MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE WITHIN EQUATORIAL LAKES OLBOLOSAT AND OLOIDEN IN KENYA

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Microbial Diversity and Community Structure within Equatorial Lakes Olbolosat and Oloiden in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my entire family; My beloved husband Dr. Robert Nesta and our lovely children Osteen Frank, Angela Sifa and Isaac Kena. My beloved mother Perister Kiama and late father Isaac Kiama for believing in me since I was a young child. You made me stronger, better and more fulfilled than I could have ever imagined. I also appreciate my brothers and sisters, Adrian Jeff and his family for the support you accorded me during the course of my studies. Sandra and Esther! you came when I needed you the most. The journey was tough but through your constant prayers, great moral support, encouragement and patience everything was made possible. Glory be to God in the highest.

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ABBREVIATIONS AND ACRONYMS

ACE	Abundance- Based Coverage Estimator
ALDEX2	Anova-Like Differential Expression 2
ANOSIM	Analysis of Similarity
ASVs	Amplicon Sequence Variants
BLASTn	Basic Local Alignment Search Tool (Nucleotide)
CAP	Constrained Analysis of Principal Coordinates
CFUs	Colony Forming Units
DADA2	Divisive Amplicon Denoising Algorithm 2
dbRDA	Distance Based Redundancy Analysis
DNA	Deoxyribonucleic Acid
GPS	Global Positioning System
ITS	Internal Transcribed Spacer
KWS	Kenya Wildlife Service
NACOSTI	National Commission for Science, Technology and Innovation
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
OTU's	Operational Taxonomic Units
PCA	Principal Component Analysis

PCR	Polymerase Chain Reaction
PERMANOVA	Permutation Multivariate Analysis of Variance
pH	Potential of Hydrogen
QIIME	Quantitative Insights into Microbial Ecology
RNA	Ribonucleic Acid

ABSTRACT

Lakes Olbolosat and Oloiden which lies along the equator are economically and ecologically important ecosystems due to their high productivity and nutrient recycling capacities. Microbes are important in the functioning and stability of lake ecosystems. Due to the complexity of the culture-dependent techniques, only a limited number of microbes have been extensively studied. Metagenomics therefore, provides detailed information on the studies of microbial communities for microbes that cannot be isolated using culture-dependent technique. This is the first comprehensive study to study microbial diversity and community structure within Equatorial lakes Olbolosat and Oloiden in Kenya. Culture-dependent technique targeted 16S rRNA gene for the identification of bacterial isolates with lakes Olbolosat and Oloiden. High throughput sequencing was performed on 15 samples obtained from the two lakes using the Illumina Miseq platform. Four different Phyla: Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes were recovered using a culture-dependent technique. Firmicutes were predominant within the two lakes. There were 17 and 10 phyla recovered using the culture-independent for the bacteria and fungi respectively. The abundant phyla included Proteobacteria (33.8 %), Firmicutes (27.3 %), Actinobacteriota (21.2 %), Chloroflexi (6.8 %), Cyanobacteria (3.8 %), Acidobacteriota (2.8 %), Planctomycetota (1.9 %) and Bacteroidota (1.1 %). Analysis of similarity (ANOSIM) revealed a significant difference in ASV composition between the two lakes ($r = 0.191$, $p = 0.048$), and between the sample types ($r = 0.6667$, $p = 0.001$). The analysis of the ITS ASVs comprised of the fungi kingdom (73.1 %) and Plantae kingdom (28.7 %), the microalgae photobionts. Among the ten highly abundant phyla, two (Chlorophyta; 56.87 % and Anthophyta; 1.2 %) belonged to kingdom Plantae, while eight (Mortierellomycota; 17.8%, Basidiomycota; 11.8 %, Chytridiomycota; 5.8 %, Monoblepharomycota; 3.4 %, Ascomycota; 2.4 %, Olpidiomycota; 0.5 %, Mucoromycota; 0.1 %, and Glomeromycota; 0.1 %) belonged to kingdom fungi. Chlorophyta (56.87 %) was the predominant phyla across the samples except for the dry sediments in both lakes that were dominated by phylum Mortierellomycota. An evaluation of the indicator ASVs correlation at the phylum level revealed a positive co-occurrence pattern among the genera of the ten major phyla for both bacteria and fungi. The interaction network for the bacterial and fungal communities within the two lakes displayed the predominant phyla to be highly positively connected with other microbes. The findings show that the microbial communities were influenced by environmental factors within the tow lakes. Lake Olbolosat a fresh water body harbored a high number of microbial taxa as compared to Lake Oloiden which is a saline-alkaline water body. Targeted bacterial and fungal isolation should be carried out to recover, characterize and identify the recovered novel microbial taxa revealed through next-generation sequencing. Novel bacteria recovered from this study could provide insights into their diversity and biotechnological applications.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Lake Olbolosat is a freshwater ecosystem formed by down-warping and it is among the lakes in Kenya outside the rift valley. Lake Oloiden is a satellite saline-alkaline of L. Naivasha (Maina *et al.*, 2018). Saline-alkaline and freshwater lakes are economically and ecologically important ecosystems due to their high productivity and nutrient recycling capacities (Krivtsov *et al*., 2020). Studies in Kenya have so much focused on salty lakes like Lake Magadi, Lake Elementaita, Lake Nakuru. Lakes Olbolosat and Oloiden are ecosystems that can serve as models for studying microbial diversity. Microbes within these ecosystems are essential to the functioning and major biogeochemical cycles (Krivtsov *et al*., 2020). The input of nutrients and fast recycling is due to active anaerobes and aerobes microorganisms (Yadav *et al*., 2019).

Microbial communities have been mostly studied using culture-dependent techniques and due to the uncultivability of most microbes, very few organisms can be isolated from these lakes. However, the culture-dependent techniques helps in better understanding microbial physiology for industrial application (Spini *et al*., 2018). Culture-dependent techniques studies from Lake Oloiden of the Kenyan Rift Valley revealed the presence of diverse populations of high G+C content belonging to the genus *Artrobacter, Dietzia* and *Terrabacter* (Duckworth *et al*., 1998). Bacteria of the genera, *Pseudomonas Paenibacillus*, *Arthrobacter*, *Bacillus*, *Fictibacillus* and *Acinetobacter*, have been isolated from L.Olbolosat (Wafula & Murunga, 2020). Fungi has been recovered from Kenyan lakes using next generation sequencing that include Ascomycota, Basidiomycota, Chytridiomycota and Glomeromycota (Salano *et al*., 2017). The culture-dependent techniques cannot be used solely for the analysis of populations within microbial communities. High-throughput sequencing is used widely to study the diversity and structure of microbial communities. Taxonomic analysis of prokaryotes and eukaryotes is regularly performed using Illumina sequencing (Oulas *et al*., 2015). Prokaryotes and eukaryotes are unique microbial communities that are important in the functioning of a

lake ecosystem (Silveira *et al*., 2020). These microbes play an important role in the decomposition of organic material into nutrients taken as food by other organisms and controlling the quality of water in the lake (Zhang *et al*., 2019). They are also important in balancing between respiration and photosynthesis in the natural cycles of oxygen, carbon, sulfur and metals (Silveira *et al*., 2020). Numerous studies of prokaryotic and eukaryotic communities have been done on different natural habitats in Kenya but despite this, exploitable microbial diversity is not exhaustive, and microorganisms represent the largest reservoir of untapped biodiversity (Kambura *et al*., 2016; Ghilamicael *et al*., 2017; Salano *et al.,* 2017). Studies on microbial communities within lakes ecosystems are important in acquiring knowledge about the prokaryotes and eukaryotes. The importance include diversity, composition of microbial genetic resources, patterns of microorganisms, the functional role of microbial diversity and the regulation of microbial diversity (Yang *et al*., 2016). Marker gene sequencing is fast and obtains community or taxonomic distribution profile or fingerprinting using PCR amplification and sequencing of evolutionarily conserved marker genes. Change in global biodiversity patterns is expected to drive the distribution and abundance of species in ways that are central to conservation efforts, food production, disease management and functioning of the ecosystem (Shoemaker *et al.,* 2017). Marker gene sequencing is fast and obtains community/ taxonomic distribution profile or fingerprinting using PCR amplification and sequencing of evolutionarily conserved marker genes. The taxonomic distribution can be associated with metadata derived from the sampling sites under investigation (Paul *et al.,* 2016).

1.2 Statement of the problem

Lakes Olbolosat and Oloiden are ecosystems that can serve as models for studying microbial communities. Extensive studies on microbial communities' functions within lakes have been done majorly on salty lakes and a few on fresh and saline-alkaline in Kenya. A large range of abiotic and biotic factors influences microbial communities which affect their diversity, abundance, composition, distribution, processes and existence within these ecosystems. Due to the complexity of the culture-dependent techniques, only a limited number of microbes have been extensively studied. This has been done through isolation, characterization and identification according to their

morphological, biochemical and genetic characteristics. No comprehensive studies have been performed on prokaryotic and eukaryotic communities using high throughput sequencing technology from lakes Olbolosat and Oloiden. This study involved the application of both culture-dependent and independent techniques to determine the community diversity and structure within samples collected from lakes Olbolosat and Oloiden in Kenya.

1.3 Justification of the study

The earth depends on biodiversity for it to sustain life. Inland water lakes are ecosystems that provide biodiversity that is economically and ecologically important due to high productivity and nutrient recycling capacities. Microorganisms play a critical role in the lake ecosystem's functioning since they are the main drivers of the biogeochemical cycling of elements. Without microbial decomposers, minerals, and nutrients important to plant and animal growth would not be made available to support other levels of the aquatic food chain. Culture-dependent technique is important in the understanding of microbial physiology for industrial application. However, this technique cannot be used solely for the analysis of microbial communities. Due to the uncultivability of most microbes, very few organisms have been isolated. Metagenomics therefore, provides detailed information on the studies of microbial communities for microbes that cannot be isolated using culture-dependent technique. The application of the culture-independent technique in the current study was expected to provide insights and significant advances in research on the diversity and community structure of prokaryotes and fungi within Lakes Olbolosat and Oloiden. The study also forms a good basis for the use of the isolated novel isolates which could be used for biotechnological applications.

1.4 Research questions

1. How diverse are bacteria within lakes Olbolosat and Oloiden as evidenced by culture-dependent techniques?
2. Is there any relationship between bacterial diversity and community structure and environmental factors as evidenced by culture-independent techniques?
3. Is there any relationship between fungal diversity and community structure and environmental factors as evidenced by culture-independent techniques?

1.5 General objective

To study microbial diversity and community structure within Equatorial lakes Olbolosat and Oloiden in Kenya.

1.6 Specific objectives

1. To isolate, characterize and identify bacterial diversity using culture-dependent techniques.
2. To determine bacterial diversity and community structure and their relationships with environmental factors using culture-independent techniques.
3. To determine fungal diversity and community structure and their relationships with environmental factors using culture-independent techniques.

1.7 Hypothesis

Lakes Olbolosat and Oloiden are not reservoirs of microbial diversity and community structure.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbial ecology

The earth depends on biodiversity for it to sustain life. Microorganisms are the most abundant, diverse, and functionally important organisms on earth (Coyte *et al*., 2015). Microbial lineages are separated by many millions of years of evolutionary time (Coyte *et al*., 2015). The domain bacteria are estimated to be approximately 3.5 billion years old, more than thirty times older than the ancestors of all birds (Barberán *et al*., 2014). Microbiologists use phylogenetic methods to determine where most of the biological diversity accumulates and how it is intrinsically structured. They also determine how phylogenetic community similarity is distributed along environmental gradients (Barberán *et al*., 2014).

Bacteria and fungi in the soil having high taxonomic diversity tend to be less phylogenetically diverse, than other habitats such as marine sediments and that, salinity is the main driver of the phylogenetic community patterns at the global scale. Bacteria and fungi are recognized as important agents in nutrient cycling (Guo *et al*., 2020). They also release inorganic matter through the decomposition of organic matter, thereby recycling nutrients to the phytoplankton (Guo *et al*., 2020). Salinity, temperature, nutrients, and numerous other environmental factors have been suggested to influence microbial diversity. Understanding how microbial communities shift in a changing environment allows one to predict the presence of particular microbial processes or the species composition (Sorenson & Drummond, 2008). A central goal of ecology is to understand the patterns and processes of biodiversity. In particular, community ecologists describe species richness at local scales (alpha diversity), differences in diversity across space and time (beta diversity) and diversity within a region (gamma diversity)(Qian & Akçay, 2020). Studies of bacteria and fungi have disclosed that marine bacterial and fungal populations are complex, widespread and often consist of unidentified or uncultivated members (Gilbert *et al*., 2012).

2.2 Bacterial communities

Studies on prokaryotic communities within tropical lakes are important in acquiring knowledge about the bacterial/ archaeal diversity and composition of microbial genetic resources, patterns of microorganisms, their functional roles and the regulation of microbial diversity (Yang *et al.*, 2016). Prokaryotes play an important role in the decomposition of organic material into nutrients taken as food by other organisms and controlling the quality of water in the lake (Zhang *et al.*, 2019). They are also important in balancing between respiration and photosynthesis in the natural cycles of oxygen, carbon, sulfur and metals (Silveira *et al.*, 2020). However, a large range of abiotic and biotic factors influences prokaryotic communities which affect their diversity, abundance, composition, distribution, processes and existence within these ecosystems. Environmental factors have been reported to promote the diversity of microorganisms. Tropical lakes exhibit considerable variation in their hydrogeology due to climate change. These lakes experience phases of being flooded with freshwater to salinity or even dried out caused by evaporation and anthropogenic activities (Luo *et al.*, 2017). Prokaryotic communities obtained from water, mats, wet and dry sediments play a very important role within saline-alkaline lakes. However, due to the complexity of the culture-dependent techniques, only a limited number of prokaryotes have been extensively studied (Kambura *et al.*, 2016).

Extensive studies on Lake Magadi have revealed various extreme alkaliphilic, moderately halophilic and benthic cyanobacteria. They were identified, by morphology to be *Synechocystis salina, Aphanothece stagnina, Chamaesiphon sublobosus, Rhabdoderma lineare, Synechococcus elongates, Phormidium ambiguum, Phormidium foveolarum, Phormidium retzii, Oscillatoria limnetica*, *Spirulina fusiformisans* and *Spirulina laxassima* (Dubinin *et al*., 1995). In 2004, eight new strains of denitrifying bacteria were found in a lagoon with a pH of (Boltyanskaya *et al*., 2004). Studies on the low saline lakes of the Kenyan Rift Valley revealed the presence of diverse populations of aerobic sulfur-oxidizing bacteria of genera *Thioalkali microbium* and *Thioalkoli vibrio* (Sorokin *et al*., 2007). The groups with relative abundance at phylum level from Lake Magadi were also identified; *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, Chloroflexi* and *Deinococcus-thermus* (Kambura *et al*., 2016). In 1996,

Thermosyntropha lipolytica anaerobic alkalithermophiles were isolated and identified from Lake Bogoria (Svetlitshny *et al*., 1996). Organotrophic bacteria of the phylum *Actinobacteria*, namely *Bogoriella caseilytica* and *Cellulomonas bogoriensis* have been isolated and identified from Lake Bogoria (Jones & Grant, 1999). Bacteria of the phylum *Firmicutes, Spirochetes, Actinobacteria, Planctomyces, Cyanobacteria, Bacteroidetes, Chloroflexi* and *Chlorobi* have been also isolated and identified from Lake Elementaita (Mwirichia *et al*., 2011). Isolation was done and novel genera and species of bacteria known as *Methylohalomonas lacus* were identified (Vavourakis *et al*., 2018). *Arthrobacter* and *Terrabacter* genus have been isolated and identified from Lake Oloiden, Kenya (Luo *et al*., 2017).

2.3 Fungal communities

Fungi are eukaryotic organisms that are highly versatile due to their physiological adaptations. Fungal communities are essential components of global nutrient cycles, where they transform inorganic nutrients and release nutrients through the decomposition of organic materials and recycling of carbon, nitrogen and phosphorus (Wahl *et al.,* 2018). They also play a key role in plant life in the form of mycorrhizae and endophytes. Fungal taxonomy undergoes major changes in its classification. The early classification is divided into four main phyla; Zygomycota, Chytridiomycota, Basidiomycota and Ascomycota (Alexopoulos & Mims, 1996). A comparison of lakes in Kenya has earlier revealed the existence of fungal communities (Kambura *et al.,* 2016; Salano *et al.,* 2017). Other studies have provided further evidence that fungal communities occur in different regions globally within the lake ecosystems (Gonçalves *et al.,* 2012; Wahl *et al.,* 2018).
Chlorophytes (photobionts) are important primary producers in humid terrestrial and aquatic ecosystems. They are however used as biological indicators in water monitoring and ecological studies. Photobiont studies have recently gained interest in biotechnological applications such as the production of chemicals, fuels, food and animal feed (Hadi *et al.,* 2016). Lichens are symbiotic eukaryotic organisms of fungi and photoautotrophic partners of algae and/or cyanobacteria (Voytsekhovich & Beck, 2016). Lichens are not single organisms like higher plants, rather they coexist inform of either- a fungus, commonly as an ascomycete (the mycobiont) and a photosynthesizing organism (the photobiont) which can either be a cyanobacterium or a eukaryotic green alga, usually a member of the genus *Trebouxia* (Voytsekhovich & Beck, 2016). Mycobiont and

photobiont are closely related to the coevolution of both components of a lichen association. Studies have reported a level of correlation between mycobionts and photobionts at the level of genera, families, orders and classes in lichen-forming fungi (Hadi *et al.,* 2016).

Most fungi grow at neutral, slightly acidic, or high pH. The phylum *Acrimonies, Scopulariopsis, Verticilium, Fusarium* and *Paecilomyces* phylum were isolated and identified from Lake Sonachi in Kenya (Ireri *et al*., 2015). Fungi belonging to the genus *Penicillium, Aspergillus, Polyzellus, Fusarium* and *Neurospora* have been isolated and identified from Lake Magadi (Salano *et al*., 2017). The groups with relative abundance at phylum level from Little Magadi were identified; *Ascomycota, Basidiomaycota, Chytridiomycota* and *Glomeromycota* (Kambura *et al.,* 2016). A study to systematically characterize the alkaliphilic tolerant filamentous fungi isolated from alkaline (soda) soils resulted in the isolation of two new obligate alkaliphilic species of *Sodiomyces* from Lake Magadi (Grum-grzhimaylo *et al*., 2015). Understanding the evolution and classification of fungi is one of the major topics for research in mycology (Wijayawardene *et al.,* 2018). More recent studies by Tedersoo *et al.* (2018) have generated a vast amount of taxonomic communities using high throughput sequencing in the classification of fungi. This has led to the adoption of a new rank accommodating this phylum: Basidiobolomycota, Calcarisporiellomycota, Mortierellomycota, Glomeromycota, Olpidiomycota. Phelidiomycota, Entomophthoromycota, Entorrhizomycota, Kickxellomycota and Monoblepharomycota. Other studies on classification by Wijayawardene *et al.* (2018) have accommodated Basidiobolomycota, Blastocladiomycota, Mortierellomycota, Calcarisporiellomycota, Caulochytriomycota, Chytridiomycota, Entomophthoromycota, Glomeromycota, Kickxellomy-cota, Monoblepharomycota, Aphelidiomycota, Mucoromycota, Neocallimastigomycota, Zoopagomycota, Rozellomycota and Olpidiomycota fungal clades. The Internal transcribed spacer (ITS) is a powerful region in the identification of fungi and photobionts in diverse and complex environmental samples such as sediments, microbial mats and water (Hadi *et al*., 2016). Metagenomics provides the identification of photobionts rapidly and consistently regardless of their life stages.

2.4 Biotechnological and industrial potential of microbes from lakes

Microorganisms especially haloalkaliphilic use several different types of organic solutes such as osmolytes. Ectoine is one of the osmolytes accumulated intracellularly by soda lake microorganisms such as methylotrophs (Antony *et al*., 2013) and sulfur-oxidizing bacteria (Sorokin & Muyzer, 2010). Ectoine has many biotechnologically relevant applications, especially in molecular biology, cosmetics and therapeutics (Lanzen *et al*., 2013). Haloalkaliphiles are thought to have significant economic potential because their enzymes are already used in detergent compositions, leather tanning and other industries. Therefore, extremophiles are foreseen to find applications in the food, waste treatment and textile industries (Hadi *et al*., 2016).

Many enzymes produced by microorganisms have relevant biotechnological applications in several industrial areas. Microbes are the preferred source of industrial enzymes because of their excellent capacity for extracellular protein production (Schaffner & Toledo, 1991). Among a large number of non-pathogenic microorganisms capable of producing useful enzymes, bacteria and filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential. These enzymes are applied in the industrialization of detergents, starch, drinks, food, textile, animal feed, baking, pulp and paper, leather, and chemical and biomedical products. The use of starch degrading enzymes was the first large-scale application of microbial enzymes in the food industry (Bennett, 1998). Amylases have applications in food, detergents, drinks, animal feed and baking (Kasana & Pandey, 2018). Gram-positive cellulose-producing bacteria have been obtained from Kenyan Soda Lakes. These microbes have been used extensively in laundry processes and textile industries (Mwirichia *et al*., 2010). Lipases and alkaline cellulases are used to manufacture detergents (Jones, 1994). Lipases and alkaline cellulases are also used in the manufacture of food ingredients and pitch control in the pulp and paper industry (Mattiasson *et al*., 2004). Cellulase enzyme-producing isolate was recovered from Lake Bogoria and used to make soft, stone-washed and bleached jeans (Lee *et al*., 2008; Aygan & Arikan, 2008). Puradax, a cellulase used for rooting out difficult stains and reducing spills on fabrics is a critical ingredient in the manufacture of Tide Alternative Bleach detergent, whose origin is from a microbe cultured from Lake Bogoria, Kenya (Lee *et al.*,2008).

2.5 Culture-dependent and culture-independent techniques

Saline and alkaline lakes in Kenya have been studied using both culture-dependent and culture-independent techniques (Kambuara *et al.,* 2016). Culture-dependent technique involves the isolation of microbial isolates from samples using culture media. These techniques are useful in understanding the physiological potential of isolated organisms. However, the culture-dependent approach does not provide comprehensive information on the composition of microbial communities it is still used as a complement to culture-independent methods. Culture-independent methods rely on the use of DNA-based molecular community analysis to describe the diversity of a particular sample from the environment (Kambuara *et al.,* 2016). The culture-dependent technique is the traditional approach used in microbiology. It involves the generation of isolates from the environmental samples by growth on substrates that mimic the growth conditions of the natural habitat of the target organisms. This method cannot favor the growth of every microorganism using the standard cultivation methods (Kambuara *et al.,* 2016). A metabolically active component of a niche may be recalcitrant to culturing for myriad reasons including an inadequate supply of nutrients, the presence of inhibitory compounds in the growth medium or accumulation of waste products from its metabolism, or because of an intrinsic slow growth rate (Simon & Daniel, 2009). Higher sequencing depth enables higher sensitivity (down to 1%) higher discovery power, higher mutation resolution, more data produced with the same amount of input DNA and higher sample throughput. There are several disadvantages to using next-generation sequencing (Huang *et al*., 2017). Next-generation sequencing requires sophisticated bioinformatics systems, fast data processing and large data storage capabilities, which can be costly. Although many institutions may have ability to purchase next-generation sequencing equipment, many lack the computational resources and staffing to analyze and clinically interpret the data (Meddeb *et al*., 2016). Culture-independent is the use of methods that are not based on cultivation to study microorganisms in a specific ecosystem. Undoubtedly, culture-independent methods offer several advantages over culture-dependent methods (Oulas *et al*., 2015). Microorganisms are studied not because they can grow on a specific microbiological medium, but because they possess DNA, RNA and proteins, which are the preferred targets for such approaches (Panzer *et al.,* 2015). Moreover, the physiological status of the microbial cell does not affect the outcome of

the investigation. Metagenomics is a culture-independent method and one of the novel fields in microbial ecology. It involves subjecting the entire microbial population within an environmental sample to DNA-based analysis (Shade *et al.*, 2018).

2.6 Marker gene sequencing

Marker gene sequencing can also be referred to as marker gene amplification metagenomics. It is a fast and good way to obtain a community/ taxonomic distribution profile or fingerprinting using PCR amplification and sequencing of evolutionarily conserved marker genes (Kumbhare *et al.*, 2016). This method of sequencing is fast and obtains a community/ taxonomic distribution profile using PCR amplification and sequencing of evolutionarily conserved marker genes. The taxonomic distribution can be associated with environmental data derived from the sampling site under study (Kumbhare *et al.*, 2016). The 16S rRNA gene fragment contains one or more variable regions marker genes for bacteria and archaea V1-V9. ITS is the marker gene for fungi while the 18S rRNA gene is the preferred target marker gene for fungi. This shows that life falls into three primary domains: bacteria, fungi and archaea (Chan *et al*., 2008). Taxonomic analysis for prokaryotes and fungi is regularly performed using data derived from varying sequencing technologies (ie, 454 pyrosequencing, Illumina, Solid and Ion Torrent) (Oulas *et al*., 2015).

Biomarkers have been used to explore microbial diversity in different habitats. In 2017, *Pseudomonadaceae, Comamonadaceae, Thermarceae, Caulobacteraceae, Sphingomonadaceae, Thermaceae, Bacillaceae* and *Rhodobacteraceae* bacterial groups were discovered from Eritrean lakes using marker gene sequencing (Ghilamicael *et al*., 2017). Kambura *et al*. (2016) discovered Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, Chloroflexi and Deinococcus-thermus from Lakes Magadi and Little Magadi hot springs. Fungal phylum namely Ascomycota, Basidiomycota, Glomeromycota fungi were recovered from hot springs of soda lakes in Kenya (Salano *et al*., 2017). *Cyanobacteria* and members of the phylum Firmicutes groups have also been studied using high throughput sequencing (Ghilamicael *et al*., 2017).

2.7 Ecological implications of microbial communities within lakes

Microbial ecology explores the diversity, distribution, and abundance of microorganisms, their specific interactions, and the effect that they have on ecosystems. Microorganisms are found everywhere in the biosphere, and their presence invariably affects the environment in which they are existing (Coyte *et al*., 2015). Microbial communities play a key role in the functioning and stability of lake ecosystems. They co-exist and develop complex interactions with each other, including commensalism, mutualism, competition, synergism, predation, parasitism, and antagonism. Microbial communities may change drastically as they adapt to environmental disturbances. Microbial co-occurrence patterns within lake ecosystems are mainly shaped by environmental filtering, interspecies interactions and dispersal limitation (Herren & McMahon, 2018). Indicator species are species playing crucial roles in a microbial community within the lake ecosystem. The removal of indicator species can lead to a notable variance in a community's structure and functioning. Seasonal alteration is one of the most representative environmental variations such as precipitation and temperature which influences microbial communities (Faust & Raes, 2012).

The most significant effect of the microbes on earth is their ability to recycle the primary elements that make up all living systems, especially oxygen, carbon and nitrogen (N). Primary production involves photosynthetic organisms which take up CO_2 from the atmosphere and convert it to organic (cellular) material. The process is also called CO_2 fixation, and it accounts for a very large portion of organic carbon available for the synthesis of cell material. Although terrestrial plants are primary producers, planktonic algae and cyanobacteria account for nearly half of the primary production on the planet (Zhao *et al*., 2016). These unicellular organisms which float in the ocean are the "grass of the sea", and they are the source of carbon from which marine life is derived. Decomposition or biodegradation results in the breakdown of complex organic materials to other forms of carbon that can be used by other organisms.

Decomposition or biodegradation results in the breakdown of complex organic materials into forms of carbon that can be used by other organisms (Qian & Akçay, 2020). There is no naturally-occurring organic compound that cannot be degraded by some microbes, although some synthetic compounds such as teflon, styrofoam, plastics, insecticides and

pesticides are broken down slowly or not at all. Through the metabolic processes of fermentation and respiration, organic molecules are eventually broken down to CO_2 which is returned to the atmosphere (Guo *et al.*, 2020). There is no naturally occurring organic compound that cannot be degraded by some microbe, although some synthetic compounds such as Teflon, plastics, insecticides, and pesticides are broken down very slowly or not at all. Through the microbial metabolic processes of fermentation and respiration, organic molecules are eventually broken down to CO_2 which is returned to the atmosphere for the continuous process of primary production (Coyte *et al*., 2015).

Biological nitrogen fixation is a process found only in some bacteria which removes N_2 from the atmosphere and converts it to ammonia (NH_3), for use by plants and animals. Nitrogen fixation also results in the replenishment of soil nitrogen removed by agricultural processes. Thus along with all these benefits, microbes greatly contribute to maintaining the sustainability of the environment (Coyte *et al*., 2015). Nitrogen fixation is a process found only in some bacteria which removes N_2 from the atmosphere and converts it to ammonia (NH_3), for use by plants and animals. Nitrogen fixation also results in the replenishment of soil nitrogen removed by agricultural processes. Some bacteria fix nitrogen in symbiotic associations in plants. Other Nitrogen-fixing bacteria are free-living in soil and aquatic habitats (Zhao *et al*., 2016). Oxygenic photosynthesis occurs in plants, algae and cyanobacteria. It is the type of photosynthesis that results in the production of O_2 in the atmosphere. At least 50 percent of the O_2 on earth is produced by photosynthetic microorganisms (algae and cyanobacteria), and for at least a billion years before plants evolved, microbes were the only organisms producing O_2 on earth. O_2 is required by many types of organisms, including animals, in their respiratory processes (Guo *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research authorization

Research authorization was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) (Research Permit Number NACOSTI/P/20/3808) (Appendix I) and permission to obtain samples for research from Lakes Olbolosat and Oloiden (Reference Number KWS/BRM/5001) was obtained from the Kenya Wildlife Service (KWS).

3.2 Study sites

Lake Olbolosat is a freshwater body and covers 43 Km^2. The lake is located at a longitude of 36º 26'E and latitude of 0º 09'S in the central part of Kenya, Nyandarua County. Lake Olbolosat is wedged in shape and is found at an altitude of about 2340 meters (Wafula & Murunga, 2020). Lake Olbolosat has a pH of 7, the temperature range of 22 °C, wind speed of 8km/h, the humidity of 71.5%, and precipitation of 1346mm per annum. Samples were collected at 5 different locations from lake Olbolosat. 1: 0° 8' 43.008" S, 36° 26' 26.664" E and 2338 m, 2: 0° 10' 45.264" S, 36° 26' 46.392" E and 2335 m 3: 0° 4' 38.352" S, 36° 24' 58.068" E and 2336 m 4: 0° 9' 28.98" S, 36° 25' 56.712" E and 2347 m 5: 0° 7' 26.976" S, 36° 25' 49.224" E and 2339 m. Lake Oloiden a saline-alkaline water body is about 4-7.5 Km^2. Lake Oloiden becomes fresh when the water overflows from L. Naivasha during the rain seasons. The lake is located at a latitude of 0º 48'S and 36º 16'E. Lake Oloiden is located at 1995 meters above sea level (Maina *et al*., 2018). Lake Oloiden has a pH of 9 and a temperature range of 25 °C wind speed of 10km/h, the humidity of 61% and precipitation of 1800mm per annum. The lake is separated from its west shore by a peninsula. The samples were collected from 4 different points; 1:0° 47' 59.496" S, 36° 16' 45.444" E and 1885 m, 2: 0° 49' 6.744" S, 36° 15' 49.392" E and 1890 3: 0° 48' 33.66" S, 36° 16' 36.624" E and 1884 m 4: 0° 49' 32.772" S, 36° 16' 38.748" E and 1895 m (Figure 3.1).

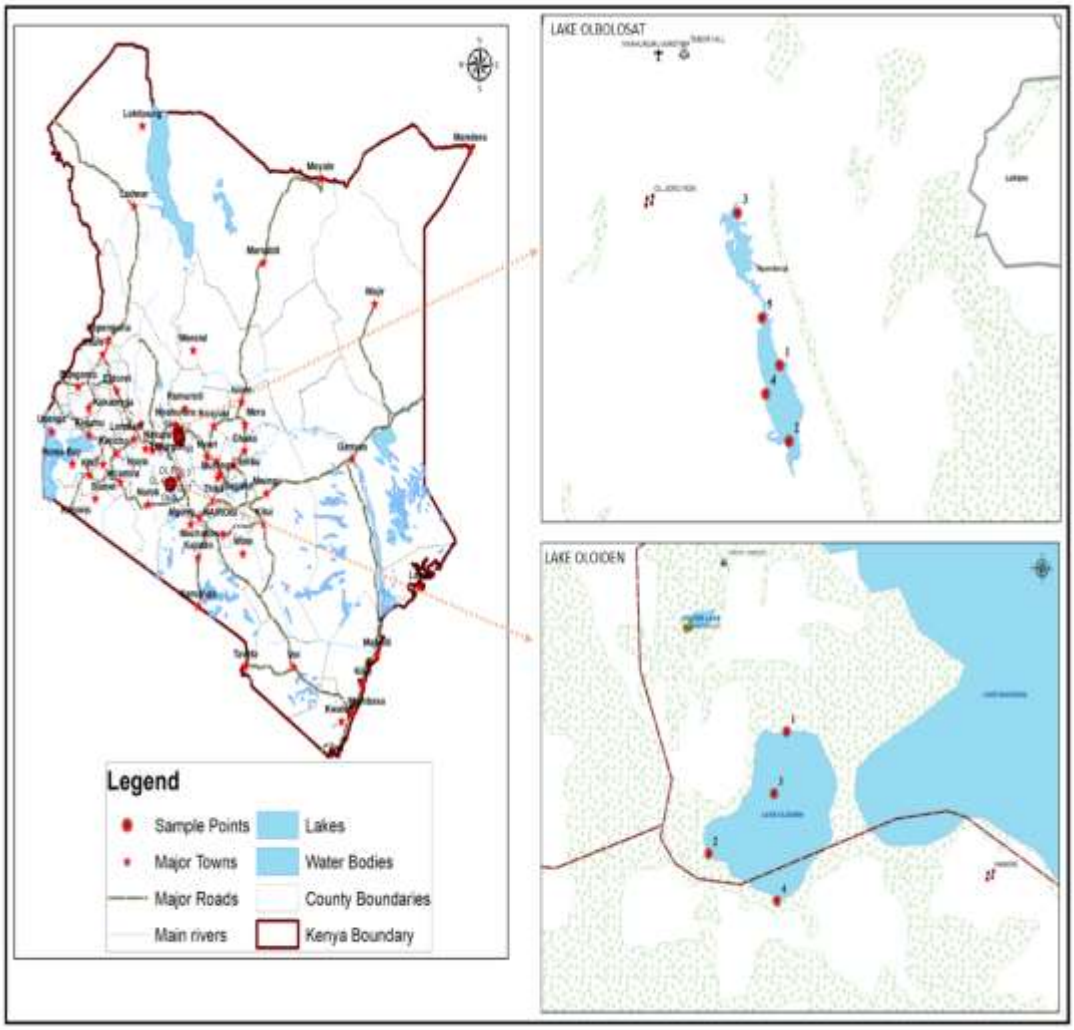

Figure 3.1: Lakes Olbolosat and Oloiden sampling points in Nyandarua and Nakuru Counties.

3.3 Measurement of physico-chemical parameters

The geographical position of the sampling sites in terms of longitude, latitude and elevation were taken using Global Positioning System (GARMIN eTrex 20). The on-site metadata for temperature, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) of each sampling point were measured using Electrical Chemical Analyzer (Jenway - 3405) The pH was measured with a portable pH-meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5-10) (Table 3.1).

Table 3.1: Summary for the onsite sampling parameters in lakes Olbolosat and Oloiden.

Lake	Sampling point	Sample code	Sample type	Latitude (°S)	Longitude (°E)	Elevation (m)	Temp (°C)	pH	Ec (mS/cm)	TDS (mg/l)	DO (mg/l)
L. Oloiden	1	EB1	Wet sediment	0° 47' 59.496" S	36° 16' 45.444" E	1885	23	9.8	16.7	58.3	3.8
	2	EA1	Water	0° 49' 6.744" S	36° 15' 49.392" E	1890	23	9.8	23.3	196.1	3.5
	2	ED1	Microbial mat	0° 49' 6.744" S	36° 15' 49.392" E	1890	23	9.1	17.7	82.8	3.2
	4	ED2	Microbial mat	0° 49' 32.772" S	36° 16' 38.748" E	1895	23	10.6	27.3	195.6	3.3
	4	EC1	Dry sediment	0° 49' 32.772" S	36° 16' 38.748" E	1895	23	9.1	14	107.2	3.7
	3	EB2	Wet sediment	0° 48' 33.66" S	36° 16' 36.624" E	1884	23	9.1	16.3	123.3	3.3
	3	EA2	Water	0° 48' 33.66" S	36° 16' 36.624" E	1884	23	9.8	24	124.6	3.6
	4	EC2	Dry sediment	0° 49' 32.772" S	36° 16' 38.748" E	1895	23	9.1	27	244.7	5
L.Olbolosat	1	ZC1	Dry sediment	0° 8' 43.008" S	36° 26' 26.664" E	2338	22	7.7	8	102.8	3.7
	2	ZB1	Wet sediment	0° 10' 45.264" S	36° 26' 46.392" E	2335	22	8	13.7	105.6	3.8
	4	ZC2	Dry sediment	0° 9' 28.98" S	36° 25' 56.712" E	2347	22	7.7	9.7	125.3	3.7
	2	ZD2	Microbial mat	0° 10' 45.264" S	36° 26' 46.392" E	2335	22	7.7	8.3	88.1	3.3
	3	ZA1	Water	0° 4' 38.352" S	36° 24' 58.068" E	2336	22	7.6	8.3	215.3	4.2
	5	ZA2	Water	0° 7' 26.976" S	36° 25' 49.224" E	2339	22	7.3	5	110.5	3.8
	5	ZB2	Wet sediment	0° 7' 26.976" S	36° 25' 49.224" E	2339	22	7.2	7.7	106.2	4.9

3.4 Experimental design and sample size

A purposive experimental design was used in this study. The sample size was determined based on the three distinct and interacting biological zones. This included Littoral, pelagic and benthic zones determined based on the unique features of the optimum coverage. There were three biological replicates for every water, microbial mat and dry and wet sediments (Kambura *et al.*, 2016).

3.5 Sample collection

Nine sampling points were selected randomly. Four from Lake Oloiden and 5 from Lake Olbolosat. Wet, dry sediments and water samples were randomly collected in triplicates. Sample collection was done by scooping wet and dry sediments with a hand shovel into sterile 250 ml plastic containers. The sterile plastic containers were used to fetch water from both lakes. All samples were transported on dry ice to the botany laboratory at Jomo Kenyatta University of Agriculture and Technology.

3.6 Bacteria isolation

3.6.1 Isolation media

Bacteria isolation was done using the ringer salt solution powder (RSSP Himedia-M525) consisting of Sodium Chloride 8.50 g, Potassium Chloride 0.20 g, Calcium chloride anhydrous 0.20 g, and Sodium bicarbonate 0.01 g. Subculturing was done using the Plate Count Medium (PCA) that consisted of Casein enzymic hydrolysate 5 g, Yeast extract 2.50 g, Dextrose 1.00 g and agar 15 g in 1 litre of water from the lakes to mimic the lake conditions.

3.6.2 Isolation and enumeration of bacterial isolate

Collected samples were homogeneously mixed and carefully sorted to remove stones and other unwanted soil debris. The PCA media was autoclaved at 121°C for 15, min allowed to cool and 20ml dispensed aseptically on the sterile disposable Petri dishes. Ampliclox 25mg/l was added to the media after autoclaving to prevent contamination by bacteria. The pure colonies were selected based on morphological characteristics. One gram of the dry and wet sediments was suspended separately in a 9 ml of ringer solution. This was followed by filtration through sterile 125 mm (Whatman ®) qualitative filter paper, Grade 1(Merck). One ml of the filtrate was transferred to 9 ml of ringer solution to make 10^{-2} and 10^{-3} dilutions. The inoculation mixture with serial dilution was then spread plated in triplicate on the plate count agar (Himedia- M091S) for bacterial diversity. Ten milliliters of water sample were suspended in 9 ml of ringer salt solution. The serial dilution for all samples was 10^{-1}, 10^{-2} and 10 dilutions. Incubation for the inoculated Petri dishes was at 30 °C for 24 to 72 hours. To measure survival efficiency, colonies were counted using the protocol by Das & Dutta (2018). To obtain pure cultures, distinctive colonies were picked, transferred to fresh PCA media and incubated at 30 °C for 24 to 72 hours. Purified colonies (80%) were grown on nutrient broth (Difco) and stored in 20 % glycerol at -75 °C.

3.7 Morphological and cellular characterization of bacterial isolates

To purify the bacterial isolates, the cultures were carefully and aseptically sub cultured on the PCA media and stored on nutrient broth for further analysis at 4 °C. Morphological characterizations of the isolates were observed using a compound microscope at a

magnification of ×1000. The observations made were as per the protocol by Cappuccino & Sherman (2014) were pigmentation of the media both top and obverse. The pigmentation is normally influenced by the temperature and the nutrients available in the media. Form shows the gross appearance for the growth pattern of the isolates in the media, elevation describes the depth of the colonies developed by microbes, margin shows how the edges of the colonies appears while cell shape is the structure of the cells. Gram-reaction was done as per the protocol by Cappuccino & Sherman (2014); the reagents used were crystal violet as the primary stain, Gram's iodine solution the mordant, acetone/ethanol, the decolorizer, 0.1% basic fuchsin solution as the counter stain and water. The gram reaction was confirmed by a 3% (w/v) KOH test to differentiate between gram positive and negative cells according to Moyes *et al*. (2009).

3.8 Biochemical characterization of isolated bacteria

3.8.1 Catalase test

Catalase test detects the activity of enzyme catalase, present in most cytochrome-containing aerobic bacteria. These microbes produce hydrogen peroxide during the aerobic breakdown of sugars. Catalase decomposes hydrogen peroxide into water and oxygen. The catalase test was done by scooping a colony of a 24-hour culture, placing it on a glass slide and adding a drop of 3 % hydrogen peroxide solution. A positive reaction was indicated by the formation of bubbles, while the absence of air bubbles indicated a negative catalase test (Cappuccino & Sherman, 2014)

3.8.2 Gelatin liquefaction

Gelatin liquefaction detects the breakdown of gelatin to polypeptides and amino acids by the enzyme gelatinase (Harold, 2002). Gelatin protein is produced by hydrolysis of a component of the connective tissues and tendons of animals known as collagen. Gelatin is solid at room temperature but above 25 °C it turns into liquid. When gelatinase hydrolyses this protein into amino acids, it remains liquefied even at the low temperatures of an ice bath (Cappuccino & Sherman, 2014). The bacterial isolates were inoculated onto nutrient broth supplemented with 12 % gelatin and 1.5 % agar, to demonstrate the hydrolytic activity of gelatinase. One uninoculated tube was used as a control for each

isolate (Cappuccino & Sherman, 2014). After incubation, cultures that remained liquefied when placed in the refrigerator at 4 °C for 30 minutes were considered positive for gelatin hydrolysis.

3.8.3 Methyl Red-Voges- Proskauer (MR-VP) test

MR-VP is used to identify mixed acid fermenting bacteria. The test detects the ability of the isolates to oxidize glucose by detecting the production of sufficient acid end products (Harold, 2002). MR test detects mixed acids, which are the characteristic end products of a particular fermentation pathway that make the medium more acidic (pH≤4.5). This is detected when an indicator is added (Cappuccino & Sherman, 2014). The VP test is used to identify bacteria that produce non-acidic or neutral end products from the organic acid products of glucose fermentation. It specifically detects an intermediate product of the fermentation pathway that yields 2, 3-butanediol known as acetoin, by the addition of Barritt's reagent (4 % KOH and 5 % alpha naphthol and 95 % ethanol). MR-VP broth was inoculated with each of the isolates, in duplicates, shaken and then incubated at 30 °C for 72 hours after which, drops of Methyl red indicator methyl red test and Barrit's reagent for VP test respectively, were added to aliquots of each culture. Positive reactions turned red while negative reactions turned pale yellow for the MR test, while for the VP test, positive tests produced a deep red coloration (Cappuccino & Sherman, 2014).

3.8.4 Indole, motility and hydrogen sulfide production tests

Indole reacts with Kovacs reagent (dimethylamino-benzaldehyde) (Harold, 2002), to form a deep red color while the Iron in the medium reacts with hydrogen sulfide to produce a black precipitate (Cappuccino & Sherman, 2014). The isolates were inoculated in Sulphur-Indole Motility (SIM) agar media by the stabbing method in duplicate for replication and then incubated at 37 °C for 48 hours. Two uninoculated tubes were used as controls. Kovac's reagent was added to each of the 48-hour cultures according to the protocol (Harold, 2002). The presence of a cherry red layer in the media indicated a positive result for Indole production while negative results were indicated by the color remaining brown (Cappuccino & Sherman, 2014). The presence of a black precipitate in the media after incubation indicated hydrogen sulfide in the media (Cappuccino &

Sherman, 2014). Lack of motility was detected by the confinement of the bacteria along the line of inoculation.

3.8.5 Citrate utilization

This test determines the ability of a microbe to use citrate as the sole source of carbon. Citrate utilization is indicated by growth accompanied by an alkaline pH Simmon's citrate agar slant containing the pH indicator bromothymol blue was inoculated by streaking with the isolates in duplicates and incubated at 37 °C for 72 hours. One uninoculated tube with the same media served as the control. A positive test was indicated by the growth of the bacteria accompanied by the color change in the medium from olive green to Prussian or deep blue. The green color medium represented a negative test (Cappuccino & Sherman, 2014).

3.8.6 Urease test

This test is used to determine the ability of a microorganism to produce a urease enzyme that splits urea, into carbon dioxide and ammonia (Harold, 2002). Ammonia makes the medium alkaline. The isolates were inoculated in urea broth media containing phenol red indicator at 37 °C color change was monitored for 4 days after incubation. A positive reaction was indicated by the presence of deep pink color, while negative tests remained yellow (Cappuccino & Sherman, 2014).

3.8.7 Starch hydrolysis

Using aseptic technique streaking was done with the respective bacteria onto the plate in a straight line within two divided sections. Incubation of the plate for 24 to 48 hours at 37 °C was done. Drops of Gram's iodine were placed on each of the lines streaked on the starch agar plate. If the area around the line of growth was clear, starch had been hydrolyzed, and the test was positive; if it was not clear or the entire medium turned blue, starch has not been hydrolyzed, and the test was negative (Cappuccino & Sherman, 2014).

3.8.8 Triple sugar iron (TSI) test

Twenty-four-hour cultures of the isolates were stabbed and streaked over a slant of TSI agar tube and incubated at 37 ºC for 24-48 hours. This test was used to determine the

ability of the bacteria colonies to ferment glucose, lactose, or sucrose and form hydrogen sulfide and gas production. A change in the color of the butt or slant from red to yellow indicated the type of sugar utilized by the organism. If the butt changed from red to yellow, it showed the ability of the isolate to utilize glucose while a change of color from red to yellow on the slant and butt indicated the ability of the isolate to glucose, sucrose and lactose. Bubbles or cracks on the medium indicated gas production. The presence of black coloration indicated the production of hydrogen sulfide from the reaction (Cappuccino & Sherman, 2014).

3.9 Physiological characterization of bacterial isolates

3.9.1 Growth of bacteria at different sodium chloride concentration

The ability of isolates to grow at different sodium chloride concentrations was determined using Plate count agar supplemented with NaCl: 0 %, 5 %, 10 %, 15 %, 20 % and 30 % sodium chloride and 1 % sodium carbonate. The media was inoculated with each of the bacterial isolates and incubated at 30 °C, then checked for growth after 48 hours by observing the extent of the growth. The diameter for the growth was measured in millimeters and recorded (Cappuccino & Sherman, 2014).

3.9.2 Growth of bacteria at different pH

The pH of each set of experiments was adjusted to 5.0, 7.0, 9.0 and 11.0 with a pH meter using HCl and NaOH. PCA media was prepared and pH is adjusted to 5, 7, 8.5 and 10 using 1 M HCl and 1 M NaOH. This was sterilized and dispensed in sterile Petri dishes. Each medium was inoculated with isolates and incubated at 30 °C. The growth of the isolates was checked after 48 hours of incubation. The diameter for the growth was measured in millimeters and recorded. Two uninoculated plates were used as controls (Venosa & Zhu, 2003).

3.9.3 Growth of bacteria at different temperatures

PCA media was prepared at pH 7.0, sterilized and dispensed in sterile Petri dishes. Each batch was inoculated with the isolates and incubated at temperatures of 20, 25, 30, 35, 40, 45 and 50 °C respectively. The growth of the isolates was checked after 48 hours of

incubation. The diameter of the growth was measured in millimeters and recorded (Cappuccino & Sherman, 2014).

3.10 Screening bacterial isolates for enzymatic activity

3.10.1 Determination of xylanolytic activity

The isolates were cultured on basal media (1 % KH_2PO_4, 0.01 % $MgSO_4.7H_2O$, 0.005 % $CaCl_2.H_2O$, 4 % NaCl and 1 % Na_2CO_3) supplemented with 1 % xylan (Fluka) as the sole carbon source, by the method described by (Lee *et al*., 2005). The medium was then inoculated with the isolates and incubated for 48 hours at 30 °C. The plates were flooded with 1 % Congo red dye. The dye was then followed with NaCl (1M) and subsequently rinsed with distilled water. The plates were observed for halos around the colonies. The diameter for the growth was measured in millimeters and recorded as an indication of positive polymer degradation (Cappuccino & Sherman, 2014).

3.10.2 Determination of amylolytic activity

The isolates were cultured on basal media (1 % KH_2PO_4, 0.0 1 % $MgSO_4.7H_2O$, 0.005 % $CaCl_2.2H_2O$, 4 % NaCl and 1 % Na_2CO_3) supplemented with 1 % starch (Merck), as the sole carbon source. The medium was then inoculated by the spotting of isolates per plate before incubation at 30 °C. After 48 hours the plates were flooded with iodine solution (Sigma–Aldrich) (Cappuccino & Sherman, 2014). Clear halos around the colonies indicated extracellular amylase production while negative results were indicated by blue-black color all over the plate (Mahmoudi *et al*., 2015).

3.10.3 Determination of lipolytic/ esterase activity

The isolates were cultured on basal media (1 % KH_2PO_4, 0.0 1% $MgSO_4.7H_2O$, 0.005 % $CaCl_2.2H_2O$, 4 % NaCl and 1 % Na_2CO_3) supplemented with 1 % olive oil (domestic grade) as the sole carbon source. The medium was inoculated and incubated for at least 48 hours at 30 °C. Positive isolates for lipase/esterase production were indicated by the precipitation of calcium crystals around the colonies (Cappuccino & Sherman, 2014).

3.10.4 Determination of the cellulolytic activity

The production of cellulose was determined using media that contains cellulose (Fluka) and carboxymethyl cellulose (CMC) - Serva, Heidelberg). The isolates were cultured on basal media (1 % KH_2PO_4, 0.01 % $MgSO_4.7H_2O$, 0.00 5% $CaCl_2.2H_2O$, 4 % NaCl and 1 % Na_2CO_3) supplemented with 1 % cellulose and 1 % CMC separately. Each medium was then inoculated by spotting of isolates per plate followed by incubation for 48 hours at 30 °C. The plates were then be flooded with 1 % Congo red dye. The dye was replaced with IM NaCl and subsequently rinsed with distilled water. The plates were observed for halos around the colonies, as an indication of positive polymer degradation (Cappuccino & Sherman, 2014).

3.10.5 Determination of the proteolytic activity

For the determination of proteolytic activity, skimmed milk was used following the method of (Lee *et al*., 2005). The isolates were cultured on basal media (1 % KH_2PO_4, 0.01 % $MgSO_4.7H_2O$, 0.005 % $CaCl_2.2H_2O$, 4 % NaCl and 1 % Na_2CO_3) supplemented with 1 % skimmed milk. The medium was then inoculated and incubated at 30 °C and observation for zones of clearing zones after 48 hours. Positive isolates for protease production normally exhibit zones of proteolysis demonstrated by clearing zones (Cappuccino & Sherman, 2014).

3.11 Screening the bacterial isolates for the production of antimicrobial compounds

Sixty bacterial isolates were screened for their ability to inhibit the growth of bacterial test organisms; *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 10788), *Bacillus subtilis* (ATCC 55732) and fungal test organism *Candida albicans* (ATCC 90028) obtained from Kenya Medical Research Institute- Centre for Microbiology research. The bacterial isolates were cultured in nutrient broth and incubated at 30 °C for 24 hours. The cultured bacterial isolates were centrifuged at 10,000× *g* for one minute and the supernatant was sieved using sterile micro membrane filters to remove any bacterial cells. The impregnated sterile Whatman ® qualitative filter papers, Grade 1(Merck) discs measuring one-centimeter paper discs were aseptically placed on Mueller Hinton agar (Himedia-M173). The media was swabbed with 0.1 ml per Petri dish of the test organisms following the Kirby-Bauer

diffusion protocol followed by incubation for 24-48 hours at 30 °C after which the results were recorded while negative control consisted of the uninoculated plate (Hudzicki, 2012).

3.12 Molecular characterization of bacterial isolates

Bacterial isolates were grown in nutrient broth media (Himedia-M002) consisting of Peptone, 5g, sodium chloride, 5 g, HM peptone B 1.5 g, Yeast extract, 1.5 g in 1 L of distilled water. The overnight cultures were centrifuged at 10,000× *g* for 1 min and the supernatant discarded remaining with the pellet. DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Germany) extraction kit according to the manufacturer's instructions. Bacterial universal primers 27F forward (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R reverse (5′-GGT TAC CTT GTT ACG ACT T-3′) were used for the amplification of the 16S rRNA gene. PCR was carried out using PEQLAB, Erlangen, Germany, 96 PCR thermocycler machine. The PCR was carried out in a 50 µl mixture containing 25 µl 3X Taq PCR Master Mix (Qiagen, Germany), 2.5 µl of each primer, 10 µl of DNA template (50 ng) and 10 µl RNase-free water. The reaction mixtures were subjected to the following PCR conditions: Initial denaturation at 95 °C for 5 minutes, 32 cycles of denaturation at 95 °C for 1 minute, primer annealing at 55 °C for 1 minute, chain extension at 72 °C for 1 minute and a final extension at 72 °C for 10 min (Roux, 1995). The amplified PCR products were checked by gel electrophoresis using 1.2% (w/v) agarose gels stained with ethidium bromide (1 µg/ml) and visualized using the Biotec-Fischer Felix6050 gel documentation system (ProfiLab24, Germany) according to the manufacturer's instructions and stored at -20 °C. The PCR products were purified using the QIAquick PCR purification Kit protocol (2010) according to the manufacturer's instructions. The shipment for the 60 bacterial isolates was done in TE buffer. Sanger sequencing of PCR products of the 60 bacterial isolates was carried out at Human Genomics Macrogen Europe (Macrogen Europe B.V, Amsterdam, Netherlands).

3.13 Phylogenetic data analysis

Sequencing of purified PCR products was done at Macrogen Netherlands. The 16S rRNA gene sequences were compared to sequences in the public database using Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology

Information (NCBI) website (*http://www.ncbi.nih.gov*) in order to determine similarity to sequences in the Gen Bank database (Engeset *et al*., 2003).

The sequences that were >320 base pairs were edited using Chromas pro software and were compared to public databases using the BLAST program (http://blast.ncbi.nlm.nih.gov/). The pairwise alignment was done using MEGA 7 software and the affiliation of the 26 isolates to the closest reference strains was determined. The phylogenetic relationship of all the partial sequences was determined in MEGA 7 using Maximum-Likelihood analyses. The evolutionary pairwise distances were estimated using the Maximum Composite Likelihood approach (Engeset *et al*., 2003).

3.14 Environmental nucleic acid extraction

Approximately 500 ml water sample for nucleic acid extraction was filtered using a 0.22 μM microfilter (Whatman) and filter papers. The obtained samples were centrifuged at 10,000× *g* for 10 minutes. Pellets obtained were resuspended in 5 ml of phosphate buffer saline solution. The pellets were placed in 2 ml Eppendorf tubes and stored at -75 °C ready for DNA extraction. DNA was extracted from 0.5 g of the microbial mat, dry and wet sediment separately. DNA from all samples was differently extracted using DNeasy® Powersoil® Kit (Qiagen, USA) following the standard manufacturer's protocol (Pearman *et al*., 2020). Extracted DNA from the 15 sample types was air-dried to prevent residual ethanol from dripping back to the DNA and stored at -20 °C. The extracted DNA samples were quantified using a NanoDrop™ 2000/c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) and evaluated with 1% agarose gel.

3.15 Amplicon library preparation

The conserved sequences flanking the hypervariable V3-V4 region of the 16S rRNA gene served as primer sites to generate PCR amplicons. The following primers were used for the prokaryotes; 341 F (5′- GTGCCAGCMGCCGCGGTAA-3′) that had a barcode and 806R (5′- GGACTACHVGGGTWTCTAAT-3′) according to (Caporaso *et al*., 2012). The PCR amplification for the ITS region was amplified using ITS1 F (5′- TCCGTAGGTGAACCTGCGG-3′) that had a barcode and ITS4 R (5′- TCCTCCGCTTATTGATATGC-3′) according to (White *et al*., (1990). The amplicon libraries were constructed under the following PCR conditions: initial denaturation at 94

ºC for 3 minutes, followed by 30 cycles of denaturation at 94 ºC for 30 seconds, annealing at 53 ºC for 40 seconds and extension at 72 ºC for 1 minute, and final elongation at 72 ºC for 5 minutes (Beckman Coulter, Korea). The PCR products were purified using the QiAmp PCR cleanup kit (QiaAamp Kit, Germany). The quality of the purified PCR products was checked on a DNA7500 BioAnalyzer chip (Agilent, Santa Clara, USA) and quantified using a Nanodrop 1000 (Thermo Fisher Waltham, MA, USA). Barcoded Amplicons were pooled in equimolar concentration and sequenced on the Illumina Miseq (paired-end 250 bases with two index reads) at Macrogen, Korea.

3.16 Sequence data analysis

The purified amplicon library was sequenced on an Illumina Miseq sequencing-by-synthesis (SBS) technology, a proprietary reversible terminator-based platform (ngs@macrogen.com, Macrogen, Inc. Seoul, South Korea). After sequencing, the barcodes and amplicon primer sequences were removed using real-time analyzer software (RTA). Low-quality sequences were identified by denoising and filtered out of the dataset. Reads with <300 base pairs after phred20-based quality trimming, sequences with ambiguous base calls and those with homopolymer runs exceeding 5 base pairs were removed using the bcl2fastq Illumina package (Reeder & Knight, 2010). The high-throughput sequencing data was analyzed by following the workflow by Callahan *et al.* (2016). Quality checking, filtering and trimming of fastq files were performed using the DADA2 v 1.16 package at 99.9 % similarity level and 1 % divergence of ASVs in R v 4.02. (Appendix II). Taxonomy was assigned to ASVs based on the naïve Bayesian classifier against the Silva v138 database while Taxonomy for the eukaryotes was against UNITE+INSD eukaryotes database v 04.02.2020 (Appendix III). Demultiplexed high-quality sequence reads were deposited in the Sequence Read Archive, the National Centre for Biotechnology Information with study accession number PRJNA723886 for prokaryotes while for the eukaryotes is PRJNA736835 freely available for download.

3.17 Statistical analysis

Statistical data on bacterial isolation was noted and recorded in an Excel sheet. Normality Test (Shapiro- Wilk) was used to compare means using *Sigma Plot* 12 v 5.0 for bacterial density. Correlation profiles of zones of hydrolysis and bacterial isolates zone of

clearance were visualized as heatmaps generated by a hierarchical clustering R script using R v 4.0.2. Sequencing was conducted in one direction using the forward primer (27 F). The Chromas pro program was used to remove ambiguity and comparisons were done with the NCBI GenBank databases using Basic Local Alignment Search Tool (BLAST) and EZBio Cloud algorithms. Sequences were submitted to the GenBank database and were assigned the accession numbers. The differences in the nucleotides were converted into distance matrices using the Maximum Likelihood method (Saitou & Nei, 1987). A phylogenetic tree was constructed using MEGA 7 (Engeset *et al*., 2003).

Statistical data for the metagenomics study was done using *phyloseq* v 1.32.0 for the general downstream analysis, *tidyverse* v 1.3.1 was used for relative abundance analysis, *compositions* v 2.0.1 used for composition analysis, *vegan* v 2.5.7 was used for venn diagrams anaysis, *ALDEX2* v 1.20.0 was used for aldex analysis, *ggcorrplot* v 0.1.3 was used for the graphs, *ggrepel* v 0.9.1 was used for the graph analysis, and *superheat* v 0.1.0. used for the heatmaps. The resulting ASVs were used to calculate alpha diversity indices (Observed, Chao1, ACE, Shannon, Simpson, and inverted Simpson). The different indices used measured species richness and the diversity for the ASVs in different samples. They were also used to show the variation of different ASVs in different sample types. The package *venerable* v 3.1.0.9000 was used to visualize the comparison of shared ASVs between the two lakes and sample types. Rarefaction curves were also visualized using the resulting ASVs to determine library sizes among the samples. *IndVal* function in the *labdsv* package (https://cran.r-project.org/web/packages/labdsv/labdsv.pdf) was used to identify taxon-habitat association patterns. *IndVal* function identifies taxa as an indicator species based on their independence abundance in the total data sets.

Principal Component Analysis (PCA) and Hierarchical clustering of both the environmental factors and taxa were used to show the relationship in microbial community composition between samples. Cluster analysis of environmental factors and taxa were based on the Euclidian and Bray-Curtis dissimilarity, respectively. Function *aldex.plot* in package *ALDEx2* v 1.20.0 was used to determine the differentially abundant ASVs and the significance was tested at we.ep < 0.05. Packages *phyloseqGraphTest* CRAN v 0.02 was used for the graphical interface analysis, *Intergraph* v 0.03 was used

for plotting, *gridExtra*, v 2.3 was used for the arrangement of multiple grid-based plots and *ggnetwork* was used to visualize the co-correlation network patterns of the indicator ASVs in the study. Edge-weighted positive values indicated the co-correlation between the ASVs (Callahan *et al*., 2017).

One-way analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) was used to explain the difference between taxa beta diversities and environmental factors. PERMANOVA was used to determine the effects of environmental factors and nutrients on the distribution of microbial communities based on standardized categories with 999 permutations. Pearson's correlation coefficient analysis between environmental factors and microbial community structure was based on the product-moment correlation on variance-stabilizing transformed counts. The significance of the predictor environmental factors was tested using Mantel test.

CHAPTER FOUR

RESULTS

4.1 Isolation of bacterial isolates obtained from Lakes Olbolosat and Oloiden

A total of 60 bacterial isolates were obtained from lakes Olbolosat and Oloiden. Lake Olbolosat recorded 35 while L. Oloiden recorded 25 bacterial isolates (Table 4.1).

4.2 Enumeration of bacterial isolates obtained from lakes Olbolosat and Oloiden

The bacterial counts for dry sediments ranged between 0 to 1.75 x 10^5 CFU/ml while for wet sediments ranged between 7.63 x 10^4 to1.16 x10^5 CFU/ml. Bacterial count for water samples ranged between 5.3 x 10^4 to 1.22 x 10^5 CFU/ml. Bacterial density significantly varied ($p<0.001$, $F=6.667$) between the sampling points (Figure 4.1).

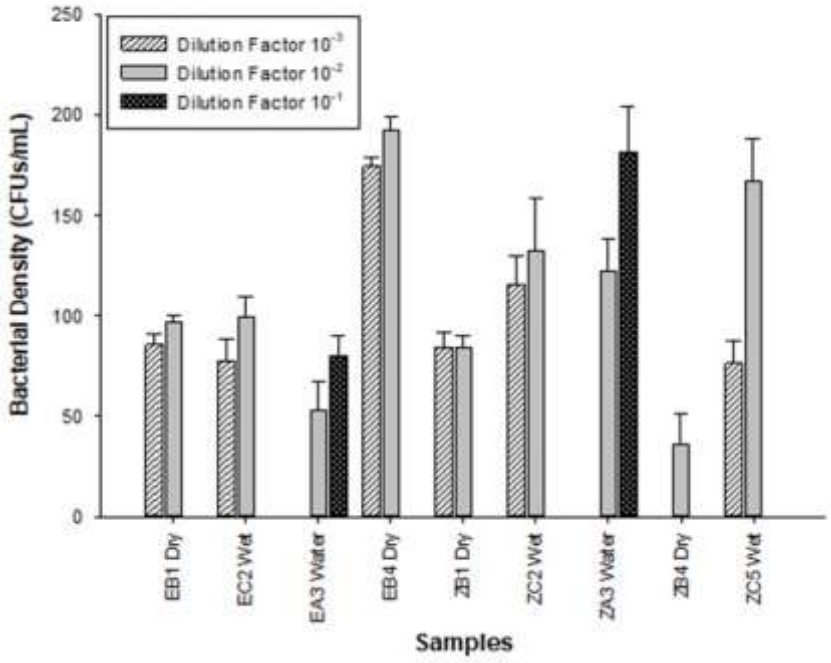

Figure 4.1: Bacterial density from lakes Olbolosat is indicated with prefix E and Oloiden with prefix Z.

4.3 Morphological and cellular characterization

The isolates had varying colony characterization characteristics. The colony color for the bacterial isolates was cream white, white, cream, cream yellow, orange, watery, reddish and brown. Bacterial isolates with circular colonies were 73 %, 20 % irregular, 5 % filamentous and only 2 % concentric in form (Table 4.1 and Appendix IV).

Table 4.1: Morphological and cellular characterization of bacterial isolates from Lakes Olbolosat and Oloiden.

	Colony Characterization				Cell characterization	
Isolate	**Pigment**	**Form**	**Elevation**	**Margin**	**Cell shape**	**Gram reaction**
EBP 8.2	Cream	Circular	Raised	Entire	Rod	+
ZCP 6.3	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 6.1	White	Circular	Umbonate	Entire	Rod	+
ZCP 6.8	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 1.2	Cream white	Circular	Flat	Entire	Rod	+
EBP 2.2	Cream	Circular	Umbonate	Entire	Rod	+
ZCP 6.7	Cream yellow	Circular	Flat	Entire	Cocci	+
ZCP 17.4	Cream	Irregular	Flat	Serrated	Cocci	+
ECP 3.1	Cream white	Circular	Flat	Entire	Rod	+
ZAP 9.6	Cream white	Irregular	Flat	Undulate	Rod	+
ZCP 1.7	White	Circular	Umbonate	Entire	Rod	+
ZCP 6.2	White	Circular	Flat	Entire	Rod	+
ZCP 1.3	White	Circular	Flat	Entire	Rod	+
EBP 2.1	Cream	Circular	Flat	Entire	Rod	+
ZAP 16.3	Cream	Irregular	Pulvinate	Entire	Rod	-
ECP 3.4	Cream white	Circular	Pulvinate	Undulate	Filamentous	+
EBP 8.1	Cream	Filamentous	Raised	Entire	Rod	+
EBP 10.1	Cream white	Filamentous	Umbonate	Entire	Rod	+
ZCP 17.2	Cream white	Irregular	Flat	Undulate	Rod	+
ZAP 16.2	Cream white	Circular	Flat	Entire	Rod	+
ZAP 10.1	Orange	Circular	Flat	Entire	Rod	+
EBP 3.9	Cream white	Circular	Flat	Entire	Rod	+
EBP 5.1	Yellow	Circular	Flat	Entire	Rod	+
EBP 8.8	Orange	Irregular	Flat	Serrated	Cocci	+
ZAP 16.1	White	Circular	Pulvinate	Entire	Filamentous	+
EBP 2.5	Watery	Concentric	Flat	Entire	Rod	+
ZAP 9.1	Reddish	Circular	Flat	Entire	Rod	-
ZAP 9.2	Cream white	Irregular	Flat	Serrated	Rod	+
ZCP 6.4	Cream white	Circular	Flat	Entire	Rod	+
ZCP 17.1	Cream white	Circular	Umbonate	Entire	Rod	+

ZCP 1.1	Cream white	Circular	Flat	Entire	Rod	+
ZCP 6.5	White	Circular	Raised	Entire	Rod	+
ZCP 17.5	White	Circular	Flat	Entire	Rod	+
ZBP 9.7	White	Irregular	Flat	Undulate	Rod	+
EBP 8.4	Cream white	Irregular	Flat	Undulate	Rod	+
EBP 8.5	Cream white	Filamentous	Pulvinate	Lobate	Rod	+
ZAP 10.2	Brownish	Circular	Flat	Entire	Rod	+
EBP 10.5	Cream white	Circular	Umbonate	Entire	Rod	+
EBP 5.3	Cream white	Circular	Flat	Entire	Rod	+
EBP 10.4	White	Circular	Raised	Entire	Rod	+
EAP 3.7	White	Circular	Flat	Entire	Rod	+
ECP 3.2	Cream white	Irregular	Flat	Serrated	Rod	+
EBP 10.2	Cream white	Circular	Flat	Entire	Rod	+
EBP 2.4	Cream white	Circular	Umbonate	Entire	Rod	+
EBP 8.3	Cream white	Circular	Flat	Entire	Rod	+
ZBP 9.2	White	Circular	Umbonate	Entire	Rod	+
ZCP 13.2	Cream white	Circular	Umbonate	Entire	Rod	+
ZCP 1.11	Cream white	Circular	Flat	Entire	Rod	+
ZCP 1.10	Cream	Circular	Umbonate	Entire	Rod	+
ZCP 1.9	Cream yellow	Circular	Flat	Entire	Rod	+
ZCP 1.8	Cream white	Irregular	Flat	Undulate	Rod	+
ZCP 1.6	White	Circular	Umbonate	Entire	Rod	+
ZCP 6.6	White	Circular	Flat	Entire	Rod	+
ZAP 9.4	White	Circular	Flat	Entire	Rod	+
ZAP 9.3	Cream white	Circular	Flat	Entire	Rod	+
EBP 6.6	Cream	Irregular	Pulvinate	Entire	Rod	+
EAP 8.9	Cream white	Circular	Pulvinate	Undulate	Rod	+
EAP 6.4	White	Circular	Flat	Entire	Rod	+
EBP 8.7	Cream white	Irregular	Flat	Serrated	Rod	+
EAP 6.6	Cream white	Circular	Flat	Entire	Rod	+

The margin for the isolates was 78 % entire,12 % undulate, 8 % serrated and only 2 % lobate. The elevation for the bacterial isolates was 62 % flat, 7 % raised, 21 % umbonate and 10 % pulvinate Cellular characterization revealed two isolates that were gram-negative out of sixty isolates. Fifty-seven isolates were rod-shaped, two were cocci-shaped and one was filamentous in shape (Table 4.1 and Appendix V). A dendrogram for morphology and cellular characteristics based on the Ward D method and the distance between characters measured using Euclidean metric for the hierarchical clustering (Appendix VI).

4.4 Biochemical characteristics of bacterial isolates

The ability of the bacterial isolates to excrete the intracellular and extracellular enzymes was done through the following tests in Table 4.2 (Catalase test, Citrate utilization test, Triple Sugar Iron test, methyl red test, Voges Proskauer, Urea test, Indole test, Gelatin liquefaction test, motility test and starch hydrolysis). Catalase test revealed that all bacterial isolates were positive for hydrogen peroxide production an end product of oxidation of sugars among the samples from lakes Olbolosat and Oloiden. There were 77% and 28% of bacterial isolates positive for methyl red-Voges Proskauer test respectively indicating their ability to ferment glucose and for the production of mixed acids. There was 16% of the bacterial isolates were positive for urease hydrolysis activity. There was 20% of the bacterial isolates positive for indole generation by reductive deamination fro tryptophan via the intermediate molecule indole pyruvic acid. There was 48% of the isolates were able to break down gelatin into polypeptides and amino acids by the enzyme gelatinase. Sixty-two percent oof the isolates showed their ability to be motile through the motility test while 73% of the isolates were positive for amylase production which was confirmed through the starch hydrolysis test. Triple-Sugar Iron test revealed that 40% of the isolates were positive for the production of acid, 35% for the production of alkalinity and only 11% for hydrogen gas production (Table 4.2).

Table 4.2: Biochemical characteristics of bacterial isolates from lakes Olbolosat and Oloiden.

	Biochemical tests											
	Cat	Cit	TSI			MR	VP	Urea	Ind	Gel	Mot	Starch
Isolate			Butt	Slant	Gas							
ZCP 9.6	+	-	+	-	-	+	-	-	-	-	-	+
ZCP 6.3	+	+	-	-	-	+	-	-	-	+	-	+
ZCP 6.2	+	+	+	-	-	+	-	-	-	+	-	+
ZCP 1.3	+	-	+	-	-	+	-	-	-	-	+	+
EBP 2.2	+	-	+	-	-	+	+	-	-	-	+	+
ZCP 1.7	+	+	+	-	-	+	-	-	-	-	+	-
ZCP 6.1	+	-	-	-	-	+	-	-	-	-	+	+
ZCP 6.7	+	-	+	+	-	-	-	-	-	-	-	+
ZCP 17.4	+	-	+	+	-	-	-	-	-	-	-	+
ECP 3.1	+	+	-	-	-	+	+	-	-	-	+	+
EBP 8.2	+	+	+	-	-	+	+	-	-	+	+	+
ZCP 1.2	+	+	+	-	-	+	-	-	-	-	-	+
ZCP 6.8	+	+	-	-	-	+	-	-	-	+	-	+
EBP 8.1	+	-	-	-	-	+	-	-	-	+	+	+
EBP 10.1	+	-	-	+	-	+	-	-	-	+	-	-
EBP 2.1	+	+	-	-	-	-	-	+	-	+	-	+
ECP 3.4	+	+	+	+	-	+	-	-	-	+	-	+
ZAP 16.3	+	+	-	-	-	+	-	-	-	-	+	+
ZCP 17.2	+	–	+	–	–		–	–	–	–	–	+
EBP 8.8	+	–	+	+	–	+	–	–	–	–	–	–
ZAP 10.1	+	–	+	+	–	+	–	–	–	+	–	+
ZAP 16.1	+	+	–	–	+	+	–	–	–	+	–	+
ZAP 16.2	+	–	+	–	–	+	+	+	–	–	–	–
EBP 3.9	+	–	+	–	–	–	–	–	–	+	–	+
ZAP 9.1	+	–	–	–	–	+	–	–	–	+	–	+
EBP 2.5	+	–	–	–	–	+	–	–	–	–	–	+
EBP 10.5	+	+	–	+	–	+	–	–	–	–	–	+
EBP 8.4	+	+	+	–	–	+	–	–	–	–	–	+
EBP 5.3	+	+	+	–	+	+	–	–	–	–	–	+
EBP 5.1	+	–	–	–	+	+	+	–	–	–	+	+
EBP 10.4	+	–	–	+	–	+	+	–	+	–	+	+
EAP 3.7	+	–	–	+	–	+	+	–	+	–	+	–
ECP 3.2	+	–	–	+	–	+	+	–	–	+	–	+
EBP 10.2	+	–	–	+	–	+	+	–	–	+	–	+
EBP 2.4	+	–	–	+	–	–	–	–	+	–	+	–
EBP 8.3	+	+	+	–	–	+	–	–	–	+	+	+
EBP 8.5	+	+	+	–	–	+	–	–	–	+	+	+

ZBP 9.2	+	−	−	−	−	+	−	−	+	+	+	−
ZCP 1.8	+	−	−	−	−	+	−	−	+	+	+	−
ZCP 17.1	+	−	−	−	−	+	−	−	+	+	+	−
ZCP 1.1	+	+	+	+	−	+	−	−	−	−	+	−
ZCP 6.5	+	−	−	−	−	−	−	+	−	−	+	−
ZAP 10.2	+	+	+	−	−	+	−	+	−	+	+	+
ZBP 9.7	+	+	+	−	−	+	−	+	−	+	+	+
ZAP 9.2	+	+	+	−	−	+	−	+	−	+	+	+
ZCP 13.2	+	+	−	+	−	+	−	+	−	+	+	+
ZCP 1.11	+	+	−	+	−	+	−	+	−	+	+	+
ZCP 1.10	+	−	−	+	−	−	+	−	−	−	+	+
ZCP 1.9	+	−	−	+	−	−	+	−	−	−	+	+
ZCP 1.8	+	+	−	+	−	−	+	−	−	+	+	+
ZCP 1.6	+	+	−	+	−	−	+	−	+	+	+	−
ZCP 17.5	+	+	−	−	+	+	−	−	−	−	+	+
ZCP 6.6	+	−	−	−	+	+	−	−	+	−	+	+
ZAP 9.4	+	−	−	−	+	+	−	+	−	+	+	−
ZAP 9.3	+	−	−	−	+	+	−	−	−	+	+	−
ZCP 6.4	+	−	−	−	−	−	+	−	+	−	+	+
EAP 8.9	+	−	−	−	−	−	+	−	+	−	+	+
EAP 6.4	+	−	−	−	−	−	+	−	+	−	+	+
EBP 8.7	+	+	+	−	+	−	−	+	−	+	+	−
EAP 6.6	+	−	−	+	−	+	+	−	+	−	+	−

Key: + indicates positive activity while – signifies negative reaction or no observable activity.

Cat-Catalase, Cit-Citrase, TSI-Triple Sugar Iron, MR-Methyl Red, VP-Voges Proskauer, Ind-Indole, Gel-Gelatin, Mot-Motility.

4.5 Physiological characterization

4.5.1 Bacterial growth at different pH

The isolates were able to grow at a wide range of pH including acidic, neutral and alkaline. There was minimal growth at pH 4 and 12 while there was optimal growth at pH 6, 8 and 10 (Figure 4.2).

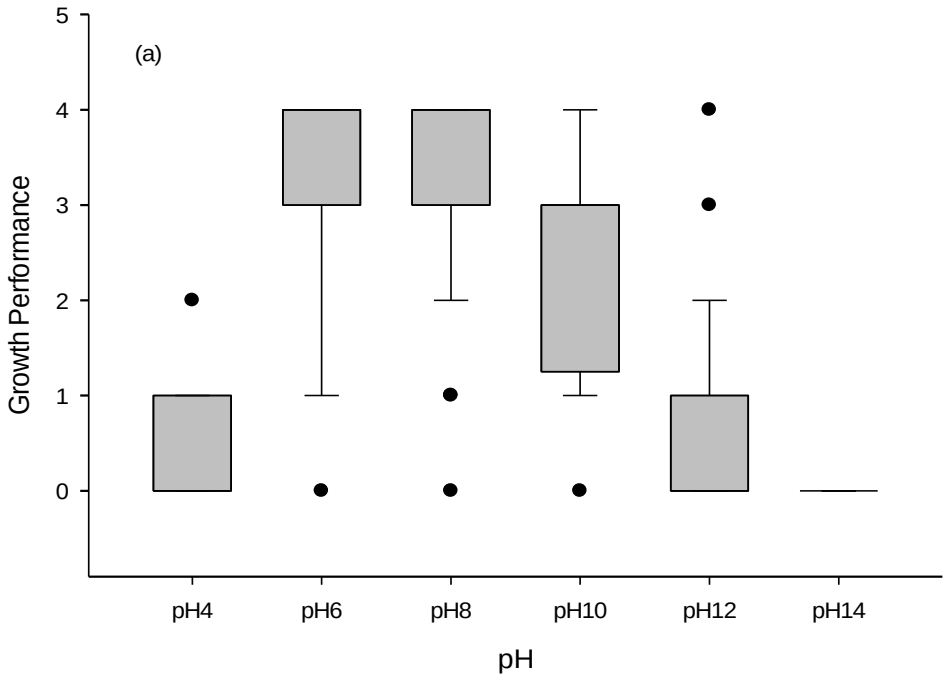

Figure 4.2: Growth performance of bacteria isolates from various sampling locations in Lake Oloiden and Olbolosat at different pH.

4.5.2 Bacterial growth at different sodium chloride concentration

All the 60 bacterial isolates were able to grow in varying sodium chloride concentrations. Growth increased with a decrease in sodium chloride concentration from 30 % to 0 %. Optimal growth was recorded at 0 % and 5 % sodium chloride concentrations (Figure 4.3).

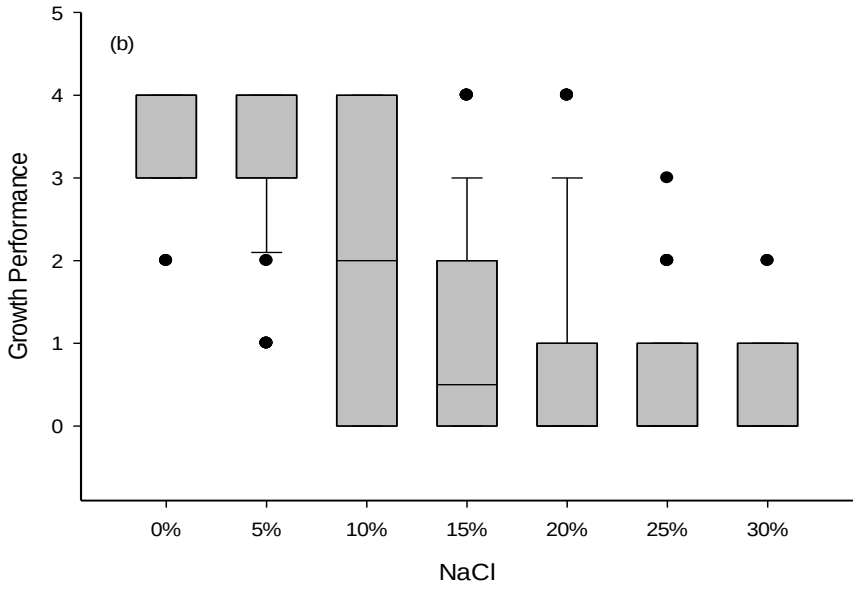

Figure 4.3:Growth performance of bacteria isolates from various sampling locations in Lake Oloiden and Olbolosat at different NaCl concentrations.

4.5.3 Bacterial growth at different temperatures

Growth of the bacterial isolates was optimal at 25 ℃ and 30 ℃ (Figure 4.4). There was a significant difference at $p<0.001$ for bacterial growth in all parameters; Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Tukey's HSD post hoc analysis.

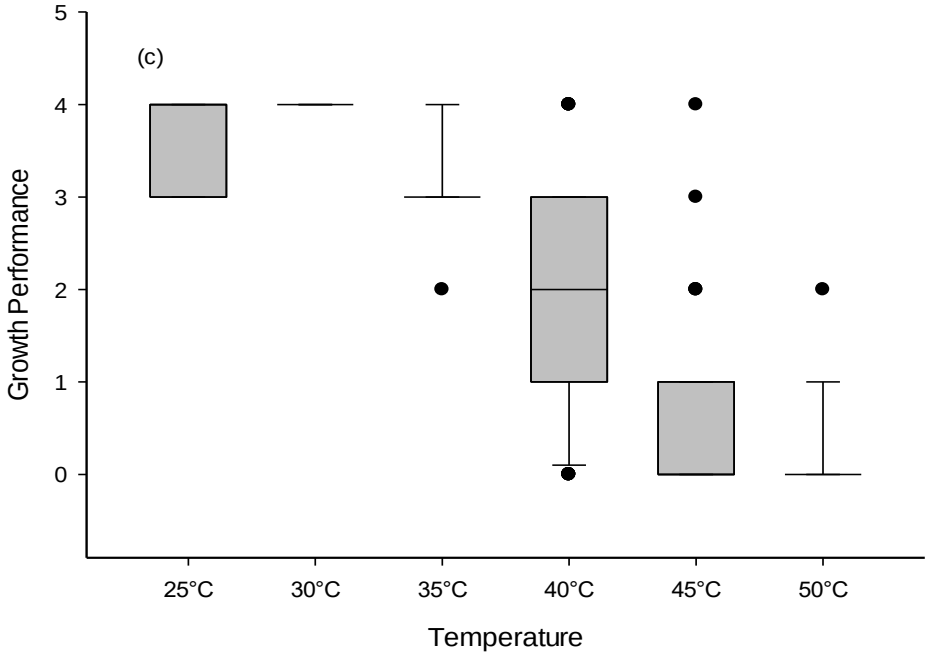

Figure 4.4: Growth performance of bacteria isolates from various sampling locations in Lake Oloiden and Olbolosat at different temperatures.

4.6 Hydrolase activity

Clustering from the heatmap shows that bacterial isolates were able to utilize different substrates indicating their ability to produce different enzymes. Correlation between enzyme hydrolysis activity and bacterial isolates revealed that there were two clusters. Fifty-nine isolates formed a single cluster while ECP 3.1 formed a solitary cluster. Among the five enzymes assayed, all others formed a common cluster while xylanase formed a solitary cluster. This shows that some of the isolates were not able to utilize xylan as a substrate. Hydrolase activity that recorded a positive result was indicated by the clear zone around the colony (Figure 4.5).

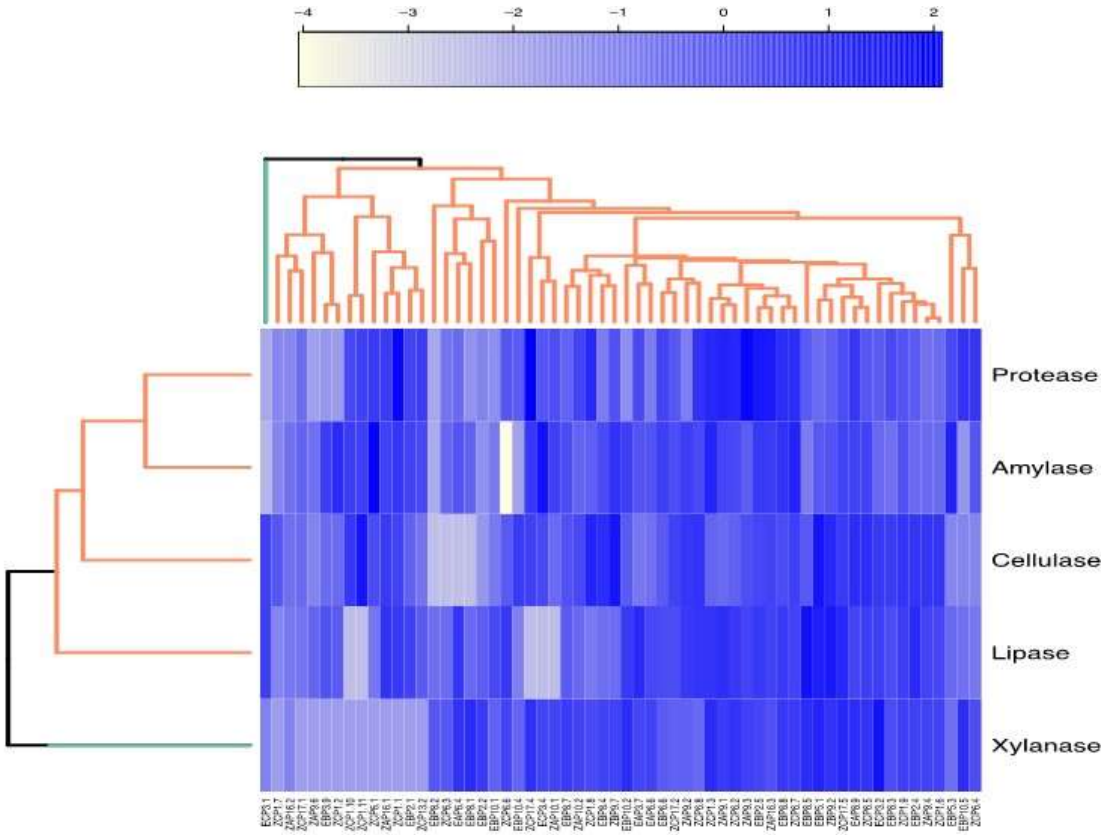

Figure 4.5: Hierarchical clustering based on Euclidean metric for bacterial isolates that produced enzymes.

4.7 Antimicrobial activity

The antagonistic activity was considered active by the formation of a zone of inhibition around the colony. Zone sizes of inhibitions were looked up on a standardized chart by following the Kirby-Bauer diffusion protocol for the sensitivity measuring above 18 mm, resistant 13 mm or less and intermediate measuring 14-16 mm. Clustering from the heatmap shows that bacterial isolates were sensitive measuring >18 mm to test organisms. Fifty-seven isolates formed one cluster, two isolates EBP 2.1 and ZCP 1.9 formed their cluster while ZCP 17.1 formed a solitary cluster. Among the five test organisms assayed *C. albicans* formed a single cluster. Correlation between antagonistic activity estimates and bacterial isolates revealed that there were two functional clusters (Figure 4.6).

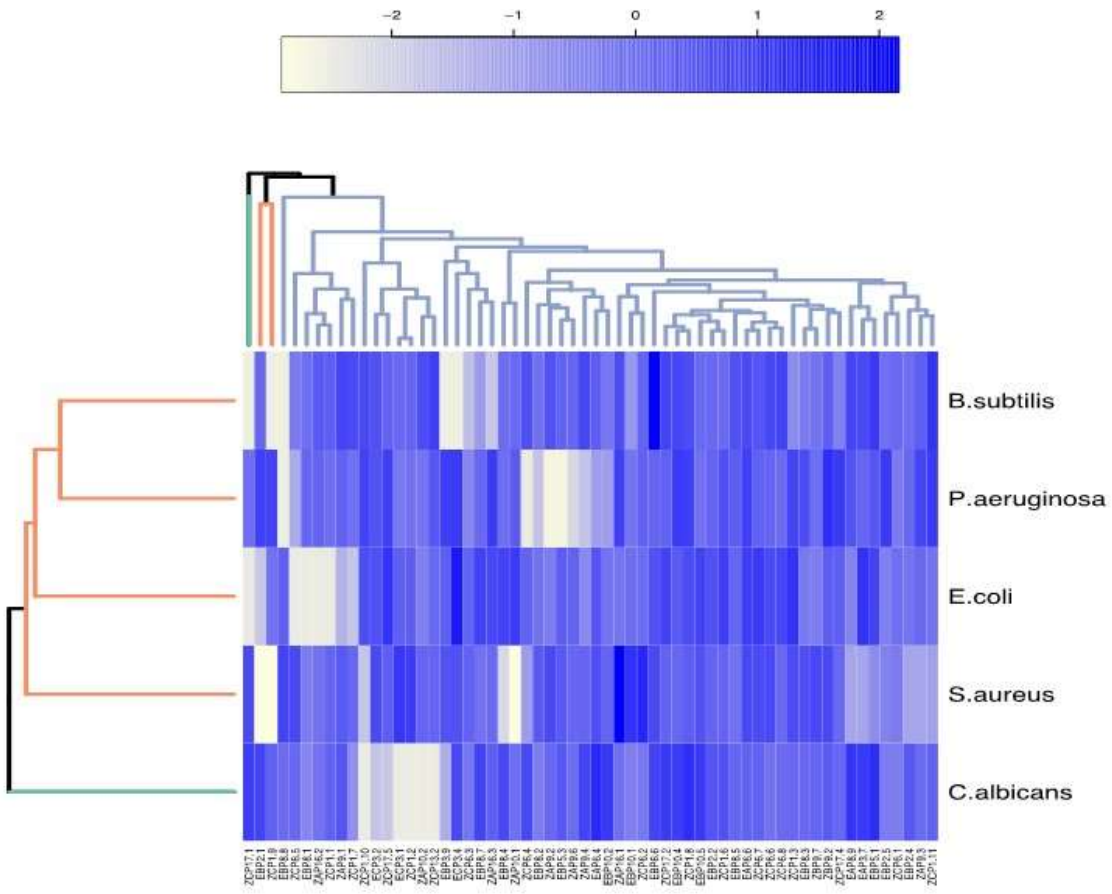

Figure 4.6: Hierarchical clustering based on Euclidean metric for bacterial isolates that produced antimicrobial activity against test organisms.

4.8 Taxonomic affiliation of partial sequences of 26 bacterial isolates from both lakes

Genomic DNA was extracted from all 60 bacterial isolates. Partial sequencing for the 16S rRNA gene using bacteria-specific primers yielded an amplification product of approximately 1500 base pairs. The 60 amplified PCR products were sequenced and only 26 were unambiguous, and their sequences were selected for phylogenetic analyses (Table 4.3).

There was one strain from the phylum Bacteroidetes closely affiliated to *Hymenobacter* sp scoring 98.56 % (Table 4.3). Eight isolates from the current study; ZCP 6.1[MT801064] closely affiliated *Bacillus* sp scored a percentage similarity of 97.43 %,), EBP 8.2 [MT801055] closely affiliated to *Bacillus pumilus* scored 97.26 %, EBP 3.9

[MT801075] closely affiliated to *Bacillus cereus* scored 96.45 %, ZCP 17.2 [MT801059] closely affiliated to *Bacillus toyoniensis* scored 97.28 %, ECP 3.4 [MT801078] closely affiliated to *Streptomyces* sp scored 98.56 %, EBP 2.5 [MT801076] closely to *Aeromicrobium* sp scored 97.79 %, ZAP 9.1[MT801072] closely affiliated to *Hymenobacter* sp scored 98.56 % while ZAP 16.1[MT801058] was closely affiliated to *Streptomyces hawaiiensis* had sequence similarity of 98.33 % (Table 4.3.) These strains represent novel genera of organisms within the lake ecosystem according to Kim *et al.*, (2014) who reported that a bacteria organism could be considered novel if the sequence similarity is <98.65 %. However whole genome sequencing should be carried out and DNA-DNA hybridization done. Out of 26 isolates submitted to the NCBI database, only 18 were assigned accession numbers MT801052-MT801078.

Bacillus group from Lake Oloiden scored >96 % sequence identity; two isolates of *B. pumilus* scored 99.69 % and 97.26 %. *Bacillus safensis* subsp *safensis* scored 99.78 %, *B. altitudinis* scored 98.79 %, *Bacillus* sp. scored 99.9 % while *B. cereus* scored 96.45 %. Among other Firmicutes were three isolates from the genus *Staphylococcus* scoring 99.79 %, 99.78 % and 99.59 % sequence identities *Staphylococcus succinus*, *S. arlettae* and *S. xylosus* respectively. Phylum Actinobacteria was affiliated to four different genera *Microbacterium oxydans* with a score of 99.47 %, *Streptomyces* sp scored 98.56 %, *Aeromicrobium* scored 97.79 % while *Streptomyces hawaiensis* scored 98.73 % sequence identities. The Phylum Proteobacteria was closely affiliated with only one isolate of *Pseudomonas* sp. scoring 99.59 % sequence identity.

Three isolates from the Firmicutes phylum (ZCP 1.3, ZCP 6.2 and ZAP 10.1) scoring 99.28 %, 99.54 % and 98.84 % were with sequence identities with known members of the genera *Paenibacillus oryzisoli*, *Bacterium* sp and *Exiguobacterium* sp respectively. Among these were *Bacillus* group from Lake Olbolosat scoring >97 % sequence identity; *Bacillus* sp recorded three different strains scoring 99.59 %,100 % and 97.43 %, *B. megaterium* scored 99.48 %, *B. aryabhattai* scored 99.89 %, *B. simplex* scored 99.6 %, *B. subtilis* scored 98.84 % while *B. toyoniensis* scored 97.28 % (Table 4.3).

Table 4.3: Taxonomic affiliation of partial sequences of 26 bacterial isolates from both lakes with closest relatives from the GenBank database.

Isolate	Sampling site	Isolation Source	Accession number	Closest taxonomic match in BLAST	Query cover	E-value	ID %	Affiliated to	G+
ZAP 16.3	Lake Olbolosat	Water	MT801074	*Pseudomonas sp.* strain LC128	100	0.0	99.59	JQ014351.1	53
ZBP 9.6	Lake Olbolosat	Dry sediment	MT801060	*Bacillus sp.* strain SC134	100	0.0	99.5	MN192133.1	53
ZCP 1.2	Lake Olbolosat	Wet sediment	MT801061	*Bacillus sp.* strain K1	100	0.0	100	MH628021.1	54
ZCP 1.3	Lake Olbolosat	Wet sediment	MT801062	*Paenibacillus oryzisoli* strain 1ZS3-15	100	0.0	99.28	NR_164873.1	55
ZCP 1.7	Lake Olbolosat	Wet sediment	MT801063	*Bacillus megaterium* strain ICMM1	100	0.0	99.48	MN889411.1	55
ZCP 17.4	Lake Olbolosat	Wet sediment	MT801069	*Staphylococcus succinus* strain cqsM8	100	0.0	99.79	MN826566.1	51
ZCP 6.1	Lake Olbolosat	Wet sediment	MT801064	*Bacillus sp.* strain ME76	100	0.0	**97.43**	LR861557.1	54
ZCP 6.2	Lake Olbolosat	Wet sediment	MT801065	*Bacterium* strain TPMX-4	100	0.0	99.54	KY427680.1	53
ZCP 6.3	Lake Olbolosat	Wet sediment	MT801066	*Bacillus aryabhattai* strain A6-P	100	0.0	99.89	MT588720.1	54
ZCP 6.7	Lake Olbolosat	Wet sediment	MT801067	*Staphylococcus arlettae* strain KTSMBNL-77	100	0.0	99.78	KM200327.1	52
ZCP 6.8	Lake Olbolosat	Wet sediment	MT801068	*Bacillus simplex* strain ER20	99	0.0	99.6	MT124545.1	54
EBP 10.1	Lake Oloiden	Dry sediment	MT801056	*Bacillus safensis subsp. safensis* strain EGI17	99	0.0	99.78	MN704393.1	55
EBP 2.1	Lake Oloiden	Dry sediment	MT801052	*Microbacterium oxydans* strain I-S-R2-2	100	0.0	99.47	MK398050.1	57
EBP 2.2	Lake Oloiden	Dry sediment	MT801053	*Bacillus pumilus* strain J8R13LARS	100	0.0	99.69	MT378474.1	55
EBP 8.1	Lake Oloiden	Dry sediment	MT801054	*Bacillus altitudinis* strain SR1-56	100	0.0	98.79	LN995455.1	55
EBP 8.2	Lake Oloiden	Dry sediment	MT801055	*Bacillus pumilus* strain PK3	100	0.0	**97.26**	MH428223.1	55
ECP 3.1	Lake Oloiden	Wet sediment	MT801057	*Bacillus sp.* strain HBUM207125	100	0.0	99.9	MT598008.1	55
ECP 3.4	Lake Oloiden	Wet sediment	MT801078	*Streptomyces sp.* strain SL37	100	0.0	**98.56**	MN812679.1	57
EBP 2.5	Lake Oloiden	Dry sediment	MT801076	*Aeromicrobium sp. strain Bt13*	41	0.0	**97.79**	KP195230.1	63
EBP 3.9	Lake Oloiden	Dry sediment	MT801075	*Bacillus cereus* strain PR37	78	0.0	**96.45**	MN232152.1	51
EBP 8.8	Lake Oloiden	Dry sediment	MT801077	*Staphylococcus xylosus* strain JM41	100	0.0	99.59	MN758801.1	55
ZAP 9.1	Lake Olbolosat	Water	MT801072	*Hymenobacter sp* strain R2A-W5	99	0.0	**98.56**	FJ627043.1	55
ZAP 10.1	Lake Olbolosat	Water	MT801071	*Exiguobacterium sp.* strain Mong-10	99	0.0	98.73	KY962739.1	55
ZAP 16.1	Lake Olbolosat	Water	MT801058	*Streptomyces hawaiiensis* strain HDJZ-ZWM-20	99	0.0	**98.33**	GU227347.1	59
ZAP 16.2	Lake Olbolosat	Water	MT801070	*Bacillus subtilis* strain MA6	78	0.0	98.84	KT758735.1	55
ZCP 17.2	Lake Olbolosat	Wet sediment	MT801059	*Bacillus toyonensis* strain HRT5	99	0.0	**97.28**	MH197375.1	54

4.9 Phylogenetic analysis for the bacterial isolates

Bacterial isolates were clustered into four different Phyla belonging to Firmicutes, Actinobacteria, Proteobacteria and Bacteriodetes for both lakes Ololosat and Oloiden (Figures 4.7 and 4.8). Firmicutes were 77 % closely affiliated with twenty isolates, Actinobacteria was 15 % closely affiliated with four isolates while Proteobacteria and Bacteriodetes were 4 % closely affiliated with each isolate from both lakes BLAST analysis of the partial sequences showed there were fourteen isolates (54 %) that were closely affiliated with the members of the genus *Bacillus* with >96 sequence identity from both lakes (Figures 4.7 and 4.8). There were 10 isolates from Lake Oloiden revealing two clusters. One cluster had strains from Firmicutes with a bootstrap value of 99 while the other one had strains belonging to Actinobacteria with a bootstrap value of 57: one strain formed a node from the latter cluster with a bootstrap value of 50 *Trichorderma* sp. (HQ630962.1) was used to root the tree. Only bootstrap values above 50 are shown. The scale bar indicates approximately 0.01 the sequence difference. Lakes Oloiden is indicated with prefix E (Figure 4.7). The phylogenetic tree of the 16S rRNA partial sequences of the 16 isolates from Lake Olbolosat revealed two major clusters. One cluster had isolates belonging to Firmicutes, proteobacteria and Bacteroidetes with bootstraps values 93, 100 and 100 respectively while the other one had isolates belonging to Actinobacteria Phyla with bootstraps values of 98. *Trichorderma* sp. (HQ630962.1) was used to root the tree. Only bootstrap values above 50 are shown. The scale bar indicates approximately 0.01 of the sequence differences. Lakes Olbolosat is indicated with prefix Z (Figure 4.8).

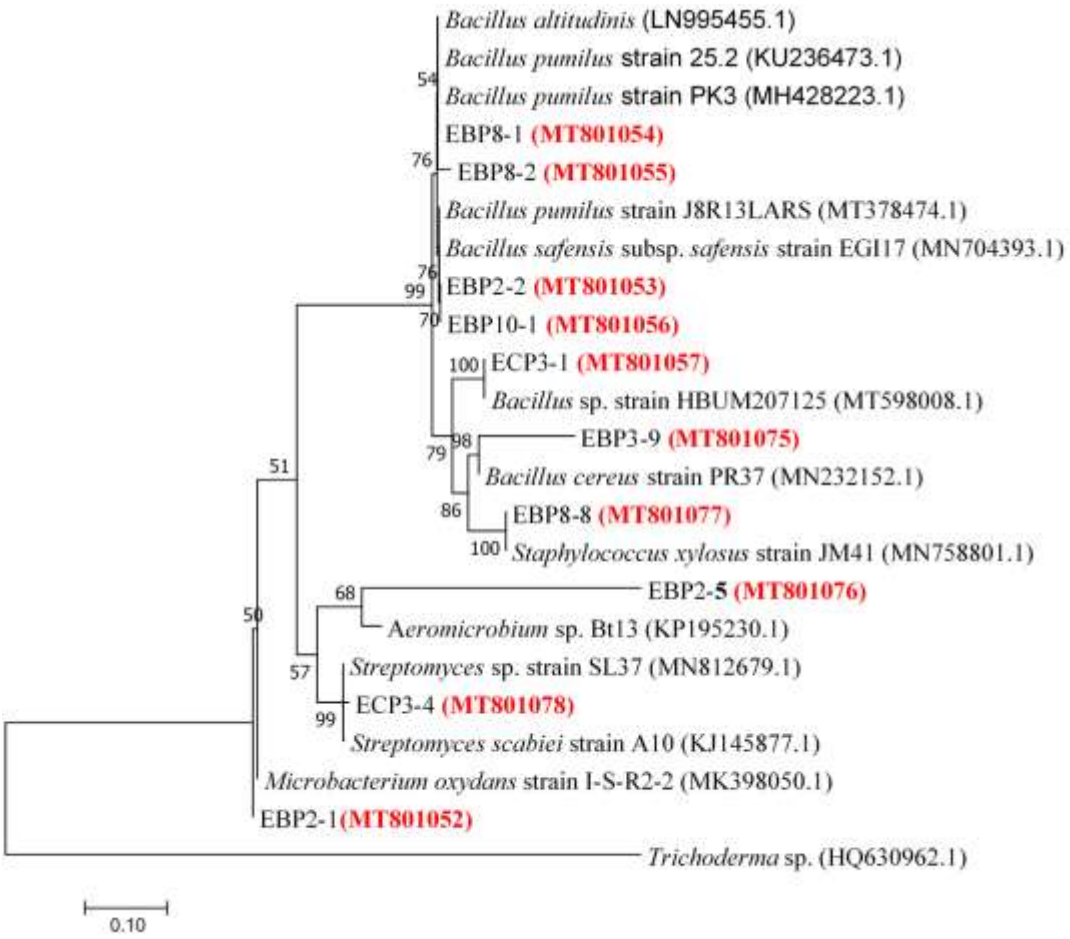

Figure 4.7: Phylogenetic tree displaying the evolutionary relationship between partial 16S rRNA gene sequences from Lake Oloiden and closest neighbor isolate.

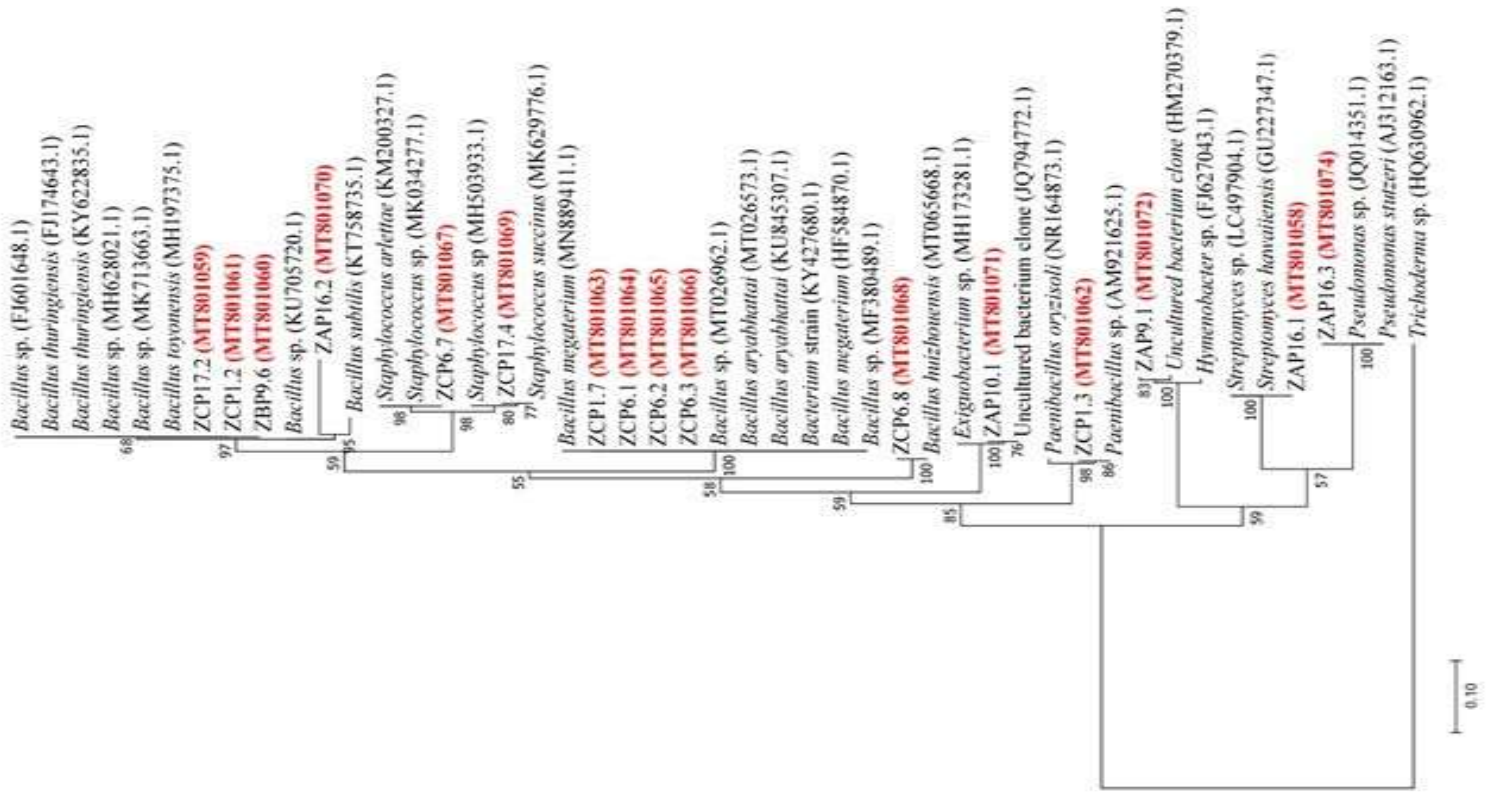

Figure 4.8: Phylogenetic tree displaying the evolutionary relationship between partial 16S rRNA gene sequences from Lake Olbolosat and closest neighbor isolate.

4.10 Diversity of bacterial communities

A total of 274,892 sequence reads were obtained after removing chimeras from 15 DNA amplicon data sets. Total ASV richness at 1 % distance amounted to 10,244 ASVs distributed among 17 highly abundant phyla and 54 highly abundant genera. The ASV per data set ranged between 1 and 392. Out of the 10,244 ASVs, 280 (2.66 %) ASVs were shared between lakes Oloiden and Olbolosat. Consequently, lakes Oloiden and Olbolosat constituted 4973 (47.27 %) ASVs and 5271 (50.09 %) ASVs, respectively (Figure 4.9 a). The distribution of shared ASVs between sample types revealed an overlap between water and wet sediments in L. Olbolosat. Dry sediments and the microbial mat had the lowest overlap in the same lake (Figure 4.9b). Wet sediments and mat had the greatest overlap while dry sediment and mat had the lowest overlap in L. Oloiden (Figure 4.9 c). Overall, wet sediments and dry sediments shared a larger number of ASVs while dry sediment and mat had the lowest overlap across lakes Olbolosat and Oloiden (Figure 4.9 d). Further, the high overlap between sample types was supported by the ANOSIM which revealed a significant difference in ASV composition between the two lakes ($r = 0.191$, $p = 0.048$), and between the sample types ($r = 0.6667$, $p = 0.001$) (Appendix VII).

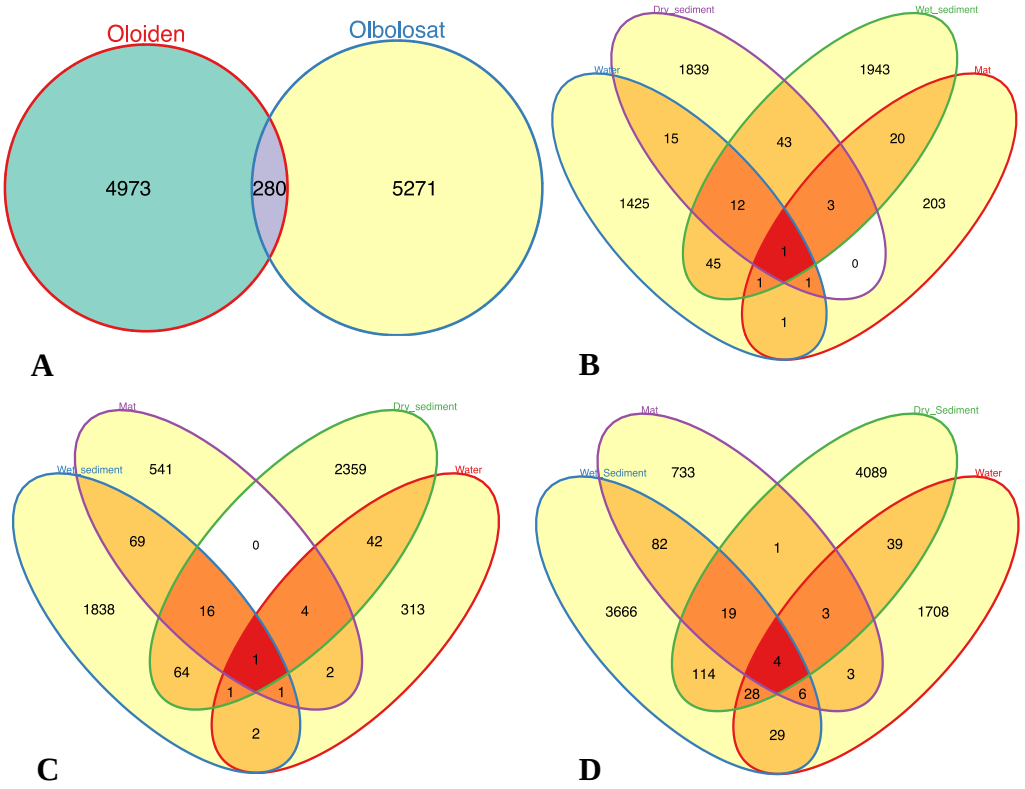

Figure 4.9: Venn diagram showing the distribution of unique and shared ASVs.

4.10.1 Bacterial alpha diversity

Alpha diversity indices were significantly ($p < 0.05$) different among samples (Figure 4.10 and Table 4.4). The number of sequences per sample ranged from 2730 to 34827 with a mean value of 18326.13 and 12533.76 standard deviations (Table 4.4). Dry sediments from both lakes had the highest number of sequences of ZC1 and EC1 (Table 4.4). All the indices except Simpson were highest in the dry sediment sample (EC1) followed by the dry sediment sample (EC2) both from lake Oloiden. The lowest alpha diversity was in the water sample (EA1) from lake Oloiden) (Table 4.4). The rarefaction curves also indicated that all the sites were far from being exhaustively sampled (Appendix VIII and Appendix IX).

Table 4.4: Alpha diversity indices computed on all ASVs-based microbial taxonomic units within 16S rRNA.

Site	No. of Sequences	Observed	Chao1	ACE	Shannon	Simpson	Inverse Simpson
EA1	2730	197	224.029	240.110	4.627	0.986	71.759
EA2	3344	212	222.150	236.312	4.832	0.990	96.863
EB1	26691	1181	1205.265	1238.172	6.375	0.997	342.655
EB2	16645	933	989.953	1053.067	6.181	0.997	351.963
EC1	33878	1331	1357.750	1401.321	6.696	0.998	623.953
EC2	28223	1259	1291.659	1343.116	6.624	0.998	582.833
ED1	5223	293	300.065	307.122	5.136	0.991	117.433
ED2	6495	343	352.220	360.925	5.492	0.995	201.261
ZA1	5146	379	400.468	415.263	5.309	0.993	142.890
ZA2	28819	1164	1189.084	1224.216	6.446	0.998	415.700
ZB1	29579	1220	1237.243	1270.553	6.555	0.998	525.634
ZB2	23268	1015	1023.797	1051.801	6.402	0.998	465.894
ZC1	34827	1187	1219.591	1245.735	6.569	0.998	531.790
ZC2	26807	886	897.938	916.577	6.271	0.998	406.068
ZD2	3217	229	255.640	255.299	4.912	0.990	100.626

4.10.2 The abundance of the bacterial taxa

Composition analysis of the 16S rRNA ASVs comprised of the kingdom Bacteria (88.24 %) and Archaea (11.76 %). These results suggest that bacteria were the dominant taxa and the prominent phyla were Proteobacteria (33.8 %), Firmicutes (27.3 %), Actinobacteriota (21.2 %), Chloroflexi (6.8 %), Cyanobacteria (3.8 %), Acidobacteriota (2.8 %), Planctomycetota (1.9 %), Bacteroidota (1.1 %), Deinococcota (0.16 %), Gemmatimonadota (0.16 %), Verrucomicrobiota (0.12 %), Bdellovibrionota (0.12 %), Armatimonadota (0.12 %), Patescibacteria (0.1 %), and Campilobacterota (0.05 %). The prominent Archaea phyla in the prokaryotic composition were Halobacterota (0.14 %), and Euryarchaeota (0.06 %). The remaining 0.93 % were occupied by other less abundant phyla (<0.002 %) phyla (Figure 4.10).

Though Proteobacteria was the dominant phyla in the bacterial kingdom, the trend changed between sample types. For instance, the microbial mat samples were dominated by the Firmicutes (66.3 %) against Proteobacteria (15.6 %) (Figure 4.10) (Appendix X and Appendix XI).

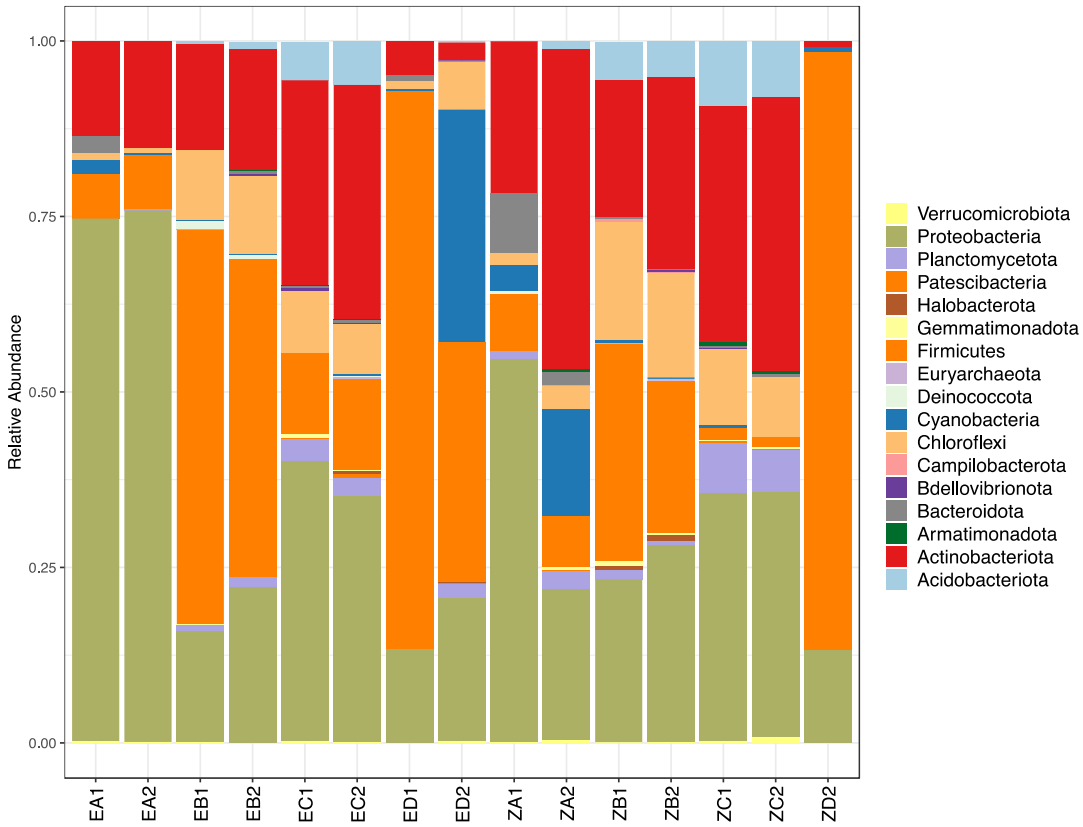

Figure 4.10: Relative abundance of the most predominant phyla in various sample types collected within Lakes Olbolosat and Oloiden.

The dominant phylum Proteobacteria was represented by two classes, α-Proteobacteria (75.3 %) and γ-Proteobacteria (24.7 %) which constituted 38 and 19 families, respectively. Among the abundant families belonging to class α-Proteobacteria were *Xanthobacteraceae* (18.3 %), *Rhodobacteraceae* (12 %), *Sphingomonadaceae* (11.5 %), and *Berinckiaceae* (9.3 %). Firmicutes was the second most abundant phylum represented by class Clostridia and four families *Peptostreptococcaceae* (74 %), Clostridia (20 %), *Lachnospiraceae* (4 %), and *Peptostreptococcales-Tissierellales_fa* (2 %). Class Clostridia dominated wet sediments from Lake Olbolosat (wet sediment,

15.54-28.66 %; Dry sediment, 0.65-13.3 %; water, 0.14-5.66 %) compared to Lake Oloiden wet sediments (wet sediment, 5.58-8.01 %; Dry sediment, 0.68-7.96 %; water, 0.49-3.16 %). Clostridia were, however, highly abundant in Lake Oloiden microbial mat samples (4.08-5.96 %) compared to the Lake Olbolosat microbial mat samples (0-0.13 %). Dominant families, *Peptostreptococcaceae* dominated microbial mat samples (1-3.1 %). *Lachnospiraceae* dominate wet sediment (1 %), and microbial mat (1.8 %), while *Peptostreptococcales-Tissierellales_fa* dominated the mat (1.1 %) (Appendix XII).

The phylum Actinobacteriota was the third as shown in Figure 4.10, most abundant among the sample types and it was represented by five classes. Among the dominant classes were Actinobacteria (78.6 %) and Acidimicrobiia (15.6 %). Actinobacteria were highly abundant in L. Oloiden (Dry sediment, 9.38-16.61 %; wet sediment, 0.44-14.57 %; water, 9.5-14.56 %; microbial mat, 0.05-0.23%) compared to L. Olbolosat (Dry sediment, 1.79-4.16 %; wet sediment, 5.15-8.4 %; water, 0.54-13.8 %; mat, 0-0.82 %) (Figure 4.11). The abundance of class Acidimicrobiia varied between sample types across the two lakes. They were highly dominant in the L. Oloiden water samples (water, 3.18-54.49 %; wet sediment, 0-5.75 %; Dry sediment, 4.12-5.38 %; mat, 0-0.31%) compared to L. Olbolosat water samples (water, 0.49-7.29 %; wet sediment, 5.4-8.69 %; Dry sediment, 1.04-3.61 %; mat, 0-0.25 %) (Figure 4.11). Phylum Chloroflexi was the fourth most dominant represented by class Anaerolineae (45.7 %), Chloroflexia (44.6 %), and Ktedonobacteria (9.5 %). Class Anaerolineae was abundant in L. Olbolosat (2.2-34.18 %) compared to L. Oloiden (0-0.84 %). They were associated with dry sediments (34.18 %), water samples (23.5 %), wet sediments (13.14 %), and lower abundance was associated with microbial mat samples (0-0.84 %) (Figure 4.11). Class Chloroflexia was highly abundant in L. Oloiden (Dry sediment, 6.95-15.23 %; wet sediment, 0.05-14.51 %; water, 7.01-8.36 %; mat, 0.00-3.46 %) compared to L. Olbolosat (Dry sediment, 0.89-9.83 %; wet sediment, 10.63-12.26 %; water, 0.08-10.71 %; mat, 0.05 %) (Figure 4.11). Similarly, class Ktedonobacteria was highly abundant in Lake Oloiden (Dry sediment, 1.03-52.82 %; wet sediment, 0.0-9.44 %; water, 0.0-35.09 %; microbial mat, 0 %) compared to L. Olbolosat (Dry sediment, 0 %; wet sediment, 0.34-0.51 %; water, 0.0-0.77 %; mat, 0.0 %). The abundant archaea were represented by four classes, Methanobacteria (33.3

%), Methanocellia (28.6 %), Methanosacinia (23.8 %), and Methanomicrobia (14.3 %) (Figure 4.11).

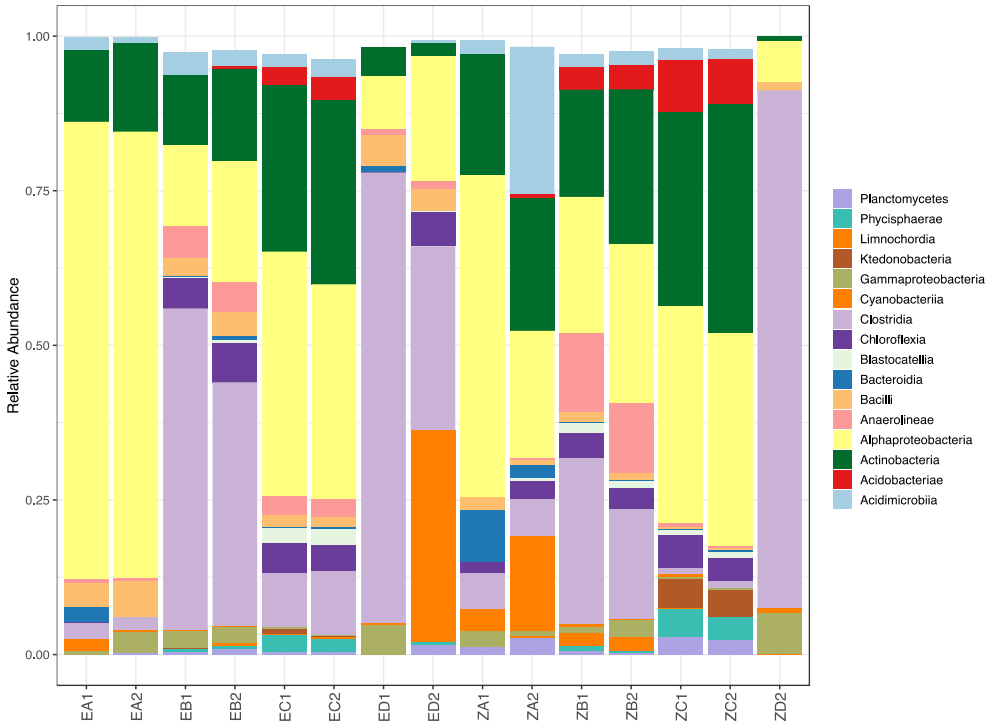

Figure 4.11: Relative abundance of the most predominant classes in various sample types collected within Lakes Olbolosat and Oloiden.

4.10.3 Bacterial indicator species analysis

Indicator species analysis using lambdsv package revealed most genera affiliated to major phyla were associated with wet sediments, dry sediments, and microbial mat samples across lakes Olbolosat and Oloiden. The representatives of the genera associated with the wet sediments were distributed among Proteobacteria (*Bradirhizobium*, *Sphingomonas*, p <0.05), Firmicutes (*Clostridium_sensu_stricto_10*, *Exiguobacterium*, p <0.05), Actinobacteriota (*Pseudonocardia*, *Streptomyces*, p <0.05), Chloroflexi (*Anaerolinea*, p <0.05), and Acidobacteriota (*Candidatus_Solibacter*, *Bryobacter*, p <0.05). Genus *Anaerolinea* was the indicator species in the wet sediments with an indicator value of 0.88 and it was closely followed by genus *Clostridium_sensu_stricto*_10 at a 0.76 indicator value.

Genus *Candidatus_Solibacter* was the indicator species in dry sediments with an indicator value of 0.84, while *Exiguobacterium* was the indicator species in the microbial mat with an indicator value of 0.98. The frequency of indicator species ranged between 6 to 10. Genera belonging to phylum Firmicutes (*Clostridium_sensu_stricto*_10 and *Exiguobacterium*) were represented with the highest (10) and lowest (6) frequency in wet sediments and microbial mat, respectively (Figure 4.12) (Appendix XIII).

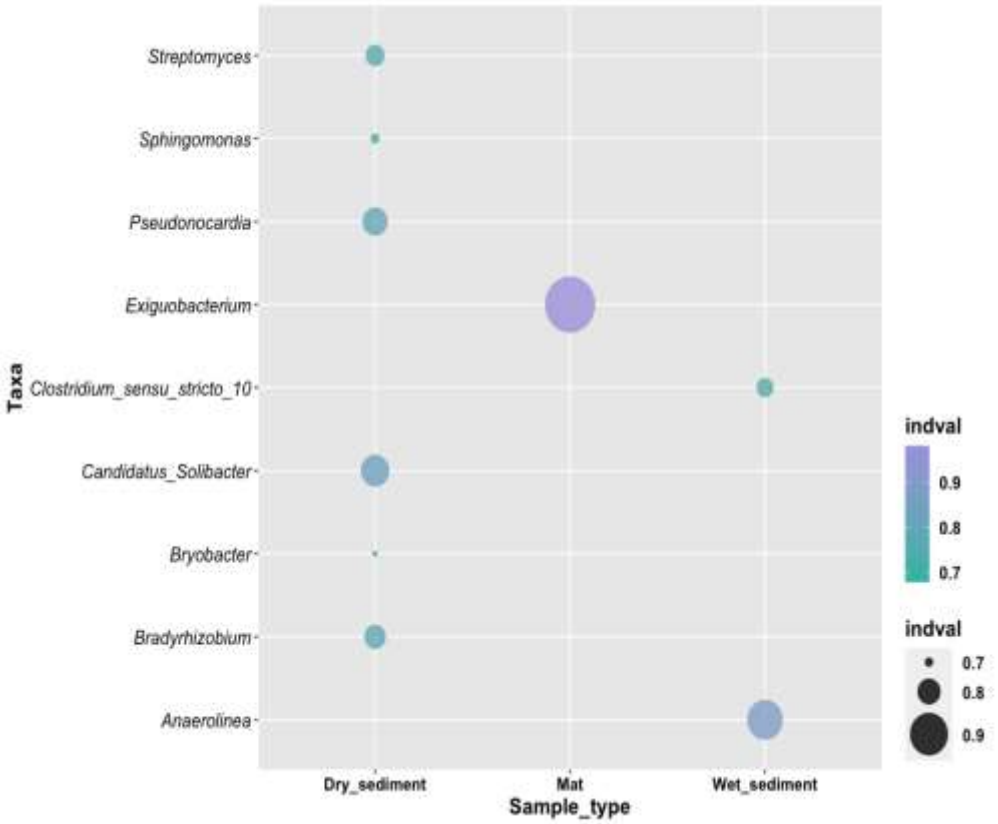

Figure 4.12: Indicator species within lakes Olbolosat and Oloiden at the genus level.

4.10.4 Bacterial community composition and correlation with environmental factors

Principal Component Analysis indicated that community compositions were clustered according to the locations (Lakes) of the sampling sites (Figure 4.13a). Bacterial community compositions were also clustered based on the sample types across the

lakes (Figure 4.13 b-d). The distribution of the ASVs in lake Olbolosat was associated with Cu^{+}, Fe^{2+}, and Mn^{2+}, while the distribution of ASVs in lake Oloiden was associated with Mg^{2+}, Na^{+}, and K^{+} (Figure 4.13a). The concentration of anions F^{-} was associated with the distribution of ASVs in the wet sediments while the distribution of ASVs in the water samples and dry sediments was associated with NO_2^{-} and PO_4^{3-}, respectively. The concentration of SO_4^{3-}, HCO_3^{-}, and Cl^{-} were positively associated with water samples (Figure 4.13b). The concentration of TDS and DO were associated with the distribution of ASVs in water samples, while the distribution of ASVs in dry sediment samples was associated with the Ec and temperature (Figure 4.13 c). Similarly, the distribution of the ASVs between water and microbial mat samples was associated with NH_4^{+}, Na^{+}, and Mg^{2+}, while Fe^{2+} concentrations were associated with the water and wet sediments. Mn^{2+} and Cu^{+} were distinctly associated with dry sediments and microbial mats, respectively. Potassium ions (K^{+}) were, however, associated with the microbial mat samples (Figure 4.13d).

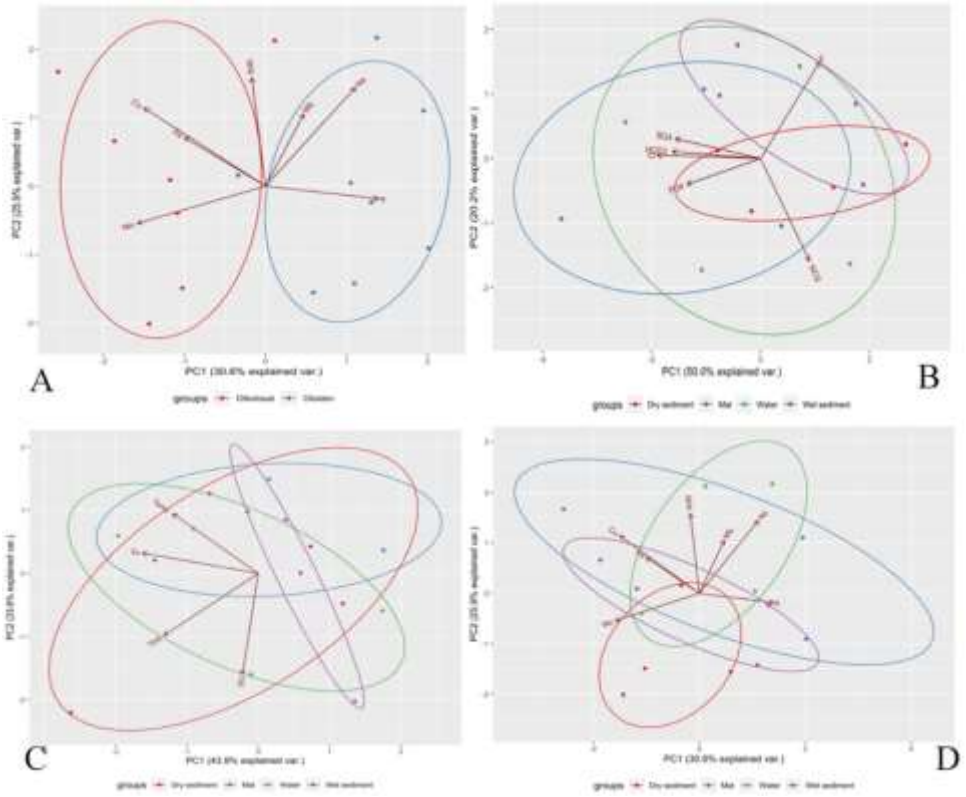

Figure 4.13: Principal component analysis based on a Bray-Curtis similarities matrix between ASVs of 16S rDNA datasets

The effects of environmental factors on bacterial diversity and community composition were supported by the Permutation Multivariate Analysis of Variance (PERMANOVA). Nine out of the seventeen factors strongly influenced the variation between samples and distribution of ASVs with R^2 ranging from 0.38 to 0.71 and $p < 0.05$ (Table 4.5). The effect of temperature on the ASV distribution in water sample was about two times ($R^2 = 0.71$, $p = 0.002$) the impact of nitrite ($R^2 = 0.38$, $p = 0.045$). The distribution of ASVs in the microbial mat and water samples were strongly influenced by Magnesium ($R^2 = 0.70$, $p = 0.005$), while the distribution of ASVs and sample variations in the wet sediment were influenced by Manganese ($R^2 = 0.58$, $p = 0.008$). A subset analysis of a correlation between the ions and the ASVs revealed Magnesium and Manganese as the greatest predictors of microbial community structures (r = 0.39). Further, analysis of physical parameters also revealed dissolved oxygen (DO) and Temperature as the best predictors (r = 0.17) of microbial community structures in dry sediments and water samples, respectively. Consequently, the subset analysis of a correlation between the cations and ASVs revealed sulfate as the predictor of the distribution of bacterial community structure in the microbial mat and water samples (r = 0.17) (Table 4.5).

Table 4.5: Results from individual PERMANOVA analyses on selected environmental factors, using Pearson's product-moment correlation on variance-stabilizing transformed counts.

Factor	R^2	*p*
Temperature (Temp)	0.71	0.002**
Magnesium (Mg^{2+})	0.70	0.005**
Manganese (Mn^{2+})	0.58	0.008**
Bicarbonate (HCO_3^-)	0.56	0.005**
Sulphate (SO_4^{3-})	0.54	0.007**
Chloride (Cl^-)	0.46	0.023*
Total Dissolve Substances (TDS)	0.44	0.031*
Dissolve Organic (DO)	0.42	0.042*
Nitrite (NO_2^-)	0.38	0.045*
Copper (Cu^+)	0.31	0.123
Electrical Conductivity (Ec)	0.20	0.249
Fluoride (F^-)	0.09	0.564
Potassium (K^+)	0.06	0.713
Sodium (Na^+)	0.04	0.778
Ammonium (NH_4^+)	0.04	0.786
Iron (Fe^{2+})	0.00	0.782
Phosphate (PO_4^{3-})	0.00	0.993

Mantle statistical test Pearson's product-moment correlation did not show any significant correlation between community similarity and spatial proximity to each of the environmental factors. Ordination cluster analysis showed that sample types that clustered together had similar bacterial community structures to the sample types that clustered independently or further away (Figure 4.14).

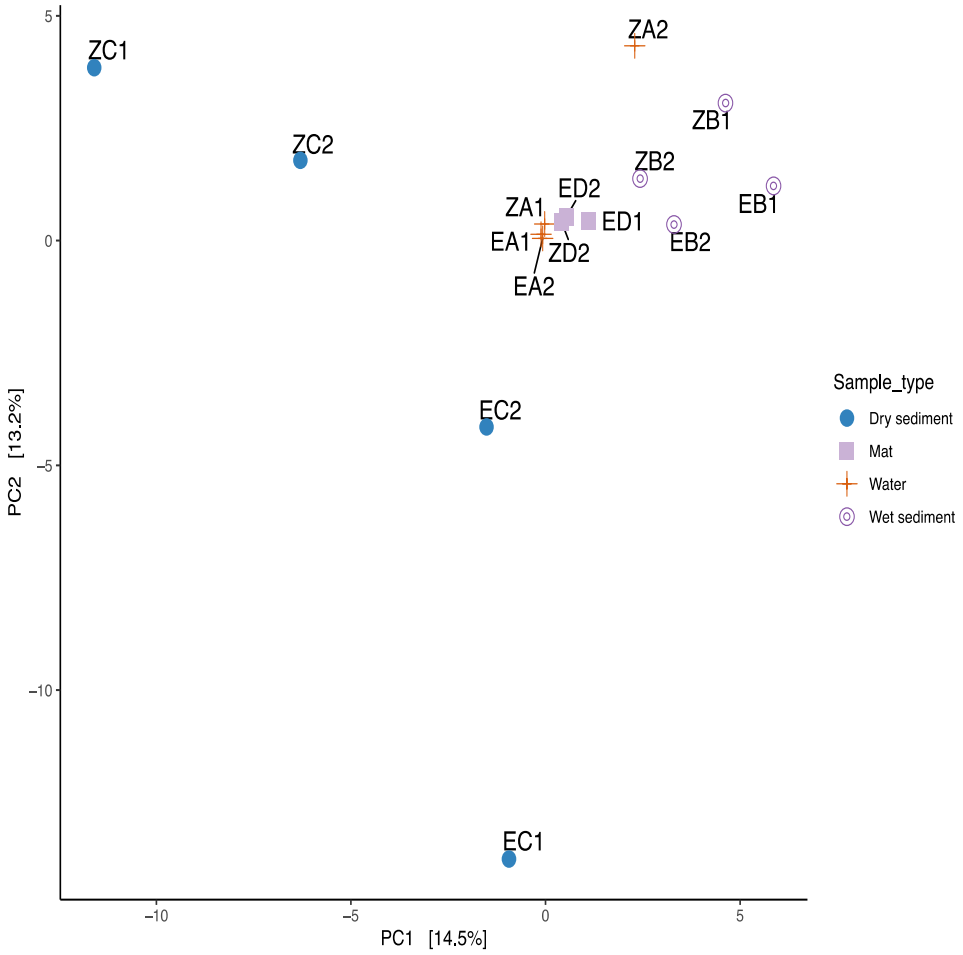

Figure 4.14: PCA ordination plot for all sample types, using Bray-Curtis dissimilarity distance, colored by sample type.

The clustering and distribution of ASVs were also supported by hierarchical clustering in which samples were clustered according to the sample types. The hierarchical cluster of the highly abundant ASVs also indicated that major phyla and minor phyla clustered separately (Figure 4.15). The ordination and hierarchical cluster results were significantly (*r = 0.07143, p = 0.041*) supported by the ANOSIM.

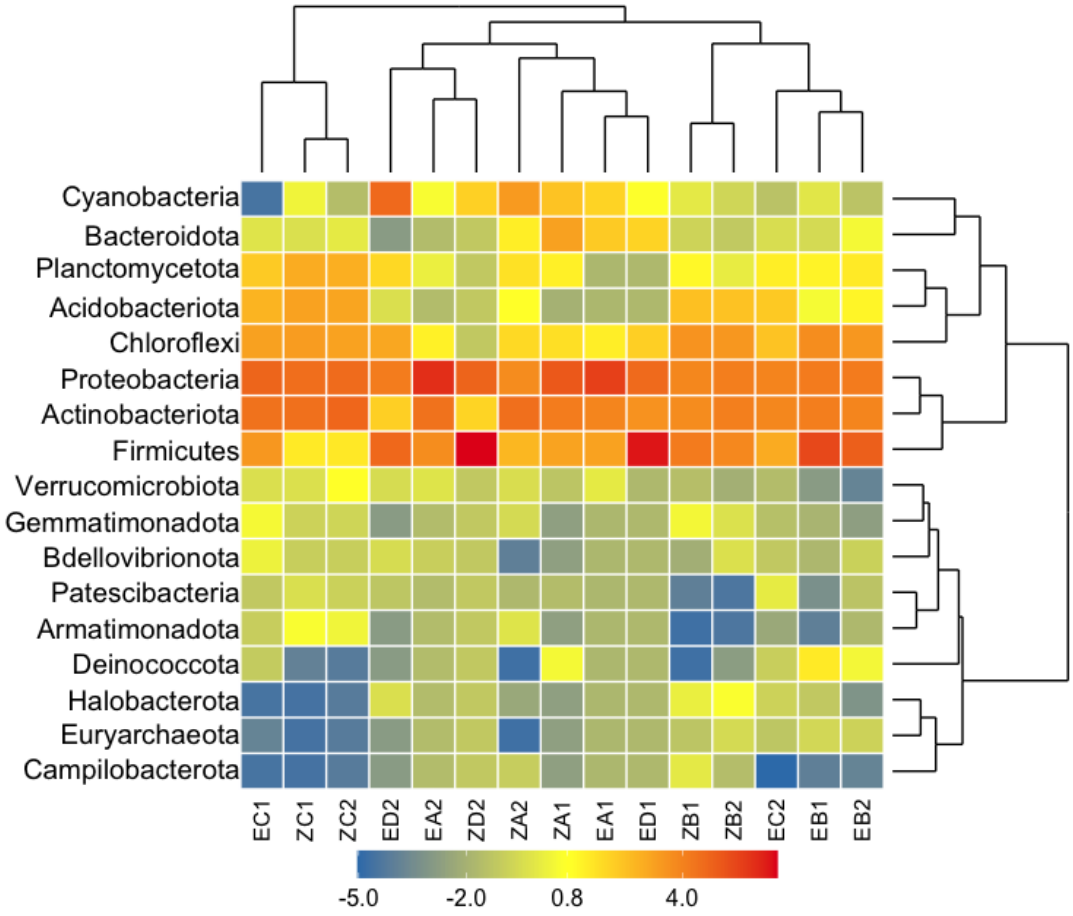

Figure 4.15: Hierarchical clustering of phyla assessing the relationship between sample types and bacterial taxa based on Bray-Curtis dissimilarity of the highly abundant taxa at the phylum.

Further, aldex.ttest and aldex.effect functions revealed a total of 5 differentially abundant (we.ep < 0.05) ASVs from 70 indicators ASVs between the sample types. Most ASVs were, however, non-differentially abundant across the samples. The differentially abundant ASVs formed 7.1% of the total indicator ASVs from the sampling sites (Figure 4.16).

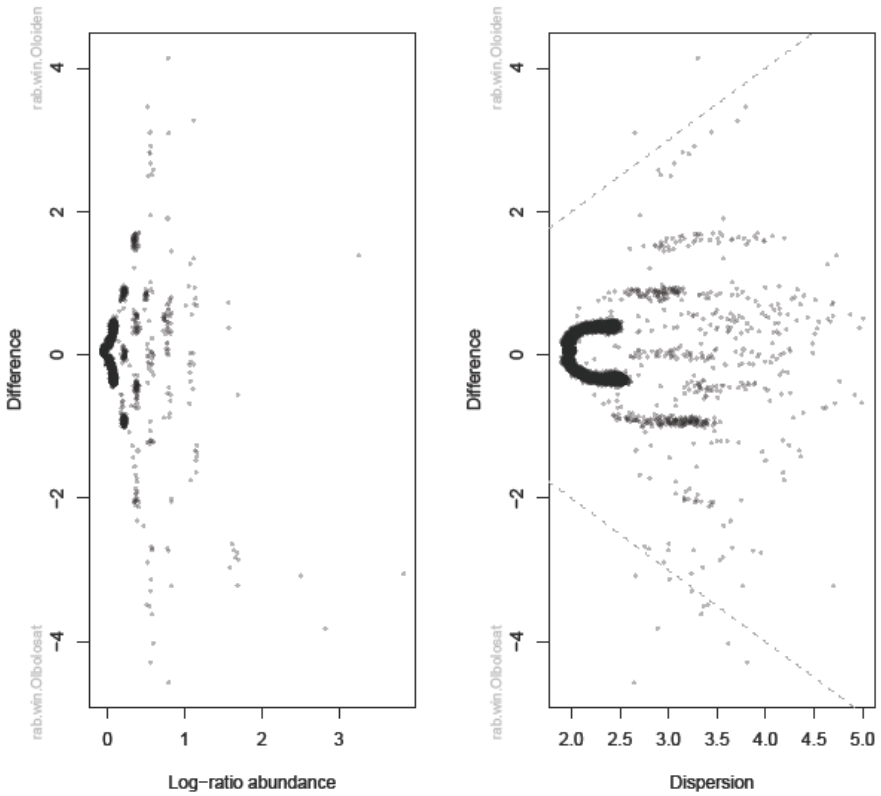

Figure 4.16: Output from aldex.plot function between samples obtained from lakes Olbolosat and Oloiden.

The left panel is a Bland-Altman or MA plot that shows the relationship between abundance and difference. The right panel is an effect plot (MW)that shows the relationship between difference and dispersion. In both plots, the grey feature represents the abundant, but not non-differentially abundant ASVs while black features represent rare and non-differentially abundant ASVs. Among the 5 differentially abundant ASVs, 80 % belonged to phylum Actinobacteriota and 20 % belonged to phylum Proteobacteria. Further, the differentially abundant ASVs were classified down to genus level representing phylum Actinobacteriota; *Pseudarthrobacter* (40 %), *Paracoccus* (20 %), *Micromonospora* (20 %), and phylum Proteobacteria; *Methylosistis* (20 %). Similarly, an evaluation of the ASV correlation at the phylum level revealed a positive co-correlation network pattern of the indicator ASVs (Figure 4.17).

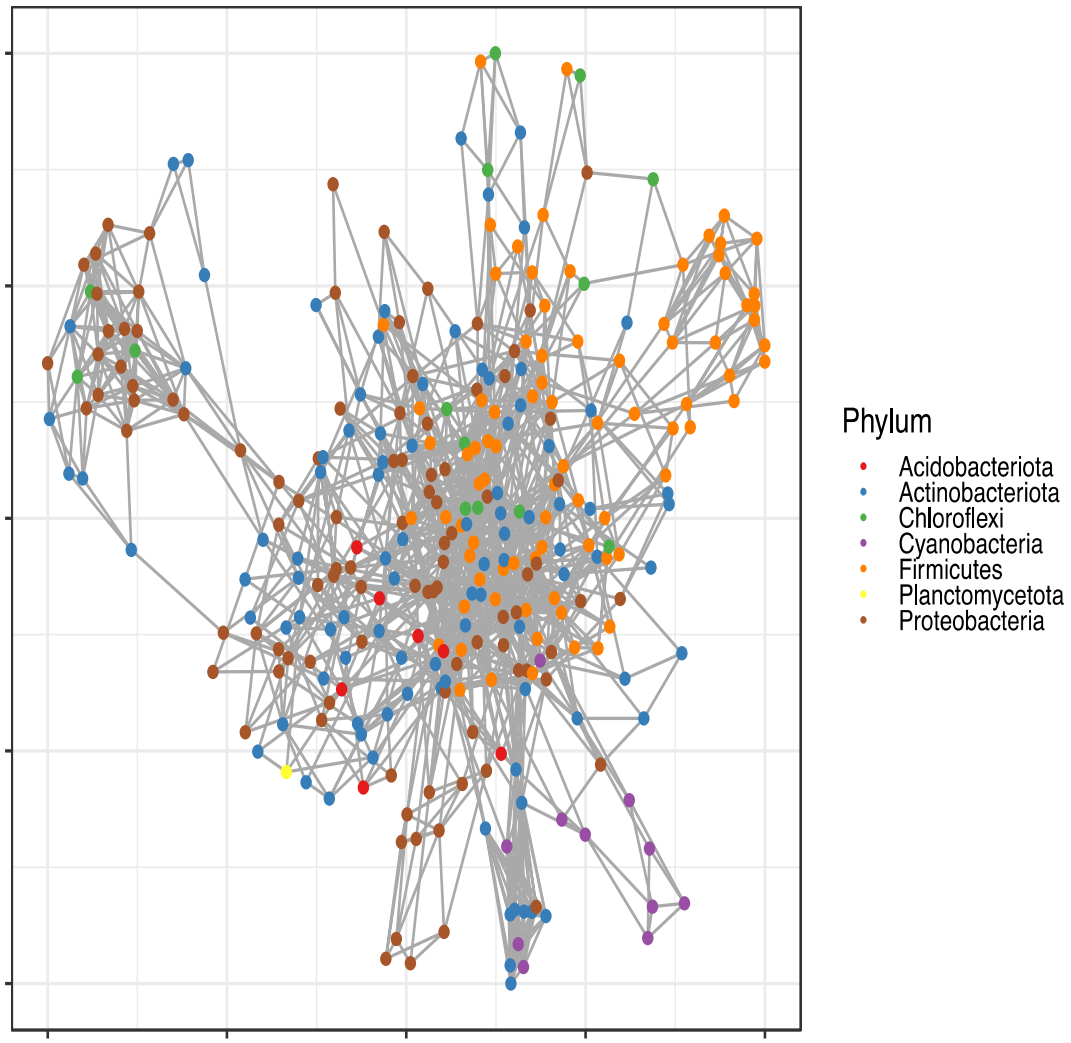

Figure 4.17: ASV interaction network according to the DNA data set of lakes Olbolosat and Oloiden samples. Each node is represented by an ASV indicating individual species.

4.11 Diversity of fungal communities

A total of 1,295,469 was obtained from sequence reads after removing chimeras from 15 DNA amplicon data sets. Total ASV richness at 1 % distance amounted to (288) 283 ASVs distributed among 10 highly abundant phyla, 16 highly abundant classes, 22 highly abundant order, 26 highly abundant families, and 45 highly abundant genera. Out of the 288 ASVs, 6 (2.08 %) ASVs were shared between lakes Oloiden and Olbolosat. Lakes Oloiden and Olbolosat constituted 120 (41.5 %) ASVs and 163 (56.4 %) ASVs, respectively (Figure 4.18a). The distribution of shared ASVs between

sample types revealed restricted overlap among samples in both lakes. Two ASVs were shared between the wet sediment and microbial mat while single ASVs were shared between water and dry sediment, microbial mat and dry sediment, and among the water, dry sediment and microbial mat in Lake Oloiden (Figure 4.18b). However, dry sediments had the highest number of ASVs (58) followed by Microbial mat and wet sediment each (25 ASVs), while water samples had the lowest number of ASVs (7). Similarly, dry sediment had the highest number of ASVs (81) in lake Olbolosat (Figure 4.18c) followed by wet sediment (38), microbial mat (24), and water (17) ASVs. Three ASVs were shared across wet sediment and microbial mat, while single ASVs were shared between microbial mat and water as well as microbial mat and wet sediment (Figure 4.18c). A similar trend of shared ASVs was also evidenced between samples across the two lakes. Dry sediment had the highest number of ASVs (134) followed by wet sediment (63), microbial mat (49), and water (23) ASVs (Figure 4.19d). Overall, wet sediments, microbial mat, and water shared a larger number of ASVs (4) while the remaining samples had the lowest overlap across lakes Olbolosat and Oloiden (Figure 4.18d). Further, the high overlap between sample types was supported by the ANOSIM that revealed a significant difference in ASV composition between the two lakes ($r = 0.2092$, $p = 0.002$).

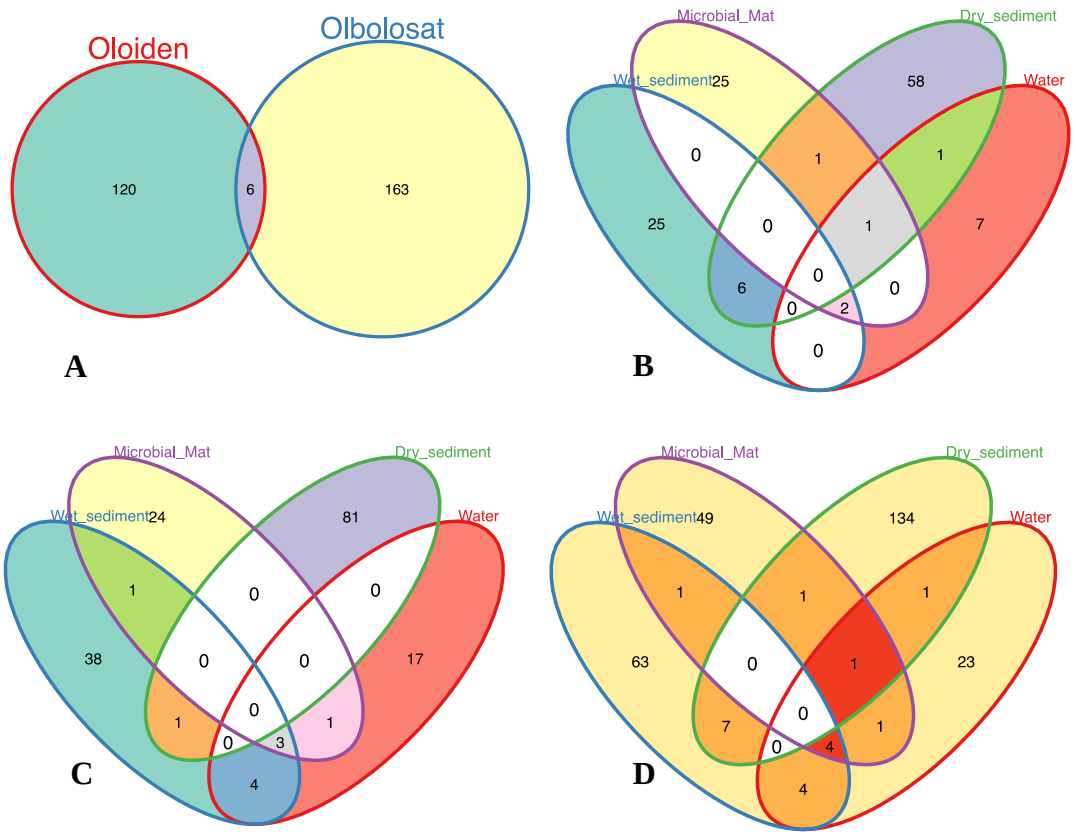

Figure 4.18: Venn diagram showing the distribution of unique and shared ASVs.

4.11.1 Fungal alpha diversity

Alpha diversity indices were significantly ($p < 0.05$) different among samples (Table 4.6). The number of sequences per sample ranged from 18394 to 242248 (Table 4.6). Dry sediments (ZC2) and water samples (EA1) from Lake Olbolosat and Oloiden, respectively had the highest number of sequences (Table 4.6). Dry sediments, however, had the lowest number of sequences in Lake Oloiden (EC1). All the indices except Simpson were highest in the dry sediment sample (EC1) in Lake Oloiden followed by the dry sediment sample (ZC1) in Lake Olbolosat. The lowest alpha diversity was in the water sample in both lakes EA1, EA2, ZA1, and ZA2 (Table 4.6). The rarefaction curve also supported the difference in α-diversity since species diversity was highest in the dry sediment sample (ZC1). The rarefaction curves were generated based on ASVs at 99.9% similarity and they supported the difference in α-

diversity indices since species diversity was highest in the dry sediment (ZC1) and lowest in the water sample EA1 and EA2 (Appendix IX and Appendix XIV).

Table 4.6: Alpha diversity indices computed on all ASVs-based microbial taxonomic units within ITS.

Site	Number of Sequences	Observed	Chao1	Shannon	Simpson	Inverse Simpson
EA1	126216	7.00	7.00	0.35	0.13	1.15
EA2	112744	4.00	4.00	0.09	0.03	1.03
EB1	31577	21.00	21.00	2.17	0.77	4.31
EB2	34622	13.00	13.00	2.04	0.80	5.03
EC1	18394	35.00	35.00	3.03	0.92	13.31
EC2	23792	40.00	40.00	3.23	0.94	18.05
ED1	77163	17.00	17.00	2.22	0.83	5.93
ED2	64981	12.00	12.00	1.30	0.64	2.77
ZA1	88949	7.00	7.00	1.14	0.54	2.17
ZA2	103535	21.00	21.00	1.28	0.57	2.32
ZB1	99905	26.00	26.00	2.40	0.85	6.87
ZB2	98221	26.00	26.00	2.06	0.77	4.35
ZC1	37163	53.00	53.00	3.05	0.89	9.34
ZC2	242248	39.00	39.00	0.24	0.07	1.07
ZD2	135959	28.00	28.00	1.25	0.50	2.00

4.11.2 Abundance of the fungal taxa

Composition analysis of the ITS ASVs comprised of the kingdom Fungi (73.1 %) and Plantae (28.7 %). Among the ten highly abundant phyla, two (Chlorophyta; 56.87 % and Anthophyta; 1.2 %) belonged to kingdom Plantae, while eight (Mortierellomycota; 17.8 %, Basidiomycota; 11.8 %, Chytridiomycota; 5.8 %, Monoblepharomycota; 3.4

%, Ascomycota; 2.4 %, Olpidiomycota; 0.5 %, Mucoromycota; 0.1 %, and Glomeromycota; 0.1 %) belonged to kingdom fungi. Chlorophyta (56.87 %) was the predominant phyla across the samples except for the dry sediments in both lakes that were dominated by the phylum Mortierellomycota (Figure 4.19). Though the dominant phyla belonged to the kingdom Plantae, the trend changed across samples and fungal phyla were homogeneously distributed across the samples (Figure 4.19). Water sample (EA2; Oloiden), dry sediment (ZC2; Olbolosat) and microbial mat (ZD2; Olbolosat) were, however, unique with specificity to Chlorophyta, Mortierellomycota, and Chlorophyta phyla composition, respectively (Figure 4.19) (Appendix X and Appendix XI).

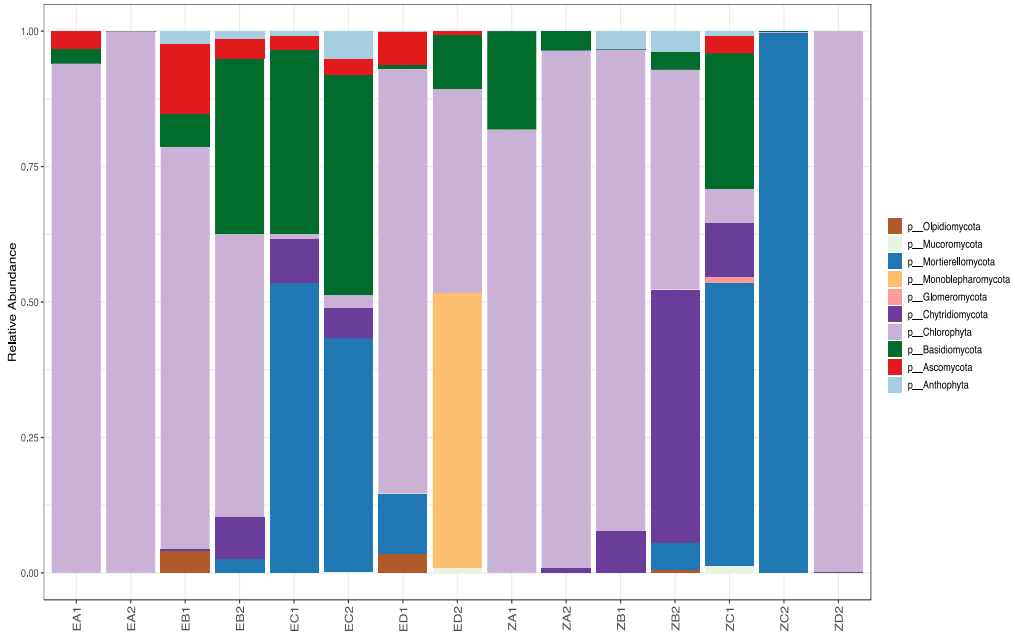

Figure 4.19: Relative abundance of the most predominant phyla in various sample types collected within Lakes Olbolosat and Oloiden.

Among the 16 highly abundant classes, there was one class Trebouxiophyceae (57.4 %) belonging to the phylum Chlorophyta. There were one class Mortierellomycetes (17.9 %) belonged to the phylum Mortierellomycota. Basidiomycota phylum was represented by three classes Agaricomycetes (6.1 %), Tremellomycetes (4.5 %), Microbotryomycetes (0.6 %). Monoblepharomycota phylum was represented by one class Monoblepharidomycetes (3.4 %). Chytridiomycota phylum was represented by four classes Lobulomycetes (2.7 %, Spizellomycetes (1.9 %, Rhizophlyctidomycetes

(1.1 %), Rhizophydiomycetes (0.2 %). Anthophyta phylum was represented by one class Monocotyledonae (1.2 %). Ascomycota phylum was represented by four classes Sordariomycetes (1.1 %) Saccharomycetes (0.5 %), Orbiliomycetes (0.4 %), Eurotiomycetes (0.3 %). The class Olpidiomycetes (0.5 %) represented the phylum Olpidiomycota (Figure 4.20) (Appendix XII).

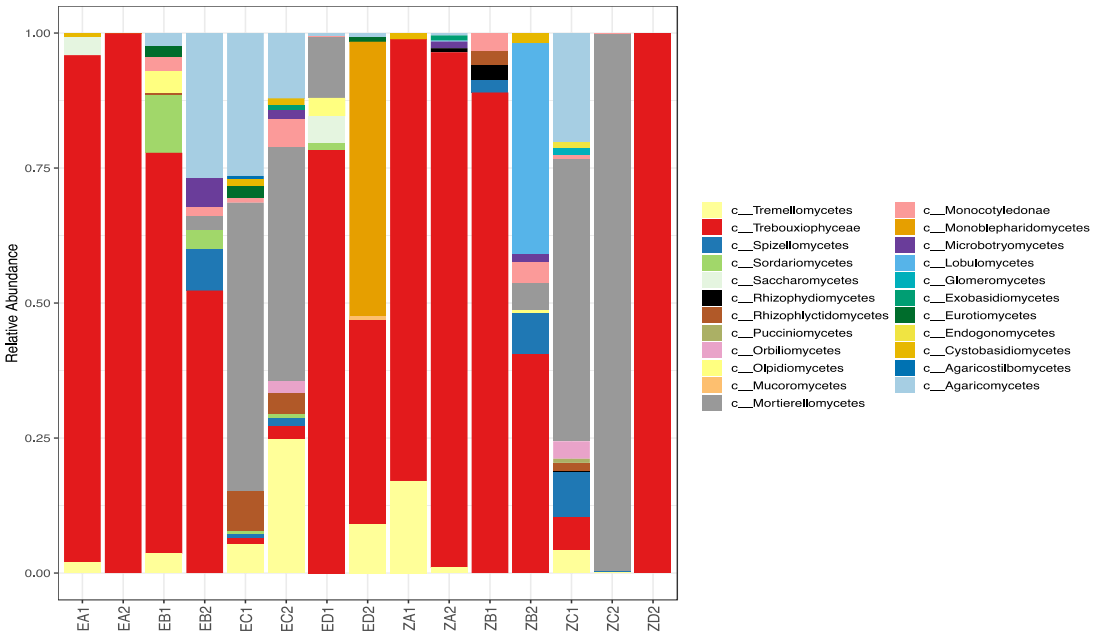

Figure 4.20: Relative abundance of the most predominant classes in various sample types collected within Lakes Olbolosat and Oloiden.

There were eight orders that represented the phylum Basidiomycota Agaricales (4.7 %), Filobasidiales (3.5 %), Cystofilobasidiales (0.8 %), Microbotryales (0.5 %), Cantharellales (0.5 %), Sebacinales (0.3 %), Tremellales (0.3 %), Hymenochaetales (0.2 %) out of the 22 highly abundant orders. There were five orders representing Ascomycota phylum Conioscyphales (1.1 %), Saccharomycetales (0.6 %), Orbiliales (0.4 %), Eurotiomycetes (0.2 %), Onygenales (0.1 %). The phylum Chytridiomycota was represented by four orders Lobulomycetales (2.7 %), Spizellomycetales (1.9 %), Rhizophlyctidales (1.1 %), Rhizophydiales (0.2 %) (Figure 4.21).

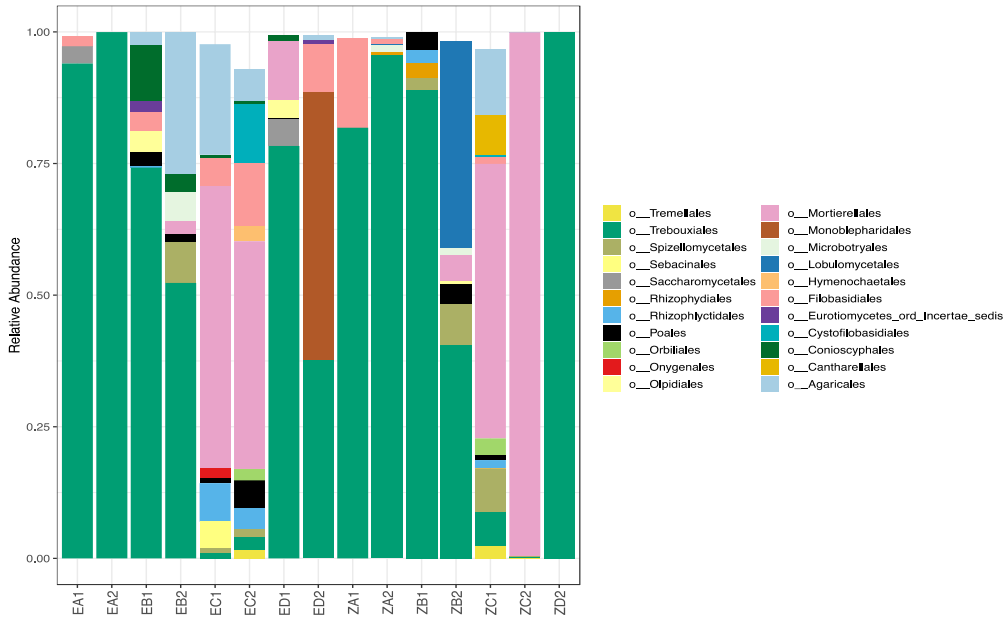

Figure 4.21: Relative abundance of the most predominant orders in various sample types collected within Lakes Olbolosat and Oloiden.

The predominant family belonged to phylum Chlorophyta *Trebouxiaceae* (57.9 %). Twelve families represented the phylum Basidiomycota *Filobasidiaceae* (3.5 %), *Stephanosporaceae* (2.1 %), *Agaricaceae* (1.1 %), *Lycoperdaceae* (0.8 %), *Cystofilobasidiaceae* (0.8 %), *Microbotryaceae* (0.5 %), *Ceratobasidiaceae* (0.5 %), *Sebacinaceae* (0.4 %), *Tremellaceae* (0.3 %), *Tricholomataceae* (0.2 %), *Hymenochaetaceae* (0.2 %), *Clavariaceae* (0.2 %). There were five families that represented the Ascomycota *Conioscyphaceae* (1.1 %), *Saccharomycetaceae* (0.6 %), *Orbiliaceae* (0.4 %), *Eurotiomycetes* (0.2 %), *Onygenaceae* (0.2 %). There were four families *Lobulomycetaceae* (2.7 %), *Spizellomycetaceae* (1.8 %), *Rhizophlyctidaceae* (1 %), *Halomycetaceae* (0.2 %) that represented the phylum Chytridiomycota (Figure 4.22).

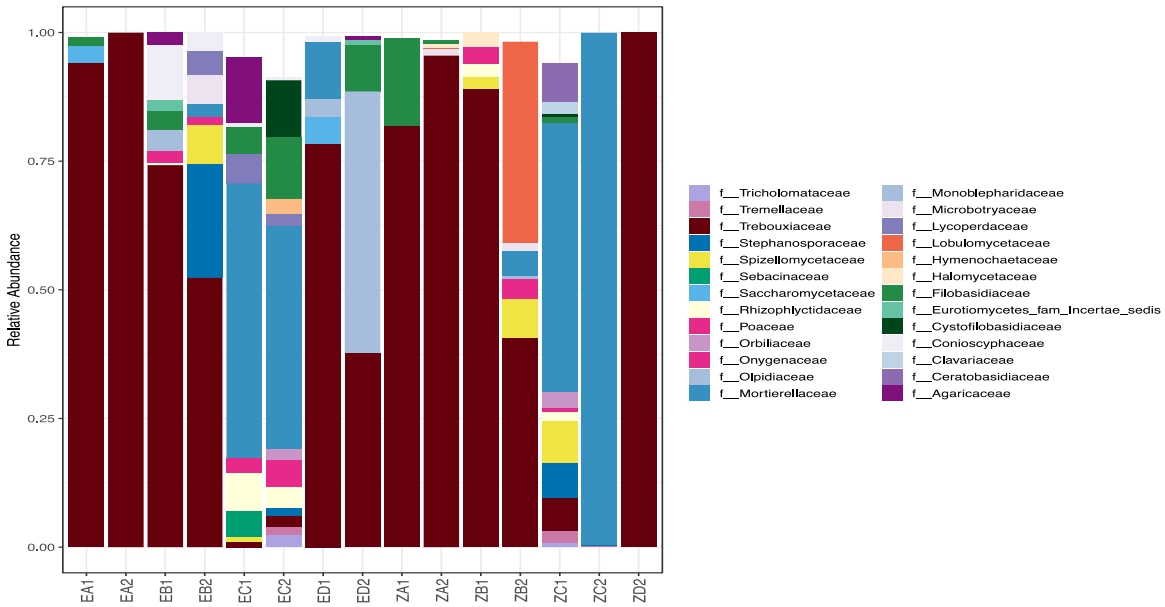

Figure 4.22: Relative abundance of the most predominant families in various sample types collected within Lakes Olbolosat and Oloiden.

4.11.3 Indicator species analysis

Indicator species analysis using lambdsv package revealed most genera affiliated to major phyla were associated with both lakes Olbolosat and Oloiden (Figure 4.23). The representatives of the genera associated with Lake Olbolosat were distributed among four major phyla Chlorophyta, Chytridiomycota, Mortierellomycota and Basidiomycota. Chlorophyta was represented by one indicator genera (*Trebouxia*, p <0.05). Chytridiomycota was represented by six indicator species *Spizellomyces*, *Clydaea*, *Halomyces*, *Rhizophlyctis*, *Paranamyces*, *Kochiomyces*, p <0.05). Mortierellomycota was represented by one indicator species (*Mortierella*, p <0.05). Basidiomycota phylum was represented by nine indicator species *Erythrobasidium*, *Clavaria*, *Lindtneria*, *Cryptococcus*, *Termitomyces*, *Septobasidium*, *Thanatephorus*, *Entoloma*, *Entyloma*, p <0.05) (Figure 4.23). Genus *Trebouxia* was the indicator species in Lake Olbolosat with an indicator value of 0.69 and it was closely followed by genus *Spizellomyces* at a 0.55 indicator value.

The representatives of the genera associated with Lake Oloiden were distributed among five major phyla Chytridiomycota, Ascomycota, Basidiomycota, Monoblepharomycota and Olpidiomycota. Chytridiomycota was represented by Powellomyces, $p < 0.05$). Ascomycota was represented by six indicator species *Conioscypha*, *Spiromastix*, *Amauroascus*, *Hyalorbilia*, *Kazachstania*, *Saccharomyces*, $p < 0.05$). Basidiomycota was represented by sixteen indicator species *Bovista*, *Naganishia*, *Filobasidium*, *Chlorophyllum*, *Tulostoma*, *Curvibasidium*, *Exidia*, *Inocutis*, *Kurtzmanomyces*, *Limonomyces*, *Marasmius*, *Microbotryum*, *Microstroma*, *Phylloporia*, *Cystofilobasidium*, *Flagelloscypha*, $p < 0.05$). Monoblepharomycota was represented by *Monoblephari*s, $p < 0.05$), while Olpidiomycota was represented by *Spiromastix*, $p < 0.05$) (Figure 4.23). Genus *Conioscypha* was the indicator species in Lake Oloiden with an indicator value of 0.63 closely followed by genus *Bovista* at a 0.38 indicator value. There was a great variation in the indicator values of the genera and the frequency of their occurrence across the two lakes. The indicator values ranged between 0.1 to 0.6, while the frequency of occurrence across the lakes ranged between 1 to 15. Genus *Trebouxia* occurred with the highest frequency of 15 in lake Olbolosat, whereas genus *Filobasidium* had the highest frequency of 6 in lake Oloiden (Figure 4.23) (Appendix XV).

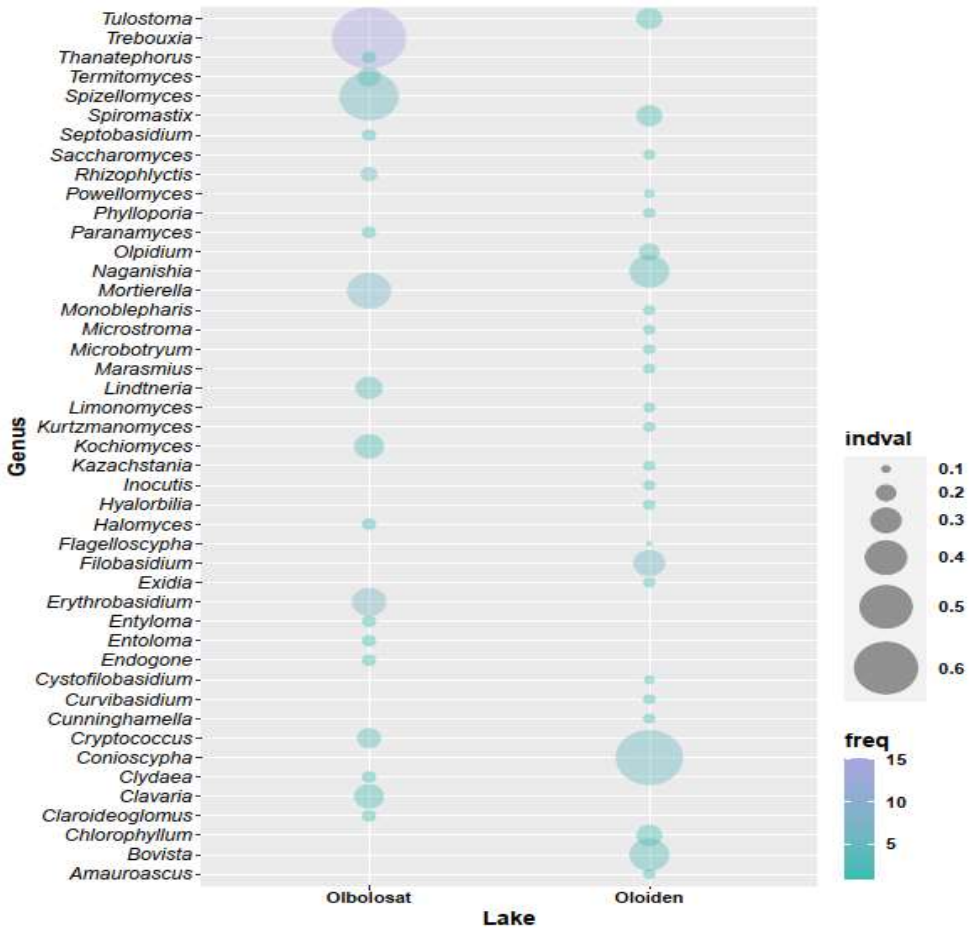

Figure 4.23: Indicator species within lake Olbolosat and Oloiden at the genus level.

Indicator species analysis also revealed most genera affiliated to major phyla were associated with wet sediments, dry sediments, water, and microbial mat samples across Lake Olbolosat and Oloiden (Figure 4.24 and 4.25 respectively). The representatives of the genera associated with the wet sediments in Lake Olbolosat were distributed among three major phyla Basidiomycota (*Paranamyces, Rhizophlyctis, Erythrobasidium,* $p < 0.05$), Chytridiomycota (*Spizellomyces, Clydaea*, $p < 0.05$), and Olpidiomycota (*Olpidium,* $p < 0.05$) (Figure 4.24). Other representatives of the genera associated with the dry sediments in Lake Olbolosat were distributed among three major phyla Basidiomycota, Chytridiomycota and Mortierellomycota. Basidiomycota was represented by *Clavaria, Lindtneria, Cryptococcus, Termitomyces, Septobasidium, Thanatephorus, Entoloma, Cystofilobasidium,* $p < 0.05$).

Chytridiomycota was represented by two indicator species *Kochiomyces,* and *Powellomyces,* p <0.05). Mortierellomycota was represented by *Mortierella,* p < 0.05) (Figure 4.24). Further, the representatives of the genera associated with the water samples in Lake Olbolosat were distributed among two major phyla Basidiomycota (*Etyloma, Filobasidium,* p <0.05), and Chytridiomycota (*Halomyces*, p <0.05) (Figure 4.24) (Appendix XVI). *Thanatephorus, Termitomyces, Septobasidium, Powellomyces, Mortierella, Lindtneria, Kochiomyces, Flagelloscpha, Entoloma, Endogone, Cystofilobasidium, Cryptococcus, Clavaria and Claroideoglomus* were the indicator species recovered from Dr sediments from lake Olbolosat. Only Trebouxia was recovered as an indicator specie from lake Olbolosat in microbial mat samples. *Halomyces, Filobasidium* and *Entyloma* were the indicator species recovered from water samples from lake Olbolosat. *Spizellomyces, Rhizophlyctis, Paranamyces, Olpidium, Erythrobasidium* and *Clydaea* were recovered from wet sediments from lake Olbolosat (Figure 4.24) (Appendix XV1).

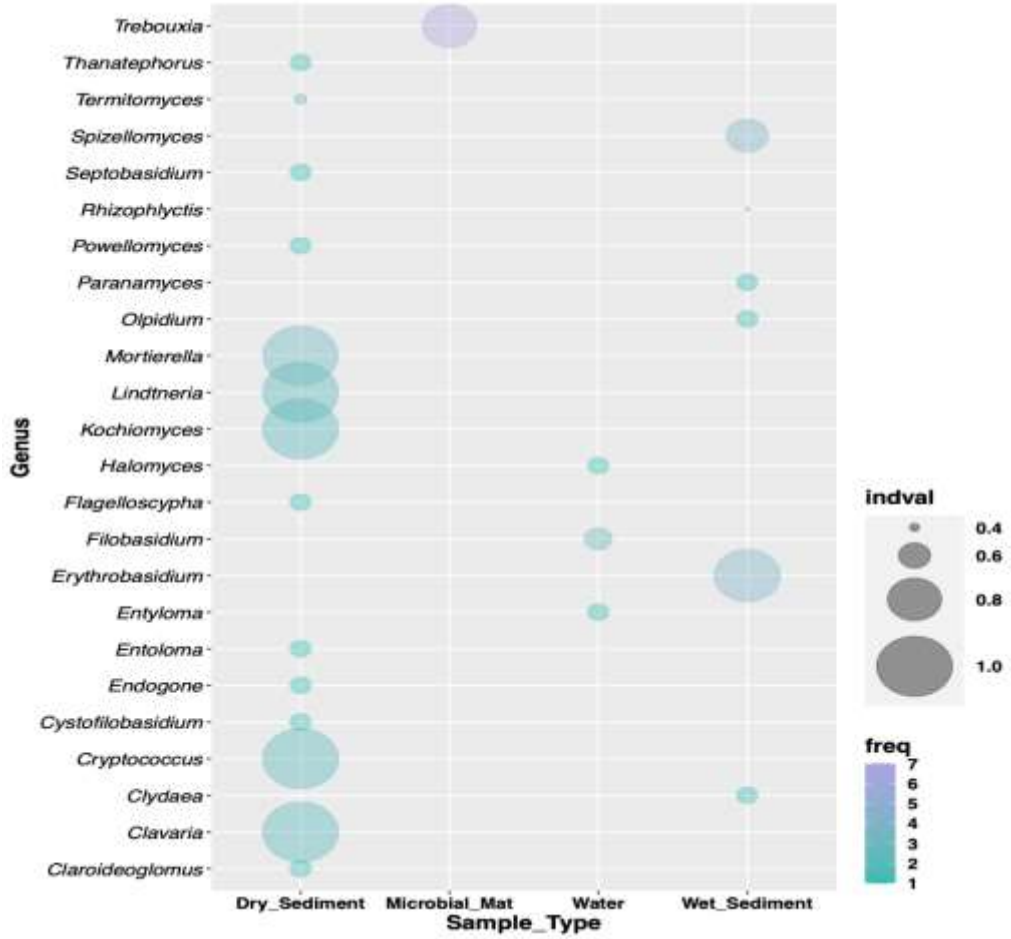

Figure 4.24: Indicator species within lake Olbolosat at the genus level.

Representatives of the genera associated with the microbial mats in Lake Olbolosat were distributed in the predominant phyla Chlorophyta (*Trebouxia*, $p < 0.05$) (Figure 4.24). The indicator genera in the wet sediment, dry sediment, water sample, and microbial mat in Lake Olbolosat were *Erythrobasidium* (0.91), *Kochiomyces* (1), *Filobasidium* (0.56), and *Trebouxia* (0.80), respectively (Figure 4.24). A similar trend for the taxa frequency was observed in Lake Olbolosat, however, the trend changes in the indicator value that were quite high (0.4 to 1) across sample types in Lake Olbolosat (Figure 4.24).

Similarly, indicator species analysis also revealed most genera affiliated to major phyla were associated with different samples in Lake Oloiden (Figure 4.25). The representatives of the genera associated with the wet sediments in Lake Oloiden were

distributed among three major phyla Basidiomycota (*Microbotryum,* p <0.05), Chytridiomycota (*Spizellomyces,* p <0.05), and Ascomycota (*Conioscypha,* p < 0.05) (Figure 4.25). Other representatives of the genera associated with the dry sediments were distributed among four major phyla Basidiomycota, Chytridiomycota, Mortierellomycota and Ascomycota. The Basidiomycota phylum was represented by seventeen indicator species; *Rhizophlyctis, Naganishia, Bovista, Cryptococcus, Curvibasidium, Cystofilobasidium, Exidia Basidiomycota, Flagelloscypha, Inocutis, Kurtzmanomyces, Limonomyces, Lindtneria, Marasmius, Microstroma, Phylloporia, Termitomyces, Chlorophyllum*, p <0.05). Chytridiomycota was represented by *Powellomyces,* p <0.05). Mortierellomycota was represented by *Mortierella,* p < 0.05) while Ascomycota was represented by *Amauroascus* and *Hyalorbilia*, p < 0.05) (Figure 4.25). Further, the representatives of the genera associated with the water samples were distributed among three major phyla Basidiomycota (*Erythrobasidium,* p <0.05), Chlorophyta (*Trebouxia,* p <0.05), and Ascomycota (*Saccharomyces,* p <0.05) (Figure 4.25). Representatives of the genera associated with the microbial mats were distributed among four major phyla Basidiomycota (*Tulostoma, Filobasidium*, p <0.05), Ascomycota (*Kazachstania, Spiromastix,* p < 0.05,), Monoblepharomycota (*Monoblepharis,* p < 0.05), and Olpidiomycota (*Olpidium,* p < 0.05) (Figure 4.25). The indicator genera in the wet sediment, dry sediment, water sample, and microbial mat in Lake Oloiden were *Conioscypha* (0.66), *Rhizophlyctis* (0.99), *Trebouxia* (0.70), and *Monoblepharis* (0.50), respectively (Figure 4.25) (Appendix XVII).

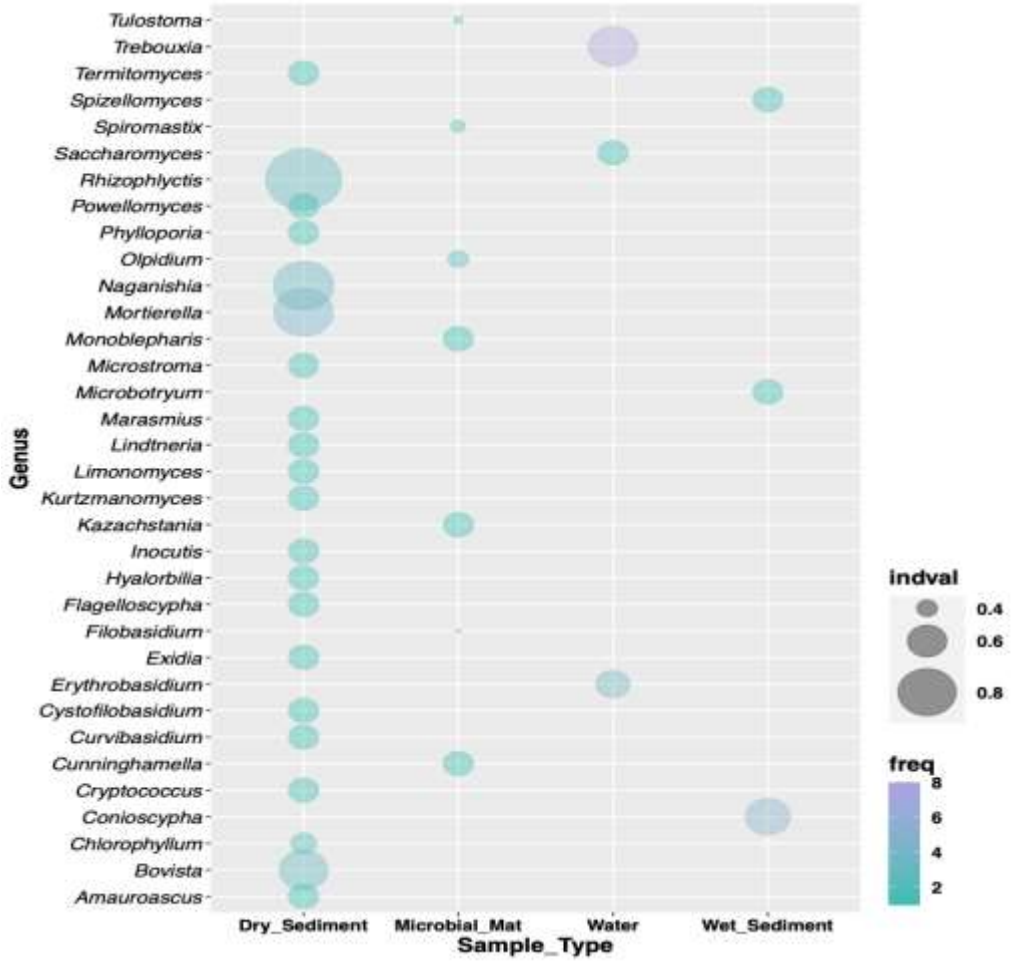

Figure 4.25: Indicator species within lake Oloiden at the genus level.

There was variation in taxa composition between the sample types and between the lakes. Indicator species changed between samples both in frequency and in indicator value. The indicator values ranged between 0.5 to 0.7 and the taxa frequency ranged between 0.2 to 1 in the wet sediments from Lake Oloiden. Within the same lake, indicator values and taxa frequency ranged between 0.5 to 0.9 and 0.1 to 1 in the dry sediments, respectively. Consequently, indicator values and taxa frequency in the water samples ranged between 0.5 to 0.7 and 1 to 8 in Lake Oloiden, respectively. The variation in indicator values ranged between 0.2 to 0.5, while the taxa frequency ranged between 1 to 3 in Lake Oloiden (Figure 4.25).

4.11.4 Fungal community composition and correlation with environmental factors.

Principal Component Analysis (PCA) indicated that fungal communities were clustered according to the regions of sampling (Lakes) (Figure 4.26). For instance, wet sediments from Lake Oloiden clustered separately from the wet sediments from Lake Olbolosat.

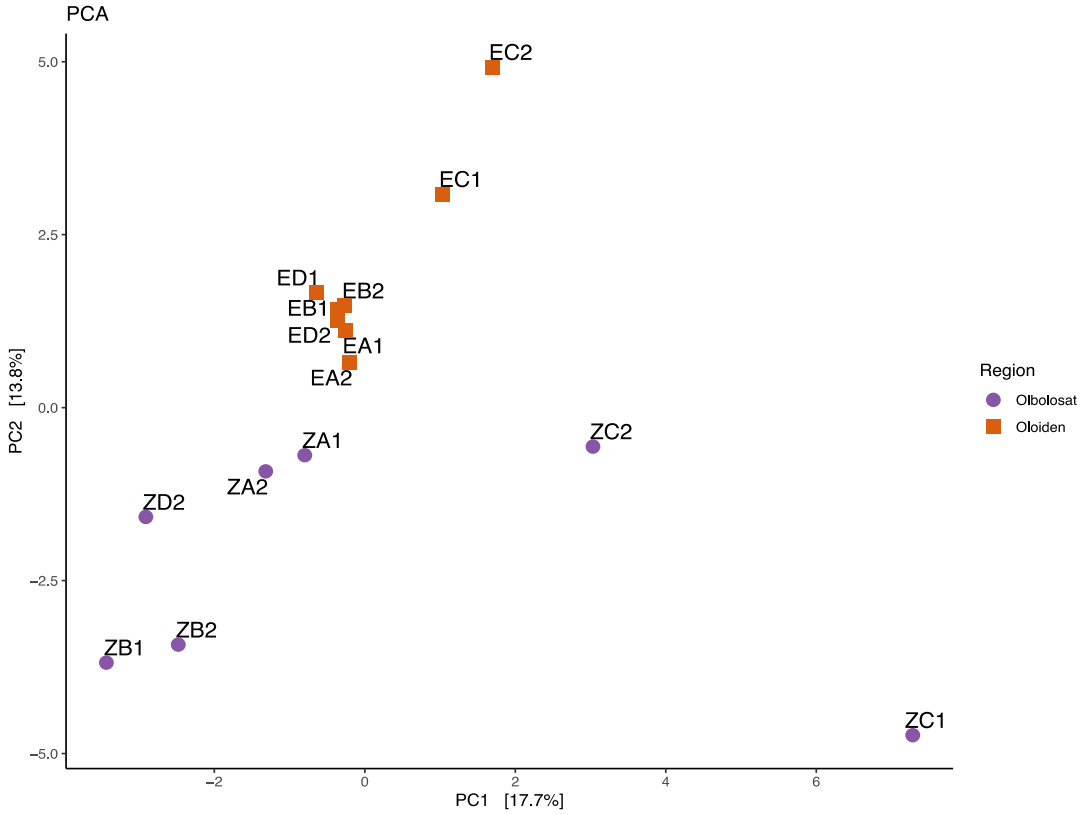

Figure 4.26: PCA ordination plot for all sample types across the two lakes, using Bray-Curtis dissimilarity distance, colored by lake.

Water samples, dry sediments, and microbial mat also clustered separately indicating diversity between the sample types and the lakes. Some of the samples, especially water samples (EC1, EC2, ZC1, and ZC2) were clustered independently from other samples across the two lakes (Figure 4.26). Similarly, ordination for PCA showed that fungal communities were clustered according to the sample type and region of sampling (Lake) (Figure 4.27). This ordination pattern was also observed among the

sample types in which similar samples were clustered independently from other samples (Figure 4.27). Ordination cluster analysis based on the PCA indicated variation in fungal community structures between samples and across the lakes. The similarity between samples depended either on the sample type or sampling region (Lake Oloiden or Lake Olbolosat) (Figure 4.26 & Figure 4.27).

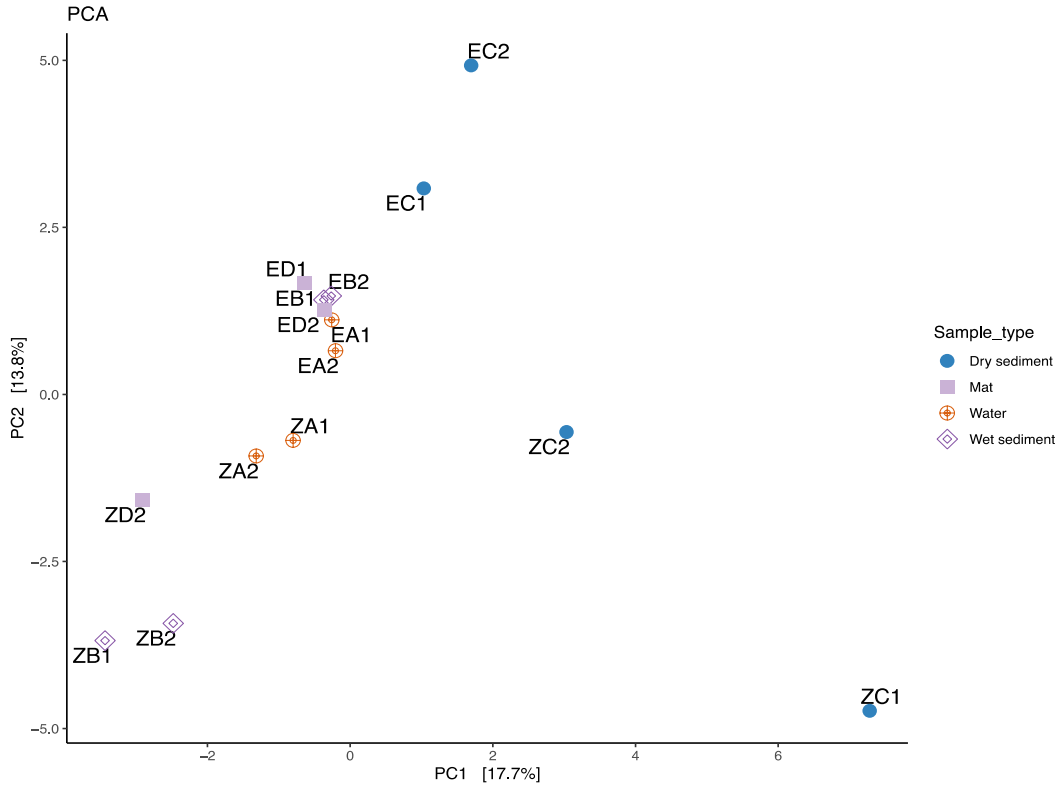

Figure 4.27: PCA ordination plot for all sample types, using Bray-Curtis dissimilarity distance, colored by sample type.

The hierarchical cluster of the highly abundant ASVs also indicated that major phyla and minor phyla clustered separately (Figure 4.28). The ordination and hierarchical cluster results were significantly ($r = 0.213$, $p = 0.002$) supported by the ANOSIM.

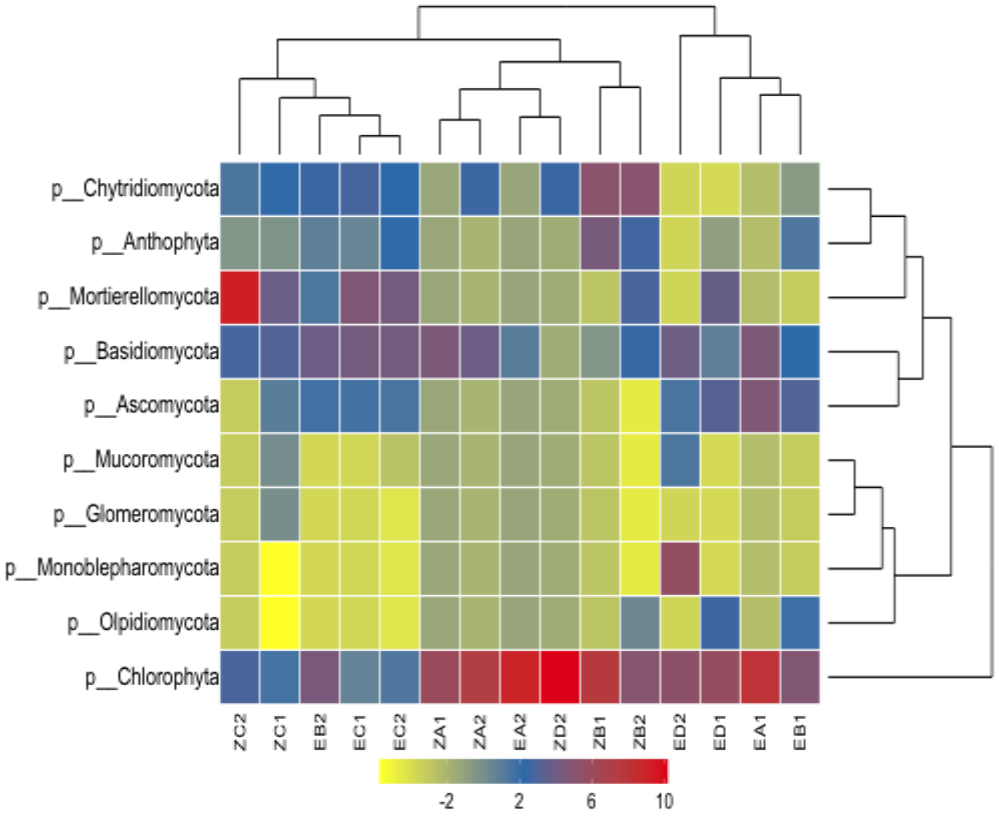

Figure 4.28: Hierarchical clustering of phyla assessing the relationship between sample types and fungal taxa based on Bray-Curtis dissimilarity of the highly abundant taxa at the phylum.

Similarly, an evaluation of the ASV correlation at the phylum level revealed a positive co-correlation network pattern of the indicator ASVs. The network analysis revealing the co-occurrence patterns among the genera of the major phyla. Node 6 and 103 were the only ASVs that were not connected with the others (Figure 4.29).

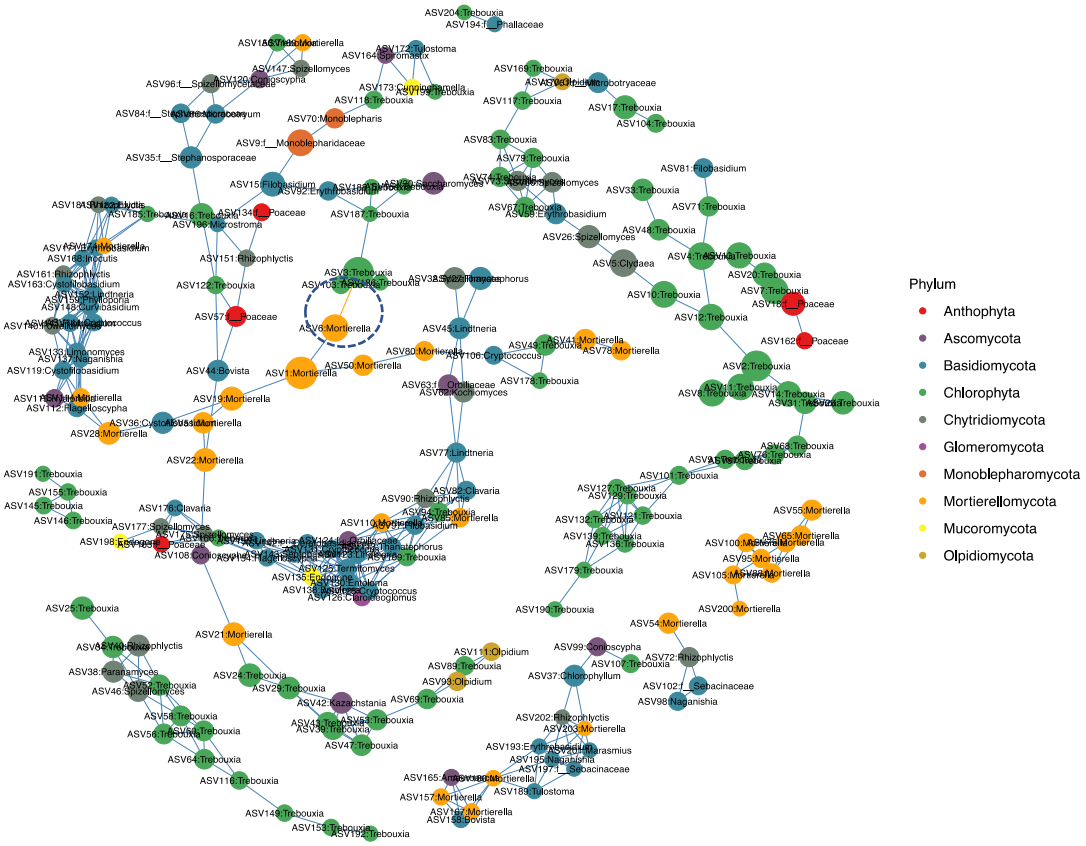

Figure 4.29: Correlation network pattern for the indicator ASVs.

Nodes were colored according to the genus and the lines connecting nodes (edges) represent positive (steelblue) or negative (orange) co-occurrence relationships. The intensity of the color and the length of the edges represent the strength of correlation. The size of each node represents the number of connections between the genera of the major phyla. The effects of environmental factors on the eukaryotic diversity and community composition were supported by the Permutation Multivariate Analysis of Variance (PERMANOVA), Constrained Analysis of Principal Coordinates (CAP), and distance-based Redundant Analysis (dbRDA). PERMANOVA revealed that four out of the seventeen factors strongly influenced the variation between samples and distribution of ASVs with R^2 ranging from 0.09 to 0.13 and $p < 0.05$ (Table 4.7). CAP analysis revealed ten out of the seventeen factors to strongly influence the variation between samples and distribution of ASVs with r^2 ranging from 0.41 to 0.93 and $p <$

0.05 (Table 4.7). The dbRDA also revealed nine out of the seventeen factors to strongly influence the variation between samples and distribution of ASVs with r^2 ranging from 0.66 to 0.93 and $p < 0.05$ (Table 4.7).

Copper and Magnesium were the most influential cations (R^2 = 0.1 to 0.13, p = 0.003; PERMANOVA, r^2 = 0.70 to 0.72, $p < 0.01$; CAP, r^2 = 0.70 to 0.72, $p < 0.001$) (Table 4.7). Manganese also influenced the distribution of ASVs across the samples based on the CAP and db-RDA (r^2 = 0.67, p = 0.003 and r^2 = 0.67, p = 0.001, respectively). Chloride ion was the most influential anion (R^2 = 0.11, p = 0.05; PERMANOVA, r^2 = 0.76, p = 0.002; CAP, r^2 = 0.76, p = 0.001) (Table 4.7). Sulphate and Bicarbonate anions also influenced the distribution of ASVs based on the CAP and db-RDA (r^2 = 0.72 to 0.82, p = 0.001 and r^2 = 0.72 to 0.82, p = 0.001, respectively). Further, Electrical Conductivity was the most influential environmental factor (R^2 = 0.09, p = 0.04; PERMANOVA, r^2 = 0.62, p = 0.001; CAP, r^2 = 0.62, p = 0.005) (Table 4.7). Dissolve organic matter and temperature also influenced the distribution of ASVs across samples based on the CAP and db-RDA (r^2 = 0.66 to 0.93, $p < 0.001$ and r^2 = 0.66 to 0.93, p = 0.001, respectively).

Further, analysis of environmental factors also revealed Iron, Magnesium, and Manganese as the best predictor cations for the distribution of fungal communities (r = 0.68) with a significant Pearson's product-moment correlation Mantle statistics (r = 0.4, p = 0.02). Analysis of anions revealed sulfate as the best predictor for the distribution of eukaryotic communities across the lakes (r = 0.29). However, the Pearson's product-moment correlation Mantle statistics were not significant for the effect of anions (r = -0.08, p = 0.68). Consequently, the analysis of the environmental factors revealed dissolved oxygen (DO) and Temperature as the best predictors (r = 0.23) of eukaryotic community structures across the sample types with a non-significant Pearson's product-moment correlation Mantle statistics (r = 0.1, p = 0.28).

Table 4.7: Results from individual PERMANOVA, CAP and dbRDA analyses on selected environmental factors, using Pearson's product-moment correlation on variance-stabilizing transformed counts.

	PERMANOVA		CAP		dbRDA	
Factor	R^2	*p*	r^2	*p*	r^2	*p*
Ammonium (NH_4^+)	0.05	0.66	0.09	0.58	0.09	0.57
Copper (Cu^+)	0.1	0.003**	0.72	0.002*	0.72	0.001***
Iron (Fe^{2+})	0.09	0.06	0.30	0.110	0.30	0.13
Magnesium (Mg^{2+})	0.13	0.003**	0.70	0.013*	0.70	0.005**
Manganese (Mn^{2+})	0.09	0.14	0.67	0.003*	0.67	0.001***
Potassium (K^+)	0.07	0.31	0.41	0.05*	0.41	0.050
Sodium (Na^+)	0.05	0.68	0.20	0.240	0.20	0.240
Chloride (Cl^-)	0.11	0.05*	0.76	0.002**	0.76	0.001***
Nitrite (NO_2^-)	0.05	0.83	0.16	0.350	0.16	0.374
Phosphate (PO_4^{3-})	0.07	0.6	0.17	0.326	0.17	0.318
Sulphate (SO_4^{3-})	0.08	0.26	0.72	0.001***	0.72	0.001***
Bicarbonate (HCO_3^-)	0.05	0.83	0.82	0.001***	0.82	0.001***
Fluoride (F^-)	0.06	0.68	0.34	0.072	0.34	0.096
Electrical Conductivity (Ec)	0.09	0.04*	0.62	0.001***	0.62	0.005***
Total Dissolve Substances (TDS)	0.05	0.85	0.30	0.118	0.30	0.109
Dissolve Organic (DO)	0.08	0.22	0.93	0.001***	0.93	0.001***
Temperature (^oC)	0.08	0.24	0.66	0.002***	0.66	0.001***

P values with an asterisk *are significant.

Spearman's correlation coefficient analysis between anions revealed bicarbonate, nitrite, and chloride to have a significant negative correlation with fluoride across all sample types except dry sediment (Figure 4.30). Chloride ions, however, had a stronger significant positive correlation with sulfate and bicarbonate except in microbial mat which had a significant negative correlation with sulfate. Conversely,

nitrite ions had a stronger significant negative correlation with sulfate and bicarbonate in wet sediments unlike in the microbial mat A red/orange circle shows a positive correlation, and a blue circle shows a negative correlation. A cross "X" on the circle indicates no significance ($p < 0.05$) (Figure 4.30).

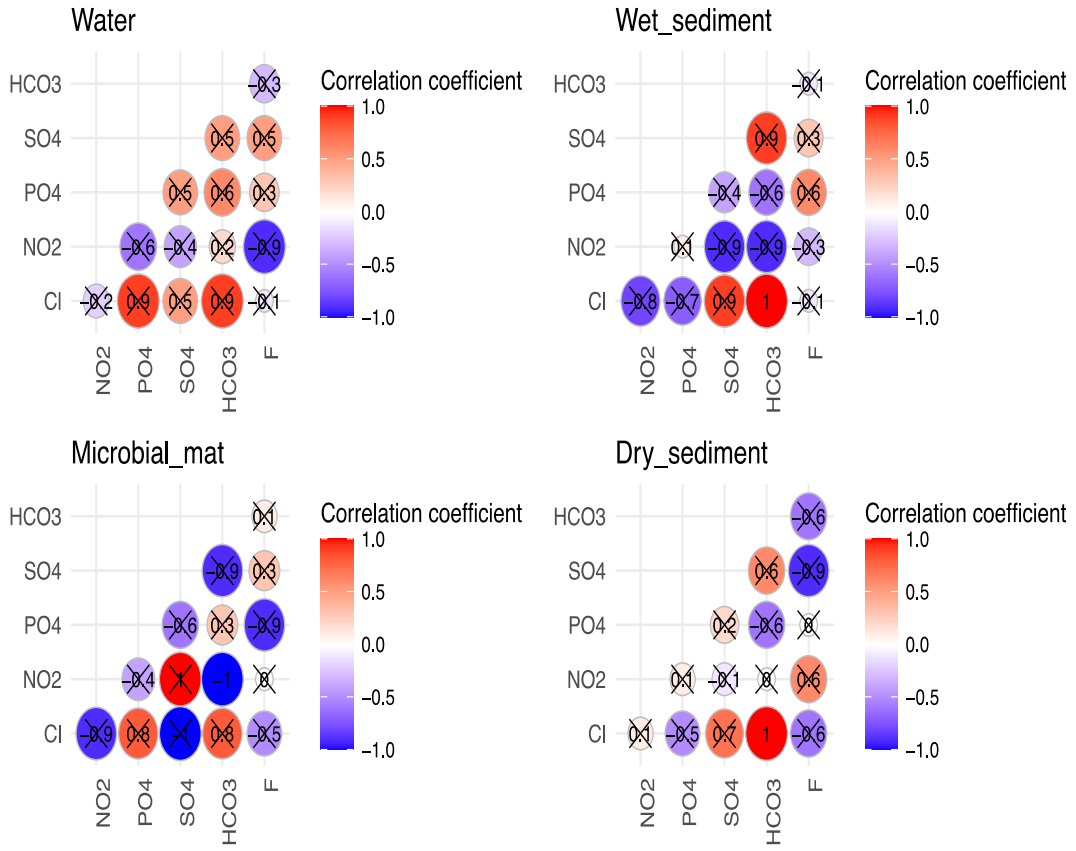

Figure 4.30: Spearman's correlation between anions.

Similarly, Spearman's correlation coefficient analysis revealed a significant coefficient correlation between cations in which a stronger significant positive correlation existed between magnesium and potassium, iron and manganese, ammonium and sodium in the water samples. Iron and potassium, copper and manganese also revealed a stronger significant positive correlation within the wet sediments. The coefficient changed in the microbial mat in which potassium had a stronger significant correlation with sodium ions. Iron and manganese also had a stronger significant correlation within the microbial mat. Further, the correlation effect

of the cations was revealed by the significant correlation between magnesium and sodium, ammonium and magnesium within the dry sediment. A red/orange circle shows a positive correlation, and a blue circle shows a negative correlation. A cross "X" on the circle indicates no significance ($p < 0.05$) (Figure 4.31).

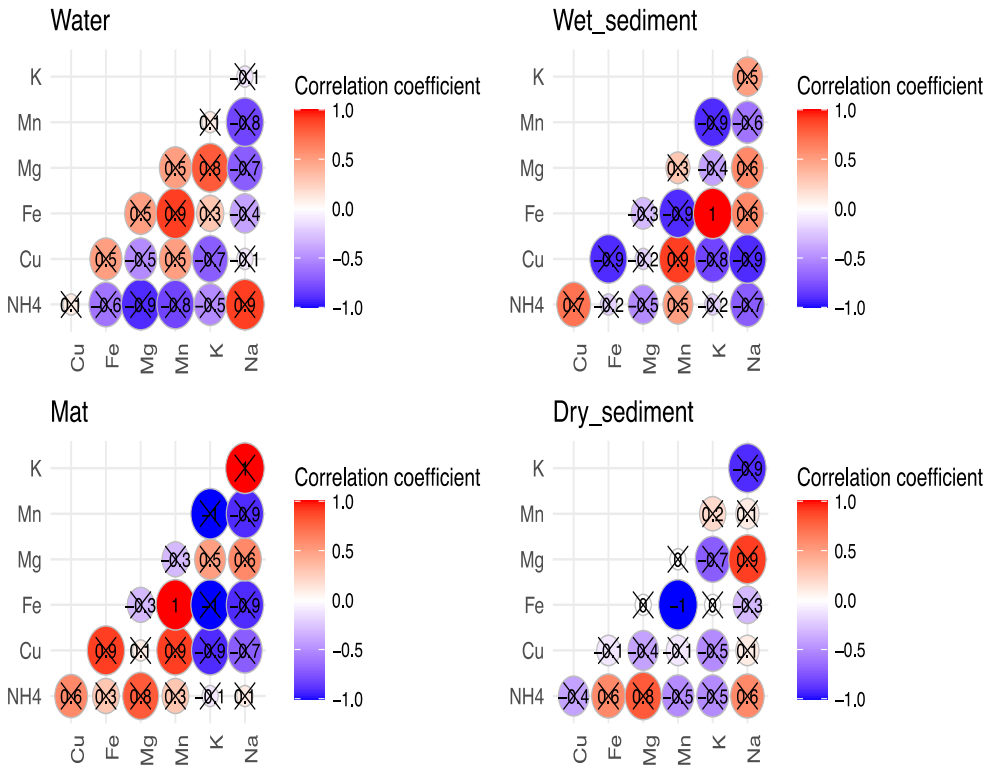

Figure 4.31: Spearman's correlation between cations.

The effect of electrical conductivity and dissolved organic matter was evidence of a stronger significant correlation in dry sediment (Figure 4.32). A similar effect also occurred between electrical conductivity and temperature in wet sediment and water samples. Electrical conductivity also had a significant positive correlation with total dissolve substances in a microbial mat. A red/orange circle shows a positive correlation, and a blue circle shows a negative correlation. A cross "X" on the circle indicates no significance ($p < 0.05$) (Figure 4.32).

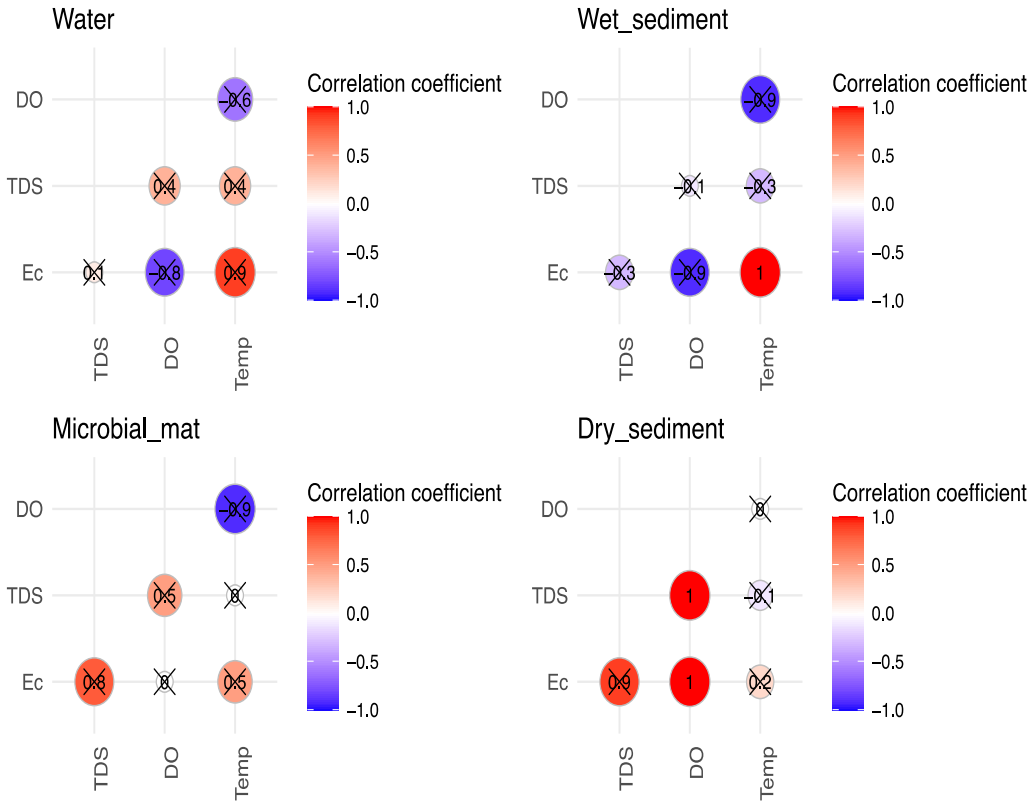

Figure 4.32: Spearman's correlation between onsite physical factors.

CHAPTER FIVE

DISCUSSION

5.1 Bacterial isolates obtained from lakes Olbolosat and Oloiden

The growth of isolates in culture mediums at different salinity, pH, and temperature ranges, indicates that they can tolerate and can adapt to adverse growth conditions in the marine ecosystem. The physiological analysis revealed that all isolates were able to grow at different pH ranges from pH 4 to 14. Some had a growing preference for either an alkaline or slightly acidic environment. Isolate MT801056 (*Bacillus safensis* subsp *safensis*), MT801070 (*Bacillus subtilis*) MT801061 (*Bacillus* sp) and MT801074 (*Pseudomonas* sp) grew optimally at pH 4, 8 and 10 ranges. Some isolates like MT801056 (*Bacillus safensis* subsp *safensis*), MT801067 (*Staphylococcus arlettae*) and MT801063 (*Bacillus megaterium*) grew optimally at a lower Nacl concentration compared to all others indicating their preference in different levels of salinity within the two lakes. There was significant growth for MT801062 (*Paenibacillus oryzisoli*) that grew optimally at all temperature ranges of 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. Other bacterial isolates like MT801065 (*Bacterium* sp) MT801068 (*Bacillus simplex*) and MT801067 (*Staphylococcus arlettae*) grew optimally up to 40 °C. The physiological conditions (pH, temperature, and sodium chloride) are important in the current study if the isolates are to be cultured in the laboratory and exploited for industrial use (O'Brien *et al.,* 2019). The pH, temperature and salinity are indicators of environmental setting that shapes microbial communities according to O'Brien *et al.* (2019) and also could affect the activities of extracellular enzymes and the breakdown of organic matter (Li *et al.,* 2019). The presence of bacteria in the lake ecosystem could be involved in the biodegradation of contaminants such as polycyclic aromatic hydrocarbons through the use of their extracellular enzymes (Yadav *et al.,* 2019). Several bacterial isolates; MT801063 (*Bacillus megatarium*) MT801075 (*Bacillus cereus*) MT801061(*Bacillus* sp) MT801064 (*Bacillus* sp) MT801052 (*Microbacterium oxydans*) utilized xylan minimally as a substrate. Lipase was also not well produced by MT801061(*Bacillus* sp), MT801069 (*Staphylococcus succinus*), MT801078 (*Streptomyces* sp),

MT801071(*Exiguobacterium* sp). Cellulose was also not well produced by MT801055 (*Bacillus pumilus*), MT801066 (*Bacillus aryabhattai*), MT801054 (*Bacillus altitudinis*), MT801053 (*Bacillus pumilus*). Starch substrate was not well utilized by MT801075 (*Bacillus cereus*), MT801061(*Bacillus* sp), MT801055 (*Bacillus pumilus*) bacterial isolates. Protease was well produced by most bacterial isolates. The production of enzymes from bacterial isolates within lakes Olbolosat and Oloiden are in line with studies by other authors that did the same analysis (Mulango *et al*., 2020). Wet samples recorded the highest number of isolates that were able to utilize most substrates within the two lakes. There as a high number of bacterial isolates that produced enzymes from lake Olbolosat a fresh water lake as compared to lake Oloiden a saline-alkaline lake. Production of extracellular enzymes by bacterial isolates in this study such as amylases, lipases, proteases, xylanases and cellulases and also intracellular enzymes among them starch, catalase, gelatinase and citrate, indicates their biotechnological potential in agriculture, food industries, detergent, medicinal formulations and wastewater management (Yadav *et al.,* 2019). Out of the 60 sequences for bacterial isolates from both lakes, 26 were without ambiguities. The 26 bacterial isolates identified in the current study belonged to the domain bacteria and four different Phyla: Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes. There were 12 bacterial isolates recovered from wet sediments, 9 from dry sediments and only 5 from water samples from both lakes. Firmicutes were predominance within the two lakes. Among the 13 isolates of firmicutes recovered, 7 isolates were from dry sediments from both lakes. A possible reason for this distribution could be more space for colonization in dry sediments due to the production of their spores (Ludwing *et al.,* 2009). They are known to produce spores that are highly resistant to environmental stress. The formation of spores explains why they may be able to easily outgrow other microorganisms after transfer to microbiological media with their repeated isolation from sediments (Ludwing *et al.,* 2009). Firmicutes biodegrade complex compounds, therefore, breaking down macromolecules entering the lake ecosystem such as plants and dead animals providing energy and carbon sources for microbial communities (Ludwing *et al.,* 2009). There were 13 isolates of Firmicutes belonging to the family *Bacillaceae* identified in this study; among these were *Bacillus safensis* subsp *safensis*, *Bacillus pumilus*, *B. altitudinis*, *B. simplex*, *B. aryabhattai*, *Bacillus megaterium*, *B.*

simplex, B. cereus, B. subtilis and *Bacillus toyoniensis*. *Bacillus pumilus* is known to play a good role in the biodegradation of macromolecules in the ecosystem (Mishra *et al.,* 2017).

Bacillus altitudinis utilizes various kinds of carbon sources in the lake ecosystem according to Mishra *et al*., (2017). *Bacillus megaterium* could be used as an industrial organism and has been used in bioremediation. They are commonly used in agriculture as plant-promoting bacteria and in health sectors (Wafula & Murunga, 2020). *Bacillus subtilis, B. cereus, B. pumilus, B. aryabhattai, Bacillus safesis* subp *safensis* and *B. simplex* have been used as plant promoting bacteria to fix nitrogen, secrete plant hormones or antibiotics, solubilize phosphates, inhibit pathogenic microbes and modify insoluble iron to soluble iron (Chiboub *et al.,* 2018). This is because they are resistant to adverse environmental conditions through the production of spores, they replicate rapidly, and they have a broad-spectrum to biocontrol ability (Chiboub *et al.,* 2018). Plant-promoting bacteria are important in enhancing seedling vigor, leaf area, and shoot and root growth. Plant-promoting hormones like GA3 and IAA are enhanced by the different species of bacteria. The hormone GA3, together with auxin plays an important role in the elongation of plant and leaf bud formation (Chiboub *et al.,* 2018). The hormone IAA helps in the emergence and origination of adventitious roots and enhancement of shoot development. Plant-promoting hormones also enhance the availability of nutrient uptake to plants helping them against abiotic and biotic stresses (Shafi *et al.,* 2017). The production of antibiotics by bacteria may help them in colonization. Both pathogenic and nonpathogenic organisms compete for space and nutrients with other organisms around them. This is because the soil has a limited amount of nutrients available to sustain them (Shafi *et al.,* 2017). *Bacillus cereus* occurs naturally and is responsible for most food poisoning (Bartoszewicz & Czyzewska, 2017). *Bacillus toyoniensis* was isolated from South Africa marine sediment by Ugbenyen1 *et al.,* (2017) for the production of flocculants used in the biodegrading of pollutants.

Staphylococcus sp belonging to Firmicutes occurs ubiquitously in nature and has been isolated from various animals such as birds and mammals (Rossi *et al.,* 2020). *Staphylococcus xylosus* and *S. succinus* identified in this study have been used in Italy

for the fermentation of traditional sausages (Ratsimba *et al.,* 2017). *Staphylococcus xylosus* produces biosurfactants an important bioactive compound used in food, cosmetics, petroleum, medicine and pharmaceutical industries (Ratsimba *et al.,* 2017). *Exiguobacterium* genus is another Firmicute that was identified in this study and has been isolated earlier from different environmental niches such as sediments, seawater, soils glaciers, hydrothermal vents and industrial effluents (Kasana & Pandey, 2018). Isolates from the *Exiguobacterium* genus can grow in extreme environments with temperatures ranging from 12-50 °C and under low nutrient conditions (Vishnivetskaya *et al.,* 2009). Different strains from the *Exiguobacterium* genus have been used in industries, in agriculture as plant-promoting bacteria and in the biodegradation of pollutants (Kasana & Pandey, 2018).

Streptomyces sp is known to produce 80% of the antibiotic compounds according to Hamid *et al.,* (2020) which are the most important secondary metabolites of the bacterial isolate. *Microbacterium oxydans* and most species in this genus inhabit diverse environments and are associated with the aquatic plants as symbionts according to (Mishra *et al.,* 2017) *Microbacterium oxydans* are also used in commercial applications such as food colorants, dietary supplements, cosmetics and pharmaceuticals purposes (Meddeb-Mouelhi *et al.,* 2016). Bacteroidetes and Proteobacteria are abundant during or following an algal bloom (Meddeb-Mouelhi *et al.,* 2016). *Hymenobacter* sp belongs to the phylum Bactroidetes and was also identified in this study by Royo-Llonch *et al.,* (2017) who reported that *Hymenobacter* sp, inhabit different environmental niches like marine, freshwater, air, soil, and glacier. *Pseudomonas* sp a Proteobacteria identified currently is common in the aquatic environment according to (Mishra *et al.,* 2017) and most strains are known to be phosphate solubilizing bacteria and also produce antagonism to other pathogens (Paul & Sinha, 2016).

5.2 Alpha diversity of the recovered bacteria using culture-independent technique

Bacterial diversity for lake Oloiden was lower than that of L. Olbolosat. This is following studies by Luo *et al*., (2017) who reported that microbial biodiversity of

soda lakes is normally lower than that of freshwater ecosystems. The of ASVs recovered was lowest in water samples as compared to other samples. This is in line with studies by (Cleary & Polónia, 2018) who reported OTU richness being the lowest in water biotopes. The results of α-diversity in the investigated lake ecosystems were in line with values for some indices reported from other tropical lakes in the same latitude (Inceoɬlu *et al.,* 2015).

5.3 Ecological roles of the recovered bacterial indicator taxa

Similar to other 16S surveys from inland water ecosystems within tropical lakes in the same latitude, the bacterial communities recovered were similar to the following studies carried out by Mwirichia *et al.* (2011; Inceoɬlu *et al.* (2015; Kambura *et al.* (2016; Ghilamicael *et al.* (2017; Cleary & Polónia (2018). The higher relative abundance of phyla Proteobacteria, Firmicutes, Actinobacteriota, Chloroflexi, Cyanobacteria, Acidobacteriota, Planctomycetota and Bacteroidota comprised most of the observed taxa, perhaps indicating either that inland water ecosystems are similar regardless of seasons and geographical locations, or that high-level taxonomic assessment harbors important species-level variation (Mhuireach *et al.,* 2019). Lake Olbolosat a fresh water body harbored a high number of microbial taxa as compared to Lake Oloiden which is a saline-alkaline water body. The reason why some microbes were predominating in dry sediments within the two lakes could be more space for colonization in dry sediments. Some were predominant within water samples due to their flagella which made them mobile. Wet sediments and microbial mats had a low abundance as compared to water and dry samples (Luo *et al*., 2017).

The phylum Proteobacteria was the predominant phylum observed across the samples within the two lakes but was highly abundant in water samples. Proteobacteria have been reported to play a crucial role in degradation and metabolism within the lake ecosystem and are also involved in biogeochemical processes in marine ecosystems (Huang *et al.,* 2017). The class α-Proteobacteria was higher within the two lakes than γ-Proteobacteria. The α-Proteobacteria was highly abundant within lake Oloiden recovered from the dry sediment samples as compared to wet sediments, water and microbial mats. *Xanthobacteraceae, Rhodobacteraceae, Sphingomonadaceae,* and

Berinckiaceae were families retrieved from the α-Proteobacteria class. These members are beneficial partners in plant-microbe interactions especially nitrogen fixation, legume nodulation and methanotrophic activities (Grube & Berg, 2015). *Pseudomonadaceae*, *Moraxellaceae*, *Comamonadaceae*, and *Oxalobacteraceae* are families that belonged to the class γ-Proteobacteria. These members have previously been reported to play a key role in the degradation of low and high-molecular-weight organic matter in the ecosystem, especially where there are low levels of oxygen concentration (Mahmoudi *et al.,* 2015).

The α-Proteobacteria and γ-Proteobacteria have been regarded as primary colonizers and they prepare the ecosystem for subsequent colonization according to Abed *et al.* (2019). They mutually coexist and establish a heterotrophic bacterial film in the mats. They sense environmental signals such as NA+flux in the surrounding water thereby shifting from motile to sessile lifestyle (Belas, 2014). Actinobacterota and Firmicutes are secondary colonizers depending on the nutrients, microbial, or decay products provided by primary colonizers (Abed *et al.,* 2019). The interaction between the primary and secondary colonizers such as competition, cooperation, mutual exclusion, limited dispersal or availability can even result in a segregated pattern of biofilm by such microbes present in biofilm over time (Abed *et al.,* 2019). The phylum Firmicutes was the second most abundant phylum recovered from lakes Olbolosat and Oloiden. They are ubiquitous in aquatic ecosystems probably due to their spores that quickly germinate during favorable conditions (Abed *et al*., 2019). Adherence of Firmicutes to the surfaces could be a strategy used for survival and evolving as a community in the lake ecosystem (Prieto-Barajas *et al.,* 2018). Firmicutes are also known to be good bio-degraders of organic pollutants such as petroleum hydrocarbons polychlorinated biphenyl and hexahydro-1,3,5-trinitro-1,3,5-triazine (Zhang *et al.,* 2015). Clostridia class was highly abundant among Firmicutes retrieved from wet sediments within L. Olbolosat as compared to L. Oloiden. Lake Oloiden's Clostridia class was highly abundant from microbial mats than from L. Olbolosat. Microbial mats comprise millions of microorganisms that belong to different taxa, that interact and exchange signals embedded in a matrix of exopolysaccharides and nutrients to enable the flow of energy, and resources for the survival of the community (Prieto-Barajas *et al.,* 2018). Earlier studies have reported Clostridia being precursors for sulfate reduction.

They are also important in the anaerobic decomposition of organic matter in aquatic ecosystems (He *et al*., 2020). Dominant families in the class of Clostridia were P*eptostreptococcaceae* dominated the microbial mat samples. *Lachnospiraceae* dominated wet sediment, and mat, while *Peptostreptococcales-Tissierellales_fa* dominated the mat samples.

Actinobacteria and Acidiimicrobiia were also retrieved from the current studies. Most members of this group are known to have a high mol G+C content because of their triple hydrogen bond (Hamid *et al.,* 2020). The high mol G+C content helps them to adapt to unfavorable environmental conditions, become tolerant to antagonism factors and with low mutation rate from the study showed that ASVs for Cyanobacteria, Acidobacteriota, Planctomycetota, Bacteroidota, Deinococcota, Gemmatimonadota, Verrucomicrobiota, Bdellovibrionota, Armatimonadota, Patescibacteria, and Campilobacterota in recovered samples were very scarce. Sequences affiliated with Halobacterota and Euryarchaeota were detected from the archaeal kingdom and have been reported to be methanogens within lake ecosystems (He *et al.*, 2020). Early studies by Cavicchioli *et al.* (2011) have reported that archaea inhabit only the extreme ecosystems only but studies by Li *et al.*, (2019) demonstrated that this group is ubiquitous and can be found in non-extreme ecosystems. This suggests why four archaeal classes; Methanobacteria, Methanocellia, Methanosacinia, and Methanomicrobia were retrieved from samples in the current studies. Studies by Laskar *et al.* (2018) indicate that methanogens are highly distributed in stringently anaerobic and natural ecosystems such as freshwater and marine sediments, rice fields, deep-sea hydrothermal vents, marine mud volcanoes, mangroves and hot springs (Li *et al.,* 2019). Global methane emission has been linked with methanogenic archaeal communities leading to a great increase in global warming (Laskar *et al.*, 2018).

The indicator representatives of the genera associated with the wet sediments were distributed among Proteobacteria (*Bradirhizobiumand* and *Sphingomonas*), Firmicutes (*Clostridium_sensu_stricto*_10 and *Exiguobacterium*), Actinobacteriota (*Pseudonocardia* and *Streptomyces*), Chloroflexi (*Anaerolinea*), and Acidobacteriota (*Candidatus_Solibacter* and *Bryobacter*) Josiah *et al.*, 2018) . This study might be limited to ecosystem functioning due to biasness brought during DNA analysis from

the environmental samples. Only dormant or inactive prokaryotes and fungi were targeted using Illumina sequencing. However, studies by Hartmann *et al.* (2012); Rime *et al.* (2015) suggests that the analysis of indicator organism can be useful in the study of the functional role of these microbes in the ecosystem. *Sphingomonas* and *Bradyrhizobium* have been reported to biodegrade environmental pollutants (Huang *et al.*, 2017). *Exiguobacterium* retrieved in this study from microbial mats as an indicator organism is a secondary colonizer that has been reported to withstand harsh environmental conditions by the formation of spores (Liao *et al.*, 2019. *Streptomyces* and *Pseudonocardia* have been reported to be important in the degradation of complex organic molecules in the lake ecosystem. They are also able to survive in harsh environmental conditions due to their high G+C content. *Candidatus_Solibacter* and *Bryobacter* are oligotrophic microbes retrieved in this study and the findings are in line with studies by Liao *et al.* (2019) who reported that they are known to adapt to low nutritional concentrations. Among the 5 differentially abundant ASVs, 80 % belonged to phylum Actinobacteriota and 20 % belonged to phylum Proteobacteria. Sediments within lakes are the main media for migration and nutrient transformation and influence the nutrient contents within the lakes (Huang *et al.*, 2017).

5.4 Bacterial community composition and correlation to environmental factors

Despite intensive anthropogenic activities that can influence changes in biogeochemical cycles and microbial ecology within inland water bodies such as lakes Olbolosat and Oloiden, these ecosystems are still rich with microbial communities. However, microbial communities are dependent on environmental factors. Prokaryotic communities within lakes Olbolosat are highly dependent on Copper, Iron, Manganese and Ammonium while those from lake Oloiden are dependent on Magnesium, Sodium, Calcium and potassium. Copper, Iron and Manganese are micronutrients that are trace elements used as enzyme cofactors by microorganisms. Magnesium, Sodium and Potassium are macro elements that have been reported to contribute to the species richness and composition of prokaryotic communities (Liu *et al.*, 2018).

5.5 Alpha diversity of the recovered fungi using culture-independent technique

The study revealed fungal diversity within lake Olbolosat is higher than that of lake Oloiden. This finding is consistent with previous studies within the same lentic habitats on prokaryotes by Kiama *et al*. (2021) where they observed lake Oloiden recording a low number of prokaryotic taxa compared to lake Olbolosat. There was a low number of fungal diversity recovered within lakes Olbolosat and Oloiden. The low levels of species diversity for the detected fungal represent a species-poor community probably due to the dominance of free-floating single-celled and highly abundant microalgae (Chlorophyta) recovered in the current studies, low nutrient levels, absence of substrate of fungal cell attachment (Bärlocher & Boddy, 2016; Khomich *et al.*, 2017). Water samples recorded the lowest diversity compared to microbial mats, dry and wet sediments from both lakes Olbolosat and Oloiden. Pre-filtering of water samples during DNA extraction could probably have led to the exclusion of some fungi or filamentous fungi attached to the phytoplankton thus reducing their detection (Khomich *et al.*, 2017). Eukaryotic community structure varied between lakes Olbolosat and Oloiden that respond to local scale productivity (Khomich *et al.*, 2017).

5.6 Ecological roles of the recovered indicator fungal taxa

Most fungi and other eukaryotes like Chlorophyta in aquatic ecosystems can decompose a wide range of organic substrates and as major consumers of the biomass produced by the bacteria (Wahl *et al.*, 2018). Aquatic fungi produce spores as dispersal propagules in the water and later become entrapped on a substrate, subsequently colonizing their new niche. The current findings are consistent with studies by Voytsekhovich & Beck, (2016) who reported Chlorophyta being highly abundant in all samples during spring and summer in Baiyangdian wetland lake in China. *Trebouxia* (green algae) was the only recovered genus in Chlorophyta phyla across all samples obtained from lakes Olbolosat and Oloiden. *Trebouxia* (green algae) was the only recovered genus in Chlorophyta phyla across all samples obtained from lakes Olbolosat and Oloiden. *Trebouxia* can exist independently or in a symbiotic relationship with fungi in the form of lichen (Wang *et al*. 2013). It is a primary producer of freshwater, marine and terrestrial ecosystems (Lu *et al.*, 2020). It also

serves as a bioindicator of climate change, habitat disturbance, air pollution, freshwater quality and carbon dioxide concentration. The algal partner is referred to as phycobiont. The green algae (*Trebouxia*) and blue-green (*Nostoc*) are the most commonly known phycobionts Romeike *et al.,* 2002). Fungi partners are referred to as mycobionts and are usually Ascomycota, Basidiomycota and anamorphic fungi. The fungi derive their nutrients in return for protecting the algae. The fungi and the algae partners lose their identity in the lichen thallus. Studies are underway on whether to place mycobionts as fungi kingdoms or as phycobionts in algae classification (Lu *et al.,* 2020).

The phylum Chytridiomycota was recovered from eight sample types. It was highest in wet sediment from lake Olbolosat ZB2. The current studies are in line with studies by Gleason *et al.* (2017) who identified Chytridiomycota from water samples within inland lakes. Members of this group have been recovered in Kenyan lakes by Odilia *et al*. (2017) and Kambura *et al.* (2016). The Zoosporic fungi such as Chytridiomycota have been known as parasites of freshwater and marine phytoplankton while mutualism symbiosis is very rare (Gleason *et al.,* 2017). In some cases, some zoosporic fungi have been known to cause infections to larger aquatic organisms such as shrimps, fish or fish eggs and frogs. *Batrachochytrium dendrobatidis* is a chytrid that has paid attention to the worldwide extinction of some unknown and known species of frogs (Tsui *et al.,* 2016). Zoosporic fungi are also known to be saprobes that colonize or grow on submerged wood or waterlogged leaves within lakes (Tsui *et al.,* 2016). Chytridiomycota is known to produce zoospores. Zoospores are motile spores characterized by a single posterior-directed whiplash flagellum. Chytridiomycota plays a key role in aquatic environments by recycling nutrients and controlling algal biomass and blooms. They mostly inhabit pelagic zones within the lake ecosystems (Panzer *et al.,* 2015). Zoospores for Chytrids are food resource to Zooplanktons by acting as food in a nutrient loop that is called mycoloop (Lepère *et al.,* 2019) Similar studies on fungal communities within sediments and water from hot springs in Kenya by (Kambura *et al.* (2016) reported low abundance of Chytridiomycota phyla.

The phylum Monoblepharomycota was only recovered from microbial mat ED2 from lake Oloiden. Monoblepharomycota has been classified together with the Chytridiomycota phylum (Lepère *et al.,* 2019). Both Monoblepharomycota and

Chytridiomycota have zoospores that are motile and are present in aquatic ecosystems. There has been a recent proposal to place them in their phylum because they have a true mycelial growth unlike their sister clade Chytridiomycota. They are also unique in their oogonic sexual cycle since the gametes are morphologically different. Monoblepharomycota phyla are free-living saprobes and parasitoids in aquatic environments especially freshwater lakes (Naranjo-Ortiz & Gabaldón, 2019).

Mortierellomycota was highest from dry sediments from lake Olbolosat ZC2. It was present in other dry sediments EC1 and EC2 from lake Oloiden, microbial mat ED2 from lake Oloiden and wet sediment from lake Olbolosat ZB2 and EB2 from lake Oloiden. It was recovered in abundance from lake Oloiden a saline-alkaline lake. Hoffmann *et al*. (2011) reported that they are important in the conversion of organic compounds into poly-unsaturated fatty acids in dry sediments. Mortierellomycota belongs to zygomycetes fungi known for the production of zygospores during sexual reproduction Wijayawardene *et al.* (2018). Zygospores lack hyphal cell walls except in the reproductive structures. There has been a recent proposal to place Mortierellomycota in their phylum because their zygospores are different from the rest of zygomycete fungi (Naranjo-Ortiz & Gabaldón, 2019). The phylum also lacks columella in its sporangiophore. They are distinguished from the rest of zygospores since they produce a garlic-like odor when grown in the media (Hurdeal *et al.,* 2021. Most species grow as filamentous saprobes within aquatic environments (Hoffmann *et al.,* 2011).

Glomeromycota phylum was classified together with zygomycetes fungi. It was separated to its phylum due to the absence of s zygospore based on early ribosomal protein phylogenies or any sexual structure. This phylum was only recovered from dry sediment from lake Olbolosat. Members of this group have been recovered in Kenyan lakes by Kambura *et al*. (2016) and Odilia *et al*. (2017). Most members in this group form an endomycorrhizal relationship with most plants and macrophytes except *Geosiphon* spp which form a symbiosis with cyanobacteria. Glomeromycota benefit the plant with the acquisition of nitrogen, phosphorus and water in exchange for photosynthesis-derived metabolites. The endomycorrhizal fungus invades cells of the cortex of the rootlets forming no sheath and hyphae (Naranjo-Ortiz & Gabaldón, 2019).

The phylogenetic position of Olpidiomycota remains unclear. However, most Olpidiomycota phyla are ubiquitous obligate parasites of roots of flowering plants, algae, mosses, pollen, fungi, nematodes and rotifers in freshwater ecosystems and soil (Barron, 2004; Meirinho *et al*., 2013). The phylum Olpidiomycota was frequently recovered from wet sediment from both EB1samples from lake Oloiden and ZB2 from lake Olbolosat. Members of this phylum were also identified from microbial mats from lake Oloiden (ED1. Studies by Meirinho *et al.* (2013) are consistent with the current studies that reported this phylum being recovered from aquatic samples. Rotifers are important zooplanktons of aquatic ecosystems especially freshwater ecosystems because of their abundance and diversity. *Olpidium* is an endoparasitic organism that produces zoosporangia that discharge zoospores via tubes. These tubes are plunged with mucus before their release into their hosts. During the infection, they digest the internal content of their hosts while forming a thallus inside where zoospores can be produced and discharged hence starting the process again (Meirinho *et al.*, 2013). Several *Olpidium* species have been reported to infect eggs or adults of rotifers and other invertebrates (Meirinho *et al.*, 2013).

Members of this phylum were recovered from microbial mats and dry sediments from lake Oloiden and Olbolosat respectively. These studies are consistent with studies by Kambura et al. (2016) and Odilia et al. (2017) who identified them from Kenyan lakes. They are filamentous zygomycetous fungi that are saprobes and occasional mycoparasites, plant pathogens, or ectomycorrhizal. The sporangiophore for Mucoromycota has well-developed columella, unlike Mortierellomycota which lacks columella (Naranjo-Ortiz & Gabaldón, 2019).

Basidiomycota and Ascomycota belong to dikarya subkingdom referred to as “higher fungi”. Basidiomycota is the second most rich in terms of species richness with approximately 32, 000 species after Ascomycota, a dikarya that describes nearly 64,000 species (Wahl *et al.*, 2018). The phylum Basidiomycota was recovered in almost all sample types within the two lakes except EAE, ZB1, ZC2 and ZD2. The current findings are in line with studies by Kambura et al. (2016) and Odilia et al. (2017) who identified Basidiomycota from Kenyan lakes. Basidiomycota is one of the complex fungi in terms of the cell cycle, especially *Puccinia* spp. The main

characteristic of this phylum is the production of basidiospores inside a basidium. Basidiomycota forms an ectomycorrhizal symbiotic relationship with the roots of aquatic macrophytes and other plants. The Basidiomycota fungus produces a covering outside the hyphae of the rootlets of the host plant all around. They are fewer than their terrestrial counterparts and colonize a wide range of substrates and are also well adapted in their habitats by having a reduced basidium (Tsui *et al.,* 2016).

The phylum Ascomycota was present in seven sample types among the fifteen samples that were collected from both lakes Olbolosat and Oloiden. The abundance of thy phylum is lower than the Basidiomycota. Studies by Kambura *et al.,* (2016) and Odilia et al. (2017) also identified members of this phylum from Kenyan lakes. Ascomycota produces non-motile ascospores with various gelatinous gel-like sheath appendages that assist ascospores' dispersal or attachment to a substrate. The hyphae are normally thick-walled. Ascomycota is known as lignicolous such as Sordiomycetes that colonize organic matter for submerged wood, aquatic plants, or fallen leaves in freshwater ecosystems (Wahl *et al.,* 2018). Lignicolous fungi play an important role within the aquatic ecosystems through carbon and nutrient cycling ecosystems functioning of freshwater ecosystems. The lignicolous fungi can decompose woody litter using lignocellulose enzymes releasing nutrients in such environments (Luo *et al*., 2019). Dikarya fungi have been reported to predominate marine environments such as oceans, seas and deep pelagic lakes (Panzer *et al.,* 2015).

5.7 Fungal community composition and correlation to environmental factors

The diversity and community structure of aquatic fungi is influenced by environmental factors (Zhang *et al*., 2016). Copper and magnesium among the cations, chlorine and anion, and electron conductivity were the best predictors for fungal diversity and structure within microbial mats, water, dry and wet sediments within lakes Olbolosat and Oloiden. Copper was the best predictor among all environmental factors within lakes Olbolosat and Oloiden and probably this could have been attributed to anthropogenic activities around these lentic ecosystems (Sutcliffe *et al.,* 2019). Studies have reported aquatic communities sensitive to low concentrations of soluble copper ions (Sutcliffe *et al*., 2019). The current study is partly in agreement with Zhang *et al.*

(2016) who reported electron conductivity being positively significant in fungal communities recovered in samples from Ny-Ålesund Region.

5.8 Community network interactions

The interaction network of bacterial communities within lakes Olbolosat and Oloiden displayed Proteobacteria to be highly positively connected with other microbes. This could be due to diverse metabolic mechanisms thus promoting the growth of the microbial community (Liao *et al*., 2019). Network analyses of the co-occurrence patterns among the genera of the major phyla also revealed species dynamics between sample types, particularly genus *Trebouxia* and genus *Mortierella* represented phyla Chlorophyta and Mortierellomycota, respectively. The difference between these two phyla is due to their life cycle and physiological functions. Chlorophyta is an efficient symbiont to other fungi within the aquatic ecosystems Lu *et al.* (2020) while Mortierellomycota is a filamentous saprobe that converts organic compounds into poly-saturated fatty acids within the dry sediments (Hoffman *et al*., 2020).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- Bacteria recovered through culture-dependent technique were 4 phyla while 17 phyla were recovered using culture-independent technique from lakes Olbolosat and Oloiden.
- The study indicated eight bacterial isolates could be novel those that are closely related to; ZCP 6.1(*Bacillus* sp), EBP 8.2 (*Bacillus pumilus*), EBP 3.9 (*Bacillus cereus*), ZCP 17.2 (*Bacillus toyoniensis*), ECP 3.4 (*Streptomyces* sp), EBP 2.5 (*Aeromicrobium* sp), ZAP 9.1(*Hymenobacter* sp) and ZAP 16.1 (*Streptomyces hawaiiensis*).
- Firmicutes were the most predominant phylum recovered using culture-dependent technique from all samples within the two lakes.
- Proteobacteria was the most predominant bacterial phylum recovered using high throughput sequencing observed across all samples within the two lakes.
- Chlorophyta a photobiont and Basidiomycota were the most predominant fungal phyla recovered using high throughput sequencing within the two lakes.
- This study provides evidence that bacterial and fungal taxa are greatly influenced by environmental factors.

6.2 Recommendations

1. An upscale for the novel bacterial isolates with industrial application in food, agriculture, textile and health could be done as a way forward.
2. Studies should be carried out to determine whether changes in microbial communities correlate with changes during dry and rainy seasons.
3. More studies should be done on bacteria and fungi from both lakes to reveal their functional role.

4. Targeted bacterial isolation should be carried out to recover, characterize and identify the recovered novel bacterial and fungal taxa revealed through next-generation sequencing.

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APPENDICES

Appendix I: A copy of the research authorization

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Appendix II: Script for upstream analysis

```
library(dada2)
path <- "C:/Cate metagenomics/Bacterial community" # CHANGE ME to the
directory containing the fastq files after unzipping.
list. files(path)
Forward and reverse fastq filenames have format: SAMPLENAME_R1_001.fastq
and SAMPLENAME_R2_001.fastq
fnFs <- sort (list.files(path, pattern="_1.fastq.gz", full.names = TRUE))
fnRs <- sort (list.files(path, pattern="_2.fastq.gz", full.names = TRUE))
Extract sample names, assuming filenames have format:
SAMPLENAME_XXX.fastq
sample.names <- sapply(strsplit(basename(fnFs), "_"), `[`, 1)
Inspect read quality profiles
plotQualityProfile(fnFs [1:2])
plotQualityProfile (fnRs [1:2])
Place filtered files in filtered/ subdirectory
filtFs <- file.path(path, "filtered", paste0(sample.names, "_F_filt.fastq.gz"))
filtRs <- file.path(path, "filtered", paste0(sample.names, "_R_filt.fastq.gz"))
names(filtFs) <- sample.names
names(filtRs) <- sample.names
out <- filterAndTrim (fnFs, filtFs, fnRs, filtRs, truncLen=c (240,200),
                maxN=0, maxEE=c (2,2), truncQ=2, rm.phix=TRUE,
                compress=TRUE, multithread=FALSE) # On Windows set
multithread=FALSE
head(out)
Learn the Error Rates
errF <- learnErrors (filtFs, multithread=TRUE)
errR <- learnErrors (filtRs, multithread=TRUE)
Error Rates
plotErrors (errF, nominalQ=TRUE)
Sample Inference
dadaFs <- dada (filtFs, err=errF, multithread=TRUE)
```

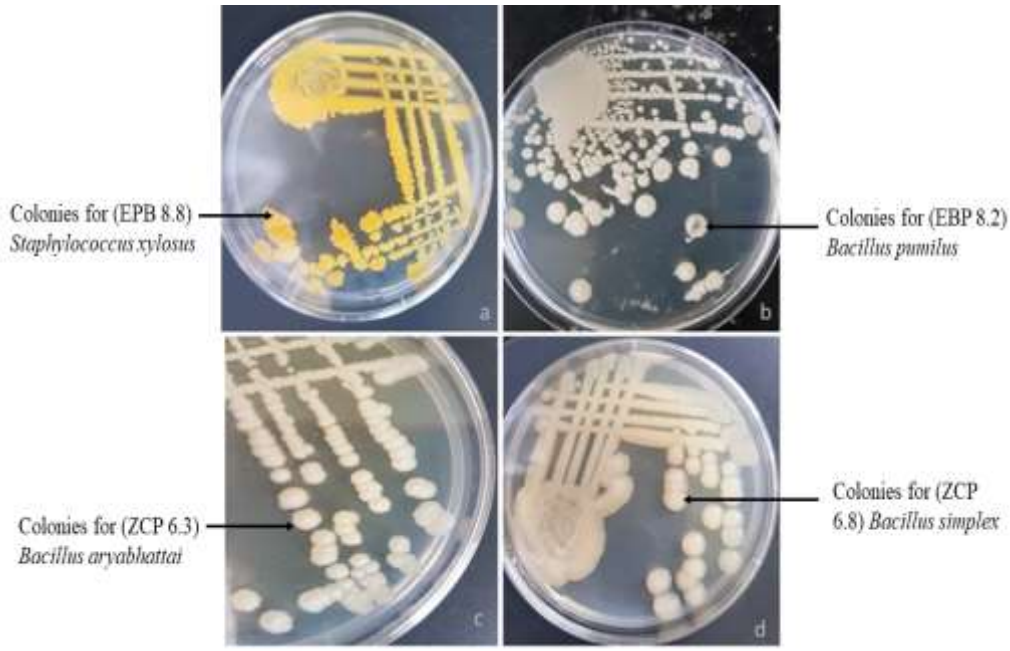
```
dadaRs <- dada (filtRs, err=errR, multithread=TRUE)
Inspecting the returned dada-class object:
dadaFs[[1]]
Merge paired reads
mergers <- mergePairs(dadaFs, filtFs, dadaRs, filtRs, verbose=TRUE)
Inspect the merger data.frame from the first sample
Head (mergers [[1]])
Construct sequence table
seqtab <- makeSequenceTable(mergers)
dim(seqtab)
Inspect distribution of sequence lengths
table(nchar(getSequences(seqtab)))
Remove chimeras
seqtab.nochim <- removeBimeraDenovo(seqtab, method="consensus",
multithread=TRUE, verbose=TRUE)
dim(seqtab.nochim)
sum(seqtab.nochim)/sum(seqtab)
Track reads through the pipeline
getN <- function(x) sum(getUniques(x))
track <- cbind(out, sapply(dadaFs, getN), sapply(dadaRs, getN), sapply(mergers,
getN), rowSums(seqtab.nochim))
If processing a single sample, remove the sapply calls: e.g. replace sapply(dadaFs,
getN) with getN(dadaFs)
colnames(track) <- c ("input", "filtered", "denoisedF", "denoisedR", "merged",
"nonchim")
rownames(track) <- sample.names
head(track)
savedata
saveRDS (seqtab.nochim, "Catebacteria.rds")
```

Appendix III: Script for the preparation of the downstream analysis

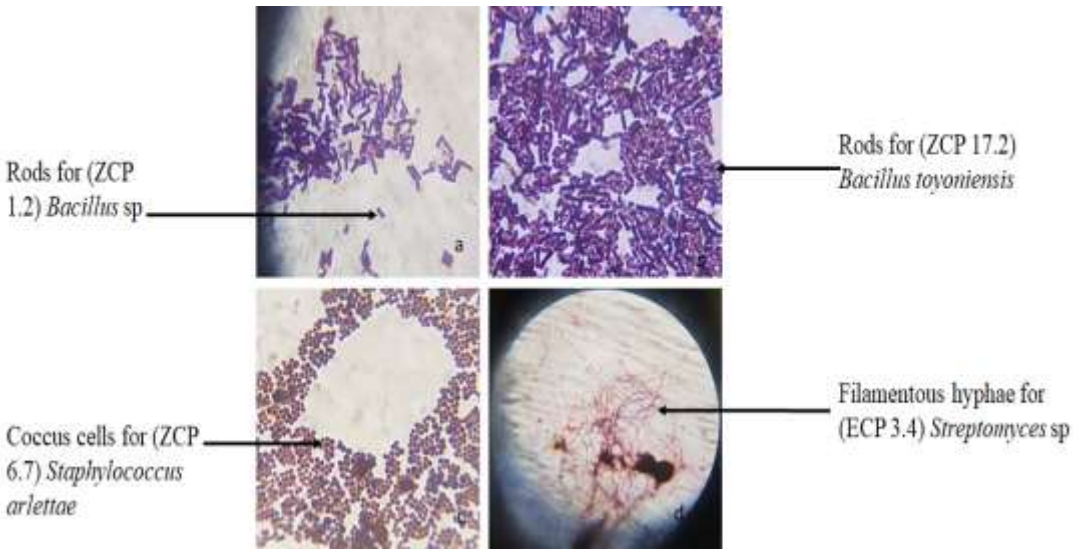
```
library(dada2)
Kenya_lakes <- readRDS("dada2output9Oct.rds")
dim (Kenya_all_lakes)
table(nchar(getSequences(Kenya_all_lakes)))
Kenya_all_lakes_filtered <- Kenya_all_lakes [,nchar(colnames(Kenya_all_lakes))
%in% seq(500)]
dim (Kenya_all_lakes_filtered)
table(nchar(getSequences(Kenya_all_lakes_filtered)))
#Create FASTA
asv_seqs <- colnames(Kenya_all_lakes_filtered)
asv_headers <- vector (dim (Kenya_all_lakes_filtered)[2], mode="character")
asv_fasta <- c (rbind(asv_headers, asv_seqs))
write (asv_fasta, "rep_set.fasta")
#Silva 138 data is available from
https://figshare.com/account/projects/60689/articles/7794296
taxa <- assignTaxonomy(Kenya_all_lakes_filtered,
"silva_nr99_v138_train_set.fa.gz", multithread=TRUE)
write.table(taxa, "dada2_taxa.txt", sep='\t', row.names=FALSE, quote=FALSE)
##Seq Table
Kenya_all_lakes_filtered_t <- t(Kenya_all_lakes_filtered) #transpose the table
Kenya_all_lakes_filtered_t <- cbind('#OTUID' =
rownames(Kenya_all_lakes_filtered_t), Kenya_all_lakes_filtered_t)#Add
'#OTUID' to the header (required by biom)
write.table(Kenya_all_lakes_filtered_t, "dada2_seq_table.txt", sep='\t',
row.names=FALSE, quote=FALSE)
#Import meta data##
Meta <- read.delim("Metadata200626.txt", row.names = 1)
library(phyloseq)
library(ggplot2)
##Mke phyloseq data table
```

```
Lakes_all <- phyloseq(otu_table(Kenya_all_lakes_filtered,
taxa_are_rows=FALSE),
                      sample_data(Meta),
                      tax_table(taxa))
Lakes_all
##remove Chloroplast and Mitochondria
Lakes_all_no_chloro<- subset_taxa(Lakes_all,
!Order=="Chloroplast"&!Family=="Mitochondria")
get_taxa_unique(Lakes_all_no_chloro, "Order")
get_taxa_unique (Lakes_all_no_chloro, "Family")
##Change row name from sequence to consequtive number
new.names <- paste0("ASV", seq(ntaxa(Lakes_all_no_chloro)))
seqs <- taxa_names (Lakes_all_no_chloro)
names(seqs) <- new.names
taxa_names(Lakes_all_no_chloro) <- new.names
#Pick up Kenyan lakes samples
Kenya2020_lakes = subset_samples(Lakes_all_no_chloro, DNA=="DNA")
##general information
sample_names (Kenya2020_lakes)
otu_table (Kenya2020_lakes) [1:5, 1:5]
rank_names (Kenya2020_lakes)
tax_table (Kenya2020_lakes)
sample_variables (Kenya2020_lakes)
##Save the data
saveRDS(Kenya2020_lakes, "taxonomyoutput9Oct.rds")
```

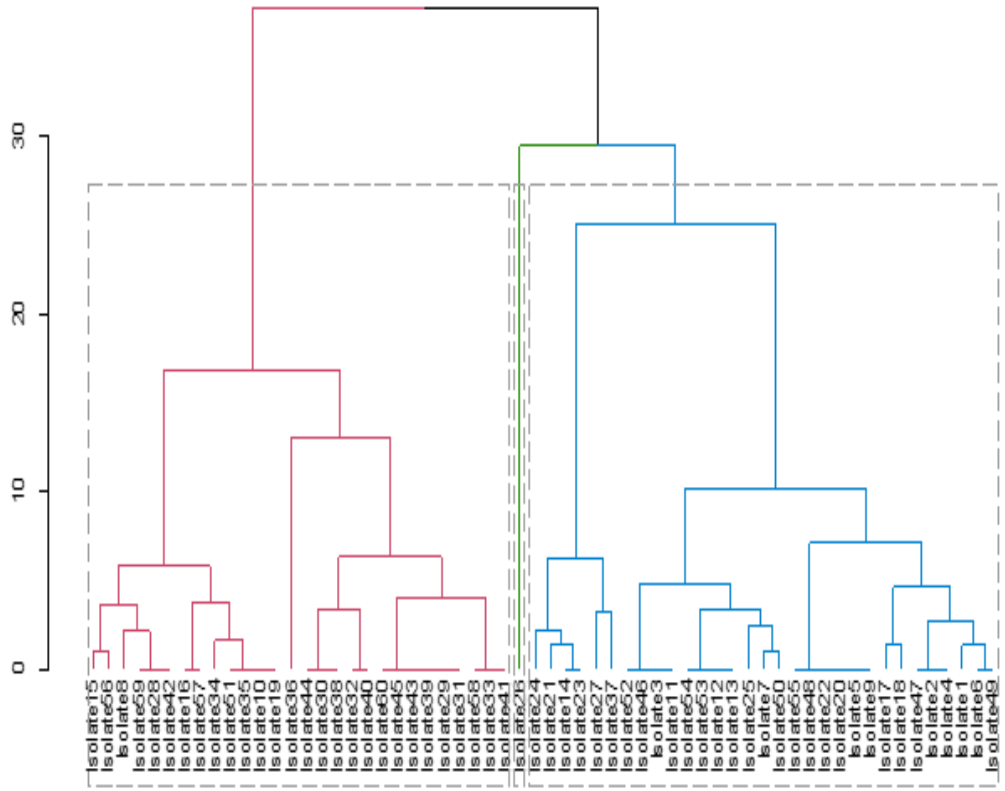
Appendix IV: A pure culture plate showing - a) irregular form and serrated margin for EBP 8.8 b) circular and entire margin for EBP 8.2 isolate c) circular and entire margin for ZCP 6.3 d) circular and entire margin for ZCP 6.8.



Appendix V: A pure culture plate showing - a) Gram-positive rods for ZCP 1.2 b) Gram-positive rods for ZCP 17.2 c) Gram-positive coccus cells for ZCP 6.7 d) filamentous hyphae for ECP 3.4.



Appendix VI: A dendrogram for morphology and cellular characteristics for the 60 bacterial isolates



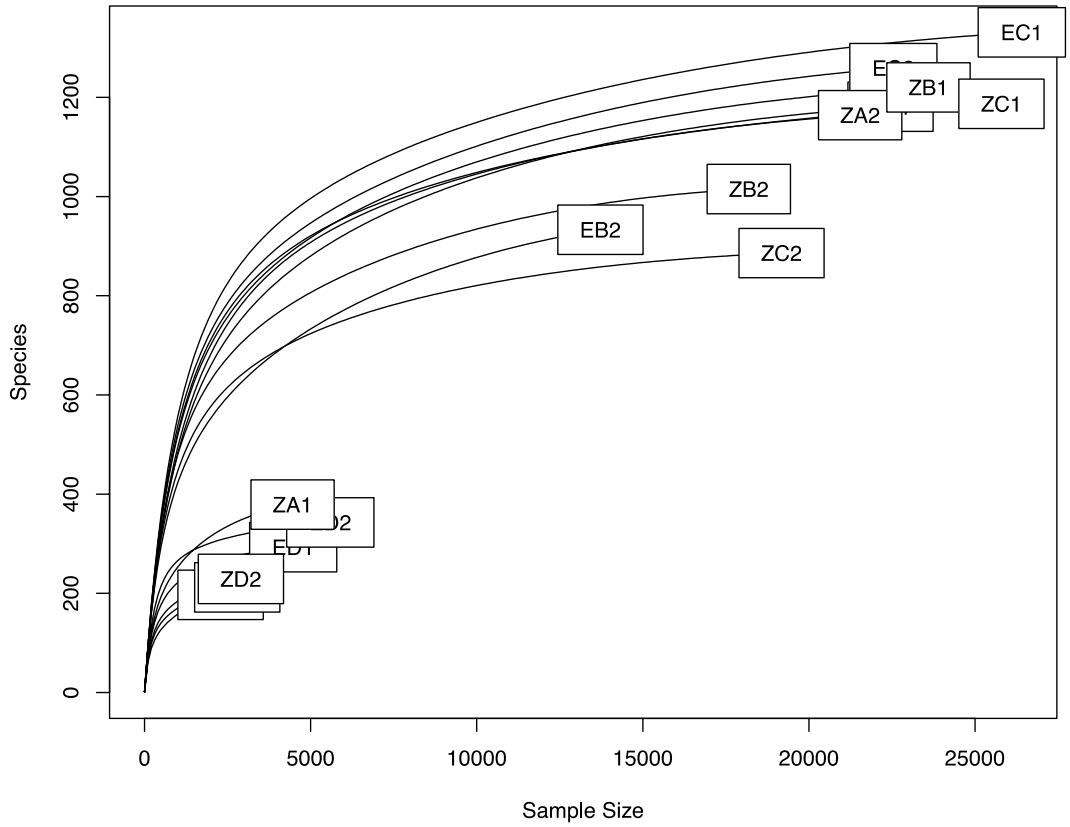
Appendix VII: Script for the ANOSIM

```
dune.dist <- vegdist(community)
attach(sample)
dune.ano <- anosim(dune.dist, SampleType)
summary(dune.ano)
plot (dune.ano, ylab = "Diversity", xlab = "Sample Type")
```

Appendix VIII: Script for the Heatmap

```
community<- read.csv ("taxa.csv", header = TRUE, sep=",")
sample<- read.csv ("2020/filter.csv", header = TRUE, sep=",", row.names=1)
write.csv (lakes, "Lakes.csv")
Cate<- read.csv ("antimicrobial.csv", header = TRUE, sep=",", row.names=1)
data.dist <- vegdist(hmfamsite, method = "bray")
col.clus <- hclust(data.dist, "aver")
data.dist.g <- vegdist(t(data.prop.1), method = "bray")
row.clus <- hclust(data.dist.g, "aver")
plot(annHeatmap2(as.matrix(t(data.prop.1)), col =
colorRampPalette(c("lightyellow", "red"), space = "rgb")(540), breaks = 541,
dendrogram = list(Row = list(dendro = as.dendrogram(row.clus)), Col =
list(dendro = as.dendrogram(col.clus))), cluster = list(Col = list(cuth = 0.98, col =
brewer.pal(10, "Set2")), Row = list(cuth = 0.97, col = brewer.pal(10, "Set2"))),
legend = 3, labels = list(Row = list(nrow = 3), Col = list(nrow
```

Appendix IX: Rarefaction curves for the prokaryotic communities in each sample.



Appendix X: Script for the alpha diversity

```
#Plot

plot_richness (Kenya2020, x="Site", measures=c("Chao1", "ACE", "Shannon",
"Simpson", "InvSimpson", "Observed", "Evenness"), color = "Site") +
  geom_boxplot() +
  geom_point(position = position_dodge(width = 0.75)) +
scale_x_discrete(limits=c("EA1","EA2","EB1","EB2","EC1","EC2","ED1","ED2
","ZA1","ZA2","ZB1","ZB2","ZC1","ZC2","ZD2")) +
  scale_color_manual(values =
c("EA1"="#d95f0e","EA2"="#d95f0e","EB1"="#d95f0e","EB2"="#8856a7","EC
1"="#8856a7","EC2"="#8856a7","ED1"="#3182bd","ED2"="#3182bd","ZA1"="
#d95f0e","ZA2"="#d95f0e","ZB1"="#d95f0e","ZB2"="#d95f0e","ZC1"="#8856a
7","ZC2"="#8856a7","ZD2"="#3182bd")) +
  theme_bw() +
  ylab("Diversity") +
  ggtitle("") +
  theme (axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.55, size=11),
        axis.title.x = element_blank(), legend.position = "none")
```

Appendix XI: Script for the Phylum composition

```
Kenya_phylum_clr <- Kenya2020 %>%
tax_glom(taxrank = "Phylum") %>%  # Transform to rel. abundance
psmelt() %>%
unite (Phylum, Phylum, col = "Phylum", sep = "_") %>%
arrange (Phylum)
Kenya_phylum_mean_table <- Kenya_phylum_clr %>% dplyr: filter (Phylum
%in% Kenya_phylum_highRA$Phylum) #pick target
Kenya_phylum_each <- Kenya_phylum_mean_table %>% dplyr: select (Sample,
Phylum, Abundance) %>% spread (Phylum, Abundance)
write.table(Kenya_phylum_each, "Kenya_phylum_each.csv", sep =",")
##Change all zero to 1
rownames(Kenya_phylum_each) <- Kenya_phylum_each$Site
Kenya_phylum_each <- Kenya_phylum_each[,-1]
Kenya_phylum_each[Kenya_phylum_each == 0] <- 1
Kenya_phylum_each[1:10,1:18]
phylum_clr <- as.data.frame(clr(Kenya_phylum_each))
phylum_clr[1:10,1:10]
superheat(as.matrix(t(phylum_clr)), # change the size of the labels
        left.label.size = 0.4,
        bottom.label.size = 0.1,# change the grid size
        grid.hline.col = "white",
        grid.vline.col = "white",
        heat.pal = c ("white", "#377eb8","#ffff33","#e41a1c"),
        heat.pal.values = c(-1, 0, 0.5, 1), #Max1 within 1 chnage the position of
colors
        bottom.label.text.angle = 90,
        left.label.text.size =5,
```

Appendix XII: Script for the relative abundance

```
####Phylum_RA####

Kenya_phylum <- Kenya2020 %>%
  transform_sample_counts(function(x) {x/sum(x)}) %>% # Transform to rel.
abundance
  tax_glom(taxrank = "Phylum") %>%     # agglomerate at phylum level
  psmelt() %>%                         # Melt to long format   # Filter out low
abundance taxa
  arrange (Phylum)
##plot
Kenya_phylum_highRA <- Kenya_phylum %>%
  group_by(Phylum, Sample) %>%
  summarise(Abundance=mean(Abundance)) %>%
  filter (Abundance > 0.002) #pick target (high RA) by their names
Kenya_phylum_highRA_table <- Kenya_phylum %>%
  filter (Phylum %in% unique (Kenya_phylum_highRA$Phylum)) #select target
by their names
write.table(Kenya_phylum_highRA_table, "RA_phyla.csv", sep =",")
Kenya_phylum_final <- Kenya_phylum_highRA_table %>%
  group_by(Phylum, Sample) %>%
  summarise(Abundance=mean(Abundance))
ggplot(Kenya_phylum_final, aes(x = Sample, y = Abundance, fill = Phylum)) +
  geom_bar(stat = "identity") +
  scale_fill_manual(values =
c("Anthophyta"="#a6cee3","p__Ascomycota"="#e31a1c","p__Basidiomycota="
#006d2c",
"p__Chlorophyta"="#878787","p__Chytridiomycota"="#6a3d9a","p__Glomerom
ycota"="#fb9a99","p__Monoblepharomycota"="#fdbf6f",
"p__Mortierellomycota"="#1f78b4","p__Mucoromycota"="#e5f5e0",
                          "p__Olpidiomycota"="#cab2d6","Firmicutes"="#ff7f00",
"Gemmatimonadota"="#ffff99",
```

```
"Halobacterota"="#b15928","Patescibacteria"="#FF8000FF","Planctomycetota"=
"#ACA4E2",

"Proteobacteria"="#ABB065","Verrucomicrobiota"="#FFFF80FF")) +
theme_bw() +
  guides (fill = guide_legend(reverse = TRUE, keywidth = 1, keyheight = 1)) +
  ylab("Relative Abundance") +
scale_x_discrete(limits=c("EA1","EA2","EB1","EB2","EC1","EC2","ED1","ED2
","ZA1","ZA2","ZB1","ZB2","ZC1","ZC2","ZD2")) +
  theme (axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.55, size=11),
       axis.title.x = element_blank(), legend.title=element_blank())
```

Appendix XII: Script for the Phylum description

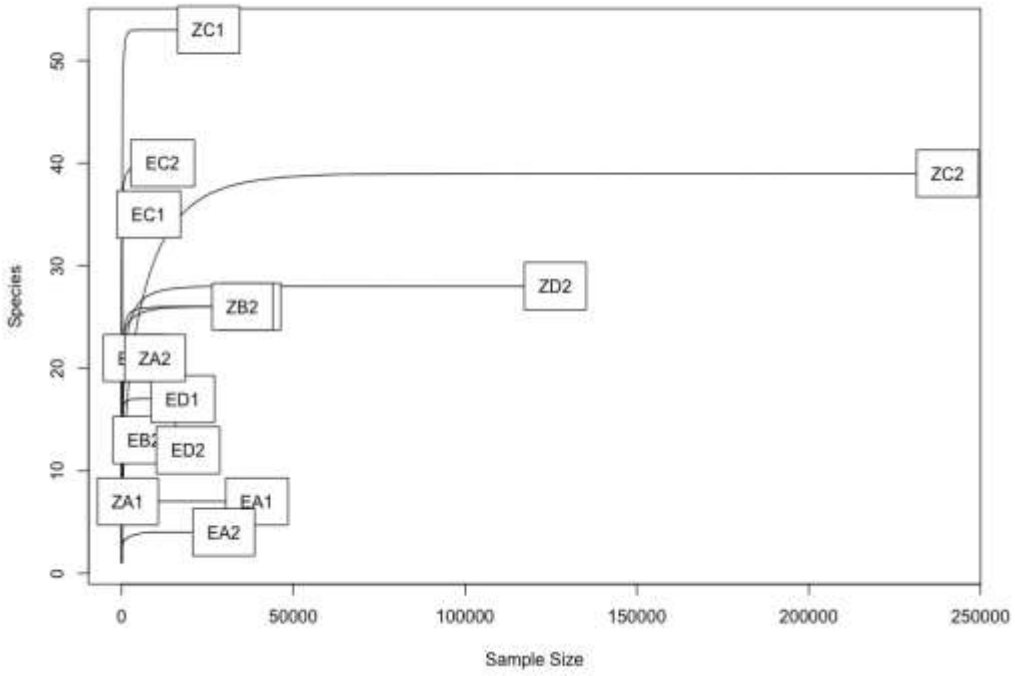
```
Kenya_phylum_detail <- Kenya_phylum_highRA_table %>%
dplyr::select(Sample, Abundance, Phylum) %>% spread(Phylum, Abundance)
Kenya_phylum_Lakes <- Kenya_phylum_detail %>% dplyr::select(-Sample)
colMeans(Kenya_phylum_Lakes, na.rm = TRUE)
sapply(Kenya_phylum_Lakes, sd, na.rm=TRUE)
write.table(Kenya_phylum_Lakes, "percentage_phyla.csv", sep=",")
```

Appendix XIII: Script for the indicator species

```
IndVal
install.packages(??labdsv)
library(labdsv)
Frequency
library(labdsv)
samples <-read.delim("indicators.csv", header = TRUE, sep=",") #XXXX??????
iva <- indval (samples [, -1], samples [,1])
gr <- iva$maxcls[iva$pval<=0.01] #0.01? P-value???
iv <- iva$indcls[iva$pval<=0.01]
pv <- iva$pval[iva$pval<=0.01]
fr <- apply (samples [, -1]>0, 2, sum) [iva$pval<=0.01]
```

```
indvalsummary <- data.frame(group=gr, indval=iv, pvalue=pv, freq=fr)
indvalsummary <- indvalsummary[order(indvalsummary$group, -
indvalsummary$indval),]
indvalsummary write.table(indvalsummary, "output.csv", quote=F, col.names=F,
append=T)
```

Appendix XIV: Rarefaction curves for the eukaryotic communities in each sample.



Appendix XV: Indicator species analysis using lambdsv package for most genera affiliated to major genera between Lake Olbolosat and Oloiden.

Genus	group	indval	pvalue	freq
Conioscypha	Oloiden	0.63	0.03	5
Bovista	Oloiden	0.38	0.20	3
Naganishia	Oloiden	0.38	0.17	3
Filobasidium	Oloiden	0.31	0.53	6
Chlorophyllum	Oloiden	0.25	0.48	2
Spiromastix	Oloiden	0.25	0.45	2
Tulostoma	Oloiden	0.25	0.47	2
Olpidium	Oloiden	0.20	0.58	3
Amauroascus	Oloiden	0.13	1.00	1
Cunninghamella	Oloiden	0.13	1.00	1
Curvibasidium	Oloiden	0.13	1.00	1
Exidia	Oloiden	0.13	1.00	1
Hyalorbilia	Oloiden	0.13	1.00	1
Inocutis	Oloiden	0.13	1.00	1
Kazachstania	Oloiden	0.13	1.00	1
Kurtzmanomyces	Oloiden	0.13	1.00	1
Limonomyces	Oloiden	0.13	1.00	1
Marasmius	Oloiden	0.13	1.00	1
Microbotryum	Oloiden	0.13	1.00	1
Microstroma	Oloiden	0.13	1.00	1
Monoblepharis	Oloiden	0.13	1.00	1
Phylloporia	Oloiden	0.13	1.00	1
Saccharomyces	Oloiden	0.13	1.00	1
Powellomyces	Oloiden	0.11	1.00	2
Cystofilobasidium	Oloiden	0.11	1.00	2
Flagelloscypha	Oloiden	0.07	1.00	2
Trebouxia	Olbolosat	0.69	0.54	15
Spizellomyces	Olbolosat	0.55	0.09	5
Mortierella	Olbolosat	0.41	0.65	7
Erythrobasidium	Olbolosat	0.33	0.73	8
Clavaria	Olbolosat	0.29	0.18	2
Kochiomyces	Olbolosat	0.29	0.21	2
Lindtneria	Olbolosat	0.26	0.45	3
Cryptococcus	Olbolosat	0.23	0.44	3
Termitomyces	Olbolosat	0.22	0.45	3
Rhizophlyctis	Olbolosat	0.17	0.97	5
Claroideoglomus	Olbolosat	0.14	0.48	1
Clydaea	Olbolosat	0.14	0.45	1
Endogone	Olbolosat	0.14	0.46	1
Entoloma	Olbolosat	0.14	0.46	1
Entyloma	Olbolosat	0.14	0.44	1
Halomyces	Olbolosat	0.14	0.46	1
Paranamyces	Olbolosat	0.14	0.44	1
Septobasidium	Olbolosat	0.14	0.47	1
Thanatephorus	Olbolosat	0.14	0.45	1

Appendix XVI: Indicator species analysis using lambdsv package for most genera affiliated to major genera in lake Olbolosat.

Genus	Sample_Type	indval	pvalue	freq
Filobasidium	Water	0.56	0.64	3
Entyloma	Water	0.50	1.00	1
Halomyces	Water	0.50	1.00	1
Erythrobasidium	Wet_Sediment	0.91	0.51	4
Spizellomyces	Wet_Sediment	0.69	0.25	4
Clydaea	Wet_Sediment	0.50	1.00	1
Olpidium	Wet_Sediment	0.50	1.00	1
Paranamyces	Wet_Sediment	0.50	1.00	1
Rhizophlyctis	Wet_Sediment	0.35	1.00	2
Clavaria	Dry_Sediment	1.00	0.12	2
Cryptococcus	Dry_Sediment	1.00	0.16	2
Kochiomyces	Dry_Sediment	1.00	0.14	2
Lindtneria	Dry_Sediment	1.00	0.15	2
Mortierella	Dry_Sediment	0.99	0.12	3
Claroideoglomus	Dry_Sediment	0.50	1.00	1
Cystofilobasidium	Dry_Sediment	0.50	1.00	1
Endogone	Dry_Sediment	0.50	1.00	1
Entoloma	Dry_Sediment	0.50	1.00	1
Flagelloscypha	Dry_Sediment	0.50	1.00	1
Powellomyces	Dry_Sediment	0.50	1.00	1
Septobasidium	Dry_Sediment	0.50	1.00	1
Thanatephorus	Dry_Sediment	0.50	1.00	1
Termitomyces	Dry_Sediment	0.42	0.85	2
Trebouxia	Microbial_Mat	0.81	0.25	7

Appendix XVII: Indicator species analysis using lambdsv package for most genera affiliated to major genera in lake Oloiden.

Genus	Sample_Type	indval	pvalue	freq
Trebouxia	Water	0.71	0.13	8
Erythrobasidium	Water	0.54	0.42	4
Saccharomyces	Water	0.50	1.00	1
Conioscypha	Wet_Sediment	0.66	0.15	5
Microbotryum	Wet_Sediment	0.50	1.00	1
Spizellomyces	Wet_Sediment	0.50	1.00	1
Rhizophlyctis	Dry_Sediment	0.99	0.15	3
Naganishia	Dry_Sediment	0.82	0.16	3
Mortierella	Dry_Sediment	0.81	0.14	4
Bovista	Dry_Sediment	0.70	0.27	3
Amauroascus	Dry_Sediment	0.50	1.00	1
Cryptococcus	Dry_Sediment	0.50	1.00	1
Curvibasidium	Dry_Sediment	0.50	1.00	1
Cystofilobasidium	Dry_Sediment	0.50	1.00	1
Exidia	Dry_Sediment	0.50	1.00	1
Flagelloscypha	Dry_Sediment	0.50	1.00	1
Hyalorbilia	Dry_Sediment	0.50	1.00	1
Inocutis	Dry_Sediment	0.50	1.00	1
Kurtzmanomyces	Dry_Sediment	0.50	1.00	1
Limonomyces	Dry_Sediment	0.50	1.00	1
Lindtneria	Dry_Sediment	0.50	1.00	1
Marasmius	Dry_Sediment	0.50	1.00	1
Microstroma	Dry_Sediment	0.50	1.00	1
Phylloporia	Dry_Sediment	0.50	1.00	1
Powellomyces	Dry_Sediment	0.50	1.00	1
Termitomyces	Dry_Sediment	0.50	1.00	1
Chlorophyllum	Dry_Sediment	0.45	1.00	2
Cunninghamella	Microbial_Mat	0.50	1.00	1
Kazachstania	Microbial_Mat	0.50	1.00	1
Monoblepharis	Microbial_Mat	0.50	1.00	1
Olpidium	Microbial_Mat	0.40	1.00	2
Spiromastix	Microbial_Mat	0.34	1.00	2
Tulostoma	Microbial_Mat	0.28	1.00	2
Filobasidium	Microbial_Mat	0.23	1.00	3