

## **Author response to Anonymous Referee #1**

**REPLY:** We thank the reviewer for raising a number of issues that stimulated us to clarify text and strengthen the manuscript.

This manuscript examines the chemical composition of DOM in the Congo River basin. The data presented is predominantly optical (CDOM/FDOM) alongside <sup>13</sup>C-DOC and DOC concentration data and I have major concerns with the optical data quality from the study that is likely unfortunately beyond the authors control. This leads to issues with data interpretation as well as clear overinterpretation of the data with respect to processing for which there is no evidence presented. The manuscript is clearly organized but there are a lot of mistakes with English language throughout that need a careful read through before it would be suitable for publication. Please find below a list of major / minor comments:

Line 17: You can't have a DOM concentration – just DOC.

**REPLY:** This has been corrected.

Line 19: “stretch in” is poor English. I'm not editing English throughout but there is a lot of minor editorial English correction required.

**REPLY:** “stretch” has been replaced by “transect”. Overall, we have carefully read the manuscript to improve English.

Line 20: “second river in the world” for what? Discharge, basin size, number of people?

**REPLY:** “in terms of drainage basin area and water discharge”. However this sentence has been removed in the revised manuscript in order to reduce the length of the abstract and to avoid repetition with the introduction and methods.

Lines 23-28: This is all speculation for which no evidence is presented. I'll come back to this below.

**REPLY:** We recognize that our manuscript doesn't include experimental evidence from incubation in controlled conditions that could confirm our interpretations regarding the impact of photodegradation and microbial degradation on DOM composition in the Congo River Basin. Such experiments pose logistical difficulties in the field and bringing large volumes of waters to our laboratories in Belgium holds the risk of modifying the natural conditions during transport and thus DOM composition. The abstract of the revised manuscript has been modified to accommodate this comment.

That being said, we respectfully disagree that our interpretation can be considered as “all speculation”. The application of PARAFAC modeling on a large spatial scale allows us to characterize the compositional features of DOM, and, more importantly, to identify specific fractions of DOM and their associated reactivity/origin (see Table 2). Thus, the identification of PARAFAC components previously identified as highly photodegradable (e.g. C3), photo-produced (e.g. C5), or produced during microbial reworking of terrestrial DOM (e.g. C2) significantly enhances the potential interpretations of our dataset. Moreover, the spatial and temporal variations in our dataset are interpreted alongside with other measurements including

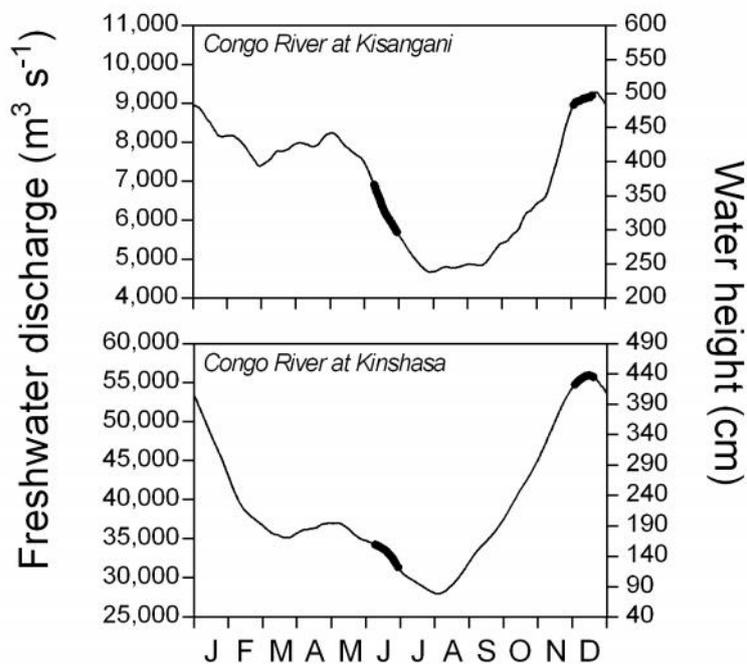
biogeochemical parameters (e.g. pelagic respiration, pH) and changes in environmental conditions (e.g. landscape morphology), and some of our conclusions are highly consistent with concepts evidenced in experimental studies (e.g. the link between photodegradation of terrestrial DOM and pelagic respiration, see Fig. 7). Consequently, despite the lack of experimental incubations, we argue that the interpretation of our data and ensuing conclusions are based and supported by recent literature. Overall, we attempted to improve the revised manuscript by pointing out how the variability observed in our dataset is consistent with recent literature (and also to enhance the general language flow as suggested by reviewer #2).

Lines 101-105: So what is the difference in WRT between the two seasons? If you are going to postulate WRT as a major driver it would be good to know at least how much it varies.

**REPLY:** WRT in the Congo River between Kisangani and Kinshasa has been roughly estimated to increase about ~5 days between FW and HW (Descy et al., 2016) but is likely not uniform along the transect: because the main tributaries are all located downstream, the increase of WRT is probably more important upstream of Oubangui compared to downstream. We have specified this explicitly in the revised manuscript.

Lines 132-135: Samples were collected during the declining limb of a secondary discharge maxima which has its source in the southern part of the Basin and so this makes sense for comparison for samples near the mouth to the major discharge maxima. As you move upstream towards Kisangani on the map how is seasonality changing there for example – does this hydrograph have any bearing on what is happening there? It is unclear to me if this hydrograph (i.e. timing of high and low waters) is the same for all the sites – it seems not from what is written so what is really been compared in terms of seasonality / WRT?

**REPLY:** Water level of the Congo River follows similar seasonal variations at Kisangani and Kinshasa (Fig. 1 (revised Fig. 2)). Thus, timing of high and low waters are similar for the sites located along the mainstem although changes in WRT are likely more important upstream of Oubangui as outlined in the previous comment. We have specified this explicitly in the revised manuscript, as well as revising Figure 2.

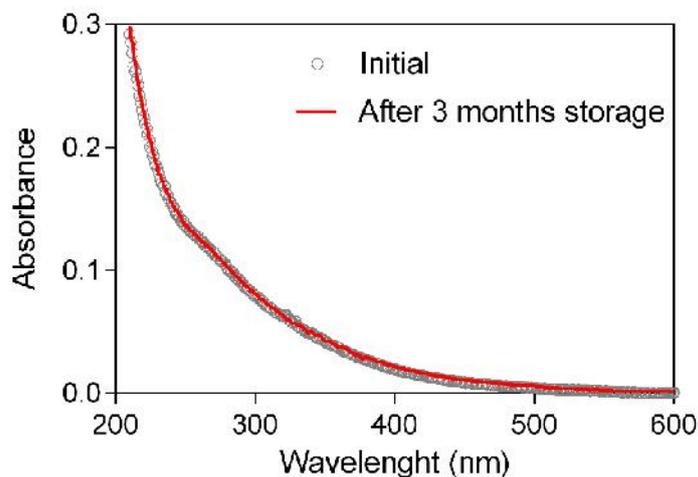


Revised Fig. 2

Lines 141-149: Samples for DOC and <sup>13</sup>C-DOC were filtered and acidified so were stable. Samples for optical analysis were just filtered – were they then refrigerated or frozen – it is unclear? A major concern here is that these samples were just filtered (even at 0.2µm we see 3% of the microbial community get through the filter and it rebounds in a few days) and then basically were degrading until they were analyzed. Please provide details of how samples were stored, I appreciate this is probably a logistically very difficult part of the world to work in but more details on sample storage and analysis are required to assess the validity of the optical data.

**REPLY:** Among the different preservation techniques, we have chosen to just filter samples without acidification and to store the samples at cold temperature. Acidification of samples (or addition of HgCl<sub>2</sub>) could have significant effects on the colored and fluorescent fractions of DOM due to change in the ionic strength (leading to flocculation or complexation) and/or through the release of DOM of biological origin due to senescence of microorganisms, and freezing has been shown to significantly affect the composition of DOM, especially when surface water are enriched in aromatic DOM (Fellman et al., 2008, STE). In order to limit potential degradation, samples were stored just after filtration at 4°C in a refrigerator placed on the boat until transport toward Belgium. Once received, samples were again stored at 4°C until analysis, the latter being performed during the week after arrival. Potential storage and degradation effects were assessed by analyzing a series of contrasting samples after 3 months (see one example of absorbance spectra in Fig. 2). The differences in optical proxies ( $a_{350}$ ,  $SUVA_{254}$  ad  $S_R$ ) were less than 5% (n=22) and no significant differences were observed by comparing excitation-emission matrices, validating

therefore both the preservation technique as well as the quality of the data. These details as well as the figure (revised supplementary Fig. 2) have been added in the revised manuscript, and should eliminate the doubts raised about sample/data integrity.



Supplementary Fig. 2 in the revised manuscript

Lines 204 -207: SUVA is a linear sliding scale, there is no hard and fast cut off between aromatic and aliphatic: higher values = greater percent aromaticity.

**REPLY:** We have modified the text in the revised manuscript.

Lines 378-383: A general increase in DOC is reported along the axial transect related to inputs from flooded forest (Fig 4). The authors are stating that flooded forest extent controls DOC export, which makes sense, and so how then does it not control DOM composition? If the composition is varying independently of DOC concentration you cannot invoke processes like photochemistry as that would also impact DOC concentration. This gives me concern about the DOM composition data as its storage history is not clear.

**REPLY:** In fact we found significant differences in the longitudinal increase in DOC concentrations between FW ( $0.0002 \text{ mgDOC km}^{-1}$ ) compared to HW ( $0.0012 \text{ mgDOC km}^{-1}$ ) in the upper part of the transect (these details have been added in the revised version), and these changes were accompanied with changes in DOM composition (already mentioned in the manuscript). We recognize that we cannot rule out that lateral exchanges between the central water masses of the Congo River and DOM-rich waters from tributaries can be driven by water level fluctuations in the Congo River (comment included in the revised manuscript). That being said, the occurrence of greater photodegradation during FW compared to HW is supported by several of our results (reformulated in the revised manuscript), including (1) the lower %C1 and %C3 during FW compared to HW (both these components are associated with highly aromatic molecules of high MW known to be sensitive to photodegradation), (2) the more important decrease in %C3 (consistent with the well documented high photosensibility of this component relative to other

terrestrial humic-like components) and (3) the PCA analysis that clearly separates stations collected in the mainstem between HW (negative scores) and FW (positive scores) along the PC2. Along PC2, PARAFAC components are distributed with C1 and C3 as negative loadings and C4 and C5 as positive loadings. C1 and C3 has been identified as photo-sensitive (Yamashita et al., 2010; Lapierre and del Giorgio, 2014) while C4 and C5 are respectively considered as photo-resistant (Massicotte and Frenette, 2010; Ishii and Boyer, 2012) and produced by photodegradation (Lapierre and del Giorgio, 2014). Therefore, we interpret positive values of PC2 as indicative of photo-degradation.

The results of the PCA analysis (i.e. the meaning of PC2) have been detailed in the revised manuscript.

Lines 397-436: This is all highly speculative and ok so WRT is changing but by how much? TSS and phytoplankton growth are leading to less light penetration but in the Congo your light penetration is likely controlled by CDOM as its low TSS year round apparently. So if you have high CDOM year round (i.e. your river is colored) you have lots of substrate for photochemical reactions but its self regulating as your absorption is so high in the water column that only the very surface will degrade...when you look at any reference except the Cory Science paper they see very little evidence for photochem in freshwaters...they are typically either mechanistic (i.e. we put this in a bottle and degraded it) or they are in coastal systems where your colored waters are diluted out in optically clear marine waters and so see more light (i.e. self-shading goes away). I'm very skeptical that in a colored river like the Congo photochem actually can meaningfully degrade DOM so it shows up compositionally between seasons – maybe though if the WRT increases by weeks / months it could but this is not described.

**REPLY:** We agree with the reviewer that light penetration is likely controlled by CDOM. However, while the depth of UV light penetration decreases with increasing level of CDOM, the average rate of light absorption by CDOM increases in parallel (Hu et al., 2002). In this case, high rate of photodegradation can occur in ecosystems rich in CDOM such as the Congo Basin even if UV light penetration is low (Cory et al., 2015). Furthermore, an increase of ~5 days in WRT between FW and HW is likely sufficient to significantly enhance DOM photodegradation as this degradation pathway can significantly processed terrestrial DOM over short time scale (e.g. ~12h of sunlight alteration; Cory et al., 2015). Clearly, further research is needed in order to quantify rate of photodegradation in the Congo River and the relative importance of this process on C cycling in the Congo Basin, but this was beyond the scope of the present study. We have mentioned this explicitly in the revised manuscript.

Moreover, without considering experimental incubation studies (such as the Cory Science paper) that represent the most easy and common way to investigate this process, it should be noted that several field studies carried out in “natural conditions” have illustrated the occurrence of photodegradation in freshwaters. The most relevant of these studies, conducted in the St Lawrence River (Massicotte and Frenette, 2011) and the Okavango Delta (Cawley et al., 2012), have thus related longitudinal changes in DOM composition to the impact of photodegradation on the aromatic and colored fraction of DOM during its downstream transport through notably the characterization of DOM by PARAFAC modeling.

Lines 467-551: Again this whole section is really speculative with just the optical data to suggest things – there are no incubations to support any of the trends. I appreciate the authors are just trying to explain their data but in reality its always fairly aromatic dominated. When I look at all the data I see a highly terrestrially dominated system and I'm not sure I'd dare get too much into seasonality with just two time periods. This needs scaling back to reflect this as a minimum.

**REPLY:** We agree with the reviewer that the Congo River Basin is fairly dominated by terrestrial and aromatic DOM during the two periods. This point has been added in the revised manuscript. That being said, this does discard our results and interpretations that are based on the patterns of PARAFAC components that can be used as tracers of source, origin and processing of DOM according to an increasing body of literature (Fellman et al., 2010; Jaffé et al., 2008 and other references cited in the manuscript). Moreover, patterns of PARAFAC components are interpreted along with measurements of environmental variables known to drive DOM reactivity (e.g. pH) that provide additional support for our interpretations. Finally, this section of the manuscript is focused on the downstream transformation of DOM during its transport in the fluvial network and not on seasonality (that would require monitoring at high frequency).

Lines 552-583: Again highly speculative – isn't the Cuvette constantly connected and so its not like it flushes like the varzea on the Amazon? If its always connected shouldn't the DOM coming out of it always look compositionally similar and so there should be no flood pulse?

**REPLY:** We respectfully disagree that the comparison made in our study with the conceptual model proposed by Creed et al. (2015) can be qualified as speculative as we used the same approach than these authors to investigate the relative influence of terrestrial inputs versus biogeochemical transformations across a gradient of Strahler order. Our results show that the Cuvette Centrale have a major control on DOM biogeochemistry at the scale of the Congo Basin. Although the seasonal variations of water level are modest in the Congo compared to the Amazon, and that the Cuvette Centrale Congolaise (CCC) does not flush as in the Amazon, there are several rivers that drain the CCC (Ruki, Likouala aux herbes, Sangha), that bring large amounts of water and DOM to the river (The Ruki is the second tributary after the Kasai). DOM is therefore fairly dominated by aromatic compounds, but shows some differences in composition between the two periods because of changes in WRT. Overall, our results highlight the critical importance of river-floodplain connectivity in lowland tropical rivers as postulated in the flood pulse concept of Junk et al. (1989) in controlling DOM concentration and composition (see also Battin, 1998; Zurbrügg et al., 2013; Lambert et al., 2016). Patterns of other data acquired during the same cruises such as CO<sub>2</sub> and CH<sub>4</sub> also show the influence of inputs of these quantities from the Cuvette Centrale Congolaise (Borges et al. 2015). This is markedly different from the Chemostat Hypothesis of Creed et al. (2015), the latter being built on the river continuum concept (Vannote et al. 1980) that is typically applicable to rivers at temperate latitudes (devoid of large wetlands). This section has been clarified, and two references have been added (Moreira-Turcq et al., 2003; Zurbrügg et al., 2013).

## **Author response to Anonymous Referee #2**

### General Comments

The submitted manuscript characterizes the downstream changes in DOM composition in the Congo River with a seasonal component. The study covers a large spatial scale in an important basin in the context of the global carbon cycle. Additionally, the African continent has been underrepresented in studies of this kind. This paper is an important contribution to the field of aquatic carbon biogeochemistry. The measurements (SUVA, PARAFAC,  $^{13}\text{C}$ -DOC) are commonly used in the field and thus can be easily compared to future studies.

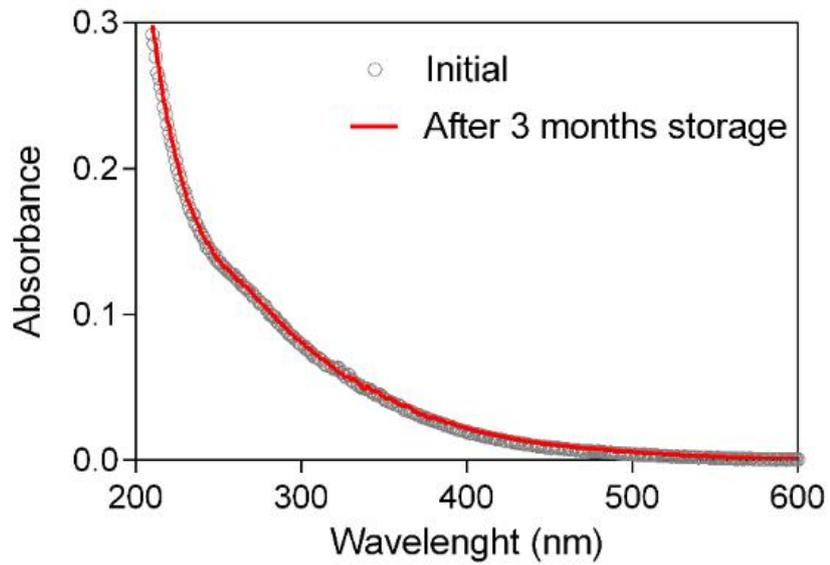
The authors did commendable work interpreting the data in the discussion. I believe their conclusions about photodegradation and production of aliphatic-like DOM are well supported by the cited literature. Additionally, the insights into the role of landscape morphology on DOM composition are sound. The incorporation/comparisons to the chemostat hypothesis and pulse-shunt concept were insightful and are supported by the authors' interpretation of the results. I would recommend the article be accepted with minor revisions. The English could be improved throughout the manuscript. It would benefit from additional proofreading for mistakes and general language flow. Additionally, the authors must address methodological concerns with regards to sample storage/preservation.

**REPLY:** We thank reviewer #2 for this positive feedback. We aimed to improve the revised manuscript by correcting English mistakes and by reformulating some parts of the discussion to improve the language flow.

### Specific Comments

L141-L151 Samples for fluorescence and absorbance were filtered and kept in the dark but how long were they exposed to ambient temperatures before refrigeration and then analysis? Given the respiration measured after 24 hour incubations at ambient temperature this is a major concern and must be addressed.

**REPLY:** In order to limit potential degradation, samples were stored just after filtration at 4°C in a refrigerator placed on the boat until transport toward Belgium. Once received, samples were again stored at 4°C until analysis, the latter being performed during the week after arrival. Potential storage and degradation effects were assessed by analyzing a series of contrasting samples after 3 months (see one example of absorbance spectra). The differences in optical proxies ( $a_{350}$ ,  $\text{SUVA}_{254}$  and  $S_R$ ) were less than 5% ( $n=22$ ) and no significant differences were observed by comparing excitation-emission matrices, validating therefore both the preservation technique as well as the quality of the data. These details as well as the figure have been added in the revised manuscript, and should eliminate the doubts raised about sample/data integrity.



Supplementary Fig. 2 in the revised manuscript

Technical Errors

L20 Should be second largest basin/discharge as mentioned in the introduction/methods

**REPLY:** This sentence has been removed in the revised manuscript in order to reduce the length of the abstract and to avoid repetition with the introduction and methods.

L225 "wwereas"? Should be were

**REPLY:** This typo was corrected.

### **Relevant changes made in the manuscript:**

Overall, the text of the revised manuscript has been carefully read and reworked in order to correct English mistakes and to improve the language flow. A specific attention has been devoted to the comments of the reviewer 1. By reformulating some entire sections of the manuscript, we aimed to better present how our results and interpretations are consistent with a large body of literature aiming to investigate DOM dynamic in fluvial systems by using PARAFAC modeling.

Major changes include: (1) a better description of the PCA results that illustrate the role of photodegradation in controlling DOM composition in the Congo (PC2), (2) a full rewriting of the sections 4.1.2, 4.2.1 and 4.2.2 (but without changing our interpretations), (3) the adding of references (especially for the dual role of microorganisms as consumers of terrigenous aromatic DOM and producers of novel compounds), (4) the adding of details regarding changes in WRT along the Congo River, based on a recent study (Descy et al., 2016, in press) and (5) the adding in the Materials and Methods section of details regarding the preservation method for CDOM samples. The figure comparing initial spectra (i.e. measured during the week after arrival in Belgium) and spectra acquired after 3 mounts has been added as a supplementary figure in the revised manuscript.

Marked-up manuscript version

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

Thibault Lambert<sup>1,\*</sup>, Steven Bouillon<sup>2</sup>, François Darchambeau<sup>1</sup>, Philippe Massicotte<sup>3</sup>,  
and Alberto V. Borges<sup>1</sup>

<sup>1</sup> University of Liège, Chemical Oceanography Unit, Liège, Belgium

<sup>2</sup> K.U. Leuven, Department of Earth and Environmental Sciences, Leuven, Belgium

<sup>3</sup> Aarhus University, Department of Bioscience, Denmark

\* Corresponding author

**Abstract.** The processing of terrestrially derived dissolved organic matter (DOM) during downstream transport in fluvial networks is poorly understood. Here, we report a dataset of dissolved organic carbon (DOC) concentrations and DOM composition (stable carbon isotope ratios, absorption and fluorescence properties) acquired along a 1700 km transect in the middle reach of the Congo River Basin. Samples were collected in the mainstem

and its tributaries during high water (HW) and falling water (FW) periods. DOC concentrations and DOM composition along the mainstem were found to differ between the two periods, because of a reduced lateral mixing between the central water masses of the Congo River and DOM-rich waters from tributaries and also likely because of a greater photodegradation during FW as water residence time (WRT) increased. Although the Cuvette Centrale wetland (one of the world's largest flooded forest) continuously releases highly aromatic DOM in streams and rivers of the Congo Basin, the downstream transport of DOM was found to result in an along stream gradient from aromatic to aliphatic compounds. The characterization of DOM through parallel factor analysis (PARAFAC) suggests that this transition results from (1) the losses of aromatic compounds by photodegradation and (2) the production of aliphatic compounds by biological reworking of terrestrial DOM. Finally, this study highlights the critical importance of the river-floodplain connectivity in tropical rivers in controlling DOM biogeochemistry at large spatial scale and suggests that the degree of DOM processing during downstream transport is a function of landscape characteristics and WRT.

## 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 (Bertilsson and Jones, 2003; Battin et al., 2008). Recent experimental and field studies have demonstrated 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; Lapierre and del Giorgio, 2014; Lambert et al., 2016). Large surveys of boreal lakes have suggested that DOM was degraded along a gradient from aromatic to aliphatic compounds and that the chemical composition of DOM was the dominant control of overall DOM reactivity (Kothawala et al., 2014; Kellerman et al., 2015). Similarly, a large survey of temperate streams and rivers has 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). However, the role of environmental factors (i.e. climatic variables, water chemistry, landscape properties) on DOM transformation in fluvial networks remains poorly constrained (Massicotte and Frenette, 2011; Marín-Spiotta et al., 2014).

The consideration of temporal dynamics in addition to the spatial dimension is poorly investigated, yet it represents 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 rivers and wetlands (Junk et al., 1989; Battin, 1998; Besemer et al., 2009; Osburn et al., 2009; 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). According to the recent pulse-shunt concept (Raymond et al., 2016) that builds off 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 hydrological events favor the transport of DOM through the drainage network and therefore reduce 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 important role in the global carbon cycle (Borges et al., 2015a; 2015b). Yet, they remain largely underrepresented in large-scale studies on DOM processing. For example, only few studies have investigated DOM biogeochemistry in the Congo Basin (Spencer et al., 2010, 2012; Bouillon et al., 2012, 2014; Mann et al., 2014) despite the fact that the Congo River – the largest river in Africa and the second largest river worldwide after the Amazon in terms of drainage basin area and water discharge (Laraque et al., 2009) – is the second major exporter of terrestrial organic carbon to the oceans, 85-90% being in the form of DOC (Coynel et al., 2005; Laraque et al., 2009). Downstream transport of DOM in the Congo River has not yet been investigated in its middle reach where is located the second largest tropical flooded forest wetland, the Congolese ‘Cuvette Centrale’. Additionally the Congo River receives in the middle reach inputs from its major tributaries such as the Kasai, Ruki and Oubangui (Fig. 1).

Emerging conceptual models that describe how inland waters transform DOM flowing down the fluvial network, such as the “chemostat” hypothesis (Creed et al., 2015) and the pulse-shunt concept (Raymond et al., 2016), need to be tested by empirical data acquired through extensive studies in tropical ecosystems. The Congo River and its tributaries were sampled along a 1700 km transect from the city of Kisangani to the city of

Kinshasa during two contrasting hydrological periods (Figs. 1 and 2). DOM was characterized through its optical properties, its stable carbon isotope composition ( $^{13}\text{C}_{\text{DOC}}$ ) and its DOC concentration. Optical measurements (including absorption and fluorescence) have been underscored as an efficient tool for characterizing the source and the reactivity of specific fractions of the DOM pool at large spatial scales (Jaffé et al., 2008; Yamashita et al., 2010; 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 aims of this study were (1) to characterize the longitudinal evolution of DOM in the Congo River in the middle reach of the basin, (2) to investigate the transformation of DOM during its downstream transport in the fluvial network and (3) to identify potential environmental drivers of DOM processing.

## 2. Materials and Methods

### 2.1 Study area.

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 ( $\sim 3.7 \times 10^6 \text{ km}^2$ ) and water discharge ( $\sim 43\,000 \text{ m}^3 \text{ s}^{-1}$ ) (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 known as the Congo. The Congo basin straddles the Equator, with major tributaries located in both hemispheres (Fig. 1). Thus, the rainy season on the northern part is compensated by the dry season on the southern part, and *vice-versa*. Consequently, seasonal water height variations are largely attenuated (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 River at Kisangani and Kinshasa is similar, with a bimodal distribution: maximum water peaks occur 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 on 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 the 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; high waters (HW)) and during falling waters (FW) following the second discharge maximum occurring in March (10-30 June 2014) (Fig. 2). As water level of the Congo River follows similar seasonal fluctuations at Kisangani and Kinshasa (Fig. 2), the timing of high and low waters was likely similar for the sites located along the mainstem. WRT between Kisangani and Kinshasa has been roughly estimated to increase by about ~5 days during FW compared to HW (Descy et al., 2016) but is likely not uniform along the transect: because the main tributaries are all located downstream, the increase of

WRT is probably more important upstream of the Oubangui. Water samples were collected in the Congo River itself as well as in small and large tributaries at the confluence (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 bank of the Congo, and the Lomami, the Lulonga, the Ikelemba, the Ruki and the Kwa/Kasai on the left bank. 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, stored 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  $\mu\text{m}$  porosity), then on 0.2  $\mu\text{m}$  polyethersulfone syringe filters. Samples for the measurement of DOC concentrations and  $^{13}\text{C}_{\text{DOC}}$  signatures were stored in 40 mL glass vials with polytetrafluoroethylene (PTFE) coated septa with 50  $\mu\text{L}$   $\text{H}_3\text{PO}_4$  (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  $\text{H}_3\text{PO}_4$  addition. Samples for CDOM and FDOM were stored just after filtration at 4°C in a refrigerator on the boat until transportation back to Belgium, where they were also stored at 4°C until analysis within one week of arrival. Potential storage and degradation effects were assessed by analyzing a series of contrasting samples after 3 months (Supplementary Fig. 2). The differences in optical proxies ( $a_{350}$ ,  $\text{SUVA}_{254}$  and  $S_R$ ) were less than 5% (n=22) and no significant changes were observed by comparing excitation-emission matrices (EEMs). Samples for major elements (including Fe) were stored in 20 mL scintillation vials and acidified with 50  $\mu\text{L}$  of  $\text{HNO}_3$  65 % prior to analysis.

Fe was measured by inductively coupled plasma spectrometry (Agilent 7700x ICP-MS). DOC and  $^{13}\text{C}_{\text{DOC}}$  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  $< \pm 5\%$  for DOC, and  $\pm 0.2\%$  for  $^{13}\text{C}_{\text{DOC}}$ . Quantification and calibration were performed with series of standards prepared in different concentrations, using both IAEA-C6 ( $^{13}\text{C} = -10.4\%$ ) and in-house sucrose standards ( $^{13}\text{C} = -26.9\%$ ). All data are reported in the ‰ 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:

$$A_{\lambda} = A_0 e^{-S(\lambda-\lambda_0)} + K \quad (1)$$

where  $A$  and  $A_0$  are the absorbance measured at defined wavelength 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 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<sub>2</sub>, and pH were measured *in situ* with portable field probes calibrated using standard protocols (YSI ProPlus probe). Pelagic respiration (R) was determined from the decrease of O<sub>2</sub> in 60 ml biological oxygen demand bottles over ~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 O<sub>2</sub> decrease was determined from triplicate measurements at the start and the end of the incubation with an optical O<sub>2</sub> probe (YSI ProODO). The respiratory quotient (RQ), defined as the molar ratio of O<sub>2</sub> consumed to CO<sub>2</sub> produced by respiration, allows the conversion of respiration measurements from O<sub>2</sub> 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, 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. 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<sub>d</sub> (m<sup>-1</sup>), 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<sub>d</sub> was derived from the slope of the semi-logarithmic regression between relative quantum irradiance and depth. Transparency of the water column was measured using a 20-cm diameter Secchi disk.

### **2.3. Characterization of DOM composition.**

The specific ultra-violet absorbance (SUVA<sub>254</sub>) was calculated as the UV absorbance at = 254 nm (A<sub>254</sub>) normalized to the corresponding DOC concentration

(Weishaar et al., 2003). The natural UV absorbance of Fe at  $\lambda = 254$  nm was estimated based on measured Fe concentrations and was then subtracted from the UV absorbance measured. The corrected value of  $A_{254}$  was then used to calculate  $SUVA_{254}$ . The  $SUVA_{254}$  was used as an indicator of the aromaticity with higher values indicative of greater percent of aromaticity (Weishaar et al., 2003).

Napierian absorption coefficients were calculated according to:

$$a_{\lambda} = 2.303 \times A_{\lambda}/L \quad (2)$$

where  $a$  is the absorption coefficient ( $m^{-1}$ ) at wavelength  $\lambda$ ,  $A$  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 spectra versus wavelength. The slope ratio  $S_R$  was then calculated as the ratio of  $S_{275-295}$  to  $S_{350-400}$ .  $S_R$  is inversely related to the molecular weight (MW) distribution of DOM (Helms et al. 2008). 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 (including removing first and second Raman scattering, standardization to Raman units, absorbance corrections and inner filter effects) was 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 build using MATLAB (MathWorks, Natick, MA, USA) and the drEEM Toolbox version 1.0 (Murphy et al., 2013). Validation of the model was performed 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 ( $n = 17$ ) 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  $\%C_X = F_{\text{Max}}(X) / F_{\text{Tot}}$ .

The positions of maximum peaks established by the model were compared to the classical excitation-emission matrices nomenclature (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 Swedish lakes (Kellerman et al., 2015), boreal streams and rivers (Stubbins et al., 2014) and a subtropical wetland (Wagner et al., 2015). Although such comparison has not been performed with our own samples, the relatively good consistency of associations between

optical and molecular linkages observed in these 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 flooded 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 differences 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 ( $a_{350}$ ), bulk composition ( $SUVA_{254}$ ,  $S_R$ , 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. DOC concentrations and DOM bulk composition.**

DOC concentrations in the mainstem were higher during HW (5.4 – 13.9 mg L<sup>-1</sup>, average 8.2±2.6 mg L<sup>-1</sup>) compared to FW (4.2 – 9.8 mg L<sup>-1</sup>, average 5.9±1.8 mg L<sup>-1</sup>) but showed similar longitudinal patterns during both hydrological periods (Fig. 3a, 3b): DOC increased slowly in the upper part of the transect (0.0012 mgDOC km<sup>-1</sup> at HW and 0.0002 mgDOC km<sup>-1</sup> at FW) and then faster as the Congo River evolved throughout the core of the Cuvette Centrale and mixed with the Kwa/Kasai River (0.0091 mgDOC km<sup>-1</sup> at HW and 0.0085 mgDOC km<sup>-1</sup> at FW) (Fig. 1). The breaking point in the DOC longitudinal increase in DOC concentrations was located around km 700 during HW, and km 500 during FW. DOC concentrations in tributaries were highly variable (from 1.8 to 67.8 mg L<sup>-1</sup>) 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.

<sup>13</sup>C<sub>DOC</sub> signatures in the mainstem were lower during HW (from -30.6 to -28.8 ‰, average -29.4±0.3 ‰, *n* = 35) compared to FW (from -29.3 to -25.2 ‰, average -27.5±0.9 ‰, *n* = 34). <sup>13</sup>C<sub>DOC</sub> during HW decreased about 0.7 ‰ from Kisangani to km ~ 1200, remained stable until km ~ 600 and then increased slightly towards Kinshasa. During FW, <sup>13</sup>C<sub>DOC</sub> decreased markedly about 3 ‰ between Kisangani and km ~ 1600. Downstream, values were variable (-27.2±0.6 ‰ between km 600 – 1600, *n* = 18) and then showed ~ 1 ‰ drops at km ~ 600 and ~ 200, coinciding with the confluence zones with the Oubangui and the Kwa/Kasai rivers, respectively. In tributaries, <sup>13</sup>C<sub>DOC</sub> values displayed a similar pattern during the two hydrological periods with low and relatively stable values (-29.7±0.5

‰,  $n = 76$ ) in streams and rivers draining dense forest areas and higher signatures in those flowing savannah areas 0-400 km upstream of Kinshasa ( $-28.1 \pm 0.8$ ,  $n = 14$ ). Stations of the mainstem located within or upstream the Cuvette Centrale were characterized by highest  $^{13}\text{C}_{\text{DOC}}$  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  $^{13}\text{C}_{\text{DOC}}$  signatures than those measured in tributaries ( $p < 0.0001$ , all periods).

SUVA<sub>254</sub> and  $S_{\text{R}}$  during both periods varied mainly between  $4.0 - 5.2 \text{ L mgC}^{-1} \text{ m}^{-1}$  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<sub>254</sub> generally decreased from Kisangani to Kinshasa in the mainstem during HW, with a slight increase between km 500 and 800 upstream of Kinshasa, while  $S_{\text{R}}$  exhibited stable values from Kisangani and then started to increase towards Kinshasa at km 700. Compared to HW, SUVA<sub>254</sub> during FW was relatively stable and lowest from Kisangani to km 500 but higher between km 0 – 500 as SUVA<sub>254</sub> increased markedly in this section ( $p < 0.0001$ ).  $S_{\text{R}}$  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<sub>254</sub> ( $p = 0.0043$ ) associated with an increase in  $S_{\text{R}}$  ( $p = 0.047$ ) was also observed between km 200 – 400. Generally, SUVA<sub>254</sub> in tributaries were slightly higher at 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 at HW ( $p = 0.0006$ ), and were 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. 3). Components C1, C3, C4, and C5 are all classified as “humic-like” but differ in terms of sources, molecular association and reactivity (Table 2). C1 and C3 are commonly reported in freshwaters 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, 2014). C4 is associated with terrigenous molecules of lower aromaticity and MW relative to C1 and C3 (Kellerman et al., 2015). C4 commonly originates from terrestrial inputs (Stedmon and Markager, 2005; Yamashita et al., 2010; Lambert et al., 2016), and has been shown to be resistant to photodegradation (Massicotte and Frenette, 2010; Ishii and Boyer, 2012). Among the humic-like compounds, C5 is associated with molecules characterized by lowest aromaticity and MW produced during the photodegradation of terrestrial DOM (Lapierre and del Giorgio, 2014; Stubbins et al., 2014). C2 and C6 are classified as microbial humic-like and tryptophan-like components, respectively (Fellman et al., 2010). In contrast 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 a fraction of DOM produced during the microbial degradation of terrestrial DOM 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 periods (Fig. 5). %C1 and %C3 during HW presented a slight downstream decrease followed by an increase 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, the longitudinal evolution of %C4 showed an opposite pattern to those of %C1 and %C3, with a higher 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 higher %C3 but lower %C2 and %C6 relative to the mainstem ( $p < 0.0001$ ). %C4 and %C1 were higher ( $p = 0.0007$ ) and lower ( $p = 0.017$ ), respectively, 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 ( $p = 0.003$ ) during FW compared to HW.

### **3.3. PCA results.**

The first two principal components (PC) accounted for 57% of the total variance (Fig. 6). The first PC (PC1) showed a transition from terrestrial aromatic DOM (%C3,  $SUVA_{254}$ , DOC,  $a_{350}$ , positive loadings) to aliphatic DOM (%C2, %C6, FI, negative

loadings). Along the second PC (PC2), PARAFAC components are distributed with C1 and C3 as negative loadings and C4 and C5 as positive loadings. C1 and C3 have been identified as photo-sensitive (Yamashita et al., 2010; Lapierre and del Giorgio, 2014) while C4 and C5 are considered as photo-resistant (Massicotte and Frenette, 2010; Ishii and Boyer, 2012) and produced by photodegradation (Lapierre and del Giorgio, 2014), respectively. Therefore, the PC2 can be interpreted as indicative of photodegradation (Cawley et al., 2012). 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.** From Kisangani to Kinshasa the Congo River continually receives DOM inputs from tributaries flowing through the Cuvette Centrale (Fig. 4), resulting in a net longitudinal increase in DOC concentrations during both periods.

However the longitudinal evolution in DOM content and composition differed between the two campaigns due to the combination of several factors.

**4.1.1. Seasonal changes in DOM sources mobilized in the upper basin.** The large variation in  $^{13}\text{C}_{\text{DOC}}$  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  $^{13}\text{C}_{\text{DOC}}$  signatures that occurred during high flow periods have 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). Higher  $^{13}\text{C}_{\text{DOC}}$  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 is further supported by an ongoing high frequency sampling carried out at Kisangani (own unpublished data).

#### **4.1.2. Reduced lateral exchanges between the Congo River and the flooded forest**

**during falling waters.** The decreasing but higher  $^{13}\text{C}_{\text{DOC}}$  signatures in the mainstem compared to tributaries during FW indicate a reduced lateral mixing between the central water masses of the Congo River and DOM-rich waters from tributaries. Although we cannot rule out that lateral exchanges are likely driven by water level fluctuations in the Congo River, our data suggest that photodegradation of terrestrial DOM contributes to limit the downstream DOC enrichment during FW. Photodegradation is known to preferentially act on the colored, photosensitive molecules associated with high MW and aromaticity (Helms et al., 2008; Spencer et al., 2009; Lapierre and del Giorgio, 2014), and is expected to be highly efficient in aquatic ecosystems rich in light-absorbing CDOM such as the Congo River (Cawley et al., 2012; Cory et al., 2014). A stronger effect of DOM photodegradation in the mainstem during FW is supported by three lines of evidence in our data. First, C1 and C3 – both associated with highly aromatic molecules of high MW (Table 2) – were lower during FW compared to HW, and this decrease occurred along with a decrease in DOM aromaticity (lower  $\text{SUVA}_{254}$ ) and increase in average MW (higher  $\text{S}_R$ ) (Fig. 3 and 5). Second, the more drastic decrease in %C3 is consistent with the well documented high photosensibility of this component relative to other terrestrial humic-like components (Yamashita et al., 2010; Cawley et al., 2012; Lapierre and del Giorgio, 2014).

Finally, the PCA analysis clearly separates stations collected in the mainstem between HW (negative scores) and FW (positive scores) along the PC2 which highlights that photodegradation is an important driver of DOM composition in the Congo Basin.

A stronger degree of DOM photodegradation during FW implies a higher exposure of CDOM to solar irradiation, either spatially (i.e. in the water column) or temporally. The higher coefficient of light attenuation in the water column ( $K_d$ ) and lower Secchi depths during FW (Table 1) indicate 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), although CDOM likely remained the main driver of light penetration. Thus, the degree of DOM photodegradation appears to be mainly driven by changes in WRT that has been estimated to increase about ~5 days in FW compared to HW (Descy et al., 2016). Although sunlight depth penetration was low and the variation in the WRT relatively small, rates of photodegradation can be elevated in CDOM-rich ecosystems (Cory et al., 2015) and this process can substantially alter terrestrial DOM over short time scale (e.g. ~12h of sunlight alteration according to Cory et al., 2015). Further research is needed in order to quantify photodegradation rate and the relative importance of this process on C cycling in the Congo Basin, but this was beyond the scope of the present study.

**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 in terms of discharge (i.e. the Lulonga, the Ruki, the Sangha, the Oubangui and the Kwa/Kasai rivers) connect the mainstem. These tributaries are also very rich in terrestrial DOM from the large flooded forest (Coynel et al., 2005; Laraque et

al., 2009) (Fig. 1 and Supplementary Fig. 1). DOC concentrations 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 was found to occur first at ~70 km downstream of the confluence zone with the Oubangui River, coinciding with a strong reduction of the channel width (Supplementary Fig. 4). The ~1 ‰ drop in  $^{13}\text{C}_{\text{DOC}}$  associated with changes in DOM composition (especially increase in  $\text{SUVA}_{254}$  and %C3) at this station indicates that the reduction of the channel width favors the lateral mixing between the mainstem and waters from the Cuvette Centrale that travel along the river ridge lined by dense forest without being significantly impacted by photodegradation (Supplementary Fig. 5). In fact, a “complete” lateral mixing with waters from the Cuvette Centrale occurs after the confluence zone with the Kwa/Kasai River (based on cross channel transects of conductivity and pH, not shown). In this part of the river, the high discharge of the Kwa/Kasai combined with a narrow mainstem channel devoid of sand bars and islands (Runge et al., 2008) forces lateral mixing. This is supported by the fact that  $^{13}\text{C}_{\text{DOC}}$  signatures of the Congo mainstem became typical of black waters only after connecting with the Kwa/Kasai during FW, and could also explain why the DOC increase is greater at this point while DOC is typically higher in tributaries located more upstream (e.g. The Ruki River).

Large tributaries also controlled the general evolution of DOM composition from Kisangani to Kinshasa. DOM aromaticity ( $\text{SUVA}_{254}$ ) decreased slightly along the transect during HW (from ~4.6 to 4.2  $\text{mgC L}^{-1} \text{m}^{-1}$  from Kisangani to Kinshasa), but increased significantly during FW (from ~4.0 to 5.3  $\text{mgC L}^{-1} \text{m}^{-1}$  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.** Strahler stream order was used as an organizing concept for the individual characterization of stream reaches within the network (Strahler, 1957, Poole, 2010), and in order to investigate DOM composition across a gradient of streams and rivers. Although DOM in the Congo Basin is fairly dominated by terrestrial inputs of aromatic DOM, the loadings plot along PC1 indicates a transition in the dominant DOM composition from aromatic (%C3, SUVA<sub>254</sub>) to aliphatic (%C2, %C6, FI) compounds (Fig. 6). It is noteworthy that similar gradients have recently been reported in Swedish lakes (Kellerman et al., 2015) and 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), while 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 influencing the transition from an aromatic to aliphatic dominant composition.

**4.2.1. Losses of aromatic DOM through photodegradation and biological activity as producer of aliphatic DOM.** The role of photodegradation in removing the aromatic fraction of DOM in the Congo Basin, strongly suggested by the PCA analysis as well as by the changes in DOM composition along the mainstem between HW and FW, is also

supported by patterns in C5. Indeed, %C5 was inversely related to %C3 (Fig. 7a), suggesting that C5 tracks molecules directly produced during the photodegradation of terrestrial aromatic molecules as previously demonstrated in boreal freshwaters (Lapierre and del Giorgio, 2014). In addition, %C5 was inversely related with 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. 7b). Given that C5 is associated with molecules of low MW and aromaticity (Stubbins et al., 2014), this observation is consistent with experimental results showing that the aromatic and high MW fraction of terrestrial DOM can be photochemically converted into more labile substances of lower MW that in turn can 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 of labile DOM. The higher concentrations of chlorophyll-a in the mainstem compared to tributaries (Table 1) suggest that this source could be phytoplanktonic exudates that have been shown to be very labile and rapidly assimilated by bacteria (Baines and Pace, 1991; Morana et al., 2014).

The dual role of microorganisms as consumers of terrigenous aromatic DOM and producers of novel compounds has recently been emphasized (Guillemette and del Giorgio, 2012; Ward et al., 2013; Fasching et al., 2014) and could explain the gain in aliphatic DOM in the Congo River. Components C2 and C6 – associated with aliphatic molecules (Table 2) – exhibited systematically higher contributions in the mainstem compared to tributaries, implying a permanent internal production. However, none of these components were correlated to chlorophyll-a concentrations (data not shown), suggesting that the phytoplankton primary production in the Congo basin was not

controlling their distribution. On the other hand, several studies carried out in a wide variety of aquatic ecosystems have attributed the origin of components like C2 and C6 to the biological degradation of terrestrial DOM within the aquatic ecosystems (Stedmon et al., 2003; Yamshita et al., 2010; Walker et al., 2013; Fasching et al., 2014; Kellerman et al., 2015). Therefore, microbial reworking of terrestrial DOM during its transport could lead to the production of these components, an assumption supported by higher FI values in the mainstem inferring greater inputs of microbially derived DOM relative to tributaries (McKnight et al., 2001).

Our results contrast with previous investigations based on lignin biomarkers suggesting that DOM transformation in the Congo basin is 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). The  $^{13}\text{C}$  signatures of POC collected during our field campaigns spanned a much wider range but were poorly correlated with  $^{13}\text{C}_{\text{DOC}}$  ( $R^2 = 0.20$ ,  $p < 0.0001$ ;  $n = 158$ , data not shown), suggesting limited exchange between these pools. However, our results are in line with previous field studies showing that downstream changes in DOM composition can be largely controlled by photodegradation (Massicotte and Frenette, 2011; Cawley et al., 2012).

**4.2.2. Role of external drivers on the aromatic towards aliphatic transition.** The distribution of data in the PCA (Fig. 6) shows that (1) DOM in the mainstem is enriched in the aliphatic fraction compared to its tributaries and (2) the distribution of tributaries for a given Strahler order is strongly heterogeneous. This highlights the combined effects of landscape morphology and water chemistry that can influence the downstream transformation of DOM independently of the size of the streams/rivers. Thus, land cover – here mostly forest and savannah – has a strong influence on the composition of DOM

released in aquatic ecosystems (Mann et al., 2014; Lambert et al., 2015), and 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. 6). 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 large quantities of aromatic DOM in inland waters (Battin et al., 1998; Hanley et al., 2013; Lambert et al., 2016) and thus obscuring variations in DOM occurring upstream. A multi-year monitoring illustrates for example that DOM in the Oubangui River at Bangui (i.e. upstream the Cuvette Centrale, Fig. 1) experiences significant changes in composition between rising waters (June, DOM has few aromatic structures) and high waters (December, DOM is highly aromatic; Bouillon et al., 2014) while our data report highly aromatic DOM during both periods as the river becomes enriched in organic material from the flooded forest (Laraque et al., 2009). Finally, the positive correlations between %C2 and pH and between %C6 and pH (Fig. 8) advocate for a limited degree of bacterial degradation of DOM in very acidic environments as previously suggested by Borges et al. (2015a) based on the CO<sub>2</sub> and DOC relationship in the Congo Basin. 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. 7).

**4.3. The chemostat hypothesis and the pulse-shunt concept.** The chemostat hypothesis suggests a decrease in DOC concentrations and a convergence in DOM composition towards lower aromaticity with increasing stream order due to the increasing influence of in-stream processes that overwhelm terrestrial inputs from headwater catchments (Creed et al., 2015). The shift from a predominantly terrestrial influence to

biogeochemical processing – assessed by the variation of  $SUVA_{254}$  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  $SUVA_{254}$  associated with a decrease with DOC concentrations was only found to occur from six to eight 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 the DOM biogeochemistry in the Congo Basin. Although the seasonal variations of water level are modest in the Congo and that the Cuvette Centrale does not flush as floodplain lakes in the Amazon (e.g. Moreira-Turcq et al., 2003), the flooded forest is drained by several large rivers (Ruki, Sangha) that bring large amounts of water and DOM to the mainstem (The Ruki is the second tributary after the Kasai). This falls in line with the “flood pulse concept” of Junk et al. (1989) that highlights the critical importance of the river-floodplain connectivity in lowland tropical rivers (Battin, 1998; Zurbrügg et al., 2013; Lambert et al., 2016), while the chemostat hypothesis builds on the river continuum concept (Vannote et al. 1980) that is typically applicable to rivers at temperate latitudes and devoid of large wetlands. Patterns of other data acquired during the same cruises such as  $CO_2$  and  $CH_4$  also showed the influence of inputs of these quantities from the Cuvette Centrale (Borges et al. 2015a). Also, an increase in DOM content and aromaticity was found to occur in nine order streams, reflecting the fact that DOM-rich waters from the Cuvette Centrale can travel along the ridge of the Congo River without completely mixing with the central water masses of the mainstem (Supplementary Figure 5).

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, yet apparently sufficient to significantly impact the degree of 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 Hemispheres with more contrasting hydrological regimes, as recently shown 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 and by additional incubation experiments.

### **Data availability**

The digital elevation model HydroSHEDS (Lehner et al., 2008) is available at <http://hydrosheds.cr.usgs.gov/index.php>. The Global Lakes and Wetlands database (Lehner and Döll, 2004) is available at <http://www.worldwildlife.org/pages/global-lakes-and-wetlands-database>. The Global Land Cover 2009 database is available at <http://landcover.usgs.gov/landcoverdata.php>.

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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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### 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, and corresponding water height at the gauging station at (a) Kisangani for the period 2013-2016, and (b) Kinshasa 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,  $^{13}\text{C}_{\text{DOC}}$ ,  $\text{SUVA}_{254}$ ,  $S_{\text{R}}$  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 ( $\text{SUVA}_{254}$ ) 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 (%O<sub>2</sub>), pH, Secchi depth, vertical light attenuation coefficient (K<sub>d</sub>), total suspended matter (TSM) and Chlorophyll-a (Chla) concentrations (data from Descy et al. 2016).

Period	n	%O <sub>2</sub> (%)	pH	Secchi (cm)	K <sub>d</sub> (m <sup>-1</sup> )	TSM (mg L <sup>-1</sup> )	Chla (µg L <sup>-1</sup> )
<b>Maximum high waters</b>							
Mainstream	35	60.3±10.6 (48.4-89.2)	6.46±0.22 (6.07-6.92)	54.6±15.6 (25-80)	1.5±0.4 (1.0-2.8)	29.4±21.9 (14.0-99.8)	0.84±0.42 (0.10-1.76)
Major tributaries	13	54.3±33.3 (8.6-111.3)	5.67±1.09 (3.91-6.87)	79.5±60.7 (25-250)	1.5±0.6 (0.4-2.5)	12.5±13.3 (0.74-44.4)	0.54±1.02 (0.01-3.57)
Minor tributaries	26	27.9±30.2 (4.2-99.8)	5.33±0.75 (3.91-6.17)	86.2±29.7 (15-140)	1.5±0.5 (0.9-2.8)	7.7±13.4 (1.7-71.4)	0.35±0.42 (0-1.85)
<b>Falling waters after second peak water discharge</b>							
Mainstream	34	84.8±7.4 (54.2-93.4)	6.82±0.32 (6.08-7.38)	46.8±5.7 (35-62)	3.9±0.6 (1.5-4.6)	31.9±9.1 (4.0-45.4)	3.99±1.54 (1.13-7.68)
Major tributaries	12	62.1±31.2 (0.3-98.2)	5.77±1.22 (3.63-7.05)	66.7±23.4 (35-106)	3.3±0.7 (2.4-5.1)	14.4±12.5 (0.73-43.0)	1.65±2.27 (0.02-6.39)
Minor tributaries	41	37.8±35.6 (0.3-103.0)	4.56±0.77 (3.6-6.1)	80.7±42.2 (38-205)	3.3±0.9 (1.5-5.2)	6.1±6.5 (0.5-34.8)	0.55±0.99 (0.01-5.12)

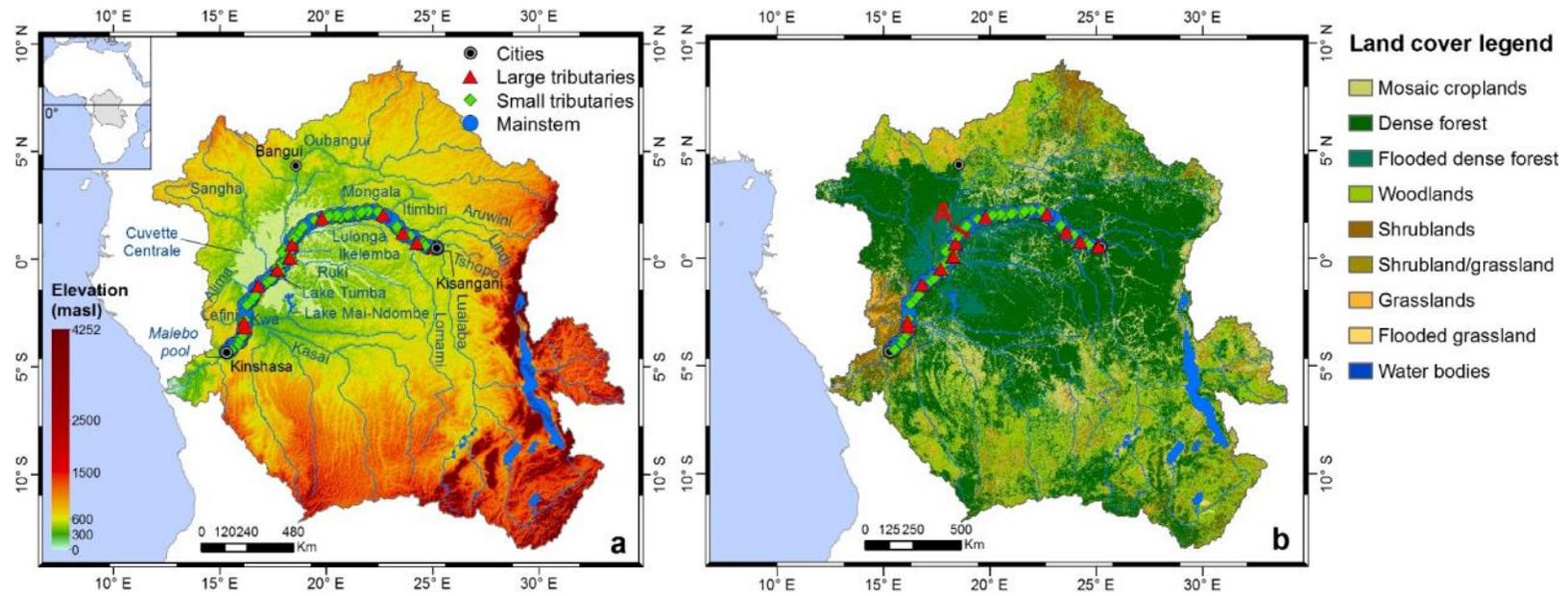
**Table 2** – Spectral properties (excitation and emission maxima ( $Ex_{max}/Em_{max}$ )) of the six components identified using PARAFAC modelling, correspondence with peak classification, general assignment and comparison with previously identified components in different environments, dominant molecular 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.

Component	$Ex_{max}$ (nm)	$Em_{max}$ (nm)	Peak(s) name <sup>1</sup>	General assignment <sup>2</sup>	Comparison with others environments							Dominant molecular association <sup>4,6,10</sup>	Potential sources and reactivity*
					Arctic Rivers <sup>3</sup>	St Lawrence River <sup>4</sup>	Boreal streams <sup>5,6</sup>	Boreal lakes <sup>7,8</sup>	Tropical wetland <sup>9,10</sup>	Zambezi basin <sup>11</sup>	Tropical wetland <sup>12</sup>		
C1	<260 (375)	488	A+/C+	Terrestrial humic-like	C3	C3	C2	C3	C5	C2	C2	High aromaticity, high MW	T <sup>1-12</sup> , P <sup>-5</sup>
C2	305 (<260)	414	M	Microbial humic-like	—	C7	C5	C2	C4	C3	C3	Aliphatic, low MW	M <sup>+8-10</sup>
C3	330 (<260)	444	A <sub>c</sub> /C	Terrestrial humic-like	C1	C2	C3	C1	C6	C1	C1	High aromaticity, high MW	T <sup>1-12</sup> , P <sup>-5,9,10</sup>
C4	<260	444	A <sub>c</sub>	Terrestrial humic-like	—	C1	—	C5	C2	C4	—	Aromatic, intermediate MW	T <sup>9-11</sup> , P <sup>+4</sup> , Pr <sup>10</sup>
C5	350	424	C	Humic-like	—	—	C4	—	—	—	—	Low aromaticity, low MW	P <sup>+5</sup>
C6	275	350	B/T	Tryptophan- like	C5	C4	C6	C6	C7	C5	C4	Aliphatic, low MW	Au <sup>4,9,11</sup> , M <sup>+3,7</sup> , M <sup>-5</sup>

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

\*Au: autochthonous production; T: terrestrial inputs; M+: microbial degradation; M-: biolabile; P+: photoproduct; Pr: photo-resistant; P-: photosensible

**Figure 1**



**Figure 2**

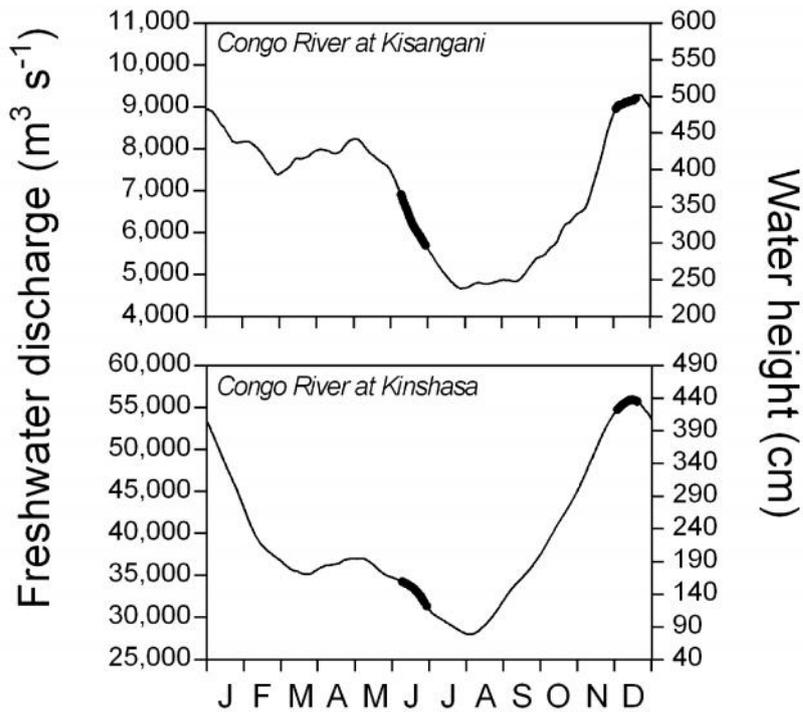


Figure 3

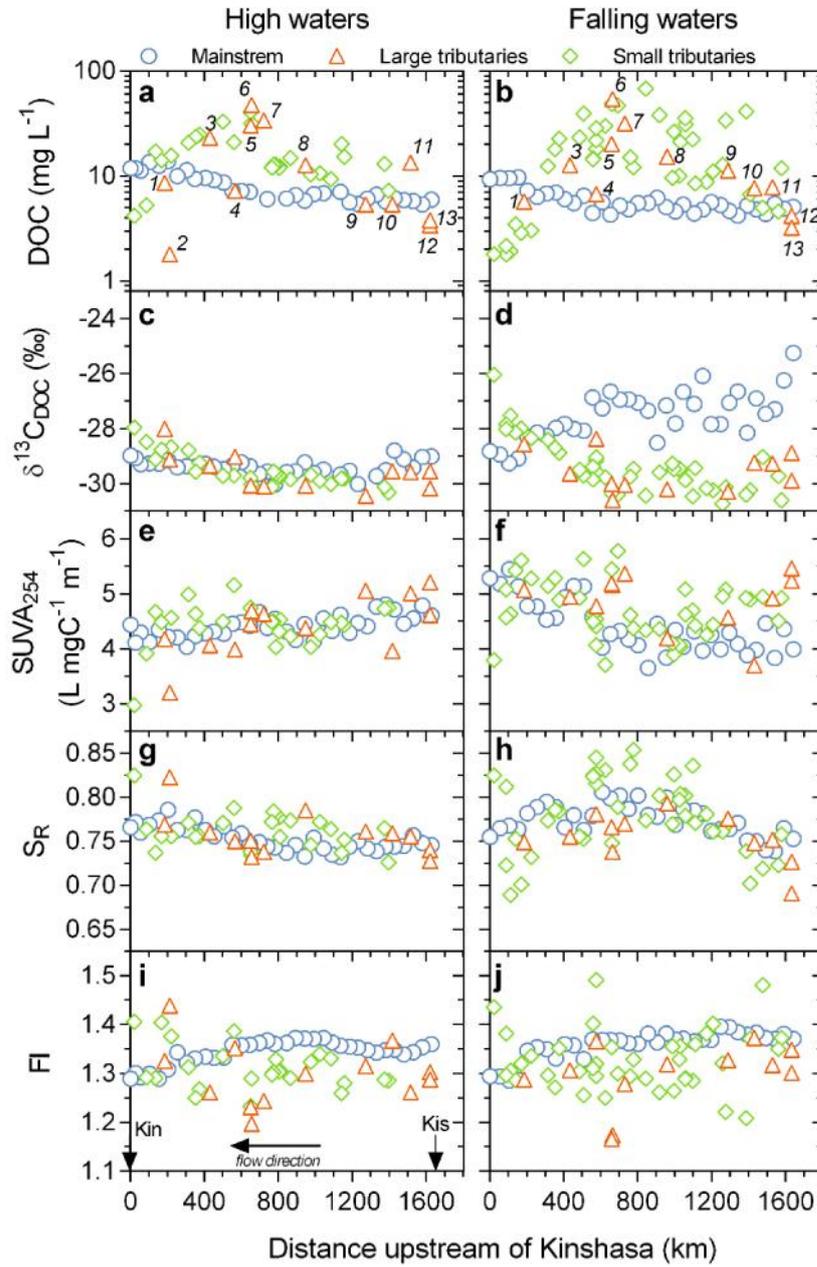


Figure 4

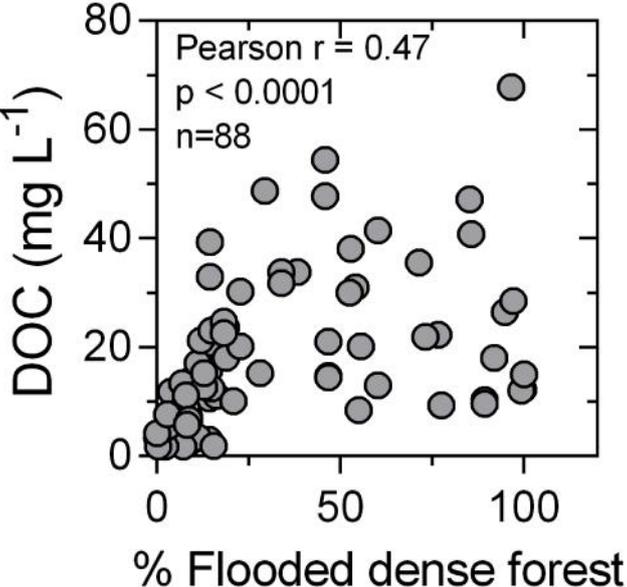


Figure 5

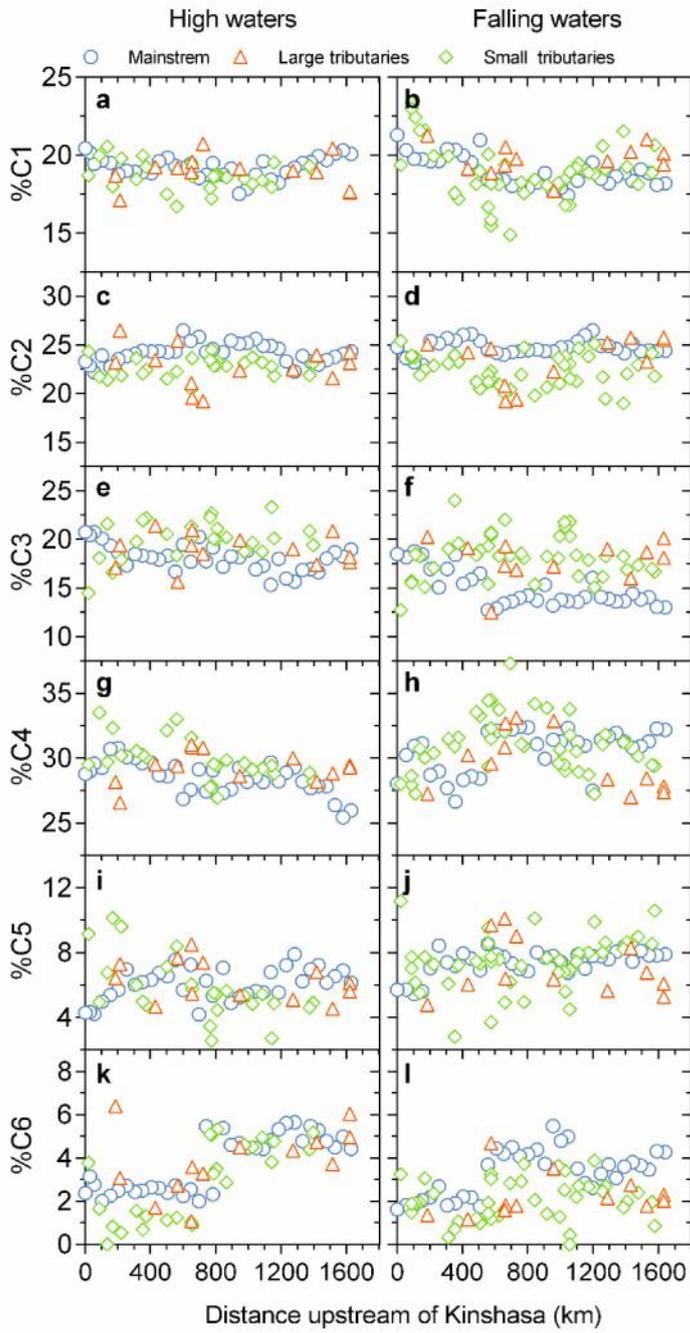


Figure 6

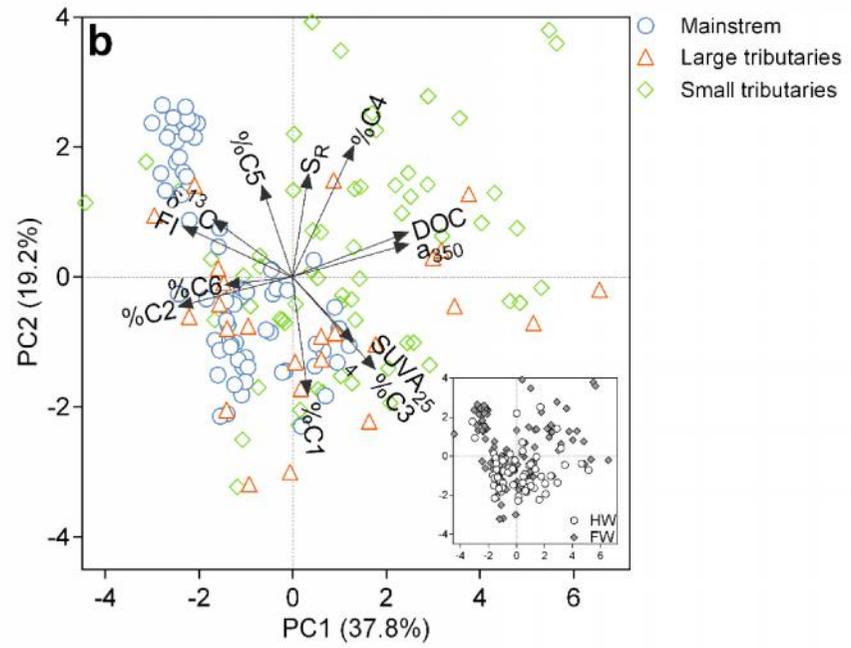
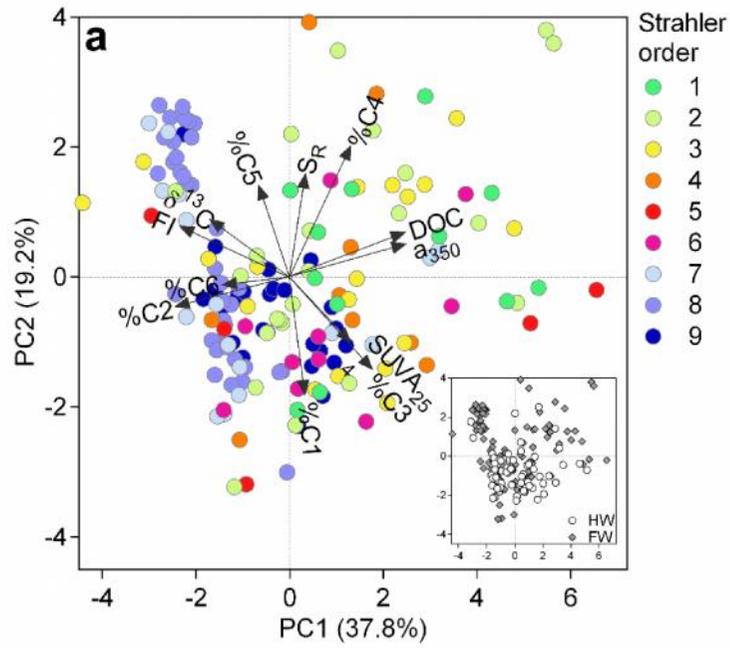


Figure 7

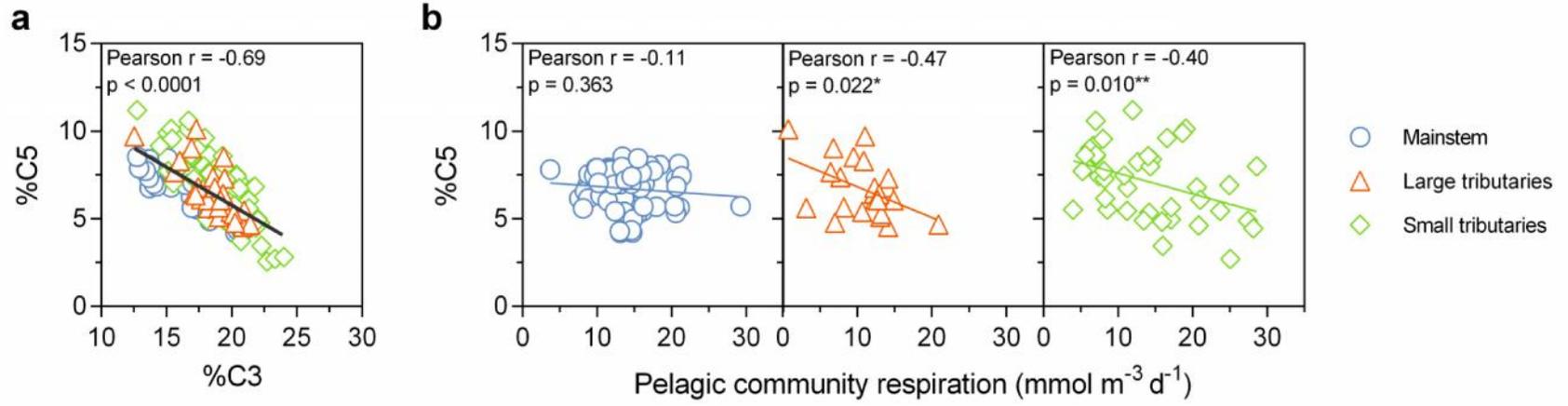


Figure 8

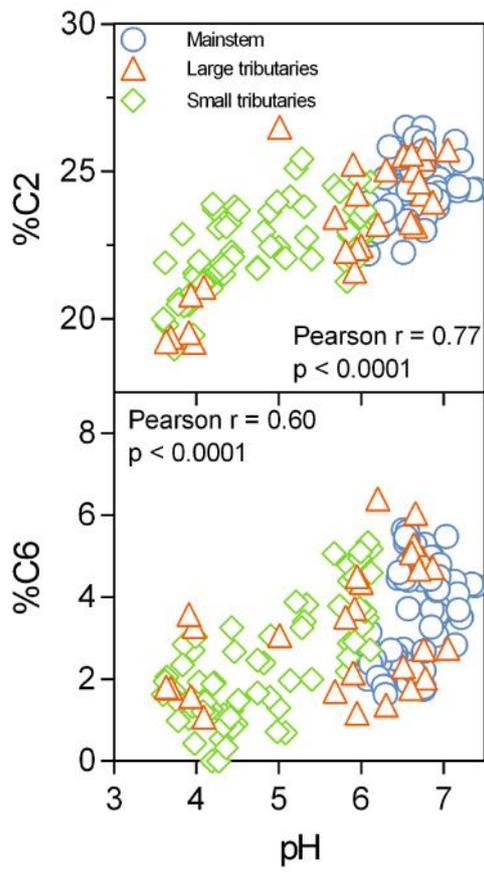


Figure 9

