



**EFFECT OF OPEN TOP CHAMBERS ON SOIL MICROBIOME AND
COMPARISON BETWEEN TROPICAL AND POLAR REGIONS**

By

NG YI CHING

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

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Abstract of thesis presented to the Senate of Universiti Putra
Malaysia in fulfillment of the requirement for the degree of Master of
Science

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July 2020

Chairman : Professor Cheah Yoke Kqueen, PhD
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Soil contains the highest microbial diversity compared to other natural habitats on the earth. The advance in the next generation sequencing (NGS) techniques have allowed scientists to explore the diversity and composition of soil microorganisms at different locations including extreme cold places such as polar regions. Global warming has become an alarming issue to public as it could bring harms to all living organisms on earth. There are many studies reporting the effect of warming on the macroorganisms. However, not many studies reported on the response of microorganisms towards warming due to complexity of microbial genome. The importance of the study is to provide the reference knowledge for researchers on the relationship between the response of soil microbes and the effect of warming. The aim of this study was to compare soil metagenome between different months affected by open top chambers (OTC). A total of 18 soil samples were collected from 3 different open top chambers (OTC) located near the Molecular Biology Laboratory at Universiti Putra Malaysia between February and August 2017. The primer pairs 341F and 805R was employed to perform PCR amplifications of 16S rRNA gene and the samples were then sequenced on IlluminaMiseq2500 platform. The results showed that the most dominant bacterial phyla found within tropical soil samples were phyla *Proteobacteria* followed by *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, *Verrucomicrobia*, and *Nitrospirae*. After 6 months of OTC warming, the results showed that there was an increase in abundance of *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Verrucomicrobia* while *Chloroflexi*, *Acidobacteria*, *Cyanobacteria*, and *Nitrospirae* showed decrease in their abundance. No significant change in soil bacterial diversity and richness but there was a shift in bacterial structure and composition. On the other hand, to study the species diversity among samples compared to available database and compare the

analysis of soil metagenome between tropical and polar regions, two soil samples from Greenwich Island, Antarctic and Hornsund, Spitsbergen, Arctic regions were utilized. These two samples were not simulated by OTC at these two polar regions. In addition, they were pre-collected and pre-sequenced separately from the current batch of tropical soil samples. The soil metagenome data between tropical and polar regions were then compared together to observe their differences in bacterial diversity and composition. The results showed that three most dominant phyla found within the tropical soil samples were phyla *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* while for both polar regions, the most dominant groups were represented by phyla *Proteobacteria*, *Actinobacteria*, and *TM7*. Additionally, the results also showed that there was presence of some commonly shared bacterial groups which were dominated in both polar and tropical soils. To conclude, short-term OTC warming did not cause any changes in soil bacterial diversity and richness but did cause changes in bacterial abundance of certain bacterial groups and in turn shift the bacterial structure and composition. Additionally, two most abundant and dominant bacterial groups, *Proteobacteria* and *Actinobacteria* were revealed from tropical and polar regions when comparing their metagenome data.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra
Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**KESAN DARIPADA RUANG ATAS TERBUKA PADA
MIKROBIOM TANAH DAN PERBANDINGAN ANTARA RANTAU
TROIKA DAN KUTUB**

Oleh

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Tanah mengandungi kepelbagaian jenis mikrob yang tertinggi berbanding dengan habitat alam semula jadi lain yang berada di bumi. Kemajuan dalam teknik “*next generation sequencing*” (NGS) membolehkan para saintis untuk meneroka kepelbagaian dan komposisi mikroorganisma tanah di lokasi yang berbeza termasuk tempat sejuk yang melampau iaitu kawasan kutub. Pemanasan global telah menjadi masalah yang membimbangkan masyarakat kerana ia boleh membawa kemudaratan kepada semua organisma yang hidup di bumi. Terdapat banyak kajian yang melaporkan kesan pemanasan pada makroorganisma. Namun begitu, tidak banyak kajian yang melaporkan tindak balas mikroorganisma terhadap pemanasan kerana kerumitan genom mikrob. Kepentingan kajian ini adalah untuk memberikan pengetahuan kepada penyelidik sebagai rujukan terhadap hubungan antara tindak balas mikrob tanah dan kesan pemanasan. Kajian ini bertujuan untuk membandingkan metagenom tanah yang dipengaruhi oleh ruang atas terbuka (OTC) di antara bulan yang berlainan. Sejumlah 18 sampel tanah daripada kajian dikumpulkan dari 3 ruang terbuka yang berbeza (OTC) yang terletak berhampiran Makmal Biologi Molekul di Universiti Putra Malaysia antara Februari dan Ogos 2017. Pasangan primer 341F dan 805R telah digunakan untuk PCR gen 16S rRNA dan seterusnya dianalisis dengan IlluminaMiseq2500. Hasil kajian ini menunjukkan bahawa Filum bakteria yang paling dominan telah ditemui dalam sampel tanah tropika adalah Filum *Proteobacteria* diikuti oleh *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, *Verrucomicrobia* dan *Nitrospirae*. Setelah pemanasan OTC selepas 6 bulan, dapatan kajian menunjukkan terdapat peningkatan jumlah *Proteobacteria*, *Actinobacteria*, *Firmicutes* dan *Verrucomicrobia* tetapi *Chloroflexi*, *Acidobacteria*, *Cyanobacteria* dan *Nitrospirae* menunjukkan penurunan jumlahnya. Tiada perubahan ketara dalam kepelbagaian dan kekayaan bakteria tanah boleh ditemui tetapi terdapat perubahan struktur dan komposisi bakteria. Sebaliknya, untuk mengkaji

kepelbagaian spesies di antara sampel berbanding dengan pangkalan data yang sedia ada dan membandingkan analisis metagenome tanah antara rantau tropika dan kutub. Dua sampel tanah dari *Greenwich Island*, Antartika dan *Hornsund, Spitsbergen, Arctic* telah digunakan. Kedua-dua sampel ini tidak disimulasikan oleh OTC di dua rantau kutub ini. Selain itu, kedua-dua sampel tersebut telah dikumpulkan dan diujukkan secara berasingan dari kumpulan sampel tanah tropika semasa. Seterusnya, data metagenom tanah antara rantau tropika dan kutub, dibandingkan untuk melihat perbezaan di antara mereka dari segi kepelbagaian dan komposisi bakteria. Hasil kajian ini menunjukkan bahawa tiga Filum yang paling dominan dalam sampel tanah tropika adalah phyla *Proteobacteria*, *Actinobacteria* dan *Chloroflexi* sementara untuk kedua-dua rantau kutub, kumpulan yang paling dominan diwakili oleh phyla *Proteobacteria*, *Actinobacteria* dan *TM7*. Tambahan pula, hasil kajian ini juga menunjukkan bahawa terdapat beberapa persamaan kumpulan bakteria di rantau tropika dan kutub. Sebagai kesimpulannya, pemanasan OTC pada masa jangka pendek tidak menyebabkan sebarang perubahan dalam kepelbagaian dan kekayaan bakteria tanah tetapi ia menyebabkan perubahan dari segi jumlah bakteria untuk kumpulan bakteria tertentu dan seterusnya mengubah struktur dan komposisi bakteria. Tambahan pula, *Proteobacteria* dan *Actinobacteria* merupakan dua kumpulan bakteria yang paling banyak dan dominan dalam sampel dari rantau tropika dan kutub ketika perbandingan data metagenom dilakukan.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
CEC	Cation exchange capacity
DNA	Deoxyribonucleic acid
EC	Electrical Conductivity
IPCC	Intergovernmental Panel on Climate Change
ITS	Internal transcribed spacer
MWC	Maximum water capacity
NGS	Next Generation Sequencing
OTC	Open Top Chambers
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PLFA	Phospholipid-derived fatty acids
PVC	Polyvinyl chloride
RDP	Ribosomal Database Project
RNA	Ribonucleic acid
SOLiD	Sequencing by Oligonucleotide Ligation and Detection
SOM	Soil organic matter
TAE	Tris-Acetate-EDTA
USDA	United States Department of Agriculture

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Soil nurtures a vast diversity of life and it contains the highest microbe diversity compared to other natural environment (Roesch et al., 2007). One teaspoon of soil consists of billions of bacterial cells and around 100,000 different bacterial species (Torsvik, Ovreas, and Thingstad, 2002). This soil microbial communities determine the key functions of the soil and directly affecting the qualities and properties of the land. They play a crucial role in plant fertility, cycling of carbon (C) and nutrients, decomposing of organic matters, controlling the production and absorption of greenhouse gases such as methane and nitrous oxides (Hu, Firestone, and Chapin, 1999). However, despite the presence of enormous bacterial diversity in soil environment, 99% of bacterial species cannot be cultured and identified through traditional way (Pham and Kim, 2012). To reveal the information about the biodiversity and functions of the soil microbes, it is believed that the employment of metagenomic approach involving direct isolation of DNA from soil environment could improve better access to these soil microbial communities.

Current advent molecular techniques that bypass prior need for extraction and lab cultivation of individual bacterial species enable the study of bacterial genome directly from natural environment (Riesenfeld, Schloss, and Handelsman, 2004; Streit and Schmitz, 2004; Handelsman, 2005). The advances in next generation sequencing (NGS) have redefined and allowed significant breakthroughs in metagenomic studies. It is a high throughput, massive parallel sequencing that allow much faster and reproductive results at an acceptable cost (Schuster, 2007; Shendure and Ji, 2008). There are two commonly used NGS technologies that have been widely applied in metagenomic studies which are the Roche 454 pyrosequencing and Illumina (solexa) sequencing. In the present study, the Illumina sequencing has been applied to sequence and analyze bacterial genome as it has been reported in most of metagenomic studies that could produce greater yield, allows significant decrease of systematic errors and low cost (Mandal et al., 2015).

Two polar regions including Arctic and the Antarctic regions that are located at the North and South poles of the earth are the coldest places in the world. From the past, it was believed that there was presence of low microbial biodiversity in these environments. However, recent studies have utilized advanced next generation sequencing (NGS) techniques to claim that the soil ecosystems at these two regions are dominated by cold tolerant microorganisms with high biodiversity level (Yergeau et al., 2007; Niederberger et al., 2008; Schutte et al., 2009). Bacterial populations are the most dominant and diverse group compared to other microorganisms in the soil ecosystem. To have a better understanding on

the unusual physiological and biochemical properties of these specific soil bacteria species adapting in these cold ecosystem, the next generation sequencing (NGS) techniques have played an important role in accessing the diversity and composition of the soil bacteria communities and revealing their specific metabolic functions in these extremely cold environments.

The term 'global warming' or climate change refers to the gradual increase in the average temperature of the Earth's atmosphere and oceans. For the last century, the global mean temperature has elevated for 1 °C globally and it is postulated that the temperature will rise again by another 2-3 °C in the next two decades (Karmakar et al., 2016). This is primarily due to pollution, industrialization, deforestation, and intensified conventional agriculture. Global warming could lead to many negative consequences to upper ground organisms like human beings, animals, and plants on terrestrial ecosystem. However, not many studies have been done on the response of the lower ground microorganisms towards the effect of global warming in the soil ecosystem due to the complexity of soil microbiome (Sheik et al., 2011). In this study, an in-situ warming experiments was conducted by using open top chambers (OTC) to explore how climatic changes affect the response of soil microbial communities.

A total of 18 tropical soil samples were collected from three different area of plots near the Molecular Biology and Bioinformatics Lab, Universiti Putra Malaysia (UPM) (2°58'39.88837"N, 101°43'6.22083"E). After that, the primer sets 341F and 805R that targeted to amplify V3 and V4 hypervariable region of 16s rRNA gene was employed to perform PCR amplifications. Illumina sequencing of 16s rRNA gene amplicons was used to reveal the bacterial community diversity and composition from the tropical soil samples at UPM. For both soil samples collected from Greenwich Island (62°27'02.1"S, 59°44'12.1"W), Antarctic and Hornsund, Spitsbergen (77°1.66 5"N, 15°10.206"E), Arctic region, they were pre-sequenced separately from these batch of tropical soil samples by other team members. During the analysis of metagenomic data, the phylogenetic relationship of different OTUs was studied and the difference of the dominant species in different samples (groups) was compared with the available Greengene database. The subsequent analysis of alpha diversity and beta diversity were performed based on the output data. By analyzing the metagenomic data obtained, the changes in abundances, structure, and diversity of bacterial communities under short-term warming effect were examined by comparing the samples on different warming OTC plots with the control plots.

To study and compare the differences in bacterial diversity and composition of the polar and tropical regions, two soil samples from Greenwich Island (62°27'02.1"S, 59°44'12.1"W), Antarctic and Hornsund, Spitsbergen (77°1.665"N, 15°10.206"E), Arctic regions were utilized in this study. Both Arctic and Antarctic soil were pre- collected by other team members from upper 15-20 cm layer from top surface of soils. Additionally, these two soil samples were not subjected to open top chamber (OTC) conditions at these two polar regions. The primer pairs 341F and 805R that targeted to amplify V3 and V4 hypervariable region of 16s rRNA gene were also used to perform the PCR amplification on

these two samples and they were then pre-sequenced separately from the current batch of tropical soil samples on an IlluminaMiSeq2500 platform by other team members. The Greengene Database was also used to annotate the taxonomic information of microbial communities from each representative sequence from both polar soil samples. Lastly, the soil metagenomic data between polar and tropical regions were compared by examining the similarities and differences in their taxonomic composition. The flow chart of summarized version of research methodology is shown at Figure 1.1 as below.

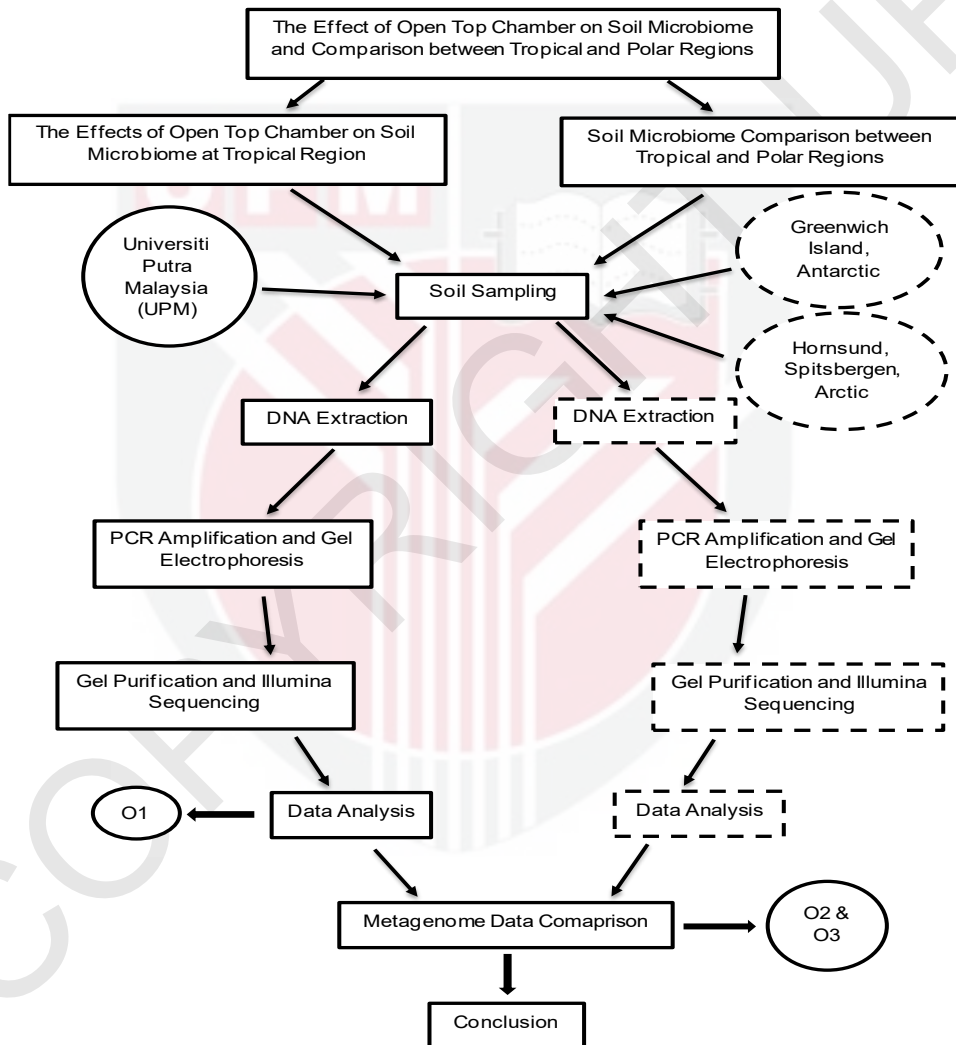


Figure 1.1: Conceptual Framework of Experimental Design. The flowchart as presented in Figure 1.1 shows the two-way direction of experimental design with the objectives achieved. Note: the solid boxes/circle represent the current research work; the dotted boxes/circles represent the pre-processed research work done by other team members. O1 represents Objective 1; O2 and O3 represent Objectives 2 and 3.

1.2 Problem Statements

Many previous studies have been done on the response of the upper-ground macro-organisms toward the effect of global warming. However, there was little information about the impact of warming on the response of the lower ground microorganisms in the soil ecosystem could be revealed from previous reported studies due to their inconsistent response under warming effect and the complexity of soil microbiome (Sheik et al., 2011). For examples, Yergeau et al. (2012) reported that there was a consistent change in Antarctic soil microbial composition under short duration warming treatment by using open top chambers. However, Xue et al. (2016) reported that there were no significant changes in bacterial composition in tundra soil under short duration warming. For better understanding on how microbial communities respond towards warming effect, the Illumina sequencing technique was employed to conduct a short-term OTC warming experiment on soil microbial communities. On the other hand, the current study was also conducted to assess and compare the soil bacterial diversity of both tropical and polar regions as there were fewer comparative studies done on soil metagenome from across different geographical locations especially Southeast Asia regions with polar regions. Therefore, in this study, the results of soil metagenomic data from tropical regions (inclusive OTC) was utilized to compare with the soil metagenomic data from polar regions (exclusive OTC) which are done by other team members.

1.3 Significance of Study

The present study is focused on the effect of warming on microbial communities in soil. Several studies have examined the response of soil microbial communities under the effect of warming in different locations such as in temperate mountain forest and alpine grassland and Arctic region (Kuffner et al., 2012, Zhang et al., 2016 and Lim et al., 2018). The outcome of the present study is to provide the knowledge on the relationship between the response of microbial communities in soil and the effect of warming. Besides, the current study also has a strong urge to create awareness among public on the consequences of global warming to the environment due to the soil microbes' composition. For examples, global warming might increase certain soil microbial abundances and respiration which in turn promote the efflux of carbon from soil to atmosphere and result in positive feedback on climate changes (Bardgett, Freeman, and Ostle, 2008).

In addition, the current study also contributes a specific experimental model in methodology whereby the soil samples were subjected to open top chamber conditions in tropical region while the samples in both polar regions, Arctic and Antarctic were not subjected to it. By applying this method, the study is able to provide valuable information and better understanding to researchers on the differences in microbial diversity and composition from multiple soil types at different locations when comparing both tropical and polar soil metagenomes. It also provides a reference for future researchers to examine further the

importance of the study of microbial communities from various type of soil metagenome at different locations such as the phylogenetic relationship in between the soil microbial communities at different locations.

1.4 Objectives:

1. To compare soil metagenome between different months affected by open top chamber (OTC).
2. To compare the analysis of soil metagenomic between polar and tropical region.
3. To study the species diversity among samples compared to the available database.

1.5 Research Hypothesis

Certain dominant soil bacterial communities would be expected to have distinct responses in a short-term warming effect by open top chamber (OTC). Besides, there would also be a shift in soil bacterial composition under warming situation. Furthermore, the taxonomic information of soil bacteria communities in tropical region would be expected almost same with polar region.

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