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Taxonomic Diversity and Metatranscriptomic Activity of Lake Fryxell's Microbial Mat and
Metabolisms at 10.1 m

By

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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in

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in the

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of the

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DAVIS

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Abstract

Lake Fryxell, a perennially ice-covered lake in the McMurdo Dry Valleys of Antarctica is an isolated extreme environment. As depth into the lake increases, the environment changes from an illuminated and oxygen-rich environment to an increasingly dark, sulfidic environment, lacking oxygen. The lake also hosts an abundance of microbial growth, characterized by photosynthetic microbial mats that grow on the lake floor to the base of the photic zone. In this study, we elucidate the taxonomic diversity and metabolic framework of the microbial mats during the Antarctic summer at 10.1 m, which is near the maximum depth of photosynthesis.

The microbial mats at 10.1 m are exposed to $\sim 3 \mu\text{mol photons/m}^2/\text{s}$ of photoactive radiation, less than $0.5 \pm 0.5 \text{ mg O}_2/\text{L}$, and $16.8 \mu\text{M}$ of hydrogen sulfide. How microbial organisms thrive in these conditions, especially what metabolisms they utilize to produce energy, is a fundamental ecological question. 16S rRNA, metagenomic and metatranscriptomic analyses described in chapter one revealed 700 - 800 different species residing in the lake, representing over 18 different phyla, with nine phyla that have not previously been reported in Lake Fryxell. High completion metagenome assembled genomes, and assembled transcriptomes reveal the metabolic capacity of the mats. Aerobic respiration, photosynthesis, fermentation, electron bifurcation, sulfide oxidation, sulfate reduction, and nitrogen cycling metabolisms are active at 10.1 m. The Wood Wjungdahl Pathway (WLP) and Calvin Cycle metabolisms were also active, indicating different methods of carbon fixation. Analysis of both the genomic capacity and transcriptomic expression of the microbial mats suggests that a diverse range of organisms with a variety of microbial metabolism can accommodate the fluctuating environmental conditions.

Photosynthetic and primary productivity is not limited to the bacterial community; diatoms in Lake Fryxell display high abundance in the microbial mats but remain largely ignored. To understand the impact that the diatom community has on the microbial mats, it is critical to not only genomically identify the organisms present but elucidate what metabolic pathways they are using to thrive in the extreme conditions of Lake Fryxell. Utilizing long read sequencing for DNA and short read sequencing for RNA, chapter two explores the diversity of diatoms in the microbial mats at 10.1 m. Six different diatom species were identified with 18S rRNA sequences extracted from raw DNA long reads; three of these species have been morphologically identified but not fully genomically characterized. One diatom 18S rRNA classification was a novel sequence, only registered as Bacillariophyceae, which could indicate novel species or genomic detection of diatoms only morphologically identified in Lake Fryxell. The two remaining 18S rRNA sequences were unidentified species of *Amphora*, *Pseudo-nitzschia* genera. RNA expression data showed transcription of oxygenic photosynthesis and aerobic respiration occurring at 10.1 m despite the presence of hydrogen sulfide and only 3 $\mu\text{mol photons/m}^2\text{s}$. There was also expression data unable to be mapped but still classified as diatom in origin. The transcript encoded components for photosynthesis, sulfide oxidation, and fermentation. This implies that the diatom population contributes to the primary productivity of the microbial mats in the presence of sulfide while also employing aerobic and anaerobic processes. Overall, the discovery of new diatom species and diverse metabolic activity establishes the diatoms as key community members in the microbial mats at 10.1 m and improves our understanding of their genomic and metabolic diversity in extreme environments.

Constructing an ecological metabolic framework as to how the microbial mats actively survive in this environment at 10.1 m would provide foundational knowledge into how microbial

communities may adapt to environmental and season changes in the lake. Utilizing the high completion metagenome assembled genomes collected in chapter one, bacterial metagenome assembled genomes (MAGs) were investigated for their specific metabolic roles in the microbial mats. Mapping expressed RNA to the assembled DNA yielded insights into both specialization and versatility among and within bacterial MAGs. Collected phyla contained complete pathways for the nitrogen cycle, sulfur cycle, aerobic respiration, fermentation, the WLP, the Calvin cycle, and lipid beta oxidation. A deeper investigation resulted in sulfate reduction expressed by forty MAGs assembled. Other bacterial metagenomes displayed notable metabolic diversity, utilizing over nine different metabolic pathways. MAGs within the the Gemmatimonadota phylum heavily expresses the enzyme critical to dissimilatory nitrate to ammonium. MAGs placed within the Desulfobacterota expressed the WLP while Pseudomonadota related metagenomes utilized the Calvin cycle, contributing to the chemoautotrophy of the lake as nonphotodependent primary producers. It was expected that the microbial communities at 10.1 m would rely primarily on anaerobic metabolisms like sulfate reduction, sulfide oxidation, anaerobic respiration, and fermentation due to the reduced dissolved oxygen and photoactive radiation. However, the established overall diversity of framework created suggested that oxygenic photosynthesis by Cyanobacteria and diatoms increased the oxidation reduction potential above and within the mat in diel cycles. This may raise the ORP and provide oxygen for aerobic respiration, nitrification, aerobic sulfide oxidation, and abiotic conversion of sulfide to sulfate for sulfate reducers. While sulfate reduction was the most prominently expressed metabolisms, the MAGs analyzed showed both diversity and specialization in aerobic and anaerobic processes, revealing the nuance and complexity of this isolated extreme environment.

Introduction

Perennially ice-covered lakes of the McMurdo Dry Valleys, Antarctica, are considered an isolated extreme environment (Laybourne-Perry et al., 2007). Lake Fryxell is characterized by permanent ice cover, strong vertical stratification, and limited physical mixing, resulting in pronounced gradients in light availability, dissolved oxygen, and nutrient chemistry with depth (Green et al., 1989; Craig et al., 1992). During the summer, meltwater streams deliver solutes and nutrients to the lake, while water loss occurs primarily through ice ablation and evaporation from the summer moat (Lawrence & Hendy, 1985). As a result, this produced a stable, density-stratified water column below 5 m. (Warton et al., 1986). While the ice cap does reduce 97% - 99% of incident irradiance, the light that does pass through provides enough photoactive radiation (Howard-Williams et al., 1998) for the photosynthetic microbial communities in the microbial mats.

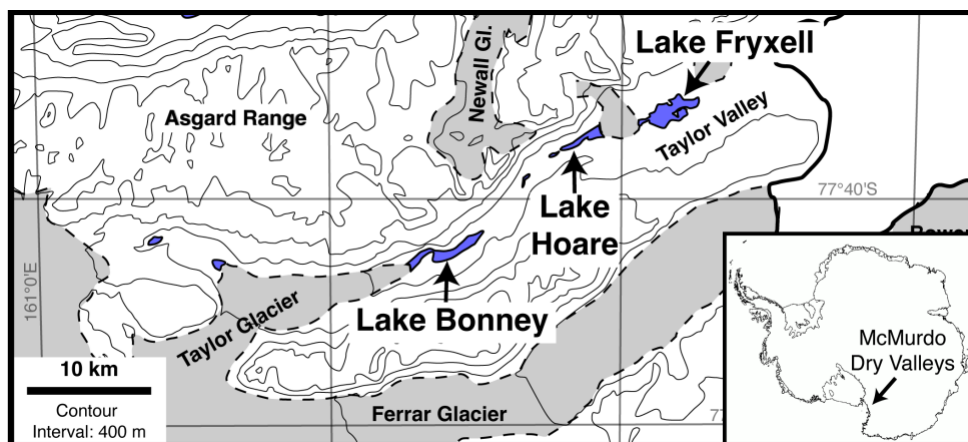


Figure 1: Location of Lake Fryxell in the McMurdo Dry Valleys, Antarctica (Sumner et al., 2015)

Microbial mats in Lake Fryxell are layered biofilms composed of diverse bacteria, archaea, and microeukaryotes (Jungblut et al., 2016; Dillon et al., 2020; Connell et al., 2018). Community composition and metabolic potential are linked to environmental gradients, with

phototrophic organisms dominating surface layers and anaerobic metabolisms, including sulfate reducers, fermenters, and methanogenic organisms, becoming increasingly important with depth into the lake and mats (van Gernerden, 1993; Bolhuis et al., 2014; Dillon et al., 2020a). While previous studies have demonstrated that oxygenic photosynthesis and aerobic respiration dominate mats between 9.0 and 9.8 m, much less is known about microbial diversity, metabolic expression, and taxonomic composition below this range, particularly near 10 m where light is minimal and oxygen availability is highly variable (Dillon et al., 2020).

Diatoms are increasingly recognized as key contributors to primary production within Lake Fryxell microbial mats, particularly at greater depths where they become dominant microeukaryotic phototrophs (Jungblut et al., 2016). However, their taxonomic diversity, genomic potential, and metabolic strategies remain poorly characterized, largely due to limited genomic resources and reliance on short-read sequencing and morphological identification (Schulte et al., 2021; Stone et al., 2024). Given their known capacity for metabolic plasticity under low-light, anoxic, and sulfidic conditions, diatoms may play a critical and underappreciated role in sustaining carbon and nitrogen cycling in deeper regions of the lake.

Understanding how microbial communities—and diatoms in particular—adapt to the combined constraints of low light, fluctuating oxygen, and complex nitrogen and sulfur chemistry is essential for elucidating the metabolic framework of Lake Fryxell's benthic ecosystem. This study aims to characterize microbial diversity and metabolic potential at ~10.1 m depth, providing new insight into how environmental gradients structure community composition and energy metabolism in one of Earth's most extreme aquatic environments.

Chapter 1: Taxonomic and Metabolic Diversity of Lake Fryxell's Microbial Mat at 10.1 m

Introduction

Microbial mats are distributed all over the globe. They can be found in hypersaline lakes, hot springs, marine sediment, intertidal zones, and polar lakes (Prieto-Barajas et al., 2018). The microbial mats of Lake Fryxell, Antarctica, McMurdo Dry Valleys are no exception. The structure of microbial mats are governed by availability of photoactive radiation (PAR), dissolved oxygen, and oxidation-reduction potential (Dillon et al., 2020). Within just millimeters of vertical space, mat structure, microbial communities and active metabolisms can change (Dillon et al., 2020b). The microbial mat structure shift from lift off mats at ~8 m, where oxygen produced by photosynthesis builds up within the mat and tears it off the lake sediment, to honeycomb mats, where ridges form honeycomb like structures (Jungblut et al., 2016). Finally, the microbial mats become flat/prostrate at ~ 10 m (Jungblut et al., 2016). The top layers of the lake's microbial mats are generally covered in phototrophic organisms such as Cyanobacteria, algae, and diatoms (Jungblut et al., 2016; Dillon et al., 2020). It is unclear though what the composition of the mats is like below 10 m. Still, these photoautotrophic organisms on the mat surface can provide oxygen and fix carbon, producing required materials for heterotrophic metabolisms and build biomass to establish an ecological foundation in different environments. Deeper into the anaerobic layers of the mats, anaerobic community members consist of anaerobic bacteria and archaea involved in sulfate reduction, methanogenesis, fermentation, and other anaerobic processes (van Gemerden, 1993; Bolhuis et al., 2014). Although metabolic expression has been previously explored at oxic depths in Lake Fryxell's mat, it is unclear how the microbial communities adapt and what strategies they use in oxic-anoxic transition depths in the lake (Dillon et al., 2020). Determining the community composition and metabolic activity at 10.1

m would provide insight into the dynamics of microbial mats at this depth that has not been investigated in detail with metagenomics and metatranscriptomics.

Prior work has investigated the taxonomic diversity of microbial communities between 9.0 m and 9.8 m. *Proteobacteria* and *Cyanobacteria* were abundant, similar to Lake Untersee and non-Antarctic microbial mats (Dillon et al., 2020; Greco et al., 2020). Yet, with the dissipation of PAR with depth and increase in hydrogen sulfide, understanding how the microbial populations change will provide valuable insights into how the microbial mats communities change in response at 10.1 m. If patterns remain consistent with previous depths, community diversity and metabolism would be determined to be influenced by the presence of dissolved oxygen and PAR (Dillon et al., 2020).

In Lake Fryxell, as depth increases, so does light. Generally, with less photons available for photosynthesis, there is a reduction of oxygenic photosynthesis, and less oxygen is produced. This, in turn reduces the availability of oxygen for aerobic and oxidative processes. It also limits microbes to using anaerobic metabolisms despite some microbes being able to utilize aerobic and anaerobic metabolisms (Jiang et al., 2012). At 9.0 m to 9.8 m, oxygen and PAR determine the metabolic diversity (Dillon et al., April 2020). It is expected that with a reduction in oxygen, there will be less diversity of metabolic expression as pathways will be confined to anaerobic reductive processes. Determining if the microbial mats follow this structured framework for metabolic diversity at 10.1 m will provide an understanding of what autotrophic and heterotrophic processes the microbial communities utilize.

Environment of Lake Fryxell

Lake Fryxell is a perennially ice-covered lake located in the McMurdo Dry Valleys of Antarctica. The lake is fed by melt water streams that provide solutes, sediment, and nutrients during the summer and into the moat that forms around the lake (Green et al., 1989, Lawrence and Hendy, 1985). This provides the lake with dissolved organic nitrogen, soluble reactive phosphorus, sulfate, calcium, magnesium, sodium, potassium, chloride, and dissolved organic matter (Herbei et al., 2016; McKnight et al., 1999). Local topography prevents the outflow of water which is only lost through ice ablation and liquid water evaporation from the summer moat. The solutes and nutrients deposited into the lake are subject to diffusion below ~5m due to density stratification in the water column caused by the long-term history of solute concentration and the lack of wind mixing (e.g. Craig et al., 1992; Warton et al., 1986). The ice-cover blocks 97-99.5% of incident PAR (Howard-Williams et al., 1998). This reduces photons during the summer at noon from 100 $\mu\text{mol photons/m}^2/\text{s}$ just below the ice. The water has a depth attenuation coefficient of 0.5-0.6/m which reduces irradiance to ~3 $\mu\text{mol photos/m}^2/\text{s}$ at about 10 m depth (Figure 1; Howard-Williams et al., 1998). The theoretical proposed minimum PAR for photosynthesis is 0.01 $\mu\text{mol photons/m}^2/\text{s}$ with contextual evidence of Arctic algae demonstrating the capacity to use as little as $0.04 \pm 0.02 \mu\text{mol photos/m}^2/\text{s}$. (Hanke K et al., 2018; Hoppe CJM et al., 2024). Similarly in Lake Hoare, also located in the McMurdo Dry Valleys, it has been demonstrated that Cyanobacteria and diatom communities within a 16.6 m depth are able to produce $\sim 100\text{-}500 \mu\text{mol m}^{-2} \text{h}^{-1}$ of oxygen with only 1.0-4.6 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ with a community compensation irradiance of 0.1 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (Hawes et al., 2006). Thus, the PAR available during summer is likely enough for photoautotrophic organisms to perform oxygenic photosynthesis at 10.1 m in Lake Fryxell.

During the winter, the sun is below the horizon for several months, and there is insufficient light for photosynthesis within the lake. This causes a complete halt of oxygenic photosynthesis (Cono et al., 2022). As a result, the water above the mats at 9.5 m and deeper becomes anoxic (Figure 1; Cortes et al. in prep). In response, photoautotrophs are expected to switch to alternative metabolic strategies, like glycogen catabolism (Welkie et al., 2018). Other non-photosynthetic bacterial phyla may utilize the remaining oxygen and or use anaerobic processes, such as but not limited to fermentation, anaerobic respiration, or dissimilatory sulfate reduction. The key goal is to understand how these environmental factors affect the microbial diversity at 10.1 m and what metabolisms the microbes use at this depth to produce energy.

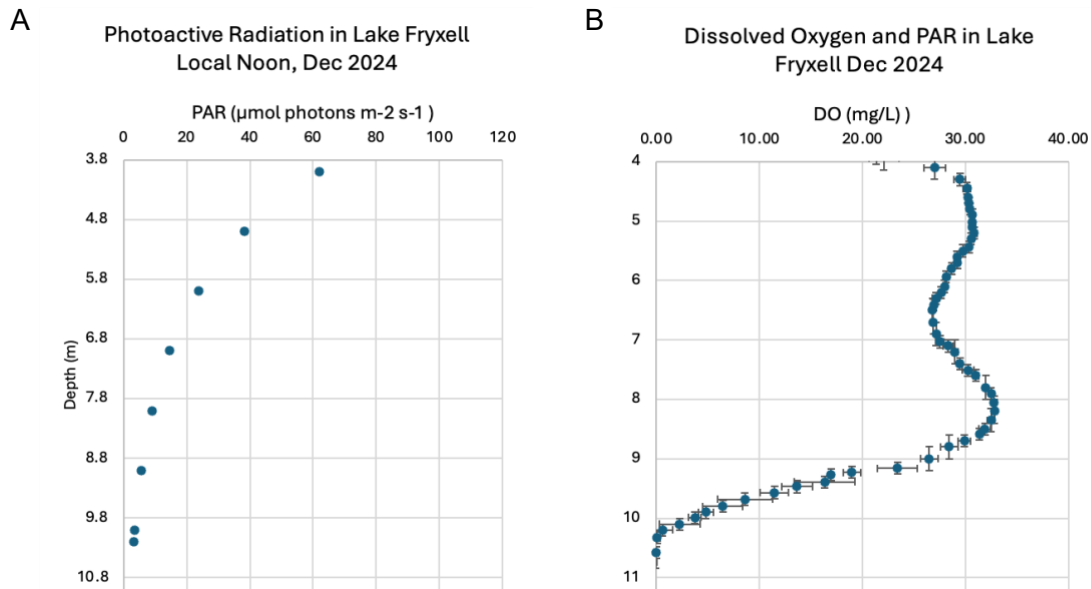


Figure 1: (a) PAR and (b) oxygen with depth below the ice-cover at Lake Fryxell. The graph depicts dissolved oxygen declines between 9 m – 10.1 m and PAR.

Lake Fryxell's Microbial Mats

The mats growing in Lake Fryxell thrive in extreme conditions. These microbial mats are complex multi-layered biofilms found in and contain a variety of different microbial communities. Previous research has identified the bacterial phyla found in the microbial mats including Cyanobacteria, Pseudomonadota (formerly Proteobacteria), Bacillota, Chloroflexota, Patescibacteria, Plantomycetes, Actinobacteria, Acidobacteria, Gemmatimonadetes, Nitrospirae, Verrucomicrobia, and Bacteroidota (Dillon et al., 2020). The microbial mats are not constrained to only bacteria; the archaea belonging to the phyla, Melainabacteria, Thaumarchaeota, Parvachaea, Crenarchaeota and Euryarchaeota can be found in the mats (Jungblut et al., 2016; Karr et al., 2006). Microeukaryotic organisms are also prevalent. Bacillariophyta are present in the microbial mats and detected between 8.9 – 9.8 m (Dillon et al., 2020; Jungblut et al., 2016). Most of the diatom community consists of *Muellaria* spp., *Navicula* spp., *Luticola* spp., *Nitzschia* spp., *Stauroneis* spp., and *Psammothidium*, below 10 m, *Diadsmis contenta* and *Navicula gregaria* become most abundant (Jungblut et al., 2016). Previous research at 9.0-9.8 m shows that alpha diversity increases with increases in both dissolved oxygen and PAR (Dillon et al., 2020). In some cases, Cyanobacteria decrease from more than 50% in abundance at the top of the mats at 9.8 m to less than 5% at the bottom, while Pseudomonadota increase from ~10% to ~25% abundance from the top to the bottom of the microbial mats (Dillon et al., 2020). It is still unclear how the microbial communities of Lake Fryxell change below 9.8 m, where the water above the mats becomes nearly permanently anoxic. It is expected that at greater depths, with reduced levels of oxygen and PAR, Cyanobacteria will decrease in abundance. With the absence of oxygenic photoautotrophs and oxygen production, facultative anaerobic organisms and obligate anaerobic organisms may increase in abundance. Chemoautotrophic metabolisms would

become more prominent and expression of pathways like the Wood Ljungdahl Pathway would be more abundant. Heterotrophic pathways would favor anaerobic metabolisms like nitrogen respiration, sulfate reduction, and or fermentation.

The organisms present in the lake have the genetic potential to perform a wide range of metabolisms including photosynthesis, aerobic respiration, anaerobic respiration, sulfur oxidation and reduction, nitrogen-based metabolisms, methanogenesis, and electron bifurcation (Karr et al., 2006; Dillon et al., April; 2020; Jungblut et al., 2016). Previous research on mats at 9.0-9.8 m depth used the high relative metagenomic abundance of Cyanobacteria to conclude that the most ecologically important process is oxygenic photosynthesis, using rubisco to fix carbon (Dillon et al., 2020; Dillon et al., April; 2020; Jungblut et al., 2016). Complimenting this factor, aerobic respiration capacity is highly abundant genomically as well, which is consistent with the measured abundance of dissolved oxygen (Dillon et al., 2020; Sumner et al., 2015). Alternative metabolisms are genomically represented as well. Genes associated with dissimilatory sulfate reduction were less prominent at 9.0-9.8 m but indicate active sulfate reducing communities (Dillon et al. 2020). Despite the presence of key enzymes, establishing what metabolic activities are occurring in higher resolution at 10.1 m is critical to understanding how autotrophic and heterotrophic processes compose the metabolic and taxonomic structure of the microbial mat's ecology.

Material and Methods

Dissolved Oxygen Measurements

Benthic variations in dissolved oxygen (DO) were measured from January 20 to November 1, 2023, at 10.1 m water depth to understand oxygen availability for the mats. Equipment was deployed and retrieved by divers operating through a hole melted in the ice cover

on the northern side of Lake Fryxell (-77.6078°, 163.114517° E). Divers deployed 3 PME miniDOT loggers (Vista, CA) at 9.99-10.35 m (7.7-8.0 m above mean sea level (a.m.s.l.)) depths by laying them horizontally with the sensor approximately 1 cm above the microbial mats coating the lake floor. The end opposite the sensor was attached to a PVC stake to counter buoyancy. The depth of each logger was measured using a Rugged TROLL pressure transducer from In-situ inc. (Fort Collins, CO), and depth is reported relative to January 2023 hydrostatic water levels. Elevation above mean sea level (a.m.s.l.) is also reported to aid in comparisons across years.

Sample Collection

Samples were collected on January 15, 2023, by divers operating through the same hole in the ice. Five samples were collected at 10.1 m water depth (7.9 m a.m.s.l.), and these samples are used for this study. Divers collected the samples in sterilized plastic boxes and brought them to the surface of the lake. At the lake surface, researchers put the samples into sterile 15 mL screw cap tubes and then into liquid nitrogen. The time between sample collection and freezing was less than 15 minutes for each sample. Samples remained in a dry shipper in liquid nitrogen temperatures until they were sent from Antarctica to the lab at UCD in a -20°C container. Once at UCD, they were stored in the laboratory at -80°C at UCD until DNA and RNA were extracted.

RNA Extraction

The RNA extractions at SeqCenter followed the ZymoBIOMICSTM Quick-RNA Miniprep Kit (Zymo Research, Irvine, CA). The preparation of microbial mat samples followed Zymo Research recommendations stated in the protocol. Samples were eluted into a final volume

of 40 µl. Final RNA concentrations were determined via Qubit3 from (Thermo Fisher Scientific Waltham, MA).

RNA Sequencing

Samples were extracted by SeqCenter (Pittsburg, PA, USA). The samples were DNase treated with Invitrogen RNase free DNase (Thermo Fisher Scientific, Waltham, MA, USA) . Library preparation was performed using Illumina's Stranded Total RNA Prep Ligation (Illumina, San Diego, CA) with Ribo-Sero Plus kit (Illumina, San Diego, CA) and 10bp unique dual indices (UDI). Sequencing was done on a NovaSeq XPlus (Illumina, San Diego, CA), producing paired end 150bp reads. Demultiplexing, quality control, and adapter trimming was performed with Illumina's bcl-convert (v4.2.4; Illumina, San Diego, CA).

DNA Extraction

The DNA extraction was performed by SeqCenter (Pittsburg, PA, USA) and followed the ZymoBIOMICS™ DNA Miniprep Kit2 (Zymo Research, Irvin, CA, USA). A single sequencing sample from bulk complete mat samples was created from five individual sample collection tubes at 10.1 m. The sample was suspended in lysis solution were transferred into the ZR BashingBead™ Lysis Tubes (Zymo Research, Irvin, CA, USA) and mechanically lysed using the MP FastPrep-24™ (MP Biomedicals, Irvine, CA, USA) lysis system with 1 minute of lysis at maximum speed and 5 minutes of rest for 5 cycles. The sample were then centrifuged at 10,000 rcf for 1 minute. 400 µl of supernatant was transferred from the ZR BashingBead™ (Zymo Research, Irvin, CA, USA) Lysis Tube to a Zymo-Spin™ III-F Filter (Zymo Research, Irvin, CA, USA) and centrifuged at 8,000rcf for 1 minute. 1,200 µl of ZymoBIOMICS™ DNA Binding Buffer (Zymo Research, Irvin, CA, USA) was added to the effluent and mixed via

pipetting. Next, 800µl of this solution was transferred to a Zymo-Spin™ IICR Column (Zymo Research, Irvin, CA, USA) and centrifuged at 10,000 rcf for 1 minute. This step was repeated until all material was loaded onto the Zymo-Spin™ IICR Column. DNA bound to the Zymo-Spin™ IICR Column was washed 3 times with 400µl and 700µl of ZymoBIOMICS™ DNA Wash Buffer (Zymo Research, Irvin, CA, USA) and then 200 µl of ZymoBIOMICS™ DNA Wash Buffer 2 (Zymo Research, Irvin, CA, USA) with a 1-minute spin down at 10,000 rcf for each, respectively. Washed DNA was eluted using 75µl of ZymoBIOMICS™ DNase/RNase Free Water (Zymo Research, Irvin, CA, USA) following a 5-minute incubation at room temperature and a 1-minute spin down at 10,000 rcf. The Zymo-Spin™ III-HRC Filter (Zymo Research, Irvin, CA, USA) was prepared using 600 µl of the ZymoBIOMICS™ HRC Prep Solution (Zymo Research, Irvin, CA, USA) and a centrifugation at 8,000 rcf for 3 minutes. Eluted DNA was then purified by running the effluent through the prepared Zymo-Spin™ III-HRC Filter. Final DNA concentrations were determined via Qubit (Thermo Fisher Scientific, Waltham, MA, USA).

DNA Sequencing

Sample libraries were prepared using the PCR-free Oxford Nanopore Technologies ONT_ Ligation Sequencing Kit SQK-NBD114.24 (Oxford Nanopore Technologies, Oxford, UK) with the NEBNext© Companion Module E7180 (Ipswich, MA, USA) to manufacturer's specification. No additional DNA fragmentation or size selection was performed. Nanopore sequencing was performed on an Oxford Nanopore, a MinION Mk1B (Oxford Nanopore Technologies, Oxford, UK) sequencer or a GridION sequencer (Oxford Nanopore Technologies, Oxford, UK) using R10.4.1 flow cells (Oxford Nanopore Technologies, Oxford, UK) in one or more multiplexed shared-flow cell runs. Run design utilized the 400 bps sequencing mode with a

minimum read length of 200bp. Adaptive sampling was not enabled. Guppy (v6.5.7; Oxford Nanopore Technologies, Oxford, UK) was used for super-accurate basecalling (SUP), demultiplexing, and adapter removal. Quality filtering was performed with fastp (v0.23.4) with a per-base quality trimming of Q20 and per-read quality filtering of Q15 (Chen et al., 2018).

Full Length 16S rRNA (V1-V9) Preparation and Sequencing Methods

Samples were prepared using Zymo “Quick-16S™ _Full-Length Library Prep Kit” using the following primers:

Table 1: RNA primers used for full length 16S rRNA sequencing by SeqCenter (Pittsburg, PA, USA)

Region	Forward Sequence	Reverse Sequence
	27f	1492r
V1-V9	AGRGTTYGATYMTGGCTCAG	RGYTACCTTGTTACGACTT

Samples were prepared via 1-step PCR and were pooled by equal volume. A MagBead cleanup was performed on the pooled library which was then prepared for sequencing using the PacBio HiFi prep kit 96 (Pacific Biosciences of California Inc, Menlo Park, CA, USA) with the SMRTbell® barcoded adapter plate 3.01 (Pacific Biosciences of California Inc, Menlo Park, CA, USA). Final libraries underwent the PacBio binding and cleanup steps using the Revio SPRQ Polymerase kit (Pacific Biosciences of California Inc, Menlo Park, CA, USA). Prepared libraries were sequenced on the PacBio Revio (Pacific Biosciences of California Inc, Menlo Park, CA, USA) with a 12hr run time. SMRTbell® (Pacific Biosciences of California Inc, Menlo Park, CA, USA) barcoded adapters were removed on instrument during CCS. Post-CCS HiFi reads

underwent secondary demultiplexing via Lima (v2.12.0; Lepik et al., 2026) using a 5bp linker plus a 10bp index with the following enabled options in Table 2.

16S rRNA Analysis

The 16S rRNA analysis was performed by SeqCenter (Pittsburg, PA, USA) to determine abundance of bacterial species. Samples were prepared using Zymo Research's Quick-16S Full-Length Library Prep Kit using the primers mentioned in Table 1. Five 10.1 m bulk microbial mat samples were processed by SeqCenter (Pittsburg, PA, USA). Samples were prepared via 1-step PCR and were pooled by equal volume. A MagBead cleanup was performed on the pooled library which was then prepared for sequencing using the PacBio HiFiPrep kit 96 with the SMRTbell barcoded adapter plate 3.0. Final libraries underwent the PacBio binding and cleanup steps using the Revio SPRQ Polymerase kit. Prepared libraries were sequenced on the PacBio Revio with a 12hr run time. SMRTbell barcoded adapters were removed on instrument during CCS. Post-CCS HiFi reads underwent secondary demultiplexing via Lima (v2.12.0) using a 5bp linker plus a 10bp index (Lepik et al., 2026). The following options were enabled:

Table 2: Options used for Lima (v2.12.0) during 16S rRNA processing.

Lima Option	Parameters
ccs	(enabled)
different	(enabled)
min-score	80
min-end-score	50
min-ref-span	0.75
min-scoring-regions	2
split-named	(enabled)
split-subdirs	(enabled)
store-unbarcoded	(enabled)

BAM files from the secondary demultiplexing were converted to fastq.gz files using samtools (v1.17) and pigz (v2.6) (Madler., 2022; Danecek et al., 2021).

Bioinformatic Analysis

16S rRNA Classification and Refraction Analysis

Sequences were imported to Qiime2 for analysis (Boylen et al., 2019). Primer sequences were removed using Qiime2's cutadapt3 plugin using the following the degenerate primer queries previously stated. Sequences were then denoised using Qiime2's dada2 (v2025.7.0) plugin4 Callahan et al., 2016; Boylen et al., 2019). Denoised whole sequences above QC 20 were placed into a feature table detailing which amplicon sequence variants (ASVs) were observed in which samples, and how many times each ASV was observed in each sample. ASVs were taxonomically assessed using the Silva (v136.2; Chuvochina et al., 2025) 138 99% full-length sequence database and the VSEARCH5 utility within Qiime2's feature-classifier plugin (Chuvochina et al., 2025; Ragnes et al., 2016). ASVs were then collapsed to their lowest taxonomic units (strain, species, genus, etc.), and their counts were converted to reflect their relative frequency within a sample.

RNA Metatranscriptomic Quality Control and Assembly

RNA sequencing for five samples recovered from 10.1 m were checked for quality with fastqc (v0.12.1) (Chen et al. 2018; Chen 2023). Reads collected were assembled with Trinity (v1.2.9) with paired end specification (Grabherr et al., 2011). Quality of the assembly was assessed with RNAquast (v2.3.1) (Bushmanova et al. 2016). The assembled RNA contigs were then annotated using kofmscan (v1.3.0) using standard settings (Arakami et al., 2019).

Taxonomic Classification of Metagenomes

DNA read quality was assessed with NanoPlot (v1.41) and low-quality reads, <15, were filtered out with fastp (v0.23.4) (De Coster et al. 2023; Chen et al. 2018; Chen 2023).

Fastp (v0.23.4) was used to clean the sequenced reads using default settings without adapter trimming (Chen et al. 2018; Chen 2023). Reads were assembled with flye (v2.9.4-b1799) using the --meta setting and checked for assembly quality with Quast (v5.2.0) using standard settings (Kolmogorov et al. 2020, Mikheenko et al. 2023). Reads were mapped back to the assembly using minimap2 (v2.28-r1209) (Li et al., 2018). After mapping, metabat2 (v2.18) was used to bin the DNA contigs which were subsequently taxonomically classified with GTDB-TK (v1.4.0) using the standard classify_wf settings (Kang et al., 2019; Chuameil et al., 2019). The metagenome assembled genomes were assessed for completion and contamination with CheckM (v1.2.3) -lineage_wf standard settings using default settings (Parks et al., 2015). High completion low contamination (HCLC) bins were selected based on a completion equal to or greater than ninety percent and contamination less than or equal to five percent. Contigs were also used for protein-based classification with kaiju(v1.10.1; Menzel et al., 2016) and the nr_euk database under greedy settings.

Autotrophic Metabolic Pathways Present in DNA MAGs and RNA-seq Assembled Transcripts

The Wood-Ljungdahl Pathway, the Calvin Cycle, the 3-hydroxypropionate bi-cycle, photo-dependent sulfide oxidation, and photosynthesis were thought to be utilized at 10.1 m. To determine this, DNA contigs were assembled as stated previously and annotated using PROKKA (v1.14.6) with standard settings (Seeman et al., 2014). RNA transcripts were converted to amino acid sequences with transdecoder (v5.7.0) and annotated using kofamscan (v1.3.0) using standard settings and an E-value less than e^{-5} (Haas., 2023; Aramaki et al., 2019). The annotation with the lowest E-value was chosen. Both annotations were investigated for enzymes used in the Wood-Ljungdahl Pathway, the Calvin Cycle, the 3-hydroxypropionate bi-cycle, photo-dependent sulfide oxidation, and photosynthesis (Table 3).

Table 3: List of autotrophic metabolisms and the transcripts/genes searched for within the KEGG ON annotation files.

Metabolism	Transcript/Gene Search List
Wood-Ljungdahl Pathway	<i>ackA, acsA, cooS, coxL, coxM, coxS, fdh, fhs, folD, pfor, pta, yitJ (metF) homologue</i>
Calvin Cycle	<i>cbbL, fbaB, fbp, gap, gap1, gapA, pgk, pgk/tpi, prkA, rbcL/S, rpe, rpiA, rpiB, sbp, tal, tkt, tpiA</i>
3-Hydroxypropionate Bicycle Cycle	<i>accA, accB, accC, accD, acul, gcl, gk, hcl, hpd, mce, mcl, mcr, mutB, pccA, pccB, pcs, scr, ssr, tsr</i>
Photodependent Sulfide Oxidation	<i>fccA, fccB, pufC, pufL, pufM, soxA, soxB, soxC, soxD, soxX, soxY, soxZ, sqr, tsdA</i>
Oxygenic Photosynthesis – Photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbO, psbT, psbU, psbX, psbY, psbZ, chlB, petB, petC, petE, petH</i>
Oxygenic Photosynthesis – Photosystem I	<i>psaA, psaB, psaC, psaD, psaE, psaF, psaI, psaJ, psaK, psaL, psaM, sqr, ycf3, ycf4</i>

Determination of Rubisco Large Chain Forms

Rubisco is a critical component in both light-dependent and light-independent carbon fixation. To find rubisco transcripts and genes, using RNA sequences that were assembled using Trinity (v1.2.9) and annotated with KOfamScan(v1.3.0) as previously stated to determine presence of expression of *rbcL* and *cbbL* (Grabherr et al., 2011; Aramkai et al., 2019). Resulting transcript sequences were subjected to BLASTX (v2.16.0) query searches using standard settings with an E-value less than e^{-15} using the nr database. Resulting matches with E-values less than e^{-15} were placed into a table displaying sample transcript, representative uniprot ID, representative phyla, and representative organism. For *rbcL* gene identification, alignments were filtered to retain only those with $\geq 90\%$ coverage against reference rubisco models, and sequences were restricted to a length between 358 and 716 amino acids to exclude partial or composite fragments. To analyze sequence diversity, clustering was performed at a 65% sequence identity threshold using MMseqs2 (Steinegger et al., 2017). To ensure that standard reference sequences—sourced from the dataset established by Kehl et al. (2025) served as cluster representatives rather than target-specific study sequences, the total dataset was partitioned into target-specific and reference subsets. Initial clustering was conducted on the reference subset using specific parameters (--min-seq-id 0.65, -c 0.8, --cov-mode 2, -s 1.5, --cluster-mode 0, and --cluster-steps 1), after which representative sequences were extracted. Target-specific sequences were then queried against these reference representatives using identical identity and coverage thresholds, with each assigned to the best-matching representative based on bitscore. This assignment to the Kehl et al. (2025) reference set was used to define the Rubisco Form and subform for the target sequence library. Any sequences failing to meet these alignment criteria were recorded as unassigned.

Results

Dissolved Oxygen

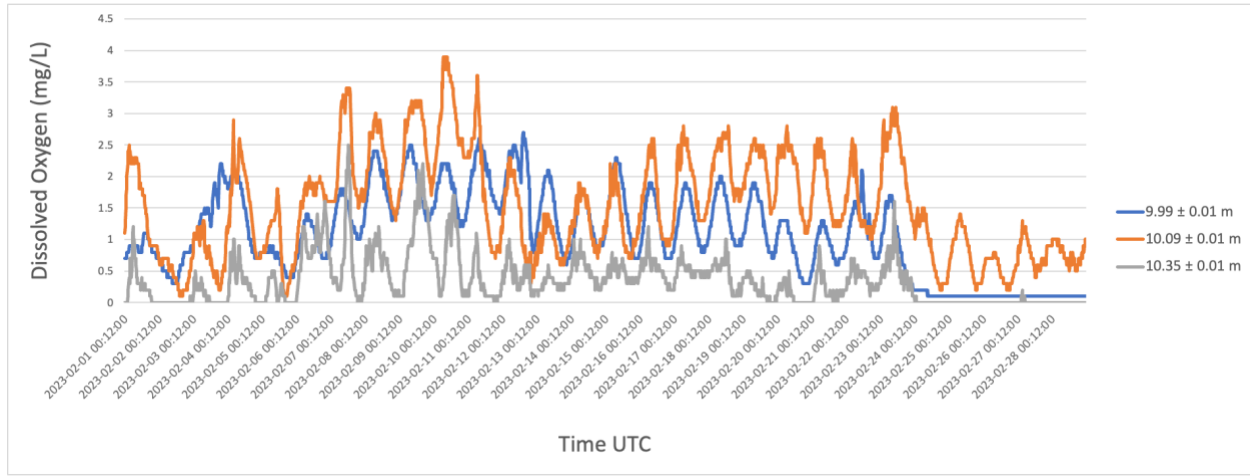


Figure 2: February 2023 Dissolved Oxygen Readings of Lake Fryxell Deep Benthic Mats. Dissolved oxygen concentrations demonstrated diel cycles that persisted into the fall at the end of February for 10.09 m while the records at 9.99 m and 10.35 declined to zero dissolved oxygen on February 24th.

At 9.99 m (8.0 m a.m.s.l.), the DO levels reach a maximum of 2.6 mg/L before falling to 0.2 mg/L at the start of winter. DO at 10.09 m (7.9 m a.m.s.l.) shows a similar cyclical pattern but reaches higher DO maximums of 3.9 mg/L and continues to cycle into late February between 0.3 mg/L and 1.3 mg/L. Lastly at 10.35 m (7.7 m a.m.s.l.), DO reading reaches a maximum of 2.2 mg/L and then becomes anoxic.

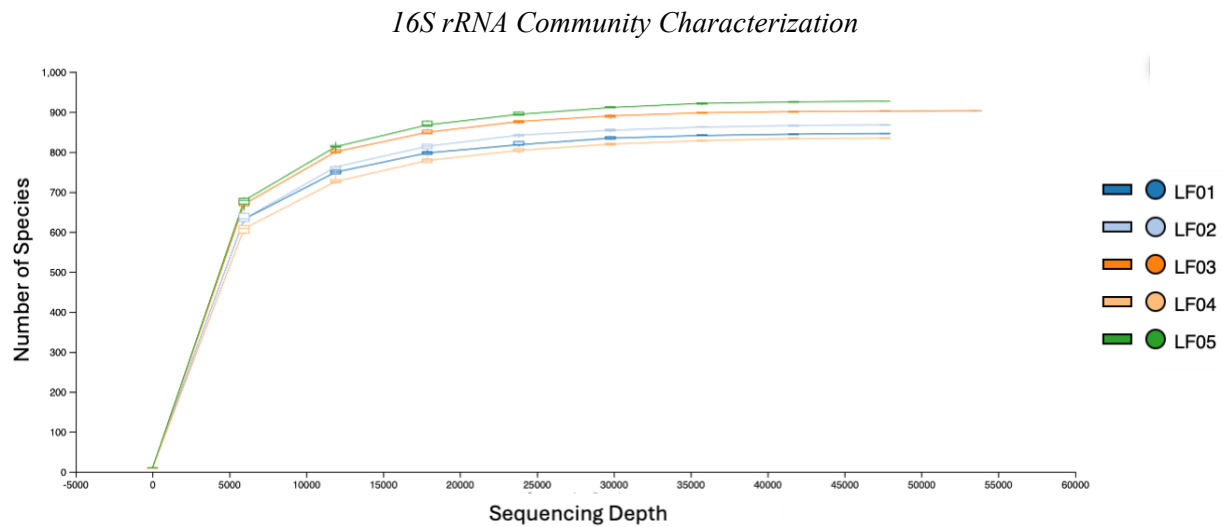


Figure 3: Rarefaction Analysis of 16 rRNA sequencing results. The sequencing depth of all 5 samples was sufficient to capture most community members.

Samples contained 835 - 927 different ASVs, averaging 876 ASVs per sample (Figure 3). Sample four contained the lowest number of species at 835. Sample 1 contained 845 followed by sample two at 868. Sample three contained the second highest number of species at 903, surpassed by sample five at 927. The difference between the largest and smallest sample is ~10%.

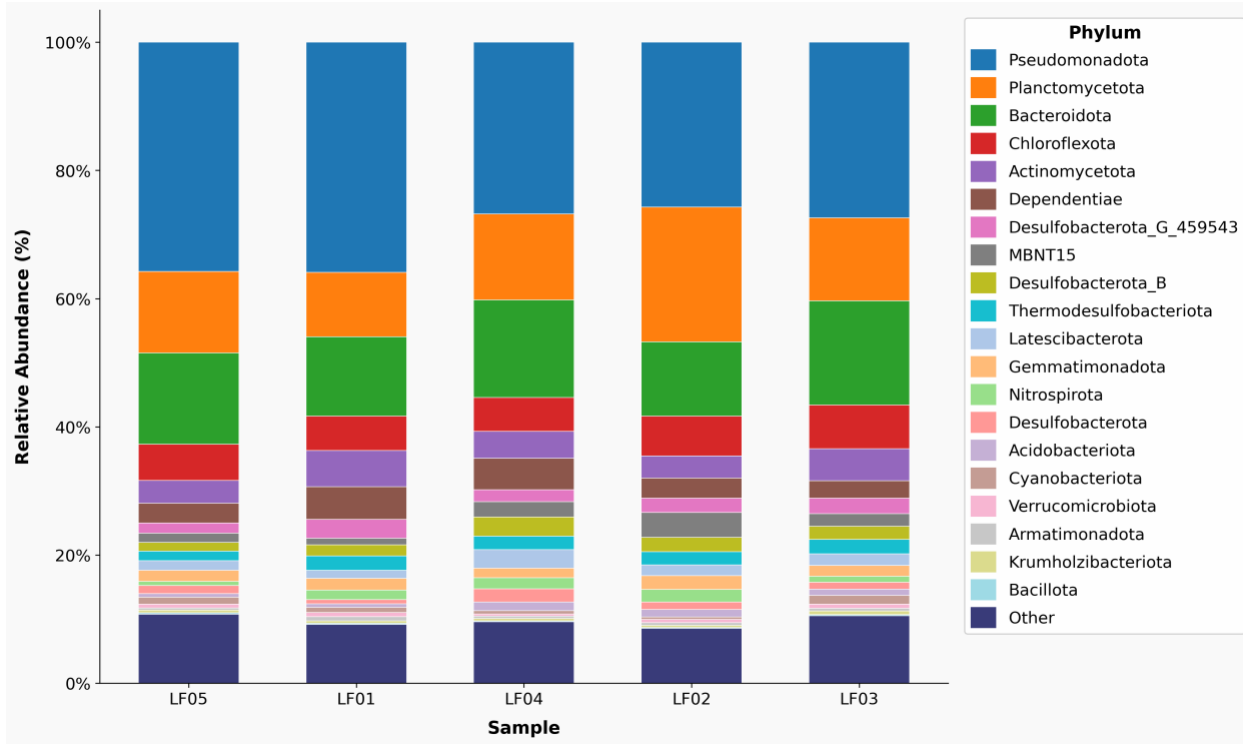


Figure 4: Microbial community composition of Lake Fryxell at the phylum level. Phyla with >0.1% abundance are shown. Pseudomonadota, Planctomycetota, and Bacteroidota were the most abundant in all five samples.

Microbial community composition was assessed across five samples (LF01–LF05) using amplicon sequencing, with relative abundances calculated at the phylum level. The community was dominated by Pseudomonadota, which accounted for the largest proportion of sequences across all samples, with a mean relative abundance of 30.34% (range: 25.72–35.92%). There was a mitochondrial sequence classified as Pseudomonadota. This sequence nor any other mitochondrial classifications were abundant, only one sequence was present, and was relegated to the “Other” category. Planctomycetota and Bacteroidota were the second and third most abundant phyla, contributing mean abundances of 14.00% (10.05–21.03%) and 13.95% (11.56–16.29%), respectively. Together, these three phyla accounted for approximately 58% of the total classified community on average. Several phyla were present at intermediate abundances. Chloroflexota constituted a mean of 5.87% (5.27–6.82%), followed by Actinomycetota at 4.36%

(3.43–5.66%) and Dependitiae at 3.80% (2.75–5.11%). Desulfobacterota and its variants (Desulfobacterota_B, Desulfobacterota_G_459543), MBNT15, Thermodesulfobacteriota, Latescibacterota, and Gemmatimonadota were each present at mean abundances between approximately 1.8% and 2.2%, indicating a persistent low-to-moderate presence across samples. Nitrospirota, Desulfobacterota, Acidobacteriota, and Cyanobacteria were collectively detected at less than 1.5% mean abundance each. The remaining classified phyla: Verrucomicrobiota, Armatimonadota, Krumholzibacteriota, and Bacillota were minor portions of the community, each contributing less than 0.6% on average. Sequences that could not be assigned to a phylum present at $\geq 0.1\%$ relative abundance were grouped as "Other," represented a mean of 9.72% (8.56–10.76%) of the community across samples. From these results, five phyla were found in Lake Fryxell for the first time, including Armatimonadota, Krumholzibacteriota, Latescibacterota, MBNT15, and Thermodesulfobacteriota. Armatimonadota has been found in Antarctic soil, and the species in these phyla are considered to consist of aerobic oligotrophs (Nou et al., 2024; Iwashita et al., 2025) Krumholzibacteriota have been documented in Antarctic soil, whereas Latescibacterota related organisms have been recorded in the Antarctic coastal sediment, but neither are cited to be in Lake Fryxell (Medeiros et al., 2026; Dutta et al., 2023). Thermodesulfobacteriota are known to primarily be sulfate reducing bacteria and have been found in subglacial lakes in Antarctica (Lee et al., 2024).

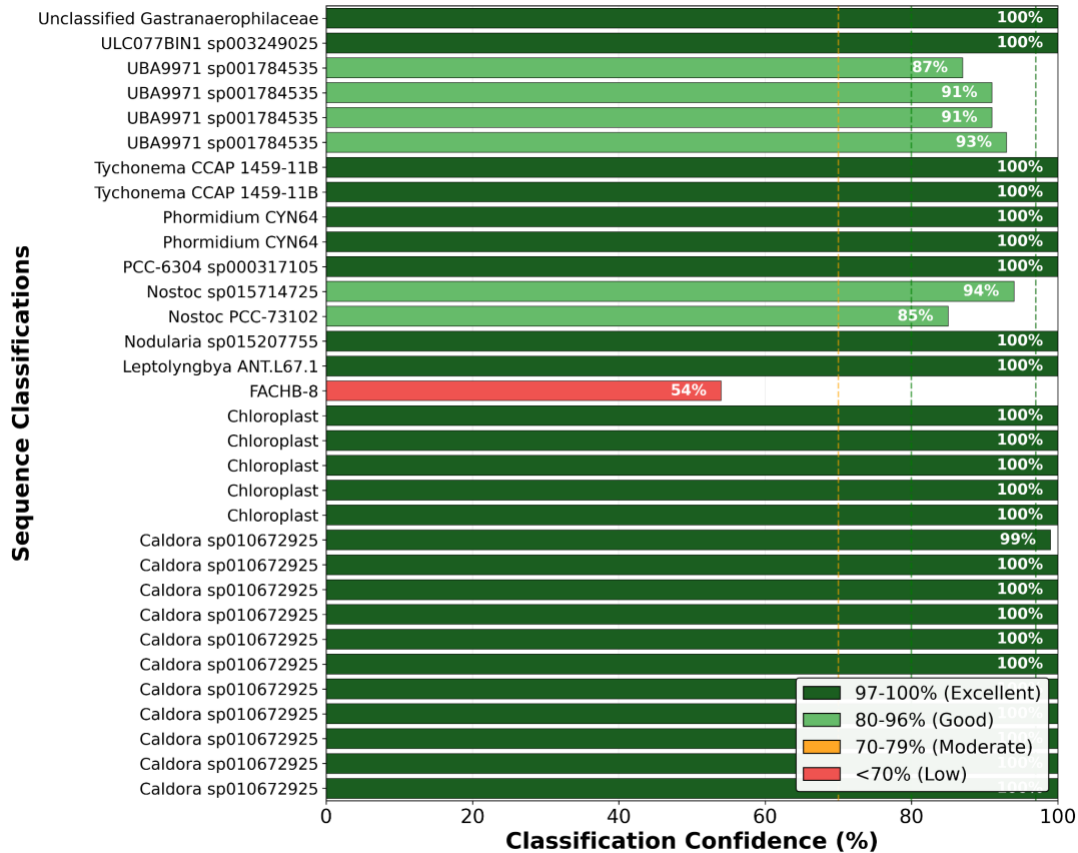


Figure 5: Classified 16S rRNA of cyanobacterial sequences. Most of the Cyanobacteria belonged to *Caldora sp010672925*. Classifications are limited to the highest taxonomic classification available. Confidence indicates percent similarity to known species.

Thirty-two cyanobacterial ASVs were identified in the 16S rRNA classification. Eleven of the classified sequences were classified as *Caldora sp010672925* with 99-100% certainty. Four classifications were determined to be chloroplast in origin with 100% confidence; they are most likely from diatoms within the mat (Chapter 2). These sequences were not high in abundance, ranging from 0.0002 in sample four to 0.0024 in sample five and did not contribute to the Cyanobacteria category in Figure 4 as it's abundance did not meet requirements for all samples. Three sequences were 87-93 % confident in being classified as *UBA9971 sp001784535*. Two were classified as an unknown *Tychonema CCAP 1459-11B* at a confidence level of 100%. *Phormidium CYN64* was also represented with two 100% confident sequences. Two sequences were identified as two different *Nostoc* species, *Nostoc PCC-73102* and *Nostoc sp015714725* at

85% and 94% confidence. A *Nodularia sp015207755* was identified at 100% confidence. The 100% classification confidence *PCC-6304 sp000317105* sequence was classified as an unknown cyanobacterium within the *Phormidiaceae* family. An unclassified *Leptolyngbya ANT.L67.1* at 100% confidence was reported along with *ULC077BIN1 sp003249025* at 100% confidence. There was one unclassified Gastranaerophilaceae sequence at 100% confidence. The lowest confidence sequence at 54% belonged to *FACHB-8*, an *Elainellaceae*.

Metagenomic Characterization

Table 5: Quality metrics of oxford nanopore flye assembly statistics using Quast (ve5.2.0).

Assembly Statistics	Assembly
# contigs (≥ 0 bp)	20232
# contigs (≥ 1000 bp)	20069
# contigs (≥ 5000 bp)	18665
# contigs (≥ 10000 bp)	16339
# contigs (≥ 25000 bp)	9820
# contigs (≥ 50000 bp)	4997
Total length (≥ 0 bp)	1139668483
Total length (≥ 1000 bp)	1139548985
Total length (≥ 5000 bp)	1135151615
Total length (≥ 10000 bp)	1117438839
Total length (≥ 25000 bp)	1007876233
Total length (≥ 50000 bp)	837608649
# contigs	20228
Largest contig	5130016
Total length	1139666869
GC (%)	57.77
N50	135543
N90	22666
auN	409755.5
L50	1545
L90	10568
# N's per 100 kbp	0

The pooled DNA assembly sample sequenced using Oxford nanopore sequencing had a mean sequence length of 4,957bp. Assembly resulted in a total 20,232 assembled contigs. In total 24.7% of all reads assembled resulted in contigs longer than 50,000bp. The longest contig was 5,130,016 bp and classified as an unknown genus of the *JAHQVL01* family within the Rhodothermales order. The MAG was 99.72% complete. Binning of the assembled contigs resulted in 419 bins. Taxonomic classification resulted in 242 classified bins, 109 were “Unclassified Bacteria” and 68 were “Unclassified” due to low completion, unreliability due to contamination, or not being archaeal or bacterial in origin. 23 different bacterial phyla were represented in the 242 MAGs classified (Figure 6). None were classified as Cyanobacteria. Bins were filtered for high completion and low contamination, which resulted in 15 phyla, 61 MAGs with $\geq 90\%$ completion and $\leq 5\%$ contamination (Figure 6).

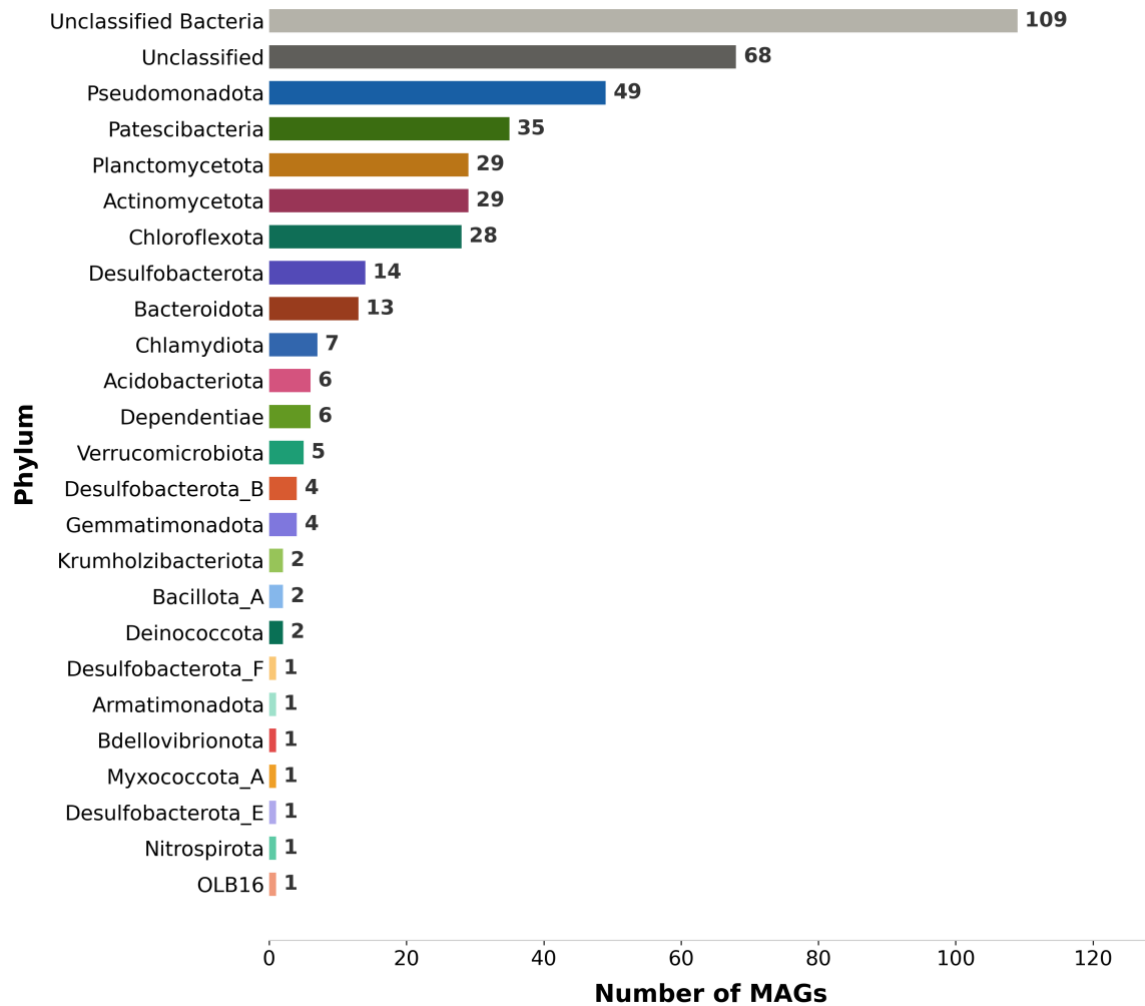


Figure 6: GTDB-tk based classification of MAGs. Most assembled MAGs belong to unclassified bacterial organisms and Pseudomonadota.

The most abundant classified phyla belonged to Pseudomonadota (49 MAGs), Patescibacteria (35 MAGs), Actinomycetota (29 MAGs), and Planctomycetota (29 MAGs). Of the highest classified phyla, Patescibacteria have not previously been recovered from Lake Fryxell. They have been found in other Antarctic environments, specifically the water column of Lake Mercer, Lake Enigma, subglacial environments, and Antarctic soil (Savaglia 2023). Chloroflexota (28 MAGs), Bacteroidota (13 MAGs), Acidobacteriota (6 MAGs), Verrucomicrobiota (5 MAGs), Gemmatimonadota (4 MAGs), Bacillota (2 MAGs),

Armatimonadota (1 MAG), Chlamydoita also known as Chlamydiae (7 MAGs), Desulfobacterota (20 MAGs), Dependientiae (6 MAGs), and Nitrospirota (1 MAG) were present at 10.1 m and have all been found in Lake Fryxell at depths shallower than 10 m (Dillon et al., 2020). The phyla Deinococcota (2 MAGs) Krumholzibacteriota (2 MAGs), Bdellovibrionota (1 MAG), Myxococcota (1 MAG) and OLB16 (1 MAG) were not previously known to be present in Lake Fryxell's mats. OLB16, a novel phylum, has been found in Ace Lake, Antarctica (Williams et al., 2022).

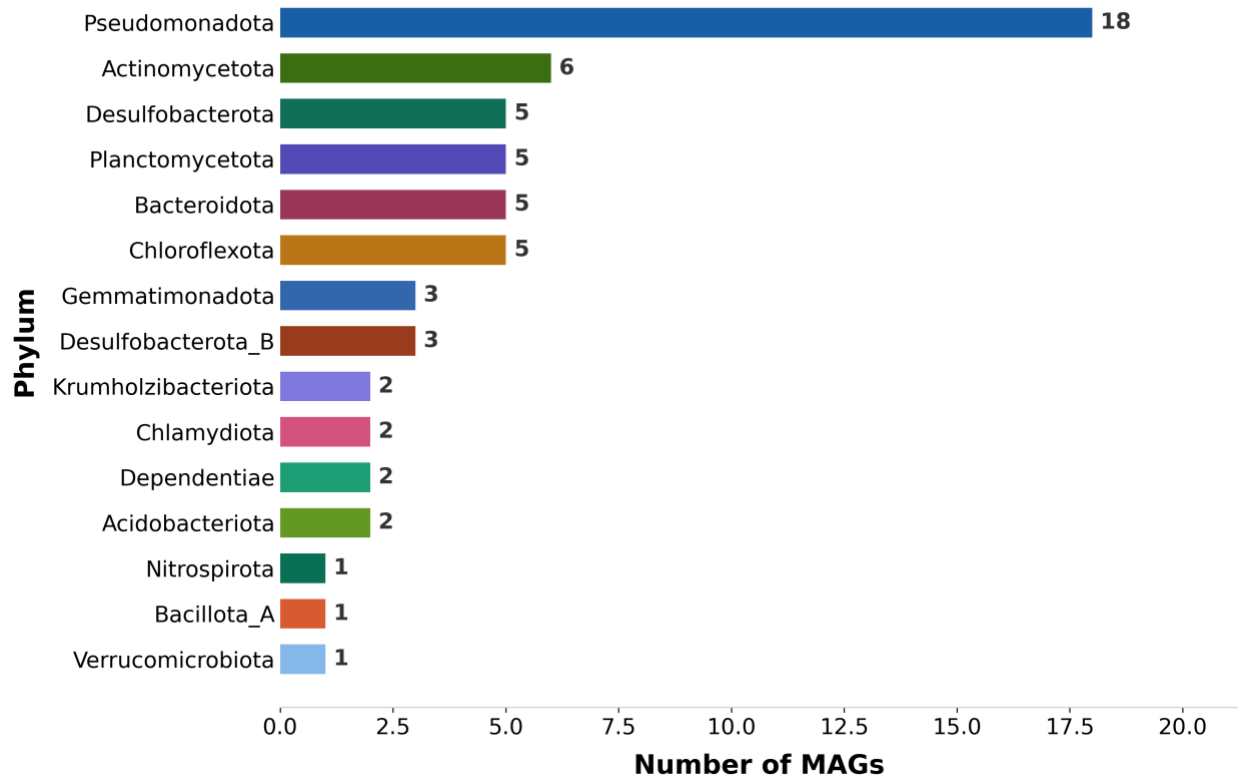


Figure 7: GTDB-tk classification of high-completion low-contamination bins of classified phyla. After filtering, Pseudomonadota is the largest phyla represented in HCLC MAGs.

There were 61 MAGs in total that met the requirements of >90% completion and <5% contamination. The HCLC MAGs contained mostly Pseudomonadota (18 MAGs) (Figure 7). Actinomycetota followed at 10% (6 MAGs). Bacteroidota (5 MAGs), Desulfobacterota (5

MAGs), Chloroflexota (5 MAGs), Planctomycetota (5 MAGs) were represented at eight percent each. Gemmatimonadota (3 MAGs) and Desulfobacterota_B (3 MAGs) were five percent of the total assembled. Krumholzibacteriota (2 MAGs), Acidobacteriota (2 MAGs), Chlamydiota (MAGs), and Dependientiae (2 MAGs) were each present at three percent of the phyla recovered. Lastly Verrucomicrobiota (1 MAG), Bacillota_A (1 MAG), and Nitrospirota (1 MAG) represented 1% each of the HCLC MAGs.

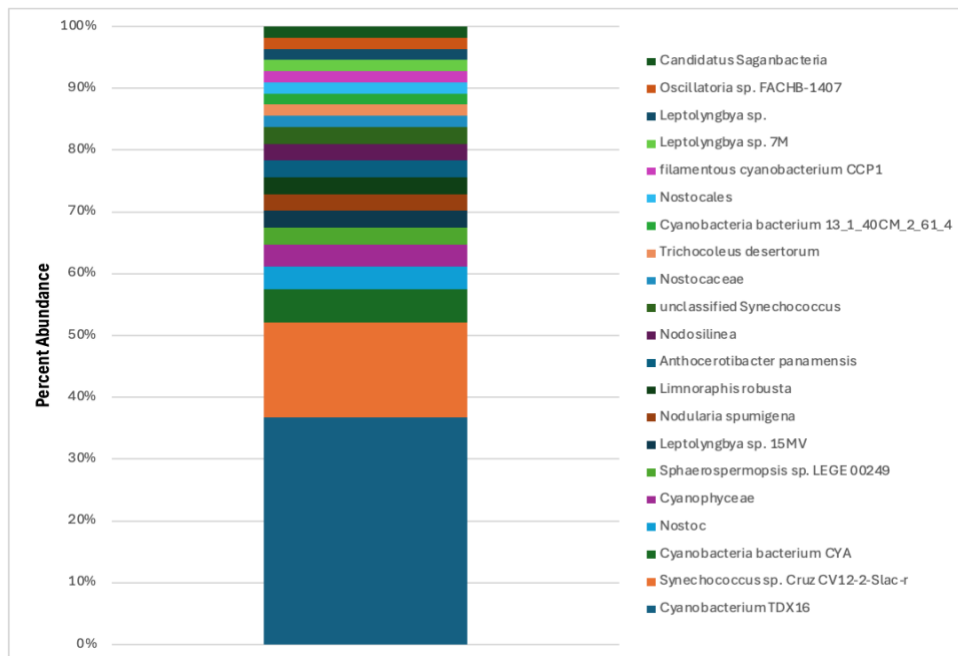


Figure 8: Relative abundance of contigs classified as cyanobacterial contigs. Cyanobacterium TDX16 was the most abundant classification.

Cyanobacteria were present in 16S rRNA analyses of the samples analyzed here, but none were found in the MAGs. To address this unexpected result, protein-based classification with *kaiju* (v1.10.1) was utilized to determine if any contigs in the metagenomic results were classified as Cyanobacteria. As expected, there were cyanobacterial contigs. The 160 different contigs were classified as 87 distinct Cyanobacteria with *kaiju* with the *nr_euk* database. The

length of these contigs ranged from 1117 to 3,110,244 bp. Twenty-one classifications were in percent abundance over 1% within the contigs identified as cyanobacterial.

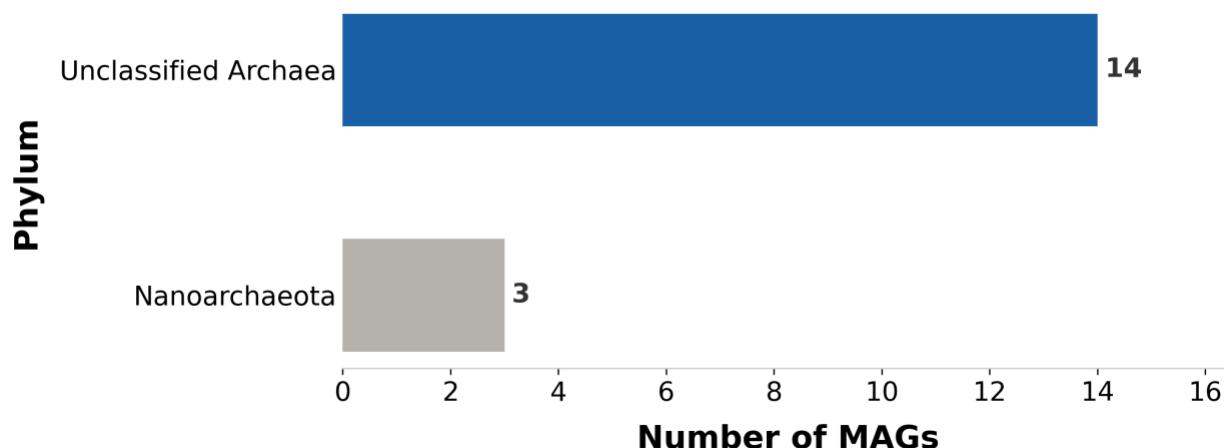


Figure 9: MAGs that were classified as archaeal. The 3 Archaeal MAGs collected were classified as Candidatus Woesearchaeota within the Nanoarchaeota phylum.

The taxonomic classification of the archaeal MAGs has added a new archaeal phylum to those previously known in Lake Fryxell. Results from our phylogenetic classification indicate that all archaeal MAGs belong to the Nanoarchaeota phylum (Figure 9). Analyses of these MAGs show that they have glycolysis-related genes (Table 6), but methanogenesis genes were not detected.

Table 6: Metabolic capacity of archaeal MAGs. The only protein encoding genes identified were utilized in glycolysis and gluconeogenesis.

Gene Symbol	Enzyme	Function
<i>eno</i>	Enolase	Catalyzes 2-phosphoglycerate to phosphoenolpyruvate
<i>ppsA</i>	Phosphoenolpyruvate synthase	Involved in gluconeogenesis and related pathways
<i>fba</i>	Fructose-bisphosphate aldolase (FBP)	Catalyzes FBP to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate
<i>pfkB</i>	ATP-dependent 6-phosphofructokinase	Key regulatory enzyme of catalyzing the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate

Metabolic Transcriptomic Assemblies

Transcriptomic assemblies reveal the metabolic activity of the community at the time of sampling (Table 7). The compiled dataset representing all five samples was searched for key metabolic enzymes related to photosynthesis, aerobic respiration, anaerobic respiration, dissimilatory nitrate reduction to ammonium (DNRA), denitrification/nitrate respiration, electron bifurcation, sulfate reduction, and sulfide oxidation. Not all genes within these processes were found. Results for photosynthesis show that the major components of PSI and PSII were being expressed including the transcript supporting the oxygen evolving complex, *psbO* (Table 3). DNRA and nitrate-based respiration were actively expressed.

Sulfate reduction and sulfide oxidation transcripts are present as well (Table 2). Dissimilatory sulfite reductase was transcribed. The presence of these transcripts suggests that the complete pathway for dissimilatory sulfate reduction was active. The sulfide oxidation *sqr* gene transcripts are present, which both facilitate the oxidation of sulfide to elemental sulfur (Marcia et al., 2009; Anwar et al., 2023). The *fccA* and *fccB* sulfide dehydrogenase enzymes were also expressed when analyzing MAG expression in chapter three but was not detected in the original scan of expressed transcripts. The *soxA* and *soxB* enzymes transcripts are present as well. The enzyme product initiates sulfur oxidation by forming a protein-bound sulfur intermediate and releasing the sulfate product (Bamford et al., 2002; Kappler et al., 2001).

Electron bifurcation transcripts were found within the community (Table 7). The *etfAB* complex is present. This complex is involved in flavin based electron bifurcation and is critical for certain anaerobic organisms (Vigil et al., 2022). The *hndABCD* transcripts were also found; they facilitate flavin-based electron bifurcation in *Desulfovibrio fructosovorans*, which belongs to the Thermodesulfobacteriota phyla (Jacq-Bailly et al., 2021). This phylum was found in the

16S rRNA results for these samples, but not in the MAGs. Lastly the *hydAC* components of the *hydABC* complex were actively transcribed in the sample.

Table 7: Metabolic transcripts from total RNA and DNA KEGG ON annotations. KO ID represent the KEGG Ontology identifier.

KO ID	Gene Symbol	Description	Present in DNA	Present in RNA
Aerobic Respiration				
K00330	<i>nuoA</i>	NADH-quinone oxidoreductase subunit A	X	X
K00331	<i>nuoB</i>	NADH-quinone oxidoreductase subunit B	X	X
K00332	<i>nuoC</i>	NADH-quinone oxidoreductase subunit C	X	X
K00333	<i>nuoD</i>	NADH-quinone oxidoreductase subunit D	X	X
K00334	<i>nuoE</i>	NADH-quinone oxidoreductase subunit E	X	X
K00335	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F	X	X
K00336	<i>nuoG</i>	NADH-quinone oxidoreductase subunit G	X	X
K00337	<i>nuoH</i>	NADH-quinone oxidoreductase subunit H	X	X
K00338	<i>nuoI</i>	NADH-quinone oxidoreductase subunit I	X	X
K00339	<i>nuoJ</i>	NADH-quinone oxidoreductase subunit J	X	X
K00340	<i>nuoK</i>	NADH-quinone oxidoreductase subunit K	X	X
K00341	<i>nuoL</i>	NADH-quinone oxidoreductase subunit L	X	X
K00342	<i>nuoM</i>	NADH-quinone oxidoreductase subunit M	X	X
K00343	<i>nuoN</i>	NADH-quinone oxidoreductase subunit N	X	X
K00404	<i>ccoN/fixN</i>	Cytochrome c oxidase cbb3-type subunit I	X	X
K00405	<i>ccoO/fixO</i>	Cytochrome c oxidase cbb3-type subunit II	X	X
K00411	<i>petB/fbcB</i>	Ubiquinol-cytochrome c reductase cytochrome b subunit	X	X
K00412	<i>petC/fbcC</i>	Ubiquinol-cytochrome c reductase cytochrome c1 subunit	X	X
K00413	<i>petA/fbcF</i>	Ubiquinol-cytochrome c reductase iron-sulfur subunit	X	
K00425	<i>cydA</i>	Cytochrome bd complex subunit I	X	X
K00426	<i>cydB</i>	Cytochrome bd complex subunit II	X	X
K02111	<i>atpA</i>	F-type ATPase subunit alpha	X	X
K02112	<i>atpB/atpD</i>	F-type ATPase subunit beta	X	X
K02113	<i>atpC/atpG</i>	F-type ATPase subunit delta	X	X
K02114	<i>atpE/atpC</i>	F-type ATPase subunit epsilon	X	X
K02115	<i>atpG/atpC</i>	F-type ATPase subunit gamma	X	X

K02116	<i>atpF/atpF</i>	F-type ATPase subunit b	X	X
K02117	<i>atpE</i>	F-type ATPase subunit c	X	X
K02118	<i>atpI</i>	F-type ATPase subunit i/j	X	X
K02136	<i>atpA/ntpA</i>	V-type ATPase subunit A	X	X
K02137	<i>atpB/ntpB</i>	V-type ATPase subunit B		X
K02138	<i>atpC/ntpC</i>	V-type ATPase subunit C		X
K02139	<i>atpD/ntpD</i>	V-type ATPase subunit D		X
K02140	<i>atpE/ntpE</i>	V-type ATPase subunit E		X
K02144	<i>atpI/ntpI</i>	V-type ATPase subunit I		X
K02145	<i>atpK/ntpK</i>	V-type ATPase subunit K		X
K02256	<i>cox1/ctaD</i>	Cytochrome c oxidase subunit 1	X	X
K02262	<i>cox3/ctaF</i>	Cytochrome c oxidase subunit 3	X	X
K02263	<i>cox4/ctaG</i>	Cytochrome c oxidase subunit 4		X
K02274	<i>coxA/ctaD</i>	Cytochrome c oxidase subunit I	X	X
K02275	<i>coxB/ctaC</i>	Cytochrome c oxidase subunit II	X	X
K02276	<i>coxC/ctaE</i>	Cytochrome c oxidase subunit III	X	X
K02300	<i>cyoA</i>	Cytochrome o ubiquinol oxidase subunit I	X	X
K02302	<i>cyoC</i>	Cytochrome o ubiquinol oxidase subunit III	X	X
K02303	<i>cyoD</i>	Cytochrome o ubiquinol oxidase subunit IV	X	X
K05572	<i>ndhA</i>	NAD(P)H-quinone oxidoreductase subunit 1		X
K05573	<i>ndhB</i>	NAD(P)H-quinone oxidoreductase subunit 2		X
K05574	<i>ndhC</i>	NAD(P)H-quinone oxidoreductase subunit 3		X
K05575	<i>ndhD</i>	NAD(P)H-quinone oxidoreductase subunit 4		X
K05576	<i>ndhE</i>	NAD(P)H-quinone oxidoreductase subunit 4L	X	X
K05577	<i>ndhF</i>	NAD(P)H-quinone oxidoreductase subunit 5	X	X
K05578	<i>ndhG</i>	NAD(P)H-quinone oxidoreductase subunit 6		X
Fermentation			DNA	RNA
K00101	<i>lldA/dld</i>	L-lactate dehydrogenase (cytochrome)	X	X
K00102	<i>lldD</i>	L-lactate dehydrogenase (FMN)	X	X
K00114	<i>adhA/adh</i>	Alcohol dehydrogenase	X	X
K00116	<i>sdhABCD/frdABCD</i>	Succinate dehydrogenase / fumarate reductase	X	
K00121	<i>adhP</i>	Alcohol dehydrogenase class IV	X	X
K00122	<i>fdh</i>	Formate dehydrogenase	X	
K00123	<i>fdhA</i>	Formate dehydrogenase major subunit	X	X
K00124	<i>fdhB</i>	Formate dehydrogenase subunit beta	X	X

K00125	<i>fdhC</i>	Formate dehydrogenase subunit gamma	X	X
K00126	<i>fdhD</i>	Formate dehydrogenase subunit delta	X	
K00127	<i>fdhE</i>	Formate dehydrogenase subunit epsilon	X	X
K00132	<i>aldH/ald</i>	Aldehyde dehydrogenase	X	X
K00925	<i>ackA</i>	Acetate kinase	X	X
K01512	<i>acyP</i>	Acylphosphatase	X	X
K04072	<i>etfA</i>	Electron transfer flavoprotein alpha subunit	X	X
K04073	<i>etfB</i>	Electron transfer flavoprotein beta subunit	X	
K13788	<i>pta</i>	Phosphate acetyltransferase	X	X
Hydrogen Metabolism			DNA	RNA
K00436	<i>hynL/hydA</i>	NiFe hydrogenase large subunit	X	X
K00437	<i>hoxF/hndA</i>	NAD-reducing hydrogenase subunit A		X
K00533	<i>hydA</i>	Ferredoxin hydrogenase large subunit		X
K00534	<i>hydB</i>	Ferredoxin hydrogenase small subunit		X
K03620	<i>hyfA</i>	Hydrogen:quinone oxidoreductase subunit A	X	X
K03621	<i>hyfB</i>	Hydrogen:quinone oxidoreductase subunit B	X	X
K05586	<i>mhyC</i>	Membrane-bound hydrogenase subunit C	X	X
K05587	<i>mhyD</i>	Membrane-bound hydrogenase subunit D	X	X
K06281	<i>hyaA/hybO</i>	Membrane-bound hydrogenase small subunit	X	X
K06282	<i>hyaB/hybC</i>	Membrane-bound hydrogenase large subunit	X	X
K06283	<i>hyaB</i>	NiFe hydrogenase Hyd-1 large subunit	X	
K06284	<i>hyaA</i>	NiFe hydrogenase Hyd-1 small subunit	X	X
K17992	<i>hoxH</i>	NADP-reducing hydrogenase subunit A	X	X
K18008	<i>hydB/hyaA</i>	NiFe hydrogenase small subunit	X	X
K18009	<i>hydC</i>	NiFe hydrogenase cytochrome b subunit	X	
K18010	<i>hypA</i>	NiFe hydrogenase maturation HypA		X
Nitrogen Cycling			DNA	RNA
K00362	<i>nirB</i>	Nitrite reductase (NADH) large subunit	X	
K00363	<i>nirD</i>	Nitrite reductase (NADH) small subunit	X	
K00366	<i>nirA</i>	Ferredoxin-nitrite reductase	X	X
K00367	<i>nirA</i>	Ferredoxin-nitrite reductase		X
K00368	<i>nirK/nirS</i>	Nitrite reductase (NO-forming)	X	X
K00370	<i>narG</i>	Nitrate reductase alpha subunit	X	X
K00371	<i>narH</i>	Nitrate reductase beta subunit	X	X
K00372	<i>narI</i>	Nitrate reductase gamma subunit	X	X
K00373	<i>narJ</i>	Nitrate reductase delta subunit	X	X

K00374	<i>narJ/narW</i>	Nitrate reductase molybdenum cofactor assembly	X	X
K00376	<i>nosZ</i>	Nitrous-oxide reductase	X	X
K00531	<i>nifH/anfH</i>	Nitrogenase delta subunit		X
K02305	<i>norC</i>	Nitric oxide reductase subunit C	X	X
K02584	<i>nifB</i>	Nitrogenase cofactor biosynthesis NifB	X	X
K02585	<i>nifQ</i>	Nitrogenase biosynthesis NifQ	X	X
K02586	<i>nifD</i>	Nitrogenase molybdenum-iron protein alpha chain	X	X
K02587	<i>nifE</i>	Nitrogenase molybdenum-cofactor synthesis NifE	X	X
K02588	<i>nifH</i>	Nitrogenase iron protein NifH	X	X
K02589	<i>glnB/nif1</i>	Nitrogen regulatory protein PII 1	X	X
K02590	<i>glnK/nif2</i>	Nitrogen regulatory protein PII 2	X	X
K02591	<i>nif3</i>	Nitrogen regulatory protein PII 3	X	X
K03385	<i>nrfA</i>	Nitrite reductase (cytochrome c-552)	X	X
K04561	<i>norB</i>	Nitric oxide reductase subunit B	X	X
K10535	<i>hdh</i>	Hydroxylamine dehydrogenase	X	X
K10536	<i>hao</i>	Hydroxylamine oxidoreductase	X	
K10944	<i>amoA</i>	Ammonia monooxygenase subunit A	X	X
K10945	<i>amoB</i>	Ammonia monooxygenase subunit B	X	X
K10946	<i>amoC</i>	Ammonia monooxygenase subunit C	X	X
K15864	<i>nirS/nirK</i>	Nitrite reductase (NO-forming) / hydroxylamine reductase	X	X
K15876	<i>nrfA/cytC552</i>	Nitrite reductase (cytochrome c)	X	X
K20932	<i>hzsA</i>	Hydrazine synthase subunit A		X
K20933	<i>hzsB</i>	Hydrazine synthase subunit B		X
K20935	<i>hdh/hzo</i>	Hydrazine dehydrogenase		X
Photosynthesis			DNA	RNA
K02291	<i>crtl/pds</i>	Phytoene desaturase	X	X
K02634	<i>petB</i>	Cytochrome b6		X
K02635	<i>petC</i>	Cytochrome b6/f complex iron-sulfur subunit	X	X
K02636	<i>petA</i>	Cytochrome f	X	X
K02637	<i>petD</i>	Cytochrome b6/f complex subunit 4		X
K02638	<i>petE/pcyA</i>	Plastocyanin	X	X
K02639	<i>petF/fdx</i>	Ferredoxin		X
K02640	<i>petH/fnr</i>	Ferredoxin-NADP+ reductase		X
K02641	<i>petG</i>	Cytochrome b6/f complex subunit 5		X
K02689	<i>psaA</i>	Photosystem I P700 chlorophyll a apoprotein A1		X

K02690	<i>psaB</i>	Photosystem I P700 chlorophyll a apoprotein A2		X
K02691	<i>psaC</i>	Photosystem I iron-sulfur center		X
K02692	<i>psaD</i>	Photosystem I subunit II		X
K02694	<i>psaE</i>	Photosystem I subunit IV		X
K02696	<i>psaF</i>	Photosystem I subunit VI		X
K02697	<i>psaJ</i>	Photosystem I subunit VII		X
K02698	<i>psaK</i>	Photosystem I subunit VIII		X
K02699	<i>psaL</i>	Photosystem I subunit IX		X
K02703	<i>psbA</i>	Photosystem II D1 protein	X	X
K02704	<i>psbB</i>	Photosystem II CP47		X
K02705	<i>psbC</i>	Photosystem II CP43		X
K02706	<i>psbD</i>	Photosystem II D2 protein		X
K02707	<i>psbE</i>	Photosystem II cytochrome b559 alpha		X
K02708	<i>psbF</i>	Photosystem II cytochrome b559 beta		X
K02709	<i>psbH</i>	Photosystem II protein H		X
K02710	<i>psbI</i>	Photosystem II protein I		X
K02711	<i>psbO</i>	Photosystem II oxygen-evolving enhancer 1		X
K02712	<i>psbP</i>	Photosystem II oxygen-evolving enhancer 2	X	
K06443	<i>chlP/bchP</i>	Geranylgeranyl diphosphate reductase	X	
K08906	<i>pscB</i>	Anoxygenic type-I reaction center subunit B		X
K08928	<i>pufM</i>	Anoxygenic type-II reaction center subunit M	X	
K08929	<i>pufL</i>	Anoxygenic type-II reaction center subunit L	X	X
K10027	<i>crtY/lyc</i>	Lycopene cyclase	X	X
K13789	<i>rpe65</i>	Retinal pigment epithelium 65 kDa protein	X	X
Sulfur Cycling			DNA	RNA
K00380	<i>cysI/sir</i>	Sulfite reductase (ferredoxin) beta subunit	X	X
K00381	<i>cysJ</i>	Sulfite reductase (ferredoxin) alpha subunit	X	X
K00387	<i>sorA/soeA</i>	Sulfite dehydrogenase (quinone)		X
K00390	<i>cysH/aprAB</i>	Phosphoadenosine phosphosulfate reductase	X	X
K00394	<i>aprA</i>	Adenylylsulfate reductase subunit A	X	X
K00395	<i>aprB</i>	Adenylylsulfate reductase subunit B	X	X
K00858	<i>sqdB</i>	Sulfolipid biosynthesis SqdB	X	X
K00956	<i>sat/cysN</i>	Sulfate adenylyltransferase subunit 1	X	
K00957	<i>sat/cysD</i>	Sulfate adenylyltransferase subunit 2	X	
K00958	<i>sat</i>	Sulfate adenylyltransferase	X	X
K05301	<i>sorAB</i>	Sulfite dehydrogenase	X	X
K07306	<i>dmsA</i>	DMSO reductase subunit A	X	X

K07307	<i>dmsB</i>	DMSO reductase subunit B	X	X
K07308	<i>dmsC</i>	DMSO reductase subunit C		X
K08352	<i>phsA</i>	Thiosulfate reductase subunit A	X	X
K08354	<i>phsC</i>	Thiosulfate reductase subunit C		X
K11180	<i>dsrA</i>	Dissimilatory sulfite reductase alpha subunit	X	X
K11181	<i>dsrB</i>	Dissimilatory sulfite reductase beta subunit	X	X
K16937	<i>doxD</i>	Thiosulfate oxidase DoxD	X	X
K16966	<i>ddhC</i>	DMS dehydrogenase subunit C		X
K17218	<i>sqr</i>	Sulfide:quinone oxidoreductase	X	X
K17222	<i>soxB</i>	Sulfur oxidation protein SoxB	X	X
K17223	<i>soxC</i>	Sulfur oxidation protein SoxC	X	X
K17224	<i>soxD</i>	Sulfur oxidation protein SoxD	X	X
K17225	<i>soxX</i>	Sulfur oxidation protein SoxX	X	
K17226	<i>soxY</i>	Sulfur oxidation protein SoxY	X	X
K17227	<i>soxZ</i>	Sulfur oxidation protein SoxZ	X	X
K17486	<i>dddL/dddP</i>	DMSP lyase	X	
K17725	<i>ethe1/persulfide dioxygenase</i>	Sulfur dioxygenase	X	X
K17993	<i>hydA/mvhA</i>	Sulphydrogenase subunit alpha		X
K17994	<i>hydB/mvhD</i>	Sulphydrogenase subunit beta		X
K19713	<i>tsdA</i>	Thiosulfate dehydrogenase (cytochrome) TsdA	X	X

Autotrophy Potential

It was decided to determine if the metabolic potential of HCLC MAGs included autotrophic metabolisms. The genomic annotations of the HCLC were compared to the general assembled and annotated RNA transcripts that were annotated to see if the potential of this subsample of the community is reflected in the larger community sample. Based on the organisms present in the HCLC MAGs, five major autotrophic pathways were investigated based on what is thought to occur at 10.1 m: the Calvin Beson-Bassam Cycle, the 3-hydroxypropionate bicycle cycle, photo dependent sulfide-oxidization, oxygenic photosynthesis, and the Wood–Ljungdahl Pathway (WLP). The WLP was fully represented in the collection of HCLC MAGs and transcripts (Figure 8).

Nearly all genes necessary for the Calvin Beson-Bassam Cycle were present in the subset of HCLC MAGs investigated. The only missing gene was *sbp*, Sedoheptulose-1,7-bisphosphatase. Similarly, 14 of 17 potential transcripts were present at the time of sampling (Figure 8). Twelve of the 18 *cbbL* large subunits that were actively transcribed were classified as cyanobacterial in origin, with 6 classified as from Pseudomonadota (Table 8). Numerous rubisco *rbcL* genes were present (Table 5). Forms I, II, IIIC, and III-like were found in the annotation of the DNA contigs. The form I rubiscos recovered belonged to Pseudomonadota, and Actinomycetota, as well as those belonging to Bacillariophyta and Dinoflagellata. Form II recovered gene sequences belonged to the Pseudomonadota bacterial phyla. Three form III rubiscos were recovered, and they were most similar to rubiscos found in the Patescibacteria phylum, an unknown bacterium, and Candidatus Dojkabacteria bacterium. There was one sequence, HMDFMLOA_68167, that was similar to a rubisco large chain found in a dinoflagellate reference organism, *Symbiodinium* sp., but its form was unable to be classified.

Since Chloroflexota and archaea were present in our metagenomic data, we looked for the presence and potential activity of the 3-hydroxypropionate bicycle cycle. Only 8 of the 19 genes were present in the metagenomic data and only two transcripts corresponding to the *accB* and *accC* genes were present. Three transcripts related to the bicycle cycle (*pccA*, *mce*, and *metB*) did not have a genomic counterpart in the HCLC MAGs. Neither of the key enzymes Malonyl-CoA reductase nor Propionyl-CoA synthase were represented in the metagenome or transcriptome

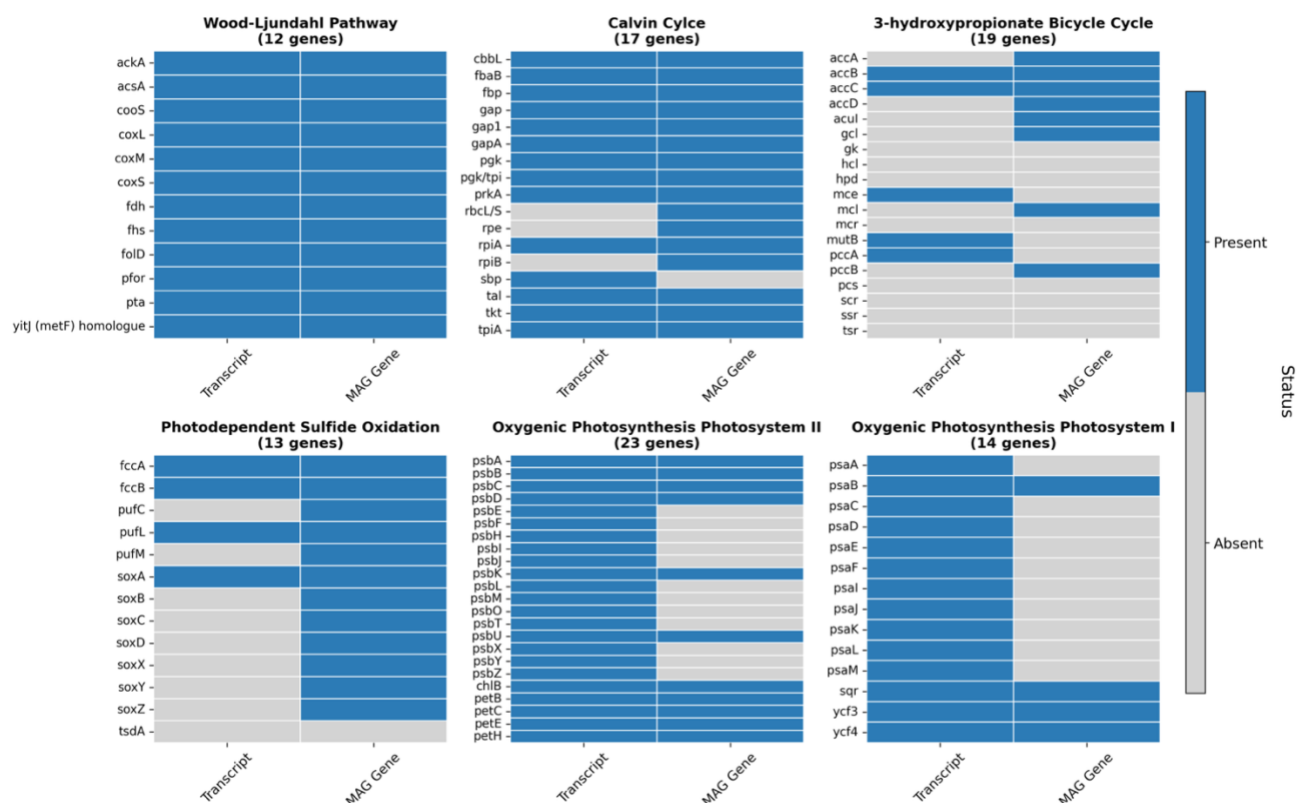


Figure 8: Presence and absence of autotrophic pathways in assembled transcripts and general MAG assembly. The Wood-Ljungdahl Pathway was the only autotrophic pathway present at 100% capacity in both RNA transcripts and within the high completion low contamination gene annotations. The second best most complete pathway is the Calvin Cycle at 76% completion in both transcript and gene presence.

Phototrophic sulfur oxidation genes and transcripts were present in our MAGs and transcriptomes. Reaction center genes, *pufL* and *pufM* were present but not the transcripts. The *fccA* and *fccB* transcripts were present, components that facilitate the oxidation of sulfide to elemental sulfur. The *soxA* transcript was expressed. All genes considered in this process were found expect for *tsdA*. The local environment contained hydrogen sulfide, which can be used as an electron donor for light dependent sulfide oxidation. (Kushkevych et al., 2021). However, sulfide oxidation may also occur in a light-independent process.

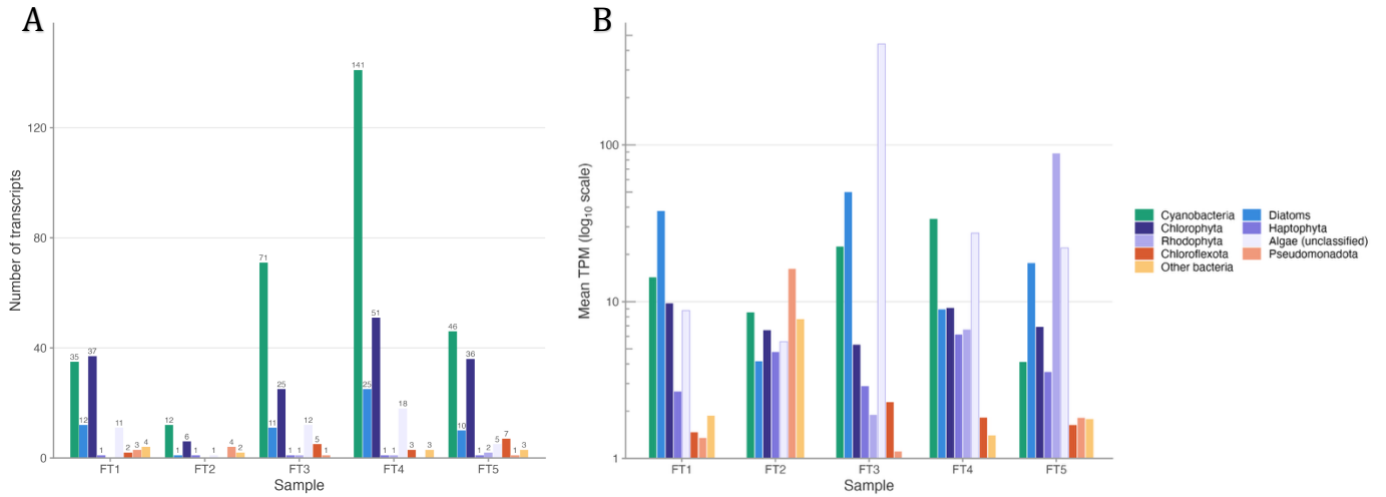


Figure 9: Transcript per million of photosynthesis related transcripts collected from 10. 1 m in Lake Fryxell. **A)** Cyanobacteria produced the highest number of photosynthesis related transcripts in all samples except for FT1 where Chlorophyta algae produced the highest number. **B)** The highest transcriptional activity varies between sample, diatoms show the highest transcriptional activity in FT1, Pseudomonadota in FT2, unclassified algae in FT3, Cyanobacteria in FT4, and Haptophyta in FT5.

No oxygenic photosynthetic organisms were found in the high completion low contamination bins. Photosynthetic genes were present in annotation files of assembled DNA contigs. The photosystem II (PSII) D1 protein encoded by *psbA* was present along with *psbB*, *psbC*, *psbD*, *psbE*, *psbF*, *psbH*, *psbI*, *psbJ*, *psbK*, *psbL*, *psbM*, *psbO*, *psbT*, *psbU*, *psbX*, *psbY*, *psbZ*, *chlB*, *petB*, *petC*, *petE* and *petH*. The only PSII genes found in the MAG assemblies were *psbA*, *psbB*, *psbC*, *psbK*, *psbU*, *chlB*, *petB*, *petC*, *petE*, and *petH*. Major photosystem I transcripts were also present, including *psaA*, *psaB*, *psaC*, *psaD*, *psaE*, *psaF*, *psaI*, *psaJ*, *psaK*, *psaL*, *psaM*, *sqr*, *ycf3*, and *ycf4*. Detection of the MAG genes were limited to *psbB*, *sqr* and *ycf3*, and *ycf4*. Those transcripts unable to be attributed to the MAGs are derived from a mixture of

photosynthetic organisms in Figure 9 (Cyanobacteria, Algae, Diatoms, Bacteria, and other unclassified organisms).

Table 8: Active *cbbL* transcripts mapped to the DNA assembly. The majority of the *cbbL* genes recovered were from Cyanobacteria. Pseudomonadota was the only non-Cyanobacterial phyla represented.

Sample Transcript	Representative UniProt ID	Representative Phyla	Representative Organism
k127_40102	Q6LBA6	Pseudomonadota	<i>Afipia carboxidovorans</i>
k127_149534	P00879	Cyanobacteria	<i>Nostoc sp.</i>
k127_26896	P00879	Cyanobacteria	<i>Nostoc sp.</i>
k127_166226	P54205	Cyanobacteria	<i>Synechocystis sp.</i>
k127_63465	Q44176	Cyanobacteria	<i>Picosynechococcus sp.</i>
k127_83407	P00879	Cyanobacteria	<i>Synechocystis sp.</i>
k127_136501	P00879	Cyanobacteria	<i>Nostoc sp.</i>
k127_54081	P22849	Pseudomonadota	<i>Allochromatium vinosum</i>
k127_44561	P54205	Cyanobacteria	<i>Synechocystis sp.</i>
k127_144119	Q6LBA6	Pseudomonadota	<i>Afipia carboxidovorans</i>
k127_64870	Q6LBA6	Pseudomonadota	<i>Afipia carboxidovorans</i>
k127_104946	P54205	Cyanobacteria	<i>Synechocystis sp.</i>
k127_55658	P00879	Cyanobacteria	<i>Nostoc sp.</i>
k127_2719	P00879	Cyanobacteria	<i>Nostoc sp.</i>
k127_62259	Q0K1E0	Pseudomonadota	<i>Cupriavidus necator</i>
k127_22577	P42721	Pseudomonadota	<i>Cupriavidus necator</i>
k127_195200	Q8DIS5	Cyanobacteria	<i>Thermosynechococcus vestitus</i>
k127_52899	Q44176	Cyanobacteria	<i>Picosynechococcus sp.</i>

Table 9: Forms of *rbcL* rubiscos DNA sequences were classified into rubisco clades were collected from *rbcL* amino acid sequences in HCLC PROKKA annotation output files. The phyla most represented in the *rbcL* sequences belonged to Pseudomonadota.

Representative UniProt Sequence	Representative Phyla	Representative Organism	DNA Annotation ID	Centroid Final Form	Sub Form
WP_132462416.1	Pseudomonadota	<i>Rhodovulum marinum</i>	HMDFMLOA_1119763	I	Ia
MCU7808652.1	Pseudomonadota	<i>Candidatus Thiodiazotropha</i>	HMDFMLOA_1119852	II	n/a
MCU7808652.1	Pseudomonadota	<i>Candidatus Thiodiazotropha</i>	HMDFMLOA_1120023	II	n/a
WP_132462416.1	Pseudomonadota	<i>Rhodovulum marinum</i>	HMDFMLOA_1177348	I	Ia
QIX03930.1	Bacillariophyta	<i>Pinnularia borealis complex sp.</i>	HMDFMLOA_171223	I	Id
MCU7808652.1	Pseudomonadota	<i>Candidatus Thiodiazotropha sp</i>	HMDFMLOA_246381	II	n/a
MCU7808652.1	Pseudomonadota	<i>Candidatus Thiodiazotropha sp.</i>	HMDFMLOA_312529	II	n/a
WP_132462416.1	Pseudomonadota	<i>Rhodovulum marinum</i>	HMDFMLOA_312554	I	Ia
MFA6534587.1	Patescibacteria	<i>Patescibacteria group bacterium</i>	HMDFMLOA_494273	IIIC	UNK
WP_132462416.1	Pseudomonadota	<i>Rhodovulum marinum</i>	HMDFMLOA_634801	I	Ia
WP_132462416.1	Pseudomonadota	<i>Rhodovulum marinum</i>	HMDFMLOA_634810	I	Ia
QIX03930.1	Bacillariophyta	<i>Pinnularia borealis complex sp.</i>	HMDFMLOA_634833	I	Id
MBP5204290.1	Bacteria	<i>Bacterium</i>	HMDFMLOA_68167	IIILike	n/a
MDD2270234.1	Candidatus Dojkabacteria	<i>Candidatus Dojkabacteria bacterium</i>	HMDFMLOA_68169	IIILike	n/a
GFG54764.1	Actinomycetota	<i>Mycolicibacterium agri</i>	HMDFMLOA_785759	I	Ie
CAE7724040.1	Dinoflagellata	<i>Symbiodinium sp.</i>	HMDFMLOA_876237	n/a	n/a
WP_097030133.1	Pseudomonadota	<i>Cereibacter ovatus</i>	HMDFMLOA_876238	II	n/a

Discussion

Classified Cyanobacteria from 16S rRNA and DNA Contigs

The abundance of Cyanobacteria in Lake Fryxell's microbial mats is well documented. At 10.1 m we have detected some organisms that have not been seen in Antarctica or at this depth in Lake Fryxell. Of the cyanobacterial 16S rRNA sequences classified at 10.1 m, *Caldora* has not previously been identified in Lake Fryxell or confirmed in Antarctica. *Nodularia* has been found in Antarctica in meltwater ponds on the McMurdo ice shelf (Jungblut et al., 2005), but it has not been reported in Lake Fryxell. Similarly, *Phormidium* CYN64 has been recovered from Antarctic ponds in Pyramid Trough (Martineau et al., 2000) and Lake Fryxell (Lumian et al., 2021). *Nostoc* has been found in Lake Fryxell and the surrounding moat, demonstrating consistency with previous studies (Dillon et al., 2020; Sohm et al., 2020; Brambilla et al., 2001). *Tychomnema* CCAP 1459-11B has been recovered on Livingston Island in Antarctica (Doytchinov et al., 2024), but not previously from Lake Fryxell. *UBA9971 sp001784535* has not been found in Lake Fryxell or Antarctica nor has PCC-6304 sp000317105. The last of the Cyanobacteria classified in this study, *FACHB-8* has not been found in Antarctica but its family, Elainellaceae, has been found in Lake Untersee (Greco et al., 2020). *Nostoc*, *Nodularia* sp, Nostocales, *Leptolyngbya* sp, and *FACHB-1407* were identified in both 16S rRNA and DNA contigs classified by kaiju (v1.10.1). The summation of these results indicate that Cyanobacteria are still a community member within the mats. With support from oxygenic photosynthesis transcripts (Figure 8) and rubisco cbbL transcripts (Table 4), there is evidence to suggest that Cyanobacteria are producing oxygen and building biomass through primary production through autotrophy. However, the discovery of diatom 18S rRNA gene sequences (Chapter 2), chloroplast 16S rRNA gene (Figure 5), and *rbcL* sequences (Table 5), and transcripts (Chapter 2)

suggests that diatoms are playing a role in primary productivity. This may increase with depth into the lake as diatoms overtake Cyanobacteria due to tolerance of hydrogen sulfide deeper in the lake which inhibits cyanobacterial oxygenic photosynthesis.

16S rRNA ASV Classification and MAG Construction

The MAGs generated could not capture and construct genomes of all organisms present in the 16S rRNA results but was able to provide information on some of the phyla represented. This shortcoming is expected due to the different purpose of each technique and the understanding that MAGs do not reflect the community due to massive data loss from reads to HCLC MAGs (Maguire et al., 2020). For our study, the 16S rRNA gene and metagenome taxonomic data contain similar phyla, with some important differences. We were able to construct MAGs of some organisms of following phyla shared with the 16S rRNA ASVs to provide a functional understanding of these microbial organisms: Verrucomicrobiota, Pseudomonadota, Plantomycetota, Nitrospirota, Latescibacterota, Krumholzibacteriota, Gemmatimonadota, Desulfobacterota, Dependuntiae, Chloroflexota, Bacteriodota, Bacillota, Actinomycetota, Acidobacteriota, and Armatimonadota. The >0.1% abundance ASVs contained four phyla absent from the MAGs: Thermodesulfobacteriota, MBNT15, Latescibacterota, and Cyanobacteria. The MAGs included four phyla not represented in the >0.1% abundance ASVs. These were Bdellovibrionota, Myxococota, OLB16, and Chlamydiae. The phyla were only in abundance below 0.1%. Low population abundance suggested by the low ASV abundance may explain the low completion of the Bdellovibrionota, Myxococota, and OLB16 MAGs. In contrast, Chlamydiae was represented by a high completion low contamination MAG despite low abundance. The highest resolution of classification in the 16S rRNA belong to the LD1-

PA32 order. The family identified from the MAG data suggests the Parachlamydiaceae family which is known to infect amoeba (Greub et al., 2002).

Overall, these results demonstrate that the 16S rRNA analyses captured the phyla represented in the MAGs. In addition, the presence of cyanobacterial ASVs confirms their presence in the community, consistent with cyanobacterial transcripts (Figure 5), even though they are not represented by any MAGs. In addition, the 68 “Unknown bacteria” classifications with low completion that represent unknown metabolic potential of organisms that were unable to be classified and did not have enough genetic information to fully annotate. This result demonstrates that while the MAGs are important for understanding the metabolic potential of organisms, they did not capture the full range of metabolically important organisms in the community.

Newly Identified Phyla in Lake Fryxell

Nine new phyla were identified in Lake Fryxell from the 16S rRNA and metagenomic results. The newly identified phyla include: Deinococcota, Krumholzibacteriota, Bdellovibrionota, Myxococcota, OLB16, Pastescibacteria, Armatimonadota, Lastescibacterota, also known as WS-3 , and Nanoarchaeota.

Deinococcota is a highly variable phylum characterized by their tolerance of extreme conditions. (Liu et al., 2023) These extremophiles variety widely in their activity because they likely do not have a metabolic core genome (Lum et al., 2024). Deinococcota has been found in Antarctic soil but not previously within Lake Fryxell (Lefentse Mashamaite et al., 2023).

Krumholzibacteriota is a relatively newly defined phylum that was first described in 2019 (Youssef et al. 2019). It was discovered in an anoxic spring, which is consistent with redox

conditions in the samples analyzed here. The phylum metabolizes monoaromatic compounds using benzoyl-CoA reductase, e.g. genes *bcrABCD* (Cabell et al., 2024), of which, *bcrABC* were found in transcript annotations of samples analyzed here. The metabolic transcript data recovered in our sample suggest active expression of aerobic respiration, fermentation, nitrification, denitrification, DNRA, sulfate reduction, and lipid beta oxidation (Chapter 3).

Bdellovibrionota is a phylum of gram-negative bacteria that contains classes of obligate bacterial predators and non-bacterial predators (Li et al., 2021). The specific genus binned was *Bacteriovorax*, a known predatory bacterium that feeds on larger gram-negative bacteria (Crossman et al., 2012). The phylum has been detected in Whaler's Bay in Antarctica and seasonally active streams flowing into Lake Fryxell (McQueen et al., 2022; Medieros et al., 2024), but not previously in Lake Fryxell. The bacteria in this phylum typically invade bacterial host cell walls using glycanases and then feed on cell contents with exozymes (Li et al., 2021). It is unknown what hosts this phylum might have been using in Lake Fryxell.

Myxococcota is another predatory bacterial phylum that has been recorded within Antarctic rhizospheres in the Ross Ice Shelf (Contreras et al., 2023; Martínez-Pérez et al., 2022) but not in Lake Fryxell. The genome of this organism within this phylum contains genes from type II reaction centers and are suspected to be able to utilize a chimeric lifestyle (Li Liuyang et al., 2023). Unfortunately, this could not be confirmed in my data, neither the *pufL* transcripts could be mapped or genes found.

OLB16 has been recovered from sediment from Ace Lake, Antarctica (Williams et al., 2022). The metabolisms of members of this phylum vary, including aerobic respiration, dissimilatory nitrate reduction to ammonium, sulfur oxidation and reduction, hydrogen oxidation, methane reduction and metal reduction (Williams et al., 2022). Published MAGs

suggest it also fixes carbon using the Wood-Ljungdahl pathway (Willaims et al., 2022), which is fully expressed in our samples.

Patescribacteria is a poorly characterized phylum, and their current taxonomic diversity is not well understood (Srinivas et al., 2024). They are typically associated with a microbial host and are considered obligate epibionts (Wang et al., 2023). Published research notes that their small genome, less than one Mbp, indicates their requirement for a bacterial host, in some cases Actinobacteria, for essential metabolic capabilities (Wang et al., 2023). Based on our 16S rRNA and metagenomic data, the Actinobacteria in the Lake Fryxell community could be acting as hosts.

Armatimonadota is a phylum found globally, including in Antarctica (Kato et al., 2022), although previous studies have not recorded this bacterial group in Lake Fryxell. The phylum contains classes that can use the Wood–Ljungdahl pathway to fix carbon and facilitate energy production through glycolysis (Carlton et al., 2023).

Latescribacterota is an uncultured phylum that has been found in a variety of environments like soil, coral, and sediment (Lan et al., 2022; Olivera et al., 2023; Hillyer et al., 2023). The metabolism utilized by this phylum includes respiration as well as fermentation (Oliver et al., 2023). There were fermentation and aerobic related transcripts active from these MAGs, which provides evidence that this organism may be anaerobically active in the community.

The only phylum of archaea found was Nanoarchaeota, which was not previously identified in Lake Fryxell. Nanoarchaeota is a metabolically diverse phylum primarily thought to utilize fermentation-based pathways (Cloarec et al., 2024). However, previous studies also suggest the phylum is capable of metal-reduction respiration, glycolysis, and flavin-based

electron bifurcation (Huang et al., 2021). Glycolysis-related genes were present in the annotated archaeal MAG (Table 2).

With a long-read sequencing and metatranscriptomic approach, we uncovered phyla have not been reported in Lake Fryxell that highlight the microbial diversity in Lake Fryxell. Specifically, some of these new phyla reframe the microbe-to-microbe interaction. The phyla Bdellovibrionota, Myxococcota, and Patescibacteria are typically bacterial predators. Thus, their presence suggests bacterial predation in the ecosystem. More specifically, Patescibacteria are potentially using Actinobacteria as hosts in Lake Fryxell as they do elsewhere (Wang, Yaxi et al., 2023).

Autotrophic Metabolisms

Despite the absence of Cyanobacteria in the HCLC MAGs, 16S rRNA sequences demonstrate the presence of Cyanobacteria, and mRNA transcripts indicated the presence of both PSII and PSI related transcripts classified as Cyanobacteria and diatom. Collected transcripts showed a complete collection of PSII including psbO, the transcript encoding for the oxygen evolving complex stabilizing protein. In addition, protein classification with kaiju (v1.10.1) revealed cyanobacterial organisms. However, one reason for the inability of Cyanobacteria to be binned could suggest that they may have lower relative abundance at 10.1 m, although they were present in the 16S rRNA community composition. Their decrease may be due to the lower light and higher hydrogen sulfide concentrations (Lumian et al., 2021). A previous hypothesis suggested that the Cyanobacteria *Phormidium pseudopreistleyi* at 9.8 m depth may have sulfide tolerance or potentially use sulfide in PSI when the concentrations are high enough (Lumian et al., 2021). However, the MAG genomic data from 10.1 m did not include this cyanobacterium,

so it is unlikely that it was a significant contributor to the community when sampling occurred. Even so, DO is in the water immediately above mats but not in the water column at this depth. Cyanobacteria most likely are contributing to the production of oxygen. Diatoms have also shown active transcription of oxygenic photosynthesis transcripts, demonstrating their importance to the autotrophic metabolisms at 10.1 m (Chapter 2).

Oxygenic photosynthesis was not the only autotrophic process. There were phototrophic sulfide oxidation genes and transcripts, indicating that some anoxygenic photosynthetic or possibly photoheterotrophic organisms were active. Since photoautotrophic sulfur bacteria utilize type-1 reaction centers to obtain energy and oxidize sulfide, if this is occurring, one of the byproducts is often elemental sulfur (Niel C., 1932). The phyla Acidobacterota and Bacillota are known to perform this process and are present in the collected samples (Hanada et al., 2016). The genomic and transcriptomic presence of the Wood–Ljungdahl pathway (WLP) is particularly interesting. Several HCLC MAGs exhibit full expression of the WLP, indicating a community actively fixing carbon without photosynthesis. Key enzymes in this pathway require anaerobic microenvironments and hydrogen as an electron donor. Hydrogen is likely produced by community members expressing *hydA*, such as the *Clostridium* MAGs, which are known to be anaerobic hydrogen producing fermenters (Latifi et al., 2018; Morimoto et al., 2005). When light becomes unavailable with depth into the mat or at deeper water depths, the WLP becomes a convenient way to fix carbon in anoxic conditions. The only other relatively complete autotrophic pathway in the metatranscriptomic study was the Calvin Cycle. Rubisco genes (*rbcL*, *rbcS* and *cbbL*) were present in the genomic data along with other enzymes of the Calvin Cycle. There are four forms of rubisco, but only forms I,II, IIIC, III-like were recovered from the annotated *rbcL* genes. Cyanobacteria and diatoms use form I, while form II is found in

Proteobacteria and some algae, form IIIC is in bacteria and lastly form III-like is found in bacteria and archaea (Kehl et al., 2025). The forms I,II, and III that were recovered from the annotated *rbcL* genes mapped to the Cyanobacteria, Pseudomonadota, Dinoflagellata phyla and Bacillariophyceae class. The identification of the different forms indicates that Cyanobacteria, diatoms, other algae, and non-cyanobacterial bacteria, can express rubisco for carbon fixation and can use it to build biomass. However, no expression of *rbcL* and *rbcS* genes was identified. Only *cbbL* was found in the metatranscriptomic data. Two thirds of the sequences recovered were similar to those found in Cyanobacteria, which indicates that they were active at 10.1 m when the samples were collected, consistent with the expression of PSII and PSI genes.

In summation, at 10.1 m, there are a few key autotrophic pathways and conservation reactions that are synthesizing chemical energy and providing an ecological foundation for a diverse range of metabolisms in Lake Fryxell. These pathways are providing a pool of biologically available carbon that is used to build the biomass of the microbial mats. When PAR is available at 10.1 m, oxygenic photosynthesis provides oxygen to aerobic organisms without removing the anaerobic community members. Phototrophic sulfur oxidizing bacteria assist in sulfur cycling which in turn produces sulfate that is utilized by sulfate reducing bacteria. Pairing this with rubisco-based carbon fixation, allows for production of biomolecules that build biomass in oxic or anoxic micro-environments (Klatt et al., 2015). In anaerobic conditions, the WLP may be a major process for carbon fixation. With an abundance of carbon dioxide and molecular hydrogen, the WLP becomes increasingly optimal as depth increases.

Heterotrophic Metabolisms

The presence of transcripts for oxygenic photosynthesis demonstrated active expression and when paired with measured changes in dissolved oxygen in the water overlying the mat, it can be assumed the process is actively occurring. The identification of certain Pseudomonadota in the 16S rRNA dataset, MAGs, and transcripts suggests the presence of organisms capable of aerobic respiration along with the expression of aerobic respiration related transcripts. Species identified included but not limited to *Methyloceanibacter* sp., *Bradyrhizobium erythrophlei*, *Aestuariivirga litoralis* as major community members with other members like Actinomycetota and some members of the Bacteriodota phyla in lesser abundance. Their activity is confirmed by the presence and expression of aerobic respiration transcripts (Chapter 3). Thus, it is likely that local oxygenic photosynthesis provides the ecological niche for aerobic respirators. Even though conventional wisdom suggests that anaerobic organisms find the presence of oxygen lethal, our data demonstrate that they are active when dissolved oxygen is present at the scale of sampling. Phyla containing anaerobic organisms and transcripts for anaerobic processes were found in the same samples as aerobes, demonstrating that anoxic micro-environments persist in the mats. In addition, fermentation was active in the samples, most likely occurring in the anoxic micro-environments of the mats along with other anaerobic processes.

Electron bifurcation was also an important discovery gathered from these results. It may provide context in how some organisms in Lake Fryxell are able to preserve energy by utilizing electrons from both high and low potential reactions. It can occur in diverse organisms as a near free energy conservation metabolism. In an environment like Lake Fryxell during the winter, it may be crucial to organisms waiting in transitioning oxic/anoxic zones for preferential conditions as (Yuly et al., 2020; Peters et al., 2019).

Summation of Metabolic Activity at 10.1 m in Lake Fryxell.

In the oxic-anoxic transition zone of Lake Fryxell, the environmental conditions provide a variety of resources to support diverse metabolisms. With PAR penetrating the ice cover and providing above the minimum threshold for photosynthesis, Cyanobacteria, diatoms, and other algae utilize the photons and split water, producing oxygen in the local microbial mat while fixing carbon dioxide with rubisco. The oxygen released in the local environment provides a critical resource for aerobic respirators. Dissolved oxygen concentrations (Figure 1), active photosynthesis transcripts (Table 3), and *cbbL* rubisco transcripts (Table 6) suggest that this primary productivity is occurring and is contributing to the micro redox potential within microbial mat and supporting aerobic metabolisms.

Hydrogen sulfide is present at 10.1 m and can be utilized by sulfide oxidizing bacteria aerobically or anaerobically in a light-dependent manner or light-independent manner (Lumian et al., 2021; Findlay et al.,

of energy with as little effort as possible, electron bifurcation is available. The known bifurcating enzyme 2022; Anwar et al., 2023; Kamp et al., 2006). With the expression of *dsrEF*, *fccA*, *fccB*, *sat*, *soxA*, *soxB*, and *sqr*, the product of this, directly and indirectly, is sulfate which in turn can be utilized for sulfate reduction which is common in oxic marine and aquatic conditions (Demin et al., 2024). The expressed transcripts *cysD*, *cysN*, *sat*, *aprAB*, *dsrAB*, and *asrB* indicate dissimilatory sulfate reduction and *dsrB* suggests that this occurs in both oxic and anoxic microenvironments. The presence of the transcripts suggests this process may be occurring in both conditions.

A nitrogen cycle is also active at this depth in Lake Fryxell. The *narG* and *napA* transcripts expressed convert nitrate to nitrite while *nirS* produces nitric oxide and ultimate molecular nitrogen with *norB* and *nosZ* which can then be converted to ammonium with *nif*. Dissimilatory nitrate reduction to ammonium is active with the previously mentioned nitrate reducing transcripts along with *nir* and *nrf*. Ammonium is also converted back into nitrite with *amoA*. With this complete cycle active, it demonstrates that alternative metabolisms that do not utilize light are active within these mats.

At 10.1 m, only locally produced oxygen is present, and anaerobic metabolisms are important compared to consistently oxic depths shallower in the lake. Fermentation is active at 10.1 m and the pathways investigated are fully transcribed (Table 3). This, along with the sulfur cycle and nitrogen cycle, shows that the microbial mats are capable of versatile metabolic pathways and can respond to changing conditions. However, if conditions change to where organisms need to produce adequate amounts transcripts *etfAB*, *hndABCD*, *hydAC* are active and can facilitate this. This method would most likely be important if bacteria or archaea are facing unfavorable thermodynamic conditions (Schut et al., 2022).

Comparisons with Shallower Depths and Water Column communities

The microbial mats at 9.0 - 9.8 m depths show similar bacterial communities to those at 10.1 m (Dillon, et al., 2020). Pseudomonadota, Cyanobacteria, Plantomycetota, Actinomycetota, Chloroflexota, Bacillota, Verrucomicrobiota, Acidobacteriota, Gemmatimonadota, and Nitrospirota are shared phyla that show changes in abundance from 9 m to 10.1 m. Pseudomonadota is the largest phyla in abundance and increases from 9 m to 10.1 m from approximately 15 -25% to nearly 30% (Dillon et al., 2020; Figure 2). Cyanobacteria decrease

from ~50% at the top of mats at 9.8 m to ~1% with respect to the 16S rRNA (Dillon et al., 2020; Figure 5). Planctomycetota increases from ~3% at 9 m to 8% at 10.1 m (Dillon et al., 2020; Figure 5). Actinomycetota marginally increases from ~2.5% at 9.8 m to 3%. Chloroflexota shows its presence increasing from ~1.5% to 8%. Bacillota formerly firmicutes do not show similar increases, only rising from 1.5% to 2%. Verrucomicrobiota shows little increase from ~1.5% to 2%. Gemmatimonadota shows a large increase from ~0.75% to 5% while Nitrospirota only shows an increase of ~1% to 2% (Dillon et al., 2020). Microbial mat 16S rRNA community studies have also shown commonality in the presence of Bacteroides, Pseudomonadota, Planctomycetes, Cyanobacteria, Chloroflexi, Chlorobi, and Verrucomicrobia but may still have distinct community compositions (Jungblut et al., 2016). In comparison to our protein-based classification of cyanobacterial contigs and 16S rRNA results, both studies verify the presence of *Oscillatoria*, *Leptolyngbya*, and *Nostoc* related organisms. Organisms found by Jungblut et al. (2016) but missing in our study at 10.1 m include *Malainabacteria*, *Pseudanabaena*, and *Phormidium pseudopriestleyi* (Lumian et al., 2021). Even though the abundance of Cyanobacteria dwindles deeper into the lake, some persist to 10.1 m depth, likely those that can adapt to lower PAR levels. Some Cyanobacteria produce chlorophyll f and incorporate it into photosystem I when growing in far-red low-light environments. The acclimation of the photosystem-chlorophyll structure may allow Cyanobacteria to perform far red light-driven photosynthesis (Gisriel et al., 2020). Although we did find transcripts or genes related to chlorophyll f in these samples. Still, of the Cyanobacteria identified, the most numerous, *Caldora sp010672925* found in the 16S rRNA microbial composition may be a good candidate if enough genetic information can be recovered from future samples. Overall, there are similarities between the previous data and our data, which show significant changes in certain populations in

Lake Fryxell with respect to depth. With environmental conditions changing, specifically PAR and oxygen, other bacterial communities are adjusting their metabolic activity to the variable microenvironments they may experience in the microbial mat.

The mats are also different from the water column in Lake Fryxell. Water column taxonomic results have some overlapping phyla with previous studies of the microbial mats (Dillon et al., 2020) at including the presence of Actinobacteria, Pseudomonadota, Chloroflexi, Planctomycetes, Verucomicrobia, and Bacteroidetes (Lei et al., 2019). However, the water column has a lower phyla diversity, with 28 phyla in the water column to 60 phyla in our below 0.01% abundance 16S rRNA results (Li et al., 2019). Among the shared phyla, their relative abundances differ significantly between the water column and the microbial mats. In the water column, Actinobacteria make up a significant amount of 16S rRNA population, >30% (Li et al., 2019), while in the mats, they make up less than 10%. Similarly, Bacteroidetes constitute almost 40% in the water column (Li et al., 2019), but less than 20% in our microbial mat samples. Pseudomonadota appear similar with abundances in both studies within 20-25% (Li et al., 2019). Previous archaeal studies of Lake Fryxell's water column revealed four archaeal phyla: Thaumarchaeota, Parvachaea, Euryarchaeota and Crenarchaeota (Jungblut et al., 2016; Karr et al., 2006). These four archaeal phyla were not detected in the microbial mat in our sample, but the Nanoarchaeota phylum was, demonstrating a difference in archaeal communities.

The distribution of phyla in Lake Fryxell is not substantially different from those found in other non-Antarctic microbial mats. Hypersaline lakes in the Atacama Desert in Chile share the phyla Pseudomonadota, Verrucomicrobia, Armatimonadetes, Cyanobacteria, Actinobacteria, Bacteroidetes, Bacillota, Planctomycetes, Acidobacteria, and Chloroflexota (Rasuk et al., 2015). Pseudomonadota, represented the most abundant phyla in the Atacama lakes, similarly to Lake

Fryxell (Vagnale et al., 2022). The same shared phyla were also major population in Dutch barrier island Schiermonnikoog, a nearly closed horizontally stratified salt marsh (Bauersachs et al., 2020). This demonstrates the broad success these phyla have in microbial mats. The marsh composition shares all the same phyla as the Atacama lake and Lake Fryxell (Bolhuis et al., 2011). Crater Lake, a sulfidic freshwater lake fed by hydrothermal springs in Oregon, shows some commonality in microbial phyla composition. The phyla shared with Lake Fryxell include Acidobacteria, Actinobacteria, Bacteroidetes, Blamydidae, Chloroflexi, Bacillota, Gemmatimonadetes, Latescibacteria, Planctomycetes, Pseudomonadota, and Verrucomicrobiota (Stromecki et al., 2022). Similarly to other lakes mentioned and Lake Fryxell, Pseudomonadota represented the largest community member in Crater Lake (Stromecki et al., 2022). The similarity among these different lakes emphasizes the remarkable microbial diversity of commonly shared phyla and how these communities can adapt to different extremes while maintaining similar community structures at the phyla level.

Conclusion

In summation, Lake Fryxell's microbial mat at 10.1 m depth is consistent with previously characterized taxa at shallower depths with the addition of new phyla. With the utilization of long read sequencing of 16S rRNA, Lake Fryxell's taxonomic profile has added five new distinct phyla at 10.1 m. The acquired data is also consistent with previous populations of prokaryotes in the lake. Verrucomicrobiota, Pseudomonadota, Planctomycetota, Nitrospirata, Gemmatimonadota, Cyanobacteria, Chloroflexota, Bacteroidota, Bacillota, Actinomycetota, and Acidobacteriota are present at this depth. In comparison to previous data, certain phyla increase

or decrease within the community composition deeper into the lake. This signals that some community members become more ecologically relevant or able to adjust to environmental changes. In total, based on our refraction analysis, this depth may contain up to 900 unique organisms at 10.1 m in this isolated extreme environment with potentially more to be discovered.

Utilizing metagenome assembled genomes provided a similar picture of microbial composition, although they are not meant to be direct representations of the community. These MAGs indicate the metabolic potential of the organisms to build a metabolic framework of Lake Fryxell at 10.1 m. Annotation of the assembled MAGs revealed an intricate mix of metabolic potential where it can be expected that aerobic respiration, fermentation, dissimilatory sulfate reduction, sulfide oxidation, and a complete nitrogen cycle may be reflected in expression data from the HCLC MAGs (Chapter 3).

Metatranscriptomic data provide insights into this metabolic framework, which is expanded in Chapter 3. Currently, it is clear, photosynthesis was occurring at 10.1 m when sampled, with major components of photosystem II, photosystem I, and cytochrome b6f being expressed. Aerobic respiration was active indicating that the oxygen produced was being utilized by heterotrophs. Fermentation, nitrogen-based processes, sulfide oxidation, sulfate reduction, and electron bifurcation transcripts demonstrate the metabolic variety organisms can apply depending on the environmental dynamics at 10.1 m to maximize energy production to produce biomass.

It is important to note that photosynthesis may be at its lowest PAR limit and still producing oxygen, but the overlying water is anoxic. Thus, the redox conditions represent a balance among microbial processes and environmental conditions, including seasonality of the region. Yet oxygen production allows aerobic metabolisms to be active in the microenvironments of the mat. It is also almost certain that the mats are not completely

oxygenated and contain anoxic zones. The presence of anaerobic transcripts suggests that these anoxic micro-environments permit the activity of obligate anaerobic fermenters, nitrogen respirators, sulfate reducers, and anaerobic sulfide oxidation. The interesting metabolic dynamics of 10.1 m from the environmental and microbial data demonstrate that this depth is a zone with interesting shifts between anoxic and oxic conditions and microbial populations that are metabolically variable to adjust to changing conditions.

Chapter 2

Taxonomic Classification and Metabolic Capacity of Lake Fryxell Diatoms at 10.1 m

Introduction

Diatoms are one of the most globally distributed photosynthetic primary producers (Malviya et al., 2016). Ecologically, they play a critical role; they produce approximately 20%-50% of the Earth's oxygen and contribute to 20% of global carbon fixation (Sethi et al., 2020). They can be found in a wide variety of environments. They are highly prevalent in oceans where they contribute up to 40% of the total marine carbon fixation and found well below the photic zone (Prierella et al., 2021). Diatoms are also found in freshwater streams and lakes in planktonic and benthic habitats (B-Béres et al., 2022). They are also not strangers to extreme environments which include sulfidic hot springs and acidic conditions below pH 3.5 (DeNicola et al., 2000; Nikulina et al., 2026).

Due to their global distribution, they experience varying conditions. This can range from changes in light, temperature, oxygen, nutrient availability, and microbial neighbors, which all in turn affect diatom physiology (Zhou et al., 2021; Zeng et al., 2020). Changes in light and temperature can affect photosynthesis, while oxygen limits respiratory capacity; alterations in nutrient limitation can cause changes in lipid composition and microbial communities may introduce complicated direct and indirect changes (Zeng et al., 2020; Zhou et al., 2020; Bromke et al., 2019). Considering these alterations in activity, diatoms in extreme environments must adapt to more than one of these conditions. This is true of diatoms found in polar regions, including those found in Antarctica (Zhai et al., 2026). Diatoms have been studied in Lake Fryxell, but what species are present at 10.1 m and their contributions to the microbial mats is unknown (Jungblut et al., 2016; Dillon et al., 2020). Their ecological setting provides able opportunities for understanding their metabolic capabilities through metagenomic approaches. At

10.1 m, the low light, oxic, anoxic, and sulfidic conditions paired with the seasonality of light availability creates an interesting environment to study the taxonomic diversity and metabolic versatility of these diatoms.

Several diatom species have been discovered in broader Lake Fryxell environments, including in lake sediment, summer streams and pools, and dispersed by the wind, but those residing in Lake Fryxell have not been fully characterized (Schulte et al., 2021; Stone et al., 2024). It is unclear how many species there are in Lake Fryxell benthic mats, though there are suspected to be at least 6-10 species based on recent morphological studies (Jungblut et al., 2016). At lake depths below 9 m, previous work suggests that two species become dominant, *Diadesmis contenta* and *Navicula gregaria* (Jungblut et al., 2016). These two organisms in Lake Fryxell have not been fully genomically categorized. To date, only 47 nucleic sequences of *Navicula gregaria* in general are available ranging in size from 27 bp to 1745 bp. A 18S rRNA sequence for *Navicula gregaria* is publicly available but *Diadesmis contenta*, also known as *Humidophila contenta* only has an unverified chloroplast sequence available. Prior metagenomic work on Lake Fryxell's diatoms has been limited to metagenomic short read DNA sequencing and protein-based classification, morphological identification, and short 18S rRNA sequencing (Spaulding et al., 1994; Jungblut et al., 2016; Dillon et al., 2020; Schulte et al., 2021; Stone et al., 2024). With currently less than 30 fully characterized diatom genomes available globally and with potentially 100,000 - 200,000 different species in existence (Wang et al., 2022; Mar, 2022), Lake Fryxell presents an opportunity to discover novel species and determine their roles within the microbial mats and their metabolic strategies.

At 10.1 m water depth, the diatoms in Lake Fryxell experience a variety of challenging environmental conditions such as low-light, anoxia, and presence of hydrogen sulfide (Howard-

Williams et al., 1998; Jungblut et al., 2016; Lumian et al., 2021). To determine their functional role in Lake Fryxell, it is important to determine how they adapt their metabolism to these conditions. The minimum photon requirement for photosynthesis is $0.01 \mu\text{mol photons/m}^2/\text{s}$ (Hanke et al., 2018; Randelhoff et al., 2020; Raven et al., 2000) and based on collected photoactive radiation (PAR) readings, the diatoms do receive enough PAR for photosynthesis, approximately $3 \mu\text{mol photons/m}^2/\text{s}$ in summer, despite the lake's ice cover blocking 97-99% of incident irradiance (Howard-Williams et al., 2013). As a response to dark-anoxic conditions, some diatoms can perform dissimilatory nitrate reduction to ammonium to persist in a long-term resting stage while utilizing internal storage supplies (Joli et al., 2024; Kamp et al., 2011). With a return of light, diatoms can quickly restart photosynthesis and resume optimal metabolic productivity (Joli et al., 2024). Similarly, some diatoms can tolerate 0.9-6.8 mM of sulfide and are stimulated by sulfide to perform nitrate reduction (Admiraal et al., 1979; Merz et al., 2022). Since diatom adaptability can vary, and diatoms are known to inhabit Lake Fryxell, it can be assumed that Lake Fryxell diatoms have acclimated to conditions at 10.1 m and deeper into the lake, including the presence of sulfide. The results in this study reveal new insights into Lake Fryxell diatoms and their contribution to the microbial ecology within microbial mats at 10.1 m water depth.

Materials and Methods

Sample Collection

Sample collection is as described in chapter one. Samples were collected on January 15, 2023, by divers operating through a hole melted in the ice cover on the northern side of Lake Fryxell

(-77.6078° latitude and 163.114517° longitude). Five samples were collected at 10.1 m water depth and were used for this study. All water depths are based on the hydrostatic water level in January 2023. Divers collected the samples in sterilized plastic boxes and brought them to the surface of the lake. At the lake surface, researchers put the samples into sterile falcon tubes and then into liquid nitrogen. The time between sample collection and freezing was less than 15 minutes for each sample. Samples remained in a dry shipper at liquid nitrogen temperatures until they were sent from Antarctica to the lab at UCD at -20°C. Once at UCD, they were stored at -80°C until DNA and RNA were extracted.

DNA Extraction and Sequencing

DNA Extraction and Sequencing are as described in chapter one. Sample libraries were prepared using the PCR-free Oxford Nanopore Technologies Ligation Sequencing Kit (Oxford Nanopore Technologies, Oxford, UK) with NEBNext Companion Module (New England Biolabs, Ipswich, MA) to manufacturer's specifications. No additional DNA fragmentation or size selection was performed. Nanopore sequencing was performed on an Oxford Nanopore a MinION Mk1B (Oxford Nanopore Technologies, Oxford, UK) sequencer or a GridION (Oxford Nanopore Technologies, Oxford, UK) sequencer using R10.4.1 flow cells (Oxford Nanopore Technologies, Oxford, UK) in one or more multiplexed shared-flow-cell runs. Run design utilized the 400bps sequencing mode with a minimum read length of 200bp. Adaptive sampling was not enabled. Guppy (v6.5.7; Oxford Nanopore Technologies, Oxford, UK) was used for super-accurate basecalling, demultiplexing, and adapter removal. Quality filtering was performed

with fastp (v0.23.4; Chen et al., 2018) with a per-base quality trimming of Q20 and per-read quality filtering of Q15.

RNA Extraction

All standard RNA extractions at SeqCenter follow the ZymoBIOMICSTM Quick-RNA Miniprep Kit (Zymo Research, Irvine, CA). The preparation of cell and tissue sample types followed Zymo Research recommendations stated in the protocol. Samples were eluted into a final volume of 40 µl. Final RNA concentrations were determined via Qubit3 from (Thermo Fisher Scientific Waltham, MA).

RNA Sequencing

Samples were DNase treated with Invitrogen DNase, RNase free (Thermo Fisher Scientific Waltham, Massachusetts). Library preparation was performed using Illumina's Stranded Total RNA Prep Ligation (Illumina, San Diego, California) with Ribo-Zero Plus kit (New England Bio Labs, Ipswich, Massachusetts) and 10bp unique dual indices. Sequencing was done on a NovaSeq X Plus (Illumina, San Diego, California), producing paired end 150bp reads. De multiplexing, quality control, and adapter trimming was performed with bcl-conver (v4.2.2; Illumina, San Diego, California).

Bioinformatic Analysis

Taxonomic Classification of Diatom DNA

Quality of the nanopore sequencing was assessed with NanoPlot (v1.46.1; De Coster Wouter et al., 2023). The reads were assembled into contigs with flye using --meta settings (v2.9.4-b1799) and quality controlled with quast (v5.2.0) (Gurevich et al., 2013; Kalgomarov et al., 2019). Reads were mapped to the assembly using minimap2 (v2.27; Li et al., 2018). To identify diatom reads, kaiju (v1.10.1; Menzel et al., 2016) was used using the nr_euk database to perform protein-based taxonomy. Contigs that were classified as “Bacillariophyceae” were extracted from the general assembly along with their taxonomic classification for additional analyses.

Identification of Diatom RNA and Annotation of Expressed Transcripts

RNA reads for every sample were inspected for quality using fastqc (v0.12.1; Andrews., 2010). Resulting reads were then assembled using Trinity(v2.5.1; Grabherr et al., 2011), specifying paired end reads. The RNA assembly was checked using RNAquast (v2.2.4; Bushmanova et al., 2016). The contigs were then taxonomically classified using kaiju (v1.10.1) with the nr_euk database (Menzel et al., 2016). Contigs identified by kaiju (v1.10.1) as belonging to Bacillariophyceae were subjected to BLASTX (ver 2.17; Altschul et al., 1990) searches with an E-value cutoff of $< e^{-15}$. For functional annotation, RNA reads were aligned to the Bacillariophyceae DNA contigs identified by kaiju (v1.10.1). Resulting mapped RNA reads were then assembled using Trinity (v2.5.1; Grabherr et al., 2011). Transdecoder (v5.7.0; Haas B.,

2023) was used to find open reading frames for annotation with Trinotate (v4.0.2; Bryant et al., 2017).

Identification of Diatom 18S rRNA Sequences in Unassembled DNA Long Reads

Assembled contigs were also analyzed for 18S rRNA using barrnap (v0.9; Seeman., 2024). Collected 18S rRNA sequences were compared to the Protist Ribosomal Reference database and used for BLASTN queries using standard settings and an E-value cutoff of $\leq e^{-10}$ to identify Bacillariophyceae (Guillou et al., 2012; Camacho et al., 2009).

Generation and Visualization of Diatom Phylogenetic Tree

The 18S ribosomal RNA (rRNA) gene sequences were identified and extracted from assembled metagenomic contigs using barrnap (v0.9; Seemann, 2013; Bioconda). The tool was run with the eukaryotic kingdom flag --kingdom euk to specifically target eukaryotic 18S rRNA genes. Predicted gene coordinates were output in GFF3 format, and the corresponding nucleotide sequences were extracted from the assembly FASTA file using bedtools (v2.31.0). All sequences annotated as 18S rRNA were saved for downstream analysis.

Extracted 18S rRNA sequences were quality filtered using mothur (v1.48.0; Schloss et al., 2009). Sequences were filtered using the screen.seqs command with the following parameters: minimum length of 200 bp minlength=200, maximum length of 2,000 bp maxlength=2000, and a maximum homopolymer run of 10 bp maxhomop=10. These thresholds

removed short fragments unlikely to classify accurately and excessively long sequences. A total of 7,562 sequences were retained after quality filtering. Quality-filtered sequences were classified against the Protist Ribosomal Reference database (v5.0.0; Guillou et al., 2013) using the `mothur classify.seqs` command with the Wang Bayesian classification method `method=wang` and a bootstrap confidence cutoff of 60% `cutoff=60`. To remove bacterial and archaeal contamination inherent in rRNA extraction pipelines, the `remove.lineage` command was applied to exclude sequences classified as Bacteria, Archaea, or unknown. This filtering step retained 965 eukaryotic sequences with a median length of 1,703 bp.

To identify diatom-affiliated sequences among the filtered eukaryote dataset, a nucleotide BLAST search was performed using BLAST+ v2.14.1 (Camacho et al., 2009; Bioconda). A BLAST database was first constructed from the PR2 v5.0.0 SSU reference sequences in `mothur` format using `makeblastdb` with the nucleotide database type flag `-dbtype nucl`. Query sequences were searched against this database using `blastn` with the following parameters: tabular output format 6 with query ID, subject ID, percent identity, alignment length, e-value, bitscore, and subject title fields `-outfmt "6 qseqid sseqid pident length evalength bitscore stitle"`; a maximum of ten target sequences per query `-max_target_seqs 10`; and a minimum percent identity threshold of 70% `-perc_identity 70`. BLAST results were cross-referenced with the PR2 taxonomy file to identify queries with significant hits to Bacillariophyta sequences, confirming the presence of one unique nuclear diatom 18S rRNA sequence.

To construct a phylogenetic reference dataset, one representative nuclear 18S rRNA sequence was selected per diatom genus from the PR2 v5.0.0 database. Sequences were sourced exclusively from nuclear-origin entries accessions with the suffix `_U`, excluding plastid and mitochondrial sequences. Where multiple sequences were available per genus, named species were

prioritized over unclassified entries *sp.*. Genera represented only by unclassified or placeholder entries were excluded. This procedure yielded a set of approximately 120 representative diatom sequences spanning 12 orders and over 100 genera. Three outgroup sequences were selected from closely related stramenopile lineages to root the phylogenetic tree: *Aureococcus anophagefferens* (Pelagophyceae; accession AF117779), *Dictyocha octonaria* (Dictyochophyceae; accession HQ646562), and *Nannochloropsis* sp. (Eustigmatophyceae; accession FJ896224). These taxa represent the closest well-resolved outgroups to the Bacillariophyta within the Stramenopiles.

The one query diatom sequence, approximately 120 PR2 reference sequences, and three outgroup sequences were combined into a single FASTA file. Multiple sequence alignment was performed using MAFFT (v7.526; Katoh & Standley, 2013) with automatic algorithm selection – *auto* and sequence reordering enabled *--reorder*. The *--auto* flag selects the most appropriate alignment strategy based on dataset size and sequence diversity, defaulting to FFT-NS-2 or L-INS-i as appropriate. The resulting alignment was trimmed using trimAl (v1.4; Capella-Gutiérrez et al., 2009) with the *-automated1* heuristic, which automatically selects trimming parameters optimized for downstream maximum likelihood phylogenetic analysis.

Maximum likelihood phylogenetic analysis was conducted using IQ-TREE2 (v2.4.0; Minh et al., 2020; Bioconda). The best-fit nucleotide substitution model was selected using ModelFinder (Kalyaanamoorthy et al., 2017) integrated within IQ-TREE2 via the *-m TEST* flag, which evaluates models using the Bayesian Information Criterion (BIC). The model General Time Reversible with gamma-distributed rate variation model was used. Branch support was assessed using the ultrafast bootstrap approximation (Hoang et al., 2018) with 1,000 replicates *-B 1000*. The tree was rooted on the three outgroup taxa using the *-o* flag specifying *OUTGROUP_Aureococcus_anophagefferens*, *OUTGROUP_Dictyocha_octonaria*, and

OUTGROUP_Nannochloropsis_sp. The consensus tree with bootstrap support values .contree was used for all downstream visualization.

The phylogenetic tree was visualized using the Interactive Tree of Life (iTOL) web platform (v6; Letunic & Bork, 2021). Tips were color-coded by diatom order using a custom color strip annotation file. The one unknown query sequence was highlighted in red and labeled as "YOUR_Unknown Diatom 18S rRNA. Outgroup taxa were labeled and colored in black. Bootstrap support values ≥ 70 were displayed on internal nodes. The tree was displayed in circular layout format with branch length values displayed.

Results

Diatom Classification

Table 1: DNA contigs identified as diatoms by kaiju (v1.10.1) along with contig length, sequencing depth, and successfully mapped RNA reads. The longest diatom contig was classified as an unknown Bacillariophycidae.

Contig ID	Contig Length	DNA Sequencing Depth	Mapped RNA Reads	Kaiju Classification
contig_10207	69461	2	3358	Bacillariophycidae
contig_10777	35518	8	808	<i>Seminavis robusta</i>
contig_11044	4528	20	0	<i>Chaetoceros tenuissimus</i>
contig_12329	38719	47	1760	<i>Fragilaria crotonensis</i>
contig_16433	10435	69	376	<i>Fistulifera solaris</i>
contig_20131	46511	3	2141	<i>Skeletonema marinoi</i>
contig_24239	39691	4	371	<i>Pseudo-nitzschia multistriata</i>
contig_24248	26993	12	9	<i>Thalassiosira oceanica</i>
contig_2563	42294	22	1962	<i>Skeletonema marinoi</i>
contig_8393	6360	9	293	<i>Cylindrotheca closterium</i>

In total, 20,228 contigs were assembled after long read nanopore sequencing. All read average quality was above Q15 after fastp (v0.23.4) quality control and filtering (Chen et al.,

2018). Ten contigs were classified as diatoms in origin with kaiju (v1.10.1) protein-based classification and the nr_euk database (Table 1.1). The contigs assembled were classified as the following diatom species and one taxonomic family: Bacillariophycidae, *Seminavis robusta*, *Chaetoceros tenuissimus*, *Fragilaria crotonensis*, *Fistulifera solaris*, *Skeletonema marinoi*, *Pseudo-nitzschia multistriata*, *Thalassiosira oceanica*, *Skeletonema marinoi*, *Cylindrotheca closterium*. The diatom contig lengths ranged from 6,360 to 69,461 bp with an average coverage spanning 2X – 69X. RNA mapping to the assembled contigs ranged from zero to 3358. The longest contig, contig 10207, had 3358 successfully mapped RNA reads.

Table 2: Blastn results of 18S rRNA sequences using the Protist Ribosomal Reference Database. Ribosomal RNA sequences were extracted using barrnap on raw nanopore long read sequences.

Sequence	Percent Similarity	Alignment Length	E-value	Database Reference Organism
Unknown Diatom 1	77	1387	0	<i>Pseudo-nitzschia australis</i>
Unknown Diatom 2	82	543	2E ⁻¹¹⁵	<i>Amphora</i> sp.

Two 18S rRNA sequences were extracted from the unassembled nanopore sequenced reads and compared to the Protist Ribosomal Reference Database. These were compared successfully as similar to diatoms in the PR² database (Table 2). One sequence was close in relation to the reference organism *Pseudo-nitzschia australis*, with a percent similarity of 77 and an alignment length of 1387 bp. The second 18S rRNA sequence was only 82% similar to an *Amphora* sp. reference sequence with an alignment length of 543 bp.

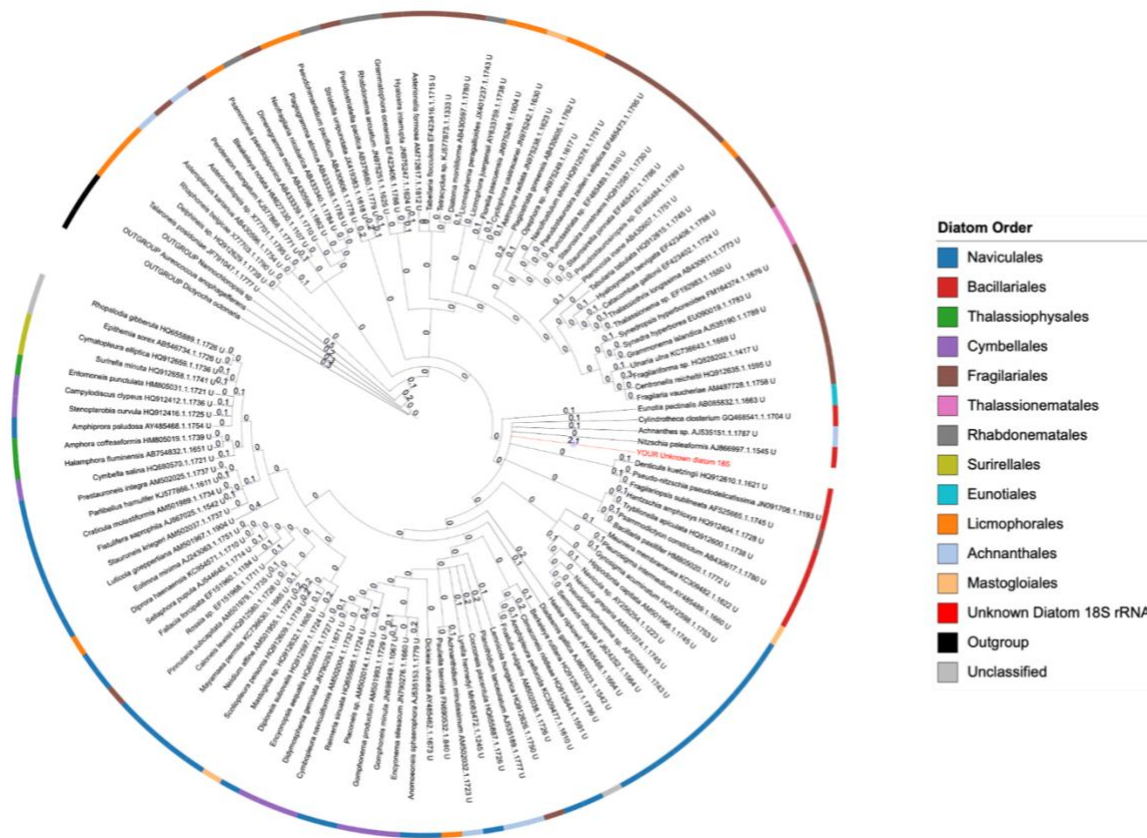


Figure 1: Construction of Bayesian model phylogeny tree using 120 unique non-plasmid diatom 18S rRNA genes from the Protist Ribosomal Reference Database and 1 unknown sequence collected from 10.1 m in Lake Fryxell. Actual branch length depictions are not displayed in favor of displaying the numeric value of the branch length. Diatom phylogenetic orders are listed in the legend. The unknown diatom 18S rRNA sequence is labeled as “YOUR Unknown diatom 18S” and depicted in red along with its branch. Outgroups are labeled in black and diatoms with an unclassified order are labeled in grey.

To determine the evolutionary relationship and divergence of the collected unknown diatom sequence a phylogenetic tree was constructed. The sequence with the largest length was chosen, 1857 bp, compared to the shorter sequence, ~600 bp, to enhance accuracy with multiple variable regions within the sequence for comparison. Both a Bayesian and Maximum Likelihood model were used to determine if the placement of the sequence would be similar in both

phylogenetic trees. In the Bayesian model, the unknown sequence was in a similar clade to the diatoms in the order Bacillariales but with a branch length of 2.1 where all other branch lengths ranged from 0 to 0.4 (Figure 1). In the Maximum Likelihood model (Figure 2), the unknown sequences appeared early in the phylogenetic branching in the same clade as a diatom belonging to the Licomophorales order. The branch length was approximately 2.8. All other branch lengths in this model ranged from 0 to 0.5. Both models produced different results displaying inconsistencies with the unknown 18s rRNA placement.

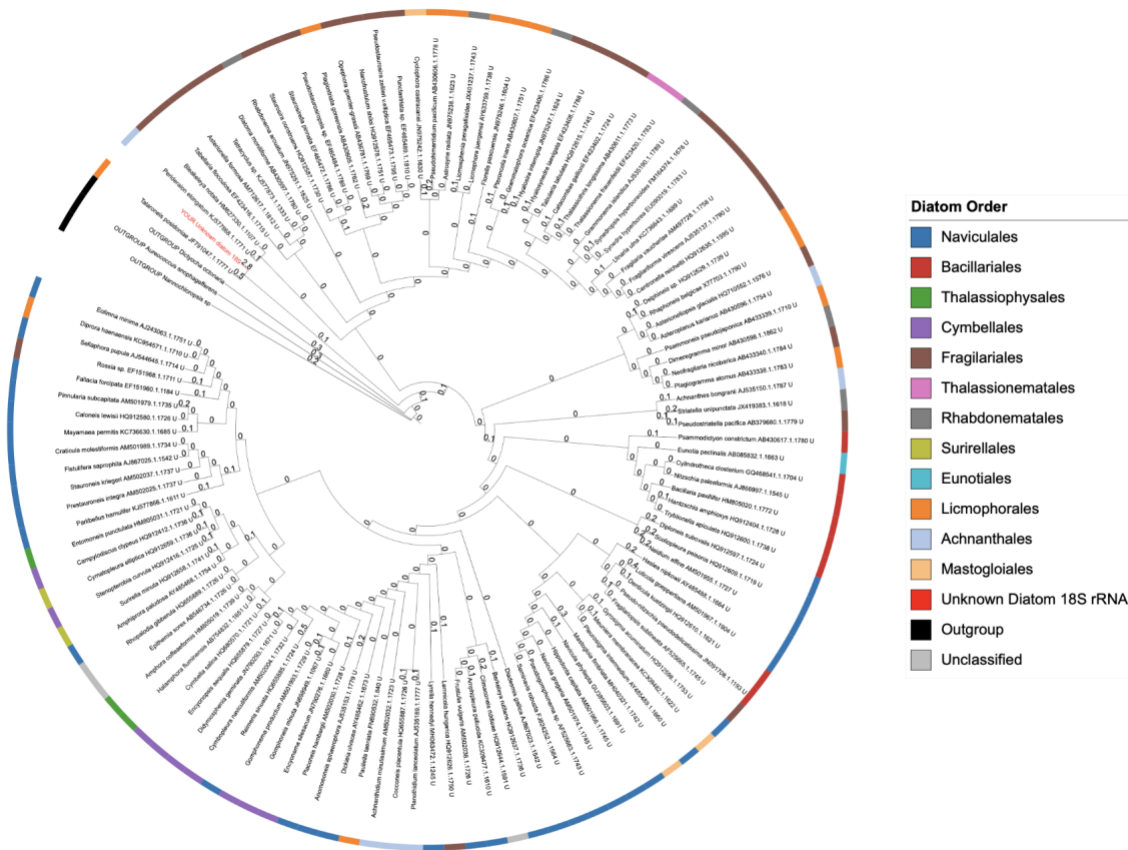
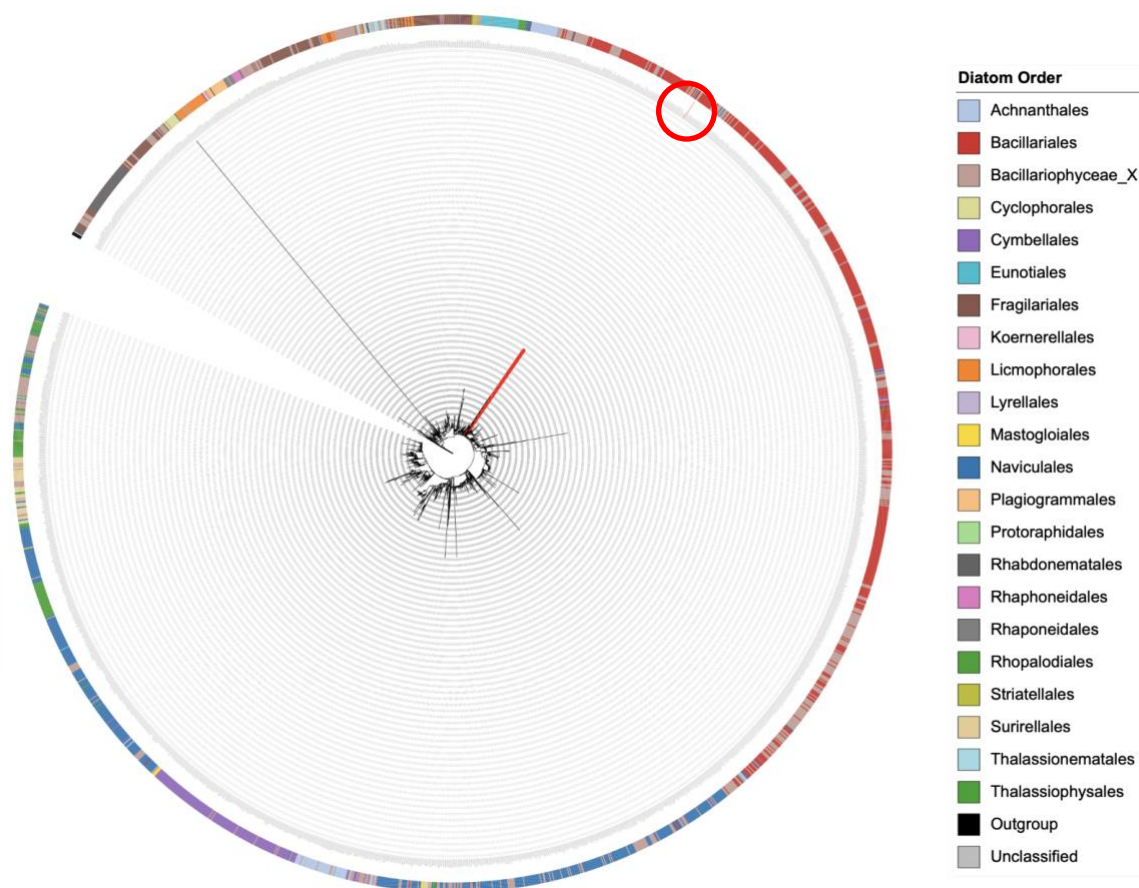


Figure 2: Construction of Maximum Likelihood model phylogeny tree using 120 unique non-plasmid diatom 18S rRNA genes from the Protist Ribosomal Reference Database and 1 unknown sequence collected from 10.1 m in Lake Fryxell. Branch length depictions are not displayed but the assigned numeric value of the branch length is. Diatom phylogenetic orders are listed in the legend. The unknown diatom 18S rRNA sequence is labeled as “YOUR Unknown diatom 18S”



and depicted in red along with its branch. Outgroups are labeled in black and diatoms with an unclassified order are labeled in grey.

Figure 3: Construction of a Maximum Likelihood phylogenetic tree with all non-plasmid or repeat diatom 18S rRNA sequences in the Protist Ribosomal Reference Database. Branch length depictions or values are displayed to illustrate the unknowns branch length. Diatom phylogenetic orders are listed in the legend. The unknown diatom 18S rRNA sequence is labeled as “YOUR Unknown diatom 18S” and depicted in red along with its branch. Outgroups are labeled in black and diatoms with unclassified orders are labeled in grey.

To address if sample size affected the placement of the unknown 18S rRNA sequence in the Maximum Likelihood model, a phylogenetic tree using all non-plasmid and only unique diatom 18S rRNA sequences, ~2500, from the Protist Ribosomal Reference Database was constructed (Figure 3). Similarly to the small sample size Bayesian model, the unknown sequence was grouped with diatoms belonging to the Bacillariales order. The branch length for

the unknown sequence in this tree and model was 1.4, with other branches ranging from 0 to 5.3. This represented at 50% reduction in branch length compared to the small-scale version. The longest branch belonged to *Navicula cryptocephala*, a freshwater diatom (Kützinger F., 1844). The unknown sequence did not group near any *Navicula gregaria*, a diatom species previously cited in Lake Fryxell. There were no *Humidophila contenta*, formerly *Diademsis contenta* a species found within Lake Fryxell, sequence available for comparison. However, *Humidophila contenta* is under the Naviculales order, as is *Navicula gregaria*, but the placement of the unknown sequence did not suggest a shared classification.

Similar to the Maximum likelihood model, a larger sample size was used to determine its effect on placement of the unknown diatom sequence (Figure 4). The Bayesian model using all non-plasmid unique diatom 18S rRNA sequences placed the unknown sequence in the same clade as a *Licomophora* sp. MH040316.1.1708 U, a diatom classified under the Licomophorales order. The branch length was 0.9 with the range of branch lengths in the entire tree ranging from 0 to 1.6. The reduction in branch size compared to the smaller scale Bayesian model was greater than 50%. The unknown sequence did not group with a collection of organisms within the same order.

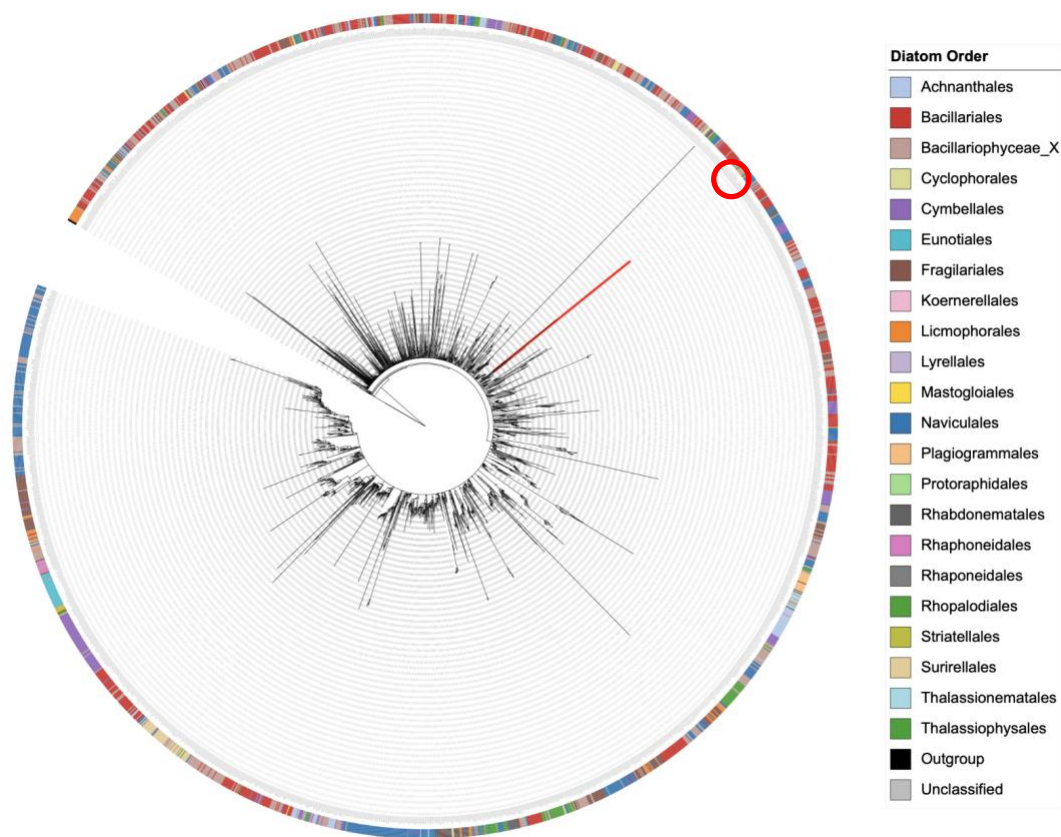


Figure 4: Construction of a Bayesian model phylogenetic tree with all non-plasmid or repeat diatom 18S rRNA sequences from the Protist Ribosomal Reference Database, 2500 sequences. Included was an unknown sequence recovered from 10.1 m found in the red circle, and three outgroup sequences from the same database. Branch length is depicted to distinguish the unknown 18S rRNA diatom sequence in red. The legend displays diatom orders, outgroup classification as black, and unknown order classifications in grey.

Table 2: BLASTX, average coverage, and average depth results of diatom specific metabolic transcripts assembled with Trinity and identified with kaiju (v1.10.1) protein classification.

Category	Protein Identification	Mean Depth	Coverage	Reads Mapped
Aerobic Respiration	cytochrome c oxidase subunit 3 [Scherffelia dubia]	77	100	425
	cytochrome c oxidase subunit 1 [Naviculales sp.]	7	100	21
	cytochrome c oxidase subunit 1 [Vischeria punctata]	29	100	182
	cytochrome c oxidase subunit 1 [Naviculales sp.]	364	100	42066
	succinate dehydrogenase flavoprotein [Phaeodactylum tricornutum CCAP 1055/1]	6	100	18
	ATP synthase subunit beta, mitochondrial [Mayamaea pseudoterrestris]	5	100	14
	NADH dehydrogenase subunit 7 [Vischeria punctata]	6	100	13
	NADH dehydrogenase subunit 7 [Naviculales sp.]	5	100	14
	NADH dehydrogenase subunit 2 [Naviculales sp.]	962	100	42821
	NADH dehydrogenase subunit 4L [Monosiga brevicollis]	6	100	13
	NADH dehydrogenase subunit 4 [Naegleria gruberi]	97	100	571
	NADH dehydrogenase subunit 4L [Schizostauron trachyderma]	9	100	42
	cytochrome oxidase subunit II [Phaeodactylum tricornutum]	17	100	105
	cytochrome c oxidase subunit II [Nannochloropsis gaditana]	10	97	119
	NADH dehydrogenase subunit 11 [Berkeleya fennica]	481	100	10023
	succinate dehydrogenase flavoprotein subunit [Mayamaea pseudoterrestris]	12	100	34
	NADH dehydrogenase [Fistulifera solaris]	4	100	12
	ATP synthase CF1 alpha subunit [Halamphora calidilacuna]	6	100	20
	NADH dehydrogenase subunit 4 [Naegleria gruberi]	18	95	77
	ATP synthase CF0 subunit IV [Cocconeopsis kantsiensis]	10	94	58
	NADH dehydrogenase [Fistulifera solaris]	6	100	17
	NADH dehydrogenase subunit 4L [Naviculales sp.]	49	100	385
	cytochrome oxidase subunit I, partial [Plantago spathulata subsp. spathulata]	13	100	52
	cytochrome c oxidase subunit 3 [Gomphonema parvulum]	19	100	162
	ATP synthase F0 subunit 6 [Naviculales sp.]	7	100	27

	NADH dehydrogenase subunit 4 [Halamphora calidilacuna]	2	90	4
Photosynthesis	fucoxanthin chlorophyll a/c protein, lhcr type [Phaeodactylum tricornutum CCAP 1055/1]	9	100	30
	subunit II of photosystem I (ferredoxin-binding protein) [Entomoneis sp.]	17	100	67
	photosystem II protein Z [Halamphora americana]	4	100	10
	protein fucoxanthin chlorophyll a/c protein [Phaeodactylum tricornutum CCAP 1055/1]	7	100	18
	photosystem II 10 kDa phosphoprotein [Fistulifera solaris]	64	88	234
	rubisco operon transcription regulator [Craticula sp.]	71	100	396
	light-harvesting complex II chlorophyll a/b binding protein 4 [Fistulifera solaris]	4	100	14
	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Gomphonema affine]	1805	100	26673
	photosystem I P700 chlorophyll a apoprotein A2 [Phaeodactylum tricornutum]	32	100	375
	photosystem I subunit XI [Phaeodactylum tricornutum]	10	98	32
	CP43 chlorophyll apoprotein of photosystem II [Nitzschia anatoliensis]	220	100	3548
	Fucoxanthin-chlorophyll a-c binding protein E, chloroplastic [Phaeodactylum tricornutum]	6	100	14
	protein fucoxanthin chlorophyll a/c protein [Phaeodactylum tricornutum CCAP 1055/1]	5	100	14
	ferredoxin-nitrite reductase [Fistulifera solaris]	4	100	10
	Fucoxanthin-chlorophyll a-c binding protein E, chloroplastic [Phaeodactylum tricornutum]	109	100	577
	cytochrome b6 [Phaeodactylum tricornutum]	10	96	89
	subunit IV of cytochrome b6/f complex [Navicula tsukamotoi]	6	100	21
	apocytochrome f [Navicula arenaria]	4	100	10
	D1 reaction center protein of photosystem II [Naviculales sp.]	6007	100	84299
	ATP synthase CF1 alpha subunit [Halamphora calidilacuna]	6	100	20
	pyruvate-ferredoxin/flavodoxin oxidoreductase [Fistulifera solaris]	3	96	10
	photosystem II CP43 protein, partial [Nitzschia fonticola]	83	100	213
	magnesium chelatase subunit of protochlorophyllide reductase [Amicula sp.]	8	100	20
	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Phaeodactylum tricornutum]	296	100	4181
	protein fucoxanthin chlorophyll a/c protein [Phaeodactylum tricornutum CCAP 1055/1]	6	100	16
	photosystem I P700 apoprotein A1 [Navicula incertata]	38	100	850
	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial [Navicula salinarum f. minima]	18	100	111

	D1 reaction center protein of photosystem II [Naviculales sp.]	5482	100	119405
	ATP synthase CF0 subunit IV [Cocconeopsis kantsiensis]	10	94	58
	photosystem I P700 chlorophyll a apoprotein A1 [Phaeodactylum tricornutum]	170	100	5844
	magnesium chelatase subunit of protochlorophyllide reductase [Schizostauron trachyderma]	61	100	394
	ferredoxin [Halamphora sp.]	7	100	31
	apocytochrome f of cytochrome b6/f complex [Naviculales sp.]	6	100	23
Anaerobic Respiration	pyruvate-flavodoxin oxidoreductase (Fragment) [Seminavis robusta]	6	100	20
	pyruvate-flavodoxin oxidoreductase (Fragment) [Seminavis robusta]	12	100	88
	pyruvate-ferredoxin/flavodoxin oxidoreductase [Fistulifera solaris]	3	96	10
	NAD(P) transhydrogenase, mitochondrial-like [Pocillopora damicornis]	31	100	303

Thirty-seven distinct RNA transcripts that were related to a major metabolic process were classified as diatom in origin by kaiju (v1.10.1). Fifteen RNA contigs were identified that were relevant to photosynthesis related processes (Table 2). Major components of the photosystems including the D1 protein, chlorophyll reaction centers, and cytochrome b6f components are expressed. Rubisco subunit and regulation operons were also obtained from the assembled RNA transcripts. Twenty-eight enzymes from unique transcripts were classified as belonging to aerobic respiration, anaerobic respiration, fermentation, and sulfide oxidation (Table 5). NADH dehydrogenases and oxidoreductases are most of the products of the collected RNA expression. Succinate dehydrogenases, ATP synthase, and cytochrome oxidases were also products expressed by the diatoms. Anaerobic transcripts belong to pyruvate-flavodoxin or ferredoxin oxidoreductases. There was one transcript belonging to ferredoxin-nitrite reductase. Sulfide oxidation transcripts only contained sulfite oxidase.

Discussion

Genomic Identification and Phylogeny of Lake Fryxell's Diatoms

Two genomic methods were used to identify the diatoms at 10.1 m depth in Lake Fryxell, protein-based classification of contigs and classification of 18S rRNA found within the raw nanopore sequence dataset. This verifies that the diatom population is present, and with expression of photosynthetic and aerobic respiration related transcripts, they are active at 10.1 m. The Bacillariophyceae 18S rRNA results suggest that the mats may contain previously undiscovered species in Lake Fryxell. Specifically, based on the generally poor representation of diatom genomes in databases, it is most likely that the resulting contig classifications do not represent the true identity of these organisms nor species that reside in Lake Fryxell. Rather, the identified species are the closest matches from incomplete databases. Even though we discuss their prior identification in Antarctic environments, we do not propose that these organisms are native to Lake Fryxell. Rather, we emphasize the need for further genomic characterization and classification of Antarctic diatoms.

Concerning the protein-based classification, the only diatom without a species-specific classification was contig 10207, classified only as a Bacillariophyceae. It is likely that this is a novel organism. The two diatom sequences represented in the 18S rRNA data but could not be mapped to the unidentified contig. Other diatom species related to those from Lake Fryxell are known to reside in Antarctica even if none have been previously reported from the lake itself. The diatom *Pseudo-nitzschia australis* has not been identified in Lake Fryxell but can be found in Australia, New Zealand, and in the Southern Ocean (Flanders Marine Institute, 2025). The 18S rRNA sequence in the lake may not be the same genus. Although the second unknown sequence was similar to the database reference but is most likely a different genus, the *Amphora*

genus has been morphologically identified in the streams of the McMurdo Dry Valleys (Stanish et al., 2024) It is unknown in general how diatoms are deposited at 10.1 m and deeper into the lake so the likelihood of an *Amphora* diatom residing in Lake Fryxell is not unlikely.

To help refine the classification and evolutionary relatedness to known diatoms, phylogenetic trees were made using the Maximum likelihood model and Bayesian model. The trees used 120 diatom reference sequences and 3 outgroup sequences. Surprisingly, the unknown sequence did not group with any *Navicula gregaria* in either tree confirming, along with previous blast results, that this 18S rRNA extracted is most likely not from this organism or even closely related. It is unclear if this organism is the other cited diatom species *Humidophila contenta* as there were no sequences belonging to said organism within the used database. Both trees did produce large branch lengths, >2, for the unknown sequence, which was initially thought to be long branch attraction. To remedy this, all non-plasmid unique diatom 18S rRNA sequences were used, ~2504 sequences (Figure 3; Figure 4). As a result, there were shorter branches, 1.4 for the Maximum likelihood model and 0.9 for the Bayesian model, confirming that sample size is a determining factor. This signaled that this unknown sequence is potentially highly divergent from known diatoms rather than an artifact from long branch attraction. This could be confirmed if more 18S rRNA sequences were collected from diatoms in Lake Fryxell. Ideally, if the diatoms in the lake are highly divergent but still of the same community, they would group together within the phylogenetic tree. This would help determine if they are in a novel family or order of diatoms. Previous studies have shown that evolutionary divergence of diatoms can be driven by local environmental conditions rather than by isolation, distance between ponds, but it is unknown if this is true for Antarctic lakes (Taurozzi et al., 2026). So it could be that the diatoms at 10.1 m may be genetically similar at this depth but may vary

between the oxic and anoxic zones of Lake Fryxell. It would also be important to determine if diatoms are genetically diverse between lakes in the McMurdo Dry Valleys and the McMurdo Dry Valleys diatom diversity with other non-polar environments. Although diatom abundance may increase with latitude, species richness has been cited to decrease with latitude, but this does not consider diatoms found within the Antarctic continent stuck under a perennial ice cap (Olguín Salinas et al., 2015).

Ultimately searching for diatoms to establish pure cultures would be an ideal way of confirming the genetic identify with matching morphology. This combined approach would provide a better understanding of the distribution of diatoms in Lake Fryxell, the genomic adaptations present, and the morphological similarities to known organisms. Comprehensively understanding how these organisms have adjusted to the region and what roles they may play within the mats would provide ecological significance in how they may shape the mat environment.

Comparisons to Prior Studies

The genomic identification of two potential diatom species agrees on previous identification of only 2 morphotypes at 10 m water depth in Lake Fryxell (Jungblut et al., 2016). The previous species identified at this depth, *Diadesmis contenta* and *Navicula gregaria*, were not represented in our 18S rRNA sequence or contig assemblies. However, the methods used to identify said species before was based on morphological identification (Jungblut et al., 2016). Regionally in Antarctica, diatom populations have been morphologically identified in the pools, streams, moat, and soil associated with Lake Fryxell (Esposito et al., 2008; Stanish et al., 2024).

However, few of these environments have been identified using 18S rRNA, and metagenomics for addition into databases (Dillon et al., 2020; Jungblut et al., 2016; Wang et al., 2022).

Our results highlight a gap between morphological and genomic characterization, which can lead to misidentification if these two methods are not paired from pure cultures. The data reflects this as prior research has identified *Diademsis contenta* and *Navicula gregaria* as prominent species deep into the lake, neither of these organisms were taxonomically classified in our sample even though these 18S rRNA sequence may belong to these species. The current genomic catalogues of diatoms are still not robust enough to categorize the species, even though efforts are currently being made to add and refine them. It is also unknown if the DNA contigs belong to the same or multiple organisms. Diatom genomes can range from ~30 Mbp to 558 Mbp, and the collected data is not enough to assemble complete genomes (Suzuki et al., 2022; Jian et al., 2024). Despite this, the only way to increase our understanding of these organisms in Lake Fryxell and diatoms in general is to isolate and classify these novel organisms with 18S rRNA and morphologically, while utilizing metagenomes to understand metabolic capacity.

Taxonomic classification of nanopore long read assemblies did identify ten different species, but none of the classifications match previously observed morphological classifications from Lake Fryxell. The 18S rRNA sequencing data provides a better picture of what organisms are present. Although one sequence was identified as 77% similar to *Pseudo-nitzschia australis*, it is not expected that this is the species present as percent. It is most likely that the Fryxell organism is a species that has yet to be added to the Protist Ribosomal Reference Database. There we no certainties collected from this data other than actively highlighting the need for cooperation between morphological and genomic classification, especially when the diatoms are

known to be present in the community and their ecological importance and potential adaptations to this extreme environment are unknown.

Active Diatom Metabolisms at 10.1 m in Lake Fryxell

Results show that diatoms are metabolically active in this cold, low light, sulfidic environment. Despite PAR being only $\sim 3 \mu\text{M photo m}^{-2} \text{ s}^{-1}$ and oxygen at a maximum of 4 mg/L, annotated transcripts indicated diatoms were producing major components of the photosynthetic pathway, aerobic respiration, anaerobic respiration, sulfite reduction, and sulfide oxidation. This may provide oxygen in a microenvironment for neighboring aerobic respirators or be utilized by the diatoms themselves. The transcription of rubisco subunits and the rubisco operon transcription regulator indicate active carbon fixation. Previous studies have stated that diatom populations increase deeper into the lake while Cyanobacteria populations decrease (Jungblut et al., 2016). It is most likely that diatoms become top contributors to oxygen production and primary productivity for biomass production at 10.1 m and below as cyanobacterial populations dwindle due to low-light and sulfide inhibition. Interestingly this oxygenic activity may have contributed to the unexpected microbial metabolic diversity recorded in the general taxonomic analysis in Chapter 1 and Chapter 3.

Since diatoms are capable of both photosynthesis as well as aerobic respiration, it was no surprise to find aerobic respiration related transcripts. The NADH dehydrogenases, ATP synthase, and succinate dehydrogenase indicate some aspects of the respiratory activity are occurring. Pyruvate-flavodoxin oxidoreductase is also present which would help in the production of acetyl-co enzyme A. Although the TCA cycle is incomplete based on our

recovered transcripts, combined with DO data, and presence of oxygenic photosynthesis transcripts, there may be enough evidence to suggest that the diatoms were performing aerobic respiration, potentially using the oxygen produced and or available in the mat.

Overall diatoms are prevalent at 10.1 m and novel and new species are likely present (Jungblut et al., 2016; Dillon et al., 2020). Even though the cyanobacterial population decreases, diatoms are still capable of conducting oxygenic photosynthesis and contributing to primary productivity. Diatom utilization of sulfite reductase produces sulfide which can be utilized to stimulate nitrate reduction (Merz et al., 2022). This creates a unique niche for the diatoms in the lake as contributors to the production of oxygen at 10.1 m by photosynthesis. They also benefit from their production of oxygen by utilizing the oxygen produced for aerobic respiration and the resulting carbon dioxide produced for carbon fixation (Sethi et al., 2020; Hopkinson et al., 2011). The oxygen they produce can also be used by other microbial communities for aerobic metabolisms like but not limited to, aerobic respiration, nitrification, or sulfide oxidation. Diatom produced oxygen also contributes to the abiotic conversion of sulfide into sulfate, providing the necessary component for sulfate reducers.

Conclusion

Lake Fryxell's sulfidic, low-light, and microenvironments varying in DO provide an opportunity to discover how diatoms that have adapted to anoxic low-light sulfidic conditions. Our results have discovered organisms that have not been previously described in Lake Fryxell. The two unknown 18S rRNA results and protein classification of long read sequences showcase the importance of building diatom genomic databases and that Lake Fryxell harbors previously undiscovered diatoms. It was also suggested that the two sequences could belong to *Diadesmis contenta* or *Navicula gregaria* based on previous observations. Although the assemble contigs,

classified by kaiju (v1.10.1), provided taxonomic results, the returned diatoms are most likely not to be present in Lake Fryxell. Still, the contigs did provide a reference for data collected in the future. Further investigation revealed that diatoms in Lake Fryxell at 10.1 m are actively transcribing photosynthesis related components and fixing carbon with rubisco, establishing the diatom community as active primary producers. Other transcripts indicated that the diatoms are involved in aerobic and anaerobic respiration processes, displaying the potential adaptability between oxic/anoxic microenvironments within the microbial mat. Overall, this work serves as an initial study into determining the taxonomic diversity and metabolic contributions of diatoms at 10.1 m depth in Lake Fryxell.

Chapter 3

Constructing a Metabolic Framework from HCLC MAGs as a Model for Lake Fryxell during Summer Months

Introduction

Microbial metabolic strategies are defined by the redox pairs in their environment; the overall redox state is important for determining how much energy they can extract from reactions (Varnam et al., 2018). Oxygen substantially increases the redox state of an environment (Gillespie L., 1920). As oxygen decreases, the redox state becomes increasingly negative, establishing a reductive environment favoring iron and sulfate reducers as well as methane and hydrogen producers (Zhu et al., 2014). The perennially ice-covered lakes in Antarctica have chemically stratified environments due to their ice covers and contain distinct oxic-anoxic zones, that can determine what electron acceptors may be available (Vincent et al., 1981; Angino et al., 1962; Lawrence et al., 1985; Karr et al., 2006; Aiken et al., 1996). In general, shallower depths are oxic due to more available PAR. As a result, oxygenic photosynthesizers produce oxygen which can abiotically remove strong reductants and increase the redox potential of the environment. Lake Fryxell at 10.1m does contain a small amount of oxygen compared to shallower depths (Chapter 1) and oxygenic photosynthesizers are active. The oxygen within the mat during the summer can be utilized by local microbes for aerobic respiration and is supported by the aerobic respiration and nitrification transcripts present (Chapter 1). Although this is occurring, it is unclear if the decrease in Cyanobacteria abundance and oxygen production affect other microbial metabolisms. 10.1 m has not been investigated to determine what metabolism are active and what microbial communities are expressing them. Understanding how the metabolic diversity at 10.1 m can provide insight into how microbial communities in Antarctic extreme environments respond to low redox potential environments that may still contain oxygen. Ideally

this research produces a framework for the summer metabolisms at this depth and constructs a reference point for how metabolisms may change at different depths and during the winter in Lake Fryxell. Previous results in chapter one show that oxygenic photosynthesis transcripts were present at 10.1 m when sampled, as well as anaerobic transcripts. However, the mat at 10.1 m may not be completely oxygenated. Thus, microbial activity suggests simultaneous aerobic environments with oxygen available for aerobic processes, and anoxic environments that favor anaerobic metabolisms. With support from the transcriptional data, the metabolisms that are active in an environment could suggest that despite the presence of strong reductants, an oxygenated reductive environment exists at 10.1 m in Lake Fryxell. This type of environment provides, due to microbial processes, a wide variety of aerobic and anaerobic electron acceptors. During the summer, oxygen is present which not only can be used as a electron acceptor but is also used to generate anaerobic electron acceptors. For example, nitrate, nitrite, and sulfate are present in the water column (Figure 1; Figure 2) and can be used as electron acceptors, seemingly in both the presence and absence of oxygen (Chapter 1). During the winter, oxygenic photosynthesis stops, eliminating oxygen production. Since the water overlying the mats at 10.1 m depth lacks oxygen (Chapter 1 Figure 1), it is reasonable that the mats would become completely anoxic over winter, severely dropping the redox state and limiting the communities to anaerobic metabolisms (Figure 3). In addition, the lack of oxygen except what is produced locally in microenvironments suggests that the whole redox state of the mat in general at 10.1 m is low in both summer and winter. This makes it more likely that anaerobic metabolisms are more widely used by bacterial communities than aerobic processes.

In this chapter, using 1) the microbial phyla recovered in the high completion low contamination bins, which represent a sample of the organism diversity present and 2)

metatranscriptomic data that displays a wide range of metabolic strategies, I aim to determine if anaerobic metabolisms are highly prevalent in the microbial community of the high completion low contamination (HCLC) MAGs by phylum. The high completion, low contamination bins provide an excellent reference to determine what critical enzymes for energy producing metabolisms were actively transcribed during summer sampling. In addition, they provide insights into whether the species represented by the HCLC MAGs rely on one metabolism or utilize multiple different strategies at once. At 10.1 m depth in Lake Fryxell, it is most likely the bacterial species that make up these phyla may display a wide distribution in the strategies rather than specialization in one or two methods of energy production.

Cycles of Interest: Nitrogen and Sulfur Chemistry of Lake Fryxell

During the summer, the active oxygenic photosynthesizers within the microbial mat provide oxygen for other metabolic processes (Chapters 1 and 2). Oxygen is needed for aerobic respiration and the oxygen supplied by photosynthesis assists aerobic bacteria and microeukaryotes. The oxygen produced also contributes to the sulfur cycle. Sulfide can be abiotically and biotically oxidized to sulfate by oxygen, which also benefits sulfate reducing bacteria utilize for energy production. Some heterotrophic bacteria able to perform sulfate reduction can also perform sulfide oxidation under aerobic conditions (Xia et al., 2017), which raises the possibility that bacteria at 10.1 m in Lake Fryxell might perform both processes. If so, it could be reflected in the collected transcripts attributed to the high completion genomes to determine what metabolisms are occurring at the taxon level. The nitrogen cycle also benefits from oxygen production. Nitrification, the conversion of ammonium to nitrate, requires oxygen and would provide nitrate for denitrification. With the production of DO, aerobic respiration

becomes feasible for other bacterial communities as well as microeukaryotes while their byproducts provide carbon dioxide for autotrophic carbon fixation.

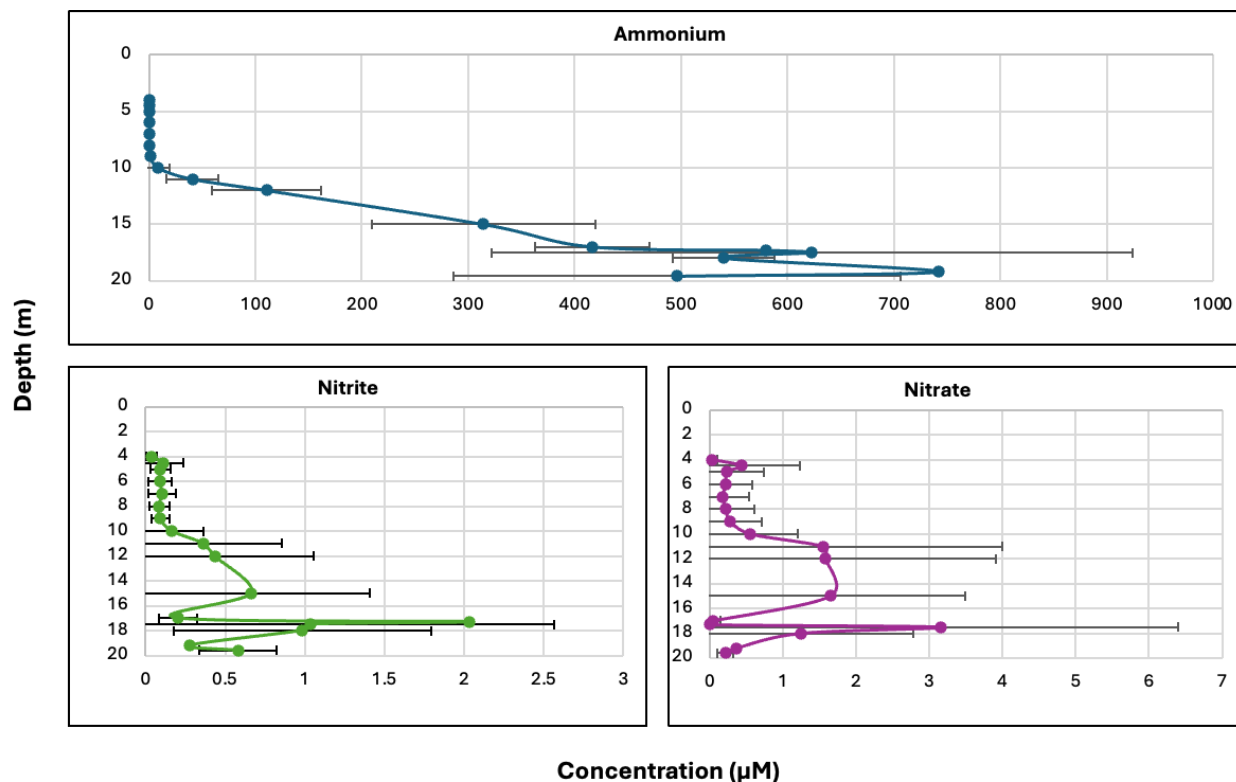


Figure 1: Averages of ammonium, nitrate, and nitrite concentrations, 2016 - 2026, collected from the Water Column near the Center of the Lake Fryxell from McMurdo LTER (Priscu et al., 2020) Error bars represent one standard deviation of collected samples per depth ranging from sample sizes of 2-49.

Photosynthetically active radiation (PAR), oxygen, nitrogen-containing molecules, phosphorus, sulfur-containing molecules, and organic carbon determine what processes are available to these microbial communities. PAR at 8 m vary from 0.083 - 6.14 photons/m²/s during the summer and is 0 photons/m²/s during the winter (McMurdo LTER 2025) oxygen at 10.1 m ranged from 0 mg/L to ~ 4.8 mg/L (Chapter 1). Nitrate, nitrite, and ammonium concentrations vary as a function of water depth (Figure 1). Nitrate concentrations in the water

are an average of 0.74, at ~10 m, increasing with depth (Figure 1; McMurdo LTER 2019). Nitrite at 10 m averages 0.15 μM and similarly nitrate increases into the lake (Figure 1; McMurdo LTER 2019). Lastly, ammonium averages a concentration of 2.42 μM at 10 m (Figure 1; McMurdo LTER 2019).

The availability of sulfur is important to microbial sulfide oxidation and sulfate reduction metabolisms. Sulfate is abundant at 10.1 m in the water column, with a mean concentration of 1.36 mM (Figure 2; McMurdo LTER 2019). Sulfide is generally absent in the water column above 9 m but gradually increases to 16.8 μM at 10.1 m and to over 1.2 mM at 18 m (Karr et al., 2006; Lumian et al., 2021). Based on the environmental data collected from the past ten years, Figure 2, and the oxygen, PAR, and transcriptomic data in Chapter 1, it is suspected that sulfate reduction and sulfide oxidation is mainly used by the organisms in samples from 10.1 m depths (Chapter 1).

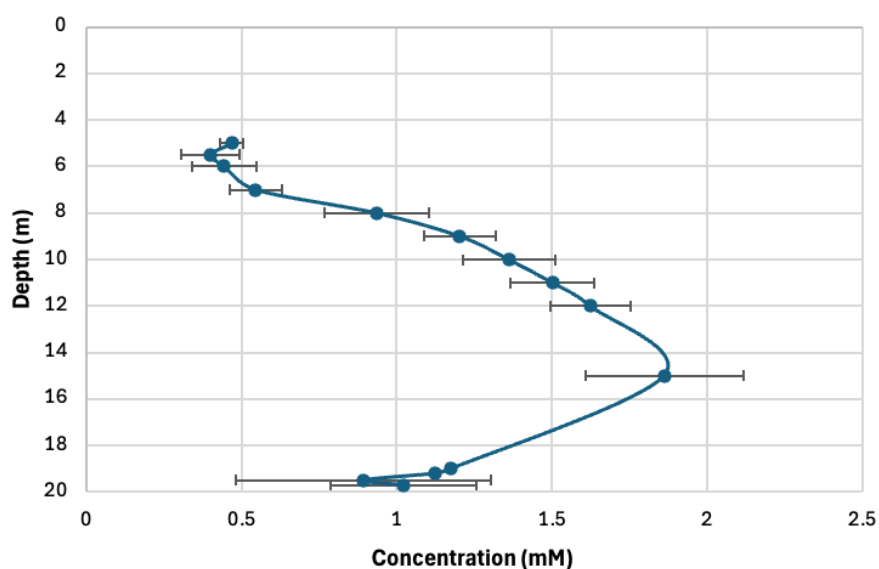


Figure 2: Sulfate Concentrations from 2016 - 2026, Collected from the Water Column near the Center of the Lake Fryxell from McMurdo LTER (Priscu 2020.) Error bars represent one standard deviation of collected samples per depth ranging from sample sizes of 3-8 based on available public data.

Metabolic Diversity of Lake Fryxell's Microbial Mats

The 16s rRNA analysis performed in chapter one indicated the presence of 700 - 900 unique species at 10.1 m from 18 phyla (Chapter 1). It is still unclear what metabolic pathways the organisms were performing. If trends remain consistent with previous research, the metabolic diversity would be structured by the availability of PAR and concentration oxygen (Dillon et al., 2020b). At 9.8 m, Cyanobacteria were over 50% of the surface mat microbial composition, and they were interpreted as producing oxygen near the surface of the mat (Dillon et al., 2020a). The production of oxygen would locally produce a positive redox environment expanding the available terminal electron acceptors. This prediction is consistent with the metabolic diversity at 9.8 m, which indicated a wider range of metabolic strategies in the top layers of the mat and decreasing diversity with depth into it. This suggests that the presence of oxygen, in this case,

due to oxygenic photosynthesis, increased the metabolic diversity (Dillon et al., 2020b). At 10.1 m, Cyanobacteria made up less than three percent of the 16S rRNA classified (Chapter 1). With light and oxygen lower in the mats at 10.1 m than shallower depths, metabolic strategies might be less diverse, since potential oxygen limitation reduces aerobic capacity, and preferential for anaerobic metabolisms like fermentation, sulfate reduction, anaerobic sulfide oxidation, anaerobic ammonium oxidation (anammox), dissimilatory nitrate reduction to ammonium (DNRA), and denitrification. Aerobic respiration transcripts were recovered from the samples analyzed here, as were those for the anaerobic metabolisms (Chapter 1). This chapter presents analyses of transcript data that characterize which phyla were active and what metabolisms they rely on for energy production. Results are used to build a metabolic model of the community in summer and predict metabolic changes over winter and with increasing depth in the lake.

Methods

Oxidation Reduction Potential Measurements

To characterize the redox state of water near the mat surface, oxidation-reduction potential (ORP) was measured using an Atlas lab-grade OPR probe and EZO circuit (Long Island City, NY) controlled by a Mayfly Data Logger from the Stroud Water Research Center (Avondale, PA) using custom Arduino-based software. The probe was deployed by a diver at ~10.2 m depth with the tip ~1 cm above the mat surface. It logged ORP every 4 hours starting from January 20th, 2025 0:00 until the system stopped on October 26th, 2025, at 16:00. The ORP data were collected two years after collection of the mat samples analyzed here and at a slightly different depth. Thus, results are not quantitatively applicable to the transcriptomic results.

Sample Collection and Sequencing

Sample collection was as described in Chapter 1.

RNA Extraction

The RNA extractions at SeqCenter used the ZymoBIOMICSTM Quick-RNA Miniprep Kit (Zymo Research, Irvine, CA). The preparation of microbial mat samples followed Zymo Research recommendations stated in the protocol. Samples were eluted into a final volume of 40 μ l. Final RNA concentrations were determined via Qubit3 from (Thermo Fisher Scientific Waltham, MA).

RNA Sequencing

The extracted samples were DNase treated with Invitrogen RNase free DNase (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation was performed using Illumina's Stranded Total RNA Prep Ligation (Illumina, San Diego, CA, USA) with Ribo-Sero Plus kit (Illumina, San Diego, CA, USA) and 10bp unique dual indices (UDI). Sequencing was done on a NovaSeq XPlus (Illumina, San Diego, CA), producing paired end 150bp reads. Demultiplexing, quality control, and adapter trimming was performed with Illumina's bcl-convert (v4.2.4; Illumina, San Diego, CA, USA).

DNA Extraction

The DNA extraction at followed the ZymoBIOMICS™ DNA Miniprep Kit2 (Zymo Research, Irvin, CA, USA). The sample was suspended in lysis solution and transferred into the ZR BashingBead™ Lysis Tubes (Zymo Research, Irvin, CA, USA) and mechanically lysed using the MP FastPrep-24™ (MP Biomedicals, Irvine, CA, USA) lysis system with 1 minute of lysis at maximum speed and 5 minutes of rest for 5 cycles. The samples were then centrifuged at 10,000 rcf for 1 minute. The volume of 400 µL of supernatant was transferred from the ZR BashingBead™ (Zymo Research, Irvin, CA, USA) Lysis Tube to a Zymo-Spin™ III-F Filter (Zymo Research, Irvin, CA, USA) and centrifuged at 8,000rcf for 1 minute. 1,200 µl of ZymoBIOMICS™ DNA Binding Buffer (Zymo Research, Irvin, CA, USA) was added to the effluent and mixed via pipetting. Next, 800µl of the solution was transferred to a Zymo-Spin™ IICR Column (Zymo Research, Irvin, CA, USA) and centrifuged at 10,000 rcf for 1 minute. This step was repeated until all material was loaded onto the Zymo-Spin™ IICR Column. DNA bound to the Zymo-Spin™ IICR Column was washed 3 times with 400µl and 700µl of ZymoBIOMICS™ DNA Wash Buffer (Zymo Research, Irvin, CA, USA)1 and then 200 µl of ZymoBIOMICS™ DNA Wash Buffer 2 (Zymo Research, Irvin, CA, USA) with a 1-minute spin down at 10,000 rcf for each, respectively. Washed DNA was eluted using 75µl of ZymoBIOMICS™ DNase/RNase Free Water (Zymo Research, Irvin, CA, USA) following a 5-minute incubation at room temperature and a 1-minute spin down at 10,000 rcf. The Zymo-Spin™ III-HRC Filter (Zymo Research, Irvin, CA, USA) was prepared using 600 µl of the ZymoBIOMICS™ HRC Prep Solution (Zymo Research, Irvin, CA, USA) and a centrifugation at 8,000 rcf for 3 minutes. Eluted DNA was then purified by running the effluent through the

prepared Zymo-Spin™ III-HRC Filter. Final DNA concentration was determined via Qubit (Thermo Fisher Scientific, Waltham, MA, USA).

Bioinformatic Analysis

Annotation of HCLC MAG Metabolic Strategies

DNA sequenced and quality controlled were used for assembly with flye using --meta settings (v2.9.4-b1799) and checked for assembly quality with Quast (v5.2.0) using standard settings (Kolmogorov et al. 2020, Mikheenko et al. 2023). The resulting assembly was then binned and filtered for completion with of 90% or higher and contamination lower than five percent and taxonomically classified according to methods described in Chapter 1. Taxonomic classifications were matched with their respective bins and grouped by phylum. The bin specific .fa files were then used as references for mapping assembled RNA transcripts with minimap2 (v2.28-r1209) to each MAG (Grabherr et al., 2011; Li et al., 2018). Successfully mapped reads were extracted, and protein coding sequences were identified and translated into amino acid sequences with prodigal (v2.6.3; Hyatt et al., 2010). Amino acid sequences were annotated with KOfamScan (v1.3.0) and identifications were filtered for matches with an E-value less than e^{-10} (Aramaki et al., 2019). The annotated .tsv files were searched for transcripts involved in aerobic respiration, fermentation, nitrification, denitrification, dissimilatory reduction of nitrate to ammonium (DRNA), nitrogen fixation, anammox, dissimilatory sulfate reduction, sulfur oxidation, lipid beta oxidation, the Wood–Ljungdahl Pathway, and the Calvin Cycle using KEGGDecoder (v1.2.2; Graham et al., 2018; Table 1). Collected transcripts were normalized by

length and depth and quantified by transcript per million with salmon (v1.10.3) using standard settings (Patro et al., 2017).

Generation of Metabolic Pathway Presence/Absence Heat Map

A heatmap was generated to highlight the presence or absence of genes and transcripts necessary for the metabolisms of interest. The criterium for this presence/absence heat map is based on enzymes that were critical for metabolic pathways to occur whether that be a single enzyme or multiple (Table 1), following an approach utilized by Du et al. (2023). Certain enzymes deemed critical to all listed pathways were only considered present if all subunits or chains were present in transcript annotations. Justification for the importance of the enzyme is provided (Table 1). The score distribution was divided evenly among transcripts classified as a critical enzymatic product and if needed, subdivided depending on importance of individual chains or subunits. A complete pathway results in a score of one, as mentioned in Table one, while incomplete pathways will be less than one but greater than zero.

Aerobic respiration was determined to be present with the expression of cytochrome c oxidase cbb3-type subunit I/II or cytochrome bd ubiquinol oxidase subunit I. Both are terminal electron acceptors specific to aerobic respiration in low oxygen environments (Hamada et al., 2014; Grund et al., 2021). Fermentation was considered present if pyruvate formate lyase activating enzyme, D-lactate dehydrogenase, phosphate acetyltransferase, acetate kinase, and alcohol dehydrogenase were present. Nitrification required ammonia monooxygenase with hydroxylamine reductase and both nitrite oxidoreductase alpha and beta units. Denitrification was considered complete if nitrate reductase (cytochromic), nitric oxide reductase FIRD-NAD(+) reductase, or periplasmic nitrate reductase, nitrite reductase (NO-forming), and nitric oxide

reductase B and C subunits or anaerobic nitric oxide reductase flavorubredoxin. DRNA was defined by the detection of the cytochrome c nitrite reductase small subunit. Nitrogen fixation was considered complete when nitrogenase iron protein, *nifH*, and the alpha and beta chains of nitrogenase molybdenum-iron protein were present. Anammox was considered complete if the hydrazine synthase was expressed along with hydrazine dehydrogenase. Dissimilatory sulfate reduction required sulfate adenylyltransferase subunit I, adenylylsulfate reductase subunit A and B, dissimilatory sulfite reductase alpha and beta subunit, and dissimilatory sulfite reductase flavoprotein beta subunit. Sulfide oxidation was complete if sulfide:quinone oxidoreductase or sulfide dehydrogenase [flavocytochrome c] flavoprotein chain were transcribed. The WLP required the presence of both anaerobic carbon-monoxide dehydrogenase and acetyl-CoA synthase to be considered fully complete. The epsilon subunit for the WLP was present in the initial KOfamScan results but did not meet the E-value requirements post filtering. The alpha subunit was used as the judgement criteria as it is crucial for enzyme function. The Calvin Cycle was determined to be defined as both the ribulose-bisphosphate carboxylase large chain and phosphoribulokinase present. Lipid oxidation was considered present with the identification of enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, acetyl-CoA acyltransferase, and 3-hydroxybutyryl-CoA dehydrogenase. With respect to these criteria, a heat map was generated using python (v3.10.8) and matplotlib.pyplot based on the scoring of critical components present for each process.

Transcript Quantification of Expression

The successfully mapped metabolic transcripts for all taxa within a phylum were normalized for read count and length and quantified for expression in transcripts per million

(TPM) with salmon (v1.10.3; Patro et al., 2017). Quantified expression data of metabolic pathways as a percentage of TPM expressed per phyla was plotted using matplotlib.pyplot.

Table 1: Grading Criteria for Assessment of Metabolic Pathway Completion. Grading was based on enzymes critical to the metabolic process to determine presence or absence of the pathway. Incomplete scores were still recorded and presented in analysis.

Total score of 1 = pathway present, < 1 = pathway not complete

Metabolic Pathway	Enzyme	Score Contribution	Justification
Aerobic Respiration	cytochrome c oxidase cbb3-type subunit I/II	1	Terminal oxidase, supports respiration under low oxygen, associated with microaerobic conditions (Hamada et al., 2014)
	cytochrome bd ubiquinol oxidase subunit I		Terminal oxidase, widely used in bacteria under low oxygen conditions (Borisov et al., 2011)
Fermentation	pyruvate formate lyase activating enzyme	0.2	Central to anaerobic metabolism, converting pyruvate into acetyl-CoA and formate (Crain et al., 2013)
	D-lactate dehydrogenase	0.2	Performs redox-balancing step, reoxidizing NADH and producing lactate (Kasai et al., 2019)
	phosphate acetyltransferase	0.2	ATP generating step working with acetate kinase (Wolfe., 2005)
	acetate kinase	0.2	ATP generating step working with phosphate acetyltransferase (Puri et al., 2014)
	alcohol dehydrogenase (NADP+)	0.2	Terminal reductase, producing ethanol from acetaldehyde (Korkhin et al., 1998)
Nitrification	ammonia monooxygenase A	0.083	First step of nitrification converting ammonia to hydroxylamine (Hodgskiss et al., 2023)
	ammonia monooxygenase B	0.083	First step of nitrification converting ammonia to hydroxylamine (Hodgskiss et al., 2023)
	ammonia monooxygenase C	0.083	First step of nitrification converting ammonia to hydroxylamine (Hodgskiss et al., 2023)
	hydroxylamine reductase	0.25	Central step, signals strong support when paired with amoABC (Soler-Jofra et al., 2021)
	nitrite oxidoreductase, alpha	0.25	Final step in producing nitrate for bioavailability (Daimes et al., 2016)
	nitrite oxidoreductase, beta	0.25	Final step in producing nitrate for bioavailability (Daimes et al., 2016)
Denitrification	nitrate reductase (cytochrome)	0.5	Initiates nitrate reduction as a primary starting point for the pathway (Duran et al., 2021)
	periplasmic nitrate reductase		Catalyzes nitrate reduction in the periplasm in low oxygen conditions (Sparacino-Watkins et al., 2015)
	nitrite reductase (NO-forming)	0.25	Key to reducing nitrite to nitric oxide (Braker et al., 2000)
	nitric oxide reductase subunit B	0.125	Catalytic core of nitric oxide reductase producing nitrogen gas (Kumita et al., 2004)
	nitric oxide reductase subunit C	0.125	Catalytic core of nitric oxide reductase producing nitrogen gas (Kumita et al., 2004)
	anaerobic nitric oxide reductase flavorubredoxin	0.25	Detoxifies nitric oxide under anoxic conditions (Gardner et al., 2002)
DNRA	cytochrome c nitrite reductase small subunit	1	Terminal step is DNRA process forming ammonium (Campeciño et al., 2020)
Nitrogen Fixation	nitrogenase iron protein NifH	0.5	One of the two component proteins widely used as a functional marker (Jiang et al., 2021)
	nitrogenase molybdenum-iron protein alpha chain	0.25	Contains the active site cofactors (Solomon et al., 2024)
	nitrogenase molybdenum-iron protein beta chain	0.25	Contains the active site cofactors (Solomon et al., 2024)

Anaerobic Ammonium Oxidation (anammox)	hydrazine synthase subunit	0.5	Produces hydrazine intermediate, distinct for anammox bacteria (Hägglund et al., 2025)
	hydrazine dehydrogenase	0.5	Oxidizes hydrazine toward nitrogen gas (Maalcke et al., 2016)
Dissimilatory Sulfate Reduction	sulfate adenylyltransferase subunit 1	0.25	Sat activates sulfate to APS, a required step in sulfate reduction (Carlson et al., 2025)
	adenylylsulfate reductase, subunit A	0.125	Core energy-metabolism step (Diao et al., 2024)
	adenylylsulfate reductase, subunit B	0.125	Core energy-metabolism step Diao et al., 2024)
	dissimilatory sulfite reductase flavoprotein subunit	0.25	Key enzyme in reducing sulfite to sulfide (Diao et al., 2024)
	dissimilatory sulfite reductase alpha subunit	0.125	Key enzyme in reducing sulfite to sulfide (Diao et al., 2024)
	dissimilatory sulfite reductase beta subunit	0.125	Key enzyme in reducing sulfite to sulfide (Diao et al., 2024)
Sulfur Oxidation	sulfide:quinone oxidoreductase	1	Considered first step into sulfide oxidation, used widely as an oxidation marker (Cherney et al., 2012)
	sulfide dehydrogenase [flavocytochrome c] flavoprotein chain		Central enzyme in sulfur-oxidizing bacteria, transferring electrons to cytochrome c (Tikhonova et al., 2021)
Wood-Ljungdahl Pathway	anaerobic carbon-monoxide dehydrogenase, subunit alpha	0.5	Core defining enzyme of the WLP (Ragsdale et al., 2009)
	acetyl-CoA synthase	0.5 (if codH/ac present)	Carbon fixing step of WLP (Ragsdale et al., 2009)
Calvin Cycle	ribulose-bisphosphate carboxylase large chain	0.5	Catalyzes the carbon dioxide fixing step, entry of inorganic carbon (Meloni et al., 2023)
	phosphoribulokinase	0.5	Regenerates ribulose-1,5bisphosphate, carbon dioxide acceptor, essential for sustained operation (Meloni et al., 2023)
Lipid β Oxidation	Enoyl-CoA hydratase	0.25	Hydration and oxidation steps of fatty-acid oxidation (Agnihotri et al., 2003)
	3-hydroxyacyl-CoA dehydrogenase	0.25	Hydration and oxidation steps of fatty-acid oxidation (Schulz et al., 2020)
	Acetyl-CoA acyltransferase	0.25	Thiolytic cleavage producing acetyl-CoA (Volp., 2018)
	3-hydroxybutyryl-CoA dehydrogenase	0.25	Gatekeeps energy generation through interconversion of acetoacetyl-CoA and 3-hydroxybutyryl-CoA (Liu Li et al., 2021)

Results

Photoactive Radiation and Oxidation Reduction Potential above the Microbial Mat at 10.2 m

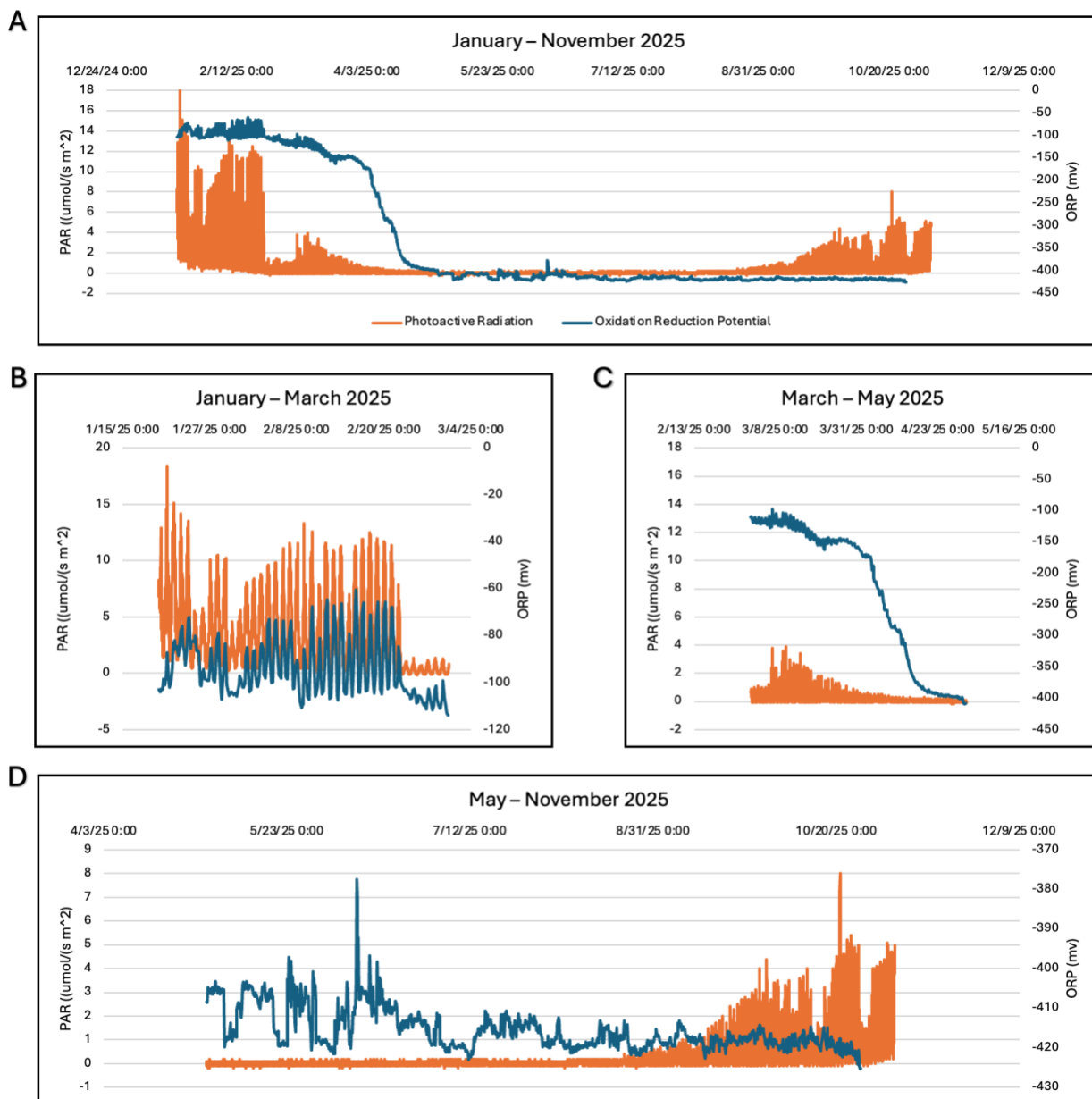


Figure 3: PAR and ORP of water above microbial mats at 10.2 m. Panel A is the complete recording from January to November in 2025. Panel B displays the PAR and ORP values from January to March. Panel C shows the ORP values from March to May and Panel D shows ORP values from May to November. Local solar noon is equivalent to 02:00:00.

The PAR ORP collected at 10.2 m in Lake Fryxell displayed changing values because of seasonal and daily change in the environment. PAR decreased from January to March from a maximum of 17.5 $\mu\text{mol}/(\text{s m}^2)$ to 0 $\mu\text{mol}/(\text{s m}^2)$ while displaying repeating oscillating cycles. PAR remained below 0.1 $\mu\text{mol}/(\text{s m}^2)$ until August and increased into the spring reaching up to 8 $\mu\text{mol}/(\text{s m}^2)$. The reduction potential measured from December 2024 to October 2025 was consistently reductive and ranged from a maximum of -60.5 mV in February to a minimum of -425.4 mV in late October (Figure 2A). ORP values in February show daily undulating patterns in between -60.5 mV and -111 mV (Figure 2B). As the fall season progressed and light declined, a decrease in ORP became apparent at the end of February at -110 mV and further decreased to -200 mV in April when no PAR was present in Winter (Figure 2C). ORP finally settled to a -400 mV to -425.4 mV range with one large ORP spike to -377.4 mV in June (Figure 2D). The ORP also remained unchanged from mid-August into October as PAR increased into the spring.

Completion of Metabolic Pathways Mapped to High Completion Low Contamination MAGs

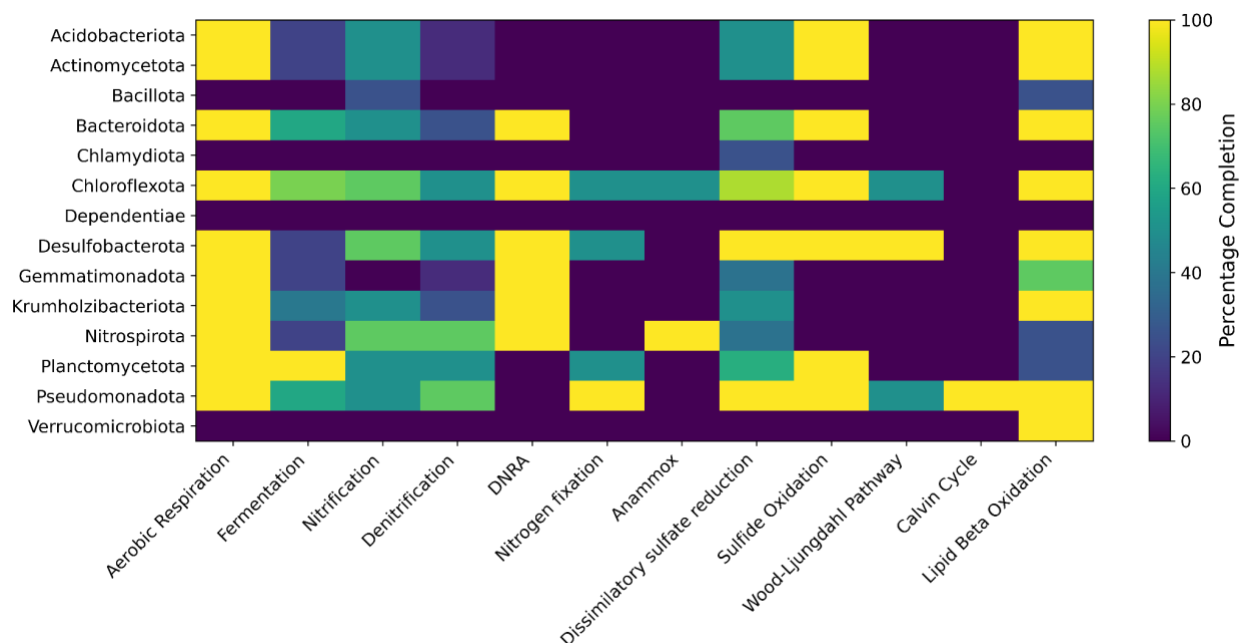


Figure 3: Heatmap visualizing the percent completion of expressed metabolic pathways in HCLC bins collected from Lake Fryxell at 10.1 m in summer 2024. The most complete pathways across phyla were aerobic respiration, DNRA, sulfide oxidation, and lipid beta oxidation.

Transcripts mapped to the MAGs within 11 of the 14 phyla contained at least three complete metabolic pathways for each phylum, with a maximum of 6 for MAGs within Desulfobacterota and Pseudomonadota (Figure 2). In contrast, Bacillota and Chlamydiota only had low completion pathways represented in the transcripts, and Dependentiae lacked any transcripts for the metabolisms investigated. Transcripts mapped to Verrucomicrobiota represented only a single, complete metabolic pathway, lipid oxidation.

Transcripts associated with complete aerobic respiration pathways were present in all MAGs within the present phyla except for Bacillota, Chlamydiota, Dependentiae, and Verrucomicrobiota. Fermentation varied in percent pathway completion, only reaching 100% completion in Planctomycetota. The next highest completion was 80% in Chloroflexota. Bacillota,

Chlamydoita, Dependientiae, and Verrucomicrobiota did not contain any mapped fermentation related transcripts.

The nitrogen cycle consisted of five different pathways: Nitrification, Denitrification, Dissimilatory Nitrate Reduction to Ammonium, Nitrogen Fixation, and Anammox. There were no MAGs in within any phyla that contained a complete nitrification pathway. Nitrification was present in nine phyla. Nitrospirota, Chloroflexota, and Desulfobacterota were at 75% completion, Acidobacteriota, Actinomycetota, Bacteroidota, Krumholzibacteriota, Planctomycetota, and Pseudomonadota were 50% complete, and Bacillota only displayed 25% completion of this pathway.

Denitrification was expressed by MAGs classified withing ten phyla. Pseudomonadota and Nitrospirota expressed 75% of the required enzymes. Chloroflexota, Planctomycetota and Desulfobacterota followed at 50%. Bacteroidota and Krumholzibacteriota had 25% complete pathways. Acidobacteriota, Actinomycetota and Gemmatimonadota only reached 12.5% completion.

The DNRA pathway was fully expressed in Bacteroidota, Chloroflexota, Desulfobacterota, Gemmatimonadota, Krumholzibacteriota, and Nitrospirota and absent from all other phyla.

Nitrogen fixation was only fully expressed in one phylum, Pseudomonadota. Chloroflexota, and Desulfobacterota expressed 50% of the required enzymes. All remaining phyla did not express any of the required key enzymes.

Anaerobic ammonium oxidation (anammox) was only expressed in two phyla. It was fully complete in Nitrospirota and only 50% complete in Chloroflexota.

Dissimilatory sulfate reduction was completely expressed in Pseudomonadota and Desulfobacterota. This pathway was expressed above 50% completion in Acidobacteriota, Actinomycetota, Bacteroidota, Chloroflexota, and Planctomycetota. Krumholzibacteriota MAGs contained 50%. Chlamydoita, Gemmatimonadota, and Nitrospirota contained less than to 50% of the necessary transcripts. This pathway was not expressed in Bacillota, Dependitiae, and Verrucomicrobiota. Dissimilatory sulfide oxidation was completely expressed in seven phyla, Acidobacteriota, Actinomycetota, Bacteroidota, Chloroflexota, Desulfobacterota, Planctomycetota, and Pseudomonadota. Sulfide oxidation was not expressed in any other phyla. Assimilatory sulfide oxidation, phosphoadenosine phosphosulfate was expressed in the total RNA sample indicating both processes are occurring within the mat. Assimilatory sulfite reductase was not expressed, only dissimilatory.

Autotrophic pathways were limited to the Wood Ljungdahl Pathway and the Calvin Cycle. Desulfobacterota expressed both enzymes required for completion of the WLP. Pseudomonadota and Chloroflexota both only expressed carbon-monoxide dehydrogenase, resulting in a 50% completion. The Calvin Cycle was only expressed in Pseudomonadota as *cbbL*. There were no *rbcL* transcripts found in the annotated metatranscriptomic sample.

Fatty acid beta oxidation was expressed in all phyla except Chlamydoita and Dependitiae. The only incomplete pathways were found in Gemmatimonadota at 75%, Nitrospirota at 25%, and Planctomycetota at 25%. Interestingly, fatty acid oxidation was the only pathway recorded for Verrucomicrobiota, the only phylum with 100% completion.

Metabolic Composition of HLCL Phyla expressed as Percent Transcripts per Million at 10.1 m in Lake Fryxell

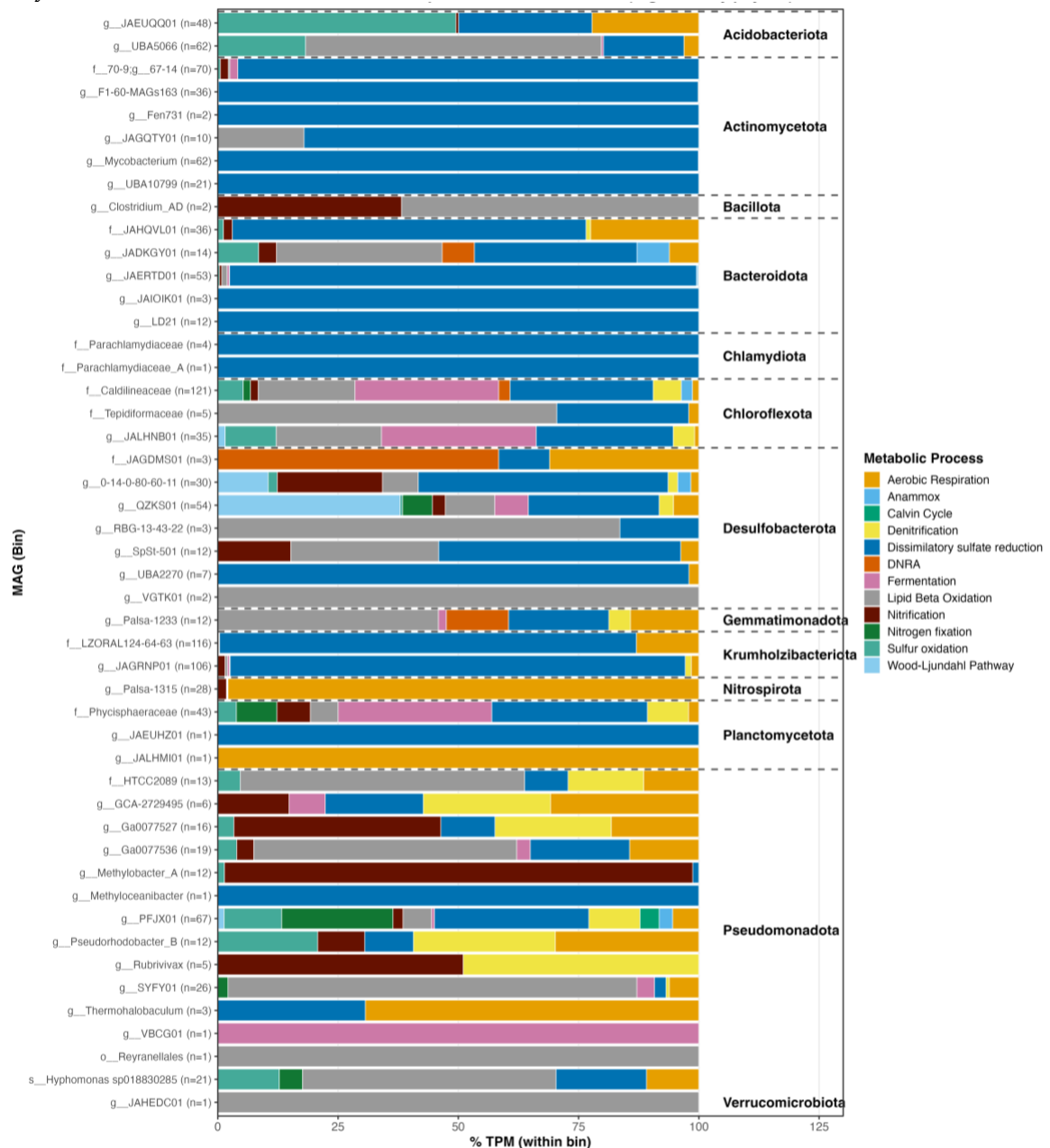


Figure 4: Metabolic pathway expression as percent transcripts per million by phylum at 10.1 m in Lake Fryxell. MAGs were labeled with their most specific taxonomic classification. In parenthesis is the number of unique transcripts of critical enzymes from table 1 that successfully mapped to the HCLC MAGs. Numbers represent the number of HCLC MAGs classified to these phyla. All metabolisms were included regardless of if they were incomplete to completely assess the composition of metabolic expression within a phylum.

The dominant metabolic process in Acidobacteriota MAGs was lipid beta oxidation, representing 61.5% of average transcript expression across these MAGs, with 3 total transcripts assigned to this pathway. However, individual MAGs showed distinct profiles: MAG g__*UBA5066* specialized in lipid beta oxidation (61.5%, 3 transcripts), with additional activity in sulfur oxidation (18.2%, 3 transcripts), dissimilatory sulfate reduction (16.7%, 43 transcripts), aerobic respiration (3.1%, 12 transcripts), and minor fermentation (0.4%, 1 transcript). This MAG appears to be a versatile heterotroph capable of lipid degradation with facultative respiratory capabilities. MAG g__*JAEUQQ01* showed a different profile dominated by sulfur oxidation (49.5%, 3 transcripts), followed by dissimilatory sulfate reduction (27.8%, 24 transcripts), aerobic respiration (22.2%, 20 transcripts), and minor nitrification (0.5%, 1 transcript).

Dissimilatory sulfate reduction was the overwhelmingly dominant metabolism in Actinomycetota, representing 96.3% of mean expression across 6 MAGs, with 187 total transcripts. This phylum showed remarkable specialization in sulfate reduction: MAG g__*Mycobacterium* exhibited near-complete specialization in dissimilatory sulfate reduction (99.9%, 61 transcripts), with only trace aerobic respiration (0.1%, 1 transcript). MAG g__*UBA10799* showed complete dedication (100.0%) to dissimilatory sulfate reduction with 21 transcripts, indicating obligate sulfate-reducing metabolism. MAG g__*Fen731* similarly showed 100.0% dissimilatory sulfate reduction with 2 transcripts. MAG f__70-9;g__67-14 was dominated by dissimilatory sulfate reduction (95.9%, 61 transcripts), with minor activities in nitrification (1.7%, 2 transcripts), fermentation (1.6%, 4 transcripts), sulfur oxidation (0.5%, 2 transcripts), and

lipid beta oxidation (0.3%, 1 transcript). MAG g__*F1-60-MAGs163* showed 99.7% dissimilatory sulfate reduction (34 transcripts), with traces of aerobic respiration (0.1%, 1 transcript) and fermentation (0.1%, 1 transcript). MAG g__*JAGQTY01* exhibited 82.1% dissimilatory sulfate reduction (8 transcripts) alongside 17.9% lipid beta oxidation (2 transcripts), suggesting coupling of sulfate reduction with organic carbon oxidation.

The single Bacillota MAG, g__*Clostridium_AD*, showed lipid beta oxidation as the dominant process (61.8%, 1 transcript), with substantial nitrification (38.2%, 1 transcript).

Dissimilatory sulfate reduction was the major metabolism in Bacteroidota, representing 80.9% of mean expression across 5 MAGs, with 65 total transcripts assigned. MAG g__*LD21* and g__*JAIOIK01* both showed 100.0% dedication to dissimilatory sulfate reduction, 12 and 3 transcripts, respectively. MAG g__*JAERTD01* exhibited 97.1% dissimilatory sulfate reduction (17 transcripts) with minor aerobic respiration (2.9%, 1 transcript). MAG f__*JAHQVL01* showed 73.5% Dissimilatory sulfate reduction (25 transcripts), with notable aerobic respiration (22.5%, 8 transcripts), and minor nitrification (1.9%, 1 transcript), Sulfur oxidation (1.2%, 1 transcript), and denitrification (1.0%, 1 transcript). This MAG demonstrates metabolic versatility with both anaerobic sulfate reduction and aerobic respiration. MAG g__*JADKGY01* diverged from the phylum pattern, showing lipid beta oxidation as dominant (34.4%, 2 transcripts), followed by dissimilatory sulfate reduction (27.4%, 6 transcripts), aerobic respiration (25.8%, 5 transcripts), denitrification (9.6%, 2 transcripts), and sulfur oxidation (2.8%, 1 transcript), indicating a facultative metabolism with diverse respiratory options.

Both Chlamydiota MAGs showed complete (100.0%) specialization in Dissimilatory

sulfate reduction. MAG f__Parachlamydiaceae had 4 transcripts and f__Parachlamydiaceae_A had 1 transcript assigned to this pathway. This represents an interesting metabolic streamlining in organisms traditionally known as obligate intracellular pathogens, suggesting either free-living sulfate-reducing Chlamydiae or symbiotic relationships where sulfate reduction benefits the host.

Lipid beta oxidation was highly expressed in Chloroflexota (37.5% mean expression, 22 total transcripts), though individual MAGs showed diverse strategies: MAG f__Tepidiformaceae showed 70.5% lipid beta oxidation (3 transcripts), with additional aerobic respiration (27.5%, 3 transcripts) and minor dissimilatory sulfate reduction (2.1%, 1 transcript), suggesting aerobic heterotrophic metabolism utilizing lipids. MAG f__Caldilineaceae was dominated by Fermentation (29.9%, 57 transcripts), followed by dissimilatory sulfate reduction (27.8%, 33 transcripts), aerobic respiration (22.3%, 16 transcripts), lipid beta oxidation (11.8%, 5 transcripts), denitrification (4.4%, 4 transcripts), and sulfur oxidation (3.8%, 4 transcripts). This highly versatile MAG demonstrates broad metabolic capabilities across multiple pathways. MAG g__JALHNB01 showed fermentation dominance (32.1%, 15 transcripts), alongside dissimilatory sulfate reduction (30.8%, 37 transcripts), aerobic respiration (21.6%, 17 transcripts), lipid beta oxidation (14.4%, 14 transcripts), and sulfur oxidation (1.2%, 1 transcript), indicating facultative anaerobic metabolism.

Desulfobacterota contained seven MAGs with diverse metabolic profiles. MAG f__JAGDMS01 was dominated by DNRA (58.40%, 1 transcript), aerobic respiration (31.03%, 1 transcript), and dissimilatory sulfate reduction (10.57%, 1 transcript). MAG g__0-14-0-80-60-11 expressed dissimilatory sulfate reduction (51.95%, 15 transcripts), nitrification (21.89%, 5 transcripts), the WLP (10.45%, 7 transcripts), lipid beta oxidation (7.43%, 1 transcript), anammox (2.74%, 1 transcript), denitrification (1.99%, 1 transcript), sulfur oxidation

(1.89%, 1 transcript), and aerobic respiration (1.67%, 1 transcript). The WLP was the highest expressed metabolism (37.88%, 6 transcripts) in MAG g__*QZKS01*, followed by dissimilatory sulfate reduction (27.20%, 23 transcripts), lipid beta oxidation (10.25%, 5 transcripts), fermentation (6.99%, 6 transcripts), nitrogen fixation (6.20%, 5 transcripts), aerobic respiration (5.28%, 4 transcripts), denitrification (2.97%, 2 transcripts), nitrification (2.72%, 3 transcripts), and sulfur oxidation (0.50%, 1 transcript). MAG g__*RBG-13-43-22* was dominated by lipid beta oxidation (83.58%, 2 transcripts) with dissimilatory sulfate reduction (16.42%, 1 transcript). MAG g__*SpSt-501* expressed dissimilatory sulfate reduction (50.34%, 6 transcripts), lipid beta oxidation (30.73%, 2 transcripts), nitrification (15.16%, 3 transcripts), and aerobic respiration (3.76%, 1 transcript). MAG g__*UBA2270* was heavily dominated by dissimilatory sulfate reduction (97.95%, 5 transcripts) with minor aerobic respiration (2.05%, 2 transcripts). MAG g__*VGTK01* only expressed lipid beta oxidation at 100% with two transcripts.

Gemmatimonadota was represented by one MAG, g__*Palsa-1233*, which expressed lipid beta oxidation (45.85%, 2 transcripts), dissimilatory sulfate reduction (20.83%, 4 transcripts), aerobic respiration (14.22%, 3 transcripts), DNRA (12.96%, 1 transcript), denitrification (4.49%, 1 transcript), and fermentation (1.66%, 1 transcript).

Krumholzibacteriota contained two MAGs, both heavily dominated by dissimilatory sulfate reduction. MAG f__*LZORAL124-64-63* expressed dissimilatory sulfate reduction (86.65%, 73 transcripts), aerobic respiration (12.99%, 38 transcripts), nitrification (0.21%, 3 transcripts), fermentation (0.09%, 1 transcript), and DNRA (0.06%, 1 transcript). MAG g__*JAGRNP01* expressed dissimilatory sulfate reduction (94.63%, 75 transcripts), aerobic respiration (1.59%, 12 transcripts),

nitrification (1.50%, 9 transcripts), denitrification (1.24%, 8 transcripts), lipid beta oxidation (0.50%, 1 transcript), fermentation (0.41%, 2 transcripts), and DNRA (0.14%, 2 transcripts).

Nitrospirota was represented by one MAG, g__*Palsa-1315*, which was dominated by aerobic respiration (97.89%, 3 transcripts) with minor nitrification (1.79%, 13 transcripts), fermentation (0.08%, 2 transcripts), anammox (0.07%, 4 transcripts), denitrification (0.07%, 3 transcripts), dissimilatory sulfate reduction (0.05%, 2 transcripts), lipid beta oxidation (0.03%, 2 transcripts), and DNRA (0.02%, 1 transcript).

Planctomycetota contained three MAGs with different metabolic profiles. MAG f__*Phycisphaeraceae* expressed dissimilatory sulfate reduction (32.36%, 11 transcripts), fermentation (31.94%, 18 transcripts), denitrification (8.53%, 5 transcripts), nitrogen fixation (8.44%, 3 transcripts), nitrification (6.95%, 1 transcript), lipid beta oxidation (5.77%, 1 transcript), sulfur oxidation (3.84%, 2 transcripts), and aerobic respiration (2.16%, 2 transcripts). MAG g__*JAEUHZ01* only expressed dissimilatory sulfate reduction at 100% with one transcript. MAG g__*JALHMI01* only expressed aerobic respiration at 100% with one transcript.

Pseudomonadota contained 14 MAGs exhibiting the greatest metabolic diversity of any phylum. MAG f__*HTCC2089* expressed lipid beta oxidation (59.11%, 5 transcripts), denitrification (15.71%, 3 transcripts), aerobic respiration (11.47%, 2 transcripts), dissimilatory sulfate reduction (9.05%, 2 transcripts), and sulfur oxidation (4.65%, 1 transcript). MAG g__*GCA-2729495* expressed aerobic respiration (30.83%, 2 transcripts), denitrification (26.45%, 1 transcript), dissimilatory sulfate reduction (20.37%, 1 transcript), nitrification (14.80%, 1

transcript), and fermentation (7.54%, 1 transcript). MAG g__*Ga0077527* was dominated by nitrification (43.05%, 7 transcripts), followed by denitrification (24.18%, 3 transcripts), aerobic respiration (18.20%, 4 transcripts), dissimilatory sulfate reduction (11.25%, 4 transcripts), and sulfur oxidation (3.32%, 1 transcript). MAG g__*Ga0077536* expressed lipid beta oxidation (54.68%, 5 transcripts), dissimilatory sulfate reduction (20.66%, 6 transcripts), aerobic respiration (14.39%, 5 transcripts), sulfur oxidation (3.91%, 1 transcript), nitrification (3.55%, 1 transcript), and fermentation (2.81%, 1 transcript). MAG g__*Methylobacter_A* was heavily dominated by nitrification (97.36%, 8 transcripts) with minor sulfur oxidation (1.37%, 2 transcripts) and dissimilatory sulfate reduction (1.26%, 2 transcripts). MAG g__*Methyloceanibacter* only expressed dissimilatory sulfate reduction at 100% with one transcript. MAG g__*PFJX01* exhibited the most diverse metabolic profile in the dataset, expressing dissimilatory sulfate reduction (32.10%, 22 transcripts), nitrogen fixation (23.15%, 7 transcripts), sulfur oxidation (11.98%, 9 transcripts), denitrification (10.63%, 16 transcripts), lipid beta oxidation (5.92%, 3 transcripts), aerobic respiration (5.44%, 6 transcripts), the Calvin Cycle (4.00%, 2 transcripts), anammox (2.80%, 2 transcripts), nitrification (2.09%, 3 transcripts), the WLP (1.28%, 4 transcripts), and fermentation (0.62%, 1 transcript). MAG g__*Pseudorhodobacter_B* expressed aerobic respiration (29.85%, 4 transcripts), denitrification (29.49%, 5 transcripts), sulfur oxidation (20.75%, 2 transcripts), dissimilatory sulfate reduction (10.13%, 2 transcripts), and nitrification (9.78%, 2 transcripts). MAG g__*Rubrivivax* expressed nearly equal nitrification (51.01%, 4 transcripts) and denitrification (48.99%, 4 transcripts). MAG g__*SYFY01* was dominated by lipid beta oxidation (85.00%, 15 transcripts), followed by aerobic respiration (6.15%, 5 transcripts), fermentation (3.66%, 2 transcripts), dissimilatory sulfate reduction (2.42%, 2 transcripts), nitrogen fixation (2.11%, 1 transcript), and

denitrification (0.66%, 1 transcript). MAG g__*Thermohalobaculum* expressed aerobic respiration (69.34%, 2 transcripts) and dissimilatory sulfate reduction (30.66%, 1 transcript). MAG g__*VBCG01* only expressed fermentation at 100% with one transcript. MAG o__*Reyranellales* only expressed lipid beta oxidation at 100% with one transcript. MAG s__*Hyphomonas sp018830285* expressed lipid beta oxidation (52.70%, 7 transcripts), dissimilatory sulfate reduction (18.83%, 5 transcripts), sulfur oxidation (12.78%, 4 transcripts), aerobic respiration (10.87%, 4 transcripts), and nitrogen fixation (4.81%, 1 transcript).

Discussion

PAR and ORP Above Microbial Mats

ORP and PAR are indicators of what metabolic processes are possible, and the contextual information it provides helps establish a metabolic framework at 10.1 m. The PAR recorded before March at 10.2 m is well above the theoretical minimum for net primary productivity, therefore there is more likely more PAR available at 10.1 m. At 10.1 m it is most likely that oxygen is being produced by oxygenic photosynthesizers. Figure 3B demonstrates the daily cycles of PAR and ORP activity suggesting that if oxygenic photosynthesis is occurring, the oxygen produced is increasing the ORP before being consumed. The oxygen reduction could be due to reacting abiotically with sulfide, through aerobic sulfide oxidation, aerobic respiration, nitrification, or aerobic lipid-beta oxidation and that is reflected in decreasing ORP in these cycles. The decrease of ORP within this cycle could also be due to the production of strong reductions like hydrogen sulfide which is a product of dissimilatory sulfide reduction. This

metabolism was widely expressed by the MAGs in this study and in pair with the environmental data of the lake could be a common process in the community.

Progression from March to May in Figure 3C shows a sharp decline in PAR due to seasonal change and most likely as a result ORP declines. With a reduction in PAR, less oxygen may be produced which creates an imbalance with processes consuming it. Strong reductants produced from sulfate reducers contribute to the creation of this highly reductive environment, reducing the ORP by more than four times the values recorded from January to March. At approximately -400 mV, sulfate reduction, fermentation, methanogenesis and hydrogen production are the processes that are likely to occur (Fleitas García et al., 2025; de Rink et al., 2022; Soule et al., 1951; Thauer et al., 1977). It would be expected that although these processes may happen year-round, they would become more prominent metabolic strategies.

Interestingly, as PAR increases into the spring, ORP remains low. During the winter, there may have been a large accumulation of hydrogen sulfide. The hydrogen sulfide could be inhibiting cyanobacterial oxygenic photosynthesis and need to be reduced or outpaced by cyanobacterial D1 protein repair. It is also likely that this is where diatoms help Cyanobacteria since diatoms can tolerate hydrogen sulfide. As the diatoms produce more oxygen, the hydrogen sulfide would be slowly removed until cyanobacterial oxygenic photosynthesis restarts. Sulfide oxidizers would contribute to the removal of sulfide aerobically when enough oxygen is present or anaerobically. Eventually it is expected that enough sulfide is removed to increase the ORP and return to a low oxygen reductive environment during the summer.

Relative Expression of Metabolic Pathways at 10.1 m

The expression of the metabolisms chosen for study attempted to determine what metabolisms were used by organisms within these phyla. Transcript per million (TPM) normalizes the sequencing data, accounting for length and abundance for all the metabolic transcripts specified within the sample. This provided us with a balanced comparison of metabolic strategies within each phylum to determine what strategy is most prominent.

Aerobic respiration is only possible in the presence of oxygen. Oxygen at 10.1 m in the mat and diversity of metabolisms present are largely influenced by the production of oxygen from oxygenic photoautotrophs, e.g. Cyanobacteria, diatoms, and other algae. The presence of cytochrome c oxidase cbb3-type subunit I/II and cytochrome bd ubiquinol oxidase subunit I indicated that within the Bacillota, Chlamydoita, Dependuntiae, and Verrucomicrobiota phyla, the taxa were able to utilize the oxygen produced by oxygenic photoautotrophs. Cytochrome c oxidase cbb3-type and cytochrome bd ubiquinol oxidase have a high affinity for oxygen and are predominantly expressed under low-oxygen conditions (Hamada et al., 2014; Brisov et al., 2011), indicating that aerobic organisms were in oxygen limited conditions and utilizing what little oxygen is available. Aerobic respiration is one of the highest energy producing metabolisms. With respect to the relative expression within each MAG, aerobic respiration highly prevalent. Surprisingly, it was a majority of sole Nitrospirota MAG expressed pathways despite it being known that organisms within Nitrospirota often rely on nitrification-based pathways which is most likely due to the low amount of successfully mapped transcripts (Kop et al., 2025). Although aerobic respiration may not be the major pathway utilized, microbial communities are still utilizing the oxygen available to produce energy. Whether or not aerobic respiration is producing the most energy at this depth is speculation, but it is still actively used.

Sulfide increases with depth in Lake Fryxell, reaching 16.8 μM , at 10.1 m (Lumian et al., 2021). The criteria for sulfide oxidation expression only consisted of sulfide oxidases (Table 1), they perform a crucial step for conversion of sulfide into sulfur. Over 50% of the MAGs analyzed expressed one of the enzymes required. This biological capacity correlates with the ecological conditions the microbial mats experience and may contribute to the increase in sulfate as depth increases (Figure 2). This interpretation is reinforced by the ecological conditions of the lake where abiotic conversion of sulfate into sulfide is abiotically impossible in these conditions and is most likely due to microbial sulfate reduction (Ohmoto et al., 1982). The HCLC MAGs classified within the phyla Actinomycetota, Bacteroidota, Desulfobacterota, Krumholzibacteriota are the main contributors to sulfide production by dissimilatory sulfate reduction within this sample. Interestingly, Chlamydoita contained dissimilatory sulfate reduction despite all cultivated organisms within this phylum being obligate intracellular bacteria. The sulfide released by these organisms can then be utilized by the MAGs expressing sulfide oxidation, leading to sulfate production and a complete return of the sulfur cycle. This reinforces the concept that sulfate reducers and sulfide oxidators could be residing near each other. Overall, the resulting percent TPM in these collected MAGs confirms the heavy reliance of sulfate reduction within this metabolic composition. Since sulfate is present in abundance in the environment and the ORP of 10.1 m is most likely reductive. Acidobacteriota, Actinomycetota, Desulfobacterota, Planctomycetota, Pseudomonadota, Chloroflexota, and Bacteroidota MAGs would be able to perform both sulfide oxidation and sulfate reduction, but it is unknown if they are performed concurrently within the same organism. Within the microbial mat, these MAGs are actively contributing to a complete sulfur redox cycle and potentially interchanging metabolism end products within and between community members for reuse.

The nitrogen cycle processes investigated consisted of five different pathways that are active in mats at 10.1 m. Nitrification, oxidation of ammonium or ammonia to nitrate, is not a dominant pathway expressed at 10.1 m. The MAGs in multiple phyla expressed components involved in the process, but only two expressed high quantities in percent TPM. These were MAGs in Bacillota, Desulfobacterota, and Pseudomonadota. The product of nitrification, nitrate, can be used for denitrification and dissimilatory nitrate reduction to ammonium, both anaerobic respiratory processes, but in an oxygen poor environment this process may be limited. Denitrification was present in similar phyla as nitrification. This does imply that these MAGs can produce nitrogen gas as a by-product of anaerobic nitrogen respiration in oxygen scarce environments, which can be used to produce ammonia and ammonium for amino acid and nucleic acid synthesis by other organisms by nitrogen fixation. DNRA, another microbial process capable of reducing nitrate, was present in Bacteroidota, Chloroflexota, Desulfobacterota, Gemmatimonadota, Krumholzibacteriota, and Nitrospirota MAGs. The Gemmatimonadota MAG highly expressed the key enzyme required in our collected sample indicating this is a viable metabolism at 10.1 m. It was expected that it was primarily diatoms performing DNRA, but that was not reflected in the data presented here. These organisms are contributing to the nitrogen cycle by production of ammonium which can then be readily available for incorporation into organic molecules or reused in the nitrogen cycle by anammox and nitrification. However, unlike nitrification, anammox results in the production of nitrogen gas. Anammox was expressed in Chloroflexota, Nitrospirota, Bacteroidota, and Pseudomonadota MAGs. However, the pathway did not make up a significant portion of any of the phyla's metabolic expressed pathways. It ranged from 0.07% to 1.57%, which may contribute to the nitrogen gas that is produced overall. If nitrogen gas is produced by denitrification or anammox, nitrogen fixation by Planctomycetota,

Desulfobacterota, and Chloroflexota can convert it back into ammonia or nitrate. The nitrogen fixation pathway was only fully complete and expressed in Pseudomonadota. Antarctic nitrogen fixing Pseudomonadota have been previously reported in Antarctic soil (Eckford et al., 2002). In the microbial mats of Lake Fryxell, it may produce ammonium for itself or other microbes in the community. Although the pathways in the other three phyla were not complete, it is still highly likely that these communities are producing biologically available forms of nitrogen as the key nitrogenase transcript, *nifH*, was being produced by all three. Overall, a complete cycle of nitrogen is present in Lake Fryxell, nitrate is produced and consumed along with ammonium and nitrogen gas.

Fermentation was expressed in ten phyla but only complete across Planctomycetota MAGs, in which it also constituted approximately 24.9% of percent TPM within the phyla and was complete in its expression. Chloroflexota also heavily expressed fermentation (23.7%) related transcripts indicating that for both these phyla were utilizing this anaerobic process heavily of their energy production. The uniqueness of it being an outlier may provide a competitive advantage to these phyla as it would only rely on carbohydrates and amino acids.

The only chemoautotrophic pathways investigated were the WLP and the Calvin Cycle. The WLP was actively transcribed in Desulfobacterota HCLC MAGs indicating that bacteria within this phylum could contribute to the microbial mats by biomass production while also using heterotrophic pathways to produce energy. The Calvin Cycle was small overall portion of the HCLC MAG activity within Pseudomonadota. The Pseudomonadota rubisco can be used in light-dependent or independent manner but overall does not seem to be highly expressed in the collected HCLC MAGs (Nishihara et al., 2026).

Lipid beta oxidation allows for these microbes to utilize free fatty acids as carbon sources when nutrients are scarce (Cambell et al, 2003). Lipid beta oxidation was found in the HCLCL MAGs, distributed among twelve phyla. The process was a sole activity collected in the metabolic composition of Verrucomicrobiota (100%) MAG. The process was a piece of the overall activity of the HCLC MAGs in Acidobacteriota (~31.4%), Bacillota (~61.8%), Chloroflexota (~20.1%), Gemmatimonadota (~45.8%), and Pseudomonadota (~23.2%). The results indicate that these microbes are utilizing fatty acids collected from their environment. This process may be utilized to work to produce energy production in aerobic microenvironments in parallel to other metabolic strategies expressed by HCLC MAGs within these phyla. Verrucomicrobiota related MAGs only containing genes for this process. In all, lipid beta oxidation appears to be an important metabolic process to several phyla and may assist in optimal energy production during the summer in Lake Fryxell.

Versatility and Specialization within Recovered Phyla

The versatility of and selective specialization of some bacterial phyla in the Fryxell mats at 10.1 m depth display marked adaptability and resourcefulness that contribute to dynamic interactions among and within the phyla. The relatively new phylum, Krumholzibacteriota, made up less than five percent of the HCLC MAGs and less than three percent of the 16S rRNA classified (Chapter 1). This phylum has been recovered from anoxic sulfidic conditions and is capable of fermentation and nitrite reduction to ammonium (Youssef et al., 2019), which is reflected in the data collected from this study. Here I demonstrated metabolic transcription of sulfate reduction, aerobic respiration, and denitrification, expanding the known metabolic

capabilities of Krumholzibacteriota. This was also determined in different extreme environments experiencing sulfidic conditions (Sun et al., 2024; Campbell et al., 2024; Youssef et al., 2019), where the phylum participated in the sulfur cycle and nitrogen cycle.

Unlike Krumholzibacteriota, Nitrospirota did not highly express any nitrogen related transcripts. It is also important to note that although Nitrospirota was one of the highest-completion MAG phyla, it represented a small portion of the microbial abundance (Chapter 1). The main pathway expressed was aerobic respiration at 97.9%. The enzyme transcribed was cytochrome bd ubiquinol oxidase, which is specifically expressed under microaerobic conditions and is tolerant to oxidative stress, hypoxic conditions, and sulfide. (D'mello et al., 1996; Forte et al., 2020; Belevich et al., 2005; Grund et al., 2021). Nitrogen-based processes, including anammox, DNRA, denitrification, and nitrification, were expressed but just were only a small portion of overall MAG activity. This does still suggest evidence for involvement in the nitrogen cycle of Lake Fryxell. Nitrification was the main nitrogen cycle process present (1.80%), which shows that the Nitrospirota collected were oxidizing ammonium, but the proportion of transcripts suggests a focus on aerobic respiration in low oxygen conditions.

Pseudomonadota displayed a wide variety of metabolic activity and was one of the most metabolically diverse and abundant phyla. Carbon fixation by the Calvin Cycle was present, and only present in Pseudomonadota, suggesting that this phylum was contributing as part of autotrophic microbial community by assimilating carbon dioxide. Specifically, the phylum demonstrated transcription and presence of sulfate reduction and sulfide oxidation pathways. Upon further investigation, the MAG bin_176 was classified as an unknown Gammaproteobacteria species. It contained transcripts critical to sulfide oxidation, sulfide quinone oxidoreductase and a dissimilatory sulfite reductase flavoprotein subunit. This result

implies the possibility that one group of organisms can recycle their own end products or utilize both pathways to maximize energy production while also contributing to the nitrogen and sulfur cycles. This also highlights that within these phyla, individual taxa can show versatility in the metabolisms they express.

Desulfobacterota was the only phylum in the high completion MAGs to express both enzymes required for the Wood-Ljungdahl Pathway. This observation indicates that nonphotoautotrophic organisms likely played a role in carbon fixation and building biomass at 10.1 m. Similar to Pseudomonadota, Desulfobacterota was not solely performing autotrophic carbon fixation; it expressed nitrogen fixation, nitrification, lipid beta oxidation, fermentation, DNRA, dissimilatory sulfate reduction, denitrification, anammox, aerobic respiration.

Summer Metabolic Framework in Lake Fryxell at 10.1 m

Characteristic of an extreme environment, the organisms in Lake Fryxell mats implemented diverse metabolic approaches. My results provide a framework for understanding the summer ecosystem. The metabolisms active during summer at 10.1 m include aerobic and anaerobic respiration, fermentation, photosynthesis, the Calvin Cycle, and the WLP. Results have shown that oxygen and ORP increase and decrease in daily cycles that reflect the activity of aerobic and anaerobic metabolisms. Using these results, we propose an ecologic structure for these mats.

The presence of sufficient light allows oxygenic photosynthesis, performed by cyanobacteria, diatoms, and other algae. This activity is confirmed with the expression of photosystem II and I from cyanobacteria and diatoms (Chapter 1; Chapter 2). Diatoms are conducting photosynthesis at 10.1 m as they can display higher resistance to hydrogen sulfide,

which inhibits cyanobacterial oxygenic photosynthesis (Admiraal et al., 1979; Kennett et al., 1985). It is possible that at 10.1 m there are Cyanobacteria resistant to or utilize sulfide in photosynthesis. The most likely candidate, from the classified Cyanobacteria in the 16S rRNA results, are the *Caldora* species as they are the most abundant Cyanobacteria. The photoautotrophy is paired with expression of enzymes for light-dependent rubisco carbon fixation and establishes that diatoms and cyanobacteria are key parts of the primary producer community, which also includes Desulfobacterota or Pseudomonadota utilizing the WLP and Calvin Cycle which converts carbon dioxide into organic matter. In addition, the oxygen produced increases ORP and supplies oxygen for oxygen dependent processes while chemoautotrophs build biomass, both supporting the microbial mat community by introducing the potential for aerobic metabolisms and biomolecules. The production of oxygen can be traced in the ORP values collected from the water above the microbial mats (Figure 1). As oxygen is produced, ORP rises; when it is consumed by oxygen-based metabolisms like aerobic respiration or nitrification, it decreases. In addition, dissimilatory sulfate reduction produces a strong reductant, sulfide, which can decrease the ORP (Pearsall et al., 1939). Despite the negative ORP, oxygen is still present, but not in high enough concentrations to establish an oxidative environment. The resulting oxygenated reductive environment in the mat provides a situation in which by ORP, reductive processes are favored but aerobic respiration is still occurring as shown by the expression of both dissimilatory sulfate reduction and aerobic respiration in 25 MAGs (Figure 4).

The oxygen produced by oxygenic phototrophs creates a thin layer of DO, an oxygen oasis, available for the aerobic metabolisms (Sumner et al., 2015). Aerobic respiration was present and expressed in nine phyla analyzed, even constituting almost 100% percent of the

Nitrospirota g_*Palsa-1315* MAG activity which is highly unlikely. Considering Nitrospirota made up a small portion of the 16S rRNA analysis, it is likely not all transcripts from MAGs classified within the phyla present have been sufficiently sampled. The enzymes expressed, cytochrome c oxidase cbb3-type and cytochrome bd ubiquinol oxidase, indicate aerobic organisms experience low-oxygen conditions but can still utilize aerobic respiration for additional energy production. The transcriptional evidence establishes that Pseudomonadota, Planctomycetota, Nitrospirota, Krumholzibacteriota, Gemmatimonadota, Desulfobacterota, Chloroflexota, Bacteroidota, and Acidobacteriota MAGs are aerobic consumers of the oxygen oasis. However, despite the presence of an oxygen oasis, anaerobic metabolisms are also active, implying that oxygen consumption produces anaerobic microenvironments and contributes to ORP decline through oxygen removal. In these anaerobic microenvironments, fermentation is a common metabolism. Fermentation does not produce as much ATP when compared to its aerobic alternative, aerobic respiration. Acidobacteriota, Actinomycetota, Bacteroidota, Chloroflexota, Desulfobacterota, Gemmatimonadota, Krumholzibacteriota, Nitrospirota, Planctomycetota, and Pseudomonadota MAGs all expressed some degree of completion of fermentation in at least one of the MAGs representing these phyla. The process was fully complete and was 27% of percent TPM in the Plactomycetota MAG f_*Phycisphaeraceae*. All the MAGs present except those within *Chlamydoita* and *Dependentiae* lipid beta oxidation was expressed. This process can be aerobic or anaerobic and is used while organisms are experiencing a stationary or stress phase (Pelikan et al., 2020; Jimenez-Diaz et al., 2017). In the Lake Fryxell framework, lipid beta oxidation can serve as an alternative metabolism to produce additional energy. Overall, 10.1 m clearly shows combinations of aerobic and anaerobic metabolisms, displaying versatility in this oxygenated reductive environment.

The presence of oxygen also supports the oxidative processes of the sulfur and nitrogen cycles. Sulfide is present at 10.1 m at 0.571 mg/L (Lumian et al., 2021). Oxygen produced in summer can be used to oxidize sulfide to produce energy and sulfate, while also reducing sulfide inhibition of oxygenic photosynthesis in cyanobacteria. Sulfide oxidation was present in seven phyla but the highest average percent TPM expression across MAGs was in the Acidobacteriota MAG g_*JAEUQQ01* at 49%. In the remaining average across MAGs within the phyla Actinomycetota, Bacteroidota, Chloroflexota, Desulfobacterota, Planctomycetota, and Pseudomonadota sulfide oxidation below 6.5%. This may indicate that bacterial sulfide oxidation is not an optimal metabolic strategy for all community members during the summer. In addition, the enzymes determined to be critical for sulfide oxidation function both aerobically and anaerobically (Jung et al., 2025; Chuanjuan et al., 2024; Xia et al., 2017). Anaerobic sulfide oxidation connects the sulfur and nitrogen cycle by the utilization of nitrate which can be produced by the nitrification activity of the Bacillota, Chloroflexota, Desulfobacterota, Krumholzibacteriota, Nitrospirota, Planctomycetota, Pseudomonadota, and Bacteroidota MAGs (Kamp et al., 2006; Nie et al., 2021). The biotic or abiotic oxidation of sulfide to sulfate provides a resource that can be reduced through dissimilatory sulfate reduction. The MAGs that showed expression greater than 50% TPM in dissimilatory sulfate reduction pathway belonged to Bacteroidota, Actinomycetota, Desulfobacterota, and Krumholzibacteriota. During the summer while oxygen is present, these organisms can capitalize on the abiotic and biotic conversion of sulfide into sulfate. Interestingly, Acidobacteriota, Actinomycetota, Desulfobacterota, Planctomycetota, Pseudomonadota, Chloroflexota, and Bacteroidota MAGs contained both sulfate reduction and sulfide oxidation within the same MAGs suggesting the organisms can use both pathways but most likely not concurrently.

Bacillota MAGs and Pseudomonada MAGs heavily expressed nitrification related enzymes. The increase in nitrate production could assist in providing anaerobic electron acceptors in anaerobic microenvironments. Anammox, which requires nitrite, was not as prevalent, only ranging from 0.07-1.6% TPM within the analyzed phyla. Overall, it does not seem to be a pathway that is particularly active during the summer or one that leads to large amounts of nitrogen gas production. Together though, nitrification and anammox convert ammonium and ammonia to nitrite and nitrate, or nitrogen gas, respectively, which can be utilized in the anaerobic process's denitrification, DNRA, and nitrogen fixation. Denitrification was widespread and heavily expressed in Pseudomonadota MAGs. The process could be used to prevent biofilm damage from reactive nitrogen species (Barraud et al., 2006). DNRA was only expressed in four MAGs. Strong inferences on the importance of this activity can't be made but it does show that this part of the nitrogen cycle is occurring within the mat. Nitrogen fixation was supported by activity in Pseudomonadota and Planctomycetota MAGs. Chloroflexota and Desulfobacterota allocated fewer resources to this process based on the successfully mapped transcripts. Still, there is reintroduction of nitrogen back into the microbial mats. Ammonium can also be produced from nitrate by DNRA, so nitrogen fixation is not necessarily the only means to produce biologically available nitrogen. In summation, a complete summer nitrogen cycle has numerous community members participating in one or multiple processes within the nitrogen cycle.

Predicted Changes in Summer Metabolisms at Shallower Depths

The 10.1 m summer metabolic framework in Lake Fryxell allows inferences to be made on how microbial communities may alter their metabolism to adjust to depths below and above 10.1 m. Above 10.1 m, oxygen increases due to increased PAR availability, sulfide also decreases, allowing more oxygenic photosynthesis by Cyanobacteria and diatoms. Increased photosynthesis should result in an increase in Calvin Cycle activity, since sulfide is considerably low in shallower depths it would not be likely to see this increase due photo-dependent sulfide oxidizers or purple sulfur bacteria (McFarlane et al., 2019). The WLP may decrease in prominence as a carbon fixation pathway on the surface of the mats compared to the Calvin Cycle utilized by Cyanobacteria, which produce oxygen that can inhibit the WLP. This may be in response to an increase in Cyanobacteria in the community that would utilize light-dependent rubisco carbon fixation. The resulting oxygenic photosynthesis oxygenates the mat and overlying water, providing more oxygen for aerobic respiration. Aerobic respiration would most likely increase following an increase in oxygen and is a high energy producing metabolism. As a result of increased oxygen, fermentation and lipid beta oxidation expression may decrease (Cho et al., 2006). Aerobic environments would be inhospitable to obligate anaerobic organisms that rely on fermentation. Still, there may be anaerobic microenvironments available deeper into the mat, s. Overall, increased oxygen would increase metabolic diversity (Jiang et al., 2012). Aerobic respiration should see a substantial increase. This would be consistent with previously collected data at 9 – 9.8 m where aerobic respiration was one of the highest gene markers recovered (Dillon et al., 2020b).

Higher oxygen will alter the sulfur cycle in shallower depths. As oxygen is produced, it can react with sulfide yielding sulfate. However, the sulfide concentration is low, < 0.01 mg/L,

above 9.5 m (Lumian et al., 2021), suggesting that it either oxidizes back to sulfate quickly or there is not a source of sulfide. However, sulfate found at shallower depths (Karr et al., 2005). Thus, dissimilatory sulfate reduction would potentially be active in the mats at the same level or even higher.

The nitrogen cycle is crucial to the production of organic molecules and would be present, but the different metabolisms involved may change. With more oxygen available, it would not be a limiting factor for oxidation of ammonium in the nitrification process. Denitrification would most likely decrease as the process is inhibited by high oxygen concentrations. Some denitrification may still occur to remove radial nitrogen species, helping prevent biofilm damage and protecting cell wall integrity (Mai et al., 2021; Nikolaou et al., 2025). As a result, nitrogen gas production would increase. Expression of DNRA would decrease at shallower depths during the summer as oxygen becomes available for aerobic respiration. Ammonium would still be produced for incorporation into biomolecules (Pandy et al., 2020; Decleyre et al., 2015). Despite the presence of ammonium and oxygen which can be used to make the required nitrite, anammox would most likely not occur as it is an anaerobic based metabolism and inhibited by the presence of oxygen (Dalsgaard et al., 2014). It may only be likely in anaerobic microenvironments with nitrite produced from denitrification.

Predicted Changes in Summer Metabolisms at Deeper Depths

Metabolic dynamics in the deeper parts of Lake Fryxell can be inferred upon with the results produced during the summer at 10.1 m. With an increase in depth, PAR is attenuated, reducing photosynthetic capacity of oxygenic phototrophs. This would reduce light-dependent Calvin Cycle carbon fixation. An increase in chemoautotrophy, via the Wood-Ljungdahl Pathway,

would become more prominent throughout the mats as it can be paired with methanogenesis and acetogenic metabolisms (Ragsdale et al., 2008; Borrel et al., 2016). As oxygenic photosynthesis decreases, lack of oxygen reduces the ORP, preventing removal of strong reductants. Once oxygen production is no longer active and remaining supply exhausted, the aerobic respiration process will cease as well, instead restricting microbes to alternative electron acceptors. Alternative strategies to maximize energy production lost by respiration may involve increased fermentation and lipid oxidation. Changes in metabolic activity would not be limited to these processes as lack of oxygen would have a notable influence on the sulfur and nitrogen cycles.

In this study involving the sulfur cycle, sulfide oxidation and sulfate reduction were active processes and most likely are used as deeper depths. In the lower parts of Lake Fryxell sulfide oxidizers would benefit from the increased amounts of sulfide available for oxidation. Sulfide oxidation itself can be performed in oxic and anoxic conditions, aerobically and anaerobically (Friedrich et al., 2001; Xia et al., 2017; Findlay et al., 2015). Without oxygen the aerobic process would not occur and below 10.1 m anaerobic sulfide oxidation would most likely be the dominant form of this metabolism. In this anaerobic environment, nitrate could be used as an oxidizing agent for sulfide oxidation, but the production of nitrate may be limited as nitrification requires oxygen. If nitrate and nitrite are no longer available this may prevent other anaerobic oxidation processes in other cycles. This may potentially present a decoupling of the sulfur cycle and nitrogen cycle if nitrate is not being produced or acquired. Sulfate reduction would most likely remain a prominent metabolism deeper into the lake as sulfate is still present and builds with depth (Figure 2). Due to the decrease in ORP by lack of oxygen and presence of hydrogen sulfide, sulfate would be a useful electron acceptor for facilitating energy production in an environment with oxygen as a higher potential electron acceptor.

The nitrogen cycle is composed of many processes of converting nitrogen species, but in an anoxic environment, mainly the anaerobic processes would increase in prevalence. Nitrification would likely decrease as oxygen would no longer be available for nitrate production while denitrification is no longer inhibited by oxygen and will increase. DNRA would increase as nitrate would be a viable electron acceptor in the absence of oxygen and has been noted to be used by diatoms in dark anoxic waters unless nitrate is depleted (Merz et al., 2020). The resulting ammonium could then be used for anaerobic ammonium oxidation, anammox, if nitrite is available. The lack of nitrogenous species due to lack of oxygen for production would limit anaerobic oxidation of sulfide and anaerobic respiratory processes.

Implications for Metabolic Changes During Winter at 10.1 m

As the season changes to winter, PAR declines and then becomes unavailable. With a loss of photons, photosynthesis ceases, reducing the oxygen output to zero. This, along with oxygen consumption and sulfide production, causes ORP to decline by over -300 mV (Figure 3A). With a lack of photoautotrophy, chemoautotrophic pathways would increase in relative importance. Desulfobacterota, Pseudomonadota, and Chloroflexota taxa may increase expression of the WLP and rubisco for light-independent carbon fixation. Aerobic respiration may remain consistent until oxygen is depleted, creating a completely anoxic environment. Fermentation would be expected to increase as a result. Evidence also suggests that sulfate reducing microbes can use ethanol, along with the *hnd* bifurcating gene family, to oxidize hydrogen for NADH and ferredoxin production (Kpebe et al., 2023). Fermentation was expressed in our sample including alcohol dehydrogenase. Alcohol dehydrogenase can perform bidirectional reactions between ethanol and acetyl-CoA, implying the presence of ethanol. Of the phyla represented,

Plactomycetota, Desulfobacterota, and Chloroflexota taxa may utilize this process to maximize energy production during the winter. The interplay between dissimilatory sulfate reduction, fermentation, and electron bifurcation may become more common.

The sulfur cycle may change from fall to winter as the lack of oxygen can permit the increase of sulfide over time. The lack of oxygen reduces the abiotic reaction of oxygen and sulfide. Without oxygen from the oxygen oasis oxidizing sulfide to sulfate, sulfide builds up, promoting anaerobic sulfide oxidation as a valid metabolic strategy. The process was present in seven phyla and would likely show an increased expression as more sulfide becomes available. Dissimilatory sulfate reduction would be expected to continue to show increased prominence in the metabolic composition of the microbial mat community.

Like the sulfur cycle, the nitrogen cycle may have attenuated oxidation reactions due to the lack of oxygen. Without oxygen, nitrification becomes highly unlikely to occur after depletion of the oxygen oasis. Expression may continue into winter but would ultimately cease when anoxia is established. Denitrification would substantially increase in anoxic environments as nitrate is used as a terminal electron acceptor. DNRA is expected to increase in expression due to anoxic dark conditions. Nitrogen fixation may increase as the nitrogen limitation of the lake may prompt communities to scavenge available nitrogen for biomolecule anabolism (Green et al., 1989). This would also be promoted by the lack of oxygen which can inhibit the nitrogenase critical to the fixation process (Asubel et al., 1984; Sakar et al., 2014; Helbleib et al., 2000). Lastly with the production of ammonium, it is likely that anammox could increase to convert excess ammonium to nitrogen gas but that is dependent on the levels of nitrite as it is required for the process to occur.

Conclusion

The metabolic framework of Lake Fryxell displays a diversity of metabolic processes with phyla diversifying or specializing their metabolic capabilities. Autotrophic expression, forming the basic biomass of the microbial mats can most likely be attributed to Desulfobacterota and some Pseudomonadota MAGs participating in the WLP and Calvin Cycle. The framework includes a complete sulfur cycle with both sulfide oxidation and sulfate reduction present and sometimes performed by the same organisms. Denitrification, nitrification, anammox, nitrogen fixation, and DNRA are represented indicating a complete nitrogen cycle at 10.1 m during the summer in Lake Fryxell. Anaerobic processes are also active as fermentation occurs as well in the microbial mats. Although both anaerobic and aerobic processes are expressed, they may also be supplemented by lipid beta oxidation to further utilize fatty acids in the extreme environment.

Overall, this study at 10.1 m extends understanding of the microbial metabolisms in the lower parts of Lake Fryxell in depth by investigating what metabolisms are present and what assembled MAGs are expressing. Previous studies have focused within the oxycline, 9.0 - 9.8 m, where cyanobacteria are highly prominent in the surface layers of the mat (Dillon et al., 2020). There they produce oxygen and it is reflected in the high prevalence of key photosynthesis genes, which benefits aerobic respirators (Dillon et al., 2020b). At 10.1 m this study, an anaerobic shift is occurring. This change explains how the communities, that were also found at shallower depths, adjust to changing environmental conditions with the respect to depth in summer. Oxygenic photosynthesizers are still contributing to oxygen production, which increases the

ORP, but most likely doesn't lead to positive ORP above the mat. Instead, the reductive environment at 10.1 m supports the prominence of anaerobic metabolisms.

Works Cited

- AADC. "Taxon Profile: *Fragilaria Crotonensis* - Biodiversity Database - AADC." *Aad.gov.au*, 2025, data.aad.gov.au/aadc/biodiversity/taxon_profile.cfm?taxon_id=116978. Accessed 10 Dec. 2025.
- Admiraal, Wim, and Harry Peletier. "Sulphide Tolerance of Benthic Diatoms in Relation to Their Distribution in an Estuary." *British Phycological Journal*, vol. 14, no. 2, June 1979, pp. 185–96, <https://doi.org/10.1080/00071617900650201>. Accessed 30 Jan. 2022.
- Agnihotri, Gautam, and Hung-wen Liu. "Enoyl-CoA Hydratase: Reaction, Mechanism, and Inhibition." *ChemInform*, vol. 34, no. 14, Wiley, Mar. 2003, <https://doi.org/10.1002/chin.200314288>. Accessed 28 Mar. 2024.
- Aiken, George, et al. "Geochemistry of Aquatic Humic Substances in the Lake Fryxell Basin, Antarctica." *Biogeochemistry*, vol. 34, no. 3, Sept. 1996, <https://doi.org/10.1007/bf00000900>. Accessed 22 June 2020.
- Altschul, Stephen F., et al. "Basic Local Alignment Search Tool." *Journal of Molecular Biology*, vol. 215, no. 3, Oct. 1990, pp. 403–10, [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Andrews, Simon. "Babraham Bioinformatics - FastQC a Quality Control Tool for High Throughput Sequence Data." *Www.bioinformatics.babraham.ac.uk*, 2010, www.bioinformatics.babraham.ac.uk/projects/fastqc.
- Angino, Ernest E., et al. "Chemical Stratification in Lake Fryxell, Victoria Land, Antarctica." *Science*, vol. 138, no. 3536, American Association for the Advancement of Science, Oct. 1962, pp. 34–36, <https://doi.org/10.1126/science.138.3536.34>. Accessed 17 July 2025.
- Anwar , Alsanea, et al. "Optimizing Autotrophic Sulfide Oxidation in the Oxygen-Based Membrane Biofilm Reactor to Recover Elemental Sulfur." *Environmental Science & Technology*, vol. 57, no. 51, American Chemical Society, Dec. 2023, pp. 21736–43, <https://doi.org/10.1021/acs.est.3c05785>. Accessed 18 Mar. 2026.

- Aramaki, Takuya, et al. “KofamKOALA: KEGG Ortholog Assignment Based on Profile HMM and Adaptive Score Threshold.” *Bioinformatics*, edited by Alfonso Valencia, vol. 36, no. 7, Nov. 2019, pp. 2251–52, <https://doi.org/10.1093/bioinformatics/btz859>.
- Ausubel, Frederick M. “Regulation of Nitrogen Fixation Genes.” *Cell*, vol. 37, no. 1, May 1984, pp. 5–6, [https://doi.org/10.1016/0092-8674\(84\)90294-0](https://doi.org/10.1016/0092-8674(84)90294-0). Accessed 2 Jan. 2020.
- B-Béres, Viktória, et al. “Ecosystem Services Provided by Freshwater and Marine Diatoms.” *Hydrobiologia*, vol. 850, Sept. 2022, <https://doi.org/10.1007/s10750-022-04984-9>.
- Bamford, V. A. “Structural Basis for the Oxidation of Thiosulfate by a Sulfur Cycle Enzyme.” *The EMBO Journal*, vol. 21, no. 21, Nov. 2002, pp. 5599–610, <https://doi.org/10.1093/emboj/cdf566>.
- Barraud, Nicolas, et al. “Involvement of Nitric Oxide in Biofilm Dispersal of *Pseudomonas Aeruginosa*.” *Journal of Bacteriology*, vol. 188, no. 21, Nov. 2006, pp. 7344–53, <https://doi.org/10.1128/JB.00779-06>.
- Bauersachs, T., et al. “Diazotrophic Microbial Community of Coastal Microbial Mats of the Southern North Sea.” *Geobiology*, vol. 9, no. 4, Apr. 2011, pp. 349–59, <https://doi.org/10.1111/j.1472-4669.2011.00280.x>. Accessed 11 Oct. 2020.
- Bayer, Barbara, et al. “Metabolic Versatility of the Nitrite-Oxidizing Bacterium *Nitrospira Marina* and Its Proteomic Response to Oxygen-Limited Conditions.” *The ISME Journal*, vol. 15, no. 4, Apr. 2021, pp. 1025–39, <https://doi.org/10.1038/s41396-020-00828-3>. Accessed 16 Apr. 2022.
- Belevich, Ilya, et al. “Oxygenated Complex of Cytochrome *Bd* from *Escherichia Coli*: Stability and Photolability.” *FEBS Letters*, vol. 579, no. 21, July 2005, pp. 4567–70, <https://doi.org/10.1016/j.febslet.2005.07.011>. Accessed 28 Jan. 2026.
- Bolhuis, Henk, and Lucas J. Stal. “Analysis of Bacterial and Archaeal Diversity in Coastal Microbial Mats Using Massive Parallel 16S rRNA Gene Tag Sequencing.” *The ISME*

- Journal*, vol. 5, no. 11, May 2011, pp. 1701–12, <https://doi.org/10.1038/ismej.2011.52>. Accessed 17 Sept. 2019.
- Bolyen, Evan, et al. “Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2.” *Nature Biotechnology*, vol. 37, no. 8, July 2019, pp. 852–57, <https://doi.org/10.1038/s41587-019-0209-9>.
- Borisov, Vitaliy B., et al. “The Cytochrome Bd Respiratory Oxygen Reductases.” *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, vol. 1807, no. 11, Nov. 2011, pp. 1398–413, <https://doi.org/10.1016/j.bbabi.2011.06.016>.
- Borrel, Guillaume, et al. “Methanogenesis and the Wood–Ljungdahl Pathway: An Ancient, Versatile, and Fragile Association.” *Genome Biology and Evolution*, vol. 8, no. 6, May 2016, pp. 1706–11, <https://doi.org/10.1093/gbe/evw114>. Accessed 12 Feb. 2022.
- Braker, Gesche, et al. “Nitrite Reductase Genes (NirK And nirS) as Functional Markers to Investigate Diversity of Denitrifying Bacteria in Pacific Northwest Marine Sediment Communities.” *Applied and Environmental Microbiology*, vol. 66, no. 5, May 2000, pp. 2096–104, <https://doi.org/10.1128/aem.66.5.2096-2104.2000>. Accessed 11 Mar. 2021.
- Brambilla, Evelyne, et al. “16S rDNA Diversity of Cultured and Uncultured Prokaryotes of a Mat Sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica.” *Extremophiles*, vol. 5, no. 1, Feb. 2001, pp. 23–33, <https://doi.org/10.1007/s007920000169>. Accessed 3 June 2021.
- Bromke, Mariusz A., et al. “Metabolic Analysis of Adaptation to Short-Term Changes in Culture Conditions of the Marine Diatom *Thalassiosira pseudonana*.” *PLoS ONE*, edited by Miguel A Blazquez, vol. 8, no. 6, June 2013, p. e67340, <https://doi.org/10.1371/journal.pone.0067340>. Accessed 25 Nov. 2019.
- Bryant, Donald M., et al. “A Tissue-Mapped Axolotl de Novo Transcriptome Enables Identification of Limb Regeneration Factors.” *Cell Reports*, vol. 18, no. 3, Jan. 2017, pp. 762–76, <https://doi.org/10.1016/j.celrep.2016.12.063>. Accessed 26 Apr. 2020.

- Bushmanova, Elena, et al. “RnaQUAST: A Quality Assessment Tool Forde Novotranscriptome Assemblies: Table 1.” *Bioinformatics*, vol. 32, no. 14, Apr. 2016, pp. 2210–12, <https://doi.org/10.1093/bioinformatics/btw218>. Accessed 27 Mar. 2019.
- Callahan, Benjamin J., et al. “DADA2: High-Resolution Sample Inference from Illumina Amplicon Data.” *Nature Methods*, vol. 13, no. 7, May 2016, pp. 581–83, <https://doi.org/10.1038/nmeth.3869>.
- Camacho, Christiam, et al. “BLAST+: Architecture and Applications.” *BMC Bioinformatics*, vol. 10, no. 1, 2009, p. 421.
- Campbell, Bronwyn C., et al. “Krumholzibacteriota and Deltaproteobacteria Contain Rare Genetic Potential to Liberate Carbon from Monoaromatic Compounds in Subsurface Coal Seams.” *MBio*, vol. 15, no. 3, American Society for Microbiology, Feb. 2024, pp. e0173523–23, <https://doi.org/10.1128/mbio.01735-23>. Accessed 20 Nov. 2025.
- Campbell, John W., et al. “A New Escherichia Coli Metabolic Competency: Growth on Fatty Acids by a Novel Anaerobic β -Oxidation Pathway.” *Molecular Microbiology*, vol. 47, no. 3, Jan. 2003, pp. 793–805, <https://doi.org/10.1046/j.1365-2958.2003.03341.x>. Accessed 16 Mar. 2022.
- Campeciño, Julius, et al. “Correction: Cytochrome c Nitrite Reductase from the Bacterium Geobacter Lovleyi Represents a New NrfA Subclass.” *Journal of Biological Chemistry*, vol. 298, no. 12, Dec. 2022, p. 102743, <https://doi.org/10.1016/j.jbc.2022.102743>. Accessed 14 Oct. 2025.
- Canfield, Donald E., and William J. Green. “The Cycling of Nutrients in a Closed-Basin Antarctic Lake: Lake Vanda.” *Biogeochemistry*, vol. 1, no. 3, Springer Science+Business Media, Sept. 1985, pp. 233–56, <https://doi.org/10.1007/bf02187201>. Accessed 26 Jan. 2025.
- Carlson, Hans K., et al. “Sulfate Adenylyl Transferase Kinetics and Mechanisms of Metabolic Inhibitors of Microbial Sulfate Respiration.” *ISME Communications*, vol. 1, no. 1,

- Springer Nature, Nov. 2021, pp. 67–67, <https://doi.org/10.1038/s43705-021-00069-1>. Accessed 3 Dec. 2025.
- Carlton, John D., et al. “Expansion of Armatimonadota through Marine Sediment Sequencing Describes Two Classes with Unique Ecological Roles.” *ISME Communications*, vol. 3, no. 1, Springer Nature, June 2023, <https://doi.org/10.1038/s43705-023-00269-x>. Accessed 13 June 2025.
- Chaumeil, Pierre-Alain, et al. “GTDB-Tk: A Toolkit to Classify Genomes with the Genome Taxonomy Database.” *Bioinformatics*, edited by John Hancock, vol. 36, no. 6, Nov. 2019, <https://doi.org/10.1093/bioinformatics/btz848>.
- Chen, Ming, et al. “Global Landscape of Total Organic Carbon, Nitrogen and Phosphorus in Lake Water.” *Scientific Reports*, vol. 5, no. 1, Oct. 2015, <https://doi.org/10.1038/srep15043>. Accessed 23 Mar. 2022.
- Chen, Nuo, et al. “Impacts of Aquaculture on Nitrogen Cycling and Microbial Community Dynamics in Coastal Tidal Flats.” *PubMed*, National Institutes of Health, Jan. 2025, pp. 120973–73, <https://doi.org/10.1016/j.envres.2025.120973>. Accessed 11 Apr. 2025.
- Chen, Shifu, et al. “Fastp: An Ultra-Fast All-In-One FASTQ Preprocessor.” *Bioinformatics*, vol. 34, no. 17, Sept. 2018, pp. i884–90, <https://doi.org/10.1093/bioinformatics/bty560>.
- Cherney, Maia M., et al. “Structure–Activity Characterization of Sulfide:quinone Oxidoreductase Variants.” *Journal of Structural Biology*, vol. 178, no. 3, June 2012, pp. 319–28, <https://doi.org/10.1016/j.jsb.2012.04.007>. Accessed 9 Feb. 2021.
- Cho, Byung-Kwan, et al. “Transcriptional Regulation of the Fad Regulon Genes of *Escherichia Coli* by ArcA.” *Microbiology*, vol. 152, no. 8, Aug. 2006, pp. 2207–19, <https://doi.org/10.1099/mic.0.28912-0>. Accessed 25 Mar. 2025.
- Chuanjuan , Lü, et al. “Cupriavidus Necator H16 Uses Flavocytochrome C Sulfide Dehydrogenase to Oxidize Self-Produced and Added Sulfide.” *Applied and Environmental Microbiology*, vol. 83, no. 22, American Society for Microbiology, Nov. 2017, <https://doi.org/10.1128/aem.01610-17>. Accessed 5 July 2024.

- Chuvochina, Maria, et al. "SILVA in 2026: A Global Core Biodata Resource for RRNA within the DSMZ Digital Diversity." *Nucleic Acids Research*, Nov. 2025, <https://doi.org/10.1093/nar/gkaf1247>.
- Cloarec, Lilian A., et al. "Lineage-Dependent Partitioning of Activities in Chemoclines Defines Woesearchaeota Ecotypes in an Extreme Aquatic Ecosystem." *Microbiome*, vol. 12, no. 1, BioMed Central, Nov. 2024, <https://doi.org/10.1186/s40168-024-01956-0>. Accessed 14 July 2025.
- Connell, Laurie, et al. "Biodiversity and Abundance of Cultured Microfungi from the Permanently Ice-Covered Lake Fryxell, Antarctica." *Life*, vol. 8, no. 3, Sept. 2018, p. 37, <https://doi.org/10.3390/life8030037>. Accessed 22 Nov. 2021.
- Cono, Violetta La, et al. "Wintertime Simulations Induce Changes in the Structure, Diversity and Function of Antarctic Sea Ice-Associated Microbial Communities." *Microorganisms*, vol. 10, no. 3, MDPI AG, Mar. 2022, p. 623, <https://doi.org/10.3390/microorganisms10030623>. Accessed 14 Mar. 2026.
- Contreras, María José, et al. "Commonalities between the Atacama Desert and Antarctica Rhizosphere Microbial Communities." *Frontiers in Microbiology*, vol. 14, Frontiers Media, July 2023, <https://doi.org/10.3389/fmicb.2023.1197399>. Accessed 20 Oct. 2025.
- Crain, Adam V., and Joan B. Broderick. "Pyruvate Formate-Lyase and Its Activation by Pyruvate Formate-Lyase Activating Enzyme." *Journal of Biological Chemistry*, vol. 289, no. 9, Dec. 2013, pp. 5723–29, <https://doi.org/10.1074/jbc.m113.496877>. Accessed 21 Nov. 2020.
- Cremer, H., et al. "The Holocene Diatom Flora of Marine Bays in the Windmill Islands, East Antarctica." *Botanica Marina*, vol. 46, no. 1, Jan. 2003, <https://doi.org/10.1515/bot.2003.010>. Accessed 30 Aug. 2021.
- Crossman, Lisa, et al. "A Small Predatory Core Genome in the Divergent Marine Bacteriovorax Marinus SJ and the Terrestrial Bdellovibrio Bacteriovorus." *The ISME Journal*, vol. 7,

- no. 1, Springer Nature, Sept. 2012, pp. 148–60, <https://doi.org/10.1038/ismej.2012.90>. Accessed 10 Nov. 2023.
- D'mello, Rita, et al. "The Cytochrome Bd Quinol Oxidase in Escherichia Coli Has an Extremely High Oxygen Affinity and Two Oxygen-Binding Haems: Implications for Regulation of Activity in Vivo by Oxygen Inhibition." *Microbiology*, vol. 142, no. 4, Apr. 1996, pp. 755–63, <https://doi.org/10.1099/00221287-142-4-755>. Accessed 27 May 2021.
- Daims, Holger, et al. "A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria." *Trends in Microbiology*, vol. 24, no. 9, Sept. 2016, pp. 699–712, <https://doi.org/10.1016/j.tim.2016.05.004>.
- Dalsgaard, Tage, et al. "Oxygen at Nanomolar Levels Reversibly Suppresses Process Rates and Gene Expression in Anammox and Denitrification in the Oxygen Minimum Zone off Northern Chile." *MBio*, edited by Douglas G. Capone, vol. 5, no. 6, Oct. 2014, <https://doi.org/10.1128/mbio.01966-14>. Accessed 11 Oct. 2020.
- Danecek, Petr, et al. "Twelve Years of SAMtools and BCFtools." *GigaScience*, vol. 10, no. 2, Jan. 2021, <https://doi.org/10.1093/gigascience/giab008>.
- Dawson, H. M., et al. "Large Diversity in Nitrogen- and Sulfur-Containing Compatible Solute Profiles in Polar and Temperate Diatoms." *Integrative and Comparative Biology*, vol. 60, no. 6, Sept. 2020, pp. 1401–13, <https://doi.org/10.1093/icb/icaa133>. Accessed 25 Aug. 2025.
- De Coster Wouter, and Rosa Rademakers. "NanoPack2: Population Scale Evaluation of Long-Read Sequencing Data." *Bioinformatics*, vol. 39, no. 5, Oxford University Press, May 2023, <https://doi.org/10.1093/bioinformatics/btad311>.
- de Rink, Rieks, et al. "Bacteria Determine the Measured Oxidation Reduction Potential in the Biological Gas Desulfurization Process." *ACS Sustainable Chemistry & Engineering*, vol. 11, no. 1, Dec. 2022, pp. 38–46, <https://doi.org/10.1021/acssuschemeng.2c03241>. Accessed 11 Feb. 2026.

- Decleyre, Helen, et al. "Dissimilatory Nitrogen Reduction in Intertidal Sediments of a Temperate Estuary: Small Scale Heterogeneity and Novel Nitrate-To-Ammonium Reducers." *Frontiers in Microbiology*, vol. 6, Oct. 2015, <https://doi.org/10.3389/fmicb.2015.01124>. Accessed 15 Feb. 2025.
- Deeg, Christoph, et al. "Chromulinavorax Destructans, a Pathogen of Microzooplankton That Provides a Window into the Enigmatic Candidate Phylum Dependistia." *PLOS Pathogens*, vol. 15, no. 5, Public Library of Science, May 2019, pp. e1007801–1, <https://doi.org/10.1371/journal.ppat.1007801>. Accessed 24 Oct. 2023.
- Demin, Konstantin A., et al. "Sulfate-Reducing Bacteria Unearthed: Ecological Functions of the Diverse Prokaryotic Group in Terrestrial Environments." *Applied and Environmental Microbiology*, vol. 90, no. 4, American Society for Microbiology, Mar. 2024, <https://doi.org/10.1128/aem.01390-23>.
- Deng, Zichao, et al. "Dynamics of Free-Living and Attached Bacterial Assemblages in Skeletonema Sp. Diatom Cultures at Elevated Temperatures." *Frontiers in Marine Science*, vol. 8, Frontiers Media, July 2021, <https://doi.org/10.3389/fmars.2021.626207>. Accessed 3 Mar. 2025.
- DeNicola, Dean M. "A Review of Diatoms Found in Highly Acidic Environments." *Hydrobiologia*, vol. 433, no. 1/3, 2000, pp. 111–22, <https://doi.org/10.1023/a:1004066620172>.
- Dharamshi, Jennah E., et al. "Marine Sediments Illuminate Chlamydiae Diversity and Evolution." *Current Biology*, vol. 30, no. 6, Mar. 2020, pp. 1032–1048.e7, <https://doi.org/10.1016/j.cub.2020.02.016>. Accessed 21 Oct. 2022.
- Diao, Muhe, et al. "Global Diversity and Inferred Ecophysiology of Microorganisms with the Potential for Dissimilatory Sulfate/Sulfite Reduction." *FEMS Microbiology Reviews*, vol. 47, no. 5, Oxford University Press, Sept. 2023, <https://doi.org/10.1093/femsre/fuad058>. Accessed 30 Sept. 2024.

- Dillon, Megan L., et al. “Energetic and Environmental Constraints on the Community Structure of Benthic Microbial Mats in Lake Fryxell, Antarctica.” *FEMS Microbiology Ecology*, vol. 96, no. 2, Feb. 2020, <https://doi.org/10.1093/femsec/fiz207>. Accessed 3 June 2021.
- Dillon, Megan L., et al. “Environmental Control on the Distribution of Metabolic Strategies of Benthic Microbial Mats in Lake Fryxell, Antarctica.” *PLOS ONE*, edited by Steven Arthur Loiselle, vol. 15, no. 4, Apr. 2020, p. e0231053, <https://doi.org/10.1371/journal.pone.0231053>. Accessed 26 Nov. 2025.
- Doytchinov, Vesselin V., et al. “Study of the Bacterial, Fungal, and Archaeal Communities Structures near the Bulgarian Antarctic Research Base ‘St. Kliment Ohridski’ on Livingston Island, Antarctica.” *Preprints.org*, Jan. 2024, <https://doi.org/10.20944/preprints202401.1172.v1>. Accessed 1 Dec. 2025.
- Du, Yi-Heng, and Jing-Hua Mu. “Metabolic-Pathway-Presence-Heatmap (MPPH): Constructing Phylogenetic Trees Based on Metabolic Pathways.” *BioRxiv*, June 2023, <https://doi.org/10.1101/2023.06.27.546232>.
- Durand, Sylvain, and Maude Guillier. “Transcriptional and Post-Transcriptional Control of the Nitrate Respiration in Bacteria.” *Frontiers in Molecular Biosciences*, vol. 8, May 2021, <https://doi.org/10.3389/fmolb.2021.667758>.
- Dutta, Avishek, et al. “Depth Drives the Distribution of Microbial Ecological Functions in the Coastal Western Antarctic Peninsula.” *Frontiers in Microbiology*, vol. 14, Frontiers Media, May 2023, <https://doi.org/10.3389/fmicb.2023.1168507>.
- Dyksma, Stefan, and Michael Pester. “Growth of Sulfate-Reducing Desulfobacterota and Bacillota at Periodic Oxygen Stress of 50% Air-O₂ Saturation.” *Microbiome*, vol. 12, no. 1, Springer Science and Business Media LLC, Oct. 2024, <https://doi.org/10.1186/s40168-024-01909-7>.
- Eckford, Ruth, et al. “Free-Living Heterotrophic Nitrogen-Fixing Bacteria Isolated from Fuel-Contaminated Antarctic Soils.” *Applied and Environmental Microbiology*, vol. 68, no.

- 10, Oct. 2002, pp. 5181–85, <https://doi.org/10.1128/aem.68.10.5181-5185.2002>. Accessed 3 Apr. 2025.
- Eddy, Sean R. “Accelerated Profile HMM Searches.” *PLoS Computational Biology*, vol. 7, no. 10, Oct. 2011, <https://doi.org/10.1371/journal.pcbi.1002195>. Accessed 25 Mar. 2021.
- Esposito, R. M. M., et al. “Inland Diatoms from the McMurdo Dry Valleys and James Ross Island, Antarctica.” *Botany*, vol. 86, no. 12, Canadian Science Publishing, Dec. 2008, pp. 1378–92, <https://doi.org/10.1139/b08-100>. Accessed 25 Apr. 2024.
- Fang, Ferric C., and Andrés Vázquez-Torres. “Reactive Nitrogen Species in Host–Bacterial Interactions.” *Current Opinion in Immunology*, vol. 60, Oct. 2019, pp. 96–102, <https://doi.org/10.1016/j.coi.2019.05.008>. Accessed 18 Oct. 2022.
- Findlay, Alyssa J., et al. “Light-Dependent Sulfide Oxidation in the Anoxic Zone of the Chesapeake Bay Can Be Explained by Small Populations of Phototrophic Bacteria.” *Applied and Environmental Microbiology*, edited by A. M. Spormann, vol. 81, no. 21, Nov. 2015, pp. 7560–69, <https://doi.org/10.1128/aem.02062-15>. Accessed 28 Aug. 2022.
- Flanders Marine Institute. “WoRMS - World Register of Marine Species - *Pseudo-Nitzschia Australis* Frenguelli, 1939.” *Www.marinespecies.org*, 10 Dec. 2025, www.marinespecies.org/aphia.php?p=taxdetails&id=246604.
- Fleitas García, Ana-Rosa, et al. “Effect of Redox Potential on Biohydrogen Production during Dark Fermentation of Food Wastes in Bioreactor.” *International Journal of Hydrogen Energy*, vol. 141, Pergamon, Feb. 2025, pp. 1199–210, <https://doi.org/10.1016/j.ijhydene.2025.02.089>.
- Forte, Elena, et al. “In *Escherichia Coli* Ammonia Inhibits Cytochrome *Bo3* but Activates Cytochrome *Bd-I*.” *Antioxidants*, vol. 10, no. 1, Multidisciplinary Digital Publishing Institute, Dec. 2020, pp. 13–13, <https://doi.org/10.3390/antiox10010013>. Accessed 23 May 2024.

- Francisco, Annelise, et al. "Mitochondrial NAD(P)⁺ Transhydrogenase: From Molecular Features to Physiology and Disease." *Antioxidants & Redox Signaling*, Aug. 2021, <https://doi.org/10.1089/ars.2021.0111>. Accessed 31 Mar. 2022.
- Friedrich, Cornelius G., et al. "Oxidation of Reduced Inorganic Sulfur Compounds by Bacteria: Emergence of a Common Mechanism?" *Applied and Environmental Microbiology*, vol. 67, no. 7, July 2001, pp. 2873–82, <https://doi.org/10.1128/aem.67.7.2873-2882.2001>.
- Gardner, Anne M., et al. "Flavorubredoxin, an Inducible Catalyst for Nitric Oxide Reduction and Detoxification in Escherichia Coli." *Journal of Biological Chemistry*, vol. 277, no. 10, Mar. 2002, pp. 8172–77, <https://doi.org/10.1074/jbc.m110471200>. Accessed 10 Jan. 2023.
- Gillespie, Louis J. "REDUCTION POTENTIALS of BACTERIAL CULTURE and of WATER-LOGGED SOILS." *Soil Science*, vol. 9, no. 4, Apr. 1920, pp. 199–216, <https://doi.org/10.1097/00010694-192004000-00001>. Accessed 6 Jan. 2022.
- Gisriel, Christopher, et al. "The Structure of Photosystem I Acclimated to Far-Red Light Illuminates an Ecologically Important Acclimation Process in Photosynthesis." *Science Advances*, vol. 6, no. 6, American Association for the Advancement of Science, Feb. 2020, pp. eaay6415–15, <https://doi.org/10.1126/sciadv.aay6415>. Accessed 16 Jan. 2026.
- Grabherr, Manfred G., et al. "Full-Length Transcriptome Assembly from RNA-Seq Data without a Reference Genome." *Nature Biotechnology*, vol. 29, no. 7, May 2011, pp. 644–52, <https://doi.org/10.1038/nbt.1883>.
- Graham, E. D., et al. "Potential for Primary Productivity in a Globally-Distributed Bacterial Phototroph." *The ISME Journal*, vol. 12, no. 7, Mar. 2018, pp. 1861–66, <https://doi.org/10.1038/s41396-018-0091-3>. Accessed 2 Jan. 2026.
- Greco, Carla, et al. "Microbial Diversity of Pinnacle and Conical Microbial Mats in the Perennially Ice-Covered Lake Untersee, East Antarctica." *Frontiers in Microbiology*, vol. 11, Frontiers Media, Dec. 2020, <https://doi.org/10.3389/fmicb.2020.607251>. Accessed 3 Apr. 2024.

- Green, William J., et al. “Geochemical Processes in the Lake Fryxell Basin (Victoria Land, Antarctica).” *Hydrobiologia*, vol. 172, no. 1, Springer Science+Business Media, Mar. 1989, pp. 129–48, <https://doi.org/10.1007/bf00031617>. Accessed 17 May 2024.
- Greub, Gilbert, and Didier Raoult. “Parachlamydiaceae: Potential Emerging Pathogens.” *Emerging Infectious Diseases*, vol. 8, no. 6, June 2002, pp. 626–30, <https://doi.org/10.3201/eid0806.010210>. Accessed 14 Sept. 2021.
- Grund, Tamara N., et al. “Mechanistic and Structural Diversity between Cytochrome *Bd* Isoforms of *Escherichia Coli*.” *PNAS*, vol. 118, no. 50, Dec. 2021, <https://doi.org/10.1073/pnas.2114013118>.
- Guillou, Laure, et al. “The Protist Ribosomal Reference Database (PR2): A Catalog of Unicellular Eukaryote Small Sub-Unit RRNA Sequences with Curated Taxonomy.” *Nucleic Acids Research*, vol. 41, no. D1, Nov. 2012, pp. D597–604, <https://doi.org/10.1093/nar/gks1160>.
- Gurevich, Alexey, et al. “QUAST: Quality Assessment Tool for Genome Assemblies.” *Bioinformatics*, vol. 29, no. 8, Feb. 2013, pp. 1072–75, <https://doi.org/10.1093/bioinformatics/btt086>.
- Haas, BJ. “TransDecoder.” *GitHub*, 24 Apr. 2023, github.com/TransDecoder/TransDecoder.
- Häggglund, Emil, et al. “Origin and Evolution of Key Enzymes in the Anammox Pathway Revisited.” *Genome Biology and Evolution*, edited by Tal Dagan, vol. 18, no. 1, Dec. 2025, <https://doi.org/10.1093/gbe/evaf244>. Accessed 23 Jan. 2026.
- Halbleib, Cale M., and Paul W. Ludden. “Regulation of Biological Nitrogen Fixation.” *The Journal of Nutrition*, vol. 130, no. 5, May 2000, pp. 1081–84, <https://doi.org/10.1093/jn/130.5.1081>.
- Hamada, M., et al. “Cbb3-Type Cytochrome c Oxidases, Aerobic Respiratory Enzymes, Impact the Anaerobic Life of *Pseudomonas Aeruginosa* PAO1.” *Journal of Bacteriology*, vol. 196, no. 22, Sept. 2014, pp. 3881–89, <https://doi.org/10.1128/jb.01978-14>. Accessed 2 Oct. 2020.

- Hamilton, Trinity L., et al. “The Role of Biology in Planetary Evolution: Cyanobacterial Primary Production in Low-Oxygen Proterozoic Oceans.” *Environmental Microbiology*, vol. 18, no. 2, Dec. 2015, pp. 325–40, <https://doi.org/10.1111/1462-2920.13118>.
- Hanada, Satoshi. “Anoxygenic Photosynthesis —a Photochemical Reaction That Does Not Contribute to Oxygen Reproduction—.” *Microbes and Environments*, vol. 31, no. 1, 2016, pp. 1–3, <https://doi.org/10.1264/jsme2.me3101rh>. Accessed 3 Oct. 2019.
- Hanke, Kasper, et al. “Extreme Low Light Requirement for Algae Growth underneath Sea Ice: A Case Study from Station Nord, NE Greenland.” *Journal of Geophysical Research: Oceans*, vol. 123, no. 2, Feb. 2018, pp. 985–1000, <https://doi.org/10.1002/2017jc013263>.
- Hanson, Thomas E., et al. “Phototrophic Sulfide Oxidation: Environmental Insights and a Method for Kinetic Analysis.” *Frontiers in Microbiology*, vol. 4, 2013, <https://doi.org/10.3389/fmicb.2013.00382>. Accessed 27 Jan. 2026.
- Hauser, Maria, et al. “MMseqs Software Suite for Fast and Deep Clustering and Searching of Large Protein Sequence Sets.” *Bioinformatics*, vol. 32, no. 9, Jan. 2016, pp. 1323–30, <https://doi.org/10.1093/bioinformatics/btw006>. Accessed 1 Feb. 2021.
- Hawes, Ian, and Anne-Maree Schwarz. “PHOTOSYNTHESIS in an EXTREME SHADE ENVIRONMENT: BENTHIC MICROBIAL MATS from LAKE HOARE, a PERMANENTLY ICE-COVERED ANTARCTIC LAKE.” *Journal of Phycology*, vol. 35, no. 3, Wiley, June 1999, pp. 448–59, <https://doi.org/10.1046/j.1529-8817.1999.3530448.x>. Accessed 31 Mar. 2025.
- Herbei, Radu, et al. “Hydrological Controls on Ecosystem Dynamics in Lake Fryxell, Antarctica.” *PLOS ONE*, edited by Judi Hewitt, vol. 11, no. 7, July 2016, p. e0159038, <https://doi.org/10.1371/journal.pone.0159038>. Accessed 3 Feb. 2021.
- Hillyer, Katie E., et al. “Multi-Omics Eco-Surveillance of Bacterial Community Function in Legacy Contaminated Estuary Sediments.” *SSRN Electronic Journal*, 2022, <https://doi.org/10.2139/ssrn.4189287>. Accessed 22 Nov. 2025.

- Hiroshi Ohmoto, and Antonio C. Lasaga. “Kinetics of Reactions between Aqueous Sulfates and Sulfides in Hydrothermal Systems.” *Geochimica et Cosmochimica Acta*, vol. 46, no. 10, Elsevier BV, Oct. 1982, pp. 1727–45, [https://doi.org/10.1016/0016-7037\(82\)90113-2](https://doi.org/10.1016/0016-7037(82)90113-2). Accessed 1 May 2023.
- Hodgskiss, Logan H., et al. “Correction To: Unexpected Complexity of the Ammonia Monooxygenase in Archaea.” *The ISME Journal*, vol. 17, no. 6, Apr. 2023, pp. 947–47, <https://doi.org/10.1038/s41396-023-01403-2>. Accessed 23 Jan. 2026.
- Hopkinson, B. M., et al. “Efficiency of the CO₂-Concentrating Mechanism of Diatoms.” *Proceedings of the National Academy of Sciences*, vol. 108, no. 10, Feb. 2011, pp. 3830–37, <https://doi.org/10.1073/pnas.1018062108>.
- Hoppe, Clara J. M., et al. “Photosynthetic Light Requirement near the Theoretical Minimum Detected in Arctic Microalgae.” *Nature Communications*, vol. 15, no. 1, Nature Portfolio, Sept. 2024, <https://doi.org/10.1038/s41467-024-51636-8>.
- Howard-Williams, Clive, et al. “Optical Properties of the Mcmurdo Dry Valley Lakes, Antarctica.” *Antarctic Research Series*, American Geophysical Union, Mar. 2013, pp. 189–203, <https://doi.org/10.1029/ar072p0189>. Accessed 18 Feb. 2026.
- Huang, Wen-Cong, et al. “Comparative Genomic Analysis Reveals Metabolic Flexibility of Woesearchaeota.” *Nature Communications*, vol. 12, no. 1, Nature Portfolio, Sept. 2021, <https://doi.org/10.1038/s41467-021-25565-9>. Accessed 2 Sept. 2023.
- Hyatt, Doug, et al. “Prodigal: Prokaryotic Gene Recognition and Translation Initiation Site Identification.” *BMC Bioinformatics*, vol. 11, no. 1, Mar. 2010, <https://doi.org/10.1186/1471-2105-11-119>.
- Iwashita, Tomoki, et al. “Diverse Members of the Phylum Armatimonadota Promote the Growth of Aquatic Plants, Duckweeds.” *International Journal of Molecular Sciences*, vol. 26, no. 19, Oct. 2025, p. 9824, <https://doi.org/10.3390/ijms26199824>. Accessed 22 Apr. 2026.
- Jacq-Bailly, Aurore, et al. “Electrochemical Characterization of a Complex FeFe Hydrogenase, the Electron-Bifurcating Hnd from Desulfovibrio Fructosovorans.” *Frontiers in*

- Chemistry*, vol. 8, Frontiers Media, Jan. 2021,
<https://doi.org/10.3389/fchem.2020.573305>. Accessed 10 June 2024.
- Jacqui, Stuart, et al. “Not so Toxic: Antarctic Pseudo-Nitzschia Isolates Give Domoic Acid the Cold Shoulder.” *UNESCO Harmful Algae News*, no. 77, Dec. 2024, pp. 13–16,
<https://doi.org/10.5281/zenodo.14364072>.
- Jian, Jianbo, et al. “A High-Quality Genome of the Early Diverging Tychoplanktonic Diatom *Paralia Guyana*.” *Scientific Data*, vol. 11, no. 1, Oct. 2024,
<https://doi.org/10.1038/s41597-024-03843-7>. Accessed 13 Mar. 2025.
- Jiang, Xi, et al. “Exploiting Genetic Diversity and Gene Synthesis to Identify Superior Nitrogenase NifH Protein Variants to Engineer N₂-Fixation in Plants.” *Communications Biology*, vol. 4, no. 1, Jan. 2021, pp. 1–11, <https://doi.org/10.1038/s42003-020-01536-6>.
 Accessed 2 June 2021.
- Jiang, Ying-Ying, et al. “The Impact of Oxygen on Metabolic Evolution: A Chemoinformatic Investigation.” *PLoS Computational Biology*, vol. 8, no. 3, International Society for Computational Biology, Mar. 2012, pp. e1002426–26,
<https://doi.org/10.1371/journal.pcbi.1002426>. Accessed 3 Mar. 2025.
- Jimenez-Diaz, L., et al. “Pathways for the Degradation of Fatty Acids in Bacteria.” *Aerobic Utilization of Hydrocarbons, Oils and Lipids*, 2017, pp. 1–23,
https://doi.org/10.1007/978-3-319-39782-5_42-1.
- Joli, Nathalie, et al. “Hypometabolism to Survive the Long Polar Night and Subsequent Successful Return to Light in the Diatom *Fragilariopsis cylindrus*.” *New Phytologist*, vol. 241, no. 5, Wiley, Dec. 2023, pp. 2193–208, <https://doi.org/10.1111/nph.19387>.
 Accessed 20 May 2025.
- Jung, Heejung, et al. “Overexpression of Sulfide:quinone Reductase (SQR) in *Acidithiobacillus ferrooxidans* Enhances Sulfur, Pyrite, and Pyrrhotite Oxidation.” *Applied and Environmental Microbiology*, edited by John R. Spear, vol. 91, no. 4, American Society

- for Microbiology, Apr. 2025, <https://doi.org/10.1128/aem.00170-25>. Accessed 6 May 2025.
- Jung, Patrick, et al. “Dark Blue-Green: Cave-Inhabiting Cyanobacteria as a Model for Astrobiology.” *Frontiers in Astronomy and Space Sciences*, vol. 10, Frontiers Media, Feb. 2023, <https://doi.org/10.3389/fspas.2023.1107371>.
- Jungblut, Anne D., et al. “Diversity within Cyanobacterial Mat Communities in Variable Salinity Meltwater Ponds of McMurdo Ice Shelf, Antarctica.” *Environmental Microbiology*, vol. 7, no. 4, Wiley-Blackwell, Apr. 2005, pp. 519–29, <https://doi.org/10.1111/j.1462-2920.2005.00717.x>. Accessed 27 Apr. 2023.
- . “Microbial Mat Communities along an Oxygen Gradient in a Perennially Ice-Covered Antarctic Lake.” *Applied and Environmental Microbiology (Print)*, vol. 82, no. 2, American Society for Microbiology, Jan. 2016, pp. 620–30, <https://doi.org/10.1128/aem.02699-15>. Accessed 3 Apr. 2024.
- Kamp, A., et al. “Diatoms Respire Nitrate to Survive Dark and Anoxic Conditions.” *Proceedings of the National Academy of Sciences*, vol. 108, no. 14, Mar. 2011, pp. 5649–54, <https://doi.org/10.1073/pnas.1015744108>.
- Kamp, Anja, et al. “Anaerobic Sulfide Oxidation with Nitrate by a Freshwater Beggiatoa Enrichment Culture.” *Applied and Environmental Microbiology*, vol. 72, no. 7, July 2006, pp. 4755–60, <https://doi.org/10.1128/aem.00163-06>. Accessed 19 Jan. 2022.
- Kanda, Tomoki, and Hiroshi Ishikita. “Redox Potentials of Iron–Sulfur Clusters in Type I Photosynthetic Reaction Centers.” *The Journal of Physical Chemistry B*, vol. 127, no. 22, May 2023, pp. 4998–5004, <https://doi.org/10.1021/acs.jpcc.3c01071>. Accessed 10 Feb. 2026.
- Kang, Dongwan D., et al. “MetaBAT 2: An Adaptive Binning Algorithm for Robust and Efficient Genome Reconstruction from Metagenome Assemblies.” *PeerJ*, vol. 7, July 2019, p. e7359, <https://doi.org/10.7717/peerj.7359>.

- Kappler, Ulrike, and Christiane Dahl. "Enzymology and Molecular Biology of Prokaryotic Sulfite Oxidation." *FEMS Microbiology Letters*, vol. 203, no. 1, Sept. 2001, pp. 1–9, <https://doi.org/10.1111/j.1574-6968.2001.tb10813.x>. Accessed 24 Apr. 2021.
- Karr, Elizabeth A., et al. "Biodiversity of Methanogenic and Other Archaea in the Permanently Frozen Lake Fryxell, Antarctica." *Applied and Environmental Microbiology*, vol. 72, no. 2, Feb. 2006, pp. 1663–66, <https://doi.org/10.1128/aem.72.2.1663-1666.2006>. Accessed 14 Aug. 2020.
- Priscu, J.C. 2022. *Nitrogen and phosphorus concentrations in discrete water column samples collected from lakes in the McMurdo Dry Valleys, Antarctica (1993-2020, ongoing)*. Environmental Data Initiative. DOI: 10.6073/pasta/5cba7e25aa687c1e989c72c3ee0a0f69. Dataset accessed 17 December 2025.
- . "Remarkable Diversity of Phototrophic Purple Bacteria in a Permanently Frozen Antarctic Lake." *Applied and Environmental Microbiology*, vol. 69, no. 8, Aug. 2003, pp. 4910–14, <https://doi.org/10.1128/aem.69.8.4910-4914.2003>.
- Kasai, Takuya, et al. "Roles of D-Lactate Dehydrogenases in the Anaerobic Growth of *Shewanella Oneidensis* MR-1 on Sugars." *Applied and Environmental Microbiology*, vol. 85, no. 3, Feb. 2019, pp. e02668-18, <https://doi.org/10.1128/AEM.02668-18>.
- Kato, Shingo, et al. "Insights into Ecological Roles of Uncultivated Bacteria in Katase Hot Spring Sediment from Long-Read Metagenomics." *Frontiers in Microbiology*, vol. 13, Nov. 2022, <https://doi.org/10.3389/fmicb.2022.1045931>. Accessed 6 Feb. 2023.
- Katsyv, Alexander, et al. "Molecular Basis of the Electron Bifurcation Mechanism in the [FeFe]-Hydrogenase Complex HydABC." *Journal of the American Chemical Society*, vol. 145, no. 10, American Chemical Society, Feb. 2023, pp. 5696–709, <https://doi.org/10.1021/jacs.2c11683>. Accessed 24 Nov. 2023.

- Kehl, Alexander J., et al. *Diversity-Driven Biochemical Survey Reveals Dimeric Structural Origin of Rubisco*. openRxiv, Nov. 2025, <https://doi.org/10.1101/2025.11.05.686826>. Accessed 7 Feb. 2026.
- Keller, Lisa M., et al. “Simultaneous Aerobic and Anaerobic Respiration in Hot Spring Chemolithotrophic Bacteria.” *Nature Communications*, vol. 16, no. 1, Nature Portfolio, Jan. 2025, <https://doi.org/10.1038/s41467-025-56418-4>. Accessed 8 Feb. 2025.
- Kennett, D. M., and P. E. Hargraves. “Benthic Diatoms and Sulfide Fluctuations: Upper Basin of Pettaquamscutt River, Rhode Island.” *Estuarine, Coastal and Shelf Science*, vol. 21, no. 4, Oct. 1985, pp. 577–86, [https://doi.org/10.1016/0272-7714\(85\)90058-7](https://doi.org/10.1016/0272-7714(85)90058-7). Accessed 25 Mar. 2021.
- Klatt, Judith M., et al. “Cyanobacteria in Sulfidic Spring Microbial Mats Can Perform Oxygenic and Anoxygenic Photosynthesis Simultaneously during an Entire Diurnal Period.” *Frontiers in Microbiology*, vol. 7, Dec. 2016, <https://doi.org/10.3389/fmicb.2016.01973>. Accessed 7 Feb. 2021.
- Klatt, Judith M., and Lubos Polerecky. “Assessment of the Stoichiometry and Efficiency of CO₂ Fixation Coupled to Reduced Sulfur Oxidation.” *Frontiers in Microbiology*, vol. 6, May 2015, <https://doi.org/10.3389/fmicb.2015.00484>.
- Koch, Hanna, et al. “Expanded Metabolic Versatility of Ubiquitous Nitrite-Oxidizing Bacteria from the Genus *Nitrospira*.” *Proceedings of the National Academy of Sciences*, vol. 112, no. 36, Aug. 2015, pp. 11371–76, <https://doi.org/10.1073/pnas.1506533112>.
- Kolmogorov, Mikhail, et al. “Assembly of Long, Error-Prone Reads Using Repeat Graphs.” *Nature Biotechnology*, vol. 37, no. 5, Apr. 2019, pp. 540–46, <https://doi.org/10.1038/s41587-019-0072-8>.
- Kop, Linnea F. M., et al. “Comparative Genome Analysis Reveals Broad Phylogenetic and Functional Diversity within the Order *Nitrospirales*.” *The ISME Journal*, Springer Nature, July 2025, <https://doi.org/10.1093/ismejo/wraf151>.

- Kpebe, Arlette, et al. “An Essential Role of the Reversible Electron-Bifurcating Hydrogenase Hnd for Ethanol Oxidation in *Solidesulfovibrio Fructosivorans*.” *Frontiers in Microbiology*, vol. 14, Frontiers Media, Mar. 2023, <https://doi.org/10.3389/fmicb.2023.1139276>. Accessed 7 June 2024.
- Kučera, Igor, and Vojtěch Sedláček. “Involvement of the Cbb3-Type Terminal Oxidase in Growth Competition of Bacteria, Biofilm Formation, and in Switching between Denitrification and Aerobic Respiration.” *Microorganisms*, vol. 8, no. 8, Aug. 2020, p. 1230, <https://doi.org/10.3390/microorganisms8081230>. Accessed 30 Aug. 2020.
- Kumita, Hideyuki, et al. “NO Reduction by Nitric-Oxide Reductase from Denitrifying Bacterium *Pseudomonas Aeruginosa*.” *Journal of Biological Chemistry*, vol. 279, no. 53, Dec. 2004, pp. 55247–54, <https://doi.org/10.1074/jbc.m409996200>. Accessed 7 Dec. 2022.
- Kushkevych, Ivan, et al. “Anoxygenic Photosynthesis in Photolithotrophic Sulfur Bacteria and Their Role in Detoxication of Hydrogen Sulfide.” *Antioxidants*, vol. 10, no. 6, June 2021, p. 829, <https://doi.org/10.3390/antiox10060829>.
- Kützing, Friedrich Traugott. *Die Kieselchaligen Bacillarien Oder Diatomeen*. Zu finden bei W. Köhne, 1844, <https://doi.org/10.5962/bhl.title.64360>. Accessed 1 May 2026.
- Lan, Jiacheng, et al. “The Shift of Soil Bacterial Community after Afforestation Influence Soil Organic Carbon and Aggregate Stability in Karst Region.” *Frontiers in Microbiology*, vol. 13, Frontiers Media, June 2022, <https://doi.org/10.3389/fmicb.2022.901126>. Accessed 30 Nov. 2023.
- Latifi, Amel, et al. “Clostridial Whole Cell and Enzyme Systems for Hydrogen Production: Current State and Perspectives.” *Applied Microbiology and Biotechnology*, vol. 103, no. 2, Nov. 2018, pp. 567–75, <https://doi.org/10.1007/s00253-018-9514-3>.
- Lawrence, M. J. F., and C. H. Hendy. “Water Column and Sediment Characteristics of Lake Fryxell, Taylor Valley, Antarctica.” *New Zealand Journal of Geology and Geophysics*, vol. 28, no. 3, Taylor & Francis, July 1985, pp. 543–52, <https://doi.org/10.1080/00288306.1985.10421206>. Accessed 14 Feb. 2025.

- Laybourn-Parry, Johanna, and David A. Pearce. “The Biodiversity and Ecology of Antarctic Lakes: Models for Evolution.” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 362, no. 1488, May 2007, pp. 2273–89, <https://doi.org/10.1098/rstb.2006.1945>.
- Lee, Hanbyul, et al. “Microbial Assemblages and Associated Biogeochemical Processes in Lake Bonney, a Permanently Ice-Covered Lake in the McMurdo Dry Valleys, Antarctica.” *Environmental Microbiome*, vol. 19, no. 1, BioMed Central, Aug. 2024, pp. 60–60, <https://doi.org/10.1186/s40793-024-00605-1>. Accessed 8 Dec. 2025.
- Lefentse Mashamaite, et al. “Microbial Diversity in Antarctic Dry Valley Soils across an Altitudinal Gradient.” *Frontiers in Microbiology*, vol. 14, Frontiers Media, July 2023, <https://doi.org/10.3389/fmicb.2023.1203216>. Accessed 23 Mar. 2024.
- Lepik, Kaido, et al. “LiMA: Robust Inference of Molecular Mediation from Summary Statistics.” *The American Journal of Human Genetics*, vol. 113, no. 1, Elsevier BV, Jan. 2026, pp. 202–20, <https://doi.org/10.1016/j.ajhg.2025.12.005>. Accessed 14 Mar. 2026.
- Li, Heng. “Minimap2: Pairwise Alignment for Nucleotide Sequences.” *Bioinformatics*, edited by Inanc Birol, vol. 34, no. 18, May 2018, pp. 3094–100, <https://doi.org/10.1093/bioinformatics/bty191>.
- Li, Liuyang, et al. “Globally Distributed Myxococcota with Photosynthesis Gene Clusters Illuminate the Origin and Evolution of a Potentially Chimeric Lifestyle.” *Nature Communications*, vol. 14, no. 1, Nature Portfolio, Oct. 2023, <https://doi.org/10.1038/s41467-023-42193-7>. Accessed 30 June 2024.
- Li, Qing-Mei, et al. “Phylogenomic Insights into Distribution and Adaptation of Bdellovibrionota in Marine Waters.” *Microorganisms*, vol. 9, no. 4, Apr. 2021, p. 757, <https://doi.org/10.3390/microorganisms9040757>. Accessed 15 June 2021.
- Li, Wei, and Rachael M. Morgan-Kiss. “Influence of Environmental Drivers and Potential Interactions on the Distribution of Microbial Communities from Three Permanently

- Stratified Antarctic Lakes.” *Frontiers in Microbiology*, vol. 10, Frontiers Media, May 2019, <https://doi.org/10.3389/fmicb.2019.01067>. Accessed 7 Mar. 2025.
- Liu, Feng, et al. “The Radioresistant and Survival Mechanisms of *Deinococcus Radiodurans*.” *Radiation Medicine and Protection*, vol. 4, no. 2, Mar. 2023, <https://doi.org/10.1016/j.radmp.2023.03.001>.
- Liu, Li, et al. “(S)-3-Hydroxybutyryl-CoA Dehydrogenase from the Autotrophic 3-Hydroxypropionate/4-Hydroxybutyrate Cycle in *Nitrosopumilus Maritimus*.” *Frontiers in Microbiology*, vol. 12, July 2021, <https://doi.org/10.3389/fmicb.2021.712030>. Accessed 23 Jan. 2026.
- Liu, Lu-Yao, et al. “Anaerobic Ammonium Oxidation Coupled with Sulfate Reduction Links Nitrogen with Sulfur Cycle.” *Bioresource Technology*, vol. 403, Elsevier BV, July 2024, pp. 130903–3, <https://doi.org/10.1016/j.biortech.2024.130903>. Accessed 8 Sept. 2024.
- Lum, Aaron, et al. “Core Genome of *Deinococcota* Phylum from 72 Strains across 40 Species Consist of Only One Gene, Beta Subunit of DNA-Directed RNA Polymerase.” *Medicon Microbiology*, vol. 3, no. 1, Jan. 2024, <https://doi.org/10.55162/mcmmi.03.047>. Accessed 17 Aug. 2024.
- Lumian, Jessica E., et al. “Metabolic Capacity of the Antarctic Cyanobacterium *Phormidium Pseudopriestleyi* That Sustains Oxygenic Photosynthesis in the Presence of Hydrogen Sulfide.” *Genes*, vol. 12, no. 3, Mar. 2021, p. 426, <https://doi.org/10.3390/genes12030426>. Accessed 2 Dec. 2025.
- Lundholm, Nina, et al. “Diversity, Toxicity, and Distribution of Potentially Toxic Diatoms in Antarctic Waters—with Description of *Pseudo-Nitzschia Meridionalis* Sp. Nov. And *P. Glacialis* Sp. Nov.” *Harmful Algae*, vol. 139, Elsevier BV, Sept. 2024, pp. 102724–24, <https://doi.org/10.1016/j.hal.2024.102724>. Accessed 10 Dec. 2025.
- Maalcke, Wouter J., et al. “Characterization of Anammox Hydrazine Dehydrogenase, a Key N₂-Producing Enzyme in the Global Nitrogen Cycle.” *Journal of Biological Chemistry*/□the

- *Journal of Biological Chemistry*, vol. 291, no. 33, Elsevier BV, Aug. 2016, pp. 17077–92, <https://doi.org/10.1074/jbc.m116.735530>. Accessed 20 June 2024.
- madler. “GitHub - Madler/Pigz: A Parallel Implementation of Gzip for Modern Multi-Processor, Multi-Core Machines.” *GitHub*, 2022, github.com/madler/pigz.
- Maguire, Finlay, et al. “Metagenome-Assembled Genome Binning Methods with Short Reads Disproportionately Fail for Plasmids and Genomic Islands.” *Microbial Genomics*, Oct. 2020, <https://doi.org/10.1099/mgen.0.000436>.
- Mai, Wenning, et al. “Advances in Studies on Microbiota Involved in Nitrogen Removal Processes and Their Applications in Wastewater Treatment.” *Frontiers in Microbiology*, vol. 12, Frontiers Media, Sept. 2021, <https://doi.org/10.3389/fmicb.2021.746293>.
- Maire, Justin, et al. “Chlamydiae as Symbionts of Photosynthetic Dinoflagellates.” *The ISME Journal*, vol. 18, no. 1, Springer Nature, Jan. 2024, <https://doi.org/10.1093/ismejo/wrae139>. Accessed 19 Nov. 2025.
- . “Chlamydiae in Corals: Shared Functional Potential despite Broad Taxonomic Diversity.” *ISME Communications*, vol. 4, no. 1, Springer Nature, Jan. 2024, pp. ycae054–54, <https://doi.org/10.1093/ismeco/ycae054>. Accessed 19 Nov. 2025.
- Malviya, Shruti, et al. “Insights into Global Diatom Distribution and Diversity in the World’s Ocean.” *Proceedings of the National Academy of Sciences*, vol. 113, no. 11, Feb. 2016, pp. E1516–25, <https://doi.org/10.1073/pnas.1509523113>.
- Marcia, Marco, et al. “The Structure of *Aquifex Aeolicus* Sulfide:quinone Oxidoreductase, a Basis to Understand Sulfide Detoxification and Respiration.” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 24, National Academy of Sciences, June 2009, pp. 9625–30, <https://doi.org/10.1073/pnas.0904165106>. Accessed 6 Nov. 2023.
- Martineau, Edouard, et al. “Characterisation of Antarctic Cyanobacteria and Comparison with New Zealand Strains.” *Hydrobiologia*, vol. 711, no. 1, Mar. 2013, pp. 139–54, <https://doi.org/10.1007/s10750-013-1473-1>. Accessed 8 Apr. 2022.

- Martínez-Pérez, Clara, et al. “Phylogenetically and Functionally Diverse Microorganisms Reside under the Ross Ice Shelf.” *Nature Communications*, vol. 13, no. 1, Nature Portfolio, Jan. 2022, <https://doi.org/10.1038/s41467-021-27769-5>. Accessed 21 Mar. 2025.
- McFarlane, Ciaran R., et al. “Structural Basis of Light-Induced Redox Regulation in the Calvin–Benson Cycle in Cyanobacteria.” *Proceedings of the National Academy of Sciences*, vol. 116, no. 42, Oct. 2019, pp. 20984–90, <https://doi.org/10.1073/pnas.1906722116>. Accessed 12 Jan. 2021.
- McKnight, Diane M., et al. “Dry Valley Streams in Antarctica: Ecosystems Waiting for Water.” *BioScience*, vol. 49, no. 12, Dec. 1999, pp. 985–95, <https://doi.org/10.1525/bisi.1999.49.12.985>.
- McKnight, Diane M., et al. “PHYTOPLANKTON DYNAMICS in a STABLY STRATIFIED ANTARCTIC LAKE during WINTER DARKNESS.” *Journal of Phycology*, vol. 36, no. 5, Wiley-Blackwell, Oct. 2000, pp. 852–61, <https://doi.org/10.1046/j.1529-8817.2000.00031.x>. Accessed 8 Jan. 2024.
- “McMurdo Dry Valleys LTER.” *Lternet.edu*, 2026, mcm.lternet.edu. Accessed 19 Jan. 2026.
- McQueen, J. Parr, et al. “Host Identity Is the Dominant Factor in the Assembly of Nematode and Tardigrade Gut Microbiomes in Antarctic Dry Valley Streams.” *Scientific Reports*, vol. 12, no. 1, Nov. 2022, p. 20118, <https://doi.org/10.1038/s41598-022-24206-5>. Accessed 13 Apr. 2023.
- Medeiros, William, et al. “Unlocking the Biosynthetic Potential and Taxonomy of the Antarctic Microbiome along Temporal and Spatial Gradients.” *Microbiology Spectrum*, vol. 12, no. 6, American Society for Microbiology, May 2024, <https://doi.org/10.1128/spectrum.00244-24>. Accessed 19 Oct. 2025.
- Medeiros, William B., et al. “Antarctic Soil Prokaryotic Diversity: A Dataset of 319 Metagenome-Assembled Genomes from Deception and Livingston Islands.” *Microbiology Resource Announcements*, edited by Simon Roux, vol. 15, no. 3,

- American Society for Microbiology, Mar. 2026, <https://doi.org/10.1128/mra.01346-25>. Accessed 22 Apr. 2026.
- Mehdizadeh Allaf, Malihe, and Hassan Peerhossaini. “Cyanobacteria: Model Microorganisms and Beyond.” *Microorganisms*, vol. 10, no. 4, Mar. 2022, p. 696, <https://doi.org/10.3390/microorganisms10040696>.
- Meloni, Maria, et al. “Ribulose-1,5-Bisphosphate Regeneration in the Calvin-Benson-Bassham Cycle: Focus on the Last Three Enzymatic Steps That Allow the Formation of Rubisco Substrate.” *Frontiers in Plant Science*, vol. 14, 2023, p. 1130430, <https://doi.org/10.3389/fpls.2023.1130430>. Accessed 6 May 2023.
- Menzel, Peter, et al. “Fast and Sensitive Taxonomic Classification for Metagenomics with Kaiju.” *Nature Communications*, vol. 7, no. 1, Apr. 2016, <https://doi.org/10.1038/ncomms11257>.
- Merz, Elisa, et al. “Nitrate Respiration and Diel Migration Patterns of Diatoms Are Linked in Sediments underneath a Microbial Mat.” *Environmental Microbiology*, vol. 23, no. 3, Wiley-Blackwell, Dec. 2020, pp. 1422–35, <https://doi.org/10.1111/1462-2920.15345>.
- . “Sulphide Stimulates Nitrate Reduction in Benthic Diatoms from a Microbial Mat.” *EGU General Assembly Conference Abstracts*, May 2022, pp. EGU22-7981.
- Momper, Lily, et al. “Energy and Carbon Metabolisms in a Deep Terrestrial Subsurface Fluid Microbial Community.” *The ISME Journal*, vol. 11, no. 10, June 2017, pp. 2319–33, <https://doi.org/10.1038/ismej.2017.94>. Accessed 13 Nov. 2020.
- Morimoto, Kenji, et al. “Overexpression of a Hydrogenase Gene in *Clostridium Paraputrificum* to Enhance Hydrogen Gas Production.” *FEMS Microbiology Letters*, vol. 246, no. 2, Autumn 2005, pp. 229–34, <https://doi.org/10.1016/j.femsle.2005.04.014>.
- Nef, Charlotte, et al. “Whole-Genome Scanning Reveals Environmental Selection Mechanisms That Shape Diversity in Populations of the Epipelagic Diatom *Chaetoceros*.” *PLOS Biology*, vol. 20, no. 11, Nov. 2022, p. e3001893, <https://doi.org/10.1371/journal.pbio.3001893>. Accessed 6 Sept. 2023.

- Nie, Wen-Bo, et al. “Simultaneous Nitrate and Sulfate Dependent Anaerobic Oxidation of Methane Linking Carbon, Nitrogen and Sulfur Cycles.” *Water Research*, vol. 194, Apr. 2021, p. 116928, <https://doi.org/10.1016/j.watres.2021.116928>. Accessed 5 Apr. 2021.
- Niel, C. B. “On the Morphology and Physiology of the Purple and Green Sulphur Bacteria.” *Archiv Für Mikrobiologie*, vol. 3, no. 1, 1932, pp. 1–112, <https://doi.org/10.1007/bf00454965>. Accessed 26 May 2020.
- Nikolaou , Athanasios, et al. “The Ratio of Reactive Oxygen and Nitrogen Species Determines the Type of Cell Death That Bacteria Undergo.” *Microbiological Research*, vol. 292, Mar. 2025, <https://doi.org/10.1016/j.micres.2024.127986>.
- Nikulina, Tatiana V., and J. Patrick Kociolek. “Diatoms from Hot Springs from Kuril and Sakhalin Islands (Far East, Russia).” *Cellular Origin, Life in Extreme Habitats and Astrobiology*, Springer Netherlands, 2011, pp. 333–63, https://doi.org/10.1007/978-94-007-1327-7_15. Accessed 20 Mar. 2026.
- Nishihara, Arisa, et al. “Exploring the Diversity and Metabolic Potential of CO₂ Fixation Mediated by RubisCO in Prokaryotes in the Japan Collection of Microorganisms.” *Microbes and Environments*, vol. 41, no. 1, 2026, p. n/a, <https://doi.org/10.1264/jsme2.me25035>. Accessed 27 Jan. 2026.
- Nishimura, Tomohiro, et al. “Update of the Planktonic Diatom Genus Pseudo-Nitzschia in Aotearoa New Zealand Coastal Waters: Genetic Diversity and Toxin Production.” *Toxins*, vol. 13, no. 9, Sept. 2021, p. 637, <https://doi.org/10.3390/toxins13090637>. Accessed 14 Apr. 2023.
- Nou, Nancy O., et al. “Genome-Guided Isolation of the Hyperthermophilic Aerobe Fervidibacter Sacchari Reveals Conserved Polysaccharide Metabolism in the Armatimonadota.” *Nature Communications*, vol. 15, no. 1, Nature Portfolio, Nov. 2024, <https://doi.org/10.1038/s41467-024-53784-3>. Accessed 9 Feb. 2025.

- Olguín Salinas, Héctor F., et al. “Latitudinal Diversity Patterns of Diatoms in the Southwestern Atlantic and Antarctic Waters.” *Journal of Plankton Research*, vol. 37, no. 4, June 2015, pp. 659–65, <https://doi.org/10.1093/plankt/fbv042>. Accessed 6 May 2025.
- Oliveira, Rafael S., et al. “Genome-Resolved Metagenomic Analysis of Great Amazon Reef System Sponge-Associated Latescibacterota Bacteria and Their Potential Contributions to the Host Sponge and Reef.” *Frontiers in Microbiomes*, vol. 2, Frontiers Media, Aug. 2023, <https://doi.org/10.3389/frmbi.2023.1206961>. Accessed 29 Apr. 2024.
- Pandey, C. B., et al. “DNRA: A Short-Circuit in Biological N-Cycling to Conserve Nitrogen in Terrestrial Ecosystems.” *Science of the Total Environment*, vol. 738, Oct. 2020, p. 139710, <https://doi.org/10.1016/j.scitotenv.2020.139710>.
- Parks, Donovan H., et al. “CheckM: Assessing the Quality of Microbial Genomes Recovered from Isolates, Single Cells, and Metagenomes.” *Genome Research*, vol. 25, no. 7, May 2015, pp. 1043–55, <https://doi.org/10.1101/gr.186072.114>.
- Patro, Rob, et al. “Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression.” *Nature Methods*, vol. 14, no. 4, Mar. 2017, pp. 417–19, <https://doi.org/10.1038/nmeth.4197>.
- Paulo, Lara M., et al. “Methanogens, Sulphate and Heavy Metals: A Complex System.” *Reviews in Environmental Science and Bio/Technology*, vol. 14, no. 4, Nov. 2015, pp. 537–53, <https://doi.org/10.1007/s11157-015-9387-1>.
- Payne, Natalie, et al. “The Electron-Bifurcating FeFe-Hydrogenase Hnd Is Involved in Ethanol Metabolism in *Desulfovibrio Fructosovorans* Grown on Pyruvate.” *Molecular Microbiology*, vol. 117, no. 4, Wiley-Blackwell, Feb. 2022, pp. 907–20, <https://doi.org/10.1111/mmi.14881>. Accessed 13 May 2024.
- Payne, Paige E., et al. “Uncovering Novel Functions of the Enigmatic, Abundant, and Active *Anaerolineae* in a Salt Marsh Ecosystem.” *MSystems*, edited by Laetitia G. E. Wilkins, vol. 10, no. 1, American Society for Microbiology, Jan. 2025, <https://doi.org/10.1128/msystems.01162-24>. Accessed 28 Mar. 2025.

- Pearsall, W. H., and C. H. Mortimer. "Oxidation-Reduction Potentials in Waterlogged Soils, Natural Waters and Muds." *Journal of Ecology*, vol. 27, no. 2, Wiley, Aug. 1939, pp. 483–83, <https://doi.org/10.2307/2256375>. Accessed 3 Nov. 2024.
- Pelikan, Claus, et al. "Anaerobic Bacterial Degradation of Protein and Lipid Macromolecules in Subarctic Marine Sediment." *The ISME Journal*, vol. 15, no. 3, Nov. 2020, pp. 833–47, <https://doi.org/10.1038/s41396-020-00817-6>.
- Peters, John W., et al. "A New Era for Electron Bifurcation." *Current Opinion in Chemical Biology*, vol. 47, Dec. 2018, pp. 32–38, <https://doi.org/10.1016/j.cbpa.2018.07.026>. Accessed 17 Dec. 2019.
- Petriglieri, Francesca, et al. "A Comprehensive Overview of the Chloroflexota Community in Wastewater Treatment Plants Worldwide." *MSystems*, vol. 8, no. 6, American Society for Microbiology, Dec. 2023, <https://doi.org/10.1128/msystems.00667-23>.
- Pierella Karlusich, Juan José, et al. "Carbon Dioxide Concentration Mechanisms in Natural Populations of Marine Diatoms: Insights from Tara Oceans." *Frontiers in Plant Science*, vol. 12, Apr. 2021, <https://doi.org/10.3389/fpls.2021.657821>.
- Pike, Jennifer, et al. "Observations on the Relationship between the Antarctic Coastal Diatoms *Thalassiosira Antarctica* Comber and *Porosira Glacialis* (Grunow) Jørgensen and Sea Ice Concentrations during the Late Quaternary." *Marine Micropaleontology*, vol. 73, no. 1-2, Elsevier BV, Oct. 2009, pp. 14–25, <https://doi.org/10.1016/j.marmicro.2009.06.005>. Accessed 11 Sept. 2023.
- Pjevac, Petra, et al. "Community Shift from Phototrophic to Chemotrophic Sulfide Oxidation Following Anoxic Holomixis in a Stratified Seawater Lake." *Applied and Environmental Microbiology*, edited by G. Voordouw, vol. 81, no. 1, Jan. 2015, pp. 298–308, <https://doi.org/10.1128/aem.02435-14>.
- Prieto-Barajas, Cristina M., et al. "Microbial Mat Ecosystems: Structure Types, Functional Diversity, and Biotechnological Application." *Electronic Journal of Biotechnology*, vol. 31, Jan. 2018, pp. 48–56, <https://doi.org/10.1016/j.ejbt.2017.11.001>.

- Puri, Pranav, et al. "Regulation of Acetate Kinase Isozymes and Its Importance for Mixed-Acid Fermentation in *Lactococcus Lactis*." *Journal of Bacteriology*, vol. 196, no. 7, Apr. 2014, pp. 1386–93, <https://doi.org/10.1128/JB.01277-13>.
- Ragsdale, Stephen W., and Elizabeth Pierce. "Acetogenesis and the Wood–Ljungdahl Pathway of CO₂ Fixation." *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1784, no. 12, Dec. 2008, pp. 1873–98, <https://doi.org/10.1016/j.bbapap.2008.08.012>.
- Randelhoff, Achim, et al. "Arctic Mid-Winter Phytoplankton Growth Revealed by Autonomous Profilers." *Science Advances*, vol. 6, no. 39, Sept. 2020, p. eabc2678, <https://doi.org/10.1126/sciadv.abc2678>.
- Rasuk, Maria Cecilia, et al. "Bacterial Diversity in Microbial Mats and Sediments from the Atacama Desert." *Microbial Ecology*, vol. 71, no. 1, July 2015, pp. 44–56, <https://doi.org/10.1007/s00248-015-0649-9>.
- Raven, J. A., et al. "Put out the Light, and Then Put out the Light." *Journal of the Marine Biological Association of the United Kingdom*, vol. 80, no. 1, Feb. 2000, pp. 1–25, <https://doi.org/10.1017/S0025315499001526>. Accessed 28 Oct. 2021.
- Robinson, David, et al. "Antarctic Lake Viromes Reveal Potential Virus Associated Influences on Nutrient Cycling in Ice-Covered Lakes." *Frontiers in Microbiology*, vol. 15, Frontiers Media, Sept. 2024, pp. 1422941–41, <https://doi.org/10.3389/fmicb.2024.1422941>. Accessed 23 Jan. 2026.
- Rognes, Torbjørn, et al. "VSEARCH: A Versatile Open Source Tool for Metagenomics." *PeerJ*, vol. 4, Oct. 2016, p. e2584, <https://doi.org/10.7717/peerj.2584>.
- Sarkar, Abhijit, and Barbara Reinhold-Hurek. "Transcriptional Profiling of Nitrogen Fixation and the Role of NifA in the Diazotrophic Endophyte *Azoarcus* Sp. Strain BH72." *PLoS ONE*, edited by Mark Gijzen, vol. 9, no. 2, Feb. 2014, p. e86527, <https://doi.org/10.1371/journal.pone.0086527>. Accessed 27 Mar. 2020.
- Schulte, Nicholas O., et al. "Blowin' in the Wind: Dispersal, Structure, and Metacommunity Dynamics of Aeolian Diatoms in the McMurdo Sound Region, Antarctica." *Journal of*

- Phycology*, vol. 58, no. 1, Wiley-Blackwell, Dec. 2021, pp. 36–54,
<https://doi.org/10.1111/jpy.13223>. Accessed 3 Apr. 2024.
- Schulz, Horst, and Wolf-H. Kunau. “Beta-Oxidation of Unsaturated Fatty Acids: A Revised Pathway.” *Trends in Biochemical Sciences*, vol. 12, Jan. 1987, pp. 403–6,
[https://doi.org/10.1016/0968-0004\(87\)90196-4](https://doi.org/10.1016/0968-0004(87)90196-4). Accessed 21 Nov. 2020.
- Schut, Gerrit J., et al. “An Abundant and Diverse New Family of Electron Bifurcating Enzymes with a Non-Canonical Catalytic Mechanism.” *Frontiers in Microbiology*, vol. 13, Frontiers Media, July 2022, <https://doi.org/10.3389/fmicb.2022.946711>. Accessed 23 Mar. 2025.
- Seemann, T. “Prokka: Rapid Prokaryotic Genome Annotation.” *Bioinformatics*, vol. 30, no. 14, Mar. 2014, pp. 2068–69, <https://doi.org/10.1093/bioinformatics/btu153>.
- Seemann, Torsten. “Tseemann/Barnnap.” *GitHub*, 4 Feb. 2024, github.com/tseemann/barnnap.
- Sethi, Deepak, et al. “Diatoms for Carbon Sequestration and Bio-Based Manufacturing.” *Biology*, vol. 9, no. 8, Aug. 2020, p. 217,
<https://doi.org/10.3390/biology9080217>.
- Shestakov, S. V., and E. A. Karbysheva. “The Origin and Evolution of Cyanobacteria.” *Biology Bulletin Reviews*, vol. 7, no. 4, July 2017, pp. 259–72,
<https://doi.org/10.1134/s2079086417040090>.
- Shibabaw, Tebkew, et al. “Diatom Community Structure in Relation to Environmental Factors in Human Influenced Rivers and Streams in Tropical Africa.” *PLOS ONE*, edited by Jean-François Humbert, vol. 16, no. 2, Feb. 2021, p. e0246043,
<https://doi.org/10.1371/journal.pone.0246043>. Accessed 12 May 2023.
- Smith, Erin P., and Raphael H. Valdivia. “*Chlamydia Trachomatis* : A Model for Intracellular Bacterial Parasitism.” *Journal of Bacteriology*, American Society for Microbiology, Feb. 2025, <https://doi.org/10.1128/jb.00361-24>.

- Sohm, Jill A., et al. “Microbial Mats of the McMurdo Dry Valleys, Antarctica: Oases of Biological Activity in a Very Cold Desert.” *Frontiers in Microbiology*, vol. 11, Frontiers Media, Oct. 2020, <https://doi.org/10.3389/fmicb.2020.537960>.
- Soler-Jofra, Aina, et al. “Hydroxylamine and the Nitrogen Cycle: A Review.” *Water Research*, vol. 190, Feb. 2021, p. 116723, <https://doi.org/10.1016/j.watres.2020.116723>.
- Solomon, Joseph B., et al. “Ammonia Synthesis via an Engineered Nitrogenase Assembly Pathway in Escherichia Coli.” *Nature Catalysis*, vol. 7, no. 10, Nature Portfolio, Sept. 2024, pp. 1130–41, <https://doi.org/10.1038/s41929-024-01229-x>. Accessed 28 Jan. 2025.
- Song, Weizhi, et al. “MarkerMAG: Linking Metagenome-Assembled Genomes (MAGs) with 16S rRNA Marker Genes Using Paired-End Short Reads.” *Bioinformatics*, vol. 38, no. 15, Oxford University Press, June 2022, pp. 3684–88, <https://doi.org/10.1093/bioinformatics/btac398>.
- Soule, Malcolm H. “Oxidation-Reduction Potentials in Bacteriology and Biochemistry.” *American Journal of Clinical Pathology*, vol. 21, no. 2, Feb. 1951, pp. 164.1-164, <https://doi.org/10.1093/ajcp/21.2.164>. Accessed 9 Apr. 2020.
- Sparacino-Watkins, Courtney, et al. “Nitrate and Periplasmic Nitrate Reductases.” *Chemical Society Reviews*, vol. 43, no. 2, Jan. 2014, pp. 676–706, <https://doi.org/10.1039/c3cs60249d>.
- Spaulding, Sarah A., et al. “Phytoplankton Population Dynamics in Perennially Ice-Covered Lake Fryxell, Antarctica.” *Journal of Plankton Research*, vol. 16, no. 5, 1994, pp. 527–41, <https://doi.org/10.1093/plankt/16.5.527>. Accessed 3 Mar. 2021.
- Srinivas, Pooja, et al. “Beyond Genomics in Patescibacteria: A Trove of Unexplored Biology Packed into Ultrasmall Bacteria.” *Proceedings of the National Academy of Sciences*, vol. 121, no. 51, National Academy of Sciences, Dec. 2024, <https://doi.org/10.1073/pnas.2419369121>.
- Stanish, Lee F., et al. “Drifting Along: Using Diatoms to Track the Contribution of Microbial Mats to Particulate Organic Matter Transport in a Glacial Meltwater Stream in the

- McMurdo Dry Valleys, Antarctica.” *Frontiers in Microbiology*, vol. 15, May 2024, <https://doi.org/10.3389/fmicb.2024.1352666>. Accessed 10 Dec. 2025.
- Stock, Willem, et al. “Thermal Niche Differentiation in the Benthic Diatom *Cylindrotheca Closterium* (Bacillariophyceae) Complex.” *Frontiers in Microbiology*, vol. 10, June 2019, <https://doi.org/10.3389/fmicb.2019.01395>.
- Stone, Michael S., et al. “McMurdo Dry Valley Lake Edge ‘Moats’: The Ecological Intersection between Terrestrial and Aquatic Polar Desert Habitats.” *Antarctic Science*, Apr. 2024, pp. 1–17, <https://doi.org/10.1017/S0954102024000087>. Accessed 31 July 2024.
- Stromecki, Amanda, et al. “Unexpected Diversity Found within Benthic Microbial Mats at Hydrothermal Springs in Crater Lake, Oregon.” *Frontiers in Microbiology*, vol. 13, Sept. 2022, <https://doi.org/10.3389/fmicb.2022.876044>. Accessed 9 Nov. 2022.
- Sumner, Dawn Y., et al. *Antarctic Microbial Mats: A Modern Analog for Archean Lacustrine Oxygen Oases*. no. 10, Oct. 2015, pp. 887–90, <https://doi.org/10.1130/g36966.1>. Accessed 30 May 2023.
- Sun, Kai, et al. “Microbial Communities Related to the Sulfur Cycle in the Sansha Yongle Blue Hole.” *Microbiology Spectrum*, vol. 11, no. 5, American Society for Microbiology, Aug. 2023, <https://doi.org/10.1128/spectrum.01149-23>. Accessed 5 Nov. 2024.
- Suzuki, Shigekatsu, et al. “Rapid Transcriptomic and Physiological Changes in the Freshwater Pennate Diatom *Mayamaea Pseudoterrestris* in Response to Copper Exposure.” *DNA Research*, vol. 29, no. 6, Oct. 2022, <https://doi.org/10.1093/dnares/dsac037>. Accessed 25 Apr. 2026.
- Takemura, Masaharu. “Genome Sequence of a New ‘ Candidatus ’ Phylum ‘Dependentiae’ Isolate from Chiba, Japan.” *Microbiology Resource Announcements*, edited by Irene L. G. Newton, vol. 11, no. 2, Feb. 2022, <https://doi.org/10.1128/mra.01123-21>. Accessed 18 Feb. 2022.
- Tallada, Sheetal, et al. “Antibiotic Resistance Genes and Taxa Analysis from Mat and Planktonic Microbiomes of Antarctic Perennial Ice-Covered Lake Fryxell and Lake

- Bonney.” *Antarctic Science*, vol. 34, no. 6, Cambridge University Press, Dec. 2022, pp. 408–22, <https://doi.org/10.1017/s0954102022000360>. Accessed 30 Apr. 2025.
- Tan X-Y, Liu X-J, Lu D-C, Ye Y-Q, Liu X-Y, Yu F, Yang H, Li F, Du Z-J, Ye M-Q. Insights into the physiological and metabolic features of *Thalassobacterium*, a novel genus of *Verrucomicrobiota* with the potential to drive the carbon cycle. *mBio*. 2025 Apr 9;16(4):e0030525. doi: 10.1128/mbio.00305-25. Epub 2025 Mar 20. PMID: 40111047; PMCID: PMC11980603.
- Taurozzi, Davide, and Massimiliano Scalici. “Environmental and Elevational Drivers of Diatom Diversity in Alpine Temporary Ponds.” *Ecohydrology & Hydrobiology*, vol. 26, no. 1, Elsevier BV, Jan. 2026, p. 100726, <https://doi.org/10.1016/j.ecohyd.2025.100726>. Accessed 4 May 2026.
- Thauer, R. K., et al. “Energy Conservation in Chemotrophic Anaerobic Bacteria.” *Bacteriological Reviews*, vol. 41, no. 1, 1977, pp. 100–80, <https://doi.org/10.1128/br.41.1.100-180.1977>.
- Tikhonova, Tamara V., et al. “Catalytic Properties of Flavocytochrome c Sulfide Dehydrogenase from Haloalkaliphilic Bacterium Thioalkalivibrio Paradoxus.” *Biochemistry (Moscow)*, vol. 86, no. 3, Mar. 2021, pp. 361–69, <https://doi.org/10.1134/s0006297921030111>. Accessed 18 Mar. 2025.
- Treangen, Todd J., and Steven L. Salzberg. “Repetitive DNA and Next-Generation Sequencing: Computational Challenges and Solutions.” *Nature Reviews Genetics*, vol. 13, no. 1, Nov. 2011, pp. 36–46, <https://doi.org/10.1038/nrg3117>.
- Tschitschko, Bernhard, et al. “Rhizobia–Diatom Symbiosis Fixes Missing Nitrogen in the Ocean.” *Nature*, May 2024, pp. 1–3, <https://doi.org/10.1038/s41586-024-07495-w>.
- Varnam, Alan H., and Malcolm G. Evans. *Environmental Microbiology*. Boca Raton Crc Press, 2018.

- Vigil, Wayne, et al. "The Reductive Half-Reaction of Two Bifurcating Electron-Transferring Flavoproteins." *Journal of Biological Chemistry*, vol. 298, no. 6, June 2022, p. 101927, <https://doi.org/10.1016/j.jbc.2022.101927>. Accessed 1 Dec. 2025.
- Vignale, Federico A., et al. "Geobiology of Andean Microbial Ecosystems Discovered in Salar de Atacama, Chile." *Frontiers in Microbiology*, vol. 12, Frontiers Media, Oct. 2021, <https://doi.org/10.3389/fmicb.2021.762076>. Accessed 4 Mar. 2024.
- Vincent, W. F., et al. "Nitrous Oxide Cycling in Lake Vanda, Antarctica." *Nature*, vol. 292, no. 5824, Springer Science and Business Media LLC, Aug. 1981, pp. 618–20, <https://doi.org/10.1038/292618a0>. Accessed 1 May 2026.
- VLIZ. "Register of Antarctic Marine Species (RAMS) - *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C.Lewin, 1964." *Marinespecies.org*, 2025, marinespecies.org/rams/aphia.php?p=taxdetails&id=149004. Accessed 10 Dec. 2025.
- Volpe, Joseph J. *Volpe's Neurology of the Newborn*. Elsevier, 2018.
- Vopel, Kay, and Ian Hawes. "Photosynthetic Performance of Benthic Microbial Mats in Lake Hoare, Antarctica." *Limnology and Oceanography*, vol. 51, no. 4, July 2006, pp. 1801–12, <https://doi.org/10.4319/lo.2006.51.4.1801>. Accessed 3 June 2021.
- Wang, Yaxi, et al. "Genetic Manipulation of *Patescibacteria* Provides Mechanistic Insights into Microbial Dark Matter and the Epibiotic Lifestyle." *Cell*, vol. 186, no. 22, Oct. 2023, pp. 4803–4817.e13, <https://doi.org/10.1016/j.cell.2023.08.017>.
- Wang, Yichao, et al. "Diatom Biodiversity and Speciation Revealed by Comparative Analysis of Mitochondrial Genomes." *Frontiers in Plant Science*, vol. 13, Mar. 2022, <https://doi.org/10.3389/fpls.2022.749982>.
- Waschulin, Valentin, et al. "Biosynthetic Potential of Uncultured Antarctic Soil Bacteria Revealed through Long-Read Metagenomic Sequencing." *The ISME Journal*, vol. 16, no. 1, July 2021, pp. 101–11, <https://doi.org/10.1038/s41396-021-01052-3>.

- Weisse, Louis, et al. “Here, There and Everywhere: Ecology and Biology of the Dependientiae Phylum.” *Environmental Microbiology*, vol. 25, no. 3, Wiley-Blackwell, Dec. 2022, pp. 597–605, <https://doi.org/10.1111/1462-2920.16307>. Accessed 26 Apr. 2024.
- Welkie, David G., et al. “A Hard Day’s Night: Cyanobacteria in Diel Cycles.” *Trends in Microbiology*, vol. 27, no. 3, Mar. 2019, pp. 231–42, <https://doi.org/10.1016/j.tim.2018.11.002>. Accessed 29 Jan. 2021.
- Williams, Timothy J., et al. “Into the Darkness: The Ecologies of Novel ‘Microbial Dark Matter’ Phyla in an Antarctic Lake.” *Environmental Microbiology*, vol. 24, no. 5, Wiley, May 2022, pp. 2576–603, <https://doi.org/10.1111/1462-2920.16026>. Accessed 30 Oct. 2025.
- Wolfe, A. J. “The Acetate Switch.” *Microbiology and Molecular Biology Reviews*, vol. 69, no. 1, Mar. 2005, pp. 12–50, <https://doi.org/10.1128/membr.69.1.12-50.2005>.
- Xia, Yongzhen, et al. “Sulfide Production and Oxidation by Heterotrophic Bacteria under Aerobic Conditions.” *The ISME Journal*, vol. 11, no. 12, Dec. 2017, pp. 2754–66, <https://doi.org/10.1038/ismej.2017.125>.
- Y. Korkhin, et al. “NADP-Dependent Bacterial Alcohol Dehydrogenases: Crystal Structure, Cofactor-Binding and Cofactor Specificity of the ADHs of *Clostridium Beijerinckii* and *Thermoanaerobacter Brockii* 1 Edited by R. Huber.” *ScienceDirect*, vol. 278, no. 5, May 1998, pp. 967–81, <https://doi.org/10.1006/jmbi.1998.1750>. Accessed 3 July 2023.
- Yao, Dan, et al. “Genome-Wide Investigation and Analysis of Microsatellites and Compound Microsatellites in *Leptolyngbya*-like Species, Cyanobacteria.” *Life*, vol. 11, no. 11, Multidisciplinary Digital Publishing Institute, Nov. 2021, pp. 1258–58, <https://doi.org/10.3390/life11111258>. Accessed 1 Dec. 2025.
- Youssef, Noha H., et al. “Candidatus *Krumholzibacterium zodlettonense* Gen. Nov., Sp Nov, the First Representative of the Candidate Phylum Krumholzibacteriota Phyl. Nov. Recovered from an Anoxic Sulfidic Spring Using Genome Resolved Metagenomics.” *Systematic and Applied Microbiology*, vol. 42, no. 1, Jan. 2019, pp. 85–93, <https://doi.org/10.1016/j.syapm.2018.11.002>. Accessed 20 Nov. 2025.

- Yuly, Jonathon L., et al. “Universal Free-Energy Landscape Produces Efficient and Reversible Electron Bifurcation.” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 117, no. 35, National Academy of Sciences, Aug. 2020, pp. 21045–51, <https://doi.org/10.1073/pnas.2010815117>. Accessed 24 Nov. 2023.
- Zeng, Xiaopeng, et al. “Light Alters the Responses of Two Marine Diatoms to Increased Warming.” *Marine Environmental Research*, vol. 154, Feb. 2020, p. 104871, <https://doi.org/10.1016/j.marenvres.2019.104871>.
- Zhai, Chuan, et al. “Comparative Genomics of an Antarctic Sea-Ice Diatom (*Nitzschia* Sp.) Provides Insights into Potential Polar Adaptation.” *Frontiers in Microbiology*, vol. 17, Frontiers Media SA, Apr. 2026, <https://doi.org/10.3389/fmicb.2026.1755917>. Accessed 26 Apr. 2026.
- Zhou, Lu, et al. “Photosynthesis Acclimation under Severely Fluctuating Light Conditions Allows Faster Growth of Diatoms Compared with Dinoflagellates.” *BMC Plant Biology*, vol. 21, no. 1, Apr. 2021, <https://doi.org/10.1186/s12870-021-02902-0>. Accessed 20 Apr. 2022.
- Zhu, Hongfang, et al. “Effects of Low Light on Photosynthetic Properties, Antioxidant Enzyme Activity, and Anthocyanin Accumulation in Purple Pak-Choi (*Brassica Campestris* Ssp. *Chinensis* Makino).” *PLOS ONE*, edited by Zhihui Cheng, vol. 12, no. 6, June 2017, p. e0179305, <https://doi.org/10.1371/journal.pone.0179305>.
- Zhu, Y., et al. “Metabolic Changes in *Klebsiella Oxytoca* in Response to Low Oxidoreduction Potential, as Revealed by Comparative Proteomic Profiling Integrated with Flux Balance Analysis.” *Applied and Environmental Microbiology*, vol. 80, no. 9, Feb. 2014, pp. 2833–41, <https://doi.org/10.1128/aem.03327-13>. Accessed 4 Dec. 2021.
- Zhuang, Wei-Qin, et al. “Incomplete Wood–Ljungdahl Pathway Facilitates One-Carbon Metabolism in Organohalide-Respiring *Dehalococcoides Mccartyi*.” *Proceedings of the National Academy of Sciences*, vol. 111, no. 17, Apr. 2014, pp. 6419–24, <https://doi.org/10.1073/pnas.1321542111>. Accessed 5 Dec. 2022.