Hydrology Research

An International Journal

Formerly Nordic Hydrology

Characterisation of dissolved organic matter in the Lower Kinabatangan River, Sabah, Malaysia

Sahana Harun, Andy Baker, Chris Bradley, Gilles Pinay, Ian Boomer and R. Liz Hamilton

ABSTRACT

Spatial patterns and trends in the concentration and quality of dissolved organic matter (DOM) are characterised across a tropical agricultural catchment using ultraviolet (UV)-visible absorbance, and fluorescence spectroscopy. Related determination of the environmental isotopes ^{18}O and ^{2}H clarify the dynamics of catchment water movement. Water samples were collected from the Kinabatangan River, Borneo, and selected tributaries in August and September 2008 in four regions with oil palm plantations (KB1, KB2, KB3 and KB4). The isotopic compositions of surface waters suggest that canals were characterised by a strong evaporative effect than tributaries and streams with more natural, forested vegetation. DOM was characterised by variations in UV absorbance and spectral slope. Individual fluorescence excitation–emission matrices were decomposed by Parallel Factor Analysis (PARAFAC) and three components extracted (C1, C2 and C3). Components C2 and C3 both appear to be derived from microbial sources and/or photo-degradation. The PARAFAC components indicate a clear trend of increasing DOM degradation as waters pass through the catchment. It is hypothesised that upstream DOM is rapidly photo and microbially degraded to less fluorescent DOM, while DOM concentration and character of DOM downstream is controlled by the hydrology, specifically by variations in the rate of water movement.

Key words | excitation–emission matrix spectroscopy, fluorescence, parallel factor analysis, stable isotopes, tropical wetlands

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INTRODUCTION

Tropical wetlands are ecologically diverse (Junk 2002; Dudgeon 2003) and characterised by rapid nutrient recycling and processing (Hader et al. 1998). Typically, wetlands function as a carbon store and possible carbon sink (Richey et al. 2002; Alkhatib et al. 2007; Limpens et al. 2008) and where closely integrated with fluvial systems, they constitute an important source of carbon for marine environments (Stephens & Rose 2005). Tropical wetlands alone have been estimated to contribute ~60% of the total water, sediment and organic carbon input to the ocean globally (Alkhatib et al. 2007).

Dissolved organic matter (DOM) is ubiquitous in aquatic systems (Baker & Spencer 2004; Evans et al. 2005; Oliveira et al. 2006) but many wetlands are characterised by the presence of particularly high quantities of DOM (Mladenov et al. 2007; Stern et al. 2007). DOM fluxes from wetlands represent an important carbon input to river systems, constituting its largest and most bioavailable pool (Wilson & Xenopoulos 2009). The presence of organic matter affects the transport of organic pollutants, particle surface and colloid chemistry, photochemistry of natural waters and nutrient availability in freshwater systems (Hope et al. 1994; Fellman et al. 2008b), while DOM also contributes to chemical processes in natural water bodies by altering surface-water acidity, and affecting metal speciation and ion-exchange between the water and sediment phase.

Significantly, however, to date DOM has been relatively poorly studied in tropical catchments. Here, DOM characteristics are likely to change substantially as a result of photochemical degradation. This reflects greater penetration of ultraviolet (UV)-B radiation, mixing within the water body, and input of terrestrial material (Findlay & Sinsabaugh 1999; Spencer *et al.* 2009). DOM may also degrade directly (by structural alteration), or indirectly (by reaction with free radicals created by the application of light). In catchment headwaters, DOM is generally dominated by inputs of terrestrially derived DOM and dissolved organic carbon (DOC) concentrations are high (Dalzell *et al.* 2009). In contrast, the controls on DOM downstream are often related to hydrological processes, particularly the rate of water movement in large rivers (Findlay & Sinsabaugh 1999). Moreover, light penetration at downstream sites is often less and mixing depths greater, decreasing the effects of photolysis. Photochemical reactions produce inorganic carbon, low molecular weight organic compounds, trace gases, phosphorus- and nitrogen-rich compounds (Cory *et al.* 2007; Winter *et al.* 2007; Kowalczuk *et al.* 2009).

DOM is an important primary food source for aquatic food webs (Pace *et al.* 2004) and ecosystem metabolism (Bradley *et al.* 2007), however, DOM quality and quantity is likely to be affected significantly by catchment management, including land use change, wetland drainage, river channelisation and flow regulation. This is particularly evident in South and Southeast Asia where significant areas of tropical forest have been recently cleared for agriculture (Mattsson *et al.* 2000; Sidle *et al.* 2006; Atapattu & Kodituwakku 2009). In Indonesia, for example, it has been estimated that ~45% of the original peat swamp forest has been lost (Rixen *et al.* 2008) following development of rubber (*Hevea brasiliensis*) and oil palm plantations (*Elaeis guineensis*) (Hooijer *et al.* 2006). In Malaysia, changes in land use have been encouraged by government (Abdullah & Nakagoshi 2006), leading to the recent (and rapid) increase in oil palm plantations in Sabah (Malaysian Borneo). Land conversion has been particularly extensive in East Sabah; however, the environmental implications of this expanding agro-forestry industry specifically on DOM quality and quantity has yet to be quantified. This is important, both for this region and elsewhere in the Tropics: the extent of oil palm has increased rapidly in recent years in

Southeast Asia, reflecting the market for oil palm both for vegetable oil and as a bio-fuel. In 2008, oil palm cultivation was estimated at $>13.5 \times 10^6$ ha (Fitzherbert *et al.* 2008), and there are increasing pressures to cultivate the crop in other agriculturally suitable areas in Africa and South America. However, at present, the hydrological impacts of converting tropical forest to agriculture are relatively poorly understood (particularly with respect to DOM). On the Tekam River, Pahang (Figure 1), the removal of forest and development of oil palm plantations led to a significant increase in annual sediment loads from $28 \, \mathrm{t \, km^{-2}}$ (mean of three years) to $414 \, \mathrm{t \, km^{-2}}$ (Douglas 1999). In the same catchment, a large and sustained increase in annual runoff was observed after the fourth year of planting (Chuan 2003). For many reasons therefore, it is essential that the environmental impacts of oil palm are fully understood by studying impacted catchments in Southeast Asia so that any effects may be mitigated elsewhere.

This urgent need is addressed in this paper by characterising DOM in a tropical catchment which is currently experiencing rapid agricultural change from the logging of primary and secondary forest and the on-going development of oil palm plantations. The objectives of the paper are to characterise and interpret spatial patterns and trends in DOM (both concentration and quality) across a tropical catchment using UV-visible absorbance and fluorescence spectroscopy and infer differences in water movement through the catchment using environmental isotopes (^{18}O and ^{2}H).

MATERIALS AND METHODS

Study sites

DOM characteristics and stable isotopic variability were determined in selected downstream reaches of the Kinabatangan River and tributaries in Sabah, Malaysia. The Kinabatangan River (560 km in length) is the largest river in Sabah, with a catchment area of 16,800 km², representing 23% of Sabah (Figure 1; Josephine *et al.* 2004). The area has a humid tropical climate with mean daily temperatures ranging from 22 to 32 °C and mean annual rainfall of between 2,500 and 3,000 mm (Boonratana 2000; Josephine
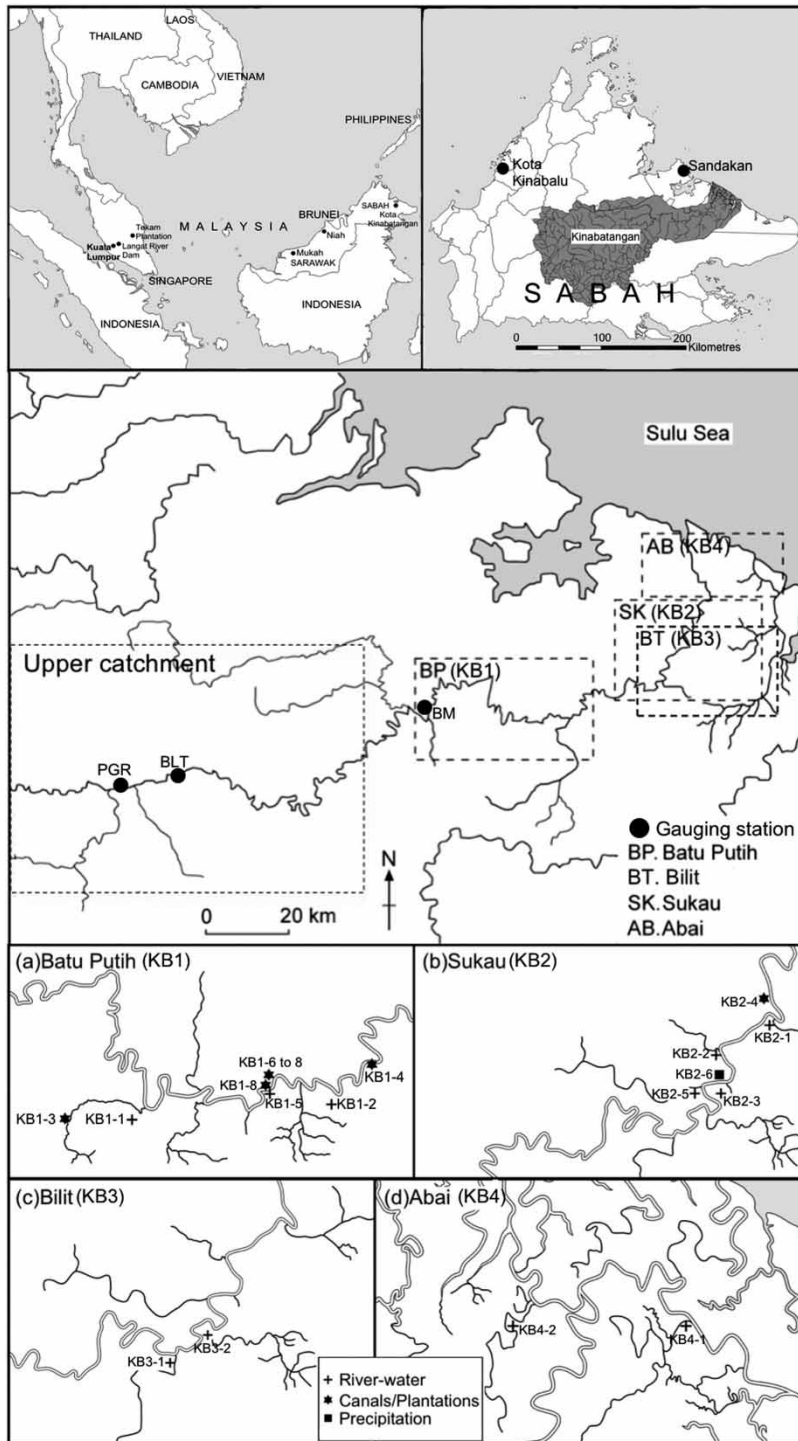
Figure 1 | The Lower Kinabatangan River catchment (above right and centre) and its regional location (top), with local site maps for: (a) Batu Putih (KB1); (b) Sukau (KB2); (c) Bilit (KB3); and (d) Abai (KB4).

et al. 2004). Heaviest rainfall occurs during the northeast monsoon between October and March at which time the floodplain and coastal plain are widely inundated and there is considerable inter-annual variability in rainfall.

The lower floodplain of the Kinabatangan extends over >2,800 km^2, and is the largest wetland in Sabah (Figure 1). There are ~20 ox-bow lakes in three main clusters: (i) above Batu Putih; (ii) between Batu Putih and Bilit; and (iii) between Bilit and Sukau, with a dense network of tributary streams and creeks that extend into an estuary downstream. The natural floodplain vegetation mainly comprises riverine and freshwater swamp forest, with some open reed swamp, and pristine lowland dipterocarp forest in areas that are not frequently inundated (Boonratana 2000). Although the catchment is relatively sparsely populated, it has been estimated that ~26% of the catchment has been developed for oil palm plantations, mainly in the floodplain of the Lower Kinabatangan River (Josephine *et al.* 2004).

The Kinabatangan River is the main source of water for local communities; some river-water is also abstracted and piped to the city of Sandakan to the north (Boonratana 2000; Mansourian *et al.* 2003). Long-term monthly mean discharge for three gauging stations in Kinabatangan, Pagar (PGR), Balat (BLT) and Barek Manis (BM), are summarised in Figure 2. From 1979 to 2013, monthly mean river flow in the upper catchment (stations PGR and BLT) (Figure 1) is within the range 14.1–1,285.8 and 26.1–1,944.2 m^3/s, respectively. Peak daily mean discharge was recorded in January 1986 at Balat (~1,944 m^3/s), and the lowest at Pagar in June 1998 (~14 m^3/s). A water quality study indicated that total suspended solids in the Lower Kinabatangan catchment, in Sukau, was Class IIB (max. conc. of 50 mg/L) according to the Malaysian Interim National Water Quality Standard (Harun 2006). This probably reflects widespread intensive commercial logging over much of the catchment since the 1980s and the increasing development of oil palm plantations (Boonratana 2000; Josephine *et al.* 2004).

Field-work design

Water samples were collected during summer baseflow conditions at selected locations in the Kinabatangan catchment in August and September 2008. Sampling design was significantly constrained by the difficulty of access, but the intention was to sample waters along a freshwater estuarine gradient and to capture the land use variations of DOM in the Lower Kinabatangan floodplain towards the Sulu Sea downstream. The nearest gauging station was at BM with an upstream catchment of 12,300 km^2, which was located about ~11 km from KB1-3 (Figure 1). The daily mean discharges observed during the fieldwork campaign are summarised in Table 1.

Waters were sampled from sites that were closely associated with oil palm plantations and processing mills, as well as the main-stem of the Kinabatangan and tributary streams with varying proportions of oil palm plantation. Sample sites were initially selected according to their accessibility (within 3 hours by boat) and included: (i) the main stem of the Lower Kinabatangan River (26 samples); (ii) tributary streams (180 samples); and (iii) irrigation ditches associated with selected oil palm plantations (28 samples).

In total, 234 water samples were collected in 2 mL glass vials (in most cases from a boat) at three points across the river channel. Given uncertainties as to whether river waters were completely mixed, at each sampling point waters were sampled at points across the channel: adjacent to both riverbanks and in the channel mid-point. The small sample sizes also enabled rapid shipment of the water samples (unfrozen) to Birmingham, UK, for laboratory analysis. There are a number of logistical problems of sample storage and preservation when working in remote areas (e.g., Burke *et al.* 2002) although freezing has been applied successfully (Fellman *et al.* 2008a). In this case, freezing of samples was not feasible, however, nutrient concentration can be stabilised using inorganic acids (Harmel *et al.* 2006). Other physicochemical parameters, including pH and water temperature, were measured *in situ* using a Hanna multi-parameter water quality meter (Model HI9828). All samples for organic matter fluorescence analysis were filtered through Whatman GF/F syringe filters (nominal pore size 0.7 μm), and stored in the dark at 4 °C until analysis, which occurred within 1 month of sampling. A comparison of the effects of filtering in the field vs. filtering post-collection using triplicate grab samples demonstrated that the fluorescence of post-collection filtered samples was more variable than for field-filtered samples (<9% coefficient of variance for Parallel Factor Analysis (PARAFAC) C1 and <20% for C2 and C3 vs.
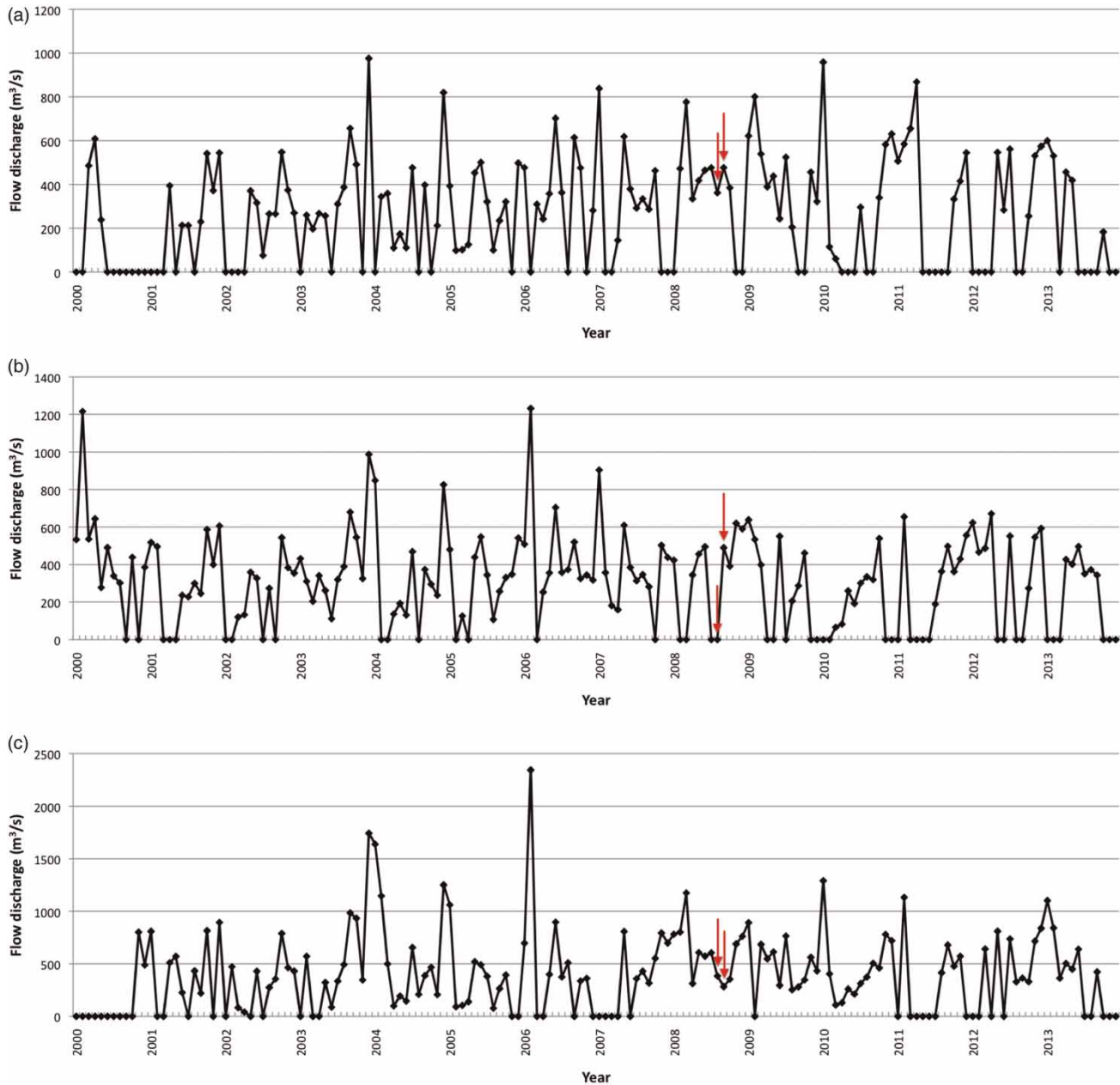
Figure 2 | Long-term mean monthly flow discharge for three gauging station at Kinabatangan: (a) PGR; (b) BLT; (c) BM. The catchment area for each station is 9,430, 10,800 and 12,300 km^2, respectively. Arrows indicate flow discharges during fieldwork campaign.

<6% for all three components). This variability is taken into consideration in the subsequent interpretation.

Surface water samples were collected from different water body types as follows: (i) tributary streams (~2–3 km above their confluence) (ST); (ii) effluent from oil palm plantation ditches (DC); (iii) main stem of the Kinabatangan (MS); and (iv) ox-bow lake. Ditches from oil palm

plantations were found to have less vegetation cover, compared to other water body types. Sample sites have been grouped into four principal sampling regions (based on upstream–downstream gradient) (Figure 1):

1. **Batu Putih (KB1):** samples were collected from tributary streams (KB1-1, KB1-2; 39 samples), effluent from an oil

Table 1 | Sampling date for each station and mean daily discharge at the nearest gauging station (BM) recorded by the Department of Irrigation and Drainage Malaysia

Sampling date (dd/mm/yy)	Sampling station	Average of daily mean discharge at BM (m³/s)
23/08/08	Sg. Pin (KB1-1)	209.5
23/08/08	Sg. Koyah (KB1-2)	209.5
24/08/08	BS Mill (KB1-3)	204.5
25/08/08	PS Plantation (KB1-4)	195.0
25/08/08	Canals (KB1-6 to 8)	195.0
27/08/08	DGFC (KB1-5)	260.2
28/08/08	Sg. Resang (KB2-1)	[a]
28/08/08	Sg. Resik (KB2-2)	[a]
28/08/08	Kuala Sukau (KB2-3)	[a]
29/08/08	Balat Damit (KB4-1)	[a]
29/08/08	Sg. Merah (KB4-2)	[a]
02/09/08	Sg. Menanggol (KB2-5)	213.7
02/09/08	Sg. Tenagang Besar (KB3-1)	213.7
02/09/08	Sg. Tenagang Kecil (KB3-2)	213.7
02/09/08	Malbumi Plantation (KB2-4)	213.7

[a]Data are unavailable.

palm plantation mill and ditches (KB1-3, KB1-4, KB1-6 to KB1-8; 19 samples) and an ox-bow lake (KB1-5; 1 sample). The local population is ~1,200–1,400, in a number of small settlements, along the river.

2. **Sukau (KB2):** samples were collected from tributary streams (KB2-1, KB2-2, KB2-3, KB2-5; 63 samples), a drainage ditch associated with an oil palm plantation (KB2-4; 9 samples) and the main river (KB2-1, KB2-2, KB2-3, KB2-4, KB2-5; 15 samples). Sukau lies on the Kinabatangan River, 70 km upstream of Sandakan. The population of ~2,000 primarily inhabit small settlements scattered along the riverbank. In places, untreated sewage discharges into the river, but some areas of riparian forest remain in the vicinity.

3. **Bilit (KB3):** samples were collected from streams (KB3-1, KB3-2; 27 samples) and the main river (KB3-1, KB3-2; 6 samples). This area is sparsely populated (~300 inhabitants) and more riparian forest remains here. Characteristic vegetation includes three forest types: seasonal freshwater swamp, mixed dipterocarp forest (MDF) and MDF-limestone forest (Sabah Forestry Department 2001). The latter vegetation is associated with limestone outcrops that are also found in this area.

4. **Abai (KB4):** samples were collected from streams (KB4-1, KB4-2; 51 samples) and the main river (KB4-1,

KB4-2; 5 samples). Abai is relatively close to the estuary of the Sulu Sea, near a recently designated (2008) Ramsar wetland: Kinabatangan – Lower Segama. Although there are small settlements in this area, the population in 1996 was estimated at only ~290 (Payne 1996). Freshwater swamp vegetation, including *Nypa fruticans*, is widespread along the estuary.

Laboratory analyses

Stable isotope analyses

Stable isotope analyses were undertaken in the Water Sciences laboratory at the University of Birmingham, UK, using a GV Instruments Isoprime isotope-ratio mass spectrometer (IRMS) connected to a Eurovector elemental analyser. Stable isotope values are expressed using the δ convention, where $\delta^{18}O = (^{18}O/^{16}O_{sample})/(^{18}O/^{16}O_{standard})-1$, and similarly for hydrogen isotopes (δ^2H) expressed as ‰ (per mil) where the standard is Vienna standard mean ocean water (V-SMOW). For hydrogen isotope analysis, ~0.3 μL of water was injected from sample vials on an autosampler into a column where reduction to hydrogen took

place at 1,050 °C over a chromium metal catalyst. At least two successive analyses were made by repeat injections from the same vial. Internal (within-run) precision is 0.4 per mil for δD and overall (external) precision is ±1 per mil.

Oxygen isotope analyses were undertaken using an equilibration technique. 200 μL water samples were left to equilibrate (CO_2) in a sealed container for 7 hours allowing the headspace CO_2 to assume the $δ^{18}O$ composition of the water. The equilibrated CO_2 was then analysed on the IRMS. The internal precision for $δ^{18}O$ is typically 0.08 per mil, external precision is better than 0.15 per mil.

Fluorescence spectroscopy and UV absorbance

DOM was characterised by fluorescence spectroscopy and UV-visible absorbance. Fluorescence intensity was measured using a Varian Cary Eclipse spectrophotometer equipped with a Peltier temperature controller. Emission scans were performed for wavelengths from 280 to 500 nm, with data collected at 2 nm intervals, and excitation wavelengths from 250 to 400 nm, at 5 nm intervals. Uncorrected spectra were combined to form an excitation–emission matrix (EEM). Water sample temperature was set to 20 °C and photomultiplier tube voltage was 725 V. Spectrophotometer output was monitored by regular determination of the Raman intensity of ultra-pure water in a sealed 10×10 mm cuvette at 348 nm excitation and 5 nm bandpass. A 1.5 mL (10×3.33 mm) cell was used to minimise both the sample volumes required. Due to the turbidity of the water samples, which remained in some samples even after filtration, manufacturer-supplied excitation filters (250–395 nm) were employed. An inner-filter effects correction has been applied to the data set following Ohno (2002):

$$I = I_0 \left(10^{-b(A_{ex}+A_{em})} \right) \tag{1}$$

where I is detected fluorescence intensity; I_0 is fluorescence in the absence of self-absorption; b is the path length for both the excitation and emission beam; A_{ex} is absorbance at excitation wavelength; and A_{em} is the absorbance at emission wavelength.

Absorption coefficients at 254 and 340 nm (a_{254} and a_{340}) and spectral slope over the interval of 275–295 nm ($S_{275-295}$) (Helms *et al.* 2008) were determined using a Lightwave spectrophotometer and 2 ml (10×5 mm; 5 mm path length) cuvettes. $S_{275-295}$ was calculated by linear regression of the log-transformed a spectra. Distilled deionised water was used as a reference, and absorbance readings were corrected, where necessary, for long-term baseline drift. An inner-filter correction was also applied (mean UV absorption coefficient across all samples at a_{340} was 37.6 m^{-1}).

Data analysis

Parallel factor analysis

PARAFAC was undertaken to separate EEMs statistically into their individual, underlying, fluorescent groups with specific excitation and emission spectra. This analysis provides a qualitative (fluorescent groups) and quantitative (fluorescent intensity of each group) model of the data and is ideally suited to detecting small, but potentially significant, differences in DOM composition.

PARAFAC modelling was undertaken following Stedmon & Bro (2008). Fluorescence EEMs were combined into a three-dimensional data array and decomposed to a set of trilinear terms and a residual array

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \quad i = 1, \dots, I \quad j = 1, \dots, J \\ k = 1, \dots, K \tag{2}$$

where x_{ijk} is the fluorescence intensity for sample i at emission wavelength j and excitation wavelength k; a_{if}, b_{if} and c_{kf} are the loading matrices. F is the number of model components, and e_{ijk} is residual noise (i.e., variability not explained by the model). Despite the use of a 250–395 nm excitation filter, initial PARAFAC analysis was confounded by scatter in the EEMs within the wavelength range of 250–280 nm (excitation) and 280–290 nm (emission). As a result, excitation wavelengths <290 nm were removed for PARAFAC analysis, and consequently it was not possible to investigate microbial fluorescence located at excitation wavelength 280 nm.

The model was defined and validated by split-half technique (Stedmon *et al.* 2003) in which the data array was divided into two halves and modelled separately. PARAFAC

models, ranging from two to seven components, were derived for both data sets independently. Model validation was carried out by comparing the spectral shape of the components derived by the models. The model returns relative intensities of derived components (scores) because the specific absorption and quantum yield of fluorescence of individual components is unknown. The intensity of the nth component in a given sample, I_n, was calculated as the fluorescence intensity at the peak excitation and emission maximum of the nth component using

$$I_n = \text{Score}_n * \text{Ex}_n(\lambda_{\max}) * \text{Em}_n(\lambda_{\max}) \qquad (3)$$

where Score_n is the relative intensity of the nth component, $\text{Ex}_n(\lambda_{\max})$ is the maximum excitation loading of the nth component, $\text{Em}_n(\lambda_{\max})$ is the maximum emission loading of the nth component derived from the model. The total fluorescence intensity of a given sample was calculated as the sum of the components present in the samples

$$I_{TOT} = \sum_{1}^{n} I_n \qquad (4)$$

RESULTS

Stable isotope analyses

The stable isotope analyses ($\delta^{18}O$ and δ^2H) are summarised in Table 2 and Figure 3. The global isotopic composition of rainfall defines the global meteoric water line (GMWL, $\delta^2H = 8 \times \delta^{18}O + 10$), while local, or regional, meteoric water lines have slightly different slopes and intercepts and can be determined from local precipitation. Araguás-Araguás *et al.* (1998) established a regional meteoric water line (RMWL) for neighbouring Sarawak (~400 km from Kota Kinabatangan) (Figure 1), which provides a reference for this study (Table 2).

The $\delta^{18}O$ of meteoric water in KB2 ranged from -9.7 to $-2.5‰$ while δ^2H values ranged from -61 to $-7.8‰$, all lying parallel to, but slightly above, the RMWL. Precipitation on 29 August 2008 has a deuterium-excess of $+11.3$ compared to the RMWL published value of 9.2, indicating

a slightly heavier vapour source for these short-term events compared to the longer-term mean. The large absolute range of values observed in Kinabatangan can be explained by the fact that the rainfall samples were not representative of complete events, but only a sub-sample.

Optical parameters

The results of the summer base-flow survey are presented here using descriptive statistics to identify trends in fluorescence and UV-visible absorption of waters sampled across the four study regions of the Lower Kinabatangan River catchment.

UV-visible absorption coefficients at 254 and 340 nm are summarised in Table 3, and varied considerably between sites. UV absorption has been found to correlate with DOC (Ahmad & Reynolds 1999; Tipping *et al.* 2009), and given the lack of direct DOC analyses (due to the small sample size) the results are interpreted here as indicative of relative variations in DOC. The greatest variability in absorption coefficients at 254 (340) nm were found at sites directly associated with oil palm plantations: KB1-6 to 8: 142.5 (47.9) m^{-1} and KB2-4: 123.7 (59.8) m^{-1}.

Several parameters can be derived from the UV-visible absorbance and the linearised gradient of absorbance, or spectral slope. Following Helms *et al.* (2008), the latter has been calculated between the wavelengths 275 and 295 nm. The spectral slope is plotted against absorption at 340 nm (a_{340}) (indicator of DOM concentration) in Figure 4. The spectral slope is lowest in KB2 (KB2-1 to KB2-6), indicating DOM of a higher molecular weight, while samples from Batu Putih have the highest spectral slope (lowest molecular weight).

PARAFAC modelling

Three fluorescent components were identified by PARAFAC using EEMs of all samples collected from the study area. The ratio of the PARAFAC component intensity to the total fluorescence intensity for each sampling site is summarised in Table 3. The excitation and emission pairs of the main peak positions for each component are presented in Figure 5. The PARAFAC model identified three terrestrial peaks as characteristic fluorescent components in the Lower

Table 2 │ Summary isotope data for the Lower Kinabatangan River catchment (standard deviations in parentheses) and comparison with data from Niah in Sarawak (from Stephens & Rose 2005)

Sampling station	No. of samples From main river	Sampling date (dd/mm/yy)	pH		Water temperature (°C)		δ^{18}O (‰) V-SMOW		δ^{2}H (‰) V-SMOW		d-Excess (‰)	
Rainfall events												
Batu Putih (KB1):												
Rainfall (KB1-5)	1 [a]	25/08/08	[a]	[a]	[a]	[a]	−4.0	[a]	−25.4	[a]	3.9	[a]
Sukau (KB2):												
Rainfall 1 (KB2-6)	2 [a]	27/08/08	[a]	[a]	[a]	[a]	−2.5	(0.1)	−7.9	(0.41)	10.7	(0.7)
Rainfall 2 (KB2-6)	1 [a]	28/08/08	[a]	[a]	[a]	[a]	−2.9	[a]	−7.8	[a]	13.2	[a]
Rainfall 3 (KB2-6)	1 [a]	29/08/08	[a]	[a]	[a]	[a]	−6.5	[a]	−36.5	[a]	11.3	[a]
Rainfall 4 (KB2-6)	1 [a]	30/08/08	[a]	[a]	[a]	[a]	−9.7	[a]	−60.8	[a]	10.4	[a]
Tributary streams												
Batu Putih (KB1):												
Sg. Pin (KB1-1)	6 [a]	23/08/08	7.9	(0.3)	30.6	(0.7)	−6.8	(0.1)	−46.6	(0.44)	3.2	(0.5)
Sg. Koyah (KB1-2)	6 [a]	23/08/08	6.9	(0.3)	29.6	(2.9)	−7.3	(0.1)	−49.3	(0.25)	4.6	(0.6)
DGFC (KB1-5)	1 [a]	27/08/08	5.0	(0.04)	25.9	[a]	−6.3	[a]	−43.9	[a]	2.3	[a]
Sukau (KB2):												
Sg. Resang (KB2-1)	8 1	28/08/08	6.5	(0.5)	29.1	(0.6)	−7.9	(0.1)	−50.0	(0.86)	8.4	(0.6)
Sg. Resik (KB2-2)	3 1	28/08/08	7.4	(0.1)	30	(0.8)	−7.9	(0.1)	−51.0	(0.16)	7.2	(0.5)
Kuala Sukau (KB2-3)	5 1	28/08/08	7.5	(0.1)	29.4	(0.6)	−7.9	(0.1)	−50.9	(1.0)	7.5	(1.3)
Sg. Menanggol (KB2-5)	5 1	02/09/08	6.4	(0.04)	27.4	(1.1)	−7.1	(0.2)	−48.0	(1.23)	4.6	(0.6)
Bilit (KB3):												
Sg. Tenagang Besar (KB3-1)	6 1	02/09/08	6.4	(0.2)	28.4	(0.4)	−6.2	(0.5)	−43.5	(4.59)	2.5	(1.7)
Sg. Tenagang Kecil (KB3-2)	3 1	02/09/08	6.4	(0.03)	27.8	(0.4)	−6.4	(0.8)	−43.6	(4.58)	3.6	(1.0)
Abai (KB4):												
Balat Damit (KB4-1)	11 1	29/08/08	6.2	(0.5)	28.5	(0.4)	−7.7	(0.4)	−49.7	(2.59)	6.9	(0.8)
Sg. Merah (KB4-2)	6 1	29/08/08	6.5	(0.2)	29	(0.6)	−7.8	(0.1)	−50.7	(0.3)	6.5	(0.6)
Ditches												
BS Mill (KB1-3)	1 [a]	24/08/08	4.9	[a]	31.2	[a]	−6.7	[a]	−43.9	[a]	5.1	[a]
PS Plantation (KB1-4)	5 [a]	25/08/08	4.9	(0.2)	27.9	(0.9)	−7.2	(0.6)	-47.2	(3.3)	6.1	(1.3)
Canal 1 (KB1-6)	1 [a]	25/08/08	5.7	[a]	29.2	[a]	−5.1	[a]	−39.7	[a]	-2.5	[a]
Canal 2 (KB1-7)	1 [a]	25/08/08	7.0	[a]	28.5	[a]	−6.7	[a]	−43.8	[a]	5.2	[a]
Canal 3 (KB1-8)	1 [a]	25/08/08	6.0	[a]	28.9	[a]	−7.0	[a]	−44.4	[a]	7.0	[a]
Malbumi Plantation (KB2-4)	3 1	02/09/08	6.5	(0.1)	28.7	(0.5)	−7.4	(0.1)	−49.0	(0.66)	5.6	(0.6)
Data from Niah (from Stephens & Rose 2005)												
S. Niah	15 [a]	26/04/01	[a]	[a]	[a]	[a]	−7.9	(0.3)	−49.0	(4.1)	13.5	(2.9)
S. Niah	1 [a]	27/04/01	[a]	[a]	[a]	[a]	−8.3	[a]	−52.4	[a]	13.0	[a]
South China Sea	1 [a]	26/04/01	[a]	[a]	[a]	[a]	−4.6	[a]	−29.3	[a]	7.0	[a]

(*continued*)

Table 2 | continued

Sampling station	No. of samples		Sampling date (dd/mm/yy)	pH		Water temperature (°C)		$\delta^{18}O$ (‰) V-SMOW		δ^2H (‰) V-SMOW		d-Excess (‰)	
	From main river												
K. Niah	3	a	26/04/01	a	a	a	a	−6.9	(3.1)	−33.0	(19.1)	6.8	(5.0)
G. Kira cave interior drip	2	a	23/04/01	a	a	a	a	−6.7	(0)	−38.9	(0.6)	14.2	(0.7)
Rain outside West Mouth	2	a	25/04/01	a	a	a	a	−10.8	(0.1)	−71.0	(0.5)	14.2	(0.8)
G. Kira cave mouth drip	2	a	23/04/01	a	a	a	a	−8.2	(0)	−53.3	(1.1)	11.8	(1.2)
Rain outside Gan Kira	1	a	23/04/01	a	a	a	a	−4.8	a	−26.0	a	11.6	a
Rainforest pool	1	a	27/04/01	a	a	a	a	−7.3	a	−44.6	a	13.3	a
Rainforest stream (S. Subis)	1	a	27/04/01	a	a	a	a	−8.4	a	−54.7	a	12.1	a

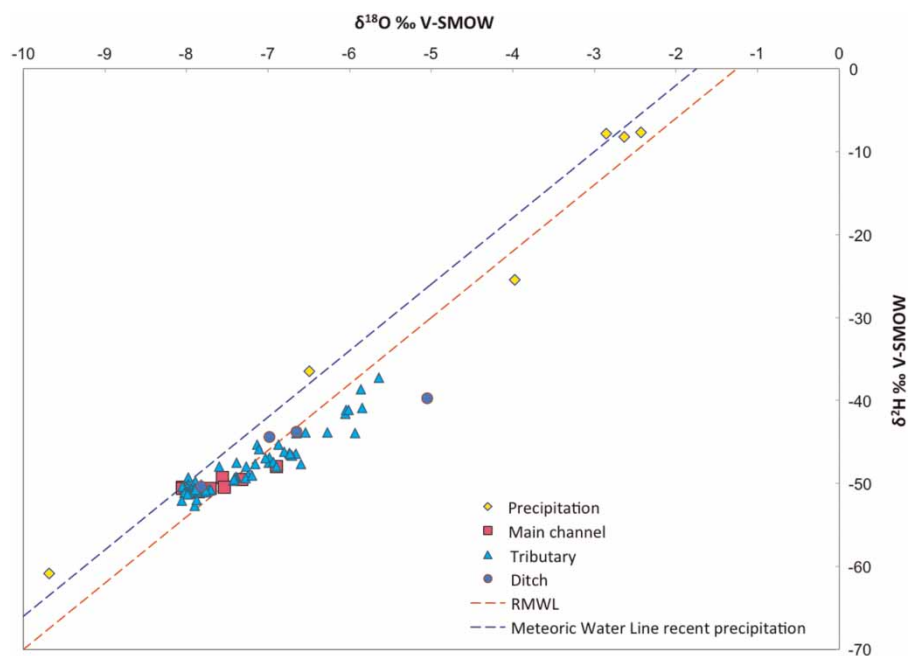[a]Data are unavailable.

Figure 3 | Plot of $\delta^{18}O$ versus δ^2H for sampling sites at the Lower Kinabatangan River catchment, and comparison with the regional meteoric water line (RMWL) and the meteoric water line for local precipitation.

Kinabatangan River catchment. The mean total fluorescence intensity of samples collected is: IC2 > IC1 > IC3.

Component C1 contributed 35% of modelled fluorescence for the samples. It represents a combination of two non-separated peaks of different excitation wavelength: with a double excitation maxima at 345 and <290 nm

(corresponding to the type A and C) and a single emission peak at 458 nm.

The excitation maximum for C2 occurred at 315 nm at 398 nm emission and the percentage contribution of modelled fluorescence is 43%, while component C3 occurred at a maximum excitation wavelength of <290 and 330 nm

Table 3 | Summary mean of absorbance, spectral slope and PARAFAC data for the Lower Kinabatangan River catchment (standard deviation in parentheses)

Sampling station	No. of samples	Absorption coefficient a_{254} (m^{-1})		Absorption coefficient a_{340} (m^{-1})		Spectral slope (nm^{-1})	PARAFAC components			
							I_{tot}	I_{C1}	I_{C2}	I_{C3}
Main stem	26	99.3	(21.4)	46.2	(12.2)	0.001	15.2 (1.2)	5.1 (0.6)	6.1 (0.7)	3.9 (0.7)
Tributary streams										
Batu Putih (KB1):										
Sg. Pin (KB1-1)	21	97.6	(3.5)	33.6	(1.8)	0.013	62.5 (9.1)	20.4 (3.9)	28.1 (5.6)	14 (5)
Sg. Koyah (KB1-2)	18	82.1	(3.4)	28.7	(1.4)	0.013	40.7 (4.8)	13.6 (1.7)	18.7 (2.3)	8.4 (1.8)
DGFC (KB1-5)	1	62.2	[a]	20.3	[a]	0.019	27.0 ([a])	5.9 ([a])	9.1 ([a])	12.0 ([a])
Sukau (KB2):										
Sg. Resang (KB2-1)	27	81.9	(3.8)	34.8	(1.8)	0.011	17.4 (5.4)	6.3 (2.5)	7.1 (2.2)	4.0 (1)
Sg. Resik (KB2-2)	12	90.0	(1.7)	40.1	(1.1)	0.010	14.6 (0.8)	5.1 (0.3)	6.1 (0.5)	3.5 (0.3)
Kuala Sukau (KB2-3)	18	86.8	(2.2)	40.6	(1.2)	0.009	15.1 (0.8)	5.3 (0.5)	6.4 (0.4)	3.4 (0.3)
Sg. Menanggol (KB2-5)	18	106.8	(8.4)	47.6	(6.9)	0.010	23.9 (6.6)	9.6 (3.1)	10.1 (2.9)	4.2 (0.7)
Abai (KB4):										
Balat Damit (KB4-1)	36	92.3	(10.5)	39.5	(3.7)	0.010	21.8 (8.8)	9.0 (4.9)	8.9 (3.7)	3.9 (0.7)
Sg. Merah (KB4-2)	21	82.1	(6.3)	34.0	(2.7)	0.012	14.2 (1.8)	4.9 (0.2)	5.8 (0.7)	3.5 (1.7)
Bilit (KB3):										
Sg. Tenagang Besar (KB3-1)	21	101.0	(5.1)	39.1	(3.9)	0.013	36.7 (11.8)	11.7 (3.7)	17.0 (5.7)	8.0 (3)
Sg. Tenagang Kecil (KB3-2)	12	73.9	(11.0)	30.3	(6.5)	0.011	23.1 (5.3)	8.2 (2)	10.3 (2.7)	4.6 (0.8)
Ditches										
BS Mill (KB1-3)	3	89.2	(2.2)	34.2	(2.0)	0.012	41.3 (0.5)	13.6 (0.5)	18.3 (0.3)	9.5 (1.0)
PS Plantation (KB1-4)	13	81.6	(5.2)	30.9	(2.0)	0.013	26.5 (11)	8.7 (4.2)	10.8 (5.2)	7.1 (1.8)
Canals (KB1-6 to 8)	3	142.5	(39.8)	47.9	(15.0)	0.013	57.4 (20.1)	24.4 (11.9)	24.4 (8.4)	8.6 (2.7)
Malbumi Plantation (KB2-4)	12	123.7	(14.5)	59.8	(9.0)	0.008	17.3 (4.1)	6.3 (1.8)	7.1 (1.8)	4.0 (0.5)

[a]Data are unavailable.

and emission wavelength of 360 nm, and contributes 22% of modelled fluorescence.

The fluorescent intensities of both PARAFAC components C1 and C2 were greatest at KB1, particularly in waters sampled from plantation ditches (highest intensity: 34.8 units) and in tributary stream KB1-1 (highest intensity: 23.2 units) (Table 3). Components C1, C2 and C3 are plotted against a_{340} in Figures 4(a)–4(f), and theoretical lines of constant fluorescence per unit absorbance are shown in Figures 4(a)–4(c). Water samples would be expected to lie on one of these lines if there were no change in DOM characteristics between sites, and if the intensity of both

parameters reflected a dilution effect. Figures 4(a)–4(f) indicate that only one subset of samples, those from the main stem of the Kinabatangan, lie along a dilution line. The results suggest that there is a loss of fluorescent DOM between the catchment tributaries and the main stem of the river. As a result, DOM in the main stem of the river is relatively less fluorescent per unit absorbance than expected. DOM hydrophilicity was found to decrease as DOM travels from Batu Putih to Abai, which may also reflect degradation of the DOM from PARAFAC component C1 (peak C) to PARAFAC component C2 (peak A). This trend is also evident in Figures 4(g)–4(i) where PARAFAC
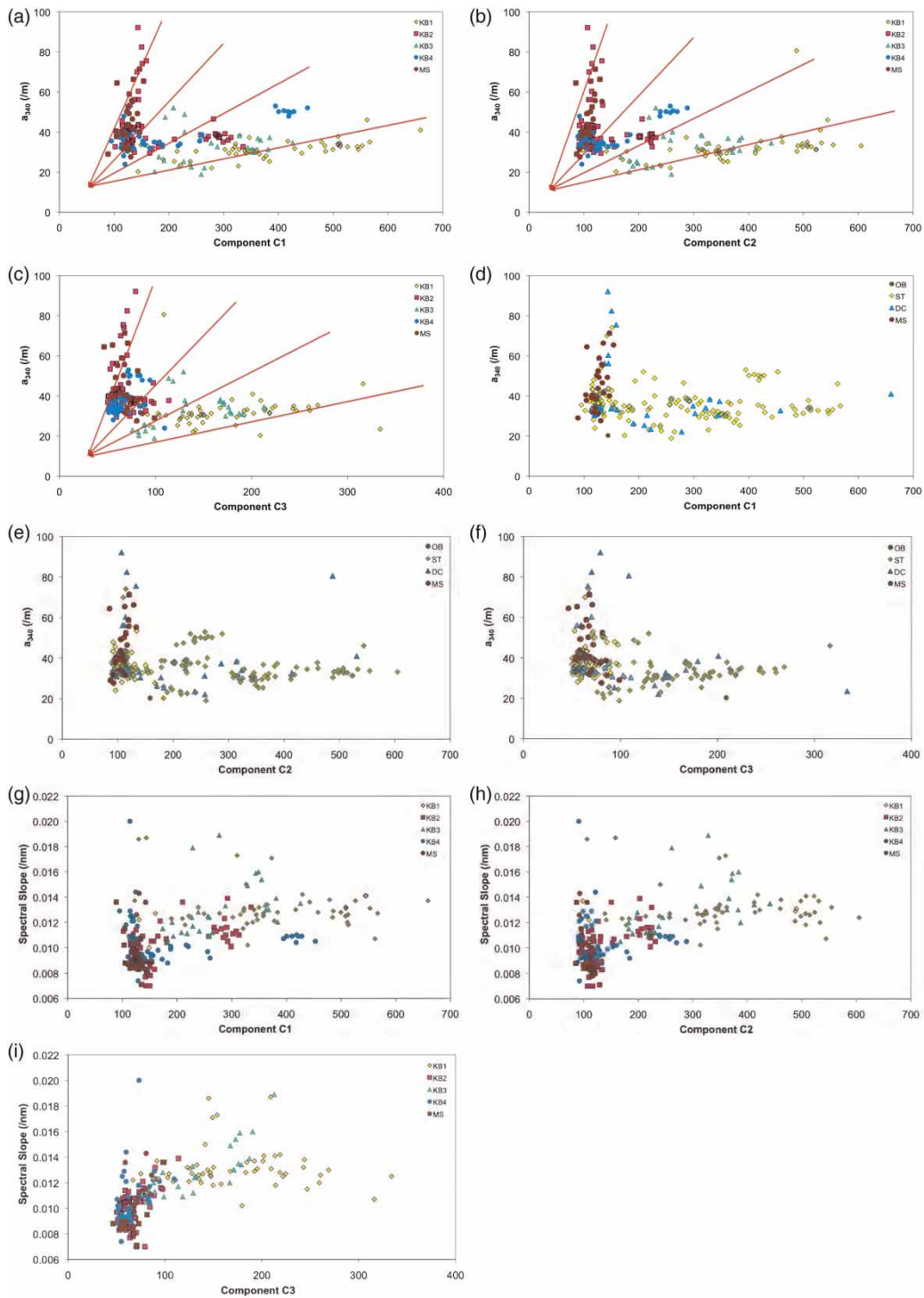
Figure 4 │ Correlation and comparison graphs between: (a) to (f) PARAFAC components C1, C2 and C3 against UV absorbance at 340 nm, respectively; (g) to (i) PARAFAC components C1, C2 and C3 against the spectral slope.
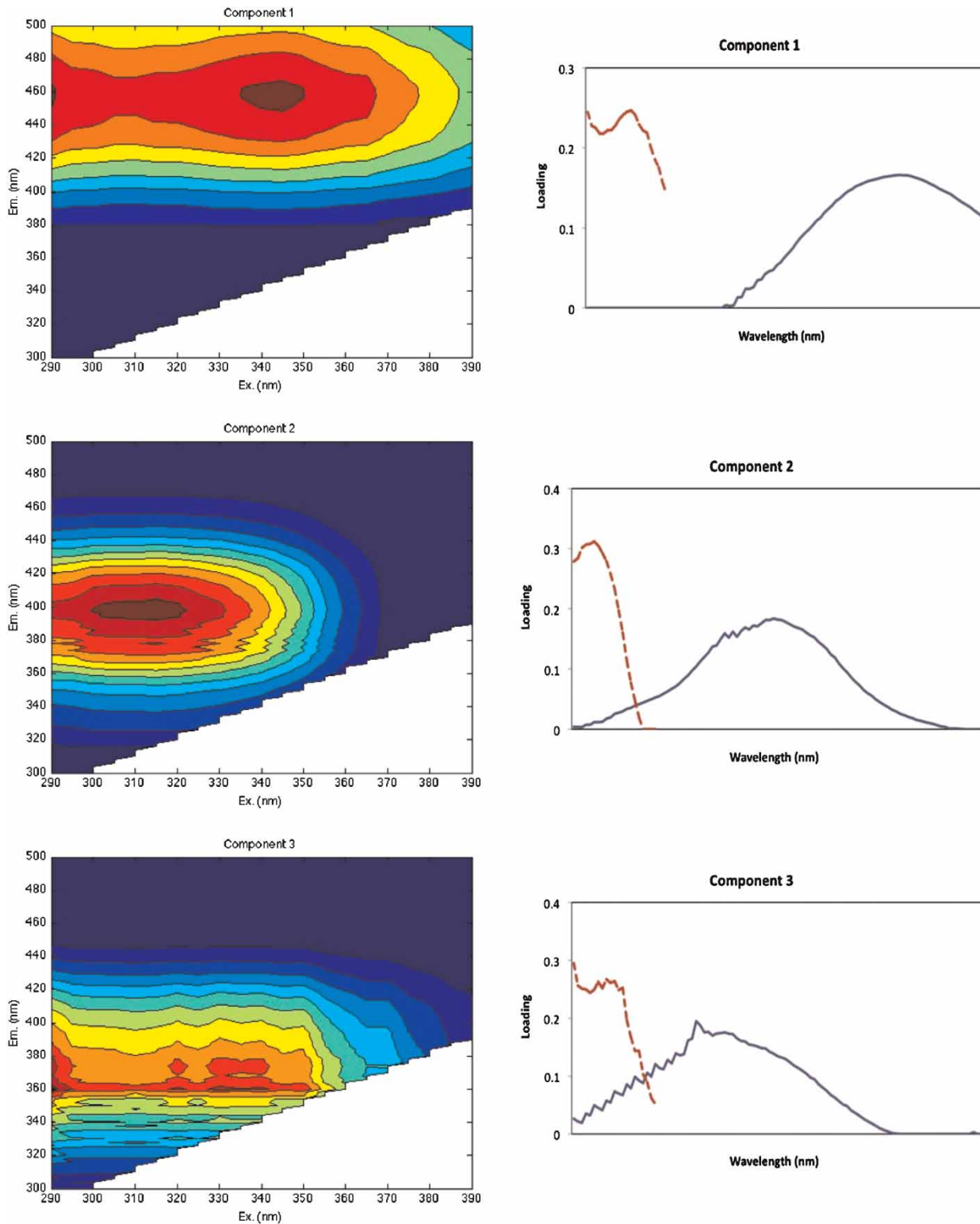
Figure 5 | Fluorescence signatures of three identified PARAFAC model components. Contour plots present spectral shapes of excitation and emission of derived components. Line plots adjacent to each contour plot present split-half validation results for each identified component. Excitation (dotted line) and emission (solid line) loadings for each component, obtained from two independent PARAFAC models on random halves of the data array.
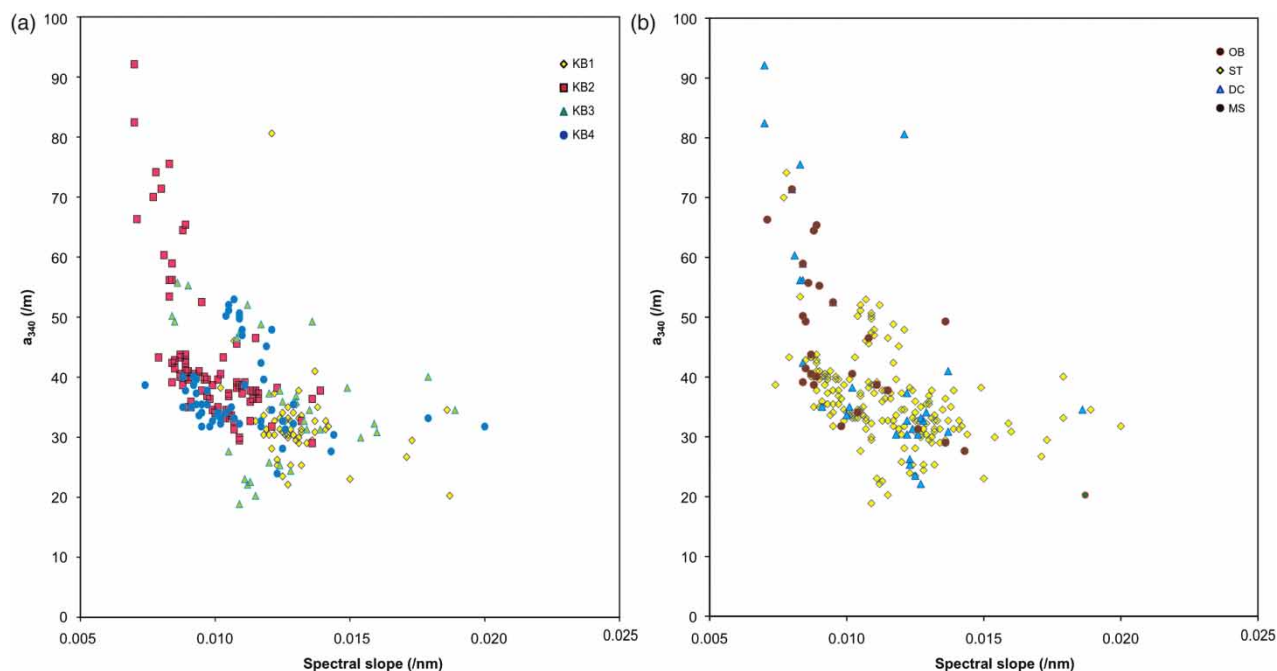
Figure 6 | UV absorbance at 340 nm against spectral slope ($S_{275-295}$) according to: (a) sampling area; (b) type of sampling site.

components C1, C2 and C3 are plotted against spectral slope.

Figures 6(a) and 6(b) present the UV absorbance at 340 nm against spectral slope for each sampling station. High UV-visible absorption coefficient of a_{340} and low spectral slopes characteristics were found in waters sampled from the main stem of the Kinabatangan and from oil palm plantation ditches (DC) in KB2 and KB4, which could be indicative of reprocessed DOM. A comparison between type of sampling sites showed that UV absorbance at 340 nm for DC was more variable than for MS and ST samples <45% coefficient of variance for DC and <27% for MS and ST). $S_{275-295}$ also exhibited similar variations where the coefficient of variance for DC was <23% and <20% for both MS and ST.

DISCUSSION

The environmental isotopes provide useful information on water movement through the catchment. Isotope data from tropical catchment are scarce (Stephens & Rose 2005), and hence the results of this study are useful in helping understand the modern stable isotope composition of Borneo.

The isotopic composition of precipitation samples varies significantly during the period of sampling, and becomes isotopically lighter over time. Deuterium isotope values within single rainfall events have been found to vary by as much as 30‰ in 15 min within a single temperate rainfall event (Dansgaard 1964). Isotopic variability within single rainfall events in tropical regions will also be strongly controlled by the isotopic composition of precipitation during the monsoonal periods reflecting 'amount effect' (Dansgaard 1964; Darling *et al.* 2005). The isotopic composition of waters sampled from the Kinabatangan River and tributaries are isotopically lighter than the annual mean composition of precipitation, intersecting the RMWL at ~50‰ ($\delta^2 H$) and -8‰ ($\delta^{18}O$). These waters evolve away from the MWL at a lower slope due to evaporation. Some tributaries respond more strongly (i.e., with a reduced slope) indicating greater evaporation across their sub-catchments which may be due to vegetation cover and/or higher evaporation. Significantly, waters sampled from drainage ditches associated with the oil palm plantations were among those waters that were isotopically evolved. This possibly reflects a reduced cover of understorey vegetation and hence a greater evaporation effect.

Spectral slope and UV absorbance at 340 nm yield useful information about DOM characteristics and have been found to correlate strongly with the molecular weight of fulvic acid isolates (Baker *et al.* 2008; Helms *et al.* 2008). UV absorbance at 340 nm has been found to be a useful surrogate for DOC concentration (Spencer *et al.* 2009). An indicative four times difference in molecular weight is seen between the results and the spectral slope of Suwannee River natural organic matter (Helms *et al.* 2008). Other studies have reported $S_{275-295}$ to be ~$13-17 \times 10^{-3} \, nm^{-1}$ in freshwater samples from Chesapeake Bay, USA (Helms *et al.* 2008), $12.2-19.9 \times 10^{-3} \, nm^{-1}$ in the Yukon River, Alaska (Spencer *et al.* 2009) and ~$18-19 \times 10^{-3} \, nm^{-1}$ from eutrophic Lake Taihu, China (Zhang *et al.* 2009b). Spectral slope has been found to increase with irradiation (e.g., Helms *et al.* 2008; Zhang *et al.* 2009a, b), but the ditches (DC) and main stem river-water samples (MS) were characterised by a lower spectral slope ($7-19 \times 10^{-3} \, nm^{-1}$), especially in the main river compared to the tributaries. This suggests that DOM in the main stem and oil palm plantation ditches of the Lower Kinabatangan River catchment in particular samples from KB2 is of relatively high DOC and molecular weight. A quantitative aquatic carbon budget for the Langat River watershed in Malaysia (Figure 1) indicated that although C3 plant-derived matter was the primary source of carbon in wetland areas, sewage treatment and landfill sites in the lower reaches of the river provided a significant additional source of organic carbon (Lee *et al.* 2013). The relatively non-fluorescent and high-molecular-weight DOM at the Lower Kinabatangan River catchment may be indicative of relatively stable organic complexes such as humic substances (HS) given the high concentration of fine sediment within the colloidal (<0.7 μm) size range, rather than as a result of photo-degradation processes. This is consistent with a wetland study in Mukah, Sarawak (Figure 1) which found DOC concentrations varied between 18.9 and 75.3 mgC/L with a significant correlation with soil-derived humic substances (Watanabe *et al.* 2012). This study also suggested that humic substances mainly drive DOC photoreactivity in wetlands and HS tend to increase with increasing precipitation and/or temperature.

The PARAFAC analysis provides further information on the origin and biogeochemistry of DOM at each site, as well as any systematic differences in DOM characteristics throughout the catchment (particularly from sites upstream to downstream; KB1 to KB4). The spectral characteristics of C1 resemble those of Kowalczuk *et al.* (2009), Luciani *et al.* (2008), Stedmon & Markager (2005) and Yamashita *et al.* (2008) and can be associated with terrestrially derived OM, which occurs in a range of aquatic environments. Both components C2 and C3 have been described by Coble (1996) and Parlanti *et al.* (2000) as marine in origin. Stedmon *et al.* (2003) suggested that this component was observed in samples which were terrestrially origin OM while more recently, Fellman *et al.* (2010) identified this peak as ultraviolet A; a low molecular weight component which they attributed to microbial processing. This component is common in marine environments associated with biological activity but has also been found in wastewater, wetland and agricultural environments. Thus, it is likely that components C2 and C3 found in samples from the Kinabatangan catchment is reprocessed DOM derived from microbial and/or photo-degradation processes.

The PARAFAC components C1, C2 and C3 identified in this study, indicate that the majority of DOM in the Kinabatangan catchment comprises terrestrially derived material, and that the relative loss of PARAFAC component C1, relative to absorbance, is due to a change in DOM characteristics. Upstream sites on the Kinabatangan (i.e., KB1) which include several sites closely associated with oil palm plantations (KB1-3, KB1-4, KB1-6 to 8), have a high fluorescence PARAFAC component C1 per unit absorbance and high spectral slope (Figures 6(a) and 6(b); Table 3), which is indicative of organic matter with relatively low molecular weight. In contrast, sites on the Kinabatangan downstream (i.e., at KB2) have a high UV-visible absorption coefficient and low spectral slope, which is indicative of a higher molecular weight DOM.

Although the effects of sunlight on DOM degradation by bacteria are various and contrasting (Cory *et al.* 2013), both components C2 and C3 could be indicative of microbial and/or photo-degradation processes, particularly given the high solar radiation that is typical of tropical areas. Significantly though, the variation in fluorescence relative to UV-visible absorbance between tributary sites and the main stem of the Kinabatangan, reveal a loss of highly fluorescent DOM within the catchment. This loss, and the general spatio-temporal variability of DOM characteristics,

will reflect the interaction between at least three sources of variability of DOM: (i) spatial variation in DOM source; (ii) the effects of transport (e.g., temporal degradation from photo-degradation); and (iii) differences among DOM in the propensity for biological and photochemical removal.

The results suggest that in the Kinabatangan catchment, rapid oxidation by photochemical and more especially microbial processes to produce carbon dioxide (Cory *et al.* 2007) preferentially breaks down the aromatic carbon-containing molecules which account for the fluorescent properties of DOM. DOM in waters sampled from low-order tributaries would be expected to be dominated by inputs of terrestrially derived DOM, which would include DOM that is derived from activities associated with oil palm plantations. This DOM is rapidly photo- and bio-degraded to less fluorescent, stable DOM, which is probably present as fine colloidal complexes. The concentration and character of DOM downstream is then likely to reflect hydrological controls, particularly the rate of water movement (Findlay & Sinsabaugh 1999).

CONCLUSIONS

The results and analyses presented in this paper provide important baseline data on variations in DOM quantity and quality in a degraded tropical catchment in the island of Borneo. The findings from this study provide further information on DOM characteristics in tropical agricultural catchments. These environments have been hitherto neglected in studies of DOM characterisation and hence the results presented here are particularly relevant to other degraded aquatic ecosystems, particularly those potentially impacted by the development of oil palm plantations. The results suggest that by characterising DOM using absorbance and fluorescence, it is possible to differentiate between the DOM characteristics of individual sub-catchments of the Kinabatangan River. The analysis is helped significantly by the additional information provided from environmental isotopes, and there is evidence of progressive photo- and microbial-degradation of DOM downstream. While this is important in itself, the focus in this study as been on baseflow conditions, and more work is urgently required to investigate any seasonal trends in DOM characteristics in catchments such as the Kinabatangan.

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