Send Orders for Reprints to reprints@benthamscience.net

512 *Current Genomics,* **2020,** *21,* **512-524**

RESEARCH ARTICLE

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

Rajiv Das Kangabam[1], Yumnam Silla[2], Gunajit Goswami[1,3] and Madhumita Barooah[1,*]

[1]*Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat-785013, India;* [2]*Advanced Computational and Data Sciences Division, CSIR- North East Institute of Science and Technology, Jorhat-785006, India;* [3]*DBT North East Centre for Agricultural Biotechnology, Assam Agricultural University, Jorhat-785013, India*

ARTICLE HISTORY

Received: January 10, 2020
Revised: April 28, 2020
Accepted: May 30, 2020

DOI:
10.2174/1389202921999200716104355

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

*Address correspondence to this author at the Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat 785013, India;
E-mail: m.barooah@aau.ac.in

1389-2029/20 $65.00+.00

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 93^0 46' - 93^0 55' E and 24^0 25' - 24^0 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 (KLNP) located on the southern part is the world's only floating national park and last natural habitat of Manipur brow-antlered deer *Rucervus eldii eldii* 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.

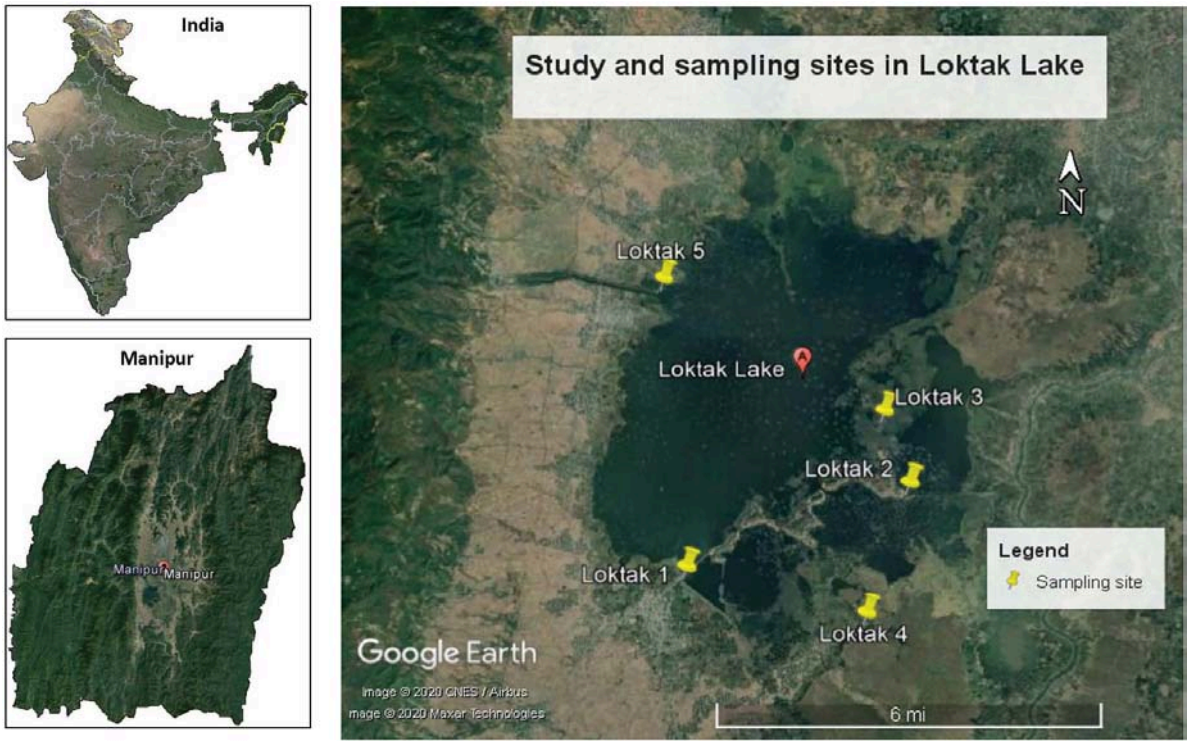

Fig. (1). Study map and sampling sites in the Loktak Lake. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Description of sampling sites across the Loktak Lake with varying land use.

Location	Latitude	Longitude	Altitude	Land Use	Description
Loktak 1	24^0 50' 74'' N	93^0 78' 47'' E	771 m	Tourist and water sports	Open water area famous among the tourist coming to see Loktak lake
Loktak 2	24^0 31' 86'' N	93^0 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	24^0 53' 86'' N	93^0 83' 40'' E	768 m	Runoff of water and floating island from the Lake	Area between Thanga Karang (island) and Thanga
Loktak 4	24^0 47' 71'' N	93^0 81' 38'' E	759 m	National Park	Floating island surrounded by human settlement
Loktak 5	24^0 34' 34'' N	93^0 46' 43'' E	768 m	Aquaculture and open water area	Aquaculture and water runoff for hydroelectric project

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 loading1 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 amplified 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 pM 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 (reads with more than 10% quality threshold < 20 phed score) from FASTQ data. A minimum length of 100 nucleotides after trimming was applied. After removing the adapter and low-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

Table 2. Details of quality of reads, GC content and observed OTUs of each sample from Post- monsoon and winter data.

Sample Name	Post-monsoon			Winter		
	Total No. of Reads	GC Content (%)	Observed OTUs	Total No. of Reads	GC Content (%)	Observed OTUs
Loktak L1	775,011	57	5377	256,843	56	4048
Loktak L2	540,600	57	3656	258,267	56	4263
Loktak L3	672,324	57	5995	230,764	56	3734
Loktak L4	615,990	57	5945	314,527	58	2636
Loktak L5	458,397	57	6250	265,306	58	2207

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 sequences that passed the QC criteria were used for the 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

Table 3. Summary of environmental variables of sampling: Means and standard deviations.

	Post Monsoon					Winter				
Measured Variables	**Loktak 1**	**Loktak 2**	**Loktak 3**	**Loktak 4**	**Loktak 5**	**Loktak 1**	**Loktak 2**	**Loktak 3**	**Loktak 4**	**Loktak 5**
pH	6.54±0.02	6.82±0.01	6.75±0.02	6.83±0.02	6.73±0.01	7.81±0.12	7.3±0.1	7.27±0.1	6.55±0.1	7.03±0.13
Temperature	28±0.00	27.4±0.01	27.3±0.13	26.3±0.01	27.2±0.11	21.2±0.13	22.8±0.1	21.5±0.13	20.4±0.06	20.7±0.05
Salinity	0.018±0.00	0.012±0.02	0.025±0.016	0.018±0.12	0.012±0.06	0.014±0.024	0.01±0.012	0.01±0.02	0.01±0.0	0.01±0.0
Turbidity	ND	ND	ND	ND	1±0.1	ND	ND	ND	2±0.02	2±0.04
Total Hardness	35±0.32	50±0.22	60±0.26	55±0.18	50±0.32	50±0.24	55±0.34	50±0.26	45±0.22	45±0.18
Calcium	0.4±0.02	0.8±0.01	0.8±0.02	0.4±0.02	0.6±0.04	0.4±0.02	0.8±0.02	0.8±0.018	0.4±0.06	0.6±0.04
Magnesium	0.6±0.03	0.7±0.12	0.9±0.16	1.1±0.04	0.8±0.13	0.9±0.06	0.85±0.12	0.7±0.21	0.85±0.04	0.7±0.02
Iron	1.6±0.06	0.5±0.2	0.68±0.04	0.5±0.03	1.1±0.10	0.015±0.01	0.077±0.021	0.087±0.04	3.8±1.2	0.6±0.3
Sulphate	< 0.5±0.0	< 0.5±0.0	< 0.5±0.0	< 0.5±0.0	< 0.5±0.0	ND	ND	ND	ND	< 5.0±0.0
Chloride	10.4±2.4	7±1.2	14±4.5	10.4±3.6	7±2.8	7.81±2.6	5.6±1.24	6.8±1.6	7.28±2.8	5.8±3.2
Phenolphthelain Alkalinity	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Methyl orange Alkalinity	35.2±4.68	48±6.0	41.6±4.6	51.2±.4.2	4.59±3.3	38.5±0.22	46.7±0.36	49.5±0.48	35.7±6.32	41.2±0.36
Dissolved Oxygen	4.46±0.24	4.09±2.2	4.45±1.67	4.19±2.32	4.55±1.68	11.98±2.54	6.99±3.16	8.04±2.12	6.89±1.86	5.99±3.2
Total dissolved solid	43.5±4.5	55.4±3.8	64.9±6.2	53.5±5.2	47.7±4.8	44.7±4.6	57.3±3.8	61.8±4.16	44.5±4.32	55±5.16
Total suspended solid	125±12.4	124±22.2	117±12,43	164±20.34	121±9.76	50±8.32	115±8.46	132±6.68	270±15.2	285±14.68
COD	147.5±60.10	200±11.31	210±14.14	146.5±54.44	239±12.72	189±12.72	183.5±12.02	205±21.21	178±14.14	224±19.79
BOD	84±6.46	85±5.12	88±6.86	83±4.28	89±7.82	87±8.46	92±6.74	91±8.68	87±5.68	90±7.68
Total Count	200 +	100+	100+	200+	200+	80+	50+	50+	80+	110+
MPN coliform	25±0.24	30±0.26	25±0.32	20±0.14	35±0.46	60±0.54	20±0.28	30±0.42	40±0.22	110±0.38
MPN *E. coli*	8±0.12	15±0.24	10±0.28	8±0.18	20±0.36	5±0.04	6±0.34	5±0.56	4±0.18	7±0.46

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. Parvarchaeotais is known for their physical interaction with thermoplasmatales, and likeacidophiles, growing optimally at pH below 2. Parvarchaeota were reported to be only detected in acid mine drainage and hot springs habitats [21]. Euryarchaeota is the physiologically diverse group, which includes halophiles, thermophiles, and methagogens, while Crenarchaeota consists of sulfur dependent hyperthermophiles. Methanosaetasps, 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].

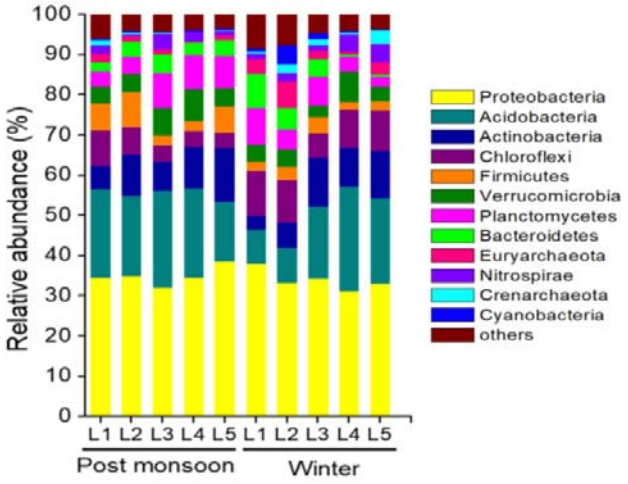

Fig. (2). Bacterial community taxonomic structure at phylum level in relation to physicochemical variables. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

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.

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

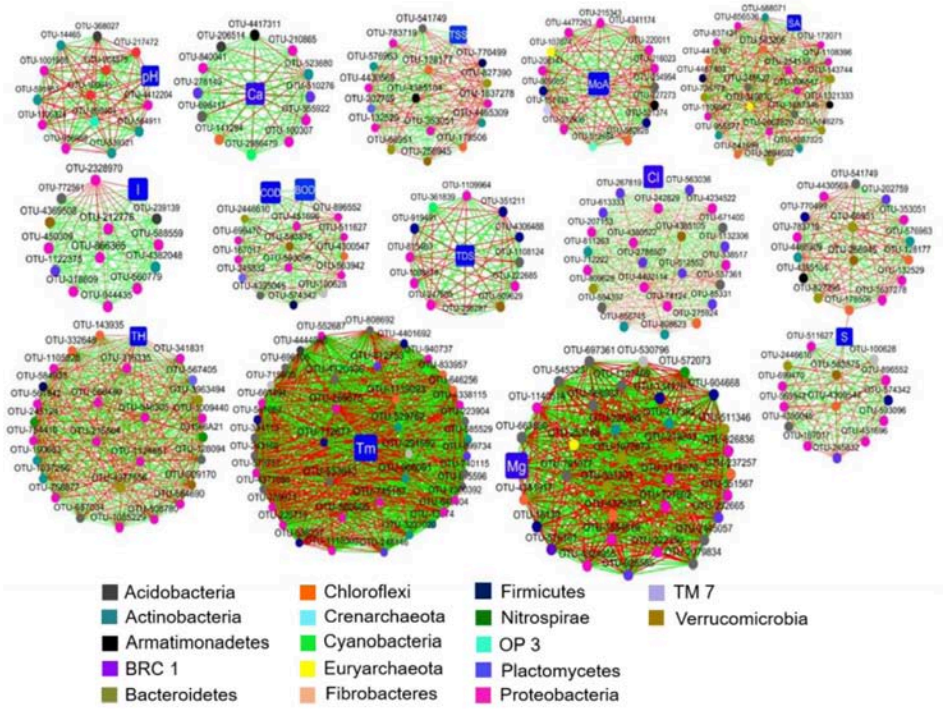

Fig. (3). Co-occurrence microbial interaction sub-network from Post-monsoon season. Blue squared nodes correspond to physicochemical parameters and circle notes correspond to OTUs. The green and red edges represent positive correlation and negative correlation respectively. Physicochemical parameters 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*).

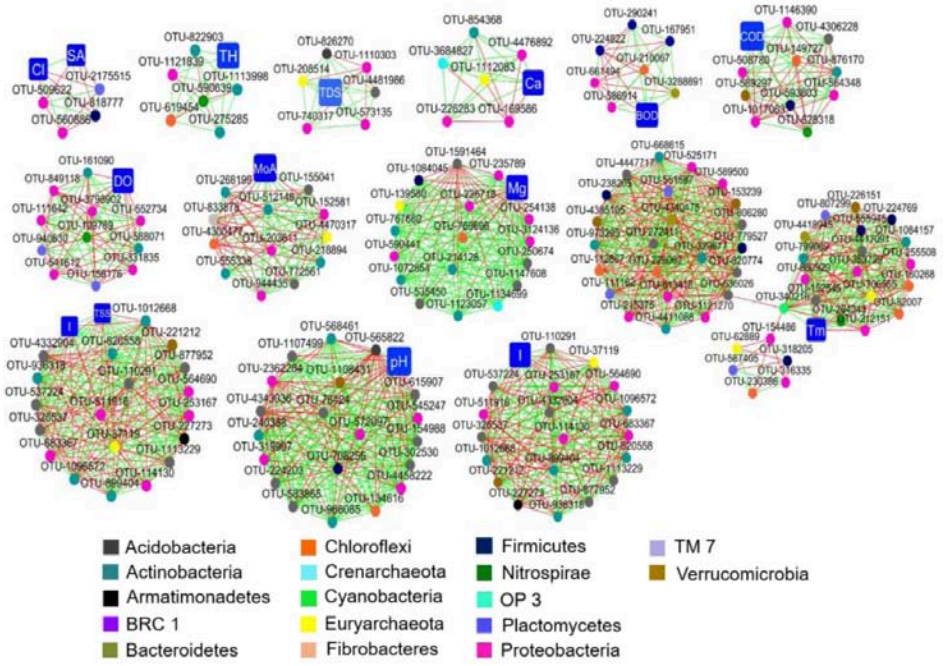

Fig. (4). Co-occurrence microbial interaction sub-network from winter season. Blue squared nodes correspond to physicochemical parameters and circle notes correspond to OTUs. The green and red edges represent positive correlation and negative correlation respectively. Physicochemical parameters represent 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*).

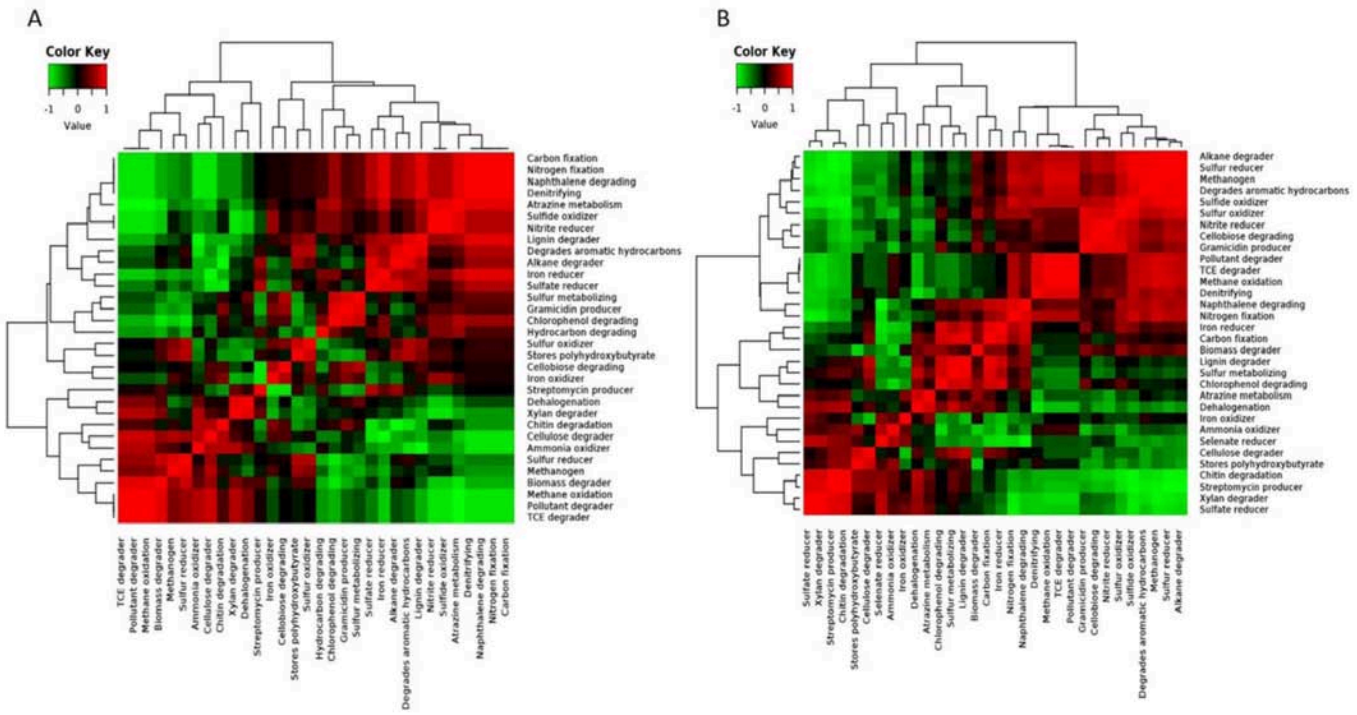

Fig. (5). Heat-map of microbial community composition with cluster analysis. **A**, post-monsoon, **B**, winter. The color variation in each panel shows the percentage in a sample, referring to color key at the top. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

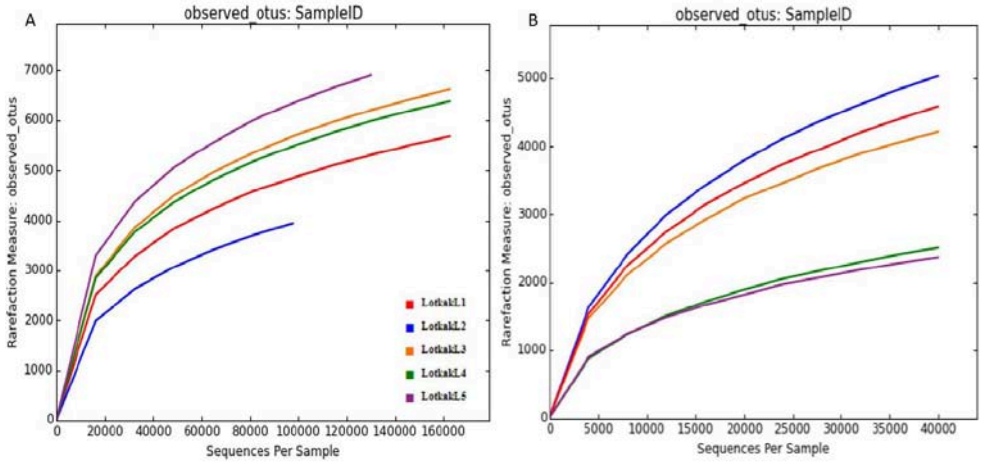

Fig. (6). Species accumulation and rarefaction curves. **A**, post-monsoon, **B**, winter. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

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 predicators 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.

Table 4. Summary of keystone taxa detected in Loktak Lake.

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; Rhodobacterales, 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, Alteromonadaceae, 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]

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

from the environment or other community members [21]. Crenothrix belonging to the family Crenotrichaceae 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 metacommunity 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 perturbances, 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

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
KLNP	=	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.

FUNDING

This work was supported by the Science and Engineering Research Board (SERB) through National Post-Doctoral Fellowship to the first author under the NPDF, PDF/2016/002849.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

We would like to thank Eurofins private Limited, Bangalore for the metagenomic sequencing.

SUPPLEMENTARY MATERIAL

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

REFERENCES

[1] Bardgett, R.D.; van der Putten, W.H. Belowground biodiversity and ecosystem functioning. *Nature,* **2014**, *515*(7528), 505-511. http: //dx.doi.org/10.1038/nature13855 PMID: 25428498

[2] Fierer, N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.,* **2017**, *15*(10), 579-590. http: //dx.doi.org/10.1038/nrmicro.2017.87 PMID: 28824177

[3] Fuhrman, J.A. Microbial community structure and its functional implications. *Nature,* **2009**, *459*(7244), 193-199. http: //dx.doi.org/10.1038/nature08058 PMID: 19444205

[4] Graham, E.B.; Knelman, J.E.; Schindlbacher, A.; Siciliano, S.; Breulmann, M.; Yannarell, A.; Beman, J.M.; Abell, G.; Philippot, L.; Prosser, J.; Foulquier, A.; Yuste, J.C.; Glanville, H.C.; Jones, D.L.; Angel, R.; Salminen, J.; Newton, R.J.; Bürgmann, H.; Ingram, L.J.; Hamer, U.; Siljanen, H.M.P.; Peltoniemi, K.; Potthast, K.; Bañeras, L.; Hartmann, M.; Banerjee, S.; Yu, R.-Q.; Nogaro, G.; Richter, A.; Koranda, M.; Castle, S.C.; Goberna, M.; Song, B.; Chatterjee, A.; Nunes, O.C.; Lopes, A.R.; Cao, Y.; Kaisermann, A.; Hallin, S.; Strickland, M.S.; Garcia-Pausas, J.; Barba, J.; Kang, H.; Isobe, K.; Papaspyrou, S.; Pastorelli, R.; Lagomarsino, A.; Lindström, E.S.; Basiliko, N.; Nemergut, D.R. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Front. Microbiol.,* **2016**, *7*, 214. http: //dx.doi.org/10.3389/fmicb.2016.00214 PMID: 26941732

[5] Hamady, M.; Knight, R. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome Res.,* **2009**, *19*(7), 1141-1152. http: //dx.doi.org/10.1101/gr.085464.108 PMID: 19383763

[6] MG. van der H.; RD, B.; NM, van S. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.,* **2008**, *11*(3), 296-310. http: //dx.doi.org/10.1111/J.1461-0248.2007.01139.X

[7] Petersen, D.G.; Blazewicz, S.J.; Firestone, M.; Herman, D.J.; Turetsky, M.; Waldrop, M. Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. *Environ. Microbiol.,* **2012**, *14*(4), 993-1008. http://dx.doi.org/10.1111/j.1462-2920.2011.02679.x PMID: 22225623

[8] Reese, B.K.; Mills, H.J.; Dowd, S.E.; Morse, J.W. Linking molecular microbial ecology to geochemistry in a coastal hypoxic zone. *Geomicrobiol. J.,* **2013**, *30*(2), 160-172. http: //dx.doi.org/10.1080/01490451.2012.659331

[9] El-Sheekh, M.M.; Hamouda, R.A. Biodegradation of crude oil by some Cyanobacteria under heterotrophic conditions. Desalin. *Water Treat.,* **2014**, *52*(7-9), 1448-1454. http: //dx.doi.org/10.1080/19443994.2013.794008

[10] National Wetland Atlas. SAC/EPSA/ABHG/NWIA/ATLAS/34/2011, **2011**.

[11] Kangabam, R.D.; Bhoominathan, S.D.; Kanagaraj, S.; Govindaraju, M. Development of a Water Quality Index (WQI) for the Loktak Lake in India. *Appl. Water Sci.,* **2017**, *7*(6), 2907-2918. http: //dx.doi.org/10.1007/s13201-017-0579-4

[12] Das Kangabam, R.; Govindaraju, M. Anthropogenic activity-induced water quality degradation in the Loktak lake, a Ramsar site in the Indo-Burma biodiversity hotspot. *Environ. Technol.,* **2019**, *40*(17), 2232-2241. http://dx.doi.org/10.1080/09593330.2017.1378267 PMID: 28893154

[13] Census of India. **2011**. Available from: http: //www.censusindia.gov.in/

[14] American Public Health Association. *Eaton, A. D. W. American Water Works Association. Standard Methods for the Examination of Water and Wastewater,* 21st ed; APHA-AWWA-WEF: Washington, D.C., **2005**.

[15] Water and Wastewater Analysis - A Guide Manual by Central Pollution Control Board, **2011**. https://www.indiawaterportal.org/articles/water-and-wastewater-analysis-guide-manual-central-pollution-control-board

[16] Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F.O. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.,* **2013**, *41*(1), e1. http: //dx.doi.org/10.1093/nar/gks808 PMID: 22933715

[17] Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* **2014**, *30*(15), 2114-2120. http: //dx.doi.org/10.1093/bioinformatics/btu170 PMID: 24695404

[18] Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.,* **2007**, *73*(16), 5261-5267. http: //dx.doi.org/10.1128/AEM.00062-07 PMID: 17586664

[19] Faust, K.; Raes, J. CoNet app: inference of biological association networks using Cytoscape. *F1000 Res.,* **2016**, *5*, 1519. http: //dx.doi.org/10.12688/f1000research.9050.1 PMID: 27853510

[20] Arndt, D.; Xia, J.; Liu, Y.; Zhou, Y.; Guo, A.C.; Cruz, J.A.; Sinelnikov, I.; Budwill, K.; Nesbø, C.L.; Wishart, D.S. METAGENassist: a comprehensive web server for comparative metagenomics. *Nucleic Acids Res.,* **2012**, *40*(Web Server issue), W88-95. http: //dx.doi.org/10.1093/nar/gks497 PMID: 22645318

[21] Chen, L.X.; Méndez-García, C.; Dombrowski, N.; Servín-Garcidueñas, L.E.; Eloe-Fadrosh, E.A.; Fang, B.Z.; Luo, Z.H.; Tan, S.; Zhi, X.Y.; Hua, Z.S.; Martinez-Romero, E.; Woyke, T.; Huang, L.N.; Sánchez, J.; Peláez, A.I.; Ferrer, M.; Baker, B.J.; Shu, W.S. Metabolic versatility of small archaea Micrarchaeota and Parvarchaeota. *ISME J.,* **2018**, *12*(3), 756-775. http: //dx.doi.org/10.1038/s41396-017-0002-z PMID: 29222443

[22] Carr, S.A.; Schubotz, F.; Dunbar, R.B.; Mills, C.T.; Dias, R.; Summons, R.E.; Mandernack, K.W. Acetoclastic Methanosaeta are dominant methanogens in organic-rich Antarctic marine sediments. *ISME J.,* **2018**, *12*(2), 330-342. http: //dx.doi.org/10.1038/ismej.2017.150 PMID: 29039843

[23] Faust, K.; Sathirapongsasuti, J.F.; Izard, J.; Segata, N.; Gevers, D.; Raes, J.; Huttenhower, C. Microbial co-occurrence relationships in the human microbiome. *PLOS Comput. Biol.,* **2012**, *8*(7), e1002606. http: //dx.doi.org/10.1371/journal.pcbi.1002606 PMID: 22807668

[24] Oremland, R.S.; Hollibaugh, J.T.; Maest, A.S.; Presser, T.S.; Miller, L.G.; Culbertson, C.W. Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: biogeochemical significance of a novel, sulfate-independent respiration. *Appl. Environ. Microbiol.,* **1989**, *55*(9), 2333-2343. http://dx.doi.org/10.1128/AEM.55.9.2333-2343.1989 PMID: 16348014

[25] Herren, C.M.; McMahon, K.D. Keystone taxa predict compositional change in microbial communities. *Environ. Microbiol.,* **2018**, *20*(6), 2207-2217. http: //dx.doi.org/10.1111/1462-2920.14257 PMID: 29708645

[26] Deng, Y.; Jiang, Y.H.; Yang, Y.; He, Z.; Luo, F.; Zhou, J. Molecular ecological network analyses. *BMC Bioinformatics,* **2012**, *13*(1), 113. http: //dx.doi.org/10.1186/1471-2105-13-113 PMID: 22646978

[27] Lupatini, M.; Suleiman, A.K.A.; Jacques, R.J.S.; Antoniolli, Z.I.; de Siqueira Ferreira, A.; Kuramae, E.E.; Roesch, L.F.W. Network

topology reveals high connectance levels and few key microbial genera within soils. *Front. Environ. Sci.,* **2014**, *2*. http: //dx.doi.org/10.3389/fenvs.2014.00010

[28] Zhou, J.; Deng, Y.; Luo, F.; He, Z.; Yang, Y. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. *MBio,* **2011**, *2*(4), e00122-11. http: //dx.doi.org/10.1128/mBio.00122-11 PMID: 21791581

[29] Banerjee, S.; Baah-Acheamfour, M.; Carlyle, C.N.; Bissett, A.; Richardson, A.E.; Siddique, T.; Bork, E.W.; Chang, S.X. Determinants of bacterial communities in Canadian agroforestry systems. *Environ. Microbiol.,* **2016**, *18*(6), 1805-1816. http: //dx.doi.org/10.1111/1462-2920.12986 PMID: 26184386

[30] Eldridge, D.J.; Woodhouse, J.N.; Curlevski, N.J.A.; Hayward, M.; Brown, M.V.; Neilan, B.A. Soil-foraging animals alter the composition and co-occurrence of microbial communities in a desert shrubland. *ISME J.,* **2015**, *9*(12), 2671-2681. http: //dx.doi.org/10.1038/ismej.2015.70 PMID: 25932616

[31] Ma, B.; Wang, H.; Dsouza, M.; Lou, J.; He, Y.; Dai, Z.; Brookes, P.C.; Xu, J.; Gilbert, J.A. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME J.,* **2016**, *10*(8), 1891-1901. http: //dx.doi.org/10.1038/ismej.2015.261 PMID: 26771927

[32] Ding, J.; Zhang, Y.; Wang, M.; Sun, X.; Cong, J.; Deng, Y.; Lu, H.; Yuan, T.; Van Nostrand, J.D.; Li, D.; Zhou, J.; Yang, Y. Soil organic matter quantity and quality shape microbial community compositions of subtropical broadleaved forests. *Mol. Ecol.,* **2015**, *24*(20), 5175-5185. http: //dx.doi.org/10.1111/mec.13384 PMID: 26363284

[33] Jiang, Y.; Li, S.; Li, R.; Zhang, J.; Liu, Y.; Lv, L.; Zhu, H.; Wu, W.; Li, W. Plant Cultivars Imprint the Rhizosphere bacterial community composition and association networks. *Soil Biol. Biochem.,* **2017**, *109*, 145-155. http: //dx.doi.org/10.1016/j.soilbio.2017.02.010

[34] Liang, Y.; Zhao, H.; Deng, Y.; Zhou, J.; Li, G.; Sun, B. Long-term oil contamination alters the molecular ecological networks of soil microbial functional genes. *Front. Microbiol.,* **2016**, *7*, 60. http: //dx.doi.org/10.3389/fmicb.2016.00060 PMID: 26870020

[35] Wang, H.; Wei, Z.; Mei, L.; Gu, J.; Yin, S.; Faust, K.; Raes, J.; Deng, Y.; Wang, Y.; Shen, Q.; Yin, S. Combined use of network inference tools identifies ecologically meaningful bacterial associations in a paddy soil. *Soil Biol. Biochem.,* **2017**, *105*, 227-235. http: //dx.doi.org/10.1016/j.soilbio.2016.11.029

[36] Gokul, J.K.; Hodson, A.J.; Saetnan, E.R.; Irvine-Fynn, T.D.L.; Westall, P.J.; Detheridge, A.P.; Takeuchi, N.; Bussell, J.; Mur, L.A.J.; Edwards, A. Taxon interactions control the distributions of cryoconite bacteria colonizing a High Arctic ice cap. *Mol. Ecol.,* **2016**, *25*(15), 3752-3767. http: //dx.doi.org/10.1111/mec.13715 PMID: 27261672

[37] Comte, J.; Lovejoy, C.; Crevecoeur, S.; Vincent, W.F. Co-occurrence patterns in aquatic bacterial communities across changing permafrost landscapes. *Biogeosciences,* **2016**, *13*(1), 175-190. http: //dx.doi.org/10.5194/bg-13-175-2016

[38] Hill, R.; Saetnan, E.R.; Scullion, J.; Gwynn-Jones, D.; Ostle, N.; Edwards, A. Temporal and spatial influences incur reconfiguration of Arctic heathland soil bacterial community structure. *Environ. Microbiol.,* **2016**, *18*(6), 1942-1953. http: //dx.doi.org/10.1111/1462-2920.13017 PMID: 26259508

[39] Yang, S.; Wen, X.; Shi, Y.; Liebner, S.; Jin, H.; Perfumo, A. Hydrocarbon degraders establish at the costs of microbial richness, abundance and keystone taxa after crude oil contamination in permafrost environments. *Sci. Rep.,* **2016**, *6*(1), 37473. http: //dx.doi.org/10.1038/srep37473 PMID: 27886221

[40] Chao, Y.; Liu, W.; Chen, Y.; Chen, W.; Zhao, L.; Ding, Q.; Wang, S.; Tang, Y.T.; Zhang, T.; Qiu, R.L. Structure, variation, and co-occurrence of soil microbial communities in abandoned sites of a rare earth elements mine. *Environ. Sci. Technol.,* **2016**, *50*(21), 11481-11490. http: //dx.doi.org/10.1021/acs.est.6b02284 PMID: 27670106

[41] Jiao, S.; Liu, Z.; Lin, Y.; Yang, J.; Chen, W.; Wei, G. Bacterial communities in oil contaminated soils: biogeography and co-occurrence patterns. *Soil Biol. Biochem.,* **2016**, *98*, 64-73. http: //dx.doi.org/10.1016/j.soilbio.2016.04.005

[42] Shi, S.; Nuccio, E.E.; Shi, Z.J.; He, Z.; Zhou, J.; Firestone, M.K. The interconnected rhizosphere: High network complexity dominates rhizosphere assemblages. *Ecol. Lett.,* **2016**, *19*(8), 926-936. http: //dx.doi.org/10.1111/ele.12630 PMID: 27264635

[43] Yan, Y.; Kuramae, E.E.; de Hollander, M.; Klinkhamer, P.G.L.; van Veen, J.A. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *ISME J.,* **2017**, *11*(1), 56-66. http: //dx.doi.org/10.1038/ismej.2016.108 PMID: 27482928

[44] Vick-Majors, T.J.; Priscu, J.C.; Amaral-Zettler, L.A. Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *ISME J.,* **2014**, *8*(4), 778-789. http: //dx.doi.org/10.1038/ismej.2013.190 PMID: 24152712

[45] Geng, H.; Tran-Gyamfi, M.B.; Lane, T.W.; Sale, K.L.; Yu, E.T. Changes in the structure of the microbial community associated with Nannochloropsis salina following treatments with antibiotics and bioactive compounds. *Front. Microbiol.,* **2016**, *7*, 1155. http: //dx.doi.org/10.3389/fmicb.2016.01155 PMID: 27507966

[46] Graham, E.B.; Crump, A.R.; Resch, C.T.; Fansler, S.; Arntzen, E.; Kennedy, D.W.; Fredrickson, J.K.; Stegen, J.C. Deterministic influences exceed dispersal effects on hydrologically-connected microbiomes. *Environ. Microbiol.,* **2017**, *19*(4), 1552-1567. http: //dx.doi.org/10.1111/1462-2920.13720 PMID: 28276134

[47] Ji, Y.; Angel, R.; Klose, M.; Claus, P.; Marotta, H.; Pinho, L.; Enrich-Prast, A.; Conrad, R. Structure and function of methanogenic microbial communities in sediments of Amazonian lakes with different water types. *Environ. Microbiol.,* **2016**, *18*(12), 5082-5100. http: //dx.doi.org/10.1111/1462-2920.13491 PMID: 27507000

[48] Musat, N.; Halm, H.; Winterholler, B.; Hoppe, P.; Peduzzi, S.; Hillion, F.; Horreard, F.; Amann, R.; Jørgensen, B.B.; Kuypers, M.M.M. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(46), 17861-17866. http: //dx.doi.org/10.1073/pnas.0809329105 PMID: 19004766

[49] Zhao, D.; Shen, F.; Zeng, J.; Huang, R.; Yu, Z.; Wu, Q.L. Network analysis reveals seasonal variation of co-occurrence correlations between Cyanobacteria and other bacterioplankton. *Sci. Total Environ.,* **2016**, *573*, 817-825. http://dx.doi.org/10.1016/j.scitotenv.2016.08.150 PMID: 27595939

[50] Steele, J.A.; Countway, P.D.; Xia, L.; Vigil, P.D.; Beman, J.M.; Kim, D.Y.; Chow, C.E.T.; Sachdeva, R.; Jones, A.C.; Schwalbach, M.S.; Rose, J.M.; Hewson, I.; Patel, A.; Sun, F.; Caron, D.A.; Fuhrman, J.A. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J.,* **2011**, *5*(9), 1414-1425. http: //dx.doi.org/10.1038/ismej.2011.24 PMID: 21430787

[51] Li, F.; Chen, L.; Zhang, J.; Yin, J.; Huang, S. Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. *Front. Microbiol.,* **2017**, *8*, 187. http: //dx.doi.org/10.3389/fmicb.2017.00187 PMID: 28232824

[52] Hajishengallis, G.; Liang, S.; Payne, M.A.; Hashim, A.; Jotwani, R.; Eskan, M.A.; McIntosh, M.L.; Alsam, A.; Kirkwood, K.L.; Lambris, J.D.; Darveau, R.P.; Curtis, M.A. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe,* **2011**, *10*(5), 497-506. http: //dx.doi.org/10.1016/j.chom.2011.10.006 PMID: 22036469

[53] Fisher, C.K.; Mehta, P. Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression. *PLoS One,* **2014**, *9*(7), e102451. http: //dx.doi.org/10.1371/journal.pone.0102451 PMID: 25054627

[54] Shetty, S.A.; Hugenholtz, F.; Lahti, L.; Smidt, H.; de Vos, W.M. Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. *FEMS Microbiol. Rev.,* **2017**, *41*(2), 182-199. http: //dx.doi.org/10.1093/femsre/fuw045 PMID: 28364729

[55] Curtis, M.M.; Hu, Z.; Klimko, C.; Narayanan, S.; Deberardinis, R.; Sperandio, V. The gut commensal Bacteroides thetaiotaomicron exacerbates enteric infection through modification of the metabolic landscape. *Cell Host Microbe,* **2014**, *16*(6), 759-769. http: //dx.doi.org/10.1016/j.chom.2014.11.005 PMID: 25498343

[56] Maldonado-Contreras, A.; Goldfarb, K.C.; Godoy-Vitorino, F.; Karaoz, U.; Contreras, M.; Blaser, M.J.; Brodie, E.L.; Dominguez-Bello, M.G. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. *ISME J.,* **2011**, *5*(4), 574-579. http: //dx.doi.org/10.1038/ismej.2010.149 PMID: 20927139

[57] Trosvik, P.; de Muinck, E.J. Ecology of bacteria in the human gastrointestinal tract-identification of keystone and foundation taxa. *Microbiome,* **2015**, *3*(1), 44. http: //dx.doi.org/10.1186/s40168-015-0107-4 PMID: 26455879

[58] Wu, S.; Rhee, K.J.; Albesiano, E.; Rabizadeh, S.; Wu, X.; Yen, H.R.; Huso, D.L.; Brancati, F.L.; Wick, E.; McAllister, F.; Housseau, F.; Pardoll, D.M.; Sears, C.L. A human colonic commensal promotes colon tumorigenesis *via* activation of T helper type 17 T cell responses. *Nat. Med.,* **2009**, *15*(9), 1016-1022. http: //dx.doi.org/10.1038/nm.2015 PMID: 19701202

[59] Ze, X.; Duncan, S.H.; Louis, P.; Flint, H.J. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J.,* **2012**, *6*(8), 1535-1543. http: //dx.doi.org/10.1038/ismej.2012.4 PMID: 22343308

[60] Garrett, W.S.; Gallini, C.A.; Yatsunenko, T.; Michaud, M.; DuBois, A.; Delaney, M.L.; Punit, S.; Karlsson, M.; Bry, L.; Glickman, J.N.; Gordon, J.I.; Onderdonk, A.B.; Glimcher, L.H. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe,* **2010**, *8*(3), 292-300. http: //dx.doi.org/10.1016/j.chom.2010.08.004 PMID: 20833380

[61] Kumar, V.; Bharti, P.K.; Talwar, M.; Tyagi, A.K.; Kumar, P. Studies on high iron content in water resources of Moradabad district (UP), India. *Water Sci.,* **2017**, *31*(1), 44-51. http: //dx.doi.org/10.1016/j.wsj.2017.02.003

[62] Björnerås, C.; Weyhenmeyer, G.A.; Evans, C.D.; Gessner, M.O.; Grossart, H-P.; Kangur, K.; Kokorite, I.; Kortelainen, P.; Laudon, H.; Lehtoranta, J.; Lottig, N.; Monteith, D.T.; Nõges, P.; Nõges, T.; Oulehle, F.; Riise, G.; Rusak, J.A.; Räike, A.; Sire, J.; Sterling, S.; Kritzberg, E.S. Widespread increases in Iron concentration in European and North American freshwaters. *Global Biogeochem. Cycles,* **2017**, *31*(10), 1488-1500. http: //dx.doi.org/10.1002/2017GB005749

[63] Kangabam, R.D. Contamination in Loktak Lake. *A Down to Earth Annual – State of India's Environment*, **2018**, pp. 77-79.

[64] Brockett, B.F.T.; Prescott, C.E.; Grayston, S.J. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in Western Canada. *Soil Biol. Biochem.,* **2012**, *44*(1), 9-20. http: //dx.doi.org/10.1016/j.soilbio.2011.09.003

[65] Zhang, Y.; Dong, J.; Yang, B.; Ling, J.; Wang, Y.; Zhang, S. Bacterial community structure of Mangrove sediments in relation to environmental variables accessed by 16S rRNA gene-denaturing gradient gel electrophoresis fingerprinting. *Sci. Mar.,* **2009**, *73*(3), 487-498. http: //dx.doi.org/10.3989/scimar.2009.73n3487

[66] Leibold, M.A.; Holyoak, M.; Mouquet, N.; Amarasekare, P.; Chase, J.M.; Hoopes, M.F.; Holt, R.D.; Shurin, J.B.; Law, R.; Tilman, D.; Loreau, M.; Gonzalez, A. The metacommunity concept: A framework for multi-scale community ecology. *Ecol. Lett.,* **2004**, *7*(7), 601-613. http: //dx.doi.org/10.1111/j.1461-0248.2004.00608.x

[67] Logue, J.B.; Mouquet, N.; Peter, H.; Hillebrand, H. Metacommunity Working Group. Empirical approaches to metacommunities: a review and comparison with theory. *Trends Ecol. Evol. (Amst.),* **2011**, *26*(9), 482-491. http: //dx.doi.org/10.1016/j.tree.2011.04.009 PMID: 21641673

[68] Gilbert, J.A.; Steele, J.A.; Caporaso, J.G.; Steinbrück, L.; Reeder, J.; Temperton, B.; Huse, S.; McHardy, A.C.; Knight, R.; Joint, I.; Somerfield, P.; Fuhrman, J.A.; Field, D. Defining seasonal marine microbial community dynamics. *ISME J.,* **2012**, *6*(2), 298-308. http: //dx.doi.org/10.1038/ismej.2011.107 PMID: 21850055

[69] Zhang, W.; Bougouffa, S.; Wang, Y.; Lee, O.O.; Yang, J.; Chan, C.; Song, X.; Qian, P.-Y. Toward understanding the dynamics of microbial communities in an estuarine system. *PLoS One,* **2014**, *9*(4), e94449. http: //dx.doi.org/10.1371/journal.pone.0094449 PMID: 24732211

[70] Shi, P.; Jia, S.; Zhang, X.X.; Zhang, T.; Cheng, S.; Li, A. Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. *Water Res.,* **2013**, *47*(1), 111-120. http: //dx.doi.org/10.1016/j.watres.2012.09.046 PMID: 23084468

[71] Liu, M.; Huang, H.; Bao, S.; Tong, Y. Microbial community structure of soils in Bamenwan mangrove wetland. *Sci. Rep.,* **2019**, *9*(1), 8406. http: //dx.doi.org/10.1038/s41598-019-44788-x PMID: 31182804

[72] Vaz-Moreira, I.; Nunes, O.C.; Manaia, C.M. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS Microbiol. Rev.,* **2014**, *38*(4), 761-778. http: //dx.doi.org/10.1111/1574-6976.12062 PMID: 24484530

[73] Henry, E.A.; Devereux, R.; Maki, J.S.; Gilmour, C.C.; Woese, C.R.; Mandelco, L.; Schauder, R.; Remsen, C.C.; Mitchell, R. Characterization of a new thermophilic sulfate-reducing bacterium *Thermodesulfovibrio yellowstonii*, gen. nov. and sp. nov.: its phylogenetic relationship to Thermodesulfo bacterium commune and their origins deep within the bacterial domain. *Arch. Microbiol.,* **1994**, *161*(1), 62-69. http: //dx.doi.org/10.1007/BF00248894 PMID: 11541228

[74] Oswald, K.; Graf, J.S.; Littmann, S.; Tienken, D.; Brand, A.; Wehrli, B.; Albertsen, M.; Daims, H.; Wagner, M.; Kuypers, M.M.M.; Schubert, C.J.; Milucka, J. Crenothrix are major methane consumers in stratified lakes. *ISME J.,* **2017**, *11*(9), 2124-2140. http: //dx.doi.org/10.1038/ismej.2017.77 PMID: 28585934

[75] Jebeli, M.A.; Maleki, A.; Amoozegar, M.A.; Kalantar, E.; Izanloo, H.; Gharibi, F. *Bacillus flexus* strain As-12, a new arsenic transformer bacterium isolated from contaminated water resources. *Chemosphere,* **2017**, *169*, 636-641. http://dx.doi.org/10.1016/j.chemosphere.2016.11.129 PMID: 27912188

[76] Armengaud, J.; Happe, B.; Timmis, K.N. Genetic analysis of dioxin dioxygenase of *Sphingomonas* sp. Strain RW1: catabolic genes dispersed on the genome. *J. Bacteriol.,* **1998**, *180*(15), 3954-3966. http: //dx.doi.org/10.1128/JB.180.15.3954-3966.1998 PMID: 9683494

[77] Abro, A.H.; Rahimi Shahmirzadi, M.R.; Jasim, L.M.; Badreddine, S.; Al Deesi, Z. *Sphingobacterium multivorum* bacteremia and acute meningitis in an immunocompetent adult patient: A case report. *Iran. Red Crescent Med. J.,* **2016**, *18*(9), e38750. http: //dx.doi.org/10.5812/ircmj.38750 PMID: 28144466

[78] Javed, S.; Gul, F.; Javed, K.; Bokhari, H. *Helicobacter pullorum*: an emerging zoonotic pathogen. *Front. Microbiol.,* **2017**, *8*(APR), 604. http: //dx.doi.org/10.3389/fmicb.2017.00604 PMID: 28443081

[79] Fouts, D.E.; Matthias, M.A.; Adhikarla, H.; Adler, B.; Amorim-Santos, L.; Berg, D.E.; Bulach, D.; Buschiazzo, A.; Chang, Y.F.; Galloway, R.L.; Haake, D.A.; Haft, D.H.; Hartskeerl, R.; Ko, A.I.; Levett, P.N.; Matsunaga, J.; Mechaly, A.E.; Monk, J.M.; Nascimento, A.L.T.; Nelson, K.E.; Palsson, B.; Peacock, S.J.; Picardeau, M.; Ricaldi, J.N.; Thaipandungpanit, J.; Wunder, E.A., Jr; Yang, X.F.; Zhang, J.J.; Vinetz, J.M. What makes a bacterial species pathogenic? Comparative genomic analysis of the genus Leptospira. *PLoS Negl. Trop. Dis.,* **2016**, *10*(2), e0004403. http: //dx.doi.org/10.1371/journal.pntd.0004403 PMID: 26890609

[80] Piette, A.; Verschraegen, G. Role of coagulase-negative staphylococci in human disease. *Vet. Microbiol.,* **2009**, *134*(1-2), 45-54. http: //dx.doi.org/10.1016/j.vetmic.2008.09.009 PMID: 18986783

[81] Mayali, X.; Azam, F. Algicidal bacteria in the sea and their impact on algal blooms. *J. Eukaryot. Microbiol.,* **2004**, *51*(2), 139-144. http://dx.doi.org/10.1111/j.1550-7408.2004.tb00538.x PMID: 15134248