Seasonal shifts in microbial diversity in the lakes of Fildes Peninsula, King George Island, Maritime Antarctica

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Abstract: Fildes Peninsula, on King George Island, has been greatly influenced by recent rapid climate warming. Lakes are pervasive features of Fildes Peninsula landscapes, some of which are used as water sources for Antarctic stations. We studied seven Fildes Peninsula lakes to explore differences among lakes and between seasons in phytoplankton and bacterioplankton communities. We measured environmental variables, analysed pigments using high-performance liquid chromatography and examined bacterial DNA through high-throughput sequencing of the 16S rRNA gene. The main driver structuring microbial communities was the season (i.e. spring *vs* autumn). Chlorophyceae were the dominant phytoplankton group in all lakes and both seasons. Indicator bacteria for each season were identified, including *Flavobacterium*, *Polaromonas* and Oxalobacteraceae as indicators of spring conditions under thick ice, whereas Frankiales and Verrucomicrobia were indicator species of autumn following the ice-free summer. The indicator species for spring are generally observed in oligotrophic conditions, whereas many of the autumn indicators are commonly found in soils. There were lesser between-lake differences in microbial communities in autumn, at the end of the open-water period, than in spring at the end of the ice-covered period. This study will act as the basis for future assessments of changes in aquatic microbial communities.

Introduction

The Antarctic Peninsula (AP) region has been subjected to some of the most rapid climate warming on Earth during the 20th and 21st centuries, with an increase of more than five times the global mean (0.6 ± 0.2°C during the 20th century; Turner *et al.* 2013). As part of the Maritime Antarctic, the AP region differs from the rest of the continent in many aspects including its climate, which is characterized by higher mean temperature and precipitation (Convey *et al.* 2014). Numerous ice-free areas with terrestrial habitats are distributed along the coastal fringes of the AP and surrounding islands, as well as in small areas protruding from the ice on the AP plateau (Oliva *et al.* 2017). Ongoing climate change in the region has occurred simultaneously with the intensification of human activities due to increases in scientific research and tourism that impose physical, chemical and biological burdens on the local environment (Schiffer 2013).

Lakes and ponds are common features of the ice-free areas of Maritime Antarctica. Although these lakes are not subject to the same harsh environments as those in Continental Antarctica, they experience pronounced annual cycles of solar irradiance and temperature caused by their high latitudes, and they are affected by ice and snow cover during much of the year (Quayle *et al.* 2003). The less extreme conditions from this region result in lakes that currently lose their ice covers completely each summer when they display a primary production peak, and they remain ice free typically from the end of December to March before refreezing in April (Izaguirre *et al.* 2021). During these ice-free periods, lakes interact with the atmosphere and receive runoff from local snowmelt and surrounding streams, as well as from permafrost thaw and precipitation (Vincent *et al.* 2008). These lakes are dominated by the microbial loop, including viruses, bacteria, phytoplankton and protozoa, generally represented by a few species of pigmented zooplankton (Vincent *et al.* 2008).

Much of our understanding of annual cycles in Maritime Antarctic lakes was developed from studies on Signy Island (South Orkney Islands), which showed
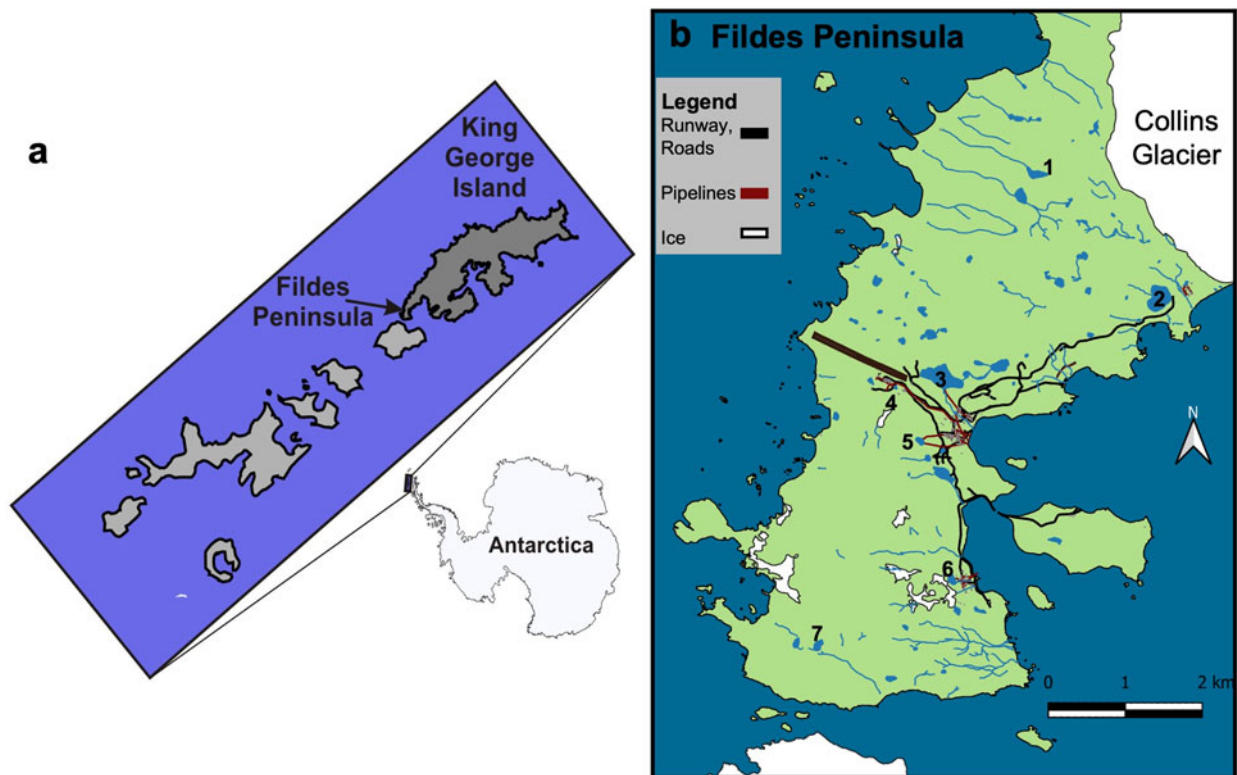
Fig. 1. Location of the study region. **a.** King George Island and Fildes Peninsula. **b**. Map of Fildes Peninsula indicating the study lakes: 1 = Mondsee, 2 = Uruguay, 3 = Kitiesh, 4 = Hotel, 5 = Las Estrellas, 6 = Xihu, 7 = Jurasico. Maps were created with geospatial data from the Scientific Committee on Antarctic Research (SCAR) Antarctic Digital Database, accessed 2021.

marked seasonal and interannual variations in planktonic population dynamics (Quayle *et al.* 2003). Limnological research is often focused near research stations, with well-studied lake clusters present near Hope Bay and Cierva Point on the AP (Izaguirre 2003, Schiaffino *et al.* 2009, Allende & Mataloni 2013), Byers Peninsula on Livingston Island (Toro *et al.* 2007), James Ross Island (Roman *et al.* 2019) and Potter and Fildes peninsulas on King George Island (Vinocur & Unrein 2000, Schiaffino *et al.* 2009, Zhang *et al.* 2022), where authors have studied phytoplankton, zooplankton and bacterioplankton dynamics. Rochera *et al.* (2017) recorded major changes in plankton communities on Byers Peninsula (Livingston Island) during the transition to the ice-free period when light availability and allochthonous nutrient fluxes were high. Studies from Byers Peninsula also examined bacterioplankton dynamics using next-generation sequencing (NGS), revealing the dominance of globally distributed freshwater bacterial classes, but also the presence of endemic clades (Picazo *et al.* 2019). However, bacterioplankton diversity studies employing this approach, which enables the detection of taxa with very low abundances, are not yet common in lakes of the AP region.

In this study, we focused on seven lakes on Fildes Peninsula, an area of King George Island (South

Shetland Islands). King George Island is among the areas of Antarctica most impacted by humans due the presence of 12 research stations constructed since 1968. Concern has been raised about environmental contamination near the stations, and studies have observed locally elevated concentrations of heavy metals due to transportation and oil pollution, as well as polycyclic aromatic hydrocarbons (Chu *et al.* 2019, Choi *et al.* 2022). The limited existing limnological research from Fildes Peninsula has focused largely on Lake Kitiesh due in part to its importance as a water supply (e.g. Montecino *et al.* 1991) or surveys of basic limnological parameters (e.g. Préndez & Carrasco 2003, Shevnina & Kourzeneva 2017), although one recent study applied NGS to the analysis of microeukaryote communities in five Fildes Peninsula lakes (Zhang *et al.* 2022). Finally, the only study of bacterioplankton diversity examined three ponds and one lake from Fildes Peninsula during five consecutive summers, employing a bacterial culture-dependent approach (Morel *et al.* 2015).

The aim of our study was to examine the phytoplankton and bacterioplankton communities of seven lakes from the Fildes Peninsula and to explore the compositional differences both among lakes and between seasons (spring and autumn).

Table I. Characteristics and variables measured in the surface water in the study lakes. NA: not available, ND: not detectable. Autumn ice thickness values are approximate. Differences in the means of the temperature, pH and specific conductivity between seasons were not significant ($P = 0.48$, $P = 0.22$ and $P = 0.56$, respectively). The difference in the mean of the total chl-a between seasons was significant ($P = 0.02$).

Lake	Latitude Longitude	Max. depth (m)	Surface area (ha)	Catchment area (ha)	Season	Temp (°C)	pH	Conductivity (μS cm^{-1})	Dissolved O$_2$ (%)	Ice thickness (m)	Snow thickness (m)	Total chl-a (μg L^{-1})	Total reads
Mondsee	62°10'21.50"S, 58°56'43.26"W	7.0	1.90	10.50	Spring	0.24	6.75	228	67.3	1.06	0.12	1.09	59877
					Autumn	0.64	7.69	172	NA	0.06	0.00	1.03	40944
Jurasico	62°13'00.0"S, 59°00'00.0"W	4.9	1.07	7.45	Spring	0.47	6.90	130	67	0.80	NA	0.24	37134
					Autumn	0.57	7.84	182	NA	0.50	0.00	0.58	49801
Uruguay	62°11'07.4"S, 58°54'39.6"W	15	7.03	18.66	Spring	0.43	7.30	108	55.9	0.96	0.02	0.36	52993
					Autumn	0.27	7.60	136	NA	0.00	0.00	0.66	43957
Kitiesh	62°11' 36.9" S, 58°58' 0.2" W	11	9.43	59.71	Spring	0.28	7.63	156	67.8	0.80	0.05	ND	58380
					Autumn	0.11	7.40	176	NA	0.20	0.00	1.28	35708
Hotel	62°11' 40.4" S, 58°58' 42.2" W	5.0	0.11	1.71	Spring	0.34	6.70	551	23.4	1.60	0.50	0.11	66507
					Autumn	0.52	7.46	461	NA	0.55	0.00	12.76	62617
Las Estrellas	62°12'2.5"S, 58°58'19.4"W	5.6	0.66	4.05	Spring	0.10	7.30	80.0	68.6	0.90	0.05	0.20	43515
					Autumn	0.64	8.15	167	NA	0.20	0.00	0.75	20254
Xihu	62°13'00.80"S, 58°57'56.41"W	10	0.98	39.32	Spring	0.13	7.94	300	69.4	0.95	0.05	0.20	47081
					Autumn	0.29	8.06	173	NA	0.20	0.00	0.82	53928

Materials and methods

Study region

At 38 km^2, Fildes Peninsula (62°11' S, 58°58' W) is the largest ice-free area of King George Island in the South Shetland Islands of the Maritime Antarctic region (Fig. 1a). The peninsula is the site of numerous lakes and is also among the Antarctic regions with the highest intensity of human activity, including six permanent stations. Fildes Peninsula also serves as the entry point and logistical hub for the South Shetland Islands, being the site of an airport and a harbourmaster station; most visitors to these islands and to the AP therefore pass through Fildes Peninsula.

This study focuses on seven lakes from around Fildes Peninsula (Fig. 1b & Table I). Five of them are or have been used as water supplies for the different stations and so are located adjacent to infrastructure and associated human activities (Fig. 1b). These include Uruguay Lake, which supplies Artigas Station (Uruguay); Kitiesh Lake, which is the source of potable water for the Escudero, Frei and Bellingshausen stations (Chile and Russia); Hotel Lake, which is adjacent to Teniente Marsh Airport and formerly was its water supply, and which has ceased to be used for drinking water due to elevated levels of heavy metals (Peter et al. 2013); Las Estrellas Lake, which previously supplied Escudero Station (Chile); and Xihu Lake, the source of drinking water for Great Wall Station (China). Two lakes more distant from stations were also studied: Mondsee Lake, located 3.5 km from Frei Station and 2.6 km from Artigas Station; and Jurasico Lake, located 2.1 km from Great Wall Station and 3.3 km from Escudero Station. The lakes are of glacial origin, having formed following the retreat

of Collins Glacier, which sits at the north-east extreme of the peninsula (Fig. 1b). They occur in catchments ranging from 1.71 to 59.71 ha and mostly occupy shallow basins, with depths ranging from 3 to 11 m, except for Uruguay Lake, which has a maximum depth of 15 m (Table I). The degree to which anthropogenic activities may have affected these lakes is unclear, as they are largely unstudied, and for some even basic limnological parameters are still unknown.

Sampling

Two sampling seasons were covered: spring 2017 (23 November–17 December), while the lakes were still completely frozen over with thick ice cover (0.80–1.60 m; Table I); and autumn 2018 (17–23 April), after the summer, when lakes were either ice free or had ice cover up to 55 cm thick (Table I). Profiles of temperature, pH, specific conductivity and dissolved oxygen in the water column were obtained using a YSI 600 QS probe. Dissolved oxygen data in autumn were not included because the probe membrane was damaged in the field.

Water samples for DNA and pigment extractions were collected from the surface at the deepest site in each lake. When lakes were ice covered, holes were drilled in the ice with a manual auger to collect water samples immediately below the ice layer. Water samples were taken with a 6.2 l Kemmerer sampler, transferred to acid-washed plastic containers and transported in the dark to the laboratory at Artigas Base. At the laboratory, the phytoplankton fraction was captured by filtering between 215 and 1000 ml of water per lake through 0.7 μm glass microfibre filters of GF/F grade in

duplicate or triplicate depending on the filtered volume. Between 250 and 1000 ml of water (in duplicate or triplicate) were filtered through 0.2 µm nitrate cellulose filters in order to capture the bacterial fraction. Filters were immediately frozen and were transported in a cooler with ice packs from King George Island to Uruguay (DNA filters) and Quebec (pigment filters), where they were stored in the dark at -80°C until analysis. Samples remained frozen at all times. The volumes of water filtered for pigment and DNA extractions are shown in Table S1 (see below).

Pigment biomass and diversity analyses

We assessed phytoplankton diversity through the analysis of photosynthetic pigments using high-performance liquid chromatography (HPLC), which enables the separation and quantification of individual chlorophylls and carotenoids (Wright & Jeffrey 2006). Broadly distributed pigments, such as chlorophyll *a* (chl *a*), serve as indicators of phytoplankton biomass, while other taxonomically diagnostic pigments may be used to examine community composition at the class level (Wright & Jeffrey 2006). In 2019, pigments were extracted at Université Laval from filters by sonication in 2.5 ml of 95% methanol and incubated under argon gas in darkness, in an ice bath, for 30 min. The extracts were then cleared by centrifugation at 4°C, filtered through 0.2 µm polytetrafluoroethylene filters into HPLC vials and placed under argon gas. Shortly following extraction, samples were injected into a Thermo Fisher Accela 600 HPLC device equipped with an autosampler, a Hypersil Gold C8 column (3 µm pore size), a photo-diode array and a fluorescence detector using the reverse-phase solvent protocol of Zapata *et al.* (2000). HPLC peaks were detected using diode-array spectroscopy (350–750 nm) set to a slit width of 1 nm, and absorbance chromatograms were obtained at 450 nm. Chlorophylls were also detected by fluorescence (excitation, 440 nm; emission, 650 nm). Pigments were identified and quantified from retention times and absorbance chromatograms using reference standards from Sigma, Inc. (St Louis, MO, USA) and DHI Water & Environment (Hørsholm, Denmark); concentrations of unidentified carotenoids were calculated using the calibration coefficient for β,β-carotene. Derivative products of chl *a*, including chlorophyllide *a*, pheophorbide *a* and pheophytin *a* (Table S2), were measured in the analysis, and total chl *a* was defined as the sum of chl *a* and chl *a* derivatives.

Marker pigments were classified into nine major phylogenetic groups at the following division levels: Chlorophyceae, Prasinophyceae, Euglenophyta, Bacillariophyceae, Prymnesiophyceae, Chrysophyceae, Dinophyta, Cryptophyta and Cyanobacteria. As certain groups share marker pigments, those of the Chlorophyceae, Prasinophyceae and Euglenophyta (Mg-3,8-divinyl-pheoporphyrin a_5 monomethyl ester (MgDVP), 9-cis-neoxanthin, violaxanthin, antheraxanthin, lutein and chlorophyll *b*) were combined, as well as those of the groups Bacillariophyceae, Prymnesiophyceae and Chrysophyceae (chlorophyll c_1, fucoxanthin, 19-hex-fucoxanthin and diadinoxanthin). The pigments dinoxanthin and peridinin were employed as indicators of the Dinophyta, alloxanthin for Cryptophyta and zeaxanthin for Cyanobacteria (Wright & Jeffrey 2006). In addition, we found several unidentified carotenoids that were not considered in the statistical analyses; their absorption maxima and retention times can be found in Table S3. No pigment data are available from Kitiesh Lake during spring, as logistical difficulties prevented sampling of this lake. In addition, no carotenoid concentrations are available from Hotel Lake in spring, as all carotenoids were below detection limits; however, our more sensitive fluorescence detector enabled the quantification of chl *a* at that time.

Bacterioplankton composition analyses

Bacterioplankton were examined through high-throughput sequencing of the V4 hypervariable region of the 16S gene, which enables sensitive and accurate molecular detection of bacterial diversity (Picazo *et al.* 2019). In 2020, at the Instituto de Investigaciones Biológicas Clemente Estable (IIBCE) laboratory in Uruguay, DNA was extracted from cellulose filters based on physical disruption of the cells and nucleic acid purification. Filters were cut into small pieces and placed in microcentrifuge tubes containing ceramic beads and an extraction buffer described Martínez de la Escalera *et al.* (2014) and homogenized with a FastPrep homogenizer. After centrifugation at 12 000 *g*, supernatants were subjected three times to chloroform: isoamyl alcohol (24:1) extractions, and the pellets were precipitated with 0.6 volumes of cold isopropanol at room temperature over 24 h. These precipitates were then subjected to centrifugation for 40 min at 12 000 *g* at room temperature, and the DNA obtained in the pellets was washed with cold ethanol, dried and suspended in water overnight at 4°C. The concentration and purity of DNA were determined spectrophotometrically at 260 and 280 nm, and the quality of the DNA was checked through the amplification of a variable region (V4) of the ribosomal 16S gene. Polymerase chain reaction products were verified by gel electrophoresis on 1% agarose gel in 0.5X Tris-borate-EDTA (TBE) buffer. DNA samples were sent to the University of Minnesota Genomics Center for Illumina MiSeq paired-end sequencing of the V4 region using the primers 515F and 806R.
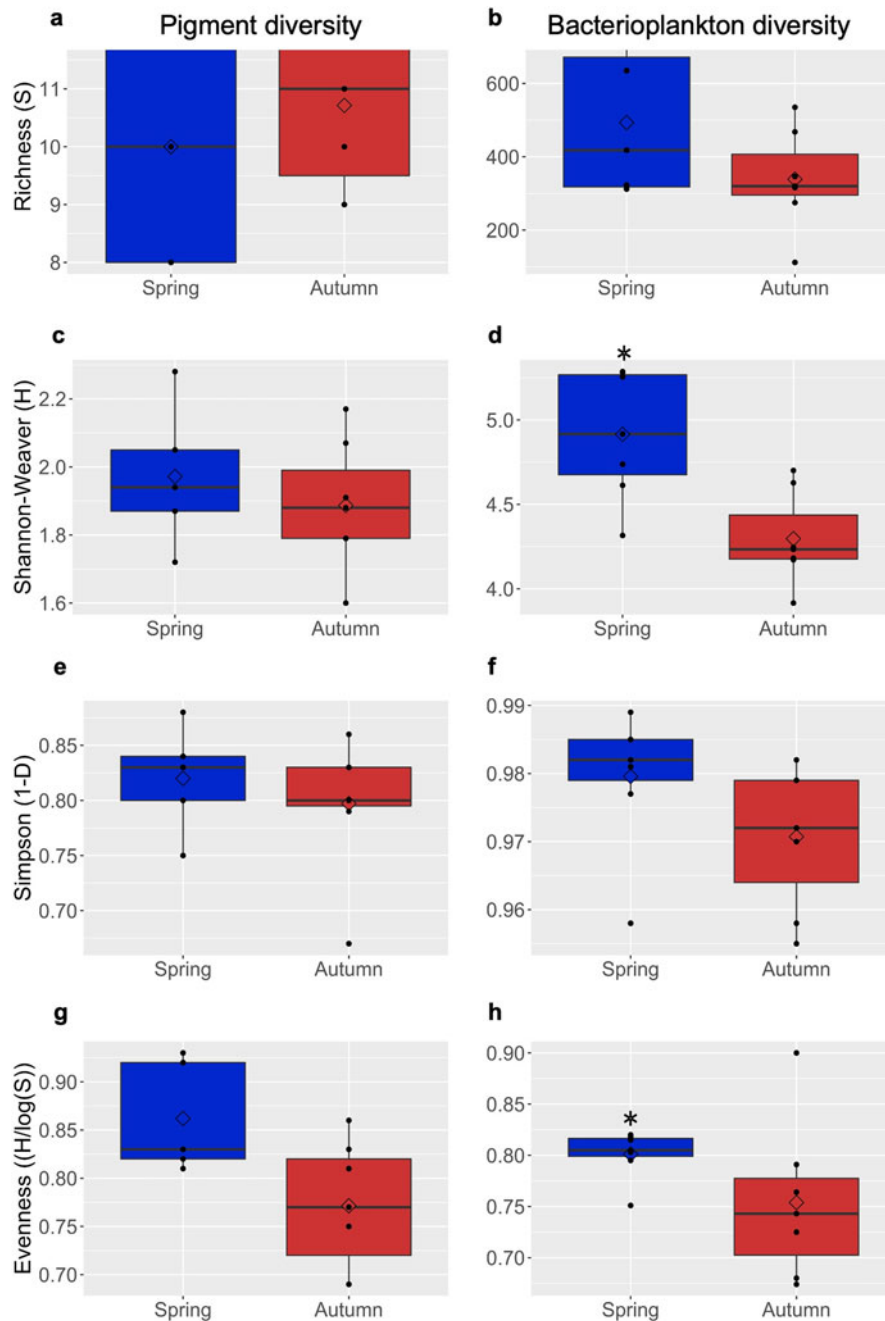
Fig. 2. Alpha-diversity for pigments and bacterioplankton measured as amplicon sequence variants (ASVs) for the two seasons. **a.** Pigment richness, **b.** ASV richness, **c.** Shannon-Weaver diversity index for pigments, **d.** Shannon-Weaver diversity index for ASVs, **e.** Simpson's index of diversity for pigments, **f.** Simpson's index of diversity for ASVs, **g.** evenness index for pigments, **h.** evenness index for ASVs. Horizontal lines inside the boxplots are median values and boundaries indicate the 25th and 75th percentiles. Vertical lines above and below indicate the 10th and 90th percentiles. Asterisks indicate means with significant differences between seasons ($P < 0.05$).

To correct for sequencing errors and create amplicon sequence variants (ASVs), reads were processed in *R* using the DADA2 pipeline following a modified version of the DADA2 Bioconductor workflow (Callahan *et al.* 2017). Briefly, reads were filtered and trimmed by the *filterAndTrim* function based on the quality score, which estimates the error probability of the DNA sequence. Reads with a maximum expected error > 2 were removed and, based on quality profiles, reads were truncated at 200 and 150 bp for forward and reverse reads, respectively. Sequence variations were inferred with the *learnerrors* and *dada* functions. Chimeric

sequences were eliminated, and taxonomy assignments from kingdom to genus were performed with *assignTaxonomy* based on the SILVA database (v138; Quast *et al.* 2012). ASVs assigned as Archaea, Chloroplasts and Mitochondria were removed. Sequences obtained were submitted to the nucleotide archive GenBank with the project ID PRJNA848662.

Data analysis

Microbial alpha-diversity was calculated and compared between seasons using different indices (richness (*S*),
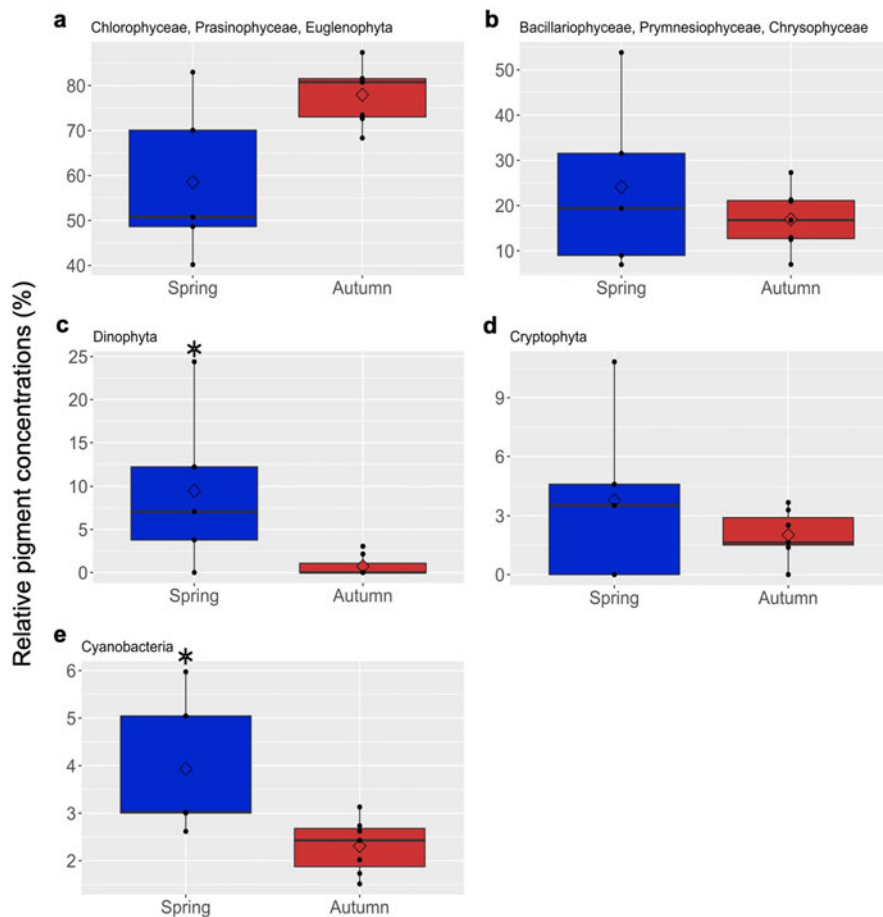
Fig. 3. Percentages of phytoplankton groups (according to taxonomic pigments) between seasons. Groups were defined according to the combination of the following pigments: **a.** Mg-3,8-divinyl-pheoporphyrin a_5 monomethyl ester (MgDVP), 9-cis-neoxanthin, violaxanthin, antheraxanthin, lutein and chlorophyll b for Chlorophyceae, Prasinophyceae and Euglenophyta; **b.** chlorophyll c_1, fucoxanthin, 19-hex-fucoxanthin and diadinoxanthin for Bacillariophyceae, Prymnesiophyceae and Chrysophyceae; **c.** dinoxanthin and peridinin for Dinophyta; **d.** alloxanthin for Cryptophyta; **e.** zeaxanthin for Cyanobacteria. Horizontal lines inside the boxplots are median values and boundaries indicate the 25th and 75th percentiles. Vertical lines above and below indicate the 10th and 90th percentiles. Asterisks indicate means with significant differences between seasons ($P < 0.05$).

the Shannon-Weaver index (H), and the Simpson dominance (1-D) and evenness ($H/\log(S)$) indexes) using the function *diversity*() of the R package *vegan* (Oksanen *et al*. 2020). These indices were calculated for taxonomic pigments and for the ASV composition table. We tested differences in the means of the measured indices as well as the means of the measured environmental variables and in chl a concentrations for each season using Kruskal-Wallis tests. Phytoplankton groups were defined by their taxonomic pigments, and their proportions were also compared between seasons and using Kruskal-Wallis tests. In addition to the calculation of alpha-diversity indices, alpha-diversity for bacterioplankton was analysed from the rarefaction curves of the ASV abundances using the *rarecurve*() function in the R package *vegan* (Oksanen *et al*. 2020).

As initial bacterioplankton diversity ordinations according to distance-based redundancy analysis (db-RDA) indicated that the major similarities were between seasons and not between individual sites across different seasons (see below), we applied the indicator value (IndVal) method (Dufrêne & Legendre 1997) to identify bioindicator bacterioplankton for a particular season (group). 'Good' indicator species are those found

only or mostly in a single group of sites (high specificity) as well as those being present at most of the sites belonging to that group (high fidelity). The IndVal of a species is expressed as the degree (%) to which it fulfils the criteria of specificity and fidelity within any group of samples. Finally, permutation tests were used to assess the significance of individual indicator species, and the P-values were corrected for multiple testing using Holm's correction (McGeoch & Chown 1998). IndVals were calculated by the function *multipatt* from the R package *indicpecies* (De Cáceres *et al*. 2010).

Beta-diversity was calculated as the variation in the community composition among lakes for each season (spatial beta-diversity). The estimation of spatial beta-diversity was based on the total variance of the data matrix separately for each season examined for taxonomic pigments and ASVs (Legendre & De Cáceres 2013), as calculated with Hellinger-transformed data using the *beta.div*() function of the R package *adespatial* (Dray *et al*. 2022). This function also computed the local contribution to beta-diversity (LCBD) index for each sample and a permutational P-value indicating the significance of each LCBD value. LCBD is calculated as the sum of the variance for each site with respect to the
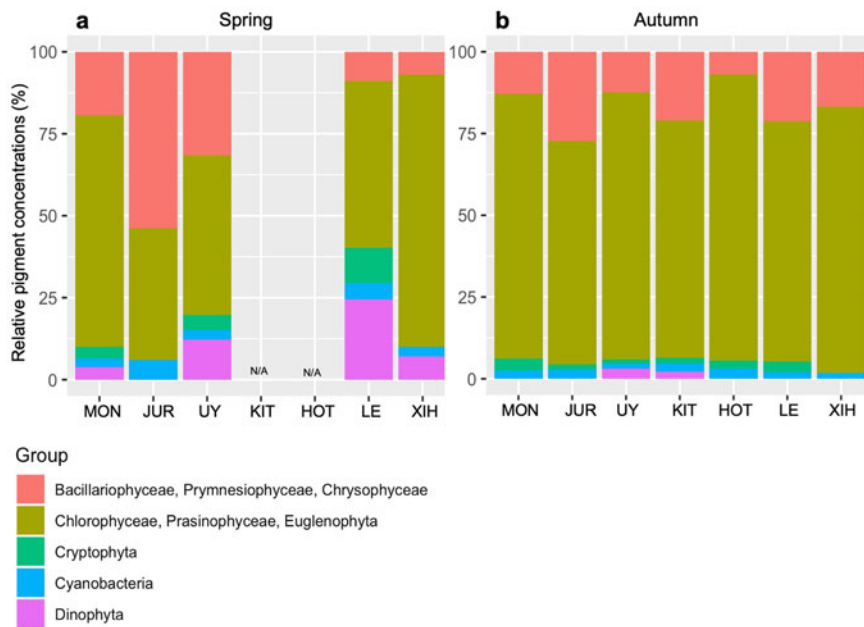
Fig. 4. Phytoplankton groups by lake between seasons: **a.** spring, **b.** autumn. N/A = not available. Lake codes: MON = Mondsee; JUR = Jurasico; UY = Uruguay; KIT = Kitiesh; HOT = Hotel; LE = Las Estrellas; XIH = Xihu.

total variance, assessing the individual contribution of each lake to the total spatial beta-diversity in the system. The P-values were then corrected for multiple testing using Holm's correction.

To relate the structure of the microbial community to lake characteristics, db-RDA was employed using the taxonomic pigment concentration matrix as the response variables for phytoplankton pigments and the ASV table for bacteria, with the environmental variable matrix as the explanatory dataset in both cases. The environmental variables considered were temperature, specific conductivity, pH and the ice-layer thickness of each lake for both seasons. Dissolved oxygen was not considered in the analysis because it could not be quantified in autumn (Table I). The significance of each db-RDA and each environmental variable was examined, as was the adjusted R^2. db-RDA was performed with the function *dbrda()* in *vegan* (Oksanen *et al.* 2020) using the percentage difference dissimilarity distances of the raw response matrices.

To explore the association of unknown carotenoids (Table S3) with taxonomic phytoplankton pigments, we used Spearman correlations to test their relationships with taxonomic marker pigments, and we report P-values as well as P-values adjusted for multiple tests using Holm's correction.

Results

Environmental characterization

There were differences in the assessed environmental variables (temperature, specific conductivity and pH) between the two sampling seasons and between lakes;

however, these differences were not significant ($P > 0.05$; Table I & Fig. S1). The profiles of environmental variables in the water column showed only slight variations in most of the lakes and in both seasons, apart from specific conductivity, which increased gradually with depth in spring (Fig. S1). Water temperature at the surface ranged from 0.10°C to 0.47°C in spring and from 0.11°C to 0.64°C in autumn. Lakes were circumneutral to weakly alkaline, with pH overall ranging from 6.75 to 8.15 (spring average: 7.22; autumn average: 7.74; Table I). The pH of two lakes did not change between seasons (lakes Uruguay and Kitiesh), while five had moderate pH increases (average: +0.63 pH; Table I). The four lakes with the lowest specific conductivities in spring increased somewhat in autumn (spring average: 118 μS cm^{-1}; average increase: 46 μS cm^{-1}), while those with the three highest spring values decreased in autumn (spring average: 360 μS cm^{-1}; average decline: 91 μS cm^{-1}; Table I). The highest specific conductivity was found in Hotel Lake in both seasons, with values of 551 μS cm^{-1} in spring and 461 μS cm^{-1} in autumn (Table I).

Pigment biomass and diversity

The lakes were generally ultraoligotrophic, with chl a concentrations indicating very low phytoplankton biomass (Tables I & S2). Median total chl a across all lakes was 0.22 μg l^{-1} in spring (minimum: 0.11 μg l^{-1}; maximum: 1.09 μg l^{-1}) and 0.82 μg l^{-1} in autumn (minimum 0.58 μg l^{-1}; maximum 12.76 μg l^{-1}), with extreme values found in Hotel Lake both in spring and in autumn (0.11 and 12.76 μg l^{-1}, respectively; Table I). While most lakes had higher chl a concentrations in

Pigment diversity

Bacterioplankton diversity
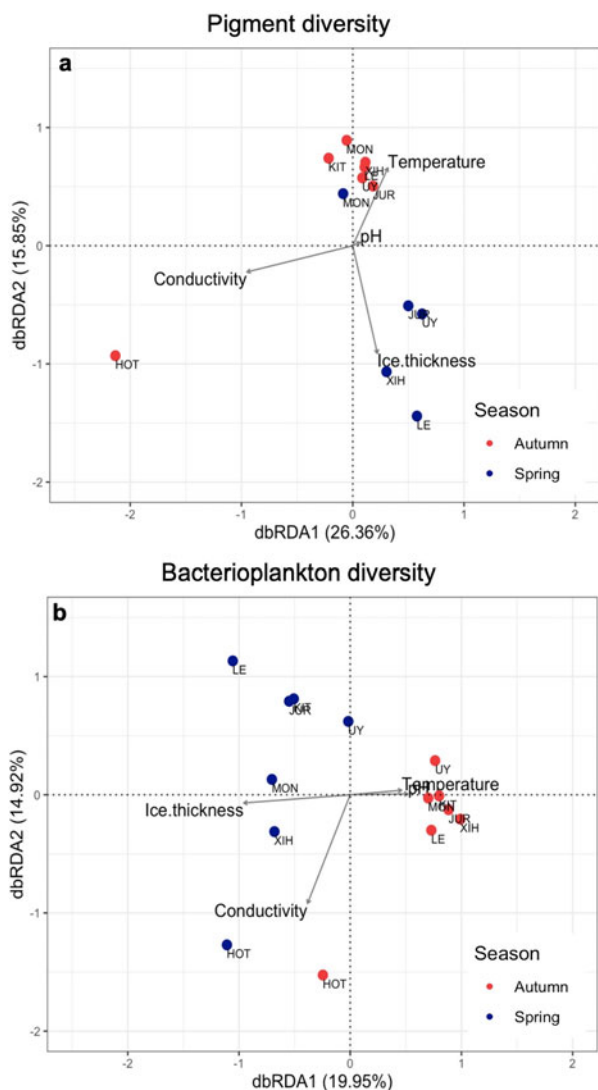
Fig. 5. Distance-based redundancy analysis (db-RDA).
a. db-RDA for pigments, $P = 0.040$, $R^2 = 0.19$. **b.** db-RDA for bacterioplankton, $P = 0.003$, $R^2 = 0.17$. Lake codes: MON = Mondsee; JUR = Jurasico; UY = Uruguay; KIT = Kitiesh; HOT = Hotel; LE = Las Estrellas; XIH = Xihu.

autumn than spring, Lake Mondsee did not differ appreciably between seasons (1.09 *vs* 1.03 µg l⁻¹; Table I).

With the exception of richness, the average indices of all phytoplankton taxonomic pigments decreased in autumn relative to spring, although the differences between seasons were not significant ($P > 0.05$; Fig. 2). Chlorophyceae, Prasinophyceae and Euglenophyta pigments dominated in both seasons and in most lakes, representing between 41% and 87% of total marker pigments (Fig. 3 & Tables S2 & S4). Chl *b* and lutein, mainly derived from Chlorophyceae, were the dominant pigments (Tables S2 & S4), and they were more abundant in autumn compared to spring, while

in spring the proportion of pigments from Dinophyta and Cyanobacteria was significantly higher ($P < 0.05$), and those from Bacillariophyceae, Prymnesiophyceae, Chrysophyceae and Cryptophyta were also higher but not significantly ($P > 0.05$; Fig. 3). This was accentuated in lakes Las Estrellas, Jurasico and Uruguay due to the proportion of pigments from the Bacillariophyceae, Prymnesiophyceae and Chrysophyceae, which reached 52% and 32% in lakes Jurasico and Uruguay, respectively, in spring, while in Las Estrellas Lake, pigments from the Dinophyta represented 26% of total marker pigments in spring (Fig. 4). Cyanobacteria, for which zeaxanthin was the only marker pigment identified, represented only a small proportion of pigments in all lakes (between 2% and 7%; Figs 3 & 4).

The spatial beta-diversity for phytoplankton taxonomic pigments varied slightly between seasons, with a higher value in spring when all lakes were ice covered (0.21) that decreased in autumn (0.10). In spring, Las Estrellas Lake had a significantly higher LCBD relative to the other lakes ($P < 0.05$), while Jurasico Lake also had a high LCBD but the difference was not significant after Holm correction ($P > 0.05$; Table S5). In autumn, Hotel Lake had the highest LCBD but the difference with the other lakes was not significant after Holm correction, if only marginally ($P = 0.08$; Table S5).

The db-RDA for phytoplankton taxonomic pigments explained 48.51% of the variance, of which 26.36% was explained by the first axis and 15.85% by the second axis (Fig. 5a). The overall permutation test of the analysis was significant ($P < 0.05$), and the adjusted R^2 was 0.19. Specific conductivity was the only environmental variable with a significant contribution to the model ($P < 0.05$), and ice thickness was marginally non-significant ($P = 0.08$). Specific conductivity was related to the first db-RDA axis, largely controlled by the influence of Hotel Lake in autumn (Fig. 5a). Ice thickness and temperature had the largest influence on the dispersion of the sites along the second db-RDA axis (Fig. 5a). Finally, pH had a very weak relationship with both axes (Fig. 5a).

The results of the multiple Spearman correlations performed between the unknown carotenoids and the taxonomic pigments are shown in Table S6. From the 11 unknown carotenoids found (Table S3), three of them had high correlation coefficients (0.93, 0.66 and 0.54) with the pigment peridinin, a further unknown carotenoid with the pigments diadinoxanthin, antheraxanthin and alloxanthin (0.66, 0.63 and 0.67, respectively), another with chl c_1 (0.74) and a last one with violaxanthin (0.58). All of these correlations were significant ($P < 0.05$); however, after the multiple testing Holm correction, only one correlation was significant (the one between one unknown pigment and peridinin). In addition, some of the different unknown pigments were highly and significantly correlated with each other even after Holm corrections ($P < 0.05$; Table S7).
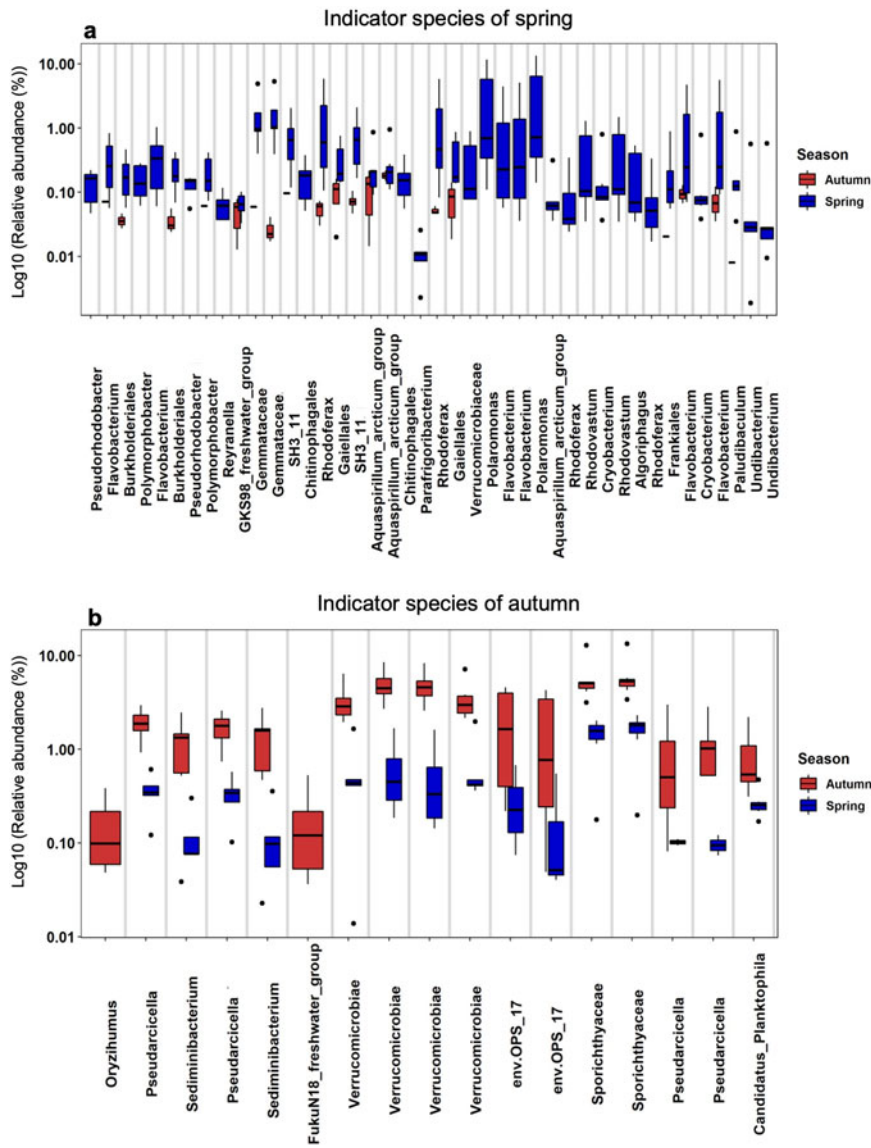
Fig. 6. Indicator bacteria species for each season: **a.** spring, **b.** autumn. Indicator species that were present only in one sample of a particular season and with a proportion ≤ 0.09 were not included in the graph. Horizontal lines inside the boxplots are median values and boundaries indicate the 25th and 75th percentiles. Vertical lines above and below indicate the 10th and 90th percentiles. The name of each amplicon sequence variant is according to the highest taxonomic rank identified.

Bacterioplankton diversity

A total of 672 606 reads were obtained, representing 3349 ASVs. Rarefaction curves based on the observed ASVs (richness) reached plateaus (Fig. S2), indicating that sequencing depth was sufficient to capture the overall diversity of all lakes. Bacterioplankton alpha-diversity was higher in spring (when all lakes were ice covered) than in autumn (Fig. 2). Shannon-Weaver diversity and evenness were significantly higher in spring relative to autumn ($P < 0.05$), while between-season differences were not significant for richness ($P > 0.05$) and were marginally non-significant for Simpson diversity ($P = 0.06$; Fig. 2).

Total bacterioplankton beta-diversity was higher than that observed for phytoplankton pigments and was higher in spring than in autumn (0.54 and 0.37, respectively). The largest contributions to this beta-diversity were from lakes Hotel and Las Estrellas, which showed significantly higher LCBDs in spring ($P < 0.05$; Table S5). Hotel Lake also had a significantly higher LCBD in autumn than in spring ($P < 0.05$).

The phyla Bacteroidota (26.24%), Proteobacteria (25.68%), Actinobacteriota (25.27%), Verrucomicrobiota (14.32%) and Planctomycetota (3.12%) had the highest total relative abundances across all samples. The classes Bacteroidia, Gammaproteobacteria (including the former Betaproteobacteria class now within the Gammaproteobacteria in SILVA), Actinobacteria and Verrucomicrobiota were the most abundant members from these phyla, respectively.

The db-RDA model for bacterioplankton explained 42.57% of the variance, with the first and second axes

explaining 19.95% and 14.92%, respectively. The overall permutation test of the analysis was significant ($P < 0.05$), and the adjusted R^2 was 0.17. Similarly to phytoplankton pigments, the samples were separated according to season along the first axis, but with significant dispersion during spring along the second axis (Fig. 5b). The variables that contributed significantly to the model were ice thickness and specific conductivity ($P < 0.05$). The first db-RDA axis was controlled by ice thickness, which had a high influence on the dispersion of spring samples, as well as by temperature and pH, which appeared to influence the dispersion of autumn samples. Finally, specific conductivity was the variable most strongly related with the second db-RDA axis, largely reflecting the higher values in Hotel Lake (Fig. 5b).

Bacterioplankton indicator species analysis selected 42 indicator ASVs in spring, with a predominance of the classes Gammaproteobacteria (14 ASVs), Alphaproteobacteria (7 ASVs; both phylum Proteobacteria) and Bacteroidia (9 ASVs; phylum Bacteroidota; Fig. 6a & Table S8). These indicator taxa included the genera *Rhodoferax*, *Aquaspirillum arcticum* group and *Polaromonas*, all from the order Burkholderiales of the Gammaproteobacteria, the genus *Flavobacterium* from the order Flavobacteriales, the genus *Algoriphagus* from the order Cytophagales and the order Chitinophagales, all from the Bacteroidia, and members from the class Alphaproteobacteria, including the orders Rhodobacterales (genus *Pseudorhodobacter*), Sphingomonadales (genus *Polymorphobacter*), Reyranellales (genus *Reyranella*) and Acetobacterales (genus *Rhodovastum*; Fig. 6a & Table S8).

For autumn, 17 indicator ASVs were identified, represented by the classes Bacteroidia (8 ASVs), Verrucomicrobiae (5 ASVs) and Actinobacteria (4 ASVs), from the phyla Bacteroidota, Verrucomicrobiota and Actinobacteriota, respectively (Fig. 6b & Table S9). Although members of the class Bacteroidia were also identified as indicator species for spring, different taxa were selected in autumn, including the orders Cytophagales (genus *Pseudarcicella*), Chitinophagales (genus *Sediminibacterium*) and Sphingobacteriales (Fig. 6b). Indicators of autumn from the class Actinobacteria were from the orders Micrococcales and Frankiales (Table S9). Finally, most of the autumn indicator taxa from the class Verrucomicrobiae were not assigned to any order (Fig. 6b & Table S9).

While the composition of the bacterioplankton was mainly structured according to season, the presence of certain groups was notable in some individual lakes, in particular lakes Hotel and Las Estrellas, as reflected in the db-RDA and LCBD values (Fig. 5b & Table S5). For example, methane-oxidizing bacteria (genus *Methylobacter* from the family Methylomonadaceae) and sulphur bacteria (sulphate-reducing forms from the families Geobacteraceae, Desulfurivibrionaceae and Desulfocapsaceae and sulphur oxidizers from the families Rhodobacteraceae, Rhodocyclaceae and Sulfurimonadaceae) were relatively more abundant in Hotel Lake in spring. These groups were observed at low relative abundances, with proportions of 0.05% for *Methylobacter* and 0.01% and 0.20% for sulphate-reducing and sulphur-oxidizing bacteria, respectively. Moreover, the greater variance in community composition observed in Las Estrellas Lake in spring (Fig. 5b & Table S5) was due to the presence of some bacteria families with relative abundances between 0.002% and 0.040%, including Acidobacteriaceae, Blastocatellaceae, Chthoniobacteraceae, Frankiaceae and Xanthobacteraceae.

Discussion

Chl *a* concentrations in the Fildes Peninsula lakes here studied were comparable to most oligotrophic lakes from Maritime and Continental Antarctica (typically < 3 µg l⁻¹), with the exception of autumn concentrations in Hotel Lake (12.76 µg l⁻¹). Conversely, eutrophic lakes described in the region are generally close to the sea and are enriched by excreta from birds and marine mammals, with concentrations in extreme cases exceeding 100 µg l⁻¹ chl *a* (Izaguirre *et al.* 1998). The higher chl *a* concentrations found in autumn relative to spring (Table I) indicate higher phytoplankton biomass. However, we note that chl *a* concentrations in Mondsee Lake did not vary between seasons (Table I), and that this was the only lake whose autumn phytoplankton community clustered together with the spring samples from the other lakes (Fig. 5a). Its microclimate is probably strongly influenced by the adjacent Collins Glacier (Fig. 1b), which may dampen the amplitude of seasonal phytoplankton shifts. The extremely low chl *a* concentration found in spring for Hotel Lake (0.11 µg l⁻¹) was unexpected given that the lake water during spring was visibly green on the lake's snow and ice. Despite this, in spring carotenoids were below the detection limit of our HPLC. This may be related to clogging of the filter with particulate matter (only 250 ml were filtered before the filter became clogged; Table S1), which may have resulted in an underestimation of chl *a*. While the volume of water filtered in Hotel Lake in autumn was also low, and even lower (215 ml) than that filtered in spring (Table S1), it was enough for the detection of high concentrations of pigments.

Phytoplankton such as small flagellates from the Cryptophyceae, Chrysophyceae and Dinophyta are dominant in most Antarctic lakes, both in the AP when they are ice covered (Izaguirre *et al.* 1998) as well as perennially ice-covered lakes (Lizotte & Priscu 1998). Their dominance under ice is attributed to the presence

of shade-adapted species capable of free movement in water columns where wind-induced mixing is absent (Priddle *et al*. 1986). However, we found Chlorophyceae, Prasinophyceae and Euglenophyta, and particularly the Chlorophyceae, to be dominant during both seasons, although Chrysophyceae, Cryptophyta and Dinophyta were more abundant in spring (Figs 3 & 4), when lakes were still ice covered. Chlorophytes have also been shown to dominate in Antarctic lakes but usually under relatively high nutrient concentrations, such as those found in summer when lakes are ice free (Izaguirre *et al*. 1998, Rochera *et al*. 2013). Nevertheless, a recent study performed in Fildes Peninsula during the ice-free period found Chrysophyta to be dominant, while Chlorophyta were the second most abundant group (Zhang *et al*. 2022). Zhang *et al*. (2022) is the first study in the region using NGS for phytoplankton analysis, and differences in the methods employed could be a key factor explaining the differences in the results. We recognize that the chemotaxonomic approach we employed by analysing pigments does not enable high-resolution determinations of phytoplankton taxa. HPLC analysis nonetheless enabled the detection of different phytoplankton pigment assemblages between seasons, similarly to that observed for bacterioplankton diversity.

The unidentified carotenoids in our dataset are probably degradation products of several marker carotenoids, as suggested by their high correlations. During degradation, structural alterations of pigment molecules result in modified retention times and absorption spectra that therefore did not match those of our pigment standards. In addition, these unidentified carotenoids were strongly related to peridinin, violaxanthin and diadinoxanthin, all highly labile pigments prone to rapid degradation in the water column (Wright & Jeffrey 2006).

Our analysis based on ASVs shows the dominance of phyla previously reported from high-latitude systems in studies employing NGS, such as in ice-free lakes from the Maritime Antarctic region (Byers Peninsula; Picazo *et al*. 2019), ice-covered Laurentian Great Lakes (North America; Beall *et al*. 2016) and the Canadian High Arctic (Marois *et al*. 2022). In addition, some of the most representative phyla in our lakes were reported to be abundant in marine and freshwater systems, including in the Antarctic, such as members of Proteobacteria and Bacteroidota (Rochera & Camacho 2019). Their dominance in oligotrophic lakes may be related to their high biogeochemical and physiological diversity that allows for adaptation to nutrient scarcity, among other stressors (Newton *et al*. 2011).

The identification of indicator bacterioplankton species by the IndVal approach suggests that there are unique portions of the microbial community in each season, probably related to confined niches under selection pressure. Both groups of indicator species showed high

gene sequence similarity, with relatives found in GenBank from environments similar to those of Fildes Peninsula, including microbial mats from King George Island, Antarctic and Arctic soil and sediment, glacier ice, cryoconite holes, permafrost, lakes in Patagonia, Antarctic lichens and lakes at high latitudes (Tables S8 & S9). Indicator species from the phylum Proteobacteria were exclusive to spring samples, which could be related to more oligotrophic conditions reflecting winter conditions. On the other hand, indicator species of Bacteroidota were present in both seasons, but taxa from the genus *Flavobacterium* were indicators only in spring (Tables S8 & S9). *Flavobacterium* and *Polaromonas* (order Burkholderiales), both indicator species of spring, appear to be common in nutrient-limited Antarctic lakes, as reported in lakes from Byers Peninsula (Livingston Island, South Shetland Islands; Rochera & Camacho 2019). In addition, the fact that *Polaromonas* has been reported as a dominant bacterium in lake ice (Veillette *et al*. 2011) may suggest that this bacterium indicates ice presence. There were other indicator species of spring from the family Oxalobacteraceae (also from the order Burkholderiales; Table S8), which contains a mix of psychrophilic species and genera adapted to oligotrophic conditions (Baldani *et al*. 2014). By comparison, Actinobacteria are commonly found in soils, and their presence in lakes is attributed primarily to runoff and aeolian deposition (Beall *et al*. 2016). The identification of members of the soil Actinobacteria Frankiales as indicator species of autumn and not of spring (Tables S8 & S9) may be because in spring there has been neither runoff for ~8 months nor delivery of aeolian particles and soils have been snow covered. By contrast, in autumn there has been runoff and aeolian delivery to the lakes for at least 3 months, with exposed soils, and their identification as autumn indicators may therefore suggest that Actinobacteria are being deposited allochthonously from soils. Verrucomicrobiota was also well represented in our lakes, and the indicator species of Verrucomicrobiota were exclusively from autumn (Table S9). Verrucomicrobia is another important representative of soil communities, but it is also present in aquatic environments where it metabolizes diverse polysaccharides (Martinez-Garcia *et al*. 2012). Finally, the unassigned members of Verrucomicrobiae (Table S9) suggest yet undescribed taxa that could imply bacterial groups with new adaptations, potentially related to the hydrolysis of compounds that could have entered lakes during the summer when they were ice free.

The microbial community (both phytoplankton and bacterioplankton) in Hotel Lake differed from the rest of the lakes (Fig. 5 & Table S5). This lake was characterized by greater specific conductivity, which showed a significant relationship with its microbial community (Fig. 5), and its chl *a* concentration in autumn was extremely elevated

compared to the rest of the lakes (Table I). This lake has been the focus of particular attention due to evidence of high concentrations of heavy metals that caused it to be abandoned as a drinking water source (Peter *et al.* 2013). Transportation is among the human activities that have most strongly altered natural environments in Fildes Peninsula (Peter *et al.* 2013), and the lake is situated adjacent to the airport as well as the peninsula's most travelled road. The only group of Methanotrophs we identified was classified as *Methylobacter* (family Methylomonadaceae) and was almost exclusively found in this lake during spring. While this bacterium is aerobic, a previous study suggested that these methanotrophs are microaerophilic, and they were found throughout the water column under ice cover in a dimictic Arctic lake (Schütte *et al.* 2016). The sequences that we identified from this group have high gene similarities with relatives from potentially anoxic environments, including sediments, tailings, pond water, a stratified freshwater lake and a methane seep (GenBank accession numbers MT067475, OK135604, AB753945 and MN602493). The sulphur-reducing and sulphur-oxidizing bacteria found in Hotel Lake indicate that they might be important for the sulphur cycle during spring. Sulphate-reducing bacteria reduce sulphate to sulphide using a variety of electron donors, including H_2, fatty acids, alcohols, metals and aromatic compounds such as those found in organic contaminants (e.g. petroleum compounds; George *et al.* 2011). In addition, because most of these bacteria are obligate anaerobes, such as members of the families Geobacteraceae and Desulfurivibrionaceae (Kuever 2014), they may indicate anoxia under spring ice, which is further supported by the presence of *Methylobacter*.

Several factors may be hypothesized to explain the fact that Hotel Lake was distinguished from the other lakes in terms of phytoplankton, bacterioplankton and the environmental variables that we measured. This lake is considerably smaller than the others, has the thickest ice cover in both spring and autumn and has the highest specific conductivity and lowest dissolved oxygen among all of the lakes. The lake is also adjacent to the airport, and its access road and was previously noted to be strongly contaminated with heavy metals (Peter *et al.* 2013). As such, this lake merits further investigation to discern the precise causes of its disparate microbial assemblages.

The decrease in the variance between the microbial community composition of the lakes in autumn (Fig. 5 & Table S5) indicated that communities were more similar at the end of the summer open-water period, whereas in spring, following the prolonged, dark winter period, lakes differed more. We hypothesize that, in spring, the thick ice cover that isolated the lakes from the atmosphere for several months may have engendered variable environments conditioned by lake-specific factors. In autumn, by contrast, the more homogeneous microbial communities may have resulted from summer conditions that resulted in free exchange with the atmosphere and more similar irradiance regimes between ice-free lakes.

Conclusions

This study explored the diversity of microbial communities in two seasons in lakes strongly affected by global warming and located near an area with high human impacts. This is the first study in lakes from Fildes Peninsula employing HPLC for phytoplankton analysis and NGS for the analysis of bacterial diversity, including under-ice communities.

We found that lakes from Fildes Peninsula showed less variation between each other than between seasons and that the main seasonal differences from spring to autumn were decreased bacterioplankton richness and evenness, increased phytoplankton biomass and changes in the community composition of both communities. Lakes were more similar in autumn, at the end of the summer open water, while in spring, at the end of the winter period, lakes differed more.

These results have important consequences given that changes in bacterial communities imply changes in ecosystem function. Moreover, longer ice-free periods such as those predicted due to future warming, in conjunction with the presence of pollutants because of the increases in human activities in the area, will generate further microbial changes that we cannot yet predict. The matching responses of phytoplankton and bacterioplankton highlight the importance of both communities as bioindicators that reflect environmental changes. These aquatic ecosystems and their microbial assemblages are thus sentinels of adaptive responses to environmental change in this rapidly warming area.

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