Benthic Microbial Mat Adaptations to Nutrient Scarcity and Nutrient Cycling Dynamics in Oligotrophic, Perennially Ice-covered Lake Vanda and Lake Fryxell in the McMurdo Dry Valleys, Antarctica

By

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Table of Contents

ABSTRACT

The primary productivity of all ecosystems is linked to biogeochemical cycles and is often limited by nutrient availability. These links and interactions result in feedback loops, where changes in biogeochemical cycling dynamics and ecosystem dynamics can profoundly influence each other. In lacustrine environments, the availability of either nitrogen or phosphorus can be limiting, as well as other environmental factors such as light availability. The McMurdo Dry Valleys are the largest ice-free region in Antarctica. They receive very little precipitation, experience freezing temperatures, and frequent freeze-thaw cycles. With very little liquid water, habitat connectivity is limited, and thus, terrestrial biomass. While there are seasonal glacial meltwater streams and rivers, liquid water only persists year-round in the perennially ice-covered lakes. Fed by glacial meltwater and many occupying closed basins, these lakes exhibit interesting physical and chemical properties. The permanent ice covers offer protection from the extreme terrestrial environmental conditions, allowing the lakes to remain mostly isolated from the atmosphere, resulting in meromictic and salinity stratified water columns. Most of the lakes are home to thick, photosynthetic, benthic microbial mats. In some lakes, the primary productivity of the benthic communities is greater than the planktonic communities.

Perennially ice-covered lakes Vanda and Fryxell are found in Wright and Taylor Valley, respectively. The geology and exposure age of the valleys differ, and due to the slow rates of weathering, nutrient (nitrogen and phosphorus) availability varies. The longest river in the MDVs is the Onyx River, which feeds Lake Vanda with multiple sources and sinks of nitrogen and phosphorus along it. Lake Fryxell is fed directly by the Canada and Commonwealth glaciers and several smaller ephemeral meltwater streams. Lake Vanda's water column has a severe orthophosphate deficiency, whereas Lake Fryxell's water column is limited in both nitrogen and

phosphorus. These limitations have been characterized for the planktonic communities; however, very little research has been conducted on constraints of primary productivity by nutrients in the benthic mat communities. The benthic microbial mats accrue biomass on annual scales as laminations that can vary in community composition. Despite the nutrient-depleted water columns, microbial mats grow along both varying nutrient and light regimes in both lakes. Metagenomic surveys of the benthic communities show a metabolic adaptation to efficiently scavenge nitrogen and phosphorus from their environment and tightly recycle nutrients within the mat through a suite of organic degradation and synthesis gene pathways, the synthesis and hydrolysis of nitrogen and phosphorus storage compounds as availability varies either seasonally or within their environment. The microbial communities are likely not limited by nutrients due to these advantageous adaptations; instead, they act as a nutrient sink in their environment and are limited by other factors.

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The diverse microbial consortia that make up microbial mats have persisted and evolved over billions of years of Earth's history and are well-adapted to survive a plethora of extreme environments. These communities flourish by sharing necessary resources, producing substances that protect against environmental stresses and adhere them to a surface ("ground them"). These communities not only survive under harsh conditions, they often thrive; and they do so because they are just that, 'communities.' This is also true of my graduate research and experience. I am profoundly grateful for the community that has supported me through this challenging yet deeply rewarding experience. I would not be where I am now without the academic, intellectual, and personal support I received throughout my tenure here at UC Davis.

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darkness; I want to acknowledge the ongoing genocide occurring in Palestine and the struggle of the Palestinian people to resist oppression and occupation. From the Black Lives Matter movement to ending apartheid in South Africa to Palestine, our struggles for liberation are interconnected. My aunt Anan Ameri is a reminder of this resistance; her family was forced from their home in Jerusalem during the *Nakba* in 1948, and she continues to fight for Palestinian liberation from Michigan. Despite a large diaspora, the Palestinian people have a deeply rooted connection to their land, and its stewardship is a crucial part of their resistance to occupation.

Connection to land is something our society often forgets. I remember my ice flight, glued to one of the three small porthole windows on a C-130 aircraft, watching our approach to the southernmost continent. The ocean turns the most gorgeous dark blue, patterned and cracked with white sea ice. The further south you fly, the more icebergs and sea ice cliffs. Then it appears: the white and black continent emerges, obliterating previous expectations. I stood at the back of the plane with my sunglasses on due to the blinding glare of the whiteness, staring out with tears pouring down my face. I was, in the truest meaning of the word, in awe. It was bigger than I expected, and it was pristine. The white snow is untouched; there was no color other than black and brown rock and white snow and ice. Despite its magnitude, you can feel how fragile and sensitive this place is. The amount of water locked up in this ice seemed like it could drown the whole world. Our fight against colonialism, capitalism, and climate change is the same; collective liberation and our survival depend on it.

Chapter 1: Nutrient sources and sinks to perennially ice-covered Antarctic Lake Vanda: Implications for microbial ecosystem structure and function

Abstract

Lake Vanda is a perennially ice-covered, endorheic, meromictic lake that lies in Wright Valley of the McMurdo Dry Valleys, Antarctica. It has one of the clearest water columns in the world due to its ultraoligotrophic nature, specifically orthophosphate, which limits the growth of planktonic communities in its upper water column. Despite this paucity of nutrients, thick, laminated, photosynthetic benthic microbial mats grow on the lake floor to depths of 50 m. While lake levels have risen >15m since 1960, the salinity-stratified water column's geochemical and physical profiles have remained remarkably stable over time. The water column is stratified into four distinct cells characterized by either diffusion or slow convection, with the thickness of the nutrient-deplete upper convecting cell only increasing with rising lake levels. This review synthesizes the sources and sinks of nitrogen and phosphorus from Wright Valley to Lake Vanda (e.g., glaciers, rivers, and soils) and the nutrient cycling dynamics within the lake, focusing primarily on Vanda's two microbial ecosystems: the planktonic community and the benthic mat community. The environmental controls of the structure and function of these communities differ. The planktonic biomass and activity maxima are deep within the diffusion zone, controlled by the diffusive upward flux of nutrients from the anoxic bottom waters and sufficient downwelling irradiance for photosynthesis. Meanwhile, the benthic microbial mats are likely not limited by nutrients, as they colonize the lake bottom from the nutrient-depleted shallow waters to the diffusion-limited bottom waters where nutrient concentrations increase; instead, benthic photosynthesis is likely limited by light availability. The effects of a changing climate on these sensitive polar ecosystems are discussed but complicated by various feedback mechanisms that

influence environmental controls.

Introduction

Nutrient stoichiometry plays a crucial role in shaping ecosystem dynamics, as the relative proportions of essential elements in organisms and ecosystems influence their structure, function, and response to environmental changes. Within lacustrine ecosystems, the availability and ratios of essential elements often limit the microbial communities. Therefore, the cycling of these nutrients often controls the primary productivity of the whole lake ecosystem (Essington & Carpenter, 2000). The hydrologic properties and physical characteristics of lakes can influence the rates and dynamics of internal nutrient cycling (Essington & Carpenter, 2000). Such characteristics include flow dynamics like turbulent mixing within lakes compared to the unidirectional flow of rivers/streams and the amount of sedimentation (Essington & Carpenter, 2000).

Nutrient dynamics are particularly interesting in endorheic lakes. These lakes occupy basins with no outlets, so nutrients are primarily lost through microbial denitrification, burial in the sediments, or to groundwater. Water loss from lakes without an outlet is primarily through evaporation and ground percolation, the former resulting in significant salinization and particularly common in desert regions. As water is lost through these processes, solutes, like salts, are left behind, ultimately increasing salinity in the water column. When coupled with variable freshwater influxes and limited mixing, this can often result in persistent water column stratification (meromixis) when an increase in freshwater input results in a less dense water layer overlying a strongly salinized, dense water layer. Stratification controls the distribution and cycling dynamics of nutrient species within the water column by forming a barrier to vertical mixing.

The MDVs are the largest ice-free region in Antarctica, a cold, arid region that receives less than 50 mm of precipitation annually (Castendyk et al., 2016). Endorheic, stratified lakes are common in the McMurdo Dry Valleys (MDVs) of Antarctica. These lakes are perennially ice-covered and fed almost exclusively by glacial meltwater, making them sensitive to minor shifts in temperature that increase or decrease meltwater volumes and lead to changes in water balance. Over recent decades, the MDVs have been experiencing conditions that have resulted in inflowing meltwater volume exceeding the loss of water from evaporation and ablation, leading to rising lake levels (Castendyk et al., 2016; P. T. Doran et al., 2008; Herbei et al., 2016). While research on the impact of climate change on this sensitive region is extensive, intricate feedback mechanisms yield variable ecosystem responses. Each of these perennially ice-covered lakes is home to rich, benthic microbial mat communities, which are hot spots of primary productivity in this region (Hawes et al., 2001).

Lake Vanda is one of these meromictic, endorheic, perennially ice-covered lakes that lies in Wright Valley of the MDVs (Figure 1). Lake Vanda is considered ultraoligotrophic, with phosphorus as the limiting nutrient to primary productivity throughout most of the lake. Despite the extreme phosphorus deficiency, the lake floor is carpeted with benthic microbial mats composed of millimeter- to decimeter-tall pinnacles that grow to depths exceeding 50m. These benthic mat communities play an important role in the cycling dynamics of carbon and nutrients in Wright Valley (Hawes et al., 2013a; Sumner et al., 2016).

Lake Vanda receives most of its water from Lower Wright Glacier's meltwater via the Onyx River (Figure 2), and water is lost from the lake through ice ablation and evaporation from the moat that forms along the perimeter of the lake during the austral summer. The lake is 8 km by 2 km in size and has a maximum depth of 75 m, making it the deepest and largest by volume in the

MDV region (Castendyk et al., 2016). The lake has a perennial ice cover that is 2-3 m thick and, compared to other Antarctic ice-covered lakes, has a remarkably clear water column (e.g., Hawes & Schwarz, 2001; Howard-Williams et al., 1998).

In this review, we expand upon Castendyk et al.'s comprehensive 2016 review of Lake Vanda's historical physical and hydrological properties and its response to climate change (Castendyk et al., 2016). We focus on the biogeochemistry of Lake Vanda, with particular attention to the sources and fate of nitrogen (N) and phosphorous (P) to the lake from the whole valley system and the cycling dynamics within the lake. We characterize the microbial communities with depth, both pelagic and benthic, and the interactions between these communities and nutrient cycling dynamics. Lake Vanda's biogeochemical water column profiles have remained fundamentally stable over the timescales they have been measured (Green & Lyons, 2009; Hawes et al., 2013b; Schutte et al., 2020); however, climate change will likely affect the processes that produce these profiles and, thus, potentially the profiles and communities themselves.

The structure of the review is as follows: 1) we provide an overview of the geography and geology of Wright Valley, followed by a summary of Vanda's hydrological history and water balance from Castendyk et al. (2016). 2) We summarize the sources (and transport) of nutrients to Lake Vanda from Wright Valley. 3) We characterize Lake Vanda's physical and chemical structure, starting with descriptions of the interlayered salinity stratified and mixed zones, including salinity, temperature, and irradiance. This focuses on consideration of the biogeochemical consequences of the unusual physical structure, including characteristics like dissolved oxygen (DO), pH, and nutrient availability. 4) We then characterize the planktonic and benthic microbial communities, including community composition and photosynthetic potential.

5) We compile and summarize the data regarding nutrient cycling dynamics of Lake Vanda across decades of published work before finally interpreting and discussing it under the lens of Vanda's ecosystems in section 6.

Geography and Geology

Located in South Victoria Land, Antarctica, the McMurdo Dry Valleys were shaped through tectonic uplift and further by fluvial and glacial processes that persisted throughout the Cenozoic, as summarized in Castendyk et al. (2016). The valleys are home to 15 perennially ice-covered lakes, most of which are meromictic. Lake Vanda sits in the east-west trending Wright Valley, to the north is the Olympus Range, and to the south is the Asgard Range. The geology of the region consists mostly of granitic intrusive rocks and metamorphosed sedimentary rocks such as marble, hornfels, and schist (Asgard Formation) (McKelvey & Webb, 1962). The valley floor is composed of bedrock and glacial deposits, a testament to paleoglaciers that once flowed eastward from the inland ice plateau toward McMurdo Sound (McKelvey & Webb, 1962). The valley glaciers retreated during the late Miocene, leaving the glacial deposits that now define the landscape (McKelvey & Webb, 1962).

Hydrological history

The hydrological history of Lake Vanda is summarized in detail in Castendyk et al. (2016). During the Last Glacial Maximum, advances in the Ross Ice Sheet blocked the mouth of Wright Valley and formed a large proglacial lake known as Glacial Wright Lake (Castendyk et al., 2016; Hall et al., 2001). Radiocarbon dating of paleo-microbial mats from the highest paleo-shorelines were found to be between 2000 and 3000 yr BP, coinciding with a warm period in the Ross Sea (Castendyk et al., 2016; Smith & Friedman, 1993). Subsequent changes in regional climate, such as colder, dryer periods, resulted in dramatic shifts in Vanda's hydrology due to its endorheic

nature (Lyons et al., 1998); Vanda likely lost its ice cover due to aridity in the late Holocene followed by subsequent evaporation and refilling events (Castendyk et al., 2016; Hall et al., 2001, 2010).

The Onyx River formed as the large proglacial lake retreated, starting at the Wright Glacier, which still blocks the eastern end of the valley, flowing westward to Lake Vanda (Figure 2). The Onyx receives essentially all of its water from meltwater from Lower Wright Glacier with very minor tributary inputs (Green & Lyons, 2009). Due to the seasonality of meltwater at its source, the Onyx only flows during the austral summer (~6-8 weeks). Discharge records for the Onyx River show a highly variable flow regime, which has been attributed to the frequency of down-valley föhn summer winds (Castendyk et al., 2016; Doran et al., 2008). Summers with higher frequency of föhn winds have warmer air temperatures, which increases meltwater production and thus stream discharge (Castendyk et al., 2016; Doran et al., 2008). The Onyx river channel is shallow with ~0.5 m water depth, and it travels over bedrock, alluvium, moraines, and a boulder pavement. The meltwater and sediment are major sources of magnesium, potassium, sulfate, and bicarbonate delivered to Lake Vanda (Green & Lyons, 2009).

Castendyk et al. (2016) characterize the physical structure of Lake Vanda and summarize its hydrological history with lake level changes and water balance. Castendyk et al. separate the lake into four distinct zones with depth, each zone characterized by the salinity gradient (Figure 3a). The deepest water, which Castendyk et al. call the “diffusion zone” (DZ), is density gradient-stabilized and is overlain by a lower salinity layer with water characteristics suggesting convective internal mixing, denoted as the “lower convection cell” (LCC). A second density gradient-stabilized layer, “upper-pycnocline” (UP), sits between this lower mixed zone and an upper freshwater zone, “upper convection cell” (UCC) that also has uniform chemistry and

extends to the ice on the lake surface. This structure is interpreted as representing a series of dry-down and re-filling events that saw salinization during periods of low meltwater influx, followed by the formation of overlying meltwater layers during periods of increased meltwater flow.

Ice-cover thickness decreased from 4 m to 3 m between 1960-75 but has since remained relatively stable (Castendyk et al., 2016). In contrast, lake levels have been rising (Figure 4), and the UCC has increased in thickness from 5m to 20m + since 1960 (Castendyk et al., 2016). Lake level rise is attributed to increased flow from the Onyx River, although lower ice ablation may also play a role. As the only source of water to the lake, the Onyx River also delivers a significant amount of Vanda's nutrients from the surrounding valley, and that supply may have changed as the flow in the Onyx changed.

Overview of nitrogen cycling dynamics

In a stratified aquatic ecosystem like Lake Vanda, nutrient cycling dynamics are primarily controlled by the linkages between microbial metabolisms and abiotic environmental factors (e.g. oxygen availability, salinity, temperature, and pH). These abiotic factors control the spatial distribution of the microbial communities and, thus, the biological processes responsible for N-transformations in aquatic ecosystems. The N cycle is primarily composed of microbially mediated redox reactions, where the electron acceptors in these reactions vary in the presence or absence of O_2. In the presence of O_2, the process of nitrification occurs, and NH_4^+ is oxidized to NO_3^- and NO_2^- with O_2 as the electron acceptor. Conversely, in the absence of O_2, the anaerobic process of denitrification occurs. The oxidized N species (NO_3^- and NO_2^-) act as the electron acceptors and are reduced producing the gaseous N species (N_2O or N_2), ultimately removing N from the system. These N-transformation processes are vertically separated along redox gradients, along with their microbial mediators. Some microbes have the genetic capability of

fixing their own N by breaking the double bonds in N_2 gas to produce ammonium, providing a source of N to the environment, particularly under N-limitation.

There is no equivalent to N fixation for mitigating phosphate scarcity, as P cannot be biologically fixed. Instead, the primary source of inorganic P is the weathering of apatite in the parent rock. In polar regions, P is often the limiting nutrient to primary productivity as low temperatures and aridity lead to slow rates of weathering of parent material (Wang et al., 2024). The affinity of di- and trivalent cations and calcium towards phosphate perpetuates this limitation as it results in larger pools of sorbed and mineral P, which is less bioavailable in the environment (Wang et al., 2024). To deal with P scarcity, some microorganisms have adapted to limit their cellular P demand, use substitute lipids in place of phospholipids (Dyhrman et al., 2012; Park et al., 2022; Shemi et al., 2016; Van Mooy et al., 2009), enhance their phosphate uptake efficiency, or utilize more recalcitrant forms of P (e.g., phosphonates, polyphosphates, phosphoesters) (Dyhrman et al., 2006; Riegman et al., 2000).

Nutrient Sources for Lake Vanda

Ecosystem stoichiometry of the MDV region provides a framework for understanding sources, sinks, and controls on nutrients to the Lake Vanda ecosystem. The balance of energy and essential nutrients (C, N, and P) in an ecosystem affects the abiotic and biotic influences on biogeochemical cycles and can provide insights into ecosystem interactions (Barrett et al., 2007; Sterner & Elser, 2002). Barrett et al. (2007) present a biogeochemical model for the MDV ecosystem using ecosystem stoichiometric approaches to evaluate nutrient cycling dynamics over time across the landscape (Barrett et al., 2007). They found that N and P varied significantly in terrestrial and aquatic ecosystems and that the controls on nutrient availability to soils and aquatic ecosystems differed. The exposure age of landscape surfaces strongly influenced N and P

availability to the valley soils, whereas the biota controlled the nutrient stoichiometry in aquatic systems (Barrett et al., 2007). Thus, lake ecosystem stoichiometry, including for Lake Vanda, is modeled as controlled by biological processes that are coupled to the long-term legacy of the geology and climate of the region (Barrett et al., 2007).

Soils

Soils are a major source of nutrients through weathering processes. However, weathering rates are slow due to the arid and cold conditions. The parent rock composition and the age of the soils of the valleys directly control the availability of N and P (Barrett et al., 2007). Geologically young soils are rich in P due to the availability of primary mineral-bound PO_4^{3-} (like apatite) and are typically limited in N (Barrett et al., 2007). Geologically old soils are more limited by P and less by N due to N input from soil N_2-fixing communities (Barrett et al., 2007) and atmospheric deposition (Wada et al., 1981). MDV soils have high inorganic N concentrations compared to organic N, specifically NO_3^-, due to atmospheric salt deposition and low potential for microbial denitrification in soils in arid, saline environments (Barrett et al., 2007; Wada et al., 1981). Nitrate concentrations can be one to two orders of magnitude higher than those of organic N (Wada et al., 1981).

An absence of higher plants and animals results in exceptionally slow rates of organic matter decay; thus, $\delta^{15}N$ can be used as a primary tracer for organic matter sources (Burkins et al., 2000). The soils of the MDV are extremely depleted in ^{15}N compared to the biogenic nitrogen sampled from the region, with $\delta^{15}N_{NO3-}$ values ranging from -23‰ to -11‰ (Burkins et al., 2000; Wada et al., 1981). Wada et al. (1981) interpret the depleted soil $\delta^{15}N_{NO3-}$ values around Lake Vanda to be derived from atmospheric precipitation carrying depleted NO_x from N isotopic fractionations occurring in the atmosphere (Wada et al., 1981). The relatively old soils of Wright

Valley have lower soil P and higher N than other valleys, such as Taylor Valley (Barrett et al., 2007; Bate et al., 2008). The lower soil P correlates with the P deficiency measured in most of Lake Vanda's water column, although transport processes also strongly affect nutrient delivery.

Transport of nutrients into the Lake

Aeolian and fluvial processes are the main modes of transport of nutrients from the surrounding landscape to Lake Vanda. Due to the extreme aridity and thus limited hydrological processes, aeolian processes are a significant transport process of sediments and nutrients in the valley system (Diaz et al., 2018). The strong, down-valley föhn winds are a major transport mechanism of aeolian material (Diaz et al., 2018). These winds deposit grains on the lake ice, some of which melt through and settle on the lake floor (Green et al., 1993; Rivera-Hernandez et al., 2019). The aeolian material that has not yet been in contact with the hydrological system also contains soluble nutrients that solubilize when deposited on the lake ice, eventually adding nutrients to the lake ecosystem (Diaz et al., 2018).

The Onyx River

Although aeolian processes provide some nutrients, most are delivered to the lake from the Onyx River. Nutrients transported by fluvial processes likely undergo numerous transformations due to the metabolism of the microbial communities living in the seasonal river. Understanding the sources, sinks, and cycling processes of nutrients in the Wright Valley soil, fluvial, and lacustrine systems provides a holistic approach to modeling N and P cycles in Lake Vanda and its microbial communities.

The Onyx River is the only inlet into Lake Vanda, making it a major source of Vanda's nutrients and sediments. The Onyx originates at Lower Wright Glacier and terminates at Lake Vanda; at 30 km in length, it is the longest of the glacial meltwater streams of the MDV (Green

et al., 2005). Sources of nutrients to the Onyx River include the Lower Wright Glacier, tributary glaciers and streams, and soil leaching. Microbial communities also remove and transform nutrients in the river water and sediments, leading to interesting relationships among river composition and water sources.

The origin of the Onyx River is Lake Brownworth, which is fed directly by meltwater from Lower Wright Glacier, located about 27 km west of Lake Vanda (Figure 2) (Canfield & Green, 1985; Clive Howard-Williams et al., 1997). Lake Brownworth, and thus the glacier, is the main source of water to the Onyx River. Lower Wright Glacier is also a major source of nitrate to the Onyx River (Canfield & Green, 1985). Nutrients within the glacial ice are concentrated in cryoconite holes, which are lows formed by sediment melting into the glacial ice surface (Bagshaw et al., 2013). Microbial communities in these holes perform significant biogeochemical cycling (Bagshaw et al., 2013; Schmidt et al., 2022); most of the dissolved carbon in the cryoconite is inorganic, while the dissolved nitrogen is primarily organic (Bagshaw et al., 2013). In Taylor Valley, the total cryoconite hole store of C and N is larger than that of the ephemeral streams feeding the local lakes (Bagshaw et al., 2013). The average area covered by cryoconite holes on Lower Wright Glacier is 3.5% and, despite their ice-lid, have been found to never truly be isolated from the drainage system, hydrologically connecting them to the surrounding area (Macdonell et al., 2016). These holes are flushed by glacial meltwater every few years (Bagshaw et al., 2013). For example, during warm periods like the 2001/2002 summer, abnormally warm temperatures led to extreme melting; most of the cryoconite holes lost their ice lids, and their nutrient stores flushed into the streams, resulting in higher nutrient loads to many MDV lakes (Bagshaw et al., 2013). However, compared to lakes with smaller, shorter stream inputs, nutrient delivery to Lake Vanda is likely complicated by microbial processes in Lake

Brownworth and along the Onyx River.

Howard-Williams et al. (1997) characterized sources and sinks of nutrients along the Onyx from Lower Wright Glacier to Lake Vanda. By calculating fluxes (g day^{-1}) of DRP, DIN, DOP, and DON along its transect, they found that Lake Brownworth acted as a sink for most nutrients due to the extensive cyanobacterial mats on the lake floor (Clive Howard-Williams et al., 1997). Fluxes were measured during early flow (1 December 1993) and peak flow (16 January 1994). Flux trends for DRP through Lake Brownworth during early flow were -40 g day^{-1} with -130 g day^{-1} during peak flow, showing that the lake was a sink for DRP (Clive Howard-Williams et al., 1997). DOP was similarly stripped from water passing through Lake Brownworth with fluxes of -3 g day^{-1} and -1200 g day^{-1} during early flow and peak flow, respectively. For DIN, fluxes were -2100 g day^{-1} during early flow and -1600 g day^{-1} during peak flow. The only flux that did not follow the same trend was DON; during early flow, the flux of DON was +400 g day^{-1} at Lake Brownworth, whereas during peak flow, it was -3700 g day^{-1} (Clive Howard-Williams et al., 1997).

Similarly, Green et al. (2005) measured nutrient concentrations along the Onyx River and saw DRP concentrations drop from ~0.15 μM to ~0.05 μM across Lake Brownworth before increasing again downstream. Their study measured a DRP maximum of 0.3 μM; however, concentrations remained relatively constant at ~0.1 μM for most of the length of the Onyx River (Green et al., 2005). Green et al. (2005) suggest that contributions of DRP to the Onyx River is primarily derived from the chemical weathering of channel rocks and valley soils.

Nitrogen processing in the Onyx River is complicated due to the uptake, release, and transformations of nitrogen species by the benthic microbial mats that colonize the riverbed. These processes create variations in N species and concentrations along the Onyx River.

Specifically, NO_3^- values range from ~0.1 μM to over 5 μM (Green et al., 2005), whereas NH_4^+ concentrations vary from a high of 156 mg/m^{-3} at the melthead to as low as 4 mg/m^{-3} in the main river (Clive Howard-Williams et al., 1997). The benthic mats play variable roles in removing and supplying nutrients to the Onyx Rivers. Benthic mats likely 'sequester' nutrients, removing dissolved species from the river water and decreasing concentrations downstream (Clive Howard-Williams et al., 1997; Green et al., 2005). However, particles of mat dislodged from the riverbed can be a source of N to the river water and downstream mats. Freeze-thaw cycles can also release nutrients from the cyanobacterial mats; DRP and NH_4^+ are released at rates of ~0.1 ug N or P cm^{-2} $cycle^{-1}$ and over 10 times this amount in the form of DON (Clive Howard-Williams et al., 1997). Conversely, very little DOP and NO_3^- are released by the mats during these cycles (Clive Howard-Williams et al., 1997). Thus, the fluxes of nutrients to and from the benthic mats in the Onyx riverbed vary significantly depending on the details of temperature, flow speed, and benthic mat properties.

The Boulder Pavement

One of the most influential environments for nutrient cycling along the Onyx is a 1.5 km stretch called the "Boulder Pavement" (Clive Howard-Williams et al., 1997; Green et al., 2005). This region is composed of large, flat rocks colonized by microbial mats with high primary productivity by cyanobacteria and algae (Clive Howard-Williams et al., 1997; Green et al., 2005). Its large surface area-to-water volume ratio allows substantial contact between the water and microbial community, allowing them to sequester nutrients efficiently (Green et al., 2005). The importance of this surface area-to-volume ratio is highlighted by changes in N:P across this area at high flow volumes even though the boulder pavement was a significant sink for N and P, the N:P ratio increased significantly across the pavement from a ratio of 0.59 to 8.59 (Green &

Canfield, 1984). This variation suggests that the mat communities are less effective at removing nitrate during high flows (Green et al., 2005). These changes in nutrient concentrations with flow volume make it difficult to calculate average nutrient changes across the boulder pavement. During early flow (1 December 1993), the Boulder Pavement was a source of DRP, DIN, DOP, and DON with fluxes of +10, +100, +10, and +60 g day^{-1}, respectively. Conversely, during peak flow (16 January 1994), the Boulder Pavement acted as a sink of DRP and DIN, with fluxes of -300 and -7800 g day^{-1}, respectively. DOP and DON still had positive fluxes passing through the Boulder Pavement with values of +12 and +720 g day^{-1} (Clive Howard-Williams et al., 1997). Thus, the mats colonizing the Boulder Pavement are capable of significant uptake of nitrate from the river and transformation of DIN into DON, similar to processes associated with temperate wetland systems (Clive Howard-Williams et al., 1997). The DON and DOP are released during freeze-thaw cycles, ultimately exported downriver from the Boulder Pavement towards Lake Vanda (Clive Howard-Williams et al., 1997).

Not all the water and nutrients that leave the Boulder Pavement reach Lake Vanda. There was no flow from the Onyx into Lake Vanda during the earliest flows across the Boulder Pavement, thus no influx of nutrients into the lake. However, during peak flow volumes, the Onyx River enters Lake Vanda carrying influxes of DRP, DIN, DOP, and DON into Lake Vanda of +90, +1900, +60, +3420 g day^{-1}, respectively (Clive Howard-Williams et al., 1997).

The datasets from the Green et al. (2005) study, however, differed significantly from that of Clive Howard-Williams et al. (1997). They found that the average N:P ratio of the Onyx River at the weir just before Lake Vanda was around 2.6, well below the Redfield Ratio (N:P of 16), suggesting N is the limiting nutrient for water entering the lake (Green et al., 2005); however, P is the limiting nutrient within Lake Vanda (Vincent & Vincent, 1982). Canfield and Green

(1985) and Green et al. (2005) hypothesize that this is due to the high efficiency of N recycling in the lake, which somewhat decouples nutrient delivery from nutrient availability. If so, nutrient limitation cannot be determined solely by valley-wide nutrient sources, but instead lacustrine nutrient cycling dynamics are heavily influenced by interactions among physical, chemical, and ecological characteristics and processes within Lake Vanda.

Lake Vanda: physical and chemical characteristics

Physical Lake Structure: salinity stratification

The density structure of Lake Vanda reflects its water balance history and strongly influences nutrient transport and availability. Lake Vanda has four interlayered salinity-stratified and mixed zones (Figure 3a,b) (Castendyk et al., 2016). The zones from deep to shallow are as follows: diffusion zone (DZ), lower convection cell (LCC), upper-pycnocline (UP), and upper convection cell (UCC). The elevations and thicknesses of the DZ, LCC, and UP have remained constant through time, while the UCC has increased in thickness (Castendyk et al., 2016; Hawes et al., 2013a). The deepest water in Lake Vanda (DZ) is a calcium-chloride brine with two proposed origins. It may represent concentrated salts from ablation loss of water from Lake Vanda, which may have evaporated into a small brine pond around 1000 to 1200 yr BP (Matsubaya et al., 1979; Wilson, 1964); however, the Onyx River does not have the proper ionic ratios to produce the observed calcium-chloride brine (Castendyk et al., 2016; Green & Lyons, 2009; Matsubaya et al., 1979). An alternative model is that Vanda received groundwater associated with the hyper-saline Don Juan Pond, which has a brine with a similar composition (Castendyk et al., 2016; Green & Lyons, 2009; Lyons & Mayewski, 1993). Irrespective of the origin of the brine, its presence produces density stratification of the lake water (Castendyk et al., 2016). The LCC and UCC are characterized by convective cells affected by both salinity and temperature. The

mechanism behind the formation and stability of these convection cells and physical structure of Lake Vanda is likely the interaction between these salinity and temperature gradients as well as the historic change in the temperature profile of Lake Vanda and its ice thickness (Castendyk et al., 2016).

Temperature and Irradiance

Temperature increases with depth in Lake Vanda, as seen in Figures 3a and 3b. The UCC has a temperature around 4°C, increasing through the UP, and the LCC with a temperature of around 6.5°C at 40m (Castendyk et al., 2016; J. Priscu, 2022a). From here, temperature increases steadily through the DZ, reaching a maximum of around 21°C at 73m depth (Castendyk et al., 2016; J. Priscu, 2022a). While the source of the heat at depth in Lake Vanda has been debated over the years, it is accepted that solar energy heats Vanda's bottom waters due to the transparency of its ice and water column (Ragotzkie & Likens, 1964; Wilson & Wellman, 1962). Over the past several decades, temperature has decreased at depth in Lake Vanda by ~ 2°C in the LCC and ~4°C in the DZ (Castendyk et al., 2016). This decrease in temperature is likely due to decreasing solar energy reaching the bottom waters due to the increased thickness of the UCC (Castendyk et al., 2016).

Due to Vanda's smooth and highly transparent ice, light penetrates to depths greater than 65 m. The water in the upper water column is exceptionally clear due to the very low primary productivity in the water column and the absence of dissolved humic substances (Goldman et al., 1967). Around 18% of incident PAR is transmitted through the ice cover, with an attenuation coefficient of 0.06 m^{-1} for downwelling irradiance through the LCC (Hawes et al., 2013a; Hawes & Schwarz, 2001; Howard-Williams et al., 1998). Red wavelengths are absorbed, and blue/green wavelengths (<550nm) are transmitted through the ice and water column (Hawes et al., 2013).

Blue wavelengths are strongly absorbed by organic particles. Thus the paucity of organic particles in Vanda allows these wavelengths to penetrate unusually deeply (Howard-Williams et al., 1998), and extinction coefficients for blue and green wavelengths from below the ice surface to 45 m are 0.031 m^{-1} and 0.058 m^{-1} respectively (Goldman et al., 1967). Absorbance increased at all wavelengths at depths greater than 55m (Goldman et al., 1967), which is due to an increase in plankton associated with the boundary between the DZ and LCC (Figure 3b) (Schutte et al., 2020).

Biogeochemical Consequences of the Physical Structure

Lake Vanda's salinity stratification promotes biogeochemical gradients within its water column, which influences the biological communities and creates distinct ecosystems.

Dissolved oxygen, salinity, and pH

The water in the UCC, UP, and LCC is fresh and oxygenated with a pH of 8-9 (J. Priscu, 2023). Both dissolved oxygen (DO) and conductivity are higher in the LCC than the UCC and increase within the UP, which lies between them (Figure 3a,b). Conductivity increases significantly in the DZ which consists of a brine with a conductivity of 1 mS cm^{-1} at the top to over 120 mS cm^{-1} at the bottom (Castendyk et al., 2016). DO begins to decrease at around 60 m; however, there is a local deep spike in DO at around 65 m, corresponding to a deep chlorophyll *a* maximum before it sharply declines to anoxia at around 70 m (Figure 3b) (Castendyk et al., 2016). Within the DZ, pH also decreases to 5-6 at 70 m (Figure 3a) (Green & Lyons, 2009).

Nutrient distribution

The nutrient distributions in Lake Vanda have inspired research over decades. Much of Lake Vanda is ultraoligotrophic, with very low nutrient concentrations in the UCC, UP, and LCC (Figure 5) (Canfield & Green, 1985; J. C. Priscu, 1995; J. C. Priscu et al., 1989; Vincent &

Vincent, 1982). Nutrient concentrations increase with depth and reach their maxima in the DZ (J. Priscu, 2022b); however, due to redox reactions, the concentration of NO_3^- decreases at the oxycline as it is reduced under anoxic conditions (Figure 5) (J. Priscu, 2022b). Similarly, N-converting enzymatic processes, such as microbial ammonia oxidation and heterotrophic denitrification, depend on pH (Blum et al., 2018). The activity of both enzymes decreases at a lower pH, potentially important in the DZ, where pH decreases from ≥8 to ~5-6 (Blum et al., 2018). Transport processes in the DZ are dominated by diffusion, thus, nutrients diffuse upwards from the DZ toward the nutrient-limited water of the LCC (Barrett et al., 2007; J. C. Priscu et al., 1989). The balance of nutrient diffusion and light transmission support the peak in planktonic primary productivity near the top of the DZ. The biogeochemical processes in this zone affect the DIN:SRP ratio, which is most extreme (>5000:1) at depth right above the anoxic water column (Vincent & Vincent, 1982). At this depth, maximum planktonic photosynthesis is occurring under a low light regime. Here, the planktonic community shows a strong cellular P deficiency (J. C. Priscu, 1995; Vincent & Vincent, 1982).

A flux of nutrients likely reaches the base of the LCC, where convection transports them throughout this zone of the lake. Even fewer make it to the base of the UP and then into the UCC. While the P limitation is less severe in these shallower depths, it remains the limiting nutrient throughout all of Lake Vanda (Vincent & Vincent, 1982).

Lake Vanda primary productivity

Planktonic microbial communities

The planktonic community is very sparse despite the bright environment, which is attributed to the strong phosphorus deficiency (Vincent & Vincent, 1982). This low productivity categorizes Lake Vanda as ultraoligotrophic, maintaining a cell density of 10^3 cells/mL throughout the UCC

(Bratina et al., 1998). Chlorophyll *a* concentrations are higher at depth with a series of maxima at 59.3 m, 63 m, and 68.2 m depths, corresponding with a DO maximum at 66 m (Schutte et al., 2020). The chlorophyll maxima can be attributed to relatively high populations of green algae, cryptophytes, cyanobacteria, and diatoms/haptophytes (Schutte et al., 2020). Even at these higher biomasses, the planktonic primary productivity is significantly less than that of the benthic microbial mats in the UP and UCC (Hawes et al., 2001). The planktonic community is highly shade-adapted, with some groups utilizing mixotrophy to supplement energy with predation under low nutrient stress (Hawes & Schwarz, 2001; Roberts & Laybourn-Parry, 1999).

Benthic microbial mat communities

Benthic microbial mats colonize Vanda's lake floor to depths of over 50 m. Those within the UP and UCC have annual laminations in both prostrate mat and millimeter to decimeter-tall pinnacles (Love et al., 1983; Sumner et al., 2016; Wharton Jr. et al., 1994). The mat structure is characterized by alternating hyaline and opaque layers up to several millimeters thick. The hyaline laminae consist of organic matter and water, and they are attributed to the secretion of extracellular polysaccharide (EPS) during cyanobacterial growth (spring and early summer) and the opaque layers form when photosynthesis stops, and cells and sediment accumulate on the mat surface (winter) (Hawes et al., 2001). Cyanobacterial trichomes are oriented vertically in the hyaline layers and horizontally in the opaque layers (Hawes et al., 2001). The annual nature of the laminations has been confirmed by repeated observations of mats and pinnacles after a large influx of sediment associated with the 2001 flood produced an unusually thick opaque layer (Hawes et al., 2013a).

Not all photosynthesis occurs at the mats' surface; some occurs within the top few laminae below the surface (Sumner et al., 2016). Sufficient irradiance for photosynthesis

penetrated about 5 mm into pinnacles at 21 m water depth, and imaging by PAM fluorometry demonstrated that subsurface cyanobacteria had the metabolic capacity to perform photosynthesis (Sumner et al., 2016). The modeled PSII yield was higher within the pinnacles compared to the pinnacle surface, which combined with the light availability, suggests an active subsurface photosynthetic community and significant internal biomass accumulation through carbon fixation (Sumner et al., 2016). Because this spatial variation in photosynthesis influences the distribution of biomass accumulation, it also influences the morphology of the pinnacles (Sumner et al., 2016).

Community composition

Benthic primary producers in Lake Vanda are almost entirely cyanobacteria. The cyanobacterial community compositions in the UCC have been characterized by morphology, 16S rRNA, and some metagenomics (Grettenberger et al., 2020, 2023; Powell et al., 2024; Ramoneda et al., 2021; Sumner et al., 2016; Wall, 2018; Zhang et al., 2015). Cyanobacteria primarily classify as *Phormidium, Pseudanabaena,* and *Leptolyngbya* (Wall, 2018; Grettenberger et al., 2023), with five *Leptolyngbya* sp. morphotypes dominating the cyanobacterial community with at least 77% of the relative abundance (Sumner et al., 2016). The N-fixing cyanobacteria, Nostocales, were absent in the UCC mats, likely due to low irradiance; however, they are also missing from the higher irradiance moats (Zhang et al., 2015; Salley, Chapter 2). The cyanobacterial community in Lake Vanda is more diverse than those of nearby Taylor Valley lakes Joyce and Hoare, and five OTUs were unique to Vanda (Zhang et al., 2015). Between lakes, Vanda also had the highest Shannon diversity index; however, perennially ice-covered lakes, in general, were found to have lower cyanobacterial diversity than seasonally frozen ponds and lake margins that experience higher summer irradiance (Zhang et al., 2015).

The structure of Lake Vanda's benthic microbial community is driven more by environmental abiotic conditions in the water column than habitat connectivity and dispersal opportunities (Ramoneda et al., 2021; Grettenberger et al., 2023). In comparison, benthic communities in Lake Brownworth and the Onyx River were found to be structurally similar, consistent with high physical connectivity and dispersal opportunities (Ramoneda et al., 2021). The benthic microbial assemblages in Vanda were structurally distinct across depths ranging from 0.1 to 31 m, changing the most from 0 to 2 m depths (Ramoneda et al., 2021). At the prokaryotic phylum level, Cyanobacterial abundance dropped from ~29.1% in shallow water mats (<1m) to 11.8% at depth (11-31 m) (Ramoneda et al., 2021). The composition of the communities forming pinnacles was generally similar but different in detail between 19 and 31 m depths (Grettenberger et al., 2023). The differences in the photosynthetic community structure are likely due to light availability, the deeper mats received less irradiance and have had a more prolonged growth history than the 19 m pinnacles due to lake level rise. Grettenberger et al. (2023) postulate that the factors that influenced the photosynthetic community structure may have further influenced the heterotrophic community.

Benthic photosynthetic potential and primary productivity

Spatial patterns of benthic photosynthetic potential and biomass accumulation are quite different from those of their planktonic equivalents and provide a framework for understanding the benthic community's influence on the lake's biogeochemistry (Hawes et al., 2001, 2013a; Sumner et al., 2016). Benthic photosynthesis and respiration vary temporally and spatially, as demonstrated by the relationship between irradiance and net O_2 production and modeled annual respiration rates (Hawes et al., 2001). Annual primary productivity is predicted to peak during late spring-early summer (November and December) with a maximum annual accrual of ~1400

ug C cm^2 at 10-12 m depth (Hawes et al., 2001). The annualized net primary productivity rate is predicted to result in ~0.1-4 mm of vertical mat accumulation per year, consistent with the observed lamina thicknesses. Benthic mat N and P demand have been estimated at 24 and 1.4 ug m^{-2} per year of N and P, respectively, based on carbon accrual rates and measured C:N (14:1) and N:P (90:1) ratios (Hawes et al., 2001, 2013a).

Benthic photosynthetic activity has been demonstrated in mats to ~40 m (Hawes and Schwarz, 2001). Vanda's under-ice spectra are predominantly blue-green wavelengths, and the benthic microbial mats have high concentrations of phycoerythrin pigments that are well-adapted to absorb these shorter wavelengths. Photosynthetic pigments absorb ~30-50% of light, depending on depth (Hawes & Schwarz, 2001). Within the mats, a considerable amount of irradiance penetrates beyond the pigmented layers, indicating that the light regimes in Vanda can support a greater biomass in the benthic mats than some other lakes (e.g., Lake Hoare) and suggests that the microbial mats in Lake Vanda may not have reached their light-determined carrying capacity (Hawes and Schwarz, 2001). Hawes and Schwarz hypothesize that this could be due to extremely low nutrient concentrations limiting photosynthetic biomass production; nitrate metabolism has been found to compete with carbon fixation for energy from photochemical processes in cyanobacteria (Hawes and Schwarz, 2001). Microbial mats in several MDV lakes have higher efficiency in using irradiance under limiting irradiance conditions, showing higher quantum yield with depth in the water column (Hawes and Schwarz, 2001).

Nitrogen cycling dynamics within Lake Vanda

Spatial Patterns

The N and P demands for photosynthesis in benthic mats are high relative to their concentrations within the water column. This mismatch suggests that both are supplied, at least in part, by remineralization of organic matter within the mats and sediments. Specifically, primary productivity calculations require that nutrients are concentrated in the interstitial waters of the benthic microbial mats and underlying sediments. Elevated nutrient concentrations have been observed in the interstitial waters of benthic microbial mats and the immediately underlying sediment of Antarctic lakes, with DIN and phosphate concentrations 3-220 and 2-102 times higher, respectively, than in the overlying lake water (Quesada et al., 2008; Tanabe et al., 2017). Similar accumulations in Lake Vanda may support primary productivity in benthic communities in an otherwise nutrient-limited environment. The high photosynthetic potential of these communities suggests that the small amounts of N and P from the Onyx River, or up from the UP, that enter the UCC are likely taken up by the benthic communities, keeping N and P concentrations in the UCC very low (Figure 5).

Below the UCC, the only sources of nutrients are remineralization of organic matter, diffusion transport of dissolved molecules, and settling sediment and organic particles. Since sediment and plankton concentrations are very low, metabolic transformations of N are extremely important, especially remineralization. These processes have been probed in the deep planktonic community by measuring nutrient concentration gradients, nitrogen isotopes, nutrient bioassay experiments, and net metabolite production and consumption rates (J. C. Priscu et al., 1989; Schutte et al., 2020; Vincent et al., 1981).

Nitrification typically happens where O_2 and NH_4^+ co-occur in the water column; however, in Lake Vanda, nitrification is not observed where the NH_4^+ diffuses upwards into oxic

water at 68 m (Schutte et al., 2020) instead peak nitrification rates occur 10 m shallower (Vincent et al., 1981). Schutte et al. (2020) attribute the lack of nitrification at the oxycline to the inhibition of ammonia monooxygenase (AMO) by methane (Schutte et al., 2020). It is possible that methane outcompetes ammonia for the enzymatic activity of AMO in nitrification when methane concentrations are high enough (Schutte et al., 2020). The substrate for AMO is ammonia, which has a very low concentration at 68 m depth due to the low pH (~6.3); while ammonium concentrations are >500 μmol L^{-1}, ammonia concentrations are only 1 μmol L^{-1}. Thus, methane concentrations (15 μmol L^{-1}) are 20 times higher than ammonia, which is consistent with observations of methane consumption and the lack of nitrate production at this depth (Schutte et al., 2020). At shallower depths, the methane concentrations are low enough for normal AMO function, resulting in nitrification (Schutte et al., 2020).

Some microbial N transformations are reflected in the $\delta^{15}N$ values of the nitrate in the water column of 10.3‰, 10.9‰, and 13.4‰ from depths of 52 m, 54 m, and 56 m, respectively (Wada et al., 1984). These depths overlap with peaks in nitrification (52.5-55 m) and photosynthesis (55-59 m) (Vincent et al., 1981). Specifically, $\delta^{15}N_{NO3-}$ values increase between 52 m and 56 m, where nitrification rates were highest. Thus, the positive nitrate $\delta^{15}N$ values are consistent with fractionations associated with nitrate assimilation via nitrification (Wada et al., 1984)

Nitrous oxide production and accumulation in Lake Vanda

Lake Vanda has unusually high nitrous oxide (>20,000% air saturation) concentrations at depths of ~61-64 m (Priscu, 1997; Schutte et al., 2020; Vincent et al., 1981), although not as high as concentrations in Lake Bonney, which has the highest concentrations of nitrous oxide yet reported for a natural aquatic system, at over 580,000% air saturation (J. C. Priscu et al., 1996).

Multiple processes for nitrous oxide production in Lake Vanda have been considered (Priscu, 1997; Schutte et al., 2020; Vincent et al., 1981). It can be produced: 1) with incomplete oxidation of hydroxylamine to nitrite during nitrification; 2) due to incomplete denitrification; 3) during nitrate assimilation; and 4) by abiotic ferrous iron reduction of nitrite. Vincent et al. (1981) hypothesized the source and sink of the nitrous oxide in Lake Vanda are nitrification and denitrification, respectively, by the planktonic communities (Vincent et al., 1981). Denitrification occurs several meters deeper than the nitrous oxide maxima and nitrous oxide concentrations were significantly reduced where *in situ* denitrification rates were highest, thus supporting denitrification as a sink for nitrous oxide rather than the source (Schutte et al., 2020; Vincent et al., 1981). Similarly, Schutte et al. (2020) postulate that nitrous oxide is unlikely to be produced as a byproduct of nitrate assimilation due to the net oxygen production depth being deeper than that of net nitrous oxide production. Abiotic nitrous oxide production is unlikely as dissolved iron is absent at the depths of the nitrous oxide maxima (Schutte et al., 2020). Thus, data from Schutte et al. (2020) support the hypothesis that Vanda's nitrous oxide was produced by nitrification. Nitrification activity was observed at the top of the salinity gradient through stable isotope incubations, and nitrate production with net nitrous oxide production was observed to be about 2% of the net nitrate production rate. This rate suggests nitrification was sufficient to produce both the net nitrous oxide and nitrate (Schutte et al., 2020).

Discussion

The ecosystem structure and function of the microbial communities inhabiting Lake Vanda's water column and substrate are shaped by the lake's optical, physical, and geochemical properties; however, the properties controlling these communities differ. Variations in the density of planktonic organisms correlate with the distribution and fluxes of nutrients, which

suggests that nutrients are a significant factor controlling planktonic communities. In contrast, benthic communities accrue biomass annually, forming elaborate pinnacles on the lake floor in the shallow nutrient-limited water, indicating a different control for benthic community structure. In deeper parts of the lake, where irradiance decreases and nutrient availability increases, microbial biomass production likely declines as irradiance becomes limiting to photosynthesis. Because light limitation is a major process shaping benthic communities, whereas the planktonic communities are mostly nutrient-limited, the impact of a changing climate on these communities will differ, and changes in both hinges on the feedback mechanisms driving their ecosystem dynamics.

The distribution and flux of nutrients are strongly influenced by the stratification and transport properties of Vanda's water column. The transport of dissolved nutrients that accumulate in the anoxic depths of the DZ is limited to the slow diffusion upwards into the overlying oxygenated water. It is here that nutrients are consumed by the deep planktonic community associated with the chlorophyll *a* maxima. The co-occurring nutrient and chlorophyll *a* maxima suggest high planktonic biomass where nutrients and light are sufficient for growth. Similarly, the low planktonic biomass and nutrient concentrations in the LCC and UCC signify nutrient limitation to the planktonic community, an interpretation consistent with prior research. Planktonic communities are constrained by the availability of nutrients necessary to support their metabolisms (Tilman et al., 1982); thus, the permanent stratification of Vanda's water column and the slow diffusive transport of nutrients in the DZ limits the spatial habitat of the planktonic communities. The deep planktonic community's consumption of nutrients diffusing upwards further limits nutrient input into shallower depths, limiting the distribution of nutrients in Vanda by both transport processes and microbial consumption. Without a significant shallow planktonic

community, light can transmit much deeper (50+ m), sufficient to support both the deep planktonic and benthic photosynthetic communities.

Benthic mats colonize Lake Vanda's substrate to depths exceeding 50 m, including the UCC, which, despite receiving nutrients from both the upward flux from the DZ and input from the Onyx River, has the lowest nutrient concentrations in the lake. The growth of benthic microbial mats in this nutrient-limited layer (Hawes et al*.,* 2013) indicates the benthic community is well-adapted to this environment, which requires efficiently obtaining and recycling nutrients. The nutrients support continuous photosynthetic primary productivity for several months in the austral summer when the mats receive ~24 hours of sunlight. The continuous irradiance, and thus carbon fixation, creates a high nutrient demand. The demand requires an internal cycling source as sufficient nutrients are unlikely to come solely from the UCC lake water given its oligotrophic nature. The uptake of nutrients from the UCC lake water is consistent with the UCC's consistently low nutrient concentrations. If the mats were releasing nutrients into the lake water through remineralization, the concentrations in the UCC would be higher, or we would see a larger shallow planktonic community. As there is no significant loss of N and P, the benthic mats must be a net nutrient sink. Nutrient concentrations remain low and relatively constant with increasing depth through the UP and LCC; however, irradiance rapidly decreases. As benthic photosynthetic activity has been demonstrated in mats to ~40 m and quantum yield slightly increases with depth in the lake due to higher efficiency utilizing irradiance, it is likely that the mats remain a sink for N and P even at depth despite decreased irradiance, but maybe less so as N concentrations increase around 50 m. The photosynthetic activity of the mats at this depth has not been characterized, thus microbial mat sequestration of nutrients is unlikely.

Microbial mats sequester nutrients with the help of an organic-rich matrix that allows the communities to tightly recycle nutrients under oligotrophic conditions. The planktonic community lacks this benefit, making obtaining nutrients more challenging. In the nutrient-limited UCC, the mat's net removal of nutrients from the water column further suppresses planktonic growth. With low planktonic biomass, more light is transmitted at depth, enabling benthic photosynthesis at depths of 40 m. As the photosynthetic capabilities of mats over 40 m are unknown, the mats may no longer be a sink for N and P. Between 45 and 50 m, the concentrations of N species slightly increase (Figure 5), which could be explained by 1) the loss of fixed N to the water column from the mats as their demand decreases or 2) byproducts from planktonic metabolisms at this depth. Schutte et al. (2020) document an increase in cryptophyte chlorophyll and chlorophyll *a* concentrations at this depth, which could be the result of increased N availability from the mats.

Interactions among the two ecosystems and their environments also influence the details of the N and P cycles. One of the sinks for N is falling organic matter particles. Due to slow or no convection, particularly at the depths with the highest planktonic biomass, these sinking particles settle downward into the deepest waters or the sediment surface. Where these particles settle determines the fate of bioavailable nutrients; if these particles settle on photosynthetically active mat, remineralization would provide the mat with bioavailable nutrients to sequester. If these particles sink below the photic zone or into the anoxic zone, they could be either buried or remineralized to provide a source of bioavailable nutrients to the planktonic community above. In contrast, P is not as readily recycled. While P can also be in dissolved and particulate organic forms, bioavailable P can be lost from the water column in the form of mineral particles, such as hydroxyapatite (Canfield and Green, 1985). The mineral hydroxyapatite is stable in the calcium

chloride-rich brine at the bottom of Lake Vanda (Canfield & Green, 1985). The absence of convection at these depths means the particles would remain locked in the sediment, permanently removing P from the ecosystem. While available P limits PP both in the planktonic and also potentially benthic communities, the benthic community's ability to recycle N into multiple bioavailable forms and the lack of a permanent N sink in anoxic waters, like with P, makes N less of a limiting nutrient. These cycling dynamics might explain why N is limiting in the Onyx River while P is limiting in Lake Vanda, where anoxic waters are a source of N (ammonia) but are a sink for P.

Lake Vanda's stratification and biogeochemical profiles have been relatively stable since researchers started documenting lake conditions. However, the effects of climate change may shift Vanda's physical and chemical properties and, thus, its ecosystem structure and function. As liquid water is of prime importance in this polar desert for nutrient mobilization (Barrett et al., 2007), an increase in liquid water would likely lead to increased landscape connectivity and weathering rates, altering the nutrient cycling dynamics of the valley ecosystem (Heindel et al., 2017). The lake's endorheic nature makes it sensitive to hydrological changes valley-wide; the Onyx River supplies Vanda with water and nutrients; thus, changes in discharge may affect nutrient cycling dynamics within the lake. Increased meltwater from Lower Wright Glacier, and the tributary glaciers, are predicted to pick up more nutrients from the surrounding soils as they are inundated (Ball et al., 2011). The nutrients will be transported downstream with the increased discharge as it floods the valley soils, eventually reaching the Boulder Pavement. The efficiency of the Boulder Pavement ecosystem in scavenging nutrients from the river before it reaches Vanda will determine how much any increase in nutrient transport will affect lake ecosystems. The Boulder Pavement's high surface area to volume ratio currently results in the removal of

most nutrients (Clive Howard-Williams et al., 1997; Green et al., 2005). Still, a higher volume of water flowing over these flat boulders may ultimately lead to higher nutrient influx into Vanda. When additional nutrients enter the water column, they are likely to be taken up by the benthic mats; however, we predict that light and space will start to limit the benthic community's growth rate and, thus, their ability to scavenge the new supply of nutrients. If nutrient influx outpaces benthic uptake, the UCC may no longer be nutrient-limiting to the planktonic community, causing blooms of the shallower planktonic community. These blooms would reduce downwelling irradiance, further limiting light for benthic communities at depth and possibly leading to the shallowing of the deep oxycline.

While blooms will likely reduce irradiance, a warming climate is expected to thin the perennial ice cover (Obryk et al., 2016), resulting in an increase in downwelling irradiance. Increased irradiance at depth would increase photosynthesis of the benthic community, thereby increasing their nutrient demand and uptake. This feedback would further limit the planktonic community, allowing for a clearer water column. Simultaneously, increased ice-cover melt is expected to expand the austral moat. Currently, the ice cover protects the water column from wave and wind action; however, as lake-atmosphere contact increases, wind and wave action will also increase. If waves erode benthic mats, they will likely release substantial amounts of dissolved and particulate N and P into the water column. Convection in the UCC could then distribute these moat-sourced nutrients throughout the lake. If they are not consumed by undisturbed benthic mats, these nutrients may result in increased planktonic biomass, in much the same way as would a greater influx of nutrients from the Onyx River.

The complexities of the feedback mechanisms driving these ecosystem processes make predicting the effects of climate change on Vanda ecosystems challenging. The balance of

nutrient supply and irradiance availability depends on environmental and ecosystem feedbacks and sensitivities. It is unknown whether the relative stability of the ecosystems since observations began represents stability against significant climate change or whether the system is poised for a dramatic change to one of substantial planktonic biomass increases. Ultimately, valley-wide changes in temperature, hydrology, and weathering will influence the biogeochemical cycles in Lake Vanda and, with those, the ecosystems themselves.

Conclusions

While Lake Vanda's physical and biogeochemical water column profiles have remained fundamentally stable over the decades they have been studied, climate change will most likely influence the processes producing these profiles. How a warming climate will affect the lake, its biogeochemical processes, and the microbial communities remains unknown. Valley-wide system dynamics strongly influence this polar, lacustrine ecosystem; thus, large hydrological shifts will likely have a profound impact on the lake's biogeochemical cycles and ecosystem structure and function.

Figure 1. Map of Lake Vanda in Wright Valley, Antarctica

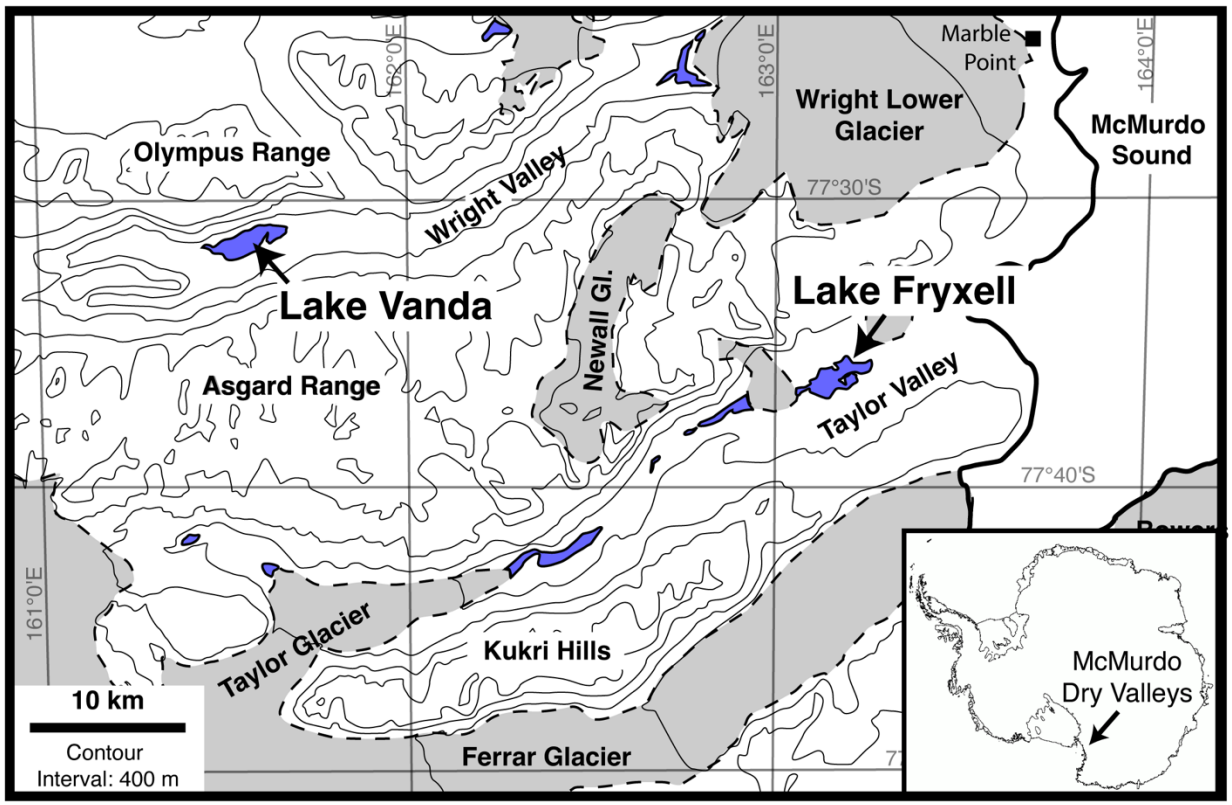


Figure 2. Map adapted from Green et al. 2005 and Howard-Williams et al. 1997. The Onyx River originates at Lower Wright Glacier and terminates at Lake Vanda; glaciers feeding the Onyx River are also shown.

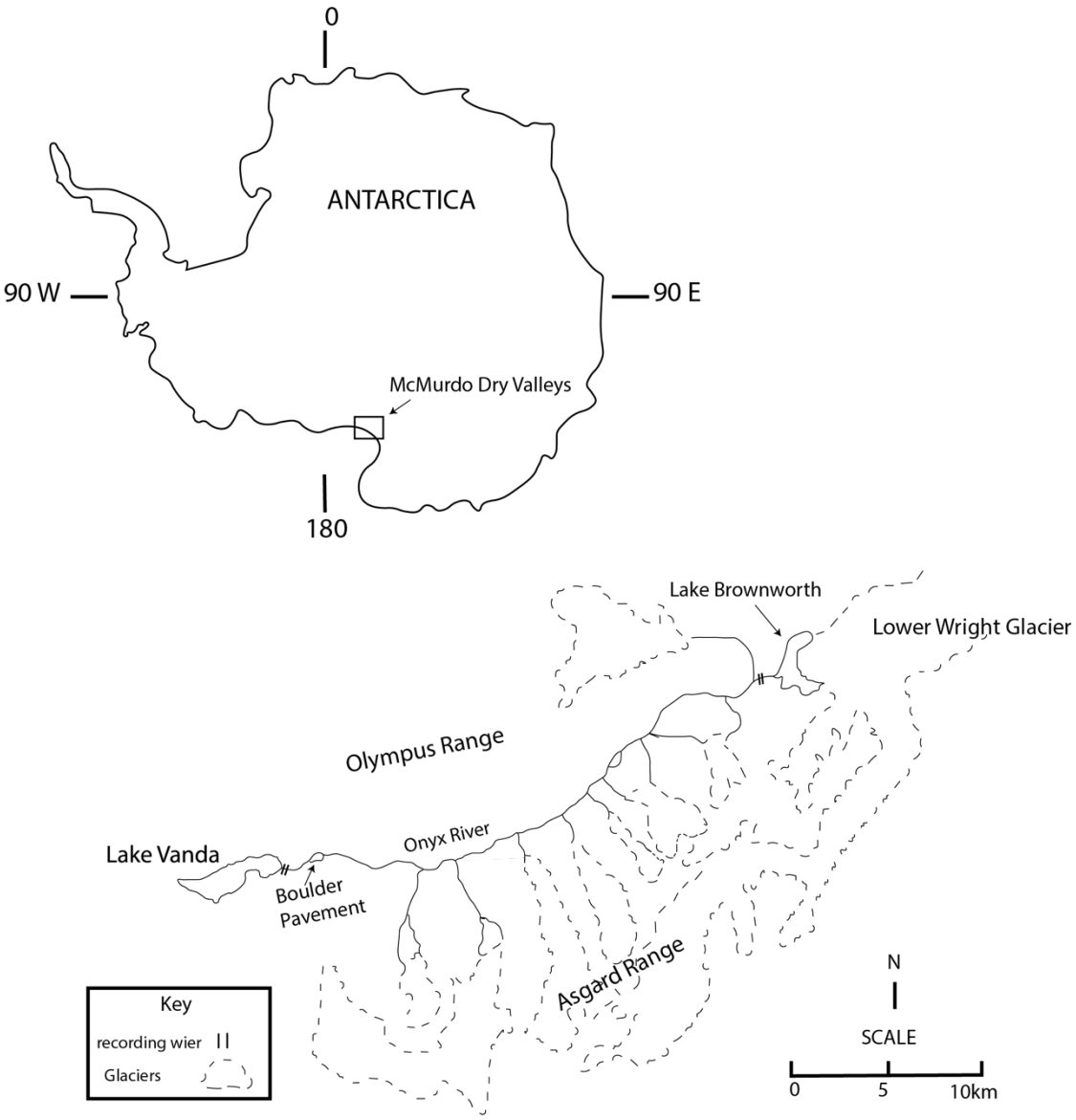
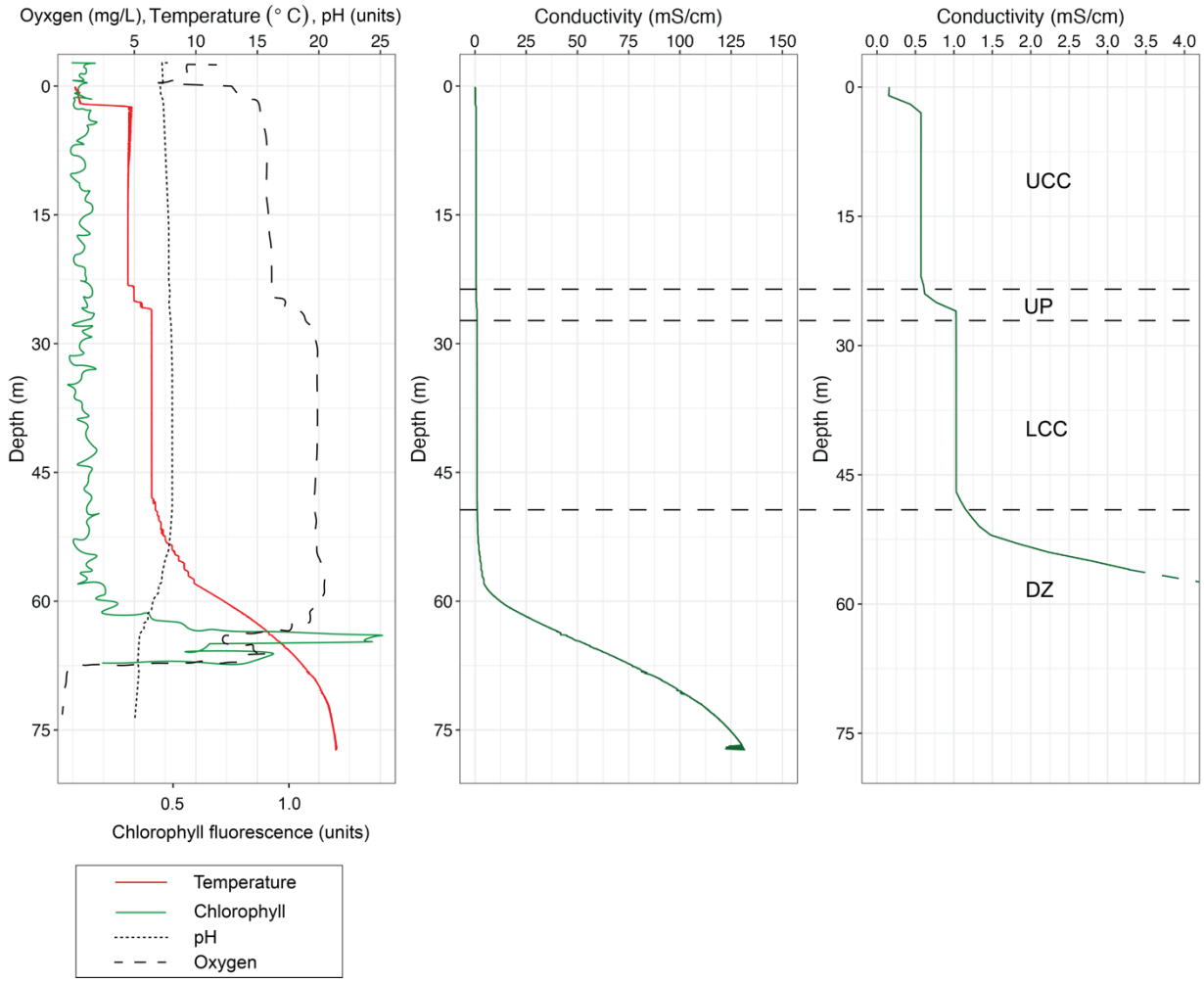


Figure 3.

a.) Temperature and conductivity profiles were collected December 16, 2013 from the lake surface (J. Priscu, 2022a). Lake structure separated into various cells, as described in Castendyk et al. (2016): upper convection cell (UCC), upper-pycnocline (UP), lower convection cell (LCC). And diffusion zone (DZ).



b.) Schematic of Lake Vanda showing physical structure of the stratified water column and the accumulation and diffusion-limited transport of nutrients from the DZ.

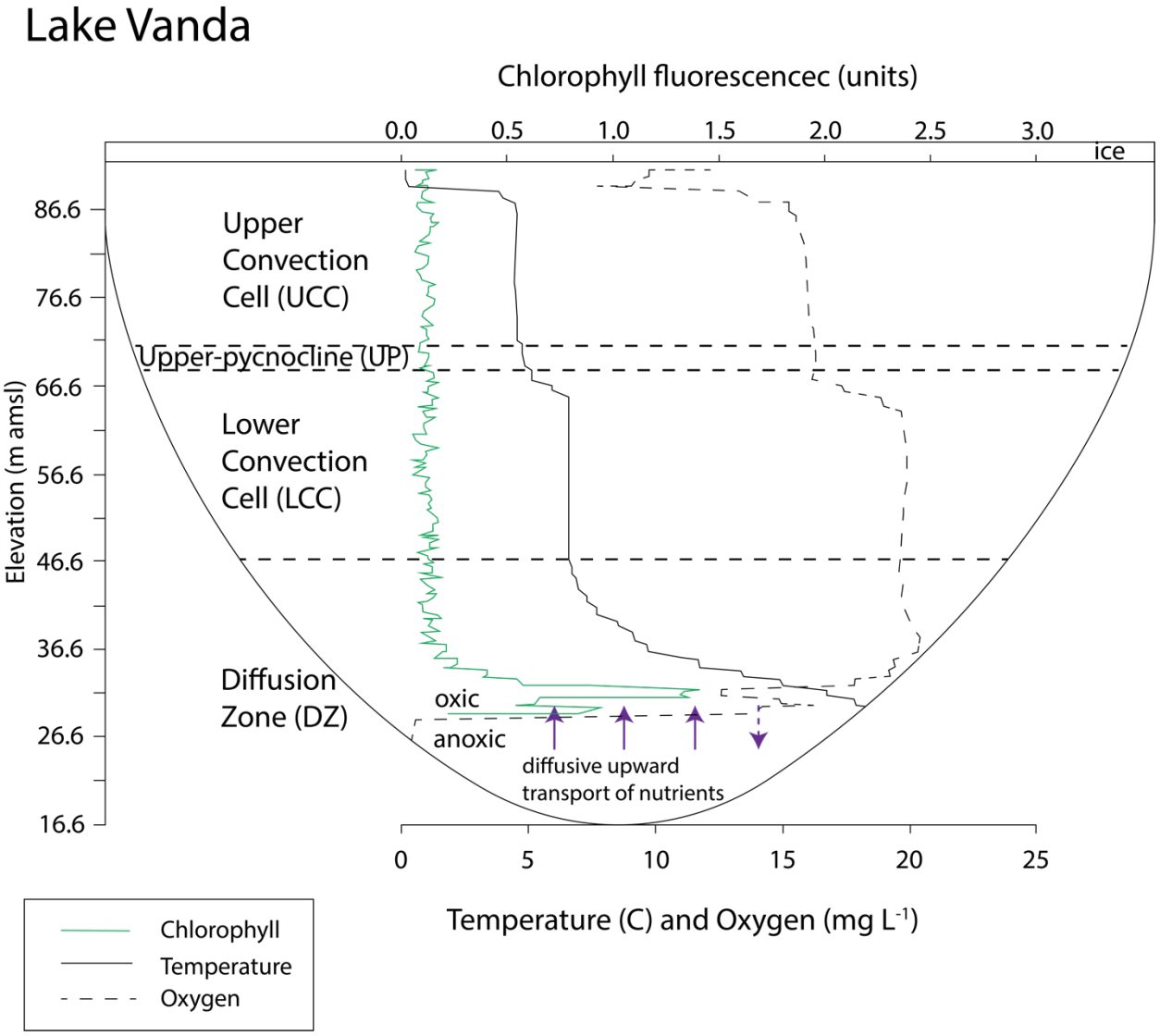


Figure 4. Lake level rise over years (meters above sea level) (P. Doran & Gooseff, 2023).

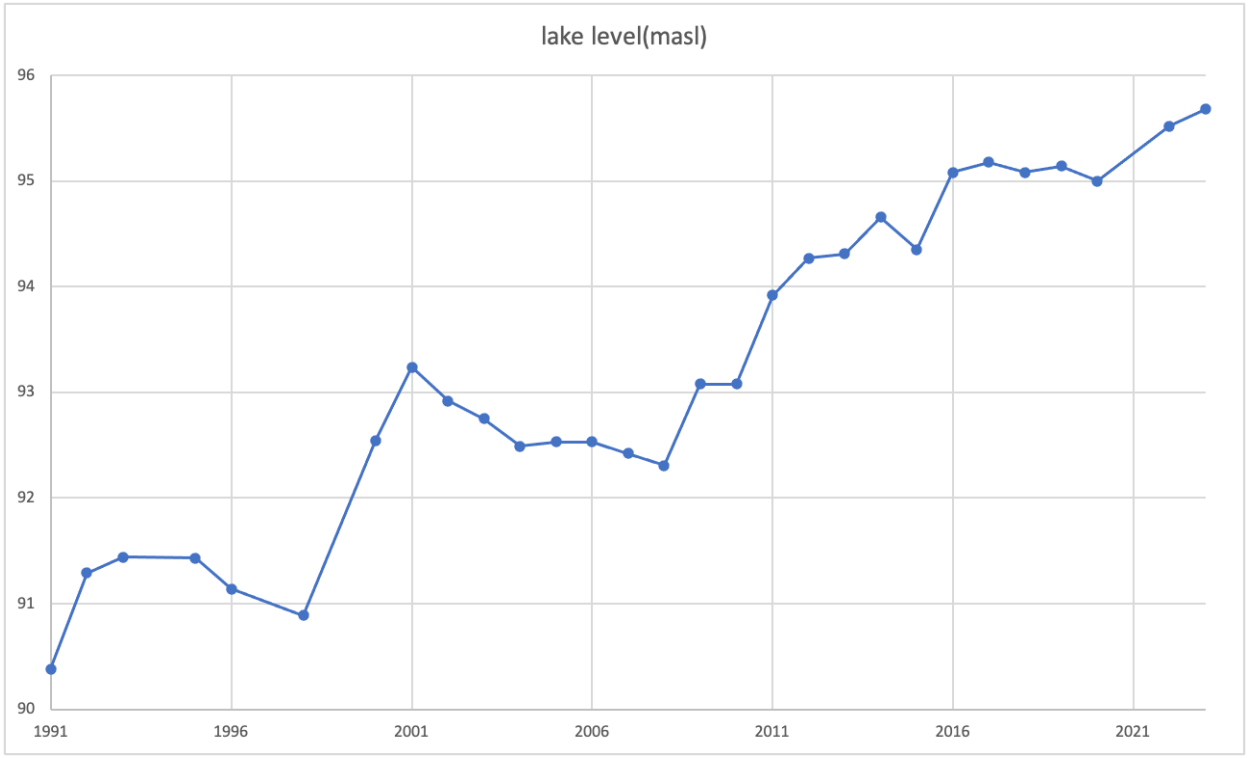
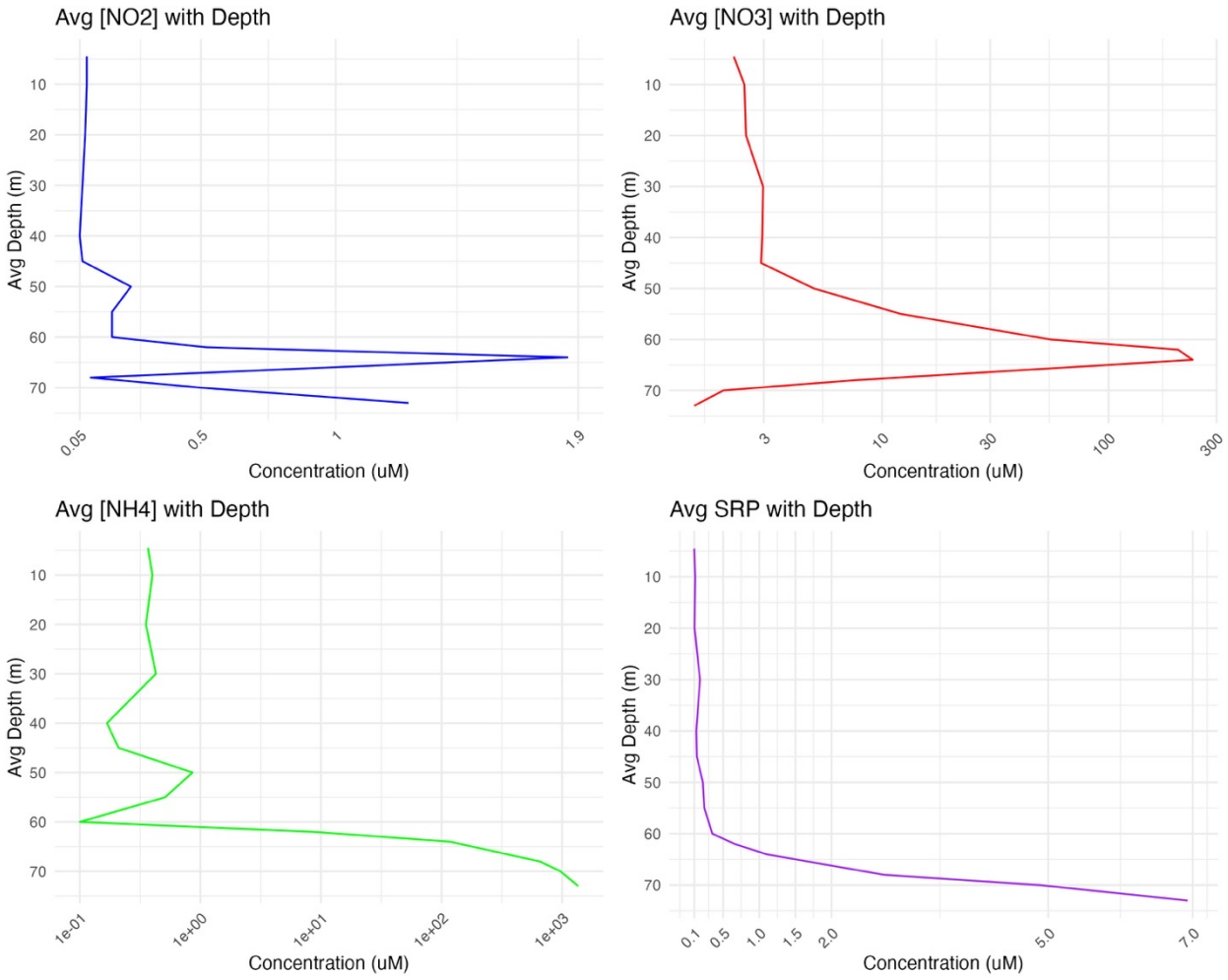


Figure 5. Nutrient concentrations with depth in Lake Vanda (J. Priscu, 2022b). Data are from 2001 and 2004-2008, the average was taken to get a more complete dataset.



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Chapter 2: Adaptations to Scarcity: Metagenomic insights into nutrient cycling dynamics of benthic microbial mats in oligotrophic, perennially ice-covered Lake Vanda

Abstract

Endorheic, meromictic, perennially ice-covered Lake Vanda of the McMurdo Dry Valleys, Antarctica, is home to thick, photosynthetic microbial mats growing on the lake floor to depths exceeding 50 m. The lake is oligotrophic, with nutrient levels low enough to limit planktonic communities in the upper water columns, making it one of the clearest lakes in the world. Despite this paucity of nutrients, the microbial mat communities accrue biomass on an annual scale, exhibiting elaborate pinnacled morphologies capable of photosynthesis ~5 mm into the pinnacles. We investigated the microbial strategies for nutrient acquisition and cycling through shotgun metagenomics, focusing on genes related to nitrogen and phosphorus metabolic pathways. We found significant gaps in the representation of several nitrogen cycling pathways, with genes missing for nitrogen fixation, and from nitrogen oxidation pathways: nitrification and anammox, suggesting constrained nitrogen cycling potential. Instead, we found the benthic communities demonstrate a robust capability for nitrogen assimilation and the degradation of organic compounds. The presence and abundance of genes related to metabolisms of alternative phosphorus sources indicate a sophisticated adaption to phosphorus scarcity. The communities exhibit a high metabolic potential for organic degradation and synthesis and a high degree of metabolic flexibility to utilize interconnected metabolic pathways (i.e., pyruvate and purine) to obtain sufficient phosphorus under orthophosphate limitation. By efficiently scavenging and tightly recycling nutrients, these mats effectively act as a sink for nitrogen and phosphorus in the surrounding water column.

Introduction

Microbial mats are self-sustained, vertically stratified, multi-layered communities of microorganisms bound together by extrapolymeric substances (Bonilla-Rosso et al., 2012). These mats, characterized by their phylogenetic and metabolic diversity, are known for their ability to thrive in a range of extreme environments, from hypersaline to ultraoligotrophic conditions. Vertical stratification results from interactions amongst microbial communities and their immediate environments that often generate steep chemical gradients that create a series of metabolic niches (Rojas et al., 2021). Oxygenic Cyanobacteria typically dominate the well-lit upper mat layers alongside other aerobic organisms, while anaerobic microbes prevail in the anoxic bottom layers (Des Marais, 1990). The close proximity of the mat layers enables complex and efficient metabolite exchange networks to develop based on diffusion alone, allowing these diverse communities to thrive (Bonilla-Rosso et al., 2012). While microbial mats are found around the globe, their distributions are restricted to environments without significant disturbances, such as erosive action (e.g., wave, wind) or eukaryotic grazing/burrowing; these environments are often found in more extreme environments where other macroscopic organisms can't survive (Des Marais, 1990). Fossilized microbial mats are some of the earliest evidence of microbial ecosystems, indicative of their adaptive success in surviving environmental change over Earth's history (Bonilla-Rosso et al., 2012). Modern mat ecosystems are often studied as analogs for life on early Earth and, potentially, on other planets.

Benthic microbial mat communities colonize the floors of most perennially ice-covered lakes and streams of the McMurdo Dry Valleys (MDV) of Antarctica. The MDVs are polar deserts with little biomass; however, these lacustrine benthic mat communities are oases where productivity often exceeds that of lacustrine planktonic communities (Hawes et al., 2001). The

MDVs receive less than 100 mm of precipitation annually and experience freezing temperatures and frequent freeze-thaw cycles (Doran et al., 2002). Despite these inhospitable conditions, the benthic mat communities are hotspots of primary productivity (PP) and key players in the MDVs' carbon and nutrient cycles (Hawes et al., 2014). In the MDV, a series of lakes are found where liquid water persists throughout the year, and here, mats accumulate substantial biomass on annual scales and exhibit elaborate morphologies due to the absence of disturbance factors such as wind and bioturbation (Sumner et al., 2016).

One of these lakes, Lake Vanda occupies a closed basin in Wright Valley and is capped with a 2.5- to 4-m-thick, perennial ice cover. Approximately 15 to 20 % of irradiance is transmitted through the ice to the salinity-stratified water column, which comprises two convection cells separated by a pycnocline and a diffusion-limited brine zone at the bottom (Figure 1) (Castendyk et al., 2016; Chapter 1). Benthic microbial mats colonize the lake floor to depths >50 m and form elaborate pinnacle morphologies ranging from millimeters to decimeters in size (Sumner et al., 2016). Most of Lake Vanda is severely limited in phosphorus (P) and has low nitrogen (N) concentrations throughout much of its water column (Canfield & Green, 1985; J. C. Priscu, 1995; J. C. Priscu et al., 1989). The benthic communities play a crucial role in the cycling of carbon and nutrients both in the lake and valley-wide (Hawes et al., 2013).

In such ultraoligotrophic environments, the microbial mat communities must be well adapted to obtain the necessary nutrients; the high biomass and elaborate morphologies of the Vanda benthic mats suggest they are meeting this demand. To better understand the mechanisms underlying the nutrient cycling of these benthic microbial communities, we conducted a metagenomic survey aimed at characterizing the metabolic potential of the benthic communities in Lake Vanda. In this study, we present the strategies employed by the benthic microbial

community to overcome extreme nutrient scarcity in this MDV lake. With a primary focus on identifying genes related to the scavenging and recycling of N and P, we hope to contribute to a broader understanding of microbial adaptations and nutrient cycling dynamics in extreme environments.

Background

Lake Vanda nutrient availability and cycling dynamics

Lake Vanda has extremely low concentrations of N and P in its water column at all depths above the anoxic water column in the DZ where nutrients accumulate (Figure 2). The microbial mats in shallower waters are still growing towards saturation biomass and accrue biomass at an average rate of 390 mg m^{-2} y^{-1} (Hawes *et al.,* 2013). These mats have C:N and N:P mass ratios of approximately 14:1 and 40:1 and have an approximate annual accrual of 24 and 1.4 ug m^{-2} y^{-1} of N and P, respectively (Hawes *et al*., 2013). The flux of nutrients from the DZ accumulation zone into the upper lake is constrained by the salinity-stratified structure of the water column, where nutrients are transported via diffusion within the DZ, before entering the convectively mixed LCC (Figure 1b). Furthermore, a layer of active phytoplankton form a deep chlorophyll maximum within the DZ salinity gradient, and largely prevent nutrients exiting the density gradient (Figure 1b) (J. C. Priscu, 1995; Vincent & Vincent, 1982; Vincent Warwick F. et al., 2003). Nutrients concentrated in the deep DZ thus enter overlying water compartments slowly, if at all (Chapter 1).

Within the lake, N is transformed through microbially mediated redox reactions. These processes are separated within the water column along oxygen gradients, with aerobic processes (i.e., nitrification) occurring in the presence of oxygen and the anaerobic processes (i.e.,

denitrification, anammox, dissimilatory nitrate reduction to ammonium) occurring under anoxic conditions. These reactions and transformations happen on a spatially smaller scale within a microbial mat.

Phosphorus limitation is often more extreme in cold environments due to slow rates of weathering of parent material and the affinity of di- and trivalent cations and calcium towards phosphate, resulting in larger pools of sorbed and mineral P (Wang et al., 2024). P cycling genes are classified into two groups: "extracellular" genes, which are responsible for inorganic P solubilization and organic P mineralization, and "intracellular" genes, responsible for the biosynthesis of P compounds in microbes (e.g., inorganic P assimilation into microbial biomass, transporter genes, P starvation regulation genes) (Wang et al., 2024). Some microbial adaptations used to deal with P scarcity include decreasing cellular P demand by substituting non-phosphorus membrane lipids (like sulfur- and nitrogen- lipids) in place of phospholipids (Dyhrman et al., 2012; Park et al., 2022; Shemi et al., 2016; Van Mooy et al., 2009). Other adaptations include mechanisms to enhance their phosphate uptake efficiency and metabolisms to utilize more recalcitrant forms of P (e.g., phosphonates, polyphosphates, phosphoesters) (Dyhrman et al., 2006; Riegman et al., 2000). Interconnected metabolic pathways prove helpful in reserving P, such as efficiently managing pyruvate (Wang et al., 2024). Pyruvate metabolism is involved in the conversion of organic P compounds like phosphoenolpyruvate (PEP), an intermediate in the glycolytic pathway in the production of ATP, which acts as a source of inorganic P as it is released into the environment, particularly important under P limitation (Wang et al., 2024).

Adaptive nutrient scavenging and recycling strategies of Vanda's benthic microbial

communities may be influenced by other environmental conditions and stressors (e.g., light availability and nutrient transport processes), resulting in interesting ecosystem dynamics.

Descriptions of microbial mats

The mats in this study were sampled from the upper convection cell (UCC) of Vanda's salinity-stratified water column. The water column at this depth is slowly convecting and fully oxic with a temperature of ~4°C and a pH of 8 to 9 (Spigel & Priscu, 1998). In 2013, the mats at 19 m received 32% of irradiance penetrating through the ice cover (C. L. Grettenberger et al., 2023). The pinnacles are laminated on millimeter scales, alternating hyaline and opaque, representing annual growth (Hawes et al., 2001). The laminae pigment changes with depth into the mat, going from brown on the surface to green to purple and then to beige on the interior (C. Grettenberger et al., 2021; Sumner et al., 2016). Based on pulse amplitude-modulated (PAM) fluorometry and 16S rRNA sequencing, brown, green, and pink pigments are associated with a significant cyanobacterial community (C. L. Grettenberger et al., 2023; Sumner et al., 2016). Deeper into the interior of the pinnacles, beige parts of the mats lack photosynthetic pigments associated with a predominantly heterotrophic community.

Methods

Sampling

In December 2013, a hole was melted in the Western Basin of the lake at 77° 31.60S, 161° 36.30E. Scientific divers collected pinnacled mat samples from 19 m depth, corresponding to an elevation of 76 meters above sea level (masl) (Castendyk et al., 2016). Samples were collected with a knife, placed in alcohol-sterilized containers, and sealed before being transported to the lake surface. At the surface, pinnacles were subsampled using aseptic sampling techniques with

ethanol and flame-sterilized forceps and scalpels. Sampled pinnacles used for this study include large (n=1), medium (n=5), and small (n=1) pinnacles; pinnacles were subsampled by pigment with depth into the mat: green, pink/purple, and inner beige for a total of 10 samples. After dissection, subsamples were placed in Zymo Xpedition buffer (Zymo Research, Irvine, CA), and cells were lysed via bead beating in the field (Zymo ZR Bashing Bead tubes). Samples were frozen on dry ice, shipped back to UC Davis frozen, and stored at -80 ℃ until downstream analysis.

DNA extraction and sequencing

In the lab, frozen mat samples were thawed, and DNA was extracted using the Zymo Soil/Fecal miniprep kit following the kit protocol. Extracted DNAs were quantified via Qubit (Life Technologies), concentrated via evaporation (>10 ng/uL), and sequenced.

Metagenomic sequences are from Grettenberger *et al.* (2020); thus, detailed DNA sequencing and bioinformatics analysis methodology can be found there. To briefly summarize the methodology, sequence data was generated by JGI using Illumina technology, and the reads were quality-controlled using the Illumina pipeline. The Illumina library was constructed using the Illumina HiSeq-2500 1TB platform and sequenced 2 x151 bp.

Samples are identified by pinnacle size: small (S), medium (M), and large, as well as pigment color: green (G), pink (P), and inner beige (IB). Metagenome sample IDs are as follows: Bulk Mat, MP5G1, MP5IB2, MP6G1, MP6IB1, MP7G1, MP7P2, MP8IB2, MP9P1, P2IB, and SP4G1.

Sequence processing and bioinformatics analysis

Details of the bioinformatic analyses can be found in Grettenberger *et al*. (2020). Quality-controlled, filtered raw data was retrieved from IMG Gold (JGI Gold ID GP0191362 and GP0191371). MEGAHIT 1.0.6 was used to assemble individual metagenomes, Bowtie2 1.2.2 was used to map back reads to the assembly, MetaBAT was used to bin the assemblies with a minimum contig length of 2500 bp. CheckM 1.0.7 was used to assess the quality of bins and identified based on phylogenetic placement, and average nucleotide identity (ANI) was calculated using OrthoANI algorithm. Prodigal V2.6.3 was used to identify protein-coding regions in CheckM, GhostKOALA and Prokka 1.12 were used for translated protein sequence annotations. The whole-genome shotgun project can be found on Genbank under accessions JAAXLU000000000 and JAAXLT000000000.

Gene families and associated processes

In order to determine metabolic potential of the microbial communities, genes were chosen from N and P cycling pathways. For N cycling, genes were chosen using NCycDB, a curated database targeting eight N cycle pathways recruiting a total of 65 gene (sub)families (Tu et al., 2019). These eight families include: N-fixation, nitrification, anammox, denitrification, assimilatory and dissimilatory nitrate reduction, hydroxylamine reduction, organic degradation and synthesis (urea metabolism, amino acids, glutamate metabolism, asparagine metabolism, and allantoin, chitin, and cyanate degradation), as well as N-storage genes (cyanophycin synthesis and degradation) that were not included in NCycDB.

Similarly, PCycDB was used to characterize P cycling metabolisms (Zeng et al., 2022). PCycDB targets 139 gene families and ten P metabolic processes. Metabolisms associated with P cycling include two-component system genes, transporter genes, phosphonate and phosphinate

metabolisms, phosphite and hypophosphite oxidation, organic phosphoester hydrolysis, and oxidative phosphorylation, as well as genes associated with purine and pyruvate oxidation.

All N and P cycling genes of interest can be found in Supplementary Table 1.

Gene relative abundance

Genes of interest were pulled from the annotated metagenomes, and the relative abundance of each gene was calculated by gene length (stop - start of codon + 1) * total average depth divided by the depth * length of the metagenome assembly size.

Phylogenetic inference

Previous work recovered and assembled metagenome-assembled genomes (MAGs) from the microbial mat metagenomes, and the bins of interest were identified based on phylogenetic placement (C. L. Grettenberger et al., 2020; J. E. Lumian et al., 2021; Powell et al., 2024). The average nucleotide identity was calculated, protein-coding regions were identified, and translated protein sequences were annotated (C. L. Grettenberger et al., 2020; J. Lumian et al., 2024). The whole-genome shotgun project can be found at Genbank under accessions JAAXLU000000000 and JAAXLT000000000 (Grettenberger *et al.* 2020). To address the question of which taxonomic groups had the metabolic potential to contribute to the various N and P cycling pathways, we investigated the taxonomic origin of organic degradation and recycling genes within the previously assembled MAGs. GTDBTk version 2.4.0+ was used for taxonomic assignment using the GTDB database release 220 (ref). The following commands were used to run GTDBTk: gtdbtk identify -- genome_dir ./-- out_dir ./-x fasta; gtdbtk align --identify_dir ./-- out_dir ./; gtdbtk ani_rep --genome_dir ./--out_dir ./-x fasta; gtdbtk classify --genome_dir ./--align_dir ./out_dir ./--mash_db ./-x fasta (ref).

Results

Many microbial mat communities are layered vertically, both phylogenetically and metabolically; thus, we expect the nutrient demand to also vary by layer. The metabolic potential and nutrient cycling dynamics within the mats were characterized by comparing the mats' photosynthetic (green and pink pigmented zones) and non-photosynthetic (inner beige pigmented zone) communities.

N and P cycling gene analysis

Presence-absence and relative abundance were determined for genes from selected metabolic pathways involved in inorganic and organic N and P cycling within the microbial mat metagenomes. There were no significant differences in gene relative abundances between the photosynthetic and non-photosynthetic communities for either N or P cycling genes.

Inorganic N cycling

The metagenomes did not represent several inorganic N cycling pathways (Figure 3ab). Out of 11 samples, five lack the *nifH* gene (essential for N-fixation). In samples where *nifH* is present, which includes four photosynthetic and two non-photosynthetic samples, the gene relative abundance was low, and all of these samples lacked the rest of the *nif* operon genes (*nifDK*) necessary for N-fixation. Marker genes associated with alternative N-fixation pathways, vanadium nitrogenase (*vnf*) and iron-only nitrogenase (*anf*), were also missing from all sample metagenomes (Figure 1). Genes associated with anammox, the anaerobic process of converting NH_4^+ and NO_2^- into N_2 gas, were missing from the metagenomes, indicating the mat communities were also incapable of this anaerobic N-oxidation process. Similarly, several of the genes required for nitrification (NH_4^+ -> NO_3^-) were either missing (*nxrAB, amoC*) or very rare

(*amoAB, hao*). Genes associated with denitrification were found in all of the Vanda metagenomes, although at low relative abundances (Figure 3ab).

Organic degradation, scavenging, and storage of N and P

Regarding organic N cycling, the genes associated with ammonium assimilation, organic degradation and synthesis, and urea metabolism were present, and in some cases highly abundant, in all metagenomes (Figure 3ab). Genes associated with the synthesis and degradation of cyanophycin (*cphAB*), a biopolymer that stores fixed N and C in most cyanobacteria and some other bacteria, were present in all metagenomes at high relative abundances. Genes associated with organic degradation (e.g., allantoin and chitin degradation and cyanate hydrolysis) were also found in the metagenomes.

Due to exceptionally low concentrations of orthophosphate in Vanda's water column, we looked for genes related to the metabolism of other forms of phosphorus (phosphonate/phosphinate and hypophosphite/phosphite), remineralization and degradation of organic forms of P, as well as genes associated with the metabolism of purine/pyrimidine and pyruvate. Genes related to the metabolism of the other inorganic phosphorus forms hypophosphite and phosphite were either missing or at very low relative abundances in all metagenomes (Figure 4). Genes associated with phosphonate/phosphinite (an organic phosphorus release) metabolism were present in all metagenomes; the genes include the *phn* gene cluster (*phnGHIJKLM*) that encodes for the C-P lyase complex to release phosphate and a hydrocarbon (Metcalf & Wanner, 1991). The gene *phnN* is present and encodes for the enzyme phosphoribosyltransferase, which is responsible for activating phosphonates for degradation. Genes for degradation enzymes were also present, specifically phosphonate dehydrogenase (*phnA*), which oxidizes phosphonates to phosphite, phosphonopyruvate decarboxylase (*ppdK*),

which converts phosphonopyruvate to phosphonoacetaldeyde, and phosphonopyruvate hydrolase (*phnX*), which cleaves the C-P bond in phosphonopyruvate to release phosphate and pyruvate.

Genes associated with organic phosphoester hydrolysis were also present in the metagenomes. These genes include *aphA*, *phoAD* (alkaline phosphatases), which hydrolyze organic phosphate esters to produce P_i and *appA* (periplasmic acid phosphatase) and *phoC* (acid phosphatase), which hydrolyze phosphate esters under acidic conditions. Phytase is encoded by *phy* and is responsible for hydrolyzing phytate for P_i and *ugpQ* is involved in scavenging P_i from glycerol-containing compounds, both of which are important sources of P under P_i limiting conditions. Genes associated with organic phosphoester hydrolysis that were missing from the metagenomes include *opd,* a gene involved in the degradation of organophosphonate compounds, as well as *phoN* (acid phosphatase) and *phoX* (Ca-dependent alkaline phosphatase), both of which are involved in the scavenging of organic phosphate compounds under P_i limitation.

At high relative abundances were genes associated with purine/pyrimidine and pyruvate metabolisms. Key genes were missing from all metagenomes, including some transporter genes (*aepPSVWX, htxB, pit, ptxABC, ugpE*) and some two-component system genes (*pgtAB, RegX3, SenX3*).

Discussion

Lake Vanda's primary producers provide carbon and nutrients to the rest of the ecosystem, and to do so, they must meet their nutrient demand for growth. The mat communities' ability to sustain the annual accrual of biomass indicates that this nutrient demand is met despite the water column's exceptionally low nutrient concentrations and the constraints of diffusion-limited

nutrient flux between lake compartments. We expected that the cycling of nutrients internal to the mats supports primary productivity in addition to any small amount that could be acquired from the overlying water, with the communities efficiently scavenging nutrients from remineralization and tightly recycling nutrients within the mat. To test this internal cycling hypothesis, we evaluated the genetic capabilities of the community with respect to key N and P cycling pathways.

Nitrogen cycling

In environments where N is limiting, microbial genetic adaptations often include N-fixation when there is enough energy to perform it. The paucity of N-fixation genes in the Vanda mat metagenomes confirms that the communities are incapable of N-fixation, indicating no *in-situ* production of bioavailable N. In a similar study looking at the metagenomes of a high Arctic ice-shelf mat, Varin *et al*. (2010) attribute the low abundance of N-fixation genes to the high N:P ratio of the mat pore water (44 to 66), consistent with a P deficiency and 'surplus' of N. Vanda also has a severe P deficiency with a mat N:P of ~40:1, similar to the Arctic mat environment. This could explain the lack of N-fixation genes in the metagenome, the low irradiance beneath the ice cover, and the lack of N-fixers in the mat communities (even in the high irradiance austral moats) previously reported (Ramoneda et al., 2021).

The presence/absence of other N cycling genes in the Vanda metagenomes sheds light on the mat's nutrient cycling dynamics. The missing or low abundance of nitrification genes is consistent with the very low nitrate and nitrite concentrations in the water column (Figure 3ab). The high relative abundance of cyanophycin synthetase (*cphA*) and cyanophycinase (*cphB*) in all metagenomes suggests that cyanophycin is an important source of N to the community- a biopolymer built from arginine and aspartate. It accumulates in opaque granules in the cell,

allowing the microbes to accumulate and store fixed N during N starvation (Watzer & Forchhammer, 2018). In nondiazotrophic cyanobacteria, cyanophycin has been found to accumulate during either time of excess N availability or under suboptimal growth conditions (e.g., phosphate, sulfate, and potassium starvation, high salinity, low light levels) and is synthesized at night (Sharon et al., 2023; Watzer & Forchhammer, 2018). The nutrients in cyanophycin are available to both those that produce it and some cyanophycin-scavenging microorganisms, which have evolved the ability to obtain nutrients from cyanophycin of lysed cells in the environment (Sharon et al., 2023). Some of these microbes use cyanophycin as their sole source of N and carbon (Sharon et al., 2023). The abundance of cyanophycin genes could indicate the mats are storing fixed N over seasonal scales as N demand is high during long periods of continuous photosynthesis during summer with much lower demand during months of winter darkness; cyanophycin could act as a significant source of fixed N to the mat community during times of N scarcity, predicted during summer.

Over the summer, the benthic mats remain oxic within 10 cm of the mat surface (Hawes et al., 2013). Oxic conditions ultimately limit the metabolic processes within the mat to aerobic metabolisms (i.e., nitrification, ammonification) and prevent anaerobic processes (i.e., denitrification). The inhibition of denitrification means there is little loss of fixed N (other than burial) to the environment during summer when the mat is oxic, thus retaining N as readily bioavailable species. Over the winter, however, the water column remains oxic, but O_2 production in the mats stops. Whether the mats go anoxic for any duration of the winter remains unknown. Either the consumption rate of O_2 is slow enough that they remain oxic, or O_2 is completely consumed internally, resulting in anoxia within the mat. In the case that the mats remain oxic, anaerobic processes would still be inhibited; however, without photosynthesis, the

metabolic activity of the active mat would be reduced as would the N demand. Without a high N demand and depending on water column N concentrations, fixed N may diffuse out of the mats and into the water column. If the mats go anoxic over winter, conditions are suitable for anaerobic processes like denitrification and anammox. The absence of anammox genes in the metagenomes leaves denitrification as the only process capable of N_2 gas production. While denitrification genes are present in the metagenomes, there is no significant source of nitrate to act as a substrate for this process. Low concentrations of nitrate in the water column and missing nitrification genes responsible for nitrate production suggest denitrification is not a dominant process.

Without denitrification, there is an insignificant loss of fixed N to the system. Despite this, the low N concentrations in the water column indicate that fixed N is likely quickly assimilated and converted to organic molecules, including storage as cyanophycin. We do not have mat or sediment porewater geochemistry to measure any accumulation of ammonium, a phenomenon seen in sediment porewaters of other oligotrophic lakes (Tanabe et al., 2017). However, the metabolically constrained N cycle and the abundance of organic degradation and synthesis genes suggest both dissolved organic and inorganic N are likely quickly assimilated and tightly recycled. The low DIN water column concentrations pose the question of sediment porewater geochemistry; remineralization at depth and no metabolic potential for loss of fixed N in the active mat indicates there could be significantly higher concentrations of DIN in underlying sediment.

Despite low N water column concentrations, the benthic community appears efficient at scavenging, storing, and recycling N. These effective N-acquisition strategies may help

overcome any potential N-limitation for the benthic communities; instead, growth might be limited by the extremely low orthophosphate concentrations in Vanda's water column.

Phosphorus cycling

As P limitation is more extreme in cold environments (particularly orthophosphate) (Wang et al., 2024), the genetic adaptations to use alternative forms of P, and strategies to conserve and recycle P, are particularly important for benthic primary productivity.

Like cyanophycin as an N storage adaptation, some microorganisms can utilize compounds to store and hydrolyze P during alternating periods of sufficiency and limitation – perhaps related to light cessation of growth in winter. The P storage genes associated with the synthesis (*ppk*) and hydrolysis (*ppx*) of the reservoir compound polyphosphate were highly abundant in all samples. Polyphosphate synthesis and hydrolysis are particularly advantageous for microorganisms living in nutrient-depleted environments as polyphosphate can be used to store available P and hydrolyze it when external P is limited. Polyphosphate granules are synthesized from ATP in microbial cells for energy and phosphate storage, which can subsequently be degraded into nucleotide triphosphate or phosphate (Achbergerová & Nahálka, 2011).

In environments where P_i is limited, organic P compounds such as phosphonates and phosphinates can be important sources of P; however, their metabolism requires enzymes that catalyze the stable C-P bond. The *phn* gene cluster is responsible for this metabolism (*phnGHIJKLM*) encoding for the C-P lyase complex, which releases phosphate and a hydrocarbon. The presence of genes affiliated with phosphonate/phosphinate metabolisms indicates the mats do have the adaptive metabolic potential to utilize alternative P sources, while

the genes associated with the hydrolysis of organic phosphoesters highlight the communities' ability to scavenge P from organic P compounds under P_i limitation.

The high abundance of pyruvate metabolism genes in all metagenomes suggests these microbial communities could utilize interconnected metabolic pathways to obtain sufficient P under P_i limitation. In a P-limited environment like Lake Vanda, the ability of microbial communities to efficiently manage pyruvate is a crucial part of the P cycle. While metagenomic data only highlights the metabolic potential, the abundance of genes associated with various pyruvate metabolic pathways, including glycolysis, gluconeogenesis, and the tricarboxylic acid (TCA) cycle, could suggest a high degree of metabolic flexibility, enabling microbes to switch between energy production and carbon storage based on availability and all can be used as adaptive mechanisms to maintain P homeostasis (Dyhrman et al., 2012). The high representation of glycolytic genes indicates that microbial mats can rapidly utilize available organic carbon sources for energy. During this process, the conversion of PEP to pyruvate (*pps* and *pyk*) liberates $P_{i,}$ which can be recycled within the cell. Conversely, genes involved in gluconeogenesis highlight the capacity to synthesize glucose from non-carbohydrate precursors, which is particularly important during periods of low external nutrient supply and replenishes the glucose required in glycolysis (Schink et al., 2022). The detection of TCA cycle genes further supports the presence of aerobic and facultatively anaerobic organisms capable of complete oxidation of pyruvate to CO_2, thus maximizing energy yield. Inorganic P is also released during this cycle, contributing to the pool of bioavailable P. These processes are important as the efficiency of the conversion and recycling of their intermediates allows the community to survive when external P_i is scarce.

Similarly to pyruvate metabolism, purine metabolism is essential for synthesizing nucleotides– the building blocks of DNA and RNA. Nucleotide metabolism genes are an integral part of purine and pyrimidine metabolism and are involved in the degradation of nucleotide molecules to release organic and inorganic P. These genes are involved in both the de novo synthesis and salvage pathways of purine metabolism; this recycling is especially advantageous in nutrient-deplete conditions where conserving and reusing cellular components is essential for survival. At the very least, the efficiency of this recycling not only supports the genetic and functional stability of microbial communities but also reflects an adaptive response to the oligotrophic environment, ensuring that vital cellular processes are sustained even under nutrient scarcity.

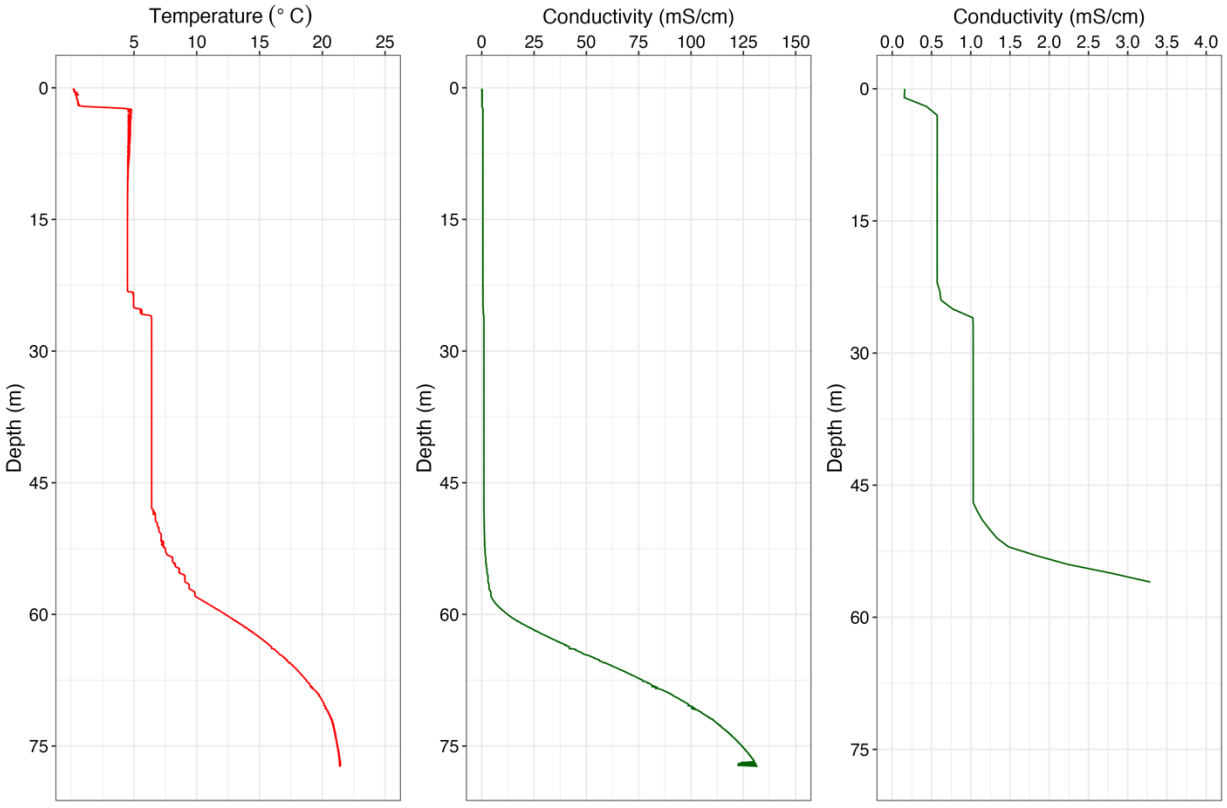
Conclusions

Lake Vanda's benthic mat communities are capable of maintaining annual biomass production to depths of 50 m under severe nutrient limitation. Based on samples from 19 m depth, the metabolic inhibition of N-fixation and the absence of genes necessary for multiple N cycling pathways indicate a constrained benthic N cycling potential, with a focus on processes that retain and recycle N. This limited N cycle, in tandem with the physiological capacity to optimize P use and retention, indicates that these microbial communities are well-adapted to acquire sufficient nutrients from organic degradation and efficient recycling and assimilation of remineralized nutrients within the mat, preventing any loss to the water column. Acting as a sink, water column N and P concentrations remain low or below detection, particularly in the shallow salinity-stratified layer (UCC). The extreme seasonality of the region adds complexity to nutrient cycling dynamics as oxygenic photosynthesis persists for several months of the year without normal diel cycles–maintaining a persistent nutrient demand in the active mat.

Similarly, winter at such high latitudes means several months without photosynthesis, driving respiration and likely fermentation as the dominant metabolisms in the active mat, altering nutrient demand. The perennial ice cover protects the lacustrine environment from significant geochemical and temperature fluctuations, preventing desiccation or freeze-thaw cycles from affecting the benthic communities. Without geochemical or genetic data, how these dynamics shift over the winter is currently unknown. However, Vanda's conditions allow the mats to maintain homeostasis over the year, and without physical disturbances, tight internal nutrient cycling dynamics capable of supporting biomass production and the growth of elaborate mat morphologies.

Figure 1. Physical, chemical, and biological profiles for Lake Vanda.

a) Temperature and conductivity profiles were collected December 16, 2013 from the lake surface (J. Priscu, 2022).



b) The lake is salinity stratified, comprising four distinct layers following the conductivity profile: a mixed upper convection cell (UCC), a diffusion-limited upper pycnocline (UP), a mixed lower convection cell (LCC), and the bottommost layer is the diffusion zone (DZ). Adapted from Castendyk *et al.* (2016).

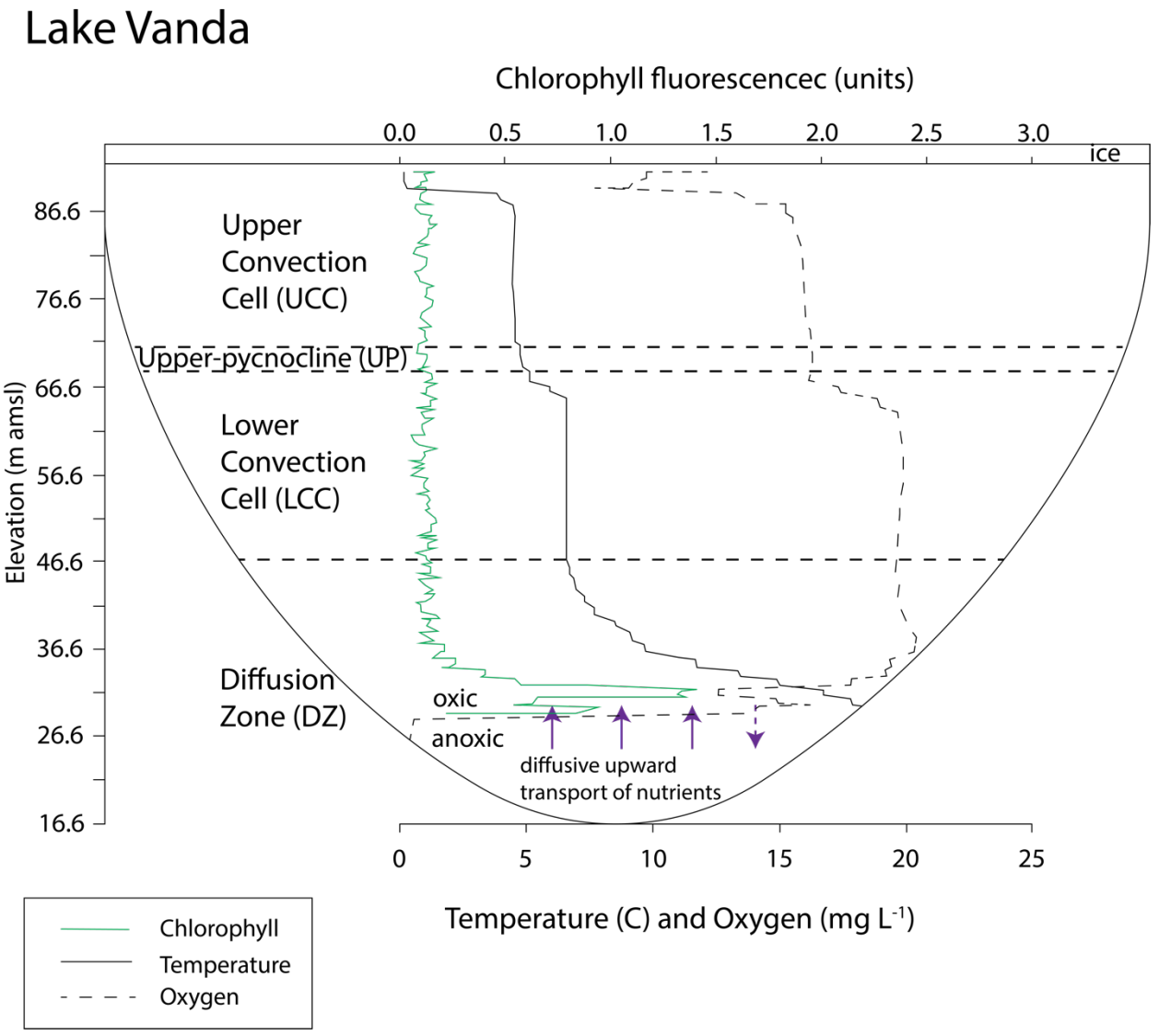


Figure 2. Nutrient concentrations with depth in Lake Vanda

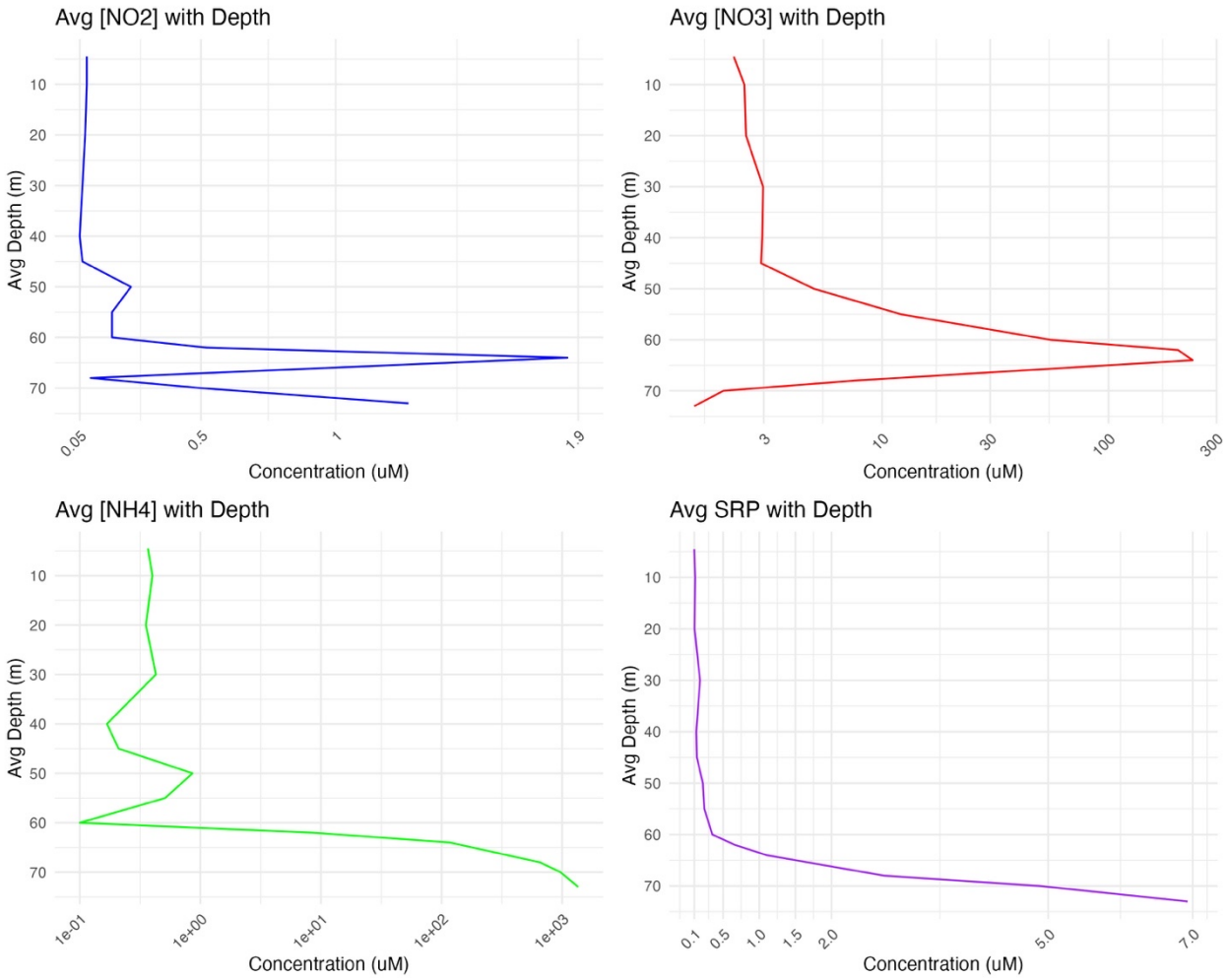


Figure 3. a) Presence-absence of N cycling genes in Vanda metagenomes.

Redox	Nitrogen Cycling Pathways	Genes	Present	Low Abundance/ Missing from some metagenomes	Absent
N-Reduction	**Dissimilatory nitrate reduction**				
	Nitrate --> nitrite	NarGHI	X		
	Nitrate --> nitrite	NapAB	napA	napB	
	Nitrite --> ammonia	NirBD	X		
	Nitrite --> ammonia	NrfAH	X		
	Assimilatory nitrate reduction				
	Nitrate --> nitrite	NarB	X		
	Nitrate --> nitrite	NR			X
	Nitrate --> nitrite	NasAB	X		
	Nitrite --> ammonia	NIT-6	X		
	Nitrite --> ammonia	NirA			X
	Nitrite --> ammonia	NasBDE	X		
	Denitrification				
	Nitrate --> nitrite	NarGHI	X		
	Nitrate --> nitrite	NapAB	napA	napB	
	Nitrite --> nitric oxide	NirK		X	
	Nitrite --> nitric oxide	NirS	X		
	Nitric oxide --> nitrous oxide	NorBC	X		
	Nitrous oxide --> nitrogen	NosZ	X		
	Nitrogen fixation				
	Nitrogen --> ammonia	NifDKH		nifH	nifDK
	Nitrogen --> ammonia	AnfG			X
	Nitrogen --> ammonia	VnfDKGH			X
N-Oxidation	**Nitrification**				
	Nitrite --> nitrate	NxrAB			X
	Hydroxylamine --> nitrite	Hao		X	
	Ammonia --> hydroxylamine	AmoCAB		amoAB	amoC
	Anammox				
	Nitrite --> nitric oxide	NirK		X	
	Nitrite --> nitric oxide	NirS	X		
	Nitric oxide + ammonia --> hydrazine	Hzs			X
	Hydrazine --> nitrogen	Hdh			X

b) Heatmap showing presence-absence and relative abundance of all N cycling genes.

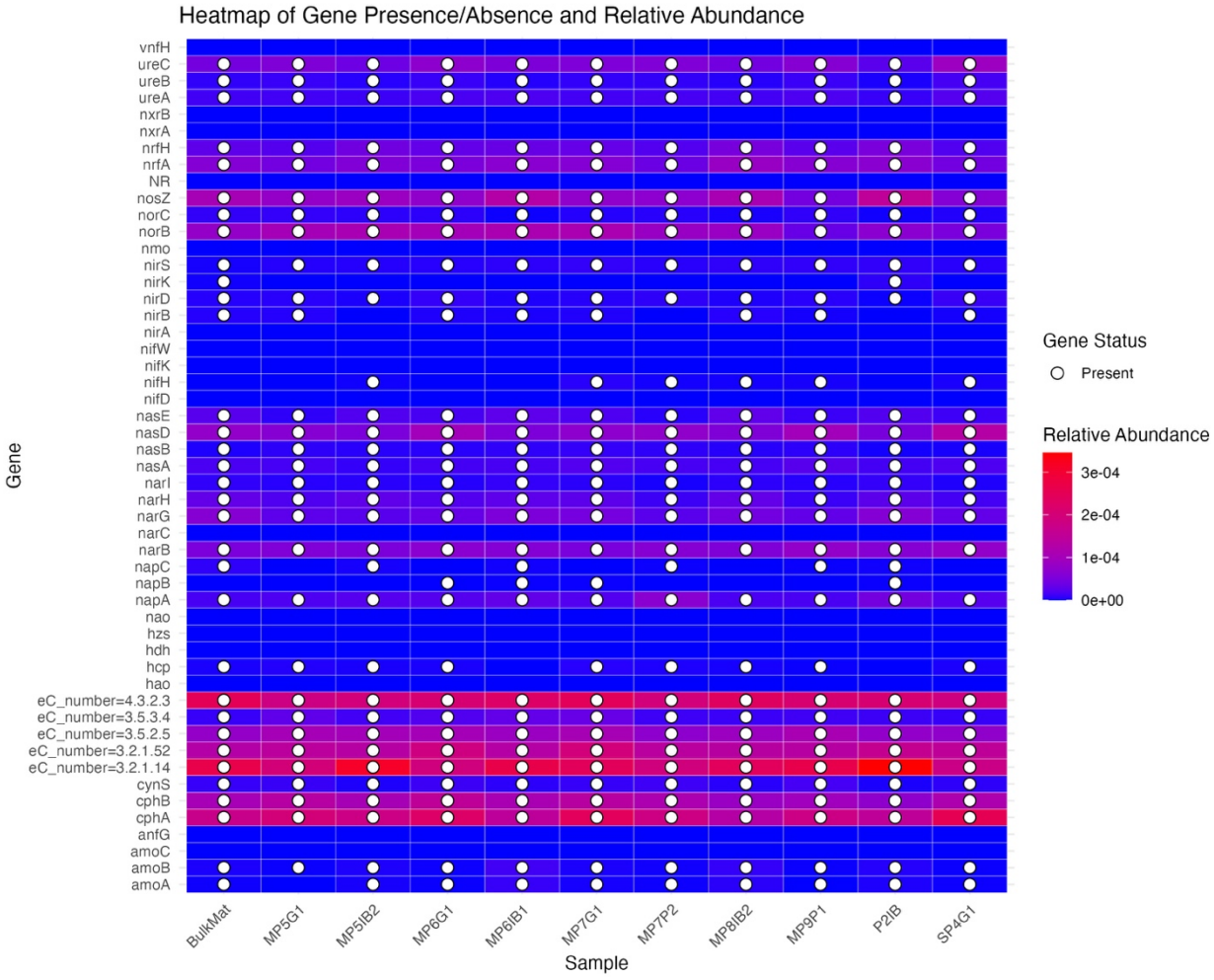
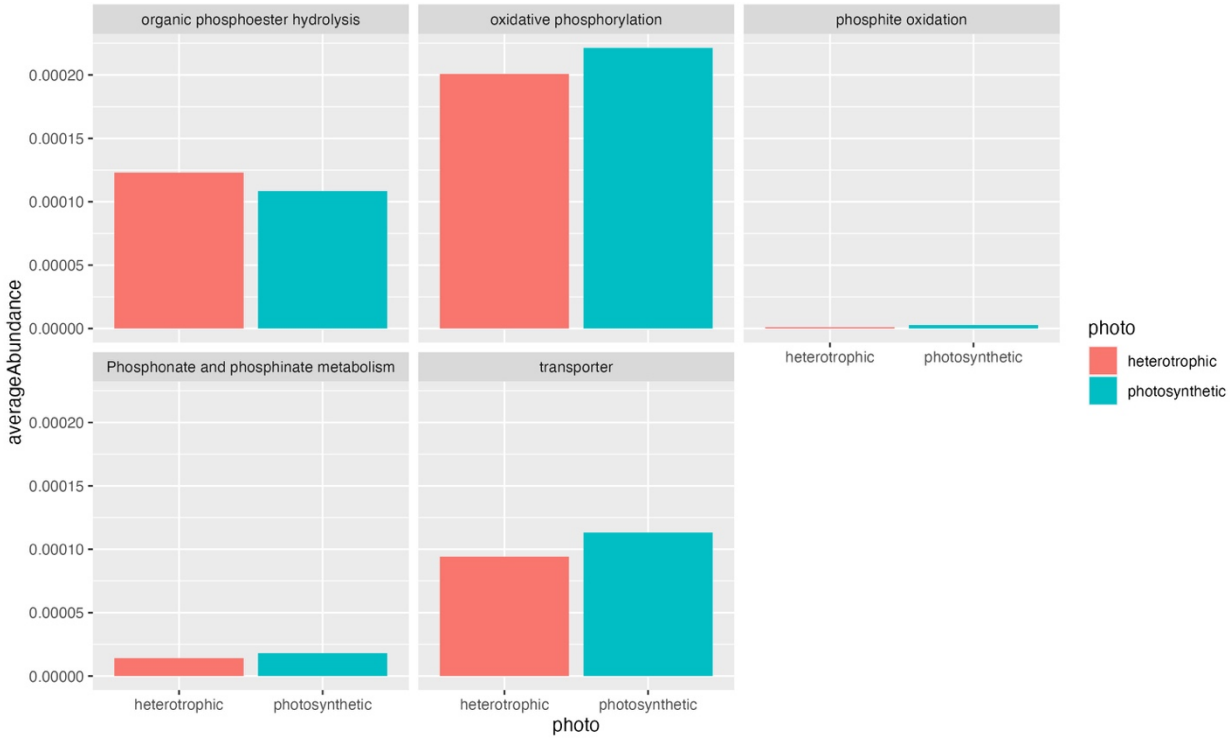


Figure 4. Relative abundances of specific phosphorus cycling gene pathways in the primarily heterotrophic (inner beige) and phototrophic (green, purple) microbial mat communities.



Supplemental 1

N and P cycling genes:

allantoin (*allABCDE*), chitin (*chiABC, nagAB*), and cyanate (*cynS*) degradation

Metabolisms associated with nitrogen cycling processes include N oxidation (*amoAB, hao*, *nxrAB*), N reduction (*narGHI, napAB, nirABD, nrfAHKS, narBGHI, NR, nasABDE, NIT-6, norBC, nosZ*), organic degradation and synthesis (urea metabolism (*ureABC*), amino acids (*ansB*), asparagine metabolism (*asnB, ansB*), and nitronate metabolism (*nmo*), as well as N-storage genes (cyanophycin synthesis and degradation *cphAB)*.

We used PCycDB to characterize P metabolic potential of the microbial community. PCycDB is a P cycling database covering 139 gene families and ten P metabolic processes. Metabolisms associated with phosphorus cycling include two component system genes (*pgtB,*

phoBPRU, *senX3*), transporter genes (*glpT, phnCDE, pstABCS, ugpABC*), phosphonate and phosphinate metabolisms (*fomC, mpnS, pbfA, phnGHIJKLMNOPWXYZ, phny, phpC, ppd, pphA*), phosphite and hypophosphite oxidation (*ptdCFG, ptxABCDE, htxA*), organic phosphoester hydrolysis (*aphA, appA, glpQ, olpA, opd, pafA, phoACDNX, phy, ugpQ*), and oxidative phosphorylation (*ppa, ppk*). Additionally, we looked at genes associated with purine and pyruvate oxidation (ADE2, *adk, gmk, guaAB, ndk, ppx, purABCDEFHKLMNOPQST, spoT, ushA, pckAG, ppc, ppdK, pyk*).

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Chapter 3: Nutrient acquisition strategies employed by microbial mat communities in oligotrophic, perennially ice-covered Lake Fryxell, Antarctica

Abstract

The McMurdo Dry Valleys of Antarctica are a polar desert with very little biomass and liquid water; despite the inhospitable valley environments, the valleys are home to several perennially ice-covered lakes. The lakes are a refuge for thick, diverse microbial mat communities that cover the lake floors year-round. Lake Fryxell has an oligotrophic, salinity-stratified water column, resulting in a steep redox gradient and oxycline within the first 10 m. While only 0.5-3% of incident irradiation makes it through the permanent ice cover, the benthic mat communities contain cyanobacteria that can photosynthesize to depths below the oxycline. The mat communities accrue biomass on an annual scale, meeting their nutrient demands despite the extremely low nitrogen concentrations in the oxygenated water column and the diffusion-limited transport of nutrients in the lake. While metagenomic data indicates the microbial communities employ a number of nutrient acquisition strategies, the nitrogen cycle of the microbial mat community appears limited as genes necessary for nitrogen oxidation and reduction metabolic pathways are either missing or at low abundance in the metagenomes. Genes required for nitrification and anammox are missing from the metagenomes across all depths sampled. Denitrification may also be limited in the benthic community, preventing a significant loss of bioavailable nitrogen to the system. Several nitrogen fixation genes are missing from the metagenomes of the mat communities in the low nitrogen, oxygenated water column, suggesting nitrogen fixation is not an adaption employed by the communities to overcome nitrogen limitation. There are diverse and abundant gene pathways related to ammonium assimilation, organic degradation and synthesis genes, and nitrogen storage compounds. The $\delta^{15}N$ values of

the mat organic matter are all positive and get progressively heavier in ^{15}N with depth in the lake and depth into the mat layers. Nitrate concentrations measured in the sediment porewaters using diffusive gradients in thin films (DGT) passive samplers were heterogeneous. However, the overall trend showed elevated nitrate concentrations within the porewaters compared to the overlying water column. Genomic data and geochemical data indicate the mats are likely nitrogen sinks in Lake Fryxell, and the benthic microbial communities have a high metabolic potential for recycling and storing fixed nitrogen within the mat.

Introduction

Research has shown that the growth of the photosynthetic organisms (primary producers) in aquatic ecosystems, as well as the overall primary productivity of an ecosystem, are intrinsically linked to biogeochemical cycles, and any changes in cycling dynamics profoundly influence ecosystem processes (Downing et al., 1999; Elser et al., 1988, 1990, 2007; Falkowski et al., 1998; Harpole et al., 2011; Hecky & Kilham, 1988; Howarth et al., 2021; Sterner, 2008). The supply of nitrogen (N) and phosphorus (P) to aquatic environments often limits primary productivity.

Phytoplankton are particularly sensitive to nutrient limitation and fluxes. In some oligotrophic lacustrine systems, the addition of both N and P is more effective at enhancing an algal growth response than the addition of only N or P (Elser et al., 1990; Harpole et al., 2011). Nutrient fluxes to and within environments also influence nutrient availability to communities. For example, Chapter 2 shows how permanently stratified water columns and diffusion-limited transport of nutrients in lacustrine environments can result in severe nutrient limitation to planktonic communities.

In contrast, while nutrient scarcity can limit benthic microbial mat productivity, previous research has shown that these communities are often adapted to grow under oligotrophic conditions (Paerl et al., 2000; Pajares et al., 2015; Peimbert et al., 2012; Sabater et al., 2000; Valdespino-Castillo et al., 2018, Chapter 2). Microbial mat communities are particularly well-adapted to many environments and have persisted through billions of years of environmental changes and represent the earliest evidence of life in the fossil record (Walter, 1983). While microbial mats are ancient and globally ubiquitous, they achieve their greatest level of complexity in environments devoid of physical and biological disturbance factors, often extreme environments where macrofauna cannot survive.

Benthic microbial mats are particularly well developed in one such extreme environment, the perennially ice-covered lakes of the McMurdo Dry Valleys (MDV) of Southern Victoria Land, Antarctica (Hawes et al., 2001; Moorhead et al., 2005; Sumner et al., 2015; Wharton Jr et al., 1983). The MDV are polar deserts with little precipitation, persistent cold temperatures, frequent freeze-thaw cycles, and exceptionally low terrestrial biomass; the MDV lakes represent some of Earth's most extreme lacustrine environments (Hawes et al., 2013). The lakes are largely free of macroscopic invertebrates and isolated from atmosphere-water column interactions due to their year-round ice cover, preventing mixing or significant gas exchange, often resulting in stratification and steep chemical gradients (Jungblut et al., 2016; Sumner et al., 2015).

While all MDV lakes' water columns have a planktonic community, phytoplankton are often not the dominant primary producers of the lacustrine ecosystems (Hawes et al., 2014). Previous research on nutrient limitations in the MDV lakes has primarily focused on the planktonic communities (Dore & Priscu, 2001; J. C. Priscu, 1995; Teufel et al., 2017; Vincent & Vincent, 1982), with limited investigations of benthic mats inferring that they are supported by

internal nutrient cycling (Quesada, Fernández-Valiente, Hawes, Howard-Williams, et al., 2008). There is, however, limited research characterizing the metabolic potential of MDV benthic communities related to N and P cycling dynamics (M. Dillon, 2018; Greco et al., 2024).

Lake Fryxell is a meromictic, endorheic, perennially ice-covered lake in Taylor Valley, Antarctica. The lake is relatively shallow compared to other MDV lakes, with a max depth of ~20m. The water column is stabilized by a salinity gradient, with density increasing progressively with depth. The upper ~9 m of water are well oxygenated, but below this, there is a steep oxycline and redox gradient, becoming immediately euxinic at the base of the oxycline, at around 10 m depth (Green et al., 1989; Vincent Warwick F., 1981). Benthic cyanobacterial mats grow down to ~11 m depth (M. L. Dillon et al., 2020; Jungblut et al., 2016; Wharton Jr et al., 1983), extending into the mostly anoxic part of the water column, where their oxygenic photosynthesis creates 1-2 mm thick O_2 "oases" of up to 50 µmol O_2 L^{-1} within the upper layers of the mat during the summer (Sumner et al., 2015). These local O_2 environments introduce a local redox gradient sandwiched between anoxic sediments and anoxic water through the austral summer, though these are likely to disappear in winter (Sumner et al., 2015). This transient oasis creates an opportunity for temporal shifts in local biogeochemical cycles.

Through nutrient bioassay experiments, previous studies have reported a co-limitation of phytoplankton growth by N and P in Lake Fryxell (J. C. Priscu, 1995; Teufel et al., 2017). No studies have yet characterized nutrient limitations for the benthic microbial communities. Previous geochemical analyses conducted in other lake environments in Antarctica and the Arctic showed significantly elevated concentrations of dissolved organic and inorganic N and P in porewaters of microbial mats and sediments under an oligotrophic overlying water column (Mueller & Vincent, 2006; Quesada, Fernández-Valiente, Hawes, & Howard-Williams, 2008;

Tanabe et al., 2017). These nutrient pools are likely not static; instead, they are being transformed by biogenic processes within the benthic mats, suggesting nutrients may not limit these microbial communities.

In the present study, we characterize the transport and availability of nutrients to Fryxell's benthic mat communities using the diffusive gradients in thin films (DGT) technique. This system's gradients and transport mechanisms are essential to nutrient remobilization and flux. We also employed stable isotopes to identify N isotopic signatures associated with N transformations occurring vertically within the benthic mats and with depth along the lake's redox gradient. Finally, to understand nutrient acquisition strategies and metabolic adaptations of the benthic community, we used shotgun metagenomics to identify N cycling metabolic pathways both along Fryxell's redox gradient and with depth into the mats. We hypothesize that the microbial mat communities are sustained by efficiently scavenging various forms of organic and inorganic nutrients and tightly recycling them within the mat, preventing significant loss to the water column. The steep geochemical gradient in Lake Fryxell and the transient O_2 oases make Fryxell particularly suitable for studying the relationships between redox chemistry and benthic microbial nutrient acquisition and cycling metabolisms. The unique interactions between microbial communities and their geochemical surroundings in these settings offer a deeper understanding of nutrient cycling mechanisms that may be obscured in more temperate, complex ecosystems.

Nitrogen Cycling Gene Pathways

The nitrogen cycle is comprised of several microbially mediated redox reactions transforming N from one species to another (Figure 1). N can be remineralized as organic matter is decayed and inorganic N is released and recycled through redox reactions. The dominant pathways are described below.

Nitrogen fixation is a common adaptation employed by microbes (diazotrophs) to deal with nutrient limitation stress. The process requires breaking the triple bonds of an N_2 gas molecule to produce ammonia, which is energetically expensive and inhibited by oxygen. Nitrogen fixation requires the presence of a cluster of genes, central to the process being nitrogenase. Three nitrogenase enzymes can fix nitrogen, differentiated by their cofactors (MoFe, VFe, and FeFe), the most common is the MoFe *nif* genes (*nifHDKW*), but alternative nitrogenases are utilized under Mo limiting environments such as *vnfH* (VFe) and *anfG* (FeFe).

Nitrification is the stepwise process of oxidation of ammonia to nitrate. The *amoABC* complex (ammonia monooxygenase) is responsible for the first step by oxidizing ammonia into hydroxylamine. Encoding for the enzyme hydroxylamine oxidoreductase, *hao* is responsible for the intermediary step converting hydroxylamine to nitrite. The final oxidation of nitrite to nitrate requires *nxrA* and *nxrB* (nitrite oxidoreductase).

Assimilatory nitrate reduction is used by some microorganisms to assimilate nitrate into organic nitrogen compounds by reducing nitrate at a cellular level. Essential genes include *narB* (nitrate reductase), *NR* (nitrate reductase), and genes that encode for assimilatory nitrate (*nasA*) and nitrite (*nasB*) reductase.

Dissimilatory nitrate reduction is a two-step process of reducing nitrate to ammonia through anaerobic respiration. The first step is nitrate reduction to nitrite, which involves genes *narGHI* (nitrate reductase) and *napAB* (periplasmic nitrate reductase). The reduction of nitrite to ammonia involves *nirBD* and *nrfAH*. *nrfAH* are involved in respiratory processes and generate a proton gradient across the membrane necessary for energy production. *nirB* and *nirD* use NADH as an electron donor and are decoupled from energy producing processes.

Denitrification reduces nitrate to N_2 gas through several intermediates. Key genes include ones also found in DNRA to reduce nitrate to nitrite, such as *narGHI* and *napAB.* As well as other genes unique to denitrification, such as nitrite reductases *nirK* and *nirS*, *norBC* (nitric oxide reductase) and *nosZ* (nitrous oxide reductase).

The **anammox** pathway (anaerobic ammonium oxidation) is characterized by converting nitrite and ammonia to N_2 gas. Genes include nitrite reductases *nirK* and *nirS*, as well as *hzs* and *hdh* .

Cyanophycin is a biopolymer used to store fixed N. The genes (*cphAB*) are related to the synthesis and degradation of cyanophycin under times of varying N availability.

Organic degradation and synthesis gene pathways include chitin, allantoin, urea, cyanate degradation genes, and ammonia/ammonium assimilation pathways. Chitinases are the enzymes responsible for chitin degradation, including both endo-chitinases (EC 3.2.1.14) and exo-chitinases (EC 3.2.1.52) (Raimundo et al., 2021). Allantoin is an N-rich organic compound; the hydrolysis of allantoin into its intermediates is catalyzed by the following enzymes (EC 3.5.2.5, EC 4.3.2.3, EC 3.5.3.4). Urease catalyzes the hydrolysis of urea (*ureABC*) into ammonia. Cyanate can serve as an N source as well as an energy source for some microorganisms, its degradation is catalyzed by cyanase (*cynS*).

Ammonium assimilation pathways include the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway and NADP-dependent glutamate dehydrogenase (GDH), which are essential for the biosynthesis of N-containing compounds like amino acids from ammonia and intermediaries (Qu et al., 2019) . Glutamine and glutamate are essential precursors for all nitrogenous compounds, and their synthesis and regulation are indicators of environmental N availability, an organism's scavenging potential, and energy conservation and usage. The only

pathway for glutamine biosynthesis is via the enzyme glutamine synthetase (GS, EC 6.3.1.2), which catalyzes the condensation of glutamate and ammonia with an energy requirement (ATP) to form glutamine, ADP, and phosphate (Schreier, 1993). GS is crucial for remobilizing protein-associated N and is coupled with glutamate synthase (GOGAT EC 1.4.1.13) in the biosynthesis of glutamate (GS/GOGAT cycle) (Schreier, 1993). Meanwhile, another glutamate synthesis pathway is via glutamate dehydrogenase (GDH EC 1.4.1.4). GDH has a lower affinity for ammonia than GS, which has a much higher affinity; thus, GDH activity is higher under ammonia-rich conditions, while GS activity increases under N limitation. So, under N-limiting conditions, the GS/GOGAT cycle allows organisms to better scavenge ammonia by expending energy (Schreier, 1993). A second ferredoxin-dependent glutamate synthase (Fd-GOGAT–EC 1.4.7.1) also plays a role in N assimilation and is found in all cyanobacteria (Seth et al., 2021); EC 6.3.5.4 (asparagine synthase (glutamine-hydrolyzing)) and EC 3.5.1.1 (asparaginase) are involved in the synthesis and hydrolysis of amino acids that either produce or recycle ammonium (Senwo & Tabatabai, 1999). EC 1.4.1.2 and EC 1.4.1.3 are both glutamate dehydrogenases (GDH) responsible for the conversion of glutamate to 2-oxoglutarate and ammonia; EC 1.4.1.3 can use either NAD+ or NADP+ while 1.4.1.2 can only use NAD+ (Dubois et al., 2003). Lastly, glutaminase (EC 3.5.1.2) catalyzes the hydrolysis of glutamine to glutamate and ammonia.

Materials and Methods

Study Site

Lake Fryxell is 5 by 1.5 km in size and lies on the eastern end of Taylor Valley. It is endorheic, where water flows into the lake but loss is only through evaporation and ablation through the ice cover (Lawrence & Hendy, 1985). It has a maximum depth of ~20 m and 3-5 m of ice on the lake surface, which prevents water column mixing (J. Priscu, 2023). The lake's

hydrologic history consists of several evaporation and refilling events over ~24,000 years–a legacy of which is evidenced by an euxinic brine at the bottom of the overlying oxygenated, salinity-stratified water column (Lyons et al., 2005). This oxycline is primarily the result of the lake's unique physical structure—its ice cover limits gas exchange with the atmosphere, allowing oxygen produced by benthic photosynthesis to accumulate to supersaturation. The absence of physical mixing and advection due to the ice cover further accentuates the stability and persistence of a steep redox gradient, and without advection, diffusion is responsible for the vertical flux of solutes in the water column. The redox gradient is defined by changes in nutrient concentrations following the oxycline (Figure 2); N species are almost immeasurable (< 1 µg NH_4-N L^{-1} and 1 µg NO_2 + NO_3 N L^{-1}) in the oxic waters but increase rapidly below 9.8 m where waters become anoxic (J. Priscu, 2022). Similarly, DRP concentrations increase with depth along the oxycline (J. Priscu, 2022).

External inputs of nutrients and Fryxell's water are supplied by 13 glacial meltwater streams; however, most water comes from the nearby Canada and Commonwealth glaciers (Aiken et al., 1996). The ephemeral streams that feed Lake Fryxell are colonized by thick microbial mats of different colors and associated microbial assemblages (McKnight et al., 2004). Some stream mat communities include the N-fixer *Nostoc* (black mat), which provides bioavailable N to the stream communities (Kohler et al., 2023). However, MDV streams with greater mat coverage were found to be nutrient sinks with significantly lower nutrient concentrations at their lake interface than upstream (Gooseff et al., 2004; Koch et al., 2010; McKnight et al., 2004). While the benthic communities in the ephemeral streams and Fryxell's austral summer moat are adapted to higher irradiance throughout the summer, benthic communities are limited to the 0.5-3% of incident irradiance transmitted through the ice cover

(Howard-Williams et al., 1998). The benthic microbial phototrophs are capable of oxygenic photosynthesis to depths of ~10 m, below which irradiance becomes too low (Sumner et al., 2015).

The microbial mat morphology and community assemblage changes with depth. Mats growing at the perimeter in the shallow, ice-free moat were typically very thin (<3 cm). Varying in texture, color, and morphology, they often contained entrapped gas bubbles, resulting in lift-off where buoyant forces rip the mat off the substrate to float upwards (Wharton Jr et al., 1983). These mats experienced ice- and wind-induced disturbances that result in regrowth cycles (Wharton Jr et al., 1983). The dominant phyla in the moat mats include Cyanobacteria (*Anabaena*, *Lyngbya, Nostoc, Phormidium*), Chlorophyta, as well as diatoms (Wharton Jr et al., 1983).

Beneath the ice cover, the shallow, brightly-lit water column is supersaturated in dissolved oxygen, resulting in benthic mats that exhibit columnar lift-off mats and float mats (Wharton Jr et al., 1983). Float mat can freeze into the newly formed ice, eventually reaching the ice surface to be exposed to the atmosphere. Following the oxycline, mats growing at depths where O_2 is supersaturated exhibit cuspate pinnacled morphology at 9.0 m depth (8.0 m sampling depth in this study) (Jungblut et al., 2016). Deeper in the water column, right above the oxic-anoxic boundary, mat morphology changed to a "honeycomb" at 9.3 m depth (8.8 m sampling depth in this study). Throughout the summer, the pits in the honeycomb were anoxic, and the tops remain oxic (Jungblut et al., 2016). Below the oxycline, mat morphology lacks topographic complexity and exhibits flat prostrate mats at 9.8 m depth (9.5 m sampling depth in this study) (Jungblut et al., 2016). Microbial community composition changed with depth into the mat and with depth in the lake (M. L. Dillon et al., 2020; Jungblut et al., 2016; Lumian et al., 2021).

Proteobacteria were the dominant phylum in the mats at all depths in the lake (M. L. Dillon et al., 2020). The cyanobacterial composition changes with depth and mat type, with *Leptolyngbya* most abundant in the pinnacled and honeycomb mats (M. L. Dillon et al., 2020; Jungblut et al., 2016). There were no heterocyst-containing cyanobacteria in the benthic mat communities, a physiological adaptation employed to physically separate the processes of oxygenic photosynthesis and N-fixation. Below the oxycline in the flat mats, *Phormidium pseudopriestleyi* became the most abundant cyanobacterium (M. L. Dillon et al., 2020; Jungblut et al., 2016). In the absence of oxygen, *Chlorobi* and *Chloroflexi* were at higher abundances in the mats at depth. The changing microbial community structure with environment suggests that microbial assemblages were correlated with different ecological niches (M. L. Dillon et al., 2020; Jungblut et al., 2016).

During our 2021/2022 field season, the lake experienced a massive ecological disturbance. Irradiance was higher in Fryxell's water column potentially due to a thinning and more transparent ice cover. Increased photosynthesis resulted in gas bubbles to become trapped within mats to depths of greater than 8 m. In many cases, bubble trapping resulted in a physical displacement of benthic microbial mats as they were lifted off the substrate. The removal of these mats exposed the underlying sediment and decayed mat, likely altering nutrient transport and availability in the lake water column.

All geochemical data (isotopes, water chemistry, and DGT sediment probes) were collected during the 2022/23 field season. Sampling depths are as follows (± 0.3 m): 4.3 (well above the oxycline), 8.0 m (supersaturated oxygen water), 8.8 m (bottom of the oxycline- oxic/anoxic transition), and 9.5 m (euxinic). Genomic data are from mats collected during the 2012 field season (M. L. Dillon et al., 2020; Jungblut et al., 2016; Sumner et al., 2016).

Metagenomics

Sampling and metagenomic sequencing methods were completed by Jungblut et al. (2016) and Dillon et al. (2020).

Sampling

The benthic microbial mats were sampled in November 2012 (see Jungblut et al 2016). Divers followed a transect installed in 2006, along which they sampled microbial mat from 9.0, 9.3, and 9.8 m depth. Sampling involved cutting into the *in-situ* mat and lifting it into plastic containers with a spatula while underwater. Divers returned to the surface with samples where they were dissected by layer pigmentation and morphology using sterile materials. The resulting subsamples are as follows: 9.0 and 9.3 m were subsampled into three layers (top, middle, bottom). The mats at 9.8 m were subsampled into four layers (film, top, middle, and bottom). The *in situ* mats at 9.0 m were pinnacled, at 9.3 m the mats had a 'honeycomb' or 'ridge-pit' morphology, with 0.5-1.0 cm deep pits. The subsampled 'tops' at this depth were the tops of the ridges separating the pits, the ridges and edges were the "middle", and the bottoms of the pits were the "bottom" (M. L. Dillon et al., 2020; Jungblut et al., 2016). In the field immediately after subsampling, samples were preserved with the buffer within Xpedition Soil/Fecal DNA MiniPrep kit (Zymo Research, Irvine, CA) and frozen. The samples were shipped frozen to UC Davis and were stored at -80°C until further downstream processing.

DNA extraction and metagenomic sequencing

See (M. L. Dillon et al., 2020; Jungblut et al., 2016) for detailed sequencing, library prep, and quality control methodologies. As per manufacturer's instructions, DNA was extracted using an Xpedition Soil/Fecal DNA MiniPrep kit (Zymo Research, Irvine, CA). Metagenomic sequencing

was performed using the Illumina HiSeq 2500, PE 250 platform at the University of California, Davis Genome Center DNA Technologies Core (http://dnatech.genomecenter.ucdavis.edu/). Metagenomic reads are available via NCBI's sequence read archive (PRJNA291280). Sequencing data were pulled from NCBI using SRA-Toolkit, and fasterq-dump was used to extract fastq files from the SRA accessions (Kodama et al., 2012; Leinonen et al., 2011).

Metagenomic read assembly and annotation

Extraction and sequencing replicates were combined prior to assembly. The trimmed and filtered reads were assembled into contigs using MEGAHIT (Li et al., 2015, 2016). Bowtie2 (Langmead & Salzberg, 2012) was used to map reads back to the assembly, and samtools (Danecek et al., 2021) was used for sequence alignment and coverage calculation. Metagenomic assemblies were annotated using Prokka (v. 1.14.6) (Seemann, 2014).

Nitrogen cycling metabolic potential

The N cycle consists of the transformation of N between several oxidation states (−3 to +5) by a series of microbially-mediated redox reactions. We examined the benthic community's metabolic potential for nitrogen transformations within Lake Fryxell using a metagenomic survey of genes responsible for N cycling processes by looking at gene presence-absence and gene relative abundance in each metagenome.

We used NCycDB, a curated database targeting gene pathways for N cycling (Tu et al., 2019). Eight pathways were targeted, recruiting a total of 65 gene (sub)families. These eight families include N-fixation, nitrification, anammox, denitrification, assimilatory and dissimilatory nitrate reduction, hydroxylamine reduction, organic degradation and synthesis (urea metabolism, amino acids, ammonia/ammonium assimilation pathways, asparagine metabolism, and allantoin, chitin,

and cyanate degradation), as well as N-storage genes (cyanophycin synthesis and degradation) that were not included in NCycDB.

All N cycling genes can be found in Supplementary Table 1.

Gene relative abundance and statistical analyses

Genes of interest were pulled from the annotated metagenomes and the relative abundance of each gene was calculated by gene length (stop - start of codon + 1) * total average depth divided by the depth * length of the metagenome assembly size.

Geochemistry of lake and mat porewater

Water column and porewater sample collection and processing

Divers collected water column samples with clean 60 mL syringe tubes from directly above the mat surface at depths of 4.3 m, 8.0 m (pinnacled mat), 8.8 m ("honeycomb" mat), and 9.5 m (flat mat). Samples were processed in the field after collection. For dissolved nutrient measurements, water samples were filtered (Whatman GF/F) into clean 50 mL falcon tubes with a maximum water sample volume of 40 mL to allow room for freezing. For total N and P concentrations, water samples were left unfiltered and stored in new clean 50 mL falcon tubes with a maximum volume of 40 mL.

Mat samples were collected for both isotopic analyses of organic matter and geochemical analyses of the porewaters. Samples were only collected from mats growing at 8.0 m, 8.8 m, and 9.5 m depth. Divers used a sterile knife and spatula to cut and transfer the mat into clean, plastic Tupperware containers. In the field lab, mats were processed to extract porewater. Wearing sterile gloves, the mats were subsampled using a sterile scalpel for porewater collection, and the remaining mat was frozen for isotopic analyses. Subsamples for porewater analyses were 3cm x

3cm. The tip of a 30mL syringe was cut, and a small metal disk sieve was placed at the bottom of the inside of the syringe. The mat subsample was placed into the syringe and the water/mat was filtered through the sieve using the syringe plunger into a second syringe placed below. An additional filtration was performed with a Whatman GF/F to collect only dissolved nutrients. Porewater was collected and stored frozen in 15mL falcon tubes.

All water samples were frozen and kept at -20°C until downstream analysis at UC Davis.

Nutrient concentration analyses

Quantitative analysis of dissolved N species (nitrate/nitrite, and ammonium) in water samples was conducted using a flow injection analyzer with a detection limit of 0.05 mg L^{-1} for all analytes. Quantitative analysis of soluble phosphorus in water was also conducted using a flow injection analyzer with a detection limit of 0.05 mg L^{-1}. Total N was quantified using a combustion method with a detection limit of 0.1 mg L^{-1}, and total P was quantified using microwave pressure/dissolution digestion and determination by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) with a detection limit of 0.1 mg L^{-1}. All analyses were performed at the UC Davis Analytical Laboratory.

Nutrient diffusion gel probe experiment

Traditional methods for characterizing chemical gradients within lake sediments, such as coring, centrifuging, and sippers, often lack the spatial resolution needed to fully understand these complex transport and nutrient transformation processes. The use of Diffusive Gradients in Thin Films (DGT) techniques provides high-resolution, in situ measurements of analytes across the sediment-water interface (Davison & Zhang, 1994). These techniques result in time-weighted average concentrations over the deployment period. In a diffusion-limited environment like Lake

Fryxell, modeling nutrient transport through sediment and across boundaries using these passive samplers can yield critical insights into the lake's nutrient dynamics.

Experimental design

Due to the massive disturbance event in water depths around the oxycline seen during the '22/'23 field season, we developed a time-series experiment to see if mat 'liftoff' released inorganic dissolved nutrients (nitrate and phosphate) sequestered in the mat and sediment interstitial water into the overlying water column. To characterize the diffusion of DIN and SRP between the sediment/mat and overlying water column, we deployed passive samplers into the mat/sediment of Lake Fryxell to determine *in situ* concentrations of nitrate and phosphate of porewaters. Additional probes were then deployed at sequential time points to observe the hypothesis of diffusion of nutrients out of the porewaters after induced 'liftoff.'

DGT sediment probes

Diffusive gradients in thin films (DGT) techniques can provide high-resolution *in situ* measurements of analytes of interest spanning the sediment-water interface and provide a time-weighted average concentration over a deployment period. We deployed DGT sediment probes (DGT®Research Ltd) to measure phosphate (LSPP-NP) and nitrate (LSPN-AP) in the water column above the microbial mat into the sediment below. The probes used to measure phosphate in solution have a polyethersulfone membrane, a 0.8 mm APA diffusive gel, and a ferrihydrite binding layer (DGT®Research Ltd). The nitrate probes have a polyethersulfone membrane, an agarose diffusive gel, and an A520E resin gel as the binding layer (DGT Research).

Plot and deployment descriptions

Three plots for the experiment were chosen and marked by divers at 8.0 m depth, where intact pinnacled microbial mat is the dominant morphology and the water column is fully oxic. The experimental design and plot descriptions are as follows (Figure 3a). The first time point of the time series experiment consists of an initial pre-induced liftoff time point (t=0), liftoff was induced 24 h later, and probes were placed into the exposed sediment immediately after the removal of mat (t=1), probes were placed three days post-liftoff (t=2) and seven days post-liftoff (t=3). All probes were removed by divers within 10 minutes of 24 h after deployment.

Plots were 20 cm x 40 cm, and each set of subsequent probes were placed within 10 cm of the previous probes. Plot one: pinnacled mat with a nearby (~ 2 ft away) 'tombstone' liftoff with bubbles (vertically displaced mat), as well as a nearby patch of tan colored mat, likely representing a previously lifted off mat where decayed mat was exposed with some new recolonization. Plot two: pinnacled mat with heterogeneously sized pinnacles with bare, exposed sediment patches around, suggesting previous mat liftoff. Plot three: pinnacles with nearby prostrate mat, suggesting newly colonized substrate. There is a common phenomenon for Lake Fryxell of aeolian deposition of sediment on the lake ice surface, which slowly melts through the ice and accumulates in pockets (c.f. Rivera-Hernandez et al., 2019). Eventually, these sediment grains fall all at once and form piles on the lake substrate (Rivera-Hernandez et al., 2019). This happened to two of the three plots throughout the experiment, creating sediment piles covering portions of the exposed plots. These piles potentially influence the diffusion of nitrate and phosphate out of the underlying sediment and may alter the accumulation of analyte on the sediment probes.

After each probe was removed from the sediment, divers shook the probes in the water column to remove any attached organic or sediment particles and immediately returned to the surface. At

the surface, probes were placed back in the original plastic sleeves before being stored at 4 °C. Probes were taken back to UC Davis for processing and analysis.

Probe processing for analysis

We followed the manufacturer's protocols (DGT Research) to process the probes. Probes were removed from their original sleeves and marked prior to cutting for subsampling (Figure 3b). Using a sterile blade, the gel was cut along the edges of the probe window and then sliced across for each subsample. Using sterile forceps, the top filter membrane was removed as well as the diffusive gel. The subsampled binding gel samples were then individually placed in clean, acid-washed 15 mL falcon tubes. Subsampling of probes differed with analyte, and probes were subsampled as follows. Phosphate probes: the t=0 phosphate probes (intact mat) were subsampled into two samples; the top 3.5 cm was one that represented the portion of the probe exposed to the overlying water column, and the remaining 11.5 cm of the probe represented the interstitial waters in the microbial mat and sediment. The phosphate probes from the remaining time points (1-3) were subsampled into three samples: the top 3.5 cm (the overlying water column), 5.5 cm in the middle, and finally, the bottom 6 cm. For the nitrate probes, the t=0 time point probes (intact mat) were subsampled into three samples: the top 3.5 cm (overlying water column), 5.5 cm in the middle, and the bottom 6 cm. For the probes from time points 1-3, the nitrate probes were subsampled into 4 samples, all of which were 3.5 cm. The probes measuring nitrate and phosphate were processed separately, and the processing protocol varied during the elution step.

The elution step followed the manufacturer's protocol (DGT Research). To elute the phosphate binding layer gels, 5 mL of high-purity water was pipetted into each tube, making sure the gel was completely submerged and sat for one hour. The water was then removed before 5

mL of 0.24 M sulfuric acid was added to each tube, again ensuring the gel was fully immersed in the solution. After 24 hrs, the gel slice was removed from the tube, leaving the remaining eluent for colorimetric analysis using the molybdenum blue method. To elute the nitrate samples, 2 mL of 2.0 M high-purity NaCl solution was added to each tube with the binding layer gel, making sure the gel was completely immersed in the solution. After 24 hrs, the gel slice was removed, and the remaining eluent was saved in the 15 mL tube for colorimetric analysis. The colorimetric analyses of the eluents by Flow Injection Analyzer were performed at the UC Davis Analytical Lab.

Calculation of mean analyte concentration

Calculation of the time-averaged mean concentration of the analyte in the deployment medium was followed using the manufacturer's protocol (DGT Research). The diffusion coefficient for nitrate used for nitrate at 1°C is 5.32 $x10^{-6}$ cm^{-2} s^{-1} (Cai et al., 2017).

Stable Isotopes of Benthic Microbial Mat

Microbial mat samples were collected by divers in January 2023 from the three sampling depths along the oxycline: 8.0 m, 8.8 m, and 9.5 m. Mats were cut with a knife before the mat was lifted by spatula into alcohol-sterilized plastic boxes and brought to the surface. Samples were kept frozen and shipped to UC Davis. Samples were kept frozen at -20°C until subsampling.

Mat sample descriptions

Mats from 8.0 m were from fully oxygenated water depths and exhibit a pinnacled morphology. The mats from 8.8 m were low in the oxycline, exhibiting a honeycomb structure with pits that are anoxic and oxic tops. These mats are predicted to become fully anoxic over the austral

winter. Mats from 9.5 m were flat and grow under the euxinic water column; they remain anoxic year-round except where local photosynthesis produces small dissolved O_2 oases.

Subsampling and sample prep

Prior to subsampling, mat samples were freeze-dried. Subsampling was conducted using sterile forceps to separate layers with depth into the mat from top, middle, and bottom. The subsamples were ground and homogenized with an agate mortar and pestle and weighed into tin capsules (~10 mg) for analysis using a continuous flow elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) at the UC Davis Stable Isotope Facility (SIF). The mean standard deviation for reference materials replicates in this project is ±0.17‰ and the mean absolute accuracy for calibrated reference materials is within ±0.07‰.

Results

Metagenomic Survey of N & P genes

Nitrogen cycling

The metagenomic survey revealed a limited yet diverse array of genes associated with nitrogen cycling. The presence-absence and the relative abundance of the genes with depth and layer in the mat communities can be found in Figure 4. Genes associated with each N cycling process are outlined below.

Nitrogen fixation Elements of the *nif* nitrogenase cluster were present in many of the metagenomes but not all. Only a few metagenomes had all four of the required genes present, and at very low abundances. The alternative nitrogenases were missing from all metagenomes.

Nitrification In the Fryxell metagenomes, nitrification is notably constrained. Critical genes were missing from all metagenomes (*amoA*, *amoC*, and *nxrAB*). The gene *hao*, necessary for

hydroxylamine oxidation, was present in only eight metagenomes at low abundances, further indicating a limited capacity for this process.

Assimilatory nitrate reduction The gene *nasA* was present in nine out of 29 metagenomes; however, *nasB*, *NR*, and *nirA* were largely absent.

Dissimilatory nitrate reduction Nitrate reductase genes *narGH* were present at low abundances, and *napA* was relatively abundant in all metagenomes. However, nitrite reductase *nirB* was absent in the majority of metagenomes, and *nirD* was not found in any of the metagenomes, while *nrfA* and *nrfH* were found in most metagenomes. *nrfAH* are involved in respiratory processes and generate a proton gradient across the membrane necessary for energy production. *nirB* and *nirD* use NADH as an electron donor and are decoupled from energy producing processes.

Denitrification In these metagenomes, *napA* was relatively abundant; however, critical genes such as *nirK* and *norBC* were missing or present at low levels across many metagenomes, but *nosZ*, the crucial final step of denitrification, was found in most metagenomes. This suggests some denitrification potential remains, albeit limited by the absence of upstream pathways.

The **anammox** pathway was poorly represented in our metagenomes, with no detection of *hzs* (hydrazine synthase) and *hdh* (hydrazine dehydrogenase), which are unique to anammox. These essential genes (*hzs* and *hdh*) were absent from all of the metagenomes. indicating the communities do not have the metabolic potential for anammox.

Cyanophycin Both genes (*cphAB*) were present in all metagenomes and more abundant in the 9.8 m mats.

Organic degradation and synthesis gene pathways include chitin, allantoin, urea, cyanate degradation genes, and ammonia/ammonium assimilation pathways. Chitinases: endo-chitinases (EC 3.2.1.14) were missing from all but two metagenomes and exo-chitinases (EC 3.2.1.52) were found in all metagenomes. Allantoin enzymes (EC 3.5.2.5, EC 4.3.2.3, EC 3.5.3.4) were present in most of the metagenomes; however, they were not present at high abundances, indicating allantoin is not a significant source of N. Ureases (*ureABC*) were found at low abundances in all metagenomes; however, *ureC* increased in abundance in the top and middle layers of the 9.8 m mats. Cyanase (*cynS*) was found in all but one metagenome but in low abundances, indicating it is likely not an important source of N to the communities.

Genes associated with ammonium assimilation pathways include the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway and NADP-dependent glutamate dehydrogenase (GDH). As seen in Figure 4, glutamate dehydrogenase (GDH) was missing from one of the 9.0 m (pinnacled mat) metagenomes and was at lower relative abundances across all depths and mat layers. In comparison, glutamine synthetase (GS) was much more abundant in all of the metagenomes, along with glutamate synthase (GOGAT), suggesting the GS/GOGAT cycle is the more likely pathway utilized for glutamate synthesis. A second glutamate synthase (Fd-GOGAT–EC 1.4.7.1) also plays a role in N assimilation and was found in all cyanobacteria (Seth et al., 2021); this ferredoxin-dependent glutamate synthase was found in all metagenomes but was significantly higher in the top and middle layers of the 9.8 m (prostrate) mats. EC 6.3.5.4 (asparagine synthase (glutamine-hydrolyzing)) and EC 3.5.1.1 (asparaginase) are involved in the synthesis and hydrolysis of amino acids that either produce or recycle ammonium (Senwo & Tabatabai, 1999) – while both were present in all metagenomes, asparagine synthase was more abundant, particularly in the bottom layers of the 9.0 m (pinnacled mat) and 9.3 m

("honeycomb") mats. EC 1.4.1.2 and EC 1.4.1.3 are both glutamate dehydrogenases (GDH) responsible for the conversion of glutamate to 2-oxoglutarate and ammonia; EC 1.4.1.3 can use either NAD+ or NADP+ while 1.4.1.2 can only use NAD+ (Dubois et al., 2003). Both GDHs were present in all metagenomes; however, EC 1.4.1.3 was more abundant than 1.4.1.2 in almost all metagenomes. Lastly, glutaminase (EC 3.5.1.2) catalyzes the hydrolysis of glutamine to glutamate and ammonia.

Overall, our results indicate a constrained N cycling potential within the studied metagenomes, characterized by the absence of several key genes associated with metabolisms of N redox reactions. This limited metabolic capacity could significantly affect N availability and cycling dynamics in Lake Fryxell.

Nutrient Concentrations

Water column and porewater nutrient concentrations

Nutrient concentrations of the water column and mat porewater were exceptionally low; many of the analytes across samples were below the detection limit.

Gel probe experiment– diffusion of nutrients

Concentrations of phosphate were below detection limits for all plots and time points.

Concentrations of nitrate were heterogeneous and varied by plot and within plots. Overall trends show higher nitrate concentrations at depth in the sediment and a diffusive nitrate flux from the sediment to the water column. Some NO3-N concentrations in the sediment were 17 to 285 times the concentration of NO3-N in the overlying water column. Figure 5a shows a positive correlation between the concentration in the sediment and the difference in concentration,

supporting the idea that nitrate concentration is usually higher in the sediment than in the water column. There is significant heterogeneity in nitrate concentrations across both plots and depth into the mat. This heterogeneity could be attributed to a couple of factors, such as variation in the amount of decay of buried mat and spatial variation in sedimentation on the lake bottom, which would ultimately affect the permeability and diffusion of nitrate. Considering the overall trends, higher concentrations of nitrate within the sediment compared to the water column suggest nitrate diffusion from sediment to the overlying mats is likely an important source of nutrients to the microbial community.

Stable Isotopes

The $\delta^{15}N$ values across the three sampling depths are detailed in Supplement 1. The values show a consistent trend of becoming, on average, progressively more enriched in ^{15}N with increasing depth in both the lake and into the mat. Averages $\delta^{15}N$ values are reported in Table 1 and $\delta^{15}N$ values with depth in the lake and with mat layer can be seen in Figure 6. For the 8.0 m mats, the average $\delta^{15}N$ values are +1.53‰ at the top, +2.63‰ in the middle, and +5.07‰ at the bottom. At 8.8 m, these values increase to +3.29‰, +5.79‰m and +5,67‰, respectively, while the mats at 9.5 m show average $\delta^{15}N$ values of +2.66‰, +5.65‰, and +5.55‰, respectively.

Discussion

Along Lake Fryxell's steep redox gradient, the availability of nutrients, PAR, and O_2 concentrations change, and with it, mat morphology, structure, and metabolic potential of the benthic microbial community. Light availability becomes limiting at depth, decreasing the rate of benthic photosynthesis and O_2 concentrations. Inversely, ammonium concentrations in the water column are significantly depleted above and within the oxycline; however, they increase

significantly where the water column becomes anoxic and oxidation potential decreases. The redox potential and primary production lead to metabolic shifts in the community from aerobic processes (e.g., nitrification, aerobic assimilatory nitrate reduction, ammonium assimilation) to anaerobic processes (e.g., DNRA, denitrification.) Metagenomics illuminates the metabolic potential of a community; thus, interpreting the microbial N cycling metabolisms relies heavily on the presence or absence of genes in the metagenomes. While a gene may be present, it does not imply transcription. In the Fryxell mat metagenomes, the absence of key genes is likely more powerful for interpreting the community's metabolic capacity to carry out a process. Complementing the metagenomic data are the $\delta^{15}N$ values of the microbial mat organic matter. Isotopic data provide information about the biogeochemical processes occurring within the mat as the isotopic signatures of the benthic OM are a mixture of the fractionations associated with the various microbial metabolisms. Hence, these isotopic values are a collective signature of the net metabolic activities of the community. The mat samples span the oxycline and redox gradient in the water column shifting the dominant metabolisms of the community. Most of the redox-sensitive microbial N cycling metabolisms are coupled along an oxygen gradient, sharing electron donors and acceptors; however, these processes can be uncoupled when the inhibition of one can lead to the suppression of others.

While some work has been done using stable isotopes to study the benthic communities in Lake Fryxell (Lawson et al., 2004; Wharton et al., 1993), none has investigated spatial variation of N isotopes within the mats themselves. An ammonium (water) sample (collected in 1979 from 15 m depth) is the only reported $\delta^{15}N$ value (+6.2‰) of a dissolved N source from Fryxell's water column, and it is significantly below the base of the oxycline (Wada et al., 1984). This value is consistent with the enriched $\delta^{15}N$ values of the benthic mat OM, suggesting it could

be a potential enriched source of N to the microbial mats. The $\delta^{15}N$ values of the soil OM along the elevation transect from the shoreline of Lake Fryxell, however, are much more depleted in ^{15}N compared to the lacustrine benthic OM, with values of +0.5‰ near the shore to -14.1‰ at higher elevations (Burkins et al., 2000). The values close to 0‰ could be attributed to N-fixation found in the moat and stream communities by *Nostoc)*.

Interpreting the isotopic patterns within the mat communities with depth involves predicting which microbially-mediated N redox reactions are occurring. Integrating the $\delta^{15}N$ values with the metagenomic data provides a more comprehensive picture of N cycling dynamics with depth in the lake and vertically into the mat. The increasingly ^{15}N enriched signatures with depth into the mats are consistent with remineralization fractionating in favor of $^{14}NH_4$. As $^{14}NH_4$ is released into the porewaters, it can be assimilated by the community in the top of the mat. Typically, under oxic conditions, the NH_4 could be used as an electron donor in the aerobic process of nitrification to produce nitrate; however, the genes (*nxrAB*) responsible for this step are missing from the metagenomes, indicating nitrification is not a dominant metabolism in the mats at this depth. Many of the genes responsible for ammonium assimilation and organic degradation and synthesis are relatively abundant across all metagenomes in Lake Fryxell, indicating that these are likely the dominant recycling processes. In the fully oxic mats, the anaerobic process of denitrification is likely limited, and thus likely little loss of fixed N to the system. The assimilation of ammonium from remineralization would result in lighter $\delta^{15}N$ values of the OM_{PP} than the decaying OM.

The mats at 8.0 m likely experience a combination of N cycling processes due to the mixed oxic and anoxic conditions within the mat. Aerobic processes such as ammonium and nitrate assimilation can occur at the surface. In contrast, anaerobic processes like denitrification

and DNRA can occur in the anoxic regions beneath the surface and in the pits. Denitrification preferentially removes ^{14}N as N_2 gas, leaving the remaining nitrate enriched in ^{15}N. As the mats lack the genetic ability to fix N_2 gas, the complete denitrification process leads to a loss of ^{14}N to the system. DNRA also reduces nitrate, producing lighter $\delta^{15}NH_4^+$ and enriched nitrate; thus, any nitrate assimilation would lead to enriched $\delta^{15}N$ OM values. The metagenomic data from these 'honeycomb' mats show the presence, but low abundance, of *norBC* genes in the metagenomes. While denitrification can occur, it might not be a significant metabolism in the mat communities. In competition with the denitrifiers are microbes performing DNRA, which also use nitrate as an electron acceptor. The rates of these processes will determine whether more isotopically light ammonium is produced or if more of the lighter isotope is lost to the system in the form of N_2 gas. The two end members of these mixing processes include 1) predominantly denitrification associated with the loss of $^{14}N_2$ from the system, leaving more enriched bioavailable N and remineralization and thus enriched OM. 2) Predominantly DNRA and remineralization would result in the diffusion of lighter ammonium up into the overlying mat, resulting in a mix of some ratio of nitrification and assimilation in the oxic mat. Metagenomic data shows higher abundances of DNRA genes in the honeycomb mat metagenomes, suggesting higher community potential for this metabolism over denitrification, with the acknowledgment that metabolic potential is not gene expression. However, this supports the idea that the mat communities are well-adapted to efficiently recycle and utilize any bioavailable N without significant loss to either the water column or the system in the form of N_2 gas.

The mats at 9.5 m, growing below a fully euxinic water column with significantly higher concentrations of ammonium, would primarily perform ammonium assimilation and the anaerobic processes of DNRA, denitrification, and remineralization. The inhibition of aerobic

nitrification would limit the nitrate available for reduction processes. In total anoxia, microbes assimilate ammonium from the water column, produced by remineralization at depth. However, evidence of localized photosynthesis within the upper layers of the mat produces transient "oxygen oases," which would allow nitrification to occur and provide a nitrate source for reduction processes. The metagenomic data indicates that nitrification is not occurring as several necessary genes are missing from these mat metagenomes. Significant complete denitrification would lead to a preferential loss of $^{14}N_2$ from the system (Deb et al., 2024), further enriching the $\delta^{15}N$ of the OM. Several denitrification genes are missing from these metagenomes, decreasing the efficiency of this process in these mats. Significantly higher DNRA metabolism would produce isotopically lighter ammonium, which could be assimilated, lowering the $\delta^{15}N$ of the OM. All genes necessary for DNRA are present, supporting the metabolic potential for DNRA in the community.

These metagenomic and isotopic data suggest nitrification is not an important metabolism in Lake Fryxell due to the missing genes necessary to complete the two-step process. This is supported by the low ammonium concentrations in the water column, particularly in the oxic depths (8.0 m) where the aerobic process can occur. The 8.0 m mats remain oxygenated throughout the summer to depths of at least 15 mm into the sediment. The lack of anoxia at depth prevents the accumulation of ammonium, a required N species to complete nitrification. The mats at 8.8 m depth are a more suitable environment for the process to occur as the mats go anoxic at depth; however, the genes responsible for nitrification are missing from these metagenomes, too. Thus, the microbial production of nitrate is likely low or not occurring. The absence of ammonium at 8.0 m depths and the absence of nitrification indicates the N source to these microbial mat communities is likely from organic degradation and supported by tight

recycling. At depths at the bottom of the oxycline, where O2 concentrations decrease to anoxia and DIN concentrations increase, ammonium becomes a more favorable and accessible source of N to the benthic mat communities. Without nitrification, there is no microbial metabolic source of nitrate for denitrification and DNRA, suggesting little loss of N to the environment. Thus, ammonium and organic degradation are likely the most important sources of N for the mat communities at these depths. This further supports the idea that the mat communities are highly efficient at scavenging and tightly recycling bioavailable N in the mat without significant loss of nutrients to the environment.

While in many other N-limited environments, N-fixation can be used to alleviate N-limitation stress, the metabolism is energetically expensive and inhibited in the presence of O_2 (Howard & Rees, 1996). Lake Fryxell's ice cover only transmits 0.5 to 3% of incident irradiance, limiting the photosynthetic activity of the community. The metabolic energy expense, the light-limiting environment, the absence of heterocystic cyanobacteria, and the low abundance of *nif* genes suggest that N-fixation is not a metabolically advantageous process. Additionally, the low gene abundances and incomplete pathways of N redox metabolisms, along with the higher abundance of organic degradation and synthesis genes, support the interpretation that the N acquisition strategy of the benthic communities is the efficient recycling and scavenging of N within the mats. This is further supported by the presence of cyanophycin genes (*cphAB*), which is used as an N storage molecule.

Across all metagenomes, the absence or low abundance of denitrification genes compared to DNRA genes suggests a higher metabolic potential for DNRA in the benthic communities, meaning there is likely little production of N_2. Two different nitrite reductases in DNRA are capable of producing ammonia; however, the absence of *nirBD* and presence of *nrfAH* is

consistent with Fryxell's geochemistry as *nrfAH* is more active at low nitrate/nitrite levels compared to *nirBD* (Wang & Gunsalus, 2000; Xie et al., 2024). With higher metabolic potential for recycling and scavenging in the benthic communities, the presence and abundance of different organic degradation and synthesis gene pathways in the metagenomes offer more interesting insights into N cycling in the mat. While not a measure of gene expression, the higher relative abundances of GS and GOGAT in the metagenomes than GDH are consistent with the low ammonium availability in Fryxell, suggesting that despite the energy requirement of the GS/GOGAT cycle, the community might be more efficient at scavenging ammonia from its environment. The Fd-GOGAT is unique to photosynthetic organisms and is found at much higher abundances in the 9.8 m metagenomes than the other glutamate synthase (NADH-GOGAT). The ferredoxin-dependent glutamate synthase may be the dominant method of ammonia assimilation in the phototrophic community at depth, highlighting the importance of ammonium as an N source in all of the mats.

As the importance of recycling and assimilation of ammonium from organic degradation is highlighted in the metagenomic data, the DGT sediment probes give insights into the other dissolved inorganic N species: nitrate. The nitrate concentration and diffusion time series data indicate an accumulation of nitrate in sediment interstitial waters. As the metagenomic data is limited to the active microbial mat, the microbial processes in the underlying sediment remain unknown. Ammonium is not an analyte included in any DGT sediment probes; thus, the concentration of ammonium in the interstitial water is also unknown. Ammonium is unlikely to accumulate in the sediment porewaters if it is oxic; however, there is no data for O_2 concentrations in the sediment below 15 mm. These data are also limited to summer conditions when oxygenic photosynthesis is maintained consistently without diel cycles for several months.

The underlying sediment likely goes anoxic throughout the austral fall through spring, allowing reduced N species to accumulate. The accumulation of nitrate in the sediment interstitial waters in the summer indicates this study is potentially missing key processes or players in Fryxell's benthic N cycle. A more complete and conclusive N-cycle model requires deeper sequencing, gene expression data, and additional geochemical data.

Conclusions

Our results highlight the important role of microbial organic degradation and recycling in microbial mat N cycling under N limitation. The unique characteristics of a polar, perennially ice-covered lake also play an influential role in the development of the benthic N cycle, as the low light and tight redox gradients shape the metabolic potential of the communities. These data show the adaptive capabilities of the microbial mat community at efficiently recycling N without major redox transformations or loss of fixed N to the system. The integration of stable isotope and geochemical data helps identify signatures and missing players in the benthic N cycle and lacustrine N cycle as a whole. Future work should address the question of gene expression to elucidate what metabolisms are actually occurring in the benthic communities. Lake Fryxell's benthic microbial communities are well-adapted to the ultraoligotrophic, low-light lake environment. The mat communities are significant contributors to the primary productivity of the MDV region, and they play a crucial role in the region's nutrient cycle. With the sensitivity of polar regions to climate change, the fate of these communities and their influence on the region remains uncertain.

Figure 1. The nitrogen cycle is comprised of microbially mediated redox reactions, transforming N from one species to another. In italics are the associated genes for each process.

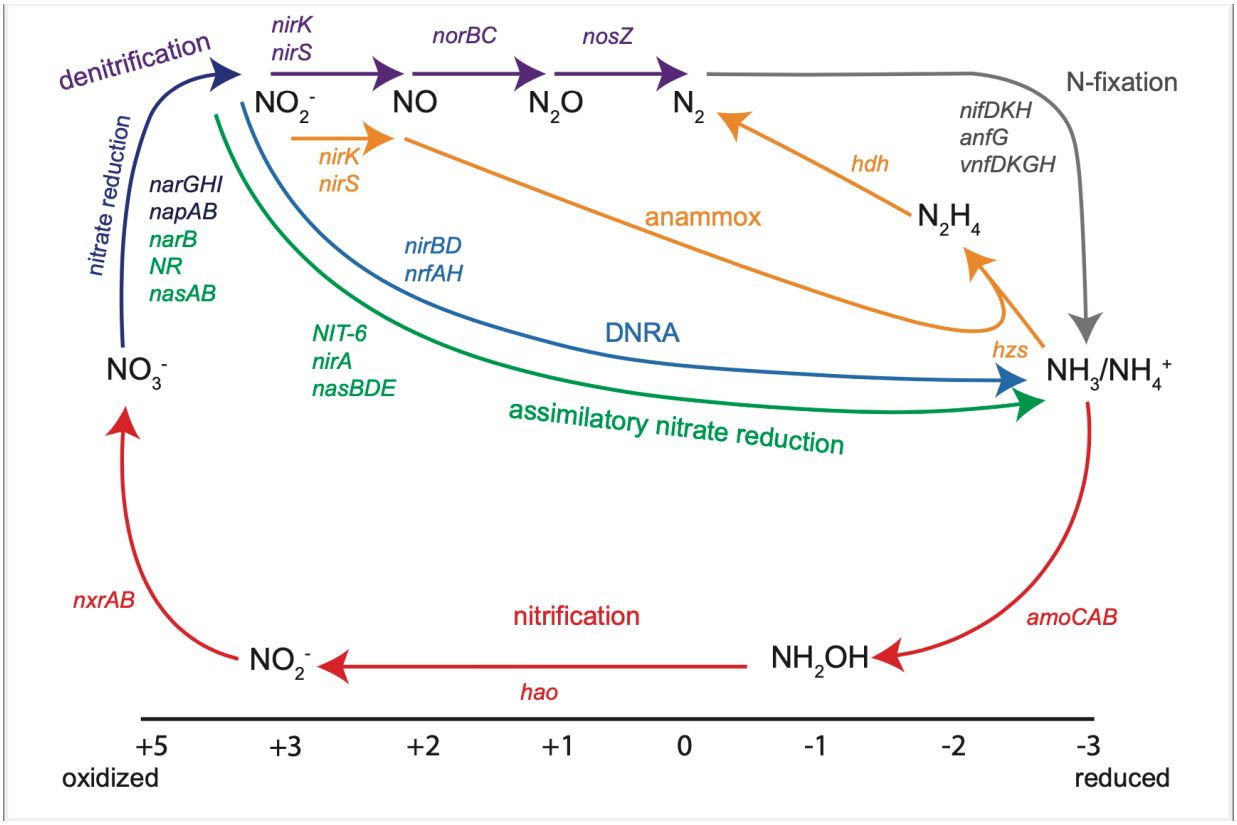


Figure 2. Nutrient (DIN and SRP) concentrations with depth in Lake Fryxell. Data are from the LTER (J. Priscu, 2022).

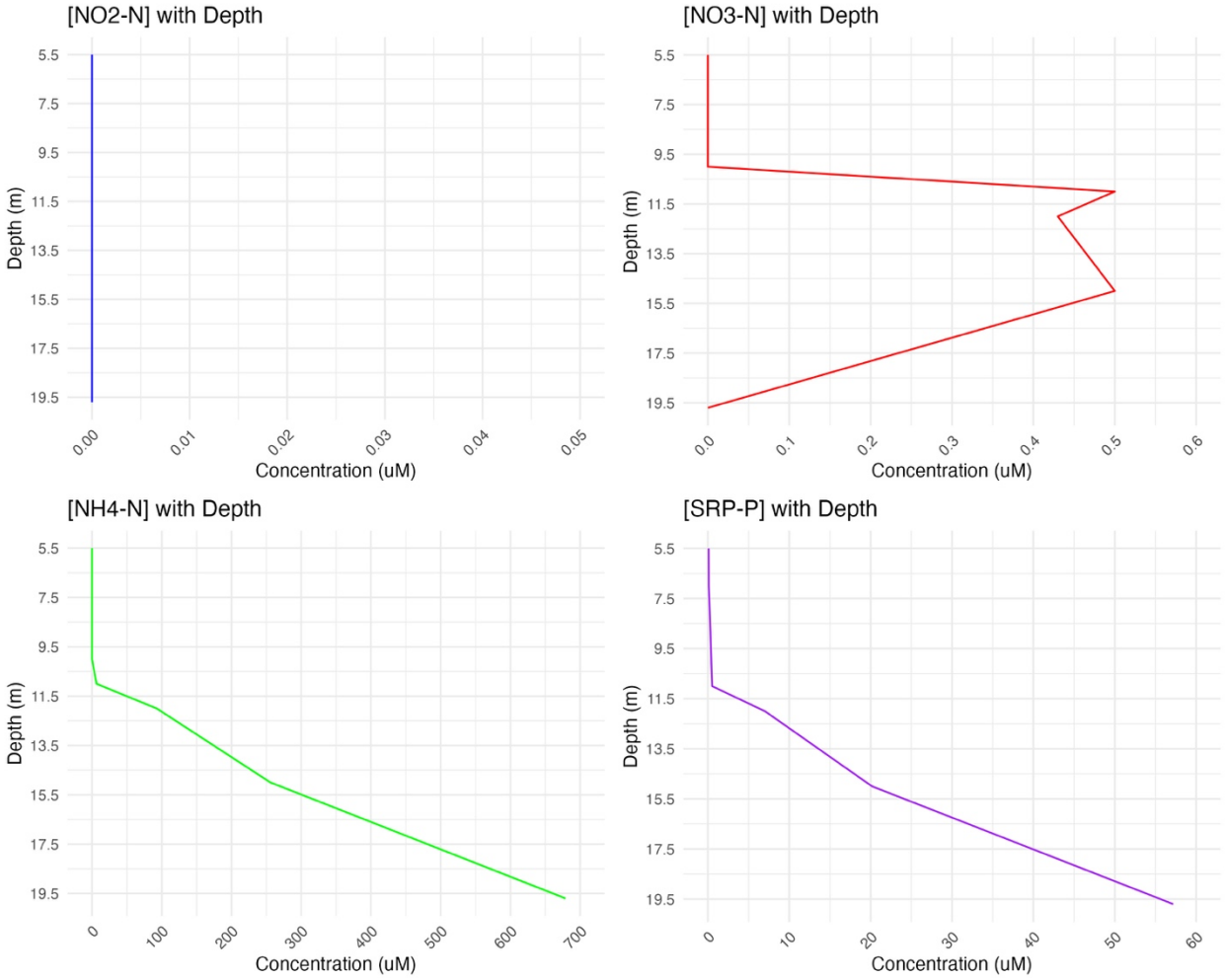
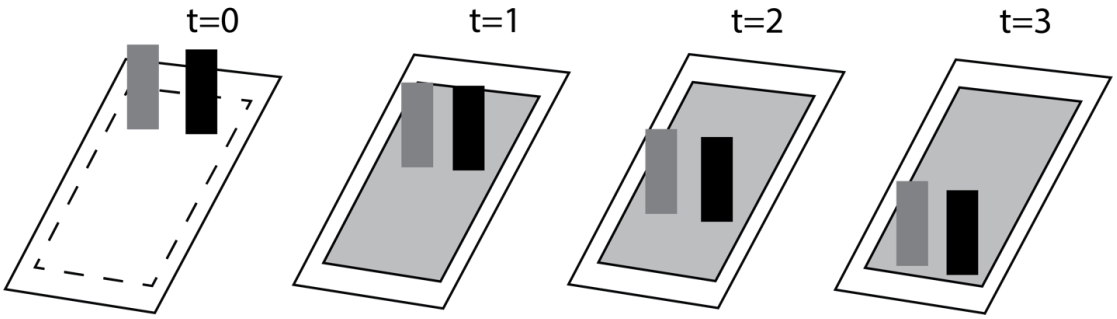


Figure 3*ab*.

a) Schematic of experimental plot and time series. The time series are represented below for one plot. The first time point (t=0) represents the intact mat before induced liftoff. The grey and black bars represent the nitrate and phosphate probes. The following are the plots post-liftoff and the associated probes' placement within the plot.



b) Diagram of the subsampling of nitrate and phosphate probes depending on time point.

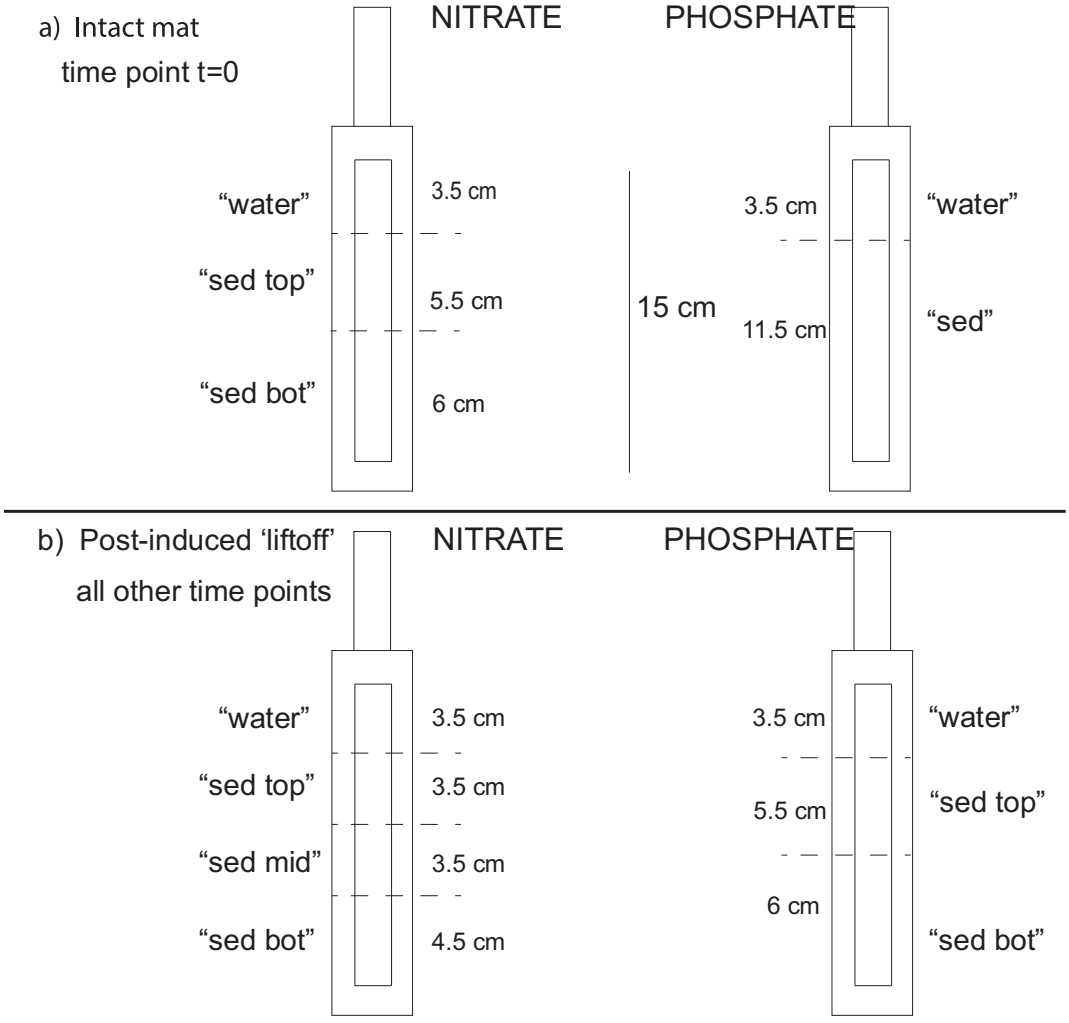


Figure 4. Heatmap showing presence-absence and relative abundance of genes responsible for N cycling pathways.

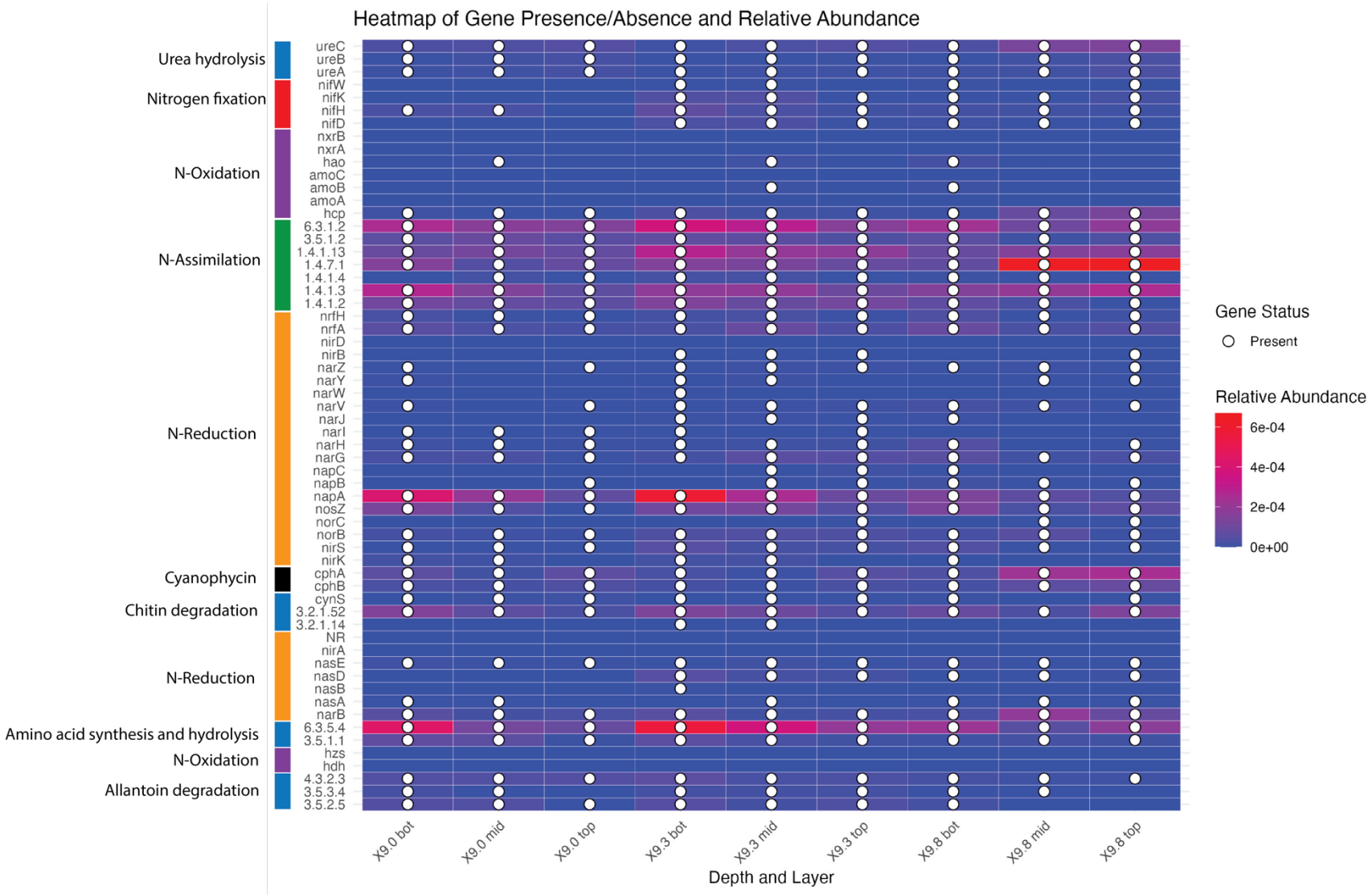
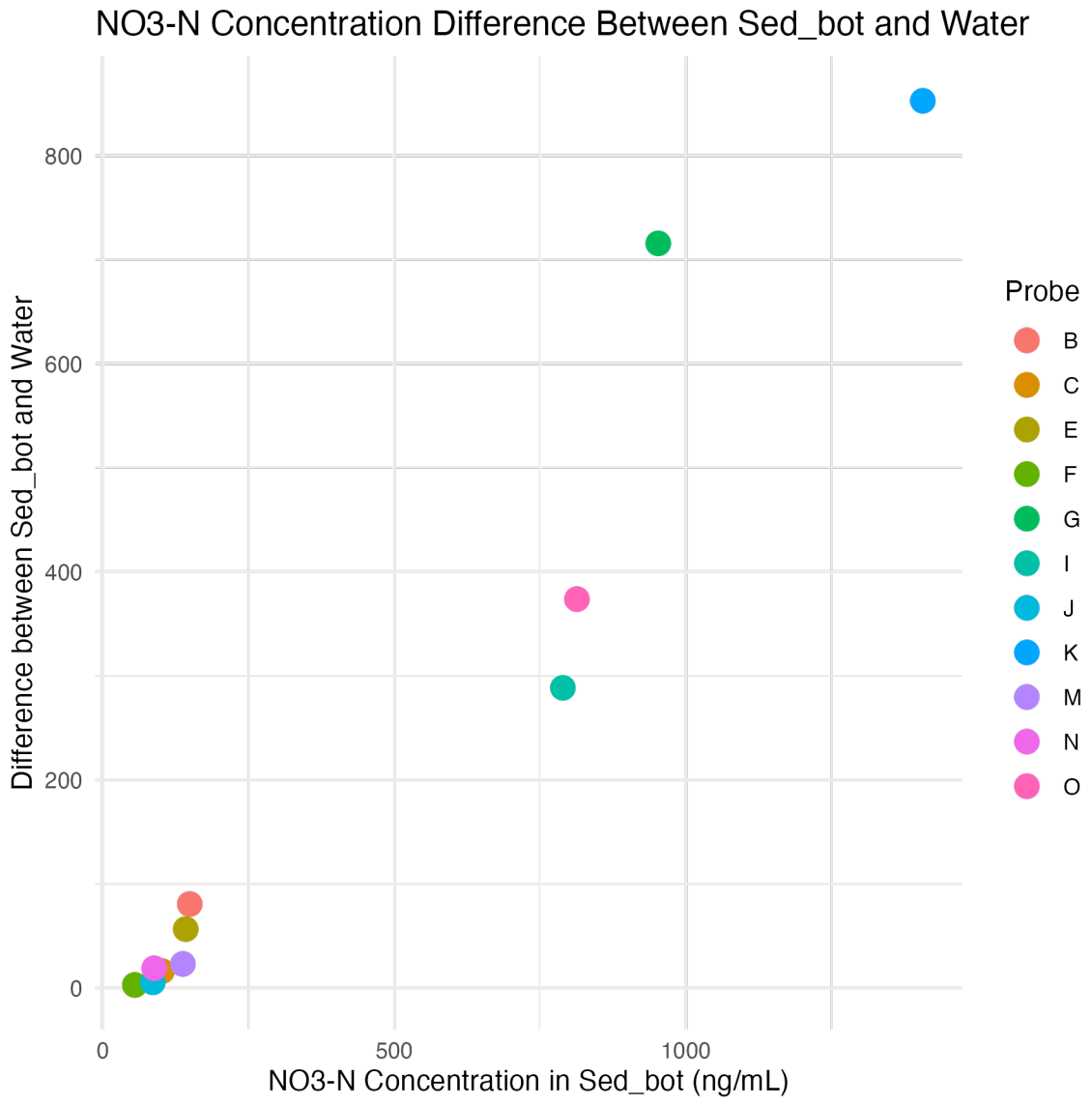


Figure 5: **a)** Scatterplot of nitrate concentration (ng/mL). The Y axis is the difference in nitrate concentration in the probe deepest in the sediment and the water column. The X axis is the concentration of nitrate in the probe deepest in the sediment. **b)** Nitrate concentration profiles with depth into the sediment.

a)



b)

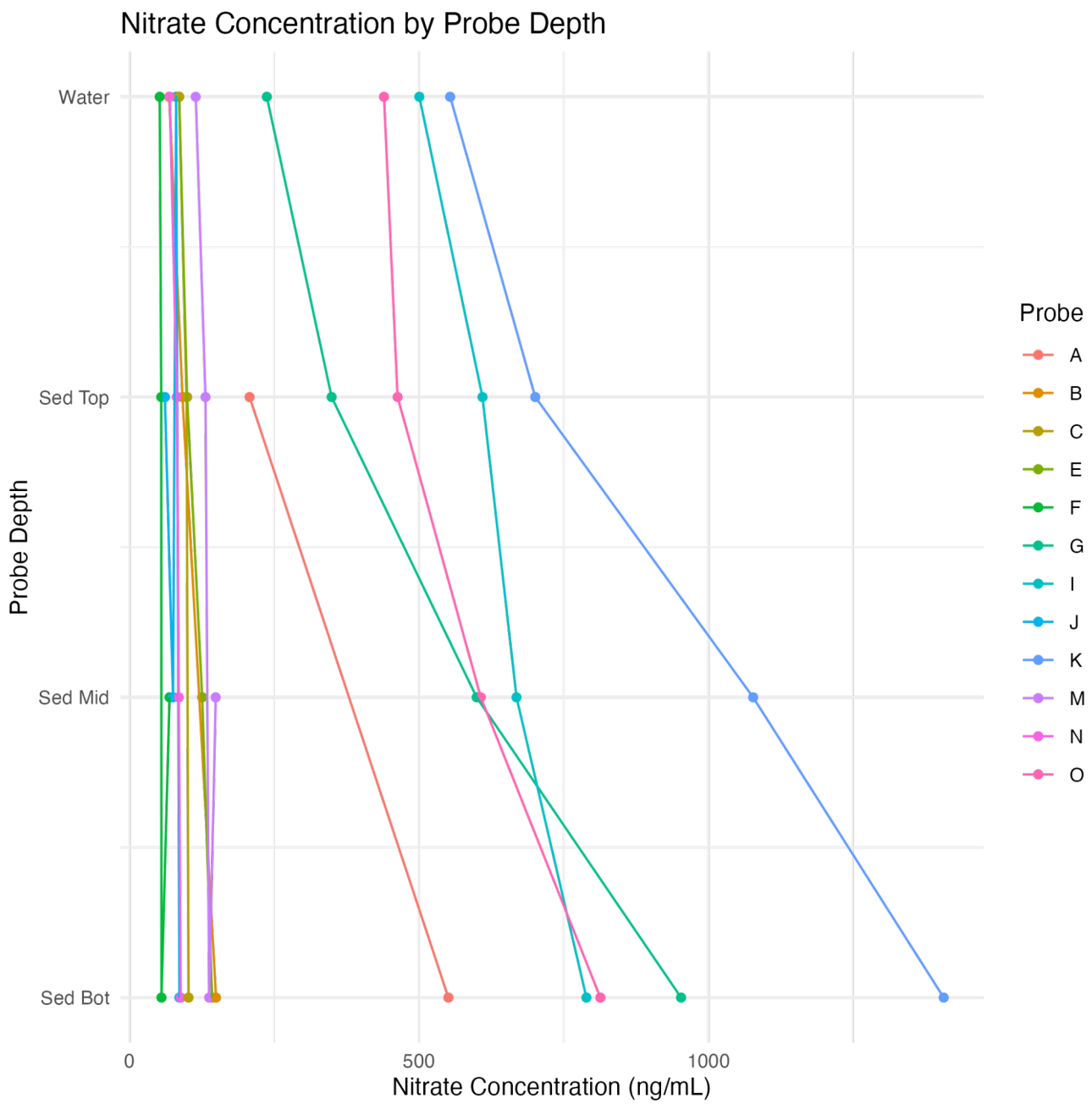

Nitrate Concentration by Probe Depth
Water
Sed Top
Sed Mid
Sed Bot
Probe Depth
0
500
1000
Nitrate Concentration (ng/mL)
Probe
A
B
C
E
F
G
I
J
K
M
N
O

Figure 6: Scatter plot showing $\delta^{15}N$ values of mat organic matter with

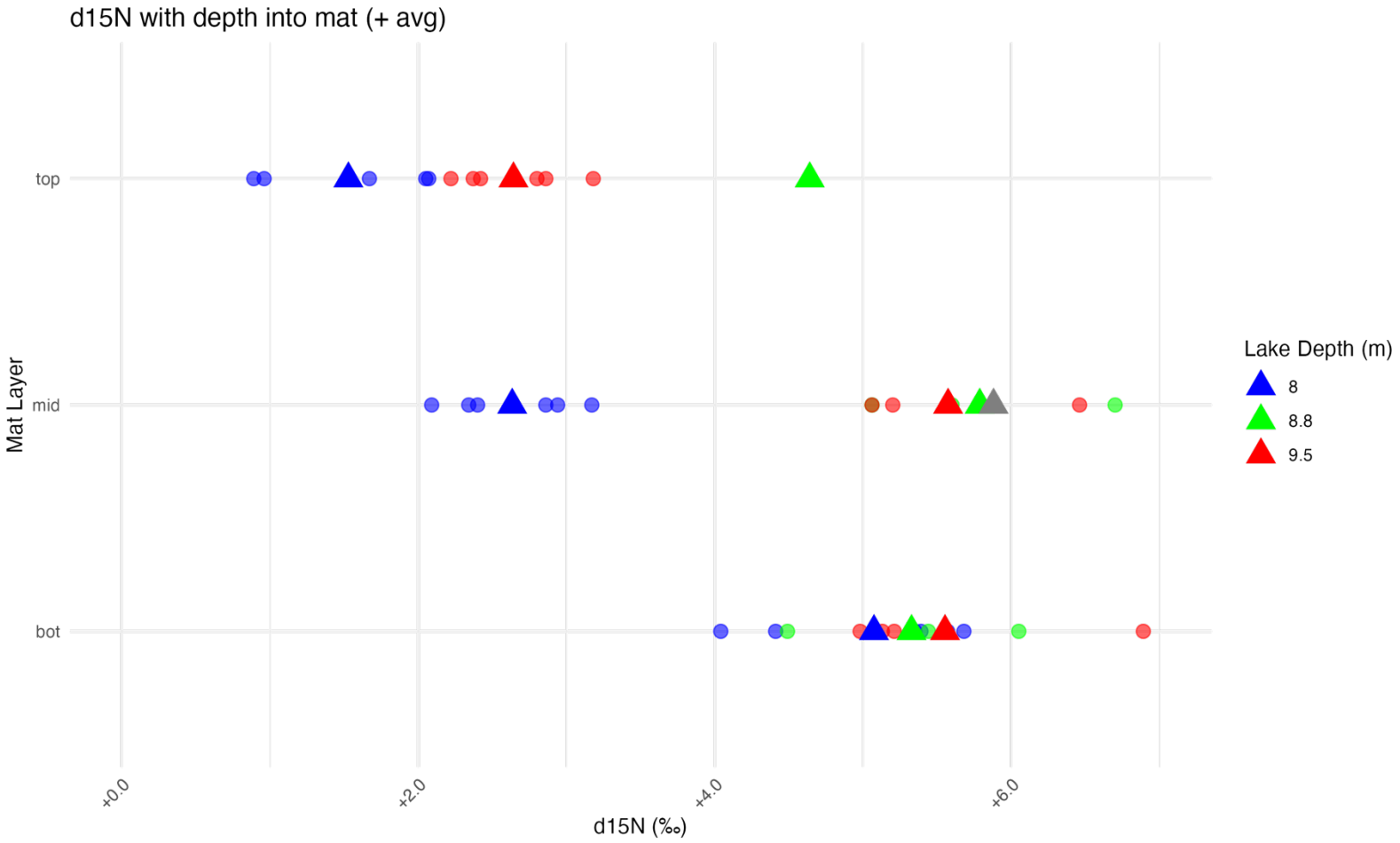


Table 1: $\delta^{15}N$ values of microbial mat organic matter from different depths along the oxycline/redox gradient.

Water depth (m)	Mat depth	$\delta^{15}N$ avg (‰)	+- $\delta^{15}N$ SD
8.0 (n=18)	top	+1.53	0.51
	middle	+2.63	0.42
	bottom	+5.07	0.68
8.8 (n=9)	top	+3.29	1.20
	middle	+5.79	0.84
	bottom	+5.67	0.79
9.5 (n=12)	top	+2.66	0.44
	middle	+5.65	0.65
	bottom	+5.55	0.89

Supplemental Table 1: Nitrogen cycling genes and pathways

Nitrogen Cycling Genes and Pathways		
		Genes/eCnum
N-Oxidation	Nitrification	amoABC
	Nitrification	hao
	Nitrification	nxrAB
	Anammox	hdh
	Anammox	hzs
N-Reduction	Hydroxylamine reductase	hcp
	Assimilatory nitrate reduction	narB
	Assimilatory nitrate reduction	NR
	Assimilatory nitrate reduction	nasAB
	Assimilatory nitrate reduction	NIT-6
	Assimilatory nitrate reduction	nirA
	Assimilatory nitrate reduction	nasBDE
	DNRA	nrfAH
	DNRA	nirBD
	DNRA/Denitrification	narGHI
	DNRA/Denitrification	napAB
	Denitrification	nirK
	Denitrification	nirS
	Denitrification	norBC
	Denitrification	nosZ
N-Fixation	MoFe nitrogenase	nifHDKW
	FeFe nitrogenase	anfG
	VFe nitrogenase	vnfH
Organic degradation and synthesis	Urease	ureaABC
	Allantoin degradation	eC_num 3.5.2.5
	Allantoin degradation	eC_num 4.3.2.3
	Allantoin degradation	eC_num 3.5.3.4
	Asparaginase	eC_num 3.5.1.1
	Asparagine synthase	eC_num 6.3.5.4
	Chitin degradation	eC_num 3.2.1.52
	Chitin degradation	eC_num 3.2.1.14
	Cyanate degradation	cynS
	Glutamate dehydrogenase	eC_num 1.4.1.2
	Glutamate dehydrogenase	eC_num 1.4.1.3
	Glutamate dehydrogenase	eC_num 1.4.1.4
	Glutamate synthase	eC_num 1.4.7.1
	Glutamate synthase	eC_num 1.4.1.13
	Glutaminase	eC_num 3.5.1.2
	Glutamine synthetase	eC_num 6.3.1.2
N Storage	Cyanophycin synthesis and hydrolysis	cphAB

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