EFFECTS OF ENVIRONMENTAL TRANSITION ON THE DIVERSITY OF ARCTIC SOIL BACTERIA

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EFFECTS OF ENVIRONMENTAL TRANSITION ON THE DIVERSITY OF ARCTIC SOIL BACTERIA

ABSTRACT

The Arctic is currently one of the most rapidly warming regions on Earth. Soil microbial communities play important roles in various ecological processes in the Arctic. Understanding how the microbial elements in Arctic soil communities are affected by climatic warming is of great concern and has been receiving increasing research attention. To date, there remains a lack of research findings which combine effects of soil warming and water availability on the High Arctic soil microbial community, despite the generally recognised significance of water impacts on soil microbial communities. The projected increase in freeze-thaw (FT) cycle frequency associated with warmer temperatures in the High Arctic could also affect the dynamics of soil bacterial communities. However, there are few studies of the impacts of FT cycles on microbial communities and diversity in the High Arctic. In this study, the first objective was to gain an understanding of the inter-seasonal dynamics of natural soil bacterial diversity at a High Arctic site near Ny-Ålesund, Svalbard, as part of a long-term field environmental manipulation study investigating the impacts of increased soil temperature and water availability on soil microbial communities. The manipulation experiment, using open-top chambers (OTC), was installed in the late summer of 2014, with the soils studied being sampled after snow melt in July 2015, and at the end of the boreal summer in September 2015 and September 2016. Analysis of high throughput sequencing of the 16S rRNA gene on the Illumina MiSeq platform showed a seasonal shift in bacterial community diversity but revealed no significant treatment effects of the open-top chamber warming and/or water addition on bacterial community diversity over the period of the manipulation. The second objective docmented the effects of FT cycles on High Arctic bacterial communities in soil samples collected under three different snow cover depths. Analysis of 16S rRNA gene amplicon

sequences showed significant differences in soil bacterial community diversity between the three different depths (low, medium and thick). Bacterial diversity in soil samples under thick snow cover was significantly lower than in those without or under medium snow cover. Experimental exposure of these communities to nine FT cycles and thawing controls led to significant declines in the diversity of soil communities sourced from under all snow cover depths. There were no significant differences in Shannon diversity between FT treatment and thawing controls throughout successive cycles, except for low snow cover soil communities which showed an increase in Shannon diversity after nine FT cycles, in contrast with the respective control which showed a consistent decrease in Shannon diversity. It is therefore likely that reduced snow cover will influence soil bacterial community resilience through exposing it to an increased frequency of FT cycling. The data presented contribute to the description of microbial diversity reported in the warming Arctic. In addition, a web-based tool, JS Euler, was developed as part of this study which can integrate up to 200 input datasets to generate Jaccard index values. This software addresses the acknowledged current limitation in making pairwise comparisons in data analyses when datasets include more than 30 samples, and can be utilized in any research field to perform pairwise comparison of datasets.

Keywords: open-top chamber, Arctic, warming, freeze-thaw, soil microbial diversity

KESAN PERALIHAN PERSEKITARAN KE ATAS KEPELBAGAIAN BAKTERIA TANAH ARTIK

ABSTRAK

Artik merupakan salah satu rantau yang mengalami peningkatan suhu pada kadar yang paling tinggi di atas bumi. Komuniti mikrob tanah memainkan peranan yang penting dalam pelbagai proses ekologi di Artik. Sedemikiannya, tujuan pertama dalam kajian ini adalah untuk menyumbang kepada pengetahuan dinamik kepelbagaian bakteria semula jadi dalam tanah Artik Tinggi, Ny-Ålesund, Svalbard. Persampelan ini dijalankan di medan kajian manipulasi jangka panjang, yang bertujuan untuk mengkaji kesan peningkatan suhu tanah dan ketersediaan air ke atas komuniti mikrob tanah. Experimen ini melibatkan penggunaan 'open-top chambers (OTC)' yang dipasang pada akhir musim panas 2014, di mana persampelan tanah dijalankan pada Julai 2015 (permulaan pencairan salji), dan akhir musim panas pada September 2015 serta September 2016. Jujukan di bahagian hipervariabel 16 rRNA gen untuk bakteria dengan menggunakan MiSeq platform Illumina menunjukkan perubahan komuniti bakteria atas peralihan musim. Namun, tindakan pemanasan OTC serta penambahan awal air ke atas tanah tidak membawa kesan kepada bakteria komuniti sepanjang kajian manipulasi kami. Di samping itu, kajian kami juga bertujuan untuk mengkaji kesan peningkatan kadar beku-cair ke atas dinamik komuniti bakteria tanah, di mana ia bersekutu dengan peningkatan suhu di rantau Artik Tinggi. Strategi kajian ini adalah mengkaji perbezaan respons komuniti bakteria tanah daripada penutup salji pada tiga tahap kedalaman ke atas kitaran beku-cair. Jujukan di bahagian hipervariabel 16 rRNA gen untuk bakteria menunjukkan perbezaan komuniti bakteria di bawah salji yang berbeza kedalaman (tebal, sederhana serta nipis). Eksperimen beku-cair juga menunjukkan perbezaan tindak balas serta kerentanan komuniti bakteria dari salji yang berlainan kedalaman ke atas mikrokosma beku-cair berturutan. Tanah daripada salji tebal mendemonstrasikan Indeks Keragaman Shannon yang berbeza

daripada salji sederhana dan nipis. Ia juga memanifestasikan perubahan kecil sahaja ke atas kitaran beku-cair berbanding dengan salji sederhana dan nipis. Sebaliknya, tanah daripada salji nipis dan sederhana menunjukkan tren kejatuhan Indeks Keragaman Shannon yang bererti sepanjang kitaran beku-cair. Tambahan pula, kami juga membina sebuah perisian bertapak web, yakni JS Euler yang dapat menampung sebanyak 200 dataset untuk menjana Jaccard index bagi kerja analisis kami. Perisian ini juga menjana Euler Diagrams serta senarai OTUs (Operational Taxonomic Unit) yang unik dan bertindih untuk perbandingan berpasangan dataset. Ia juga berunsur antara-muka unit graphic sertai berciri-ciri penyesuaian mudah mengikut pengguna. Perisian ini mengatasi batasan maksimum untuk perbandingan berpasangan iaitu maksimumnya 30 input sampel pada masa kini.

Kata kunci: open-top chamber, Artik, peningkatan suhu tanah, beku-cair, komuniti bakteria tanah

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List of Symbols and Abbreviations

α	:	Alpha
bp	:	Base pairs
β	:	Beta
<	:	Less than
≤	:	Less than or equal
>	:	More than
%	:	Percent
cm	:	Centimetre
DNA	:	Deoxyribonucleic acid
dsDNA	:	Double-stranded deoxyribonucleic acid
g	:	Gram
HS	:	High sensitivity
et al.	:	Latin word of "*et* alii" which means "and other"
i.e.	:	Latin term of "id est" which means "that is"
µm	:	Micrometer
mg	:	Milligram
mL	:	Milliliter
mM	:	Millimolar
min	:	Minute(s)
M	:	Molar
ng	:	Nanogram
NGS	:	Next generation sequencing
n.a.	:	Not applicable

OTUs	:	Operational taxonomic units
pg	:	Picogram
psi	:	Pounds per square inch
QIIME	:	Quantitative Insights into Microbial Ecology
qPCR	:	Real-time PCR
sec	:	Second(s)
×g	:	Times gravity

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Environmental conditions in the terrestrial ecosystems of the polar regions are amongst the most extreme on the planet (Ávila-Jiménez *et al*., 2010; Convey, 2012, 2014; Peck *et al*., 2006). In the Northern Hemisphere, the Arctic consists of the Arctic Ocean and its archipelagos, and parts of Alaska (United States), Canada, Greenland, Iceland, Scandinavia and Russia. Global climate change is predicted to have higher impacts at high latitudes, particularly in the Arctic, which is already rapidly warming and is expected to continue warming more quickly than other regions of the globe at lower latitudes, a phenomenon known as Polar Amplification (IPCC, 2018; ACIA, 2004). Climate projections give an expectation of warmer winters in the Arctic with increased mean temperatures, with specific consequences including both increases and decreases in snow cover and changes in frequency of freeze-thaw cycles (Convey *et al*., 2015; Cooper, 2014; Elmendorf *et al*., 2012), along with more general consequences of annual warming such as glacier retreat and thawing of permafrost. Together, at local scales, these changes can cause both increases and decreases in water availability to biota owing to the thawing of ice and greater drainage, respectively (AMAP, 2011). Biodiversity of terrestrial ecosystems in the polar regions is generally regarded as being structurally simple due to the relatively limited diversity of biotic components in comparison with ecosystems at lower latitudes. Thus, Arctic terrestrial ecosystems are considered to be sensitive indicators of climate change (Callaghan *et al*., 1995; Walther *et al*., 2002) and provide excellent models to study the impacts of climatic warming and other environmental changes on ecosystems.

Soil micro-organisms play fundamental roles in major global biogeochemical cycles (Falkowski *et al*., 2008; Prosser *et al*., 2007; Remenant *et al*., 2009) and this is particularly the case in the polar regions, where larger multicellular organisms become

progressively less important with increasing environmental extremes (Convey, 2013; Vincent *et al*., 1998). Despite the magnitude of ongoing climate change in both polar regions, little is known, in particular, of how the microbial elements of polar ecosystems are affected by these changes. A number of studies have provided information on bacterial diversity in a range of Arctic soils, including for example peats (Lipson *et al*., 2013), diesel-contaminated soils (Yergeau *et al*., 2012a), permafrost (Gittel *et al*., 2014b), buried soils (Gittel *et al*., 2014a), vegetation-influenced soils (Shi *et al.,* 2015a), and tundra tussock and shrub soils (Wallenstein *et al*., 2007). Understanding the effects of climatic warming on bacterial community composition and diversity in Arctic soils is therefore an increasingly active area of research.

Experimental simulations of warming in field studies have commonly been conducted using passive warming open-top chambers (OTCs). This type of chamber was developed for the International Tundra Experiment (ITEX) (Henry *et al.,* 1997). Warming effects of Open-top chambers deployed in high latitude ecosystems in the summer were documented by Marion *et al*. (1997). Although exerting considerably more complex manipulative effects than simply affecting temperature (Bokhorst *et al*., 2013, 2016), the ITEX Open-top chamber mains a standard approach used in field manipulations mimicking the predicted effects of climate warming. Open-top chambers simulate a warming scenario by increasing soil mean temperatures consistent with climatic predictions over the next several decades (Hollister *et al*., 2000; Marion *et al*., 1997). This method has been employed in multiple studies (e.g. Aronson *et al*., 2009; Bokhorst *et al*., 2011, 2015; De Boeck *et al*., 2012) in both polar regions.

Studies of the effects of soil warming have shown the importance of water availability, which has strong impacts on bacterial population size and diversity (Sheik *et al*., 2011). However, to date, few studies have utilised open-top chambers in long-term

studies of Arctic soil microbial communities (Deslippe *et al*., 2005; Fujimura *et al*., 2008; Michelsen *et al*., 2012; Weedon *et al*., 2012), and there is still lack of research utilizing open-top chambers to study the combined effects of soil warming and water availability on High Arctic soil bacterial communities. This study investigated effects of open-top chamber (OTC) warming and supplementary water input on a High Arctic soil bacterial community in a representative tundra habitat near Ny-Ålesund, Svalbard.

Current warming trends in Arctic regions may increase the incidence of soil temperatures above 0°C, particularly at the height of summer, and result in a decrease in snow cover and depth. Reduced or absent snow cover may make the surface and shallow layers of soils more vulnerable to temperature fluctuations, leading to increased numbers of freeze-thaw (FT) cycles (Henry, 2008; Førland *et al*., 2011; Convey *et al*., 2014). In the polar regions, freeze-thaw cycles are common during autumn before snow accumulation as well as in spring (Convey *et al*., 2018), and changes in their frequency have been suggested to have substantial effects on soil microbial communities and associated nutrient cycling functions (Kumar *et al*., 2013; Larsen *et al*., 2002; Sawicka *et al*., 2010; Yergeau & Kowalcuk, 2008).

Several microcosm studies have investigated the effects of freeze-thaw cycles on Arctic soil microbial communities and activities (Guicharnaud *et al*., 2009; Kumar *et al*., 2013; Larsen *et al*., 2002; Schimel & Mikan, 2005; Sawicka *et al*., 2010). However, a majority of these studies focus on freeze-thaw effects on microbial processes (e.g. respiration, microbial biomass carbon, enzymatic activities) rather than changes in microbial diversity and composition.

Snow cover provides an excellent shield from temperature fluctuations, insulating the underlying soil and vegetation (Convey *et al*., 2014, 2018; Tan *et al*., 2014; Zhang, 2005). Greater insulation is typically provided with increasing snow depth, which leads

to more stable and less extreme soil temperatures and a reduced occurrence of soil freeze-thaw cycles (Pauli *et al.,* 2013; Convey *et al*., 2014; Tan *et al*., 2014; Zhang, 2005). Generally, depth of snow is a proxy for snow accumulation period. Shallow snow cover is likely to have shorter accumulation period and melt completely (hence higher freeze-thaw frequency) and thick snow cover more likely to persist (hence lower freeze-thaw frequency). The current study used a laboratory-based microcosm experimental approach to apply freeze-thaw cycles (simulating natural freeze-thaw events) to soils originally sampled on Svalbard (High Arctic) under different snow depths, and assess their effects on the bacterial community diversity.

The current study obtained data on soil bacterial community diversity using a high-throughput 16S rRNA gene amplicon sequencing approach, using the Illumina MiSeq platform. This approach has proven useful in the assessment of microbial biodiversity, particularly in polar and other extreme environments where logistical constraints and other practical issues are of great concern (Czechowski *et al*., 2017). Analysis of diversity indices and taxonomic composition in microbial communities often involves comparison among the OTUs (Operational Taxonomic Units) obtained from multiple biological datasets. The Jaccard similarity index (Jaccard, 1912) is one of the most widely used similarity indices to express compositional similarity of biological assemblages. However, to the best of our knowledge, there are no available graphic user-interface (GUI) web-based tools which can cater for more than 30 input datasets for generating Jaccard similarity among all input samples simultaneously. Thus, this study included development of a web-based tool, JS Euler, which could incorporate up to 200 input datasets was developed to generate Jaccard indices for use in the required pairwise analyses. This software was utilised extensively in Sections 4.1 and 4.2 for generating Jaccard indices used in beta diversity analyses between samples. It was also used by Yew (2017) as a tool to calculate Jaccard indices.

The main objectives of this study were:

(a) To study the effects of experimental warming and water amendment on the High Arctic soil bacterial community diversity in the field.

(b) To investigate the impacts of *ex situ* exposure to repeated freeze-thaw cycles on High Arctic soil bacterial community diversity in communities sampled from soils which had experienced different freeze-thaw cycle frequency in nature.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to the Arctic

The Arctic is a large, ice-covered ocean surrounded by tree-less, frozen continental landmasses which are seasonally covered with ice and snow. There are many approaches to defining the Arctic, which include latitude (region north of the Arctic Circle), political declarations (where the definition of borders is often steered by national political or economic goals), a set of natural science-based characterizations, using climate, ecosystems and ecoregions, flora, fauna, sea ice, permafrost, etc, as well as self-perceptions of indigenous populations (Aksnes *et al*., 2016). According to Callaghan *et al*. (2004), the region situated above 66.78°N can be considered as the 'Arctic' geographically, whereby the sun stays above the horizon at midnight on midsummer's day (cool and short summers with continuous daylight); and is below the horizon at midday on midwinter's day (cold and continuous dark, long winters).

From south to north along a northern latitudinal transect, vegetation becomes increasingly sparse and lower stature. The classification of vegetation zones also differs between western and Russian literature. 'Taiga', 'Low Arctic' and 'High Arctic' are common terms used in western studies (Callaghan *et al*., 2004), whereas different vegetation zonation schemes (e.g. cushion forb, prostrate dwarf shrub, erect dwarf shrub and low shrub) are used in the Russian literature (Walker, 2000).

According to the Circumpolar Arctic Vegetation Map (CAVM), the total land surface of the Arctic is approximately 7.11 x 10^6 km^2, which includes approximately 5.05 x 10^6 km^2 of vegetated area, with the remainder being ice-covered. In the vegetated area, erect shrublands contribute the highest proportion (~26%), followed by peaty graminoid tundra (~18%), mountain complexes (~13%), barrens (~12%), prostate-shrub and mineral graminoid tundras (~11% of each), and wetlands (7%) (Walker *et al*., 2005). Most plant

species are cryptogamic taxa, including liverworts, lichens, mosses and algae, rather than vascular plants, which contribute less than half of the plant species (around 1800 recorded species) (Matveyeva & Chernov, 2000; Callaghan *et al*., 2004). Algae and cyanobacteria are also found in Arctic terrestrial and wetland habitats, and exhibit a wide range of adaptive strategies for coping with living in the harsh environment, including avoidance, protection and association with other organisms such as fungi (Elster & Benson, 2004).

According to Callaghan *et al*. (2004), the most diverse group of Arctic fauna are insects (around 3300 species), including Diptera (~50%), beetles (Coleoptera, ~10%), butterflies and moths (Lepidoptera, ~10%) and Hymenoptera. There are approximately 315 species of vertebrates, which include 75 mammals, 240 birds, five amphibians and two reptiles. The Arctic also has about 700 species of mites (Acarina), 500 species of nematodes, 400 species of springtails (Collembola), 300 species of spiders (Arachnida), 70 species of oligochaetes (the majority being Enchytraeidae), a few molluscs, and an uncertain number of protozoans. Species diversity of Arctic fauna shows similar latitudinal patterns to those of Arctic plants, decreasing in parallel with increasing latitude and declining temperatures in most animal groups, including birds, butterflies and ground beetles (Chernov, 1995). Nonetheless there are some exceptions, such as sawflies and peat-land birds, which have higher species diversity and population density in more northern than southern areas, although this is difficult to differentiate from the suitability of different habitat types. Nonetheless, the decline in faunal diversity is generally more pronounced (frequently greater than 2.5 times) than that in vascular plants (Chernov & Matveyeva, 1997; Callaghan *et al*., 2004).

2.2 Microbial research with Arctic terrestrial samples

The polar regions are more strongly affected by global warming and environmental changes than are lower latitude temperate and tropical regions (ACIA, 2004; Yoshimori *et al*., 2017; Lovejoy, 2013), while biological and chemical processes are more temperature-sensitive in cold regions (Kirschbaum, 1995). Together with alpine regions, much of the Arctic can be broadly characterised as the tundra biome (Thomas *et al*., 2008; Chapin & Körner, 2013). The biological consequences of climate warming in Arctic tundra ecosystems include shifts in flowering seasons and a concomitant decline in flower-visiting insect abundance (CaraDonna *et al*., 2014; Høye *et al*., 2013), phenological changes in High Arctic plants and arthropods (Bjorkman *et al*., 2015; Hegland *et al*., 2009; Høye *et al*., 2008; Hülber *et al*., 2010), diversity changes and altered community compositions (Lang *et al*., 2012; Myers-Smith *et al*., 2011; Sturm *et al*., 2005), species migration and extinction (Baldwin *et al*., 2014; Bässler *et al*., 2010; Clausen & Clausen, 2013; Tombre *et al*., 2008), shifts in land surface vegetation and ecosystem C cycling (Post & Forchhammer, 2007; Pearson *et al*., 2013; Leffler *et al*., 2016). In turn, these biological responses cause climate feedbacks through increased decomposition of organic carbon from the thawing carbon pools in permafrost and soils (Grosse *et al*., 2011; Schuur *et al*., 2015), impact on biogeochemical cycles (Anderson *et al*., 2017; Yde *et al*., 2014), lead to reduction of biodiversity (Pauli *et al*., 2012), and affect other organisms through alteration in trophic dynamics (Post & Forchhammer, 2007; Wookey *et al*., 2009).

The Arctic environment hosts a wide diversity of cold-adapted microorganisms encompassing the three domains of life: Bacteria, Archaea and Eukarya. These include a wide range of nutritional types, such as autotrophs and heterotrophs, aerobes and anaerobes, as well as chemoautotrophs and chemolithrotrophs (Rampelotto, 2014). As noted above, major changes are already observed at large scale in different polar

ecosystems as well as in specific macroscopic species, and the microbial food webs which support higher trophic levels are also vulnerable to environmental change (Lovejoy, 2013). In polar ecosystems, the microbial elements are most probably those likely to experience the earliest and greatest impacts from climatic warming. Thus, it is important to understand if climate change will cause significant changes in microbial diversity (abundance and structure) and the ecosystem functions and services they provide, (Rampelotto, 2014). Microbial responses towards warming have therefore formed a particular focus of research in recent years (Deslippe *et al*., 2012; Mackelprang *et al*., 2011; Natali *et al*., 2012; Sistla *et al*., 2013; Bracho *et al*., 2016; Xue *et al*., 2016; Yang *et al*., 2017).

Microbial community composition in soils across a broad range of global ecosystems, including the Arctic, are strongly structured by local environmental conditions (Chu *et al*., 2010; Crump *et al*., 2004; Horner-Devine *et al*., 2004; Fierer & Jackson, 2006; Lozupone & Knight, 2007; Cermeño & Falkowski, 2009). The above-ground vegetation (Buckeridge *et al*., 2010a; Chu *et al*., 2011; Wallenstein *et al*., 2007), soil chemistry (Frey *et al*., 2004; Nilsson *et al*., 2007; Lauber *et al*., 2008), water availability (Convey *et al*., 2014) and soil pH (Chong *et al*., 2012; Chu *et al*., 2010; Lauber *et al*., 2009; Männistö *et al*., 2007) are amongst the important drivers of microbial community distributions. Isolation, or dispersal limitation, is a less important driver in Arctic marine environments (Darling *et al*., 2000; Cermeño & Falkowski, 2009; Hubert *et al*., 2009), although some studies have found that dispersal limitations or other historical contingencies may be important in structuring bacterial taxa at lower taxonomic levels in other environments (Cho & Tiedje, 2000; Martiny *et al*., 2006; Reche *et al*., 2005).

Studies of polar microbiology provide useful insights into a variety of microbial cell adaptation strategies to the extreme environments at molecular or cellular levels. This can provide a useful model to illuminate the fundamental properties of cellular design and the evolutionary changes in the cells challenged with extreme environmental conditions. In terms of biotechnological applications, ice-associating proteins, for instance the antifreeze proteins (Kim *et al*., 2017; Zhang *et al*., 2007), ice-nucleation proteins (Cochet & Widehem, 2000) as well as proteins which provide resistance to osmotic and cold stresses (Sakamoto *et al*., 1998; Hernandez-Lopez *et al*., 2003), have found various useful applications in, for instance, the food, energy and medical industries (Wilson & Walker, 2010).

The dominating bacterial phyla in various heath tundra Arctic soils are Acidobacteria, Alphaproteobacteria, Actinobacteria, Betaproteobacteria and Bacteroidetes (Chu *et al*., 2010; Neufeld & Mohn, 2005; Wallenstein *et al*., 2007). Proteobacteria dominate Arctic shrub soils, of significance as shrub abundance and extent has been increasing due to climate change in recent years (Sturm *et al*., 2001; Tape *et al*., 2006), often displacing tussock and intertussock tundra, which are dominated by Acidobacteria (Wallenstein *et al*., 2007; Weintraub & Schimel, 2005). In Arctic marine sediments, Gammaproteobacteria, Deltaproteobacteria, Actinobacteria and Alphaproteobacteria are the dominating phyla (Li *et al*., 2009, Teske *et al*., 2011, Bienhold *et al*., 2012). Betaproteobacteria and freshwater clades of Bacteroidetes are the most commonly found phyla in freshwaters. Microbial communities within sea ice vary within the fine-scale structure of the ice, and those of multiyear ice may be different from more biologically active first year ice (Bowman *et al*., 2012). These ice-associated communities are characterized by multiple trophic levels, including chemoautotrophic bacteria and archaea, photosynthetic bacteria and algae, heterotrophic bacteria, fungi,

archaea and flagellates (Bowman *et al*., 2012, Comeau *et al*., 2013; Horner *et al*., 1992; Lizotte, 2003; Staley & Gosink 1999).

In addition to bacteria, archaea are also known to be phylogenetically and functionally crucial members of cold regions (Cavicchioli, 2006; Murray & Grzymski, 2007). Both are known to perform the oxidation of ammonia (NH_3), the rate-limiting step of nitrification (Prosser & Nicol, 2008; Schleper & Nicol, 2010). Nitrogen is the major limiting nutrient in Arctic ecosystems (Shaver & Chapin, 1980; Nordin *et al*., 2004) and its availability relies on nitrogen cycling by microorganisms. Nitrification has been documented in Arctic soils (Binkley *et al*., 1994; Chapin, 1996) and also involves the emission of the ozone-depleting and greenhouse gas nitrous oxide (N_2O) (Conrad, 1996; Ravishankara *et al*., 2009), contributing most of the N_2O released from soils, including those of the Arctic (Ma *et al*., 2007; Siciliano *et al*., 2009). Members of the phylum *Thaumarchaeota*, which are ammonia-oxidizing archaea (AOA), are widely distributed, often greatly outnumbered ammonia-oxidizing bacteria (AOB) (Leininger *et al*., 2006; Wuchter *et al*., 2006). However, research on AOA, including their community structure and functional diversity, is much more limited than for AOB (Arp *et al*., 2007). Nonetheless, research on AOA has increased in recent years (Abell *et al*., 2010; Bernhard *et al*., 2010; Tourna *et al*., 2011), including in the Arctic (Alonso-Sáez, *et al*., 2012; Alves *et al*., 2013; Pouliot *et al*., 2009; Santoro *et al*., 2010). Since nitrification rates could increase in warmer Arctic soil, this could have important implications for community composition and primary productivity in the Arctic (Chapin III, 2012).

The presence of yeasts has also been reported in the Arctic. *Cryptococcus* and *Rhodotorula* are the predominant genera and also contribute the highest number of species across a wide range of habitats including glacier ice, subglacial environments, soil, permafrost and Arctic marine habitats (Gunde-Cimerman *et al*., 2003; Starmer *et al*.,

2005; Zalar & Gunde-Cimerman, 2014). The dominant yeasts in the Arctic, including up to 24 genera as described by Zalar and Gunde-Cimerman (2014), are basidiomycetes, similar to those which occur frequently in Antarctic soils and offshore polar marine waters.

Various microfungi have been reported from a wide range of habitats in the Arctic (Gawas-Sakhalkar & Singh, 2011; Hafizah *et al*., 2013; Idid *et al*., 2014; Meyling *et al*., 2012; Singh & Singh, 2012). Arctic fungal and other microbial ecosystems, and associated functions such as nutrient cycling, have received increasing research attention in recent decades (Behie & Bidochka, 2014; Buckeridge *et al*., 2010a; Lavoie *et al*., 2011; Siciliano *et al*., 2014). Motivated by the well-documented trends of climate change in the Arctic, various studies of change impacts on microbiota (Deslippe *et al*., 2011; Geml *et al*., 2015, 2016; Morgado *et al*., 2015; Mundra *et al*., 2016; Semenova *et al*., 2015) have been carried out, in order to try to understand and predict the possible responses and vulnerability of microbial communities and ecosystems to climate warming.

2.3 Diversity of bacteria in Arctic soils

Many organisms that inhabit cold environments such as the polar regions are termed as 'cold-adapted'. They are generally categorised into two overlapping groups: psychrophilic and psychrotrophic/psychrotolerant. True psychrophiles grow optimally at temperatures of less than 15°C and with an upper limit of 20°C (Morita, 1975). They predominate in the marine environment and particularly in abyssal oceanic waters which are permanently cold (< 5°C) (Bölter, 2004; Helmke & Weyland, 2004). Psychrotrophs grow optimally at 20-25°C, however can still function well at lower temperatures (Morita, 1975). They are often the most dominant microbiota in terrestrial environments, which tend to experience larger and more rapid temperature fluctuations than does the marine environment (Bölter, 2004; Helmke & Weyland, 2004; Morita, 1975; Peck *et al*., 2006).

It is often difficult to define a particular strain as psychrotolerant or psychrophilic due to lack of adequate data. Thus, in some studies describing cold adaptation strategies of microbes, both groups are often if inaccurately termed as psychrophiles (Casanueva *et al*., 2010; De Maayer *et al*., 2014).

Various studies have investigated bacterial diversity in Arctic and sub-Arctic regions (e.g. Campbell *et al*., 2010; Ganzert *et al*., 2014; Neufeld & Mohn 2005; Männistö *et al*., 2007; Schuette *et al*., 2010; Steven *et al*., 2007; Wallenstein *et al*., 2007). Some studies have concluded that vegetation controls community composition (Shi *et al*., 2015a; Wallenstein *et al*., 2007) as plants supply nutrients to below-ground microbial communities (Bardgett & Wardle, 2010; Kowalchuk *et al*., 2002), and nutrient availability has been shown to control Arctic soil bacterial diversity (Campbell *et al*., 2010; Coolen *et al*., 2011; Koyama *et al*., 2014). Spatial distribution and types of vegetation are also influential in determining soil bacterial diversity (Banerjee *et al*., 2011; Shi *et al*., 2015a).

Chu *et al.* (2010) carried out a comprehensive biogeographical analysis of the bacterial diversity in the Arctic soils from the Canadian, Alaskan and European Arctic using a high-resolution bar-coding pyrosequencing approach. The study sampled 29 heath tundra sites close to the top of exposed ridges. The dominant phyla across all sampled Arctic soils were Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria and Bacteroidetes. The results obtained strongly suggested that the presence of particular bacterial phylotypes was strongly determined by soil pH, and that relative abundance of these dominant phyla were also strongly correlated with soil pH. In contrast, in a lower latitude study, the presence of Alphaproteobacteria and Betaproteobacteria was not significantly correlated with soil pH (Lauber *et al.*, 2009). The importance of soil pH in structuring soil bacterial communities has been

demonstrated in a range of recent studies (Chong *et al.*, 2012; Fierer & Jackson, 2006; Baker *et al*., 2009; Jones *et al*., 2009; Lauber *et al*., 2009; Männistö *et al*., 2007), all of which have shown the importance of soil pH as a regional-scale control on soil bacterial community structure. Other soil characteristics have relatively smaller impacts on bacterial community structure and phylogenetic composition (Lauber *et al*., 2009).

Zhou *et al*. (1997) and Neufeld *et al*. (2004) surveyed bacterial diversity in Arctic tundra soils using a 16S rRNA gene clone library approach applied to the melted active layer of Siberian permafrost soils and pristine soils in the Canadian Arctic, respectively. Both revealed high bacterial diversity, including high frequencies of sequences assigned to Proteobacteria, Actinobacteria, Acidobacteria and Bacteroidetes. A striking difference between the two studies was that the Siberian soil had a high proportion (26%) of sequences from Deltaproteobacteria, whereas the Canadian soil included a high percentage of sequences (13%) of Gammaproteobacteria. Nemergut *et al.* (2005) compared Arctic and alpine bacterial communities during the summer season, noting that the alpine communities contained notably higher frequency of sequences assigned to Verrucomicrobia. This taxon may be involved in plant root interactions, thus the inter-regional differences in abundance may mirror differences in timing of plant root carbon inputs or rooting depth between Arctic and alpine plants (Nemergut *et al*., 2005).

Seasonal shifts in bacterial community structure and functions have been demonstrated in the Arctic (Buckeridge *et al*., 2013; Wallenstein *et al*., 2009; McMahon *et al*., 2011), possibly linked to seasonal changes in availability of soil nutrients (Buckeridge & Grogan, 2008). A recent study (Hill *et al*., 2016) examined the confounding seasonal and spatial effects on Arctic heathland soils in response to simulated climate change, recording significant temporal changes but with no detectable spatial effects. Betaproteobacteria, particularly *Burkholderia*-affiliated OTUs, declined

in proportional contribution between spring and summer. This is consistent with previous findings that *Burkholderia* confer improved cold tolerance to plant species faced with low temperature stress (Theocharis *et al*., 2012). In the Antarctic, *Burkholderia* species have also been found in the coastal area of the Ross Sea (reviewed in Giudice & Fani (2015)). In contrast, Alphaproteobacteria and Acidobacteria increased in relative abundance in summer, consistent with a transition from r-selected (copiotrophs) to K-selected (oligotrophs) taxa in bacterial communities (Thomson *et al*., 2013). However, a soil fertilization experiment which mimicked warming in the Arctic tundra (Koyama *et al*., 2014), reported increased abundance of copiotrophic α-Proteobacteria and β-Proteobacteria and reduced abundance of oligotrophic Acidobacteria. These studies raise the prospect that stability of Arctic soil bacterial communities could be influenced by perturbation of environmental conditions, as expected under climate warming scenarios.

Environmental heterogeneity and dispersal limitations are among the most important factors governing biogeographic patterns exhibited by plants and animals (Yang *et al*., 2015). While dispersal limitations have been claimed to be the dominant force in structuring bacterial taxa at finer levels of taxonomic classification in several studies (Cho & Tiedje, 2000; Green *et al*., 2004; Reche *et al*., 2005), other studies disagree (Cermeño & Falkowski, 2009; Finlay & Clarke, 1999; Finlay, 2002). Rather, local environmental conditions are considered more important than dispersal limitations in structuring biogeographic patterns of microorganisms, as observed in Antarctica (Chong *et al.,* 2013, 2015). Across Arctic terrestrial ecosystems, local environmental selection associated with variation in soil pH has been reported to be the primary driver structuring overall soil bacterial community composition, rather than historical contingency or dispersal limitation. Notably, although environmental stresses are perceived to be more severe in the Arctic, richness and phylotype diversity of bacterial communities in Arctic soils were not significantly lower than in temperate regions,

showing that bacterial community diversity in Arctic soils is not fundamentally different from other soil biomes (Chu *et al*., 2010).

2.4 Environmental stresses and Arctic soil bacteria

2.4.1 Effects of warming and water stresses on bacterial diversity

Arctic soils sequester a large proportion of the global organic carbon (C) pool, estimated to be 1500-1672 Pg in the permafrost region, nearly twice the amount of atmospheric C (Schuur *et al*., 2015; Tarnocai *et al*., 2009). Atmospheric temperature increases in high latitude regions are already larger than in other lower latitude regions (Fyfe *et al*., 2013; Hassol *et al*., 2004), and future climate projections estimate an increase of 7 - 8°C by the end of the 21st Century (IPCC, 2018; Trenberth *et al*., 2007). This warming leads to permafrost thaw (Ramanovsky *et al*., 2010; Koven *et al*., 2013) and a thicker seasonal active layer, which increases the availability of previously frozen C for microbial decomposition (Harden *et al*., 2012). Increased microbial activity (higher mineralization rates and respiration rates) could lead to the release of significantly higher amounts of CH_4 and CO_2 to the atmosphere (Koven *et al*., 2011; Schaefer *et al*., 2014), generating a positive feedback to climatic warming.

Climate change affects both soil temperature and moisture content, which in turn directly and indirectly influence the soil microbial community and microbially-mediated processes and ecosystem functions (Wan *et al*., 2007). Soil warming can drive changes in the continuum of C decomposing pools that contribute to soil organic matter (Amundson, 2001; Schädel *et al*., 2014). Such changes result in changes in microbial biomass (Frey *et al*., 2008) and in community structure or activity (Deslippe *et al*., 2011; Hartley *et al*., 2008; Jonasson *et al*., 1999; Rinnan *et al*., 2011; Sistla *et al*., 2013), with

some reports of shifts in microbial communities towards the dominance of Gram-positive bacteria and actinomycetes (Frey *et al*., 2008; Bell *et al*., 2009) as they adapt to the new chemical and physical environment. Warming effects can also be dependent upon water availability, as together they have been reported to strongly influence bacterial population size and diversity (Sheik *et al*., 2011), microbial biomass C and N, community composition and substrate utilization patterns (Guanlin *et al*., 2017). Experimental water addition to a high Arctic semi-desert led to a substantial increase in microbial biomass and microbial activity (Illeris *et al*., 2003).

Improved understanding the impacts of changes in temperature and moisture on soil microbial community structure would greatly enhance the the prediction of impacts of environmental perturbations, including climatic warming. This is because microbial communities are crucial to carbon and nutrient fluxes in the ecosystem, particularly the very sensitive large carbon pool in the Arctic, with great potential impacts on global atmospheric carbon pools (Schuur *et al*., 2008, Tarnocai *et al*., 2009). Likewise, warming-induced changes in microbial community structure have the potential to cause sustained change in microbial activity associated with global carbon and trace gases (Schimel & Gulledge, 1998; Bardgett *et al*., 2008).

In the Arctic, functional adaptations of the soil microbial community corresponding to changes in abiotic factors have been reported, such as to biogeochemical conditions (Buckeridge & Grogan, 2010b). Seasonal shifts are also reported, whereby bacteria dominate in the summer growing season and fungi dominate in the winter (Buckeridge *et al*., 2013; Schadt *et al*., 2003; Wallenstein *et al*., 2007), corresponding to a shift of microbial substrate decomposition from labile to recalcitrant (Lipson & Schmidt, 2004). Such changes in these communities could have significant effects on soil C dynamics in the Arctic (Davidson & Janssens, 2006, Fan *et al*., 2008), feeding back to

further changes in microbial community composition or biomass (Biasi *et al*., 2005; Sistla *et al*., 2013).

In field warming experiments microbes have shown a variety of responses. Soil warming experiments (0.3 °C to 6 °C) in various ecosystems consistently led to an immediate increase in microbial respiration (Rustad *et al*., 2001). However, in the longer term, warming-induced respiration decreases over time (Hartley *et al*., 2007; Rinnan *et al*., 2011), eventually leading to no or only small differences between soils at different temperatures (Rustad *et al*., 2001). One possible explanation for this decline in soil respiration rate over time in warmed soil is lies in microbial acclimation to warming by adjusting of metabolism and growth rate to the new temperature conditions, improving carbon use efficiency (CUE) (Bárcenas-Moreno *et al*., 2009; Zogg *et al*., 1997). Changes in microbial community structure can also be involved (Luo *et al*., 2014; Bradford *et al*., 2008; Allison *et al*., 2010; Bradford, 2013). Alternatively, some studies suggest that decreasing availability of labile substrates was the primary factor underlying decreased respiration and growth rates (Kirschbaum, 2004, Eliasson *et al*., 2005, Hartley *et al*., 2007).

In Arctic and sub-Arctic ecosystems, soil warming causes changes in the diversity or abundance of both bacterial and fungal communities (Clemmensen *et al*., 2006; Deslippe *et al*., 2012; Geml J. *et al*., 2015, 2016; Semenova *et al*., 2015), shifts in the balance of fungal or bacterial domination, increased decomposition of more recalcitrant C (Rinan *et al*., 2008), changes in plant community composition (Deslippe & Simard, 2011; Liu *et al*., 2018; Ylänne, *et al*., 2015), shifts from above-ground to below-ground plant community productivity (Deslippe & Simard, 2011), and in plant–microbial associations (Bragazza *et al*., 2013; Deslippe *et al*., 2011; Jonasson *et al*., 2004). Over long-term (7 and 17 years) experimental field warming of 1 - 2°C in the sub-Arctic,

bacterial community growth decreased significantly, by 28% and 73%, respectively (Rinnan *et al*., 2011), interpreted as decreasing availability of labile substrates, while no evidence was found supporting temperature adaptations in these soil bacterial communities.

Field warming effects have been shown to be influenced by the availability of water. For example, warming of a High Arctic coastal lowland caused significant changes in microbial community composition after 13 years of treatment (Walker *et al*., 2008). However, warming of wet heath plots over six years resulted in significantly higher soil carbon loss (Rinnan *et al*., 2007a) although did not affect microbial community composition (Rinnan *et al*., 2008). According to Strathdee *et al*. (1995), the effects of experimental warming were different between sites on High Arctic Svalbard and in sub-Arctic Abisko, emphasizing that research findings cannot be generalised between these regions. To the best of our knowledge, no published studies are available using field warming experiments to study the interactive effects of soil warming and water availability. Given the importance of understanding microbial community responses to soil warming and changes in water availability in the Arctic, and the potential additive or antagonistic effects of multiple factors on soil microorganisms (Hayden *et al*., 2012; Haugwitz *et al*., 2014; Guanlin *et al*., 2017; Sheik *et al*., 2011), long-term multifactorial field experiments are required.

2.4.2 Effects of freeze-thaw (FT) stress on bacterial diversity

In the polar regions, including the Arctic, freeze-thaw cycles are common phenomena and changes in the frequency of such events have been shown to have substantial effects on soil microbial communities and associated nutrient cycling functions (Kumar *et al*., 2013; Larsen *et al*., 2002; Sawicka *et al*., 2010; Yergeau &

Kowalchuk, 2008). Most previous studies have focused on the impacts of freeze-thaw cycles on microbial-associated soil nutrients, and have reported sometimes contradictory effects of freeze-thaw cycles. Freeze-thaw cycles have been reported to cause significant changes in dissolved soil organic carbon (DOC), NH_4^+-N, NO_3-N and total dissolved phosphorus (TDP), but with much variation beteween sampling sites and soil layers (Herrmann &Witter, 2002; Schimel & Clein, 1996; Zhao *et al*., 2010). Freeze-thaw cycles have also been shown to affect soil carbon dynamics, with changes observed in CO_2 emissions, total DOC and microbial biomass (Schimel & Clein, 1996; Laresen *et al*., 2002; Grogan *et al*., 2004; Matzner & Borken, 2008). In contrast, Foster *et al*. (2016) reported that repeated short freeze-thaw cycles have no significant effects on carbon mineralization in Arctic soils. Several studies have shown that exposure to freeze-thaw cycles increases the expression of denitrifying genes (Sharma *et al*., 2006) and also results in an increase in nitrous oxide (N_2O) release in the thawing period (Koponen *et al*., 2006; Ludwig *et al*., 2006; Müller *et al*., 2002; Yu *et al*., 2011). Wang *et al*. (2007) also reported that freeze-thaw cycles increase phosphorous (P) absorption rate in soil, and that this is more pronounced in soils with a high concentration of organic matter or clay. However, increasing numbers of freeze-thaw cycles decreased absorption capacity of P in soils (Wang *et al*., 2017).

Soil freeze-thaw events have been found to increase concentrations of low-molecular weight DOC (LMW-DOC) available for microbial uptake by causing damage and physical disruptions to roots and soil aggregates (Edwards & Cresser, 1992; Tierney *et al*., 2011; Herrmann & Witter, 2002; Henry, 2007). Yergeau *et al*. (2007) argued that the frequency of freeze-thaw cycles was a potentially significant driving force for the microbial community structure involved in the C-cycle in Antarctic soils. Nonetheless, the effects of freeze-thaw-induced gas release have been documented to decrease with increasing numbers of freeze-thaw cycles (Schimel & Clein, 1996; Priemé & Christensen,

2001; Koponen & Martikainen, 2004). Schimel and Clein (1996) suggested that this is due to depletion in nutrients available for microbial respiration or cell death after repeated freeze-thaw exposure.

Soil freeze-thaw cycles have also been shown to damage some microbial cells. The released nutrients were then taken up by the surviving microbes which became very active with a drastic increase in microbial respiration (Pesaro *et al*., 2003; Schimel & Clein, 1996; Skogland *et al*., 1988) and vigorous production of N_2O (Christensen & Tiedje, 1990; Koponen *et al*., 2006; Röver *et al*., 1998) during periods of soil thawing. In the event of severe freeze-thaw-induced microbial mortality, large changes in microbial community size and structure can occur, as reported by Skogland *et al*. (1998), Stres *et al*. (2010) and Wilson and Walker (2010). Sharma *et al*. (2006) reported that, during the transition from winter to spring, freeze-thaw cycles enhanced denitrification and caused a surge in N_20 and CO_2 emissions from soil. Studies on Arctic (Wallenstein *et al*., 2007) and alpine (Lipson, 2007; Lipson & Schmidt, 2004) soils have indicated seasonal changes in the bacterial community composition. Furthermore, results from the Canadian Arctic suggested the incidence of freeze-thaw cycles may increase at high latitudes as global temperature increase (Henry, 2008). It is thus important to identify what effects freeze-thaw cycles may have on the Arctic soil microbial community to better understand the potential feedbacks to climate change.

A number of microcosm studies have investigated the impacts of freeze-thaw cycles on the soil microbial community and associated functions using a variety of methodologies. For example, Walker *et al*. (2006a), Vishnivetskaya *et al*. (2007), Stres *et al*. (2010) and Wilson *et al*. (2012) focused on microbial survival and adaptations to freeze-thaw cycles using culture-dependent methods. However, cultivated isolates inevitably provide limited information (representing < 1% of the diversity present) about

the total microbial community (Douterelo *et al*., 2014). Studies have also used phospholipid fatty acid profiles (Feng *et al*., 2007; Koponen *et al*., 2006; Männisto, 2009) and DNA-based methods (Yergeau & Kowalchuk, 2008, Sawicka *et al*., 2010) for determining bacterial community structure. Although informative, studies using different soils and approaches have reported conflicting effects of freeze-thaw on microbial biomass and activities. In some cases, a significant decline in soil microbial abundance, community structure or activity was reported (Schimel & Clein, 1996; Stres *et al*., 2010; Yu *et al*., 2011), whereas other studies reported insignificant freeze-thaw effects (Lipson & Monson, 1998; Sharma *et al*., 2006).

A common feature of experimental studies to date is that the extreme freeze-thaw treatments used often did not resemble the natural freeze-thaw cycle conditions in the field. For instance, the large range in the number applied (up to 50 diurnal freeze-thaw cycles in Stres *et al*., 2010), rates (up to 48 freeze-thaw cycles day^{-1} in Walker *et al*. (2006a) and Wilson *et al*. (2012)), and amplitude (from -20 to +10°C in Sharma *et al*. (2006), do not match natural field conditions. Hence, although the findings of such studies are informative, they provide limited insights into the impacts on microbial community composition and function under natural freeze-thaw scenarios in the field. However, it has been suggested that exposure to freeze-thaw cycles selects for freeze-thaw-tolerant community (Walker *et al*., 2006a; Wilson *et al*., 2012).

Männistö *et al*. (2009) examined effects of long term (up to 60 days) field-simulated freeze-thaw events on bacterial community composition using terminal restriction fragment length polymorphism (T-RFLP) profiles of reverse transcribed 16S rRNA coupled with clone analysis. Results showed that there were no significant changes in dominant phyla as compared with the control groups. The data obtained, however, did not conclusively show that freeze-thaw treatments had no significant effects on the

microbial community. Previously, such long-term incubations have been criticized as neglecting sensitivity to depletion of labile microbial substrates (Boone *et al*., 1998; Reichstein *et al*., 2000). Moreover, mineral transformations in long-term incubations may exaggerate measured microbial responses, thus compromising experimental sensitivity (Mikan *et al*., 2002). Short-term incubations in comparison, have some preference as they overcome such effects (Boone *et al*., 1998; Reichstein *et al*., 2000).

Warmer winters in Arctic regions may increase the incidence of above 0°C soil temperatures and result in a decreasing trend in snow cover depth. Due to reduced or absent snowpack, this may make soils more vulnerable to temperature fluctuations and lead to more freeze-thaw cycles being experienced (Henry, 2008; Førland *et al*., 2011). Snow cover provides an excellent shield from temperature fluctuations, insulating the underlying soil and vegetation. Greater insulation is typically provided with increasing snow depth, which leads to more stable and less extreme soil temperatures and a reduced occurrence of soil freeze-thaw cycles (Pauli *et al*., 2013; Convey *et al*., 2014; Tan *et al*., 2014; Zhang, 2005). Generally, depth of snow is a proxy for snow accumulation period. In simple terms, at least across a site of small area, shallow snow cover is likely to result from a shorter accumulation period and to melt completely (hence exposing the ground to higher F/T frequency) and thick snow cover is likely to persist for longer (hence lower F/T frequency). Zinger *et al*. (2009) reported spatial and temporal variation in the soil microbial community under different snow depths, with bacterial and fungal communities showing different seasonal shifts in their community structure at early snowmelt locations and lower diversity at late snowmelt locations. This study provided insights into the distribution of bacterial diversity in relation to snow cover dynamics, but did not include further investigation of the resilience of the soil bacterial community towards exposure to freeze-thaw cycles.

Thus, it is important to investigate if snow cover depth affects soil bacterial community diversity in the High Arctic. Experimentally subjecting soils obtained from different snow cover depths to the same freeze-thaw cycle regime and comparing their bacterial community response will provide insight into how snow depth affects the bacterial community response towards freeze-thaw exposure.

2.5 Methods used for analysis of bacterial diversity

The current study utilised an amplicon-based high throughput sequencing method to assess soil bacterial community diversity, using the Illumina MiSeq platform. The increased availability of high-throughput sequencing technology in recent years has enabled large-scale comparative studies of samples from multiple environments. As compared to non-selective metagenomic approaches (shotgun sequencing), targeted metagenomics provides higher sequence coverage and extensive redundancy of sequences for targeted genes, thus giving a better resolution of microbial community composition (Suenega, 2012).

The 16S rRNA gene is an excellent phylogenetic marker for bacterial community diversity studies (Pace, 1997; Konstantinidis & Tiedje, 2005), and has been utilised in many studies analyzing the diversity, composition, and dynamics of microbial communities from different habitats (e.g. Andersson *et al*., 2010; Campbell *et al*., 2010; Eilers *et al*., 2010), and also microbial community changes in response to environmental perturbations (Deslippe *et al*., 2012; Lu *et al*., 2012; Fierer *et al*., 2012; Goldfarb *et al*., 2011). Among all nine variable regions (V1-V9) in the bacterial 16S rRNA gene, the V3-V4 region was selected in this study due to its ability to distinguish all bacterial taxa to the genus level, except for closely related *Enterobacteriaceae*, in various environmental communities (Chakravorty *et al*., 2007; Claesson *et al*., 2010).

In recent years, there has been increasing use of high-throughput sequencing in various research areas resulted, resulting in a growing amount of sequence data (Stephens *et al*., 2015). Managing thethese expanding datasets becomes a challenge in data analysis, which often involves visualisation of comparisons across a wide range of datasets to discover their relationships. Two-circle Euler Diagrams are often used to show intersection and complementarity between pairs of datasets, with the numbers of overlapping and unique OTUs indicated in the diagram. These values are also important components in the calculation of similarity indices based on presence/absence of data. In ecological and taxonomic studies, similarity indices are often used to indicate compositional (dis)similarity of assemblages.

Among available similarity indices, the Jaccard Index and Sørensen Index are two classic and widely used similarity indices (Jaccard, 1912; Sørensen, 1948), and have been used in the analyses of many high-throughput sequencing datasets (e.g. Baptista *et al*., 2015; Besemer *et al*., 2012; Davydenko *et al*., 2013; Lin *et al*., 2016; Menkis *et al*., 2015). Both indices are based on the absence or presence of OTUs, and quantify (a) the number of OTUs unique to Sample A, (b) Number of OTUs unique to Sample B, and (c) Number of overlapping OTUs between Sample A and Sample B, which are equivalent to the three incidences illustrated in a two-circle Euler Diagram comparing two samples. Consequently, when dealing with large datasets, the process of determining these three incidences becomes a rate-limiting step in data analysis.

Software/web programmes which generate a two-circle Venn and/or Euler Diagram and display the unique and overlapping OTUs are widely available (e.g. Oliveros, 2007; Hulsen *et al*., 2008; Heberle *et al*., 2015; Bardou *et al*., 2014; Chen & Boutros, 2011). Nonetheless, there is currently no available graphic-user interace (GUI) software that integrates and analyses large numbers biological datasets for pairwise

comparison, which is needed in the current study for OTU comparisons between samples. To the best of our knowledge, a maximum of 30 samples can currently be interpreted simultaneously (see http://bioinformatics.psb.ugent.be/webtools/Venn/). In order to generate accurate Jaccard index values for a larger number of samples (1 - 200 samples) in this study for pairwise comparison of bacterial community composition, this project included development of a new software, JS Euler (Jaccard, Sørensen and Euler Diagram).

CHAPTER 3: MATERIALS AND METHODS

3.1. Description of location and sampling on Brøggerhalvøya Peninsula

3.1.1 Sampling location and experimental designs

The open-top chamber (OTC) experiment was established in September 2014 on the Brøggerhalvøya Peninsula at Kvadehuken (78° 58.002' N, 11° 28.446'E) on the west coast of Spitsbergen in Svalbard, shortly before the commencement of the High Arctic winter period (Figure 3.1).This experimental set-up was established by Dr. Kevin Newsham from British Antarctic Survey in an extensive area of periglacial patterned ground (soil polygon), with his research focus on the effects of field warming and/or water amendment on the High Arctic soil fungal communities. The polygons were colonised (primarily around their margins) by a vegetation composed of *Salix polaris*, *Bistorta vivipara* and *Saxifraga oppositifolia*, and by microbial soil crusts (Lim *et al*., 2018). Mosses were not frequently encountered. Warming by open-top chambers and water amendment were applied in factorial combination to the polygons, comprising four treatment groups: (1) Control; (2) Warming; (3) Water amendment and (4) Warming + Water amendment. Triplicate plots for each treatment in three blocks were sampled on three occasions (Sampling 1-June 2015, Sampling 2- September 2015 and Sampling 3- September 2016) as indicated in Table 3.1. The open-top chambers used were ITEX chambers (1.2 m basal diameter, 0.75 m aperture diameter, 400 mm height, constructed as described in Fig. 6 of the ITEX manual (Henry & Molau, 1997), and secured with tent pegs attached to cable ties. Water amendment consisted of adding 1 L of deionised water onto the polygons. Soil temperatures were recorded using Tinytag Plus 2 temperature loggers (Gemini Data Loggers Limited, Chichester, UK) inserted at 1 - 2 cm depth into the soil in both chambered and control plots.

Figure 3.1: The sampling location on Brøggerhalvøya Peninsula is represented by the blue point. The red point represents Longyearbyen.

Figure 3.2: The general environment of the open-top chamber study location (Lim *et al*., 2018). Permission from the Current Science Association.

Table 3.1: Summary of the treatments applied and sample names in the field warming and water amendment experiment across all three Sampling Occasions (Sampling 1, Sampling 2 and Sampling 3) at each block (Block 1, Block 2 and Block 3).

Block 1

Sample name	Sampling[a]	Control	Warming	Water	Sample name	Sampling[b]	Control	Warming	Water	Sample name	Sampling[c]	Control	Warming	Water
C1_1_1	1	√			C1_2_1	2	√			C1_3_1	3	√		
C2_1_1	1	√			C2_2_1	2	√			C2_3_1	3	√		
C3_1_1	1	√			C3_2_1	2	√			C3_3_1	3	√		
O1_1_1	1		√		O1_2_1	2		√		O1_3_1	3		√	
O2_1_1	1		√		O2_2_1	2		√		O2_3_1	3		√	
O3_1_1	1		√		O3_2_1	2		√		O3_3_1	3		√	
W1_1_1	1			√	W1_2_1	2			√	W1_3_1	3			√
W2_1_1	1			√	W2_2_1	2			√	W2_3_1	3			√
W3_1_1	1			√	W3_2_1	2			√	W3_3_1	3			√
M1_1_1	1		√	√	M1_2_1	2		√	√	M1_3_1	3		√	√
M2_1_1	1		√	√	M2_2_1	2		√	√	M2_3_1	3		√	√
M3_1_1	1		√	√	M3_2_1	2		√	√	M3_3_1	3		√	√

[a] represents sampling period at June 2015 (Sampling 1); [b] represents September 2015 (Sampling 2); [c] represents September 2016 (Sampling 3)

Table 3.1, continued.

Block 2

Sample name	Sampling[a]	Control	Warming	Water	Sample name	Sampling[b]	Control	Warming	Water	Sample name	Sampling[c]	Control	Warming	Water
C1_1_2	1	√			C1_2_2	2	√			C1_3_2	3	√		
C2_1_2	1	√			C2_2_2	2	√			C2_3_2	3	√		
C3_1_2	1	√			C3_2_2	2	√			C3_3_2	3	√		
O1_1_2	1		√		O1_2_2	2		√		O1_3_2	3		√	
O2_1_2	1		√		O2_2_2	2		√		O2_3_2	3		√	
O3_1_2	1		√		O3_2_2	2		√		O3_3_2	3		√	
W1_1_2	1			√	W1_2_2	2			√	W1_3_2	3			√
W2_1_2	1			√	W2_2_2	2			√	W2_3_2	3			√
W3_1_2	1			√	W3_2_2	2			√	W3_3_2	3			√
M1_1_2	1		√	√	M1_2_2	2		√	√	M1_3_2	3		√	√
M2_1_2	1		√	√	M2_2_2	2		√	√	M2_3_2	3		√	√
M3_1_2	1		√	√	M3_2_2	2		√	√	M3_3_2	3		√	√

[a] represents sampling period at June 2015 (Sampling 1); [b] represents September 2015 (Sampling 2); [c] represents September 2016 (Sampling 3)

Table 3.1, continued.

Block 3

Sample name	Sampling[a]	Control	Warming	Water	Sample name	Sampling[b]	Control	Warming	Water	Sample name	Sampling[c]	Control	Warming	Water
C1_1_3	1	√			C1_2_3	2	√			C1_3_3	3	√		
C2_1_3	1	√			C2_2_3	2	√			C2_3_3	3	√		
C3_1_3	1	√			C3_2_3	2	√			C3_3_3	3	√		
O1_1_3	1		√		O1_2_3	2		√		O1_3_3	3		√	
O2_1_3	1		√		O2_2_3	2		√		O2_3_3	3		√	
O3_1_3	1		√		O3_2_3	2		√		O3_3_3	3		√	
W1_1_3	1			√	W1_2_3	2			√	W1_3_3	3			√
W2_1_3	1			√	W2_2_3	2			√	W2_3_3	3			√
W3_1_3	1			√	W3_2_3	2			√	W3_3_3	3			√
M1_1_3	1		√	√	M1_2_3	2		√	√	M1_3_3	3		√	√
M2_1_3	1		√	√	M2_2_3	2		√	√	M2_3_3	3		√	√
M3_1_3	1		√	√	M3_2_3	2		√	√	M3_3_3	3		√	√

[a] represents sampling period at June 2015 (Sampling 1); [b] represents September 2015 (Sampling 2); [c] represents September 2016 (Sampling 3)

3.1.2 Sampling, transport and storage

Sampling was carried out at three time points: (1) Sampling 1-July 2015; (2) Sampling 2-September 2015 and (3) Sampling 3-September 2016. Soil samples (approximately 1 g) were collected from targeted plots using sterile spatulas and stored in individual 15 mL sterile tubes. The samples were initially packed with ice and stored in an insulated container, and returned to the NERC Arctic station at Ny-Ålesund within 3 h wherethe samples were immediately frozen at -20 °C. The soils were subsequently transported frozen to the University of Malaya, Kuala Lumpur, where they were again stored at -20°C until further use.

3.2 Description of sampling in Longyearbyen and laboratory freeze-thaw (FT) treatments

3.2.1 Site description, sample collection

At Longyearbyen, the snow pack and underlying soils warm to become isothermal at close to 0°C in the spring season, and the snow pack melts in late May or early June, exposing the ground surface to 24 h insolation (mid-end April until mid-end August) (Convey *et al*., 2018). The insulating effects of snow cover are well appreciated, particularly in the spring as snow cover is greater in this season than in summer. In this study, the sampling site was a snow bank close to the University Centre in Svalbard (UNIS), Longyearbyen, Norway (78° 14.874'N, 15° 24.313'E) (Figure 3.3). Soils were sampled in triplicate under three different summer snow depths (no snow cover, low snow cover and high snow cover) on 25 July 2015, giving a total of nine soil samples. Snow depth was estimated based on ground surface topography. High snow cover was covered in the most protected soil. Low snow cover was over a rather flat soil surface nearer to the edge of the bank. No snow cover soil was sampled from an exposed ridge adjacent to the remaining bank. Soil samples were collected into sterile tubes using sterile spatulas.

The collected soils were placed immediately in an insulated container and then stored frozen at -20 °C at UNIS within < 1 h. The samples were then transported frozen to the University of Malaya, Kuala Lumpur, and stored at -20°C until further processing.

3.2.2 Soil chemical analyses.

All nine baseline soil samples were subjected to basic chemical analyses by Forest Research Institute Malaysia, which included organic carbon using Walkley-Black chromic acid wet oxidation method (Walkley & Black, 1934), available phosphorus by extraction with sodium bicarbonate (Bray & Kurtz, 1945), ammonium and nitrate using wet oxidation method (Bremner, 1965; Keeney & Nelson, 1982), and total nitrogen using Micro-Kjeldahl method digestion and titration method (Ma & Zuazaga, 1942).

Figure 3.3: The sampling site on a snow bank with descriptions of sampled soils estimated to experience three different snow depths. (A) **High** (Soils below the remaining snow), from a hollow surface, assumed to experience the lowest number of freeze-thaw (FT) cycles. (B) **Low**, soils in between two remaining snow patches, assumed to experience mediuman intermediate number of FT cycles. (C) **Bare** (soils from the ridge), assumed to experience the highest number of FT cycles.

3.2.3 Laboratory freeze-thaw (FT) experiment

All nine baseline soil samples were subjected to freeze-thaw (FT) treatments over nine successive freeze-thaw cycles or a complementary thawing control (incubated under 'thaw conditions' for the duration of treatment group being exposed to FT cycles). For each soil sample (n = 9), a total of 3 g was distributed into four 2.0 ml microcentrifuge tubes, for subsampling at four occasions: (1) after 1st FT, (2) after 9th FT, (3) 1st Control, and (4) 9th Control. During the frozen phase the tubes were held at -1.4°C for 12 h followed by 4.0°C for 12 h to achieve one freeze-thaw cycle (based on average freezing and thawing temperatures and diurnal freeze-thaw fluctuations in High Arctic Shrub; data obtained from Convey *et al*. (2018)). The tubes were immersed in a temperature-regulated water bath (Haake SC-100-A25, Thermo Scientific) filled with 50% of antifreeze liquid, propylene glycol. The experimental design and individual sampling names are illustrated in Figure 3.4, Figure 3.5 and Figure 3.6.

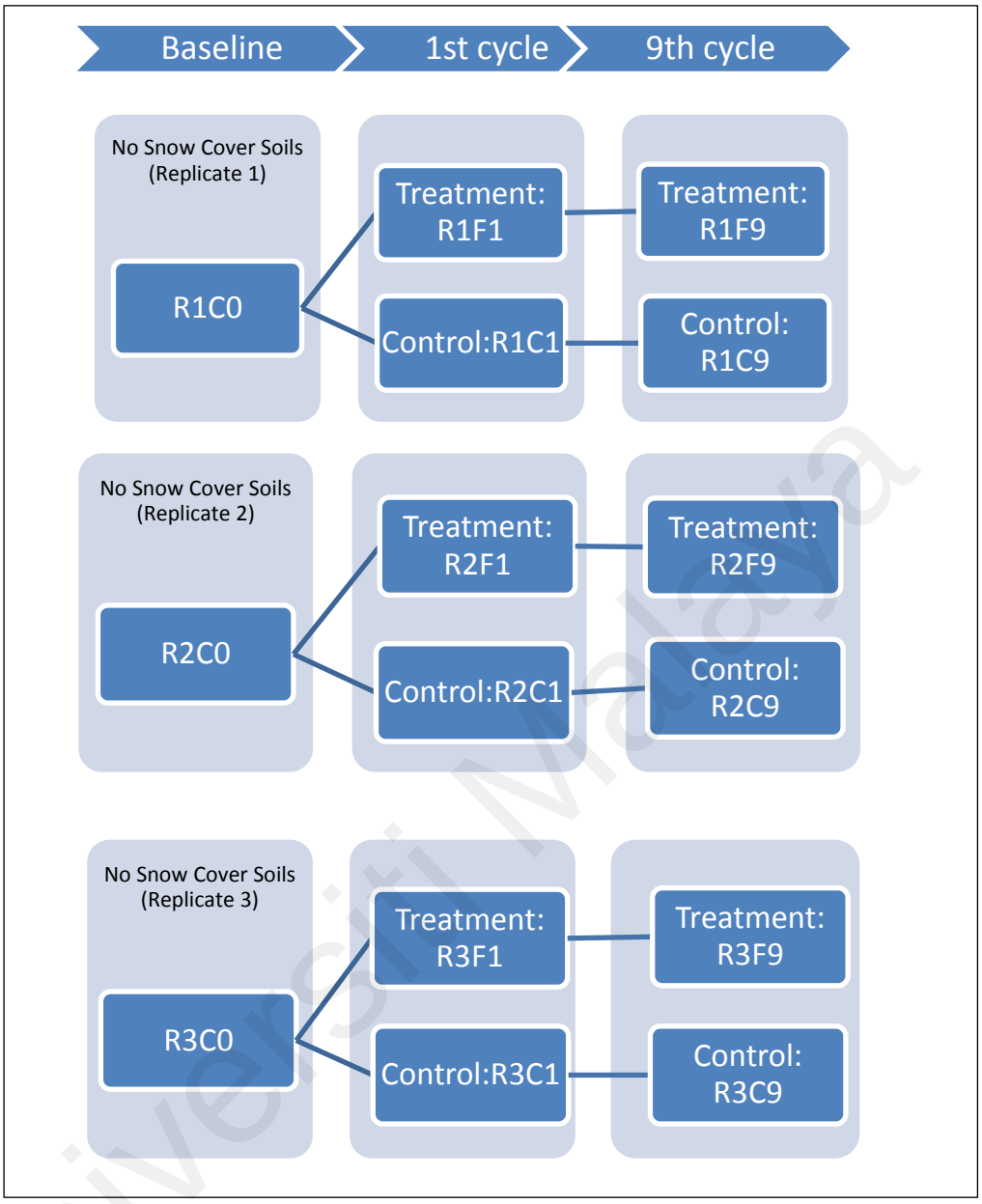

Figure 3.4: Sampling regime and sample codes for freeze-thaw treatments and complementary controls for no snow cover soils.

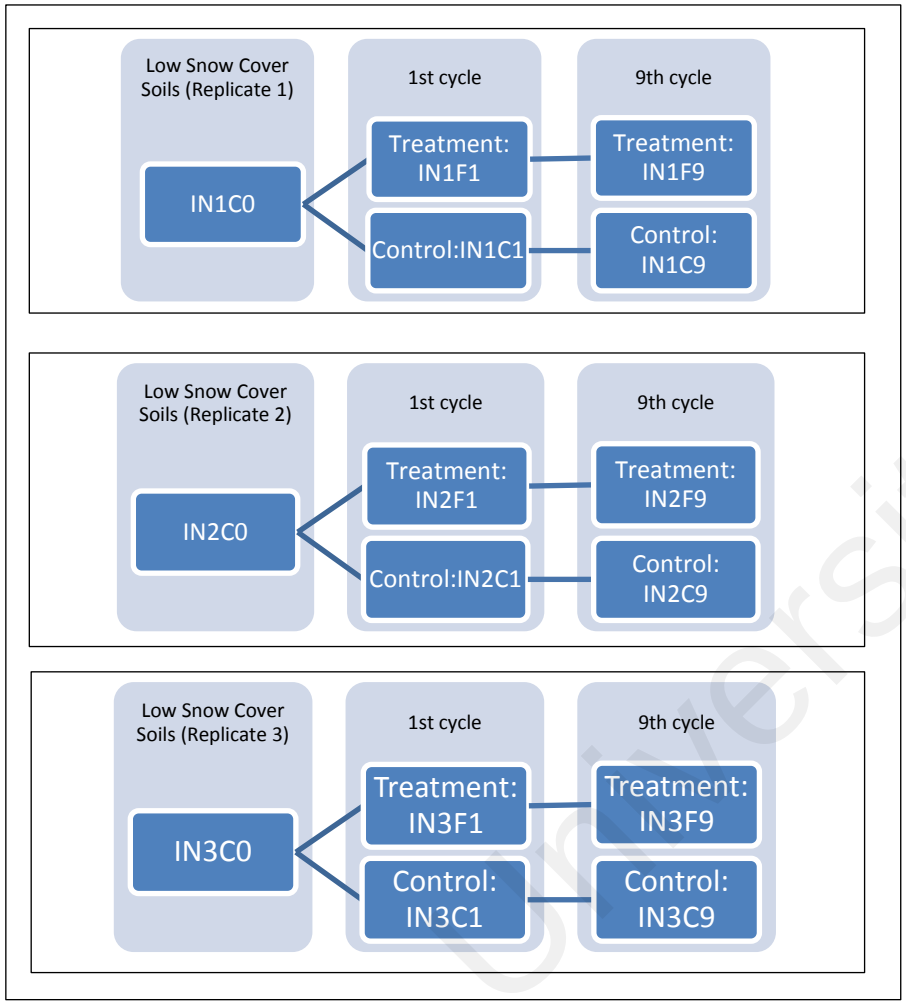

Figure 3.5: Sampling regime and sample codes for freeze-thaw treatments and their complementary controls for Low Snow Cover soils.

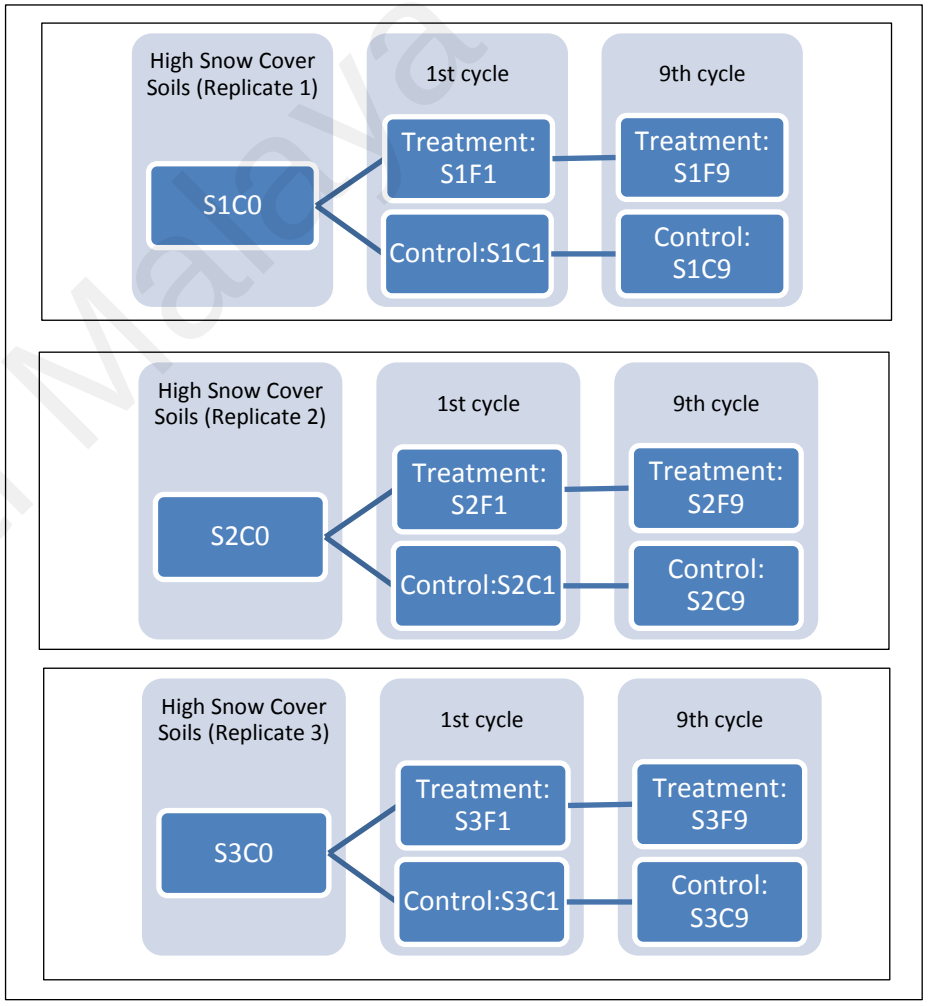

Figure 3.6: Sampling regime and sample codes for freeze-thaw treatments and their complementary controls for High Snow Cover soils.

3.3 16S rRNA amplicon library preparation and sequencing

3.3.1 DNA extraction from soil samples, quality examination and normalization

DNA was extracted from each soil sample (0.25 g) using the MoBio PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instructions (MO BIO PowerSoil DNA Isolation Kit Handbook). The quality and quantity of DNA were examined using the Qubit dsDNA HS Assay Kit (Invitrogen, Belgium) on an EnSpire Multimode Plate Reader (PerkinElmer, USA) incorporated with Janus® Automated Workstation (PerkinElmer, USA) for samples in Section 3.1, and DropPlate16-D+ Chips on LabChip DS (PerkinElmer, USA) for samples in Section 3.2. The high-quality DNA samples were normalized to 5 ng/µµ L using Janus® Automated Workstation (PerkinElmer, USA), followed by 16S rRNA amplicon library preparation following the manufacturer's instructions (Illumina, USA). DNA samples were stored at -20°C before processing.

3.3.2 Amplification and purification of bacterial 16S rRNA (V3-V4 gene fragments)

The V3 and V4 hypervariable regions of the bacterial 16SrRNA genes were targeted and amplified by polymerase chain reaction (PCR) using forward primer 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3', and the reverse primer 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC 3'. (Klindworth *et al*., 2013). PCR was performed using 25 µ L assays: 12.5 µ L of 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystem, Woburn, MA, USA), 5 µ L of each primer (1 µ M) and 2.5 µ L of microbial genomic DNA (5 ng L^{-1}). The PCR thermal profile was in accordance with the manufacturer's instructions (Kapa Biosystem, Woburn,

MA, USA), with an initial denaturation at 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final elongation step at 72°C for 5 min. Samples were immediately transferred to 1.5 mL microcentrifuge tubes and subjected to PCR clean-up using AMPure XP beads.

The AMPure XP beads were vortexed for 30 s to ensure that the beads were evenly dispersed. A volume of 20 µ L of AMPure XP beads (Beckman Coulter, Brea, California, United States) was pipetted into each amplicon sample individually and shaken with Eppendorf MixMate (Fisher Scientific, USA) at 1800 rpm for 2 min, followed by incubation at room temperature without shaking for 5 min. Sample tubes were placed on a magnetic stand for 2 min or until the supernatant had cleared. With the sample tubes on the magnetic stand, the supernatant was discarded using a pipette. Tips were changed between samples. Samples tubes remained on the magnetic stand for the subsequent steps (two-step ethanol wash and air-dry). The first ethanol wash was performed by pipetting 200 µ L of freshly prepared 80% ethanol into each sample and incubating for 30 sec. The supernatant was removed and discarded carefully. The second ethanol wash repeated the procedure of the first. Beads were air-dried for 10 min. Sample tubes were then removed from the magnetic stand. A volume of 52.5 µ L of 10 mM Tris pH 8.5 was pipetted into each sample and vortexed with Eppendorf MixMate (Eppendorf, North America) at 1800 rpm for 2 min, followed by incubation at room temperature without shaking for 2 min. Samples were then placed on a magnetic stand for 2 min or until the supernatant had cleared. A volume of 50 µ L of the supernatant was carefully transferred from the sample tubes to new 1.5 mL microcentrifuge tubes and stored at -20°C. Samples were subjected to Indexing PCR within 5 d as described below.

3.3.3 Indexing and purification of amplified bacterial 16S rRNA (V3-V4 gene fragments)

The Illumina sequencing adapter and dual-index barcode (Nextera XT Index Kit, Illumina, USA) were then incorporated into the initial PCR product via a second round of 8-cycle PCR to generate a library construct consisting of the complete Illumina adapter, dual 8 bp Nextera-like barcode and the 16S rRNA fragment in accordance with the manufacturer's instructions (Kapa Biosystem, Woburn, MA, USA).

PCR was performed using 50 µL assays: 25.0 µL of 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystem, Woburn, MA, USA), 5 µL of each primer, 5 µL of purified initial PCR products from Section 3.3.2 and 10 µL of Milli-Q water (Merck, USA). The PCR thermal profile followed the manufacturer's instructions (Kapa Biosystem, Woburn, MA, USA), with an initial denaturation at 95°C for 3 min, followed by 8 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final elongation step at 72°C for 5 min.

A volume of 56 µL of AMPure XP beads (Beckman Coulter, Brea, California, United States) was pipetted into each sample individually. Prior to this, the AMPure XP beads were vortexed for 30 s to make sure that they were evenly dispersed before adding to samples. The mixed samples were then shaken with Eppendorf MixMate (Eppendorf, North America) at 1800 rpm for 2 min, followed by incubation at room temperature without shaking for 5 min. Sample tubes were placed on a magnetic stand for 2 min or until the supernatant had cleared. With the sample tubes on the magnetic stand, the supernatant was discarded using a pipette. Separate tips were used for each sample to avoid cross contamination. Samples tubes remained on the magnetic stand for subsequent steps (two-step ethanol wash and air-dry). Both ethanol-wash steps were performed using the same procedure. First, pipetting a volume of 200 µL of freshly prepared 80% ethanol into each sample and incubating for 30 sec. The supernatant was removed and discarded carefully. The beads were to air-dried for 10 min. Sample tubes were removed from the

magnetic stand. A volume of 52.5 µL of 10 mM Tris pH 8.5 was pipetted into each sample and shaken with Eppendorf MixMate (Fisher Scientific, USA) at 1800 rpm for 2 min, followed by incubation at room temperature without shaking for 2 min. Samples were then placed on a magnetic stand for 2 min or until the supernatant had cleared. A volume of 25 µL of the supernatant was then carefully transferred from the sample tubes to new 1.5 mL microcentrifuge tubes and stored at -20°C.

3.3.4 Quantification and normalization of Index-PCR products

The amplified Index-PCR products were quantified with the Qubit dsDNA HS Assay Kit (Invitrogen, Belgium) on an EnSpire Multimode Plate Reader (PerkinElmer, USA) and normalized to 10 nM using a Janus® Automated Workstation (PerkinElmer, USA). The concentration of the amplifiable libraries was determined using an Illumina Eco qPCR machine, as described in the KAPA Library Quantification Kits for Illumina sequencing platforms (KAPA BioSystems, Boston, USA), prior to paired-end sequencing using the MiSeq Reagent Kit (v2) with the read length set to 2 × 250 bp. By calculation from the qPCR reading, the library template was diluted to 4 nM in Tris-HCl buffer (10 mM, pH 8.5 with 0.1% v/v Tween 20). DNA concentration in ng/µL was converted into nM based on the following formula:

$$\frac{concentration\ in\ ng/\mu L}{\frac{660g}{mol}\ x\ average\ library\ size^{a}} \times 10^{6} = concentration\ in\ nM$$

[a]Average library size used was 500 bp

3.3.5 Preparation of pooled libraries for Illumina Miseq paired-end sequencing

Exactly 5 µL of each library (4 nM) were subsequently pooled into one microcentrifuge tube (1.5 mL). The pooled library was denatured and further diluted to

4.5 pM with PhiX control spike-in of 25%, following the manufacturer's instructions (16S rRNA Metagenomic Sequencing Library Preparation, Illumina, USA). The mixed sample library and PhiX control were heat-denatured by incubating on a heat block at 96°C for 2 min. After incubation, the tube was inverted 1-2 times to mix properly and immediately placed in iced water for 5 min. The denatured and diluted pooled libraries were then loaded immediately into a MiSeq v2 reagent cartridge (Illumina, USA), guided by the MiSeq Control Software Interface.

3.4 Quality control (QC) of Miseq raw datasets

Quality trimming and merging were carried out using CLC Genomics Workbench 8.5. The demultiplexed sequences generated by the Illumina Miseq sequencer underwent adapter trimming, and merging of overlapping pairs using default parameters (Mismatch cost: 2; Gap cost: 3; Max unaligned end mismatches: 0; Minimum score: 8) in CLC Genomic Workbench 8.5. Unmerged reads with a minimum length of 150 bp were retained. Filtering of sequences less than 150 bp, ambiguous nucleotides and low-quality reads according to base quality (q = 20) was carried out. Chimeric sequences were identified and removed using UCHIME2 based on the Greengenes Reference Database-Greengenes version 13_8.

3.5 Operational taxonomic unit (OTU) picking and taxonomic classification

The QC-passed sequences were then subjected to downstream analysis using QIIME 1.8.0 software (Caporaso *et al*., 2010). Operational taxonomic unit (OTU) picking was performed based on an open reference approach using *pick_open_reference_otus.py* script. The representative read of each OTU underwent taxonomic profiling based on the the Greengenes Reference Database-Greengenes version 13_8.

3.6 Individual sample coverage

To ensure the OTU data provide sufficient coverage for comparison, rarefaction analysis measured by the number of observed unique OTUs in a sample was performed using *alpha_rarefaction.py* script in QIIME. In addition, the coverage percentage of each sample was calculated based on Good's coverage (Good, 1953) using *alpha_diversity.py* script.

3.7 Diversity analyses in this study

Alpha diversity refers to the microbial diversity within an environment (Lozupone & Knight, 2008). The Shannon diversity index was used as a measure of alpha diversity as it takes into account both richness and evenness of OTUs in the environment studied (Shannon, 1948; Haegeman *et al*., 2013). The Shannon diversity index for each sample was obtained using *alpha_diversity.py* script in QIIME.

Beta diversity refers to the comparison of the microbial diversity among two or more environments (Lozupone & Knight, 2008). For beta diversity, comparison of bacterial community composition was conducted at genus level between different groups of samples. Data were generated using *summarize_taxa.py* script in QIIME, followed by pairwise comparison between samples using Jaccard similarity indices, generated using the software JS Euler, as described in Section 3.7.3.

A principal coordinate analysis (PCoA) was applied based on un-weighted and weighted Unifrac distances (Lozupone *et al*., 2007) in QIIME using *beta_diversity.py* and *principal_coordinates.py*, followed by *make_2d_plots.py* scripts. The un-weighted Unifrac distances were based on the presence/absence of annotated phylotypes, whilst the weighted Unifrac distances took into account of both presence/absence and relative abundance of annotated phylotypes. In PCoA plots, the points of bacterial samples which were phylogenetically more similar to each other (shorter phylogenetic distance) were

clustered closer together, whereas samples which shared longer phylogenetic distance were visualized by points which were further apart.

The relationships of co-occurring bacterial genera between sample groups were also assessed, based on the relative abundance of shared co-occurring genera, using Spearman rank order correlation analysis. Correlation strength was categorized based on the correlation coeeficient, i.e. 0.71 - 1.00 is strong correlation, 0.51 - 0.70 is moderate correlation and 0.31 - 0.50 is weak correlation (Chua, 2013).

Statistical tests were included in all comparison and correlation tests (described in Section 3.7.1 and Section 3.7.2), using IBM SPSS Statistics for Windows version 19.0 (IBM Corp., USA). A significance level of $p < 0.05$ was applied to all statistical tests used in this study.

3.7.1 Diversity analysis of samples from the open-top chambers and water amendment study

In order to identify the significance of the experimental field manipulation warming and/or water treatments on bacterial alpha diversity at each of the three sampling points/seasons, an average Shannon index of soil replicates were used to represent the alpha diversity of the corresponding treatment group, and the average Shannon diversity values were compared among groups using one-way ANOVA. The seasonal effects on Shannon diversity of each treatment group were assessed using Repeated-Measures ANOVA, where the significance of seasonal changes were evaluated by combining alpha diversity values of all samples in the respective season, followed by one-way ANOVA to compare mean alpha diversity between seasons. Normality tests and Levene's test of heterogeneity were carried out to confirm data normality prior to the use of ANOVA.

In each season, the Jaccard similarity indices were computed between different treatment groups (control, water amendment, warming, water amendment + warming), followed by one-way ANOVA to determine pairs of treatment groups with significantly higher or lower Jaccard similarity than the other groups. Soil bacterial beta diversity of each treatment group across seasons was examined using Repeated-measured ANOVA. LSD (Least Significant Difference) post-hoc comparison tests were performed if ANOVA was significant.

Correlations among co-occurring phylotypes between treatments were assessed by comparing changes in average relative abundance among all co-occurring genera between each experimental group in each season. The correlations of co-occurring genera between seasons were examined by comparing changes in average relative abundance of all co-occurring genera between seasons. Spearman rank order correlation tests were used in these correlation analyses. Co-occurring predominant bacterial taxa (≥ 1%) at genera level were represented in line graphs. Bar charts were also created to display bacterial community composition of all samples in each season at the phylum level. Beta diversity for all 36 samples were visualised using PCoA plots in QIIME (Caporaso *et al.,* 2010).

3.7.2 Diversity analysis of samples from the freeze-thaw (FT) treatments

For baseline soils, one-way ANOVA test was again employed to assess for any significant differences between the three baseline groups (low, medium and thick snow cover) in Shannon diversity and Jaccard similarity indices of bacterial community composition at genus level. Bar charts were created to display bacterial community phyla composition of all samples for baseline soils.

For each snow depth group (low, medium and thick), Shannon diversity indices were compared among samples which received experimental freeze-thaw treatments and the thawed control (Baseline, 1st cycle, 9th cycle). Split-plot ANOVA with post-hoc tests

were used to examine for any significant changes in Shannon index throughout the treatments. Mauchly's test of sphericity was performed to confirm variances of the differences between all possible pairs were equal prior to the ANOVA test.

The Jaccard index for all possible pair of samples calculated by JS Euler was used to assess for variation in bacterial community composition between different treatment groups, followed by one-way ANOVA. The correlations of co-occurring phylotypes among baseline, treatments and control groups at 1st cycle and 9th cycle were assessed by comparing changes in average relative abundance among all co-occurring genera at each snow depth group using Spearman rank order correlation tests. Co-occurring dominant bacteria genera were visualised in line graphs. Bar charts were also created to display bacterial community composition at phylum level for all samples across freeze-thaw treatments and thawing controls at each snow depth level. Beta diversity for all 45 samples was visualised using PCoA plots.

3.7.3 Development of JS Euler software for generating Jaccard index between samples

JS Euler was developed with HTML 5.0 and functions from the JavaScript Library. It is a web-based software with simple graphic user interface, which can be used in both Windows and Unix/Linux Operating Systems. JS Euler was created to provide accurate information for comparative analyses in this study (particularly the Jaccard index), for all possible pairwise comparisons between samples. The open source libraries used were Blob.js, Bootstrap, canvas-toBlob.js, D3, FileSaver.js, jQuery, jquery.arrayUtilities, jquery-csv, jscolor, and venn.js. The original source codes written were index.html, logic_documented.js and mystyle.css (see Appendix A). Figure 3.7 illustrates the flowchart of JS Euler.

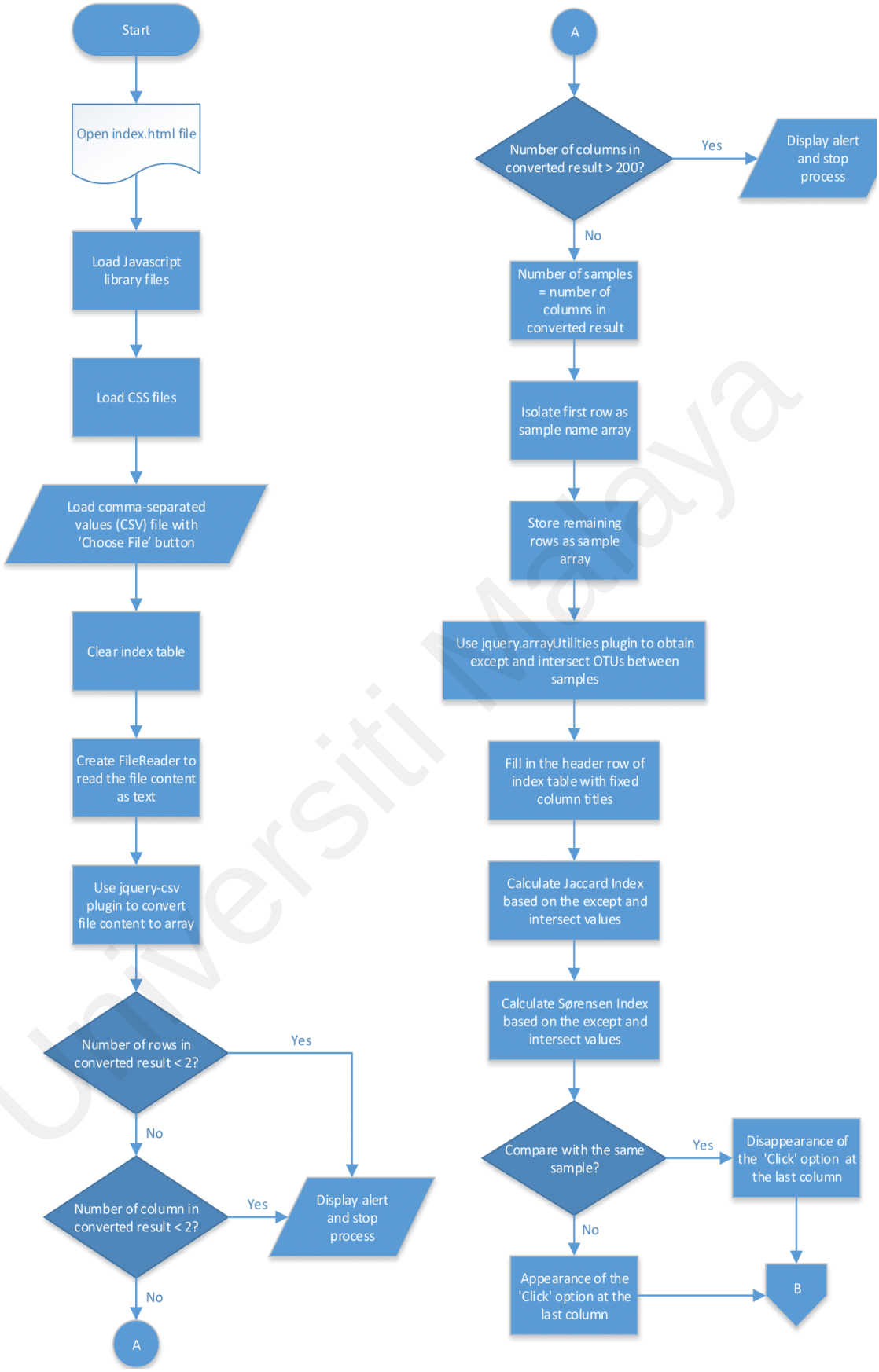

Figure 3.7: Flow chart of JS Euler software.

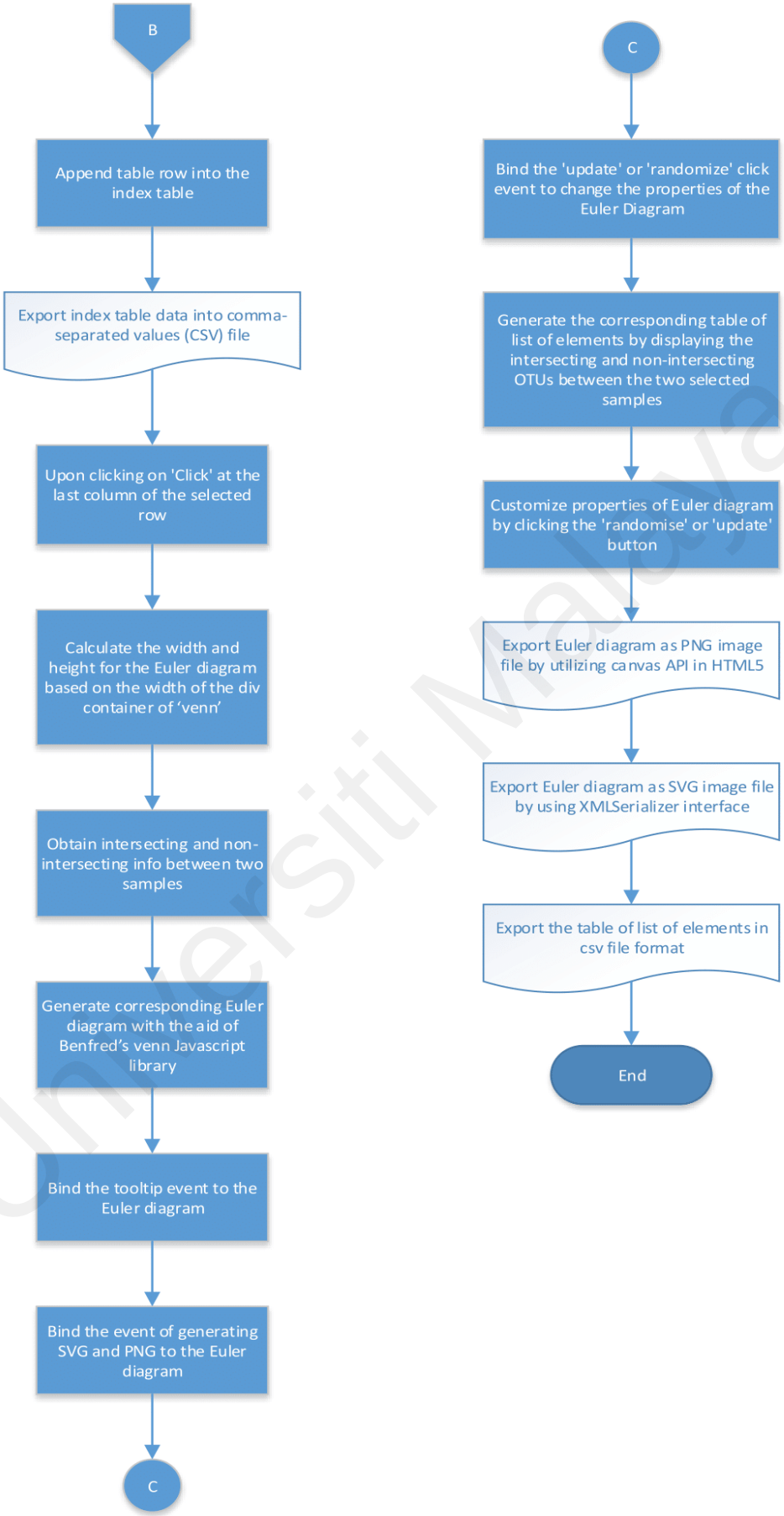

Figure 3.7, continued.

3.7.3.1 Input file and software interface

Figure 3.8 shows the input page of JS Euler before uploading a sample file. To upload biological datasets into the software, users are required to prepare data in Excel or LibreOffice in .csv or .txt format. The organisation of input data is: each column corresponds to one sample, with the title name of each sample written in the first row of the column, followed by OTUs in the subsequent rows (one OTU per row) in the respective column (one column represents for one sample). Thus, the number of OTUs corresponds to the number of filled rows in each column. The first column represents Sample 1 and the following columns represented successive sample sets, to a maximum of 200 input samples. A sample of input sample excel sheet is shown in Figure 3.9. To upload sample lists, click on 'Choose File' and select a single file (.csv or .txt) which contains all sample lists prepared in Excel Sheet or LibreOffice as shown in Figure 3.9.

Figure 3.8: JS Euler input page.

	A	B	C	D	E	F	G
1	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5		
2	Bacteria A	Bacteria F	Bacteria G	Bacteria A	Bacteria D		
3	Bacteria B	Bacteria A	Bacteria D	Bacteria C	Bacteria E		
4	Bacteria D	Bacteria S	Bacteria B	Bacteria D	Bacteria C		
5	Bacteria E	Bacteria C		Bacteria V	Bacteria J		
6	Bacteria F	Bacteria E					
7	Bacteria G						
8	Bacteria H						
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							

Figure 3.9: An input sample sheet with five samples. Each column corresponds to one sample dataset. The first cell in each column contains the sample name for each dataset. The OTUs for that sample are contained in the subsequent cells in that column. 'Soil 1', 'Soil 2', 'Soil 3', 'Soil 4' and 'Soil 5' are the sample names. Bacterial identities represent the OTUs.

3.7.3.2 Output display features

After uploading the input file, the JS Venn generated output is shown in Figure 3.10, representing the five samples as shown in Figure 3.9. Datasets were converted into variables and inserted into an array. The array differentiated headers and data values. Datasets were compared pairwise to generate numbers and identities of overlapping and unique OTUs for all samples in pairs. The Jaccard Index and Sørensen Index were also computed based on the following formulas (Chao *et al*. 2006)

Indices	Incidence- based data in terms of a, b and c
Jaccard	$\frac{a}{a+b+c}$
Sørensen	$\frac{2a}{2a+b+c}$

a= Number of overlapping OTUs between Sample 1 and Sample 2
b= Number of OTUs only present in Sample 1
c= Number of OTUs only presents in Sample 2

These output numbers are summarized in a table chart on the left part of the output interface. When the cursor hovers on a particular text box, the text box is highlighted in light yellow as shown in Figure 3.10. A two-circled Euler Diagram is displayed depending on which pair of datasets is selected by clicking the respective button in the table. The identities of the OTUs in each region in the Euler Diagram are displayed in separate text boxes on the lower right part of the output interface.

JS Euler provided customization on the generated Euler Diagram relating to particular features as indicated in Figure 3.10, including both circles' filled colours, font sizes and colours. There are two ways to customise these features: (1) Select a colour or font size and click on 'Update' button, or (2) Click on 'Randomize' button for a random selection for colour and font size by the software.

The generated Index Table on the left part of the output interface is easily exported into CSV format by clicking the button 'Export Index Table Data into CSV'. The generated Euler Diagram for the selected pair of samples can also be easily exported into SVG or PNG format by clicking the relevant buttons 'Generate SVG' or 'Generate PNG'. The lists of OTUs in each area of the Euler Diagram (unique and overlapping) situatedon the lower right part of the output interface can be exported into CSV format by clicking the button 'Export Lists of OTU(s) into CSV'.

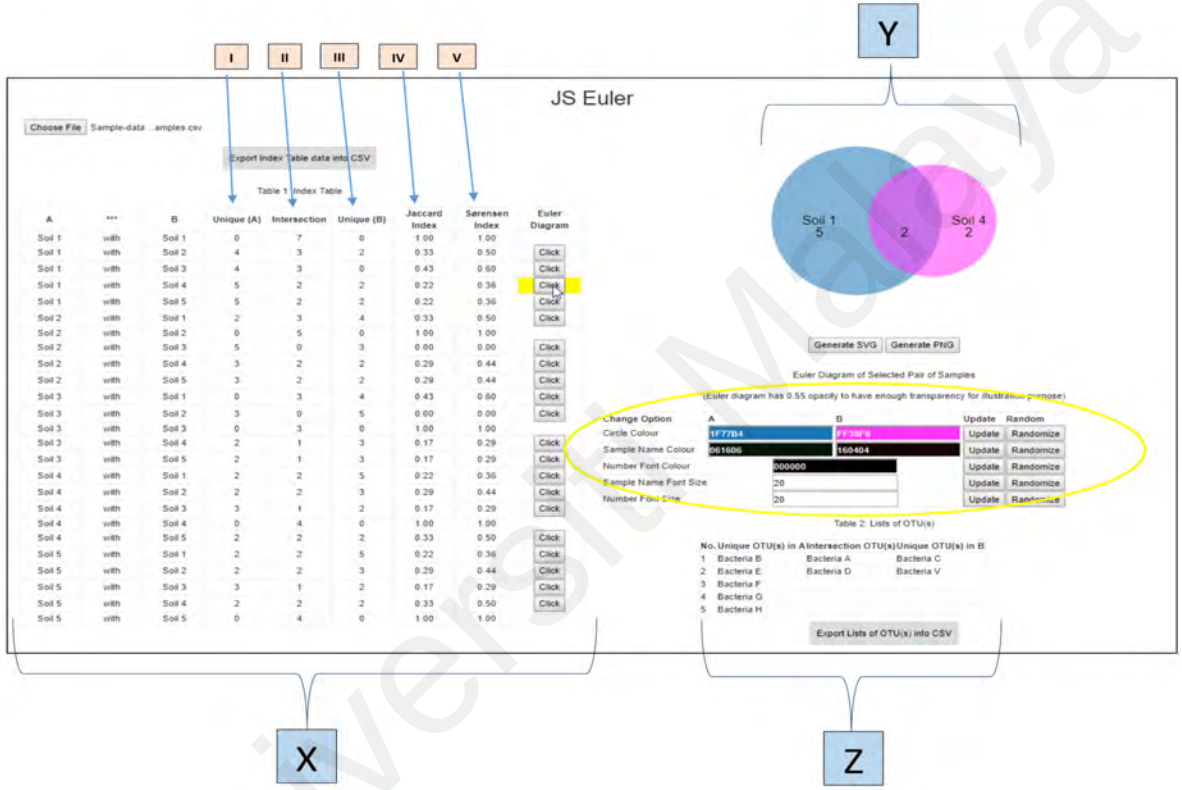

Figure 3.10: Data Output Interface and User Interface-Customization Features**.** JS Euler data output interface's main sections after uploading sample sheets are indicated by lettering X, Y and Z, as follows: (X) Index table which contains five elements of information - (i) Number of OTUs unique to Sample A, termed as Unique (A); (ii) Number of OTUs unique to Sample B, termed as Unique (B); (iii) Number of OTUs Overlapping between Sample A and Sample B, termed as Intersection; (iv) Jaccard Index; (v) Sørensen Index; (Y) Euler diagram of selected pair of samples; (Z) List of OTUs for each region of the Euler diagram. The section circled in yellow includes control buttons to customize the Euler diagram.

3.7.3.3 Advantages of JS Euler software in pairwise comparison of OTUs between samples

JS Euler is a user-friendly online webtool with a graphical user Interface (GUI). It does not require programming knowledge to operate, and is time-efficient, providing accurate comparison for up to 200 samples. This new software extends the maximum capacity of currently available software used for pairwise comparisons, where a maximum of only 30 samples can be interpreted simultaneously (http://bioinformatics.psb.ugent.be/webtools/Venn/). This software also provides more features in pairwise comparisons than other software packages as stated in Table 3.2, whereby it has extra functions in generating Jaccard and Sørensen indices for all possible combinations among the samples. A comparison of JS Euler with other available software is given in Table 3.2.

Table 3.2: Comparison of features across software used to make pairwise comparisons, including the production of Venn and/or Euler diagrams.

Software	Maximum No. of input lists	Input data Format/ Requirement	Indices for Pairwise Comparison	Output data format	Venn size Proportionality	Environment
JS Euler	200	Excel/Spreadsheet (.csv or .txt) containing all sample lists in one page	Jaccard, Sørensen	(i) Euler Diagram- SVG, PNG (ii) Number & List of OTUs for each subset - CSV (iii) Index - CSV	Yes	Web application
Pangloss [Retrieved 10 Jan 2018, from http://www.panglo ss.com/seidel/Proto cols/venn.cgi]	4	Input list in text boxes	None	GIF	None	Web application
Venny [Oliveros, 2007]	4	Input list in text boxes)	None	PNG	None	Web application
Biovenn [Hulsen *et al*., 2008]	3	Input list /one file for each list in text boxes (.ivenn)	None	SVG, PNG	Yes	Web application

Table 3.2, continued.

InteractiVenn [Heberle *et al*., 2015]	6	Input list in text boxes	None	SVG, PNG, txt	None	Web application
Jvenn [Bardou *et al*., 2014]	6	Lists, intersection counts and count lists	None	(i) Diagram- PNG, SVG (ii) List- CSV	None	Web application and Javascript library
VennDiagram [Chen & Boutros, 2011]	4	Lists	None	TIFF and R objects	Yes	Command Line interface (R package)
VennDis [Ignatchenko *et al*., 2015]	5	Lists, excel style table, precalculated values for overlap and differences	None	PNG, SVG, JPG, BMP	Yes	JavaFX software
Draw Venn Diagram [http://bioinformatics.psb.ugent.be/webtools/Venn/ Accessed 10 Jan 2018]	30	Input list/one file for each list in textboxes	None	Diagram- PNG, SVG List- txt	No	Web Application

CHAPTER 4: RESULTS

4.1 Open-top chambers (OTCs) and water amendment study

4.1.1 Effects of Open-top chambers on soil temperature

The deployment of open-top chambers led to increases in monthly mean soil temperatures (Sept 2014 to Sept 2016). The minimum and maximum increases in monthly mean soil temperatures were 0.04°C and 1.74°C, respectively (Figure 4.1). Overall, there was significant increase in monthly mean soil temperature in all months relative to controls, except for May 2015, March 2016 and May 2016.

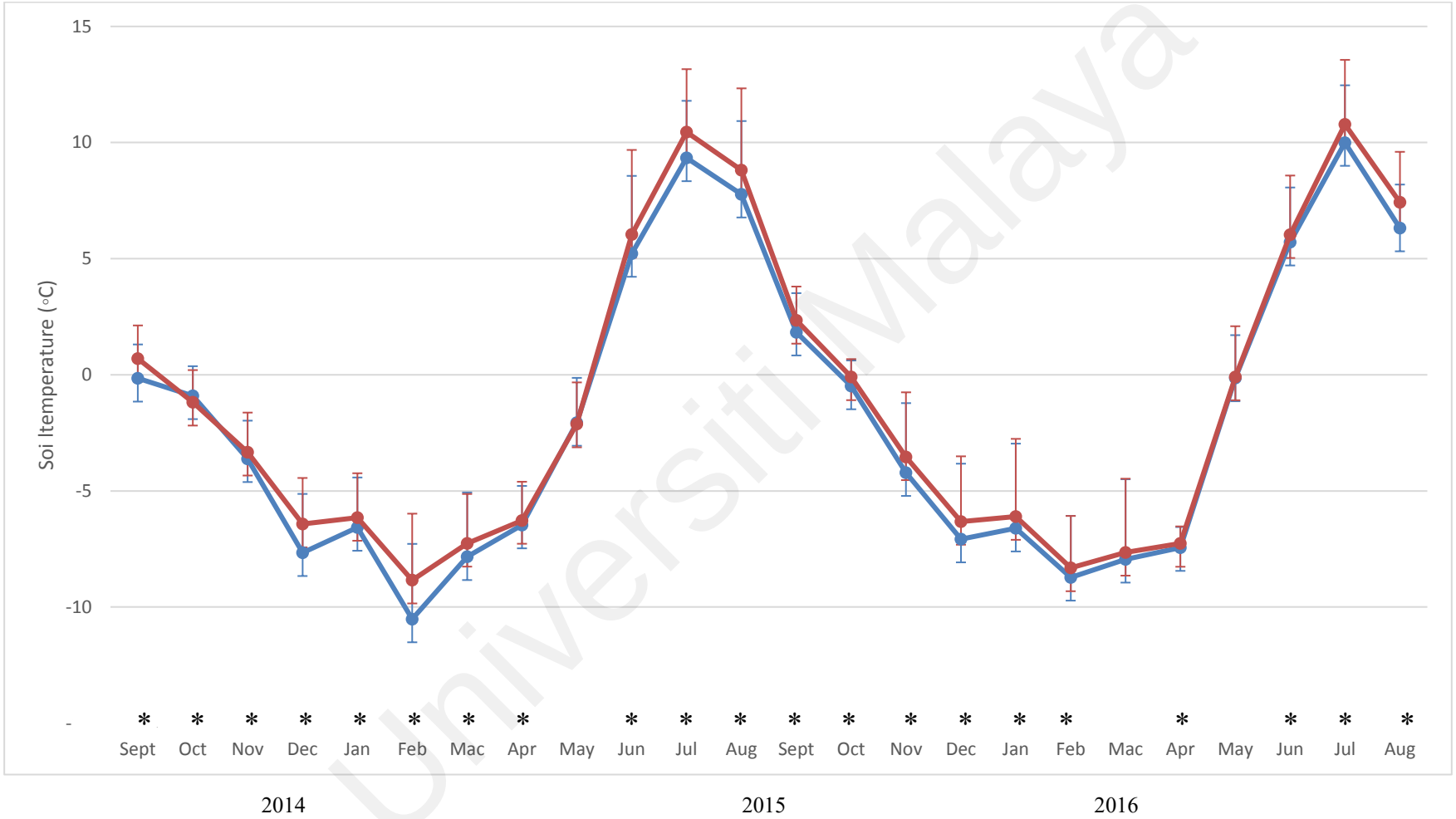

Figure 4.1: Mean monthly soil temperatures in control plots and Open-top chambered-manipulated plots. Values are means of hourly temperatures over each month (Mean ± SE). Significant differences (P <0.05, independent t-test) between control and manipulated plots are denoted by *.

4.1.2 Individual sample coverage and alpha diversity

Among the three blocks, samples from Block 2 has the highest number of raw reads after sequencing and after quality checks. Thus, samples from this block were selected for downstream analyses using QIIME (Table 4.1).

Taxon diversity across all 36 soil samples examined in this study consisted of an average relative abundance of 99.15% bacteria, followed by 0.845% unassigned taxa and 0.000045% archaea. Bacteria across all 36 soil samples examined in this study were classified into 46 phyla, 174 classes, 321 orders, 500 families and 760 genera. The details of the taxonomic assignments for each season are described below. Rarefaction analyses were performed at a depth of 24512 sequences per sample. The rarefaction curves of all samples (Figure 4.2) do not reach saturation, indicating that these High Arctic soil samples contained high levels of bacterial diversity. The Shannon diversity index curve reached a plateau at less than 5000 sequences, indicating that the standardized rarefied reads at 24512 sequences had sufficient diversity coverage for subsequent downstream diversity analysis. This was also confirmed by high values of Good's Coverage, between 0.904 and 0.933 (Table 4.1).

Table 4.1: Alpha diversity indices of samples in field warming and water amendment study for Block 2 of Sampling1, Sampling 2 and Sampling 3.

Sample	Good's Coverage	Shannon Index	Sample	Good's Coverage	Shannon Index	Sample	Good's Coverage	Shannon Index
	Sampling 1			**Sampling 2**			**Sampling 3**	
C1_1_2	0.92	10.28	**C1_2_2**	0.93	9.82	**C1_3_2**	0.92	10.11
C2_1_2	0.92	10.13	**C2_2_2**	0.92	10.03	**C2_3_2**	0.93	9.81
C3_1_2	0.92	9.98	**C3_2_2**	0.92	10.11	**C3_3_2**	0.92	9.96
O1_1_2	0.92	10.18	**O1_2_2**	0.91	10.54	**O1_3_2**	0.92	10.32
O2_1_2	0.93	9.88	**O2_2_2**	0.93	9.53	**O2_3_2**	0.93	9.93
O3_1_2	0.92	10.14	**O3_2_2**	0.91	10.28	**O3_3_2**	0.93	9.82
W1_1_2	0.92	10.11	**W1_2_2**	0.92	10.30	**W1_3_2**	0.90	10.69
W2_1_2	0.92	10.18	**W2_2_2**	0.93	9.77	**W2_3_2**	0.92	10.27
W3_1_2	0.92	10.23	**W3_2_2**	0.92	10.02	**W3_3_2**	0.92	10.06
M1_1_2	0.91	10.27	**M1_2_2**	0.91	10.39	**M1_3_2**	0.91	10.67
M2_1_2	0.93	9.95	**M2_2_2**	0.91	10.39	**M2_3_2**	0.91	10.10
M3_1_2	0.91	10.23	**M3_2_2**	0.92	10.17	**M3_3_2**	0.91	10.29

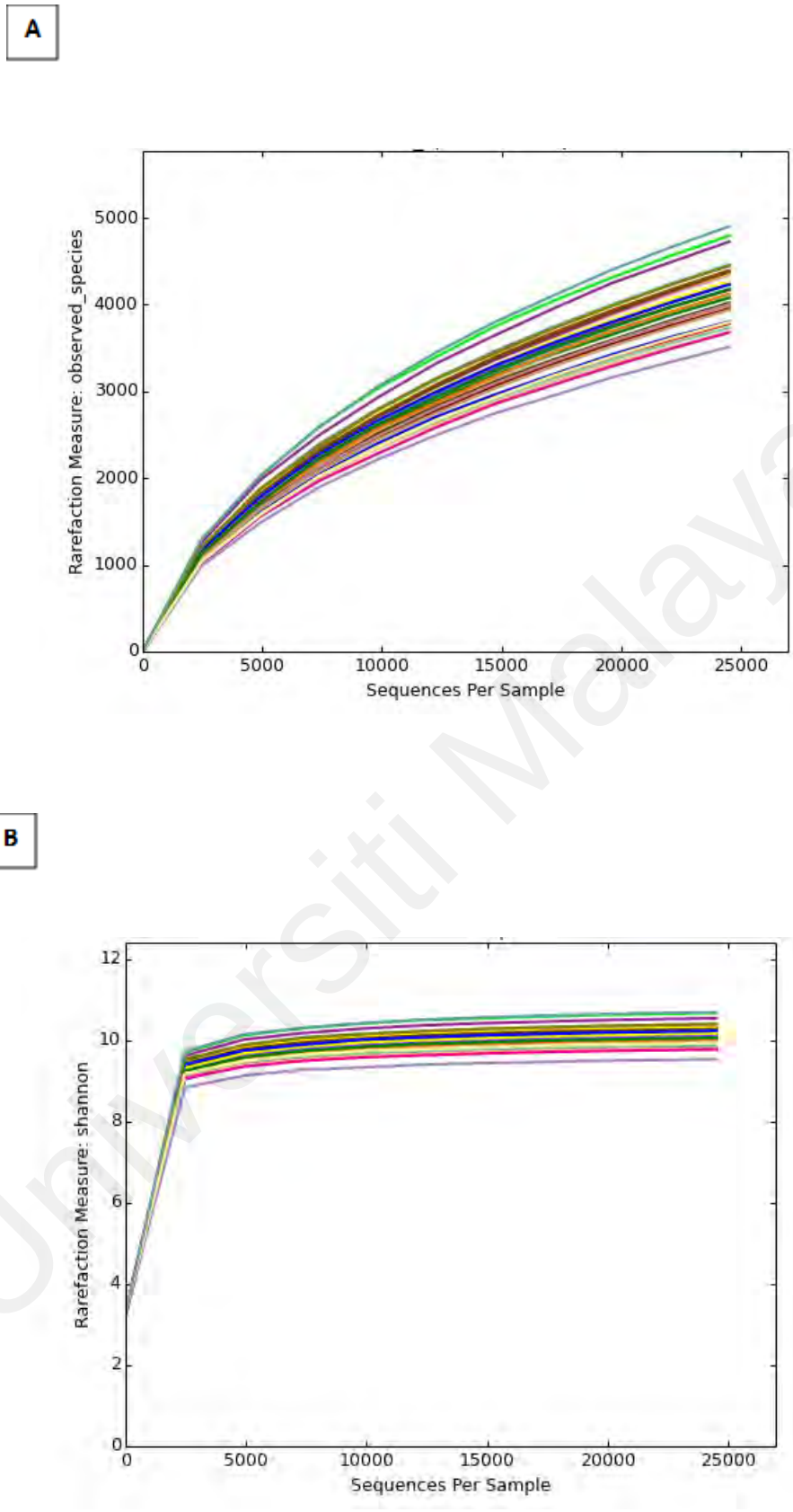

Figure 4.2: Rarefaction plots of samples in field warming and water amendment experiment based on (A) observed OTUs and (B) Shannon diversity index.

4.1.3 Effects of warming and/or water treatment on bacterial diversity in Block 2 across three sampling periods (Sampling 1, Sampling 2 and Sampling 3)

4.1.3.1 Alpha Diversity

The taxonomic assignments for each sampling period are tabulated in Table 4.2. In Sampling 1, One-way ANOVA showed no significant treatment effects on bacterial alpha diversity (Shannon index) in all four groups of soils (control, warming, water and warming*water) [$F(3,8) = 0.3$, $p = 0.825$]. For Sampling 2, One-way ANOVA again showed no significant treatment effects on bacterial alpha diversity among the four groups [$F(3,8) = 0.68$, $p = 0.59$]. Similarly, in Sampling 3, One-way ANOVA showed no significant treatment effects on bacterial alpha diversity across all groups [$F(3, 8) = 1.87$, $p = 0.213$].

Table 4.2: Number of OTUs at different taxonomic levels across all three sampling occasions (Sampling 1, Sampling 2 and Sampling 3)

	Number of OTUs				
	Phylum	**Class**	**Order**	**Family**	**Genus**
Sampling 1	42	162	299	459	669
Sampling 2	43	158	288	449	667
Sampling 3	43	169	301	460	668

4.1.3.2 Bacterial community composition similarity between treatment groups

For Sampling 1, the average Jaccard index of the bacterial community composition (generic level) between treatment groups ranged from 0.71 - 0.74 (Table 4.3). Overall, there was no significant difference in Jaccard index across the groups [$F(5, 48) = 0.773$, $p = 0.574$].

Table 4.3: Mean Jaccard index (mean ± S.D.) between treatment groups (Control_S1, Warming_S1, Water_S1, Warming*Water_S1) in Sampling 1. S1 represents Sampling 1.

	Control_S1	Warming_S1	Water_S1	Warming*Water_S1
Control_S1				
Warming_S1	0.73 ± 0.03			
Water_S1	0.73 ± 0.02	0.74 ± 0.02		
Warming*Water_S1	0.71 ± 0.02	0.72 ± 0.04	0.73 ± 0.03	

In Sampling 2, the average Jaccard index between different groups ranged from 0.72 - 0.76 (Table 4.4). ANOVA identified a significant difference overall in the Jaccard index obtained between the groups [$F(5, 48) = 3.728$, $p = 0.006$]. The LSD post-hoc test (Table 4.5) identified a number of pairwise significant differences in Jaccard similarity among the groups. Nonetheless, the largest difference of average Jaccard index between groups was only 2.7%.

Table 4.4: Mean Jaccard index (mean ± S.D.) between treatment groups (Control_S2, Warming_S2, Water_S2 and Warming*Water_S2) in Sampling 2. S2 represents Sampling 2.

	Control_S2	Warming_S2	Water_S2	Warming*Water_S2
Control_S2				
Warming_S2	0.72 ± 0.02			
Water_S2	0.75 ± 0.02	0.73 ± 0.03		
Warming*Water_S2	0.74 ± 0.01	0.73± 0.02	0.76 ± 0.01	

Table 4.5: Outcome of LSD post-hoc tests on Jaccard index between all treatment groups in Sampling 2 (S2). Significant differences between groups ($p < 0.05$) are denoted by *.

	Control_S2 with Warming_S2	**Control_S2 with Water_S2**	**Control_S2 with Warming*Water_ S2**	**Warming_S2 with Water_S2**	**Warming_S2 with Warming*Water_ S2**	**Water_S2 with Warming*Water_ S2**
Control_S2 with Warming_S2						
Control_S2 with Water_S2	0.011*					
Control_S2 with Warming*Water_S2	0.114	0.307				
Warming_S2 with Water_S2	0.494	0.057	0.363			
Warming_S2 with Warming*Water_S2	0.569	0.044*	0.307	0.909		
Water_S2 with Warming*Water_S2	0.001*	0.363	0.057	0.006*	0.004*	

In Sampling 3, the Jaccard index between treatment groups ranged from 0.71 - 0.73 (Table 4.6). Overall, there was no significant difference in Jaccard index among the groups [F (5, 48) = 0.259, p = 0.933].

Table 4.6: Mean Jaccard index (mean ± S.D) between treatment groups in Sampling 3 (Control_S3, Warming_S3, Water_S3, Warming*Water_S3). S3 represents Sampling 3.

	Control_S3	Warming_S3	Water_S3	Warming*Water_S3
Control_S3				
Warming_S3	0.73 ± 0.03			
Water_S3	0.73 ± 0.02	0.72 ± 0.03		
Warming*Water_S3	0.71 ± 0.02	0.72 ± 0.04	0.72 ± 0.03	

4.1.3.3 Bacterial community structure

Bacterial composition at the phylum level in all 12 samples from Sampling 1 is illustrated in Figure 4.3, followed by Sampling 2 (Figure 4.4) and Sampling 3 (Figure 4.5). All three seasons shared similar dominant phyla (average relative abundance ≥ 1% in each season), which were Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Cyanobacteria and Nitrospirae. The three most dominant phyla - Proteobacteria, Actinobacteria and Acidobacteria - consistently contributed over 60% of the total relative abundance in each treatment group across all three seasons. Chloroflexi had a relatively lower relative abundance in Sampling 2 (average relative abundance = 4.30%), as compared to Sampling 1 (average relative abundance = 6.62%) and Sampling 3 (average relative abundance = 6.20%). Apart from this, Sampling 3 had an additional dominant phylum (WS3) with average relative abundance of 1.16%. This phylum only appeared in low relative abundance (< 1%) in samples from Sampling 1 (average relative abundance = 0.8%) and Sampling 2 (average relative abundance = 0.49%).

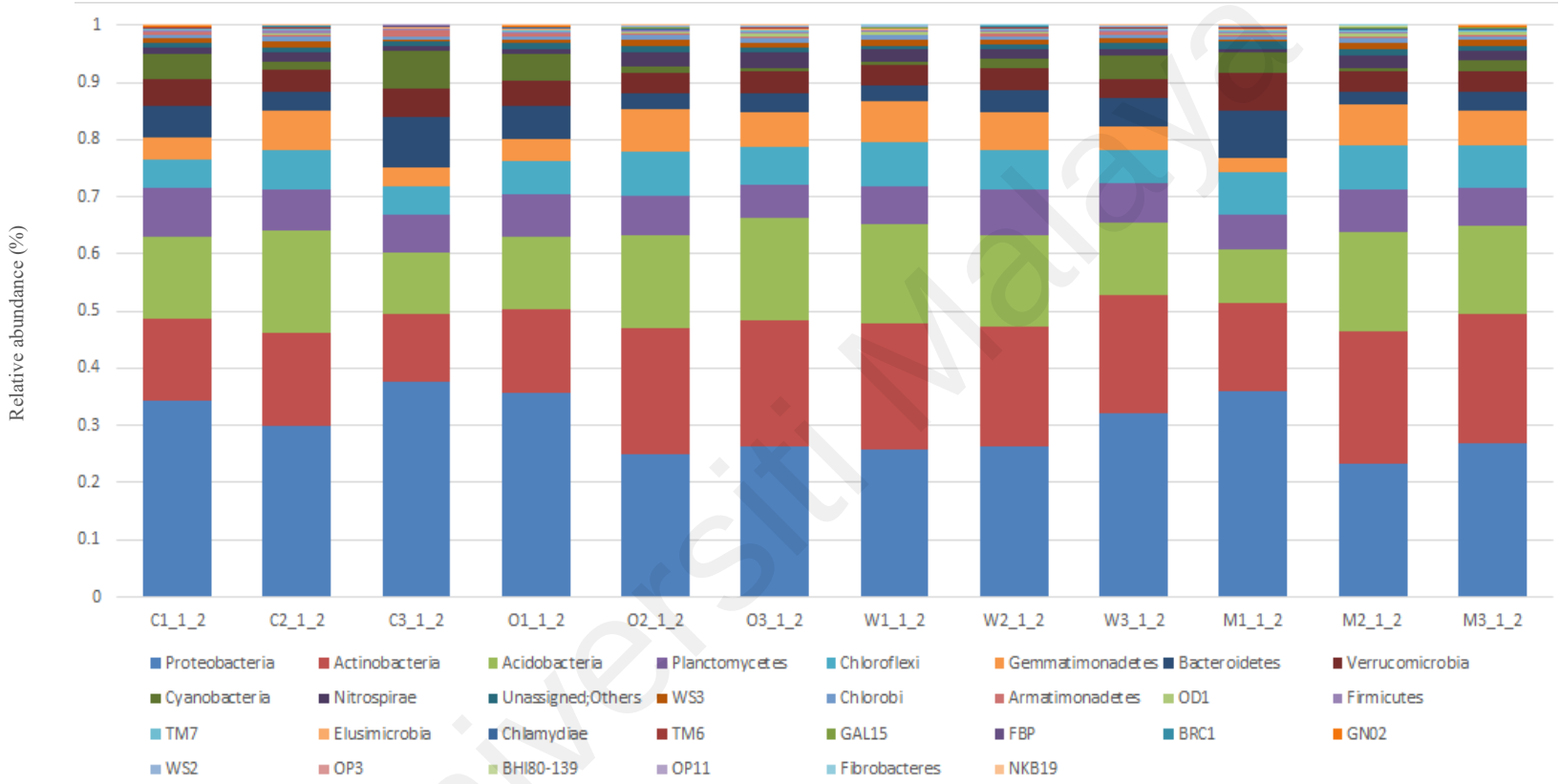

Figure 4.3: Relative abundance of different bacterial phyla among soil samples in Sampling 1. C represents Control Group with three replicates (C1_1_2; C2_1_2; C3_1_2); O represents OTC Warming Group with three replicates (O1_1_2; O2_1_2; O3_1_2). W represents Water Amendment Group with three replicates (W1_1_2; W2_1_2; W3_1_2) and M represents Warming*Water group with three replicates (M1_1_2; M2_1_2; M3_1_2) . Details of samples are tabulated in Table 3.1.

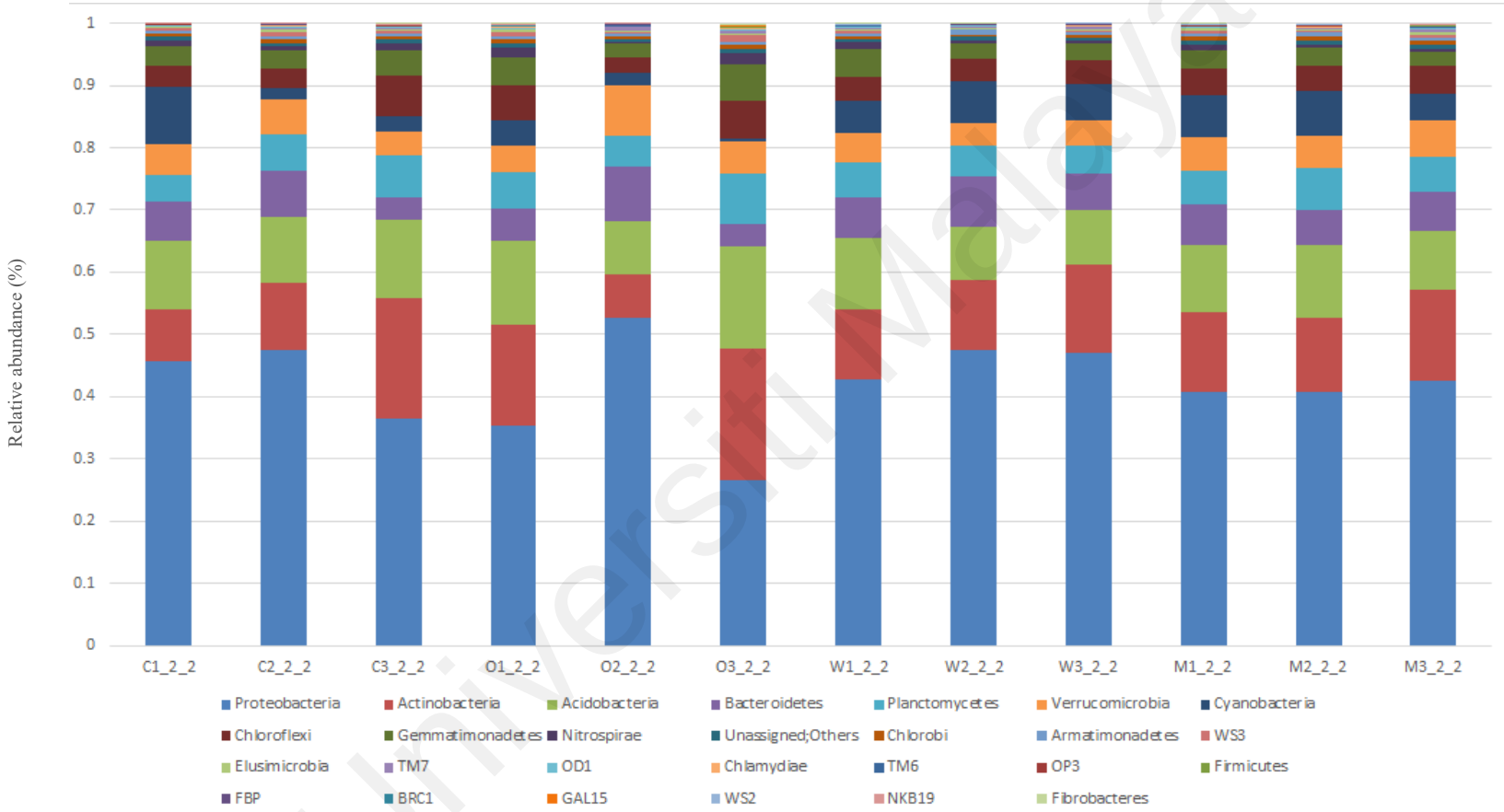

Figure 4.4: Relative abundance of different bacterial phyla among soil samples in Sampling 2. C represents Control Group with three replicates (C1_2_2; C2_2_2; C3_2_2); O represents OTC Warming Group with three replicates (O1_2_2; O2_2_2; O3_2_2). W represents Water Amendment Group with three replicates (W1_2_2; W2_2_2; W3_2_2) and M represents Warming*Water group with three replicates (M1_2_2; M2_2_2; M3_2_2) . Details of samples are tabulated in Table 3.1.

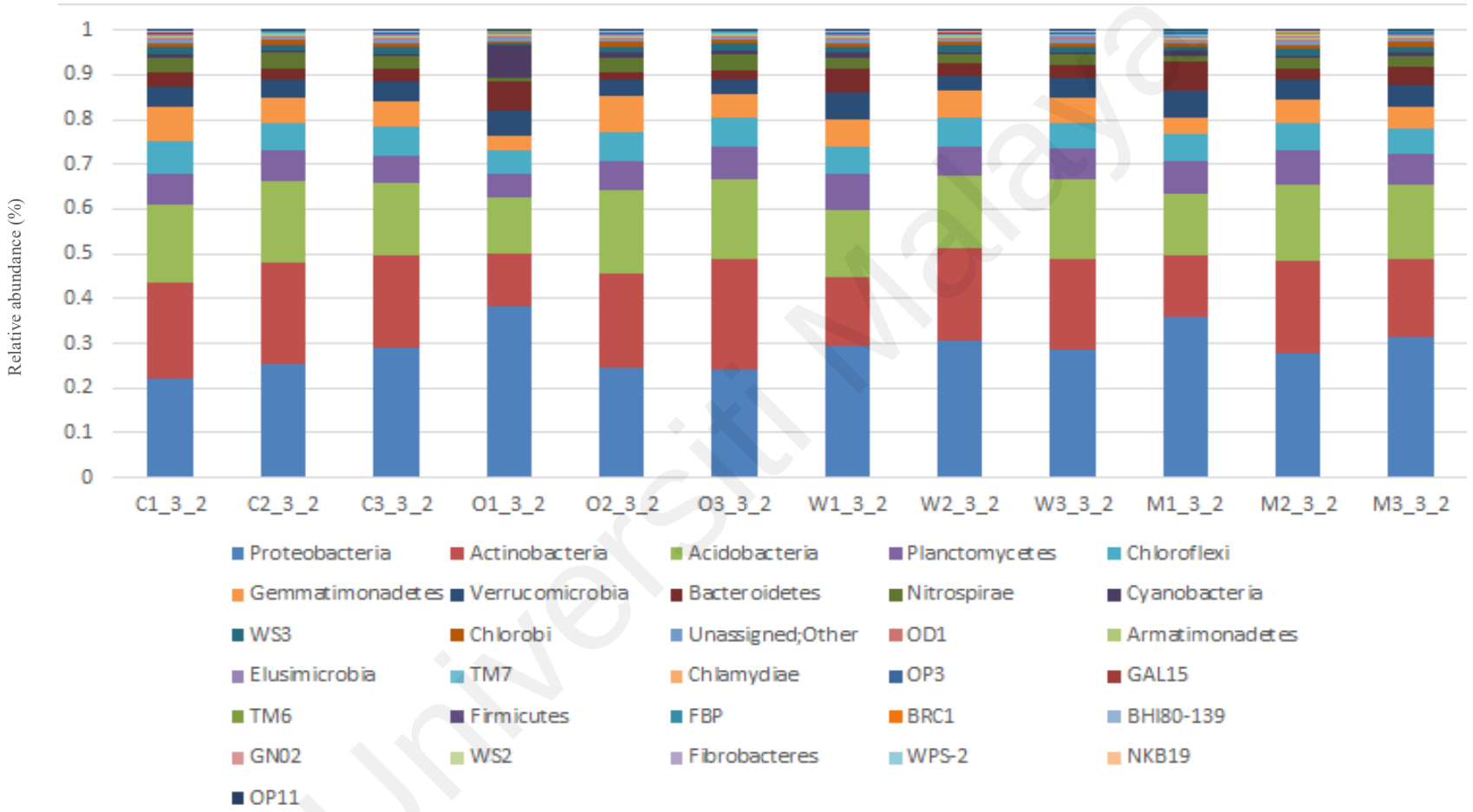

Figure 4.5: Relative abundance of different bacterial phyla among soil samples in Sampling 3. C represents Control Group with three replicates (C1_3_2; C2_3_2; C3_3_2); O represents OTC Warming Group with three replicates (O1_3_2; O2_3_2; O3_3_2). W represents Water Amendment Group with three replicates (W1_3_2; W2_3_2; W3_3_2) and M represents Warming*Water group with three replicates (M1_3_2; M2_3_2; M3_3_2) . Details of samples are tabulated in Table 3.1.

4.1.3.4 Assemblages of co-occurring bacterial community members between treatment groups

(a) Sampling 1

The unique and overlapping genera across the four experimental groups in Sampling 1, (control_S1, warming_S1, water_S1 and warming*water_S1) are represented in Figure 4.6). The dominant phylotypes in all experimental groups are represented in Figure 4.7, including a total of 32 unique genera. Co-occurring phylotypes comprised 431 of the 681 (63.3%) at genera recorded in total (Figure 4.6) across all four groups, amongst which 16 phylotypes (3.7%) were categorized as dominant genera (>1% mean relative abundance in at least one experimental group, as represented in Figure 4.7).

All four experimental groups, in different group combinations, included a wide range of both unique and overlapping genera, which ranged from 6 to 431 phylotype (Figure 4.6). However, when considering the dominant genera within the groups the differences in the numbers of unique and overlapping genera were less striking, ranging from 0 to 16 genera only (Figure 4.7).

Spearman rank order correlation (Table 4.7) showed a strong significant positive correlation in the assemblages of all co-occurring phylotypes between the four experimental groups ($r \geq 0.917$, $p < 0.001$). Consistent with this observation, the dominant bacterial taxa shared similar assemblage patterns among the experimental groups (Figure 4.8). The *Gaillaceae* family was the most dominant family in Sampling 1. The *Gaiellaceae* family, Solirubrobacterales order and 0319-7L14 order had higher relative abundance in all three treatment groups (Warming_S1, Water_S1 and Warming*Water_S1) than in the control group. In contrast, *Sphingomonadaceae* (family), *Chitinophagaceae* (family) and *Kaistobacter* (genus) were more abundant in the control group than in the three treatment groups.

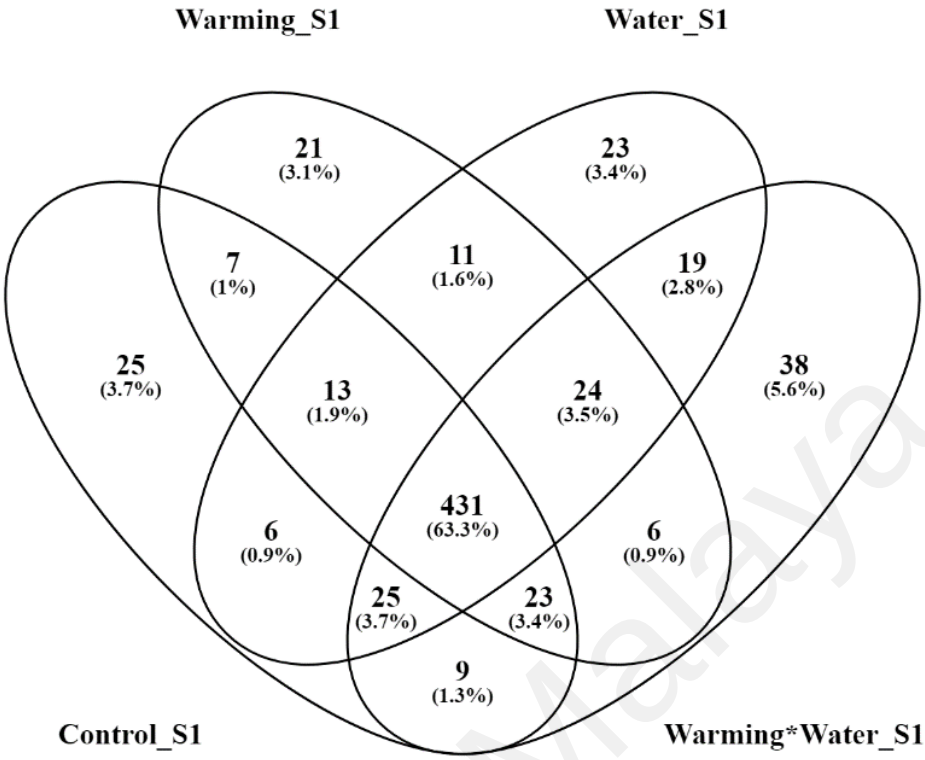

Figure 4.6: Venn diagram showing the proportion of unique and shared phylotypes across all four experimental groups in Sampling 1 (Control_S1, Warming_S1, Water_S1 and Warming*Water_S1), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). S1 represents Sampling 1.

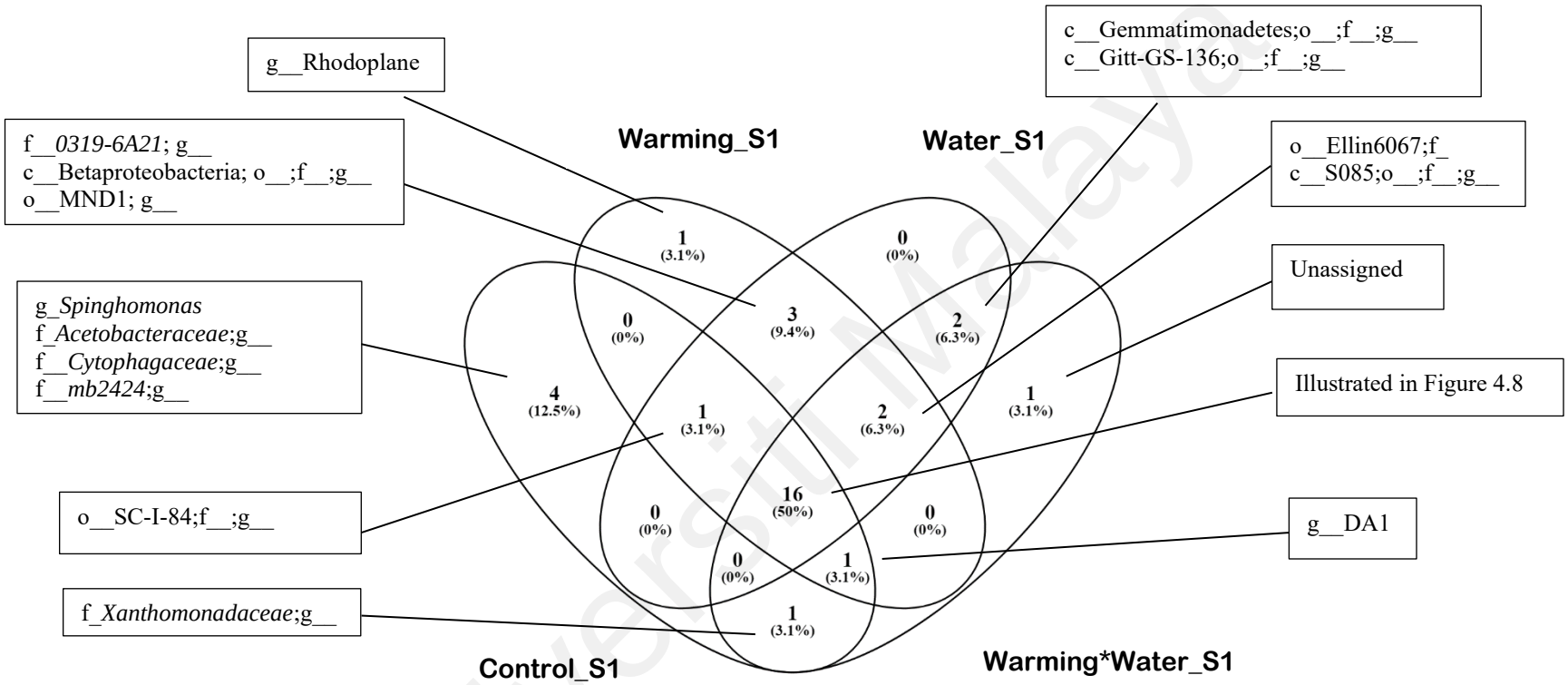

Figure 4.7: Venn diagram showed the proportion of unique and shared phylotypes which were dominant (>1% mean relative abundance in at least in one experimental group) across all four experimental groups in Sampling 1 (Control_S1, Warming_S1, Water_S1 and Warming*Water_S1), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). S1 represents Sampling 1. Annotation indicated lowest taxonomic level identified.

Table 4.7: Spearman rank order correlation (rho) of the assemblages of co-occurring bacterial genera in all four experimental groups (Control_S1, Warming_S1, Water_S1, Warming*Water_S1) in Sampling 1. S1 represents Sampling 1. Significant differences between groups ($p < 0.05$) are denoted by *. S1 represents Sampling 1.

	Control_S1	**Warming_S1**	**Water_S1**	**Warming*Water_S1**
Control_ S1				
Warming_S1	0.933, $p < 0.001$*			
Water_ S1	0.917, $p < 0.001$*	0.993, $p < 0.001$*		
Warming* Water_S1	0.938, $p < 0.001$*	0.986, $p < 0.001$*	0.986, $p < 0.001$*	

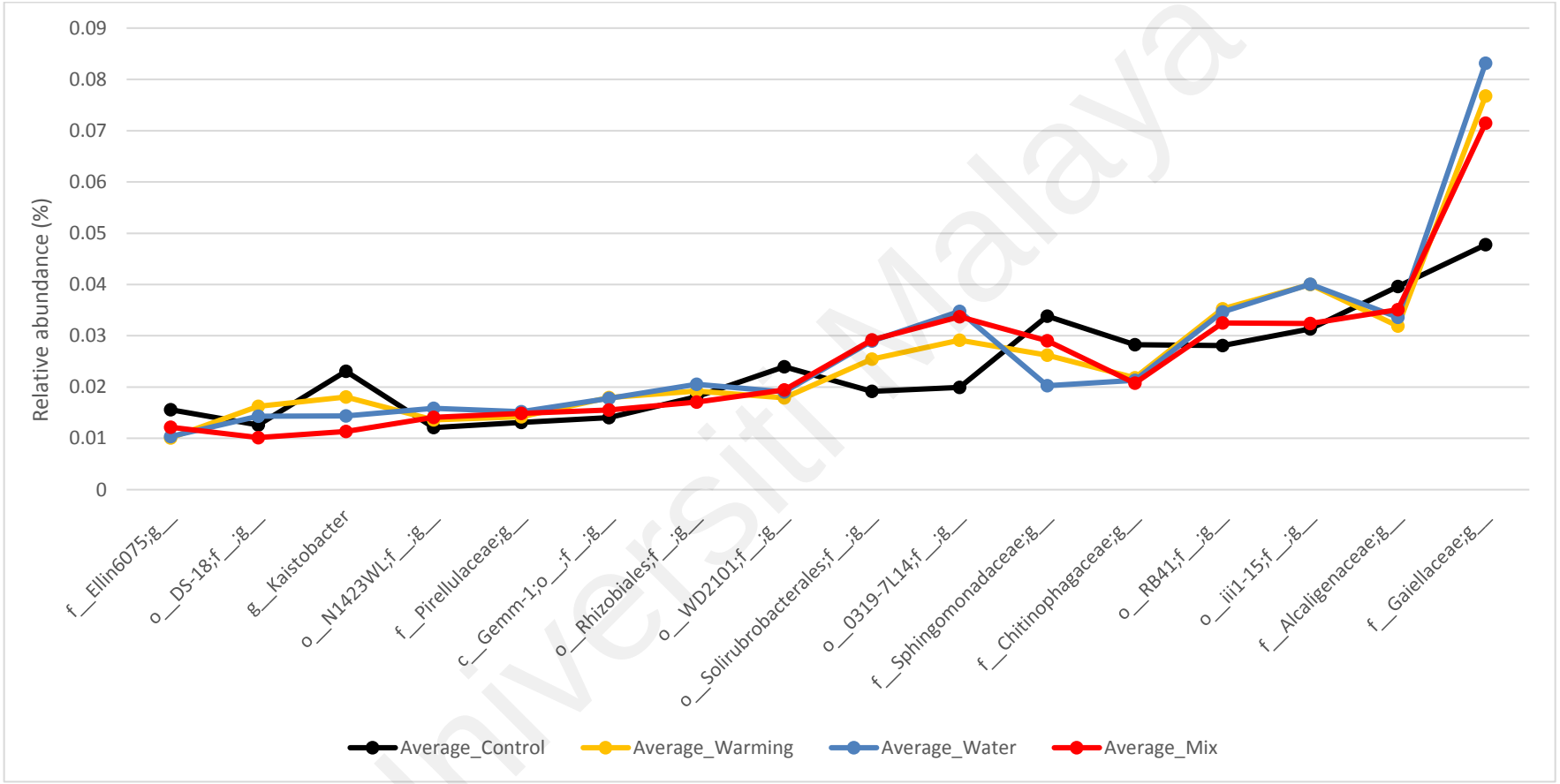

Figure 4.8: Average relative abundance of the co-occurring bacterial genera that are dominant in at least one experimental group in Sampling 1. Annotations indicate phylotypes named at the lowest taxon level. Average_control represents average relative abundance of Control Group. Average_Warming represents average relative abundance of Warming Group. Average_Water represents average relative abundance of Water Amendment Group. Average_Mix represents average relative abundance of Warming +Water Amendment group.

(b) Sampling 2

In Sampling 2, the unique and overlapping genera across all four experimental groups (control_S2, warming_S2, water_S2 and warming*water_S2), are represented in Figure 4.9. There were 457/667 (68.5%) of co-occurring genera among all groups. The dominant phylotypes in all four groups are represented in Figure 4.10. There were 19 dominant phylotypes (4.16%) which co-occurred in all four groups.

Spearman rank correlation (Table 4.8) showed that there were significant strong positive correlations in abundance among the co-occurring phylotypes in all four groups ($r \geq 0.921$, $p < 0.001$; Figure 4.11). Control and water groups had higher relative abundances of the genus *Kaistobacter* and family *Comamonadaceae* than the warming and warming*water groups. The genus *Sphingomonadaceae* accounted for the highest relative abundance among all genera across four treatment groups. Interestingly, the water group had the highest relative abundance of the family *Sphingomonadaceae* than the other three groups. The family *Gaillaceae*, on the other hand, showed higher relative abundance in the warming group, followed by the control group, and abundance decreased in the water and warming*water groups.

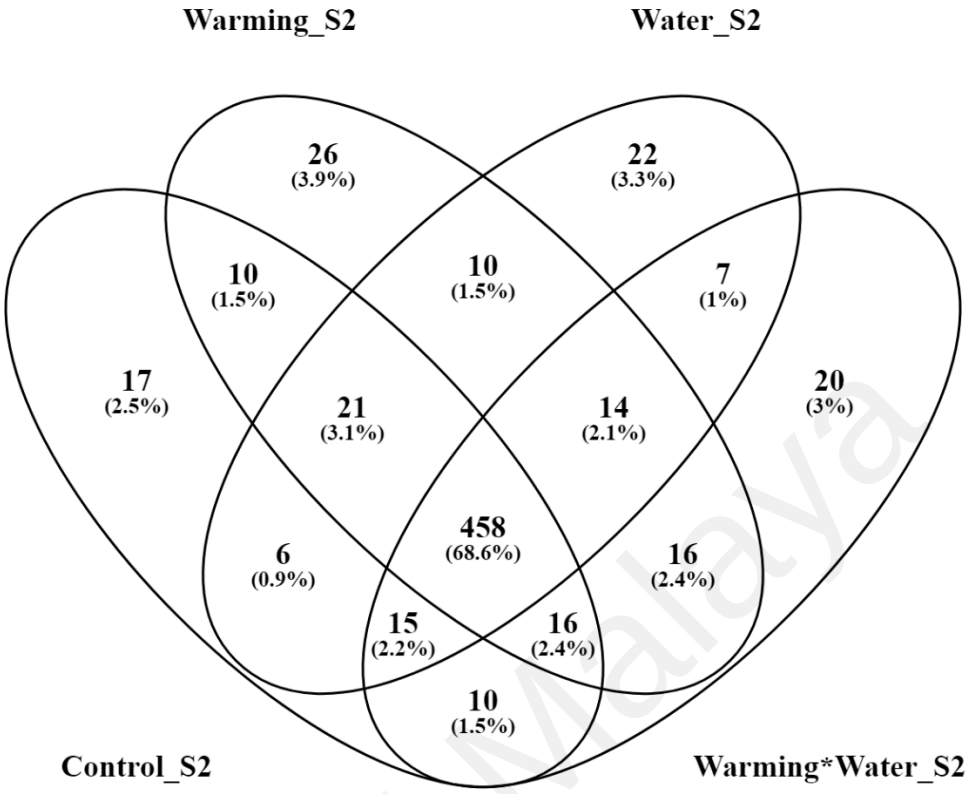

Figure 4.9: Venn diagram showing the proportion of unique and shared phylotypes across all four experimental groups in Sampling 2 (Control_S2, Warming_S2, Water_S2 and Warming*Water_S2), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). S2 represents Sampling 2.

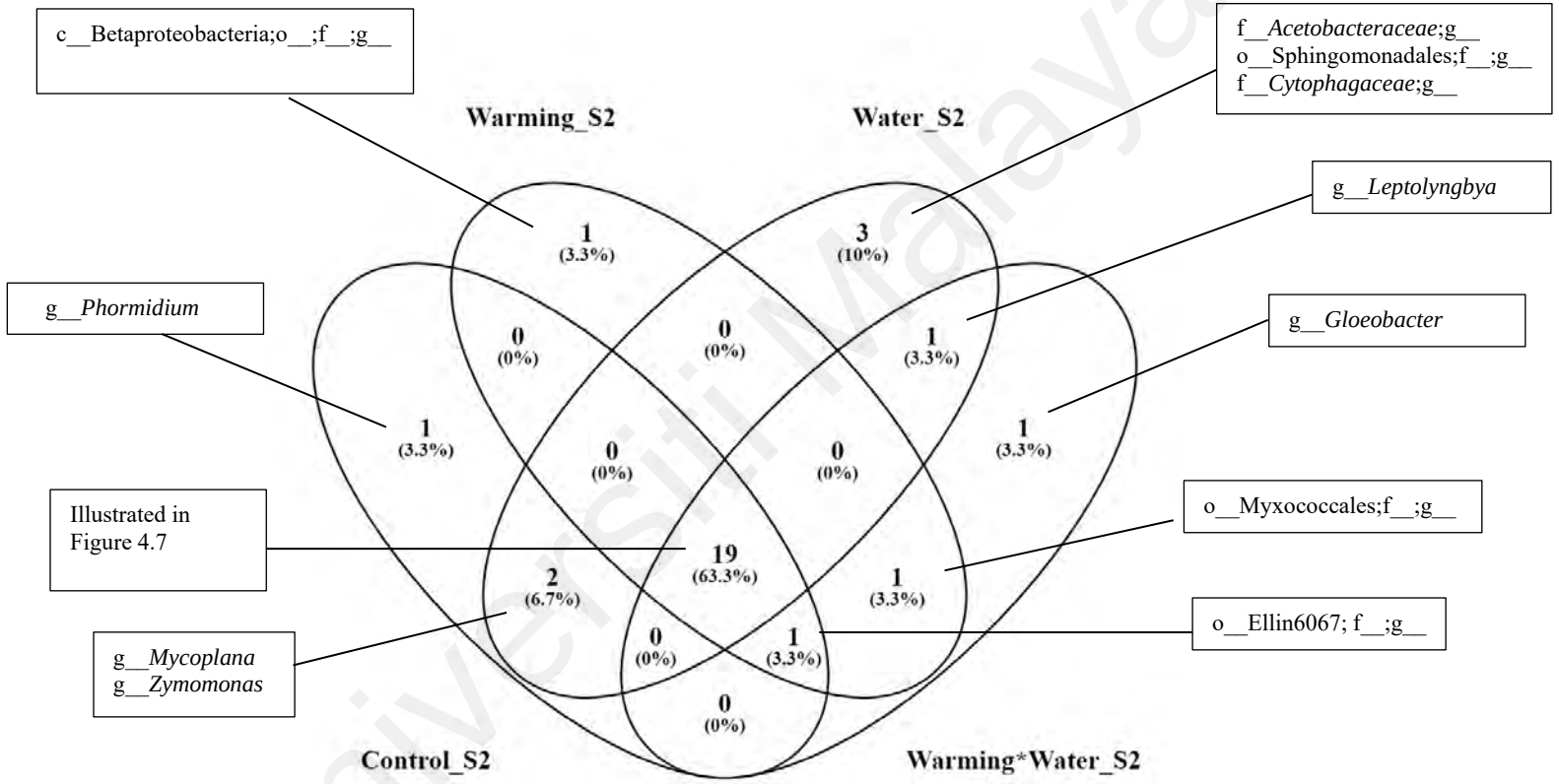

Figure 4.10: Venn diagram showing the proportion of unique and shared phylotypes that were dominant (>1% mean relative abundance in at least in one experimental group) across all four experimental groups (Control_S2, Warming_S2, Water_S2 and Warming*Water_S2) in Sampling 2, generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). S2 represents Sampling 2. Annotation indicates lowest taxonomic level identified.

Table 4.8: Spearman rank order correlation (rho) of the assemblages of co-occurring bacterial genera in all four experimental groups (Control_S2, Warming_S2, Water_S2, Warming*Water_S2) in Sampling 2. S2 represents Sampling 2. Significant differences between groups ($p < 0.05$) are denoted by *.

	Control_S2	**Warming_S2**	**Water_S2**	**Warming*Water_S2**
Control_S2				
Warming_S2	0.957, p <0.001*			
Water_S2	0.964, p < 0.001*	0.921, p < 0.001*		
Warming*Water_S2	0.960, p < 0.001*	0.964, p < 0.001*	0.961, p < 0.001*	

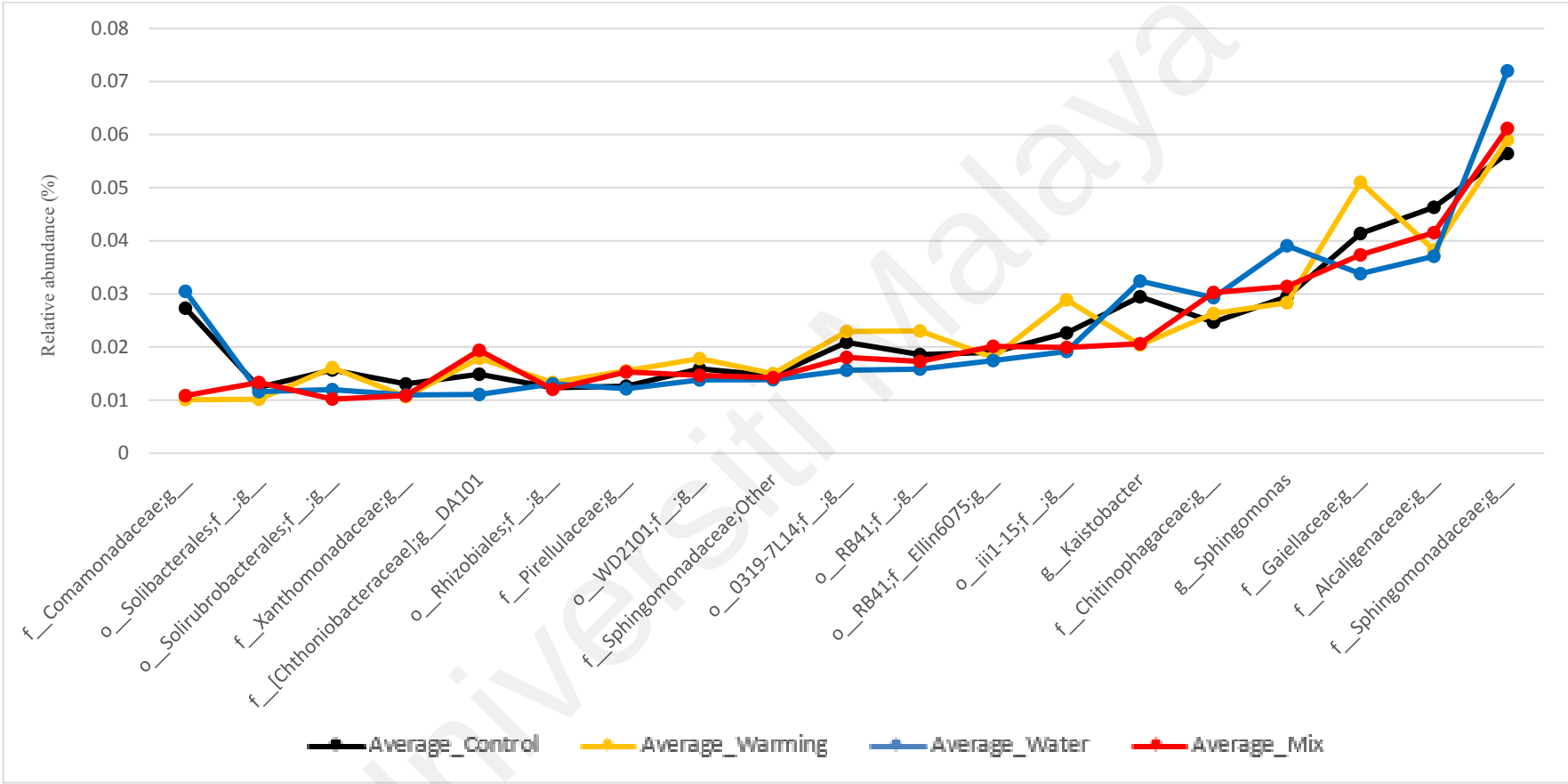

Figure 4.11: Average relative abundance of the co-occurring bacterial genera that are dominant in at least one experimental group in Sampling 2. Annotations indicate phylotypes named at the lowest taxon level. Average_control represents average relative abundance of Control Group. Average_Warming represents average relative abundance of Warming Group. Average_Water represents average relative abundance of Water Amendment Group. Average_Mix represents average relative abundance of Warming +Water Amendment group.

(c) Sampling 3

In Sampling 3, the unique and overlapping genera across all four experimental groups (control_S3, warming_S3, water_S3 and warming*water_S3) are represented in Figure 4.12. There were 438/668 genera (65.3%) that co-occurred across all experimental groups. The dominant phylotypes in all four groups are represented in Figure 4.13. There were 19 dominant phylotypes (4.16%) which co-occurred in all four groups.

Spearman rank correlation (Table 4.9) again showed a significant strong positive correlation in mean abundance across co-occurring genera ($r \geq 0.967$, $p < 0.001$; Figure 4.14). The family *Gaillaceae* was the most dominant family in Sampling 3, and was most abundant in the warming group, followed by the control group, and representation decreased in the water and warming*water groups. The control group had lowest relative abundance of the family *Spingomonodaceae* and highest abundance of the order 0319-7L14 (Acidobacteria).

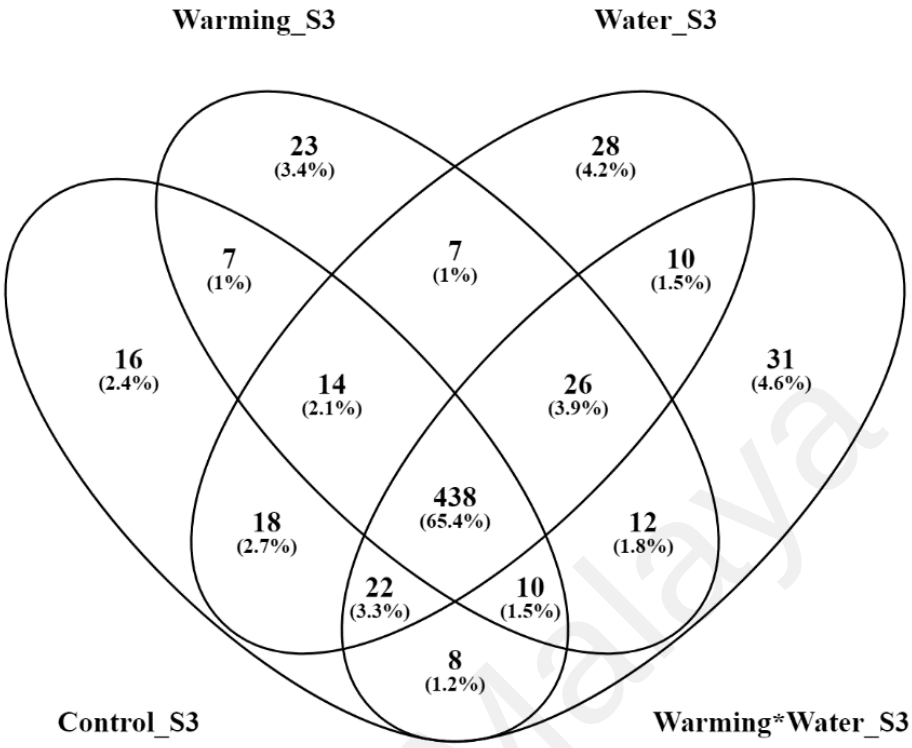

Figure 4.12: Venn diagram showing the proportion of unique and shared phylotypes (>1% mean relative abundance in at least in one experimental group) across all four experimental groups in Sampling 3 (Control_S3, Warming_S3, Water_S3 and Warming*Water_S3), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). S3 represents Sampling 3. Phylotypes are named at the lowest taxonomic level identified.

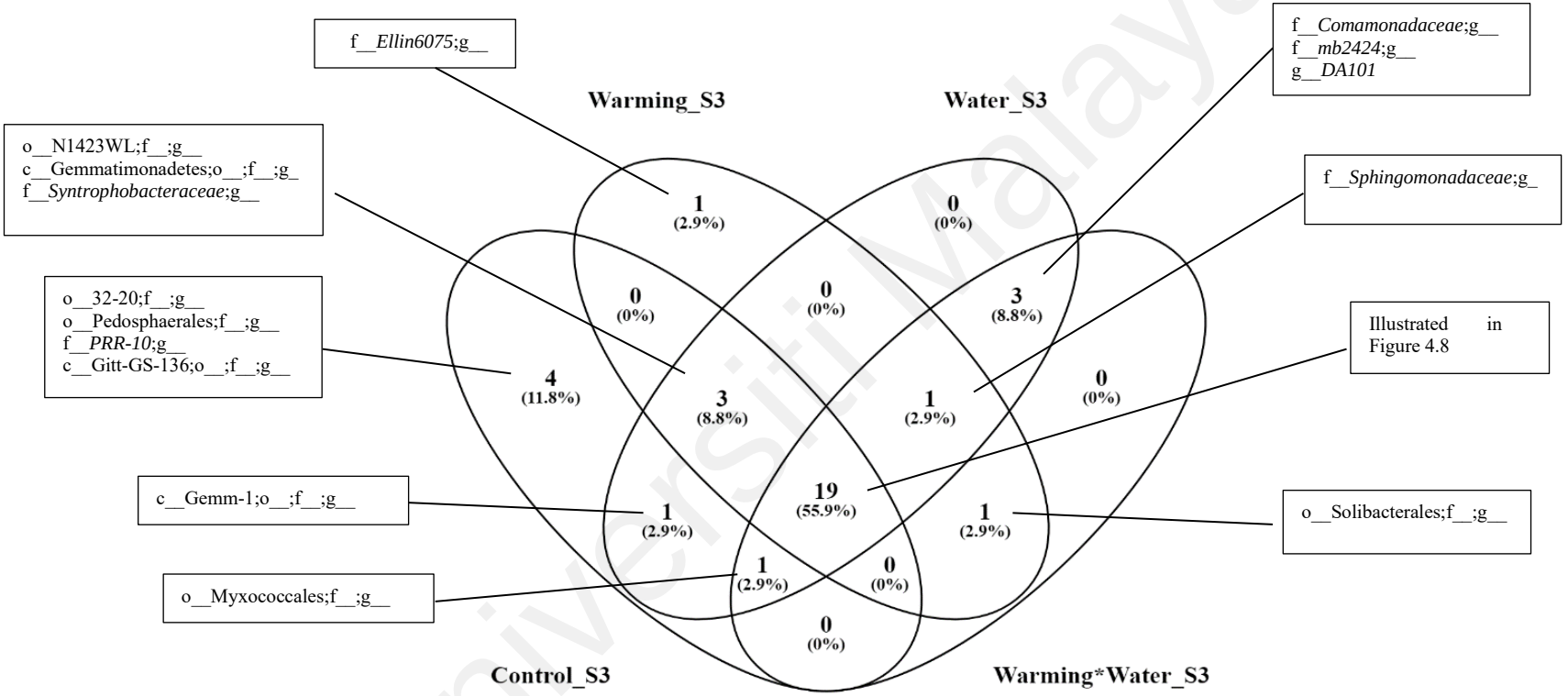

Figure 4.13: Venn diagram showing the proportion of unique and shared phylotypes that were dominant (>1% mean relative abundance in at least in one experimental group) across all four experimental groups in Sampling 3 (Control_S3, Warming_S3, Water_S3 and Warming*Water_S3), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). S3 repreents Sampling 3. Phylotypes are named at the lowest taxonomic level identified.

Table 4.9: Spearman rank order correlation (rho) of the assemblages of co-occurring bacterial genera in all four experimental groups (Control_S3, Warming_S3, Water_S3, Warming*Water_S3) in Sampling 3. S3 represents Sampling 3. Significant differences between groups ($p < 0.05$) are denoted by *.

	Control_S3	**Warming_S3**	**Water_S3**	**Warming*Water_S3**
Control_S3				
Warming_S3	0.974, $p < 0.001$*			
Water_S3	0.984, p <0 .001*	0.977, $p < 0.001$*		
Warming*Water_S3	0.967, $p < 0.001$*	0.976, p <0.001*	0.988, $p < 0.001$*	

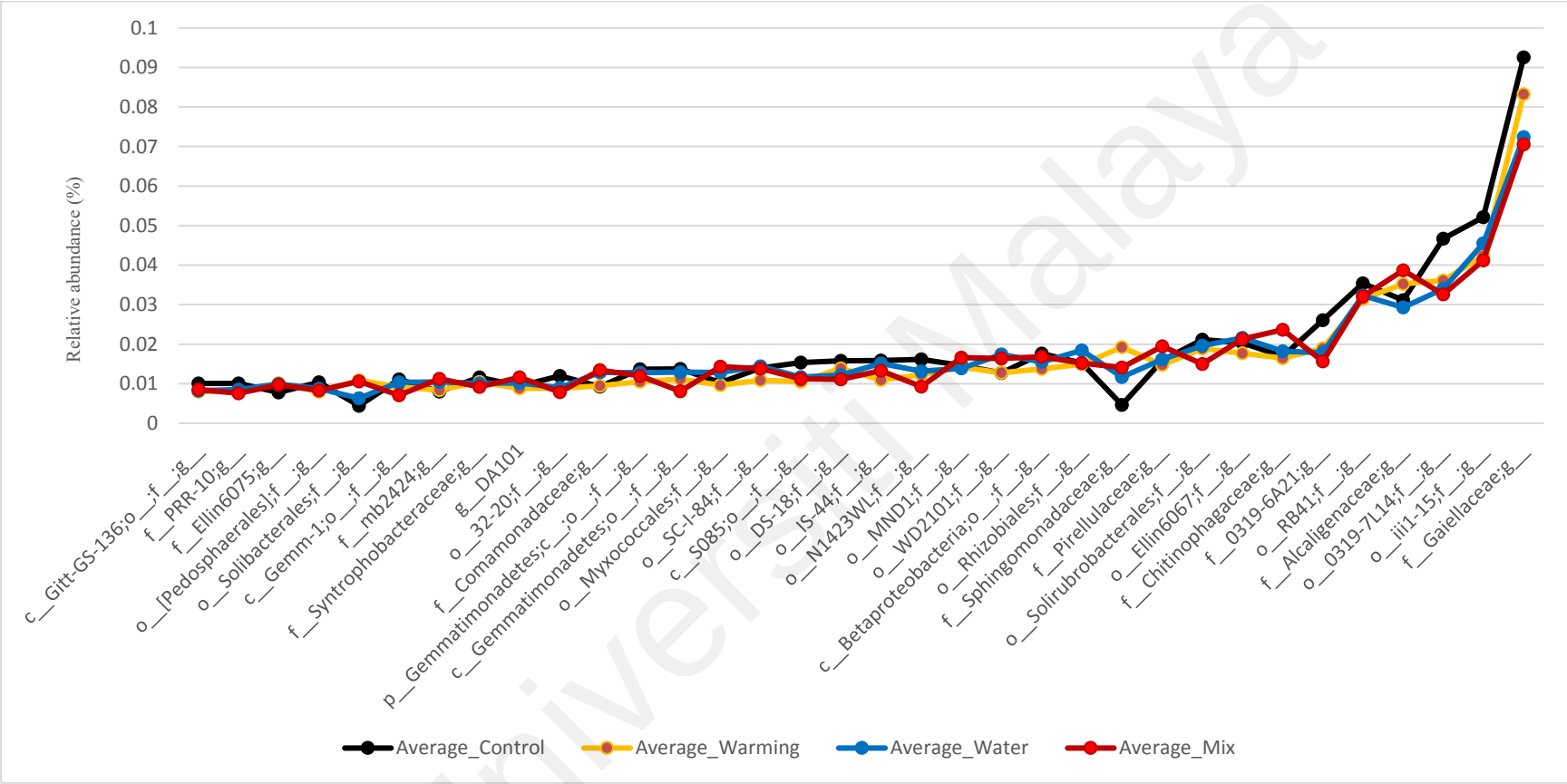

Figure 4.14: Average relative abundance of the co-occurring bacterial genera that are dominant in at least one experimental group in Sampling 3. Annotations indicate phylotypes named at the lowest taxon level. Average_control represents average relative abundance of Control Group. Average_Warming represents average relative abundance of Warming Group. Average_Water represents average relative abundance of Water Amendment Group. Average_Mix represents average relative abundance of Warming +Water Amendment group.

4.1.4 Seasonal variations in bacterial diversity

4.1.4.1 Alpha diversity

A summary of average alpha diversity for all four experimental groups across the three sampling occasions is given in Table 4.10. The assessment of seasonal effects on bacterial alpha diversity for each individual experimental group across the three sampling occasions was made using Repeated-Measured ANOVA (Table 4.11). Repeated-measures ANOVA identified no significant seasonal effects [$F(2, 33) = 0.164$, $p = 0.85$] on the Shannon diversity indices across the three sampling occasions under any of the experimental treatments. The One-way ANOVA also showed no significant differences in Shannon diversity between the experimental groups [$F(11, 24) = 0.913$, $p = 0.54$].

Table 4.10: Summary of average alpha diversity of the four experimental groups (Control, Warming, Water and Warming*Water) across the three sampling occasions (Sampling 1, Sampling 2 and Sampling 3).

Experimental Group	Sampling 1		Sampling 2		Sampling 3	
	Average number of distinct genera	Average Shannon index	Average number of distinct genera	Average Shannon index	Average number of distinct genera	Average Shannon index
Control	536	10.13	552	9.99	532	9.96
Warming	533	10.07	570	10.12	536	10.02
Water	549	10.17	552	10.03	562	10.34
Warming*Water	622	10.15	555	10.32	556	10.35
Average	560	10.13	557	10.12	547	10.17

Table 4.11: Results of Repeated-Measures ANOVA statistical analysis (P-values) for Shannon index of bacterial communities for each experimental group across the three sampling occasions.

Experimental Group	Across 3 sampling occasions	
	F-ratio	**P-value**
Control	0.77	0.52
Warming	0.10	0.91
Water	1.46	0.33
Warming * Water	1.23	0.38
Overall	0.164	0.85

4.1.4.2 Bacterial community composition similarity between seasons

In each sampling occasion (termed as 'within sampling occasion'), the mean Jaccard similarity indices of all samples, and also between experimental groups are summarized in Table 4.12. Across the three sampling occasions (termed as 'between sampling occasions'), the mean Jaccard similarity between samples and experimental groups from different sampling occasions (termed as 'between sampling occcasions') are also tabulated in Table 4.12.

Repeated measures ANOVA identified that there were significant seasonal effects [F (2, 429) = 3.36; p = 0.036] on the Jaccard index of overall samples across the three sampling occasions (Table 4.13). The LSD post-hoc test results indicated that Sampling 1 and Sampling 2 had significantly higher Jaccard index values than other season pairs (Sampling 1 and Sampling 3; Sampling 2 and Sampling 3) (Table 4.14).

When each treatment group was considered separately across seasons, only the control group showed significant changes in average beta diversity (Table 4.13). Within the control group, Sampling 1 and Sampling 2 had significantly higher Jaccard index than any other pair of seasons (Table 4.15).

The range of Jaccard indices obtained for 'between sampling occasions' (0.7039 to 0.7125) was less than that 'within sampling occasion' (0.7248 to 0.7578). This, again supported that seasonal shifts caused larger differences in beta diversity of the soil bacterial community than treatment-induced variations (warming, water and warming*water) in each sampling occasion (Table 4.12).

Table 4.12: Mean Jaccard Index within sampling occasion and between sampling occasions (Sampling 1, Sampling 2 and Sampling 3).

	Mean Jaccard index	
	Overall samples	**Between different experimental groups**
Within sampling occasion		
Sampling 1	0.7495 ± 0.0804	0.7248 ± 0.0253
Sampling 2	0.7578 ± 0.0763	0.7370 ± 0.0209
Sampling 3	0.7475 ± 0.0806	0.7233 ± 0.0265
Between sampling occasions		
Sampling 1 and Sampling 2	0.7122 ± 0.0238	0.7125 ± 0.0236
Sampling 2 and Sampling 3	0.7043 ± 0.0356	0.7042 ± 0.0357
Sampling 1 and Sampling 3	0.7039 ± 0.0316	0.7047 ± 0.0308

Table 4.13: Results of Repeated-Measures ANOVA statistical analysis (P-values) for Jaccard index of bacterial communities of all experimental groups across three sampling occasions (Sampling 1, Sampling 2 and Sampling 3).

Experimental Group	**Across 3 sampling occasions**	
	F-ratio	**P-value**
Control	9.93	0.002*
Warming	0.43	0.658
Water	1.07	0.368
Warming x Water	1.01	0.387
Overall	3.36	0.036*

Table 4.14: The p-values of LSD post-hoc tests. Significant differences of overall samples between sampling occasions ($p < 0.05$) are denoted by *. Sampling 1 is represented by S1. Sampling 2 is represented by S2. Sampling 3 is represented by S3.

Mean Jaccard ± S.D.	S1 and S2 (0.712±0.024)	S2 and S3 (0.704±0.036)	S1 and S3 (0.704±0.032)
S1 and S2 (0.712±0.024)			
S2 and S3 (0.704±0.036)	0.029*		
S1 and S3 (0.704±0.032)	0.022*	0.908	

Table 4.15: The p-values of LSD post-hoc tests. Significant differences in the control group between sampling occasions ($p < 0.05$) are denoted by *. Sampling 1 is represented by S1. Sampling 2 is represented by S2. Sampling 3 is represented by S3.

Mean Jaccard ± S.D.	S1 and S2 (0.727±0.017)	S2 and S3 (0.689±0.021)	S1 and S3 (0.683±0.038)
S1 and S2 (0.727±0.017)			
S2 and S3 (0.689±0.021)	0.007*		
S1 and S3 (0.683±0.038)	0.004*	0.617	

4.1.4.3 Assemblages of the co-occurring bacterial community members between seasons

Across all samples obtained, a total of 572/760 genera (75.3%) co-occurred in all seasons. Among these, 35 (6.12%) genera were dominant genera (> 1%) in at least one experimental groups. Spearman rank correlation of relative abundance of these shared genera across the three seasons (Table 4.16) identified a significant positive correlation between all seasons ($r = 0.737$ to $r = 0.956$, $p < 0.001$), although somewhat lower than within each season separately (cf. Tables 4.1.6 - 4.1.8). As illustrated in Figure 4.9, many of the dominant bacterial genera demonstrated similar patterns of relative abundance across the three sampling periods. Nonetheless, there were a few exceptions. The most dominant family in Sampling 1 and 3 was *Gaiellaceae*, whereas in Sampling 2, it was *Sphingomonadaceae*. The *Gaiellaceae* was less abundant while the family *Sphingomonadaceae* and genus *Sphingomonas* were more abundant in Sampling 2 than Sampling 1 and Sampling 3.

Table 4.16: Spearman rank order correlation (rho) of the relative abundance of co-occurring bacterial genera across all three sampling periods (Sampling 1, Sampling 2 and Sampling 3). Significant differences between groups ($p < 0.05$) are denoted by *.

	Sampling 1	**Sampling 2**	**Sampling 3**
Sampling 1			
Sampling 2	0.843, $p < 0.001$*		
Sampling 3	0.956, $p < 0.001$*	0.737, $p < 0.001$*	

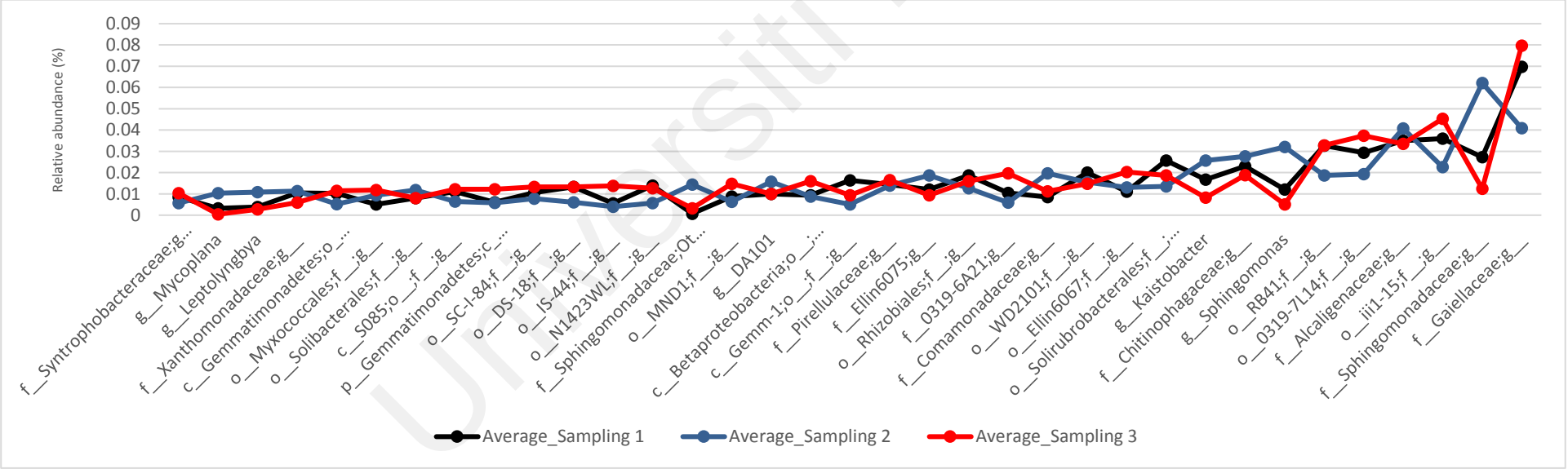

Figure 4.15: Inter-seasonal comparison: the average relative abundance of the co-occurring bacterial genera that were dominant in at least one Sampling Occasion (Sampling 1, Sampling 2 and Sampling 3). Annotations are used to name phylotypes at the lowest taxon level.

4.1.4.4 Overall diversity

The beta diversity of all samples across the three sampling periods was evaluated using weighted and unweighted PcoAs (Figure 4.16). These demonstrated distinct clustering in a majority of the samples from the same season in both metrics. There were no apparent clusterings of samples from the same experimental treatment, either within or between seasons. This was consistent with assessment of community beta diversity (Jaccard index) which indicated that sampling period was the primary driver of changes in bacterial community composition. In the ordination using unweighted UniFrac distances (qualitative difference, i.e. absence or presence of bacterial phylotypes), the highest principal component accounted for only 11.15% of the total variation. In contrast, in the weighted UniFrac ordination (quantitative difference, i.e. differences in relative abundances), the main principal component accounted for 66.23% of the total variation. This suggested that seasonal shifts had the strongest effects on relative abundances (quantitative difference) of bacterial phylotypes.

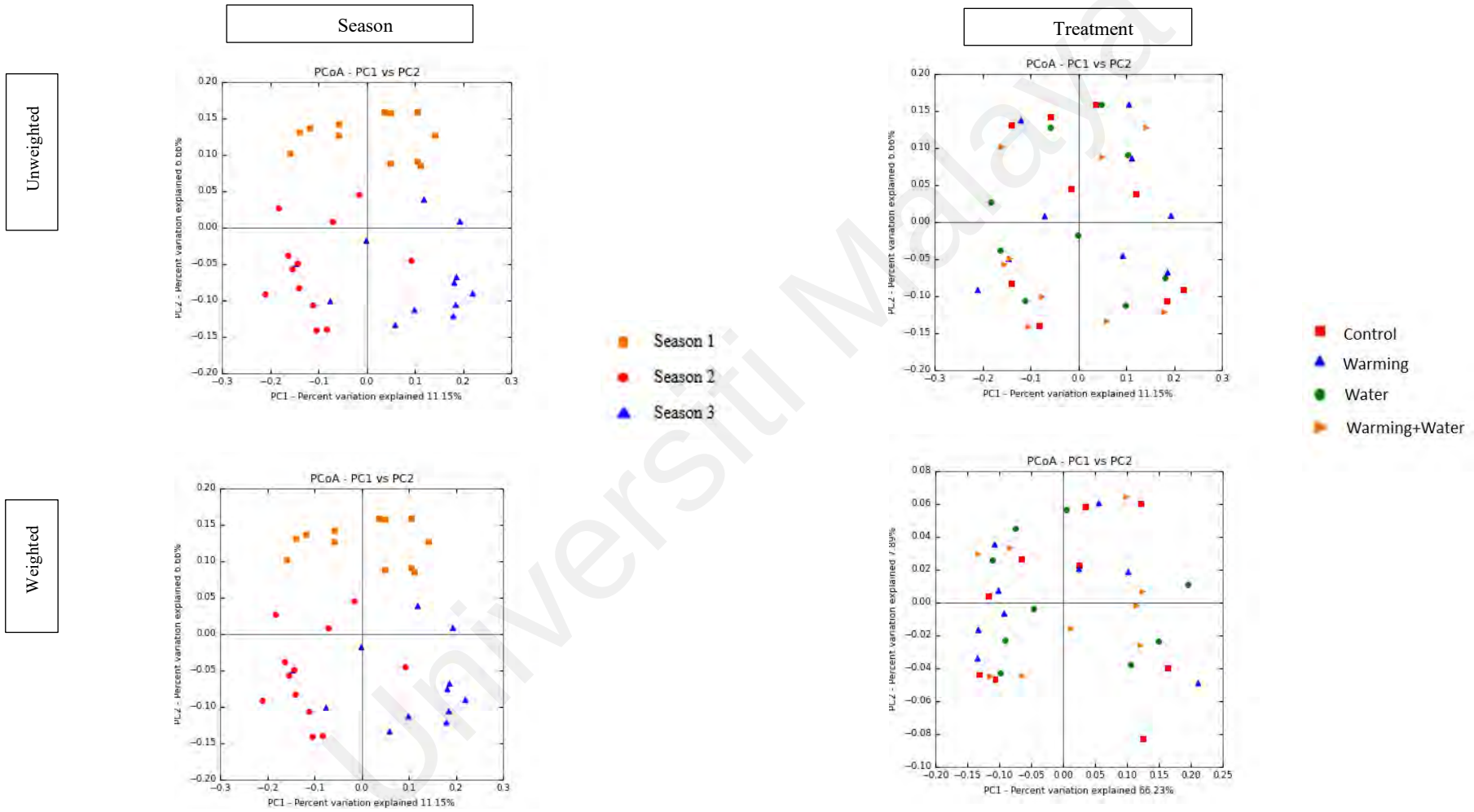

Figure 4.16: Principal coordinate analyses of bacterial communities based on weighted and unweighted UniFrac distance metrics for all three Sampling Occasions (Sampling 1, Sampling 2 and Sampling 3).

4.2 Freeze-thaw study

4.2.1 Individual sample coverage and alpha diversity

Rarefaction plots were generated using two metrics: Observed OTUs and Shannon Index (Figure 4.17; Table 4.17). The Shannon Index rarefaction reached a plateau at less than 10,000 sequences, indicating that standardized sequencing depth at 53,016 reads was sufficient for subsequent analysis of microbial diversity. This was also confirmed by Good's coverage (Good, 1953) which ranged between 94.99 and 96.88%.

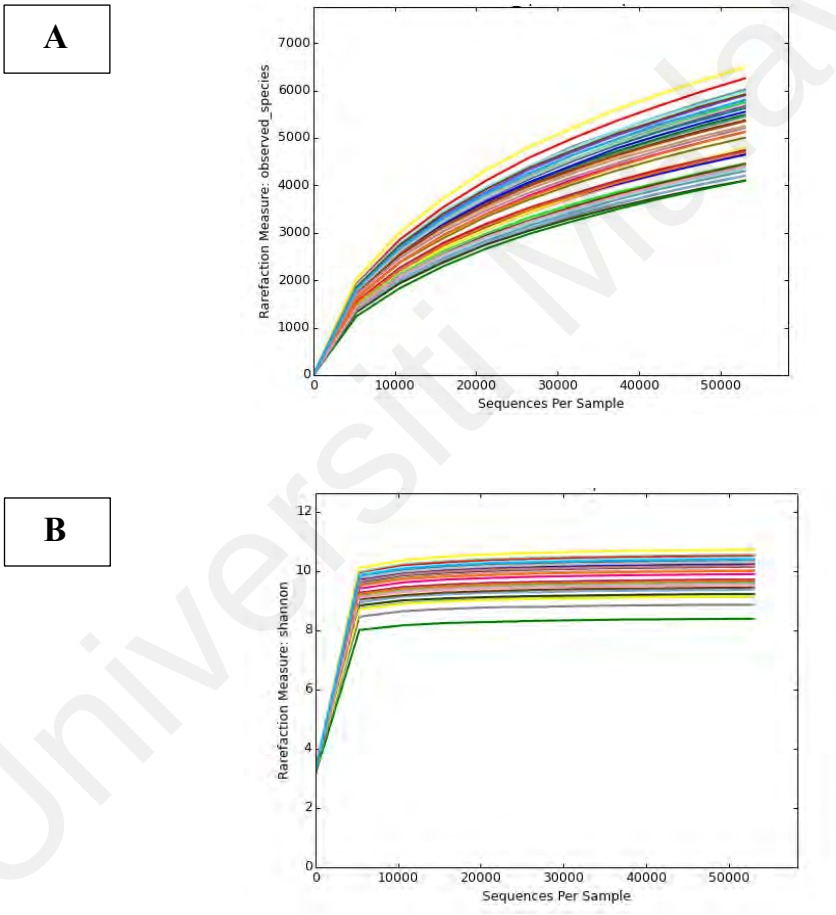

Figure 4.17: Rarefaction plots of samples in freeze-thaw study. Rarefaction curves based on A) Observed OTUs and B) Shannon diversity index.

Table 4.17: Shannon diversity index and Good's Coverage for all 45 samples in freeze-thaw study.

Sample	Good's Coverage	Shannon Index	Sample	Good's Coverage	Shannon Index	Sample	Good's Coverage	Shannon Index
High Snow Cover Soils			**Low Snow Cover Soils**			**No Snow Cover Soils**		
S1C0	0.97	9.65	**IN1C0**	0.95	10.52	**R1C0**	0.95	10.51
S1C1	0.96	9.66	**IN1C1**	0.96	10.29	**R1C1**	0.96	10.21
S1C9	0.97	8.39	**IN1C9**	0.96	9.53	**R1C9**	0.96	9.98
S1F1	0.96	9.70	**IN1F1**	0.96	10.31	**R1F1**	0.96	10.20
S1F9	0.96	9.12	**IN1F9**	0.96	9.89	**R1F9**	0.95	10.21
S2C0	0.97	9.40	**IN2C0**	0.96	10.40	**R2C0**	0.95	10.72
S2C1	0.96	9.54	**IN2C1**	0.97	10.18	**R2C1**	0.95	10.44
S2C9	0.96	9.14	**IN2C9**	0.96	10.03	**R2C9**	0.96	10.05
S2F1	0.96	9.44	**IN2F1**	0.96	10.39	**R2F1**	0.96	10.18
S2F9	0.97	8.87	**IN2F9**	0.96	10.18	**R2F9**	0.95	10.35
S3C0	0.97	9.65	**IN3C0**	0.96	10.31	**R3C0**	0.95	10.39
S3C1	0.97	9.64	**IN3C1**	0.96	10.16	**R3C1**	0.95	10.21
S3C9	0.97	9.37	**IN3C9**	0.97	9.23	**R3C9**	0.96	9.63
S3F1	0.97	9.59	**IN3F1**	0.96	10.18	**R3F1**	0.95	10.14
S3F9	0.97	9.59	**IN3F9**	0.96	9.71	**R3F9**	0.96	10.12

4.2.2 Comparative diversity analyses among baseline soils before experimental freeze-thaw (FT) exposure

4.2.2.1 Alpha diversity

Taxon diversity across all 45 soil samples examined in this study consisted of an average relative abundance of 98.3% bacteria, followed by 1.65% unassigned taxa and 0.000004% archaea. Bacterial sequences obtained from three snow cover baseline soils are shown in Table 4.18. High snow cover baseline soils clearly possessed lowest number of genera, along with slightly fewer orders and classes, among the three groups.

Table 4.18: Taxonomic assignments of baseline soils (no snow cover, low snow cover, high snow cover).

	Phyla	Class	Order	Genus
Baseline no snow cover	37	145	260	425
Baseline low snow cover	39	133	247	548
Baseline high snow cover	39	125	230	361

Across all three groups of baseline soils (no snow cover, low snow cover and high snow cover), ANOVA ($F = 39.33$, $df = 2, 6$, $p < 0.001$) demonstrated significant differences in Shannon index among the three groups of soils. Bonferroni post-hoc pairwise comparisons showed that significant differences were present between (1) high and no snow cover soils ($p = 0.001$) and (2) high and low snow cover soils ($p = 0.001$), but that there was no significant difference between the no snow cover and low snow cover soils ($p = 0.95$) (Figure 4.18).

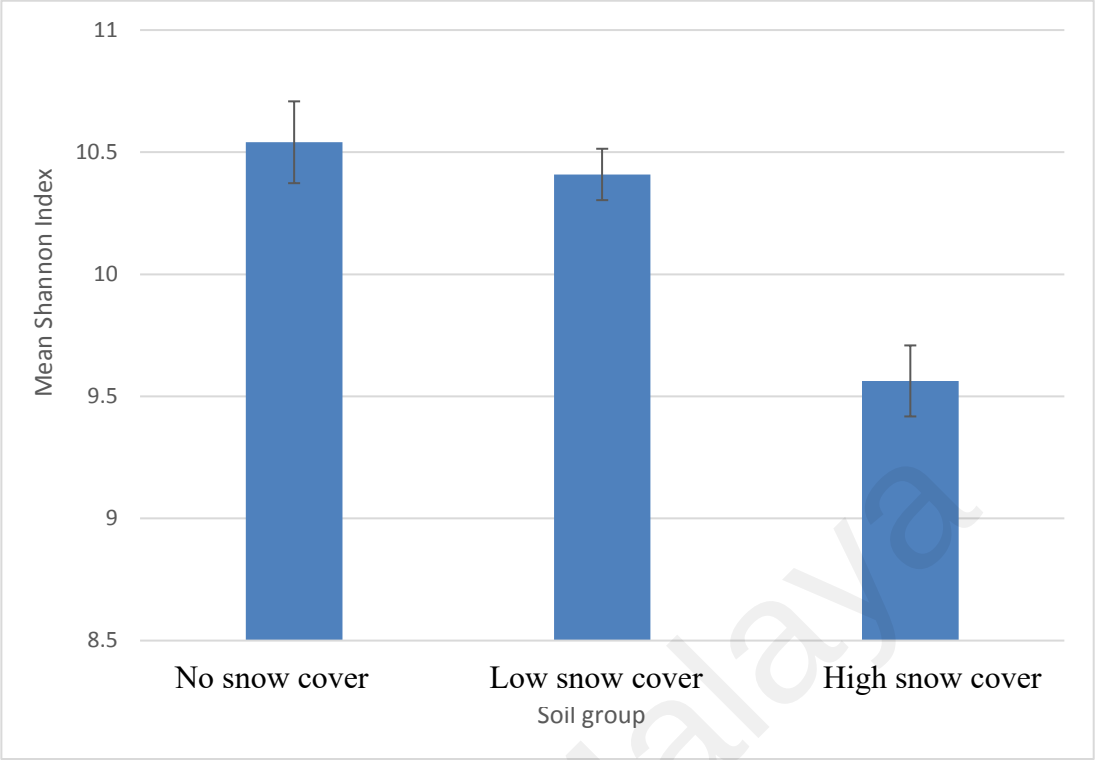

Figure 4.18: Mean Shannon index of bacterial diversity in no snow cover, low snow cover and high snow cover baseline soils.

4.2.2.2 Bacterial community composition similarity between snow depth groups

The OTUs assigned at generic level from the triplicate samples obtained in each group were pooled in order to assess for compositional similarity between groups using the Jaccard index (Table 4.19). Low and no snow cover soil showed the highest Jaccard similarity (0.70 ± 0.02). High snow cover soil shared the lowest Jaccard similarity with no snow cover soil (0.60 ± 0.02). ANOVA indicated that there were significant differences between the three pairs of soil groups in terms of their Jaccard similarity ($F = 2.92$, $df = 2$, $p < 0.001$). The LSD post-hoc test (Table 4.20) showed that no snow cover and high snow cover soils had significantly lower compositional similarity as compared to all other pairwise comparisons of soils.

Table 4.19: Mean Jaccard index (mean ± S.D.) between baseline soil groups (No, Low and High snow cover). C0 represents baseline soils.

	No _C0	Low _C0	High_C0
No _C0			
Low_C0	0.70 ± 0.02		
High_C0	0.60 ± 0.02	0.68 ± 0.04	

Table 4.20: The results of LSD post-hoc tests on Jaccard index between baseline soil pair groups. Significant differences between soil groups ($p < 0.05$) are denoted by *. C0 represents baseline soils.

	No_C0 with Low_C0	No_C0 with High_C0	Low_C0 with High_C0
No_C0 with Low_C0			
No_C0 with High_C0	< 0.001*		
Low_C0 with High_C0	0.179	< 0.001*	

4.2.2.3 Bacterial community structure

Figure 4.19 illustrates the mean abundances of all phyla that occurred in the three baseline soils. No snow cover and low snow cover soils shared similar dominant bacterial phyla, which were also present in high snow cover soils. The dominant phyla were Armatimonadetes, Gemmatimonadetes, Chloroflexi, Cyanobacteria, Planctomycetes, Verrucomicrobia, Bacteroidetes, Acidobacteria, Actinobacteria and Proteobacteria. Proteobacteria, Actinobacteria, Acidobacteria and Bacteroidetes together made up over

60% of the total bacterial diversity in all three soil groups. *Actinobacteria* were present in decreasing relative abundance as depth of snow cover increased. The high snow cover soils included a further four dominant phyla (OD1, WPS-2, Firmicutes and TM7), which appeared in lower relative abundance (< 1%) in low and no snow cover baseline soils.

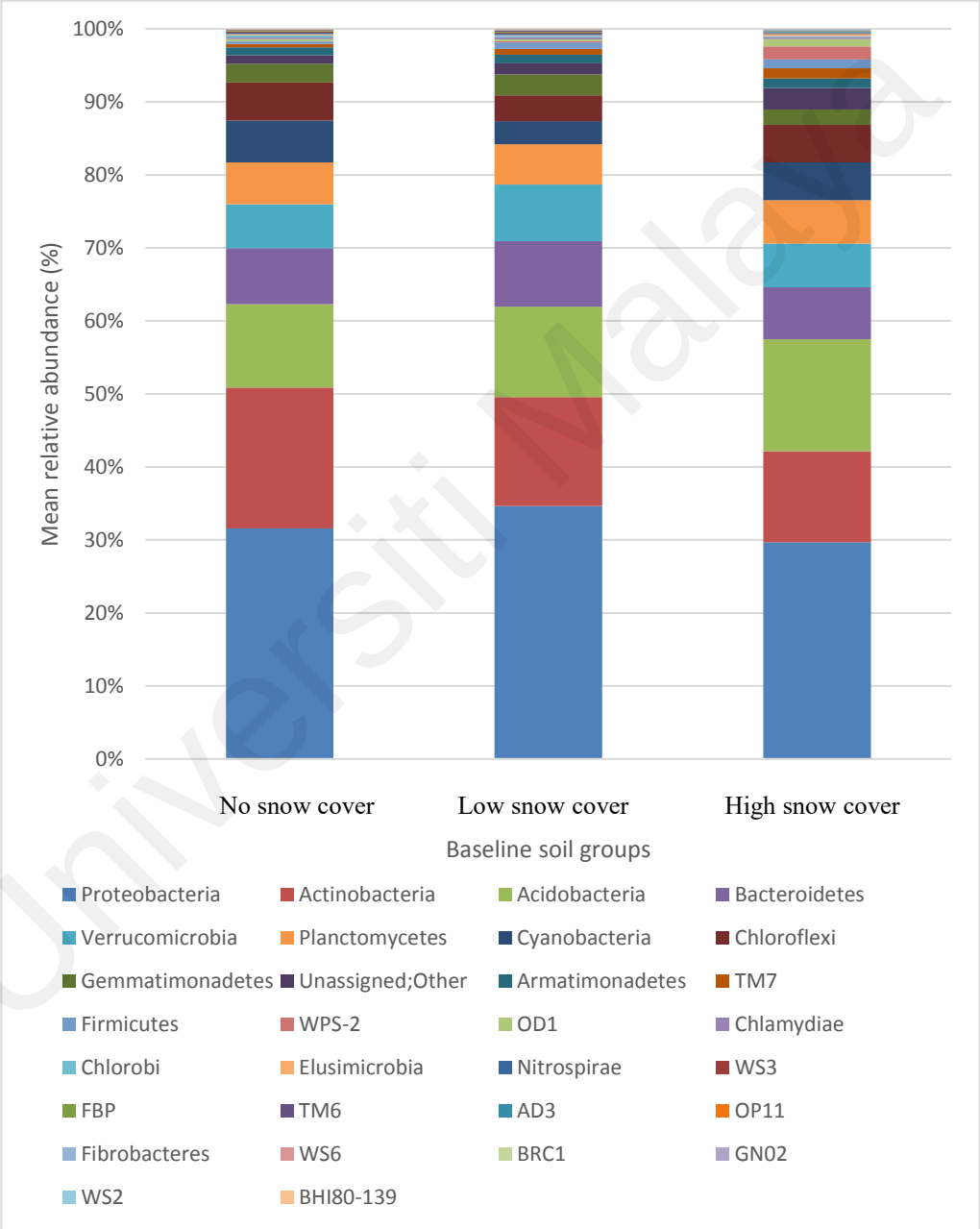

Figure 4.19: Mean relative abundance of the bacterial phyla present in baseline soil samples.

4.2.2.4 Assemblages of co-occurring bacterial community members between snow depth groups

The unique and overlapping genera across the three snow cover depth groups are represented in Figure 4.20. Overall, 415/701 genera (58.9%) co-occurred in all soil groups. The dominant phylotypes present in the three groups are represented in Figure 4.21. There were 38/701 dominant genera (5.42%). Both Figure 4.20 and Figure 4.21 clearly illustrate that there was a higher number of phylotypes that co-occurred in no snow cover group and low snow cover group, than high snow cover group and no snow cover group, or, high snow cover group and low snow cover group.

Spearman rank correlation of the relative abundance of co-occurring genera (Table 4.21) showed that there were significant positive correlations among the genera between the no snow cover and low snow cover groups ($r = 0.578$, $p < 0.001$)]. High snow cover soils, in contrast, showed no significant correlation with low snow cover soil, and a significant negative correlation with the no snow cover group ($r = -0.339$, $p = 0.037$).

Figure 4.22 illustrates the mean relative abundances of dominant genera (>1% mean relative abundance at least in one group among replicates in all three soil groups). There were a few dominant phylotypes which showed a decreasing trend in relative abundance as snow cover depth increased. For instance, the genus *Paludibacter* was a dominant genus (1.0%) in high snow cover soil. However, its relative abundance decreased to 0.012% in low snow cover soil, and it was completely absent in no snow cover soil. Similarly, Solirubrobacterales (order), Rhizobiales (order), RB41 (order) and iii1-15 (order), *Solirubrobacteraceae* (family), *C111* (family), *Hyphomicrobiaceae* (family), *Ellin6075* (family) and *Sphingomonadaceae* (family), *Pseudonocardia* (genus), *Kaistobacter* (genus) and *Sphingomonas* (genus) also showed decreasing abundance from no snow cover, through low to high snow cover soil.

In contrast, there were also a few dominant phylotypes which showed highest abundance in high snow cover soil and reduced abundance in low and no snow cover soil. These included Streptophyta (order), *Acidobacteriaceae* (family), *Ktedonobacteraceae* (family) and *Candidatus Koribacter* (species level). *DA101* (genus) and *Chitinophagaceae* (family) had their highest relative abundance in low snow cover soil.

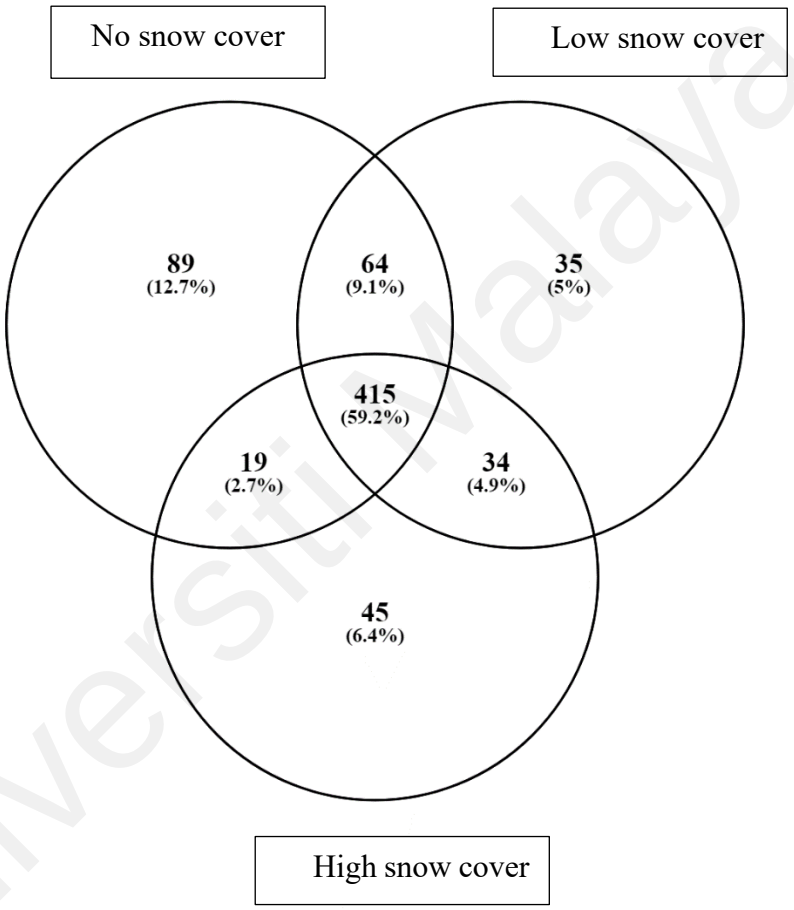

Figure 4.20: Venn diagram showing the proportion of unique and shared phylotypes across all three snow depths groups (no, low and high), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/).

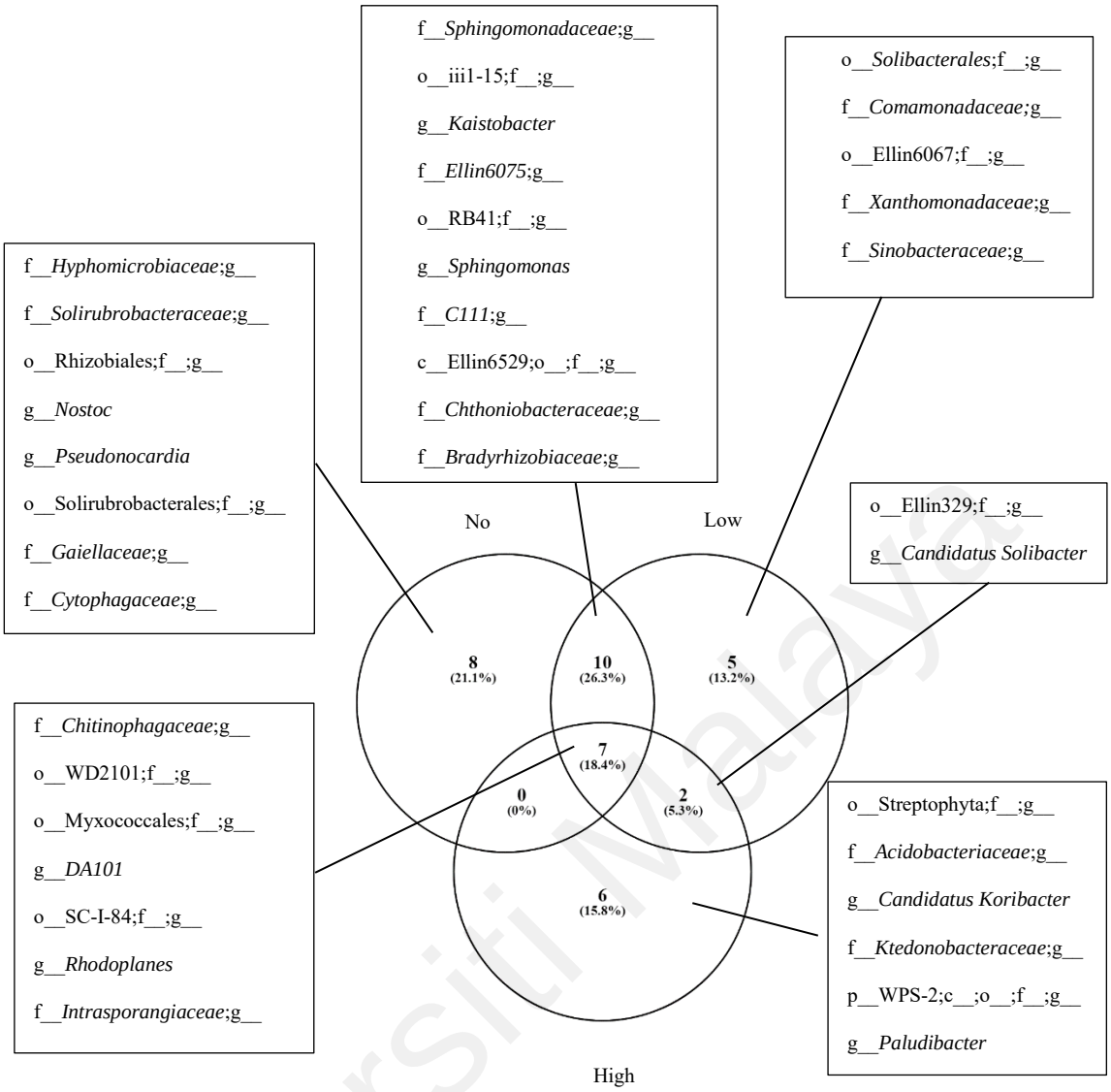

Figure 4.21: Venn diagram showing the proportion of unique and shared dominant (>1% mean relative abundance in at least in one experimental group) phylotypes across all three snow depths groups (No, Low and High), generated using Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/). Annotation indicates the lowest taxonomic level identified.

Table 4.21: Spearman rank correlation (rho) between the assemblages of co-occurring bacterial genera in all three baseline soil groups (no, low and high snow cover). Significant differences between groups ($p < 0.05$) are denoted by *.

	No	**Low**	**High**
No			
Low	0.578, $p < 0.001$*		
High	-0.339, $p < 0.037$*	0.250, $p < 0.129$	

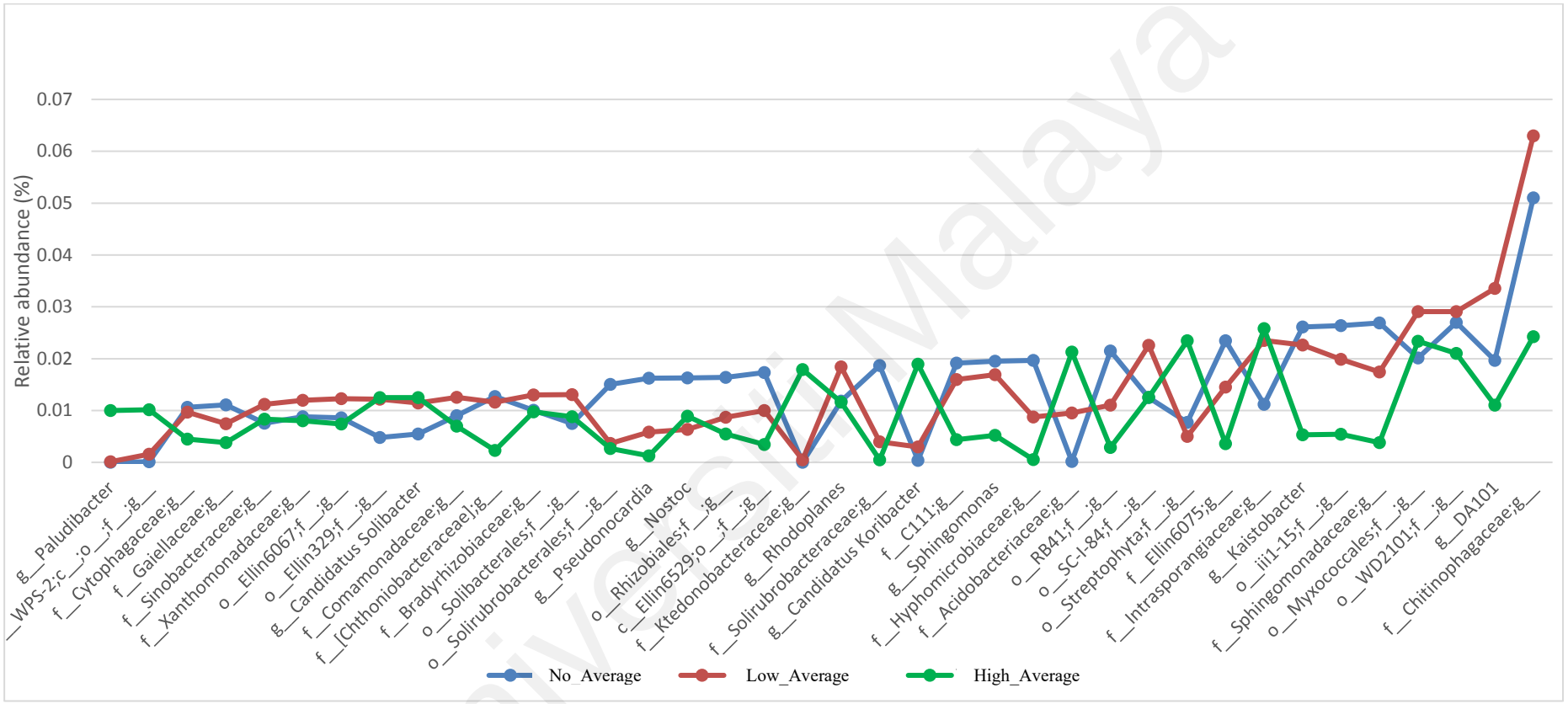

Figure 4.22: Average relative abundance of co-occurring bacterial genera that were dominant in at least one snow depth baseline soil groups (No, Low and High). Phylotypes are named at the lowest taxonomic level identified.

4.2.2.5 Soil chemical analyses

Soil carbon, nitrogen, ammonium and phosphorus contents in all baseline soils are tabulated in Table 4.22. Across all three groups of baseline soils (No, Low and High snow cover), One-Way ANOVA demonstrated no significant difference in all four soil chemical parameters among the three groups of soils (Table 4.23).

Table 4.22: Values of soil chemical parameters in the three baseline soil groups (No, Low and High).

Group	Organic carbon (%)	Total nitrogen (%)	Ammonium, NH_4 (%)	Available phosphorus (ppm)
No	11.86, 6.8, 12.0	0.52, 0.31, 0.52	7.38, 3.10, 7.00	33.75, 31.50, 28.25
Low	14.68, 13.49, 7.60	0.47, 0.60, 0.34	6.39, 10.29, 6.95	32.45, 27.80, 28.13
High	0.85, 8.36, 7.62	0.43, 0.35, 0.33	4.19, 2.96, 2.73	26.03, 21.28, 26.78

Table 4.23: Results of One-Way ANOVA on the soil chemical parameters of all baseline soil groups (No, Low and High).

Organic Carbon		Total Nitrogen		Ammonnium, NH_4		Available phosphorus	
F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
0.77	0.50	0.73	0.52	4.44	0.07	4.15	0.07

4.2.3 Bacterial diversity responses in no, low and high snow cover soils to exposure to experimental freeze-thaw (FT) cycles

4.2.3.1 Alpha diversity

Experimental exposure to freeze-thaw cycles had significant overall effects on Shannon diversity indices in no snow coer [$F(2, 8) = 49.48$, $p < 0.001$], low snow cover [$F(2, 8) = 20.49$, $p < .001$] and high snow cover soils [$F(1.04, 4.15) = 7.28$, $p = 0.05$] (Figure 4.23). For both no and low snow cover soils, tests of within-subjects contrasts (Table 4.24) further indicated significant decreases ($p < 0.05$) in Shannon diversity after one and then nine freeze-thaw cycles. However, high snow cover soils only demonstrated a marginally significant decrease ($p = 0.049$) between exposure to single and nine freeze-thaw cycles.

Table 4.24: Comparison of Shannon diversity index values obtained under different freeze-thaw treatments using tests of within-subjects contrasts in split-plot ANOVA. Significant values are indicated by *.

	Baseline with 1st cycle		**1st cycle with 9th cycle**		**Baseline with 9th cycle**	
	F ratio	**p-value**	**F ratio**	**p-value**	**F ratio**	**p-value**
No	40.21	0.003*	8.37	0.040*	179.50	< 0.001*
Low	25.49	0.007*	15.58	0.017*	24.68	0.008*
High	1.22	0.332	7.80	0.049*	6.91	0.058

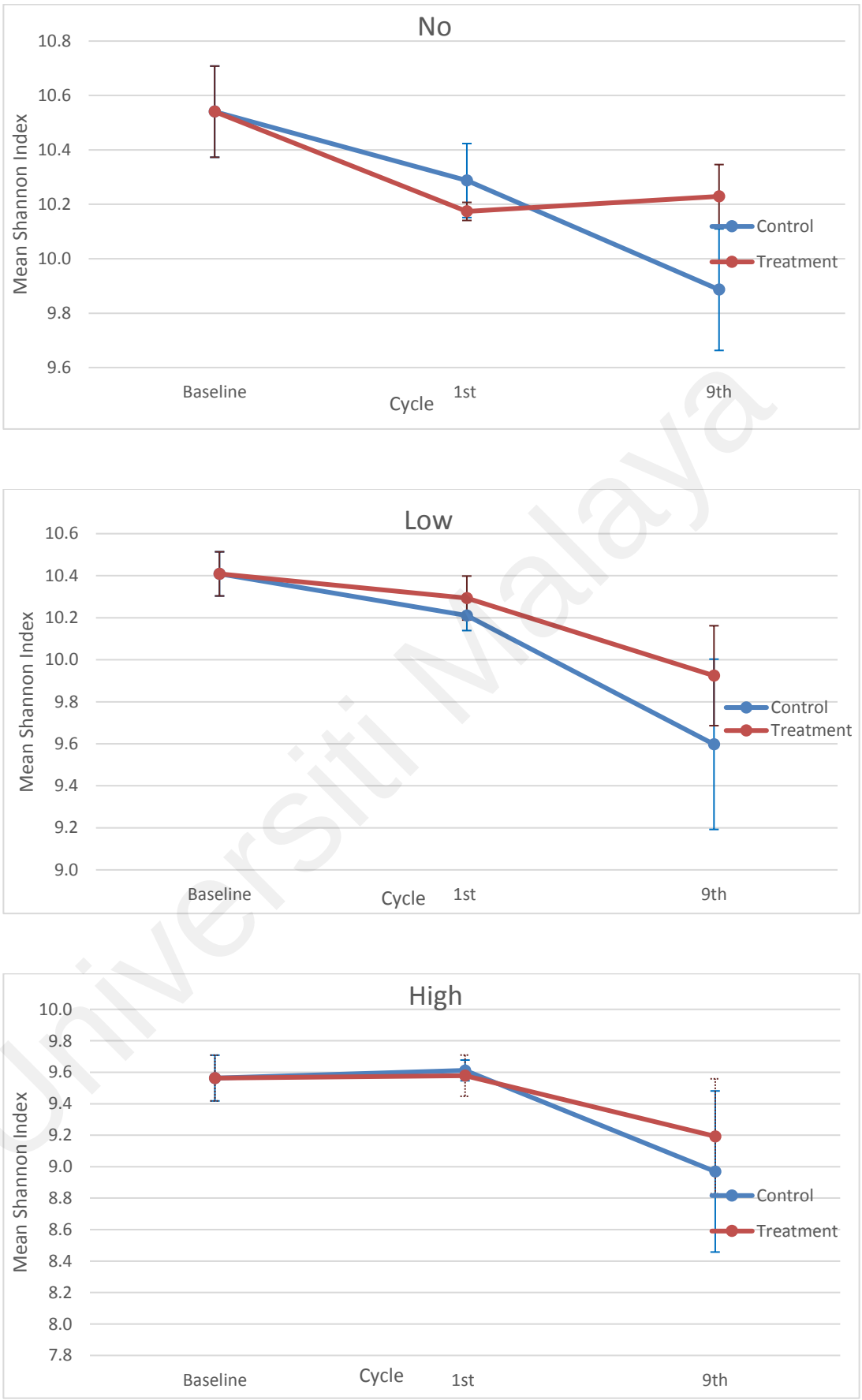

Figure 4.23: Mean Shannon index of no, low and high cover soils over the course of experimental freeze-thaw exposure.

Experimental exposure to single or multiple freeze-thaw cycles had little or no detectable effect on bacterial diversity as measured by the Shannon diversity index in comparison with the control treatment (no freeze-thaw cycles). Diversity in no snow cover soil differed significantly between treatment and control groups [$F(2, 8) = 11.69$, $p < 0.004$] only between 1st cycle and 9th cycle (Table 4.25) Low snow cover soils demonstrated no significant difference between treatment and control groups [$F(2, 8) = 1.29$, $p > 0.33$]. Similarly, high snow cover soils showed no significant difference between treatment and control groups [$F(1.04, 4.15) = 0.43$, $p = 0.554$] over the course of the experiment (Table 4.25).

Table 4.25: Results of statistical analysis on Shannon diversity between treatment and control groups for all three snow cover depths (No, Low and High) throughout freeze-thaw cycles for tests of within-subjects contrasts in split-plot ANOVA. Significant values are indicated by *.

	Statistical tests of difference in Shannon diversity index between treatment and control groups at three sampling points					
	Baseline with 1st cycle		1st cycle with 9th cycle		Baseline with 9th cycle	
	F ratio	p-value	F ratio	p-value	F ratio	p-value
No	1.35	0.31	14.61	0.019*	22.68	0.009*
Low	1.79	0.25	0.96	0.38	1.57	0.280
High (degrees of freedom corrected using Greenhouse-Geisser correction)	0.34	0.59	0.49	0.52	0.37	0.580

4.2.3.2 Bacterial community composition similarity between samples

For no snow cover soils (treatment and control groups), pairwise comparison between groups (Baseline, 1st FT, 9th FT, 1st Control, 9th Control) generated a range of mean Jaccard similarity between 0.74 and 0.77 (Table 4.26). One-Way ANOVA indicated no significant difference in Jaccard index among the groups [$F(9, 80) = 1.84$, $p = 0.074$]. This support a conclusion that, in terms of compositional similarity (qualitative), both treatment and control groups did not change in a significantly different manner to each other throughout the experimental cycles.

For low snow cover soils (treatment and control groups), bacterial compositional similarity between the five groups of pooled samples (Baseline, 1st FT, 9th FT, 1st Control, 9th Control) demonstrated a small range of Jaccard similarity indices, between 0.73 and 0.77 (Table 4.27). Overall, there was no significant difference in Jaccard index between the groups [$F(9, 80) = 1.42$, $p = 0.194$].

For high snow cover soils (treatment and control groups), bacterial community composition among the five groups of pooled high snow cover soil samples (Baseline, 1st FT, 9th FT, 1st Control, 9th Control) again demonstrated a small range of Jaccard similarity indices, between 0.72 and 0.76 (Table 4.28). Overall, there was no significant difference in Jaccard index between the groups ($F(9, 80) = 1.21$, $p = 0.298$).

Overall, bacterial communities among the five groups of high snow cover soil samples (baseline, 1st FT, 9th FT, 1st control, 9th control) demonstrated a slightly higher range of Jaccard similarity index (0.80 to 0.86) than those of no (0.79 to 0.84) and low (0.79 to 0.83) soil samples. ANOVA indicated that there were significant differences among the three groups in their Jaccard similarity ($F = 6.93$, $df = 2$, $p = 0.004$). The LSD post-hoc test (Table 4.29) showed that high snow cover had significantly lower compositional similarity than no and low depth cover.

Table 4.26: Mean Jaccard index (mean ± S.D.) between groups in no snow cover soil groups (Baseline, 1st FT, 9th FT, 1st Control and 9th Control).

	Baseline	1st Control	1st FT	9th Control	9th FT
Baseline					
1st Control	0.74 ± 0.02				
1st FT	0.77 ± 0.02	0.77 ± 0.02			
9th Control	0.76 ± 0.03	0.76 ± 0.02	0.75 ± 0.01		
9th FT	0.77 ± 0.01	0.77 ± 0.03	0.76 ± 0.03	0.77 ± 0.02	

Table 4.27: Mean Jaccard index (mean ± S.D.) between low cover soil groups (Baseline, 1st FT, 9th FT, 1st Control and 9th Control)

	Baseline	1st Control	1st FT	9th Control	9th FT
Baseline					
1st Control	0.73 ± 0.04				
1st FT	0.76 ± 0.03	0.76 ± 0.03			
9th Control	0.75 ± 0.03	0.73 ± 0.04	0.73 ± 0.03		
9th FT	0.77 ± 0.03	0.74 ± 0.03	0.75 ± 0.03	0.74 ± 0.03	

Table 4.28: Mean Jaccard index (mean ± S.D.) between high snow cover soil groups (Baseline, 1st FT, 9th FT, 1st Control and 9th Control)

	Baseline	1st Control	1st FT	9th Control	9th FT
Baseline					
1st Control	0.76 ± 0.04				
1st FT	0.75 ± 0.02	0.74 ± 0.03			
9th Control	0.75 ± 0.02	0.74± 0.03	0.73 ± 0.03		
9th FT	0.73 ± 0.03	0.73 ± 0.03	0.73 ± 0.03	0.72 ± 0.04	

Table 4.29: Outcome of LSD post-hoc tests on Jaccard index between baseline soil groups. Significant differences between soil groups ($p < 0.05$) are denoted by *.

	No	**Low**	**High**
No			
Low	0.77		
High	0.003*	0.005*	

4.2.3.3 Bacterial community structure

Bacterial composition at the phyla level in all 15 samples from no snow cover soils is illustrated in Figure 4.24, followed by low (Figure 4.25) and high cover snow soils (Figure 4.26). All three snow depth levels shared similar dominant phyla (average relative abundance ⩾ 1% at each snow depth level), these being Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Cyanobacteria and Armatimonadetes.

In no snow cover soils, the dominant genera (≥ 1%) in all three groups (Low Baseline, Low 9th FT and Low 9th Control) were *Pseudonocardia, Sphingomonas, DA101* and *Kaistobacter.* Exposure to nine freeze-thaw cycles (9th FT and 9th Control) caused *Psuedomonas* and *Oxalobacteraceae* to increase in relative abundance sufficiently to be classified as dominant genera. *Rhodoplane*, on the other hand, which was a dominant genus (1.2%) in baseline soils, declined in relative abundance to ≤1% in the 9th FT and 9th Control samples.

In low snow cover soils, the dominant genera were *Sphingomonas, DA101*, *Kaistobacter* and *Rhodoplanes. Clostridium* became a dominant genus (1.3%) after the 9th FT, having been a rare genus (≤ 1%) in the baseline and 9th Control.

In high snow cover soils, the dominant genera were *Geobacter, Nostoc, DA101, Rhodoplane, Candidatus Solibacter* (species) and *Candidatus Koribacter* (species). As with low depth cover, *Clostridium* showed a large increase in relative abundance over the experiment, from being a rare genus (≤ 1%) in the baseline, to becoming a dominant genus in the 9th Control (8.5%) and 9th FT (9.7%).

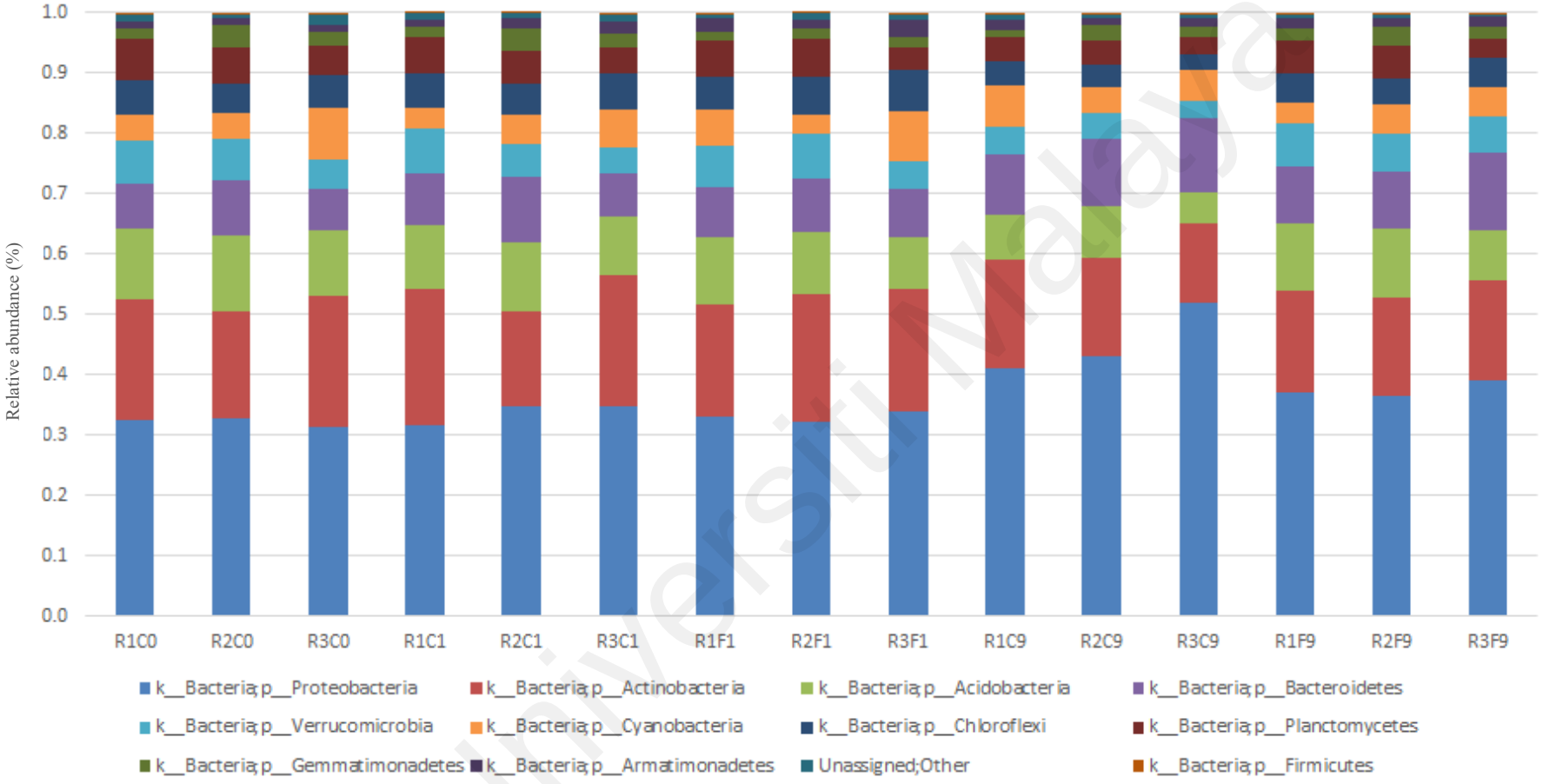

Figure 4.24: No snow cover soils. Relative abundance of bacterial phyla among soil samples exposed to experimental freeze-thaw (FT) cycles and in control. R stands for no snow cover, C0 stands for baseline soil groups with three replicates (R1C0, R2C0 and R3C0). C1 stands for 1st Control with three replicates (R1C1, R2C1 and R3C1). F1 stands for 1st FT with three replicates (R1F1, R2F1 and R3F1). C9 stands for 9th Control with three replicates (R1C9, R2C9 and R3C9). F9 stands for 9thFT with three replicates (R1F9, R2F9 and R3F9).

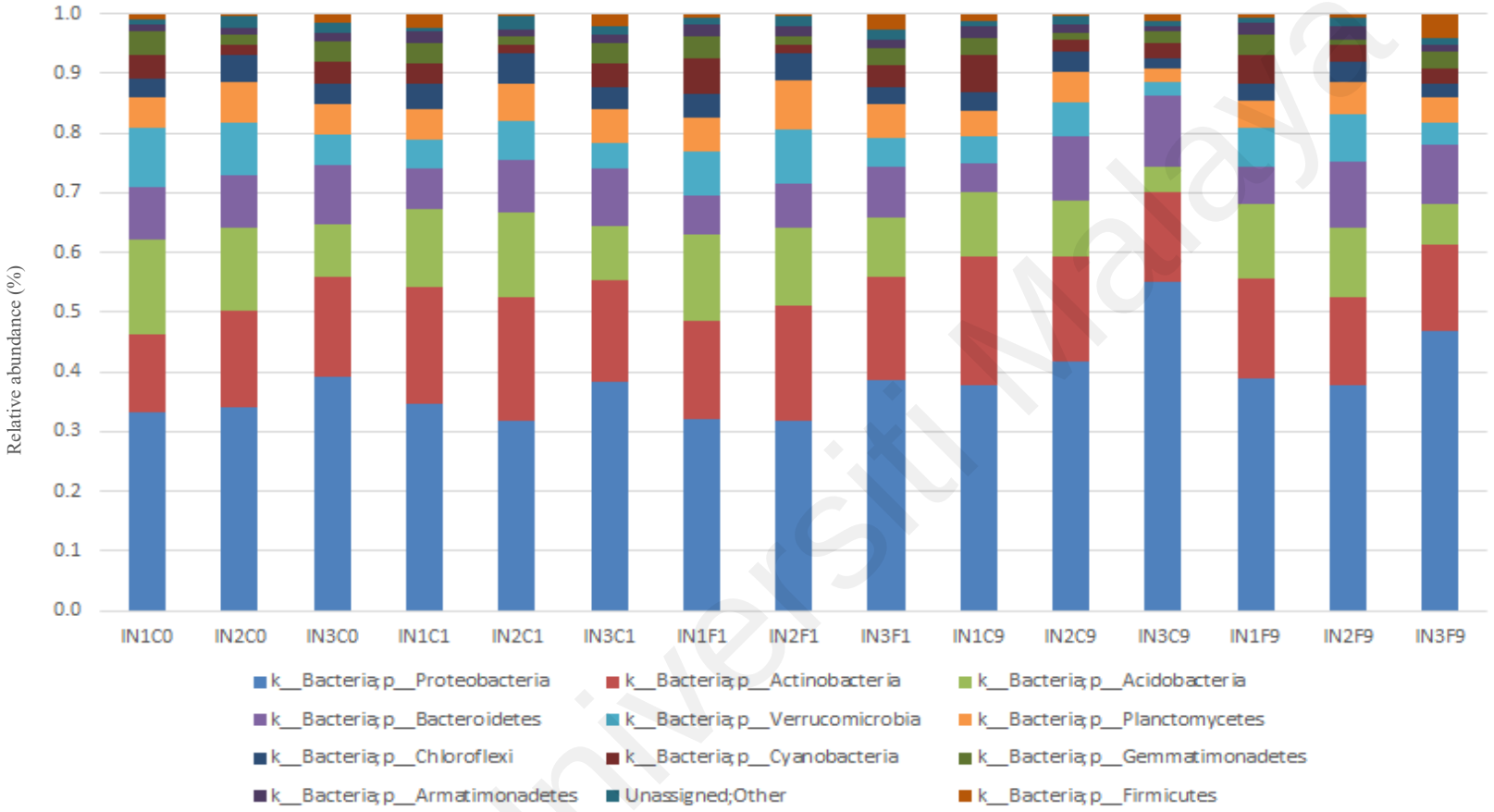

Figure 4.24: Low snow cover soils. Relative abundance of bacterial phyla among soil samples exposed to experimental freeze-thaw (FT) cycles and in control. IN stands for low snow cover, C0 stands for baseline soil groups with three replicates (IN1C0, IN2C0 and IN3C0). C1 stands for 1st Control with three replicates (IN1C1, IN2C1 and IN3C1). F1 stands for 1st FT with three replicates (IN1F1, IN2F1 and IN3F1). C9 stands for 9th Control with three replicates (IN1C9, IN2C9 and IN3C9). F9 stands for 9th FT with three replicates (IN1F9, IN2F9 and IN3F9).

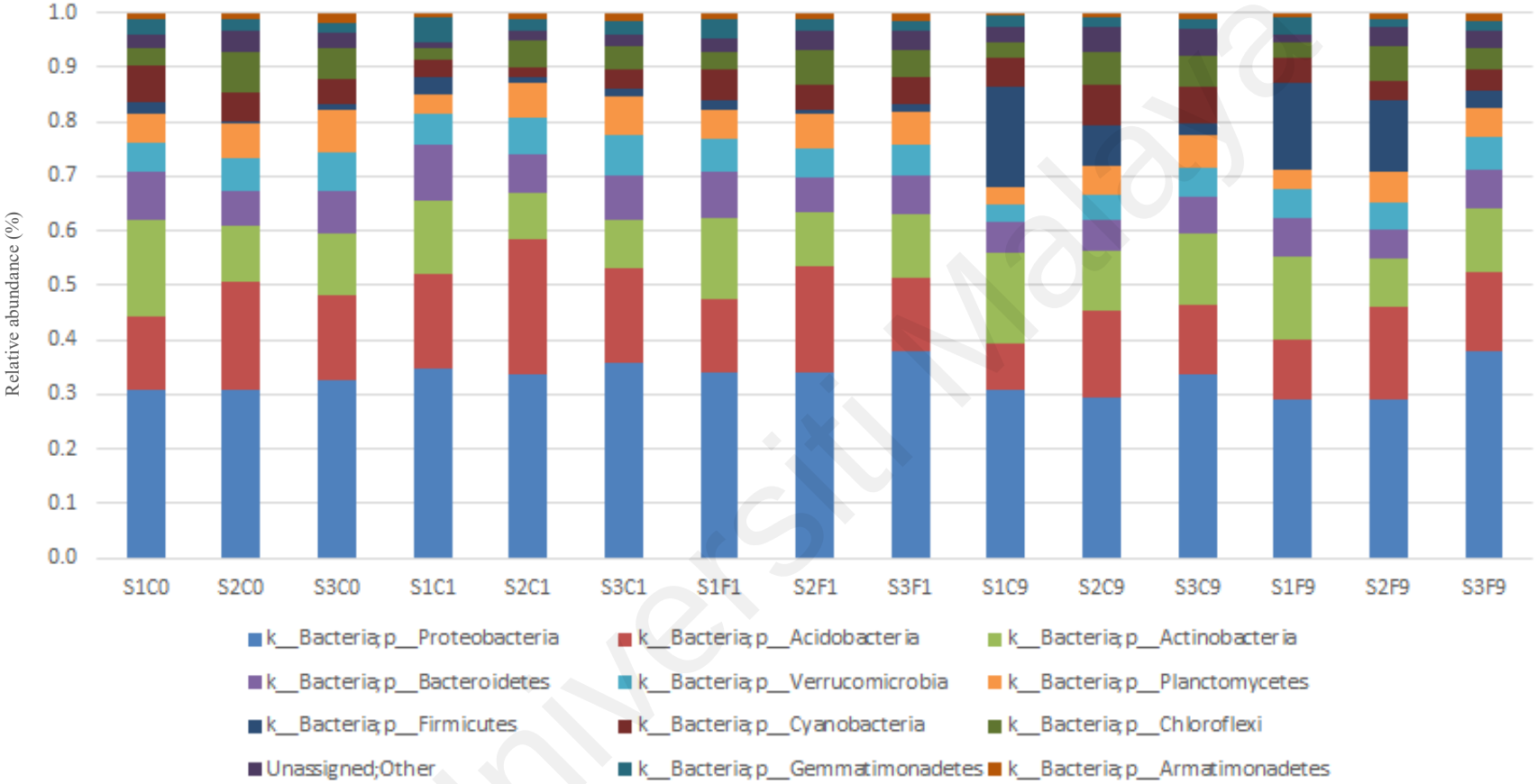

Figure 4.26: High snow cover soils. Relative abundance of bacterial phyla among soil samples exposed to experimental freeze-thaw (FT) cycles and in thaw controls. Relative abundance of bacterial phyla among soil samples exposed to experimental FT cycles and in control. S stands for high snow cover, C0 stands for baseline soil groups with three replicates (S1C0, S2C0 and S3C0). C1 stands for 1st Control with three replicates (S1C1, S2C1 and S3C1). F1 stands for 1st FT with three replicates (S1F1, S2F1 and S3F1). C9 stands for 9th Control with three replicates (S1C9, S2C9 and S3C9). F9 stands for 9th FT with three replicates (S1F9, S2F9 and S3F9).

4.2.3.4 Comparison of co-occurring bacterial community members between snow depth groups

A total of 450 genera co-occurred in all no snow cover groups (Baseline, 1st FT, 9th FT, 1st Control and 9th Control). Among these, 36 (8%) were dominant genera (> 1%) in at least one group. Mean relative abundances of each co-occurring dominant taxon in each group are presented in Figure 4.27. Spearman rank correlation (Table 4.30) showed that there were significant strong positive correlations in relative abundance among these genera in all five groups ($r \geq 0.918$, $p < 0.001$). This further supported the lack of influence of freeze-thaw cycle exposure on the relative abundance of genera in soil with no snow cover.

Kaistobacter was the most dominant genus among all five groups. Its relative abundance was very high after the 9th FT, having been lower after the 1st FT and lowest at Baseline. Similar patterns of shifts were observed in several other taxa, including *Oxalobacteraceae* (family), *Chitinophagaceae* (family), *Pseudomonas* (genus), *Janthinobacterium* (genus), *Hymenobacter* (genus) and *Flavobacterim* (genus). In contrast, Myxococcales (order) and *Rhodoplane* (genus) had highest relative abundance in the Baseline group.

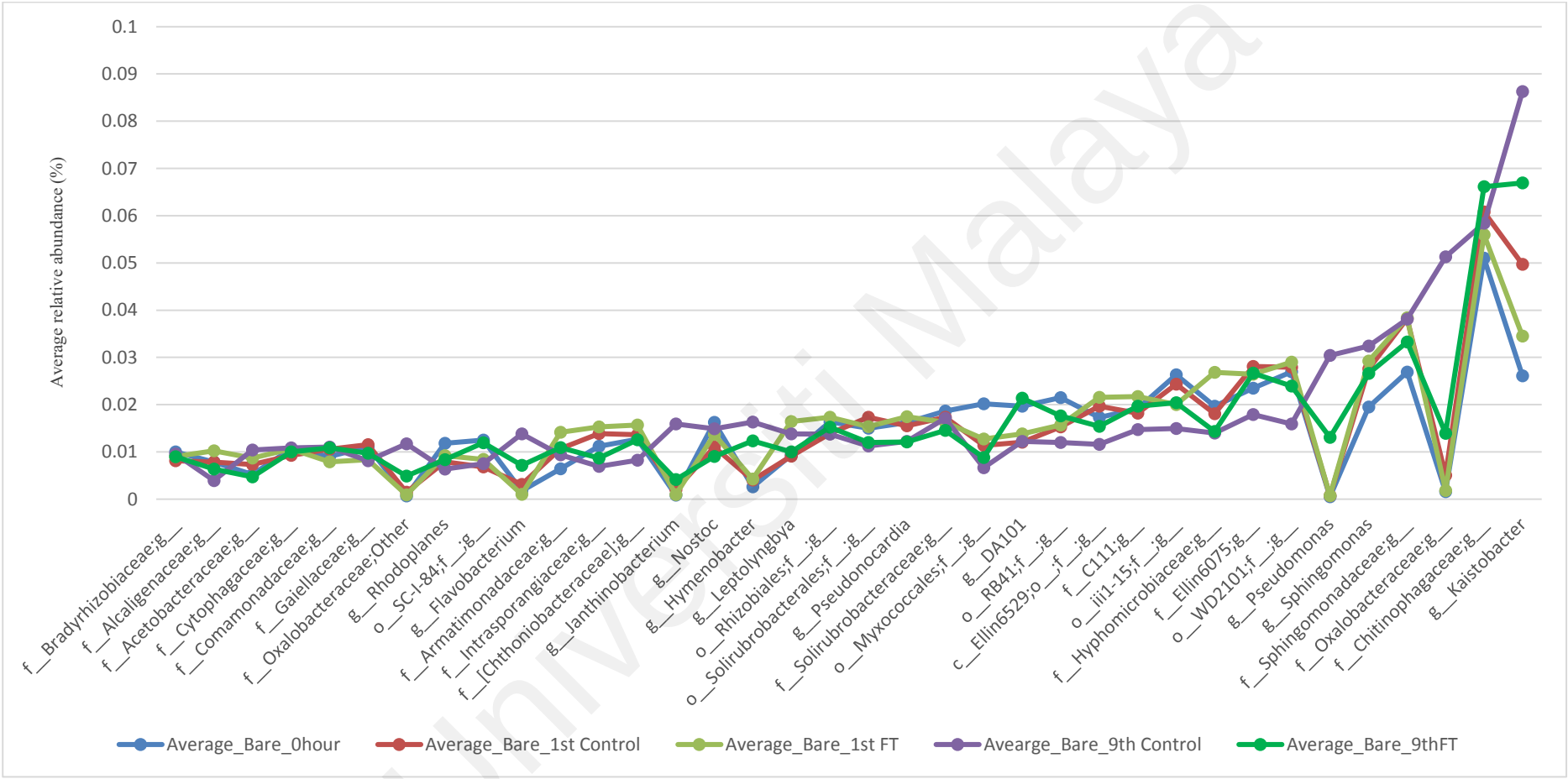

Figure 4.27: No snow cover soils (control and freeze-thaw groups). Bare represents no snow cover. Average relative abundance of co-occurring bacterial genera that were dominant in at least one group in no snow cover soils. Phylotypes are named at the lowest identified taxon level.

Table 4.30: Spearman rank order correlation (rho) of relative abundance of co-occurring bacterial genera in all five no snow cover soil groups (Baseline, 1st Control, 1st FT, 9th Control and 9th FT). Significant differences between groups ($p < 0.05$) are denoted by *.

	Baseline	1st Control	1st FT	9th Control	9th FT
Baseline					
1st Control	0.959, p < 0.001*				
1st FT	0.948, p < 0.001*	0.961, p < 0.001*			
9th Control	0.898, p < 0.001*	0.935, p < 0.001*	0.918, p < 0.001*		
9th FT	0.934, p < 0.001*	0.958, p < 0.001*	0.943, p < 0.001*	0.959, p < 0.001*	

A total of 440 genera co-occurred across all low snow cover soils, of which 35 (7.95%) were dominant in at least one group. Mean relative abundances of each taxon are presented in Figure 4.28. There were again significant strong positive correlations in relative abundance among the genera in all five groups ($r \geq 0.921$, $p < 0.001$) (Table 4.31). *Chitinophagaceae* (family) had high relative abundance (5.2% to 6.3%) across all five groups. *Oxalobacteraceae* (family) and *Pseudomonas* (genus) showed a large increase in relative abundance after the 9th Cycle in both freeze-thaw and control samples, increasing from an average of 0.2 - 0.7% (baseline and 1st Cycle) to 4.3% - 7.5% (9th Cycle). Similarly, *Janthinobacterium* (genus), *Hymenobacter* (genus) and *Microbacteriaceae* (family) also showed increased abundance after the 9th Cycle than at the Baseline and 1st Cycle.

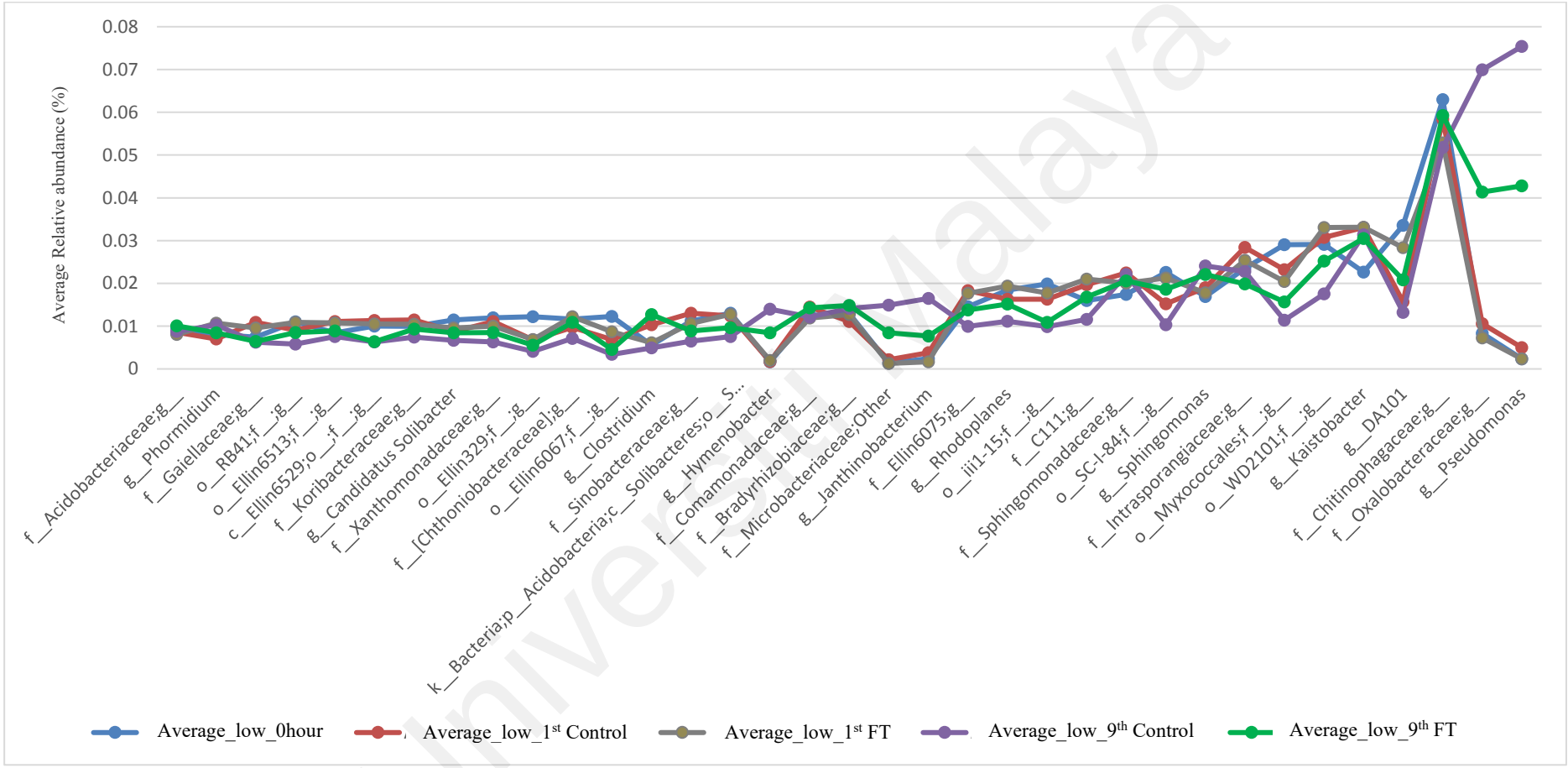

Figure 4.28: Low cover soils (control and freeze-thaw groups). Average relative abundance of co-occurring bacterial genera that were dominant in at least one group. Phylotypes are named at the lowest identified taxa level.

Table 4.31: Spearman rank order correlation (rho) of relative abundance of co-occurring bacterial genera in the five low snow cover soil groups (Baseline, 1st Control, 1st FT, 9th Control and 9th FT). Significant differences between groups ($p < 0.05$) are denoted by *.

	Baseline	**1st Control**	**1st FT**	**9th Control**	**9th FT**
Baseline					
1st Control	0.972, p < 0.001*				
1st FT	0.977, p < 0.001*	0.976, p < 0.001*			
9th Control	0.921, p < 0.001*	0.940, p < 0.001*	0.930, p < 0.001*		
9th FT	0.937, p < 0.001*	0.949, p < 0.001*	0.949, p < 0.001*	0.966, p < 0.001*	

Finally, a total of 419 genera co-occurred among all treatment groups in high snow cover soils, of which 34 (8.1%) were dominant genera. Relative abundances of each dominant taxon in each group are presented in Figure 4.29. Spearman rank correlation (Table 4.32) again showed significant strong positive correlation in relative abundance among co-occurring genera ($r \geq 0.967$, $p < 0.001$) in all groups.

The genera *Pseudomonas* and *Nostoc* showed increased relative abundance in the 9th Control. The genus *Clostridium* showed a strikingly high relative abundance (8.5% to 9.7%) after the 9th Cycle compared with its very low abundance (0.86% to 1.26%) at Baseline and 1st Cycle. The family *Intrasporangiaceae* maintained constant relative abundance (2.6% to 2.9%) throughout freeze-thaw cycle and control treatments.

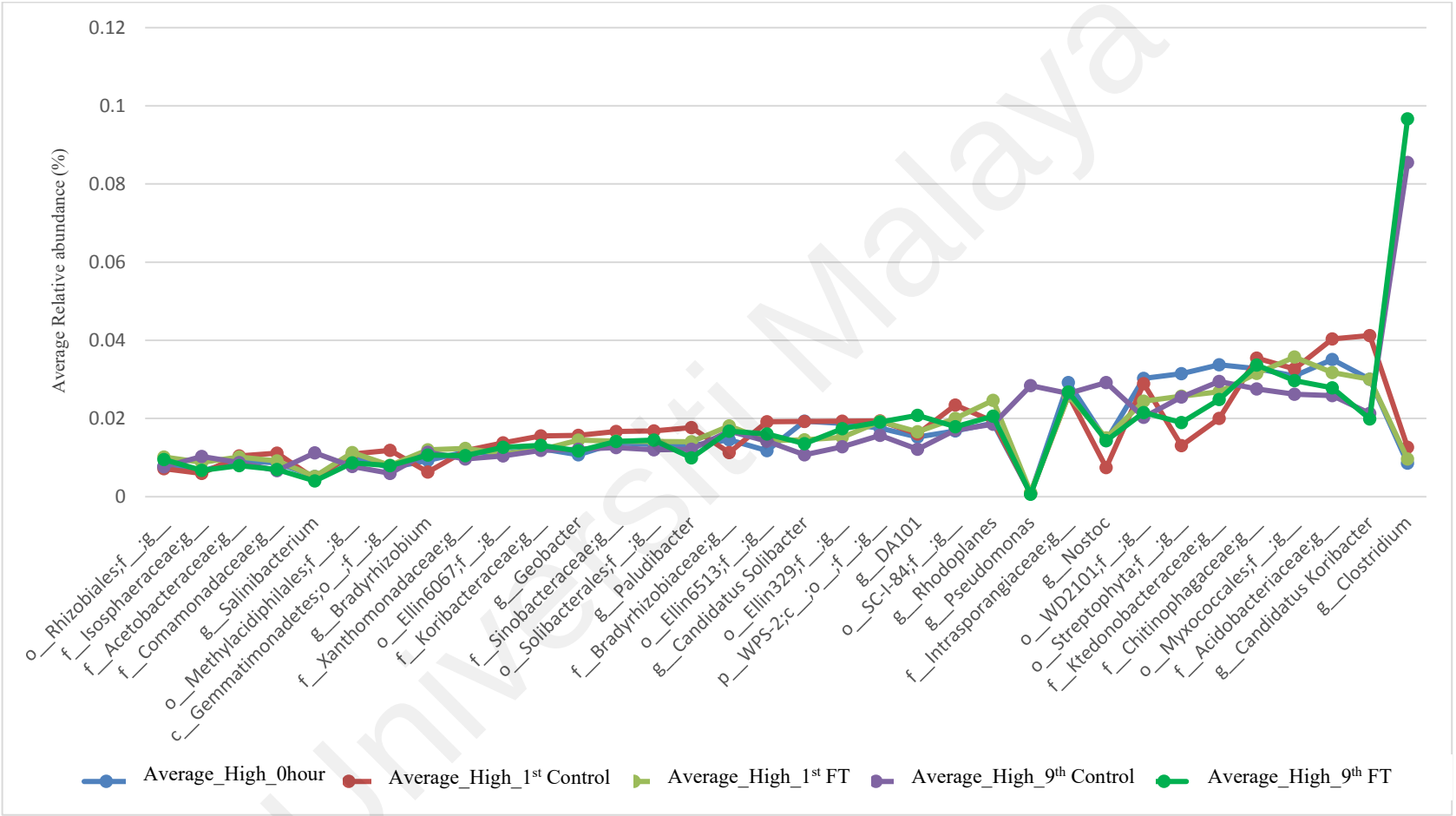

Figure 4.29: High snow cover soils (control and freeze-thaw groups). Average relative abundance of co-occurring bacterial genera that were dominant in at least one group. Phylotypes are named at the lowest identified taxon level.

Table 4.32: Spearman rank order correlation (rho) of relative abundance of co-occurring bacterial genera in all five high snow cover soil groups (Baseline, 1st Control, 1st FT, 9th Control and 9th FT). Significant differences between groups ($p < 0.05$) are denoted by *.

	Baseline	**1st Control**	**1st FT**	**9th Control**	**9th FT**
Baseline					
1st Control	0.965, p < 0.001*				
1st FT	0.968, p < 0.001	0.964, p < 0.001*			
9th Control	0.949, p < 0.001*	0.942, p < 0.001*	0.961, p < 0.001*		
9th FT	0.961, p < 0.001*	0.962, p < 0.001*	0.969, p < 0.001*	0.956, p < 0.001*	

4.2.3.5 Overall diversity

Both weighted and un-weighted UniFrac PCoAs (Figure 4.30) showed distinct separations between soils of different snow cover depth. However, there was no apparent clustering of samples in terms of control or freeze-thaw treatments at each snow cover level. Irrespective of typical level of snow cover, experimental exposure to nine consecutive freeze-thaw cycles did not lead to any detectable diversity change in the bacterial communities of the soils examined.

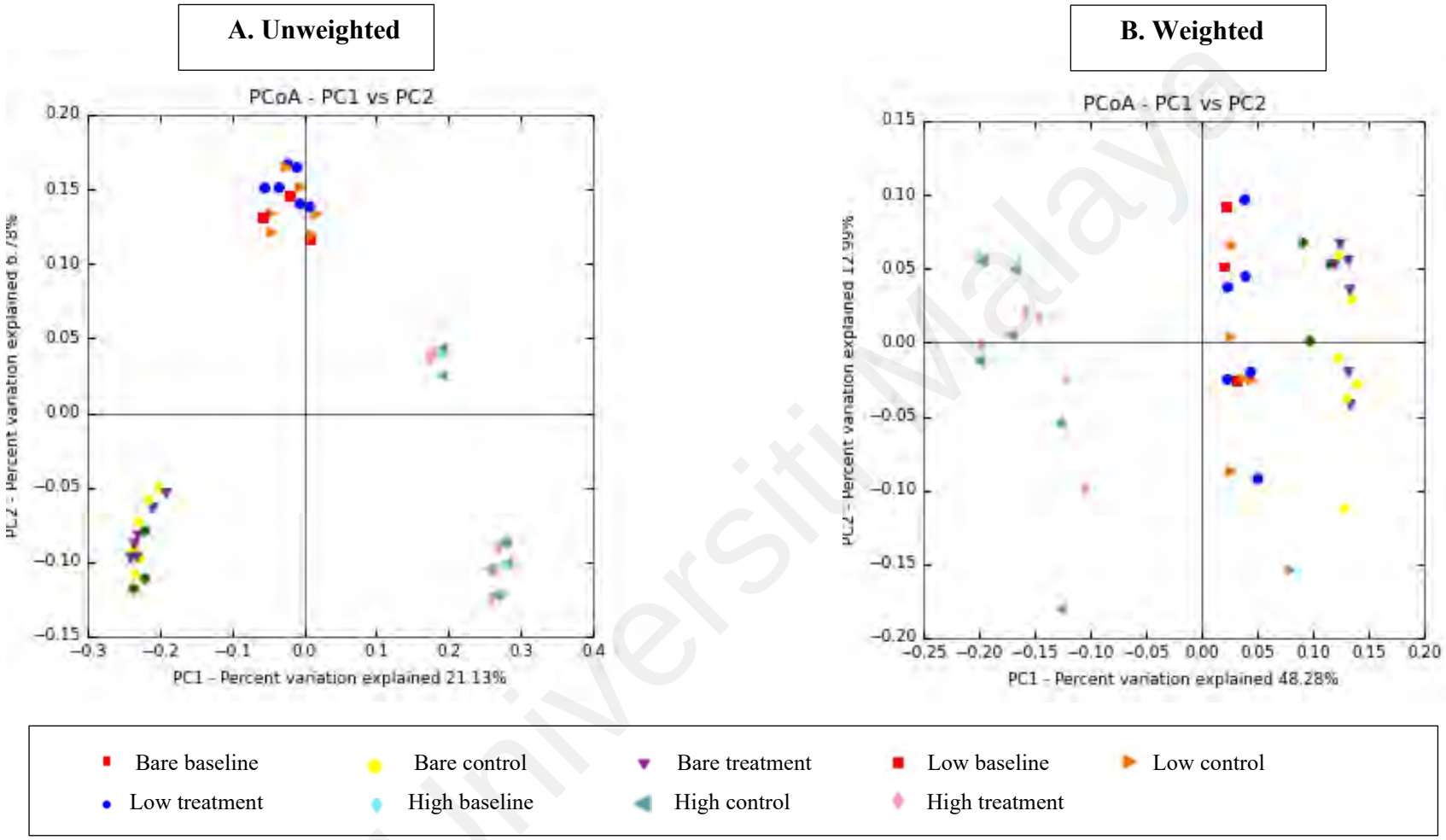

Figure 4.30: Principal coordinates analysis of bacterial communities based on weighted and unweighted UniFrac distance metrics of all samples in the freeze-thaw (FT) study. Bare stands for no snow cover soil. Low stands for low cover soil. High stand for high cover soil. Control groups includes 1st Control and 9th Control. Treatment Groups include 1st FT and 9th FT.

CHAPTER 5: DISCUSSION

5.1 Overall bacterial community composition in Open-top chamber and/or water amendment study

The dominant bacterial phyla recorded from soil on Svalbard in this study, namely Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, Cyanobacteria, Chloroflexi, Bacteroidetes, and Verrucomicrobia, are consistent with the phyla recorded in previous studies of Arctic soils. For example, Acidobacteria, Bacteroidetes and Proteobacteria were all frequent in permafrost of north-east Greenland (Ganzert *et al*., 2014), with Acidobacteria, Actinobacteria, Proteobacteria, Bacteroidetes and Firmicutes being frequent in the active layer of permafrost sampled from the Canadian High Arctic (Yergeau *et al.,* 2010). Similarly, in studies of soils in drained lake basins of Arctic Alaska, Actinobacteria, Bacteroidetes and Proteobacteria were the dominant taxa of bacteria present (Yergeau *et al.,* 2010). The OP11 lineage, which was recorded at low frequencies in the present study, was abundant in the Arctic wetland soils studied by Kao-Kniffin *et al*. (2015). We documented no taxa switching between dominant (⩾1%) and rare (<1%) categories at the phylum level, with the exception of the "Latescibacteria" WS3 lineage which was recorded at low frequencies (<1%) in Sampling 1 and Sampling 2 but increased up to 1.2% in Sampling 3. This taxon was abundant in the wetland and lake sediments studied by Stoeva *et al*. (2014).

At lower taxonomic levels (i.e. genus, family and order level), across all three sampling occasions, there were a wide range of both unique and overlapping phylotypes between seasons, ranging from 6 to 38 phylotypes (Sampling 1), 6 to 26 phylotypes (Sampling 2), and 7 to 31 phylotypes (Sampling 3). However, when restricting consideration to dominant phylotypes (≥ 1%), the ranges were much smaller, from 0 to 4 phylotypes (Sampling 1), 0 to 3 phylotypes (Sampling 2), and 0 to 4 phylotypes

(Sampling 3). This suggested that seasonal shifts did not cause large changes in the presence/absence of dominant phylotypes between seasons.

The order Solirurobrobacterales had higher abundance in all three treatment groups than the control group in Sampling 1. This order belongs to the class Thermoleophilia, which largely consists of various thermophilic and thermotolerant genera and species (reviewed in Shivlata & Tulasi, 2015). This is perhaps consistent with its higher abundance in open-top chambered warming (with or without water) treatment groups in Sampling 1. However, its abundance was more uniform across all four experimental groups in Sampling 2 and Sampling 3. These data might imply that Open-top-chambered warming in different seasons would have different impacts on this thermophilic/thermotolerant group of bacteria. In contrast, the family *Spingomonadaceae* had increased abundance in Sampling 2 and Sampling 3 in open-top chambered warming (with or without water) treatment groups than the control group, which contrasted with Sampling 1. These data suggest that open-top chambered warming leads to different impacts on different groups of bacteria.

Warming and/or water addition increased relative abundance of the family *Gaillaceae* in Sampling 1 and Sampling 2. *Gaillaceae* is within the class Actinobacteria and its members are strictly aerobic and chemoorganotrophic (Albuquerque *et al.,* 2011). Under elevated atmospheric CO_2, this family have been reported to have higher abundance, suggested to be primarily driven by increased C inputs into the soil (Raut *et al.,* 2018). The family is also highly abundant in fertile black soils in the Amazon rainforest (Lima *et al*., 2015). Taken together, these observations suggest indirect impacts of open-top chambered warming on the family *Gaillaceae*, regulated by increased C input to the soil. Subsequently, in Sampling 3, the abundance of this family was lower in all three treatment groups than in control plots. This may be due to declining amounts of

available C in the treated soil plots due to continual warming throughout Sampling 1 and Sampling 2. Further sampling points in future years are required to confirm whether this indicates a general trend.

Recently, Luo *et al*. (2014) reported that warmed soils which had undergone infrared warming of 2°C over 10 years did not show significant changes in Shannon diversity. However, they inferred that long-term warming most probably caused pre-existing abundant taxa to adapt to this environmental stress rather than there being short-term and/or pulse-like responses of a few (perhaps new) genotypes. Consistent with this interpretation, in the current study the bacterial community composition in control and warmed plots in all three sampling occasions was very similar, with some but only subtle differences in relative abundance.

5.2 Effects of open-top chambered warming and water amendments on bacterial diversity

Overall, the data obtained demonstrated that open-top chambered warming and/or initial water addition for 10 months (Sampling 1), 13 months (Sampling 2) and 2 years (Sampling 3) did not generally lead to significant changes in bacterial alpha diversity or beta diversity in the studied soils, as indicated by the Shannon index and Jaccard index respectively. Although some significant treatment effects were detected in Sampling 2 they only represented small changes in average Jaccard index, with a maximum difference of only 2.7% between treatment groups. There were, however, significant seasonal shifts in bacterial beta diversity independent of the experimental manipulation, as indicated by Jaccard index and phylogenetic divergence between bacterial communities. Another central finding of this study was the consistency in

relative abundances of co-occurring bacterial genera, which were strongly positively associated across the three sampling seasons.

In the experimental plots studied here, the average increase in mean monthly temperature within open-top chambers was 1.67°C. This increment is within the range of mean open-top chambers temperatures across polar and alpine regions (0.9 to 2.1 °C) as reported by Bokhorst *et al.* (2013). The lack of warming and/or water addition effects may be unsurprising, although rapid community changes have been reported in temperate soils upon exposure to temperature maxima exceeding local 'climate history' (Waldrop & Firestone, 2006), and in particularly vulnerable Antarctic soils (Yergeau *et al*., 2012b). A possible reason for the lack of open-top chambered warming effects is that this increment might not be extreme enough to lie outside the annual variability experienced by the microbial community. The importance of short-term temperature extreme events on soil biota had been discussed by Marchand *et al.* (2006) and Jentsch and Beierkuhnlein (2008). Moreover, Kennedy (1995) argued convincingly that the biological consequences noted during application of passive warming methodologies might not always be accountable to the mean warming, but rather to extreme temperature events and other non-temperature microclimate factors. In addition, another explanation for the lack of treatment effects in our open-top chamber study could be that there was too short a period for responses to develop. Previous experiments using open-top chambers to warm High Arctic soils have shown significant changes in community structure and genotype richness of nitrous oxide-reducing and atmospheric nitrogen-fixing bacterial genes in soil, but only after 13 years (Walker *et al*., 2008).

Sampling 2 took place about two months after Sampling 1, whereas sampling for Sampling 3 took place one year after Sampling 2. This could underlie the higher bacterial compositional similarity identified between Sampling 1 and Sampling 2, as revealed by

Jaccard index, which was significantly higher than those of Sampling 1 and Sampling 3, and, Sampling 2 and Sampling 3. Although this indicated that there was no summer shift in bacterial community composition during the study period, we cannot ignore the possibility of summer or winter community shifts over longer timeframes.

The seasonal variations detected in bacterial community composition were mainly due to changes in relative abundances among bacterial taxa, as revealed by the high percentage of phylogenetic divergence of the weighted PCoA metric (66.23%), a measure which takes into account both OTU identities and relative abundances. The patterns observed resemble the study of Buckeridge *et al*. (2013), who reported changes in Arctic soil microbial communities in relation to variation in soil nutrient status and environmental conditions between seasons, based on PLFA analysis. Lipson *et al*. (2007) and Kuffner *et al*. (2012) also reported seasonal shifts in bacterial community composition, interpreted as responses to seasonal fluctuations in carbon substrate supply from plant litter and root exudates. On the other hand, the seasonal changes in bacterial relative abundance of multiple phylotypes were not accompanied by significant changes in alpha diversity across three seasons in current study. This is plausible, as multiple studies (e.g. Ge *et al*., 2010; Ramirez *et al*., 2010) have also reported independent variation of soil bacterial diversity and community composition, suggesting these two community parameters were likely to be controlled by different factors.

An absence of treatment effects after 10 months (Sampling 1) on bacterial alpha and beta diversity is consistent with the treatment effects of open-top chambers usually being weak during winter seasons due to much reduced biological activity at consistently low temperature and low or zero light levels. Bokhorst *et al*. (2013) confirmed that, during the summer months, the trapping of incoming energy from the sun induces greater open-top chambered warming effects on soil microclimates than is achieved in the winter

season. Thus, the summer warming achieved by the time sampling took place in Sampling 2 may have underlain the very small but significant change in beta diversity. Samples obtained in the next summer (Sampling 3) showed no significant difference with Sampling 2 in either alpha and beta diversity. Continued monitoring of the seasonal dynamics of bacterial community composition over a longer period would be required to confirm these seasonal effects in open-top chambered warming studies.

The data showing limited or no detectable effects of warming on bacterial community alpha or beta diversity are consistent with previous studies which have demonstrated that changes in climate change drivers such as soil temperature and moisture do not always result in significant changes in bacterial or fungal communities (Chen *et al*., 2007; Schindlbacher *et al.,* 2011; Williams, 2007; Puissant *et al*., 2015). Furthermore, several previous studies (Zhang *et al*., 2011; Xia *et al*., 2010; Borkhorst *et al*., 2013, 2015; Hayden *et al*., 2012) have shown increased soil temperature alone to have limited or no direct effects on soil microbial community composition. Increased soil temperature, together with other effects induced by open-top chambers (Bokhorst *et al*., 2013, 2015) are the main interacting factors which can cause shifts in microbial community composition. Zhang *et al*. (2011) observed an increase in fungal relative abundance in soils due to enhancement of overlying plant growth, and not due to a direct effect caused by increased soil temperatures. Meanwhile, Xia *et al*. (2010) reported that vegetation cover reduced energy and rainfall input into soils, and microbial community diversity was observed to be influenced by climate change factors in association with plant functional type and soil chemistry (Hayden *et al*., 2012). Along with these observations, many studies have also demonstrated vegetation changes occurring in the Arctic in response to warming or nutrient amendments (e.g. Sturm *et al*., 2001; Epstein *et al*., 2004; Chapin *et al*., 2005; Shaver *et al*. 2001; Tape *et al*., 2006). However, these indirect plant-associated warming effects on the soil microbial community manifested

only after a considerable delay. For example, more than 10 years were required for significant responses in microbial community composition and biomass following slow changes in above-ground plant biomass and community composition (Deslippe *et al*., 2011; Rinnan *et al*. 2007a; Rinnan *et al*., 2007b). In the present study, although the experimental plots were colonized by vegetation, it is likely that a longer time period would be required for detectable changes to develop in the microbial community composition.

Changes in the soil microclimate induced by the experimental open-top chambered treatments may have resulted in microbial physiological responses, for example changes in substrate utilization patterns, that were not associated with microbial community changes. Some studies have suggested that an increase in soil temperature could trigger such changes in substrate use. For instance, Biasi *et al*. (2005) reported that a large temperature increase from 2°C to 12°C caused changes in carbon substrate utilization while not leading to any change in bacterial community composition as revealed by PLFA. In line with this, Bölscher *et al*. (2017) and Schindlbacher *et al*. (2011) found that substrate-use efficiency decreased significantly and hence metabolic activity increased with increasing temperature, with only a minimal shift in microbial community composition, and concluded that changes in microbial physiology were primarily responsible for changes in substrate-use efficiency. Some theoretical model studies have concluded that carbon utilization efficiency will vary at different soil incubation temperatures, generally decreasing with increasing temperatures (Manzoni *et al*., 2012, Sinsabaugh *et al*., 2013; Steinweg *et al*., 2008; Tucker *et al*., 2013). In agreement with this finding, Allison *et al*. (2010), Manzoni *et al*. (2012), and Tucker *et al*. (2013) reported reduced carbon allocation to microbial biomass as a result of decreased carbon-use efficiency at higher temperatures. Several studies have reported alteration in microbial carbon use in response to warming, as well as reduced soil microbial biomass (Bradford

et al., 2008; Frey *et al*., 2008; Rousk *et al*., 2012; Weedon *et al*., 2012). Schimel and Schaeffer (2012) suggested that carbon cycling could be affected by the life history strategies and functional traits of different microbes, which play critical roles in organic matter (OM) processing that are rate-limiting in overall OM breakdown. Such explanations could underlie the differences in response of various taxa towards warming and/or water amendment, as demonstrated by variation in relative abundances over time, seen in the present study.

Several studies have highlighted the importance and impacts of water amendment on field warming experiments. For instance, Guanlin *et al*. (2017) in a field manipulation experiment which ran for 18 months before soil sampling, reported that altered precipitation caused different magnitudes of decline in soil microbial biomass carbon and nitrogen when compared between warmed and unwarmed plots. Labile substrates were degraded faster in warmed plots, but only in those with increased precipitation. In the same study, Shannon diversity was reported to decline under warming and/or altered precipitation treatments, in contrast with the results of the present study. Other field warming studies have reported a lack of response to water amendment. Dennis *et al*. (2013) found no apparent effects on bacterial biomass resulting from a single application of water to maritime Antarctic soils, simulating a short-term increase in moisture availability due to melting snow and ice. Similarly, Newsham *et al*. (2010) reported that soil moisture level had minor effects on the bacterial community composition of maritime Antarctic soils. The lack of a water response in the present study could be due to the low frequency of water application, with 10 and 12 month periods between single applications. According to Illeris *et al*. (2003), altered precipitation in the Arctic had direct effects on below-ground microbial respiration, with the magnitude of responses being dependent on both the amount and frequency of precipitation events. Thus, more

frequent water application might have more significant effects on soil bacterial community than was observed here.

The data shown here are consistent with other studies that have shown Arctic soil microbial communities respond only slowly to warming treatments. In experiments at Alexandra Fiord on Ellesmere Island, Lamb *et al*. (2011) found that, despite significant changes in the above-ground plant community, bacterial taxa responsible for methane consumption or nitrous oxide cycling were unaffected by warming treatments after 16 years. Biasi *et al*. (2008), in a 2-year open-top chambered warming study of a lichen-rich dwarf shrub tundra in Siberia, reported that despite a significant increase in soil nitrogen turnover rates and changes in plant biomass, there were no major changes in below-ground microbial community composition. Schindlbacher *et al.* (2011), in a 4-year field warming of a mature spruce forest in Austria, also found that soil warming by 4°C did not affect microbial biomass, nor did it change the abundance of most microbial groups. Similar results were also reported by Fu *et al*. (2012) and Guanlin *et al*. (2017), who suggested the lack of significant changes in microbial biomass was due to the short period of experimental warming (< 3 years).

Guanlin *et al*. (2017), in a 18-month field warming experiment, found no significant changes in Shannon diversity caused by the treatments applied. However, warming and/or altered precipitation reduced the relative abundance of dominant bacterial groups, similar to the findings of the present study. Biasi *et al*. (2005) reported that Siberian tundra soils incubated at 2°C and 12°C showed no differences in PLFA composition. However, there was an increase in the abundance of Gram-positive bacteria when the incubation temperature was increased to 24°C, although these changes are most likely to be due to the drastic and environmentally unrealistic temperature alteration in the laboratory relative to the study location's mean annual temperature (-9.8°C). A more

realistic open-top chambered field warming experiment over three years (< 1 °C temperature increase) (Rinnan *et al*. 2009) reported no significant changes in soil bacterial community composition. The open-top chambers deployed in the present study increased soil temperatures by an average of 0.58°C. These different study outcomes highlight the need for care in comparing research studies into the warming effects on soil bacterial diversity.

Eighteen years of experimental warming led to a dominance of Actinobacteria and reductions in Gemmatimonadaceae and Proteobacteria in Arctic soils (Deslippe *et al*., 2012). Experiments using open-top chambers to warm High Arctic soil have also shown significant changes in the community structure and genotype richness as indicated by nitrous oxide-reducing and atmospheric nitrogen-fixing bacterial genes in soil after 13 years. However, the most pronounced effects were recorded in wet meadows (Walker *et al*., 2008), suggesting the importance of water in determining microbial responses to warming treatments. Such water-dependent effects of warming have also been observed in studies of the response of fungal communities to warming treatments in Arctic soils (Geml *et al*., 2015; Morgado *et al*., 2015).

In contrast with the long term studies discussed above, several studies have reported rapid shifts in microbial community composition after only short periods of experimental warming. For instance, Zogg *et al*. (1997) reported that, within 16 weeks, soil microbial communities shifted significantly in composition in response to experimental warming. Biasi *et al*. (2005) and Wei *et al*. (2014) found that relative abundances of Gram-negative bacteria and fungi decreased significantly whereas Gram-positive bacteria increased significantly after 12 or 6 week incubations, respectively. Likewise, Wu *et al*. (2010) reported an increasing saturation of PLFAs (categorized mainly as Gram-positive bacteria) with increasing temperature, although no change (Biasi

et al., 2008; Schindlbacher *et al*., 2011) and decreases (Zhang *et al*., 2005; Castro *et al*., 2010; Ziegler *et al*., 2013) have also been reported in other studies.

In comparison with the limited number of Antarctic studies available (Dennis *et al*., 2013; Yergeau *et al*., 2012b), Arctic studies have generally demonstrated slower responses of soil microbial communities to warming in the natural environment. This discrepancy between Antarctic and Arctic studies may be associated with the differences in plant cover between the two regions. A majority of experimental warming studies in the Arctic, including the present study, have been based on vegetated soils (Shaver *et al*., 2001; Walker *et al*., 2006b; Wookey *et al*., 2009). In contrast, plants have generally been absent in Antarctic studies (Dennis *et al*., 2013), allowing direct warming effects on soil microclimates and associated microbial processes. Ma *et al*. (2018) concluded that soil microbial responses to warming were dependent on plant communities. Warming treatments involving plant removal and undisturbed plots were found to significantly alter soil microbial community structure and other variables in different ways. As plant cover intercepts incoming radiation (Harte *et al*., 1995) and thereby reduces the energy input into soils (Xia *et al*., 2010; Wan *et al*., 2009), Arctic studies have generally demonstrated a longer lag phase for the warming effects to be manifested as compared to Antarctic studies.

5.3 Overall bacterial community composition in freeze-thaw study

The predominant bacteria taxa found in the communities studied here resemble the bacterial community composition reported from Svalbard snow samples (Larose *et al*., 2010; Amato *et al*., 2007). In both cases, Actinobacteria, Proteobacteria (alpha, beta and gamma) and Bacteroidetes were commonly-encountered taxa. On the other hand, in comparison with Ny-Ålesund soil samples Actinobacteria, Proteobacteria (alpha, beta

and gamma), Acidobacteria, Verrucomicrobia, Bacteroidetes, Planctomycetes, Chloroflexi, Gemmatimonadetes and Cyanobacteria were commonly-encountered taxa, for the only exception being Nitrospirae which contributed about 0.15% on average across all 12 Svalbard snow samples. All these are representative of the phyla reported during previous studies of Arctic soils (Ganzert *et al*., 2014; Yergeau *et al*., 2010). In terms of overall Shannon diversity of the baseline soils, Svalbard's soil samples (10.17) showed marginally higher average diversity values to those of Ny-Ålesund's control samples (10.13). This might be due to human population impacts on the town soil (cf. Wang *et al*., 2018) as compared to the more pristine soil in Ny-Ålesund.

The weak negative correlation between co-occurring bacterial genera of baseline no and high snow cover soils suggests that the two soils possessed distinct bacterial diversity, with the community of low baseline soils being intermediate, as revealed by Shannon index, Jaccard similarity and Spearman rank correlation. At the family level, *Chitinophagaceae* had high abundance, and remained as a dominant taxon throughout the nine freeze-thaw cycles and complementary controls, across all three levels of snow cover depths. This group of bacteria had been reported as one of the dominant bacterial groups on a contaminated Greenland ice sheet using a shotgun metagenomics sequencing approach (Hauptmann *et al*., 2017) and in polluted farmland soil using a culture-dependent approach (Zhang *et al*., 2012). This is again consistent with its high abundance in our study sites in the town. At a High Arctic tundra site in Svalbard, a culture-dependent approach has also isolated a novel bacterial strain (*Terrimonas arctica*) from the family *Chitinophagaceae* (Jiang *et al.,* 2014).

On the other hand, the family *Sphingomonadaceae*, and its member genera *Sphingomonas* and *Kaistobacter*, were dominant (>1%) in no and low snow cover baseline soils, and increased in abundance over the nine freeze-thaw cycles and controls, but were in low abundance (<1%) in high snow cover baseline soils. Various strains of

these genera that are known to have the ability to use contaminants as sources of nutrients have been isolated from toxin-contaminated environments (Chaudhary & Kim, 2016; Zhou *et al*., 2016). This may underlie their high abundance at this study's sampling site in the town of Longyearbyen, particularly in more exposed soils (no and low snow cover baseline soils) than in high snow cover baseline soils.

In no and low depth cover soils, exposure to multiple freeze-thaw cycles led to an increase in abundance of the genus *Janthinobacterium*, but this was not seen in high snow cover soils. In contrast, freeze-thaw exposure caused the genus *Clostridium* to increase drastically in abundance in high snow cover soils, but had no detectable effects in no and low snow cover soils. Members of the genus *Janthinobacterium* are capable of producing violacein, which provides resistance to freeze-thaw cycles, thus protecting from freezing damage (Mojib *et al.,* 2013; Pantanella *et al.,* 2007). Members of the genus *Clostridum* have also shown the ability to survive exposure in soil to up to 48 consecutive freeze-thaw cycles (Wilson *et al*., 2012).

As reported in other Arctic soil ecosystem studies (Männistö *et al*. 2007; Wallenstein *et al*. 2007; Chu *et al*., 2010), Acidobacteria and Proteobacteria were the dominant bacterial phyla in all soils in this study. These phyla also dominate in alpine tundra soils (Lipson & Schmidt, 2004; Zinger *et al*., 2009). Acidobacteria remained the dominant phylum throughout the freeze-thaw experiments in all soils, consistent with its high resilience towards freeze-thaw exposure (Männistö *et al*., 2009). The results reported here were also consistent with those of Männistö *et al*. (2013), who reported the dominance of Acidobacteria and Proteobacteria in both windswept soils (equivalent to low and medium cover soils in the current study) and snow accumulated soils (equivalent to thick cover soils). The current study's data, however, contrast with those of Fierer *et al*. (2007), who reported that *Acidobacteria* dominated especially in more oligotrophic

windswept soils which experienced larger temperature fluctuations, and copiotrophic phylotypes (e.g. Betaproteobacteria) dominated in snow-accumulated soils with higher nutrient levels.

5.4 Effects of freeze-thaw cycles on bacterial diversity

Overall, the data obtained in this study illustrated that there are likely to be different susceptibilities towards freeze-thaw stress in bacteria from soils reliably covered by different depths of snow cover. No and low snow cover baseline soils had similar bacterial community composition at genus level. Conversely, high snow cover baseline soils had significantly distinct bacterial diversity from no baseline and low baseline soils. Soil chemistry (organic carbon, total nitrogen, ammonium and phosphorus) was not significantly different among the samples. This suggested that snow cover depth could possibly be a factor driving the different diversity observed among soil groups. Similarly, Aanderud *et al*. (2013) revealed significant legacy impacts of winter snow depth on bacterial richness in the growing season at a mid-latitude study site, and communities in soils with shallow (ambient) or no snow cover (snow removal) were more closely clustered in comparison with soils that underwent snow addition. Zinger *et al*. (2009) also reported that soil microbial community composition was different at two extremes of the snow cover gradient, with strong spatial and temporal correlation between snow dynamics and the microbial community. Similar snow-covered habitats to those examined here are known to harbour large microbial communities (Hauptmann, 2014), and the duration and depth of snow cover is known to have dramatic impacts on Arctic and alpine ecosystem structure and functioning (Edwards *et al*., 2007; Fisk *et al*., 1998; Welker *et al*., 2000).

Previous work has shown that effects of freeze-thaw cycles on soil microbial communities can range from mild to detrimental (Edwards *et al*., 2007; Ren *et al*., 2018; Sawicka *et al*., 2010; Walker *et al*., 2006a). The Shannon index values for bacterial diversity of baseline soils, particularly in no and low snow cover soils, were significantly higher than those measured after exposure to 1st FT Cycle and 9th Cycle in both treatment and control groups. This finding is consistent with previous freeze-thaw studies (Larsen *et al*., 2002; Schimel & Clein, 1996; Stres *et al*., 2010; Wilson *et al*., 2012) which have reported significant decreases in soil microbial abundance, diversity or activities due to freeze-thaw treatments. The bacterial community in high snow cover soils, in contrast, demonstrated only a marginal decrease in bacterial diversity after freeze-thaw treatment.

The data obtained here suggest that factors other than the historical environmental exposure of the microbial community can control its response to environmental perturbations. For example, the cold adaptation strategies (e.g. production of cold-induced proteins and cold-associated general stress-responsive proteins) developed by the high snow cover soil microbiome as a result of exposure to consistently (but not extremely) cold microclimates were probably the main contributing factor to this community's high resilience towards FT exposure. Adaptations to cold conditions include increased membrane fluidity (Kumar *et al*., 2002), synthesis of cold-adapted enzymes (Groudieva *et al*., 2004) and the production of cold-induced proteins including cold-shock, antifreeze and ice-nucleation proteins (Chong *et al*., 2000; Nemecek-Marshall *et al*., 1993; Sun *et al*., 1995). De Bruijn (2016) reported up to 11 types of cold-induced and cold-associated general stress-response proteins produced by the bacteria of Arctic snow pack. Among these proteins, cryoprotectants, antifreeze proteins, ice-nucleation proteins, and cold-shock proteins (Goldstein *et al*., 1990; Jones *et al*., 1987; Xu *et al*., 1998) are known to contribute to low-temperature survival and protect microbial cells from freeze-induced injury.

In this experimental study, in low and high snow cover baseline soils, both freeze-thaw-treated and thawing controls showed significant declines in diversity, with no significant difference between the freeze-thaw-treated and control responses. In contrast, no snow cover soil showed a significantly different response between freeze-thaw-treated and control treatments, particularly after the 9th cycle, with freeze-thaw-treated soil showing an increase in Shannon diversity, while the control showed a significant decline in diversity. This may suggest that adaptations towards freeze-thaw stress are shaped by local climate, as the soils obtained from sites subjected to harsher and more variable environmental conditions appeared to show signals of recovery.

Shannon diversity index values of all baseline soil groups in the current study (high, 9.56 ± 0.15; low, 10.41 ± 0.12 and no, 10.55 ± 0.17) were generally higher than reported in previous studies on Arctic tundra soil (± 0.29, McCann *et al*., 2016; 7.4 – 8.0, Shi *et al*., 2015a; ±7.5, Koyama *et al*., 2014; 4.8-6.0, Neufeld & Mohn, 2005; 6.4 – 7.2, Steven *et al*., 2013). The higher diversity observed in this study is possibly due to the greater human impact of its location within the town of Longyearbyen as noted above, whereas the previous studies took place at more pristine locations, especially those from the polar deserts (McCann *et al*., 2016; Steven *et al*., 2013) and undisturbed Arctic tundra sites and boreal forest sites (Neufeld & Mohn, 2005). High snow cover baseline soils had the lowest diversity, possibly a consequence of the more constant and less extreme environmental conditions experienced in these than in the no and low snow cover soils.

High snow cover soils, which would have a more stable soil microclimate due to the thicker snow cover than the low cover and no snow cover soils, demonstrated the smallest changes in alpha diversity when exposed to experimental freeze-thaw cycles as compared to no and low snow cover soils. This contrasts with data presented by Waldrop and Firestone (2006), who noted that bacterial communities from soils with more stable

microclimates tended to be more sensitive to environmental perturbations, and that microbial communities which had experienced large and frequent temperature fluctuations were hardly responsive to small temperature changes. Similar findings were also reported by Balser and Firestone (2005), Fierer and Schimel (2002) and Schimel and Gulledge (1998) in studies of the impacts of changes in soil abiotic variables (e.g. soil temperature and moisture content) on microbial community composition. These studies suggested that soil microbial communities were acclimatised to the local environmental conditions they typically experience, and only environmental perturbations which were beyond this typical envelope would trigger changes in community composition and function. The no snow cover soil microbial community showed signs of recovery after exposure to nine experimental freeze-thaw cycles which were not apparent in low snow cover soil communities. This could possibly be due to the the presence of some taxa which are freeze-thaw resistant. These bacteria, when exposed to cumulative freeze-thaw cycles, may then increase in relative abundance throughout the experiment. Examples of such bacterial taxa in the current study could include *Pseudomonas* and *Oxalobacteraceae*, whose initial relative abundance of less than 1% increased by more than an order of magnitude after exposure to nine freeze-thaw cycles. Representatives of these two bacterial taxa have also been recovered after extensive laboratory freeze-thaw selection (Wilson *et al*., 2012). Previous studies have suggested that decreased snow cover advanced the timings of soil freezing and melting, and thus increased frequency of freeze-thaw cycles and daily soil temperature variations (Hardy *et al*., 2001; Groffman *et al*., 2011; Tan *et al*., 2014). Indeed, the more frequent freeze-thaw events experienced by no snow cover soils in the natural state would select for phylotypes which can produce freeze-thaw -induced proteins (Sharma *et al*., 2006; Walker *et al*., 2006a).

5.5 Discrepancies between studies

Differences in experimental designs and protocols may underlie some of the contrasting results reported in different studies. For example, to provide a representative sample, different soil homogenization methods have been deployed in various studies. Thomson *et al*. (2010) reported that different homogenization methods including soil sieving, drying and rewetting significantly altered the bacterial community structure and dominant bacterial taxon abundance subsequently described, as reflected by molecular fingerprinting (T-RFLP) of bacterial communities. Similarly, sieving (Hartley *et al*., 2007; Petersen & Klug, 1994), drying and rewetting (Fierer & Schimel, 2002; Franzluebbers, 1999; Griffiths *et al*., 2003) were reported to affect microbial community structure and function. These studies set out to elucidate specific microbial community and/or functional dynamics rather than providing a more comprehensive assessment of the impacts of environmental fluctuations, including global climate trends. In contrast, the current study aimed to clarify the influence of climatic warming on the soil microbial community by utilizing a field-based open-top chamber approach, without involving soil disturbance or plant removal.

Another methodological difference which could plausibly account for discrepancies between field warming studies could be differences in sampling time leading to varying exposure time to experimental treatments. This can be particularly important during growing seasons when microbial biomass can fluctuate rapidly (Lipson *et al*., 1999). Ma *et al*. (2018) reported that, in an alpine shrub ecosystem, changes in microbial community structure were evident between seasons. Thus, sampling period could be an important factor causing differences in warming results among studies.

Furthermore, different temperature sensitivities and thermal acclimation of individual microbial species in different environments (Malcolm *et al*., 2008; Crowther

et al., 2013; Frey *et al*., 2008; Balser & Wixon, 2009) could also lead to different responses in both open-top chamber and freeze-thaw studies. According to Burns *et al*. (2013) and Henry (2013), soil microbial community functioning, assessed by enzyme activities in relation to nutrient cycling, was temperature-dependent. Consistent with this finding, enzyme activities have been reported to be closely related to microbial community structure (Waldrop *et al*., 2000; Kourtev *et al*., 2002; Snajdr *et al*., 2013; Weand *et al*., 2010; Wei *et al*., 2014). Thus microbiomes in different environments might respond differently to environmental stress. Carbon-use patterns and efficiencies can also differ between microbial groups (Allison *et al*., 2010; Schindlbacher *et al*., 2011, Manzoni *et al*., 2012), and thermal adaptation of microbial processes which are related to microbial community structure have also been reported (Bradford *et al*., 2008; Balser & Wixon, 2009; Allison *et al*., 2010; Conant *et al*., 2011; Bradford, 2013). Such factors can all contribute to the inconsistencies reported among studies.

A further potential contributing factor to the lack of consensus among studies is that of different climatic histories, which might lead to variability of responses among soil microbial groups towards the same magnitude of experimental warming (Wu *et al*., 2010). This could apply even within the same taxonomic group due to geographical or ecological scale (Waide *et al*., 1999; Gross *et al*., 2000). A similar interpretation was also reported by Whitby & Madritch (2013) and Davidson & Janssens (2006), that the native temperature regimes of soils influenced soil respiration, such that soils from colder native climates showed higher soil respiration than did soils from warmer native climates when both were incubated under projected warming. Furthermore, Wei *et al*. (2014) reported different shifts in microbial community structure and temperature sensitivity of SOM decomposition in an experimental warming study comparing soils pre-incubated under different temperatures.

CHAPTER 6: CONCLUSIONS

This study focused on investigating the effects of climatic change on soil bacterial diversity in the High Arctic, a region which is expected to continue warming more quickly than other regions on Earth at lower latitudes. Two main elements or consequences of climatic warming were addressed, (1) general soil warming and its interaction with water availability, examined over a winter and two summer seasons using an open-top chamber (OTC) field manipulation experiment, and (2) snow cover depth and its interaction with freeze-thaw (FT) events, considered in both field and laboratory scenarios. The data obtained extend the limited previous studies of the influence of these factors on Arctic soil microbial communities specifically, and the wider literature on the impacts of climatic warming on High Arctic terrestrial soils. The detailed study of 16S rRNA (V3-V4 regions) gene amplicon sequencing using high-throughput Illumina MiSeq platform revealed a diverse and distinct landscape of bacterial communities from the distinct terrestrial soils sampled in this study of the data obtained indicated more than 90% coverage (indicated by Good's Coverage) of bacterial community diversity in both warming and freeze-thaw studies.

The study suggested significant differences in bacterial community diversity in soils covered by different snow depths (a proxy for both the duration of snow cover, and the environmental condtions experienced over the year). The study also generated evidence of different susceptibility of these communities towards freeze-thaw stress. The soil bacterial community under continuous thick snow cover appeared to be relatively insensitive towards freeze-thaw stress, as demonstrated by the maginally significant changes in alpha diversity (Shannon index) during experimental exposure to repeated freeze-thaw cycles. Soils with less or no snow cover demonstrated a contrasting response, with a significant decline in community diversity on exposure to freeze-thaw cycles.

Winter snow cover depth is therefore likely to be a key determinant of the susceptibility of the soil bacterial community towards temperature fluctuations.

The current freeze-thaw study gives new insights on the potential influence of snow depth cover on the response and resilience of the soil bacterial community towards exposure to repeated freeze-thaw cycles. In summary, the current study found no effects of short-term warming and water amendment on the bacterial community composition of soils sampled from Svalbard. Further studies, over longer timeframes than those employed here, will be required to deduce the long-term seasonal effects and treatment effects of warming and water amendment on the composition of soil bacterial communities on Svalbard. In addition, increased replication of all experimental groups, as well as sampling at different depths of snow bank before thawing period is also recommended for future studies to further elucidate the impacts of repeated freeze-thaw cycles on High Arctic soil bacterial communities of different snow depths. With more holistic sampling approaches, larger coverage of sampling area and in-depth planning for more collaborative research work in the Arctic, stronger and more robusts conclusions can be drawn in future research studies.

In the open-top chamber field experimental manipulation, inter-seasonal variation (early and late summer 2015, late summer 2015 and 2016) appeared to be a significant element of variation in bacterial community diversity. Neither warming nor water addition led to significant changes in bacterial community diversity over the timescale studied in this experiment. Nonetheless, longer-term study is required to draw robust conclusions about the impacts of open-top chambered warming on High Arctic soil bacterial diversity. Moreover, as there is poor documentation of natural interannual variability on the soil bacterial diversity, good controls and more longer-term studies are required in future studies to provide more robust conclusions on the effects of such

environmental manipulation studies. In addition, other than microbial community diversity, other aspects of studies for instance, the soil microbial respiration, microbial biomass, soil nutrient dynamics and associated vegetation changes are also worth to be investigated. This would contribute to a more holistic insights of the effects of such field warming studies.

Both open-top chamber and freeze-thaw studies revealed relatively strong correlations among the co-occurring genera present in samples obtained under different treatments, even though there were significant differences in Shannon diversity. The approach used in this study was DNA-based, relying on the short region of 16S rRNA genes, which restricted identification of actively-functioning bacteria. Future studies targeting other genes to explore the active bacterial community diversity, and also their roles in different environments, will make a very valuable contribution to this research area.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

A Publications

Lim, P. P. J., Newsham, K. K., Convey, P., Gan, H. M., Yew, W. C., & Tan, G. Y. A. (2018). Effects of field warming on a High Arctic soil bacterial community: a metagenomic analysis. *Current Science*, *115*(9), 1697-1700.

B Presentations

Effect of Freeze Thaw Cycles on Bacterial Diversity in Polar and Tropical Soils (Poster). Presented at The Scientific Committee on Antarctic Research, 20-30 Aug 2016, Kuala Lumpur Convention Centre (International)

Rigorous Selection of Ice-Nucleating Bacteria by Freeze-Thaw Cycles (Poster). Presented at the International Congress of the Malaysian Society for Microbiology, 7-10 Dec 2015, Bayview Beach Resort, Penang (International)