Author response to Anonymous Referee #1

The submitted MS presents a detailed analysis of transport and transformation of DOM along the main stem of the Zambesi and its largest tributary. A particular focus is put on the effects of floodplains/wetlands and reservoirs as well as low-flow vs high flow conditions on the longitudinal patterns in DOM concentration and composition. It is the first study to present such a detailed analysis for a whole, large river system, and in particular for a tropical river other than the Amazon. Thus, the subject of the study will be of interest for the readership of Biogeosciences. Methods and results are presented in a clear, comprehensive way. The discussion features a satisfying review of the literature and compares results of this study to the state of the art in that field. The manuscript is well written and tables and figures are mainly of good quality. I suggest publication of the MS after minor revisions.

- Reply: We thank the reviewer for the positive evaluation of our manuscript and for his/her comments and suggestions.

Major comment:

In addition to spectral properties, the authors measured delta13C of the DOM. They present the results, but they do not interpret and discuss the values. I suggest that the authors include a short interpretation of these delta13C values based on an isotopic mixing model to estimate the proportions of different terrestrial and autochtonous sources.

- Reply: We have added in the revised manuscript a new section that focuses more in depth on the results of δ^{13}C of DOC (section 4.2 in the revised manuscript). First, we compared our data with previously published data from other African tropical rivers. Secondly, we discussed the possible reasons leading to the increase of values along the Zambezi mainstem. Based on the lack of marked ^{13}C-depletion DOC in the reservoirs, we suggest that phytoplankton production has little effect on the δ^{13}C of DOC and that the increased in $\delta^{13}C_{DOC}$ is to a large extent due to increased contribution from C_4 vegetation. Finally, we performed a mass balance calculation to estimate the relative contribution of C3 and C4 plants on the DOM pool in the Zambezi basin. End-members values were fixed at -27.1 ‰ for C3 plants and -12.1 ‰ for C4 plants. The value of -27.1 ‰ was calculated in a geographical information system (ArcGIS), based on the equation of Kohn (2010) that estimates the δ^{13}C signature of C3 vegetation based on mean annual precipitation, altitude and latitude. Available and public datasets for annual rainfall (Hijmans et al., 2005) and digital elevation model (HydroSHEDs) were used. The value of -12.1 ‰ was chosen based on a study conducted in the Tana River basin (Kenya) which presents similar shift in vegetation cover (Tamooh et al., 2012).
We have also added another supplementary figure that shows the spatial variability of the estimated δ^{13}C signature of C3 plants in the Zambezi basin. Also, the first paragraph of the section 4.3.1 (previously 4.2.1) has been slightly modified in order to avoid repetition with the previous section.

General comments

L100: Maybe I am wrong, but wouldn't that rather be a unimodal distribution? Bimodal would mean that there is a second maximum. Is there a second, smaller maximum? If yes, please clarify.

- Reply: Indeed it is a unimodal distribution. The text has been corrected.

L114-118 & L121-123: Please, give a reference for these values (volumes and surface areas).

- Reply: We have added references for each reservoir.

L150-151: Please, replace 'most cases' by a number of cases or the percentage. Or report e.g. the 95th percentile of the reproducibility.

- Reply: The percentage of samples with a reproducibility higher than 5% for DOC and 2% for $\delta^{13}C_{DOC}$ was lower than 5%. This precision has been added in the revised manuscript.

Section 2.6: You should start this section with one to two sentences explaining what the aim of this PCA is.

- Reply: This has been made.

Section 3.1: When you describe the longitudinal and seasonal patterns of all these indices, you should shortly repeat what each of these indices indicates. That would increase the comprehensibility for the broader readership. That is in particular true for the delta13C values. Here, you should maybe cite some typical end-member values.

- Reply: This has been made.

L350-351: Where do you show the correlation between dominant land cover and DOM gradients?

- Reply: In fact the effect of land cover and DOM gradient is discussed just below, in the section 4.3.1. In order to make the manuscript clearer, this sentence has been removed and we reworked the paragraph 4.3.

L355: You should discuss the delta13C values. What does a low delta13C indicate? What are the endmembers?

- Reply: This comment has been been addressed by adding the new section 4.2, see also our reply to earlier comments above.

Figure 3: Overall, the figures are of a very good quality. However, in Figure 3, at least when printed, it is hard to distinguish between the numbers I, II, III.

- Reply: We appreciate this comment. The figure 3 has been modified.

Author response to Anonymous Referee #2

Comments to Author
Summary: In this manuscript the authors present new DOC and DOM composition data from one of the World's largest tropical rivers: the Zambezi River. Samples were collected during both dry and wet seasons and along the river and one of its tributaries. The results indicated clear seasonal differences in sources and processing of DOM as well as down-river shifts in concentrations and composition. "Humic"-like DOM dominated in headwaters close to forests and at wet conditions when wetlands were dominating sources of DOM. In contrast, at dry conditions the DOM composition shifted towards more aquatically produced, or influenced, material. The authors claim that these differences are primarily driven by shifts in discharge, which influences connectivity with e.g. wetlands, and water residence times. As has been noted before, the effect of reservoirs or lakes have a particularly significant role in increasing water residence times and thereby DOM composition and concentrations.

Contributions: Although the patterns presented and conclusions drawn are not revolutionary, they are indeed important since this type of data from tropical rivers is rare. In addition, the results largely confirm previous interpretations of DOM dynamics in boreal and temperate areas. This is interesting since it suggests that, although the details may differ (e.g. microbial community composition), the large-scale governing processes and functioning are similar across biomes. The manuscript was a pleasure to read. After having reviewed several poorly written manuscripts recently, it was a joy to see a well written and logically organized text. Still, I do have some minor remarks detailed in a number of general and technical comments below.

General comments:
-The description of some of the methodology requires additions and clarifications.

-The use of some terminology is confusing (not uncommon when it comes to this type of terminology) and I suggest clarification. One clear example is the apparent dichotomy between terrestrial and microbial, which is clearly misleading since substantial portions of DOM may be of terrestrial microbial origin.

-The relationships between DOM properties and landscape characteristics is interesting, but presented in the Discussion section. I suggest the authors add a paragraph or two about these results in the Results section.

Altogether, this manuscript is a valuable addition to the scientific field and I support its publication in Biogeosciences. The science is as far as I can tell sound and well communicated. I recommend minor revisions of the manuscript before the editor considers publication of the manuscript.

- Reply: We thank the reviewer for the positive evaluation of our manuscript and for his/her comments and suggestions. We are also grateful to the reviewer for his/her numerous corrections and suggestions for improving the readability of the manuscript.

Technical comments:

Abstract (why no line numbers in the abstract?)

- Reply: We made an error during the upload process.

Line 13-14: You write "terrestrial DOM dynamics shifted from transport-dominated during the wet seasons towards degradation". I don't think this terminology matches; what do you mean "towards degradation"? Do you mean that it shifted to a state dominated by in-stream processing?

- Reply: What we meant is that during high flow periods, the downstream transport of DOM dominates relative to degradation because of higher water velocities (i.e. lower water residence time). The situation is inversed during low flow periods because decreasing water velocities enhances the degradation of DOM during its transport. The sentence has been modified in order to clarify this point: "Thus, high water discharge promotes the transport of terrestrial DOM downstream instead of its degradation while low water discharge allows the degradation of DOM during its transport.".

Introduction
Line 41: This is only partly true. Sure, DOM composition controls reactivity but there are other factors that may be equally important. You identify one: water residence times. However, there are others as well, see e.g. Marín-Spiotta, E., K. E. Gruley, J. Crawford, E. E. Atkinson, J. R. Miesel, S. Greene, C. Cardona-Correa, and R. G. M. Spencer (2014), Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries, Biogeochemistry, 117(2-3), 279-297, doi: 10.1007/s10533-013-9949-7.

- Reply: We agree with this comment. The text has been modified to make this clarification, and the reference of Marín-Spiotta et al., (2014) has been added.

Line 71: ultraviolet

- Reply: This has been corrected.

Line 74-75: I know this terminology is common, but it is rather misleading, which I often point out. Terrestrial vs. microbial is not a dichotomy. On the contrary, much DOM from the terrestrial environment is of microbial origin. I think it is better to call them terrestrial and aquatic inputs.

- Reply: We have modified the sentence as follow: “terrestrial versus aquatic microbial inputs”.

Line 86-88: I don’t know if the use of prepositions is correct here. I suggest changing to “…drivers of downstream patterns in DOM at the scale of a large tropical river, with a specific attention to the…”

- Reply: This has been corrected.

Materials and methods

Line 91: northwestern Zambia

- Reply: This has been corrected.

Line 100: If it is a single peak it is not bimodal. A bimodal distribution has two peaks.

- Reply: Indeed the hydrological regime of the Zambezi is unimodal. This has been corrected.

Line 103: I suggest changing the comma to a semi-colon: …whole catchment; forests (20%)…”

- Reply: This has been corrected.

Line 128-130: I suggest you move the year before the parentheses. Now you interrupt “the flow”. So e.g. “…wet season 2013 (6 January to 21 March, n = 41) and dry season 2012 (…”

- Reply: This has been corrected.

Line 140: what do you mean by “conditioned”?

- Reply: This means the preservation of the samples for the different analyses, e.g. the addition of H_3PO_4 for samples for DOC measurements. The text has been modified for clarity.

Line 141: Did you use any blanks? I am always suspicious when filters made by organic compounds are used for DOM analyses.

- Reply: No blanks were used on the field. However, the filters were rinsed with at least 100 ml prefiltered sample water (which was collected for analysis of total alkalinity) before collection of the DOC samples in order to “flush” the potential amount of DOC present in filters.

Line 148: Were the DOM samples kept cold during sampling and transport? Due to logistical reasons I guess not (and you added phosphoric acid) but could be worth noting. Any potential effects of this sample handling? In addition, where were the analyses (concentrations, isotopes, FDOM, CDOM) performed? In Belgium?

- Reply: The DOM samples were processed within 10 minutes of water collection and sampling bottles were kept away from direct sunlight. It was not possible to keep the samples d cold uring the transport to Belgium where the analysis were performed. However, the filtration through 0.2 µm, the addition of H3PO4, and storage in the dark should guarantee good preservation of DOM concentration and composition during the storage. This has been verified previously on samples from the Oubangui River, analyzed immediately after fieldwork and after several months of dark storage at room temperature (Bouillon et al., 2014); and also clearly illustrated when comparing CDOM properties for the Zambezi sample set: the same sample analyzed upon return in Belgium (red line) and after 3 months of storage at room temperature (blue line) give identical results (see figure 1), with differences in optical values (a350, SUVA ad SR) less than 3%.

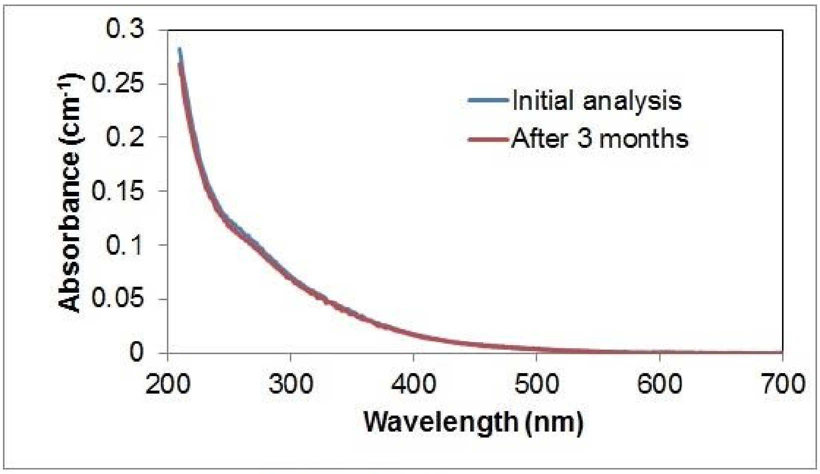

FIGURE 1

Line 151: Do these uncertainty bounds include both accuracy and precision? Relative which standard are carbon isotope values reported?

- Reply: The uncertainty bounds correspond to precision, the word "reproducibility" was replaced by "precision". Text now reads: Quantification and calibration was performed with series of standards prepared in different concentrations, using both IAEA-C6 ($\delta^{13}C$ = -10.4 ‰) and in-house sucrose standards ($\delta^{13}C$=-26.99 ‰). All data are reported in the δ notation relative to VPDB (Vienna Pee Dee Belemnite)"

Line 171-173: Again a somewhat confusing terminology. Is there a dichotomy between aromatic and hydrophobic? Is it aromatic vs. aliphatic?

- Reply: The text has been modified for clarity: "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 more aliphatic compounds (Weishaar et al., 2003)."

Line 172: "…indicative of the presence…"

- Reply: This has been corrected.

Line 193: Should this be "Raman units"?

- Reply: Indeed. This has been corrected.

Line 196: "The PARAFAC model was using…"

- Reply: This has been corrected: "PARAFAC model was build using…"

Line 197: This is repetitious so I suggest adding "Furthermore, the PARAFAC…"

- Reply: The sentence has been reworked to avoid repetition: "Validation of the PARAFAC model was performed by split-half analysis and random initialization"

Line 200: "…a two-year monitoring…"

- Reply: This has been corrected.

Line 210-211: Here is terrestrial vs. microbial again. I suggest changing this terminology.

- Reply: We added the term "aquatic microbial DOM" in the revised version.

Line 214: Define PCA

- Reply: This has been done.

Results

Line 223-224: "…one dry season; the two wet seasons' data…"?

- Reply: We have made two distinct sentences in the revised manuscript.

Line 226: "…during the dry period…"

- Reply: This has been corrected.

Line 262-263: Remove "a" before "maximum" and "minimum"

- Reply: This has been corrected.

Line 266: “globally” seems strange here

- Reply: We have replaced “globally” by “generally”.

Line 267: I guess this should read “except”

- Reply: Yes. This has been corrected.

Line 277-278: Here is terrestrial vs. microbial again. “aquatic microbial” would be fine

- Reply: This has been corrected.

Line 283: I found “corresponding river sections” unclear. Could you clarify?

- Reply: The “corresponding river sections” refer to the sections of the river that crosses wetlands and floodplains. We modified the text in order make this sentence clearer: “FMax of the C4 component presented the higher percentage of increase compared to the other component in river sections flowing through wetlands/floodplains in the upper and lower Zambezi.”

Line 288: “as” seems out of place here. Perhaps “…downstream concurrent with DOC concentrations…”

- Reply: This has been corrected according to the suggestion of the reviewer.

Line 318: Do you mean “all samples during the dry season”? I found this unclear.

- Reply: We referred only to the samples collected in the middle and lower Zambezi. The sentence has been modified: “Samples collected in the middle and lower Zambezi during both the wet and dry seasons…”

Line 319: what other variables?

- Reply: The other variables are those used in the PCA, i.e. the DOM concentration (DOC concentration) and composition (isotopic and optical proxies). The sentence has been modified: “…defined by PARAFAC components and DOM concentration and composition.”.

Discussion

Line 328: Do you mean “conversely” instead of “inversely”?

- Reply: Yes. The sentence has been modified.

Line 340-343: Perhaps, but from the figure it looks like C1, C2 and C3 are more related to PC2.

- Reply: We agree that C1 and C2 seem to be opposite to C3 along the PC2 also. However, we found that C1 and C2 are much opposed to $\delta^{13}C_{DOC}$ along this axis. Considering the effect of the vegetation gradient on $\delta^{13}C_{DOC}$ values (see the new section 4.2) and the fact that C1 and C2 are highest at the source of the Zambezi, this suggests that changes in land cover control the distribution of samples along PC2. We have added 2 sentences at the end of the section 4.1 to discuss this point.

Line 348: “in” instead of “of”?

- Reply: Yes. The sentence has been modified.

Line 356-357: “…in the northern part of the basin at the headwaters of the Zambezi to grasslands…”

- Reply: Following the major comment of the other reviewer regarding the lack of interpretation for $\delta^{13}C_{DOC}$ values, a new section has been added where we discussed about the transition of land cover along the Zambezi River. This sentence has been removed in order to avoid repetition with the new section.

Line 370-371: Aren’t these results and should therefore be presented in the Results section?

- Reply: We have moved this figure in the Results section as suggested. We have also added a sentence in the text to introduce this figure. The numbering of figures 4-8 have been checked in the revised manuscript.

Line 393: This only applies to water residence times, not necessarily solute residence times since they are dependent on vertical fluxes and in-stream recycling as well.

- Reply: Indeed. We have made the correction.

Line 397: Why only photodegradation? This should also include microbial degradation.

- Reply: According to the literature and personal unpublished experimental data, the preferential losses of a_{350} compared to DOC associated with a decrease in $SUVA_{254}$ and increase in S_R values are the typical expression of losses of DOM by photodegradation. Even if microbial degradation is capable of degrading aromatic compounds of terrestrial DOM, this degradation pathway is not expected to have a similar impact on DOM composition. Please note however that the microbial degradation of DOM is also taken into account in the next sentence.

Line 403-404: “… (1) increasing water levels mobilizes a greater proportion of terrestrial DOM and (2) higher water velocities…”

- Reply: This has been corrected.

Line 409: What does “in which” refer to? I found this sentence unclear.

- Reply: We replaced “in which” by “where” and moved “independently of water level fluctuations” at the end of the sentence.

Line 420-422: Is it more likely that this is due to macrophytes than to algae? What about CO2 evasion?

- Reply: We are not able to estimate precisely the role of macrophytes or algae due to the lack of adequate measurements. Therefore, we have modified the sentence in the revised manuscript to include a potential effect of algae. We have also provided more details regarding the difference between CO2 concentrations in reservoirs and rivers. The modified sentence is “The level of fluorescence of C5 could be additionally sustained by the FDOM from primary producers such as macrophytes (Lapierre and Frenette, 2009) or phytoplankton (Yamashita et al., 2008), that also lead to low values of the partial pressure of CO2 below atmospheric equilibrium in the Kariba and Cahora Bassa reservoirs while rivers (i.e., excluding reservoirs) displayed CO2 supersaturated conditions with respect to atmospheric equilibrium (Teodoru et al., 2015).”.

Line 434-436: This agrees with work in temperate/boreal systems, see e.g. Winterdahl, M., M. Erlandsson, M. N. Futter, G. A. Weyhenmeyer, and K. Bishop (2014), Intra-annual variability of organic carbon concentrations in running waters: Drivers along a climatic gradient, Global Biogeochemical Cycles, 28(4), 451-464, doi:10.1002/2013GB004770.

- Reply: This reference has been added. “The role of lakes/reservoirs in lowering the seasonality of DOC in river network has also been evidenced in temperate and boreal streams and rivers in Sweden (Winterdahl et al., 2014).”

Line 437: According to Table 2 this is a 1.5 year long monitoring.

- Reply: In fact, it is a 21 month long monitoring. We have modified the text as follow: “…data from an almost two-year monitoring”, and also in the Material and Methods section.

Line 446-448: This is interesting! Could you then estimate the loss/production of C in the reservoir by using CO2 and CH4 data?

- Reply: We thank the reviewer for the valuable suggestion. Please note that in the reservoirs CO2 (and CH4) will be also affected by phytoplanktonic primary production as testified by the reported under-saturation of CO2 (Teodoru et al. 2015). This will complicate any mass balance budgets, and the available data does not allow us to investigate this, since the aim of the project was to describe the biogeochemistry in all aquatic compartments, not addressing in detail the C processing rates in specific environments, as reservoirs.

Line 450: “…sources to sinks…”
Reply: This has been modified.

Line 461-462: See also Fiebig et al. (1990), Dosskey & Bertsch (1994) or Hinton et al. (1998). Fiebig, D. M., M. A. Lock, and C. Neal (1990), Soil water in the riparian zone as a source of carbon for a headwater stream, Journal of Hydrology, 116(1-4), 217-237
Dosskey, M. G., and P. M. Bertsch (1994), Forest sources and pathways of organic matter transport to a blackwater stream: a hydrologic approach, Biogeochemistry, 24(1), 1-19.
Hinton, M. J., S. L. Schiff, and M. C. English (1998), Sources and flowpaths of dissolved organic carbon during storms in two forested watersheds of the Precambrian Shield, Biogeochemistry, 41(2), 175-197

- Reply: All these references have been included in the revised manuscript.

Line 465-466: There are several references for this; the Winterdahl et al. (2014) paper referred to above is another.

- Reply: We have included this reference.

Figure captions

Line 728: “…upstream of their…”

- Reply: This has been modified.

Line 746: Remove “wet”

- Reply: This has been modified.

Line 754: This is really exports. Fluxes are technically export per unit area.

- Reply: This has been modified.

Line 755-756: “…exports at the same location between wet and dry seasons.”

- Reply: This has been modified.

Table 1: Very interesting!

- Reply: Thank you!

Table 2 Line 763: “…during a one and a half year monthly…”

- Reply: We have modified as follow: “…during an almost two-year monthly sampling”.

Figure 7: Are these all sites? The number of sites in the Zambezi River seems few compared to other figures. Is this a selection of sites? If so, based on what?

- Reply: This relationship has been obtained by considering only samples collected during the wet periods, i.e. when the hydrological connectivity between the mainstem rivers and wetlands are strong. Also, for the Zambezi, only the samples collected in the upper part of the basin have been considered due to the effect of the Kariba and Cahora Bassa reservoirs on the longitudinal pattern of DOC concentrations. These points have been added in the caption as well as in the text (Results section).

Figure 8: This is rather DOC export. Flux is export per unit area.

- Reply: The figure has been modified.

Relevant changes made in the manuscript are:

- The adding of a new section 4.2 in which we discussed more in depth the variability of $\delta 13C_{DOC}$ within the Zambezi basin (Reviewer 1). In this new section, we estimated the relative contribution of C3 and C4 plants to the DOM pool, and also discussed about the role of vegetation cover in controlling the gradient of $\delta 13C_{DOC}$ in the Zambezi River. This leads to slight changes in the first and second paragraph of the 4.3 section in order to avoid repetition in the text.
- Clarifications required by the reviewer 2 regarding the methods used in our study (sampling, storage, accuracy of measures,…).
- Clarifications suggested by the reviewer 2 regarding some points of terminology, especially terrestrial *versus* aquatic microbial inputs.
- The move and presentation in the Results section of the figure showing the relationship between DOC concentrations and wetland extents in the Zambezi Basin (reviewer 2), leading to a new numbering of our figures in the revised version.

Marked-up manuscript version

Along-stream transport and transformation of dissolved organic matter in a large tropical river

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Abstract - Large rivers transport considerable amounts of terrestrial dissolved organic matter (DOM) to the ocean. However, downstream gradients and temporal variability in DOM fluxes and characteristics are poorly studied at the scale of large river basins, especially in tropical areas. Here, we report longitudinal patterns in DOM content and composition based on absorbance and fluorescence measurements along the Zambezi River and its main tributary, the Kafue River, during two hydrological seasons. During high

flow periods, a greater proportion of aromatic and humic DOM was mobilized along rivers due to the hydrological connectivity with wetlands, while low flow periods were characterized by lower DOM content of less aromaticity resulting from loss of connectivity with wetlands, more efficient degradation of terrestrial DOM and enhanced autochthonous productivity. Changes in water residence time due to contrasting water discharge were found to modulate the fate of DOM along the river continuum. Thus, high water discharge promotes the transport of terrestrial DOM downstream relative to its degradation while low water discharge enhances the degradation of DOM during its transport. The longitudinal evolution of DOM was also strongly impacted by a hydrological buffering effect in large reservoirs in which the seasonal variability of DOM fluxes and composition was strongly reduced.

1. Introduction

The composition, transport and transformation of dissolved organic matter (DOM) in large rivers are key aspects for determining regional and global carbon (C) budgets (Schlesinger and Melack, 1981), the fate of terrigenous DOM flowing to the oceans (del Giorgio and Pace, 2008; Massicotte and Frenette, 2011), the influence of fluvial inputs on DOM biogeochemistry in coastal and oceanic environments (Holmes et al., 2008), and the functioning of inland waters as active pipes with regards to the global C cycle (Cole et al., 2007; Borges et al., 2015a). Riverine DOM is mainly derived from terrestrial soils (e.g. Weyhenmeyer et al., 2012), but can also be fueled by sources within the aquatic system (Lapierre and Frenette, 2009; Massicotte and Frenette, 2011). Longitudinal patterns of riverine DOM, both in terms of concentration and composition, are controlled by numerous environmental drivers including connectivity with surrounding wetlands (Battin, 1998; Mladenov et al., 2007), lateral inputs from tributaries (Massicotte and Frenette, 2011) and shifts in dominant land cover (Ward et al., 2015). Once in the aquatic ecosystem, terrestrial DOM is exposed to in-stream processing such as photodegradation (Cory et al., 2007; Spencer et al., 2009), microbial respiration (Amon and Benner, 1996; Fasching et al., 2014), and flocculation (von Wachenfeldt and Tranvik, 2008), that usually operate simultaneously and lead to the removal and the transformation of DOM during its transport (Massicotte and Frenette, 2011; Cawley et al., 2012). The composition of DOM has been identified as a major driver determining its reactivity in freshwaters (Weyhenmeyer et al., 2012; Kothawala et al., 2014; Kellerman et al., 2015). For example, the selective loss of the colored fraction of terrestrial DOM is a common pattern observed in a wide variety of ecosystems (Moran et al., 2000; Cory et al., 2007; Spencer et al., 2009; Weyhenmeyer et al., 2012). However, aquatic ecosystem properties (e.g., temperature, oxygen availability or composition of aquatic microbial community) may also play an equal role in determining the fate of DOM (Marín-Spiotta et al., 2014). Thus, the extent of DOM decay depends on the water residence time (WRT) of the aquatic ecosystem (Cory et al., 2007; Hanson et al., 2011; Köhler et al., 2013). In large rivers, WRT varies spatially, increasing in reservoirs and lakes compared to river channels, and seasonally, being higher during low flow compared to high flow. Considering that changes in water level also control the hydrological connectivity with wetlands, it is likely that the downstream gradient in DOM composition drastically differs in relation to spatial and temporal changes in hydrodynamic conditions.

Longitudinal patterns of DOM in large rivers are often assessed in one specific environment, such as wetlands/floodplains (Mladenov et al., 2007; Yamashita et al., 2010; Cawley et al., 2012; Zurbrügg et al., 2013) or lakes (Parks and Baker, 1997; Massicotte and Frenette 2013; Stackpoole et al., 2014), or limited to a subsection of large rivers (del

Giorgio and Pace; 2008; Massicotte and Frenette, 2011; Ward et al., 2015), and mostly carried out during one specific hydrological period. Our understanding of rivers as a continuum in which DOM is simultaneously transported from terrestrial soils to oceans, produced and degraded is thus fundamentally limited by a lack of basin-scale studies taking into account seasonal variations. This is especially true for tropical waters that have the highest riverine dissolved organic carbon (DOC) flux to the oceans (Meybeck, 1993) but for which DOM cycling has received less attention than rivers in other climate zones with the exception of the Amazon River (Mayorga et al., 2005; Johnson et al., 2011; Ward et al., 2013; 2015).

The study of DOM biogeochemistry at large spatial and temporal scales requires analytical tools that are simple to implement but have a large sample throughput while providing pertinent information about the DOM chemical composition. Spectroscopic methods, primarily based on ultraviolet-visible and fluorescence measurements, fulfill these criteria (Jaffé et al., 2008). Optical properties of colored DOM (CDOM) and fluorescent DOM (FDOM) can be used to calculate several indices related to DOM composition. These include the specific ultraviolet absorbance at 254 nm ($SUVA_{254}$), positively related to the degree of DOM aromaticity (Weishaar et al., 2003), the spectral slope ratio (S_R), inversely related to the average DOM molecular weight (Helms et al., 2008) and the fluorescence index (FI), related to the contribution of terrestrial versus aquatic microbial inputs (McKnight et al., 2001). FDOM measurements acquired as three-dimensional excitation-emission matrices (EEMs) and coupled with the parallel factor analysis (PARAFAC) provide additional benefits for the characterization of DOM (Stedmon et al., 2003; Stedmon and Markager, 2005; Yamashita et al., 2008). In addition, the carbon stable isotope composition of DOC ($\delta^{13}C_{DOC}$) can provide information about the terrestrial or aquatic origin of DOM (Mladenov et al., 2007; Lambert et al., 2015).

The Zambezi River basin, the fourth largest river in Africa, was extensively sampled from its source to its mouth during three field campaigns carried out over wet and dry seasons (Teodoru et al., 2015; Fig. 1 and 2). Longitudinal patterns of DOM were assessed through measurements of DOC concentrations and characterization of DOM ($\delta^{13}C_{DOC}$ coupled with CDOM and FDOM) along the Zambezi River (>3000 km) and its main tributary, the Kafue River (>1500 km). The aim of this study was to determine the main drivers on downstream patterns of DOM at the scale of a large tropical river, with a specific attention to the role of WRT in modulating the fate of DOM.

2. Materials and methods

2.1. Study site. The Zambezi River has a drainage area of 1.4 × 10^6 km², originates in northwestern Zambia and flows southeast over 3000 km before it discharges into the Indian Ocean in Mozambique (Fig. 1). The climate of the Zambezi Basin is classified as humid subtropical and is characterized by two main seasons, the rainy season from October/November to April/May and the dry season from May/June to September/October. Annual precipitation strongly varies with latitude, from > 2000 mm in the northern part and around Lake Malawi to less than 500 mm in the southern part of the basin. The mean annual rainfall over the entire catchment is ~940 mm (Chenje, 2000). Up to 95% of the annual rainfall occurs during the rainy period while the dry period presents irregular and sporadic rainfall events. Consequently, water discharge in Zambezi River has a unimodal distribution with a single maximum peak discharge occurring typically in April/May and a minimum in November (Fig. 2).

Woodlands and shrublands are the dominant (55%) land cover and stretch over the whole catchment; forests (20%) and grasslands (9%) areas are mainly confined to the northeast part of the basin and croplands represents 13% of the total area (Mayaux et al., 2004). Wetlands, including swamps, marshes, seasonally inundated floodplains and mangroves cover 5% of the total basin area (Lehner and Döll, 2004).

Based on distinct geomorphological characteristics, the Zambezi Basin can be divided into three major segments: (1) the upper Zambezi from the headwaters to Victoria Falls; (2) the middle Zambezi, from Victoria Falls to the edge of the Mozambique coastal plain (below Cahora Bassa Gorge); and (3) the lower Zambezi, the stretch crossing the coastal plain down to the Indian Ocean (Wellington, 1955). The upper Zambezi covers about 40% of the total area of the Zambezi basin but comprises the highest fraction of wetlands and floodplains (about 60% of the total wetlands/floodplains areas of the Zambezi Basin), including the Barotse Floodplain and the Chobe Swamps (Fig. 1). The middle stretch of the Zambezi River is buffered by two major man-made impoundments, namely the Kariba Reservoir (volume: 167 km^3; area: 5364 km^2 (Magadza, 2010)) and the Cahora Bassa Reservoir (volume: 63 km^3; area: 2739 km^2 (Davies et al., 2000)). The Kafue River (drainage area: 1.56×10^5 km^2) joins the Zambezi River ~ 70 km downstream of the Kariba Dam. Similarly to the upper Zambezi, the Kafue River comprises a high density of wetlands/floodplains (about 26% of the total wetlands/floodplains areas of the Zambezi basin), including the Lukanga Swamps and the Kafue Flats (Fig. 1). It also comprises two smaller reservoirs, the Itezhi Tezhi Reservoir (volume: 5.4 km^3; area: 365 km^2 (Kunz et al., 2011)) and the Kafue Gorge Reservoir (volume: ~1 km^3; area: 13 km^2 (Teodoru et al., 2015)). In its lower part, the Zambezi River and its tributary the Shire River both drain narrow but ~ 200 km long wetlands areas before their confluence zone. At the end of its course, the river forms a large, 100 km long floodplain-delta system of swamps and meandering channels.

2.2. Sampling and analytical methods. Sampling was conducted during two consecutive years and over two climatic seasons: wet season 2012 (1 February to 5 May, n=40), wet season 2013 (6 January to 21 March, n=41), and dry season 2013 (15 October to 28 November, n=24) (Fig. 2). Sites in the Zambezi and the Kafue rivers were located 100 – 150 km apart from the spring to the outlet (Fig. 1) except during the 2013 dry season when sampling in the Zambezi River ended before its entrance in the Cahora Bassa Reservoir due to logistical constraints.

Water sampling was mainly performed from boats or dugout canoes in the middle of the river. In few case (n=10), in the absence of boats/canoes, sampling was carried out either from bridges or directly from the shore and as far as possible away from the shoreline, but without discernable effects on the longitudinal patterns on DOM or other biogeochemical variables (Teodoru et al., 2015). Approximately 2 L of water were collected 0.5 m below the surface, kept away from direct sunshine and filtered within 2 h of sampling. The samples preparation for the different analysis was performed just after filtrations. Filtrations were performed successively on pre-combusted GF/F glass fiber filters (0.7 µm porosity), then on 0.2 µm polyethersulfone syringe filters. The 0.2 µm filters were rinsed with at least 100 ml prefiltered sample water before collection in order to "flush" the potential amount of DOC present in filters. Samples for the measurement of DOC concentration and $\delta^{13}C_{DOC}$ signatures were stored in 40 mL glass vials with polytetrafluoroethylene (PTFE) coated septa with 50 µL H_3PO_4 (85%). Samples for CDOM/FDOM analyses were stored in 20 mL amber glass vials with PTFE-coated septa but without H_3PO_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. Samples were brought back to Belgium for analysis. For logistical reasons, it was not possible to store the samples cold, but the effects of room temperature storage over several months on samples collected using our preservation technique has been found to preserve both DOC, $\delta^{13}C_{DOC}$, and CDOM properties (own unpublished results).

2.3. DOC analysis. DOC and $\delta^{13}C_{DOC}$ were analyzed with an Aurora1030 total organic carbon analyzer (OI Analytical) coupled to a Delta V Advantage isotope ratio mass spectrometer (KU Leuven, Belgium). Typical precision observed in duplicate samples was in >95% cases < ± 5 % for DOC, and ± 0.2 ‰ for $\delta^{13}C_{DOC}$. Quantification and calibration was performed with series of standards prepared in different concentrations, using both IAEA-C6 ($\delta^{13}C$ = -10.4 ‰) and in-house sucrose standards ($\delta^{13}C$=-26.9 ‰). All data are reported in the δ notation relative to VPDB (Vienna Pee Dee Belemnite).

2.4. CDOM analysis and calculations. Absorbance was recorded on a Perkin-Elmer UV/Vis 650S spectrophotometer (Université Libre de Bruxelles) using a 1 cm quartz cuvette. Absorbance spectra were measured between 190 and 900 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 absorption spectra to the data over the 200-700 nm range according to the following equation:

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

where A_λ and A_0 are the absorbance measured at defined wavelength λ and at reference wavelength λ_0 = 375 nm, respectively, S the spectral slope (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).

The $SUVA_{254}$ was calculated as the UV absorbance at λ = 254 nm (A_{254}) 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 A_{254} was then used to calculate $SUVA_{254}$. The $SUVA_{254}$ was used as an indicator of the aromaticity of DOC with high values (>3.5 l mgC^{-1} m^{-1}) indicating of the presence of more complex aromatic moieties and low values (<3 l mgC^{-1} m^{-1}) indicative of the presence of more aliphatic compounds (Weishaar et al., 2003).

Napierian absorption coefficients were calculated according to:

$$a_{\lambda} = 2.303 \times A_{\lambda}/L \qquad (3)$$

where a_{λ} is the absorption coefficient (m^{-1}) at wavelength λ, A_{λ} the absorbance corrected at wavelength λ 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 nm and 350-400 nm 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 $S_{275\text{-}295}$ to $S_{350\text{-}400}$ according to Helms et al. (2008). S_R is related to the molecular weight 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 (Helms et al., 2008).

2.5. FDOM analysis and PARAFAC modeling. Fluorescence intensity was recorded on a Perkin-Elmer LS55 fluorescence spectrometer (Université Libre de Bruxelles) 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 $A_{254} < 0.2\ m^{-1}$ to avoid problematic inner filter effects (Ohno, 2002). Before each measurement session (i.e. each day), a Milli-Q water sample was also analysed. EEMs preprocessing such as removing first and second Raman scattering, standardization to Raman units, absorbance corrections and inner filter effects were performed prior the PARAFAC modelling. 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 DOM Fluorescence Toolbox 1.7. Validation of the PARAFAC model was performed by split-half analysis and random initialization (Stedmon and Bro, 2008). Additional samples analysed in the same manner and collected from (1) tributaries of the Zambezi and the Kafue rivers as well as during an almost two-year monitoring period of the Zambezi and the Kafue rivers (n = 42; data not published), and (2) the Congo Basin (n = 164; data not published) were added to the dataset. This was done to increase the variability of DOM fluorescence signatures and therefore help detect components that could have been present in insufficient quantity to be detected in our environment (Stedmon and Markager, 2005). The maximum fluorescence F_{Max} values of each component for a particular sample provided by the model were summed to calculate the total fluorescence signal F_{Tot} of the sample in Raman's unit (R.U.). The relative abundance of any particular PARAFAC component X was then calculated as $\%C_X = F_{Max}(X)/ F_{Tot}$. The FI index 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 microbial terrestrial source; intermediate values indicate a mixed DOM source.

2.6. Statistical Analysis

A principal component analysis (PCA) was initially used as a diagnostic tool to examine relationships between PARARAC results, DOM concentration and composition assessed by optical proxies and isotopic measurements in order to better characterize the origin and source of the PARAFAC components identified in the study. The PCA was performed on scaled variables using the prcomp function in R software. DOC concentrations, stable carbon isotopic composition, optical indices ($SUVA_{254}$, S_R, FI), a_{350}, F_{Max} and the relative abundance of PARAFAC components were used as the variables for the PCA. Given the different units of the variables used in the PCA, data were scaled to zero-mean and unit-variance as recommended (Borcard et al., 2011). The PCA was then performed on the correlation matrix of the scaled variables.

3. Results

3.1. Longitudinal patterns in DOC concentration, composition and DOM optical properties

Data were acquired during two wet seasons and one dry season. The two wet season datasets are discussed together hereafter. DOC concentrations in the Zambezi River ranged from 1.9 ± 0.1 to 4.9 ± 1.0 mg L^{-1} during the wet periods and from 1.2 to 2.9 mg L^{-1} during the dry period (Fig. 3A). Along the upper Zambezi DOC increased downstream during the wet seasons, while DOC gradually decreased downstream during the dry season. In the Kariba Reservoir, DOC variability between wet and dry seasons was relatively low, and concentrations ranged from 2.4 ± 0.3 to 2.9 ± 1.4 mg L^{-1}. DOC exhibited relatively small variability downstream of the Kariba Reservoir and along the lower Zambezi, with the exception of a slight increase during the wet seasons downstream of the confluence with the Shire River (outlet of Lake Malawi). In the Kafue River, DOC was generally higher during the wet seasons (from 3.1 ± 0.1 to 5.4 ± 0.7 mg L^{-1}) compared to the dry season (from 1.3 to 3.6 mg L^{-1})(Fig. 3B). Despite this seasonal difference, DOC increased gradually downstream during both wet and dry seasons. DOC concentrations in the Itezhi Tezhi Reservoir showed a decrease (~25%) during the wet seasons but an increase (~20%) during the dry season compared to the upstream station. During the wet periods, DOC concentrations in the upper Zambezi and the Kafue River were closely correlated with the extent of wetlands (Fig. 4).

The a_{350} values (Fig. 3C and 3D), used to assess the level of CDOM, were higher during the wet seasons (1.7 to 16.6 m^{-1} in the Zambezi and 3.9 to 11.5 m^{-1} in the Kafue) than during the dry season (1.3 to 10.7 m^{-1} in the Zambezi and 1.2 to 4.7 m^{-1} in the Kafue). They followed similar spatial and seasonal patterns as DOC concentrations, with some differences. First, decreases in a_{350} values were more pronounced than for DOC, especially in the upper Zambezi during the dry season and in the Kariba and Itezhi Tezhi reservoirs during the wet season. For example, while DOC decreased by a factor ~2 as the Zambezi enters the Kariba Reservoir during the wet periods, a_{350} decreased by a factor ~4. Secondly, while DOC concentrations were higher at the outlet of reservoirs compared to upstream stations during the dry season, a_{350} values were lower.

$\delta^{13}C_{DOC}$ values in the Zambezi basin ranged from -28.1 to -19.6 ‰ over the study period, i.e. from typical C_3 dominated values (C_3 end-member was estimated at -28.5 ‰ according to Kohn(2010) to values representing mixed C_3-C_4 vegetation($\delta^{13}C$ value for the C_4 end-member -12.1 ‰ (Tamooh et al., 2012)). $\delta^{13}C_{DOC}$ showed a gradual increase along the Zambezi River during all periods, from -28.1 and -26.5 ‰ at the source to -21.4 to -20.1 ‰ near its delta, the latter being especially marked between the two first sampling sites in the upper Zambezi (Fig. 3E), while no significant pattern was observed along the Kafue River (values between -25.9 and -20.5 ‰, Fig. 3F).

DOM at the source of the Zambezi exhibited the highest $SUVA_{254}$ (> 4 L mgC^{-1} m^{-1}, indicating strong aromaticity), and lowest S_R (< 0.8, indicative high molecular weight) values during both wet and dry seasons (Fig. 3G and 3I). During the wet seasons, the upper Zambezi was characterized by stable $SUVA_{254}$ (3.5 – 4.0 L mgC^{-1} m^{-1}) and low S_R (0.85 – 0.91) values. In the middle Zambezi, $SUVA_{254}$ and S_R values were lowest (2.2 ± 0.2 – 2.9 ± 0.1 L mgC^{-1} m^{-1}) and highest (1.22 ± 0.09 – 1.41 ± 0.01) in the Kariba and the Cahora Bassa reservoirs compared to samples collected in-between (2.6 ± 0.1 – 3.1 ± 0.02 L mgC^{-1} m^{-1} for $SUVA_{254}$ and 0.97 ± 0.1 – 1.10 ± 0.08 for S_R). Overall, $SUVA_{254}$ increased from 2.1±0.5 to 2.9±0.9 L mgC^{-1} m^{-1} whereas S_R decreased from 1.08±0.09 to 0.97±0.04 in the lower Zambezi, with maximum (3.3±0.9 L mgC^{-1} m^{-1}) and minimum (0.88±0.006) values recorded below the confluence with the Shire River, respectively. During the wet periods, FI values ranged between 1.24 and 1.41 in the mainstream, and between 1.43 and 1.58 in reservoirs (Fig. 3K). FI values during the dry season were generally higher than during the wet periods with values ranging from 1.29 to 1.72, except at the source of the Zambezi, where an FI value of 1.19 was observed.

In the Kafue River, variations in DOM composition were marked between the wet and dry seasons, but minimal along the longitudinal transect (Fig. 3H, 3J and 3L). $SUVA_{254}$ and S_R ranged from 3.5 to 4.0 L mgC^{-1} m^{-1} and from 0.79 to 1.05, respectively, during the wet seasons, except in the Itezhi Tezhi Reservoir where $SUVA_{254}$ decreased to 2.4 L mgC^{-1} m^{-1} and S_R increased up to 1.16. Values were quite stable during dry periods, and ranged between 2.2 and 2.8 L mgC^{-1} m^{-1} for $SUVA_{254}$ and from 1.11 to 1.22 for S_R. FI values ranged between 1.27 and 1.42 during the wet seasons, and between 1.41 and 1.74 during the dry season.

3.2. Longitudinal patterns in FDOM

PARAFAC modelling identified three terrestrial humic-like components (C1, C2 and C4), one aquatic microbial humic-like component (C3) and one protein tryptophan-like (C5) component (Table 1 and Supplementary Fig. 1). In the Zambezi River, the fluorescence intensities (F_{Max}) of PARAFAC components during the wet seasons presented patterns similar to DOC concentrations with some exceptions (Fig. 5). F_{Max} of the C4 component presented the higher percentage of increase compared to the other component in river sections flowing through wetlands/floodplains in the upper and lower Zambezi (data not shown). All terrestrial and microbial humic-like components showed a systematic and marked decrease in their F_{Max} values in reservoirs, while F_{Max} of C5 decreased in a smaller proportion in the Kariba Reservoir and increased in the Cahora Bassa Reservoir. During the dry season, F_{Max} of terrestrial humic-like components decreased downstream concurrent with DOC concentrations, while F_{Max} remained stable for C3 or increased for C5. In the Kafue River, F_{Max} of all components followed similar spatial and temporal patterns as those of DOC concentrations. The main difference observed was that while F_{Max} values of humic-like compounds were lower during the dry season compared to the wet seasons, F_{Max} of C5 exhibited similar values accross the hydrological cycle.

As a direct consequence of the spatial and temporal differences in F_{Max} of PARAFAC components, the relative contribution of each component to the total fluorescence signal F_{TOT} showed distinct patterns (Fig. 6). Thus, the downstream decrease of %C1 and %C2 observed in the upper Zambezi during the wet seasons can be related to the parallel increase of %C4, the latter being due to the more pronounced increase in F_{Max} of C4 relative to the other components. The same patterns for %C1 and %C2 observed during the dry season, however, reflect the fact that F_{Max} values of C3 and C5 were stable or increased during the dry season, respectively, while F_{Max} of C1 and C2 decreased. %C5 was higher during the dry season compared to the wet seasons, and reached highest values in reservoirs during the wet periods due to its specific spatial and temporal variations in F_{Max} values. No longitudinal changes in the relative abundance of PARAFAC components were observed along the Kafue River. Similar to what was observed along the Zambezi River, the dry season was marked by a decrease in %C4 and an increase in %C5, while %C1, %C2 and %C3 were equivalent to values recorded during the wet seasons.

3.3. Principal component analysis (PCA)

The first two components of the PCA explained 71.7% of the variance and regrouped the variables in three main clusters (Fig. 7). The first includes %C1, %C2 and samples collected at or near the source of the Zambezi. The second group was defined by %C4 and several variables including DOC, F_{Max}, $SUVA_{254}$ and a_{350}. Samples from the upper Zambezi and from the Kafue rivers (excluding reservoirs) were mainly located in this cluster. Finally, %C3 and %C5 were clustered with S_R and FI. Samples from reservoirs (including Kariba, Cahora Bassa and Itezhi Tezhi) were almost all in this cluster. Samples collected in the middle and lower Zambezi during both the wet and dry seasons were located between the distinct clusters defined by PARAFAC components and DOM concentration and composition.

4. Discussion

4.1. Identification of PARAFAC components. Humic-like components C1 and C2 are among the most common fluorophores found in freshwaters and are associated with high molecular weight and aromatic compounds of terrestrial origin (Stedmon and Markager, 2005; Yamashita et al., 2008; Walker et al., 2013). Component C4 has been reported to be of terrestrial origin (Stedmon and Markager, 2005; Kothawala et al., 2015) or to be a photoproduct of terrestrially derived DOM (Massicotte and Frenette, 2011). The association of %C4 with DOC concentrations and terrestrial optical indices including a_{350} and $SUVA_{254}$ advocates for a terrestrial origin of this component (Fig. 7). Conversely, %C3 and %C5 were negatively correlated with a_{350} and $SUVA_{254}$. C3 and C5 components are respectively classified as microbial humic-like and tryptophan-like components related to the production of DOM within aquatic ecosystems (Kothawala et al., 2014; Kellerman et al., 2015). Both fluorophores can originate from autochthonous primary production (Yamashita et al., 2008; 2010; Lapierre and Frenette, 2009) or from degradation of terrestrial DOM in the water column as previously reported in a wide variety of environments as marine (Jørgensen et al., 2011) and lake waters (Kellerman et al., 2015) for C3, and large Arctic rivers (Walker et al., 2013) or small temperate catchment (Stedmon and Markager, 2005) for C5. The opposite relationship of %C1 and %C2 versus %C3 (Fig. 7) suggests that C3 would be the result of the transformation of terrestrial components C1 and C2 through biological activity in the water column as suggested by Jørgensen et al. (2011). The distribution of samples along PC1 is thus likely controlled by the transition from terrestrial DOM with a high degree of aromaticity and humic content (negative loadings) to less aromatic DOM produced within the aquatic ecosystem by the degradation of terrestrial DOM during transport and/or by autochthonous sources (positive loadings). Regarding PC2, %C1 and %C2 were also strongly opposed to $\delta^{13}C_{DOC}$. Considering the highest level of %C1 and %C2 at the source of the Zambezi and suggesting that $\delta^{13}C_{DOC}$ was primarily controlled by the vegetation gradient along the mainstem from C_3 forest toward mixed C_3/C_4 savannah, this suggests that land cover influences DOM in the river network.

4.2. Relative contribution of C_3 and C_4 plants to the DOM pool. The $\delta^{13}C_{DOC}$ values in the Zambezi basin were in the range of data reported for other African river systems, being higher than those measured in C_3 tropical rainforest catchments such as the Congo (Spencer et al., 2009; Bouillon et al., 2012, 2014), the Ogooué (Lambert et al., 2015) or the Nyong rivers (Brunet et al., 2009), but similar to catchments with significant areas of C_4 vegetation (e.g. savannah) such as the Tana (Tamooh et al., 2012), the Niger (Lambert et al., 2015) or the Betsiboka and Rianilia rivers (Marwick et al., 2014).

The increase in $\delta^{13}C_{DOC}$ in the Zambezi, especially marked along the first stations, was consistent with the vegetation gradient along the mainstem, where upstream C_3 forest ecosystems quickly shift towards mixed C_3-C_4 grassland and woodland/shrubland ecosystems that dominate in the basin (Supplementary Fig. 2). $\delta^{13}C_{DOC}$ did not show marked depletion in surface waters of reservoirs, suggesting that phytoplankton production had little net effect on $\delta^{13}C_{DOC}$ (Bouillon et al., 2009; Tamooh et al., 2012). In addition to an increased contribution from C_4 vegetation, the downstream increase in $\delta^{13}C_{DOC}$ could also partially result from differences in the $\delta^{13}C$ composition of C_3 vegetation at the basin scale. Indeed, $\delta^{13}C$ values of C_3 plants increase with decreasing mean annual precipitation (MAP) (Kohn, 2010) and MAP in the Zambezi strongly varies from > 2000 mm yr^{-1} in the northern part of the basin to < 500 mm yr^{-1} in the southern part (Chenje, 2000). Using high resolution maps of MAP (Hijmans et al., 2005), a digital elevation model at 3 arcsec resolution computed by the HydroSHEDS mapping product (http://hydrosheds.cr.usgs.gov/index.php), and the proposed equation of Kohn (2010) that estimates the $\delta^{13}C$ signature of C_3 vegetation based on MAP, altitude and latitude, we estimated an average value of -27.1 ‰ and a range of variation from -29.3 to -26.0 ‰ for the C_3 vegetation of the Zambezi basin (Supplementary Fig. 3). This shift of 3.3% is smaller than the observed shift of ~8 % in the Zambezi River, indicating that the increase of $\delta^{13}C_{DOC}$ is to a large extent due to increased contribution from C_4 vegetation. As a first approximation and using values of -27.1 ‰ for C_3 plants (calculated above) and -12.1 ‰ for C_4 plants (Tamooh et al., 2012), we found that DOM in the Zambezi basin was mainly from C_3 origin, with a relative contribution of ~69% and ~75 % for DOC during the wet and dry period, respectively.

4.3. Seasonal and spatial variability in downstream gradients in DOM concentration and composition. Altogether data showed clear changes in the downstream gradients of DOM concentration and composition, both seasonally and spatially. In addition to the vegetation gradient, these changes were essentially controlled by three main factors: WRT and connectivity with wetlands/floodplains, both highly dependent on seasonal variations in water level (and discharge), and water retention by lakes/reservoirs that is more independent from seasonal variations of water level.

4.3.1 Land cover and hydrological connectivity with wetlands/floodplains. The DOM at the source of the Zambezi was clearly distinct from the rest of the basin, independently of the hydrological period (Fig. 6), with a strong aromatic character (highest $SUVA_{254}$), a high degree of molecules with elevated molecular weight (lowest S_R) and low $\delta^{13}C_{DOC}$.

The shift in land cover (see Supplementary Fig. 2) was reflected in the DOM gradient from the source station of the Zambezi to the next sampling site, and marked by an increase in S_R, $\delta^{13}C_{DOC}$ and a decrease in $SUVA_{254}$. This pattern is consistent with the role of forest in releasing more aromatic DOM of high molecular weight than other vegetation types in tropical freshwaters (Lambert et al., 2015).

Downstream, the variability in the optical properties of DOM between wet and dry seasons indicated seasonal changes in the sources of riverine DOM in relation with changes in water level and connectivity with wetlands/floodplains. The high $SUVA_{254}$ and low S_R values during the wet seasons indicate the mobilisation of fresh aromatic DOM of high molecular weight due to the increased water flow through DOM-rich upper soil horizons during high flow periods (Striegl et al., 2005; Neff et al., 2006; Mann et al., 2012; Bouillon et al., 2014). Wetlands and floodplains were the main sources of terrestrial DOM at the basin scale during wet seasons, as shown by the relationships between DOC and wetland extent (Fig. 4). Among the different terrestrial humic-like components, C4 was the most affected by fluctuations in the connectivity with wetlands/floodplains. The increase in the relative contribution of C4 suggests that this component was mobilized in greater proportion relative to others (Fig. 6). This observation is consistent with a recent study conducted in boreal streams, in which a component similar to C4 was found to increase relative to other humic-like fluorophores (equivalent to C1 and C2) in stream waters during the peak spring melt due to the higher abundance of this component in uppermost soil horizons of wetlands (Kothawala et al., 2015). The longitudinal and seasonal variations in %C4 in the upper Zambezi are consistent with the hypothesis that C4 is mainly produced in the upper soil horizons of wetlands/floodplains and therefore preferentially mobilized during high flow periods.

4.3.2 WRT modulates the downstream patterns of DOM. During the dry season, DOM was characterized by lower $SUVA_{254}$ and higher S_R values, indicating the transport of compounds of lower aromaticity and lower average molecular weight compared to high flow periods. The difference in downstream gradients of DOM compared to the wet seasons can be explained in part by the loss of connectivity between rivers and riparian wetlands/floodplains and the deepening of hydrological flowpaths through DOM-poor deeper subsoil horizons during the dry season (e.g. Striegl et al., 2005; Bouillon et al., 2014). Changes of connectivity with wetland during the dry season was also found to strongly impact CO_2 and CH_4 distribution in the Zambezi (Teodoru et al., 2015). That being said, the considerable decrease in water discharge during dry/base flow period compared to wet/high flow periods (Fig. 2) likely leads to a decrease in water velocities and subsequently to an increase in water residence time, allowing a more efficient degradation of terrestrial DOM along a given section. For illustration, the preferential downstream loss of a_{350} compared to DOC in the upper Zambezi, associated with a gradual decrease of $SUVA_{254}$ and increase of S_R, is a strong evidence of the preferential loss of the terrestrial and aromatic fraction of DOM through photodegradation (e.g. Spencer et al., 2009; Weyhenmeyer et al., 2012). The stable level of F_{Max} of C3 suggests a continuous supply of this component, likely due to microbial degradation of terrestrial DOM. In addition, the increase in WRT could favour the accumulation of DOM from autochthonous sources as suggested by higher values of FI and the gradual increase in F_{Max} for C5 (Fig. 3 and 4). Flushing during high flow periods perturbs the downstream gradient of DOM established during base flow because (1) increasing water level mobilizes a greater proportion of terrestrial DOM and (2) higher water velocities increases the travel distance of humic and aromatic terrestrial compounds before removal due to microbial and photochemical degradation processes and limits the accumulation of autochthonous DOM in the water column.

4.3.3. Retention of water by lakes/reservoirs. Longitudinal patterns of DOM were affected by the presence of reservoirs where DOM was characterized by low aromaticity and molecular weight and higher microbial contribution independently of water level fluctuations (Fig. 5 and 7). The net loss of DOC and the preferential loss of the coloured and aromatic fraction of DOM (based on a_{350} and $SUVA_{254}$, Fig. 3) in lakes and reservoirs have been previously documented (Hanson et al., 2011; Köhler et al., 2013) and attributed to the combination of several processes including flocculation, photochemical and microbial degradation (Cory et al., 2007; von Wachenfeldt and Tranvik, 2008; Köhler et al., 2013; Kothawala et al., 2014). Although we were not able to estimate the relative contribution of these mechanisms, our results indicate that the humic-like fractions of DOM (C1-C4) were more susceptible to degradation compared to the protein-like fraction (C5), an observation consistent with recent studies carried out in boreal lakes (Kothawala et al., 2014). The level of fluorescence of C5 could be additionally sustained by the FDOM from primary producers such as macrophytes (Lapierre and Frenette, 2009) or phytoplankton (Yamashita et al., 2008), that also lead to low values of the partial pressure of CO_2 below atmospheric equilibrium in the Kariba and Cahora Bassa reservoirs while rivers (i.e., excluding reservoirs) displayed CO_2 supersaturated conditions with respect to atmospheric equilibrium (Teodoru et al., 2015).

In agreement with others studies (e.g. Hanson et al., 2011), the effects of reservoirs on the fate of DOM were related to their specific WRT. The Itezhi Tezhi Reservoir had little effect on longitudinal patterns of DOM, as also suggested by a recent study (Zürbrugg et al., 2013), likely due to its relatively low WRT (0.7 yr, Kunz et al., 2011) compared to the Kariba (5.7 yr, Magadza, 2010) and the Cahora Bassa (~2 yr, Davies et al., 2000) reservoirs. The DOC concentrations upstream and downstream of the Cahora Bassa Reservoir were similar but DOM composition exhibited significant changes within the reservoir compared to upstream and downstream stations, suggesting a balance between loss and production of new compounds. In fact, the Kariba Reservoir was the most important reservoir responsible for the perturbation of the longitudinal DOM gradient. The seasonal variability of DOM at the outlet of the Kariba Reservoir, both in terms of concentration and composition, was drastically reduced compared to the seasonal patterns observed in the upper Zambezi (Fig. 3 and 5). This was also illustrated by data from an almost two-year monitoring of the Zambezi River 70 km downstream of the Kariba Dam, showing that the terrestrial fraction of DOM leaving the reservoir has undergone extensive transformation (Table 2). The role of lakes/reservoirs in lowering the seasonality of DOC in river network has also been evidenced in temperate and boreal streams and rivers in Sweden (Winterdahl et al., 2014).

Beyond their role as hotspots for DOM processing and mineralization, lakes/reservoirs act as a hydrological buffer and reduce the temporal variability of downstream water flow (Goodman et al., 2011; Lottig et al. 2013). Except for some isolated events, water discharge remained constant at Kariba Dam due to hydropower management (Fig. 2). Combined with the low temporal variability in DOM content (Table 2), DOC fluxes at the outlet of the Kariba Reservoir were relatively invariant and ranged between 8.3×10^7 and 9.7×10^7 kg yr^{-1}. This results in a twofold decrease of DOC fluxes during the wet seasons between upstream inputs from the upper Zambezi and export at the outlet of the Kariba Reservoir, but in the increase by a factor of 12 during the dry season (Fig. 8). On a longitudinal perspective, lakes/reservoirs can thus shift from DOM sources to sinks relative to upstream ecosystems while reducing the temporal variation of DOM fluxes and composition to downstream ecosystems. That being said, DOM losses were largely offset during the wet seasons by inputs from the Kafue and the Shire rivers as well as from wetlands in the lower Zambezi (Fig. 3 and 8). Therefore, the spatial arrangement of the different elements that constitute large river networks such as lakes/reservoirs, wetlands/floodplains and large tributaries is a key aspect in controlling DOM export at the basin scale.

4.4. Comparison with others rivers. The results of this study are similar to those reported in large rivers from other biomes regarding (1) the role of peak flow periods in exporting a greater portion of terrestrial aromatic and humic DOM (Neff et al., 2006; Duan et al., 2007; Holmes et al., 2008; Walker et al., 2013; Bouillon et al., 2014), (2) the disproportionate importance of riparian wetlands and floodplains in regulating in-stream chemistry (Fiebig et al. 1990; Dosskey and Bertsch, 1994; Hinton et al. 1998; Battin, 1998; Hanley et al., 2013; Abril et al., 2014; Borges et al., 2015b) and (3) the reactivity of terrestrial DOM during its transport (Massicotte and Frenette, 2011; Cawley et al., 2012; Wehenmeyer et al., 2012). However, while changes in temperature have been suggested as a secondary factor impacting DOM patterns in temperate and boreal streams and rivers (Kothawala et al., 2014; Winterdahl et al., 2014; Raymond et al., 2015), changes in longitudinal DOM patterns in the Zambezi Basin were only controlled by changes in hydrology. Indeed, water temperatures were systematically elevated with values mainly ranging from 25 to 29°C (data not shown) and no significant patterns were apparent between the contrasting seasons.

Our study clearly illustrates that the DOC in a given station is the legacy of upstream sources and their degree of processing during transport, and suggests that WRT is a major driver controlling the fate of DOM in freshwaters (the latter resulting from the competition between transport and degradation processes). Seasonal changes in DOM concentration and composition in large rivers assessed by monitoring programs are often explained by vertical changes in DOM sources mobilized during high flow and base flow conditions, i.e. shallow versus deep sources along the soil profile (Neff et al., 2006; Mann et al., 2012; Bouillon et al., 2014). Our results show that the upstream degradation history of DOM during transit should also be taken into consideration, especially during base flow periods. Given the strong reactivity of fresh terrestrial humic DOM exported during high flow periods (e.g. Holmes et al., 2008; Mann et al., 2012) and the ability of large hydrological events to transport DOM downstream over large distances (Raymond et al., 2015), the functioning of large rivers at the seasonal scale and their impacts on receiving ecosystems (e.g. coastal waters) should deserve more attention.

Author contributions

The research project was designed by AVB and SB, field data collection was done by CRT and FCN. CDOM and FDOM measurements were done by TL with the help of FD. Data analysis was done by TL with the help of PM for PARAFAC modelling. Manuscript was drafted by TL and was commented, amended and approved by all co-authors.

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Figure captions

Figure 1 – Map of the Zambezi basin showing elevation, wetlands and floodplains areas (data from Lehner and Döll, 2004), the main hydrological network and the distribution of sampling sites along the Zambezi and the Kafue rivers.

Figure 2 – Water discharge between January 2012 and January 2014 for (a) the Zambezi River at Victoria Falls and at Kariba Dam, and (b) for the Kafue River at Hook Bridge located upstream of the Itezhi Tezhi Reservoir and at the Kafue Gorge Dam (data from Zambia Electricity Supply Corporation Limited, ZESCO). Bars refer to the three periods during which field campaigns were performed.

Figure 3 – Longitudinal variations of DOM properties along the Zambezi River (left panels) and the Kafue River (right panels) during the wet and the dry seasons. From top to bottom the panels represent: DOC, a_{350}, $\delta^{13}C_{DOC}$, $SUVA_{254}$, S_R and FI. Dark gray and light gray rectangles in background represent the approximate position along the mainstream of wetlands/flooplains areas and reservoirs, respectively. Roman numerals refer to (I) Barotse Floodplain, (II) Chobe Swamps, (III) Kariba Reservoir, (IV) Cahora Bassa Reservoir, (V) lower Zambezi wetlands for the Zambezi River and (VI) Lukanga Swamps, (VII) Itezhi Tezhi Reservoir and (VIII) Kafue Flats for the Kafue River. The diamonds

represent samples collected from main tributaries upstream of their confluence with mainstreams: (IX) the Kabompo, (X) the Kafue, (XI) the Luangwa, (XII) the Mazoe and (XIII) Shire River for the Zambezi River and (XIV) the Lunga River for the Kafue River. Symbols and error bars for data collected during the wet seasons represent the average and standard deviation between the two field campaigns performed in 2012 and 2013, respectively.

Figure 4 – Relationships between DOC and % wetlands in the catchement in the Zambezi and the Kafue rivers during the wet periods, with *:$p<0.1$, and ***:$p<0.001$. For the Zambezi, only the samples collected in the upper part of the basin have been considered due to the effect of the Kariba and Cahora Bassa reservoirs on the longitudinal pattern of DOC concentrations.

Figure 5 – Longitudinal variations of FDOM along the Zambezi River (left panels) and the Kafue River (right panels) during the wet and the dry seasons. From top to bottom the panels represent: F_{Tot} and F_{Max} for each PARAFAC component. The diamonds represent samples taken from main tributaries upstream of their confluence with mainstreams.

Figure 6 – Longitudinal variations of the relative contribution of PARAFAC component along the Zambezi River (left panels) and the Kafue River (right panels) during the wet and the dry seasons. The diamonds represent samples taken from main tributaries upstream of their confluence with mainstreams.

Figure 7 – Graphical representation of PCA results, including loadings plot for the input variables and scores plot for water samples collected during the wet (circles) and the dry (triangles) seasons. Water samples from the Zambezi River (ZBZ) were classified according to its source and the three major segments of the Zambezi basin. Samples from reservoirs (i.e. Kariba, Cahora Bassa and Itezhi Tezhi reservoirs) were classified together.

Figure 8 – DOC exports calculated at different locations along the Zambezi River during the wet and the dry seasons. Vertical arrows represent changes in DOC exports at the same location between wet and dry seasons. Diagonal changes represent longitudinal variations.

Table 1– Spectral characteristics of the five fluorophores identified by PARAFAC modelling, correspondence with previously identified components in different environments, general assignment and possible source. Numbers in brackets refer to the second peak of maximal excitation.

Component	Maximum Excitation (nm)	Maximum Emission (nm)	Comparison with others environments								Assig
			St Lawrence River[1]	Large Arctic rivers[2]	Boreal Lakes[3,4]	Subtropical wetlands[5,6]	Tropical wetland[7]	Temperate estuary[8]	Coastal waters[9]	Marine waters[10]	
C1	<240 (325)	443	C2	C1	C4	C1	C1	C4	—	C1	Terrestria
C2	<240 (365)	517	C3	C3	C3	C5	C4	C2	C3	—	Terrestria
C3	<240 (305)	383	C7	—	C2	C4	C3	C6	C6	C4	Microbial
C4	<240	405	C1	—	C5	C2	C2	C1	C1	—	Terrestria
C5	275 (<240)	337	C4	C5	C6	C8	—	C7	C4	C2	Tryptop

[a] T: Terrestrial inputs; Au: Autochthonous primary production; An: Anthropogenic origin; M: Microbial degradation; P: Photochemical degradation.

1) Massicotte and Frenette (2011); 2) Walker et al. (2013); 3) Kothawala et al. (2014); 4) Kellerman et al. (2015); 5) Yamashita et al. (2010); 6) Cawley al. (2013); 8) Stedmon and Markager (2005); 9) Yamashita et al. (2008); 10) Jørgensen et al. (2011).

Table 2 – Temporal variations of DOM properties measured at the outlet of the Kariba Reservoir during an almost two-year monthly sampling (from February 2012 to November 2013).

	DOC (mg L^{-1})	$\delta^{13}C_{DOC}$ (‰)	a_{350} (m-1)	$SUVA_{254}$ (L mgC-1 m-1)	S_R	%C1	%C2	%C3	%C4	%C5
Min	2,00	-23,96	1,00	1,39	1,010	27,7	12,2	16,1	4,0	12,3
Max	2,60	-22,26	2,50	3,11	1,428	36,5	16,6	26,2	13,8	35,9
Mean	2,22	-23,08	1,60	2,02	1,185	34,1	15,2	24,1	9,3	17,3
S.D.	0,17	0,37	0,44	0,43	0,141	2,4	1,2	2,7	3,1	6,2
n	*20*	*20*	*12*	*12*	*12*	*12*	*12*	*12*	*12*	*12*