

Low Biodegradability of Dissolved Organic Matter From Southeast Asian Peat-Draining Rivers

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Key Points:

- Low biodegradability of peatland dissolved organic carbon 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
- Autoxidation of the phenol oxidase assay substrate L-3,4-dihydroxyphenylalanine occurs at pH ≥ 7 ; we recommend ultrafiltration to generate enzyme-free controls

Supporting Information:

Supporting Information may be found in the online version of this article.

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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 for tDOC biodegradation across peat-draining rivers and the coastal waters of Sarawak, Borneo, and report experimental measurements of microbial tDOC remineralization rates from this region. We first show that phenol oxidase assays in aquatic samples are problematic because of the rapid, pH-dependent autoxidation of the assay substrate. Our field measurements of phenol oxidase activity detected only substrate autoxidation, 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 colored dissolved organic matter) during several 56-day incubation experiments at an in situ temperature of $\sim 30^{\circ}\text{C}$, 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.

Plain Language Summary Southeast Asia's extensive tropical peatlands deliver around 10% of the global flux of riverine terrestrial dissolved organic carbon (tDOC) to the ocean, but we still have a limited understanding of what happens to this tDOC. In the ocean, microbes are often responsible for decomposing tDOC to CO_2 . To decompose peat-derived tDOC, microbes are thought to require enzymes called phenol oxidases. Our study consists of two parts: first, we assess the potential for using phenol oxidase assays as a measure of microbial tDOC decomposition activity in northwestern Borneo. Second, we experimentally measured the rate of microbial tDOC decomposition in this region. We first show that phenol oxidase assays in aquatic samples are problematic because of the high pH-dependent background oxidation rate of the assay substrate molecule. Second, we show that tDOC from one of the few intact peatlands in Borneo is resistant to microbial degradation, even after diluting it with seawater or adding nutrients. Our results suggest that direct microbial respiration is perhaps less important for peatland tDOC decomposition in Southeast Asia than photooxidation.

1. Introduction

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

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However, it is usually thought that the DOC pool in the deep ocean only contains a relatively minor contribution from tDOC (Dittmar & Stubbins, 2014; Opsahl & Benner, 1997), even though the flux of tDOC to the ocean is sufficient to account for the entire oceanic DOC turnover (Williams & Druffel, 1987). This implies that tDOC is biogeochemically labile over relatively short timescales. Microbial degradation is recognized to be a key pathway for tDOC remineralization in aquatic environments (Bianchi, 2011; Cai, 2011; Ward et al., 2013) and was shown to quantitatively dominate over photodegradation in the Arctic Ocean and on the Louisiana Shelf (Fichot & Benner, 2014; Kaiser et al., 2017). Aarnos et al. (2018) recently estimated that 71% of the global riverine tDOC flux is remineralized within 1 year of entering the ocean by a combination of biodegradation and photodegradation. However, some studies report tDOC to be resistant to biodegradation and/or photodegradation (Shirokova et al., 2017; Stubbins et al., 2017). 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 CO₂ 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 O₂ 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 CO₂, 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, 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, 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 and Peduzzi (2014) found that flooding of the Danube River initially promoted the activity of sugar-degrading 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 community switched to lignin degradation. Sieczko and Peduzzi (2014) therefore proposed that changes in the ratio of hydrolytic enzyme activity to POx activity could be used as a proxy for tracking the utilization of allochthonous versus autochthonous DOM. These two studies (Mann et al., 2014; Sieczko & Peduzzi, 2014) thus suggest that POx activity measurements hold promise as a proxy for tDOC biodegradation in aquatic environments.

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

2. Materials and Methods

2.1. Sites and Sample Collection

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

Water samples for enzyme activity assays were collected from the upper 1 m of water using a bucket or handheld jug and immediately frozen unfiltered in dry shippers (−190°C) for transporting back to the laboratory and then stored at −80°C 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 and 500 m depth. All samples were frozen immediately after collection in a dry shipper (−190°C) and stored at −80°C until analysis within 3 months. Additional synthetic solutions to measure the pure autoxidation of the POx assay substrate were prepared from ultrapure Elga water (here and below, 18.2 $\text{M}\Omega \text{ cm}^{-1}$) either alone or with added NaHCO_3 (Sigma-Aldrich S6014) or synthetic sea salt (Sigma-Aldrich S9883) to manipulate the pH and salinity.

2.2. Enzyme Assays and Data Analysis

Leucine aminopeptidase (LAP) activity was measured to provide an index of the 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 10 m microwell plate reader. Proteinase activity was measured as LAP activity and was assayed using the substrate L-leucine-7-amido-4-methylcoumarin (Sigma-Aldrich, A9891) at a final concentration of 40 $\mu\text{mol l}^{-1}$ by measuring the fluorescence (excitation 365 nm, emission 450 nm) at 10-min intervals for 5 h (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 the quenching of sample fluorescence were corrected by adding a known concentration of the standard to each sample and calculating the quench factor compared to the standard curve.

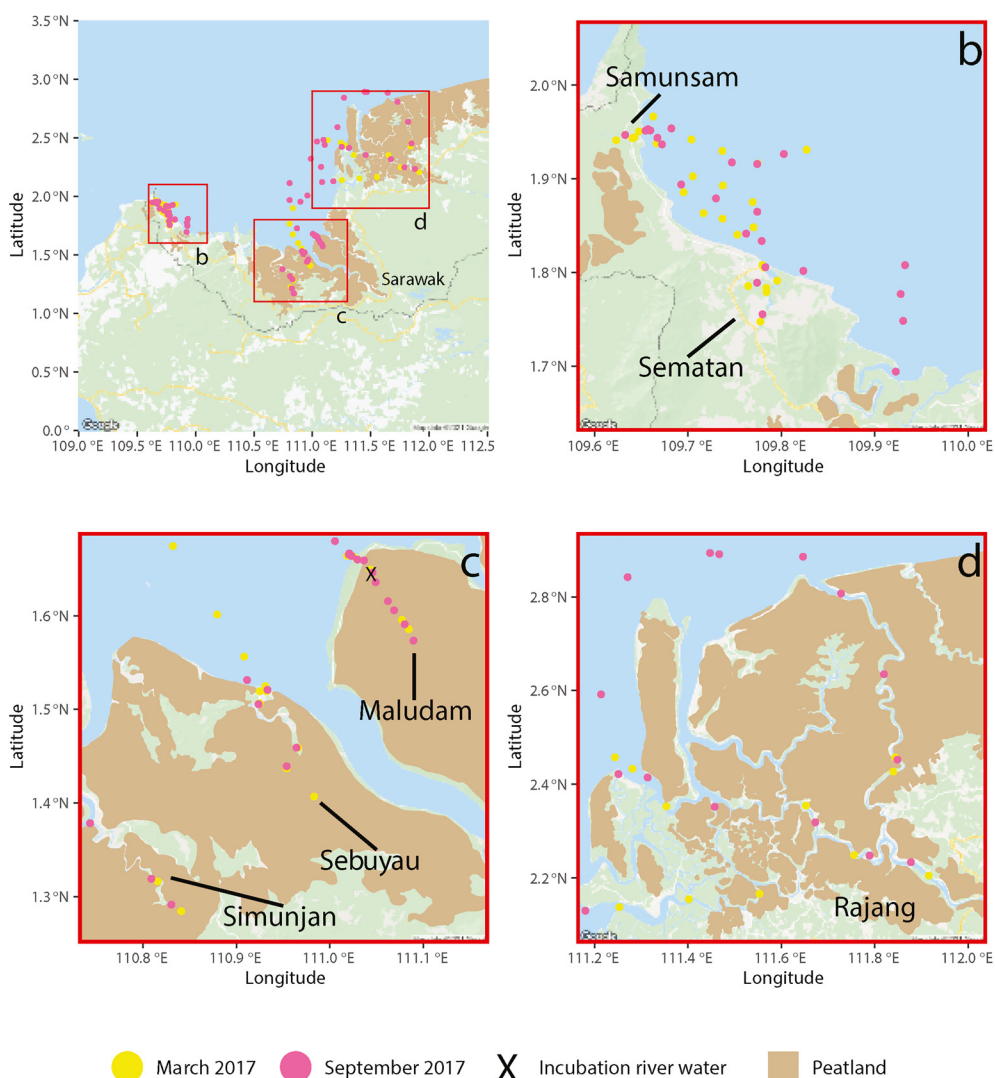


Figure 1. Locations of March (yellow circles) and September (pink circles) sampling locations for phenol oxidase and Leucine-aminopeptidase activity measurements in Sarawak, 2017. The sampling location of river water collected for all biodegradation incubations is shown (X). Labeled boxes correspond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

POx activity was measured with the intention of providing a relative measure of tDOC degradation activity. POx was assayed using the substrate L-3,4-dihydroxyphenylalanine (L-DOPA, 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-min intervals for 6 h (Figure S1), and the substrate oxidation rates were calculated from the maximum linear slope of absorbance or fluorescence over at least five consecutive time points. The steepest slope for POx assays was typically found between 1 and 2 h 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 making a known quantity of L-DOPA react 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 ($0.1832 \mu\text{mol l}^{-1} \text{m}^{-1}$ at 460 nm) to convert the absorbance to moles of oxidized L-DOPA.

Enzyme activity assays require matrix-matched controls to correct for possible nonenzymatic changes in the 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, the controls for autoclaving were collected only 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 min at 121°C, and they never showed any significant change in fluorescence. Controls for POx were autoclaved for 1 h at 121°C. As discussed below, all autoclaved controls for the POx activity 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 prefiltering 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 all the autoclaved controls showed uniformly minimal change in fluorescence such that this correction was negligible. Because the autoclaved controls for POx assays had high substrate oxidation rates similarly to 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 example of a pristine tropical peat-draining river within our sample area, and it 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 prebaked at 450°C for 4 h or acid-washed and dried before use. All filters were prewashed 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, the 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 was left unfiltered. The samples were stored in separate 10-L HDPE jerry cans and shipped to Singapore. Incubations were started 7 days after collection. The unfiltered water was then further split into two biodegradation treatments, one of which was amended with nutrients (5 $\mu\text{mol l}^{-1}$ KNO_3 [Sigma-Aldrich, product number 221295] and 1 $\mu\text{mol l}^{-1}$ 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 the DOC and colored 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), three sacrificial replicates were taken per treatment to measure the 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 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 was 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 the background DOC remineralization rate), filtered seawater + filtered Maludam water (as sterile control), and filtered seawater + filtered Maludam water + unfiltered seawater inoculum (to measure the remineralization rate of tDOC). The two treatments with added tDOC received 1.25% of the final volume of Maludam water, which raised the DOC concentration by $\sim 45 \mu\text{mol l}^{-1}$. The two treatments to which the seawater inoculum was added received 5% of the final volume of unfiltered seawater. After taking the initial DOC and CDOM samples from each treatment, the remaining water was equally split into 250-mL Duran bottles per treatment to give three 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. The mixing ratios for all incubations are shown in Table S1.

Oxygen measurements were not taken during the incubations. However, a headspace of air of at least 40% of the total bottle volume was left in all the incubation flasks. We estimate that this provided an O_2 :DOC molar ratio of at least 1.9 even in the treatment with the highest DOC concentration, which contained $\sim 470 \mu\text{mol DOC}$ in 150 ml sample volume while the headspace of 100 ml would have contained $\sim 840 \mu\text{mol O}_2$. While the rate of O_2 diffusion from the headspace into the sample could have slowed the rate of biodegradation, carbon decomposition was not expected to be limited by O_2 over the duration of our experiments.

2.4. Chemical Analyses

2.4.1. DOC Analysis

DOC samples (30 mL) for all experiments were syringe-filtered ($0.22 \mu\text{m}$ Acrodisc, prerinsed with 180 ml Elga water and flushed with the sample before collection) into EPA vials, acidified with $100 \mu\text{L}$ 50% H_2SO_4 , and stored at 4°C . The samples were analyzed within one month on a Shimadzu TOC-L system with a high-salt kit, with an injection volume of $150 \mu\text{l}$, a sparge time of 5 min, and 5–7 replicate injections to ensure a coefficient of variance $\leq 2\%$. Calibration and drift monitoring were performed using potassium hydrogen phthalate standards. The analytical accuracy was determined using deep-sea Certified Reference Material from the University of Miami, USA ($42\text{--}45 \mu\text{mol l}^{-1}$ DOC), which returned a long-term mean and standard deviation of $48 \pm 3.9 \mu\text{mol l}^{-1}$.

2.4.2. CDOM Analysis

Samples for CDOM were syringe-filtered ($0.22 \mu\text{m}$ Acrodisc, prerinsed with 180 ml Elga water, and flushed with the sample before collection) into EPA vials, which were then either run immediately or stored at 4°C and then warmed to room temperature before analysis. The absorbance was measured at 230–900 nm at 1-nm resolution against an Elga water reference on a Thermo Evolution 300 dual-beam spectrophotometer, using either a 10 or 0.2-cm quartz cuvette. Instrument performance was checked prior to the analysis, according to Mitchell et al., (2000).

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

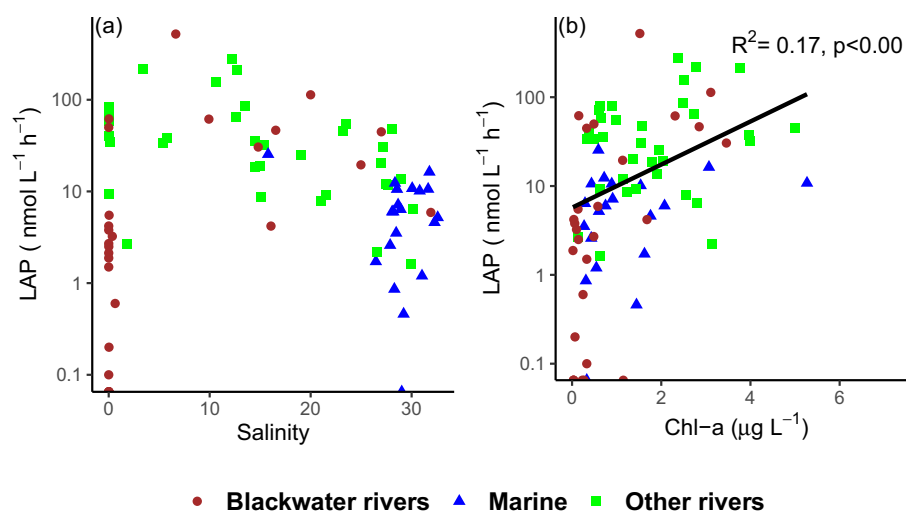


Figure 2. Leucine-aminopeptidase (LAP) of blackwater rivers (Maludam, Sebuyau, Samunsam, and Simunjan, all $>500 \mu\text{M}$ DOC), other rivers (Rajang, Sematan and Lundu, all $<500 \mu\text{M}$ DOC) and marine waters from Sarawak, Borneo during surveys in March and September 2017. (b) Linear regression of LAP (this study) and chlorophyll-*a* concentrations as reported by Martin et al. (2018). All data are presented as the control-corrected LAP activities.

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 & Sergio, 2020). All concentrations and activity rates are quoted as mean ± 1 standard deviation unless otherwise stated.

3. Results

3.1. 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 ($57 \pm 26.7 \text{ nmol l}^{-1} \text{ h}^{-1}$, $n = 8$, DOC concentration $100\text{--}400 \mu\text{mol l}^{-1}$, $n = 8$, DOC concentration $1,100\text{--}4,400 \mu\text{mol l}^{-1}$), except for two outliers (59.9 and $49.9 \text{ nmol l}^{-1} \text{ h}^{-1}$) which were collected next to the villages in the Maludam and Sebuyau blackwater rivers. LAP activities reached the highest values in the estuaries at salinities between 3 and 12 ($159 \pm 158 \text{ nmol l}^{-1} \text{ h}^{-1}$, $n = 10$), then decreased with increasing salinity, and only averaged $11.1 \pm 11.5 \text{ nmol l}^{-1} \text{ h}^{-1}$ ($n = 30$) at stations with salinity >26 . Across the entire data set, there was a statistically significant relationship between the LAP activity and chlorophyll-*a* concentration ($R^2 = 0.17$, $p < 0.001$, Figure 2b; chlorophyll-*a* data taken from Martin et al., 2018).

3.2. POx Activity and Autoxidation 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 $140 \mu\text{mol l}^{-1} \text{ h}^{-1}$ (Figure 3a). However, our autoclaved controls showed essentially the same substrate oxidation rates as the unautoclaved samples, which means that the L-DOPA oxidation rate in our assays was due to nonenzymatic oxidation of the substrate (Figure 3b).

To verify that the L-DOPA is oxidized nonenzymatically 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 Gyreshowed similarly high L-DOPA oxidation rates in untreated water as in 3-kDa ultrafiltered and autoclaved controls (Figures 3c–3f). The small decrease in oxidation rate observed in the 3 kDa ultrafiltered controls from the Johor Strait (Figure 3c) was most likely due to the slight dilution with residual Elga water used to rinse the filters. Notably, the L-DOPA oxidation rates were very

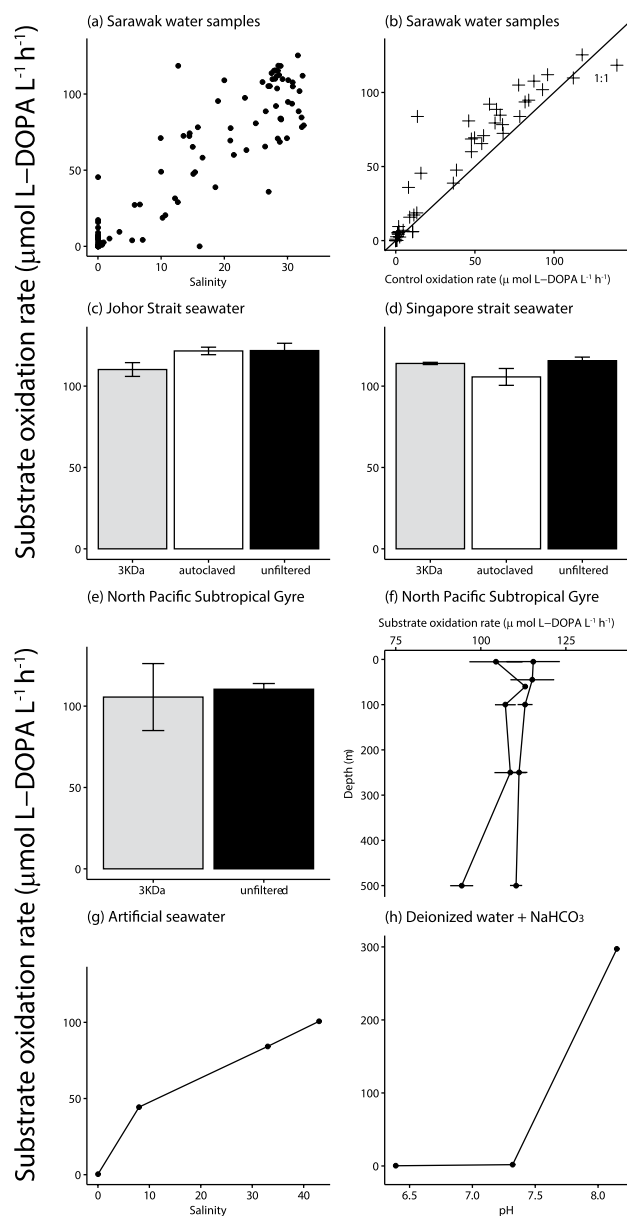


Figure 3. Phenol oxidase assay substrate oxidation rate (L-3,4-dihydroxyphenylalanine oxidation rate) in: (a, b) Water samples (substrate oxidation rate), autoclaved controls (control oxidation rate) collected from Sarawak, Borneo during surveys in March and September 2017. Comparisons of unfiltered water, autoclaved samples and 3 KDa ultrafiltration of water from the (c) Johor Strait (Singapore, salinity 31), (d) Singapore Strait (Singapore, salinity 31), (e, f) two depth profiles (0–500 m) 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–e) three replicate measurements of one water sample; (f) eight individual water samples from differing depths.

similar for all Sarawak samples with salinity >30 ; all the samples were from Singapore and Station ALOHA from the surface down to 500 m (all between $100\text{--}140 \mu\text{mol l}^{-1} \text{h}^{-1}$). Additional assays with solutions of artificial sea salts and sodium bicarbonate in Elga water showed that the L-DOPA oxidation rate increases approximately linearly with salinity up to $\sim 100 \mu\text{mol L-DOPA l}^{-1} \text{h}^{-1}$ (salinity 44) and that substrate oxidation is observed above $\text{pH} \sim 7$ (Figures 3g and 3h), similar to the trend observed in our samples from Sarawak.

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 the Maludam River water with and without nutrient addition showed minor DOC variation in treatment means by $<90 \mu\text{mol l}^{-1}$, corresponding to $<3.5\%$ (Figure 4a). The data could not be fit with exponential decay curves. Linear regressions of mean DOC over time were nonsignificant in the filtered controls, but they showed small significant decreases in unfiltered and unfiltered + nutrients river water treatments (Figure 4a). These corresponded to the total tDOC decreases over 56 days by $0\%\text{--}4.4\%$ in the untreated river water ($61 \pm 73 \mu\text{mol l}^{-1}$ DOC) and $2.5\%\text{--}6\%$ in the river water with added nutrients ($131 \pm 53 \mu\text{mol l}^{-1}$ DOC) (Figure 4a), calculated as the mean change in DOC after 56 days \pm the combined standard deviation (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 (Figures 4b–4e).

Incubations in which the tDOC-rich Maludam River water was mixed with coastal seawater showed a small decrease in DOC in both treatments, by $6.8 \pm 2.5 \mu\text{mol l}^{-1}$ (8%) in the unamended seawater and by $7 \pm 3.0 \mu\text{mol l}^{-1}$ (5.5%) in the seawater treatment with the added Maludam River water (Figure 4f). However, neither the linear nor exponential regressions of DOC over time were significant. This indicates that the added Maludam tDOC was not being remineralized. A small, significant linear decrease in a_{350} was observed in both the unfiltered + tDOM and filtered control + tDOM treatments, amounting to $0.413 \pm 0.02 \text{ m}^{-1}$ (11% decrease) and $0.253 \pm 0.018 \text{ m}^{-1}$ (7% decrease), respectively (Figure 4g). A much smaller (but significant) decrease in a_{350} was observed in the unfiltered seawater without the added Maludam River water ($0.04 \pm 0.01 \text{ m}^{-1}$), with no significant change in the filtered seawater control. Little change was observed in slope parameters during incubations, few of these changes were statistically significant (Figure 4, Table S2).

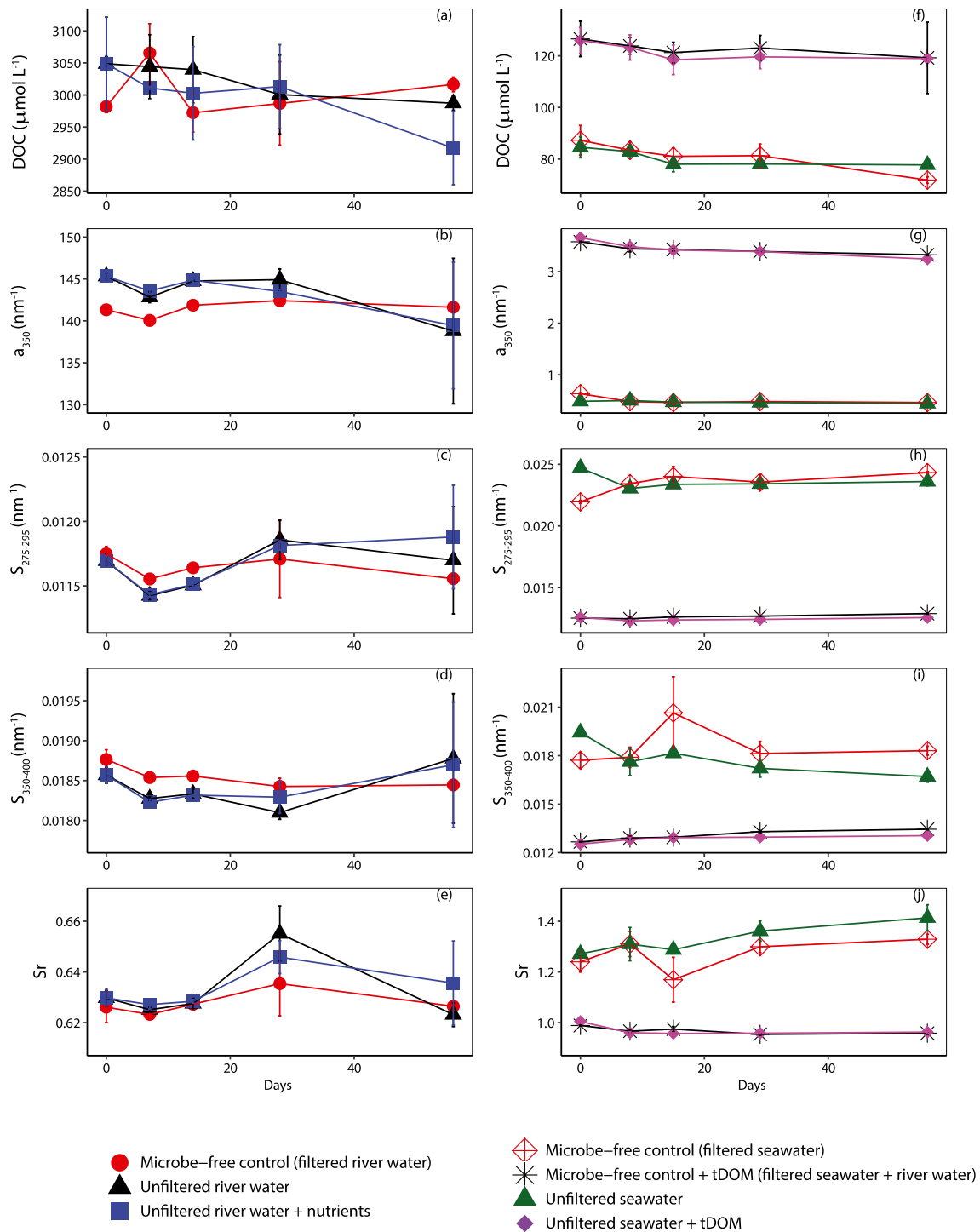


Figure 4. Biodegradation incubation experiments of terrigenous dissolved organic carbon rich river water collected from the peat draining Maludam river, Sarawak, Borneo. Maludam river water, with and without the addition of nutrients (Left column, a–e), and seawater with and without the addition of Maludam river water (right column, f–j). Results shown are dissolved organic carbon, absorption coefficient at 350 nm (a_{350}), spectral slopes $S_{275-295}$ nm and $S_{350-400}$ 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 full natural microbial community. Nutrient additions consisted of $5 \mu\text{mol L}^{-1}$ nitrate and $1 \mu\text{mol L}^{-1}$ phosphate. All data are mean ± 1 standard deviation of three replicate incubations. Significant regressions are detailed in Table S2.

4. Discussion

Our results overall demonstrate that (a) phenol oxidase activity assays in aquatic samples are problematic because of the pH-dependent auto-oxidation rate of L-DOPA and (b) that peatland tDOC in Southeast Asia is relatively refractory to microbial degradation. We discuss both of these points in turn below, starting with the results for both of the enzyme activity assay measurements.

4.1. 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 and highest in the estuaries (Figure 2) and the Rajang River. 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 $1 \mu\text{g l}^{-1}$ and mostly $<2 \mu\text{g l}^{-1}$; Martin et al., 2018), and LAP activities were consistently low at high salinities (Figure 2). 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 data set indicates instead, that LAP activity was most likely related to the degradation of autochthonous organic matter, and therefore it does not imply that microbial processing of tDOC was taking place (Figure 2b). This is consistent with the interpretation by Sieczko and 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

Overall, our POx results clearly suggest that this assay did not return data that reflect microbial tDOC remineralization across the region but rather, autooxidation of the phenol oxidase assay substrate L-DOPA related to changes in water pH and salinity. The trend in the substrate oxidation rate with salinity could be interpreted as evidence in support of our 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 (Figures 3a and 3b), 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, 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 (Figure 3). 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 (Figures 3g and 3h). Significant autooxidation of L-DOPA at alkaline pH was also reported in two previous studies (Tahvanainen & Haraguchi, 2013; Zhou et al., 2012). Autoclaving causes significant changes in the 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 unlikely to reflect real enzymatic

activity and is more likely the result of small changes in the autoxidation rate between the samples and controls.

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

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

We would therefore recommend that POx activities also be measured in regions where rapid rates of tDOC biodegradation have been observed. It is possible that in such environments, real microbial POx activity could significantly overwhelm the autoxidation rate, in which case L-DOPA-based POx assays could prove to be valuable, after all, for tracing tDOC biodegradation in aquatic environments. When using ultrafiltration to overcome this, we found little or no difference between substrate oxidation rates in filtered and unfiltered water if enough sample is collected to avoid any dilution during the filtration process (Figures 3c–3e). We therefore recommend that ultrafiltration be used to generate enzyme-free controls without altering the water chemistry in a way that can affect the L-DOPA autoxidation rate.

4.3. Lack of Peatland tDOC Biodegradation in Sarawak

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

Porewater DOC in Southeast Asian peatlands likely undergoes significant processing by the soil microbial community, resulting in DOC that has a very young radiocarbon age (~10 years) but already shows chemical and optical characteristics of being highly degraded by the time it enters rivers (Gandois et al., 2014; Müller et al., 2015; Zhou et al., 2019). Such predegradation 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 CO₂ 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 of Sumatra also concluded that nearly 80% of the total carbon leached from soils in Indonesia is emitted as CO₂ in the rivers, estuaries, and coastal ocean, which was attributed to respiration (Wit et al., 2018). This apparent discrepancy between our results and CO₂ outgassing measurements could potentially be explained by the lateral transport of CO₂ and CH₄ 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 around 74% of tDOC from the Maludam River was lost during experimental irradiation in a solar simulator (Zhou et al., 2021). 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 the unfiltered water from a Sumatran blackwater river to natural sunlight and reported that ~27% of DOC was labile to combined photodegradation and biodegradation, mostly within 8 days (Rixen et al., 2008). However, the relative importance of photodegradation versus biodegradation was not determined, and it is possible that these results primarily reflect photo-chemical remineralization. In addition, Zhou et al. (2021) reported that coastal seawater containing tDOC input from Sumatra showed less than 6% loss of DOC over a 109-day biodegradation experiment; although this study could not determine whether the DOC loss represented microbial remineralization of marine-produced DOC or of peatland tDOC.

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

5. Conclusions

We found that the commonly used phenol oxidase enzyme assay substrate L-DOPA undergoes significant autoxidation at typical environmental pH values in estuaries and coastal seas, which could significantly confound results unless adequate care is taken to prepare appropriate enzyme-free controls. Furthermore, our data suggest that autoclaved samples are not appropriate controls because autoclaving can change the sample chemistry; we therefore recommend that ultrafiltration as a necessary procedure. We conclude that there was no measurable POx activity across our study region in Sarawak but recommend that this assay also be tested (with ultrafiltered controls) in aquatic environments where substantial tDOC biodegradation is anticipated.

LAP data indicated that microbial heterotrophic activity follows the expected trends with low activity in blackwater rivers and the highest activities in estuaries. However, this most likely reflects the heterotrophic processing of autochthonous organic matter, not tDOC biodegradation. Our incubation experiments further demonstrated that tDOC from one of the few pristine peat-draining rivers in Southeast Asia was largely refractory to biodegradation over eight weeks at in situ temperatures, even after adding nutrients or diluting the DOC with coastal seawater. We surmise that the remineralization of Southeast Asian peatland DOC in the aquatic environment is likely dependent on photodegradation.

Data Availability Statement

Nichols Robert Scott; Patrick Martin, 2021, "Related data for: Low biodegradability of dissolved organic matter from Southeast Asian peat-draining rivers (Nichols & Martin, 2021)," <https://doi.org/10.21979/N9/ORIGHW>

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