Effect of hydrological regime on the sediment bacterial diversity and community structure in Sultanpur Lake, Gurugram, Haryana

Sandhya Bhat1,2 and Pamposh1

1University School of Environment Management GGSIP University, Sector-16C, Dwarka, New Delhi, India
2E-mail: sandhyabhatambardar@yahoo.com

Abstract. The present study focuses on the changes in sediment bacterial community structure and diversity of Sultanpur Lake due to changes in its hydrological regime. The assessment of the bacterial community structure was done by using the Next Generation Sequencing of 16S rRNA amplicon, a bacterial phylogenetic marker. Sultanpur Lake is located in Sultanpur National Park Gurugram, in the state of Haryana, India. This place harbors great plant and animal biodiversity and serves as an important feeding, roosting and breeding ground for both resident and migratory avian fauna. Increasing pollution level and scarcity of water during summer months impose a great threat to the lake. Sediment bacteria are considered as reliable recorders of nutrient cycling and active mediums of biogeochemical processes and thus were assessed in this study. Wet and dry sediment samples were taken and analyzed for their bacterial community structure and diversity. The amplicon sequencing generated 881,118 reads which corresponded to 93 known species in case of dry sediments and 8,71,102 reads corresponding to 44 species in case of wet sediments. In dry sediments the top ten phyla were Firmicutes (26%), Bacteroidetes (14%), Proteobacteria (15%), Cyanobacteria (12%), Parcubacteria (7%), Euryarcheota (6%), Actinobacteria (5%), Armatimonadetes (5%), Chloroflexi (2%) and Planctomycetes (2%). In case of wet sediments, the top ten phyla were Firmicutes (35%), Bacteroidetes (22%), Proteobacteria (22%), Actinobacteria (7%), Chloroflexi (5%) Parcubacteria (3%), Verrucomicrobia (3%) and Deinococcus-Thermus (3%). There was change in the community structure at species level too with Clostridium species as the most dominant species in case of wet sediments and Lactobacillus species as the predominant one in dry sediments. This study provides an insight into the changes in the bacterial communities due to changes in hydrology and how this change will affect the health of this wetland as irregular water supply is a major stressor of this wetland.

Keywords: Bacterial Diversity, bacterial community, 16S rRNA amplicon, Next generation sequencing

1. Introduction

Wetlands are one of the most important ecosystems and are distributed in almost all regions of the world. These are active sites of biogeochemical processes which regulate flux of material between living and non-living components
of the biosphere [1-3]. Microbial communities are the drivers of biogeochemical processes and these microbial activities are crucial to the functions and health of wetland systems [4-6]. Sediments bacterial communities are less variable than the bacterial communities present in surface and interstitial waters [7-9] and thus are usually used as reliable recorders of ecological responses to pollution [10-12]. Changes in nutrients and pollution significantly impact the diversity, function and bacterial biomass [13-14]. Water, temperature and organic matter are limiting factors for sediment bacteria [1], so alternate drying and wetting cycles of a wetland will have an impact on the sediment bacteria. To quantify the impact of availability of water on sediment bacteria 16S rRNA marker was used. 16S rRNA as marker is routinely being used by microbial ecologists for assessment of the biodiversity of microbial communities. [15]. Microbial species in environmental samples are identified by the comparative analysis of 16S rRNA sequences for microbial phylogenetic analysis [16-19].

2. Materials and methods

2.1. Study site

Sultanpur National Park (28°28′N 76°53′E, c.25 km southeast of Delhi) is an important bird habitat and a hot spot for biodiversity in urbanized area near New Delhi. It is located in a predominantly agricultural landscape in Gurgaon district of Haryana state Figure 1. The region was notified as a bird sanctuary by the Haryana state government in 1970 [20]. SNP covers an area of approximately 13.727 ha, including its core area of 1.43 ha [21]. The core area, is a seasonal freshwater wetland with irregular margins of drastically fluctuating water.

Given the current climate change scenarios valuable wetland ecosystems especially in semi-arid regions are becoming more fragile and vulnerable to hydrological stresses. SNP falls in the region which is semi-arid and hot and is mainly characterized by extremely dry air throughout the year except during monsoon months [22]. In addition to its location and global climate scenarios, there are a number of factors which influence the hydrology of this wetland like changes in land use, natural drainage of the adjoining area in the past, low precipitation, the shallow nature of the wetland.

All the above factors influence the retention of water in this wetland. This wetland retains water until March or April and only a few small pools remain by May-July [23]. So, to maintain the habitat for waterfowl and to prevent loss of floral and faunal biodiversity, input of water from irrigation canals and groundwater is undertaken each year.

This study was undertaken to assess the impact of the alternating drying and wetting cycles on the sediment bacterial diversity in this stressed wetland.

2.2. Sampling strategy

Water and sediment samples from Sultanpur were collected in the month of May, 2018 from six different sampling sites as shown in figure 1. Within each site two plots were chosen for sampling. One plot which was permanently inundated and another that was inundated only for some months. (from about July to April). Water and sediment samples were collected in triplicates from all the sites. A sediment corer was used for collecting the sediments. The sediments were sieved by using a 2mm mesh. Both sediment and water samples were stored in sterile containers. All the samples were kept on ice and transported back to the laboratory. Water was checked for pH, specific conductance and temperature in the field using portable meters (WTW, Germany). In the laboratory all the samples were refrigerated. Sediment samples were analyzed within 24 hours. Water samples were tested for DO (Modified Winkler’s Method), TKN (Kjeldahl’s method) Ammonia (Distillation and Volumetric analysis) Nitrate (UV method) and Nitrite (Colorimetric method). Sediment was tested for water content (Drying method), Total organic matter content, TKN (Kjeldahl’s method). All these analyses were done according to APHA 1999 [24]. Total carbon was estimated using (Elemental Analyzer), C:N ratio was calculated. Sediment samples were
subjected to DNA extraction. OM content was determined on a subsample of dry sediment as loss on ignition at 550 °C for 8 h and expressed as a percentage.

2.3. DNA extraction and amplicon library preparation
Bacterial and Archaeal 16S rRNA gene q PCR was done as described in [25]. Mo Bio Power Soil® DNA Isolation Kit was used for e-DNA extraction. 25ng of sediment metagenomic DNA was used to amplify 16S rRNA hyper variable region V3-V4 and create a single amplicon of approximately 460 base pairs. [26] (Berry et al.2011). Library preparation of this 16S rRNA amplicon was done and the libraries were quantitated using Qubit DNA HS quantitation assay (Thermo Scientific, MA, USA) and Bioanalyzer 2100 (Agilent, CA, USA).[27-28].

2.4. Sequence processing and analysis
Illumina MiSeq platform was used to sequence these libraries. The read data generated was subjected to Operational Taxonomic Unit (OUT) Clustering. OTU picking was performed with the default UCLUST algorithm. The most abundant read in each OTU cluster was selected as the representative sequence using 97% identity. Green gene reference database (Version gg_13_5) was used to assign taxonomy to the representative sequence. Bioinformatics software the “Quantitative Insights into Microbial Ecology” (QIIME v1.9.1) [29] was used for the analysis and an OTU table was generated. Abundance for each sample are subjected to Cumulative Sum Scaling (CSS) normalization. Diversity profiling was done at alpha and beta level. Alpha diversity which calculates within-sample diversity was calculated using three indices namely Chao1, Shannon index, Simpson index. Beta diversity which calculates diversity for a group of samples was calculated between all the samples by using Euclidean metric [30].

2.5. Data Availability
The sequence data from Sultanpur Lake was submitted to NCBI sequence read archive (SRA) under accessions numbers SRX6085350 to SRX6085355.

3. Results and discussion
3.1. Water and sediment analysis
The physio chemical parameters for water column in the lake and the sediments are shown in the Table 1. As the sampling was done during summer the surface water temperature of the lake was high at around 30°C which influences the growth of bacteria that are more tolerant to heat. The pH of water recorded
during the study was slightly alkaline (8.04). The lake waters showed low DO (5.59 mg/L) high nitrate (0.34 mg/l) and ammonia (2.713mg/l). Low dissolved oxygen in the water column may be due to shallow and stagnant nature of the water column and the pollution. High ammonia when found in natural waters is regarded as indicative of sanitary pollution [11] which is collaborated by the fact that this waterbody has lot of sanitary waste flowing in it due to adjoining drains and human and animal defecation that is prevalent along its shores. Nitrite concentration was moderate for freshwater wetland.

Table 1. Water and sediment parameters of water and sediment in Sultanpur Lake.

| S No. | Water Characteristics | Values | Sediment Characteristics | Values (%) |
|-------|------------------------|--------|--------------------------|-----------|
| 1     | pH                     | 8.04   | Sediment Carbon          | 1.97      |
| 2     | Temperature (°C)       | 30.3   | Nitrogen Content         | 0.07      |
| 3     | Specific Conductance(us/cm) | 288.78 | Water content           | 4.1       |
| 4     | Dissolved oxygen(mg/L) | 5.59   | C:N ratio                | 28.14     |
| 5     | Ammonium(mg/L)         | 2.713  | Total Organic matter    | 4.9       |
| 6     | Nitrates(mg/L)         | 0.34   |                          |           |
| 7     | Nitrites(mg/L)         | 0.01   |                          |           |
| 8     | Nitrogen (TKN)(mg/L)   | 15.10  |                          |           |

3.2. DNA Analysis
The sequencing of the dry sediment samples generated 8,811,18 total reads which corresponded to 28805 OTUs whereas the wet samples generated 8,71,102 reads and 23179 OTUs. Relative abundance of different OUTs was used in diversity analysis. Diversity profiling was done for all sites buy calculating Chao1, Simpson Index and Shannon index. (Table 2). Chao1 Shannon and Simpson estimates were higher in Dry sediments pointing to greater Evenness, richness and abundance of different bacterial species in Dry sediments. Dry sediments contained 93 different bacteria at species level and wet samples contained 44 bacterial species.

3.3. Diversity of bacteria
The community structure of Sultanpur Dry sediment revealed Firmicutes (26), Proteobacteria (15%) Bacteroidetes (14%) and as the dominant phyla. Cyanobacteria (12%), Parcubacteria (7%), Euryarcheota
Actinobacteria (5%), Armatimonadetes (5%), Chloroflexi (2%) and Planctomycetes (2%) are the other major phyla present in dry sediments phyla. In case of wet sediments, the top phyla were Firmicutes (35%), Bacteroidetes (22%), Proteobacteria (22%) Actinobacteria (7%), Chloroflexi (5%) Parcubacteria (3%), Verrucomicrobia (3%) and, Deinococcus-Thermus (3%) as shown in Figure 2. Both these sediment types contained more or less same major phyla but phyla like cyanobacteria, Euryarcheota, Armatimonadetes and planctomycetes are absent in wet sediments while as phyla Verrucomicrobia and, Deinococcus-Thermus are absent in dry sediments.

Figure 2. Comparison of Bacterial communities at phylum level in Dry and Wet sediments of Sultanpur Lake.

Euryarcheota is the only archaeal phyla present in Sultanpur. Absence or low diversity of archaeal population in freshwater sediments is associated pollution. [31].

Phylum Firmicutes consists of saprophytic microbes which are very resistant and produce endospores under stressful environmental conditions. Usually firmicutes are not the dominant bacteria present in freshwater sediments and are associated with polluted water. Dominance of this phylum in both wet as well as dry sediments points to our wet land being polluted. Clostridium is the major species present in wet sediments and lactobacillus as the major species in case of dry sediments. Both clostridia and bacilli are abundant taxa in sewage sludge.[32-33].

Phylum Proteobacteria is the largest and most diverse group of bacteria. Proteobacteria were represented by all the five classes, Alpha proteobacteria, Beta Proteobacteria, Gamma Proteobacteria, Delta Proteobacteria and Epsilon Proteobacteria (Figure 3). Epsilon Proteobacteria were present in wet sediments only whereas Alpha Proteobacteria were present in dry sediments only. Alpha proteobacteria are widely distributed in wetland sediments and these have the ability to survive in low conc of nutrients. Presence of all classes of phylum proteobacteria confirms ample biodiversity in the wetland sediments [34-35].
Figure 3. Comparison of observed Proteobacteria taxon in dry and wet sediments of Sultanpur Lake.

4. Conclusion
This study revealed a change in bacterial community structure between sediment samples that are permanently inundated sediments and sediments submitted to seasonal dewatering. However, this compositional shift was confined to differences in just a few phyla. The findings suggest there is change in the bacterial community structure and that change is due to the pollution in the wetland. So, the process of pumping water into this wetland to restore it can be continued but the quality of water that is pumped into the wetland must improve.

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