Low biodegradability of dissolved organic matter from Southeast Asian peat-draining rivers

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Key points

- Low biodegradability of peatland DOC during 56-day incubations, including when diluted with seawater or amended with nutrients
- No measurable phenol oxidase activity in the peat-draining rivers and coastal waters of Sarawak
- Auto-oxidation of the phenol oxidase assay substrate L-DOPA occurs at pH ≥7; we recommend ultrafiltration to generate enzyme-free controls

Abstract

Southeast Asia's extensive tropical peatlands account for a significant proportion of the global riverine dissolved organic carbon (DOC) flux to the ocean. Peat-derived DOC is rich in polyphenolic compounds, the microbial degradation of which is thought to rely on extracellular phenol oxidases. Despite substantial interest in the biogeochemical fate of terrigenous DOC (tDOC), few studies have quantified phenol oxidase activity in aquatic environments, and microbial remineralization rates of tDOC have never been measured in Southeast Asia. Here, we assess the potential for using phenol oxidase assays as a proxy of tDOC biodegradation across peat-draining rivers and coastal waters of Sarawak, Borneo, and report experimental measurements of microbial tDOC remineralization rates from this region. We show first that phenol oxidase assays in aquatic samples are problematic because of the rapid, pH-dependent auto-oxidation of the assay substrate. Our field measurements of phenol oxidase activity detected only substrate auto-oxidation, suggesting that real phenol oxidase activity was low or absent. Second, we report that peatland tDOC, collected from one of the few remaining intact peatlands on Borneo, showed at most very limited biodegradation (0–6% loss of DOC, and 0–12% loss of coloured dissolved organic matter) during several 56-day incubation experiments at in-situ temperature of ~30°C, even when diluted with seawater or amended with nutrients. Our results suggest that direct microbial respiration is perhaps not a major pathway for peatland tDOC remineralization in Southeast Asia, and that photo-oxidation is more likely to control the fate of this carbon.

41 **1 Introduction**

42 Peatlands in Southeast Asia contain approximately 69 Gt of soil organic carbon, mainly in
43 Sumatra and Borneo (Dommain et al., 2014; Page et al., 2011). Rivers draining Southeast Asian
44 peatlands have amongst the highest dissolved organic carbon (DOC) concentrations reported
45 globally (Alkhatib et al., 2007; Baum et al., 2007; Martin et al., 2018; Moore et al., 2011;
46 Müller et al., 2015), and are thought to account for around 10% of the global land-to-ocean
47 flux of terrigenous DOC (tDOC) (Baum et al., 2007; Moore et al., 2011). tDOC derived from
48 soils has traditionally been thought of as largely refractory to microbial decomposition, owing
49 to its inherent chemical properties (Alexander, 1965; Sollins et al., 1996). Peat-derived tDOC
50 is rich in phenolic lignin degradation products that are generally considered resistant to
51 degradation (Gandois et al., 2014; Moore et al., 2013), and which inhibit extracellular enzymes
52 (Freeman et al., 2001; Mann et al., 2014). Tropical peat may in fact be particularly phenol-rich
53 compared to peat at high latitudes (Hodgkins et al., 2018, Yule et al., 2018).

54 However, it is usually thought that the DOC pool in the deep ocean only contains a relatively
55 minor contribution from tDOC (Dittmar & Stubbins, 2014; Opsahl & Benner, 1997), even
56 though the flux of tDOC to the ocean is sufficient to account for the entire oceanic DOC
57 turnover (Williams & Druffel, 1987). This implies that tDOC is biogeochemically labile over
58 relatively short time-scales. Microbial degradation is recognized to be a key pathway for tDOC
59 remineralization in aquatic environments (Bianchi, 2011; Cai, 2011; Ward et al., 2013) and
60 was shown to quantitatively dominate over photodegradation in the Arctic Ocean and on the
61 Louisiana Shelf (Fichot & Benner, 2014; Kaiser et al., 2017). Aarnos et al. (2018) recently
62 estimated that 71% of the global riverine tDOC flux is remineralised within 1 year of entering
63 the ocean by a combination of bio- and photodegradation. However, some studies report tDOC
64 to be resistant to bio- and/or photodegradation (Stubbins et al., 2017; Shirokova et al., 2017).
65 Moreover, both Follett et al. (2014) and Zigah et al. (2017) argued, based on carbon isotopic

data, that a greater proportion of DOC in the ocean is tDOC than previously believed. Resolving whether tDOC in different regions of the world is labile to biodegradation and/or to photodegradation is therefore important for improving our understanding of the global carbon cycle. Moreover, it is now increasingly thought that the biodegradability of organic matter is not purely determined by its chemical properties, but depends also on the microbial community composition, physical protection of the organic matter, environmental drivers such as temperature and pH, and enzyme kinetics (Bianchi, 2011; Kleber, 2010). Consequently, it is also necessary to determine whether the biodegradability of tDOC changes across the land–ocean aquatic continuum.

In Southeast Asia, several studies have concluded from measurements of air–water CO_2 fluxes that the majority of the peatland tDOC is remineralized within rivers, estuaries, and the coastal ocean (Müller et al., 2015, 2016; Wit et al., 2015, 2018). Low O_2 concentrations have also been taken as evidence for remineralization of tDOC within the peat-draining rivers (Müller et al. 2015; Rixen et al., 2008). However, tDOC often appears to mix conservatively across peat-draining estuaries in Southeast Asia (Alkhatib et al., 2007; Martin et al., 2018; Rixen et al., 2008; Zhou et al., 2019). Such conservative mixing suggests that tDOC actually experiences limited biogeochemical processing at least until it reaches the coastal sea. Moreover, Southeast Asian shelf seas have been classed both as sources (Borges et al., 2005; Kartadikaria et al., 2015) and as sinks (Laruelle et al., 2014) for atmospheric CO_2, highlighting the need for a better understanding of carbon biogeochemistry in this region.

Microbial processing of organic matter relies on enzymes, and enzyme activity rates are therefore widely measured as indices of microbial processes in terrestrial and aquatic environments (Arnosti et al., 2014; Hoppe, 1991; Sinsabaugh et al., 2008). Phenol oxidases (POx) are a key class of enzymes that oxidize phenolic groups, and are released by a broad range of microbes to detoxify metal ions and degrade phenolic and humic compounds

(Sinsabaugh, 2010). POx activity is often measured in soil biogeochemical studies because POx are thought to exert a key control over soil organic matter degradation rates (Allison & Vitousek, 2004; Carreiro et al., 2000; Freeman et al., 2001; Freeman et al., 2004; Mazzon et al., 2018; Prescott, 2010; Sinsabaugh, 2010; Sinsabaugh & Shah, 2011; Stursova & Sinsabaugh, 2008; Wang et al., 2015). POx activity in soils is strongly inhibited by lack of oxygen (Freeman et al., 2001), low pH (Stursova & Sinsabaugh, 2008; Tahvanainen & Haraguchi, 2013), and high concentrations of phenolic compounds (Williams et al., 2000). The environmental conditions in peatlands have therefore been hypothesized to act as a positive feedback that promotes further organic matter accumulation by inhibiting POx activity (Freeman et al., 2001; Freeman et al., 2004). This "enzymatic-latch hypothesis" (Freeman et al., 2001) would predict that POx rates, and thus the potential for biodegradation of tDOC, should increase as phenol-rich tDOC is diluted, and as oxygen and pH increase during transit through estuaries and into coastal seas.

Yet despite the interest in the biogeochemical fate of tDOC in aquatic environments (Bauer et al., 2013; Cai, 2011; Ciais et al., 2013), to our knowledge only two studies have attempted to measure POx in aquatic samples. Mann et al. (2014) reported that POx and other extracellular enzyme activities increased after experimental removal of phenolic DOC from samples taken in the permafrost-draining Kolyma River (Mann et al., 2014). This result is similar to the reported effect of reducing the phenol concentration in soil samples (Freeman et al., 2004). Moreover, POx activity in the Kolyma River was correlated with the microbial respiration rate (Mann et al., 2014). These results are consistent with a link between POx activity and tDOC biodegradation rate in rivers. A second study by Sieczko & Peduzzi (2014) found that flooding of the Danube river initially promoted the activity of sugar- and protein-degrading enzymes (glucosidases and protease) due to the introduction of fresh DOM, but this was later followed by increasing POx activity after the water table started to drop, suggesting that the microbial

116 community switched to lignin degradation. Sieczko & Peduzzi (2014) therefore proposed that
117 changes in the ratio of hydrolytic enzyme activity to POx activity could be used as a proxy to
118 track the utilization of allochthonous versus autochthonous DOM. These two studies (Mann et
119 al., 2014; Sieczko & Peduzzi, 2014) thus suggest that POx activity measurements hold promise
120 as a proxy of tDOC biodegradation in aquatic environments.

121 Here, we test whether POx activity can be measured as a proxy of tDOC biodegradation across
122 salinity gradients from peatland-draining rivers to coastal waters of Sarawak, Borneo.
123 Moreover, we report experimental measurements of tDOC biodegradability from Southeast
124 Asian peatland-draining rivers.

125

126 **2 Materials and Methods**

127 2.1 Sites and sample collection

128 Six rivers, their estuaries, and surrounding coastal seawater were sampled in Sarawak (Borneo)
129 during March and September 2017 (Figure 1). The rivers Maludam, Simunjan, Sebuyau, and
130 Samunsam are blackwater rivers that drain catchments containing large areas of peatland, and
131 have DOC concentrations of 1,000–4,400 µmol l^{-1}. In contrast, the rivers Rajang and Sematan
132 drain catchments that consist to a greater proportion of mineral soils, and have DOC
133 concentrations below 500 µmol l^{-1} (Martin et al., 2018). Data for DOC concentration, coloured
134 dissolved organic matter, and fluorescent dissolved organic matter for these sampling
135 campaigns were already reported by Martin et al. (2018) and Zhou et al. (2019). March
136 corresponds to the end of the wettest season of the year (northeast monsoon), while September
137 marks the end of the drier southwest monsoon. Rainfall in Sarawak is relatively high at all
138 times of the year though, averaging >100 mm per month year-round (Sa'adi et al., 2017). Water
139 temperature averaged 28.5–29.5ºC in both seasons.

Water samples for enzyme activity assays were collected from the upper 1m of water using a bucket or hand-held jug and immediately frozen unfiltered in dry shippers (-190ºC) for transport back to the laboratory, and then stored at -80ºC until analysis up to 3 months later. Salinity and temperature were measured at all stations using either a YSI CastAway or a Valeport FastCTD. Water pH was measured at most stations using a YSI Aquaread AP-2000.

To further test the POx assay method, water samples were collected from two sites around Singapore (Singapore Strait: 1.226°N 103.746°E, 5 m depth, December 2018; Johor Strait: 1.403°N 104.002°E 1 m depth, March 2019). Depth profiles of water were also collected at two oceanic sites north of Hawaii (22.76°N -158.077°E and 22.77°N -158.056°W) using a Niskin rosette between 10 m and 500 m depth. All samples were frozen immediately after collection in a dry shipper (-190 ºC) and stored at -80ºC until analysis within 3 months. Additional synthetic solutions to measure pure auto-oxidation of the POx assay substrate were prepared from ultrapure Elga water (here and below, 18.2 MΩ cm^{-1}) either alone or with added $NaHCO_3$ (Sigma-Aldrich S6014) or synthetic sea salt (Sigma-Aldrich S9883) to manipulate the pH and salinity.

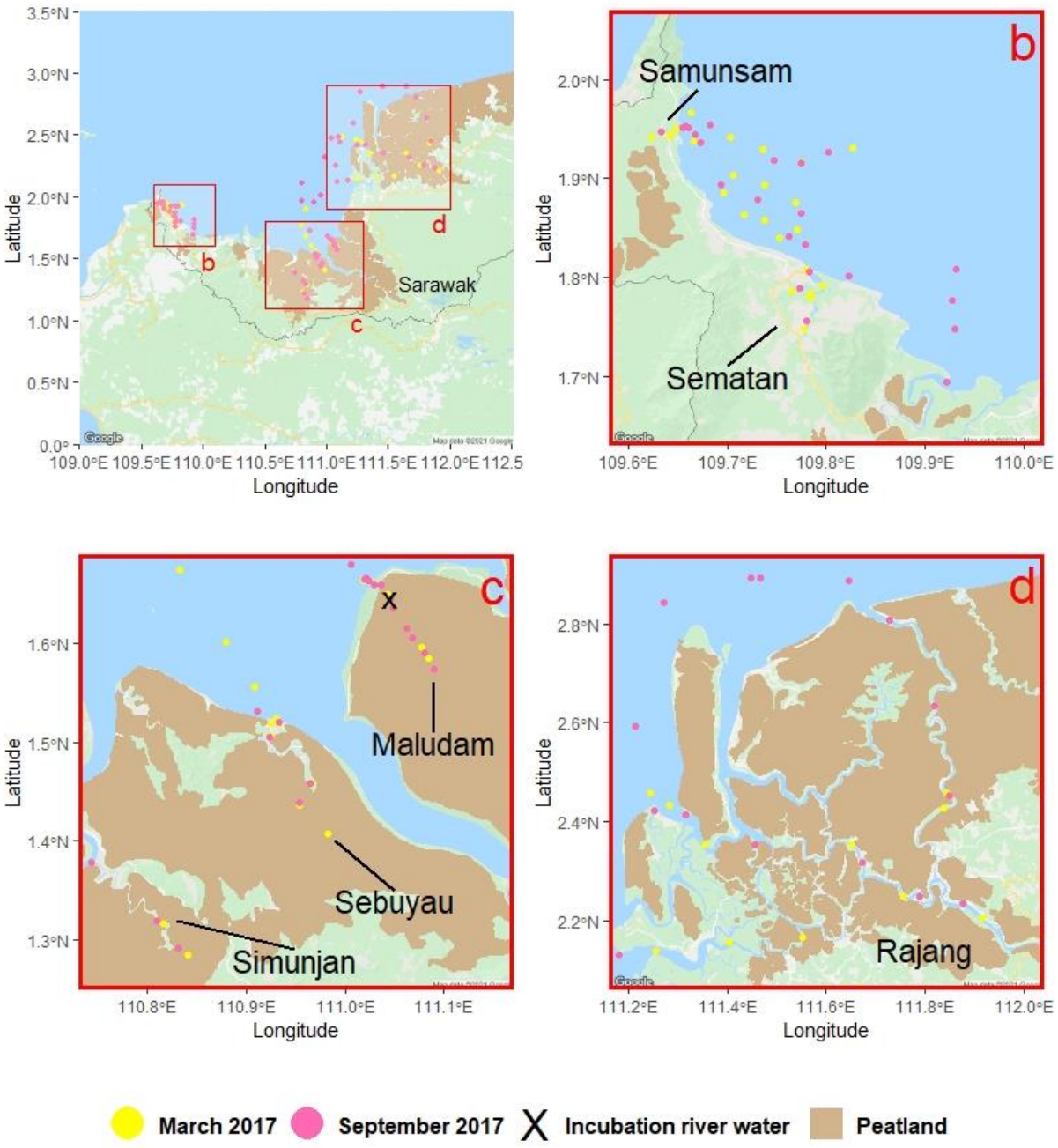


Figure 1. Locations of March (yellow circles) and September (pink circles) sampling locations for phenol oxidase and Leucine-aminopeptidase activity measurements in Sarawak, 2017. The sampling location of river water collected for all biodegradation incubations is shown (X). Labelled boxes correspond to the maps in panels (b-d) and annotated with rivers sampled. Brown shading delineates peatland as reported by Dommain et al., 2014.

2.2 Enzyme assays and data analysis

LAP activity was measured to provide an index of total heterotrophic microbial activity. Note that the presence of measurable LAP does not imply that tDOC biodegradation is taking place,

as LAP activity might simply reflect the processing of autochthonous organic matter. All enzyme assays were conducted with unfiltered water (200 µl) in triplicate with a Spark Tecan 10m microwell plate reader. Proteinase activity was measured as leucine aminopeptidase activity (LAP) and was assayed using the substrate L-leucine-7-amido-4-methylcoumarin (Sigma-Aldrich, A9891) at a final concentration of 40 µmol l^{-1} by measuring fluorescence (excitation 365 nm, emission 450 nm) at 10-minute intervals for 5 hours (Hoppe 1993). Standard curves were created for each assay in Elga water using the fluorescent standard 7-Amino-4-methylcoumarin (Sigma-Aldrich, A9891), and differences in quenching of sample fluorescence were corrected for by adding a known concentration of standard to each sample and calculating the quench factor compared to the standard curve.

POx activity was measured with the intention of providing a relative measure of tDOC degradation activity. POx was assayed using the substrate L-DOPA (L-3,4-dihydroxyphenylalanine, Sigma-Aldrich D9628) at a final concentration of 1 mM by measuring the change in absorbance at 460 nm. POx assays consistently showed sigmoidal reaction kinetics (Figure S1), which is a known feature of L-DOPA-based POx assays (Sinsabaugh 2010). To account for this, absorbance measurements were taken at 10-minute intervals for 6 hours (Figure S1), and the substrate oxidation rates were calculated from the maximum linear slope of absorbance or fluorescence over at least 5 consecutive time points. The steepest slope for POx assays was typically found between 1 and 2 hours after starting the assay. Because the oxidation product of L-DOPA (Dopachrome) is not commercially available, we determined the extinction coefficient empirically during each assay by reacting a known quantity of L-DOPA with a solution of mushroom tyrosinase (0.2 mg/ml final concentration, Sigma-Aldrich CAS 9002-10-2). We then applied the average extinction coefficient of all assays (0.1832 µmol l^{-1} m^{-1} at 460 nm) to convert absorbance to moles of L-DOPA oxidized.

Enzyme activity assays require matrix-matched controls to correct for possible non-enzymatic changes in sample absorbance or fluorescence. Duplicate samples for POx and LAP samples were collected in September 2017 to provide individual autoclaved controls for each sample, but in March 2017, controls for autoclaving were only collected from four stations covering the main gradients in water chemistry across our study region (i.e., peatland-draining blackwater rivers, mid-salinity estuaries, and coastal seawater). Controls for LAP assays were autoclaved for 20 minutes at 121°C, and never showed any significant change in fluorescence. Controls for POx were autoclaved for 1 hour at 121°C. As discussed below, autoclaved controls for POx activity all showed similar L-DOPA oxidation rates to samples, prompting additional experiments to distinguish between enzymatic and chemical oxidation of L-DOPA. Ultrafiltration to create enzyme-free samples was performed by pre-filtering samples with 0.2 µm Acrodisc syringe filters, followed by 3 kDa Amicon centrifugal filtration.

For the LAP assays, all data are presented as the autoclaved control-corrected LAP activities, but the autoclaved controls all showed uniformly minimal change in fluorescence so that this correction was negligible. Because the autoclaved controls for POx assays had similarly high substrate oxidation rates as the samples, we present the POx data simply as the measured L-DOPA oxidation rates in the samples and in the controls (Substrate oxidation rate).

2.3 Biodegradation incubations

We conducted two separate experiments to test whether tDOC from an intact Southeast Asian peatland is labile to microbial remineralization. Water for experimental incubations was collected from the upper 1 m in the Maludam River (at salinity 0, upstream of any human infrastructure in and around the village of Maludam, 1.645°N, 111.046°E) in July 2019 and December 2019. Coastal seawater was collected from the Singapore Strait in December 2019. The Maludam River was selected because it is a fully peat-draining river that originates within an intact peat dome and drains a catchment that is a designated national park (Müller et al.,

2015). The Maludam therefore represents the closest to a pristine example of a tropical peat-draining river within our sample area, and is one of the few peat swamp forests in Southeast Asia that is still intact (i.e. has not been subjected to large-scale drainage or deforestation), unlike most peatlands in the other river catchments we studied.

All glassware and containers used for biodegradation and DOC analysis were either pre-baked at 450ºC for 4 hours or acid-washed and dried before use. All filters were pre-washed with ~300 ml of Elga water before use. 250-ml Duran bottles with polypropylene screw caps were used for all incubations. Incubation bottles were kept in a dark box in a covered location outdoors at ambient temperature (ranging from 26°C at night to 31°C during the day) and swirled gently every 2-3 days. All incubation bottles were kept tightly sealed to avoid evaporation.

To quantify tDOC biodegradation rates in undiluted river water, and test whether tDOC biodegradation might be limited by nutrients, Maludam River water was collected in July 2019. Half of the water was immediately filtered (0.2 µm Whatman Polycap TC 75 capsule filter) upon collection and half left unfiltered. The samples were stored in separate 10-L HDPE jerry cans and shipped to Singapore. Incubations were started 7 days after collection. Unfiltered water was then further split into two biodegradation treatments, one of which was amended with nutrients (5 µmol l^{-1} KNO_3 (Sigma-Aldrich, product number 221295) and 1 µmol l^{-1} KH_2PO_4 (Fisher Scientific, catalog number P285)). The filtered water was then re-filtered (0.22 µm Supor membrane, Merck Millipore) as a microbe-free control. Triplicate initial samples were taken from each homogenized treatment to measure DOC and coloured dissolved organic matter (CDOM). The remaining water was then equally split into 250-ml Duran bottles with PTFE-lined caps each with ~150 mL water and 100 mL headspace. At each sampling point (after 7, 14, 28, and 56 days), 3 sacrificial replicates were taken per treatment to measure DOC & CDOM.

To test the hypothesis that biodegradation of peatland tDOC might occur after the tDOC has been substantially diluted with coastal seawater and exposed to a coastal marine microbial community, water was again collected from the Maludam river in December 2019. The water was immediately filtered as in July 2019, shipped to Singapore, and then re-filtered through a 0.22 µm Supor membrane. Coastal surface seawater was then freshly collected in the Singapore Strait (1.228°N, 103.750°E), part of which was kept unfiltered as an inoculum and the rest filtered through a 0.22 µm Supor filter. Incubations were started 21 days after the Maludam water was collected, and one day after the seawater was collected, by creating four treatments: filtered seawater only (as sterile control), filtered seawater + unfiltered seawater inoculum (to measure background DOC remineralization rate), filtered seawater + filtered Maludam water (as sterile control), and filtered seawater + filtered Maludam water + unfiltered seawater inoculum (to measure remineralization rate of tDOC). The two treatments with added tDOC received 1.25% of final volume of Maludam water, which raised the DOC concentration by ~45 µmol l^{-1}. The two treatments to which the seawater inoculum was added received 5% of final volume of unfiltered seawater. After taking initial DOC and CDOM samples from each treatment, the remaining water was equally split into 250-mL Duran bottles per treatment to give 3 sacrificial replicates of ~150 mL for each sampling point (after 8, 15, 29, and 56 days) with single DOC and CDOM samples taken from each replicate. Mixing ratios for all incubations are shown in table S1.

Oxygen measurements were not taken during incubations. However, a headspace of air of at least 40% of the total bottle volume was left in all incubation flasks. We estimate that this provided an O_2:DOC molar ratio of at least 1.9 even in the treatment with highest DOC concentration, which contained ~470 µmol DOC in 150 ml sample volume, while the headspace of 100 ml would have contained ~840 µmol O_2). While the rate of O_2 diffusion from headspace into the sample could have slowed the rate of biodegradation, we can confidently

263 rule out that O_2 limitation prevented carbon decomposition over the duration of our
264 experiments. This is also demonstrated by the fact that river water samples from Sarawak lost
265 up to 400 µmol l^{-1} DOC during five-day photodegradation experiments using lower
266 headspace:sample volume ratios in sealed bottles (Martin et al., 2018).

267 2.4 Chemical analyses

268 *2.4.1 Dissolved organic carbon (DOC) analysis*

269 DOC samples (30 mL) for all experiments were syringe-filtered (0.22 µm Acrodisc, pre-rinsed
270 with 180 ml Elga water and flushed with sample before collection) into EPA vials, acidified
271 with 100 µL 50% H_2SO_4 and stored at 4°C. Samples were analysed within one month on a
272 Shimadzu TOC-L system with high-salt kit, with an injection volume of 150 µl and a sparge
273 time of 5 min, and 5-7 replicate injections to ensure a coefficient of variance ≤2%. Calibration
274 and drift monitoring were performed using potassium hydrogen phthalate standards. The
275 analytical accuracy was determined using deep-sea Certified Reference Material from the
276 University of Miami, USA (42–45 µmol l^{-1} DOC), which returned a long-term mean and
277 standard deviation of 48 ± 3.9 µmol l^{-1}.

278 *2.4.2 Coloured dissolved organic matter (CDOM) analysis*

279 Samples for CDOM were syringe-filtered (0.22 µm Acrodisc, pre rinsed with 180 ml Elga
280 water and flushed with sample before collection) into EPA vials, which were then either run
281 immediately, or stored at 4˚C and then warmed to room temperature before analysis. The
282 absorbance was measured at 230-900 nm at 1-nm resolution against an Elga water reference
283 on a Thermo Evolution 300 dual-beam spectrophotometer, using either a 10-cm or 0.2-cm
284 quartz cuvette. Instrument performance was checked prior to analysis according to Mitchell et
285 al., (2000).

286 Absorbance spectra were converted to Napierian absorbance coefficients as:

$$a_\lambda = \frac{2.303 \times A_\lambda}{l}$$

Where a_λ is the absorption coefficient at wavelength λ, A_λ is the absorbance at wavelength λ, and l is the pathlength of the cuvette in meters. Spectra were baseline-corrected by subtracting the mean absorbance from 700-800 nm (Green and Blough, 1994). The absorption coefficient at 350 nm (a_{350}) was determined, and CDOM spectral slopes calculated for the intervals 275-295 nm ($S_{275–295}$) and 350-450 nm ($S_{350–400}$) as the absolute value of the linear regression of log-transformed Napierian absorption coefficients against wavelength (Helms et al., 2008). The spectral slope ratio S_R was calculated as the ratio of $S_{275–295}$ to $S_{350–400}$.

2.5 Data analysis

All statistical analyses and CDOM spectral calculations were conducted using R (R core team 2020) and the R packages 'tidyverse' (Wickham et al., 2019), and 'hyperspec' (Beleites & Sergo, 2020). All concentrations and activity rates are quoted as mean ± 1 standard deviation unless otherwise stated.

3 Results

3.1 Leucine aminopeptidase (LAP) activity

Extracellular LAP activity spanned four orders of magnitude (Figure 2a). Freshwater stations (i.e. salinity = 0) showed higher LAP activity in the Rajang and Sematan (57 ± 26.7 nmol l^{-1} h^{-1}, n=8, DOC concentration 100–400 µmol l^{-1}) than in the blackwater rivers (2.5 ± 5.4 nmol l^{-1} h^{-1}, n=20, DOC concentration 1100-4400 µmol l^{-1}), except for two outliers (59.9 and 49.9 nmol l^{-1} h^{-1}), which were collected next to villages in the Maludam and Sebuyau blackwater rivers. LAP activities reached highest values in the estuaries at salinities between 3 and 12 (159 ± 158 nmol l^{-1} h^{-1}, n = 10) and then decreased with increasing salinity, and only averaged 11.1 ± 11.5 nmol l^{-1} h^{-1}(n=30) at stations with salinity>26. Across the entire dataset, there was a statistically

significant relationship between LAP activity and chlorophyll-*a* concentration (R^2 = 0.17, $p<0.001$, Figure 2b; chlorophyll-*a* data taken from Martin et al., 2018).

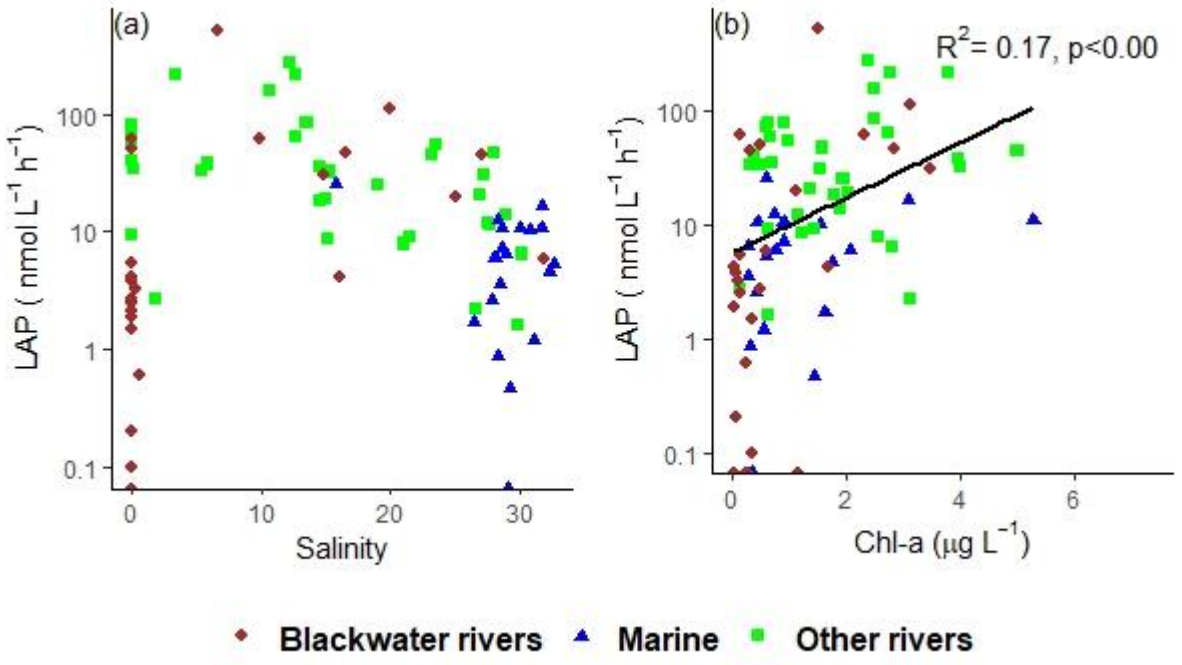


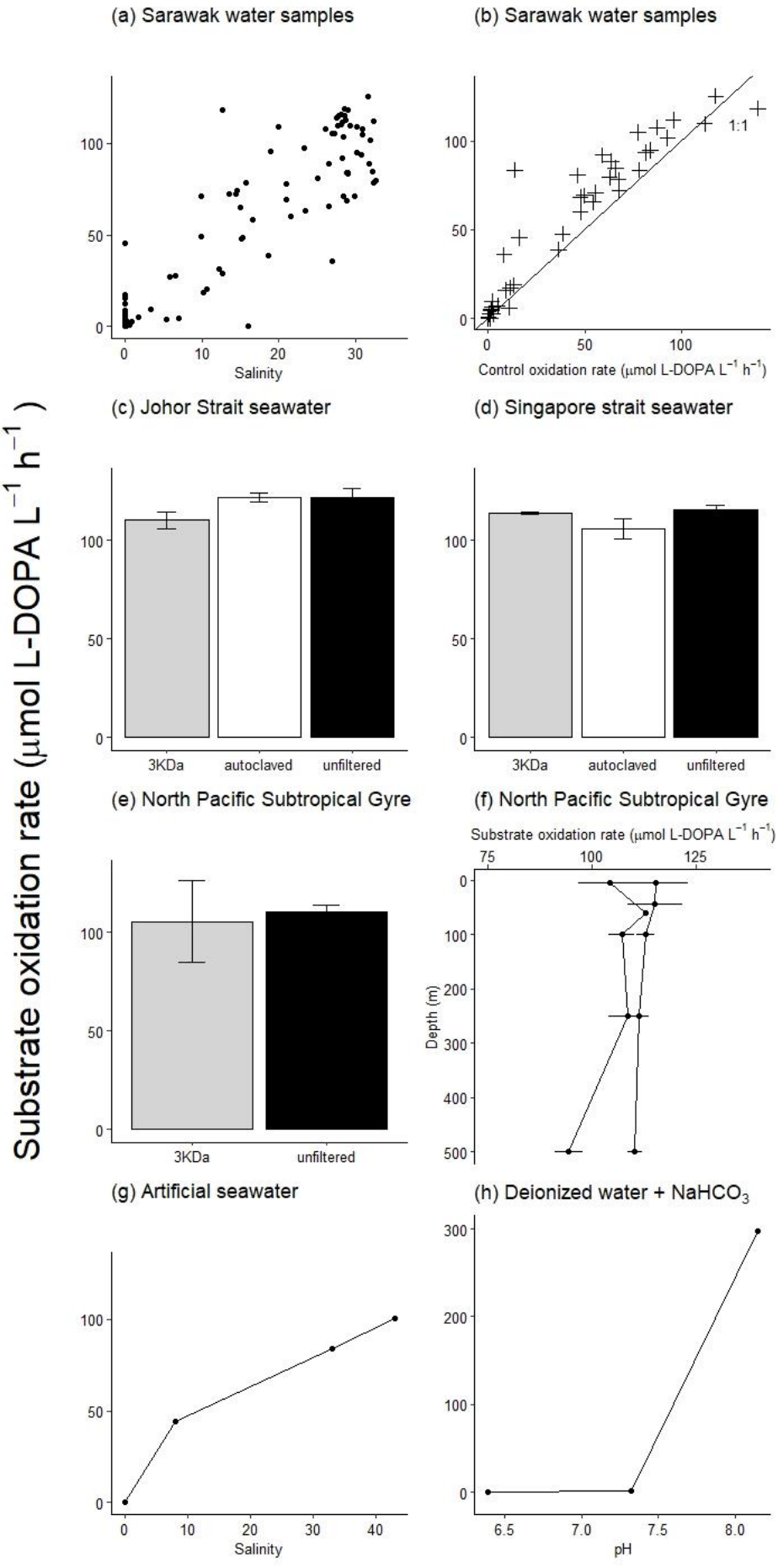
Figure 2. Leucine-aminopeptidase (LAP) of blackwater rivers (Maludam, Sebuyau, Samunsam and Simunjan, all >500µM DOC), other rivers (Rajang, Sematan and Lundu, all <500µM DOC) and marine waters from Sarawak, Borneo during surveys in March and September 2017. (b) Linear regression of LAP (this study) and chlorophyll-a concentrations as reported by Martin et al. (2018).

3.2 Phenol oxidase (POx) activity and auto-oxidation of L-DOPA

Strikingly, we observed a strong increase in the oxidation rate of the POx assay substrate, L-DOPA, with increasing salinity, ranging from 0 to about 140 µmol l^{-1} h^{-1} (Figure 3a). However, our autoclaved controls showed essentially the same substrate oxidation rates as the un-autoclaved samples, which means that the L-DOPA oxidation rate in our assays was due to non-enzymatic oxidation of the substrate (Figure 3b).

To verify that L-DOPA is oxidized non-enzymatically in seawater, we used ultrafiltration to generate enzyme-free seawater samples. We found that samples collected in the Johor and Singapore Straits (two coastal sites around Singapore with salinity ≥31), as well as from two depth profiles north of Station ALOHA in the North Pacific Subtropical Gyre, all showed

328 similarly high L-DOPA oxidation rates in untreated water as in 3-kDa ultrafiltered and
329 autoclaved controls (Figure 3c-f). The small decrease in oxidation rate observed in the 3 kDa
330 ultrafiltered controls from the Johor Strait (Figure 3c) was most likely due to slight dilution
331 with residual Elga water used to rinse the filters. Notably, the L-DOPA oxidation rates were
332 very similar for all Sarawak samples with salinity >30, all samples from Singapore, and all
333 samples from Station ALOHA from the surface down to 500 m (all between 100–140 µmol l^{-1}
334 h^{-1}). Additional assays with solutions of artificial sea salts and sodium bicarbonate in Elga
335 water showed that the L-DOPA oxidation rate increases approximately linearly with salinity
336 up to ~100 µmol L-DOPA l^{-1} h^{-1} (salinity 44), and that substrate oxidation is observed above
337 pH ~7 (Figure 3g,h), similar to the trend observed in our samples from Sarawak.


(a) Sarawak water samples
(b) Sarawak water samples
1:1
Salinity
Control oxidation rate (μmol L-DOPA L^{-1} h^{-1})
(c) Johor Strait seawater
(d) Singapore strait seawater
3KDa
autoclaved
unfiltered
(e) North Pacific Subtropical Gyre
(f) North Pacific Subtropical Gyre
Substrate oxidation rate (μmol L-DOPA L^{-1} h^{-1})
Depth (m)
(g) Artificial seawater
(h) Deionized water + NaHCO$_3$
pH
Substrate oxidation rate (μmol L-DOPA L^{-1} h^{-1})

338

Figure 3. Substrate oxidation rate (phenol oxidase assay) in: (a,b) Water samples (substrate oxidation rate), autoclaved controls (control oxidation rate) collected from Sarawak, Borneo during surveys in March and September 2017. Comparisons of unfiltered water, autoclaved samples and 3KDa ultrafiltration of water from the (c) Johor Strait, (Singapore, salinity 31), (d) Singapore Strait (Singapore, salinity 31), (e,f) two depth profiles (0-500m) from the North Pacific Subtropical Gyre. (g) Artificial seawater. (h) Deionized water with pH manipulation ($NaHCO_3$ addition). Data show means ± 1 standard deviation of (c,d,e) 3 replicate measurements of one water sample; (f) 8 individual water samples from differing depths.

3.3 Biodegradation of tDOC

Both incubation experiments showed few significant changes in DOC and CDOM parameters, and where these changes were significant, they were small. Statistical parameters for those treatments and measurements that did show significant changes are given in Table S2. Incubations of Maludam river water with and without nutrient addition showed minor DOC variation in treatment means by <90 µmol l^{-1}, corresponding to <3.5% (Figure 4a). The data could not be fit with exponential decay curves. Linear regressions of mean DOC over time were non-significant in the filtered controls, but showed small significant decreases in unfiltered and unfiltered + nutrients river water treatments (Figure 4a). Corresponding to total tDOC decreases over 56 days by 0–4.4% the untreated river water (61 ± 73µmol l^{-1} DOC), and of 2.5–6% in the river water with added nutrients (131 ± 53 µmol l^{-1} DOC) (Figure 4a); calculated as the mean change in DOC after 56 days ± the combined sd of the three initial and three final replicates for each treatment.

No significant changes in CDOM parameters were observed in the unfiltered or filtered river water. The nutrient-amended treatment showed a significant decrease in a_{350} over 56 days (by 5.9 m^{-1}, or 4%), but CDOM spectral slopes and slope ratios did not show any significant change (Figure 4b–e).

Incubations in which tDOC-rich Maludam river water was mixed with coastal seawater showed a small decrease in DOC in both treatments, by 6.8 ± 2.5 µmol l^{-1} (8%) in the unamended seawater and by 7 ± 3.0 µmol l^{-1} (5.5%) in the seawater treatment with added Maludam river water, Figure 4f). However, neither linear nor exponential regressions of DOC over time were

368 significant. This indicates that the added Maludam tDOC was not being remineralized. A small,
369 significant linear decrease in a_{350} was observed in both the unfiltered + tDOM and filtered
370 control + tDOM treatments, amounting to 0.413 ± 0.02 m^{-1} (11% decrease) and 0.253 ± 0.018
371 m^{-1} (7% decrease), respectively (Figure 4g). A much smaller (but significant) decrease in a_{350}
372 was observed in the unfiltered seawater without added Maludam river water (0.04 ± 0.01 m^{-1}),
373 with no significant change in the filtered seawater control. Little overall change was observed
374 in slope parameters during incubations. No significant changes were observed in $S_{275–295}$ except
375 for a small significant increase in the filtered seawater controls (Figure 4h). In the unamended
376 seawater $S_{350\text{-}400}$ decreased slightly but not significantly, and increased very slightly (but
377 significantly) in the filtered and unfiltered treatments with added Maludam river water (Figure
378 4i). A significant increase in slope ratio was observed in the unfiltered seawater treatment,
379 which was not observed in the other treatments and controls (Figure 4j).

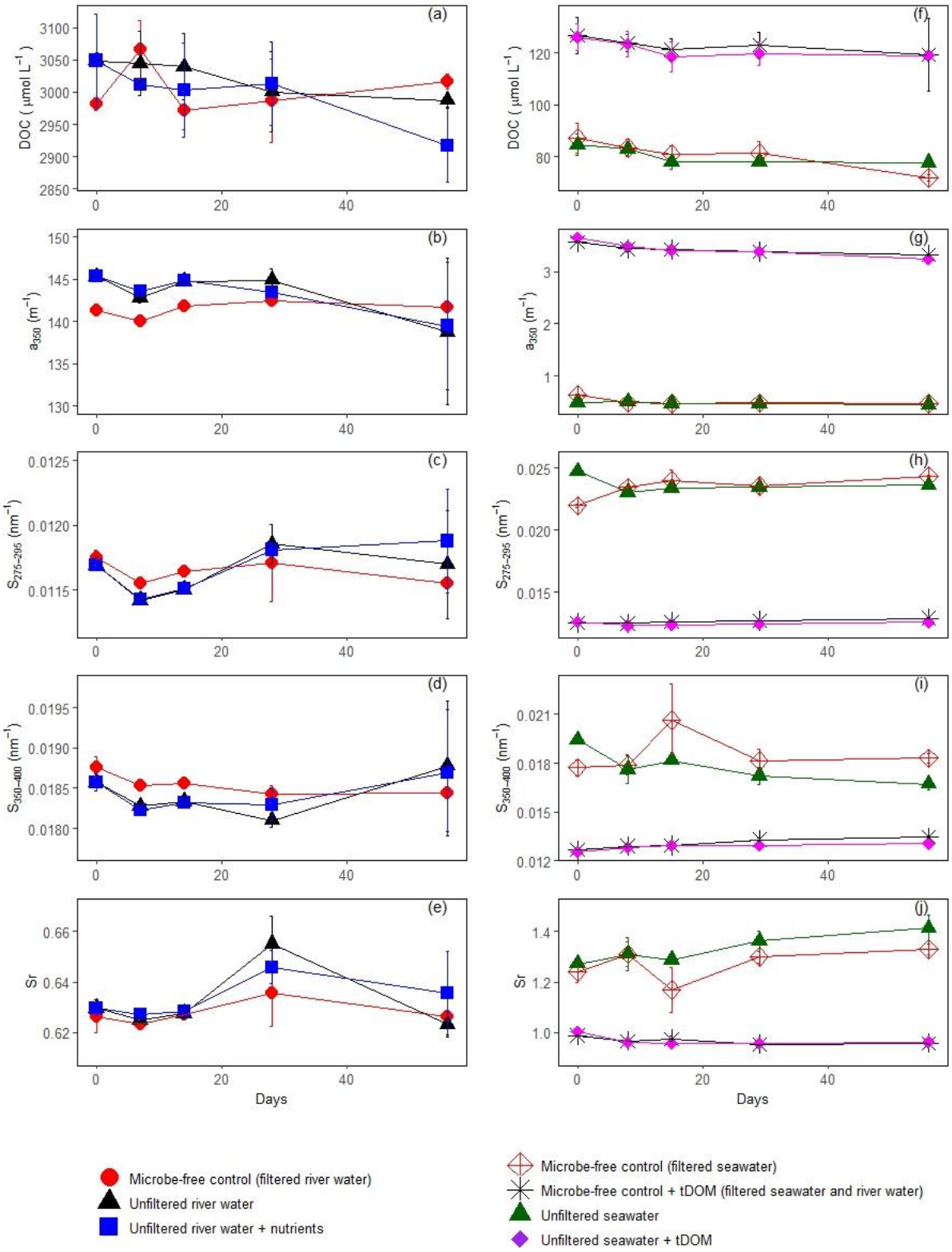


Figure 4. Biodegradation incubation experiments of tDOC rich river water collected from the peat draining Maludam river, Sarawak, Borneo. Maludam river water, with and without the addition of nutrients (Left column, a-e), and seawater with and without the addition of Maludam river water (right column, f-j). Results shown are dissolved organic carbon, absorption coefficient at 350 nm (a_{350}), spectral slopes $S_{275\text{-}295}$nm and $S_{350\text{-}400}$nm, and the slope ratio of $S_{275–295}$:$S_{350–400}$ (Sr). In all incubations, filtered controls represent microbe free controls whereas unfiltered water contains the natural microbial community. Nutrient additions consisted of 5 µmol L^{-1} nitrate and

1 µmol L^{-1} phosphate. All data are mean ± 1 standard deviation of three replicate incubations. Significant regressions are detailed in Table S2.

4 Discussion

4.1 Leucine aminopeptidase (LAP) activity

Leucine aminopeptidase is typically associated with the activity of heterotrophic bacteria degrading organic matter for protein synthesis (Kirchman et al., 1985). Our data therefore suggest that heterotrophic microbial activity was lower in blackwater rivers than the less DOC-rich Rajang River, and highest in the estuaries. Estuaries are generally recognized as areas of high biogeochemical activity (Cai, 2011), so this result was not unexpected. The higher LAP activity in the Rajang River compared to the blackwater rivers is likely a consequence of the differences in river chemistry promoting higher microbial activity in the Rajang, with higher pH and lower DOC concentrations than blackwater rivers (Martin et al., 2018) and high dissolved inorganic nitrogen levels likely due to anthropogenic inputs (Jiang et al., 2019). Conversely, chlorophyll concentrations were relatively low at all marine stations (on average around 1 µg l^{-1} and mostly <2 µg l^{-1}; Martin et al., 2018), and LAP activities were consistently low at high salinities. This indicates that LAP is likely an accurate index of relative variation in heterotrophic microbial activity across the study region. Crucially, however, this enzyme plays no role in the degradation of lignin or other phenolic molecules and therefore these results do not indicate biodegradation of peatland derived tDOC. The fact that we observed a significant relationship between LAP and chlorophyll-*a* across the entire dataset indicates instead that LAP activity was most likely related to the degradation of autochthonous organic matter, and therefore does not imply that microbial processing of tDOC was taking place. This is consistent with the interpretation by Sieczko & Peduzzi, (2014) that LAP provides an index of the processing of labile DOM but not of more refractory, terrigenous DOM.

4.2 Phenol oxidase assay methodological limitations

Our POx results clearly suggest that this assay did not return data that reflect microbial tDOC remineralization across the region. It could be tempting to interpret the trend in substrate oxidation rate with salinity as evidence in support of the hypothesis that dilution of phenol concentration and increasing pH over the salinity gradient promote POx activity and tDOC remineralization (Sinsabaugh, 2010; Williams et al., 2000). However, because the oxidation rate in our autoclaved controls accounted for most of the substrate oxidation rate, these data cannot be interpreted as representing enzymatic activity. Moreover, we observed very similar rates of L-DOPA oxidation in the marine samples from Sarawak, from Singapore, and from the surface down to 500 m depth in the North Pacific Subtropical Gyre, which are biogeochemically very different environments, and, in the case of the depth profiles, span a large gradient in expected microbial activity. This further indicates that our POx assay results cannot be interpreted as representing enzymatic activity, since we would expect far more variable oxidation rates across such different sampling locations.

The additional experiments we conducted clearly demonstrated that the L-DOPA oxidation rate in POx assays is highly sensitive to pH and ionic concentration. Significant auto-oxidation of L-DOPA at alkaline pH was also reported by two previous studies (Tahvanainen & Haraguchi, 2013; Zhou et al., 2012). Autoclaving causes significant changes to solution chemistry: although autoclaved seawater usually becomes more alkaline because CO_2 is released (Harrison & Berges, 2005), autoclaving of soil extracts can release organic acids and result in a more acidic pH (Skipper & Westermann, 1973). In our coastal water samples, the presence of varying quantities of organic and inorganic particulate matter of terrestrial and aquatic origin may either have led to small decreases in pH or otherwise changed the solution chemistry in a way that led to small reductions in L-DOPA oxidation rate in most controls. Consequently, we conclude that any POx activity rate we could calculate from our data is

437 unlikely to reflect real enzymatic activity, and is more likely the result of small changes in auto-
438 oxidation rate between samples and controls.

439 Although POx assays can also be conducted with the alternative substrates pyrogallol and
440 ABTS, L-DOPA is much more commonly used because it has a more suitable redox potential
441 and can be used over a much wider pH range (Bach et al., 2013). Thus, there are no alternative
442 substrates known at present that could overcome the limitations of L-DOPA and be used over
443 the range of chemical gradients that are found across the land–ocean aquatic continuum.

444 Our data do not preclude the possibility that genuine microbial POx activity might be
445 measurable in aquatic samples from other regions using L-DOPA. However, our results clearly
446 show that great care must be taken to ensure that such assays are not confounded by the auto-
447 oxidation of L-DOPA. Moreover, because autoclaving alters solution chemistry in a way that
448 is likely to influence the L-DOPA auto-oxidation rate, we would strongly recommend that
449 ultrafiltered controls are used. Because POx are larger than ~40 kDa (Dean & Eriksson, 1994;
450 Van Gelder et al., 1997; Goulart et al., 2003; Thurston 1994; Weemaes et al., 1998),
451 ultrafiltration through a suitably small pore size can generate enzyme-free controls without
452 altering the L-DOPA auto-oxidation rate.

453 We would therefore recommend that POx activities should also be measured in regions where
454 rapid rates of tDOC biodegradation have been observed. It is possible that in such
455 environments, real microbial POx activity could significantly overwhelm the auto-oxidation
456 rate, in which case L-DOPA-based POx assays could prove to be valuable after all for tracing
457 tDOC biodegradation in aquatic environments. However, we recommend that ultrafiltration
458 should be used to generate enzyme-free controls without altering the water chemistry in a way
459 that could affect the L-DOPA auto-oxidation rate.

460 4.3 Lack of peatland tDOC biodegradation in Sarawak

461 The fact that we only observed 0–4.4% DOC loss in river water incubations over 56 days at
462 around 30°C indicates very low biodegradability of tDOM from an intact tropical peatland. In
463 comparison, around 5-20% of DOC in temperate peat-draining rivers is typically biodegradable
464 over time-scales of 5–55 days at incubation temperatures of 10–22°C (Asmala et al., 2014;
465 Fovet et al., 2020; Hulatt et al., 2014; Stutter et al., 2013). Biodegradability of tDOC is
466 determined not only by inherent chemical characteristics but also by environmental constraints
467 (Guggenberger et al., 2011; Kleber, 2010). In Southeast Asian peat-draining rivers, these
468 constraints might include low concentrations of nutrients (Alkhatib et al., 2007; Bange et al.,
469 2019; Gandois et al., 2020; Wickland et al., 2012) and low pH due to organic acids (Borges et
470 al., 2015; Müller et al., 2015). However, our nutrient-amended treatment showed a maximum
471 of 6% DOC loss, indicating that microbial degradation of peatland tDOM in our region is not
472 simply limited by nutrients. Moreover, addition of tDOM-rich Maludam River water to coastal
473 seawater did not result in any excess DOC loss compared to the unamended seawater
474 treatments, with only ~7 µmol l^{-1} lost in both cases, indicating that Maludam river tDOM does
475 not become more biodegradable when mixed with coastal seawater and microbes. A linear
476 decrease in CDOM concentration (a_{350}) was observed in both treatments and filtered controls
477 where Maludam River tDOM was added to coastal seawater, and $S_{350-400}$ showed a very small
478 increase typically associated with a shift towards lower molecular weight and/or decreasing
479 aromaticity (Hansen, et al., 2016). Because these changes occurred in both unfiltered and
480 filtered treatments, this possibly reflects slow, abiotic transformations of CDOM after mixing
481 with seawater, although this was clearly not associated with a decrease in the added tDOC.

482 Porewater DOC in Southeast Asian peatlands likely undergoes significant processing by the
483 soil microbial community, resulting in DOC that has a very young radiocarbon age (~10 years)
484 but already shows chemical and optical characteristics of being highly degraded by the time it
485 enters rivers (Gandois et al., 2014; Müller et al., 2015; Zhou et al., 2019). Such pre-degradation

would likely reduce the biodegradability of the DOC that is ultimately exported to rivers and coastal seas. A lack of biodegradability was also recently reported for DOC in surface waters from peat bogs in the permafrost zone of Siberia (Shirokova et al., 2019).

Our data are consistent with the predominantly conservative mixing pattern of tDOC that has been reported for peat-draining rivers in Southeast Asia (Alkhatib et al., 2007; Baum et al., 2007; Martin et al., 2018; Zhou et al., 2019), as well as with the absence of measurable POx enzyme activity across our study region in Sarawak. However, these results contrast with reports of consistent outgassing of CO_2 from peat-draining rivers in Southeast Asia, including the Maludam (Müller et al., 2015; Müller-Dum et al., 2019; Wit et al., 2015). A recent analysis of the riverine and coastal carbon budget off Sumatra also concluded that around 60% of the total carbon exported by rivers is emitted as CO_2 in the estuaries and coastal ocean, which was attributed to respiration (Wit et al., 2018). This apparent discrepancy between our results and CO_2 outgassing measurements could potentially be explained by lateral transport of CO_2 and CH_4 from peat pore water into rivers (Clymo & Pearce, 1995; Johnson et al., 2008; Jones & Mulholland, 1998), and subsequent methanotrophy consuming the appreciable methane concentrations in these rivers (Bange et al., 2019). Moreover, tDOC from Borneo has been shown to be highly photolabile: filtered blackwater samples exposed for five days to natural sunlight lost up to 26% of DOC (Martin et al., 2018), and fluorescence spectra from a blackwater river system in Borneo were also interpreted recently as showing evidence of photodegradation (Gandois et al., 2020). To our knowledge, the only other tDOC degradation experiment in Southeast Asia exposed unfiltered water from a Sumatran blackwater river to natural sunlight and reported that ~27% of DOC was labile to combined photo- and biodegradation, mostly within 8 days (Rixen et al., 2008). However, the relative importance of photo- *versus* biodegradation was not determined, and it is possible that these results primarily reflect photo-chemical remineralization.

511 It is possible that biodegradation of tDOC in Southeast Asia takes place after partial
512 photodegradation: it is now widely recognized that photodegradation can render DOC more
513 labile to biodegradation (Cory & Kling, 2018; Ward et al., 2017). Overall, our data suggest that
514 photodegradation may be more likely to act as the main control over peatland tDOC
515 remineralization in coastal waters of Southeast Asia, as has been shown for carbon processing
516 in freshwater systems in the Arctic (Cory et al., 2014; Bowen et al., 2020).

517 **5 Conclusions**

518 We found that the commonly used phenol oxidase enzyme assay substrate L-DOPA undergoes
519 significant auto-oxidation at typical environmental pH values in estuaries and coastal seas,
520 which could significantly confound results unless adequate care is taken to prepare appropriate
521 enzyme-free controls. Our data suggest that autoclaved samples are not appropriate controls
522 because autoclaving can change sample chemistry; we therefore recommend that ultrafiltration
523 is necessary. We conclude that there was no measurable POx activity across our study region
524 in Sarawak, but recommend that this assay should also be tested (with ultrafiltered controls) in
525 aquatic environments where substantial tDOC biodegradation is anticipated. LAP data
526 indicated that microbial heterotrophic activity follows the expected trends with low activity in
527 blackwater rivers and highest activities in estuaries. However, this most likely reflects
528 heterotrophic processing of autochthonous organic matter, not tDOC biodegradation. Our
529 incubation experiments further demonstrated that tDOC from one of the few pristine peat-
530 draining rivers in Southeast Asia was largely refractory to biodegradation over eight weeks at
531 in-situ temperatures, even after adding nutrients or diluting the DOC with coastal seawater. We
532 surmise that the remineralization of Southeast Asian peatland DOC in the aquatic environment
533 is likely dependent on photodegradation.

534

535

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Availability of data and material

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