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

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

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

Keywords: Arctic, Spitsbergen, Freshwater bacterial community, Bacterial diversity, Nutrients

1. Introduction

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

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

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

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

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

2. Materials and Methods

2.1. Study area

The Revelva catchment (Wedel-Jarlsberg Land, southwestern Spitsbergen) is located in the vicinity of the Polish Polar Station Hornsund (77º0'0"N, 15º33'0") (Figure 1). The main river (Revelva) and the lake (Revvatnet) are fed both directly by atmospheric precipitation, snow-fed streams and a river originating from a glacier (Ariebreen), as well as permafrost thaw, especially once the snow has melted. The permafrost thaw in the area is pronounced, as this region is characterised by the highest active layer depth in the Svalbard archipelago, exceeding 2 m (Dolnicki et al. 2013). Furthermore, in the Hornsund station, long-term monitoring (1990-2009) of the active layer temperature at 1 m depth has shown an increasing trend (Dolnicki et al. 2013), leading to the conclusion that permafrost degradation was advancing in that period.

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

2.2. Sampling

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

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

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

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

2.3. Chemical Analysis

All technical specifications of the analytical equipment and methods, including basic validation parameters of the analytical procedures, are given in Table 1. The basic parameters of electrical conductivity (EC) and pH were measured immediately upon return from the field. The concentrations of the following inorganic ions: Li^+, Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, Br^-, NO_2^-, NO_3^-, PO_4^{3-} and SO_4^{2-} were determined with the use of ion chromatography technique. Phosphorus concentration was also determined in elemental form, as were the concentrations of iron and boron, all with the use of and ICP-MS (Inductively Coupled Plasma Mass Spectrometer). The element concentration CVs of the obtained triplicate results ranged from 0.5 to 1.5%. Carbon, in all organic forms, was measured as non-purgeable organic carbon with a Total Organic Carbon Analyzer TOC-$V_{CSH/CSN}$, (Shimadzu, Japan) method of catalytic combustion (oxidation) with the application of the NDIR detector. All blanks were prepared with Milli-Q deionised water.

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

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

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

2.5. Bacterial Abundance Analysis

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

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

2.6. Bacterial Community Structure Analysis

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

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

2.7. Principal Component Analysis (PCA)

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

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 μS cm^{-1} to 102.1 μS cm^{-1} in June
227 2016, and from 76.9 μS cm^{-1} to 174.5 μS 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 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, 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.

Nitrogen occurred in the Revelva catchment in ionic form, especially as NH_4^+ and NO_3^-. Nitrate occurred at concentrations ranging from 1.27 to 3.22 mg L^{-1}, with an increase in September (June median concentration amounted to 1.55 mg L^{-1}, while September median equaled 1.99 mg L^{-1}). Ammonium concentrations spanned 0.03 – 0.43 mg L^{-1}, with somewhat higher concentrations in June (June median = 0.11 mg L^{-1}, September median = 0.08 mg L^{-1}). Of other ionic nutrients, neither nitrite nor phosphate was detected, which implies their concentrations were below 0.06 mg L^{-1}.

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

3.1.3. Elemental nutrients

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

Table 2. Concentrations (±standard deviation, SD) of elemental nutrients in the collected freshwater samples.

3.2. Microbial Community

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

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

3.3. Bacterial Taxonomy

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

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

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

Figure 5. Cluster analysis of bacterial community structures.

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

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

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

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

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

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

In *Bacteroidetes* phylum, the *Flavobacteriaceae* family formed from 2.24% to 5.57% of total 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,

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

4.1.2. Inorganic ions

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

The content of ammonium and nitrate ions in aquatic environment is an important factor in the development of microorganisms, especially in low-nutrient environment (Rivkina et al. 2000), and the interesting fact found for the Revelva catchment was their reverse pattern of seasonal change in concentration. McNamara et al. (2008) reported similar temporal patterns of NH_4^+ and NO_3^- concentrations in the Kuparuk river system in Alaska, connecting them to the origin of ammonium from snowmelt (including leaching the top layer of soil by snowmelt, which in anoxic conditions produces more NH_4^+). Such a mechanism is corroborated by the finding of ammonia oxidising archaea and bacteria in the microbial population of the Revelva system. As McNamara et al. (2008) point out, the following increase in nitrate concentration could a be result of nitrification occurring in the well-mixed stream waters. Also in this study, the microbial communities responsible for nitrogen transformation were detected, which was 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

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

4.2. Microbial Community

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

What is more interesting, however, in the Revelva catchment, are the spatial patterns observed at a smaller scale. In these, nutrient supply is likely to play a significant role. For example, in September 2016, in sampling points R5, R6 and R10, bacteria were more abundant than in June 2016, which is consistent with the greater availability of NO_3^- and Fe at the time. Furthermore, ACV was higher upstream from TBN maxima, and this disparity between the two indices may be interpreted assuming the organisms to represent various ecological tactics

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

4.3. Bacterial Taxonomy

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

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

phylum found here is considered an indicator of the tundra influence (Männistö et al. 2013), although it was detected at a lower relative abundance than is typical for Arctic settings. The *Acidobacteria* phylum was reported as predominant for instance in Canadian, Alaskan, and Siberian Arctic soils (Neufeld and Mohn 2005; Wallenstein et al. 2009; Rawat et al. 2012; Männistö et al. 2013), but not in Kongsfjorden tundra soil, which has pH close to neutral. In Kongsfjorden tundra soil, the dominance of the *Proteobacteria* over the *Acidobacteria* was reported by Tveit et al. (2013). Moreover, water samples taken from the Revelva catchment were slightly alkaline, characterized by pH from 7.1 to 7.9, while the pH growth optima for *Acidobacteria* is in the range from 3 to 6 (Jones et al. 2009). This can explain why, in this study the *Acidobacteria* phylum accounted for a small share of the population from 0.22% to 0.6%, while the most abundant were *Proteobacteria* (from 43% up to 53%). In the Arctic rivers and lakes, biochemical carbon cycling may be limited by the availability of N and P. In this study, specialist bacteria were detected, utilising various nitrogen sources. The ammonia oxidizing bacteria (AOB) and archaea (AOA) as well as anammox bacteria, which use ammonia as a substrate for metabolism, occurred in the studied points at concentrations which can substantially influence the experienced nutrient levels. Despite the limited robustness of gene-fragment assignment to a certain species or even genus, it has to be noted that the ammonia-metabolising organisms were very likely represented in the studied catchment. In particular, the abundance of AOB in the tested samples was at a comparable level as obtained in wastewater processes, where besides the higher temperature also ammonia concentrations are several times higher. Despite the nitrite concentration below detection limit in the water samples (<0.06 mg L^{-1}), the nitrite oxidising bacteria (NOB) were also detected, although at a very low level. Furthermore, *Nitrospira*, besides being an NOB, was reported to convert 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); NH_4^+ is oxidized to N_2 gas
464 using 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 Canion 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 $\mu g\ L^{-1}$, 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.

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

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

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

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

The seasonal difference in nutrient abundance was clearly depicted by the PCA. Only ammonium and boron showed higher concentrations in June samples than in September. The closely correlated variable groups in the whole dataset were: [Fe]-[TOC], [SO_4^{2-}]-[Cl^-], and [Mg^{2+}]-[K^+]-[NO_3^-]. However, in June the strongest correlations were found between [Na^+]-[Cl^-]-[B] and [Fe]-[SO_4^{2-}]-[TOC]. The Na^+ and Cl^- clearly indicate the sea spray source, which is also present in the local precipitation (including snow cover). Their presence in

surface drainage may be modified at this time by elution intensity from snowpack. Boron is therefore likely to originate mostly from seawater as well, through elevated concentrations in precipitation (Kozak et al. 2015). Indeed, in several samples the B/Cl^- ratio was close to the 0.000241 value reported as the mean for North Atlantic and North Pacific water (Lee at al. 2010), and the mean ratio has dropped from 0.000210 to 0.000163 (from June to September). Since rock sources nomally contain proportionally more B than seawater (Arnórsson and Andrésdóttir 1995), such a drop can be interpreted as an indication or boron being depleted by the local microbial community. The connection between Fe and SO_4^{2-} corresponds well to their common source in pyrite and chalkopyrite decomposition, yet their increased concentrations occur mainly in the downstream part of the catchment ('river' on Figure 7 middle), and the simultaneous accumulation of TOC is in agreement with the fact that pyrite decomposition is microbially mediated.

In September, the variables with the closest relationship were $[Mg^{2+}]$-$[Ca^{2+}]$-$[SO_4^{2-}]$, [B]-[TOC] and [Fe]-[pH]. The first group likely corresponds to rock weathering, and these ions achieved the highest concentrations in the waters supplied from the glacier and near the river mouth. The boron and TOC association, combined with their close correlation to TBN, confirms the likely use of boron in biological processes enabling the bacterial community to grow and release organic substances. Finally, the Fe concentration can be regulated by the pH of the environment, however the direction of the relationship found here is contrary the one based on solubility of iron (and its speciation forms) only. Potentially, the oxidation-reduction potential of the water and the presence of iron bacteria modify the pattern more significantly here (Hem and Cropper 1962). Such an interpretation is confirmed by the difference between lake and flowing water iron concentrations, with the lowest concentrations found in the Revvatnet (large lake) and some main river samples (below that lake), while the highest values were noted in the headwaters of the upper part of the catchment (data not shown).

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

5. Final remarks and conclusions

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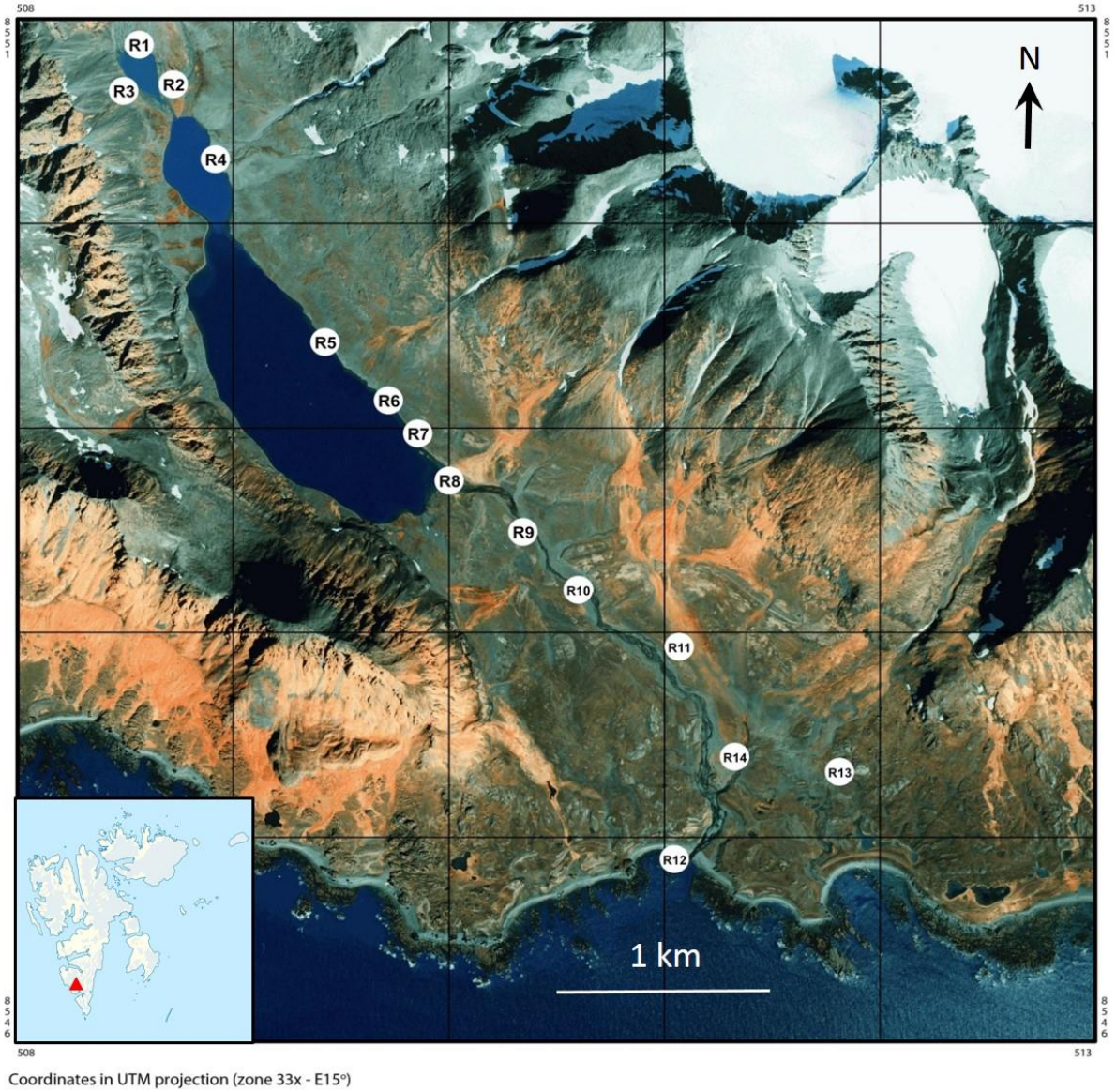
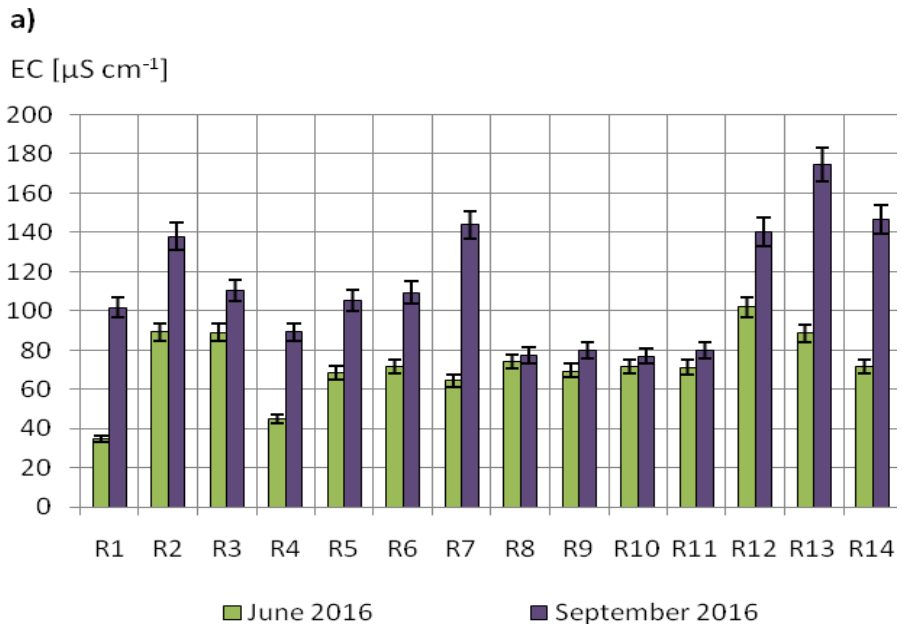
833 **Figures**



834

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

836 catchment.



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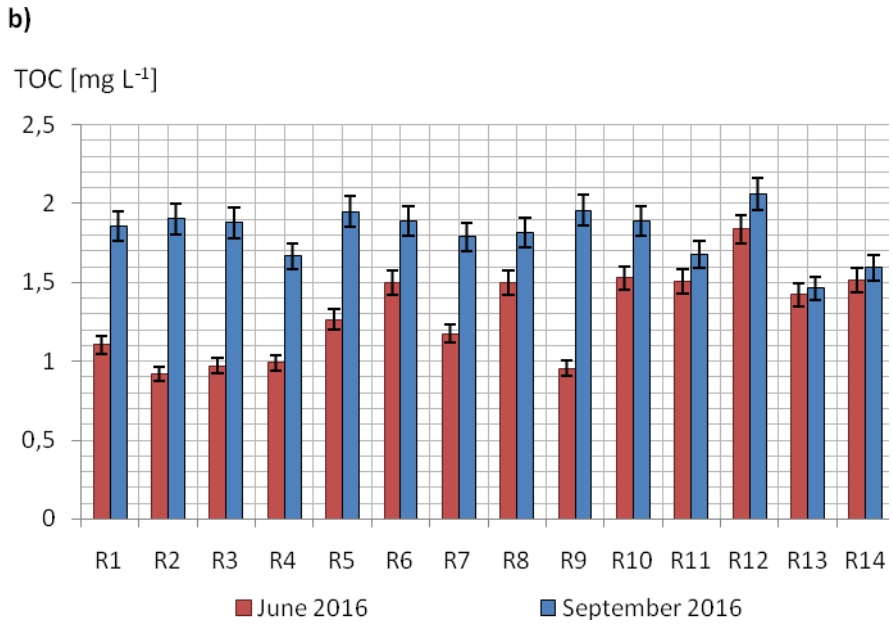

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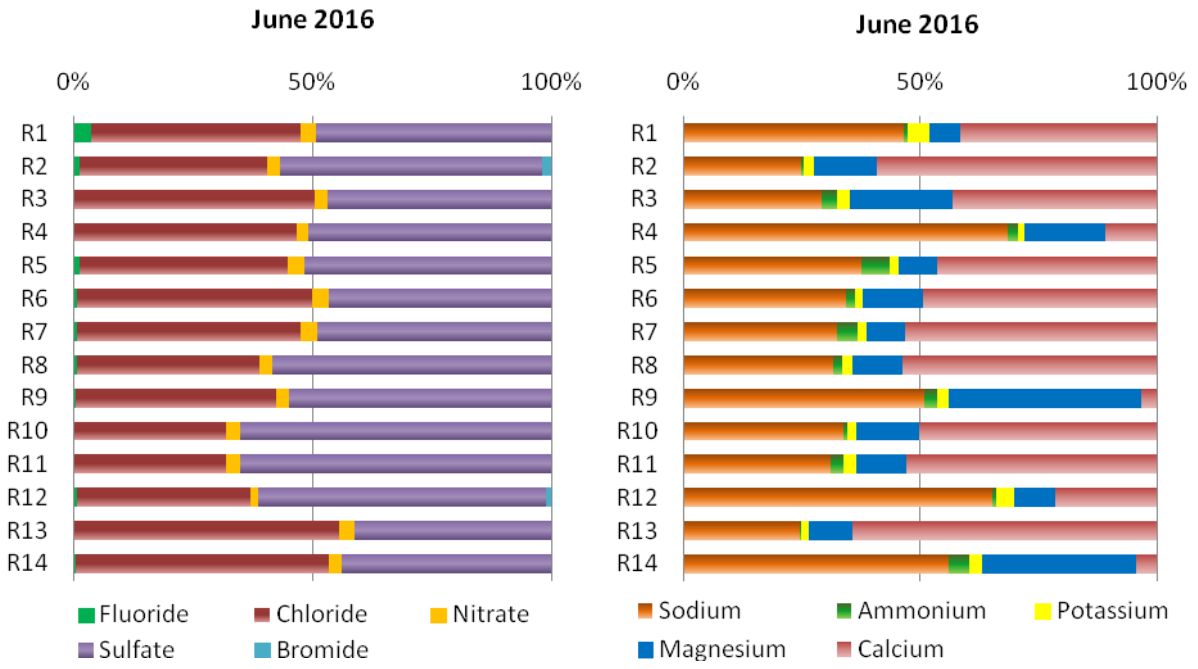
840 **Figure 2.** Concentration levels of electrical conductivity and total organic carbon determined
841 in the collected freshwater samples, compared between the studied periods; a) electrical
842 conductivity (EC), b) total organic carbon (TOC).



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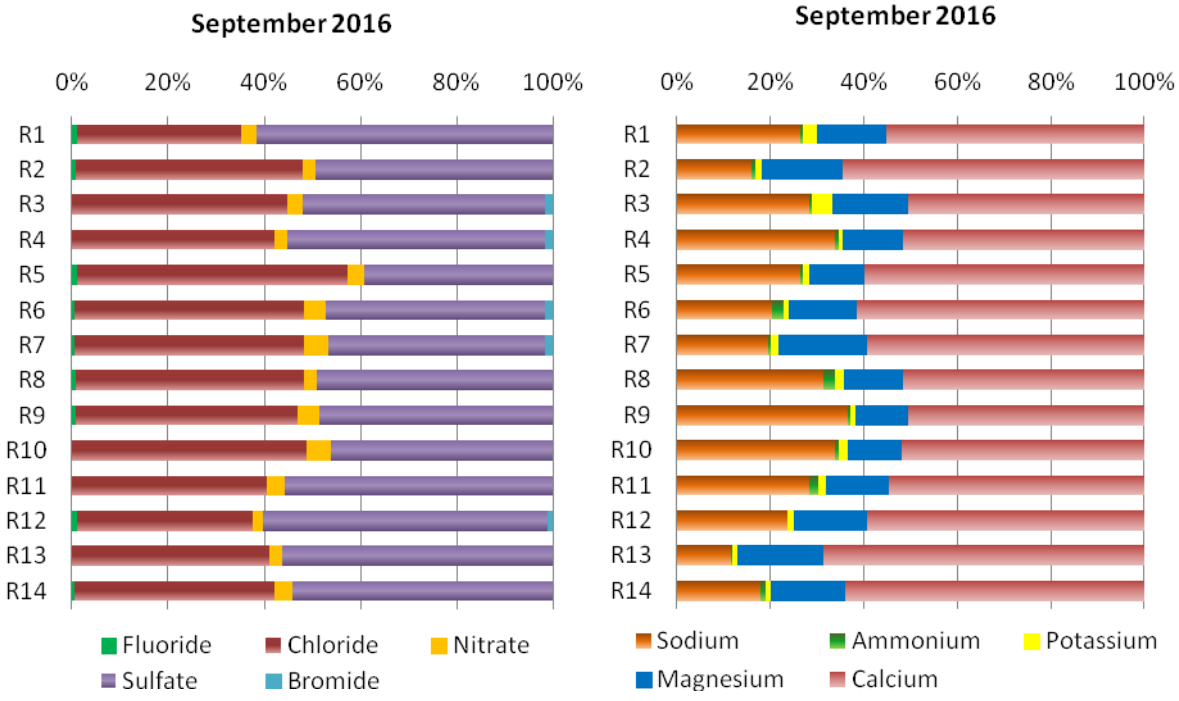
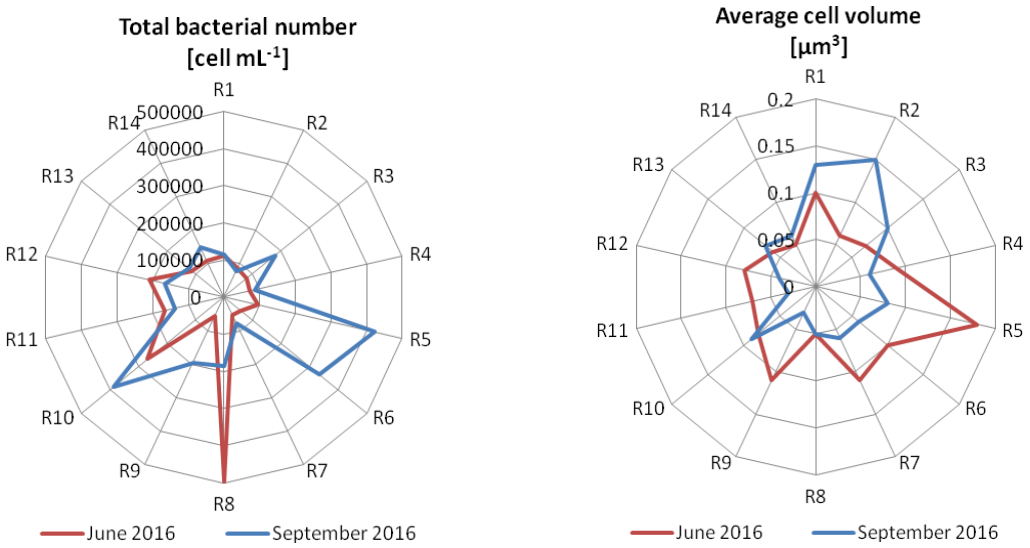


844

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



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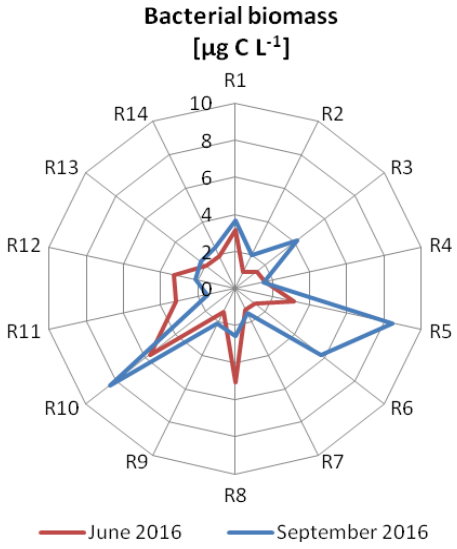
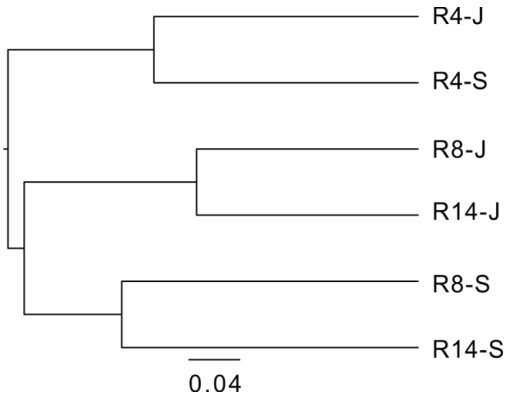


847

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.

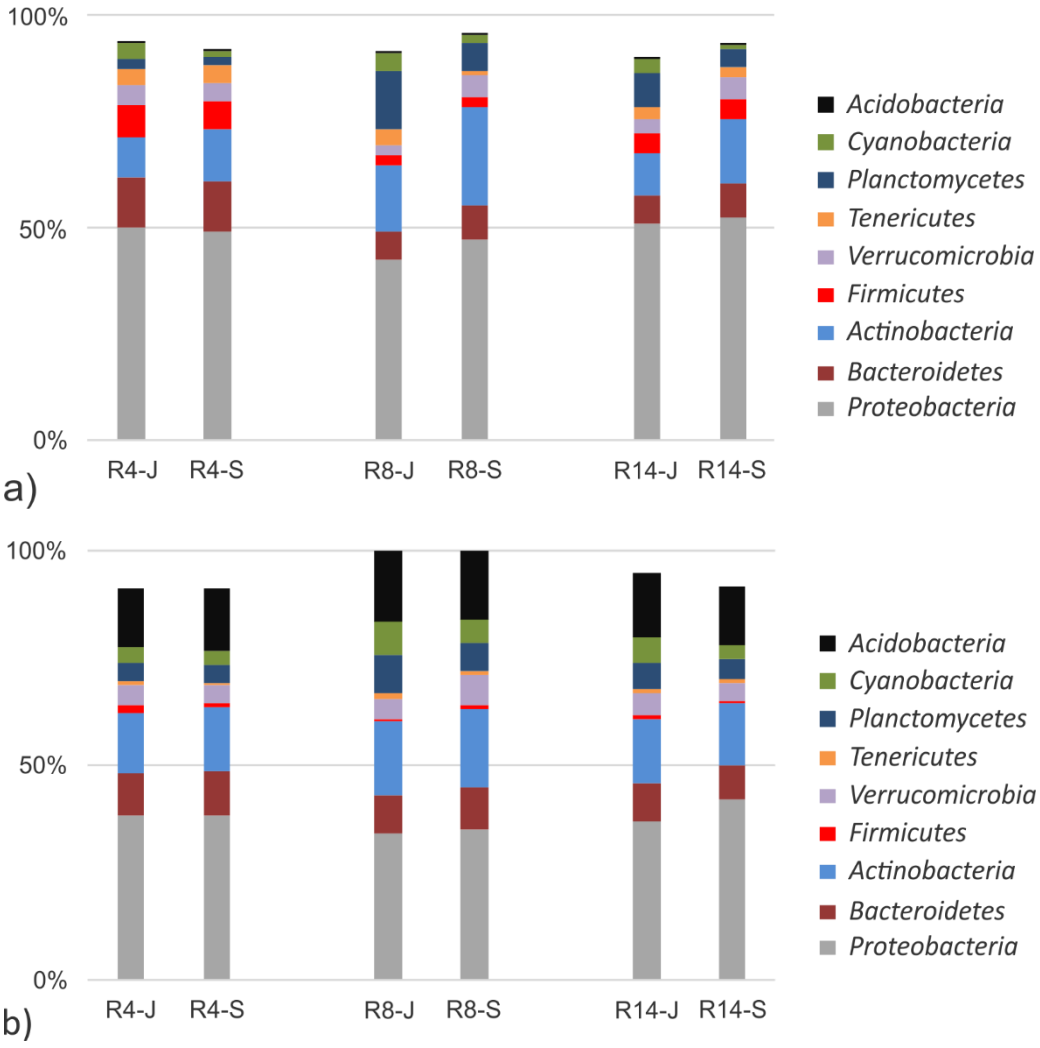
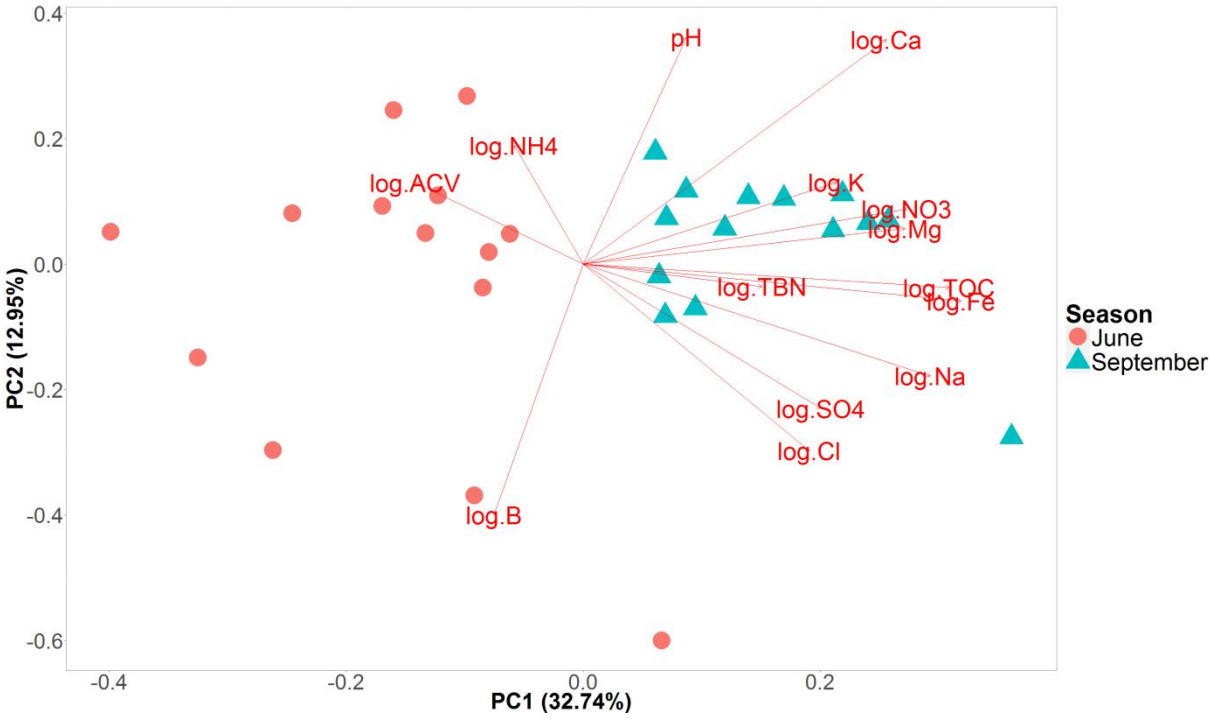


Figure 6. Bacterial community structures and relative abundances based on the number of reads (a) and OTUs (b) for major phyla (>1%) identified in tested water samples in June and September 2016.



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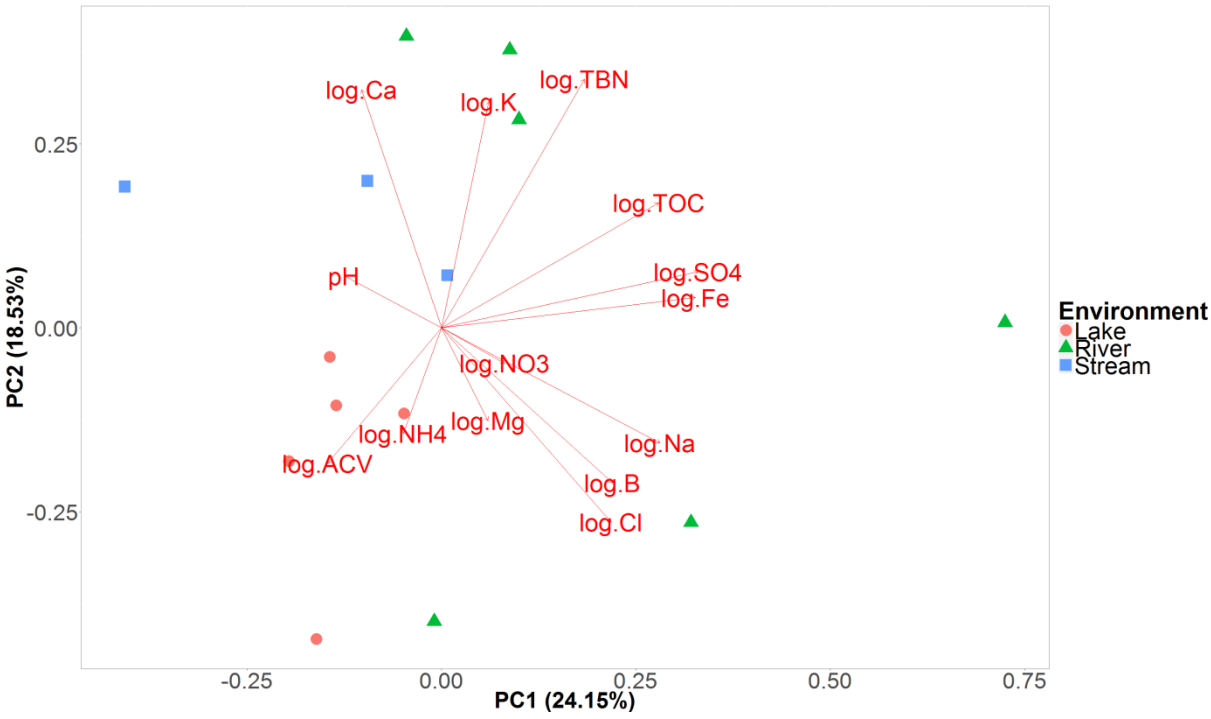

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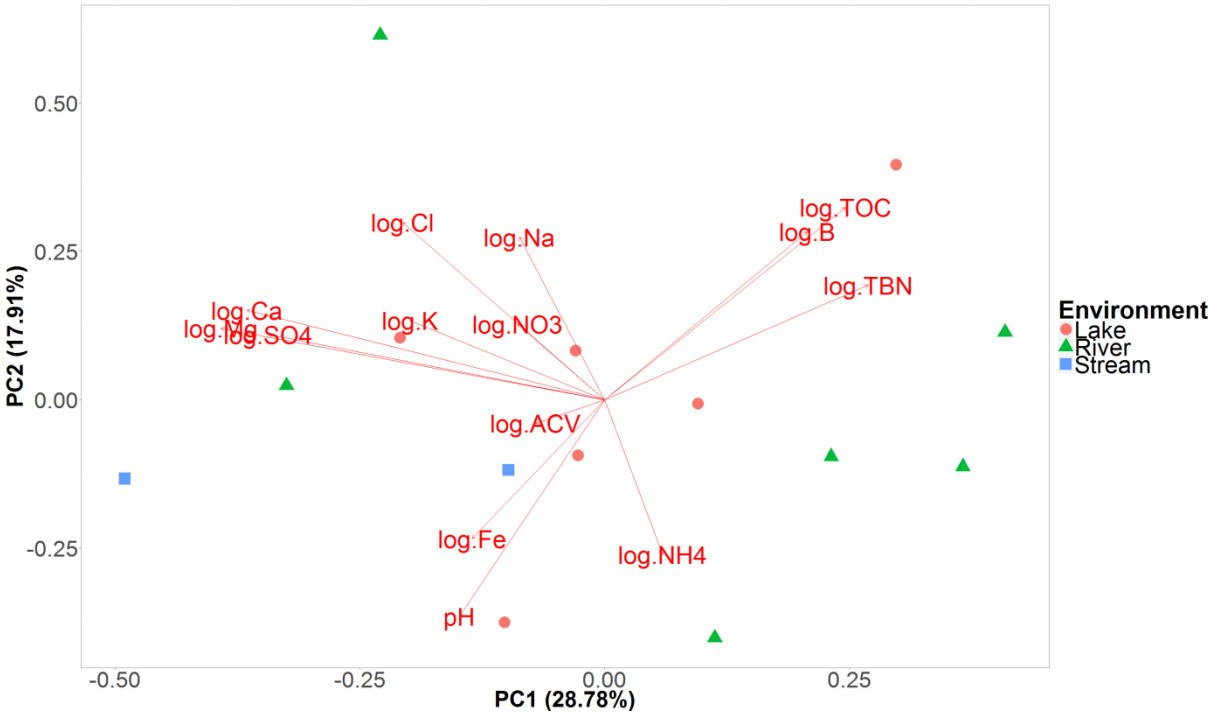

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860 **Figure 7.** Principal component analysis results for nutrient concentrations and the bacterial
861 community parameters (top). The two graphs below represent the two studied periods (June in
862 the middle and September at the bottom), which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

Tables

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

Determined compounds/parameters		Measurement range	LOD[4]	LOQ[4]	Measurement method/technique
Electrical conductivity[1]		-	-	-	Electrochemical method: CPC-411 conductometer (Elmetron), conductivity sensor EC60
pH		-	-	-	Electrochemical method: microcomputer pH-meter(Elmetron), electrode type EPS-1
TOC[2]		0.150-10.0	0.030	0.100	Total Organic Carbon Analyzer, TOC-$V_{CSH/CSN,}$method of catalytic combustion (oxidation) with the application of the NDIR detector
Anions[2]		0.030-250	0.060	0.180	Ion Chromatography technique with the application of the conductivity detector (DIONEX ICS-3000)
Cations[2]		0.030-250	0.010	0.030	
Micronutrients[3]	**Fe**	0.010-1000	0.010	0.030	Inductively Coupled Plasma Mass Spectrometry technique (Thermo Scientific XSERIES 2 ICP-MS)
	B	0.100-1000	0.100	0.300	
	P	1.00-1000	1.00	3.00	

[1][µS cm^{-1}], [2][mg L^{-1}], [3][µg L^{-1}], [4]the limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (b), according to the formulas: LOD=3.3(s/b), LOQ=10(s/b)

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

		June 2016	September 2016
Elemental nutrients	**B**	1.701±0.041 – 4.513±0.024	1.888±0.038 – 2.714±0.016
[µg L^{-1}]	**P**	<LOD – 1.91±0.47	<LOD
	Fe	0.0100±0.0050 – 0.279±0.017	0.1210±0.0010 – 1.32±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
R4-J	154022	6500	6755	10.32	0.995
R4-S	170636	6732	7026	10.42	0.996
R8-J	118556	3838	4914	7.28	0.971
R8-S	119228	3936	5485	6.82	0.948
R14-J	99569	5576	6284	9.13	0.987
R14-S	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**
Comamonadaceae	11.66%	13.00%	*Comamonadaceae*	*Isosphaeraceae*	11.53%	13.45%	*Kiloniellaceae*	*Comamonadaceae*	6.48%	8.19%	*Comamonadaceae*
Flavobacteriaceae	5.57%	3.59%	*Flavobacteriaceae*	*Streptomycetaceae*	7.68%	11.21%	*Cellulomonadaceae*	*Isosphaeraceae*	6.35%	5.22%	*Cellulomonadaceae*
Oxalobacteraceae	4.46%	3.16%	*Flexibacteraceae*	*Comamonadaceae*	6.35%	10.64%	*Comamonadaceae*	*Oxalobacteraceae*	5.30%	3.79%	*Kiloniellaceae*
Clostridiaceae	2.48%	2.67%	*Sphingomonadaceae*	*Oxalobacteraceae*	5.64%	5.99%	*Isosphaeraceae*	*Kiloniellaceae*	4.76%	3.43%	*Oxalobacteraceae*
Flexibacteraceae	2.22%	2.56%	*Intrasporangiaceae*	*Kiloniellaceae*	5.31%	4.16%	*Verrucomicrobiaceae*	*Flavobacteriaceae*	3.74%	3.12%	*Verrucomicrobiaceae*
Sphingobacteriaceae	1.97%	2.54%	*Sphingobacteriaceae*	*Flavobacteriaceae*	4.73%	3.86%	*Oxalobacteraceae*	*Cellulomonadaceae*	2.68%	2.92%	*Isosphaeraceae*
Sphingomonadaceae	1.90%	2.15%	*Acholeplasmataceae*	*Rhizobiaceae*	2.93%	3.52%	*Pseudonocardiaceae*	*Legionellaceae*	1.88%	2.64%	*Legionellaceae*
Geobacteraceae	1.74%	2.12%	*Xanthomonadaceae*	*Cellulomonadaceae*	2.01%	2.85%	*Flavobacteriaceae*	*Pseudonocardiaceae*	1.86%	2.39%	*Intrasporangiaceae*
Bifidobacteriaceae	1.72%	1.97%	*Cellulomonadaceae*	*Mycoplasmataceae*	1.93%	2.76%	*Chitinophagaceae*	*Rhizobiaceae*	1.78%	2.24%	*Flavobacteriaceae*
Xanthomonadaceae	1.69%	1.83%	*Oxalobacteraceae*	*Pseudonocardiaceae*	1.76%	1.90%	*Microbacteriaceae*	*Sphingomonadaceae*	1.33%	2.23%	*Rhodospirillaceae*
Legionellaceae	1.53%	1.67%	*Pseudonocardiaceae*	*Brocadiaceae*	1.58%	1.42%	*Flexibacteraceae*	*Mycoplasmataceae*	1.26%	2.10%	*Sphingomonadaceae*
Intrasporangiaceae	1.49%	1.66%	*Verrucomicrobiaceae*	*Verrucomicrobiaceae*	1.52%	1.13%	*Legionellaceae*	*Brocadiaceae*	1.21%	2.04%	*Pseudonocardiaceae*
Chitinophagaceae	1.37%	1.61%	*Chitinophagaceae*	*Legionellaceae*	1.37%	1.10%	*Veillonellaceae*	*Verrucomicrobiaceae*	1.10%	1.80%	*Sphingobacteriaceae*
Rhodospirillaceae	1.27%	1.49%	*Rhodocyclaceae*	*Rivulariaceae*	1.18%	1.00%	*Methylophilaceae*	*Hyphomicrobiaceae*	1.08%	1.75%	*Acholeplasmataceae*
Hyphomicrobiaceae	1.10%	1.40%	*Rhodospirillaceae*	*Halothiobacillaceae*	1.01%	0.99%	*Alcaligenaceae*	*Rhodospirillaceae*	1.05%	1.68%	*Chitinophagaceae*
Thermoanaerobacteraceae	1.03%	1.40%	*Paenibacillaceae*	*Alcaligenaceae*	0.68%	0.97%	*Bogoriellaceae*	*Geobacteraceae*	0.97%	1.54%	*Flexibacteraceae*
Pseudonocardiaceae	1.01%	1.32%	*Legionellaceae*	*Enterobacteriaceae*	0.66%	0.89%	*Sphingomonadaceae*	*Rhodocyclaceae*	0.94%	1.26%	*Xanthomonadaceae*
Puniceicoccaceae	1.01%	1.23%	*Clostridiaceae*	*Cyanobacteriaceae*	0.65%	0.64%	*Caulobacteraceae*	*Flexibacteraceae*	0.92%	1.18%	*Caulobacteraceae*
Coxiellaceae	1.00%	1.21%	*Hyphomicrobiaceae*	*Micromonosporaceae*	0.62%	0.64%	*Rhodobacteraceae*	*Coxiellaceae*	0.90%	1.15%	*Hyphomicrobiaceae*
Rhodocyclaceae	0.99%	1.09%	*Thermoanaerobacteraceae*	*Geobacteraceae*	0.57%	0.50%	*Rhodocyclaceae*	*Alcaligenaceae*	0.89%	1.05%	*Microbacteriaceae*
Verrucomicrobiaceae	0.99%	0.97%	*Microbacteriaceae*	*Caulobacteraceae*	0.55%	0.50%	*Streptomycetaceae*	*Sphingobacteriaceae*	0.86%	0.97%	*Rhodocyclaceae*
Cellulomonadaceae	0.92%	0.96%	*Geobacteraceae*	*Flexibacteraceae*	0.55%	0.49%	*Intrasporangiaceae*	*Chitinophagaceae*	0.84%	0.92%	*Geobacteraceae*
Brocadiaceae	0.91%	0.85%	*Chromatiaceae*	*Planctomycetaceae*	0.50%	0.43%	*Hyphomonadaceae*	*Intrasporangiaceae*	0.78%	0.79%	*Coxiellaceae*
Paenibacillaceae	0.89%	0.84%	*Caulobacteraceae*	*Coxiellaceae*	0.50%	0.42%	*Cyanobacteriaceae*	*Caulobacteraceae*	0.77%	0.75%	*Paenibacillaceae*
Chromatiaceae	0.88%	0.78%	*Puniceicoccaceae*	*Sphingobacteriaceae*	0.47%	0.40%	*Polyangiaceae*	*Rivulariaceae*	0.76%	0.70%	*Chromatiaceae*
Chthoniobacteraceae	0.84%	0.75%	*Brocadiaceae*	*Sphingomonadaceae*	0.45%	0.37%	*Thermogemmatisporaceae*	*Enterobacteriaceae*	0.74%	0.67%	*Clostridiaceae*
Acholeplasmataceae	0.80%	0.74%	*Methylophilaceae*	*Hyphomicrobiaceae*	0.45%	0.36%	*Armatimonadaceae*	*Xanthomonadaceae*	0.71%	0.66%	*Brocadiaceae*
Acetobacteraceae	0.77%	0.63%	*Chthoniobacteraceae*	*Chthoniobacteraceae*	0.42%	0.34%	*Rhodospirillaceae*	*Puniceicoccaceae*	0.68%	0.65%	*Alcaligenaceae*
Caulobacteraceae	0.75%	0.57%	*Bifidobacteriaceae*	*Methylophilaceae*	0.42%	0.33%	*Xanthomonadaceae*	*Paenibacillaceae*	0.68%	0.63%	*Enterobacteriaceae*
Microbacteriaceae	0.74%	0.55%	*Gemmatimonadaceae*	*Phormidiaceae*	0.41%	0.32%	*Bacteriovoracaceae*	*Chromatiaceae*	0.65%	0.61%	*Mycoplasmataceae*
Peptococcaceae	0.67%	0.50%	*Pedosphaeraceae*	*Pasteurellaceae*	0.41%	0.31%	*Sphingobacteriaceae*	*Clostridiaceae*	0.65%	0.61%	*Bifidobacteriaceae*

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

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