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1 **Differential impact of top-down and bottom-up forces in structuring**
2 **freshwater bacterial communities**

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16 **Running title: Bottom-up and top-down effects on bacterial community**

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31

32 **ABSTRACT**

33

34 Limited data exists on the simultaneous impact of bottom-up (nutrients) and top-down
35 (viruses and heterotrophic nanoflagellates) forces in shaping freshwater bacterial
36 communities. In our laboratory microcosms, nutrient additions (organic and inorganic) and
37 viral reduction approach led to the proliferation of high nucleic acid (HNA) bacterial
38 subpopulation without an increase in phage abundance. High viral-mediated bacterial lysis in
39 the presence of nanoflagellates yielded high proportion of low nucleic acid bacterial
40 subpopulation. 16S rRNA gene sequence analysis indicated that members of class
41 Proteobacteria and Bacteroidetes evoked differential responses to nutrients and mortality
42 forces thereby resulting in differences ($p < 0.001$) in bacterial community composition and
43 diversity, as observed from ANOSIM and UniFrac analysis. Bacterial species richness (Chao)
44 and diversity (Shannon) index was significantly higher ($p < 0.001$) in the presence of both the
45 top-down factors and viruses alone, whereas lower host diversity was observed under nutrient
46 relaxation of growth limiting substrates due to the explosive growth of opportunistic HNA
47 bacterial subpopulation. Our results are in agreement with the theoretical model of 'Killing
48 the Winner' where the availability of growth limiting substrates can act as a stimulating factor
49 for host community composition while top-down forces can operate in the control of host
50 diversity.

51

52 **Keywords:** bacteria, viruses, nutrients, bacterial community, 16S rRNA gene sequencing,
53 freshwater microcosms

54

55 INTRODUCTION

56

57 Top-down control of bacterial populations especially by viruses has received attention in
58 recent years since the finding of its high numbers in a wide variety of aquatic systems (Weitz
59 and Wilhelm 2012; Sime-Ngando 2014). Together with bacterivorous nanoflagellates, viruses
60 are now considered to be a major ecological determinant in regulating the structure and
61 function of heterotrophic microbial communities (Suttle 2007; Sandaa *et al.* 2009). The
62 recognition of viruses as dynamic components of planktonic communities through their
63 destruction up to 60% of daily produced bacterial biomass in freshwater systems has
64 challenged the conventional view that bacterial communities were largely thought to be
65 impacted by bottom up factors. Among the interacting forces structuring bacterial
66 communities, most attention has been on bottom-up effects (physical and chemical
67 parameters), with lesser focus given to bacterial interaction with other segments of a
68 microbial community such as viral or protistan activity (Bouvy *et al.* 2011; Pradeep Ram *et*
69 *al.* 2016).

70 Within the microbial part of aquatic systems, bottom-up (nutrient supply) and top-
71 down (protistan grazing and viral lysis) processes are known to influence and control bacterial
72 community composition and diversity in time and space. Viruses and protists can directly
73 impact bacterial communities either through their host specific lysis and size selective grazing
74 respectively or indirectly through the alteration of organic pools by mortality processes (Liu *et*
75 *al.* 2015). Previous studies carried out in freshwater systems have revealed that under nutrient
76 limiting conditions, synergistic interactions between grazing and viral lysis tend to generate
77 growth resources for fueling bacterial activity (Simek *et al.* 2001; Pradeep Ram and Sime-
78 Ngando 2008), however their net influence on the taxonomical resolution of bacterial
79 communities is less clearly understood. Detail examination of how the trophic interactions
80 such as the supply of resources and mortality factors that lead to alteration of bacterial
81 community composition and their diversity have been less studied. In seawater microcosms,
82 relatively few moderate changes in bacterial community amidst of high viral abundance and
83 their apparent high turnover made it difficult to distinguish statistically significant effects of
84 viruses on bacterial community composition (Schwalbach *et al.* 2004). In perialpine lakes,
85 multivariate ordination analyses suggested that bottom-up factors (nutrients and temperature)
86 was the driving force behind the bacterial community shifts in spite of prevailing high viral
87 abundances (Berdjeb *et al.* 2011). Considering the complexity and the importance of
88 interactions between bottom-up and top-down factors, we used a reconstitution approach

89 using incubation of bacteria with viruses and virus-free treatments together with nutrient
90 manipulation, and both the sources of mortality in parallel to simultaneously examine the
91 effect of both top-down and bottom-up control on bacterial community.

92 In this study we extend the line of investigation to a natural freshwater system (Lake
93 Pavin, France) to investigate the variable effect and impact of bottom-up (substrate) and top-
94 down (viruses and heterotrophic nanoflagellates) on the bacterial community. Lake Pavin is a
95 high altitude, meromictic freshwater lake where its late season (autumn) is characterized by
96 lowest annual concentration of nutrient (carbon and inorganic) and chlorophyll pigments, both
97 of which are known to have direct influence on bacterial physiology (inferred from the
98 nucleic acid content, high versus low) and henceforth on viral life styles, namely lytic and
99 lysogeny (Palesse *et al.* 2014). Therefore the strategies adopted by the viruses, whether to kill
100 or not to kill can have variable impacts on certain members of bacterial community (Bouvier
101 *et al.* 2007; Pradeep Ram and Sime-Ngando 2008; Chen *et al.* 2019). Several bacterial groups
102 are known to have specific preferences for nutrient uptake especially during the nutrient
103 depleted season, henceforth it can be expected that different members of the group with
104 varying physiology and activity which tend to occupy different niches could signal changes in
105 host-virus interactions (Øvreås *et al.* 2003; Liu *et al.* 2015).

106 Here we hypothesize that the relative abundances of low and high nucleic acid content
107 bacterial subpopulation possessing differing physiological state and metabolism can strongly
108 be influenced by the presence or type of mortality factors and nutrient supply, which
109 eventually can evoke significant patterns and variations in bacterial community composition
110 and diversity estimates. We also investigated the extent to which we can link bacterial
111 diversity estimates and community composition to top-down and nutrient availability
112 respectively. In this study, high throughput sequencing of 16S rRNA genes using Illumina
113 MiSeq platform was the method of preference for capturing the effect of top-down and
114 bottom-up forcing's on host taxonomical changes in bacterial community because they not
115 only facilitates a rapid and high resolution of host community but also provides information
116 on bacteria-virus interactions at the community level.

117

118 **MATERIALS AND METHODS**

119

120 **Study site, sampling and experimental design**

121 Lake water samples for the experiment was collected from oligo-mesotrophic Lake Pavin (see
122 Palesse *et al.* [2008] for detailed characteristics) at euphotic depth (5m) using Van Dorn bottle

123 at the deepest central point of the lake (45°29'N ; 2°56'E) on 16 October 2015 (autumn
124 season). The water samples collected in triplicates (i.e. three replicate hauls) were filled in
125 clean insulated carboys and transported to laboratory well protected from heat and light
126 radiations. Upon arrival, the samples were immediately processed for microcosm set up.

127 Preparation of water samples for the experimental setup is shown in **Fig. 1**. The
128 experiment involved three fractions, 1). “grazer fraction” (containing bacteria, viruses and
129 heterotrophic nanoflagellates, BVF) via filtration through a succession of nylon fibre sieves
130 (150, 63 and 25 μm porosities), 2). “grazer free fraction” (containing bacteria and viruses,
131 BV) via filtration through 1 μm pore-size filter (cellulose acetate, Sartorius) and 3). “viral
132 reduced fraction” (containing bacteria with depleted viral abundance, B) obtained from
133 washing and flushing out the viruses in 10 fold concentrated samples (by ultrafiltration, Model
134 DC 10LA, Amicon®) with ultrafiltrate (virus free water) according to Weinbeuer and Suttle
135 (1996). For obtaining bacteria and viruses twice that of *in situ* abundances (BV2x and B2x),
136 the concentrated samples were diluted accordingly with ultrafiltrate to obtain the desired
137 concentration. Both grazer free (BV) and viral reduced fractions (B), which received organic
138 and inorganic nutrients, individually and in combinations, including organic carbon (glucose,
139 100 μM C), nitrate (NaNO_3 , 25 μM N) and phosphate (NaH_2PO_4 , 5 μM P) in three different
140 treatments were designated as BV-C, BV-NP, BV-CNP (containing bacteria and viruses at *in*
141 *situ* abundances) and B-C, B-NP, B-CNP (represents viral reduced fraction) respectively. The
142 experimental samples were equally distributed in polycarbonate bottles (5L) and incubated in
143 the dark at *in situ* temperature ($14.7 \pm 1^\circ\text{C}$). The samples were taken at every 12 hour interval
144 from time zero (T0) to 108 hours (T108) for bacterial and viral abundances, and at 24 hour
145 interval from T0 to T96 for viral lytic infection and community composition. Prior to use, all
146 glass wares, tubings and filtration devices coming in contact with the experimental samples
147 were acid washed (10% HCl) and thoroughly rinsed with de-ionized water. The experimental
148 microcosms were set up within 4 hours from the time of sampling.

149 Our reconstitution approach involving filtration and dilution procedures were
150 successful in obtaining desired concentration of bacterial and viral abundances among
151 different microcosms. Viral reduction treatment was chosen to observe the response and
152 development of bacterial communities under reduced viral top-down pressure. The followed
153 procedure was effective in reducing viral abundance by about two fold when compared to its
154 *in situ* concentration.

155

156 **Flow cytometry**

157 Single-celled bacteria and viruses were counted using flow cytometer (FACS Calibur, Becton
158 Dickinson, Franklin Lake, NJ, USA). The optimized flow cytometry protocol (different
159 sample dilutions, threshold levels, flow rates etc.) that was previously validated for the
160 enumeration of bacteria and viruses from Lake Pavin was adopted (Palesse *et al.* 2014).
161 Samples (2 ml each) were fixed with paraformaldehyde (0.5% final concentration) for 30
162 minutes in the dark at 4°C. Following fixation, the samples were diluted in 0.02 µm filtered
163 TE buffer (10 mM Tris–HCl and 1 mM EDTA, pH 8), and stained with SYBR Green I
164 (10,000 fold dilution of commercial stock, Molecular Probes, Oregon, USA). The mixture
165 was incubated for 5 min at ambient temperature, heated for 10 min at 80°C in the dark, and
166 cooled for 5 min at room temperature prior to analysis. The samples were then passed through
167 flow cytometer equipped with an air-cooled laser providing 15 mW at 488 nm with the
168 standard filter set-up (Brussaard *et al.* 2010). Bacteria and viruses were discriminated based
169 on side light scatter versus green fluorescence (530 nm wave- length, fluorescence channel 1
170 of the instrument) plot. Flow cytometry list modes were analyzed using CellQuest Pro
171 software (BD Biosciences, San Jose, CA, USA; version 4.0). TE buffer blanks were routinely
172 examined to control for contamination of the equipment and reagents.

173 Flow cytometry allowed the identification and quantification of two major bacterial
174 fractions: low nucleic acid content (LNA cells) and high nucleic acid content (HNA cells)
175 based on the differences in the individual cell fluorescence (related to nucleic acid content)
176 and in the side and forward light scattering signal on the cytogram (Gasol *et al.* 1999; Bouvier
177 *et al.* 2007).

178

179 **Heterotrophic nanoflagellate abundance**

180 Samples for the measurements of heterotrophic nanoflagellate (HNF) abundance were fixed
181 immediately after sampling with 1% glutaraldehyde (final concentration). Primulin-stained
182 HNF collected on 0.8-µm polycarbonate black filters (25 mm diameter) were counted under
183 UV excitation under a LEICA epifluorescent microscope as described by Caron (1983). A
184 total of 200-400 nanoflagellates from each slide were counted along several transects (SD <
185 10%). All solutions were filter-sterilized, and a blank was routinely examined to control for
186 contamination of equipment and reagents.

187

188 **Viral lytic infection**

189 Viral lytic infection was inferred from the frequency of visibly infected cells (FVIC)
190 according to Pradeep Ram and Sime-Ngando (2008). Bacterial cells contained in 8 ml of

191 glutaraldehyde fixed water samples (final concentration 1%) were collected on copper
192 electron microscope grids (400-mesh, carbon-coated Formvar film) by ultracentrifugation
193 (Beckman coulter SW 40 Ti Swing-Out-Rotor at 70 000 x g for 20 minutes at 4°C). Each grid
194 was stained at room temperature (ca. 20°C) for 30 s with Uranyl acetate (2 % w/w, pH = 4),
195 rinsed twice with 0.02 µm-filtered distilled water, and dried on a filter paper. Grids were
196 examined using a JEOL 1200Ex transmission electron microscope (TEM) operated at 80kV at
197 a magnification of 20,000 to 60,000x to distinguish between bacterial cells with and without
198 intracellular viruses. A bacterium was considered infected when at least five viruses,
199 identified by their shape and size, were clearly visible inside the host cell. At least 400-600
200 bacterial cells were inspected per grid to determine FVIC. Because mature phages are visible
201 only late in the infection cycle, FVIC counts were converted to the frequency of infected cells
202 (FIC) using the equation $FIC = 9.524 FVIC - 3.256$ (Weinbauer *et al.* 2002).

203

204 **DNA Extraction**

205 Bacteria from the experimental water samples (150-200 ml) were collected onto 0.2µm white
206 polycarbonate membrane filters (Sartorius, Germany) by applying low vacuum and stored for
207 a week at -20°C. The total DNA was then extracted from the filters by using Fast DNA spin
208 kit for soil (MP Biomedicals, Germany), which included two consecutive bead-beating steps,
209 first, for 40 s, followed by a second bead beating 60 s to disrupt additional, previously un-
210 lysed cells. Remaining steps were followed as per manufacturer's protocol. Yield and quality
211 of the extracted DNA samples were checked on 0.8% agarose gel, and the extracted genomic
212 DNA was quantified, checked for purity at A260/280 nm by NanoDrop spectrophotometer
213 (Thermo Scientific, USA.). The DNA was stored at -20 °C until further processing.

214

215 **16S rRNA gene amplification and sequencing**

216 PCR amplification was carried out to amplify V3–V4 conserved regions of bacterial 16S
217 rRNA gene sequences in triplicate using the 16S rRNA gene specific primers (forward primer
218 5'-CTTCCCTACACGACGCTCTTCCGATCTACGGRAGGCAGCAG-3' and reverse 5'-
219 GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT-3') (Liu *et al.*
220 2007). These primers include the Illumina adapter overhang nucleotide sequences (underlined
221 segment) as well as V3-V4-specific sequences producing an amplicon of 460 bp in length.
222 The PCR library preparation was carried out using KAPA Hi Fi Hot Start Ready-mix PCR Kit
223 (KAPA BIOSYSTEMS® U.S.A.). Briefly, each 25 µL of PCR reaction contains 10 ng/µL (1
224 µL) of genomic DNA template, 12.5 µL 2X Mastermix KAPA Hi Fidelity DNA polymerase

225 (1 U), 10 μ M each primer and nuclease free water. PCR reactions were carried out with initial
226 denaturation step at 95 °C for 3 min followed by 24 cycles of 98 °C for 30 s, 65 °C for 30 s,
227 and 72 °C for 30 s and ended with an extension step at 72 °C for 5 min. The PCR products
228 were confirmed by 1.5% agarose gel electrophoresis. Amplicons from respective samples
229 were pooled together in order to decrease the PCR biasness led by stochastic fluctuations in
230 amplification efficiencies and sequenced (paired-end at a length of 300 nucleotides in each
231 direction) on the Illumina MiSeq platform at the INRA, Toulouse (France). Pooling of PCR
232 amplicons has not been significantly shown to influence Illumina based sequencing data and
233 such procedure has been previously done in samples obtained from freshwater systems
234 (Pascualt *et al.* 2014). Sequence data were received as .fastq files and has been archived in
235 NCBI Sequence Read Archive under the accession number PRJNA479735.

236

237 **Sequence data analysis**

238 Sequence processing was performed using mothur software, version 1.39.1 (Schloss *et al.*
239 2009). The paired-end MiSeq Illumina reads from the samples were aligned and converted to
240 contigs yielding 2,007,708 reads. Sequence analysis of each combined single fasta file was
241 processed using the mothur MiSeq SOP accessed on the 10 December 2016 (Kozich *et al.*
242 2013). Sequences having ambiguous characters, homopolymers longer than 8 bp were
243 removed. High-quality sequences were aligned against the mothur version of SILVA bacterial
244 reference sequences (Pruesse *et al.* 2007) pre clustered, chimeras and singletons removed and
245 OTUs were defined at 97% identity. Sequence processing was followed as described in
246 Keshri *et al.* (2017). Alpha and Beta diversity was calculated on rarified data. Non-metric
247 multidimensional scaling (NMDS) was used to visualize the similarity in bacterial
248 communities between samples. Similarity among bacterial communities among different
249 experimental treatments was also assessed using theayc distance matrix (Yue and Clayton
250 2005).

251

252 **Physico chemical parameters**

253 Water temperature and dissolved oxygen profiles were determined *in situ* using an YSI Pro
254 ODOTM probe (Yellow Springs, Ohio, USA). Phytoplankton concentration was estimated *in*
255 *situ* as μ g equivalent Chlorophyll-*a* L⁻¹ (Chl) using a submersible fluorescence photometer
256 (Fluoroprobe; BBE-Moldaenke, Kiel, Germany). Total organic carbon and total nitrogen
257 concentrations were determined by high temperature catalytic oxidation method (680°C)
258 using a TOC analyzer (Shimadzu TOC-V CPN, Japan).

259

260 **Statistical analysis**

261 The response of bacterial and viral parameters to different nutrient (inorganic and organic)
262 additions was tested by analysis of variance (ANOVA), with a significant response at $p <$
263 0.05 . Potential relationships among various measured variables were tested by Pearson's
264 correlation analysis. All statistical analyses were performed using Minitab's software for
265 Windows (Release 17, Minitab). Differences in bacterial community composition among the
266 experimental treatments were assessed using UniFrac weighted distance metric (Lozupone *et*
267 *al.* 2006). The randomization/permutation procedure analysis of similarities (ANOSIM) was
268 used to evaluate the significant differences between groups. The ANOSIM statistic R displays
269 the degree of separation between groups and is calculated by the difference of the between-
270 group and within-group mean rank similarities. The value of $R = 1$ denotes complete
271 separation whereas $R = 0$ suggests no separation (Clarke and Gorley 2001).

272

273 **RESULTS**

274

275 **Initial conditions**

276 The experiment was carried out during the clear water phase where the water temperature (14
277 $\pm 0.2^\circ\text{C}$), dissolved oxygen ($8.2 \pm 0.4 \text{ mg l}^{-1}$), organic carbon ($1.8 \pm 0.3 \text{ mg l}^{-1}$), total nitrogen
278 ($0.2 \pm 0.02 \text{ mg l}^{-1}$) and chlorophyll *a* ($1.7 \pm 0.3 \text{ mg l}^{-1}$) concentrations were typical of the
279 studied season. Phosphate concentration was below the detection limit. At the start of the experiment
280 (T0), the mean viral and bacterial abundance were $1.8 \pm 0.2 \times 10^7 \text{ ml}^{-1}$ and $0.9 \pm 0.1 \times 10^6$ cells
281 ml^{-1} respectively, with virus to bacteria ratio of 20. Low nucleic acid bacterial cells (LNA)
282 were 8 times higher than high nucleic acid bacterial cells (HNA). Actinobacteria (69.1 %),
283 Proteobacteria (17.3 %), and Bacteroidetes (10.5 %) together accounted for 97% of the total
284 bacterial phyla at start of the experiment. HNF abundance estimated the start of the
285 experiment was $1.7 \pm 0.2 \times 10^3 \text{ cells ml}^{-1}$.

286

287 **Bacterial and viral abundance**

288 The results presented here correspond to mean value (triplicates) of different incubation times
289 (T0 to T108). Addition of nutrients (carbon+nitrogen+phosphorous) evoked a strong and
290 significant ($F_{1,26}=29.4$, $p < 0.0001$) increase in bacterial abundance from T48 and T12 in BV-
291 CNP and B-CNP compared to T0 (**Fig. 2A&B**). The maxima in bacterial abundance that was
292 observed at T108 ($12.6 \pm 0.5 \times 10^6 \text{ cells ml}^{-1}$) and T72 ($15.0 \pm 0.8 \times 10^6 \text{ cells ml}^{-1}$) in BV-

293 CNP and B-CNP respectively was one order of magnitude higher than the abundance at the
294 start of the experiment. In both the cases the increase in bacteria was never accompanied by
295 proportional increase in viral abundances, thereby resulting in significantly lower ($F_{1,24}=27.5$,
296 $p < 0.0001$) virus to bacteria ratio (range = 1.1-2.2) compared to microcosms without nutrient
297 additions (range = 11.1-23.1). Except in CNP treatments, viruses followed host abundance in
298 the grazer and grazer free fraction resulting in a significant correlation ($r = 0.88$, $p < 0.001$)
299 between the two. Generally, in the viral reduced microcosms continuous or moderate decline
300 in viral abundance was observed from start to end of the experiment (**Fig. 2B**)

301 Addition of nutrient limiting substrates and viral reductions contributed to the
302 variability of HNA and LNA populations with incubation time. In the microcosms without
303 nutrient additions (BVF, BV and BV2X), LNA was always found to dominate HNA cells
304 (LNA:HNA = 1.4 to 13.9), whereas in microcosms with carbon supplements the reverse was
305 observed (HNA:LNA = 1.0 to 15.2) (**Fig. 3**). In all viral reduced microcosms, HNA was the
306 dominant subpopulation (**Fig. 3**).

307

308 **Phage infection**

309 Viral lysis was more evident in the grazer treatment where the highest percentage of viral
310 infection (30.3%) coincided with the maxima of HNF abundance at T48 (**Supplementary**
311 **material, Fig. S1**). Overall, viral lysis in the grazer and grazer free fraction varied by a factor
312 of 10 and was significantly ($F_{1,18}=30.1$, $p < 0.0001$) higher compared viral reduced treatments
313 (**Fig. 4A&B**). High viral lysis did not bring about significant variability in virus to bacteria
314 ratio suggesting a constant production and loss. The significant increase in host population
315 following nutrient addition in grazer free fraction was never accompanied by increase in lytic
316 infection.

317

318 **16S rRNA gene sequencing analysis**

319 A total of 1,168,347 effective sequences after quality and chimera removal were analyzed
320 further with over 15,000 sequences in each library. Clustering at 97% similarity resulted in a
321 total of 9,183 OTUs. All of the rarefaction curves reached saturation indicating that
322 sequencing depth was considerable enough to cover the whole bacterial diversity
323 (**Supplementary material, Fig. S2**).

324

325 **Bacterial community diversity and composition in different microcosms**

326 Incubations of the resident bacterial assemblage with the presence of viruses and grazers
327 (BVF), viruses alone (BV) and under viral reduction (B) yielded distinct signatures in
328 bacterial diversity and community composition. The variation in bacterial richness (Chao =
329 328-383) and diversity (Shannon = 3.58-3.75) was higher in the presence of both the loss
330 factors throughout the experiment. Overall, the bacterial diversity under reduced viral
331 concentrations was significantly lower ($p < 0.001$) compared to microcosms with *in situ* viral
332 abundances (**Supplementary material, Table S1**). Addition of glucose and inorganic
333 nutrients in BV and B microcosms promoted shifts in bacterial community dominants as a
334 function of carbon source preference, resulting in lower bacterial diversity.

335 In microcosms, the distribution and relative abundance of different bacterial groups
336 which was visible at the genus level. Out of 16 different phyla along with unclassified
337 bacterial members with varying abundance, 3 phyla namely Actinobacteria, Proteobacteria
338 and Bacteroidetes, approximately accounted for 99% of the total sequences with varying
339 abundances. The members of Actinobacteria (represented by genera *clade hgcI*, *CL500-29*
340 *marine group*, *Candidatus Planktophilia*) which were the dominant group at the start of the
341 experiment decreased linearly with time. Proteobacterial members such as
342 Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Bacteroidetes were
343 highly dynamic and showed variable response with progress in incubation time in different
344 microcosms.

345 Within the class Betaproteobacteria, *Limnohabitans* increased 3 to 5 fold in BV-CNP
346 treatment, whereas in the viral reduced fractions a notable increase in the relative abundance
347 of genera *Delftia*, *Duganella* and *Pelomonas* was observed (**Fig. 5**). Similarly the increase of
348 *Caulobacter* belonging to class Alphaproteobacteria by 6.7 fold compared to start of the
349 experiment was evident in B-NP microcosms. The strong increase of Gammaproteobacteria
350 by 53 and 70.3 fold in the BV-CNP and B-CNP treatment respectively eventually coincided
351 with high percentage increase of HNA cells. This response of Gammaproteobacteria to
352 carbon addition was represented by members belonging to genera *Pseudomonas* and
353 *Acinetobacter* in viral reduced and *Pseudomonas* only in microcosm containing *in situ*
354 abundances. Among the class Bacteroidetes, the genera *Flavobacterium* and
355 *Sediminibacterium* which showed ten and two-fold increase respectively in the presence of
356 both the loss factors only. Overall the sequences from all the microcosms were classified into
357 29 classes, 69 orders, 135 families and 281 genera.

358 Two dimensional non-metric multidimensional scaling accounted for bacterial
359 community differences (stress = 0.08, $r^2 = 0.97$) (**Fig. 6**). The variations towards first axis of

360 non-metric multidimensional scaling is mostly contributed by OTU2 (*Pseudomonas*) OTU43
361 (env.OPS_17_ge from family Sphingobacteriales), OTU122 (*Polaromonas*) and OTU100
362 (*Rhodococcus*). However, OTU10 (*Sediminibacterium*), OTU66 (Microbacteriaceae), OTU5
363 (*Pseudomonas*) and OTU3 (*Pseudomonas*) contributed most to the variations of second axis.
364 Based on the distribution of OTUs, ANOSIM and weighted UniFrac analyses indicated
365 significant differences ($p < 0.001$) in bacterial community composition in grazer, grazer free
366 and viral reduced microcosms (**Supplementary material Table S2A&B**). The clustering of
367 samples from microcosms with ambient and reduced viral abundance is clearly visible except
368 in the case of CNP supplemented samples. The dendrogram based on thetacy calculators
369 confirmed the hierarchical clustering of bacterial community composition in samples in two
370 clades of the tree and raw samples branched closely with samples from ambient viral
371 abundances microcosms (**Supplementary material Fig. S3**).

372

373 **DISCUSSION**

374

375 This study provides evidence on the differential impact of top-down (viruses and grazers) and
376 bottom-up (nutrients) forces on bacterial community structure (diversity and composition)
377 which caused shifts in bacterial subpopulation with differing physiological state and
378 metabolism. Two dimensional non-metric multidimensional scaling accounted for structural
379 differences in bacterial community, whereas ANOSIM and UniFrac analysis highlighted for
380 significant differences in bacterial communities in experimental microcosms.

381

382 **Nutrient impact on bacterial physiology**

383 The response of natural bacterial communities to nutrients additions can be linked
384 closely to the state of the ecosystem (Øvreås *et al.* 2003). During the studied period of the
385 year, multiple nutrient limitation of bacterial community was evident with large proliferation
386 of high nucleic acid content bacterial subpopulation in experimental microcosms
387 supplemented with organic and inorganic nutrients (Fig. 2). However, the inability of bacterial
388 cells to divide but increase in their biovolume in microcosms with addition of carbon source
389 alone is suggestive of strong co-limitation of phosphate source (Carlsson and Caron 2001).
390 Phosphate is known to enhance cell division, whereas their strong limitation could result in
391 the storage of organic carbon in cells without dividing and thus maintain higher carbon
392 metabolism (del Giorgio and Cole 1998). The dominance of LNA at the start of the
393 experiment and subsequent increase of HNA cells following the addition of carbon source

394 suggest that these two groups may differentiate according to the prevailing nutrient
395 environment and thus play different ecological roles. The explosive growth of HNA cells with
396 nutrient additions is consistent with the reports that an enrichment of copiotrophic bacterial
397 phylotypes (such as those comprise the HNA subpopulation) coincides with bacterial
398 productivity and biomass maxima (Nelson *et al.* 2014; Mojica *et al.* 2019). Therefore, nutrient
399 conditions which are bound to vary at different time periods especially in natural freshwater
400 system could serve as a potential influencing factor in altering the bacterial physiological state
401 with high and low concentrations favoring HNA and LNA cells respectively (Palesse *et al.*
402 2014; Liu *et al.* 2015).

403 The expression of HNA cells under nutrient relaxation could be a part of growth
404 strategy where certain bacteria such as *Vibrio* are capable of surviving extended periods of
405 carbon starvation and grow rapidly at high substrate concentrations (Øvreås *et al.* 2003). Our
406 results support and suggest the fact that HNA cells could be fast growing, diverse and be more
407 adapted to changing environments, using their repertoire of transport and regulation genes
408 (Philippot *et al.* 2010). Such versatility and fast growing nature of HNA cells which possess
409 more flexible genes allow them to exploit pulses of nutrients (Mojica *et al.* 2019) and occupy
410 more ecological niches (Schattenhofer *et al.* 2011). Cytometric signatures have revealed that
411 both HNA and LNA cells may represent physiologically and ecologically distinct bacterial
412 populations that have components which are intrinsic to themselves and this distribution may
413 in fact be linked to more general adaptive processes within the bacteria (Bouvier *et al.* 2007;
414 Mojica *et al.* 2019). This bimodal distribution could probably play a key role in the
415 functioning of bacterioplankton communities.

416

417 **Linking top-down and bottom-up effects to bacterial community dynamics**

418 Top-down forces and nutrients had variable impact on bacterial community. High viral lysis
419 in the presence of top-down forces in non-amended microcosms containing microbial
420 populations at *in situ* abundances and low viral impact on bacterial population with nutrient
421 additions brought about changes in bacterial community composition and diversity. The
422 explosive increase of fast-growing opportunistic bacteria (i.e. HNA cells) in nutrient spiked
423 microcosms was never accompanied by the increase in phage abundance and infection, thus
424 differing with the reports from marine systems where the limiting nutrient was found to
425 constrain bacterial growth and consequently phage-mediated host cell lysis (Williamson and
426 Paul 2004). Low viral impact on bacterial communities could likely be due to tradeoff which
427 can exists between bacterial traits of antiviral defense (reduction and masking of receptors or

428 viral adsorption) and resource competition, where organic enrichment may lower resource
429 competition and allow bacterial communities to pay the costs involved in increasing antiviral
430 defense (Thingstad *et al.* 2005). Moreover extracellular proteases produced by rapidly
431 growing bacteria can largely play a role in the hydrolytic destruction or modification of viral
432 capsids thus alleviating viral lytic pressure leading to uncoupling between bacterial and viral
433 production (Motegi and Nagata, 2009). In microbial model systems, the magnitude of trade-
434 off between host and phages are largely influenced by environmental factors especially
435 resources (nutrients) where its limitation can result in the coexistence of both susceptible and
436 resistant hosts in natural communities (Friman and Buckling 2013; Record *et al.* 2016). Such
437 a scenario of viral defense trade-offs through the expression of host defense mechanisms such
438 as CRISPR-Cas (Barrangou *et al.* 2007) and restriction enzymes (Labrie *et al.* 2010) can have
439 predictable effects on their community structure, properties, dynamics and also on their
440 response to environmental changes (Stearns 1989; Jessup and Bohannan 2008). Host defense
441 strategies which tend to contribute to the shape of trade-off curve in a predator-prey system
442 can be either concave (cheap defense) or convex (costly defense) depending on the presence
443 of ancestral and coevolved predators respectively (Huang *et al.* 2017). The presence of such
444 dynamical trade-off between host and phage, which vary with the environment can eventually
445 affect their ecological interactions and subsequently drive evolutionary changes (Jessup and
446 Bohannan 2008).

447 The application of next generation sequencing to sequence 16S rRNA genes facilitated
448 in obtaining greater information on taxonomic resolution of bacterial community and their
449 diversity pattern in microcosms. Bacterial diversity was highest in the presence of both the
450 top-down factors indicating that they had tighter control on bacterial communities (Zhang *et al.*
451 *et al.* 2007). Contrastingly, the addition of carbon source resulted in lower bacterial diversity
452 estimates due to the proliferation of certain communities which have the ability to rapidly
453 consume available resources (Sandaa *et al.* 2009; Topper *et al.* 2012). It has been previously
454 demonstrated that both nutrient and loss factors tend to operate simultaneously in freshwater
455 systems where the former can evoke strong changes in the physiology and metabolic activity
456 of certain members of bacterial population which can eventually make those susceptible
457 communities selectively vulnerable to the later, thus impacting their diversity (Storesund *et al.*
458 2015; Pradeep Ram *et al.* 2016). The observed differences in bacterial communities in
459 response to top-down and bottom-up factors in different treatments with the progress of
460 experiment was highlighted by ANOSIM and weighted UniFrac analyses which is based on
461 the distribution of OTUs where significant difference between the bacterial groups are

462 reflected in the differences in their community composition. This was duly supported by two
463 dimensional non-metric multidimensional scaling based on thetacyc dissimilarity scores to
464 account for community differences.

465 The members of class Gamma-, Beta- and Alphaproteobacteria which evoked
466 contrasting and differential responses to nutrient additions and predators are known to belong
467 to HNA subpopulation (Schattenhofer *et al.* 2011; Mojica *et al.* 2019), while lineages from
468 Actinobacteria were selected against in all the treatments. The low detection of
469 Gammaproteobacteria under high viral infection rates in the presence top-down factors could
470 be explained by their potential vulnerability to loss factors, whereas their sharp increase in
471 with nutrient supplements is suggestive of their opportunistic lifestyle and adaptability to
472 grow under high-nutrient concentrations (Øvreås *et al.* 2003; Pinhassi and Berman 2003),
473 much quicker than the viruses could infect them. It was interesting to note that even within
474 *Gammaproteobacteria*, different genera such as *Pseudomonas* and *Alteromonas* had variable
475 responses to nutrient additions and viral reductions respectively.

476 Although it is very well known that Betaproteobacteria are more substrate responsive,
477 their significant presence of genus *Limnohabitans* amid both the top-down factors are justified
478 by their ability to efficiently utilize regenerated nutrients from grazing and viral lysis under
479 nutrient limiting conditions (Simek *et al.* 2001; Pradeep Ram and Sime-Ngando 2008). The
480 high expression of minor genera *Pelomonas*, *Delftia*, and *Duganella* (Betaproteobacteria) and
481 genus *Caulobacter* (Alphaproteobacteria) under reduced viral conditions are suggestive of
482 their vulnerability to viral infection wherein the scenario of host characteristics could prevail
483 over density (Bouvier and del Giorgio 2007). The increased representation of *Sphingobacteria*
484 and *Flavobacteria* (Bacteroidetes) in the presence of both the top down factors are suggestive
485 of its ability to change their morphology (filamentous and floc formation) to escape grazing
486 and at the same time efficiently use regenerated nutrients (Hornak *et al.* 2005).

487 The generalizations from a single microcosm experiment should be done with care,
488 even when the experiment was carried out with a defined set of controls, parallels and
489 factorial design. The initial bacterial community composition that was determined at the start
490 of the experiment in the grazer fraction was also applied to other fractions (grazer free and
491 viral reduced) assuming that the initial differences between treatments may overall be small.
492 However such assumption could perhaps be associated with certain amount of bias in our
493 experimental microcosms. Despite of methodological constraints and pit falls associated with
494 our chosen experimental approach, we were able to demonstrate the variable influence of top-
495 down factors especially viral lysis and nutrients on bacterial diversity and community

496 composition. The expression of HNA over LNA subpopulation under nutrient enriched and
497 viral depletion was reflected in the changes in bacterial community composition at the genus
498 level. Our study is in agreement within the conceptual idea of simple model version of
499 ‘Killing the Winner (KtW)’ theory, which postulates that bacterial community diversity is a
500 feature that is essentially top-down controlled by viruses, while community composition is
501 bottom-up controlled by competition for limiting nutrients (Töpper *et al.* 2012; Storesund *et*
502 *al.* 2015). Such a model where the two separate control mechanisms for composition and
503 diversity could be envisaged in our study and therefore can be extended for freshwater
504 bacterial communities. Therefore accordingly, our findings indicate that the competition for
505 growth limiting nutrients can act as a structuring factor for the host community composition
506 which occupies available niches, while top-down factors control the identity and abundance of
507 bacterial hosts within each niche.

508

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515

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517

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