Bacterial and Viral Communities of Wetland Ecosystems

A Dissertation for

Course code and course title: MIC- 651 Discipline Specific Dissertation

Credits: 16

Submitted in partial fulfilment of Master's Degree

Master of Science in Microbiology

by

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Roll Number: 22P0420007

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Under the supervision of

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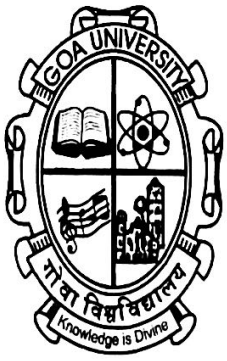

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I hereby declare that the data presented in this Dissertation report entitled, **"Bacterial and viral communities of wetland ecosystems"** is based on the results of investigations carried out by me in the Microbiology Programme at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Judith M. Noronha and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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This is to certify that the dissertation report **"Bacterial and viral communities of wetland ecosystems"** is a bona fide work carried out by **Ms. Eden Kimberly D'Souza** under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of Master of Science in Microbiology, in the Discipline Microbiology, at the School of Biological Sciences and Biotechnology, Goa University.

Date: 8th April 2024

Dr. Judith M. Noronha

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School stamp

Date: 8th April 2024

Place: Goa University

School/Dept stamp

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Date: April 2024 Dr. Judith M. Noronha

Dr. Lakshangy Charya School/Dept stamp

School stamp

Date: April 2024

Place: Goa University

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PREFACE

Scientists and environmentalists have long been fascinated by the complex web of life found in wetland habitats. Wetlands are essential components of our worldwide ecosystem, fulfilling functions in nutrient cycling, water purification, and flood control, as well as providing a home for a wide variety of plants and animals and also offering important ecological services to both the natural world and people. My interest in wetland habitats originated from my great appreciation for their ecological significance and the urgent need to comprehend and protect these delicate yet resilient ecosystems. This dissertation explores the vital roles that bacterial and viral communities play in wetland ecosystems. A thorough review of wetland ecosystems, emphasizing their ecological significance, distinctive qualities, and range of functions, is the basis of the first chapter. Through an exploration of wetlands in Goa, including the Ramsar-designated Nanda Lake, the dissertation delves into the intricate interplay between wetland ecosystems and human activities, emphasizing the critical need for conservation efforts. The techniques used to separate and analyze bacterial and virus populations from soil and water samples further highlight the need for thoroughness and accuracy in order to solve the challenges surrounding wetland microbial ecology. Every stage, from membrane filtering to DNA extraction and bioinformatics analysis, advances our knowledge. The results of this dissertation offer practical implications for environmental management, conservation measures, and the sustainable use of wetland resources in addition to adding to the expanding body of knowledge in wetland ecology. Through establishing a connection between scientific investigation and real-world application. This dissertation essentially serves as a tribute to the complex beauty and profound importance of wetland ecosystems, showcasing the delicate balance of life in these aquatic environments and the urgent need to protect and preserve them in the face of growing environmental challenges

CONTENTS

LIST OF ABBREVIATIONS

CTAB Cetyl trimethyl ammonium bromide

dsDNA Double stranded Deoxyribonucleic acid

DNA Deoxyribonucleic acid

EDTA Ethylenediaminetetraacetic acid

NCBI National Center for Biotechnology Information

OTU Other taxonomic units

PEG Polyethylene glycol

PVDF Polyvinylidene fluoride

rpm Revolutions per minute

SDS Sodium Dodecyl Sulfate

TBE Tris borate EDTA

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ABSTRACT

The bacterial and viral communities in wetland ecosystems are diverse and play key roles in various processes; hence the importance of investigating these communities in different seasons. This study aimed to carry out metagenomic sequencing of bacterial community DNA isolated from the aquatic phase of wetlands. The samples were collected and processed from water and soil by various methods and metagenomic DNA was successfully isolated. The results showed that the bacterial communities in Nanda Lake and Sarzora Lake exhibit distinct taxonomic compositions between the wet and dry seasons. Proteobacteria, Actinobacteria, and Bacteroidetes dominate bacterial communities, with notable variations between seasons influenced by environmental factors. Cyanobacteria, Fibrobacteres, Planctomycetes, and Chlamydiae exhibit distinct abundance patterns in response to seasonal fluctuations. Comamonadaceae, a family known for denitrification and organic matter degradation, exhibited increased dominance during wet seasons, possibly due to heightened nutrient availability. These findings deepen our understanding of wetland microbial ecosystems, emphasizing the critical interplay between microbial communities and environmental conditions. Such insights are crucial for effective wetland conservation and management, ensuring the preservation of these vital ecosystems and their invaluable ecological contributions.

INTRODUCTION

1.1 Brief overview of wetland ecosystems and their ecological significance

Wetland ecosystems are very important components of the global environment as they support a rich biodiversity and various ecological services [1]. These wetland ecosystems are either permanently or seasonally flooded or saturated by water and that support a high frequency of plant and animal life. Wetlands are among the world's most productive ecosystems and provide a variety of beneficial services for people, fish and wildlife. Wetlands are characterized by unique hydric soils and hydrophytes, which are plants that have adapted to wet conditions. Depending on the resident plants, wetlands are classified into marshes – those that are floated over by emergent vegetation (reeds, cattails, and sedges); and swamps – those dominated by woody vegetation (trees and shrubs). Wetland ecosystems are vital to the ecology of watersheds, providing habitat for a variety of organisms, including fish, shellfish, birds and mammals [2]. They also provide directly observable ecosystem services such as water purification, groundwater losses to recharge, storm shelter, water storage, flood control, the processing of carbon and nutrients and pollutants, and global cycles of water, nitrogen and sulfur. Wetland ecosystems are important for human economies as natural products such as fish, shellfish, timber, wild rice, and medications come from wetlands. However, wetlands are also among the most endangered ecosystems on earth and are often strained, filled, or converted to various usages, making them vulnerable to the erosion of biodiversity and ecosystem services [3]. Wetland conservation and protection are vital to preserving humanity and our planet [4]

1.2 Wetlands of Goa

Some of the important lakes in Goa that are notified wetlands are Carambolim Lake, Mayem Lake and Nanda Lake (1). In South Goa, Curchorem is the place where Nanda Lake is located, which is the first Ramsar site in Goa. A Ramsar site is a wetland area designated under the

Ramsar Convention, officially known as the Convention on Wetlands of International Importance. These sites are listed on the List of Wetlands of International Importance and are recognized for meeting specific criteria that emphasize the conservation of biodiversity and the sustainable use of wetland resources[5]. The criteria include the presence of representative, rare, or unique wetland types, support for vulnerable species, and the importance of the site for maintaining biological diversity, among others[6]. Ramsar sites play a crucial role in protecting wetlands globally and promoting their sustainable management.

. With its designation as a Ramsar site, Nanda Lake has gained international recognition for its significance in delivering ecological services and biodiversity values for the local communities and society at large. Nanda Lake has an area of 42 hectares and is one of Goa's largest wetlands. It was approved as a Ramsar site after it passed nine criteria including its services as a habitat for several species of birds like the common kingfisher, black-headed ibis, etc. Most of the area is intermittent freshwater marshes that lie adjacent to one of the major rivulets of the Zuari River. This enables the residents to store the water during the off-monsoon season, which can be used to cultivate paddy. It also supports fishing and recreation. In the monsoons when the sluice gate is opened and the water is released, the character of the lake changes into a marshland (10). Research into the physicochemical and biological characteristics of Nanda Lake is currently gaining focus.

1.3 Bacterial and viral communities of wetland ecosystems

Bacterial and viral communities are significant components of wetland ecosystems, in terms of their numerical dominance and ecological effects. Bacterial communities found in wetlands play an important role in biogeochemical processes such as the recycling of elements like carbon, nitrogen and sulphur. However very little is known about their community structure, dynamics, and interactions in wetlands [3, 7]

Studies have shown that lakes support a variety of bacterial phyla like Actinobacteria, Bacteroidetes, Proteobacteria, and Cyanobacteria [8, 9]. These bacterial communities are influenced by various environmental factors such as trophic status, salinity, and geographical location as a result of which the composition and functioning may vary between different types of lakes[9, 10].

Further, various studies conducted in wetland ecosystems indicate that the artificially constructed wetlands have a higher diversity of microbial taxa in comparison to the natural wetlands as stated by [4, 11].like for example in the subtropical coastal wetlands the bacterial and fungal communities are different between wet and dry seasons and also between the mangrove and mudflat regions where else the archaeal communities remain relatively stable[7].

In wetlands, as in other aquatic ecosystems, viruses influence biogeochemical and carbon cycles. The viruses present in the wetland ecosystem infect a large range of organisms, including prokaryotes, algae, protozoa, and plants. Studies have shown that bacteriophages likely represent the dominant category of viruses in freshwater wetlands, although viruses infecting other organisms may also be present[12].

Viruses found in lakes especially those in recreational lakes, may include viruses pathogenic to humans. For example, Adenovirus, enterovirus, norovirus, and rotavirus have all been found in both recreational and other lakes. Most water quality monitoring protocols do not include the detection of such viruses. The presence of *E. Coli* and other widely used faecal indicator bacteria is not a definitive indicator of viral pollution [13]

In summary, the bacterial and viral communities of wetland ecosystems are diverse and play key roles in various processes; hence the importance of investigating these communities in different seasons.

Aim and objectives

1) To carry out metagenomic sequencing of bacterial and viral community DNA isolated from the aquatic phase of wetland ecosystems

2) To characterize the bacterial and viral communities taxonomically and functionally using bioinformatics analysis.

3) To compare the bacterial and viral communities in wet and dry seasons

Hypothesis

Wetland ecosystems are dynamic, undergoing various physicochemical and biological changes over wet and dry seasons. The seasonal changes are expected to be reflected in changes in community structure of bacteria and viruses. Moreover, the microbial communities of a pristine wetland (Sarzora Lake) are expected to be distinct from those of an urban wetland (Nanda Lake).

LITERATURE REVIEW

2.1 Metagenomic studies on viral communities in wetland ecosystems

Recent metagenomic studies have focused on uncovering viral communities in various environments. Research has delved into extreme environments, terrestrial geothermal sites, freshwater lakes, and wetlands, revealing the diversity and functions of viral communities[14–17]. These studies have employed advanced techniques like metagenomic assembly, virome analysis, and taxonomic classification to identify novel viruses and understand their interactions with microbial hosts[14–16]. The exploration of viral metagenomics in different ecosystems provides valuable insights into viral diversity, ecological roles and the complex relationships between viruses and their hosts. The most common viral species found in wetland ecosystems using metagenomics are bacteriophages, particularly belonging to the order Caudovirales. Studies have shown that Caudovirales, with families like Siphoviridae, are prevalent in wetlands, indicating their significant presence and potential impact on microbial communities in these environments[18]. These bacteriophages play a crucial role in regulating bacterial populations and biogeochemical processes within wetland ecosystems.

A recent metagenomic study of a high-altitude wetland, Salar de Huasco, Chile, examined two viral-enriched metagenomes from freshwater ponds[19]. The study classified DNA viruses and identified potential new bacteriophages. The results revealed that the viral metagenomes were dominated by bacteriophages, with Siphoviridae being the most abundant family. The study also identified several potential host microbes, including Proteobacteria, Actinobacteria, Bacteroidetes, and Cyanobacteria.

Studying the most common viral species found in wetland ecosystems through metagenomics has several potential applications. Firstly, it can provide insights into the diversity and abundance of viruses in these ecosystems, contributing to a better understanding of viral ecology and their role in biogeochemical cycles[18, 20]. Secondly, metagenomics can help

identify potential new bacteriophages and their putative hosts, which can lead to the discovery of novel viruses and their interactions with microbial communities[18]. Thirdly, analyzing genes encoding enzymes related to the lytic and lysogenic cycles can shed light on viral replication pathways and their impact on microbial populations[18]. Lastly, classifying auxiliary metabolic genes (AMGs) related to metabolic pathways of biogeochemical cycles can reveal the role of viruses in elemental cycling within wetland ecosystems[18]. Overall, metagenomics can significantly advance our knowledge of viral communities in wetland ecosystems, with implications for ecological, biogeochemical, and evolutionary research.

2.2 Metagenomic studies on bacterial communities in wetland ecosystems

Wetland environments are unique due to their combination of terrestrial and aquatic characteristics. A comparison of bacterial diversity in freshwater and intertidal wetlands along China's Pearl River highlighted the existence of distinct bacterial communities in different habitats, emphasizing the importance of understanding microbial compositions in wetland ecosystems [21]. Additionally, a study focused on an acidic wetland dominated by sphagnum revealed vertical stratification of bacterial communities between surface and subsurface peat layers [22].

Lakes act as important freshwater ecosystems which host diverse bacterial communities crucial for assessing water quality, ecosystem dynamics, and human health risks [23]. Pyrosequencing-based assessments in northern wetlands demonstrated the diverse nature of bacterial communities, with the presence of various phyla such as Acidobacteria, Alphaproteobacteria, Actinobacteria, and others [4].

Various factors like soil type, vegetation, and environmental conditions influence bacterial community composition in wetlands. For instance, the effects of salt on soil microbial communities in coastal estuarine wetlands highlight the impact of environmental factors [7].

Metagenomic studies have also proven valuable in uncovering the composition and functional potential of microbial populations in wetlands, identifying predominant phyla like Proteobacteria, Firmicutes, and Actinobacteria [25] [16]

These discoveries extend to potential applications, including the identification of novel antibiotics and insights into biotechnological applications [18]. However, metagenomic analysis demands significant processing power and bioinformatics expertise, which is facilitated by various bioinformatics pipelines [19].

Recent metagenomic studies in wetlands have revealed significant findings regarding sediment microbial communities in freshwater lakes [20], microbial communities in wetland soil samples in the Florida Everglades [21] and shifts in bacterial populations and ecosystem functions in different wetland types [22]. Collectively, these studies contribute to our understanding of bacterial diversity, functions, and ecological dynamics in wetland ecosystems.

In conclusion, metagenomic research has substantially enhanced our understanding of bacterial communities in wetlands, shedding light on their diversity, composition, and potential ecological functions.

METHODOLOGY

Water samples were collected from Nanda Lake (15.241116°, 74.105465°) and Sarzora Lake (15.219167°, 74.003037°) located in Goa, India, during two seasons, specifically wet and dry seasons, and processed for bacterial and viral metagenomic DNA, as outlined in Table 1 and sections 3.1-3.3.

Table 1: Details of sampling sites

(A) Bacteria

Sample	Sampling site	Date
16S-NL1	Nanda Lake	7th July 2023
16S-SL1	Sarzora Lake	7th July 2023
16S-NL2	Nanda Lake	28th November 2023
16S-SL2	Sarzora Lake	28th November 2023

(B) Viruses

Sample	Sampling site	Date
SM-NL1	Nanda Lake	7th July 2023
SM-SL1	Sarzora Lake	7th July 2023
SM-NL2	Nanda Lake	23rd October 2023
SM-NL3	Nanda Lake	28th November 2023
SM-SL2	Sarzora Lake	28th November 2023
SM-NL4	Nanda Lake	15th January 2024
SM-SL3	Sarzora lake	25th February 2024

Requirements: Screw capped bottles, magnetic flea, membrane filter, falcon tubes, Eppendorf tubes, tips, syringe filter, syringes,

Instruments: membrane filtration unit, vacuum pump, centrifuge, autoclave, hot air oven, incubator, laminar air flow, Eppendorf centrifuge, weighing balance, electrophoresis unit, micropipette (10 µl, 100 µl, 1000 µl), refrigerator, pH meter.

Chemicals: ethanol, lysis buffer, proteinase K, SDS, phenol, chloroform, isoamyl alcohol, isopropanol, Tris Cl, EDTA, CTAB, NaCl, agarose, ethanol, TBE, sterile milliQ water.

3.1 Isolation of viral community DNA from water sample[26]

One liter of water was drawn from the lakes of Nanda and Sarzora, pre-filtered through a GF/A filter (pore size 1.6 µm) and filtered through an autoclaved PVDF filter with a pore size of 0.22 µm. 10% PEG (100g) and 1M NaCl (58.44g) were added to the filtered sample, dissolved slowly on a magnetic stirrer, and kept at 4°C overnight. Concentrated viral particles were recovered by centrifugation at 9000 rpm, followed by resuspension of the concentrate in a minimum volume of 10 mM Tris chloride. Subsequently, the sample was treated with 2.5 U of DNase per ml od sample, and incubated for 90 minutes at 37°C. The following was then added to the sample: 1 volume of formamide, 100 µl of 0.5M EDTA/10ml, and 0.1 volumes of 2M Tris-HCl/0.2M EDTA. After incubation for 30 minutes at 25°C, two volumes of 100% ethanol was added and the tube was centrifuged for 20 minutes at 9000 rpm. The supernatant was discarded and the pellet washed with 70% ethanol, suspended in 567µl of 10mM TrisCl and stored at – 20°C until the next step. 3 µl of proteinase K (20 mg/ml stock) and 30 µl of 10% (wt/vol) SDS was added to the sample and incubated at 37°C for 90 minutes. Following this, 80 µl of the CTAB/NaCl solution was added to the tube and mixed, followed by 100 µl of 5M NaCl. After that, the tube was incubated for ten minutes at 65°C. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added, mixed by gentle inversion and centrifuged

for ten minutes at 4°C at 10,000 rpm. The aqueous layer was transferred carefully to a fresh tube. An equal volume of chloroform: isoamyl alcohol (24:1) was and the mixture was centrifuged for 10 minutes at 4°C at 10,000 rpm. The aqueous layer was transferred carefully to a fresh tube. 0.7 volumes of isopropanol were added and incubated for 60 minutes at room temperature, followed by centrifugation at 10,000 rpm for 20 minutes After discarding the supernatant, the pellet was washed with 70% ethanol and finally resuspended in 20µl of 10mM TrisCl. The sample was stored at –20°C.

3.2 Isolation of bacterial community DNA from water sample[27]

1 litre water sample from Nanda and Sarzora Lakes was acquired and filtered through a 0.22 µm membrane filter. The filter was suspended in lysis buffer and incubated in a water bath at 37°C for 45 minutes. Following this, the procedure described above for extraction of viral DNA was followed, comprising treatment with SDS and Proteinase K, CTAB/NaCl, phenol:chloroform extraction of DNA and finally precipitation with isopropanol.

3.3 Isolation of metagenomic DNA via kit method

DNA was extracted from soil using HiPurA Soil DNA Purification Kit (HiMedia) according to the manufacturer's instructions. Around 0.5g of soil sample was taken in the HiBead Tube and 750µl of Soil Lysis Solution was added to it. Contents were subjected to two cycles of vortexing for 10 minutes followed by incubation in a water bath at 75°C for 10 minutes. The sample was centrifuged at 12000 rpm for 1 minute at room temperature. Supernatant was transferred to a 2 ml collection tube to which 250µl of Inhibitor Removal Solution was added, vortexed and incubated at 4°C for 5 minutes. Tube was centrifuged at 10000 rpm for 1 minute at room temperature and supernatant was transferred to 2ml collection tube. To that, 1.2ml of Binding Solution was added and vortexed. 650µl of the sample was then loaded onto HiElute

Miniprep Spin Column and centrifuged at 12000 rpm for 1 minute at room temperature. Flow-through was discarded and the step was repeated for the remaining of the sample. To HiElute Miniprep Spin Column, 500µl of Wash Solution was added and centrifuged at 8000 rpm for 1 minute at room temperature. The column was transferred to the capped 2ml collection tube and 40µl of elution buffer was added to the center of the column and kept for 5 minutes. The tube was then centrifuged at 10000 rpm for 1 minute at room temperature. The column was discarded and DNA in the collection tube was stored at –20°C.

3.4 Visualization of DNA obtained

The DNA extracted in each case, was loaded on a 0.8% agarose gel, run till 3/4th the total length of the gel and visualized under a UV transilluminator.

3.5 Bioinformatics analysis

The bioinformatic analysis was performed on the raw reads obtained after metagenomic sequencing by using the tools Kraken, Convert Kraken and Krona pie chart.

3.5.1 Kraken 2 (Version 2.1.1)

Kraken is a very quick and precise tool that helps metagenomic DNA sequences to be assigned with taxonomic labels.[28]

3.5.2 Convert Kraken (Version 1.2)

The purpose of this utility is to convert Kraken metagenomic classifier findings into a complete NCBI taxonomy representation. It uses the Taxonomic ID field that Kraken provides to accomplish this. According to[28], the output of this tool can also be directly viewed by Krona.

3.5.3 Krona pie chart (Version 2.7.1)

This program uses Krona to create a zoomable pie chart based on the findings of metagenomic profiling.[29]

ANALYSIS AND CONCLUSIONS

The samples were collected and processed, and metagenomic DNA was successfully isolated.

1) Sarzora Lake during the Wet season, hereafter referred to as WS Sarzora
2) Sarzora Lake during the Dry season, hereafter referred to as DS Sarzora
3) Nanda Lake during the Wet season, hereafter referred to as WS Nanda.
4) Nanda Lake during Dry season, hereafter referred to as DS Nanda.

Figure 1: Sampling sites:Sarzora Lake (1) (2) Nanda Lake (3) (4)

The pH and salinity of the samples were measured. (Table 2)

Table 2: Physicochemical parameters of water samples

	WS SARZORA	DS SARZORA	WS NANDA	DS NANDA
pH	6.7	6.64	6.48	6.38
Salinity	0	0	0	0

Visualization of metagenomic DNA

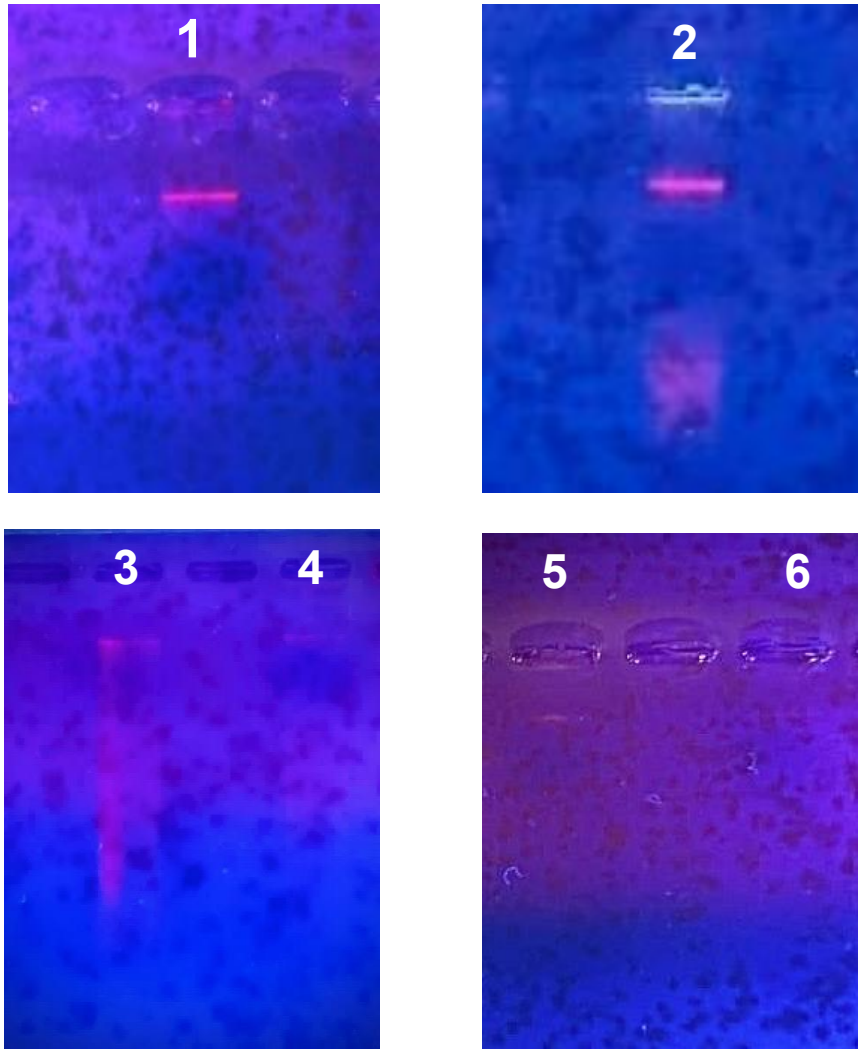

Figure 2: Bacterial metagenomic DNA from: (1) WS Nanda lake (2) WS Sarzora lake (3) DS Nanda lake (4) DS Sarzora lake ;

Virus-enriched metagenomic DNA from: (5) WS Nanda lake (6) WS Sarzora lake

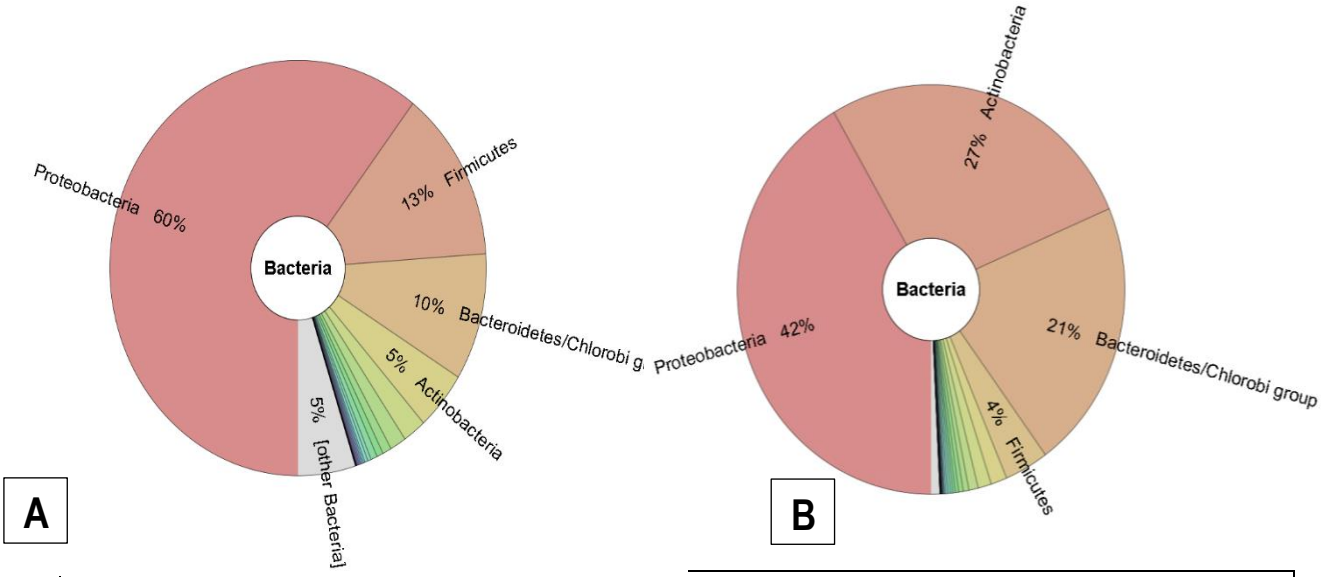

Figure 3: Phylum level classification of bacteria from Nanda Lake during (A)Wet season and (B)Dry season

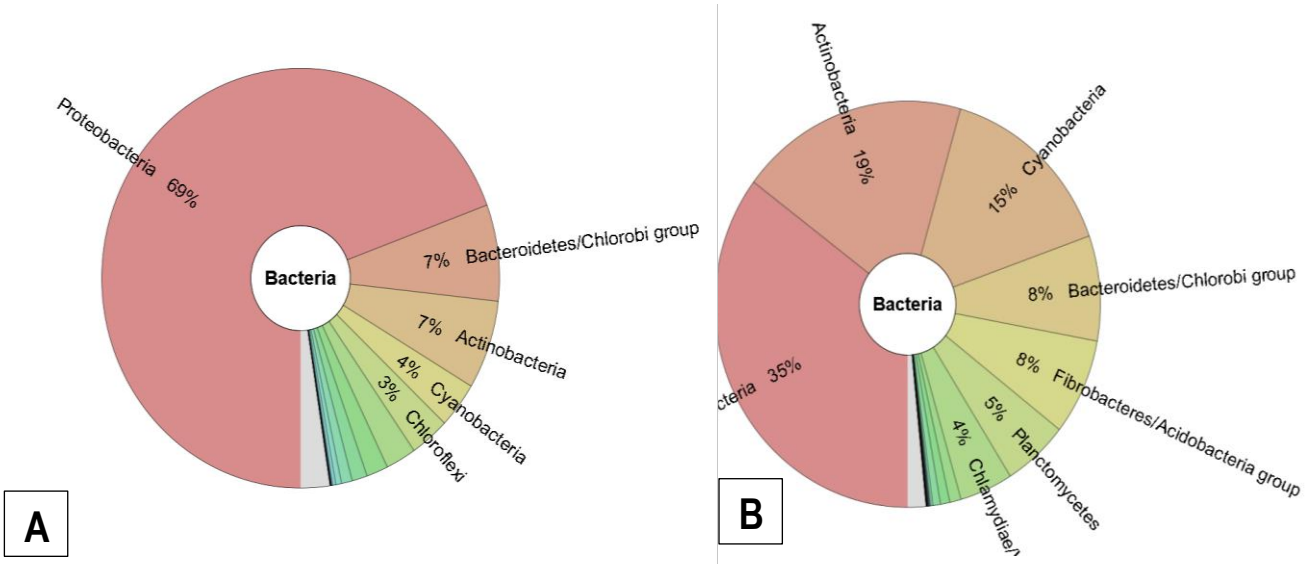

Figure 4: Phylum level classification of bacteria from Sarzora Lake during (A)Wet season and (B)Dry season

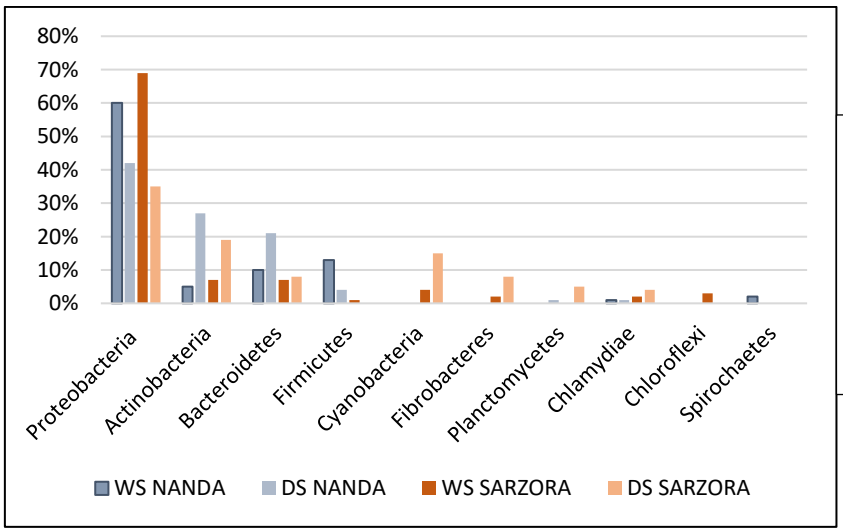

Figure 5: Overall comparison between bacterial communities of Nanda and Sarzora Lakes, in wet and dry seasons

As we can observe, the bacterial communities in Nanda Lake and Sarzora Lake exhibit distinct taxonomic compositions between wet and dry seasons. In the wet season, Proteobacteria dominate in both lakes, with a higher relative abundance in Sarzora Lake (69%) compared to Nanda Lake (60%). Actinobacteria, Bacteroidetes, and Firmicutes are also prevalent, with varying proportions between the two lakes. In contrast, the dry season shows shifts in bacterial phyla, with Proteobacteria decreasing in abundance and Actinobacteria becoming more dominant, especially in Nanda Lake. Bacteroidetes and Firmicutes remain significant, while Cyanobacteria and other phyla emerge in different proportions in each lake.

Coming to the comparison of the bacterial communities across the two seasons we can observe that the bacterial communities in Nanda Lake during the wet and dry seasons show that Proteobacteria is the most abundant phylum, accounting for 60% during the wet season and 42% during the dry season. Actinobacteria is the second most abundant phylum, accounting for 5% during the wet season and 27% during the dry season. Bacteroidetes is the third most abundant phylum, accounting for 10% during the wet season and 21% during the dry season. Firmicutes is the fourth most abundant phylum, accounting for 13% during the wet season and 4% during the dry season. Chlamydiae is present in low abundance, accounting for 1% during both the wet and dry seasons.

These results suggest that there is a shift in the bacterial community composition between the wet and dry seasons, with an increase in the relative abundance of Actinobacteria and Bacteroidetes during the dry season, and a decrease in the relative abundance of Proteobacteria and Firmicutes[30]. This shift may be due to changes in environmental conditions, such as temperature, nutrient availability, and pH, which can influence the growth and survival of different bacterial taxa[4, 31].

Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes are commonly found in wetland ecosystems and play important roles in nutrient cycling, organic matter decomposition, and other ecosystem processes[32]. Chlamydiae are also present in wetland ecosystems, but their ecological roles are less well understood.

Similarly, the results for the comparison of bacterial communities in Sarzora Lake during the wet and dry seasons indicate significant variations. In the wet season, Proteobacteria dominated with 69%, followed by Actinobacteria at 7%, Bacteroidetes at 7%, Cyanobacteria at 4%, Fibrobacteres at 2%, and Chlamydiae at 2%. Contrastingly, during the dry season,

Proteobacteria decreased to 35%, Actinobacteria increased to 19%, Bacteroidetes remained at 8%, Cyanobacteria rose to 15%, Fibrobacteres stayed at 8%, and Chlamydiae increased to 4%

These results also suggest a shift in the bacterial community composition between the wet and dry seasons in Sarzora Lake. One possible reason for the high percentage of cyanobacteria during dry season may be nutrient enrichment, leading to increased nutrient concentrations in water bodies due to reduced dilution from rainfall and runoff. This nutrient enrichment, especially phosphorus and nitrogen, can promote the growth of cyanobacteria, leading to blooms[33]. Warmer temperatures during the dry season can favor the growth and reproduction of cyanobacteria. Cyanobacteria thrive in warm conditions, and higher temperatures can accelerate their growth rates, contributing to an increase in their abundance[34].Changes in environmental conditions during the dry season, such as increased temperatures and nutrient availability, can favor cyanobacteria over other phytoplankton species. This competitive advantage can lead to an increase in cyanobacterial abundance relative to other algae groups[35].

According to similar studies, the most common bacterial taxa found in wetland ecosystems include Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, and Verrucomicrobia[36].These phyla are consistently found in various wetland types, including coastal wetlands, freshwater wetlands, and intertidal wetland[37]. The functions of these bacterial taxa in wetland ecosystems are diverse and crucial for the biogeochemical cycles and overall health of the ecosystem. For example, Proteobacteria in the wet season suggests a response to nutrient availability and environmental conditions conducive to their growth as well as play a key roles in denitrification and nutrient cycling, while Acidobacteria are involved in carbon cycling and organic matter decomposition[38]. Chloroflexi are known for their anaerobic respiration capabilities, while Bacteroidetes are involved in the degradation of complex organic matter[39, 40]. Actinobacteria are important for nutrient cycling and the degradation of recalcitrant organic matter and its increased prevalence in the dry season may indicate adaptations to lower nutrient levels or specific ecological niches during this period[37]. The higher abundance of Actinobacteria, Bacteroidetes and Firmicutes play essential roles in both seasons, potentially involved in nutrient cycling and organic matter degradation. [39, 41, 42] The variations in Cyanobacteria, Fibrobacteres, Planctomycetes, and Chlamydiae highlight the complexity of wetland ecosystems and the diverse microbial interactions occurring in response[43]. Overall, the functions of these bacterial taxa in wetland ecosystems are closely linked to the unique environmental conditions found in wetlands, including the proximity of

oxic-anoxic conditions, nutrient input, and hydrological to seasonal changes[44]. These findings align with previous studies emphasizing the importance of seasonal dynamics in shaping bacterial communities in wetlands[42].

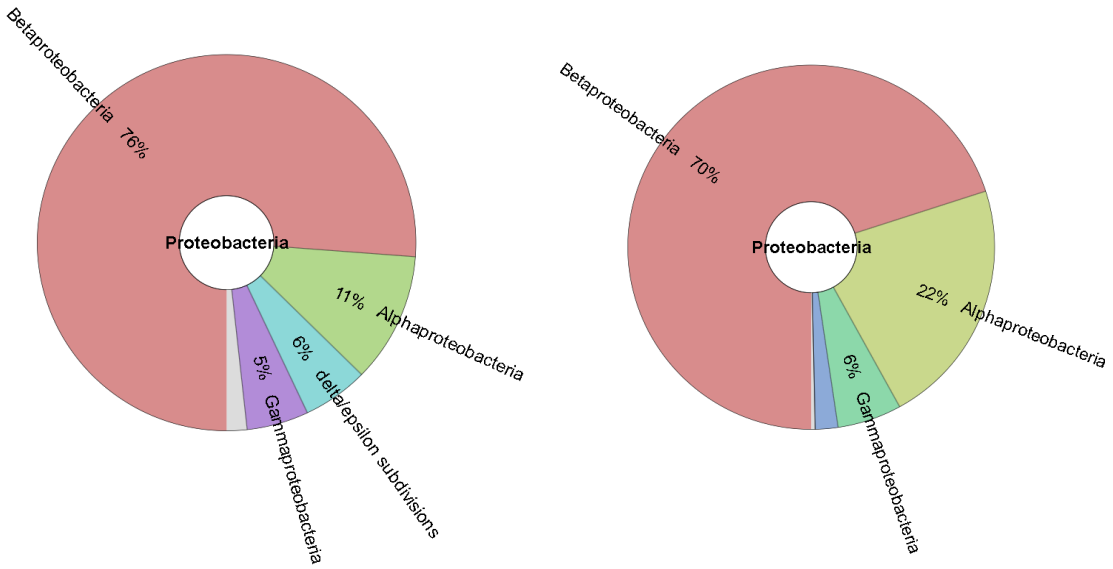

Figure 6: Various classes under the Proteobacteria phylum from Nanda Lake during (A)Wet season and (B)Dry season

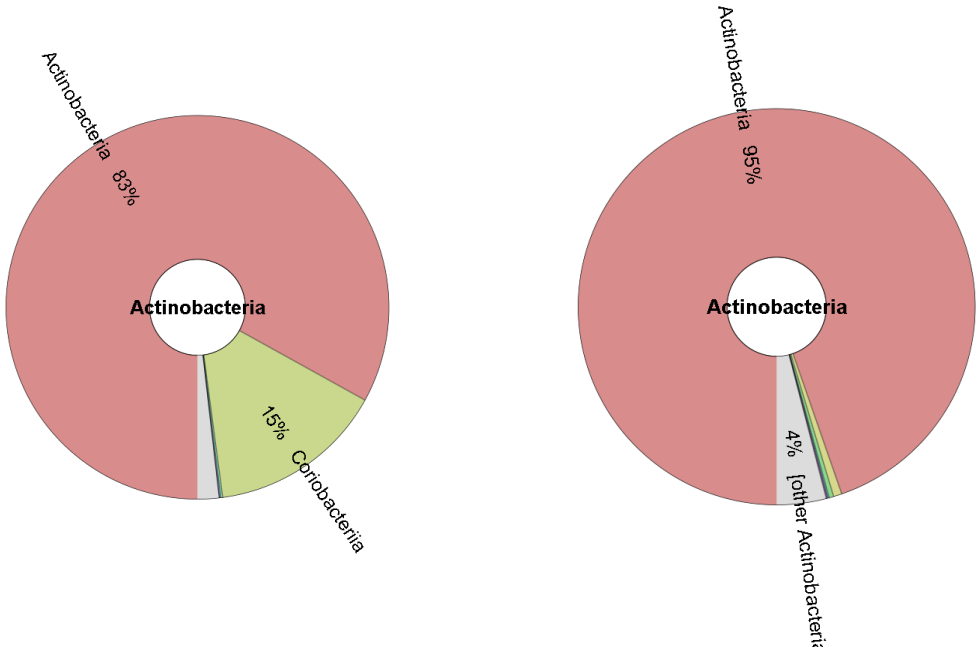

Figure 7: Various classes under Actinobacteria phylum from Nanda Lake during (A)Wet season and (B) Dry season

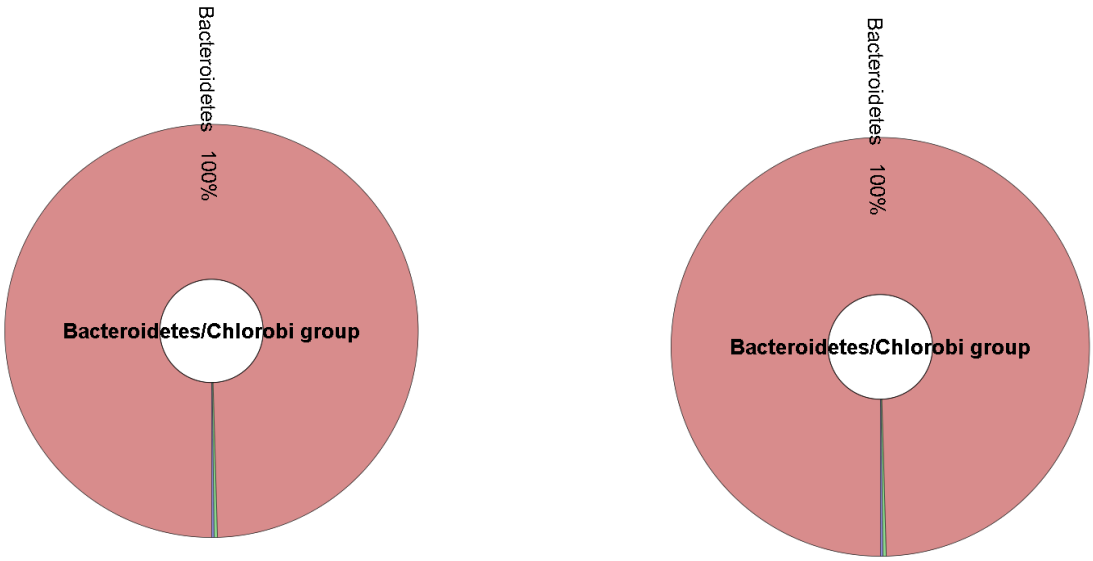

Figure 8: Various classes under Bacteroidetes phylum from Nanda lake during (A)Wet season and (B)Dry season

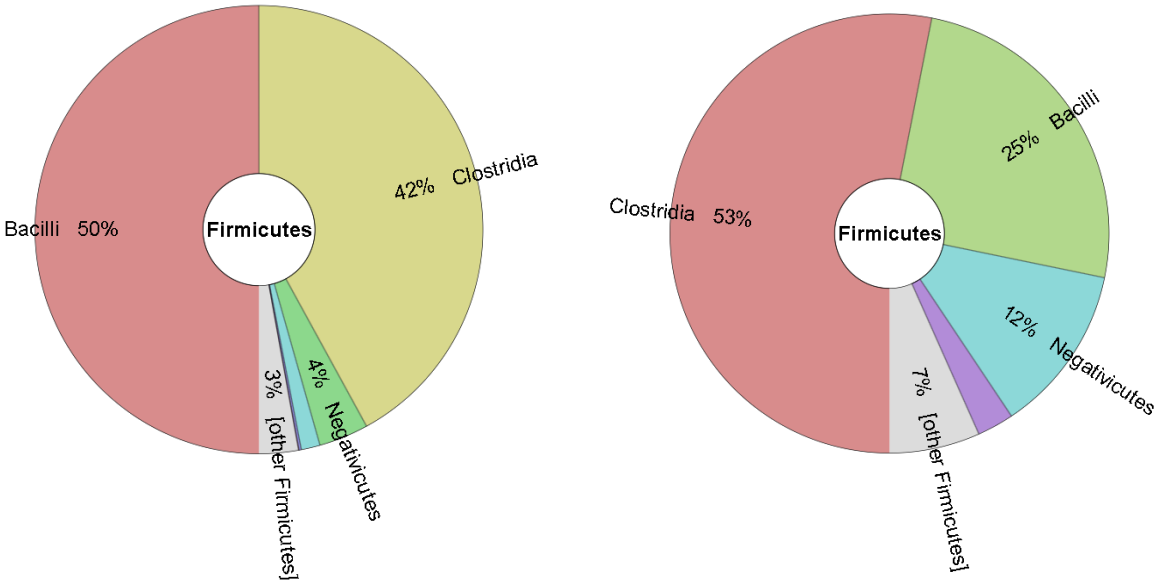

Figure 9: Various classes under Firmicutes phylum from Nanda lake during (A)Wet season and (B)Dry season

The Class level distribution within various bacterial phyla is depicted in fig 5-14. Proteobacteria is a large and diverse phylum of bacteria that can be divided into five major classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria[38]. These classes have different ecological roles in lakes. Alphaproteobacteria are commonly found in illuminated anoxic zones of lakes, particularly meromictic lakes, and in sulfur springs [38]. They are known to play a key role in the formation of stromatolites in alkaline lakes, where they contribute to the formation of aragonite and hydromagnesite[37]. Betaproteobacteria are commonly found in freshwaters and are known to play a role in denitrification and the nitrogen cycle [45]. They are also known to be associated with the decomposition of organic matter in sediments [45].Gammaproteobacteria are known to be associated with the decomposition of organic matter in sediments, particularly in sediments with high nutrient concentrations [42]. They are also known to be involved in the sulfur cycle and the degradation of pollutants in sediments [45].Deltaproteobacteria are known to be involved in the sulfur cycle and the degradation of organic matter in sediments [45]. They are also known to be associated with the decomposition of organic matter in sediments, particularly in sediments with high nutrient concentrations [45]Epsilonproteobacteria are known to be associated with the decomposition of organic matter in sediments, particularly in sediments with high nutrient concentration[45]They are also known to be involved in the sulfur cycle and the degradation of pollutants in sediments [45].The relative abundance of these classes can vary depending on the season and environmental conditions in the lake. For example, Alphaproteobacteria have been found to be more abundant in the dry season, while Betaproteobacteria and Gammaproteobacteria have been found to be more abundant in the wet season[46]. This variation in relative abundance is likely due to differences in environmental conditions, such as nutrient availability and temperature, which can affect the growth and survival of different classes of Proteobacteria.

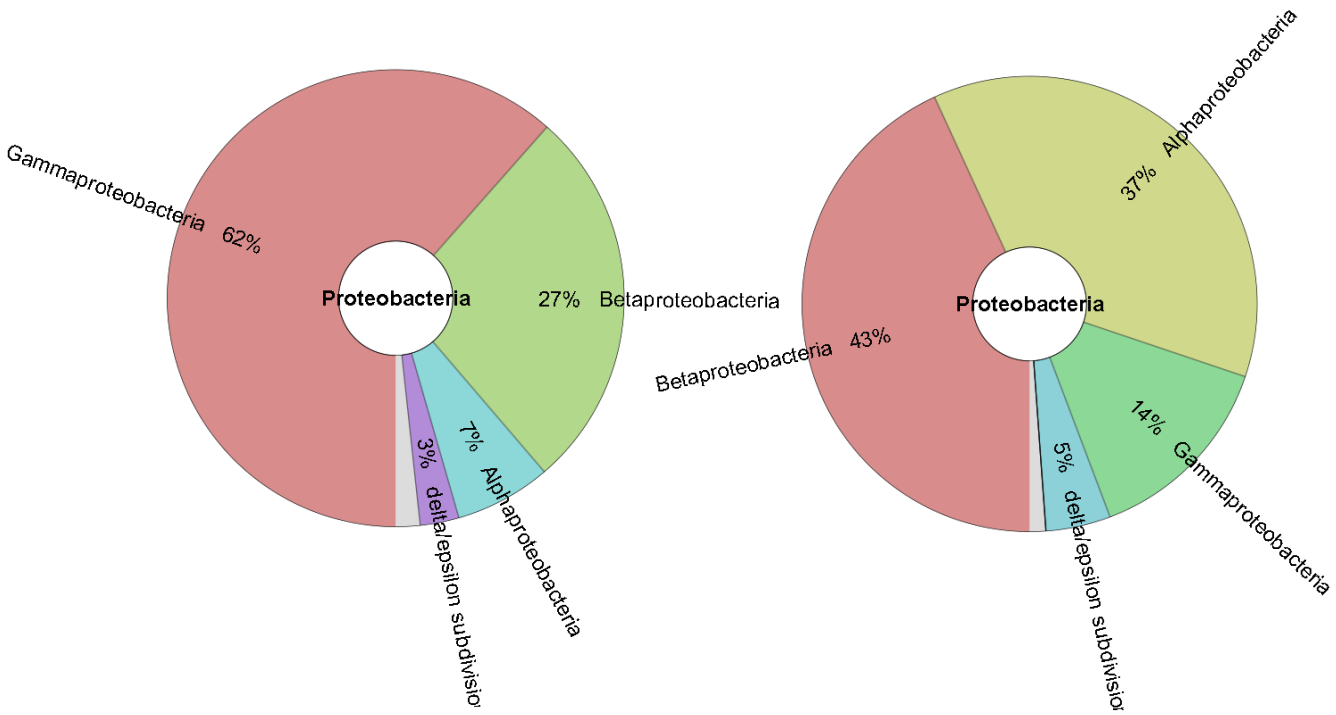

Figure 10: Various classes under Proteobacteria phylum from Sarzora lake during (A)Wet season and (B) Dry season

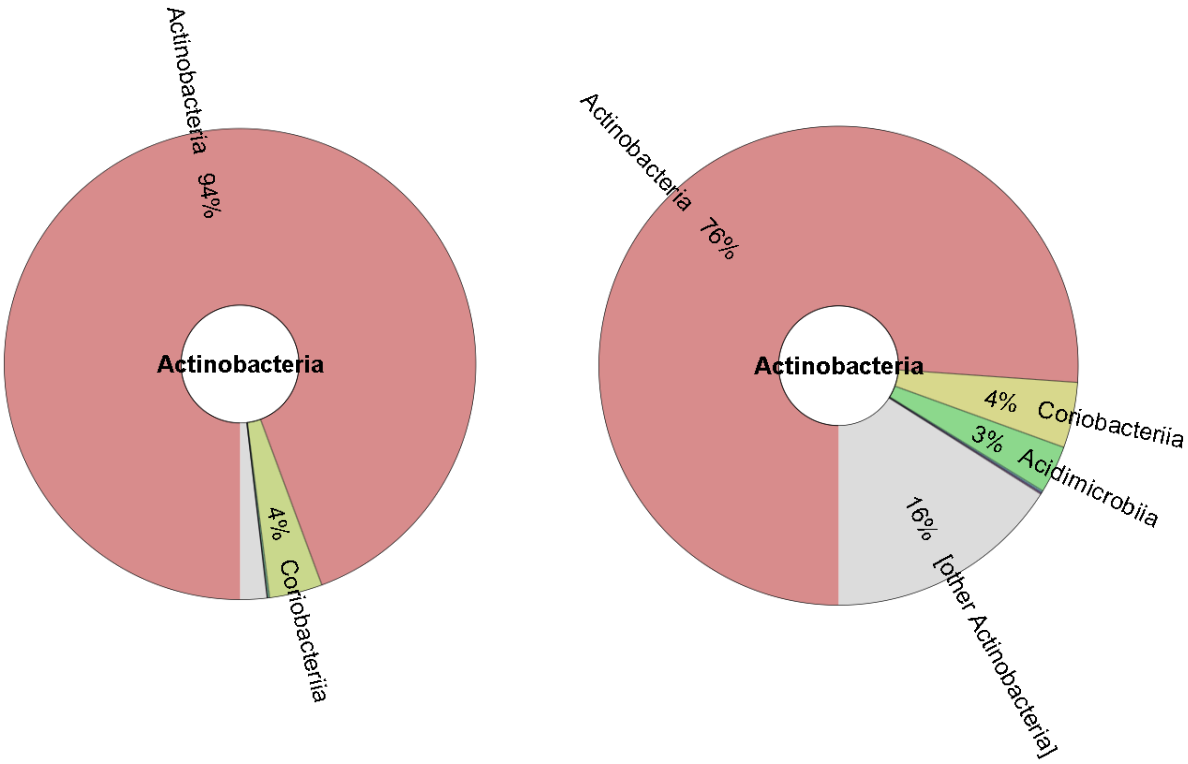

Figure 11: Various classes under Actinobacteroia phylum from Sarzora lake during (A)Wet season and (B) Dry season

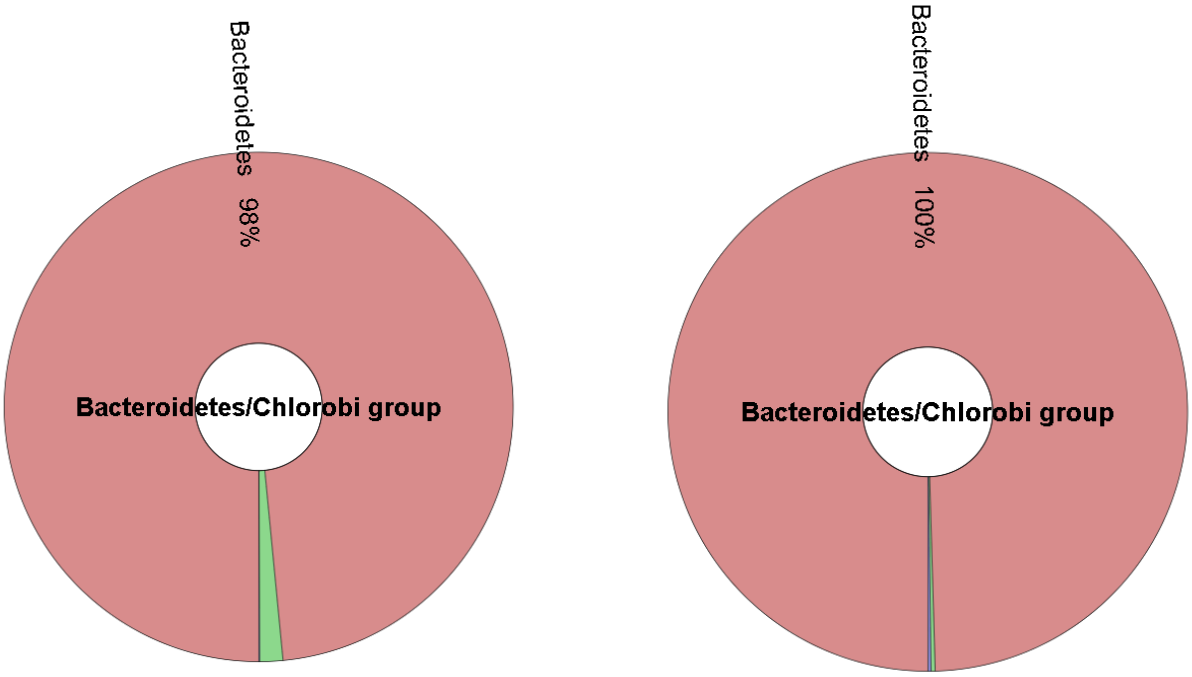

Figure 12: Various classes under Bacteroidetes phylum from Sarzora lake during (A)Wet season and (B) Dry season

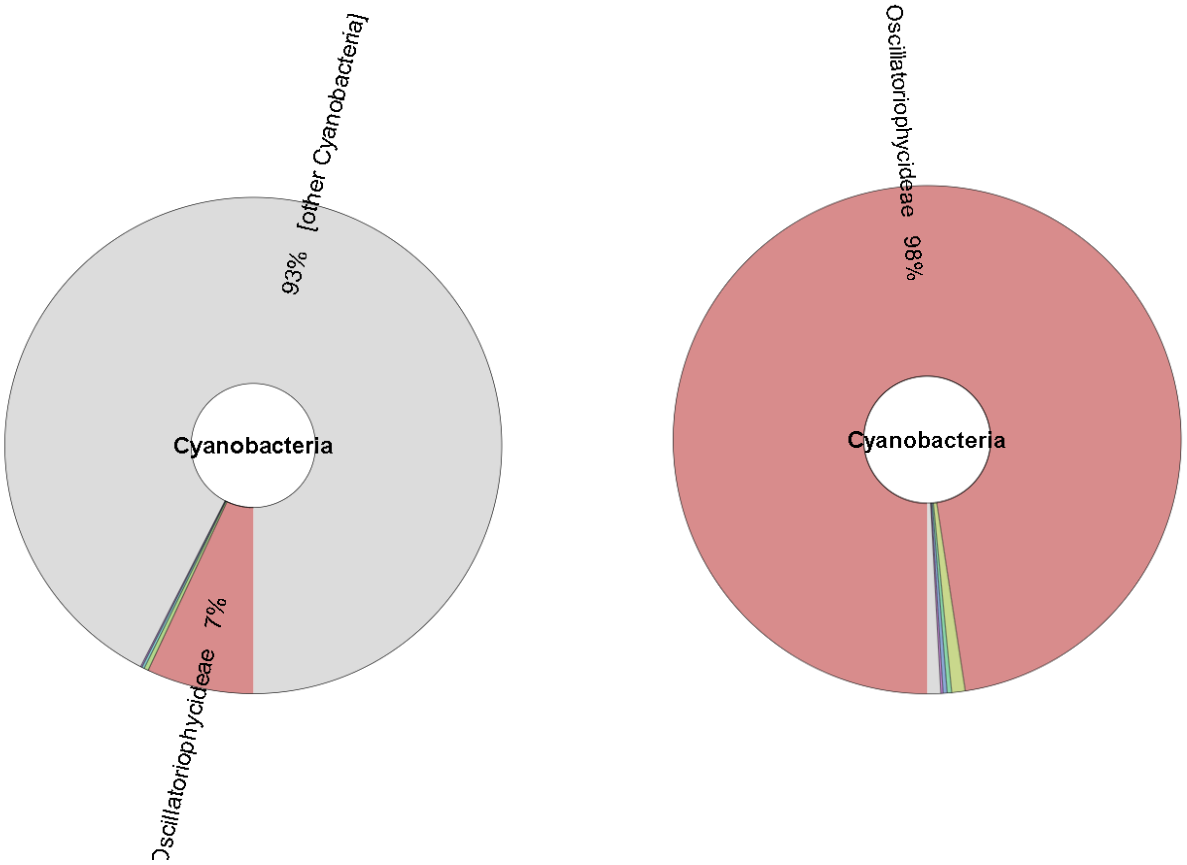

Figure 13: Various classes under Cyanobacteria phylum from Sarzora lake during (A)Wet season and (B) Dry season

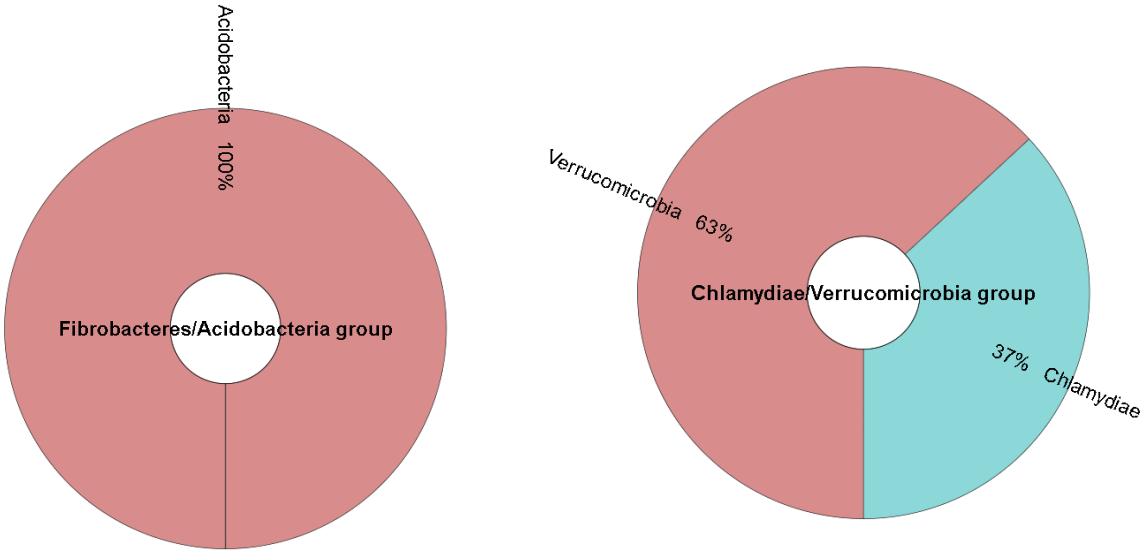

Figure 14: Various classes under Fibrobacteres and Chlamydiae phylum from Sarzora lake during Wet season

Table 3: Classification of families found during the dry and wet seasons in Nanda and Sarzora Lake

WS NANDA	DS NANDA	WS SARZORA	DS SARZORA
Comamonadaceae 39%	Comamonadaceae 23%	Moraxellaceae 26%	Pelagibacteraceae 8%
Others less than 4%	Microbacteriaceae 16%	Enterobacteriaceae 12%	Microbacteriaceae 5%
	Others less than 4%	Chromobacteriaceae 7%	Planctomycetaceae 4%
		Comamonadaceae 5%	Burkholderiaceae 4%
		Others less than 4%	Others less than 4%

As we can observe from Table 3 the four major bacterial families observed across wet and dry seasons are Comamonadaceae, Moraxellaceae, Pelagibacteraceae, Microbacteriaceae, Enterobacteriaceae, Chromobacteriaceae, Planctomycetaceae, and Burkholderiaceae. These families play diverse roles in the biogeochemical processes of lake ecosystems and showcase distinctive abundance patterns in response to seasonal fluctuations. Comamonadaceae has a reported high prevalence in freshwater environments [23, 47] and members of this family are known for denitrification and organic matter degradation activities. Comamonadaceae was the most abundant family in Nanda Lake during both seasons. This could possibly be due to the increased nutrient content in Nanda Lake, which is an urbanised environment, compared to Sarzora Lake, which is a pristine ecosystem. This observation aligns with previous studies indicating their prevalence in environments with ample organic material for degradation and nitrogen compounds for denitrification [1]. In previous studies, Moraxellaceae and Enterobacteriaceae, associated with pollution indicators, were more prevalent during dry seasons, correlating with lower nutrient levels [23, 47]however we observed higher abundance of this family during the wet season. Pelagibacteraceae, adapted to oligotrophic environments, showed increased prominence in dry seasons characterized by lower organic matter content. This finding supports their role as key players in carbon cycling and other biogeochemical processes in nutrient-scarce conditions [47, 48].Microbacteriaceae and Chromobacteriaceae, are involved in nitrogen fixation and denitrification, demonstrated varying abundances linked to nutrient availability and oxygen levels.[47, 48]These bacterial families collectively contribute to crucial biogeochemical processes in lake ecosystems, with their abundances dynamically responding to seasonal variations in environmental conditions. The dominance of specific families during wet or dry seasons underscores the intricate interplay between microbial communities and their surrounding habitats [47, 48][49].

Table 4: The most abundant genera found in Nanda and Sarzora lake during the wet and dry season

WS NANDA	DS NANDA	WS SARZORA	DS SARZORA
Candidatus Symbiobacter sp. 26%	*Limnohabitans sp.* 19%	*Acinetobacter sp.* 26%	*Candidatus Pelagibacter sp.* 8%
Ramlibacter sp. 8%	*Rhodoluna sp.* 15%	*Cronobacter sp.* 9%	*Cyanobium gracile* 13%
Others Less than 4%	Others Less than 4%	Others Less than 4%	Others Less than 4%

As it can be observed that there are distinct bacterial communities present in Nanda and Sarzora lakes during different seasons. In Nanda lake, *Candidatus Symbiobacter sp*. is the dominant bacterium in the wet season, while *Limnohabitans sp. and Rhodoluna sp*. are more prevalent in the dry season. On the other hand, *Acinetobacter sp. and Cronobacter sp*. are the dominant bacteria in Sarzora lake during the wet season, while *Candidatus Pelagibacter sp.* and *Cyanobium gracile* are more abundant in the dry season.

Conclusion

The results of this study, which concentrated on Nanda Lake and Sarzora Lake during the wet and dry seasons, offer us a better understanding of the microbial populations in wetland environments. Significant taxonomic diversity and seasonal fluctuations among the bacterial communities were revealed by metagenomic sequencing and bioinformatics analysis.

These findings contribute significantly to our knowledge of wetland microbial ecology and have implications for wetland conservation and management strategies.

Understanding the seasonal dynamics and taxonomic variations in microbial communities is crucial for preserving ecosystem health and functionality, particularly in light of the continuous environmental challenges.

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APPENDIX

1)CTAB/NaCl solution

Ingredient	Quantity
CTAB	2.5g
NaCl	1g
MiliQ water	15ml

Dissolve 1g NaCl in 15 mL MiliQ water. Add CTAB to it and vortex it to make a uniform mixture. Keep in 60°C water bath if necessary

2) Dnase I (1mg/Ml)

Pancreatic DNase I	0.002g
NaCl	0.00029g(50mM)
10mM Tris Cl	1mL
$MgCl_2$	0.00009g(1mM)
Glycerol	1mL

Dissolve NaCl and $MgCl_2$ in 1mL in 10mM TrisCl (DNase Buffer). Dissolve 0.002g crude pancreatic DNase in DNase Buffer. Add 1mL of glycerol to it, mix gently and store at -20°C.

3) Proteinase K (20mg/mL)

Proteinase K	0.020g
$CaCl_2$	0.0016(3mM)
200mM Tris-Buffer (pH 8.0)	5mL

Dissolve $CaCl_2$ in Tris-Buffer to make Tris-CaCl2 buffer. Measure 0.5 mL of Tris- CaCl2 buffer and add 0.020g of Proteinase K to it. Mix gently and filter sterilize through 0.22µm filter. Make the final volume up-to 1mL with glycerol. Store at -20°C.

4) TBE Buffer (5X)

Tris Base	54g
Boric acid	27.5g
0.5M EDTA (pH 8)	20mL
MiliQ water	1000mL

7) 2M TrisCl (pH 8.5)/0.2 M EDTA

TrisHCl	315.12g
Na_2EDTA	74.44g
MiliQ water	1000 mL

Dissolve TrisCl in 200 mL MiliQ water and adjust the pH to 8.5 using NaOH/HCl. Dissolve Na2EDTA separately in 200 mL and adjust the pH to 8.0. Mix both the solutions and make up the volume up to 1 Litre. Autoclave and Store at room temperature

8) Lysis Buffer

1M TrisHCl (pH 8.0)	0.05mL
0.5 M EDTA (pH 8.0)	1mL
SDS 10%	0.5mL