Assessment of the humification degree of peat soil under sago (*Metroxylon sagu*) cultivation based on Fourier Transform Infrared (FTIR) and Ultraviolet-Visible (UV-Vis) spectroscopic characteristics

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SUMMARY

Sago palm (*Metroxylon sagu*) is a tropical crop that can survive the acidic conditions of peat soil, which is cultivated at large scale in Sarawak (Malaysia). The performance of sago palm on deep peat is variable, and not all specimens are able to grow to maturity and produce a trunk. It is hypothesised that sago growth may be influenced by peat humification because a positive relationship between the fertility of peat soil and its degree of humification has been well reported. This article investigates the humification degree of peat soil used for cultivation of sago palms, as indicated by spectroscopic characteristics. The peat soil adjacent to trunking and non-trunking palms was sampled and compared with exposed uncultivated peat. The results showed that, where largely undecomposed woody material predominated in the underlying peat, degree of humification decreased with increasing depth. Uncultivated peat was more highly humified than cultivated peat because the latter was continuously replenished with new plant matter. On the basis of FTIR spectroscopy, no significant difference was found between cultivated peat sampled adjacent to trunking and non-trunking palms. On the other hand, the UV-Vis and FTIR data suggested lower humification degree in the underlying peat which may have led to inconsistent growth.

KEY WORDS: decomposition, Histosol, Malaysia, non-trunking, Sarawak, trunking

INTRODUCTION

Sago palm is a tropical crop that can survive in the low-pH and waterlogged environment of peat. In Sarawak there is a total of 1.7 million hectares of peatland, of which a considerable area has been designated for large-scale sago plantations. Dalat and Mukah Plantations are two established estates that cover areas of 1,600 ha and 20,000 ha, respectively. With this scale of plantation, sago export is ranked the fourth largest agricultural revenue earner in Sarawak (Chew *et al*. 1999).

Sago palms are cultivated for the starch stored in their trunks. Hence, palms that produce trunk are often referred to as ‘trunking’ specimens. Although sago is well known as one of the crops that adapt to the extreme conditions of peat, the cultivation of sago has not been straightforward. Sago palms grow slowly on peat soil, with lower production than on mineral soils (Purwanto *et al*. 2002). Jong *et al*. (2006) compared the growth performance of sago palms cultivated on deep peat and shallow peat. Their results showed that only 20 % of the palms produced trunks on deep peat; whilst on shallow peat they grew better, with more than 80 % trunking in 5–6 years and attaining maturity in ten years. Sim *et al*. (2005) associated the poor growth of sago on deep peat with limiting nutrients. In fact, the challenge of sago cultivation on deep peat is similarly encountered in oil palm plantations, where lower yield is reported as a result of less humified haemic peat in the deeper horizons of the soil (Veloo *et al*. 2015).

Peat soil is composed primarily of humic substances, which are complicated heterogeneous compounds resulting from the process of decomposition. This process is also referred to as humification. The fertility of peat is closely associated with degree of humification. It is found that peat soil with higher humification displays a lower C:N ratio, indicating a larger mobile nitrogen reserve (Sári & Forró 2008). Venegas-González *et al*. (2013) further report that blackberry (*Rubus* spp.) experiences enhanced nutrient uptake when grown in substrate with increased humification, whilst Requena *et al*. (1997) provide evidence of improved growth performance in relation to humification degree.

For this reason, peat immaturity is expected to associate with retarded sago growth on deep peat. However, there is limited information on the humification profiles of peat soils in Sarawak, particularly those in sago palm plantations. Hence, the objective of our study was to determine the

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humification degree of peat soil under sago cultivation, based on spectroscopic characteristics. The techniques applied were Fourier Transform Infrared (FTIR) and Ultraviolet-Visible (UV-Vis) spectroscopy, which are well known rapid and efficient approaches for characterisation of humification degree (Artz *et al*. 2008, Tivet *et al*. 2013, Biester *et al*. 2014, Merlin *et al*. 2014). Cultivated peat obtained from different depths adjacent to trunking and non-trunking sago palms was compared with uncultivated peat.

METHODS

Study area and sampling

Peat soil samples were collected from Sungai Talau Sago Research Station in Mukah, Sarawak (Figure 1), by experienced sago plantation staff. The Station is characterised by peat soil of varying thickness, from less than 1 m to more than 6 m. In this study, soil samples were collected from an area (plot) with 5–6 m of peat and nine-year-old sago palms which had not all produced trunks (Figure 2). The peat soil was cored to a depth of 1 m using an Eijkelkamp gouge auger with extension rod (core diameter 52 mm, length 0.5 m) under the canopies of seven trunking and seven non-trunking palms (selected randomly). Each core was sliced into 20 cm sections, measuring from the ground surface, then each section was removed from the auger and placed in a separate 'ziplock' bag with gloved hands. The samples were labelled to indicate the greatest depth included, *i.e*. the peat from 0–20 cm depth was labelled '20 cm', peat from 20–40 cm depth was labelled '40 cm', *etc*. The diameters at breast height of the seven trunking palms ranged from 45 to 57 cm, whilst no trunk formation was observed in the seven non-trunking palms. For comparison, a core was collected in the same way from an unplanted plot nearby (Figure 1), which had been left undisturbed for five years since its last reclamation for sago cultivation. The water table was maintained at 20–40 cm below the ground surface in both plots. The soil samples were oven dried at 30 °C overnight, then ground into fine particles with a mortar and pestle.

Fourier Transform Infrared (FTIR) spectroscopy

The soil samples were analysed using an ATR-FTIR equipped with diamond crystal, controlled by OMNIC software (Thermo Nicolet Analytical Instruments, Madison, WI). A flat tip powder press was used to achieve even distribution and contact. All spectra were collected at 32 scans with a resolution of 4 cm^{-1} in the range 4000–670 cm^{-1}. The spectrum of each sample was ratioed against a fresh background spectrum recorded from the bare ATR crystal. The ATR crystal was cleaned with ethanol. The spectrum for each soil sample was replicated three times. Thus,

Figure 1. Sungai Talau sago plantation and the sampling plots.

in total, 225 spectra were acquired, of which 15 were for uncultivated peat samples and 210 were for cultivated peat samples collected at five depths near trunking and non-trunking palms. The spectra in .csv format were baseline corrected and analysed with a peak detection and matching algorithm using Matlab R2013a. Briefly, the algorithm detects and integrates the peak areas of the absorption bands present in each spectrum; the bands identified are then matched across samples to produce a peak table with rows corresponding to samples and columns to variables (wavenumber, cm^{-1}) (Sim & Ting 2012). The ratio intensities of 1700/1030 (carboxyl C=O and aromatic esters/ polysaccharides) and 1610/1030 (aromatic C=C or COO^-/polysaccharides) were calculated as the index of humification (Broder *et al*. 2012).

Figure 2. Photographs of non-trunking (above) and trunking (below) sago palms.

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Ultraviolet-Visible (UV-Vis) spectroscopy

The humic acid fraction was extracted from the dried peat soil for UV-Vis characterisation (Jasco V-360 Spectrophotometer) according to Khan *et al*. (2006), with slight modification. A 0.5 g sample of soil was mixed with 60 mL of 0.5 % NaOH and centrifuged at 1100 rpm for 15 minutes. The supernatant was collected and the pH was adjusted to unity with HCl. Humic acid was allowed to separate under room temperature for one hour and centrifuged at 4000 rpm for ten minutes. The humic acid was then re-dissolved in 5 mL of 0.1 M NaOH. One millilitre of the solution was transferred to a 100 mL volumetric flask and brought up to the mark with distilled water. This solution was scanned from 200 to 800 nm, recording the absorbance at 465, 540 and 665 nm. The ratio of absorbances at 465 and 665 nm is referred to as E_4/E_6.

Statistical analyses

Principal Component Analysis (PCA) was used to determine whether different sample origins were discernible in the underlying pattern of the data. The peak table values obtained from the peak detection and matching algorithm were square rooted and standardised prior to PCA to ensure that all variables were comparable. Fisher weight was used to identify variables with significant discriminatory ability. The Fisher weight of each variable was calculated according to the following equation:

$$f_m = \frac{\sum_{c=1}^{C} N_c(\bar{x}_{mc} - x_m)^2}{s_m^2 \sum_{c=1}^{C}(N_c - 1)} \qquad [1]$$

where f_m is the Fisher weight of Variable m, $\bar{x}_{mc}$ is the mean of Variable m in Class c, $\bar{x}_m$ is the overall mean of the variable, S_m is pooled standard deviation, and N_c is the number of samples in Class c. Variables were ranked in descending order of f_m value, those with higher weight having stronger discriminatory ability (Brereton 2009). T-test was used to compare the means of the two different sample groups. All calculations were performed using Matlab 2013a (The MathWorks Inc., Natick, MA, USA).

RESULTS

The FTIR spectra of peat soil obtained from the uncultivated and cultivated plots are shown in Figure 3. The spectra exhibit similarities, with common absorption bands at 3400, 2920, 2850, 1700, 1600, 1500, 1458, 1376, 1230, 1159 and 1030 cm^{-1}, in agreement with the findings of Silamiķele *et al*. (2010).

Figure 4 shows the scores plot of PC2 *versus* PC1 for the peak tables of FTIR spectra obtained from cultivated and uncultivated peat at 0–20 cm depth. In the scores plot, cultivated samples are clearly distinguishable from uncultivated samples, implying the presence of characteristic functional properties for each group.

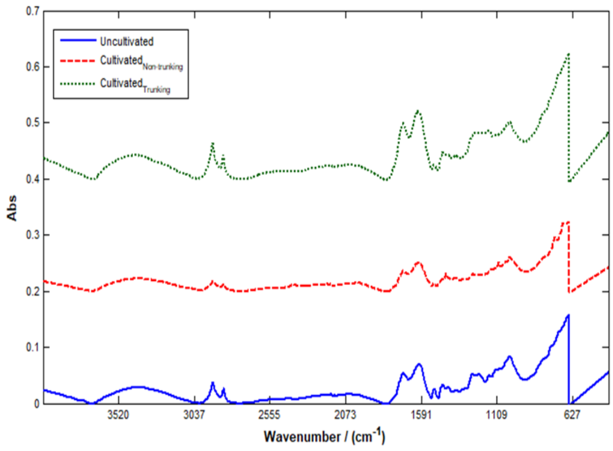


Figure 3. The FTIR profiles of uncultivated peat (bottom trace), and cultivated peat sampled adjacent to non-trunking (middle trace) and trunking (top trace) sago palms.

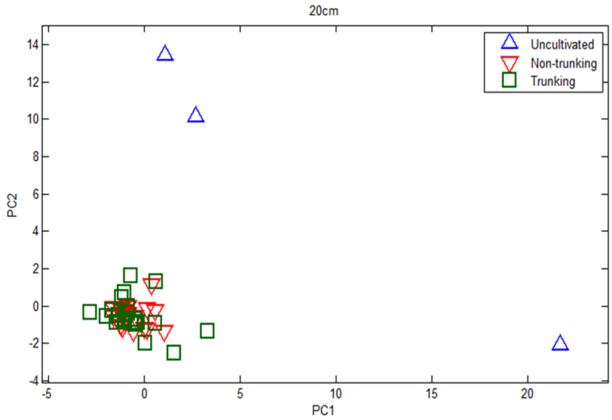
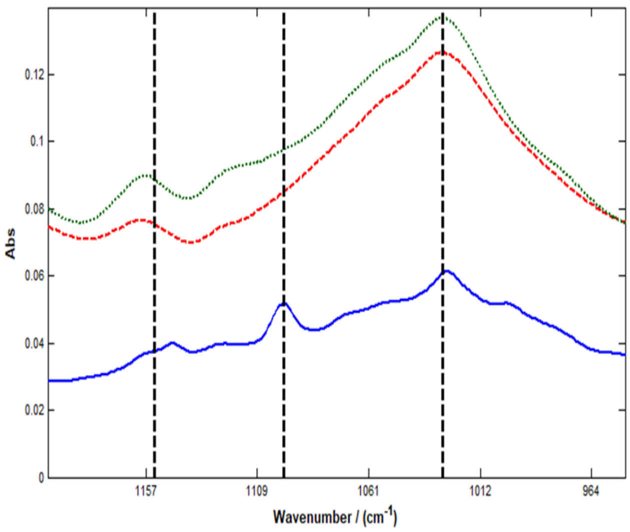
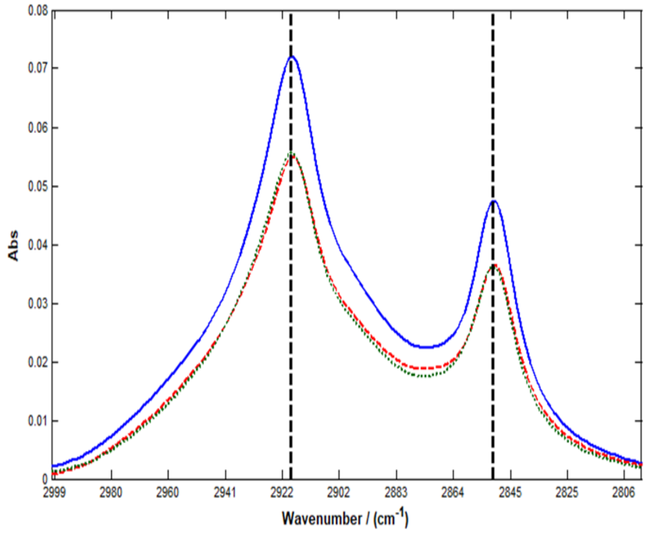


Figure 4. The scores plot of PC2 *versus* PC1 for the peak table of FTIR spectra obtained from cultivated and uncultivated peat at 0–20 cm depth.

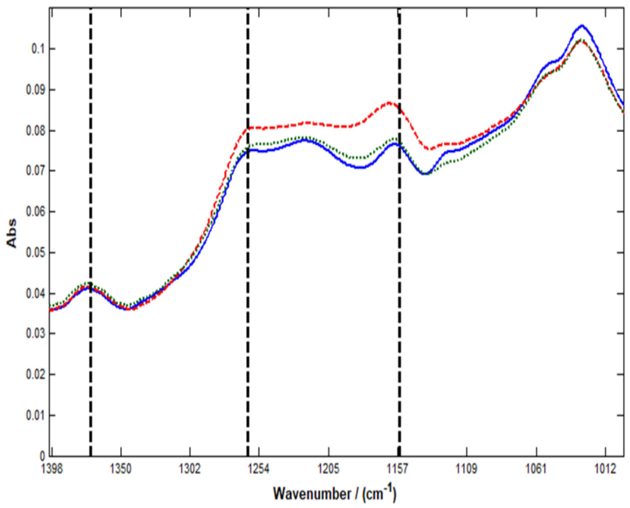
The absorption bands at 1030, 1097, 1157, 1265, 1370 and 2850 cm^{-1} were identified as discriminatory variables according to Fisher weight. For cultivated peat at 20 cm depth, the weak absorption band at 1097 cm^{-1} (ascribed to C–O stretching of polysaccharide-like compounds) was absent, whereas the band at 1030 cm^{-1} was more pronounced, suggesting a predominance of cellulose and hemicellulose compounds indicative of less-humified materials (Figure 5(a)). At 40 cm depth, the C–H absorption bands at 2850 and 2920 cm^{-1} were more prominent in uncultivated peat (Figure 5(b)).



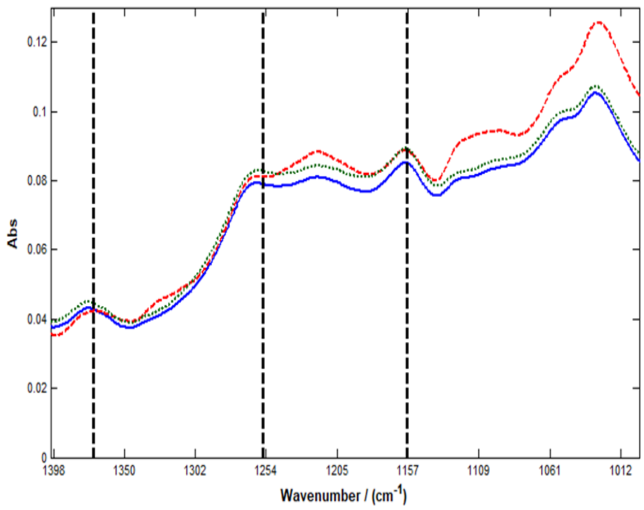
(a) 1100–1000 cm^{-1} (at 20 cm depth).



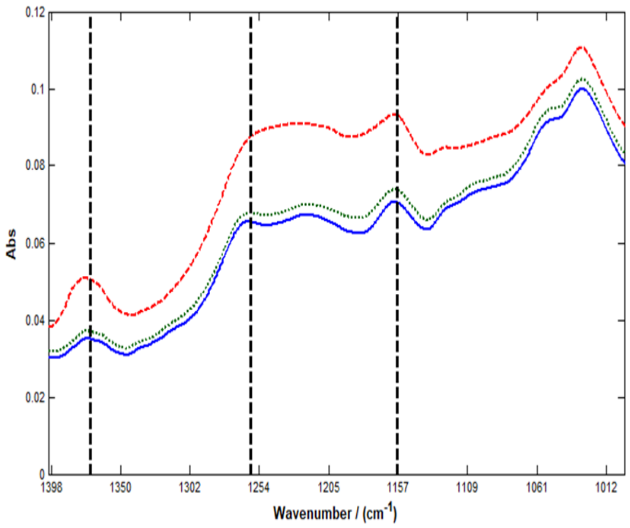
(b) 3000–2800 cm^{-1} (at 40 cm depth).



(c) 1400–1000 cm^{-1} (at 60 cm depth).



(d) 1400–1000 cm^{-1} (at 80 cm depth).



(e) 1400–1000 cm^{-1} (at 100 cm depth).

Figure 5. Spectral regions of uncultivated and cultivated peat sampled from different depths at Sungai Talau. The vertical dashed lines (black) indicate the absorption bands detected.

According to Krumins *et al*. (2012), a higher concentration of aliphatic compounds suggests a higher degree of decomposition. Besides, at depths of 60–100 cm, the absorption bands at 1370 (C–O, phenolics), 1265 (C–O, lignin-like) and 1157 cm^{-1} (C–O, cellulose) were consistently less abundant in the uncultivated peat (Figure 5(c–e)), corroborating the presence of compounds with fewer cellulose and lignin features.

Evaluating the vertical profiles of peat soil from the three different origins showed that the characteristic bands for cellulose and hemicellulose compounds in the region 1400–1000 cm^{-1} were relatively weaker for shallower samples. Figure 6 shows the spectral region 1400–1000 cm^{-1} for uncultivated peat sampled at the five different depths. The humification degree was further characterised on the basis of ratio intensities of 1700 and 1610 cm^{-1} against 1030 cm^{-1}. Greater index values signify peat of higher humification degree, whose structural features are dominated by aromatic C=C and carboxyl groups characteristic of a more humified fraction. Table 1 summarises the ratio intensities of the selected bands for uncultivated and cultivated peat according to depth. The ratio indices were higher for uncultivated than for cultivated peat, but the index values were rather consistent within the individual vertical profiles.

Other changes in FTIR spectra that are used to probe increase in humification include a reduction in intensity at 1700 cm^{-1} and the shifting of bands at 3420 and 1650 cm^{-1} to lower wavenumbers (El Fels *et al*. 2015). In this study, the bands at 1705 cm^{-1} and 1157 cm^{-1} were significantly less abundant in uncultivated peat, especially at 80 and 100 cm depth ($p < 0.05$). This confirmed the greater humification degree of the exposed peat. Besides, the band at 1620–1600 cm^{-1} was found to shift between uncultivated and cultivated peat at all depths. In uncultivated peat, this band was detected at the lower wavenumber of 1607 cm^{-1}; whilst in cultivated peat it was identified at 1620 cm^{-1}, indicating lower peat maturity. Cultivated and uncultivated peat were plausibly discernible, but cultivated samples collected adjacent to trunking and non-trunking palms appeared to cluster on the scores plot with no significant difference in the marker bands, suggesting that they shared spectroscopic properties ($p > 0.05$).

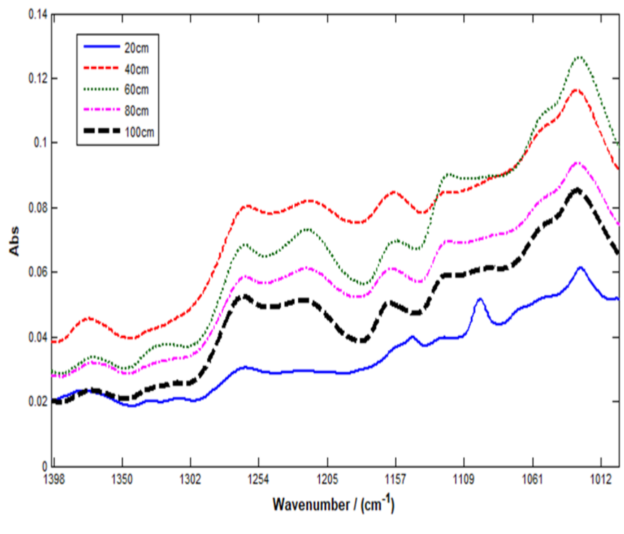


Figure 6. The spectral region of 1400–1000 cm^{-1} for uncultivated peat, distinguished according to depth.

Table 2 summarises the E_4/E_6 of humic acid extracted from uncultivated and cultivated peat according to depth. The E_4/E_6 of humic acid from surface peat was generally lower than that in deeper horizons for all three of the peat origins. The lower E_4/E_6 implies greater condensation structure which, in turn, suggests higher humification degree in the surface peat (0–20 cm). This postulation agrees with our findings using IR spectra nonetheless; there was no significant difference in E_4/E_6 between cultivated and uncultivated peat samples. The absorbance at 540 nm (A_{540}) was measured in addition, as an index of humification. Humic acid from the cultivated plot exhibited higher absorbance than that from the exposed (uncultivated) peat (Table 3).

Table 1. The ratio intensities of 1700/1030 and 1610/1030 for uncultivated and cultivated peat, according to depth.

Depth (cm)	Sample origin	1700/ 1030	1610/ 1030
	Uncultivated	0.16	0.10
20	Non-trunking	0.08	0.05
	Trunking	0.09	0.05
	Uncultivated	0.11	0.07
40	Non-trunking	0.10	0.06
	Trunking	0.10	0.07
	Uncultivated	0.11	0.07
60	Non-trunking	0.09	0.05
	Trunking	0.10	0.06
	Uncultivated	0.12	0.09
80	Non-trunking	0.10	0.05
	Trunking	0.10	0.07
	Uncultivated	0.12	0.09
100	Non-trunking	0.08	0.05
	Trunking	0.10	0.06

Table 2. The E_4/E_6 values of humic acid extracted from uncultivated and cultivated peat, according to depth.

Depth (cm)	Uncultivated	Cultivated	
		Trunking	Non-trunking
20	5.96	6.16	5.78
40	7.04	6.34	6.14
60	7.07	6.09	6.47
80	6.64	6.28	7.12
100	6.47	6.44	6.91

Table 3. Absorbance at 540 nm for humic acid extracted from uncultivated and cultivated peat, according to depth.

Depth (cm)	Uncultivated	Cultivated	
		Trunking	Non-trunking
20	0.03	0.09	0.05
40	0.04	0.09	0.07
60	0.04	0.10	0.07
80	0.05	0.11	0.06
100	0.04	0.11	0.06

DISCUSSION

FTIR has been commonly used to evaluate soil humification degree. The frequencies of absorption bands are used to probe the functional groups present, whilst the abundance and ratios of relative intensities are integrated to provide information on the degree of humification. Typically, during humification, polysaccharides and phenolic moieties of parent materials are transformed into non-lignin aromatic structures. As a result, more humified materials are usually enriched with aromatic groups (Zech *et al*. 1997). The bands at 3400 and 1030 cm^{-1} are attributable to polysaccharide markers whilst those at 1500, 1458, 1376, 1265 cm^{-1} are assigned to lignin-like compounds. The aliphatic structures are typically represented by the absorption bands at 2920 and 2850 cm^{-1} (Artz *et al*. 2008). The more pronounced absorption band at 1030 cm^{-1} along with absence of the band at 1097 cm^{-1} in the uncultivated peat suggests that the exposed sample is more humified. It is likely that this is because the uncultivated peat is subject to weather conditions with minimal input of fresh plant residues, which could accelerate the process of humification. This suggestion is supported by the work of Yule *et al*. (2016), who found that tropical peat was almost completely decomposed in the absence of canopy and decomposition was reduced with increasing shelter. The 1700/1030 and 1610/1030 ratio intensities further support this inference, with the cultivated peat demonstrating lower index values, especially at 20 cm.

Consideration of the spectra of peat samples collected from various depths suggests that samples from deeper horizons exhibit stronger bands in the region 1400–1000 cm^{-1}, indicating less-decomposed cellulose and hemicellulose characteristics. Particularly for ‘uncultivated’ samples, the ratio value is lower in the underlying peat (> 40 cm), again inferring its less-decomposed nature. However, this difference is indistinctive in the ‘cultivated’ samples. Carlson *et al*. (2015) have reported that reclaimed peatland with drainage is more susceptible to decomposition, especially in the upper layer; and at Sungai Talau the practice of maintaining the water table at a depth of 20–40 cm below the ground surface could have enhanced decomposition in the 0–20 cm soil layer. The observation of higher humification in the surface peat layer contradicts numerous reports in the literature relating to temperate peat (Bravard & Righi 1991, Purmalis & Klavins 2012, Szajdak *et al*. 2007, Reiche *et al*. 2010, Broder *et al*. 2012, Krumins *et al*. 2012, Hodgkins *et al*. 2014). It also conflicts with the findings of Kawahigashi & Sumida (2006), who found that the deeper horizons of Mukah and Dalat peat had a higher degree of humification. According to Wüst *et al*. (2003), temperate peat is derived primarily from bryophytes and dwarf shrubs, whilst tropical peat originates from various woody species with roots that usually penetrate to depths of several metres. This is confirmed by Lim *et al*. (2013) and Veloo *et al*. (2014) who describe the underlying tropical peat in Sarawak as consisting largely of undecomposed woody material with recognisable plant remains. It concurs well with our experience of the auger often landing on undecomposed tree trunk during field sampling.

Humic substances, comprised of humic acids, fulvic acids and humin, are major constituents of peat soil. The characteristics of humic acids can be used to indicate the humification degree of peat. The UV-Vis spectrum of humic acids is generally featureless with absorbance gradually reducing from 200 to 800 nm (spectra not shown). Despite this uninformative spectral profile, UV-Vis spectroscopy has been used widely for characterisation of humic molecules, on the basis of absorbance at various wavelengths such as 280, 465, 540 and 665 nm. The ratio of absorbance at 465 and 665 nm, E_4/E_6, is often calculated to demonstrate the condensation degree and aromaticity of humic compounds. The ratio value is inversely related to aromaticity; the greater the E_4/E_6 value (which implies predominace of proteins and carbohydrates), the less humified the soil (Vieyra *et al*. 2009). The E_4/E_6 values obtained in this research are typical for humic acids, with the surface peat consistently demonstrating lower values than the underlying peat. This implies that the former has a more humified nature, corroborating our findings using IR spectra. Although the surface peat is indicatively more humified, there is no pattern in humification degree between 40 and 100 cm depth that is interpretable as an analogue of the ratio indices of FTIR spectroscopy.

The compounds are expected to become more conjugated with increasing humification degree, giving rise to absorption at 540 nm (A_{540}) (Tsutsuki & Kuwatsuka 1979). It is assumed that samples with higher humification degree, reflected in lower E_4/E_6 values, will in turn demonstrate lower A_{540}. However, the results we obtained do not show the predicted direct relationship between E_4/E_6 and A_{540}. In fact, inconsistent correlation between E_4/E_6 and A_{540} has been reported; Bonnett *et al*. (2017) demonstrated an inverse relationship between the two indices whilst Klavins *et al*. (2013) reported a significant positive correlation.

The IR spectra indicate that the uncultivated peat was more humified than the cultivated peat, but trunking and non-trunking peat samples were not differentiable on this basis. Surface peat (0–20 cm depth) displayed characteristics of greater humification than the underlying peat (> 40 cm) but there was no interpretable pattern between 40 and 100 cm. Based on E_4/E_6, there was no significant difference between the humic acid extracted from uncultivated and cultivated peat but the surface peat appeared to demonstrate more profound condensation structure implying higher humification degree; this observation is in agreement with the findings based on IR spectra. As for FTIR, no conclusive humification pattern could be derived from the vertical profile of E_4/E_6 and A_{540}. Although a vertical humification profile was not clearly demonstrated by this study, the fact that the deeper peat was overlain by more-humified surface peat suggests that planting on deep tropical peat is not viable. Veloo *et al*. (2015) claim that the growth of oil palm is affected when its roots are in contact with undecomposed substrates. However, the palm grows and produces normally if no woody material is present. This condition may apply similarly in sago plantations, causing the inconsistent growth that we observed on a single plot. Unfortunately, the spectroscopic data obtained in this study were insufficient to reveal any relationship between decomposition degree and the trunking performance of the palms. It is recommended that other information such as elemental composition is sought to complement the spectroscopic data.

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