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Temporal controls on dissolved organic matter and lignin biogeochemistry in a pristine tropical river, Democratic Republic of Congo

Robert G. M. Spencer,[1] Peter J. Hernes,[2] Rosmarie Ruf,[3] Andy Baker,[4] Rachael Y. Dyda,[1,2] Aron Stubbins,[5] and Johan Six[1]

[1]   Dissolved organic carbon (DOC), lignin biomarkers, and the optical properties of dissolved organic matter (DOM) were measured in the Epulu River (northeast Democratic Republic of Congo) with the aim of investigating temporal controls on the quantity and chemical composition of DOM in a tropical rainforest river. Three different periods defined by stages of the hydrologic regime of the region, (1) post dry flushing period, (2) intermediary period, and (3) start of the dry period/post flush, were sampled. Temporal variability in DOM quantity and quality was observed with highest DOC, lignin concentration (Σ_8) and carbon-normalized (Λ_8) values during the flushing period attributed to greater surface runoff and leaching of organic-rich horizons, with lowest values in the dry period/post flush once source materials were well leached. Chromophoric DOM (CDOM) was strongly correlated to DOC and Σ_8 ($r^2 = 0.85$ and 0.83, respectively; $p < 0.001$), and CDOM quality measurements (SUVA$_{254}$, spectral slope ratio and fluorescence index) were strongly correlated to Λ_8 values ($r^2 = 0.77$, 0.69, and 0.75, respectively; $p < 0.001$), demonstrating the ability to derive DOC and lignin export and to track DOM quality in tropical riverine systems from simple optical measurements. This study demonstrates similar effects in the variability of DOM quantity and quality due to changing hydrologic inputs for a tropical river as has been previously reported for temperate and northern high-latitude rivers. Therefore, flushing periods in tropical rivers warrant further study, as they are critical toward understanding ecosystem biogeochemistry as maximal export of freshly leached plant material occurs during this time period.

1.   Introduction

[2]   Dissolved organic matter (DOM) represents a fundamental link between terrestrial and aquatic carbon cycles and plays an essential role in aquatic ecosystem biogeochemistry. The majority of DOM studies in catchments have focused on measuring bulk concentrations such as dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in order to calculate export fluxes and observe temporal trends. Such bulk measurements, however, provide little information about the chemical composition or quality of DOM and, thus, its biogeochemical role [*Hernes et al.*, 2008; *Baker et al.*, 2008a; *Fellman et al.*, 2009]. To examine biogeochemical processes in relation to DOM quality previous studies have focused on measurements of bulk DOM properties such as C/N ratios, isotopic composition, and chromophoric (colored) DOM (CDOM) [*Hood et al.*, 2005, 2007; *Neff et al.*, 2006] or measurements of the molecular composition of DOM such as amino acids, carbohydrates, and lignin phenols [*Hedges et al.*, 1994, 2000; *Lobbes et al.*, 2000]. CDOM optical properties, such as absorption and fluorescence, have been the focus of numerous current studies in aquatic sciences due to their potential as proxies for understanding biogeochemical processes such as lability and photochemical degradation [*Cory et al.*, 2007; *Stubbins et al.*, 2008; *Fellman et al.*, 2009].

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Figure 1. Map of the Epulu River study site. The black box in (a) study area within Africa and (b) study area within the Congo River Basin represents the study area shown in (c) inset.

A number of simple optical proxies related to DOM aromaticity and molecular weight, including the spectral slope ratio (S_R) [*Helms et al.*, 2008], specific UV absorbance at 254 nm ($SUVA_{254}$) [*Weishaar et al.*, 2003], and the fluorescence index (FI) [*McKnight et al.*, 2001; *Cory and McKnight*, 2005], have provided information on the chemical composition of DOM. These simple proxies can be garnered from small volumes of sample at relatively low cost, therefore opening up the possibility of real-time monitoring of DOM dynamics in aquatic ecosystems.

[3] The development of optical proxies to elucidate DOM biogeochemical processes also benefits from the "ground truthing" of CDOM optical properties with established biomarker techniques for examining the chemical composition of DOM. Molecular level analyses of lignin phenols are diagnostic tracers of vascular plant inputs in aquatic systems and, when normalized to the DOC concentration, can be used to calculate the relative contribution of vascular plant-derived material to the DOM pool [*Hedges et al.*, 2000; *Hernes et al.*, 2008]. In riverine systems, dissolved

lignin measurements have been employed to highlight changes in DOM sources with variation in hydrologic conditions [*Dalzell et al.*, 2005; *Hernes et al.*, 2008; *Spencer et al.*, 2008]. Previous studies in high-latitude northern rivers dominated by allochthonous DOM inputs have shown that temporal variability in lignin carbon-normalized yields is linked to DOM lability and photoreactivity [*Holmes et al.*, 2008; *Spencer et al.*, 2008; *Osburn et al.*, 2009]. Thus, utilizing optical proxies for biomarkers such as lignin phenols increases our capacity to characterize ecosystem functioning by identifying predominant DOM sources and processes that drive observed variability in aquatic systems at relevant temporal and spatial scales.

[4] Recent DOM studies have focused disproportionately on quantity and quality in temperate and northern high-latitude rivers due to pertinent questions about anthropogenic and climate-driven changes in these systems [*Worrall et al.*, 2003; *Evans et al.*, 2007; *Raymond et al.*, 2007]. However, tropical rainforest systems have by far the highest riverine DOC fluxes to the oceans [*Aitkenhead and McDowell*,
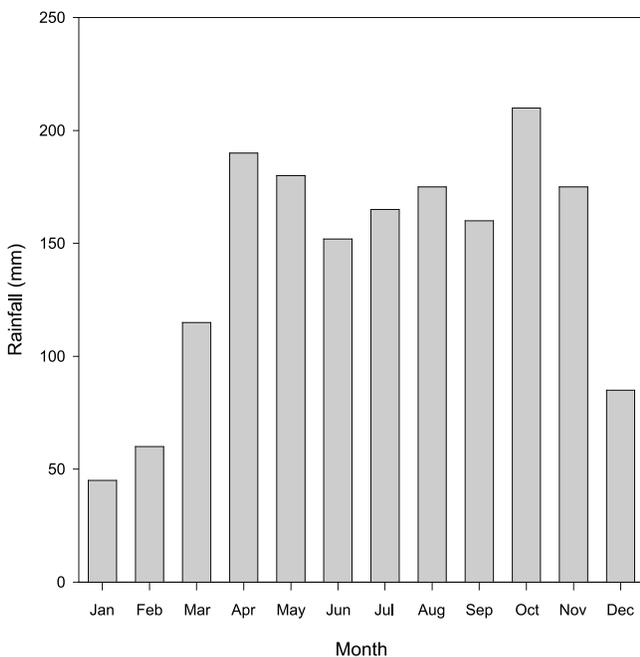
Figure 2. Annual variation in mean monthly rainfall data [adapted from *Katuala et al.*, 2005] in the Okapi Wildlife Reserve (Ituri Forest).

2000] and have received little attention with respect to temporal variability in DOM quality. The second largest area of tropical rainforest in the world is found in central Africa, and within this rainforest, the Congo River Basin includes vast and unbroken regions from the Atlantic Ocean to the Albertine Rift [*Duveiller et al.*, 2008]. Political instability and limited infrastructure have severely inhibited research efforts in this region [*Baccini et al.*, 2008], thereby limiting our fundamental knowledge of biogeochemical cycling in the Congo River Basin. This study examined the Epulu River, a typical river draining pristine tropical rainforest in central Africa. The aim of this study was to investigate temporal variation in the quantity and chemical composition of DOM, which in turn was used to identify source materials and to address how important seasonality is in the biogeochemical cycling of DOM derived from tropical riverine ecosystems.

2. Materials and Methods

2.1. Study Site

[5] The Epulu River was sampled near the Okapi Breeding and Research Station (1°24′N, 28°34′E) near Epulu in the Okapi Wildlife Reserve in the northeast of the Democratic Republic of Congo (Figure 1). The Okapi Wildlife Reserve is a World Heritage Site that covers an area of ~13,700 km^2 in the Ituri Forest. The Epulu River is one of two main rivers that drain the Okapi Wildlife Reserve in the Ituri Forest. It joins the other main river (the Ituri) and takes its name as it flows westward and later becomes the Aruwimi River, which continues westward and joins the Congo River at Basoko. The Ituri Forest (~70,000 km^2) exists in a pristine state and due to the density of its vegetation, has among the highest carbon content per hectare of any rainforest [*Koenig*, 2008]. Vegetation within the Ituri Forest is dominated by

two types of tropical moist forest (mixed forest and mono-dominant forest) with small areas of swamp forest in areas of poor drainage [*Makana et al.*, 2004; *Katuala et al.*, 2005]. Human impacts within the region are minimal (e.g., traditional hunting-gathering), and the population density is low (~1.5 persons/km^2). A lack of transportation and recent conflict in the region has restricted logging to the forest edge and the banks of navigable rivers [*Makana et al.*, 2004]. Within the central African countries, the Democratic Republic of Congo has the lowest amount of forest disturbance within its borders, with just 1% currently disturbed [*Laporte et al.*, 2007].

[6] Climatic records for the region are not detailed, and no discharge data exist for the rivers in the Ituri Forest; however, hydrologic regimes can be examined by utilizing existing rainfall data. The annual rainfall is estimated to be between ~1300 and 2100 mm (typically 1650–1800 mm), with April, May, and August through October as the wettest months while December, January, and February (the dry season) typically receive less than 100 mm of precipitation per month [*Makana et al.*, 2004; *Katuala et al.*, 2005, and references therein]. Mean monthly rainfall data collected in Epulu from 2003 to 2008 follows the long-term trends observed previously [*Katuala et al.*, 2005, and references therein] (Figure 2) and the mean annual rainfall for 2003–2008 was ~1670 mm. Temperature shows little variation in the region with a mean annual temperature of ~24°C (mean monthly temperature ~ 22.8–24.6°C) [*Katuala et al.*, 2005, and references therein].

2.2. Water Sample Collection and Processing

[7] Water samples were collected during three different periods of the year (9 or 10 days either consecutive to one another or within a 12 day period). The three sampling periods corresponded to different stages of the hydrologic regime of the area (flushing period, intermediary, and the start of the dry period/post flush). The high-discharge flushing period was sampled at the start of the April/May wet period (16–27 April 2008) for 10 days out of the 12 day period. The intermediary period was sampled daily toward the end of the dry season (February) when individual small storms occurred (5–13 February 2008). The post flush/onset of the dry period was sampled for 10 days after some of the wettest months (August through October) at the onset of the dry season, typically during the middle to end of November/ beginning of December (28 November to 6 December 2007). Water samples were collected from the surface of the river in midchannel via a precleaned and prerinsed 1 L Nalgene bottle and filtered immediately through precombusted (450°C) 0.7 μm glass fiber filters (Whatman, GF/F). Samples were stored frozen, kept in the dark, and shipped on ice to the University of California-Davis (USA) for further analyses.

2.3. Dissolved Organic Carbon and Chromophoric Dissolved Organic Matter Analyses

[8] Dissolved organic carbon was measured on filtered water samples using a high-temperature catalytic oxidation (HTCO) Shimadzu TOC 5000A instrument and methodology as described in *Spencer et al.* [2007a]. All reported DOC data are the mean of three to five replicate injections

Table 1. Epulu River Dissolved Organic Carbon, Chromophoric Dissolved Organic Matter, Specific UV Absorbance at 254 nm, and Fluorescence Index Data[a]

Sampling Period	Date	DOC (mg L^{-1})	$S_{275-295}$	$S_{350-400}$	S_R	SUVA$_{254}$ (L mg C^{-1} m^{-1})	FI
Wet/flushing	16 Apr 2008	9.0	0.01453	0.01824	0.796	3.57	1.22
Wet/flushing	17 Apr 2008	8.7	0.01464	0.01836	0.797	3.45	1.28
Wet/flushing	18 Apr 2008	8.5	0.01476	0.01828	0.807	3.34	1.24
Wet/flushing	19 Apr 2008	7.6	0.01481	0.01794	0.826	3.38	1.29
Wet/flushing	20 Apr 2008	6.8	0.01520	0.01771	0.858	3.34	1.30
Wet/flushing	21 Apr 2008	6.9	0.01512	0.01775	0.852	3.35	1.30
Wet/flushing	23 Apr 2008	8.0	0.01487	0.01806	0.823	3.43	1.27
Wet/flushing	25 Apr 2008	7.2	0.01492	0.01711	0.872	3.36	1.28
Wet/flushing	26 Apr 2008	6.9	0.01479	0.01707	0.867	3.34	1.29
Wet/flushing	27 Apr 2008	6.6	0.01463	0.01735	0.843	3.29	1.33
Intermediary	05 Feb 2008	7.4	0.01484	0.01716	0.865	3.33	1.30
Intermediary	06 Feb 2008	6.8	0.01459	0.01600	0.912	3.30	1.31
Intermediary	07 Feb 2008	6.0	0.01475	0.01604	0.920	3.27	1.38
Intermediary	08 Feb 2008	5.2	0.01491	0.01562	0.954	3.21	1.41
Intermediary	09 Feb 2008	5.5	0.01533	0.01571	0.976	3.17	1.44
Intermediary	10 Feb 2008	5.4	0.01497	0.01614	0.928	3.19	1.43
Intermediary	11 Feb 2008	7.8	0.01485	0.01662	0.893	3.29	1.27
Intermediary	12 Feb 2008	8.4	0.01443	0.01569	0.920	3.31	1.25
Intermediary	13 Feb 2008	8.0	0.01454	0.01595	0.912	3.29	1.25
Dry period/post flush	28 Nov 2007	5.3	0.01470	0.01518	0.968	3.09	1.45
Dry period/post flush	29 Nov 2007	6.0	0.01431	0.01464	0.978	3.12	1.42
Dry period/post flush	30 Nov 2007	5.5	0.01463	0.01484	0.986	3.08	1.45
Dry period/post flush	01 Dec 2007	5.3	0.01417	0.01354	1.046	3.09	1.45
Dry period/post flush	02 Dec 2007	5.4	0.01440	0.01352	1.066	3.13	1.43
Dry period/post flush	03 Dec 2007	5.4	0.01479	0.01409	1.050	3.11	1.42
Dry period/post flush	04 Dec 2007	6.6	0.01473	0.01498	0.983	3.15	1.39
Dry period/post flush	05 Dec 2007	5.6	0.01469	0.01459	1.007	3.16	1.41
Dry period/post flush	06 Dec 2007	5.7	0.01412	0.01497	0.944	3.20	1.41

[a]DOC, dissolved organic carbon; CDOM, chromophoric dissolved organic matter (spectral slope $S_{275-295}$ and $S_{350-400}$ and their ratio S_R); SUVA$_{254}$, specific UV absorbance at 254 nm; FI, fluorescence index.

with a coefficient of variance of less than 2%. For both CDOM absorbance and fluorescence measurements, all samples were measured in a 10 mm quartz cuvette at a constant laboratory temperature of 20°C, in duplicate and with ultrapure laboratory water (Milli-Q, Millipore) used as a blank. CDOM absorbance was measured with a Shimadzu UV-2501PC UV/Vis dual-beam spectrophotometer and Naperian absorbance coefficients (a m^{-1}) were determined following *Hu et al.* [2002]. SUVA$_{254}$ values were calculated by dividing the UV absorbance (A) at $\lambda = 254$ nm by the DOC concentration (mg L^{-1}) [*Weishaar et al.*, 2003]. Spectral slope (S) was calculated using a nonlinear fit of an exponential function to the absorption spectrum over the ranges 275–295 and 350–400 nm. These ranges were chosen because *Helms et al.* [2008] observed in an extensive study of S across a range of aquatic ecosystems and DOM sources that the first derivative of the natural log spectra indicated the largest variations in these ranges. The spectral slope ratio (S_R) was calculated as the ratio of $S_{275-295}$ to $S_{350-400}$ [*Helms et al.*, 2008]. DOM fluorescence spectra were measured on a Varian Cary Eclipse spectrofluorometer with a xenon excitation source and slits were set to 5 nm for both excitation and emission. A single emission scan was obtained from 400 to 550 nm at an excitation wavelength of 370 nm, and fluorescence intensity was blank corrected and also corrected for instrument specific bias and any inner filter effects [*Cory and McKnight*, 2005; *Spencer et al.*, 2007a]. Fluorescence index (FI) was then calculated as the ratio of fluorescence emission wavelength intensities at 470

and 520 nm at an excitation wavelength of 370 nm [*McKnight et al.*, 2001; *Cory and McKnight*, 2005]. Samples ranged in pH from 5.68 to 5.97 and, thus, were within the natural range typically observed in freshwaters in which the response of fluorescence to pH has been previously shown to be limited [*Ahmad and Reynolds*, 1999; *Spencer et al.*, 2007b]. As samples were stored frozen, the impact of freeze/thaw (thawing was conducted in the dark in a refrigerator at 4°C) was examined on DOC and CDOM parameters. After freezing and subsequent thawing DOC, a_{350}, $S_{275-295}$, $S_{350-400}$, S_R, SUVA$_{254}$, and FI exhibited changes typically within analytical error and always less than ±2%.

2.4. Lignin Phenol Analysis

[9] Lignin phenols were measured on whole water samples (~150 mL) acidified to pH 2 with 12 N HCl to minimize precipitation during rotary evaporation, and then rotary evaporated to ~3 mL. The concentrate was transferred to monel reaction vessels (Prime Focus, Inc.) and dried under vacuum centrifugation in preparation for lignin analysis. Lignin phenols were determined by alkaline CuO oxidation, followed by acidification and ethyl acetate extraction [*Hedges and Ertel*, 1982; *Spencer et al.*, 2008]. Quantification was carried out on a GC-MS (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector and a DB5-MS capillary column; 30 m, 0.25 mm inner diameter, Agilent) using cinnamic acid as an internal standard and a five-point calibration scheme
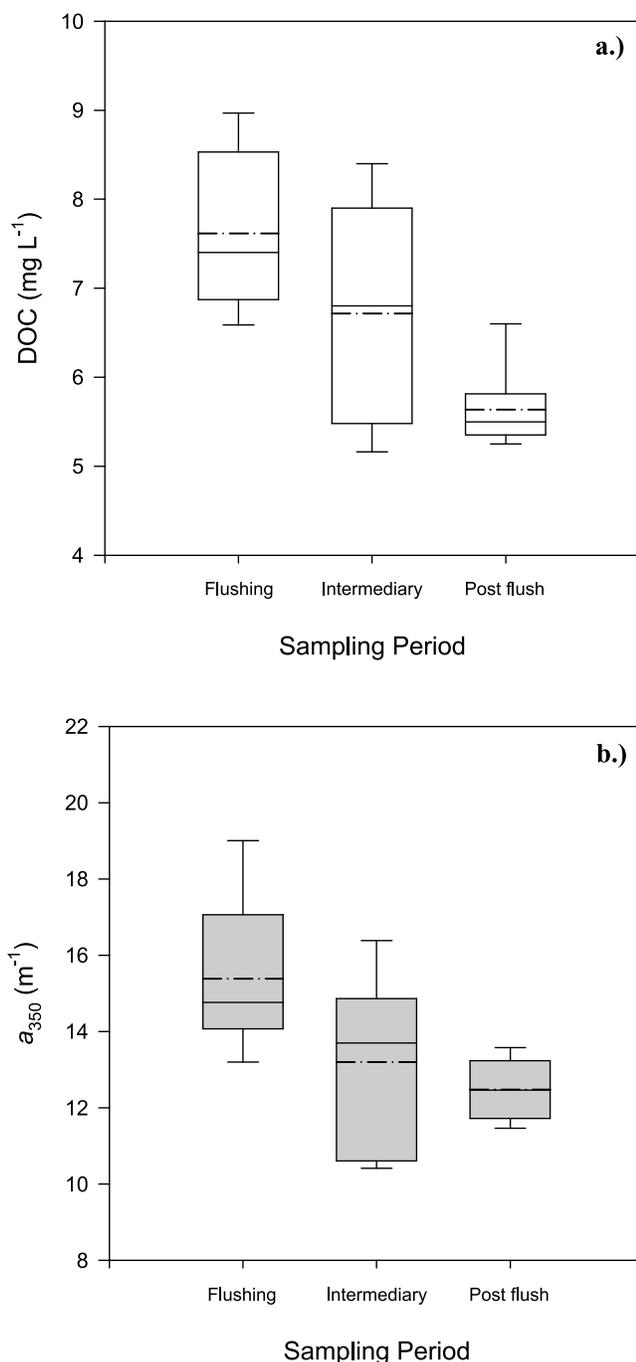
Figure 3. Box plots of (a) DOC and (b) a_{350} during the flushing, intermediary, and post flush sampling periods. The black dash-dotted line and the solid black line in the box represent the mean and the median, respectively. The horizontal edges of the box denote the 25th and 75th percentiles, and the error bars denote the 10th and 90th percentiles.

[*Hernes and Benner*, 2002]. Eight lignin phenols were quantified for all samples, including three vanillyl phenols (vanillin, acetovanillone, and vanillic acid), three syringyl phenols (syringaldehyde, acetosyringone, and syringic acid), and two cinnamyl phenols (*p*-coumaric acid and ferulic

acid). One blank was run for every 10 sample oxidations, and all samples were blank corrected. Blank concentrations of lignin phenols were low (30–50 ng) and, thus, never exceeded 2% of the total lignin phenols in a sample.

3. Results

3.1. Dissolved Organic Carbon

[10] Epulu River DOC concentrations ranged from 5.2 to 9.0 mg L^{-1} (mean = 6.7 mg L^{-1}; n = 28; Table 1 and Figure 3a) dependent on the time of sampling in the annual hydrological cycle. Concentrations of DOC from the dry period/post flush were consistently lower, ranging from 5.3 to 6.6 mg L^{-1} (mean = 5.6 mg L^{-1}; n = 9), increasing in the intermediary period to 5.2–8.4 mg L^{-1} (mean = 6.7 mg L^{-1}; n = 9), with highest DOC values in the flushing period ranging from 6.6 to 9.0 mg L^{-1} (mean = 7.6 mg L^{-1}; n = 9) (Table 1 and Figure 3a).

3.2. Chromophoric Dissolved Organic Matter

[11] Chromophoric DOM absorption coefficients at 350 nm ranged from 10.41 to 19.20 m^{-1} (mean = 13.75 m^{-1}; n = 28; Figure 3b) in the Epulu River. Similar to DOC, CDOM varied seasonally, with a_{350} exhibiting highest values during the flushing period ranging from 13.12 to 19.20 m^{-1} (mean = 15.38 m^{-1}; n = 10), lowest values in the dry period/post flush ranging from 11.47 to 13.58 m^{-1} (mean = 12.48 m^{-1}; n = 9), and with the intermediary period in between, with values ranging from 10.41 to 16.39 m^{-1} (mean = 13.20 m^{-1}; n = 9) (Figure 3b).

[12] Spectral slope calculated over the range 275–295 nm ($S_{275–295}$) showed little variation in the Epulu River samples ranging from 0.01533 to 0.01412 nm^{-1} (mean = 0.01472 nm^{-1}; n = 28; Table 1) and no significant difference between samples from different times of year. However, $S_{350–400}$ ranged from 0.01836 to 0.01352 nm^{-1} (mean = 0.01618 nm^{-1}; n = 28; Table 1) over the year. In contrast to $S_{275–295}$, $S_{350–400}$ showed seasonal variability with steeper S values associated with the flushing period ranging from 0.01836 to 0.01707 nm^{-1} (mean = 0.01779 nm^{-1}; n = 10) but shallower in the intermediary period ranging from 0.01716 to 0.01562 nm^{-1} (mean = 0.01610 nm^{-1}; n = 9). The shallowest $S_{350–400}$ values were observed in the dry period/post flush ranging from 0.01518 to 0.01352 nm^{-1} (mean = 0.01448 nm^{-1}; n = 9). The S_R in the Epulu River ranged from 0.796 to 1.066 (mean = 0.916; n = 28; Table 1 and Figure 4a), with seasonal variations driven by changes in $S_{350–400}$. The lowest S_R values were observed in the flushing period ranging from 0.796 to 0.872 (mean = 0.834; n = 10) and the highest values in the dry period/post flush ranging from 0.944 to 1.066 (mean = 1.003; n = 9), whereas S_R ranged from 0.865 to 0.976 (mean = 0.920; n = 9) in the intermediary period.

[13] SUVA$_{254}$ values ranged from 3.08 to 3.57 L mg C^{-1} m^{-1} (mean = 3.26 L mg C^{-1} m^{-1}; n = 28; Table 1 and Figure 4b). Seasonal variability was observed in SUVA$_{254}$ with highest values during the flushing period, ranging from 3.29 to 3.57 L mg C^{-1} m^{-1} (mean = 3.39 L mg C^{-1} m^{-1}; n = 10), whereas the lowest values were observed in the dry period/post flush ranging from 3.08 to 3.20 L mg C^{-1} m^{-1} (mean = 3.13 L mg C^{-1} m^{-1}; n = 9). In the intermediary period SUVA$_{254}$ values fell in between the range
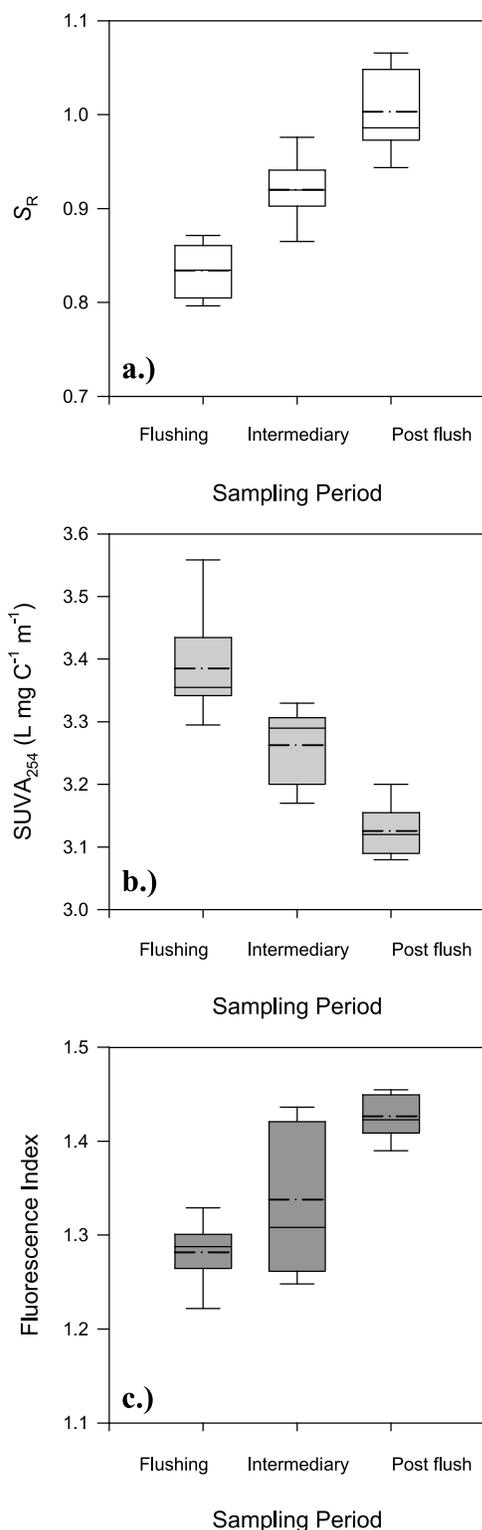
Figure 4. Box plots of (a) spectral slope ratio S_R, (b) SUVA$_{254}$, and (c) fluorescence index during the flushing, intermediary, and post flush sampling periods. The black dash-dotted line and the solid black line in the box represent the mean and the median, respectively. The horizontal edges of the box denote the 25th and 75th percentiles, and the error bars denote the 10th and 90th percentiles.

shown in the flushing and dry/post flush periods ranging from 3.17 to 3.33 L mg C^{-1} m^{-1} (mean = 3.26 L mg C^{-1} m^{-1}; $n = 9$).

[14] The fluorescence index (FI) ranged from 1.22 to 1.45 (mean = 1.35; $n = 28$; Table 1 and Figure 4c). As with the parameters derived from CDOM absorbance, the FI also showed seasonal variability in the Epulu River samples. Samples taken during the flushing period had the lowest values ranging from 1.22 to 1.33 (mean = 1.28; $n = 10$) and samples from the dry period/post flush had the highest values ranging from 1.39 to 1.45 (mean = 1.43; $n = 9$). As with the CDOM absorbance-derived parameters, the FI of samples from the intermediary period was within the ranges shown during the flushing and dry/post flush periods ranging from 1.25 to 1.44 (mean = 1.34; $n = 9$).

3.3. Lignin Concentrations and Carbon-Normalized Yields

[15] Lignin concentrations (Σ_8) and carbon-normalized yields (Λ_8) are presented as the sum of three vanillyl phenols, three syringyl phenols, and two cinnamyl phenols. Σ_8 for the Epulu River samples ranged from 41.9 to 131.9 μg L^{-1} (mean = 77.8 μg L^{-1}; $n = 26$; Table 2 and Figure 5a). Λ_8 and the carbon-normalized vanillyl yields (V) for Epulu River samples ranged from 0.78 to 1.47 (mg (100 mg OC)$^{-1}$) (mean = 1.14 (mg (100 mg OC)$^{-1}$); $n = 26$) and 0.39 to 0.81 (mg (100 mg OC)$^{-1}$) (mean = 0.59 (mg (100 mg OC)$^{-1}$); $n = 26$), respectively (Table 2 and Figures 5b and 5c). Σ_8, Λ_8, and V all exhibited temporal variation with highest concentrations or yields in the flushing period (mean = 97.6 μg L^{-1}, 1.27 (mg (100 mg OC)$^{-1}$) and 0.68 (mg (100 mg OC)$^{-1}$), respectively), declining in the intermediary period (mean = 76.1 μg L^{-1}, 1.15 (mg (100 mg OC)$^{-1}$) and 0.60 (mg (100 mg OC)$^{-1}$), respectively) and with lowest values in the dry period/post flush (mean = 54.6 μg L^{-1}, 0.96 (mg (100 mg OC)$^{-1}$) and 0.46 (mg (100 mg OC)$^{-1}$), respectively) (Table 2 and Figure 5).

3.4. Lignin Composition

[16] Cinnamyl/vanillyl (C/V) and syringyl/vanillyl phenol (S/V) ratios in the Epulu River samples ranged from 0.10 to 0.16 (mean = 0.12; $n = 26$) and 0.71 to 0.84 (mean = 0.79; $n = 26$), respectively (Table 2). The ratios of vanillic acid to vanillin (Ad/Al)$_v$ and syringic acid to syringaldehyde (Ad/Al)$_s$ ranged from 1.08 to 1.17 (mean = 1.13; $n = 26$) and 0.80 to 0.91 (mean = 0.86; $n = 26$), respectively (Table 2). The C/V, S/V, (Ad/Al)$_v$, and (Ad/Al)$_s$ ratios did not show any significant temporal trends.

4. Discussion

4.1. Epulu River Dissolved Organic Matter Temporal Trends

[17] The DOC and dissolved lignin phenol concentrations (Σ_8) in the Epulu River are high in comparison to other studied rivers (Tables 1 and 2 and Figures 3a and 5a) and highlight the organic-rich nature of the Ituri tropical rainforest ecosystem [Koenig, 2008]. Epulu River DOC concentrations are comparable to previously reported DOC concentrations in the Congo River and its tributaries [Coynel et al., 2005; Spencer et al., 2009a] and in other rivers draining tropical rainforest [Hedges et al., 1994; Battin,

Table 2. Epulu River Dissolved Organic Carbon, Lignin Phenol Concentration, Carbon-Normalized Yield, Vanillyl Carbon-Normalized Yield, Cinnamyl/Vanillyl Phenol Ratios, Syringyl/Vanillyl Phenol Ratios, Vanillic Acid/Vanillin Ratios, and Syringic Acid/Syringaldehyde Ratios[a]

Sampling Period	Date	DOC (mg L^{-1})	Σ_8 (μg L^{-1})	Λ_8 (mg (100 mg OC)$^{-1}$)	V (mg (100 mg OC)$^{-1}$)	C/V	S/V	(Ad/Al)$_v$	(Ad/Al)$_s$
Wet/flushing	16 Apr 2008	9.0	131.9	1.47	0.81	0.10	0.80	1.11	0.86
Wet/flushing	17 Apr 2008	8.7	125.2	1.44	0.76	0.10	0.79	1.15	0.88
Wet/flushing	18 Apr 2008	8.5	107.8	1.27	0.71	0.10	0.71	1.15	0.87
Wet/flushing	19 Apr 2008	7.6	94.4	1.24	0.66	0.10	0.79	1.14	0.91
Wet/flushing	20 Apr 2008	6.8	83.0	1.21	0.66	0.10	0.76	1.14	0.83
Wet/flushing	21 Apr 2008	6.9	84.5	1.23	0.66	0.10	0.77	1.12	0.84
Wet/flushing	23 Apr 2008	8.0	111.9	1.39	0.72	0.12	0.81	1.12	0.85
Wet/flushing	25 Apr 2008	7.2	91.4	1.27	0.66	0.12	0.81	1.11	0.85
Wet/flushing	26 Apr 2008	6.9	77.3	1.12	0.59	0.12	0.80	1.13	0.86
Wet/flushing	27 Apr 2008	6.6	68.8	1.05	0.60	0.10	0.78	1.09	0.82
Intermediary	05 Feb 2008	7.4	78.6	1.07	0.57	0.10	0.77	1.11	0.82
Intermediary	06 Feb 2008	6.8	90.0	1.32	0.68	0.11	0.84	1.14	0.84
Intermediary	07 Feb 2008	6.0	64.8	1.09	0.57	0.12	0.80	1.08	0.84
Intermediary	08 Feb 2008	5.2	54.1	1.05	0.54	0.14	0.82	1.08	0.83
Intermediary	09 Feb 2008	5.5	51.8	0.94	0.49	0.12	0.79	1.12	0.81
Intermediary	10 Feb 2008	5.4	60.4	1.11	0.57	0.14	0.80	1.10	0.85
Intermediary	11 Feb 2008	7.8	101.6	1.31	0.69	0.11	0.78	1.12	0.88
Intermediary	12 Feb 2008	8.4	107.2	1.28	0.68	0.11	0.76	1.17	0.89
Intermediary	13 Feb 2008	8.0	-	-	-	-	-	-	-
Dry period/post flush	28 Nov 2007	5.3	46.8	0.88	0.45	0.12	0.79	1.16	0.89
Dry period/post flush	29 Nov 2007	6.0	60.5	1.02	0.49	0.15	0.81	1.11	0.88
Dry period/post flush	30 Nov 2007	5.5	56.0	1.02	0.48	0.11	0.78	1.17	0.86
Dry period/post flush	01 Dec 2007	5.3	-	-	-	-	-	-	-
Dry period/post flush	02 Dec 2007	5.4	41.9	0.78	0.39	0.16	0.78	1.12	0.86
Dry period/post flush	03 Dec 2007	5.4	52.0	0.96	0.49	0.11	0.79	1.16	0.80
Dry period/post flush	04 Dec 2007	6.6	66.2	1.00	0.45	0.11	0.80	1.15	0.89
Dry period/post flush	05 Dec 2007	5.6	54.2	0.96	0.44	0.13	0.80	1.11	0.90
Dry period/post flush	06 Dec 2007	5.7	59.3	1.04	0.50	0.13	0.79	1.10	0.85

[a]DOC, dissolved organic carbon; Σ_8, lignin phenol concentration; Λ_8, carbon-normalized yield; V, vanillyl carbon-normalized yield; C/V, cinnamyl/vanillyl phenol ratios; S/V, syringyl/vanillyl phenol ratios; (Ad/Al)$_v$, vanillic acid/vanillin ratios; (Ad/Al)$_s$, syringic acid/syringaldehyde ratios.

1998]. The mean Σ_8 value for the Epulu River of 77.8 μg L^{-1} is comparable to concentrations for the main stem of the Congo River (76.5 μg L^{-1}) [*Spencer et al.*, 2009a], blackwater tributaries of the Amazon (up to 72 μg L^{-1}) [*Ertel et al.*, 1986], and the Yukon River at the height of the spring flush (up to 73.5 μg L^{-1}) [*Spencer et al.*, 2008]. Maximum DOC concentrations during flushing events as observed in the Epulu River have been reported previously in a diverse range of watersheds including other tropical rivers [*Newbold et al.*, 1995; *Coynel et al.*, 2005], temperate rivers [*Inamdar et al.*, 2006; *Fellman et al.*, 2009], and northern high-latitude rivers [*Raymond et al.*, 2007; *Striegl et al.*, 2007]. As with DOC, the highest Σ_8 values for the Epulu River occurred during the April flushing period (up to 131.9 μg L^{-1}; Table 2 and Figure 5a) and are comparable to values from blackwater tributaries of the Yukon River (e.g., Hess Creek, 124.4 μg L^{-1} and Black River, 114.9 μg L^{-1}) during the spring flush period [*Spencer et al.*, 2008] and storm event samples from a Californian agricultural catchment (107 μg L^{-1}) [*Hernes et al.*, 2008]. To further assess relative contributions of vascular plant-derived material to organic matter pools, carbon-normalized lignin yields (Λ_8) are frequently used. The mean Λ_8 value of 1.14 (mg (100 mg OC)$^{-1}$) in the Epulu River is high when compared to previously studied rivers, including the main stem of the Congo River (0.72 (mg (100 mg OC)$^{-1}$)) [*Spencer et al.*, 2009a]. This elevated Λ_8 is indicative of high vascular plant inputs to the Epulu River.

[18] Carbon-normalized yields based on solely the vanillyl phenols (V) has recently been suggested by *Hernes et al.* [2007] as a representative end-member for estimation of vascular plant inputs to DOM in riverine systems, as it removes the variation in sources and reactivity associated with syringyl and cinnamyl phenols. In the Epulu River samples, V was typically greater than the combined sum of the carbon-normalized syringyl and cinnamyl phenol yields (Table 2) as has been observed in other rivers [*Lobbes et al.*, 2000; *Spencer et al.*, 2008, 2009a]. The Epulu River mean V yield of 0.59 (mg (100 mg OC)$^{-1}$) is comparable to that of 0.62 (mg (100 mg OC)$^{-1}$) observed in storm events in a Californian agricultural catchment by *Hernes et al.* [2007] and 0.67 (mg (100 mg OC)$^{-1}$) in high molecular weight (HMW) DOC in the Amazon River [*Hedges et al.*, 2000]. Employing the mean leachate/sorption end-member V yield of 1.53 (mg (100 mg OC)$^{-1}$) from an assorted range of angiosperm and gymnosperm sources [*Hernes et al.*, 2007] and the Epulu River mean V yield, it is estimated that the vascular plant component of DOC is 39% in the Epulu River. Utilizing this end-member V yield for samples from the flushing period (mean V yield = 0.68 (mg (100 mg OC)$^{-1}$)) and dry period/post flush (mean V yield = 0.46 (mg (100 mg OC)$^{-1}$)) results in an estimated vascular plant component of DOC as 44% and 30%, respectively, demonstrating temporal variation in the Epulu River with highest inputs during the flushing period in April. However, the actual vascular plant component of the Epulu River
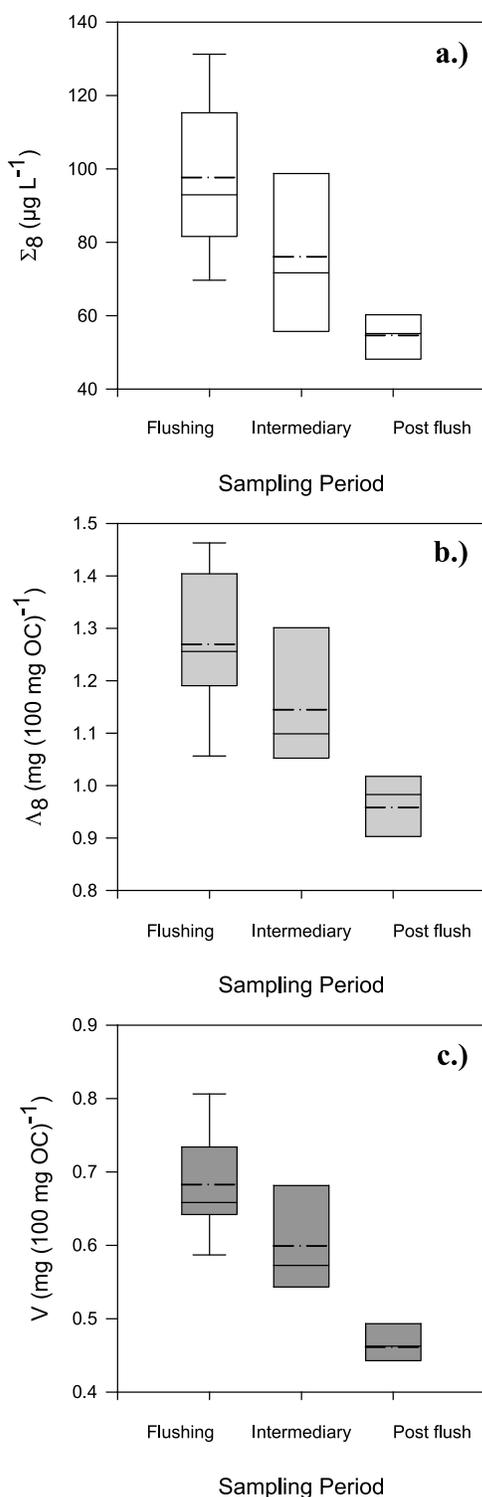
Figure 5. Box plots of (a) lignin phenol concentration (Σ_8), (b) carbon-normalized yield (Λ_8), and (c) vanillyl carbon-normalized yield (V) during the flushing, intermediary, and post flush sampling periods. The black dash-dotted line and the solid black line in the box represent the mean and the median, respectively. The horizontal edges of the box denote the 25th and 75th percentiles, and the error bars denote the 10th and 90th percentiles.

could be even higher than that estimated here if DOM sources within the catchment generate lower carbon-normalized yields [*Spencer et al.*, 2008], and this seems likely in an organic-rich tropical river such as the Epulu.

[19] The ratio of spectral slopes (S_R) was developed as a rapid and easily reproducible method for characterizing CDOM and has been correlated to molecular weight and source, i.e., samples of greater allochthonous character with higher molecular weight DOM have lower S_R values [*Helms et al.*, 2008]. The range of S_R in the Epulu River samples (0.796–1.066; Table 1 and Figure 4a) is comparable to that from other allochthonous dominated freshwater systems [*Helms et al.*, 2008; *Spencer et al.*, 2009a, 2009b]. SUVA$_{254}$ has been positively correlated to the percentage of aromaticity of DOM [*Weishaar et al.*, 2003] and the range of values observed in the Epulu River are comparable to the temporal variation observed in other rivers (Table 1 and Figure 4b) [*Hood et al.*, 2005; *Spencer et al.*, 2008; *Fellman et al.*, 2009]. The fluorescence index (FI) changes with aromaticity and indicates the relative contribution of lower molecular weight nonaromatic DOM versus HMW aromatic DOM [*McKnight et al.*, 2001; *Cory and McKnight*, 2005; *Asrat et al.*, 2007]. For example, fulvic acids with aromaticities of 12%–17% yielded FI values of 1.7–2.0, while fulvic acid samples including Suwannee River with aromaticities of 25%–30% yielded FI values of 1.3–1.4 [*McKnight et al.*, 2001]. FI is conceptually similar to utilization of the emission wavelength of the fulvic-like fluorophore (excitation 300–350 nm, emission 400–460 nm), which has also been linked to DOM hydrophobicity and its functional properties [*Baker et al.*, 2008a] and DOM source [*Spencer et al.*, 2007a]. The range of FI values in the Epulu River (1.22–1.45) are typical of those observed in allochthonous dominated rivers (Table 1 and Figure 4c) [*McKnight et al.*, 2001; *Cory et al.*, 2007]. Fluorescence index was observed to be linearly correlated to SUVA$_{254}$ and S_R ($r^2 = 0.81$; $p < 0.001$ and 0.70; $p < 0.001$, respectively; $n = 28$) and SUVA$_{254}$ and S_R were linearly correlated to one another ($r^2 = 0.83$; $p < 0.001$; $n = 28$), emphasizing the common drivers of aromaticity and molecular weight underlying these measurements.

[20] The observed temporal variation in Epulu River DOC, Σ_8, Λ_8, and V with elevated values in the flushing period, declining in the intermediary period and exhibiting lowest values in the dry period/post flush (Tables 1 and 2 and Figures 3a and 5) is indicative of different DOM source pools, hydrologic flow paths, and residence times between the sampling periods [*McGlynn and McDonnell*, 2003; *Striegl et al.*, 2005]. The highest riverine DOC, Σ_8, Λ_8, and V values are observed during the period of high rainfall after the dry period due to increased surface runoff and leaching of organic rich horizons [*Neff et al.*, 2006; *Spencer et al.*, 2008] during the April flushing period. For example, during this flushing period, the highest Λ_8 values (Table 2 and Figure 5b) are comparable to those observed by *Hernes et al.* [2008] (up to 1.6 (mg (100 mg OC)$^{-1}$)), during storm events in a Californian agricultural catchment concurrent with strong leaching of surface vegetation and organic rich soil layers. Conversely, lower DOC, Σ_8, Λ_8, and V values are observed in the dry period/post flush likely due to increased flow path, residence time, and, thus greater microbial mineralization of DOM [*Striegl et al.*, 2005], as well as due to previous extensive flushing of the organic rich

horizons during the preceding wet period (August through October; Figure 2). The extensive flushing of the organic-rich surface layers in the August through October wet period subsequently contributes to lower Λ_8 and V yields during the dry period/post flush sampling period, as lignin phenols have been shown to leach at a faster rate than the bulk DOC pool [*Spencer et al.*, 2009b]. The intermediary period shows a large range of DOC, Σ_8, Λ_8, and V values driven by storm pulses as this period undergoes flushing and drying cycles during the transition out of the dry season. Such variation in the DOM source pools and processing has previously been shown in temperate and northern high-latitude rivers to impact DOC concentration and to cause shifts in the biochemical composition, age, and reactivity of DOM [*Holmes et al.*, 2008; *Fellman et al.*, 2009; *Sanderman et al.*, 2009].

[21] The larger relative terrestrial signature as evidenced by the higher Λ_8 and V values in the Epulu River during the April flushing period is supported by the trends in the CDOM data. The lowest S_R, highest SUVA$_{254}$, and lowest FI values (Table 1 and Figure 4) observed during the post dry flushing period indicate that DOM in this period is enriched in HMW compounds and aromatic moieties compared to other times of year due to the leaching of fresh organic rich layers [*Neff et al.*, 2006; *Spencer et al.*, 2008]. The highest FI and S_R values and lowest SUVA$_{254}$ values were found in the dry period/post flush, indicating that DOM is relatively less aromatic in nature at this time due to a shift in source pools (i.e., this material is already heavily leached from the earlier wet period of August through October), hydrologic flow paths, and residence times between the sampling periods [*McGlynn and McDonnell*, 2003; *Striegl et al.*, 2005]. Therefore DOM in the Epulu River exhibits temporal variations in its composition, which presumably have a significant impact upon its reactivity in aquatic ecosystems.

4.2. Epulu River Lignin Composition Temporal Trends

[22] The ratios of syringyl (S) and cinnamyl (C) phenols to the ubiquitous vanillyl phenols (V) are used to discriminate between angiosperm and gymnosperm sources (S/V) and between nonwoody and woody tissues (C/V) as syringyl phenols and cinnamyl phenols are specific to angiosperms and nonwoody tissues, respectively [*Hedges and Mann*, 1979]. In the Epulu River samples, these ratios showed no temporal trends and mean ratios of C/V (0.12) and S/V (0.79) are comparable to those observed in the Congo River [0.15 and 0.68, respectively; *Spencer et al.*, 2009a]. The C/V and S/V ratios in the Epulu River point toward the dominance of angiosperm sources in the region [*Hart*, 1995; *Makana et al.*, 2004], if no phase change fractionation or degradation has taken place [*Hedges and Mann*, 1979; *Hernes et al.*, 2007]. The ratios of vanillic acid to vanillin (Ad/Al)$_v$ and syringic acid to syringaldehyde (Ad/Al)$_s$ have been shown to have increased relative yields in degradation experiments [*Opsahl and Benner*, 1995; *Hernes and Benner*, 2003; *Spencer et al.*, 2009a]. *Hernes et al.* [2007] also showed that the processes of leaching and sorption can result in the fractionation of lignin phenols including the relative increase of (Ad/Al)$_v$ and (Ad/Al)$_s$, thus, these ratios may not be indicative solely of degradation in riverine studies. Epulu River (Ad/Al)$_v$ and (Ad/Al)$_s$ ratios showed no

temporal trends and mean ratios of 1.13 and 0.86, respectively. These (Ad/Al)$_v$ and (Ad/Al)$_s$ ratios are lower than those observed in the Congo River main stem (1.38 and 1.11, respectively) [*Spencer et al.*, 2009a] and may reflect fresher DOM in the small tributaries with lower residence times and degradation or other sources throughout the Congo Basin with elevated (Ad/Al)$_v$ and (Ad/Al)$_s$ ratios. Previous riverine studies employing lignin have shown (Ad/Al)$_v$ and (Ad/Al)$_s$ ratios that are comparable to each other from a diverse range of systems [*Lobbes et al.*, 2000; *Hedges et al.*, 2000; *Hernes et al.*, 2008], thus suggesting that (Ad/Al)$_v$ and (Ad/Al)$_s$ are primarily governed by common sources and physicochemical processing as opposed to degradation.

4.3. Relationships Between DOC, Lignin, and CDOM

[23] The absorption coefficient of CDOM has previously been utilized as a proxy for DOC in rivers as it is rapid and inexpensive to measure [*Worrall et al.*, 2003; *Baker et al.*, 2008b] and can also be measured in situ to allow for increased spatial and temporal resolution [*Spencer et al.*, 2007c; *Downing et al.*, 2009]. Epulu River a_{350} values exhibited the same temporal trend as DOC (Figure 3) and a strong linear relationship was found between a_{350} and DOC ($r^2 = 0.85$; $p < 0.001$; $n = 28$). This further demonstrates the potential of CDOM absorption measurements to derive fluxes of DOC from rivers that have been "ground truthed" [*Spencer et al.*, 2009b].

[24] The utility of molecular biomarkers such as lignin phenols for investigating organic biogeochemical sources and processing has been extensively demonstrated. However, analysis of lignin phenols is expensive and analytically intensive, thus making it poorly suited for the detailed temporal and spatial measurements that are often required to understand complex highly dynamic natural systems. Temporal and spatial coverage of DOM dynamics can be greatly improved by combining CDOM measurements that have been "ground truthed" to molecular biomarkers such as lignin, as has been shown in estuarine [*Hernes and Benner*, 2003], agricultural river [*Hernes et al.*, 2008], and high-latitude northern river [*Spencer et al.*, 2008, 2009b] systems. In this tropical riverine system, a_{350} was strongly linearly correlated with Σ_8 ($r^2 = 0.83$; $p < 0.001$; $n = 26$), indicating that Σ_8 and DOC fluxes (see above) from tropical rivers can be reliably derived from CDOM absorption coefficients. Such measurements will allow for the quantification of fluxes of DOC and Σ_8 from CDOM but do not assist in delineating its role in the environment because the spectral parameters of CDOM are largely independent of concentration [*Del Vecchio and Blough*, 2004; *Helms et al.*, 2008]. In order to "ground truth" CDOM measurements associated with compositional properties, we investigated relationships between lignin carbon-normalized yields (Λ_8 and V) and CDOM measurements related to aromaticity and molecular weight (i.e., S_R, SUVA$_{254}$, and FI). Linear correlations were observed between Λ_8 and S_R, SUVA$_{254}$, and FI ($r^2 = 0.69$, 0.77, and 0.75, respectively; $p < 0.001$; Figure 6). Linear correlations were also observed between V and S_R, SUVA$_{254}$ and FI ($r^2 = 0.78$, 0.84, and 0.82, respectively; $p < 0.001$). These relationships indicate that lignin has a fundamental role in CDOM measurements related to aroma-
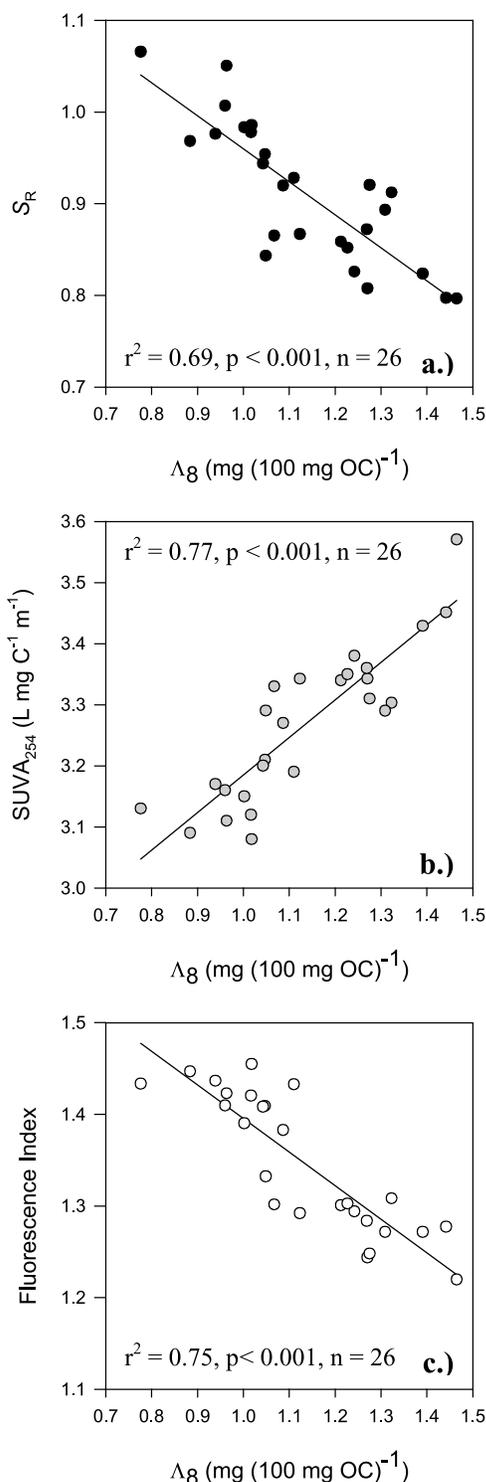
$r^2 = 0.69$, $p < 0.001$, $n = 26$ **a.)**

S_R

Λ_8 (mg (100 mg OC)$^{-1}$)

$r^2 = 0.77$, $p < 0.001$, $n = 26$

$SUVA_{254}$ (L mg C^{-1} m^{-1})

b.)

Λ_8 (mg (100 mg OC)$^{-1}$)

Fluorescence Index

$r^2 = 0.75$, $p < 0.001$, $n = 26$ **c.)**

Λ_8 (mg (100 mg OC)$^{-1}$)

Figure 6. Relationships between lignin carbon-normalized yields (Λ_8) and CDOM measurements related to aromaticity and molecular weight: (a) spectral slope ratio (S_R), (b) $SUVA_{254}$, and (c) fluorescence index.

ticity, which are used to assess DOM quality. As such these simple and inexpensive optical properties show great potential for future studies aiming to assess the quality and composition of DOM in tropical riverine systems. The prospective of "ground truthing" CDOM measurements to an array of biomarkers and indicators of DOM reactivity (e.g., lability) serves to increase our fundamental understanding of DOM catchment dynamics [*Cory et al.*, 2007; *Fellman et al.*, 2009].

5. Conclusions

[25] To date there has been little work on DOM dynamics in tropical ecosystems, and there is especially a lack of data on temporal variation in DOM quality. Although tropical rainforest systems do not undergo the temporal variability with respect to seasonal climatic extremes observed in temperate and northern high-latitude rivers, this study clearly shows both temporal variation in DOM quantity and more significantly DOM quality. Previous studies in temperate and northern high-latitude rivers have shown the potential to use CDOM-derived optical measurements to examine temporal changes in DOM composition [*Hernes et al.*, 2008; *Spencer et al.*, 2008, 2009b] and to examine lability [*Fellman et al.*, 2009] in riverine systems. Here we show the possibility of deriving DOC and lignin phenol fluxes from CDOM measurements in a tropical river system. This study also shows the ability to track DOM quality by relating lignin carbon-normalized yields (which indicate the contribution of vascular plant-derived material in the DOM pool) to simple optical measurements (e.g., S_R, $SUVA_{254}$, and FI). CDOM-derived measurements, such as S_R and FI, can be measured in situ and, thus, allow for increased temporal and spatial coverage to elucidate DOM catchment dynamics.

[26] The temporal variability in DOM quality in the Epulu River is attributed to greater surface runoff and leaching of the organic-rich horizons during the April wet period and increased flow path, residence time, and, therefore, greater microbial mineralization in the intermediary period in conjunction with an already well-leached source material in the dry period/post flush [*Strigel et al.*, 2005; *Spencer et al.*, 2008]. This shift in DOM source and processing results in DOM exported during the flushing period with greater HMW and more aromatic character (as indicated by the CDOM quality measurements and the higher Λ_8 and V yields) versus DOM exported during the dry period/post flush. In high-latitude northern rivers, DOM exported during the spring flush has a greater proportion derived from vascular plant sources than at other times of the year (i.e., higher Λ_8 and V yields as well as relatively lower S_R and higher $SUVA_{254}$ values [*Neff et al.*, 2006; *Spencer et al.*, 2008, 2009b]) and has also been shown to be younger, more labile, and more photoreactive [*Raymond et al.*, 2007; *Holmes et al.*, 2008; *Osburn et al.*, 2009]. Thus, it seems likely that the Epulu River and other tropical rivers during wet periods are exporting a greater proportion of freshly leached plant material that tends to be highly labile and more susceptible to photochemical degradation. Therefore, temporal variations in DOM quality in tropical rivers will impact the fundamental role the material plays in the receiving ecosystem's biogeochemistry. Future work is urgently required to better constrain and understand the dynamics and ultimate fate of tropical riverine DOM, which represents >28% of all terrestrial DOC inputs to the oceans [*Coynel et al.*, 2005].

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