

RESEARCH ARTICLE

Energetic and Environmental Constraints on the Community Structure of Benthic Microbial Mats in Lake Fryxell, Antarctica

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One sentence summary: Energy input affects the composition and diversity of microbial mats growing at the bottom of an Antarctic lake.

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ABSTRACT

Ecological communities are regulated by the flow of energy through environments. Energy flow is typically limited by access to photosynthetically active radiation (PAR) and oxygen concentration (O_2). The microbial mats growing on the bottom of Lake Fryxell, Antarctica, have well-defined environmental gradients in PAR and (O_2). We analyzed the metagenomes of layers from these microbial mats to test the extent to which access to oxygen and light controls community structure. We found variation in the diversity and relative abundances of Archaea, Bacteria and Eukaryotes across three (O_2) and PAR conditions: high (O_2) and maximum PAR, variable (O_2) with lower maximum PAR, and low (O_2) and maximum PAR. We found distinct communities structured by the optimization of energy use on a millimeter-scale across these conditions. In mat layers where (O_2) was saturated, PAR structured the community. In contrast, (O_2) positively correlated with diversity and affected the distribution of dominant populations across the three habitats, suggesting that meter-scale diversity is structured by energy availability. Microbial communities changed across covarying gradients of PAR and (O_2). The

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comprehensive metagenomic analysis suggests that the benthic microbial communities in Lake Fryxell are structured by energy flow across both meter- and millimeter-scales.

Keywords: Antarctica; microbial mat; energy; Lake Fryxell; Oxygen; Photosynthetically Active Radiation

INTRODUCTION

The composition and diversity of biological communities respond strongly to energy availability, as evidenced by the positive correlation between net primary productivity (NPP) and diversity (Gillman et al. 2015). This correlation led to the development of species-energy theory, which posits that habitats with greater energy capture by a community have more niche diversity (Hurlbert and Stegen 2014). The relationships between communities and environmental characteristics have historically been studied in the presence of macroflora and -fauna because in most habitats, microbial niches and those of plants and animals are interdependent. Exceptions, where microorganisms grow independently of macroorganisms include extreme environments such as hot springs (Amin et al. 2017), the deep biosphere (Ino et al. 2018) and snowfall (Brown and Jumpponen 2019). Studies these habitats have been foundational to evaluating the application of ecological principles to microbial ecosystems.

Species-energy theory is likely applicable to microbial ecosystems based on recent experimental work investigating the effects of increased carbon substrate availability on community composition and diversity in a groundwater system (Zhou et al. 2014). Other laboratory and field studies also suggest that microbial diversity increases with energy availability (Bernstein et al. 2017) and (O_2) (Spiezia et al. 2015). However, the actual energy available to a community is a complex function of both the biogeochemistry of the environment (e.g. Marlow et al. 2014) and the metabolisms that can be utilized by the microbes present (Tilman 1994). When and where specific groups of microbes are highly efficient at capturing available energy, the effects of energy flow on microbial diversity are complex. One important source of energy for many microbial ecosystems is photosynthetically active radiation (PAR). Where PAR is high, species-energy theory predicts that ecosystems will have increased taxonomic richness. For example, where PAR is available, oxygenic photosynthesizers are particularly abundant, and should drive NPP in their habitats. These primary producers simultaneously affect the redox state of electron donors and acceptors (Macalady et al. 2013), thus increasing resource availability for heterotrophic community members. However, ecosystems with a large population of oxygenic photosynthesizers sometimes show negative correlations between alpha diversity and primary productivity (Bernstein et al. 2017). In these communities, oxygenic photosynthesizers capture the majority of the energy in a habitat resulting in greater population sizes of oxygenic photosynthesizers and reduced relative abundance of heterotrophs, anaerobes and overall alpha diversity, in conflict with the species-energy theory (Hurlbert and Stegen 2014).

An alternative to the species-energy theory – the maximum power principle – argues that communities may be structured to optimize productivity (Odum and Pinkerton 1955; DeLong 2008). The maximum power principle posits that communities that maximize the capture of energy will be at a selective advantage over those that do not, and the relative abundances of populations in an ecosystem will adjust accordingly. In contrast to species-energy arguments, the maximum power principle predicts that optimization of energy capture may result in

decreased diversity (Odum and Pinkerton 1955; DeLong 2008). Both species-energy theory and the maximum power principle assert that ecological communities are structured by resource heterogeneity (spatial, temporal or both) and both hypotheses are therefore variations of the species sorting model according to metacommunity theory (Leibold and Chase 2017).

Understanding relationships between energy flow and diversity in microbial ecosystems is crucial to distinguishing what structures them. The ecological theories discussed above were developed from well-studied macroscopic ecosystems. If they are universal, species-energy theory and the maximum power principle should apply to microbial ecosystems. By tracking microbial diversity across energy gradients, we can gain insights into the similarities and differences in how ecosystems are structured without macroscopic organisms. Specifically, we can use such gradients to determine whether the diversity of microbial ecosystems is structured by, and correlated with, energy availability and harvesting. However, we can expect the composition and diversity of microbial communities to also be constrained by the biogeochemistry of the local environment, given the diversity of metabolic strategies employed by microorganisms. Thus, heterogeneity of resources may be more important than total energy input in determining alpha diversity of a microbial ecosystem.

The processes determining the structure of microbial ecosystems have been investigated largely in extreme environments such as those with high salinity (Kunin et al. 2008; Schneider et al. 2013) or high temperatures (Vick et al. 2010; Schneider et al. 2013; Fortney et al. 2016), where macroorganisms are excluded and microorganisms predominate. Similarly, ice-covered lakes in Antarctica are extensive habitats for microbial mats that grow in the absence of most plants and animals (Van Trappen et al. 2002; Taton et al. 2003; Rios et al. 2004). The circumpolar Southern Ocean currents and the freezing temperatures of the continent isolate Antarctica from much of the terrestrial plant and animal life in the rest of the world (Cavicchioli 2015), and the extreme aridity of the McMurdo Dry Valleys (MDVs) further limits the diversity of organisms that might otherwise inhabit the lakes (Cary et al. 2010).

MDVs Lake Fryxell is a perennially ice-covered, density-stratified lake with a steep oxycline and gently sloping basin (Lawrence and Hendy 1985). Steep PAR and (O_2) gradients existed from 9 to 10 m depth in the lake in 2012 (Fig. 1). The persistent density gradients in the water column lead to stratification in Lake Fryxell's pelagic community (Spaulding et al. 1994): NPP in the photic zone is dominated by oxygenic photosynthesis, but the redox structure of the water column and the distribution of irradiance allows anoxygenic photosynthesis to contribute to primary productivity as well (Klatt et al. 2015). The distribution of pelagic chemolithoautotrophs in Lake Fryxell is also limited to depths greater than 11 m by redox potential (Dolhi et al. 2015).

Together, the irradiance and oxygen gradients in Lake Fryxell, and the stratification of the pelagic community, indicate that PAR and (O_2) are key environmental characteristics that influence the community composition and diversity. Lake Fryxell is particularly informative, as the distinct gradients in PAR and (O_2) do not co-vary across all habitats, allowing for comparison of

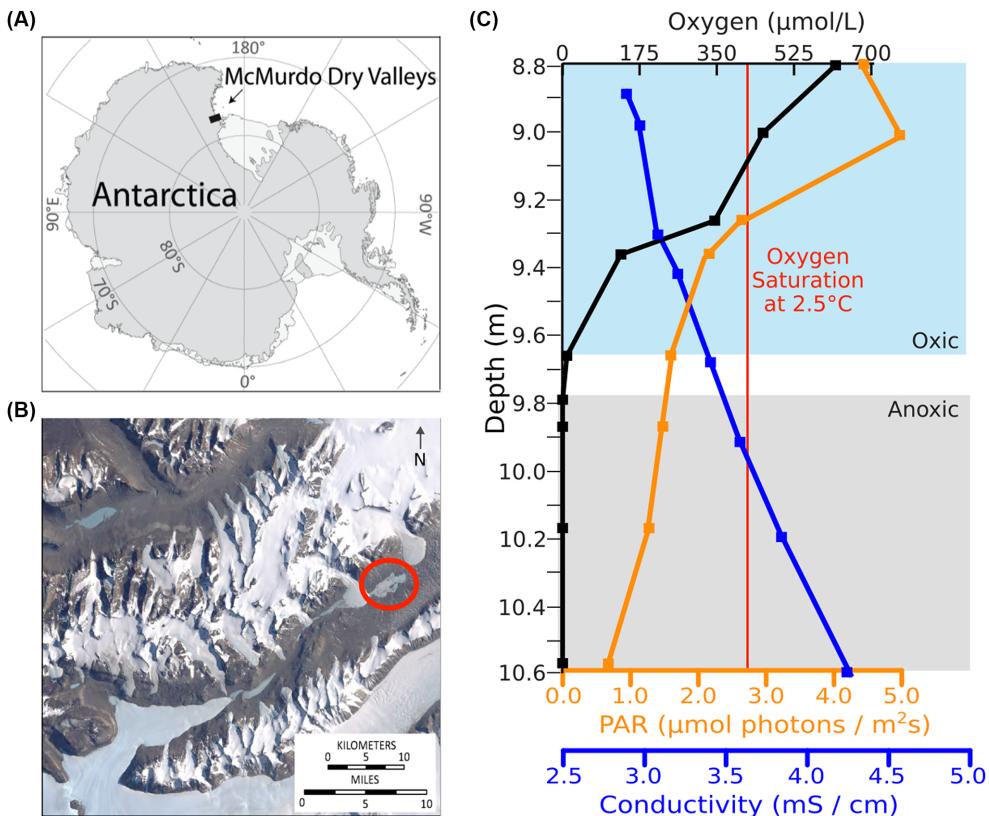


Figure 1. (A) The McMurdo Dry Valleys are in Southern Victoria Land, East Antarctica. (B) Location of Lake Fryxell in Taylor Valley (circled in red) (Herried 2010). (C) Oxygen concentration and conductivity, PAR and oxygen saturation at 0°C along a benthic mat transect in Lake Fryxell in November 2012 (Jungblut et al. 2016).

these parameters. The variation of PAR and (O_2) in Lake Fryxell provides an opportunity to study how the structure of benthic microbial mat communities differ according to habitat, specifically energy availability. In this study, we present the benthic community diversity and composition in Lake Fryxell with the aim of providing insights into the relative importance of PAR and (O_2) in shaping community structure and testing ecological theories of energy use in a microbial ecosystem.

METHODS

Site description

Lake Fryxell ($77^{\circ} 36'S$ $162^{\circ} 6'E$) is a density stratified and oligotrophic freshwater ecosystem in the MDVs, Antarctica. It is 5×1.5 km in extent and the maximum depth is approximately 20 m (Green and Lyons 2009). Water is supplied to Lake Fryxell by 13 glacial melt-water streams, with most water coming from the Canada and Commonwealth glaciers (McKnight et al. 1999). Water is lost by evaporation and ablation from the surface, and changes in lake level reflect net water balance; there are no outflowing streams (Lawrence and Hendy 1985).

Environmental conditions in Lake Fryxell are strongly affected by a 4–5 m thick perennial ice cover (Obryk et al. 2016). The ice cover inhibits wind mixing and prevents gas equilibration between lake water and the atmosphere. A lack of vertical mixing is due to a stable density stratification, as demonstrated by conductivity profiles (Priscu 2014; Jungblut et al. 2016) (Fig. 1). Stratification limits the transport of nutrients and redox pairs to diffusion and creates stable redox and nutrient gradients in the water column (Lee et al. 2004; Jungblut et al. 2016).

Oxygen and other gases are excluded as ice freezes onto the underside of the ice cover during winter, building to gas supersaturation in shallow waters (Hood, Howes and Jenkins 1998). Oxygen concentration declines rapidly with depth below 8 m, and oxygen is absent from the water column below approximately 9.7 m (Sumner et al. 2015; Jungblut et al. 2016). In addition to contributing to gas buildup in shallow waters, the ice cover also limits the amount of light reaching the lake waters. During the summer, the ice cover transmits approximately 1% of incident irradiance (Jungblut et al. 2016), which provides the lake's primary energy influx. Due to the low slope of the Fryxell basin, PAR is generally consistent across the lake ice cover seasonally without topographic shading effects (Dana, Wharton and Dubayah 1998; Acosta 2016). Light reaching the floor of Lake Fryxell declines with increasing depth in the water column but is adequate to support photosynthesis at the top of benthic mats to depths of 11 m during the summer months (Sumner et al. 2015; Jungblut et al. 2016). PAR, as measured by percent surface irradiance, was collected at the transect mat-water interface, and (Jungblut et al. 2016). Temperature varies from $2.4^{\circ}C$ to $2.7^{\circ}C$ and pH varies from 7.44 to 7.52 and from 8.9 to 11.0 m depth in the lake along an established transect (Hillman 2013; Jungblut et al. 2016). All depths are in relation to lake level during November 2012.

Lake Fryxell supports a rich microbial community. The pelagic community thrives near the deep chlorophyll maximum, located just above the oxic-anoxic transition (9–10 m), which is localized where nutrients diffuse up into the photic zone (Roberts 2000). Benthic microbial mats grow to depths of at least 10.5 m, forming cm-to-dm-scale thick mats (Jungblut et al. 2016). Mat pigmentation and morphology transition from purple

pinnacles to orange ridges and pits to bright green flat mats with depth, as PAR and (O_2) decline (Jungblut et al. 2016). Oxygenic photosynthesis raises redox conditions seasonally within mats near the oxic-anoxic transition in the water column (Sumner et al. 2015). Microelectrode profiles taken in late spring through the benthic microbial mats showed that (O_2) varied at different water depths (Sumner et al. 2015). At 9.0 m, (O_2) ranged from $\sim 650 \mu\text{mol O}_2/\text{L}$ in the water immediately above and just below the surface of the mat, to $\sim 825 \mu\text{mol O}_2/\text{L}$ at 2 mm below the mat surface. The mats still contained $> 500 \mu\text{mol O}_2/\text{L}$ at 17 mm below the surface. In contrast, at 9.8 m, there were $0 \mu\text{mol O}_2/\text{L}$ in the overlying water column, but up to $50 \mu\text{mol O}_2/\text{L}$ were present within the upper mat due to photosynthetic O_2 production, falling back to $0 \mu\text{mol O}_2/\text{L}$ by 6 mm depth into the mat (Sumner et al. 2015).

Sampling

The benthic microbial mats in Lake Fryxell, Antarctica were sampled in November 2012, as described by (Jungblut et al. 2016). Sampling was performed at 9.0, 9.3 and 9.8 m depths along a transect that was installed in 2006 (Hillman 2013). Divers retrieved material from the bottom of the lake by cutting blocks out of *in situ* mats using a spatula and lifting them into plastic boxes underwater. Upon delivery to the surface, samples were dissected according to layer pigmentation and morphology using sterile sampling equipment, resulting in three layers at 9.0 m (top, middle and bottom), three layers at 9.3 m (top, middle and bottom), and four layers at 9.8 m (film, top, middle and bottom). Mats at 9.3 m had complicated topography with 0.5–1.0 cm deep pits between ridges. At this depth, the tops of the ridges were separated as the top layer, the ridges including their edges were separated as the middle layer, and the bottoms of pits were collected as the bottom layer (Jungblut et al. 2016, Supporting Information). The samples were preserved in the field immediately after sampling using an Xpedition Soil/Fecal DNA MiniPrep kit (Zymo Research, Irvine, CA) frozen and shipped frozen to UC Davis where they were stored at -80°C until DNA was extracted.

Metagenomic sequencing

DNA was extracted using an Xpedition Soil/Fecal DNA MiniPrep kit (Zymo Research, Irvine, CA) as per manufacturer instructions from biological and technical replicates of 10 sample types (Table S1, Supporting Information). Metagenomic sequencing was performed at the University of California, Davis Genome Center DNA Technologies Core (<http://dnatech.genomecenter.ucdavis.edu>) using the Illumina HiSeq 2500, PE 250 platform. Library preparation was performed using Illumina's Nextera DNA Kit (Oligonucleotide sequences © 2007–2013 Illumina, Inc.). Reads were quality filtered to Q20 using FASTX-Toolkit v0.0.13 (Gordon, Hannon and Others 2010), and forward and reverse reads were joined using PEAR v0.9.6 (Zhang et al. 2014). Fastq files with fewer than 10 000 reads were determined to be outliers and were therefore removed from downstream analyzes. Metagenomic reads are available via NCBI's sequence read archive (PRJNA291280).

Taxonomic assignment

Unassembled reads were assigned to Bacteria, Archaea, and Eukaryotes. Protein-level classifications for taxonomic assignments were made per replicate using NCBI's non-redundant protein database (accessed 3/2/2017), including Bacteria, Archaea, Eukaryotes and Viruses. All assignments, from domain to

operational taxonomic units (OTUs), were made using Kaiju's greedy mode with 5 allowed substitutions, a minimum match score of 70, and low-complexity query sequence filtering (Menzel, Ng and Krogh 2016). Taxa in relative abundance less than 0.5% per sample were removed from downstream analyses.

Phylogenetic diversity analyzes

Phylosift v1.0.1 (Darling et al. 2014) was used to generate phylogenetic placements for the marker genes (<https://phylosift.wordpress.com/tutorials/scripts-markers/>) per replicate using default settings. Guppy v1.1 was used to calculate balance weighted phylogenetic diversity (bwpd, alpha diversity) (Matsen, Kodner and Virginia Armbrust 2010), which provides alpha diversity based on phylogenetic relationships using jplace files generated by Phylosift as input, as opposed to community count data (Nipperess and Matsen 2013). Beta diversity was measured using Kantorovich–Rubinstein distance (kr distance) (Evans and Matsen 2012), calculated using guppy (v 1.1) (Matsen et al. 2010). Guppy v1.1 was also used to perform edge principal components analysis (EPCA). Guppy addresses uncertainty in the placement of reads by generating a collection of weighted placements (pqueries). When generating the EPCA, every pquery was treated as a point mass concentrated on the highest-weight placement and rotating three dimensions for support overlap minimization. Unrooted phylogenetic diversity rarefaction curves were generated using Guppy (McCoy and Matsen 2013) (Fig. S1, Supporting Information).

Statistical analyses

Significant differences in taxonomic classification, alpha diversity, and beta diversity between samples were determined using Permutational Multiple Analysis of Variance (PERMANOVA) in R v3.3.2 (Warton, Wright and Wang 2012; Anderson and Walsh 2013; R Core Team and Others 2013) using R package vegan v2.4–2 (Oksanen et al. 2017). Samples determined to differ significantly in alpha diversity (bwpd), as per PERMANOVA implemented via the adonis function, were then subjected to Tukey's Honest Significant Difference (Tukey's HSD) test (Tukey 1949) in R v3.3.2 (R Core Team and Others 2013) to establish which lineages differed between depths and between layers at each depth. Partial distance-based redundancy analysis (RDA) (Jari Oksanen, F. Guillaume Blanchet, ...) was used to determine the relationships among lake depth, mat layer, (O_2) and PAR and between these environmental characteristics and differences in community composition as measured by kr distance.

RESULTS

Taxonomic annotation

After quality filtering, the total amount of metagenomic sequence data came to approximately 47.1 billion bp from approximately 177 million reads (Table S1, Supporting Information). Taxa from Bacteria, Archaea, and Eukarya were present in all samples (Fig. 3; Tables S3–S6, Supporting Information). The most relatively abundant organisms across all samples are from the domain bacteria and the phyla Proteobacteria (19.2%) and Cyanobacteria (7.5%) (Fig. 3, Tables S3–S6, Supporting Information). The genera with the highest relative abundance across all layers at all depths belong to the phylum Cyanobacteria, and genera in Cyanobacterial subsection III (Oscillatoriaceae, filamentous Cyanobacteria that lack cell differentiation



Figure 2. Relative abundance of phyla and families from all samples. The relative proportion of Cyanobacteria to Proteobacteria decrease through mat layers at all depths and mats at 9.8 m are dominated by the family Oscillatoriaceae. Family labels: Pr, Proteobacteria; C, Cyanobacteria; Ba, Bacteroidetes; Pl, Planctomycetes; V, Verrucomicrobia; G, Gemmatimonadetes; N, Nitrospirae; Bp, Bacillariophyta; T, Thaumarchaeota.

(Tomitani et al. 2006) are dominant. One genus is found in greater relative abundance ($P < 0.01$, Table S5, Supporting Information) than any other identified taxon in the film, top, and middle layers of the microbial mat growing at 9.8 m, which was identified as a species of *Phormidium* in previous 16S rRNA gene analyses (Jungblut et al. 2016, Supporting Information). We therefore also refer to this genus as *Phormidium*, even though reads in this genus were annotated as both *Phormidium* and *Oscillatoria* (Altschul et al. 1990). In this study, 55 *Phormidium* OTUs occur across all ten sample types, but *Phormidium pseudopristleyi* is found in higher relative abundance in the film at 9.8 m than in any other sample (Table S5 and S6, Supporting Information).

Alpha diversity

Alpha diversity varies both with mat layer and lake depth (Fig. 2; Table 1). When layers are grouped by depth, alpha diversity decreases with increasing depth in the lake, which correlates with decreasing PAR and (O_2) (Fig. 2; Fig. S2, Supporting

Information). In contrast, alpha diversity increases into the mat from the top to bottom layers when depths are pooled (Fig. 2; Fig. S3, Supporting Information), corresponding to decreasing PAR. In 9.0 m mats, where oxygen is supersaturated and effectively constant throughout the mat and PAR decreases through the mat, alpha diversity increases from the top to the bottom layer (Fig. 2). The diversity of the dark bottom layers from mats at each depth differ by (O_2) . At 9.0 m, where (O_2) is highest, the alpha diversity of the bottom layer is greater than the diversity of the bottom layer at 9.3 m and the bottom layer at 9.8 m. Considering only the bottom layers, where PAR is negligible, alpha diversity is lowest where (O_2) is lowest (Fig. 2). Simpson's diversity indices do not vary significantly between depths or layers (Table S2, Supporting Information).

Beta diversity

Edge Principal Components Analysis (EPCA) illustrates variations in community composition among samples (Fig. 4). At 9.0 and 9.3 m, mat layers were well separated in ordination space

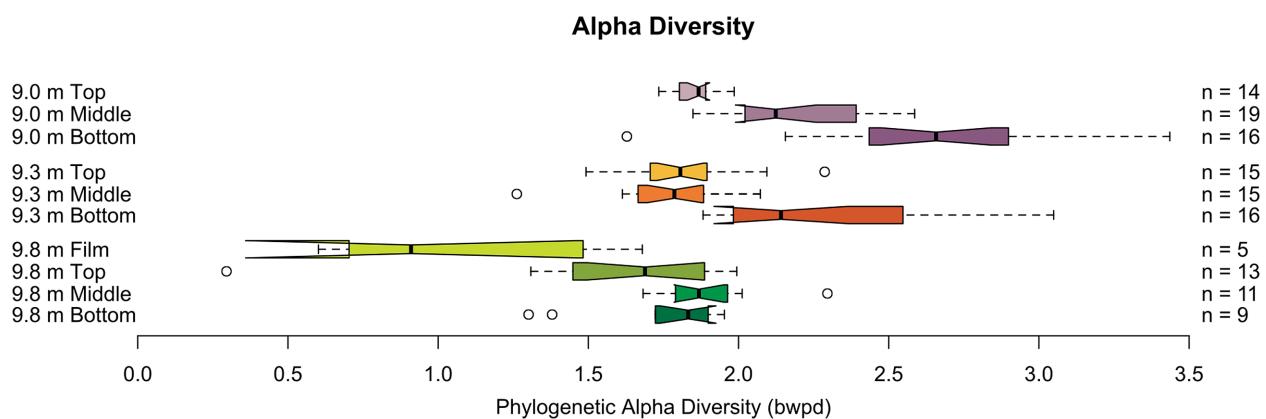


Figure 3. Alpha diversity of microbial mat communities by lake depth and mat layer as measured by balance-weighted phylogenetic diversity, which provides alpha diversity based on phylogenetic relationships as opposed to community count data. Whiskers represent extreme values, and boxes represent the interquartile range.

Table 1. Adjusted P-values of alpha diversity (bwpd, based on phylogenetic relationships as opposed to community count data) comparisons between samples, calculated using Tukey's HSD. Mat layer communities were considered significantly different at P-values < 0.01. Comparisons without significant differences are marked – and self-comparisons are marked x.

	9.0 m Top	9.0 m Middle	9.0 m Bottom	9.3 m Top	9.3 m Middle	9.3 m Bottom	9.8 m Film	9.8 m Top	9.8 m Middle	9.8 m Bottom
9.0 m Top	x									
9.0 m Middle	–	x								
9.0 m Bottom	0.00E+00	1.85E-03	x							
9.3 m Top	–	–	0.00E+00	x						
9.3 m Middle	–	5.10E-03	0.00E+00	–	x					
9.3 m Bottom	–	–	–	9.54E-03	1.96E-03	x				
9.8 m Film	1.66E-04	0.00E+00	0.00E+00	4.00E-04	1.34E-03	0.00E+00	x			
9.8 m Top	–	7.80E-06	0.00E+00	–	–	3.10E-06	–	x		
9.8 m Middle	–	–	1.00E-07	–	–	–	1.34E-04	–	x	
9.8 m Bottom	–	–	0.00E+00	–	–	7.33E-03	–	–	–	x

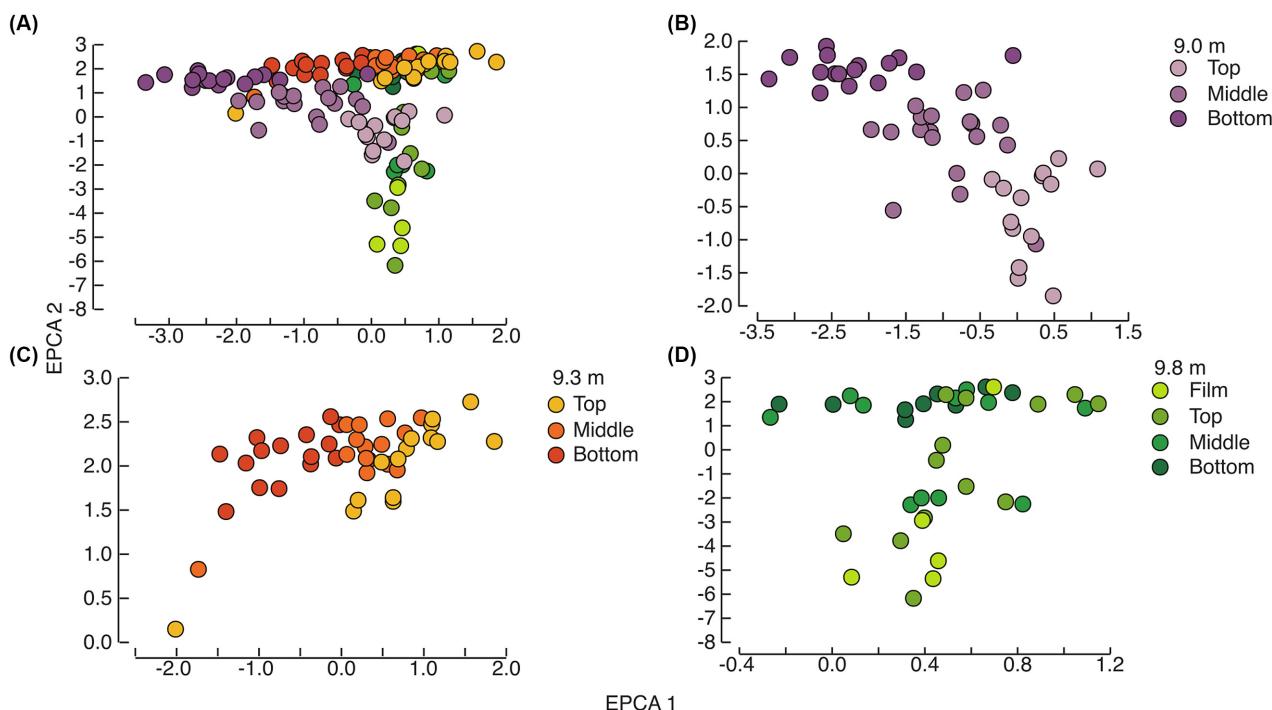


Figure 4. Beta diversity analysis using EPCA of mat layer communities by depth as tested by PERMANOVA and Tukey's HSD (Tukey 1949) (Tables 2–4). (A) All samples, (B) samples from all mat layers at 9.0 m (C) samples from all mat layers at 9.3 m and (D) samples from all mat layers at 9.8 m.

Table 2. Effect of mat layer and lake depth calculated using partial distance-based redundancy analysis in R package vegan. Effects were considered significantly different at P-values ≤ 0.01 .

kr distance ~ Layer \times Depth						
Permutation: free						
Number of permutations: 999						
Source of variation	Degrees of freedom	Sums of squares	Mean squares	F statistic	R ²	Pr(>F)
Layer	1	22.555	22.5548	40.951	0.18699	0.001
Depth	1	10.309	10.3093	18.718	0.08547	0.001
Layer \times Depth	1	9.545	9.5449	17.33	0.07913	0.001
Residuals	142	78.211	0.5508		0.64841	
Total	145	120.62			1	

(Fig. 4B and 4C), while at 9.8 m, the separation was much less obvious (Fig. 4D).

Partial RDA and Tukey's HSD test revealed the OTUs contributing to statistically significant differences between samples (Tables S3–S5, Supporting Information). Performing partial RDA on the subset of samples for which percentage surface irradiance was able to be collected revealed that lake depth can be used as a proxy for (O₂) and mat layer can be used as a proxy for PAR for the purposes of disentangling the effects of PAR and (O₂) on microbial community composition and diversity (Fig. S4, Supporting Information). (O₂) decreases as lake depth increases and PAR decreases with mat layer, top to bottom (Fig. S4, Supporting Information). Partial RDA calculated on samples from all depths and layers showed that lake depth and mat layer significantly affect community composition (Table 2).

At 9.0 m, 54 OTUs (6% of the OTUs identified throughout the 9.0 m mats) vary significantly (P < 0.01) between layers (Table S3, Supporting Information; Fig. 3), including several bacterial OTUs as well as diatoms and one archaeal genus (*Nitrosopumilis*). At 9.3 m, only eight bacterial and archaeal OTUs (0.1% of the OTUs identified throughout the 9.3 m mats) vary significantly (P < 0.01) between the layers (Table S4, Supporting Information; Fig. 3). At 9.8 m, one OTU (0.5% of the OTUs identified throughout the 9.8 m mats), *Phormidium pseudopristleyi*, varies significantly (P < 0.01) between the layers (Table S5, Supporting Information; Fig. 3).

In summary, oxygen-supersaturated mats (9.0 m) have higher alpha diversity (approximately 1.7–2.6, Fig. 2) and larger phylogenetic differences in community composition between layers (Fig. 4) as compared to anoxic mats (9.8 m), which have lower alpha diversity (approximately 0.8–1.9, Fig. 2) and less phylogenetic difference in community composition between layers (Fig. 4). In contrast, top layers have lower alpha diversity across lake depths than subsurface layers (Fig. S3, Supporting Information).

DISCUSSION

Effects of (O₂) and PAR on community composition and diversity

In Lake Fryxell, PAR and (O₂) influence the microbial community in the various layers of the benthic mats differently, depending on the lake depth. The data suggest that the 9.0 m mats were structured by PAR. All layers grew at supersaturated (O₂) (Sumner et al. 2015), and the mat communities from the 9.0 m habitat likely did not experience physiologically relevant variation in (O₂) during the austral summer. The decrease in putative

photoautotrophic Cyanobacteria through the layers at 9.0 m (Fig. 2; Fig. S5, Supporting Information) can be explained by the top layer of the mat being illuminated and underlying layers being shaded. Thus, differences in community compositions (Fig. 2) and diversity (Figs 3 and 4) from the top to the bottom samples at 9.0 m can be attributed to a decline in PAR with depth into the mat.

The mats at 9.3 m had complex topography with pits and ridges, and all layers (top, middle and bottom) were exposed to lake water (Jungblut et al. 2016, Supporting Information Figure). The mat topography likely affected PAR and (O₂) exposure by shading the bottoms of pits, limiting phototrophy and oxygen production. Although PAR was not measured in the pits, some light penetrated into them, based on downward-looking photographs in which pit bottoms were visible (Jungblut et al. 2016). Thus, all three sample layers were likely exposed to at least some PAR. Oxygen gradients were measured in pits versus ridges (unpublished data), and the bottoms of pits were anoxic in contrast to the presence of 7.4 μ mol O₂/L in the water column immediately above the mat (Jungblut et al. 2016). Thus, the mat spanned the local oxycline, although specific (O₂) for each sample type is not well constrained. Even though PAR and (O₂) are poorly constrained in detail, some illumination of all layers is consistent with the presence of cyanobacteria, diatoms and anoxygenic phototrophs in all layers. Furthermore, at 9.3 m there are no statistically significant variations in photoautotrophs, specifically Cyanobacteria, among the mat layers (Fig. 2; Table S4, Supporting Information). Rather, the OTUs that vary significantly between layers at 9.3 m are putative aerobes (physiology as reported in (Finster, Liesack and Tindall 1997; Schneiker et al. 2007); Table S7, Supporting Information). These aerobes are more abundant in the top samples, which were assumed to be exposed to more O₂. Thus, (O₂) likely structured the community composition at 9.3 m, with the communities constructing both mat topography and the local redox gradient.

The film, top, middle and bottom mat layers at 9.8 m hosted similar communities to each other, although the film samples were dominated by *Phormidium pseudopristleyi*. The seasonal variability in (O₂) at 9.8 m in Lake Fryxell, as well as the proximity of the oxycline, allow for the presence of sulfide (Jungblut et al. 2016). The dominance of *P. pseudopristleyi* as a primary producer at 9.8 m suggests that it is well adapted to the low light and anoxia in this habitat. Specifically, it appears to tolerate the presence of sulfide (Jungblut et al. 2016), unlike most cyanobacteria (Cohen et al. 1986; Miller and Bebout 2004). The lack of variation in other microbial guilds at 9.8 m and the paucity of *P. pseudopristleyi* at other water depths (Fig. 3; Fig. S5, Table S7,

Supporting Information) suggest that PAR structured the communities and that the small amount of seasonal O₂ (Sumner et al. 2015) had little effect on the distribution of OTUs.

The effects of PAR on microbial mats are well documented, and many microbial mats are structured by redox cycles that depend on photosynthetic organisms (e.g. Whitton 2012 and references therein). Within this low-temperature and high latitude setting, changes in dissolved oxygen vary little daily due to small and slow changes in PAR, low absolute rates of respiration and photosynthesis and slow diffusion through benthic microbial mat layers (Hawes, Giles, Doran 2014). The microbial mats at 9.0 m and 9.8 m provide insights into the extent that PAR affects the composition and diversity of a microbial community in the absence of physiologically meaningful variations in (O₂). All layers at 9.0 m are supersaturated with oxygen, and PAR decreases from the top to bottom of the microbial mat. Similarly, layers at 9.8 m mostly lack oxygen, and PAR decreases from the top to bottom of the microbial mat. In both habitats, the decrease in the ratio of photoautotrophs to heterotrophs with depth into the mats suggests that PAR affects the microbial community by allowing photoautotrophs, specifically cyanobacteria, to dominate the illuminated top layers while heterotrophs, specifically proteobacteria, dominate the darker underlying layers (Fig. S5, Supporting Information). These results are therefore consistent with PAR structuring communities largely by influencing the distribution of photoautotrophs. This trend of cyanobacteria in top layers and proteobacteria in underlying layers is found in other microbial mat ecosystems (Kunin et al. 2008; Harris et al. 2013; Bernstein et al. 2017; Maier et al. 2018), raising the possibility that alpha diversity in microbial ecosystems is generally more dependent on the distribution of net primary producers than heterotrophs.

PAR further modulates microbial communities via the photosynthetic conversion of PAR into NPP. Most organic carbon in MDV lakes is autochthonous (Laybourn-Parry and Pearce 2007), implying a direct relationship between NPP and PAR in Lake Fryxell. In these benthic mats, PAR and alpha diversity are negatively correlated (Fig. 2), consistent with the hypothesis that microbial ecosystems in the photic zone function more efficiently with a less diverse community, one dominated by oxygenic photosynthesizers (e.g. Hurlbert and Stegen 2014).

Scale-dependent relationships between energy and diversity

We observed variable relationships between diversity, community composition, PAR and (O₂) in Lake Fryxell. Diversity decreases with decreasing energy (PAR) input at the meter-scale across the lake floor in accordance with the species-energy theory, which states that diversity increases with greater energy capture by a community (Hurlbert and Stegen 2014). However, at the millimeter-scale of the layers, the dominance of photoautotrophs reduces alpha diversity in two of the habitats (the 9.0 m top layer and the 9.8 m film and top layer), resulting in a negative correlation between PAR and diversity. Thus, across large-scale changes in PAR and (O₂) in Lake Fryxell, species energy-theory explains community composition and diversity. However, at a smaller scale, increases in diversity into mat layers may follow the maximum power principle, by which community diversity and composition shift to optimize energy consumption under prevailing biogeochemical constraints (DeLong 2008). Specifically, photoautotrophs provide the bulk of the NPP, but their abundance reduces the diversity of the top mat

layer; the absence of oxygen in deeper layers requires that anaerobic metabolisms optimise energy use. The community structure of mats in Lake Fryxell therefore reflects the optimization of energy use across small-scale PAR and (O₂) gradients as well as increased diversity in response to more energy availability across large-scale PAR and (O₂) gradients.

Chase and Leibold (2002) suggested a highly variable relationship between productivity and diversity that changes depending on how large a landscape is considered, a notion recently supported by studies of terrestrial plant communities (Grace et al. 2016). Similar to plant and animal communities, microbial mat communities in Lake Fryxell are structured in a scale-dependent manner. The differences in Lake Fryxell between meter-scale and millimeter-scale community diversity and composition support the hypothesis that integrating ecological theories at different spatial scales may best explain observed communities (Leibold and Chase 2017). Microbial mats therefore provide tractable systems within which to study the complex feedbacks among organisms and between communities and their environment.

In lakes of the MDVs generally, and Lake Fryxell specifically, seasonal changes in PAR appear to affect the trophic interactions in microbial communities, including cell counts, bacterial productivity and photoautotrophic primary productivity (Vick and Priscu 2012). Furthermore, temporal variation in environmental conditions has been found to be important to microbial community structure in other ecosystems, especially those supported by photoautotrophs. For example, chemostat experiments using Cyanobacteria isolated from the Baltic Sea found that the balance of competition and coexistence depended on the temporal variability of PAR, which varied approximately 40 $\mu\text{mol photons/m}^2\text{s}$ over the course of a week (Stomp et al. 2008). The temporal variability in PAR and (O₂) in Lake Fryxell effectively means that there is more resource heterogeneity across environments in the lake than observed at any given point in time. PAR transitions from sufficient for photosynthesis during the spring and summer to insufficient for photosynthesis during the fall and winter. Oxygen concentration declines at 9.3 and 9.8 m during fall and winter due to the reduced oxygen production of oxygenic phototrophs (Lawrence and Hendy 1985). Therefore, in any one location, the habitat experiences temporal resource heterogeneity on an annual timescale. Coexistence of taxa across PAR and (O₂) gradients in Lake Fryxell may be attributable to temporal fluctuations in those gradients on seasonal scales. The annual variation in PAR and (O₂) in Lake Fryxell also likely leads to changes in population abundances in the benthic microbial mats, as they do in the pelagic community, however, this has never been measured to due difficulties of field logistics during the Antarctic winter (McKnight et al. 2000).

Overall, in Lake Fryxell, the benthic microbial mats contain diverse taxa and they respond to the spatially heterogeneous features of their habitats, specifically PAR and (O₂), both in terms of phylogenetic diversity and relative population abundances. Alpha diversity is greatest where (O₂) is highest and PAR is absent. Differences in communities are also strongly correlated to (O₂), with greater beta diversity in oxygenated habitats and at low PAR. One OTU, *P. pseudoprysti*, dominates the film layer at 9.8 m, which suggestss that it is better suited to live in the presence of trace hydrogen sulfide near the oxycline (Jungblut et al. 2016) than the majority of other Cyanobacterial genera living in other parts of the lake. These results, based in PCR-free sequencing, more biological replicates than previously recovered, and protein-level taxonomic characterization, support the patterns discovered in recent amplicon-based surveys

(Jungblut et al. 2016). Where (O_2) varies minimally in Lake Fryxell, either where it saturates the microbial mats (9.0 m) or where it is absent (middle and bottom layers at 9.8 m), PAR structures the overall microbial community from top to bottom. But where (O_2) varies spatially, the microbial community is structured by (O_2) . The main source of energy in Lake Fryxell is PAR, but (O_2) influences phylogenetic diversity and taxonomic composition to a greater degree. Additional environmental parameters likely contribute to the taxonomic and phylogenetic patterns seen here. Future direct measurement of the O_2 and H_2S gradients, as well as pH and redox state within the mats, in addition to the collection of mat cores for nutrient analyzes would allow for better models of the factors driving the microbial populations. Even so, the response of microbial mat communities to their geochemical environment is clearly scale-dependent, as is the case in plant and animal communities and microbial ecosystems can provide important test cases for ecological theories.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](#) online.

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