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

4

5 **Selvakumar Dhandapani^{a(#)}, Karl Ritz^a, Stephanie Evers^{b,c,d}, Catherine M. Yule^{e,f}, Sofie**
6 **Sjögersten^a**

7 **Affiliations**

8 ^aSchool of Biosciences, University of Nottingham, Sutton Bonington, UK.

9 ^bSchool of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool,
10 UK.

11 ^cSchool of Biosciences, University of Nottingham Malaysia Campus, Semenyih, Malaysia.

12 ^dTropical Catchment Research Initiative (TROCARI)

13 ^eSchool of Science, University of the Sunshine Coast, Queensland, Australia

14 ^fSchool of Science, Monash University, Malaysia

15 ^(#) **Corresponding author**

16 **Selvakumar Dhandapani,**

17 **Postal address:** School of Natural Sciences and Psychology, James Parsons building,

18 Liverpool John Moores University, 3 Byrom Street, Liverpool L3 3AF.

19 **Telephone:** +44 7413649444

20 **Email address:** s.dhandapani@ljmu.ac.uk

21 Abstract

22 Tropical peatlands are globally important ecosystems with high C storage and are endangered
23 by anthropogenic disturbances. Microbes in peatlands play an important role in sustaining the
24 functions of peatlands as a C sink, yet their characteristics in these habitats are poorly
25 understood. This research aimed to elucidate the responses of these complex ecosystems to
26 disturbance by exploring greenhouse gas (GHG) emissions, nutrient contents, soil microbial
27 communities and the functional interactions between these components in a primary and
28 secondary peat swamp forest in Peninsular Malaysia. GHG measurements using closed
29 chambers, and peat sampling were carried out in both wet and dry seasons. Microbial
30 community phenotypes and nutrient content were determined using phospholipid fatty acid
31 (PLFA) and inductively-coupled plasma mass spectrometry (ICSPM) analyses respectively.
32 CO₂ emissions in the secondary peat swamp forest were >50% higher than in the primary
33 forest. CH₄ emission rates were *ca.* 2 mg m⁻² hr⁻¹ in the primary forest but the secondary
34 forest was a CH₄ sink, showing no seasonal variations in GHG emissions. Almost all the
35 nutrient concentrations were significantly lower in the secondary forest, postulated to be due
36 to nutrient leaching via drainage and higher rates of decomposition. Cu and Mo
37 concentrations were negatively correlated with CO₂ and CH₄ emissions respectively.
38 Microbial community structure was overwhelmingly dominated by bacteria in both forest
39 types, however it was highly sensitive to land-use change and season. Gram-positive and
40 Gram-negative relative abundance were positively correlated with CO₂ and CH₄ emissions
41 respectively. Drainage related disturbances increased CO₂ emissions, by reducing the nutrient
42 content including some with known antimicrobial properties (Cu & Na) and by favouring
43 Gram-positive bacteria over Gram-negative bacteria. These results suggest that the
44 biogeochemistry of secondary peat swamp forest is fundamentally different from that of

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

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

49 1. Introduction

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

57 There is a considerable cover of peatlands in the tropics, approximating to 0.25% of land
58 surface area yet accounting for 3% of global soil C or 18% of the total peat C (Hapsari et al.,
59 2017; Hergoualc'h and Verchot, 2014; Strack, 2008). These are most likely an
60 underestimation due to insufficient information on tropical peatlands in general, along with
61 the new discovery of tropical peatlands in Africa (Dargie et al., 2017), and other recent
62 estimates showing increased cover in South America (Gumbrecht et al., 2017; Xu et al.,
63 2018). Unlike most northern peatlands, tropical peatlands are forested, thus they are C-rich
64 both above- and below-ground (Dargie et al., 2017). Additionally tropical peatlands are
65 biologically active throughout the year and accumulate 200% more C each year per area than
66 northern peat bogs (Guo and Gifford, 2002; Strack, 2008). Most of these C-rich tropical
67 peatlands are located in South East Asia (SEA), and here they store *ca.* 69 Gt of C and absorb

68 about 2.6 t of CO₂ per hectare each year (Dohong et al., 2017; Miettinen and Liew, 2010;
69 Norwana et al., 2011).

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

84 Almost all of the remaining peat forests in Peninsular Malaysia are secondary forests (about
85 22.5% of peat cover), which were either drained or selectively logged (Miettinen et al., 2012;
86 Yule, 2010). Logging is often a pathway for other degrading land-uses such as oil palm
87 expansion (Dhandapani, 2015; Koh and Wilcove, 2008; Woodcock et al., 2011). The
88 construction of roads for the transportation of logged timber gives access to the remote
89 forests and has significant indirect effects on forest degradation and land-use change (Dohong
90 et al., 2017; Forman and Alexander, 1998; Perz et al., 2008). Illegal logging is also a major
91 concern, threatening the remaining forests in SEA (Dohong et al., 2017; Indrarto et al., 2012;
92 Yule, 2010). The process of logging and associated disturbance results in soil compaction

93 (Chung et al., 2000). Selectively logged forests were also found to have lower leaf litter
94 density, even after 25 years past cessation of timber extraction (Bruhl, 2001; Chung et al.,
95 2000). Selective logging also reduces the complexity of the canopy with some regions of
96 logged forests having an open canopy (Floren and Linsenmair, 2005), which may not provide
97 a stable microclimate otherwise provided by a multi-layered canopy in primary forest. All
98 these changes will affect highly sensitive tropical peatlands, where hydrology, canopy cover,
99 leaf litter inputs, above ground vegetation and substrate quality are all inter dependent and
100 crucial (Yule, 2010).

101 Timber extractions from peatlands in SEA are mostly associated with drainage of peatlands,
102 affecting their ecology and function (Dohong et al., 2017). Peatlands in their natural state can
103 hold very high quantities of water, up to 5-10 times the weight of peat (Yule, 2010), thus
104 playing an important role in regional flood governance. This high water content is crucial for
105 peatland functioning, as it creates anoxic conditions that prevent aerobic decomposition,
106 resulting in accumulation of dead above-ground vegetation and peat formation. Therefore,
107 draining these peatlands will expose the C stored over years to aerobic decomposition,
108 resulting in breakdown (hence emissions of CO₂) and subsidence, also reducing their water
109 holding capacity (Tonks et al., 2017; Yule, 2010). Prolonged drainage may result in the
110 disappearance of peat even in forested land, as higher water tables are necessary for peat
111 formation (Evers et al., 2017). Drainage also makes peatlands highly susceptible to fire, as
112 dried peat is extremely flammable (Evers et al., 2017; Posa et al., 2011).

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

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

120 To understand the differences between pristine and secondary peat swamp forests in terms of
121 greenhouse gas emissions, and associated microbial community structure, nutrient
122 concentration and peat physico-chemical characteristics, we tested the hypothesis that peat
123 characteristics, nutrient content and microbial phenotypic community structure are affected by
124 historical drainage and logging in a secondary forest and they exhibit significant functional
125 correlations with GHG emissions. We anticipated that there are changes in microbial
126 community structure between primary and secondary forests due to the differences in
127 hydrology, nutrient and oxygen availability, which would result in lower CH₄ emissions and
128 higher CO₂ emissions in the secondary forest.

129 2. Materials and Methods

130 2.1 Study sites

131 2.1.1 Terengganu Setiu peat swamp forest - primary forest

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

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

156 2.1.2 North Selangor peat swamp forest – secondary forest

157 This historically drained and selectively logged peat swamp forest is the largest area (81,304
158 ha) of peatlands in the state of Selangor at the central western part of Peninsular Malaysia
159 (Figure 1). The North Selangor peatlands are divided and managed as four natural reserves,
160 which have been protected since 1990 (Tonks et al., 2017). The sampling site (3°41'39.5"N
161 101°11'05.4"E) was located in the northern part of the peatlands in Sungai Karang forest
162 reserve and was managed by Kelang forestry office. North Selangor peat forest had
163 undergone drainage for logging and also irrigation for nearby oil palm and paddy fields
164 (Irvine et al., 2013). This site has not been logged since the 1980s and contains old channels
165 for timber extraction, many of which remain blocked. The site is bordered with oil palm
166 plantations that are surrounded by paved roads. The forest vegetation includes of *Macaranga*

167 *pruinosa* (Miq.) Müll. Arg, *Camposperma coriaceum* (Jack) Hallier f., *Blumeodendron*
168 *tokbrai* (Blume) Kurz, *Shorea platycarpa* F.Heim, *Parartocarpus venenosus* Becc., *Ixora*
169 *grandiflora* Ker Gawl, *Pternandra galeata* Ridl., *Stenoclaena palustris* (Burm. f.) Bedd.,
170 *Asplenium longissimum* Baker, *Nephrolepis biserrata* (Sw.) Schott, *Cryptostachys* sp.,
171 *Cyperus rotundus* L., and *Pandanus atrocarpus* Griff. (Yule and Gomez, 2009). Above
172 ground biomass in North Selangor peat swamp forests ranged between 126.96 – 443.27 mg
173 ha⁻¹ with an average of 319.52 mg ha⁻¹, while the breast height diameter ranged from 17 to 375
174 cm with an average of 46.39 cm (Brown et al., 2018). The observed ground vegetation was
175 generally less dense in comparison to the Terengganu primary forest. The rainfall patterns
176 around the year in North Selangor peatlands have two distinct peaks in March-April period
177 and October-November period with rainfall greater than 200 mm, while the rainfall is low in
178 the months between May and August at just under 125 mm, with lowest rainfall in June
179 (Global Environmental Centre, 2014). The peat depth was roughly 2 m at the sampling
180 region. The water table was below the surface on both the wet and dry season sampling
181 periods. The maximum water table drawdown during the drought period is *ca.* 50-60 cm
182 below surface and the water table is close to the surface for most of the year (Tonks et al.,
183 2017). More information on North Selangor peatlands is given by Tonks et al. (2017).

184 2.2 Sampling strategy

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

192 sub plot of 10 m diameter where 5 measurements are made. The gas analyser was moved 50-
193 70 m from each sub plot, 5 times each visit making a total of 25 measurements per visit
194 including all the sub plots. The measurements within that 10 m diameter circle is at-least
195 1.5m away from each other. At each sampling point, greenhouse gas measurements were
196 taken and surface (0-5cm) soil samples were collected using a spoon for laboratory analyses.
197 This resulted in 150 independent sampling points in the secondary forest, with 75 samples
198 from each season. For the primary forest a total of 50 samples, with 25 samples from each
199 season were taken. Of these samples, 5 random samples were taken from each visit for PLFA
200 analysis and a different 10 random samples from each visit were used for nutrient analysis.

201 2.3 Greenhouse gas measurements

202 CO₂ and CH₄ emissions from the soil surface were measured using a Los Gatos (San Jose,
203 California, USA) ultraportable greenhouse gas analyser. The gas analyser works on the
204 principle of laser absorption spectroscopy and gives readings of CH₄ and CO₂ ppm as well as
205 gas temperature. The measurements were made using the closed chamber method using a
206 chamber with a height of 15 cm and inner diameter of 13.5 cm. The chamber had an inlet and
207 an outlet port that were connected to the gas analyser, using 6.35 mm outer diameter
208 polytetrafluoroethylene (PTFE) tube. During each measurement about 1 cm of the chamber
209 was inserted into the ground until it was sealed to the ground surface, and gas measurements
210 were taken for 5 min. There was no surface vegetation in any of the measurement points. The
211 gas analyser was set to record gas flux every 20 seconds, resulting in at least 12 recorded
212 measurement points for each plot. The first minute of each measurement was ignored
213 allowing the gas flux to settle down after initial disturbance of placing the chambers. The gas
214 measurements in ppm were converted to mg CO₂ m⁻² hr⁻¹ and µg CH₄ m⁻² hr⁻¹ for CO₂ and
215 CH₄ respectively, as described in (Samuel and Evers, 2016), using the ideal gas law
216 PV=nRT. Where: P = atmospheric pressure; V = volume of headspace; n = number of moles

217 (mol); R = universal Gas Constant law ($8.314\text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$) and T = temperature (K), with
218 conversion factor, 1 mol of $\text{CO}_2 = 44.01\text{g}$ and 1 mol $\text{CH}_4 = 16.02\text{g}$.

219 2.4 Soil properties

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

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

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

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

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

248 2.5 Phospholipid fatty acid analysis

249 2.5.1 PLFA extraction

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

259 2.5.2 Gas chromatography and peak identification

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

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

269 The fatty acids were represented by a fatty acid shorthand, showing the number of carbon
270 atoms, followed by the number of double bonds separated by colon. The position of the
271 double bond is defined by the letter 'n' followed by the number of carbons from the methyl
272 end of the fatty acid molecule. The prefixes 'i' and 'a' were used to represent isomers and
273 anti-isomers. 10me indicates a methyl group on the 10th carbon atom from the carboxyl end
274 of the molecule. The prefix cyc refers to cyclopropyl fatty acids. The fatty acids i15:0, a15:0,
275 i16:0, i17:0, a17:0 were considered as Gram-positive biomarkers (Wilkinson et al., 2002).
276 The fatty acids 10me16:0 and 10me18:0 were described as the biomarkers for actinomycetes
277 (Wilkinson et al., 2002, Moore-Kucera & Dick, 2008), a group that belongs to Gram-positive
278 bacteria. The relative abundances of Gram-negative bacteria were calculated using 16:1n9,
279 16:1n7, cyc17:0, 18:1n7 and cyc19:0 as biomarkers (Kaiser et al., 2010; Wilkinson et al.,
280 2002). 18:2n6 and 18:1n9 were used as fungal biomarkers (Kaiser et al., 2010; Vestal and
281 White, 1989; Wilkinson et al., 2002). 14:0, 16:0, 18:0, a17:1 and 20:0 were non-specific fatty
282 acids (Wilkinson et al., 2002). The fatty acids with similar mass spectra 18:1n9 and 18:1n7
283 were differentiated with the help of neutral lipid fatty acid analysis, by the findings that
284 fungal biomarker 18:1n9 should have much higher NLFA/PLFA ratio than the Gram-negative
285 biomarker 18:1n7 (Baath, 2003). The ratio of cyclopropane fatty acids (cyc17:0 & cyc19:0) to
286 their monoeionic precursors (16:1n7 & 18:1n7) and the ratio of total saturated fatty acids
287 (14:0, 16:0, 18:0, 20:0) to mono-unsaturated fatty acids (16:1n9, 16:1n7, a17:1n, 18:1n9,

288 18:1n7) were used indicators of stress and other ecological conditions (Bossio and Scow,
289 1998).

290 2.6 Statistical analysis

291 All the statistical analyses were carried out using Genstat® 17th edition (VSN international,
292 2017). The significance of differences between sites for greenhouse gas emissions and other
293 environmental parameters were evaluated using linear mixed models with restricted
294 maximum likelihood (REML) incorporating seasons and sites as fixed affects. For the data
295 sets that were not normally distributed, the data were log transformed. For data that did not
296 meet normality assumption after log transformation, non-parametric Kruskal- Wallis test was
297 performed.

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

304 Backward stepwise multiple regression was performed with CO₂ and CH₄ as response
305 variables and other environmental parameters as fitted terms. Similar backward stepwise
306 multiple regressions were also performed with macronutrient and micronutrients separately as
307 fitted terms. To meet the normality assumptions considering the dramatic differences between
308 the primary forest and secondary forest in terms of CH₄ emissions, each individual data point
309 was divided by the calculated variance of CH₄ for each site, and the variance adjusted data
310 were used for CH₄ multiple regression with environmental parameters. The variance adjusted
311 CH₄ was used to find correlations between CH₄ and other environmental parameters.

312 Backward stepwise multiple regression was also carried out to determine the relationship for
313 CO₂ and CH₄ emissions with relative abundance of different microbial groups.

314 3. Results

315 3.1 Greenhouse gas emissions

316 CO₂ emissions in North Selangor secondary forest was twice as high as the CO₂ 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 CO₂ emissions in either of the forest types ($F_{(1,195)}=$
319 0.02 , $P=0.9$; Fig.2a).

320 CH₄ 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 $\mu\text{g m}^{-2} \text{hr}^{-1}$ 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 $2 \text{ mg m}^{-2} \text{hr}^{-1}$ and
324 showed no significant variations between seasons (Kruskal 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°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 CO₂ 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 CH₄ emissions: moisture, pH and
347 temperature were combined significant predictors of CH₄ emissions, $F_{(3,160)}=21.9$, $p<0.001$,
348 $R^2=0.28$: $CH_4 = -0.697 + 0.0115(\text{pH}) + 0.0217(\text{Temperature}) + 0.00115(\text{Moisture})$

349 The C:N ratio was positively correlated with LogCH₄ (Fig. 4b). Cu was negatively related to
350 LogCO₂ (Fig 4c), and Mo was negatively related to LogCH₄ (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

359 in the secondary forest, resulting in significant interactions. The loadings for individual
360 PLFAs associated with PC1 showed two groupings (Fig. 5b). All the fungal biomarkers
361 (18:2n6, 18:1n9) and all the monenoic Gram-negative biomarkers (16:1n9, 16:1n7, 18:1n7)
362 were grouped together and were associated with separation of primary forest with respect to
363 PC1. The separation of secondary forest with PC1 was aided by the grouping of all
364 actinomycetes biomarker (10me18:0, 10me16:0) and most of the Gram-positive biomarkers
365 (i16:0, i17:0, i15:0). Non-specific biomarkers particularly a17:1 and 18:0 were associated
366 with distinctness of wet season for both forest types with respect to PC2. Likewise, Gram-
367 positive biomarkers a17:0 and a15:0 were strongly associated with distinctness of dry season
368 for both forest types with respect to PC2.

369 3.5.2 Relative abundance and microbial community structure

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

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

391 3.6 Microbial communities and GHG emissions

392 Gram-positive and Gram-negative relative abundance showed a positive relationship with
393 CO₂ emissions and LogCH₄, respectively (Fig. 8a & b). Among ratios, cyc:pre was positively
394 correlated with CO₂, and sat:mono ratio was negatively correlated with logCH₄ (Fig. 8c & d).
395 None of the other microbial groups or ratios, was a statistically significant predictor of GHG
396 emissions.

397 4. Discussion

398 4.1 Lower total GHG emissions in primary forest

399 The peat properties such as loss on ignition, pH and temperature showed minimal differences
400 between the forest types; however, there was a stark difference in CO₂ emissions. High CO₂
401 emissions in the drained forest were expected as the water levels in the North Selangor peat
402 swamp forests were reduced due to the legacy of logging and the resultant drainage ditches
403 that still run through the forest. This drainage exposes the surface peat to aerobic
404 decomposition, unlike the primary forest in Terengganu which remained water-logged
405 throughout the seasons, with no history of drainage. Water level was the significant predictor
406 of CO₂ emissions, showing a strong negative correlation, which is in agreement with previous
407 studies (Couwenberg et al., 2010; Sangok et al., 2017; Wakhid et al., 2017). In mineral soil

408 systems, secondary forests were found to have greater heterotrophic respiration than primary
409 forests (Shi et al., 2015). In tropical peat forest systems, Murdiyarso et al. (2010) found that
410 heterotrophic respiration contributed >50% of the total respiration in restored secondary
411 forest, while Hergoualc'h et al. (2017) found *ca.* 55% of the total CO₂ emissions were
412 heterotrophic emissions in a primary forest with water table 25 cm below surface. Indeed,
413 CO₂ emissions in the primary forest were 50.6% and 56.5% lower than that in the secondary
414 forest during the wet and dry season respectively, equivalent to the heterotrophic contribution
415 reported by Hergoualc'h et al. (2017) and Murdiyarso et al. (2017) in drier peat forest
416 systems in SEA.

417 CH₄ emissions of 2 mg m⁻² hr⁻¹ in the primary forest here is within the previously observed
418 range for peat swamp forests in South East Asia (Couwenberg et al., 2010; Sjögersten et al.,
419 2014). CH₄ emissions in pristine peat swamp forests in SEA is markedly lower than what is
420 observed in peatland ecosystems in other regions, including the neotropics (up to 143 mg m⁻²
421 hr⁻¹) (Sjögersten et al., 2014). This stark difference in CH₄ emissions between climatically
422 similar SEA peatlands and neotropical peatlands is possibly due to the difference in microbial
423 community structure, with Gram-negative dominance in peat surface in neo-tropics unlike
424 Gram-positive dominance in Malaysian tropical peatlands (Troxler et al., 2012). Differences
425 in aboveground vegetation may influence CH₄ emissions via root exudate composition, as
426 root exudates contribute large amount of labile carbon input into tropical peatlands and the
427 composition of root exudates can significantly impact greenhouse gas emissions in tropical
428 peatlands (Girkin et al., 2018) . However, the secondary forest in North Selangor peatlands
429 might oxidise methane due to far lower moisture levels observed in the site. Moreover
430 potential CH₄ oxidation in North Selangor peatlands complements the finding of Jackson et
431 al. (2009) that methanogenic bacteria were absent in the top 50 cm. The same study found
432 Proteobacteria and Verrucomicrobia, the phyla that contain methanotrophic bacteria, in a

433 North Selangor peatland, plausibly explaining higher methane oxidation than production in
434 the secondary forest. Our study also showed positive correlation of CH₄ emissions with
435 moisture, temperature and pH. These positive correlations with CH₄ were observed in several
436 other studies for moisture (Inubushi et al., 2005; Melling et al., 2005a), pH (Inubushi et al.,
437 2005), and temperature (Aben et al., 2017; Melling et al., 2005a). The secondary forest had
438 higher temperatures and similar pH to the primary forest, yet methane was absorbed likely
439 due to moisture limitations.

440 4.2 Higher nutrient content in primary forest and their impact on GHG emissions
441 North Selangor peat swamp forest has undergone severe drainage over the past four decades
442 (Irvine et al., 2013), which is likely to have resulted in leaching and a reduction in nutrients
443 (Kløve et al., 2010) in contrast to the primary forest in Terengganu which had higher nutrient
444 content. Loss of nutrients with drainage has been observed in northern peatlands, where the
445 nutrients further decreased with increasing age of drainage (Laiho and Laine, 1995;
446 Sallantaus, 1992). Although most macronutrients were about 50% lower in the secondary
447 forest, P was a notable exception, due to leaf litter addition, as P was found to be more
448 rapidly released into the environment from leaf litter than other nutrients at the secondary
449 forest (Ong et al., 2017). In turn, the reductions in concentrations of micronutrients and trace
450 elements except Mo and Se might favour aerobic activity due to their strong antimicrobial
451 properties on this process (Rietz and Haynes, 2003; Sederholm, 2016; Wilke, 1987). On the
452 other hand, previous evidence has indicated that decreases in these elements such as Na
453 might not be favourable to anaerobic microbes (Lassiter et al., 1963).

454 Cu has long been considered, and proven to have antimicrobial properties (Berg et al., 2005;
455 Gajjar et al., 2009; Wheeldon et al., 2008), and is also found to commonly alter soil microbial
456 communities (Nunes et al., 2016; Smit et al., 1997), which could have impacted the microbial
457 communities involved in aerobic decomposition, resulting in reduced CO₂ emissions with

458 increased concentration of Cu. This element is also known to affect anaerobic microbial
459 communities, however the effects of Cu on methanotrophs are greater than their effects on
460 methanogens (Mao et al., 2015), and in environments with high C content, Cu concentrations
461 were also found to favour CH₄ emissions (Jiao et al., 2005). Therefore higher Cu
462 concentrations likely significantly impacted CO₂ emissions but not CH₄ emissions in our
463 study.

464 However CH₄ emissions were negatively correlated with Mo concentrations. Mo is an
465 essential micronutrient that influences N₂ fixation in peatlands (Warren et al., 2017). In
466 peatlands, methanotrophs are considered to play a major part in N₂ fixation (Larmola et al.,
467 2014), which is dependent on Mo for nitrogenase activity (Kaiser et al., 2005). It is plausible
468 that increased presence of Mo supports N₂ fixing bacteria that are predominantly also
469 methanotrophs in peatlands (Vile et al., 2014). Mo limitation may act as a mechanistic
470 control on biological N₂ fixation, which likely affected the peatland methanotrophic
471 communities (Vile et al., 2014; Warren et al., 2017). Mo is also an essential element in the
472 activity of sulphite oxidase, the enzyme that reduces sulphite to sulphate (Kaiser et al., 2005).
473 Such increases in sulphate concentration could stimulate sulphate-reducing bacteria that
474 compete with methanogenic archaea for substrate (Dowrick et al., 2006). Attributing to this,
475 increase in sulphate concentrations are known to suppress CH₄ emissions in peatlands
476 (Fowler et al., 1995). Therefore, higher Mo concentrations may have benefited the
477 methanotrophic community and suppressed methanogenic archaea in the secondary forest,
478 resulting in higher CH₄ oxidation in that site.

479 4.3 Tropical peatland microbial community structures and their relationship with 480 GHG emissions

481 This study has demonstrated that microbial communities in natural tropical peat swamp
482 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 Krashevskaya et al. (2015) in
485 tropical mineral soil systems, who also found that Gram-positive relative abundance
486 increased with disturbance (Krashevskaya 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 CH₄ 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 CO₂ 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

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

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

516 Even though the changes in environmental parameters were minimal between seasons, the
517 microbial community structure was significantly different between the seasons for both forest
518 types. The seasonal changes in secondary forest that had water table below the surface may
519 be due to sudden heavy downpours during the wet season, as the response of microbial
520 communities to sudden flooding by rainfall can be very quick ranging from minutes to days
521 (Bardgett and van der Putten, 2014). However the water table was above the surface during
522 both seasons for the primary forest, yet microbial community structure was significantly
523 different between seasons, indicating the possibility that there are more factors influencing
524 the seasonal variations than flooding. Nevertheless, the sat:mono ratio that responded to
525 flooding was higher in the wet season than in the dry season in the primary forest (Bossio and
526 Scow, 1998), indicates increased physiological stress in the wet season (Willers et al., 2015).
527 Most of the saturated fatty acids were non-specific biomarkers in our study, also explaining
528 the increased relative abundance of non-specific PLFAs in wet season for both forest types,
529 and also explaining the higher proportion of non-specific fatty acids in submerged primary
530 forest peat. Higher sat:mono ratio is used as an indication of nutritional stress (Kieft et al.,
531 1994; Moore-Kucera and Dick, 2008), and the ratio is also known to increase with flooding
532 (Bossio and Scow, 1998). The ratio was higher in the secondary forest, despite the flooded

533 conditions in the primary forest. This might be due to greater nutrient content and greater C
534 input, resulting in higher substrate availability in the primary forest (Bossio and Scow, 1998).
535 Similarly, greater nutrient availability may be the factor that influenced greater fungal
536 abundance in primary forest than the secondary forest, resulting in higher F:B ratio in
537 primary forest. Confirming the hypothesis, peat characteristics, nutrient content and
538 microbial phenotypic community structure were significantly different between primary and
539 secondary forest, and these changes exhibited significant functional correlations with GHG
540 emissions, resulting in lower CH₄ emissions and higher CO₂ emissions in the secondary
541 forest.

542 5. Conclusions

543 Secondary peat swamp forests are certainly distinct from primary peat forest systems in terms
544 of nutrient status, peat microbial community structure and GHG emissions, and it is clear that
545 secondary peat forest systems cannot be taken as a benchmark to study the other land-use
546 classes and the effects of disturbances in tropical peatlands. Historic drainage and selective
547 logging of peat swamp forests severely affect a peatlands' ability to function as a carbon sink,
548 as the CO₂ emissions were 115% higher in the secondary forest. The drier conditions in the
549 secondary forest favoured Gram-positive bacteria over Gram-negative bacteria, thereby
550 potentially increasing aerobic microbial activity and CO₂ emissions in the secondary peat
551 swamp forest. Historic drainage in the secondary forest also possibly resulted in the loss of
552 essential nutrients including some containing antimicrobial properties that inhibit aerobic
553 activity. Though the secondary forest oxidised methane contrasting with CH₄ emissions in the
554 primary forest, it was insignificant in comparison to the scale of increase in CO₂ emissions in
555 the secondary forest. The results have provided new insights into the interlinking
556 relationships between different spheres of the environment such as peat nutrient content,
557 microbial community structure and GHG emissions in tropical peatlands, and the ways

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

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819 Figure captions

820 **Figure 1:** Location of the study sites.

821 **Figure 2:** Effects of forest type and season upon (a) CO₂ emissions, (b) CH₄ emissions
822 (Black- primary forest, grey- secondary forest) during wet and dry season. Bars denote mean
823 values (For primary forest n= 25; For secondary forest, wet season n=76 and dry season n=
824 75) and whiskers denote standard errors.

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

829 **Figure 4:** Relationship between (a) CO₂ and moisture, $F_{(1,190)}= 190.06$, $p<0.001$, $R^2=0.497$
830 $CO_2= 1583.2-11.520$ (moisture), (b) LogCH₄ and C:N, $F_{(1,18)}= 8.34$, $p=0.010$, $R^2 = 0.279$
831 $LogCH_4= -0.113+0.1046(C:N)$, (c) LogCO₂ and Cu concentration, $F_{(1,18)}=17.79$, $p<0.001$, R^2
832 $=0.469$ $LogCO_2=2.9935-0.0452(Cu)$, (d) LogCH₄ and Mo concentration, $F_{(1,18)}= 11.76$,
833 $p=0.003$, $R^2=0.361$ $LogCH_4=2.665-1.005(Mo)$.

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

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

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

849 **Figure 8:** Relationship between (a) CO₂ emissions and Gram-positive (G+) relative
850 abundance, $F_{(1,18)}= 10.85$, $p=0.004$, $R^2=0.341$ $CO_2= -243+32.30(G+)$, (b) LogCH₄ and Gram-
851 negative (G-) relative abundance, $F_{(1,18)}= 15.95$, $p<0.001$, $R^2= 0.44$ $CH_4= -1.114+0.1160(G-)$,
852 (c) CO₂ emissions and cyclopropane fatty acids (cyc17:0, cyc19:0) to their monenoic
853 precursors (16:1n7 & 18:1n7) ratio (cyc:pre), $F_{(1,17)}= 21.82$, $p<0.001$, $R^2=0.523$
854 $CO_2= 243+251.9(cyc:pre)$, (d) LogCH₄ and saturated fatty acids (14:0, 16:0, 18:0, 20:0) to
855 mono-unsaturated fatty acids (16:1n9, 16:1n7, a17:1n, 18:1n9, 18:1n7) ratio (sat:mono),
856 $F_{(1,18)}=17.11$, $p<0.001$, $R^2=0.459$ $LogCH_4= 4.273-1.889(sat:mono)$.

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