

Sources and Distribution of *n*-Alkanes in Borneo Peat Core, Sarawak, Malaysia

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This work reported the *n*-alkanes contents in a 10 m peat core collected at Timbarap, Sarawak. Different biomarker proxies were used to understand the predominance of sources present in the tropical peatland of Borneo. The total concentrations of *n*-alkanes in selected depths of Timbarap peat core ranged between 0.01 and 12.60 $\mu\text{g/g}$ with an average of 1.58 $\mu\text{g/g}$. The presence of strong odd-to-even predominance from higher molecular weight *n*-alkanes homologues with high values of carbon preference index (CPI) provide clear evidences of the terrestrial plant wax assemblages. Increment of low-molecular-weight *n*-alkanes provide an indicator of biogenic-aquatic organic materials. The ratios of isoprenoids (pristane and phytane) have shown that the peat core was under anaerobic and reducing-depositional conditions, which could result the predominance source that alternated between aquatic-biogenic and terrestrial-derived materials.

Keywords: Tropical peatland, GC-MS, Peat lithology, Deposition, Terrestrial.

INTRODUCTION

Tropical peatland has contributed significantly as a carbon storage reservoir for the past years, which supplies at least 88.6% (528,000 megatonnes) of carbon pool globally. Southeast Asia tropical peatland covers ~25 Mha, providing at least 50 to 60% of global carbon source [1]. With distinct features such as thick underlying deposit, rapid carbon and peat accumulation, these make tropical peatland capable of providing a higher percentage of carbon storage capacity pools compared to other temperate peatlands. Being a dynamic ecosystem, tropical peatland is influenced by local changes of hydrology and climate variability which can affect the carbon exchange between peat and environment [2-4].

n-Alkane is a straight-chain hydrocarbon and can be found in synthesis of bacteria, plants, algae, phytoplankton in marine environment [5], modern and fossil leaves in soils and paleosols [6]. The establishment of *n*-alkane as a proxy has earned recognition due to its stability towards degradation, fossilization [7] and resistance towards decomposition and diagenesis [8]. *n*-Alkane is widely used in source determination and deci-

phering environmental and climatic condition in the past years at various Borneo peatland regions [9-11]. This is the reason why *n*-alkane provides an important understanding in the determination of environmental and depositional changes to infer the oxic-anoxic condition and behaviour in Borneo peatland.

Previous study has shown that Borneo tropical region is commonly exposed to intensive environmental changes such as monsoonal variability and seasonal precipitation [12]. The formation of peat in the peatland is dependable on these factors with the aid from biotic and abiotic components [13]. Consequently, studies related to the understanding of environmental changes have increased throughout the years [14-16]. In Malaysia, the research on peat core still limited mostly focused on peatland degradation [17] and community structure [18]. Hence, to understand the source preference of peatland by using geolipid biomarker proxies information to decipher the preference required in our tropical ecosystem regions is aimed. This study was carried out by determining the concentration of *n*-alkanes in the peat core of Timbarap, Sarawak, Malaysia in order to elucidate the source predominance of tropical peatland using different molecular proxies.

EXPERIMENTAL

Study area: The climates in Borneo Island are influenced by the tropical Pacific and Indian Oceans, where strong winter wind prevails during boreal winter driving higher Asian monsoon derived precipitation occurrences [19]. Sarawak experiences average annual precipitation ranging between 3,300 mm and 4,600 mm with temperature ranging between 23 °C and 32 °C and humidity exceeding 68% throughout the year [20]. The peatland deposits in Sarawak, Northwest of Borneo Island are composed of a large basin swamps and small inner valleys, which are formed in proximity to the coastal areas [21]. Evidences have shown that the radiocarbon-dated (^{14}C) sediments collected from Baram delta (northern part of Sarawak) was derived from the inland margins of peat swamps about 5400 years ago [22].

The study area is located at Timbarap site (04°03'01.47"N, 114°15'02.45"E) near Marudi town under Miri division, Sarawak, situated in the Northwest of Borneo at an altitude of 18 m above sea level (Fig. 1a). The study area is situated at the summit of Baram peat dome, which formed by paludal deposits that composed of ombrogenic peat, concentrated at the central part of the dome [23]. These ombrogenic peats are made of disintegrated and broken leaves and wooden branches fragments [24–27]. The formation of peat dome is controlled by factors such as hydrology and nutrient content, which can be influence the peat thickness and vegetation community from marginal towards central of the peat dome [28]. The study site is surrounded with

low-level oligotropic vegetation [25] and has been converted to oil palm plantation in recent years.

Sampling and sample preservation: A 10 m peat core (Fig. 1b) with 5 cm internal diameter was collected at Timbarap, from an undisturbed and isolated peat dome adjacent to the oil palm plantation using a Russian core sampler. The peat colouration was comprised of weathered peat (0–50 cm), brown (50–700 cm), dark brown (700–900 cm), dark grey mud (950–1000 cm) and a core gap is observed in 900–950 cm. Here, the peat lithology of Timbarap peat core collected was found resembled from Kota Samarahan-Asajaya, which composed mainly of undegraded roots and wood fragments found throughout the entire core, with the exception for the core gap and dark grey mud sections [11]. Based on the core colouration, a total of 52 samples were selected to represent each lithology covering the whole the peat core. The core collected was sliced into 5 cm intervals, wrapped in polythene film and transported to the laboratory. Each sample was kept into pre-cleaned aluminium foil and placed in –80 °C freezer before proceeded with chemical analysis.

Extraction and fractionation of *n*-alkane: The method of extraction and separation was adopted from Tolosa *et al.* [29] with some modifications. A deuterated standard ($^2\text{H}_{50}$) tetracosane was spiked into a blank sample for recovery assessment before extraction. Approximately 1 g of freeze-dried and homogenised peat sample was extracted three times using ultrasonic agitation technique with 100 mL dichloromethane/methanol (3:1, v/v) mixture for 45 min. The extraction was

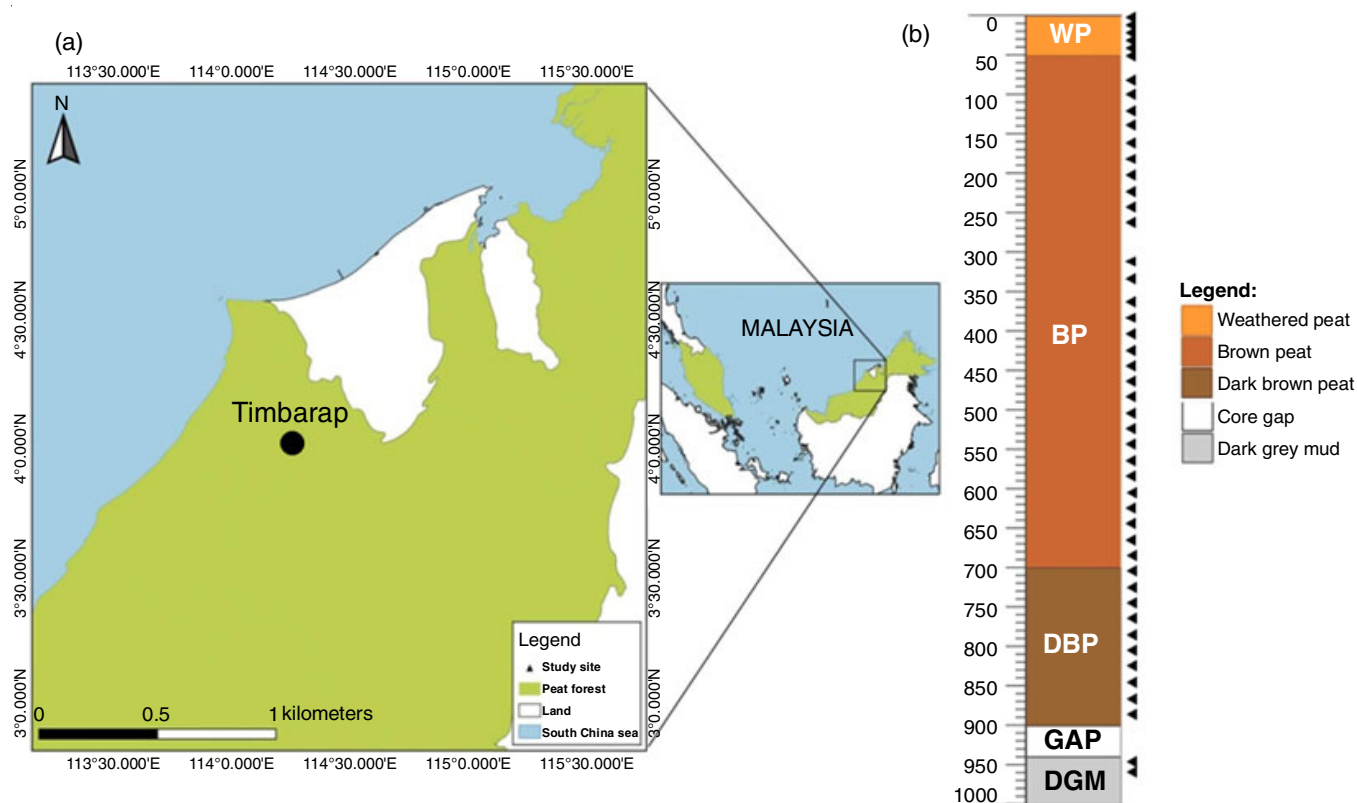


Fig. 1. (a) Map location of study site, Timbarap, Sarawak, Malaysia; (b) the lithology colouration of Timbarap peat core are as follow: WP = weathered peat, BP = brown peat, DBP = dark brown peat, GAP = core gap, DGM = dark grey mud, with black triangles showing the depths of selected sample sections chosen for analysis

again repeated twice, and the pooled extracts were then evaporated using a rotary evaporator to approximately 3 mL. The total extract underwent saponification using 1 mL of 6% KOH in methanol-water (80:20) and 1 mL of mili-Q water, heated in a water bath at 80 °C for 60 min. *n*-Hexane was added into the saponified mixture three times in total to collect the neutral lipid layer component from the non-saponifiable lipid component. The combined neutral lipid extract was concentrated under a gentle stream of nitrogen prior to fractionation. The concentrated lipid extract was fractionated using 5% deactivated slurry silica gel (40-60 µm mesh) column and the *n*-alkane fraction was eluted with 8 mL of *n*-hexane (1:1, v/v) from the column chromatography.

Identification and quantification: The identification and quantification of *n*-alkanes were carried out using Shimadzu-QP2010 ultra gas chromatograph-mass spectrometer (GC-MS) fitted with DB-5 fused silica capillary column (30 m length × 0.32 mm i.d.) with a film thickness of 0.25 µm. The MS was operated using a full scan in electron impact mode (70 eV) with mass range between 45 and 600 Da at a scan speed of 1.25 s. Helium with 99.9% purity was used as a carrier gas. Using splitless mode, the temperature of injector, ion source and interface was set to 300, 200 and 300 °C, respectively. The column temperature was programmed as follows: hold for 1 min at 50 °C, followed by a temperature ramp of 50 to 300 °C at 5 °C/min and then maintained at 300 °C for 20 min, which resulted a total run time of 74 min. The confirmation of *n*-alkane peaks was based on the comparison of retention time with injected external standards that comprised of *n*-decane (nC_{12}) to *n*-hexatriacosane (nC_{36}). The average recovery result obtained from the step-by-step procedures of the spiked standard was 81.12%, which is within the acceptable range for *n*-alkane analysis recovery assessment [30] and the recovery corrections were made for the calculated concentration. The contaminant (*i.e.* nC_{10} and nC_{11}) detected in blanks were deducted from the sample concentration.

RESULTS AND DISCUSSION

Distribution of total *n*-alkanes (TNA): In this study, the total concentrations of *n*-alkanes (TNA) in the analyzed samples (Fig. 2) with carbon number between nC_{12} and nC_{38} have values

varied between 0.01 and 12.60 µg/g with an average value of 1.58 µg/g. However, most of TNA values were low with a range of 0.01-5.50 µg/g (mean: 0.80 µg/g; median 0.28 µg/g). Exceptions of higher TNA concentrations (> 5.50 µg/g) were observed at brown (55-60 cm, 341-346 cm) and dark brown (701-706 cm, 821-826 cm) sections, which could be due to high organic materials at the depth. Nevertheless, the high concentrations recorded in four depths of TNA profile (Fig. 2) were also dominated with nC_{12-33} (55-60 cm, 341-346 cm, 821-826 cm) and nC_{12-37} (701-706 cm) ranges. Overall, *n*-alkanes homologous demonstrated either a bimodal pattern (*e.g.* shorter-chain predominance in Fig. 3a and longer-chain predominance in Fig. 3b) or unimodal pattern (*e.g.* longer-chain predominance in Fig. 3c). This observation could suggest a possible contribution of different sources in this study.

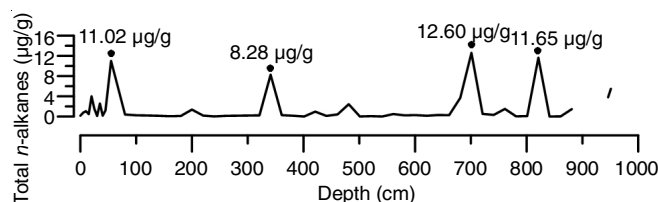


Fig. 2. Distribution profile of total *n*-alkanes (TNA) concentrations in selected depths of Timbarap peat core

Sources of *n*-alkane: The studied core received *n*-alkanes from higher vascular plant inputs [31-33], which displayed an odd-to-even nC_{12-38} carbon-chain preference. The C_{max} was maximized at nC_{29} , nC_{31} (weathered peat), nC_{17} , nC_{27} , nC_{29} , nC_{31} (brown peat), nC_{27} , nC_{31} (dark brown peat) and nC_{31} (dark grey mud). The four marked depths of TNA values (Fig. 4) also maximized at nC_{31} peak. Thus, the overall prominence of C_{max} with depth profile showed that nC_{31} is the major peak occurred in Timbarap core.

The C_{max} at nC_{29} or nC_{31} are commonly reported in woody or ligneous-type vegetation such as mangrove plants like *Rhizophora apiculata*, *Avicennia alba*, *Sonneratia alba* [34-36] and grass or herbaceous-type vegetation such as marsh grass like *Spartina alterniflora* [37,38]. Land vascular plants in warm tropical climate region are adapted to biosynthesise longer-carbon chain compounds for waxy coating to minimize the water loss from leaf cuticle during transpiration [39].

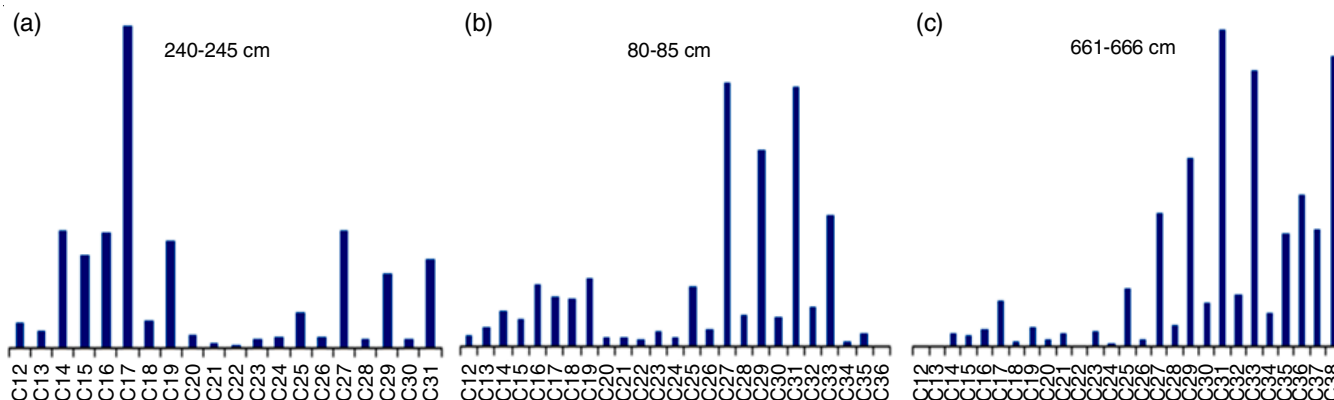


Fig. 3. Examples of *n*-alkanes distribution pattern of biogenic-dominated: (a) bimodal peaks with marine/bacterial-dominated (b) bimodal peaks of terrestrial dominated and (c) unimodal peaks of terrestrial-dominated from Timbarap peat core

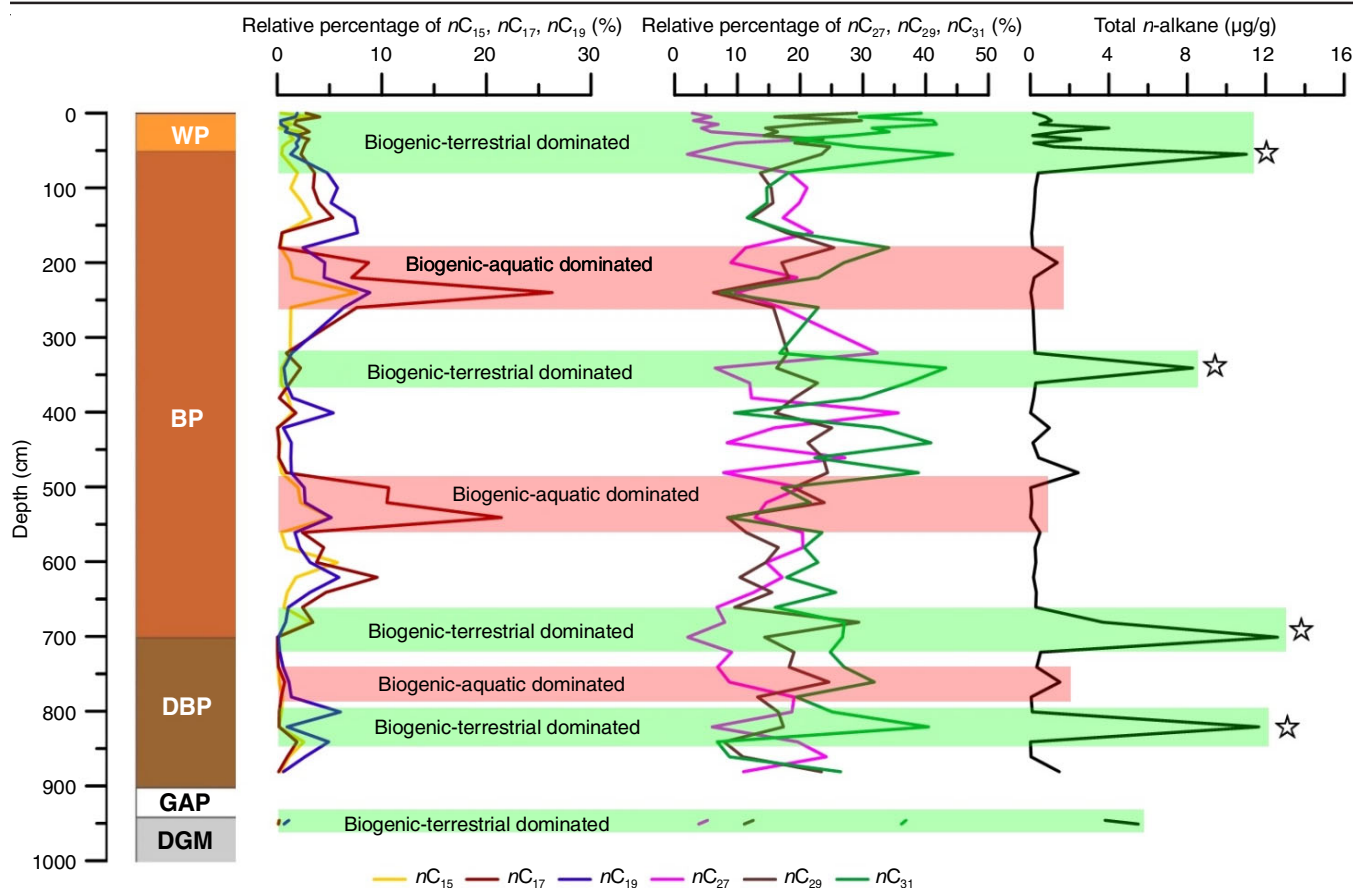


Fig. 4. Depth profiles of relative percentages of nC_{15} , nC_{17} , nC_{19} , nC_{27} , nC_{29} and nC_{31} to the total of n -alkanes

On the other hand, the C_{max} at nC_{15} , nC_{17} or nC_{19} is associated to aquatic source-dominance for floating organisms such as algae, plankton, fungi and aquatic bacteria [40,41], while nC_{16} and nC_{18} could be linked to microbial-source input [42-44]. Here, prominent peaks of nC_{17} (at depths of 240-245 cm and 541-546 cm) could point to the participation of biogenic-aquatic materials from microbial or algal inputs. The microbial diversity in tropical peat forest has been reported in Malaysia [18,45], Brunei [46] and Thailand [47]. Their results revealed that Proteobacteria and Acidobacteria are among the most dominant phyla in tropical peat regions. Hence, nC_{17} n -alkanes could indicate the inputs of microbial setting during the formation of peat environment [48,49]. To decipher these source variations, the biogenic aquatic-dominated (nC_{15} , nC_{17} and nC_{19}) and biogenic terrestrial-dominated (nC_{27} , nC_{29} and nC_{31}) proxies were plotted against the depth profile (Fig. 4). The distribution of nC_{27} and nC_{31} profiles showed opposite trend from each other while nC_{29} seemed to behave neutrally in comparison to nC_{27} and nC_{31} .

Four remarkable periods were recognized with the increment of TNA concentrations and also higher plant n -alkanes homologous, especially with the abundance of nC_{31} . These findings provide an insight of n -alkanes contribution from higher vascular plants and aquatic sources, and the presence of these peaks dominated at nC_{31} or nC_{17} peak at respective depth could represent the alternating sources from higher plant to aquatic inputs.

Distribution of biomarker proxies: Table-1 shows the formula of selected n -alkane proxies as source indicators. These ratios were calculated based on the measured concentrations determined in each analyzed samples and the results were shown in Fig. 5.

Average chain length of the n -alkanes (ACL) measures the weighted average number of carbon-based on the abundance of long-chain n -alkanes nC_{27} , nC_{29} , nC_{31} [52], to relate the plant growth factors which could be experienced by n -alkane precursors [67]. Other than that ACL also reflect the variability of vegetation types [68-70] such as herbaceous (non-woody) and ligneous (woody) plants [71]. Lipid distributions in woody and non-woody plants were varied [72] whereby modern plants such as grasses and woody plants able to be distinguished due to difference in climate variability [6]. Smaller ACL ratio can be seen when the formation of organic matter is dominated by ligneous plants and while larger ACL ratio is when the organic matter formation is dominated by herbaceous plants [69]. In this study, ACL varied between 28.27 and 31.37 averaging at 29.90 (std_{dev} = 0.68). The highest ACL ratio was recorded at the deepest layer of the core in the dark grey mud section (951-956 cm). The overall values of the analyzed samples showed a constant profile with a median value of 30.07. Similar ACL range was reported in the Peruvian lowland tropical rainforest (range: 27.20-32.60) [73] and sediments of Ashtamudi estuary (range: 28.50-30.5) [74]. The ACL ratio could infer from the perspective of temperature, where higher ACL indicative of

TABLE-1
THE LIST OF *n*-ALKANES DIAGNOSTIC PROXIES THAT WAS USED TO
EVALUATE THE SOURCE PREDOMINANCE OF TIMBARAP PEAT CORE

Proxy	Formula	Ref.
Carbon number maximum (C_{max})	Number of carbon with the highest concentration	[50-52]
Average chain length (ACL)	$ACL = [27(nC_{27}) + 29(nC_{29}) + 31(nC_{31}) + 33(nC_{33})] / (nC_{27} + nC_{29} + nC_{31} + nC_{33})$	[52]
Carbon preference index (CPI)	$CPI_{13-37} = 0.5[(\text{odd } \Sigma nC_{13-37} / \text{even } \Sigma nC_{14-38}) + (\text{odd } \Sigma nC_{13-37} / \text{even } \Sigma nC_{12-36})]$ $CPI_{13-21} = 0.5[(\text{odd } \Sigma nC_{13-21} / \text{even } \Sigma nC_{14-22}) + (\text{odd } \Sigma nC_{13-21} / \text{even } \Sigma nC_{12-20})]$ $CPI_{23-37} = 0.5[(\text{odd } \Sigma nC_{23-37} / \text{even } \Sigma nC_{24-38}) + (\text{odd } \Sigma nC_{23-37} / \text{even } \Sigma nC_{22-36})]$	[53-56]
Ratio of isoprenoids (Pr/Ph)	Pr/Ph = [Pristane]/[Phytane]	[57]
Ratio of sub emergent/aquatic and emergent/terrestrial vegetation (P_{aq})	$P_{aq} = [nC_{23} + nC_{25}] / [nC_{23} + nC_{25} + nC_{29} + nC_{31}]$	[58,59]
Ratio of low-molecular weight and high-molecular weight (LMW/HMW)	$\Sigma LMW / \Sigma HMW = \Sigma nC_{13-20} / \Sigma nC_{21-37}$	[60]
Percentage of terrestrial input in <i>n</i> -alkanes (%Terr Alk)	$\% \text{Terr Alk} = [\Sigma nC_{27} + nC_{29} + nC_{31} + nC_{33}] / [\Sigma \text{Alk}]$	[61,62]
Abundance of terrestrial waxy plant (Wax C_n)	$Wax C_n = C_n - 0.5(C_{n+1} + C_{n-1})$, where $n = 27, 29, 31, 33$ and negative values are considered as zero	[63,64]
Ratio of isoprenoids with <i>n</i> -alkanes	Pr/ nC_{17} = [Pristane]/[nC_{17}]; Ph/ nC_{18} = [Phytane]/[nC_{18}]	[65,66]

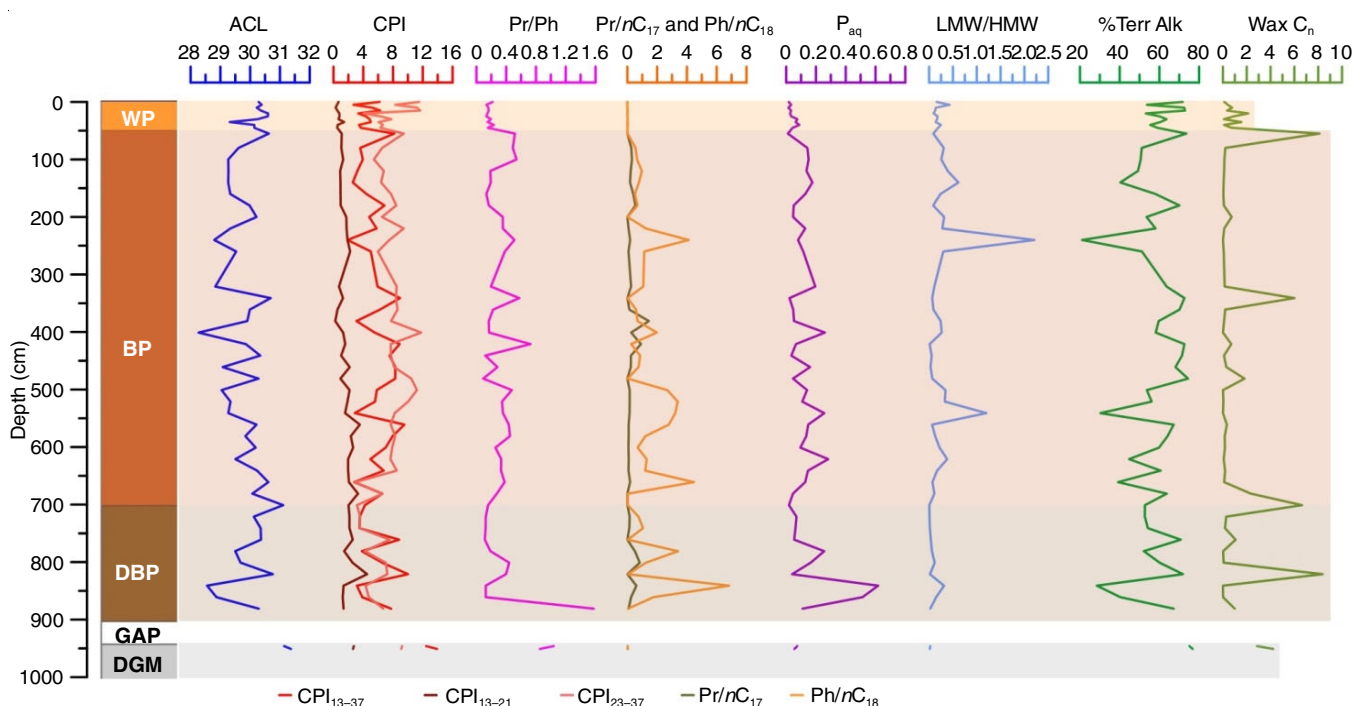


Fig. 5. Depth profile of selected diagnostic proxies used in evaluating source predominance in Timbarap peat core. The code names for the core lithology are as follow: WP = weathered peat, BP = brown peat, DBP = dark brown peat, GAP = core gap, DGM = dark grey mud

warmer condition or *vice-versa*. Thus, the high and narrow ACL ratios indicated the prominence of longer-chain *n*-alkanes in Borneo peatland under warmer climate [52,75].

Carbon preference index (CPI) is used to evaluate the possible sources of organic matter based on their odd-even chain length characteristics [53,56]. The CPI for *n*-alkanes of higher land plants have an odd-numbered predominance, with ratio > 5 [31,76], whereas microbial and petrogenic inputs have no odd/even carbon number preference with ratio approaching to 1 [77]. In this study, the CPI_{13-37} of the core showed values ranged from 1.89 to 13.92 (avg. = 5.76), indicating *n*-alkanes derived mostly from leaf wax of higher plants. Since CPI of short- and long-chain has different odd-even predominance of *n*-alkanes,

CPI_{13-21} (short-chain) and CPI_{23-37} (long-chain) were calculated, whereby CPI_{13-21} showed range between 0.24 and 4.52 averaging at 1.57 (median = 1.39) while long-chain CPI_{23-37} showed range between 2.91 and 11.78 averaging at 7.47 (median = 7.69). The CPI_{13-21} exhibited oppositely with CPI_{23-37} profile, whereby decrease CPI_{13-21} values and increase CPI_{23-37} values as going down the core (Fig. 5). The shifting of CPI profile towards longer chain homologues indicating enrichment of organic matter made up of higher terrestrial plants. Study conducted in Moloka'i tropical island also showed the CPI core ranged between 3 and 19 [78], which revealed the predominance of biogenic-terrestrial origin. In contrast, the CPI_{13-21} of this core showed more than 1 coherently with nC_{17} occurrence could also accounted

for inputs mixture from biogenic-aquatic with higher terrestrial plants. The similarity and slight fluctuation showed between ACL and CPI profiles also demonstrated environmentally stable condition supported by natural organic matter input.

Pristane (C_{19}) and phytane (C_{20}) are isoprenoids produced through hydrolysis of phytols in chlorophylls [79], where oxidation-decarboxylation and dehydration-reduction form pristane and phytane as their by-products [57]. The Pr/Ph ratio is useful in deciphering changes of redox condition in the depositional environment [80]. They are also useful to discriminate autochthonous and allochthonous origins [81] and the nature of contributing organic matter reflected in an environment [61,82]. The depositional conditions can be interpreted when Pr/Ph < 1 implying anoxic environment; ~1 indicating interchange of oxic-anoxic environment; and >1 showing oxic environment [79,80,83]. The Pr/Ph > 3.0 indicates terrigenous plant input deposited under oxic to suboxic condition, while Pr/Ph < 0.8 indicates saline to hypersaline condition associated with evaporate and carbonate deposition [7]. The isoprenoids core, with Pr/Ph ratio ranged between 0.09 and 1.58, indicating robust anoxic, mild saline depositional environment. Most Pr/Ph < 1 observed in the peat core, but an abrupt increased of Pr/Ph value of 1.58 at 881-886 cm was recorded at the end of DBP section, showing a slight suboxic condition as depth goes deeper. Zulkifley *et al.* [11] have been reported that Pr/Ph were about 0.97 in West Sarawak peat core. Similarly, Pr/Ph ranges of 0.88-1.50 were also reported from Winsconsin river, USA [84]. This observation indicated both findings shared a similar characteristic of tropical lowland peat dome under strong anaerobic conditions.

The ratios of Pr/ nC_{17} and Ph/ nC_{18} are used in petroleum correlation studies as these ratios able to relate with thermal maturation when decreasing of these ratios. The depositional condition could be determined when aerobic bacteria degrades the n -alkanes before isoprenoids as the ratio increase [82]. Pr/ nC_{17} < 0.5 shows depositional condition from open water environment; > 1 originates from inland peat swamp environment; intermediate of 0.5-1.0 indicates source rock deposited in an alternating peat swamp and open water conditions [85,86]. Here, the Pr/ nC_{17} and Ph/ nC_{18} recorded median values of 0.09 and 0.67, respectively (Fig. 6). This observation indicates a strong anoxic condition in the Timbarap peatland with both ratios < 1 [87]. Apart from that, this area could experience biodegradation process of substrate during thermal immaturation condition.

Submergent or aquatic *versus* emergent or terrestrial vegetation ratio (P_{aq}) has been developed to understand and distinguish the contributions of n -alkanes in the environments [88,89] and historical water level change [68]. Ficken *et al.* [88] proposed that P_{aq} in range of 0.01-0.23 (< 0.1) indicates for terrestrial-type vegetation; 0.07-0.61 (0.1-0.4) for emergent; and 0.48-0.94 (0.4-1.0) for submerged/floating-type vegetation. Here, P_{aq} in Timbarap peat core ranged from 0.01 to 0.62 with an average of 0.12. Most of the P_{aq} in the core are < 0.1, corresponded to the terrestrial organic matter, except at the depth of 841-846 cm near to the core gap with P_{aq} > 0.6 could indicate emergent/submerged vegetation.

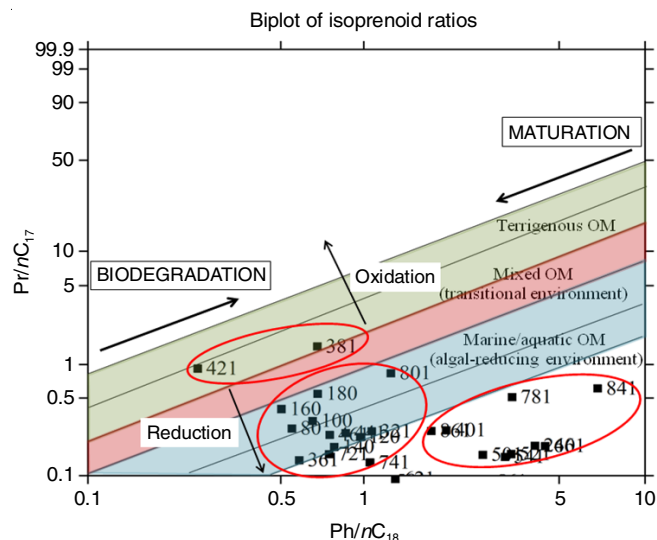


Fig. 6. Relationship plot of Pr/ nC_{17} against Ph/ nC_{18} , showing deposition environment of the organic matter in Timbarap peat core

Low- *versus* high-molecular-weight ratio (LMW/HMW) is utilized to differentiate ratio between the total of lower molecular weight ($\leq nC_{20}$) and higher molecular weight ($\geq nC_{21}$). LMW/HMW ratios nearing to 1 demonstrate for algae, plankton and petroleum inputs, while < 1 displays origin from sedimentary bacteria, marine animals, higher plants, seawater and sediments [90]. In this study, the LMW/HMW values averaged at 0.17, however, tended towards median value of 0.14, showing reliable indicator of higher plant influences. Therefore, this observation justifies that higher molecular weight n -alkanes are the principal biogenic inputs from terrestrial plants. C_{max} at nC_{17} and LMW/HMW > 1 at the depth of 240-245 cm and 541-546 cm are indicative of the presence of biogenic microbial/bacteria inputs, which could play an essential role in the formation of Borneo peat dome.

Percentage of terrestrial n -alkanes (%Terr Alk) and plant waxes (Wax C_n) are used to infer the contribution of n -alkanes from epicuticular higher plant waxes, with high values providing strong evidence of terrestrial n -alkanes origin [91,92]. In this study, %Terr Alk and Wax C_n values approached at 75.96% and 0.17, respectively. High %Terr Alk and Wax C_n were observed at the depths of 55-60 cm (73.61%; 8.11), 341-346 cm (72.63%; 6.01), 701-706 cm (52.70%; 6.64) and 821-826 cm (71.87%; 8.37). The %Terr Alk profile showed an interesting opposite trend with LMW/HMW profile, whereby depths which recorded %Terr Alk < 50% exhibited higher LMW values. Apart from that the Wax C_n profile resembled to TNA profile in Fig. 2, showing a strong influence of higher plant inputs. Therefore, high %Terr Alk and Wax C_n ratios provide strong evidence of biogenic-terrestrial input, consistently with the prominent peaks of nC_{27} , nC_{29} and nC_{30} [93].

Conclusion

The distribution of n -alkanes as a proxy biomarker was successfully used to interpret the predominance source of peat collected from Timbarap tropical peat dome, Northwestern of Borneo. The finding revealed the predominance of n -alkanes from terrestrial higher plants. The waxy coating on the terres-

trial higher plants and their strong hydrophobic and affinity properties, making them less mobilized and biodegradable, contributed to the strong odd-even preference higher molecular weight *n*-alkanes and significantly high CPI ratios. The isoprenoid Pr/Ph ratios have demonstrated the anaerobic conditions of the studied peat. The prominent peaks of *n*C₁₅, *n*C₁₇ and *n*C₁₉ and increase of LMW/HMW ratios at specific depths could be corresponded to the biogenic-aquatic inputs of algal and microbial reworking activities. At present, evidences have suggested that the tropical peatland able to respond towards disturbance dynamically. In-depth study will be carried with measurement of radiocarbon dating to evaluate the deposition change with past climate events in this study area.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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