# PROKARYOTIC BIODIVERSITY IN MATURED OIL PALM PLANTATION COMPARED TO LOGGED-OVER AND PRIMARY FOREST IN DEEP PEAT, SARAWAK, MALAYSIA

# MOHD SHAWAL THAKIB MAIDIN\*; SAKINAH SAFARI\*; NUR AZIEMAH ABU GHANI\*; SHARIFAH AZURA SYED IBRAHIM\*; SHAMSILAWANI AHAMED BAKERI\*; MOHAMED MAZMIRA MOHD MASRI\* and SITI RAMLAH AHMAD ALI\*

## ABSTRACT

Soil microbes are the unseen living organisms which are involved in various roles for oil palm productivity. Matured oil palm plantation peat soil may be inhabited by different bacterial populations compared to logged-over and primary forest. Thus, conversion of peat forests into oil palm plantations might cause changes in the prokaryotic population in soil. The differences in the prokaryotic population were analysed to assess the profile of the prokaryotic population in each ecosystem. In this study, Denaturing Gradient Gel Electrophoresis of the purified 16 rDNA amplicon (16S PCR-DGGE) was analysed. Berger-Parker and Shannon-Weaver Species Index indicated that Durafarm Plantation, DF (0.10, 8.05) had the most diverse soil population followed by Maludam primary forest, MD (0.11, 7.75) and Cermat Ceria loggedover forest, CC (0.19, 7.63). The 16S rDNA sequence analysis showed that all ecosystems were dominated by unclassified Bacteria (62%, 65%, 69%), followed by Acidobacteria (8%, 19%, 4%), Actinobacteria (8%, 6%, 12%),  $\alpha$ -Proteobacteria (8%, 3%, 8%) and Firmicutes (5%, 2%, 2%), respectively for MD, CC and DF. Increased number of species was recorded in Durafarm Plantation with the emergence of the forest microbes such as Acidipila rosea, Acidimicrobium sp., Actinomycete sp., Mycobacterium sp., Afipia sp., Acetobacteraceae bacterium, Rhizobium sp., Rhodoplanes sp. and Sphingomonadaceae bacterium. It is interesting to note that planting oil palm on deep peat can assist in rejuvenating some bacterial population that were missing during forest clearing and thus contributing to the improvement of soil bacterial biodiversity.

Keywords: prokaryotic diversity, deep peat, primary forest, logged-over forest, 16S PCR-DGGE.

Date received: 12 October 2017; Sent for revision: 13 October 2017; Received in final form: 18 January 2018; Accepted: 5 July 2018.

## **INTRODUCTION**

Growing demand of oil palm products in Malaysia and Indonesia for domestic and international usage had resulted in increment of oil palm plantation

\* Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. E-mail: shawal@mpob.gov.my (Laurance, 2007; Tiku and Bullem, 2015, Kushairi *et al.*, 2017). Challenges surface to manage public perception and stay sustainable (Kushairi *et al.*, 2017). It was reported that, carbon dioxide emission had increased by 0.5 Pg C yr<sup>1</sup> in peatlands due to deforestation, degradation and fires (Baccini *et al.*, 2012). Furthermore, Conrad *et al.* (2012) reported that methane emission in paddy cultivation had elevated by 33% compared to the secondary forests. Drier northern peatlands of Finland had also

recorded increased rates of soil microbial nitrous oxide production and organic matter mineralisation (Oertel *et al.*, 2016). Water content reduction, cramped pores and declining organic particle sizes due to increased rate of decomposition have also disrupted the peatland environmental amenities in term of its ecological function (Huat *et al.*, 2011).

The diversity and functionality of microbial communities in an ecosystem have direct connectivity to the environmental chemical, physical and biological components, thus making these parameters highly feasible for detecting changes (Mandic-Mulec *et al.*, 2014). Soil fertility, structure and quality maintenance were highly influenced by the activity of microorganisms underneath the ground (Hu *et al.*, 2016). Bowles *et al.* (2014) reported that the activities of soil microorganisms were highly influenced by the different management practices, quality and quantity of plant materials in the soil. Therefore, natural or anthropogenic soil disruption could influence the microbial diversity of peatlands (Preston and Basiliko, 2016).

Nevertheless, information on the influence of physico-chemical changes in tropical peatlands was still limited as opposed to the boreal and temperate peatlands (Jauhiainen et al., 2016). Moreover, the microbial population diversity, activity and functionality in boreal and temperate peatlands have also been extensively studied (Jackson et al., 1995; Jackson and Vallaiere, 2007; Merino et al., 2016). It is important to investigate the microbial changes during the transition from forest to agricultural ecosystem, to understand its immediate impact to the microbial community sustainability in tropical peatlands. Therefore, this research was aimed to compare the tropical peat soil microbial diversity in matured oil palm plantation (Durafarm) developed from the primary (Maludam) and logged-over forests (Cermat Ceria) that had been previously reported in Maidin et al. (2016). The diversity of soil microorganisms as affected by land use change before and after land clearing was also observed. This research was also targeted to obtain information on bacterial strains and construct a prokaryotic data on the studied peatland. Therefore, the relationships between the microbial diverse communities in tropical peatlands in those aforementioned ecosystems were investigated.

## MATERIALS AND METHODS

#### **Peat Soil Sampling**

Soil samples were collected from peat areas in Sri Aman, Sarawak (*Figure 1*). The sampling locations included Maludam National Park (MD), Cermat Ceria logged-over forest (CC) and Durafarm Plantation (DF), each representing the primary peat swamp forest, logged-over peat swamp forest and oil palm plantation, respectively. Ten global positioning system (GPS) points encompassing the sampling plots were recorded using Trimble Juno 3D handheld GPS device (Trimble Navigation Limited, Sunnyvale, CA) (*Table 1*). The soil within 0-30 cm depth in between the topsoil and water table was collected in 50 ml falcon tubes. A total of 25 g of soil sample was collected in triplicates from each coordinate point. The samples were chilled and stored at 10°C prior to DNA isolation.

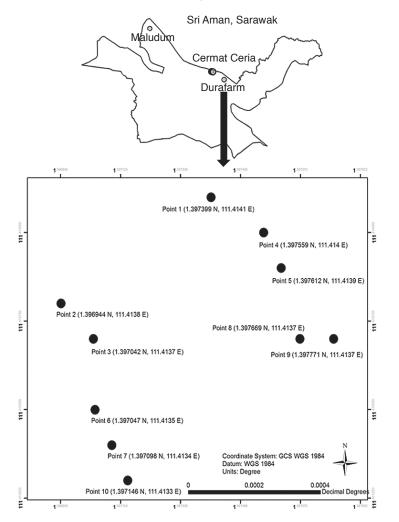
## **DNA Extraction**

The total DNA was extracted from the peat soil samples by using GeneMATRIX Soil DNA Purification Kit (Eurx Ltd, Gdansk, Poland). The amount and quality of the DNA were quantified using Nanophotometer TM P360 (Implen GmbH, Schatzbogen, Germany). The minimum concentration of DNA for optimal amplification was established at 10 ng  $\mu$ <sup>1</sup> with OD<sub>260</sub>/<sub>280</sub> purity of 1.7-1.8. Extracted genomic DNA was stored at -20°C until further analysis.

## Polymerase Chain Reaction (PCR)-Denaturing Gradient Gel Electrophoresis (DGGE) Analysis

The V3-V5 region (550 bp) of 16S ribosomal ribonucleic acid (rRNA) gene of the bacteria was amplified using universal bacterial primer set 341F-GC clamp (5'-cgc-ccg-cgc-gcg-gcg-ggc-ggg-gcgggg-gca-cgg-ggg-gcc-tac-gg-agg-cag-cag-3') and 907R (5'-ccc-cgt-caa-ttc-att-tga-gtt-t-3') (Muyzer et al., 1997; Overmann and Tuschak, 1997). Total PCR reaction buffer volume of 25 µl containing a series of 10 pmol of each primer, 100 mM dNTP, 1X PCR buffer, 50 mM Mg<sub>2</sub>Cl, 0.3% BSA, 2.5 units of Taq polymerase and template DNA was prepared and performed using a thermocycler (Gradient). The thermocycling condition was set for initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s and final extension at 72°C for 2 min and held at 10°C. The PCR products were subsequently subjected to DGGE analysis.

The amplicons of the V3-V5 region were utilised for separation of DNA sequences by DGGE (Simpson *et al.*, 2004). The gradient of denaturants and running conditions were optimised as follows: 40 µl of guanine-cytosine (GC) clamped-amplicons were resolved in 6% acrylamide (37.5:1, acrylamide:Bisacrylamide) perpendicular gels in a 40%-70% gradient of denaturants (where 100% denaturant concentration was equal to 7 M urea (Winkler, Ltd) and 40% (v/v) of deionised formamide (Amresco<sup>®</sup> Solon Ind., Ohio). Tetramethylethylenediamine (TEMED) and ammonium persulphate (APS) were added to a final concentration of 0.1%. Each gel



#### Biodiversity Sampling Points at Durafam

Figure 1. Biogeographical points located at Durafarm Oil Palm Plantation, Sri Aman, Sarawak, Malaysia.

was loaded with 100 bp marker as reference for normalisation of differences among different gels. Gel electrophoreses were run in 1× Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.0) solution at a constant voltage of 65 V for 17 hr at 60°C using Bio-Rad D-Code<sup>TM</sup> Universal

TABLE 1. LOCATION OF SAMPLING POINTS STUDIED IN DEEP PEAT SARAWAK, MALAYSIA

Estates	Sampling points	Location on GPS
Durafarm	1	N, 1°23' 50.63697" E, 111°24' 50.59624"
Plantation	2	N, 1°23' 48.99708" E, 111°24' 49.62261"
	3	N, 1°23' 49.35106" E, 111°24' 49.30407"
	4	N, 1°23' 51.21340" E, 111°24' 50.47918"
	5	N, 1°23' 51.40201" E, 111°24' 50.03203"
	6	N, 1°23' 49.55455" E, 111°24' 48.16225"
	7	N, 1°23' 51.60689" E, 111°24' 49.45209"
	8	N, 1°23' 51.97572" E, 111°24' 49.26872"
	9	N, 1°23' 49.72718" E, 111°24' 47.85654"
	10	N, 1°23' 49.72718" E, 111°24' 47.85654"

Mutation Detection System. The gels were soaked in 0.1% (vol/vol) SYBR staining gel diluted in 1× TAE buffer for 1 hr and visualised under UV illumination using AlphaImager HP (Alpha Innotech, San Leandro, CA).

#### **Band Matching Analysis of PCR-DGGE Profiles**

The analysis of PCR-DGGE patterns was performed using Phoretix 1D Gel Analysis software (Total Lab Quant Ltd, United Kingdom). Dice similarity coefficient (Dsc) was used to compare the whole image profiles. Unweighted pair group with mathematical averages (UPGMA) at 1% position tolerance was performed to generate the dendogram tree. The DGGE gels were numbered 1B until 10B which represented the 10 different points.

#### Sequencing of DGGE Band

High intensity DGGE bands were excised eluted with 50  $\mu$ l of Tris-EDTA (TE) buffer and incubated

overnight at 4°C. Eluted DNA from excised gel was utilised as DNA templates for re-amplification using 16S rDNA primers, 341f (with no GC-clamp) (5' –cctacg-gga-ggc-agc-ag-3') and 907R reverse(r) (Muyzer *et al.*, 1997). The PCR products were purified using QIAquick gel extraction kits (QIAGEN, Inc., Valencia, CA) according to the manufacturer's instruction.

The PCR products were sent to First Base Laboratories (Malaysia) for sequencing. Sequence similarity searches were conducted using the nucleotide-nucleotide basic logic alignment search tool (BLASTn) of the National Centre for Biotechnology Information (NCBI) GenBank database to identify the nearest relatives of the partially sequenced 16S rRNA genes of excised bands.

## **Phylogenetic and Statistical Analysis**

The nucleotide sequences were aligned and Neighbor-Joining Tree was generated using MEGA version 4.0 (Molecular Evolutionary Genetics Analysis [http://www.megasoftware. net]). The Neighbor-Joining phylogenetic tree was reconstructed based on the position of the 16S rRNA gene by using the Kimura two-parameter substitution model evaluated by 1000 bootstrap resampling of the data, and nodes with bootstrap values were indicated.

Alpha diversity index (Shannon-Weaver biodiversity index, Berger-Parker dominance index and Simpson's diversity index) of the abundance, dominance and evenness of the species present in the three different ecosystems were calculated based on method adapted from Hill (1973) and Hammer (2015) by using Paleontological statistics (PAST).

## **RESULTS AND DISCUSSION**

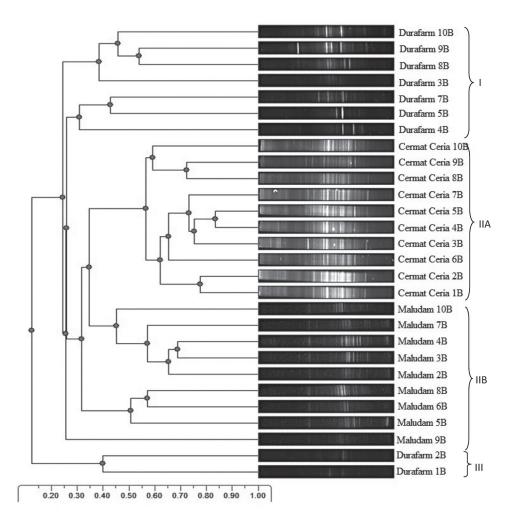
The DGGE fingerprints were analysed using dice coefficient and UPGMA algorithms performed in Phoretix 1D software (*Figure 2*). The dendogram was reconstructed based on the 16S rDNA bacterial community fingerprints of MD, CC and DF. The differences between DGGE band profiles were indicated by the percentage of similarity. The DGGE-

DGGE profile analysis has shown that the total bacterial communities of the two deep peat forests and the oil palm plantation were grouped into three clusters. Cluster II formed the major cluster with two separate minor clusters consisting of peat forests CC and MD whilst cluster I and cluster III, comprising the oil palm plantation and DF, remained as two distinct clusters. Cluster IIA and Cluster IIB consisting the two deep peat forests revealed about 33% of genetic similarity. MD in cluster IIB showed distinct profiles than CC in cluster IIA, as described by Maidin et al. (2016). Cluster II was dissimilar to Cluster I, which comprised of bacterial fingerprints of DF, by a lower similarity percentage of 25%. Cluster III remained having the least similarity of lower than 20% with other clusters. Overall, analysis on DGGE profiles revealed high genetic similarity in the deep peat forests which grouped them into a major cluster, compared to the oil palm plantation which formed distinct clusters due to low genetic similarity.

Shannon-Weaver prokaryotic biodiversity index showed that the sampling location DF had the most diverse bacterial species, calculated at 8.05 followed by MD, 7.75 and CC, 7.63 (Table 2). Berger-Parker and Simpson-1D indices also showed that the bacterial community in DF was more diverse than in MD and CC (*Table 2*) (*Figure 3*) even though a relatively large number of unclassified bacteria were recorded. The population of bacterial community was presented on a pie chart (Figure 4) in comparison to previous study done by Maidin et al. (2016). The chart showed that at least 12 phyla were present in DF (*Figure 4c*), representing the highest number of phyla compared to only 10 phyla in MD (*Figure 4a*) and CC (*Figure 4b*). High diversity of bacterial phyla in DF suggested that conversion of peatland forests into oil palm plantations did not reduce the bacterial population in peat soil. The unclassified bacteria dominated the largest section in the pie chart projection in all three peat ecosystems with percentage range of 62%-69% (*Figure 4*). These unclassified bacterial clusters were reportedly lacking proper taxonomic placement which eventually lead to enormous listing in the database (Tuzhikov et al., 2014). It is also important to note on the probable presence of undescribed novel taxa being listed among these (Maidin et al., 2016; Logan et al., 2009).

TABLE 2. MICROBIAL BIODIVERSITY INDICES AND SOIL pH FOR TOTAL MICROBES ON PEAT SOIL SAMPLED FROM MALUDAM PRIMARY PEAT FOREST, CERMAT CERIA LOGGED-OVER FOREST AND DURAFARM OIL PALM PLANTED ECOSYSTEM

Ecosystem	Shannon-Weaver biodiversity index	Berger-Parker dominance index	Source	pH (average)
Maludam	7.748	0.11	Maidin <i>et al.</i> (2016)	3.58
Cermat Ceria	7.627	0.19	Maidin <i>et al</i> . (2016)	3.68
Durafarm	8.046	0.09	This study	3.37



Note: B - peat level at above water table (Phoretix 1D).

Figure 2. Dendogram constructed with the 16S rDNA bacterial community fingerprint of Maludam reserved deep peat forest, Cermat Ceria logged-over forest and Durafarm Oil Palm Plantation. The differences between profiles are indicated by percentage of similarity. The dendrogram was based on Dice Coefficient index and cluster analysis by the unweighted pair group method using arithmetic averages (UPGMA).

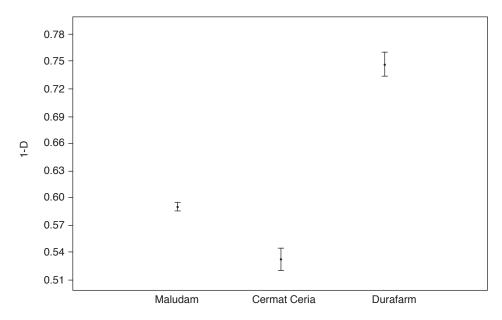


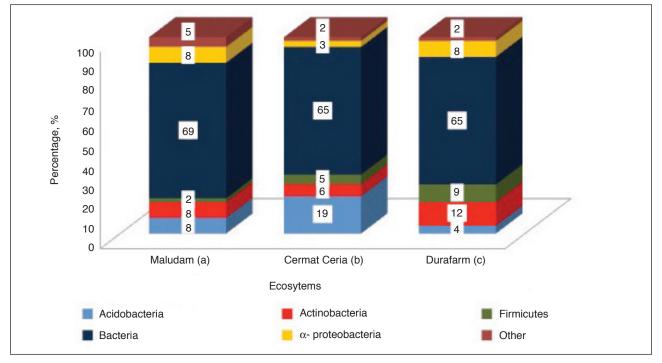
Figure 3. Simpson-1D diversity index of Maludam primary forest, Cermat Ceria logged-over forest and Durafarm oil palm planted ecosystem.

Lower percentage of Acidobacteria population in the main forest and oil palm plantation ecosystems (Figures 4a and 4c) compared to CC (Figure 4b) indicated that these bacterial phyla were more dominant in logged-over forests. DF also recorded a higher diversity of Actinobacteria,  $\alpha$ -Proteobacteria and Firmicutes (Figure 4c) in comparison to the logged-over forest (Figure 4b), even though the abundance of Actinobacteria,  $\alpha$ -Proteobacteria and Firmicutes were lower in MD (Figure 4a). While both Actinobacteria and  $\alpha$ -Proteobacteria showed similar abundance in MD, the population of Acidobacteria increased from 8% in MD to 19% in CC and 13% in DD, making it the second most prevalent phyla after the unclassified bacteria phyla in CC. In addition, the Firmicutes population had steadily increased from 2% (MD), to 5% (CC) and 9% (DF). On the other hand, the bacterial diversity showed decrement in Actinobacteria and  $\alpha$ -Proteobacteria phyla. Nitrospirae was only found in CC while Cyanobacteria were identified in both MD and DF whereas Chloroflexi and ε-Proteobacteria were only present in DF.

The total number of Acidobacteria individuals was the lowest in plantation soil DF with only 321 individuals recorded, preceded by MD and CC with 660 and 1668 individuals recorded, respectively (*Figure 5b*). The species diversity in Acidobacteria in DF was also less prevalent compared to MD and CC (*Figure 5b*). Acidobacteria played an important role in plant and fungal polysaccharides degradation in acidic logged-over forests, which explained their

high abundance in logged-over forests (Lladó *et al.*, 2016; Maidin *et al.*, 2016). It was also known as the most common bacteria in soil, hence its regular presence in every ecosystem (Kanokratana *et al.*, 2011; Poerschmann *et al.*, 2012; Stamps *et al.*, 2014; Moore *et al.*, 2015; Maidin *et al.*, 2016; Lynn *et al.*, 2017). The prominent species consistently recorded in all studied ecosystems with average soil pH of 3.58–3.37 (*Table 2*) was identified as *Candidatus koribacter* sp. with 100% identity similarity to *Candidatus koribacter* sp. clone HLA (accession No. KF225961) (Hu *et al.*, 2014). Constant occurrence of this bacterial species in all ecosystems indicated that the land-use conversion did not affect the bacteria's natural population.

highest number of Actinobacteria The individuals was recorded in DF (958), followed by MD (506) and CC (218) (Figure 5c) and was also supported by the relative percentage among the ecosystems studied (Figure 4). Therefore, Actinobacteria might have a preference over the oil palm plantation soil compared to cleared land or involved in activities related to plant physiology such as the degradation of macromolecules including cellulose, lignin, chitin and starch by producing extracellular enzyme (Bakeri et al., 2015; Rajagopal and Kannan, 2017). Significant difference was also observed in the number of uncommon bacterial species, recorded at 93 different species in DF as compared to only six species in CC and 53 species in MD (*Figure 5c*). All three ecosystems were dominated by the genus Mycobacterium spp. (Figure 5c) with MD



Sources: Adapted from Maidin et al. (2016).

*Figure 4. Prokaryotic phyla for deep peat forest, logged-over deep peat and oil palm planted area. a) Primary deep peat forest at Maludam, b) logged-over deep peat forest at Cernat Ceria and c) oil palm planted area at Durafarm.* 

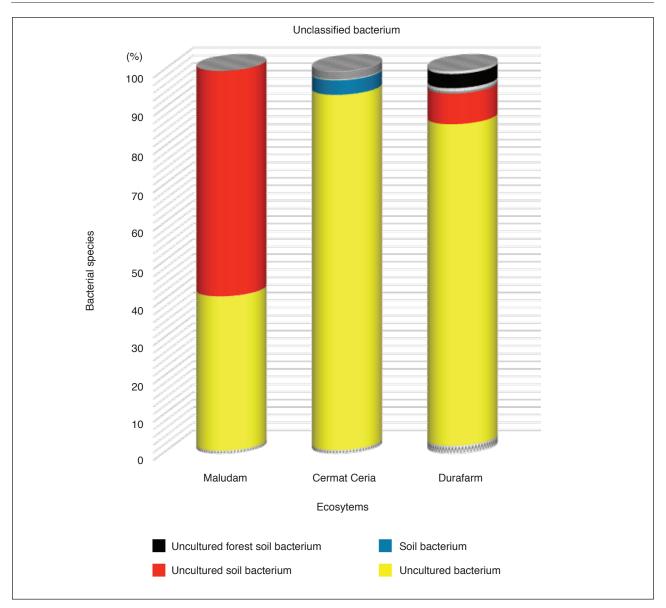


Figure 5a. Unclassified bacteria distribution for Maludam deep peat forest, Cermat Ceria logged-over deep peat and Durafarm oil palm planted ecosystem.

harbouring more diversified *Mycobacterium* species. Previous research done by Maidin *et al.* (2016) had successfully identified 27 Mycobacterium species in MD and only three in CC. This finding showed that the *Mycobacterium* species had increased to a total of 11 in DF. *Actinobacterium* spp. was dominant in DF with 537 similarity occurrences which was similar to MD that was dominated by 225 similarities.

Actinobacteria (*Figure 5c*) and  $\alpha$ -Proteobacteria (*Figure 5d*) were the two phyla that showed a clear number of microbial species in different ecosystems. Both phyla shared the same trend of population differences with species reduction in CC (Maidin *et al.*, 2016). However, these bacterial populations had increased along with the plantation of oil palms from 6% to 12% for Actinobateria (*Figure 4*) and from 3% to 8% for  $\alpha$ -Proteobacteria species. The uncommon bacterial species of  $\alpha$ -Proteobacteria

had also increased from 9 species to 34 species (*Figure 5d*) from CC to DF. Furthermore, the number of Mycobacterium species in the phylum of Actinobacteria that was largely affected during logging activity (Maidin *et al.*, 2016) have showed increment in the oil palm plantation (*Figure 5c*). The *Mycobacterium* species have increased from only three species in CC to 11 in DF.

Compared to MD and CC which were dominated by  $\alpha$ -Proteobacterium, phyla  $\alpha$ -Proteobacteria in DF was dominated by *Rhodoplanes* sp. with 281 similarity occurrences. The  $\alpha$ -Proteobacteria population was more diverse in DF compared to CC with 34 species in DF and 18 species in CC (*Figure* 5*d*). DF has shown less diversity compared to 66 different species in MD (*Figure* 5*d*). These results might indicate that the  $\alpha$ -Proteobacteria population have been increasing as the young palm were

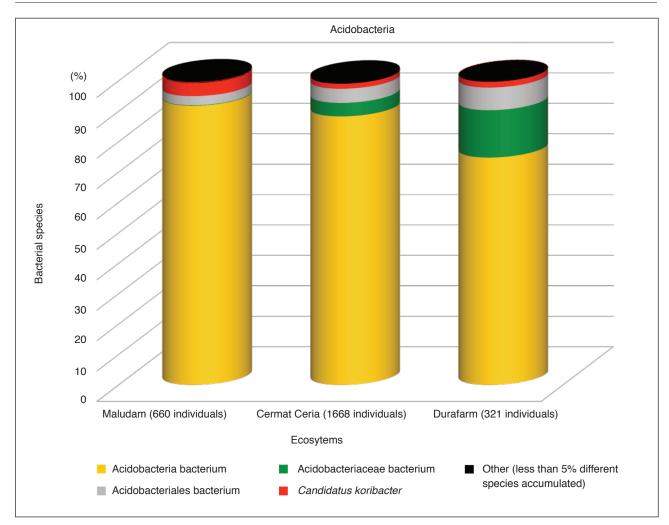


Figure 5b. Acidobacteria distribution for Maludam deep peat forest, Cermat Ceria logged-over deep peat and Durafarm oil palm planted ecosystem.

growing into maturity. Continuous monitoring on the stability of  $\alpha$ -Proteobacteria phyla during the transition state of the forest into oil palm plantation have also shown that the bacterial population was not conserved even after oil palm plantation. The recovery of bacterial population in oil palm plantation was evident through the increment of Hyphomicrobiaceae bacterium and Rhizobiales bacterium abundance in DF compared to CC and MD (Figure 5d). Both bacteria were vital in C and N cycles in soil (Maidin et al., 2016; Acosta-Martinez et al., 2010). Furthermore, abundance of Rhodoplanes sp. with 281 occurrences (Figure 5d) in Durafarm also showed that this bacteria species might function as plant growth promoting bacteria (Pershina et al., 2015).

The dominant bacterial species in DF was *Paenibacillus* sp. with 105 similarity occurrences compared to unidentified Firmicutes bacterium in MD and CC (Maidin *et al.*, 2016). DF also recorded a more diverse Firmicutes population compared to MD and CC with 28 different species compared to 15 and 19, respectively (*Figure 5e*). This showed that Firmicutes was dominant in oil palm plantation soil.

Koberl *et al.* (2011) reported that Firmicutes were well-characterised with antagonistic properties towards plant pathogens such as fungi. *Paenibacillus polymyxa, Paenibacillus peoriae* and *Paenibacillus jamilae* were the dominant Paenibacillus species in DF (*Figure 5e*). The abundance of these species might relate to the nature of the soil, which was rich in humus and plant materials as well as their ability of nitrogen-fixation and association with the rhizosphere of plants (Priest, 2015). Some of the Paenibacillus strains might act as plant protector and as important pathogen to insect pests (Chowdhury *et al.*, 2013; Priest, 2015; Grady *et al.*, 2016).

All sites had a total number of 32 common bacterial species, which was equivalent to their population in forest and logged-over forest (Maidin *et al.*, 2016). However, DF has shown increment of distinctive species number for unclassified Bacteria (*Figure 5a*), Actinobacteria (*Figure 5c*),  $\alpha$ -Proteobacteria (*Figure 5d*) and Firmicutes (*Figure 5e*). This was also supported by the diversity profiles in *Figure 4* that shows higher alpha diversity (number of taxa) in DF followed by MD and CC. Only Acidobacteria phyla have recorded slightly distinctive species

reduction (Figure 5b). The bacteria Acidipila rosea that was originally identified in the forest had reoccurred in the oil palm plantation along with Acidimicrobium sp., Actinomycete sp., Mycobacterium celatum, Mycobacterium avium, Mycobacterium branderi, Mycobacterium fragae, Mycobacterium intracellulare, Mycobacterium kyorinense, Afipia sp., Acetobacteraceae bacterium, Rhizobium sp., Rhodoplanes sp. and Sphingomonadaceae bacterium. Therefore, this finding suggests that oil palm plantation could recover the natural bacterial populations that were lost during the forest clearance and enhance soil bacterial biodiversity. Denitrifying bacteria such as Rhodoplanes sp. and Rhizobium sp. were renowned in nitrogen cycling ability and energy transformation in soil (Allen et al., 2015).

The development and conservation of peat soils for oil palm plantations should be closely monitored. The knowledge gap on the microbial diversity in oil palm plantation development was resolved in this study. The data obtained showed that oil palm plantation did not reduce the microbial biodiversity. However, the environmental differences could be clearly observed in the reduction of bacterial population in logged-over forests. Microorganisms were a useful indicator for soil health (Ferris and Tuomisto, 2015). Therefore, maintaining healthy soil is crucial for the vitality of living system within the ecosystem and land use boundaries, which also promotes clean water and air for the well-being of humanity (Doran and Safley, 1997; Cardoso *et al.*, 2013).

Peat degradation could affect soil fertility (Agus, 2015). Therefore, proper management techniques such as regulation of the micro and macronutrient cycles, pH and water table in the soil could be implemented to improve soil fertility (Hasnol *et al.*, 2011). The effect of the changes could be analysed through PCR-DGGE technique. Nevertheless, this technique could be improved through the

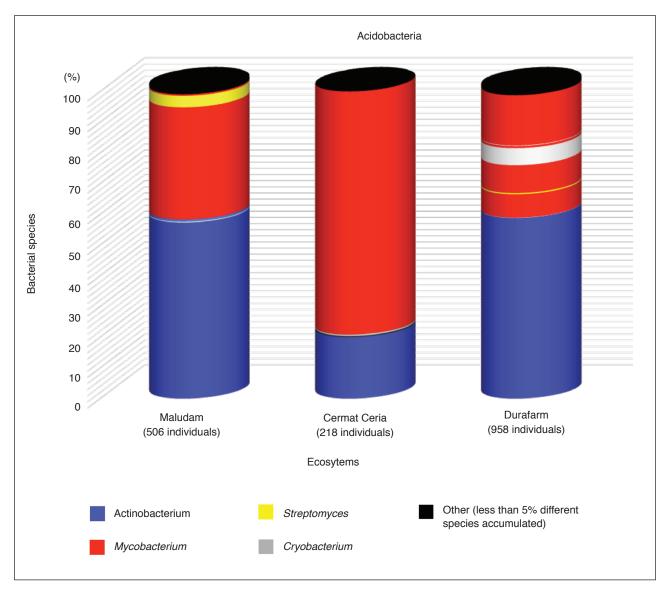


Figure 5c. Actinobacteria distribution for Maludam deep peat forest, Cernat Ceria logged-over deep peat and Durafarm oil palm planted ecosystem.

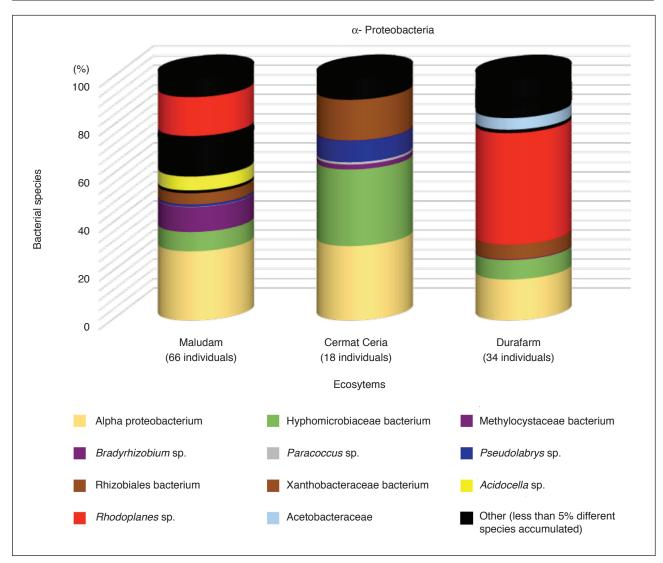


Figure 5d. The  $\alpha$ -Proteobacteria distribution for Maludam deep peat forest, Cermat Ceria logged-over deep peat and Durafarm oil palm planted ecosystem.

application of high throughput Next Generation Sequencing by performing amplicon sequencing to resolve the biasness in the multicopy DNA band excision of DGGE (Bokulich and Mills, 2012).

## CONCLUSION

Microbial biodiversity in oil palm plantation DF showed significant differences in the number of microbial groups compared to the primary deep peat forest, MD and the logged-over forest, CC. The findings from this study suggested that oil palm plantations had not affected the soil microbial diversity. The impact of management practices related to the microbial diversities should be investigated to gather more information on its sustainability for soil fertility. Furthermore, the application of Next Generation Sequencing should be utilised to relate the microbial diversity and its function in the soil.

# SUPPLEMENTARY INFORMATION

Supplementary information for *Figure 5* on the distribution of unclassified Bacteria, Acidobacteria, Actinobacteria,  $\alpha$ -Proteobacteria and Firmicutes for Maludam deep peat forest, Cermat Ceria logged-over deep peat forest and Durafarm oil palm planted ecosystems is available via http://jopr.mpob.gov.my/wp-content/uploads/2018/08/joprv30sept2018-Figure5shawal.pdf.

## ACKNOWLEDGEMENT

We would like to thank the Director-General of MPOB for permission to publish this article. We also would like to express our gratitude to Mohamad Izzuddin Anuar for his guidance in the preparation of this manuscript. This research is supported by the MPOB Research Fund: R009711000.

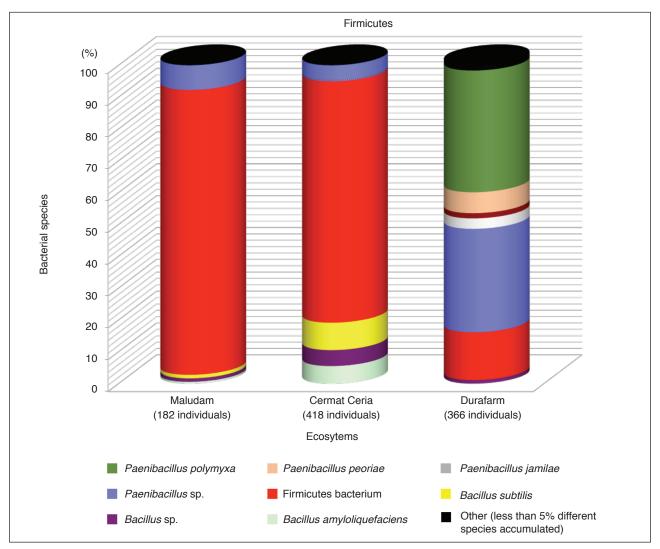


Figure 5e. Firmicutes distribution for Maludam deep peat forest, Cermat Ceria logged-over deep peat and Durafarm oil palm planted ecosystem.

#### REFERENCES

ACOSTA-MARTINEZ, V; DOWD, S E; BELL, C; LASCANO, R; BOOKER, J D; ZOBECK, T M and UPCHURCH, D R (2010). Microbial community comparison as affected by dryland cropping systems and tillage in a semiarid sandy soil. *Diversity*, 2: 910-931.

AGUS, F (2015). Degradation and sustainable management of peat soils in Indonesia. *MARCO Symposium Tsukuba International Congress*. 26-28 August 2015. Tsukuba, Japan. p. 7.

ALLEN, K; CORRE, M D; TJOA, A and VELDKAMP, E (2015). Soil nitrogen-cycling responses to conversion of lowland forests to oil palm and rubber plantations in Sumatra, Indonesia. *PLoS ONE*, *10*(7): e0133325.

BACCINI, A G S J; GOETZ, S J; WALKER, W S; LAPORTE, N T; SUN, M; SULLA-MENASHE, D; HACKLER, J; BECK, P S A; DUBAYAH, R; FRIEDL, M A and SAMANTA, S (2012). Estimated carbon dioxide emissions from tropical deforestation improved by carbon-density maps. *Nature Climate Change*, *2*(3): 182-185.

BAKERI, S A; ALI, S R A; SAFARI, S and MAIDIN, M S T (2015). Underground prokaryotic biodiversity under oil palm plantation on alluvial soil at Belaga, Sarawak. *Proc. of the PIPOC 2015 International Palm Oil Congress - Biotechnology and Sustainability Conference*. 6-8 October 2015. MPOB, Bangi. p. 315-323.

BOKULICH, N A and MILLS, D A (2012). Nextgeneration approaches to the microbial ecology of food fermentations. *BMB Reports*, *45*(7): 377-389.

BOWLES, T M; ACOSTA-MARTÍNEZ, V; CALDERÓN, F and JACKSON, L E (2014). Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biology and Biochemistry,* 68: 252-262.

CARDOSO, E J B N; VASCONCELLOS, R L F; BINI, D; MIYAUCHI, M Y H; SANTOS, C A D; ALVES, P R L; PAULA, A M D; NAKATANI, A S; PEREIRA, J D M and NOGUEIRA, M A (2013). Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola*, 70(4): 274-289.

CHOWDHURY, S P; DIETEL, K; RÄNDLER, M; SCHMID, M; JUNGE, H; BORRISS, R; HARTMANN, A and GROSCH, R (2013). Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. *PLoS ONE*, *8*: e68818.

CONRAD, R; KLOSE, M; LU, Y and CHIDTHAISONG, A (2012). Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. *Frontiers in Microbiology*, *3*: 4.

DORAN, J W and SAFLEY, M (1997). Defining and assessing soil health and sustainable productivity. *Biological Indicators of Soil Health* (Pankhurst, C E; Doube, B M and Gupta, V V S R eds.). CAB International, Wallingford, United Kingdom. p. 1-28.

FERRIS, H and TUOMISTO, H (2015). Unearthing the role of biological diversity in soil health. *Soil Biology and Biochemistry*, 85: 101-109.

GRADY, EN; MACDONALD, J; LIU, L; RICHMAN, A and YUAN, Z C (2016). Current knowledge and perspectives of Paenibacillus: A review. *Microbial Cell Factories*, *15*(1): 203.

HAMMER, Ø (2015). PAST 3 (Paleontological Statistics) version. 3.11. http://folk.uio.no/ ohammer/past/.

HASNOL, O; TARMIZI, A M; DARUS, F M; HARUN, M H and ZAMBRI, M P (2011). Best management practices for oil palm cultivation on peat: Ground water-table maintenance in relation to peat subsidence and estimation of  $CO_2$ emissions at Sessang, Sarawak. J. Oil Palm Res. Vol. 23: 1078-1086.

HILL, M O (1973). Diversity and evenness: A unifying notation and its consequences. *Ecology*, 54: 427-431.

HU, L; CAO, L and ZHANG, R (2014). Bacterial and fungal taxon changes in soil microbial community

composition induced by short term biochar amendment in red oxidized loam soil. *World J. Microbiol Biotechnol.*, *30*: 1085-1092.

HU, N; LI, H; TANG, Z; LI, Z; LI, G; JIANG, Y; HU, X and LOU, Y (2016). Community size, activity and C: N stoichiometry of soil microorganisms following reforestation in a Karst region. *European J. Soil Biology*, *73*: 77-83.

HUAT, B B; KAZEMIAN, S; PRASAD, A and BARGHCHI, M (2011). State of an art review of peat: General perspective. *International J. Physical Sciences*, *6*: 1988-1996.

JACKSON, C R; FOREMAN, C M and R L (1995). Microbial enzyme activities as indicators of organic matter processing rates in a Lake Erie Coastal wetland. *Freshwater Biology*, 34: 329-342.

JACKSON, C R and VALLAIRE, S C (2007). Microbial activity and decomposition of fine particulate organic matter in a Louisiana Cypress swamp. *J. North American Benthological Society*, 26: 743-753.

JAUHIAINEN, J; PAGE, S E and VASANDER, H (2016). Greenhouse gas dynamics in degraded and restored tropical peatlands. *Mires and Peat*, 17: 1-12.

KANOKRATANA, P; UENGWETWANIT, T; RATTANACHOMSRI, U; BUNTERNGSOOK, B; NIMCHUA, T; TANGPHATSORNRUANG, S; PLENGVIDHYA, V; CHAMPREDA, V and EURWILAICHITR, L (2011). Insights into the phylogeny and metabolic potential of a primary tropical peat swamp forest microbial community by metagenomic analysis. *Microbial Ecology*, 61: 518-528.

KOBERL, M; MULLER, H; RAMADAN, E M and BERG, G (2011). Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. *PLoS ONE*, *6*: e24452.

KUSHAIRI, A; SNGH, R and ONG-ABDULLAH, M (2017). The oil palm industry: Thriving with transformative technologies. *J. Oil Palm Res. Vol.* 29(4): 431-439.

LAURANCE, W F (2007). Forest destruction in tropical Asia. *Current Science*, 93: 1544-1550.

LLADÓ, S; ŽIFČÁKOVÁ, L; VĚTROVSKÝ, T; EICHLEROVÁ, I and BALDRIAN, P (2016). Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition. *Biology and Fertility of Soils*, 52(2): 251-260. LOGAN, N A; BERGE, O; BISHOP, A H; BUSSE, H J; DE VOS, P; FRITZE, D; HEYNDRICKX, M; KÄMPFER, P; RABINOVITCH, L; SALKINOJA-SALONEN, M S and SELDIN, L (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *International J. Systematic and Evolutionary Microbiology*, 59: 2114-2121.

LYNN, T M; LIU, Q; HU, Y; YUAN, H; WU, X; KHAI, A A; WU, J and GE, T (2017). Influence of land use on bacterial and archaeal diversity and community structures in three natural ecosystems and one agricultural soil. *Archives of Microbiology*: 1-11.

MAIDIN, M S T; SAFARI, S; GHANI, N A; AZURA, S; IBRAHIM, S; BAKERI, S A; MASRI, M M M and ALI, S R A (2016). Differences in prokaryotic species between primary and logged-over deep peat forest in Sarawak, Malaysia. *J. Oil Palm Res. Vol.* 28(3): 281-295.

MANDIC-MULEC, I; AUSEC, L; DANEVCIC, T; LEVICNIK-HOEFFERLE, S; JERMAN, V and KRAIGHER, B (2014). Microbial community structure and function in peat soil. *Food Technology and Biotechnology*, *52*(2): 180.

MERINO, A; OMIL, B; FONTURBEL, M T; VEGA, J A and BALBOA, M A (2016). Reclamation of intensively managed soils in temperate regions by addition of wood bottom ash containing charcoal: SOM composition and microbial functional diversity. *Applied Soil Ecology*, 100: 195-206.

MOORE, E K; VILLANUEVA, L; HOPMANS, E C; RIJPSTRA, W I C; METS, A; DEDYSH, S N and DAMSTÉ, J S S (2015). Abundant trimethylornithine lipids and specific gene sequences are indicative of planctomycete importance at the oxic/anoxic interface in sphagnum-dominated Northern wetlands. *Applied and Environmental Microbiology*, *81*: 6333-6344.

MUYZER, G; BRINKHOFF, T; NÜBEL, U; SANTEGOEDS, C; SCHÄFER, C and WAWER, C (1997). Denaturing gradient gel electrophoresis (DGGE) in microbial ecology: *Molecular Microbial Ecology Manual*. Kuluwer Academic Publishers, Netherlands. p. 1-27.

OERTEL, C; MATSCHULLAT, J; ZURBA, K; ZIMMERMANN, F and ERASMI, S (2016). Greenhouse gas emissions from soils - A review. *Chemie der Erde-Geochemistry*, *76*(3): 327-352.

OVERMANN, J and TUSCHAK, C (1997). Phylogeny and molecular fingerprinting of green sulfur bacteria. *Archieve Microbiology*, *167*: 302-309.

PERSHINA, E; VALKONEN, J; KURKI, P; IVANOVA, E; CHIRAK, E; KORVIGO, I; and ANDRONOV, E (2015). Comparative analysis of prokaryotic communities associated with organic and conventional farming systems. *PloS ONE*, *10*(*12*): e0145072.

POERSCHMANN, J; KOSCHORRECK, M and GÓRECKI, T (2012). Organic matter in sediments of an acidic mining lake as assessed by lipid analysis. Part I: Fatty acids. *Sci. Total Environ.*, *414*: 614-623.

PRESTON, M D and BASILIKO, N (2016). Carbon mineralization in peatlands: Does the soil microbial community composition matter? *Geomicrobiology J.*, 33(2): 151-162.

PRIEST, F G (2015). Paenibacillus. *Bergey's Manual of Systematics of Archaea and Bacteria*.

RAJAGOPAL, G and KANNAN, S (2017). Systematic characterization of potential cellulolytic marine actinobacteria *Actinoalloteichus* sp. MHA15. *Biotechnology Reports*, *13*: 30-36.

SIMPSON, P; FITZGERALD, G; STANTON, C and ROSS, R (2004). The evaluation of a mupirocinbased selective medium for the enumeration of bifidobacteria from probiotic animal feed. *J. Microbiology Methods*, *57*: 9-16.

STAMPS, B W; LOSEY, N A; LAWSON, P A; BRADLEY, S and STEVENSON, B S (2014). Genome sequence of Thermoanaerobaculum aquaticum MP-01T, the first cultivated member of Acidobacteria subdivision 23, isolated from a hot spring. *Genome Announcements*, 2: e00570-14.

TIKU, N E and BULLEM, F A (2015). Oil palm marketing, Nigeria - Lessons to learn from Malaysia experience, opportunities and foreign direct investment in Cross River State. *J. Development and Agricultural Economics*, 7(7): 243-252.

TUZHIKOV, A; PANCHIN, A and SHESTOPALOV, V I (2014). TUIT, A BLAST-based tool for taxonomic classification of nucleotide sequences. *Biotechniques*, *56*(2): 78.