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Growth dynamics of a laminated microbial mat in response to variable irradiance in an Antarctic lake.

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Summary

1. Laminated microbial mats are important ecosystem components of perennially ice-covered Antarctic dry valley lakes. In order to understand better their response to changing environment, we made observations and carried out a manipulation experiment to determine their response to variations in irradiance in Lake Hoare (77°38' S, 162°53' E).

2. Ice transparency was the most variable parameter that affected benthic light dose, both spatially and between years. Patterns of lamina accrual corresponded to irradiance history, with laminae that were initiated in high transmission years thicker than those from low transmission years.

3. A shading experiment confirmed that accrual of lamina thickness, calcite precipitation and ash-free dry mass were determined by irradiance, but photosynthetic biomass and phototrophic species composition were less affected.

4. Buried laminae decomposed only slowly over time, with potentially viable phototrophs many laminae down into the microbial mat. Decay rate increased only slightly with shading.

5. We conclude that the microbial mats in Lake Hoare are characterised by remarkable stability, with slow accumulation rates and turnover of biomass over time. Photosynthetic biomass and species composition appeared to be stable across long time periods, with inter-annual variation in lamination pattern due to differential accumulation of extracellular polysaccharide and representing the visible expression of annual growth conditions.
Introduction

The McMurdo Dry Valleys (MDV) of Southern Victoria Land, Antarctica experience one of the coldest and driest climates on Earth, with mean annual valley bottom temperatures ranging from -14.8°C to -30.0°C and with < 100 mm yr\(^{-1}\) of precipitation (Doran et al., 2002; Fountain et al., 2010). Within this desert are a number of perennially ice-covered lakes that act as “oases”, supporting rich microbial communities (Wharton Parker & Simmons, 1983; Priscu et al., 1999; Roberts et al., 2004). One of the biggest effects of perennial ice cover on lakes is the interception of solar energy. Reflection and attenuation of light by and within the ice cover typically results in only a few percent of the irradiance that reaches the ice surface penetrating to the underlying water column (Howard-Williams et al., 1998). The degree of attenuation, however, varies both spatially and temporally. The optical properties of the ice change over the course of the austral summer (Howard-Williams et al., 1998) and Quesada et al., (2008) assembled data to show inter-annual variation in the transmission of the ice cover of Lake Hoare, one of the MDV lakes, from 0.1 to 2.5% of surface incident. More recently, an order of magnitude spatial variability of light penetration has been documented in Lake Joyce (Hawes et al., 2011) and the west lobe of Lake Bonney (Obryk, Doran & Priscu, 2014), and these authors note that this heterogeneity propagates to depths of at least ten metres. Such variability, in a severely light limited environment (Priscu et al., 1999; Hawes et al., 2001; Moorhead Schmeling & Hawes, 2005), can be expected to lead to substantial changes in community photosynthesis and potential for growth over time. For example, Doran et al., (2002) related the gradual reduction in planktonic photosynthesis over more than a decade in Lake Bonney to a corresponding increase in ice thickness and reduction in irradiance.

Variability in irradiance regimes in MDV lakes is not restricted to ice properties. In recent years, climate amelioration has resulted in increased volumetric inflow (Fountain et al., 2014) which currently exceeds volumetric outflow in the form of ablation and evaporation (the lakes mostly occupy closed basins and have no outlet), resulting in a gradual increase in level. Documented impacts on communities mediated through increasing depth of the water column include loss of deep growing photosynthetic organisms (Hawes et al., 2011), and abrupt increases in meltwater...
delivery can both decrease and increase planktonic production, through impacts on water turbidity and enhancement of nutrient concentrations (Foreman, Wolf & Priscu 2004).

As enhanced monitoring systems deliver more information on environmental variability, it is increasingly clear that an understanding of how ecosystems integrate, accommodate and respond to variability in key climatic drivers is necessary, particularly with the current focus on the effects of directional, climate-related change. In the case of polar desert systems, where hydrological regime drives the ecological systems (Foreman et al., 2004; Fountain et al., 2014) such changes will be amplified. In this study we use a combination of multi-year observation and manipulation experiments to examine how variability in irradiance regime affects primary producers in MDV lakes. While the best known of the primary production pathways in the MDV lakes is the phytoplankton (Priscu et al., 1999; Laybourn-Parry & Pearce, 2007), these have a limited ability to record change. In contrast, the microbial mats are less well known, but are conspicuous contributors to diversity and productivity in the euphotic zones of these lakes, are perennial and contain laminations that record annual net accumulation (Hawes & Schwarz 1999; Hawes et al., 2001; 2013; 2014; Quesada et al. 2008). Furthermore, there is evidence that irradiance directly limits the rate of photosynthesis in the mat communities (Vopel & Hawes 2006, Hawes, Giles & Doran, 2014), and hence their potential for growth. The limited information currently available suggests that these mats may be responsible for 50% or more of the carbon sequestration, on a lake-wide basis, through accumulation of organic carbon (Moorhead et al., 2005) and precipitation of carbonates (Sutherland & Hawes 2009). We therefore elected to examine the response of benthic communities in Lake Hoare to variable irradiance as a measure of community response to changing resource availability.

Quesada et al. (2008) argued that responses to environmental variability in Antarctic lake benthic communities would vary from physiological responses to short-term variability, through biomass accrual, to community composition with lengthening time scale of change. They suggested that year-to-year environmental variations are most likely to result in changes to productivity and biomass. We therefore focused our investigations around the hypotheses that biological responses to variable irradiance in these energy-limited environments are such that short-term
(year-by-year) fluctuations are accommodated by physiological and growth-related change in the mats that are recorded by each year’s growth performance. We used repeat sampling at a fixed location in Lake Hoare to examine the development of microbial mats over time. We supplemented these temporal observations with an experimental manipulation of light at the site, and combined these data with measurements of lake level, ice thickness and irradiance in order to determine how fluctuating irradiance affects the accrual of benthic mats and their biochemical characteristics over time.

Methods

Study site.

Lake Hoare (77°38’ S, 162°53’ E) is a closed-basin lake near the eastern end of Taylor Valley in southern Victoria Land, Antarctica (Fig. 1). The Lake is 4.2 km long, 1.0 km wide and has maximum and mean depths of 34 and 14 m (Spigel & Priscu, 1998). It is dammed to the north-east by the Canada Glacier, which provides an inflow of glacial meltwater (Wharton et al., 1992). Other sources of inflow come from Andersen Creek entering the north-east corner of Lake Hoare, and drainage from Lake Chad in the south-east. No surface outflows from Lake Hoare exist, and water loss is restricted to sublimation of ice and evaporation of melt water during summer (Doran, Wharton & Lyons, 1994), although recent geophysical evidence suggests the possibility of groundwater drainage towards the coast (Mikucki et al 2015). The ice cover of Lake Hoare is perennial, except for small areas at the lake margins, which melt during summer. The ice cover was 3.5 m thick in 1983 (Wharton et al., 1992) and increased to ~5 m thick by the end of the 20th century (Doran et al., 2002). There are 3 months of complete darkness during the austral winter and 3 months of continuous light during summer (Dana, Wharton & Dubaya, 1998). Howard-Williams et al. (1998 showed that the net transmission of solar radiation to the bottom of the ice cover ranged from <1 – 3 %, with a spectral transmission peak at wave lengths between 450 – 550 nm. Vertical extinction coefficients (Kd) for PAR within the water column from beneath the ice to a depth of 33 m were typically 0.12 – 0.22 m⁻¹ (Howard-Williams et al., 1998).
Lake Hoare shows weak density stratification; the concentrations of dissolved ions increase with depth, while temperature decreases slightly. Although there is controversy over the extent of mixing of surface water deeper into the water column (Spigel & Priscu 1998, Tyler et al., 1998), the density gradient is continuous to at least 13 m depth, and transport of solutes is likely to be slow. There is a pronounced inflection in the density-depth profile at 13 – 15 m from the surface (the depth of the lake varies temporally), which divides the lake into upper and lower compartments. The upper compartment is characterized by lower concentrations of dissolved nutrients, particularly nitrate (Lizotte & Priscu 1992), and higher concentrations of dissolved oxygen (0.94 – 1.25 × 10^{-3} mol L^{-1} compared to 0.63 – 0.94 × 10^{-3} mol L^{-1}) (Wharton et al., 1986). Upper waters contain lower bicarbonate and have a higher pH (up to 8.6; Cathey et al., 1981) than the lower compartment (pH 7.9). The lake is anoxic below 25 – 26 m.

**Microbial mats**

Benthic microbial mats, comprised primarily of cyanobacteria, diatoms and bacteria, line the lake from the wetted edge into the anoxic zone. Mats differ in their structure, which is driven by surface micro-topography and the quantity and quality of the incident irradiance (Wharton et al., 1983). Two benthic mat morphologies predominate under ice in Lake Hoare: columnar lift-off and prostrate mats. Gas ebullition within lift-off mats makes them buoyant; hence they lift away from the underlying sediment until they rise up and freeze into the ice. Prostrate mats at approximately 10 m depth are the focus of this study; they are persistent as they occur at depths below those where large-scale gas ebullition and lift-off occurs, and tend to have smooth surfaces suitable for quantitative sampling, though surface irregularities in the form of pinnacles up to 3 cm high, can occur (Wharton et al., 1986).

The matrices of the mats are dominated by narrow, filamentous cyanobacteria, attributable on a morphological basis to *Leptolyngbya antarctica* (West & West) Anagnostidis & Komárek. *Leptolyngbya fragilis* (Gomont) Anagnostidis & Komárek., *Pseudanabaena frigida* (Fritsch) Anagnostidis and *Leptolygbya angustissima* (West & West) Anagnostidis & Komárek (Wharton et al., 1983; Sutherland and Hawes, 2009). Recent molecular analysis of Lake Hoare mats has provided a similar suite of
dominant cyanobacteria (Zhang et al., 2015). Diatoms comprise only 10–15% of the mat community, though this increases at depth. Sixteen taxa of diatoms have been recognised, with *Psammothidium chlidanos* (Horn & Hellerman) Lange-Bertalot, *Diadesmis contenta* var. *parallela* Petersen and *Navicula gregaria* Donkin being most frequent (Sutherland & Hawes, 2009).

Microbial mats in Lake Hoare are adjusted to low irradiance through efficient light harvesting, and utilization, and compensation points of <1 μmol photons m\(^{-2}\) s\(^{-1}\) allow net production to occur to >20 m depth in this lake (Hawes & Schwarz, 1999, 2000; Vopel & Hawes, 2006). Consumer organisms are limited to protozoa, rotifers, tardigrades and nematodes, and macro-invertebrates are absent (Cathey et al., 1981; Parker et al., 1981). Mats are laminated on millimetre scales (Wharton et al., 1983) and these horizontal laminations represent annual growth layers (Hawes et al., 2001). Hawes & Schwarz (1999) recognized an ‘active layer’ comprising the upper three to nine laminae as containing all of the photosynthetically active components of the microbial mat, and this has been confirmed experimentally (Vopel & Hawes, 2006; Hawes et al., 2014). Areal photosynthetic rates appear to be almost linearly related to irradiance under ambient conditions (Vopel & Hawes, 2006; Hawes et al., 2014).

**Irradiance, lake level and ice thickness**

Incident irradiance at the Lake Hoare site is monitored continuously using a LiCor Li190 cosine-corrected photosynthetically active radiation (PAR) sensor recording onto a CR10X datalogger (Campbell Scientific, Logan, USA). This monitoring is part of a comprehensive network operated by the McMurdo Dry Valleys Long Term Ecological Research programme (MDV LTER) and is fully described and available at [http://www.mcmlter.org](http://www.mcmlter.org). Sub-ice irradiance is monitored at 15 minute intervals by the MDV LTER using a LI194 spherical PAR sensor (Li-Cor, Lincoln, USA) moored at 10 m depth, equipped with a pneumatic cleaning device and connected to a surface datalogger. Underwater PAR data are corrected for changes in lake level, using a measured extinction coefficient calculated each season. Data and metadata are also available at [http://www.mcmlter.org](http://www.mcmlter.org). From these data we calculated summer irradiance dose for each annual growth season (September to March) for incident and under-ice irradiance.
We also carried out synoptic surveys of spatial variability of ice transparency. A diver using surface-supplied air swam a series of transects from a ~1 m diameter dive hole melted through the lake ice. The diver carried a Li-Cor LI1400 data logger, in a waterproof housing, connected via a 1 m cable to a LI192 cosine corrected underwater PAR sensor. At horizontal intervals of approximately 3 m the diver held the sensor to the underside of the ice and noted the irradiance. A simultaneous record of incident irradiance was made using a Li 190 PAR sensor connected to a second meter, and percent transmission was calculated.

To determine whether PAR spatial variability at the ice-water interface propagates to the microbial mat surface, in December 2010 a diver followed a transect from shallow to deep, along the lake bottom, and obtained a profile of irradiance vs depth by recording depth and irradiance at 5 m horizontal intervals. From the depth-specific data, a vertical attenuation coefficient for downwelling irradiance was calculated by log-linear regression, according to Kirk (1994). Deviations about this relationship were interpreted as due to differences in ice transmission overhead the sampling point. The attenuation rate of irradiance through the water column, was determined by log-linear regression of % irradiance against depth.

Lake level and ice thickness are monitored annually by the MDV LTER and data are available from [http://www.mcmlter.org](http://www.mcmlter.org).

**Microbial mat sampling**

Microbial mats were sampled in November-December of 2002, 2004, 2006, 2008 and 2010 by divers operating through a ~1 m diameter hole melted through the ice, in the same location every year. All mats sampled fell into the aerobic prostrate mat category (*sensu* Wharton *et al.*, 1983). Hawes & Schwarz (1999) showed that the upper part of the mats was cohesive and could be lifted away from underlying, less cohesive material. They termed this the “active layer” and noted that it contained upper pigmented and lower unpigmented zones, and that together the pigmented and unpigmented zones contained 10-20 accumulated annual growth layers, depending on sample depth (Fig.2). Our sampling targeted potentially photosynthetic biomass, and was therefore confined to the active layer. The diver cut a haphazard series of 10 cm diameter core samples of this layer from within a 3 m horizontal by 1 m vertical
sampling area, at 10 m depth. These were carefully separated from underlying materials using a spatula, transferred into an opaque polyethylene box, and the lid of the box was closed to make a watertight seal. These samples were returned to the ice surface, where they were wrapped in black cloth to protect them from excess irradiance, placed into insulated boxes and immediately transferred to a lakeside laboratory. Due to logistic restrictions, only one site was sampled at each occasion.

On return to the lakeside laboratory, 3-4 mm wide vertical sections of mat were cut using a razor and laid on their sides in lake water in plastic petri dishes. Measurements were made of laminations and digital photographs taken as permanent records. Three replicate mat photographs from each year were imported into Image–J (http://imagej.nih.gov/ij) and the thickness of each lamina was measured after calibration against a photographed scale bar. Three sets of lamina measurements were averaged from each cross section, and these averages used to compare between years.

Samples were taken using a 47 mm diameter corer, and frozen for return to New Zealand, where they were freeze-dried and gently ground to a fine powder. Weighed aliquots were then taken for analysis of chlorophyll-a (chl-a) and phycobilin pigments. Chl a, was extracted in 4°C, 90% acetone, in a high-speed Teflon grinder (30 s). Absorption of extracts was measured at 665 nm, and correction for phaeophytin was by acidification with 0.1 N HCl after Marker et al. (1980).

Phycobilins were extracted by sonication of aliquots (20 W for 30 s on a 50% duty cycle in an ice-water bath) in 0.1 M Tris buffer. We determined the concentrations of phycoerythrin (Pe) in extracts by means of fluorometry using an excitation/emission wavelength combination of 430/550 nm and a calibration curve constructed from pure pigment (Downes & Hall, 1998).

Further aliquots were taken to determine the proportion of carbonate and organic material in freeze-dried mat material. Aliquots of freeze-dried microbial mat were weighed into 2-mL plastic centrifuge tubes and excess 10% HCl was added to displace carbonates. Acidified samples were then centrifuged, rinsed three times in distilled water to remove the resulting chlorides, re-dried and re-weighed. Weight loss, termed loss on acidification (LOAc) was taken to indicate the carbonate content of the original sample. In 2002, 2004, 2006 and 2008, residues were then carefully transferred to crucibles, combusted at 450 °C for 4 h, cooled in desiccators and the
organic matter was estimated as ash free dry mass (AFDM) (Sutherland & Hawes, 2009). Results were converted to mg cm\(^{-2}\) using the total sample and aliquot weights.

**Manipulation experiment**

In order to examine the specific relationship between irradiance and microbial mat development, a relatively flat and homogenous area of lake bottom was selected at 10 m depth in December 2008 and three shades, each of 1 m\(^2\), were placed over mats removing 50% of incident light. Shades were suspended 20 cm above the lake bottom. Shade location was selected at random and control areas were identified close by. While shades initially reduced incident irradiance by 50%, this increased to 80% over the course of the two year deployment due to growth of cyanobacteria and accumulation of fine sediment on the shade screens, though at an unknown rate. Because of this, great care is taken in interpreting experimental results.

Two years after shades were placed over the mats, samples of active layer from the three replicate treatments and controls were collected (three samples from each of the replicates), as described above. On return to the laboratory, each sample was split horizontally into three parts based on colour. In all samples, two differently pigmented layers, an orange-brown upper layer and a pink middle layer were recognized, underlain by a colourless/beige lower layer (Hawes et al., 2014; Fig. 2). Each layer contained several laminae (Fig. 2). Samples were split on the basis of removing the orange-brown layers and then separating the pink layers at the conspicuous sediment horizon indicated in Fig. 2. Where there was no clear distinction between the upper (orange-brown) and middle (pink) layers in specific samples, the cut was made after following laminae through the mat to the nearest location where a clear transition was evident. Samples were frozen and returned to NZ, freeze-dried, ground to a fine powder, weighed and aliquots taken for measurements of Chl-\(\alpha\), Pe and Pc, acid soluble and LOI, as described above.

In addition to bulk pigment analyses, three acetone extracts were randomly selected from each layer of each treatment and transferred to sealed glass vials and stored at -80\(^\circ\)C under nitrogen for up to 3 months before a more detailed analysis of pigments was undertaken by High Performance Liquid Chromatography (HPLC). A Dionex HPLC system, with PDA-100 diode array detector (300-800 nm), separated...
pigments according to the chromatographic method of Zapata, Rodriguez & Garrido (2000). Pigments were quantified at 436 nm by reference to commercially available standards. Full details are given in Hawes et al., (2011).

Samples were also examined using a Leitz microscope at up to 1000x magnification at a lakeside laboratory, and dominant diatom and cyanobacterial morphotypes identified as described by Sutherland & Hawes (2009). To supplement morphometric descriptions of cyanobacteria, representative samples of each layer from the two treatments were taken and frozen (-20°C) for return to the UK for molecular analysis. Molecular analysis was primarily through the production and characterization of clone libraries. Samples of microbial mat from 10 m lake depth were vertically divided into three horizontally split sections, again based on pigmentation (Fig. 2), for 16S rRNA gene sequence analysis. PCR amplification of cyanobacterial 16S rRNA gene was performed using the well-established cyanobacteria-specific primers 27F and 809R, 1 unit Platinum Taq DNA Polymerase High Fidelity (Life Technologies, Warrington, UK) in a 20 µL reaction as described in Jungblut, Lovejoy & Vincent. (2010). PCR products, of approximately 750 bp length, were purified (Quiagen, Hilden, Germany) and cloned using the Stragegene clone kit (Stragagene, La Jolla, USA) following the manufacturer’s instructions. Sequencing was carried using the vector-specific T7 universal primer (single read) at the Natural History Museum sequencing facility using an Applied Biosystems 3730xl DNA analyser (Applied Biosystems, Waltham, USA). Sequences are available under accession numbers shown in Table S3.

PAM fluorometry
We used Imaging Pulse Amplitude Modulated (PAM) fluorometry to assess the distribution of photosynthetic capacity within samples from the two treatments in the manipulation experiment. Imaging PAM fluorometry was applied to vertical sections of microbial mat to obtain 2-dimensional identification of where pigment systems were present and where photosystem II (PSII) was active (c.f. Vopel & Hawes, 2006; Hawes et al., 2011).

A Walz Imaging PAM fluorometer (Walz Mess- und Regeltechnik, Effeltrich, Germany), fitted with a lens imaging an area of 30 x 23 mm, was used throughout.

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The saturation pulse method was used to determine the minimum ($F_o$) and maximum ($F_m$) fluorescence yields of dark-adapted samples to a pulsed blue light. For a detailed description of the saturation pulse method see Schreiber et al., (1996), and for the use of spatially resolved PAM imaging see Grunwald & Kühl (2004) and Ralph et al., (2005), and for application to similar Antarctic microbial mats, Vopel & Hawes (2006) and Hawes et al. (2011).

The Imaging PAM was used to derive two parameters. Firstly, $F_o$ is a direct measure of fluorescence yield of photosystem II of the imaged target. It integrates to some extent to the depth to which measuring light penetrates (e.g. Kühl et al., 2001) and as such is biased towards pigment arrays close to the surface. The returned value of $F_o$ is also sensitive to changing excitation intensity, but by standardising the settings of the instrument and the focal distance, some degree of cross-image continuity in false colour:fluorescence yield was maintained across samples. The imaging PAM measures $F_o$ as the fluorescence yield in response to a series of high frequency, low amplitude pulses. The second parameter $\phi_{II}$, which provides an index of quantum yield of PSII, is determined during application of a 0.6 s bright saturating pulse, at the end of which the maximum fluorescence yield ($F_m$) is measured. $\phi_{II}$ is then derived as ($F_m - F_o$)/$F_m$, a dimensionless ratio and can be directly compared between images. The Imaging PAM generates false-colour images that describe these fluororescence properties in two dimensions. Three types of false-colour fluorescence images were used here to characterise each sample, $F_o$, and $\phi_{II}$ together with a composite image that shows both $\phi_{II}$ as a false colour, and $F_m$ as the intensity of colour. In addition, profiles of $F_o$ and $\phi_{II}$ with depth into the mat vertical sections were obtained from three replicate images for each treatment using the analysis package in the Imaging-PAM software.

**Statistical analyses**

Differences between quantitative metrices were determined using Analysis of Variance (ANOVA), within the Sigmaplot 13 package (Systat Software, San Jose, USA). Multivariate analyses of HPLC pigment data were undertaken after standardisation to total pigment content and square-root transformation, using the multidimensional scaling (MDS) component of Primer-E 6 (Primer-E, Ivybridge).
The BEST routine in Primer-E was used to identify the pigments most significant to the MDS structure.

In analyzing the molecular data generated from clone libraries, diversity indices and unweighted Unifrac pair-wise significance tests were used to determine significance of differences between samples, and these were calculated using 100-permutations and Bonferroni correction using MOTHUR (Schloss et al., 2009).

Results

Ice thickness, lake level and irradiance

Since 2001, the ice on Lake Hoare has thinned by 1.7 m from 5.1 to 3.4 m (Table 1). Much of the loss of ice thickness occurred between 2000 and 2002. After 2002 ice thinned more gradually to 2008, and since then has increased slightly. Accompanying the thinning of ice cover has been an increase in lake level. Between 2001 and 2002, which coincided with a major flood event in the catchment (Doran et al., 2008), lake level rose by 0.5 m and in the second half of the decade rose by a further 0.4 m (Fig. 3).

There was little year-to-year variation in the amount of PAR incident to the surface of the lake (Fig. 4a); annual light dose varied between years by less than 10% of the median with no obvious trend over time. However, PAR reaching 10 m depth was considerably more variable (Fig. 4b). Under-ice PAR data are incomplete, due to failed sensors, but the data suggest that irradiance at 10 m varied more than five-fold, from ~0.5 to 2.5% surface, with the brightest recorded year being 2002-03, followed by 2008-09. The irradiance reaching 10 m appears to have been low during the middle years of the decade.

However, these observations are limited to a single site on the lake and we found that transmission through ice varied considerably over short distances, with ranges about the median typically approaching 100% (Table 1). In 2000, we repeated measures after a light snowfall, which showed a temporary 70% decline in mean transmission and an increase in variability, though snow rarely persists for long periods on this lake. ANOVA indicated that differences were significant, and a post-hoc Holm-Sidak procedure indicated a transmission minimum in the middle of the decade and 2002-03 year as the clearest.
In 2010, spatial variation in irradiance, exemplified by deviation from a simple exponential decline in irradiance with depth, propagated to at least 20 m depth (Fig. 5). The attenuation coefficient of downwelling irradiance for these data was estimated as 0.15 m$^{-1}$ by log-linear regression ($r^2 = 0.78$).

**Microbial Mat Laminations**

Cross sections of microbial mats taken from the same location at ~10 m depth in Lake Hoare in November of 2004, 2006, 2008 and 2010 showed the development of annual laminations. Fig. 6a aligns these cross sections using a natural marker provided by the influx of fine sediments that accompanied a pulse of large water influx in 2001-2002 season (Foreman et al., 2004). A consistent pattern was evident of a relatively thin translucent lamina at the surface if the mat, in the process of being added, overlying two to three orange-brown and four to six pink-purple laminae, then innumerable colourless laminae. Two laminae were added above the marker sediment horizon by 2004, increasing to four by 2006 etc, with the number of laminae indicating annual accrual. Comparison of specific laminae in successive samplings suggested that they tend to continue to thicken for one, occasionally two, years after inception, thereafter thickness begins to decline (Fig. 6b). The two laminae immediately above the 2001-2002 season marker layer, laid down in 2003 and 2004, had decreased in thickness by 45 and 40% by 2010.

Laminae were not of equal thickness between years. For example, the two laminae evident below the surface layer in 2004 were considerably thicker (2.6 mm each) than the two below the surface in 2006 and 2008 (2.2 mm each). In 2010 sub-surface laminae were again rather thick (2.8 and 3.2 mm) and, while uncertainties about irradiance at the collection sites due to spatial variability preclude quantification of the effect of irradiance, this pattern is consistent with the trend for the middle period of the 2000’s decade having lower ice transparency than at either end.

The effect of >50% shading on the development of laminae was to substantially reduce vertical growth where the shades were applied (Fig. 7 a-c). Cross sections show that neither the two upper orange-brown laminae characteristic of mats at this depth were evident under shading, despite the fact that they were present when shades were applied in 2008. Quantification showed that buried as well as surface
laminae were thinner under shades than controls (Fig. 7d) Observation of the shaded areas by SCUBA divers immediately after the removal of the shades confirmed that their surfaces were overall purple while the unshaded control areas were orange-brown.

**Pigments.**

Inter-annual variation in chlorophyll-a at 10 m depth suggests a chlorophyll minimum during the middle of the decade (Table 1). Broad variance prevented significant differences being identified using ANOVA, though the power of the test was low (F = 2.84; p = 0.051; power = 0.46) resulting in a high probability of ANOVA failing to identify ecologically significant differences.

The manipulation experiments, however, also indicated that pigments respond little to light variability, the chlorophyll-a and phycoerythrin contents of mats from under the shade treatments (Table 2) not being significantly different from control values, for the upper (orange-brown) or lower (pink) zones. MDS of HPLC data indicated that all samples showed a high degree of similarity (>70%), but separated the samples according to their treatment, with all except the shaded pink zones showing >80% similarity within replicates (Supporting Information Fig. S1). BEST analysis suggested that the separation of the orange-brown and pink zones in the control samples was affected by higher amounts of the breakdown products chlorophyllide-a and phaeophorbide-a in the pink layer, and there was no evidence of a higher concentration of diatom-specific pigments in the orange-brown layer. The effect of shading on the orange-brown layer was an increase in the amount of phaeophytin-a relative to the control orange layer, while the control and shaded pink layers grouped together. The shaded orange-brown layer did not converge on the pink layers (Supporting Information Fig. S1).

**Acid soluble and ash-free dry weights**

Unlike pigment concentrations, significant differences between controls and shaded treatments were seen for both acid-soluble and ash-free dry weight (ANOVA p>0.05) in the manipulation experiment. Acid-soluble weight was significantly lower in the upper layers of shaded treatments than in controls, though not in the pink layer (Table...
The ash-free dry weight was significantly lower in both layers in the shade treatment than in controls, but there were no significant differences in the residual (ash) weight.

**Microbial mat assemblage**

We also divided the mats into orange-brown, pink and beige layers for comparison of microbial assemblage composition. By field microscopy, twelve cyanobacteria morphotypes were recognized (Table S1). *Leptolyngbya* was the dominant genus in all of the three layers, and five morphotypes were attributable to this genus, one of which was consistent with *L. antarctica*. *Phormidium* was the second most frequent genus, while *Pseudanabaena* was also detected, together with some coccoid unicellular cyanobacteria. The orange and purple layers had a similar diversity, and the abundance of the individual morphotypes between the two was very similar. The diversity in the beige layer underlying the pink was less than the upper layers, with only two *Leptolyngbya* morphotypes, two *Phormidium* and one *Chroococcales* morphotype (Supporting Information Table S1). The majority of biomass in the beige layer appeared to comprise of empty sheath material. No difference was apparent between the morphotypes present in the shaded and unshaded material. Diatom frustules were seen in all layers, though intact cells were most abundant in the orange-brown layer, with *Luticola muticopsis* and *Navicula gregaria* co-dominant.

Based on 16S rRNA cyanobacteria clone libraries, the orange-pigmented mat layer had the highest operational taxonomic unit (OTU) richness with 7 OTUs followed by the pink (6 OTUs) and beige (4 OTUs) (Table S2). Diversity indices also suggested a higher OTU diversity than detected by clone library surveys. All OTUs had BLASTn highest matches to similarities of ≥99% to uncultured cyanobacterial sequences from Antarctica or other permanently cold environments, and matched with ≥ 97% to cultured representative of the oscillatorian genera *Leptolyngbya*, *Phormidium*, *Limnothrix*, together with *Chaemosiphon* belonging to the unicellular order Chroococcales (Table S3). The 16S rRNA gene sequences grouping with *Leptolyngbya* and *Phormidium* were found in all clone libraries. OTUs with highest BLASTn match to *Leptolyngbya* (*Leptolyngbya antarctica*) dominated all three clone
libraries. OTU 8 and 2 had highest BLASTn match to cultured representatives of the
genus *Phormidium*, and were also found in all clone libraries, whereas OTU9
(*Phormidium priestleyi*) was only found in the orange layer. OTU7 (98% *Limnothrix*
*redekei* CCAP 1443/1) was found in orange and beige layer, and OTU4 and 5
(*Chaemosiphon* spp.) only in the purple layer (Fig. S2). Both unweighted Unifrac
pair-wise test and Libshuff suggested that 16S rRNA gene cyanobacterial
communities were not significantly different in the orange, pink and beige zones of
the microbial mats.

Both molecular analysis and microscopy suggested that *Leptolyngbya* and
*Phormidium* were the most abundance genera in the three differently coloured mat
layers. The molecular diversity was lower than the morphotype diversity, likely due to
incomplete sampling of the cyanobacterial composition in clone libraries. Both
methods detected a decrease in richness with depth into the mat. The composition in
the three layers was overall similar, and further sampling is needed to exclude that
apparent differences were due to incomplete sampling of the composition.

**Variable chlorophyll fluorescence.**

Images of variable fluorescence from control and shaded treatments in the
manipulation experiment show consistent differences (Fig. 8, Fig. S3). The control
section showed a broader band of fluorescence, extending to more than 15 laminae,
with a slight decline in fluorescence intensity into the mat. The upper two laminae had
a lower $\phi_{II}$ than middle laminae, while $\phi_{II}$ declined from approximately lamina 8
downwards. The effects of shade include (i) a thinner overall fluorescent thickness,
which is consistent with the absence of lamina accrual at the surface, (ii) a tendency
for $F_{m}$ to show a pronounced surface maximum, declining with depth into the mat,
and (iii) a narrowing of the band of high $\phi_{II}$ and positioning of this band at the mat
surface (Figs S3, S4).

**Discussion**

A major motivation of this research was to determine how variability in irradiance
affects carbon accrual of microbial mats in a perennially ice covered Antarctic lake.
Underlying this objective was the expectation that the gradual rise in lake water level

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Currently experienced in all lakes in this region (Bomblies, McKnight & Andrews, 2001; Hawes et al., 2013) would steadily impact on benthic communities, as inferred in nearby Lake Joyce (Hawes et al., 2011), through a gradual reduction in light as levels rise and water columns deepen. In fact, our results confirm previous findings that such a relationship is compounded by other variables that affect the irradiance reaching the lake floor, in particular ice transparency (Quesada et al., 2008). While incident irradiance varies by only 10% between years, the transmission of light through the ice cover shows a 400% variability, both between years and within years on a spatial basis. Neither between nor within years was this variability in ice clarity linked to ice thickness, and in previous studies the importance of the albedo and sediment burden of the upper part of the ice layer on transmission, rather than thickness per se, has been emphasised (McKay, Andersen & Wharton, 1994; Obryk et al., 2014).

This is the first time that the depth of propagation of the patchiness of lake ice light transmission has been measured in Lake Hoare, and it is clear that it extends sufficiently deep to create several-fold spatial variability to at least 20 m. A similar finding has been recorded in nearby Lake Joyce, though to lesser depth (Hawes et al., 2011). While this patchiness in irradiance in Antarctic dry valley lakes is particularly relevant to the spatially fixed phytobenthos, where it means that depth alone is not a reliable indicator of irradiance, it will also have implications in modelling of phytoplankton productivity, which to date has not accommodated spatial variability (eg Priscu et al., 1999).

While ice properties are the dominant determinants of PAR transmissivity, the combined effect of the 2.4 m thinning of ice and 0.9 m rise in lake level over the decade also resulted in a net 3.3 m increase in the thickness of the layer of water overlying mats. This is likely to have reduced irradiance reaching the lake floor. At an average attenuation rate for downwelling irradiance of 0.15 m$^{-1}$ for Lake Hoare, seen in our data and previously (Howard-Williams et al., 1998), such an increase could have led to a decrease in irradiance by up to 40%. This calculation is an overestimate, as it assumes that the “missing” ice would not have absorbed any light, which is not the case. However, McKay et al. (1994) stressed that optically dense ice is confined to the upper 1-2 m of the ice column in Lake Hoare and the lower layers are exceptionally clear. Since ice thinning is associated with loss of clear bottom ice,
increasing water column height will have reduced irradiance to the lake floor by
several tens of percent. However, given the multi-fold spatial and year-to-year
variability in ice transparency and the propagation of such effects to considerable
depth in the lake, the irradiance that is experienced by microbial mats growing on the
lake floor appears to be affected more by the clarity of the immediately overlying ice
than by the <40% reduction associated with the increasing lake depth. This estimate is
sensitive to variability in the turbidity and hence attenuation properties of the water
column, which does occur (Howard-Williams et al., 1998).

Within the region from which microbial mats were sampled, changes in
average ice transparency correspond to the accrual of thickness in annual laminations.
Thinnest lamina accrual was seen in the middle of the decade, when the ice was least
transparent and maximum at the start and end when ice was relatively clear. That
laminae continuously accrued throughout the study period, even when light
penetrating the ice averaged <1% incident, attests to the capacity of these mats to
grow and accumulate at extremely low irradiance (Vopel & Hawes, 2006; Hawes et
al., 2014). Our inability to precisely link mat lamina thickness with the light climate
experienced at that location, due to spatial variability, precludes a correlation
approach. However, the conclusion that reduction in light results in a decrease in
carbon accrual is supported by the mechanistic explanation that emerges from other
measurements.

Experimental reduction in irradiance reduced both the thickness and the
AFDM, but not the LOAc, of deeper laminae. An increase in the net consumption of
organic carbon in these zones is perhaps more likely to have been due to a decrease in
gross photosynthesis than an increase in respiration, and this is consistent with the
observations of (a) a slight increase in lamina thickness up to 2 years after burial and
(b) a gradual thinning of older laminae over many years under unshaded conditions.
That new laminae forming in shaded mats were thinner, with less organic carbon and
less precipitation of calcite are consistent with populations where light limits
photosynthetic carbon accrual (Vopel & Hawes, 2006; Hawes et al., 2014). One of the
unexpected results from our manipulation experiment was that, while vertical growth
and accrual of ash free dry mass declined under shading, there was no significant loss
of either chlorophyll or phycoerythrin from the two pigmented layers. Moreover,
variable fluorescence images suggest that these layers retained their photosynthetic

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capacity. This implies either that similar abundances of pigmented, active cells remained in the upper lamina after 2 years of shading, albeit concentrated in a thin lamina, or that residual cells contained higher pigment complements.

The persistence of potentially photosynthetically active cells deep into mats (in laminae that were formed up to eight years ago) also implies an ability of these organisms to tolerate prolonged periods of adverse growth conditions, perhaps in vegetative form. Persistence of cyanobacteria requires that they are not consumed by grazers, lysed by viruses or succumb to autolysis or asymmetric division (Franklin, 2013). The absence of macroscopic grazing animals from the MDV lakes (Cathey et al. 1981) is consistent with low grazing pressure, though the feeding habits of the tardigrades, nematodes and protists that are present are not known. Viruses are, however, known from the water columns of MDV lakes (Säwström et al., 2008) and have been reported from Arctic mat communities (Varin et al., 2010) and Phormidium-dominated microbial mats in other regions (Voorhies et al., 2015). It must be expected that viruses and microinvertebrates play a role in cyanobacterial turnover in Lake Hoare, though the apparent longevity of cyanobacteria suggests that this may be a minor effect. Autolysis under nutrient stress is also increasingly recognised in cyanobacteria, and has been compared to programmed cell death, where individual death favours population survival by releasing nutrients to survivors (Berman-Frank et al., 2004). Likewise, cyanobacterial mats have the ability to degrade phycobilisomes during conditions of nitrogen starvation (Voorhies et al., 2015). A mechanism for population advantage from autolysis or phycobilisome degradation under light stress is less immediately apparent, and we suggest that the simplest explanation for sustaining biomass under increased light stress and deep into old mat laminae is the ability of cyanobacteria to persist at low temperature and low irradiance with minimal metabolic activity. Some loss of cell integrity in the deeper laminae may, however, be indicated by the increase in chlorophyllide-a and phaeophorbide-a in the pink layers, compared to the orange, which may be breakdown products of chlorophyll-a. A slightly different impact on pigment complement from shading to burial appears to result in the pigment transition to an increased phaeophytin-a content.

The mechanisms of accrual of laminae that emerges from our data suggest that the summer pulse of growth sees accrual of extracellular polysaccharide to form the...
new lamina, and precipitation of calcite, and both are limited by reduced irradiance.
The assemblage forming this new lamina is the same as that left behind in the buried
laminae, and while some move up vertically as they grow, others, or their
descendants, remain and may be photosynthetically active or many years in the
shaded lower laminae. Those lower laminae slowly decompose, at a net rate that is
enhanced by shading and after 8-10 years lack all photosynthetic pigmentation and
thereafter gradually lose their cohesiveness.

The change in appearance between the pink and orange layers within the mat
was not associated with a shift in cyanobacterial composition, nor with an increase in
any specific carotenoid pigment relative to chlorophyll-a. This is despite poorly
quantified lakeside microscope observations that suggested that diatoms were more
common in the orange-brown layer than the pink. Surface increases in carotenoids in
Antarctic microbial mats, that produce an orange-brown colour, have previously been
associated with protection from high irradiance (Vincent et al., 1992), but this is
unlikely to be the case under ice in limiting irradiance, where carotenoids are more
likely to have a light harvesting role (Bertrand, 2010). We suspect that the different
visual appearance may be slightly illusory and a function of the overall concentration
of pigments. When normalised to AFDM, concentrations of phycoerythrin and
chlorophyll-a were more than twice as high in the orange as the pink layers.

At the outset of this research, and based on observations that mat growth rates
in Lake Hoare are very slow and light limited, we hypothesised that accrual of benthic mat biomass was dependent on the irradiance reaching the mats. Further, as
pigment concentrations and species composition have tended to be stable over long
time periods as lake level rose and irradiance declined, we hypothesised that there
would be a gradual decline in accrual, but that responses would be sufficiently slow to
be evident primarily as physiological rather than compositional shifts over annual to
decadal time scales. Our observations show that benthic mats respond as predicted to
variable irradiance, with a greater accumulation at higher light. However, lake level
per se was a poor predictor of irradiance, which is much more affected by ice
transparency. Observations confirm that at such low irradiance and temperature, mats
turn over very slowly. Deep layers within the mat that were established many years
ago retain apparently photosynthetically viable organisms, that differ little from those
at the mat surface, for eight or more years. During favourable growth-years mats
accumulate organic material, but cyanobacteria persist through at least two years of unfavourable conditions without any obvious loss of photosynthetic potential or taxonomic diversity. Thick accumulations of benthic mats in Antarctic lakes are a consequence of gradual accumulation of remarkably stable microbial communities in an environment where rates of physical and biotic disturbance are extremely low.

Acknowledgments

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References


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**Supporting Information**

Additional supporting Information may be found in the online version of this article:
Table S1. Cyanobacteria morphotypes identified in the orange, purple and beige layers of microbial mat from 10 m depth in Lake Hoare.

Table S2. Number of sequences and OTUs, Good’s coverage and diversity indices for three mat layers, Lake Hoare, Antarctica.

Table S3. OTUs defined from the 16S rRNA gene clone libraries of the orange, purple and beige pigmented layers in benthic microbial mats of Lake Hoare. OTUs were defined at 97% 16S rRNA gene similarity. Representative sequences for each OTU are available under the Genbank accession numbers KU230333-KU230341.

Figure S1. Multidimensional scaling plot based on HPLC analysis of pigments in Top and Middle layers of mats after two years of shading and controls.

Figure S2. Relative abundance of cyanobacterial 16S rRNA genes grouped into genera based on the clone libraries in the Orange, Pink and Beige layers of Lake Hoare microbial mats.

Figure S3. Fluorescence images of vertical sections through control and shaded treatments.

Figure S4. Profiles of fluorescence parameters through replicated vertical sections of microbial mat.

Table 1. Ice thickness, transmission of irradiance around the Lake Hoare dive hole, and benthic biomass as chlorophyll-a at ~10 m depth. In 2000, irradiance measurements were repeated after a fall of snow. All values are arithmetic mean ± standard deviation. ANOVA groupings for transmission means are indicated by letter superscripts; no significant differences were seen in chlorophyll-a, and ice thickness is based on one measure per year. N indicates samples for PAR. For chlorophyll-a N = 5. 2008-09 chlorophyll-a samples were lost through tectonic activity. Ice thickness data from Priscu (2015).
Table 2. Composition of the upper two layers of microbial mats in Lake Hoare under control and shaded conditions. Each value is the mean of 4 replicates (± standard deviation). The upper layer corresponds to the three orange-brown-pigmented laminae, the middle layer to the six pink laminae. Where control-shaded values within a layer are significantly different (ANOVA, p>0.05), they are italicised. LOAc = loss on acidification, AFDM = ash free dry mass.

<table>
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<th>Mean Chlorophyll-a (%</th>
<th>Max Chlorophyll-a (%)</th>
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**Figure Legends**

Figure 1. Map showing the location of Lake Hoare in the Taylor Valley, Antarctica
Figure 2. A vertical section of microbial mat from Lake Hoare, showing mm-scale lamination superimposed on zonation from orange-brown at the surface, though pink, to beige below. The scale bar represents 10 mm.

Figure 3. Water level of Lake Hoare over the study period. Line indicates measurements made using automated sensors, symbols those made by surveying to a local benchmark.

Figure 4. Photosynthetically available radiation incident on the lake surface (A) and reaching a moored sensor at 10 m depth (B) in Lake Hoare.

Figure 5. Relationship between lake floor irradiance and depth along a transect from 8 to 22 m depth in Lake Hoare. The fitted line is a best fit by least-squares regression of the exponential decline that would normally be expected in homogenous natural waters.

Figure 6. Top: Superimposition of cross sections of microbial mat from a site at ~10 m depth in Lake Hoare over a period of six years. The sections are set to the same scale and aligned using the heavy line of sedimentation associated with a large pulse of melt water during 2001-2002 season. Below: Thickness of laminae laid down in specific years when sampled in 2004, 2006, 2008 and 2010. Bars show average, ± standard deviation (N = 3).

Fig. 7. Cross sections of microbial mat from 8-9 m depth in Lake Hoare taken through the boundary between shade and no shade. The arrow marks the boundary. A and B are magnifications of the boxed areas in C. The scale bar is 10 mm. D indicates the thickness of laminations in three vertical transects through each of three samples from shaded and control regions.

Fig. 8. Composite images of variable fluorescence properties of vertical sections of microbial mats from under shades (left) and control (right). In these images the false colour indicates the yield ($\phi_H$) of the mat section after dark adaptation, and the intensity of fluorescence is indicated by the brightness. The arrow indicates the approximate position of the mat surface at the start of the two-year experiment. The images are scaled similarly and the lower bar is 10 mm. See supplemental material for more details.
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