



Phototrophic pigment diversity and picophytoplankton in permafrost thaw lakes

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Abstract. Permafrost thaw lakes (thermokarst lakes) are widely distributed across the northern landscape, and are known to be biogeochemically active sites that emit large amounts of carbon to the atmosphere as CH₄ and CO₂. However, the abundance and composition of the photosynthetic communities that fix CO₂ have been little explored in this ecosystem type. In order to identify the major groups of phototrophic organisms and their controlling variables, we sampled 12 permafrost thaw lakes along a permafrost degradation gradient in northern Québec, Canada. Additional samples were taken from five rock-basin reference lakes in the region to determine if the thaw lakes differed in limnological properties and phototrophs. Phytoplankton community structure was determined by high-performance liquid chromatography analysis of their photoprotective and photosynthetic pigments, and autotrophic picoplankton concentrations were assessed by flow cytometry. One of the black-colored lakes located in a landscape of rapidly degrading palsas (permafrost mounds) was selected for high-throughput 18S rRNA sequencing to complement conclusions based on the pigment and cytometry analyses. The results showed that the limnological properties of the thaw lakes differed significantly from the reference lakes, and were more highly stratified. However, both waterbody types contained similarly diverse phytoplankton groups, with dominance of the pigment assemblages by fucoxanthin-containing taxa, as well as chlorophytes, cryptophytes and cyanobacteria. Chlorophyll *a* concentrations

(Chl *a*) were correlated with total phosphorus (TP), and both were significantly higher in the thaw lakes (overall means of 3.3 µg Chl *a* L⁻¹ and 34 µg TPL⁻¹) relative to the reference lakes (2.0 µg Chl *a* L⁻¹ and 8.2 µg TPL⁻¹). Stepwise multiple regression of Chl *a* against the other algal pigments showed that it was largely a function of alloxanthin, fucoxanthin and Chl *b* ($R^2 = 0.85$). The bottom waters of two of the thaw lakes also contained high concentrations of bacteriochlorophyll *d*, showing the presence of green photosynthetic sulphur bacteria. The molecular analyses indicated a relatively minor contribution of diatoms, while chrysophytes, dinoflagellates and chlorophytes were well represented; the heterotrophic eukaryote fraction was dominated by numerous ciliate taxa, and also included Heliozoa, Rhizaria, chytrids and flagellates. Autotrophic picoplankton occurred in biovolume concentrations up to $3.1 \times 10^5 \mu\text{m}^3$ picocyanobacteria mL⁻¹ and $1.9 \times 10^6 \mu\text{m}^3$ picoeukaryotes mL⁻¹, with large variations among lakes. Both groups of picophytoplankton were positively correlated with total phytoplankton abundance, as measured by Chl *a*; picocyanobacteria were inversely correlated with dissolved organic carbon, while picoeukaryotes were inversely correlated with conductivity. Despite their net heterotrophic character, subarctic thaw lakes are rich habitats for diverse phototrophic communities.

1 Introduction

Degradation of ice-rich permafrost leads to the formation of thaw lakes, which are among the most abundant aquatic habitats in high-latitude regions (Pienitz et al., 2008; Jones et al., 2012). These environments have attracted increasing scientific interest because of their biogeochemical reactivity. However, although there is rapidly increasing knowledge about their role in greenhouse gas (GHG) emissions (Laurion et al., 2010; Walter et al., 2006), little is known about their photosynthetic communities. Phototrophic organisms consume CO₂ and thereby reduce the net emission to the atmosphere; however, few studies have examined phytoplankton or other phototrophs in these waters. Early studies in the US Tundra Biome Program at Barrow, Alaska, recorded 105 species of algae in tundra lakes and ponds, with dominance of cryptophytes and chrysophytes (Alexander et al., 1980). More recent studies have focused on thaw lake diatoms as paleolimnological indicators, but the dominants in these records are often benthic taxa such as *Pinnularia* and *Fragilaria* (Bouchard et al., 2013). A lake survey in the western Hudson Bay lowlands, including in permafrost catchments, showed that the phytoplankton had diverse communities, primarily composed of cyanobacteria, chrysophytes, chlorophytes, cryptophytes, dinoflagellates and diatoms (Paterson et al., 2014).

Picophytoplankton (PP), consisting of picocyanobacteria and picoeukaryotes (nominally defined as cells 1 to 3 µm in diameter), contribute a major fraction of the total phototrophic biomass across a wide range of aquatic ecosystems (Richardson and Jackson, 2007), including northern lakes and rivers (Waleron et al., 2007; Vallières et al., 2008). In subarctic (Bergeron and Vincent, 1997) and high arctic (van Hove et al., 2008) lakes, picocyanobacteria may dominate the phytoplankton community in terms of biomass as well as cell abundance. For example, in the large oligotrophic Clear Water Lake (Lac à l'eau claire, Nunavik, Canada), small cell phytoplankton (cell fraction that passed through a 2 µm filter) accounted for 75 % of the total phytoplankton Chl *a* (Bergeron and Vincent, 1997). However, the suitability of permafrost thaw lakes as a habitat for picophytoplankton has not been explored.

Our overall aim in the present study was to evaluate the major groups of phytoplankton in subarctic thaw lakes, and to relate this abundance and community structure to environmental variables. For this we employed phototrophic pigment analysis by high-performance liquid chromatography (HPLC), an approach that has been applied with success to describe phytoplankton community structure at the phylum level in a wide range of freshwater (e.g., Fietz and Nicklisch, 2004) and marine (e.g., Ansotegui et al., 2001) studies.

A secondary objective was to determine the abundance and distribution of picocyanobacteria and picoeukaryotes. As a further guide to the composition of the eukaryotic plankton, and in support of the pigment and picoeukaryote observa-

tions, we also applied high throughput 18S rRNA sequencing to surface and bottom waters from one selected lake that was strongly influenced by permafrost degradation. Our study included a wide range of small lakes across the gradient of permafrost degradation in subarctic Québec, Canada, from sporadic permafrost landscapes in the south (less than 10 % of the area containing permafrost) to discontinuous permafrost in the north (10–90 % permafrost). We also took comparative samples from a set of shallow rock-basin lakes that are unaffected by thermokarst processes. Given their limnological variability, as indicated by the variety of water colors among thaw lakes, we hypothesized that there would be large variations in total phytoplankton pigment concentration, pigment diversity and picophytoplankton biovolume. Degrading permafrost soils release dissolved organic carbon (DOC) and fine inorganic particles into the thaw lakes, and these constituents determine the attenuation of light down the water column and the variability in color (Watanabe et al., 2011). DOC also influences the near-surface thermal and stratification regime (Caplanne and Laurion, 2008), and temperature is known to exert a direct effect on phytoplankton community structure, particularly favoring cyanobacterial dominance (Paerl and Huisman, 2008). We therefore hypothesized that DOC and temperature would be the primary drivers of variations in phytoplankton pigmentation and picophytoplankton biovolume.

2 Materials and methods

2.1 Study sites

Twelve thaw lakes (small perennial waterbodies created by thermokarst erosion of the permafrost) were sampled in subarctic Québec during the period of warm open-water conditions, in August 2011 and 2012 (Table S1 in the Supplement). The lakes were distributed along a north–south permafrost degradation gradient and across four geographically distinct locations: the Sasapimakwananisikw River valley (SAS) and the Kwakwatanikapistikw River valley (KWK) near Whapmagoostui–Kuujuarapik; and the Sheldrake River valley (BGR) and the Nastapoka River valley (NAS) near Umiujaq. The KWK and SAS valleys occur within the sporadic permafrost landscape, while the BGR and NAS valleys are located in the discontinuous permafrost landscape (Fig. 1). Each valley is characterized by distinct vegetation cover and soil structure. Lakes located within the KWK valley are situated on impermeable clay-silt beds where the drainage basin is covered with dense shrub vegetation (Breton et al., 2009), whereas lakes in the SAS valley are located in peatlands in which permafrost mounds (palsas) are thawing and degrading rapidly (Bhiry et al., 2011). The lakes located in the northern valleys (BGR, NAS) are situated on marine clay-silt beds and are surrounded by forest and shrub tundra. In addition to 12 permafrost thaw lakes, a



Figure 1. Location of the study area in subarctic Québec.

set of five shallow rock-basin lakes (SRB) located on basalt bedrock was sampled in the vicinity of Whapmagoostui–Kuujuarapik. These provided a set of reference lakes that are located at the same latitude and climatic setting, but without the direct influence of degrading permafrost that is experienced by the thaw lakes. The dates of sampling are given in Table S1.

2.2 Physicochemical analyses

Profiles of temperature, dissolved oxygen, conductivity and pH of the 17 lakes were recorded with a 600R multiparametric probe (Yellow Springs Instrument Co.). Additionally, temperature and conductivity were recorded with an RBR XR620 conductivity–temperature–depth profiler (Richard Brancker Research Ltd). Near-surface water samples (0.2 m depth) were collected into dark polyethylene bottles, previously washed with 10 % hydrochloric acid and rinsed in Milli-Q (MQ) water. The samples were stored in coolers and transported to the laboratory within 4 h of collection. The total nitrogen (TN) and total phosphorus (TP) measurements were performed on unfiltered water samples collected in 125 mL bottles, acidified with sulfuric acid (0.2 % final concentration), and stored at 4 °C until persulfate digestion. TN concentrations were then measured with a Lachat flow injection analyzer and TP concentrations were measured using a Genesys 10UV spectrophotometer (Thermo Spectronic) and standard techniques (Stainton et al., 1977). Total suspended solids (TSS) were collected onto pre-combusted and pre-weighed glass-fiber filters (Advantec MFS) that were dried for 2 h at 60 °C and weighed on a Sartorius high-precision balance. Dissolved organic carbon (DOC), colored dissolved organic matter (CDOM), soluble reactive phosphorus (SRP)

and nitrate (NO_3^-) measurements were performed on water filtered through 0.2 μm cellulose acetate filters (Advantec MFS). Samples for DOC analyses were stored in 45 mL dark glass bottles that had been previously burned at 450 °C for 4 h and rinsed with MQ water to remove any traces of organic substances. The DOC analysis was done with a Shimadzu TOC-5000A carbon analyzer calibrated with potassium biphthalate. CDOM was determined by spectrophotometric absorbance of the filtrates at 320 nm, blanked against filtered MQ water and converted to absorption values. SRP and NO_3^- were measured in the filtrates using standard colorimetric methods (Stainton et al., 1977), and major ions were measured using a Dionex ICS 2000 ion chromatograph.

2.3 Pigment analysis

Near-surface (0.2 m depth) and near-bottom water samples (0.2 m above sediments; 50–500 mL) from each lake were filtered onto 25 mm diameter GF/F glass-fiber filters, and immediately frozen and stored at -80°C until pigment extraction in methanol. Pigments were analyzed by high-performance liquid chromatography (HPLC) following the protocols and standards described in Bonilla et al. (2005). For some of the statistical analyses, two groups of algal accessory pigments were separated as in Bonilla et al. (2005): photoprotective pigments (canthaxanthin, diadinoxanthin, echinenone, lutein, violaxanthin and zeaxanthin) and light-harvesting, photosynthetic pigments (alloxanthin, Chl *b*, fucoxanthin and peridinin). Standards for identification and quantification of pigments (Chl *a*, Chl *b*, Chl *c*₂, alloxanthin, β , β -carotene, canthaxanthin, crocoxanthin, diadinoxanthin, echinenone, fucoxanthin, lutein, peridinin, violaxanthin and zeaxanthin) were obtained from Sigma Inc. (St. Louis, MO, USA) and DHI Water & Environment (Hørsholm, Denmark) to calibrate the HPLC. The photodiode array spectrum of each peak was checked against the reference spectra in Roy et al. (2011). No standards were available for bacteriochlorophyll *d*; and the main chromatogram peaks for this pigment at 430 nm were expressed as Chl *a*-equivalent concentrations.

2.4 Picophytoplankton enumeration

Near-surface (0.2 m depth) unfiltered water samples from each lake were transferred to 5 mL Cryovials, fixed with glutaraldehyde (1 % final concentration) and stored at -80°C until analysis for picophytoplankton abundance. The cells were enumerated using a Becton Dickinson flow cytometer (BD FACSCalibur), equipped with an argon laser. Analyses were done at the lowest flow rate (12 $\mu\text{L min}^{-1}$), using a solution of 1 μm diameter, yellow-green microspheres (Polysciences, Inc.) as an internal standard. Bead concentrations in the calibration solution were controlled using TruCount™ absolute counting tubes (BD Biosciences). Picocyanobacteria and picoeukaryotes were distinguished based on their chloro-

phyll and phycoerythrin fluorescence. Detection of the two groups was performed by the comparison of flow cytograms where cells were discriminated based on their side scatter signals (SSC) and both red (FL3) and orange fluorescence (FL2) as well as FL3 versus FL2. The cytograms were analyzed using the Cell Quest Pro software, with manual gating to discriminate the different populations. For the picophytoplankton biovolume estimates, the diameters of 20 cells of each group in a sample from thaw lake KWK12 were measured under epifluorescence microscopy at 1000× magnification, and were then converted to spherical biovolumes. The measured cell diameters (\pm SD) were $1.0 \pm 0.2 \mu\text{m}$ for picocyanobacteria and $2.0 \pm 0.5 \mu\text{m}$ for picoeukaryotes, giving biovolumes per cell of 0.52 and $4.19 \mu\text{m}^3$, respectively.

2.5 RNA sampling and analysis

Water samples from the near surface (0.2 m depth) and near bottom (0.2 m above sediments) of the black palsa lake SAS2A were first pre-filtered through a $20 \mu\text{m}$ mesh to remove larger organisms and then filtered sequentially through a $3 \mu\text{m}$ pore-size, 47 mm diameter polycarbonate filter (DHI) and a $0.2 \mu\text{m}$ Sterivex unit (Millipore) with a peristaltic pump. From 100 to 300 mL of water were filtered and the filtration was stopped after 2 h to minimize RNA degradation. The $3 \mu\text{m}$ filter for larger cells (L fraction) and the $0.2 \mu\text{m}$ filter for the smaller fraction (S fraction) were both preserved in RNA later (Life Technologies) and then stored at -80°C until extraction.

Samples were extracted with the AllPrep DNA/RNA Mini Kit (Qiagen). This protocol was modified by the addition of cross-linked polyvinylpyrrolidone (PVP, Alfa Aesar) (UV light-sterilized) to a final concentration of 10 % before loading the samples onto the lysate homogenization column. For all samples, the extracted RNA was converted to cDNA immediately with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems-Ambion) and stored at -80°C until analysis. The V4 region of the eukaryotic 18S rRNA that had been converted to cDNA was amplified using the 454 primers as described in Comeau et al. (2011). PCR was carried out in a total volume of $50 \mu\text{L}$, the mixture contained HF Buffer 1X (NEB), $0.25 \mu\text{M}$ of each primer, $200 \mu\text{M}$ of each dNTP (Life Technology), 0.4 mg mL^{-1} BSA (NEB), 1 U of Phusion[®] High-Fidelity DNA polymerase (NEB) and $1 \mu\text{L}$ of template cDNA. Two more reactions with 5X and 10X diluted template were also carried out for each sample, to minimize potential primer bias. Thermal cycling began with an initial denaturation at 98°C for 30 s, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 270 s. The three dilution reactions were pooled and purified with a magnetic bead kit Agencourt AMPure XP (Beckman Coulter) and then quantified spectrophotometrically with the Nanodrop 1000 (Thermo Fisher Scientific). The amplicons were sequenced on 1/8 plates of the Roche 454 GS-FLX

using the “PLUS” chemistry at the IBIS/Laval University, Plate-forme d’analyses génomiques (Québec City, QC). The reads have been deposited in the NCBI short read archive as SRP060634.

Sequences were analyzed using the UPARSE pipeline (Edgar, 2013). For quality filtering, the sequences were truncated at 245 bp to keep 50 % of the reads at the 0.5 expected error rate. Singletons as well as chimeras were then removed, and operational taxonomic units (OTUs) were determined at the $\geq 98\%$ similarity level. These OTUs were classified using the mothur classifier (Schloss et al., 2009) with a 0.8 confidence threshold based on the SILVA reference database (Pruesse et al., 2007) modified to include sequences from our in-house, curated northern 18S rRNA gene sequence database (Lovejoy et al., 2015). In order to compare samples, the OTU tables were each subsampled 100 times at 2200 reads, which corresponded to the lowest number of reads per sample minus 10 %; this subsampling used the command `multiple_rarefaction_even_depth.py` in QIIME (Caporaso et al., 2010). The most abundant and unclassified OTUs were subsequently submitted to a BLASTn search to the nr database in NCBI GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the nearest match.

2.6 Statistical analysis

The normal distribution of environmental variables was tested using the Kolmogorov–Smirnov test, and right-skewed variables were normalized by natural logarithm transformation. Given the order of magnitude differences in picophytoplankton abundances and pigment concentrations among samples, the HPLC and flow cytometry data were also normalized by logarithmic transformation. Correlations within and among the phytoplankton, pigment and environmental variables were tested by Pearson correlation analysis, with correction for multi-testing using the false discovery rate procedure as in Benjamini and Hochberg (1995). To investigate the extent to which environmental variables drove the distribution of pigment diversity among the different water bodies, a redundancy analysis (RDA, Legendre and Legendre, 2012) was run. This was based on Bray–Curtis distances for the pigment matrix (db-RDA) and the data were log-transformed prior to analysis. The significance of the model was assessed via 1000 permutations, and the analysis was performed in R (R Core Team, 2014) using the Vegan package (Oksanen et al., 2015). Stepwise multiple linear regression models were performed using Past 3.04, with secondary cross-correlated variables removed prior to analysis.

3 Results

3.1 Environmental heterogeneity

The thaw lakes spanned a wide range of environmental conditions, including water color and CDOM, with the latter

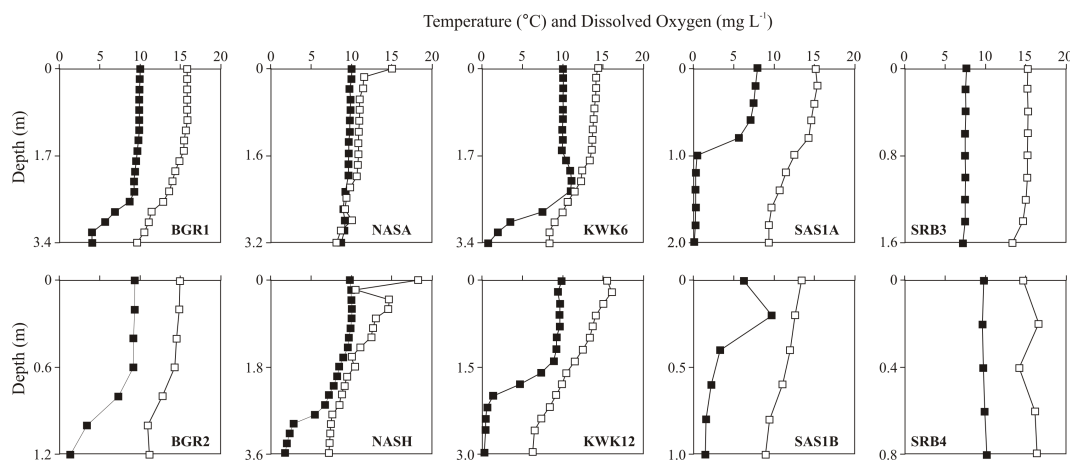


Figure 2. Temperature (white squares) and oxygen (black squares) stratification in the permafrost thaw lakes (BGR, KWK, NAS, SAS) and shallow rock-basin lakes (SRB) in summer 2012. The codes at the bottom of each graph refer to specific lakes.

strongly correlated with DOC ($R = 0.67$, $p < 0.0001$). The highest DOC concentrations (up to 17 mg L^{-1}) and CDOM (up to 117 m^{-1}) were recorded in the SAS lakes (Table 1). These waters were black in color and also had the lowest pH values (6.0–6.6). The highest total nutrient concentrations (up to $125 \text{ } \mu\text{g TPL}^{-1}$ and 4 mg TNL^{-1}) were recorded in lakes located within the KWK and NAS valleys, and the values were lowest in the shallow rock-basin waters (minima of $1.6 \text{ } \mu\text{g TPL}^{-1}$ and 0.1 mg TNL^{-1}). Nitrogen to phosphorus ratios varied greatly among the 17 lakes, from 4 to 131 (g g^{-1}), and total suspended solids were similarly variable, from 1 to 320 mg L^{-1} (Table 1). The NAS valley waters contained especially high concentrations of suspended clay particles, producing an opaque milky appearance. Despite their shallowness and small size, the thaw lakes were highly stratified in terms of temperature and oxygen (Fig. 2), with anoxic bottom waters in the SAS and KWK lakes. Some had pronounced thermal gradients, with temperature differences up to $10 \text{ } ^\circ\text{C}$ between the surface and bottom waters. In contrast, the reference lakes showed more homogenous conditions, indicative of mixing (Fig. 2).

3.2 Planktonic pigments

Phytoplankton abundance, as measured by Chl *a* concentrations, varied greatly among the waterbodies (Table 1), from 0.4 (SRB1) to 6.8 (KWK6) $\mu\text{g L}^{-1}$ in 2011 and from 0.2 (SRB1) to 9.1 (KWK1) $\mu\text{g L}^{-1}$ in 2012. There was also a small but significant difference in Chl *a* concentrations between years, with means of 3.7 and $2.6 \text{ } \mu\text{g L}^{-1}$, respectively (paired *t* test, $t = 2.5$, $p = 0.02$). On average, Chl *a* was significantly higher in the thaw lakes than the reference rock-basin waters: the overall means were 3.3 and $2.0 \text{ } \mu\text{g Chl } a \text{ L}^{-1}$, respectively.

The pigment analyses of the phytoplankton (Table 2) showed that there were diverse communities including

fucoxanthin-containing groups (potentially diatoms, chrysophytes and certain dinoflagellates), chlorophytes (Chl *b*, lutein and violaxanthin), cryptophytes (alloxanthin), dinoflagellates (peridinin) and cyanobacteria (zeaxanthin, canthaxanthin, echinenone). The pigments Chl *c*₁, *c*₂, *c*₃ and crocoxanthin were also present, but generally at trace concentrations, and only in certain lakes. The abundance of cyanobacterial populations in KWK, BGR and NAS lakes was indicated by high concentrations of zeaxanthin (e.g., NASH) and echinenone (SRB3). The KWK lakes had high concentrations of zeaxanthin (up to 0.7 nmol L^{-1} in KWK23), accompanied by high concentrations of fucoxanthin and green algal pigments (lutein and violaxanthin), as well as high concentrations of diadinoxanthin (e.g., KWK1). In the SAS lakes, a dominance of dinoflagellates was indicated by high concentrations of peridinin. Echinenone was present in KWK and SRB lakes and high concentrations of violaxanthin were also recorded in BGR lakes. Fucoxanthin groups were abundant in SRB and SAS as well as in NASH and BGR2. The turbid thaw lakes within the NAS valley had high concentrations of β , β -carotene. Relatively high levels of ancillary photosynthetic pigments were present in NASA and SAS lakes as well as in some waters of shallow rock-basin lakes (Table 2). Photoprotective pigments were relatively more abundant in KWK lakes (notably KWK1 and KWK6) as well as in the SRB waters (violaxanthin), and less abundant in the DOC-rich SAS lakes (Table 2). The bottom waters of the thaw lakes also contained diverse planktonic pigments, including high levels of diadinoxanthin and alloxanthin in KWK lakes, fucoxanthin in BGR2 and Chl *b* in SRB lakes. High levels of bacteriochlorophyll *d* indicating abundant populations of green photosynthetic sulfur bacteria were recorded in the deeper, anoxic waters of KWK lakes (Table 3, Fig. 3).

For the overall data set, Chl *a* concentrations were significantly correlated with TP ($R = 0.47$; $p = 0.05$), and with TSS ($R = 0.55$; $p = 0.03$), which were themselves

Table 1. Limnological characteristics of the subarctic lakes. Surface values for temperature (T), pH, dissolved organic carbon concentration (DOC), colored dissolved organic matter (CDOM), total suspended solids (TSS), soluble reactive phosphorus (SRP), total phosphorus (TP), total nitrogen (TN), nitrate (NO_3) and Chlorophyll a (Chl a). The values are means from 2011 and 2012 (\pm range in brackets; nd denotes no data from 2011).

Sites	T (°C)	pH	DOC (mg L^{-1})	CDOM (m^{-1})	TSS (mg L^{-1})	SRP ($\mu\text{g L}^{-1}$)	TP ($\mu\text{g L}^{-1}$)	TN (mg L^{-1})	NO_3 (mg N L^{-1})	Chl a ($\mu\text{g L}^{-1}$)
Thaw lakes on northern clay soils										
BGR1	15.1 (0.7)	7.5 (0.2)	3.9 (0.4)	6.1 (1.7)	2.9 (0.5)	1.8 (0.6)	17.3 (3.5)	0.1 (0.0)	0.05 (0.0)	1.8 (1.0)
BGR2	14.5 (0.4)	7.0 (0.3)	9.0 (0.3)	39.1 (5.7)	19.2 (6.1)	2.4 (1.0)	45.7 (0.2)	0.3 (0.0)	0.05 (0.0)	3.4 (1.4)
NASA	15.6 (nd)	7.0 (nd)	3.0 (nd)	12.3 (nd)	319.3 (nd)	2.9 (nd)	124.5 (nd)	3.7 (nd)	0.25 (nd)	4.1 (nd)
NASH	18.3 (nd)	7.6 (nd)	4.1 (nd)	22.6 (nd)	18.2 (nd)	6.2 (nd)	28.5 (nd)	0.4 (nd)	0.04 (nd)	1.7 (nd)
Thaw lakes on southern clay soils										
KWK1	17.1 (4.4)	6.1 (0.8)	9.2 (2.8)	39.0 (16.4)	14.1 (12.0)	2.2 (1.5)	36.8 (26.7)	0.3 (0.1)	0.05 (0.0)	8.0 (1.2)
KWK6	14.9 (0.8)	6.8 (0.4)	5.2 (0.0)	10.7 (0.7)	9.3 (1.1)	1.0 (0.4)	30.9 (3.0)	0.2 (0.0)	0.06 (0.0)	4.4 (1.6)
KWK12	16.8 (0.8)	8.0 (1.1)	8.6 (0.7)	39.6 (3.7)	13.8 (2.6)	1.6 (0.0)	27.7 (2.1)	0.2 (0.0)	0.08 (0.0)	2.5 (0.1)
KWK23	14.9 (0.2)	6.7 (0.2)	7.2 (0.6)	35.8 (1.9)	10.2 (1.9)	4.9 (0.6)	47.7 (5.6)	0.2 (0.0)	0.04 (0.0)	3.5 (1.9)
Thaw lakes on peatlands										
SAS1A	14.3 (0.2)	6.6 (0.3)	10.7 (0.8)	68.7 (2.7)	7.6 (2.6)	1.6 (0.3)	14.3 (0.9)	0.5 (0.1)	0.17 (0.0)	3.6 (1.2)
SAS1B	13.6 (0.1)	6.3 (0.3)	15.9 (0.4)	109.5 (3.9)	21.8 (5.4)	1.9 (0.4)	12.7 (2.2)	0.6 (0.1)	0.07 (0.0)	4.3 (0.1)
SAS2A	19.9 (nd)	6.2 (nd)	14.9 (nd)	98.4 (nd)	2.6 (nd)	3.1 (nd)	9.6 (nd)	0.7 (nd)	0.04 (nd)	1.2 (nd)
SAS2B	16.0 (nd)	6.0 (nd)	17.1 (nd)	116.9 (nd)	5.2 (nd)	1.3 (nd)	10.3 (nd)	0.5 (nd)	0.11 (nd)	0.9 (nd)
Shallow rock-basin lakes										
SRB1	15.8 (2.2)	7.6 (0.6)	9.9 (0.0)	46.0 (6.5)	2.6 (1.2)	2.1 (0.6)	7.9 (2.8)	0.2 (0.1)	0.06 (0.0)	0.3 (0.1)
SRB2	13.8 (0.5)	7.6 (1.1)	13.2 (2.7)	68.8 (21.5)	1.3 (0.3)	1.4 (0.5)	11.2 (2.6)	0.2 (0.1)	0.14 (0.1)	1.4 (0.4)
SRB3	15.6 (0.2)	6.6 (0.2)	7.8 (1.4)	34.5 (9.5)	5.4 (2.0)	1.1 (0.1)	13.4 (2.8)	0.3 (0.1)	0.05 (0.0)	5.2 (0.6)
SRB4	15.0 (0.3)	7.9 (0.5)	10.4 (1.6)	20.0 (0.5)	5.0 (1.9)	0.8 (0.1)	5.7 (2.6)	0.8 (0.4)	0.44 (0.4)	2.4 (1.0)
SRB5	18.7 (1.8)	7.1 (0.9)	3.7 (0.1)	9.4 (2.7)	0.7 (0.1)	0.5 (0.2)	2.9 (1.3)	0.1 (0.0)	0.32 (0.2)	0.8 (0.0)

strongly correlated ($R = 0.87$; $p < 0.0001$). A forward stepwise linear regression showed that Chl a was best described by a combination of the accessory pigments alloxanthin ($p < 0.014$), fucoxanthin ($p < 0.001$) and Chl b ($p < 0.001$): $\ln \text{Chl } a = 1.774 + 0.161 \ln \text{Allo} + 0.380 \ln \text{Chl } b + 0.341 \ln \text{Fuco}$ ($R^2 = 0.85$; $p < 0.001$).

Several pigments were highly cross-correlated. These included alloxanthin and lutein ($R = 0.81$, $p < 0.001$) and both pigments with Chl b ($R = 0.71$; 0.92 , $p < 0.001$). The chlorophyte pigment violaxanthin was also correlated with Chl b ($R = 0.60$, $p < 0.001$) and fucoxanthin ($R = 0.58$, $p < 0.001$). Fucoxanthin itself was most strongly correlated with diadinoxanthin ($R = 0.77$, $p < 0.001$). The cyanobacterial pigments echinenone and canthaxanthin were significantly correlated ($R = 0.57$, $p < 0.001$), but not with zeaxanthin ($p > 0.05$). The summations within the two categories of pigments, photoprotective and photosynthetic, were also positively correlated ($R = 0.63$; $p < 0.001$). Consistent with the multivariate analyses, the accessory pigments were uncorrelated with individual environmental variables (all corrected p values were > 0.05), with the exception of lutein. This chlorophyte pigment was significantly correlated with TP ($R = 0.53$; $p = 0.01$), but this may simply reflect the strong

correlation between lutein and Chl a ($R = 0.79$; $p < 0.0001$), which itself correlated with TP.

The db-RDA model showed a clear separation of the different valleys in terms of phytoplankton pigment composition. These distinct patterns in pigment composition were partially explained by the influence of valley-specific environmental variables. In addition, the results from db-RDA reaffirmed the large lake-to-lake heterogeneity in each of the valleys, even among nearby lakes. The first two canonical components were related to TP, DOC and pH and explained 11 % of the total variance in pigment composition (Fig. 4). The db-RDA model as a whole explained 16.4 % of the variance ($R^2 = 0.164$, $F = 1.71$, $df = 8$, $p = 0.012$).

3.3 Picophytoplankton abundance and biovolume

Picophytoplankton concentrations varied greatly among the lakes (Fig. 5). The picocyanobacterial abundances ranged from 1.8×10^3 cells mL^{-1} (SAS1B) to 5.9×10^5 cells mL^{-1} (KWK23), equivalent to biovolume concentrations of 9.5×10^2 (SAS1B) to $3.1 \times 10^5 \mu\text{m}^3 \text{mL}^{-1}$ (KWK23), while the picoeukaryote abundances ranged from 1.35×10^2 cells mL^{-1} (SAS2B) to 4.6×10^5 cells mL^{-1} (KWK1), equivalent to biovolume concentrations of

Table 2. The dominant photosynthetic and photoprotective accessory pigments (nmol L^{-1}), their sums (Σ) and ratio in each of the subarctic water bodies sampled in 2012.

Sites	Photosynthetic					Photoprotective							Ratio
	Allo	Chl <i>b</i>	Fuco	Perid	Σ	Cantha	Diadino	Echin	Lut	Viola	Zea	Σ	
Thaw lakes on northern clay soils													
BGR1	0.282	0.108	0.174	0.034	0.598	0.000	0.113	0.045	0.126	0.132	0.080	0.497	1.2
BGR2	0.053	0.159	0.611	0.343	1.167	0.073	0.483	0.088	0.372	0.442	0.677	2.134	0.5
NASA	2.078	0.401	0.101	0.000	2.580	0.000	0.000	0.000	0.745	0.308	0.000	1.053	2.4
NASH	0.161	0.204	0.740	0.000	1.106	0.184	0.464	0.208	0.208	0.568	1.692	3.324	0.3
Thaw lakes on southern clay soils													
KWK1	0.641	1.681	0.702	0.458	3.481	0.147	3.836	0.202	1.435	0.847	0.375	6.843	0.5
KWK6	0.248	0.760	0.429	0.075	1.513	0.093	0.398	0.000	1.335	0.771	0.561	3.158	0.5
KWK12	0.290	0.202	0.569	0.151	1.213	0.046	0.358	0.089	0.337	0.354	0.111	1.295	0.9
KWK23	0.281	0.236	0.499	0.057	1.073	0.046	0.443	0.026	0.644	0.450	0.688	2.296	0.5
Thaw lakes on peatlands													
SAS1A	0.498	0.130	1.080	0.110	1.817	0.083	0.291	0.000	0.161	0.366	0.092	0.993	1.8
SAS1B	1.066	0.271	1.484	0.363	3.184	0.044	0.435	0.000	0.294	0.420	0.083	1.275	2.5
SAS2A	0.342	0.054	0.199	0.000	0.594	0.000	0.047	0.000	0.047	0.000	0.000	0.093	6.4
SAS2B	0.606	0.163	0.502	0.000	1.271	0.036	0.049	0.045	0.220	0.171	0.000	0.521	2.4
Shallow rock-basin lakes													
SRB1	0.018	0.038	0.051	0.017	0.124	0.000	0.026	0.008	0.045	0.069	0.026	0.175	0.7
SRB2	0.196	0.220	0.360	0.040	0.816	0.037	0.078	0.057	0.229	0.183	0.062	0.646	1.3
SRB3	1.026	0.352	2.011	0.261	3.650	0.103	0.460	0.219	0.557	0.924	0.530	2.792	1.3
SRB4	0.094	0.203	0.557	0.050	0.905	0.016	0.159	0.080	0.333	0.340	0.101	1.028	0.9
SRB5	0.066	0.048	0.420	0.004	0.537	0.010	0.085	0.031	0.064	0.199	0.087	0.477	1.1

Key: Allo, alloxanthin; Chl *b*, chlorophyll *b*; Fuco, fucoxanthin; Perid, peridinin; Cantha, canthaxanthin; Diadino, diadinoxanthin; Echin, echinenone; Lut, lutein; Viola, violaxanthin; Zea, zeaxanthin.

Table 3. The relative concentration of bacteriochlorophyll *d* (BChl *d*, $\mu\text{g L}^{-1}$) based on chromatogram peak area at 430 nm. The KWK thaw sites have been arranged from lowest to highest concentrations of pigment.

Lake	Date	Depth (m)	BChl <i>d</i> ($\mu\text{g L}^{-1}$)
KWK6	4 Aug 2012	3.1	1.2
KWK6	21 Aug 2011	3.1	1.3
KWK23	4 Aug 2012	2.0	1.9
KWK12	3 Aug 2012	2.0	8.2
KWK12	19 Aug 2011	2.5	24.6
KWK1	19 Aug 2011	2.0	28.9
KWK23	4 Aug 2012	3.3	36.2
KWK23	21 Aug 2011	3.3	44.3
KWK1	3 Aug 2012	2.0	44.7
KWK12	3 Aug 2012	2.5	47.3

5.6×10^2 (SAS2B) to $1.9 \times 10^6 \mu\text{m}^3 \text{mL}^{-1}$ (KWK1). In general, the lakes located on clays (KWK and BGR) contained the highest cell concentrations and biovolume of total picophytoplankton. The shallow rock-basin (SRB)

and peatland lakes (SAS) were apparently less favorable, with picocyanobacterial and picoeukaryote biovolume concentrations below $10^4 \mu\text{m}^3 \text{mL}^{-1}$. Picoeukaryotes were generally less numerically abundant than picocyanobacteria, but because of their larger cell size, they often dominated the total picophytoplankton biomass. Picoeukaryotes accounted on average ($\pm\text{SD}$) for 65 (± 28) % of total picophytoplankton biovolume, however there was a wide range among lakes in this contribution, from 8 % (SAS2B) to 99 % (SAS1A).

Total picophytoplankton biovolume increased with Chl *a* concentration ($R = 0.52$; $p = 0.03$), but this relationship was only significant for the eukaryotic component ($R = 0.53$; $p = 0.02$). Picocyanobacteria correlated negatively with DOC ($R = -0.47$; $p = 0.05$), while picoeukaryotes correlated negatively with conductivity ($R = -0.48$; $p = 0.05$). Picocyanobacteria were highly correlated with zeaxanthin ($R = 0.72$; $p = 0.0002$), and there was also a significant, albeit less strong correlation between picoeukaryotes and zeaxanthin ($R = 0.54$; $p = 0.02$). Stepwise multiple linear regression models showed that picophytoplankton (picoeukaryotes, PEuk; picocyanobacteria, PCyan) biovolumes were statistically correlated to certain limnological variables according to the relationships: $\text{PEuk} = 14.9 + 2.9 \times \text{Chl } a - 1.7 \times \text{TN}$

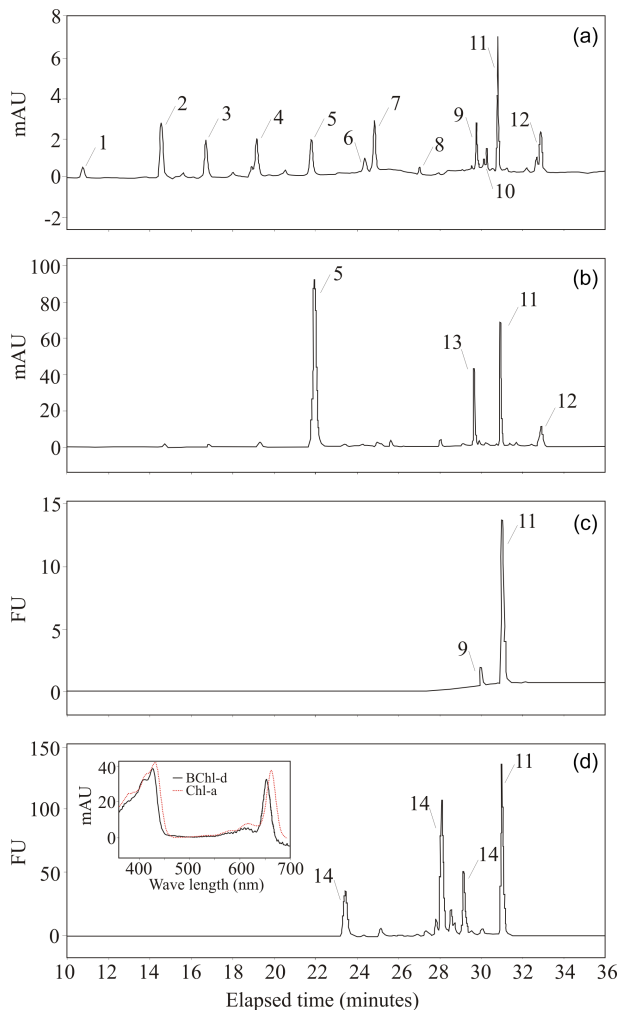


Figure 3. High-performance liquid chromatograms of KWK12 sampled on 3 August 2012: absorbance for the surface (a) or bottom (b) water layers and fluorescence for the surface (c) or bottom (d) water layers (mAU denotes measured absorption units; FU denotes fluorescence units). Pigments from left to right: 1. Perid, 2. Fuco, 3. Viola, 4. Diadino, 5. Allo, 6. Zea, 7. Lut, 8. Cantha, 9. Chl *b*, 10. Echin, 11. Chl *a*, 12. β , β -Carotene, 13. Croco and 14. BChl *d*. Insert in (d): Bacteriochlorophyll *d* (Bchl *d*, black line) and Chl *a* (red line) absorption spectra.

($R^2 = 0.56$, $p = 0.001$), and $\text{PCyan} = -2.9 + 4.3 \text{ Temp} + 1.1 \times \text{Chl } a - 1.1 \times \text{TSS} + 1.5 \times \text{TP} - 1.2 \times \text{DOC}$ ($R^2 = 0.67$, $p = 0.001$).

3.4 Molecular analyses

The 18S rRNA data set from the palsa thaw lake (SAS2A) contained large numbers of rotifer sequences (400 to 1350 reads per sample, all with closest matches to the genus *Ascomorpha*) and these were removed prior to further analysis. This left a total of 3857 and 3128 reads for the surface L ($> 3.0 \mu\text{m}$) and S ($< 3.0 \mu\text{m}$) fractions, and 3522 and 2457

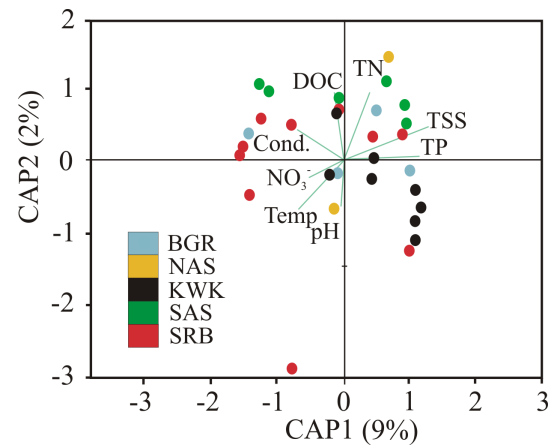


Figure 4. Multivariate analysis by db-RDA of phototrophic pigment composition in the thaw and SRB lakes. Each circle represents an individual lake. The labeled lines represent the significant environmental vectors resulting from correlation analysis.

reads for the bottom L and S fractions; 84 to 93 % of these eukaryotic sequences could be assigned (≥ 98 % identity) to phylum in the modified SILVA database. The largest fraction of total reads was attributable to ciliates (up to 33 % in the surface waters and 74 % in the bottom waters; Table 4), including the genus *Stokesia*, especially in the surface waters, and the genera *Cryptocaryon*, *Halteria*, *Peniculida* and *Cyclidium*, especially in the bottom waters (Table 5). Among the groups nominally considered as phytoplankton were dinoflagellates, chrysophytes and chlorophytes, with lesser proportions of reads associated with katablepharids, bacillariophytes (diatoms) and cryptophytes (Table 4). Analysis of the dissimilarity distances (Bray–Curtis distance on the subsampled data set) showed that community structure greatly differed with depth (Bray–Curtis dissimilarity index of 0.795 for the large fraction and 0.820 for the small fraction), and to a much lesser extent between large and small fractions (Bray–Curtis dissimilarity index of 0.423 for the surface samples and 0.312 for the bottom samples). Chlorophytes, dinoflagellates, katablepharids and diatoms were more represented in the large surface water fraction.

4 Discussion

Each of the subarctic thaw lakes contained pigments from several phytoplankton phyla, revealing that these abundant waters provide habitats for diverse phototrophic groups. The most abundant accessory pigment (apart from β , β -carotene present in all algal groups) was fucoxanthin, indicating the possible presence of diatoms, chrysophytes or certain dinoflagellates. Peridinin and alloxanthin were also present in many of the samples, indicating the presence of dinoflagellates and cryptophytes, respectively (Jeffrey et al., 2011). Diatoms would be less favored in these stratified wa-

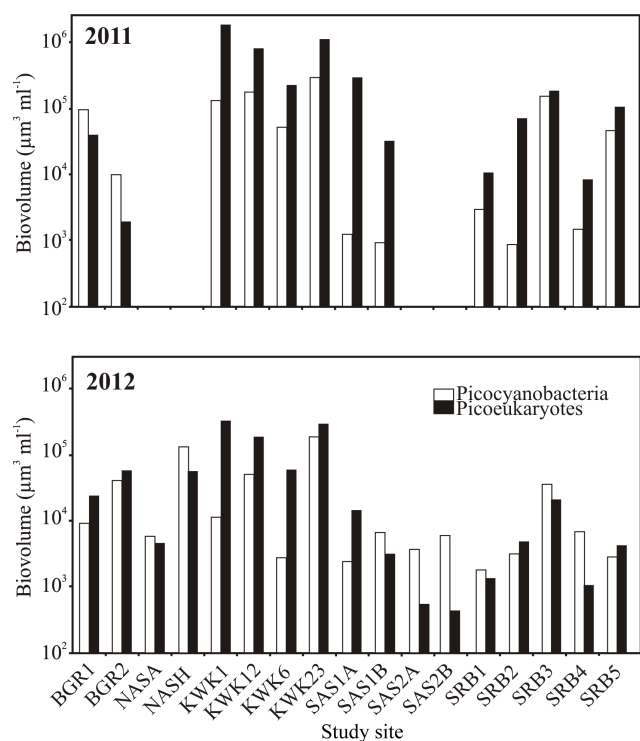


Figure 5. Picophytoplankton biovolume in the surface water of shallow rock-basin (SRB) and permafrost thaw lakes located on clays (KWK, BGR, NAS) and peatlands (SAS).

ters given their relatively high sinking rates, while flagellated taxa including chrysophytes, dinoflagellates and cryptophytes would be able to maintain their position in the euphotic zone. Mixotrophic chrysophytes and dinoflagellates have been observed in many high-latitude lakes (Charvet et al., 2012; and references therein), and may be additionally favored by the high biomass concentrations of heterotrophic bacteria that occur in some of these waters (Breton et al., 2009; Roiha et al., 2015). Green algae were also well represented at most sites, indicating that despite the strong light attenuation by CDOM and TSS (Watanabe et al., 2011), there is adequate light availability for obligate phototrophs. Another conspicuous feature of the pigment data was the large variation in pigment characteristics among lakes within the same valley, even between adjacent waterbodies. This large within-valley variation has also been observed in bacterial communities in the region (Crevecoeur et al., 2015; Comte et al., 2015).

The concentrations of photoprotective pigments were conspicuously high in NASH relative to photosynthetic pigments (Table 2). This was unexpected given that this lake contained elevated concentrations of suspended solids, which indicate a low light availability for photosynthesis, and a lack of need for protection against bright light. It is possible, however, that in this lake, cells suspended in the mixed layer are adapted to intermittent exposure to bright light

Table 4. RNA sequence analysis of eukaryotes in samples from permafrost thaw lake SAS2A, sampled in 2012. Each value is the % number of reads of the total for each sample (total number of reads minus rotifer sequences). The large fraction was retained on a 3 µm filter and the small fraction was on a 0.2 µm filter after passing through the 3 µm pre-filter.

Taxonomic group	Percentage of reads			
	Surface		Bottom	
	Large	Small	Large	Small
Phytoplankton groups				
Dinophyta	17.11	4.02	8.71	1.90
Chrysophyta ^a	14.47	14.81	9.07	3.60
Chlorophyta ^b	9.97	2.03	2.11	0.40
Cryptophyta	3.10	1.71	2.39	0.65
Katablepharidophyta	2.71	1.00	0.11	0.20
Bacillariophyta	2.47	0.44	0.96	0.45
Raphidophyceae	0.51	0.33	0.00	0.04
Pavloales	0.44	0.19	0.18	0.04
Prymnesiales	0.15	0.03	0.06	0.00
Other groups				
Ciliophora	22.94	43.55	62.95	83.90
Cercozoa	6.17	12.37	2.50	1.22
Fungi ^c	1.47	0.61	0.32	0.04
Centromeliozoa	1.44	0.80	0.11	0.12
Choanoflagellida	1.32	3.12	0.23	0.20
Perkinsea	0.00	0.00	0.03	0.12
Unknown affinities	15.75	15.00	10.28	7.11

^a includes Chrysophyceae, Synurophyceae and Bicosoecida; ^b includes Chlorophyceae and Trebouxiophyceae; ^c includes Chytridiomycota, Oomycota and Ascomycota.

rather than the average water column irradiance. Such conditions have been observed in a turbid estuarine environment, where the phytoplankton were photosynthetically adapted to high near-surface irradiances rather than the overall shade or dark conditions experienced on average by the cells as they were circulated by turbulent mixing through the water column (Vincent et al., 1994). In contrast, the ratio of photosynthetic to photoprotective pigments was high in NASA and the SAS lakes, indicating acclimation to low irradiances in their strongly light-attenuating water columns.

The pigment analyses also indicated the abundant presence of cyanobacteria. Echinonone and canthaxanthin are well-known photoprotective pigments in cyanobacteria, with the latter especially prevalent in Nostocales, which may suggest the presence of nitrogen-fixing taxa. These results are consistent with bacterial 16S rRNA analyses, which showed the presence of cyanobacterial taxa in some of these lakes that had strong affinities (>99% sequence similarity) to the Nostoclean *Dolichospermum curvum* (Crevecoeur et al., 2015). Zeaxanthin can potentially occur in high cellular concentrations in cyanobacteria, but it also is found in eukaryotic

Table 5. Closest identity (ID) of eukaryotic RNA sequences from permafrost thaw lake SAS2A to GenBank sequences (following a BLASTn search), at the lowest taxonomic level identified.

GenBank taxonomy	Accession number	Isolation source	%	Percentage of reads	
				ID	Surface
Phytoplankton groups					
Chrysophyta					
<i>Uroglena</i> sp.	EU024983	FU44-26	99	6.03	0.03
<i>Paraphysomonas</i> sp.	JQ967316	Freshwater	99	0.67	3.78
<i>Dinobryon divergens</i>	KJ579346	WO33_4	99	0.40	0.00
<i>Spumella</i> -like flagellate	AY651098	Lake Mondsee	99	0.03	0.76
Cryptophyta					
<i>Cryptomonas tetrapyrenoidosa</i>	KF907407	Deokam032610	99	1.71	0.87
<i>Cryptomonas pyrenoidifera</i>	KF907397	CNUCRY 166	99	0.33	0.00
<i>Cryptomonas curvata</i>	KF907377	CNUCRY 90	99	0.06	0.28
Dinophyta					
<i>Dinophyceae</i> sp.	GQ423577	Lake Baikal	99	1.31	0.58
<i>Peridinium wierzejskii</i>	KF446619	Baikal region	99	1.03	2.07
<i>Gyrodiniellum shiwhaense</i>	FR720082	Shiwha Bay	98	0.49	0.01
Bacillariophyta					
<i>Urosolenia eriensis</i>	HQ912577	Y98-8	98	1.22	0.58
Chlorophyta					
<i>Lemmermannia punctata</i>	JQ356704	SAG 25.81	99	1.07	0.08
<i>Chlorella</i> sp.	Y12816	OvS/Ger1	99	1.00	0.00
<i>Choricystis</i> sp.	AY195972	AS-29	99	0.89	0.11
<i>Koliella longiseta</i>	HE610126	SAG 470-1	99	0.72	0.04
<i>Monoraphidium</i> sp.	KP017571	LB59	99	0.39	0.00
Raphidophyta					
<i>Gonyostomum semen</i>	KP200894	Freshwater	100	0.23	0.02
Prymnesiales					
<i>Chrysochromulina parva</i>	EU024987	FU44-40	100	0.09	0.03
Other groups					
Ciliophora					
<i>Stokesia vernalis</i>	HM030738	Freshwater	99	8.65	0.04
<i>Cryptocaryon</i> sp.	JF317699	Drinking water	99	0.89	5.61
<i>Peniculida</i> sp.	GQ330632	Peat-bog water	98	0.83	1.85
<i>Halteria</i> sp.	GU067995	Lake water	99	0.49	6.38
<i>Cyclidium marinum</i>	JQ956553	Marine coast	99	0.00	34.42
Rhizaria					
<i>Cercozoa</i>	AB771834	Lake Kusaki	99	3.83	0.28
Fungi					
<i>Saprolegnia</i> sp.	FJ794911	Lake (parasite)	99	0.11	0.00
<i>Penicillium brevicompactum</i>	KP981369	ATCC 16024	99	0.00	0.10

algal groups. This pigment is a component of photoprotective xanthophyll cycles, and may co-occur with other components of these cycles. For example, studies on the diatom *Phaeodactylum tricornerutum* have shown the co-occurrence of the diadinoxanthin cycle and the violaxanthin cycle (Lohr and Wilhelm, 1999). Consistent with this co-occurrence, we found a strong correlation between diadinoxanthin and vio-

laxanthin in the studied lakes ($R = 0.72$, $p < 0.001$). We also observed high concentrations of zeaxanthin, which is often associated with cyanobacteria but also chlorophytes (Jeffrey et al., 2011). Given the molecular analyses results of thaw lake bacterial communities (Crevecoeur et al., 2015) and our flow cytometry data, zeaxanthin was likely to be at least in part associated with the abundant picocyanobacteria in the

order Synechococcales. The strong correlation between picocyanobacteria and zeaxanthin further supports this relationship.

HPLC analysis has been used in a variety of aquatic ecosystems to not only identify major algal groups, but also to quantify their proportional representation using the software program CHEMTAX (Mackay et al., 1996). However, given the large known variation in pigment ratios in algal cells, this method requires extensive calibration for each class of waters. For example, in a shallow eutrophic lake, CHEMTAX gave a reliable estimation of cyanobacterial and chlorophyte biomass, but not chrysophytes and dinoflagellates (Tamm et al., 2015). The latter were two of the dominant groups in the permafrost thaw lakes, and although CHEMTAX offers a potentially useful approach for future analyses of these waters, further work will be required before it can be calibrated and reliably applied.

The presence of bacteriochlorophyll *d* in high concentrations in KWK lakes containing anoxic bottom waters indicate that these environments are favorable habitats for photosynthetic sulfur bacteria. These results were unexpected given the strong attenuation of light by the CDOM and suspended particles in these waters, and the low photosynthetically available radiation at depth. However, the results are consistent with molecular analyses of the bacterial assemblages. 16S rRNA gene clone library analysis of KWK lakes detected the presence of green sulfur bacteria (Rossi et al., 2013), and high throughput 16S rRNA sequencing revealed that the green sulfur bacterium *Pelodictyon* (Chlorobi) was one of the most abundant sequences in KWK waters (Crevecoeur et al., 2015). The high concentrations of bacteriochlorophyll *d* suggest that these populations could play an important role in the overall primary production of certain thaw lakes, albeit restricted to anoxic conditions well below the surface, and our observations extend the range of environments in which this pigment has been detected.

Picophytoplankton occurred in all of the sampled lakes, but with large differences among waters in terms of the relative abundance of prokaryotes versus eukaryotes, and probably also in terms of their contribution to the total phytoplankton community biomass. As a first estimate of the relative contribution of picocyanobacteria, their cell concentrations may be converted to equivalent Chl *a* by an appropriate cell conversion factor. Analysis of *Synechococcus* in culture under different irradiance regimes gave a median value around 7.5 fg Chl *a* per cell (Moore et al., 1995), and applying this value as a first-order estimate, picocyanobacteria would contribute 0.3 % (SAS1B) to 80 % (KWK23) of the measured total community Chl *a*. Such estimates are highly approximate given the known variation in the cellular content of this pigment among strains and with growth conditions; for example, by a factor of 4 as a function of irradiance (Moore et al., 1995). However these calculations imply large lake-to-lake variations in the percentage contribution of picophyto-

plankton to the total community, ranging from negligible to major.

In general, the concentrations of both picocyanobacteria and picoeukaryotes increased with increasing total phytoplankton biomass, as measured by Chl *a* concentrations. However, the two groups differed in their correlative relationships with other limnological variables. In partial support of our initial hypothesis that DOC would be a controlling variable, picocyanobacteria, but not eukaryotes, were negatively correlated with DOC. An inverse relationship with DOC was also found for picophytoplankton in Swedish lakes (Drakare et al., 2003). Similarly in Lake Valkea-Kotinen, in the boreal zone of Finland, variations in autotrophic picoplankton were most closely correlated with water column stability, which in turn was strongly regulated by DOC concentration (Peltonmaa and Ojala, 2012). High-DOC waters are often characterized by low pH, which may be a constraint on certain cyanobacteria, however acid-tolerant picocyanobacteria are known (Jasser et al., 2013). Even in the low-pH SAS waters, picocyanobacteria were always present, although in low concentrations (e.g., the minimum of $1.8 \times 10^3 \text{ mL}^{-1}$ in SAS2B in 2011). Other factors such as zooplankton grazing may also have played a role in controlling picocyanobacteria (Rautio and Vincent, 2006), although this seems less likely for picocyanobacteria given that they are a nutritionally deficient food source for zooplankton in thaw lakes (Przytulska et al., 2015).

Picocyanobacteria did not show the expected relationship with temperature in the correlation analyses, although temperature was one of the variables retained in the multiple linear regression analysis. Temperature has often been identified as a key variable for cyanobacterial growth and dominance in lakes elsewhere. For example, in reservoirs in the southeastern USA, there was a strong, positive correlation between picocyanobacterial cell concentrations and temperature, while picoeukaryotes showed an inverse correlation, and dominance of the picophytoplankton community shifted from picoeukaryotes in winter to picocyanobacteria in summer (Ochs and Rhew, 1997). Similarly, increasing temperature favored picocyanobacteria over picoeukaryotes in German lakes (Hepperle and Krienitz, 2001). In experiments with subarctic lake and river water at 10 and 20 °C, the concentration of Chl *a* in the picoplankton fraction increased substantially at the warmer temperature (Rae and Vincent, 1998). The temperature range in the present study may have been too restricted to observe such effects.

The molecular data provided insight into the large diversity of microbial eukaryotes that occur within the plankton of thaw lake ecosystems, including heterotrophic components such as ciliates and flagellates that may exert grazing pressure on some of the phototrophs. When rotifers were excluded from the analyses, ciliates were dominant in the RNA sequences of SAS2A. This likely reflects not only their cellular abundance but also their large cell sizes with a concomitantly large number of ribosomes; for example, *Stokesia ver-*

nalis, the most abundant sequence identified in the surface waters (Table 5), can be $> 100 \mu\text{m}$ in length. Ciliates are also known to be fragile cells that are easily broken up during manipulation like pre-filtration through a $20 \mu\text{m}$ mesh, which could account for their highly abundant sequences in the S as well as L fractions.

Chrysophytes and chlorophytes were well represented in the RNA sequences, particularly in the surface water L fraction, consistent with their abundance as indicated by the pigment data. Dinoflagellates constituted the dominant fraction of the phytoplankton sequences, yet were not detected as peridinin in SAS2A, although this pigment was present in two other SAS lakes. This may indicate the presence of large dinoflagellate cells, for example rigid *Ceratium* cells that can extend up to $100 \mu\text{m}$ in length, but may also be due to the presence of dinoflagellates that lack the accessory pigment peridinin. It should also be noted that the relative abundance of taxa in these analyses may additionally reflect PCR biases in amplification (Potvin and Lovejoy, 2009). Diatoms can also include large cell types, but their representation in the sequences was small, suggesting that most of the fucoxanthin that we measured was associated with chrysophytes such as *Uroglena* (Table 5) rather than diatoms. It is of interest that diatoms from the genus *Urosolenia* were the closest match following a BLAST search. This diatom is lightly silicified and may be less susceptible to sedimentation in these well-stratified waters. The cryptophyte pigment alloxanthin was in high concentration in SAS2A, as in the other thaw lakes, yet cryptophyte sequences accounted for $< 1.5\%$ of the total RNA reads. This might reflect the small cell-size of certain cryptophyte taxa, for example *Chroomonas*.

The molecular data also provided insight into the nature of the picoeukaryotic communities in the SAS lakes. The taxonomic identities (Table 5) indicated the presence of several chlorophyte genera that are known to produce small cells, notably *Choricystis*, *Lemmermannia*, *Monoraphidium* and *Chlorella*. For example, in subalpine Lake Tahoe (USA), *Choricystis coccoides* produces cells that are only $0.5 \mu\text{m}^3$ in volume, too small to be grazed by calanoid copepods in that lake (Vincent, 1982). Among the chrysophytes, *Spumella* and related genera are known to produce small cells. For all of these analyses, the many unidentified eukaryotic reads add an extra element of uncertainty to the interpretation, but collectively, these data underscore the eukaryotic diversity of the thaw lake ecosystem. This parallels the large prokaryotic diversity that has been observed in these lakes, including bacterial phototrophs (Crevecoeur et al., 2015; Comte et al., 2015).

Permafrost thaw lakes receive large quantities of allochthonous organic carbon from their surrounding catchments and this is reflected in their high DOC and CDOM concentrations, as observed in the present study. These waters have high respiratory oxygen demands and are net heterotrophic, resulting in prolonged hypoxia or anoxia in the bottom waters during summer, and anoxia throughout the

water column once ice covers the lake in the winter (Deshpande et al., 2015). The abundant ciliate and nanoflagellate sequences in our molecular analyses also point to high productivity by bacterial heterotrophs, their likely prey in these waters. However, despite these multiple signs of intense heterotrophy, the pigment, cytometry and molecular results in the present study show that these ecosystems are also the habitats for abundant phototrophs from diverse taxonomic groups.

5 Conclusions

The wide range of thaw lakes sampled in the present study significantly differed from the reference rock basin lakes in their limnological properties. On average, they contained higher phytoplankton (Chl *a*) and TP concentrations than the reference lakes, but had a comparable diversity of pigments, dominated by chlorophyte, chrysophyte and dinoflagellate pigments. Cyanobacteria and cryptophytes were also well represented, but the thaw waters appeared to be less favorable for diatoms, at least during the highly stratified late-summer period. Picophytoplankton occurred in all of the thaw lakes, in some of the waters at high biovolume up to $10^6 \mu\text{m}^3 \text{mL}^{-1}$, but the proportion of eukaryotes and prokaryotes and their contribution to total phytoplankton biomass varied greatly among the lakes. Molecular analysis of samples from one thaw lake in the degraded permafrost, peatland valley indicated that small cell chlorophytes may be among the dominants in the picoeukaryotic fraction. Despite the heterotrophic nature of these organic-rich ecosystems, with respiration likely exceeding photosynthesis throughout the year, permafrost thaw lakes contain abundant, diverse phototrophs that potentially support higher trophic levels, and that will lessen the net- CO_2 release from these waters to the atmosphere.

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