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# Bacterial Diversity in High-Altitude Chandra Taal Lake: A Pilot Study

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# Abstract

Chandrataal Lake is a high-altitude lake and a popular tourist destination in the Lahaul and Spiti district of Himachal Pradesh. The lake is least studied except its bathymetry and few physicochemical properties. The harsh environment and extreme UV radiation may harbour the unique microbial composition in the lake. In the following study, the water sample was collected in June 2022 to study culture independent bacterial composition and basic physicochemical properties. The microbial diversity was studied by targeting V3–V4 regions of 16S rRNA gene sequenced by using Illumina Miseq Platform. The results indicated that the lake is dominated by Proteobacteria (47–48%) followed by Bacteroides (19–20%). The dominant families/genus are Xanthomonadaceae, Chitinophagaceae, *Chitinimonas taiwanensis, Exiguobacterium sp.*, Sphingomonadaceae, Comamonadaceae, Cytophagaceae, Acidimicrobiales, *Luteolibacter sp.*, Aeromonadaceae, *Flavobacterium sp., Rhodobacter sp.* Other dominating members are Acidobacteria, Nitrospirea, Firmicutes etc. Some basic physicochemical properties like dissolved oxygen, pH, ammonia, temperature, conductivity (EC), and total dissolved solids (TDS) were recorded. The dissolved oxygen was 4–5 mg/L, likely contributed by photosynthetic activity as the water samples were collected at around 11.00 am with bright sunlight. The average temperature was 15–18˚C. The average ammonia level was 0.5-1 mg/L. The pH of the water is alkaline which might be due to constant weathering. These findings were like the previous reports from high-altitude lakes.


# Introduction

India is blessed with various type of water bodies, like various riverine systems, freshwater, brackish water and, saline lakes etc. The presence of glacier lakes in the northern states of India is common. One of such lake is Chandrataal situated at an altitude of about 4,300 m (14,100 ft) on the Samudri Tapu plateau, with the co-ordinates of 32.475180°N and 77.617060°E in the Lahaul and Spiti district of Himachal Pradesh. This lake has a contour interval of 8.5 m and maximum depth of 42 m. The dimensions of the lake have been measured as the maximum length and maximum effective length are 1.76 and 1.75 km respectively. The orientation of the lake is NW-SE, steeper on the southwestern part and flatter to gently sloping towards the northern and southern banks. Lake can be attributed to rapid accumulation of sediments from the catchment area of Chenab (Rout et al. 2022). This lake is declared as Ramsar site (As per Ramsar site declaration on November 08, 2005). Chandrataal lake is a one of the offroad popular destination for tourists in the summer season.

The microbial composition in any habitat is a product of the complex interactions between organic and inorganic substances, influenced by factors like bedrock, sediments, solar radiation, and glacial meltwater (Hodson et al. 2008; Chuvochina et al. 2011; Lutz et al. 2015; Boetius et al. 2015). The high altitude may create the variations in different environmental parameters like temperature, nutrients, pH, electrical conductivity, etc. which may harbour the random composition of microbial diversities (Wilhelm et al. 2013). Generally, a large amount of water, nutrients, and microorganisms are found to accumulate in such proglacial lakes formed by the moraine's damming during glacier retreat, a glacial dam, or melting of glacial affecting the microbial composition in lakes due to the variation in different

physicochemical parameters (Jacobsen et al. 2012; Liu et al. 2017). These conditions may facilitate a unique microbial population.

Studies from other alpine lakes showed the endemic microbial diversity of bacterial and archaeal metacommunities consisting of autotrophic, chemolithotrophic, and heterotrophic bacteria. Most of the heterotrophic bacteria belong to the phylum Proteobacteria, Bacteroidetes, and Actinobacinteria and they play a key role in the nutrient cycle (Lutz et al. 2017; Franzetti et al. 2016). Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes, Cytophagae–Flavobacteriae–Bacteroides (CFB group), Planctomyces and Gemmatimo-nadetes were found to be cold adapted communities found in diverse of cold adapted habitats (Carpenter et al. 2000; Neufeld et al. 2004; Cheng and Foght 2007; Steven et al. 2007; Amato et al. 2007; Gangwar et al. 2009). Above reports from the glacier lake showed different structure of microbes, mostly dominating by Proteobacteria. Thus, representing a different structure of microbial biota from tropical lakes.

The lake is least investigated except bathymetry and physicochemical properties of the lake water only by Rout and Vasudev (2022). However, other aspects of the lake, particularly its microbial life, remain largely unexplored. This may be due to the lake's remote location, making frequent sampling difficult. The lake exhibits distinctive chemical and biological characteristics

## Materials and Method

The water samples of the lake were collected in the month of June 2022 which is the peak summer season in northern India. The basic physicochemical properties were studied in the field by using kits at three sites (TRM AQUATEK PRIVATE LIMETED, INDIA) while EC/TDS and pH was measured by digital meter (Hanna, Itali). The water samples were collected from the exit site as (32.475231°N, 77.616971°E). The collected water samples were stored in BPA-free plasticware designed for sample storage (Tarsons, India).

The basic physicochemical properties of the water samples were analysed at the collection sites like pH, temperature, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solid and ammonia-N following the manufacture’s instruction.

# Microbial analysis:

# Extraction of total DNA from soil and PCR amplification of the 16S rRNA gene

DNA isolation and downstream analysis were carried out by Eurofins Genomics India Pvt. Ltd. India. Metagenomic DNA was isolated from the water samples using commercially available Nucleospin kit following the manufacture’s kit. The qualities of the isolated metagenomic DNA samples were quantified using NanoDrop. The isolated metagenomic DNA samples were amplified against the 16S rRNA using

the primers F- GCCTACGGGNGGCWGCAG and R-ACTACHVGGGTATCTAATCC (V3-V4 region). These region of 16s rRNA (V3-V4) region is highly variable and highly useful in microbial diversity study (Cardoso et al. 2017). The amplicon libraries were prepared using Nextera XT Index Kit (Illumina Inc.). The PCR-enriched libraries were analysed in a 4200 Tape station system (Agilent Technologies). Then the libraries were loaded onto MiSeq platform at an appropriate concentration for cluster generation and sequencing at Eurofins Genomics India Pvt. Ltd. India.

For further downstream analysis, high- quality clean reads were obtained using Trimmomatic v0.38 by removing the adapter sequences, ambiguous reads (reads with unknown nucleotides “N” larger than 5%), and low-quality sequences. The paired-end (PE) data was stitched into single end reads using FLASH (v1.2.11). The obtained high quality (HQ) reads were subjected to operational taxonomic unit (OUTs) identification using QIIME (Quantitative Insight into Microbial Ecology) based on the sequence similarity. Operational Taxonomic Units were picked from the Greengenes database (version 13_8) and clustered by using UNCLUST at 97% sequence similarity. The alpha diversity and rarefactions curves were calculated. Heatmap images are generated to visualize the OTU table at different taxonomic levels.

## Results and Discussions

# Physicochemical properties

The physicochemical properties of the sampling sites are given in Table 1. The average temperature of the sampling sites was 19–20°C, while the average pH was around 8. The dissolved oxygen was fair enough to support the livelihood as it is present around 5 mg/L. The concentration of ammonia was ranged around 0.5-1 mg/L. The other parameters are presented in Table 1. The average temperature of surface water was high enough to sustain planktons and other aquatic organisms as the sample were collected during peak summer as the sun rises around 11.00 am. Fair dissolved oxygen levels may be attributed to photosynthetic activity by plankton in the lake. Low ammonia concentrations could be due to organic decay of nearby plants and plankton. This level is below toxic thresholds for aquatic life. The presence of nitrifying bacteria indicates active nutrient cycling. The slightly alkaline pH is likely due to the weathering of carbonate rocks in the surrounding area. Previous research by Rout and Vasudev (2022) also confirmed the lake's slightly alkaline nature, making it suitable for drinking and industrial uses.

Table 1
Physicochemical properties of Chandrataal Lake

| Sl. No | Parameters | Site.1 | Site.2 |
|---|---|---|---|
| 1 | Average temperatures | 22 ± 2°C | 21 ± 1°C |
| 2 | pH | 8.3 ± 1°C | 8 ± 0.2°C |
| 3 | Dissolved oxygen | 5 ± 1mg/L | 5 ± 1mg/l |
| 4 | EC | 169 ± 10 | 171 ± 1 |
| 5 | TDS | 84 ± 10 | 86 |
| 6 | $NH_3$-N | 0.5-1mg/L | 1 mg/l |

# Microbial diversity

A total of 190,176 high-quality DNA sequences were obtained from water samples collected from Chandra taal lake. Analysis revealed 19 major phyla, with Proteobacteria being the most abundant, followed by Bacteroidetes, Verrucomicrobia, and others (Fig. 1). Within Proteobacteria, Betaproteobacteria, Alphaproteobacteria, and Gammaproteobacteria were the most dominant classes (Fig. 2). At the order and family levels, Xanthomonadales, Sphigomonadales, Niesseriales, Xanthomonadaceae, Chitinophagaceae, and *Chitinimonas taiwanensis* were prominent (Figs. 3 & 4). This microbial composition aligns with findings from other glacial and high-altitute lakes, such as the Wuli cold springs, where Proteobacteria and Crenarchaeota were prevalent (Wang et al. 2020). Common phyla in other temperate lakes, like those on the Qinghai-Tibetan Plateau, include Acidobacteria, Deinococci, Sphingobacteria, Flavobacteria, Nitrospirae, Actinobacteria, Gemmatimonadetes, and unclassified bacteria (Li et al. 2012). Similar microbial diversity has been observed in cold sulfur-rich springs near Lake Erie (Chaudhary et al. 2009). Other temperate lakes, such as Lake Yukidori-Ike and Lake Nurume-Ike, also showed dominance of Proteobacteria, specifically Betaproteobacteria and Alphaproteobacteria, respectively (Chaya et al. 2019).

Chitinophagaceae were detected in the samples. These bacteria are crucial in breaking down complex organic matter like chitin and cellulose, exhibiting β-glucosidase activity (Bailey et al. 2013; Rosenberg 2014). *Chitinimonas taiwanensis*, also found in the sample which plays a vital role in chitin cycling. *Exiguobacterium* species were also present, and they are known for their involvement in nitrate reduction, starch hydrolysis, gelatin liquefaction, and catalase activity (Yumoto et al. 2004; Kim et al. 2005; López-Cortés et al. 2006). They likely contribute to nutrient cycling and starch hydrolysis. Sphingomonadaceae and Cytophagaceae were other abundant families. Many Cytophagaceae members can degrade macromolecules like cellulose and other polysaccharides or proteins (McBride et al. 2014). The presence of abundant nearby plants may have contributed to their presence. Actinobacteria were also present in the sample. The previous study identified that isolates of cold Himalayan region were identified based on 16S rRNA gene sequencing and showed phylogenetic relationship to *Arthrobacter*

belonging to the class Actinobacteria, *Bacillus*, *Exiguobacterium*, *Paenibacillus*, and *Planomicrobium* to Bacilli, and *Pseudomonas*, *Serratia*, and *Stenotrophomonas* to Gammaproteobacteria (Salwan et al. 2020). Presence of *Proteobacteria* (56%), *Firmicutes* (16%), *Actinobacteria* (12%), and *Bacteroidetes* (8%), were reported from other high-altitude lake in Sikkim, India (Sherpa et al. 2021). Other microbial communities isolated from the cold areas are *Arthrobacter*, *Pseudomonas*, *Janthinobacterium*, *Sphingobacterium*, *Exiguobacterium*, *Planococcus*, *Psychrobacter*, *Sporosarcina* and *Paenibacillus glacialis* (Sahay et al. 2013). The sample of Chandrataal Lake is similar with other cold environments.

The observed phyla in Chandrataal Lake align with previous findings from other cold-adapted lakes, suggesting a strong link between microbial diversity and ecological factors. Chandrataal Lake's oligotrophic nature (low nutrient content) influences its microbial community. The present data indicates that most phyla are involved in plant-related processes or nutrient cycling. Microorganisms thriving in such harsh, isolated environments possess unique adaptations and competitive advantages (Khosla et al. 2017). Further exploration beyond the periphery regions may reveal even greater microbial diversity within Chandrataal Lake

## Conclusions

Temperate lakes exhibit unique ecological characteristics. The observed microbial diversity in Chandrataal Lake shows similarities to other glacial lakes, with a predominance of plant-associated microbes. The lake's alkaline pH suggests potential suitability for drinking and may indicate the presence of various minerals. Further in-depth studies are needed to fully understand the microbial composition of this high-altitude lake. Nutrient cycling microorganisms often face challenges in regions with fluctuating temperatures, as these variations can impact their efficiency. Identifying new microbial populations involved in nutrient cycling, such as nitrogen and phosphorus, could significantly rescue the ammonia accumulation in wastewater treatment plants during colder seasons.

## Declarations

# Competing Interests

The authors declare no competing financial interests.

# Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Harit Ajay Kumar, Ingochouba Meetei Lukram and Khangembam Cherita Devi. The first draft of the manuscript was written by Khangembam Cherita Devi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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# Figures

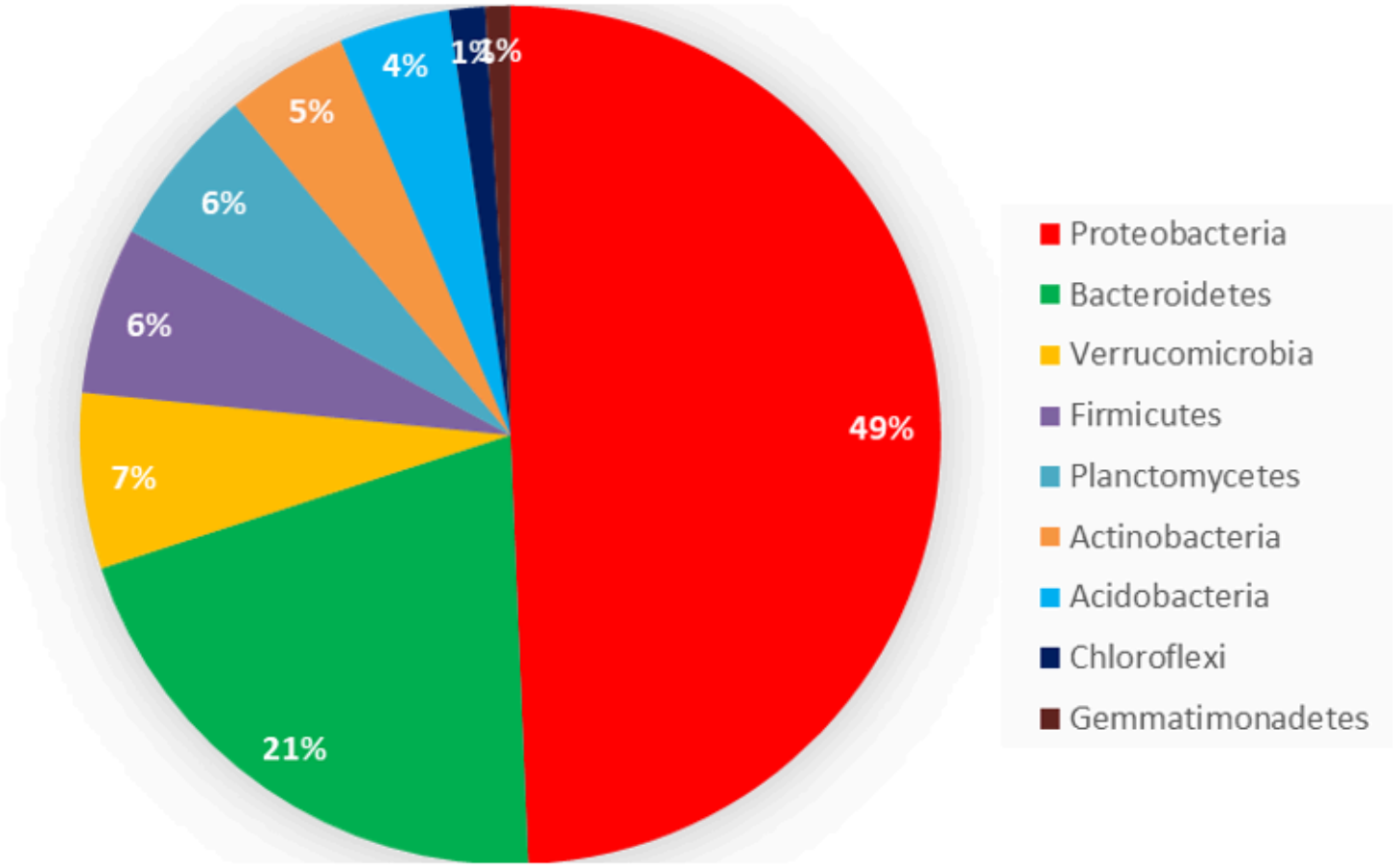
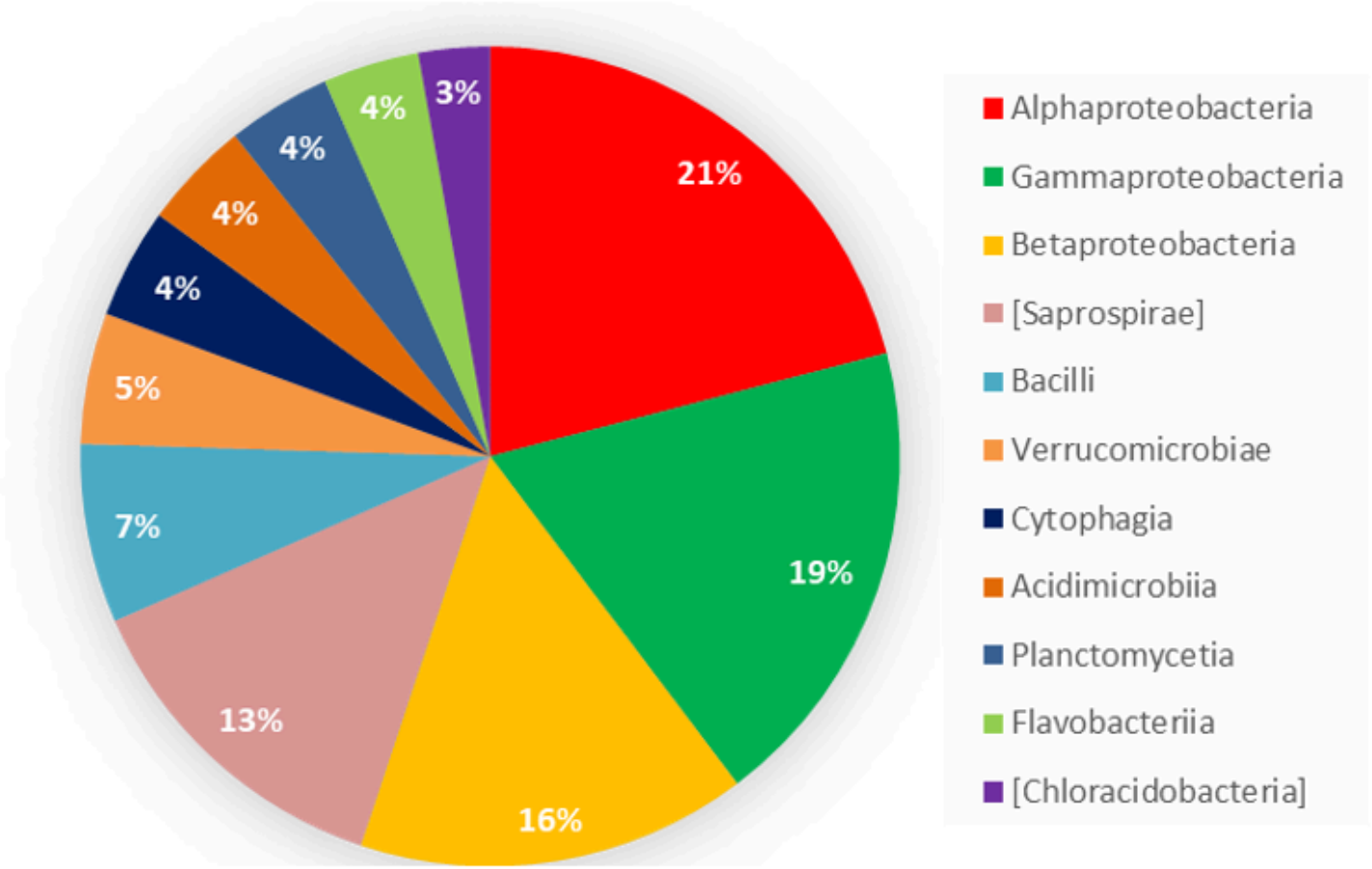



**Figure 1**

Bacterial diversity at the level of major phylum




## Figure 2

Bacterial diversity at the level of major classes

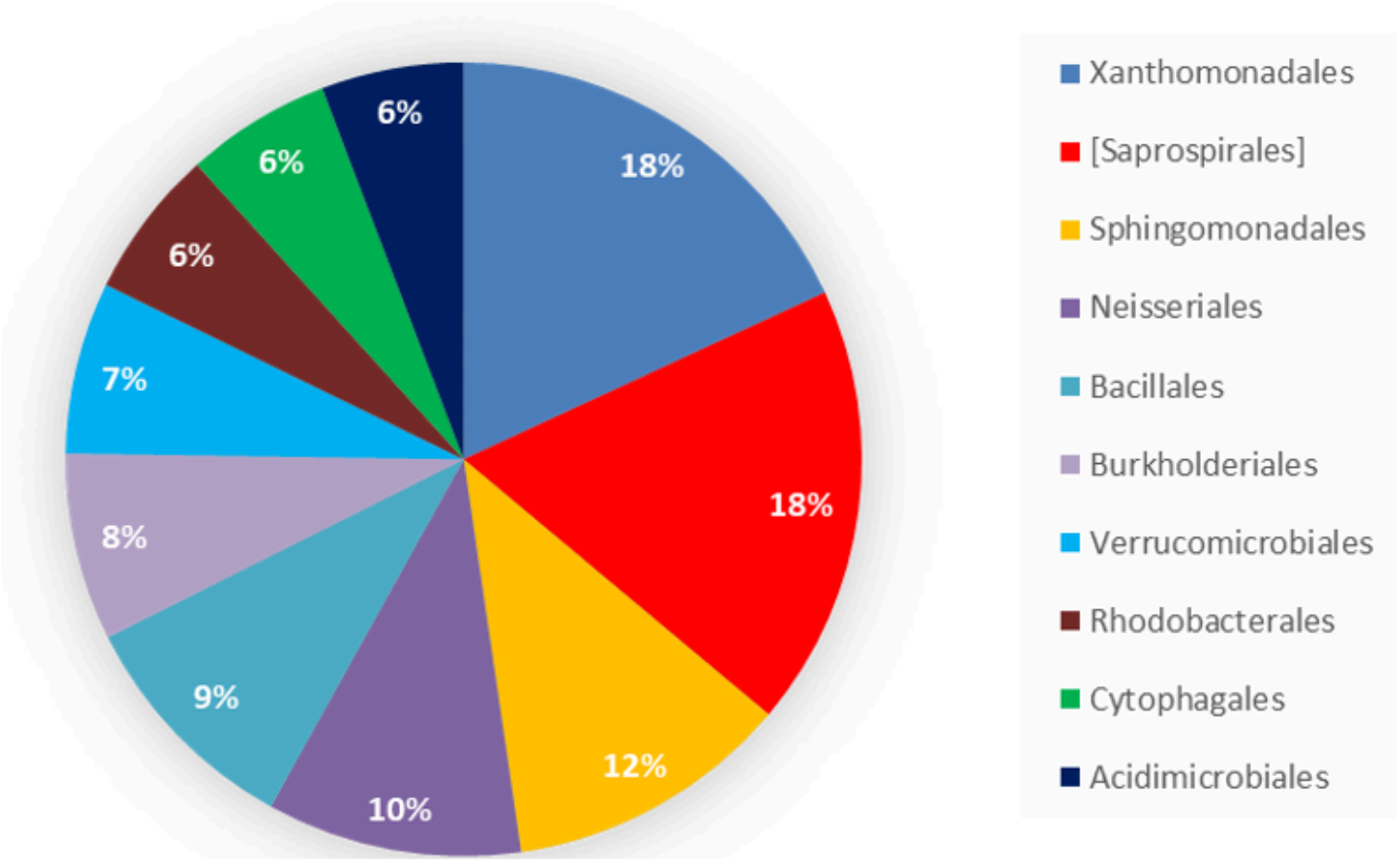



**Figure 3**

Bacterial diversity at the level of orders

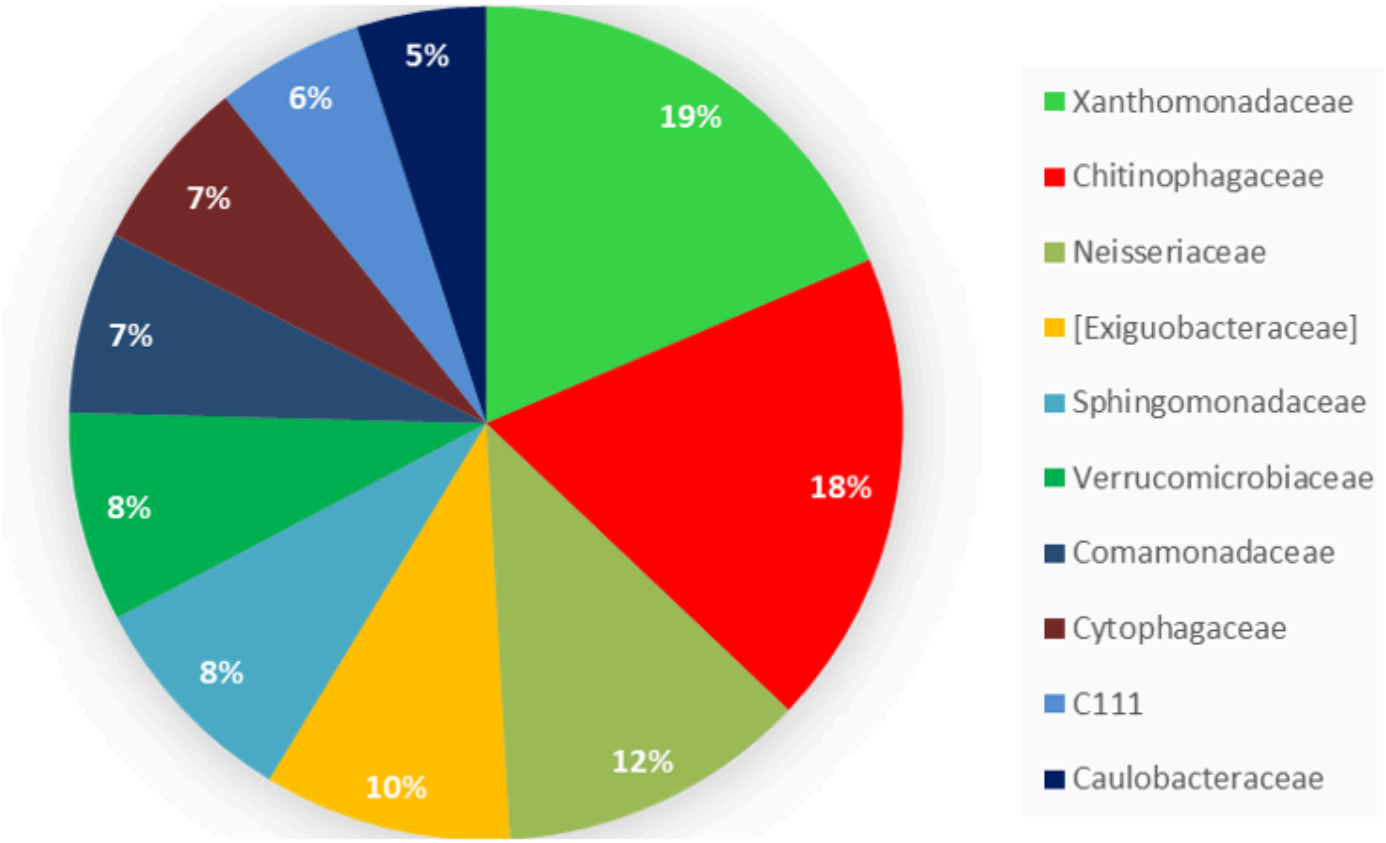



**Figure 4**

Bacterial diversity at the level of major families