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Soil Microbiomes in Suthamalli Reservoir, Tirunelveli District, Tamil Nadu, Using 16S rRNA Gene Sequencing

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Abstract

Soil microbial diversity encompasses the variety of microorganisms found within the soil, including bacteria, fungi, archaea, and viruses. Bacteria are among the many different types of microorganisms found in soil. In Suthamalli Reservoir, Tirunelveli District, Tamil Nadu, India, soil samples were used in this study to investigate the variety of bacteria. DNA from the environment was extracted. The diversity indices have been calculated and the Illumina MiSeq sequencing was conducted. Illumina MiSeq sequencing revealed 24 Phyla, five of which were abundant: Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Bacillus. The investigated samples' soil microbial communities had a stable community structure whereas becoming phylogenetically varied. 56 classes are represented with relative abundances of Gamma Proteobacteria, Alpha proteobacteria, Beta proteobacteria, Actinobacteria, Chitinophgia, and Bacilli. Totally 253 genera were listed in the suthamalli soil sample among these *Proteus, Hydrogenophage, Gemmatimonas, Sphingomonas, Pseudomonas, Magnetospirillum, Flavobacterium, Serratia, Prevotella, Lactobacillus, Enterococcus, Streptococcus,* and *Geobacter* were most dominant in the study area.

Keywords: Environmental Microbiology, Metagenomic, 16S rRNA Gene Sequencing, Microbiome.

Introduction

Soil microbial communities are essential components of terrestrial ecosystems, playing crucial roles in nutrient cycling, organic matter decomposition, soil structure formation, and plant health. These communities consist of a diverse array of microorganisms, including bacteria, archaea, fungi, viruses, and protozoa, each contributing uniquely to soil functions. Among these, bacteria are the most abundant and diverse, often studied through 16S ribosomal RNA (rRNA) gene sequencing, a powerful technique that allows for the identification and classification of bacterial species based on variations in their 16S rRNA genes (Torsvik, V., *et al*., 2002).

Microorganisms in the soil interact in complex networks, influencing and being influenced by various biotic and abiotic factors such as soil pH, moisture, temperature, organic matter content, and plant root exudates. These interactions affect the composition, structure, and functionality of microbial communities, which in turn impact soil fertility and ecosystem productivity (Fierer, N., *et al*., 2006). Understanding the diversity and dynamics of soil microbial communities is critical for several reasons. First, these microorganisms are integral to the decomposition of organic matter and the recycling of nutrients, including carbon, nitrogen, phosphorus, and sulfur, which are vital for plant growth. Second, soil microbes play a significant role in the suppression of soil-borne diseases and the enhancement of plant immunity through various mechanisms, including competition, antibiotics, and induced systemic resistance. Third, microbial communities contribute to the stabilization of soil structure by producing extracellular polymeric substances that bind soil particles together, enhancing soil porosity and water retention (Van der Heijden, M. G. A., *et al*., 2008).

Recent advancements in high-throughput sequencing technologies, particularly 16S rRNA gene sequencing, have revolutionized our ability to study soil microbial communities. These techniques provide comprehensive insights into microbial diversity, enabling the identification of both culturable and unculturable microorganisms. Comparative studies using 16S rRNA sequencing can reveal how different environmental conditions, land management practices, and ecological disturbances affect microbial community composition and function (Caporaso, J. G., *et al*., 2011).

The study of soil microbial communities not only enhances our understanding of soil ecosystem functioning but also informs sustainable agricultural practices, soil conservation

strategies, and climate change mitigation efforts. By delving deeper into the complexities of these microbial ecosystems, we can develop innovative approaches to harness their potential for improving soil health and productivity, ultimately contributing to global food security and environmental sustainability (Singh, B. K., *et al*., 2010).

Soil microbiomes play a crucial role in ecosystem functioning. The Suthamalli Reservoir in Tirunelveli District, Tamil Nadu, provides a unique environment to study these microbial communities due to its specific geographical and climatic conditions. Using 16S rRNA gene sequencing allows for a detailed analysis of the bacterial diversity and community structure present in the soil of this region.

In this connection the current study, focused on to explore bacterial soil diversity, thus determining through the taxonomy of different phyla, classes, orders, families and operational taxonomic units, the metagenomic sequencing of 16S RNA gene has been used on the specific illumina. So this study have to improve our knowledge of microbial ecology and how it affects ecosystem health and management. In addition to expanding scientific understanding, the results are expected to have practical implications for conservation, agriculture, and climate change mitigation.

Materials and Methods

GPS Coordinates of the study area

As per the plan and objective of the study, the three different Environmental sampling sites at different GPS loaction were selected in the Suthamalli Reservoir, Tirunelveli District, Tamil Nadu for this study. They are, 1. Suthamalli Dam (37 msl), Tirunelveli District, 2. Thenkarai Pond, Thirupparankundram (136 msl), Madurai District, 3. Manjalar Dam, Theni District (311 msl) Theni District.

Sample Collection

Three soil samples were taken from the study area using a sterile spatula in clean, dry, and sterile glass falcon tubes. The three samples were mixed to make a composite soil, which was intended to increase the bacterial diversity at the study area. In the same study area, sample collection sites were separated by 150 m. The collected soil samples were brought to the Environmental studies Laboratory, Madurai Kamaraj University, Tamil Nadu under sterile conditions for analysis.

Environmental parameter of soil

The pH was the sole environmental parameter tested for this investigation. Hydrogen ion concentration is the measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in soil. Hydrogen ion concentration is generally measured on a log scale and equals to negative log $_{10}$ of hydrogen ion concentration.

$$pH = -\log 10 [H]$$

Hydrogen ion concentration scale ranges from 0 to14 with 7 as neutral, below 7 being acidic and above 7 as alkaline. Thus, Hydrogen ion concentration plays a vital role in soil quality assessment. Soil pH of the samples were measured using a digital pH meter available in Laboratory of Environmental Studies, Madurai Kamaraj University.

Characterization of Soils Bacterial Diversity

DNA Extraction

DNA extraction is done using the suitable method for the sample type from commercially available kits such as for soil sample soil gDNA Xploregen kit. According to the manufacturer's recommendation, DNA extraction had been carried out. The extracts that contained DNA were stored at -20˚C in preparation for further amplifying using PCR.

PCR Amplification of V3-V4 Region of 16s Gene

16S rRNA gene amplification and high throughput sequencing were performed. The universal 16S rRNA gene primers were 16sF:- 5’ AGAGTTTGATGMTGGCTCAG3’ 16sR:- 5’ TTACCGCGGCMGCSGGCAC3’. The bar code and adapter have been incorporated between the adapter and the front primers. The PCR was carried out in Biokart India Pvt.Ltd., Karnataka. The Composition of PCR TAQ Master MIX are High-Fidelity DNA Polymerase, 0.5mM dNTPs, 3.2mM MgCl2, PCR Enzyme Buffer. 40ng of Extracted DNA is used for amplification along with 10pM of each primer. The PCR process consisted of an Initial Denaturation at 95 degree C for15 sec, Annealing at 60 degree C for 15 sec, Elongation at 72 degree C for 2 mins and Final Extension at 72 deg C for10 mins and Hold at 4 deg C.

The same sample was mixed with the PCR product with detection by electrophoresis on 2% agar. Using the Axy Prep DNA gel extraction kit (Biokart, Karnataka), PCR products were cut, eluted with Tris-HCl and subjected to detection of 2% agarose electrophoresis at the same time. The Quanti FluorTM-ST blue fluorescence quantitative system (Biokart, Karnataka)

for PCR products was used for detection. The final DNA fragments completed with the primer base and were attached to a chip, for a laser. Chemical cutting of "fluorescent groups" and "end groups" was performed, and the viscosity at the end of 3' was restored. During this time, the second nucleotide was aggregated and the fluorescent signal results in each round were recalculated and collected, while the template DNA obtained has been illumina Mi sequenced.

Overview of Sequencing Method

The amplicons from each sample were purified with Ampure beads to remove unused primers and an additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the sequencing libraries. Libraries were purified using Ampure beads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing wasper formed using Illumina Mi seq with 2x300 PE v3 sequencing kit.

Bioinformatic Analyses of Sequences and Statistics

The Fast QC (v0.11.2) and MultiQC (version1.9) was used to quality control checks on raw sequence data coming from high throughput sequencing pipelines. Multi qc consolidates fast qc results into single report. Trimming of adapters and low quality reads was perfomed by TRIMGALORE. The trimmed reads are further taken for processing which includes merging of paired end reads, chimera removal and OTU abundance calculation and estimation correction was achieved by QIIME and KRAKEN workflows. This work flow enables highly accurate investigations at genus level.

OTU taxonomy plot analysis was performed after distinguishing the samples. The OTU table gives an overall microbial community present in the samples. From the generated OTU the stacked bar charts are pivoted based on taxonomic levels such as Phylum, Class, Order, Families and GENUS level using Microsoft excel 2010.

The analysis of the community structure in each classification level was conducted by the taxonomy plot information. Based on the above analysis, the study was carried out on a serial analysis of community structure, system development and visualization. According to the similarity levels, all the sequences were taken using the OTU division. All optimized sequences were mapped to the representative OTU sequence. The analysis of the community The frequency curve was obtained from the sequencing depth of the sample. The heat map representation of the relative abundance of bacterial OTUs among the samples was developed using Excel (Rappe, M.S, *et al*., 2003).

Results

The GPS coordinates of different sites of soils sample and the pH values of each site before the composite soil samples are showed in Table 1 and 2. The pH values are all comprise in between 6.00 and 7.00, around the neutral composition. Controlling the pH of the soil between 6 and 7 is needed for healthy soil and maximum plant development. Regular monitoring and appropriate amendments can help achieve and sustain this pH range, ensuring that nutrients are available to plants and microbial activity is supported. The sample contain 55.5% of GC content. This is because the 16S rRNA gene is highly conserved and its sequence composition tends to fall within this range for the majority of bacterial species. The some variation in the GC content of 16S rRNA genes among different bacteria, a typical GC content range for 16S rRNA sequences is around 50%-60%. Values significantly outside this range might warrant further investigation to ensure the accuracy and integrity of the sequencing data (Klindworth, A *et al*., 2013).

Table 1: GPS Coordinates for the Suthamalli Reservoir sampling sites

Station	Composites	GPS Coordinates
Suthamalli Reservoir, Tirunelveli District	Site-1	08˚41'15.93"N 77˚38'14.93"E
	Site-2	08˚41'17.18"N 77˚38'10.31"E
	Site-3	08˚41'18.42"N 77˚38'05.52"E

Table 2: pH Values for the different composite soil sample

Station	Composite	pH value
Suthamalli Reservoir, Tirunelveli District	Site-1	6.5
	Site-2	6.4
	Site-3	7.0

Table 3: Sample Details for V3-V4 amplicon region

S.No	Sample Name	No. of reads (in Million)	GC Content (%)
1	SM01	0.4M	55.5%

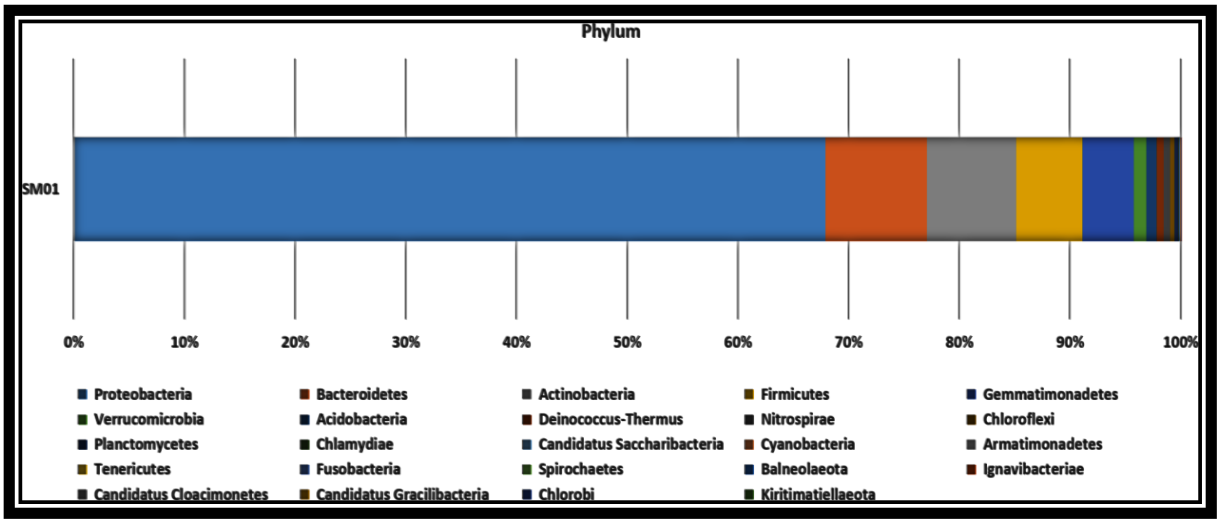
Composition of the Bacterial Community

A totally of 71,756 OTUs were obtained for the soil sample of Suthamalli Reservoir, Tamil Nadu's Tirunelveli District. These OTUs are divided into 24 phyla, 56 classes, 113 orders, 253 families, and 253 genera.

Relative Abundance of Phyla

The relative abundances phyla level of the soil sample shown in the Figure 1. The most abundant phyla are Proteobacteria with 68% followed by Bacterioidetes 9%, Actinobacteria 8%, Firmicutes 4% and Bacilli 3% respectively. On the other hand, the least abundant are unclassified Armatimonades, Kiritimatiellacota with parent abundances of less than 1%.

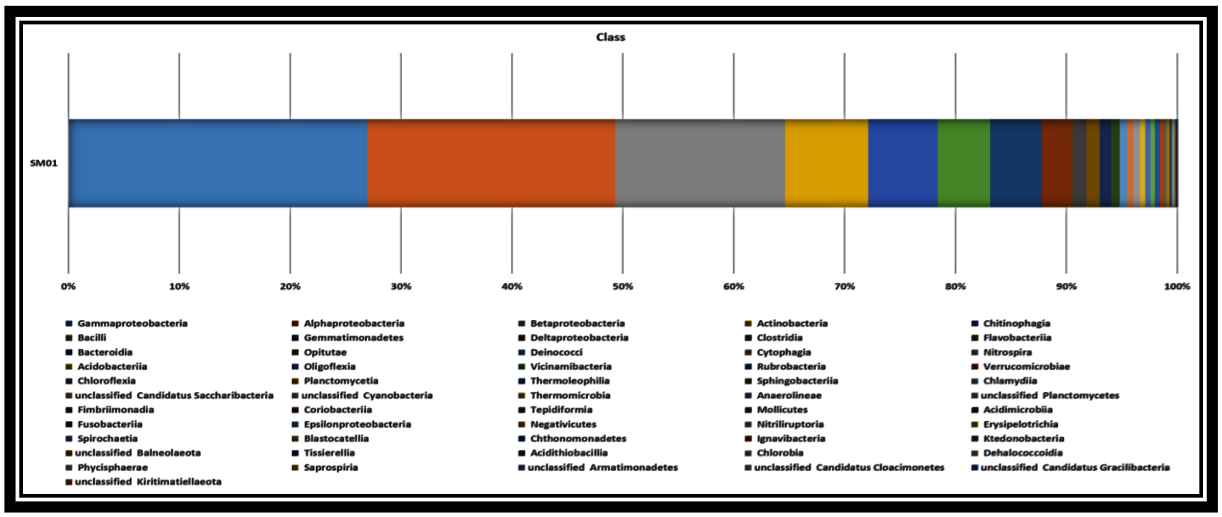
Fig. 1: The taxonomy and relative abundances of the most prevalent phyla in Suthamalli Reservoir soil samples.



Relative Class Abundance

The relative abundances at class level of the soil sample shown in the Figure 2. The most abundant class are Gamma Proteobacteria with 28% followed by Alpha proteobacteria 21%, Beta proteobacteria 16%, Actinobacteria 8%, Chitinophgia 9% and Bacilli 3% respectively. On the other hand, the least abundant are unclassified Armatimonades, unclassified candidatus cloacimonetes and unclassified candidatus Gracilibacteria with parent abundances of less than 1%.

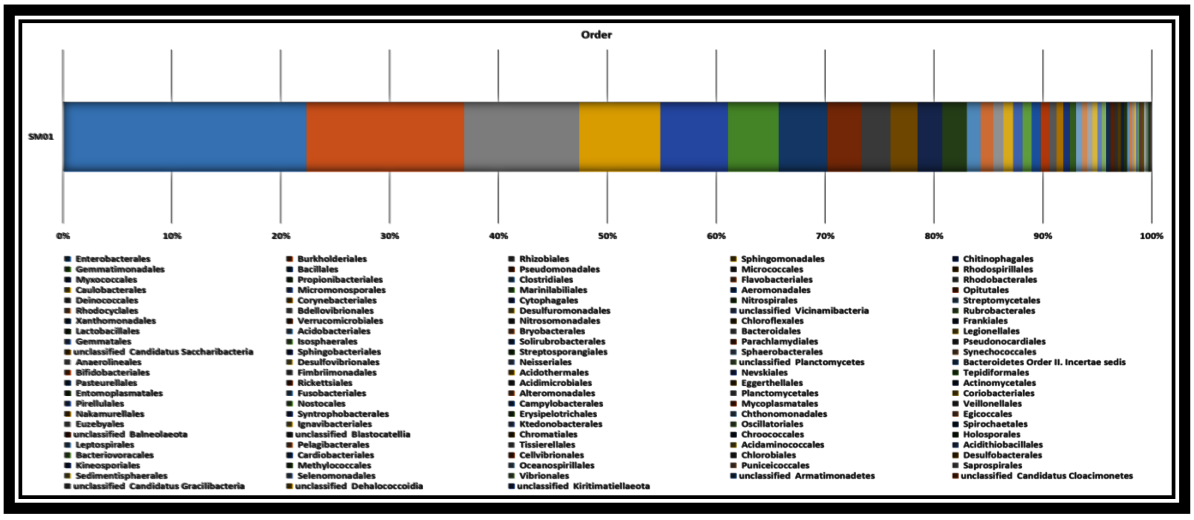
Fig. 2: The taxonomy and relative abundances of the most prevalent classes in Suthamalli Reservoir soil samples.



Relative Abundance of Orders

In Figure 3 shows the relative abundance of orders present in the soil. Of the 113 orders listed, Enterobacterales, Burkholderiales, Rhizobiales, Sphingomonadales, Chitinophagales, Gemmatimonadales, Bacillales, are the most abundant orders. The relative abundance of Enterobacterales is 23% in the sample, Burkholderiales is 14%, Rhizobiales is 10% respectively. Bacillales, Chitinophagales are less then 5% and unclassified Kiritimatiellaeota has the lowest abundances in the soil sample.

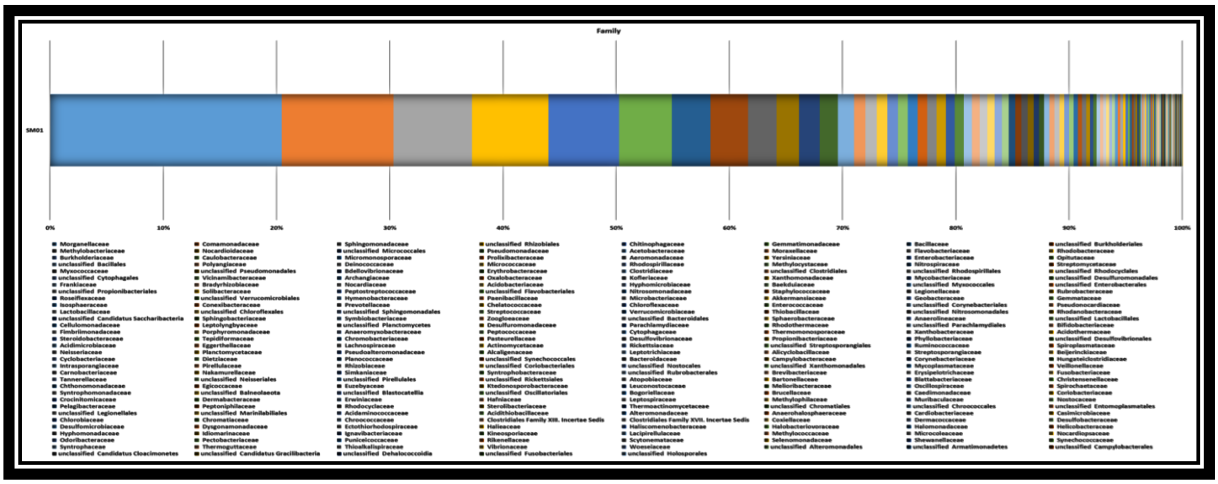
Fig. 3: The taxonomy and relative abundances of the most prevalent orders in Suthamalli Reservoir soil samples.



Relative Abundance of Families

Figure 4 presents 253 families with the highest abundances: Morganellaceae have a higher relative abundance 21%, Comamonadaceae are more abundant in the sample is 8%, the Sphingomonadaceae and Unclassified Rhizobiales are have 7% and Chitinophagaceae is in 6%. In the soils, the Unclassified Holosporales are the least common.

Fig. 4: The taxonomy and relative abundances of the most prevalent Families in Suthamalli Reservoir soil samples.



Relative Abundance of Genera

Figure 5 shows that among the listed genera, Based on the taxonomical classification almost the ten genus were predominantly present in sample *Proteus, Hydrogenophage, Gemmatimonas, Sphingomonas, Pseudomonas, Magnetospirillum, Flavobacterium, Serratia, Prevotella, Lactobacillus, Enterococcus, Streptococcus, Geobacter,* and 10 more unidentified genera. Among the genera, *Unclassified Cyclobacteriaceae* is least common.

Fig. 5: The taxonomy and relative abundances of the most prevalent Geneus in Suthamalli Reservoir soil samples.

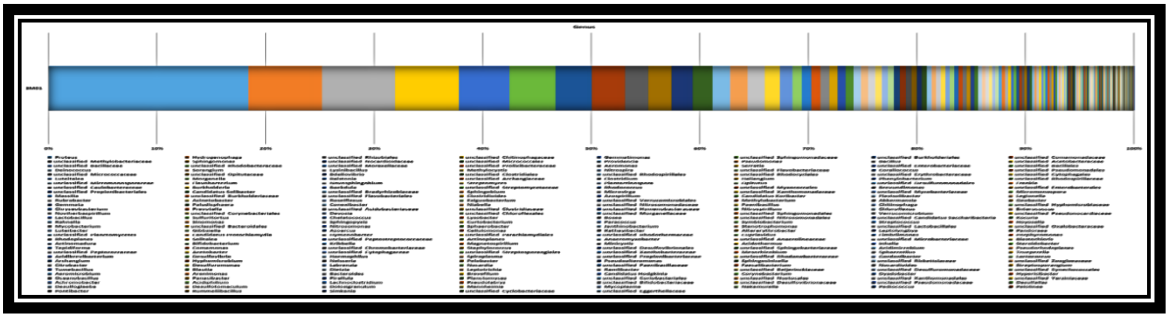
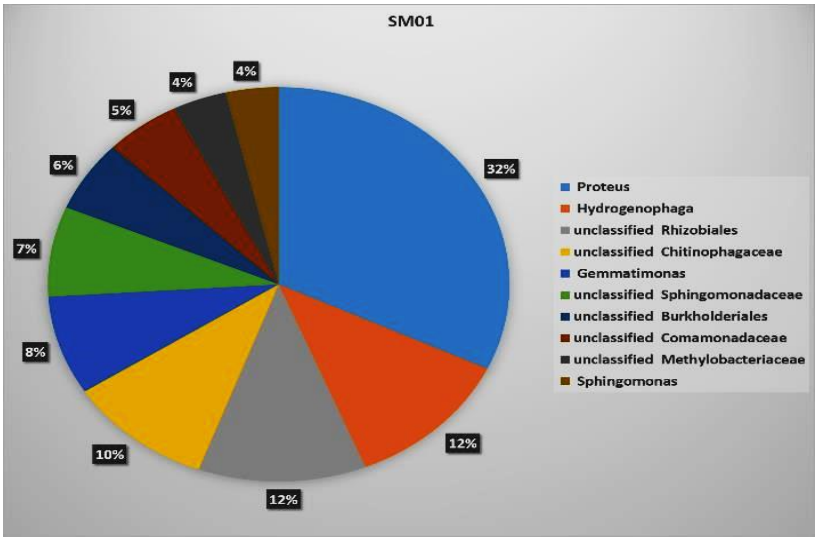


Fig. 6: Top Ten Genus in the soil sample of Suthamalli Reservoir



Discussion

The comprehensive analysis of soil microbiomes in the Suthamalli Reservoir, Tirunelveli District, Tamil Nadu, using 16S rRNA gene sequencing has revealed a rich and diverse microbial community. This study has highlighted the presence of various bacterial taxa, including those involved in key ecological functions such as nutrient cycling, organic matter decomposition, and nitrogen fixation. The predominant phyla identified include *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Bacillus* reflecting a complex interplay of microbial communities adapted to the unique environmental conditions of the reservoir. In the soils that were studied, these five phyla accounted for almost 90% of the sequences. Our findings corresponded to those presented in Janssen's meta-analysis (Janssen, P.H., 2006). The same author claims that the reason the soils were identical to one another was that the rarer phyla never had an abundance of 10% and that all soils had the same five bacterial families.

Many other studies have reported the same type of observation (Ding, G.C *et al*, 2013) . This result is to be expected; a number of other studies have used modeling approaches (Sun, R., *et al.*, 2015) or more in-depth surveys than those described here (Coolon, J.D. *et al*., 2013) to show that the bacterial communities in soil are habitat to a vast array of different species.

Since most bacterial soil taxa can be considered rare (He, Z.L., *et al*., 2010). It is difficult to adequately document the diversity of bacteria present in a given soil, even if one

solution obtains a complete pyrosequencing (Yun, J.L *et al*., 2015). Again, this is not unexpected, given previous studies (Zhang, W. *et al*., 2016) have also shown a notable level of endemism at higher taxonomic resolutions. It is crucial to recognize that there would likely be more overlap across soil categories as a consequence of a thorough investigation regarding specific soils.

Soils that have a pH range of 6 to 7 are the most active for soil microorganisms, including fungus and bacteria. These microorganisms play a crucial role in organic matter decomposition and nutrient cycling, thereby enhancing soil fertility. Controlling the pH of the soil between 6 and 7 is essential for healthy soil and optimum plant development. Regular monitoring and appropriate amendments can help achieve and sustain this pH range, ensuring that nutrients are available to plants and microbial activity is supported. (Chandrasekaran, M., *et al*., 2013).

Pfeiffer, F., Oesterhelt, D., *et al.* (2019): This study discusses the significance of GC content in quality control of sequencing data and highlights how unusual GC content values can indicate potential issues such as contamination or sequencing errors. Soil microbial diversity is a cornerstone of ecosystem health and functioning. Advances in molecular techniques have revolutionized our ability to study and understand these complex communities. By integrating knowledge of soil microbial diversity into land management and agricultural practices, we can enhance soil health, promote sustainable agriculture, and address environmental challenges.

Way Forward

Future research should focus on the interactions between soil microbes and other environmental factors, and how these interactions can be harnessed to improve soil and ecosystem resilience. They are,

Undiscovered Diversity: Many soil microorganisms are still unknown and unculturable, making it challenging to fully understand soil microbial diversity.

Functional Diversity: Understanding the functional roles of different microorganisms in soil processes is crucial for managing soil health.

Impact of Climate Change: Investigating how climate change and environmental stressors affect soil microbial communities and their functions.

Sustainable Land Management: Developing practices that enhance and preserve soil microbial diversity to promote

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Author Contribution

Ms. M.Mathar Fathima have designed the experiment and executed experiment, organised the data, and prepared the manuscript. Dr.S.Kannan has reviewed the data along with the whole manuscript.

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