



# Characterization of fungi from different ecosystems of tropical peat in Sarawak, Malaysia

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## Abstract

Conversion of logged-over peatlands in Malaysia to oil palm plantations have led to changes in the microbial community in peat soil, mainly fungi. Therefore, this study was conducted to identify and compare the fungal species in four sites with different gradient of disturbances, namely a primary forest (Maludam NP), a logged-over forest (Cermat Ceria LOF), a 7.5-year-old oil palm plantation (Durafarm OPP), and an 11.5-year-old oil palm plantation (Naman OPP) by using a culture-dependent method. Fungi were isolated and identified based on morphological and molecular characterizations. The 18S rRNA gene was amplified, sequenced, and compared for the closest match in GenBank. Quantification of total fungal viable count, soil nutrients, and environmental aspects was also carried out. The total fungal viable count was not significantly different in all sites. Phylum Ascomycota was the dominant taxon isolated from all sites, which was about 90%. Basidiomycota and Zygomycota were also identified but found in low percentages. The most prevalent species for the different sites were *Aspergillus fumigatus* (20.6%) in Maludam NP, *Penicillium chrysogenum* in Cermat Ceria LOF (24.3%), *Hypocrea muroiana* in Durafarm OPP (27.4%), and *Hypocrea atroviridis* in Naman OPP (20%). Most of the species isolated were saprophytic fungi involved in decomposition of peat. In conclusion, fungal species composition in Sarawak peatlands with different gradient of disturbances was different in each site.

**Keywords** 18S rDNA · Culture-dependent · Fungi · Malaysia · Oil palm plantation · Tropical peat

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## 1 Introduction

Tropical peatlands in Malaysia are located at low-elevation areas and characterized by a wet tropical climate with high annual rainfall and temperature (Page et al. 2006). Peatlands in Malaysia are unique, having a combination of rainforest and peatland ecosystems. In Malaysia, peatlands are located along the coastal areas of Peninsular Malaysia, Sabah, and Sarawak. Sarawak possesses about 70% (1.6 million hectares) of total peatlands in Malaysia (Melling 2016). These areas represent a pool of biodiversity for various flora and fauna, including microorganisms (Yule 2010; Adila et al. 2017; Shuhada et al. 2017).

Peat is formed from the accumulation of organic materials over the decomposition rate in soil due to the acidic environment and waterlogged conditions (Zinck 2011; Cobb et al. 2017). In tropical areas, peat is formed by the accumulation of partially decomposed organic matter from trees and dead fauna over thousands of years (Kanokratana et al. 2011). Peatlands in Sarawak have a high annual rainfall and are ombrogenous,

acidic (pH 2.5–4.7), and dominated by dipterocarp trees (Melling 2016).

Despite their environmental conditions, peatlands provide a habitat for a wide range of animals and plants including soil microorganisms. Soil microorganisms such as fungi, bacteria, protists, and archaea have important ecological roles in regulating the ecosystem function via global carbon cycling (Winsborough and Basiliko 2010) and nutrient mineralization in peatlands (Andersen et al. 2013). Fungi are the primary decomposers (Thormann 2006; Myers et al. 2012) and dominant microbes found in peat; they are involved in biogeochemical processes such as the carbon cycle (Tveit et al. 2013) and emission of greenhouse gases (Toma et al. 2011). Fungi have the ability to sequester soil carbon and consequently provide ecosystem equilibrium (Treseder and Holden 2013). Therefore, the classification of fungi in peat is important in order to forecast carbon dynamics in the future.

Currently, the molecular approach is widely used to identify fungal species and resolve the taxonomic relationship among taxa (Hibbet et al. 2011; Asemaninejad et al. 2016; Sanders and Rodriguez 2016). Molecular identification provides more reliable results of identification compared to morphological characterization alone. Several markers of fungi such as protein-coding gene regions and nuclear ribosomal DNA (rDNA) are used in species identification. Ribosomal DNA that consists of a large subunit (28S), internal transcribed spacer (ITS), and small subunit (18S) are conserved within the fungi kingdom (Seifert 2009; Bellemain et al. 2010). The 18S rRNA gene is commonly used as a universal marker that can amplify across a wide range of fungi species with variations at the intraspecific level (Liu et al. 2015). The amplification of 18S rRNA gene has been used in many studies of diversity to assess fungal communities in a particular ecological niche such as forests and agricultural soils (Lim et al. 2010; Siles and Margesin 2016; Turrini et al. 2017).

Recently, peatlands in Sarawak are subjected to anthropogenic disturbances when logged-over degraded peatlands are converted to oil palm plantations. These disturbances may affect the diversity of soil microorganisms, mainly fungi, which later causes alteration of soil functions (Kerfahi et al. 2014; Mcguire et al. 2014; Nurulita et al. 2015). Therefore, the aim of this study was to identify and compare the fungal diversity among sites with different gradients of disturbances. The sites consisted of a primary forest, a logged-over forest, and two oil palm plantations.

## 2 Materials and methods

### 2.1 Sampling sites

Sampling sites were selected in four different forms of peat swamp ecosystems, which consisted of a primary forest

(Maludam NP), a logged-over forest (Cermat Ceria LOF), and two oil palm plantations (Durafarm OPP and Naman OPP) (Fig. 1). Maludam NP (1°37' 43.15"N, 111°02' 22.12"E) represents the least disturbed ecosystem of peat swamp forest. Cermat Ceria LOF (1°23' 58.85"N, 111°24' 08.61"E) is a logged-over forest which has been recovered naturally after timber was harvested years ago. Durafarm OPP (1°23' 50.63"N, 111°24' 50.59"E) with 7.5 years old oil palm while Naman OPP (2°09' 47.03"N, 111°55' 22.32"E) with oil palm aged 11.5 years (Fig. 2). Durafarm OPP and Naman OPP have represented the highly disturbed areas. Maludam NP, Cermat Ceria LOF, and Durafarm OPP were located in Sri Aman, Sarawak while Naman OPP in Sibul, Sarawak. The distance between each site was ranged between 4 km up to 102 km.

Sampling was carried out in October 2012 during the wet season (monthly precipitation > 200 mm). Peat samples were collected at ten sampling points for each site with depth between 0 and 60 cm, using peat auger (Eijkkelkamp, The Netherlands). The sampling points were at least 10 m apart from each other. 50 ml tubes were used to keep the peat samples and transported in boxes containing ice packs to the laboratory for analysis. Based on soil survey conducted by Param Agricultural Soil Surveys (M) Sdn. Bhd. (Paramanathan 2012a), peat in Maludam NP is classified as decomposed sapric organic materials (sapric; fibre content less than 33%). Meanwhile, peat in Cermat Ceria LOF, Durafarm OPP and Naman OPP consisted of sapric with undecomposed wood (Paramanathan 2012b).

### 2.2 Fungal isolation

Fungi from peat samples were isolated on potato dextrose agar (PDA) and malt extract agar (MEA) using spread plate method. A volume of 100 µl of dilutions,  $10^{-4}$  and  $10^{-5}$  were spread onto PDA and MEA using hockey stick and incubated at  $25 \pm 2$  °C for 3 days. Each different colony grown was subcultured onto new PDA to obtain pure isolates. The pure isolates were stored as the conidial disc made using cork borer in mineral oil for future use.

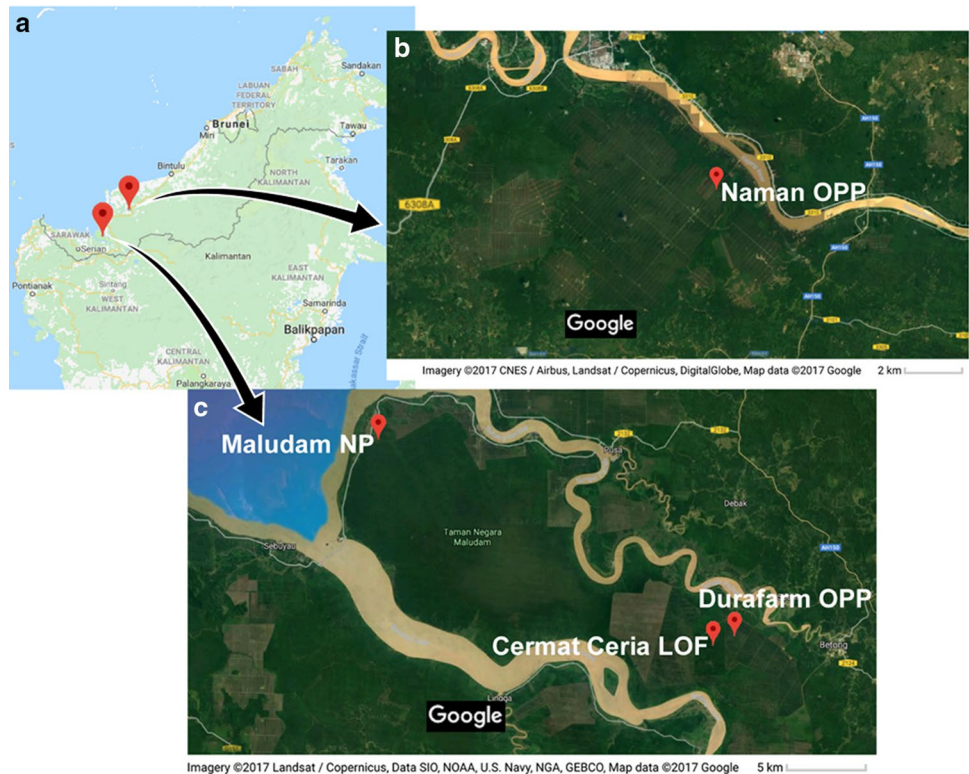
### 2.3 Enumeration of total fungal viable count

The soil dilutions of  $10^{-4}$  and  $10^{-5}$  were plated on PDA and MEA and incubated at  $25 \pm 2$  °C. The viable colonies on the plate were counted and recorded after 3 days of incubation. The total fungal viable count was calculated as colonies forming unit per 1 g of dry soil (cfu/g).

### 2.4 Morphological characterization

The macroscopic characteristic such as colony appearance was observed on PDA after 5 days' incubation at  $25 \pm 2$  °C.

**Fig. 1** Maps showing the location of study sites. **a** Borneo Island where the sites are located in Sarawak, Malaysia. **b** Naman OPP is located at Sibu and **c** Maludam NP, Cermat Ceria LOF and Durafarm OPP are located at Sri Aman, Sarawak. Cermat Ceria LOF is adjacent to the Durafarm OPP. Source of maps: Google maps Imagery©2017 <https://www.google.com.my/maps>



For microscopic characteristics, the conidia, conidiophores and phialides shape were examined using slide culture method. *Hypocrea* sp. were identified based on taxonomy key by Gams and Bissett (1998). The characteristics of *Aspergillus* and *Penicillium* species were observed and identified according to Diba et al. (2007) and Pitt (1979), respectively. Besides, morphological characteristics of other species were identified according to Watanabe (2002).

## 2.5 Amplification of 18S rDNA

The species identification of all isolates was done using DNA sequencing of 18S rDNA region. The pure isolates were grown on PDA for 3 days (fast-growing fungi) and 7 days (slow-growing fungi) before proceeding with the DNA extraction. The mycelium was harvested and extracted for genomic DNA using Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp., Canada) according to manufacturer's instruction. The 18S rDNA was amplified using a pair of primer: EF4-F (5'-GGAAGGG[G/A]TGTATTTAT TAG-3') and fung5-R (5'-GTAAAAGTCCTGGTTCCC C-3') (Smit et al. 1999). The PCR amplification was conducted in 25  $\mu$ l reaction master mix that consists of 1  $\times$  PCR buffer, 1.0  $\mu$ M of each primer, 0.2 mM of each dNTPs, 0.3 U of GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA), 2.5 mM MgCl<sub>2</sub>, 0.3% of bovine serum albumin (BSA) and 1.0  $\mu$ l DNA template. The amplification was performed in a thermal cycler (Applied Biosystems Veriti™

Thermal Cycler, USA) with an initial denaturation at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, extension at 72 °C for 1 min and 5 min final extension at 72 °C.

## 2.6 DNA sequencing

The PCR products were loaded on 1% agarose gel added with gel stain (FloroSafe DNA Stain, First BASE Laboratories, Malaysia). The gel electrophoresis was run at 80 V for 30 min and viewed under UV light. The amplicon sized 550 bp was excised from agarose gel and purified using EasyPure® Quick Gel Extraction Kit (TransGen Biotech, China). The purified PCR products were sent to First BASE Laboratories (Malaysia) for sequencing. DNA sequences obtained were analysed and compared using Basic Local Alignment Search Tool (BLAST) to determine the closest match sequences from a database (<http://www.ncbi.nlm.nih.gov>).

## 2.7 Phylogenetic tree

The sequences were aligned using CLUSTAL W in Molecular Evolutionary Genetics Analysis version 6.0 (MEGA 6.0). The aligned sequences then were used to construct a phylogenetic tree. The tree was generated based on neighbour-joining (NJ) method using Jukes–Cantor with 1000 bootstrap replications.



**Fig. 2** Sites with different gradient of disturbances. **a** Primary peat swamp forest, Maludam NP. **b** Cermat Ceria LOF is a logged-over forest which has been recovered naturally after logging. **c** Durafarm

oil palm plantation with 7.5-year-old oil palm and **d** Naman oil palm plantation with oil palm aged 11.5 years

## 2.8 Soil physiochemical analysis

Soil pH was determined by adding 10 g of peat samples to 50 ml of distilled water and stirring for 30 min before measuring using a pH meter (Mettler Toledo, Switzerland) (Gheda and Ahmed 2015). The moisture content of soil was determined by drying 5 g of peat samples in the oven at 105 °C for 24 h (Kelly and Sivakumar 2014). After 24 h, the peat samples were weighed again and the moisture content was calculated following Latiffah et al. (2011). Rainfall data were recorded for the sampling month (October 2012). On field measurement such as peat depth was also recorded for all sites. For carbon (C) and nitrogen (N) determination, peat samples were dried at 25 °C for 2 weeks until constant weight achieved. The peat samples were combusted at 1040 °C using a model of Primacs SNC analyser (Skalar, UK). Total C was determined by using infrared detection and total N using a thermal conductivity detector. For micro-nutrients, peat samples were dried at 60 °C for 2–3 days until constant weight was reached. About 0.3 g of dry samples was extracted with 95% nitric acid, digested in Titan MPS Microwave Sample Preparation System (PerkinElmer

SCIEX, USA) and diluted with MiliQ water before determination using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ELAN DRC (PerkinElmer SCIEX, USA). Analysis was conducted according to manufacturer's protocol.

## 2.9 Statistical analysis

The mean value of soil physiochemical properties for each site was calculated. The data of soil physiochemical properties and total fungal viable count were analysed by one-way ANOVA using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) to determine variation between each site.

## 3 Results

### 3.1 Total fungal viable count

Based on total fungal viable count (Fig. 3), Cermat Ceria LOF recorded the highest culturable fungal population on MEA with  $5.0 \times 10^5$  cfu/g compared to other sites. Meanwhile, fungal population was the highest on PDA for Naman

OPP with  $7.4 \times 10^5$  cfu/g. Statistical analysis revealed no significant difference in total fungal viable counts on PDA and MEA when compared between sites.

### 3.2 Species identification

A total of 294 isolates were recovered from peat samples in all sites. A total of 72, 74, 73, and 75 isolates were obtained from Maludam NP, Cermat Ceria LOF, Durafarm OPP, and Naman OPP, respectively (Table 1). Based on a BLAST search, all isolates showed similarity between 73 and 100% with sequences in GenBank (Online Resource 1). Three phyla which comprised of Ascomycota, Basidiomycota, and Zygomycota were identified. Peat soils in Sarawak are dominated by filamentous ascomycetes. Basidiomycota and Zygomycota were found in Cermat Ceria LOF and Durafarm OPP.

The total number of fungal species varied among sites. Naman OPP had the highest species richness with 18 species. Durafarm OPP accounted for 17 species, followed by Maludam NP (16 species) and Cermat Ceria LOF (15 species).

Among the 36 species identified, *Aspergillus fumigatus* (40 isolates) was commonly found across the sites. The second most common species was *P. chrysogenum* (37 isolates) followed by *Aspergillus* sp. (30 isolates) and *A. niger* (21 isolates). *Hypocrea* sp. identified as *H. atroviridis* (34 isolates) and *H. muroiana* (31 isolates) were the most common species in plantation areas, Durafarm OPP and Naman OPP. Other than that, *Penicillium roqueforti* (19 isolates) was found only in oil palm plantations.

Three species of higher fungi (*Antrodia variiformis*, *Byssomerulius albostramineus*, and *Ganoderma australe*) were isolated from Cermat Ceria LOF, and an isolate of *Pulcherricium caeruleum* was found in Durafarm OPP. A species of Zygomycota, *Mortierella chlamydospora* was

obtained from all sites except Naman OPP, while *Umbelopsis isabellina* was only recorded from Maludam NP.

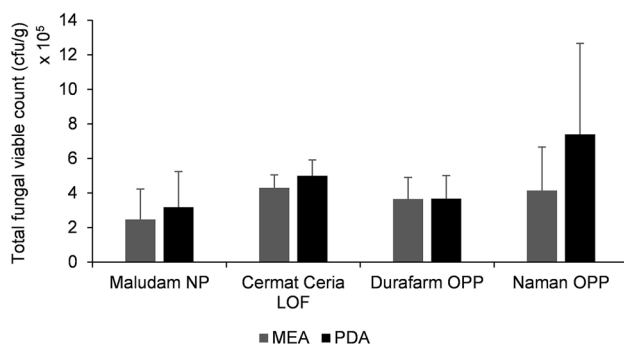
In Maludam NP, about 20.6% of total fungal isolates was dominated by *A. fumigatus* followed by *Penicillium corylophilum* (17.8%). *Penicillium corylophilum* can also be found in Naman OPP at a low percentage. A teleomorphic species of *Penicillium* known as *Talaromyces purpureogenus* was isolated and found in Maludam NP. The other species of *Penicillium* identified were *P. chrysogenum*, *P. citrinum*, and *P. namyslowskii*.

The most prevalent fungus in Cermat Ceria LOF was *P. chrysogenum* (24.3%) followed by *Aspergillus* species including *Aspergillus* sp. (21.6%), *A. fumigatus* (20.3%), and *A. niger* (16.2%). *Trichoderma* sp. and *T. harzianum*, the anamorphic stage of *Hypocrea*, were also identified in Cermat Ceria LOF. Other fungi species found were *Cladosporium cladosporioides*, *P. javanicum*, *Sarcladium* sp., *T. leycettanus*, and *M. chlamydospora*.

The fungal species in the two oil palm plantations were relatively the same. *Hypocrea muroiana* and *H. atroviridis* were commonly isolated from Durafarm OPP (27.4 and 26%) and Naman OPP (15 and 20%). About 17.8% of the isolates in Durafarm OPP consisted of *P. roqueforti*, while only 8% of *P. roqueforti* was found in Naman OPP. Besides that, *P. chrysogenum* was isolated from both sites with 9.3% in Naman OPP and 2.7% in Durafarm OPP. The uncommon species found in Durafarm OPP were *Daldinia* sp., *Hypoxyton haematostroma*, *Phomopsis* sp., *Pulcherricium caeruleum*, and *M. chlamydospora*. On the other hand, the least isolated species in Naman OPP were *Eurotiomycetes* sp., *Fusarium oxysporum*, *Neurospora crassa*, *P. namyslowskii*, *P. purpureogenum*, and *Purpureocillium lilacinum*.

Based on observations of isolates on PDA, the colony morphology varied. *Aspergillus fumigatus* and *A. niger* produced white-to-yellowish mycelia which turned into dark green and black with the formation of spores (Fig. 4a, b). Meanwhile, *Aspergillus* sp. produced black colonies and abundant white-to-yellowish mycelia (Fig. 4c). *Hypocrea muroiana* and *H. atroviridis* formed whitish-to-green circular colonies on PDA (Fig. 4d, e). *Penicillium corylophilum* produced yellow and light green colonies towards the edge of the colonies (Fig. 4f). The colonies of *Penicillium* sp. were dark green in colour (Fig. 4g). On the other hand, *P. javanicum* showed flat colonies of grey to whitish on PDA (Fig. 4h). The morphology of *P. chrysogenum* was different from other *Penicillium* species. It produced flat mycelia with yellow-and-white colony on PDA (Fig. 4i). *Umbelopsis isabellina* produced white sparse aerial mycelia (Fig. 4j), while *M. chlamydospora* formed fluffy white mycelia after five days of incubation (Fig. 4k).

For microscopic characterization, conidiophore of *A. niger* was long with dark spore masses at the tip (Fig. 5A). The conidia of *A. niger* were globose and found in clusters



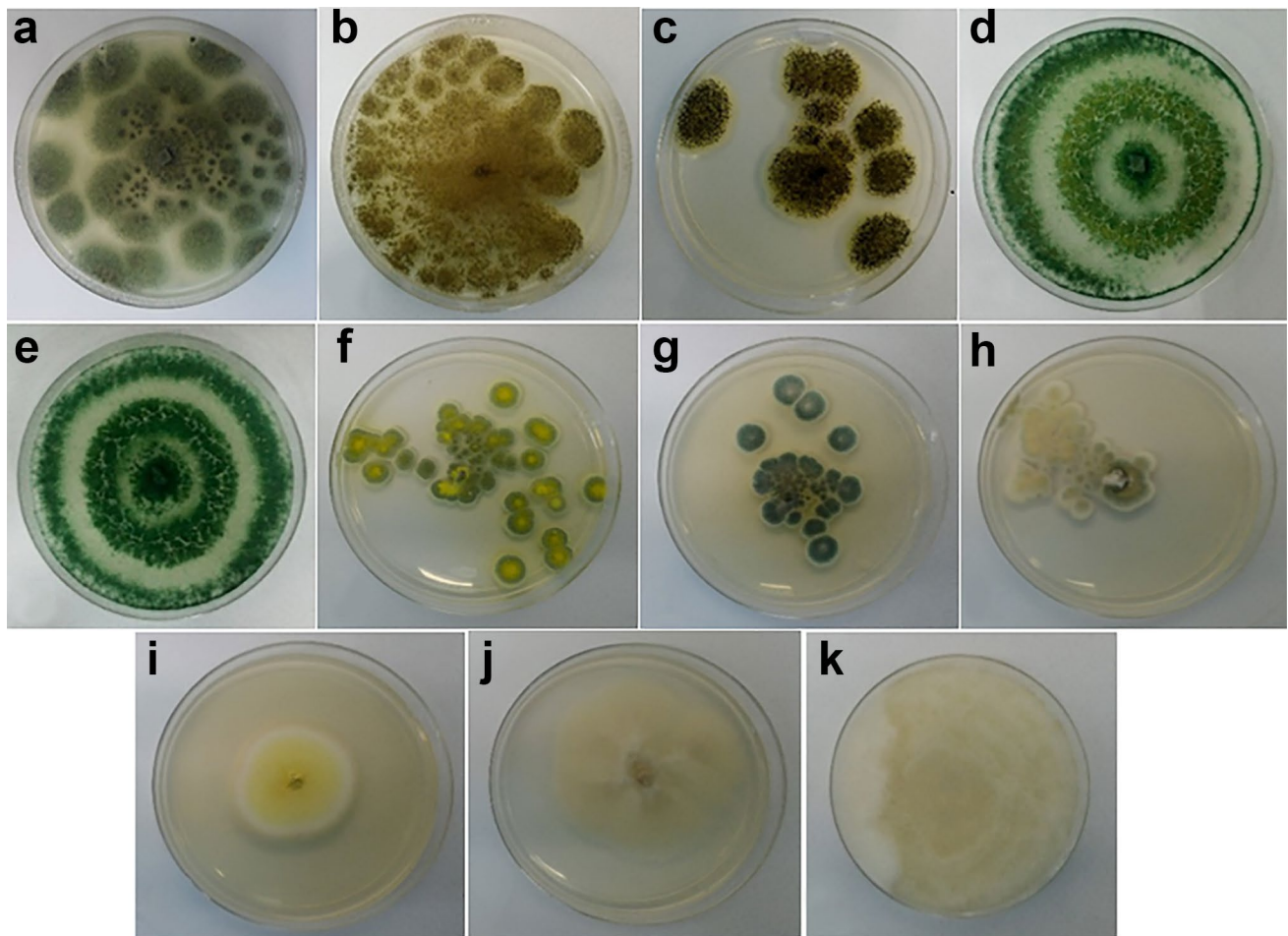
**Fig. 3** Mean of total fungal viable count on PDA and MEA in different sites. There was no significant difference of total fungal viable count between sites

**Table 1** Distribution and frequency of fungal species in different sites

Phylum	Species	Number of isolates (number of isolates in percentage, %)			
		Maludam NP	Cermat Ceria LOF	Durafarm OPP	Naman OPP
Ascomycota	<i>Aspergillus fumigatus</i>	15 (20.6)	15 (20.3)	4 (5.5)	6 (8.0)
	<i>Aspergillus niger</i>	3 (4.1)	12 (16.2)	2 (2.7)	4 (5.3)
	<i>Aspergillus</i> sp.	2 (2.7)	16 (21.6)	1 (1.4)	11 (14.7)
	<i>Aspergillus nomius</i>	–	–	–	1 (1.3)
	<i>Aspergillus ustus</i>	–	–	2 (2.7)	–
	<i>Cladosporium cladosporioides</i>	1 (1.4)	2 (2.7)	–	–
	<i>Daldinia</i> sp.	–	–	1 (1.4)	–
	<i>Eurotiomycetes</i> sp.	–	–	–	1 (1.3)
	<i>Fusarium oxysporum</i>	–	–	–	2 (2.7)
	<i>Hypocrea atroviridis</i>	–	–	19 (26.0)	15 (20.0)
	<i>Hypocrea muroiana</i>	–	–	20 (27.4)	11 (15.0)
	<i>Hypoxyton haematostroma</i>	–	–	1 (1.4)	–
	<i>Neurospora crassa</i>	–	–	–	1 (1.3)
	<i>Penicillium</i> sp.	3 (4.1)	1 (1.4)	2 (2.7)	–
	<i>Penicillium chrysogenum</i>	10 (13.7)	18 (24.3)	2 (2.7)	7 (9.3)
	<i>Penicillium citrinum</i>	2 (2.7)	–	1 (1.4)	–
	<i>Penicillium corylophilum</i>	13 (17.8)	–	–	2 (2.7)
	<i>Penicillium javanicum</i>	–	1 (1.4)	–	–
	<i>Penicillium namyslowskii</i>	2 (2.7)	–	–	1 (1.3)
	<i>Penicillium purpurogenum</i>	–	–	–	1 (1.3)
	<i>Penicillium roqueforti</i>	–	–	13 (17.8)	6 (8.0)
	<i>Penicillium spinulosum</i>	–	–	1 (1.4)	–
	<i>Phomosis</i> sp.	–	–	1 (1.4)	–
	<i>Pseudallescheria</i> sp.	1 (1.4)	–	–	–
	<i>Purpureocillium lilacinum</i>	–	–	–	1 (1.3)
	<i>Sarocladium</i> sp.	–	1 (1.4)	–	–
	<i>Simplicillium lamellicola</i>	4 (5.5)	–	–	–
	<i>Talaromyces leycettanus</i>	–	1 (1.4)	–	–
	<i>Talaromyces purpureogenus</i>	8 (11.0)	–	1 (1.4)	3 (4.0)
	<i>Trichoderma</i> sp.	–	2 (2.7)	–	–
	<i>Trichoderma harzianum</i>	–	1 (1.4)	–	2 (2.7)
	Uncultured <i>Eurotium</i>	1 (1.4)	–	–	–
Zygomycota	<i>Mortierella chlamydospora</i>	3 (4.1)	1 (1.4)	1 (1.4)	–
	<i>Umbelopsis isabellina</i>	4 (5.5)	–	–	–
Basidiomycota	<i>Antrodia variiformis</i>	–	1 (1.4)	–	–
	<i>Byssomerulius albostramineus</i>	–	1 (1.4)	–	–
	<i>Ganoderma australe</i>	–	1 (1.4)	–	–
	<i>Pulcherricium caeruleum</i>	–	–	1 (1.4)	–
	Total isolates	72 (100)	74 (100)	73 (100)	75 (100)
	Total species	16	15	17	18

(Fig. 5a). *Hypocrea muroiana* produced branch conidiophores and phialides (Fig. 5B) while the conidia were round (Fig. 5b). The conidiophore of *H. atroviridis* grew from the main hyphae and stout phialides were formed from the branch (Fig. 5C). The conidia shape was ovoid to round (Fig. 5c). *Penicillium chrysogenum* produced biverticillate branching and bore conidia in chains (Fig. 5D). The conidia

were found in clusters and chains with an ovoid shape (Fig. 5d). *Penicillium corylophilum* formed long monoverticillate conidiophores with chains of conidia (Fig. 5E). The conidia of *P. corylophilum* were phialosporous and globose (Fig. 5e). *P. roqueforti* formed long conidiophore that bore oval-to-round conidia in chains (Fig. 5F, f). *Umbelopsis isabellina* produced round and globose sporangiospores



**Fig. 4** Morphological characteristics of fungi isolated from different sites in Sarawak based on macroscopic observation on PDA after 5 days. **a** *A. fumigatus*, **b** *A. niger*, **c** *Aspergillus* sp., **d** *H. muroiana*, **e**

*H. atroviridis*, **f** *P. corylophilum*, **g** *Penicillium* sp., **h** *P. javanicum*, **i** *P. chrysogenum*, **j** *U. isabellina*, and **k** *M. chlamydospora*

(Fig. 5g). The special thick wall of resting structure known as chlamydospore was observed in the *M. chlamydospora* culture. The chlamydospores were globose and granulate, occurred singly, and were found in the intercalary and terminal sections of the hyphae (Fig. 5h).

### 3.3 Phylogenetic tree analysis

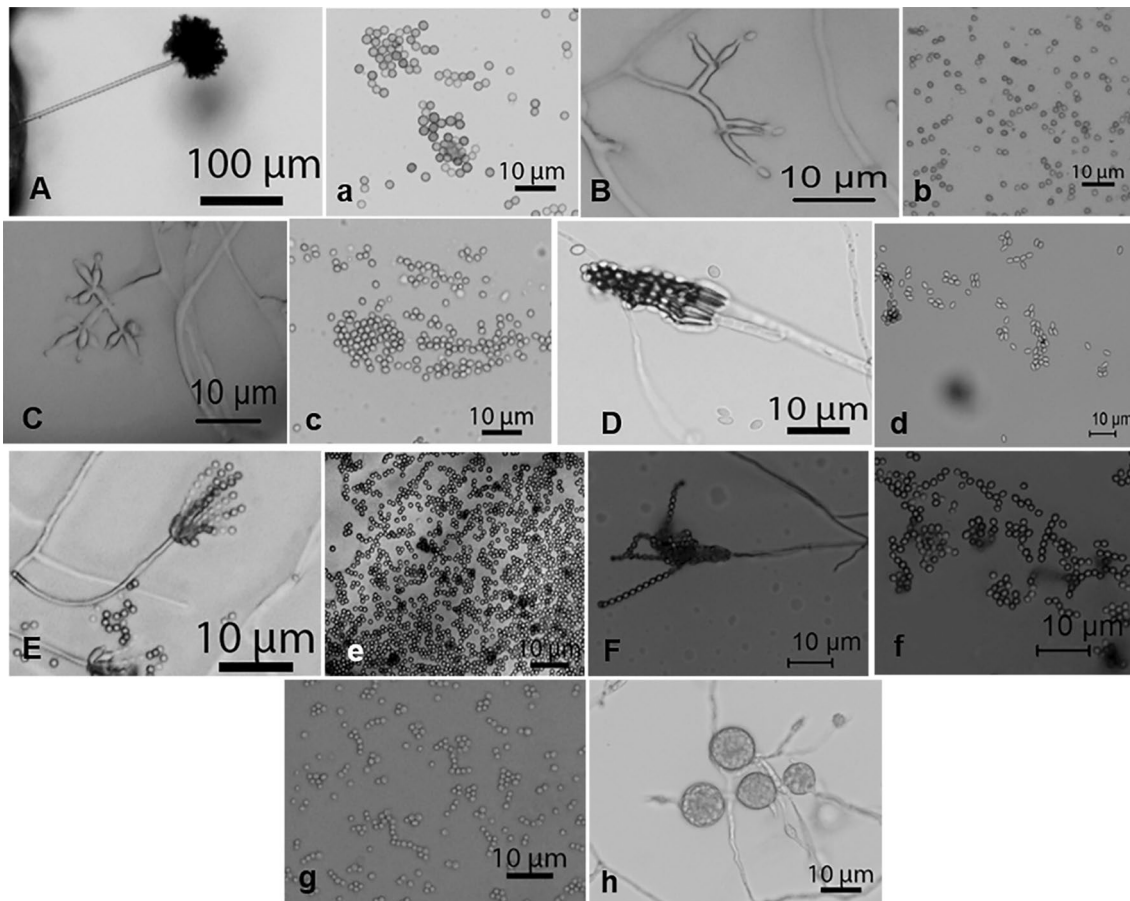
The tree was divided into four clades (Fig. 6). Clade A consisted of Ascomycota with a bootstrap value of 65%. Clades B and C consisted of isolates of Basidiomycota, while clade D consisted of Zygomycota. Clade A was divided into several subclades of Eurotiales (*Aspergillus*, *Penicillium*, and *Talaromyces*), Hypocreales (*Fusarium*, *Hypocrea* and its anamorphic stage, *Trichoderma*, and *Purpureocillium*), Xylariales (*Hypoxylon haematostroma*), Capnodiales (*Cladosporium cladosporioides*), and Diaporthales (*Phomopsis* sp.). Clade B consisted of an isolate of *Byssomerulius albostramineus*, a basidiomycete. Several species of

basidiomycetes obtained from tropical peat soil were placed on clade C. On the other hand, *M. chlamydospora*, a zygomycete, was located on clade D with a high bootstrap value of 100%. In contrast, two isolates of *U. isabellina* (Zygomycota) were placed distinctly from the other clades.

### 3.4 Soil physiochemical analysis

Total C content was the highest at the oil palm plantations, Naman OPP ( $55.77 \pm 0.27\%$ ) and Durafarm OPP ( $53.42 \pm 0.44\%$ ) compared to Maludam NP and Cermat Ceria LOF (Table 2). Meanwhile, total N content in peat soil was the highest at Durafarm OPP ( $1.88 \pm 0.44\%$ ) and lowest at Cermat Ceria LOF ( $1.28 \pm 0.48\%$ ). Concerning the C:N ratio, Cermat Ceria LOF had a higher value ( $38.88 \pm 18.11$ ) than other sites.

A comparison of the soil pH showed no significant difference among sites. The soil pH ranged between 3.37 and 3.41. The moisture content in all sites was significantly different



**Fig. 5** Morphological characteristics of fungi based on microscopic characteristics. Conidiophores (capital letter) and conidia (small letter) of **A** *A. niger*, **B** *H. muroiana*, **C** *H. atroviridis*, **D** *P. chrysogenum*, **E** *P. corylophilum*, **F** *P. roqueforti*, **g** *U. isabellina*, and **h** chlamydospores of *M. chlamydospora*

*num*, **E** *P. corylophilum*, **F** *P. roqueforti*, **g** *U. isabellina*, and **h** chlamydospores of *M. chlamydospora*

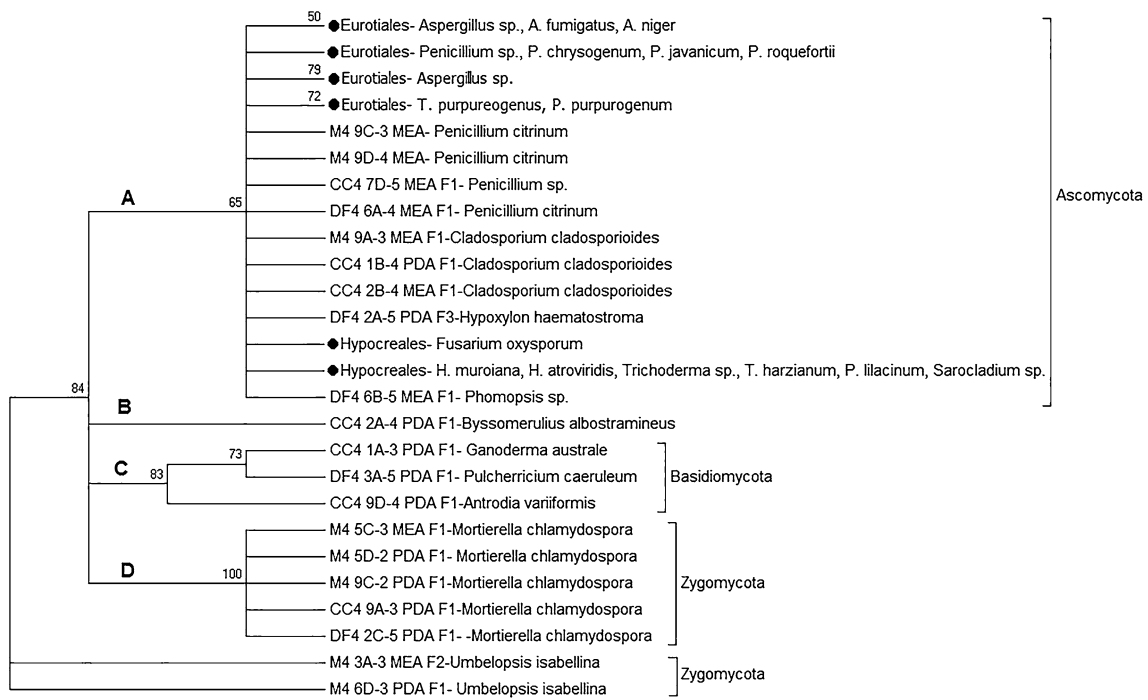
between each other. The moisture content in Durafarm OPP and Naman OPP was the highest with  $65.06 \pm 1.14\%$  and  $65.09 \pm 1.41\%$ , respectively. On the contrary, Maludam NP had the lowest moisture content with  $49.5 \pm 1.4\%$ . For peat depth, there was no significant difference and it ranged from 104.20 to 113.70 cm.

The concentrations of B, Mg, P, K, Ca, Mn, Fe, and Ni were significantly higher in Durafarm OPP compared to the other sites (Table 3). On the other hand, peat soil of Naman OPP showed a high content of trace elements such as Cu and Zn. In terms of heavy metal contents, Mo, Cd, and Pb were significantly greater in Cermat Ceria LOF compared to the other sites. In Maludam NP, Mg, P, K, Ca, Ni, Cu, Zn, Mo, and Cd showed the lowest value.

## 4 Discussion

Amongst sites, Naman OPP had the highest population of fungi on PDA, which was reflected by total fungal viable count. In this study, the oil palm plantations supported a large number of fungi compared to the forest. Fertilizer application in the plantations provides a favourable condition for fungal growth and enhances their population (Kerekes et al. 2013; Krashevskaya et al. 2013). Nurulita et al. (2015) also showed that fungal population was higher in oil palm and rubber plantation than in forest. Cermat Ceria LOF showed slightly higher fungal population on MEA as compared to the other sites. Nevertheless, both





**Fig. 6** A neighbour-joining tree showing the relationship between all isolates based on 18S rRNA gene. The tree was constructed using Jukes–Cantor method with 1000 replications of bootstrap value. The analysis involved 294 nucleotide sequences and the length of nucleotide for aligned sequences was 891 nucleotides. The tree was applied cut-off 50% with bootstrap values shown next to the branch. Symbol of ‘filled circle’ represented condensed clades which are consisted of the isolates in the same order

... (This text is merged into the caption above for better flow and to avoid redundancy.)

**Table 2** Soil physiochemical properties and soil measurement in field

Measurement	Maludam NP	Cermat Ceria LOF	Durafarm OPP	Naman OPP
Total C (%)	48.35 ± 1.30 <sup>ab</sup>	45.68 ± 3.09 <sup>a</sup>	53.42 ± 0.44 <sup>bc</sup>	55.77 ± 0.27 <sup>d</sup>
Total N (%)	1.71 ± 0.44 <sup>b</sup>	1.28 ± 0.48 <sup>a</sup>	1.88 ± 0.44 <sup>c</sup>	1.60 ± 0.32 <sup>b</sup>
C:N	28.61 ± 5.43 <sup>a</sup>	38.88 ± 18.11 <sup>b</sup>	29.13 ± 4.79 <sup>a</sup>	35.43 ± 5.36 <sup>b</sup>
Rainfall (mm)	296.5	276.0	213.5	327.5
pH	3.39 ± 0.02 <sup>a</sup>	3.41 ± 0.02 <sup>a</sup>	3.37 ± 0.02 <sup>a</sup>	3.41 ± 0.02 <sup>a</sup>
Moisture content (%)	49.5 ± 1.4 <sup>a</sup>	56.06 ± 0.76 <sup>b</sup>	65.06 ± 1.14 <sup>c</sup>	65.09 ± 1.41 <sup>c</sup>
Peat depth (cm)	112.10 ± 4.77 <sup>a</sup>	104.20 ± 5.65 <sup>a</sup>	113.70 ± 5.17 <sup>a</sup>	108.83 ± 2.40 <sup>a</sup>

Data reported as mean and standard error for different sites. <sup>a,b,c,d</sup>In the same row indicated the significant different (ANOVA  $p < 0.05$ )

media used in this study were rich with carbon and nitrogen, allowing the growth of sporulating species from all sites.

In this study, Ascomycota was recorded as the common taxon isolated from peatlands in Sarawak. This phylum was reported as the dominant phylum in bog and fen peat (Lin et al. 2012), as well as in permafrost peatlands (Tveit et al. 2013). They are usually fast-growing fungi with a high spore production rate, which enables them to thrive in the peat soil.

Several species of *Aspergillus* are common in Sarawak peat soil and were found in all sites. *Aspergillus* species are ubiquitous soil fungi that globally play a role in carbon

and nitrogen cycle (Dagenais and Keller 2009). *Aspergillus fumigatus* was the most abundant species in the primary forest as compared to the other sites. Conversely, *A. niger*, a soft rot fungus, was prevalent in Cermat Ceria LOF. A study by Hamed (2013) showed that *A. niger* degrades cellulose, rather than lignin, in wood decomposition. Thus, *A. niger* would have the same role to degrade simple structures during slow litter decomposition in logged-over forest. *Aspergillus fumigatus* and *A. niger* are tolerant to acid and temperature and are involved in phosphate solubilization process in mineral soil (Rinu and Pandey 2010).

The genus of *Penicillium* is a cosmopolitan fungus found in a wide range of ecosystems including peatlands.

**Table 3** Micronutrients content analysis of peat soil in different sites

Nutrient (mg kg <sup>-1</sup> )	Maludam NP	Cermat Ceria LOF	Durafarm OPP	Naman OPP
B	2.30 ± 0.05 <sup>ab</sup>	2.39 ± 0.03 <sup>b</sup>	2.81 ± 0.07 <sup>c</sup>	2.19 ± 0.03 <sup>a</sup>
Mg	715.38 ± 49.11 <sup>b</sup>	1553.81 ± 41.81 <sup>c</sup>	2248.44 ± 120.30 <sup>d</sup>	505.56 ± 14.10 <sup>a</sup>
P	205.08 ± 10.48 <sup>a</sup>	276.86 ± 12.05 <sup>b</sup>	442.40 ± 35.90 <sup>c</sup>	278.92 ± 9.72 <sup>b</sup>
K	511.99 ± 81.41 <sup>a</sup>	860.35 ± 26.99 <sup>b</sup>	1581.20 ± 74.08 <sup>c</sup>	997.83 ± 79.66 <sup>b</sup>
Ca	403.34 ± 21.47 <sup>a</sup>	1831.34 ± 160.99 <sup>c</sup>	3153.08 ± 179.91 <sup>d</sup>	1207.27 ± 31.74 <sup>b</sup>
Cr	7.57 ± 0.69 <sup>b</sup>	13.32 ± 0.98 <sup>c</sup>	16.02 ± 1.03 <sup>d</sup>	5.33 ± 0.18 <sup>a</sup>
Mn	14.84 ± 1.67 <sup>a</sup>	24.93 ± 2.88 <sup>b</sup>	64.82 ± 6.21 <sup>c</sup>	12.91 ± 0.98 <sup>a</sup>
Fe	1899.58 ± 251.17 <sup>ab</sup>	1344.92 ± 110.16 <sup>a</sup>	2395.09 ± 300.57 <sup>b</sup>	2246.53 ± 127.20 <sup>b</sup>
Ni	4.23 ± 0.52 <sup>a</sup>	9.47 ± 0.48 <sup>b</sup>	12.00 ± 0.74 <sup>c</sup>	4.70 ± 0.67 <sup>a</sup>
Cu	3.37 ± 0.21 <sup>a</sup>	10.08 ± 0.32 <sup>ab</sup>	23.05 ± 6.18 <sup>b</sup>	62.33 ± 11.84 <sup>c</sup>
Zn	6.77 ± 0.91 <sup>a</sup>	26.34 ± 2.18 <sup>b</sup>	54.06 ± 5.15 <sup>c</sup>	57.27 ± 5.42 <sup>c</sup>
Mo	0.25 ± 0.01 <sup>a</sup>	9.98 ± 0.05 <sup>d</sup>	8.84 ± 0.32 <sup>c</sup>	1.61 ± 0.007 <sup>b</sup>
Cd	0.04 ± 0.0005 <sup>a</sup>	9.41 ± 0.07 <sup>d</sup>	8.18 ± 0.31 <sup>c</sup>	1.85 ± 0.15 <sup>b</sup>
Pb	3.48 ± 0.71 <sup>a</sup>	11.56 ± 0.36 <sup>b</sup>	12.73 ± 0.25 <sup>b</sup>	2.67 ± 0.14 <sup>a</sup>

Data reported as mean and standard error for different sites. <sup>a,b,c,d</sup>In the same row is indicated the significant different (ANOVA  $p < 0.05$ )

*Penicillium chrysogenum* is a soft rot fungus, which decomposes hardwood rather than softwood trees (Hamed 2013). This is supported by the result in this study where *P. chrysogenum* was highly prevalent in both primary and logged-over forests. This species is able to live in saline and acidic soil (Hujšlová et al. 2010). *Penicillium corylophilum* has been reported to inhabit mangroves, where oxygen is depleted (Arfi et al. 2012). Thus, it can survive in peat soil. *Penicillium roqueforti* was present in peat soil of both oil palm plantations. However, *P. roqueforti* has only been widely used in cheese production before; other information on its association in peat soil remains unknown. *Talaromyces purpurogenus* is able to solubilize insoluble phosphorus in soil for plant utilization (Vieira and Nahas 2005). This species was isolated from tropical forests in India (Devi et al. 2012) and in acidic soils located in the Czech Republic (Hujšlová et al. 2010).

The *Hypocrea* genus is ubiquitous, widely distributed, and associated with a wide range of substrates (Chaverri et al. 2015). *Hypocrea* spp. are also the predominant species of soil mycobiota (Jaklitsch and Voglmayr 2015). In the agricultural industry, *Hypocrea* species are economically important as biocontrol agents against diseases and pests, as well as agents to promote nutrient uptake in plants (Druzhinina et al. 2011; Harman et al. 2012). In the present study, *H. atroviridis* (= *Trichoderma atroviride*) and *H. muroiana* (= *T. koningii*) were abundantly distributed in oil palm plantations. *Hypocrea atroviridis* was commonly found in peat soil of oil palm plantations (Ramlah Ali et al. 2016). Besides that, *H. atroviridis* produces laccase enzymes that are used in lignin biodegradation (Chakroun et al. 2010). In the denitrification process, *H. muroiana* is involved in nitrous oxide

(N<sub>2</sub>O) production that occurs in soil (Mothapo et al. 2015). Both species work as an extensive network to increase the nutrient uptake of oil palm. The anamorphic stage of *Hypocrea*, namely *Trichoderma* sp. and *T. harzianum*, were found in Cermat Ceria LOF and Naman OPP. *Trichoderma harzianum* is widely reported as having antagonistic properties against plant pathogens, such as *Ganoderma boninense* in oil palm plantations (Naher et al. 2012). Presence of *T. harzianum* in Naman OPP has a positive impact on oil palm health, reducing the basal stem rot incidence in this site.

In a matured oil palm plantation like Naman OPP, a few isolates of *Fusarium oxysporum* were isolated. This species causes vascular wilt disease (Rusli et al. 2015) and crown disease (Hafizi et al. 2013) on oil palm. *Fusarium oxysporum* was previously isolated from peat bog (Golovchenko et al. 2013) and tropical peat (Nurul Farah et al. 2016) as it is involved in the N<sub>2</sub>O-producing activity in soil (Mothapo et al. 2015).

Basidiomycetes were found in Cermat Ceria LOF and Durafarm OPP in low frequency. Basidiomycetes may take the role as polymer decomposers in logged-over forests and plantations for decomposition process. These fungi, widely known as complex polymer degraders, degrade lignin and plant biomass polysaccharide (Lundell et al. 2010). *Antrodia variiformis*, found in Cermat Ceria LOF, is a polypore; the same species was isolated from an ombrophilous mixed forest in Brazil (Westphalen and Silveira 2012).

Other species of basidiomycetes, such as *Byssomerulius albostramineus* and *Ganoderma australe* isolated in this study, are commonly reported to be associated with plants as wood-decay fungi (Stokland and Larsson 2011; Schwarze et al. 2012). The population of Basidiomycetes

was underestimated since this study focused on soil samples. The culture-dependent method generally isolates sporulating fungi in the Ascomycota and Zygomycota divisions as compared to Basidiomycota. Besides that, the fruiting body production of basidiomycetes is erratic and influenced by moist weather (Rydin and Jeglum 2013).

Two species of Zygomycota were found in Sarawak peat. Members of *Mortierella* are common saprobic fungi associated with soil (Wagner et al. 2013). Based on micromorphological analysis of *M. chlamydospora*, it was observed to have a special thick wall structure and chlamydo-spores that can survive and remain dormant in peat soil. The percentage of occurrence of *Mortierella* species in peat is low, as reported by Dobrovol'skaya et al. (2012). Meanwhile, another species of zygomycetes, *U. isabellina*, was isolated from the root of conifers in peatlands and act as an endophytic fungus (Terhonen et al. 2014).

The molecular data resolved three divisions of fungi, indicating a variation of intraspecific level as shown in the neighbour-joining tree. In this study, isolates of *T. purpurogenum* formed a monophyletic group with *P. purpurogenum* (synonym of *T. purpurogenum*) and were placed on different clade from *Penicillium* genus. This is because the *Talaromyces* genus was introduced to accommodate the *Penicillium* subgenus *Biverticillium*, which is phylogenetically distinct from *Penicillium* (Yilmaz et al. 2014). Thus, isolates of *T. purpurogenum* were not grouped together with other *Penicillium* species. Besides, *U. isabellina* was placed apart from *Mortierella* group as they formed a new family, Umbelopsidaceae (Meyer and Gams 2003).

Molecular identification in this study also supported the morphological characterization based on macroscopic and microscopic characteristics. Most of the isolated fungi, such as *Penicillium* sp., *Aspergillus* sp., and *Hypocrea* sp., were prolific spore producers on media (Thormann 2006). The characteristics of the conidia with minute size enabled them to colonize and distribute well in peat soil.

Fungal abundance and richness increased with high abundance of plant diversity and richness, especially in forest soil (Costa et al. 2012; Dassen et al. 2017). However, the present study showed slightly higher species richness and abundance of fungi in oil palm plantations as compared to forest sites. This result did not reflect the previous report by Zak et al. (2003), where the abundance of plant diversity enhanced the fungal community in the soil. The difference in results was probably because of the characteristics of peat itself and the abundance of organic materials in oil palm plantations to provide a good niche to fungi. Moreover, there were limitations and biases of the culture-dependent method, since this method overestimated sporulating species as compared to slow-growing fungi and fungi which cannot grow on media. The nutrient contents in soil might contribute to high species richness

in oil palm plantation ecosystems, which are scarce in the forests.

Abiotic factors such as pH, organic contents, and moisture influence the distribution and diversity of fungal species (Devi et al. 2012). Generally, fungi favour acidic conditions for growth and reproduction (Nurul Farah et al. 2016). Most of the species isolated in this study were previously reported to be tolerant to low pH and high temperature, such as *Penicillium*, *Aspergillus*, and *Trichoderma* (Rinu and Pandey 2010). Nutrients in peat soil influenced the fungal species composition. A direct relationship showed that oil palm plantations have higher nutrient contents in soil and a higher number of fungal species as compared to the forests.

The rate of decomposition is reflected by the C:N ratio in the soil (Hazelton and Murphy 2007). A high total C and low N indicate a slow decomposition rate in soil (Melling 2016). Maludam NP showed the highest decomposition rate, followed by Durafarm OPP, Naman OPP, and Cermat Ceria LOF. According to Banerjee et al. (2016), the decomposition rate in an ecosystem is determined by the abundance of the keystone taxa, which is associated with organic matter degradation. Even though Maludam NP exhibited a low number of fungal species (16 species, 72 isolates), the abundance of the same species, such as *A. fumigatus* and *P. chrysogenum*, was large enough to carry out a high decomposition rate as compared to oil palm plantations, Durafarm OPP and Naman OPP. Besides, the peat in Maludam NP (decomposed sapric materials) was associated with high decomposition rate in peat soil. On the other hand, Cermat Ceria LOF showed the slowest decomposition rate, which may be due to chemical recalcitrant in plant debris (Edmonds 1991). Other factors, such as climate may also affect the rate of soil decomposition (Lutzow et al. 2006).

In this study, peat soil in oil palm plantations has a high moisture content as compared to primary and logged-over forests. This result contradicts with a study by Nurulita et al. (2015), where forests showed higher moisture content than oil palm plantations. This was because the samples were taken from disturbed edges of Maludam NP (Online Resource 2). Microbes respond to moisture content changes to survive and carry out their functional activities in the soil (Manzoni et al. 2012).

## 5 Conclusions

In the peatlands of Sarawak, Malaysia, different species of fungi were identified from the phyla Ascomycota, Basidiomycota, and Zygomycota. Most of the species isolated were saprophytic fungi that are able to survive in peat soil. These fungi had prominent roles in maintaining peat ecosystem function. Fungi species' composition in peat soil of Sarawak differed among sites and this might be driven by the nutrient

contents and abundance of organic material in the sites. Species of *Aspergillus* and *Penicillium* were predominant in peat forests while *Hypocrea* species were commonly found in oil palm plantations. However, the culture-dependent method still showed no clear difference for fungal species richness and abundance in Sarawak peatlands. Therefore, a different approach such as metagenomics is needed to study the fungal diversity, richness, and composition in peat soil comprehensively.

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