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

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32 Abstract

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

53

54 Keywords

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

57 **Introduction**

58

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

71 Generally high concentrations of organic matter in reservoirs tend to support higher
72 standing stock of bacteria due to their increased growth rate and activity. Bacteria inhabiting
73 in reservoir systems are highly dynamic and diverse (Llirós et al. 2014; Yu et al. 2014; Zhang
74 et al. 2015), therefore in such systems bacterial community structure can be expected to vary
75 in accordance with the prevailing environmental conditions with time and space. Compared to
76 bottom up control (i.e. resources), knowledge on the modulation of biotic factors especially
77 by viruses in the control of bacterial communities is scarce, owing to the lack of appropriate
78 tools to explore biological interactions. Bottom-up factors are known to have variable impact
79 on different bacterial communities, but still the net effect is largely dependent on mortality
80 forces such as viral lysis or protistan grazing (Sandaa et al. 2009; Pradeep Ram et al. 2016a).
81 Although many studies have underlined the importance of including viruses as one of the
82 potential factors influencing bacterial community structure in freshwaters (Berdjeb et al.
83 2011; Llirós et al. 2014) relatively fewer investigations have been conducted in complex
84 reservoir systems (Weinbauer et al. 2007, Pradeep Ram and Sime-Ngando 2008).

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

91 2008), although their net influence on bacterial communities has been variable (Schwalbach et
92 al. 2004) or limited (Jardillier et al. 2005; Berdjeb et al. 2011).

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

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

112

113 **Materials and methods**

114

115 **Site description and sampling**

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

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

127 The sampling was carried out in Grangent (20 April, 13 July and 5 October) and
128 Villerest (7 May, 30 July and 26 October) in 2015, covering three distinct seasons namely,
129 spring, summer and autumn respectively. Triplicate water samples were collected from
130 Villerest (45°55'N, 4°02'E) and Grangent (45°27', 4°15'E) reservoirs using Van Dorn bottle
131 (10 L capacity) at three different depths namely, epilimnion (0.5 m below the surface),
132 metalimnion (7 m below the surface), and hypolimnion (1 m above the sediments). The
133 collected water samples were immediately pre-screened through a 150 µm pore size nylon
134 filter to exclude predatory metazoan zooplankton and then transferred to sterile containers.
135 The samples were transported to laboratory in refrigerated boxes and processed immediately
136 upon arrival.

137

138 **Physico-chemical analyses**

139 Secchi measurements (Z_s) were used to estimate the euphotic depth (Z_{eu}) according to the
140 relationship: $Z_{eu} = 2.42Z_s$ (Wetzel and Likens 1995). Water temperature was measured
141 directly with a field thermometer and dissolved oxygen concentration was determined by the
142 Winkler method (Carpenter, 1965). The pH was determined with a standard pH probe (pH
143 1000L, VWR, UK). Total organic carbon were determined by high temperature catalytic
144 oxidation method (680°C) using a TOC analyzer (Shimadzu TOC-V CPN, Japan) (Lønborg
145 and Søndergaard 2009) and total nitrogen using the same analyzer with an attached measuring
146 unit (Mahaffey et al. 2008) with values reported after correction with instrument blank.

147

148 **DNA Extraction**

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

158

159 **PCR Amplification of 16S rRNA gene and Sequencing**

160 PCR amplification was carried out to amplify V3–V4 conserved regions of bacterial 16S
161 rRNA gene sequences in triplicate using the 16S rRNA gene specific primers (forward primer
162 5'-CTTTCCCTACACGACGCTCTTCCGATCTACGGRAGGCAGCAG-3' and reverse 5'-
163 GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT-3') (Liu et al.
164 2007). These primers include the Illumina adapter overhang nucleotide sequences (underlined
165 segment) as well as V3-V4-specific sequences producing an amplicon of 460 bp in length.
166 The libraries for 16S rRNA were prepared according to procedure as detailed in Keshri et al.
167 (2017). Negative PCR controls were also run using Milli-Q water instead of template DNA to
168 confirm that there was no contamination of the reagents or extraction protocol. Triplicate
169 amplicons from respective samples were pooled together in order to decrease the PCR bias
170 led by stochastic fluctuations in amplification efficiencies (Polz and Cavanaugh 1998) and
171 sequenced (paired-end at a length of 300 nucleotides in each direction) on the Illumina MiSeq
172 platform at the INRA, Toulouse, France. Pooling of PCR amplicons has been done in samples
173 obtained from aquatic systems (Pascault et al. 2014, Li et al. 2017) without significantly
174 influencing Illumina based sequencing data (Kennedy et al. 2014). Sequence data which were
175 received as .fastq files was submitted to the National Center for Biotechnology Sequence
176 Read Archive under Bio Project accession number PRJNA395988.

177

178 **Sequence data analysis**

179 Sequence processing was performed using mothur software, version 1.39.1 (Schloss et al.
180 2009). The paired-end MiSeq Illumina reads from the 18 samples were aligned and converted
181 to contigs yielding 693,384 reads. Sequence analysis of each combined single fasta file was
182 processed using the mothur MiSeq SOP accessed on the 10 December 2016 (Kozich et al.
183 2013). The high quality of sequences were filtered using following parameters; base quality
184 score ≥ 20 , deltaq=6, match=1, mismatch= -1, gapopen = -2 and gapextend = -1. Sequences
185 having ambiguous characters and homopolymers longer than 8 bp were removed. High-
186 quality sequences were aligned against the mothur version of SILVA bacterial reference
187 sequences (Pruesse et al. 2007), pre clustered, chimeras and singletons removed and OTUs
188 were defined at 97% identity. After normalizing the number of sequences in each sample
189 based on rarefied or subsampled data i.e. the minimum number of remaining sequences
190 (6,569) in any of the samples); rarefaction was performed on each sample to assess sampling
191 adequacy, using a 100 sequence increment. Alpha diversity was assessed by calculating the
192 richness Chao1 estimator, Shannon diversity, and Evenness indices. The Shannon index is the

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

201

202 **Bacterial and viral abundances**

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

221

222 **Viral lytic infection**

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

227 (Beckman coulter SW 40 Ti Swing-Out-Rotor at 70 000 x g for 20 minutes at 4°C). Each grid
228 was stained at room temperature (ca. 20°C) for 30 s with Uranyl acetate (2 % w/w, pH = 4),
229 rinsed twice with 0.02 µm-filtered distilled water, and dried on a filter paper. Grids were
230 examined using a JEOL 1200Ex transmission electron microscope (TEM) operated at 80kV at
231 a magnification of 20,000 to 60,000x to distinguish between bacterial cells with and without
232 intracellular viruses. A bacterium was considered infected when at least five viruses,
233 identified by their shape and size, were clearly visible inside the host cell. At least 400-600
234 bacterial cells were inspected per grid to determine FVIC (**Supplementary material, Figure**
235 **S1**). Because mature phages are visible only late in the infection cycle, FVIC counts were
236 converted to the frequency of infected cells (FIC) using the equation $FIC = 9.524 FVIC -$
237 3.256 (Weinbauer et al. 2002).

238

239 **Statistical analysis**

240 Differences in physicochemical and biological variables between the reservoirs were tested by
241 one-way analysis of variance (ANOVA). We used two-way ANOVA to test the potential
242 effects of seasons and depths on the variability in the targeted biological variables.

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

253

254 **Results**

255

256 **Limnological characteristics of the reservoirs**

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

261 sampled seasons (**Table 2**). Clear differences in the vertical structure of the water column
262 with consistent thermal stratification and formation of anoxia in the bottom waters during
263 summer were evident, similar to our previous reports (Pradeep Ram et al. 2009; 2016b).
264 Overall pH ranged between 7.2 and 9.3. The concentration of potentially limiting inorganic
265 nutrients, notably dissolved reactive phosphate in both the reservoirs was well above the
266 threshold concentration ($>15 \mu\text{g l}^{-1} \text{PO}_4\text{-P}$) to induce any kind of limitation for bacterial
267 growth (Pradeep Ram et al. 2009; 2016b). Total organic carbon was significantly ($p < 0.005$)
268 higher in Villerest (range = 6.4 to 9.5 mg l^{-1}) compared to Grangent (range = 5.1 to 7.6 mg l^{-1})
269 with both lakes showing highest values in summer (**Table 2**). Unlike organic carbon, total
270 nitrogen did not show any significant differences between the reservoirs; however bottom
271 waters showed significantly higher ($p < 0.01$) concentrations than surface waters. Viral and
272 bacterial abundances in Villerest were significantly higher ($p < 0.001$) than Grangent, which
273 was eventually reflected in virus to bacteria ratio (VBR) (**Table 2**). Like abundances, the
274 frequency of viral infected bacterial cells also reflected higher infection in Villerest (mean =
275 18.8%) compared to Grangent (mean = 12.1%). Two-way ANOVA indicated that FIC
276 exhibited strong variability ($p < 0.0001$) with depths and seasons and significant interaction (p
277 < 0.04) between depths and seasons were observed (**Table 3**).

278

279 **Sequence processing and analysis**

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

289

290 **Bacterioplankton Community Diversity and its Taxonomical Distribution**

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

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

301 The bacterial community of the reservoirs Grangent and Villerest comprised 22
302 different phyla along with unclassified bacterial members with varying abundance at different
303 depths and seasons. Among these 22 phyla, 20 were recorded in both lakes and only one
304 phylum, namely Latescibacteria and Gracilibacteria, was exclusive to Grangent and Villerest
305 reservoirs, respectively. The bacterial phyla were largely represented by Actinobacteria,
306 Proteobacteria and Bacteroidetes, which covered more than 98% of the total sequences in
307 both reservoirs. Actinobacteria was the most abundant phylum covering 21.9% to 67.1%
308 sequences across the different depths, followed by Proteobacteria which accounted for 17.6%
309 to 55.1% of the total sequences. Actinobacteria were most abundant in all the individual
310 samples except in hypolimnion samples of Villerest during summer and autumn where
311 Proteobacteria were the most abundant. The sequences from the reservoirs were classified into
312 41 classes, 82 orders, 172 families and 297 genera. At class level, the analyzed reservoirs
313 presented bacterial community composition which is typical of freshwater bacterial groups
314 (**Supplementary material, Figure S3**). Taxonomical affiliations showed Actinobacteria
315 (range: 18.3%-51.3%), Betaproteobacteria (range: 9.7%-32.1%), Acidimicrobiia (range:
316 6.1%-17.0%), Sphingobacteriia (range: 5.2%-17.0%), Alphaproteobacteria (range: 1.9-14.3%)
317 and Flavobacteriia (range: 0.4%-14.7%) as the six most abundant bacterial classes recovered
318 in each reservoir.

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

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

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

345

346 **Viral effect on bacterial diversity and composition**

347 Overall, viral lytic infection was negatively correlated ($p < 0.05$) to bacterial diversity indexes
348 such as bacterial species richness ($R^2 = 0.54$), OTUs ($R^2 = 0.61$) and species diversity ($R^2 =$
349 0.32). Furthermore, the total richness as assessed by number of species per sample was
350 significantly higher ($p < 0.001$) in Villerest which displayed higher mortality rates than
351 Grangent. Permutation procedure analysis using pairwise adonis function revealed distinct
352 differences in bacterial community composition with reservoir and seasons (**Table 5**).
353 Irrespective of the depths and seasons, pairwise distance matrices from the weighted UniFrac
354 analysis supported the claim that bacterial phylogenetic composition showed significant
355 variability ($p < 0.001$) in samples which were grouped based on threshold viral infection rates
356 (FIC = below10% and above10%) and virus-bacteria ratio (VBR = below10 and above 10)
357 (**Table 6**).

358

359 **Discussion**

360

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

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

371

372 **Bacterial community composition**

373 In both reservoir systems, next generation Illumina MiSeq sequencing analysis revealed the
374 existence of a typical freshwater bacterial community (Actinobacteria, Proteobacteria and
375 Bacteroidetes), as reported from numerous freshwater systems (Llirós et al. 2014; Zhang et al.
376 2015; Avila et al. 2017). Members of Actinobacteria mainly belonging to genus *hgcI clade*
377 represented the majority of the total sequences in Grangent (22%) and Villerest (17.8%)
378 which is higher than those obtained in freshwater lakes from the same geographical region
379 (Keshri et al. 2017). The numerical dominance of free living *hgcI clade* comprising of
380 different tribes are previously known to occupy several ecological niches in freshwater
381 systems (Ghylin et al. 2014; Llirós et al. 2014; Hayden and Beman 2016). Despite its high
382 abundances in the studied reservoirs, this clade showed small seasonal variations when
383 compared to other freshwater groups, similar to observations made in other freshwater
384 systems (Allgaier and Grossart 2006, Salcher et al. 2010). Previous reports suggest that the
385 members of *hgcI clade* are characterized by slower growth rates which are indicative of
386 oligotrophic lifestyles and also known to possess several genes that likely provide a
387 competitive advantage when scavenging for energy, carbon, nitrogen and phosphorus in
388 freshwater habitats (Ghylin et al. 2014). Moreover, the large presence of *hgcI clade* in our
389 reservoirs and elsewhere could be related to the fact that they have been qualified as defense
390 specialists due to their small cell size and cell wall structure which protects them from
391 protistan grazing (Jezbera et al. 2006).

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

397 2013; Keshri et al. 2017). Members of *Limnohabitans*, belonging to the R-BT cluster of
398 family Comamonadaceae are known to contribute over-proportionally to total
399 bacterioplankton biomass. Their ability to efficiently utilize algal derived substrates to sustain
400 high growth makes them one of the important players in the carbon flow to plankton grazer
401 food chain (Šimek et al. 2010). However their opportunistic lifestyle with resource
402 specialization makes them highly vulnerable to predation (Salcher 2014). The significant
403 presence of free living bacteria *Polynucleobacter* (family Burkholderiaceae) belonging to
404 BET II tribe in both reservoirs are suggestive of their adaptation to heterogeneous
405 combination of allochthonous (terrestrial) and autochthonous (aquatic) organic carbon
406 compounds that are likely to occur in these waters (Jezbera et al. 2012; Llirós et al. 2014).
407 The variability of *Flavobacterium* with sampled months and depth in both the reservoirs could
408 be related to the concentration of organic carbon and inorganic nutrients, as they have been
409 previously reported to appear during episodes of high bacterial production, emphasizing that
410 resource availability is an important driver for their succession in such eutrophic lakes
411 (Kirchman 2002; Eiler and Bertilsson 2007).

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

423

424 **Viral impact on bacterial community structure**

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

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

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

464 Although nutrients have largely been viewed to be a major environmental factor
465 controlling bacterial community, our findings provides insight on the potential role of viruses
466 in influencing bacterial community structure. Such changes in bacterial community structure
467 driven by viral lysis can eventually alter the overall bacterial carbon metabolism which is
468 essentially linked to the functioning of aquatic systems. Results from the present investigation
469 not only lends growing support to increasing evidences of viral impact on bacterial
470 community structure from aquatic environments (Bouvier and del Giorgio 2007; Chow et al.
471 2014; Keshri et al. 2017), but also advances our understanding in the microbial ecology of
472 these man-made lakes.

473

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483

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485

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487

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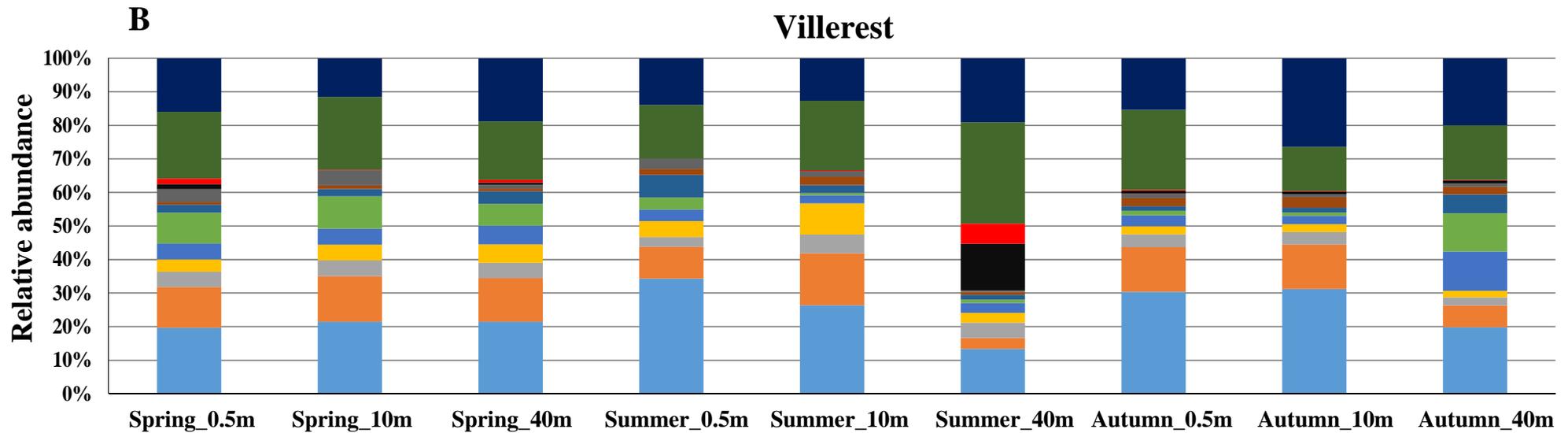
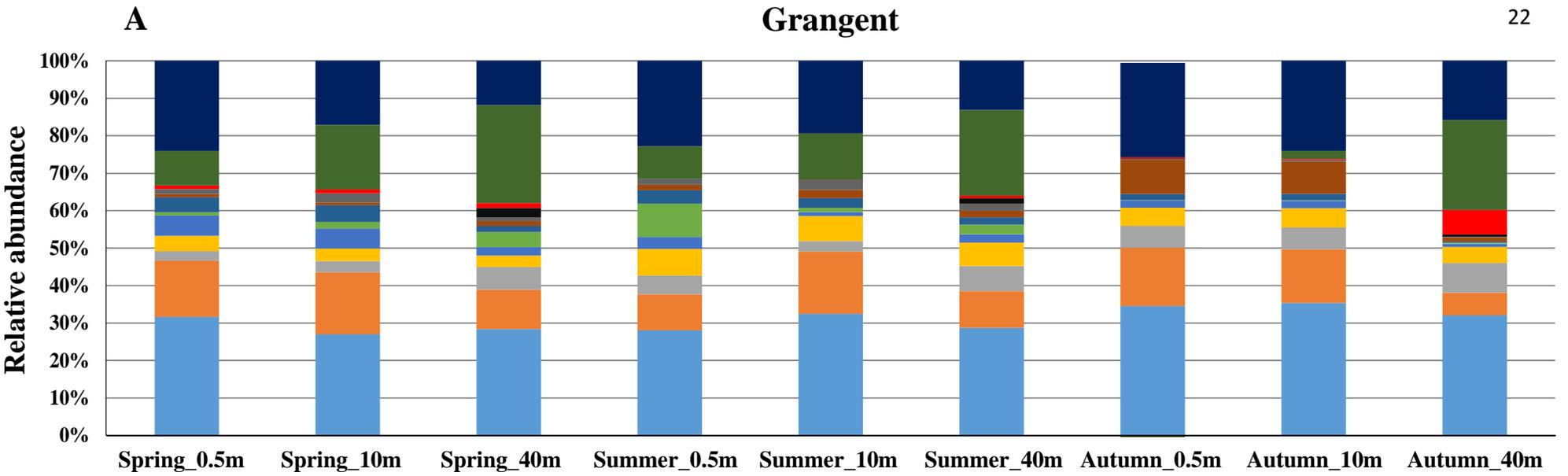
638 **Figure Legends**

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, $r^2 = 0.79$).



- *hgcI_clade*
- *CL500-29_marine_group*
- *Sediminibacterium*
- *Candidatus_Planktophila*
- *Limnohabitans*
- *Flavobacterium*
- *Polynucleobacter*
- *LD28_freshwater_group*
- *Fluviicola*
- *Methylobacter*
- *Methylothera*
- *Others*
- *Unclassified*

Fig. 1. Pradeep Ram et al.

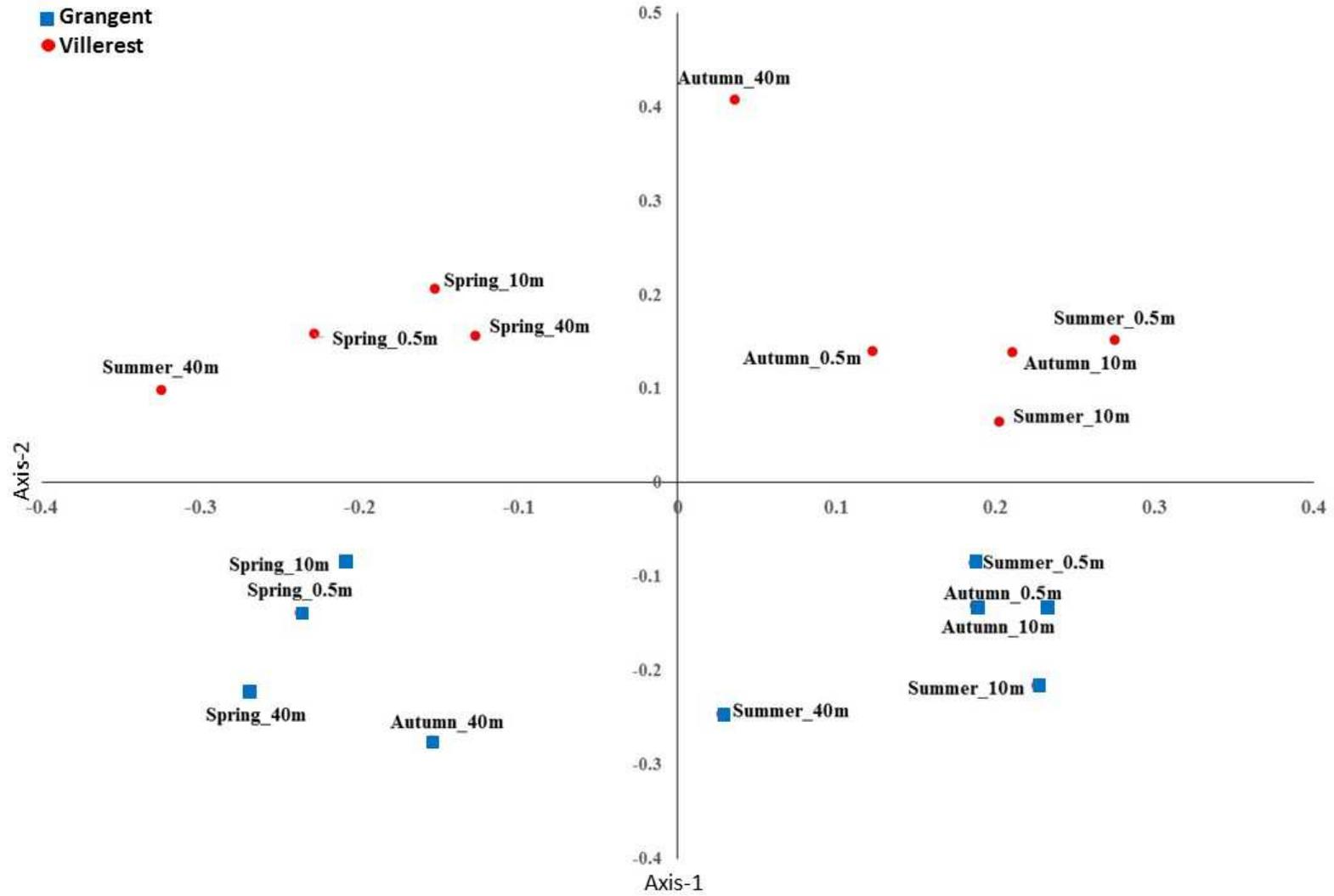


Fig. 2. Pradeep Ram et al.

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	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
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	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
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	R²	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