source and that both physical mixing and other alteration processes (e.g., photochemical or microbial decomposition) acted at nearly identical rates (Murphy et al., 2008). The equatorial location of the Amazon River means that plume water receives substantial solar irradiation as water meanders northward, likely supporting an enhanced photochemical removal of terrestrial humic-like fluorophores (i.e., C1 and C3) during mixing processes.

The protein-like component C4 displays an overall significant, but relatively weak correlation with C1 ($r = 0.17$, $p < 0.05$, Fig. 5(c)) when data from the entire mixing regime is evaluated together. This indicates that while related to C1, C4 was at least partly regulated by

different processes, especially evident in the decoupling at the river mouth and in the plume core (salinity < 30) ($r = 0.02$, $p = 0.65$). The less correlation between C4 and C1 in the plume core further substantiates our previous suggestion that C4 experienced processes (e.g., microbial reworking) in the plume distinct from simple mixing. The increasing concentration of C4 toward the outer-plume and in oceanic water may indicate the changing bioavailability and biochemical composition of DOM.

3.5. Assessing fluorescent components using EEM-PARAFAC-PCA

To better evaluate the spatiotemporal variability of DOM fluorescence, we performed PCA using the relative abundances of the four identified PARAFAC fluorescent components ($n = 50$). The bi-plot in Fig. 6 illustrates the distribution of all four fluorescent components on the first two PCs, which together accounted for more than 98% of the total variance. The humic-like components (C1–C3), with negative loadings on both PCs (ranges = -0.53 to -0.40 on PC1 and -0.29 to -0.18 on PC2, respectively), were clearly separated from the protein-like component (C4), which has negative (~ -0.39) PC1 loading and a positive (~ 0.92) loading on PC2. The large degree of variance explained by PC1 ($\sim 85\%$), the less variable loadings of the four components on this PC, and the highly significant correlation of PC1 scores with DOC ($r = 0.79$, $p < 0.001$) collectively suggest that PC1 separates samples according to their gradient in terrestrial signature. Compared to PC1, PC2 accounts for a relatively small amount of the observed variance ($\sim 14\%$) and does not significantly correlate with DOC ($r = 0.12$, $p > 0.05$), indicating that PC2 may be related to *in situ* processes that alter DOM composition across the continuum as well as in deep oceans. Clustering of the three humic-like components and their separation from the protein-like component on PC2 again confirms that C1–C3 were similar in origin but different from that of C4. In addition, the close grouping of C2 (marine humic-like) to

C1 and C3 (both terrestrial humic-like) supports the suggestion that C2 experienced modification processes similar to those affecting C1 and C3. Hence, PC2 most likely indicates a difference in source material within the fluorescent DOM fraction.

The first two PC scores (Fig. 6) also show a clear difference between plume and oceanic waters. Oceanic water samples, regardless of discharge conditions and sampling depths, tended to group together (range = -2.3 to 0.6 and -1.4 to 1.3 on PC1 and PC2, respectively). Conversely, scores for plume waters, including the plume core, were more dispersed and variable, especially on PC1 (range = -1.4 to 4.1). To be more specific, the most positive PC1 scores (~ 4.1) were associated with the plume core samples having the lowest salinity (salinity ~ 0.05), while alongshore outflow exhibited slightly higher scores (range = -1.4 to 2.2 on PC1). The distribution pattern of PC1 scores was similar to that of DOC, further highlighting the contribution of fluorescent DOM to the bulk organic pool on the first PC. PC2 scores were more constrained than those for PC1, with most samples ranging from -1 to 1 . Moreover, PC2 scores may be indicative of an evolution of different fluorescent components during the mixing process, progressing from the more negative scores in the plume core at the river mouth, characterized as humic-rich but with protein-like component starting low (C4 $\sim 6\%$ of total fluorescence during high flow) and gradually increasing along the plume pathway while humic-like components are diluted by physical mixing and removed by biogeochemical process (C4 $\sim 18\text{--}19\%$). However, PC2 scores for oceanic waters, having proportionally more protein-like components (C4 $\sim 27\%$ and 38% during low and high flow, respectively), are evenly distributed along the PC2 axis, suggesting that C4 was comparable under the two flow conditions examined. The observed distribution of PC2 scores further supports the idea that PC2 may be an indicator of fluorescent

DOM sources, further serving as an indicator of the relative contribution of the protein-like component in this dynamic ecosystem.

3.6. DOM changes during photo-irradiation and microbial incubation experiments

Levels of DOC varied slightly under three different incubation conditions: (1) a combined photochemical + microbial treatment, (2) a photochemical-only sunlight exposure (“sterile” filtered) and (3) a microbial-only dark incubation. However, our 5-day light treatment of the river mouth water did not result in a statistically significant DOC loss for any treatment. This is contrary to large photochemical (compared to bacterial) losses of DOC previously reported for 70-day exposures by Vähätalo and Wetzel (2004) and Moran et al. (2000) using sunlight and solar simulation respectively. This could be interpreted as the specific DOC compounds found within the Amazon plume being far less photochemically reactive for CO₂ production (the dominant photochemical loss term for DOC). Alternatively, differences from previous studies more likely reflect the large difference in cumulative photon dose to CDOM in our relatively short exposures (on the order of days) relative to those in longer incubations (over several months to years) done by others. Our deck exposure system also circulated the highly attenuating surrounding waters to cool the samples, also reducing the overall photon flux within the exposure tanks relative to other studies.

Regardless of the immeasurable DOC changes found for all treatments, photochemistry was a significant process as demonstrated by the dramatic changes noted for the light-absorbing fraction of DOM (Table 2). Over 5-days, water from the Amazon River mouth lost ~ 25 to 30% of CDOM absorption when irradiated ($a_g(350) = 7.3 \text{ m}^{-1}$ after 5-day light exposure, compared to initial $a_g(350)$ of 10.43 m^{-1}) and 9% when kept in the dark ($a_g(350) = 9.5 \text{ m}^{-1}$ after 5 days). We

also found that both irradiation treatments produced the same changes in CDOM-related proxies ($p > 0.05$) with lower $SUVA_{254}$, and higher $S_{275-295}$ and S_R values. $S_{275-295}$ increased from 0.0139 nm^{-1} to 0.0169 nm^{-1} and S_R from 0.827 to 0.986 after 5-days of photochemical + microbial incubation. These changes were accompanied by a significant decrease in $SUVA_{254}$ ($p < 0.05$), from 10.867 to 8.8 $\text{mgC L}^{-1}\text{m}^{-1}$, clearly reflecting the loss of CDOM at a rate faster than the loss of DOC during irradiation. Taken together, these changes in optical proxies with no measurable DOC loss may indicate a shift to smaller MW compounds with lower aromaticity in the DOM pool. This agrees well with the general notion that, through photochemical ring cleavage and loss of aromaticity, large DOM molecules are broken down into smaller organic compounds. Compared to photochemistry, spectral characteristics were not altered in the dark microbial incubation (Table 2). Furthermore, the 24% reduction of CDOM absorptivity from solar irradiation relative to the 9% decrease in the dark incubation demonstrates that photochemical processes are more effective in removing CDOM than microbial consumption alone. Previous observations made in aquatic environments, from temperate forested and human-modified headwater streams (Lu et al., 2013) to Arctic waters (Mann et al., 2012) have shown the same result.

Changes in fluorescent properties from incubation experiments further emphasize the dominance of photodegradation in modifying fluorescent DOM (Table 2). Light exposure with or without the presence of bacteria resulted in a decrease in total FI ($> 30\%$) similar to CDOM loss by the end of our 5-day exposure. There were also significant changes in the accompanying fluorescent components, with decreases of 30 to $> 40\%$ for the C1, C2, and C3 components, and an increase of more than 150% for C4. Like CDOM and total FI, the humic-like fluorophores were similarly degraded by photochemistry. In contrast, dark microbial degradation did not result

in significant changes of total FI or alterations in relative abundances of the four individual components, supporting the notion that the fluorescent-active fraction was not available for bacterial removal over a 5-day period. This is consistent with many temperate studies that have found humic-rich compounds within the DOC pool to be biologically refractory in nature over short time scales (e.g., Cory and Kaplan, 2012; Lu et al., 2013).

Our result agrees well with prior findings of the removal of the humic-like fraction while the protein-like fluorophores increase during light exposure of northern high-latitude waters (Mann et al., 2012). Of course with a preferential removal of humic-like fluorophores, the protein-like component C4, even with no change, can account for a greater proportion in total FI after photochemical treatment (Cory et al., 2007). However, this does not fully explain our result, with the actual C4 fluorescence increased from an initial of 1.4 QSU to 4.1 after 5-day exposure. Since 0.2 μm filtered water is not entirely sterile, it is possible that the increase of the protein-like component may be due to bacterial cell lyses resulting from a limitation of bioavailable carbon sources over the 5-day incubation period (Fasching, 2009). Overall, results from photochemical and microbial incubations highlight the important influence of photochemistry in altering DOM optical character and emphasize the preferential removal of terrestrial humic-like substances, relative to the loss of microbial humic-like and gain of protein-like fluorophores, via photoprocesses.

4. Conclusions and implications

In the present study, DOM optical properties were characterized within, and adjacent to the Amazon River plume. Four distinct fluorescent components were resolved with an EEM-PARAFAC model: two terrestrial humic-like components (C1 and C3) that dominated the total

fluorescence, one marine humic-like component (C2), and one protein-like component (C4). Variability of CDOM and terrestrial humic-like components was mainly governed by mixing processes, and appear to be depleted due to photochemical degradation during transport and/or dilution with seawater. Regression analysis further confirms that the distributions of humic-like components (i.e., C1, C2, and C3) were mainly described with conservative mixing, although other mechanisms, such as autochthonous primary production, cannot be neglected. Conversely, protein-like component C4, which showed no conservative mixing pattern in the study area, was altered by different processes, with microbial activities presumably contributing substantially to its distribution. PCA allowed a clear separation of samples based on terrestrial signatures and enabled some differentiation of source materials associated with the four fluorescent components.

We also found that photochemistry, in short, 5-day surface exposures, is more effective than microbial processes in degrading CDOM and humic-like fluorescent DOM, removing roughly 30% of these optical properties in filtered Amazon river mouth water. In the same samples, no measurable changes were seen in bulk DOC, confirming the utility of simple optical proxies to examine potentially important transformations within the DOC pool. This result also argues that over short time scales, a significant synergistic removal of DOC does not occur when bacteria and sunlight are together in our experimental exposures. Since microorganisms are known to respond to DOM changes resulting from photochemical processing (Moran et al., 2000), these results may have ecological implications within the Amazon continuum. Of course the *rates* found in these filtered samples do not translate directly to the complex optical soup of the Amazon River outflow since the heavy sediment load close to shore blocks much of the UV radiation required for DOM photochemistry. However, the relative importance of the processes

studied will provide a backdrop on which to interpret *in situ* observations. Our optical result validates previous fading experiments and shows that photochemistry controls optics and even when bacteria is present, the EEMS components are largely still controlled by photochemistry. This study allows us to better understand how and to what extent the optically active DOM changes with photoprocessing in this highly dynamic system. Results in this work provide additional information about potential photoreactivity of DOM, and should encourage further efforts to better characterize the bacterial species and functional responses of the microbial community to photochemical transformations in the DOM pool.

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Table Captions

Table 1. Spectral characteristics of the four fluorescent components identified by PARAFAC.

Table 2. Results of DOM photo- and microbial degradation experiments performed with water from the Amazon River mouth (Stn. 10, salinity = 0.05). Incubation duration was 5 days for each treatment.

Figure Captions and Legends

Figure 1: Map of study area and sampling locations with bathymetry (water depth (in meters)) and topography (land elevation (in meters)) color coded. Circles in purple represent sampling locations during September - October of 2011 (low water discharge) and gray circles denote station locations in July 2012 (high flow discharge). The location of water collection for photo- and microbial incubation experiments is marked in white (Stn. 10).

Figure 2: Contour plots of the four components resolved by PARAFAC (upper panel) and the spectral characteristics of each component (lower panel). Gray lines in each excitation and emission spectra, mostly coincident with the full data result, represent the results of split-half validation.

Figure 3: Box and whisker plots of salinity, DOC, and DOM optical properties ($a_g(300)$, fluorescence intensity (FI) of components 1 to 4) during low flow (LF) in plume (salinity < 35) and surface seawater (salinity > 35); and during high flow (HF) discharge in the plume core (salinity < 30), plume (30 < salinity < 35), and surface seawater (salinity > 35).

Figure 4: Relationships between salinity and (a) DOC, (b) $a_g(300)$, (c-f) fluorescence intensity of Component 1-4 in surface waters (water depth < 20 m). The inset plot in (b) gives enhanced resolution of the relationship for salinities > 30.

Figure 5: Correlations for log-transformed fluorescence intensities of Component 1 with (a) Component 2, (b) Component 3, and (c) Component 4 in the plume (beige, $n = 17$); surface seawater (light blue, $n = 1$) during low flow discharge (2011); plume core in the vicinity of river mouth (dark orange, $n = 7$); plume (yellow, $n = 5$); and surface seawater (dark blue, $n = 3$) in high flow condition (2012). Solid black line represents the 1:1 correlation.

Figure 6: PCA bi-plot of PARAFAC components with loadings (top and right axes, gray arrows with cross symbols) and scores (bottom and left axes, circles color-coded as in Fig. 5, $n = 50$) for the first two PCs derived from all the 50 survey samples at all depths.

Table 1Spectral Characteristics of the Four Fluorescent Components Identified by PARAFAC^a.

Component	Ex _{max} /Em _{max} ^b	Compare with previous studies	Description and Possible Source
C1	<250(345)/460	Peak A: 230-260/380-460 (Coble, 1996) C1: 250/452 (Kowalczyk et al., 2009) C1: <250/448 (Stedmon and Markager, 2005) C1: < 260/458 (Yamashita et al., 2008)	UVC humic/fulvic-like substance, ubiquitous
C2	<250(305)/397	Peak M: 290-310/370-420 (Coble, 1996) C3: 250(310)/400 (Kowalczyk et al., 2009) C4: 325(250)/416 (Stedmon et al., 2003) C6: 325(< 260)/385 (Yamashita et al., 2008)	Marine humic-like substance, of microbial/biological origins
C3	280/490	C4: 270(390)/508 (Kowalczyk et al., 2009) C3: 270(360)/478 (Stedmon et al., 2003)	Terrestrial humic-like components, widespread
C4	260/320	Peak T: 275/340 (Coble, 1996) C5: 270/332 (Kowalczyk et al., 2009) C7: 270/299 (Yamashita et al., 2008) C5: 265/<320 (Mann et al., 2012)	Amino acid, free or bound in proteins Autochthonous protein-like fluorescence

^aSecondary excitation maxima are given in parentheses.^bEx_{max}/Em_{max} stands for excitation maximum wavelength (in nm)/ emission maximum wavelength (in nm).

Table 2

Results of DOM photo- and microbial degradation experiments of the water at Amazon River mouth (Stn. 10, Salinity = 0.05).

Incubation duration was 5 days for each treatment.

	<u>Initial</u> <i>t</i> = 0	<u>Combined light+microbial incubation</u> <i>t</i> = final (mean ± SD ^a)	<u>Light-only photo irradiation</u> <i>t</i> = final (mean ± SD ^a)	<u>Dark microbial incubation</u> <i>t</i> = final (mean ± SD ^a)
DOC (μMC)	277	278.5 ± 3.5	274 ± 6.1	280.5 ± 0.7
<i>a_g</i> (350) (m ⁻¹)	10.43	7.15 ± 0.14	7.3 ± 1.2	9.5 ± 0.05
<i>S₂₇₅₋₂₉₅</i> (nm ⁻¹)	0.0139	0.0169	0.0163	0.0142
<i>S_R</i>	0.827	0.986	0.992	0.827
SUVA ₂₅₄ (mg C L ⁻¹ m ⁻¹)	10.9	8.8	9.2 ± 0.9	10.1 ± 0.1
Component 1 (ppb QSU)	64.6	41.2 ± 0.11	42 ± 1.56	66.0 ± 0.2
Component 2 (ppb QSU)	24.6	16.4 ± 0.12	16.9 ± 0.69	25 ± 0.01
Component 3 (ppb QSU)	29.0	16.4 ± 0.07	16.8 ± 0.66	29 ± 0.11
Component 4 (ppb QSU)	1.4	3.9 ± 0.33	4.1 ± 0.9	1.4 ± 0.03

^aSD standard deviation was calculated from the replicate bottles.

Results after treatments (*t* = final) significantly different from the initial condition (*t* = 0) at confidence level 0.05 are marked bold.

Figure1

Figure2

Figure 3

Figure 4

Figure 5

Figure 6