

# Temperature effects on carbon and nitrogen metabolism in some Maritime Antarctic freshwater phototrophic communities

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**Abstract** Biofilms growing on ice and benthic mats are among the most conspicuous biological communities in Antarctic landscapes and harbour a high diversity of organisms. These communities are consortia that make important contributions to carbon and nitrogen input in non-marine Antarctic ecosystems. Here, we study the effect of increasing temperatures on the carbon and nitrogen metabolism of two benthic communities on Byers Peninsula (Livingston Island, Maritime Antarctica): a biofilm dominated by green algae growing on seasonal ice, and a land-based microbial mat composed mainly of cyanobacteria. Inorganic carbon photoassimilation, urea and nitrate uptake and  $N_2$ -fixation (acetylene reduction activity) rates were determined in situ in parallel at five different temperatures (0, 5, 10, 15, 25°C) using thermostatic baths. The results for the cyanobacterial mat showed that photosynthesis and  $N_2$ -fixation responded positively to increased temperatures, but urea and  $NO_3^-$  uptake rates did not show a significant variation related to temperature. This microbial mat exhibits relatively low activity at 0°C whereas at higher temperatures (up to 15°C),  $N_2$ -fixation rate increased significantly. Similarly, the maximum photosynthetic activity increased in parallel with temperature

and showed no saturation up to 25°C. In contrast, the ice biofilm displayed higher photosynthetic activity at 0°C than at the other temperatures assayed, and it showed elevated photoinhibition at warmer temperatures.

**Keywords** Antarctica · Benthic community · Photosynthesis · Temperature dependence · Cyanobacteria · Chlorophyta · Nitrogen uptake

## Introduction

Microorganisms that grow at low temperatures and nutrient availability, as well as cope with months of near complete darkness during winter, are often the dominant life forms in Antarctic freshwater ecosystems (Laybourn-Parry et al. 2002). These life forms have occupied the Continent since the Last Glacial Maximum (Convey and Stevens 2007). During the last century, warming of Antarctic Peninsula has significantly exceeded that of the continental area, as reported by Steig et al. (2009). The potential effects of this warming on living organisms need more thorough investigation. From this perspective, knowing the eco-physiological consequences of rising temperatures for different species assemblages can help us to estimate the impact of warming trends on communities and therefore on ecosystems.

Cyanobacterial mats consist of a matrix of mucilage in which dominant cyanobacteria and other algal cells are embedded together with heterotrophic and chemoautotrophic microorganisms, sand grains and other inorganic and organic materials (de los Ríos et al. 2004). Accordingly, the mats can be seen as multilayered structured ecosystems with relatively complex food webs. In Antarctica, these microbial communities usually dominate the benthic

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habitats of ponds and other shallow freshwater ecosystems. They often account for almost all biological productivity in some polar environments (Vincent and Howard-Williams 2000). In Maritime Antarctica, microbial mats located in the catchment area of oligotrophic lakes are also an important allochthonous source of nutrients for the lakes via run-off (Toro et al. 2007). These communities are found where some liquid water is available during the thawing period every year and have a time span of several years. In this sense, they can be viewed as perennial communities. Other typical benthic communities are those dominated by green algae growing in or on the surface of seasonal ice, communities well known from alpine areas at temperate latitudes (Hoham and Duval 2001; Hoham et al. 2008), throughout the Antarctic region (Ling and Seppelt 1993, 1998; Ling 1996; Novis 2002), and from the Maritime Antarctic (Mataloni and Tesolín 1997). These communities only become conspicuous if the mean summer air temperature reaches or exceeds 0°C (Ellis-Evans 1997). For this reason, they are only evident when ice is present. When the ice thaws, they shift to the next stage of their life cycle. Accordingly, they are considered temporary communities. They persist as resistant forms until the next favourable period.

Different adaptive strategies are found in these two ecosystems. One strategy is to become a cold-condition specialist. Such specialists exhibit evolutionary adaptations for thriving at low temperatures. The phytoflagellate *Chlamydomonas* and other green algae are good examples of this strategy. Some of their strains possess a photosynthetic apparatus adapted to function under low-temperature conditions (Morgan et al. 1998). These so-called psychrophilic organisms can grow only at temperatures below 15°C. In contrast, other groups of microorganisms that inhabit polar regions, including cyanobacteria, simply tolerate low temperatures and can grow despite the fact that such conditions put them far below their growth optimum (Tang et al. 1997). These organisms are called psychrotrophs.

Some limnological studies on microbial communities inhabiting shallow ecosystems have been performed in the South Shetland Islands and Antarctic Peninsula (Tell et al. 1995; Vinocur and Pizarro 2000), Continental Antarctica (Sabbe et al. 2004) and also in the Arctic (Bonilla et al. 2005). Few studies have investigated the effects of temperature variation in situ on microbial communities (Nadeau and Castenholz 2000; Pringault et al. 2001). The present work, a physiological study performed in situ, aims to compare the carbon and nitrogen metabolism of a psychrophilic biofilm and a psychrotolerant cyanobacterial mat growing in a mild Antarctic region where air temperature exceeds 0°C in summer.

## Materials and methods

### Study site

Byers Peninsula (Livingston Island, South Shetland Islands), the largest ice-free area in Maritime Antarctica, supports very abundant and diverse aquatic ecosystems (Toro et al. 2007). The area is located at the west end of Livingston Island (62° 34'35"–62°40'35"S and 60°54'14"–61°13'07"W) (Fig. 1). The peninsula, 60.6 km<sup>2</sup> in area, is largely free of ice and snow cover during almost the entire austral summer season (December to March). The low-lying areas of the peninsula favour the retention of surface water and include temporary waterlogged areas, lakes and pools (López-Martínez et al. 1996). The microbial mat studied here grew on the plateau of the peninsula. In contrast, the ice biofilm grew on the seasonally accumulated icy snow at the South Beaches (Fig. 1).

### Microscopic study

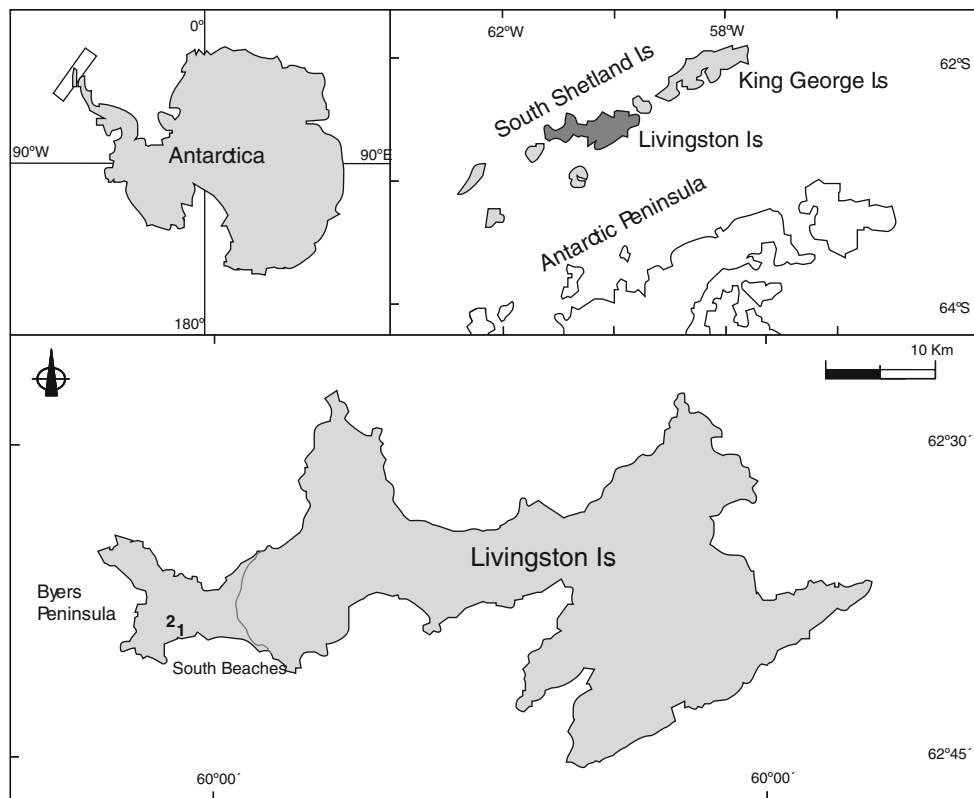
Samples for microscopic observations and taxonomic determinations were taken with 15- or 22-mm-inner diameter metal core samplers. Stones and gross-sized sediments were removed, and the samples were kept frozen at –20°C for microscopic determination. Observations were made using both bright field and epifluorescence microscopy at the field camp immediately after collection. The samples were subsequently sent to the laboratory in Spain for further microscopic study.

The composition of the phototrophic assemblages within the communities was studied with fresh samples, using two different fluorescence filters and Nomarski interference microscopy. For each microscopic field, observations were made 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.

Although recent taxonomic literature of the taxonomy for polar cyanobacteria was consulted (Komarek and Anagnostidis 1999, 2005), the Broady and Kibblewhite (Broady and Kibblewhite 1991), morphotype definitions were followed to provide simple morphological assignments. Ling and Seppelt (1998), Duval et al. (1999) and Mataloni and Tesolín (1997) were also used for taxonomical identification of the biofilm community.

### Experimental setting and analytical methods

The purpose of our experimental design was to allow physiological comparisons between two distinct communities found on Byers Peninsula: an ice biofilm (IB)



**Fig. 1** Location of Byers Peninsula on Livingston Island in the South Shetland Islands of Maritime Antarctica. (1) Site location of Ice Biofilm at South Beaches (5 m asl). (2) Site location of Purple Mat (70 m asl)

growing on the accumulated seasonal icy snow on the southern beaches of Byers Peninsula and dominated mainly by green algae and a microbial mat, the purple mat (PM), a well-developed cyanobacterial consortium growing on a shallow pond and highly stable across years.

Experiments were carried out in situ during the last week of January with material collected on the day of the experiment. Samples of both communities were obtained using a metal corer of 22 mm inner diameter. For each of the assayed temperature and light conditions, three replicates of each community were placed inside Whirl-pak<sup>®</sup> bags (60 ml, Nasco) filled with 10 ml of water (pond water or melted snow, for PM and IB, respectively) filtered through GF/F glass fibre filters (Whatman).

Uptake assays for inorganic carbon, nitrate, urea and nitrogenase activity were performed by means of the stable isotope technique (see below for further details) at the camp site using five thermostatic baths at temperatures of 0, 5, 10, 15 and 25°C. All the incubations were made for 2 h under natural light exposure. The temperatures logged on the mat surface where samples were collected during the week the study was performed were from 0 to 10°C for PM and from 1 to 4°C for IB.

Different irradiance conditions were also assayed in the determinations of inorganic carbon uptake. The different

light intensities for this assay were achieved by using neutral filters. The resulting light levels during incubations were 100, 63, 24, 15, 7.8, 4.5, 1.5, 0.8, 0.5 and 0% of incident light. The transmitted light was measured with a  $2\pi$  quantum sensor (Li-Cor, LI-192) connected to a data-logger (Li-Cor, LI-1000). After the experiments were finished, samples were washed to remove the non-fixed isotopes, frozen and transported to Spain.

In the laboratory, cores were weighed and dried at 60°C for 24 h. After grinding, the isotopic enrichment of samples relative to non-treated controls was determined using an IRMS Micromass-Isochrom mass spectrometer. The particulate organic carbon (POC) and nitrogen (PON) contents of both communities were measured from the dried samples using an Elemental Analyser LECO CHNS-932.

For chlorophyll *a* (Chl-*a*) analyses, the lipophilic pigments were extracted twice in dark conditions with 90% aqueous methanol for 24 and 1 h, consecutively. The concentration of extracted pigments was then measured in a diode array spectrophotometer (MultiSpec-151, Shimadzu). Absorbance was measured at two wavelengths: 750 nm, as a turbidity reference, and 665 nm, as a main peak of Chl-*a* absorbance. The Chl-*a* concentration values were expressed in  $\mu\text{g Chl-}a \text{ cm}^{-2}$  determined using the coefficient of Marker et al. (1980).

### Inorganic carbon uptake

Photosynthetic carbon assimilation was measured as  $^{13}\text{C}$  (98%  $^{13}\text{C}$ , Isotec) incorporation in samples, with  $\text{NaH}^{13}\text{CO}_3$  as a tracer (Ariosa et al. 2006) from a stock solution of  $1 \text{ mgC ml}^{-1}$ . The dissolved inorganic carbon (DIC) was calculated from water alkalinity (by considering pH and temperature), measured after titration with HCl using a pH shift indicator (phenolphthalein) of equivalence endpoint pH. Incubations were carried out in thermostatic baths at the temperatures and light conditions mentioned above. The incubations were started by adding the  $^{13}\text{C}$  tracer to the bags at an approximate concentration of 10% of the natural concentration of  $^{12}\text{C}$ . After 2 h, the incubations were stopped by adding 1 ml of 1 N HCl per bag to exclude non-assimilated  $^{13}\text{C}$  to the atmosphere in  $^{13}\text{CO}_2$  form. During this step, the bags were kept open. Then, 1 ml of 1 N NaOH was added to neutralize HCl, and samples were rinsed immediately with GF/F-filtered pond water. Samples were dried, frozen and transported to the laboratory.

### Nitrate and urea uptakes

Nitrate and urea uptake rates were measured by using urea- $^{15}\text{N}$  (98%  $^{15}\text{N}$ , CIL) and  $\text{K}^{15}\text{NO}_3$  (99.9%  $^{15}\text{N}$ , Isotec). The stock solutions used in the assays were 30 and  $4 \mu\text{gN ml}^{-1}$ , respectively. From these stocks, an appropriate volume was added to bags to reach 10% of the natural concentration of the light isotope, as measured the previous year in the same area. The urea was not measured in the environment and was added at the same concentration as DIN.

### Nitrogenase activity

The rates of  $\text{N}_2$ -fixation (nitrogenase activity) were determined as the acetylene reduction activity (ARA) after collecting the biomass, as described by Fernández-Valiente et al. (2001), with the same light intensity as the PvsI curves described above. In brief, two cores of 22 mm inner diameter from each community were immersed in 100 ml of GF/F-filtered surrounding water in 250-ml flat tissue culture flasks (IWAKI, Asahi Glass Co., Ltd) sealed with rubber stoppers. The experiments were run in triplicates. To each flask, pure acetylene obtained from calcium carbide was added at 10% (v/v) concentration. After 4 h of incubation, ethylene produced by the nitrogenase from acetylene reduction was collected by extracting air from the incubation flasks and transferred to 10-ml vacuum tubes (Vacutainer). The tubes were wrapped with film and stored at  $4^\circ\text{C}$  for shipping to the laboratory. There, ethylene

concentration was determined by a gas-chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector using a Porapak N80/100 column.

### Mathematical and statistical analyses

Photosynthesis versus irradiance curves for purple mat (PM) were fitted to the Platt photosynthesis model (Platt et al. 1980), which follows a hyperbolic tangent equation at each temperature, using Sigmaplot software (Systat Software Inc.). Here,  $P_s$  is defined as the estimated maximum photosynthetic rate,  $\alpha$  as the initial slope of the curve (a measure of photosystems efficiency) and  $E$  as the irradiance in each interval of the curve. The ice biofilm (IB) data did not fit this equation. Our experimental data unexpectedly showed extremely quick saturation of photosynthesis and photoinhibition, even under very low irradiances. In this case, the experimental data were fit to the exponential difference equation proposed by Ritchie (2008). Here, plotted values were the values predicted by least square fitting. Correlation was expressed using the Pearson correlation coefficient. To compare the different assays for both communities,  $Q_{10}$  values were calculated. The  $Q_{10}$  value obtained over the range from 0 to  $25^\circ\text{C}$  was used as a standard indicator of the cold adaptation of the physiological activities measured. This value reflects activity variation within a given temperature increment (Vincent 1988) as follows:

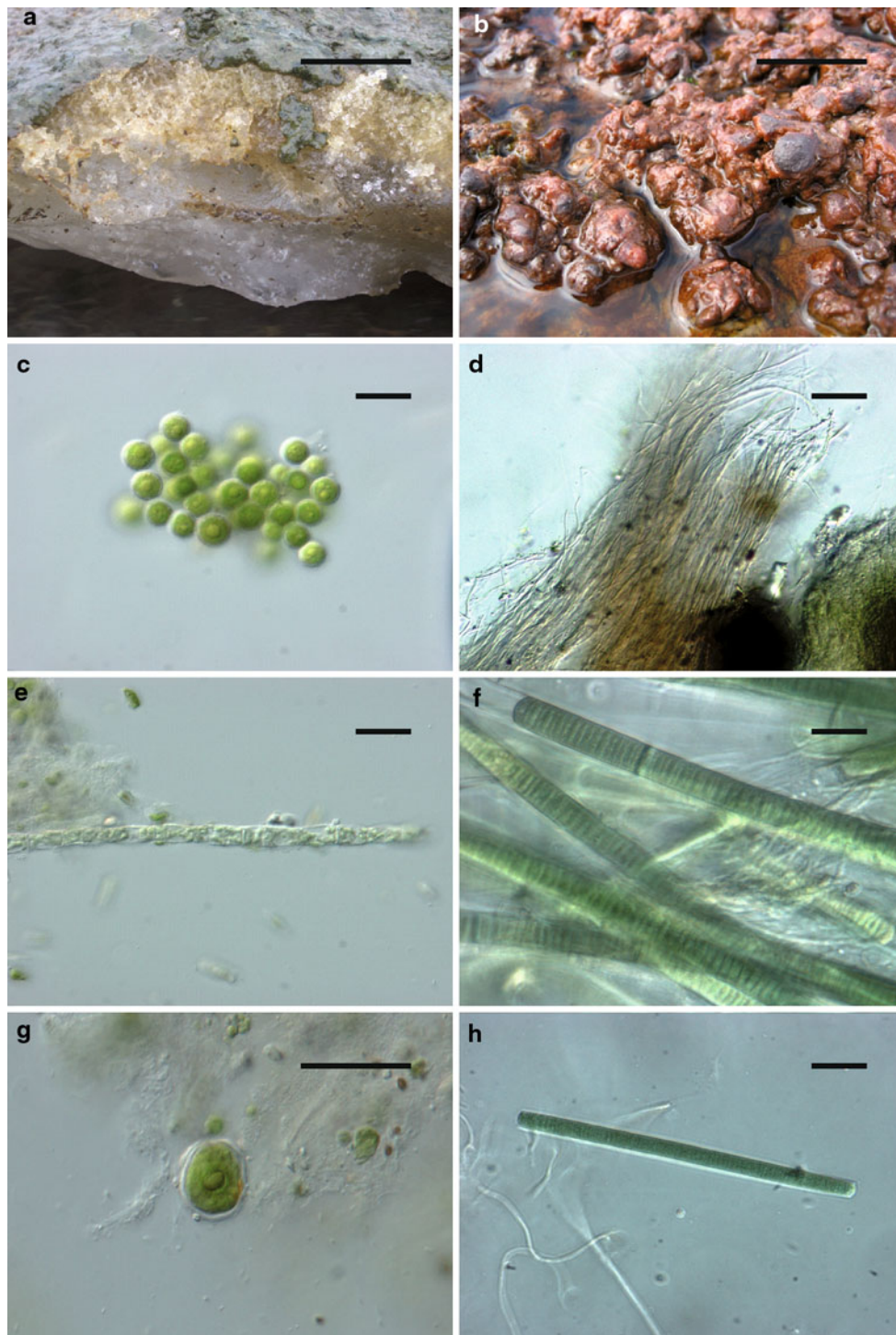
$$Q_{10} = (k_2/k_1)^{10/(t_2-t_1)}$$

where the expression  $(k_2/k_1)$  is the activity ratio at both temperatures assayed and  $(t_2 - t_1)$  is the difference in temperature. If appropriate, a paired  $t$ -test or ANOVA with the Tukey post hoc test was used to compare the means of different treatments. SPSS Statistics 17.0 (SPSS Inc., 2008) and Sigmaplot 11.0 (Systat Software Inc.) were used for all statistical procedures.

## Results

### Phototrophic community structure

The two communities studied differed macroscopically. The ice biofilm (IB) was dark green at the surface, thinner (1–1.5 mm) and had a gelatinous texture (Fig. 2a). In contrast, the microbial mat (PM) showed a purple-greenish surface colour, was 3–4 mm in thickness, and had a rugged surface (Fig. 2b). The substrates for the communities are also different. IB occurred in seasonal ice from precipitation, whereas PM occurred in semipermanent shallow ponds.



**Fig. 2** Microphotographs of the communities assayed. *Scale bar* represents 10  $\mu\text{m}$  in (a, c, e, g) and 1 cm in (a, b). a, c, e, g Ice biofilm. b, d, f, h Purple Mat. a Macroscopic mat structure of IB.

b Macroscopic mat structure of PM. c Cryobiontic *Chlorophyta*. d PM matrix. e *Klebsormidium* sp. g *Chlamydomonas* sp. f, h cf. *Phormidium* spp

Microscopic observations showed marked differences in composition among the communities. The matrix of PM consisted of two thin cyanobacteria of the morphotypes I (1  $\mu\text{m}$  in diameter) and J (3  $\mu\text{m}$  in diameter) *sensu* Broady and Kibblewhite (1991) (Fig. 2d). The work of these

authors is based on the Oscillatorian diversity of Ross Island and Southern Victoria Land in Continental Antarctica. Intermixed with the matrix filaments were many unicellular cyanobacteria (1.5  $\mu\text{m}$  in diameter). Thicker cyanobacteria from morphotype C (5.5–6  $\mu\text{m}$  in diameter)

were also present within the mat. According to Anagnostidis and Komarek (1988), all these morphotypes can be assigned to different species of the genus *Phormidium* (Broady and Kibblewhite 1991), but morphotype I presumably belonged to genus *Leptolyngbya*. Other filamentous cyanobacteria present in the surface layer included different *Phormidium* species assigned to morphotypes B and K (4–5  $\mu\text{m}$  in diameter) (Fig. 2h) and morphotype E (11  $\mu\text{m}$  in diameter), which were also abundant (Fig. 2f). Furthermore, an unidentified filamentous cyanobacterium with a dark thick sheath (18  $\mu\text{m}$  in diameter) was observed profusely at the mat surface layer. Cyanobacterial cells surrounded by thick sheaths and 5.5  $\mu\text{m}$  in diameter were observed as well. These cyanobacteria probably belong to the family *Phormidiaceae* because they exhibited a thick, lamellated and coloured sheath. Diatoms were also present in this mat but at low densities. On the other hand, abundant microcolonies of *Nostoc* sp. appeared in the deepest layer. In contrast, the IB photosynthetic community was dominated by the forms of the genera *Chloromonas* sp., *Chlamydomonas* sp. and *Klebsormidium* sp. (green algae) (Fig. 2c, g, e). Moreover, some filamentous cyanobacteria presumably of the genus *Phormidium* (assigned to the morphotypes B and K, 4–5  $\mu\text{m}$  in diameter) and thinner filamentous cyanobacteria of the genus *Leptolyngbya* were found within the community.

The values of dry weight per unit surface area were very similar in both communities investigated (Table 1). Results for chemical composition were as follows: PM showed slightly higher C content per dry weight than IB (*t*-test:  $P < 0.001$ ) (Table 1). In contrast, N content, as a fraction of dry weight, was almost twofold higher in IB than in PM (*t*-test:  $P < 0.001$ ). This difference in N content resulted in much higher C/N ratios for the PM community than for the IB community (ANOVA:  $P < 0.001$ ). The chlorophyll

*a* (Chl-*a*) content per surface area did not show statistical differences between communities (ANOVA:  $P$  value = 0.381). Both communities showed differences in their carbon and nitrogen isotopic composition, and this result suggests some metabolic differences. Hence, although the  $^{13}\text{C}$  natural abundance was significantly more negative in PM (ANOVA:  $P$  value  $< 0.001$ ), the  $^{15}\text{N}$  isotopic signatures were very similar for both communities (ANOVA:  $P$  value = 0.809).

#### Photosynthetic features under different temperatures

Because the experiments were conducted under natural conditions on different days for both communities, the irradiance regimes were different for both assayed communities. Maximum irradiance values during the incubations experiments were 380 and 1,852  $\mu\text{E m}^{-2} \text{s}^{-1}$  for the purple mat (PM) and the ice biofilm (IB), respectively.

Carbon assimilation in PM increased with temperature (Fig. 3a). Indeed, at 25°C, the maximum photosynthetic rates were twofold higher than at 0°C. The PvsI curves for this community were, however, similar from 0 to 10°C and showed significantly lower values of maximum estimated photosynthesis ( $P_s$ ) compared with those found at 15 and 25°C (Fig. 3a). Photoinhibition was not found at any temperature at the irradiances assayed (Fig. 3a). Photosynthetic efficiency values ( $\alpha$ ) were all very similar at the different temperatures assayed and ranged between 0.01 and 0.02  $\mu\text{g C cm}^{-2} \text{h}^{-1} (\mu\text{E m}^{-2} \text{s}^{-1})^{-1}$  (Table 2). Results for this community data indicate low rates of photosynthesis at lower temperatures. Increases in temperature were accompanied by increased rates of carbon assimilation, ca. a twofold increase (Table 2). In this sense,  $Q_{10}$  values calculated between 0 and to 25°C for each carbon uptake ( $P_s$ ) assay exhibited a relevant temperature effect, with  $Q_{10} = 1.8$ .

The different PvsI curves obtained as a function of the temperature in the ice biofilm (IB; Fig. 3b) were fitted to the exponential difference equation proposed by Ritchie (2008). This formulation accounts for the photoinhibition of the system more accurately than other previously proposed models (Platt et al. 1980; Jassby and Platt 1976; Webb et al. 1974; Frenette et al. 1993). Probably, owing to the extreme thinness and heterogeneous composition of this community, the data dispersion was quite high. Uncertainty in the estimated parameters could be excessively high in some cases. Nevertheless, photoinhibition was evident at 0°C, a naturally occurring temperature for organisms growing on ice. The  $P_s$  values increased with irradiance (Fig. 3b) up to the highest level and then decreased at the highest irradiance. The PvsI curves at 5°, 10°, 15° and 25°C were likewise very similar and exhibited clear photoinhibition effects at very low irradiance

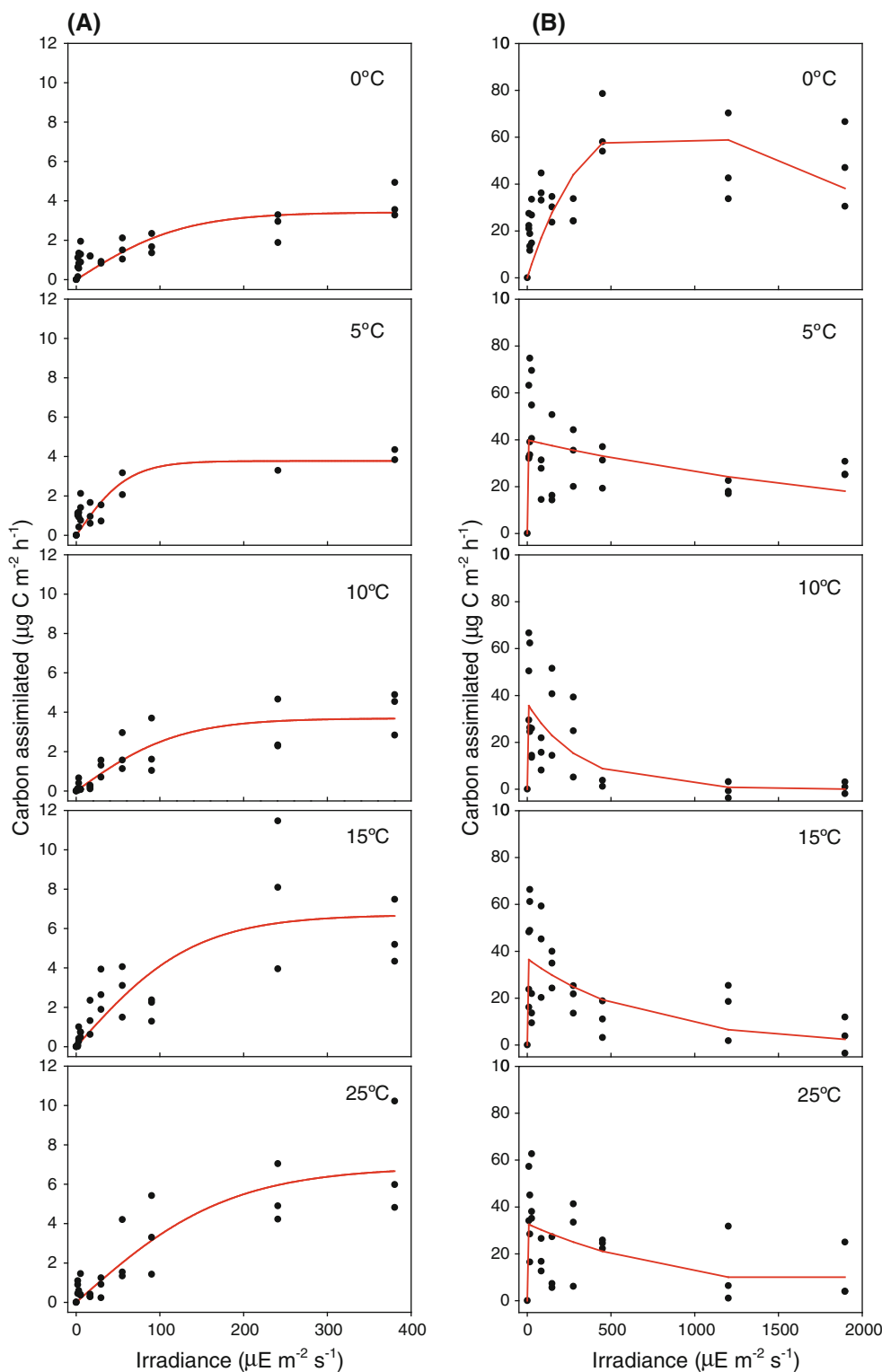
**Table 1** Biochemical characterization of the surveyed microbial communities

Variable	Purple mat (PM)	Ice biofilm (IB)
DW ( $\text{mg cm}^{-2}$ )	153 $\pm$ 78	151 $\pm$ 99
Carbon ( $\text{mg gdw}^{-1}$ )	228.4 $\pm$ 3.4	210 $\pm$ 2
Nitrogen ( $\text{mg gdw}^{-1}$ )	18.3 $\pm$ 0.2	35.9 $\pm$ 0.97
C/N	12.5 $\pm$ 0.1	5.9 $\pm$ 0.2
Chlorophyll <i>a</i> ( $\mu\text{g cm}^{-2}$ )	20.0 $\pm$ 3.5	16.1 $\pm$ 5.9
DIN ( $\mu\text{g N l}^{-1}$ ) <sup>a</sup>	50	162
DIC ( $\text{mgC l}^{-1}$ ) <sup>a</sup>	1.44	1.88
$\delta^{13}\text{C}_{\text{PDB}}$	-12.1 $\pm$ 0.6	-9.3 $\pm$ 0.3
$\delta^{15}\text{N}_{\text{air}}$	2.5 $\pm$ 0.4	2.4 $\pm$ 0.8

DW dry weight, DIN dissolved inorganic nitrogen, DIC dissolved inorganic carbon. Data are averages of three or more replicates for most analysis

<sup>a</sup> Community-overlying water for PM and snow in the IB case

**Fig. 3** Photosynthesis versus irradiance curves for each incubation temperature in both communities studied. **a** Purple mat (*PM*), **b** ice biofilm (*IB*). Mean irradiance values during the assays were  $1,852 \mu\text{E m}^{-2} \text{s}^{-1}$  for the Purple mat and  $380 \mu\text{E m}^{-2} \text{s}^{-1}$  for the ice biofilm. The line shown for PM represents the fitted values obtained using the Platt et al. model (1980). The line shown for IB represents smoothing values of the data



(Fig. 3b). At higher temperatures,  $P_s$  values were very similar (an average of  $36.3 \mu\text{g C cm}^{-2} \text{h}^{-1} \pm 2.9$ ) and they took place under very low irradiance (between 15 and  $26 \mu\text{E m}^{-2} \text{s}^{-1}$ , values extracted from the raw data), followed by an intense photoinhibition, except in the case of

the 0°C assay. Those  $P_s$  values were one order of magnitude higher than those found in the PM, even when expressed as specific activity ( $\mu\text{g C} (\mu\text{g Chl } a \text{ h})^{-1}$ ). Moreover, in IB, there were no relevant differences in C uptake between low and higher temperature treatments, and

**Table 2** Estimated photosynthetic parameters from both communities

Community	Temperature (°C)	$\alpha$	$P_s$	$P_s^{\text{Chl}}$	$r^2$	$E_k$
PM	0	0.010	3.42	0.17	0.57	342.0
	5	0.023	3.77	0.19	0.61	163.9
	10	0.011	3.69	0.18	0.78	335.5
	15	0.018	6.70	0.33	0.67	372.2
	25	0.014	6.88	0.34	0.78	491.4
IB	0	0.23	65.5	4.07	0.71	284.8
	5	80.1	39.8	2.47	0.53	0.50
	10	77.8	36.3	2.25	0.70	0.46
	15	74.1	36.7	2.19	0.63	0.49
	25	71.0	32.7	2.03	0.51	0.46

Maximum photosynthesis ( $P_s$ ) values are in  $\mu\text{g C cm}^{-2} \text{h}^{-1}$ . Photosynthetic efficiency ( $\alpha$ ) units are  $\mu\text{g C cm}^{-2} \text{h}^{-1} (\mu\text{E m}^{-2} \text{s}^{-1})^{-1}$ . These values were fitted to Platt et al. (1980) model for each photosynthesis versus irradiance curves at given temperature for PM. IB data were fitted to an exponential difference equation proposed by Ritchie (2008). Chlorophyll-specific photosynthetic rates ( $P_s^{\text{Chl}}$ ) are measured as  $\mu\text{g C} (\mu\text{g Chla h})^{-1}$ .  $r^2$  is identified by the fitted boundary of the models

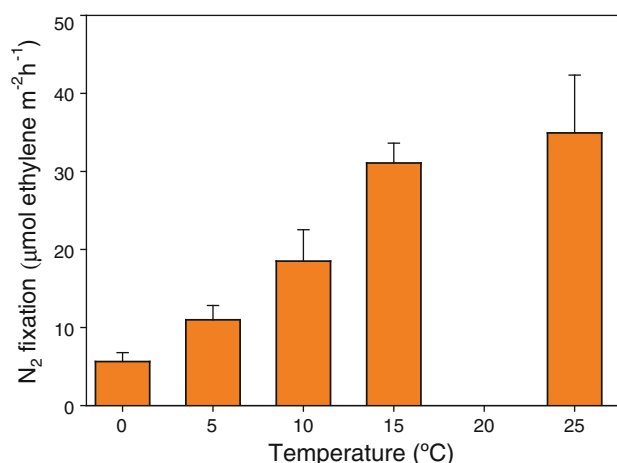
the  $Q_{10}$  for  $P_s$  between 0 and 25°C was 0.5, indicating a lower activity rate.

The parameters derived from the light-dependent curves difference significantly between communities. This result indicates a relative difference in photosynthetic light efficiency at low irradiances (Fig. 3; Table 2). The irradiance of saturation ( $E_k$ ) values (defined as  $P_s/\alpha$ ) obtained in PM was somewhat similar at all the temperatures assayed (Table 2), averaging  $341 \pm 117 \mu\text{E m}^{-2} \text{s}^{-1}$ . In contrast,  $E_k$  for IB averaged  $0.48 \pm 0.02 \mu\text{E m}^{-2} \text{s}^{-1}$  (excluding the  $E_k$  value for 0°C,  $284.8 \mu\text{E m}^{-2} \text{s}^{-1}$ ). For the photosynthetic efficiency ( $\alpha$ ), IB shows higher values, reflected in the near vertical slopes of the PvsI curves. Owing to the strong differences in natural light conditions when the experiments were carried out, these results should be interpreted cautiously.

Chlorophyll-specific photosynthetic rates ( $P_s^{\text{Chl}}$ ) were one order of magnitude higher in IB compared with PM (Table 2), ranging respectively  $2.06\text{--}4.07 \mu\text{gC} (\mu\text{g Chla h})^{-1}$  and  $0.17\text{--}0.34 \mu\text{gC} (\mu\text{g Chla h})^{-1}$ . These differences parallel those observed for  $P_s$ .

#### Temperature dependence of nitrogenase activity

Significant acetylene reduction activity (ARA) was found only in the PM. Likewise, heterocystous  $\text{N}_2$ -fixing cyanobacteria (Nostocales) were only found in this community, whereas non-heterocystous cyanobacteria, also thought to be potentially  $\text{N}_2$ -fixing, were a minority in IB. Our results indicated that  $\text{N}_2$ -fixation depended strongly on temperature (Fig. 4), with a  $Q_{10}$  value defined between 0 and 25°C



**Fig. 4** Results of the acetylene reduction assays (nitrogenase activity) at each assayed incubation temperature for PM. The assay was performed during the afternoon. Irradiance during the experiment ranged from 255 to 755  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Data are means  $\pm$  SD of three replicates

of 5.8. ARA increased up to 15°C, showing no significant differences from the activity measured at 25°C (ANOVA:  $P$  value = 0.823); on the contrary, significant differences were found when the values from 0 to 10°C were compared with those of 15 and 25°C (ANOVA:  $P$  value <0.001). On average,  $\text{N}_2$ -fixation measured at 25°C was sevenfold higher than that found at 0°C.

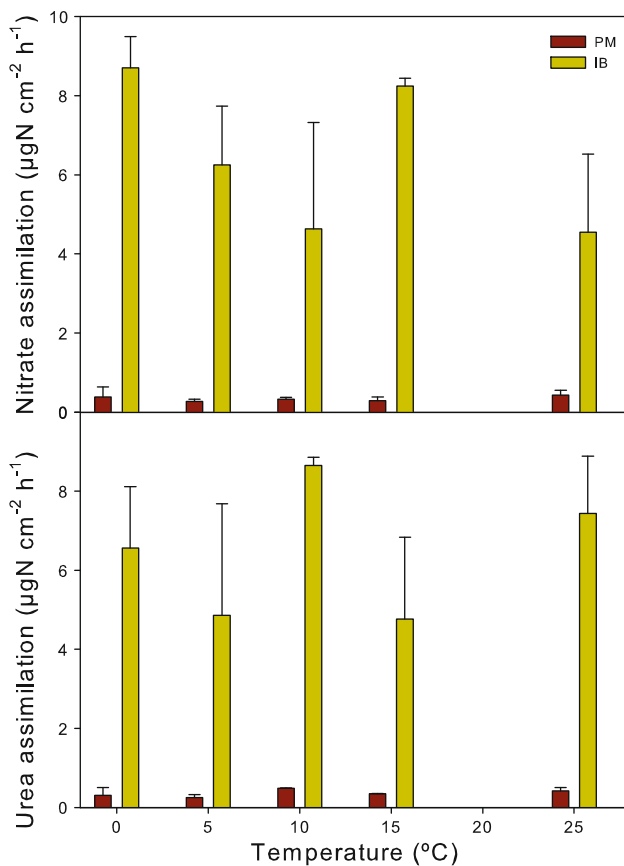
#### Urea and nitrate temperature-dependent uptake

Trends in the assimilation of urea and nitrate at different temperatures under natural light were assayed in parallel with PvsI incubations (see “Materials and methods”). Uptake rates of nitrogen compounds for both communities suggested that nitrogen uptake was independent of temperature regime (paired  $t$ -test;  $P$  value <0.0001 and  $P$  value <0.0001 for urea and nitrate uptake, respectively, in PM; and  $P$  value <0.0001 and  $P$  value = 0.0002 for urea and nitrate uptake, respectively, in IB) (Fig. 5). As shown in Fig. 5, urea and nitrate uptake ratios were almost one order of magnitude lower in PM than in IB at each temperature evaluated.

#### Discussion

Maritime Antarctica is one of the regions of the Earth where climate change is most pronounced (Quayle et al. 2002). Nevertheless, studies of the effects of temperature change on the physiological activity of aquatic organisms from this region are scarce. Our research addressed the need for such additional studies. We sought to analyse and to compare the short-term effects of temperature shifts on





**Fig. 5** Nitrate and urea uptakes at different temperatures expressed per unit surface area in two microbial communities at Byers Peninsula, Livingston Island (Antarctica). Data are means  $\pm$  SD of three replicates

carbon and nitrogen metabolisms in two Antarctic microbial communities, one dominated by cyanobacteria (purple mat, PM) growing on a shallow pond and the other dominated by chlorophytes (ice biofilm, IB) growing over annual ice. The experiments were run without preincubation periods in order to measure the organisms' physiological status at the moment of the experiment. We chose this approach because experiments including previous acclimation can produce strain-dependent results (Hoham et al. 2008). Such results can be difficult to interpret for natural mixed communities.

PM is a perennial mat-forming community that has remained viable for several years (personal observation). It is entirely exposed to fluctuating physical conditions at daily and seasonal scales as temperature or liquid water availability varies, but it is covered by snow and ice from fall until spring. The main components of PM, cyanobacteria, are considered to be psychrotrophs instead of psychrophiles. Psychrotrophy has been already described by Mueller et al. (2005) for other cyanobacterial mats in the high Arctic. In contrast, Nadeau and Castenholz (2000) isolated a cyanobacterium from a pond on the

McMurdo Ice Shelf (Antarctica) and found that this organism appeared to be psychrophilic. Their cyanobacterium exhibited higher physiological activity at lower temperatures characteristic of different polar ecosystems (Taton et al. 2003). Nevertheless, truly psychrophilic strategies would not be suitable in shallow water ecosystems in the non-continental region of Antarctica, where summer water temperatures very often reach 15°C. In contrast, IB is a seasonal community that disappears after the ice melt and emerged only under particular conditions. Indeed, on Byers Peninsula, this type of community developed completely only once in six summers. The environmental conditions in late spring, when this community grows, are quite stable with similarly cold temperatures provided by the ice-melt-water interphase. Chlorococcales and Volvocales are the primary constituent taxa of IB. This cryosestonic community has some taxa in common with the assemblages previously described by Mataloni and Tesolín (1997). The organisms in those assemblages are generally considered psychrophilic. As long as the ice remains, this community enjoys optimal conditions. When temperatures rise, cells differentiate into resistant spores (Hoham et al. 2008).

Our main hypothesis about the differing strategies found in the two types of communities agrees well with the  $P_s$  curves obtained. In fact, these curves show completely different patterns.  $P_s$  values for PM exhibit a remarkable increase in response to increasing temperatures up to 15°C. In contrast, the values of this parameter in IB decreased dramatically from 5°C upwards. A possible explanation of these observations is that the cold-tolerant organisms forming the PM community survive under suboptimal temperatures and can thrive under a broad range of conditions, even though their photosynthetic activity slows down at colder temperatures. In contrast, the organisms inhabiting the IB are stenothermal, displaying an optimal temperature for maximum photosynthesis close to 0°C; indeed, at temperatures slightly above 0°C the  $P_s$  in this biofilm is markedly reduced by 45%. Nevertheless, at least in the short term, temperatures as high as 25°C did not inhibit photosynthetic activity under moderate photon flux rates. Differences between the two communities are even more evident from a comparison of the  $Q_{10}$  values. Although IB shows a negative  $Q_{10}$  value, PM shows a positive (1.8) value. These results clearly indicate that the IB organisms are well suited to cold temperatures, whereas the PM organisms do best under warmer conditions. Other Antarctic communities dominated by Oscillatoriaceae exhibited  $Q_{10}$  values between 2.3 and 1.7 (Goldman et al. 1963; Vincent and Howard-Williams 1986). A  $Q_{10}$  value of 2 is the assumed theoretical value for temperature-dependent kinetic effects (Falkowski and Raven 1997) for non-cold-adapted organisms. So far as we know, no other findings

on this topic for Antarctic benthic communities composed of chlorophytes have been published to date.

The results for photosynthetic efficiency ( $\alpha$ ) indicate higher values in the IB community than in PM. This finding suggests that the photosynthetic mechanism of chlorophytes captures light more efficiently than does that of cyanobacteria (Table 2). Furthermore, this higher photosynthetic efficiency might explain the greater amount of photoinhibition exhibited by the IB community with increasing temperature (Fig. 3b). If the majority of the IB organisms are truly psychrophilic, then they should be heat stressed under high temperatures. Photoinhibition in vascular plants is known to be greater under high light regimes. Under these circumstances, the xanthophyll cycle plays a crucial role in protecting PSII against heat-induced photoinhibition (Yin et al. 2010). However, our findings seem to disagree with the results obtained by Remias et al. (2005) for the snow algae communities from a high-mountain environment. That study found no photoinhibition up to  $1,800 \mu\text{E m}^{-2} \text{s}^{-1}$ . Furthermore, the highest photosynthetic rate found was in higher temperatures. In contrast, other studies, e.g. Ling and Seppelt (1998, 1993), found that Antarctic snow algae were killed by temperatures above  $10^\circ\text{C}$ .

Although the IB community showed no detectable nitrogenase activity, the rates of  $\text{N}_2$ -fixation in the PM community were in the same range as that suggested by previously published data from the area (Fernández-Valiente et al. 2007). Indeed, the dominance of heterocystous cyanobacteria (Nostocales) in the PM mat was confirmed by microscopic observation, even though these organisms were absent from the IB biofilm. The temperature dependence of  $\text{N}_2$ -fixation was higher for Byers Peninsula than for other parts of Antarctica (e.g. East Antarctica) (Howard-Williams et al. 1989). This difference is evident from the relative  $Q_{10}$  values, despite the fact that the analyses involved different types of communities (streams vs. ponds). The dependence of  $\text{N}_2$ -fixation on temperature has also been described in tropical oceans (Staal et al. 2003). The temperature independence of N uptake in both communities indicates that even if one of them is psychrophilic for a given metabolic activity, the same is not necessarily the case for others.

The low fixation rates of both inorganic carbon and  $\text{N}_2$  observed in the PM community at around  $5^\circ\text{C}$ , added to its sensitivity to temperature changes, suggest that this cyanobacteria-based community accumulates biomass during the brief periods of warmer summer temperatures. This strategy contrasts notably with that observed for the IB biofilm. The latter community exhibits explosive growth when conditions are optimum. The extremely high inorganic C and combined N uptake rates observed during colder periods permit extremely rapid growth rates in this

cold-adapted community (authors' observations). Indeed, during this short period of appropriate conditions, the IB community can produce as much biomass as the PM, measured by carbon and chlorophyll *a* contents. But, in contrast to the PM mat, the development of this biofilm is greatly restricted to short time periods and continues only until the ice thaws.

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