Are secondary forests second-rate? Comparing peatland greenhouse gas emissions, chemical and microbial community properties between primary and secondary forests in Peninsular Malaysia.

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Abstract

Tropical peatlands are globally important ecosystems with high C storage and are endangered by anthropogenic disturbances. Microbes in peatlands play an important role in sustaining the functions of peatlands as a C sink, yet their characteristics in these habitats are poorly understood. This research aimed to elucidate the responses of these complex ecosystems to disturbance by exploring greenhouse gas (GHG) emissions, nutrient contents, soil microbial communities and the functional interactions between these components in a primary and secondary peat swamp forest in Peninsular Malaysia. GHG measurements using closed chambers, and peat sampling were carried out in both wet and dry seasons. Microbial community phenotypes and nutrient content were determined using phospholipid fatty acid (PLFA) and inductively-coupled plasma mass spectrometry (ICSPM) analyses respectively. CO_2 emissions in the secondary peat swamp forest were >50% higher than in the primary forest. CH_4 emission rates were *ca.* 2 mg m^{-2} hr^{-1} in the primary forest but the secondary forest was a CH_4 sink, showing no seasonal variations in GHG emissions. Almost all the nutrient concentrations were significantly lower in the secondary forest, postulated to be due to nutrient leaching via drainage and higher rates of decomposition. Cu and Mo concentrations were negatively correlated with CO_2 and CH_4 emissions respectively. Microbial community structure was overwhelmingly dominated by bacteria in both forest types, however it was highly sensitive to land-use change and season. Gram-positive and Gram-negative relative abundance were positively correlated with CO_2 and CH_4 emissions respectively. Drainage related disturbances increased CO_2 emissions, by reducing the nutrient content including some with known antimicrobial properties (Cu & Na) and by favouring Gram-positive bacteria over Gram-negative bacteria. These results suggest that the biogeochemistry of secondary peat swamp forest is fundamentally different from that of

primary peat swamp forest, and these difference have significant functional impacts on their respective environments.

Keywords: Pristine tropical peatlands, Land use change, Drained and logged peatlands, GHG emissions, Nutrient content, Microbial community structure.

1. Introduction

Peatlands are globally important ecosystems that support high C storage, unique endemic biodiversity and distinct ecosystem services (Strack, 2008; Xu et al., 2018). Peatlands are formed as a result of primary production exceeding soil microbial decomposition, due to a unique blend of environmental conditions such as hydrology, topography, climate and microbial ecology (Miettinen et al., 2012; Page et al., 1999). Owing to the variations in the source of these environmental conditions, diverse range of peatlands exist around the globe covering 423 million hectare or about 2.9% of land surface (Xu et al., 2018).

There is a considerable cover of peatlands in the tropics, approximating to 0.25% of land surface area yet accounting for 3% of global soil C or 18% of the total peat C (Hapsari et al., 2017; Hergoualc'h and Verchot, 2014; Strack, 2008). These are most likely an underestimation due to insufficient information on tropical peatlands in general, along with the new discovery of tropical peatlands in Africa (Dargie et al., 2017), and other recent estimates showing increased cover in South America (Gumbricht et al., 2017; Xu et al., 2018). Unlike most northern peatlands, tropical peatlands are forested, thus they are C-rich both above- and below-ground (Dargie et al., 2017). Additionally tropical peatlands are biologically active throughout the year and accumulate 200% more C each year per area than northern peat bogs (Guo and Gifford, 2002; Strack, 2008). Most of these C-rich tropical peatlands are located in South East Asia (SEA), and here they store *ca.* 69 Gt of C and absorb

about 2.6 t of CO_2 per hectare each year (Dohong et al., 2017; Miettinen and Liew, 2010; Norwana et al., 2011).

In spite of their global importance, tropical peatlands remain relatively poorly understood ecosystems (Posa et al., 2011; Yule, 2010). The interest and importance of tropical peatlands became apparent only after most of the peat forests in SEA were degraded for logging and agricultural plantations, creating global attention on endangerment of iconic species such as orangutans and tigers (Swarna Nantha and Tisdell, 2009), along with persistent smog created by burning of peatlands (McKirdy, 2015). However despite this, anthropogenic disturbance in peatlands continues. Malaysia, which contains a sizeable portion of tropical peatlands, has the world's highest deforestation rate in the 21st century (Hansen et al., 2013) and natural undisturbed peatlands are almost extinct from Peninsular Malaysia (Miettinen et al., 2016; Yule, 2010). This has led several researchers to use secondary forests as a yardstick to study natural peat habitats (Melling et al., 2005b; Tonks et al., 2017). Therefore, there is a need to study and understand the last remaining pristine peatlands in Peninsular Malaysia, to fully assess the impacts of forest conversion on peat characteristics and their consequent effects on GHG emissions.

Almost all of the remaining peat forests in Peninsular Malaysia are secondary forests (about 22.5% of peat cover), which were either drained or selectively logged (Miettinen et al., 2012; Yule, 2010). Logging is often a pathway for other degrading land-uses such as oil palm expansion (Dhandapani, 2015; Koh and Wilcove, 2008; Woodcock et al., 2011). The construction of roads for the transportation of logged timber gives access to the remote forests and has significant indirect effects on forest degradation and land-use change (Dohong et al., 2017; Forman and Alexander, 1998; Perz et al., 2008). Illegal logging is also a major concern, threatening the remaining forests in SEA (Dohong et al., 2017; Indrarto et al., 2012; Yule, 2010). The process of logging and associated disturbance results in soil compaction

(Chung et al., 2000). Selectively logged forests were also found to have lower leaf litter density, even after 25 years past cessation of timber extraction (Bruhl, 2001; Chung et al., 2000). Selective logging also reduces the complexity of the canopy with some regions of logged forests having an open canopy (Floren and Linsenmair, 2005), which may not provide a stable microclimate otherwise provided by a multi-layered canopy in primary forest. All these changes will affect highly sensitive tropical peatlands, where hydrology, canopy cover, leaf litter inputs, above ground vegetation and substrate quality are all inter dependent and crucial (Yule, 2010).

Timber extractions from peatlands in SEA are mostly associated with drainage of peatlands, affecting their ecology and function (Dohong et al., 2017). Peatlands in their natural state can hold very high quantities of water, up to 5-10 times the weight of peat (Yule, 2010), thus playing an important role in regional flood governance. This high water content is crucial for peatland functioning, as it creates anoxic conditions that prevent aerobic decomposition, resulting in accumulation of dead above-ground vegetation and peat formation. Therefore, draining these peatlands will expose the C stored over years to aerobic decomposition, resulting in breakdown (hence emissions of CO_2) and subsidence, also reducing their water holding capacity (Tonks et al., 2017; Yule, 2010). Prolonged drainage may result in the disappearance of peat even in forested land, as higher water tables are necessary for peat formation (Evers et al., 2017). Drainage also makes peatlands highly susceptible to fire, as dried peat is extremely flammable (Evers et al., 2017; Posa et al., 2011).

Microbes mineralize nutrients that accumulate within peat which are required for high primary production, thereby cycling C and N (Andersen et al., 2013). Considering the quantity of C stored in tropical peatlands, the activity of peat microbial communities can strongly influence the path of global climate change. As soil microbial communities are responsive to soil moisture status and substrate quality (e.g. Martinez-Garcia et al., 2018) , it

is plausible that microbial community structure and function differ between pristine and secondary peat swamp forests.

To understand the differences between pristine and secondary peat swamp forests in terms of greenhouse gas emissions, and associated microbial community structure, nutrient concentration and peat physico-chemical characteristics, we tested the hypothesis that peat characteristics, nutrient content and microbial phenotypic community structure are affected by historical drainage and logging in a secondary forest and they exhibit significant functional correlations with GHG emissions. We anticipated that there are changes in microbial community structure between primary and secondary forests due to the differences in hydrology, nutrient and oxygen availability, which would result in lower CH_4 emissions and higher CO_2 emissions in the secondary forest.

2. Materials and Methods

2.1 Study sites

2.1.1 Terengganu Setiu peat swamp forest - primary forest

This pristine pocket of tropical peat swamp forest is located in Terengganu state, in the north eastern part of Peninsular Malaysia (Figure 1). The site is roughly 842 hectares and ~11.3 km from the coast, located in Kampung Mat Jintan (5°25'16.2°N 102°55'46.2°E) in the boundary between Kuala Nerus and Setiu districts. This peatland area was previously unrecorded and is yet to be given national protection (WWF Malaysia, pers. comm.). To date, there are no published studies on the site and many of the site characteristics were unexplored (WWF Malaysia, pers. comm.). There is no known history of disturbance other than the local villagers collecting timber for household uses. There were no large scale oil palm plantations or major roads bordering the peatland area. The forest vegetation was composed of mostly trees up to ~40 m tall and 40-50 cm dbh completely closing the canopy with complex

multiple layers. The tree species include *Antisoptera* sp., *Shorea* sp., *Calophyllum sclerophyllum* Vesque, *Calophylum* sp., *Blumeondendron tokbrai* (Blume) Kurz, *Durio carinatus* Mast, *Gonystylus bancanus* (Miq.) Kurz, *Elateriospermum tapos* Blume, *and Syzgium* sp. Trees typical of secondary peat swamp forests *Macaranga pruinosa* (Miq.) Müll.Arg and *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg were present on the forest edges (Yule C., pers. obs.). *Pandanus helicopus* Kurz ex Miq (palms), *Nepenthes ampullaria* Jack (pitcher plants) and *Stenocleaena palustris* (Burm. f.) Bedd. (ferns) were common understory vegetation (Yule C. pers. obs.). The rainfall in the region is high from October to February period where it remains higher than 300 mm, with highest rainfall in November at nearly 1200 mm and low in June to September period remaining well below 200 mm, with lowest recorded rainfall in June at less than 50 mm (Suratman et al., 2017). The peat depth was *ca.* 2 m. The water table is generally above surface throughout the forest all around a year (WWF Malaysia, pers. comm.) The water table was *ca.* 10 cm and 5 cm above surface during wet and dry seasons respectively.

2.1.2 North Selangor peat swamp forest – secondary forest

This historically drained and selectively logged peat swamp forest is the largest area (81,304 ha) of peatlands in the state of Selangor at the central western part of Peninsular Malaysia (Figure 1). The North Selangor peatlands are divided and managed as four natural reserves, which have been protected since 1990 (Tonks et al., 2017). The sampling site (3°41'39.5"N 101°11'05.4"E) was located in the northern part of the peatlands in Sungai Karang forest reserve and was managed by Kelang forestry office. North Selangor peat forest had undergone drainage for logging and also irrigation for nearby oil palm and paddy fields (Irvine et al., 2013). This site has not been logged since the 1980s and contains old channels for timber extraction, many of which remain blocked. The site is bordered with oil palm plantations that are surrounded by paved roads. The forest vegetation includes of *Macaranga*

pruinosa (Miq.) Müll. Arg, *Campnosperma coriaceum* (Jack) Hallier f., *Blumeodendron tokbrai* (Blume) Kurz, *Shorea platycarpa* F.Heim, *Parartocarpus venenosus* Becc., *Ixora grandiflora* Ker Gawl, *Pternandra galeata* Ridl., *Stenoclaena palustris* (Burm. f.) Bedd., *Asplenium longissimum* Baker, *Nephrolepsis biserrata* (Sw.) Schott, *Crytostachys* sp., *Cyperus rotundus* L., and *Pandanus atrocarpus* Griff. (Yule and Gomez, 2009). Above ground biomass in North Selangor peat swamp forests ranged between 126.96 – 443.27 mg ha^{-1} with an average of 319.52 mg ha^{-1}, while the breast height dimeter ranged from 17 to 375 cm with an average of 46.39 cm (Brown et al., 2018). The observed ground vegetation was generally less dense in comparison to the Terengganu primary forest. The rainfall patterns around the year in North Selangor peatlands have two distinct peaks in March-April period and October-November period with rainfall greater than 200 mm, while the rainfall is low in the months between May and August at just under 125 mm, with lowest rainfall in June (Global Environmental Centre, 2014). The peat depth was roughly 2 m at the sampling region. The water table was below the surface on both the wet and dry season sampling periods. The maximum water table drawdown during the drought period is *ca.* 50-60 cm below surface and the water table is close to the surface for most of the year (Tonks et al., 2017). More information on North Selangor peatlands is given by Tonks et al. (2017).

2.2 Sampling strategy

Sampling were carried out during both the wet and dry seasons. The wet season sampling was carried out during December 2016 and October 2017 for the secondary and primary forest respectively, while the dry season sampling was done during July 2017 for both forest types. The secondary forest was visited three times during each season, while the primary forest was visited just once during both seasons. At each time, samples were collected from 25 random points distributed over an area of *ca.* 100×100 m, that is at least 200 m away from the forest edges. The gas analyser was connected with the chamber with 5 m long tube, thus making a

sub plot of 10 m diameter where 5 measurements are made. The gas analyser was moved 50-70 m from each sub plot, 5 times each visit making a total of 25 measurements per visit including all the sub plots. The measurements within that 10 m diameter circle is at-least 1.5m away from each other. At each sampling point, greenhouse gas measurements were taken and surface (0-5cm) soil samples were collected using a spoon for laboratory analyses. This resulted in 150 independent sampling points in the secondary forest, with 75 samples from each season. For the primary forest a total of 50 samples, with 25 samples from each season were taken. Of these samples, 5 random samples were taken from each visit for PLFA analysis and a different 10 random samples from each visit were used for nutrient analysis.

2.3 Greenhouse gas measurements

CO_2 and CH_4 emissions from the soil surface were measured using a Los Gatos (San Jose, California, USA) ultraportable greenhouse gas analyser. The gas analyser works on the principle of laser absorption spectroscopy and gives readings of CH_4 and CO_2 ppm as well as gas temperature. The measurements were made using the closed chamber method using a chamber with a height of 15 cm and inner diameter of 13.5 cm. The chamber had an inlet and an outlet port that were connected to the gas analyser, using 6.35 mm outer diameter polytetrafluoroethylene (PTFE) tube. During each measurement about 1 cm of the chamber was inserted into the ground until it was sealed to the ground surface, and gas measurements were taken for 5 min. There was no surface vegetation in any of the measurement points. The gas analyser was set to record gas flux every 20 seconds, resulting in at least 12 recorded measurement points for each plot. The first minute of each measurement was ignored allowing the gas flux to settle down after initial disturbance of placing the chambers. The gas measurements in ppm were converted to mg CO_2 m^{-2} hr^{-1} and µg CH_4 m^{-2} hr^{-1} for CO_2 and CH_4 respectively, as described in (Samuel and Evers, 2016), using the ideal gas law $PV=nRT$. Where: P = atmospheric pressure; V = volume of headspace; n = number of moles

(mol); R = universal Gas Constant law (8.314J. $K^{-1}mol^{-1}$) and T = temperature (K), with conversion factor, 1 mol of CO_2 = 44.01g and 1 mol CH_4 = 16.02g.

2.4 Soil properties

Soil temperature and moisture were measured *in situ*, using a digital thermometer from Fischer Scientific (Loughborough, UK) and a theta probe® (Delta-T Devices, Cambridge, UK) digital volumetric moisture meter, respectively.

For pH measurements, about 5 ml volume of peat sample was diluted in 10 ml deionised water in a centrifuge tube and shaken in a rotary shaker for 30 minutes. The pH of the supernatant was then measured using a pH meter (Mettler Toledo Leicester, UK).

Oven dried peat samples (105^{o}C for 48 h) were used to calculate the organic matter content. Dried peat samples were placed in silica crucibles and then transferred to a muffle furnace and maintained at 550^{o}C for 4 h. The organic matter content was then determined by calculating the loss on ignition as follows, organic matter content (%) = [(weight of oven dried soil – weight of ash) / weight of oven dried soil] ×100.

For analysing total C and N content, all samples were oven dried (105^{o}C for 48 h) and finely ground using a ball mill. Approximately 10 mg of sample was weighed into a Al foil cup and the exact weight was recorded. The samples were then transferred to an auto sampler on Flash 2000 CHNS-O elemental analyser supplied by Thermo Scientific (Loughborough, UK) to measure total C and N. The analyser was set at 55^{o}C oven temperature, with helium as the carrier gas at the flow rate of 140 ml min^{-1}. L-aspartic acid supplied by Sigma Aldrich (St Louis, USA) was used as quality control and peaty soil standard supplied by Elemental Microanalysis (Okeham, UK) was used as a standard.

The soil nutrient content were analysed using inductively coupled plasma mass spectroscopy (ICP-MS). For this, approximately 0.1g of oven dried (105^{o}C for 48 h) and ball-milled peat were weighed in digitubes. The digitubes were then placed in the heating blocks and 8 ml of nitric acid was added to each sample. The samples were left overnight and then 2 ml of hydrogen peroxide was added, the tubes were closed with watch glasses. Samples were then heated at 95^{o}C for 2 h. After the heat block digestion, the samples were diluted by filling milliQ water up to 50 ml, 1 ml of each sample was transferred in to 10ml tube and further diluted with 9ml of milliQ water. The samples were then analysed using 'Thermo Scientific (Loughborough, UK) ICAP Q' ICP-MS fitted with 'CETAC™ A5X- 520' auto sampler.

2.5 Phospholipid fatty acid analysis

2.5.1 PLFA extraction

Microbial community phenotypic structure was determined by phospholipid fatty acid (PLFA) analysis. PLFAs were extracted from replicate 1 g freeze-dried tropical peat samples using a modification of the method described by (Frostegard et al., 1991). The lipids from peat were extracted using Bligh & Dyer extraction (Bligh and Dyer, 1959). The extracted lipids were then separated into neutral lipids, glycol lipids and polar lipids (containing phospholipids) fractions using Megabond Elut® silica gel column supplied by Agilent (Santa Clara, USA). The extracted polar lipids were then methylated by mild alkaline methanolysis and converted into fatty acid methyl esters, which were then analysed using gas chromatography.

2.5.2 Gas chromatography and peak identification

The dried fatty acid methyl esters were suspended in 200 µl of hexane, ready for GC injection. One µl of each sample was injected into the GC in split-less mode. The column used in the GC for phospholipid analysis was 'ZB-FFAP' column, supplied by Phenomenex (Torrance, USA). The column was 30 m length x 0.25 mm inner diameter x 0.25 µm film

thickness. The carrier gas was helium with a constant pressure of 18 psi. The initial oven temperature in GC was 120°C; this was maintained for 1 min and then programmed to 250°C at the rate of 5°C min^{-1}. The constant temperature of 250^{o}C was maintained throughout the run. The results were displayed as a chromatogram of retention times of the compounds and the mass spectroscopy provides the ion profile of each compounds.

The fatty acids were represented by a fatty acid shorthand, showing the number of carbon atoms, followed by the number of double bonds separated by colon. The position of the double bond is defined by the letter 'n' followed by the number of carbons from the methyl end of the fatty acid molecule. The prefixes 'i' and 'a' were used to represent isomers and anti-isomers. 10me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule. The prefix cyc refers to cyclopropyl fatty acids. The fatty acids i15:0, a15:0, i16:0, i17:0, a17:0 were considered as Gram-positive biomarkers (Wilkinson et al., 2002). The fatty acids 10me16:0 and 10me18:0 were described as the biomarkers for actinomycetes (Wilkinson et al., 2002, Moore-Kucera & Dick, 2008), a group that belongs to Gram-positive bacteria. The relative abundances of Gram-negative bacteria were calculated using 16:1n9, 16:1n7, cyc17:0, 18:1n7 and cyc19:0 as biomarkers (Kaiser et al., 2010; Wilkinson et al., 2002). 18:2n6 and 18:1n9 were used as fungal biomarkers (Kaiser et al., 2010; Vestal and White, 1989; Wilkinson et al., 2002). 14:0, 16:0, 18:0, a17:1 and 20:0 were non-specific fatty acids (Wilkinson et al., 2002). The fatty acids with similar mass spectra 18:1n9 and 18:1n7 were differentiated with the help of neutral lipid fatty acid analysis, by the findings that fungal biomarker 18:1n9 should have much higher NLFA/PLFA ratio that the Gram-negative biomarker 18:1n7 (Baath, 2003). The ratio of cyclopropane fatty acids (cyc17:0& cyc19:0) to their monoeionic precursors (16:1n7 & 18:1n7) and the ratio of total saturated fatty acids (14:0, 16:0, 18:0, 20:0) to mono-unsaturated fatty acids (16:1n9, 16:1n7, a17:1n, 18:1n9,

18:1n7) were used indicators of stress and other ecological conditions (Bossio and Scow, 1998).

2.6 Statistical analysis

All the statistical analyses were carried out using Genstat® 17th edition (VSN international, 2017). The significance of differences between sites for greenhouse gas emissions and other environmental parameters were evaluated using linear mixed models with restricted maximum likelihood (REML) incorporating seasons and sites as fixed affects. For the data sets that were not normally distributed, the data were log transformed. For data that did not meet normality assumption after log transformation, non-parametric Kruskal- Wallis test was performed.

Principal component (PC) analysis was performed on PLFA data using Mol% normalised spectra. Relative abundance of individual microbial groups, and ratios between groups, were calculated and were subjected to statistical analysis using restricted maximum likelihood (REML) models, to identify the interactions of individual microbial groups with forest type, season and combination of forest type and season. Similar REML were also performed for PCs. REML was carried out using 'forest type' and 'season' as fixed model.

Backward stepwise multiple regression was performed with CO_2 and CH_4 as response variables and other environmental parameters as fitted terms. Similar backward stepwise multiple regressions were also performed with macronutrient and micronutrients separately as fitted terms. To meet the normality assumptions considering the dramatic differences between the primary forest and secondary forest in terms of CH_4 emissions, each individual data point was divided by the calculated variance of CH_4 for each site, and the variance adjusted data were used for CH_4 multiple regression with environmental parameters. The variance adjusted CH_4 was used to find correlations between CH_4 and other environmental parameters.

312 Backward stepwise multiple regression was also carried out to determine the relationship for
313 CO_2 and CH_4 emissions with relative abundance of different microbial groups.

314 3. Results

315 3.1 Greenhouse gas emissions

316 CO_2 emissions in North Selangor secondary forest was twice as high as the CO_2 emissions
317 from the Terengganu primary peat swamp forest ($F_{(1,195)}$= 93.7, P<0.001) and there was no
318 significant variation between seasons in CO_2 emissions in either of the forest types ($F_{(1,195)}$=
319 0.02, P=0.9; Fig.2a).

320 CH_4 emissions also varied significantly between the forest types (Kruskal Wallis H=80.08,
321 p<0.001). The secondary forest absorbed methane at -10 and -30 µg m^{-2} hr^{-1} during wet and
322 dry season monitoring periods respectively, showing statistical difference between seasons
323 (Kruskal Wallis H=21.15, p<0.001), while the primary forest emitted about 2 mg m^{-2} hr^{-1} and
324 showed no significant variations between seasons (Kruskall Wallis H=0.0036, p=0.95)
325 (Fig.2b).

326 3.2 Peat properties

327 Peat organic matter content was >90% in both forests, with primary forest showing
328 significantly higher percentage of organic matter content ($F_{(1,164)}$=4.03, p=0.046; Table 1).
329 Volumetric moisture was significantly lower in the secondary forest at <55% during both
330 seasons ($F_{(1,190)}$=344, p<0.001) with no statistically significant differences between seasons
331 ($F_{(1,190)}$=1, p=0.32). The water table of the primary forest was above the surface during both
332 seasons. pH did not vary significantly between primary and secondary forest
333 ($F_{(1,167)}$=0.01,p=0.93). The secondary forest had significantly higher temperatures than the
334 primary forest ($F_{(1,196)}$=527, p<0.001). The secondary forest was more than 1^{o}C greater than

335 the primary forest. Both forest types had significantly lower temperatures in the dry than in
336 the wet season ($F_{(1,196)}$=58.3, p<0.001).

337 ### 3.3 Nutrient content

338 Total C and N were at *ca.* 50%, and 2.5% respectively, in both forest types (Fig. 3a). All the
339 other studied macronutrients were substantially higher in the primary forest than in the
340 secondary forest, with P being a notable exception, being present at similar levels in both
341 forest types (Fig. 3b). Similarly all the micronutrients and trace elements, except
342 molybdenum were substantially higher in the primary forest (Fig 3c).

343 ### 3.4 GHG emissions and environmental controls

344 Backward step-wise multiple regression analysis showed that CO_2 emissions were
345 significantly predicted by peat moisture level (Fig.4a), while none of the other measured
346 environmental parameters was a significant predictor. For CH_4 emissions: moisture, pH and
347 temperature were combined significant predictors of CH_4 emissions, $F_{(3,160)}$=21.9, p<0.001,
348 R^2=0.28: CH_4= -0.697+0.0115(pH)+0.0217(Temperature)+0.00115(Moisture)

349 The C:N ratio was positively correlated with LogCH_4 (Fig. 4b). Cu was negatively related to
350 LogCO_2 (Fig 4c), and Mo was negatively related to LogCH_4 (Fig 4d).

351 ### 3.5 Peat microbial communities

352 #### 3.5.1 Effect of forest type and season

353 PC1 & 2 collectively accounted for 64% of the total variations. PC1 significantly
354 discriminated the two forest types from each other, while PC2 significantly discriminated the
355 two seasons from each other (Fig. 5a). Both PC1 & 2 showed significant interaction between
356 forest type and season (Table 2). PC1 significantly discriminated seasons in secondary forest,
357 but not in the primary forest, resulting in significant interactions between site and season.
358 Similarly for PC2, the difference between the seasons were greater in the primary forest than

in the secondary forest, resulting in significant interactions. The loadings for individual PLFAs associated with PC1 showed two groupings (Fig. 5b). All the fungal biomarkers (18:2n6, 18:1n9) and all the monenoic Gram-negative biomarkers (16:1n9, 16:1n7, 18:1n7) were grouped together and were associated with separation of primary forest with respect to PC1. The separation of secondary forest with PC1 was aided by the grouping of all actinomycetes biomarker (10me18:0, 10me16:0) and most of the Gram-positive biomarkers (i16:0, i17:0, i15:0). Non-specific biomarkers particularly a17:1 and 18:0 were associated with distinctness of wet season for both forest types with respect to PC2. Likewise, Gram-positive biomarkers a17:0 and a15:0 were strongly associated with distinctness of dry season for both forest types with respect to PC2.

3.5.2 Relative abundance and microbial community structure

Both forest types were overwhelmingly dominated by bacteria over fungi (Fig. 6). Among bacterial groups, Gram-positive was the dominant group in both seasons and forest types, except the pristine forest in the dry season, which was dominated by Gram-negative bacterial group. The relative abundance of all the microbial groups significantly varied between the forest types. All the microbial groups, except fungi, significantly varied between seasons, and the interactions between site and season were significant only for Gram-negative and actinomycetes microbial groups (Table 3). The relative abundance of Gram-negative, fungi and non-specific fatty acids were greater in the pristine forest than the secondary forest. Gram-positive and actinomycetes relative abundances were greater in the secondary forest than the primary forest. The respective relative abundances of both Gram-positive and Gram-negative groups were greater in the dry than the wet season. Relative abundance of non-specific fatty acids were greater in the wet season. Relative abundance of actinomycetes was greater in the wet season for secondary forest, but did not differ between seasons at the primary forest site.

All the studied ratios namely, G+:G-, F:B, cyc:pre and mon:sat, were significantly different between the forest types (Fig. 7 and Table 3). All the ratios except cyc:pre, were significantly different between seasons and also exhibited significant interactions between season and forest type. All the ratios except F:B, were greater in secondary forest than primary forest in both seasons (Fig. 7 and Table 3). The Secondary forest did not exhibit seasonal variations in any of the ratios, while in the primary forest, all the ratios were greater in the wet season than dry season, resulting in statistically significant interactions between season and forest type.

3.6 Microbial communities and GHG emissions

Gram-positive and Gram-negative relative abundance showed a positive relationship with CO_2 emissions and LogCH_4, respectively (Fig. 8a & b). Among ratios, cyc:pre was positively correlated with CO_2, and sat:mono ratio was negatively correlated with logCH_4 (Fig. 8c & d). None of the other microbial groups or ratios, was a statistically significant predictor of GHG emissions.

4. Discussion

4.1 Lower total GHG emissions in primary forest

The peat properties such as loss on ignition, pH and temperature showed minimal differences between the forest types; however, there was a stark difference in CO_2 emissions. High CO_2 emissions in the drained forest were expected as the water levels in the North Selangor peat swamp forests were reduced due to the legacy of logging and the resultant drainage ditches that still run through the forest. This drainage exposes the surface peat to aerobic decomposition, unlike the primary forest in Terengganu which remained water-logged throughout the seasons, with no history of drainage. Water level was the significant predictor of CO_2 emissions, showing a strong negative correlation, which is in agreement with previous studies (Couwenberg et al., 2010; Sangok et al., 2017; Wakhid et al., 2017). In mineral soil

systems, secondary forests were found to have greater heterotrophic respiration than primary forests (Shi et al., 2015). In tropical peat forest systems, Murdiyarso et al. (2010 found that heterotrophic respiration contributed >50% of the total respiration in restored secondary forest, while Hergoualc'h et al. (2017) found *ca.* 55% of the total CO_2 emissions were heterotrophic emissions in a primary forest with water table 25 cm below surface. Indeed, CO_2 emissions in the primary forest were 50.6% and 56.5% lower than that in the secondary forest during the wet and dry season respectively, equivalent to the heterotrophic contribution reported by Hergoualc'h et al. (2017) and Murdiyarso et al. (2017) in drier peat forest systems in SEA.

CH4 emissions of 2 mg m^{-2} hr^{-1} in the primary forest here is within the previously observed range for peat swamp forests in South East Asia (Couwenberg et al., 2010; Sjögersten et al., 2014). CH_4 emissions in pristine peat swamp forests in SEA is markedly lower than what is observed in peatland ecosystems in other regions, including the neotropics (up to 143 mg m^{-2} hr^{-1}) (Sjögersten et al., 2014). This stark difference in CH_4 emissions between climatically similar SEA peatlands and neotropical peatlands is possibly due to the difference in microbial community structure, with Gram-negative dominance in peat surface in neo-tropics unlike Gram-positive dominance in Malaysian tropical peatlands (Troxler et al., 2012). Differences in aboveground vegetation may influence CH_4 emissions via root exudate composition, as root exudates contribute large amount of labile carbon input into tropical peatlands and the composition of root exudates can significantly impact greenhouse gas emissions in tropical peatlands (Girkin et al., 2018) . However, the secondary forest in North Selangor peatlands might oxidise methane due to far lower moisture levels observed in the site. Moreover potential CH_4 oxidation in North Selangor peatlands complements the finding of Jackson et al. (2009) that methanogenic bacteria were absent in the top 50 cm. The same study found Proteobacteria and Verrucomicrobia, the phyla that contain methanotrophic bacteria, in a

North Selangor peatland, plausibly explaining higher methane oxidation than production in the secondary forest. Our study also showed positive correlation of CH_4 emissions with moisture, temperature and pH. These positive correlations with CH_4 were observed in several other studies for moisture (Inubushi et al., 2005; Melling et al., 2005a), pH (Inubushi et al., 2005), and temperature (Aben et al., 2017; Melling et al., 2005a). The secondary forest had higher temperatures and similar pH to the primary forest, yet methane was absorbed likely due to moisture limitations.

4.2 Higher nutrient content in primary forest and their impact on GHG emissions

North Selangor peat swamp forest has undergone severe drainage over the past four decades (Irvine et al., 2013), which is likely to have resulted in leaching and a reduction in nutrients (Kløve et al., 2010) in contrast to the primary forest in Terengganu which had higher nutrient content. Loss of nutrients with drainage has been observed in northern peatlands, where the nutrients further decreased with increasing age of drainage (Laiho and Laine, 1995; Sallantaus, 1992). Although most macronutrients were about 50% lower in the secondary forest, P was a notable exception, due to leaf litter addition, as P was found to be more rapidly released into the environment from leaf litter than other nutrients at the secondary forest (Ong et al., 2017). In turn, the reductions in concentrations of micronutrients and trace elements except Mo and Se might favour aerobic activity due to their strong antimicrobial properties on this process (Rietz and Haynes, 2003; Sederholm, 2016; Wilke, 1987). On the other hand, previous evidence has indicated that decreases in these elements such as Na might not be favourable to anaerobic microbes (Lassiter et al., 1963).

Cu has long been considered, and proven to have antimicrobial properties (Berg et al., 2005; Gajjar et al., 2009; Wheeldon et al., 2008), and is also found to commonly alter soil microbial communities (Nunes et al., 2016; Smit et al., 1997), which could have impacted the microbial communities involved in aerobic decomposition, resulting in reduced CO_2 emissions with

increased concentration of Cu. This element is also known to affect anaerobic microbial communities, however the effects of Cu on methanotrophs are greater than their effects on methanogens (Mao et al., 2015), and in environments with high C content, Cu concentrations were also found to favour CH_4 emissions (Jiao et al., 2005). Therefore higher Cu concentrations likely significantly impacted CO_2 emissions but not CH_4 emissions in our study.

However CH_4 emissions were negatively correlated with Mo concentrations. Mo is an essential micronutrient that influences N_2 fixation in peatlands (Warren et al., 2017). In peatlands, methanotrophs are considered to play a major part in N_2 fixation (Larmola et al., 2014), which is dependent on Mo for nitrogenase activity (Kaiser et al., 2005). It is plausible that increased presence of Mo supports N_2 fixing bacteria that are predominantly also methanotrophs in peatlands (Vile et al., 2014). Mo limitation may act as a mechanistic control on biological N_2 fixation, which likely affected the peatland methanotrophic communities (Vile et al., 2014; Warren et al., 2017). Mo is also an essential element in the activity of sulphite oxidase, the enzyme that reduces sulphite to sulphate (Kaiser et al., 2005). Such increases in sulphate concentration could stimulate sulphate-reducing bacteria that compete with methanogenic archaea for substrate (Dowrick et al., 2006). Attributing to this, increase in sulphate concentrations are known to suppress CH_4 emissions in peatlands (Fowler et al., 1995). Therefore, higher Mo concentrations may have benefited the methanotrophic community and suppressed methanogenic archaea in the secondary forest, resulting in higher CH_4 oxidation in that site.

4.3 Tropical peatland microbial community structures and their relationship with GHG emissions

This study has demonstrated that microbial communities in natural tropical peat swamp forests were dominated by bacteria, regardless of hydrology and level of disturbance. Greater

483 abundance of Gram-negative forms in primary forest was in accordance with the findings of
484 Troxler et al. (2012) in neotropical peatlands and those of Krashevska et al. (2015) in
485 tropical mineral soil systems, who also found that Gram-positive relative abundance
486 increased with disturbance (Krashevska et al., 2015). Fungi were generally observed to be
487 more tolerant to acidic conditions than bacteria, and found to be more dominant in afforested
488 systems, with low N inputs, as bacteria require more N per C biomass than fungi for substrate
489 utilization (Bardgett, 2005; Bossuyt et al., 2001; Fierer et al., 2009). However, this did not
490 hold true for tropical peat forest systems even though the C:N ratio was low and the
491 conditions were highly acidic. Bacteria often depend on fungal decomposition products in
492 ecosystems with complex substrate input (Moore-Kucera and Dick, 2008). The bacterial
493 dominance found here despite the complexity of SEA peat swamp forests, might be due to the
494 availability of more labile C through leaf litter and dissolved organic C in these ecosystems
495 (Yule, 2010), and may also be due to fungal decomposition activity in leaf litter and plant
496 parts before they are incorporated into peat soil.

497 The wetter conditions in undrained primary forest and in deeper layers in other disturbed
498 ecosystems in SEA peatlands might be the factor favouring Gram-negative over Gram-
499 positive bacteria (Dhandapani et al., unpublished manuscript; Jackson et al., 2009). This is
500 further supported by the observation in this study that Gram-negative relative abundance was
501 positively correlated with CH_4 emissions, which is a by-product of submerged anaerobic
502 decomposition (Aben et al., 2017), and Gram-positive relative abundance was positively
503 correlated with CO_2 emissions, which is a by-product of aerobic decomposition. Indeed,
504 results from Jackson et al. (2009) show both extracellular microbial activity and Gram
505 positive relative abundance decreased with depth in North Selangor secondary forest. In
506 addition, all the aerobic surface peat layers in different agricultural and forest systems were
507 dominated by gram-positive bacteria (Dhandapani et al., unpublished manuscripts), strongly

suggesting that Gram positive bacteria are an important contributor in aerobic decomposition in SEA tropical peatlands.

The cyc:pre ratio that were used as stress indicators were substantially higher in the secondary forest. The fatty acids *16:1n7* and *18:1n7* are transformed into *cyc17:0* and *cyc19:0* in stressful conditions as this adaptation helps maintaining functional living membrane under stressful conditions, and the cyclopropane fatty acids are more stable than their monoenoic precursors (Kaur et al., 2005). This likely indicates that the secondary forest conditions were more stressful for the peat microbial communities.

Even though the changes in environmental parameters were minimal between seasons, the microbial community structure was significantly different between the seasons for both forest types. The seasonal changes in secondary forest that had water table below the surface may be due to sudden heavy downpours during the wet season, as the response of microbial communities to sudden flooding by rainfall can be very quick ranging from minutes to days (Bardgett and van der Putten, 2014). However the water table was above the surface during both seasons for the primary forest, yet microbial community structure was significantly different between seasons, indicating the possibility that there are more factors influencing the seasonal variations than flooding. Nevertheless, the sat:mono ratio that responded to flooding was higher in the wet season than in the dry season in the primary forest (Bossio and Scow, 1998), indicates increased physiological stress in the wet season (Willers et al., 2015). Most of the saturated fatty acids were non-specific biomarkers in our study, also explaining the increased relative abundance of non-specific PLFAs in wet season for both forest types, and also explaining the higher proportion of non-specific fatty acids in submerged primary forest peat. Higher sat:mono ratio is used as an indication of nutritional stress (Kieft et al., 1994; Moore-Kucera and Dick, 2008), and the ratio is also known to increase with flooding (Bossio and Scow, 1998). The ratio was higher in the secondary forest, despite the flooded

conditions in the primary forest. This might be due to greater nutrient content and greater C input, resulting in higher substrate availability in the primary forest (Bossio and Scow, 1998). Similarly, greater nutrient availability may be the factor that influenced greater fungal abundance in primary forest than the secondary forest, resulting in higher F:B ratio in primary forest. Confirming the hypothesis, peat characteristics, nutrient content and microbial phenotypic community structure were significantly different between primary and secondary forest, and these changes exhibited significant functional correlations with GHG emissions, resulting in lower CH_4 emissions and higher CO_2 emissions in the secondary forest.

5. Conclusions

Secondary peat swamp forests are certainly distinct from primary peat forest systems in terms of nutrient status, peat microbial community structure and GHG emissions, and it is clear that secondary peat forest systems cannot be taken as a benchmark to study the other land-use classes and the effects of disturbances in tropical peatlands. Historic drainage and selective logging of peat swamp forests severely affect a peatlands' ability to function as a carbon sink, as the CO_2 emissions were 115% higher in the secondary forest. The drier conditions in the secondary forest favoured Gram-positive bacteria over Gram-negative bacteria, thereby potentially increasing aerobic microbial activity and CO_2 emissions in the secondary peat swamp forest. Historic drainage in the secondary forest also possibly resulted in the loss of essential nutrients including some containing antimicrobial properties that inhibit aerobic activity. Though the secondary forest oxidised methane contrasting with CH_4 emissions in the primary forest, it was insignificant in comparison to the scale of increase in CO_2 emissions in the secondary forest. The results have provided new insights into the interlinking relationships between different spheres of the environment such as peat nutrient content, microbial community structure and GHG emissions in tropical peatlands, and the ways

anthropogenic disturbance impacts these complex and globally important ecosystems. It is also evident that primary peat swamp forest play a vital role in carbon sequestration and that it is important to conserve the last few remaining unprotected pristine peatlands under national law with a new conservation unit of higher importance before they become extinct. There is also need for promotion of sustainable forest management in secondary forests.

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Figure captions

Figure 1: Location of the study sites.

Figure 2: Effects of forest type and season upon (a) CO_2 emissions, (b) CH_4 emissions (Black- primary forest, grey- secondary forest) during wet and dry season. Bars denote mean values (For primary forest n= 25; For secondary forest, wet season n=76 and dry season n= 75) and whiskers denote standard errors.

Figure 3: Effect of forest type upon (a) C and N content, (b) essential macronutrients, (c) essential micronutrients and trace elements, between the primary forest (black) and the secondary forest (grey), Bars denote mean values (n=10) and whiskers denote standard errors. Note scale breaks in y-axis for (a) and (c) to allow effective visualisation of wide-range data.

Figure 4: Relationship between (a) CO_2 and moisture, $F_{(1,190)}= 190.06$, $p<0.001$, $R^2=0.497$ $CO_2= 1583.2-11.520$ (moisture), (b) $LogCH_4$ and C:N, $F_{(1,18)}= 8.34$, $p=0.010$, $R^2 = 0.279$ $LogCH_4= -0.113+0.1046$(C:N), (c) $LogCO_2$ and Cu concentration, $F_{(1,18)}=17.79$, $p<0.001$, $R^2 =0.469$ $LogCO_2=2.9935-0.0452$(Cu), (d) $LogCH_4$ and Mo concentration, $F_{(1,18)}= 11.76$, $p=0.003$, $R^2=0.361$ LogCH4=2.665-1.005(Mo).

Figure 5: Effects of forest type and season upon phenotypic structure of soil microbial communities determined by PLFA analysis, as shown by principal component (PC) analysis. (a) ordination of PC1 and 2 and (b) associated loadings for individual PLFAs. For (a) points denote means (n=5), whiskers denote standard errors. Explanation for PLFA shorthand were given in section 2.5.2.

Figure 6: Relative abundance of different microbial groups as determined by PLFA analysis. Mean values are presented (n=5). Mol% is calculated by dividing the individual PLFA's peak area by the sum of the peak areas of all PLFAs and multiplying it by 100.

Figure 7: The difference in (a) fungi to bacteria relative abundance ratio (F:B), (b) Gram-positive to Gram-negative relative abundance ratio (G+:G-), (c) cyclopropane fatty acids (cyc17:0, cyc19:0) to their monenoic precursors (16:1n7 & 18:1n7) ratio (cyc:pre), and (d) saturated fatty acids (14:0, 16:0, 18:0, 20:0) to mono-unsaturated fatty acids (16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7) ratio (sat:mono) (Black- primary forest, grey- secondary forest) during wet and dry season. Bars denote mean values (n=5) and whiskers denote standard errors.

Figure 8: Relationship between (a) CO_2 emissions and Gram-positive (G+) relative abundance, $F_{(1,18)}$= 10.85, p=0.004, R^2=0.341 CO_2= -243+32.30(G+), (b) LogCH_4 and Gram-negative (G-) relative abundance, $F_{(1,18)}$= 15.95, p<0.001, R^2= 0.44 CH_4= -1.114+0.1160(G-), (c) CO_2 emissions and cyclopropane fatty acids (cyc17:0, cyc19:0) to their monenoic precursors (16:1n7 & 18:1n7) ratio (cyc:pre), $F_{(1,17)}$= 21.82, p<0.001, R^2=0.523 CO_2= 243+251.9(cyc:pre), (d) LogCH_4 and saturated fatty acids (14:0, 16:0, 18:0, 20:0) to mono-unsaturated fatty acids (16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7) ratio (sat:mono), $F_{(1,18)}$=17.11, p<0.001, R^2=0.459 LogCH_4= 4.273-1.889(sat:mono).