LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 65, 2020, 143–156

doi: 10.1002/lno.11257

Methane production in the oxygenated water column of a perennially ice-covered Antarctic lake

Wei Li,[1] John E. Dore,[1] August J. Steigmeyer,[2] Yong-Joon Cho,[3] Ok-Sun Kim,[3] Yongqin Liu,[4] Rachael M. Morgan-Kiss,[5] Mark L. Skidmore,[2] John C. Priscu[1]*

[1]Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana
[2]Department of Earth Sciences, Montana State University, Bozeman, Montana
[3]Division of Polar Life Sciences, Korea Polar Research Institute, Incheon, Republic of Korea
[4]Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing, China
[5]Department of Microbiology, Miami University, Oxford, Ohio

Abstract

Aerobic methane production in aquatic ecosystems impacts the global atmospheric budget of methane, but the extent, mechanism, and taxa responsible for producing this greenhouse gas are not fully understood. Lake Bonney (LB), a perennially ice-covered Antarctic lake, has cold hypersaline waters underlying an oxygenated freshwater layer. We present temporal methane concentration profiles in LB indicating methane production in the oxygenated (> 200% air saturation) water. Experiments amended with methylphosphonate (MPn) yielded methane generation, suggesting in situ methanogenesis via the carbon-phosphorus (C-P) lyase pathway. Enrichment cultures from the lake were used to isolate five bacterial strains capable of generating methane when supplied with MPn as the sole P source. Based on 16S rRNA gene sequencing, the isolates belong to the Proteobacteria (closely related to *Marinomonas*, *Hoeflea*, and *Marinobacter* genera) and Bacteroidetes (*Algoriphagus* genus). 16S rRNA metagenomic sequencing confirms the presence of these taxa in LB. None of the isolated species were reported to be capable to produce methane. In addition, orthologs of the phosphoenolpyruvate mutase gene (*PepM*) and methylphosphonate synthase (*MPnS*), enzymes involved in phosphonate and MPn biosynthesis, were widely spread in the LB shotgun metagenomic libraries; genes related to C-P lyase pathways (*phn* gene clusters) were also abundant. 16S rRNA and *mcrA* genes of anaerobic methanogens were absent in both 16S rRNA and metagenomics libraries. These data reveal that in situ aerobic biological methane production is likely a significant source of methane in LB.

Methane is a potent greenhouse gas whose atmospheric concentration has increased 2.5 times since industrialization (Intergovernmental Panel on Climate Change 2013). Despite the global importance of this gas, assessment of the interplay between methane sources and sinks remains tentative (Dlugokencky et al. 2011; Rigby et al. 2017; Turner et al. 2017). Biological and geological methane sources are considered to be the major natural source to the atmosphere (Kirschke et al. 2013; Zhang et al. 2017). Recent analysis of methane trapped in ancient glacial ice from Antarctica indicates that geological sources contribute relatively little to past atmospheric methane levels during periods when it was rising abruptly (Petrenko et al. 2017), emphasizing the contribution of biotic methane sources to the global atmospheric pool. Anaerobic microbial methanogenesis has been widely accepted as the major mechanism of biologically produced atmospheric methane (Bender et al. 2015). Recent observations of methane oversaturation in oxic ocean and lake water columns have led others to question this view (del Valle and Karl 2014; Tang et al. 2016; Donis et al. 2017). Initially, the "methane paradox" was explained as the result of production from the anoxic sediments followed by upward transportation (i.e., ebullition and diffusion; DelSontro et al. 2016) or within the anoxic digestive tracts of fish or zooplankton (van der Maarel et al. 1999; Schmale et al. 2018). Grossart et al. (2011) suggested that methane oversaturation in oxic lake water might be caused by oxygen-tolerant methanogens associated with phytoplankton. Bacteria have also been reported to produce methane as byproduct in oxic conditions, utilizing various methylated organic compounds (Karl et al. 2008; Damm et al. 2010; del Valle and Karl 2014; Wang et al. 2017). Recent studies have also shown that certain photosynthetic organisms can produce methane in oxic environments (Lenhart et al. 2016; Teikari et al. 2018). Clearly, many questions remain regarding methane production in oxic marine and

*Correspondence: jpriscu@montana.edu

Additional Supporting Information may be found in the online version of this article.

freshwater environments. Additional data addressing these questions will provide a more thorough understanding of global methane dynamics (Bastviken et al. 2011).

A portion of the dissolved organic matter pool in marine and freshwater environments resides as dissolved organic phosphorus (DOP), a poorly characterized organic carbon and phosphorus reservoir (Kolowith et al. 2001). Phosphonates, organic phosphorus molecules with C-P bonds, are noticeably abundant in marine waters (Sannigrahi et al. 2006; Young and Ingall 2010) and their biosynthesis has been recently characterized (Metcalf et al. 2012; Yu et al. 2013; Born et al. 2017). Although the utilization of phosphonates as a phosphorus source for microbial growth requires additional enzyme systems and more free energy than direct utilization of inorganic phosphorus (P*i*), phosphonates can be an alternative phosphorus source for microbial growth under phosphorus-limited conditions (Karl and Björkman 2014; Sosa 2018). Methylphosphonate (MPn) is the simplest phosphonate that occurs in the bulk DOP pool and within microbial cells (Metcalf et al. 2012; Repeta et al. 2016).

Microbial MPn demethylation using the C-P lyase pathway has been shown to be a potential mechanism for methane production in aerobic conditions in various marine and freshwater microorganisms (Metcalf et al. 2012; Wang et al. 2017; Sosa 2018; Teikari et al. 2018). Repeta et al. (2016) estimated that demethylation of only a fraction of the phosphonate reservoir in surface ocean water could support the observed aerobic methane flux to the atmosphere. Despite the potential importance of the MPn demethylation pathway, little is known about the geographic extent and organisms responsible for it.

Our study focused on methane production in the oxic (> 200% oxygen saturation) waters of Lake Bonney, a permanently ice-covered and phosphorus deficient lake in McMurdo Dry Valleys, Antarctica (Priscu 1995; Dore and Priscu 2001). We hypothesized that methane is generated via microbial demethylation of MPn via the C-P lyase pathway in the oxygenated lake water. This hypothesis was tested by (1) amending lake water with MPn and measuring rates of methane production, (2) examining the capability of bacterial strains isolated from the lake to generate methane when supplied with MPn as the sole P source, and (3) using metagenomic sequencing to provide information on functional genes capable of demethylating MPn via the C-P lyase pathway.

Materials and methods

Sampling site

The McMurdo Dry Valleys (MDV) in Southern Victoria Land, Antarctica, is the largest ice-free region on the continent with mean annual temperatures at the valley bottoms ranging between −14.8°C and −30.0°C (Doran et al. 2002) and annual precipitation less than 50 mm in water equivalent per year (Fountain et al. 2010). The permanently ice-covered (3–5 m thick) lakes located in the MDV region provide the only year-round water bodies for life in this polar desert (Priscu et al. 1999; Morgan-Kiss et al. 2016). Lake Bonney, which abuts the snout of the Taylor Glacier in the western end of Taylor Valley, is one of the McMurdo Long Term Ecological Research (MCM-LTER; (https://www.mcmlter.org)) study sites and has been sampled systematically since 1993. Its physical and chemical characteristics have been thoroughly described by Spigel and Priscu (1998) and Spigel et al. (2018). The absence of wind mixing resulting from the ice cover, saline bottom waters, and low stream inflow result in strong permanent vertical stratification in the water column: molecular diffusion is the dominant mechanism for vertical transport of heat and chemical constituents (Spigel and Priscu 1998). Gas exchange between the atmosphere and the lake water is also limited by the permanent ice cover in this lake (Wharton et al. 1986; Craig et al. 1992; Prisu et al. 1996).

Lake Bonney is divided into East and West lobes (ELB and WLB, respectively), each being ~ 40 m deep and separated by approximately a 13-m-deep shallow sill that effectively isolates the highly saline bottom waters of the two lobes (Fig. 1). The salinities in the deep water are between three and five times seawater (Spigel and Priscu 1998; Spigel et al. 2018) forming steep chemoclines occurring at ~ 20 and ~ 18 m in ELB and WLB, respectively. The trophogenic zone in the lake basins is confined to the relatively freshwater layer above the chemoclines. Bioassay experiments and alkaline phosphatase activity revealed that the phyto- and bacterioplankton in the upper photic zone of the lakes are extremely P-deficient (Priscu 1995; Dore and Priscu 2001; Teufel et al. 2017). Less than ~ 5% of the ambient irradiance reaches the liquid water column of the lake due to attenuation by the thick lake ice. Shade adapted photosynthetic protists (i.e., chlorophytes, cryptophytes, and haptophytes) are abundant in the water columns and are thought to provide the majority of newly

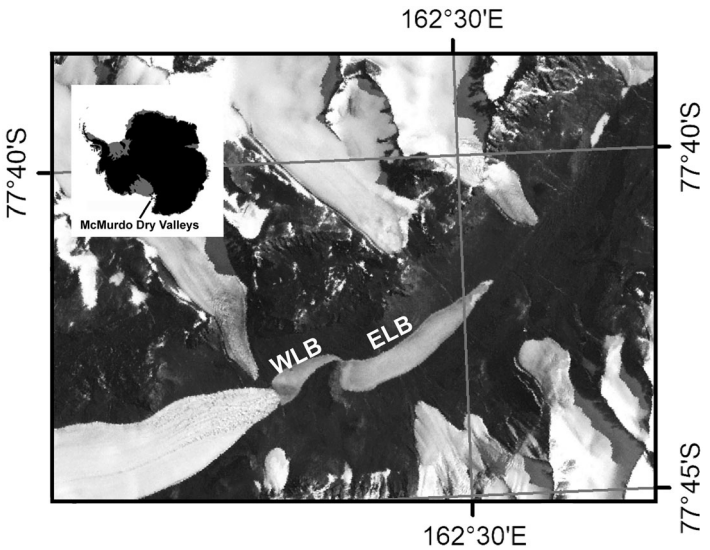

Fig. 1. Map of McMurdo Dry Valleys, Antarctica, and location of study site, east (ELB) and west (WLB) Lake Bonney in this study.

fixed organic carbon to the lake (Priscu et al. 1999; Kong et al. 2012, 2014; Dolhi et al. 2015). The water columns of the lakes lack metazoans; protozoans occupy the highest trophic level of the truncated food web (Priscu et al. 1999; Bowman et al. 2016).

Water sampling and dissolved methane gas measurements

Temperature and conductivity profiles were measured with an SBE 25 Sealogger CTD as outlined by Spigel and Priscu (1998). Discrete lake water samples were obtained during the austral summer in November 2016 and 2017 for dissolved oxygen (DO), microbial cultivation, and all methane measurements and experiments. All of our sampling depths corresponded with depths sampled routinely by the MCM-LTER to provide context for our methane work. Water samples were obtained through ~ 35-cm-diameter bore holes in the lake ice using a 5-L Niskin bottle (General Oceanics). Water for dissolved gas measurements (DO and methane) was gently decanted through a teflon hose into the bottom of sterile serum bottles which were allowed to overflow by about one bottle volume minimizing contact of sample water with the atmosphere. DO concentration in the serum bottles was measured immediately using the azide modification of the mini-Winkler titration (limit of detection = 0.09 mg O_2 L^{-1}) (American Public Health Association 1995). Serum bottles for methane measurements were preserved with NaOH (final concentration 0.1 M), capped with chlorobutyl stoppers, and inverted for transport to Montana State University (MSU) at 4°C in the dark. Once at MSU, a 20 mL headspace was introduced in each bottle using ultra-high pure N_2 gas followed by agitation to allow gas concentrations in the headspace to equilibrate with the liquid sample. Methane concentration in the headspace was then measured with an HP 5890 gas chromatography (Hewlett Packard) equipped with a flame ionization detector (FID) and 1 mL sampling loop. The original methane concentration in the lake water was derived using Henry's Law and appropriate solubility equations (Wiesenburg and Guinasso 1979). All limnological metadata used in this study are available on the MCM-LTER website (https://www.mcmlter.org).

Microbial activity

Based on gas profiles in WLB and ELB, we selected 15, 20, and 25 m to conduct experiments to assess the lake microbial community response to MPn addition. These depths were selected because they represent layers in the water columns with various combinations of oxygen and methane concentration (Fig. 2) and are depths where long-term data have been collected by the MCM-LTER.

Lake water for the preliminary experiment in 2016 was collected from the Niskin bottle into 1-L sterile amber bottles and transferred to MSU at 4°C within 3 weeks after collection. Water samples were then aliquoted into 60 mL serum vials and amended with MPn (10 μM), P*i* (10 μM), pyruvate (10 μM), glucose (50 μM), glycine (5 μM), carbon, and nitrogen sources according to the matrix in Table 1 (Carini et al. 2014). All stock chemical solutions were filter sterilized with 0.2 μm syringe filters. Vials were then sealed with chlorobutyl stoppers and aluminum crimps, and 20 mL of 0.2 μm filtered ambient air was added to create a headspace. All vials were then incubated at 5°C in the dark. Methane concentration in the headspace was measured at day 0, 3, 21, and 70 by gas chromatography as described previously. Although oxygen in the headspace gas during the course of the cultivation experiment was not quantified, its presence was confirmed by the small peak produced on the chromatograms due to its effect on combustion within the FID.

Similar experiments were conducted during the 2017 season with the exception that all work and incubations were conducted in a lake side laboratory which allowed for experimental setup within 2 h of sample collection and to extend our sampling intervals to 0, 1, 3, 6, 14, 21, and 30 d. NaOH (0.1 M final concentration) preserved samples were inverted and shipped to MSU at 4°C in the dark for gas measurement as described above.

Cultivation, taxonomy, and physiology of bacterial isolates

Solid media plates were prepared using autoclaved lake water amended with MPn (10 μM), glucose (50 μM), pyruvate (10 μM), glycine (5 μM), and agar (1% w/v) to selectively cultivate bacteria that can utilize MPn. Following inoculation with 200 μL of raw lake water, plates were incubated at 8°C in the dark. After about one month of growth, single colonies from the plates were inoculated into 5 mL of MPn liquid media in 20 mL air-tight culture tubes with 0.2 μm filtered ambient air as head space. The tubes were incubated at 8°C in the dark with shaking at 150 rpm for 14 d. The MPn liquid medium was prepared with an oligotrophic culture medium with P*i* replaced by MPn as the sole P source using the formula described in Carini et al. (2014). The salinity of all media was adjusted with NaCl to match that of the lake water. Methane concentrations were measured in the headspace gas by the chromatographic method described previously. After methane production was confirmed from the isolates, cultures were inoculated into larger volumes of media in 150-mL flasks to obtain larger biomass for sequencing and physiology tests.

DNA of each bacterial culture was extracted using Wizard Genomic DNA Purification Kits (Promega) following the manufacturer's instructions. Near full length 16S rRNA genes were amplified using 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) prokaryotic universal primers. PCR reactions were carried out using 5 min of initial denaturation at 95°C, 25 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min with a final extension step at 72°C for 10 min (Weisburg et al. 1991). Amplicons were cloned into pCR2.1 plasmid (Invitrogen) with a TA Cloning Kit (Invitrogen). Sequencing reactions were performed using the BigDye Terminator v3.1 cycle sequencing kit (ABI) with M13R primer and the fragments were sequenced on an Applied Biosystems 3730×l DNA Analyzer (ABI) located in the Center for Bioinformatics and Functional Genomics (CBFG) at Miami

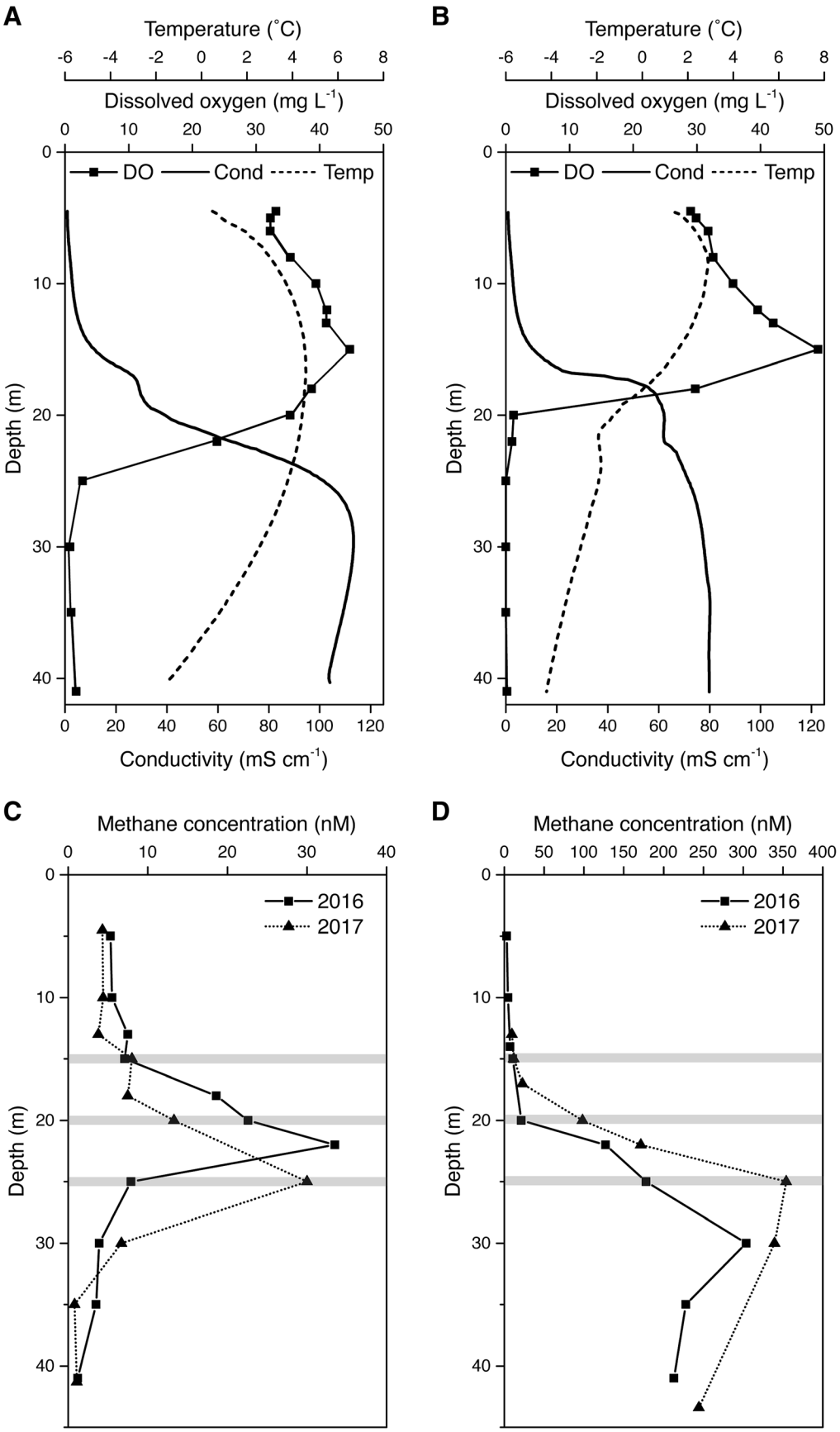

Fig. 2. Temperature, conductivity, and dissolved oxygen profiles in the water columns of east (ELB, **A**) and west (WLB, **B**) Lake Bonney. Date were collected on 18 and 22 November 2018 for ELB and WLB, respectively. Methane concentration measured from samples collected in 2016 and 2017 of ELB (**C**) and WLB (**D**). Horizontal lines in **C** and **D** indicate discrete sampling depths for cultivation experiment in the study.

Table 1. Experiment setup of lake water amended with methylphosphonate (MPn), various organic C and N sources, and phosphate (P*i*).

Treatment	Setup
Water	Lake water
Filtered water	0.2-μm filtered lake water
+ MPn	Lake water + 10 μM MPn
+ MPn, C, N	Lake water + 10 μm MPn, 10 μM pyruvate, 50 μM glucose, 5 μM glycine
+ P*i*	Lake water + 10 μM phosphate
+ P*i*, C, N	Lake water + 10 μm phosphate, 10 μM pyruvate, 50 μM glucose, 5 μM glycine
+ MPn, P*i*, C, N	Lake water + 10 μm MPn, 10 μM phosphate, 10 μM pyruvate, 50 μM glucose, 5 μM glycine

University. The resulting sequences were searched and aligned in SILVA database (v123) using SINA Aligner (v1.2.11, https://www.arb-silva.de) to identify closely related organisms (Pruesse et al. 2012), and subjected to a maximum-likelihood phylogenic tree construction using RAxML (v8.2.11) (GTRGAMMA model of nucleotide substitution and 1000 bootstraps).

To investigate the natural presence of the bacterial isolates, the natural bacterial community information was obtained via existing 16S rRNA gene amplicon libraries (SRR5893985) from a previous study (Li and Morgan-Kiss 2019). Briefly, we used the Quantitative Insights Into Microbial Ecology (MacQIIME v 1.9.1) pipeline (Caporaso et al. 2010) to analyze the community composition in the natural water column data. Paired-end reads were randomly subsampled to 5000 reads per sample. Selection of operational taxonomic unit (OTU) and taxonomic classification were both performed following the open-reference clustering procedure. Sequences were clustered against the SILVA (v 123) database using the UCLUST algorithm with a similarity cutoff of 97%. Sequences without any hits from the reference databases were clustered de novo to assign new OTUs (Edgar 2010; Rideout et al. 2014). OTUs with one sequence in samples were discarded to reduce the potential diversity inflation due to sequencing errors.

A series of duplicated cultures was established for each isolated bacterial strain using four culturing temperatures (4°C, 8°C, 12°C, and 25°C) and four NaCl concentrations in the culturing media (0.2, 0.5, 0.7, and 0.9 M). Cell concentrations were monitored over time using a PhytoCyt flow cytometer (Turner Designs) with SYBR Green I (Molecular Probes) staining following the protocol described in Santibáñez et al. (2016).

Community DNA isolation and shotgun metagenomic sequencing

Water samples (1–2 L) collected at 5, 10, 13, 20, and 30 m in ELB and 5, 8, 14, 17, and 30 m in WLB were concentrated onto 0.2 μm PES filters (PALL Corporation). Filters were immediately frozen and stored at −80°C until DNA extraction. Library preparation for shotgun metagenomic sequencing was conducted with a Truseq Nano DNA LT Kit (Illumina) following manufacturer's instructions. DNA libraries were sequenced on an Illumina MiSeq platform (Illumina) at LAS (Gimpo) (GenBank SRA accessions: SRR9199117-9199120). We also included a WLB sample (from 15 m) collected in November 2012 (NCBI Bioproject accessions: PRJNA367371 - PRJNA367373).

Metagenomic data analysis

The raw sequences were quality filtered using Skewer (v0.2.2) (Jiang et al. 2014) to obtain reads that are longer than 200 bp with average quality scores of at least 30 after adapter trimming. Contigs were assembled using Ray (v.2.3.1, Ray Meta) assembler (Boisvert et al. 2012) with a k-mer length of 41. Contigs with a length < 1 kb were discarded and the rest were binned based on composition and differential coverage using the CONCOCT package (Alneberg et al. 2014). Coding genes (CDS) were predicted using Prodigal (v.2.6.3) (Hyatt et al. 2010), and were then annotated with the COG database (Tatusov et al. 2000; Overbeek et al. 2013) via RPS-BLAST. We used Checkm (v1.0.11) (Parks et al. 2015) and a small set of 36 universal single copy genes to evaluate the coverage of generated genome bins. We then used CDS homologous to the 36 single copy genes to assign taxonomy to each genome bin based on BLAST searching against GenBank. Coverages of each CDS were evaluated by mapping the reads with Bowtie2 (Langmead and Salzberg 2012) and HTseq-count programs. The coverage values were transferred to reads per kilobase per million mapped values calculated using in-house Perl scripts. To identify CDS or genes involved in methane producing and consuming from these assembled data, BLAST-searches (McGinnis and Madden 2004) and HMM-searches (Johnson et al. 2010) were performed with an in-house database consisting of amino acid sequences of methyl coenzyme M reductase (mcrA), particulate methane monooxygenase, and proteins involved in C-P lyase pathway retrieved from the GenBank database.

Results

Physical and chemical properties of the Lake Bonney water column

Conductivity in both lobes of Lake Bonney increased precipitously below distinct chemoclines near 20 and 17 m in ELB and WLB, respectively (Fig. 2A,B). These chemoclines, together with the permanent ice covers and low advective stream input, produce highly stable water columns where mixing is dominated by molecular diffusion (Spigel and Priscu 1998). Conductivities in the deep water of ELB and WLB exceed seawater levels by more than 2 times in ELB and 1.5 times in WLB. Water column temperatures never exceed 5°C in either lobe and were below 0°C in the salty bottom water. Oxygen levels in the trophogenic waters above the chemoclines were supersaturated reaching maximum levels

between 40 and 50 mg L^{-1}. DO dropped precipitously beneath the chemoclines to suboxic levels. Despite suboxia in the deep waters of both lobes, ELB contains high levels of nitrate, as well as oxidized sulfur and iron compounds (Ward and Priscu 1997; Lee et al. 2004).

Methane oversaturation in the oxic water column

Methane concentration in the water column above 30 m in ELB and the entire water column in WLB exceeded air saturation (~ 2.9 nM, Fig. 2C,D). The highest levels of dissolved methane occurred in ELB between 15 and 30 m with peak concentration of 33.5 nM at 22 m and 30.0 nM at 25 m in 2016 and 2017, respectively (Fig. 2C). These peak concentrations were over 1100% of ambient air saturation. DO concentrations in ELB were ~ 23 and 3 mg L^{-1} at 22 and 25 m, respectively. In contrast, WLB exhibited consistently high methane concentrations (> 4400% air saturation) below the chemocline (18–20 m) with peaks at 30 m (303.8 nM) and 25 m (354.1 nM) for 2016 and 2017, respectively. In WLB, the methane concentrations at the chemocline of (18–20 m) were close to those of ELB, while methane concentrations were significantly higher than those of ELB below the chemocline. DO concentration was oversaturated above 18 m in WLB and suboxic below 20 m in WLB (Fig. 2B).

Production of methane from MPn in the lake water

MPn amendment experiments were conducted with water samples collected at 15, 20, and 25 m in both ELB and WLB in 2016 and 2017 seasons, respectively. Methane production was not detected in unamended lake water or lake water with P*i* additions (i.e., +P*i*, +P*i*, C, N or +MPn, P*i*, C, N). Methane production in treatments amended with MPn (ELB 15 and 20 m and WLB 15 m) typically occurred from 15 to 20 d and reaching maximal values near 30 d (Fig. 3). In 2016 experiments, maximal yields were 248.0 and 945.9 nM for ELB at 15 and 20 m, respectively, and 529.7 nM for WLB at 15 m. Maximal methane yields in the 2017 experiments were significantly higher (*t*-test, $p < 0.001$) than that of corresponding samples in 2016 experiments, with 909.0 and 1954.3 nM for ELB at 15 and 20 m, respectively, and 1459.2 nM for WLB at 20 m.

Owing to limited sample volume during the 2016 experiments only four time points (over 70 d) were included in the time-course experiments whereas seven time points were included (over 30 d) in 2017. Methane production rates were only estimated in 2017 based on the assumption of linearity between the time of initial production and the time of maximum yield. Methane production rates in MPn amended treatments were 66.2, 210.8, and 73.8 nM d^{-1} for ELB 15 m, 20 m and WLB 15 m, respectively. Methane production rates in

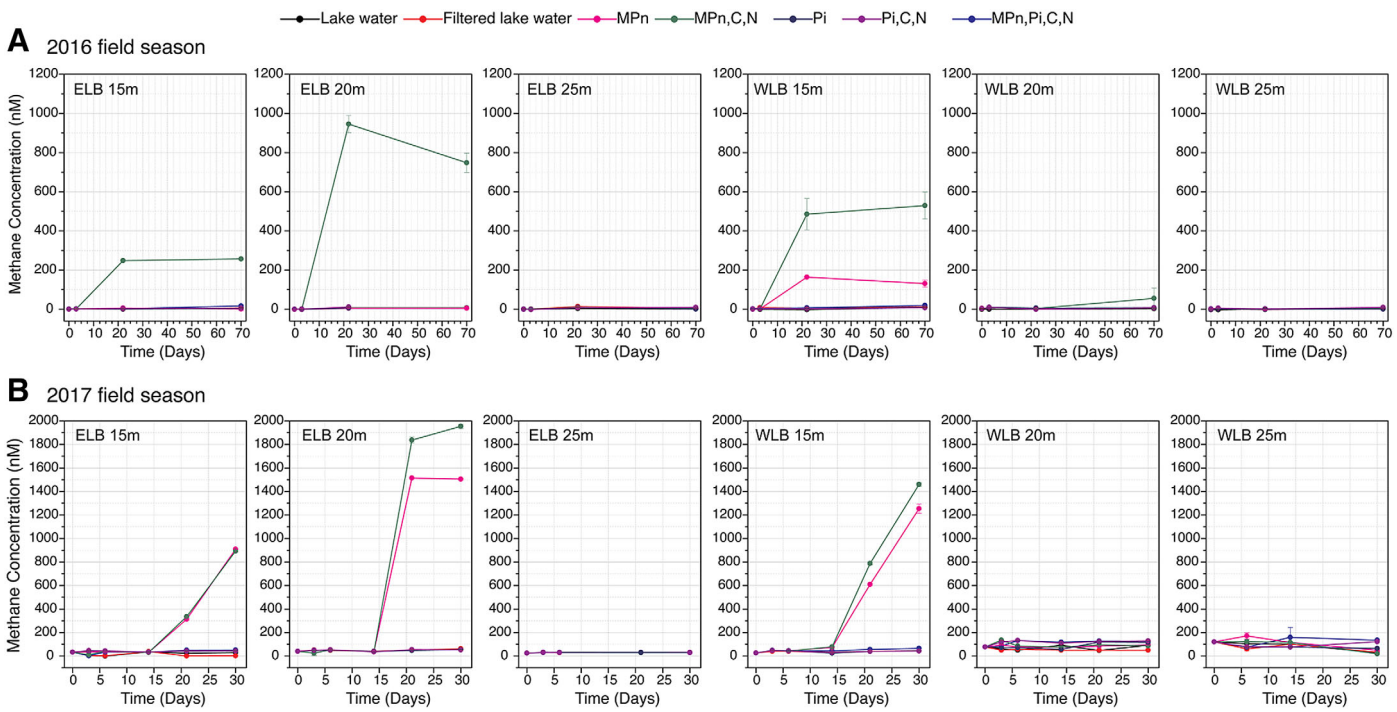

Fig. 3. Methane generation of lake water amended with various phosphorus compounds and/or organic carbon and nitrogen sources. Methane concentrations were measured in the headspace of sealed serum vials and converted to equivalent concentration in the liquid cultures. Filtered lake water: lake water filtered using syringe filters with a 0.22 μm pore size; MPn, 10 μM methylphosphonate; P*i*, 10 μm phosphate; C, organic carbon source: 10 μM pyruvate and 50 μm glucose; N, organic nitrogen source: 5 μM glycine. Data points are the mean and the bars indicate the range of replicated samples ($n = 2$). Experiments were conducted on samples collected during the 2016 (A) and 2017 (B) field seasons.

MPn, C, N triple-amendments were 53.9, 257.4, and 86.3 nM d^{-1} for ELB 15 m, 20 m and WLB 15 m, respectively.

Bacterial isolates

Five bacterial isolates who were able to use MPn as the sole P source and produce methane under oxic conditions were obtained from ELB 20 m (isolates E20_1_1, E20_4_1 and E20_9_1), as well as WLB 15 m (isolate W15_3_1) and 20 m (isolate W20_7_3). We sequenced near full length 16S rRNA gene PCR amplicons from the isolates. Phylogenetic analysis identified isolates E20_4_1 and E20_9_1 as two *Hoeflea* species, while E20_1_1 were closely related to *Marinomonas*. Bacteria strains derived from WLB were closely related to *Marinobacter* (W15_3_1) and *Algoriphagus* (W20_7_3) (Fig. 4).

The MPn-utilizing strains exhibited variable tolerances to temperature and salinity. Growth conditions for isolates were investigated by culturing the bacterial strains in various temperatures (4°C, 8°C, 12°C, and 25°C) and salinities (0.2, 0.5, 0.7, and 0.9 M of NaCl) conditions (Table 2). Isolate E20_1_1 reached maximum cell density in low salinity and low temperature; however, it grew at all the temperatures tested and up to 0.7 M NaCl, which was equivalent to 115% of salinity at the depth of isolation in the lake). Both *Hoeflea* species (E20_4_1 and E20_9_1) grew in all tested temperatures and salinity conditions and had optimal growth temperatures of 25°C and 12°C, respectively, well above the temperatures at the depth of isolation of 4.4°C. E20_4_1 culture reached to higher cell density in higher salinity. Isolates W15_3_1 (*Marinobacter*) and W20_7_3 (*Algoriphagus*) both grew in low temperatures (< 12°C) and required high salt (≥ 0.7 M NaCl) concentrations.

Community and functional gene analysis

We aligned the isolated bacteria strain sequences with the 16S rRNA gene (V4 region) amplicon sequencing libraries to identify OTUs matches the isolates (> 97% sequence similarity) and investigated their relative abundance in the prokaryotic

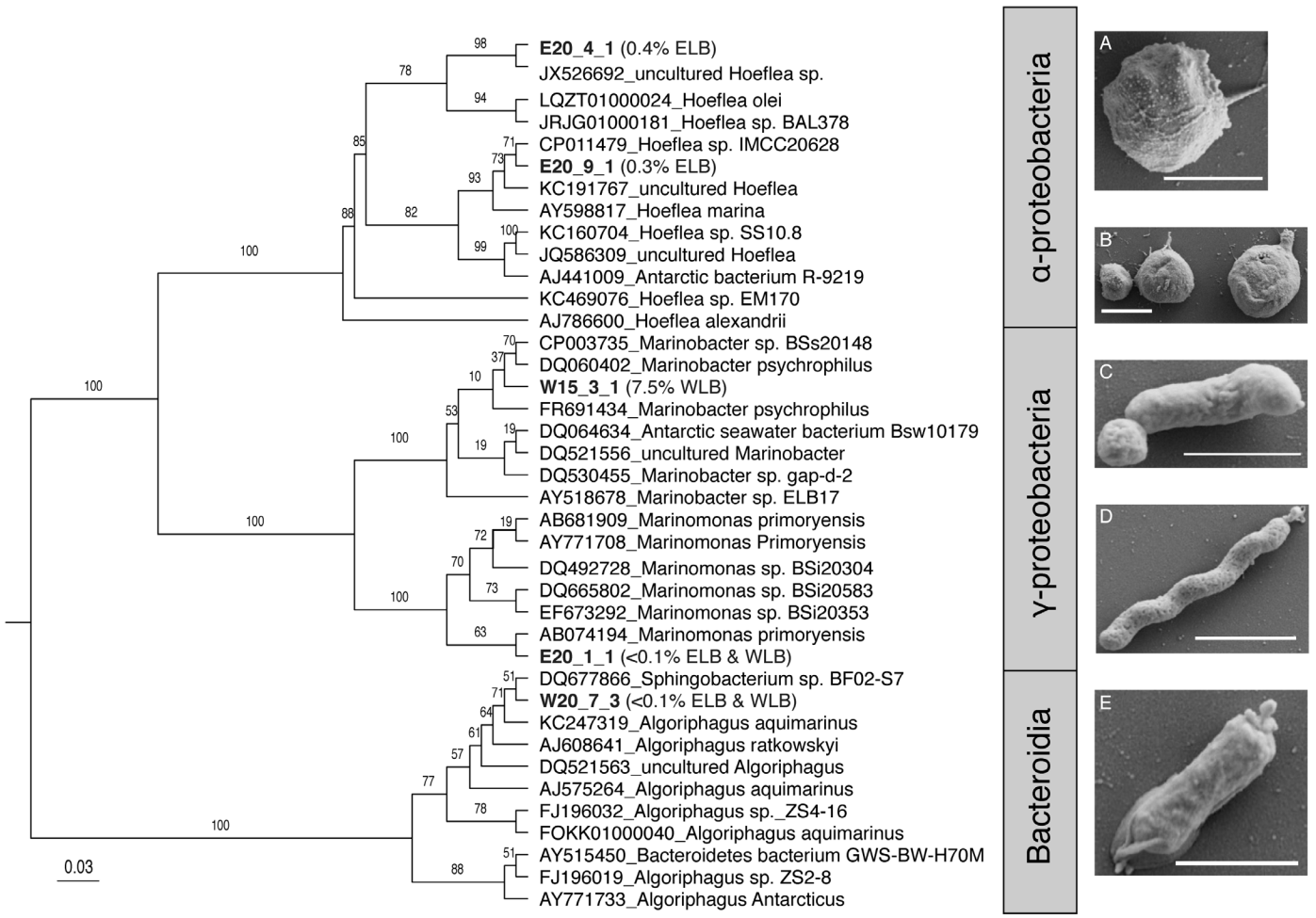

Fig. 4. A maximum-likelihood tree illustrating the phylogenetic relationships of Lake Bonney isolates. RAxML with 1000 bootstrap replicates. Numbers in the parentheses indicated relative abundance of isolates in the lake water column based on 16S rRNA gene sequencing libraries. Inserts are representative scanning electron microscopy images of isolated bacteria. (**A**) E20_4_1, *Hoeflea* sp.; (**B**) E20_9_1, *Hoeflea* sp.; (**C**) W15_3_1, *Marinobacter* sp.; (**D**) E20_1_1, *Marinomonas* sp.; and (**E**) W20_7_3, *Algoriphagus* sp. bars: 1 μm.

Table 2. Growth characteristics of isolated bacterial strains from Lake Bonney. Isolates: 1, E20_1_1, *Marinomonas* sp.; 2, E20_4_1, *Hoeflea* sp.; 3, E20_9_1, *Hoeflea* sp.; 4, W15_3_1, *Marinobacter* sp.; and 5, W20_7_3, *Algoriphagus* sp. Temperature experiments were carried out with media with 0.5 M of NaCl. Salinity experiments were carried out at optimal temperature of individual isolates. Salinity is equivalent to NaCl concentration.

Isolate	1	2	3	4	5
Temperature (°C)			Growth rate (day^{-1})		
4	0.085	0.128	0.042	0.068	0.062
8	0.024	0.141	0.045	0.045	0.109
12	0.014	0.098	0.145	0.036	0
25	0.033	0.326	0.131	0	0
Optimal temperature (°C)	4	25	12	4	8
Ambient temperature (°C)	4.4	4.4	4.4	1.3	−1.5
Salinity (M)			Growth rate (day^{-1})		
0.2	0.690	0.231	0.094	0.071	0
0.5	0.084	0.366	0.123	0.071	0.065
0.7	0.118	0.552	0.072	0.071	0.135
0.9	0.107	0.520	0.150	0.196	0.102
Optimal salinity (M)	0.7	0.7	0.9	0.9	0.7
Ambient salinity (M)	0.5	0.5	0.5	0.2	0.7

community of the lake water (shown in parentheses in Fig. 4). Among all five isolates, sequences of isolate W15_3_1 matched the sequence (99% similarity) of an OTU, which was one of dominant OTUs in the Gammaproteobacteria family and represented in approximately 7.5% of the total sequences in the whole water column. Searching results in GenBank database via BLASTn indicated that W15_3_1 is closely related to *Marinobacter psychrophilus* sp. nov. isolated from Artic sea ice (Zhang et al. 2008) and a bacterium isolated from Antarctic sea water (DQ064634) with 98% sequence similarity. Other isolates each represented less than 0.5% of the community.

On average, 22.8 million paired-end reads of 300 bp were generated in the metagenomic sequencing on samples collected in 2017 (ELB 5, 10, 13, 20, and 30 m; WLB 5, 8, 14, 17, and 30 m). Frequencies of several predicted functional gene families in the metagenomic libraries were calculated to compare the potential community functions in methane production and oxidation (Table 3). Predicted proteins related to C-P

Table 3. Frequency of predicted functional gene families in the lake metagenomic sequences. Normalized ratio (Ra) = total reads related to function/(gene sequence length/1000) × total number of reads/1,000,000).

	East Lake Bonney					West Lake Bonney				
Depth	5 m	10 m	13 m	20 m	30 m	5 m	8 m	14 m	17 m	30 m
Predicted function	Normalized abundance (Ra)									
Methyl coenzyme M reductase (mcrA)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phosphoenolpyruvate phosphomutase (pepM)	5.08	4.14	19.80	21.74	10.93	4.49	7.78	13.86	15.00	11.88
Methylphosphonate synthase (mpnS)	1.75	2.55	0.00	0.24	0.00	1.10	3.49	1.82	0.00	0.00
Alkylphosphonate utilization operon (PhnA)	4.27	6.47	104.82	172.46	28.18	3.00	9.32	37.82	94.22	98.36
PhnB	34.76	43.78	60.60	77.53	181.80	23.75	34.83	37.78	49.13	74.65
PhnG	2.34	2.02	10.09	10.89	14.51	1.91	3.19	12.65	2.16	0.92
PhnH	1.63	4.79	15.43	9.56	13.06	1.71	4.50	8.61	1.71	0.65
PhnI	6.52	8.28	30.31	34.58	19.62	5.06	7.75	17.91	3.93	1.99
PhnJ	3.98	7.21	28.68	33.00	18.34	2.58	6.08	17.23	5.79	1.64
PhnO	0.53	0.77	16.91	23.69	0.98	0.65	1.44	2.88	6.63	12.35
Phosphonates transport ATP-binding (PhnK)	3.95	6.30	27.09	28.10	20.13	3.02	7.96	18.98	3.51	1.62
Phosphonates transport ATP-binding (PhnL)	5.16	3.02	23.35	23.38	15.87	3.52	4.97	15.86	5.18	2.36
Methane monooxygenase (mmoX)*	1.62	1.71	3.34	0.48	0.65	3.28	4.26	6.08	0.00	0.66
Methane monooxygenase (mmoY)*	1.51	0.73	1.19	0.00	0.00	1.74	1.65	2.12	0.15	0.00

*Only soluble methane monooxygenase (mmoX and mmoY) sequences were identified in the lake metagenomic libraries.

lyase pathway were abundant, with the greatest number found in deeper water layers (i.e., 13–30 m in ELB and 14–30 m in WLB) relative to 5 and 10 m in ELB and 5 and 8 m in WLB. Previous studies suggested that the *phnCDEFGHIJKLMNOP* operon encoded the C-P lyase pathway in *Escherichia coli* to utilize organophosphonate. Phn C-E is considered as the phosphonate transporters while Phn G-P as functions as the C-P lyase complex, two fundamental components for this pathway (Jochimsen et al. 2011). Orthologs of the phosphonate transporters and C-P lyase complex in 42 and 27 metagenome bins, respectively, were identified in the metagenomic sequence library derived from WLB 15 m collected in 2012. In addition, the phophoenolpyruvate mutase gene (*PepM*), encoding a key enzyme involved in most known phosphonate biosynthetic pathways (Seidel et al. 1988), and the orthologs of methylphosphonate synthase (*MPnS*), the only gene known so far directly synthesizing Mpn in microorganisms (Born et al. 2017), were identified throughout the water columns. Grossart et al. (2011) proposed that methanogenic archaea associated with photosynthetic organisms could be responsible for oxic methanogenesis. In our metagenomic libraries from Lake Bonney, we detected no orthologs for the methyl coenzyme M reductase (*mcrA*) gene, a marker gene for methanogens. Moreover, no OTUs related to methanogens were found in the 16S rRNA gene tag sequencing library neither. We also surveyed major methane oxidation genes (i.e., soluble methane monooxygenase clusters) in the metagenomic libraries, and only fragments of *mmoX* and *mmoY* were identified. These fragments occurred primarily in samples from shallow layers.

Discussion

Methane oversaturation in aerobic waters has been reported in various marine and freshwater environments (Damm et al. 2010; Grossart et al. 2011; del Valle and Karl 2014; Donis et al. 2017; Teikari et al. 2018), however, the geographic extent of this paradox remains unclear (Tang et al. 2016). Conventional explanations for the oxic methane paradox include diffusion from anoxic sediments or oxygen-tolerance methanogens in the oxic waters (Hofmann et al. 2010; Grossart et al. 2011). A study on the sediments in Lake Bonney revealed no methanogens in the sediment core (Tang et al. 2013). Moreover, a previous study based on 16S rRNA gene Illumina tag sequencing indicated that methanogens were absent in the water column of Lake Bonney (Kwon et al. 2017). The methanogenesis marker gene, *mcrA* was also lacking in our metagenomic libraries (Table 2). Therefore, Archaeal methanogens are likely not major contributors to the methane in Lake Bonney, despite the fact that bacteria-specific 16S rRNA gene primers (Kwon et al. 2017) and metagenomic sequencing might not exclude the present of Archaeal methanogens at low abundances (Borrel et al. 2012). Collectively, these results indicate that methane production in Lake Bonney is largely independent of methanogenesis and is likely associated with an unconventional pathway.

Lake Bonney is an extremely phosphorus deficient system (Dore and Priscu 2001). Organisms could potentially overcome phosphate starvation by sequestration of organic phosphonate compounds (Metcalf and Wanner 1993; Carini et al. 2014). Using a metagenomic approach, we identified the presence of amino acid sequences of phophoenolpyruvate mutase (*pepM*), an important enzyme involving all known phosphonate biosynthesis (Seidel et al. 1988), and MPnS (Born et al. 2017), an enzyme that is responsible for MPn production, in Lake Bonney (Table 3). These results indicate the potential for bio-production of methylated phosphonate substrate for oxic methane production in both lobes of Lake Bonney. The Lake Bonney amendment experiments on whole lake water indicated that bacterial lysis of MPn could be a potential in situ source of methane in the oxygen supersaturated layers in this lake (Fig. 3). This contention is supported by the orthologs of C-P lyase gene cluster in our metagenomic sequencing libraries, for example, phosphonate transportors (*phnCDE*), C-P lyase complex (*phn*G-M), and ATP-binding cassette transporters (*phnK* and *phnL*) (Table 3; Jochimsen et al. 2011), suggesting the existence of a complete C-P lyase pathway in the lake water column.

Although rare methanotroph OTUs presented in the 16S rRNA gene libraries (Li and Morgan-Kiss 2019) and methanotrophic genes were identified from the metagenomic libraries (Table 3), constant methane concentrations were observed in the lake water control and Pi-amended samples over the course of the amendment experiments, indicating no significant methane consumption occurred (Fig. 3). Also, methane oxidation rates in the chemocline of either lobes of Lake Bonney were not measurable during a campaign in 2013 (Supporting Information Fig. S1). Therefore, methanotrophic activity was considered absent or minor in the depths of this study, and the methane increases in the MPn amendment treatment should be considered as gross methane production. The conversion rate of MPn to methane reached a height of 18% in all the positive methane production treatments. These rates were close to or exceeded values reported in similar experiments conducted in other oligotrophic aquatic environments (Murase and Sugimoto 2005; Wang et al. 2017). Because excess levels of MPn were added in our time-course experiments, the methane yields should be considered as community potential. Despite this caveat, the relatively high rates of MPn conversion that we measured in Lake Bonney suggest that the microbes are capable of utilizing this substrate in this P-limited environment. Although sources and sinks of MPn in natural aquatic environment are equivocal and the natural concentration of MPn in Lake Bonney remain unknown, our experiments indicated that with sufficient amount of substrate, natural microbial communities are capable of producing the levels methane detected in the oxic zones of both lobes of the lake. Collectively, our results from whole lake water amendment experiments lead us to contend that bacterial lysis of MPn

contributes the formation of methane over saturation in the oxic zone in the Lake Bonney basins.

To complement our amendment experiments on natural lake water, we cultivated MPn-utilizing bacteria from WLB and ELB and showed that five bacterial isolates were able to both utilize MPn as the sole P source and produce methane in the presence of MPn as a substrate. Based on molecular data from natural samples, the Gammaproteobacterium W15_3_1, *Marinobacter* sp., was the most abundant OTU in the water column of WLB. The WLB *Marinobacter* was closely related to a psychrophile isolated from Arctic sea ice, *M. psychrophilus* (Zhang et al. 2008), which agreed with our growth physiology data indicating that the WLB isolate is a halophilic (optimum salinity > 0.7 M NaCl) psychrophile (optimum growth temperature ≤ 12°C). *Marinobacter* is one of the most ubiquitous genera in global marine environment because of their metabolic versatility (Handley and Lloyd 2013) and resistance to environmental stresses (Polz et al. 2006). Although methane production from MPn metabolism has not been reported in any *Marinobacter*, it has been shown that *Marinobacter aquaeolei* VT8 possesses multiple putative C-P lyase-encoding operons in its genome (Handley and Lloyd 2013). Handley and Lloyd also showed that *M. aquaeolei* VT8 could utilize organic phosphorus (i.e., glyphosate) as a sole P source. To our knowledge, we are the first to show directly that *Marinobacter* sp. (W15_3_1) can produce methane via the lysis of MPn at a rate that can yield significant quantities of methane in the oxic waters of a P-deficient lake.

The isolate *Marinomonas* sp. E20_1_1 obtained in our study represents a second Gammaproteobacteria capable of utilizing MPn. OTUs related to the ELB *Marinomonas* were detected at low levels in both lobes of Lake Bonney (Fig. 4), and were most closely related to a second sea-ice psychrophile, *Marinobacter primoryensis* (Romanenko et al. 2003). Unlike the WLB *Marinobacter* isolate, the ELB *Marinomonas* is not a psychrophilic halophile: cultures grew under a wide range of temperature and salinity conditions, with an optimal of 4°C in relatively freshwater (Table 2). Our study is also the first to show that *Marinomonas* has the potential to produce methane from MPn. Genome mining predicted that *Marinomonas* sp. MED121, a strain isolated from the northwestern Mediterranean Sea, can potentially utilize 2-aminoethylphosphonate via C-P lyase pathway, however, the genome only contained truncated *phn* operons (Martinez et al. 2010).

Our culture work also isolated *Hoeflea* spp. E20_4_1 and E20_9_1 from ELB, and *Algoriphagus* sp. W20_7_3 (class Bacteroidia) from WLB, two strains belonging to Alphaproteobacteria. E20_4_1 is related to an uncultured *Hoeflea* sp. from the Southern Ocean (Singh et al. 2015), while E20_9_1 is related to an isolate with a sequenced genome (*Hoeflea* sp. IMCC20628). Both ELB *Hoeflea* spp. strains exhibited broad temperature and salinity tolerances (Table 2). The WLB isolate was most closely related to an uncultured *Sphingobacterium* from samples collected from the subglacial outflow from the Taylor Glacier known as Blood Falls, which flows into WLB (Mikucki and Priscu 2007). The WLB *Algoriphagus* isolate exhibited psychrophily (optimum growth temperature ≤ 8°C) and a requirement for high salinity (0.7 M NaCl). Neither *Hoeflea* nor *Algoriphagus* genera have been reported to exhibit the capacity to produce methane via MPn metabolism. Taken together, the physiological capacities of these newly isolated bacteria reveal the utilization of alternative phosphorus sources such as organic phosphonate via C-P lyase pathway with the subsequent production of methane.

Experimental results on whole lake water and cultures, in concert with genomic data on both basins of Lake Bonney, reveal that biological methane production via the C-P lyase pathway is likely responsible for methane oversaturation in the oxic waters of the lake. Additional sources of methane to Lake Bonney may be derived from metabolic lysis of organosulfur compounds or comminution of Taylor Glacier basal sediments. Damm et al. (2010), proposed that certain phytoplankton may produce methane using other lytic pathways such as that mediated by C-S lyase, and Althoff et al. (2014) demonstrated abiotic methane production from

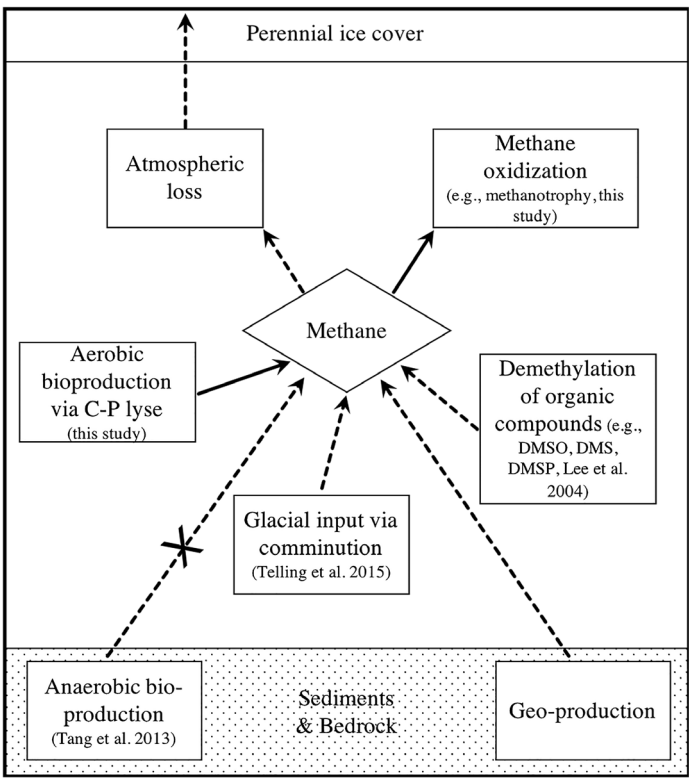

Fig. 5. A conceptual model for methane sources and sinks in Lake Bonney. The solid arrows indicate sources or sinks that have known evidence. Dashed arrows indicate sources or sinks that have not been tested. X indicates that an experiment in the lake has been performed and no evidence of anaerobic bio-production has been found (Tang et al. 2013).

organosulfur compounds including methionine, dimethyl sulphoxide (DMSO), and dimethylsulphoniopropionate (DMSP) in the presence of ferrihydrite and another chemicals under ambient temperature condition. The high levels of dimethylsulfide (DMS), DMSP, and DMSO in the water column of Lake Bonney (Lee et al. 2004) in concert to the iron rich brines derived from subglacial brine discharge (Mikucki et al. 2004, 2015) may provide the substrate for these methane-yielding lytic reactions. In addition, this subglacial discharge may also contain methane produced via comminution of basal sediments as shown by Telling et al. (2015) (Fig. 5) and demethylation of other methylated organic compounds (i.e., methyl amines) has been shown as a potential source of methane in other lakes (Bižić-Ionescu et al. 2018) and is an unexplored potential source in Lake Bonney. Clearly, a thorough understanding of methane sources in our study lake requires knowledge of sources other than conventional methanogenesis.

Reference

Alneberg, J., and others. 2014. Binning metagenomic contigs by coverage and composition. Nat. Methods **11**: 1144.

Althoff, F., and others. 2014. Abiotic methanogenesis from organosulphur compounds under ambient conditions. Nat. Commun. **5**: 4205.

American Public Health Association. 1995. WPCF, standard methods for the examination of water and wastewater. American Public Health Association.

Bastviken, D., L. J. Tranvik, J. A. Downing, P. M. Crill, and A. Enrich-Prast. 2011. Freshwater methane emissions offset the continental carbon sink. Science **331**: 50–50.

Bender, J. M. M. K. S., D. H. B. Madigan, T. Michael, and D. A. Stahl. 2015. Brock biology of microorganisms. Pearson.

Bižić-Ionescu, M., D. Ionescu, M. Günthel, K. W. Tang, and H. P. Grossart. 2018. Oxic methane cycling: New evidence for methane formation in Oxic lake water, p. 1–22. *In* Biogenesis of hydrocarbons. Springer.

Boisvert, S., F. Raymond, É. Godzaridis, F. Laviolette, and J. Corbeil. 2012. Ray meta: Scalable de novo metagenome assembly and profiling. Genome Biol. **13**: R122.

Born, D. A., E. C. Ulrich, K.-S. Ju, S. C. Peck, W. A. Van Der Donk, and C. L. Drennan. 2017. Structural basis for methylphosphonate biosynthesis. Science **358**: 1336–1339.

Borrel, G., A. C. Lehours, O. Crouzet, D. Jézéquel, K. Rockne, A. Kulczak, E. Duffaud, K. Joblin, and G. Fonty. 2012. Stratification of Archaea in the deep sediments of a freshwater meromictic lake: Vertical shift from methanogenic to uncultured archaeal lineages. PLoS One **7**: 43346.

Bowman, J. S., T. J. Vick-Majors, R. Morgan-Kiss, C. Takacs-Vesbach, H. W. Ducklow, and J. C. Priscu. 2016. Microbial community dynamics in two polar extremes: The lakes of the McMurdo Dry Valleys and the West Antarctic peninsula marine ecosystem. Bioscience **66**: 829–847.

Caporaso, J. G., and others. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods **7**: 335–336.

Carini, P., A. E. White, E. O. Campbell, and S. J. Giovannoni. 2014. Methane production by phosphate-starved SAR11 chemoheterotrophic marine bacteria. Nat. Commun. **5**: 4346.

Craig, H., R. Wharton, and C. McKay. 1992. Oxygen supersaturation in ice-covered Antarctic lakes: Biological versus physical contributions. Science **255**: 318–321.

Damm, E., and others. 2010. Methane production in aerobic oligotrophic surface water in the central Arctic Ocean. Biogeosciences **7**: 1099–1108.

del Valle, D. A., and D. M. Karl. 2014. Aerobic production of methane from dissolved water-column methylphosphonate and sinking particles in the North Pacific Subtropical Gyre. Aquat. Microb. Ecol. **73**: 93–105.

DelSontro, T., L. Boutet, A. St-Pierre, P. A. del Giorgio, and Y. T. Prairie. 2016. Methane ebullition and diffusion from northern ponds and lakes regulated by the interaction between temperature and system productivity. Limnol. Oceanogr. **61**: S62–S77.

Dlugokencky, E. J., E. G. Nisbet, R. Fisher, and D. Lowry. 2011. Global atmospheric methane: Budget, changes and dangers. Philos. Trans. A Math Phys. Eng. Sci. **369**: 2058–2072.

Dolhi, J. M., A. G. Teufel, W. Kong, and R. M. Morgan-Kiss. 2015. Diversity and spatial distribution of autotrophic communities within and between ice-covered Antarctic lakes (McMurdo Dry Valleys). Limnol. Oceanogr. **60**: 977–991.

Donis, D., S. Flury, A. Stockli, J. E. Spangenberg, D. Vachon, and D. F. McGinnis. 2017. Full-scale evaluation of methane production under oxic conditions in a mesotrophic lake. Nat. Commun. **8**: 1661.

Doran, P. T., and others. 2002. Valley floor climate observations from the McMurdo Dry Valleys, Antarctica, 1986–2000. J. Geophys. Res **107**: 4772. doi:10.1029/2001JD002045

Dore, J. E., and J. C. Priscu. 2001. Phytoplankton phosphorus deficiency and alkaline phosphatase activity in the McMurdo Dry Valley lakes, Antarctica. Limnol. Oceanogr. **46**: 1331–1346.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics **26**: 2460–2461.

Fountain, A. G., T. H. Nylen, A. Monaghan, H. J. Basagic, and D. Bromwich. 2010. Snow in the McMurdo Dry Valleys. Antarctica. Int. J. Climatol. **30**: 633–642.

Grossart, H. P., K. Frindte, C. Dziallas, W. Eckert, and K. W. Tang. 2011. Microbial methane production in oxygenated water column of an oligotrophic lake. Proc. Natl. Acad. Sci. USA **108**: 19657–19661.

Handley, K. M., and J. R. Lloyd. 2013. Biogeochemical implications of the ubiquitous colonization of marine habitats and redox gradients by *Marinobacter* species. Front. Microbiol. **4**: 136.

Hofmann, H., L. Federwisch, and F. Peeters. 2010. Wave-induced release of methane: Littoral zones as source of methane in lakes. Limnol. Oceanogr. **55**: 1990–2000.

Hyatt, D., G.-L. Chen, P. F. LoCascio, M. L. Land, F. W. Larimer, and L. J. Hauser. 2010. Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinform. **11**: 119.

Intergovernmental Panel on Climate Change. 2013. Climate change 2013: The physical science basis: Working group I contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press.

Jiang, H., R. Lei, S.-W. Ding, and S. Zhu. 2014. Skewer: A fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinform. **15**: 182.

Jochimsen, B., and others. 2011. Five phosphonate operon gene products as components of a multi-subunit complex of the carbon-phosphorus lyase pathway. Proc. Natl. Acad. Sci. USA **108**: 11393–11398.

Johnson, L. S., S. R. Eddy, and E. Portugaly. 2010. Hidden Markov model speed heuristic and iterative HMM search procedure. BMC Bioinform. **11**: 431.

Karl, D. M., L. Beversdorf, K. M. Björkman, M. J. Church, A. Martinez, and E. F. Delong. 2008. Aerobic production of methane in the sea. Nat. Geosci. **1**: 473–478.

Karl, D. M., and K. M. Björkman. 2014. Dynamics of dissolved organic phosphorus, p. 233–334. *In* Biogeochemistry of marine dissolved organic matter, 2nd ed. Elsevier.

Kirschke, S., and others. 2013. Three decades of global methane sources and sinks. Net. Geosci. **6**: 813–823.

Kolowith, L. C., E. D. Ingall, and R. Benner. 2001. Composition and cycling of marine organic phosphorus. Limnol. Oceanogr. **46**: 309–320.

Kong, W., W. Li, I. Romancova, O. Prášil, and R. M. Morgan-Kiss. 2014. An integrated study of photochemical function and expression of a key photochemical gene (psbA) in photosynthetic communities of Lake Bonney (McMurdo Dry Valleys, Antarctica). FEMS Microbiol. Ecol. **89**: 293–302.

Kong, W., D. C. Ream, J. C. Priscu, and R. M. Morgan-Kiss. 2012. Diversity and expression of RubisCO genes in a perennially ice-covered Antarctic lake during the polar night transition. Appl. Environ. Microbiol. **78**: 4358–4366.

Kwon, M., and others. 2017. Niche specialization of bacteria in permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica. Environ. Microbiol. **19**: 2258–2271.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods **9**: 357–359.

Lee, P. A., J. C. Priscu, G. R. DiTullio, S. F. Riseman, N. Tursich, and S. J. de Mora. 2004. Elevated levels of dimethylated-sulfur compounds in Lake Bonney, a poorly ventilated Antarctic lake. Limnol. Oceanogr. **49**: 1044–1055.

Li, W., and R. M. Morgan-Kiss. 2019. Influence of environmental drivers and potential interactions on the distribution of microbial communities from three permanently stratified Antarctic lakes. Front. Microbiol. **10**: 1067.

Lenhart, K., and others. 2016. Evidence for methane production by the marine algae *Emiliania huxleyi*. Biogeosciences **13**: 3163–3174.

Martinez, A., G. W. Tyson, and E. F. DeLong. 2010. Widespread known and novel phosphonate utilization pathways in marine bacteria revealed by functional screening and metagenomic analyses. Environ. Microbiol. **12**: 222–238.

McGinnis, S., and T. L. Madden. 2004. BLAST: At the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. **32**: W20–W25.

Metcalf, W. W., and others. 2012. Synthesis of methylphosphonic acid by marine microbes: A source for methane in the aerobic ocean. Science **337**: 1104–1107.

Metcalf, W. W., and B. L. Wanner. 1993. Evidence for a fourteen-gene, phnC to phnP locus for phosphonate metabolism in *Escherichia coli*. Gene **129**: 27–32.

Mikucki, J. A., E. Auken, S. Tulaczyk, R. A. Virginia, C. Schamper, K. I. Sørensen, P. T. Doran, H. Dugan, and N. Foley. 2015. Deep groundwater and potential subsurface habitats beneath an Antarctic dry valley. Nat. Commun. **6**: 6831.

Mikucki, J. A., C. M. Foreman, B. Sattler, W. B. Lyons, and J. C. Priscu. 2004. Geomicrobiology of blood falls: An iron-rich saline discharge at the terminus of the Taylor Glacier, Antarctica. Aquat. Geochem. **10**: 199–220.

Mikucki, J. A., and J. C. Priscu. 2007. Bacterial diversity associated with blood falls, a subglacial outflow from the Taylor Glacier, Antarctica. Appl. Environ. Microbiol. **73**: 4029–4039.

Morgan-Kiss, R., M. Lizotte, W. Kong, and J. Priscu. 2016. Photoadaptation to the polar night by phytoplankton in a permanently ice-covered Antarctic lake. Limnol. Oceanogr. **61**: 3–13.

Murase, J., and A. Sugimoto. 2005. Inhibitory effect of light on methane oxidation in the pelagic water column of a mesotrophic lake (Lake Biwa, Japan). Limnol. Oceanogr. **50**: 1339–1343.

Overbeek, R., and others. 2013. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res. **42**: D206–D214.

Parks, D. H., M. Imelfort, C. T. Skennerton, P. Hugenholtz, and G. W. Tyson. 2015. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. **25**: 1043–1055.

Petrenko, V. V., and others. 2017. Minimal geological methane emissions during the Younger Dryas-Preboreal abrupt warming event. Nature **548**: 443–446.

Polz, M. F., D. E. Hunt, S. P. Preheim, and D. M. Weinreich. 2006. Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. Philos. Trans. Royal Soc. B **361**: 2009–2021.

Priscu, J. C. 1995. Phytoplankton nutrient deficiency in lakes of the McMurdo Dry Valleys, Antarctica. Freshw. Biol. **34**: 215–227.

Priscu, J. C., and others. 1999. Carbon transformations in a perennially ice-covered Antarctic lake. Bioscience **49**: 997–1008.

Prisu, J. C., M. T. Downes, and C. P. McKay. 1996. Extreme supersaturation of nitrous oxide in a poorly ventilated Antarctic lake. Limnol. Oceanogr. **41**: 1544–1551.

Pruesse, E., J. Peplies, and F. O. Glöckner. 2012. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics **28**: 1823–1829.

Romanenko, L. A., N. V. Zhukova, A. M. Lysenko, V. V. Mikhailov, and E. Stackebrandt. 2003. Assignment of 'Alteromonas marinoglutinosa'NCIMB 1770 to Pseudoalteromonas mariniglutinosa sp. nov., nom. rev., comb. nov. Int. J. Syst. Evol. Microbiol. **53**: 1105–1109.

Repeta, D. J., and others. 2016. Marine methane paradox explained by bacterial degradation of dissolved organic matter. Nat. Geosci. **9**: 884–887.

Rideout, J. R., and others. 2014. Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. PeerJ **2**: 545.

Rigby, M., and others. 2017. Role of atmospheric oxidation in recent methane growth. Proc. Natl. Acad. Sci. USA **114**: 5373–5377.

Sannigrahi, P., E. D. Ingall, and R. Benner. 2006. Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter. Geochim. Cosmochim. Acta **70**: 5868–5882.

Santibáñez, P. A., J. R. McConnell, and J. C. Priscu. 2016. A flow cytometric method to measure prokaryotic records in ice cores: An example from the West Antarctic ice sheet divide drilling site. J. Glaciol. **62**: 655–673.

Schmale, O., and others. 2018. The contribution of zooplankton to methane supersaturation in the oxygenated upper waters of the central Baltic Sea. Limnol. Oceanogr. **63**: 412–430.

Seidel, H. M., S. Freeman, H. Seto, and J. R. Knowles. 1988. Phosphonate biosynthesis: Isolation of the enzyme responsible for the formation of a carbon–phosphorus bond. Nature **335**: 457–458.

Singh, S. K., and others. 2015. Response of bacterioplankton to iron fertilization of the Southern Ocean, Antarctica. Front. Microbiol. **6**: 863.

Sosa, O. A. 2018. Phosphorus redox reactions as pinch hitters in microbial metabolism. Proc. Natl. Acad. Sci. USA **115**: 7–8.

Spigel, R. H., and J. C. Priscu. 1998. Physical limnology of the McMurdo Dry Valleys lakes, p. 153–187. *In* Ecosystem dynamics in a polar desert: The McMurdo Dry Valleys, Antarctica, v. **72**. American Geophysical Union.

Spigel, R. H., J. C. Priscu, M. K. Obryk, W. Stone, and P. T. Doran. 2018. The physical limnology of a permanently ice-covered and chemically stratified Antarctic lake using high resolution spatial data from an autonomous underwater vehicle. Limnol. Oceanogr. **63**: 1234–1252.

Tang, C., M. T. Madigan, and B. Lanoil. 2013. Bacterial and archaeal diversity in sediments of west Lake Bonney, McMurdo Dry Valleys, Antarctica. Appl. Environ. Microbiol. **79**: 1034–1038.

Tang, K. W., D. F. McGinnis, D. Ionescu, and H.-P. Grossart. 2016. Methane production in Oxic lake waters potentially increases aquatic methane flux to air. Environ. Sci. Technol. Lett. **3**: 227–233.

Tatusov, R. L., M. Y. Galperin, D. A. Natale, and E. V. Koonin. 2000. The COG database: A tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. **28**: 33–36.

Teikari, J. E., and others. 2018. Strains of the toxic and bloom-forming *Nodularia spumigena* (cyanobacteria) can degrade methylphosphonate and release methane. ISME J. **12**: 1619–1630.

Telling, J., and others. 2015. Rock comminution as a source of hydrogen for subglacial ecosystems. Nat. Geosci. **8**: 851–855.

Teufel, A. G., W. Li, A. J. Kiss, and R. M. Morgan-Kiss. 2017. Impact of nitrogen and phosphorus on phytoplankton production and bacterial community structure in two stratified Antarctic lakes: A bioassay approach. Polar Biol. **40**: 1007–1022.

Turner, A. J., C. Frankenberg, P. O. Wennberg, and D. J. Jacob. 2017. Ambiguity in the causes for decadal trends in atmospheric methane and hydroxyl. Proc. Natl. Acad. Sci. USA **114**: 5367–5372.

van der Maarel, M. J., W. Sprenger, R. Haanstra, and L. J. Forney. 1999. Detection of methanogenic archaea in seawater particles and the digestive tract of a marine fish species. FEMS Microbiol. Lett. **173**: 189–194.

Wang, Q., J. E. Dore, and T. R. McDermott. 2017. Methylphosphonate metabolism by *Pseudomonas* sp. populations contributes to the methane oversaturation paradox in an oxic freshwater lake. Environ. Microbiol. **19**: 2366–2378.

Ward, B., and J. Priscu. 1997. Detection and characterization of denitrifying bacteria from a permanently ice-covered Antarctic lake. Hydrobiologia **347**: 57–68.

Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173**: 697–703.

Wharton, R. A., C. P. McKay, G. M. Simmons, and B. C. Parker. 1986. Oxygen budget of a perennially ice-covered Antarctic lake1. Limnol. Oceanogr. **31**: 437–443.

Wiesenburg, D. A., and N. L. Guinasso Jr. 1979. Equilibrium solubilities of methane, carbon monoxide, and hydrogen in water and sea water. J. Chem. Eng. Data **24**: 356–360.

Young, C. L., and E. D. Ingall. 2010. Marine dissolved organic phosphorus composition: Insights from samples recovered

using combined electrodialysis/reverse osmosis. Aquat. Geochem. **16**: 563–574.

Yu, X., and others. 2013. Diversity and abundance of phosphonate biosynthetic genes in nature. Proc. Natl. Acad. Sci. USA **110**: 20759–20764.

Zhang, D.-C., H.-R. Li, Y.-H. Xin, Z.-M. Chi, P.-J. Zhou, and Y. Yu. 2008. *Marinobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from the Arctic. Int. J. Syst. Evol. Microbiol. **58**: 1463–1466.

Zhang, Z., and others. 2017. Emerging role of wetland methane emissions in driving 21st century climate change. Proc. Natl. Acad. Sci. USA **114**: 9647–9652.

Acknowledgment

This work was supported by NSF Office of Polar Programs Grant (OPP-1115245, 1637708, and 1543537) and Korea Polar Research Institute (PE18340).

Conflict of Interest

None declared.

Submitted 12 April 2019
Revised 05 June 2019; 27 June 2019
Accepted 28 June 2019

Associate editor: Bo Thamdrup