

Glacier melt-down changes habitat characteristics and unique microbial community composition and physiology in alpine lake sediments

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One sentence summary: When glaciers recede, lakes that lose hydrological connection to the glacier get clearer and warmer and microbes associated with glacial lake sediments alter in community composition and physiology.

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Abstract

Glacial melt-down alters hydrological and physico-chemical conditions in downstream aquatic habitats. In this study, we tested if sediment-associated microbial communities respond to the decrease of glaciers and associated meltwater flows in high-alpine lakes. We analyzed 16 lakes in forefield catchments of three glaciers in the Eastern Swiss Alps on physico-chemical and biological parameters. We compared lakes fed by glacier meltwater with hydrologically disconnected lakes, as well as “mixed” lakes that received water from both other lake types. Glacier-fed lakes had a higher turbidity (94 NTU) and conductivity (47 $\mu\text{S}/\text{cm}$), but were up to 5.2°C colder than disconnected lakes (1.5 NTU, 26 $\mu\text{S}/\text{cm}$). Nutrient concentration was low in all lakes (TN < 0.05 mg/l, TP < 0.02 mg/l). Bacterial diversity in the sediments decreased significantly with altitude. Bacterial community composition correlated with turbidity, temperature, conductivity, nitrate, and lake age and was distinctly different between glacier-fed compared to disconnected and mixed water lakes, but not between catchments. Chemoheterotrophic processes were more abundant in glacier-fed compared to disconnected and mixed water lakes where photoautotrophic processes dominated. Our study suggests that the loss of glaciers will change sediment bacterial community composition and physiology that are unique for glacier-fed lakes in mountain and polar regions.

Keywords: aquatic microbiology, biofilms, climate change, cryosphere, high-altitude, Switzerland

Introduction

The ongoing global melt-down of glaciers is one of the most striking examples of current climate change impacts. In the European Alps, small and low altitude glaciers (below 3000 m) already have disappeared or are predicted to disappear within this century (Zemp et al. 2009, Huss and Fischer 2016). The decline of glaciers is expected to affect glacier-associated ecosystems and ecosystem services especially in downstream hydrological systems (Hotelling et al. 2017, Cauvy-Fraunié and Dangles 2019, Grima and Campos 2020). The loss of hydrological connection of a catchment to a glacier changes the hydrodynamic regime (runoff) and a number of physico-chemical characteristics that are important for downstream habitats (Elser et al. 2020). Especially, transported sediments (glacial flour) are diminished as a consequence of reduced glacial influence (Bavay et al. 2013, Bliss et al. 2014). Turbidity in glacial meltwater stems from the erosive activity of the glacier on the underlying rock (Hallet et al. 1996) that mobilizes fine sediment particles. In contrast to that, water from precipitation and snow-melt that feed disconnected lakes is low in suspended solids. This leads to a regime shift from turbid to clear conditions in glacial lakes (Elser et al. 2020). With a continued receding of glaciers worldwide more lakes will be affected, which will

have a fundamental impact not only lake chemistry but also ecology (Sommaruga 2014). Ecological consequences of glacial melt-down have been demonstrated in glacier-fed alpine streams (Miller et al. 2009, Brighenti et al. 2019), and the planktonic and littoral fraction of alpine lakes (Sommaruga 2014, Peter and Sommaruga 2016, Tiberti et al. 2020).

Lakes located in glacier forefields at high altitudes (> 2000 m), however, are usually small, relatively shallow, and generally ice-covered for prolonged periods of time (Rogora et al. 2018). Due to the harsh conditions, prokaryotic organisms often dominate over eukaryotes in these habitats (Sommaruga 2014). High alpine and similar lakes in the polar regions are inhabited by benthic and sediment-associated microorganisms and act as diversity hotspots compared to the surrounding barren ground (Ji et al. 2015, Kleinteich et al. 2017). These microbial communities consist of a unique diversity of bacteria, archaea, and microeukaryotes that drive key biogeochemical cycles (Christoffersen et al. 2008, Quesada et al. 2008). Nevertheless, their ecology and biodiversity especially in alpine regions remain largely unknown (Tiberti et al. 2020, Fodelianakis et al. 2021). The relatively simple trophic structure in such lakes limits top-down control and complex biotic interactions (Cary et al. 2010, Tiberti et al. 2020) simplify-

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ing the correlation with environmental parameters. Moreover, microorganisms have short generation cycles and are, thus rapid indicators of environmental change. Benthic and sediment microbial community composition and physiology are, thus ideal indicators for environmental changes in polar and alpine habitats (Pearce 2005). However, benthic communities have been neglected in alpine studies (Rott et al. 2006, Ren et al. 2019) and their response to environmental changes in alpine lakes during glacial receding has not been studied to date.

Glacial meltwater provides constant but cold conditions in glacier-fed alpine lakes. Suspended sediments from glaciers increase the albedo-effect and, therefore, decrease light penetration and the extent of the euphotic zone (Elser et al. 2020), which limits primary productivity in glacier-fed lakes (Slemmons et al. 2013, Tiberti et al. 2020). Suspended sediments, however, also provide protection from harmful UV radiation especially at high altitudes (Tartarotti et al. 2017, Elser et al. 2020). Due to the lower albedo (Elser et al. 2020) and a missing inflow of cold glacial meltwater, nonglacier fed lakes usually show a relatively warmer temperature than glacier-fed lakes (Tiberti et al. 2020) and organisms therein are exposed to higher UV radiation (Elser et al. 2020). Contrary to expectations, high latitude and high-altitude lakes and ponds can reach relatively high temperatures of up to 20°C and may have standing stocks of biomass that are not necessarily smaller than in temperate lakes (Vincent et al. 2009, Kleinteich et al. 2012). In fact, temperatures above 0°C under the ice in winter may sustain a higher abundance and diversity of life compared to the surrounding terrestrial habitats that are exposed to much lower temperatures (Vincent et al. 2009). Temperature seems to be a major driver of community diversity and physiology in polar and alpine aquatic systems and ecological consequences of temperature increase in polar and alpine freshwater habitats were demonstrated in a number of studies (Sommaruga-Wögrath et al. 1997, Pringault et al. 2001, Velázquez et al. 2011, Kleinteich et al. 2012).

Glacier meltwater and suspended sediments therein are regarded as a source of carbon, iron and nutrients (Singer et al. 2012, Slemmons and Saros 2012), and thus contribute to the nutrient pool in an otherwise nutrient-poor environment (Sommaruga 2014, and references therein). Nutrients stem from atmospheric deposition on the glacier, glacial abrasion of phosphorus-rich substrates, or biological accumulation in glacier-associated habitats (Ren et al. 2019). Decreasing glaciers may, thus not only impact the quantity but also the stoichiometry of supplied nutrients in downstream habitats, albeit this seems to vary geographically (Elser et al. 2020). Lakes receiving glacier runoff were shown to have higher concentrations of nitrate and total dissolved phosphorus than lakes without glacial inflow (Saros et al. 2010, Peter and Sommaruga 2016, Warner et al. 2017). In the latter study, turbidity (i.e. suspended sediments) correlated with a higher planktonic bacterial diversity, albeit a decrease in metabolic functionality (Peter and Sommaruga 2016). Similarly, in a study by Slemmons and Saros (2012), higher reactive nitrogen concentration caused higher primary productivity in turbid than in clear lakes.

In summary, modified glacier runoff impacts a variety of hydrological and physico-chemical parameters and can, thus change downstream ecology, representing a potential bottleneck for the established local diversity (Peter and Sommaruga 2016). Due to the above-described sensitivity of high alpine lake ecosystems and the indicator function of benthic and sediment microbial communities, we hypothesize that benthic microbial community composition and physiology in high mountain lake sediments is impacted by the disappearance of glaciers and the resulting loss of glacier meltwater inflow. We tested this hypothesis by compar-

ing sediment-associated bacterial communities of 16 proglacial lakes located at three alpine glaciers in Switzerland that either receive glacial meltwater or lost connectivity to the glacier in the past 150 years. We correlated biological data with chemical and physical lake properties to identify the main drivers for observed biological and physiological changes.

Methods

Study site

Sediments and water of 16 lakes located in three glacier forefields in the Swiss Alps, namely the Joeri and the Silvretta glacier close to Davos, and the Tambo glacier, close to the Splügen pass (Table S1, Supporting Information) were analyzed. Field trips were conducted in August and/or September in each of the years 2016, 2017, 2018, and 2019. All water and sediment samples were collected and measurements were taken close to the lake shore in a water depth of 10–30 cm as well as in the run-off of the glacier. All three glaciers have decreased in size in the past 150 years (compare time shift function at <https://map.geo.admin.ch/>). In 2020, the Joeri as well as the Tambo glacier have almost disappeared. In each glacier forefield, several lakes have formed in a chronosequence during glacial retreat and are interconnected by small streams (Figure S1, Supporting Information). During glacier retreat several of the lakes have lost hydrological connectivity to the glacier melt-flow (disconnected) and are exclusively fed by precipitation and groundwater or thawing permafrost. Environmental properties of the 16 sampled lakes are summarized in Table S1 (Supporting Information). The lakes are located at an altitudinal gradient from 2300 to 2750 m a.s.l. and vary in size between 117 and 94 000 m² of surface area. Topographic data and minimum lake age were derived from swisstopo <https://map.geo.admin.ch/>. Based on the time shift function of that tool, minimum lake age and minimum time since disconnected was estimated as difference between the year of first occurrence on the maps and the year 2016 when the study started.

Lake types

A total of three categories of lakes are distinguished in relation to their hydrological connectivity with glacial meltwater: six lakes had a direct inflow (with max one lake in between) of glacial meltwater (“glacier-fed lake”), seven lakes were hydrologically disconnected from the glacier (“disconnected lakes”), and three lakes were located further downstream and received inflow from both of the above-described lake types (“mixed lakes”). While glacier-fed lakes were highly turbid and appeared in bright turquoise color, isolated lakes were clearer and had a dark blue color. Lakes located at a higher altitude have usually formed more recently, are shallower and have a high abundance of benthic phototrophic communities (Julia Kleinteich, personal observation). Deeper and older lakes (i.e. Joeri Lakes 1 and 3) located at lower altitudes are known to contain phyto- and zooplankton (Hinder et al. 1999) as well as fish that are stocked up by the local fishing community.

Water parameters

Physical parameters (water temperature, conductivity, pH, dissolved oxygen, and turbidity) were recorded *in situ* with portable sensors. For the analysis of chemical parameters lake water was sampled in triplicates. Filters (VWR™ 0.22 µm PES syringe filters) were rinsed with lake water on site and the filtrate stored in sterile plastic tubes. Dissolved organic carbon (DOC) was measured on an elemental vario TOC CUBE, nitrate, nitrite, and am-

monium were measured using the Seal Analytical Auto Analyzer 3, and inorganic ions (including nitrate, nitrite, ammonium, and phosphate) were measured with a DIONEX DX120 ion chromatograph. Total bound nitrogen (TN_b), total phosphorus (TP), and orthophosphate phosphorus (orthoP) were measured by Eurofins Institut Jäger GmbH according to ISO norms (DIN 12260, 6878, and 17294–2).

Temperature dynamics

Summer temperature dynamics were recorded using temperature loggers (iButton Measurement Systems Ltd, Newbury, UK) that were placed at the sediment surface in various lakes of the Jöeri catchment at a similar depth (~20 cm) and covered by stones to protect them from direct sunlight. The sensors recorded water temperature in 20-min intervals between 26 August 2016 and 14 September 2016 in lakes 1, 2, 16, 17, and 23 and in 30-min intervals between 15 August 2019 and 11 September 2019 in lakes 12, 14, 16, 17, 18, 23, and 24.

Benthic chlorophyll

To estimate benthic primary productivity (chlorophyll concentration), we sampled sediment and attached phototrophic communities in a standardized area of 5 cm² in triplicates by placing a metal ring on the sediment and using a sterile spatula to scoop up the top 1 cm layer into a sterile bag. The triplicate samples were located at least in 1 m distance at a depth of approximately 10 cm at the lake shores. The samples were stored cool and in the dark and were stored frozen at –20°C within 8 h after collection. Chlorophyll concentration of triplicate sediment material was determined based on cold acetone-extraction and spectrophotometry. Sediment material was freeze-dried and a preweighted amount of dried material was covered with 37 ml of ice-cold acetone (90:10 (v/v) acetone:water) saturated with MgCO₃. Samples were shaken for 1 h and ultrasonicated on ice for 10 min. After incubation over night at –20°C the samples were thoroughly vortexed and centrifuged at 2000 r/m for 5 min. The supernatant was decanted into a new tube. Absorption was measured using a spectrophotometer (VARIAN Cary 50 Bio UV–visible spectrophotometer) at 665 and 750 nm wavelength before and after acidification with 5 drops of 0.1 N HCl. Chlorophyll and phaeophytin content were calculated based on Lorenzen (1967).

Sediment parameters

Sediment cores were taken in duplicates (2016) or triplicates (2017) at the lake shores in at least 1 m distance from each other using a sterile syringe (60 ml) with a cut-off front end resulting in approximately 5–10 cm long cores. Cores were sealed and stored individually in sterile plastic bags. Cores were transported cool and in the dark and were stored frozen at –20°C within 36 h after collection. In the laboratory, sediment cores were thawed, removed from the plastic tubes, and transferred to sterile petri dishes. Each core was longitudinally cut into two halves using a sterile scalpel. Sediment (0.1–8 g) for DNA extraction was sampled from a complete longitudinal transect of the center of the halved core using a sterile spatula. The material was placed in a microcentrifuge tube and stored frozen at –20°C until further use. The remnants of the sediment cores (2016 samples only) were transferred in a 50 ml centrifugation tube and centrifuged at 2500 g for 10 min to separate solid and liquid phase for pore water collection. Porewater was decanted, filtered (Milli-Q prewashed 0.45 μm MCE Merck Millex™-HA Sterile Syringe Filter Unit) and DOC was measured as described above. The remaining sediment was dried on

a sand bath for 48 h and ground to a fine powder (planetary mill). The total organic carbon (TOC) in the sediment was measured by titration with HCl and subsequent analysis on an elemental vario TOC CUBE.

DNA extraction, amplification, sequencing, and bioinformatics

DNA was extracted from the thawed and homogenized (grinding with sterile pastel) material from duplicate (2016 samples) or triplicate (2017 samples) sediment cores using the PowerSoil DNA extraction Kit (Qiagen, Germantown, USA). DNA concentrations were determined using NanoDrop™ Spectrophotometer ND-1000 (Thermo Fisher Scientific, Waltham, USA). Bacterial community composition in lake sediments was determined by genetic fingerprinting as well as high-throughput amplicon sequencing as follows: ARISA (Automated Ribosomal Intergenic Spacer Analysis) was performed on duplicate core samples from 2016 as described previously (Kleinteich et al. 2014). Specifically, polymerase chain reactions (PCRs) were performed in technical duplicates with 20 ng of DNA and the Q5 Hot Start polymerase (New England Biolabs, Ipswich, USA) in a 2-strep protocol on a BIORAD S1000 thermal cycler as follows: 98°C 2 min [98°C 25 s, 70°C 30 s (10 cycles)/20 s (25 cycles), 72°C 50 s] × 35, 72°C 2 min. The quality of the product was checked on an ethidium-bromide stained agarose gel. Technical duplicates of each sediment core were combined and purified using the Monarch PCR purification kit (New England Biolabs). Purified PCR products were normalized to 100 ng/μl and sent to Eurofins Genomics (Ebersberg, Germany) for fragment length analysis. ARISA fragment length data were manually sorted, i.e. double peaks and peaks with a fluorescent signal lower than 100 or a shorter read length than 100 base pairs (bp) were removed. The data were subsequently transformed into a presence/absence classification.

For high-throughput amplicon sequencing, 50 ng DNA extract of each replicate core was combined. Of this replicate mix 15 ng of DNA was used for amplicon PCRs targeting the V3–4 variable regions of the 16S rRNA gene using the primer pair F319 and R806 (Fadrosh et al. 2014) and the Q5 polymerase (New England Biolabs) with addition of BSA (bovine serum albumin) and DMSO (dimethyl sulfoxide). PCR conditions were as follows: 98°C 2 min [98°C 15 s, 63°C 15 s, 72°C 18 s] × 20, 72°C 2 min. In a second-step-PCR, tags and barcodes were attached to the amplicons and used for High-Seq Illumina Sequencing. The second-step PCR and sequencing were performed by a sequencing company (Microsynth, Germany and Switzerland).

Bioinformatic analysis of Illumina sequencing data was performed on raw sequences using the Lotus v 2.00 Pipeline (Özkurt et al. 2021) that includes clustering, chimera and singleton removal resulting in 33–115 k sequences per sample. Amplicon sequence variants (ASVs) were clustered on a high level of similarity using the DADA2 Pipeline (Callahan et al. 2016) and taxonomically assigned by alignment to SILVA (Quast et al. 2013) using a least common ancestor (LCA) approach (Bedarf et al. 2021). ASVs assigned to chloroplast, archaea, and those without assigned phylum were removed manually. Data were rarefied to the lowest number of reads using the R package rarefaction tool kit (RTK) (Saary et al. 2017).

Faprotax analysis

Based on the created 16S rRNA gene ASV table and assigned taxa we assigned physiological traits with the faprotax pipeline (Louca et al. 2016). It assigns functions based on the identified taxa and

on a limited reference database. This approach uses maximum likelihood estimates, but cannot be as accurate as metagenomic sequencing and direct identification of functions from sequence reads. Significant differences between pathways occurring in lake types on log+1 transformed data were calculated with a 2-way ANOVA ($P < .05$) using GraphPad Prism.

Data analysis

Sequencing data were submitted to NCBI under accession (BioProject) number PRJNA801858 (Kleinteich 2022b). Environmental and ARISA data were deposited at the eScience-Center of the University of Tübingen, and are accessible under <http://hdl.handle.net/10900.1/2cd2fcc2-9b23-40b6-8c2f-6de56ec109b0> (Kleinteich 2022a). Statistical calculation and comparison of environmental parameters between the three lake types (glacier-fed, disconnected from the glacier, and mixed lakes) was performed with the software GraphPad Prism (version 8.1.0) and using a two-way ANOVA ($P < .05$) on log+1 transformed data and Tukeys' post-test. All measurements collected for each lake type were considered as replicates in this analysis. The same software was used to correlate environmental parameters and lake properties by calculating Pearson r ($P < .05$). In this case, only data pairs (i.e. measurement of the same lake and date) were correlated.

Numerical ecology calculations were conducted in R version 4.0.3. Alpha diversity indices (Richness, Chao1, Shannon, and Simpson) of ARISA, and NGS data were calculated using the vegan package in R. Statistical comparison of alpha diversity indices between lake types was calculated using GraphPad Prism and a two-way ANOVA as described for environmental parameters. Linear correlations of alpha indices with environmental parameters were calculated using GraphPad Prism. Microbial community composition (beta-diversity) between lake types and statistical analysis perMANOVA were calculated based on a Bray–Curtis dissimilarity matrix in R. A canonical correspondence analysis was performed and plotted with the software PAST on the 1000 most abundant ASVs and six environmental parameters, that were selected from the environmental dataset based on their relevance for ecological processes in aquatic environments. To estimate the relation between microbial composition and environmental parameters, a Mantel test (999 permutations) was calculated between ASV beta-diversity (Bray–Curtis) and a Euclidian distance matrix of all environmental and physico-chemical parameters using R. Relative abundances of individual phyla were compared and statistically tested using GraphPad Prism and a one-way ANOVA with Tukey's post-test ($P < .05$).

Results

In the 16 lakes, physical and chemical properties were recorded in the water column, pore water, and sediment in the months of August and September between 2016 and 2019 (Table 1). Turbidity was highest in glacier-fed lakes ($94(\pm 123)$ NTU) while lakes with mixed inflow ($24(\pm 29)$ NTU) and disconnected lakes ($1.5(\pm 1.8)$ NTU) were significantly lower in turbidity (Table 1; 2-way ANOVA and Tukeys post-test, $P < .01$). These differences were caused by the suspended sediment load in the glacier run-off that had the highest turbidity measured (up to 900 NTU). Turbidity decreased with increasing flow distance to the glacier (Figure S2, Supporting Information; Pearson r , $P < .01$) due to sedimentation of suspended particles. Also, conductivity was significantly lower in disconnected compared to glacier-fed and mixed lakes. In accordance with that, most ions were lower concentrated in the water

of disconnected lakes compared to glacier-fed or mixed lakes (Table 1). This was statistically significant for sulfate, magnesium and calcium. As can be expected, lake water conductivity correlated positively with ion concentrations (Figure S2, Supporting Information; significant for nitrate, sulfate, sodium, magnesium, and calcium) as well as with pH. Lower conductivity and ion concentration may be due to the relatively larger influence of ion-poor water from precipitation in disconnected lakes. This was supported by a negative correlation of conductivity and the time of separation of the lakes to the glacier (Figure S2, Supporting Information; Pearson r , $P < .01$). Dissolved oxygen and pH did not differ significantly between the three lake types.

In situ water temperature was slightly elevated in isolated lakes compared to glacier-fed lakes ($10.2(\pm 3.5)^\circ\text{C}$ and $8.3(\pm 2.8)^\circ\text{C}$, respectively) at the day of sampling, but this difference was not statistically significant. Nevertheless, *in situ* water temperature correlated significantly with flow distance and lake age, but decreased with higher altitude and turbidity (Figure S2, Supporting Information; Pearson r , $P < .01$). Seasonal and continuous records (Joeri catchment, mid-August to mid-September in 2016 and 2019, Fig. 1) showed considerable day–night temperature fluctuations of up to 15°C and overall temperature variations between 0 and 19.5°C depending on weather conditions. Isolated lakes showed significantly elevated temperatures compared to glacier-fed lakes at similar altitudes (Mixed-effects model, $P < .001$, Fig. 1). Disconnected Lake 16 was in 2016 on average 3.1°C and in 2019 5.2°C colder than glacier-fed Lake 17, i.e. located at a similar altitude. An exception from this pattern were glacier-fed Lake 24 and disconnected Lake 23, which did not show a significant difference, since the latter received cold water inflow from permafrost layers and showed continuously low temperatures.

The lakes were generally low in nutrients. Ortho-phosphate and TP in lake water were around or below the limits of detection (< 0.01 and 0.02 mg/l) and slightly higher only in glacial run-off (0.01 and 0.035 mg/l). DOC in lake water was similar in all three lake types with ~ 1 mg/l DOC and a range of 0.4 – 4.8 mg/l among all samples. Dissolved inorganic nitrogen (NH_4^+ , NO_3^-) in lake water was on average between 0.1 and 0.2 mg/l ΣNH_4^+ and NO_3^- in all lake types. Our data show that the glacier was a source of inorganic nitrogen with $0.23(\pm 0.12)$ mg N/l measured for NO_3^- and NH_4^+ in the glacier run-off (Table 1). With increasing distance from the glacier, lower altitude and older lake age, NO_3^- in lake water significantly decreased (Figure S2, Supporting Information). TOC and total nitrogen (TN) in the sediment, as well as DOC in the pore water of the sediment were not significantly different between the lake types, whereas TN in the pore water was significantly higher (Table 1; $P < .01$, 2-way ANOVA) in mixed lakes than in the other two lake types.

Benthic primary productivity (chlorophyll concentration) was not significantly different between glacier-fed and disconnected lakes (Table 1; 2-way ANOVA and Tukeys post-test, $P > .05$), but dead phototrophic biomass (pheophytin concentration) was significantly higher ($P < .05$) in disconnected than in glacier-fed lakes. Lakes of the mixed type, that were generally older and located at lower altitudes, seemed to have a generally higher primary productivity (Table 1). However, this could not be tested statistically due to the low number of replicates. Neither chlorophyll nor pheophytin concentration were significantly correlated to environmental parameters (Pearson r , $P > .05$, data not shown). In some glacier-fed lakes such as Lake 24 at Joeri as well as at Lake B at Tambo, we observed thick cyanobacterial-dominated benthic biofilms that were detached from the sediment and floating in the lake. They were not included in the sediment cores and,

Table 1. Physico-chemical parameters [average \pm standard deviation (SD)] in water, pore water, or sediment according to the different lake types. All measurements were taken in August or September in the years 2016–2019. Values refer to lake water samples if not indicated otherwise. Statistically significant differences (two-way ANOVA, Bonferroni corrected, $P < .05$) of log-transformed ($\log x+1$) data are shown between ^aglacier-fed and disconnected, ^bglacier-fed and mixed, and ^cdisconnected and mixed lakes. Glacier run-off was not included in the statistical test. n.a.—not applicable, +SD cannot be calculated due to low number of replicates. D/TOC—dissolved/total organic carbon. TN—total nitrogen. AFLs—ARISA fragment lengths, and ASV—amplicon sequence variant.

Water parameters	Glacier-fed	Disconnected	Mixed	Glacier run-off
Turbidity [NTU] ^{a,b,c}	93.6(\pm 123)	1.5(\pm 1.8)	24.3(\pm 28.7)	364(\pm 318)
Conductivity [μ S/cm] ^{a,c}	47.1(\pm 15.7)	25.8(\pm 12.8)	37.5(\pm 7.4)	40.5(\pm 35.5)
pH [-]	8.9(\pm 1.4)	8.15(\pm 1.2)	7.9(\pm 0.3)	8.1(\pm 1.1)
Oxygen [mg/l]	9.2(\pm 0.7)	9.4(\pm 0.7)	8.9(\pm 0.5)	10.9(\pm 0.4)
Oxygen [%]	110(\pm 3.9)	115(\pm 10.9)	112(\pm 6.0)	109(\pm 3.5)
In situ temperature [$^{\circ}$ C]	8.3(\pm 2.8)	10.2(\pm 3.5)	10.4(\pm 2.6)	0.9(\pm 0.8)
Fluoride [mg/l]	0.14(\pm 0.06)	0.12(\pm 0.04)	0.36(\pm 0.11)	0.13(\pm 0.04)
Chloride [mg/l]	0.16(\pm 0.18)	0.18(\pm 0.30)	0.12(\pm 0.04)	0.09(\pm 0.02)
Sulphate [mg/l] ^{a,b,c}	3.74(\pm 2.19)	2.27(\pm 3.04)	6.17(\pm 2.39)	6.27(\pm 3.36)
Sodium [mg/l]	0.77(\pm 0.31)	0.54(\pm 0.29)	0.67(\pm 0.12)	0.78(\pm 0.54)
Potassium [mg/l]	0.53(\pm 0.20)	0.40(\pm 0.42)	0.58(\pm 0.41)	0.60(\pm 0.16)
Magnesium [mg/l] ^a	1.19(\pm 0.42)	0.38(\pm 0.24)	0.55(\pm 0.21)	1.67(\pm 0.87)
Calcium [mg/l] ^{a,c}	8.25(\pm 1.77)	3.56(\pm 1.99)	5.58(\pm 1.01)	9.15(\pm 6.46)
DOC [mg/l]	1.00(\pm 0.81)	1.09(\pm 0.67)	1.27(\pm 1.76)	1.14(\pm 0.79)
TN _b as N [mg/l]	< 0.5	< 0.5	< 0.5	< 0.5
NO ₂ as N [mg/l]	< 0.005	< 0.005	< 0.005	< 0.005
NH ₄ as N [mg/l]	0.02(\pm 0.04)	0.02(\pm 0.02)	0.02(\pm 0.00)	0.23(\pm 0.12)
NO ₃ as N [mg/l]	0.18(\pm 0.08)	0.11(\pm 0.08)	0.10(\pm 0.01)	0.23(\pm 0.06)
TP as P [mg/l]	< 0.02	< 0.02	0.01 ⁺	0.035 ⁺
PO ₄ as P [mg/l]	0.017 ⁺	< 0.01	< 0.01	0.01 ⁺
Sediment parameters				
TOC sediment [% dry weight]	0.12(\pm 0.04)	0.45(\pm 0.19)	0.23(\pm 0.07)	n.a.
TN sediment [% dry weight]	0.011(\pm 0.004)	0.029 (\pm 0.012)	0.021(\pm 0.008)	n.a.
DOC pore water as C [mg/l]	46.5(\pm 39.4)	25.1(\pm 19.7)	35.0(\pm 15.7)	n.a.
TN pore water as N [mg/l] ^{b,c}	2.05(\pm 0.42)	2.84(\pm 2.03)	7.10(\pm 1.97)	n.a.
Benthic chl.-a [μ g/cm ²]	7.8(\pm 2.6)	8.6(\pm 3.2)	11.9 ⁺	n.a.
Benthic phaeophytine [μ g/cm ²] ^{a,b}	4.8(\pm 0.9)	13.1(\pm 6.9)	12.2 ⁺	n.a.
Richness fingerprinting [# AFLs]	17(\pm 5.7)	24(\pm 6.4)	23(\pm 10.4)	n.a.
Chao1 fingerprinting [-] ^{a,b}	168(\pm 50)	313(\pm 79)	329(\pm 140)	n.a.
Richness sequencing [# ASVs]	1600(\pm 150)	1598(\pm 108)	1984(\pm 53)	n.a.
Shannon sequencing [-]	5.6(\pm 0.46)	5.6(\pm 0.34)	5.9(\pm 0.19)	n.a.

thus in this analysis. Benthic primary productivity in these lakes may, therefore, be underestimated. This is supported by a relatively high pH of > 9 in those lakes (individual data not shown), i.e. likely caused by high rates of photosynthesis.

In the lake sediment, alpha-diversity indices of the microbial community did not differ between the three lake types for the dataset generated by high-throughput sequencing. However, the Chao1 index as a measure of species richness of the fingerprinting dataset was significantly lower in glacier-fed lakes compared to disconnected and mixed lakes (Table 1; $P < .05$). Linear correlation showed that Shannon diversity (NGS data) of bacterial taxa in the sediment decreased significantly with increasing altitude (Fig. 2). Even though there was a positive trend of Shannon diversity and lake age especially among glacier-fed lakes this correlation was not significant. Alpha-diversity did not correlate with any of the other environmental and physico-chemical lake properties (data not shown).

Bacterial community composition was significantly different in glacier-fed and disconnected lakes based on NGS results (Bray–Curtis similarity and perMANOVA $P < .001$, Bonferroni corrected, Fig. 3). Bacterial communities in glacier-fed lakes showed a high homology to each other, reflected by the close cluster in the canonical correspondence graph (Fig. 3). In contrast, bacterial

community composition in disconnected lakes was more variable and clustered more loosely. Those lakes that had been disconnected from the glacier only recently (< 20 years) such as Lake 23 at Joeri were relatively more similar to glacier-fed lakes than other lakes in the same category. Community composition in lakes receiving water from both catchments was more similar to disconnected than to glacier-fed lakes and was significantly different only to the latter (Bray–Curtis similarity and perMANOVA $P < .001$, Bonferroni corrected).

Albeit our samples originated from three distant glacial catchments, we did not observe a significant difference in the microbial community composition between these sites (Bray–Curtis similarity and 2-way perMANOVA $P < .01$ on lake type and catchment). The latter suggests that environmental factors were more important for bacterial community composition than the geographic location. A Mantel-test confirmed that environmental and physico-chemical parameters correlated with microbial community composition (based on Bray–Curtis, $P < .01$). In glacier-fed lakes microbial community composition correlated strongest with a high turbidity, conductivity and nitrate (Fig. 3). Microbial community composition in disconnected and mixed lakes covered a broader habitat range and was correlated with an older lake age, as well as warmer temperature (Fig. 3). In contrast to the above described

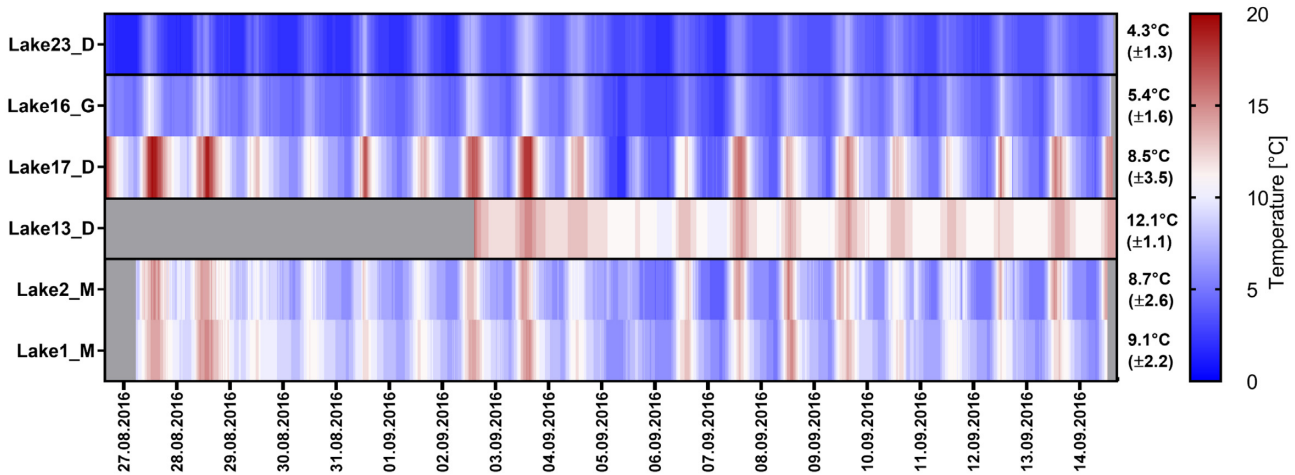
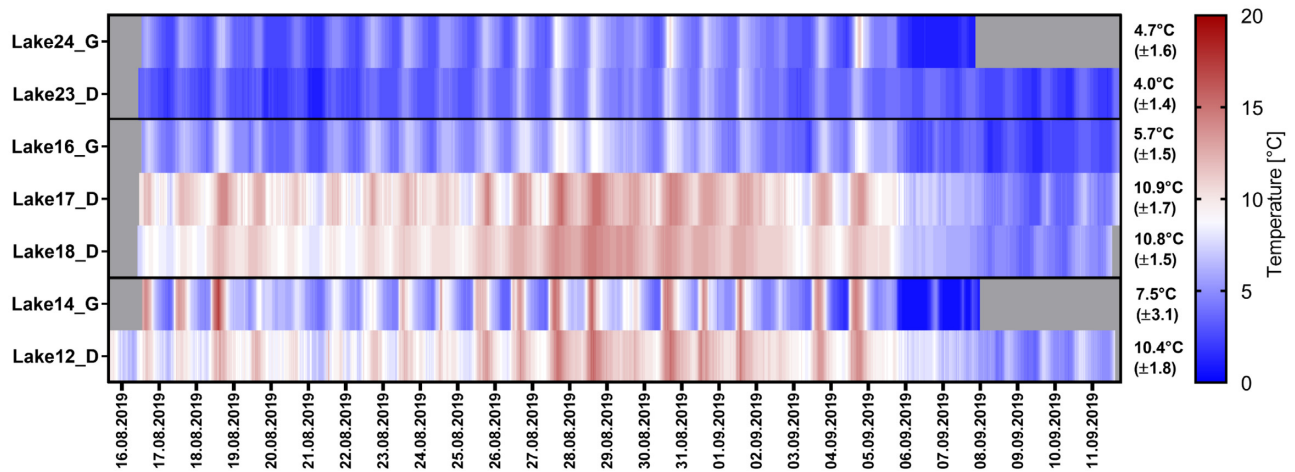
(A) Lake water temperature 2016**(B)** Lake water temperature 2019

Figure 1. Seasonal summer temperature records [°C] in selected lakes of the Joeri catchment in (A) 2016 and (B) 2019, recorded between the mid of August and mid of September. Lakes are arranged according to their relative altitude with lakes located at higher altitudes being displayed further up in the graph. Lakes located at similar altitudes are surrounded by a black box. Gray areas = no records. Average temperature and standard deviation are displayed at the right side of each lake temperature record. The data show a general temperature gradient from the warmer water temperature at lower altitude to colder water temperature at higher altitude. Lakes at a similar altitude with active glacial meltwater inflow (G-lakes) or mixed lakes (M-lakes) are distinctly colder than disconnected lakes without meltwater inflow (D-lakes).

parameters, surface area, depth, or altitude only had a relatively small influence on beta-diversity and are, thus not displayed in Fig. 3.

In all lake types, proteobacteria were the most abundant phylum, followed by Chloroflexi, Acidobacteria, and Bacteroidetes (Fig. 4). Chloroflexi were significantly less and Proteobacteria more abundant in glacier-fed compared to disconnected lakes. Nevertheless, the overall relative abundance on phylum level did not show major differences between the three lake types. The most abundant ASVs in the dataset belonged to the aerobic and chemoheterotrophic genus *Sphingomonas* of the Proteobacteria that was 4.8 times more abundant in glacier-fed compared to disconnected lakes (Table S2, Supporting Information). Another abundant Proteobacterium genus *Arenimonas* was 14 times more abundant in glacier-fed compared to disconnected lakes. The difference in Chloroflexi between lake types was mostly due to the third and fourth most abundant ASVs that could not be identified further. Both of these ASVs were almost absent in glacier-fed lakes but

highly abundant in disconnected lakes. Also different types of cyanobacteria were detected. In glacier-fed lakes, *Pseudanabaena* was the most abundant genus, while *Leptolyngbya* and *Tychonema* were more abundant in disconnected lakes.

We used our 16S rRNA data to compare assigned taxa with known physiological pathways using Faprotax (Louca et al. 2016). This analysis suggested that lake types varied in some of their key physiological pathways (Fig. 5). Chemoheterotrophy was the most common physiological pathway we detected in all lake types, but it was significantly elevated in glacier-fed lakes (Two-way ANOVA and Tukeys post-test $P < .05$). In disconnected lakes physiological pathways related to cyanobacteria and photosynthesis were significantly elevated compared to mixed lakes, suggesting that phototrophic organisms were abundant in sediments of lakes without connection to the glacier and corroborating above described results about higher pheophytin content in disconnected lakes. As can be expected in a nitrogen-poor environment, we found a high proportion of nitrogen fixation pathways in all lake types,

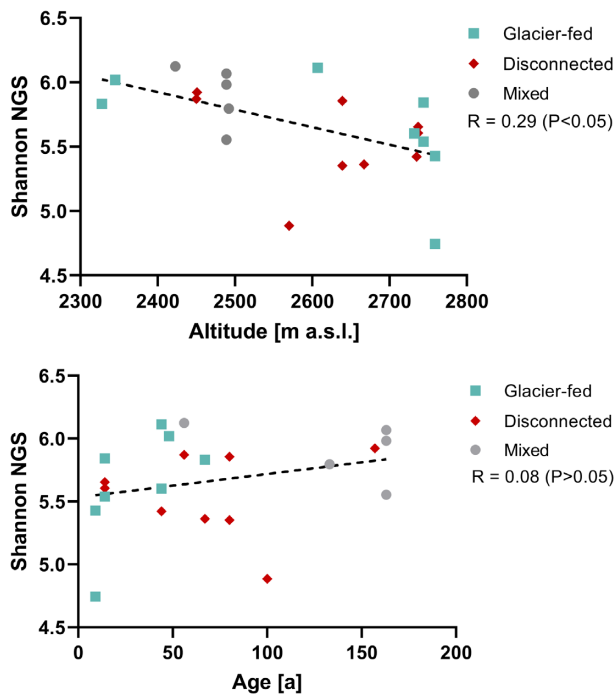


Figure 2. Correlation of Shannon diversity based on Illumina amplicon sequencing data from sediments of glacier-fed (blue), disconnected (red), and mixed (gray) alpine lakes with lake altitude in meters above sea level and estimated age of the lake. Shannon diversity decreased significantly in lakes at higher altitudes. The correlation with lake age was not significant but the overserved trend of increasing diversity with lake age was stronger for glacier-fed than for disconnected or mixed lakes.

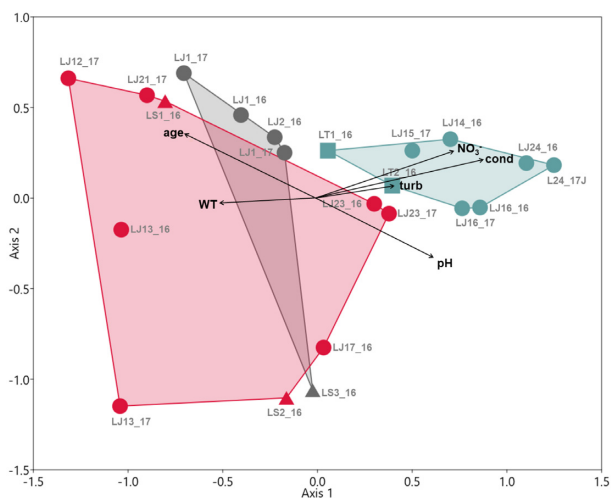


Figure 3. Canonical correspondence analysis of bacterial community composition based on Illumina amplicon sequencing data and environmental lake parameters; only the 1000 most abundant ASVs were considered. Glacier-fed lakes (blue) cluster separately from disconnected (red), and mixed lakes (gray). Conductivity, turbidity, nitrate, and altitude correlated with glacier-fed lakes, while disconnected lakes were correlated with temperature, flow distance and lake age. Environmental parameters were more important than geographical origin (circles—Joeri (LJ), triangles—Silvretta (LS), and squares—Tambo (LT)). Labels and numbering are according to Table S1 (Supporting Information) and additionally indicate the year of sampling. WT—water temperature, age—minimum lake age, turb—turbidity, and cond—conductivity. IDs of each lake can be found in Table S1 (Supporting Information).

but slightly lower in glacier-fed lakes, supporting above-described findings that the glacier acts as an external source of nitrogen for those lakes. Whereas nitrification was detected quite often, denitrification was rarely present. We also found the potential for alternative energy pathways such as respiration of sulfur compounds, or iron or manganese respiration. In glacier-fed lakes, fermentation, aromatic compound degradation, and iron respiration were observed to be significantly elevated compared to the other lake types. In contrast, methanotrophy was elevated in disconnected compared to glacier-fed lakes.

Discussion

The decline of glaciers in mountain regions worldwide will affect associated environments and organisms living therein, especially in downstream aquatic habitats (Brown et al. 2007, Brighenti et al. 2019, Tiberti et al. 2020). In this study, we used lake sediment microbial communities as in situ sentinels for changes associated with the loss of glacier meltwater inflow and, thereby gained a glance into the future of high alpine lakes in a potential post-glacial era. We could show that the melt-down of glaciers and associated changes in physico-chemical conditions will lead to the loss of a unique habitat and a specialized microbial community composition associated with mountain lake sediments in favor of a more diverse community with a broader habitat range.

When glaciers recede, new lakes form in the glacier forefield and lakes that formerly received glacial meltwater inflow become hydrologically disconnected from the glacier. We observed that this disconnection is associated with a distinct shift in physico-chemical parameters. As expected, turbidity along with conductivity and ion concentration were the most apparent differences between glacier-fed and disconnected lake types. Similar to our findings, Thies et al. (2013) found a higher electrical conductivity and ion concentration in streams originating from rock glaciers than from unaffected streams. In glacier-fed lakes of the present study, the constant flow of cold meltwater provided more stable, albeit cold temperatures. Disconnected lakes were more sensitive to external temperature fluctuations with higher temperatures during the day and lower temperatures during the night, but were in general warmer than glacier-fed lakes. Water temperature is an important factor for many physiological processes especially during the growth period (Elser et al. 2020). In mountain lakes, e.g. pH showed a response to a temperature increase indicating physiological changes (Sommaruga-Wögrath et al. 1997). Similarly, benthic microbial community composition and physiology in polar lakes and streams changed at elevated temperatures (Pringault et al. 2001, Velázquez et al. 2011, Kleinteich et al. 2012). In mountain streams, elevated temperature is expected to increase nutrient cycling and heterotrophic processes (Milner et al. 2009). It has been shown that with climate change high alpine and polar lakes show a disproportional temperature increase in water-to-air ratio, concurring with an elongation of the ice-free period (Sommaruga-Wögrath et al. 1997, Quayle et al. 2002, Adrian et al. 2009). It can be suspected that due to the reduced buffering with cold water inflow and a lower albedo, disconnected lakes and their associated communities may be more susceptible to such a climate change related temperature increase, especially in dry and warm summer months than glacier-fed lakes.

High alpine as well as polar aquatic systems are generally nutrient-poor and assumed to be limited by the availability of nitrogen but also phosphorus (Darcy et al. 2018b, Slemmons et al. 2013, Kohler et al. 2016). Glacial meltwater is described to be a source of nutrients (Saros et al. 2010, Slemmons et al. 2013). Pre-

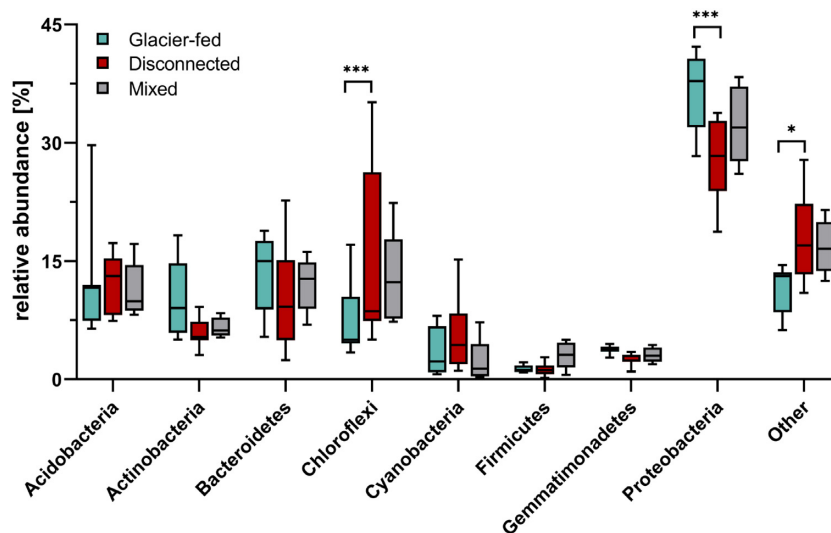


Figure 4. Relative abundance of bacterial phyla as observed by Illumina amplicon sequencing according to lake type. Bar charts represent mean, quartiles, and range. Proteobacteria show significantly higher (two-way ANOVA and Tukey's post-test) and Chloroflexi lower abundance in glacier-fed compared to disconnected lakes. Acidobacteria and Bacteroidetes were present at similar relative abundances in all lake types.

vious deposition and conservation of atmospheric nitrogen in the ice delivers nitrogen in glacial meltwater that can affect abundance and composition of present biological taxa (Saros et al. 2010, Kohler et al. 2016). Although nutrient concentrations in lake water were generally low in our study, TP and nitrate were slightly elevated in the glacial meltwater outflow as well as glacier-fed lakes supporting these previous studies. In addition, we observed a strong positive gradient in lake water nitrate concentration with increasing altitude. The same was observed in the Rocky Mountains for a cascade of glacier-fed lakes, but not in snow-fed lakes (Warner et al. 2017), suggesting that a glacier can indeed act as a source for nitrogen. The disconnection from its glacial inflow may, thus lead to a deprivation of nitrogen in a lake, which may be counteracted by nitrogen-fixing cyanobacteria as indicated in our study by a slightly higher abundance of nitrogen-fixation pathways in disconnected and mixed lakes. Moreover, older lakes at lower altitude had a lower concentration of nitrogen in the water phase, but a higher concentration of TN in the pore water of the sediment, suggesting that during the lake ageing, nutrients were transferred by biological processes from the water phase to the sediment. In general, nutrient-poor conditions support benthic over planktonic life (Bonilla et al. 2005, Cantonati and Lowe 2014) and benthic algal biomass was high also in nutrient-poor mountain lakes in another study (Saros et al. 2010). It can be suspected that sediment-bound nutrients and internal nutrient cycling in the sediment supported high biomass development as observed in some lakes.

The above-described changes in physico-chemical parameters were related to a distinct shift in the sediment-associated microbial community and suggests strong environmental selection pressures and unique habitat characteristics. In our study, microbial richness was lower and community composition was more similar between glacier-fed lakes than between disconnected lakes. While a high suspended sediment load provided nutrients in glacier-fed lakes, constant flow of cold and turbid water may pose challenging conditions for benthic communities in these habitats. Disconnected lakes in contrast, provide a habitat with warmer temperatures, albeit nutrient-poor and probably with high UV exposure and, thus light stress. Conditions in disconnected lakes may support the abundance of benthic graz-

ers that can impact microbial diversity (Camacho 2006). In addition, grain size may impact sediment microbial community composition. Nevertheless, neither benthic grazers nor grain size were assessed in the present study. Shifts in community composition associated with glacier-loss were also observed for alpine lake plankton (Sommaruga 2014, Peter and Sommaruga 2016, Warner et al. 2017), as well as stream and lake diatoms (Saros et al. 2010, Thies et al. 2013). Less relative influence of glacier water shifts seasonal peak flows, elevates water temperature and increases channel stability in alpine stream (Milner et al. 2009) and sustains more complex communities (Rott et al. 2006, Milner et al. 2009). While Peter and Sommaruga (2016b) observed a positive relationship between diversity and turbidity in lake bacteria, benthic diatom communities had a lower diversity in glacier-fed streams and lakes (Saros et al. 2010, Thies et al. 2013). Another study stated that phytoplankton communities in glacier-fed lakes were more similar to one another than their snow-fed counterparts (Warner et al. 2017, Ren et al. 2019). The latter supports the results of our study and also suggests that processes in relatively small lakes as studied here can be scaled up to habitats with a higher socio-economic impact.

Most lakes that became isolated from the glacier lacked a permanent in- or outflow. This isolation of lakes may have supported the higher variability in community composition detected there. Disconnected lakes seemed to act as more or less isolated environments in each of which a unique microbial community could establish, similar to isolated microbial communities in cryoconite holes (Darcy et al. 2018a). Lake type also affected bacterial physiology, with chemoheterotrophs being more abundant in glacier-fed and photoautotrophs in disconnected lakes. Even though benthic chlorophyll-a content did not differ between the lakes, this was corroborated by a higher pheophytin concentration in disconnected lake that suggest an accumulation of phototrophic biomass. Such functional shifts in a turbidity gradient were also observed in lake plankton that showed a decrease in metabolic activity with increasing turbidity (Peter and Sommaruga 2016). Similarly, more simple trophic food-webs were observed in glacier-fed vs. nonglacier fed lakes (Tiberti et al. 2020). Overall, this suggests that glacier-fed lakes provide narrow habitat conditions (i.e. tem-

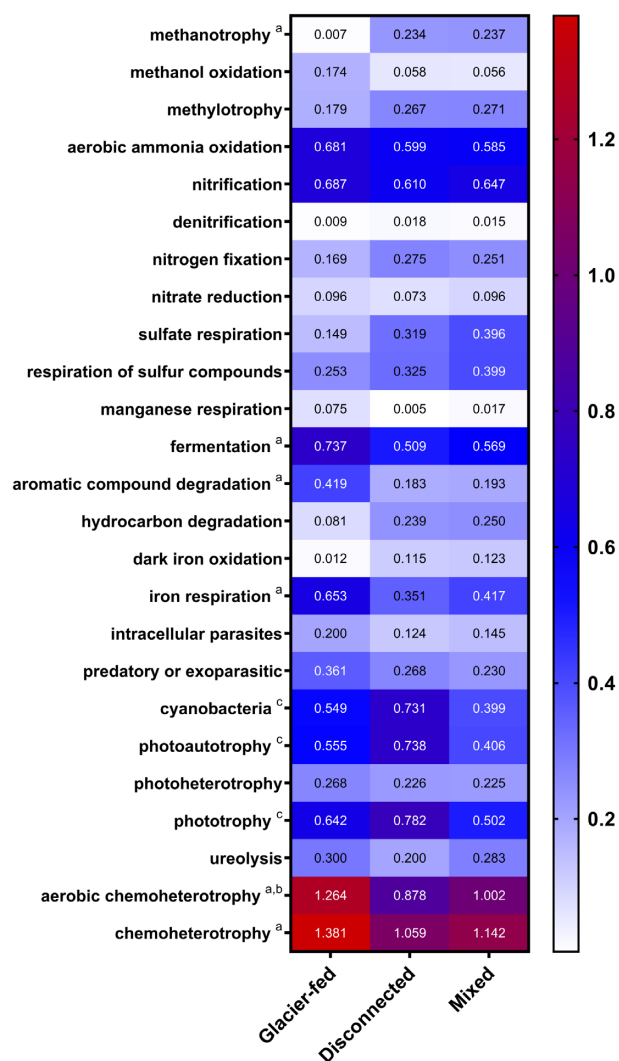


Figure 5. Physiological pathways in relative abundance [%] on log+1 transformed data and based on an assignment to identified taxa with FAPROTAX. Data are shown on a color gradient: blue = low genomic potential and red = high genomic potential for the particular lifestyle. For better visibility, pathways with an average abundance < 0.25% across all lake types were removed (except for denitrification due to its relevance in the nitrogen cycle).

perature and turbidity) and are inhabited by a unique but simplified community, while disconnected and isolated lakes show broader habitat range and a greater taxonomic and physiological diversity. It can be anticipated that when glaciers continue to disappear, we will observe a diversification in high alpine lake microbial communities at the expense of a local, unique, and highly specialized community.

Lake type (glacier-fed vs. disconnected) and environmental properties were more important for bacterial community composition than geographical distance even between distant catchments. Similar results were reported for glacial stream microbial diversity in the Rocky Mountains (Hotaling et al. 2019). The fact that habitat type was more selective than geographic distance supports our previous hypothesis that microbes in cold habitats are not dispersal limited (Kleinteich et al. 2017). Terrestrial and freshwater habitats in polar and high alpine regions are usually inhabited by similar groups of microbial organisms (Vincent 2000, Pointing et al. 2015, Biersma et al. 2017). Other studies concluded

that the same species of cyanobacteria, fungi, lichen, and mosses occur Arctic and Antarctic habitats (Zakhia et al. 2008, Cox et al. 2016, Biersma et al. 2017, Garrido-Benavent et al. 2017, Pessi et al. 2018). In line with those studies, several of the ASVs detected here showed high sequence similarity to taxa originating from the Arctic or Antarctic (data not shown). The degree to which high alpine and polar regions share the same microorganisms and how they disperse remains to be evaluated.

In this study, we could show that high alpine lakes, despite the harsh environmental conditions, are inhabited by a diverse set of sediment-associated microbial organisms that can be present in high biomass. Glacial retreat and disappearance and related changes in environmental parameters (i.e. suspended sediment and temperature) lead to a distinct shift in sediment-associated bacterial community composition, with impacts on their physiological function. It can be expected that when worldwide glacial melt-down continues, many high alpine, glacier-fed, and polar lakes will experience the observed loss of the unique microbial community composition that differs significantly from the composition in isolated and mixed lakes, but is similar across geographic regions.

Supplementary data

Supplementary data are available at [FEMSEC](https://femsec.org) online.

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Conflicts of interest. None declared.

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