

Trophic interactions in microbial mats on Byers Peninsula, maritime Antarctica

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Abstract Cyanobacteria-based microbial mats are common in Antarctic terrestrial freshwater ecosystems such as the extensive wetland seepages that cover Byers Peninsula on Livingston Island (South Shetland Islands), maritime Antarctica, where they play an important role in biomass generation and productivity. Although cyanobacteria, microfauna and fungal communities have been described for such microbial mats, to date, little is known about trophic interactions within the mats, which are likely important to overcome nutrient constraints in oligotrophic polar freshwater ecosystems. We therefore carried out a biomass assessment of the different taxonomic components and their trophic interactions using DNA analysis and stable isotope analysis, as well as physiological activities such as primary and secondary production and nitrogen uptake within the mat food web throughout an austral spring and summer season. Our results suggested, based on a Bayesian mixing model, that carbon flow from

cyanobacteria to upper trophic levels was limited to tardigrades and rotifers, whereas fungal and bacterial activity were likely the main connectors between consumers and producers via a heterotrophic loop. This suggests that homeostatic state displayed in freshwater microbial mats from maritime Antarctica provides stability to the microbial mats under the fluctuating environmental conditions commonly found in permanently cold shallow terrestrial aquatic ecosystems in Antarctica.

Keywords Antarctic · Microbial mats · Cyanobacteria · Fungi · Trophic interactions · Food web

Introduction

In polar terrestrial landscapes, highly pigmented multilayered microbial mats can cover much of the benthos of shallow lakes, ponds and wetlands. These microenvironments comprise a myriad of organisms including bacteria, diatoms, green algae, fungi, ciliates and microfauna, as well as viruses (Vincent 2000a; Jungblut et al. 2005). These mats are often macroscopically dominated by filamentous oscillatorian cyanobacteria and can achieve high standing stocks and often account for most ecosystem productivity in Antarctic shallow terrestrial aquatic ecosystems (Hawes and Schwarz 1999; Quesada and Vincent 2012).

In comparison with other climate zones, aquatic food webs of high-latitude ecosystems are thought to be relatively simple and dominated by microorganisms (Vincent et al. 2008). Previous stable isotope work has shown that zooplankton may use microbial mats as a carbon source in the absence of sufficient phytoplankton in high-latitude shallow lakes and ponds (Rautio and Vincent 2007). While

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a number of publications investigate the relationship between microbial mats' biodiversity and their interactions with the physico-chemical environment (e.g. Sutherland 2009; Varin et al. 2012), less is known about the food webs of freshwater benthic communities in polar regions.

Here, we therefore investigated cyanobacteria-based microbial mats growing in an Antarctic wetland at Byers Peninsula (Livingston Island, South Shetland Islands), located at the northern limit of maritime Antarctica. Byers Peninsula is considered a main biodiversity hotspot in maritime Antarctica (Rautio et al. 2008) and represents a key monitoring spot for climate change because of the variety of freshwater bodies (Quesada et al. 2009; Lyons et al. 2013a) colonized mostly by microbial mats. Our objectives in the present study were to investigate and model, for the first time, the different taxonomic components within the mat food web and their interactions using a combination of microscopy, DNA and stable isotope analyses. Our results are based on multiple time points from spring to late summer, allowing an evaluation of taxonomic succession and shifts in productivity patterns. The use of Bayesian mixing models of those trophic interactions within cyanobacterial-based microbial mats in shallow, often oligo- to ultraoligotrophic polar terrestrial aquatic ecosystems, will expand current knowledge about the relationship between the environment and the organisms present in benthic habitats (Wilkins et al. 2012).

Materials and methods

Study site

The study took place from spring (November 2006) to the end of the austral summer in (February 2007) (Table 1), and the sampling was conducted on microbial mats that covered an open-water seepage area (SW Pond, 62°39'10.00"S, 61°06'21.48"W) on Byers Peninsula (Livingston Island, South Shetland Islands) (see more details in: Fernández-Valiente et al. 2007; Toro et al. 2007) (Fig. 1). The microbial mats were multi-layered purple pigmented, with a brittle and non-uniform surface following the microtopography of the gravel underneath with an average thickness of 0.7 ± 0.4 cm. The deep green basal layer combined microorganisms with fine-grained sand and mineral deposits. Mats sheltered a variety of recognizable cyanobacterial morphotypes being the non-heterocystous taxa dominant in biomass.

Physical parameters

Irradiance and air temperature measurements were taken during the season by using an automatic weather station

(Campbell Scientific Co.) placed within 400 m of the sampling site (Bañón et al. 2013). When microbial mats were snow-covered, light conditions above the microbial mats were calculated by fitting data from snow light profiles measured by a 2π quantum sensor (Li-COR, LI-192) connected to a Li-COR LI-1000 data-logger (Stibal et al. 2007). Automatic temperature data-loggers (Tidbit. Onset Co.) were placed inside the microbial mat community throughout the season, registering at 30 min intervals. The raw data for light and irradiance measurements were smoothed by way of LOESS algorithm and cubic polynomials in order to identify trends.

Photosynthesis versus irradiance curves

Photosynthesis versus irradiance curves (PvsI) were determined during ice-covered conditions, early ice-free patches (both sampled on 23 November of 2006) and ice-free conditions (17 January 2007). Photosynthesis and irradiance datasets followed a hyperbolic tangent equation at each temperature and were fitted to Platt's photosynthesis model (Platt et al. 1980). Here, photosynthesis (Ps) is defined as estimated maximum photosynthetic rate, i.e. the initial slope of the curve, which reflects the photosystems' efficiency, and E as the irradiance in each interval of the curve. SPSS Statistics 17.0 (SPSS Inc. 2008) and SigmaPlot 11.0 (Systat Software Inc.) were used for statistical procedures and graphical representations.

C and N uptake measurements

Throughout the season, inorganic C and N uptake rates were measured in triplicate. Photosynthetic carbon assimilation was measured as ^{13}C (98% ^{13}C . Isotec) incorporation in samples, with $\text{NaH}^{13}\text{CO}_3$ as a tracer from a stock solution of 1 mg C ml^{-1} . The dissolved inorganic carbon (DIC) was calculated from water alkalinity (by considering pH and temperature), measured after titration with HCl using a pH shift double indicator (phenolphthalein) of equivalence endpoint pH. The incubations were launched by adding the ^{13}C tracer to the samples at an approximate concentration of 10% of the natural concentration of DIC. After 2 h, the incubations were acidified with 1 ml of 1 N HCl per bag to exclude non-assimilated tracer. Then, 1 ml of 1 N NaOH added to neutralize HCl and rinsed immediately with GF/F-filtered pond water (Velázquez et al. 2011). The POC contents of samples were measured from dried samples using an elemental analyser (Perkin-Elmer 2400CHN) with a thermal conductivity detector. Nutrient analyses were carried out following the standard APHA methods (APHA 1992). Soluble reactive phosphorous of the pond water was measured using the phosphomolybdic

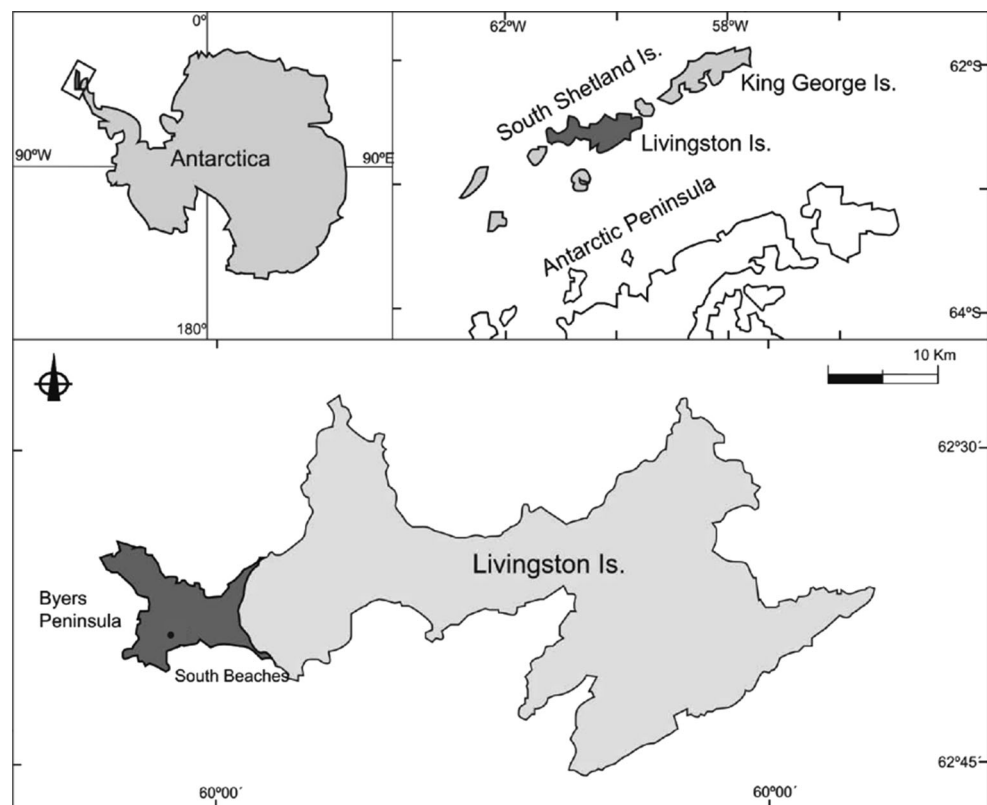
Table 1 Activities and analyses carried out during the 2006–2007-fieldwork season at SW pond (Byers Peninsula, Livingston Is., Antarctica)

Measurements/analyses	Southern hemisphere spring			Southern hemisphere summer		
	November 23rd, 2006	December 3rd, 2006	December 13th, 2006	January 17th, 2007	February 1st, 2006	February 10th, 2007
Photosynthesis versus Irradiance assays	x	n.a.	n.a.	x	n.a.	n.a.
Primary production	x	x	x	x	x	x
Secondary production	x(a)	n.a.	x	x	x	x
NH ₄ ⁺ uptake	x	x	x	x	x	x
NO ₃ ⁻ uptake	x	x	x	x	x	x
Cyanobacterial clone library	x	n.a.	n.a.	x	n.a.	x
Fungal biomass estimation	x	x	x	x	x	x
Nutrient analyses (overlying water)	x	x	x	x	x	x
Metazoa quantification	x	n.a.	x	x	x	x
Particulate organic carbon	x	x	x	x	x	x
Dissolved inorganic carbon	x	x	x	x	x	x
Dissolved inorganoc nitrogen	x	x	x	n.a.	x	x

(a) This measurement was the baseline for the subsequent secondary production measurements

n.a. not analysed

Fig. 1 Location of Byers Peninsula at Livingston Island in the South Shetland Islands of maritime Antarctica. *Black dot* in the lower diagram marks the sampling point



acid—ascorbic acid method after the pH neutralization of the samples.

Ammonium and nitrate uptake rates were measured by using (¹⁵NH₄)SO₄ (99% ¹⁵N, CIL) and K¹⁵NO₃ (99.9% ¹⁵N, Isotec). The stock solutions used in the assays were 4 l and 30 mg N ml⁻¹, respectively. From these stocks,

respective volumes were added to samples to reach 10‰ of the natural concentration of the light isotope, as measured the previous year in the same area. Mat samples for biomass estimation, C and N uptake, particulate organic carbon (POC), and taxonomic determinations were collected every two weeks from early

November until mid February and kept frozen until further analysis.

Cyanobacterial-specific polymerase chain reaction (PCR), cloning, restriction fragment length polymorphism analysis (RFLP), sequencing and diversity analysis

The surveyed microbial mat community was sampled for triplicate for genetic analysis at the same site, at three time points throughout the growing season in Byers Peninsula 2006–2007: 11 November 2006 (SWmat-01, spring, ice-covered), 17 January 2007 (SWmat-02, summer, ice-free) and 10 February 2007 (SWmat-03, summer, ice-free) using ethanol-sterilized metal cores (15 mm inner diameter).

DNA was extracted using MoBio PowerBiofilm DNA extraction kits (Carlsbad, CA) following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using cyanobacterial-specific primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 809R (5'-GCTTCGGCACGGCTCGGGTCGATA-3') as described in Jungblut et al. (2012). Correct-sized amplicons were subjected to RFLP screening using the enzymes AluI and HpaII as described in Jungblut et al. (2012). At least two clones for each unique RFLP pattern were sequenced. Sequencing was carried out using the vector-specific T7 universal forward primer (single read), with an Applied Biosystems 3730xl DNA analyser (Applied Biosystems, Foster City, CA). Sequences generated by this study were deposited to GenBank under the accession numbers JX893036–JX893148.

Sequences were checked for chimeras using Bellerophon (Huber et al. 2004), and chimeras were removed from further analysis. Only high-quality sequences were edited using 4Peaks (Version 1.7.2) and aligned using Clustal X (version 2.0.9). Operational Taxonomic Units (OTUs) were delineated on the basis of 97% sequence similarity by using Mothur (Schloss et al. 2009). BLASTN search (Altschul et al. 1990) to GenBank was performed to determine the closest cultured and uncultured match. Sampling effort was assessed by calculation of rarefaction curves, while estimates of library richness and α -diversity were made using the nonparametric estimators: ACE, Chao1, Sobs, Shannon and Simpson by using Mothur (Schloss et al. 2009). β -diversity was analysed by estimators Jaccard and Sorenson, and γ -structural diversity was performed by Yu and Clayton estimator (Online Resource Table 1).

Microscopy identification of microfauna

Microfauna biomass fractions of the community were determined at two-week intervals from 23 November 2006

to 10 February 2007. Triplicates of cores of 5 mm of inner diameter were gently disaggregated, mounted on microscope slides, and microscope photographs were taken to count organisms belonging to the different taxonomic groups and to determine biovolumes. Biovolume and carbon content of nematodes, tardigrades and rotifers were estimated following Meyer (1989), Burgherr and Meyer (1997), Benke et al. (1999), Nielsen et al. (2011), respectively. The biovolume of ciliates was calculated by measuring cell dimensions and assuming simple ovoid forms and a correspondence of $0.19 \text{ pg C } \mu\text{m}^{-3}$ (Putt and Stoecker 1989). Ciliate genera identification was based on Petz (2003). Net secondary production by the different taxa monitored was calculated by extracting their carbon content differences from the previous measurements.

Microscopic identification of cyanobacteria and phototrophic community

The composition of the phototrophic assemblages within the communities was qualitative studied with fresh samples, using two different fluorescence filters and Nomarsky interference microscopy along the season. For each microscopic field, observations were done under white illumination and using an Olympus® blue filter set (EF 400–490 nm, DM 570, FB 590) where chlorophyll a was excited, whereas for excitation of cyanobacterial phycobiliproteins an Olympus® green filter set (EF 530–545 nm, DM 570, FB 590) was used. We followed the Broady and Kibblewhite cyanobacterial morphotype definitions (Broady and Kibblewhite 1991) to provide simpler morphological assignments.

Fungal hyphal length and biomass

Total fungal hyphal length and biomass were estimated by fluorescence microscopy from 5-mm-diameter mat cores that had been disaggregated and re-suspended in distilled water using calcofluor white stain (Fluka), which binds to chitin and cellulose in cell walls. In brief, samples were incubated in calcofluor white stain for 5 min, washed with KOH 5% (v/v) final concentration, and 0.05 ml of re-suspended mat material was mounted in a Neubauer chamber slide (Brand GmbH) and analysed using an Olympus BX10 microscope, excited by blue light with the fluorescence set up B2A (EX: 450, DM: 505, BA: 520). Up to 100 microscopic fields were counted for hyphal length estimation according to Newman (1966), and biovolume was estimated assuming a hyphal cylindrical mean diameter of $1.5 \mu\text{m}$ through the total hyphal length. Carbon content of the fungal section of the microbial mats was calculated according to Bloem et al. (1995).

Isotopic tracing and food web modelling

To assess trophic relationships between different members of the microbial mat community, microbial mat samples were disaggregated and the different compartments of the community (tardigrades, nematodes, rotifers, cyanobacteria, diatoms, mosses and fungi) were sorted using a stereozoom microscope (Leica MZ75). Samples were dried at 65.5 °C for about 48 h, and the natural abundances of $\delta^{13}\text{C}$ were analysed by triplicate in a mass spectrometer (IRMS Micromass-Isochrom) with the aim of displaying the trophic connection between these organism groups. Natural abundances of $\delta^{15}\text{N}$ into the community were also surveyed to assess the different trophic levels of the mat ecosystem (Post 2002). The $\delta^{13}\text{C}$ signal for dissolved organic carbon (DOC) and particulate organic matter (POM) between 30 and 0.22 μm was determined by quadruplicate as follows: a piece of mat was pressed gently against a Nylal sieve of 30- μm pore size diameter, and the fraction <30 μm was centrifuged (5 min, 10,000 rpm). Then, the supernatant was filtered through a 0.22 μm hydrophilic membrane, concentrated by evaporation at 40 °C under vacuum and analysed by mass spectrometry (IRMS Micromass-Isochrom).

Data analysis and modelling

To assess the trophic pathways, a Bayesian isotopic mixing model, available as an open source R package, SIAR (Stable Isotope Analysis in R; Jackson et al. 2009; Parnell et al. 2010), was used. The SIAR model is fitted via Markov Chain Monte Carlo (MCMC) method, producing simulations of plausible values of dietary proportions of sources consistent with the data using a single data point prior distribution (Jackson et al. 2009; Parnell et al. 2010). The SIAR MCMC was run for 200,000 iterations, discarding the first 32,000 samples and then thinning by 32 to reduce sample autocorrelation. Prior to running SIAR, we assumed similar stoichiometry for C and N fractionation through the food web.

Results

Light and temperature profiles

During spring ice-covered conditions, an ice-snow layer of 33-cm thickness covered the microbial mats and reduced incident irradiance to 2% above the microbial mat. Incident irradiance gradually increased with melting of the ice cover, and microbial mats were completely ice-free from mid-January until the end of the summer season (Fig. 2). Daily average surface temperature of microbial mats was 9.52 ± 4.8 °C (Fig. 2). Absolute maximum and minimum

temperature were 18.9 and -3.1 °C, respectively. Temperatures within the microbial mat surface were consistently higher than air temperature, and this difference was on average 3.5 °C after LOESS fitting.

Nutrient dynamics

The water overlying the microbial mats analysed displayed an oligotrophic profile based in its nutrient composition (Online Resource Figure 1). Soluble reactive phosphorus (SRP) concentrations were between 0.04 and 0.29 μM , while dissolved inorganic nitrogen (DIN) was mainly composed of oxidized species (nitrate + nitrite) of variable concentrations ranging from 1.61 to less than 0.01 μM . The N/P molar ratios based on inorganic forms (i.e. DIN and SRP) varied notably during the season, from 2.95 ± 1.7 at

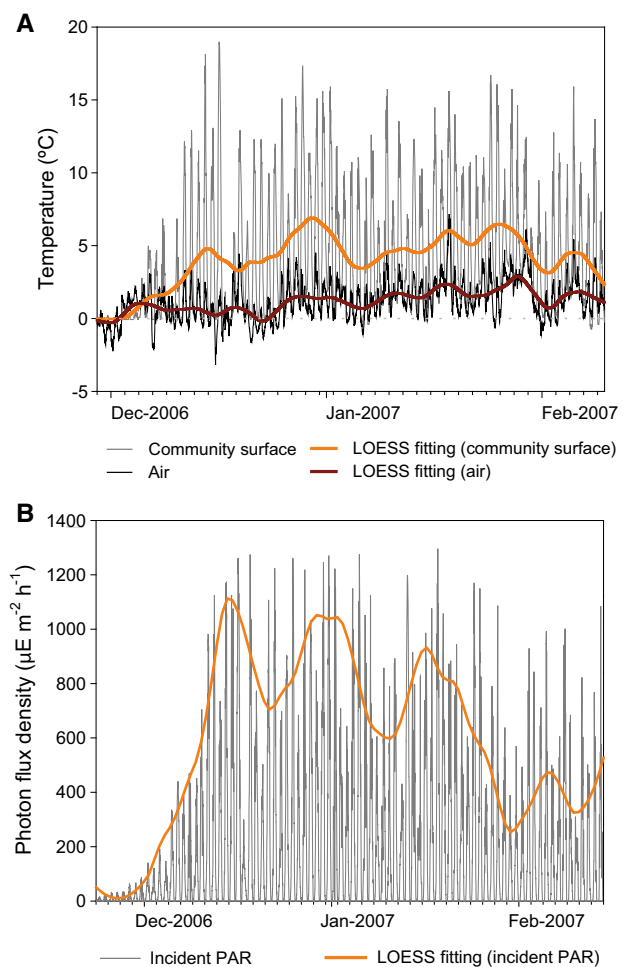


Fig. 2 **a** Temperature of the air and at the surface of microbial mats at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica). LOESS lines represent locally weighted scatterplot smoothing models for each of the datasets collected. **b** Variations in incident Photosynthetic Active Radiation (PAR). Values were corrected by extinction coefficient of the snow cover, which remained until the 1st week of December 2006

the beginning of the season to 13.5 ± 1.9 at midsummer, which is close to the Redfield ratios (16/1). The mean C/N ratios along the season were 10.8 ± 1.3 , almost twice the Redfield ratio (6.6/1). Total N to total P ratio increased about one hundred times through the season.

The ammonium and nitrate uptake showed similar patterns throughout the season both declining over the course of the summer (Online Resource Figure 1). Although dissolved inorganic nitrogen (DIN) was mainly composed of reduced nitrogen compounds (ammonium) at the beginning of the summer season, later in the study period the trend switched and nitrate drove DIN concentration. Similarly, ammonium values reveal higher uptake rates of reduced N relative to oxidized species.

Primary and secondary production

Primary and secondary production decreased from early to late summer conditions (from 1.56 to $0.59 \mu\text{gC cm}^{-2} \text{h}^{-1}$, and 2.71 to $0.61 \mu\text{gC cm}^{-2} \text{d}^{-1}$, respectively). The PvsI curves (Online Resource Figure 2) provided good descriptors of the photosynthetic activity within the mat. The photosynthetic parameters estimated by different models exhibited similar behaviours (Online Resource Table 2); however, the fitting based on the Platt's model showed the best results, with Pearson correlation coefficients (r) varying between 0.94 and 0.97. The photosynthetic efficiency (α values) calculated for spring (ice-covered) and summer (ice-free) was 0.32 ± 0.10 and $1.41 \pm 0.45 \mu\text{gC cm}^{-2} \text{h}^{-1} (\text{Me m}^{-2} \text{s}^{-1})^{-1}$, respectively. In contrast, spring (ice-free) experiments showed a lower photosynthetic efficiency ($0.03 \pm 0.01 \mu\text{gC cm}^{-2} \text{h}^{-1} (\mu\text{E m}^{-2} \text{s}^{-1})^{-1}$). In general, the curves saturated asymptotically, showing no photoinhibition under maximum natural irradiances. Comparison of curve shapes showed photosynthetically active mats throughout the season. However, P_m values differed between the three studied situations (ice-covered spring, ice-free spring and ice-free summer mats). Maximum modelled photosynthesis (P_m) of the ice-free stages during spring and summer was in the same range, with 10.36 ± 1.36 and $11.56 \pm 0.87 \mu\text{gC m}^{-2} \text{h}^{-1}$, respectively, whereas maximum photosynthesis of the ice-covered community was only $7.98 \pm 0.55 \mu\text{gC m}^{-2} \text{h}^{-1}$. The variation of light saturation index (E_k) suggested changes of photosynthetic groups dominating in the assemblage. E_k of spring ice-covered and ice-free (24.65 and $8.22 \mu\text{E m}^{-2} \text{s}^{-1}$, respectively) remain lower than values for the ice-free mat in summer ($357.7 \mu\text{E m}^{-2} \text{s}^{-1}$).

Moreover, POC values, with some variability, remained in a dynamic equilibrium between 300 and 500 mg C cm^{-2} through the whole season, but no significant differences between ice-covered and ice-free periods were observed (ANOVA; p value = 0.27) (Fig. 3).

Microscopic characterization of the phototrophic community

The microbial community matrix was dominated by two thin cyanobacterial morphotypes, I ($1 \mu\text{m}$ in diameter, *Leptolyngbya* cf. *antarctica*) and J ($3 \mu\text{m}$ in diameter) sensu Broady and Kibblewhite (1991). Thicker oscillatorioid cyanobacteria were also observed in the surface layer and included morphotype C ($5.5\text{--}6 \mu\text{m}$ in diameter, cf. *Tychonema* sp.), morphotype B, which was assigned to *Phormidium autumnale*, morphotype K ($4\text{--}5 \mu\text{m}$ in diameter, *Phormidium* cf. *pseudopriestleyi*) and morphotype E ($11 \mu\text{m}$ in diameter, *Phormidium* cf. *subproboscideum*). Nostoclean morphotypes cf. *Tolypothrix* were also present (Komarek and Anagnostidis 2005). An oscillatorioid Phormidiaceae, with a dark thick lamellate and coloured sheath and a cell diameter of $5.5 \mu\text{m}$, was observed emerging from broken sheaths. Unicellular cyanobacteria (Chroococcales) ($1.5 \mu\text{m}$ in diameter) were also relatively abundant and occurred intermixed within the mat matrix. Abundant microcolonies of *Nostoc* cf. *commune* appeared in the deepest layer at the beginning of summer. Thicker ($>5.5 \mu\text{m}$) oscillatorioid morphotypes such as *Phormidium* spp. and *Tychonema* sp. became more abundant throughout the summer, despite the fact that these taxa have recently been proposed for a taxonomical reassignment into genus *Microcoleus* (Strunecký et al. 2013) that opens a discussion out of the scope of the present paper and will be analysed separately hereafter. The composition of matrix-forming thin cyanobacteria remained unchanged with an absolute dominance of morphotypes I and J.

Chlorophytes, especially coccoid green algae, were relatively abundant in the microbial mat at the beginning of

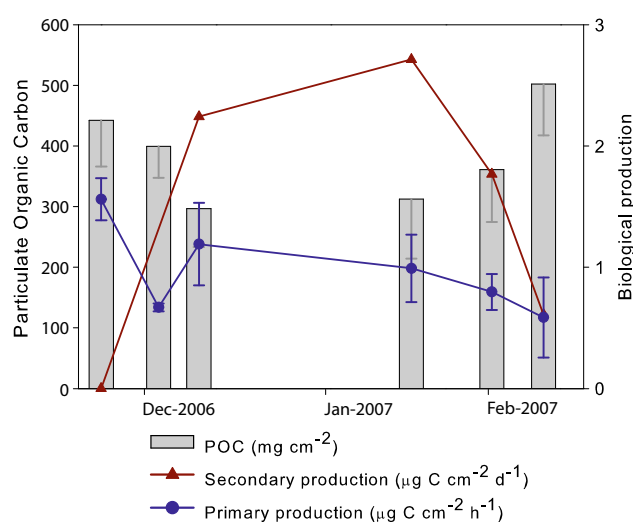


Fig. 3 Carbon dynamics of SW Pond benthic microbial community (Byers Peninsula, South Shetland Islands, Antarctica) during the 2006–2007 field season

the season during ice-covered condition. However, their abundance decreased with increasing ice-free conditions, whereas number of algae resting forms increased and remained abundant through the summer (data not shown). Pigmented diatoms belonging to the genera *Navicula*, *Nitzschia*, *Psammothidium* and *Pinnularia* were present in relatively low density throughout the study. There were no major changes in diatom abundances throughout the season based on semi-quantitative assessment.

Cyanobacterial 16S rRNA gene composition

A total of 112 clones were analysed by way of clone library surveys of the microbial mat samples. We identified a total of 48 OTUs at 97% similarity with the highest diversity being observed at the end of the season (SWmat-03, late summer conditions) with up to 18 cyanobacterial OTUs. SWmat-01 (November, spring conditions) and SW-02 (January, summer conditions) were comprised both of 15 OTUs (Fig. 4; Table 1). Rarefaction curves did not reach an asymptote, indicating incomplete sampling of the cyanobacterial OTU diversity (data not shown). Coverage for SWmat-01 and SWmat-03 was 70 and 66%, respectively, whereas only 33% of diversity was covered in SWmat-02. Richness estimators such as nonparametric Chao-1 and parametric ACE were generated and were higher than clone library diversity for most samples. Shannon index ranged between 1.46 for SWmat-02 to 2.74 for SWmat-01 (Online Resource Table 3).

The cyanobacterial community in the spring (SWmat-01) and late summer samples (SWmat-03) was dominated by OTUs matching the genus *Leptolyngbya*, comprising up to 62.8% in relative abundance (Fig. 4), whereas

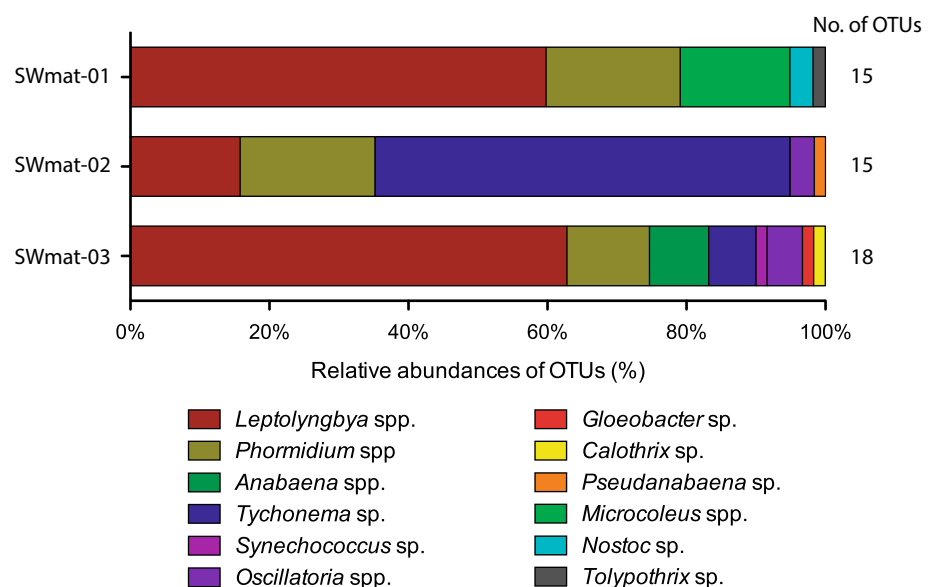
Leptolyngbya sp. was less than 20% of relative abundance in the mid-summer sample (SWmat-02). Most of these OTUs matched to *L. antarctica* and *L. frigida*. The most abundant group of OTUs in SWmat-02 matched to *Tychonema*. Similarity of the community structure was compared throughout the season, and estimators also indicated shifts in the cyanobacterial community composition (Online Resource Figure 3). Communities were significantly different based on Cramer-von Mises test with correction for multiple comparisons (1000 randomizations, $p < 0.005$) (Online Resource Table 4).

Phormidium ranged from 20 to 30% relative abundance in all samples. Sequences assigned to *Oscillatoria* spp. were absent in the spring sample (SWmat-01) but gradually increased in abundance in ice-free conditions (SWmat-02 and SWmat-03). OTUs matching the oscillatorian genera *Microcoleus* and *Pseudanabaena*, and the heterocyst-forming *Tolypothrix* were also detected. For Nostocales, an OTU with highest match to *Nostoc* sp. 152 (AJ133161) was detected in SWmat-01, whereas several OTUs grouping within Nostocales were detected in the sample from late summer (SWmat-03, *Anabaena* (AJ630458, AJ293110) and *Calothrix* (HQ847581)) (Online Resources Table 1).

Microfauna, bacterial and fungal biomass

A diverse assemblage of organisms was found including nematodes, ciliates, tardigrades, rotifers, fungi and Bacteria. Bacterivorous nematodes, *Geomonhystera* spp., *Plectus* spp., *Teratocephalus* spp., and fungi-consuming nematodes, *Aphelenchoides* spp., were identified during the community seasonal survey. The identified Tardigrades belonged to the genera *Macrobiotus*, *Minibiotus* and

Fig. 4 Ribotype survey along the 2006–2007 season at SW Pond (Byers Peninsula, Antarctica). Relative percentage abundance of the major groups of cyanobacterial ribotypes at the three time points during the spring–summer season. SWmat-01, SWmat-02 and SWmat-03 samples represent ice-covered, transition and ice-free conditions, respectively



Dactilobiotus. Tardigrades gut content surveys exhibited high concentration of chlorophytes and cyanobacteria especially at the ice-melting stage. Rotifers belonged to the classes *Bdelloidea* and *Monogononta*. Ciliates were previously described for the same community by Petz (2003) and mainly belonged to the groups *Stichotrichia*, *Litostomatea* and *Peritrichia*.

In terms of biomass, nematodes had the highest relative abundance of organic C fraction amongst microfauna during ice-free conditions, whereas rotifers and tardigrades dominated during ice melting. Ciliates contribution to POC was always below 5% (Fig. 5). Fungal taxonomical identification was not performed, but relative abundance of fungi in the mat community was estimated by microscopy. The fungal hyphal biomass increased from 2 to 8% of total (POC) during ice melting and displayed a strong positive correlation with surface temperature records on the community (Pearson correlation coefficient = 0.97, p value = 0.003) (Fig. 6).

Food web tracing and SIAR model interpretation

Variations in stable isotope fractionation allowed carbon pathways and food web structure to be traced. The isotopic ^{13}C signals of primary producers cyanobacteria and diatoms were -11.95 ± 0.87 and -13.49 ± 1.05 ($\delta^{13}\text{C}_{\text{VPDB}}$), respectively (Fig. 7), whereas $\delta^{15}\text{N}$ signals were -3.2 ± 0.56 and $+1.98 \pm 0.01$ ($\delta^{15}\text{N}_{\text{AIR}}$), respectively. Mosses around the mat plotted outside of the trophic web, with -23.16 ± 0.46 ($\delta^{13}\text{C}_{\text{VPDB}}$) and -2.8 ± 0.11 ($\delta^{15}\text{N}_{\text{AIR}}$), and were therefore not considered for the modelling. DOC $\delta^{13}\text{C}$ values were assumed to reflect most of the bacterial activity within the community. DOC $\delta^{13}\text{C}$ (-11.23 ± 0.19 $\delta^{13}\text{C}_{\text{VPDB}}$) was the highest of the observed isotopic signals. POM smaller than $30\ \mu\text{m}$ ($<30\ \mu\text{m}$) comprised the whole fraction of the community, including

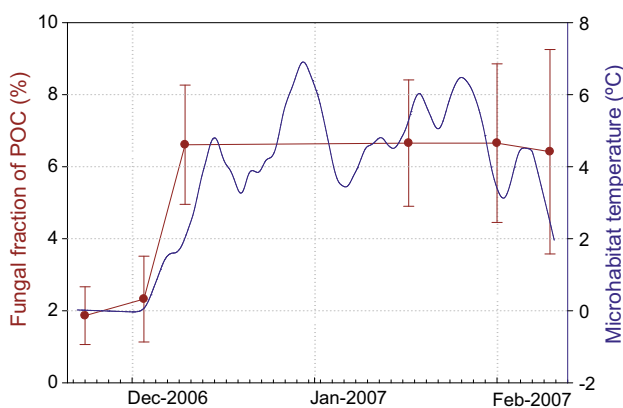


Fig. 5 Total carbon percentage of microfauna estimated during summer season of 2006–2007 at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica)

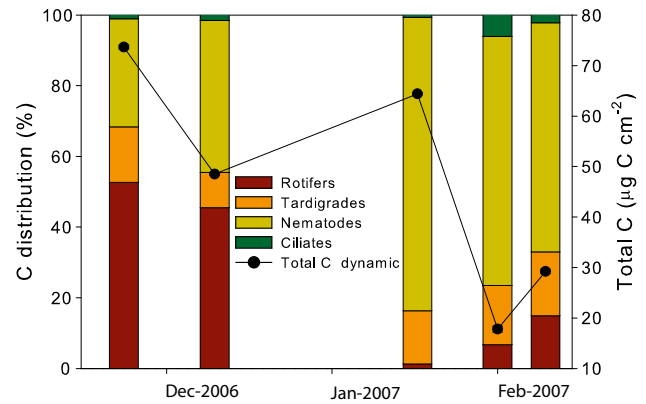


Fig. 6 Fungal hyphal fraction of the particulate organic carbon (POC) estimated during summer season of 2006–2007 at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica) compared to average temperatures in the microbial mats

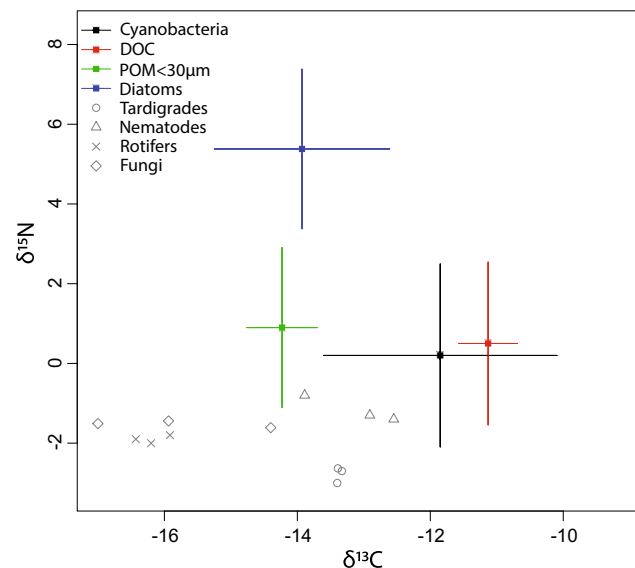


Fig. 7 Bivariate model plot of isotopic signatures of heterotrophic organisms in the community (except Bacteria) and potential food sources of the microbial mat food web from SW Pond (Byers Peninsula, South Shetland Islands, Antarctica). Points are mean trophic enrichment factor, and error bars are ± 1 standard deviation regarding to the trophic enrichment factor

chlorophytes, ciliates and partially decomposed matter (-14.33 ± 0.24 $\delta^{13}\text{C}_{\text{VPDB}}$ and -2.5 ± 0.06 $\delta^{15}\text{N}_{\text{AIR}}$). Fungi and rotifer $\delta^{13}\text{C}$ displayed the most negative values of the consumers' fraction within the food web. SIAR modelling suggested that cyanobacteria and diatoms were the main C sources of rotifers, while fungi C sources were influenced by cyanobacteria, DOC and diatoms in different proportions (Fig. 8).

As major grazers, tardigrades and nematodes presented -2.84 ± 0.24 , -1.13 ± 0.34 ($\delta^{15}\text{N}_{\text{AIR}}$) and

-12.92 ± 0.76 , -13.12 ± 0.69 $\delta^{13}\text{C}_{\text{VPDB}}$, respectively (Fig. 7). SIAR model explained tardigrades' $\delta^{13}\text{C}$ signal as shared by cyanobacteria (ca. 90%) and POM (ca. 5%) proportions (Fig. 8). Correlation coefficient (-0.8) between both food sources indicated a diet mainly based on cyanobacteria and eventually later in the summer on POM. The nematodes' food sources are more dispersed: cyanobacteria (ca. 20%), DOC (ca. 22%) and POM (ca. 52%). Correlation between nematodes' C sources revealed divergent consumption behaviour: they select either cyanobacteria or POM (correlation coefficient = -0.96), and the POM consumers correlate positively with DOC sources (0.68). In both cases, diatoms represent a residual fraction of C sources (Fig. 8). Nonetheless, rotifers diet seems to be mainly based on diatoms and occasionally on cyanobacteria.

Discussion

Seasonal dynamics of the phototrophic community

Early in the season, snowpack and ice were the parameters driving the light, temperature and liquid water availability. The microbial mat studied showed a microbial community progressively adjusting its photosynthetic metabolism to the environmental conditions, as has been described by observations in previous studies (Harding et al. 1982; Velázquez et al. 2011). The lower photosynthetic efficiency values displayed during the spring period (ice-free conditions) reflect shifts in community's photosynthetic metabolism responding to less severe environmental conditions including warmer temperatures, higher incident PAR and higher nutrient inputs from the catchment.

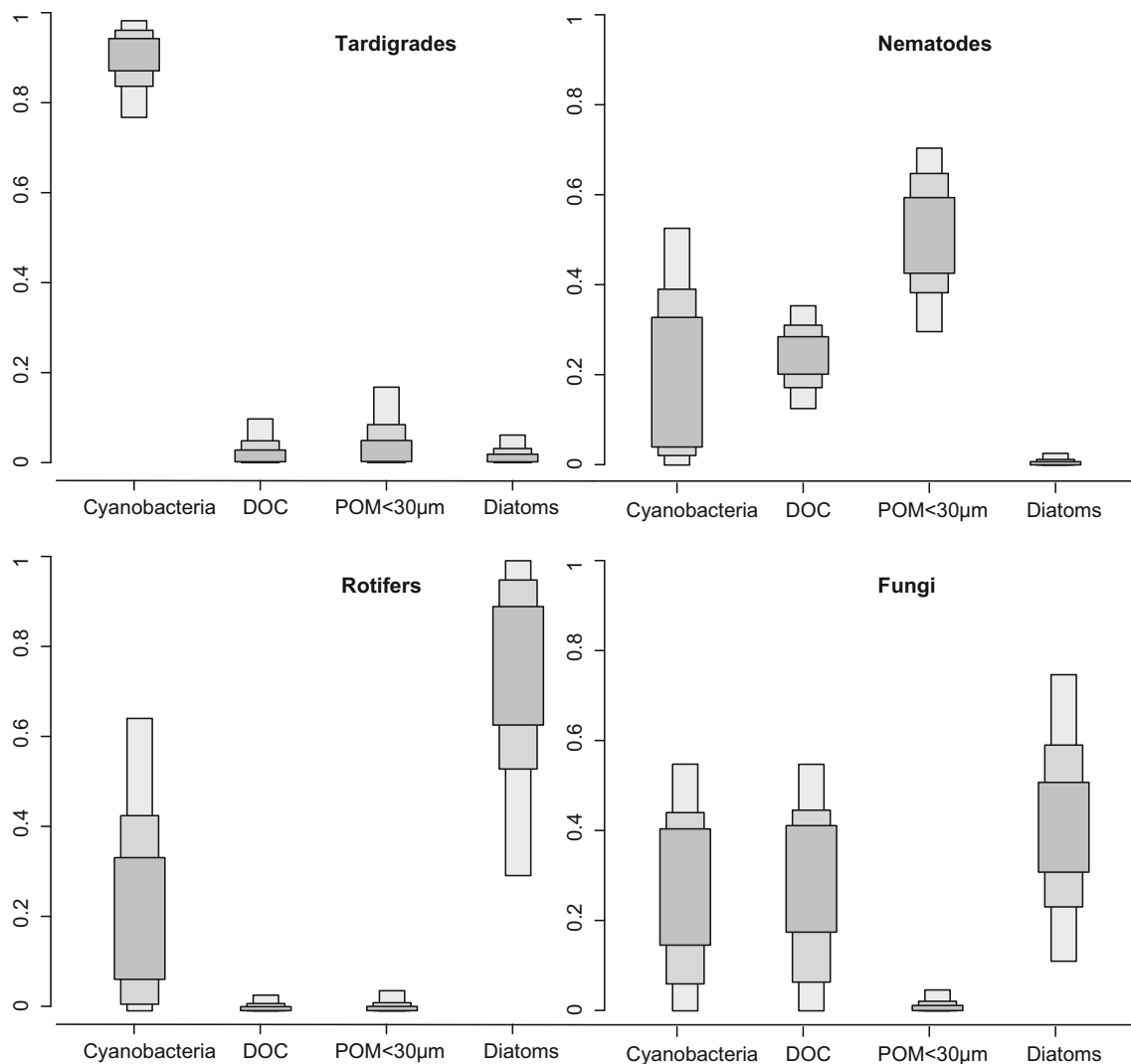


Fig. 8 Results of SIAR Bayesian mixing model showing the contribution of each C source to the different heterotrophic fractions of the microbial mat community from SW Pond. Different grey

coloured boxes indicate confidence intervals of 25, 75 and 95%. DOC is Dissolved Organic Carbon, and POM is Particulate Organic Matter

Modelled parameters suggest the phototrophic community could be in an optimal stage by mid-January, with highest photosynthetic rates during the most favourable environmental conditions in terms of temperature and liquid water availability during ice-free conditions. The liquid water availability, which is intimately linked to temperature regimes, acts as the most limiting factor for organism zonation along Antarctica (Convey et al. 2014). Although, metabolism and primary production are not completely inhibited at temperatures near 0 °C, that is likely due to the presence of effective photosynthetic performances, able to manage large amounts of electrons at low temperatures (Vincent 2000b) and to the presence of active psychrophilic Chlorophyta in the presence of snow (Velázquez et al. 2011).

Parallel to changes in the photosynthetic metabolism over the season, community analysis indicated a shift in the relative abundance of chlorophytes and cyanobacteria. However, this finding requires additional confirmation using next generation sequencing or quantitative DNA/RNA methods, especially as cyanobacterial mats have been seen as relatively static ecosystems, in contrast to phototrophic biofilms growing in highly dynamic habitats such as snowbanks or ephemeral streams (Vincent 2000a).

Trophic interactions

Antarctic microbial mats have been shown to grow well in nutrient poor freshwater ecosystems and maintain nutrients within the mats (Bonilla et al. 2005). However, it is not well understood how the different taxonomic groups are connected to comprise a food web that appears to be able to overcome the nutrient deplete conditions of the environment. Stable isotope analysis was therefore applied, since the $\delta^{13}\text{C}$ signal of organisms allows the origin and transfer of carbon across members of biological communities to be traced within a food web, while the $\delta^{15}\text{N}$ isotope signal can help to establish the number of trophic levels due it high level of fractionation from one trophic level to another (Post 2002).

Cyanobacteria and diatom $\delta^{13}\text{C}$ values indicated a combined C dependence source from allochthonous inputs and atmospheric CO_2 fixation (Post 2002), whereas moss $\delta^{13}\text{C}$ signal indicated a main aerial C source by fixation of CO_2 . Moss $\delta^{13}\text{C}$ signal plotted aside of the microbial mat food web, but likely related to primary producer fractionation by high C content exudates. Tardigrades and rotifers represented a significant fraction of the consumers during ice melting, and their activity transferred matter directly to upper trophic levels. SIAR model suggested mixed C sources for rotifers, including diatoms and cyanobacteria, which agrees with studies on other Antarctic and temperate freshwater ecosystems (Battaglia and Valencia 1997;

Mialet et al. 2013). Nematodes, the main grazers of the community, may have fed mostly on organic matter sized between 30 and 0.22 μm (Fig. 8) and occasionally on cyanobacteria, as described by the Bayesian mixing model (correlation coefficient = -0.86). Nematode feeding on cyanobacteria has been described by Gaudes et al. (2006), who suggested that cyanobacterial proteins might compensate for a lack of polyunsaturated fatty acids in nematode's diet.

Heterotrophic bacteria and fungi likely played an important role in the food web by allowing alternative carbon pathways within the mat, by scavenging organic matter, and transfer of carbon from primary producers to consumers. It would explain the unequal dynamics observed between primary and secondary productivity. The relation of fungal biomass with total POC suggests its dependence on mat temperature, which is in agreement with the observed psychrotrophic profiles of Antarctic fungi (Robinson 2001) and supports the idea that a suitable physical and chemical environment is needed for heterotrophic metabolisms (Yergeau et al. 2007). This would explain the relatively stable POC values observed throughout the whole season, if bacteria and fungi can only tap into the carbon pool to a limited extent due to prevailing low temperature regimes.

Metagenomic studies in Arctic microbial mats have demonstrated that genes involved in bacterial mineralization processes represent an important portion of the total gene pool present in the microbial mats (Varin et al. 2010). Nutrient recycling by bacteria might be extremely relevant for microbial mat survival and regeneration, but at the same time, might be accelerated by the concurrence of other organisms. This could be particularly relevant in these polar environments where the input of allochthonous organic C into the system is limited due the unproductive surrounding catchment (Lyons et al. 2013b). Likewise, N and P inputs are considered low because of the ultraoligotrophic characteristics of the running waters (Davey 1993). Similarly, our overlying water nutrients analyses confirm an oligotrophic profile of running water inputs. However, in situ assimilation bioassays and C:N:P ratios in the studied mat do not display a strong limitation. This agrees with previous polar studies where growth of microbial mats could not be increased through additional nutrients, and enriched nutrient concentrations were identified in the interstitial water of High Arctic (Bonilla et al. 2005) and Antarctic mats (Tang et al. 1997; Villeneuve et al. 2001). It also suggests that the studied community can be considered as a self-contained ecosystem with extremely low matter inputs, with likely a maximization of recycling processes.

Our findings suggest that Cyanobacteria, especially filamentous oscillatorian taxa, likely play an important role in

structuring the three-dimensional organization of the mat to provide a more suitable substrate to other more specialized organisms (de los Ríos et al. 2004) and likely represent the larger standing stock of C in polar ecosystems. However, they are likely not a direct driver of the food web in the microbial mats on Byers Peninsula, as most of their C is directly transferred to selected members of the upper trophic levels. The occurrence of unicellular cyanobacteria during ice-free conditions (e.g. *Synechococcus* spp.), however, may represent a direct C source to tardigrades. Furthermore, the prevalence of chlorophytes during ice-covered conditions may act as alternative C source, sustaining metazoan population under icy conditions, as was seen in the gut content of tardigrades and nematodes by fluorescence microscopy observations (E. Rico pers. comm.). Our results indicate cyanobacterial DOC exudates, also referred to as exopolymeric substances (EPS), are only partially consumed by fungi and may contribute to the overall mat community homeostasis by providing protection against temperatures and desiccation (De Los Ríos et al. 2014; De Maayer et al. 2014).

In summary, cyanobacterial-based mats from Byer Peninsula generated high C standing stocks, which had the potential to be reincorporated into the food web by fungal and bacterial activity. The overall productivity of the microbial mat is likely limited by number of trophic relationships, where a bacterial and fungal loop connects primary production with heterotrophic fractions of the community by grazing and detritivory. This implies that the microbial mat structure and function with large C standing stocks may represent an ecological adaptation of the microbial mat community to overcome fluctuating conditions across seasonal scales (Moyer et al. 1994) with high recycling rates that maximize the system stability by the occurrence of S-strategies sensu Grime (1979), where individuals maintain their metabolic performances.

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