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Spatial and seasonal variations in the composition of dissolved organic matter in a tropical catchment: the Lower Kinabatangan River, Sabah, Malaysia

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Abstract

Dissolved organic matter (DOM) was characterised in waters sampled in the Lower Kinabatangan River Catchment, Sabah, Malaysia between October 2009 and May 2010. The study sought to: i. distinguish between the quality of DOM in waters draining palm oil plantations (OP), secondary forests (SF) and coastal swamps (CS) and, ii. identify the seasonal variability of DOM quantity and quality. Surface waters were sampled during fieldwork campaigns that spanned the wet and dry seasons. DOM was characterised optically by fluorescence Excitation Emission Matrix (EEM), the absorption coefficient at 340 nm and the spectral slope coefficient (S). Parallel Factor Analysis (PARAFAC) was undertaken to assess DOM composition from EEM spectra and five terrestrial derived components were identified: (C1, C2, C3, C4 and C5). Components C1 and C4 contributed most to DOM fluorescence in all study areas during both the wet and dry seasons. The results suggest that component C4 could be a significant (and common) PARAFAC signal found in similar catchments. Peak M (C2 and C3) was dominant in all samples collected in wet and dry seasons, which could be anthropogenic in origin given active land use change in the study area. In conclusion, there were significant seasonal and spatial variations in DOM which demonstrated the effects of land use cover and precipitation amount in the Kinabatangan catchment.

Keyword: Dissolved organic matter, tropical river, Excitation-emission matrix, Kinabatangan, PARAFAC model

Introduction

A recent synthesis and re-evaluation of the global carbon cycle suggested that approximately 3 Pg C per year of CO_2 is outgassed from global inland waters[1], while the estimated global riverine total carbon flux is 0.80-1.33 Pg C per year.[2] Given that it has been estimated that approximately half of the carbon is consumed within river systems before reaching the ocean[3], in-stream and near-stream processing of organic matter is a fundamental component of the carbon cycle. This corroborates a research which found that Amazonian rivers outgassed more than ten times the quantity of carbon exported to the ocean in the form of total organic carbon or dissolved organic carbon (DOC).[4] Significantly, determination of the carbon isotopic composition of DOC suggests that contemporary organic carbon (i.e. carbon < 5 years in age) was the dominant source of excess CO_2 that drives outgassing in Amazonian first order streams and large rivers.[5] Together, these results emphasize the importance of land-derived, biologically available carbon, for heterotrophic microbial processes in river systems.

Tropical wetlands provide important ecosystem services including flood mitigation, coastal and wildlife protection, carbon sequestration and respiration.[6] Tropical wetland ecosystems include a variety of landforms such as lowland floodplains, forested peatlands, swamps and mangroves.[7] The latter are particularly important carbon sinks which have been reported to store ~49-98% ecosystem carbon in the organic soils.[8] Tropical wetlands also experience periodic (prolonged) inundation[9], reflecting marked seasonal variations in precipitation[10], while evapotranspiration rates are high.[11] Tropical wetlands have been associated with the release of an estimated ~60% of total (global) water, sediment and organic carbon input to the ocean[12]. However, these wetlands are seriously threatened by environmental deterioration as many catchments have experienced rapid conversion of land to agriculture[13-15] with a concomitant reduction in wetland extent.

Among other agricultural threats, conversion of tropical forest to oil palm (*Elaeis guineensis*) cultivation is a major concern given the recent growth of the palm oil industry.[16] Oil palm plantations are now estimated to extend over >13.5 million ha of the tropics[17] and have contributed to the drainage of floodplain wetlands, and the loss of primary and secondary forest.[18] At present, the majority of oil palm plantations are confined to South East Asia, as Malaysia and Indonesia produce ~80% of the world's palm oil. However, substantial areas of the Congo and Amazon Basin are suitable for oil palm plantation, and further plantation developments are likely in these areas. This situation emphasizes the urgent need to understand the environmental implications of oil palm development. This is particularly important as the full implications of recent and, in some places accelerating, changes in oil palm cover have yet to be considered in detail and these land use changes are likely to affect the quantity and quality of dissolved organic matter (DOM) and DOC.[19,20]

Recent advances in fluorescence spectroscopy have considerable potential as we seek to address this research gap, as they have significantly enhanced our ability to characterize DOM.[21] DOM fractions possess fluorescent properties enabling DOM monitoring in soils[22], rivers[23,24], lakes[25], estuarine and coastal environments.[26,27] Reassessment of fluorescence excitation emission matrix (EEM) spectra using Parallel Factor Analysis (PARAFAC) has been invaluable in characterising and quantifying changes in DOM fluorescence. By decomposing an EEM dataset into several, mathematically independent components parameterized by concentrations (loadings) and excitation and emission spectra, different DOM fractions have been traced through the natural environment.[28] For example, in southern Ontario, Canada, DOM production and transformation processes were successfully studied in areas of different use.[24] Specifically in a tropical catchment, DOM export was found to be greater during the April flush (inter-monsoonal period), and it has been suggested that tropical rivers are likely to export more labile DOM during periods of high

precipitation.[29] This is supported by a study in the sub-tropical Jiulong River catchment, China, where increased DOM concentrations were observed after storms, as a result of terrestrial DOM export to the river; with a decrease in the protein-like fraction of DOM over the same period.[30] In sub-tropical Uruguay also, DOM characteristics have been found to vary temporally in catchments with intensive farming practices which was positively related to microbial processing.[19] These results have implications for downstream and marine ecosystems, however, the importance of this research has yet to be more widely established.

The potential utility of fluorescence spectroscopy, specifically in SE Asia, was demonstrated in a preliminary study that sought to characterize spatial trends in DOM in the Lower Kinabatangan River Sabah, Malaysian Borneo.[31] River flow and photodegradation were found to have a significant effect on DOM properties, however, the extent to which DOM varies seasonally was not considered. This provides the motivation for this paper particularly as, in common with other catchments in this region, there has been a rapid recent increase in the areal extent of oil palm plantations. This, and the conservation of riparian secondary forest and coastal wetlands within the catchment, provides an opportunity to determine the degree to which DOM quantity and quality is affected: first by land-use change, and second by the seasonal flood pulse.[32] Accordingly, the objectives of this study were to: i. characterize DOM quality in waters associated with palm oil plantations, secondary forests and coastal wetlands using fluorescence spectroscopy and PARAFAC;[33,34] and ii. determine the seasonal variability of DOM quantity and quality, and its attribution to each land cover type.

METHODS AND ANALYTICAL PROCEDURES

Study area - DOM characteristics 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 total catchment area of 16,800 km^2 (Fig. 1).[35] Geologically, the Kinabatangan area is predominantly covered by sandstone and shales, with minor occurrence of cherts and limestones, while the igneous rocks are mainly basalts, serpentinites, gabbros, volcanic breccias and tuffs.[36] Four groups of soil parent material were identified by surveys conducted in the early 1950s: undifferentiated alluvium, peat, sandstone and mudstone and limestone.[37,38] Recent alluvium, originating mainly from sedimentary rocks, is found widely on floodplains and in freshwater swamps.[38]

The area has a humid tropical climate with mean daily temperatures ranging from 22°C to 32°C and mean annual rainfall of 2,500 - 3,000 mm.[35,39] Rainfall is greatest between November and March particularly during the northeast (NE) monsoon, and to a lesser degree during the southwest (SW) monsoon.[39,40] Transition periods, referred to as the 'inter-monsoonal periods', normally occur in April and October and generally correspond with the period of lowest rainfall[41] although significant precipitation events may still occur at this time.[40,42] Typically, the floodplain and coastal plain are widely inundated during the rainy season but rainfall totals exhibit considerable inter-annual variability.

The lower floodplain of the Kinabatangan is >2,800 km^2 in area (Fig. 1) with two principal land uses: (i) forest (mangroves and peat swamps); (ii) agriculture (primarily oil palm plantations and other crops); with relatively little urban development and only occasional small water bodies.[43] Approximately 74% of the Kinabatangan catchment is tropical forest, including floodplains with open reed swamp, and lowland dipterocarp forest in areas that are inundated frequently.[39,43] Mean river flow in the upper catchment, recorded at Pagar (PGR) and Balat (BLT) (Fig. 2), varied from ~14.0 to 1944 m^3/s (26-1944 m^3/s) between 1979 to 2013 (peak daily discharge was recorded in January 1986 at BLT; the lowest flow was observed at PGR in June 1998). Only limited sediment data are available, but a survey at Sukau (at points upstream of coastal swamps) (Fig. 1) in 2005 and 2006 indicated

that maximum sediment concentrations were 96 mg/l equating to Class IIB of the Malaysian Interim National Water Quality Standard (INWQS).[43,44] This appears to reflect commercial logging in the catchment since the 1980s and the development of oil palm plantations which currently extend over ~4,200 km^2 which represents approximately 25% of the basin.[43]

Sampling and analyses – Waters were characterized throughout the lower catchment through the manual collection of 510 water samples during five sampling periods in 2009-2010. One period corresponded with the inter-monsoonal period (IM): October 2009; three corresponded with the wet season (WS): November, December 2009 and February 2010; and one the dry season (DS): May 2010. Fieldwork design was constrained by difficulties of access; however, waters were sampled along a freshwater – estuarine gradient to determine seasonal trends in DOM in the Lower Kinabatangan floodplain including across the freshwater-marine interface between the Kinabatangan River and the Sulu Sea. The nearest gauging station was at Barek Manis (BM), situated ~11km from sample point SF-1, at which point the upstream catchment is 12,300 km^2 (Fig. 1). Monthly mean discharges during the fieldwork campaign are presented in Table 1.

Waters were sampled from streams or creeks situated entirely within: i. oil palm (*Elaies guineensis*) plantations: OP-1, OP-2, OP-3 and OP-4 (220 samples); ii. secondary forests: SF-1, SF-2 and SF-3 (139 samples) and iii. coastal swamps of *Nypa fruticans* (nipa palm): CS-1 and CS-2 (151 samples) (Fig. 1). The vegetation characteristics for each land cover type are summarised in Table 2 (after Abram et al.[45]). At each point, a 200 ml water sample was collected from the middle of the river / stream from a boat at three points in the water profile: the surface, the mid-point and near the riverbed using a WaterMark Horizontal Polycarbonate water sampler. Samples were stored in high-density polyethylene (HDPE) bottles, pre-washed with hydrochloric acid 10% and deionised water. pH and salinity were determined using a

Hanna Water Quality Multiparameter (Model HI 9828) immediately prior to filtering the water samples (within six hours of sample collection) using 47 mm pre-combusted Whatman glassfiber GF/C filter papers (nominal pore size 1.2 μm). Filtered water samples were kept in the dark and stored at 4ºC before shipment to the UK for laboratory analysis, which occurred within seven days of the end of the field-campaign.

Spectral measurements and DOC – Fluorescence analyses of samples were performed at the University of Birmingham, UK, using a Varian Cary Eclipse spectrophotometer. Excitation-emission matrices (EEMs) were generated for each sample over excitation wavelengths 250 to 400 nm at 5-nm intervals and emission wavelengths 280 to 500 nm at 5-nm intervals, with 2-nm bandwidths on excitation and emission modes. Spectrophotometer output was monitored by regular determination of the Raman intensity of ultra pure water in a sealed 10 x 10 mm cuvette at 348 nm excitation and 5 nm bandpass. No significant changes were observed in the EEMs, particularly in samples associated with the secondary forest and coastal swamps (the mean salinity for all samples collected was 1.27‰), although Yang & Hur[46] suggested the potential impact of salinity on fluorescence DOM peaks A and M. An inner-filter effect (IFE) correction was applied to the data set[47]:

$$I = I_0 \, (10^{-b(Aex+Aem)}) \qquad (1)$$

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 absorbance at emission wavelength.

Absorption coefficients at 340 nm and spectral slope over the interval of 275-295 nm ($S_{275-295}$)[48] were determined using a Lightwave (WPA) spectrophotometer in a

10 mm quartz cuvette. Absorption measurements were corrected against Milli-Q water blanks and the slope of the absorption curve was calculated by linear regression of the log-transformed *a* spectra.

Dissolved organic carbon (DOC) was determined using a Shimadzu TOC-V-SCH analyser with auto-sampler TOC-ASI-V. Samples were acidified to pH ~2 with HCl and analysed within one month collection. Acidified samples (pH ~2) were sparged for 8 minutes at 75 or 100 ml/min^{-1} with either ultra-pure oxygen to remove all inorganic carbon from samples prior to measurement.

PARAFAC modelling – Fluorescence excitation emission matrix (EEM) spectra were reassessed using Parallel Factor Analysis (PARAFAC).[33,34] Fluorescence EEMs were combined into a 3-dimensional data array and decomposed to a set of trilinear terms and a residual array:

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

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 components in the model, and e_{ijk} is the residual noise (i.e. the variability that is not explained by the model). Despite the use of a 250-395 nm excitation filter, the initial PARAFAC analysis was confounded by scatter in individual EEMs, which occurred within the excitation (emission) wavelength ranges of 250 to 280-nm (280 to 290-nm). In this study, mean suspended sediment concentrations of the Lower Kinabatangan River varied between ~7 and ~9800 mg/l (data not presented). As a result, of filtration the signal / noise ratio for excitation wavelengths <290 nm was not acceptable and consequently the microbial peak, which corresponds to an excitation wavelength of 280 nm excitation, was removed in the PARAFAC analysis.

A PARAFAC model with non-negativity constraint on all modes (samples, emission and excitation) was implemented in MATLAB. The data were split into two random halves each comprising 254 EEMs, representing a calibration data array and a validation array. The appropriate number of components (the model rank) was determined by comparing the excitation and emission spectra of components between the calibration and validation data arrays and from split-half analysis, a total of five components were validated. Two categories of independent data sets were successfully validated: first, an inter-seasonal comparison between the wet and dry season, and second a land use comparison: oil palm plantations (OP), secondary forests (SF) and coastal swamps (CS). This compares with an earlier PARAFAC model for the Kinabatangan catchment which had validated three components.[31] In the earlier model, however, all sampling stations were situated in the immediate vicinity of oil palm plantations while in the current study, sampling sites distinguished between three land use types (oil palm plantations, secondary forests and coastal swamps). Thus the five components presented in this study could potentially reflect differences in DOM composition according to land use.

The PARAFAC model returns the relative intensities of derived components, and the intensity of the n^{th} component in a given sample remains unknown. Hence I_n was estimated by determining the fluorescence intensity at the peak excitation and emission maximum of the n^{th} component[49]:

$$I_n = Score_n * Ex_n(\lambda_{max}) * Em_n(\lambda_{max}) \quad (3)$$

where: $Score_n$ is the relative intensity of the nth component, $Ex_n(\lambda_{max})$ is the maximum excitation loading of the n^{th} component, $Em_n(\lambda_{max})$ is the maximum emission loading of the n^{th} component derived from the model. The total fluorescence intensity (I_{tot}) was calculated as the sum of the components.

Statistical Analysis – Precipitation data were analysed using a paired-sample t test to determine whether there were significant differences between inter-monsoonal period (IM), wet (WS) and dry seasons (DS). The *p*-value ($p<0.05$) for pairs of IM-WS, IM-DS and WS-DS are 0.950, 0.142 and 0.018 respectively. This analysis also sought to verify whether the rainfall data used in the study were free from precipitation anomalies, potentially caused by irregular synoptic forcing associated with the El Niño Southern Oscillation (ENSO) and changes in the seasonality of the monsoon in SE Asia.[44] Discriminant analysis was applied to characterise DOM according to land use type and seasonal variations. Calculations of the fluorescence intensities (I_n) of the individual components indicated that: IC4 > IC2 > IC3 > IC1 > IC5, suggesting that the terrestrially derived peak A had the most abundant spectral characteristics, followed by peak M (IC2 and IC3), peak C (IC1) and peak M (IC5). UV absorbance at 340 nm and fluorescence DOM (FDOM) were normalized to IC4 and fluorescence indices (FI) (ratios as detailed below) were used to determine the pre-dominance of each parameter in each land use type to gain more insight on DOM characterisation: $IC4/a_{340}$ (peak A/a_{340}), IC2/IC4 (peak M/peak A), IC3/IC4 (peak M/peak A) and IC5/IC4 (peak M/peak A). Both paired-sample t test and discriminant analysis were undertaken using SPSS version 21.0.

Results and Discussion

The data presented here provide the first evidence of seasonal changes in DOM composition in a catchment affected by the recent development of oil palm plantations. In the following section we compare our results with recent studies of other tropical catchments and consider the wider significance of this work.

Characterisation of PARAFAC Components – Five fluorescent components were identified by PARAFAC from analysis of the 510 sample dataset (Fig. 3). The excitation and emission pairs of the main peak positions for each component are summarised in Table 3, and individual components are plotted in Fig. 3. Table 3 also compares the results with components identified in selected studies that have modelled DOM in marine, oceanic and estuarine environments.

Our PARAFAC model identified five terrestrially-derived substances: component 1 (C1) to component 5 (C5). Our terrestrially-derived components (C1 and C4) have been observed in other tropical and sub-tropical studies: these are ubiquitous, fulvic-acid representing fluorophores that have the longest excitation (and emission) wavelength and broadest excitation (and emission) band. Our components were found to relate specifically to the Component 1 described by Luciani et al.[50], Stedmon and Markager[51] and Yamashita et al.[52]; to the Component 2 of Fellman et al.[21], Component 3 of Yao et al.[53] and to Component 4 of Kowalczuk et al.[49] Our earlier DOM characterisation study in the Lower Kinabatangan also reported a terrestrial-derived Component 1 (peak A and C).[31] Our C1 (identified here) is similar to the humic-like fluorophore in the visible region defined by Coble.[54]

Our components C2, C3 and C5 have been previously reported as peak M; they have shorter emission wavelengths and were initially attributed to a marine source of DOM.[54,55] Subsequently Stedmon et al.[56] suggested that this component is found in 'terrestrially dominated end-member samples', and Fellman et al.[57] described this peak as ultraviolet A (UVA), a low molecular weight component related to microbial activities. While peak M is common in marine environments and is apparently related to biological activity, it is also found in wastewater, in wetlands and agricultural environments. Peak M production could be partly due to photobleaching of terrestrial FDOM or autochthonous production from microbial processes.[58] Our C2 resembles Component 3 found by Murphy et al.[59], and Components 4 and 6 of

Stedmon et al.[56] and Yamashita et al.[52]. This component was also reported by Zhang et al.[60]: their Component 1; Luciani et al.[50]: their Component 2; Stedmon et al.[56]: their Component 3; Yao et al.[53]: their Component 3; and Stedmon et al.[56]: their Component 5.

Comparison of the fluorescence intensities, $I_{n,}$ indicated that terrestrially-derived peak IC4 (peak A) had the most abundant spectral characteristics. The peak component has been described as ubiquitous, photo-labile, terrestrially-derived OM which originates from agricultural activities[52] but it could also represent a photodegradation processing pathway.[61] Natural forest cover in the Lower Kinabatangan river catchment has declined from ~91% in 1970's to ~47% in 1995[62]; and at present ~25% of the catchment is largely cultivated with oil palm plantations[39], which could explain the spectral characteristics and abundance of component IC4.

The PARAFAC components summarised in this paper are similar to those outlined in other studies of tropical catchments[31,50,52] indicating that common attributes can be identified. However, the DOM characteristics described in most previous studies, are of DOM that has a very different origin (including subtropical wetlands[50] and enclosed coastal water bodies[52]) to that found in our study in NE Sabah[31]. Consequently the results and the implications for both the Kinabatangan catchment, and tropical regions generally, should be interpreted with caution, as there might be site-specific contributions of natural organic matter from other land use and vegetation types might be only applicable in a local context[23]. It might also be possible for the fluorescence characteristics to appear 'identical' in different catchments, albeit associated with a different DOM composition.[63]

Seasonal and Land Use Variations – Discriminant analyses of the DOM data-set and land use type yielded two discriminant functions as summarised in Table 4 and Fig. 4. The ratios of i. IC3/IC4 and ii. IC2/IC4 were found to always correlate positively with

IC5/IC4. They were classified in discriminant function 1 (DF1) and explained 79.2% of the variance. These results suggest that DF1 corresponds to fluorescence properties arising through photodegradation thus representing a DOM processing signature. Moreover, samples from coastal swamps (CS) were found to comprise DOM which was less processed (i.e. the DOM was fresher or younger), while DOM in waters sampled from the oil palm plantations (OP) showed evidence of greater processing, particularly in those samples collected from canals with stagnant water.

Seasonal trends in DOM characteristics were also evident in the discriminant analysis: DF1 suggests that the ratio of IC5/IC4 correlated positively with IC2/IC4 and explained 84.1% of the variance (Table 4). IC4/a_{340} was dominant in waters sampled during the wet season and (Fig. 4), suggesting that DOM was fresher during the wet season (WS) compared with the dry season (DS) when DOM was more processed. While no seasonal variations in EEMs were observed by Baker & Spencer[64] in their study in a temperate maritime catchment with anthropogenic DOM inputs in the Tyne, UK, other studies highlighted seasonal variability in EEMs. For example, Zhao et al.[65] observed seasonal variations in EEMs from semi-arid lakes in NE China. Seasonal patterns of DOM distribution also have been found in subtropical Florida Bay, USA where relative abundance of humic-like (Ex/Em=<260, 345/462 and protein-like component (Ex/Em=275/326) were higher during the early wet season (June to August).[66]

The ratios IC2/IC4 and IC5/IC4 were high in samples from the oil palm plantations (OP) during the dry season, suggesting that the DOM was more processed in the OP samples and could have been affected by microbial activities and/or photo-degradation during this period. Preliminary $\delta^{13}C$ and molar C:N values of both DOM and particulate organic matter (POM) in an Australian tropical rainforest catchment suggested that exports of microbially processed organic matter were higher from upper soil horizons during the dry season.[67] Subsequently, Lee-Cruz et al.[68] investigated soil bacterial communities in logged forest and oil palm plantations

in Sabah and found a high abundance of *Actinomycetales*, which are dominant in cultivated areas.[69] Their study indicated that oil palm plantation soils have a higher bacterial diversity and turnover and are more heterogeneous. A study in Jambi, Indonesia revealed a high abundance of the genus *Burkholderia, Cupriavidus* and *Acinetobacter* in bacteria isolates from oil palm plantation aquatic sediments.[70] *Burkholderia* and *Cupriavidus* are nitrogen-fixing[71,72] plant growth promoting bacteria[72] while *Acinetobacter* has been reported ubiquitous in soil and surface waters[73], is a nonmotile, agent for biodegradation, leaching and removal of organic and inorganic waste.[74] An earlier water quality study in the Sukau area of the Kinabatangan catchment (Fig 1) during a weak La Niña event (2005 to 2006) indicated that the Biochemical Oxygen Demand (BOD) of a stream in an oil palm plantation ranged from 1.3 to 2.1 mg/l.[44] Dry season water samples from downstream reaches on the Sg. Langat in Selangor, Malaysia, which were also located within oil palm plantations, had mean BOD values ranging from 2.1 to 2.6 mg/l[75]. Therefore, we hypothesize that the peak M we found in the Lower Kinabatangan River catchment, which varied seasonally and according to land use, could be derived from microbial and/or photo-degradation processes.

The variation in DOM according to season and land cover is illustrated in Fig. 5 by plotting DOC against PARAFAC component IC4 for each land use type. Tabulated DOC concentrations varied from 9.88 to 12.85 mg/l (Table 1). Samples from secondary forests (SF) and coastal swamps (CS) showed a strong positive correlation between DOC and PARAFAC component C4 (r^2 of 0.6 and 0.7 respectively). It also showed that DOM composition in both SF and CS were moderately constrained by monsoon and flow, compared to samples from the oil palm plantations (OP), which were highly constrained in particular during the inter-monsoonal period and wet season. This is consistent with the discriminant analysis (Fig. 4), which indicated the ratio of IC4 to a_{340} was dominant in samples collected in SF during the wet season, while the spectral slope (275-295 nm) was found to be

dominant in CS during the inter-monsoonal period (October 2009). There were positive correlations between UV absorbance a_{340} nm and PARAFAC component C4 (peak A) with regression value of 0.5 for all types of land cover (Fig. 6). UV absorbance at ~340 nm and spectral slope have been showed to be indicative of DOM molecular weight[48,76], and to correlate positively with lignin concentration.[29] Lignin concentration in aquatic ecosystems was strongly influenced by seasonal hydrology, river catchment discharge, flooding events and types of vegetation and land use.[77] A quantitative aquatic carbon budget for the Langat River in Malaysia indicated that although C3 plant-derived matter was the primary source of carbon in wetland areas, sewage treatment and landfill sites in the lower catchment provided significant additional inputs of organic carbon.[78] Nedwell et al.[79] demonstrated that carbon mineralisation in a subtropical mangrove swamp in Jamaica was higher compared to other areas, indicating abundant OM availability. Mangrove forests also typically have rich tannins, which is likely to be the main source of protein-like fluorescence.[80] They are also associated with decreasing bacterial counts[81] and hydrophobic acids[82], which could explain observations of low molecular weight DOM in CS samples in the Lower Kinabatangan River catchment during the inter-monsoonal period.

Our results indicated that the ratio IC4 (peak A) to a_{340} was high and the spectral slope ($S_{275-295}$) was low in waters sampled from secondary forests during the wet season. This could be associated with DOM inputs that were fresher and of higher molecular weight. There was also evidence of DOM degradation (bio- and photo-degradation) in river reaches downstream, including the estuary. The consistent high DOC concentrations that we observed in our study are indicative of high concentrations of humic material in the waters sampled. Previous work has demonstrated that secondary forests have the potential to absorb and store a large proportion of the carbon and nutrients lost as a result of changes in land use and

particularly deforestation.[83-85] Secondary forests can be effective nutrient sinks in which nutrients can accumulate rapidly over time. With respect to organic matter production, secondary forests can return significant OM in litter fall although they store less nutrients in their litter.[86,87] This results in high nutrient cycling rates in litter, facilitating nutrient recycling but potentially contributing to nutrient loss.[86]

Conclusion

We conclude that the characteristics of DOM in the Lower Kinabatangan River catchment, Malaysia are dominated by the fluorescence peaks A (IC4) and M (IC2, IC3 and IC5). These peaks indicate the importance of microbial and photo-degradation processes, particularly during the dry season, which break down the aromatic carbon molecules that account for DOM fluorescence. Discriminant analysis of the PARAFAC data set indicated that OP samples could be distinguished by plotting peak M (IC2, IC3 and IC5) against a_{340}, confirming the importance of microbial activity and photo-degradation processes in streams associated with oil palm plantations. The ratio IC4/a_{340} and spectral slope successfully distinguished secondary forests, followed by oil palm plantations and coastal swamps, suggesting that DOM with higher MW are found in SF. This also suggests variations in the quality of DOC production in different land use types, modified by the monsoonal cycle. This is supported by the PARAFAC model presented here which yielded three peak M components. Hence the results presented here demonstrate that analysis of EEMs, supported by PARAFAC, are a useful tool to determine and characterise the humic and fulvic substances in aquatic ecosystems, which correlate strongly with DOC.

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Table 1 Descriptive statistics of environmental conditions, average monthly discharge at BM station and PARAFAC model of selected sampling stations in the Lower Kinabatangan River Catchment.

	Average of monthly discharge at Barek Manis (BM) (m^3/s)	Land cover		pH	Salinity	DOC (mg/l)	a_{340} (/m)	$S_{275-295}$ (/nm)	IC1	IC2	IC3	IC4	IC5	I total
Inter-monsoonal (IM)	Oct. 2009 346.6	**OP**	Mean	6.94	0.07	15.30	42.14	0.0128	8.17	10.93	13.95	8.92	5.72	47.69
			Std. dev.	0.52	0.03	9.50	23.25	0.0018	3.68	3.83	5.75	3.10	2.96	15.88
			Variance	0.27	0.00	90.24	540.63	0.0000	13.53	14.66	33.05	9.59	8.76	252.27
		SF	Mean	6.89	0.04	10.20	55.75	0.0113	8.06	9.44	13.29	7.62	3.23	41.63
			Std. dev.	0.25	0.01	7.64	13.17	0.0012	3.31	3.64	5.07	2.93	1.66	15.86
			Variance	0.06	0.00	58.35	173.43	0.0000	10.97	13.26	25.75	8.59	2.75	251.64
		CS	Mean	6.20	1.33	10.36	45.49	0.0125	8.48	10.34	14.23	8.14	2.83	44.03
			Std. dev.	0.76	2.15	5.03	17.49	0.0018	3.07	3.32	4.88	2.52	0.95	14.16
			Variance	0.57	4.62	25.27	305.76	0.0000	9.41	11.02	23.84	6.35	0.91	200.60
Wet Season (WS)	Nov. 2009 561.4	**OP**	Mean	6.77	0.06	11.59	62.37	0.0104	10.46	14.94	11.70	25.63	3.39	66.11
			Std. dev.	0.66	0.02	3.58	35.46	0.0016	5.03	6.18	4.96	10.70	0.87	26.85
			Variance	0.44	0.00	12.82	1257.48	0.0000	25.28	38.24	24.64	114.55	0.75	721.17
	Dec. 2009 432.5	**SF**	Mean	6.93	0.04	11.17	75.88	0.0100	9.90	13.28	10.08	23.59	2.89	59.75
			Std. dev.	0.30	0.02	5.46	65.67	0.0019	6.55	7.69	5.95	13.77	0.75	33.96
			Variance	0.09	0.00	29.79	4312.30	0.0000	42.85	59.07	35.37	189.74	0.57	1153.60
	Feb. 2010 404.4	**CS**	Mean	5.95	0.08	14.70	100.49	0.0112	14.34	18.16	14.14	33.12	2.71	82.70
			Std. dev.	0.76	0.04	4.14	62.52	0.0023	6.15	6.50	5.45	12.16	0.85	29.65
			Variance	0.57	0.00	17.15	3908.20	0.0000	37.88	42.27	29.74	147.76	0.73	879.15
Dry Season (DS)	May 2010 260.5	**OP**	Mean	6.90	0.09	7.26	20.28	0.0125	7.13	11.21	9.11	17.61	6.27	51.33
			Std. dev.	0.75	0.03	1.61	5.80	0.0015	1.92	3.44	2.87	5.13	3.26	13.96
			Variance	0.56	0.00	2.58	33.59	0.0000	3.68	11.85	8.23	26.27	10.61	194.99
		SF	Mean	6.87	0.07	7.42	28.04	0.0113	7.74	11.48	8.84	18.45	4.51	51.03
			Std. dev.	0.24	0.04	2.40	6.40	0.0012	3.54	5.17	4.09	8.36	3.18	22.99
			Variance	0.06	0.00	5.77	40.93	0.0000	12.52	26.71	16.70	69.90	10.13	528.59
		CS	Mean	7.11	2.39	6.21	14.03	0.0135	6.16	10.23	7.58	16.66	5.92	46.55
			Std. dev.	0.30	2.09	0.95	4.59	0.0019	1.47	2.57	1.84	4.65	2.58	10.66
			Variance	0.09	4.38	0.91	21.02	0.0000	2.15	6.62	3.40	21.64	6.68	113.55

Table 2 List of sampling stations based on land cover and its vegetation characteristics (after Abram et al.[45]).

Sampling station	Vegetation characteristics
Oil palm plantation:	**Palm oil classes:**
Sg. Pin (OP-1)	i. Young mature: Palms were planted from 3-6 years.
Sg. Koyah (OP-2)	ii. Prime mature and full stand: Prime yield and planted within the range from 7-24 years.
Malbumi Plantation (OP-3)	iii. Underproductive at 75%: Palm capacity is within 51-75% palms per ha. Older palm with natural mortality start to occur.
Sg. Resang (OP-4)	iv. Underproductive at 50%: Palm capacity is ranged from 26-50% palms per ha.
Secondary forest:	**Forest type:**
[a]Danau Kaboi (SF-1)	i. Lowland dry forest: Secondary forest, preceding dipterocarp forest with species include *Nauclea subdita, Neolamarckia cadamba, Glochidion rubrum.*
[b]Danau Kalinanap (SF-2)	ii. Lowland dry dipterocarp forest: Preceding logged lowland mixed dipterocarp forest, dominated by *Dipterocarp sp.*
Sg. Menanggol (SF-3)	
Coastal swamp:	**Mangrove forest:**
Balat Damit (CS-1)	Nipah palm forest: *Nypa fruticans* are dominant within the mangrove system. Can be found either in mono-stand or coexist with *Rhizophora apiculata*.
Sg. Merah (CS-2)	

[a,b] SF-1 and SF-2 are oxbow lakes.

Table 3 Spectral characteristics of excitation and emission maxima of five components identified by PARAFAC modelling for the whole EEMs data set collected in the Lower Kinabatangan River catchment compared to previously identified sources.

Component in this study	Excitation maximum (nm)	Emission maximum (nm)	Coble et al.[54]	Description and probable source
C1	345	466	Peak C 320-360 / 420-480	Ubiquitous humic-like substances, widespread Hydrophobic acid fraction (HPOA) Component 1: 350 / 400-450[50] Component 1: 345 / 462[52] Component 4: 350 / 420-480[49]
C2	305	426	Peak M 290-312 / 370-420	Terrestrial humic-like substances, widespread Hydrophobic acid fraction (HPOA), suggested as photo-refractory Component 2: 255 / 380-460[50] Component 3: 255 (330) / 412[60] Component 3: 270 (360) / 478[56] Component 3: 250 (355) / 461[53]
C3	325	408		
C4	290	464	Peak A 260 / 380-460	Terrestrial humic-like substances, widespread Hydrophobic acid fraction (HPOA), suggested as photo-refractory Component 1: 270 (365) / 453[60] Component 2: 255 / 380-460[50] Component 3: 270 (360) / 478[56] Component 3: 250 (355) / 461[53]
C5	290	338	Peak M 290-312 / 370-420	Ditto with description for C2.

Table 4 Factor structure coefficients from the discriminant analysis for both land use and seasonal data sets.

Fluorescence indices	**Discriminant Function (DF)**	
Land use:	1	2
IC3/IC4 (Peak M/Peak A)	.913*	-.015
IC2/IC4 (Peak M/Peak A)	.618*	.095
IC5/IC4 (Peak M/Peak A)	.405*	-.054
IC1/IC4 (Peak C/Peak A)	-.285*	-.043
IC4/a_{340} (Peak A/a_{340})	-.374	.746*
Spectral slope	.144	-.626*
45.9% of original group cases correctly classified		
Seasonal:	1	2
IC5/IC4 (Peak M/Peak A)	.678*	.003
IC4/a_{340} (Peak A/a_{340})	-.672*	.133
IC2/IC4 (Peak M/Peak A)	.538*	.503
IC3/IC4 (Peak M/Peak A)	.406	.586*
IC1/IC4 (Peak C/Peak A)	-.108	-.384*
Spectral slope	.205	.253*
63.9% of original group cases correctly classified		

List of figure

Figure 1.

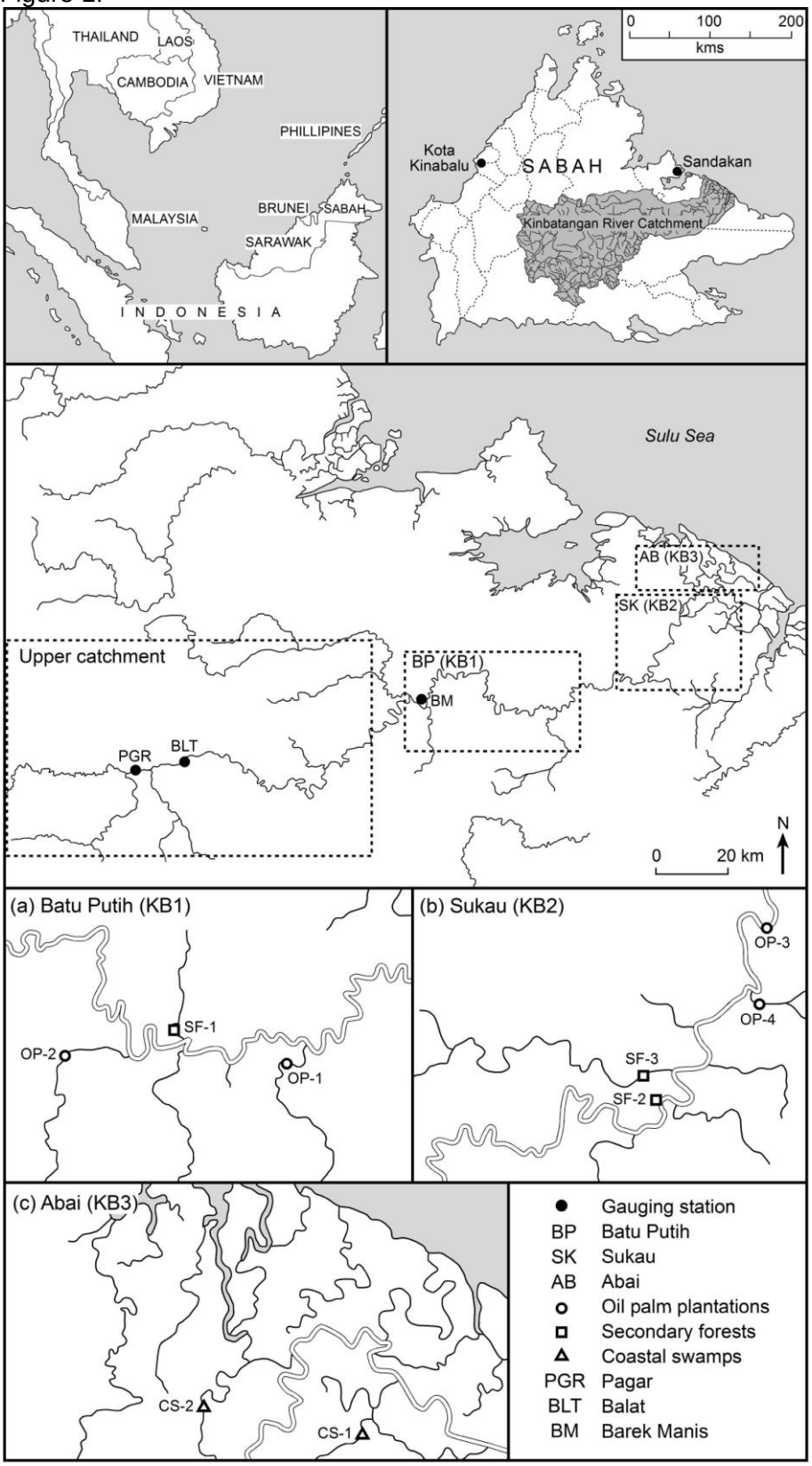
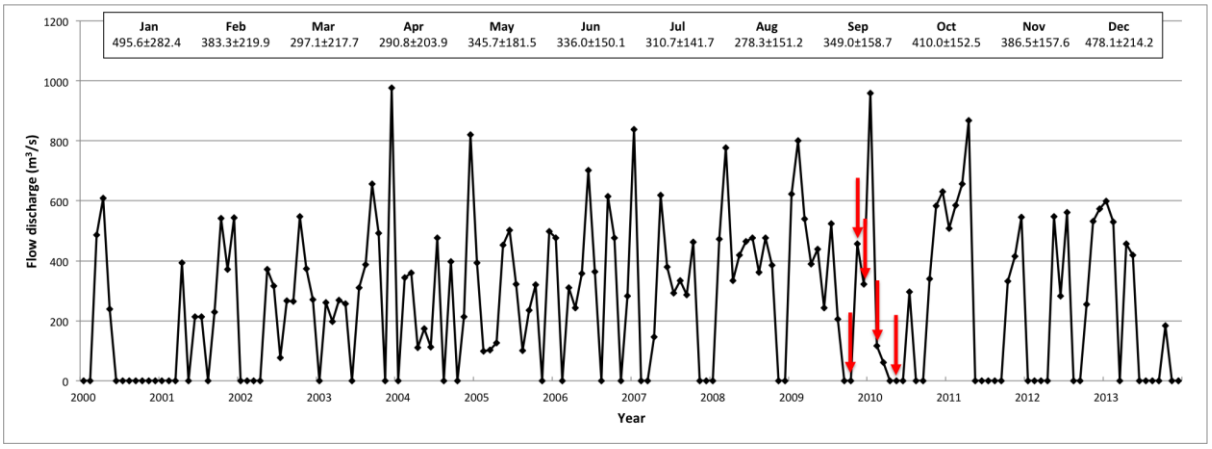
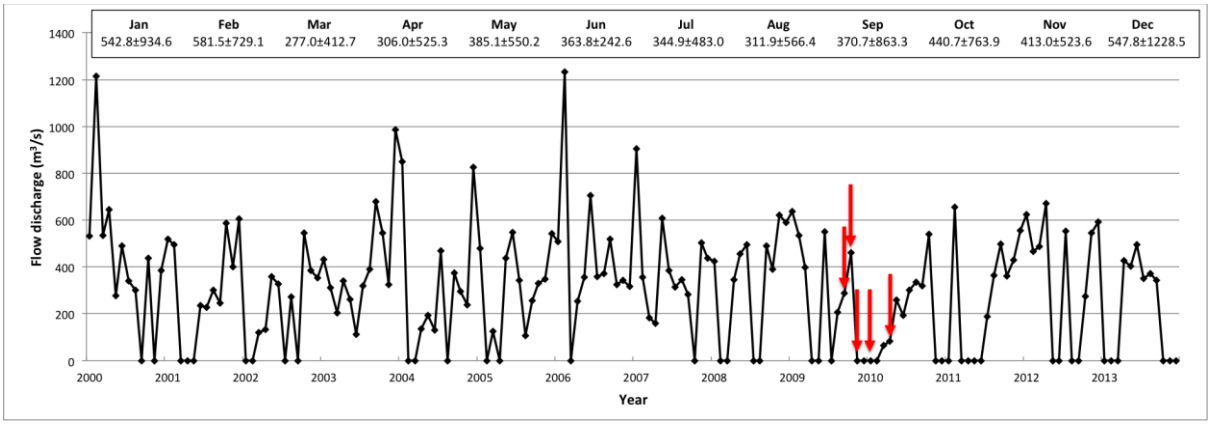

THAILAND
LAOS
CAMBODIA
VIETNAM
PHILLIPINES
MALAYSIA
BRUNEI
SABAH
SARAWAK
I N D O N E S I A
0 100 200
kms
Kota Kinabalu
S A B A H
Sandakan
Kinbatangan River Catchment
Sulu Sea
AB (KB3)
SK (KB2)
Upper catchment
BP (KB1)
BM
PGR
BLT
N
0 20 km
(a) Batu Putih (KB1)
SF-1
OP-2
OP-1
(b) Sukau (KB2)
OP-3
OP-4
SF-3
SF-2
(c) Abai (KB3)
CS-2
CS-1
Gauging station
BP Batu Putih
SK Sukau
AB Abai
Oil palm plantations
Secondary forests
Coastal swamps
PGR Pagar
BLT Balat
BM Barek Manis

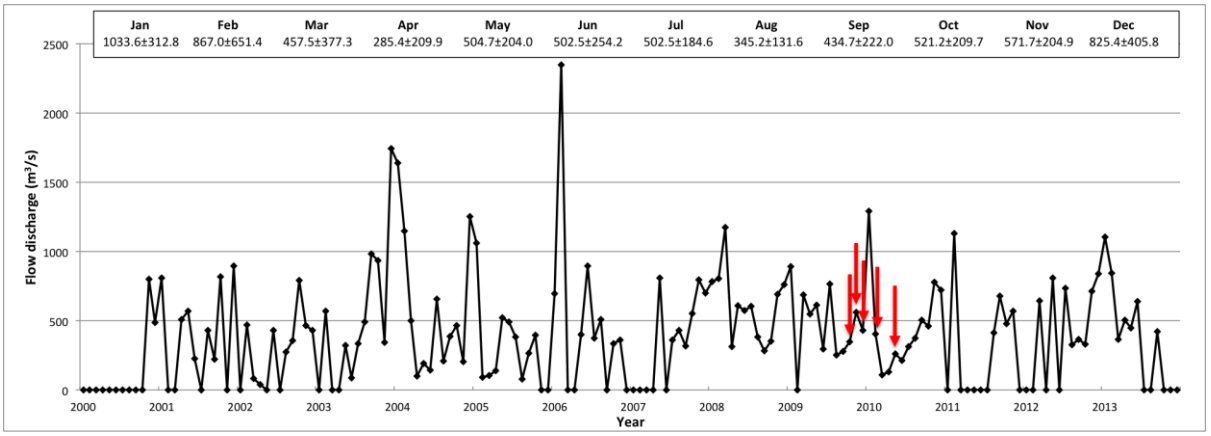
Figure 2



(a) Pagar (PGR)



(b) Balat (BLT)



(c) Barek Manis (BM)

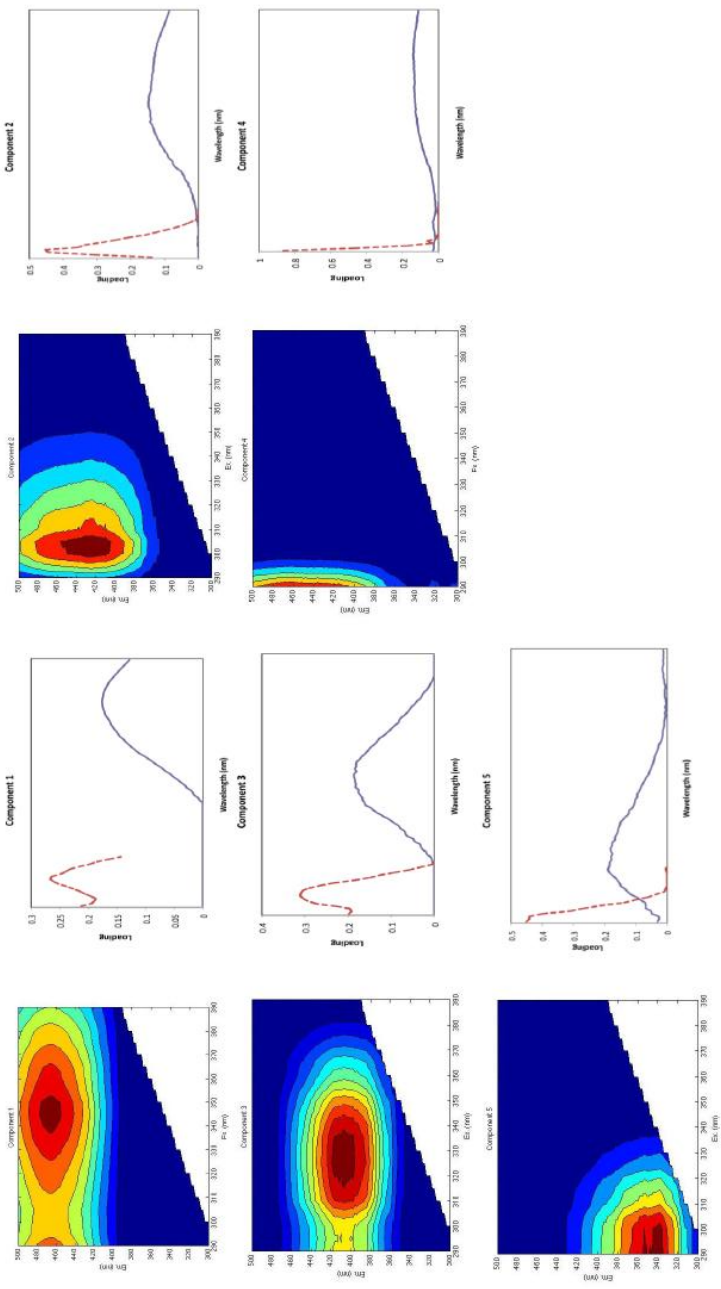
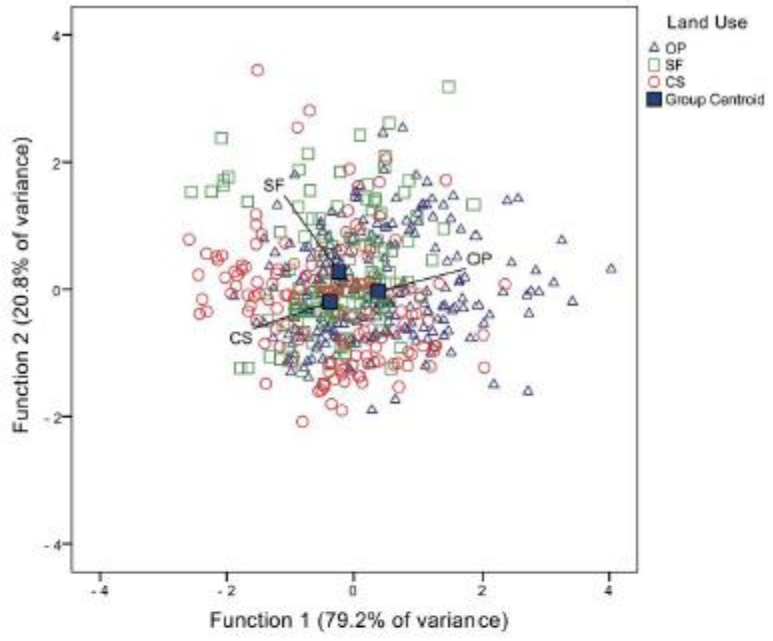
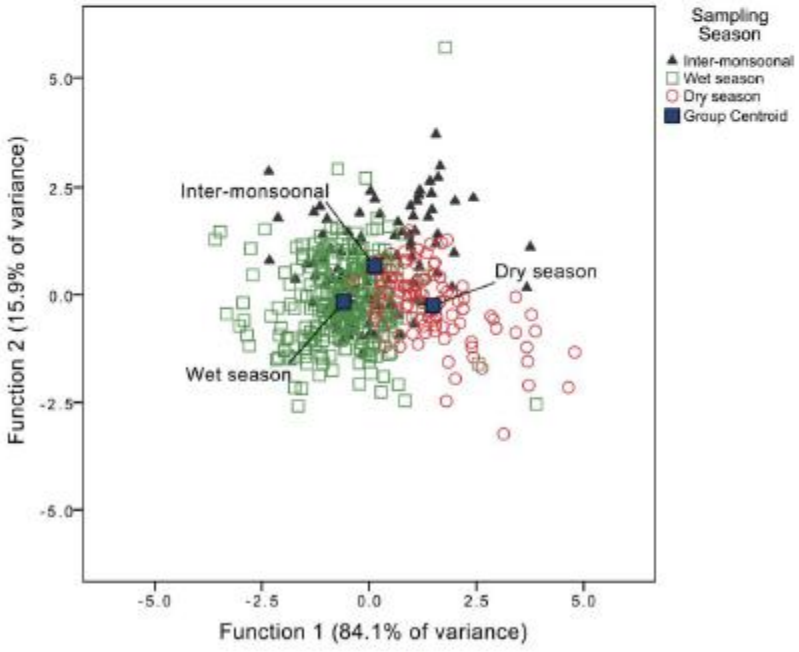

Component 1
Component 2
Component 3
Component 4
Component 5
Loading
Wavelength (nm)
Em. (nm)
Ex. (nm)

Figure 3

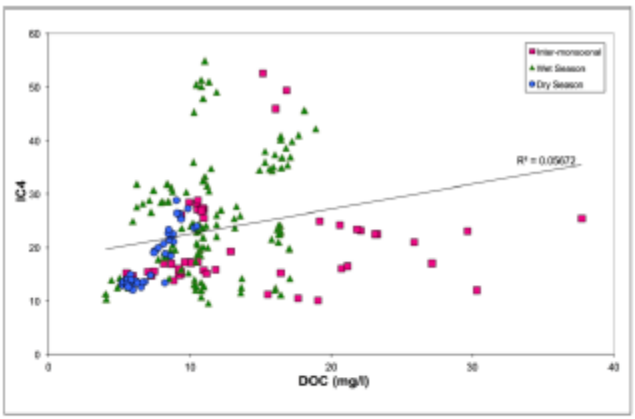

Land Use
OP
SF
CS
Group Centroid
SF
OP
CS
Function 2 (20.8% of variance)
Function 1 (79.2% of variance)
4
2
0
-2
-4
-4
-2
0
2
4

(a)

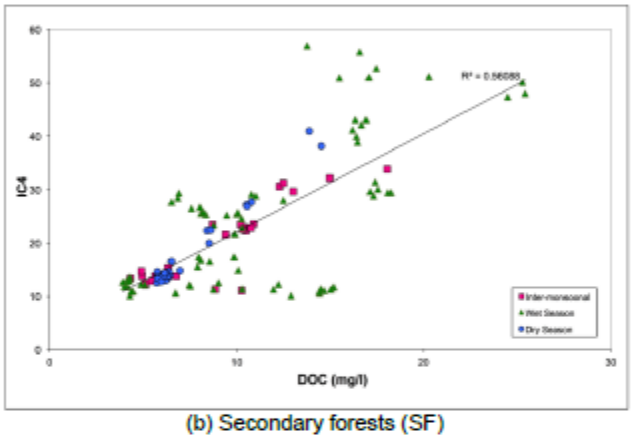

Sampling Season
Inter-monsoonal
Wet season
Dry season
Group Centroid
Inter-monsoonal
Dry season
Wet season
Function 2 (15.9% of variance)
Function 1 (84.1% of variance)
5.0
2.5
0.0
-2.5
-5.0
-5.0
-2.5
0.0
2.5
5.0

(b)

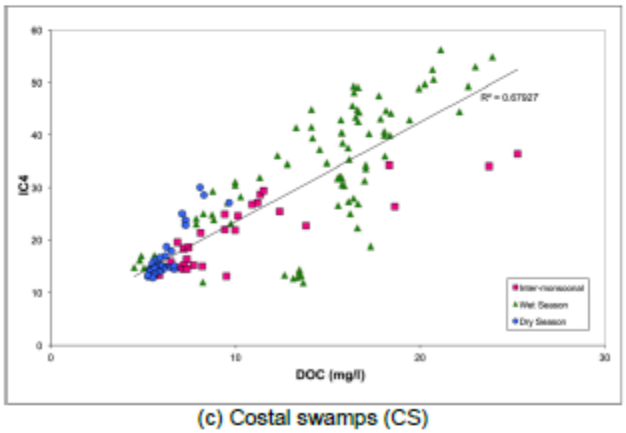
Fig 4



(a) Oil palm plantations (OP)

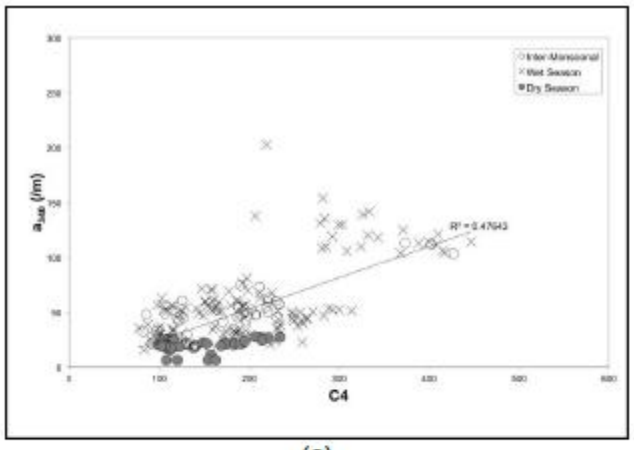


(b) Secondary forests (SF)

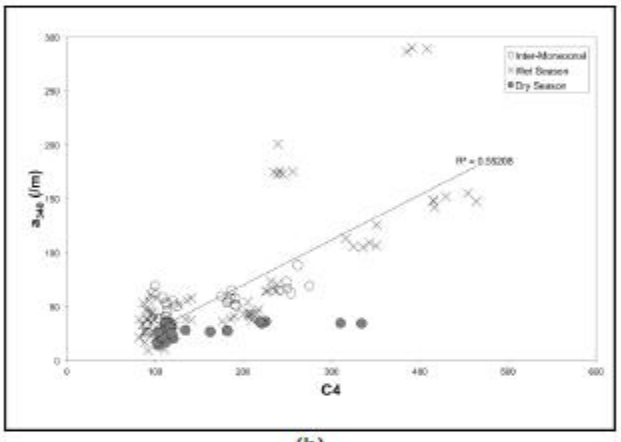


(c) Costal swamps (CS)

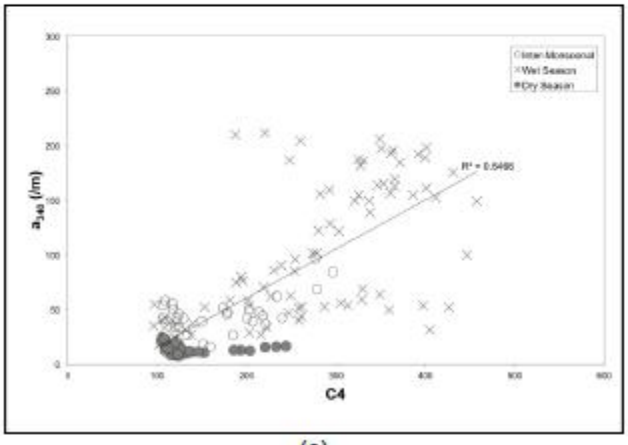
Fig. 5


Inter-Monsoonal
Wet Season
Dry Season
a_{340} (/m)
C4

(a)


Inter-Monsoonal
Wet Season
Dry Season
a_{340} (/m)
C4

(b)


Inter-Monsoonal
Wet Season
Dry Season
a_{340} (/m)
C4

(c)

Fig. 6