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7 **Environmental characteristics of a tundra river system in Svalbard. Part 1: bacterial**  
8 **abundance, community structure and nutrient levels**

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20

21 **Abstract:** The Arctic hosts a set of unique ecosystems, characterised by extreme  
22 environmental conditions and undergoing a rapid change resulting from the average  
23 temperature rising. We present a study on an aquatic ecosystem of the Revelva catchment  
24 (Spitsbergen), based on samples collected from the lake, river and their tributaries, in the  
25 summer of 2016. The landscape variety of the study site and the seasonal change in the  
26 hydrological regime modify the availability of nutrients. In general, the upper part of the  
27 catchment consists of the mountain rocky slopes which are especially abundant in iron  
28 minerals, sulphides and phosphorus minerals. The lower part of the catchment is covered by  
29 plants - lichens, saxifrages and bryophytes, which are a different source of nutrients. In the  
30 analysed water samples, the maximum concentrations of nutrients such as iron, boron and

31 phosphorus were  $0.28 \mu\text{g L}^{-1}$ ,  $4.52 \mu\text{g L}^{-1}$  and  $1.91 \mu\text{g L}^{-1}$ , respectively, in June, while in  
32 September, Fe and B reached the concentrations of  $1.32 \mu\text{g L}^{-1}$  and  $2.71 \mu\text{g L}^{-1}$ , respectively.  
33 The concentration of P in September was below the detection limit of  $1.00 \mu\text{g L}^{-1}$ , which may  
34 be explained by the necessity of bacteria to consume it immediately on current needs. We  
35 noted also an increase in TOC concentration between the June and September samples, which  
36 could originate both from the biomass accumulation in the catchment and the permafrost  
37 melting contributing to the hydrological regime of the river. The bacterial community  
38 developed in this environment consisted mainly of *Proteobacteria*, *Actinobacteria*,  
39 *Bacteroidetes* and *Firmicutes* phylum, while the presence of *Acidobacteria* was less  
40 pronounced than in other tundra-related environments. The described catchment shows that  
41 despite the relatively small amount of bioavailable nutrients, the Revelva system is biodiverse  
42 and one of the most significant biogeochemical changes occurs there in response to seasonally  
43 switching water sources.

44 **Keywords:** Arctic, Spitsbergen, Freshwater bacterial community, Bacterial diversity,  
45 Nutrients

## 46 **1. Introduction**

47 The unique Arctic ecosystems, adapted to the extreme environmental conditions of this area,  
48 are under pressure due to environmental changes following more than twice as intensive  
49 warming of this area as the global average temperature rise (ACIA 2005; AMAP 2017).  
50 Rising temperatures affect water supply from shrinking glaciers (Gardner et al. 2013) and  
51 permafrost thaw (Frey and McClelland 2009), and they decrease the extent and duration of the  
52 snow cover (AMAP 2017), effectively modifying the hydrological regime of the Arctic rivers.  
53 The associated landscape changes encompass the exposure of formerly glaciated land and  
54 significant shifts in vegetation (Elmendorf et al. 2012; Bjorkman et al. 2018). Another

55 demonstration of such change may be an increased frequency of extreme precipitation events  
56 (Łupikasza 2007). The Arctic rivers, draining the catchments changing in these various ways,  
57 are predicted to experience a biogeochemical shift towards a groundwater-dominated system,  
58 as opposed to one dominated by surface water supply (Frey and McClelland 2009). Since a  
59 similar change in supply proportions occurs in the tundra rivers across the summer season  
60 (Pulina et al. 1984), we sampled the June and September waters from a Svalbard lake-river  
61 system as two biogeochemical composition endmembers, hypothesising their differences will  
62 reflect a likely direction in future Arctic river biogeochemistry.

63 A river and its headwaters capture various biogeochemical elements originating from the  
64 landscapes it encompasses. The bacterial composition in the Arctic rivers and lakes is linked  
65 to the transport of microorganisms from other habitats containing developed microbiota. The  
66 changing sources of water supply affect also the pathways of transporting chemical  
67 compounds and bacteria into the catchment, which can originate from the airborne pool,  
68 glaciers, sea aerosols and permafrost thaw (Houghton et al. 2001; Pomeroy and Wiebe 2001;  
69 Hodson et al. 2005, Adams et al. 2010, Kühnel et al. 2013, Górniak et al. 2016). Although the  
70 riverine nutrient concentrations and fluxes in the Arctic in inorganic form are relatively low,  
71 such catchments usually discharge high amounts of organic matter (Dittmar and Kattner  
72 2003) and the microbial communities harboured by these watercourses may be very diverse  
73 (Crump et al. 2012). Carbon, nitrogen and phosphorus can all be limiting nutrients, as related  
74 to individual cell physiology and environmental factors (Fagerbakke et al. 1996; Göransson et  
75 al. 2011). Nutrient limitation can influence not only elemental ratios in biomass, but also cell  
76 volume and shape (Vrede et al. 2002). Phosphorus has been found a common limiting element  
77 in the Arctic lakes and ponds, although its enhancing effect on bacterial abundance and  
78 production is usually only seen in sites with an increased temperature. A combined effect of

79 phosphorus and organic carbon or nitrogen in water samples may result in increased  
80 productivity signals (Graneli et al. 2004; Mindl et al. 2007; Edwards et al. 2014).

81 The main objective of the conducted research was to observe the biogeochemical diversity of  
82 the studied aquatic environments with respect to seasonal change, anticipating similar changes  
83 with the future shift towards a groundwater-fed system (Frey and McClelland 2009).  
84 Furthermore, we investigate whether the nutrients present in the studied catchment are  
85 sufficient and available for the development of the bacteria living in it, by studying the  
86 interactions between the nutrients, such as phosphate, nitrate, ammonia, or organic carbon,  
87 and the bacterial abundance. A background factor influencing them is the variety of  
88 hydrological environments and landscapes in this catchment. This information was compared  
89 to the quantitative and qualitative data on the local bacterial community composition, showing  
90 its variety and adaptation to the environment.

## 91 **2. Materials and Methods**

### 92 2.1. Study area

93 The Revelva catchment (Wedel-Jarlsberg Land, southwestern Spitsbergen) is located in the  
94 vicinity of the Polish Polar Station Hornsund (77°0'0"N, 15°33'0") (Figure 1). The main river  
95 (Revelva) and the lake (Revvatnet) are fed both directly by atmospheric precipitation, snow-  
96 fed streams and a river originating from a glacier (Ariebreen), as well as permafrost thaw,  
97 especially once the snow has melted. The permafrost thaw in the area is pronounced, as this  
98 region is characterised by the highest active layer depth in the Svalbard archipelago,  
99 exceeding 2 m (Dolnicki et al. 2013). Furthermore, in the Hornsund station, long-term  
100 monitoring (1990-2009) of the active layer temperature at 1 m depth has shown an increasing  
101 trend (Dolnicki et al. 2013), leading to the conclusion that permafrost degradation was  
102 advancing in that period.

103 Revelva's estuary drains into the bay of Ariebukta. In the upper part of the catchment, the  
104 tributary streams originate from rocky mountain slopes. A series of three lakes occupies the  
105 valley bottom and contributes to the hydrological diversity of this site. The catchment is  
106 characterised by an asymmetry, with a predominance of left tributaries, of which the largest is  
107 the proglacial Ariebekken. The sampling was performed mainly on the left side of the river  
108 and lake, to reflect this asymmetry in water input and the influence of the lush tundra  
109 vegetation in the valley bottom, visited by birds and reindeer herds – biological vectors of  
110 chemical species. A further characterisation of this catchment is provided in former  
111 publications of our research group (Kozak et al. 2016; Kosek et al. 2018).

## 112 2.2. Sampling

113 Water sampling in the Revelva catchment was repeated in June and September 2016 in 14  
114 locations representing the distribution of water inflows and chemical species into the Revelva  
115 system (Figure 1), which were directly comparable to our former study sites (Kosek et al.  
116 2018). The choice of sampling months reflected the timespan of the vegetation season and a  
117 change in hydrological regime in the area. In June, snow cover is melting and on the sampling  
118 date residual snow patches remained in the valley bottom, hence the Revelva system was still  
119 influenced chemically and biologically by snowmelt. In September, there occurs an increase  
120 in atmospheric precipitation and permafrost thaw, while vegetation season is at an end.

121 **Figure 1.** Location of the studied area in Svalbard and the sampling points in the Revelva  
122 catchment.

123 Freshwater samples were collected manually from the Revvatnet (lake) and the Revelva  
124 (river) at a distance of 1.5 m from the shore with no headspace into air-tight, chemically clean  
125 1L bottles (daily blank sample confirming the purity of the procedure). Pre-cleaning  
126 procedure for the bottles included week-long soaking with Milli-Q deionised water and

127 removing the water from the sampling containers several times. The running water was taken  
128 from the main stream at depths 20-50 cm below water level. For microbiological analysis,  
129 separate sub-surface 50 mL samples were taken and preserved by injecting formaldehyde  
130 solution (2% final concentration), then stored at 4°C. An aliquot was taken from the 1 L  
131 chemical sample and stored frozen for nutrient analysis (the remaining volume was stored for  
132 polycyclic aromatic hydrocarbons analysis – see Part 2, Kosek et al. accepted). The  
133 metagenomics samples of 1.5 L were frozen and maintained under such conditions until  
134 analysis.

### 135 2.3. Chemical Analysis

136 All technical specifications of the analytical equipment and methods, including basic  
137 validation parameters of the analytical procedures, are given in Table 1. The basic parameters  
138 of electrical conductivity (EC) and pH were measured immediately upon return from the field.  
139 The concentrations of the following inorganic ions:  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  
140  $\text{Br}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  were determined with the use of ion chromatography  
141 technique. Phosphorus concentration was also determined in elemental form, as were the  
142 concentrations of iron and boron, all with the use of and ICP-MS (Inductively Coupled Plasma  
143 Mass Spectrometer). The element concentration CVs of the obtained triplicate results ranged  
144 from 0.5 to 1.5%. Carbon, in all organic forms, was measured as non-purgeable organic carbon with  
145 a Total Organic Carbon Analyzer TOC-V<sub>CSH/CSN</sub>, (Shimadzu, Japan) method of catalytic combustion  
146 (oxidation) with the application of the NDIR detector. All blanks were prepared with Milli-Q  
147 deionised water.

148 **Table 1.** Validation parameters and technical specifications used in the applied analytical  
149 procedures.

### 150 2.4. Quality Assurance / Quality Control (QA/QC)

151 The analytical procedures used to determine individual components in the studied samples  
152 have been validated against certified reference materials (CRMs) concordant with ISO Guide  
153 34:2009 and ISO/IEC 17025:2005. The data obtained here were subject to strict QC  
154 procedures. Prior to pH measurements, a three-point calibration of the electrode was  
155 performed with temperature compensation, using MERCK Millipore Certipur® buffer  
156 solutions of pH 4.00, 7.00 and 9.00 (25°C). The analysis of elemental nutrients involved the  
157 application of Standard Reference Material (RM) NIST 1643e Trace Elements in Water, and  
158 RM Enviro MAT ES-L-2CRM, ES-H-2 CRM SCP SCIENCE. The calibration of the  
159 apparatus was based on RMs by Inorganic ventures ANALITYK: CCS-4, CCS-6, CCS-1, IV-  
160 ICPMS-71A. Potassium hydrogen phthalate by NacalaiTesque (Japan) was used for the  
161 calibration of the TOC Analyser. The sensitivity of the applied methods was tested by  
162 injecting standard mixtures of the analytes in the measured concentration range. Linear  
163 calibration curves of the peak area against standard concentration showed correlation  
164 coefficients ( $R^2$ ) in the range of 0.898–0.999 for all standards. Each sample was analysed in  
165 triplicate. The instrumental background was checked by inserting Milli-Q water blanks once  
166 per every six samples.

## 167 2.5. Bacterial Abundance Analysis

168 For the determination of total bacterial number, average bacterial cell volume and bacterial  
169 biomass, the collected water samples have been stained with DAPI (4,6-diamidino-2-phenyl-  
170 indol) in a final concentration of  $2 \mu\text{g mL}^{-1}$  and filtered through a polycarbonate membrane  
171 filter with a pore diameter of  $0.2 \mu\text{m}$ . The samples prepared for bacteria detection have been  
172 analysed using the epifluorescence microscope Nikon Microscope 80i with NIS-Elements BR  
173 3.0 and MultiScan automated image analysis system. The analysis was carried out using  
174 appropriate excitation filters adapted to the used fluorochrome. The total useful microscope  
175 magnification was 1200. The image analysis system consisted of a snap-in to the microscope



176 Epifania Mda monochrome high resolution color digital camera (Nikon DS-5Mc-U2).  
177 Structure indicators of bacteriocenosis were estimated based on the results obtained in 20  
178 consecutive fields of view.

## 179 2.6. Bacterial Community Structure Analysis

180 The water samples were filtered through sterile 0.2- $\mu$ m membrane filters. The total genomic  
181 DNA was extracted using the commercially available Sherlock AX kit (A&A Biotechnology,  
182 Poland). The membrane filters were first transferred into microcentrifuge tubes containing 0.5  
183 g of 0.5 mm zirconia beads and supplemented with 300  $\mu$ l of sterile water, 300  $\mu$ l of L 1.4  
184 buffer and 20  $\mu$ l of proteinase K. Next, the samples were placed in a Beadbeater for 60 s. The  
185 isolation protocol was then followed according to the manufacturer's instructions. The DNA  
186 concentrations of the samples were determined using a ND-1000 UV-Vis spectrophotometer.  
187 The extracted DNA was stored at 4°C. The microbial community in the tested samples was  
188 analysed using high-speed multiplexed 16S microbial sequencing on a MiSeq platform  
189 (Illumina). The microbial community was analysed using the hypervariable regions V3-V4 of  
190 the 16S rRNA, regarded as the most appropriate for the Illumina sequencing (Klindworth et  
191 al. 2013). The region was amplified using the following primer set: 341F –  
192 CCTACGGGNGGCWGCAG and 785R – GACTACHVGGGTATCTAATCC. PCR was  
193 conducted using Q5 Hot Start High Fidelity 2X Master Mix (New England Biolabs, Ipswich,  
194 MA, USA). Each library was prepared with a two-step PCR protocol based on Illumina's '16S  
195 metagenomic library prep guide'. Paired-end (PE, 2 $\times$ 250 nt) sequencing with a 5% PhiX  
196 spike-in was performed with an Illumina MiSeq (MiSeq Reagent kit v2) at Genomed  
197 (Warsaw, Poland) following the manufacturer's run protocols (Illumina, Inc., San Diego, CA,  
198 USA). The primary automatic analysis and the de-multiplexing of the raw sequences were  
199 performed with MiSeq, with the use of MiSeq Reporter (MSR) v. 2.6 (BaseSpace). Next  
200 sequences were analysed using the bioinformatics pipeline Qiime (Quantitative Insights Into

201 Microbial Ecology) v. 1.8.0. Raw paired-end reads were subjected to the following process:  
202 (1) searching and removing both forward and reverse primer sequences using CutAdapt, with  
203 no mismatches allowed in the primer sequences, (2) the removal of the low quality sequences  
204 not having an average quality of 20 over a 30 bp sliding window based on the *phred* algorithm  
205 and a 97% overlap identity, (3) quality-filtered reads were merged based on the overlap of PE  
206 read with the use of *fastq-joint*, (4) the sequence reads were classified into OTUs (Operational  
207 Taxonomic Units) on the basis of sequence similarity using the UCLUST algorithm, (5) the  
208 chimera sequences were detected and removed using the Chimera Slayer algorithm, (6)  
209 clustering of operational taxonomic units (OTUs) was performed at 97% similarity using the  
210 *uclust* method, based on GreenGenes v. 13.8 database, (7) additionally, samples were  
211 hierarchically clustered using Unweighted-Pair Group Method with Arithmetic mean  
212 (UPGMA). It is important to note that the software used here limits the identification of  
213 taxonomical level to the lowest unequivocally assigned one, i.e. to family if genus and species  
214 cannot be recognised. Based on clusters, the diversity indices were estimated, including the  
215 Chao1, Shannon, and Simpson indices.

## 216 2.7. Principal Component Analysis (PCA)

217 Principal Component Analysis (PCA) is a multivariate statistical analysis that allows  
218 revealing internal relations in the data set. PCA finds linear combinations of the original  
219 variables, referred to as principal components, which provide better descriptors of the data  
220 pattern than the original (chemical or physical) measurements and account for most of the  
221 dataset variation. The PCA for this study was performed using R v. 3.4.4, using the *prcomp*  
222 function, on a log-transformed dataset, except the pH value which is a logarithm.

## 223 3. Results

### 224 3.1. The chemical composition of freshwater samples

#### 225 3.1.1. Electrical conductivity (EC), pH and total organic carbon (TOC) concentration

226 The EC values in the collected samples ranged from  $34.8 \mu\text{S cm}^{-1}$  to  $102.1 \mu\text{S cm}^{-1}$  in June  
227 2016, and from  $76.9 \mu\text{S cm}^{-1}$  to  $174.5 \mu\text{S cm}^{-1}$  in September 2016 (Figure 2a), while pH  
228 ranged from 7.0 to 8.0 in both months (data shown in the Part 2 of this article, Kosek et al.  
229 accepted).

230 Figure 2b shows that the concentrations of total organic carbon (TOC) in September 2016  
231 were higher than in June at all locations. The maximum value of  $2.06 \text{ mg L}^{-1}$  was measured in  
232 September at the R12 site, in the river estuary, while the lowest concentrations occurred in  
233 June in the upper part of the catchment (R2-R4 sites); similar concentration was found also at  
234 site R9 in June. The smallest seasonal difference in TOC measurements was found at site  
235 R13.

236 **Figure 2.** Concentration levels of electrical conductivity and total organic carbon determined  
237 in the collected freshwater samples, compared between the studied periods; a) electrical  
238 conductivity (EC), b) total organic carbon (TOC).

#### 239 3.1.2. Inorganic ions

240 Figure 3 shows the percentage of total anion and cation concentrations detected in the  
241 collected samples. Chloride and sulfates dominate the anion composition both in June and  
242 September 2016. In the collected freshwater samples,  $\text{Cl}^-$  constituted almost 46% of all  
243 detected ions both in June and September 2016. Sodium and calcium were predominant  
244 cations in all samples except sites R4, R9 and R14 in June, when magnesium exceeded  
245 calcium concentrations. A marked change in the cation composition occurred from June to  
246 September, with calcium becoming the most abundant cation in all September samples.

247 Nitrogen occurred in the Revelva catchment in ionic form, especially as  $\text{NH}_4^+$  and  $\text{NO}_3^-$ .  
248 Nitrate occurred at concentrations ranging from 1.27 to 3.22  $\text{mg L}^{-1}$ , with an increase in  
249 September (June median concentration amounted to 1.55  $\text{mg L}^{-1}$ , while September median  
250 equaled 1.99  $\text{mg L}^{-1}$ ). Ammonium concentrations spanned 0.03 – 0.43  $\text{mg L}^{-1}$ , with somewhat  
251 higher concentrations in June (June median = 0.11  $\text{mg L}^{-1}$ , September median = 0.08  $\text{mg L}^{-1}$ ).  
252 Of other ionic nutrients, neither nitrite nor phosphate was detected, which implies their  
253 concentrations were below 0.06  $\text{mg L}^{-1}$ .

254 **Figure 3.** Percentage anion and cation composition of the collected samples.

### 255 3.1.3. Elemental nutrients

256 Among nutrients, elemental B, P and Fe were analysed here in this speciation form (Table 2).  
257 The maxima in both boron and phosphorus concentrations occurred in June, while iron  
258 concentrations exhibited a marked increase in September.

259 **Table 2.** Concentrations ( $\pm$ standard deviation, SD) of elemental nutrients in the collected  
260 freshwater samples.

### 261 3.2. Microbial Community

262 In the collected freshwater samples, the highest bacterial biomass (BB) was detected in  
263 September 2016, in the sampling point R5, at 8.47  $\mu\text{g C L}^{-1}$ . BB was strongly linked to total  
264 bacterial number (TBN) which at this point was also the biggest ( $42.1 \cdot 10^4 \text{ cell mL}^{-1}$ ). Figure  
265 4 presents the TBN, BB and average cell volume (ACV) detected in freshwater samples in  
266 both months. Notably, the ACV increased in areas where the number of bacteria was lower in  
267 both June and September.

268 **Figure 4.** Comparison of bacterial abundance (total number), average bacterial cell volume  
269 and bacterial biomass in the Revelva catchment in June and September 2016.



### 270 3.3. Bacterial Taxonomy

271 In the three tested points (R4, R8 and R14) at two occasions (in June and September), 840348  
272 sequences (reads) were detected. Among them, 805 000 were linked to the bacterial and 708  
273 to the archaeal domain, while 34640 were not identified (not found in the conventional  
274 databases). Samples R4 and R14 showed the highest bacterial diversity, while samples R8  
275 were the least diverse (Table 3), regardless of the period of sampling.

276 **Table 3.** Number of reads and OTUs as well as species richness estimate (Chao1) and  
277 diversity indices (Shannon and Simpson) for the sampling points.

278 A cluster analysis of sequence data for the examined samples and bacterial community  
279 structures with relative abundances at the phylum level (based on the number of Illumina  
280 MiSeq-based method) are given in Figure 5 and Figure 6. The predominant bacterial phyla  
281 found in the studied catchment were: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*,  
282 *Firmicutes* and *Planctomycetes*; *Verrucomicrobia*, *Tenericutes*, *Cyanobacteria* and  
283 *Acidobacteria* were also identified.

284 **Figure 5.** Cluster analysis of bacterial community structures.

285 **Figure 6.** Bacterial community structures and relative abundances based on the number of  
286 reads (a) and OTUs (b) for major phyla (>1%) identified in tested water samples in June and  
287 September 2016.

288 The bacterial taxonomic composition at the family level is presented in Table 4. We found the  
289 ammonia oxidizing bacteria (AOB) and archaea (AOA), as well as the nitrite-oxidizing  
290 bacteria (NOB) in each sampling point, at a total concentration below 0.5% of the total reads.  
291 The identified AOA were mainly represented by *Nitrosopumilus* and *Candidatus*  
292 *Nitrososphaera*, while among AOB, *Nitrosococcus* from *Gammaproteobacteria* class, and

293 *Nitrospira*, *Nitrosovibrio* from *Betaproteobacteria* class were detected. The nitrite-  
294 oxidizing taxa (*Nitrospira* and *Nitrobacter*) in the tested water samples were at a very low  
295 level (<0.08%).

296 **Table 4.** Family level taxonomic composition in the Revelva catchment (among 274 families  
297 reported in this study, first 40 are presented).

298 Within the predominant (at the study site) *Proteobacteria* phylum, *Alphaproteobacteria*  
299 constituted 8.83% – 18.57% of total reads, and were mainly represented by genus  
300 *Thalassospira* (*Rhodospirillaceae* family), which was reported as involved in the phosphorus  
301 cycling in nutrient-limited environments (Hütz et al. 2011). In this study, *Thalassospira* was  
302 mainly reported in point R8 (R8-J – 5.31% and R8-S – 13.45%) and R14 (R14-J – 4.76% and  
303 R14-S – 3.79%), influenced by the tundra soil active-layer controls, while in R4 it reached  
304 less than 0.5%.

305 Among the *Betaproteobacteria* class (*Proteobacteria*), the predominant genera were from  
306 *Comamonadaceae* family: *Polaromonas* (from 1.8% to 5.1%), *Rhodoferax* (from 1.6% to  
307 5.6%), from *Oxalobacteraceae* family: *Polynucleobacter* (from 0.2% to 3.4%) and  
308 *Herminiimonas* (from <0.1% to 2.5%). The *Rhodoferax* genus was represented in this study  
309 mainly by *R. ferrireducens* sp. nov., a facultatively anaerobic bacterium that oxidizes acetate  
310 with the reduction of Fe (III) (Finneran et al. 2003). Another dissimilatory iron reducing  
311 bacteria, *Geobacter*, was also found in the studied river-lake system (at abundances up to  
312 1.7%). This is a mesophilic bacteria from the *Geobacteraceae* family, class  
313 *Deltaproteobacteria*. Sulfate-reducing bacteria were also detected in this study, e.g.  
314 *Desulfovibrio* spp. from *Deltaproteobacteria* (up to 0.3%).

315 In *Bacteroidetes* phylum, the *Flavobacteriaceae* family formed from 2.24% to 5.57% of total  
316 reads, represented mainly by genus *Flavobacterium* (from 1.55% to 4.54%). *Bacteroidetes*



317 phylum was also represented by *Sphingobacteriaceae* family (from 0.31% to 2.54%), as well  
318 as by *Flexibacteraceae* family (from 0.55% to 2.22%). Interestingly, a higher abundance of  
319 *Bacteroidetes* phylum was noted in the sampling point R4 (up to 11.8%), when compared  
320 with R8 (up to 8.0%) and R14 (up to 7.7%), while in the case of the *Actinobacteria* phylum,  
321 an opposite pattern was found (R4 - up to 11.97%; R8 - up to 22.9% and R14 - up to 15.39%).

## 322 4. Discussion

### 323 4.1. The chemical composition of freshwater samples

#### 324 4.1.1. Electrical conductivity (EC), pH and total organic carbon (TOC) concentration

325 The noted EC and pH values do not deviate significantly from the former measurements in  
326 hydrochemical studies of the Hornsund fjord area (including the Revelva catchment),  
327 although the area is characterised by a marked hydrochemical variability. For example, all  
328 samples collected in the previous years in the Revelva catchment, as well as those collected  
329 this study, were characterised by a near-neutral pH (Ruman et al. 2012; Kozak et al. 2016;  
330 Kosek et al. 2018). They also resembled in this respect a nearby lake-stream system in the  
331 Brategg Valley (Górniak et al., 2016), however in the Revelva catchment the EC was higher,  
332 approximately doubling the values noted by Górniak et al. (2016).

333 The TOC concentrations in this fluvial system showed a spatial pattern of higher values in the  
334 lower part of the river system, indicating the likely transport of TOC downstream and its  
335 accumulation from the biological production in the lakes and the surrounding tundra. Such  
336 spatial distribution was especially visible in the beginning of the season, when the upper parts  
337 of the catchment were still partly snow-covered. The maximum TOC value in Revelva  
338 approximately doubled the maximum DOC (dissolved organic carbon) value noted in the  
339 biggest lake of the Brategg Valley (in the first half of August, Górniak et al., 2016). We noted  
340 also a temporal increase in TOC concentration between the June and September samples,

341 which could originate from the biomass accumulation in the catchment, but also from the  
342 permafrost melting contributing to the hydrological regime of the river. The point in which  
343 such a change was the least notable (R13) was fed by glacial meltwater.

#### 344 4.1.2. Inorganic ions

345 The high concentrations of chloride and sodium in the collected samples testify to the  
346 important influence of sea spray on the local precipitation (Kosek et al. 2018), which feeds the  
347 surface waters, especially as snow melt in June. Another important contributor to the ion  
348 composition of the Revelva system waters is rock weathering, which increases the  
349 concentrations of calcium, magnesium and potassium ions. It is the most likely source of the  
350 predominant concentration of  $\text{Ca}^{2+}$  in September, when groundwater related to permafrost  
351 thaw feeds the surface waters in a significant proportion (McKenzie and Voss 2013;  
352 Szumińska et al. 2018).

353 The content of ammonium and nitrate ions in aquatic environment is an important factor in  
354 the development of microorganisms, especially in low-nutrient environment (Rivkina et al.  
355 2000), and the interesting fact found for the Revelva catchment was their reverse pattern of  
356 seasonal change in concentration. McNamara et al. (2008) reported similar temporal patterns  
357 of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations in the Kuparuk river system in Alaska, connecting them to  
358 the origin of ammonium from snowmelt (including leaching the top layer of soil by snowmelt,  
359 which in anoxic conditions produces more  $\text{NH}_4^+$ ). Such a mechanism is corroborated by the  
360 finding of ammonia oxidising archaea and bacteria in the microbial population of the Revelva  
361 system. As McNamara et al. (2008) point out, the following increase in nitrate concentration  
362 could be a result of nitrification occurring in the well-mixed stream waters. Also in this study,  
363 the microbial communities responsible for nitrogen transformation were detected, which was  
364 described in details in points: 3.2 and 4.2.





#### 365 4.1.3. Elemental nutrients

366 The nutrients B, Fe and P in the studied catchment waters may originate from local rock  
367 weathering, however B was also found to occur at higher concentrations in precipitation than  
368 in surface waters, in the neighbouring Fuglebekken catchment (Kozak et al. 2015).  
369 Geologically, the studied part of Spitsbergen is built of Proterozoic crystalline rocks that in  
370 the coastal zone of the valley are covered by Quaternary clastic formations. The crystalline  
371 bedrock is formed of various kinds of metamorphic rocks, mainly gneisses, mica-schists,  
372 quartzites, migmatites, marbles, amphibolites and calcareous-silicate rocks (Marszałek and  
373 Wąsik 2013). These rocks are characterised by various degrees of fissuring and they are  
374 markedly weathered in the upper parts of the catchment. The river valleys are filled with  
375 coarse clastic material, interdigitating with moraine till formations from local glaciers. The  
376 coastal zone is covered by coarse gravels and boulders (Marszałek and Wąsik 2013).  
377 Throughout the rock formations of the Revelva catchment, ore-bearing mineral veins occur,  
378 which are especially abundant in iron minerals: many are ankerite or quartz-ankerite veins,  
379 and they contain other iron minerals, such as pyrite, chalcopyrite, pyrrhotite, sometimes also  
380 magnetite and haematite (Wojciechowski 1964). Thus the increase in Fe concentrations in  
381 September may be caused by the occurrence of groundwater associated with the active layer  
382 of permafrost, which gains more importance in the hydrological regime of the Revelva once  
383 snow patches disappear in the catchment, and leaches iron from ore-bearing layers. Some of  
384 these minerals are sulphides, and these occur on the whole left side of the Revelva, which  
385 would contribute to the formation of abundant sulphate in the runoff. In the top part of the  
386 catchment (Gangpasset), Smulikowski (1965) mentions also the occurrence of phosphorus  
387 minerals (apatite), although this did not raise elemental phosphorus or phosphate  
388 concentrations in the studied samples to the detection level. In fact, the only points and  
389 sampling occasion when we detected elemental phosphorus was the lowest part of the

390 catchment in June. This could reflect the elevated concentrations of phosphorus-containing  
391 particles in snowpack and the correlation of inorganic phosphorus removal with runoff, as  
392 was observed in a catchment in Alaska (McNamara et al. 2008). The general pattern matches  
393 the low concentration levels of inorganic phosphorus in other Arctic rivers, which tend to  
394 carry nitrogen and phosphorus mainly as organic compounds (Dittmar and Kattner 2003).

#### 395 4.2. Microbial Community

396 The parameters such as the TBN, ACV and BB, provide means for a general monitoring of  
397 temporal and spatial changes of the bacterial abundance in river-lake systems. In this study,  
398 the observed values were lower than in a neighbouring valley. Both the maximum TBN and  
399 BB values were slightly less than the minimum values of these parameters noted by Górnjak  
400 et al. (2016), factoring in the presence of <4% archaea in their estimations of biomass. As the  
401 Revelva system is only glacially fed in a small proportion, while the Bratæg system was a  
402 typical proglacial succession sequence, this can reflect the influence of nutrient and cell  
403 supply from the glacier, magnified by the increasing temperature downstream, on the Bratæg  
404 data (*cf.* Graneli et al. 2004; Mindl et al. 2007). However, the contrast is not very strong. In  
405 fact, the values found in Mackenzie river by Vallières et al. (2008), as well as in the Kuparuk  
406 river and the Toolik lake by Hobbie et al. (1983) encompassed the range of values found in  
407 Revelva, and they were not different from the values characteristic for temperate rivers.

408 What is more interesting, however, in the Revelva catchment, are the spatial patterns observed  
409 at a smaller scale. In these, nutrient supply is likely to play a significant role. For example, in  
410 September 2016, in sampling points R5, R6 and R10, bacteria were more abundant than in  
411 June 2016, which is consistent with the greater availability of  $\text{NO}_3^-$  and Fe at the time.  
412 Furthermore, ACV was higher upstream from TBN maxima, and this disparity between the  
413 two indices may be interpreted assuming the organisms to represent various ecological tactics



414 (Golovlev 2001) which depend on the nutrient availability in the catchment. In oligotrophic  
415 environments, organisms are less likely to reproduce fast, so the remaining cells may grow in  
416 size (Cole et al. 1993; Šimek 1994).

#### 417 4.3. Bacterial Taxonomy

418 The tested points differ significantly in terms of nutrient sources originating from bedrock,  
419 local plant tissue, or supplied by animal vectors and water inflow. Points R4 and R8  
420 experience low nutrient input from local vegetation, while point R14 is located in a boggy  
421 area, rich in cyanobacteria and bryophytes, assisted by lichens and saxifrages in varying  
422 proportions (Kumar et al. 2017). Additionally it should be noted that R8 is located at the  
423 drainage point of the Revvatnet lake to the river, R14 is located in a small stream on a raised  
424 marine terrace, while R4 represents stagnant water of a small lake. These environmental  
425 factors corroborated the changes in bacterial biodiversity, which indicated the main river at  
426 the lake drainage point to be the least diverse, confirming the generally observed drop in  
427 bioersity from headwaters for main watercourses (Crump et al. 2012; Górnjak et al. 2016). It  
428 should also be noted that across the summer season the predominant bacterial families have  
429 changed in the tested points (Table 4), especially at the lake drainage point, where the  
430 abundance of a certain family could change by as much as 8%.

431 The obtained results are in agreement with those previously presented by Ntougias et al.  
432 (2016), where members of the *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes*  
433 were found predominant for the Revelva catchment. Interestingly, members of those phyla  
434 were also predominant in the Arctic tundra (Nissinen et al. 2012). Several identified genera  
435 are psychrofiles or psychrotolerant (*Rhodoferrax ferrireducens* sp. nov., Finneran et al. 2003),  
436 typically found on glacier surfaces (*Polaromonas*, Hell et al. 2013; Gawor et al. 2016) or in  
437 Arctic fjord sediments (*Herminiimonas*, Canion et al. 2013). Furthermore, the *Acidobacteria*

438 phylum found here is considered an indicator of the tundra influence (Männistö et al. 2013),  
439 although it was detected at a lower relative abundance than is typical for Arctic settings. The  
440 *Acidobacteria* phylum was reported as predominant for instance in Canadian, Alaskan, and  
441 Siberian Arctic soils (Neufeld and Mohn 2005; Wallenstein et al. 2009; Rawat et al. 2012;  
442 Männistö et al. 2013), but not in Kongsfjorden tundra soil, which has pH close to neutral. In  
443 Kongsfjorden tundra soil, the dominance of the *Proteobacteria* over the *Acidobacteria* was  
444 reported by Tveit et al. (2013). Moreover, water samples taken from the Revelva catchment  
445 were slightly alkaline, characterized by pH from 7.1 to 7.9, while the pH growth optima for  
446 *Acidobacteria* is in the range from 3 to 6 (Jones et al. 2009). This can explain why, in this  
447 study the *Acidobacteria* phylum accounted for a small share of the population from 0.22% to  
448 0.6%, while the most abundant were *Proteobacteria* (from 43% up to 53%). In the Arctic  
449 rivers and lakes, biochemical carbon cycling may be limited by the availability of N and P. In  
450 this study, specialist bacteria were detected, utilising various nitrogen sources. The ammonia  
451 oxidizing bacteria (AOB) and archaea (AOA) as well as anammox bacteria, which use  
452 ammonia as a substrate for metabolism, occurred in the studied points at concentrations which  
453 can substantially influence the experienced nutrient levels. Despite the limited robustness of  
454 gene-fragment assignment to a certain species or even genus, it has to be noted that the  
455 ammonia-metabolising organisms were very likely represented in the studied catchment. In  
456 particular, the abundance of AOB in the tested samples was at a comparable level as obtained  
457 in wastewater processes, where besides the higher temperature also ammonia concentrations  
458 are several times higher. Despite the nitrite concentration below detection limit in the water  
459 samples ( $<0.06 \text{ mg L}^{-1}$ ), the nitrite oxidising bacteria (NOB) were also detected, although at a  
460 very low level. Furthermore, *Nitrospira*, besides being an NOB, was reported to convert  
461 ammonia directly to nitrate in comammox process (Daims et al., 2015).

462 Another possible metabolic path, where nitrogen serves as both an electron acceptor and an  
463 electron donor, is anaerobic ammonium oxidation (anammox);  $\text{NH}_4^+$  is oxidized to  $\text{N}_2$  gas  
464 using  $\text{NO}_2^-$  (Lotti et al. 2014). In the conducted study, anammox bacteria could be found in  
465 the *Brocadiaceae* family. However, the occurrence of anammox bacteria in the studied  
466 samples does not confirm their anammox activity, due to the oxygen presence. The detected  
467 anammox bacteria can catalyse other oxidation/reduction processes or be transported with  
468 runoff from an occasionally deoxygenated area of the boggy biological soil crust.

469 Finally, there could be even nitrogen-fixing bacteria in the studied catchment, as the second-  
470 most abundant phylum in terms of read numbers, *Actinobacteria*, includes members linked to  
471 the symbiotic nitrogen-fixing associations with plants (Cernava et al. 2015). On the other  
472 hand, denitrifying bacteria could be found in *Betaproteobacteria* class (*Proteobacteria*),  
473 *Comamonadaceae* family, genus *Herminiimonas*, detected in this study. Such were described  
474 before by Canon et al. (2013) in samples from Svalbard fjord sediments. Overall, the  
475 bacterial community of this river-lake system has members occupying various niches in the  
476 nitrogen cycle.

477 In Arctic aquatic ecosystems, the degradation of permafrost typically increases phosphorus  
478 export to surface waters, although it can be consumed immediately on current needs. Thus,  
479 the presence of *Rhodospirillaceae* supports the explanation of the provenance of extra  
480 phosphorus supplies to maintain the described microbial abundance. Furthermore, the second-  
481 most abundant phylum in terms of read numbers, i.e. *Actinobacteria*, includes a large number  
482 of taxa exhibiting P solubilization and mineralization ability, which seems to be crucial in  
483 Arctic lakes and waters. In this study, mineral forms of phosphorus were mostly below the  
484 detection limit of  $1.0 \mu\text{g L}^{-1}$ , and the only sites with phosphorus detected were located near  
485 the river mouth in June. This highlights the low availability of this nutrient once the microbial  
486 activity has increased in the summer season.

487 Further families detected in the studied catchment contain species and genera capable of iron  
488 and sulphur compound reduction. The *Comamonadaceae* and *Geobacteraceae* family contain  
489 iron-reducing genera. The higher concentration of Fe noted in September coincided with the  
490 higher abundance of iron reducing bacteria from the *Comamonadaceae* family, but not from  
491 the *Geobacteraceae* family. The latter contains species possibly reducing both ferric iron  
492 and/or sulphur compounds at a low temperature (Nixon et al. 2017), and other sulfate-  
493 reducing bacteria were likely found in the studied catchment, although at low abundances.

494 Further links between the bacterial taxonomy and the utilised sources of organic carbon can  
495 be found. The phylum *Planctomycetes*, commonly detected in permafrost-affected soil  
496 ecosystems as minor microbial components (Steven et al. 2007; Wagner et al. 2009; Kim et  
497 al. 2014; Hultman et al. 2015) and predominant in lichen-covered soil (Ivanova et al. 2016),  
498 was represented in abundance at points R8 and R14, which were surrounded by an area  
499 covered by mat-forming cyanobacteria, bryophytes, lichens and saxifrages. Together with  
500 *Planctomycetaceae* family, bacteria from *Flavobacteriaceae* (*Bacteroidetes* phylum) family  
501 are common inhabitants of detrital aggregates, linked to algal bloom and the degradation of  
502 algal sulfated polysaccharides (Kolton et al. 2016; Ivanova et al. 2016). The abundance of  
503 *Alphaproteobacteria* members in the analysed water samples can also be explained by their  
504 participation in degradational and symbiotic relationships with lichens, and also in the  
505 nitrogen fixing, since nitrogenases are known to be ubiquitous among endophytes (Grube and  
506 Berg 2009). Local wildlife (especially birds, reindeer, and other terrestrial mammals, such as  
507 the polar fox and the polar bear) may also act as nutrient vectors (Mindl et al. 2007), in which  
508 their gut microbiota play a yet poorly understood role. Few studies have examined the gut  
509 microbiome of animals living in the polar environments to date (Glad et al. 2010). In the case  
510 of arctic-breeding shorebirds (Grond et al. 2017), gut microbiota were dominated by  
511 *Clostridia* and *Gammaproteobacteria*, but the environment of their nesting area was

512 comprised predominantly of *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Verrucomicrobia*  
513 and to a lesser extent of *Bacteroidetes*, the core bacteria noted also in this study. Gut of the  
514 wildlife is an important source of organic nitrogen and phosphorus in the oligotrophic  
515 conditions of the Arctic. As bioindicators of fecal contamination serve the following bacteria:  
516 *Escherichia coli*, *Clostridium perfringens*, members of *Enterococcus* and *Bifidobacterium*  
517 genera. In this study, the key fecal indicators were detected in all sampling points:  
518 *Enterococcus* spp. by less than 0.01% of total reads, *Escherichia* up to 0.28%, while  
519 *Bifidobacterium* up to 1.71% and *Clostridium* up to 2.29%. Among other *Firmicutes*,  
520 *Candidatus Phytoplasma*, *Acholeplasma* and *Mycoplasma* were present in each sampling  
521 point at up to 0.67%, 1.47% and 1.94%, respectively. Their role and abundance need further  
522 study, yet their presence points to the influence of animals supplying nutrients throughout the  
523 catchment with faeces, a fact confirmed by the observations of reindeer herds and various bird  
524 species in the catchment.

#### 525 4.4. Statistical analysis on the nutrient-dependence of the bacterial abundance in the Revelva 526 catchment

527 An increasing number of studies, e.g. Stibal et al. 2008; Petrone and Richards 2009;  
528 Jørgensen et al. 2014; Ntougias et al. 2016, have shown that despite the small amount of  
529 bioavailable nutrients, persistent subfreezing temperatures, prolonged darkness during winter,  
530 and exposure to sunlight during summer, aquatic bacteria lead a relatively abundant life in the  
531 Arctic (Chu et al. 2010). To explore patterns in the correlations between nutrient levels and  
532 bacterial abundance, we have conducted principal component analysis (PCA) on a set of  
533 chosen variables. In a coordinate system described by the two first principal components,  
534 there was a clear division between samples collected in the early and late summer (Figure 7  
535 top). Moreover, the clear division between the bacterial communities in these two periods can  
536 be read from the performed cluster analysis (Figure 5).

537 This seasonal division was consistent with the higher concentration of TOC in September  
538 (likely originating from both the decomposing plant tissue and permafrost thaw), as well as  
539 the higher bacterial cell counts (but not higher cell volumes). The organic matter and most  
540 ionic concentrations are typically higher in permafrost thaw waters than in melting snowpack,  
541 hence the seasonal division in sample chemical composition can be interpreted as a change in  
542 hydrological regime over the summer (Pulina et al. 1984). The PCA showed a less distinct  
543 division between hydrological environments, however there were variables in each separate  
544 season that differentiated lake and flowing water as well (Figure 7 middle and bottom). The  
545 PCA demonstrated also that the variability connected to bacterial volume was disconnected  
546 from the variability related to the bacterial number, which may represent the application of  
547 different ecological tactics in the bacterial community at conditions of nutrient abundance and  
548 shortage. In general, the lake samples were more likely to contain bacteria with high cell  
549 volume, while the stream and river environments facilitated higher bacterial numbers and  
550 most likely also higher biodiversity, as could be observed in the taxonomic characterisation of  
551 the selected few samples.

552 **Figure 7.** Principal component analysis results for nutrient concentrations and the bacterial  
553 community parameters (top). The two graphs below represent the two studied periods (June in  
554 the middle and September at the bottom), which were clearly divided in the analysis of the  
555 whole dataset.

556 The seasonal difference in nutrient abundance was clearly depicted by the PCA. Only  
557 ammonium and boron showed higher concentrations in June samples than in September. The  
558 closely correlated variable groups in the whole dataset were: [Fe]-[TOC], [SO<sub>4</sub><sup>2-</sup>]-[Cl<sup>-</sup>], and  
559 [Mg<sup>2+</sup>]-[K<sup>+</sup>]-[NO<sub>3</sub><sup>-</sup>]. However, in June the strongest correlations were found between [Na<sup>+</sup>]-  
560 [Cl<sup>-</sup>]-[B] and [Fe]-[SO<sub>4</sub><sup>2-</sup>]-[TOC]. The Na<sup>+</sup> and Cl<sup>-</sup> clearly indicate the sea spray source,  
561 which is also present in the local precipitation (including snow cover). Their presence in



562 surface drainage may be modified at this time by elution intensity from snowpack. Boron is  
563 therefore likely to originate mostly from seawater as well, through elevated concentrations in  
564 precipitation (Kozak et al. 2015). Indeed, in several samples the B/Cl<sup>-</sup> ratio was close to the  
565 0.000241 value reported as the mean for North Atlantic and North Pacific water (Lee et al.  
566 2010), and the mean ratio has dropped from 0.000210 to 0.000163 (from June to September).  
567 Since rock sources normally contain proportionally more B than seawater (Arnórsson and  
568 Andrésdóttir 1995), such a drop can be interpreted as an indication of boron being depleted by  
569 the local microbial community. The connection between Fe and SO<sub>4</sub><sup>2-</sup> corresponds well to  
570 their common source in pyrite and chalcopyrite decomposition, yet their increased  
571 concentrations occur mainly in the downstream part of the catchment ('river' on Figure 7  
572 middle), and the simultaneous accumulation of TOC is in agreement with the fact that pyrite  
573 decomposition is microbially mediated.

574 In September, the variables with the closest relationship were [Mg<sup>2+</sup>]-[Ca<sup>2+</sup>]-[SO<sub>4</sub><sup>2-</sup>], [B]-  
575 [TOC] and [Fe]-[pH]. The first group likely corresponds to rock weathering, and these ions  
576 achieved the highest concentrations in the waters supplied from the glacier and near the river  
577 mouth. The boron and TOC association, combined with their close correlation to TBN,  
578 confirms the likely use of boron in biological processes enabling the bacterial community to  
579 grow and release organic substances. Finally, the Fe concentration can be regulated by the pH  
580 of the environment, however the direction of the relationship found here is contrary to the one  
581 based on solubility of iron (and its speciation forms) only. Potentially, the oxidation-reduction  
582 potential of the water and the presence of iron bacteria modify the pattern more significantly  
583 here (Hem and Cropper 1962). Such an interpretation is confirmed by the difference between  
584 lake and flowing water iron concentrations, with the lowest concentrations found in the  
585 Revvatnet (large lake) and some main river samples (below that lake), while the highest  
586 values were noted in the headwaters of the upper part of the catchment (data not shown).

587 In conclusion, the catchment chemical state and the abundance of bacteria undergo a notable  
588 shift during the summer season, which indicates features of increased groundwater supply, but  
589 also an increased microbial activity and resulting nutrient depletion. The parameters with  
590 elevated concentrations in September samples are likely candidates for more important  
591 biogeochemical factors in the future of the Arctic rivers. However, the bacterial activity may  
592 revert some of the typically observed patterns, e.g. by depleting phosphorus and nitrogen in  
593 inorganic forms. The complex feedbacks between such processes require further  
594 investigations.

## 595 **5. Final remarks and conclusions**

596 The rapid environmental change in the Arctic is likely to bring complex biogeochemical  
597 shifts, some of which can be anticipated by studying changes in a catchment with a seasonally  
598 changing water supply. The results obtained here confirm that freshwater environments in the  
599 Arctic contain a low amount of bioavailable forms of nutrients (especially phosphorus)  
600 needed for bacterial growth, an amount that is altered as the summer season progresses, in  
601 connection to switching nutrient sources and microbial activity. This requires applying  
602 various ecological tactics to survive (e.g. investing in cell growth or reproduction in different  
603 environments / periods). Despite this, a number of bacterial phyla occupy the studied  
604 catchment (mainly *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes* and  
605 *Firmicutes* by the order of abundance, however by the number of OTUs *Acidobacteria* were  
606 at least the third most important phylum in all samples). The determined bacteria were  
607 characterised by high biodiversity indices and a development of multiple survival strategies,  
608 as well as a variety of metabolic pathways developed to utilise the existing small nutrient  
609 concentrations. The community has also shown a remarkable ability to adjust to the existing  
610 conditions changing over the summer season, showing a change in taxonomical composition  
611 and relative family abundances between the June and September samples (by up to 8%). For

612 future studies, we recommend especially the studies of this complex relationships between the  
613 bacterial community and their chemical environment at a higher temporal resolution,  
614 combined with the determination of activity of various bacterial groups.

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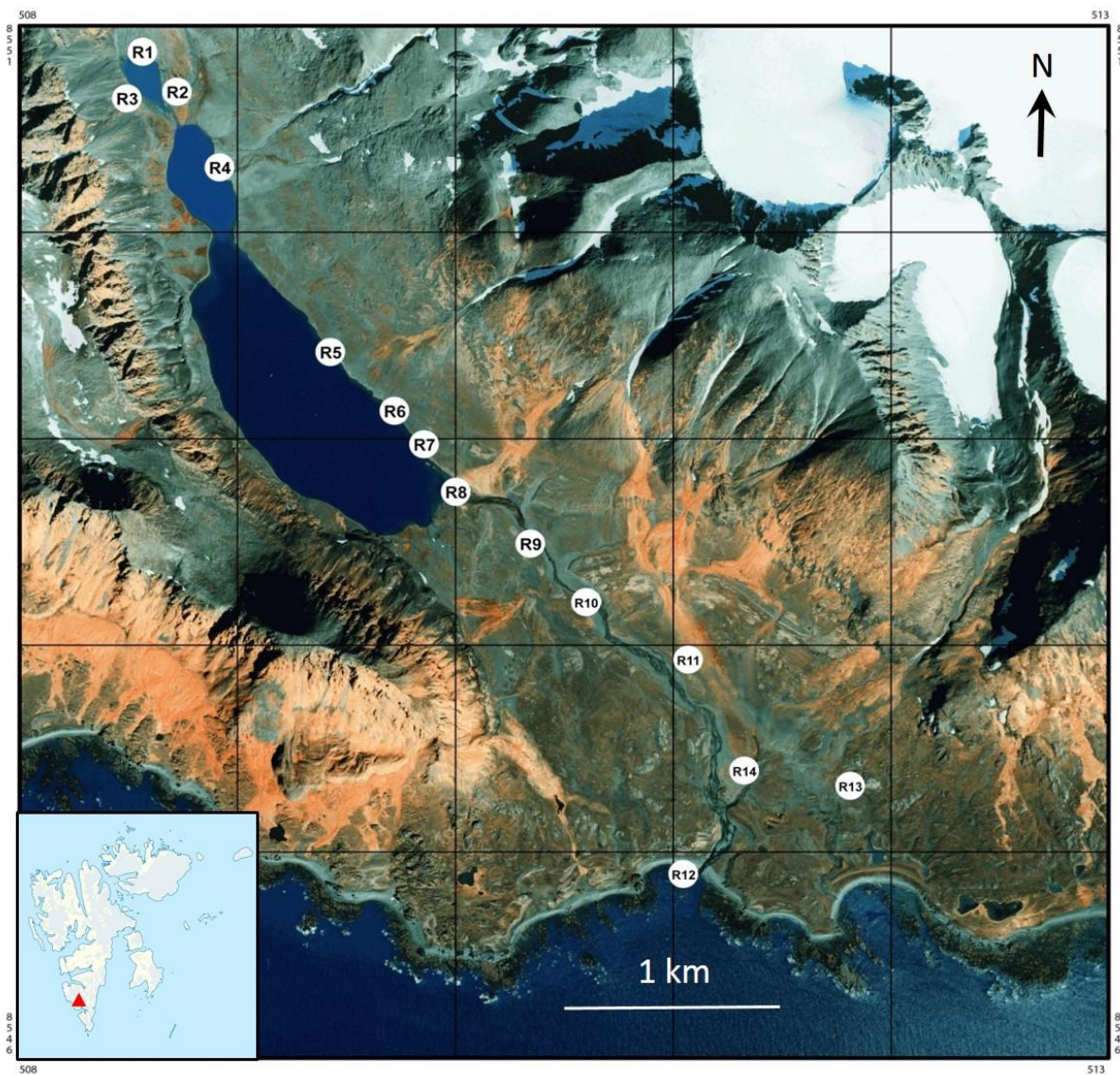
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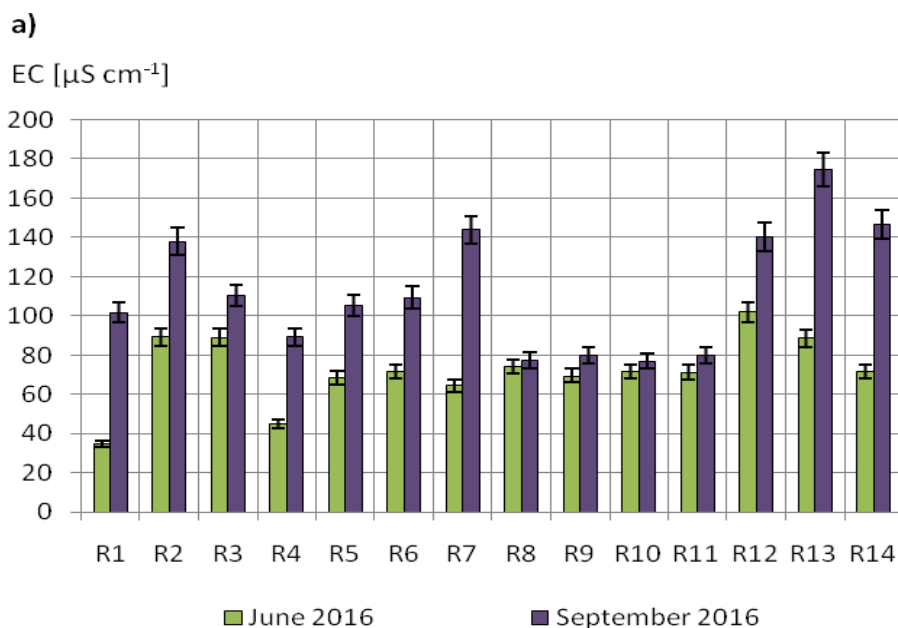


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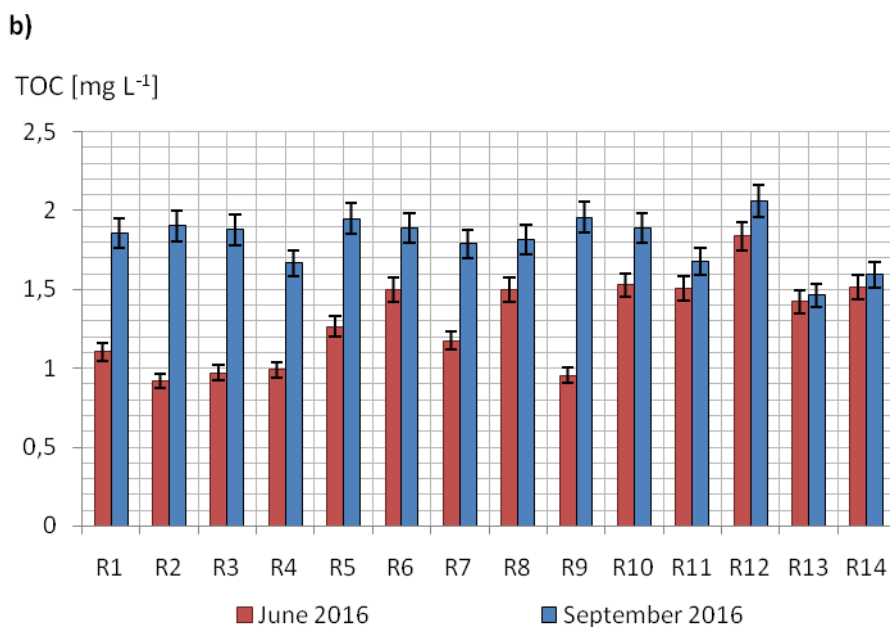
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835 **Figure 1.** Location of the studied area in Svalbard and the sampling points in the Revelva

836 catchment.



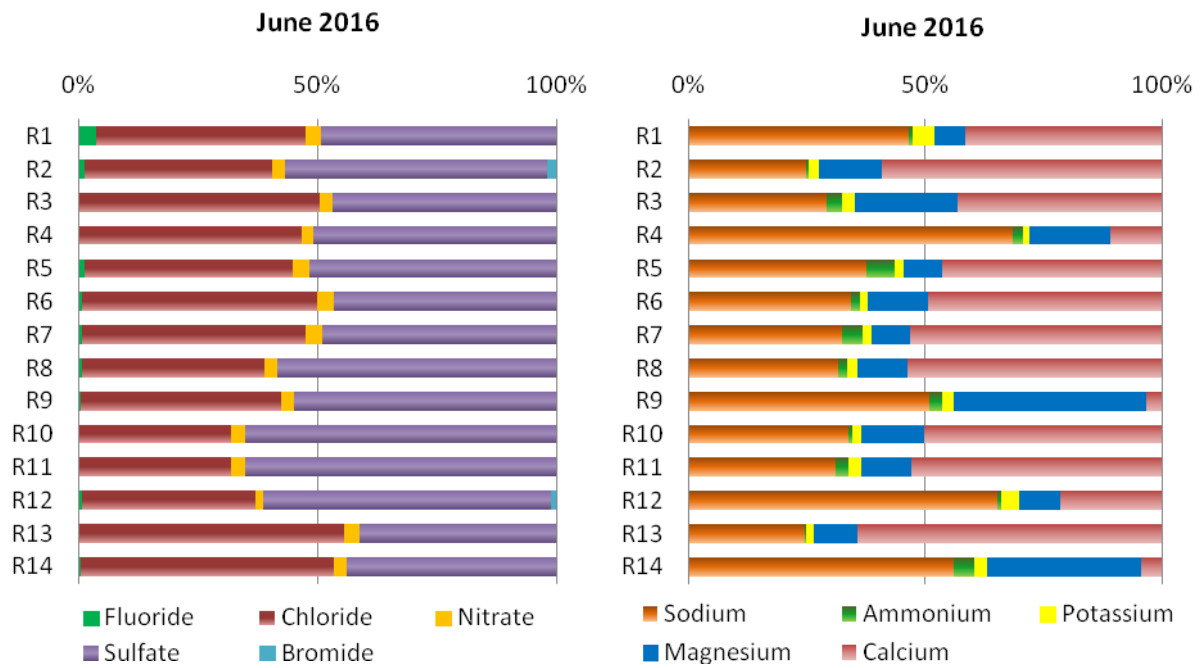
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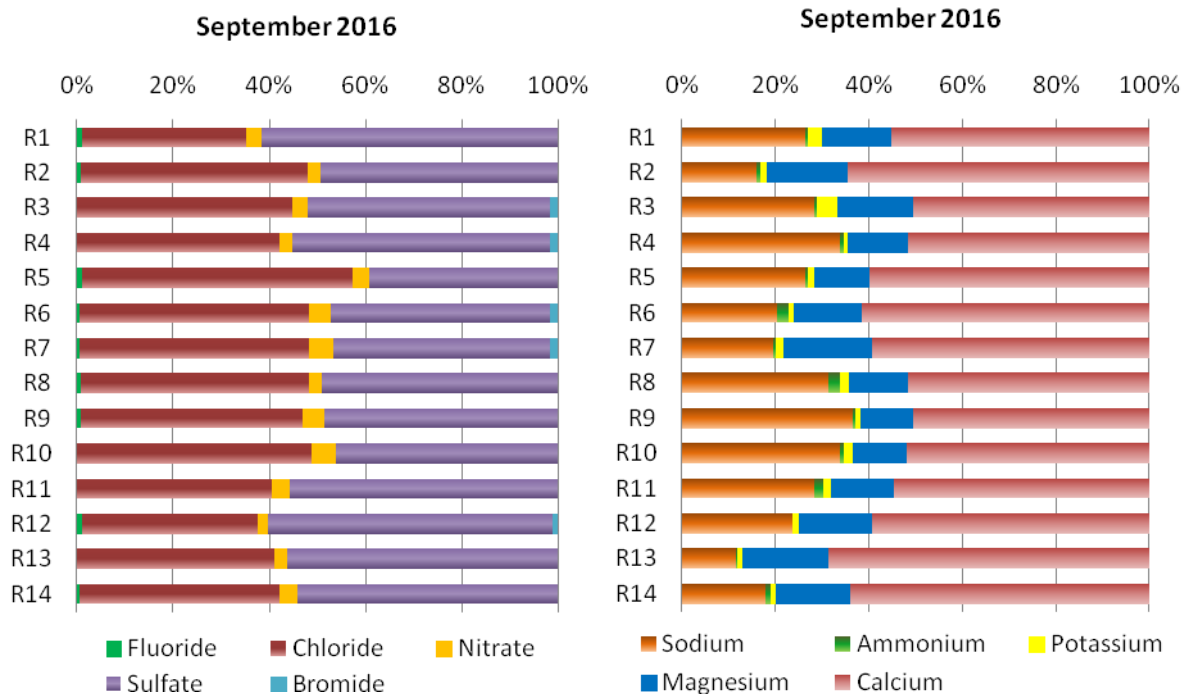
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840 **Figure 2.** Concentration levels of electrical conductivity and total organic carbon determined  
 841 in the collected freshwater samples, compared between the studied periods; a) electrical  
 842 conductivity (EC), b) total organic carbon (TOC).



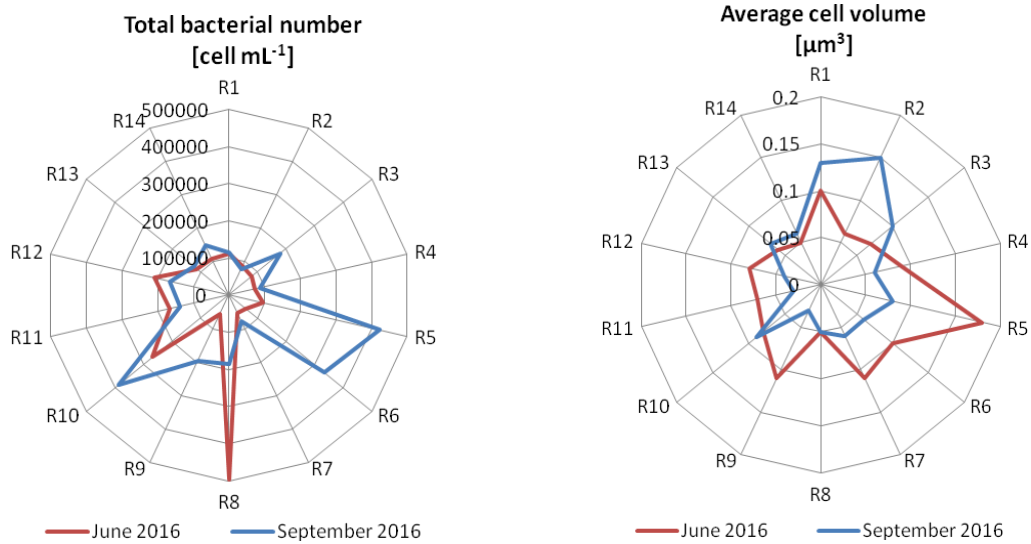
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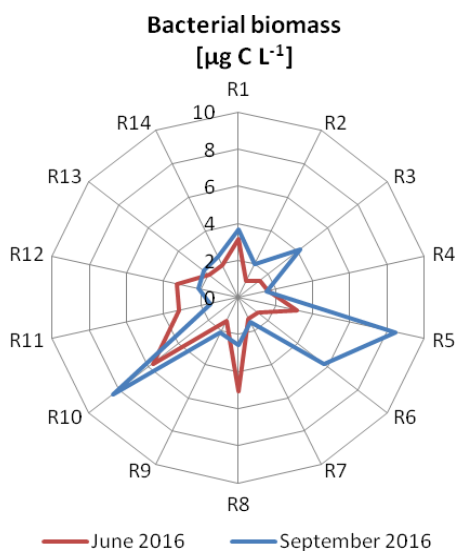
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845 **Figure 3.** Percentage anion and cation composition of the collected samples.



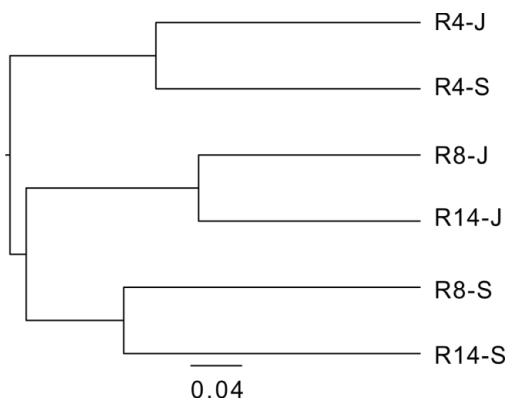


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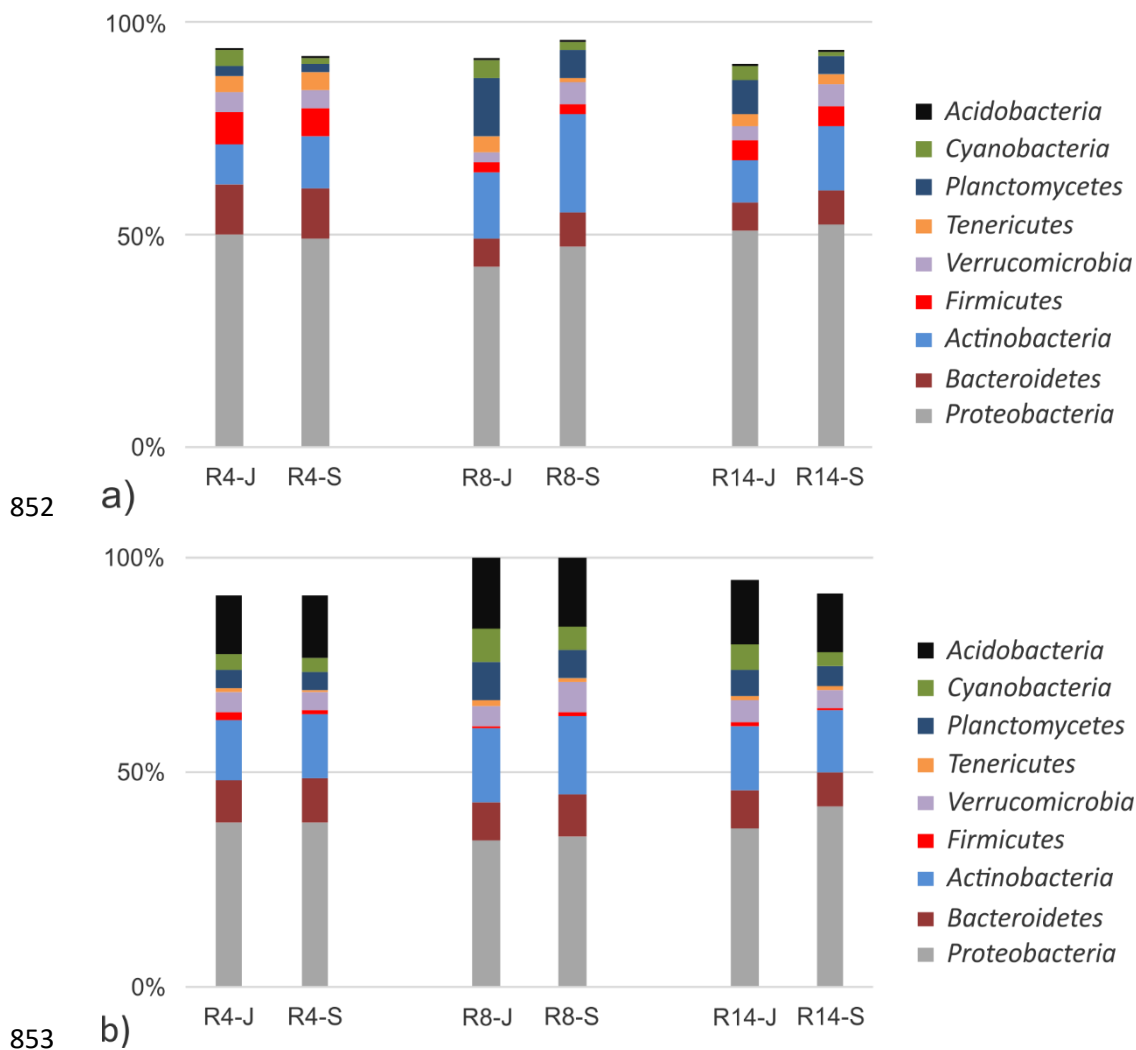
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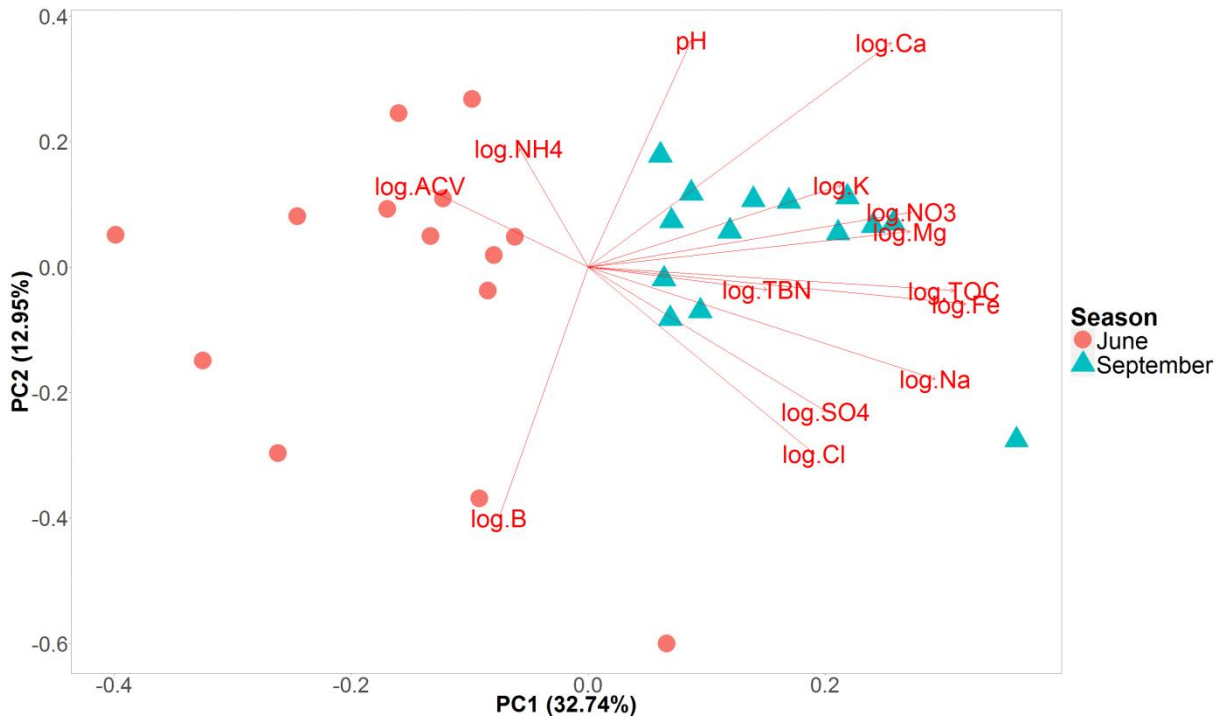
848 **Figure 4.** Comparison of bacterial abundance (total number), average bacterial cell volume  
 849 and bacterial biomass in the Revelva catchment in June and September 2016.



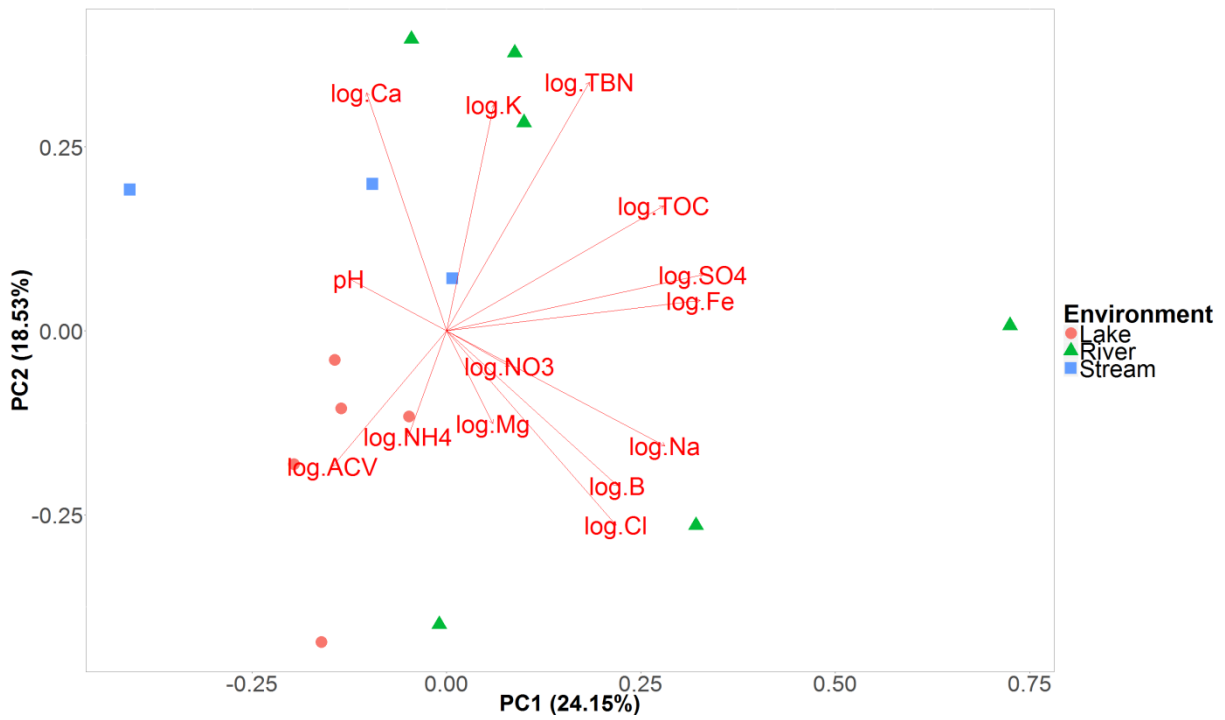
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851 **Figure 5.** Cluster analysis of bacterial community structures.

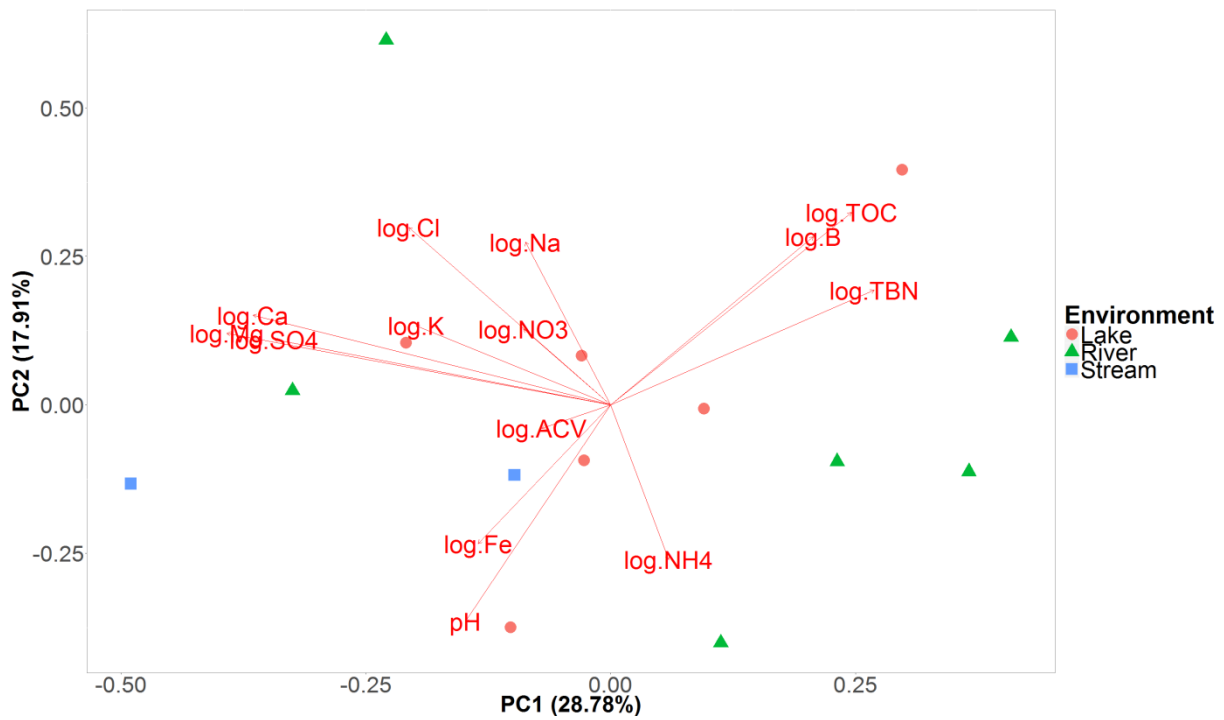




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860 **Figure 7.** Principal component analysis results for nutrient concentrations and the bacterial  
 861 community parameters (top). The two graphs below represent the two studied periods (June in  
 862 the middle and September at the bottom), which were clearly divided in the analysis of the  
 863 whole dataset.

864 **Tables**865 **Table 1.** Validation parameters and technical specifications used in the applied analytical procedures.

Determined compounds/parameters	Measurement range	LOD <sup>4</sup>	LOQ <sup>4</sup>	Measurement method/technique
<b>Electrical conductivity</b> <sup>1</sup>	-	-	-	Electrochemical method: CPC-411 conductometer (Elmetron), conductivity sensor EC60
<b>pH</b>	-	-	-	Electrochemical method: microcomputer pH-meter(Elmetron), electrode type EPS-1
<b>TOC</b> <sup>2</sup>	0.150-10.0	0.030	0.100	Total Organic Carbon Analyzer, TOC-V <sub>CSH/CSN</sub> , method of catalytic combustion (oxidation) with the application of the NDIR detector
<b>Anions</b> <sup>2</sup>	0.030-250	0.060	0.180	Ion Chromatography technique with the application of the conductivity detector (DIONEX ICS-3000)
<b>Cations</b> <sup>2</sup>	0.030-250	0.010	0.030	
<b>Micronutrients</b> <sup>3</sup>	<b>Fe</b>	0.010-1000	0.010	Inductively Coupled Plasma Mass Spectrometry technique (Thermo Scientific XSERIES 2 ICP-MS)
	<b>B</b>	0.100-1000	0.100	
	<b>P</b>	1.00-1000	1.00	

866 <sup>1</sup>[ $\mu\text{S cm}^{-1}$ ], <sup>2</sup>[ $\text{mg L}^{-1}$ ], <sup>3</sup>[ $\mu\text{g L}^{-1}$ ], <sup>4</sup>the limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the response (s) and the867 slope of the calibration curve (b), according to the formulas:  $\text{LOD}=3.3(\text{s}/\text{b})$ ,  $\text{LOQ}=10(\text{s}/\text{b})$ 

868 **Table 2.** Concentrations ( $\pm$ standard deviation, SD) of micronutrients in the collected freshwater samples.

		June 2016	September 2016
<b>Elemental nutrients</b>	<b>B</b>	1.701 $\pm$ 0.041 – 4.513 $\pm$ 0.024	1.888 $\pm$ 0.038 – 2.714 $\pm$ 0.016
<b>[<math>\mu\text{g L}^{-1}</math>]</b>	<b>P</b>	<LOD – 1.91 $\pm$ 0.47	<LOD
	<b>Fe</b>	0.0100 $\pm$ 0.0050 – 0.279 $\pm$ 0.017	0.1210 $\pm$ 0.0010 – 1.32 $\pm$ 0.14

869 **Table 3.** Number of reads and OTUs as well as species richness estimate (Chao1) and diversity indices (Shannon and Simpson) for the sampling  
870 points.

Sampling point	Reads	OTU	Chao 1	Shannon	Simpson
<b>R4-J</b>	154022	6500	6755	10.32	0.995
<b>R4-S</b>	170636	6732	7026	10.42	0.996
<b>R8-J</b>	118556	3838	4914	7.28	0.971
<b>R8-S</b>	119228	3936	5485	6.82	0.948
<b>R14-J</b>	99569	5576	6284	9.13	0.987
<b>R14-S</b>	178337	6663	6920	9.89	0.992

871 OTUs were defined at a 97% sequence identity threshold

872

873 **Table 4.** Family level taxonomic composition in the Revelva catchment (among 274 families reported in this study, first 40 are presented).

R4 in June			R4 in September			R8 in June			R8 in September			R14 in June			R14 in September			
Family	%	%	Family	Family	%	%	Family	Family	%	%	Family	Family	%	%	Family	Family	%	%
<i>Comamonadaceae</i>	11.66%	13.00%	<i>Comamonadaceae</i>	<i>Isosphaeraceae</i>	11.53%	13.45%	<i>Kiloniellaceae</i>	<i>Comamonadaceae</i>	6.48%	8.19%	<i>Comamonadaceae</i>	<i>Comamonadaceae</i>	6.48%	8.19%	<i>Comamonadaceae</i>	<i>Comamonadaceae</i>	6.48%	8.19%
<i>Flavobacteriaceae</i>	5.57%	3.59%	<i>Flavobacteriaceae</i>	<i>Streptomyetaceae</i>	7.68%	11.21%	<i>Cellulomonadaceae</i>	<i>Isosphaeraceae</i>	6.35%	5.22%	<i>Cellulomonadaceae</i>	<i>Isosphaeraceae</i>	6.35%	5.22%	<i>Cellulomonadaceae</i>	<i>Cellulomonadaceae</i>	6.35%	5.22%
<i>Oxalobacteraceae</i>	4.46%	3.16%	<i>Flexibacteraceae</i>	<i>Comamonadaceae</i>	6.35%	10.64%	<i>Comamonadaceae</i>	<i>Oxalobacteraceae</i>	5.30%	3.79%	<i>Comamonadaceae</i>	<i>Oxalobacteraceae</i>	5.30%	3.79%	<i>Kiloniellaceae</i>	<i>Oxalobacteraceae</i>	5.30%	3.79%
<i>Clostridiaceae</i>	2.48%	2.67%	<i>Sphingomonadaceae</i>	<i>Oxalobacteraceae</i>	5.64%	5.99%	<i>Isosphaeraceae</i>	<i>Kiloniellaceae</i>	4.76%	3.43%	<i>Isosphaeraceae</i>	<i>Kiloniellaceae</i>	4.76%	3.43%	<i>Oxalobacteraceae</i>	<i>Oxalobacteraceae</i>	4.76%	3.43%
<i>Flexibacteraceae</i>	2.22%	2.56%	<i>Intrasporangiaceae</i>	<i>Kiloniellaceae</i>	5.31%	4.16%	<i>Verrucomicrobiaceae</i>	<i>Flavobacteriaceae</i>	3.74%	3.12%	<i>Verrucomicrobiaceae</i>	<i>Flavobacteriaceae</i>	3.74%	3.12%	<i>Verrucomicrobiaceae</i>	<i>Verrucomicrobiaceae</i>	3.74%	3.12%
<i>Sphingobacteriaceae</i>	1.97%	2.54%	<i>Sphingobacteriaceae</i>	<i>Flavobacteriaceae</i>	4.73%	3.86%	<i>Oxalobacteraceae</i>	<i>Cellulomonadaceae</i>	2.68%	2.92%	<i>Oxalobacteraceae</i>	<i>Cellulomonadaceae</i>	2.68%	2.92%	<i>Isosphaeraceae</i>	<i>Isosphaeraceae</i>	2.68%	2.92%
<i>Sphingomonadaceae</i>	1.90%	2.15%	<i>Acholeplasmataceae</i>	<i>Rhizobiaceae</i>	2.93%	3.52%	<i>Pseudonocardiaceae</i>	<i>Legionellaceae</i>	1.88%	2.64%	<i>Pseudonocardiaceae</i>	<i>Legionellaceae</i>	1.88%	2.64%	<i>Legionellaceae</i>	<i>Legionellaceae</i>	1.88%	2.64%
<i>Geobacteraceae</i>	1.74%	2.12%	<i>Xanthomonadaceae</i>	<i>Cellulomonadaceae</i>	2.01%	2.85%	<i>Flavobacteriaceae</i>	<i>Pseudonocardiaceae</i>	1.86%	2.39%	<i>Flavobacteriaceae</i>	<i>Pseudonocardiaceae</i>	1.86%	2.39%	<i>Intrasporangiaceae</i>	<i>Intrasporangiaceae</i>	1.86%	2.39%
<i>Bifidobacteriaceae</i>	1.72%	1.97%	<i>Cellulomonadaceae</i>	<i>Mycoplasmataceae</i>	1.93%	2.76%	<i>Chitinophagaceae</i>	<i>Rhizobiaceae</i>	1.78%	2.24%	<i>Chitinophagaceae</i>	<i>Rhizobiaceae</i>	1.78%	2.24%	<i>Flavobacteriaceae</i>	<i>Flavobacteriaceae</i>	1.78%	2.24%
<i>Xanthomonadaceae</i>	1.69%	1.83%	<i>Oxalobacteraceae</i>	<i>Pseudonocardiaceae</i>	1.76%	1.90%	<i>Microbacteriaceae</i>	<i>Sphingomonadaceae</i>	1.33%	2.23%	<i>Microbacteriaceae</i>	<i>Sphingomonadaceae</i>	1.33%	2.23%	<i>Rhodospirillaceae</i>	<i>Rhodospirillaceae</i>	1.33%	2.23%
<i>Legionellaceae</i>	1.53%	1.67%	<i>Pseudonocardiaceae</i>	<i>Brocadiaceae</i>	1.58%	1.42%	<i>Flexibacteraceae</i>	<i>Mycoplasmataceae</i>	1.26%	2.10%	<i>Flexibacteraceae</i>	<i>Mycoplasmataceae</i>	1.26%	2.10%	<i>Sphingomonadaceae</i>	<i>Sphingomonadaceae</i>	1.26%	2.10%
<i>Intrasporangiaceae</i>	1.49%	1.66%	<i>Verrucomicrobiaceae</i>	<i>Verrucomicrobiaceae</i>	1.52%	1.13%	<i>Legionellaceae</i>	<i>Brocadiaceae</i>	1.21%	2.04%	<i>Legionellaceae</i>	<i>Brocadiaceae</i>	1.21%	2.04%	<i>Pseudonocardiaceae</i>	<i>Pseudonocardiaceae</i>	1.21%	2.04%
<i>Chitinophagaceae</i>	1.37%	1.61%	<i>Chitinophagaceae</i>	<i>Legionellaceae</i>	1.37%	1.10%	<i>Veillonellaceae</i>	<i>Verrucomicrobiaceae</i>	1.10%	1.80%	<i>Veillonellaceae</i>	<i>Verrucomicrobiaceae</i>	1.10%	1.80%	<i>Sphingobacteriaceae</i>	<i>Sphingobacteriaceae</i>	1.10%	1.80%
<i>Rhodospirillaceae</i>	1.27%	1.49%	<i>Rhodocyclaceae</i>	<i>Legionellaceae</i>	1.37%	1.10%	<i>Methylophilaceae</i>	<i>Hyphomicrobiaceae</i>	1.08%	1.75%	<i>Methylophilaceae</i>	<i>Hyphomicrobiaceae</i>	1.08%	1.75%	<i>Acholeplasmataceae</i>	<i>Acholeplasmataceae</i>	1.08%	1.75%
<i>Hyphomicrobiaceae</i>	1.10%	1.40%	<i>Rhodospirillaceae</i>	<i>Rivulariaceae</i>	1.18%	1.00%	<i>Alcaligenaceae</i>	<i>Rhodospirillaceae</i>	1.05%	1.68%	<i>Alcaligenaceae</i>	<i>Rhodospirillaceae</i>	1.05%	1.68%	<i>Chitinophagaceae</i>	<i>Chitinophagaceae</i>	1.05%	1.68%
<i>Thermoanaerobacteraceae</i>	1.03%	1.40%	<i>Paenibacillaceae</i>	<i>Halothiobacillaceae</i>	1.01%	0.99%	<i>Bogoriellaceae</i>	<i>Geobacteraceae</i>	0.97%	1.54%	<i>Bogoriellaceae</i>	<i>Geobacteraceae</i>	0.97%	1.54%	<i>Flexibacteraceae</i>	<i>Flexibacteraceae</i>	0.97%	1.54%
<i>Pseudonocardiaceae</i>	1.01%	1.32%	<i>Legionellaceae</i>	<i>Alcaligenaceae</i>	0.68%	0.97%	<i>Sphingomonadaceae</i>	<i>Enterobacteriaceae</i>	0.66%	0.89%	<i>Sphingomonadaceae</i>	<i>Enterobacteriaceae</i>	0.66%	0.89%	<i>Xanthomonadaceae</i>	<i>Xanthomonadaceae</i>	0.66%	0.89%
<i>Puniceococcaceae</i>	1.01%	1.23%	<i>Clostridiaceae</i>	<i>Enterobacteriaceae</i>	0.66%	0.89%	<i>Caulobacteraceae</i>	<i>Cyanobacteriaceae</i>	0.65%	0.64%	<i>Caulobacteraceae</i>	<i>Cyanobacteriaceae</i>	0.65%	0.64%	<i>Caulobacteraceae</i>	<i>Caulobacteraceae</i>	0.65%	0.64%
<i>Coxiellaceae</i>	1.00%	1.21%	<i>Hyphomicrobiaceae</i>	<i>Cyanobacteriaceae</i>	0.65%	0.64%	<i>Rhodobacteraceae</i>	<i>Micromonosporaceae</i>	0.62%	0.64%	<i>Rhodobacteraceae</i>	<i>Micromonosporaceae</i>	0.62%	0.64%	<i>Hyphomicrobiaceae</i>	<i>Hyphomicrobiaceae</i>	0.62%	0.64%
<i>Rhodocyclaceae</i>	0.99%	1.09%	<i>Thermoanaerobacteraceae</i>	<i>Geobacteraceae</i>	0.57%	0.50%	<i>Rhodocyclaceae</i>	<i>Geobacteraceae</i>	0.57%	0.50%	<i>Rhodocyclaceae</i>	<i>Geobacteraceae</i>	0.57%	0.50%	<i>Microbacteriaceae</i>	<i>Microbacteriaceae</i>	0.57%	0.50%
<i>Verrucomicrobiaceae</i>	0.99%	0.97%	<i>Microbacteriaceae</i>	<i>Caulobacteraceae</i>	0.55%	0.50%	<i>Streptomyetaceae</i>	<i>Caulobacteraceae</i>	0.55%	0.50%	<i>Streptomyetaceae</i>	<i>Caulobacteraceae</i>	0.55%	0.50%	<i>Rhodocyclaceae</i>	<i>Rhodocyclaceae</i>	0.55%	0.50%
<i>Cellulomonadaceae</i>	0.92%	0.96%	<i>Geobacteraceae</i>	<i>Caulobacteraceae</i>	0.55%	0.49%	<i>Intrasporangiaceae</i>	<i>Flexibacteraceae</i>	0.55%	0.49%	<i>Intrasporangiaceae</i>	<i>Flexibacteraceae</i>	0.55%	0.49%	<i>Geobacteraceae</i>	<i>Geobacteraceae</i>	0.55%	0.49%
<i>Brocadiaceae</i>	0.91%	0.85%	<i>Chromatiaceae</i>	<i>Planctomycetaceae</i>	0.50%	0.43%	<i>Hyphomonadaceae</i>	<i>Planctomycetaceae</i>	0.50%	0.43%	<i>Hyphomonadaceae</i>	<i>Planctomycetaceae</i>	0.50%	0.43%	<i>Coxiellaceae</i>	<i>Coxiellaceae</i>	0.50%	0.43%
<i>Paenibacillaceae</i>	0.89%	0.84%	<i>Caulobacteraceae</i>	<i>Coxiellaceae</i>	0.50%	0.42%	<i>Cyanobacteriaceae</i>	<i>Coxiellaceae</i>	0.50%	0.42%	<i>Cyanobacteriaceae</i>	<i>Coxiellaceae</i>	0.50%	0.42%	<i>Paenibacillaceae</i>	<i>Paenibacillaceae</i>	0.50%	0.42%
<i>Chromatiaceae</i>	0.88%	0.78%	<i>Puniceococcaceae</i>	<i>Sphingobacteriaceae</i>	0.47%	0.40%	<i>Polyangiaceae</i>	<i>Sphingobacteriaceae</i>	0.47%	0.40%	<i>Polyangiaceae</i>	<i>Sphingobacteriaceae</i>	0.47%	0.40%	<i>Chromatiaceae</i>	<i>Chromatiaceae</i>	0.47%	0.40%
<i>Chthoniobacteraceae</i>	0.84%	0.75%	<i>Brocadiaceae</i>	<i>Sphingomonadaceae</i>	0.45%	0.37%	<i>Thermogemmatimonadaceae</i>	<i>Sphingomonadaceae</i>	0.45%	0.37%	<i>Thermogemmatimonadaceae</i>	<i>Sphingomonadaceae</i>	0.45%	0.37%	<i>Clostridiaceae</i>	<i>Clostridiaceae</i>	0.45%	0.37%
<i>Acholeplasmataceae</i>	0.80%	0.74%	<i>Methylophilaceae</i>	<i>Hyphomicrobiaceae</i>	0.45%	0.36%	<i>Armatimonadaceae</i>	<i>Hyphomicrobiaceae</i>	0.45%	0.36%	<i>Armatimonadaceae</i>	<i>Hyphomicrobiaceae</i>	0.45%	0.36%	<i>Brocadiaceae</i>	<i>Brocadiaceae</i>	0.45%	0.36%
<i>Acetobacteraceae</i>	0.77%	0.63%	<i>Chthoniobacteraceae</i>	<i>Chthoniobacteraceae</i>	0.42%	0.34%	<i>Rhodospirillaceae</i>	<i>Chthoniobacteraceae</i>	0.42%	0.34%	<i>Rhodospirillaceae</i>	<i>Chthoniobacteraceae</i>	0.42%	0.34%	<i>Alcaligenaceae</i>	<i>Alcaligenaceae</i>	0.42%	0.34%
<i>Caulobacteraceae</i>	0.75%	0.57%	<i>Bifidobacteriaceae</i>	<i>Chthoniobacteraceae</i>	0.42%	0.34%	<i>Xanthomonadaceae</i>	<i>Methylophilaceae</i>	0.42%	0.33%	<i>Xanthomonadaceae</i>	<i>Methylophilaceae</i>	0.42%	0.33%	<i>Enterobacteriaceae</i>	<i>Enterobacteriaceae</i>	0.42%	0.33%
<i>Microbacteriaceae</i>	0.74%	0.55%	<i>Gemmatimonadaceae</i>	<i>Methylophilaceae</i>	0.42%	0.33%	<i>Bacteriovoracaceae</i>	<i>Phormidiaceae</i>	0.41%	0.32%	<i>Bacteriovoracaceae</i>	<i>Phormidiaceae</i>	0.41%	0.32%	<i>Mycoplasmataceae</i>	<i>Mycoplasmataceae</i>	0.41%	0.32%
<i>Peptococcaceae</i>	0.67%	0.50%	<i>Pedosphaeraceae</i>	<i>Pasteurellaceae</i>	0.41%	0.31%	<i>Sphingobacteriaceae</i>	<i>Pasteurellaceae</i>	0.41%	0.31%	<i>Sphingobacteriaceae</i>	<i>Pasteurellaceae</i>	0.41%	0.31%	<i>Bifidobacteriaceae</i>	<i>Bifidobacteriaceae</i>	0.41%	0.31%

<i>Alcaligenaceae</i>	0.65%	0.48%	<i>Leuconostocaceae</i>	<i>Chitinophagaceae</i>	0.38%	0.30%	<i>Acetobacteraceae</i>	<i>Chthoniobacteraceae</i>	0.63%	0.58%	<i>Thermoanaerobacteraceae</i>
<i>Opitutaceae</i>	0.61%	0.48%	<i>Coxiellaceae</i>	<i>Lactobacillaceae</i>	0.37%	0.28%	<i>Brocadiaceae</i>	<i>Microbacteriaceae</i>	0.56%	0.56%	<i>Veillonellaceae</i>
<i>Polyangiaceae</i>	0.59%	0.46%	<i>Acetobacteraceae</i>	<i>Rhodocyclaceae</i>	0.36%	0.28%	<i>Chthoniobacteraceae</i>	<i>Veillonellaceae</i>	0.56%	0.56%	<i>Puniceicoccaceae</i>
<i>Mycoplasmataceae</i>	0.56%	0.45%	<i>Polyangiaceae</i>	<i>Nostocaceae</i>	0.35%	0.27%	<i>Micromonosporaceae</i>	<i>Thermoanaerobacteraceae</i>	0.54%	0.49%	<i>Bacteriovoracaceae</i>
<i>Veillonellaceae</i>	0.55%	0.44%	<i>Kiloniellaceae</i>	<i>Veillonellaceae</i>	0.34%	0.27%	<i>Acholeplasmataceae</i>	<i>Micromonosporaceae</i>	0.50%	0.49%	<i>Rhodobacteraceae</i>
<i>Conexibacteraceae</i>	0.53%	0.44%	<i>Isosphaeraceae</i>	<i>Microbacteriaceae</i>	0.33%	0.26%	<i>Hyphomicrobiaceae</i>	<i>Methylophilaceae</i>	0.48%	0.47%	<i>Leuconostocaceae</i>
<i>Enterobacteriaceae</i>	0.52%	0.42%	<i>Opitutaceae</i>	<i>Mycobacteriaceae</i>	0.32%	0.26%	<i>Cerasicoccaceae</i>	<i>Acholeplasmataceae</i>	0.47%	0.47%	<i>Chthoniobacteraceae</i>
<i>Pedosphaeraceae</i>	0.51%	0.42%	<i>Rhodobacteraceae</i>	<i>Rhodospirillaceae</i>	0.31%	0.25%	<i>Saprospiraceae</i>	<i>Halothiobacillaceae</i>	0.47%	0.47%	<i>Acetobacteraceae</i>
<i>Gemmatimonadaceae</i>	0.46%	0.41%	<i>Peptococcaceae</i>	<i>Conexibacteraceae</i>	0.29%	0.25%	<i>Nostocaceae</i>	<i>Bifidobacteriaceae</i>	0.41%	0.45%	<i>Rhizobiaceae</i>

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