Bacterial Operational Taxonomic Units Replace the Interactive Roles of Other Operational Taxonomic Units Under Strong Environmental Changes

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Abstract: Background: Microorganisms are an important component of an aquatic ecosystem and play a critical role in the biogeochemical cycle which influences the circulation of the materials and maintains the balance in aquatic ecosystems.

Objective: The seasonal variation along with the impact of anthropogenic activities, water quality, bacterial community composition and dynamics in the Loktak Lake, the largest freshwater lake of North East India, located in the Indo-Burma hotspot region was assessed during post-monsoon and winter season through metagenome analysis.

Methods: Five soil samples were collected during Post-monsoon and winter season from the Loktak Lake that had undergone different anthropogenic impacts. The metagenomic DNA of the soil samples was extracted using commercial metagenomic DNA extraction kits following the manufacturer's instruction. The extracted DNA was used to prepare the NGS library and sequenced in the Illumina MiSeq platform.

Results: Metagenomics analysis reveals Proteobacteria as the predominant community followed by Acidobacteria and Actinobacteria. The presence of these groups of bacteria indicates nitrogen fixation, oxidation of iron, sulfur, methane, and source of novel antibiotic candidates. The bacterial members belonging to different groups were involved in various biogeochemical processes, including fixation of carbon and nitrogen, producing streptomycin, gramicidin and perform oxidation of sulfur, sulfide, ammonia, and methane.

Conclusion: The outcome of this study provides a valuable dataset representing a seasonal profile across various land use and analysis, targeting at establishing an understanding of how the microbial communities vary across the land use and the role of keystone taxa. The findings may contribute to searches for microbial bio-indicators as biodiversity markers for improving the aquatic ecosystem of the Loktak Lake.

Keywords: Wetlands, metagenomics, microbial diversity, land use, biogeochemical, keystone, bio-indicators.

1. INTRODUCTION

Wetlands cover about 5-8% of the earth’s land surface and are important as providers of ecosystem services such as initiation of the food web, nutrient (re)cycling capacities and providing habitat for several wildlife species. Increasing anthropogenic activities including land use and climate change have affected the global wetland in terms of vulnerability and threatened their very existence. The role of microbial communities in the ecosystem functioning is unequivocal [1, 2]. They are the key drivers of multiple ecosystem processes, including nutrient cycling of soil, plant growth, marine biogeochemical processes and human health maintenance [3-6]. The microbial communities associated with the sediment also provide important biogeochemical processes in surface water systems [7, 8] and bioremediation of organic contaminants [9]. Given the importance of microbes in the aquatic ecosystem, monitoring the change in microbial community structure and functioning in response to environmental perturbations including anthropogenic activities such as land use pattern is essential for sustainable wetland management practices.

We used an integrative approach including community-level physiological profiling, culture-dependent and independent method for identification of indigenous bacterial communities and functions associated with various land use pattern in the Loktak Lake, the largest freshwater lake in North East India located within the Indo-Burma biodiversity hotspot region. Measurement of microbial community and
gene transcript composition within a diverse ecosystem is key to understanding the mechanisms of adaptation, and functional potential. We hypothesized that the indigenous microbial communities play an important role in sustaining natural resources especially, biogeochemical cycling and reclamation of the various land use areas across seasons. We used metagenomics approach to analyze the microbial communities across various land use patterns, ascertain the role of the isolated microbial community across the seasonal profiles to gain insights into the impact of anthropogenic activities and environmental factors on their composition and function.

2. MATERIALS AND METHODS

2.1. Study Area

Loktak Lake is a unique natural ecosystem designated under the Ramsar Convention (Fig. 1), covering an area of 246.72 km² [10] and located between 93° 46’ - 93° 55’ E and 24° 25’ - 24° 42’ N. The study sites have been described previously by Kangabam et al., 2017 [11, 12]. About 12 towns and 52 settlements are located in and around the Loktak Lake with a population of 2, 20,017 people, i.e. 9% of the total population of the state of Manipur [13]. The lake is unique for its floating island locally known as Phumdis. The lake is one of the 48 wetland sites in the world under the Montreux record of Ramsar. The Keibul Lamjao National Park (KLN) located on the southern part is the world’s only floating national park and last natural habitat of Manipur brow-antlered deer Rucervus eldi eldi locally known by Sangai.

2.2. Sample Collection

The samples were collected during late September of 2017 and early February of 2018 from the various location of the Loktak Lake: Sendra (Loktak 1), Thanga (Loktak 2), Karang (Loktak 3), KLNP (Loktak 4) and Ningthoukhong (Loktak 5). All the sample sites had indirect and direct exposure to anthropogenic activities including, tourist, aquaculture, household waste and hydroelectricity project (Table 1). The soil/sediment samples of ~500 g from the upper 20 cm depth were collected and pooled together from each of the five sites in sterilized polythene bags and the samples were transported to the laboratory in an icebox.

2.3. Physicochemical Analysis of Water

The lake water samples were collected from five locations from the central and southern areas from a depth of 0.5 m during post-monsoon and winter of September 2017 and February 2018. Water samples in triplicates were collected at each sampling site by random sampling. The collected samples were preserved in pre-rinsed 2-L PET (Polyethylene terephthalate) bottles at 4°C in darkness. Each container was clearly labeled with the name and date of sampling. All analysis was done following the standard method [14, 15]. Physicochemical parameters like temperature, pH, Turbidity, Dissolved Oxygen, Total hardness, Calcium, Magnesium, Iron, sulphate, chloride, Alkalinity, Total Dissolved solids, Total suspended solids, Chemical Oxygen demand, Biochemical Oxygen demand and Bacteriological water analysis like total viable count, and most probable number for coliform and E. coli were analyzed using standard procedures.

Fig. (1). Study map and sampling sites in the Loktak Lake. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
2.4. Metagenomic DNA Isolation and Qualitative/Quantitative Analysis

The metagenomic DNA from the soil samples was extracted using the commercial soil extraction kits (Nucleospin Soil) using the manufacturer's instruction. The quality of the isolated genomic DNA samples was checked by loading 1 µl of samples in NanoDrop for determining the A260/280 ratio. The QC pass DNA samples were processed for the first amplicon generation followed by NGS library preparation.

2.5. Preparation of MISEQ Library

The amplicon libraries were prepared using the Nextera XT Index kit (Illumina Inc.) per the 16S metagenomic sequencing library preparation protocol. The primers for the amplification of the 16S rDNA gene-specific for bacterial V3-V4 were designed and synthesized at Eurofins Genomics Laboratory. The forward PCR 16S rDNA primer 5’-GCCTACGGGNGGCWGCAG-3’ and the PCR reverse primer 5’-ACTACHVGGGTATCTAATCC- 3’ were used to amplify the V3 and V4 regions of the 16S rDNA gene [16]. The total volume of PCR mixture was 25 µl containing 2.5 µl of microbial DNA, 5 µl of each primer (1 µM), and 12.5 µl of ReadyMix. The PCR was performed in a thermal cycler program: 95 °C for 3 min followed by 25 cycles at 95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec and a final extension at 72 °C for 5 min with a holding at 4 °C. The mean of the library fragment size distributions ranged between 567 bp to 586 bp for all the five samples. The libraries were sequenced on MiSeq using 2x300 bp. The QC passed amplicons with the adapters were amplicons with the adapters were sequenced using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation. The amplicon libraries were purified by AMPureXP beads and quantified using a Qubit fluorometer.

Further, the quantity and quality of the amplified libraries were analyzed in 4200 Tape Station System (Agilent Technologies) using D1000 Screen tape following the manufacturer’s instructions.

2.6. Cluster Generation and Sequencing

Based on the data obtained from library concentration and the mean of the library fragment size distributions ranging from 567 bp to 586 bp for all the samples, 10 µM of the library was loaded onto Illumina MiSeq (2x300 bp) for cluster generation and sequencing. Paired-end sequencing allows the template fragments to be sequenced in both the forward and reverse directions on MiSeq. The raw sequenced data were processed to obtain the high-quality clean reads using the Trimmomatic (Version 0.35) [17] to remove the adapter sequences, ambiguous reads (reads with unknown nucleotides larger than 5%) and low-quality sequences from the raw data, high-quality sequences from the raw data, high-quality reads were obtained for each sample (Loktak L1, L2, L3, L4 and L5), respectively for both the seasons as shown in Table 2.

2.7. Analysis of Metagenomics Data

For metagenomics data analysis, we used QIIME (version 2.0) pipeline. QIIME is a comprehensive software comprising tools and algorithms to explore phylogenetic inferences and assignment of taxonomic data using naïve Bayesian classifier [18]. After processing the high-quality reads (in FASTQ) into QIIME, the Operational Taxonomic Units (OTUs) were generated and assigned the OTUs to a taxonomic identity using reference databases. Microbial community composition and relative abundance profiles of OTUs of each sample's taxonomic distribution at the phylum level

| Location | Latitude | Longitude | Altitude | Land Use | Description |
|----------|----------|-----------|----------|----------|-------------|
| Loktak 1 | 24° 50’ 74” N | 93° 78’ 47” E | 771 m | Tourist and water sports | Open water area famous among tourist coming to see Loktak lake |
| Loktak 2 | 24° 31’ 86” N | 93° 41’ 102” E | 759 m | Aquacultures and human settlement | Area inside the Loktak lake inhabited by the local people with mostly aquaculture activities |
| Loktak 3 | 24° 53’ 86” N | 93° 83’ 40” E | 768 m | Runoff of water and floating island from the Lake | Area between Thanga Karang (island) and Thanga |
| Loktak 4 | 24° 47’ 71” N | 93° 81’ 38” E | 759 m | National Park | Floating island surrounded by human settlement |
| Loktak 5 | 24° 34’ 34” N | 93° 46’ 43” E | 768 m | Aquaculture and open water area | Aquaculture and water runoff for hydroelectric project |

Table 1. Description of sampling sites across the Loktak Lake with varying land use.
were analyzed. Alpha diversity and rarefaction curves were analyzed to calculate species richness as a function of the number of samples. The co-occurrence network of ecological interaction of microbial distinctive 16S rDNA sequences and seasonal physicochemical parameters were constructed using the CoNet algorithm [19]. The phenotype correlation heatmap of the OTUs was analyzed at the metabolism level using METAGENAssist packages [20].

3. RESULTS

3.1. Surface Water Characteristic

The major physicochemical parameters of the lake water were analyzed and shown in Table 3. The pH of the surface water ranged from 6.54 to 7.81, while the temperature of the surface water was in the range of 20 °C to 28 °C. The highest value of pH was observed in Loktak 1 which is similar to our earlier finding [11]. Dissolved Oxygen values ranged from 4.09 mg/l to 11.98 mg/l. The highest concentration was recorded at Loktak 1 during winter and lowest at Loktak 2 during post-monsoon. The low DO value indicates a slow rate of photosynthesis by the phytoplankton present in the Loktak Lake and overall poor water quality. Turbidity was found to vary across season and sampling locations. The level of turbidity was more in winter with Loktak 4 and Loktak 5 recording 2 NTU which is beyond the permissible limit of IS:10500 of 2012. The highest and lowest concentrations of Iron were recorded at Loktak 4 (3.8 mg/l) and Loktak 1 (0.015 mg/l).

Total hardness values ranged from 35 mg/l to 60 mg/l with Loktak 3 and Loktak 1 recording the highest and lowest respectively. The concentration of sulphate was low with less than 0.5 mg/l in all the locations during post-monsoon. Chloride concentration ranged from 5.8 mg/l to 14 mg/l while TDS ranged from 43.5 mg/l to 64.5 mg/l. The TSS ranged between 50 mg/l and 285 mg/l with the lowest recorded in Loktak 1 and highest at Loktak 5. Phenolphthalein Alkalinity (PA) was not detected in any of the samples collected, while Methyl Orange alkalinity (MA) was tested positive in all the collected samples with values ranging from 48 mg/l to 51.2 mg/l. The difference between these two alkalinity tests is that PA determines alkalinity of all hydroxyl and half of carbonate, while the MA determines alkalinity of all hydroxyl, carbonate, and bicarbonates. The BOD in the Loktak ranged from 83 mg/l to 92 mg/l, while COD of the same lake ranged between 5 mg/l to 239 mg/l. The higher concentration of COD indicates a greater amount of oxidizable organic material, which will reduce the dissolved oxygen level. Further, the bacteriological analysis showed the presence of Coliform in all the samples beyond the permissible limit of the BIS range of not detectable in any of 100 ml of sample. This indicates the contamination of fecal materials in the lake water. The total viable count values ranged from 50 ml to 200 ml in all the five locations. The most probable number of coliforms and E. coli was also found to be present in all the locations. The values of MPN coliform ranged from 20 to 110 per 100 ml, while the MPN E. coli were found to be in the range from 5 to 20 per 100 ml of water.

3.2. Metagenome

Metagenomic sequencing of free-living bacterial communities collected seasonally (post-monsoon and winter) from five locations under different land use patterns was performed with 10 samples collected from different sites viz., tourist spots, aquaculture activity, human habitat, national park, and water runoff for hydroelectric project (Table 1). Loktak 5- the sites with aquaculture and runoff and Loktak 4- the Keibul Lamjao National Park has the highest concentrations of nutrients and were the most distinct in terms of water chemistry during post-monsoon and winter while Loktak 1- the tourist and water sports area and Loktak 2-aquaculture and human settlement had the lowest concentrations of nutrients during post-monsoon and winter. The bacteriological analysis for both the seasons identified a higher concentration of harmful microbes at Loktak 5 in both the seasons, while lowest concentration was detected at Loktak 2 and Loktak 3 during winter and post-monsoon. The sequenced reads met the QC criteria for analysis. The percentages of the archaeal sequences in Amplicon 16S sequencing were low in all the sites.

3.3. Microbial Community Composition

Bacteria and Archaea were the dominant species detected across the various land use areas in the Loktak Lake. Out of 955 phyla detected, 928 were bacteria. The domain bacteria dominated the microbial composition in the lake samples (Fig. 2). Among the bacteria, Proteobacteria was the most abundant occurring phylum accounting for 32.86 % of the total bacterial population followed by Actinobacteria (13.03 %) and Firmicutes (9.91 %) during the post-monsoon. Both Proteobacteria and Acidobacteria accounted for 45.89 % of the total microbial abundance during the
post-monsoon. Deltaproteobacteria, Betaproteobacteria and Gammaproteobacteria accounting for 4.2%, 4.55%, and 4.02%, respectively were the major classes belonging to the phylum Proteobacteria (Supplementary Table 1). A comparison of the seasonal profile of Acidobacteria and Actinobacteria population revealed an increased by 0.66% and 2.37% during winter (Supplementary Table 2), while Proteobacteria decreased by 1.91% during the same period.

Acidobacteria belonging to Acidobacteria is the major class contributing only 1.44% of the total class, remaining microbes represent only one class, and the majority of the class belongs to the unclassified group. Archaea have been shown to be ubiquitous among the microbial communities coexisting with other microorganisms in a niche environment. The dominant Archaea are Euryarchaeota (66.66%) followed by Crenarchaeota (25.92%) and Parvarchaeota (7.4%) during post-monsoon and winter, respectively. The Euryarchaeota diversity decreased during winter compared with post-monsoon by 16.66%, while there was a slight increase in the population of Crenarchaeota (14.08%) and Parvarchaeota (2.6%), respectively. The four major classes belonging to the phylum Proteobacteria detected in the Loktak Lake were Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria.

The Archaea, Crenarchaeota, Euryarchaeota, and Parvarchaeotawere are the distinct lineages detected in the Loktak
Lake. Parvarchaeota is known for their physical interaction with thermoplasmatales, and like acidophiles, growing optimally in acid mine drainage and hot springs habitats [21]. Euryarchaeota is the physiologically diverse group, which includes halophiles, thermophiles, and methanogens, while Crenarchaeota consists of sulfur dependent hyperthermophiles. Methanosaetaps, belonging to Euryarchaeota was detected in all the locations at high frequency in Loktak 2. They play a key role in controlling the methane emission from the wetland [22].

![Relative abundance (%) vs Post monsoon vs Winter](image)

**Fig. (2).** Bacterial community taxonomic structure at phylum level in relation to physicochemical variables. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

In winter, Acidobacteria was the most dominant bacteria phyla with 12.62 % followed by Chloroflexi (8.08 %) and Actinobacteria (4.04 %), respectively (Supplementary Table 1).

### 3.4. Co-Occurrence of Microbial Networks Across Seasonal Variation

The microbial ecological interaction inference was obtained from patterns of occurrence of distinctive 16S rDNA sequences and seasonal physicochemical parameters. The networks of co-occurrence were constructed using the CoNet algorithm, as described earlier [19, 23]. The network of OTUs from the post-monsoon (Fig. 3) showed 1564 nodes (1550 OTUs and 14 variables) connected by 12381 edges (6626 co-presence and 5755 mutual exclusions), while for the winter (Fig. 4), the network contained 701 nodes (686 OTUs and 15 variables) and 5937 edges (3443 co-presence and 2494 exclusions).

Network attributes like the clustering coefficient or the degree, to which graph tends to cluster as the path length and the shortest path were found to be preserved, while other parameters were observed to change remarkably (Supplementary Table 3). It was observed that the network density and heterogeneity alter across the season; the post-monsoon network shows more average connections and a higher density of hub nodes compared to the winter season network.

![Network diagram](image)

Among the water variables used in the network constructions, monsoon data indicated that temperature (42 nodes with 821 edges) and Mg (34 nodes with 720 edges) had the maximum number of directly connected OTUs. The BOD and COD were found to be directly connected by 14 OTUs nodes and 125 edges, in which 10 negative edges and 4 positive edges were connected to BOD and COD, while other parameters like pH, temperature (T), manganese (Mg), total hardness (TH), iron (I), TDS, Sulphur (S), dissolved oxygen (DO), calcium (Ca) and chlorine (Cl) were found as individual clusters (Supplementary dataset Table 4).

The co-occurrence network was constructed from the data of winter; temperature showed maximum OTUs connected (43 nodes with 250 edges). The two cluster networks were found in which salinity (S) and chloride (Cl), TSS and I were directly connected forming 2 cluster networks. S and Cl network consists of total 6 nodes with 15 edges (3 negative edges and 3 positive edges), while TSS and I have 21 nodes with 190 edges (11 negative edges and 9 positive edges) (Supplementary dataset Table 5). Other water parameters formed individual cluster networks as shown in Figs. (3 and 4).

Taxonomic and phylogenetic relationships between the networks were visualized at the phylum level because the proportion of unclassified bacteria was high. Heat map analysis was carried out to compare the taxonomic composition at metabolism level and identified as being positively or negatively correlated with each other (Fig. 5A and 5B). A positive correlation is indicated by red, while a negative correlation is represented by green color. It was found that the microbes in the Loktak Lake play a number of important and diverse roles like fixation of carbon, nitrogen, degradation of naphthalene, hydrocarbon, xylan, biomass, pollutant, reducer of nitrite, sulfur, iron, and production of streptomycin, gramicidin, etc. The major functions of the microbes are similar across the season with variation in correlation. Selenate reducer was found only in the winter season, which can reduce selenate to elemental selenium [24].

The species richness was calculated to analyze a known number of individual samples based on the rarefaction curves. The curves reflect the average number of various species annotations for subsamples of the complete dataset (Fig. 6A and 6B). The curve is a plot of the total number of distinct species annotations as a function of the number of sequences sampled. The steep slope on the left indicates the presence of a large fraction of species diversity, which needs to be discovered. The vertical axis displays the diversity of the community, while the horizontal axis displays the number of sequences considered in the diversity calculation. In the post-monsoon season, Loktak 5 had the highest observed OTUs followed by Loktak 3 and Loktak 4, respectively. However, in the winter season, Loktak 2 recorded the highest OTUs followed by Loktak 1 and Loktak 3, respectively. It was found that the observed OTUs and sequence per sample were much higher during post-monsoon compared to the winter season.

### 3.5. Keystone Taxa

Microbial keystone taxa are highly connected taxa, which influence the assembly and microbial community through
Fig. (3). Co-occurrence microbial interaction sub-network from Post-monsoon season. Blue squared nodes correspond to physicochemical parameters and circle notes correspond to OTUs. The green and red edges represent positive correlation and negative correlation respectively. Physicochemical parameters represent as pH; power of hydrogen, BOD; Biological Oxygen Demand, Ca; Calcium, COD; Chemical Oxygen Demand, Cl; Chlorine, DO; Dissolved oxygen, MoA; Methyl Orange Alkalinity, Mg; Magnesium, SA; Salinity, TDS; Total Dissolved Solids, TH; Total Hardness, Tm; Temperature, TSS; Total Suspended Solids. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Fig. (4). Co-occurrence microbial interaction sub-network from winter season. Blue squared nodes correspond to physicochemical parameters and circle notes correspond to OTUs. The green and red edges represent positive correlation and negative correlation respectively. Physicochemical parameters represent as pH; power of hydrogen, BOD; Biological Oxygen Demand, Ca; Calcium, COD; Chemical Oxygen Demand, Cl; Chlorine, DO; Dissolved oxygen, MoA; Methyl Orange Alkalinity, Mg; Magnesium, SA; Salinity, TDS; Total Dissolved Solids, TH; Total Hardness, Tm; Temperature, TSS; Total Suspended Solids. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
biotic interactions thereby helping in microbial community composition. The population of keystone taxa is a small subset of highly connected keystone taxa of 1-5 % richness, which can be the optimal predictors of whole community compositional changes [25]. It was observed that Loktak Lake harbors several important keystone taxa found in the different ecosystems or habitats like grasslands, forest, agricultural lands, arctic and Antarctic, contaminated soil, plant-associated, aquatic ecosystems, human gut microbiome and human oral microbiome.

Loktak Lake has the maximum number of keystone taxa, including the Burkholderiales, Sphingobacteriales, Clostridiales, Actinomycetales, and Acidobacteria, etc. (Table 4). The presence of numerous keystone taxa in the Loktak Lake may help in engaging the microbes in synergistic relationships thereby effecting the community and structure performance. In order to achieve this, keystone taxa adopt various strategies to nurture the microbiota in their favor, while the selection of a particular strategy would depend on the surrounding microenvironment [6]. There is a need for a detailed study of these keystone taxa to identify the important role they play in Loktak Lake ecosystems functioning.
4. DISCUSSION

The study of the water quality of the Loktak Lake indicated a poor status of the lake water in terms of pollution and bacterial contamination. The concentration of iron was found to be beyond the permissible limit in all the locations in post-monsoon while in winter, the concentration of iron in Loktak 4 and Loktak 5 was beyond the permissible limit of 0.3 mg/l. An increase in anthropogenic influences including discharges of municipal waste was brought down by important rivers like Nambul and Nambol. Rivers may be the primary reason for the poor water quality [11, 12] which might have also led to the change in the color of the water to brown as the higher concentration of iron binds with environmental toxins like lead and arsenic [61, 62]. The bacteriological study of the water identified the presence of harmful microbes including Coliform and *E. coli* beyond the permissible limit. The values of MPN coliform ranged from 20 to 110 per 100 ml, while the MPN *E. coli* were found to be in the range from 5 to 20 per 100 ml of water. This value is more than the prescribed guidelines of IS 10500, which stipulates that the population of *E. coli* and coliforms should not be more than 10 CFU. The presence of coliforms and other pollutants indicate poor quality of the Lake and may have serious health issues for human and the aquatic fauna [63].

Our results show Proteobacteria to be the most abundant in the lake during the post-monsoon. Several studies have reported that microbial composition is influenced by environmental factors [64]. The diversity and abundance of microbes vary across seasons and the most favorable season for microbes are wet and warm climates which are associated with high bacterial abundance and diversity while cold and dry seasons resulted in low abundance and diversity [65]. The local microbial communities within the metacommunity were connected through multiple potentially interacting species. Interaction at the local and regional level determines how the microbial community assembles [66, 67]. The analysis of beta diversity revealed the dominant communities to be similar between Loktak 1 and Loktak 2 as well as Loktak 3 and Loktak 4. The bacterial composition is known to be influenced more by season rather than by the location of the site [68, 69], although our study reveals Loktak 5 site to have a different composition. This may be due to the fact that the sampling site lies downstream of a less populated area. Previous studies have reported the predominance of Proteobacteria in freshwater bodies [70] and mangrove sediments [71].

The detection of *Acinetobacter* sp. and *Pseudomonas* sp., in the present study, may have been contributed by the municipal waste, agricultural runoff, and aquaculture activities in and around the lake. Both the detected species are known to be pathogenic to humans and animals. *Acinetobacter* sp. and *Pseudomonas* sp. are known as antibiotic-resistant in aquatic environments [70, 72]. The family of Thermodesulfovibrio plays an important function in the oxidation of hydrogen and other organic matter through the reduction of sulfate in their original ecosystems [73].

Although, the genome of Parvarchaeota is small (0.64-1.08 Mb), they are reported to play an important role in carbon and nitrogen cycling by degrading multiple saccharides and proteins and produce ATP via aerobic respiration and fermentation. Although, Parvarchaeota lack biosynthetic pathways for amino acids and nucleotides, they play an important role in iron cycling by scavenging the biomolecules.

| Ecosystem or Habitat          | Keystone Taxa                                                                 | Refs.          |
|-------------------------------|-----------------------------------------------------------------------------|----------------|
| **Computational Inference**   |                                                                             |                |
| Grasslands                    | Burkholderiales, Sphingobacteriales, Clostridiales, Actinomycetales, Acidobacteria | [26-28]        |
| Forest or woodlands           | Actinomycetales, Acidobacteria, Rhizobiales, Burkholderiales, Clostridiales, Sphingobacteriales; Rhodobacteriales, Verrucomicrobia | [27, 29-32]    |
| Agricultural lands            | Gemmatimonas, Acidobacteria, Xanthomonadales, Rhizobiales, Burkholderiales, Solirubrobacterales, Verrucomicrobia | [27, 33-35]    |
| Arctic and Antarctic ecosystems| Rhizobiales, Burkholderiales, Actinobacteria, Alphaproteobacteria             | [36-39]        |
| Contaminated soil             | Rhizobiales, Nitrospira, Pseudomonadales, Actinobacteria                      | [40, 41]       |
| Plant-associated microbiota   | Acidobacteria, Rhizobiales, Burkholderiales, Pseudomonadales, Bacteroidetes, Frankiales | [33, 42, 43]    |
| Aquatic ecosystems            | Pelagibacter, Oceanospirillales, Flavobacteriaceae, Nitrospira, Alteromonadaeae, Chromatium, Rhizobiales, Burkholderiales, Verrucomicrobia, Chloroflexi, Candidatus | [44-50]        |
| **Empirical Evidence**        |                                                                             |                |
| Agricultural lands            | Gemmatimonas, Acidobacteria                                                  | [29, 51]       |
| Human oral microbiome         | Porphyromonas                                                                | [52]           |
| Human gut microbiome          | Helicobacter pylori, Actinobacteria, Bacteroides fragilis, Bacteroides stercoris, Bacteroides thetaiotaomicron, Ruminococcus bromii, Klebsiella pneumoniae | [53-60]        |
from the environment or other community members [21]. Crenothrix belonging to the family Crenothrickaceae was detected in all the locations of the lake. Although Crenothrix bacteria have been known as contaminants of drinking water supplies, they play an important role in oxidizing methane [74] and can act as a relevant biological sink for methane in stratified lakes. A strain of Bacillus flexus is reported as arsenic transformers which oxidizes As (III) to As (V) [75]. The S. wittichii bacterium is Gram-negative, rod-shaped, monotrichous, and asporogenous first isolated from the Elbe River in Germany. It was noted for its ability to degrade dioxins, chemicals that are produced as byproducts in industrial processes [76]. Although several bacteria with important functional properties were detected in the lake, many bacteria with harmful properties also exist in the lake including Sphingobacterium multivorum known to cause bacteremia and acute meningitis in human [77], Helicobacter pullorum causing gastroenteritis [78], Leptospira, causing Leptospirosis that affects humans and animals [79]. Staphylococcus associated with nosocomial and health-care related infections [80]. Further, a number of pathogenic bacteria belonging to Bacillus, Bacteroides, Brucella, Campylobacter, Chlamydia, Clostridium, Helicobacter, Klebsiella, Legionella, Leptospira, Listeria, Mycobacterium, Mycoplasma, Neisseria, Pseudomonas, Nocardia, Rickettsia, Staphylococcus, Treponema were detected in our studies.

We constructed the co-occurrence network representing the seasonal variations that grouped sites with water quality as high or low: post-monsoon and winter. The network analysis was carried out to study the changes in the ecological interactions across the land use among the microbial OTUs as well as their association with the physicochemical and nutritional variables of the water. The analysis of the seasonal co-occurrence microbial networks of post-monsoon and winter showed variation in some of the parameters measured like connectivity and network attributes, as both followed the Erdos-Renyi degree of distribution [31].

The seasonal network of the most connected OTUs (10 % higher centrality values), which are used as keystone components for network stability [81] belonged to the phyla Proteobacteria, Acidobacteria and Actinobacteria. These hub nodes were dominant in abundance, indicating that the central and abundant nodes belong to the dominant genera. These are critical for network dynamics and stability and have a pivotal role in network dynamic properties.

CONCLUSION

Our study provides an insight into the seasonal profile of bacterial metagenomes across different land uses patterns in Loktak Lake. Different members of the microbial meta-community were associated with multiple physicochemical activities during the post-monsoon and winter season in the Loktak Lake. The individual physicochemical parameters were directly associated with the microbial diversity, which correlated both positively and negatively with the changes in the values of individual parameters. Among the highly connected taxa in networks were the OTUs of Proteobacteria, Acidobacteria and Actinobacteria. Phylogenetically related bacterial OTUs correlated with a similar set of bacterial and microbial OTUs. The positive correlations between the bacterial OTUs with the physicochemical parameter point to a mutualistic interaction whereas the negative correlations may indicate competition. When faced with environmental perturbations, some of the important OTUs replace the interactive roles played by other OTUs in the bacterial network. The resilience and adaptation of microbial communities are modulated by the change in water quality and land use patterns in the Loktak Lake.

LIST OF ABBREVIATIONS

| Abbreviation | Description                  |
|--------------|------------------------------|
| APHA         | American Public Health Association |
| BIS          | Bureau of Indian Standards    |
| BOD          | Biochemical Oxygen Demand     |
| C            | Carbon                        |
| Ca           | Calcium                       |
| Cl           | Chlorine                      |
| CFU          | Colony-Forming Unit           |
| COD          | Chemical Oxygen Demand        |
| CPCB         | Central Pollution Control Board |
| DO           | Dissolved Oxygen              |
| DNA          | Deoxyribonucleic Acid         |
| I            | Iron                          |
| KLNK         | Keibul Lamjao National Park   |
| MA           | Methyl Orange Alkalinity      |
| Mb           | Megabyte                      |
| Mg           | Manganese                     |
| mL           | Milliliter                    |
| MPN          | Most Probable Number          |
| NGS          | Next-Generation Sequencing    |
| NTU          | Nephelometric Turbidity Unit  |
| OUT          | Operational Taxonomic Unit    |
| PA           | Phenolphthalein Alkalinity    |
| PET          | Polyethylene Terephthalate    |
| pH           | Hydrogen Ion Concentration    |
| PCR          | Polymerase Chain Reaction     |
| rDNA         | Recombinant DNA               |
| S            | Sulphur                       |
| QC           | Quality Control               |
| T            | Temperature                   |
| TH           | Total Hardness                |
| TDS          | Total Dissolved Solids        |
| TSS          | Total Suspended Solids        |

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.
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**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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**SUPPLEMENTARY MATERIAL**

Supplementary material is available on the publisher’s website along with the published article.

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