EFFECTS OF TEMPERATURE AND WATER CONTENT ON BACTERIAL COMMUNITY COMPOSITION IN A TROPICAL AND AN ANTARCTIC SOIL, BASED ON MICROCOSM STUDIES IN THE LABORATORY

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KUALA LUMPUR

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ABSTRACT

Environmental factors such as temperature and water content play crucial roles in shaping the dynamics of soil bacterial community which in turn influences the ecosystem functioning. Alteration in any of these factors might alter the structure, composition and abundance of soil bacterial community. In this study, effects of temperature and water content on bacterial community in a tropical soil and an Antarctica soil were elucidated using laboratory-based microcosm studies. Tropical soil microcosms were incubated at 25°C, 30°C, 35°C, and subjected to low or high water treatments (2 ml or 5 ml respectively). The microcosms were analysed at Weeks 1, 2 and 4. Antarctic soil microcosms were incubated at 5°C, 10°C, 15°C with no variation in water treatment, and analysed at Weeks 4, 8 and 12. Bacterial richness, abundance and composition were analysed by terminal restriction fragment length polymorphism (T-RFLP) and high-throughput next generation sequencing. Functional genes (*nifH, amo-A, nirS, nirK*, *nosZ* and *Chitinase GA*) abundance was determined by quantitative polymerase chain reaction (Q-PCR). Abiotic parameters (pH, electrical conductivity, moisture, nitrate, nitrite and phosphate) in the microcosms were also measured.Results indicated that both structure and composition of tropical soil bacterial community differed significantly across the treatments. The relative abundance of *Firmicutes*, the dominant phylum, correlates positively with temperature and water content, and the highest compositional shifts were observed in the Week 2 microcosms. On the other hand, only subtle difference in Antarctic soil bacterial community structures was detected across temperature. Nevertheless, bacterial assemblages were strongly structured by period of incubation. Antarctic soil samples were dominated by *Proteobacteria* which responded positively to temperature upshift. Distance-based linear

model (DISTLM) analysis showed that pH, electrical conductivity, nitrate, nitrite and moisture content were the most significant parameters that correlated with the tropical soil bacterial community. In contrast, soil nitrate content was the sole parameters found to correlate with the Antarctic soil bacterial community. Significant correlations were found between tropical soil bacterial communities and the nitrogen fixation gene (*nifH*) and denitrification gene (*nosZ*) whereby an increase of *nifH* gene copies was observed with increase in temperature. In the Antarctic soil microcosms, the *nosZ* and *GA* genes showed the highest correlation to the bacterial community. Collectively, the above findings indicate that changes in temperature and water content induced shifts in soil bacterial community composition, abiotic parameters and functional gene abundance.

ABSTRAK

Faktor-faktor persekitaran seperti suhu dan kandungan air memainkan peranan penting dalam membentuk dinamik masyarakat bakteria tanah yang seterusnya mempengaruhi fungsi ekosistem. Perubahan dalam mana-mana faktor-faktor ini mungkin membawa kesan yang mendalam kepada struktur, komposisi dan kuantiti tanah komuniti bakteria. Dalam kajian ini, kesan kandungan air dan suhu terhadap masyarakat mikrob tanah dari hutan hujan tropika dan tanah mineral Antartika telah dijelaskan menggunakan berasaskan makmal kecilnya pengeraman eksperimen. Kekayaan bakteria, kuantiti dan komposisi dalam kedua-dua tanah tropika dan Antartika telah dipantau sehingga empat dan 12 minggu masing-masing di bawah tiga suhu yang berbeza setiap satu (Tropical : 25°C, 30°C, 35°C; Antartika :5°C, 10°C , 15°C). Untuk rawatan air, sampel tanah tropika tertakluk kepada dua tahap yang berbeza air (2 dan 5 ml) dan sampel tanah Antartika tertakluk kepada 0.5 ml samping air. Di samping itu, gen berfungsi (*nifH, amo-A, nirS, nirK, nosZ* dan *Chitinase GA*) telah ditentukan dengan menggunakan kuantitatif tindak balas rantai polymerase (Q-PCR). Faktor abiotik termasuk pH, kemasinan, kelembapan, nitrat, nitrit dan fosfat juga diukur. Menggunakan gabungan kaedah molekul *"terminal restriction fragment length polymorphism"* (T-RFLP) dan pemprosesan tinggi penjujukan, keputusan kami menunjukkan bahawa kedua-dua struktur dan komposisi tanah tropika masyarakat bakteria berbeza dengan ketara di seluruh rawatan, didorong terutamanya oleh peningkatan dalam kandungan relatif *Firmicutes* dengan peningkatan kandungan air suhu dan dan perubahan komposisi tertinggi diperhatikan di Minggu 2. Sebaliknya, hanya perbezaan halus dalam Antartika struktur masyarakat bakteria itu dikesan di seluruh suhu. Walau bagaimanapun, assemblages bakteria telah kuat berstruktur oleh tempoh pengeraman.

Sampel tanah Antartika dikuasai *Proteobacteri*a yang bertindak balas secara positif kepada peningkatan suhu. Distance-based (DISTLM) analisis berdasarkan jarak jauh menunjukkan bahawa pH, kekonduksian elektrik, nitrat, nitrit dan kandungan lembapan adalah parameter yang paling penting yang berkait rapat dengan komuniti bakteria tanah tropika. Sebaliknya, kandungan nitrat tanah adalah parameter tunggal dipilih untuk model DISTLM untuk tanah Antartika komuniti bakteria. Di samping itu, analisis kami gen berfungsi kuantitinya mendedahkan perkaitan yang signifikan antara tanah tropika masyarakat bakteria dengan gen nitrogen *(nifH)* dan gen denitrification *(nosZ).* Peningkatan seiring *nifH* salinan gen dengan suhu juga didapati di dalam tanah tropika. Untuk sampel tanah Antartika, gen *nosZ* dan *GA* menunjukkan korelasi yang paling tinggi kepada komuniti bakteria. Secara kolektif, penemuan ini menunjukkan bahawa perubahan dalam faktor-faktor alam sekitar perubahan dalam komposisi masyarakat bakteria yang boleh mengubah tanah faktor abiotik dan berfungsi gen banyak teraruh.

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LIST OF SYMBOLS

℃	: Degree celcius
µl	: microliter
<	: less than

LIST OF ABBREVIATIONS

bp	: Base pairs
CAP	: Canonical Analysis
DGGE	: Denaturing gradient gel electrophoresis
DISTLM	: Distance-based linear model
DNA	: Deoxyribonucleic acid
EDTA	: Ethylenediaminetetraacetic acid
HW	: High water
LW	: Low water
NA	: Undetected
ng	: Nanogram
NGS	: Next generation sequencing
PCR	: Polymerase chain reaction
PERMANOVA	: Permutational multivariate analysis of variance
rDNA	: ribosomal deoxyribonucleic acid
rRNA	: ribosomal ribonucleic acid
Taq	: *Thermus aquaticus*
TE	: Tris-EDTA
TGGE	: Temperature gradient gel electrophoresis
T-RFLP	: Terminal restriction fragment length polymerase
UV	: Ultraviolet
w/v	: weight per volume

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

The soil is a part of complex ecosystem and known as the primary reservoir of biodiversity (Jangid *et al.,* 2010). Among this biodiversity, soil microorganisms represent a considerable fraction and are highly diversified (Allison & Martiny, 2008; Torsvik *et al*., 1998). Bacteria are the most prevalent prokaryotic organisms in soil (Ramirez *et al*., 2014; Bates *et al*., 2013; Daniel, 2005) and it has been shown that each gram of soil harbors more than 10^8 bacterial cells. (Torsvik *et al*., 1996). Scientific evidence suggested that a gram of soil may consisted of 13103 to 13106 individual group of bacteria (Gans *et al*., 2005; Tringe *et al*., 2005; Torsvik & Øvreås, 2002). To date, 52 of bacteria phyla were identified globally (Rappe & Giovannoni, 2003) with *Acidobacteria* and *Proteobacteria* as the most common phyla found in various soils (Chodak *et al*., 2015; Janssen, 2006).

Bacteria have wider dispersal potential due to the large population size and therefore found in different environments (Fierer & Jackson, 2006; Fenchel & Finlay, 2004; Whitman *et al*., 1998). For instance, the bacterial community have also been identified in several extreme environments such as hot thermal springs, cold Antarctic and Arctic regions using different molecular techniques such as DGGE, T-RFLP, clone libraries, and next-generation sequencing (Chong *et al*., 2012; Deslippe *et al*., 2012; Yergeau *et al*., 2012; Chong *et al*., 2009; Lauber *et al*.,2009; Mannisto *et al*., 2009; Margesin, 2009; Wallenstein & Vilgalys, 2005). Bacteria are involved in many soil functions such as decomposition process, biogeochemical cycling and plant productivity (Li *et al*., 2014; Zhao *et al*., 2014; Fierer *et al*., 2012; Schneider *et al*., 2012). Plant growth promoting rhizobacteria which are known as PGPR bacteria, such as *Pseudomonas fluorescens* can increase the nutrient input to plants (Maurhofer *et al.,* 1998). Furthermore, soil bacteria are

able to produce extracellular enzymes to digest organic matters into carbon (C), nitrogen (N), and phosphorus (P) content, thus regulating the availability of nutrients in the soil. Due to such crucial roles of bacterial community to the functioning of wider related ecosystems (Aislabie & Deslippe, 2013; Dominati *et al*., 2010; Nannipieri *et al*., 2003), many bacterial taxa have been proposed as proxy indicator of soil disturbances (Rutgers *et al*., 2016; Silva *et al*., 2013).

Community diversity can be defined as the species richness and evenness in an ecosystem (Torsvik *et al*., 1998; Torsvik *et al*., 1996). In general, an increase in community unique may equate to greater community-level traits or functions. Indeed, a high level of diversity is considered an important factor as it begets ecosystem stability by acting as a genetic and functional reservoir that increases community resilience toward perturbations (DeAngelis *et al*., 2013; Bissett *et al*., 2007). Thus, loss of diversity has been identified as a major threat to soil ecosystems especially the loss of keystone species which would certainly affect the functional stability of the community (Singh *et al*., 2014; Griffiths & Philippot, 2013).

The stability of a community is often related to resistance (insensitivity of a population towards perturbation) and resilience (the rate of recovery and ability to return to pre-disturbance condition) (Griffiths & Philippot, 2013; Wertz *et al*., 2007; Griffiths *et al*., 2000; McNaughton, 1994). Besides, it has been postulated that a community with the higher level of diversity and functional redundancy is more resistance and resilience towards disturbances (Allison & Martiny, 2008; Wertz *et al.,* 2007). For instance, Fierer *et al*. (2003) found that the bacteria taxonomic diversities and richness from grassland were not affected by the drying-rewetting frequency and this is incongruent with the growth response of bacteria community obtained from Mediterranean pasture soil which demonstrated high

resistance and resilience to fire (Velasco *et al*., 2011). Further, it has been reported that the diversities and activities of denitrifier, nitrifiers and decomposers were remained unaffected under constant environmental states (Wertz *et al*., 2007; Griffiths *et al*., 2000). Together, these findings imply that resistance and resilience of a community are not solely owing to the diversity but influenced by other environmental factors.

1.2 Global Climate Change

Greenhouse gasses such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (NO_2) emitted via anthropogenic activities are able to absorb long-wave infrared radiation and therefore expected to increase the global temperature (Motavalli *et al.*, 2003). Recent reports from the International Panel on Climate Change (IPCC, 2007) forecasted that global temperature would increase by 0.3°C to by the year 2035. Warming had increased the evaporation rate, which resulted in alteration of rainfall events such as reduction in rainfall volumes in the Tropics (IPCC, 2007; Huntington, 2006; Seneviratne *et al*., 2006). As the climate continues to change, it becomes more necessary to predict the response of soil ecosystems to climate drivers (McMahon & Boucrot, 2011; Dillon *et al*., 2010). However, the majority of the current studies mainly focussed on higher organisms while relatively less emphasis was given to soil microorganisms (Bellard *et al*., 2012). The fact that soil microorganisms are highly sensitive to environmental factors suggested that changes in climatic-related stressors are able to alter the real diversity of populations via directional selection or compositional shifts which could subsequently affect the overall soil ecosystem functioning (Mokany & Ferrier, 2011; Botkin *et al.,* 2007). For instance, intensified rainfalls increased the amount of water flow into soils. Such conditions created periodic anaerobic zones in the soil that preferentially selects for taxa with lower oxygen demand (Hueso *et al*., 2012; Schimel *et al*., 2007). Thus, it is the critical to assess the impacts of

warming and moisture fluctuations on the soil bacterial community independently and in concert to further enhance our understanding on the ecosystem functions.

1.3 Why Tropical

Tropical regions are the major hot spot of biodiversity of the Earth system (Lewis, 2006). Besides, tropical forests covered up 15% of the Earth's land surface and they sustained for more than 2/3 of the world's biodiversity (Cavaleri *et al*., 2015). Also, tropical soil provides essential ecosystem services such as carbon storage and regulation of water level (Poorter *et al.,* 2015; Costanza *et al.,* 1997). The ecosystem contributes to the highest carbon dioxide efflux into the atmosphere (Tarnocai, 2009; Luo *et al*., 2001). Besides, tropical soil which is characterized by high nitrogen and clay content promote emissions of nitrous oxide via nitrification process. Therefore, this ecosystem plays crucial roles in both carbon and nitrogen cycling and act as an important regulator of climate change globally. Despite of their importance, tropical ecosystem is one the most understudied biomes in the world (Cavaleri *et al.,* 2015) particularly the knowledge of their responses to changes in environmental factors (e.g. temperature) is still lacking (Clark *et al*., 2013; Randerson, 2013; Lloyd & Farquhar, 2008; Lewis, 2006). It has also been hypothesized that microbial communities from diverse ecosystem are more resistance towards perturbations than those from simple ecosystems (Griffiths & Philippot, 2013). However, to our best knowledge, there are limited studies which elucidated the differences in microbial responses of tropical soil with other soil community from other regions.

1.4 Why Antarctica?

Antarctica is one of the most extreme environments on the Earth. It is characterized by low temperature, precipitations and nutrient availability, periodic freeze-thaw cycles and experience high solar radiations in summer (Severin *et al*., 2010). The harshness of Antarctic soil conditions which are inhospitable to many insects, mammalians and higher organisms (Heal & Block, 1987) have resulted in a simplified trophic and food chain with most of the biogeochemical cycles are solely driven by soil microorganisms. Therefore, Antarctic soil act as a perfect model to study the direct impact of climatic drivers (e.g. temperature) to the bacterial community and the consequences to ecosystem functioning. Besides this, human activities (e.g. research and tourism) in Antarctica are imposing significant changes to the soil microbial communities. For example, it was found that soils with high anthropogenic impact harbored lower bacterial diversity than the undisturbed sites (Chong *et al*., 2010; Chong *et al*., 2009; Saul *et al*., 2005). However, only a few studies have discussed the impacts of ongoing warming on the Antarctic soil bacterial community (Dennis *et al*., 2013; Yergeau *et al*., 2012; Rinnan *et al*., 2009). Numerous findings have reported that the Antarctic Peninsula is undergoing the largest global warming at around 0.56°C per decade (Steig *et al.,* 2009) and similar warming trends were also observed in other parts of Antarctica. Additionally, the average temperature of Antarctica has continuously increased at a rate of 0.1°C over the past 50 years. Since most of the Antarctic regions were covered with ice sheets, such warming had resulted in melting which further increases water and nutrient availability in soils (Wang *et al*., 2015; Steig *et al*., 2009). Apart from this, studies have demonstrated that such continued warming increases vegetation density and consequently changes the soil properties which have already been detected throughout the Antarctic Peninsula (Frenot *et al*., 2005; Smith *et al*., 1994). Thus, rapid and continuous warming might have profound consequences for the

Antarctic terrestrial ecosystems (Cowan *et al.,* 2011; Tin *et al.,* 2008) which would affect the structures, diversities and activities of soil bacterial community. Separately, the geographic isolation and evolutionary history of Antarctica may also lead to endemism (Makhalanyane et al., 2015; Fernandez-Carazo *et al*., 2011; Taton *et al*., 2003). A growing number of studies have indicated the presence of endemic taxa in Antarctic regions (Casamatta *et al*., 2005; Jungblut *et al*., 2005; Taton *et al*., 2003). For instance, some unique *Cyanobacteria* taxa and genera (e.g., *Psychrobacter*) have been identified in Antarctic ecosystem (Taton *et al.,* 2003). In a recent molecular approach (combination of T-RFLP and high-throughput sequencing), Lacap-Bugler *et al*. (2017) observed *Phormidium* as the most abundant cyanobacterial taxon in Antarctic. Besides, this phylum had been reported to govern the hypolithic communities from Antarctica (Wei *et al*., 2016; De los Ríos *et al*., 2014; Yung *et al*., 2014; Taton *et al*., 2003). The fundamental ecological role of this group in producing exopolymer matrix which provides cryoprotection and desiccation protection to other bacterial taxa is well known (De Los Ríos *et al*., 2014).

Additionally, it have been reported that the sustainability of certain heterotrophic bacterial taxa such as *Firmicutes*, *Proteobacteria* and *Bacteroidetes* in a particular niche in Antarctica is closely associated with the existence of autotrophs *Cyanobacteria* (Yung *et al*., 2014; Makhalanyane *et al.*, 2013; Chan *et al*., 2012; Aislabie *et al*., 2006). Consistently, the former group has been identified as primary producers in Victoria Land (Aislabie *et al*., 2006). Since endemic species are found small in numbers and highly vulnerable to extinction (Gaston *et al*., 2003), it is paramount importance to study the impacts of warming on the Antarctica soil bacterial community to recuperate biodiversity and ecosystem services in future.

1.5 Research Objectives

The primary objectives of this study were;

1. To assess and compare the effects of temperature and moisture content on the bacterial assemblage patterns and diversity in soils collected from tropical and Antarctic regions
2. To evaluate the effect of temperature and water content on the soil bacterial functional gene stability.
3. To identify and correlate some environmental drivers with changes in bacterial structures in the tropical and Antarctic soil.

CHAPTER 2: LITERATURE REVIEW

2.1 Tropical biodiversity

The Tropics are divided into three major regions: The Neotropical, Paleotropical and Southeast Asia. This region covered an area of 1.6 million square miles and ranged from Brunei to Vietnam. Tropical soils are considered to harbour various and a high number of rare bacteria species (Giller, 1996). A wide variety of animal and plant communities ranging from highly specialized to generalized species resides in this region, hence lead to extensive research of communities (Kurten, 2013; Ollerton *et al*., 2011; Wunderle Jr, 1997; Hölldobler & Wilson, 1990; Myers, 1988). It was found that organisms at higher trophic positions were also impacted due to environmental disturbances which subsequently change the distribution and composition of organisms at the bottom level in the food web structures (Freedman *et al.,* 2014). This phenomenon is known as top-down effects (Hairston *et al*., 1960).

Figure 2:1: Map indicating Southeast Asia of the tropical region. Retrieved from (http://www.merrytravelasia.com/admin/Administrator/images/users_images/map2.gift)

2.2 Antarctic biodiversity

Though Antarctica is the fifth largest continent in the world, the contact with human occurred fairly recently (Turner *et al*., 1997). In general, Antarctic is divided into three major geographic regions: East Antarctica, West Antarctica and the Antarctic Peninsula (Hale, 2014) (Figure 2.2). The thermal and climatic conditions are different in each region (Selkirk & Skotnicki, 2007). For instance, soils from Ross Sea regions were exposed to high climate variation in which soils along the coastal areas tend to receive a higher volume of precipitation in comparison with inland (Aislabie *et al*., 2009; Bockheim & McLeod, 2008).

Besides, soils in the Antarctic Peninsula have recorded higher level of moisture content contributed by evaporation rates and this condition leads to rapid microbial growth (Campbell & Claridge, 1987). Although soils in the regions are quite heterogeneous, most soils in Antarctica are classified as Gelisols (Bockheim & McLeod, 2006) and the formation of soils is dominated by environmental factors (e.g. temperature; moisture) than chemical processes (Campbell & Claridge, 1987). Gelisols are known as permafrost-affected soils and contained low organic matters (Ugolini & Bockheim, 2008). In contrast, high level of nitrogen in the form ammonium and nitrate were detected in soils from maritime Antarctic due to the presence of native marine vertebrates (e.g. penguins and elephant seals) and colonization of birds (Yergeau *et al*., 2012). Similarly, high levels of organic matters and occurrence of Podzols were reported in Casey Stations, East Antarctica (Ugolini & Bockheim, 2008). As a result, some soil-

borne organisms or invertebrates such as Protozoa, nematodes and Acari were observed in these areas (Velasco-Castrillon *et al.,* 2014; Nkem *et al.,* 2005). Antarctica is dominated by lower plants such as mosses and lichens (Bubach *et al*., 2015; Azeem *et al*., 2013). In maritime Antarctica, there are only two vascular plants, namely *Deschampsia Antarctica* (Antarctic hair grass) and *Colobantus quitensis* (Antarctic pearlwort) (Teixeira *et al.,* 2010). Though the rate of nitrogen mineralization is slow in maritime Antarctic, the ability of these vascular plants to access nitrogen via their roots ensures their survivability in nutrient-limited conditions (Hill *et al*., 2011). Interestingly, several studies on Antarctic soils revealed that plant coverage and composition influenced the diversity of soil microorganisms (Bolter *et al*., 1997). Specifically, Bokhorst *et al*. (2007) identified different rates of cellulose degradation by microbial decomposers under various types of plant species thereby proves that types of the plant could influence metabolic activities of soil microorganisms in Antarctica (Yergeau *et al.,* 2007). On the other hand, Teixeira *et al*. (2010) found that the bacterial diversity in rhizosphere soil from King George Island was unaffected by types of vegetation. Such discrepancies among studies could be possibly due to the non-homogenous distribution of vegetation and soils in Antarctic. Further, it has been reported that variation in vegetation parameters such as density and coverage is strongly associated with warming effects (Yergeau & Kowalchuk, 2008; Vishniac, 1993) and such changes subsequently could altered the composition of bacterial community in individual sites of Antarctic regions (Walker *et al*., 2008). Hence, further research evaluating the factors (e.g. warming) that structure bacterial community in the Antarctic ecosystem is required.

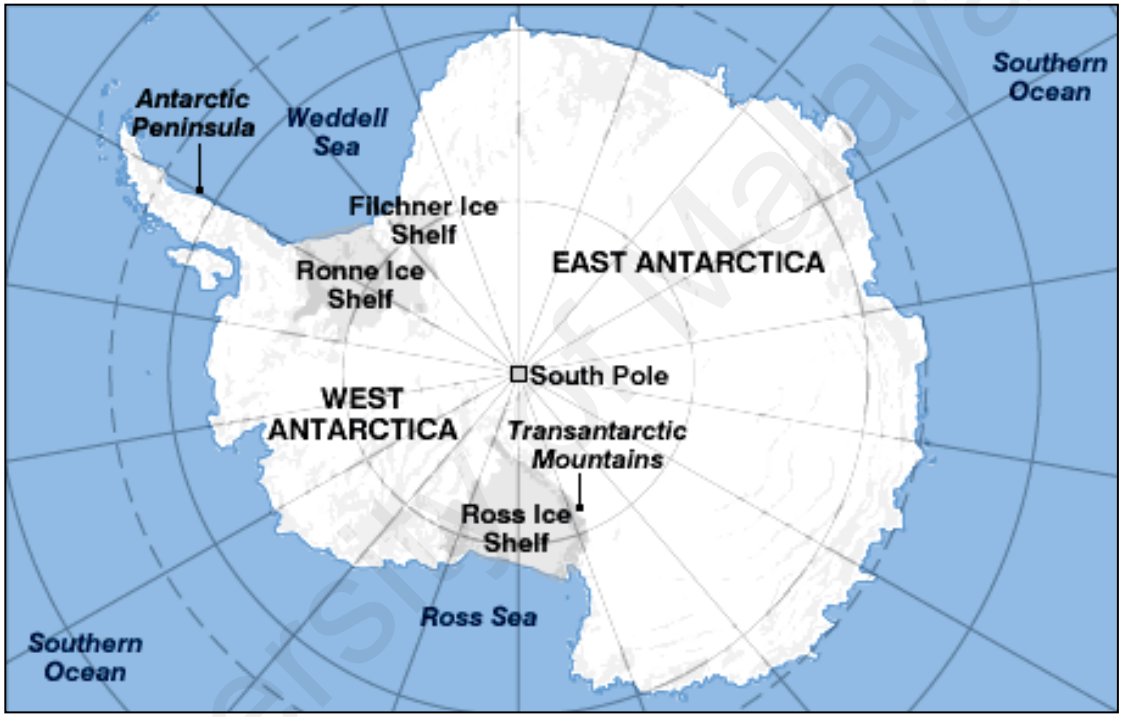


Figure 2.2: Map indicating three major regions in Antarctica: East, West and Antarctic Peninsula. Retrieved from Lima Project.

2.3 Tropical and Antarctic soil bacterial community

Bacterial community of both tropical and Antarctic soils are distributed heterogeneously (Chau *et al*., 2011; Aislabie *et al*., 2009). Many soil investigations have shown the existence of distinct sets of bacterial taxa within a single type of ecosystem (Nemergut *et al.,* 2011; Martiny *et al.,* 2006). For example, Ghosh *et al*. (2010) reported the dominance of *Proteobacteria* in tropical mangrove sediments while (Garcia-Pichel & Pringault, 2001) identified widespread of *Cyanobacteria* in tropical desert soils. Similar patterns were also observed for Antarctic soils. *Actinobacteria* was the dominant phylum in Dry Valleys while *Bacteroidetes* was abundant in Victoria Land (Aislabie *et al*., 2006; Smith *et al*., 2006). In parallel, high abundance of *Cyanobacteria* was detected in most Antarctic mineral soils (Brinkmann *et al*., 2007; Smith *et al*., 2006). Such observed trends have been linked to a range of soil-physiochemical factors such as pH (Fierer & Jackson, 2006), the amount of organic matters (Vishniac, 1993), redox potential (Braker *et al*., 2001) and vegetation types (Bolter *et al.,* 1997).

Nevertheless, several major bacterial phyla such as *Actinobacteria* and *Proteobacteria* are found to be prevalence in both continents (Wang *et al*., 2015; Aislabie *et al*., 2006). One possible reason for cosmopolitan distribution of these phyla is that bacteria are distributed globally via human activities and several vectors such as water, wind and animals (Pearce *et al*., 2009; Vanormelingen *et al*., 2007; Griffin *et al*., 2002). For instance, identification of *Escherichia coli* and *Staphylococcus epidermis* in human-impacted sites in Antarctica (Tow & Cowan, 2005; Sjöling & Cowan, 2000) and dispersal of several bacterial phyla such as *Actinobacteria* and *Sphingobacteria* through windblown across tropical regions (Yamaguchi *et al.,* 2014) therefore abetted the

previous statement. It may be noteworthy that the tropics are environmentally different from Antarctica. Unlike the former, Antarctica is considered one of the most isolated and hostile environments on Earth, resulting in low rates of colonization by external bacteria as they tend to lose viability in the long distance transport (Pearce *et al.,* 2016; Pearce *et al.,* 2009). Therefore, certain bacterial phyla such as *Acidobacteria* and *Firmicutes* were reported to be more abundant in tropical soils as compared to Antarctic soil samples (DeAngelis *et al*., 2011; Otsuka *et al*., 2008).

Based on the culture-independent molecular techniques, higher bacterial diversity was recorded in the tropical soils as opposed to temperate and Antarctic soils (Lyngwi *et al*., 2013; Fierer & Jackson, 2006). Besides, the Shannon diversity index (H') of tropical soils were reported to be much higher (H' ranging from 3 to 7) as compared to Antarctic soils (H' ranging from 1 to 4) (Kim *et al*., 2013; Aislabie *et al*., 2006; Smith *et al*., 2006; Saul *et al*., 2005). The low diversity could be due to adverse environmental conditions in Antarctica which act as a strong selection factor in reducing the soil biodiversity (Nielsen & Wall, 2013; Smith *et al.,* 1992), thus resulting in species-poor or depauperate communities (Kennedy *et al*., 2004).

2.4 Environmental Factors

A large body of evidence has documented the importance of environmental factors such as temperature, moisture, salinity and pH in structuring the soil bacterial community (Braker *et al*, 2010; Wallenstein *et al*., 2006). Although several studies have described soil pH as the best predictor of bacterial community composition and diversity across various soil ecosystems (Tripathi *et al.,* 2013; Lauber *et al.,* 2009; Wakelin *et al.,* 2008), temperature and moisture content are also known as key determinants of the

bacterial community composition and diversity globally. It should be noted that temperature and moisture may have interactive effects on the bacterial community (Classen *et al.,* 2015). For instance, increase in temperature can reduce the soil water availability to the underlying microbial communities (Zhang *et al*., 2013). Therefore, temperature and water content are known to be proximal factors that could result in significant compositional shifts in bacterial community (Brockett *et al*., 2012).

2.4.1 Temperature

Generally, microbes are classified according to their growth response at different temperatures. For instance, psychrophiles microorganisms are able to grow at temperature below than 15°C, mesophiles are able to survive at moderate temperatures (20°C to 40°C) while thermophiles are able to grow at temperatures above than 50°C (Krishnan *et al*., 2011; Feller & Gerday, 2003; Norris *et al*., 2002). Though microbes are classified according to their growth response at different temperatures, metabolic activities such as exoenzyme production, protein synthesis and membrane permeability (De *et al*., 1997; Feller *et al*., 1994) are altered with the increase of temperature. Therefore, it has been proposed that the use of growth rate to define optimum growth temperature is inappropriate (Feller & Gerday, 2003). The authors have suggested terms such as stenothermal and eurythermal to classify organisms that grow in a narrow and wide range of temperatures respectively. For instance, obligate psychrophiles are regarded as stenothermal psychrophiles while facultative psychrophiles are regarded as eurythermal psychrophiles. Such classification, therefore, suggests that microorganisms with a wide range of growth temperatures are more abundant in cold-environments as opposed to microorganisms with the narrow range of temperatures. Therefore, at given temperature gradients, the activity of eurythermal enzymes (e.g. facultative

psychrophiles) is less modified than stenothermal enzymes (e.g. obligate psychrophiles) (Cipolla *et al*., 2012).

The Arrhenius equation used to describe the temperature relationships with the rate of reaction is alternatively known as enzyme kinetics (Wallenstein *et al.,* 2010). The Arrhenius theory: k= Ae-E/ (R(t + 273.15)) where k is the rate constant, A is the pre-exponential factor, Ea is the activation energy, R is the gas constant, and the T is the Kelvin (K) temperature can also be expressed in the form of log (log k = (- Ea/ 2.303 RT) + log A) which allows the determination of the activation energy throughout the reaction (Wallenstein *et al*., 2010). It has been proposed that the rate of enzymatic reaction will increase 2-fold with every 10°C rise (Wu *et al*., 2015; Hamdi *et al*., 2013) thereby suggesting that enzymatic reactions are temperature-dependent and this could affect the metabolism of the bacterial community. For instance, the cold-adapted enzyme is having more flexible active site due to the weak intermolecular forces that result in low activation energy (Wallenstein *et al*., 2010). Such adaptation allows the enzyme to catalyze reactions at lower temperatures (Gerday *et al*., 1997). Indeed, cold-adapted bacteria are able to produce enzymes that functionally efficient at lower temperatures (Feller & Gerday, 2003). Conversely, heat-adapted enzymes possess rigid active site and this promotes thermal stability. It has been proposed that the activity of heat-adapted enzymes increase drastically in response to increase in temperature thereby explaining the adaptation of thermophiles at high temperatures (Cowan *et al*., 2014). This could explained the adaptation of thermophilic bacteria in extreme environments (Cowan *et al*., 2014).

Temperature fluctuation can induce bacteria to modify their membrane fatty acid compositions (Russell & Fukunaga, 1990) to maintain cellular integrity and functional metabolisms (Atlas & Bartha, 1993) and therefore have potential to cause changes in their composition and functional traits frequently used (Hartley *et al*., 2008; Norris *et al*., 2002). Recent studies have demonstrated the influence of warming treatments on the size, diversity, and composition of the microbial community (Newsham *et al*., 2016; Wu *et al*., 2015; Dennis *et al*., 2012). For example, several studies employing different assessment approaches detected the dominance of Actinobacteria in response to warming treatments (Wu *et al*., 2015; Frey *et al*., 2008). Such warming-induced compositional shift is associated with the metabolic activity (e.g.enzymatic activity and respiration rate) of survived population (Laucidina *et al*., 2015; Dennis *et al*., 2013; Zogg *et al*., 1997). For instance, temperate soil incubation at 50°C for 10 years led to a significant compositional shift that increases the abundance of Actinobacteria and Bacteriodetes and reduces other phyla such as Acidobacteria and Proteobacteria (Riah-Anglet *et al*., 2015). The shift was also accompanied by a reduction in enzymatic activities (e.g. cellulase and β-glucosidase) and microbial biomass. Therefore, it is essential to study how resilient would tropical and Antarctic soil systems be to external perturbations and whether the soil systems harbour populations that could adapt to variation in environmental drivers.

It was proposed that high temperature promotes cell degradation that could reduce microbial biomass (Riah-Anglet *et al.,* 2015) and subsequently affects the production of enzyme molecules (Wallenstein *et al*., 2012; Allison *et al*., 2005). For example, warming causes conformational changes in catabolic enzymes that suppress the catalytic rate (Hochachka & Somero, 2002) and efficiency of carbon utilization (Steinweg, 2008). At low temperature (6°C), Karhu *et al*. (2014) observed that the rate of soil

microbial respiration declined in a range of cooled soils (arable, grassland, deciduous and evergreen broadleaf forest, coniferous forest and heath) thereby reflecting the incompetence of microbial community to degrade soil organic matters at low temperatures (Auffret *et al*., 2016).

However, the increase in temperature is beneficial to microbial life in the cold environments such as Antarctica. Yergeau *et al*. (2008) noted that the enzymatic activity (e.g. laccase and cellulase) from maritime Antarctic increases with warming particularly at 15°C. Similarly, the activity of oxidative (e.g. phenol oxidase) and exoenzymes that involve in the decomposition of phenolic component and organic carbon respectively was observed to increase with warming thereby increases the nutrient content (e.g. carbon) in soils from colder regions (Fraser *et al*., 2013; Li *et al*., 2012; Wallenstein *et al*., 2011). It was reported that warming promotes niche selection (Zhou *et al*., 2012; Schimel *et al*., 2007) that increases specialists with adaptative traits (e.g. nutrient utilization) to changes in soil conditions (Riah-Anglet *et al*., 2015; Xiong *et al*., 2014c). For instance, warming (open top chamber, OTC) of maritime Antarctic soils increases the ratio of Alphaproteobacteria to Acidobacteria (Yergeau *et al*., 2011). Such shifts are due to increase in soil carbon content in response to warming treatment which preferentially select for taxa (e.g. Alphaproteobacteria) that are able to metabolize the available resources (e.g.carbon) (Xiong *et al*., 2014; Fierer *et al*., 2007). Nevertheless, Sun *et al*. (2014) reported that the combined effect of warming and substrate addition (e.g. glucose and carbon) on the rate of soil microbial respiration and nitrogen mineralization from maritime Antarctica was greater than warming alone. Such divergent response explaining the effect of temperature on the bacterial community is dependent on the availability of substrate that may constrain the metabolic activity (e.g.

respiration rates and enzymatic activities), consequently accelerating physiological responses upon the nutrient addition (Dennis *et al*., 2013; Sparrow *et al*., 2011).

2.4.2 Moisture Content

The availability of water in soils is depending on the water pulses (e.g. rainfalls) and matric potential (Rodriguez-Iturbe & Porporato, 2004; Tyree, 2003). Water molecules in soil ecosystems are connected to soil particles via adhesive and cohesive forces that led to the formation of matric potential which determines water flow in unsaturated soil (Manzoni *et al.,* 2014). As soil matric potentials become more negative, soil microbes are required to regulate osmotic pressure in order to maintain cell functionality (Manzoni *et al*., 2014; Schimel *et al*., 2007). Besides, it was found that when soil matric potential is more than -0.01 MPa, the rate of nitrification in soils declined due to oxygen limitation (Stark & Firestone, 1995). Moisture content is an important factor in controlling soil properties (e.g. oxygen content) that may affect the dynamic of soil microbial activities and the associated ecological processes (DeAngelis *et al*., 2010). Consistently, microbial activities were reported to be optimum at moisture levels ranging from 50% to 70% of water holding capacity (Franzluebbers, 1999). Therefore, in this study, the soil water content was measured and correlated with the observed pattern of the bacterial community structures.

The water content in tropical soils had been reported to be in the range of 2% to 40 % (Cardenas *et al*., 1993). Most of the tropical soils are classified as Oxisols and Ultisols which contain cations (Tomasella & Hodnett, 2004). The leaching of these cations during rainfalls results in higher concentration of hydrogen ions that consequently decreases the pH of the soil and contributes to an acidic nature (Tomasella

& Hodnett, 2004). The acidity of tropical soils further increases the binding of aluminium oxides that enhance the stability of micro-aggregates and such features create a loamy texture (Tomasella & Hodnett, 2004).

The water content in Antarctic displayed a strong gradient with soils along the coastal regions have recorded higher moisture content as opposed to inland soils (Bockheim & McLeod, 2008) attributed to the differences in volume of precipitation received in each region. Another source of moisture in Antarctic soils is the melting of snowfields (Barrett *et al*., 2006). As a result of low precipitation, the humidity and availability of liquid water are much lower in Antarctic soil (Hopkins *et al*., 2008; Campbell & Claridge, 1987) as compared to tropical soils. Nevertheless, the presence of permafrost layer within Antarctic soil subsurface leads to occurrence of wetted zone that contributes to the availability of liquid water content in mineral soils even at low temperatures (Cary *et al*., 2010; Burkins *et al*., 2001). Besides, the rise of soil temperature above the freezing point was found to increase the availability of liquid water content in Antarctic soils (Barrett *et al*., 2008). As soil temperature in Casey areas has been reported to exceed 5°C (Petz, 1997), it could be possible to measure the liquid water content in our Antarctic microcosms. Therefore, in this study, the standard gravimetric method was used to determine the average liquid water content in tropical and Antarctic soil samples. Besides, this technique is inexpensive as compared to other techniques such as gamma ray attenuation, hence more suitable to measure the water content in our large number of replicates. A wide range of soil types is reported across the Antarctic continent; dry mineral soils occurring on glacial till, ornithogenic soils in coastal zones and desert soils around the Mc Murdo Dry Valleys. Though the soils had beenreported to be distinct in term of nutrient and water content (Niederberger *et al*., 2008; Cowan & Tow, 2004), Antarctic soils are generally coarse-grained sands

(Campbell & Claridge, 1987) and less acidic (pH>5) (Wang *et al*., 2015; Chong *et al*., 2009; Niederberger *et al*., 2008). However, it was observed that penguin-impacted soils are more acidic and exhibited a high value of carbon and nitrogen as compared to other soil in Antarctica (Aislabie *et al*., 2009; Chong *et al*., 2009; Barrett *et al*., 2006a). Apart from matric potential, the type of soil is one of the important factors in determining water availability (Stark & Firestone, 1995). For instance, at given water pressure, coarse-textured soils such as sand have lower water content as opposed to fine-textured soils (Stark & Firestone, 1995). This therefore explained the significant water-holding potential within micro-aggregates in tropical soils (loamy texture) (Tomasella & Hodnett, 2004). Due to sandy texture and low clay content in Antarctic soils, the soil generally exhibited low water-holding capacity.

Soil bacterial community that frequently experienced large temperature and moisture fluctuations like low water availability (Fierer & Jackson, 2006) and freeze-thaw cycles (Stres *et al*., 2008; Schimel *et al*., 2007) on the other hand are less responsive to changes in moisture regimes (Evans & Wallenstein, 2011) as compared to those from stable environmental conditions (Waldrop & Firestone, 2006). For instance, a comparison study of bacterial community at three different sites in Alexander Island, Antarctica revealed that the bacterial diversity or community structure was similar among these sites regardless of variation in the soil moisture content (Newsham *et al*., 2010). Using Illumina-based amplicon sequencing, Armstrong *et al*. (2016) found that the taxonomic profile of bacterial community from Namib Desert soils subjected to precipitation events repeatedly becomes more similar with pre-rainfalls community structure within 30 days of time period. Hence, similarities in community diversity, structures and composition among sites (e.g. Antarctica) or after treatments (e.g. the

Namib Desert) may indicate the development of community resistance towards disturbances (Allison & Martiny, 2008).

2.5 Molecular Methods

Early studies of the soil bacterial community were hampered by the availability and accessibility of appropriate methods. Traditionally, bacterial community was characterized by using the culture-dependent approach which involves the isolation of microorganisms based on their chemotaxonomic and biochemical characteristics (Coleman & Whitman, 2005). However, based on this method, the actual diversity of prokaryotes remains unexplored as only 1% of the bacterial members in soil is cultivable (Janssen, 2006; Kirk *et al*., 2004; Schoenborn *et al*., 2004). The "great plate anomaly" is usually associated with the difficulty in replicating the actual growth condition in culture media (Vartoukian *et al*., 2010; Kopke *et al*., 2005). To circumvent such barriers, many culture-independent or molecular methods have been used to identify and examine complex soil bacterial community (Borneman & Triplett, 1997; Rheims *et al*., 1996; Liesack & Stackebrandt, 1992). Molecular methods directly interpret the phylogenetic information of targeted communities based on the extraction, amplification, and identification of nucleic acids, fatty acids and proteins that are specific to individual microorganism groups (Rastogi & Sani, 2011).

2.5.1 16S rDNA-based on molecular methods

The PCR-based 16S DNA has been regarded as one of the core methods employed to study soil bacterial diversities by microbiologists due to some reasons. Firstly, the identification of bacterial communities based on 16S DNA gene sequencing

needs lesser time because this technique eliminates cultivation of microorganisms thereby has resulted in the direct and more accurate estimation (Amann *et al*., 1995; Muyzer *et al*., 1993; Ward *et al*., 1990). Secondly, in comparison to cultivation methods, this technique is sensitive and requires a smaller quantity of starting materials (Kalle *et al*., 2014) .

16S DNA is a highly conserved gene in prokaryotes (Rappe & Giovannoni, 2003). This feature allows universal PCR primers or hybridization probes to be designed for various taxa (Head *et al*., 1998). Apart from this, the presence of variable regions which have unique sequences from each other also permits taxonomic identification of bacterial phyla (Vetrovsky & Baldrian, 2013). The availability of 16S DNA gene database for comparison studies makes it as the "gold standard" choice in microbial ecology studies. It have been shown that 16S DNA genes was able to recover more than 90% of microorganisms (Das *et al*., 2014) and these findings further enhanced our understanding about prokaryotes organisms that inhabit in soils and the roles in maintaining the ecosystem stability.

There are several pitfalls of 16S DNA genes studies despite the advantages. For example, incomplete lysis of nucleic acids could result in underestimation of microbial richness (Kirk *et al.,* 2004). Besides, the number of copies of 16S DNA genes varies from 1 to 15 or more copies (Vetrovsky & Baldrian, 2013). Other barrier of the molecular method is the bias associated with PCR amplifications such as contamination of DNA templates by inhibitors like humic acids, differences in primer affinities and formation of primer dimers (Kirk *et al.,* 2004). Nevertheless, as compared to cultivation, molecular-based method still generates vital information about the bacterial community. Further, such limitations can be overcome with the utilization of appropriate approaches

and kits. For instance, the contamination of humic acids can be eliminated by using soil extraction kit which has proven to increase the efficiency of DNA yielding and recovery (Maarit Niemi *et al*., 2001).

2.5.2 Terminal restriction fragment length polymorphism (T-RFLP)

T-RFLP is a genetic fingerprinting technique used to study the structure and diversity of microbial community without the formation of clone libraries (Schutte *et al*., 2008; Ranjard *et al*., 2000). In this approach, the PCR primers are tagged with fluorescent dyes such as 6-HEX (4, 7, 2′, 4′, 5′, 7′-hexachloro-6-carboxyfluorescein) and 6-FAM (phosphoramidite fluorochrome 5-carboxyfluorescein) (Kirk *et al.,* 2004). The resulting fluorescently labeled amplicons are then digested with restriction enzymes (Liu *et al*., 1997) to produce terminal restriction fragments (T-RFs). Also, in a comparison study of 18 different types of restriction enzymes revealed that *BstU1*, *DdeI*, *Sau961* and *Msp1* were able to differentiate most of the specific populations from complex communities (Engebretson & Moyer, 2003). The T-RFs are separated by capillary electrophoresis using an automated sequence analyzer which subsequently generates electropherograms (Fakruddin & Mannan, 2013). Automatisation permits analysis of a vast number of samples within a short span of time, therefore, proves high reproducibility and sensitivity of this method in comparison with other techniques such as DGGE, TGGE and cloning (Torsvik & Øvreås, 2002). Each T-RF is classified as the operational taxonomic unit (OTU) (Fakruddin & Mannan, 2013; Ranjard *et al.,* 2000). The community diversity is then determined based on the size, number and heights of the resulting T-RFs fragments (Culman *et al*., 2009). Like any other method, T-RFLP analysis has its pitfalls, therefore need to be handled with care. For instance, Dunbar *et al*. (2006) used the smallest peak height as a base to standardize the total peak heights

generated in electropherogram profiles to create a correction factor for each profile. Therefore, bias introduced due to variation in height sizes will be reduced. Recently, several techniques such as fixed threshold, the proportional threshold (Dunbar *et al.,* 2006) and statistical threshold (Abdo *et al.,* 2006) have been developed to determine this baseline. Despite the limitation, T-RFLP is still considered a useful approach in determining community structure (Fierer & Jackson, 2006).

2.5.3 Quantitative Polymerase Chain Reaction (Q-PCR)

Q-PCR works in the same manner as conventional PCR, however, the former involves quantification and detection of amplicons in each PCR cycles rather than end-point detection. Therefore, Q-PCR is also known as real-time PCR. In traditional PCR, the products formed do not inform the actual quantity of sequences present due to bias introduced during polymerization (Su *et al*., 2012). The SYBR green and TaqMan probe are two different fluorescence chemistries are commonly used in Q-PCR (Dupouey *et al*., 2014; Tajadini *et al*., 2014). SYBR green binds to DNA strands which generate fluorescence signals. Hence, the intensity of fluorescence signals is directly proportional to PCR amplicons produced (Valasek & Repa, 2005). As SYBR Green binds to DNA, optimization of specific PCR primers is important to ensure efficient amplification. Even though these barriers are able to be mitigated with the usage of TaqMan probe which binds specifically to the sequence of interest, this probe is costly and requires the existence of conserved site within the sequence (Smith & Osborn, 2009). Dissociation curve analysis can further increase the accuracy and specificity of the results. This curve consists of four phases: background noise, exponential amplification, linear amplification and a plateau stage (Smith & Osborn, 2009). Absolute quantification of the targeted gene is obtained at the exponential stage as product formations are highest

at this phases (Sharma, 2006). The output of several different peaks during this analysis indicates productions of non-specific PCR amplicons (Valasek & Repa, 2005). This feature eliminates the needs of post-PCR procedures thus reduce the possibility of cross-contamination of amplicons (Heid *et al*., 1996).

The Q-PCR assay is mostly exploited to study the bacterial community in soils (DeAngelis *et al*., 2015; Wan *et al*., 2014) and interestingly some studies have used this approach to investigate the functional genes of bacterial community from polar regions (Yergeau *et al*., 2007). Besides, genes involved in ammonia oxidation (Li *et al*., 2012; Prosser & Nicol, 2012; Zhang *et al*., 2011; Mincer *et al*., 2007), nitrate reduction and denitrification (Chen *et al*., 2015; Ligi *et al*., 2014; Sanford *et al*., 2012; Smith *et al*., 2006) have been quantified in several literatures. Q-PCR assay which quantifies the functional genes abundance that mediates biogeochemical processes further enhanced our knowledge in understanding the direct relationship between variation in functional gene expression and changes in composition, rates and activity of microbial communities. Real-time detection of taxonomic markers expression which is not afforded by any other conventional methods proves that Q-PCR is a useful molecular tool to analyze microbial community from the complex environment (Pereyra *et al*., 2010; Smith & Osborn, 2009).

2.6 Next-Generation Sequencing (NGS)

First-generation automated Sanger sequencing was introduced by Edward Sanger in 1975 and adopted as the standard approach in microbial ecology studies (Sanger *et al*., 1977). However, this technique is time-consuming, expensive and such limitations allowed identification of several clones. Emergence of next-generation sequencing

(NGS) technologies has provided a new window into bacterial diversity and composition from various environments (Wang *et al*., 2015; Han *et al*., 2013; Roesch *et al*., 2007; Sogin *et al*., 2006) as these approaches are able to detect millions of DNA sequences at one time (Shokralla *et al*., 2012; Metzker, 2010). NGS technologies are also known as 'high-throughput,' 'ultra-deep' or 'massive parallel' sequencing (Marguerat *et al.,* 2008). Besides, the ability of NGS methods in generating the rapid, economical, reproducible and unprecedented scale of outputs ease the analysing of multiplex environmental samples (Caporaso *et al.,* 2012; Roh *et al.,* 2010). For instance, the application of these technologies has proved that the diversity and population of bacterial community that inhabit extreme environments such as Antarctic soils are much greater than reported previously (Wang *et al*., 2015; Tiao *et al*., 2012; Teixeira *et al*., 2010; Yergeau *et al*., 2007). The advance of high-throughput sequencing technology permits whole-genome sequencing of bacterial strains in a matter of days (Chun & Rainey, 2014) thereby may further sharpen our understanding of bacterial compositions in soil in response to changes in environmental drivers (Zumsteg *et al*., 2013; Brockett *et al*., 2012; Naether *et al*., 2012; Zhang *et al*., 2011).

The high sensitivity of NGS platforms in detecting even small shifts in community structure due to environmental stressors (Fierer *et al*., 2007; Leininger *et al*., 2006) therefore boost the utilization of NGS methods. Less significant shifts are difficult to be identified with traditional molecular techniques (e.g., Sanger sequencing) (Xu *et al*., 2013; Sogin *et al*., 2006). Hence, it is not surprising that extensive usage of NGS approaches in comparison to traditional techniques lately (Ledford, 2008). Besides, these technologies eliminate the need of cultivation and thus reduce the associated bias (Royo *et al*., 2007). NGS approaches can be classified into two different categories. The first type is PCR-oriented approaches while the second is based on the single molecule

sequencing (SMS) (Shokralla *et al.,* 2012). Although it was reported that NGS platforms produced shorter reads (max~ 600 bp), studies have also shown that amplicons as small as 100 ~ 150 bp can resolve and provide accurate identification of individual taxa (Liu *et al*., 2013; Caporaso *et al*., 2012; Hao & Chen, 2012). Besides, the use of barcoded primers to target hypervariable regions of 16S rDNA increases sample throughput ands analyze of multiple samples in a single flow cell (Xu *et al*., 2013; Lauber *et al*., 2009; Anderson *et al*., 2008).

Several studies have utilized Illumina and Roche 454 platforms to characterize bacterial communities from various environments (Wu *et al*., 2015; Hutalle-Schmelzer & Grossart, 2009; Jones *et al*., 2009). For instance, Roesch *et al*. (2007) employed 454 Roche platform for the first time to study bacterial community from Brazilian forest soils and found a significant number of bacterial 16S rRNA sequences from this region. Further, this platform was used to evaluate the effect of warming on the soil bacterial community from temperate steppe (Zhang *et al*., 2013) and also to study bacterial community in soils over spatial and temporal scales (Mao *et al*., 2011). Recent studies have extensively used Illumina platform to assess the effect of warming on the ammonia-oxidizing prokaryotic communities from Antarctic soils (Han *et al.,* 2013), to study impact of logging and land uses on the soil bacterial community composition (Lee-Cruz *et al.,* 2013) and to explore bacterial diversity from Eastern Himalayas (De Mandal *et al*., 2015). However, only limited number of studies have utilized both of these approaches simultaneously to analyze and compare bacterial community composition (Liu *et al*., 2015; Sinclair *et al*., 2015).

2.6.1 Illumina Sequencing

Although Illumina works in similar concept (sequencing by synthesis) as pyrosequencing, this system employed reversible termination chemistry of nucleotide (Bentley *et al*., 2008; Turcatti *et al*., 2008). To date, there are four different Illumina sequencers available in the market: Hi Seq 2500, Hi Seq 2000, Genome Analyzer IIx and Miseq platform. In this study, MiSeq platform was adopted to snapshots the bacteria taxa present in the tropical soil. This platform was introduced recently with a total throughput of 1.5-2 Gb per run (Shokralla *et al.,* 2012).

The Illumina sequencing involves ligation of DNA amplicons to specific adapters on both ends (Mardis, 2008) which covalently attached to the flow cell of the microfluidic cluster station. This reaction is followed by bridge amplification to form groups which contain several thousand of amplified DNA fragments (Bentley *et al*., 2008; Fedurco *et al*., 2006). The flow cell is then placed in a sequencer and each cluster is provided with a polymerase and four differentially labeled fluorescent nucleotides that have their 3'-OH chemically inactivated. This is to ensure that single base incorporation at one time and followed by imaging step to identify the incorporated nucleotide (McElhoe *et al.*, 2014; Mardis, 2008). The chemical deblocking step allows insertion of next nucleotide is thereby enabling the extension of the sequence. The end outputs of each cluster is computed and filtered to discard poor quality reads (Shendure & Ji, 2008).

2.6.2 Barcoded Pyrosequencing

The 454 Genome Sequencing operated based on the sequencing-by-synthesis concept to produce large DNA sequence reads (Liu *et al*., 2013; Shokralla *et al*., 2012). Each nucleotide incorporations result in the release of a pyrophosphate molecule which serves as a substrate that triggers downstream enzymatic reactions (Roh *et al.,* 2010). This produce light signals and the intensity is directly proportional to nucleotide incorporation. Therefore, this approach is known as 454 pyrosequencing.

Pyrosequencing data accession is based on the detection of the light signal by a camera (Bona *et al*., 2015; Roh *et al*., 2010). An enzymatic reaction begins with attachment of DNA fragments to the oligonucleotides which are immobilized onto beads (Margulies *et al.,* 2005). These fragments are then amplified in an oil-water emulsion generating billions of identical copies (Dressman *et al*., 2003). This step is then followed by an enrichment step in which beads without amplification are removed. The successful beads are annealed to primer and arrayed into a picotiter plate (PTP) containing more than one million wells. Finally, this PTP is sequenced by 454 GS pyrosequencing instrument (Shokralla *et al.,* 2012). The most outstanding feature of Roche technology is the sequence length its offers. As compared to any other NGS platforms, this platform provides the longest read length (Egan *et al*., 2012; Tamaki *et al*., 2011) thereby increase the accuracy of estimation of bacterial richness in samples.

2.7 Choice of Methods

Most studies examined the bacterial diversity using various culture-independent techniques. The selection of the method utilized in each study is dependent on the experimental designs and objectives that need to be achieved. Thus, the technique employed in each research is very subjective. It is known that fingerprinting techniques such as DGGE and T-RFLP will only identify the dominant taxa (Rudi *et al*., 2007). Such barriers can be overcome by exploiting high-throughput sequencing as minor populations can also be detected thereby provide more comprehensive and robust insights into complex soil communities (Fakruddin & Mannan, 2013). Therefore, a combination of bacterial community fingerprinting techniques and next generation sequencing has recently been applied to address and characterize the communities in soil. It has been shown that utility of multiple techniques in resolving bacteria communities can reduce error rates and produce more reliable data. For instance, Cleary *et al*. (2012) utilized both pyrosequencing and DGGE analysis to evaluate bacterial community composition from mangrove environment while Gozdereliler *et al*. (2013) used the similar combination of techniques to detect shifts in community composition in response to the herbicide.

In this study, a combination of various molecular methods such as T-RFLP, Q-PCR together with NGS technologies was employed to analyze the shifts in bacterial community compositions in response to changes in temperature and moisture contents and further linked the observed shifts with functional roles of communities via quantification of functional genes. Such combinations provide detailed information about taxonomic profiles of bacterial community present as well as their vital roles in ecosystem functioning and further enhance our understanding of the changes in the primary environmental processes.

Table 2.1: Comparison of IlluminaMiseq and Roche 454 technologies (Pareek *et al*., 2010).

Platform	**IlluminaMiseq**	**Barcoded Pyrosequencing**
Sequencing mechanism	Cyclic reversible termination	Pyrosequencing
Amplification method	Bridge Amplification	Emulsion PCR
Read length (bp)	100-150	400-800
Cost per Mb	$ 5.97	$ 84.39
Output per run (Gb)	120-600	0.7
Run time	2-11 days	24 hours
Detection Method	Fluorescent emission from incorporated dye-labeled nucleotides.	Light emission from secondary reaction upon released of pyrophosphate molecule.
Advantages	High throughput	Longest read length, fast

2.8 Functional Genes

The study of the expression of functional genes that encode for the key metabolic enzymes allows evaluation of the genetic potential of a particular community within an environment (Levy-Booth *et al*., 2014) as changes in gene abundance may indicate an alteration in specific ecological functions (Douterelo *et al.,* 2014). Both carbon and nitrogen cycles which influence the ecosystem functioning are entirely driven by soil microbes (Silva *et al*., 2013; Zhang *et al*., 2013; Schimel & Schaeffer, 2012; Wallenstein & Vilgalys, 2005). In general, nitrogen cycle consisted of three main processes: nitrogen fixation, nitrification, and denitrification. The bacterial groups that are responsible for catalyzing each of the steps are known as 'nitrogen fixers,' 'nitrifiers' and 'denitrifiers' respectively.

2.8.1 Nitrogen fixation

Nitrogen fixation involves the reduction of atmospheric nitrogen into ammonia by diazotroph organisms (Levy-Booth *et al*., 2014; Chowdhury *et al*., 2009). This reaction is catalyzed by nitrogenase which is a complex enzyme with two components; heterotetrameric (encoded by *nifD* and *nifK*) and nitrogenase reductase (encoded by the *nifH*) (Hoffman *et al*., 2014). Studies have shown that the *nifH* is most often used biomarker for studying diazotrophic community as this gene is highly conserved in these organisms (Deslippe & Egger, 2006; Jenkins, 2003; Rosch *et al*., 2002; Rosado *et al*., 1998). *NifH* gene has been characterized from various environments including marine (Farnelid *et al*., 2011; Langlois *et al*., 2006), hydrothermal sites (Mehta *et al.*, 2003) and different types of soils such as cold polar (Zhang & Xu, 2008; Deslippe & Egger, 2006)

and agricultural soils (Zou *et al.,* 2011; Coelho *et al.,* 2009). It was also found that the diversity of nitrogen-fixing bacteria depends on the ecosystem types (Zehr *et al*., 2003) as the structure of *nifH* communities is shaped by both abiotic and biotic factors (Tai *et al.,* 2013).

2.8.2 Nitrification

Nitrification is the key process in the nitrogen cycle as it involves transformation of ammonia (NH_3) into nitrate (NO_3^-) with nitrite (NO_2^-) as an intermediate product (Merbt *et al*., 2012; Huang *et al*., 2011). The first step of ammonia oxidation into nitrite is known as the rate-limiting step because it is carried out by ammonia-oxidizing bacteria (AOB) that exhibited slow growth rate and this reaction is highly sensitive to disturbances (Srithep *et al.,* 2014; Alves *et al.,* 2013). The oxidation reaction is catalyzed by ammonia monooxygenase which is encoded by three different genes: *amoA*, *amoB*, and *amoC*. Among these genes, *amoA* is used frequently as a marker for studying AOB communities because this gene encodes the active site of ammonia monooxygenase (Wan *et al*., 2014; Francis *et al*., 2005; Rotthauwe *et al*., 1997). Besides, the sequences of *amoA* are found to be highly conserved within AOB communities (Norton *et al*., 2002). Correspondingly, several studies have used this particular gene for studying ammonia oxidizers from environmental samples (Long *et al.,* 2012; Petersen *et al.,* 2012; Okano *et al.,* 2004). Besides, the nitrate content and diversity of AOB communities are known as a good indicator of soil quality and health (Huang *et al*., 2013; Wessén & Hallin, 2011; Nyberg *et al*., 2005). This is because any changes in the soil properties are highly attributed to metabolism activities of AOB communities (Ke *et al*., 2013). Therefore, nitrification process has received much

attention in the past decades due to crucial roles in ecological functions (Zhang *et al*., 2014; Shen *et al*., 2012).

2.8.3 Denitrification

Denitrification process is a chain reduction of oxidized N compounds (NO_3^-, NO_2^-) into nitrogen (N_2) with nitric oxide (NO) and nitrous oxide (N_2O) as the intermediate products (Braker *et al*., 2010; Wallenstein *et al*., 2006). Due to the release of such gaseous products into environments, denitrification process is certainly important in climate regulation (Hallin *et al.,* 2012). For instance, N_2O generated through this pathway is an important greenhouse gas (Levy-Booth *et al*., 2014; Wertz *et al*., 2013). Therefore, it is paramount important to understand and study this bacterial-mediated process as this step completes nitrogen cycle by releasing N_2 into the atmosphere (Saggar *et al.,* 2013). Further, incomplete denitrification pathways may result in the release of gaseous products into the air.

Denitrification process occurs under anaerobic condition and is carried out by the denitrifier community which accounting for 5% of the total bacterial population (Bru *et al*., 2011; Henry *et al*., 2006; Wallenstein *et al*., 2006). This process begins with the reduction of NO_3^- into NO_2^- catalyzed by nitrate reductase, a molybdoenzyme (NAR) (Zumft, 1997), encoded by the *napA* gene (Saggar *et al.,* 2013). However, the presence of nitrate reductase in nitrate respires and dissimilatory reducers of nitrate to ammonia does not permit the utilization of *napA* gene to quantify the exact denitrifier community (Cheneby *et al*., 2003; Philippot *et al*., 2002). The second step involves the reduction of NO_2 to NO which is the critical step in denitrification. Two functionally redundant

enzymes: copper reductase (Gschwendtner *et al*., 2014; Ye *et al*., 1993) and cytochrome cd_1 nitrite reductase (Zumft, 1997) and encoded by *nirK* and *nirS* respectively (Gschwendtner *et al.,* 2014; Braker *et al.,* 2010) are catalyzing denitrification step. However, it was found that the two genes (*nirK* and *nirS*) do not appear together in the same organism (Gschwendtner *et al*., 2014; Jones & Hallin, 2010). Surprisingly, both of these genes were recently found to occur together in a bacterial strain namely, *Thermusoshimai* JL-2 from hot spring environment (Murugapiran *et al.,* 2013). The ability of a denitrifier to possess either of a copper reductase or a cytochrome cd_1 nitrite reductase explains the utilization of the *nirK* and *nirS* genes as standard molecular markers to study denitrifier community in soils (Gschwendtner *et al*., 2014; Saggar *et al*., 2013; Braker *et al*., 2010; Smith & Osborn, 2009). The last step of the denitrification process is the reduction of N_2O into nitrogen gas, catalyzed by nitrous oxide reductase (NOS) which is encoded by *nosZ* gene (Iribar *et al*., 2015; Richardson *et al*., 2015; Jones *et al*., 2013; Jung *et al*., 2013; Sanford *et al*., 2012). Functional gene analysis of denitrification process demonstrate a complex interaction between the denitrifying community and soil environments as this pathway is influenced by numbers of factors like nitrogen availability and types of cultivation (Ning *et al*., 2015; Senbayram *et al*., 2012; Kandeler *et al*., 2009; Rasche *et al*., 2006).

2.8.4 Organic compound degradation

Bacterial chitin degradation in soils is one of the major contributors to carbon cycling (Wieczorek *et al*., 2014). Chitin (1-4)-β-linked N-acetylglucosamine (GlcNAc) is the second most prevalent biopolymer, and it was reported to structurally support many unicellular and multicellular eukaryote organisms (Martínez *et al*., 2009). The complete lysis of chitin involves three different steps and the first two steps are

catalyzed by a degrading enzyme known as chitinase (Beier & Bertilsson, 2013). Chitinases are grouped into hydrolases family GH 18 and GH 19 (Saito *et al*., 2003), the latter is found mainly in plants. The chitinolytic bacterial community is dominated by GH 18 which is subsequently divided into three subfamilies A, B and C (Cantarel *et al.,* 2009; Karlsson & Stenlid, 2009). However, the majority of chitinase-producing bacterial community have been dominated by group A (Kielak *et al*., 2013; Metcalfe *et al*., 2002), therefore *chiA* has been adopted as phylogenetic marker to target chitin degraders and used to study carbon cycling as well (Yergeau *et al*., 2007; Hobel *et al*., 2005; Xiao *et al*., 2005). This chitin-degrading community is reported to be affected by several factors such as water content, temperature, substrate availability and pH (Wieczorek *et al*., 2014; Kielak *et al*., 2013; Manucharova *et al*., 2011).

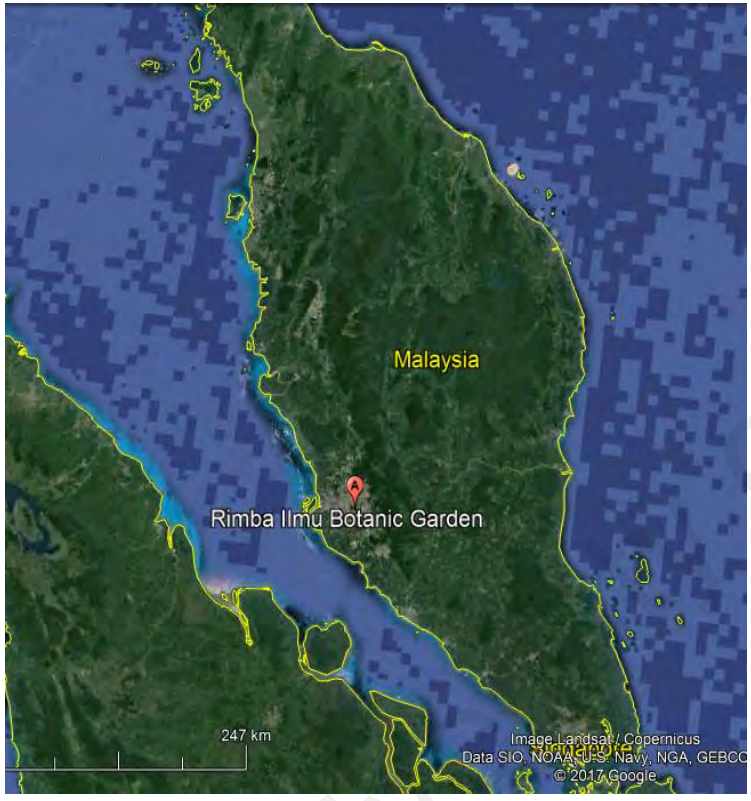
CHAPTER 3: MATERIALS AND METHODS

3.1 Sites descriptions and sampling procedures

3.1.1 Rimba Ilmu

Tropical soil samples were collected from Rimba Ilmu (Figure 3.1). The Rimba Ilmu is a botanical garden located within the University of Malaya campus in Kuala Lumpur, Malaysia. It is modeled after a rain forest and harbor more than 1600 of microfauna and macrofauna species (Jusoff, 2010). Rimba Ilmu is a protected area with minimal impact of human activity (Dzulhelmi & Norma-Rashid, 2014). The soil samples were collected at 3° 7'51.85 N; 101° 39'28.67 E, using a sterile spade at a depth of 0-20 cm. The collected soil was placed in sterile polythene bags and transported to the laboratory at the National Antarctic Research Center which is also within the University of Malaya in Kuala Lumpur, Malaysia. The collected soil was sieved through a 2-mm mesh sieve on the same day and stored at 4°C for less than 24 h before the start of the soil microcosm experiment. The bare soil was fine-textured and dark brown in color. The climate in Malaysia is typically tropical, consists of dry and wet seasons throughout the year with the average temperatures range from 21°C to 32°C (Manap *et al*., 2011; Suhaila *et al*., 2010; Wong *et al*., 2009). However, the annual rainfall pattern in Malaysia is strongly influenced by two rainy seasons associated with the Southeast Monsoon and the Northeast Monsoon (Juneng & Tangang, 2005; Tangang & Juneng, 2004; Tangang, 2001). The mean annual rainfall recorded is around 2400mm (Dominic *et al*., 2015) while the average daily rainfall is between 10 and 25 mm (Althuwaynee *et al*., 2014).

a



b

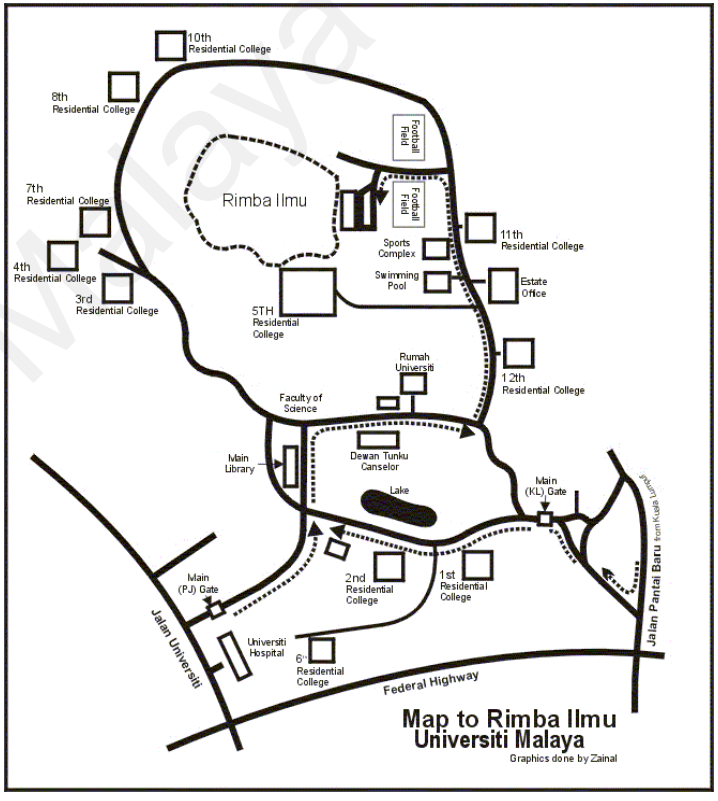


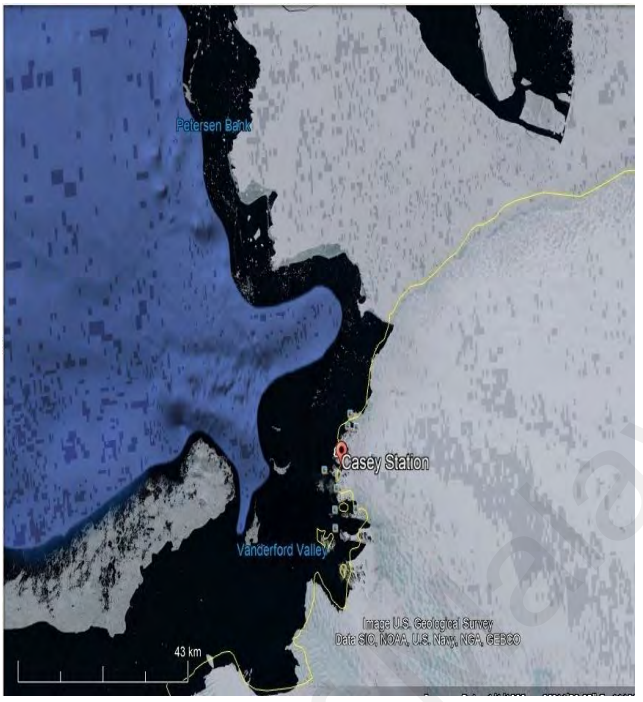
Figure 3.1: Maps indicating the Rimba Ilmu of University of Malaya. Tropical soil samples were collected from Rimba Ilmu.

3.1.2 Casey Station, East Antarctica

The Antarctic soil samples were obtained from Casey Station, situated on the shore of Newcomb Bay in the Windmill Islands region of East Antarctica (Figure 3.2). The Casey Station is located at 66° 14'52.92 S; 110° 31'59.50 E. Some sites around Casey Station are highly impacted by human activities (e.g. Thala Valley) while other sites have lower impact (e.g. Browning Peninsula), and there are also protected areas (e.g. ASPA 136) (Chong *et al*., 2009). Since in this study the soil was collected in the vicinity of Casey Station, there is high level of human impact too. After collection, the soil samples were sealed in sterile polythene bags and packed with dry ice to keep cold. These samples were subsequently shipped to the laboratory at the National Antarctic Research Center, Kuala Lumpur, Malaysia, taking 3 weeks in transit. Once received, the Antarctic soil samples were stored at -20°C until use.

It has been well recognized that soil from Casey Station is profoundly impacted by marine (Scouller *et al*., 2006; Stark *et al*., 2003). Besides, the climate conditions at Casey station exhibited high seasonal variation as the temperatures range from approximately 0°C in the summer to -15°C in the winter (Nielsen & King, 2015; Deprez *et al*., 1999). The mean annual temperatures and precipitation are about -9.3°C and 180 mm yr^{-1} respectively (Beyer, 2000).

a



b

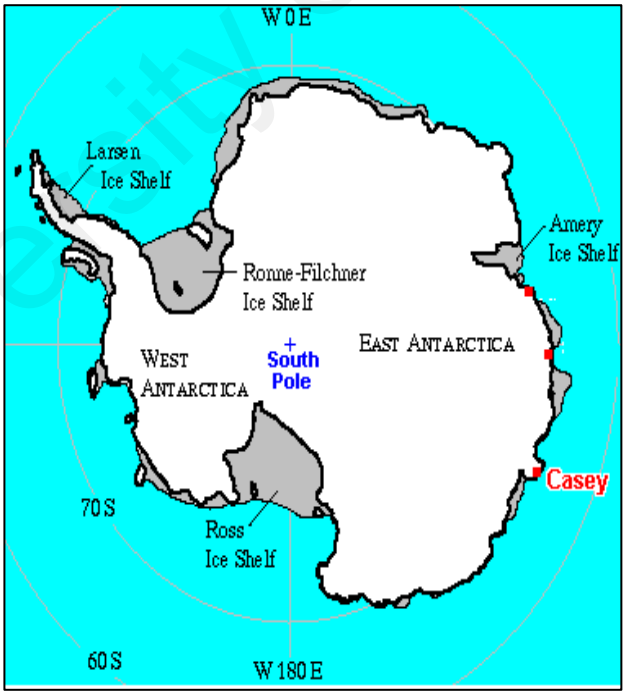


Figure 3.2: Maps indicating Casey Station, East Antarctica.

3.2 Establishment of soil microcosms

Soil microcosms were prepared using sterile falcon tubes (3 cm in diameter, 50 mL, n=114). Each tube was filled with 50g (± 0.5g) of sieved soils. The headspace of each microcosm was fitted with a well-rolled cotton wool to trap dust and allow aeration during incubation. The bottom part of each falcon tube was filled with sterilized silicone glass beads in approximate height of 2 cm. This setup is to simulate the natural soil leaching effect. In tropical region, variation in soil temperature is relatively smaller compared to the Polar region (Takada *et al*., 2015; Kosugi *et al*., 2007). For instance, Malaysian soil temperature generally ranges from 25°C to 31°C (Fazli *et al*., 2016; Takada *et al*., 2015; Sanusi *et al*., 2013). In contrast, soil temperatures in Casey Station, Antarctica range from -10°C to + 20°C (Ferguson *et al*., 2003), with maximum temperature recorded was 30.4°C (Bölter, 1992). Such fluctuations are highly associated with soil water content (Lopez-Velasco *et al*., 2011; Revill *et al*., 2007). It is noteworthy that annual temperature in both tropical and Antarctic ecosystems is projected to increase in the range of 0.3 to 0.7°C per year (Yau & Hasbi, 2013; Christensen *et al*, 2007; IPCC, 2007). The increase in annual temperature is predicted to result to drastic changes to soil conditions (Groffman *et al.,* 2001). In order to simulate such changes, we have designed two microcosm experiments to study the effects of variation in temperature and water content on both tropical and Antarctic soil bacterial community structure and diversity.

Tropical soil microcosms were incubated at three different temperatures (25°C, 30°C and 35°C). In Malaysia, the average daily rainfall was found to be in the range of 10-25 mm (1-16 mL) (Althuwaynee *et al.,* 2014). Based on this data, water treatments subjected in present study were within the range of the rainfalls reported as tropical microcosms were treated with 2 and 5 mL for every three days interval. The former was termed the low water treatment (LW) while the latter was known as high water treatment (HW). Besides, it has been reported that 1mL of water weighs 1g (Berdanier & Zempleni, 2008), therefore adding more than 6 mL of water for four weeks may result in waterlogging as each microcosm contained 50g of soil samples. These microcosms were analyzed at weeks 1, 2 and 4.

The Antarctic soil microcosms were incubated at 5°C, 10°C and 15°C. Although the average daily rainfalls in East Antarctica is less than 0.1ml (Fujita & Abe, 2006), increased of air temperatures was found to increase the precipitation volumes and melt Antarctic ice sheets (Schlosser *et al.,* 2016; Steig *et al.,* 2009). Such events further increase the water content in Antarctic soils (Steig *et al.,* 2009). Therefore, to stimulate the effect of high moisture content, 0.5 ml of sterile water was added to each sample at every three days interval. However, due to the limited Antarctic soil samples obtained, only a single water treatment was administered to the microcosms. It is well recognised that enzymatic activities are low under cold condition, as such, the Antarctic bacterial community generally showed a slow response to warming (Rinnan *et al*., 2009; Yergeau & Kowalchuk, 2008). To account for the longer response time, Antarctic soil samples were collected at week 4, 8 and 12. For each temperature and water treatment subjected to tropical soils, there were 18 replicates. Six replicates were removed from the incubators at each of three incubation periods: 1, 2 and 4 weeks. While for Antarctic soil samples, there were 15 replicates for each temperature treatment. Five replicates were

removed from the incubators at each of three incubation periods: 4, 8 and 12 weeks. In total there were 114 and 50 replicates for tropical and Antarctic soil samples respectively. Untreated tropical (6 replicates) and Antarctic (5 replicates) soil were used as controls.

3.3 Analysis of soil abiotic factors

Soil water content was determined gravimetrically by oven-drying the samples at 70°C and weighed until a constant mass was obtained. The soil pH was measured using a pH meter (Eutech Instrument, Singapore) in ratio of 1:2 (w/v) of dry soil in distilled water. The soil salinity was determined as electrical conductivity (µS/cm) and quantified using a conductivity meter (Milwaukee MI360, USA) in 1:5 (w/v) suspensions of dry soil in distilled water (Chong *et al*., 2012). The soil nitrate and nitrite content were extracted by adding 5 g of dried soil into a mixture of calcium chloride (0.025 mol/L) and activated charcoal. After shaking the mixture for an hour and the final filtrate obtained was used for quantification. This technique is known as Griess method (Melchert *et al*., 2007). For determination of soil phosphate content, Perhydrol® decomposition method (Stanisławska-Glubiak *et al.,* 2014) which involves digestion of 5 g of fresh soil samples with concentrated sulphuric acid and hydrogen peroxide was utilized. All nitrate, nitrite and phosphate content were determined photometrically using spectroquant photometer (MERCK, USA).

3.4 Extraction of genomic DNA

The soil DNA was extracted from each replicate (1.0g fresh weight) using the Mo BioPower Soil DNA extraction kit (MoBio, USA), following the manufacturer's recommendations. This method involved mechanical lysis of bacterial cells with gentle bead-beating. The free DNA was bound to a silica spin filter which is subsequently washed. After a series of washings, the DNA was recovered in 50 µl TE buffers (10 mMTris-HCI, 1mM EDTA, pH 8.0). The DNA yield and quality were checked using UV spectrophotometry at 260 and 280nm (Biophotometer, Eppendorf, Hamburg, Germany). The extracted DNA was stored at -20°C for downstream analysis.

3.5 Polymerase chain reaction (PCR) and terminal restriction fragment length polymorphism (T-RFLP) analysis of soil bacterial community

The bacterial community structure was determined by amplification of 16S DNA genes from extracted DNA of each soil replicate using bacteria-specific 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3') primers labeled with carboxyfluorescein (6-FAM) and hexa-chloro derivative (6-HEX) respectively (Chong *et al*., 2012). PCR amplification was achieved using a PCR thermocycler (Bio-Rad,USA) in a total volume of 50 uL containing 5 µL of DNA templates (~30 ng of extracted DNA), 1 µl of 0.25 mM dNTP, 5 µL of 1 × PCR buffer, 5 µl of 5µM of each primer 27F/1492R, 1µL of 1.25 units of Taq DNA polymerase (Invitrogen, USA) and 28µL of PCR grade water. Amplification was accomplished byinitial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for the 30s, 52.5°C for 45s and 72°C for 2 min. The final extension was performed at 72°C for 10

min. Each DNA sample was amplified in duplicates and the amplicons were pooled and run on a 1.2 % agarose gel stained with ethidium bromide (EtBr). The PCR products were purified using MEGAquick-spin^{TM} PCR Product Purification Kit (iNtRON Biotechnology, Korea). Restriction digestion was carried out separately with 10 U of Msp-I (Fermentas, USA) at 37°C for 4 hours. Each terminal restriction fragment (T-RFs) profile was analyzed by FirstBase Laboratories (Selangor, Malaysia). Fragment analysis was achieved by capillary electrophoresis (ABI 3100 and ABI 3730 XL DNA analyzer; Applied Biosystems, CA), using a GeneScan ROX-labeled GS500 internal size standard. The community diversity is estimated by analyzing the size, numbers and peak heights of the resulting T-RFs. Each T-RF represents an Operational Taxonomic Unit (OTU) or a ribotype (Tiedje *et al*., 1999). T-RFLP patterns were inferred using the GeneMapper software (Applied Biosystems), peaks within the range of 50 base pairs (bp) and 500 bp were selected and grouped into a T-RF. Fingerprints were aligned to reduce run-to-run variability before further statistical analyses.

3.6 Quantitative PCR (Q-PCR)

In this study the presence of six different genes:- including *nifH* (nitrogen fixation gene), *amoA* (nitrification gene), *nirS, nirK* and *nosZ* (denitrification gene) and *chiA* (carbon degradation gene) were quantified. These genes were targeted specifically due to their importance in nitrogen and carbon cycles. The quantification was carried out via fluorometric detection system in a 25 µl of reaction volume containing 12.5 µL of Absolute Q-PCR SYBR green mix (AbGene, UK), 1.25 µL of each primer (5µM) and 5.3 µL of sterile DNA-free water. Soil DNA concentrations were standardized to 10 ng μl^{-1}, and 1.0 µl of DNA sample was added to each PCR reaction. *Pseudomonas fluorescens* isolates were prepared via serial dilution (known concentration) and used as

the standard to quantify all six functional genes. Melting curve analysis was conducted to confirm the specificity of the amplified products. The standard curve obtained was used as a comparison to quantify the gene copy number per nanogram (copies/ng) for each gene. The primers and Q-PCR conditions employed in this study were summarized in Table 3.1.

3.7 Statistical analyses of T-RFLP community profiling

The T-RFLP profiles were filtered and analyzed using a web-based program T-REX (Culman *et al*., 2009; Smith *et al*., 2005). For clarity, peaks within the range of 50 base pairs (bp) and 500 bp were selected and grouped into a T-RF. Peaks outside of this range were regarded as background noise and excluded from the analysis. The peak alignment was carried out using the method suggested by Smith *et al*. (2005) to reduce run-to-run variability before further statistical analyses. The relative abundance of a T-RF was determined based on the peak height as suggested by Osborn *et al*. (2001). In this study, T-RF profiles were normalized based on the peak height as this method was reported to increase the similarities among the replicates and results reproducibility as opposed to normalization based on the peak areas (Fredriksson *et al*., 2014). The minimum peak heights was set at 100 fluorescence units to minimize false T-RFs and artifacts (Fredriksson *et al*., 2014; Osborn *et al*., 2001). The total of peak heights in each replicate profile obtained from forward and reverse probe was calculated, indicating the total number of individuals (Sessitsch *et al*., 2001).

Multivariate statistical analyses were conducted using Primer 6 multivariate data analysis package (Plymouth Marine Laboratory, UK) (Anderson & Wills, 2003; Anderson, 2001). The Bray-Curtis similarity was chosen as this index will consider two samples without species similarity (Rees *et al*., 2004). In this study, different statistical tools such as permutational multivariate analysis of variance (PERMANOVA), canonical analysis of principal component (CAP), principal coordinate analysis (PCO) and distance-based linear model (DISTLM) were used. The PERMANOVA was conducted to evaluate the effect of each factor (temperature, water content or weeks) and their interaction on the bacterial community composition. For all PERMANOVA tests, type III (adjusted) sums of squares was used and p-values were obtained by 1000 permutations under a reduced model (Anderson & Terbraak, 2003). The significant values from PERMANOVA can be visualize by CAP, a constrained ordination (Fernández *et al.,* 2014; Anderson & Wills, 2003). CAP find axes that maximizes the different among a *priori* groups (Cookson *et al*., 2007; Anderson and Wills, 2003). The model calculated the number of m (axes) and misclassification error or successful classfication of samples across the dataset (Anderson & Wills, 2003; Anderson, 2001). The square of first canonical correlation (δ^2) indicates the strenght of observed differences between dependent and independent variables (Goetze *et al*., 2011). As suggested by Ingels and Vanreusel (2013), estimated component of variation was used as a percentage of total variation to describe the magnitude of variation in bacterial assemblages at each treatment. The PCO is an unconstrained ordination that extract major variance component from multivariate dataset by reducing dimensionality (Gower, 2005). PCO is used to display broad pattern of variables across the treatments (Anderson & Wills, 2003).

Further, to evaluate the correlations between bacterial community structure with soil abiotic properties and functional genes, a step-wise distance-based linear model (DISTLM) (Mc Ardle & Anderson, 2001) analysis was carried out. The DISTLM analysis was conducted with Akaike Information Criterion (AICc) (Anderson *et al.,* 2008) as a selection criterion. Statistical analysis on T-RFLP-derived community profiles was conducted as follows: Alpha diversity indices (Shannon's Index-H'), Simpsons Diversity Index [D], Pielou's Evenness [J'], the number of species (Sobs) and the total number of individuals [N] were calculated.

Table 3:1: Primers and real-time PCR conditions used in this study

Gene	Process/Enzyme	Primers	Cycling conditions	References
N-cycle				
nifH	N-fixation/ dinitrogenase reductase	*nifHF/nifHRb*	95°C for 15 min, followed by 45 cycles of the 30s at 95°C, 45s at 53°C and 30s at 72°C	(Rösch & Bothe, 2005)
nirK	Denitrification/ nitrate reductase	*nirK1F/nirK5R*	95°C for 10 min, followed by 35 cycles of the 30s at 94°C, 90s at 57°C, 2 min at 72°C and 7 min at 72°C	(Braker *et al.,* 2000)
nirS	Denitrification/ nitrate reductase	*cd3aF/R3cd*	95°C for 10 min, followed by 35 cycles of the 30s at 94°C, 90s at 57°C, 2 min at 72°C and 7 min at 72°C	(Throback *et al*., 2004)
nosZ	Denitrification/ Nitrous oxide reductase	*nosZF/nosZ1622R*	95°C for the 90s, followed by 35 cycles of 24s at 95°C, 24s at 56°C, 24s at 58°C and final extension 7 min at 72°C	(Throbäck *et al.,* 2004)
amoA	Nitrification/ ammonia monooxygenase subunit A	*amoA-1F/amoA-2R*	50°C for 2 min, followed by 40 cycles 10 min at 95°C, 45s at 95°C, 1min at 55°C and 45s at 72°C	(Rotthauwe *et al*., 1997)
C-cycle				
chiA	Carbon Degradation	*GA1F/GA1R*	95°C for 15 min, followed by 40 cycles of 1min at 94°C, 1min at 56°C, 1min at 72°C and final extension 10 min at 72°C	(Williamson *et al*., 2000)

3.8 Next Generation Sequencing

Illumina Miseq sequencing was conducted on Tropical soil samples while 454 Pyrosequencing was carried out on Antarctic soil to elucidate the taxonomic identity of the soil bacterial community composition.

3.8.1 Illumina Miseq Sequencing

Seven different tropical soil samples were sequenced using paired-end Illumina Miseq sequencing: untreated soil, 25°C + LW, 25°C + HW, 30°C + LW, 30°C + HW, 35°C + LW, 35°C + HW. Based on the T-RFLP analysis, soil microcosms treated at week 2 showed the highest variation in bacterial community structure. Thus samples from this time point were selected. DNA extracted from each replicate was pooled and approximately 50 ng of pooled DNA was used for amplification of bacterial 16S rRNA gene. The hypervariable regions (V1) and (V3) were amplified using designed primers 341F and 518R containing flow-cell adapter sequences (Bates *et al*., 2011). Besides, each reverse primer was tagged with specific 6-base barcodes to distinguish each sample after multiplex sequencing analysis (Gloor *et al.,* 2010). Each PCR reaction mixture contained 2 × KAPA HiFi Hot Start Ready Mix (Life Technologies, USA), 0.3µM of each primer and 50 ng template DNA, making up a total volume of 25-µl. The reaction was performed in a PCR thermocycler (Bio-Rad, USA). The PCR protocols begin with an initial denaturation step at 95°C for 5 min, and 20 cycles of 98°C for 20 s, 63°C for 15 s and 72°C for 15 s, with a final extension at 72°C for 1 min. The PCR products were purified with a QIAquick Gel Extraction Kit (QIAGEN Sciences, USA). The quality and concentration of the purified product were determined by NanoDrop ND2000 (Thermo Scientific, USA) and sequencing was performed on GAII × Genome Analyzer (Illumina,

USA).

A total of 14582627 raw sequences with mean length of 176 bp were retrieved. The sequence reads were further analyzed and processed using MOTHUR V.1.22.2 (Schloss *et al.,* 2009). Sequences were analyzed based on the MiSeq standard protocols (SOP) with the exception of beta diversity measurements. The average merged reads for all the template was 170 bp. Screening and filtering of low-quality sequences and chimera detection were conducted using UCHIME (Edgar *et al*., 2011). The assignment of bacterial phylotypes into taxonomic ranks was based on the naive Bayesian classification (RDP classifier; 32) (Preem *et al.,* 2012). Sequences affiliated with mitochondria, archaea, chloroplasts and unclassified were removed. Operational taxonomic units (OTUs) were selected at 97% sequence similarity (Lemos *et al*., 2012). The sequences were also used to calculate alpha diversity with several indices: the observed richness (Sobs), Chao1 estimator, Invsimpson and Shannon index. The sequences read generated for each OTUs were classified based on the bacterial reference alignment (SILVA) (Preem *et al.,* 2012).

3.8.2 454 Pyrosequencing

Ten different Antarctic soil samples were selected and analyzed using 454 Pyrosequencing. Based on the T-RFLP analysis, variation in bacterial community structure was almost similar across the treatment. Therefore, an untreated sample and a replicate from each treatment were selected from week 4, 8 and 12. The hypervariable regions of V3-V4 from each DNA template was amplified using primers 27F and 518R, and each reverse primer was tagged with ten different bases for multiplexing. The PCR protocols consisted of initial denaturation at 94°C for 3 min, 35 cycles of denaturation at

94°C for 15s, primer annealing at 55°C for 45s and extension at 72°C for 1 min with the final extension at 72°C for 8 min. PCR amplification and purification was performed by FirstBase Laboratories (Selangor, Malaysia).

Sequencing of ten Antarctic soil samples yielded a total of 158499 raw pyrosequence reads which were quality-filtered, trimmed and analyzed using MOTHUR V.1.22.2 (Schloss *et al.,* 2009), followed by the 454 SOP. Overall the average reads for all samples were 444 bp. Reads with ambiguous bases and chimera were removed using UCHIME algorithm (Edgar *et al.,* 2011) and the qualified sequences were clustered into OTUs (97% similarity) using naive Bayesian classification (RDP classifier; 32) (Preem *et al.,* 2012). The resulting sequences were used to calculate alpha-diversity, invsimpson and Shannon index, Chao1 estimator. The Good's coverage was used to determine the coverage of sequences generated. Representative sequences from mitochondria, archaea, chloroplasts and unclassified reads were discarded. The identity of each bacterial phylotypes was determined based on the bacterial reference alignment (SILVA) (Preem *et al.,* 2012).

CHAPTER 4: RESULTS

4.1 Responses of T-RFLP derived bacterial community to treatments

4.1.1 Responses of T-RFLP derived bacterial community to temperature and moisture treatments in the tropical soil microcosms

The PERMANOVA analysis of T-RFLP profiles across the treatments showed significant effects of temperature (Pseudo-$F_{2,113}$= 3.26, P_{MC} =0.001, P=0.001) and water content (Pseudo-$F_{1,113}$ = 9.71, P_{MC} = 0.001, P=0.001) on bacterial community structure. Besides, a significant interaction between the two factors also was found (Pseudo-$F_{2,113}$= 4.06, P_{MC} = 0.001, P=0.001). This was confirmed by CAP ordination derived to visualize the overall soil bacterial distribution patterns in relation to temperature and water treatments (Fig. 4.1a). Based on this diagram, the most explicit shifts in community composition were detected in microcosms incubated at 35°C. The total explained variation for the CAP 1 and CAP 2 is 9.42 % and 15.12% respectively. The ordination comparing temperature and water induced bacterial community separation was further repeated according to weeks (Figure 4.1b-d). As shown in Figure 4.1a-d, there were profound differences in the T-RF profiles of community structure across the treatments. The weightage of CAP axes was calculated according to weeks ((Week 1: CAP1= 14.17%, CAP2= 23.23 %), (Week 2: CAP1= 17.15 %, CAP2= 22.76%), (Week 4: CAP1 = 16.6 %, CAP2= 19.42 %)). The square of canonical correlation δ^2 for first two axes is 0.793 and 0.677 with 70.18 % of correct classification. Further, the ordination comparing temperature and water induced bacterial community separation was further repeated according to weeks (Figure 4.1b-d). Regardless of incubation temperature, for Week 1, clustering was observed for samples receiving lower water enrichment as opposed to samples with higher water enrichment (Figure 4.1b). In Week

4 however, such separation was less apparent for microcosms incubated at 25°C and 30˚C (Figure 4.1d). In comparison, the highest variation and maximum separation between temperature and water treatments was observed in Week 2 (Figure 4.1c). Therefore, tropical soil samples from Week 2 were selected for Illumina sequencing.

The overall community diversity (measured by species richness, the total number of individuals, Shannon diversity, Margalef diversity) and evenness calculated for each microcosm are summarized in Table 4.1. Statistical analysis of bacterial community from tropical soil microcosms revealed that a total number of species (S), species richness (d) and evenness (J') (low dominance) were found to be the highest in untreated samples. Similarly, examination of Shannon Diversity Index indicated that untreated samples contain the highest bacterial diversity (H'= 1.96±0.29). Notable reduction in community evenness (high dominance) was observed for microcosms treated at 35°C and high water content (HW). On the other hand, the total number of individuals (N) remained stable across the treatments.

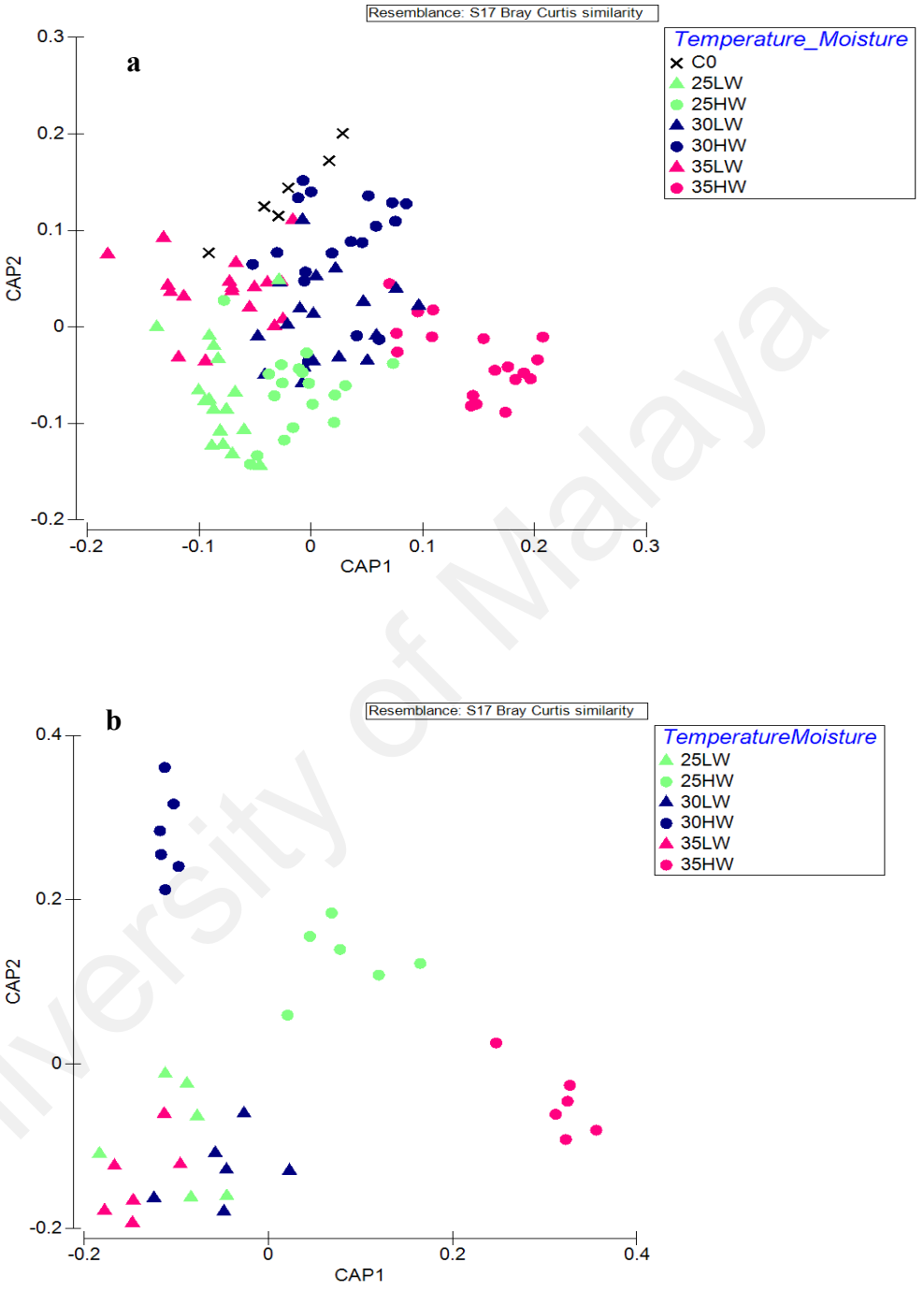


Figure 4.1: Effect of incubation temperature and water addition regimes on the tropical soil bacterial community structure at different sampling times. Bacterial community was analyzed by terminal restriction fragment length polymorphism (T-RFLP) of the 16S rRNA gene. The axes indicate canonical analysis of principal coordinates (CAP) ordination of bacterial community composition based on different incubation weeks (Week 1, 2 and 4). (a) Overall bacterial community structure, (b) Week 1, (c) Week 2, (d) Week 4. C0 represents the untreated samples. Overall includes all untreated samples and treated sample (Week 1, 2 & 4).

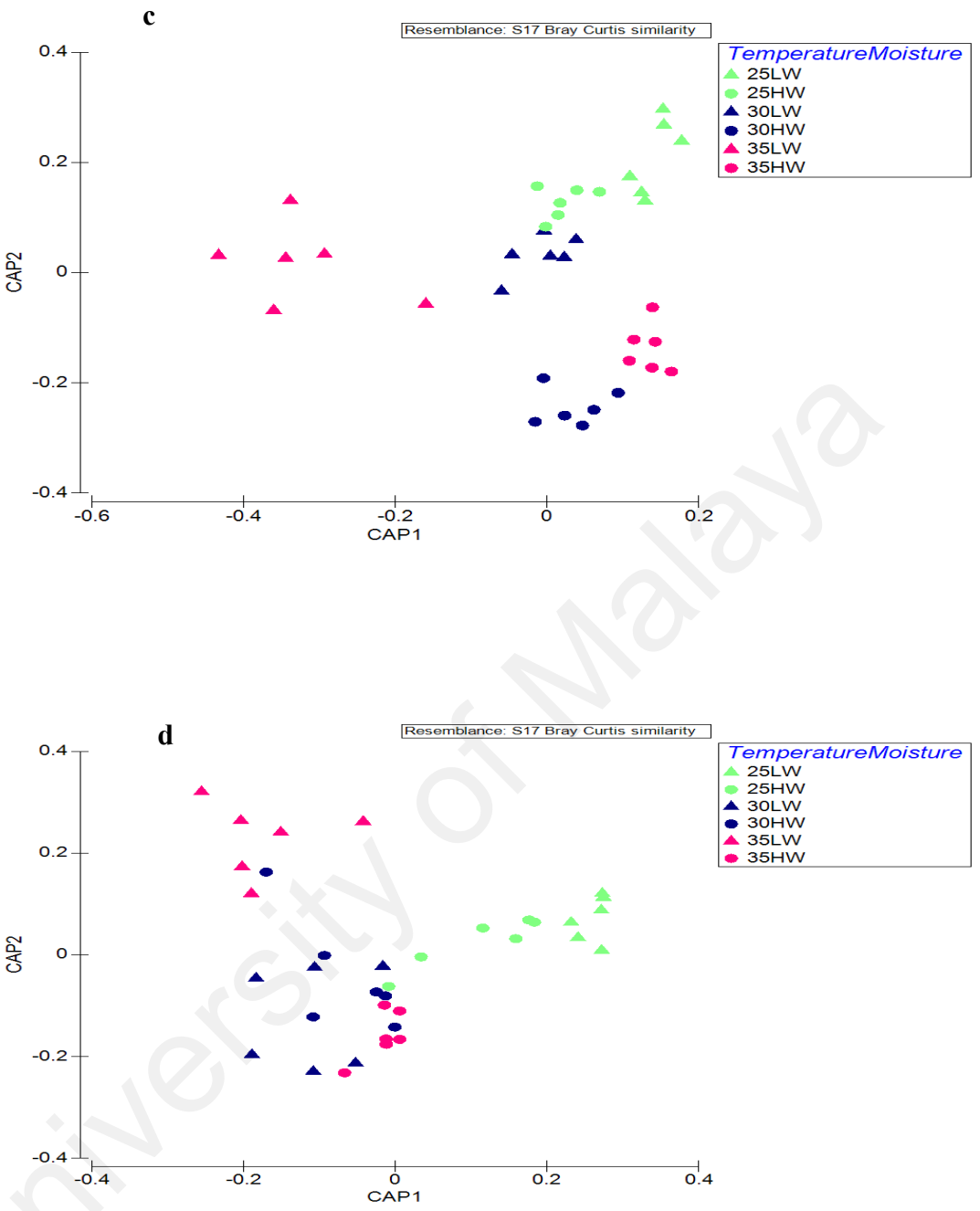


Figure 4.1, continued.

Table 4.1: Estimated bacterial richness, evenness and diversity indices (mean ± standard deviation) from T-RFLP data of the tropical soil microcosms at different temperatures and water regimes.

Group	Total Species (S)	Total Individuals (N)[b]	Species Richness Margalef (d)[c]	Pielou's evenness (J')[d]	Shannon Diversity Index H
Untreated	199.71± 131.4	199.96±0.07	37.51±24.8	0.88±0.01	1.96±0.29
25°C LW	76.39±88.05	200±0.02	14.23±16.63	0.82±0.06	1.38±0.24
25°C HW	41.06±27.34	200±0.01	7.56±5.16	0.82±0.04	1.24±0.26
30°C LW	70.59±87.98	200±0.02	13.13±16.61	0.86±0.05	1.31±0.43
30°C HW	113.17±122.75	200±0.05	21.17±23.17	0.85±0.04	1.60±0.57
35°C LW	52.56± 33.90	200±0.02	9.73±6.40	0.85±0.04	1.38±0.25
35°C HW	85.5± 47.31	199.99±0.03	15.95±8.93	0.76±0.06	1.36±0.22

[a] (S)= the number of species in each group
[b] (N)= total number of individual in each group
[c] (J')= H'/ *InS*
[d](d) = (S-1)/ Log (N)

4.1.2 Responses of bacterial community to temperature treatments in the Antarctic soil microcosms

The PERMANOVA result showed significant effects of temperature (Pseudo-$F_{2,49}$= 2.98, P_{MC} =0.005, P=0.001) and incubation periods (Pseudo-$F_{2,49}$= 10.95, P_{MC} =0.001, P= 0.001) on the bacterial community composition in the Antarctic soil microcosms. Though there was a significant interaction between the factors (Pseudo-$F_{2,49}$= 1.63, P_{MC} =0.044, P=0.006), the effect of incubation period on the bacterial community structures outweighed the effect of temperature. The PCO analysis was conducted for Antarctic soil microcosms to visualize the effect of temperature and incubation periods on the overall bacterial community structures. PCO was chosen to determine the total explained variation by incubation period as the weightage of this factor could not be determined by PERMANOVA (degree of freedom, df= 0) when the ordination was repeated according to weeks.

Based on the overall community distribution (Figure 4.2 a), there were only little detectable changes in the bacterial community structures across the treatment. The first two axes (PCO1 and PCO2) which explained 36.4 % and 19.3 % of total variation respectively were included. The weightage of PCO axes was calculated according to weeks ((Week 4: PCO1= 69.7%, PCO2= 9.6 %), (Week 8: PCO1= 56.3 %, PCO 2= 18.1%), (Week 12: PCO1 = 57.2 %, PCO2= 14.9 %)). The square of canonical correlation δ^2 for first two axes is 0.912 and 0.81 respectively with 60% of correct classification. The ordination comparing temperature induced bacterial community separation was further repeated according to weeks (Figure 4.2 b-d). Though variation in

the bacterial community structures were observed in Week 4 and 8 microcosms, the community separation was not as clear as tropical soil microcosms (Figure 4.1 b-c). For Week 12 microcosms (Figure 4.2d), the community profiles for all tested temperatures separated clearly along the PCO2 axis. Perhaps at this incubation period the slight variation observed between the groups (e.g. 5°C and 10°C) is correlated to PCO2 rather than PCO1. The community diversity derived from T-RFLP data are summarized in Table 4.2. As shown in the Table 4.2, the total number of species (S) and evenness (J') were higher in untreated samples as compared to treated samples. Though the highest effect of temperature was observed in Week 4 microcosms (Figure 4.2b), it should be stressed that the effect of incubation period on the overall community structure outweighed the effect of temperature. Therefore, for 454 pyrosequencing analysis, a replicate from each temperature and incubation period was chosen to identify bacteria groups that are responsible for the observed shifts in bacterial community composition.

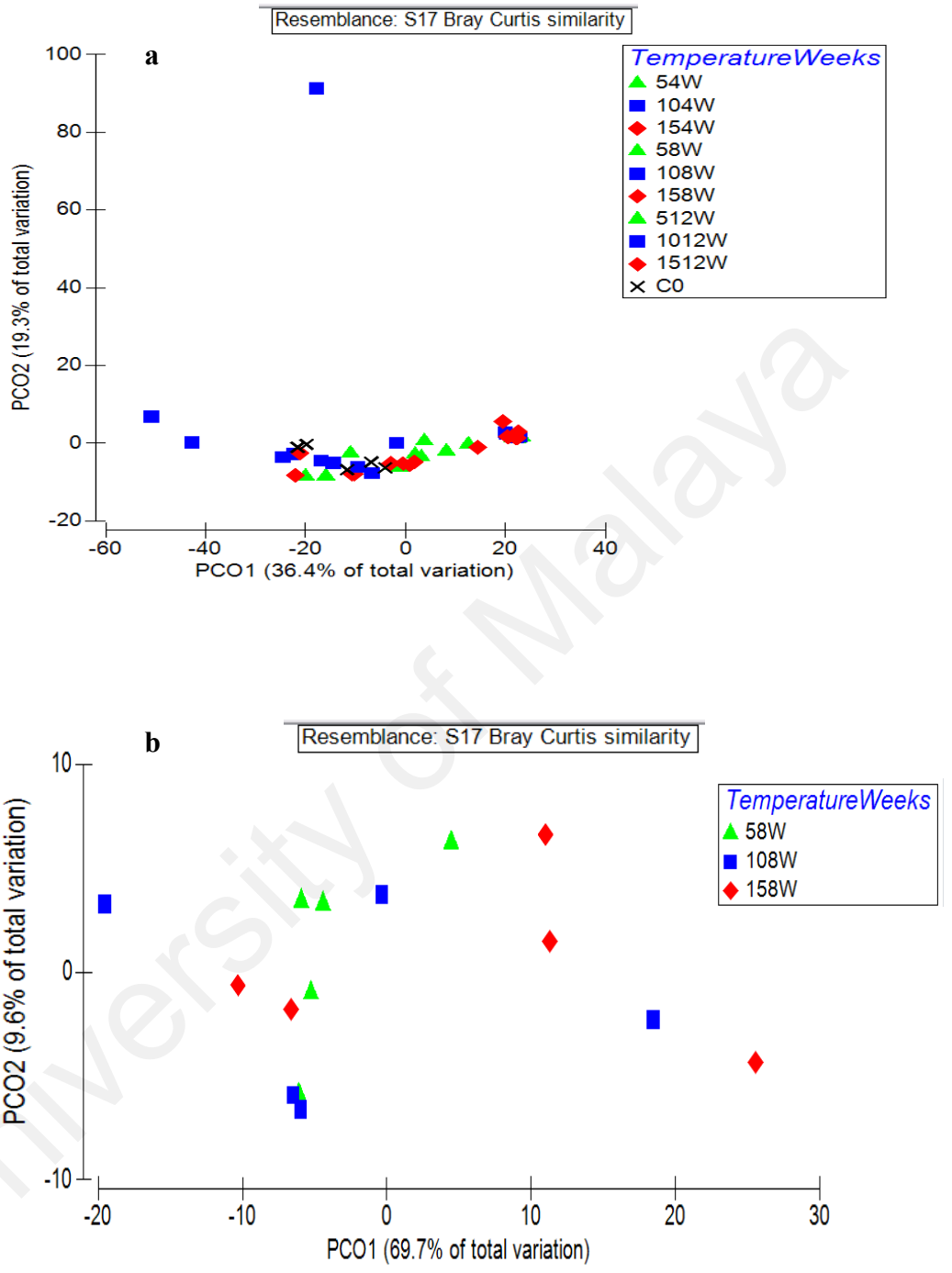


Figure 4.2: Effect of incubation temperature on the Antarctic soil bacterial community structure at different sampling times. Bacterial community was analyzed by terminal restriction fragment length polymorphism (T-RFLP) of the 16S rRNA gene. The axeof bacterial community based on different incubation periods (Week 4, 8 and 12).(a) Overall bacterial community composition, (b)Week 4, (c) Week 8, (d) Week 12.C0 represents the untreated samples. Overall includes all untreated samples and treated samples(Week 4,8 and 12)

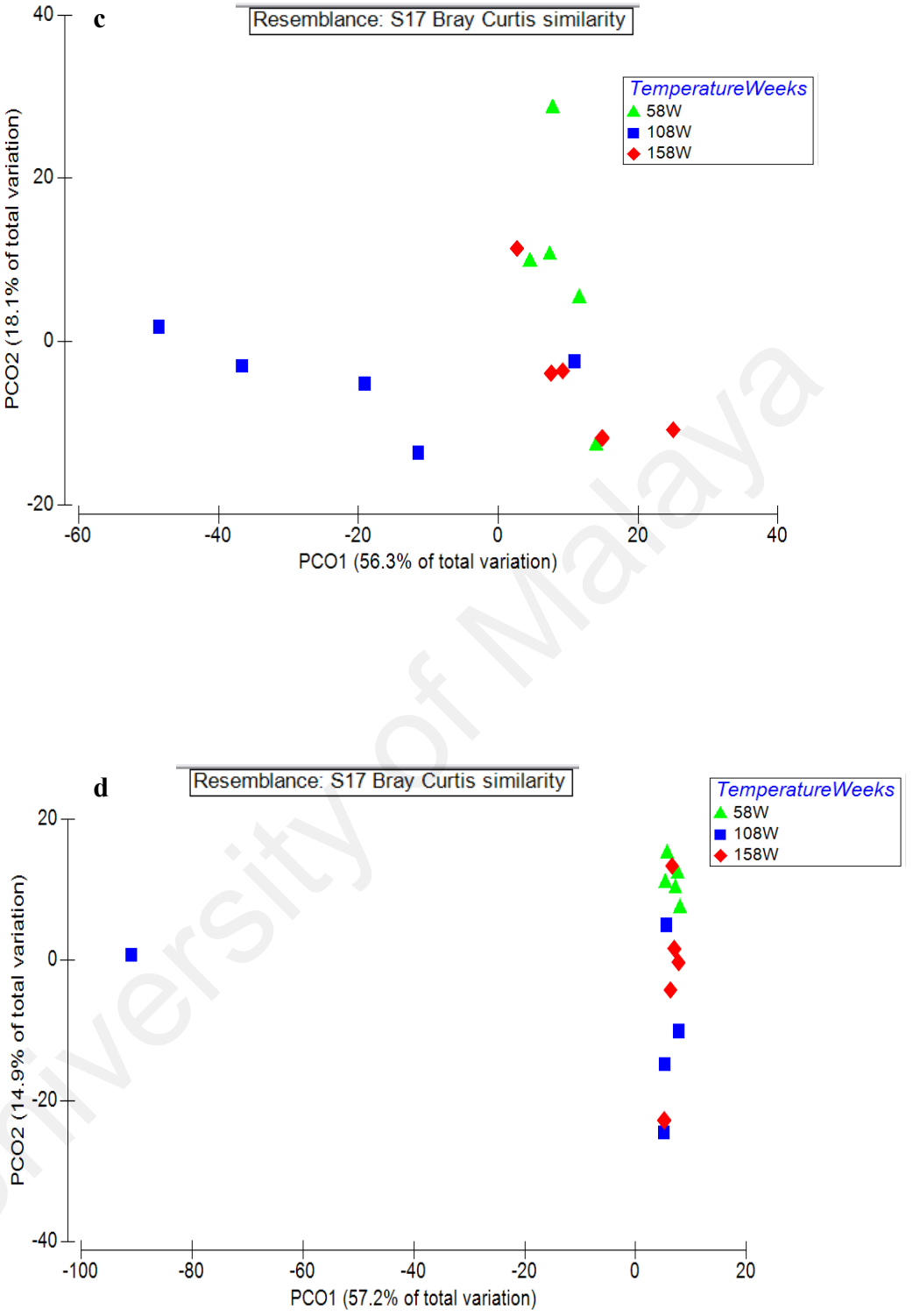


Figure 4.2, continued.

Table 4.2: Estimated bacterial richness, evenness and diversity indices (mean ± standard deviation) from T-RFLP data of the Antarctic soil microcosms at different temperatures and incubation periods.

Group	Total Species (S)[a]	Total Individuals(N)[b]	Species Richness Margalef (d)[c]	Pielou's evenness (J')[d]	Shannon Diversity Index H'
Untreated	143.4±114.52	199.96±0.05	26.87±21.61	0.83±0.01	1.68±0.29
5°C 4W	87.8±37.12	200±0.02	16.38±7	0.73±0.02	1.41±0.11
5°C 8W	50.2±28.51	199±0.02	9.29±5.38	0.82±0.01	1.34±0.20
5°C 12W	100.4±39.90	200.01±0.01	18.76±7.53	0.78±0.02	1.54±0.20
10°C 4W	82.8±45.06	199.99±0.02	15.44±8.51	0.75±0.04	1.39±0.23
10°C 8W	45.2±18.19	199.99±0.02	8.34±3.43	0.74±0.07	1.22±0.23
10°C12W	110.4±66	199.99±0.02	20.65±12.46	0.78±0.09	1.39±0.50
15°C 4W	122.2±61.41	199.99±0.01	22.87±11.59	0.76±0.05	1.56±0.25
15°C 8W	63.4±9.66	199.99±0.02	11.78±1.82	0.80±0.05	1.44±0.12
15°C12W	138.8±61.83	200±0.05	26±11.67	0.75±0.05	1.55±0.20

[a](S) = the number of species in each group
[b](N) = total number of individual in each group
[c](d) = (S-1)/ Log (N)
[d](J') =H'/*InS*

4.2 Taxonomic profiles of the bacterial community based on sequencing

4.2.1 Taxonomic profiles of the bacterial community from tropical soil microcosms

The taxonomic analysis of the bacterial community from tropical soil microcosms indicates significant changes in OTU richness (Sobs) and diversity indices values across treatments (Table 4.3). For instance, the overall community richness (Sobs) was found to be the highest in untreated samples. Both Simpson and Shannon evenness also demonstrated the highest diversity evenness (low dominance) in untreated samples. Striking differences were noted in microcosms treated at 35°C and high water level (HW) which exhibited the lowest bacterial diversity and evenness (Table 4.3).

The classified sequences based on the naive Bayesian classifier were affiliated with five phyla and 25 genera. The identified phyla were *Firmicutes* (64.22%), *Acidobacteria* (16.11%), *Proteobacteria* (13.24%) and *Actinobacteria* (0.13%) (Figure 4.3). As shown in Figure 4.3, distinct community shifts across the treatments were observed. In the tropical soil used in this study, *Firmicutes* was the dominant phylum of bacteria across all the treatments. The relative abundance of *Acidobacteria* was found to be higher in dryer soil (LW) as compared to wet soil (HW). The largest shifts in the bacterial community structure occurred in microcosms incubated at 35°C, attributed to increase in the proportion of *Firmicutes* (>90 %) with a concomitant reduction in the other phyla. Such changes in the distribution of bacterial taxa, therefore, supported the result of T-RFLP analysis which indicates similar compositional shifts across the treatments (Fig. 4.1). Similarly, at the genus level, significant differences in community composition between untreated and treated samples were also evident (Figure 4.4). The *Tumebacillus* genus (phylum *Firmicutes)* accounted for the majority of the sequences detected

(63.55%), followed by Acidobacteria subgroup 13 (*Gp 13*) (15.69%) and *Aquicella* (11.78 %). We found that the proportion of *Tumebacillus* increased up to 90 % in microcosms incubated at 35°C. Additionally, *Gp13* was found to be more abundant in LW-treated microcosms.

Table 4.3: Estimated diversity indices from 16S rRNA gene libraries of the tropical soil microcosms at different temperatures and watering regimes.

Group	Sequence Coverage	Sobs†	Inverse Simpson	Invsimpson (lci††, hci†††)	Shannon	Shannon (lci, hci)	Simpson evenness	Shannon evenness
Untreated	0.92	124.00	16.23	(14.28,18.80)	3.59	(3.48,3.71)	0.13	0.75
25°C LW	0.90	119.34	14.04	(12.58,15.89)	3.36	(3.24,3.48)	0.12	0.70
25°C HW	0.93	91.65	6.91	(6.10,7.96)	2.88	(2.75,3.01)	0.08	0.64
30°C LW	0.90	117.21	11.98	(10.62,13.75)	3.29	(3.16,3.41)	0.10	0.69
30°C HW	0.91	105.55	6.81	(6.05,7.80)	2.92	(2.78,3.06)	0.06	0.63
35°C LW	0.98	25.92	2.50	(2.36,2.66)	1.26	(1.17,1.36)	0.10	0.39
35°C HW	0.97	30.94	2.14	(2.01,2.30)	1.16	(1.05,1.26)	0.07	0.34

† Sobs, number of observed species
††Low confidence interval
†††High confidence interval

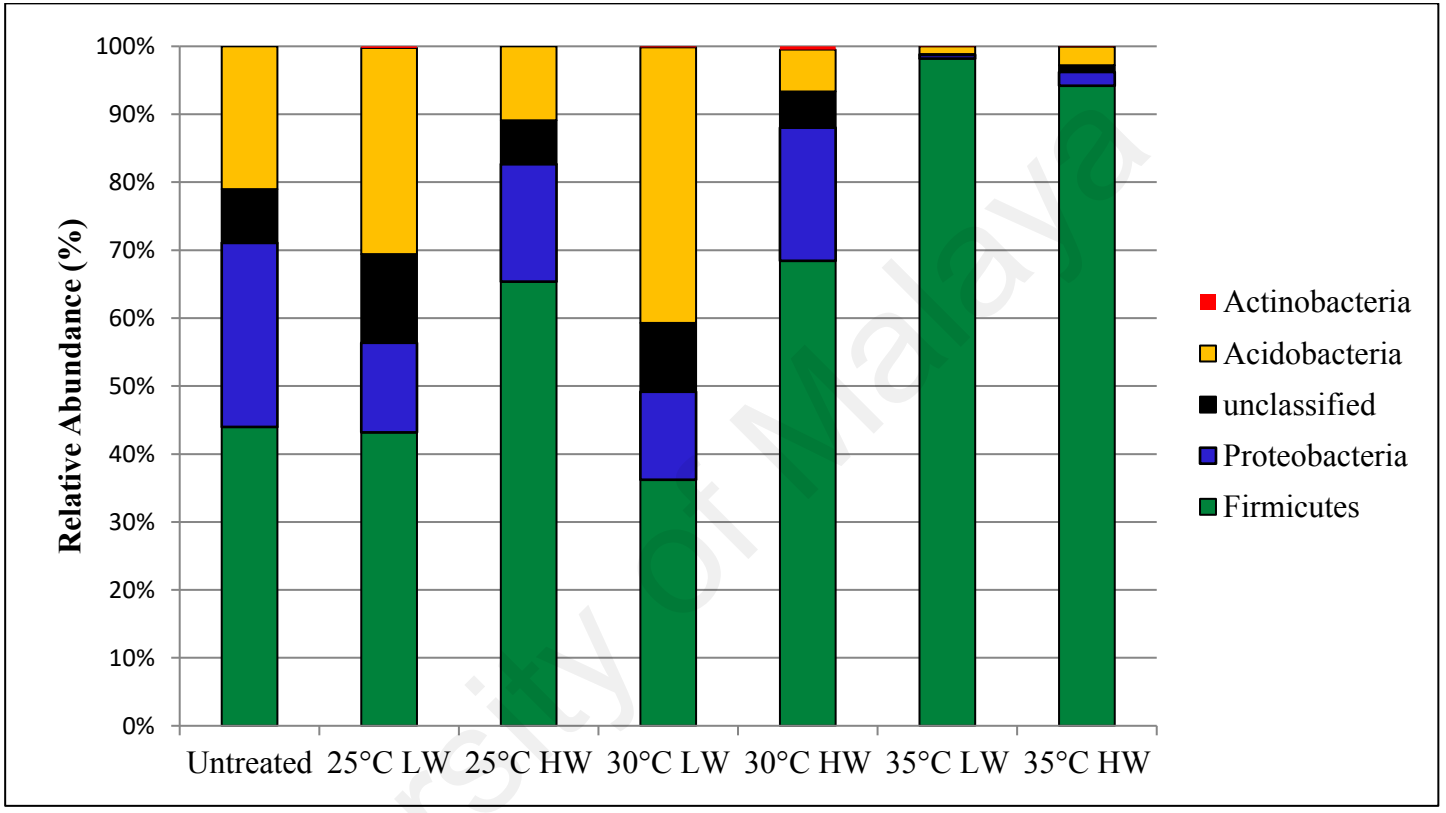


Figure 4.3: Relative abundance of bacterial phyla in tropical soil incubated for two weeks at different temperatures (25°C, 30°C and 35°C) and watering regimes (LW and HW), identified by Illumina Miseq analysis of the 16S rRNA gene. Taxonomic assignments of the 16S rRNA gene sequences to the phylum level were carried out by using the RDP-II Classifier tool. Sequences not aligned to any known phylum (at 97% homology) are placed under "unclassified.

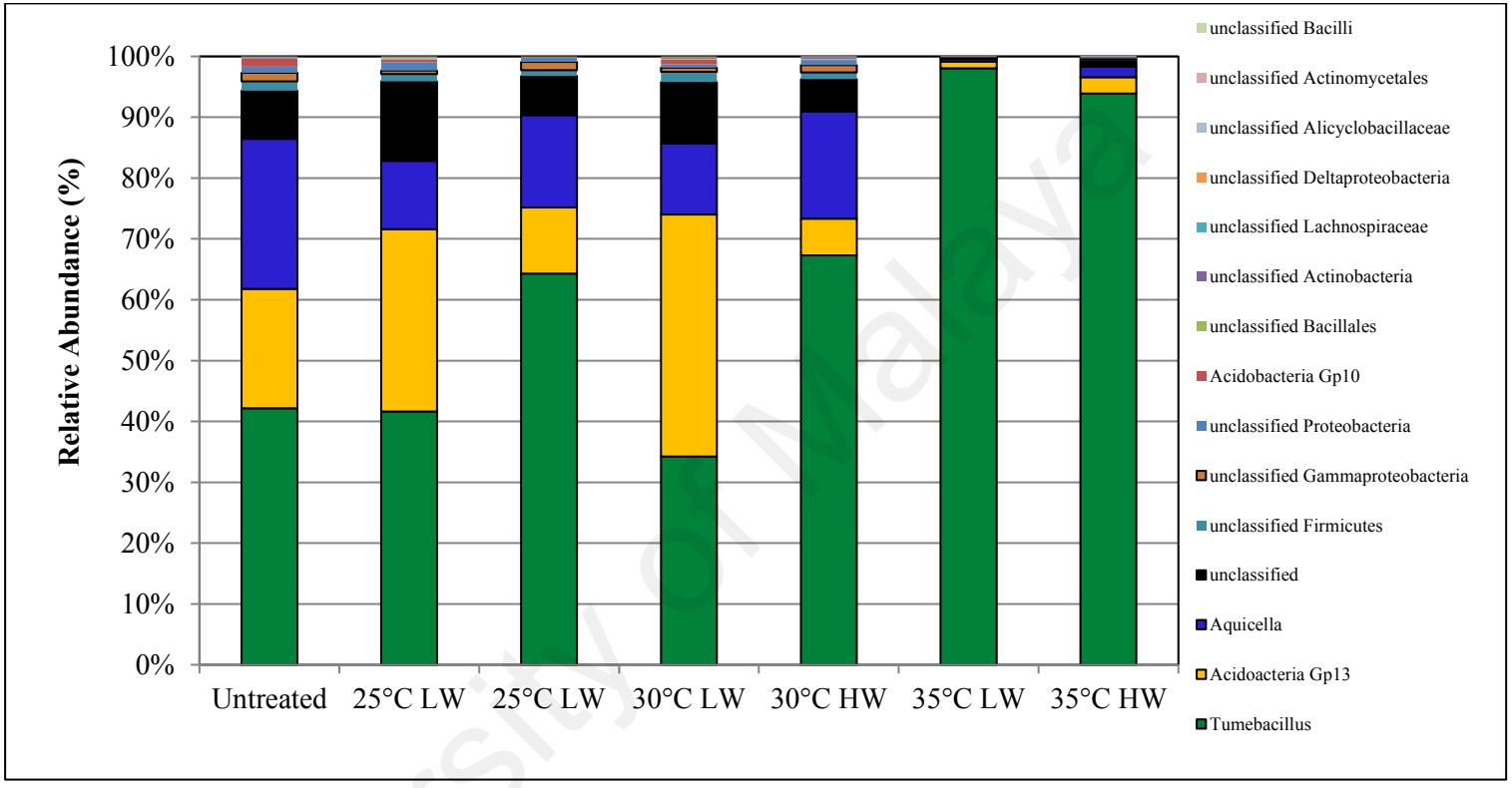


Figure 4.4: Relative abundance of dominant genera in tropical soil incubated for two week at different temperatures (25°C, 30°C and 35°C) and watering regimes (LW and HW), identified by Illumina Miseq analysis of the 16S rRNA gene. Taxonomic assignments of the 16S rRNA gene sequences to the genus level were carried out by using the RDP-II Classifier tool. Sequences not aligned to any known genus (at 97% homology) are placed under "unclassified.

4.2.2 Taxonomic profiles of the bacterial community from Antarctic soil microcosms

Based on the pyrosequencing analysis of the Antarctic soil 16S DNA gene libraries, the OTU richness (Sobs) and diversity indices did not change significantly across the treatments (Table 4.4). It is important to note that the patterns observed based on T-RFLP analysis (Table 4.2) were different from pyrosequencing analysis. The community diversity and evenness obtained based on T-RFLP data was higher in untreated samples as opposed to treated samples. The bacterial community from Antarctic soil microcosms was dominated by five main phyla (Figure 4.5). Our untreated soil samples were dominated by *Proteobacteria* which accounted for more than 70 % of the sequences detected, followed by *Gemmatimonadetes* (4.31%), *Planctomycetes* (2.94%) and *Actinobacteria* (1.37%). Substantial effects of warming on the relative abundance of bacterial phyla were detected as the proportion of *Proteobacteria* increased up to 95% in microcosms incubated at 15°C. Conversely, *Planctomycetes* and *Actinobacteria* were significantly affected by warming treatments as the proportions of these phyla decreased from 15.7 % and 9.8 % respectively in untreated samples to less than 3% in treated samples. *Gemmatimonadetes* composition appeared to be varied across the treatments. The relative abundance of the top 25 bacterial families and genera detected in the Antarctic soil microcosms is shown in Figure 4.6. Unclassified *Erythrobacteraceae* belonging to the *Proteobacteria* phylum, accounted for 50% of all sequences, followed by unclassified *Rhodobacteraceae* (14.1%), unclassified *Alphaproteobacteria* (5.7%) and *Gemmatimonas* (5.5%). The other bacterial genera were less than 5%.

The warming treatment had significantly increased the proportion of *Erythrobacteraceae* from 39 % (in untreated samples) to more than 70 % (in microcosms incubated at 15°C for 8 and 12 weeks). The relative abundance of other genera varied across the treatments.

Table 4.4: Estimated diversity indices from 16S rRNA gene libraries of the Antarctic soil microcosms at different temperatures.

Group	Sequence Coverage	Sobs†	Inverse Simpson	Invsimpson (lci††,hci†††)	Shannon	Shannon (lci, hci)	Simpson evenness	Shannon evenness
Untreated	0.70	22.57	7.24	(4.51,18.59)	2.50	(2.14,2.86)	0.32	0.80
5°C4W	0.63	29.00	22.37	(13.27,71.02)	3.10	(2.84,3.36)	0.77	0.92
5°C8W	0.57	29.89	22.09	(13.07,73.14)	3.11	(2.83,3.38)	0.74	0.91
5°C12W	0.80	18.80	11.29	(7.93,19.63)	2.54	(2.28,2.81)	0.60	0.87
10°C 4W	0.68	22.66	6.70	(4.21,16.69)	2.47	(2.10,2.84)	0.29	0.79
10°C 8W	0.75	21.30	12.87	(8.49,26.67)	2.69	(2.42,2.96)	0.60	0.88
10°C12W	0.53	27.84	9.06	(5.51,26.54)	2.72	(2.35,3.09)	0.32	0.82
15°C 4W	0.65	23.38	5.17	(3.28,12.42)	2.36	(1.95,2.77)	0.22	0.75
15°C 8W	0.71	17.98	2.69	(1.90,4.71)	1.72	(1.27,2.17)	0.15	0.59
15°C12W	0.60	27.29	9.04	(5.34,31.72)	2.73	(2.37,3.10)	0.33	0.83

†Sobs, number of observed species
††Low confidence interval
†††High confidence interval

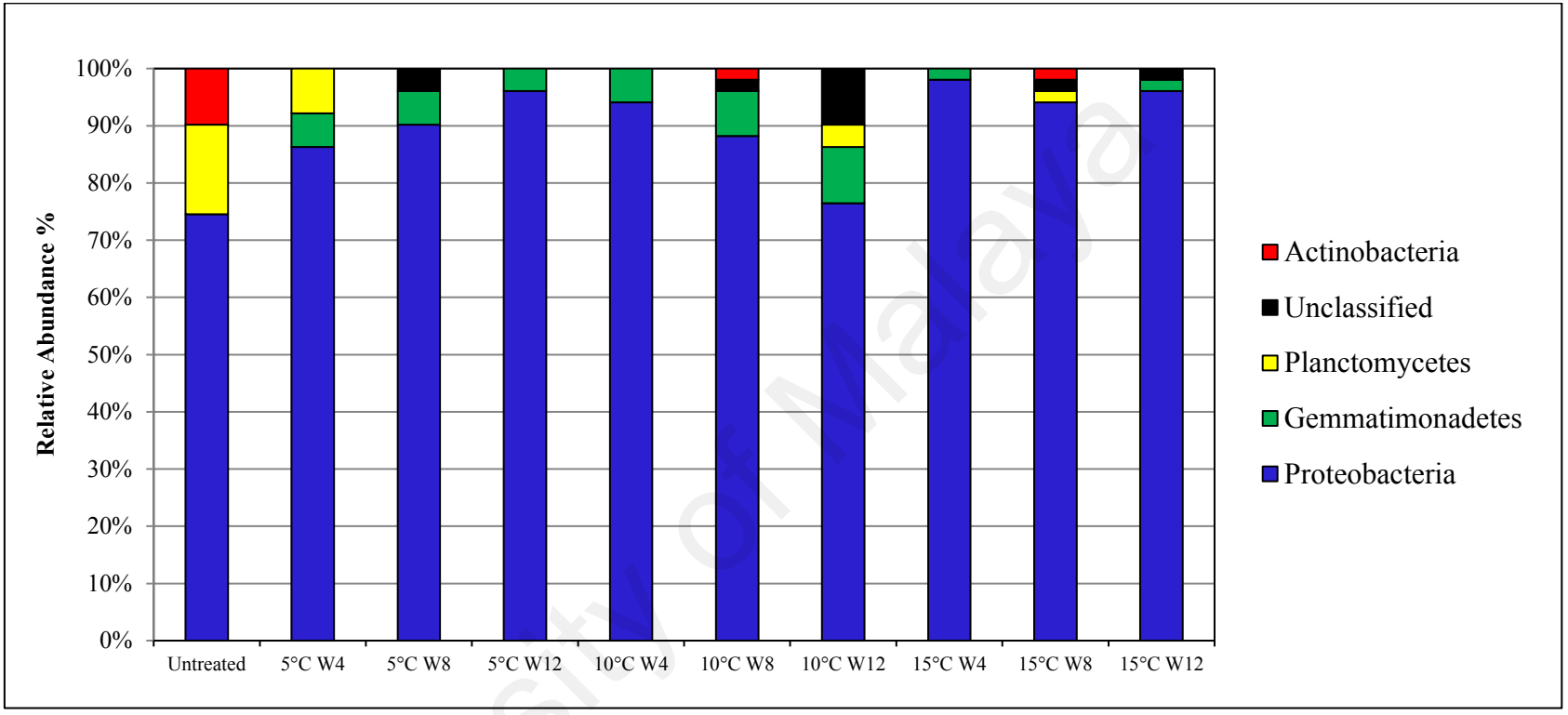


Figure 4.5: Relative abundance of bacterial phyla in Antarctic soil incubated at three different temperatures (5°C, 10°C and 15°C) and incubation periods (4, 8 and 12 weeks), identified by pyrosequencing analysis of the 16S rRNA gene. Taxonomic assignments of the 16S rRNA gene sequences to the phylum level were carried out by using the RDP-II Classifier. Sequences not aligned to any known phylum (at 97% homology) are placed under "unclassified."

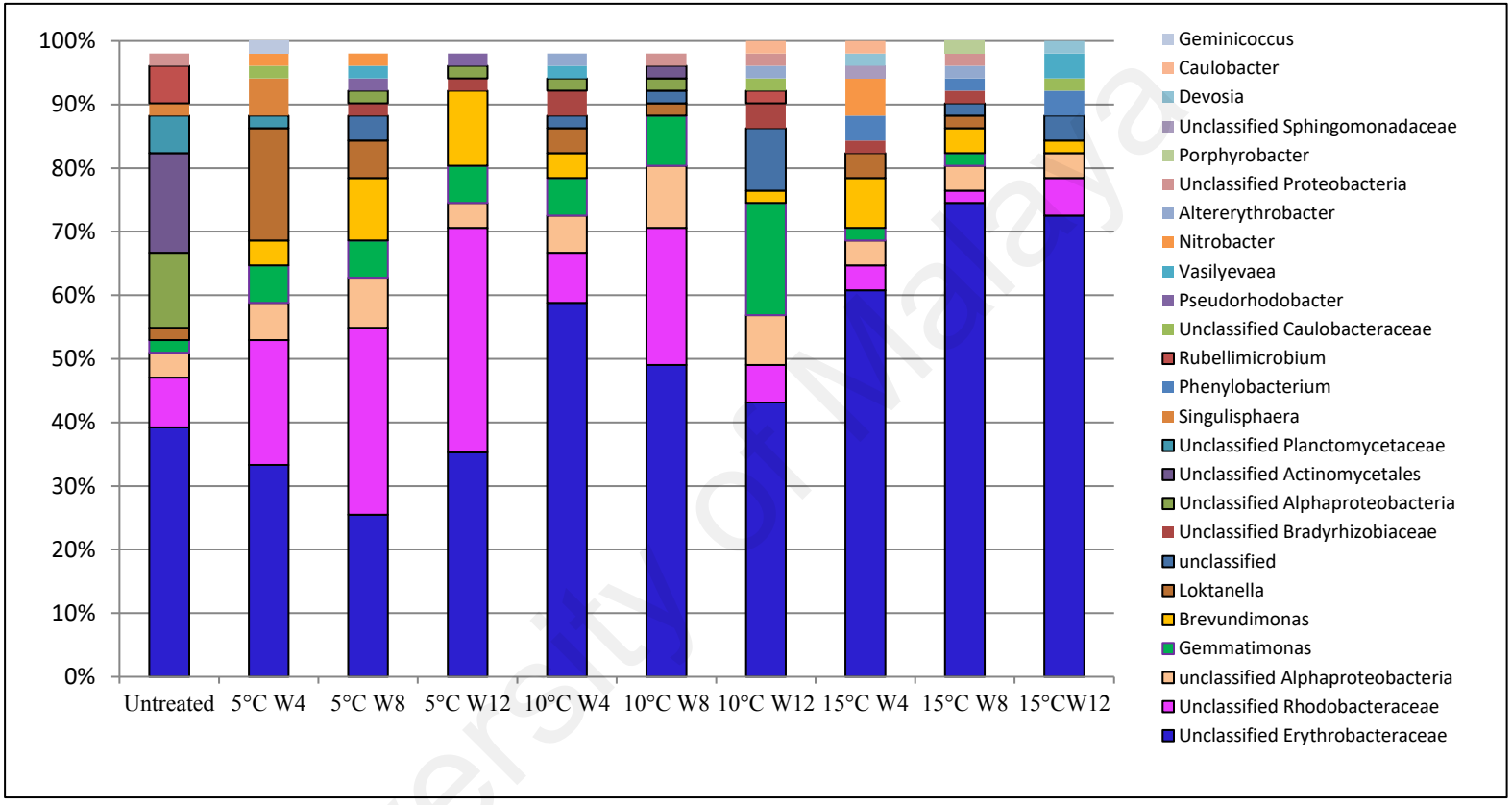


Figure 4.6: Relative abundance of dominant families and genera in Antarctic soil incubated at three different temperatures (5°C, 10°C and 15°C) and incubation periods (4, 8 and 12 weeks), identified by pyrosequencing analysis of the 16S rRNA gene. Taxonomic assignments of the 16S rRNA gene sequences to the genus level were carried out by using the RDP-II Classifier tool. Sequences not aligned to any known genus (at 97% homology) are placed under "unclassified."

4.3 Soil chemical properties in the microcosms

4.3.1 Effect of temperature and water content on some chemical properties of tropical soil microcosms

The tropical soil characteristics including pH, electrical conductivity (EC), water content, nitrate, nitrite, and phosphate content changed significantly across temperature (PERMANOVA pseudo-$F_{2,113}$= 3.18, P_{MC}= 0.03) and water levels (PERMANOVA pseudo-$F_{1,113}$= 8.20, P_{MC}=0.01). The changes in patterns of soil chemical properties across the treatments are illustrated in Fig. 4.7 (a-f). The pH of tropical soils was highly acidic (pH =3.3) (Figure 4.7a). On the other hand, EC showed considerable variability (EC= 84-123 µs/cm) (Figure 4.7b) with higher conductivity detected in week 4 microcosms in comparison to Week 1 and 2 microcosms. Regardless of the volume of water added, the water content in microcosms incubated at 35°C was found to be the lowest as compared to microcosms incubated at 25°C and 30°C (Figure 4.7c). Such observations could be due to the higher evaporation and metabolic rates in microcosms incubated at 35°C. In comparison to the low water content (LW) groups, the high water content (HW) groups recorded higher EC (Figure 4.7b) and phosphate content (Figure 4.7d). Soil nitrate content increased linearly with temperature in both LW and HW groups (Figure 4.7 e). In contrast, nitrite content remained low throughout the treatments in all microcosms (Figure 4.7f).

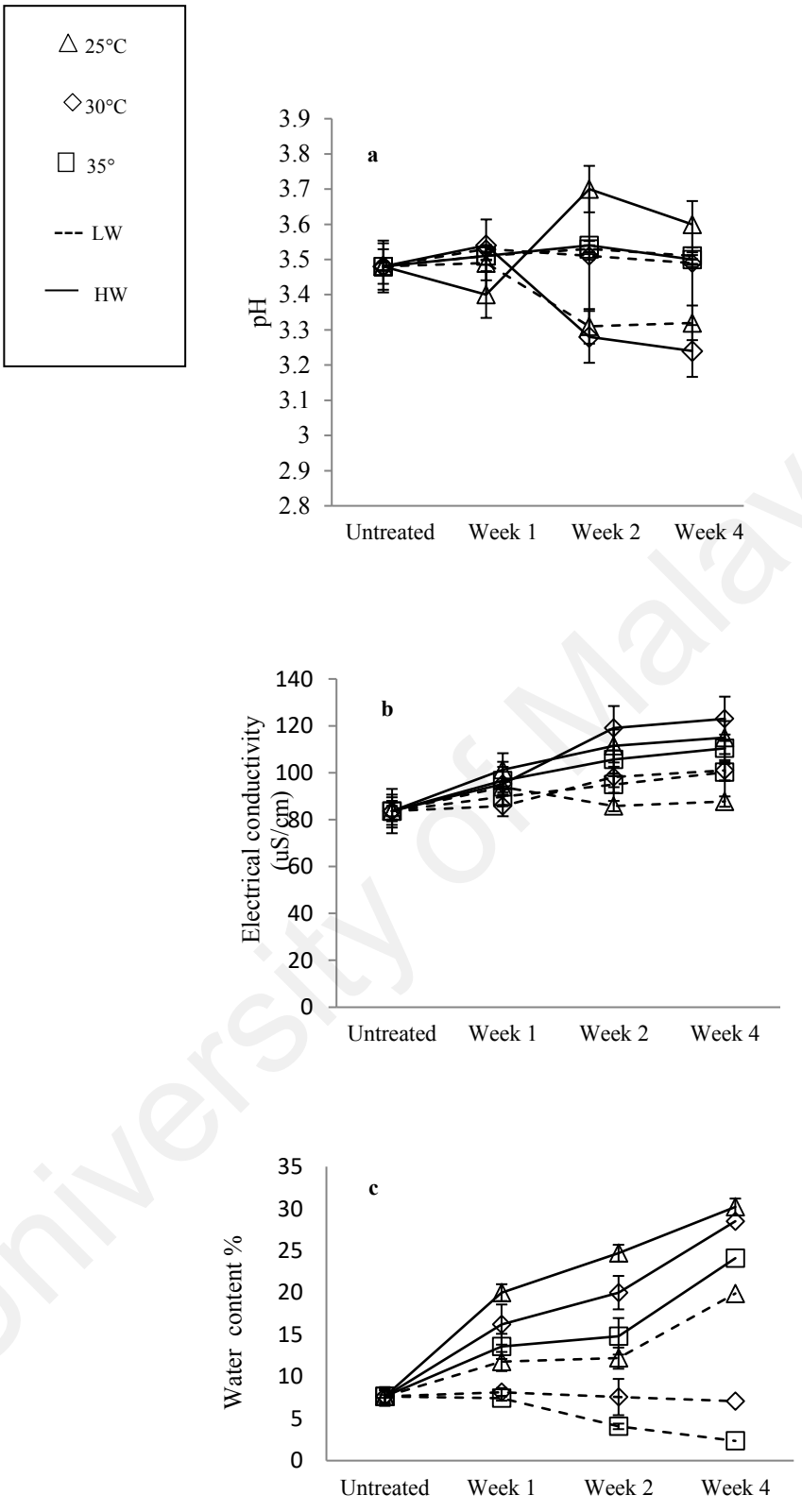


Figure 4.7: Changes in tropical (a) soil pH, (b) electrical conductivity, (c) water content, (d) phosphate content, (e) nitrate, (f) nitrite after 1, 2 and 4 weeks of incubation. Results represent the means ± standard error (n=6).

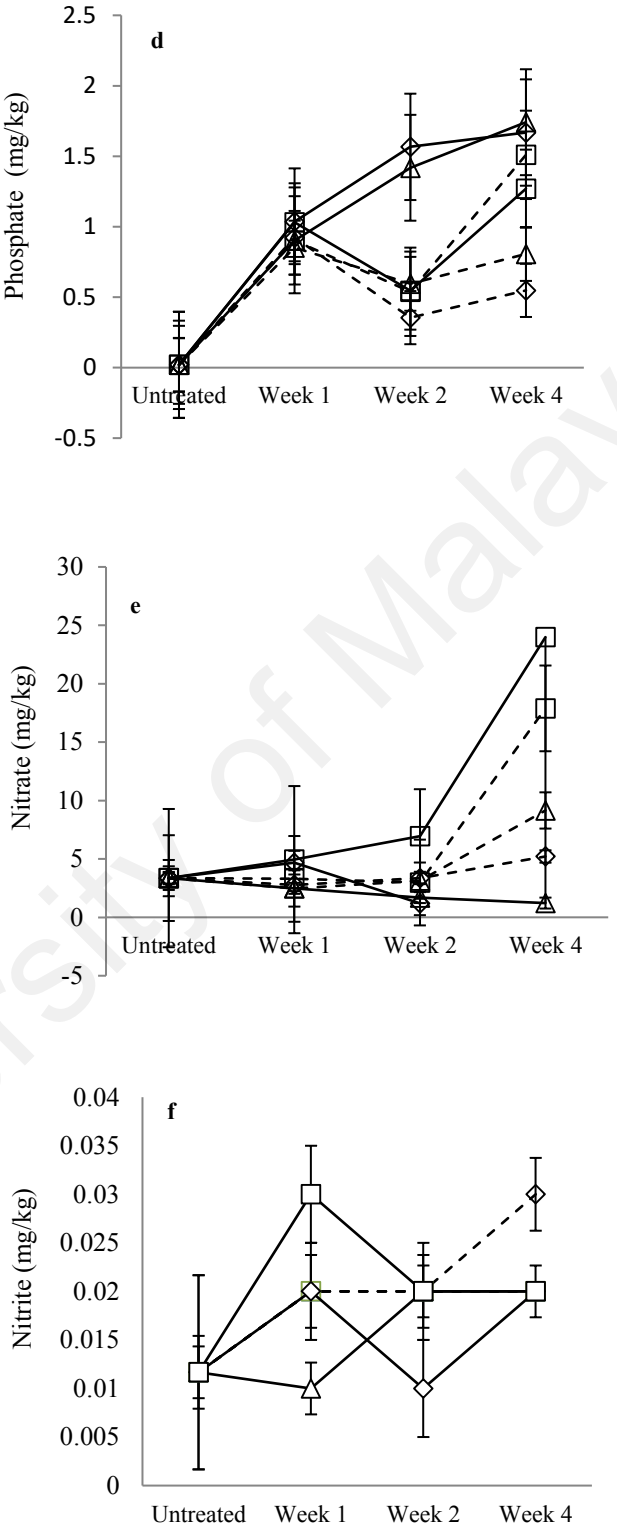


Figure 4.7, continued.

4.3.2 Effect of temperature and incubation periods on some chemical properties of Antarctic soil microcosms

The chemical characteristics of Antarctic soils changed with temperature (PERMANOVA pseudo-$F_{2,\ 49}$= 2.48, P_{MC}= 0.05) and increase of incubation periods (PERMANOVA pseudo-$F_{2,\ 49}$= 8.95, P_{MC}= 0.001). The changes in Antarctic soil characteristics across the treatments are shown in Fig. 4.8 (a-f). The soils were slightly acidic (pH = 6.0-6.4) (Figure 4.8a) and non-saline (EC= 40-70 μs cm^{-1}) (Figure 4.8b) throughout the duration of the treatments. The water content in the Antarctic soil microcosms decreased with the increase of incubation periods for all three temperatures tested and the lowest water content was recorded in microcosms incubated at 12 weeks (Figure 4.8c). The phosphate content showed a more complex pattern (Figure 4.8d). On the other hand, the highest nitrate content was observed in week 12 microcosms for all three temperatures tested (Figure 4.8e). Nitrite content in the studied soil samples was found to be low throughout the incubation (Figure 4.8f).

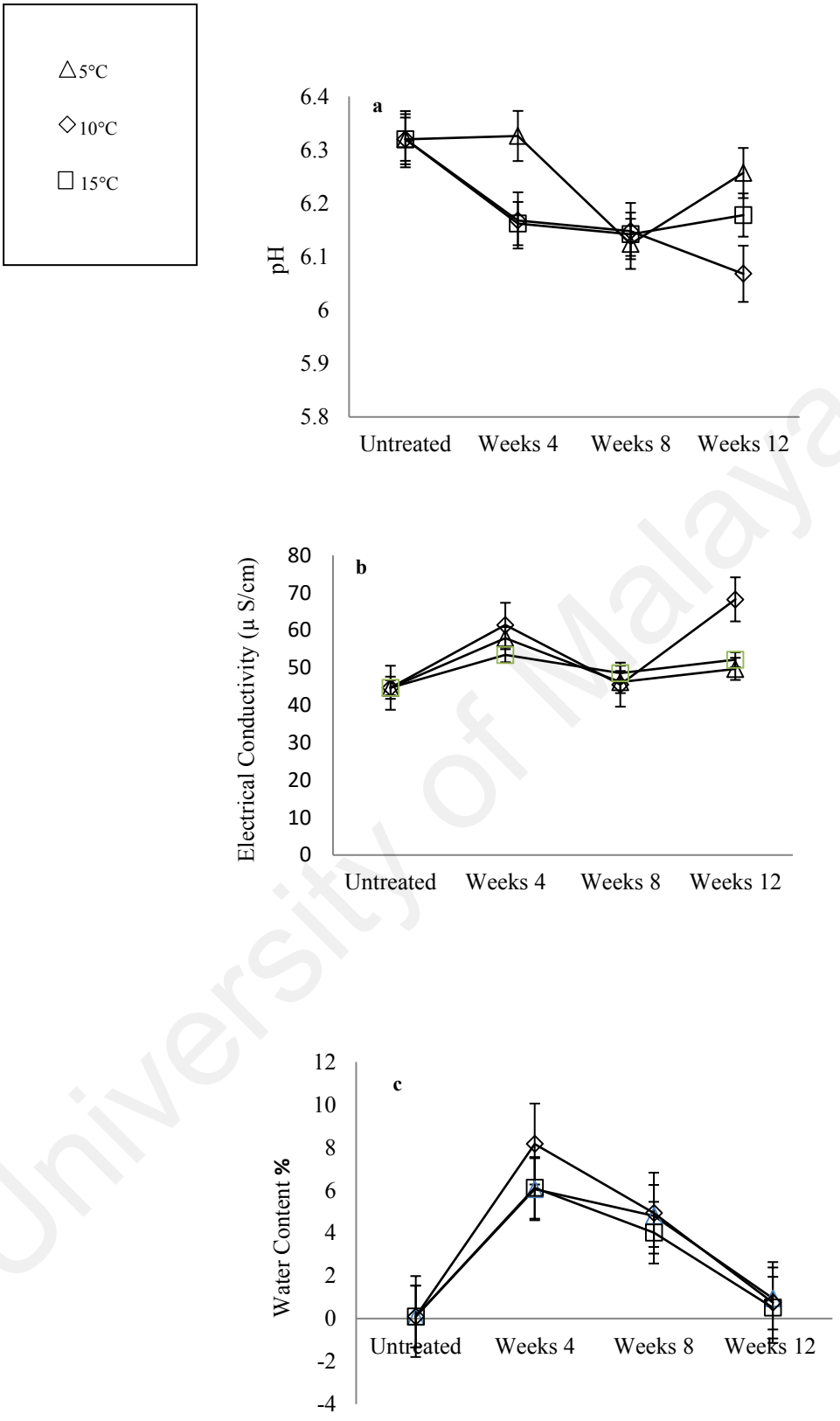


Figure 4.8: Changes in Antarctic (a) soil pH, (b) electrical conductivity, (c) water content, (d) phosphate content, (e) nitrate, (f) nitrite after 4, 8 and 12 weeks of incubation. Results represent the means ± standard error (n=5)

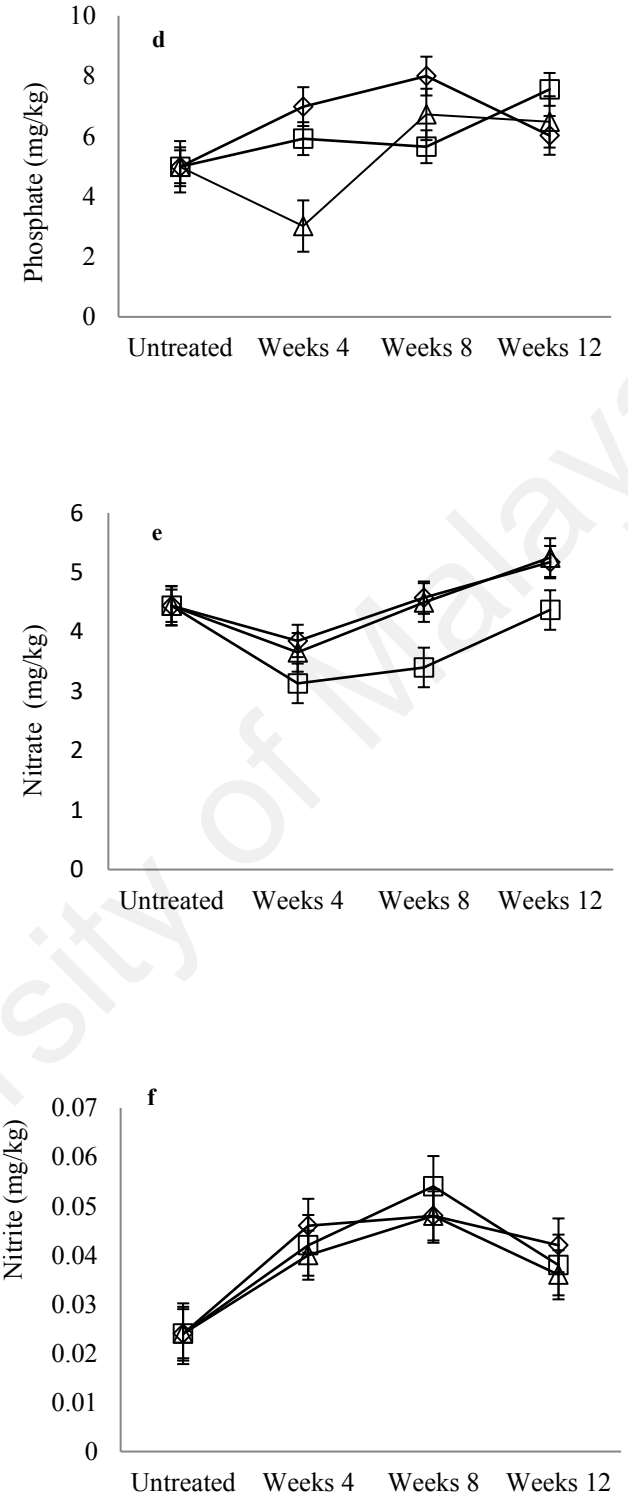


Figure 4.8, continued.

4.4 Associations between bacterial community structure and soil abiotic factors

4.4.1 Associations between tropical bacterial community structure and soil abiotic factors

The DISTLM was used to correlate the observed variation in the bacterial community structure in response to changes in temperature and water content with soil abiotic factors (Table 4.5). Among the six measured soil parameters, nitrite accounted for the highest correlation (R^2= 17%, *P*=0.064) with the observed T-RFLP derived bacterial community patterns. When the analysis is repeated using sequential test, a combination of electrical conductivity, water content, nitrite, nitrate and pH were selected as the best parameters. On the other hand, a weak correlation was detected between phosphate content and bacterial community structure.

4.4.2 Associations between Antarctic bacterial community structure and soil abiotic factors

The DISTLM analysis was also used to determine abiotic factors that strongly explain the changes observed in Antarctic bacterial community composition in response to warming treatments (Table 4.6). The result of this analysis revealed that nitrate was the single best predictor that correlated significantly with observed shifts in the bacterial community (R^2= 8.50%, *P*=0.01).

The cumulative variation was much lower (8.50 %) as nitrate was sole parameter selected as the best parameter in the sequential test. Based on the marginal test, a weak correlation between nitrite and bacterial community structures was identified as well.

4.5 Functional Genes Abundance

4.5.1 Functional genes abundance in the tropical soil microcosms

Six functional genes were studied from the tropical soil microcosms. PERMANOVA analysis revealed significant effects of temperature (Pseudo-$F_{2,113}$ = 11.279, P_{MC} = 0.001) and water content (Pseudo-$F_{1,113}$ = 4.24, P_{MC} = 0.001) on the functional genes abundance. A significant interaction was also identified between temperature and water content (Pseudo-$F_{2,113}$=1.81, P_{MC}=0.05). However, based on DISTLM, only two genes (*nifH* and *nosZ*) were significantly correlated with variation in bacterial community structure (P<0.05). When considered singly, each functional gene contributed to 2-3 % of the variation in bacterial assemblage pattern. The parsimonious model consisting *nifH* and *nosZ* showed an explanatory value of 4.80 % (Table 4.7). The changes in each gene copy numbers are shown in Table 4.8.

Table 4.5: DISTLM marginal and sequential test results for the T-RFLP derived tropical bacterial community patterns correlated to six soil abiotic factors.

Variables	Marginal Tests			Step-wise selection sequential tests			
	Pseudo-F	P	Prop	Pseudo-F	P	Prop	Cumulative variation
EC†	**8.53**	**0.001**	**0.071**	**8.531**	**0.001**	**0.071**	**0.0710**
Nitrate	**5.58**	**0.001**	**0.065**	**3.830**	**0.007**	**0.031**	**0.1018**
WC††	**7.78**	**0.001**	**0.020**	**3.691**	**0.009**	**0.029**	**0.1309**
pH	**1.47**	**0.179**	**0.013**	**2.876**	**0.022**	**0.022**	**0.1532**
Nitrite	2.33	0.054	0.024	2.172	0.064	0.017	0.1700
Phosphate	1.91	0.093	0.016				

Prop is the proportion of explained variation (999 permutations)
Bold values indicate the significance difference.
† Electrical conductivity (μS cm^{-1}) was measured in 1:5 (w/v) suspensions of soil in distilled water
†† Water content is expressed as the percentage of dry soil mass.

Table 4.6: DISTLM marginal and sequential test results for the T-RFLP derived Antarctic bacterial community patterns correlated to six soil abiotic factors.

Variables	Marginal Tests			Step-wise selection sequential tests			
	Pseudo -F	**P**	**Prop**	**Pseudo -F**	**P**	**Prop**	**Cumulative variation**
EC†	0.65	0.504	0.013				
Nitrate	**4.44**	**0.018**	**0.084**	**4.44**	**0.01**	**0.085**	**0.85**
WC††	1.12	0.368	0.023				
pH	0.41	0.582	0.008				
Nitrite	2.22	0.076	0.044				
Phosphate	0.86	0.446	0.018				

'Prop' is the proportion of explained variation (999 permutations).
Bold values indicate the significance difference
† Electrical conductivity (μS cm^{-1}) was measured in 1:5 (w/v) suspensions of soil in distilled water.
†† Water content is expressed as the percentage of dry soil mass.

Table 4.7: DISTLM marginal and sequential test results for the functional genes abundance correlated with the bacterial community structure in the tropical soil.

Variables	Marginal Tests			Step-wise selection sequential tests			
	Pseudo-F	**P**	**Prop**	**Pseudo-F**	**P**	**Prop**	**Cumulative variation**
nirS	1.225	0.255	0.011				
amoA	1.091	0.341	0.001				
nirK	1.753	0.123	0.015				
chiA	1.724	0.113	0.015				
nosZ	3.193	0.008	0.028	3.193	0.005	0.028	0.028
nifH	**2.306**	**0.015**	**0.024**	**2.306**	**0.036**	**0.020**	**0.048**

'Prop' is the proportion of explained variation (999 permutations) correlated with temperature and water variation
Bold values indicate the significance difference.

Table 4.8: Gene copy numbers of *nifH, amoA,nirK,nirS, nosZ*, and *chiA* in the tropical soils incubated at different temperatures (25°C, 30°C and 35°C) and water levels (LW, HW)

Treatments				Functional gene (copies/ng)					
Samples	Temp(°C)	Water (LW=2, HW=5)	Incubation (Weeks)	*nifH*	*amoA*	*nirK*	*chiA*	*nirS*	*nosZ*
C0	Untreated	0	0	2.51×10^{8}	1.36×10^{12}	1.41×10^{4}	1.8×10^{8}	5.8×10^{10}	2.13×10^{11}
25LW1	25	2	1	2.33×10^{6}	7.68×10^{12}	0.0108	3.44×10^{7}	1.96×10^{11}	5.53×10^{13}
25LW2	25	2	2	2.33×10^{6}	1.66×10^{11}	5.78×10^{4}	3.74×10^{8}	3.96×10^{11}	5.80×10^{11}
25LW4	25	2	4	4.76×10^{7}	1.58×10^{11}	8.39×10^{5}	9.64×10^{5}	1.74×10^{11}	3.78×10^{14}
25HW1	25	5	1	1.50×10^{9}	1.16×10^{9}	5.69×10^{15}	5.32×10^{4}	3.74×10^{12}	6.70×10^{12}
25HW2	25	5	2	1.67×10^{6}	6.28×10^{9}	3.29×10^{12}	3.41×10^{8}	3.80×10^{13}	2.98×10^{15}
25HW4	25	5	4	1.73×10^{7}	3.06×10^{11}	1.99×10^{13}	5.75×10^{6}	6.87×10^{11}	8.13×10^{13}
30LW1	30	2	1	6.90×10^{7}	2.83×10^{8}	4.58×10^{8}	8.89×10^{9}	8.45×10^{7}	3.36×10^{14}
30LW2	30	2	2	1.47×10^{8}	8.61×10^{22}	7.13×10^{8}	2.78×10^{2}	3.13×10^{8}	3.35×10^{10}
30LW4	30	2	4	2.04×10^{8}	6.56×10^{18}	3.29×10^{9}	1.77×10^{8}	1.56×10^{8}	7.53×10^{15}
30HW1	30	5	1	3.54×10^{8}	1.54×10^{5}	2.05×10^{9}	5×10^{9}	2.21×10^{9}	3.49×10^{12}
30HW2	30	5	2	2.08×10^{10}	2.54×10^{19}	4.01×10^{9}	7.75×10^{5}	1.72×10^{8}	3.48×10^{15}
30HW4	30	5	4	8.77×10^{9}	2.27×10^{14}	2.14×10^{10}	4.29×10^{8}	4.6×10^{8}	3.98×10^{10}
35LW1	35	2	1	3.69×10^{9}	6.23×10^{15}	2.56×10^{6}	6.36×10^{8}	1.8×10^{12}	3.35×10^{10}
35LW2	35	2	2	6.33×10^{9}	7.39×10^{10}	7.33×10^{8}	3.33×10^{8}	1.25×10^{14}	3.35×10^{10}
35LW4	35	2	4	4.43×10^{10}	5.21×10^{16}	6.17×10^{7}	5.35×10^{7}	3.03×10^{8}	3.35×10^{9}
35HW1	35	5	1	8.35×10^{10}	5.42×10^{12}	4.25×10^{5}	3.39×10^{6}	7.99×10^{4}	2.33×10^{9}

Treatments				Functional gene (copies/ng)					
Samples	Temp(°C)	Water (LW=2, HW=5)	Incubation (Weeks)	*nifH*	*amoA*	*nirK*	*chiA*	*nirS*	*nosZ*
35HW2	35	5	2	5.21×10^{11}	1.56×10^{15}	2.11×10^{6}	5.23×10^{5}	1.34×10^{7}	3.35×10^{9}
35HW4	35	5	4	7.33×10^{10}	1.16×10^{15}	5.64×10^{7}	3.21×10^{12}	5.82×10^{3}	3.35×10^{8}

Table 4.8, continued.

Note: 35HW4 means samples incubated at 35°C, high water content (5ml) for 4 weeks; C0 means untreated sample

4.5.2 Functional genes abundance in the Antarctic soil microcosms

In comparison to the tropical soil, *nirS* (cytochrome cd1 reductase) is not detectable from the Antarctic soil samples. Based on the PERMANOVA analysis, temperature (Pseudo-$F_{2,49}$ = 0.7285, P_{MC} = 0.651) and period of incubation (Pseudo-$F_{2,49}$ = 1.654, P_{MC} = 0.107) had no significant on the abundance of functional genes. However, the fluctuation in *nosZ* and *chiA* were significantly correlated with T-RFLP derived bacterial assemblage patterns (DISTLM P <0.05).

The sequential tests consisting of *nosZ* and *chiA* showed an explanatory value of 9.7 % (Table 4.9). The changes in each gene copy numbers are shown in Table (4.10).

Table 4.9: DISTLM marginal and sequential test results for the functional genes abundance correlated with the bacterial community structure in the Antarctic soil microcosms.

Variables	Marginal Tests			Step-wise selection sequential tests			
	Pseudo-F	**P**	**Prop**	**Pseudo-F**	**P**	**Prop**	**Cumulative variation**
amoA	0.910	0.481	0.018				
nifH	1.049	0.386	0.021				
nirK	0.773	0.558	0.015				
nosZ	**2.289**	**0.042**	**0.046**	**2.289**	**0.037**	**0.046**	**0.046**
chiA	2.210	0.075	0.044	2.700	0.023	0.052	0.097

'Prop' is the proportion of explained variation (999 permutations)
Bold values indicate the significance difference

Table 4.10: Gene copy numbers of *nifH, amoA, nirK, nirS, nosZ,* and *chiA* found in the Antarctic soils incubated at different temperatures (5°C, 10°C and 15°C) and incubation periods (4,8 and 12 weeks). Water added (0.5ml) was the same for all treatments.

Treatments				**Functional gene (copies/ng)**					
Samples	Temp (°C)	Water (ml)	Weeks	*nifH*	*amoA*	*nirK*	*chiA*	*nirS*	*nosZ*
C0	Untreated	0.5	0	4.3×10^{11}	3.44×10^{11}	2.55×10^{12}	1.75×10^{13}	NA	9.39×10^{9}
5°C M4	5	0.5	4	2×10^{11}	1.72×10^{11}	29502.28	58337725	NA	2.08×10^{11}
5°C M8	5	0.5	8	5.5×10^{13}	4.33×10^{13}	1065189	5.28×10^{12}	NA	4.2×10^{10}
5°CM12	5	0.5	12	6.2×10^{15}	8.6×10^{15}	2.18×10^{14}	1.87×10^{17}	NA	1.06×10^{10}
10°C M4	10	0.5	4	2×10^{10}	1.57×10^{10}	4.4×10^{7}	2.83×10^{21}	NA	2×10^{10}
10°C M8	10	0.5	8	3.4×10^{9}	3.24×10^{9}	2.68×10^{7}	1.78×10^{14}	NA	4.47×10^{10}
10°CM12	10	0.5	12	3.7×10^{12}	2.97×10^{12}	1.49×10^{8}	8.61×10^{14}	NA	3.2×10^{12}
15°C M4	15	0.5	4	2.4×10^{8}	1.33×10^{8}	2.36×10^{11}	2.63×10^{22}	NA	3.16×10^{10}
15°C M8	15	0.5	8	7.5×10^{12}	1.01×10^{14}	4646093	3.42×10^{13}	NA	3.89×10^{10}
15°CM12	15	0.5	12	9.6×10^{16}	1.66×10^{17}	73265.5	5.07×10^{10}	NA	3.01×10^{10}

Note :5°CM4 means samples incubated at 5°C for 4 weeks; NA means undetected; C0 means untreated samples.

CHAPTER 5 : DISCUSSION

5.1 T-RFLP analysis of bacterial community structure

5.1.1 T-RFLP analysis of bacterial community structure in the tropical soil microcosms

T-RFLP profiling of tropical soil microcosms indicated significant compositional shifts in bacterial community in response to warming and water content (Figure 4.1). For each temperature cluster, separation in accordance to moisture content was observed. The most significant shifts in structures of bacterial community occurred at 35°C as two distinct community groups in accordance to water treatments were observed. Similarly, Zogg *et al.* (1997) reported that 16 weeks of warming of soil from temperate region rapidly increased the ratio of Gram-positive over Gram-negative bacteria. Indeed, shifts in bacterial community structures in response to warming and watering from various ecosystems had been reported (Riah-Anglet *et al*., 2015; Wu *et al*., 2015; Xiong *et al*., 2014; Kuffner *et al*., 2012). These findings support the belief that changes in environmental factors (e.g. temperature) may alter the structure and composition of soil bacterial community.

In this study, it was observed that water enrichment (Pseudo-$F_{1,\ 113}$ = 9.71, P_{MC} = 0.001) impacted bacterial community structure greater than warming (Pseudo-$F_{2,113}$= 3.26, P_{MC} =0.001). Similarly, Waring & Hawkes (2015) found that the proportion of bacterial phyla such as *Proteobacteria* and *Elusimicrobia* from tropical forest soils had increased in response to the addition of water. Additionally, Zhang *et al*. (2013) reported significant compositional shifts in six different types of bacterial phyla attributed to high moisture content. Castro *et al.* (2010) also showed that the proportions of

Syntrophobacterales (phylum *Proteobacteria*) increased in waterlogged soils. Thus, these findings supported the central roles of water content in structuring the soil bacterial community and activity. A proposed mechanism that may explain the observed shifts is physiological acclimatization that governs the changes in bacterial community composition in response to environmental perturbation (Schimel *et al.,* 2007). For instance, dry soil conditions tend to reduce soil water potential, connectivity of soil particles and limit nutrient availability (Chodak *et al*., 2015; Uhlirova *et al*., 2005). In such conditions, bacterial community that can synthesize solutes such as polyols and amino acids might be preferentially selected. However, this selection imposes physiological stresses on the bacterial community as they are required to dispose of these solutes rapidly during high water potential (Schimel *et al*., 2007; Schjonning *et al*., 2003).

Besides, it has been shown that rupturing of the bacterial cells due to water pulses increases the soil carbon content which may, in turn, modify the nutrient availability (Fierer *et al.,* 2003). In contrast, wet soil conditions would deplete oxygen content and develop an anoxia state which favours facultative or obligate anaerobic bacteria (Drenovsky *et al*., 2010). Dormancy is another physiological strategy adopted by bacterial taxa during osmolyte demand (Manzoni *et al*., 2014) which allows them to sustain in a drought condition (Manzoni *et al*., 2014; Jones & Lennon, 2010). However, it has been reported that this strategy often results in inefficient utilization of nutrient available due to delay in the recovery processes (Placella *et al*., 2012). Such responses may well explain the observed shifts in bacterial community structures subjected to both low and high water content in the current study.

As shown in T-RFLP profiles, other than the effect of water, the degree of compositional shifts was also temperature dependent (Figure 4.1a). Also warming was found to decrease the community diversities and evenness dramatically (Table 4.1) as microcosms incubated at 35°C displayed the lowest community richness (Sobs) and evenness (Table 4.3). The results suggest that warming at higher temperatures intensifies soil dryness (Zhang *et al*., 2011; Allison & Martiny, 2008) and therefore select for bacterial subsets that are tolerant and resilient to both drought and elevated temperature (Wallenstein & Hall, 2011; Allison & Martiny, 2008). In line with our results, a microcosm study by (Wu *et al*., 2015) showed pronounced compositional shifts and a drastic reduction in bacterial diversities in soils incubated at the highest temperature treatment (40°C) as opposed to microcosms at lower temperatures. A possible explanation for the reduction in diversity is that warming accelerates the consumption of resources and increases internal competitions among members. Besides, warming may suppress the decomposition rate, bacterial activity and carbon cycling (Wu *et al*., 2015; Zhang *et al*., 2011; Hartley *et al*., 2009). Such phenomenon leads to survival and proliferation of a community with adaptative traits to altered condition (Riah-Anglet *et al*., 2015; Evans & Wallenstein, 2014; Deslippe *et al*., 2012). Hence, these studies corroborate the lowest number of species observed in our microcosms incubated at 35°C (Table 4.3).

Among the soil parameters studied, nitrite was the best predictor for the changes in bacterial community structure (Table 4.5). The activity of soil nitrifiers and denitrifiers are expected to increase with warming (Bai *et al*., 2013). Indeed, acceleration in the rate of nitrification with warming had been highlighted in a number of studies (Li *et al*., 2014; Gelfand & Yakir *et al*., 2008). Besides, it has been reported that 35°C is the optimum temperature for nitrifiers in warmer climatic regions (Bai *et al*., 2013; Dalias *et*

al., 2002). Such assertation, therefore, supports the linear increase in nitrate content with temperature, as detected in this study (Figure 4.7e). It could be that rapid conversion of nitrite into nitrate content with warming may attribute to a low nitrite content observed throughout the incubation periods (Figure 4.7f). However, additional works such as measurement of nitrogen gases are required for comprehensive comparisons of the rate of nitrification with soil warming. Soil pH indirectly affects the soil conditions by altering the nutrient availability, cationic metal availability and organic C characteristics (Lauber *et al*., 2009) and such changes may induce compositional shifts in certain bacterial taxa (e.g. *Acidobacteria*). Besides, the increase of soil pH may imposes physiological constraint on the bacterial community by selecting for taxa that are able to grow in acidic environments (Lauber *et al*., 2009).

5.1.2 T-RFLP analysis of bacterial community structure in the Antarctic soil microcosms

Though the effect of temperature was significant (Pseudo-$F_{2,49}$= 2.98, P_{MC} =0.005) on the bacterial community in the Antarctic soil microcosms, the compositional shifts were not as distinct as tropical soil microcosms (Figure 4.2). The results are expected as bacterial responses are closely associated with the prevailing environmental conditions (Evans & Wallenstein, 2011;Waldrop & Firestone, 2006; Fierer *et al*., 2003). There were evidence that bacterial community which experienced frequent disturbances such as water stress (Fierer *et al*., 2003) and freeze-thaw cycle (Rinnan *et al*., 2009) are more resistant to perturbation than those from constrained climatic conditions (Waldrop & Firestone, 2006). Indeed, several studies from the Arctic (Larsen *et al*., 2002) and Antarctica (Wallenstein & Hall, 2011; Rinnan *et al*., 2009; Bokhorst *et al*., 2007; Larsen *et al*., 2002) have highlighted the insensitivity of soil bacterial community in response

to warming treatments. It could be that the bacterial community from the Antarctic ecosystems may compose of a higher proportion of temperature generalists (species that can tolerate broader ranges of temperature and water content) than specialists (species adapted to specific conditions and have narrow functional capacities). A community of generalists can endure thermal fluctuations (Wallenstein & Hall, 2011) and therefore may require a longer period to respond to the changes in temperatures. Consistent with this speculation, we observed that period of incubation (Pseudo-$F_{2,49}$= 10.95, P_{MC} =0.001) had caused a greater effect on the bacterial community structure than temperature (Pseudo-$F_{2,49}$= 2.98, P_{MC} =0.005).

Similarly, it has been shown that long-term soil warming (> 2 years) was needed to induce community shift in polar regions (Deslippe *et al*., 2012; Yergeau *et al*., 2012; Rinnan *et al*., 2009). Furthermore, other factors such as vegetation density (Yergeau *et al*., 2007), water content (Newsham *et al*., 2010) and nutrient availability (Dennis *et al*., 2013) seem to influence and shape the local Antarctic bacterial diversity strongly than temperature. For instance, Yergeau *et al.* (2007) observed that vegetated plots from the maritime Antarctic harbored higher level of bacterial diversity as opposed to non-vegetated plots. The author proposed that the presence of vegetation could decrease the severity of soil environmental conditions (e.g. increase soil water content) and selection pressure thereby resulting in a greater bacterial richness. Newsham *et al.* (2010) indicated that changes in moisture content highly influenced soil bacterial community from the maritime Antarctic. It has also been shown that the combined effects of warming (open top chambers, OTC) and nutrient addition (e.g. glycine) outweighed the impact of warming alone in reducing the ratio of Gram-positive bacteria in sites from Mars Oasis (Dennis *et al.,* 2013). These studies, therefore, confirmed that direct effects of warming were less significant than indirect effects (e.g. vegetation density) on the

bacterial community from Antarctic soils thereby supported the assertion by Vishniac (1993).

In general, the bacterial diversity in Antarctic ecosystems is recorded to be low as a result of extreme climatic conditions (Smith *et al*., 2006). For instance, bacteria diversity was found to be low in soils collected from Signy Island (Yanai *et al*., 2004). Similarly, we also found that the bacterial diversity in our Antarctic soil microcosms remained low throughout the treatments (Table 4.4). Conversely, several molecular studies revealed high levels of bacterial diversity in Antarctic ecosystems (Wang *et al*., 2015; Yergeau *et al*., 2012; Teixeira *et al*., 2010; Chong *et al*., 2009). It is not surprising as bacterial diversity from Antarctic soils indicates high levels of spatial and regional heterogeneity (Van Horn *et al*., 2013; Yergeau *et al*., 2007), suggesting that the diversity is strongly structured by local climatic drivers (Barrett *et al*., 2006). Nitrate content was the sole predictor that significantly correlated ($P < 0.05$) with variation in bacterial community structure (Table 4.6). The result is in agreement with a study from King George Island of Antarctica (Wang *et al*., 2015). As discussed earlier, warming may accelerate nitrogen mineralization thereby increase the availability of nitrate content (Figure 4.8 e).

5.2 Alpha diversity of bacterial community

5.2.1 Alpha diversity of bacterial community from tropical soil microcosms

The community diversity estimated based on the T-RFLP data for tropical soil microcosms indicated higher number of species (S), total number of individuals (N), richness and evenness in untreated samples than treated samples (Table 4.1). Similar trends were observed for community diversity recovered using MiSeq sequencing (Table 4.3). In this study, it is noted that community richness and diversity obtained from T-

RFLP data were much higher than data generated by high-throughput sequencing. Such variation could be due to some factors. Firstly, it is important to note that in this study all replicates were included for T-RFLP analysis while only some replicates were analyzed for the high-throughput sequencing as inclusion of all replicates for sequencing will be costly. Secondly, as statistically evident, high variability in terms of community evenness and richness observed among replicates in a particular treatment may contribute to variation in T-RFLP data. Therefore, community parameters (e.g. evenness) estimated from T-RFLP data only allow a coarse comparison of the effect of treatments on the samples studied. Thirdly, while number of replicates is one issue, PCR-associated bias and overestimation of diversity due to incomplete digestion as proposed by Kirk *et al*. (2004) and Osborn *et al*. (2000) may also cause variation in data obtained from T-RFLP.

5.2.2 Alpha diversity of bacterial community from Antarctic soil microcosms

The community diversity, evenness, and richness obtained from pyrosequencing analysis were much lower than T-RFLP data (Table 4.4). This is surprising as it has been reported that Antarctic bacterial community diversity recovered using pyrosequencing was much greater (Wang *et al*., 2015; Yergeau *et al*., 2012; Teixeira *et al*., 2010). The observed differences in community parameters (e.g. community richness) in this study between T-RFLP and pyrosequencing data are in agreement with a microbial study conducted in several sites from the Antarctic ecosystem (Van Dorst *et al*., 2014). Additionally, the authors also detected an insignificant correlation between T-RFLP and pyrosequencing (p>0.05). Besides, comparisons of cultivation and pyrosequencing analysis revealed that the latter was unable to identify and detect culturable bacterial community attributed by shorter sequencing depth (Tytgat *et al.*, 2014). In this study,

the Good coverage (the percentage of individuals sampled in a bacterial community) that provide the similar information as rarefaction analysis was estimated for each sample (Table 4.3 & 4.4). It is warrant to note that the sampling intensity for Antarctic microcosms was lower than 90 % and this could lead to underestimation of true diversity (Lemos *et al*., 2011). Consistently, community richness was low in Antarctic microcosms (Table 4.4) Nevertheless, low sequence coverage (< 80 %) was also reported in a study assessing the bacterial community diversity from Antarctic soil based on pyrosequencing analysis at 97% of sequence homology (Lemos *et al*., 2011). The authors concluded that more than 90% of sequence coverage is needed for analysis based on shared OTUs as vast numbers of species are present in low abundance (Martiny *et al*., 2006). Based on the previous study, it could be speculated that analysis of Antarctic soil using a platform with deeper sequencing depth (e.g. MiSeq or Hiseq platform) is essential to capture the actual diversity of the bacterial community in Antarctic soil. Besides, in this study only several replicates were analyzed based on the high-throughput sequencing and this could also results in underestimation of community richness in an ecosystem that is highly complex like soil. Since the bacterial diversity in Antarctic soils exhibited high levels of spatial and regional heterogeneity (Van Horn *et al*., 2013; Yergeau *et al*., 2007), a further study with higher number of replicates from several different locations therefore is required to increase the reliability in estimation of bacterial community attributes (e.g. richness and evenness).

5.3 Changes in the relative abundance of bacterial phyla

5.3.1 Changes in the relative abundance of bacterial phyla in the tropical soil microcosms

Based on the T-RFLP profiles, it was observed that bacterial communities from Week 1 microcosm was only impacted by water stress but not by temperature (Figure 4.1b). Community composition from Week 2 microcosms indicated the largest variation (Figure 4.1c), suggesting accomplishment of the environmental threshold which subsequently promotes community shifts from "specialists" toward "generalists" (Chong *et al*., 2015; Fierer, *et al*., 2012; Rinnan *et al*., 2009). Therefore, Week 2 microcosms (time point in which greatest community separation was observed-Figure 4.1c) were selected for Illumina next generation sequencing to evaluate the interactive effects of warming and watering on the bacterial community composition.

Interestingly, the effect of treatments was not apparent in community profiles incubated for four weeks, suggesting compositional recovery and adaptation to the treatments subjected (Figure 4.1d). This observation suggests that recovery of bacterial community to environmental stress might have occurred within four weeks. Norris *et al*. (2002) showed that bacterial community from the temperate region was able to recover compositionally and stabilize within three weeks of warming (35 to 65°C).

In our study, the tropical soil was dominated by *Firmicutes* followed by *Acidobacteria*, *Proteobacteria* and *Actinobacteria* (Figure 4.3). These phyla are commonly found in soils from various ecosystems (Chodak *et al*., 2015; Aislabie & Deslippe, 2013; Teixeira *et al*., 2010; Lauber *et al*., 2009; Janssen, 2006). The changes

in bacterial community structures detected here were consistent with the different survival mechanisms employed by the members of each phylum (Figure 4.3). Specifically, *Acidobacteria, Actinobacteria* and *Firmicutes* identified here have been proposed as k-strategists or oligotroph while *Proteobacteria* as r-strategist or copiotroph (Cleveland *et al*., 2007; Fierer *et al*., 2007). It has been shown that oligotroph tends to be prevalent in nutrient-poor soils (Philippot *et al*., 2009; Ward *et al*., 2009). Conversely, copiotroph is regarded as a fast-growing bacterial group with the ability to degrade carbon compounds in nutrient-rich soil (Chodak *et al*., 2015; Pascault *et al*., 2013; Fierer *et al*., 2007). The presence of both types of bacterial groups in untreated soil samples (Figure 4.3) therefore proves that the soil ecosystem is highly complex and diversified (Torsvik & Øvreås, 2002). Notably, warming at 35°C showed significant compositional shifts toward Gram-positive bacteria (Figure 4.3), as had been reported elsewhere (Wu *et al*., 2010; Frey *et al*., 2008). One plausible explanation is that Gram-positive bacteria possess thicker and hardier peptidoglycan cell walls that confer higher survivability of this group under osmotic stress (Lennon *et al*., 2012; Aislabie *et al*., 2009; Zhang & Xu, 2008; Schimel *et al*., 2007). Following this speculation, the relative abundance of *Firmicutes* was found to increase dramatically in microcosms incubated at 35°C (Figure 4.3). Nevertheless, it was reported that 15 months of field warming at (+ 1 and + 2°C) beyond ambient could significantly reduce the proportion of *Firmicutes* in temperate soils (Xiong *et al.,* 2014). Ecosystem types and the experimental approaches employed could attribute such contradictory findings. Singh *et al*. (2007) has described the ability of *Firmicutes* to sporulate under adverse conditions and such adaptive traits could decrease the metabolic rate and enhance their resistance under warming and drought conditions (Mandic-Mulec *et al.*, 2015). The ability to resist drought have also made this phylum capable of long distance dispersal (Acosta-Martínez *et al*., 2015). *Firmicutes* is frequently detected in soils from Antarctic, arid, desert and temperate ecosystems

(Chodak *et al*., 2015; Teixeira *et al*., 2010; Aislabie *et al*., 2009; Chowdhury *et al*., 2009).

At the genus level, *Bacillus* (phylum *Firmicutes*) is known as a phosphate solubilizer in soils (Chung *et al.,* 2005; Rodriguez & Fraga, 1999) therefore able to thrive in phosphate-limited environments as compared to other genera (Cleveland *et al*., 2007). These reports further support our data as *Bacillus* showed the highest prevalence in microcosms incubated at 35°C, which recorded low amount of phosphate content (Figure 4.4). Besides, most of *Bacillus* are regarded as anaerobic bacteria thereby able to thrive in oxygen-limited environments (Logan & Halket, 2011). This behaviour attributed to the high prevalence of this group in microcosms treated with high water level (Figure 4.4).

The second most abundant phylum in our tropical microcosms was *Acidobacteria*. To date, 26 subdivisions of *Acidobacteria* were identified globally (Barns *et al*., 2007). Although *Acidobacteria* is a cosmopolitan group present in most soils (DeAngelis *et al*., 2010; Kielak *et al*., 2009; Ward *et al*., 2009), the exact ecological roles of this phylum are still unknown due to the lack of pure isolates (Jones *et al*., 2009). *Acidobacteria* possesses ATP-binding cassette (ABC) which allows them to absorb nutrients from resource-poor soils and contain genes that inhibit them from synthesizing protein and DNA under stressful condition (Chong *et al*., 2009; Ward *et al*., 2009). It has been shown that this phylum tends to be prevalent in nutrient-poor soils (Ward *et al*., 2009; Fierer *et al*., 2007). Such behaviors may explain the high occurrence of this population in tropical soil samples which contain a low amount of nitrite content (Figure 4.7f).

Surprisingly, we found that *Acidobacteria* subgroups 10 and 13 were the most abundant in our soil samples opposing the observation of commonly reported subgroups such as *Gp 1, Gp 2* and *Gp 6* (Naether *et al.,* 2012; Janssen, 2006). In our study, we found that the proportion of *Acidobacteria* decreased with water addition (Figure 4.3 & 4.4). Our results are in agreement with findings by Castro *et al*. (2010) and Ward *et al*. (2009) which also revealed a significant reduction in the relative abundance of this phylum in response to water addition. A high proportion of *Gp 13* was detected in microcosms with lower water content (2 ml) which also recorded a low amount of nitrate and phosphate content. Similarly, *Acidobacteria* was found to deprive with carbon addition, soil organic matters, as well as nutrient mineralization rates (Cleveland *et al.,* 2007). We observed that this bacterial phylum declined significantly in our microcosms incubated at 35°C. This observation was not surprising as this population is reported to be highly vulnerable towards warming treatments (Riah-Anglet *et al.*, 2015).

Apart from *Firmicutes* and *Acidobacteria*, our tropical soil samples also contain *Proteobacteria*; a ubiquitous group found globally (Aislabie & Deslippe, 2013; Spain *et al*., 2009). This phylum has been reported as one of the largest groups that can survive in both aerobic and anaerobic environmental conditions (Gupta, 2000). In general, *Proteobacteria* composed of 5 different subphyla including *Alphaproteobacteria*, *Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria* and *Epsilonproteobacteria* (Gupta, 2000). Most of the subphyla are Gram-negative bacteria and found to be very vulnerable to warming and variation in water content (Wu *et al*., 2015; Barnard *et al*., 2013; Schimel *et al*., 2007; Singh *et al*., 2007; Lu *et al*., 2006; Uhlirova *et al*., 2005). For instance, six weeks of laboratory soil incubation led to significant reduction in the proportion of Gram-negative bacteria with a concomitant growth of Gram-positive bacteria (Biasi *et al*. (2005). This evidence supported our results of drastic reduction of *Proteobacteria* in microcosms incubated at 35°C.

At the genus level, the similar patterns were also observed. The genus *Aquicella* belongs to the *Gammaproteobacteria* severely decreased with increase of temperature. Chodak *et al*. (2015) reported that water exclusion for eight weeks severely reduced the relative abundance of *Gammaproteobacteri*a in temperate soils. It has been reported that *Gammaproteobacteria* are able to degrade carbon, aliphatic and aromatic compounds (Zhang *et al*., 2016; Cleveland *et al*., 2007; Padmanabhan *et al*., 2003). Therefore, shifts of *Proteobacteria* and the associated subphyla may reflect an alteration in the nutrients content and abiotic factors in soil ecosystems (Xiong *et al.,* 2014).

A small number of sequences detected in the current study were affiliated with *Actinobacteria*. The lower abundance of *Actinobacteria* detected in our tropical microcosms could be attributed to the high abundance of *Acidobacteria* as these groups are known to share similar niches (Sheik *et al.,* 2011). *Actinobacteria* are involved in decomposition process in soil (Kopecky *et al.,* 2011) and frequently isolated from extreme habitats such as dry volcanic soils and deserts (Costello *et al*., 2008).

5.3.2 Changes in the relative abundance of bacterial phyla in the Antarctic soil microcosms

Based on the T-RFLP profiles, it was observed that the bacterial composition from Week 12 microcosms separated along PCO 2. At this point, the variability within group in response to temperature was lesser as clustering could not be observed (Figure 4.2 d). The results suggest that recovery of bacterial community in response to temperature might have occurred within 12 weeks. As discussed earlier, compositional recovery and stabilization of bacterial community from temperate region occurred within three weeks of warming period (35 to 65°C) (Norris *et al*., 2012).

The 16S pyrosequencing analysis of the Antarctic soil microcosms applied in this study showed that the bacterial composition was relatively stable as shifts in the proportion of major phyla in response to warming were only detected at 15°C (Figure 4.2). A possible reason for the observed patterns is that the shift in bacterial community structure was likely due to the intensity of warming. For instance, Zogg *et al*. (1997) noted significant changes in the structure of bacterial community incubated at higher temperatures (>15°C) while only minor compositional shifts were detected at low temperatures (5-15°C) as observed in this study. Furthermore, it has been reported that soil temperatures in Antarctic ecosystems are highly variable and daily fluctuations of >20°C are common (Cary *et al*., 2010; Barrett *et al*., 2008; Aislabie *et al*., 2006). Such changes are much greater than the magnitude of warming treatments (5°C) utilized in this study thereby explained the small shifts in the structure of bacterial community observed in our microcosms.

It is known that the prokaryote diversity in Antarctic soils is highly heterogeneous and influenced by factors like plant communities and nutrient availability (Teixeira *et al*., 2010; Yergeau & Kowalchuk, 2008). For instance, analysis of bacterial community from the Dry Valleys showed the prevalence of *Deinococcus-Thermus, Gemmatimonadetes* (Cary *et al*., 2010) and, to a lesser extent, *Proteobacteria* (Aislabie *et al*., 2009; Smith *et al*., 2006). In contrast, other Antarctic soils, particularly from the Antarctic Peninsula are dominated by *Proteobacteria* (Yergeau *et al*., 2007). Notwithstanding the differences, several bacterial phyla such as *Actinobacteria*, *Acidobacteria, Bacteriodetes, Proteobacteria* and *Cyanobacteria* are frequently detected across Antarctic regions (Chong *et al*., 2009; Aislabie *et al*., 2006). In the present study,

bacteria in Antarctic soils were dominated by Gram-negative phyla such as *Proteobacteria* followed by *Gemmatimonadetes*, *Planctomycetes* and *Actinobacteria* (Gram-positive bacteria) (Figure 4.5).

High abundance of *Proteobacteria* (> 70%) was detected in our untreated soil samples (Figure 4.5). This finding is consistent with other studies conducted in Antarctic ecosystems (Bajerski & Wagner, 2013; Han *et al*., 2013; Yergeau, *et al*., 2007; Saul *et al*., 2005) . Casey Station (Windmill Island) located in the coastal region is considered to receive marine input (e.g. high water content) and support large plant communities that composed of 36 species of lichens and five species of bryophytes (Melick & Seppelt, 1997). Further, it has been shown that soil samples in the vicinity of Casey Station particularly from Antarctic Specially Protected Area (ASPA) 135 and 136 are enriched with nutrients contributed by birds and Adelie penguin colony (Chong *et al*., 2010). Soils colonized by penguins are categorized as ornithogenic soils due to high levels of nutrient content (Balks *et al.,* 2013) and *Proteobacteria* was found to dominate these soils (Yergeau *et al*., 2012; Aislabie *et al*., 2009). Besides, soils from Oil Spill site around Casey Station are subjected to hydrocarbon contamination due to oil spillage occurred in September 1999 (Snape *et al*., 2006) that could lead to the enrichment of hydrocarbon-degrading bacteria such as *Proteobacteria* and *Actinobacteria* (Aislabie *et al*., 2006; Saul *et al*., 2005). Such factors may contribute to the high occurrence of *Proteobacteria* in soils around Casey Station. The support for this assertion comes from a finding by Chong *et al*. (2009). The authors found that soil bacteria between ASPA 136 (a protected area) and Wilkes Tip (an abandoned waste disposal site) are highly similar attributed by dispersal of soils and bacteria between the sites by wind and movement of penguins. Although the Antarctic soils used to establish microcosms in this study are non hydrocarbon-contaminated and exhibited the low amount of nitrate, nitrite

and water content, the former phenomenon such as dispersal of soil bacteria may explain the high occurrence of *Proteobacteria* in our untreated soil samples.

In this study, taxonomic assignments of the 16S rRNA gene sequences at phylum level indicated an apparent increase in the relative abundance of *Proteobacteria* with the elevation of temperatures (Figure 4.5). At the genus level, microcosms were dominated by *Erythrobacteraceae* (a member of *Alphaproteobacteria*) and this class tends to grow rapidly at the higher temperature (15°C) (Fig 4.6). Previous studies have shown the dominance of *Proteobacteria* over *Acidobacteria* in response to warming (Xiong *et al*., 2014; Yergeau *et al*., 2012). This is because warming was found to increase soil carbon content that subsequently favours copiotroph bacteria (e.g. *Proteobacteria*) over oligotrophs (e.g. *Acidobacteria*) (Thomson *et al*., 2010; Fierer *et al*., 2007). Similarly, Xiong *et al*. (2014) observed a linear increase in soil nitrate and ammonium content with the ratio of *Alphaproteobacteria*. For Antarctic soil microcosms, a concomitant increase in nitrate content with the relative abundance of *Proteobacteria* was observed with temperature upshifts. Therefore, increase in the ratio of *Alphaproteobacteria*-to-*Acidobacteria* is often related to changes in nutrient content in soil ecosystems (Thomson *et al.,* 2010).

The proportion of bacterial phyla such as *Planctomycetes* and *Gemmatimonadetes* varied across the treatments (Fig 4.5). Due to lack of cultured representatives of these groups, the knowledge of their physiology and ecological roles in soil ecosystems remained scarce (Aislabie & Deslippe, 2013; Bouskill *et al*., 2012). However, these phyla were found to be prevalent in Antarctic cryoconite holes (Christner *et al.,* 2003) and hyperarid regions of the Atacama Desert (De Bruyn *et al.,* 2011). The ability of *Gemmatimonadetes* to adapt to low water content has been proposed by De Bruyn *et al*.

(2011). This assertion therefore supports the presence of *Gemmatimonadetes* in Antarctic soil microcosms which have less than 10 % of water content (Figure 4.8 e).

Planctomycetes are regarded as slow-growing aerobic bacteria with a few unique features. This group possesses proteinaceous instead of peptidoglycan cell wall (Jeske *et al*., 2015), shorter 5S rRNA (Fuerst, 1995) and their inner membranes are divided into several compartments (Buckley & Durbin, 2006). Though some studies have highlighted the responses of this phylum towards water fluctuation (e.g. drought) (Chodak *et al*., 2015; Bouskill *et al*., 2012), studies on the effect of temperature on this taxon are still limited. Sheik *et al*. (2011) found that the population size of *Planctomycetes* decreased severely in response to warming treatments. Similarly, in this study, the relative abundance of this community was found to be higher in untreated samples than treated samples (Figure 4.5), suggesting high vulnerability of this taxon to warming.

Gemmatimonadetes are categorized as one of the main bacterial phyla found in semiarid and arid soils (Costello *et al*., 2008; Kim *et al*., 2008). At the genus level, *Gemmatimonas* has been detected in Antarctic soil microcosms (Figure 4.6). Further, this group proliferated in microcosms incubated at 5°C and 10°C and reduced at 15°C accentuating their adaptability to lower temperatures.

5.4 Functional gene abundance and the association with changes in bacterial community

5.4.1 Functional gene abundance and the association with changes in bacterial community from tropical soil microcosms

In present study, it is important to note that changes in community composition were measured at the DNA level. Since DNA occurs in both active and dead cells, changes in DNA may not directly relate to ecosystem processes and environmental factors (Nocker & Camper, 2009). Thus, research based on the RNA quantification is necessary as it measures the active members that drive ecological processes (Nocker & Camper, 2009; Vestergård *et al.,* 2008). Six functional genes (*nifH, amoA, nirK, nirS, nosZ* and *chiA*) were analyzed from tropical soil microcosms. Significant effects of temperature (Pseudo-$F_{2,113}$ = 11.279, P_{MC} = 0.001) and water content (Pseudo-$F_{1,113}$ = 4.24, P_{MC} = 0.001) on some of these genes were observed. In a T-RFLP analysis of nitrate reductase genes (*nirK* and *nirS*) revealed that warming led to the enrichment of denitrifiers community and such shifts subsequently have resulted in enhancement of denitrification rates in temperate regions (Braker *et al*., 2010). Additionally, Xiong *et al*. (2014) observed that warming treatment increased the average ratio of *Alphaproteobacteria*-to *Acidobacteria* with a simultaneous increase in the rates of CO_2 efflux. Therefore, it can be hypothesized that perturbations (e.g. warming) could alter community dynamics that would, in turn, alter functional genes expressions that drive biogeochemical cycling and ecosystem feedbacks (Singh *et al*., 2010; Bell *et al*., 2009).

In the present study, we found that *nifH* and *nosZ* genes that encode for nitrogenase reductase and nitrous oxide reductase respectively correlated significantly with the T-RFLP derived bacterial community patterns (Table 4.5). The *nifH* gene which depicts

the presence of nitrogen-fixing or diazotrophic communities was found to increase with warming, and microcosms treated at 35°C recorded the highest abundance of the *nifH* gene (Table 4.8), thereby in agreement with notion that warming increases the rate of biogeochemical processes such as nitrogen fixation, nitrification, and nitrogen mineralization (Luo *et al*., 2007). This is because the rates of enzymatic and metabolic processes are found to increase under warming treatments (Braker *et al*., 2010; Deslippe & Egger, 2006). Similarly, warming has been shown to elicit structural shifts in nitrogen-fixing communities that contain the *nifH* gene (Deslippe & Egger, 2006). Similarly, warming has been shown to increase the abundance *nifH* gene in tall-grass prairie soils (Zhou *et al*., 2012). The author also found that such increase could curb the loss of nitrogen via denitrification and nitrate leaching and consequently retained the quantity of nitrogen content across the treatment. Furthermore, it has been proposed that warming may potentially increase the rate of nitrogen-fixing in Arctic ecosystems by 1.5-2 folds (Chapin *et al*., 1991). Previous studies therefore suggest that increase in the functional genes (e.g. *nifH*) under warming may potentially accelerate the rate of biogeochemical cycles. The *nifH* was high in our microcosms treated at high water content (HW). Such observations could reflect the ability of diazotrophic communities to survive in oxygen-deprived conditions (Kathiresan & Bingham, 2001). Further, a correlation of this gene with *Firmicutes* was detected in this study. This finding was not surprising as a strong association between this gene and *Firmicutes* had been reported (Gaby & Buckley, 2014). Besides, it was found that increase in *nifH* gene concurred with the increase in nitrate content (Figure 4.7e). Our results are in agreement with other study reported elsewhere (Bothe *et al*., 2002). The abundance of the *nosZ* gene which reduced the nitrous oxide (N_2O) into nitrogen (N_2) (Jung *et al*., 2011) was found to decrease significantly with the increase of temperature (Table 4.8). It has been reported that the rate of denitrification is low in tropical soils attributed by the higher level of

redox potential (Zhang *et al*., 2009) and lower soil pH (Aulakh *et al*., 1992) that could hamper growth and activity of denitrifying communities. Further, several factors such as nitrate content, carbon availability, oxygen levels and soil temperature were found to impact *nosZ* gene and consequently alter the rate of denitrification (Gschwendtner *et al*., 2014; Saggar *et al*., 2013; Wallenstein *et al*., 2006). Therefore, in the present study, changes in soil chemical properties in response to treatments subjected such as decrease in pH (Figure 4.7a) may contribute to reduction in number of *nosZ* genes.

5.4.2 Functional gene abundance and the association with changes in bacterial community from Antarctic soil microcosms

The abundance of six functional genes (*nifH, amoA, nirK, nirS, nosZ* and *chiA*) in Antarctic soil microcosms were not significantly affected by temperature (Pseudo-$F_{2,49}$ = 0.7285, P_{MC} = 0.651) and incubation periods (Pseudo-$F_{2,49}$ = 1.654, P_{MC} = 0.107). These results imply that bacterial community (e.g. denitrifier community) from extreme environmental conditions is more resistant toward warming. Such insignificant correlation between temperature and the functional genes abundance from Antarctic soils had been reported by Yergeau *et al*. (2008). However, *nosZ* and *chiA* genes were found to correlate significantly with observed variation in bacterial community patterns (Table 4.9). Even though denitrifier communities account for 5% of total bacterial populations (Henry *et al*., 2006), they are a cosmopolitan group and frequently detected in various environments (Yergeau *et al*., 2012; Braker *et al*., 2010; Stres *et al*., 2008). Jung *et al*. (2011) proposed that high abundance of the *nosZ* gene is an indicator of nitrous oxide emission from the Antarctic ecosystems. The *nosZ* gene was highly abundant in both our untreated and treated samples, and was relatively stable across the treatments (Table 4.10). Similarly, Stres *et al*. (2008) found that *nosZ* communities in

temperate regions were insensitive after soil incubation at 4°C and 28°C for 12 weeks. It has been reported that majority sequences recovered for *nosZ* communities were affiliated to the *Proteobacteria* phylum (Jung *et al*., 2013) particularly *Alphaproteobacteria* (Wang *et al*., 2013). Thus, the increase of *Proteobacteria* with warming as observed in this study may contribute to the significant interaction between *nosZ* genes with T-RFLP derived bacterial community patterns.

In the present study, *chiA* gene (encodes for chitinase) which represents chitinolytic community correlated significantly with variation in bacterial community structure (Table 4.9). In this study, bacterial *chiA* was not affected by the warming treatments and displaying high stability to warming treatments. Some bacterial taxa such as *Proteobacteria* and *Planctomycetes* were found to correlate closely with *chiA* genes (Wieczorek *et al*., 2014; Kielak *et al*., 2013). Hence, shifts in these phyla across the treatments explained the significant correlation of this gene (*chiA*) with the bacterial community patterns. In this study, the absence of *nirS* gene which encodes for cytochrome cd_1 nitrite reductase could be due to methodology issues such as primer coverage.

5.5 Improvisation on the experimental designs of this study

This study provides an insight of bacterial community shifts in tropical and Antarctic soil microcosms in response to temperature and water variation. However, a direct comparison between tropical and Antarctic microcosms (using same temperature range and water content) was not intended. We used temperature ranges that reflect tropical (25°C, 30°C and 35°C) and Antarctic (5°C, 10°C and 15°C) climate variations. We did not have sufficient Antarctic soil to test more than one water-addition regime and more

replicates per sampling. We acknowledge that a wider range of incubation temperatures and microcosm replicates would enhance understanding of bacterial community responses in tropical and Antarctic soil towards temperature variation. Therefore, future works encompassing similar and more levels of temperature and water gradients are required to provide a better understanding on the effect of temperature and water variation on the bacterial community composition from tropical and Antarctic microcosms. We observed that the bacterial community composition (Figure 4.2) and the abundance of functional genes (Table 4.10) from Antarctic microcosms did not change prominently in response to experimental warming, based on DNA analysis. Therefore, RNA or protein-level analysis is required to link the community composition to functional attributes. To increase the resolution of functional genes abundance, quantification based on the whole genome sequencing can be performed. Examination of community profiles using Stable Isotope probing which utilizes stable isotope (e.g. 13C) labeled substrates (e.g. RNA) is enabled to capture the changes in the active members. Such assessment is vital to evaluate the actual effect of warming and water variation on the bacterial composition. Measurement of the rate of reaction of specific enzymes would help to address the effect of treatments on the bacterial metabolic rate that would help to address the effect the soil ecological processes (e.g. denitrification) and dynamics of nutrient cycling (e.g. nitrogen cycling).

CHAPTER 6: CONCLUSIONS

The short-term impacts of water and temperature to tropical and Antarctic soil bacterial communities were rarely tested under controlled laboratory settings. Understanding the short-term effects of variations in temperature and watering on tropical and Antarctic soil can facilitate prediction for long-term environmental responses. We showed that the structures, diversities, and composition of the bacterial community from tropical soil microcosms were altered significantly in response to warming and water enrichment (2 and 5 ml) (Figure 4.1; 4.3; 4.4: Table 4.1; 4.3). The largest partition in community composition in accordance to water content was observed for microcosms incubated at 35°C. The bacterial diversity and evenness decreased drastically at 35°C (Table 4.3). PERMANOVA analysis revealed that water content contributed to 9.71% of the total variation in bacterial community while warming treatments only explained 3.26% variation. Conversely, the structures, composition, and diversities of bacterial community from Antarctic soil microcosms were more stable under warming treatments (Figure 4.2; 4.5; 4.6), perhaps a longer period is required to induce community shifts. The community diversity and evenness remained stable across the treatments (Table 4.2; 4.4). The structural shifts were not significant at the three temperatures (5°C, 10°C & 15°C) in the early stages. The largest partition in community composition in accordance to water content was observed for microcosms incubated at 35°C". Based on the T-RFLP profiles (Figure 4.1 a), a 5°C changes in temperature were able to induce significant compostional shifts in the bacterial community in tropical soil microcosms. While the bacterial community from Antarctic soil microcosms did not change significantly across the treatments. It could be that the bacterial community in Antarctic ecosystem is more influenced by other unmeasured factors such as nutrient content (Dennis *et al*., 2013).

Firmicutes was the most prevalent population in tropical soil microcosms followed by *Acidobacteria, Proteobacteria*, and *Actinobacteria* (Figure 4.3). The number of phyla decreased in accordance with increasing temperature and water content. Specifically, microcosms under highest incubation temperature at 35°C was dominated by *Firmicutes*. Antarctic soil microcosms were dominated by *Proteobacteria* followed by *Gemmatimonadetes, Planctomycetes,* and *Actinobacteria* (Figure 4.5). Strikingly, we observed that majority of bacterial sequences in microcosms treated at 15°C were dominated by *Proteobacteria*. While Antarctic experience large and frequent fluctuations in environmental conditions (e.g. periodic freeze-thaw cycles and experience high solar radiations in summer), the tropical regions experience much less environmental variations in terms of temperature and moisture. Tropical soil bacteria would have adapted to this low climatic variation and be more sensitive to shift in environmental drivers (e.g. temperature) than bacterial community from the Antarctic ecosystem. Hence, the differences in bacterial responses (e.g. structural shifts) from the tropical and Antarctic microcosms identified in this study may closely associate with the prevailing environmental conditions.

Variations in the bacterial community composition in tropical soil microcosms were correlated with electrical conductivity, water content, nitrate, nitrite and pH (Table 4.5). On the other hand, nitrate was the only parameter that significantly correlated with variation in Antarctic soil bacteria community (Table 4.6). With the use of Q-PCR to quantify specific functional genes, it was found that *nifH* and *nosZ* genes were significantly associated with structural changes in tropical bacteria community (Table 4.7). Shifts in the tropical bacterial community were also accompanied by substantial changes in the functional gene abundance particularly for *nifH* and *nosZ* genes (Table

4.8). The abundance of *nifH* gene increased linearly with the increase of temperature while *nosZ* was found to decrease under warming treatments. Such observations on specific gene numbers could be used as a proxy to reflect the ecological function that the genes mediate. For the Antarctic soil microcosms, the *nosZ* and *chiA* genes showed the highest correlation to the bacterial community (Table 4.9). Nevertheless, functional genes abundance in Antarctic soil samples did not vary much in response to warming treatments (Table 4.10).

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Effect of short term variation in temperature and water content on the bacterial community in a tropical soil

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ABSTRACT

The community dynamics of environmental bacteria is dependent on micro-climatic factors such as temperature and water potentials. A shift in any one of these factors can have a major impact on the richness and composition of the microbes in the soil. However, microbial responses towards these climate stressors are poorly characterised. Here, we undertook a microcosm study to assess the effect of temperature and water content on the bacterial community structure in a tropical soil over a four week period. The microcosms were incubated at three different temperatures (25 °C, 30 °C, and 35 °C) and maintained under two different water levels (2 and 5 mL). Using a combination of molecular assessment tools and numerical inference, we showed that short-term variation in both temperature and water content induced significant changes to the soil bacterial community composition. The greatest difference in community structure between treatments was observed in the Week 2 microcosms, but in the Week 4 microcosms, the community structure between treatments became more similar. Compared to temperature, water content exerted a greater effect on the bacterial diversity. *Firmicutes* was the most abundant phylum in all the analysed samples, and its relative abundance increased with elevation in temperature and water content. Out of six functional genes analysed, the nitrogen fixation gene (nifH) and denitrification gene (nosZ) showed significant correlations to the bacterial community structures.

1. Introduction

Bacteria are the dominant members of soil biota and they play crucial roles in the biogeochemical cycling of elements in the environment, thus contributing to the functioning of wider related ecosystems (Aislabie and Deslippe, 2013; Dominati et al., 2010; Nannipieri et al., 2003). It has been estimated that each gram of soil contained more than 10^8 bacterial cells (Torsvik et al., 1998). A high level of diversity is considered a vital aspect of ecosystem health as it begets ecosystem stability by acting as a genetic and functional reservoir that increases community resilience toward perturbations (Bissett et al., 2007; DeAngelis et al., 2013). Therefore, the loss of community richness provides a proxy indicator of deterioration in ecosystem health and functions (Allison and Martiny, 2008; Griffiths and Philippot, 2013).

The complexity of bacterial community in soil is dependent on underlying environmental stressors such as temperature and water potential (Schimel et al., 2007). Shifts in any of these modulators which are usually controlled by both short and long term local climatic gradients have the potential to change the structure and composition of soil bacteria (Bond-Lamberty et al., 2016; Brockett et al., 2012; Deslippe et al., 2012; Evans and Wallenstein, 2014; Manzoni et al., 2012; Rinnan et al., 2009; Yergeau et al., 2012). Separately, the degree to which microbial community structure can be perturbed by future environmental fluctuations is influenced by the contemporary environmental conditions (Evans and Wallenstein, 2011; Fierer et al., 2003; Waldrop and Firestone, 2006). For instance, bacterial communities that experienced frequent disturbances such as water stress (Fierer et al., 2003) or redox fluctuations (DeAngelis et al., 2010) were more resistant to perturbation than those subjected to narrow climatic conditions (Cavaleri et al., 2015). Understanding the short-term effects of variations in temperature and watering on tropical soil can therefore facilitate prediction for long-term environmental responses.

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Appendix

APPENDIX A

Solutions for agarose gel electrophoresis

10X Tris-acetic (TAE) buffer

A total volume of 1 L 10X TBE buffer, pH 8.3 (890 mM Tris, 890 mM Boric acid, 20 mM EDTA) was prepared:

Tris-base, (Molecular weight: 121.4 g/mol) 108 g

Boric acid, (Molecular weight: 61.83 g/mol) 55 g

EDTA (Molecular weight: 292.24 g/mol) 5.8 g

Sterile distilled water top up to 1 L

pH adjusted to 8.3

The 10 X TBE stock solutions was stored at room temperature and used as soon as possible to avoid precipitation.

1.5 % agarose gel

A volume of 50 ml agarose gel prepared using 50 ml of 0.5X TBE buffer:

Agarose powder	0.75 g
0.5X TBE buffer	50 ml