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Distribution patterns of bacterial communities and their potential link to variable viral lysis in temperate freshwater reservoirs

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Abstract

Man-made reservoirs which receive substantial inputs of terrestrial organic matter are characterized by physiologically diverse and distinct bacterial communities. Here we examined bacterial community structure using Illumina MiSeq sequencing of 16S rRNA genes and evaluated the potential role of viruses in influencing them in two productive freshwater reservoirs namely, Villerest and Grangent (Central France). Two dimensional non-metric multidimensional scaling analyses indicated that bacterial communities in both reservoirs were structurally different in time and space, with Villerest harboring more diverse communities than Grangent reservoir. The bacterial communities in both reservoirs were dominated by *hgcI clade* (Actinobacteria) and *Limnohabitans* (Betaproteobacteria) which are known to have adaptive life strategies towards top-down mechanisms and resource utilization. In Villerest, thermal stratification of water column which resulted in temporary anoxia especially during summer promoted the occurrence of anoxygenic phototrophic and methanotrophic bacteria. Overall, low bacterial richness which was linked to viral lytic infection possibly suggests that a relatively small number of highly active bacterial populations sustained high bacterial activity and viral abundances. Weighted UniFrac analysis indicated that a minimum threshold viral infection and virus-to-bacteria ratio (serve as a proxy) of 10% and 10, respectively, is required to exert its impact on phylogenetic structure of bacterial community. Therefore depending on the levels of viral infection we suggest that viruses at times can prevail over other trophic or top-down factors in shaping and structuring bacterial communities in such man-made artificial freshwater systems.

Keywords

freshwater reservoirs, bacterial community, viral lysis, 16S rRNA sequences, Illumina sequencing, microbial ecology

Introduction

Hydroelectric reservoirs represent up to 25% of the area used for human water supply systems (irrigation, drinking water, energy generation etc.) and are identified as potentially important sources of greenhouse gas emissions (Kosten et al. 2010; Barros et al. 2011). When compared to natural lakes, these man-made lakes are considered to have different physical, chemical and biological characteristics, high disturbance frequencies with rapid, often irregular and large changes in water level and water column stability (Wetzel, 1990). Reservoirs often receive substantial amounts of anthropogenic (terrestrial) inputs from their surrounding catchment area. These interventions affect not only the water quality but also create unique habitats, enabling a broad and diverse community of bacterioplankton to thrive, which can have implications on the functioning of the ecosystems. Therefore assessing the bacterial diversity and its interactions with environmental factors is crucial to determine their ecological and biogeochemical roles in reservoir systems.

Generally high concentrations of organic matter in reservoirs tend to support higher standing stock of bacteria due to their increased growth rate and activity. Bacteria inhabiting in reservoir systems are highly dynamic and diverse (Llirós et al. 2014; Yu et al. 2014; Zhang et al. 2015), therefore in such systems bacterial community structure can be expected to vary in accordance with the prevailing environmental conditions with time and space. Compared to bottom up control (i.e. resources), knowledge on the modulation of biotic factors especially by viruses in the control of bacterial communities is scarce, owing to the lack of appropriate tools to explore biological interactions. Bottom-up factors are known to have variable impact on different bacterial communities, but still the net effect is largely dependent on mortality forces such as viral lysis or protistan grazing (Sandaa et al. 2009; Pradeep Ram et al. 2016a). Although many studies have underlined the importance of including viruses as one of the potential factors influencing bacterial community structure in freshwaters (Berdjeb et al. 2011; Llirós et al. 2014) relatively fewer investigations have been conducted in complex reservoir systems (Weinbauer et al. 2007, Pradeep Ram and Sime-Ngando 2008).

Top-down control by viruses is believed to be more host-specific and thus acts more on the community composition than size structure of the bacterial community. Studies have indicated that viruses can exert control on the most competitive and numerically dominant bacteria, thus preventing them from displacing competing taxa (Thingstad and Lignell 1997). Several lines of evidence have shown viruses to control the abundance of rare organisms through selective mortality (Bouvier and del Giorgio 2007; Pradeep Ram and Sime-Ngando

2008), although their net influence on bacterial communities has been variable (Schwalbach et al. 2004) or limited (Jardillier et al. 2005; Berdjeb et al. 2011).

Since these man-made lake ecosystems are characterized by nutrient rich conditions, such systems could serve as potential environments for phages to attack microbes. It has been reported in reservoirs that phage lysis is responsible for up to 62% of bacterial mortality, which can contribute to recycling of carbon and nutrients through viral shunt (Pradeep Ram et al. 2016b). The impact of viruses on bacterial community dynamics is less studied compared to effects of nutrient availability. It is still unclear whether the influence of viruses on bacterial community is similar across different trophic systems. Henceforth, in the light of previously conducted studies in reservoir systems, we hypothesize that phage-host dynamics and interactions should largely influence patterns of bacterial community composition and diversity.

In the present study, two man-made lakes, namely Grangent and Villerest reservoirs which are situated across a major river (Loire, Central France) were used as model systems to evaluate viral impact on bacterial community structure in time and space. The main objectives of our study are 1) to characterize differences in bacterial community composition and dynamics (richness and composition) at a finer taxonomic scale using Illumina MiSeq sequencing of 16S rRNA amplicons over space and time and 2) to evaluate the potential impact of viral lysis on these bacterial communities. Results from the present study would add to the growing knowledge of linking bacterial community structure to the functioning of these man-made artificial ecosystems.

Materials and methods

Site description and sampling

The Grangent and Villerest reservoirs were created by dams built in 1957 and 1984 respectively across the Loire River for the purpose of generating electricity by hydroelectric plants. Both reservoirs are located in the same geographical region (Central France) with Villerest reservoir situated at a distance of 70 km downstream of Grangent reservoir (see Sabart et al. 2009 for map and study locations). Compilation of detailed hydrological and morphometric characteristics of the study reservoirs are presented in **Table 1.** These thermally stratified reservoirs experience fluctuation in water levels (up to 15 meters) due to release of water for the purpose of summertime irrigation and also to prevent deoxygenation in the deeper waters. Due to the large catchment area, both reservoirs receive high inputs of organic

matter from the terrestrial sources which annually promote toxic bloom forming freshwater bacteria *Microcystis aeuroginosa*, especially during summer.

The sampling was carried out in Grangent (20 April, 13 July and 5 October) and Villerest (7 May, 30 July and 26 October) in 2015, covering three distinct seasons namely, spring, summer and autumn respectively. Triplicate water samples were collected from Villerest (45°55'N, 4°02'E) and Grangent (45°27, 4°15'E) reservoirs using Van Dorn bottle (10 L capacity) at three different depths namely, epilimnion (0.5 m below the surface), metalimnion (7 m below the surface), and hypolimnion (1 m above the sediments). The collected water samples were immediately pre-screened through a 150 µm pore size nylon filter to exclude predatory metazoan zooplankton and then transferred to sterile containers. The samples were transported to laboratory in refrigerated boxes and processed immediately upon arrival.

Physico-chemical analyses

Secchi measurements (Zs) were used to estimate the euphotic depth (Zeu) according to the relationship: Zeu = 2.42Zs (Wetzel and Likens 1995). Water temperature was measured directly with a field thermometer and dissolved oxygen concentration was determined by the Winkler method (Carpenter, 1965). The pH was determined with a standard pH probe (pH 1000L, VWR, UK). Total organic carbon were determined by high temperature catalytic oxidation method (680°C) using a TOC analyzer (Shimadzu TOC-V CPN, Japan) (Lønborg and Søndergaard 2009) and total nitrogen using the same analyzer with an attached measuring unit (Mahaffey et al. 2008) with values reported after correction with instrument blank.

DNA Extraction

Bacteria from the lake water samples (200 ml) were collected onto 0.2µm white polycarbonate membrane filters (Sartorius, Germany) in triplicates by applying gentle vacuum. Total community DNA was then extracted from filters using Fast DNA spin kit for soil (MP Biomedicals, Germany) by using two consecutive bead-beating steps, first, for 40 s, followed by a second bead beating for 60 s to disrupt additional, previously un-lysed cells. Remaining steps were followed as per manufacturer's protocol. Yield and quality of the extracted DNA samples were checked on 0.8% agarose gel, and extracted genomic DNA was quantified, checked for purity at A260/280 nm by NanoDrop spectrophotometer (Thermo Scientific, USA.). The DNA was stored at −20 °C until further processing.

PCR Amplification of 16S rRNA gene and Sequencing

PCR amplification was carried out to amplify V3–V4 conserved regions of bacterial 16S rRNA gene sequences in triplicate using the 16S rRNA gene specific primers (forward primer 5′-CTTTCCCTACACGACGCTCTTCCGATCTACGGRAGGCAGCAG-3′ and reverse 5′-GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT-3′) (Liu et al. 2007). These primers include the Illumina adapter overhang nucleotide sequences (underlined segment) as well as V3-V4-specific sequences producing an amplicon of 460 bp in length. The libraries for 16S rRNA were prepared according to procedure as detailed in Keshri et al. (2017). Negative PCR controls were also run using Milli-Q water instead of template DNA to confirm that there was no contamination of the reagents or extraction protocol. Triplicate amplicons from respective samples were pooled together in order to decrease the PCR bias led by stochastic fluctuations in amplification efficiencies (Polz and Cavanaugh 1998) and sequenced (paired-end at a length of 300 nucleotides in each direction) on the Illumina MiSeq platform at the INRA, Toulouse, France. Pooling of PCR amplicons has been done in samples obtained from aquatic systems (Pascault et al. 2014, Li et al. 2017) without significantly influencing Illumina based sequencing data (Kennedy et al. 2014). Sequence data which were received as .fastq files was submitted to the National Center for Biotechnology Sequence Read Archive under Bio Project accession number PRJNA395988.

Sequence data analysis

Sequence processing was performed using mothur software, version 1.39.1 (Schloss et al. 2009). The paired-end MiSeq Illumina reads from the 18 samples were aligned and converted to contigs yielding 693,384 reads. Sequence analysis of each combined single fasta file was processed using the mothur MiSeq SOP accessed on the 10 December 2016 (Kozich et al. 2013). The high quality of sequences were filtered using following parameters; base quality score $\geq$ 20, deltaq=6, match=1, mismatch= -1, gapopen = -2 and gapextend = -1. Sequences having ambiguous characters and homopolymers longer than 8 bp were removed. High-quality sequences were aligned against the mothur version of SILVA bacterial reference sequences (Pruesse et al. 2007), pre clustered, chimeras and singletons removed and OTUs were defined at 97% identity. After normalizing the number of sequences in each sample based on rarefied or subsampled data i.e. the minimum number of remaining sequences (6,569) in any of the samples); rarefaction was performed on each sample to assess sampling adequacy, using a 100 sequence increment. Alpha diversity was assessed by calculating the richness Chao1 estimator, Shannon diversity, and Evenness indices. The Shannon index is the

parametric diversity measure commonly used to characterize species diversity in a community which combines estimates of richness (total number of OTUs) and evenness (relative abundance). Beta diversity (variation in community structure between samples) is determined using the same subsampling approach with an index that accounts for proportional abundances of both shared and non-shared OTUs (Yue and Clayton 2005). Non-metric multidimensional scaling (NMDS) based on Bray-curtis distance matrix was used to visualize the similarity between samples. Similarity among bacterial community structure was assessed using Sorensen's coefficient for occurrence data (Legendre and Legendre 1998).

Bacterial and viral abundances

Samples (1 ml each) for enumeration of bacterial and viral abundances were fixed with paraformaldehyde (0.5% final concentration) and kept in dark at 4°C for 30 minutes. Following fixation, the samples were immediately passed through FACS Calibur flow cytometer (Becton Dickinson, Franklin Lake, NJ, USA) equipped with an air-cooled laser providing 15 mW at 488 nm with the standard filter set-up (Brussaard et al. 2010). Briefly the extracted samples were diluted in 0.02 μm filtered TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8), and stained with SYBR Green I (10,000 fold dilution of commercial stock, Molecular Probes, Oregon, USA). Since dilution of water samples with TE buffer depends on the *in situ* viral and bacterial abundances, and sensitivity of the flow cytometer, validity check and standardization was carried out before proceeding with counts. This methodology has been previously validated and adopted for reporting bacterial and viral counts in these reservoir systems (Pradeep Ram et al., 2016b). The mixture was incubated for 5 min at ambient temperature, heated for 10 min at 80°C in the dark, and cooled for 5 min at room temperature prior to analysis. Bacteria and viruses differing in fluorescence intensity were detected by their signature in a side scatter versus green fluorescence (530 nm wave- length, fluorescence channel 1 of the instrument) plot. Flow cytometry list modes were analyzed using CellQuest Pro software (BD Biosciences, San Jose, CA, USA; version 4.0). A blank was routinely examined to control for contamination of the equipment and reagents.

Viral lytic infection

Viral lytic infection was inferred from the frequency of visibly infected cells (FVIC) according to Pradeep Ram and Sime-Ngando (2008). Bacterial cells contained in 8 ml of glutaraldehyde fixed water samples (final concentration 1%) were collected on copper electron microscope grids (400-mesh, carbon-coated Formvar film) by ultracentrifugation

(Beckman coulter SW 40 Ti Swing-Out-Rotor at 70 000 x g for 20 minutes at 4°C). Each grid was stained at room temperature (ca. 20°C) for 30 s with Uranyl acetate (2 % w/w, pH = 4), rinsed twice with 0.02 µm-filtered distilled water, and dried on a filter paper. Grids were examined using a JEOL 1200Ex transmission electron microscope (TEM) operated at 80kV at a magnification of 20,000 to 60,000x to distinguish between bacterial cells with and without intracellular viruses. A bacterium was considered infected when at least five viruses, identified by their shape and size, were clearly visible inside the host cell. At least 400-600 bacterial cells were inspected per grid to determine FVIC (**Supplementary material, Figure S1**). Because mature phages are visible only late in the infection cycle, FVIC counts were converted to the frequency of infected cells (FIC) using the equation FIC = 9.524 FVIC – 3.256 (Weinbauer et al. 2002).

Statistical analysis

Differences in physicochemical and biological variables between the reservoirs were tested by one-way analysis of variance (ANOVA). We used two-way ANOVA to test the potential effects of seasons and depths on the variability in the targeted biological variables. Interactions terms were always included in the analyses. Since the data were log-transformed to satisfy the requirements of normality and homogeneity of variance necessary for parametric statistics, potential relationships between the studied environmental and microbial variables were tested by Pearson correlations analysis using Statistica 13 software package (Statsoft, Dell Software, Tulsa, OK, USA). Bacterial community composition in both the reservoirs in different seasons altogether were compared using pairwise adonis function (Martinez 2017) under devtools package in RStudio, with Bray similarity method and Bonferroni corrected p value. Differences in bacterial phylogenetic composition in relation to the variation in viral parameters (infection rate and viral to bacterial abundance) were assessed using weighted UniFrac distance metric (Lozupone et al. 2006) using all data combined.

Results

Limnological characteristics of the reservoirs

The physico-chemical and microbial characteristics of Grangent and Villerest during the studied months at different depths are shown in **Table 2**. During the sampled seasons, the euphotic depth (Zeu) was never found to exceed 7m and 6m for Grangent and Villerest, respectively. Water temperature and dissolved oxygen concentrations were typical of the

sampled seasons (**Table 2**). Clear differences in the vertical structure of the water column with consistent thermal stratification and formation of anoxia in the bottom waters during summer were evident, similar to our previous reports (Pradeep Ram et al. 2009; 2016b). Overall pH ranged between 7.2 and 9.3. The concentration of potentially limiting inorganic nutrients, notably dissolved reactive phosphate in both the reservoirs was well above the threshold concentration (>15 µg l^{-1} PO_4-P) to induce any kind of limitation for bacterial growth (Pradeep Ram et al. 2009; 2016b). Total organic carbon was significantly ($p < 0.005$) higher in Villerest (range = 6.4 to 9.5 mg l^{-1}) compared to Grangent (range =5.1 to 7.6 mg l^{-1}) with both lakes showing highest values in summer **(Table 2)**. Unlike organic carbon, total nitrogen did not show any significant differences between the reservoirs; however bottom waters showed significantly higher ($p < 0.01$) concentrations than surface waters. Viral and bacterial abundances in Villerest were significantly higher ($p < 0.001$) than Grangent, which was eventually reflected in virus to bacteria ratio (VBR) **(Table 2)**. Like abundances, the frequency of viral infected bacterial cells also reflected higher infection in Villerest (mean = 18.8%) compared to Grangent (mean = 12.1%). Two-way ANOVA indicated that FIC exhibited strong variability ($p < 0.0001$) with depths and seasons and significant interaction ($p < 0.04$) between depths and seasons were observed **(Table 3)**.

Sequence processing and analysis

The paired-end sequencing of 16S rRNA gene produced a total of 655,779 raw sequences which after quality filtering, singletons and chimera removal yielded 310,220 effective sequences from Grangent and Villerest reservoirs with over 6,500 sequences in each library. These effective sequences were normalized for further analysis of diversity indices. Overall the effective sequences were clustered into 1,518 OTUs at 97% similarity. Rarefaction curves for the samples reached saturation, which indicated that sequencing depth was sufficient to cover the whole bacterial diversity (**Supplementary material, Figure S2**). The Good's coverage values at the 3% dissimilarity level were 98-100% among all samples indicating that the libraries could represent most of the species in the natural habitat.

Bacterioplankton Community Diversity and its Taxonomical Distribution

Between the lakes, Villerest recorded higher number of OTUs (range = 206-400) than Grangent (range = 107-293) **(Table 4)**. For all the samples, ANOVA indicated that Villerest harbored significantly higher estimates of species richness (Chao, $p < 0.02$) and diversity (Shannon, $p < 0.008$), suggesting that the former supported more diverse bacterial population

at the sampled months and depths. In the reservoirs, significant difference ($p < 0.003$) in the number of observed OTUs between epilimnion and hypolimnion could be a consequence of prevailing environmental conditions. The bacterial species were most evenly distributed in the epilimnion of Villerest in spring with the highest evenness index of 0.74, whereas in Grangent the highest dominance of particular species was observed in the hypolimnion during autumn with an index of 0.59. (**Table 4**).

The bacterial community of the reservoirs Grangent and Villerest comprised 22 different phyla along with unclassified bacterial members with varying abundance at different depths and seasons. Among these 22 phyla, 20 were recorded in both lakes and only one phylum, namely Latescibacteria and Gracilibacteria, was exclusive to Grangent and Villerest reservoirs, respectively. The bacterial phyla were largely represented by Actinobacteria, Proteobacteria and Bacteroidetes, which covered more than 98% of the total sequences in both reservoirs. Actinobacteria was the most abundant phylum covering 21.9% to 67.1% sequences across the different depths, followed by Proteobacteria which accounted for 17.6% to 55.1% of the total sequences. Actinobacteria were most abundant in all the individual samples except in hypolimnion samples of Villerest during summer and autumn where Proteobacteria were the most abundant. The sequences from the reservoirs were classified into 41 classes, 82 orders, 172 families and 297 genera. At class level, the analyzed reservoirs presented bacterial community composition which is typical of freshwater bacterial groups (**Supplementary material, Figure S3**). Taxonomical affiliations showed Actinobacteria (range: 18.3%-51.3%), Betaproteobacteria (range: 9.7%-32.1%), Acidimicrobiia (range: 6.1%-17.0%), Sphingobacteriia (range: 5.2%-17.0%), Alphaproteobacteria (range: 1.9-14.3%) and Flavobacteriia (range: 0.4%-14.7%) as the six most abundant bacterial classes recovered in each reservoir.

It was observed that Frankiales was the most abundant order (35.5% of total sequences) in all the individual samples. The second most abundant order was Acidimicrobiales followed by Burkholderiales, Sphingobacteriales, Flavobacteriales, Methylophilales, Micrococcales, Cytophagales, Sphingomonadales, Methylococcales, Rhizobiales, and Nitrosomonadales (**Supplementary material, Figure S4**). Genus could not be assigned to 19.3% and 21% of the total sequences in Grangent and Villerest, respectively, which underlines the hitherto untapped bacterial diversity in these lakes. Out of 293 observed genera, only 125 could account for more than 0.01% of sequences and 11 could represent for more than 1% of total sequences. Both reservoirs had 154 common genera with 113 and 26 genera exclusive to Villerest and Grangent, respectively. The two most abundant genera in

Grangent and Villerest belonged to Actinobacterial *hgcI clades* (27.6% of sequences), and *CL500-29 marine group* (11.9% of sequences). The temporal changes of abundant genera varied at specific depth in both reservoirs (**Fig. 1A and B**). Another notable feature in this study is the marked presence of sequences belonging to the genera *Methlylotenera* in the bottom waters (autumn) of Grangent and the presence of both *Methylobacteria* and *Methlylotenera* in Villerest when temporary anoxia occurred due to thermal stratification in the bottom waters during summer.

Differences in bacterial community was demonstrated by two dimensional NMDS analyses based on bray-curtis dissimilarity scores (stress = 0.16, $r^2 = 0.79$) (**Fig. 2**). Similarity among bacterial communities between the reservoirs with seasons and depths were assessed on the basis of Sorensens's similarity coefficient (**Supplementary material, Figure S5**). Core microbiome of both reservoirs was constituted by 4 OTUs (*hgcI clade*, *Planktophila*, *clade CL500-29 marine group*, *Sediminibacterium*) which were present with relative abundance more than 1% in all the samples of Grangent and Villerest. However an additional OTU was observed separately in both the reservoirs as part of the core microbiome (another OTU from *hgcI clade* in Grangent and OTU representing *Limnohabitans* in Villerest).

Viral effect on bacterial diversity and composition

Overall, viral lytic infection was negatively correlated ($p < 0.05$) to bacterial diversity indexes such as bacterial species richness ($R^2 = 0.54$), OTUs ($R^2 = 0.61$) and species diversity ($R^2 = 0.32$). Furthermore, the total richness as assessed by number of species per sample was significantly higher ($p < 0.001$) in Villerest which displayed higher mortality rates than Grangent. Permutation procedure analysis using pairwise adonis function revealed distinct differences in bacterial community composition with reservoir and seasons (**Table 5**). Irrespective of the depths and seasons, pairwise distance matrices from the weighted UniFrac analysis supported the claim that bacterial phylogenetic composition showed significant variability ($p < 0.001$) in samples which were grouped based on threshold viral infection rates (FIC = below10% and above10%) and virus-bacteria ratio (VBR = below10 and above 10) (**Table 6**).

Discussion

This study is one among the few conducted in temperate freshwater reservoirs (Villerest and Grangent) with an aim of characterizing bacterial community composition and diversity on a

fine taxonomic level and evaluating the potential role of viruses to influence them. Two dimensional NMDS analysis suggested that the bacterial communities in both reservoirs were structurally different from each other in time and space, with Villerest harboring more diverse communities than Grangent reservoir. The variable impact of viruses on bacterial communities was evident when they were potentially linked to bacterial diversity indices. Furthermore, weighted UniFrac analysis also indicated that a minimum threshold viral infection and virus-to-bacteria ratio of 10% and 10 respectively is required to exert its impact on bacterial community structure.

Bacterial community composition

In both reservoir systems, next generation Illumina MiSeq sequencing analysis revealed the existence of a typical freshwater bacterial community (Actinobacteria, Proteobacteria and Bacteroidetes), as reported from numerous freshwater systems (Llirós et al. 2014; Zhang et al. 2015; Avila et al. 2017). Members of Actinobacteria mainly belonging to genus *hgcI clade* represented the majority of the total sequences in Grangent (22%) and Villerest (17.8%) which is higher than those obtained in freshwater lakes from the same geographical region (Keshri et al. 2017). The numerical dominance of free living *hgcI* clade comprising of different tribes are previously known to occupy several ecological niches in freshwater systems (Ghylin et al. 2014; Llirós et al. 2014; Hayden and Beman 2016). Despite its high abundances in the studied reservoirs, this clade showed small seasonal variations when compared to other freshwater groups, similar to observations made in other freshwater systems (Allgaier and Grossart 2006, Salcher et al. 2010). Previous reports suggest that the members of *hgcI* clade are characterized by slower growth rates which are indicative of oligotrophic lifestyles and also known to possess several genes that likely provide a competitive advantage when scavenging for energy, carbon, nitrogen and phosphorus in freshwater habitats (Ghylin et al. 2014). Moreover, the large presence of *hgcI clade* in our reservoirs and elsewhere could be related to the fact that they have been qualified as defense specialists due to their small cell size and cell wall structure which protects them from protistan grazing (Jezbera et al. 2006).

Among the Proteobacteria, the class Betaproteobacteria comprising mainly the genera *Limnohabitans* and *Polynucleobacter* was the most abundant followed by class Alpha-, Gamma- and Deltaproteobacteria. The adaptability of Betaproteobacteria to outgrow other bacterial groups under nutrient relaxed conditions make this bacterial class one of the most dynamic, dominant and important groups in various freshwater environments (Salcher et al.

2013; Keshri et al. 2017). Members of *Limnohabitans*, belonging to the R-BT cluster of family Comamonadaceae are known to contribute over-proportionally to total bacterioplankton biomass. Their ability to efficiently utilize algal derived substrates to sustain high growth makes them one of the important players in the carbon flow to plankton grazer food chain (Šimek et al. 2010). However their opportunistic lifestyle with resource specialization makes them highly vulnerable to predation (Salcher 2014). The significant presence of free living bacteria *Polynucleobacter* (family Burkholderiaceae) belonging to BET II tribe in both reservoirs are suggestive of their adaptation to heterogeneous combination of allochthonous (terrestrial) and autochthonous (aquatic) organic carbon compounds that are likely to occur in these waters (Jezbera et al. 2012; Llirós et al. 2014). The variability of *Flavobacterium* with sampled months and depth in both the reservoirs could be related to the concentration of organic carbon and inorganic nutrients, as they have been previously reported to appear during episodes of high bacterial production, emphasizing that resource availability is an important driver for their succession in such eutrophic lakes (Kirchman 2002; Eiler and Bertilsson 2007).

Seasonality induced changes in physico-chemical gradient in the water column especially during the period of summer stratification resulted in stark distinction in the distribution and emergence of certain bacterioplankton communities between epilimnion and hypolimnion in both reservoirs. In Villerest, stratification and oxygen depletion in hypolimnion promoted the appearance of unusual species of anoxygenic phototrophs (*Rhodoferax, Brevundimonas,*) and methylotrophs (*Methylobacter,Methylotenera*) which are important for carbon and nutrient cycling. Although, light is a primary requisite for growth and activity of aerobic anoxygenic phototrophic bacteria, their significant presence in the dark hypolimnion is less clear. However reports suggest that they can remain viable for a limited period of time and are capable of significant growth when exposed to favorable conditions (Koblížek 2015).

Viral impact on bacterial community structure

Numerous studies have highlighted the importance of the quality and quantity of organic matter to influence bacterial community composition and diversity in reservoir systems (Llirós et al. 2014; Yu et al. 2014; Iliev et al. 2017). Therefore, under nutrient enriched conditions the rapid growth of opportunistic bacterial groups could eventually be checked by viral lysis, since viral production is linked to enhanced growth conditions of the host community. Therefore viruses through their host specificity can ultimately impact community

size and diversity (Auguet et al. 2009; Sandaa et al. 2009; Storesund et al. 2015). In this study, significant correlation of bacterial species richness with lytic viral infection corroborates our hypothesis that viruses could serve as a potential top-down factor influencing bacterial community composition. Our data suggests that the decrease in bacterial richness with viral abundance and lytic infection contradicts the specific prediction of "killing the winner hypothesis" which presumes that viral lysis should sustain high bacterial richness (Thingstad and Lignell 1997). Low bacterial richness linked to high viral abundance has been reported from marine environments when a relatively small number of highly active bacterial populations were found to sustain high bacterial activity and also high viral abundances (Winter et al. 2005). Several possible mechanisms such as resistance, decay, and masking of receptors or viral adsorption can allow some populations to become more dominant. Moreover a longer latent period of viruses can reduce bacterial species richness by increasing the risks that viruses in the host cells will be killed by grazing.

Significant differences in bacterial community phylogenetic structure were observed in samples when the frequency of viral infected bacterial cells and virus to bacteria ratio was above a threshold of 10% and 10 respectively. Such changes in bacterial community in relation to viral abundances and their infection rates with time and space in both the reservoirs were suggestive of viral effects on host bacterial community. We propose that both viral lysis and virus to bacteria ratio could be a good proxy as a measure of top-down control of host bacterial community. Viral infection rates of more than 10% have previously been found to have antagonistic effects on the bacterial carbon metabolism through preferential lysis of active cells in reservoir (Pradeep Ram et al. 2016b) and freshwater systems (Pradeep Ram et al. 2015). Studies from a deep-silled fjord ecosystem (Norway) have also suggest bacterial diversity to be top-down controlled by viruses under conditions of higher bacterial viral lysis (virus to bacterial ratio > 10) (Storesund et al. 2015), thus reflecting our main conclusion. However more studies are required to test our claim on the variable impact of viral lysis on bacterial communities in other aquatic systems with differing trophic levels. Viral lysis can either directly influence bacterial community structure by lysing certain members of bacterial community or impact it indirectly through the utilization of viral lysate by non-infected cells. Thus, viral infection does not seem to have an equal effect on all species in a bacterial community. In the light of our above findings, there exists a possibility that both viral infection and bacterial community structure can separately respond to some individual external or environmental factor(s) rather than viruses driving bacterial communities.

Although nutrients have largely been viewed to be a major environmental factor controlling bacterial community, our findings provides insight on the potential role of viruses in influencing bacterial community structure. Such changes in bacterial community structure driven by viral lysis can eventually alter the overall bacterial carbon metabolism which is essentially linked to the functioning of aquatic systems. Results from the present investigation not only lends growing support to increasing evidences of viral impact on bacterial community structure from aquatic environments (Bouvier and del Giorgio 2007; Chow et al. 2014; Keshri et al. 2017), but also advances our understanding in the microbial ecology of these man-made lakes.

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638 **Figure Legends**

639

640 Fig 1. Taxonomical distribution of major bacterial genera with seasons and depths in
641 Grangent (A) and Villerest (B) reservoirs based on the relative abundances obtained from 16S
642 rRNA gene sequencing analysis of DNA. Remaining genera are presented as others.

643

644 Fig 2. Non-metric multidimensional scaling plots of bacterioplankton communities from
645 investigated reservoirs (stress = 0.16, $r^2 = 0.79$).

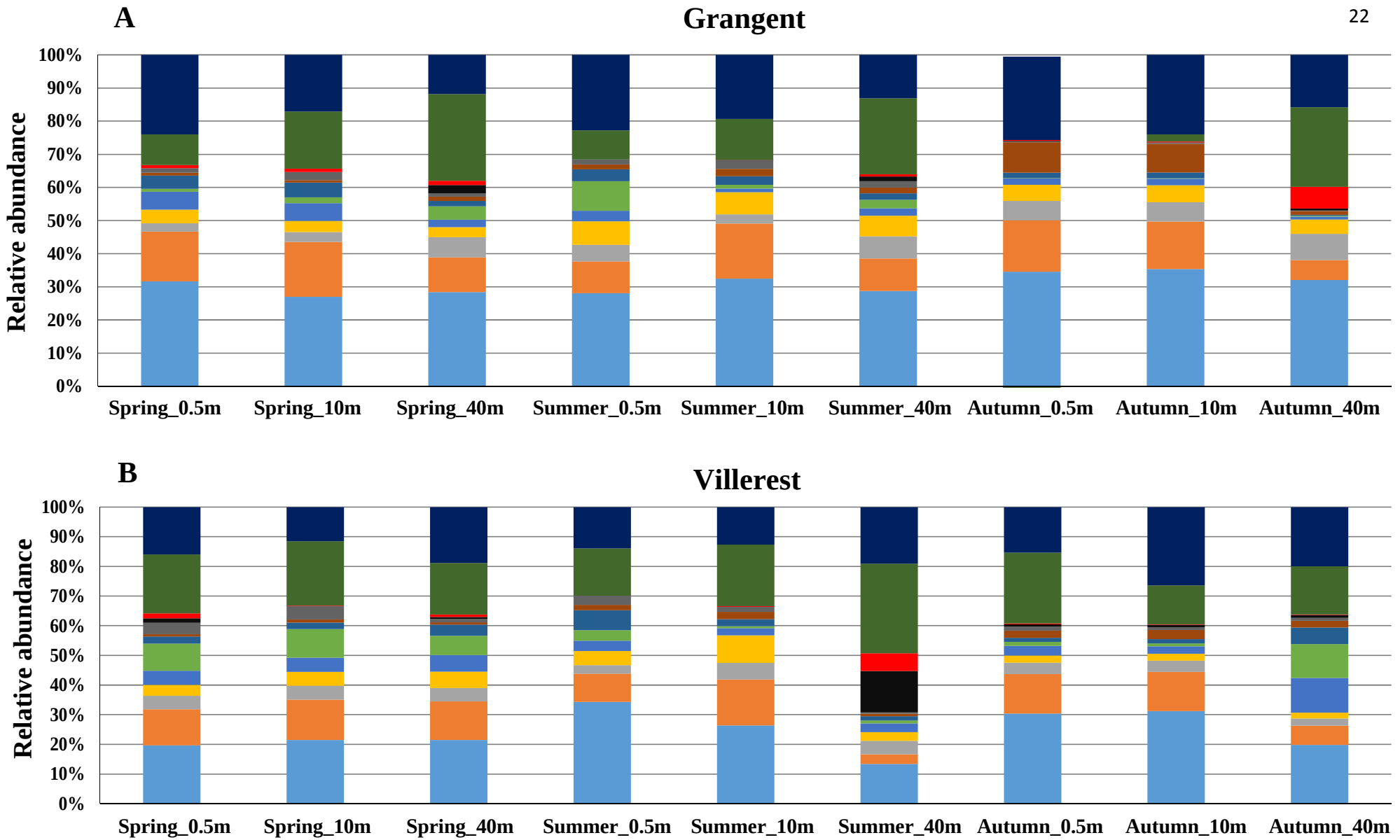


Fig. 1. Pradeep Ram et al.

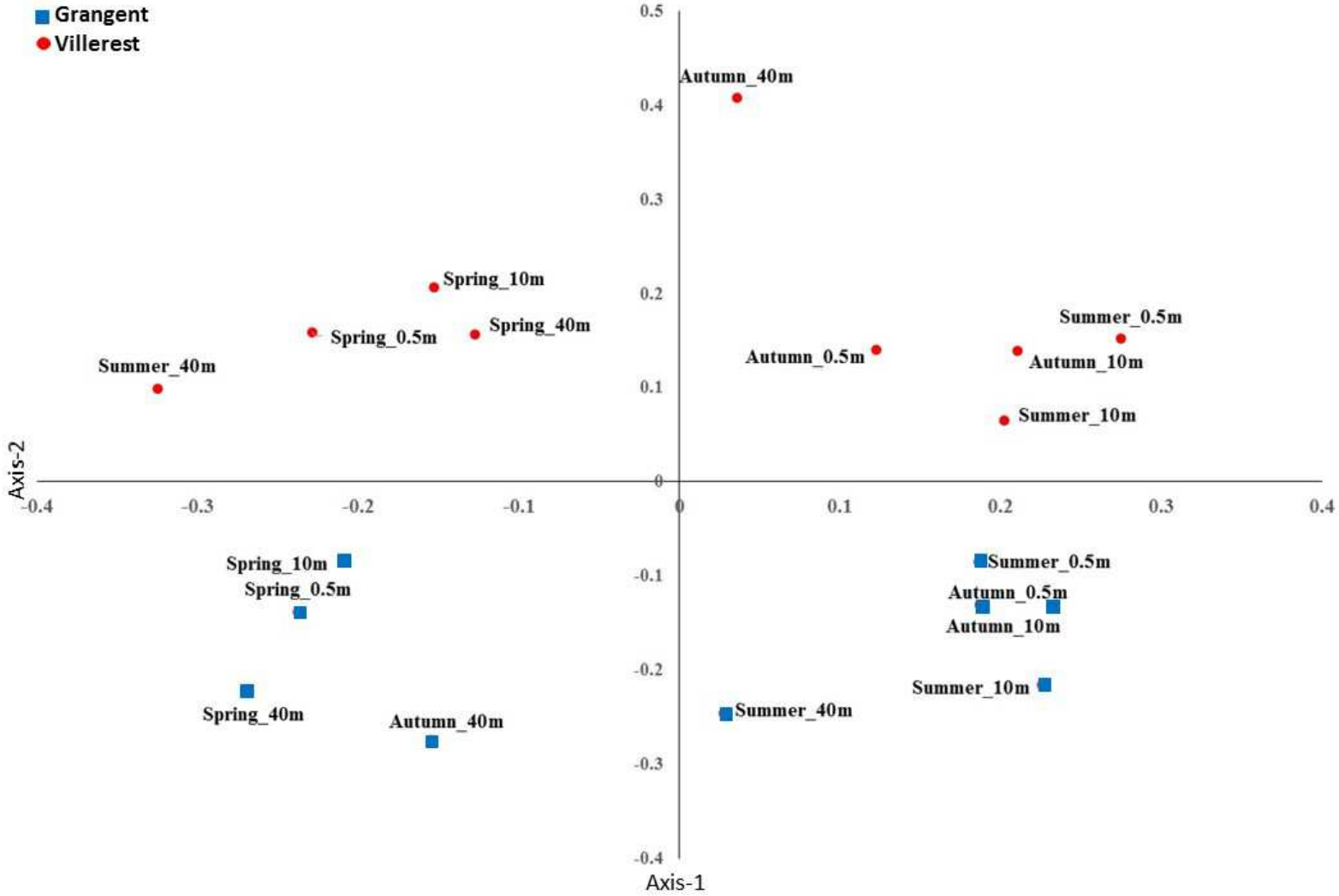


Fig. 2. Pradeep Ram et al.

Table 1. Hydrological and morphometric characteristics of Grangent and Villerest reservoirs

Characteristic	Grangent	Villerest
Altitude (m)	420	315
Trophic status	Eutrophic	Hyper-eutrophic
Maximum depth (m)	50	45
Maximum length (km)	21	40
Maximum width (m)	400	900
Water circulation	holomictic	holomictic
Water residence time (months)	1	3
Surface area (ha)	365	3000
Storage capacity (10^6 m^3)	57.4	128
Water shed area (km^2)	3850	6520
Catchment/lake area ratio	1055	217
Sedimentation rate (cm yr^{-1})	2.0	3.4

Source: Sabart et al. (2009) and author's compilation

Table 2. Mean (SD) environmental characteristics of the water column of Grangent and Villerest reservoirs.

Lake	Seasons	Depth (m)	Temp (°C)	DO (mg l^{-1})	pH	TOC (mg l^{-1})	TN (mg l^{-1})	Viruses (x 10^7 virus like particles ml^{-1})	Bacteria (x 10^6 cells ml^{-1})	VBR	FIC (%)
GRANGENT	Spring	0.5	14.3 ± 0.2	11.2 ± 0.4	7.3 ± 0.2	5.4 ± 0.3	1.2 ± 0.2	0.7 ± 0.1	2.5 ± 0.1	2.8 ±0.2	19.6 ± 0.9
		5	12.4 ± 0.2	10.8 ± 0.4	7.6 ± 0.3	5.3 ± 0.2	1.3 ± 0.1	0.7 ± 0.1	2.7 ± 0.2	2.6 ± 1.3	9.1 ± 0.8
		40	5.8 ± 0.1	8.1 ± 0.3	7.3 ± 0.1	5.1 ± 0.3	1.6 ± 0.2	0.7 ± 0.1	2.1 ± 0.2	3.3 ± 0.4	8.2 ± 0.7
	Summer	0.5	20.3 ± 0.2	7.3 ± 0.5	7.8 ± 0.2	7.7 ± 0.3	0.8 ± 0.1	5.9 ± 0.3	2.8 ± 0.3	21.1 ± 0.9	27.2 ± 1.0
		5	20.2 ± 0.3	7.0 ± 0.4	7.7 ± 0.3	7.6 ± 0.2	0.8 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	10.0 ± 0.5	12.6 ± 0.9
		40	6.6 ± 0.1	ND	7.5 ± 0.2	7.2 ± 0.3	1.3 ± 0.1	1.8 ± 0.1	1.2 ± 0.1	15.0 ± 0.9	7.5 ± 0.4
	Autumn	0.5	15.6 ± 0.1	6.2 ± 0.4	7.6 ± 0.1	7.0 ± 0.2	1.5 ± 0.2	0.8 ± 0.05	1.2 ± 0.2	6.7 ± 0.4	13.7 ± 0.5
		5	15.7 ± 0.2	6.0 ± 0.3	8.3 ± 0.2	6.5 ± 0.3	1.4 ± 0.2	0.8 ± 0.03	1.1 ± 0.2	7.3 ± 0.3	7.2 ± 0.5
		40	7.2 ± 0.1	0.4 ± 0.1	7.8 ± 0.2	5.2 ± 0.4	1.9 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	8.4 ± 0.5	5.3 ± 0.2
VILLEREST	Spring	0.5	13.4 ± 0.2	10.2 ± 0.6	7.6 ± 0.3	6.4 ± 0.3	1.6 ± 0.2	22.3 ± 2.3	29.2 ± 1.5	7.6 ± 0.3	14.3 ± 1.1
		5	12.1 ± 0.2	8.4 ± 0.4	7.6 ± 0.1	6.8 ± 0.2	1.6 ± 0.2	49.2 ± 2.1	29.1 ± 1.2	16.9 ± 0.9	26.0 ± 1.3
		40	11.7 ± 0.1	7.5 ± 0.5	7.2 ± 0.1	7.2 ± 0.3	1.9 ± 0.3	27.9 ± 1.5	26.3 ± 2.3	10.6 ± 0.8	17.0 ± 1.1
	Summer	0.5	23.9 ± 0.2	13.3 ± 0.5	9.3 ± 0.3	8.8 ± 0.5	0.8 ± 0.1	17.3 ± 1.2	21.4 ± 2.1	8.1 ± 0.3	12.5 ± 0.8
		5	20.5 ± 0.2	9.8 ± 0.4	8.3 ± 0.2	8.9 ± 0.6	1.2 ± 0.1	23.3 ± 2.3	22.1 ± 1.9	10.5 ± 0.8	13.0 ± 0.5
		40	12.7 ± 0.1	ND	7.1 ± 0.1	7.9 ± 0.2	1.9 ± 0.2	16.0 ± 1.5	16.2 ± 1.2	10.0 ± 0.9	12.5 ± 0.7
	Autumn	0.5	17.2 ± 0.2	7.0 ± 0.3	7.4 ± 0.2	7.8 ± 0.3	0.5 ± 0.01	12.2 ± 1.9	16.4 ± 1.2	7.4 ± 0.5	7.5 ± 0.5
		5	17.1 ± 0.1	6.0 ± 0.2	7.5 ± 0.2	8.1 ± 0.2	0.8 ± 0.05	10.9 ± 1.4	15.2 ± 1.5	7.2 ± 0.4	9.0 ± 0.4
		40	15.6 ± 0.2	5.9 ± 0.3	7.5 ± 0.1	9.5 ± 0.4	2.7 ± 0.2	16.4 ± 1.2	20.3 ± 1.4	8.1 ± 0.3	11.5 ± 0.6

Temp: water temperature, DO: dissolved oxygen, TOC: total organic carbon, TN: total nitrogen, VBR: virus to bacteria ratio, FIC: frequency of infected cells

ND : not detected

Table 3. Two-way ANOVA values for the effects of seasons and depths on viruses, bacteria, virus to bacteria ratio and frequency of infected cells in Grangent and Villerest Reservoir. Degrees of freedom are 2, 2, 4, and 18 for A, B, AB and error, respectively. *F*, F ratio and *P*, probability.

GRANGENT

Source	Viruses		Bacteria		Virus to Bacteria ratio		Frequency of infected cells	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Seasons (A)	17.88	0.0001	25.27	0.0001	57.64	0.0001	20.57	0.0001
Depths (B)	4.86	0.018	7.00	0.004	4.99	0.016	76.74	0.0001
Interactions (A x B)	450.03	0.0001	80.00	0.0001	39.85	0.0001	40.67	0.0001

VILLEREST

Source	Viruses		Bacteria		Virus to Bacteria ratio		Frequency of infected cells	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Seasons (A)	25.67	0.0001	51.17	0.0001	11.25	0.0001	25.70	0.0001
Depths (B)	76.4	0.003	0.84	0.444	10.00	0.001	6.66	0.005
Interactions (A x B)	994.49	0.0001	123.27	0.0001	140.52	0.0001	67.01	0.0001

Table 4. Bacterial diversity indices along the vertical water column of Grangent and Villerest.

Lake	Seasons	Depth (m)	Goods Coverage	OTUs	Chao	Shannon index	Shannon evenness
GRANGENT	Spring	0.5	0.99	193	256	3.38	0.64
		5	0.99	182	257	3.60	0.69
		40	0.99	198	231	3.64	0.69
	Summer	0.5	1.00	107	107	3.38	0.72
		5	0.99	211	238	3.71	0.69
		40	0.99	273	309	3.98	0.71
	Autumn	0.5	0.99	246	294	3.74	0.68
		5	0.98	293	428	3.82	0.67
		40	0.98	280	391	3.34	0.59
VILLEREST	Spring	0.5	0.99	308	372	4.22	0.74
		5	0.99	265	332	4.03	0.72
		40	0.99	314	374	3.98	0.69
	Summer	0.5	0.99	206	250	3.60	0.68
		5	0.99	266	312	3.84	0.69
		40	0.98	388	457	4.09	0.69
	Autumn	0.5	0.99	393	458	4.28	0.72
		5	0.99	400	447	4.31	0.72
		40	0.98	400	498	4.37	0.73

Table 5. Pairwise Adonis on Bray-Curtis dissimilarities showing the differences in bacterial community structure between the reservoirs in different seasons

Test for differences between lakes and sampling dates	R^2	P
Grangent versus Villerest (Global)	0.11	0.03
Spring versus Summer	0.24	0.003
Summer versus Autumn	0.16	0.147
Spring versus Autumn	0.25	0.015

Table 6. Weighted UniFrac analysis of lakes exhibiting variable viral infection and virus to bacteria ratio.

UniFrac	WScore	p
Viral infection rate at above and below the threshold value of 10%	0.53	0.001
Virus to bacteria ratio at above and below the threshold value of 10	0.46	0.001