The non-conservative distribution pattern of organic matter in Rajang, a tropical river with peatland in its estuary

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Abstract

South-east Asian peatland-draining rivers have attracted much attention due to their high dissolved organic carbon (DOC) yield and high CO₂ emissions under anthropogenic activities. In August 2016, we carried out a field investigation of the Rajang river and estuary, a tropical system located in Sarawak, Malaysia. The Rajang has peatland in its estuary while the river basin is covered by tropical rainforest. DOC δ¹³C in the Rajang ranged from -28.7‰ to -20.1‰ and a U-shaped trend from river to estuary was identified. For particulate organic carbon (POC), the δ¹³C ranged between -29.4‰ to -31.1‰ in the river and a clear increasing trend towards more δ¹³C -enriched with higher salinity existed in the estuary. In the estuary, there was a linear conservative dilution pattern for dissolved organic matter composition (as quantified by D/L amino acids enantiomers) plotted against DOC δ¹³C, whereas when plotted against salinity dissolved D/L amino acids enantiomers values were higher than the theoretical dilution value. Together, these data indicate that the addition of DOC in estuary (by peatland) not only increased the DOC concentration, but also altered its composition, by adding more bio-degraded, ¹³C-depleted organic matter into the bulk dissolved organic matter. Alteration of organic matter composition (adding of more degraded subpart) was also apparent for the particulate phase, but patterns were less clear. The Rajang was characterized by DOC/DON ratios of 50 in the river section, with loss of DON in the estuary increased the ratio to 140, suggesting the unbalanced export pattern for organic carbon and nitrogen, respectively. Under anthropogenic activities, further assessment of organic carbon to nitrogen ratio is needed.

Keyword

Amino acids enantiomers, DOC, POC, stable carbon isotope, Rajang, peatland
1. Introduction

Fluxes and cycling of organic matter (OM) in rivers and estuaries are important influences on global biogeochemical cycles and climate change. In river basins, vascular plants are the ultimate sources of organic matter (Hedges and Man, 1979), but algae, moss and bacteria are also important (Hernes et al., 2007). As well as providing a source of OM, bacteria may also strongly modify the composition of organic matter within a river and its resistance to degradation. The lability of organic matter determines how rapidly organic carbon will be transformed into inorganic carbon (CO$_2$), which can vary from hours to millions of years. The lability of organic matter therefore plays a role in determining whether organic matter is either a source or a sink of carbon in the atmosphere (Zhang et al., 2018). Based on $^{14}$C of organic carbon, Mayorga et al. (2005) determined that the degradation of recently synthesized organic matter in the river basin was the main reason Amazonian river waters were supersaturated in CO$_2$, and hence the a source of atmospheric CO$_2$. This highlights the potential importance of organic matter stability for carbon cycling within river systems. Nitrogen is another important element in organic matter, which is not independent from carbon, but instead is closely combined with carbon in various chemical compounds (like amino acids). Due to the nature of these specific compounds, the behavior of bulk carbon and nitrogen can differ substantially. In basins with peatland, the leaching of DOC is related to the status of peatland (disturbed vs undisturbed), whereas the leaching of dissolved organic nitrogen (DON) is controlled by the soil inorganic nitrogen content (Kalbitz and Geyer, 2002). The different leaching mechanisms of organic carbon and nitrogen indicates that the comparison of these two elements would deepen our understanding of organic matter cycles.

Tropical south-east Asian rivers play an important role in both dissolved and particulate organic
matter export (Baum et al., 2007; Huang et al., 2017; Müller et al., 2016). Located in Sarawak, Malaysia (Fig. 1a), the turbid Rajang river (hereafter refer to as the Rajang) is the longest river in Malaysia. The Rajang flows through tropical rainforest, and peatland and mangroves are distributed in the estuary (downstream of Sibu; Fig. 1b). A dam was constructed in the upper reaches of the Rajang in 2015, but the total suspended matter (TSM) in the river downstream of Kapit remains at 100 – 200 mg/L in recent years (Müller-Dum et al., 2019). Dilution of terrestrial organic matter in the adjacent coast is expected, while turbid river water strongly limits apparent organic matter photo-degradation within the river and estuary, leaving the stage of fluvial organic matter alteration to bacteria utilization and abiotic process like desorption/adsorption between particulate and dissolved phase (Martin et al., 2018). Further, dissolved oxygen is negatively related to pCO$_2$, likely due to in-stream heterotrophic respiration (Müller-Dum et al., 2019). In the Rajang brackish estuary, where peatland is located, addition of peatland DOC into river water is suggested by the non-conservative mixing pattern of DOC with increasing salinity (Martin et al., 2018), whereas removal of DON in the Rajang estuary is suggested by nitrogen stable isotopes (Jiang et al., 2019).

While stable isotopes of carbon and nitrogen are useful tools for tracing organic matter, amino acids (AAs) are the most important organic carbon and nitrogen carriers that have been chemically identified, accounting for up to ~100% of the particulate nitrogen in aquatic environments, and up to nearly half of the particulate organic carbon pool (Jennerjahn et al., 2004). Due to the selective removal and accumulation of certain amino acids, amino acids are important biomarkers in early diagenesis, allowing quantification of organic matter lability/resistance (Dauwe and Middelburg, 1998; Kaiser and Benner, 2009). With the exception of glycine, amino acids are chiral. L forms of amino acids are from animals, plants and plankton, whereas D forms mainly come from bacteria,
and are key chemical compounds in peptidoglycan, which forms the basic structure of bacterial cell membranes (Vollmer et al., 2008). Due to the key role of bacteria in OM alteration and early diagenesis, D-AAs (D forms AAs) tend to accumulate during OM degradation. A higher ratio of D- to L-AAs (D/L ratio) therefore indicates more that OM is more refractory (Davis et al., 2009). As a non-protein amino acid, accumulation of GABA (γ-aminobutyric acid) is also highly related to OM degradation (Davis et al., 2009). Conversely, a lower D/L ratio and GABA% indicates that OM is relatively less degraded, and hence more labile. In river waters, elevated D-AAs also indicates the presence of soil humic substances, which is a product of bacteria and their detritus (Kimber et al., 1990).

Tropical rivers are dominated by refractory (or bio-degraded) organic matter, yet labile OM is also known to play a role in river carbon cycles (Mayorga et al., 2005). It is hence expected that the fluvial organic matter in the river would be a mixture of labile organic matter (that can be respired to support pCO₂) and refractory terrestrial organic matter (that will be diluted/degraded after entering the sea (Martin et al., 2018), while in the estuary there would be addition of dissolved OM from peatland/mangrove (Dittmar et al., 2001b; Müller et al., 2016). Previous studies of OM in south-east Asian rivers mainly focused on its bulk concentrations, ages, or optical properties (Martin et al., 2018 and ref. therein). The use of biomarker approaches has been very limited (Baum et al., 2007; Gandois et al., 2014). Given the processes described above and their potential contribution to the carbon (Müller-Dum et al., 2019) and nitrogen cycles (Jiang et al., 2019), it is somewhat surprising that there has been limited application of amino acid approaches, including D-AAs, to investigate organic matter composition and the role of estuarine peatland/mangrove in OM regulation (Jennerjahn et al., 2004). South-east Asian rivers are subject to multiple stressors due to
increasing anthropogenic activities in both their riverine (e.g., damming, logging/secondary plantation) and estuarine sections (e.g., drainage, and oil palm plantations) (Hooijer et al., 2015). AAs enantiomers and carbon/nitrogen isotopes have the ability to provide molecular level evidence for the impact of these stressors on carbon and nitrogen cycling and bulk biogeochemistry, as well as insight into the mechanisms underlying such changes.

In this study, we carried out a field investigation in the Rajang in August 2016, from Kapit to S1 station, located on the coast of the South China Sea adjacent to the Rajang (Fig. 1b). AAs enantiomers and δ¹³C of DOC were used to elucidate the succession of organic matter sources/composition from the fresh water to the estuarine sections of the Rajang. Our aim was to address the following questions: 1) Given that peatland contributes additional DOC to fluvial DOC (Müller et al., 2016), does the composition of dissolved OM change from river to estuary? 2) Do changes in organic nitrogen mirror changes in organic carbon? 3) And hence what is the role of peatland/mangroves on OM composition and lability in the Rajang? Globally, rivers in low latitudes receive much less attention relative to temperate and polar rivers (36 vs. 958 studies)(Cloern et al., 2014), while they could equally important in carbon cycle (Cloern et al., 2014). Our work, together with other tropical studies, would enrich the understandings for organic carbon and nitrogen cycles in tropical rivers/estuaries.

2. Materials and methods

All abbreviations, together with the amino acids measured in this study, are listed in table 1.

2.1 Brief background

The Rajang river and estuary is located in Sarawak, Malaysia. The climate is wet year-round,
but the main precipitation typically occurs in winter (November to February). Climate is influenced by El Niño-Southern Oscillation (ENSO) and Madden-Julian Oscillation. In August 2016, the discharge was estimated as 2440 m³/s, in comparison with an annual mean discharge of 4000 m³/s for 2016 and 2017 (Müller-Dum et al., 2019).

Based on salinity, Sibu is regarded as the boundary of the fresh and estuarine water of the Rajang (Fig. 1b). In this work all samples with a salinity of 0 were regarded as fresh water, while samples with salinity >0 were regarded as estuarine. In the estuary, there are several branches, namely Igan, Lassa, Paloh, and Rajang itself (Fig. 1b). Since water in all these branches are from Rajang river (i.e., upstream of Sibu), in this work all these branches are regarded as the Rajang estuary. Peatland and mangroves are commonly distributed in the estuary (shown in Fig. 1b) while tropical rainforest is widely distributed upstream of Sibu (not shown in Fig. 1b). The peatland is under strong pressure of draining and change of use for oil palm, while in the basin logging and secondary growth is very common (Hooijer et al., 2015). Compared with other peatland-draining tropical blackwater rivers, the Rajang is more like a turbid tropical rainforest river (Müller-Dum et al., 2019), but with notable peatland/mangrove in its estuary (Fig. 1b).

2.2 Field sampling

The field work was carried out in August 2016. The sampling stations covered from Kapit (the upper most station in this study) to S1 on the coast. At each station, a pre-cleaned and sample-rinsed bucket was used to collect surface water from the center of the channel in a boat. After sample collection, pretreatment was done immediately on board in the boat. For DOC and its stable carbon isotope ratios ($\delta^{13}$C), water samples were collected by syringe filtering (pre-combusted Whatman GF/F; 0.7 µm) approximately 30 ml of sample water into a pre-combusted 40 ml borosilicate vial.
Samples were preserved with five drops of concentrated phosphoric acid and sealed with a lid containing a Teflon-coated septa. For total dissolved amino acids (TDAA), water samples were filtered through a 0.4 μm nylon filter. For particulate OM samples (TSM, POC, POC-δ13C, PN and PN-δ15N, and total particulate amino acids (TPAA)), suspended particles were concentrated onto glass fiber membrane (pre-combusted Whatman GF/F; 0.7 μm). The GF/F filters were folded and packed in pre-combusted aluminum. All samples were immediately stored frozen (-20°C) until analysis. A portable meter (Aquaread, AP-2000) was used to obtain conductivity/salinity, temperature, dissolved oxygen and pH.

2.3 Laboratory analyses

Concentrations and δ13C of DOC were measured via continuous-flow wet oxidation isotope-ratio mass spectrometry using an Aurora 1030W total organic carbon analyzer coupled to a Thermo Delta V IRMS (Oakes et al. 2010). Glucose of known isotopic composition dissolved in He-purged Milli-Q was used as a standard to correct for drift and to verify sample concentrations and δ13C values. Reproducibility for concentrations and δ13C was ± 0.2 mg l⁻¹ and ± 0.4 ‰. DOC concentrations and δ13C were measured at the Centre for Coastal Biogeochemistry at Southern Cross University (Lismore, Australia). For the determination of POC, samples (GF/F glass fiber filter) were freeze-dried and analyzed with a CHNOS analyzer (Model: Vario EL III) after removing the inorganic carbon by reaction with HCl vapor. For PN, a similar procedure like POC was followed, but no acid was used in pre-treatment. The detection limit for POC was 7.5×10⁻⁶ g, with precision better than 6%, based on repeated determinations (Zhu et al., 2006). The POC-δ13C and PN-δ15N were determined using a DELTAplus/XL isotopic ratio mass spectrometer (Finnigan MAT Com. USA) interfaced with a Carlo Erba 2500 elemental analyzer. The standard for δ13C was PDB and
the precision of the analysis was ± 0.2‰. For δ15N, the standard was air and precision was ± 0.3‰.

Total hydrolyzable AAs were extracted and analyzed following the method of Fitznar et al., (1999) with slight modifications (Zhu et al., 2014). Briefly, samples were first hydrolyzed with HCl at 110°C. After pre-column derivatization with o-Phthaldialdehyde (OPA) and N-Isobutyryl-L/D-cysteine (IBLC/IBDC), AAs and their enantiomers were analyzed using an HPLC (Agilent 1200) comprising of an online vacuum degasser, a quaternary pump, an auto-sampler, a thermostatted column and a fluorescence detector (excitation 330 nm, emission 445 nm). The analytical column was a Phenomenex Hyperclone column (BDS C18, 250×4mm, 5μm) with a corresponding pre-column. To eliminate the influence of racemization of L-type AAs in the hydrolysis process, the concentration of D/L AAs measured in actual samples was corrected according to the formula obtained by Kaiser and Benner (2005). The detection limit for glycine (Gly) and individual AAs enantiomers were in the lower picomolar level. Asx and Glx were used for aspartic acid + asparagine and glutamic acid + glutamine, respectively (Table 1), as the corresponding acids are formed via deamination during hydrolysis.

A few samples (e.g., TDAA in S1 station) were not measured due to instrument hardware problem. And hence the measured particulate and dissolved sample stations did not exactly match.

3. Results

In August 2016, the TSM concentration in the Rajang ranged from 22 mg/L (mean for the fresh water section) to 161 mg/L (mean for the estuarine section) (Table 2). Throughout the system DOC concentrations exceeded POC concentrations. DOC and POC in the fresh water section averaged 337 μM and 86 μM, and in the estuarine section 345 μM and 64 μM, respectively (Table 2). While
DOC concentration was slightly higher in the estuary than in the fresh water (Table 2), a maximum of both DOC and POC can be found at around salinity 15 to 20 in the estuary (Fig. 2).

DOC $\delta^{13}C$ ranged from -28.7‰ to -20.1‰ (Table 2). A U-shaped trend from fresh water section to estuary section can be identified for DOC $\delta^{13}C$, with one outlier from the Rajang main stream at a salinity of 5 (S2 station; Fig. 3a). The minimum value of DOC $\delta^{13}C$ (bottom of the U) was detected at a salinity of ~10 (Fig. 3a). For particulate OM, $\delta^{13}C$ ranged between -29.4‰ to -31.1‰ in the fresh water section. In the estuary section, there was a clear increasing trend with increasing salinity, from -30‰ (S=1.1) to values close to -24‰ (S>30) (Fig. 3b).

In the fresh water section, the mean TDAA and TPAA concentrations were 0.3 μM and 2.5 μM, respectively (Table 3). For TDAA, the AA carbon yield (the carbon from AA divided by bulk DOC or POC, in %) in both fresh water and estuary sections were very similar, namely 0.40% and 0.38% (mean), respectively (Table 3), whereas AA nitrogen yield was higher in the estuary (11%) than in the fresh water section (4.8%) (Table 3). For TPAA, there was little difference between the fresh water and estuary sections in AA carbon yield (13.5% and 16.8%, respectively) and nitrogen yield (66% and 62%, respectively) (Table 3).

With respect to individual AA compounds, in both dissolved and particulate phase, Gly, Glx, Ala and Asx were the most abundant four AAs. These four AAs together accounted for 66% of TDAA and 47% of TPAA in the fresh water section, 59% of TDAA and 48% of TPAA in the estuary. The non-protein AA GABA was detected in trace amounts, but was accumulated in the dissolved phase relative to the particulate phase, as indicated by the higher GABA% in the dissolved phase (Table 3). GABA% decreased from 2% (fresh water section mean) to 1.3% (estuarine section mean) in the dissolved phase, and decreased from 0.7% (fresh water section mean) to 0.4% (estuarine
section mean) in the particulate phase (Table 3). In the estuary, GABA% in the dissolved phase remained stable (~1.5%) in brackish water (salinity 5 to 20) and quickly dropped to <1% where salinity was over 30 (Fig. 4a). Most of the GABA% dots were above the theoretical dilution line (Fig. 4a). In the particulate phase, there was an overall decrease in GABA% with increasing salinity within the estuary (Fig. 4b).

As for the AA enantiomers, the D/L ratio of AA in the dissolved phase averaged 12% for both fresh water and estuarine section. The most abundant D-form AAs in the dissolved phase were Glx and Asx. For the particulate phase, the D/L ratio of AA was much lower, decreasing from a mean of 4.4% in the fresh water section to a mean of 3.3% in the estuary (Table 3). And patterns in the variation of D/L Glx (Fig. 5) along with conductivity/salinity gradient in the Rajang were similar to those for GABA% (Fig. 4) for both dissolved and particulate phase. For example, for dissolved phase, a similar platform can be identified at salinity range of 5 to 20 (Fig. 5a), whereas for particulate phase the decreasing pattern along with salinity is very clear in the estuary (Fig. 5b). Also, for dissolved phase in the estuary, all the data were above the theoretical dilution line for D/L Glx.

4. Discussion

4.1 Distribution patterns of OM composition

Dissolved OM

Terrestrial OM usually has a more negative δ¹³C value (~−32‰ to −26‰ for C3 plants), whereas marine OM has more positive value values (δ¹³C, ~−20‰) values (Lamb et al., 2006; Mayorga et al., 2005). Overall, the very negative δ¹³C values for DOC (~−26‰) in the river part of the Rajang
indicates that the OM had a very clear C3 plant source (e.g., mangroves and oil palms (Jennerjahn et al., 2004; Lamade et al., 2009; Wu et al., 2019)), whereas DOC $\delta^{13}$C values $> -24\‰$ in the estuary (salinity $>30$) suggest a mixture of terrestrial and marine OM (Fig. 3a). The most depleted $\delta^{13}$C values for DOC occurred at a salinity of 10 (Fig. 3a). Above this salinity, the influence of marine OM became more overwhelming, and the bulk DOC $\delta^{13}$C signal was more enriched (Fig. 3a).

Among samples in the fresh water section, the sample of most enriched DOC-$\delta^{13}$C value (S10 and S15; DOC-$\delta^{13}$C: $-25\‰$; Fig. 3a) although initially appearing to be outliers, were characterized by very elevated D/L amino acids ratios (Fig. 6a). This was particularly the case for the sample from S10 (the upper most station in this study; Fig. 1b), which showed a maximum D/L Glx ratio of 0.57 (Fig. 6a). In addition, these samples from S10 and S15 also showed a higher D/L ratio for Asp (S10: 0.49, S15: 0.38; figure not shown) when compared to all fresh water or estuary samples (mean: 0.34; Table 3). On land, D form amino acids can be derived from abiotic racemization process (which requires a very long time scale) by which L form amino acids slowly changed into their corresponding D form (Schroeder and Bada, 1976). More significantly in contemporary environments, D form amino acids are widely synthesized by bacteria during their cell membrane construction (Schleifer and Kandler, 1972). D/L Glutamic acid and D/L Aspartic acid ratios of pure peptidoglycan (Staphylococcus aureus, Gram-positive) are 0.49 and 0.30, respectively (Amon et al., 2001). Though $\delta^{13}$C values for bacteria in the Rajang remains unclear, bacteria have been reported to have $\delta^{13}$C values from $-12\‰$ to $-27\‰$ (Lamb et al., 2006). Contribution of OM derived from bacteria may therefore explain the relatively enriched $\delta^{13}$C values observed at inland S10 station and S15. A possible OM source at these stations is soil humic substances, which are expected to be under strong impact of bacteria, and have a high contribution of D-form amino acids (Dittmar et al., 2004; Lamade et al., 2009; Wu et al., 2019).
2001a). A more depleted pattern of DOC δ¹³C from mountain to lowland is suggested to be due to dilution and mixing with younger OM in the lowland (Mayorga et al., 2005). This is consistent with our findings that, depleted pattern of riverine DOC δ¹³C within the fresh water section was corresponding to a lowering D/L ratio pattern, which indicates the dilution with less degraded OM (Fig. 6a). Whether the dissolved samples with elevated D/L ratio and relatively positive δ¹³C in the fresh water section (S10 and S15; Fig. 6a) reflect the presence of soil humic substances, or instead reflect the direct presence of bacteria, requires further study.

In the estuarine section, it was very clear that terrestrial bio-degraded OM (indicated by elevated D/L ratios and more negative δ¹³C) is diluted with more labile OM (lower in D/L ratio but more positive δ¹³C)(Fig. 6a). However, this apparent dilution trend became very vague (or showed no trend) when D/L ratio was plotted against salinity (Fig. 5a). This was also confirmed by the GABA% distribution pattern which showed a platform-like pattern at a salinity between 5 and 20 (Fig. 4a). Though TDAA at S1 is missing, the composition of TDAA at S2 (salinity = 31.2) was very typical of marine OM (i.e., very low D/L ratio and relatively enriched DOC-δ¹³C; see Fig. 6a). Hence in the estuary there is a conservative distribution pattern for dissolved OM when plotted against δ¹³C (Fig. 6a) but such pattern disappeared when plotted against salinity (Fig. 4a&5a). The location above the conservative dilution line of all OM data in the brackish estuary (salinity between 10 and 25; Fig. 4a&5a), indicates that the OM in the estuarine section was more degraded than theoretically expected. The combination of degraded OM with the observed DOC concentration increase in the estuary (345 μM in the estuary vs. 337 μM in the fresh water section; or Fig. 2b), suggests the addition of degraded DOC to the Rajang. Non-conservative dissolved OM behavior in the estuary has previously been reported based on an optical approach (Martin et al., 2018), and
minimal OM alteration during estuarine transport was suggested (Martin et al., 2018). Hence, it is reasonable that changes in dissolved OM composition (Fig. 4a&5a) may largely take place in land/estuary (e.g., in pore waters of soil) and impact the Rajang riverine dissolved OM via leaching from soils.

**Particulate OM**

As for dissolved OM, depleted POC-δ^13C in the river part of the Rajang indicated the strong influence of terrestrial OM (e.g., C3 plant Dittmar et al., 2001b) whereas in the estuary, particulate OM was diluted with marine particulate OM, as indicated by the seawards enrichment of δ^13C (Fig. 3b). In the sediment, a clear woody angiosperm C3 plants as the OM source is found based on a lignin approach (Wu et al., 2019), and similar increases in carbon and nitrogen isotopes in suspended particles in brackish water have also been observed in other estuaries (Cifuentes et al., 1996; Raymond and Bauer, 2001). Unlike dissolved OM, there were no samples with unusually enriched δ^13C values in the fresh water section (Fig. 6b&c). D/L Glx ratio in the fresh water section is higher when compared with that in the estuary section (Table 3), and overall, when compared with dissolved OM, particulate OM basically became more labile when transporting seawards, as indicated by its composition shift along with salinity (Fig. 4b&5b) or isotope (Fig. 6b&c).

Although particulate OM had a lower D/L ratio than dissolved OM (Fig. 6), it should be noted that this does not mean dissolved OM is more aged or degraded than particulate OM. Rather, as observed in other estuaries (Dittmar et al., 2001a), bacteria and their detritus simply tend to accumulate in the dissolved phase, relative to the particulate phase.

**4.2 Different fate of bulk organic carbon and nitrogen**

Leaching of DOC and DON from peatlands is driven by difference mechanisms; whereas DOC
release is related to the status of peatland (pristine vs. degraded), DON release is determined by the
DIN content of peatland soil (Kalbitz and Geyer, 2002). In the Rajang, bulk DOC and DON
concentrations were not coupled, as suggested by the DOC/DON ratio variation pattern (Fig. 7).
The average DOC concentration in the estuary part was slightly higher (345 μM) than in the river
part (337 μM; Table 3), which indicates the addition of DOC in the estuary. In comparison, the
removal of DON in the estuary is suggested (Jiang et al., 2019).

In the Rajang, non-conservative dilution behavior from optical properties was observed for
estuarine DOC (Martin et al., 2018), which is consistent with other peatland-draining rivers in
Sarawak (Müller et al., 2016). The contribution of marine sources to dissolved OM is reflected in
the increasing DOC-$\delta^{13}$C in the estuary part (Fig. 3a). Peatland, however, is known for its high
contribution to fluvial DOC and has been suggested to contribute to the DOC in the Rajang (Martin
et al., 2018). In peatland-draining rivers west of the Rajang, the DOC concentration endmember can
be as high as 3690 μM (Müller et al., 2015). Under such high DOC background, a simple three-
point mixing model (i.e., a model that based on 1 observed fresh water DOC endmember, 2 peatland
DOC endmember and 3 calculated fresh water DOC endmember) suggests that peatland-DOC
addition accounts for 3% of the fluvial DOC in the Saribas river and 15% in the Lupar river (Müller
et al., 2016). Assuming that peatland in the Rajang estuary has a comparable endmember DOC
concentration to other peatland in Sarawak (i.e., 3690 μM; Müller et al., 2015), and given our
observed Rajang fresh water DOC endmember value of 337 μM (DOC concentration at S5 station)
and a marine DOC endmember of 238 μM (S1 station), a similar model approach suggests peatland
DOC addition contributed 4% of the Rajang fluvial DOC, which is comparable to Saribas river and
much lower than Lupar river (Müller et al., 2016). In the meantime, as mentioned in the previous
section, there is a non-conservative dilution pattern, with dissolved OM in the estuary part more
degraded than expected based on simple dilution with a marine endmember (Fig. 4a&5a). Hence it
is reasonable that peatland not only contributed to the fluvial DOC in concentration (Martin et al.,
2018), but also modified the dissolved OM composition (more bio-degraded) in the estuary. In
another tropical river study, mangrove in the estuary exerted a stronger influence on fluvial
dissolved OM than hinterland vegetation (Dittmar et al., 2001b). This is consistent with the Rajang,
for which estuarine processes apparently impact the dissolved OM in terms of both DOC
concentration (by increasing the bulk amount) and composition (by adding bio-degraded DOC). The
estuarine dissolved OM showed higher bio-degraded feature (e.g., elevated GABA% and D/L ratio;
Fig. 4a&5a), but this subpart may be photolabile (Martin et al., 2018). When TSM decreases and
light condition in the water column becomes good (e.g., entering the sea), photodegradation is
expected (Martin et al., 2018). Other oceanic degradation mechanisms include the priming effect
(Bianchi, 2011). The fate of the terrestrial OM in the sea requires further study. As we lack the DON
concentration endmember in peatland, peatland impact on DON in the estuary is not estimated.
In contrast to DOC, which was apparently added to the estuary, DON was removed,
contributing to a remarkable increase of dissolved inorganic nitrogen in the estuary (Jiang et al.,
2019). In the fresh water section, the nitrate concentration was not related to the ratio of D/L
dissolved AAs, nor related to dissolved GABA% (Fig. 8), and in the estuarine section, nitrate was
not related to D/L AAs but it indeed was related to GABA% in the estuarine section (Fig. 8b). This
indicates that fluvial nitrate in the fresh water section was not derived from remineralization of
fluvial organic matter in the river channel, but more likely from other sources (e.g., leaching of soil).
In the estuarine section, there may be some DON transformation occurred (Jiang et al., 2019), while
the leaching from soil process still cannot be eliminated (Fig. 8). For particulate phase, no relation can be found between nitrate and particulate OM composition (figure not shown).

The atomic DOC/DON in Rajang averaged 50 in the river part, and increased to 140 (mean value) in the estuary part (Fig. 7). Although the DOC/DON ratio was much higher when compared to other tropical peatland river waters (around 10; Sjögersten et al., 2011), the ratio is comparable with other peatland-draining rivers in Sarawak like the Lupar, Saruba and Maludan rivers (Müller et al., 2015; Müller et al., 2016), which all enter the South China sea. The ratio is also within the reported C/N ratio of peatland and leaves (Müller et al., 2016). For the Amazon river, the DOC versus total nitrogen ratio ranges from 27 to 52 (Hedges et al., 1994). Given their reported total nitrogen includes inorganic nitrogen, the DOC/DON ratio for the Amazon river would be even higher. Under the background of such high C/N ratios (e.g., 50), transformation of DON to DIN in the estuary further enhanced the high DOC/DON ratio (to 140), and hence a deficiency in terrestrial organic nitrogen output is expected. We noted that dissolved inorganic nitrogen for Rajang is on the order of 10 μM, comparable to DON (Jiang et al., 2019). Terrestrial nitrogen output is an important source for coastal primary production (Jiang et al., 2019), but peatland-impacted rivers may have relatively lower nitrogen input to the South China Sea when compared with their very high river basin DOC yields (Baum et al., 2007). On one hand, logging and secondary growth has been found to play a negative role in the nitrogen output efficiency of forest soils (Davidson et al., 2007). On the other hand, disturbed tropical peatlands could release more DOC in comparison to an undisturbed site (Moore et al., 2013) while the DOC/DON ratio may also decrease along with disturbance of peatland (Kalbitz and Geyer, 2002). Given that secondary growth in river basin and anthropogenic disturbance of peatland (e.g., drainage and conversion for oil palm) are both common
(Hooijer et al., 2015), changes of DOC/DON ratios in the Rajang are complex and further assessment is needed in the future.

5. Summary and Conclusion

In August 2016 in the Rajang, we observed that dissolved OM composition (as D/L Glx ratio) was conservatively diluted along with increasing DOC δ13C, indicating that the sources of dissolved OM have a very conservative impact on the OM composition. When D/L Glx ratio was plotted against salinity (as is usually done for an estuarine OM behavior check in many studies), such linear conservative dilution pattern disappeared (Fig. 4a&5a). This implies that the addition of DOC in the estuary (peatland/mangrove) had an impact on dissolved OM composition, adding more bio-degraded OM, and resulting in data above the theoretical dilution line (Fig. 4a&5a). For particulate OM, though the data was variable, the overall decreasing GABA% or ratio along with salinity was much clearer relative to that of dissolved OM (Fig. 4b&5b). Particulate D/L Glx ratio in the estuary was usually lower when compared with that in the fresh water section (Fig. 6b&c), whereas for dissolved OM, the majority of the samples in the estuary had a D/L Glx ratio similar to that in the fresh water (Fig. 6a). The difference in OM composition between fresh water and estuarine section suggests that dissolved OM became more degraded while particulate OM became less degraded in the estuary.

The Rajang is characterized by DOC/DON ratios of 50 in the fresh water section, and the further loss of DON in the estuary increased the ratio to 140. Peatland draining and logging/secondary growth are reported to have conflicting impacts on carbon and nitrogen cycling (Davidson et al., 2007; Moore et al., 2013), which may increase fluvial DOC and limit basin nitrogen
output, resulting in even larger DOC/DON. Mismatch in carbon and nitrogen loss from tropical rivers due to anthropogenic activities plays a role in material cycle in both land and marine systems, enhancing the tropical river as a direct carbon source to atmosphere while for nitrogen change and its further feedback on carbon cycle needs further monitoring and assessment.

At last, this work is based on a dry season investigation (August). Though the seasonality for Rajang OM may be moderate (Martin et al., 2018), for biomarkers like amino acids enantiomers further investigation in the wet season is needed.

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Table 1. Measured amino acids (the L- and D- enantiomers are not listed) and all abbreviations in this study. Note that glycine has no enantiomer.

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>organic matter</td>
<td>OM</td>
</tr>
<tr>
<td>dissolved organic carbon</td>
<td>DOC</td>
</tr>
<tr>
<td>dissolved organic nitrogen</td>
<td>DON</td>
</tr>
<tr>
<td>total suspended matter</td>
<td>TSM</td>
</tr>
<tr>
<td>amino acid</td>
<td>AA</td>
</tr>
<tr>
<td>total hydrolysable dissolved amino acids</td>
<td>TDAA</td>
</tr>
<tr>
<td>total hydrolysable particulate amino acids</td>
<td>TPAA</td>
</tr>
<tr>
<td>Alanine</td>
<td>Ala</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asx</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>Glx</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
</tr>
<tr>
<td>γ-aminobutyric acid</td>
<td>GABA</td>
</tr>
</tbody>
</table>
Table 2. TSM, DOC, POC and stable carbon isotopes in the freshwater and estuary of the Rajang (mean (min-max)).

<table>
<thead>
<tr>
<th>Unit</th>
<th>Fresh water</th>
<th>Estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSM mg/L</td>
<td>61 (22 - 126)</td>
<td>73 (25 - 161)</td>
</tr>
<tr>
<td>DOC μM</td>
<td>337 (217 - 658)</td>
<td>345 (214 - 587)</td>
</tr>
<tr>
<td>DOC δ¹³C %</td>
<td>-26.7 (-27.7 - -25.0)</td>
<td>-26.1 (-28.7 - -20.1)</td>
</tr>
<tr>
<td>POC μM</td>
<td>86 (46 - 125)</td>
<td>64 (22 - 153)</td>
</tr>
<tr>
<td>POC δ¹³C %</td>
<td>1.9 (1.2 - 2.5)</td>
<td>1.0 (0.6 - 1.9)</td>
</tr>
</tbody>
</table>

Table 3. The Rajang AAs result (mean (min-max)) in August 2016 (*total D/TDAA means total D form AA versus TDAA, the same for total D/TPAA).

<table>
<thead>
<tr>
<th>Unit</th>
<th>Fresh water</th>
<th>Estuary</th>
</tr>
</thead>
<tbody>
<tr>
<td>dissolved</td>
<td>TDAA nM</td>
<td>317 (131 - 486)</td>
</tr>
<tr>
<td></td>
<td>TDAA carbon yield %</td>
<td>0.40 (0.08 - 0.65)</td>
</tr>
<tr>
<td></td>
<td>TDAA nitrogen yield %</td>
<td>4.8 (1.3 - 15)</td>
</tr>
<tr>
<td></td>
<td>GABA %</td>
<td>2.0 (1.3 - 4.1)</td>
</tr>
<tr>
<td></td>
<td>total D/total TDAA* %</td>
<td>12 (8 - 15)</td>
</tr>
<tr>
<td></td>
<td>D/L Glx</td>
<td>0.35 (0.16 - 0.57)</td>
</tr>
<tr>
<td></td>
<td>D/L Asx</td>
<td>0.34 (0.23 - 0.48)</td>
</tr>
<tr>
<td>particulate</td>
<td>TPAA μM</td>
<td>2.5 (1.4 - 3.6)</td>
</tr>
<tr>
<td></td>
<td>TPAA carbon yield %</td>
<td>14 (9.5 - 19)</td>
</tr>
<tr>
<td></td>
<td>TPAA nitrogen yield %</td>
<td>66 (36 - 82)</td>
</tr>
<tr>
<td></td>
<td>GABA %</td>
<td>0.7 (0.6 - 0.9)</td>
</tr>
<tr>
<td></td>
<td>total D/total TPAA* %</td>
<td>4.4 (3.6 - 5.2)</td>
</tr>
<tr>
<td></td>
<td>D/L Glx</td>
<td>0.09 (0.08 - 0.10)</td>
</tr>
<tr>
<td></td>
<td>D/L Asx</td>
<td>0.04 (0.03 - 0.05)</td>
</tr>
</tbody>
</table>
Figure 1. Study area and sampling stations. a) Location of Sarawak, Malaysia; and b) the Rajang with its estuary/river mouth background shown. Samples upstream of Sibu showed 0 salinity while downstream of Sibu showed salinity >0. Hence from Sibu to Kapit is regarded as the fresh water section, and downstream of Sibu is regarded as the estuarine section.

Figure 2. Distribution pattern of (a) TSM, (b) DOC and (c) POC along with conductivity/salinity in the Rajang. The location of salinity = 0 is at Sibu (Fig. 1b). The legend indicates the branches that the samples were from and marine corresponds to S1 station.

Figure 3. Distribution pattern of (a) DOC δ13C and (b) POC δ13C along with conductivity/salinity in the Rajang. The legend indicates the branches that the samples were from and marine corresponds to S1 station.

Figure 4. GABA% distribution pattern from fresh water to estuary in the Rajang: a) dissolved and b) particulate. The legend indicates the branches that the samples were from and marine corresponds to S1 station.

Figure 5. D/L ratio of Glx from fresh water to estuary in the Rajang: a) dissolved and b) particulate. The legend indicates the branches that the samples were from and marine corresponds to S1 station.

Figure 6. D/L ratio of AAs (as Glx) plotted against a) DOC δ13C b) POC δ13C, and c) PN δ15N.

Figure 7. DOC/DON ratio distribution pattern along with salinity in the Rajang. For fresh water and estuary, the mean DOC/DON value was 50 and 140, respectively. DON is from Jiang et al., (2019)

Figure 8. Dissolved OM composition (a: D/L Glx, b: GABA%) and its relation with nitrate. Nitrate is derived from Jiang et al., (2019).
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