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DIFFERENCES IN PROKARYOTIC SPECIES BETWEEN PRIMARY AND LOGGED-OVER DEEP PEAT FOREST IN SARAWAK, MALAYSIA

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ABSTRACT

Peatland has an important role in environmental sustainability which can be used for agricultural purposes. However, deforestation in the logged-over forest may disrupt the diversity of microbial population in peat soil. Therefore, this study focuses on the differences of microbial populations in Maludam primary forest and Cermat Ceria logged-over forest in Sarawak, Malaysia. The prokaryotic 16S rDNA region was amplified followed by denaturing gradient gel electrophoresis (16S PCR-DGGE) analysis. Berger-Parker and Shannon-Weaver Biodiversity Index showed that Maludam (0.11, 7.75) was more diverse compared to Cermat Ceria (0.19, 7.63). Sequence analysis showed that the bacterial community in Maludam and Cermat Ceria were dominated by unclassified bacteria, followed by Acidobacteria, Actinobacteria, Firmicutes and α-Proteobacteria. Based on the findings, the distinct species that can be found in Maludam were Acidobacterium capsulatum, Solibacter *sp.*, Mycobacterium intracellulare, Rhodoplanes *sp.*, Clostridia bacterium, Exiguobacterium *sp. and* Lysinibacillus fusiformis*. While, the distinct species that can be found in Cermat Ceria were* Telmatobacter, Mycobacterium tuberculosis *and* Bacillus tequilensis*. Overall, the findings showed that microbial population in the logged-over forest are less diverse compared to primary forest. Higher prokaryotic diversity identified in the primary forest compared to logged-over forest showed that deforestation might cause prokaryotic population changes to both ecosystems.*

Keywords: microbial diversity, deep peat, primary forest, logged-over forest.

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INTRODUCTION

Peatlands are formed by the accumulation and anaerobic processes of incomplete plant decay in water logged area for over hundreds of years (Cole *et al*., 2015). Around 50%-70% of wetlands are covered by the peatlands worldwide (Mishra *et al*., 2013). Still, it remains a major source of world's soil carbon at approximately one-third around the globe (Freeman *et al*., 2012). Tropical peatlands in South-east Asia is covered with 77% total carbon pool which is nearly 25 million hectares of land area (Page *et al*., 2011). Page *et al*. (2011) also stated that the density of carbon is higher in tropical peatlands compared to boreal or temperate peatlands. This is due to the deeper peat layers up to 20 m thickness compared to the other two peatlands.

The nutrient recycling, organic matter dynamics and decomposition processes in the soil are highly influenced by the microbial community that makes the ecosystem works perfectly (Talbot *et al*., 2014). Furthermore, the availability of nutrients from the

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decomposition and rhizodeposition processes also offer a great influence to the prokaryotic community in the soil (Trivedi *et al*., 2013). Environmental changes and nutrient distribution due to the land conversion can contribute to the abundance and diversification of the bacterial communities (Baldrian *et al*., 2012). Other variable aspects such as soil moisture and temperature were identified to regulate the microbial diversity in a particular soil ecosystem (Lauber *et al*., 2013). These features determine the availability of the peatland as growing media, biota habitat, biodiversity and hydrotopography. The information gained from the microbial diversity evaluation is also crucial for plant pathogen and beneficial microbes detection for agricultural purposes. Thus, good land management practice can effectively control the microbial ecosystem in the agricultural soil. Conversely, the structure of the below ground ecosystems can be badly deteriorated due to the depletion of the soil carbon and biodiversity loss if poorly managed (Lauber *et al*., 2013).

Development of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique gives the opportunity to unravel the population dynamics of the culture independent bacterial species living in the soil (Zhang *et al*., 2014). This technique basically utilised by screening the bacterial fingerprint through molecular approach on the DGGE that enables the separation of different DNA sequences that have indistinguishable band length in the selected sampling site (Piterina and Pembroke, 2013). This fingerprint can be compared through numerous analysis tools such as cluster analysis, non-metric multidimensional scaling, visual inspection and moving window analysis that will directly bring the data into quantitative and qualitative biodiversity indices (Gomez-Balderas *et al*., 2014). Furthermore, almost 95%-99% of the bacterial community can be detected and potentially to be utilised as a genetic marker (Piterina and Pembroke, 2013).

The evolution of microbial populations in the soil is directly dependent on the structural features and physico-chemical changes that occurred in the ecosystem (Menezes-Oliveira *et al*., 2014) that can be analysed through PCR-DGGE technique. Therefore, this study was conducted to investigate the effect of logging on the prokaryotic population in the primary deep peat forest in Sarawak where microbial biodiversity in Maludam primary deep peat forest was compared to Cermat Ceria logged-over forest. The application of DNA fingerprinting methods enables the detection of diverse members of soil bacterial communities, including the unculturable microbes. The PCR coupled with DGGE has been used as culture-independent methods to determine species richness and differences of microbial communities between the primary and logged-over forest for this study.

MATERIALS AND METHODS

Peat Soil Sampling

Two peat areas consisting of deep peatland forest and logged-over forest at Sri Aman, Sarawak were selected as shown in *Table 1*. Maludam National Park and Cermat Ceria are primary and logged-over forest, respectively. Sampling of peat soil was taken from 10 GPS points in triplicates. The GPS points in Maludam were divided by two transects. The first transect consisted of point 1 until point 6 while the second transect consisted of point 7 until point 10. The distance between each GPS point was separated approximately 10 m on average except for points 6 and 7 which were separated by 100 m apart (*Figure 1a*). The GPS points at Cermat Cermat were separated approximately 100 m apart (*Figure 1b*). A total of 25 g of soil samples were taken in 50 ml falcon tube in each coordinate at a depth of 0-30 cm, midway between topsoil and water table. The samples were chilled and stored at 10°C prior to DNA extraction.

DNA Extraction

Total DNA was extracted from peat soil samples using GeneMATRIX Soil DNA Purification Kit (Eurx Ltd, Gdansk, Poland). The concentration and quality of DNA were measured using a Nanophotometer™ P360 (Implen GmbH, Schatzbogen, Germany) with minimum concentration and purity of 10 ng μl^{-1} and OD260/280 of 1.7-2.0, respectively. The extracted DNA was stored at -20°C prior to analysis.

PCR-DGGE Analysis

PCR amplifications of the V3-V5 region (550 bp) of the *16S rRNA* gene were performed with universal bacterial primers 341F-GC clamp (5'-cgc-ccg-ccg-cgc-gcg-gcg-ggc-ggg-gcg-ggg-gca-cgg-ggg-gcc-tac-gg-agg-cag-cag-3') and 907R (5'-ccc-cgt-caa-ttc-att-tga-gtt-t-3') using the PCR program (Muyzer *et al*., 1997; Overmann and Tuschak, 1997). The PCR was performed in 25 ul of reaction volume with a thermocycler (gradient) containing succession of 10 pmol of each primer, 100 m*M* dNTPs, 1X PCR buffer, 50 m*M* Mg_2Cl, 0.3% BSA and 2.5 units of Taq polymerase. The PCR started with initial denaturation at 94°C for 2 min, 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s, final extension at 72°C for 2 min and held at 10°C. Then the PCR products were subsequently subjected to DGGE analysis.

DGGE was performed using a D-code DGGE system (Bio-Rad, USA). The gradient of denaturants and running conditions were optimised as follows: 40 μl of GC clamped-amplicons were resolved in 6% acrylamide (37.5:1, acrylamide:Bis-acrylamide)

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

No.	Estates	Sampling points	Location on GPS
1	Maludam National Park		
		1	N, 1°37′ 43.15897″ E, 111°02′ 22.12806″
		2	N, 1°37′ 43.14684″ E, 111°02′ 22.10784″
		3	N, 1°37′ 43.13676″ E, 111°02′ 22.10172″
		4	N, 1°37′ 43.11048″ E, 111°02′ 22.09380″
		5	N, 1°37′ 43.09068″ E, 111°02′ 22.08912″
		6	N, 1°37′ 43.08420″ E, 111°02′ 22.08408″
		7	N, 1°37′ 47.89410″ E, 111°02′ 23.28638″
		8	N, 1°37′ 48.31230″ E, 111°02′ 22.79459″
		9	N, 1°37′ 48.40347″ E, 111°02′ 22.83173″
		10	N, 1°37′ 48.52168″ E, 111°02′ 22.79459″
2	Cermat Ceria logged-over forest		
		1	N, 1°23′ 58.85626″ E, 111°24′ 08.61675″
		2	N, 1°23′ 58.29805″ E, 111°24′ 08.52634″
		3	N, 1°23′ 57.46257″ E, 111°24′ 13.68159″
		4	N, 1°23′ 56.50727″ E, 111°24′ 17.37404″
		5	N, 1°23′ 56.11111″ E, 111°24′ 21.85227″
		6	N, 1°23′ 55.39797″ E, 111°24′ 27.35565″
		7	N, 1°23′ 54.86120″ E, 111°24′ 31.39397″
		8	N, 1°23′ 52.45833″ E, 111°24′ 39.79472″
		9	N, 1°23′ 42.87224″ E, 111°24′ 41.60620″
		10	N, 1°23′ 53.80569″ E, 111°24′ 43.18166″

TABLE 2. MICROBIAL BIODIVERSITY INDICES FOR TOTAL MICROBES ON PEAT SOIL SAMPLED FROM LOGGED-OVER AND PRIMARY PEAT FOREST

Shannon-Weaver biodiversity index		Berger-Parker dominance index	
Maludam	Cermat Ceria	Maludam	Cermat Ceria
7.747788	7.62656	0.11	0.19

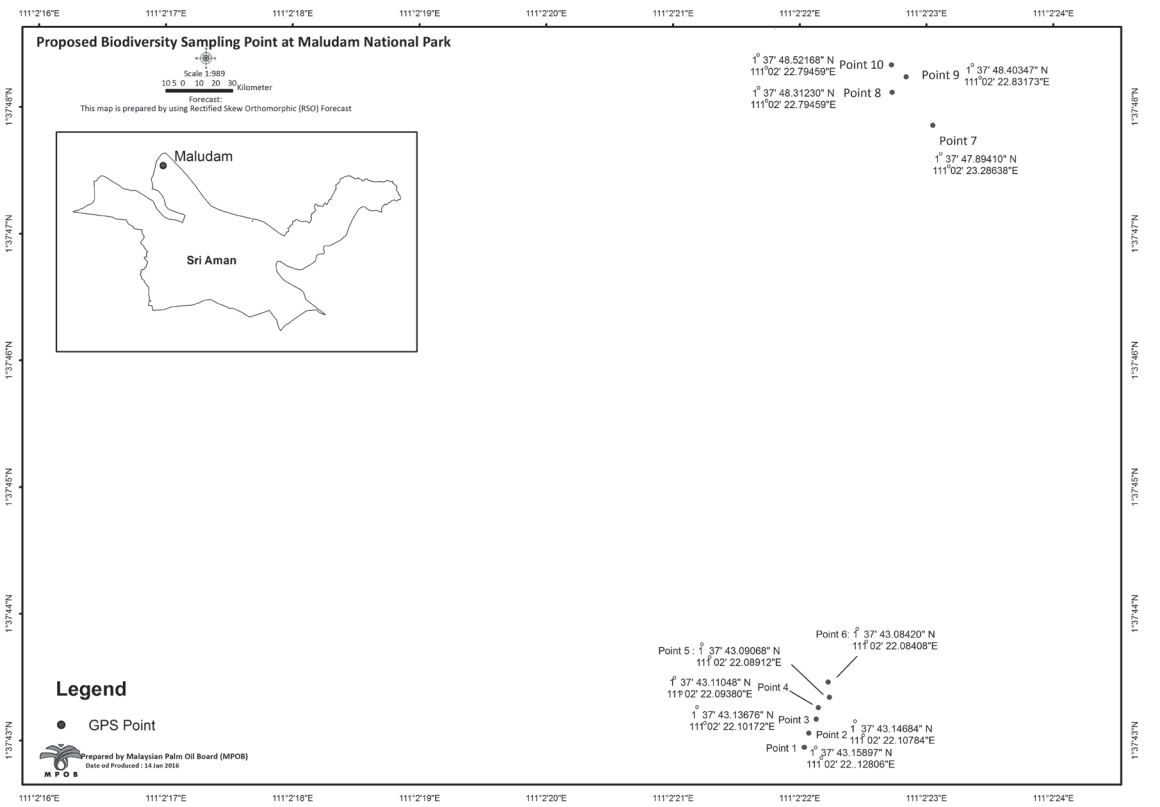
perpendicular gels in a 40%-70% gradient of denaturants (where 100% denaturant concentration) was equal to 7 M urea (Sigma, USA) and 40% (v/v) of deionised formamide (Amresco® Solon Ind., Ohio, USA). TEMED and ammonium persulfate were added to a final concentration of 0.1% each. Each gel was loaded with 100 bp marker (Invitrogen, USA) as reference lane. Electrophoresis was run in 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.0) at constant 65 V for 17 hr and at 60°C using the Bio-Rad D-Code™ Universal Mutation Detection System. Gels were stained with 0.1% (v/v) SYBR gel stain and visualised under UV using AlphaImager HP (Alpha Innotech, San Leandro, CA).

Band Matching Analysis of PCR-DGGE Profiles

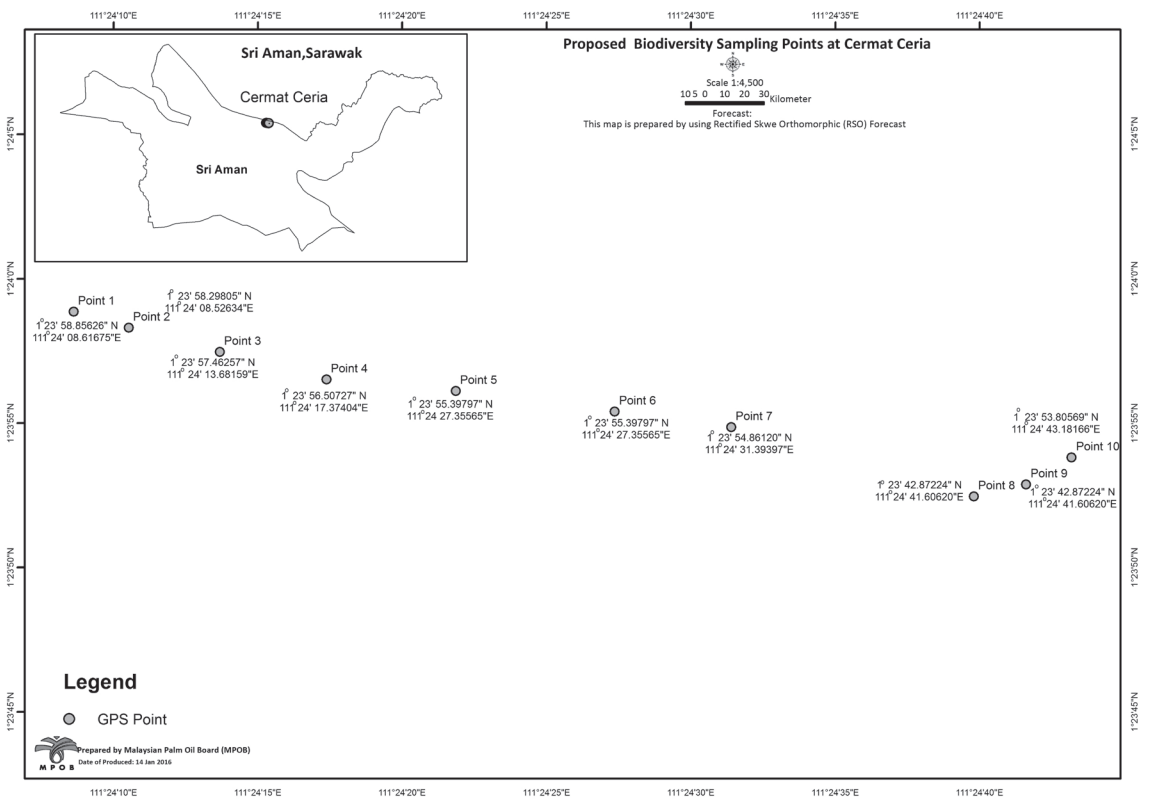
PCR-DGGE patterns were analysed using Phoretix 1D Gel Analysis software (Total Lab Quant Ltd, United Kingdom). All the images were normalised using the internal control and the comparison of whole profiles was performed using the Dice similarity coefficient (D_{sc}). The dendrogram was generated using the method of unweighted pair group with mathematical averages (UPGMA) at 1% position tolerance. The 10 different points were represented as numbering of 1B until 10B on DGGE gels.

Sequencing of DGGE Band

The intense DGGE bands were excised, eluted with 50 μl of TE buffer and incubated overnight at 4°C. Eluted DNA from excised gel was used as DNA template for re-amplification using 16S rDNA primers, 341f (with no GC-clamp) (5′-cct-acg-gga-ggc-agc-ag-3′) and 907R reverse(r) (Muyzer *et al*., 1997). The PCR products were purified using



(a)



(b)

Figure 1. Biogeographical points located at Sri Aman, Sarawak, Malaysia. Maludam primary forest (a). Cermat Ceria logged-over forest (b).

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 NCBI GenBank database to identify the nearest relatives of the partially sequenced *16S rRNA* genes of excised bands.

Phylogenetic Analysis

The nucleotide sequences determined in this study were aligned, and Neighbor-Joining Trees were constructed using MEGA version 4.0 (Molecular Evolutionary Genetics Analysis) (http://www.megasoftware.net). Neighbor-Joining phylogenetic trees were constructed based on the position of the *16S rRNA* gene by using the Kimura two-parameter substitution model evaluated by 1000 bootstrap resamplings of the data, and nodes with bootstrap values were indicated.

Statistics for Biodiversity Index

The Shannon-Weaver biodiversity index *(H')*, is calculated as follows:

$$H' = -\sum_{i=1} p_i \ln p_i$$

where *H* is the Shannon's diversity index, *S* is the total number of species in the community (richness), *Pi* is the proportion of *S* made up of the *i*th species. Shannon's index accounts for both abundance and evenness of the species present. The proportion of species *i* relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species, and multiplied by -1 (Hill, 1973).

Berger-Parker Dominance Index

The Berger-Parker dominance index expresses the proportional importance of the most abundant species (the dominant species). It is a simple measure of the numerical importance of the most abundant species. $d = N_{max}/N$ where, N_{max} is the number of individuals in the most abundant species, and *N* is the total number of individuals in the sample (Hill, 1973).

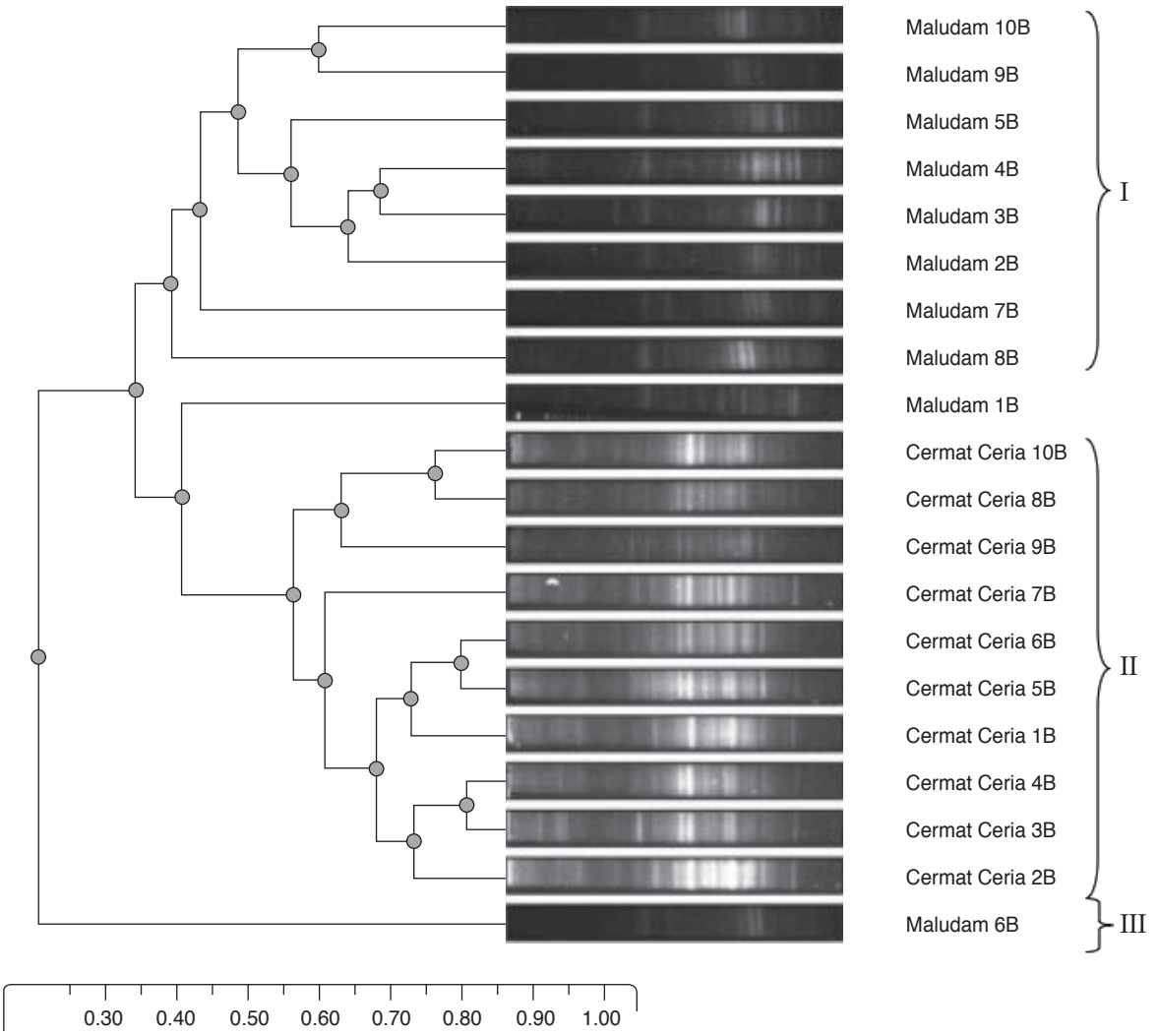
RESULTS AND DISCUSSION

Phoretix 1D software has been utilised to analyse the DGGE fingerprints by using dice coefficient and UPGMA in *Figure 2*. The dendrogram was constructed with the 16S rDNA bacterial communities fingerprint of Maludam and Cermat Ceria. The differences between DGGE band profiles were indicated by the percentage of similarity. The major cluster (cluster I and II) shows 40% genetic similarity while the minor cluster (cluster III) showed the indistinguish profile with lower genetic similarity at 20% in Maludam, lane 6B.

Shannon-Weaver prokaryotic biodiversity index in *Table 2* showed that Cermat Ceria was less diverse at 7.63 compared to Maludam, 7.75. The results for Berger-Parker concurred, where the most diversified population was Maludam compared to Cermat Ceria. Total bacteria identified in Maludam was also higher compared to Cermat Ceria with 17 501 and 11 236 similarities respectively (*Table 3*). The similarity species indicates sequences producing significant alignments (Madden, 2002). This suggests higher diversity of bacterial population in the primary forest compared to logged-over forest as shown in *Table 3*. The uncultured soil bacterium in *Table 3* was the dominant bacteria in Maludam with 9232 similarities while, the uncultured bacterium was the dominant bacteria in Cermat Ceria with 5416 similarities.

The distribution of microbial phyla for both peat ecosystems is shown in *Figure 3* and the pie charts were constructed by calculating the percentage of frequency as indicated in *Figure 4*. Unclassified bacteria was found to be the dominant bacterial phyla in both peat ecosystems (*Figure 3*). The unclassified bacteria dominated up to 69% and 65% of the total bacterial community in Maludam and Cermat Ceria, respectively (*Figures 3a* and *3b*). These findings suggested that unclassified bacteria could potentially be classified as a novel taxa candidate (Logan *et al*., 2009). Acidobacteria, Actinobacteria and α-Proteobacteria recorded the same percentage, 8% from the total bacterial population in Maludam. However, the population of Acidobacteria was at 8% in Maludam compared to 19% in Cermat Ceria. Therefore, Acidobacteria was the second most prevalent phyla after unclassified bacteria in Cermat Ceria while, Firmicutes population was lower at 2% in Maludam compared to 5% in Cermat Ceria. In addition, the population of Actinobacteria and α-Proteobacteria also decreased from both 8% to 6% and 3% in Maludam and Cermat Ceria, respectively. Nitrospirae was found in Cermat Ceria while Cyanobacteria was found in Maludam. However, the populations of Nitrospirae and Cyanobacteria cannot be related to the forest clearing since only one species can be identified in the respective area.

In the phyla of Acidobacteria (*Table 3*), Acidobacteria bacterium was the dominant prokaryotes in Maludam and Cermat Ceria with 606 and 1473 similarities occurrence, respectively. Even though Cermat Ceria harboured a higher number of similarities with 1668 compared to Maludam with 660, the diversity of bacterial species was comparable



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

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

with 12 species at Maludam (*Figure 4*) and 11 species at Cermat Ceria (*Figure 4*). Acidobacteria is a common group amongst the richest and universal bacterial phyla found in worldwide soil situations (Naether *et al*., 2012). This group of bacterial species usually can be found in low pH soils (Urbanová *et al*., 2015) and usually can be separated into distinct cluster phylogenetically based on pH differences (Zhalnina *et al*., 2015). The presence of Acidobacteria in both Maludam and Cermat Ceria was due to the low pH in peat soil (Omar *et al*., 2011; Vaessen *et al*., 2011). Acidobacteria plays an important ecological role by degrading polysaccharides of microbial origin in the important ecosystems of acidic soil (Lladó *et al*., 2015). *Candidatus koribacter* were the most prevalent Acidobacteria species. This bacterial species made up 3.03% and 1.44% of the total Acidobacteria species in Maludam and Cermat Ceria, respectively (*Table 3*). Based on BLAST analysis, the 16S rDNA sequence was closely related to *Candidatus koribacter* sp. clone HLA with 100% identity (accession No. KF225961) isolated from forest soil (red oxidised loam soil) in China (Hu *et al*., 2014). Individuals from this phylum have been distinguished by using molecular technique via 16S rDNA screening in a wide assortment of situations, including soils and residue (Foesel *et al*., 2013), hot springs (Stamps *et al*., 2014), peat swamps (Kanokratana *et al*., 2011), acidic mining lakes (Poerschmann *et al*., 2012) and acidic sphagnum peat (Moore *et al*., 2015).

The prokaryotic phyla in *Figure 3a* showed the percentage distribution of Actinobacteria occurrence at 8% of the total bacterial community in Maludam. The land clearing effect caused the percentage reduction to 6%, resulted from the physical changes to the surrounding of the bacterial population in the

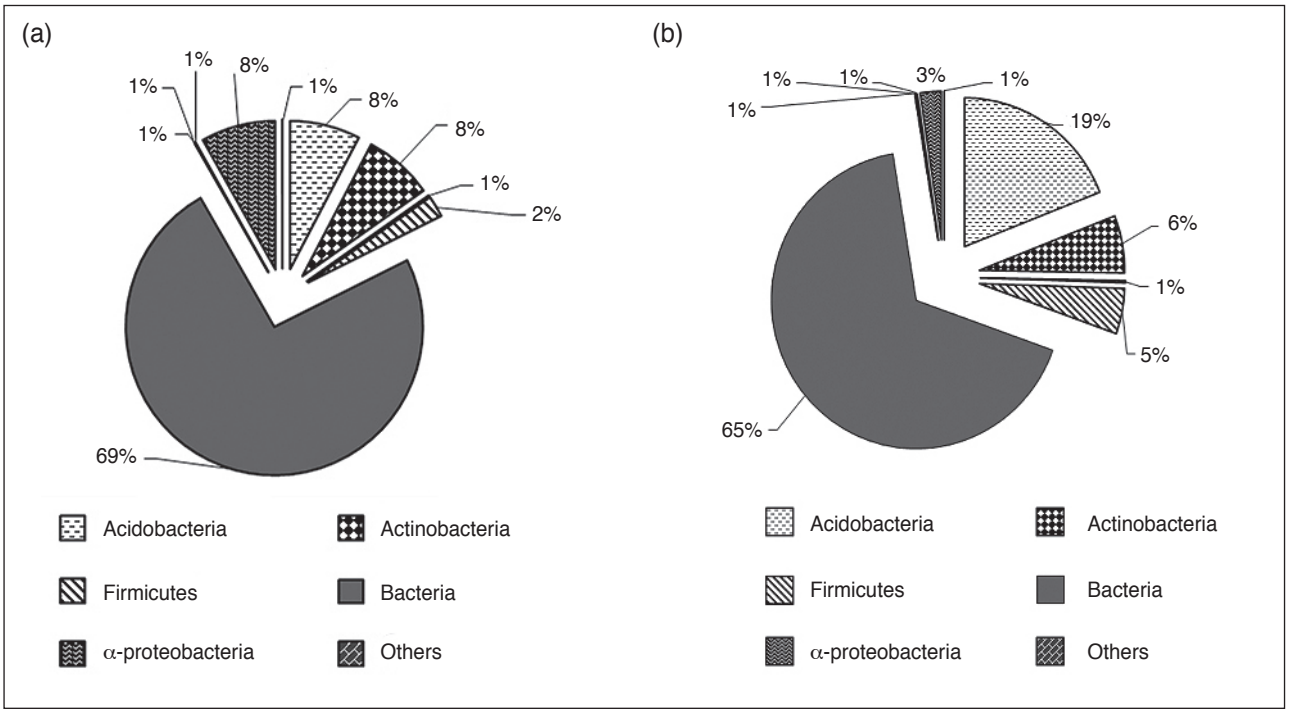


Figure 3. Prokaryotic phyla for deep peat forest and logged-over deep peat forest in Sarawak. Primary deep peat forest at Maludam (a). Logged-over deep peat forest at Cermat Ceria (b).

clean cleared area. In this study, the changes can be seen with the presence of Actinobacteria species as shown in *Table 3*. Clear differences in the number of uncommon bacterial species, which differed from 53 species in Maludam to six species in Cermat Ceria. The majority of known soil Actinobacteria is aerobic (Kutilek and Nielsen, 2015) but their richness in peat indicates at least a tolerance to anaerobic conditions, as viable aerobic Actinobacteria have been isolated from deep and anaerobic peat (Kotiaho *et al*., 2013). The genus *Mycobacterium* spp. (*Table 3*) dominated in both locations with Maludam harboring more diversified *Mycobacterium* species. A total of 27 *Mycobacterium* species were identified in Maludam compared to three in Cermat Ceria. However, *Mycobacterium tuberculosis* was the most dominant species with 141 similarities occurrence while, *Actinobacterium* spp. was the most dominant in Maludam with 225 similarities occurrence. *Actinobacteria* is a large phylum of physiologically heterogeneous bacteria found abundant in diverse environments (Stevenson and Hallsworth, 2014). It is an important decomposers of organic matter, especially cellulose, in Sphagnum peat (Moore *et a*l., 2015). *Mycobacterium avium* and *M. intracellulare* present in Maludam were the common non-tuberculous *Mycobacteria* detected in soil (Pribylova *et al*., 2011). The abundance of these *Mycobacterium* species in Maludam is because of the richer animal diversity in the forest. The forest soil is easily contaminated by fertilisation with manure or liquid dung or by water contaminated by animal faeces.

The changes in microbial diversity can be clearly observed in the phyla of *Actinobacteria* (*Table 3*) and α-Proteobacteria (*Table 3*). These two phyla had highest differences in bacterial species. The logging activity has clearly resulted in the reduction of the Actinobacteria and α-Proteobacteria species diversity in the peat forest from 506 to 218 and 475 to 101 species, respectively. The uncommon bacterial species in the phylum of α-Proteobacteria reduced significantly from 57 to nine species. Interestingly, this trend was different for *Actinobacterium* (*Table 3*). The genus *Mycobacterium* was largely affected by the logging activity as shown in *Table 3*. The *Mycobacterium* species was reduced from 29 species to only three species in Cermat Ceria.

The phyla α-Proteobacteria was dominated by *Alpha proteobacterium* in both Maludam and Cermat Ceria with 164 and 31 similarities occurrence, respectively. The α-Proteobacteria population was more diverse in Maludam compared to Cermat Ceria with 66 different species (*Figure 4*) in Maludam compared to 18 species in Cermat Ceria (*Figure 4*). Analysis of the stability of phyla before and after the logging activity showed that the Proteobacteria, mainly dominated by *α-Proteobacteria* was not conserved after the forest clearing. The number of *Hyphomicrobiaceae* bacterium which is the dominant bacterial species of α-Proteobacteria was higher in Maludam, 27.72% compared to Cermat Ceria, 7.51% (*Table 3*). This trend was also similar with *Rhizobiales* bacterium which was also higher in Maludam, 14.85% compared to Cermat Ceria, 5.26% (*Table 3*).

TABLE 3. UNCLASSIFIED BACTERIUM, ACIDOBACTERIUM, ACTINOBACTERIUM, α-PROTEOBACTERIUM AND FIRMICUTES SPECIES ISOLATED FROM PRIMARY DEEP PEAT, MALUDAM AND LOGGED-OVER FOREST, CERMAT CERIA AT SRI AMAN, SARAWAK, MALAYSIA

Phyla	Common/ Uncommon	Sites	
		Maludam	Cermat Ceria
Unclassified Bacterium	(a) Common	Bacterium enrichment culture (13) Forest soil bacterium (101) Uncultured bacteria (5) Uncultured bacterium (6 311)	Bacterium enrichment culture (9) Forest soil bacterium (42) Uncultured bacteria (1) Uncultured bacterium (5 416)
	Total similarity species	6 430	5 468
	(b) Uncommon	Bacterium SCS_30Z1_39 (1) Prokaryote clone (13) Uncultured endolithic bacterium (1) Uncultured eubacterium (1) Uncultured soil bacterium (9232)	Bacterium ADV403 (1) Bacterium ADV70 (1) Iron-reducing bacterium (1) Soil bacterium (222) Uncharacterised organism (14) Uncultured prokaryote (3) Uncultured proteobacterium (124)
	Total similarity species	9 248	3 363
	Overall	15 678 similarities	8 831 similarities
Acidobacteria	(a) Common	Acidobacteria bacterium (606) Acidobacteriaceae bacterium (1) Acidobacteriales bacterium (11) Acidobacterium (2) Bacterium Ellin (7) *Candidatus koribacter* sp. (20) *Holophaga* sp. (6)	Acidobacteria bacterium (1473) Acidobacteriaceae bacterium (74) Acidobacteriales bacterium (24) Acidobacterium (41) Bacterium Ellin (13) *Candidatus koribacter* sp. (24) *Holophaga* sp. (3)
	Total similarity species	653	1 652
	(b) Uncommon	*Acidipila rosea* (1) *Acidipila* sp. (1) *Acidobacterium capsulatum* (2) *Geothrix* sp. (1) *Solibacter* sp. (2)	*Edaphobacter* sp. (1) *Rhodanobacter* sp. (5) Sphingobacteriales bacterium (1) *Telmatobacter* sp. (9)
	Total similarity species	7	16
	Overall	660 similarities	1 668 similarities
Actinobacteria	(a) Common	*Actinoallomurus* sp. (7) *Actinobacterium* (225) *Actinomyces* sp. (3) Actinomycetales bacterium (3) Sporichthyaceae bacterium (2) Thermomonosporaceae bacterium (3)	*Actinoallomurus* sp. (2) *Actinobacterium* (32) *Actinomyces* sp. (4) Actinomycetales bacterium (1) Sporichthyaceae bacterium (2) Thermomonosporaceae bacterium (1)
	Total similarity species	324	42
	(b) Uncommon	Acidimicrobidae bacterium (1) *Acidimicrobium* sp. (1) Actinomycetales bacterium (3) Actinomycete species (1) *Dactylosporangium* sp. (1) *Kitasatospora niigatensis* (1) *Mycobacterium celatum* (6) *Mycobacterium haemophilum* (3) *Marmoricola* sp. (3) Mycobacteriaceae bacterium (1)	*Geothrix* sp. (2) *Mycobacterium bovis* (15) *Mycobacterium canettii* (13) *Mycobacterium tuberculosis* (141) *Rhodococcus erythropolis* (1) Unknown Actinomycete (4)

TABLE 3. UNCLASSIFIED BACTERIUM, ACIDOBACTERIUM, ACTINOBACTERIUM, α-PROTEOBACTERIUM AND FIRMICUTES SPECIES ISOLATED FROM PRIMARY DEEP PEAT, MALUDAM AND LOGGED-OVER FOREST, CERMAT CERIA AT SRI AMAN, SARAWAK, MALAYSIA (continued)

Phyla	Common/ Uncommon	Sites	
		Maludam	Cermat Ceria
		Mycobacterium aemonae (1)	
		Mycobacterium alsiensis (1)	
		Mycobacterium angelicum (2)	
		Mycobacterium asiaticum (2)	
		Mycobacterium avium (17)	
		Mycobacterium bourgelatii (3)	
		Mycobacterium branderi (2)	
		Mycobacterium celatum (6)	
		Mycobacterium colombiense (1)	
		Mycobacterium fragae (1)	
		Mycobacterium haemophilum (3)	
		Mycobacterium intracellulare (31)	
		Mycobacterium kansasii (1)	
		Mycobacterium kyorinense (6)	
		Mycobacterium malmoense (3)	
		Mycobacterium marinum (7)	
		Mycobacterium noviomagense (1)	
		Mycobacterium pseudoshottsii (1)	
		Mycobacterium riyadhense (2)	
		Mycobacterium shimoidei (1)	
		Mycobacterium sp. (20)	
		Mycobacterium szulgai (3)	
		Mycobacterium timonense (2)	
		Mycobacterium ulcerans (3)	
		Mycobacterium yongonense (2)	
		Nocardioidaceae bacterium (1)	
		Nocardioides jensenii (3)	
		Nocardiopsis alba (1)	
		Nocardiopsis quinghaiensis (1)	
		Nonomuraea sp. (1)	
		Streptomyces albovinaceus (3)	
		Streptomyces californicus (1)	
		Streptomyces corchorusii (1)	
		Streptomyces enissocaesilis (1)	
		Streptomyces globisporus (1)	
		Streptomyces goraiensis (1)	
		Streptomyces lusitanus (1)	
		Streptomyces mutabilis (1)	
		Streptomyces parvulus (1)	
		Streptomyces sp. (16)	
		Streptomyces sulphureus (1)	
		Streptomyces thermocarboxydus (1)	
		Uncultured Actinomycete (4)	
	Total similarity speciesl	182	176
	Overall	506 similarities	218 similarites
α-Proteobacteria	(a) Common	Alpha proteobacterium (164)	Alpha proteobacterium (31)
		Bradyrhizobiaceae bacterium (8)	Bradyrhizobiaceae bacterium (4)
		Bradyrhizobium sp. (57)	*Bradyrhizobium* sp. (1)
		Hyphomicrobiaceae bacterium (38)	Hyphomicrobiaceae bacterium (28)
		Methylocystaceae bacterium (2)	Methylocystaceae bacterium (1)
		Paracoccus sp.(1)	*Paracoccus* sp. (1)
		Pseudolabrys sp. (5)	*Pseudolabrys* sp. (9)
		Rhizobiales bacterium (25)	Rhizobiales bacterium (15)
		Xanthobacteraceae bacterium (2)	Xanthobacteraceae bacterium (2)
	Total similarity species	302	92

TABLE 3. UNCLASSIFIED BACTERIUM, ACIDOBACTERIUM, ACTINOBACTERIUM, α-PROTEOBACTERIUM AND FIRMICUTES SPECIES ISOLATED FROM PRIMARY DEEP PEAT, MALUDAM AND LOGGED-OVER FOREST, CERMAT CERIA AT SRI AMAN, SARAWAK, MALAYSIA (continued)

Phyla	Common/ Uncommon	Sites	
		Maludam	Cermat Ceria
	(b) Uncommon	Acetobacteraceae bacterium (4) *Acidocella aluminiidurans* (2) *Acidocella facilis* (1) *Acidocella* sp. (32) *Afipia* sp. (1) *Agrobacterium albertimagni* (1) *Albidovulum xiamenense* (1) *Bartonella elizabethae* (1) *Bartonella* sp. (2) *Blastochloris* sp. (1) *Bradyrhizobium elkanii* (18) *Bradyrhizobium genosp* (1) *Bradyrhizobium japonicum* (1) *Brevundimonas diminuta* (1) *Brevundimonas* sp. (1) *Brucella* sp. (1) *Chelativorans multitrophicus* (3) *Chelativorans* sp. (1) *Dongia* sp. (1) *Ensifer adhaerens* (9) *Ensifer* sp. (14) *Green Bay ferromanganous* (2) Micronodule bacterium (2) *Hyphomicrobium* sp.(1) *Labrenzia* sp.(1) *Mesorhizobium loti* (1) *Methylobacterium* sp.(1) *Methylocystis* sp. (3) *Methyloligella solikamskensis* (1) *Neorhizobium alkalisoli* (1) *Novosphingobium subterraneum* (1) *Ochrobactrum intermedium* (1) *Ochrobactrum* sp. (2) *Ochrobactrum tritici* (1) *Oligotropha* sp. (1) Rhizobiaceae bacterium (9) *Rhizobium herbae* (1) *Rhizobium* sp.(5) Rhodobacteraceae bacterium (2) Rhodobacterales bacterium (1) *Rhodoplanes roseus* (1) *Rhodoplanes serenus* (1) *Rhodoplanes* sp. (92) Rhodospirillaceae bacterium (4) Rhodospirillales bacterium (3) *Rhodovulum* sp.(1) *Roseobacter* sp. (1) *Shinella kummerowiae* (1) *Shinella* sp. (6) *Sinorhizobium fredii* (3) *Sinorhizobium medicae* (3) *Sinorhizobium meliloti* (5) *Sinorhizobium* sp. (13) Sphingomonadaceae bacterium (1) Thermophilic bacterium (1)	Alphaproteobacteria bacterium (1) *Altererythrobacter* sp. (1) *Beijerinckia* sp. (1) *Erythrobacter* sp (1) *Magnetococcus* sp. (1 Mesorhizobium sp. (1) *Methylocapsa acidiphila* (1) *Methylocapsa* sp. (1) *Rhodoblastus acidophilus* (1)

TABLE 3. UNCLASSIFIED BACTERIUM, ACIDOBACTERIUM, ACTINOBACTERIUM, α-PROTEOBACTERIUM AND FIRMICUTES SPECIES ISOLATED FROM PRIMARY DEEP PEAT, MALUDAM AND LOGGED-OVER FOREST, CERMAT CERIA AT SRI AMAN, SARAWAK, MALAYSIA (continued)

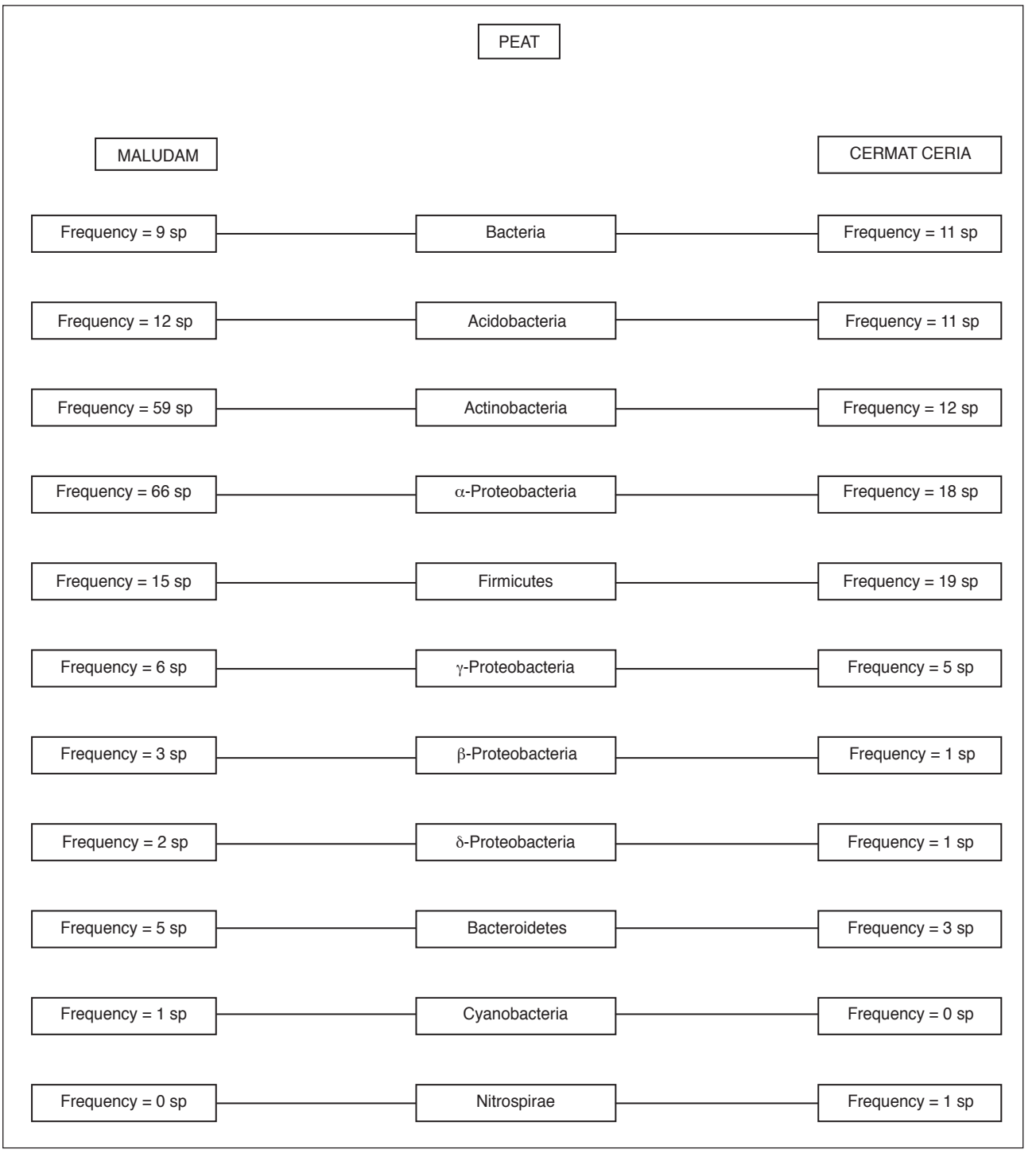
Phyla	Common/ Uncommon	Sites	
		Maludam	Cermat Ceria
		Thermovum composti (2) Uncultured *Stella* sp. (1)	
	Total similarity species	173	9
	Overall	475 similarities	101 similarities
Firmicutes	(a) Common	*Bacillus amyloliquefaciens* (1) *Bacillus* sp. (3) *Bacillus subtilis* (2) *Enterococcus* sp.(2) Firmicutes bacterium (160) *Paenibacillus* sp. (2)	*Bacillus amyloliquefaciens* (23) *Bacillus* sp. (21) *Bacillus subtilis* (36) Enterococcus sp. (1) Firmicutes bacterium (316) *Paenibacillus* sp. (1)
	Total similarity species	170	398
	(b) Uncommon	*Acidithiobacillus* sp. (1) *Anoxybacillus* sp. (1) Bacillaceae bacterium (1) *Carnobacterium viridans* (1) Clostridia bacterium (2) *Desulfotomaculum acetoxidans* (1) *Exiguobacterium* sp. (2) *Lysinibacillus fusiformis* (2) Thermoanaerobacteraceae bacterium (1)	*Bacillus atrophaeus* (1) *Bacillus licheniformis* (1) *Bacillus methylotrophicus* (2) *Bacillus polyfermenticus* (1) *Bacillus stearothermophilus* (1) *Bacillus tequilensis* (7) *Bacillus thuringiensis* (1) Endophytic bacterium (1) *Lactobacillus plantarum* (1) *Paenibacillus borealis* (1) *Streptococcus agalactiae* (1) *Streptococcus sanguinis* (1) *Virgibacillus* sp. (1)
	Total similarity species	12	20
	Overall	182 similarities	418 similarities

Note: Number indicates total similarity species.

Rhizobiales bacterium which is in the families of *Methylocystaceae* and *Hyphomicrobiaceae* together with *Bradyrhizobiaceae* represent as key bacteria involved in soil C and N cycling (Acosta-Martinez *et al*., 2010). This metabolically diverse group of organisms can be recognised in several subphyla of four, which are α-, β-, γ- and δ-Proteobacteria. The predominance of many bacteria under pasture, including Acidobacteriaceae, may reflect the combined effects of every different root systems and their exudates (*i.e*., type and amounts), and differences in soil properties (*i.e*., lower soil pH, less disturbance).

The dominant species in the phylum Firmicutes is shown in *Table 3*. Unidentified Firmicutes bacterium was the dominant species found in both ecosystems with 160 similarities occurrence in Maludam and 316 similarities occurrence in Cermat Ceria. *Bacillus amyloliquefaciens* was the dominant bacteria species identified in Cermat Ceria with 23 similarities occurrence. This bacteria was known as growth promoting bacteria, plant protector and involves in induction of Systemic Resistance (ISR) (Chowdhury *et al*., 2013). In fact, the *Bacillus* genus was more diverse in Cermat Ceria compared to Maludam with nine different species compared to Maludam with three different species (*Table 3*). Firmicutes such as *Bacillus* and *Paenibacillus* were well-characterised plant-associated genera with antagonistic properties towards fungal plant pathogens (Koberl *et al*., 2011).

Both sites had a total number of 32 common bacterial species of the prevalent phylum. Each phylum - α-Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes and unclassified Bacteria comprised of nine, seven, six, six and four common species, respectively (*Table 3*). Conversely, the distinctive species of α-Proteobacteria, Actinobacteria and Acidobacteria at Maludam and Cermat Ceria reduced from 57 to 9, 53 to 6 and 5 to 4, respectively (*Table 3*). However, the distinctive species of Firmicutes and unclassified Bacteria increased



Note: sp - species.

Figure 4. Species frequency within prokaryotic phyla in primary deep peat Maludam and Cermat Ceria at Sri Aman, Sarawak, Malaysia.

from 9 to 13 and 5 to 7, respectively (*Table 3*). The distinct species were found in Maludam such as *Acidobacterium capsulatum, Solibacter, Mycobacterium intracellulare, Rhodoplanes* sp., *Clostridia bacterium, Exiguobacterium* sp. and *Lysinibacillus fusiformis*. Whereas, the distinct species were found in Cermat Ceria such as *Telmatobacter, Mycobacterium tuberculosis* and *Bacillus tequilensis*. These data showed that many species of α-Proteobacteria and Actinobacteria disappeared after forest clearing. For example, the genus *Rhodoplanes* can be isolated from pond water (Okamura *et al*., 2009) and soil (Lakshmi *et al*., 2009) were consisted of a group of purple non-sulphur, denitrifying bacteria. The members of this genus can be found widely in the soil which shows that they are potentially involved in nitrogen cycling and energy transformation in soils (Okamura *et al*., 2009).

Species frequency within prokaryotic phyla in *Figure 4* showed the presence of bacteria that occurred more than one time as one single species. *Figure 4* was constructed based on the phylogenetic tree (data not shown) constructed for the analysis processes. Results showed the reduction of bacterial species in α-Proteobacteria, Actinobacteria and Acidobacteria from 66, 59 and 16 species to 18, 12 and 11 bacterial species for Maludam and Cermat Ceria, respectively. Conversely, the diversity of Firmicutes increased from 15 different bacterial species to 19 species. This data also proved that the logging activity affects the population diversity of the microbial species in the primary forest.

The effect of peat area degradation on the development of the peat area for agricultural purposes should be completely monitored. The physical environmental changes might also affect the microbial population before and after forest clearing. Firdaus *et al*. (2011) stated that the percentage of fibre content, volumetric water content and saturated hydraulic conductivity were not significantly affected by the logging process. However, the gravimetric water content, loss on ignition and total porosity were significantly higher in the drained secondary peat swamp forest, while ash content, bulk density, surface soil temperature and bearing capacity were significantly higher in the cleared site of drained secondary peat swamp forest. Therefore, the understanding of the bacterial diversity of soil in the affected area is essential since the bacterial diversity and soil function are correlated. The microbes can be phosphate solubilising, nitrogen fixing and plant disease suppressing which help to improve the crop yield and protection. Hence, PCR-DDGE technique was performed in order to conduct such studies (Lucena-Padrós *et al*., 2015). This is due to the immediate result that can be provided in a qualitative and semi-quantitative way. However, a single species of DGGE band might also have multiple copies of *16S rRNA* gene. Therefore, the application of cloning vector can be applied as reported by Sekiguchi *et al*. (2001). The cloning vector is highly specific to one single strain which enables the differentiation of the *16S rRNA* gene copies because one gene can only be ligated with one cloning vector (Sekiguchi *et al*., 2001). Hence, the multicopies of *16S rRNA* gene can be differentiated to distinguish different individual of the same species. Therefore, the application of DGGE coupled with cloning vector can be utilised to overcome this issue.

CONCLUSION

Microbial biodiversity in Maludam showed significant differences in the number of bacterial species compared to Cermat Ceria. The bacterial population was also more diverse in Maludam compared to Cermat Ceria. The application of DGGE provides an early indicator that the logging activity can alter the bacterial population in the peat soil. However, the overall picture of the taxonomic composition can be made broader and more accurate through the application of next generation sequencing (NGS) since it avoids the biasness during PCR amplification and DGGE gel excision.

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