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Drivers of protistan community autotrophy and heterotrophy in chemically stratified Antarctic lakes

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ABSTRACT: Single-celled, eukaryotic microorganisms, known as protists, are responsible for 2 important, yet opposing, metabolic activities within aquatic food webs. They are major primary producers and highly active predators in marine and fresh water systems. While genomics has accelerated in recent years for this taxonomically diverse group, our understanding of the metabolic capabilities of most protists remains limited. It is also poorly understood how protist trophic mode is affected by biotic and abiotic factors, and therefore it is difficult to predict how events such as global climate change will affect the balance between autotrophic and heterotrophic activities in protist communities. To address open questions regarding how protist metabolic versatility is influenced by their environment, we characterized the potential for carbon fixation versus organic carbon degradation using enzymatic assays (RubisCO and β-D-glucosaminidase, respectively) within the water columns of ice-covered lakes in McMurdo Dry Valleys (MDV), Antarctica. Steep physical and chemical gradients in the water columns, microorganism domination and minimal allochthonous inputs makes the MDV lakes uniquely suited to investigate environment–microbe interactions. Spatial trends in RubisCO and β-D-glucosaminidase activities were lake-specific and vertically stratified within the water columns. Moreover, bottom-up drivers controlling the activity of C-fixation vs. organic C-degradation among the MDV protist communities were distinct between the upper photic vs. the deep, aphotic zones. We conclude that differential controls over major C-cycling enzymes have important implications on the influence of environmental change on the carbon and nutrient cycles in the MDV lakes.

KEY WORDS: Aquatic protists · Autotrophy · Heterotrophy · β-D-glucosaminidase · RubisCO · McMurdo Dry Valleys · Antarctic lakes

INTRODUCTION

Permanently ice-covered lakes in the McMurdo Dry Valleys (MDV) of South Victoria Land, Antarctica, have closed basins and extremely stable strata that vary physically, chemically, and biologically within and between stratified water columns. The ice-covers (3–4 m in thickness) restrict water column mixing and external inputs. Ephemeral glacial melt streams, which flow for only a few weeks in the austral summer, are the primary mechanism of water and nutrient input to the lakes (Lyons et al. 2000). This study focused on 3 MDV lakes (Lake Bonney, Lake Fryxell and Lake Vanda), which have been sites of a long-term study since 1993 (www.mcm.lter.org). All 3 lakes are light-limited and either oligotrophic (Lakes Bonney and Vanda) or mesotrophic (Lake Fryxell) (Priscu 1995). Low light and limited nutrients combined with year-round low temperatures and a lack of sunlight in the winter greatly limit yearly production. The food webs in MDV lakes are microbially dominated, and lack crustacean zooplankton and fish.

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Microbial eukaryotes (or protists) play dual roles in MDV food webs as primary producers and top predators (i.e. consumers of bacteria and small phytoplankton) (Priscu et al. 1999, Bielewicz et al. 2011). Recent applications of 18S rRNA sequencing provided the first insight into the diversity and function of the microbial eukaryote populations (Bielewicz et al. 2011, Vick-Majors et al. 2014, Rojas-Jimenez et al. 2017). Spatial and seasonal trends in MDV protist community structure are complex and exhibit strong variability among lakes (Bielewicz et al. 2011, Kong et al. 2012b, 2014, Vick-Majors et al. 2014, Dolhi et al. 2015). Lake Bonney is dominated by chlorophytes *Chlamydomonas* in the shallow layers, and haptophytes *Isochrysis* and stramenopiles *Nannochloropsis* in the chemocline. Lake Fryxell is dominated by cryptophytes *Geminigera* (Bielewicz et al. 2011, Kong et al. 2012b, Dolhi et al. 2015) and prasinophytes *Pyramimonas* (Vincent 1981, Roberts et al. 2000). The least productive lake, Lake Vanda, harbors nanoplankton related to non-flagellated chlorophytes *Nannochloris* and stramenopiles *Heterococcus* (Dolhi et al. 2015).

Heterotrophic grazing by protists was measured in several MDV lakes (Roberts & Laybourn-Parry 1999, Marshall & Laybourn-Parry 2002, Thurman et al. 2012), suggesting that heterotrophic protists might play important roles in top-down control of phytoplankton and bacterioplankton populations. Mixotrophy is widespread in ice-covered Antarctic lakes, allowing exploitation of alternative nutrient and energy sources (Nygaard & Tobiesen 1993, Laybourn-Parry et al. 2005, Li et al. 2016). Recent studies in Lake Bonney and Lake Fryxell supported these observations with phylogenetic evidence of heterotrophic nanoflagellates (e.g. choanoflagellates and heterotrophic chrysophytes) (Bielewicz et al. 2011, Vick-Majors et al. 2014). Recent reports also found evidence for the important role of Cryptomycota and Chytridiomycota fungi (Rojas-Jimenez et al. 2017) and heterotrophic nanoflagellates (Li et al. 2016) as parasites and predators in the food webs in MDV lakes.

Lakes and reservoirs cover the globe, forming a network of environmental sensors which can be exploited as sentinels of climate change (Williamson et al. 2009). Seasonally and perennially ice-covered, permanently low-temperature lakes are highly prevalent in polar and alpine environments. Unlike temperate ecosystems, the MDV lakes are relatively low temperature ecosystems which have experienced low human impact, representing end members of the global lake network (Morgan-Kiss & Dolhi 2012). Relative to lower latitude watersheds, Antarctic, Arctic and high alpine aquatic habitats such as the Tibetan Plateau are more responsive to both climate warming and increased episodic events; however, physiological responses of polar microbial communities are also influenced by local environmental conditions (Morgan-Kiss & Dolhi 2012). Thus, predicting the impact of climate change on functional diversity of the polar lake protist communities is complex.

Several studies have shown that trophic modes of protist communities are sensitive to biotic and abiotic factors. Environmental drivers, including solar radiation (Batistas Navarro et al. 2011, Izaguirre et al. 2012), temperature (O'Connor et al. 2009, Wohlers et al. 2009), nutrients and other physicochemical conditions (Brown et al. 2000, Phillips et al. 2008) can have strong effects on protist activities and therefore their roles in the aquatic carbon and nutrient cycles. The impact of protist trophic mode is likely amplified in stratified, microbial-driven systems such as the MDV lakes; however, an understanding of the relationship between environmental factors and protist trophic activities in the MDV lakes is currently lacking. Improved quantification of trophic activities for MDV protists would contribute to a better understanding of polar lake food webs, building more reliable and predictive models on plankton community interactions, carbon and nutrient fluxes, and the sensitivity of these to environmental change.

Most photosynthetic protists utilize the Calvin-Benson-Bassham (CBB) cycle for autotrophic carbon fixation. The enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) catalyzes the first and rate-limiting reaction. The radiometric ^{14}C RubisCO carboxylase activity assay has conventionally been used as a measure of maximum potential of autotrophic carbon fixation and has been adapted for use in natural phytoplankton community samples (Tortell et al. 2006, Dolhi et al. 2012, Kong et al. 2012a).

In phagotrophic and pinocytic protist food uptake, prey cells and other food particles are sequestered in acidic food vacuoles where digestive enzymes such as lysozymes and proteases degrade complex organic compounds into simpler substrates for subsequent heterotrophic metabolism (Vrba et al. 1996, Verni & Gualtieri 1997). While several methods are available for estimating protistan grazing rates using fluorescently labeled bacterial or artificial prey (Sherr & Sherr 1993, Sherr et al. 1997, Smalley et al. 1999), quantifying protist heterotrophic activity in natural environments is challenging. Accurate estimates can be confounded by selective grazing of the predators and/or spatiotemporal shifts in prey abundance. Low productivity systems such as the MDV lakes are also near the limit of detection. As an alternative approach, planktonic phagotrophic activity can be quantified based on specific lysozyme activity associated with β-D-glucosaminidase (βGAM) enzymatic activity (Zubkov & Sleigh 1998, Štrojsová & Dyhrman 2008, Sintes & del Giorgio 2010, 2014). The optimal pH condition for protist vacuolar βGAM is lower (pH <5) compared to the exoenzymes of copiotrophic bacteria (pH ≥7) (Vrba et al. 1996, Arnosti 2011).

In the microbially dominated MDV lakes, protistan carbon fixation and phagotrophic activity are critical to carbon and nutrient cycling. Several studies have reported on the distribution and activity of mixotrophic and heterotrophic eukaryotes in these lakes based on artificial prey feeding experiment or microscopic numeration (Laybourn-Parry et al. 1995, James et al. 1998, Roberts & Laybourn-Parry 1999, Roberts et al. 2004a, Thurman et al. 2012), but direct rate measurements of these 2 critical processes in the Dry Valley lakes are currently lacking. We hypothesize that (1) the trophic activities of protists are stratified in the MDV lake water columns due to highly stratified environmental conditions; (2) these activities are correlated with specific environmental drivers. In this study, we investigated autotroph and protist-specific heterotroph distribution using RubisCO and acid-βGAM enzyme activities, respectively, in the physically and chemically distinct MDV lakes and correlated activity with specific biotic and abiotic factors.

MATERIALS AND METHODS

Field sampling and limnological parameters

Locations of study sites within the Taylor (Lakes Bonney and Fryxell) and Wright (Lake Vanda) Valleys of the MDV, Antarctica, are shown in Fig. 1. Water column samples were collected during the austral summer (November–December) of the 2012 field season with the exception of samples for Lake Vanda RubisCO activity, which were collected in December 2011. All sampling depths were measured from the piezometric water level in the ice hole using a depth-calibrated hand winch. Water samples were collected with a 5 l Niskin bottle and stored under low temperature and dark conditions until processing. Water samples for RubisCO carboxylase activity (2–5 l) and βGAM activity (0.75–2 l) were vacuum filtered (0.3 mbar) onto 47 mm GF/C filters (CAS#: 1822-047, Whatman) and hydrophilic nylon membrane filters (CAS#: 7404-004, Whatman) with a

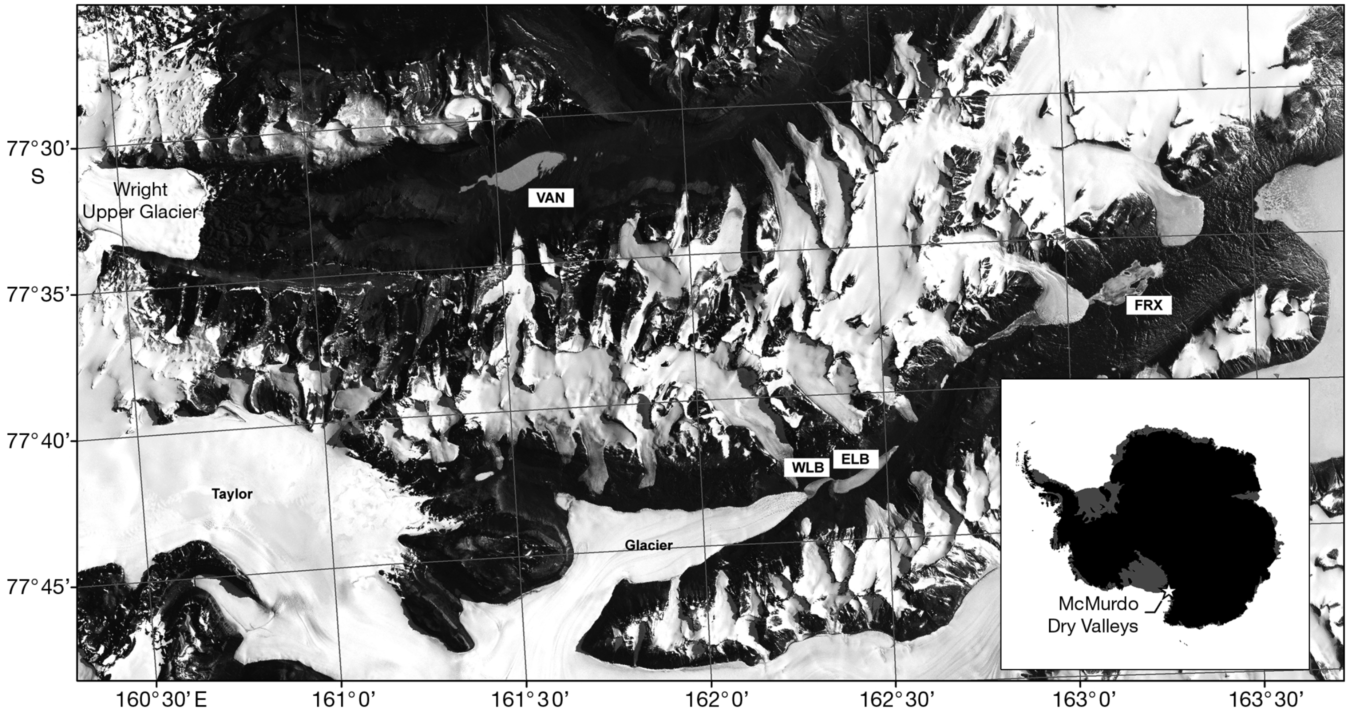

Fig. 1. Map showing locations of study sites within the McMurdo Dry Valleys, Antarctica (see inset): Lake Bonney east (ELB) and west (WLB) lobes, Lake Fryxell (FRX) and Lake Vanda (VAN). Image was edited from a LIMA based map from the Polar Geospatial Center (www.agic.umn.edu/maps/Antarctic)

0.45 µm pore size, respectively. Filters were immediately flash frozen in liquid N_2 and shipped to our US laboratory on dry ice. Samples were stored at −80°C for less than 2 mo before determining enzyme activity. Prior to the field season, both enzymatic assays were optimized. Fresh versus frozen mock filters were also compared to assess the effect of freezing on enzymatic activity.

Photosynthetically active radiation (PAR), temperature, chlorophyll *a* (chl *a*), primary productivity (PPR), and nutrient (NH_4^+, NO_3^- and soluble reactive phosphorus [SRP]) concentrations were determined through the water columns of the east and west lobes of Lake Bonney (ELB and WLB, respectively), Lake Fryxell, and Lake Vanda. PAR was measured with a LI-193 spherical quantum sensor (LI-COR Biosciences). Temperature was measured with a Seabird model 25 profiler (Spigel & Priscu 1998). Chl *a* concentrations were determined using a profiling spectrofluorometer (FluoroProbe, BBE Moldaenke) (Carraro et al. 2012). The abundance of major phytoplankton groups including green algae (Chlorophyta and Euglenophyta), Cryptophyta, Haptophyta, Chrysophyceae and Cyanobacteria were discriminated using the FluoroProbe based on their specific light harvesting pigment fluorescence (Beutler et al. 2002). This method has been routinely applied into other studies in these lakes (Winslow et al. 2014, Dolhi et al. 2015). Light-mediated PPR was determined by measuring $NaH^{14}CO_3$ incorporation in duplicate over a 24 h *in situ* incubation. Nutrients were measured as part of the NSF-funded McMurdo long-term ecological research (MCM-LTER) program according to the methodology outlined in the MCM-LTER limnological manual (www.mcm.lter.org). Briefly, inorganic nitrogen species were determined with a Lachat autoanalyzer and SRP was measured manually using the antimony-molybdate method (Strickland & Parsons 1972).

RubisCO carboxylase activity assay

GF/C filters were thawed on ice and cut in half. All subsequent extraction steps were carried out on ice. Each cut half filter was transferred to a 1.5 ml screw cap tube containing 0.1 mm zirconia/silica beads and 650 µl ice cold RubisCO extraction buffer (50 mM bicine, 10 mM $MgCl_2$, 1 mM EDTA, 5 mg ml^{-1} bovine serum albumin [BSA], and 0.1% triton X-100; add freshly made: 20 mM $NaHCO_3$, 10 mM DTT, 0.1 mg ml^{-1}, pH 7.8). Filters were disrupted with a Minibead-Beater-16 (Biospec Products) for 3 cycles of 30 s at speed setting 48 with alternating 1 min on ice incubations. The lysate was transferred to a cold 1.5 ml microcentrifuge tube and cleared by centrifugation at 4°C for 2 min at 15 000 × *g*. The soluble lysate was used in RubisCO carboxylase assays.

RubisCO carboxylase activity was measured in the soluble cell lysates (200 µl) within 30 min of extraction. Soluble cell lysates were incubated at 25°C for 1 min before RubisCO activity was measured using a modified $NaH^{14}CO_3$ assay from Tortell et al. (2006) as described previously (Dolhi et al. 2012, Kong et al. 2012a). Briefly, RubisCO was activated with $MgCl_2$ (8 mM) and $NaH^{14}CO_3$ (ViTrax, Placentia; specific activity in final reaction: 0.03 µCi mmol^{-1}) for 5 min at 25°C. The carboxylase assay was initiated by addition of 20 µl of the substrate, Ribulose-1,5-bisphosphate (RuBP; CAS #: 24218-00-6, Sigma-Aldrich; 15 mM) and the reaction was allowed to proceed for 5 min at 25°C. The reaction was stopped with 100 µl of 100% propionic acid and unincorporated ^{14}C was exhausted by centrifugation in a fume hood for 1.25 h at 2000 × *g*. Soluble cell lysate with no RuBP substrate added was used in the assay as a negative control. A multipurpose scintillation counter LS6500 (Beckman Coulter) was used to quantify acid stable end products. RubisCO carboxylase activity in the Lake Bonney psychrophillic alga *Chlamydomonas* sp. UWO241 was previously shown to be stable and optimal at 25°C (Morgan-Kiss & Dolhi 2012).

Glucosaminidase activity assay

Acidic βGAM activity was measured as particulate enzyme activity retained on filters. Nylon filters were thawed on ice and extraction was carried out on ice. Filters were disrupted by 3 cycles of bead beating in 2 ml of ice cold extraction buffer (0.1 M acetate, 0.1% 23 lauryl ether [Brig 35], pH 4.6 with glacial acetic acid). βGAM activity was measured in soluble lysates (250 µl) using a β-*N*-Acetyl-glucosaminidase Assay Kit (CAS#: 9012-33-3, Sigma-Aldrich). Cell lysates were incubated with the fluorogenic substrate 4-methylumbelliferyl-*n*-acetyl-α-D-glucosaminide (4-MUF) in the dark at 20°C for 4 h. Nano-pure water and purified β-*N*-Acetyl-glucosaminidase from Jack beans (standard with assay kit) were used as negative and positive controls, respectively. Fluorescence intensity was quantified in a Lambda-35 spectrofluorometer (Perkin-Elmer). A standard curve was created with known amounts of the product of the enzymatic reaction, 4-MUF (Vrba et al. 1993, Zubkov & Sleigh 1998).

βGAM activity was also measured in 3 psychrophilic phytoplankton strains, *Chlamydomonas* sp. ICE-MDV, *Isochrysis* sp. MDV and *Geminigera cryophila* to test for temperature sensitivity and specificity of the βGAM activity. *Chlamydomonas* sp. ICE-MDV and *Isochrysis* sp. MDV were isolated from Lake Bonney (Li et al. 2016, Raymond & Morgan-Kiss 2017), while *G. cryophila* (CCMP 2564) was isolated from Ross Sea near McMurdo Sound. All 3 algae were grown in either autotrophic (F/2 or L1) or heterotrophic media (F/2 supplemented with 5% cereal grass). βGAM activity was measured in the soluble lysate across several incubation temperatures in a homemade temperature gradient apparatus.

In order to select filter material with optimal enzyme recovering capability, various filter types (glass fiber, polyethersulfone, and nylon) were also tested using water samples from a local lake (Acton Lake, OH). The test results indicated that sample extraction from nylon filters produced the highest enzyme yield (data not shown).

Protein concentration determination

Protein concentration was determined in the soluble cell lysate extracted for RubisCO and βGAM assays according to the Bradford method using BSA (CAS #: 9048-46-8, Sigma-Aldrich) as a standard (Bradford 1976).

Bacterial enumeration

Quantitation of free-living bacteria in the lake water columns was carried out using a method modified from Lebaron et al. (1994). Lake water (2 ml) from each depth was fixed with paraformaldehyde at a final concentration of 2% v/v for 30 min. Fixed samples were filtered on 25 mm black polycarbonate membrane with a pore size of 0.2 µm. Bacteria cells were stained with a fluorochrome (4′,6-diamidino-2-phenylindole, [DAPI], CAS #: 28718-90-3, Sigma-Aldrich) followed by epifluorescent microscopic enumeration. Filters were examined using an Olympus AX-70 Multi-mode System with a specific filter set (EX 360/40 nm, EM 460/50 nm) for DAPI staining, and digital images of at least 15 random views were taken. Images were then calibrated, and bacterial counts from each image were recorded using ImageJ software (V1.47, National Institutes of Health). The bacterial concentration of each depth was determined by using the average bacterial counts of each image.

Statistical analyses

Abiotic parameter data including PAR, temperature, NH_4^+, NO_3^-, SRP^-, dissolved inorganic N:P ratio (N:P), dissolved inorganic carbon (DIC), and conductivity were normalized using z-scores. Principal component analysis (PCA) was carried out in PAST software (V3.07, Hammer et al. 2001). All other statistical analyses were performed using JMP Pro 12 software (SAS Institute). Data of physical (except temperature), chemical, and biological parameters were log-transformed, and the correlation between these parameters and enzyme activities were determined using Pearson correlation analysis. In order to identify linear models of enzyme activities (respondents) with abiotic and biotic factors (variables), we generated all possible linear models and selected the models with lowest Akaike Information Criterion (AIC) value to obtain the models with the least number of parameters and the maximum explanatory power (Akaike 1974, Sakamoto et al. 1986).

RESULTS

Optimization of enzyme assays

We have previously reported on optimization of the RubisCO enzyme assay in natural samples from Lake Bonney (Dolhi et al. 2012, Kong et al. 2012a) and tested the temperature range of RubisCO activity in a Lake Bonney isolate (*Chlamydomonas* sp. UWO241) (Morgan-Kiss & Dolhi 2012). This latter study showed that despite the fact that *Chlamydomonas* sp. UWO241 is a psychrophile (Morgan et al. 1998), RubisCO is not cold-adapted and exhibits maximum activity at assay temperatures ≥20°C (Morgan-Kiss & Dolhi 2012).

Since the βGAM assay has not been previously applied to protist communities of the MDV lakes, we tested βGAM activity in 3 psychrophilic, polar algal isolates: 2 from Lake Bonney (*Chlamydomonas* sp. ICE-MDV and *Isochrysis* sp. MDV), and a third from a polar marine environment (*Geminigera cryophila*). *Chlamydomonas* sp. ICE-MDV and *Isochrysis* sp. MDV are dominant phytoplankton of Lake Bonney, while a cryptophyte closely related to the marine *G. cryophila* dominates the water column in Lake Fryxell (Bielewicz et al. 2011, Kong et al. 2012b, Dolhi et al. 2015). In addition, previous studies have reported that *Isochrysis* sp. MDV and *G. cryophila* are mixotrophic, while *Chlamydomonas* sp. ICE-MDV is an obligate photoautotroph (Gast et al. 2014, Li et al. 2016). We compared βGAM across these 3 psychrophile isolates grown under autotrophic vs. heterotrophic conditions. As expected βGAM activity was below detectable levels in the pure photoautotroph, *Chlamydomonas* sp. ICE-MDV (Table S1 in the Supplement at www.int-res.com/articles/suppl/a082p225_supp.pdf). In contrast, βGAM activity was detectable in the mixotrophs *Isochrysis* sp. MDV and *G. cryophila,* and was maximal when both strains were grown in the presence of cereal grass (Table S1).

We also tested the effect of assay temperature on βGAM activity in *Isochrysis* sp. MDV and *G. cryophila* (Fig. S1 in the Supplement). Over a range of incubation temperatures βGAM activity of both psychrophiles increased slightly with increasing temperature; although, no significant difference was observed between 10 to 20°C (Fig. S1). Therefore, we concluded that similar to RubisCO, βGAM activity in the psychrophiles does not cold-adapt. Thus, since the temperature in the MDV lakes can widely vary (from −6 to over 20°C), we chose 20°C as the temperature for enzyme assays on environmental samples.

Lake Bonney

Lake Bonney is separated into 2 lobes, east and west. The water columns of each lobe remain isolated from each other for most of the year, with the exception of a narrow passage which connects the upper photic zones for a few weeks during the austral summer. Thus, ELB and WLB share some common characteristics, including strong thermohaline stratification (Fig. 2) and phosphorus limitation (Fig. 3A,C); however, the deeper layers of each lobe are permanently separated. For both lobes, maximum PAR values were at or below 20 µmol photons m^{-2} s^{-1} (Fig. 3B,D). Temperatures in the shallow depths and the chemoclines ranged between 2 and 4°C in both lobes, but declined to below 0°C in the monimolimnion of WLB (Fig. 3B,D). Both lobes exhibit SRP levels near the limit of detection throughout the water columns and early studies reported that phytoplankton and bacterial communities are limited by phosphorus (Priscu 1995, Ward et al. 2003). Nitrogen (NH_4^+, NO_3^-) was low in the surface waters of both lobes. Spatial trends in several chemical variables are distinct in the monimolimnia below the chemoclines, in particular salinity (Fig. 2) and concentration of several inorganic nitrogen species (Fig. 3A,C). For example, ELB exhibits high levels of NO_3^- below the chemocline, suggesting a lack of significant rates of denitrification (Ward et al. 2005). Chl *a* maxima were present in the shallow and deep layers of both lobes of Lake Bonney; the deep chlorophyll maximum (DCM) was well defined in WLB, reaching a maximum chl *a* concentration of 15 µg l^{-1} (Fig. 4A,B). Spatial trends in bacterial biomass were uncoupled from phytoplankton in WLB. Maximum bacterial biomass occurred above the chemocline in both lobes, but peaked at different depths (5 m for ELB and 10 m for WLB; Fig. 4A,B).

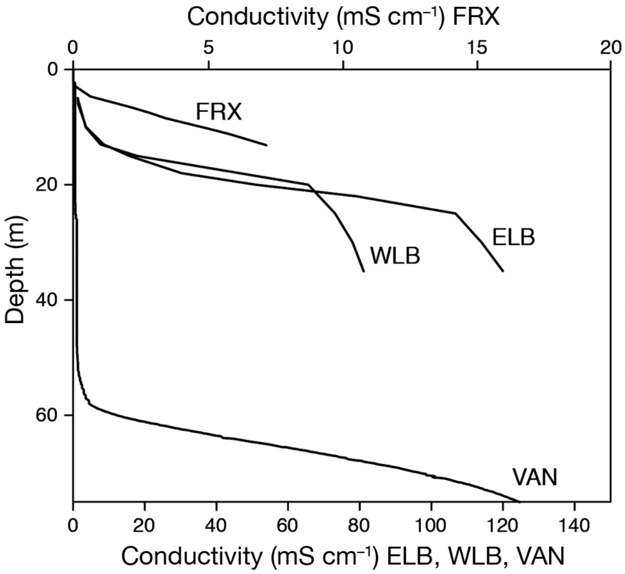

Fig. 2. Salinity profiles for Lake Bonney (east lobe, ELB; west lobe, WLB), Lake Fryxell (FRX) and Lake Vanda (VAN). Note that Lake Fryxell is plotted on a secondary *x*-axis

We used RubisCO and βGAM enzyme activities to understand how spatial variability in physiochemical and biological parameters impacts relative contributions of autotrophic and heterotrophic metabolism, respectively, in the food webs of MDV lakes. Enzyme activity was expressed on the basis of either total extracted protein (Fig. 5A–D) or volume of filtered lake water (Fig. 5E–H). Activity for both enzymes was higher in ELB relative to WLB. On a protein basis, RubisCO activity peaked at 13 m in both lobes. Spatial trends in βGAM activity were distinct from RubisCO activity, indicating that the importance of the 2 metabolic processes is variable at different layers within the lakes. Both lobes exhibited a peak in βGAM activity in shallow depths (5 m); however, WLB also exhibited a distinct peak in the chemocline (15 m depth). Spatial trends in βGAM were comparable on a protein or lake water basis; however, trends in RubisCO activity were

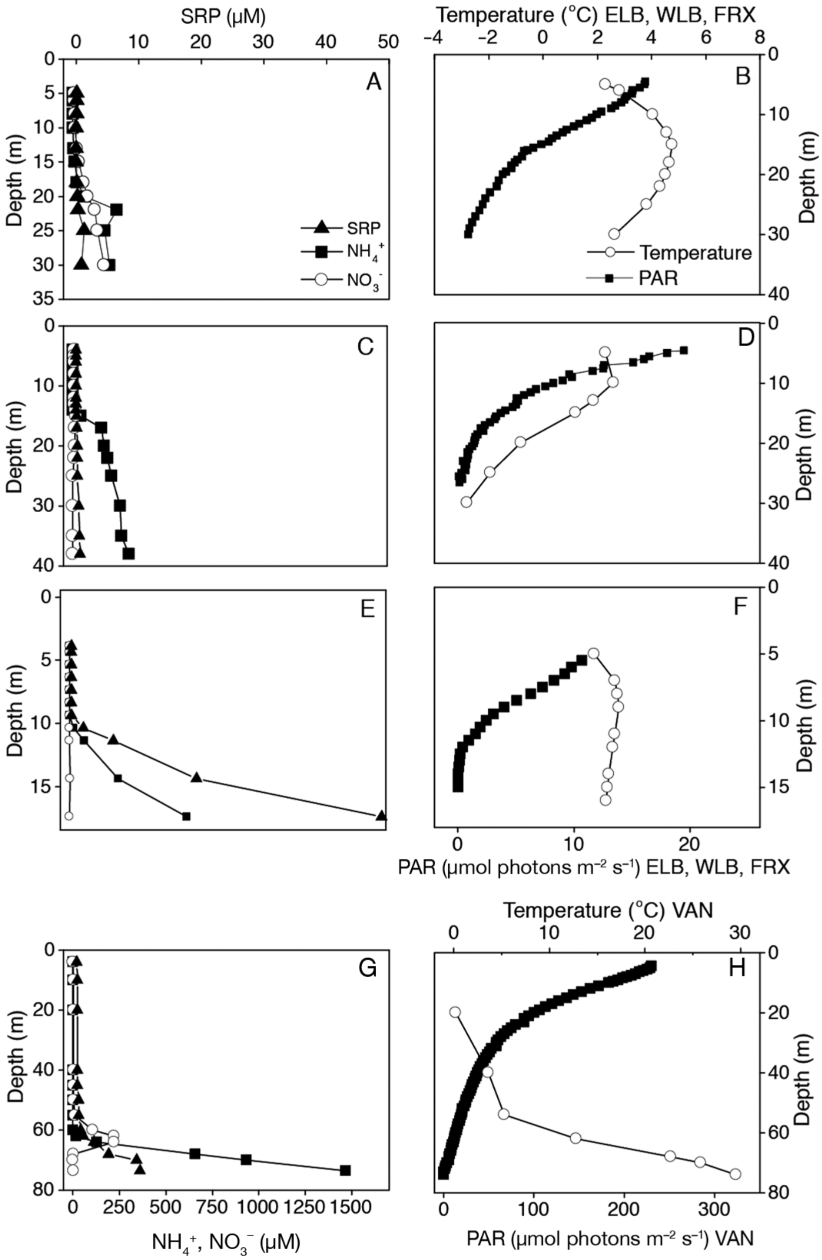

Fig. 3. Depth profiles of physical and chemical characteristics for the 3 lakes in this study: (A,B) Lake Bonney east lobe (ELB) and (C,D) west lobe Lake Bonney (WLB), (E,F) Lake Fryxell (FRX), and (G,H) Lake Vanda (VAN). Note that photosynthetically active radiation (PAR) and temperatures for Lake Vanda are plotted on secondary *x*-axes. SRP: soluble reactive phosphorus

dependent upon which variable was used for normalization of the data.

Lake Fryxell

Lake Fryxell is the least saline (maximum conductivity ~7 mS cm^{-1}) and shallowest (20 m maximum depth) of the 3 lakes in this study (Fig. 2), and exhibited very low PAR levels (maximum PAR <10 µmol photons m^{-2} s^{-1}) (Fig. 3F). In common with Lake Bonney, nutrient concentrations were low in the upper oxic zone. SRP levels were significantly higher in the deeper zones of Lake Fryxell relative to Lake Bonney and a recent study showed that Lake Fryxell phytoplankton communities are co-limited for nitrogen and phosphorus (Teufel et al. 2017). Higher nutrient levels supported the highest levels of chl *a* and bacterial biomass in the water column of Lake Fryxell compared with other MDV lakes (Fig. 4C), peaking at 9 m and 7 m for chl *a* and bacterial biomass, respectively.

On a protein basis, RubisCO activity peaked above the chemocline (7 m); however, on a lake water volume basis RubisCO activity peaked in the deep anoxic zone (14 m) (Fig. 5C,G). βGAM activity levels in Lake Fryxell were comparable with Lake Bonney; however, no spatial trends were noted.

Lake Vanda

Lake Vanda is the deepest lake (maximum depth 80 m) in our study and exhibited fresh shallow water and hypersaline bottom water which was comparable to Lake Bonney (Fig. 2). PAR levels under the ice were 5–10 × higher relative to Lakes Bonney and Fryxell, and the maximum temperature was above 20°C at the bottom of the water column (Fig. 3F). Nutrient concentrations for most of the water column (above 60 m depth) were low or at undetectable levels, and SRP levels in Lake Vanda were comparable with Lake Bonney (i.e. maximum SRP <10 µM) (Fig. 3G). Lake Vanda is considered one of the most oligotrophic bodies of water in the world (Vincent et al. 1981); both chl *a* and bacterial biomass levels peaked within the chemocline (68 m depth) and were the lowest of the 3 study lakes (Fig. 4D). In contrast, both RubisCO and βGAM activities peaked in the deeper layer of Lake Vanda (72 m) (Fig. 5D,H).

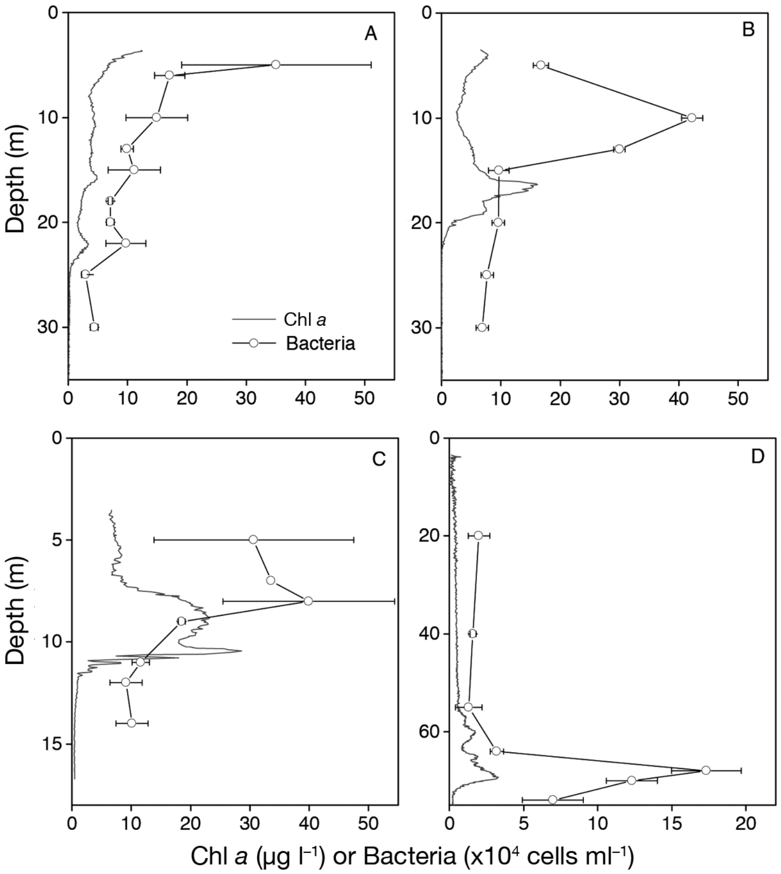

Fig. 4. Depth profiles for chl *a* concentration and bacterial abundance in (A) Lake Bonney east lobe (ELB), (B) Lake Bonney west lobe (WLB), (C) Lake Fryxell (FRX) and (D) Lake Vanda (VAN)

Cluster analyses of lake physicochemical parameters

In agreement with past reports (Vincent 1981, Priscu 1995, Roberts et al. 2000), lake physicochemistry and biology exhibited strong vertical stratification as well as lake-specific differences (Figs. 2–4). This spatial complexity in lake biogeochemistry was reflected in spatial trends of both RubisCO and βGAM enzyme activities (Fig. 5). In an effort to determine whether specific environmental and biological factors influence protist autotrophic or heterotrophic activity, we first performed PCA of major abiotic factors (i.e. temperature, PAR, conductivity and dissolved inorganic nutrients) across all sampling sites. Despite the lake-specific differences in several physicochemical parameters, the PCA results indicated that physicochemical parameters collected from 93 total samples clustered strongly into 2 major groups representing sampling depths located in the shallow layers (above chemocline) versus the deep layers (chemocline and deeper) (Fig. 6). In the PCA, the first 3 principal components explained the majority of the variation between samples (34.6, 23.4 and 20.2% for PC1, PC2, and PC3, respectively) (Fig. 6). The shallow and deeper layers were majorly separated on the first axis (PC1); the environmental parameter loadings of the first 3 principal components are shown in Table S2 in the Supplement. The first component largely represented a gradient of nutrient balance (N:P ratio) and conductivity levels. It also positively correlated with concentrations of DIC, NO_3^-, NH_4^+ while PAR levels had a weakly negative effect. In addition, the separation between samples within shallow and deeper layers was majorly in the second and third principal components. The second component majorly represented a gradient of temperature and concentrations of NH_4^+ and SRP. The third component represented a gradient of NO_3^-, SRP and DIC, and weakly correlated with PAR and temperature. Further, NO_3^- PAR and temperature were at the positive end of the gradient while SRP and DIC were at the negative end.

Correlation of autotrophic and heterotrophic activities with physicochemical parameters

Given that lake physicochemical parameters strongly clustered into 2 groups (i.e. the upper water column and at/below the chemocline), we performed Pearson correlation coefficient (PCC) analysis between autotrophic and heterotrophic enzyme activity and a suite of biological and environmental parameters in shallow and deep layers separately. Several environmental and biological parameters exhibited significant correlations with RubisCO and βGAM (Table 1). Spatial trends in Log RubisCO activity in the shallow water column layers weakly correlated with abiotic parameters such as conductivity, temperature, NO_3^-, SRP^- and N:P, as well as biotic parameters such as PPR and abundance of cryptophytes. However, in deep water columns Log RubisCO activity negatively corresponded with the environmental parameter conductivity as well as NH_4^+, NO_3^- and

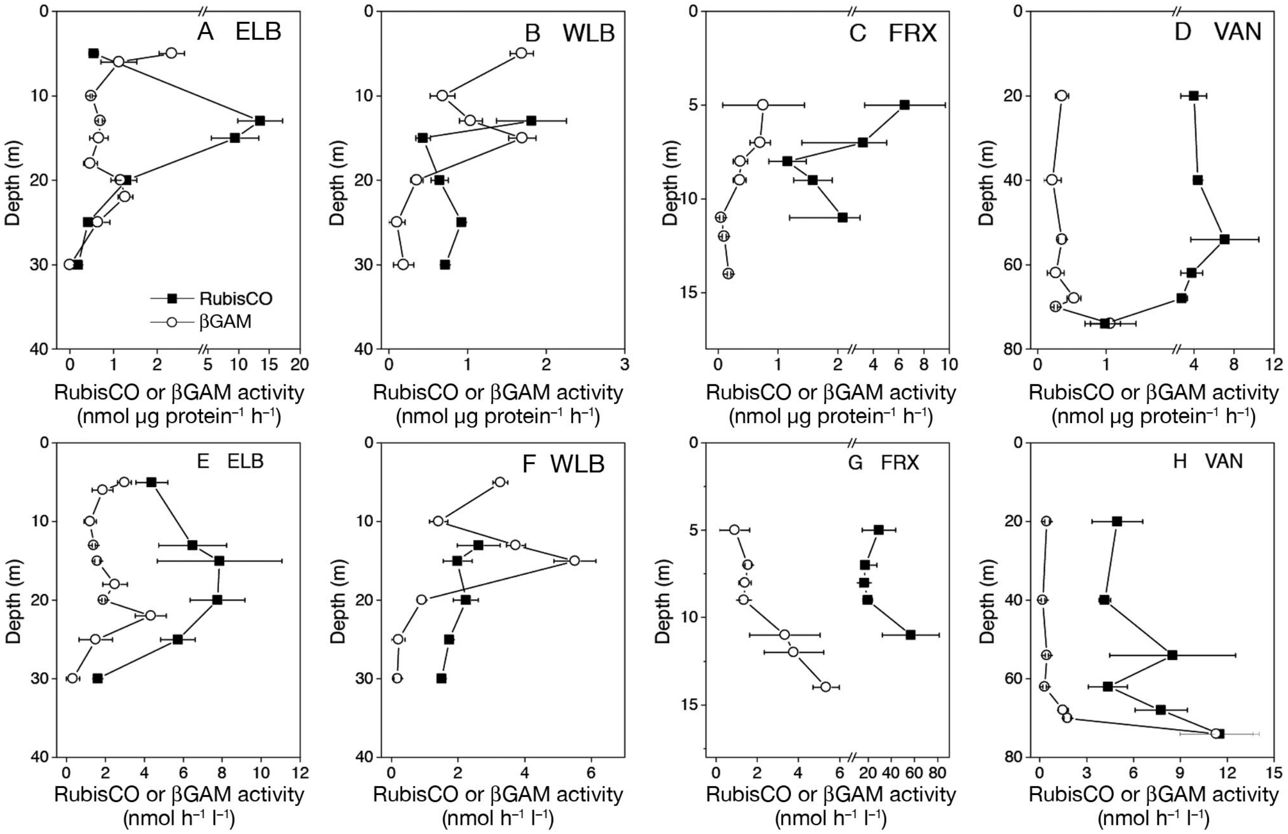

Fig. 5. Spatial variability in enzyme activity of RubisCO and β-D-glucosaminidase (βGAM) in study sites: (A,E) Lake Bonney east lobe, (B,F) Lake Bonney west lobe, (C,G) Lake Fryxell and (D,H) Lake Vanda. Enzyme activity is represented as either nmol µg protein^{-1} h^{-1} (A–D) or nmol h^{-1} l^{-1} lake water (E–H). Note that the scales of the *x*-axes are different across all panels, and also note the break in the *x*-axis in (A,C,D) and (G)

N:P, and positively corresponded with the biological parameters chl *a*, protein concentration, as well as abundance of cryptophytes and haptophytes.

The relationship between spatial distribution of heterotrophic activity (i.e. βGAM) and various lake parameters was distinct from that of RubisCO. Statistically significant correlations for βGAM activity in shallow water column layers include those with PAR, temperature, bacteria counts, protein concentration, chl *a* as well as green algae. In contrast, the enzyme activity in deep water column layers showed correlations with conductivity, temperature, NH_4^+ and SRP$^-$, protein concentration (Table 1). Interestingly, in the deep layers βGAM activity was positively correlated with temperature while in the shallow layers, it had negative correlation with this parameter.

We selected linear models of RubisCO and βGAM activities and their significant predictors (i.e. abiotic and biotic factors) based on the lowest AIC values (Table 2). RubisCO activity in the shallow layers was predicted mostly by abiotic variables (i.e. DIC concentration, conductivity and ammonia concentration) ($R^2 = 0.49$, $p = 0.02$). In the deep lake layers, irradiance (PAR), conductivity, major nutrient ratio (N:P) and total protein concentration were the significant predictors of RubisCO activity ($R^2 = 0.80$, $p < 0.001$). On the other hand, physicochemical conditions were the significant predictors of βGAM activity in both shallow and deep layer models, with $R^2 = 0.65$, $p < 0.001$ and $R^2 = 0.80$, $p < 0.001$, respectively. Temperature and N:P were present in both models while chl *a* and phosphate concentrations were important to explain βGAM activity in shallow layers, and conductivity and irradiance were significant predictors of the activity in deep layers.

DISCUSSION

MDV lakes exhibit strong gradients in the physicochemistry which drives variations in microbial distribution, diversity and abundance within and between the lakes (Laybourn-Parry et al. 1997, Roberts et al. 2004b, Kong et al. 2012b). Recent studies combining

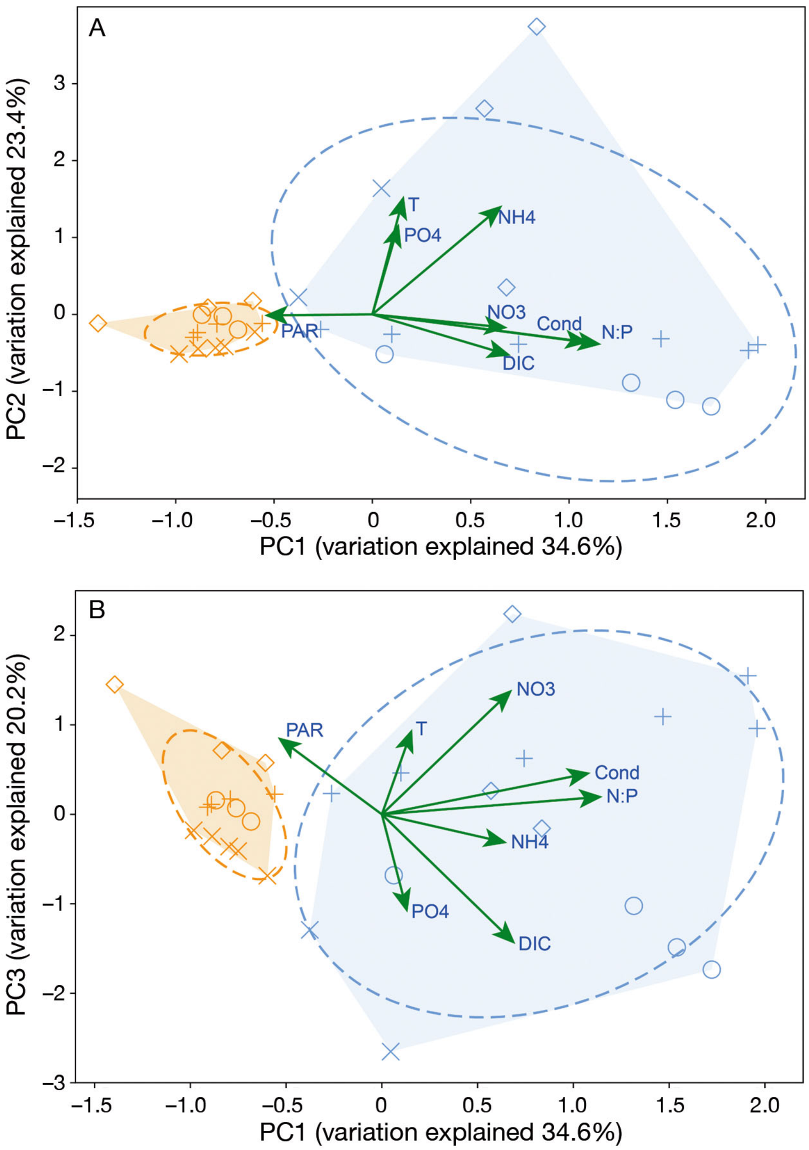

Fig. 6. Principal component analysis (PCA) based on major abiotic factors of 4 study lakes. Cond: conductivity; PAR: photosynthetically available radiation; T: temperature; DIC: dissolved inorganic carbon; NO3: NO_3^-; NH4: NH_4^+; PO4: soluble reactive phosphorus; N:P: dissolved inorganic N:P ratio. Shallow and deep layers were coded as orange and blue respectively. Circles: 75% similarity within each group. Symbols indicated samples from Lake Bonney east lobe (plus), Lake Bonney west lobe (circle), Lake Fryxell (cross) and Lake Vanda (diamond)

sequencing and quantitative PCR (qPCR) approaches have described the diversity and vertical distribution of the protistan communities residing in MDV lakes (Bielewicz et al. 2011, Kong et al. 2012a,b, Dolhi et al. 2015). Based on gene and transcript abundance of form I and II RubisCO genes (*rbcL* and *cbbM*, respectively), these studies predicted that light is a strong environmental driver for the obligate photoautotrophs (e.g. chlorophytes). On the other hand, spatial and seasonal variability in mixotrophic protist populations (e.g. haptophytes and cryptophytes) appears to be independent of PAR availability (Kong et al. 2012b, Dolhi et al. 2015). The present study extends the findings from the molecular studies by measuring autotrophic and heterotrophic enzyme activities in the MDV lake communities.

Spatial trends in autotrophic (RubisCO) activity peaked at or near the chemocline in both lobes of Lake Bonney as well as Lake Vanda. WLB exhibited relatively low RubisCO activity compared to ELB despite the presence of a well-defined chl *a* maximum. The apparent uncoupling of chl *a* from carbon fixation in WLB was also noted by Kong et al. (2012b), who observed that transcriptional activity of 2 types of RubisCO large subunit (*rbcL*) genes was 25 and 500× lower in WLB compared with ELB (Kong et al. 2012b). In support of the molecular evidence, PPR was 4 to 5× lower in WLB compared with ELB (Kong et al. 2012b); however, expression of the photosystem II reaction center gene *psbA* was comparable between the 2 lobes. These reports combined with results presented here indicate that phytoplankton in WLB have a relatively high light harvesting capacity combined with a lower capacity for fixation of CO_2, relative to ELB. This uncoupling between light energy capture and C-fixation may be due to photoacclimation in obligate photoautotrophs and/or heterotrophic metabolism in mixotrophic phytoplankton (Morgan-Kiss et al. 2016, Obryk et al. 2016).

Permanent chemoclines are steep redox transitions associated with high microbial activity and biomass in meromictic lakes (Van Gemerden & Mas 1995), and represent a rich source of POC for heterotrophic and mixotrophic protists. While mixotrophy in the chemoclines is likely a strategy for energy acquisition under limiting light levels, peaks in βGAM activity in the shallow depths of both lobes of Lake Bonney may reflect digestion of bacterial prey as an alternative mechanism for the acquisition of additional nutrients (nitro-

Table 1. Pearson correlation coefficient values (*r*) for average RubisCO and β-D-glucosaminidase (βGAM) activity with lake physical, chemical, and biological parameters for all samples (n = 57–93). The significance of the correlation coefficient is indicated by *0.01 < p < 0.05; **0.001 < p < 0.01; ***p < 0.001. Cond: conductivity; PPR: primary productivity; PAR: photosynthetically available radiation; Temp: temperature; SRP: soluble reactive phosphorous; DIC: dissolved inorganic carbon; Bact: bacteria counts; Prot: protein; Chl *a*: chlorophyll *a*; Galg: green algae; Cyan: cyanobacteria; Hapt: haptophytes; Crypt: cryptophytes

	Cond	PPR	PAR	Temp	NH_4^+	NO_3^-	SRP	N:P	DIC	Bact	Prot	Chl *a*	Galg	Cyan	Hapt	Crypt
Log RubisCO																
Shallow (n = 20)	−0.53*	0.5*	0.27	−0.49*	−0.35	−0.48*	−0.52*	−0.49*	−0.04	0.3	−0.08	0.46	−0.29	−0.31	0.26	0.51*
Deep (n = 37)	−0.66***	−0.21	0.26	−0.19	−0.52**	−0.42**	−0.23	−0.69***	−0.14	0.25	0.42**	0.57***	−0.36*	0.04	0.5**	0.75***
βGAM																
Shallow (n = 42)	0.1	−0.26	−0.47**	−0.39*	0.03	0.04	0.06	0.08	−0.01	0.54***	0.39*	0.67***	0.54***	0.15	0.17	−0.15
Deep (n = 51)	−0.47**	−0.05	−0.22	0.41**	0.5***	−0.18	0.45***	−0.22	−0.22	−0.06	0.32*	0.01	0.03	0.25	−0.09	−0.09

Table 2. Linear regression models explaining the enzyme activities in different portions of water columns. The models were selected by lowest AIC value from all possible linear models to explain log-transformed enzyme activities in shallow and deep layers as dependent variables and log-transformed abiotic (except temperature) and biotic parameters as independent variables. βGAM: β-D-glucosaminidase; Cond: conductivity; PAR: photosynthetically available radiation; Temp: temperature; DIC: dissolved inorganic carbon; Chl *a*: chlorophyll *a*; Prot: protein. The significance of the correlation coefficient is indicated by *0.01 < p < 0.05; **0.001 < p < 0.01; ***p < 0.001

No.	Dependent variable	Linear model	AIC	R^2	n
1	Log $\text{RubisCO}_{\text{shallow}}$	1.8*** + 0.27** DIC − 1.16*** Log Cond + 0.15 Log NH_4^+	52	0.49	18
2	Log $\text{RubisCO}_{\text{deep}}$	−2.83 + 0.14* PAR + 1.55* Log Cond − 0.65*** Log SRP − 0.66* Log N:P + 0.11 Chl *a* + 0.07*** Prot	78	0.80	37
3	$\beta\text{GAM}_{\text{shallow}}$	1.41** − 0.22*** Temp − 0.002 Chl *a* + 0.68*** Log SRP + 0.58*** Log N:P	95	0.65	39
4	$\beta\text{GAM}_{\text{deep}}$	8.84*** − 1.11*** PAR + 0.37*** Temp − 3.89*** Log Cond + 2.32*** Log N:P	228	0.61	54

gen and phosphorus) in the highly oligotrophic surface layers (Laybourn-Parry et al. 2005). In support of this hypothesis, βGAM activity correlated with bacterial abundance (Table 1), and N:P explained βGAM variation (Table 2) in the shallow zones.

Spatial trends in RubisCO and βGAM in both ELB and WLB matched that of haptophyte *rbcL* abundance in a previous study (Kong et al. 2012b). The dominant haptophyte in Lake Bonney is an *Isochrysis* sp. (Bielewicz et al. 2011). While this genus is not typically associated with mixotrophy, the Lake Bonney strain was isolated and shown to be capable of mixotrophy (Table S1) (Li et al. 2016). Thus, it is likely that haptophytes contribute significantly to both RubisCO and βGAM activity within the chemoclines of ELB and WLB. In addition, a recent report identified *Isochrysis* as a major prey for a heterotrophic nanoflagellate, *Pteridomonas* sp. (Li et al. 2016). Thus, *Isochrysis* sp. represents a keystone in Lake Bonney, playing a significant role in the transfer of carbon and nutrients within the food web of MDV lakes.

Of the 4 lakes in this study, Lake Fryxell exhibited the highest ratio of RubisCO to βGAM activity (RubisCO: βGAM range of 3–36). Higher PPR in Lake Fryxell correlates with its status as one of the most productive lakes in the MDV (Priscu 1995). Mixotrophic cryptophytes related to *Geminigera cryophila* dominate the photic zone (Dolhi et al. 2015) and are likely to be major contributors to the carbon cycle in Lake Fryxell. The contribution of cryptophytes to primary production in all the lakes was evident in the significant correlation between RubisCO activity and cryptophyte abundance (Table 1). The contribution of cyanobacteria to RubisCO activity cannot be ruled out, as *Oscillatoria* spp. and *Phormidium* spp. have been dominant members of the under-ice and chemocline communities, respectively, in certain years (Spaulding et al. 1994). Conversely, βGAM activity remained low in Lake Fryxell, suggesting that the Lake Fryxell mixotrophs were not reliant on heterotrophy during the sampling time of this experiment (mid-summer). Thus, while all MDV lakes are limited for light by the permanent ice-covers, we suggest that nutrient availability is a strong driver of protist community trophic status. This hypothesis is also supported by a recent nutrient bioassay study mimicking a climate-related summer flood event which showed that the diversity and abundance of phytoplankton communities in Lake Bonney, but less so in Lake Fryxell, was highly responsive to nitrogen and phosphorus concentrations (Teufel et al. 2017).

The PCC analysis of enzyme activity correlating with biotic and abiotic environmental parameters provided further insights on drivers of auto- and hetero-trophic groups in shallow and deep lake depths. The correlation between RubisCO activity and biotic parameters (protein concentration, chl *a*, and haptophyte and cryptophyte abundance) in deep layers indicates that haptophytes and cryptophytes are likely major contributors to inorganic carbon fixation within the chemoclines of Lakes Bonney and Fryxell, respectively. On the other hand, βGAM activity correlated strongly with biotic parameters (bacterial counts, protein concentration, chl *a*, and green algae abundance) in shallow lake layers, whereas βGAM activity correlated strongly with abiotic parameters (temperature, salinity, NH_4^+, and PO_4^{3-}) in deep lake layers. This correlation fits well with the assumption that heterotrophic protists heavily graze on bacterioplankton and small phytoplankton (Laybourn-Parry et al. 1995, 1997, Laybourn-Parry 1997). The positive correlation between βGAM activity and biotic parameters in these depths suggests that prey availability might be a limiting factor of heterotrophic activity in shallow layers. In contrast, PCC analysis indicates that conductivity was negatively correlated with heterotrophic activity, which can be explained by the fact that higher salinity depths (e.g. hyper-saline water in deep Lake Bonney) may impact the physiology of heterotrophic protists (Greenwald & Hurlbert 1993). Since many heterotrophic organisms are known for their delicate cell structure, it is not surprising that MDV heterotrophic protists could be adversely affected by the extreme salinity levels in the deep zone of Lake Bonney. A recent paper on the diversity and distribution of fungal communities in the MDV lakes also reported salinity as a strong driver of abundance and diversity (Rojas-Jimenez et al. 2017).

RubisCO activity was measurable at depths below the photic zones in all MDV lakes studied, indicating that there is the potential for carbon fixation using alternative energy sources, such as reduced organic or inorganic compounds (i.e. chemolithoautotrophic metabolism). Evidence for chemolithoautotrophic bacteria in the MDV aquatic ecosystems has been reported for several MDV lakes. The anoxic, sulfidic bottom waters of Lake Fryxell have yielded culture-dependent (Sattley & Madigan 2006) and independent (Dolhi et al. 2015) evidence for sulfur-oxidizing bacteria (SOB) related to *Thiobacillus thioparus*. Evidence for the existence of a SOB community below the photic zone was also found for WLB (Kong et al. 2012a, Dolhi et al. 2015). It is predicted that these organisms utilize dimethyl sulfoxide (DMSO), a breakdown product of dimethylsulfoniopropionate (DMSP) which is produced by algae including haptophytes, chrysophytes, and dinoflagellates as a cryo- or osmo-protectant (Trevena et al. 2000), as an electron acceptor. A second breakdown product, dimethylsulfide (DMS) may be utilized as an electron donor (Raina et al. 2010). The potential for dark carbon fixation by ammonia-oxidizing bacteria also exists in ELB and Lake Vanda (Vincent et al. 1981, Voytek et al. 1999).

While the RubisCO enzyme assay measured the potential for a community to fix inorganic carbon, βGAM activity is a proxy for community respiration, specifically breakdown of complex organic carbon sources by acidic food vacuoles. As the result of local climate change, it has been reported that water levels of the MDV lakes have risen significantly in the past 30 yr due to greater input of glacial melt and streams (Fountain et al. 2016). Continuous fresh water inflow into these closed basin lakes could potentially affect the lake physiochemical conditions. For example, our linear model predicts that a 10 % decrease of whole lake conductivity would cause a 12 % RubisCO activity increase in the water column above the chemocline or a 16 % RubisCO enzyme activity decrease and a 14 % increase of average βGAM activity below the chemocline.

Some fraction of the βGAM activity could be attributable to other microorganisms. Sherr & Sherr (1999) observed that both heterotrophic bacteria and phytoplankton exhibit βGAM activity. However, a more recent paper tested the contribution of heterotrophic bacteria to particulate βGAM activity and reported that particulate activity was magnitudes higher compared with that of the filtrate (Sintes & del Giorgio, 2010). In addition, βGAM activity is below detection limits in a strict photosynthetic alga, *Chlamydomonas* sp. ICE-MDV (Table S1), a dominant member of the Lake Bonney phytoplankton community (Bielewicz et al. 2011).

Using functional enzyme activity, the present study showed that autotrophic and heterotrophic spatial trends within the MDV lakes are complex. While autotrophic metabolism was generally dominant at depths at and below the chemocline, heterotrophic metabolism was dominant at both shallow, nutrient-deficient depths (Lake Bonney), within the chemocline (Lake Bonney and Vanda), or below the chemocline (Lake Fryxell). Previous studies of RubisCO gene diversity and distribution (Kong et al. 2012a,b) helped to attribute enzyme function to groups of organisms. Pairing diversity and function studies with environmental parameters will improve understanding of microbial community structure and how this may be impacted by climate change, an area of research with many unanswered questions (Caron & Hutchins 2013). Polar microbial communities on the cusp of environmental change are important predictors for future changes in low-latitude aquatic environments.

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