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*Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

- Natural and anthropogenic impact on Arctic lake ecosystems was studied
- Nutrient-rich runoff from bird colony was retained by surrounding tundra vegetation
- The core phyla of treated wastewater were mirrored in its recipient – Arctic lake
- Human-related bacteria and their resistome are disseminated in Arctic lake ecosystem
- Sustainable wastewater management is a challenge for polar human settlements

1 **The microbial community, its biochemical potential, and the antimicrobial resistance of**
2 ***Enterococcus* spp. in Arctic lakes under natural and anthropogenic impact (West**
3 **Spitsbergen)**

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13

14 **Abstract:** The sustainable management of small human communities in the Arctic is
15 challenging. In this study, both a water supply system (Lake 1) under the natural impact of a
16 bird-nesting area, and a wastewater receiver (Lake 2) were analysed in the vicinity of the
17 Polish Polar Station on West Spitsbergen. Microbial community composition, abundance and
18 activity were assessed in samples of the treated wastewater, lake water and sediments using
19 next-generation sequencing and direct microscope counts. Special attention was given to the
20 faecal indicator, *Enterococcus* spp., whose occurrence and antimicrobial resistance were
21 tested in water and wastewater samples. The results indicate that Lake 1, at a tundra stream
22 discharge (L-TS) and at a water supply point (L-WS) were dominated by three phyla:
23 *Proteobacteria* (57–58%) *Bacteroidetes* (27–29%) and *Actinobacteria* (9–10%) showing
24 similar microbial composition up to the genus level. This suggests that nutrient-rich runoff

25 from the bird colony was retained by surrounding tundra vegetation and reached Lake 1 at L-
26 TS to a limited extent. Lake 2, being the wastewater recipient (WW-R), mirrors to some
27 extent the core phyla of treated wastewater (WW-E), but in different shares. This suggests the
28 possible washout of wastewater-related bacteria with activated sludge flocs, which was also
29 supported by the microscopic observations. Compared to Lake 1, in WW-R an increase in all
30 tested parameters was noted: total prokaryotic cell number, average cell volume, prokaryotic
31 biomass and live cell percentage. The presence of *Enterococcus* spp. antibiotic resistance
32 patterns highlights the importance of human associated microbiome and resistome
33 dissemination via wastewater discharge. Additionally, it can be expected that temperature-
34 related biochemical processes (e.g. nutrient cycling) may be accelerated by the ongoing
35 climate change. Thus, proper wastewater treatment requires locally adapted solutions in
36 increasingly visited and inhabited polar regions. Additionally, microbial community
37 discharged to the environment with the treated wastewater, requires critical attention.

38 **Keywords:** Arctic freshwater; Bird-nesting area impact; Treated wastewater discharge;
39 Bacterial community and diversity; Nutrients; *Enterococcus* spp. antimicrobial susceptibility

40 **List of abbreviations**

L-TS	Lake – Tundra Stream
L-WS	Lake – Water Supply
SED-TS	Sediments – Tundra Stream
WW-E	Wastewater Effluent
WW-R	Wastewater Recipient
SED-R	Sediments - Recipient
TCN	Total (Prokaryotic) Cell Number
PB	Prokaryote Biomass
ACV	Average Cell Volume
SBR	Sequencing Batch Reactor
OTU	Operational Taxonomic Unit

41 **1. Introduction**

42 Wastewater discharged to the surface waters can influence their physicochemical parameters
43 (Hassan and Egozi, 2001; Igbinosa and Okoh, 2009), microbial community (Okoh et al.,
44 2010) and lead to accumulation of chemical substances in their sediments (Marti et al., 2014).
45 Besides the clinical settings, also wastewater is suggested to be an important pool of both
46 resistance determinants and residues of antimicrobial agents (Łuczkiwicz et al., 2010;
47 Mahfouz et al., 2018), which are introduced to sewage systems from intestinal and/or urinary
48 tracts. Current wastewater treatment methods are insufficient in removing antimicrobial
49 agents and are even suspected of increasing resistance rates among bacteria due to enhanced
50 horizontal gene transfer (von Wintersdorff et al., 2016). This phenomenon can be promoted
51 by high cell density and different selective pressures (sub-inhibitory concentrations of
52 antimicrobial agents, heavy metals or other biocides and oxidative stress) occurring during
53 wastewater treatment. But in these terms little is known about the development of resistance
54 via wastewater, especially in polar areas. To date, the anthropogenic influence was regarded
55 as negligible in these regions. However, nowadays, the increasing number of people
56 (inhabitants, researchers and cruise tourists) visiting the Arctic and Antarctica raises the risk
57 of human-associated microorganisms being introduced, with unknown consequences for local
58 wildlife (Hernández and González-Acuña, 2016).

59 Besides human beings, in polar regions other vectors of antibiotic resistant bacteria
60 dissemination should also be considered, e.g. migrating birds. Clinically-emerging resistance
61 phenotypes, such as vancomycin-resistant enterococci (VRE) and extended spectrum beta-
62 lactamase (ESBL) producing Gram-negative bacteria were isolated from glaucous gulls
63 (Hernández and González-Acuña, 2016). These birds breed in the Arctic, but are also a
64 regular visitor to urban areas, such as city dumps and sewage outlets close to human habitats.
65 But still only a few studies have focused on the topic (Perron et al., 2015).

66 A clinical approach is generally followed when defining antibiotic resistance, even in
67 environmental research. However, it is based on the bacterial susceptibility to antimicrobial
68 agent concentrations used during therapy (EUCAST 2020; CLSI 2011), and not naturally
69 occurring in the environment. Thus, bacteria that have evolved a resistance mechanism as a
70 response to naturally occurring antimicrobial agents (Davies, 1994; Perry et al., 2016) usually
71 remain susceptible from the clinical point of view. Therefore in environmental studies, the
72 resistant isolates should instead be tested using the so-called epidemiological cut-off
73 (ECOFF) concept. ECOFF is defined based on the normal distribution of minimal inhibitory
74 concentrations (MICs) for a given bacterial species and provides the upper MIC value for
75 wild-type population (EUCAST, 2020). Thus, ECOFF allows wild-type species lacking the
76 acquired and/or mutational mechanisms of resistance to be distinguished from non-wild ones
77 with resistance mechanisms.

78 Besides non-indigenous microorganisms, nutrients and organic carbon too are released with
79 wastewater to the receiver body. In polar regions it was originally thought that due to the
80 limited number of taxa, the microbial loop there is simplified. Currently, however, the role of
81 bacterioplankton in biogeochemical cycles has been recognised as crucial (Buchan et al.,
82 2014). Additionally, changes in bacterial community structure and cell size are expected as a
83 result of climate change, higher temperatures, decreasing ice cover and higher primary
84 production (Peter and Sommaruga, 2016; Rui et al., 2015). Knowledge of microbial behaviour
85 and susceptibility to different stressors, including antibiotics, can increase the understanding
86 of the links between population dynamics at different trophic levels.

87 Polar lakes' microbial communities are still poorly investigated (Stoeva et al., 2014) and have
88 only recently been studied using various metagenomic methods (Górniak et al., 2016; Wang
89 et al., 2016), mostly in terms of bacterial productivity (Adams et al., 2014) or survival of
90 microbial populations in extreme conditions (Comeau et al., 2012). Similarly, little is known



91 about bacterial composition of treated wastewater and polar lakes under the impact of faecal
92 bacteria and nutrient-rich discharge. Therefore, this study aims to fill this knowledge gap on
93 the example of the wastewater treatment plant effluent and two Arctic lakes chosen as model
94 areas. One is influenced by a bird nesting area (natural impact) and another receiving treated
95 wastewater from the Polish Polar Station (anthropogenic impact). The neighbourhood of the
96 Polish Polar Station in Hornsund, West Spitsbergen, was chosen because this area has been
97 identified by the European Union as one of the six locations on the European continent
98 suitable for biological and geophysical research due to its minimal transformation and
99 environmental pollution (7th Environment Action Programme; EEAS). Additionally, Polish
100 Polar Station wastewater treatment plant is an unique object that can serve as an example of
101 the treated wastewater influence on polar environment. It is especially valuable in the era of
102 increasing tourism and ongoing climate changes.

103 To better elucidate the ecological roles of bacterial groups, various methods were combined:
104 metagenomic analysis (next-generation sequencing [NGS]), microscopic analysis and
105 cultivation methods. Additionally, the identification and antimicrobial susceptibility testing of
106 *Enterococcus* spp. was employed. This faecal indicator was chosen due to its frequency in
107 causing multi-resistant infections and its high adaptability to harsh conditions: extreme
108 temperatures, pH and salinity (Fisher and Phillips, 2009; Gaca and Lemos, 2019).
109 Simultaneously, this study will help to evaluate the current biochemical properties of the
110 microbial community in Arctic lakes and to assess the antimicrobial resistance among human-
111 related *Enterococcus* spp., which could be used as a reference point for future research,
112 including in the context of ongoing climate changes and increasing human impact on the polar
113 areas. We hypothesize that treated wastewater discharge can significantly shape nutrient
114 cycling, as well as taxonomic composition and antibiotic resistance of microbial community

115 of the recipient. Apart from anthropogenic factor, also bird migration and nesting may
116 facilitate these changes.

117 **2. Materials and Methods**

118 2.1. Research area and sampling

119 The Stanislaw Siedlecki Polish Polar Station is situated in the South Spitsbergen National
120 Park (West Spitsbergen), at the Isbjornhamna Bay of the Hornsund Fjord (Fig. 1) since 1957,
121 and is inhabited all year round by 10–11 crew members and up to 35 additional people
122 (mainly researchers and technical service) during the summer season. There are no other
123 permanent human settlements in this area.

124 **Figure 1.** Sampling area in the vicinity of Polish Polar Station in Hornsund, Spitsbergen;
125 Lake 1 serving as a source of drinking water – sampling points: L-TS (water) and SED-TS
126 (sediment) at tundra stream inflow and L-WS (water) at water supply area; Lake 2 serving as
127 a receiver of treated wastewater – sampling points: WW-R (water) and SED-R (sediments) at
128 treated wastewater discharge point; additionally, effluent from the wastewater treatment plant
129 (WW-E) was collected; photo by Kajetan Deja

130 Water and sediments were collected from two lakes near the Polish Polar Station: Lake 1
131 (supplier) serves as a source of potable water for the Polish Polar Station, while Lake 2
132 (receiver) receives treated wastewater (Fig. 1). Lake 1 was sampled at the tundra stream
133 inflow (samples of water: L-TS, and sediments: SED-TS) and at the area of a water pumping
134 station (water: L-WS). The tundra stream flows through a nesting area for birds, mainly little
135 auk colonies, which are expected to be an important source of nutrients and faecal
136 contamination.

137 Lake 2, being a treated wastewater receiver, was sampled at the discharge point (water WW-R
138 and sediments SED-R). Additionally, the effluent (WW-E) of the Polish Polar Station
139 wastewater treatment plant was also collected. Therefore, in this study the anthropogenic
140 (human) and natural (birds) contributions to the faecal contamination of two Arctic lakes were
141 studied.

142 In the Polish Polar Station, wastewater was treated mechanically by screens, and biologically
143 by a fill-and-draw activated sludge system (two sequencing batch reactors, SBRs, 3 m³ each,
144 Fig. S1 – supplementary materials). To obtain high organic matter and nitrogen removal, the
145 SBRs were working in parallel, in 180-minute cycles (aerobic/anaerobic phase). Additionally,
146 the nitrification/denitrification process was supported by the constant temperature inside the
147 building (set at 20 °C). Excess sludge was removed from the reactors, dewatered and dried in
148 the tanks. Note that most of the year only one SBR operates, while two SBRs are used when
149 the number of visitors increases.

150 Samples were collected three times, during three consecutive weeks in August 2013, and
151 analysed in triplicates. Unless specified otherwise, the results have been presented as a mean
152 with a standard deviation. Only samples for NGS analysis were pooled together on account of
153 the low DNA content in a single sample.

154 2.2. Physicochemical parameters

155 Basic physical parameters (pH, temperature, electrical conductivity) were measured *in situ*
156 using a pH meter combined with a temperature and conductivity meter (WTW pH/oxi 340i).
157 Additionally, a set of samples was stored at -20 °C and further analysed at Gdansk University
158 of Technology. Chemical oxygen demand (COD) and concentrations of nitrite nitrogen (N-
159 NO₂), nitrate nitrogen (N-NO₃), ammonia nitrogen (N-NH₄), and total nitrogen (TN), as well
160 as phosphorus phosphate (P-PO₄) and total phosphorus (TP), were determined using

161 spectrophotometric methods (XION 500 spectrophotometer Dr. Lange, GmbH, Germany)
162 after transport to Poland.

163 2.3. Microscopic observations

164 2.3.1 DAPI staining

165 Freshwater and wastewater samples were fixed immediately after sampling with buffered
166 formalin to a final concentration of 2% and stored at +4°C until further analysis at Gdansk
167 University of Technology. Total prokaryotic cell number (TCN), average cell volume (ACV)
168 and prokaryote biomass (PB) were determined using DAPI direct counting method (Porter
169 and Feig, 1980). Samples were stained in 1 µg mL⁻¹ final DAPI concentration for 10 minutes
170 in darkness, filtered through 0.2 µm polycarbonate Whatman filters (Merck, Germany) and
171 then rinsed twice: with 1 mL of bacterium-free distilled water and 1 mL of particle-free 80%
172 ethanol. Filters were examined under UV light (BO-103W high-pressure mercury burner,
173 330–380 nm excitation filter, 420 nm barrier filter and 400 nm dichroic mirror) with an
174 epifluorescence microscope (Nikon Eclipse 80i) under 1000-fold magnification. Bacteria in
175 2 repeats of 10 fields were counted. The image analysis system of Świątecki (Świątecki,
176 1997) was applied. Bacterial biomass was estimated using conversion factors by Norland
177 (Norland, 1993).

178 2.3.2 Live/Dead staining

179 Staining for Live/Dead analysis was performed immediately after sample collection. The
180 fluorescent dyes SYTO9 and PI from the LIVE/DEAD BacLight Bacterial Viability Kit
181 (Molecular Probes, USA) were used in combination by mixing identical volumes of 0.1 mL of
182 each dye and adding 0.5 mL of water sample. After dye addition, samples were incubated in
183 darkness for approx. 30 min and filtered through 0.2 µm polycarbonate Whatman filters

184 (Merck, Germany). Filters were kept at -20 °C until further examination. The ratio of live to
185 dead cells was determined using epifluorescence microscope (EX 400–440 nm, DM 455 nm,
186 BA 470 nm and EX 450–490 nm, DM 505 nm, BA 520 nm) under 1000-fold magnification.
187 The bacteria in 2 repeats of 10 fields were counted and the percentage of live cells was
188 established. Live bacteria with undamaged cell membrane were seen as giving green
189 fluorescence (ex/em: ~495 nm / ~515 nm), while damaged (dead) cells produced a bright red
190 fluorescence (ex/em: ~495 nm / ~635 nm). The outcome of Live/Dead staining (L/D) is given
191 in percentage of live bacteria.

192 2.4. Isolation, identification and resistance profile of *Enterococcus* spp.

193 Enterococci were immediately cultivated from the tested water samples using the membrane
194 filtration method (in triplicates) on 0.45 µm cellulose-acetate filters (EMD Millipore
195 Corporation, USA) and Slanetz-Bartley *Enterococcus* selective agar (Merck, Germany). After
196 incubation at 37 °C for 48 h (ISO 7899–2:2000) dark red or maroon colonies, assumed to
197 represent *Enterococcus* spp., were counted and presented as colony forming units (CFU) per
198 100 mL. Next, for further investigations, 76 representative isolates of enterococci were taken
199 from membranes presenting less than 20 typical colonies. For further analysis, isolates were
200 stored in nutrient broth supplemented with 50% glycerol at -80 °C. The species identification
201 (ID) and antimicrobial susceptibility testing (AST) of enterococci were determined by the
202 Phoenix™ Automated Microbiology System (BD Phoenix, USA) according to the
203 manufacturer's instructions. For ID and AST the commercially available panels (BD Phoenix,
204 USA) were applied and *Enterococcus faecalis* ATCC 20212 was used as a quality control.
205 The antibiotic susceptibility analyses, based on the microdilution tests, were carried out
206 against the antimicrobial agents representative for drugs important in treating human
207 enterococcal infection (EUCAST, 2017). The identification of minimum inhibitory
208 concentration (MIC) for certain strains was done based on epidemiological cut-off value

209 (ECOFF) and clinical breakpoints provided by EUCAST (accessed 15.03.2020). Note that the
210 Phoenix system does not distinguish between *E. casseliflavus* and *E. gallinarum*, but it
211 assigns the two organisms to the overlap category: *E. casseliflavus/gallinarum*.

212 2.5. DNA extraction and PCR amplification of bacterial 16S rRNA gene

213 Water samples were filtered on polycarbonate filters (0.2 µm pore diameter, Millipore GTTP,
214 Merck, Germany) immediately after sample collection and stored at -20 °C until the DNA
215 extraction. Triplicates of the filtered material for each sampling point were merged for DNA
216 extraction and considered as one sample in further taxonomic analysis. The DNA was isolated
217 using Sherlock AX Kit (A&A Biotechnology, Poland) according to the manufacturer's
218 instruction. The DNA concentration was determined by a Qubit 2.0 fluorometer (Invitrogen,
219 USA).

220 The presence of bacterial DNA was confirmed by Real-Time PCR with SYBR Green
221 fluorochrome, in Mx3000P thermocycler (Stratagene, USA). The following PCR conditions
222 were used: initial denaturation at 95 °C for 3 min, followed by 40 cycles consisting of
223 denaturation (95 °C for 15 s), annealing (58 °C for 30 s), fluorescence measurement and
224 extension (72 °C for 30 s). For amplification of 16S rDNA fragment universal primers were
225 applied: 1055F (5'-ATGGCTGTCGTCAGCT-3') and 1392R (5'-ACGGGCGGTGTGTAC-3')
226 (Ferris et al., 1996). Final check on the DNA quality was done by determination of the PCR
227 product melting curve and measuring fluorescence at temperatures from 65 °C to 95 °C. The
228 PCR products were stored at -20 °C for sequencing.

229 2.6. Sequencing, taxonomic assignment and data analysis

230 Bacterial V3-V4 hypervariable regions of 16S rRNA gene were amplified and prepared for
231 sequencing according to the 16S Metagenomic Sequencing Library Protocol. The following

232 primer pair was used for amplification: 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R
233 (5'-GACTACHVGGGTATCTAATCC-3'). The targeted gene regions have been shown to be
234 the most suitable for Illumina sequencing (Klindworth et al., 2012). Paired-end sequencing
235 was performed with an Illumina MiSeq by the Macrogen company (Macrogen Inc., South
236 Korea) and following manufacturer's run protocols. Raw sequence data can be accessed from
237 MG-RAST database (accession numbers from mgm4900959.3 to mgm4900970.3).

238 Samples were processed and analysed by using the Quantitative Insights Into Microbial
239 Ecology (QIIME) pipeline v.1.8.0 software. Raw sequence reads were quality trimmed using
240 the QIIME suite of tools, version 1.8.0 (Caporaso et al., 2010). Low-quality paired-end reads
241 and chimera reads were discarded in operational taxonomic units (OTU) clustering analysis
242 using CD-HIT-OTU. Paired-end reads were assembled using FLASH (Magoč and Salzberg,
243 2011). Sequences shorter than 120 bp were excluded from further analysis. OTUs were
244 clustered at 97% similarity threshold using UCLUST (v.1.2.22). Taxonomy assignment was
245 performed using GreenGenes (v13.8) as a reference (McDonald et al., 2012). Various alpha
246 diversity indices were estimated based on clusters using Shannon (H'), Simpson (D) and
247 Chao1 and observed species metrics in QIIME software. Clone library coverage based on
248 Good's coverage for an OTU definition (Good, 1953) was determined using 97% identity
249 level.

250 The R program was used to plot a double hierarchical dendrogram and a heatmap depicting
251 the relative abundance of the top 20 phyla (abundance higher than 1% in at least one sample).
252 The Bray–Curtis dissimilarity matrix was calculated on the full dataset. Average linkage
253 hierarchical clustering was opted for. The heatmap was generated using the “Heatplus”
254 library. Hierarchical clustering was performed using the “vegan” library. The Principal
255 Coordinate Analysis (PCoA) plot was based on Bray–Curtis distance and relative abundances
256 of bacterial and archaeal OTUs, which were used as the dataset.

257 3. Results and discussion

258 Nowadays, the Arctic is undergoing massive transformations, including temperature increase,
259 glacier melting, milder winters, less snow and ice cover of land, fiords and Arctic Ocean, and
260 many others. All the above, together with rising tourism and related anthropogenic impact
261 (emissions from ships, planes, growing permanent human settlements and scientific bases)
262 lead to significant, yet still not fully understood changes in the polar environment. It is clear,
263 however, that they result in shifts in the ecosystem: introduction of nutrients (Qu et al., 2017)
264 and other pollutants (Eckert et al., 2018), as well as suspected changes in microbial
265 community (Wang et al., 2017) and resistome structure (Alexander et al., 2020). In this study,
266 two Arctic lakes were chosen as model objects to reveal differences between “pristine” lake
267 under natural pressure of a tundra stream and runoff from bird nesting area, versus
268 “anthropogenically influenced” lake being a treated wastewater receiver. Reservoirs near the
269 Polish Polar Station, Hornsund, West Spitsbergen were analyzed regarding physicochemical
270 parameters, microbial community composition and antimicrobial resistance of *Enterococcus*
271 spp..

272 3.1. Physicochemical analysis

273 Electrical conductivity (EC) generally shows the presence of dissolved salts and in some
274 cases can be used as an indirect indicator of pollution (Ribeiro De Sousa et al., 2014).
275 Biologically productive freshwater typically present EC values of 100–500 $\mu\text{S cm}^{-1}$, while
276 lower values ($<100 \mu\text{S cm}^{-1}$) usually suggest oligotrophic (nutrient-poor) conditions (Stewart,
277 2001). In this study, samples collected from the tested lakes showed EC values from 120 μS
278 cm^{-1} to 211 $\mu\text{S cm}^{-1}$, with slightly higher EC observed in the sampling points subjected to
279 either anthropogenic (WW-R) or natural, bird-related (L-TS) inflow, up to 211 $\mu\text{S cm}^{-1}$ and
280 up to 155 $\mu\text{S cm}^{-1}$, respectively (Table 1). Nonetheless, the EC values in lake-related samples



281 fall in the range noted for other aquifers in the area of the Polish Polar Station (Kosek et al.,
282 2019; Nowiński and Wojtasik, 2006) and, as suspected, were significantly lower than those
283 noted for treated wastewater (WW-E, up to $1,115 \mu\text{S cm}^{-1}$), which is also in the range of
284 typical treated wastewater EC values (see e.g. Prieto et al., 2001).

285 The temperature of the samples collected from the lakes (L-TS, L-WS and WW-R) was about
286 7°C and to some extent, as with other shallow water bodies of this kind, it was linked to the
287 air temperature (Woelders et al., 2018). Mean air temperature during the sampling period
288 (August 2013) was equal to $+5.8^\circ\text{C}$, which was 1.7°C higher than the multiannual mean for
289 this month (Polish Polar Station Meteorological Bulletin, 2013). The WW-E temperature was
290 about 18°C and resulted from the thermal conditions inside the wastewater treatment plant
291 building (set at 20°C). The pH values ranged from 7.0 to 7.8 in samples collected from
292 Lake 1 and Lake 2, and from 7.2 to 7.5 in WW-E (Table 1). Note that lake acidification was
293 reported as a particular sign of inflow related to the birds' breeding area (González-
294 Bergonzoni et al., 2017; Zwolicki et al., 2013). In this study, the decrease in pH was less
295 profound but was observed at Lake 1 in point L-TS. This site is under direct influence of the
296 tundra stream, collecting surface runoff from little auk colonies ($\text{pH}=7.1\pm 0.08$ versus
297 $\text{pH}=7.7\pm 0.08$ at the L-WS point at the water supply area). In this study, nitrogen and
298 phosphorus in Lake 1 were mostly below level of detection (Table 1), except ammonia (up to
299 $0.77 \text{ mg N-NH}_4/\text{L}$) and nitrates (up to $0.40 \text{ mg N-NO}_3/\text{L}$), which at the L-TS point
300 constituted the main share of total nitrogen (Table 1). This suggests that influence from runoff
301 that is nutrient rich due to bird droppings was either retained by the surrounding tundra
302 vegetation or diluted by intense rainfalls. In August 2013, during the sampling campaign,
303 exceptionally high rainfall was noted: 179.5 mm per month. It was more than three times the
304 average multiannual (1978–2012) precipitation for August (51.9 mm) and over 50 mm higher

305 than the previous maximum noted in August 2012 (123.8 mm, for more details see
306 Supplementary materials).

307 Much more excessive input of nutrients was observed in Lake 2, which serves as a WW-E
308 receiver. In such oligotrophic lake, it can highly influence the biochemical potential and
309 microbial community, which is discussed further. According to the obtained data, the
310 requirements of treated wastewater discharge were not met, especially in the case of total
311 nitrogen content (up to 80 mg N/L in WW-E, Table 1). Efficiency of this wastewater
312 treatment plant before modernisation was investigated in another study (Wilk and
313 Cimochoicz-Rybicka, 2018). The disturbances observed in the wastewater treatment plant
314 operation were connected with the summer season and full occupancy of the Polish Polar
315 Station (up to 45 people in total). As a result, the decrease in hydraulic retention time, weak
316 floc formation and settling, and finally activated sludge biomass washout was observed (for
317 details, see sections 3.2 and 3.3, Supplementary Figures S2 and S3). In consequence, a drop in
318 nitrification/denitrification effectiveness was noted. The findings and data obtained in this
319 study were later used to modify the wastewater treatment system (done in 2016). However,
320 small scale wastewater treatment plants are generally more prone to failures and problems.
321 They are difficult to operate – partly due to high variability of inflow and load that leads to
322 lower stability of the system, not only in the polar areas, but even in the mid latitudes.

323 **Table 1.** Physicochemical parameters of water collected from Lake 1 (L TS: tundra stream
324 inflow and L-WS: water supply area) and from Lake 2 (WW-R: treated wastewater recipient);
325 the results of wastewater treatment plant effluent (WW-E) were compared with the discharge
326 requirements.

327 3.2. Direct microscopic quantification of prokaryotic community

328 In general, a clear relationship was confirmed between the amount of available biogenic
329 compounds and bacterial abundance, cell volume and biomass (Danovaro and Fabiano, 1997;
330 La Ferla et al., 2014, 2010). Therefore, the physical appearance of prokaryotic cells carries
331 (unspecific) information about the trophic status of the aquatic environment. In polar regions,
332 apart from the deficit of nutrients, also low temperature and consecutive periods of very high
333 and very low exposure to solar radiation (polar day and night) are important factors
334 influencing bacterial development (Kirchman et al., 2005; Mueller et al., 2005; Rublee and
335 Bettez, 1995). But even in high Arctic lakes, classified as oligotrophic, bacterioplankton
336 activity is observed, the highest in mid-August (Laybourn-Parry and Marshall, 2003). Thus,
337 microscopic observations play an important role in evaluation of the activated sludge
338 condition. Incorporated into environmental impact assessment of the wastewater receivers,
339 such analyses could also provide rough information about the microbiological water quality.

340 In this study, as suspected, all analysed parameters: total prokaryote cell number (TCN),
341 average cell volume (ACV), prokaryote biomass (PB) and Live/Dead ratio (L/D) were lowest
342 in Lake 1 (L-WS, L-TS), followed by the wastewater-related points: the treated wastewater
343 recipient (WW-R) and the wastewater treatment plant effluent (WW-E, Fig. 2). Importantly,
344 the above parameters obtained for lake-related samples (L-TS, L-WS and WW-R), were
345 higher than in other fresh water samples collected in the Arctic. For instance, the average
346 values of TCN and PB in this study were: 1.16×10^6 cells mL⁻¹ and 28.75 $\mu\text{g C dm}^{-3}$ in L-TS,
347 1.21×10^6 cells mL⁻¹ and 25.04 $\mu\text{g C dm}^{-3}$ in L-WS and 2.31×10^6 cells mL⁻¹ with mean
348 biomass 61.24 $\mu\text{g C dm}^{-3}$ in WW-R, respectively (Fig. 2). Values reported by Górnjak (2016)
349 and Kosek (2019, 2018) in cold proglacial lakes and a brisk glacial river were even one
350 magnitude lower, which reflects the difference with the less turbulent, warmer and more
351 fertile Lake 1 and Lake 2.

352 Compared with Lake 1, Lake 2 (WW-R) showed higher availability of nutrients (Table 1),
353 probably due to the treated wastewater discharge (WW-E). This can result in intensification of
354 the primary and bacterial production. Additionally, in both lakes (Lake 1 and Lake 2) the
355 bacterial growth could have been additionally supported by the relatively high temperature
356 noted during the sampling campaign (for details see Supplementary Materials), as several
357 studies underline the influence of temperature on the physical properties of prokaryotic
358 communities (La Ferla et al., 2010; Ntougias et al., 2016). In the case of treated wastewater
359 effluent (WW-E), values of bacterial abundance (up to 5.07×10^6 cells mL⁻¹) and biomass (up
360 to 152.52 $\mu\text{g C dm}^{-3}$) were the highest among tested samples. The Live/Dead assay showed
361 also the highest ratio of live cells in WW-E (15.5% on average), followed by wastewater
362 recipient (WW-R, 10.2%), tundra stream inflow (L-TS, 8.1%) and water supply point (L-WS,
363 5.9%).

364 Live and active cells typically constitute up to 80% of the bacterial community in activated
365 sludge biomass (Kocwa-Haluch and Woźniakiewicz, 2011) and are mainly concentrated in
366 sludge flocs. Thus, elevated abundance of active bacterial cells in the wastewater effluent is
367 usually a sign of biomass washout. In this study, both free-swimming and flocs-related
368 bacteria were observed in WW-E (Supplementary materials, Fig. S2 and S3). It is suspected
369 that, in the studied wastewater treatment plant, small and weak flocs of activated sludge were
370 formed, then easily sheared and subjected to flotation in the final clarifier. This can be
371 principally caused by short hydraulic retention time and insufficient sludge age, causing
372 endogenous metabolism, lack of floc-forming species and/or low production or destruction of
373 extracellular polymers substances. A high concentration of readily degradable substrates
374 and/or the presence of some toxic or inhibitory compounds in wastewater also matters. Those
375 technological problems were confirmed in this study not only by the continuous biomass
376 washout to effluent but also by the deterioration of effluent quality (increase in turbidity, TN,

377 TP, COD and BOD values; see Table 1). It is also suggested that the fluorescence microscopy
378 observations of the wastewater treatment plant effluent can serve as a method for identifying
379 treatment efficiency or technological problems, related to, for example, activated sludge
380 washout.

381 Average cell volume (ACV) is another indicator, which can be linked to the bacterial
382 population activity and dynamics (Cole et al., 1993; Šimek et al., 1994), as well as availability
383 of nutrients. Different bacteria size classes dominate in various environments: small forms
384 prevail in oligotrophic waters, and larger rods in eutrophic (Billen et al., 1990). Small cells
385 (around $0.12 \mu\text{m}^3$) are considered to be the most active (Gasol et al., 1995). Also, limited
386 residence time of bacteria in the ecosystem influences their development (Lew et al., 2016) –
387 in this study prokaryotic ACV around $0.14 \mu\text{m}^3$ was noted in WW-E, which reflects both
388 intensive bacterial development in a nutrient-rich environment and the impact of continuous
389 flow conditions. The largest ACV range, which is observed in L-TS and WW-R samples,
390 seems to result from nutrient supply of natural (L-TS) or anthropogenic (WW-R) origin and
391 more stagnant conditions than in the wastewater treatment system, which favour growth of
392 microorganisms. Cell volume variability (Fig. 2b), which is especially noted in WW-R and L-
393 TS, could also reflect the presence of two kinds of prokaryotic cells in the Arctic lake:
394 autochthonous and discharge-related allochthonous bacteria.

395 **Figure 2.** Microscopic analysis results in water and wastewater samples: a) total prokaryotic
396 cell number (TCN), b) average cell volume (ACV), c) prokaryotic biomass (PB) and d)
397 prokaryotic activity – live cells expressed as percentage of total community (L/D).

398 3.3. Microbial community composition and diversity indices

399 For Illumina sequencing, Shannon and Simpson diversity indices were determined. They are a
400 proxy for richness and evenness and were found to be lowest for L-TS and L-WS samples



401 (3.6–4.0 and 0.83–0.85, respectively), intermediate for WW-E (5.6 and 0.95) and highest for
402 WW-R and both sediment samples (SED-TS and SED-R; 6.6–7.0 and 0.97–0.98, Figure 3d).
403 The Chao1 richness estimator predicts the total number of OTUs, but it also takes into
404 account the numbers of singletons and doubletons (species represented by exactly one or two
405 individuals, respectively), so it is highly influenced by rare OTUs and presents a slightly
406 different pattern than Shannon and Simpson indices. Chao1 was lowest for WW-E (310) and
407 highest for sediment samples: SED-TS and SED-R (496–540, Fig. 3d). In each sample the
408 Good's coverage indicates that almost the whole range of bacterial diversity is represented
409 (over 99%).

410 A total of 2,760 OTUs were identified from 314,486 sequences (average length of 428 bp),
411 which were achieved in the present study for 6 analysed samples. For water and wastewater
412 samples, 47 OTUs were common (Fig. 3a) and sediment samples shared 214 OTUs (Fig. 3b).
413 Among all the OTUs, 23 were present in all the samples (Fig. 3c) and they belonged to
414 *Actinobacteria*, *Bacteroidetes*, *Parcubacteria/OD1*, *Proteobacteria*, as well as
415 *Saccharibacteria/TM7* and *Verrucomicrobia*, which are present in the samples in lower
416 relative abundance. The highest amount of unique OTUs was observed in sediments: 579 out
417 of 918 OTUs in SED-TS and 526 out of 1,022 OTUs in SED-R (Fig. 3c). One hundred and
418 fifty OTUs were unique to the wastewater sample, and represented mainly the phyla
419 *Dojkabacteria/WS6* and *Parcubacteria/OD1*, as well as *Chloroflexi*, *Firmicutes*,
420 *Proteobacteria* and *Microgenomates/OP11* in smaller shares (Fig. 4 and Fig. 5).

421 **Figure 3.** Venn diagrams displaying the number of OTUs shared between the samples: a)
422 water and wastewater samples only, b) sediment samples only, c) all samples. Numbers in
423 brackets refer to the total number of identified OTUs in the sample. Diversity and richness
424 estimators for Illumina libraries are shown in Fig. 3d.

425 Taxonomy-based analysis indicated that *Bacteria* constituted a majority, and *Archaea* less
426 than 0.02% of the total microbial community in each sample, except for sediments collected
427 from tundra stream inflow (SED-TS), where *Archaea* accounted for 2.54%. In the case of
428 *Bacteria*, their community consisted of 55 phyla, 37 of which were abundant only in minor
429 shares of less than 1% in each sample. Unassigned sequences (not assigned to any Kingdom)
430 represented fewer than 0.6% and were most abundant in sediment samples, which is in
431 agreement with the literature that indicates under-representation of the soil taxonomy in the
432 databases (Bulgarelli et al., 2012; Gans et al., 2005).

433 In the case of wastewater effluent (WW-E), 10 core phyla constituted over 96% of the
434 community. The most abundant were *Actinobacteria* and *Proteobacteria* (21% each),
435 followed by *Dojkabacteria*/WS6 (14%), *Chloroflexi* and *Planctomycetes* (10% each), with
436 smaller shares of *Bacteroidetes*, *Firmicutes*, *Parcubacteria*/OD1, *Microgenomates*/OP11 and
437 *Saccharibacteria*/TM7 (3–6% each, Fig. 4c). Some of those phyla and their representatives
438 were also detected in major shares in wastewater treatment plant bioreactors (Saunders et al.,
439 2016), including those serving municipalities in the Arctic Circle (eg. *Bacteroidetes*,
440 *Firmicutes* and *Rhizobiales* from *Alphaproteobacteria*, as well as *Comamonadaceae* from
441 *Betaproteobacteria*) (Gonzalez-Martinez et al., 2018). Others (e.g. *Microgenomates*/OP11,
442 *Parcubacteria*/OD1 and *Saccharibacteria*/TM7) were also found in various environments
443 other than in activated sludge systems, under anoxic (nitrate and sulphate reducing) and
444 anaerobic conditions (Elshahed et al., 2007; Gihring et al., 2011; Harris et al., 2004; Peura et
445 al., 2012).

446 Interestingly, the recipient (WW-R) to some extent mirrors the core phyla from WW-E, but in
447 different shares (Fig. 4d), suggesting that, besides affecting the chemical characteristic (see
448 section 3.1), the wastewater discharge also influenced the microbiology of Lake 2. In WW-R,
449 *Proteobacteria* (29%) and *Bacteroidetes* (15%) were followed by *Actinobacteria* and

450 *Cyanobacteria* (10.5% each), *Chloroflexi* (8%), *Saccharibacteria*/TM7 and
451 *Parcubacteria*/OD1 (6-7%), with smaller shares of *Dojkabacteria*/WS6, *Planctomycetes*,
452 *Firmicutes* and *Verrucomicrobia* (2–4%). The influence of treated wastewater (WW-E) on the
453 recipient (WW-R) can be seen not only at the phylum level, but also at lower taxonomic
454 levels (258 shared OTUs, among which 165 were unique to WW-E and WW-R, Fig. 3a).
455 Particularly high abundances (2–5%) in both samples were noted for orders from *Alpha*-
456 subdivision (*Proteobacteria* phylum): *Rhizobiales* as well as *Caulobacterales* with the
457 activated-sludge-related genus *Phenylobacterium*. *Isosphaeraceae* and *Pirellulaceae* families
458 (*Planctomycetes* phylum) were most abundant in WW-E, WW-R and SED-R (0.7–5.5%).
459 They are usually related to multistage activated sludge process and found mainly in aeration
460 basins (Zheng et al., 2016), so their presence indirectly confirms their possible washout from
461 the wastewater treatment plant with activated sludge flocs (See supplementary materials,
462 Fig. S2 and S3). The *Nocardioideaceae* family from the *Actinobacteria* phylum were most
463 abundant in WW-E (12.5%) and WW-R (3.4%). Their representatives are widespread in
464 natural and polluted environments and are known for their ability to decompose a wide range
465 of organic matter (including at low temperatures). Therefore, they are suspected of playing a
466 significant role in degradation processes (Tóth and Borsodi, 2014). However, in this study,
467 mostly unclassified genera of *Nocardioideaceae* family have been noted. Non-phototrophic
468 *Caldilineaceae* and *Anaerolinaceae* families of *Chloroflexi* phylum, related to municipal and
469 domestic wastewater treatment systems (Saunders et al., 2016, Zhang et al., 2017) were
470 abundant (6-7% and 1%, respectively) in wastewater related samples (WW-E and WW-R),
471 while in Lake 1 they did not exceed 0.1%. A similar tendency was observed for gut-related
472 *Clostridia* (phylum *Firmicutes*) and potentially human-associated clade TM7-3 of the
473 *Saccharibacteria*/TM7 phylum. The B142 class from the *Dojkabacteria*/WS6 phylum
474 constituted over 14% of WW-E and 3.6% of WW-R, but was present only in minor shares

475 (<0.3%) in Lake 1 (L-TS and L-WS samples). The order *Sphingobacteriales* (phylum
476 *Bacteroidetes*) was present in similar shares in Lake 1 and Lake 2, as well as in treated
477 wastewater (~5%), though wastewater-related samples (WW-E and WW-R) contained mostly
478 unknown taxa, whereas Lake 1 was dominated by the *Sphingobacteriaceae* family, including
479 unknown species from the *Pedobacter* genus, which are common in various habitats, from
480 soil and freshwater to alpine glaciers (Gordon et al., 2009; Margesin et al., 2003; Roh et al.,
481 2008; Shivaji et al., 2005).

482 In Lake 2, the aforementioned influence of WW-E on WW-R was visible also in terms of its
483 more diversified microbial community than Lake 1, which was indicated by biodiversity
484 indices (Figure 4d). In the case of Lake 1, points L-TS and L-WS were dominated by only
485 three phyla: *Proteobacteria* (57-58%) *Bacteroidetes* (27%) and *Actinobacteria* (9-10%),
486 altogether constituting over 93% of the community (Fig. 4a and 4b). It was, however,
487 suspected that microbial community, at least at point L-TS, would mirror to some extent the
488 impact of tundra stream inflow and the nearby bird breeding area (mainly of a little auk
489 colony). Nevertheless, chemical data were similar for both water samples from Lake 1. It was
490 also confirmed by the taxonomic data showing that L-WS and L-TS core microbiota were
491 characterised by similar microbial composition up to genus level, with minor differences
492 noted for the *Cyanobacteria* phylum. This indicated that the bird-droppings-related runoff
493 was retained by the tundra vegetation surrounding Lake 1 (mainly lichens and mosses) or
494 diluted by intense rainfalls (see section 3.1).

495 Interestingly, the core phyla of both fresh waters (Lake 1 and Lake 2) were mostly
496 represented by *Alpha-* and *Beta-* subdivisions of *Proteobacteria*; *Flavobacteria* and
497 *Sphingobacteria* belonging to *Bacteroidetes*, as well as *Actinobacteria* classes (Fig. 4a, 4b,
498 4d, Table S1). These taxa dominate in freshwater (Michaud et al., 2012; Rozmarynowycz et
499 al., 2019), as well as in Arctic river-lake systems located around the Polish Polar Station

500 (Kosek et al., 2019; Ntougias et al., 2016). The prevalence of *Actinobacteria*, *Alpha-* and
501 *Betaproteobacteria* with high relative abundance of *Burkholderiales* and *Sphingomonadales*
502 was also found in an endophyte population in the Arctic tundra (Nissinen et al., 2012).
503 *Acidobacteria* have frequently been reported as predominant taxa in Canadian, Alaskan and
504 Siberian Arctic soils (Campbell et al., 2010; Neufeld and Mohn, 2005; Rawat et al., 2012;
505 Wallenstein et al., 2009) and are regarded as an indicator of tundra influence (Männistö et al.,
506 2013), but in this study they did not exceed 0.5% in Lake 1 and Lake 2. The Lake 2 (WW-R)
507 microbial community, however, contained significant shares of endophytic classes
508 *Oscillatoriothycidae* (3.9%, *Phormidium* genus), *Synechococcophycidae* (1.2%, mostly
509 genus *Leptolyngbya*) and other unclassified *Cyanobacteria* (5.4%), which were less abundant
510 in Lake 1 (Table S1). The presence of these bryophyte and plant-related taxa, as well as
511 *Pseudanabena* species, was also noted by Richter (2018) in the fertile, ornithogenic and
512 moss-dominated area around the Polish Polar Station. Undoubtedly, in Lake 2 (WW-R point)
513 cyanobacteria growth could be supported by the release of nutrients with wastewater
514 treatment plant effluent (WW-E), which was confirmed by the presence of the aforementioned
515 nitrophilous taxa.

516 Note that, despite continuous ammonia discharge with the WW-E (up to 40 mg N-NH₄/L), it
517 was not accumulated in Lake 2 (<1.2 mg N-NH₄/L in point WW-R). This can be related to the
518 dilution factor as well as microbial activity. The ammonia- and nitrite-oxidising
519 microorganisms were present in very low shares in both lakes, and did not exceed 0.1% in
520 WW-R and 0.01% in L-TS and L-WS. However, even in ammonia-rich niches such as
521 wastewater, relative abundance of the ammonia/nitrate-oxidising community is low (Saunders
522 et al., 2016). According to the obtained results the main role in the oxidation of ammonia to
523 nitrite in WW-R was played by *Nitrosomonas* spp, with *Nitrospira* as possible nitrite-
524 oxidising bacteria (NOB). However, a metabolic function of *Nitrospira* in the environment is

525 difficult to assign, since *Nitrospira* members could perform full nitrification, nitrite oxidation,
526 or other alternative pathways beyond the nitrogen cycle (Koch et al., 2015). Anaerobic
527 ammonium oxidation (anammox) bacteria *Candidatus Brocadia* were detected only in Lake 1,
528 which reflects its possible origin from occasionally deoxygenated tundra soil and
529 decomposing plants transported by surface runoff (Kosek et al., 2019). The absence of
530 *Nitrobacter*, noted in our study, was reported in the Arctic freshwater system also by Ntougias
531 (2016), but the significant shares of unknown genera of the *Bradyrhizobiaceae* family (up to
532 1% in WW-E) and the *Rhizobiales* order (up to 3.6% in WW-E, Table S1) suggests that NOB
533 were very likely represented in the samples and their low detection could mainly be ascribed
534 to the limited robustness of gene-fragment assignment to lower taxonomic levels.

535 Besides anammox, denitrification is another process releasing nitrogen to the atmosphere. A
536 wide variety of heterotrophic facultative anaerobes are capable of oxidising organic
537 compounds via nitrate respiration. Thus, in this study possible denitrifiers may belong to
538 genera such as *Flavobacterium* (2.4–2.8% in WW-R, L-TS and L-WS) and/or *Clostridium*
539 (0.6–1.5% in WW-R and sediment samples, Table S1), and also to representatives of the
540 *Actinomycetales* family (phylum *Actinobacteria*), the *Bacillus* genus (phylum *Firmicutes*) or
541 the *Alpha*-, *Beta*-, *Gamma*- and *Deltaproteobacteria* class of the *Proteobacteria* phylum.

542 In the studied lakes, apart from biogenic compounds, non-indigenous microorganisms (e.g.
543 human- and animal-related bacteria) too can be introduced. Among faecal indicators, bacteria
544 from *Escherichia* genus were noted in each sample, with the highest relative abundance in
545 WW-E (0.1%) followed by WW-R (0.06%), while in Lake 1 samples (L-TS and L-WS) they
546 did not exceed 0.01%. A similar tendency was noted for other faecal indicators – members of
547 *Enterococcus* spp. (WW-E – 0.09%, WW-R – 0.04% and <0.01% in L-TS and L-WS).
548 Additionally, one of the most abundant commensal bacteria in the human gut microbiota,
549 constituting even 5% of the intestine community in a healthy adult (Miquel et al., 2013),

550 *Faecalibacterium prausnitzii*, was also found, but only in WW-E and in a minor share
551 (<0.01%). Its absence in WW-R can be due to the fact that long survival of *F. prausnitzii*
552 outside the human gut is very unlikely, mainly due to sensitivity to oxygen (El Hage et al.,
553 2017). Cellulose-degrading *Ruminococcus*, possibly associated both with human- and
554 reindeer-gut microbiota, was found in similar abundances in both lakes (up to 0.01%) and
555 treated wastewater (0.04%). Bacterial sequences potentially associated with bird faeces
556 contained species identified as responsible for fish infections (*Acinetobacter johnsonii* or
557 *Vagococcus salmoninarum*), indicating the possible guano impact of some piscivorous bird
558 species other than the planktivorous little auk.

559 In this study, sediments from a tundra stream (Lake 1, SED-TS) and wastewater discharge
560 (Lake 2, SED-R) were also collected. According to the obtained data, the microbial
561 communities of SED-TS and SED-R differed from each other and from the other samples
562 (treated wastewater [WW-E] and lake waters [WW-R, L-TS and L-WS]). This was indicated
563 by the largest share of unique OTUs in SED-TS and SED-R (Fig. 3c) and the highest value of
564 diversity indices (Shannon, Simpson and Chao1, Fig. 3d). The bacterial communities in both
565 sediment samples were composed mainly of *Proteobacteria* (20–26%), *Actinobacteria* (11–
566 12%), *Parcubacteria/OD1* (7–8%) and *Chloroflexi* (9–12%, Fig. 4e, f). However, in SED-TS,
567 *Bacteroidetes* represented 17% of the community, while in SED-R they were replaced by
568 other phyla: *Cyanobacteria* (16%), *Verrucomicrobia* (9%) and *Acidobacteria* (11%). Soil-
569 and tundra-related *Acidobacteria* were more abundant in the wastewater-discharge-related
570 sediments (SED-R, 11%) than in the tundra stream inflow (SED-TS, 1.5%). The development
571 of *Cyanobacteria* (14%) in SED-R, can be favoured by the supply of nutrients by the treated
572 wastewater. In the SED-TS sample, where *Cyanobacteria* were rare (0.12%), anaerobic
573 sediment-related archaeal methanogens were noted (genus *Methanosaeta*, 1.4%, and
574 *Methanoregula* – 0.49%, Table S1). Similarly, sulphate-reducing *Deltaproteobacteria* were

575 particularly abundant at the tundra stream inflow (9.7% in SED-TS vs 1% in SED-R),
576 consisting mainly of *Desulfobacterales* (*Desulfobulbaceae* family), *Desulfuromonadales*
577 (*Geobacteraceae* family members, including the iron-reducing *Geobacter* genus) and
578 *Syntropobacterales* (*Desulfobacca* and *Desulfomonile* genera).

579 **Figure 4.** Bacterial community composition of the samples on phylum (inner ring) and class
580 level (outer ring)

581 The hierarchical heatmap at the bacterial phylum level reveals a dominance of *Proteobacteria*
582 among all the samples, as well as the site-specific presence of *Actinobacteria* and
583 *Dojkabacteria*/WS6 phyla in WW-E, and *Cyanobacteria* in SED-R. Two clusters confirm the
584 closest resemblance between Lake 1 water samples (L-TS and L-WS), these being different
585 from sediment and wastewater-related samples (Fig. 5a), which is also shown by PCoA
586 analysis (Fig. 5b). Sediment samples differ from the water and wastewater samples, though
587 neither is closely related to the other.

588 **Figure 5.** a) Heatmap of microbial community richness at the phylum level. Colour code
589 indicates relative abundance, ranging from yellow (low) to red (high). b) Principal
590 Coordinates Analysis for microbial community OTUs.

591 3.4. Prevalence and identification of *Enterococcus* spp.

592 The transmission of human and animal-related bacteria and their genetic elements is possible
593 mainly by faecal contamination of the environment, and thus in this study the presence of
594 faecal indicator *Enterococcus* spp. was tested in wastewater treatment plant effluent (WW-E)
595 and in lake-related samples (WW-R, L-TS and L-WS). As suspected, among the studied
596 points *Enterococcus* spp. were the most abundant in WW-E – from 0.7×10^3 CFU/100 mL to
597 1.9×10^3 CFU/100 mL. This is, however, rather low compared to other wastewater treatment
598 plants' effluents (Sadowy and Luczkiewicz, 2014). In the treated wastewater receiver (WW-

599 R) enterococci varied from 11 to 150 CFU/100 mL and their abundance was in general higher
600 than in Lake 1: up to 30 CFU/100 mL in L-TS, and occasionally noted in L-WS,
601 (<1 CFU/100 mL). Note that the presence of *Enterococcus* spp. was confirmed not only by
602 culture-dependent approach but also by metagenomic approach (minor shares, less than 0.1%,
603 see section 3.3). Nonetheless, compared to the New Bathing Directive (2006/7/EC)
604 requirements, both tested lakes (points L-TS, L-WS, WW-R) represented excellent water
605 quality in terms of enterococcal presence (below 200 CFU/100 mL).

606 Among cultivated enterococcal strains, 76 were isolated from the samples (17 from L-TS, 23
607 from L-WS, 16 from WW-E and 20 from WW-R), then biochemically identified (Fig. 6) and
608 tested for antimicrobial susceptibility (Fig. 7). Of 76 isolates, 36 were identified as *E. faecalis*
609 (47.4%), 32 as *E. faecium* (42.1%) and the remaining as *E. avium* (n = 3; 3.9%), *E. hirae* (n =
610 2; 2.6%), *E. durans* (n = 1; 1.3%) and *E. casseliflavus/gallinarum* (n = 2; 2.6%). According to
611 the obtained results, two species, *E. faecalis* and *E. faecium*, comprised 76–95.6% of all
612 enterococcal isolates in a single sample, as they belong to the autochthonous microbiota of
613 human and animal gastrointestinal tracts (Lebreton et al., 2014; Wu et al., 2019).
614 Interestingly, *E. avium*, commonly related to birds' intestinal tract (Yu et al., 2019), was
615 observed mainly in Lake 1 at the tundra stream discharge (L-TS). *E. faecium* was
616 predominant in WW-E (62.5%) and WW-R (80%), while *E. faecalis* dominated in the L-TS
617 (70.5%) and L-WS (73.9%) samples. The reason of such dominance is not fully clear and can
618 be related to the limited number of isolates. However in general, this is in agreement with
619 Zaheer et al. (2020), who suggested that to some extent enterococci show niche specificity,
620 and for this reason they can be used as indicator bacteria in antimicrobial resistance studies.

621 **Figure 6.** Identification of *Enterococcus* spp. isolated from wastewater effluent (WW-E) and
622 two lakes: under natural (L-TS, L-WS) and anthropogenic (WW-R) impact

623 3.5. Antimicrobial resistance of *Enterococcus* spp.

624 The dissemination of antimicrobial resistance in polar regions requires attention, due to the
625 observed rapid increase in human activity in this area, and other environmental changes. Wild
626 birds that migrate annually to the Arctic for breeding are also increasingly studied as vectors
627 for the transmission of resistant bacteria and resistance genes (Hernandez et al., 2010;
628 Radimersky et al., 2010).

629 In this study the susceptibility of *Enterococcus* spp. isolates was assessed against 10
630 antimicrobial agents and categorised according to the clinical breakpoints and
631 epidemiological cut-off values (ECOFFs) provided by EUCAST (EUCAST 2020, Fig. 7). The
632 main purpose of clinical breakpoints is to predict clinical efficacy of antimicrobial therapy,
633 while the ECOFF is defined as MIC differentiating the wild-type bacteria from those that
634 have an acquired form of resistance. The clinical resistance among tested enterococci was
635 noted for Nitrofurantoin (MIC > 64 mg/L); note that clinical breakpoints for nitrofurantoin are
636 valid only for *E. faecalis*. Nitrofurantoin is a bactericidal antimicrobial agent used in
637 uncomplicated urinary-tract infections (Schmiemann et al., 2012). Clinical breakpoints
638 obtained in this study indicated that resistance to nitrofurantoin was detected only in the
639 wastewater treatment plant effluent (WW-E) in 14.3% of *E. faecalis* isolates. However, MIC
640 distribution evaluated for nitrofurantoin (Fig. 7) showed that *E. faecalis* isolates with MIC >
641 32 mg/L (above ECOFF value) constituted 59.2% of isolates in WW-E and 33.3% in WW-R
642 (treated wastewater recipient), which is followed by tundra stream discharge (8.3% in L-TS).
643 None of the *E. faecalis* isolates with MIC above the ECOFF value were noted in the area of
644 the water supply system (L-WS).

645 In the case of *E. faecium*, clinical isolates have already been reported to rarely be resistant to
646 nitrofurantoin (Toner et al., 2016), as also confirmed in this study (Fig. 7), since no isolate



647 with acquired resistance (MIC > 256 mg/L) to nitrofurantoin was detected. Interestingly,
648 resistance to nitrofurantoin can be mediated via plasmids and chromosomal mutations, and
649 resistance among clinically isolated *Enterococcus* spp. has increased in recent years from near
650 zero to 40% (Toner et al., 2016). Additionally, both resistance genes and mobile genetic
651 elements have shown similarity in animals and humans, so transmission of resistance through
652 zoonotic pathogens and through commensal food-borne bacteria is possible.

653 **Figure 7.** Distribution of Minimal Inhibitory Concentration (MIC), in milligrams per litre, for
654 the studied *E. faecium* and *E. faecalis*. Clinically susceptible strains are shown on grey field.
655 ECOFFs (epidemiologic cutoff values) for both species are marked as dotted lines. For
656 daptomycin, different ECOFF values are set for *E. faecalis* (4 mg/L) and *E. faecium* (8 mg/L).
657 For nitrofurantoin, clinical breakpoint and ECOFF that are shown on the graph are valid only
658 for *E. faecalis*; ECOFF for *E. faecium* is 256 mg/L, while clinical breakpoints are not defined.

659 In this study, isolates with MIC above the ECOFF value were also noted for moxifloxacin
660 (MIC > 1 mg/L, *E. faecalis* in L-TS: 8.3% and WW-E: 14.3%) and erythromycin (MIC >
661 4 mg/L) among *E. faecalis* (14.3%) and *E. faecium* (22.2%) in WW-E. Note that the
662 remarkable capacity of *Enterococcus* spp. to acquire resistance to macrolides caused that
663 antimicrobial agents from this chemical class (including erythromycin) are no longer used to
664 treat enterococcal infections (lack of clinical breakpoints, Fig. 7), but they are still in use to
665 treat other emerging infections (EUCAST, 2020). Additionally, data of this study shown that
666 regardless of sampling point, isolates of *E. faecalis* tend to be more susceptible than *E.*
667 *faecium* to tested beta-lactam agent – ampicillin – similarly as reported among clinical
668 isolates, where most *E. faecium* isolates are ampicillin-resistant (MIC \geq 8 mg/L).

669 Due to the limited number of isolates tested in this study, no general conclusion can be drawn.
670 But bacteria related to humans and wildlife (including migratory birds) should be monitored

671 to better elucidate both their survival and possible dissemination of antimicrobial resistance.
672 This is of special importance in polar areas, where bacterial fitness cost connected with the
673 collection of resistance determinants could be justified by the presence of other environmental
674 stressors (e.g. UV light presence/absence). All the above are also important in terms of
675 climate change and increasing anthropogenic impact in polar regions.

676 **4. Conclusions**

677 Nutrient transport and cycling in polar lakes is highly influenced by catchment area. In this
678 study, the microbial communities at the tundra stream discharge and at the water supply point
679 in Lake 1 were confirmed to bear the closest resemblance, and suggested that the nutrient-rich
680 runoff from bird nesting area was retained by the surrounding tundra vegetation or diluted by
681 intense rainfalls. In the case of Lake 2, the effluent from the wastewater treatment plant
682 directly increased the diversity of the microbial community, both by introducing wastewater-
683 related bacteria and by supplying the receiver in nutrients, which may play a significant role
684 in typically oligotrophic Arctic lakes. Also, as most microbiological processes are
685 temperature-related, we can expect that climate changes can accelerate biochemical cycles in
686 Arctic lakes being amended by nutrient inflow. The microscopic observations also confirmed
687 an increase in all tested parameters in Lake 2, such as: total prokaryotic cell number, average
688 cell volume, prokaryotic biomass and live cell percentage. The presence of *Enterococcus* spp.
689 and their antibiotic resistance highlights the importance of wastewater treatment processes in
690 the dissemination of human-associated microbiome and resistome. In polar areas in particular,
691 which are increasingly being visited and inhabited by people, the introduction of wastewater-
692 related, non-indigenous microorganisms justifies the need for advanced treatment methods in
693 treatment processes. Analysis of microbial community structure combined with bacterial
694 antibiotic resistance analysis (in wastewater as well as water and sediments of the recipient),
695 provide an insight into the short- and long-term changes posed on the aquatic ecosystems by

696 the wastewater discharge. Detailed monitoring should help to identify and understand how
697 anthropogenic and natural factors impact the functioning of polar niches. Defining so called
698 'baseline conditions' is crucial in implementing the necessary regulations related to local
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706 **References**

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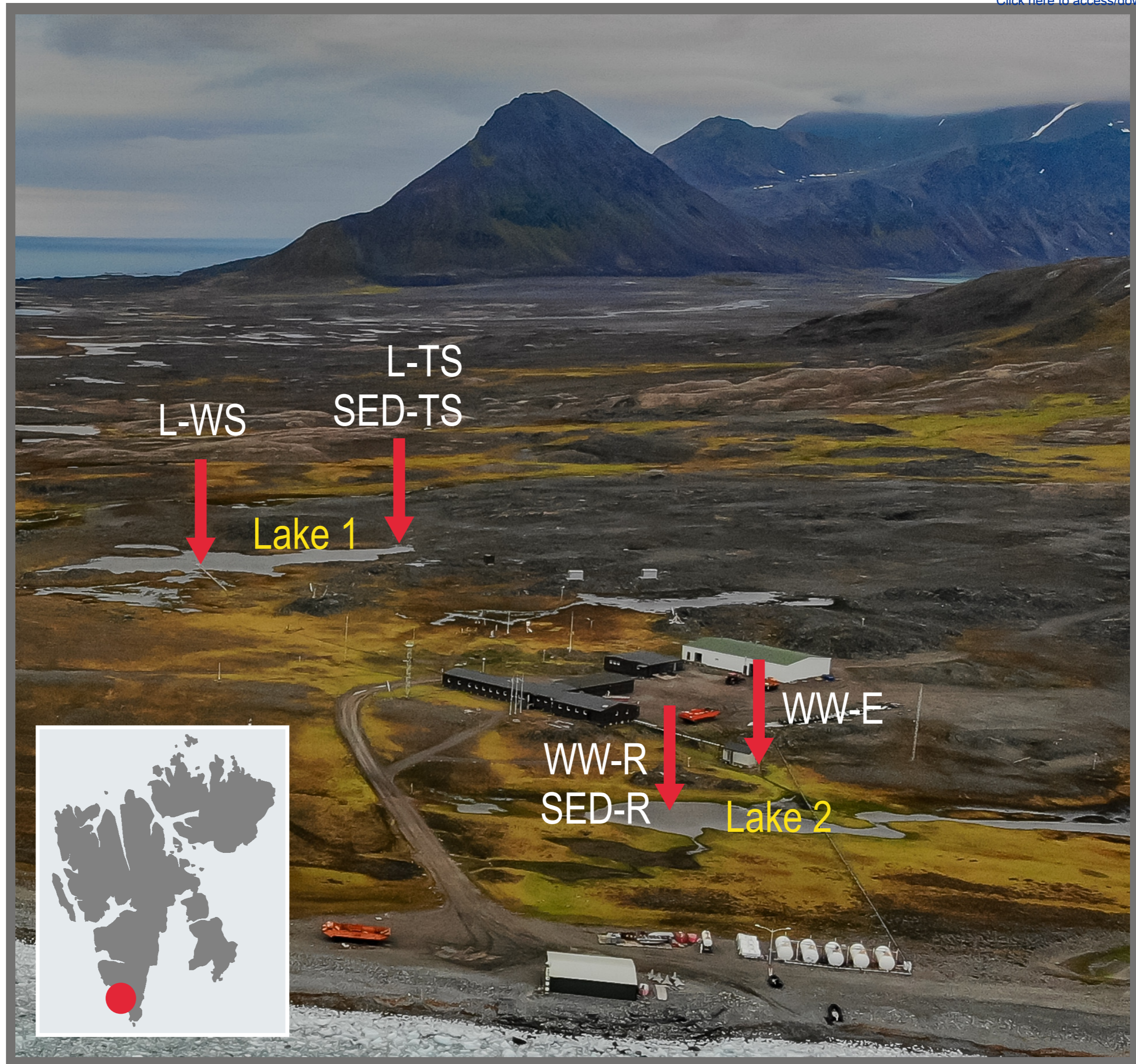
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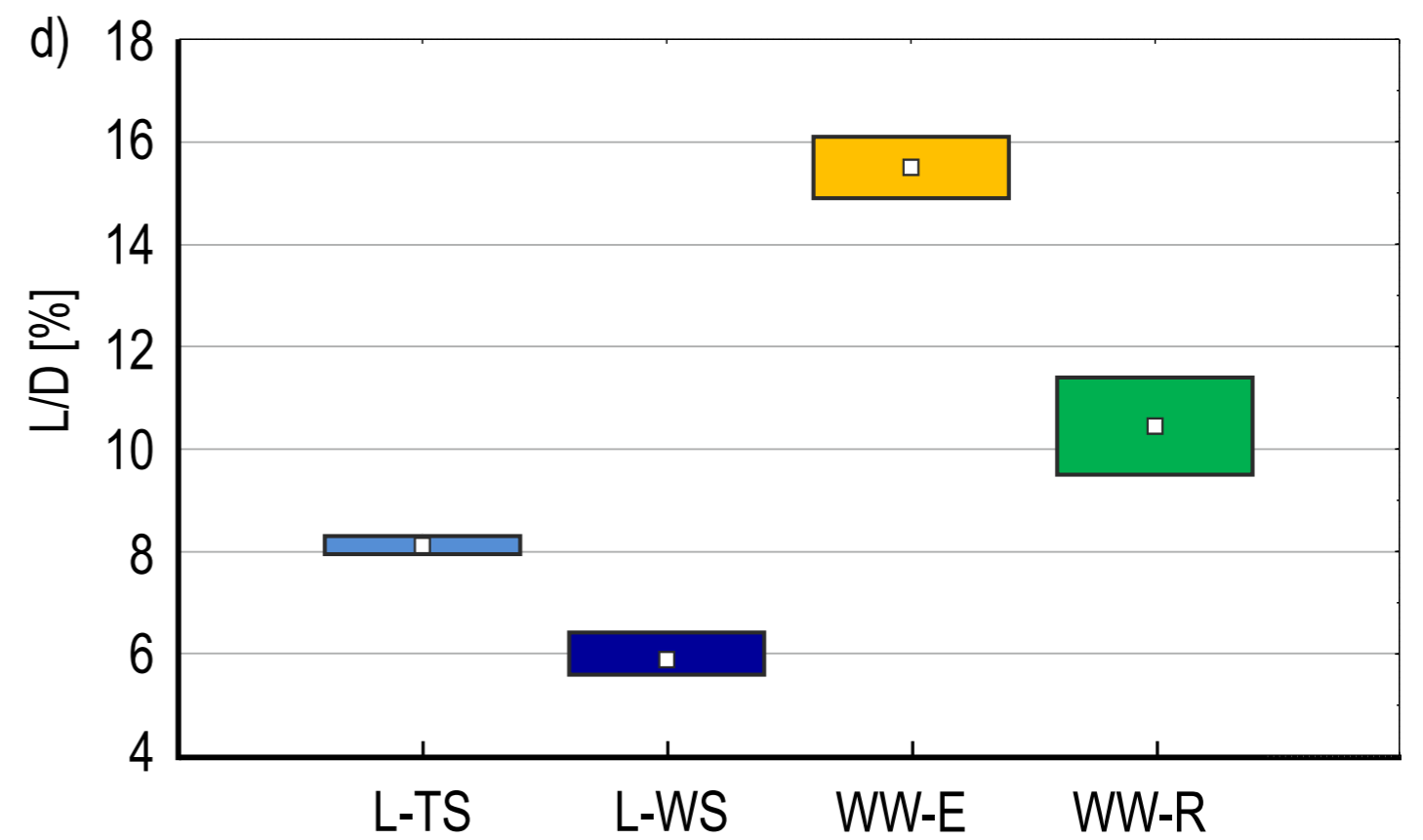
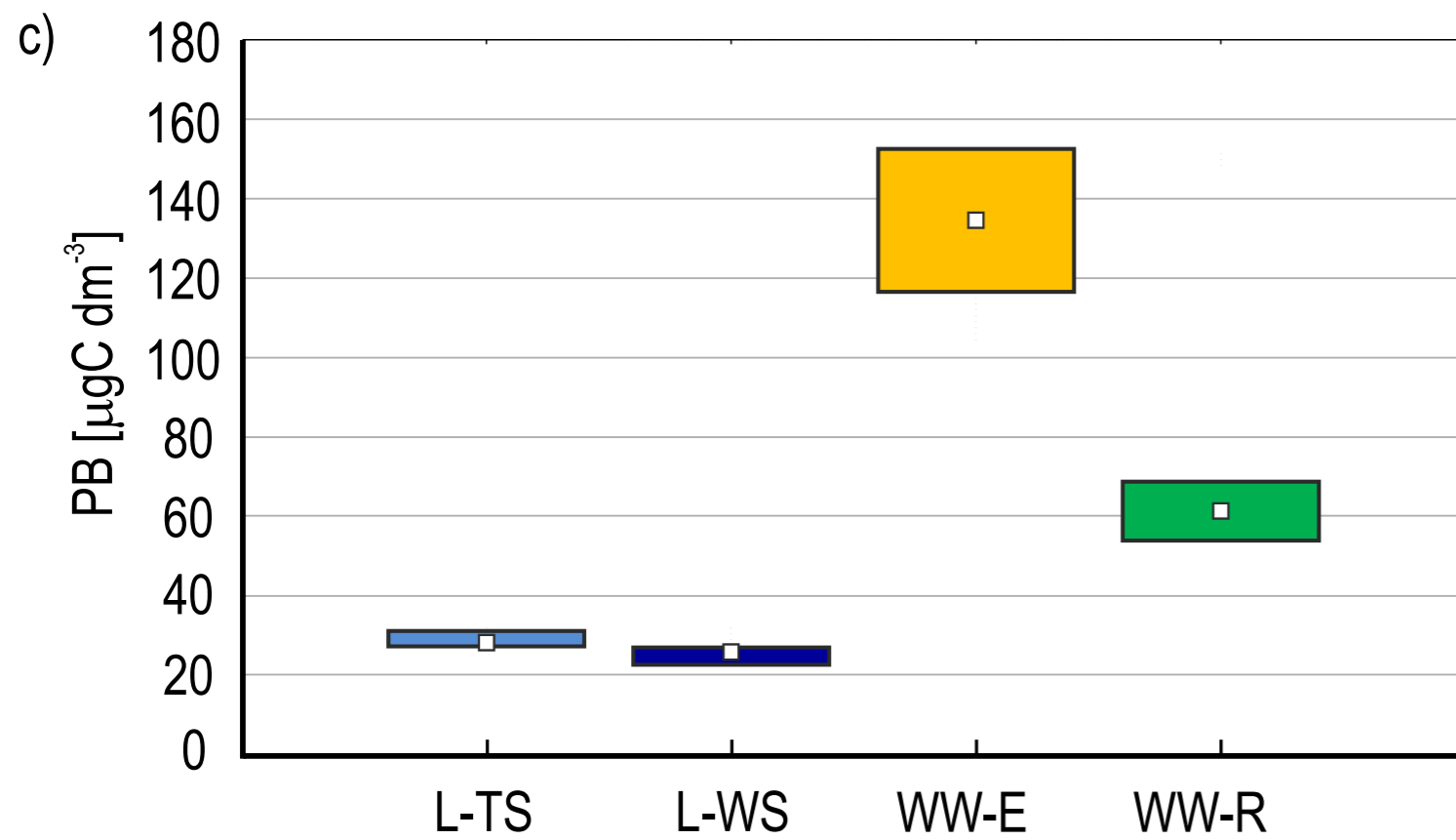
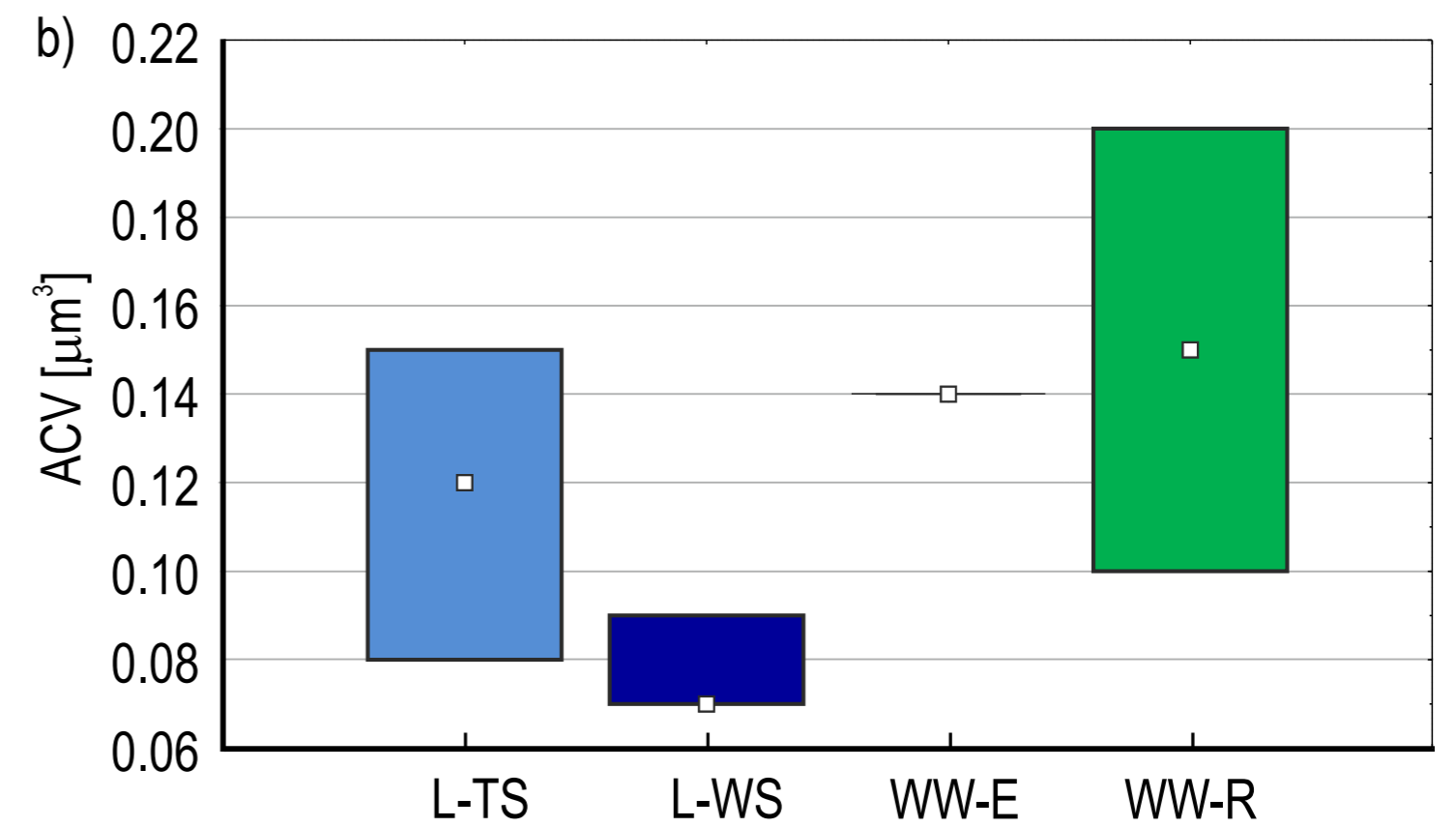
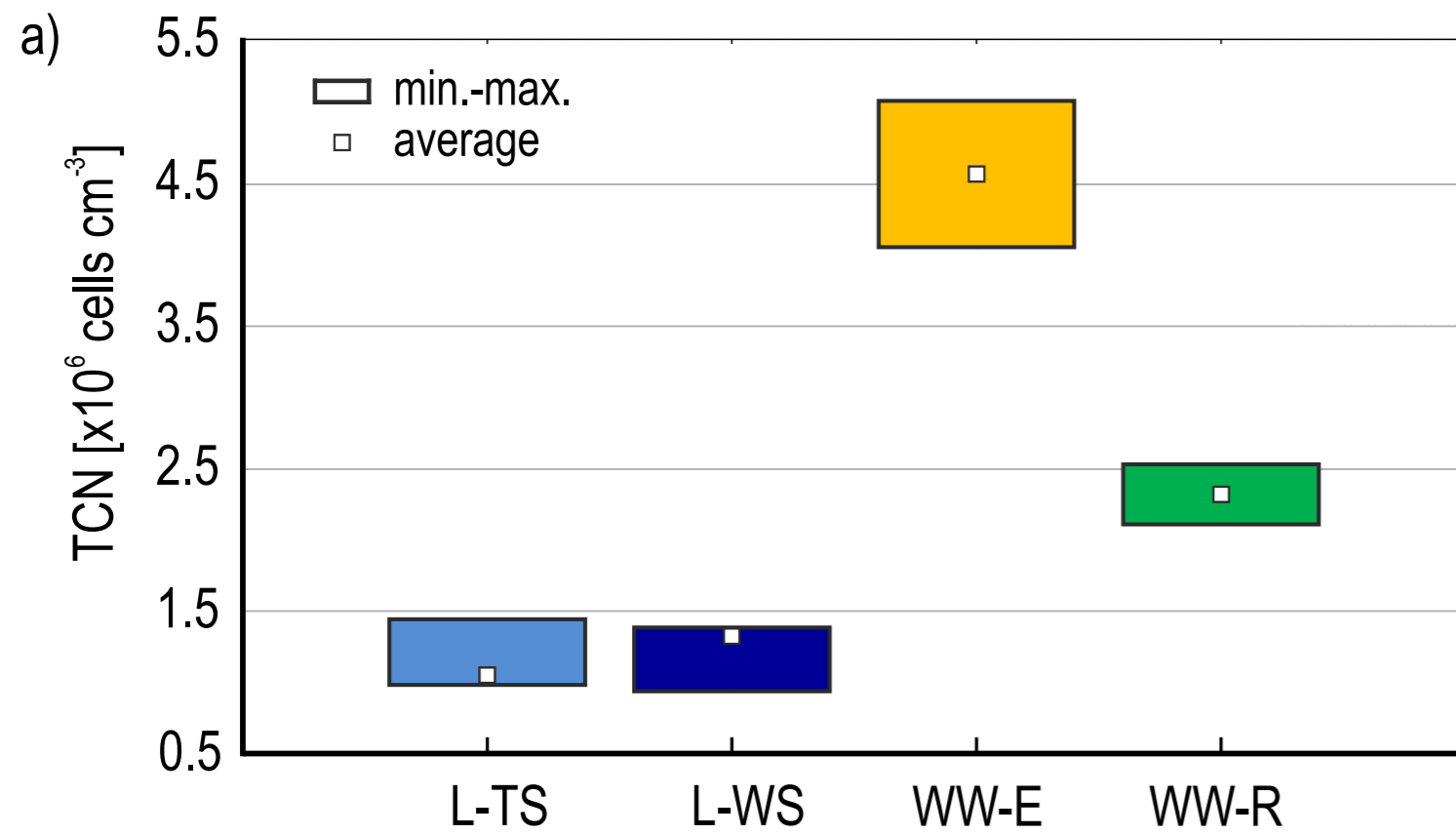
Table 1 Physicochemical parameters of water collected from the Lake 1 (L-TS: tundra stream inflow and L-WS: water supply area) and from Lake 2 (WW-R: treated wastewater recipient); the results of wastewater treatment plant effluent (WW-E) were compared with the discharge requirements.

parameter	unit	L-TS	L-WS	WW-R	requirements	
					for treated wastewater*	WW-E
T	°C	7.10±0.60	6.90±0.09	6.30±0.20	≤35	18.3±0.60
pH	[-]	7.10±0.08	7.70±0.08	7.30±0.14	6.5-9.0	7.30±0.12
EC	µS/cm	148.5±5.9	129.2±7.6	191±16	-	1 074±46
N-NH₄		0.56±0.21	0.12±0.08	1.12±0.40	-	34.2±5.6
N-NO₃		0.29±0.11	< LOD (<0.25)	0.85±0.20	-	6.7±2.1
TN		1.04±0.71	<LOD (<1.0)	2.03±0.55	≤ 30	71.6±9.2
P-PO₄	mg/L	<LOD (<0.05)	< LOD (<0.05)	0.19±0.09	-	7.4±2.0
TP		<LOD (<0.05)	< LOD (<0.05)	0.25±0.09	≤ 5	8.9±1.9
COD		< 5	< 5	30.0±5.3	≤ 150	168.±21

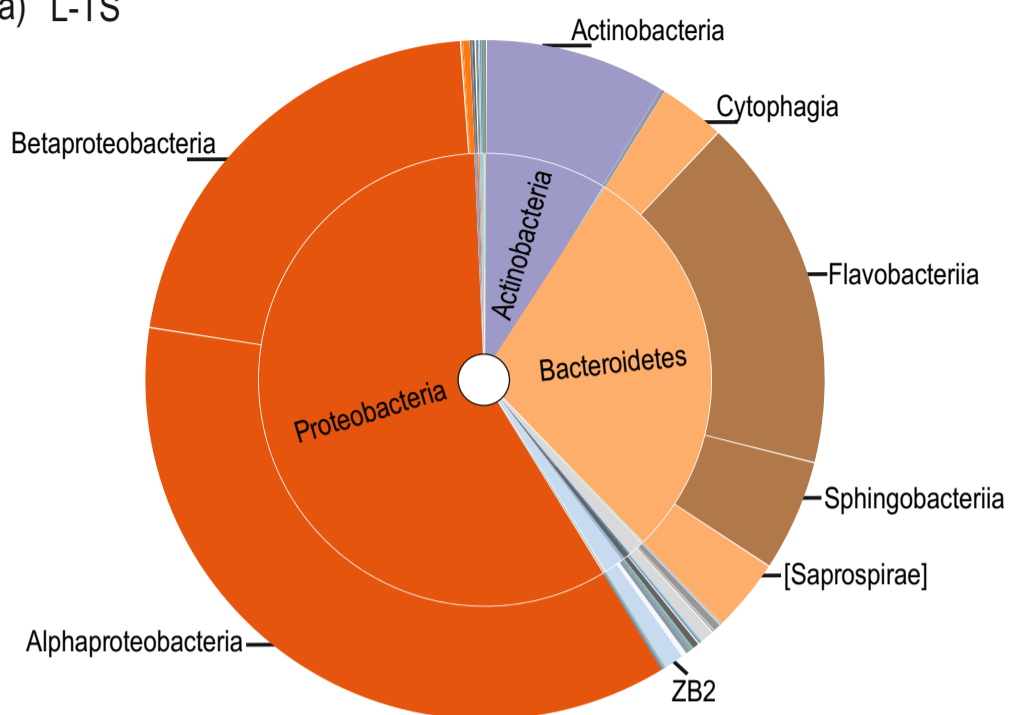
*according to Ministry of Maritime Economy and Inland Navigation (2019)

LOD – limit of detection

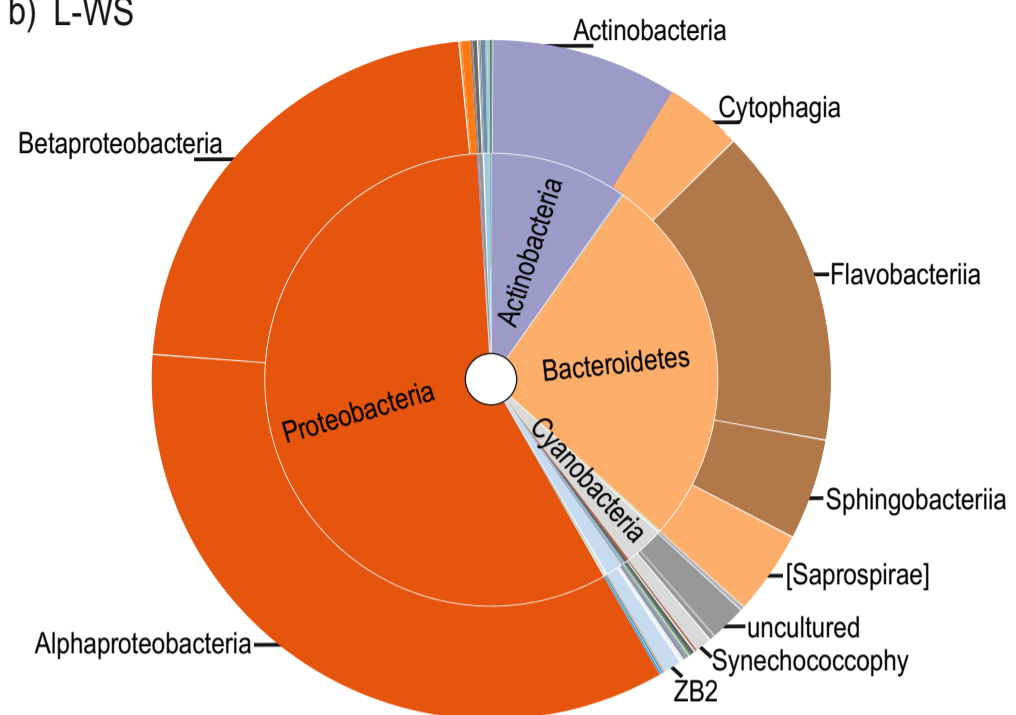




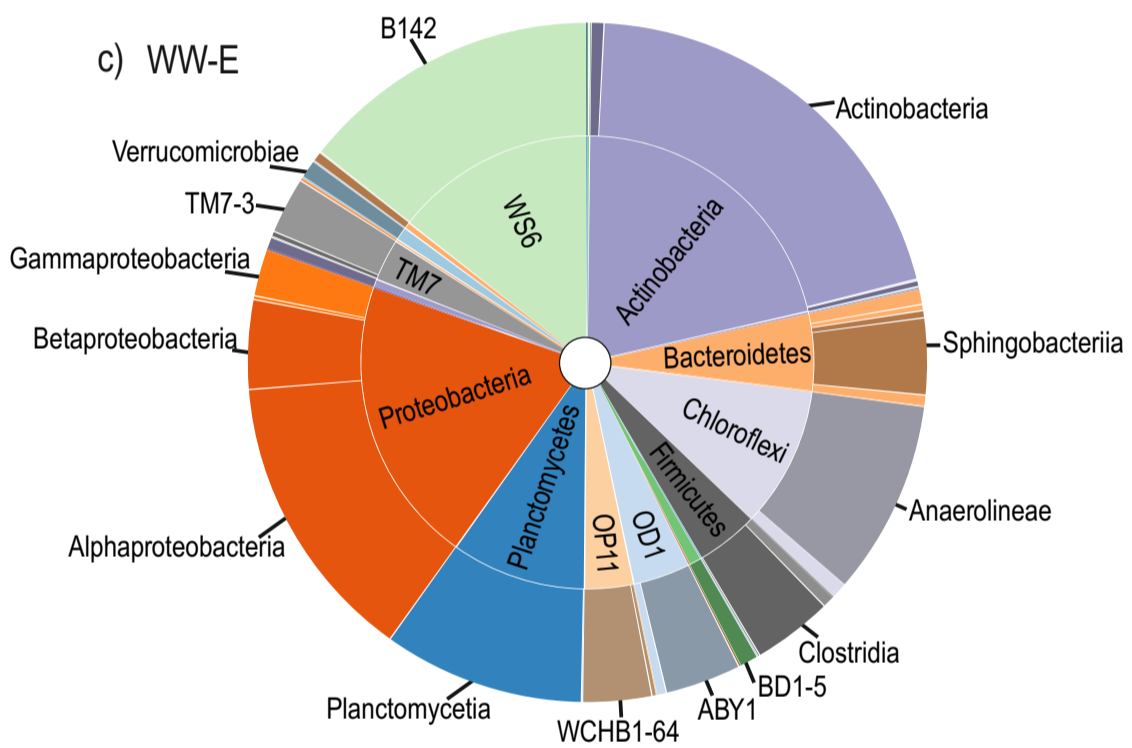
a) L-TS



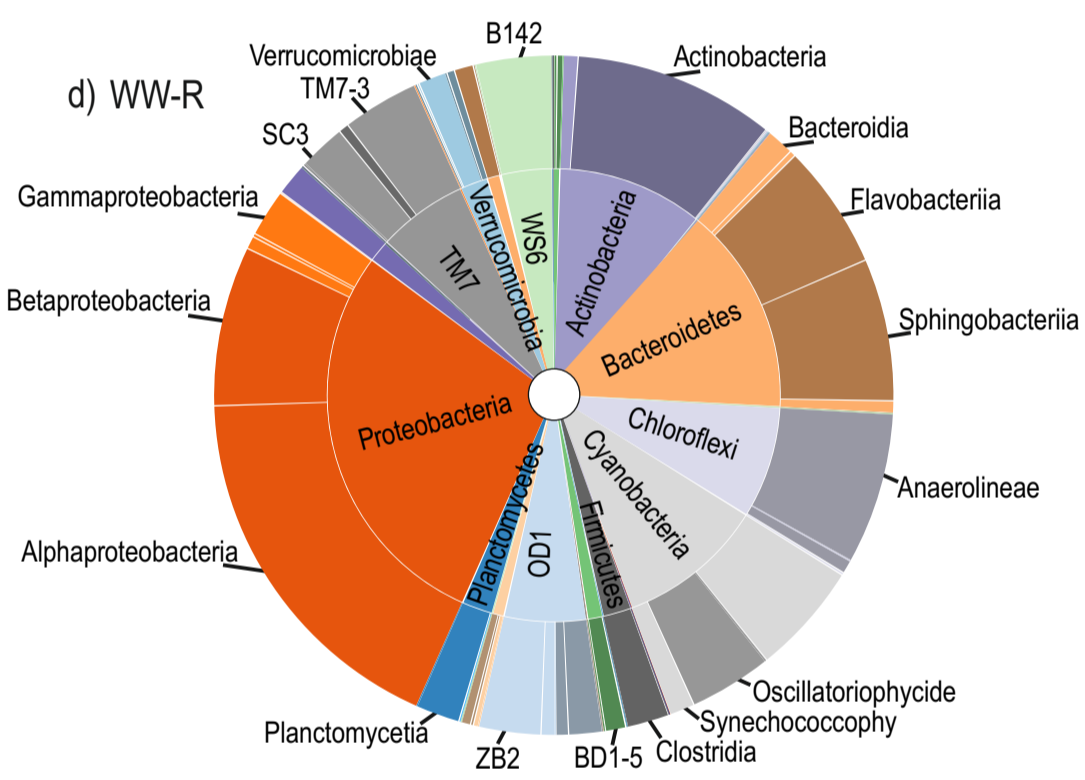
b) L-WS



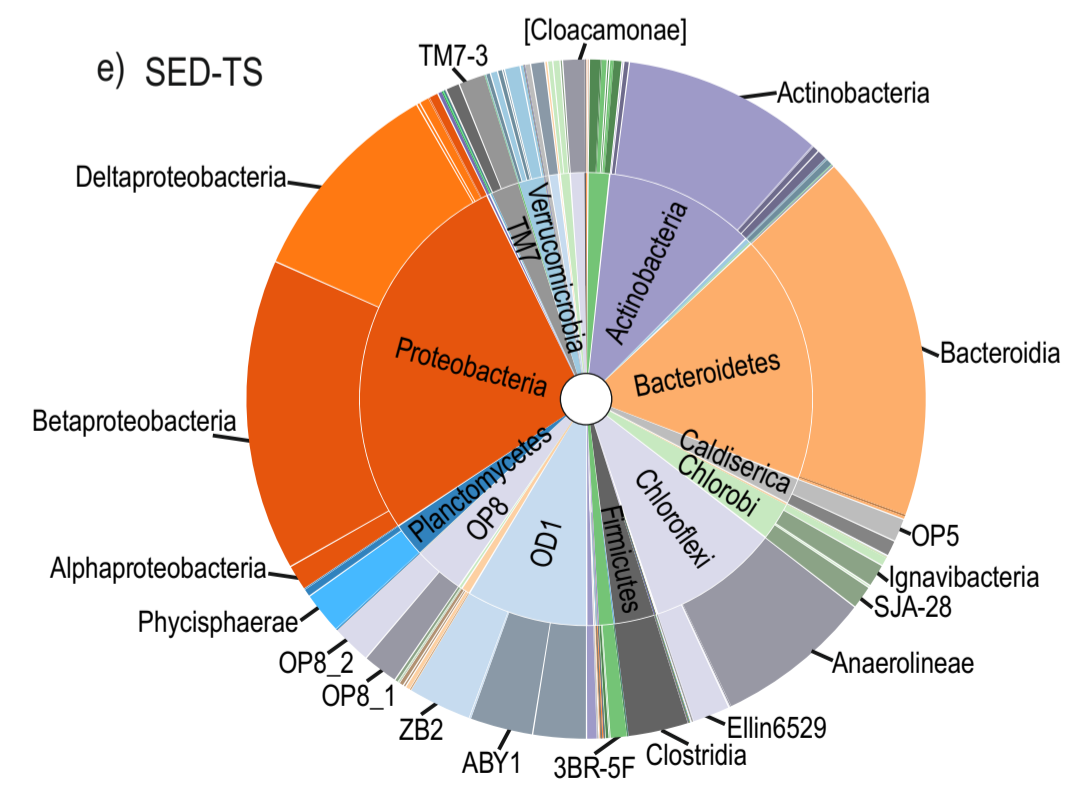
c) WW-E



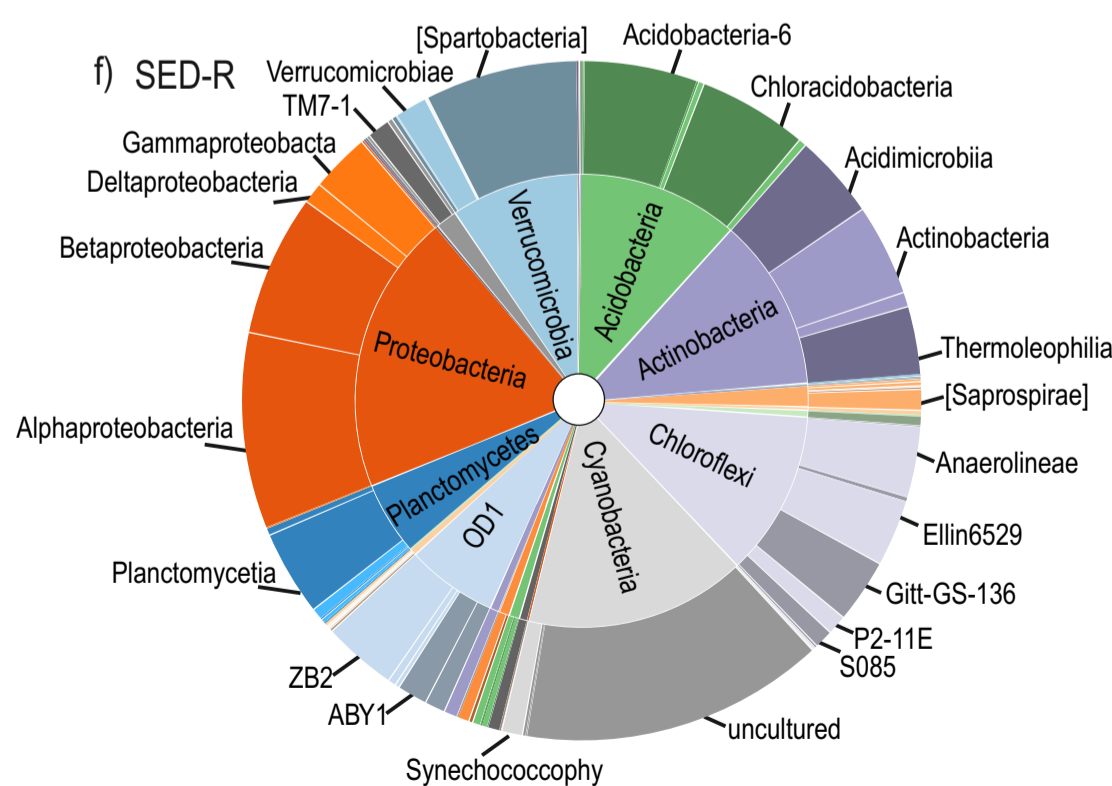
d) WW-R

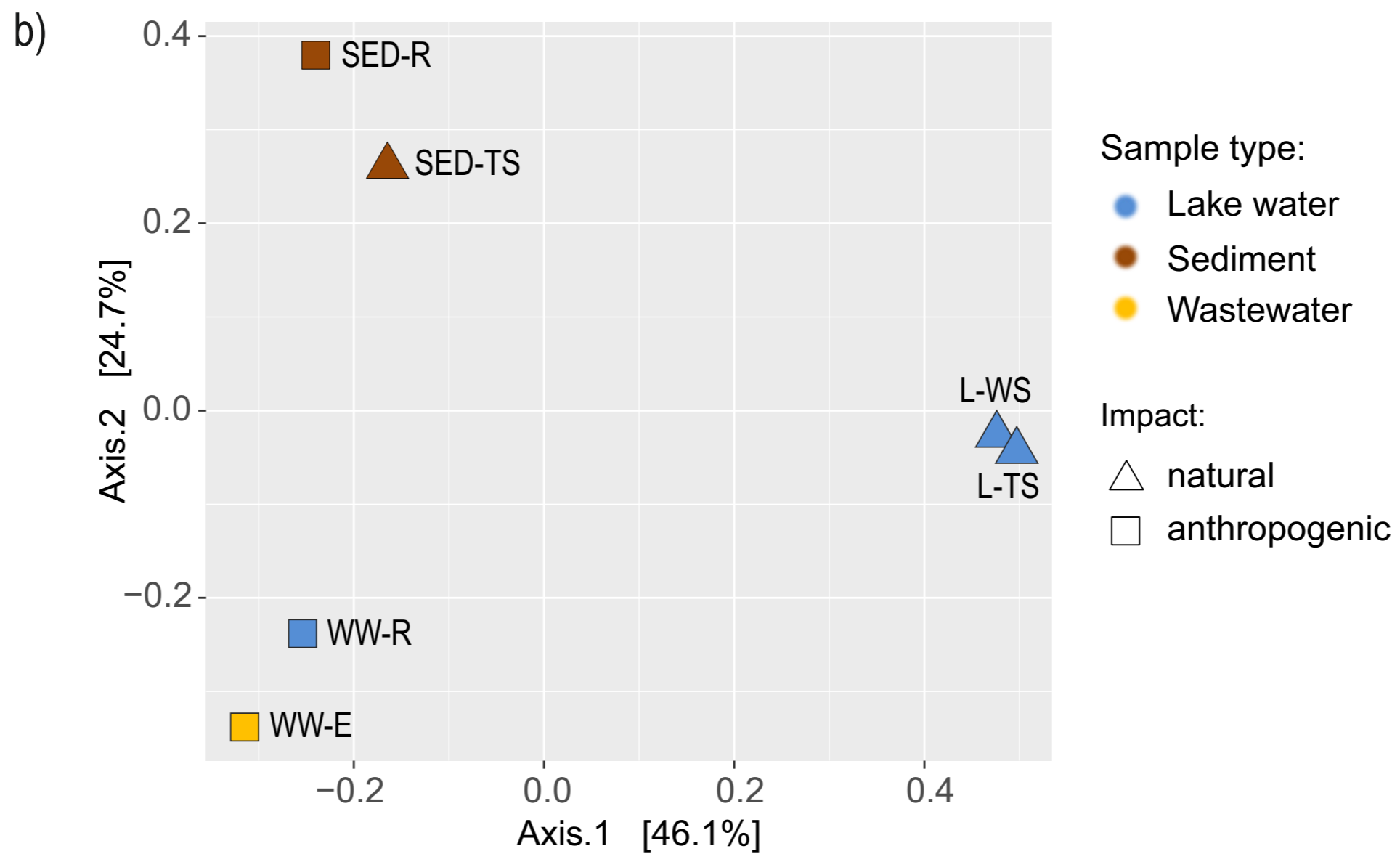
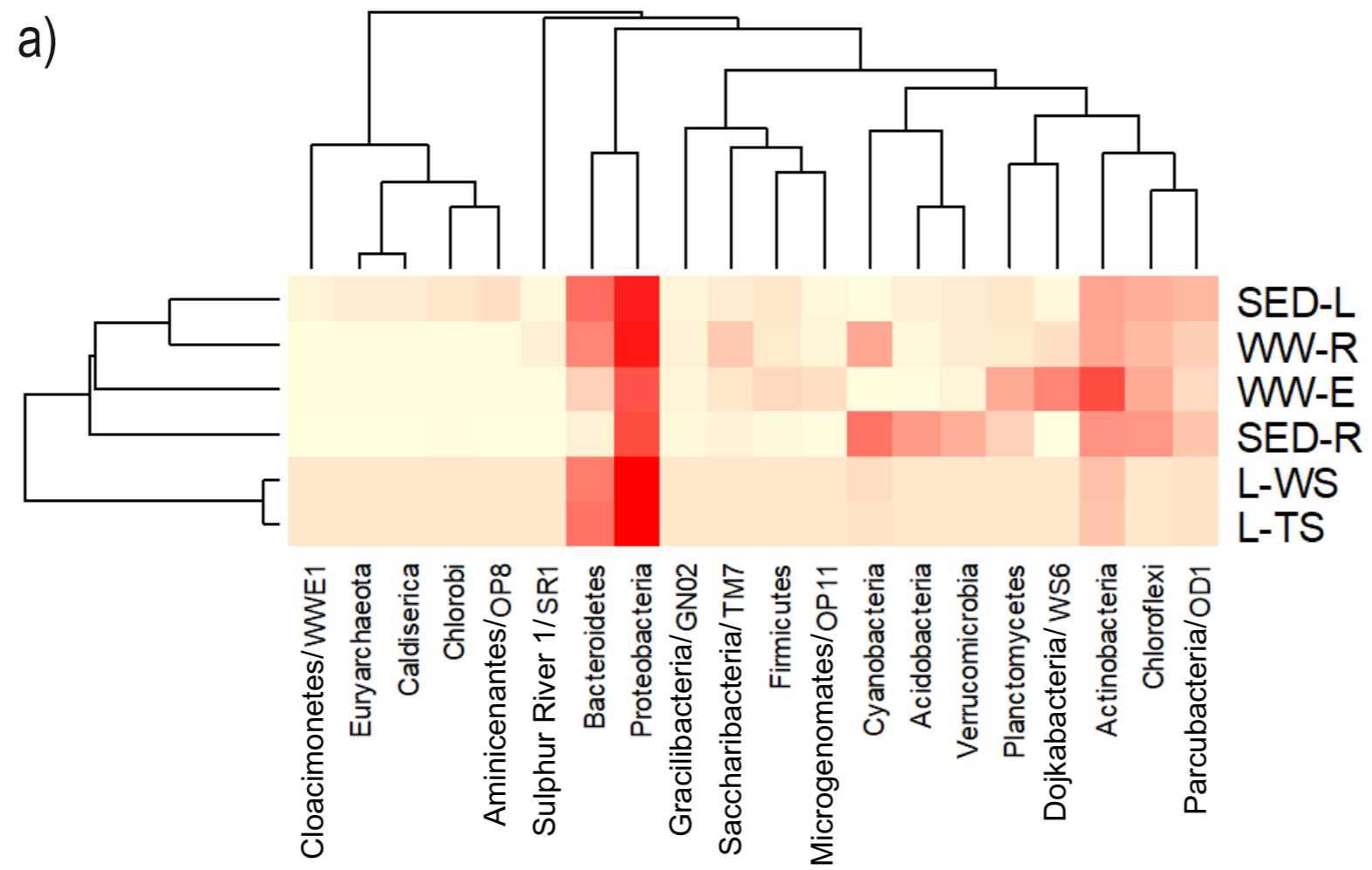


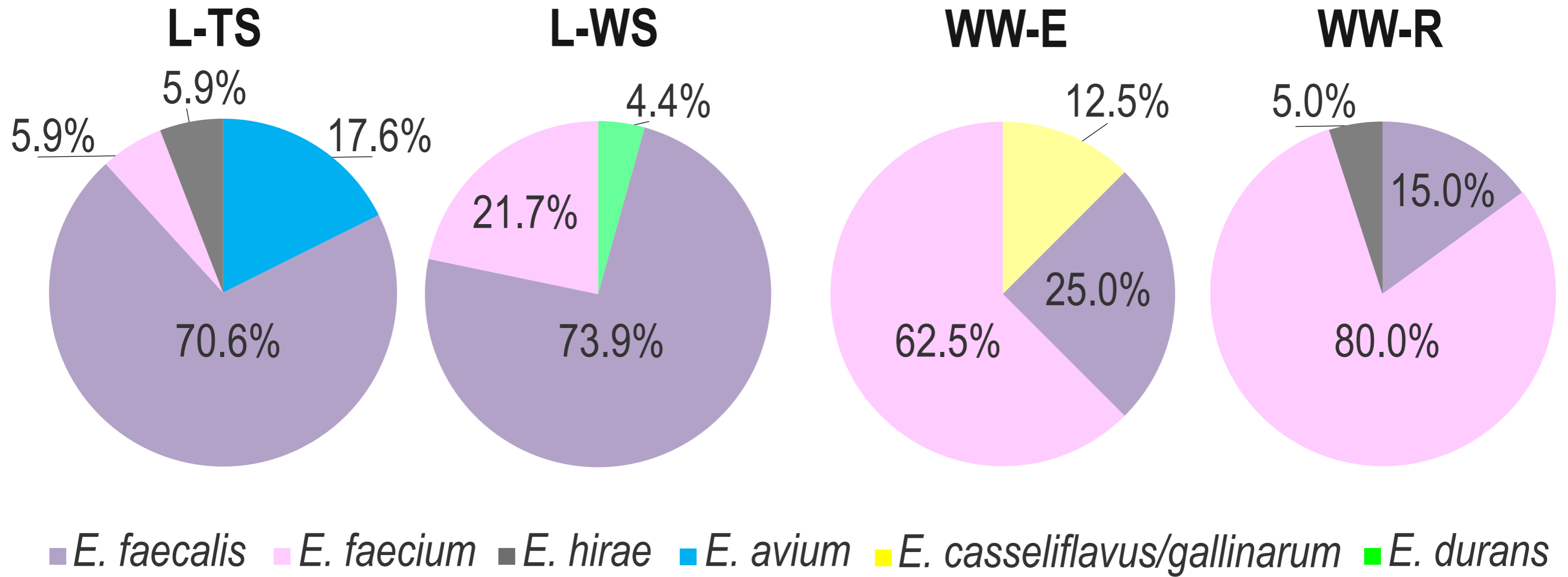
e) SED-TS



f) SED-R







MIC distribution: ■ *E. faecium* ■ *E. faecalis* susceptible according to clinical breakpoints ECOFF

