



**UNIVERSITI PUTRA MALAYSIA**

***ENVIRONMENTAL CHARACTERIZATION AND METAGENOMICS  
ANALYSIS OF BIOTIC AND ABIOTIC FACTORS IN NORTH  
SELANGOR PEAT SWAMP FOREST, MALAYSIA***

**SAADU HAMIDU**

**FS 2021 22**



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PEAT SWAMP FOREST, MALAYSIA**

By

**SAADU HAMIDU**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**September 2020**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**ENVIRONMENTAL CHARACTERIZATION AND METAGENOMICS  
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**September 2020**

**Chairman : Associate Professor Mohammad Noor Amal Azmai, PhD**  
**Faculty : Science**

The North Selangor Peat Swamp Forest (NSPSF), like all other peatland ecosystems are subjected to various of threats such as forest fire, wild animal poaching, expansion of area for agricultural and industrial activity, canal construction, deforestation, and air, water and land pollutions. Moreover, limited fundamental studies have been conducted in this unique ecosystem, especially in the aspect of microbial ecology that associated with the continuous anthropogenic activities of the protected forest.

In general, this study determines the environmental characterization and metagenomics analysis of biotic and abiotic factors in North Selangor Peat Swamp Forest, Selangor, Malaysia. Firstly, this study determines the current environmental variables of undisturbed and disturbed areas. Secondly, the composition and diversity of bacterial communities in the fish gut contents, water and soil of undisturbed and disturbed areas were identified. Thirdly, the taxa and functional genes biomarkers of bacteria in soil samples of undisturbed and disturbed areas were determined. Lastly, this study identified the relationships between the environmental variables and bacterial taxa biomarker from soil of undisturbed and disturbed areas.

In order to understand the impacts of natural and human induced factors on the bacterial communities in NSPSF, two areas namely NSPSF (undisturbed) and its nearby (disturbed) areas were selected in this study. In each area, three sites were selected, namely Sungai Karang Peat Swamp Forest site 1 (SKPSF-1), Raja Musa Peat Swamp Forest (RMPSF) and Sungai Karang Peat Swamp Forest site 2 (SKPSF-2) for undisturbed area, while for disturbed area, paddy field (PF), forest fire (FF) and oil palm plantation (OP) areas were selected. The environmental variables of anthropogenic activities, forest characteristics and water physico-chemical parameters



in undisturbed and disturbed areas were determined. Following that, the metagenomics techniques was utilized to assess the composition, diversity, taxonomical biomarkers and functional genes of bacteria in the undisturbed (U) and disturbed (D) samples of fish gut contents (UF and DF), water (UW and DW) and soil (US and DS) in the study areas, as well as to relate these metagenomics findings with the measured environmental variables.

The findings on anthropogenic activities revealed that the disturbed areas have higher scores in water (WP), land (LP) and noise (NP) pollutions and agricultural activity (AA). Other anthropogenic activities that recorded in disturbed areas were distance from human settlement (DHS), state of accessibility (SA), and deforestation (DL), and these were the predominant anthropogenic activities in disturbed area. Whereas, construction activities (CR) cut across all the sites. The higher seedling percentage (PS) in OP was observed and conversely, the percentage of trees (PT) and number of falling trees (FT) appeared to be correspondingly higher in SKPSF-1. The PF in the disturbed area was characterized by high relative light intensity (RLI). Conversely, RLI was relatively lower in the undisturbed area. The undisturbed area indicates lower dissolved oxygen (DO) values and notably increases from undisturbed to disturbed areas, particularly in PF and OP. The FF was an exception, which was observed to have comparatively lower DO concentration. Some of anthropogenic activities such as DHS, SA, DL and AA correlated closely with  $\text{NH}_3\text{-N}$  and Fe in the disturbed area, while sign of fire (SF) in the undisturbed area was associated with  $\text{NO}_3^-$ .

The metagenomics analyses in undisturbed and disturbed areas revealed a rich and diverse bacterial community across the 18 samples of fish gut contents, water and soil. Moreover, the alpha indexes showed significant difference ( $p < 0.05$ ) between the disturbed and undisturbed sites of each sample. The rarefaction among the samples showed curves, at points where the number individual organisms increase, but the number of species remain constant in the study area. The Proteobacteria appeared to be the most dominated phyla, followed by unassigned taxa, Firmicutes and Actinobacteria, and they were relatively significance in disturbed area of DF and DS samples. The finding showed significant differences in relative abundance of some phyla between undisturbed and disturbed areas. The higher relative abundance of some taxa such as *Novosphingobium* in UW and *Desulfobacter* in DS indicates the presence of pollutants in undisturbed and disturbed areas. Similarly, phylum Acidobacteria was apparently present throughout the undisturbed samples and was the most common bacterial group detected in undisturbed area.

Furthermore, among the three samples (soil, water and fish gut content), the soil samples recorded higher difference between the OTUs in undisturbed and disturbed areas. Hence, differential features of bacterial community in the soil were predicted. The metagenomics taxonomic features of soil samples showed ACK\_M1, Hydrogenophilaceae and *Thiobacillus* were taxa biomarker that prevalent in undisturbed samples, whereas, the taxa biomarker identified in disturbed area were p\_WPS\_2, Planctomycetaia and Gemmatales. The discriminatory metagenomics features detected KO biomarkers through LEfSe which were notably higher in DS

samples, and most of them were associated with chemotaxis and related electron transfer. Whereas, majority of the KO biomarkers in US samples were linked with transport, adhesion of proteins and iron. The functional biomarkers detected in DS samples were connected to synthesis of proteins and amino acid related enzymes such as DNA replication, translation factors, RNA polymerase and aminoacyl\_tRNA biosynthesis were observed. In contrast, most of the functional biomarkers depicted in US samples related to metabolism and degradation of chemicals like ascorbate and alderate metabolism, C5\_branched dibasic acid metabolism, biosynthesis and biodegradation of secondary metabolites, and dioxin degradation. Metagenome contribution by gene family in soil samples revealed Enterobacteriaceae and Aeromonadaceae, to be associated with K03088. However, only Moraxellaceae was predicted in DS sample which was linked to K03406. The higher number of taxa biomarker and metagenomic contribution by genes family were predicted in the DS samples. Thus, it suggests the presence of consortium of bacteria with synergistic or antagonistic actions of bacteria in the disturbed area, and this could have been the source of ecological difference between disturbed and undisturbed area of NSPSF.

Redundancy analysis was used to analyse and summarise the relationships between the anthropogenic activities, forest characteristics and water physico-chemical factors with bacterial taxa biomarker of soil samples in undisturbed and disturbed areas. The taxa biomarker connected to five anthropogenic activities of SA, AA, CR, DL and DHS including c\_TK17, *Legionella*, p\_WPS\_2, *Spirochaeta* and *Acinetobacter* in DS samples. Interestingly, similar taxa biomarker particularly C\_TK17, g\_*Legionella*, p\_WPS\_2, g\_*Spirochaeta* and g\_*Acinetobacter* as obtained in anthropogenic factors were also were strongly linked to the two forest characteristics of RLI and PS that found in DS samples. Whereas, the members of Hydrogenophilaceae, *Thiobacillus*, *Candidatus Rhodoluna* and f\_ACK\_M1 and Methylococcales were the biomarkers that more localized in US samples with closer association to NO<sub>3</sub><sup>-</sup>, turbidity and water depth.

In this study, some recommendations were proposed such as to regulate indiscriminate construction of canals, roads, and other buildings in the both undisturbed and disturbed areas. As such, environmental impact assessment should be carried out and this recommendation should be adhered prior to any project in and around NSPSF. On the forest characteristics, the seedlings could be complemented through artificial nurseries in undisturbed areas which recorded low PS. Despite the differences in environmental factors between disturbed and undisturbed areas, no significant difference obtained between the two areas in term of the bacterial diversity and composition. Interestingly, NO<sub>3</sub><sup>-</sup>, turbidity and water depth have strong associations with taxa biomarker in undisturbed area. Thus, suggesting that change in these three factors could likely affect the distribution and abundance of species within the bacterial biomarker identified in NSPSF. Indeed, further study on metagenomics analysis of biotic and abiotic factors could be carried out to exploit the effects of seasonal variation on environmental conditions of NSPSF. Additionally, the unclassified taxa of bacterial community in NSPSF could also be investigated to bring out its taxonomical and functional features. This study could potentially provide guidelines for effective identification,

remediation and restoration of degraded peat swamp forest, as well as give the insight in ecological monitoring and conservation of NSPSF and its environment.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN PERSEKITARAN DAN ANALISIS METAGENOMIK FAKTOR BIOTIK DAN ABIOTIK DI HUTAN PAYA GAMBUT UTARA, MALAYSIA**

Oleh

**SAADU HAMIDU**

**September 2020**

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Hutan Paya Gambut Selangor Utara (NSPSF) adalah salah satu ekosistem tanah gambut yang mengalami pelbagai ancaman seperti kebakaran hutan, pemburuan haiwan liar, perluasan kawasan untuk kegiatan pertanian dan perindustrian, pembinaan terusan, penebangan hutan, dan pencemaran udara, air dan tanah. Tambahan pula, kajian asas yang telah dilakukan di dalam ekosistem unik ini adalah terhad, terutamanya di dalam aspek ekologi mikrobiologi yang berkaitan dengan aktiviti antropogenik yang berterusan dalam hutan terlindung ini.

Secara umumnya, kajian ini menentukan pencirian persekitaran dan analisis metagenomik faktor biotik dan abiotik di Hutan Paya Gambut Utara Selangor, Selangor, Malaysia. Pertama, kajian ini menentukan pembolehubah persekitaran semasa kawasan tidak terganggu dan terganggu. Kedua, komposisi, diversiti dan penanda bio taksa bakteria oleh bakteria komuniti dalam kandungan usus ikan, air dan tanah di kawasan tidak terganggu dan terganggu dikenalpasti. Ketiga, taksa dan gen berfungsi bakteria di dalam kandungan tanah di kawasan tidak terganggu dan terganggu ditentukan. Akhir sekali, kajian ini mengenalpasti perhubungan di antara pembolehubah persekitaran dan penanda bio taksa bakteria dari tanah, dari kawasan tidak terganggu dan terganggu.

Untuk memahami kesan faktor semula jadi dan manusia pada komuniti bakteria di NSPSF, dua kawasan, iaitu NSPSF (tidak terganggu) dan kawasan berdekatnya (terganggu) telah dipilih dalam kajian ini. Di setiap kawasan, tiga lokasi dipilih, iaitu kawasan Hutan Paya Gambut Sungai Karang 1 (SKPSF-1), Hutan Paya Gambut Raja Musa (RMPSF) dan Hutan Paya Gambut Sungai Karang 2 (SKPSF-2) untuk kawasan yang tidak terganggu, manakala untuk kawasan yang terganggu, kawasan sawah padi (PF), kebakaran hutan (FF) dan penanaman kelapa sawit (OP) telah dipilih.

Pembolehubah persekitaran oleh aktiviti antropogenik, pencirian hutan dan parameter fizikokimia air di kawasan yang tidak terganggu dan terganggu telah ditentukan. Selepas itu, teknik metagenomik telah digunakan untuk menilai komposisi, diversiti, taksonomi penanda bio dan gen berfungsi bakteria kawasan tidak terganggu (U) dan terganggu (D) dari sampel kandungan usus ikan (UF dan DF), air (UW dan DW) dan tanah (US dan DS) di kawasan kajian, dan juga mengaitkan penemuan metagenomik ini dengan pembolehubah persekitaran yang telah ditentukan.

Hasil kajian ke atas aktiviti antropogenik mendapati kawasan yang terganggu mempunyai skor pencemaran air (WP), tanah (LP) dan kebisingan (NP) dan aktiviti pertanian (AA) yang lebih tinggi. Aktiviti antropogenik lain yang juga dicatatkan di kawasan terganggu adalah jarak dari penempatan manusia (DHS), tahap akses (SA) dan penebangan hutan (DL), dan merupakan aktiviti antropogenik utama di kawasan terganggu. Manakala aktiviti pembinaan (CR) merangkumi semua kawasan kajian. Semakin tinggi peratusan anak pokok (PS) dalam OP dan sebaliknya telah dicatatkan, manakala peratusan pokok (PT) dan jumlah pokok tumbang (FT) adalah lebih tinggi dalam SKPSF-1. PF di kawasan terganggu dicirikan oleh intensiti cahaya relatif (RLI) yang tinggi. Sebaliknya, RLI secara relatif adalah lebih rendah di kawasan yang tidak terganggu. Kawasan yang tidak terganggu menunjukkan nilai oksigen larut yang lebih rendah (DO) dan ketara meningkat dari kawasan yang tidak terganggu ke kawasan yang terganggu, terutamanya di PF dan OP. FF adalah pengecualian, yang dilihat memiliki kepekatan DO yang secara relatif lebih rendah. Beberapa aktiviti antropogenik seperti DHS, SA, DL dan AA berkorelasi rapat dengan  $\text{NH}_3\text{-N}$  dan Fe di kawasan yang terganggu, manakala pencemaran udara (AP) di kawasan yang tidak terganggu berkait dengan  $\text{NO}_3^-$ .

Analisis metagenomik di kawasan tidak terganggu dan terganggu menunjukkan kekayaan dan kepelbagaian komuniti bakteria pada 18 sampel kandungan usus ikan, air dan tanah. Tambahan pula, indeks alpha menunjukkan perbezaan yang signifikan ( $p < 0.05$ ) di antara kawasan terganggu dan tidak terganggu untuk setiap sampel. Penipisan di antara sampel menunjukkan lekukan pada titik di mana bilangan organisma meningkat, tetapi jumlah spesies tetap sama di kawasan kajian. Proteobacteria merupakan filum yang paling mendominasi, diikuti oleh taksa *unassigned*, Firmicutes dan Actinobacteria, di mana mereka menunjukkan relatif kepentingan di kawasan terganggu pada sampel DF dan DS. Penemuan ini menunjukkan perbezaan yang signifikan dalam kelimpahan relatif beberapa filum di antara kawasan yang tidak terganggu dan terganggu. Kelimpahan relatif beberapa taksa seperti *Novosphingobium* dalam UW, *Desulfobacter* dalam DS menunjukkan kehadiran bahan pencemar di kawasan yang tidak terganggu dan terganggu. Begitu juga, filum Acidobacteria nampaknya hadir di seluruh sampel kawasan tidak terganggu dan merupakan kumpulan bakteria yang paling biasa dikesan di kawasan yang tidak terganggu. Pseudomonadales dan Desulfobacteraceae adalah taksa penanda bio yang lazim terdapat pada sampel yang tidak terganggu, manakala, penanda bio taksa yang dikenalpasti di kawasan terganggu adalah *Methylobacterium*, *Ralstonia* dan *Bacillus*.



Di samping itu, di antara ketiga sampel iaitu tanah, air dan kandungan usus ikan, sampel tanah merekodkan perbezaan OTU yang tinggi di antara kawasan tidak terganggu and terganggu. Demikian, perbezaan ciri komuniti bakteria di dalam sampel tanah telah diramalkan. Ciri metagenomik taksa di dalam sampel tanah menunjukkan ACK\_M1, Hydrogenophilaceae dan *Thiobacillus* adalah antara taksa penanda bio yang lazim dijumpai di kawasan tidak terganggu, manakala taksa penanda bio di kawasan terganggu adalah p\_WPS\_2, Planctomycetaia and Gemmatales. Perbezaan ciri metagenomik mengesan penanda bio KO melalui LEfSe, dimana ia menunjukkan ciri yang tinggi di dalam sampel DS, dan kebanyakan daripada KO tersebut adalah berkaitan dengan kemotaksis dan pemindahan elektron. Kebanyakan penanda bio KO di sampel US adalah berkaitan dengan pemindahan, lekatan protein dan besi. Penanda bio fungsi yang hadir di dalam sampel DS menunjukkan hubungkait dengan protein sintesis dan enzim berkaitan dengan asid amino, seperti replikasi DNA, faktor translasi, polimeras RNA dan aminoacyl\_tRNA biosintesis. Sebaliknya, kebanyakan penanda bio fungsi yang ditunjukkan di dalam sampel US adalah berkaitan dengan metabolisme dan degradasi kimia seperti metabolisme askorbat dan alderat, C5\_branched dibasic metabolisme asid, biosintesis dan biodegradasi metabolit sekunder dan degradasi dioxin. Sumbangan metagenom oleh keluarga gene menunjukkan Enterobacteriaceae dan Aeromonadaceae adalah berkait rapat dengan K03088. Namun demikian, hanya Moraxellaceae yang diramal berkait rapat dengan K03406. Bilangan penanda bio taksa dan sumbangan metagenom oleh keluarga gen adalah antara yang tertinggi direkodkan di dalam sampel DS. Oleh yang demikian, ia menunjukkan bahawa bakteria konsortium hadir dengan sinergi atau aksi antagonistik di kawasan yang terganggu. Ini boleh menjadi salah satu sumber yang mendorong kepada perbezaan ekologi di antara kawasan terganggu dan kawasan tidak terganggu di NSPSF.

Analisis berlebihan telah digunakan untuk menganalisis dan meringkaskan hubungan di antara aktiviti antropogenik, pencirian hutan dan fizikokimia air dengan taksa penanda bio bakteria pada sampel tanah di kawasan tidak terganggu dan terganggu. Penanda bio taksa menghubungkan lima aktiviti antropogenik iaitu SA, AA, CR, DL dan DHA dengan c\_TK17, *Legionella*, p\_WPS\_2, *Spirochaeta* and *Acinetobacter* di dalam sampel DS. Menariknya, penanda bio taksa yang sama seperti C\_TK17, g\_*Legionella*, p\_WPS\_2, g\_*Spirochaeta* dan g\_*Acinetobacter* menunjukkan hubungan yang kuat dengan dua ciri hutan iaitu RLI dan PS yang dijumpai di dalam sampel DS. Selain itu, Hydrogenophilaceae, *Thiobacillus*, *Candidatus Rhodoluna* dan f\_ACK\_M1 merupakan antara penanda bio yang terdapat di dalam sampel US yang berhubung kait rapat dengan NO<sub>3</sub><sup>-</sup>, kekeruhan dan kedalaman air.

Di dalam kajian ini, beberapa cadangan telah dikemukakan, termasuklah untuk mengatur urus pembinaan terusan, jalan raya dan bangunan lain di kawasan yang tidak terganggu dan terganggu. Oleh itu, penilaian terhadap kesan persekitaran harus dilakukan dan cadangan ini perlu dipatuhi sebelum sebarang projek dijalankan di dalam dan sekitar NSPSF. Di bawah kategori ciri hutan, pembenihan boleh dilakukan melalui nurseri tiruan di kawasan yang tidak terganggu dimana ia merekodkan PS yang rendah. Walaupun terdapat perbezaan di antara faktor alam sekitar di antara kawasan terganggu dan kawasan tidak terganggu, tiada perbezaan yang ketara

ditunjukkan di kedua-dua kawasan tersebut dari segi diversiti dan komposisi bakteria. Menariknya,  $\text{NO}_3^-$ , kekeruhan dan kedalaman air menunjukkan kesatuan yang kuat dengan penanda bio taksa di kawasan tidak terganggu. Dengan itu, ia menunjukkan bahawa perubahan diantara tiga faktor tersebut akan memberi kesan kepada distribusi dan kebanyakan spesis di dalam penanda bio bakteria yang boleh dikesan di kawasan NSPSF. Tambahan pula, kajian lebih lanjut mengenai analisis metagenomik ke atas faktor biotik dan abiotik perlu dilakukan untuk mengeksplorasi pengaruh variasi musim terhadap keadaan persekitaran NSPSF. Tambahan itu, komuniti bakteria berstatus *unassigned* atau taksa yang tidak dapat diklasifikasi di NSPSF juga perlu disiasat untuk mencari ciri taksonomi dan fungsinya. Informasi dari kajian ini akan menambah nilai terhadap pengkalan data komuniti bakteria sedia ada di NSPSF. Tambahan pula, kajian ini berpotensi untuk memberi garis panduan untuk mengenalpasti, remediasi dan mengembalikan hutan paya gambut yang merosot dengan lebih efektif, di samping memberikan gambaran mengenai isu kesihatan umum, pemantauan ekologi dan konservasi NSPSF dan alam sekitarnya.

## ACKNOWLEDGEMENTS

I want to express my profound gratitude to almighty Allah S.W.T for the successful completion of my PhD programme within the stipulated time frame. Foremost, my sincere gratitude goes to the chairman of my supervisory committee, Assoc. Prof. Dr. Mohammad Noor Amal Azmai for not only funding the research work, but also for his guidance, patience and constructive criticism during the conduct of this study. Besides that, I would like to thank the rest of my supervisory committee, Prof. Dr. Ahmad Ismail, Dr. Syaizwan Zahmir Zulkifli and Dr. Amalia Mohd Hashim, for their encouragement, insightful comments, and mentorship which guided me throughout the period of the research.

I would like to thank the effort of Federal College of Education Katsina, Nigeria, most especially the provost and deputy provost; Dr. Aliyu Idris Maikaji and Dr. Sam Ohanado respectively, for giving me the opportunity to further my study under the Tertiary Education Trust Fund staff training and development programme. I must salute Alhaji Danjuma Muhammad, Dr. Maharazu Mamman, Dr. Sabiru Dahiru Yusuf, Alhaji Badamasi Lurwan Gafai, and the entire staffs of School of Science, Federal College of Education, Katsina, Nigeria for all the support and encouragement.

I am grateful to all the participants of this research work, especially my fellow postgraduate students for their kind support and of course friendship particularly Jumria Sutra, Saba Abdulwakil and Dr. Nurliyana Mohamad who assisted me throughout this study. I must thank you all for the encouragement and support received. It really helped me to get the results of better quality. Similarly, thanks also to all staffs at the Department of Biology, Faculty of Science, and Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, who have directly or indirectly contributed to the success of this work.

Last but not the least, I would also like to thank my father, Malam Saadu and my mother, Hajia Saratu, brothers and sisters for all the encouragement received throughout this most challenging time in my academic career. Kudos to my children, Fatima, Ummi-salma, Muhammad, Aliyu, Alhassan, Khadija, Aminatu, Alhusain, Zainab, Amira and Abdallah for being so tolerant even when being 'without dad' was tough and coupled with their willingness to engage with the struggle and ensuing discomfort during my absence throughout the period of this study. A very special thanks to my wives, Nafisa and Fatima for their practical and emotional support, and for being not only dedicated, but also a loyal partner.



This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 10 June 2021

## TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	i
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENT</b>	ix
<b>APPROVAL</b>	x
<b>DECLARATION</b>	xii
<b>LIST OF TABLES</b>	xix
<b>LIST OF FIGURES</b>	xxi
<b>LIST OF ABBREVIATIONS</b>	xxvii
 <b>CHAPTER</b>	
 <b>1 INTRODUCTION</b>	 <b>1</b>
1.1 Background of the study	1
1.2 Research problems	1
1.3 Objectives of the study	4
1.4 Limitation of the study	4
 <b>2 LITERATURE REVIEW</b>	 <b>5</b>
2.1 Peat land and peat swamp	5
2.2 Roles of peat land and peat swamp	7
2.3 Biodiversity of peat land and peat swamp	8
2.4 Threats of peat land and peat swamp	10
2.5 Strategies of peat swamp rehabilitation	13
2.6 North Selangor Peat Swamp Forest (NSPSF)	14
2.6.1 Environmental characterizations of NSPSF	15
2.6.2 The importance of NSPSF	17
2.6.3 Biodiversity in NSPSF	19
2.6.4 Fish habitat and feeding modes in NSPSF	21
2.6.5 Problems and threats of NSPSF	21
2.6.6 Conservation of NSPSF	25
2.7 Metagenomics in ecological study	27
 <b>3 ENVIRONMENTAL CHARACTERIZATION OF NORTH SELANGOR PEAT SWAMP FOREST, SELANGOR, MALAYSIA</b>	 <b>31</b>
3.1 Introduction	31
3.2 Materials and methods	32
3.2.1 Study area	32
3.2.2 Sampling sites	33
3.2.2.1 Undisturbed area of NSPSF	34
3.2.2.2 Disturbed area adjacent to NSPSF	34
3.2.3 Data collection	35
3.2.4 Scoring of anthropogenic activities	35
3.2.5 Characterization of forest characteristics	38

	3.2.5.1	Number of animals, plants and fallen trees	38
	3.2.5.2	Tree height	39
	3.2.5.3	Canopy cover	39
	3.2.5.4	Relative light intensity	39
	3.2.6	Physico-chemical water parameters	40
	3.2.7	Statistical analysis	40
3.3	Results		41
	3.3.1	Anthropogenic activities	41
	3.3.2	Forest characteristics	45
	3.3.3	Water physico-chemical parameters	48
	3.3.4	Associations between anthropogenic activities, forest characteristics and water physico-chemical parameters	54
3.4	Discussion		57
3.5	Conclusion		60
<b>4</b>	<b>COMPOSITION, DIVERSITY AND POTENTIAL TAXA BIOMARKER OF BACTERIAL COMMUNITIES IN FISH GUT CONTENTS, WATER AND SOIL IN NORTH SELANGOR PEAT SWAMP FOREST</b>		<b>61</b>
	4.1	Introduction	61
	4.2	Materials and methods	62
	4.2.1	Sampling sites	62
	4.2.2	Sampling procedures	62
	4.2.3	Fish samples	62
	4.2.4	Water samples	63
	4.2.5	Soil samples	63
	4.2.6	DNA extraction	63
	4.2.7	Polymerase Chain Reaction	64
	4.2.8	High-throughput Miseq Illumina sequencing platform	64
	4.2.9	Analysis of 16S rRNA amplicon sequencing by using Quantitative Insights into Microbial Ecology (QIIME)	65
	4.2.9.1	Trimming of adapter sequences, reads merging and length filter	65
	4.2.9.2	Formation of BIOM file and BIOM table	65
	4.2.9.3	Core diversity analysis	65
	4.2.10	Statistical analysis	66
	4.2.11	Data availability	66
4.3	Results		66
	4.3.1	Analysis of metadata and OTU number of fish gut contents, water and soil samples	66
	4.3.2	Alpha diversity of bacterial communities of fish gut contents, water and soil samples	68
	4.3.3	Beta diversity of bacterial community in among sites	71

4.3.4	Rarefaction curves patterns of bacterial communities in undisturbed and disturbed samples	77
4.3.5	Structure of bacterial communities in fish gut contents, water and soil samples of undisturbed and disturbed areas	81
4.3.6	Comparisons of bacterial diversity among sites and between disturbed and undisturbed areas	82
4.3.7	Composition of bacterial community between disturbed and undisturbed fish gut contents, water and soil samples	84
4.3.8	Composition and taxonomy of bacterial relative abundance in fish gut contents, water and soil samples of undisturbed and disturbed areas	86
4.4	Discussion	91
4.4.1	Taxonomic profile of bacteria in undisturbed and disturbed areas	91
4.4.2	Bacterial composition and activities in undisturbed and disturbed areas	92
4.5	Conclusion	95
<b>5</b>	<b>DIFFERENTIAL FEATURES AND CORE BACTERIAL COMMUNITIES IN SOIL OF NORTH SELANGOR PEAT SWAMP FOREST</b>	96
5.1	Introduction	96
5.2	Materials and methods	97
5.2.1	Sampling sites	97
5.2.2	Bacterial community and its metagenome prediction using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)	98
5.2.3	Detection of biomarkers among taxa and functional genes using Liner Discriminant Analysis Effective Size (LEfSe)	98
5.2.4	Analysis of metagenome contribution by gene family	99
5.2.5	Statistical analysis	99
5.3	Results	99
5.3.1	Detection of potential biomarkers in soil samples among taxa using Liner Discriminant Analysis Effective Size (LEfSe)	99
5.3.2	Functional genes prediction in undisturbed and disturbed soil samples	104
5.3.3	Detection of potential KEGG Ortholog (KO) biomarkers among disturbed and undisturbed areas	105
5.3.4	Detection of potential biomarkers for encoded genes based on KEGG level 1, 2 and 3 among disturbed and undisturbed areas	109

5.3.4.1	Potential biomarkers at level 1 of metagenome prediction	110
5.3.4.2	Potential biomarkers at level 2 of metagenome prediction	111
5.3.4.3	Biomarkers for the level 3 of metagenome prediction	112
5.3.5	Comparison of metagenome contribution by gene family and OTU differential abundance	114
5.3.6	Reference genome coverage of samples and accuracy of metagenomic predictions	118
5.4	Discussion	118
5.4.1	Potential biomarkers among taxa between undisturbed and disturbed areas in soil samples	118
5.4.2	Predominant genes in the undisturbed and disturbed areas	119
5.4.3	KO biomarkers among disturbed and undisturbed areas	120
5.4.4	Detection of potential biomarkers for encoded genes based on KEGG levels 1, 2 and 3 among disturbed and undisturbed areas	121
5.4.5	Metagenome contribution by gene family	122
5.5	Conclusion	123
<b>6</b>	<b>RELATIONSHIPS BETWEEN THE ENVIRONMENTAL VARIABLES AND BACTERIAL TAXA BIOMARKER IN SOIL IN NORTH SELANGOR PEAT SWAMP FOREST</b>	<b>124</b>
6.1	Introduction	124
6.2	Materials and methods	125
6.2.1	Statistical analysis	125
6.3	Results	127
6.3.1	Relationships between anthropogenic activities and bacterial taxa biomarker in disturbed and undisturbed areas	127
6.3.2	Relationships between forest characteristics and bacterial taxa biomarker in disturbed and undisturbed areas	128
6.3.3	Relationships between water physico-chemical parameters with bacterial taxa biomarker in disturbed and undisturbed areas	129
6.4	Discussion	131
6.4.1	Relationships between anthropogenic activities and diversity of bacterial taxa biomarker in soil samples	131
6.4.2	Relationships between forest characteristics and diversity of bacterial taxa biomarker	131
6.4.3	Relationships between water physico-chemical parameters and diversity of bacterial taxa biomarker	132
6.5	Conclusion	132

<b>7</b>	<b>GENERAL CONCLUSION AND RECOMMENDATION</b>	<b>133</b>
7.1	General conclusion	133
7.2	Recommendation	137
	<b>REFERENCES</b>	<b>138</b>
	<b>APPENDICES</b>	<b>164</b>
	<b>BIODATA OF STUDENT</b>	<b>194</b>
	<b>LIST OF PUBLICATIONS</b>	<b>195</b>



## LIST OF TABLES

Table	Page
2.1 Southeast Asia peat swamp forests with the estimates of major peat swamp forest areas (in square kilometres). (Posa et al., 2011)	6
2.2 Number of flora and fauna species recorded from peat swamp forests of Southeast Asia (Posa et al., 2011)	9
2.3 Net changes of forest area in some selected countries in the world	12
2.4 Landmass occupy by peat swamp in Malaysia	15
2.5 Total area of North Selangor Peat Swamp Forest as included in formal gazettelement documents (GEC, 2014a)	15
2.6 Good and services valued at Raja Musa Forest Reserve (GEC, 2014a)	18
2.7 Recent fire history in North Selangor Peat Swamp Forest by months of April 2011 to February 2014 (GEC, 2014a)	24
3.1 The coordinates of sampling sites in this study	33
3.2 Modified scoring guides to determine the anthropogenic activities in this study	37
3.3 Anthropogenic activities scoring from all of the sampling sites	42
3.4 Anthropogenic activities scoring between undisturbed NSPSF and its adjacent disturbed area	44
3.5 Correlations within the anthropogenic activities in the study area	45
3.6 Forest characteristics scoring from all of the sampling sites	47
3.7 Forest characteristics between undisturbed NSPSF and its adjacent disturbed area	47
3.8 Correlations within the forest characteristics in the study area	48
3.9 Water physico-chemical parameters from all of the sampling sites	50
3.10 Water physico-chemical parameters between undisturbed NSPSF and its adjacent disturbed area	51
3.11 Correlational analysis between water physico-chemical parameters	53

3.12	Correlation matrix from PCA on the relationships between anthropogenic activities, forest characteristics and water physico-chemical parameters	56
4.1	Metadata statistics obtained from fish gut contents, water and soil samples in undisturbed and disturbed areas	67
4.2	Alpha diversity metrics of bacterial communities in fish gut content, water and soil samples of undisturbed and disturbed areas	70
4.3	Cross-sections of bacterial composition at various level of taxa: phylum, class, order, family and genus	82
5.1	Summary on the frequencies of KEGG Ortholog, metagenome predictions and bacterial relative abundance across soil samples	103
5.2	KEGG Ortholog description of potential biomarkers among disturbed and undisturbed soil (DS and US) samples	109
5.3	Top 10 contributing KEGG orthologs of soil samples in disturbed and undisturbed area	116
5.4	Top percentage of metagenome contribution by gene family of soil samples	116
5.5	Weighted NSTI of soil samples in undisturbed and disturbed areas	118



## LIST OF FIGURES

Figure	Page
2.1 Map of Southeast Asia showing major peat swamp forest areas	6
2.2 Location of North Selangor Peat Swamp Forest, Selangor, Malaysia	14
2.3 Undisturbed area in North Selangor Peat Swamp Forest	17
2.4 Fire risk map of North Selangor Peat Swamp Forest	23
2.5 Human settlement and drainage for agricultural purposes close to North Selangor Peat Swamp Forest	25
2.6 Map showing proposed rehabilitation zones in North Selangor Peat Swamp Forest	26
3.1 Map of Selangor and North Selangor Peat Swamp Forest, Selangor	32
3.2 The North Selangor Peat Swamp Forest that covers Raja Musa Forest Reserve, Sungai Karang Forest Reserve, Sungai Dusun Forest/Wildlife Reserve and part of Bukit Belata Forest Reserve Extension. The sampling sites for undisturbed area as indicated in blue coloured star (SKPSF-1: Sungai Karang Peat Swamp Forest site 1; RMPSF: Raja Musa Peat Swamp Forest; SKPSF-2: Sungai Karang Peat Swamp Forest site 2), while green coloured star for disturbed area adjacent to NSPSF (PF: paddy field; FF: forest fire; OP: oil palm plantation).	34
3.3 Principal Component Analysis plot on anthropogenic activities between disturbed and undisturbed areas (PCA1= 50.40% and PCA2= 16.99%). Distance from human settlement = DHS; state of accessibility = SA; deforestation level = DL; sign of fire= SF; water pollution = WP; land pollution = LP; soil erosion = SE; agricultural activity = AA; construction of road/building = CR; mining activity = MN; logging/lumbering = LL. Thirty set of observation for each of the anthropogenic factor were obtained from six sites, five in each site across of disturbed area in blue color (PF = star; FF = plus; OP = dot) and undisturbed area of NSPSF in green color (SKPSF-1= square; RMPSF = triangle; SKPSF-2 = oval).	45
3.4 Principal Component Analysis plot of forest characteristics between disturbed and undisturbed areas (PCA1 = 81.20% and PCA2 = 10.67%). RLI = relative light intensity; TP = total plants; FT = fallen trees; PS = percentage of seedling; PT= percentage of trees; TH= tree height; CC = canopy cover and AS = animal sighted. Disturbed area = blue color (PF = star, FF = plus and OP = dot) and undisturbed area = green color (SKPSF-1 = square, RMPSF = triangle and SKPSF-2 = oval).	48

- 3.5 Principal Component Analysis plot of water physico-chemical parameters in both undisturbed and disturbed areas (PCA2 = 2.20% and PCA3 = 0.51%). Six sites across disturbed area = blue color (PF = star, FF = plus and OP = dot) and undisturbed area = green color (SKPSF-1 = square, RMPSF = triangle and SKPSF-2 = oval). WD = water depth, Turbid = turbidity, DO = dissolved oxygen, TDS = total dissolved solid,  $\text{PO}_4^{2-}$  = phosphate,  $\text{NH}_3\text{-N}$  = ammonia-nitrogen,  $\text{NO}_2^-$  = nitrite and  $\text{NO}_3^-$  = nitrate. WD, Turbid and DO appeared to be three water physico-chemical parameters with highest contribution. However, there is slight overlap between the undisturbed and disturbed areas. 52
- 3.6 Canonical Component Analysis plot showing relationships between anthropogenic activities and water physico-chemical parameters. The disturbed area = blue color (PF = plus, FF = star and OP = dot) and undisturbed area = green color (SKPSF-1 = square, RMPSF = triangle and SKPSF-2 = oval). Anthropogenic activities including DHS = distance from human settlement, SA = state of accessibility, DL = deforestation level, SF = sign of fire, WP = water pollution, LP = land pollution, AA = agricultural activity, and CR = construction/building. The water physico-chemical parameters including Temp = temperature, WD = water depth, pH = water pH, Turbid = turbidity, DO = dissolved oxygen, TDS = total dissolved solid,  $\text{PO}_4^{3-}$  = phosphate,  $\text{NH}_3\text{-N}$  = ammonia-nitrogen,  $\text{NO}_2^-$  = nitrite,  $\text{NO}_3^-$  = nitrate and Fe = iron. 54
- 3.7 Principal Component Analysis (PCA) plot of environmental factor of significance different. Anthropogenic activities including SA = state of accessibility, DL = deforestation level, SF = sign of fire, AA = agricultural activity and CR = construction of road/building. Forest characteristics including RLI = relative light intensity, TP = total number of plants, FT = fallen trees, PS = percentage of seedlings and PT = percentage of trees. Water physico-chemical parameters including WD = water depth, Turbid = turbidity of water, DO = dissolved oxygen,  $\text{NH}_3\text{-N}$  = ammonia-nitrogen,  $\text{NO}_3^-$  = nitrate and Fe = iron. Green = undisturbed, while blue = disturbed areas. The vector with arrows depicted significant level of correlation. 55
- 4.1 Shannon and Simpson alpha diversity of the bacteria in samples from undisturbed and disturbed areas. DW: disturbed water samples; UW: undisturbed water samples; DF: disturbed fish gut contents; UF: undisturbed fish gut contents; DS: disturbed soil samples; US: undisturbed soil samples. 69
- 4.2 NMDS ordination of the bacterial communities in between undisturbed (UF, UW and US) and disturbed (DF, DW and DS) areas. PERMANOVA at  $p = 0.5364$  and ANOSIM at  $R = -0.03086$ . 72

4.3	NMDS ordination of the bacterial communities, comparing the undisturbed and disturbed samples of fish gut contents (DF1, DF2, DF3, UF1, UF2 and UF3), water (DW1, DW2, DW3, UW1, UW2 and UW3) and soil (DS1, DS2, DS3, US1, US2 and US3) samples. PERMANOVA at $p = 0.3152$ and ANOSIM at $R = 0.2025$ .	73
4.4	NMDS ordination of the bacterial communities in comparing undisturbed fish gut content samples (UF1 = Sungai Karang Peat Swamp Forest site 1; UF2 = Raja Musa Peat Swamp Forest; UF3 = Sungai Karang Peat Swamp Forest site 2) and disturbed fish gut content samples (DF1 = paddy field; DF2 = forest fire; DF3 = oil palm plantation) and. PERMANOVA at $p = 1$ and ANOSIM at $R = 0.2222$ .	74
4.5	NMDS ordination of the bacterial communities in comparing undisturbed water samples (UW1 = Sungai Karang Peat Swamp Forest site 1; UW2 = Raja Musa Peat Swamp Forest; UW3 = Sungai Karang Peat Swamp Forest site 2) and disturbed water samples (DW1 = paddy field; DW2 = forest fire; DW3 = oil palm plantation) and. PERMANOVA at $p = 1$ and ANOSIM at $R = 0$ .	75
4.6	NMDS ordination of the bacterial communities in comparing undisturbed soil samples (US1 = Sungai Karang Peat Swamp Forest site 1; US2 = Raja Musa Peat Swamp Forest; US3 = Sungai Karang Peat Swamp Forest site 2) disturbed soil samples (DS1 = paddy field; DS2 = forest fire; DS3 = oil palm plantation). PERMANOVA at $p = 0.3116$ and ANOSIM at $R = 0.2222$ .	76
4.7	The rarefaction curves show the increase in bacterial operational taxonomic unit's (OTU's) and the number of randomly sampled sequences of the bacteria in disturbed and disturbed sites for fish gut contents (DF and UF). The OTU's detected per sample as a function of the sequencing effort.	78
4.8	The rarefaction curves show the increase in bacterial operational taxonomic unit's (OTU's) and the number of randomly sampled sequences of the bacteria in disturbed and undisturbed sites for water samples (DW and UW). The OTU's detected per sample as a function of the sequencing effort.	79
4.9	The rarefaction curves show the increase in bacterial operational taxonomic unit's (OTU's) and the number of randomly sampled sequences of the bacteria in disturbed and disturbed sites for soil (DS and US). The OTU's detected per sample as a function of the sequencing effort.	80
4.10	Chart of bacterial community structure between disturbed and undisturbed samples of fish gut contents, water and soil across taxa phylum, class, order, family and genus displayed in the absolute abundance.	81

4.11	Venn diagram summarizing the overlap between disturbed and undisturbed areas in the three samples. The number of bacterial OTU is indicated by the overlap between the two circles. (a) fish gut content samples between disturbed and undisturbed (DF and UF) areas, (b) water samples between disturbed and undisturbed (DW and UW) areas, and (c) soil samples between disturbed and undisturbed (DS and US) areas.	83
4.12	Comparison of absolute abundances of top 10 bacterial community in DF and UF (disturbed and undisturbed areas of fish gut contents), DW and UW (disturbed and undisturbed areas of water), and DS and US (disturbed and undisturbed areas of soil) samples. a) phyla level; b) family level; c) genera level.	86
4.13	Relative abundance (at $\geq 2$ ) families of bacterial community in undisturbed and disturbed and samples, a) fish gut contents (DF and UF), water (DW and UW) and soil (DS and US), and b) combined samples of disturbed (DF, DW and DS) and undisturbed (UF, UW and US) areas.	88
4.14	Relative abundance (at $\geq 2$ ) genera of bacterial community in undisturbed and disturbed and samples, a) fish gut contents (DF and UF), water (DW and UW) and soil (DS and US), and b) combined samples of disturbed (DF, DW and DS) and undisturbed (UF, UW and US) areas.	90
5.1	(a) Differentially abundant bacterial clades detected by LEfSe, (b) heatmaps of relative abundant OTU biomarkers in soil samples (g_Ente = g_Enterococcus, f_Hydr = f_Hydrogenophilaceae, g_Spir = g_Spirochaeta, g_Acin = g_Acinetobacter, g_CndRh = g_Candidatus Rhodoluna, g_Legi = g_Legionella, o_Hydr = o_Hydrogenophilales, g_Kleb = g_Klebsiella, c_TK17 = c_TK17, o_Methy = o_Methylococcales, f_Methy = Methylobacteriaceae c_Plan = c_Planctomycetia and o_Spir = o_Spirochaetales) and (c) cladogram among disturbed (DS) and undisturbed (US) soil samples.	101
5.2	Normalized relative abundance of metagenome prediction of (a) level 1, (b) level 2 and (c) level 3 at top 20 bacterial communities between disturbed and undisturbed soil samples (DS and US).	105
5.3	(a) Linear Discriminant Analysis (LDA) Effect Size (LEfSe) plot of KEGG Orthology (KO) number biomarkers between disturbed (DS) and undisturbed (US) soil samples. The threshold for the logarithmic discriminant analysis (LDA) score was 2.0. (b) Heatmaps of KOs number obtained from LEfSe based on log2 (+1 transformed values) among all samples. (c) Extended error bar of KOs between DS and US obtained from LEfSe based on log2 (+1 transformed values).	108

- 5.4 Heatmaps analysis of metagenomic prediction level 1 (a) between disturbed and undisturbed soil samples and (b) among disturbed and undisturbed soil samples. The results based on log<sub>2</sub> (+1 transformed values). The function prediction between groups was tested using bootstrap Mann-Witney U-test with cut off  $p < 0.05$ . 110
- 5.5 Potential biomarkers encoded genes for soil samples. (a) LEfSe plot at level 2 between undisturbed (US1, US2 and US3) and disturbed (DS1, DS2 and DS3) soil samples. (b) Heatmaps analysis of metagenomic prediction level 2 of soil samples between disturbed and undisturbed areas. The results were based on log<sub>2</sub> (+1 transformed values). The function prediction between groups was tested using bootstrap Mann-Witney U-test with cut off at  $p < 0.05$ . (c) Extended error bar of metagenomic prediction level 2 of soil samples obtained from LEfSe based on log<sub>2</sub> (+1 transformed values) between undisturbed and disturbed samples. 112
- 5.6 Potential biomarkers encoded genes for soil sample. (a) LEfSe plot at level 3 between disturbed (DS1, DS2 and DS3) and undisturbed (US1, US2 and US3) soil samples. The encoded genes prediction between groups was tested using bootstrap Mann-Witney U-test with cut value off  $p < 0.05$ . (b) Heatmaps at level 3 of potential biomarkers encoded genes of soil samples. Results based on log<sub>2</sub> (+1 transformed values). (c) Chart of extended error bar of LEfSe result of biomarkers at level 3 among soil disturbed soil (DS1, DS2 and DS3) and undisturbed (US1, US2 and US3) samples. 114
- 5.7 Top relative percentage contribution by gene for bacterial families in inputted metagenome for RNA polymerase sigma-70 factor, ECF subfamily, methyl-accepting chemotaxis protein and enoyl-CoA hydratase [EC:4.2.1.17. Heatmap representing the top percentage contribution by bacterial family for KEGG orthologs of K03088, K03406 and K01692 across each sample of the soil in disturbed (DS1, DS2, DS3) and undisturbed (US1, US2 and US3) areas. Four families were grouped into two phyla; A= Firmicutes and B = Proteobacteria. 117
- 6.1 RDA plots showed relationships between anthropogenic factors (DHS = Distance from human settlement, SA = State of accessibility, DL = Deforestation level, AP = Air pollution, WP = Water pollution, LP = Land pollution, AA = Agricultural activity and CR = Construction of road/building) and OTU biomarkers soil (f\_Aeromonadaceae = f\_Aero, o\_Gemmatales = o\_Gemm, f\_Ruminococcaceae = f\_Rumi, f\_ACK\_M1 = f\_ACK, p\_WPS\_2 = p\_WPS, g\_Enterococcus = g\_Ente, f\_Hydrogenophilaceae = f\_Hydr, g\_Spirochaeta = g\_Spir, g\_Acinetobacter = g\_Acin, g\_Candidatus Rhodoluna = g\_CndRh, g\_Legionella = g\_Legi, o\_Hydrogenophilales = o\_Hydr, g\_Klebsiella = g\_Kleb, c\_TK17 = c\_TK17, o\_Methylococcales = o\_Methy, c\_Planctomycetia = c\_Plan and o\_Spirochaetales = o\_Spir). Blue colour (UA) = undisturbed area and red colour (DA) =



disturbed areas. The vector with arrows depicted significant level of correlation.

128

6.2 RDA plots showed relationships between forest factors (RLI = Relative light intensity, TP = Total Plants, FT = Fallen trees, PS = Percentage Seedlings, PT = Percentage Trees, TH = Tree height, CC = Canopy cover and AS = Animals sighted) and OTU biomarkers soil (f\_Aero = f\_Aeromonadaceae, f\_Rumi = f\_Ruminococcaceae, f\_ACK = f\_ACK\_M1, p\_WPS = p\_WPS\_2, f\_Hydr = f\_Hydrogenophilaceae, g\_Spir = g\_Spirochaeta, g\_Acin = g\_Acinetobacter, g\_CndRh = g\_Candidatus Rhodoluna, g\_Legi = g\_Legionella, g\_Kleb = g\_Klebsiella and c\_TK17 = c\_TK17). Blue colour (UA) = undisturbed area and red colour (DA) = disturbed areas. The vector with arrows depicted significant level of correlation.

129

6.3 RDA plot of physicochemical factors (Water depth = WD, Turbidity of water = Turbid, Dissolved oxygen = DO, Ammonia = NH<sub>3</sub>, Nitrate = NO<sub>3</sub> and Iron = Fe) and OTU biomarkers of soil (f\_Aeromonadaceae = f\_Aero, o\_Gemmatales = o\_Gem, f\_Ruminococcaceae = f\_Rum, f\_ACK\_M1 = f\_ACK, p\_WPS\_2 = p\_WPS, g\_Enterococcus = g\_Ent, f\_Hydrogenophilaceae = f\_Hyd, g\_Spirochaeta = g\_Spir, g\_Acinetobacter = g\_Act, g\_Klebsiella = g\_Kle, o\_Hydrogenophiles = o\_Hyd, g\_Methylobacter = g\_Met, c\_TK17 = c\_TK17, g\_Thiobacillus = g\_Th, g\_Legionella = g\_Le, g\_Candidatus Rhodoluna = g\_Ca, o\_Methylococcales = o\_Me, c\_Planctomycetia = c\_Pl and o\_Spirochaetales = o\_Sp). Blue color (UA) = undisturbed & red color (DA) = disturbed areas, the vector with arrows depicted significant level of correlation.

130

## LIST OF ABBREVIATIONS

NSPSF	North Selangor Peat Swamp Forest
PSF	Peat swamp forests
NGS	next-generation sequencing
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
DNA-Seq	deoxyribonucleic acid- Sequencing
16S rRNA	16 Svedberg unit/16 Subunit of ribosomal RNA
MGE	mobile genetic elements
PPFM	pink-pigmented facultative methylotrophic
ACC	aminocyclopropane-1-carboxylic acid
PAH	Polycyclic Aromatic Hydrocarbon
HTS	high throughput sequencing
PCR	polymerase chain reaction
O <sub>2</sub>	oxygen
CO <sub>2</sub>	carbon dioxide
CH <sub>4</sub>	methane
NO <sub>2</sub>	nitrite
NO <sub>3</sub>	nitrate
Fe	iron
TDS	total dissolved solids
PO <sub>4</sub>	phosphate oxide
SO <sub>4</sub>	sulphate oxide
NH <sub>3</sub>	ammonia
DO	dissolved oxygen
DOC	dissolved organic carbon
HCH	hexachlorocyclohexane
T	temperature

TU	turbidity
WD	water depth
ha	hectare
mm	millimeter
km <sup>2</sup>	Kilometer square
km <sup>2</sup> /year	Kilometer square per year
Mha	million hectare
m	meters' square
mL	millilitre
°C	degree centigrade
pH	presence of hydrogen ion concentration
Gt	Giga tonne
<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
μ	micro
μL	microlitre
μm	micrometer
\$	American Dollar
RM	Malaysian Ringgit
SE	standard error
SD	standard deviation
p	p- value/probability value
ANOVA	analysis of variance
PCA	Principal Component Analysis
CCA	Canonical Component Analysis
RDA	Redundancy Analysis



PERMANOVA	permutational multivariate ANOVA
ANOSIM	analysis of similarity
PAST	PAleontological STatistics
STAMP	statistical analysis of metagenomic profiles
SPSS	Statistical Package for Social Science
IBM SPSS	International Bussiness Machine Statistical Package for Social Science
NMDS	Non-Parametric Dimensional Scaling
n	Population size
$H_0$	Null hypothesis
$H_a$	Alternate hypothesis
IUCN	International Union for Conservation of Nature
ESA	Endangered Species Act
GEC	Global Environmental Conservation
NOS	National Ocean Service
UNDP	United Nations Development Programme
CDDEP	Center for Disease Dynamics Economics Policy
SERAS	Scientific Engineering Response and Analytical Servives
LULUCF	Land Use, Land-Use Change and Forest
GHG	greenhouse gas
FAO	Food Agricultural Organisation
BBFRE	Bukit Belata Forest Reserve Extension
SDFWR	Sungai Dusun Forest/Wildlife Reserve
RMPSF	Raja Musa Peat Swamp Forest
SKPSF	Sungai Karang Peat Swamp Forest
BLAST	Basic Local Alignment Search Tool
QIIME	Quantitative Insights Into Microbial Ecology
GG	GreenGenes
OTU	operational taxonomic unit

PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
NSTI	Nearest Sequenced Taxon Index
RDP	Ribosomal Database project
COG	Clusters of Orthologous Grou
LEfSe	Linear Discriminant Analysis (LDA) Effective Size
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	KEGG Ortholog
SRA	Sequence Read Archive
NCBI	National Center for Biotechnology Information
MS-222	(tricaine)methanesulfonate
EDTA	ethylenediaminetetraacetic acid
GH	glycoside hydrolase
H	actual tree height
$h_1$	eye height
$h_2$	height of tree above researcher's eye level
DBH	diameter at breast height
$\tan \theta$	$\tan$ theta
x	distance between the tree and researcher observing position
FC	Foot-Candle
LIC	Light intensity net under canopy
LIO	Light intensity net open space
CRD	Complete randomized design

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of the study

North Selangor Peat Swamp Forest (NSPSF), like all other peatland ecosystems is subjected to various threats such as forest fire, expansion of area for industrial and agricultural activities, canal construction, deforestation, and air, water and land pollution (Elliott et al., 2015). Muda et al. (2012) reported that between the periods of 1997-1998, about 630 hectares (ha) of forest reserve was destroyed. Altogether, the landmass has been estimated to be about 6,500 ha is subjected to repeated forest fires (Turetsky et al., 2015). Thus, proactive actions should be taken to conserve and protect the area, in order to maintain the remaining endangered and endemic species of flora and fauna there (Muda et al., 2012).

Population growth leads to increasing agricultural activities in NSPSF (Adila et al., 2017). Other human related activity such deforestation and land clearing for agricultural activity might have been the cause of fire incidence in NSPSF, which lead to subsequently habitat degradation (Muda, et al, 2012). In this way, peat swamp forest (PSF), including the NSPSF, that use to be pristine is gradually being loss for good. In conjunction with canal excavation and other related of drainage, it initiates peat decomposition which subsequently lead to carbon emissions in PSF of South East Asia (Lampela et al., 2017). Following drainage and related human activity, an estimated of 600 tetragrams of carbon is being loss annually, while burning of peat due to fire incidence had also led to the emission of 640 tetragrams of carbon annually (Hooijer et al., 2006). As such, ecological and conservational management is important, and this require monitoring as core activity in biodiversity conservation (Marsh and Trenham, 2008).

The characterization of anthropogenic factors and microbial communities of polluted and degraded PSF could potentially provide guidelines for effective identification, remediation and restoration of such environments. The research findings could form the basis for library construction and the study of bacterial composition and abundance and its relationships between anthropogenic, forest and physicochemical factors of NSPSF.

#### 1.2 Research problems

Peat swamp forest is an important ecosystem which serve many significance purposes, but it is constantly being disturbed, degraded and sometimes loss completely. Anthropogenic activities and other environmental related agents such as flooding (Hugron et al., 2013), fire and climate change (Xu et al., 2018) leading to

contamination and destruction of such habitat are the major issues especially in countries of southeast Asia such as Vietnam, Thailand, Indonesia and Malaysia (Fitzherbert et al., 2008; Simpson, 2014; Lampela et al., 2017; Xu et al., 2018). Mining, logging, construction of buildings, roads, canals, agriculture and other related activities that could alter or lead to addition of substances consider to be harmful to the peat swamp ecosystem. As water pollution was observed along Tengi River of NSPSF following mining of tin, sand and clay (Irvine et al., 2013; GEC 2014a), pollution from manure and waste application in paddy field and oil palm plantation were also reported in NSPSF (Sule et al., 2019). Appropriate technologies and strategies are being devised toward remediation, rehabilitation and restoration of these polluted and degraded environments. The fact that the majority of the technologies developed and in use for the correction of the environmental abnormality in question exploit the potential of biological systems, in particular microbial systems (Mukherjee et al., 2017). Microbes is often grown on pollutants produced by anthropogenic related agents and much of the research conducted on bioremediation has concentrated on the capabilities of a single or couple of microbes exhibiting robust and effective growth on such pollutants (Mukherjee et al., 2017). Peat accumulates following inhibition of the microbial activities due to waterlogged anaerobic conditions and the recalcitrant plant detritus, resulting in an acidic, toxic and phenol-rich peat substrate (Too et al., 2018). However, anthropogenic activity may likely lead to change in bacterial community, thereby affecting the accumulated peat of NSPSF. For instance, construction and excavation are processes which requires both removal of the primary vegetation and soil drainage, the latter resulting in peat shrinkage through a combination of water loss, enhanced aerobic decomposition as the diversity and composition of bacterial community in PSF is altered. As at 2010, 233 million tonnes of CO<sub>2</sub> have been produced and loss through decomposition (CO<sub>2</sub> production) of organic matter losses resulting from subsidence of drained and cultivated peat in Malaysia (Page and Rieley, 2006). This imply that maintaining the status quo of the bacterial community in NSPSF means protecting it against degradation and destruction.

The forest vegetation is an important component of the PSF as it covers and protect the forest floor against excessive temperature (GEC, 2014b). This could adversely affect the functioning of bacteria. And bearing in mind the ecological roles of bacteria as an agent of disintegration, decomposition of dead organisms and subsequently recycling organic nutrients (De Boer, 2017). Notably, the nutrient deficient condition of PSF is evident by the low elemental content of the peat, while forest growth is dependent upon the supply of nutrients (Page and Rieley, 2006). Thus, it's prompted the need for better understanding of bacterial community which recycle the existing nutrient pool within the NSPSF ecosystem.

Additionally, bioremediation could be employed as alternative solution in converting degraded and polluted environment (Mukherjee et al., 2017). Environmental bioremediation is usually a complex process involving co-metabolism, cross-induction, inhibition and non-interaction among microbes. The synergistic and sometimes antagonistic actions of microbes is possibly due to nature of the pollutants, which come from a mixture of different substances and therefore are used differently

by different microbes. Thus, bioremediation is necessary. Bioremediation is a conversation of pollutants to less harmful substances through a process mediated by living organisms including consortium of microbes rather than a few, and hence, characterization of microbial communities of NSPSF could potentially provide guidelines for effective remediation and restoration of such environments (Mukherjee et al., 2017). Furthermore, available research and knowledge of the bacteria in NSPSF is often confined to traditional culture methods (Yooseph et al., 2013). Moreover, there is limited literature on the metagenomic study of bacterial community particularly in relation to environmental characteristics of NSPSF.

Until recently, microbes were studied traditionally by using a few numbers of microorganisms of interest, where it will be isolated from source materials (such as blood, soil, water or air), given the restrictions of the composition of culture media which cannot reflect and mimic the dynamic nutrient fluxes of the source environment. Indeed, only 1% of microorganisms were found to be cultivable using a set of media from the highly characterized soil rhizosphere (Mukherjee et al., 2017). Therefore, using traditional culture methods will only capture minute representation of those microbes in their habitats (Vieira and Nahas, 2005).

Another vital role play by peat swamp forest is housing of rare, threaten and endangered fish species (Ismail, 2015). There are many distinctive fish species collected and use as food, and ornamental and other recreational purposes (Song et al., 2013). The bacteria may be a cheap source of pathogens causing infectious diseases to fish living in the wild (Sudheesh, et al., 2012). The disease outbreaks against aquatic animal have increased in an alarming rate (Chen et al., 2018). The bacterial related infections from such outbreak could result in massive fish deaths. Since ecosystem is an interconnected process, which linked biotic elements among themselves and connects them with abiotic components hence, the degradation which affects one or more organisms could invariably affect all the dwelling organisms in the ecotone. Notably, increasing residential and agricultural activities could directly affect the flowing water thereby increase in potential water pollutant which most likely ends in NSPSF (Adila et al., 2017). Moreover, some bacteria in the fish gut help maintain metabolic homeostasis in the fish (Li et al., 2020). While some bacteria are source of fish feeds as they may provide vital nutritional sources of diet to fish. Thus, many bacteria activate beneficial biological activities and promote healthy growth of the host (Banerjee et al., 2000; Wang et al., 2019). Having said that, then it's vital to study the environmental factors, the bacterial community in the soil, water as well as host organism in the NSPSF. Study of bacterial community in the host organism such as fish gut contents could give information and better picture of beneficial and harmful bacteria. The intruding and threatening bacteria which could subsequently endangered fish host. Therefore, this research will enhance knowledge of fish gut microbiota in the study area.

The next-generation sequencing methods (NGS) include a variety of procedures to study any biological system such as amplicon sequencing (for various identification and phylogenetic surveys), whole genome shotgun sequencing (for single organism genome and metagenomes) and DNA-Seq. This technique allowed us to investigate



the entire complement of organisms inhabiting a certain environment. The availability of bioinformatics tools for prediction of functions i.e. metagenomes, from 16S DNA gene sequences is particularly attractive to microbial ecologists, as it allows them to study the metabolomes of complex microbial communities with reasonable precision and confidence at a high taxonomic resolution, while being able to construct robust hypotheses for further works. It has been reported that soil microbial communities respond to environmental change by changing their taxonomic and functional composition, and any functional shift means alteration in the physiological responses of the bacteria which can be detected using metagenomics signatures of the study area (Pold, 2019). The identification of the environmental parameters influencing the composition and functions of bacteria in NSPSF will give insight toward a better understanding of bacterially mediated processes.

Unfortunately, despite the large amount of work done on microbial community composition, no attempt has been made to find differential metagenomics signatures through DNA sequencing in soil, water and fishes of NSPSF. The aim of the present study is to assess the profile of bacterial community using metagenomic analysis and characterize environmental factors in and nearby NSPSF, as well as to evaluate the relationship between the significant environmental factors and functional genes of the bacterial community.

### **1.3 Objectives of the study**

The specific objectives include:

1. To identify the environmental characteristics of undisturbed NSPSF and its adjacent disturbed area.
2. To determine the composition and diversity of bacterial communities in fish gut contents, water and soil of undisturbed NSPSF and its adjacent disturbed area, Selangor, Malaysia.
3. To determine the differential features and core bacterial communities in soil samples from undisturbed NSPSF and its adjacent disturbed area.
4. To evaluate the relationships between the environmental variables with bacterial taxa biomarker in soil samples from undisturbed NSPSF and its adjacent disturbed area.

### **1.4 Limitation of the study**

Metagenomics sequencing used to be expensive, which can be a limiting factor for microbial ecology research in limited funded projects. Analysis of metagenomics sequencing datasets is manifold computationally intensive and gene analyses will require many advanced software. It thereby escalates further the operational costs. Moreover, pooling the fish gut content of all fish's species for determination of bacterial diversity and composition may alter the fish gut metagenomic data as different fish species could have different microbiome diversity.

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