

THE MICROBIAL DIVERSITY AND STRUCTURE IN PEAT LAND FOREST IN INDONESIA

Talukder, M. J. H. and Hui Sun¹

Forest Department, Ministry of Forest and Environment, Bangladesh; ¹Department of Forest Protection, College of Forestry, Nanjing Forestry University, Jiangsu, China

Abstract

Soil of tropical forest ecosystem plays very crucial part in controlling the universal carbon cycle. The isolation of microorganisms and their identification are important for understanding their vital role on transformation of organic matter of this ecosystem. Soil storage maximum microbial genetic diversity because of it's a multilayered environment. No complete methods were discovered yet to cultivate majority of soil microorganisms. A little is known about microbial structure and their essentiality in tropical peat lands compared to most other terrestrial and oceanic habitats. In providing insight to the impacts of land-use of peat land on microbes in Central Kalimantan, Borneo Indonesia, we examined the community structure and diversity of bacteria and fungi in different peat forest soil including: i) natural peat swamp forest (well mixed swamp forest type); ii) disturbed peat soil and iii) mineral soils by using 454 pyrosequencing technology. The results showed that no significant difference was found for diversity and evenness among the sites of fungal community. However, natural peat swamp forest had the highest species richness (Chao1), which was significantly higher than the other two sites ($P < 0.05$ and $P < 0.05$). According to the OTUs analysis four fungi phyla were obtained of which 45 species were classified. The *Ascomycota* was the most abundant phylum, followed by *Basidiomycota*, *Zygomycota* and *Glomeromycota*. The natural peat swamp forest and disturbed peat soil harbored the maximum number *Ascomycota*. On the other hand, mineral soil and natural peat soil contained the highest number of *Basidiomycota*. The top species in natural peat swamp forest included *Sugiyamaella paludigena*, *Polyancora globosa* and *Ganoderma gibbosum*. The mineral soil enriched the abundance of *Penicillium herquei*, *Sugiyamaella paludigena* and the disturbed peat soil contained the highest frequency of *Polyancora globosa*, *Gymnopilus lepidotus*. According to the PCoA analysis, the community structure of fungus in natural peat soil differed significantly from mineral soil ($P = 0.04$) and disturbed peat soil ($P = 0.039$). No significant difference was found for bacterial species richness (Chao1) among the sites. The diversity of bacteria in disturbed peat soil significantly differed from the other sites ($P < 0.05$ and $P < 0.05$). eleven bacterial phyla and 53 genera were examined. All of the three sites contained the similar abundance of *Proteobacteria*. The natural peat swamp forest and disturbed peat soil harbored the most abundant *Acidobacteria*. Genera *Mycobacterium*, *Gp1*, *Gp13*, *Gp2*, *Burkholderia*, *Actinospica*, *Aciditerrimonas* were found in all the sites. Genera *Granulibacter*, *Gp4*, *Acidisoma*, *Clostridium_sensu*, *Clostridium_XI* were only observed in natural peat swamp forest. Genera *Rudaea*, *Rhodopila*, *Streptomyces* were found only mineral soil. The PCoA analysis showed that the structure of bacteria in natural peat swamp forest significantly differed from the disturbed peat soil ($P = 0.045$). Overall, the bacterial species richness and diversity are more among the sites than of the fungi.

Key words: Tropical peatland; Fungi; Bacteria; Community structure; Diversity; 454 Pyrosequencing.

INTRODUCTION

Tropical Peat swamp forests are important ecosystems for conserving unique biodiversity, moderating climate and energy instabilities through carbon sequestration, reducing erosion of soil and promoting ecosystem stabilization (Harrison 2013, Rieley *et al.* 2008, Sorensen 1993). Due to population explosion unfortunately, this fragile ecosystem is under pressure from agriculture, agroforestry and silviculture (Pretty *et al.* 2011, Wosten *et al.* 1997). So the alteration of peatlands from virgin forest to alternative uses is usually accompanied by many negative effects on this environment already observed. The ominous results include increased discharge of greenhouse gases (Oleszczuk *et al.* 2008), expanded carbon loss through aerobic peat disintegration and reduction in carbon assimilation by photosynthesis (Nakane *et al.* 1996) also we loss its vivacious purposes one example a storage for

unique flora and fauna (Puglisi *et al.* 2014). Due to high population growth in many tropical countries and associated land-use pressures, this ecosystem faces greater risk. Compare to tropical peatland, boreal and temperate peatlands face lesser risk (Rieley *et al.* 1996, Vijarnsorn 1996). 11% of the global peatland area occupies by tropical peatland (441,025 km²), with 56% of it is Southeast Asia (247,779 km²) (Page *et al.* 2011). However, in the early 2000s, approximately 8800 km² of Malaysian and Indonesian peatlands were converted to cultivate of oil palm and 230,000 km² of dense peat area was clear-felled and is currently listed as despoiled lands (Koh *et al.* 2011). These disturbances have resulted in peat degradation. Peat burning associated with agricultural practices releases CO₂ and increases drainage. It can also increase the giving out of CO₂ gradually the advancement of aerobic peat decomposition. Vegetation uptake CO₂ through photosynthesis is also declined by shading due to thick smoke from peat flames ignited accidentally or deliberately for purposes of agriculture (Hirano *et al.* 2012). It is clear that, without due care, valuable tropical peatlands can switch from their traditional roles as carbon sinks to sources of carbon to the atmosphere. As the consequences of these negative impacts current research focuses on developing measures to reduce peat swamp deforestation and to restore damaged peat forests. Several restoration activities have been conducted in Indonesia and Malaysia (Holden *et al.* 2007, Page *et al.* 2009b).

MATERIAL AND METHODS

The study area is located in the jurisdiction of Central Kalimantan in Indonesian Borneo. The physiognomies of the areas primarily cover with tropical rain forest vegetation with average annual temperature of 27°C and the annual rainfall of approximately 2600 mm with dryer period started from June and end in October. Two locations were chosen for sampling. The first location is in the natural peat swamp forest in the Sabangau National park in the so-named Natural laboratory field research area managed by CIMTROP (Center for International Cooperation in Sustainable Management of Tropical Peatland). The distance of the area is 150 km inland from the Java Sea between rivers Sabangau and Katingan 13 km south from provincial capital Palangaka Raya. The distance of ground surface elevation is at highest 30 m a.s.l. The Natural laboratory area is nearly pristine with some signs of choosy logging during 1970-90s' in the forest structure especially closer to forest margins near the rivers. The site of Sabangau peat swamp forest is an ombrotrophic nature dome-shaped forested peat bog with 12 m peat depth at the epicenter of the dome. The basic forest type in the investigation zone is mixed swamp forest where the highest trees reach 35m. The uniqueness of forest ground is that the area remains under flooding approximately for 8 months a year. The earth exterior surface is uneven with variation of developed raised exteriors and depressions i.e. hummocks and hollows with a maximum range at about 110 cm (Lampela *et al.* 2014). The biodiversity of the area consist certain extent completely of trees and tree seedlings with very few other vascular plants or mosses. Considering the flora, there are more than 150 tree species in the Sabangau peat swamp forest (Waldes and Page 2002) and the underlying peat is formed of woody debris such as leaf litter, decaying roots, branches and fallen tree trunks (Shepherd *et al.* 1997, Page *et al.* 1999, Lampela *et al.* 2014). The samples were taken from two nearby spots (S 219.376', E 113°54.244' and S 219.433', E 113°54.268') within the forest of 1.5 km remoteness from the forest edge. In the perfect position of the sampling place, the peat depth is between 2 and 3m. We collected three samples from each spot representing pristine peat forest. The second location is near the village of Bawan about 70 km north from Palangka Raya. Bawan village forest areas consist of both mineral soil and disturbed peat soil with areas used for small-scale plantations and natural forests with some signs of selective logging. Three samples were collected from mineral soil (S 135.344', E 113°59.475') and disturbed peat forest (a shallow peat swamp forest with some trees and bushes, < 1 m depth) (S 134.344', E 113°59.940'). The peatland is quite open with small trees (<10 m height) such as

Combretocarpus rotundatus and *Camposperma coriaceum* and the forest floor is partly covered with *Sphagnum* mosses and herbal vegetation such as *Nepenthes sp.*, sedges, clubmosses (*Lycopodiaceae*) and ferns. All the samples were taken into 50 ml sterile tubes using rubber glove and consisted of topsoil from the foraging region with some leaf litter. The samples were kept in -20°C for further analysis.



Fig. 1. Area map: **a.** South-East Asian archipelago, island of Borneo and Palangka Raya, the capital of the Indonesian province of Central Kalimantan; **b.** Two sampling locations in Central Kalimantan: forests in the Sabangau National Park, drained and clear-cut areas near village and Bawan village forests in the north part of the river Kahayan.

Permitting to the recommendation of manufacturer, genomic DNA was extracted from 0.5 g of the homogenized soil per sample using the 'PowerSoilTM DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). The DNA was quantified with a Nanodrop-1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA). The Internal Transcribed Spacer (ITS) region was amplified using the fungus-specific primers ITS1-F (containing 454 pyrosequencing A adapter and a 6-bp barcode) and ITS4 (containing 454 pyrosequencing B) (Terhonen E et al., 2013, Gardes M, Bruns TD, 1993). In this process, the probable intensification of pollutants was evaluated with a negative PCR control, in which the template DNA was replaced with sterile water. These remained free of PCR amplicons. The bacterial primers 27 F (5'-AxxxxxxAGAGTTTGATCCMTGGCTCAG-3') and (5'-B GTATTACCGCGGCTGCTG-3') were used to generate 16S rRNA gene fragments of ca. 500 bp from the variable regions 1-3. A total of 100ng of template DNA was used for a 50 μl PCR amplification reaction. The following thermal cycling scheme was used: 98°C for 30s (pre-denaturation), 28 cycles of 10s at 98°C (denaturation), 59 $^{\circ}\text{C}$ for 30s (annealing) and 72°C for 30s (extension), followed by 10min at 72°C (final extension). Possible amplification of impurities was calculated with a negative PCR regulator in which the template DNA was exchanged with sterile H_2O . These stayed free of PCR amplicons. The attendance of PCR produces was determined by analyzing 5 μl of product on 1.5% agarose gel to confirm DNA amplicons. Amplicons were cleaned by means of Agencourt AMPure XP beads (Beck-man Coulter) to remove amplification primers and reaction buffer. After refinement, DNA concentration was calculated by Nano-drop ND-1000 spectrophotometer and equivalent quantities (~ 100 ng) of all amplicons were mixed in a solo tube. The PCR amplicons were sequenced using ITS1-F primer and 27 F primer for fungus and bacterial, respectively at the Institute of Biotechnology (University of Helsinki Finland) using the 454 GS-FLX titanium protocol (Life Science/Roche Diagnostics, CT,USA), which yielded read lengths of 400 bp.

Sequence data were analyzed using the mother pipeline v. 1.39 .2 (Schloss *et al.* 2009) with some modification. To sum up, the raw reads were exposed to quality control, and each sequence was separated for a matching to the sequencing primer (ITS1-F or 27F) and valid DNA tag. Sequences were discarded if they had (i) ambiguous (N) bases; (ii) homopolymers lengthier than eight nucleotides; or (iii) an average Phred quality score lower than 25. Each sequence that passed the quality filtering was truncated to a 250-bp length after the primer (ITS1-F for fungi or 27F for bacteria) and tag were

removed. Due to lacking of fungal reference database for alignment, the fungal data were pairwise aligned within the dataset. The remaining of the sequences was preclustered within a distance of 1 bp using a pseudo algorithm implemented in mothur. All potential chimeric sequences were categorized by using the mothur-embedded uchime *de novo* algorithm (Edgar *et al.* 2011) and discarded. The high quality and unique sequences were aligned by using the Needleman method (Needleman and Wunsch 1970) and then were clustered into operational taxonomic units (OTUs) using the average neighbour joining algorithm 97% similarity. Because of their uncertain origin, all global singletons (OTUs containing only one sequence across all samples) were omitted (Tedersoo *et al.* 2010). The whole sequence data set of fungi was phylogenetically classified using a mothur-formatted copy of the full UNITE+INSD data sets version 6 with an 80% confidence threshold in mothur. At this stage, by using the remove lineage command inn mother, non-fungal sequences were discarded. We also determined functional groups of fungal taxonomic affiliation by using FunGuild (Koljala *et al.* 2013). The sequences for bacterial dataset were aligned to the SILVA alignment database (Pruesse *et al.* 2007) and were clustered into operational taxonomic units (OTUs) defined by a 3% distance level using the average neighbor algorithm in Mothur Ver. 1.39.2 (Schloss *et al.* 2009). To determine the taxonomic groups, sequences were phylogenetically classified with classifier tool version 2.0 (Wang *et al.* 2007) with an 80% confidence threshold (Wang *et al.* 2007) using the RDP Naïve Bayesian. Observed OTUs, species evenness (simpsonevenness) species richness or (Chao1) diversity (Inverse Simpson's complement) and Good's coverage (complement of the ratio between local singleton OTUs and the total sequence count) were calculated in Mothur. The number of sequences of the lowest size (1,000 reads/sample for fungi, 1886 for bacteria) among all samples was randomly subsampled (rarefaction) and used for calculation the diversity index ensure comparable estimators across samples, estimators and for fungal structure comparison between communities.

Principle coordinate analysis (PCoA) was used visualizing the bacterial or fungal community structure based on the relative abundance of fungal or bacterial OTUs abundances in PRIMER 7+ (Anderson 2001). Permutational multivariate analysis analysis of variance (PerMANOVA) tests (Anderson 2001) was used to test the structure difference. Mann-Whitney analysis was used to determine the minimum significance difference ($p < 0.05$) in diversity index Chao1 and Shannon between (Peat swamp forest soil, disturbed peat soil and mineral soil) with SPSS 15.0 for Windows.

RESULTS AND DISCUSSION

Information about pyrosequencing data

Due to technical reason, one sample from mineral soil and one sample from disturbed peat soil were unable to be sequenced in 454 pyrosequencing. After sequence denoising and quality control, 17,616 fungal sequence reads in total were obtained and the number of reads from each sample ranged from 1022 to 2142 with an average of 1762 ± 360 per sample. For comparative analysis among sites, a total of 1000 sequences were randomly subsampled from each sample. The species accumulations (rarefaction) with sequencing efforts were showed that in Figure S1 in the supplementary material. 22,195 bacterial sequences read were obtained in total after cleaning. The number of sequences per sample ranged from 2229 to 3103 with an average 2466 ± 788 reads. For comparative analysis among sites, a total of 1886 sequences were randomly subsampled from each sample.

Fungal and bacterial community diversity

For fungi, a total of 664 fungal OTUs were observed across the three sites. The natural peat swamp forest, harbored the highest species richness (Chao1), which significantly differed from the other two sites ($P < 0.05$ and $P < 0.05$) whereas the mineral soils had the least species richness (Fig. 2). The evenness

for natural peat swamp forest, mineral soil and disturbed peat soil were 0.0819, 0.0581 and 0.11 respectively. No significant difference for evenness was found between the three sites. The disturbed peat soil had the highest fungal diversity with 9.91, followed by natural peat swamp soil with 9.58 and mineral soil with 6.51. However, no significant difference was found for diversity between the sites.

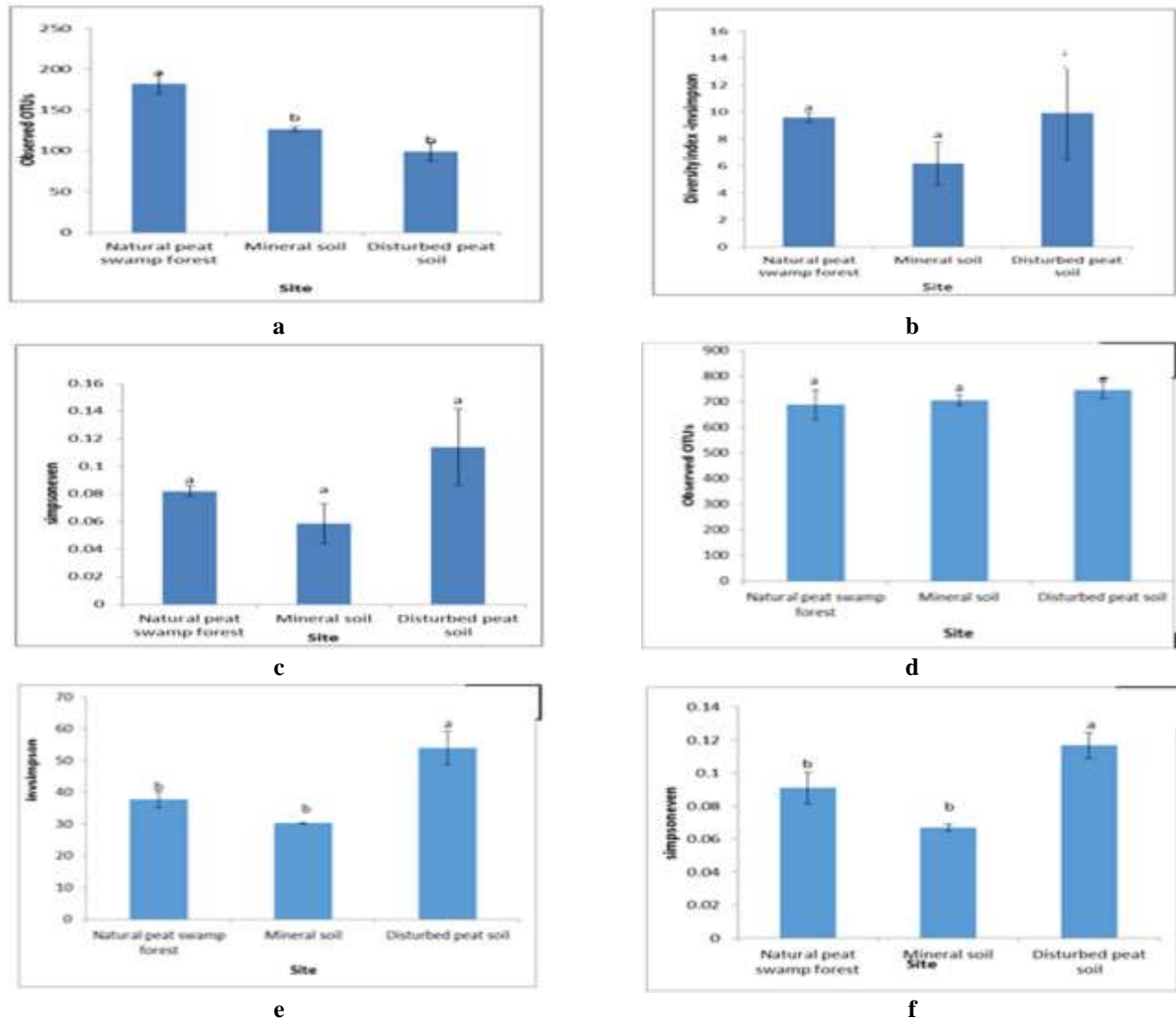


Fig. 2. Fungal and bacterial community estimators across the natural peat swamp forest, mineral soil and disturbed peat soil: **a.** observed OTU richness of fungi; **b.** inverse Simpson's diversity of fungi; **c.** evenness based on Simoson's diversity index of fungi; **d.** observed OTU richness of bacteria; **e.** inverse Simpson's diversity of bacteria; and **f.** evenness based on Simoson's diversity index of bacteria. The letter above the columns representing the Tukey's significance at a P value of 0.05.

For bacteria, the disturbed peat soil had the highest species richness (Chao1) (747) followed by mineral soil (706) and natural peat swamp forest (689). No significant difference was observed for species richness between sites. The diversity (inverse simpson index) was the highest in disturbed peat soil (54.05) followed by natural peat swamp forest (37.71) and mineral soil (30.31) (Fig. 2). The diversity in disturbed peat soil was significantly different with the other sites ($P < 0.05$, respectively). The disturbed peat soil had the highest evenness, which was significantly higher than that in the other two

sites ($P < 0.05$, respectively) and the mineral soil had the least bacterial evenness (0.066) (Fig. 2). The disturbed peat soil had the highest diversity (5.1) followed by mineral soil (4.9) and natural peat swamp soil (4.8).

Fungal and bacterial community composition

The fungal OTUs were classified into four fungal phyla (Table 1). The *Ascomycota* was the most abundant phylum, followed by *Basidiomycota*, *Zygomycota* and *Glomeromycota*, (Fig. 3). The majority of the OTUs (33% of the total 664 OTUs) belonged to the *Ascomycota* covering 7133 sequence reads (40% of the total 17616 sequences reads). *Basidiomycota* represented 17% (115 OTUs) of the total OTUs accounting for 19% of the sequences reads (33030 reads). *Zygomycota* and *Glomeromycota* made up 0.15% and 0.15% of the OTUs and counting for 0.011% and 0.011% of the sequences reads respectively. We were failed to classify nearly forty percent (7176 sequences reads) of the fungal sequences were for any fungal phylum (Fig. 3).

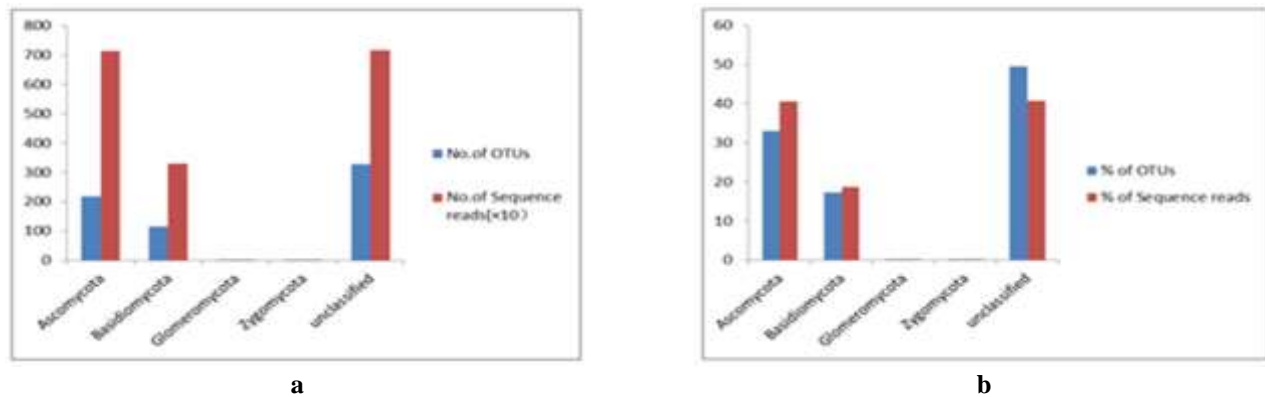


Fig. 3. Bar charts showing the phylum-level assignment for operational taxonomic units (OTUs) from the three sites: **a.** the number of OTUs ($n=664$) and sequences reads ($n=17616$); and **b.** the relative proportions of OTUs and sequence.

Class *Eurotiomycetes* was the prominent class at natural peat swamp forest and class *Leotiomycetes* was prominent in mineral soil. On the basis of frequencies, class *Saccharomycetes*, *Agaricomycetes* were also distinct at all sites (Fig. 4). Genus *Metarhizium* strongly dominated the community in disturbed peat soil. Genus *Penicillium* was also observed in natural peat swamp forest and mineral soil with relative abundance of 0.54% and 8 OTUs. Genus *Phlebia* was observed only in natural peat swamp forest with relative abundance of 0.70%. Interestingly genus *Russula* was found only in mineral soil and disturbed peat soil but completely absent from natural peat swamp forest.

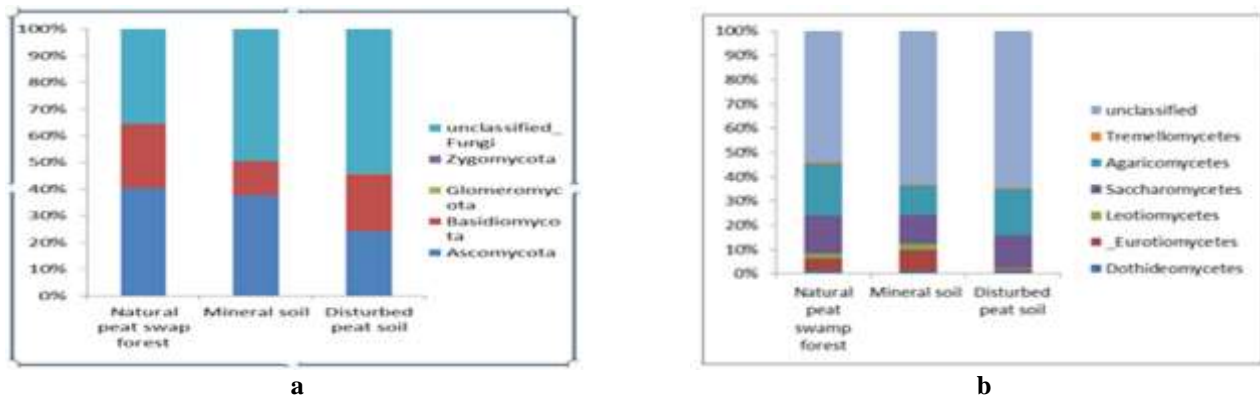


Fig. 4. Relative abundances of the three sites: **a.** phylum; and **b.** classes.

Table 1. Fungus OTUs classification.

Taxon	Natural peat swamp	Mineral soil	Disturbed peat	Total
Phyla	3	3	2	4
Class	6	6	6	6
Order	24	13	14	15
Family	30	12	16	32
Genus	38	13	31	45
Species	39	8	14	45

Genus *Lauriomyces* was also observed in mineral soils and disturbed peat soil only. In addition, genus *Candida* was only found in natural peat swamp forest and disturbed peat soil exclusively. The relative abundance of genus *Sugiyamaella* was 0.82% in natural peat swamp forest and mineral soil. Genus *Tomentella* genus was also presented in the entire three site. Unclassified affinities associated with 35% to 59% of the sequences at each sites. The top of species in natural peat swamp forest was *Basal lineages*, *Scytalidium sp*, *Sugiyamaella paludigena*, *Polyancora globosa*, *Ganoderma gibbosum*. Moreover, the mineral soil enriched the abundance of *Penicillium herquei*, *Basal lineages*, *Sugiyamaella paludigena*, *Chloridium sp*, *Heliscus submerses*. On the other hand the disturbed peat soil contained the most frequency genera of *Metarhizium sp*, *Polyancora globosa*, *Gymnopilus lepidotus*. PCoA analysis showed 39.7% variation in total among sites and the first axel explained 21.8% of total variation and the second axel explained 17.9%. The fungal community structure differed between natural peat soil and mineral soil ($P=0.04$). In addition, the fungal community structure also differed significantly between natural peat soil and disturbed peat soil ($P=0.039$). However no significance different in community structure were observed between mineral soil and disturbed peat soil ($P=0.342$).

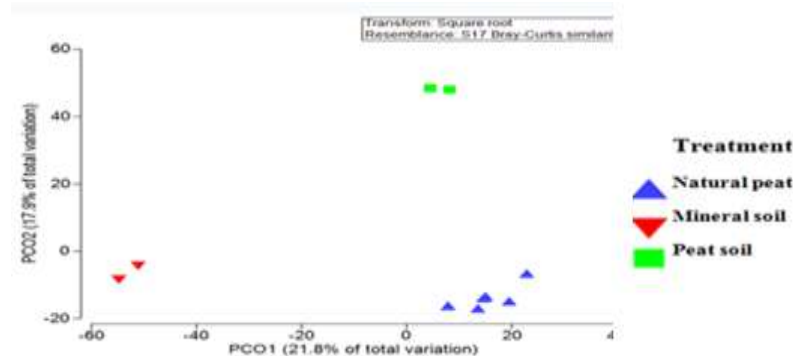


Fig. 5. Principal co-ordination analyses showing the fungal community structure in different soil types.

For the soil symbiotrophs, the abundant genera were *Serendipita*, *Williopsis*, *Russulaceae* and *Chloridium*. The soil saprotrophs included genera *Scytalidium*, *Phlebia*, *Gliocephalotrichum* and *Blastobotrys*. Pathotrophs were associated only with natural peat swamp forest. Among the top most 18 fungal genera 4 genera belonged to the mycorrhizal/symbiotroph, whereas 4 genera belonged to soil saprotrophic fungi. Mycorrhizal fungi were also the most prominent group based on the number of sequence reads. Pathotroph-saprotroph contained 4 genera. The pathotroph-saprotroph-symbiotroph contained 3 genera. Also pathotrophs contained 3 genera.

1886 sequences were randomly subsampled from each sample for comparable analysis between sites. The bacterial OTUs were classified into 11 phyla (Table 2). *Proteobacteria* was the most abundant phylum followed by *Acidobacteria*, *Actinobacteria*, *Planctomycetes* and *Verrucomicrobia* (Fig. 6). The majority of the OTUs (38% of the total 1408 OTUs) belonged to the *Proteobacteria*, covering 9728

sequence reads (44% of the total 22195 sequences reads). *Acidobacteria* represented 21% (299 OTUs) of the total OTUs and accounting for 33% (7243) of the sequences read. Moreover, *Actinobacteria* counted for 12.5% (176 OTUs) of the total OUTs and represented 10% (2165) of sequences.

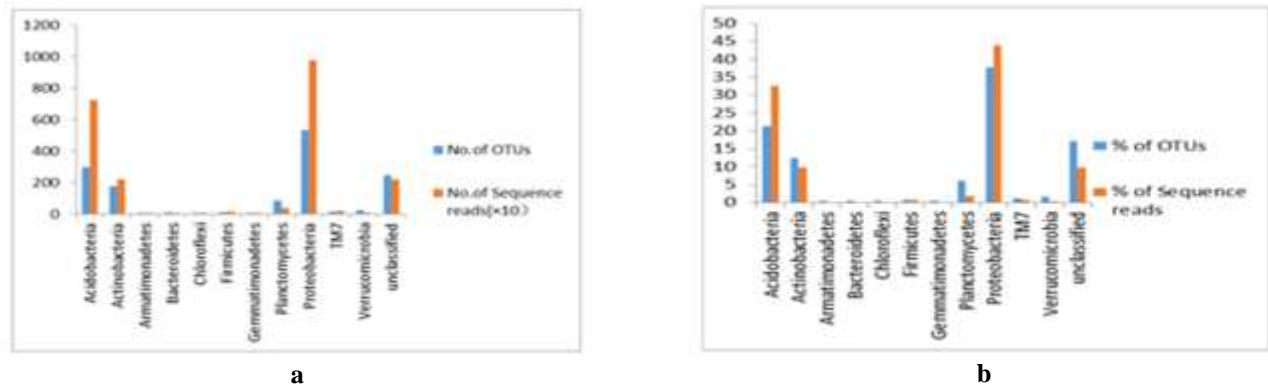


Fig. 6. Bar charts showing the phylum-level assignment for operational taxonomic units (OTUs) from the three sites: **a**. the number of OTUs (n=664) and sequences reads (n=17616); and **b**. the relative proportions of OTUs and sequence.

Twenty two bacterial classes were observed across the entire samples. The class of *Alphaproteobacteria* had the highest abundance with 36.68% across all the samples followed by *Acidobacteria* 32%, *Actinobacteria* 9.75%. Unknown class represented nearly 15% of the entire sequences. 53 genera were found across all the samples. Within *Acidobacteria* Gp1, Gp2, Gp3, Gp4, Gp5, Gp13 were identified as the most abundant genera (on average 28.78%, 2.76% and 0.73%, 0.01%, 0.01%, 0.17%, respectively).

In natural peat forest soil *Acidobacteria*, *Actinobacteria* and *Proteobacteria* were dominant all the samples comprising 85-90% of all the sequences. Unclassified or unknown affinities accounted for 12-25% of the sequences at each sites (Fig. 7). PCoA analysis showed 49.9% variation in total among the sites and the first axel explained 35.1% of total variation and the second axel explained 14.8%.

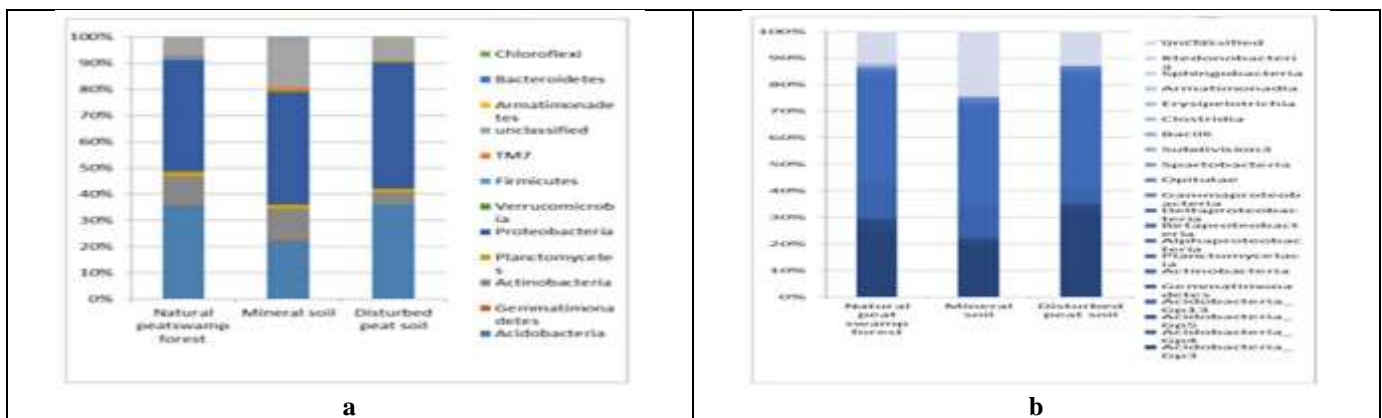


Fig.7. Relative abundances of phylum (a) and the classes (b) of the three sites.

The bacterial community structure differed significantly between natural peat soil and disturbed peat soil ($P=0.046$). No difference in community structure were observed between mineral and natural peat soil ($P=0.054$). No significant difference in community structure was observed between mineral soil and disturbed peat soil ($P=0.327$).

Table 2. Bacterial OTUs classification.

Taxon	Natural peat swam	Mineral soil	Disturbed peat soil	Total
Phyla	10	10	11	11
Class	21	17	19	22
Order	21	17	18	23
Family	25	22	21	32
Genus	41	32	34	53

Compare to the microbial studies in boreal peatland forest, the studies in tropical peatland forest are not so intensive. Presently the information is very few on how the distribution and characteristics of tropical peat lands e.g. land management practices impact on microbial diversity (Zinck 2011). Jackson *et al.* (2008) in Malaysian peat swamp forest studied the bacterial community by using denaturing gradient gel electrophoresis (DGGE) profiles. Most of the studies in tropical peatland were focused on the release of greenhouse gases burning of peatland (Langner and Siegert 2009). Few researches have been focused microbial diversity (Turjaman *et al.* 2011).

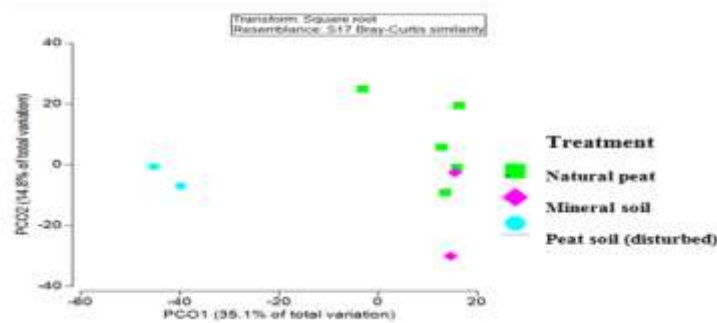


Fig. 8. Principal co-ordination analysis showing the bacterial community structure in the three types of soil.

In the current study, we analyzed both fungal and bacterial community in a natural and disturbed peat soil with one mineral soil by using next generation sequencing technology (454 pyrosequencing). Using microbial community assessment could assist in considering the regulation of the activities of a microbial population. Such can lead to the next generation of ecological theories which may help predict tropical peatland response to global change and human made disturbance (disturbed peat soil). 45 fungus genera and 53 bacterial genera were obtained. We get very few results to determine the microbial diversity using culture-dependent method, however, this method gives false result of total diversity of fungi and bacteria, also the microbes which grow rapidly in traditional culture method provides biased result (Hering 1967, Frankland 1998). There are some several limitations (Amend *et al.* 2010), next-generation sequencing seems to be better suitable to explore the microbial community because it can deliver information at higher quantitative resolution and is not biased towards easily cultural and fast growing taxa. The diversity of bacterial populations was considerably higher than that of fungal populations. Nearly 150 OTUs were found in bacteria, compared with 70 OTUs in fungi per sample. The special feature likely physical, chemical and biological properties make peat swamp forest unique consider from all other terrestrial and wetland area. Some species of bacteria properly distributed across all the sites. In our three sites of different samples *Proteobacteria* and *Acidobacteria* comprised 60%-80% of all sequences. The *Alpha-proteobacteria* and *Acidobacteria-GpI* were the dominant class in all sites. Some researchers recommended that certain bacteria phyla, such as the *Acidobacteria* and *Proteobacteria* can be used as signs of nutrient status owing to differences their lifestyles (Hartman *et*

al. 2008). If the soil contains more, that is one of the indicators of high abundance of *Proteobacteria* (McCaig *et al.* 2005). *Acidobacteria* prefers to grow one special condition. This condition is called oligotrophic and richness of the *Acidobacteria* indicates the conditions are acidic (Philipot *et al.* 2010). For fungi, sequencing yielded 17616 sequences. The fungal diversity did not differ among sites. The bacterial diversity, however, significantly differed among sites. This indicates that bacteria community diversity is more vulnerable to disturbance than fungi community. One very impressive result, we identified many taxonomic group of *Acidobacteria* (Gp1, 2, 3, 4, 5 and 13) than the previous study (Gp1, 2 and 3), which depicted high diversity of this bacteria in this acidic tropical peatland ecosystems (Pattanop *et al.*, 2010). The environment of poor nutrient humid wetland forests are thought to favor *Acidobacteria* (Gp1, 2, and 3) (Jckson 2008), that is another indicator of extreme conditions in this ecosystem. It was observed the lowest diversity in mineral soil. An increase in microbial species may increase the contribution of these organisms to ecosystem services (Ferris and Tuomisto 2015). The current study assessed only the microbial community diversity and composition. More studies are necessary to investigate microbial function. High number of OTUs and diversity indexes (the Shannon's and Simpson's indexes) suggest a high number of species in all tested samples. Still, the maximum plentiful phyla, in all verified samples, were: *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*, with variable percentage in the whole segment between the samples. Such richness and composition of portion in bacterial communities is collective for greatest soil samples of different origin considered so far (Janssen 2006). Furthermore, peat soil bacterial community structure is related to native environmental issues, especially with soil acidity, correspondingly as it was detected for the fungal community composition in this tropical region (Zhang *et al.* 2016). However, the relative abundance of these three groups (*Proteobacteria*, *Acidobacteria*, and *Actinobacteria*) and bacterial structure among three communities at the three sites were different found. The results go along with other studies which indicate that land use is one of the key factors in determining the structure of bacterial communities (Zhang *et al.* 2016). PCoA analysis indicated the fungal community structure differed between natural peat soil and mineral soil ($P=0.04$) indicates tropical peat soil harbors different microbial community. The fungal community structure also differed significantly between natural peat soil and disturbed peat soil ($P=0.039$) suggesting that type of land uses statistical significantly affect microbial community. Similarly, the bacterial community structure differed significantly between natural peat soil and disturbed peat soil ($P=0.046$), which confirming the conclusion that land use in tropical peat forest will shift the microbial community. In many cases we did not identify of fungal sequences (nearly 50%) at any of our study sites to any fungal phylum. It is unclear to which functional groups in FunGuild (ERM, ECM, and SAP) these unidentified sequences belong. Further research into the function and diversity of soil fungal communities is clearly needed. The *Basidionmycete* was one of the dominant fungi at all sites which fulfill a central role in most land-based ecosystems. They play very important role as decomposer disintegrate organic matters, such as twigs and animals and helping detrivore animals. This type of animals feed decomposed materials to get their nutrients. Some other fungi together with *Basidionmycete* can disintegrate large molecules some example lignin or cellulose and play very important roles in nutrient and carbon cycling (Deacon 2005). The fruiting bodies of the *Basidionmycete* are the great source of food for many animals ranging from slugs, snails and insects (*Gastropoda*) to the large mammals such as wild boars and deer also small rodents (Deacon 2005). Many *Basidionmycete* also have strong symbiotic relationships with other organisms, including not only animals but also plants. Genera *Ganoderma*, *Coniophora*, *Tinctoporellus*, *Tomentella*, *Trichoderma*, *Pestalotiopsis*, *Perenniporia*, *Gymnopilus* were present in all sites. The tropical peatland forest possessed a various diverse fungal community with high variation across the sampled plots. Still now of our interest growing about this ecosystem and dynamics of their role, with nearly 650 different species of micro

fungi having been isolated and identified from peatland globally (Thormann 2006). Most of these fungi are taken part in carbon cycling dynamics because of their saprobic nature. How these communities respond to natural and anthropogenic disturbances remains uncertain. For the FunGuild, among the top 18 fungal genera the mycorrhizal/ symbiotroph fungi were represented by 4 genera, whereas 4 genera belonged to soil saprotrophic fungi. Mycorrhizal fungi were also the most prominent group based on the number of sequence reads. Saprotroph-symbiotroph type of nutritional mode contained only 2 genera. 4 genera were found in the mode of nutrition likely pathotroph-saprotroph-symbiotroph and pathotroph-saprotroph. *Ganoderma*, *Gliocephalotrichum*, *Metarhizium*, *Sugiyamaella*, *Williopsis* were the most prominent genera observed. The tropical peat forest supports many unique habitats of important flora and fauna like microbes and are the last asylum for many vulnerable species. Tropical peat swamp forest is neither resistance nor resilient to alarms, particularly fires drainage and logging. The ecosystem of peatland entirely depends on the substrate of peat and regularly maintains adequate water, good canopy cover and continuous litter inputs which are completely or partially decomposed by microbes. Combustion of peat is the major cause of CO₂ emissions and also damage of microbes from regional peat swamp forests, but clearly even slight disturbance of peat results in release of stored carbon, and thus impacts ecosystem worldwide by contributing climate change. Our study highlighted microbial community in peat swamp soil in tropical area and showed high microbial diversity, to compare the tropical peat swamp forest the soil bacterial community is more diverse than fungi in our research. Indonesian peatland maintains an extraordinary biological diversity with an important implication for biogeochemical cycling and carbon sequestration of nutrients. The study depicts a starting point for the exploration of the dynamics of the microbial community in the tropical peatland soil in Indonesia. The large percentage of unidentified sequences at genre level suggests that the huge number of fungi and bacteria in tropical peatland forest are unknown. Therefore, we need more researches. This study has contributed to our understanding of the impact of different land management practices on this unique ecosystem, providing basic information relating to how tropical peat swamp forest microbial diversity changes if we convert this land for other uses.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Li-na Dong, Department of Forest Protection, College of Forestry, Nanjing Forestry University, Jiangsu, China for helping in off-campus experimentations. They also remember the help offered by Ph.D. fellow Mr. Lin Bing of the department for analyzing the data of the experiments.

REFERENCES

- Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. **27**(16): 2194-2200.
- Harrison, M. E. 2013. Using Conceptual Models to Understand Ecosystem Function and Impacts of Human Activities in Tropical Peat-swamp Forests. *Wetlands*. **33**: 257-267.
- Holden, J., L. Shotbolt, A. Bonn, T. Burt, P. Chapman, A. Dougill, E. Fraser, K. Hubacek, B. Irvine and M. Kirkby. 2007. Environmental change in moorland landscapes. *Earth-Science Reviews*. **82**: 75-100.
- Koh, L. P., J. Mittinen, S. C. Liew and J. Ghazoul. 2011. Remotely sensed evidence of tropical peatland conversion to oil palm. *Proc. Natl. Acad. Sci.* **108**(12): 5127-5132.
- Lampela, M., J. Jauhiainen and H. Vasander. 2014. Surface peat structure and chemistry in a tropical peat swamp forest. *Plant and Soil*. **382**: 329-347.

- Nakane, K., T. Ohno and T. Horikoshi. 1996. Root respiration rate before and just after clear-felling in a mature, deciduous, broad-leaved forest. *Ecol. Res.* **11**: 111-119.
- Needleman, S. B. and C. D. Wunsch. 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol.* **48**:443-453.
- Oleszczuk, R., K. Regina, L. Szajdak, H. Hoper and V. Maryganova. 2008. Impacts of agricultural utilization of peat soils on the greenhouse gas balance. In: STRACK, M. (ed.) *Peatlands and climate change.*, pp. 70-97.
- Page, S., A. Hoscilo, H. Wosten, J. Jauhiainen, M. Silvius, J. Rieley, H. Ritzema, K. Tansey, L. Graham and H. Vasander. 2009. Restoration ecology of lowland tropical peatlands in Southeast Asia: current knowledge and future research directions. *Ecosystems.* **12**: 888-905.
- Page, S. E., C. Banks and J. O. Rieley. 2007. Tropical peatlands: distribution, extent and carbon storage uncertainties and knowledge gaps. *Peatlands Int.* **2**: 26-27.
- Pretty, J., C. Toulmin and S. Williams. 2011. Sustainable intensification in African agriculture. *Int. J. Agric. Sustainability.* **9**: 5-24.
- Puglisi, E., C. Zaccone, F. Cappa, P. S. Cocconcelli, W. Shoty, M. Trevisan and T. M. Miano. 2014. Changes in bacterial and archaeal community assemblages along an ombrotrophic peat bog profile. *Biology and Fertility of Soils.*, pp. 1-12.
- Rieley, J., A. Ahmad-Shah and M. Brady. 1996. The extent and nature of tropical peat swamp. In: E. Maltby, C. Immirzi and R. Safford (eds.). *Tropical lowland peatlands of Southeast Asia.*, pp. 17-53.
- Rieley, J., R. Wust, J. Jauhiainen, S. Page, H. Wosten, A. Hooijer, F. Siegert, S. Limin, H. Vasander and M. Stahlhut. 2008. Tropical peatlands: carbon stores, carbon gas Emissions and contribution to climate change Processes. *Peatlands and Climate Change.* **12**: 148-182.
- Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn and C. F. Weber. 2009. Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**: 7537-7541.
- Shepherd, P., J. O. Rieley and S. E. Page. 1997: The relationship between forest vegetation and peat characteristics in the upper catchment of Sungai Sabangau, Central Kalimantan. In: J. O. Rieley and S. E. Page (eds.). *Biodiversity and sustainability of tropical peatlands.* Samara Publishing, Cardigan, UK., pp. 191-210.
- Sorensen, K. W. 1993. Indonesian peat swamp forests and their role as a carbon sink. *Chemosphere.* **27**: 1065-1082.
- Tedersoo, L., R. H. Nilsson, K. Abarenkov, T. Jairus, A. Sadam, I. Saar, M. Bahram, E. Bechem, G. Chuyong and U. Koljalg. 2010. 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytol.* **188**: 291- 301.
- Vijarnsorn, P. 1996. Peatlands in Southeast Asia: a regional perspective. In: E. Maltby, C. P. Immirzi and R. J. Safford (eds.). *Tropical Lowland Peatlands of Southeast Asia.* Workshop on integrated planning and management of tropical lowland peatlands. IUCN, Gland, Switzerland.
- Waldes, N. and S. E. Page. 2002. Forest structure and tree diversity of a peat swamp forest in Central Kalimantan, Indonesia. In: J. O. Rieley, S. E. Page and B. Setiadi (eds.). *Proc. Int. Symposium Tropical Peatland.* pp. 2223.