Evidence of form II RubisCO (cbbM) in a perennally ice-covered Antarctic lake

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Abstract
The permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica, harbor microbially dominated food webs. These organisms are adapted to a variety of unusual environmental extremes, including low temperature, low light, and permanently stratified water columns with strong chemo- and oxy-clines. Owing to the low light levels during summer caused by thick ice cover as well as 6 months of darkness during the polar winter, chemolithoautotrophic microorganisms could play a key role in the production of new carbon for the lake ecosystems. We used clone library sequencing and real-time quantitative PCR of the gene encoding form II Ribulose 1, 5-bisphosphate carboxylase/oxygenase to determine spatial and seasonal changes in the chemolithoautotrophic community in Lake Bonney, a 40-m-deep lake covered by c. 4 m of permanent ice. Our results revealed that chemolithoautotrophs harboring the cbbM gene are restricted to layers just above the chemo- and oxi-cline (≤ 15 m) in the west lobe of Lake Bonney (WLB). Our data reveal that the WLB is inhabited by a unique chemolithoautotrophic community that resides in the suboxic layers of the lake where there are ample sources of alternative electron sources such as ammonium, reduced iron and reduced biogenic sulfur species.

Introduction
The McMurdo Dry Valleys represent the largest ice-free region (c. 4000 km2) on the Antarctic continent (Priscu, 1998). A number of permanently ice-covered lakes located in the dry valleys have been investigated since the International Geophysical Year (1957–1958). While environmental conditions within these lakes can be extreme (including year-round low temperatures, hypersalinity, extreme shade, and seasonal extremes in light availability), the water column beneath the ice cover is one of the few sources of perennial liquid water on the Antarctic continent. As such, these lakes provide the only year-round habitable environments on the continent. Three lakes (Bonney, Fryxell, and Hoare) located in Taylor Valley, a major valley within the McMurdo Dry Valleys, have been studied intensively as part of the McMurdo Dry Valleys Long-Term Ecological Research (McM-LTER; http://www.mcm-lter.org/) program since 1993. The water columns of each of the dry valley lakes are isolated by a year-round ice cover (3–6 m thick) which prevents wind-driven turbulence and produces strong vertical stratification in biogeophysical parameters to exist. Each lake supports a distinct stratified microbial community containing bacteria, microalgae, as well as flagellated, and ciliated protozoans that interact to form truncated food webs dominated almost exclusively by microorganisms (Priscu et al., 1999). A stratified phototrophic population, including cryptophytes in the shallow waters as well as chlorophytes, haptophytes, and stramenopiles within the deeper photic waters, plays a key role in primary productivity in the food webs of these lakes during the summer (Lizotte & Priscu, 1998; Priscu et al., 1999; Bielewicz et al., 2011; Kong et al., 2012). Owing to minimal allochthonous inputs and atmospheric gas exchange as well as a lack of higher trophic levels, the microorganisms residing in this environment strongly influence the biogeochemistry of the carbon, nitrogen, and sulfur cycles in the lakes (Lee et al., 2004a, b).

Priscu et al. (1999) showed that photosynthetic primary production (P)-to-respiration (R) ratios in Lake Bonney were < 0.5 on an annual basis, indicating that photoautotrophic carbon production was inadequate to support the
level of respiration in the lakes. However, a water column 
P/R ratio of < 1 should eventually lead to a system with 
low dissolved oxygen and little to no reduced carbon: phe-
nomena which have not been observed in this lake. This 
conundrum, in concert with high levels of nitrous oxide, 
and reduced iron and biogenic sulfur compounds, indicates 
that chemolithoautotrophic fixation of carbon dioxide may 
plan an important role within this lake and perhaps others 
in the McMurdo Dry Valleys (Voytek et al., 1999; Priscu 
et al., 2008). These reports led to a recent study of the 
abundance and diversity of the rbcL gene encoding the 
major subunit of the enzyme RubisCO (Kong et al., 2012). 
The study by Kong et al. (2012) revealed rbcL sequences 
related to chemolithoautotrophic Proteobacteria from form 
I A/B rbcL sequence libraries generated from sampling 
depths collected below the chemocline (i.e. 15 and 20 m) 
in the west lobe of Lake Bonney (WLB). In contrast, no 
putative chemolithoautotroph rbcL sequences were recov-
ered from libraries generated from the east lobe of Lake 
Bonney (ELB; Kong et al., 2012). In other Antarctic lakes, 
chemolithoautotrophic bacteria have been detected based 
on cultivation and molecular methods (Karr et al., 2003, 
2005; Clocksin et al., 2007; Sattley & Madigan, 2007). The 
presence of chemolithoautotrophic bacteria is also sug-
gested by high rates of light independent fixation of inorganic 
carbon in the dry valley lakes (J. Priscu, unpublished).

Form II RubisCO is one of two forms of RubisCO that are 
directly involved in fixation of CO₂ in autotrophic organ-
isms through the Calvin–Benson–Basham (CBB) cycle, and 
this gene is adapted to functioning in low-O₂ and high-CO₂ 
environments (Tabita, 1999; Tabita et al., 2007). The form 
II RubisCO gene, cbbM, has been used in a variety of envi-
ronments as a functional marker for chemolithoautotrophic 
organisms (Giri et al., 2004; Naganuma et al., 2005; Hall 
et al., 2008; Chen et al., 2009; Tourova et al., 2010). Given 
recent reports of chemolithoautotrophs in nearby Blood 
Falls, which flows into WLB (Mikucki & Priscu, 2007; 
Mikucki et al., 2004, 2009), as well as the detection of 
putative chemolithoautotrophic rbcL sequences in the WLB 
water column (Kong et al., 2012), we designed our current 
study to focus on the diversity and abundance of the form II 
RubisCO gene in the WLB.

Materials and methods

Site description

Lake Bonney is separated into two 40-m-deep basins by a 
shallow (c. 13 m) sill that allows exchange of oxygenated 
surface waters between the basins but eliminates exchange 
of deeper nutrient rich, suboxic waters. The water 
columns lack wind-driven turbulent mixing, which has 
produced stable gradients in temperature and conductiv-
ity, with bottom waters being saline and cold. Less than 
0.1% of incident radiation reaches the depth of the 
chemocline, and no light penetrates the ice cover during 
the period of polar darkness (c. 6 months). The two lobes 
of Lake Bonney have a complex history. Long separation 
and differential evaporative histories between the two 
lobes have led to distinctive water chemistry in the iso-
lated bottom waters: in WLB, oxygenated surface waters 
overlay anoxic layers where measurable rates of denitrifi-
cation occur (Priscu et al., 1996; Priscu, 1997; Ward & 
Priscu, 1997), while ELB exhibits suboxic waters below 
the chemocline with high nitrate and supersaturated 
nitrous oxide levels (> 700 000% over air saturation; 
Voytek et al., 1999; Ward & Priscu, 1997). WLB is also 
fed by glacial melt water during the summer from the 
terminus of the Taylor Glacier, a major outlet glacier of the 
East Antarctic Ice Sheet. A unique geochemical fea-
ture known as Blood Falls is located at the northern end 
of the Taylor Glacier terminus and delivers iron-rich, hy-
persaline subglacial brine to the deep waters of WLB. 
Blood Falls is a subglacial outflow thought to originate 
from an ancient pool of marine brine located under the 
Taylor Glacier (Mikucki et al., 2004, 2009; Mikucki 
& Priscu, 2007). The saline deep waters of WLB are thought 
 to be very old (> 10^4 years) while the east lobe has 
undergone recent evaporative and refilling events and has 
been ice-covered for < 300 years (Poreda et al., 2004).

Field sampling

Water samples were collected over three field seasons 
(2008, 2009 and 2011) at selected depths throughout the 
water column of WLB. Water samples were collected 
daily during the summer–winter transition between 2 
and 30 March 2008, a period when incident photosyn-
thetically available radiation (PAR) was dropping rapidly 
and averaged 1-μmol photons m⁻² s⁻¹ at 10 m in the 
lake. To assess the presence of chemolithoautotrophs dur-
ing the polar summer, samples were also collected during 
mid-summer (16 December 2009, 1 January 2010, and 23 
November 2011). All sampling depths were measured 
from the piezometric water level in the ice hole (c. 30 cm 
below the ice surface). Water samples were collected 
using a 5-L Niskin bottle (General Oceanics, FL) and 
were filtered (1–5 L; n = 2–4 replicate filters) through 
47-mm 0.45-μm Durapore polyvinylidene fluoride mem-
brane filters (Millipore, MA) or 47-mm GF/C filters 
(Whatman, UK) for phylogenetic analyses or enzyme 
assays, respectively, using a vacuum of 0.3 mBar. The fil-
ters were frozen immediately in liquid nitrogen before 
being transported on dry ice to our US laboratory, where 
they were stored at −80 °C until processing. Conductiv-
ity, dissolved organic carbon (DOC), dissolved inorganic
carbon (DIC) PAR, light-mediated primary productivity (PPR), and nutrients (SO$_4^{2-}$, NH$_4^+$, NO$_3^-$, NO$_2^-$, and PO$_4^{3-}$) were measured as part of the NSF-funded McM-LTER program using methods described in Priscu (1997) and outlined in the McM-LTER limnology manual (http://www.mcm.lter.org). Briefly, inorganic nitrogen species were determined with a Lachat autoanalyzer, and soluble reactive phosphorus was analyzed manually using the antimony-molybdate method (Strickland & Parsons, 1972). PAR was measured with a LICOR LI-193 spherical quantum sensor (LI-COR Biosciences, NE). PPR was measured as $^{14}$C-bicarbonate incorporation into particulate matter over a 24-h in situ incubation (Priscu, 1997).

Nucleic acid isolation and real-time quantitative PCR

Environmental DNA was isolated using FastDNA spin kit for soil (MP Biomedicals, OH) following the manufacturer’s protocol and according to Kong et al. (2012). The PCR products for Form II RubisCO gene (cbbM) were amplified using the primer set (cbbM-F: TTC TGG CTG GGB GGH GAY TTY ATY AAR AAY GAC GA and cbbM-R: CCG TGR CCR GCV CGR TGG TAR TG) (Campbell & Cary, 2004). Primer specificity and cbbM sequence verification were confirmed in sequence clone libraries.

Gene copy number (i.e. copies of cbbM DNA in 1 L of lake water) was quantified by real-time quantitative PCR (qPCR) according to Kong et al. (2012) on a Bio-Rad iCycler coupled with SYBR Green kit (Bio-Rad, CA). The PCR conditions were an initial 5-min period at 95 °C, followed by 40 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and 78 °C for 10 s to collect data. To determine the PCR product specificity, a melting curve was acquired by heating from 50 to 95 °C. Data analysis was carried out using iCycler IQ Optical System software version 3.01 (Bio-Rad). The threshold cycle (Ct) was defined as the cycle number at which a statistically significant increase in fluorescence was detected.

Standard curves for qPCR were developed from plasmids containing the target inserts according to Kong & Nakatsu (2010). Copy numbers of the target genes were calculated directly from the concentration of the isolated plasmid DNA assuming 1.096 x 10$^{-12}$ g per bp. All standard curves were generated from tenfold serial dilutions of DNA with known copy numbers and were subjected to qPCR assay in duplicate.

Clone library construction and sequencing

The PCR products containing the target fragment (328 bp in length) of cbbM gene were amplified from environmental DNAs to generate clone libraries from environmental samples collected during the 2008 field season. A total of 16 transformants from each library were randomly selected and sequenced on an Applied Biosystems 3730 × 1 DNA Analyzer (Applied Biosystems, CA). All sequences obtained from each library were aligned using ClustalW from the MEGA 4.1. The resulting alignment was used to calculate rarefaction curve with a cutoff value at 0.02 (sequence differences do not exceed 2%) using the MOTHUR program (Schloss et al., 2009). Sequences with more than 98% nucleotide similarity were grouped into the same operational taxonomic unit (OTU). BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/) was used to search GenBank for nearest relative sequences to OTUs. Phylogenetic trees were constructed by neighbor-joining method with a Kimura two-parameter distance model using MEGA 4.1 software. Bootstrapping was used to estimate reliability of phylogenetic trees with 1000 replicates. Sequences generated in this study have been deposited in the National Center for Biotechnology GenBank database under the accession numbers JN091926–JN091960.

RubisCO carboxylase activity filter assay

Maximum carboxylase activity of the enzyme RubisCO was estimated using a modified radioisotope assay for filtered samples (Tortell et al., 2006; Dolhi et al., 2012). Enzyme activities from flash-frozen field samples were assayed within 2 months after collection. Briefly, frozen GF/C filters were extracted in bicine extraction buffer and soluble fractions were produced using a Minibead beater (Biospec, CA) followed by centrifugation. Soluble fractions were collected and used for enzyme assays. Maximum RubisCO activity was performed using a $^{14}$C-based assay which measured rate of $^{14}$C-incorporation into acid-stable products. A detailed protocol as well as a video of the RubisCO filter assay is described in Dolhi et al. (2012).

Results and discussion

Water column chemical characteristics

Physical and chemical characteristics of the WLB water column have changed relatively little over the past decade (compare Priscu, 1995, Spigel & Priscu, 1996, Priscu et al., 2008). A dominant feature of the water column is the steep salinity gradient between 13 and 18 m where salts (primarily NaCl) increase from freshwater levels to about 2.3 times seawater (Fig. 1a). Temperatures above the chemocline are near 2 °C and decrease to −4 °C in the deep saline waters coinciding with a sharp decrease in oxygen from supersaturated levels (1 mM) to suboxic
conditions below 14 m. The suboxic conditions within the deep water are reflected by lower redox (Eh) levels, which decrease from a maximum of 800 mV in the surface to 105 mV in the deep saline waters (Fig. 1b). The reducing conditions in the deep waters result in high levels of ferrous iron, NH$_4^+$, and reduced biogenic sulfur (Fig. 1c; see also Lee et al., 2004a, b). Active denitrification and the presence of denitrifying bacteria have been shown to be present below the chemocline (Priscu, 1997; Ward & Priscu, 1997), leading to depletion of NO$_3^-$ in the deeper waters. Despite the suboxic reducing environment that exists below the chemocline, the suboxic waters are not sulfidic; H$_2$S is not measurable below the chemocline despite high levels of SO$_4^{2-}$ (> 40 mM; Fig. 1b and c) and reducing conditions. We currently have no simple biochemical explanation to explain the lack of SO$_4^{2-}$ reduction to H$_2$S, but a similar scenario has been shown to exist in subglacial water from the Taylor Glacier that flows into WLB via Blood Falls (Mikucki & Priscu, 2007; Mikucki et al., 2009). These authors were unable to detect dissimilatory sulfate reductase genes and concluded that SO$_4^{2-}$ was reduced to reduced sulfur intermediates which were then oxidized by ferric iron back SO$_4^{2-}$ by a consortium of unknown microbial species. The geochemical gradients in the region of the chemocline (shaded area in Fig. 1), in concert with a strong gradient in dissolved inorganic carbon, provide appropriate redox couples to support chemolithoautotrophic metabolism driven by dimethyl sulfide (DMS), reduced iron, and ammonium (Lee et al., 2004a, b; Priscu et al., 2008). Any chemolithoautotrophs metabolizing within this geochemically distinct ecotone must be able to cope with cold and saline conditions.

### Analysis of form II RubisCO gene diversity and distribution

Few studies have reported on functional gene diversity in the McMurdo Dry Valley lakes, and even less regarding genes associated with chemautotrophy, despite prolonged winter periods where the water column is completely dark and respiratory carbon oxidation has been shown to exceed photosynthetic production of new carbon (Priscu et al., 1999). In a recent paper, we detected RuibsCO form IA gene (rbcL) sequences related to known chemolithoautotrophic Proteobacteria in WLB waters at depths below the chemocline (i.e. 15 and 20 m). These rbcL sequences were related to an endosymbiont of Oligobrachia haakonmosbiensis as well as Thiobacillus sp. (Kong et al., 2012). In this current study, we further investigated the presence of chemolithoautotrophic organisms in WLB waters by developing clone libraries for the form II RubisCO gene large subunit encoded by the cbbM gene.

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**Fig. 1.** Typical water column characteristics in the WLB. Depths were measured from the piezometric water level within the sampling hole. The ice cover was between 3.5 and 4.0 m thick.
Sequence libraries were constructed from depths spanning the chemocline (15 and 20 m) during the polar night transition from three sampling time points (T1 = 2 March; T3 = 16 March; T5 = 30 March). Rarefaction curves revealed a relatively high diversity of cbbM genes in WLB (Fig. 2). The cbbM gene diversity was fairly constant in all samples collected from a depth of 15 m but higher in 20-m samples, with the highest diversity observed in the last time point (i.e. 30 March; Fig. 2). A total of 35 representative OTUs across all sequences from the six clone libraries were revealed with > 98% similarity in cbbM gene sequence (Fig. 3). The phylogenetic relationships of the cbbM sequences in WLB waters fell into four groups related to *Rhodopseudomonas* and *Rhodovulum* spp. (Accession No. AF416674 and HQ877080; 79–82% similarity), *Thiobacillus* sp. (Accession No. EU746412; 82–86% similarity), *Thiomicrospira* sp. (Accession Nos. DQ272537 and DQ272535; 75–82% similarity), as well as an endosymbiont of the Arctic tubeworm, *O. haakonmosbiensis* (Accession No. AM883191; 82% similarity). The first three groups belong to *Alpha*, *Beta* and *Gammaproteobacteria*, respectively, and together with the endosymbiont have been associated with biogeochemical transformations in extreme environments (Badger & Bek, 2008; Rogers & Schulte, 2012). *Thiomicrospira*, frequently detected in extreme environments such as deep-sea hydrothermal vents (Brinkhoff et al., 1999; Dobrinski et al., 2005, 2010; Ghosh & Dam, 2009; Niederberger et al., 2009), are wide-spread sulfur-oxidizing bacteria inoxic zones of these extreme environments. The *Thiomicrospira*-like cbbM sequences dominated the 15-m water in WLB (> 70% of the clone libraries; Fig. 4). Previous studies have detected the presence of sulfur-oxidizing and sulfur-reducing bacteria in other dry valley lakes (Karr et al., 2003, 2005; Jung et al., 2004; Clocksin et al., 2007). Moreover, Mikucki & Priscu (2007) reported that the most abundant 16S rRNA gene sequence in clone libraries constructed from Blood Falls samples (which flows into WLB) were related to a psychrophilic marine *Thiomicrospira arctica*. Our results as well as others collectively indicate that sulfur might be the dominant element in supporting chemolithoautotrophic communities in WLB and other dry valley lakes in waters with high levels of SO$_4^{2-}$. In the deeper waters (20 m), both *Thiomicrospira*- and *Thiobacillus*-like Proteobacteria dominated in WLB. *Thiobacillus* are obligately autotrophic, obtaining energy for CO$_2$ fixation by oxidizing iron and sulfur with O$_2$ and have been reported to combine inorganic sulfur-compound oxidation with denitrification (Beller et al., 2006). A psychrotolerant *Thiobacillus thioparus*-like bacterium has been isolated from nearby Lake Fryxell (Sattley & Madigan, 2006) which utilizes hydrogen sulfide and elemental sulfur as electron donors. Cell numbers of the *Thiobacillus* sp. peaked in the oxycline of the water column in Lake Fryxell where both dissolved oxygen and sulfide are present (Sattley & Madigan, 2006). Lake Fryxell differs from WLB in that high levels of H$_2$S are present below the chemocline in the former, whereas no measurable H$_2$S is present in WLB, despite low redox and oxygen levels (Fig. 1a and b).

There are several reports of *Thiobacillus* strains utilizing DMS as an electron source under both anaerobic and aerobic conditions (Visscher & Taylor, 1993; Arellano-Garcia et al., 2009; Ramirez et al., 2011). DMS in the deep waters of WLB (> 330 nM) are among the highest recorded in a natural aquatic ecosystem, yet no simple biogeochemical explanation exists for its presence (Lee et al., 2004a). Lee et al. (2004b) used thermodynamic constraints to examine potential biogeochemical transformations of biogenic sulfur in WLB and hypothesized that the microbial reduction of dimethyl sulfoxide (DMSO) produced by phytoplankton was the most feasible source of DMS in the suboxic waters of this lake. Oxidation of DMS by *Thiobacillus* generally produces carbon dioxide and sulfate, and certain strains can oxidize DMS to carbon dioxide while reducing nitrate to nitrite (e.g. Kim et al., 2007; Schafer, 2007; Schafer et al., 2010). These reports, together with our results, indicate that biogenic sulfur may provide an important energy source for chemolithoautotrophic metabolism within the chemocline of WLB.

Form II RubisCOs related to purple nonsulfur bacteria (*Rhodopseudomonas* and *Rhodovulum* spp.) and a chemolithoautrophic endosymbiont were the least abundant sequences (< 10%) in the cbbM sequence libraries (Fig. 4). *Rhodopseudomonas palustris* is a highly metaboli-
Fig. 3. Neighbor-joining phylogenetic tree of cbbM gene sequences (328 bp) retrieved from environmental DNA (in bold) in WLB. Scale bar indicates 0.2 substitutions per nucleotide position. The bootstrap consensus tree was inferred from 1000 replicates. Clones are named by sampling time point_lake name_depth_primer name_template type_index number. GenBank accession numbers are listed after each sequence name. Sampling times were as follows: T1, 2 March; T3, 16 March; T5, 30 March. Bootstrap values of < 50% are not shown.
cally versatile bacterium capable of anoxygenic photosynthesis under anaerobic conditions as well as oxidative respiration under aerobic and anaerobic conditions using a variety of carbon sources (Larimer et al., 2004). Rhodovulum sulfidophium is found in marine and high salt environments and can utilize both reduced sulfur compounds such as sulfide and thiosulfate as well as oxidize DMS to DMSO (McDevitt et al., 2002; Creevey et al., 2008). Two cbbM sequences detected in the current study were most closely related to O. haakonmosbiensis endosymbiont (Fig. 3). These endosymbiont Proteobacteria have been reported in chemolithoautotrophic sulfur oxidation (Pimenov et al., 2000; Lenk et al., 2011).

Abundance of the form II RubisCO gene

Seasonal dynamics for form II RubisCO gene were monitored during the summer–winter transition in 2008 (2–30 March) and during mid-summer in the following year (10 December 2009 and 1 January 2010) using real-time qPCR (Fig. 5). Distinct vertical patterns in cbbM gene copy number were observed in different field seasons, with maximum levels occurring at 15 m in the 2008 field season and at 20 m in the 2009 season. Levels of cbbM were 100–1000 times lower in 2009 field season than 2008 field season (Fig. 5). The dramatic differences between cbbM levels in the 2008 versus 2009 samples are difficult to explain but may be related to seasonal differences in the succession of microorganisms caused by differences in under-ice light levels (Lizotte, et al., 1996) or by diffusive flux of important redox couples and episodic inflow from Blood Falls (Mikucki et al., 2004) which may directly impact microbial populations and associated biogeochemistry in WLB.

The cbbM gene copy remained relatively constant throughout the polar night transition (Fig. 5a). Seasonal trends in form II RubisCO gene differed that of form I RubisCO over the same sampling period: form I RubisCO gene generally declined during the summer–winter transition and exhibited a positive correlation with PAR (Kong et al., 2012). The relatively stable cbbM levels during the summer–winter transition suggests that the chemolithoautotrophic community harboring form II RubisCO gene is not impacted by the declining light availability during this seasonal transition implying that the availability of favorable redox couples has a dominant role in the selection of these organisms.

Carbon fixation potential

Given our molecular evidence that a chemolithoautotrophic community of bacteria harboring form II RubisCO resides in WLB at depths where light is extremely low or absent (i.e. below the chemocline at 13 m), we investigated whether we could detect RubisCO carboxylase activity in vitro at sampling depths where the cbbM gene was detected (see Figs 4 and 5). As expected, the highest levels of RubisCO-specific activity correlated with the depth where
maximum levels of light-dependent primary productivity were detected (13 m, Table 1). This sampling depth occurs just above the chemcline and also correlates with maximum levels of chlorophyll a (Kong et al., 2012). However, we also detected Rubisco activity at sampling depths (20 and 25 m) where PPR was below the level of detection. Rubisco-specific activity represents maximum carboxylase levels, thus these data are estimators of carbon fixation potential, rather than in situ carboxylation rate. As there are little to no active phytoplankton at these sampling depths, we suggest that carboxylase activity is likely evidence of Rubisco activity in the chemolithoautotrophic community.

Lake Bonney has a unique geological evolution that has changed significantly the geochemical gradients in the water column over time. WLB is also influenced by efflux from Blood Falls at depths in and below the chemocline. Thus, the influence of Blood Falls on lake biota and chemistry would be restricted to WLB. Our findings suggest that the unique biogeochemical status of WLB as well as the interactions between Blood Falls and layers at and below the WLB chemocline may regulate the abundance and distribution of chemolithoautotrophs harboring form II Rubisco in Lake Bonney.

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