Shift in the chemical composition of dissolved organic matter in the Congo River network

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Abstract. The role of river ecosystems in processing inputs of dissolved organic matter (DOM) from the terrestrial environment during downstream transport in river networks is poorly constrained. In this study we report a data-set of DOM concentrations (dissolved organic carbon) and composition (stable carbon isotopic composition, absorption and fluorescence properties) acquired along a 1700 km stretch in the Congo River Basin, the second river in the World. Samples were collected in the main river and its tributaries in the central part of the Basin during high waters (HW) and falling waters (FW) periods. The
longitudinal increase in DOC concentrations and changes in DOM characteristics along the mainstem was found to differ between the two periods, especially because of greater photodegradation of terrestrial inputs from the DOM-rich waters from the Cuvette Centrale during FW as water residence time (WRT) increased. DOM degradation within the Congo Basin was found to result in the transition from aromatic to aliphatic DOM, resulting from the losses of aromatic compounds by photodegradation and the production of aliphatic compound by biological degradation. This study highlights that landscape properties and changes in WRT can play a major role on the functioning of river ecosystems in processing DOM during its downstream transformation in river networks.

1. Introduction

Dissolved organic matter (DOM) is composed of thousands of heterogeneous compounds that differ in origin and reactivity (Leehneer and Croué, 2003) and is a central component of the global carbon cycle (Battin et al., 2008). DOM in streams and rivers mainly originates from the terrestrial ecosystem, but can also be fueled by internal sources as stream order increases (Battin et al., 2008; Creed et al., 2015). Recent experimental and field studies have evidenced that sorption, photochemical and biodegradation processes continuously degrade and transform DOM throughout fluvial networks (Massicotte and Frenette, 2011; Ward et al., 2013; Cory et al., 2014; Fasching et al., 2014; Lapi erre and del Giorgio, 2014). Large surveys of boreal lakes have suggested that DOM was degraded along a gradient from aromatic to aliphatic compounds and that the chemical properties of DOM pool were the dominant control of overall DOM reactivity (Kothawala et al., 2014; Kellerman et al., 2015). Similarly, a large survey of temperate streams and rivers have reported a preferential loss of aromatic DOM and parallel gain in
aliphatic DOM with increasing stream order, resulting in a diminution of the variability in dissolved organic carbon (DOC) concentration and DOM composition from small headwater streams to large rivers (Creed et al., 2015; see also Vannote et al., 1980).

However, the role of environmental factors (i.e. climatic variables, water chemistry, landscape properties) on the DOM transformation in fluvial networks remains poorly studied (Massicotte and Frenette, 2011; Marín-Spiotta et al., 2014; Creed et al., 2015).

The consideration of temporal dynamics in addition to the spatial dimension is poorly investigated yet a crucial step towards a better understanding of DOM transport and processing in fluvial networks. Temporal dynamics refer here to the changes of the hydrological state of catchments that occur between high flow and low flow periods and are susceptible to alter DOM dynamics for at least two reasons. First, the concentration, the composition and the reactivity of DOM in streams and rivers are largely determined by seasonal changes in water levels that control the hydrological connectivity between fluvial networks and wetland sources (Besemer et al., 2009; Osburn et al., 2009; Bouillon et al., 2012). Hydrological connectivity is particularly relevant regarding the role of fringing wetlands that can regulate DOM inputs and composition along the river-floodplain continuum (Junk et al., 1989; Battin, 1998; Cawley et al., 2012; Lambert et al., 2016).

Secondly, the increase in water discharge during high flow periods induces a decrease in water residence time (WRT) within catchments due to increasing water velocities. Beyond the role of external and intrinsic drivers on DOM degradation, WRT represents a major control that regulates the degree of DOM transformation in aquatic ecosystems (Cory et al., 2007; Battin et al., 2008; Weyhenmeyer et al., 2012; Lambert et al., 2016). According to the recent pulse-shunt concept (Raymond et al., 2016) that builds on the “active pipe” concept (Cole et al., 2007), the degree of DOM processing in fluvial networks should be
reduced during high flow periods as hydrologic events favor the downstream DOM transport through the drainage network and therefore reducing the time where dynamic processes can take place.

African tropical rivers have among the highest specific flux of DOC worldwide (Meybeck, 1993) and have an intense role in the global carbon cycle (Borges et al., 2015a; 2015b). Yet, they remain largely underrepresented in large-scale studies on DOM processing (Lambert et al., 2015). The Congo is the largest river in Africa and the second largest river in the world after the Amazon in terms of drainage basin area and water discharge (Laraque et al., 2009). The Congo is also the second major exporter of terrestrial organic carbon to the oceans after the Amazon, of which 85-90% being in the form of DOC (Coynel et al., 2005), and drains the second largest tropical forested wetland area, the Congolese ‘Cuvette Centrale’ (Bwangoy et al., 2010). Until now, the biogeochemistry of DOM in the Congo and has been investigated in the Oubangui catchment (Bouillon et al., 2014), in the western part of the basin (Mann et al., 2014), along the 350 km final stretch of the river to the head of its estuary (Spencer et al., 2012), and in a small catchment (Epulu River) on the Eastern part of the basin (Spencer et al. 2010). Downstream gradient of DOM in the mainstem of the Congo is thus poorly constrained in its central part, where the river drains the Cuvette Centrale and receives inputs from its major tributaries (Fig. 1).

Emerging concepts aiming to describe how inland waters transform DOM flowing down the river continuum, namely the “chemostat” hypothesis (Creed et al., 2015) and the pulse-shunt concept (Raymond et al., 2016), need to be tested to extensive field studies in tropical ecosystems. Indeed, ~60% of the global riverine C transport is thought to occur in the tropical zone (Ludwig et al. 1996). The Congo mainstem and its tributaries were
sampled along a 1700 km stretch from the city of Kisangani to the city of Kinshasa during two contrasted hydrological periods (Fig. 1 and 2). DOM was characterized through its optical properties, its stable carbon isotope composition ($\delta^{13}$C$_{\text{DOC}}$) and its content in DOC. Optical measurements (including absorption and fluorescence) have been underscored as an efficient tool for the characterization of the chemical structure and reactivity of DOM at large spatial scales (Massicotte and Frenette, 2011; Cawley et al., 2012; Kothawala et al., 2014; Lambert et al., 2016), notably with the development of multicomponent deconvolution techniques such as the parallel factor analysis (PARAFAC) (Stedmon et al., 2003; Murphy et al., 2013). The aim of this study was to (1) characterize the longitudinal evolution of DOM in the Congo River during its passage through the Cuvette Centrale and (2) investigate the role of environmental drivers and WRT on DOM processing across a gradient of streams and rivers in the second largest river in the tropics and in the World.

2. Material and Methods

2.1 Study site.

The Congo is the largest river in Africa and the second largest river in the world after the Amazon in terms of drainage basin area ($\sim 3.7 \times 10^6$ km$^2$) and water discharge ($\sim 43,000$ m$^3$ s$^{-1}$) (Laraque et al., 2009). The river originates in the southeastern part of the basin, and is called the Lualaba until it crosses the city of Kisangani and becomes officially known as the Congo. The Congo basin straddles on the equator, with major tributaries located on both hemispheres (Fig. 1). Thus, the rainy season on the northern part of the basin is compensated by the dry season on the southern part of the basin, and vice-versa, leading to an attenuation of seasonal water height variations (Runge, 2008), in stark...
contrast with the Amazon river, leading to marked differences in biogeochemistry (e.g. \(\text{CH}_4\) dynamics, Borges et al. 2015b) and aquatic ecology (e.g. phytoplankton development, Descy et al. 2016) between these two rivers. The hydrological cycle of the Congo is bimodal, with maximum water flow occurring in December and May and minimum flow in August and March (Fig. 2). The center of the basin is covered by evergreen forest (~50% of the total area), and surrounded by savannah in the northern and southern rims of the catchment. The Cuvette Centrale is located in the central part of the basin on both side of the equator and consists mainly in a vast permanently flooded forested area of \(360 \times 10^3 \, \text{km}^2\) (Bwangoy et al., 2010). The core of the Cuvette Centrale corresponds to a net increase in the wetland fraction along the Congo River as the mainstem connects with large tributaries flowing through the flooded forest (Fig. 1 and Supplementary Fig. 1). The most important tributaries of the Congo in terms of discharge are the Oubangui (4200 \(\text{m}^3 \, \text{s}^{-1}\)) and the Sangha (2220 \(\text{m}^3 \, \text{s}^{-1}\)) on the northern side, the Kasai (9000 \(\text{m}^3 \, \text{s}^{-1}\)) on the southern side, and the Ruki (3950 \(\text{m}^3 \, \text{s}^{-1}\)) and the Lulonga (2040 \(\text{m}^3 \, \text{s}^{-1}\)) along the equator (Bricquet, 1995; Coynel et al., 2005; Laraque et al., 2009).

2.2. Field data collection.

Samples were collected during the yearly discharge maximum in December (03-19 December 2013) and during falling waters following the second discharge maximum occurring in March (10-30 June 2014) (Fig. 2). The sampling concerned the Congo River itself as well as its small and large tributaries (Table 1). Stations along the mainstem were located ~50 km apart from Kisangani to Kinshasa. Major tributaries included the Tshopo, the Lindi, the Itimbiri, the Aruwini, the Mongala, the Oubangui, the Sangha and the Lefini on the right side of the Congo, and the Lomami, the Lulonga, the Ikelemba, the Ruki and
the Kwa/Kasai on the left side. The Lefini was sampled only during the first campaign (high waters).

Water sampling was performed from a 22 m boat on the mainstem and with a canoe in the tributaries. Approximately 2 L of water were collected 0.5 m below the surface, kept away from direct sunshine and filtered and conditioned typically within 15 min of sampling. Filtrations were performed successively on pre-combusted GF/F glass fiber filters (0.7 µm porosity), then on 0.2 µm polyethersulfone syringe filters. Samples for the measurement of DOC concentration and δ^{13}C_{DOC} signatures were stored in 40 mL glass vials with polytetrafluoroethylene (PTFE) coated septa with 50 µL H_{3}PO_{4} (85%). Samples for colored DOM (CDOM) and fluorescent DOM (FDOM) analyses were stored in 20 mL amber glass vials with PTFE-coated septa but without H_{3}PO_{4} addition. Samples for major elements (including Fe) were stored in 20 mL scintillation vials and acidified with 50 µL of HNO_{3} 65 % prior to analysis.

Fe was measured by inductively coupled plasma spectrometry (Agilent 7700x ICP-MS). DOC and δ^{13}C_{DOC} were analyzed with an Aurora1030 total organic carbon analyzer (OI Analytical) coupled to a Delta V Advantage isotope ratio mass spectrometer. Typical precision observed in duplicate samples was in >95% cases < ± 5 % for DOC, and ± 0.2 % for δ^{13}C_{DOC}. Quantification and calibration was performed with series of standards prepared in different concentrations, using both IAEA-C6 (δ^{13}C = -10.4 %o) and in-house sucrose standards (δ^{13}C=-26.9 %o). All data are reported in the δ notation relative to VPDB (Vienna Pee Dee Belemnite). Absorbance was recorded on a Perkin-Elmer UV/Vis 650S spectrophotometer using a 1 cm quartz cuvette. Absorbance spectra were measured between 200 and 700 nm at 1 nm increment and instrument noise was assessed measuring ultrapure (Type 1) Milli-Q (Millipore) water as blank. After subtracting the blank
spectrum, the correction for scattering and index of refraction was performed by fitting the
absorbance spectra to the data over the 200-700 nm range according to the following
equation:

\[ A_\lambda = A_0 e^{-S(\lambda-\lambda_0)} + K \]  \hspace{1cm} (1)

where \( A_\lambda \) and \( A_0 \) are the absorbance measured at defined wavelength \( \lambda \) and at reference
wavelength \( \lambda_0 = 375 \) nm, respectively, \( S \) the spectral slope (\( \text{nm}^{-1} \)) that describes the
approximate exponential decline in absorption with increasing wavelength and \( K \) a
background offset. The fit was not used for any purpose other than to provide an offset
value \( K \) that was then subtracted from the whole spectrum (Lambert et al., 2015).

Fluorescence intensity was recorded on a Perkin-Elmer LS45 fluorescence spectrometer
using a 1 cm quartz cuvette across excitation wavelengths of 220-450 nm (5 nm
increments) and emission wavelengths of 230-600 nm (0.5 nm increments) in order to
build excitation–emission matrices (EEMs). If necessary, samples were diluted until \( A_{254} < 0.2 \text{ m}^{-1} \) to avoid problematic inner filter effects (Ohno, 2002). Before each measurement
session (i.e. each day), a Milli-Q water sample was also measured and subtracted from
EEMs.

Water temperature, \%O\(_2\), and pH were measured \textit{in situ} with portable field probes
calibrated using standard protocols (YSI ProPlus probe). Pelagic respiration (R) was
determined from the decrease of \( \text{O}_2 \) in 60 ml biological oxygen demand bottles over \( \sim 24 \)
h incubation periods. The bottles were kept in the dark and close to in situ temperature in
a cool box filled with in situ water. The \( \text{O}_2 \) decrease was determined from triplicate
measurements at the start and the end of the incubation with an optical \( \text{O}_2 \) probe (YSI
ProODO). The respiratory quotient (RQ) is defined as the molar ratio of \( \text{O}_2 \) consumed to
CO₂ produced by respiration, and allows the conversion of respiration measurements from O₂ to C units. The RQ value is in theory equal to 1 for the oxidation of glucose, but higher than 1 for more complex and reduced organic molecules containing nitrogen and phosphorous, such as lipids and proteins (e.g. 1.3 in a temperate stream with a catchment dominated by pastures (Richardson et al., 2013), or lower than 1 for highly oxidized and oxygen-rich molecules (e.g. pyruvic, citric, tartaric, and oxalic acids) (e.g. 0.8 in boreal lakes, Berggren et al. 2012). Given the range of RQ values, we adopted a RQ value of 1.0. The vertical light attenuation coefficient, K_d (m⁻¹), was calculated from simultaneous measurements of surface irradiance with a Li-Cor LI-190 quantum sensor and underwater photosynthetically active radiation (PAR) measurements with a submersible Li-Cor LI-193SA spherical quantum sensor. K_d was derived from the slope of the semi-logarithmic regression between relative quantum irradiance and depth. Transparency of water column was measured using a 20-cm diameter Secchi disk.

2.3. Characterization of DOM composition.

The specific ultra-violet absorbance (SUVA₂₅⁴) was calculated as the UV absorbance at λ = 254 nm (A₂₅⁴) normalized to the corresponding DOC concentration (Weishaar et al., 2003). The natural UV absorbance of Fe at λ = 254 nm was estimated based on measured Fe concentrations and was then subtracted from the UV absorbance measured. The corrected value of A₂₅⁴ was then used to calculate SUVA₂₅⁴. The SUVA₂₅⁴ was used as an indicator of the aromaticity of DOC with high values (>3.5 l mgC⁻¹ m⁻¹) indicating the presence of more complex aromatic moieties and low values (<3 l mgC⁻¹ m⁻¹) indicative the presence of mainly hydrophobic compounds (Weishaar et al., 2003).

Napierian absorption coefficients were calculated according to:

\[ a_\lambda = 2.303 \times A_\lambda / L \]  

(2)
where $a_\lambda$ is the absorption coefficient ($m^{-1}$) at wavelength $\lambda$, $A_\lambda$ the absorbance corrected at wavelength $\lambda$ and L the path length of the optical cell in m (0.01 m). CDOM was reported as the absorption coefficient at 350 nm ($a_{350}$). Spectral slopes for the intervals 275-295 (S$_{275-295}$) nm and 350-400 nm (S$_{350-400}$) were determined from the linear regression of the log-transformed $a$ spectra versus wavelength. The slope ratio $S_R$ was calculated as the ratio of S$_{275-295}$ to S$_{350-400}$ (Helms et al. 2008). $S_R$ is related to the molecular weight (MW) distribution of DOM with values less than 1 indicative of enrichment in high molecular weight compounds and high values above 1 indicative of a high degree of low molecular weight compounds. The fluorescence index (FI) was calculated as the ratio of the emission intensities at 470 nm and 520 nm at an excitation wavelength of 370 nm (McKnight et al., 2001). A higher FI value (e.g., 1.8) indicates an aquatic microbial DOM source while a lower value (e.g., 1.2) indicates a terrestrial source. Intermediate values indicate a mixed DOM source.

2.4. PARAFAC modeling.

EEMs preprocessing steps (removing first and second Raman scattering, standardization to Raman units, absorbance corrections and inner filter effects) were performed prior the PARAFAC modeling. The scans were standardized to Raman units (normalized to the integral of the Raman signal between 390 nm and 410 nm in emission at a fixed excitation of 350 nm) with a Milli-Q water sample run the same day as the samples (Zepp et al., 2004). PARAFAC model was built using MATLAB (MathWorks, Natick, MA, USA) and the drEEM Toolbox version 1.0 (Murphy et al., 2013). Validation of the model using normalized EEMs was performed through by split-half analysis and random initialization. The normalization step was applied to scale each EEM to its total signal, thus ensuring the model focused entirely on compositional rather than
concentration gradients. Additional samples analyzed in the same manner and collected from the Kwa/Kasai river basin \((n = 104)\), Lago Janauacá (a central Amazon floodplain lake, \(n = 17\)), the Niger River \((n = 19)\) and the Okavango delta were added to the dataset to increase the variability of DOM fluorescence signatures and help detect components that could have been present in insufficient quantity to be detected in our environment. The maximum fluorescence \(F_{\text{Max}}\) values of each component for a particular sample provided by the model were summed to calculate the total fluorescence signal \(F_{\text{Tot}}\) of the sample in Raman unit (R.U.). The relative abundance of any particular PARAFAC component \(X\) was then calculated as \(\%C_X = \frac{F_{\text{Max}}(X)}{F_{\text{Tot}}}\).

The positions of maximum peaks established by our model were compared to the classical excitation-emission matrices nomenclatures (Fellman et al., 2010; Coble et al., 2014) and with other reported PARAFAC models built in a large variety of freshwater ecosystems (Table 2). Additionally, each PARAFAC component was associated to a dominant molecular class based on recent studies aiming to correlate individual molecular formula with different PARAFAC components through Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). Such studies have been carried out in high latitude lakes (Kellerman et al., 2015), boreal rivers (Stubbins et al., 2014) and subtropical wetland (Wagner et al., 2015). Although such comparison has not be carried out with our own samples, the relatively good consistency of associations between optical and molecular linkages observed in contrasting environments suggests that PARAFAC components can track dominant DOM molecular composition similarly across different biomes in terms of DOM MW and enrichment in aliphatic or aromatic molecules (Wagner et al., 2015).

2.5. Landscape analysis.
The total drainage area and the Strahler stream order (Strahler, 1957) were calculated at each station in the geographic information system (GIS) software ArcGis® (ESRI 2011, ArcGis Desktop 10.3.1), using the ArcHydro tools (v. 2.0) and the hydrological data and maps based on shuttle elevation derivatives at a 3” resolution (Lehner et al., 2008). The extent of wetland areas and dense forest cover were extracted from the Global Lakes and Wetlands Database (Lehner and Döll, 2004) and from Global Land Cover 2009 database (Bontemps et al. 2011), respectively.

2.6 Statistical Analysis.

Mann-Whitney t-tests were performed to investigate the difference in DOM properties spatially (mainstem versus tributaries) and temporally (HW versus FW). A principal component analysis (PCA) was also performed to explore DOM evolution during its transport through the Congo fluvial network. The optical properties of DOM including level of CDOM ($\alpha_{350}$), bulk composition (SUVA$_{254}$, Sr, FI) and the relative abundance of PARAFAC components were used as the variables. Given the different units of these variables, data were scaled to zero-mean and unit-variance as recommended (Borcard et al., 2011). The PCA was performed using the prcomp function in R software.

3. Results

3.1. DOM concentration and bulk composition.

DOC concentrations in the mainstem were higher during HW (5.4 – 13.9 mg L$^{-1}$, average 8.2±2.6 mg L$^{-1}$) compared to FW (4.2 – 9.8 mg L$^{-1}$, average 5.9±1.8 mg L$^{-1}$) but showed similar longitudinal trends during both hydrological periods (Fig. 3a, 3b): DOC increased slowly in the upper part of the transect and then faster as the Congo River evolves throughout the core of the Cuvette Centrale and mixes with the Kwa/Kasai River.
The breaking point of the DOC longitudinal evolution increase in DOC concentrations is located at km 700 during HW, and around km 500 during FW. DOC in tributaries were highly variable (from 1.8 to 67.8 mg L\(^{-1}\)) and were found to be correlated with the extent of flooded forest (Fig. 4), resulting in highest concentrations in tributaries draining the Cuvette Centrale and lowest concentrations in those draining savannah areas upstream of Kinshasa (Fig. 1). Tributaries located downstream of the Cuvette Centrale were also characterized by lowest DOC concentrations during FW compared to HW while no clear pattern was observed for those located upstream.

\(\delta^{13}C_{\text{DOC}}\) signatures in the mainstem were lower during HW (from 30.6 to -28.8 \(\%_\circ\), average -29.4±0.3 \(\%_\circ\), \(n = 35\)) compared to FW (from -29.3 to -25.2 \(\%_\circ\), average -27.5±0.9 \(\%_\circ\), \(n = 34\)). \(\delta^{13}C_{\text{DOC}}\) during HW decreased about 0.7 \(\%_\circ\) from Kisangani to km ~ 1200, remained stable until km ~ 600 and then increased slightly towards Kinshasa. During FW, \(\delta^{13}C_{\text{DOC}}\) decreased markedly about 3 \(\%_\circ\) between Kisangani and km ~ 1600. Downstream, values were variable (-27.2±0.6 \(\%_\circ\) between km 600 – 1600, \(n = 18\)) and then showed ~ 1 \(\%_\circ\) drops at km ~ 600 and ~ 200, coinciding with the confluence zones with the Oubangui and the Kwa/Kasai rivers, respectively. In tributaries, \(\delta^{13}C_{\text{DOC}}\) values displayed a similar pattern during the two hydrological periods with lowest and relatively stable values (-29.7±0.5 \(\%_\circ\), \(n = 76\)) in streams and rivers draining dense forest areas and higher signatures in those flowing savannah areas 0-400 km upstream of Kinshasa (-28.1±0.8, \(n = 14\)). Stations of the mainstem located within or upstream the Cuvette Centrale were characterized by highest \(\delta^{13}C_{\text{DOC}}\) values than those measured in tributaries collected along the same transect, both during HW (\(p < 0.004\)) and FW (\(p < 0.0001\)). Downstream the Cuvette Centrale, stations of the mainstem had lower \(\delta^{13}C_{\text{DOC}}\) signatures than those measured in tributaries (\(p < 0.0001\), all periods).
SUVA$_{254}$ and SR during the two hydrological periods varied mainly between 4.0 – 5.2 L mgC$^{-1}$ m$^{-1}$ and 0.734 – 0.802 among all stations, respectively (10% – 90% percentiles, n=160), indicating that DOM in the Congo basin was dominated by aromatic compounds of high MW during both periods (Fig. 3e-3h). SUVA$_{254}$ generally decreased from Kisangani to Kinshasa in the mainstem during HW, with a slight increase between km 500 and 800 upstream of Kinshasa, while SR exhibited stable values from Kisangani and then started to increase toward Kinshasa at km 700. Compared to HW, SUVA$_{254}$ during FW was relatively stable and lowest from Kisangani to km 500 but higher between km 0 – 500 as SUVA$_{254}$ increased markedly in this section (p < 0.0001). SR exhibited a hump-shaped pattern during FW, with increasing values from Kisangani to km 500 and decreasing value between km 0 – 500. A slight decrease in SUVA$_{254}$ (p = 0.0043) associated with an increase in SR (p = 0.047) was also observed between km 200 – 400. Generally, SUVA$_{254}$ in tributaries were slightly higher in FW than at HW (p = 0.035), similar to the mainstem in HW but higher in FW (p = 0.0113). FI in the mainstem gradually decreased from Kisangani to Kinshasa during both hydrological periods, with higher values during FW than in HW (p = 0.0006), but were generally highest than in tributaries (p < 0.0001). No distinct seasonal variation was apparent in tributaries.

3.2. PARAFAC results.

Six PARAFAC components were determined to adequately model our dataset (Table 2, Supplementary Fig. 2). Components C1, C3, C4, and C5 are all classified as "humic-like" but have been shown to differ in terms of sources, molecular association and reactivity (Table 2). C1 and C3 are commonly reported in freshwaters ecosystems and are associated with a group of high MW and aromatic molecules of terrestrial origin (e.g. Wagner et al., 2015). Both are susceptible to photodegradation (Lapierre and del Giorgio,
C4 is associated with terrigenous molecules of lower aromaticity and MW relative to C1 and C3 (Kellerman et al., 2015). In freshwaters, C4 can originate from terrestrial inputs (Stedmon and Markager, 2005; Yamashita et al., 2010), especially from wetland areas (Lambert et al., 2016), but can also be produced by photodegradation of terrestrial organic matter (Massicotte and Frenette, 2010). Among the humic-like compounds, C5 is associated with molecules characterized by lowest aromaticity and MW (Stubbins et al., 2014) and has been found to be a photoproduct derived from terrestrial DOM (Lapierre and del Giorgio, 2014). C2 and C6 are respectively classified as microbial humic-like and tryptophan-like component (Fellman et al., 2010). By opposition to the other components, C2 and C6 are associated with low MW DOM fractions enriched in aliphatic molecules biologically produced within aquatic ecosystems (Kellerman et al., 2015; Wagner et al., 2015). Both C2 and C6 can be assigned to fraction of DOM resulting from the microbial degradation of terrestrial organic matter within freshwaters (Stedmon et al., 2003; Walker et al., 2013), although autochthonous primary production represents another potential source for C6 (Yamashita et al., 2010).

The relative contribution of C1 and C3 showed similar patterns along the mainstem during both hydrological periods (Fig. 5). %C1 and %C3 presented a slight decrease then increased during HW with minimal contribution recorded around km 1100. %C1 and %C3 were lowest during FW (p = 0.017 and p < 0.0001, respectively), with low variability upstream of km 500 and highest contribution downstream. %C4 displayed a general increase along the transect at HW especially marked between Kisangani and km ~1100 and between km 600 to km 150. During FW, %C4 was opposite to the longitudinal evolution of %C1 and %C3, with highest contribution than during HW (p > 0.0001). Overall, %C5 was higher during FW than during HW (p < 0.0001) and exhibited longitudinal
patterns opposite to those of %C3 during both periods. %C2 was relatively stable along
the mainstem during both periods, with higher contribution during FW compared to HW (p
= 0.0076). C6 exhibited the lowest contribution to FDOM signal and %C6 trended to be
lower during FW compared to HW. Longitudinal evolution of C6 was characterized by a
strong drop along the mainstem, occurring around km 800 at HW and km 500 at FW.

Overall, tributaries were characterized by lower %C2, %C3 and %C6 relative to the
mainstem (p < 0.0001). %C4 and %C1 were respectively higher (p = 0.0007) and lower
(p = 0.017) in tributaries than in the mainstem during HW, and no difference was observed
at FW. No difference was observed for %C5 between tributaries and the mainstem for
both periods. The seasonal variability within tributaries was characterized by higher
contribution of C4 (p = 0.025) and C5 (p < 0.0001) and lower contribution of C3 (p =
0.0015) and C6 (p = 0.003) in FW compared to HW.

3.3. PCA results.

The first two principal component (PC) accounted for 57% of the total variance (Fig.
6). The first PC (PC1) showed a transition from terrestrial aromatic DOM (%C3, SUVA254,
DOC, a350, positive loadings) to aliphatic DOM (%C2, %C6, FI, negative loadings). The
second PC (PC2) suggests a transition from highly aromatic terrestrial DOM (%C1, %C3
and SUVA254, negative loadings) to DOM of lower aromaticity and MH (%C4, %C5, S_R,
positive loadings). The distribution of sampling stations for a given Strahler order was
highly heterogeneous (Fig. 6a). However, a global pattern emerges along PC1 with
stations collected in the mainstem showing mainly negative scores (Fig. 6b). Furthermore,
stations of the mainstem collected during HW had negative scores along PC2, but positive
scores during FW. Overall, stations collected during HW had mainly negative scores along PC2 while those sampled at FW showed large variability along PC2.

4. Discussion

4.1. Longitudinal evolution of DOM in the Congo River. The Congo River from Kisangani to Kinshasa continually receives organic matter inputs from inflowing tributaries enriched in DOM from the flooded forest (Fig. 4), resulting in a net increase in DOC concentrations along the longitudinal axis during both periods. Our data showed however that the longitudinal evolution in DOM content and composition differed between the two campaigns. These differences result from the combination of several factors.

4.1.1. Seasonal changes in DOM sources mobilized in the upper basin. The large variation in $\delta^{13}$C$_{\text{DOC}}$ values in the mainstem at Kisangani between HW (-29.0‰) and FW (-25.2‰) can be related to a shift in the source of DOM mobilized in the upper part of the basin due to differences in water routing during the hydrograph. Thus, decreasing $\delta^{13}$C$_{\text{DOC}}$ signatures that occurred with increasing water discharge during high flow periods has been attributed to the mobilization of fresh DOM from superficial soil horizons in wide variety of catchments (Neff et al., 2006; Sanderman et al., 2009; Lambert et al., 2011; Bouillon et al., 2012). Inversely, highest $\delta^{13}$C$_{\text{DOC}}$ values during low flow periods reflect the deepening of water flow paths and the subsequent mobilization of more degraded DOM from deeper soil horizons. This seasonal change in DOM composition at the start of the Kisangani – Kinshasa transect are further supported by an ongoing high frequency monitoring carried out à Kisangani (unpublished data).

4.1.2. Impact of WRT and photodegradation on lateral exchanges between the Congo River and its tributaries. The lateral mixing between the central water masses
of the Congo River and DOM-rich water from the Cuvette Centrale was likely reduced during FW due to a greater photodegradation of terrestrial DOM. The downstream evolution of δ\(^{13}\)C\(_{DOC}\) showed indeed that the lateral mixing between the mainstem and its tributaries was strong during HW (Fig. 3c), but limited at FW during which δ\(^{13}\)C\(_{DOC}\) in the Congo remained ~3 – 4 ‰ higher than values recorded in tributaries from km ~ 1600 to ~ 600 (Fig. 3d) despite slight increase in DOC concentrations (Fig. 3b). Photodegradation has been assumed to be a major pathway to remove terrigenous DOM from aquatic ecosystems (Cory et al., 2014) and mainly acts on colored, photosensitive molecules associated with high MW and aromaticity (Spencer et al., 2009; Cawley et al., 2012; Lapierre and del Giorgio, 2014). Greater photodegradation of DOM during FW was supported by several lines of evidence. %C1 and %C3, both associated with highly aromatic molecules (Table 2), were lower during FW compared to HW, and this decrease occurred along with a decrease in DOM aromaticity (lower SUVA\(_{254}\)) and increase in average MW (higher S\(_R\)) (Fig. 3 and 5). The more significant decrease in %C3 relative to %C1 was also consistent with the well documented high photosensibility of this component relative to other terrestrial humic-like component (Cawley et al., 2012; Lapierre and del Giorgio, 2014). The role of DOM photodegradation in controlling the longitudinal evolution of DOM in the Congo River during FW was also evidenced by the different distribution of stations collected in the mainstem between HW (negative scores) and FW (positive scores) along the PC2 of the PCA (Fig. 6).

Greater DOM photodegradation during FW implies a better exposure of CDOM to sunlight irradiation, either spatially (i.e. in the water column) or temporally. The higher coefficient of light attenuation in the water column (K\(_d\)) and associated lower Secchi depths (Table 1) during FW indicates that the penetration of sunlight in the water column...
was reduced compared to HW. This was likely due to the greater total suspended matter (TSM) concentrations (Table 1) and phytoplanktonic development (Descy et al. 2016). It is therefore more likely that the degree of DOM photodegradation was mainly driven by changes in WRT. Decreasing water discharge and flow velocity during FW should lead to an increase in WRT, allowing consequently more time for sunlight to degrade terrestrial DOM.

The fact that %C4 was opposite to %C1 and %C3 along PC2 could either indicate a photoproduction of this component (Massicotte and Frenette, 2011) or could simply result from the fact that this component has been identify as photo-resistant to sunlight irradiation (Ishii and Boyer, 2012). The longitudinal enrichment in %C4 reported during HW along the mainstem rather advocate for a terrestrial origin from wetland areas. This assumption is consistent with a recent study carried out in the Zambezi basin showing that wetland areas exported greater proportion of similar C4 component towards river channels relative to other terrestrial humic-like component during high flow periods (Lambert et al., 2016).

4.1.3. Role of large tributaries and channel width in controlling the longitudinal evolution of DOM from Kisangani to Kinshasa. DOM enrichment was more pronounced within the core of the Cuvette Centrale (Fig. 2) that corresponds to the region where the major tributaries of the Congo in terms of discharge (i.e. the Lulonga, the Ruki, the Sangha, the Oubangui and the Kwa/Kasai rivers) connect the mainstem after receiving great inputs of terrestrial DOM from the large flooded forest (Coynel et al., 2005; Laraque et al., 2009) (Fig. 1 and Supplementary Fig. 1). DOC concentrations in the mainstem increased faster immediately as the Congo enters in this central part of the Cuvette Centrale during HW, reflecting the strong lateral mixing between water masses. However,
the net rise in DOC concentrations during FW were found to occurred first at ~70 km
downstream of the confluence zone with the Oubangui River, coinciding with a strong
reduction of the channel width (Supplementary Fig. 3). The ~1 ‰ drop in δ¹³C_{DOC}
associated with changes in DOM composition (especially increase in SUVA₂₅₄ and %C₃)
at this station evidenced that the reduction of channel width favors the lateral mixing
between the mainstem and waters from the Cuvette Centrale that have traveled along the
river ridge lined by dense forest without being significantly impacted by photodegradation
(Supplementary Fig. 4). In fact, a “complete” lateral mixing with waters from the Cuvette
Centrale likely occurs only at the confluence zone with the Kwa/Kasai River. The high
discharge of this tributary combined with a narrow channel width of the mainstem in this
part of the basin devoid of sand bars and islands (Runge et al., 2008) likely force lateral
exchanges. This is supported by the fact that δ¹³C_{DOC} signatures of the Congo mainstem
became typical of black waters from the Cuvette Centrale only after connecting with the
Kwa/Kasai during FW, and could also explain why DOC increase is greater at this point
while DOC are largely higher in tributaries located upstream (e.g. The Ruki River).

Large tributaries also controlled the general evolution of DOM composition from
Kisangani to Kinshasa. Thus, DOM aromaticity (SUVA₂₅₄) decreased slightly along the
transect during HW (from ~4.6 to 4.2 mgC L⁻¹ m⁻¹ from Kisangani to Kinshasa), but
increased significantly during FW (from ~4.0 to 5.3 mgC L⁻¹ m⁻¹ from Kisangani to
Kinshasa) due to an increase in DOM aromaticity in large tributaries flowing through or
connected to the Cuvette Centrale.

4.2. DOM transformation during its downstream transport in the Congo River
network. Strahler stream order was used as an organizing concept for characterizing
individual stream reaches within the network (Strahler, 1957, Poole, 2010), and investigate DOM composition across a gradient of streams and rivers. The loadings plot along PC1 (Fig. 6) indicates a transition in the dominant DOM composition from aromatic (%C3, SUVA254) to aliphatic (%C2, %C6, FI) compounds. It is noteworthy that a similar gradient in DOM composition has recently been reported in high-latitude lakes (Kellerman et al., 2015) and in U.S. rivers networks (Creed et al., 2015), suggesting that the large-scale governing processes controlling DOM in freshwater are similar across biomes. However, the underlying mechanisms remain to be elucidated. Thus, the gain in aliphatic DOM has been attributed to the increasing influence of autochthonous sources (Creed et al., 2015) or to the degradation of terrestrial DOM (Kellerman et al., 2015), and external factors (i.e. not related to DOM composition) have been suggested to have little influence on this pattern (Kellerman et al., 2015). Our study supports the hypothesis that the degradation of terrestrial DOM is the main driver on DOM transformation in aquatic systems, but also highlights the role of landscape morphology and environmental conditions in mitigating the transition from an aromatic to an aliphatic dominant composition.

4.2.1. Losses of aromatic DOM through photodegradation and biological activity as producer of aliphatic DOM. The preferential losses of aromatic molecules through terrestrial DOM photodegradation was evidenced by the longitudinal evolution of DOM along the mainstem during FW. Besides resulting in the removal of terrigenous DOM from the river network, photodegradation was found to have a direct impact on the aquatic metabolism in the Congo Basin. Indeed, %C5 was inversely correlated with (1) %C3 and (2) measurements of pelagic community respiration (R) performed concurrently with DOM sampling (Borges et al., 2015a) and attributed to bacterial respiration since phytoplankton
biomass is generally low (Descy et al. 2016) (Fig. 7). These relationships suggest that C5 was a direct photoproduct of terrestrial aromatic molecules tracked by C3 and that the photoproduced organic molecules served as substrate for bacterial growth. This assumption was supported by an experimental study showing that the formation of a component similar to C5 in boreal freshwaters was mediated by photodegradation (Lapierre and del Giorgio, 2014) and is consistent with experiments claiming that the aromatic and high MW fraction of terrestrial DOM can be photochemically converted into more labile substances of lower MW that support the aquatic bacterial metabolism (Bano et al., 1998; Tranvik and Bertilsson, 2001; Remington et al., 2011; Cory et al., 2014). The lack of correlation between %C5 and R in the mainstem likely indicates an additional source for labile DOM. The higher concentration of chlorophyll-a in the mainstem compared to tributaries (Table 1) suggests that this source could be phytoplanktonic exudation (Baines and Pace, 1991). Indeed, phytoplankton exudates have been shown to be very labile and rapidly assimilated by bacteria in tropical lake waters (Morana et al., 2014).

The gain in aliphatic DOM can be explained by the microbial reworking of terrestrial DOM during its transport. Indeed, several studies carried out in a large variety of aquatic ecosystems have attributed the origin of C2 and C6 to the biological degradation of terrestrial DOM (Stedmon et al., 2003; Yamshita et al., 2010; Walker et al., 2013; Fasching et al., 2014; Kellerman et al., 2015). The fact that %C2 and %C6 remained systematically higher in the mainstem along the Kisangani – Kinshasa transect and did not decreased to level similar to that of the tributaries strongly advocate for an internal production of these components. This was supported by the higher FI values in the mainstem, indicating greater inputs of microbially derived DOM in the Congo River compared to tributaries.
Additionally, none of these components were correlated to chlorophyll-a concentration (data not shown), suggesting that the phytoplankton primary productivity in the Congo basin was not controlling their distribution contrary to what was suggested in U.S. rivers (Creed et al., 2015). The dual role of microorganisms as consumers of terrigenous DOM and producers of novel compounds has recently been emphasized in DOM-rich black waters (Ward et al., 2013; Fasching et al., 2014).

It should be noted that previous investigations based on lignin biomarkers have suggested that DOM transformation during transport in the Congo basin was mainly driven by dynamic exchanges with the particulate organic carbon (POC) pool via sorption or leaching processes (Spencer et al., 2012; Mann et al., 2014). This assumption was however not supported by the weak relationship observed between δ¹³C of DOC and δ¹³C of POC (Pearson’s r = 0.20, n = 158, data not shown), suggesting limited exchange between DOC and POC pools.

4.2.2. External drivers on the aromatic towards aliphatic transition. The enrichment of the mainstem in the aliphatic fraction compared to the majority of its tributaries advocates for a transition occurring during the downstream DOM transport in the fluvial network. However, the large heterogeneity in the distribution of tributaries for a given Strahler order indicates that landscape morphology and environmental properties can mitigate downstream DOM transformation. Thus, DOM photodegradation is likely more pronounced in catchments with large open areas, as suggested by the lower %C3 in savannah-dominated catchments compared to forest-dominated catchments (Supplementary Fig. 5, see also Lambert et al., 2015). Also, a strong connectivity with terrestrial sources can maintain a greater aromatic character to DOM independently of the size of the rivers. This typically refers to the well-known role of wetland areas in delivering
great quantity of aromatic DOM in inland waters (Hanley et al., 2013; Mann et al., 2014; Lambert et al., 2016) and was illustrated by the comparison of DOM biogeochemistry in the Oubangui River before and after it crosses the Cuvette Centrale. A multi-year monitoring carried out at Bangui (Fig. 1) has indeed illustrated that the Oubangui transported DOM of low aromaticity at the beginning of its rising water period occurring in June (Bouillon et al., 2014) while our study reports highly aromatic DOM for the same period. Finally, DOM bacterial degradation is likely limited in very acidic environments (Borges et al., 2015a). This assumption is supported by the fact that %C2 and %C6 were positively correlated with the pH of stream waters (Fig. 8). Such streams and rivers typically correspond to the DOM-rich so-called “black-waters” originating from the Cuvette Centrale, with pH between 3.6 and 5.9 and average 4.4 (Supplementary Fig. 6).

4.3. The chemostat hypothesis and the pulse-shunt concept. The chemostat hypothesis suggests a decreasing of DOC concentrations and a convergence in DOM composition towards lower aromaticity with increasing stream order because of the increasing influence of in-stream processes that overwhelm terrestrial inputs from headwater catchments (Creed et al., 2015). The shift from dominant terrestrial influence to biogeochemical processing – assessed by the variation of SUVA254 as a function of stream order – has been estimated to occur in third- or fourth-order streams in river networks across the United States (Creed et al., 2015). A net decrease in SUVA254 associated with a decrease with DOC concentrations was only found to occur from six to height order streams in our study (Fig. 9), reflecting the influence of the Cuvette Centrale (i.e. strong connectivity with the flooded dense forest, acidic waters) on DOM biogeochemistry in the Congo Basin. This falls in line with the “flood pulse concept” that highlights the critical importance of the river-floodplain connectivity in lowland tropical
rivers such as the Amazon (Junk et al. 1989), while the chemostat hypothesis builds on the river continuum concept (Vannote et al. 1980) that is typically applicable to rivers at temperate latitudes (devoid on large wetlands). Also, an increase in DOM content and aromaticity was found to occur at nine order streams, reflecting the fact that DOM-rich waters from the Cuvette Centrale can travel along the ridge of the Congo River without mixing totally with the central water masses of the mainstem (Supplemental Figure 4). Overall, these observations illustrate how landscape properties can impact the functioning of river ecosystems on DOM downstream transformation in river networks.

Our study also supports the "pulse-shunt" conceptual model that states that the removal of terrestrial DOM in fluvial networks is a function of the hydrological regime of the basin (Raymond et al., 2016). It should be noted that the seasonal variation in water discharge is relatively low in the Congo Basin compared to other large rivers (Runge, 2008), but however enough to significantly impacts DOM photodegradation between FW and HW. The switch between active and passive pipes is likely to be more pronounced in large drainage basins in northern and southern Hemisphere with more contrasted hydrological regimes, as recently showed in the adjacent Zambezi Basin (Lambert et al., 2016). Our results also suggest that the photodegradation pathway is more sensitive to changes in WRT compared to the biological pathway, but this hypothesis needs to be verified in other environments.

Data availability

Acknowledgements

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Author Contributions

A.V.B., F.D., S.B. designed the study; A.V.B., and F.D. collected the field data; S.B. and T.L. performed sample analysis; T.L. carried out the geographical system information (GIS) analysis and performed the PARAFAC model with help of P.M.; T.L. analyzed the data and drafted the manuscript that was revised and approved by all co-authors.

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at http://ionia1.esrin.esa.int/docs/GLOBCOVER2009_Validation_Report_2.2.pdf),


Figures captions

**Figure 1** – Maps of the Congo Basin showing (a) the elevation (Lehner et al., 2008), the main hydrological network, the extent of the Cuvette Centrale (Lehner and Döll, 2004), the distribution of sampling sites along the Kisangani – Kinshasa transect and (b) the dominant land cover (Bontemps et al., 2011). The red line “A” indicates the entrance of the Congo River within the core of the Cuvette Centrale (see text for details and supplementary figure 1).

**Figure 2** – Average freshwater discharge of the Congo River at Kinshasa, and corresponding water height at the gauging station, for the period 2003-2013. Timing of the two cruises is indicated by thicker lines.

**Figure 3** – Longitudinal evolution of DOM properties in the mainstem, large and small tributaries along the Kisangani-Kinshasa transect during HW (left panels) and FW (right panels). From top to bottom the panels represent: DOC, $\delta^{13}$C$_{\text{DOC}}$, SUVA$_{254}$, $S_R$ and FI. Numbers refer to large tributaries: (1) the Kwa/Kasai, (2) the Lefini, (3) the Sangha, (4) the Oubangui, (5) the Ruki, (6) the Ikelemba, (7) the Lulonga, (8) the Mongala, (9) the Itimbiri, (10) the Aruwini, (11) the Lomami, (12) the Lindi and (13) the Tshopo River.

**Figure 4** – Relationships between DOC concentrations in tributaries and the extent of flooded dense forest.

**Figure 5** – Longitudinal evolution of the relative contribution of PARAFAC component in the mainstem, large and small tributaries along the Kisangani-Kinshasa transect during HW (left panels) and FW (right panels).

**Figure 6** – Graphical representation of PCA results, including loadings plot for the input variables and scores plot for stations based on (a) their Strahler stream order or (b) sampling location. PCA results based on the hydrological period is included in each plot.
Figure 7 – (a) Relationship between %C5 and %C3 and (b) relationships between %C5 and pelagic community respiration (R) in the Congo Basin.

Figure 8 – Relationship between the relative contribution of aliphatic components (C2 and C6) and pH of stream waters in the Congo Basin.

Figure 9 – DOC concentrations and DOM aromaticity (SUVA$_{254}$) across a gradient of streams and rivers in the Congo Basin as a function of stream order. The box spans the interquartile range (25–75 percentiles), whiskers correspond to min-max values, horizontal bar to median, cross to average.
Table 1 – Selected attributes (mean±standard deviation, min-max) of sampling sites during the field campaigns: oxygen saturation level (%O₂), pH, Secchi depths, vertical light attenuation coefficient (K₅), total suspended matter (TSM) and Chlorophyll-a concentrations.

<table>
<thead>
<tr>
<th>Period</th>
<th>n</th>
<th>%O₂ (%)</th>
<th>pH</th>
<th>Secchi (cm)</th>
<th>K₅ (m⁻¹)</th>
<th>TSM (mg L⁻¹)</th>
<th>Chla (µg L⁻¹)</th>
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<td>Maximum high waters</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Mainstream</td>
<td>35</td>
<td>60.3±10.6</td>
<td>6.46±0.22</td>
<td>54.6±15.6</td>
<td>1.54±0.37</td>
<td>29.4±21.9</td>
<td>0.84±0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(48.4-89.2)</td>
<td>(6.07-6.92)</td>
<td>(25-80)</td>
<td>(1.06-2.83)</td>
<td>(14.0-99.8)</td>
<td>(0.10-1.76)</td>
</tr>
<tr>
<td>Major tributaries</td>
<td>13</td>
<td>54.3±33.3</td>
<td>5.67±1.09</td>
<td>79.5±60.7</td>
<td>1.55±0.61</td>
<td>12.5±13.3</td>
<td>0.54±1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8.6-111.3)</td>
<td>(3.91-6.87)</td>
<td>(25-250)</td>
<td>(0.44-2.46)</td>
<td>(0.74-44.4)</td>
<td>(0.01-3.57)</td>
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<tr>
<td>Minor tributaries</td>
<td>26</td>
<td>27.9±30.2</td>
<td>5.33±0.75</td>
<td>86.2±29.7</td>
<td>1.51±0.49</td>
<td>7.7±13.4</td>
<td>0.35±0.42</td>
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<tr>
<td></td>
<td></td>
<td>(4.2-99.8)</td>
<td>(3.91-6.17)</td>
<td>(15-140)</td>
<td>(0.89-2.79)</td>
<td>(1.7-71.4)</td>
<td>(0-1.85)</td>
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<td>Falling waters after second peak water discharge</td>
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<td></td>
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<tr>
<td>Mainstream</td>
<td>34</td>
<td>84.8±7.4</td>
<td>6.82±0.32</td>
<td>46.8±5.7</td>
<td>3.86±0.58</td>
<td>31.9±9.1</td>
<td>3.99±1.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(54.2-93.4)</td>
<td>(6.08-7.38)</td>
<td>(35-62)</td>
<td>(1.52-4.65)</td>
<td>(4.0-45.6)</td>
<td>(1.13-7.68)</td>
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<tr>
<td>Major tributaries</td>
<td>12</td>
<td>62.1±31.2</td>
<td>5.77±1.22</td>
<td>66.7±23.4</td>
<td>3.34±0.66</td>
<td>14.4±12.5</td>
<td>1.85±2.27</td>
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<td></td>
<td>(0.3-98.2)</td>
<td>(3.63-7.05)</td>
<td>(35-106)</td>
<td>(2.44-5.09)</td>
<td>(0.72-43.0)</td>
<td>(0.017-6.59)</td>
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<td>4.56±0.77</td>
<td>80.7±42.2</td>
<td>3.36±0.95</td>
<td>6.1±6.5</td>
<td>0.55±0.99</td>
</tr>
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<td></td>
<td></td>
<td>(0.3-103.0)</td>
<td>(3.6-6.1)</td>
<td>(38-205)</td>
<td>(1.48-5.16)</td>
<td>(0.5-34.8)</td>
<td>(0.009-6.12)</td>
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</tbody>
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Table 2 – Spectral properties (excitation and emission maxima (Ex\textsubscript{max}/Em\textsubscript{max})) of the six components identified using PARAFAC modelling, correspondence with peak classification, general assignment and comparison with previously identified components in different environments, dominant molecular association and possible source and reactivity. Dominant molecular association is based on FTICR-MS studies. Numbers in brackets refer to the second peak of maximal excitation.

<table>
<thead>
<tr>
<th>Component</th>
<th>Ex\textsubscript{max} (nm)</th>
<th>Em\textsubscript{max} (nm)</th>
<th>Peak(s) name\textsuperscript{1}</th>
<th>General assignment\textsuperscript{2}</th>
<th>Comparison with others environments</th>
<th>Dominant molecular association\textsuperscript{4,6}</th>
<th>Potential sources and reactivity\textsuperscript{5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>&lt;260 (&lt;260)</td>
<td>488</td>
<td>A+/C+</td>
<td>Terrestrial humic-like</td>
<td>C3</td>
<td>C3</td>
<td>C2</td>
</tr>
<tr>
<td>C2</td>
<td>305 (&lt;260)</td>
<td>414</td>
<td>M</td>
<td>Microbial humic-like</td>
<td>—</td>
<td>C7</td>
<td>C5</td>
</tr>
<tr>
<td>C3</td>
<td>330 (&lt;260)</td>
<td>444</td>
<td>A/C</td>
<td>Terrestrial humic-like</td>
<td>C1</td>
<td>C2</td>
<td>C3</td>
</tr>
<tr>
<td>C4</td>
<td>&lt;260 &lt;260</td>
<td>444</td>
<td>A</td>
<td>Terrestrial humic-like</td>
<td>—</td>
<td>C1</td>
<td>—</td>
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<tr>
<td>C5</td>
<td>350</td>
<td>424</td>
<td>C</td>
<td>Humic-like</td>
<td>—</td>
<td>—</td>
<td>C4</td>
</tr>
<tr>
<td>C6</td>
<td>275</td>
<td>350</td>
<td>B/T</td>
<td>Tryptophan-like</td>
<td>C5</td>
<td>C6</td>
<td>C6</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Coble et al., 2014; \textsuperscript{2} Fellman et al., 2010; \textsuperscript{3} Walker et al., 2013; \textsuperscript{4} Massicotte and Frenette, 2011; \textsuperscript{5} Lapierre and del Giorgio, 2014; \textsuperscript{6} Stubbs et al., 2014 (FTICR-MS); \textsuperscript{7} Kothawala et al., 2014; \textsuperscript{8} Kellerman et al., 2015 (FTICR-MS); \textsuperscript{9} Yamashita et al., 2010; \textsuperscript{10} Cawley et al., 2012; \textsuperscript{11} Lambert et al., 2016; \textsuperscript{12} Wagner et al., 2015 (FTICR-MS)

\textsuperscript{4,6} Au: autochthonous production; T: terrestrial inputs; M+: microbial degradation; M-: biolabile; P+: photoproduct; Pr: photo-resistant; P-: photosensible
Figure 2
Figure 3
Figure 4

![Graph showing the relationship between DOC and the percentage of flooded dense forest. The Pearson correlation coefficient is $r = 0.47$, with $p < 0.0001$ and $n=88$. The graph plots DOC (mg L$^{-1}$) on the y-axis against the percentage of flooded dense forest on the x-axis. Data points are distributed across the graph, indicating a positive correlation.]
Figure 5
Figure 8

![Graph showing the relationship between pH and %C2, %C6 for Mainstem, Large Tributaries, and Small Tributaries. The graph includes correlation coefficients: Pearson r = 0.77, p < 0.0001 for %C2, and Pearson r = 0.60, p < 0.0001 for %C6.](image-url)
**Figure 9**

![Figure 9](image-url)