

Community structure and physiological characterization of microbial mats in Byers Peninsula, Livingston Island (South Shetland Islands, Antarctica)

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Abstract

The community structure and physiological characteristics of three microbial mat communities in Byers Peninsula (Livingston Island, South Shetland Islands, Antarctica) were compared. One of the mats was located at the edge of a stream and was dominated by diatoms (with a thin basal layer of oscillatorian cyanobacteria), whereas the other two mats, located over moist soil and the bottom of a pond, respectively, were dominated by cyanobacteria throughout their vertical profiles. The predominant xanthophyll was fucoxanthin in the stream mat and myxoxanthophyll in the cyanobacteria-dominated mats. The sheath pigment scytonemin was absent in the stream mat but present in the soil and pond mats. The stream mat showed significantly lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ values than the other two mats. Consistent with the $\delta^{15}\text{N}$ values, N₂ fixation was negligible in the stream mat. The soil mat was the physiologically most active community. It showed rates of photosynthesis three times higher than in the other mats, and had the highest rates of ammonium uptake, nitrate uptake and N₂ fixation. These observations underscore the taxonomic and physiological diversity of microbial mat communities in the maritime Antarctic region.

Introduction

Microbial mats are dense communities of vertically stratified microorganisms comprising largely cyanobacteria. These mats typically consist of a matrix of mucilage in which cyanobacterial trichomes and other algal cells are embedded together with other heterotrophic and chemoautotrophic microorganisms and with sand grains and other inorganic materials (De los Ríos *et al.*, 2004). The morphology, structure and colour of microbial mats are determined by the dominant species, sediment features and other environmental factors (Stal, 2000). The stratification of these communities can often be recognized by some degree of vertical colour zonation due to the different pigmentation of phototrophic microorganisms (Vincent *et al.*, 1993a). Cyanobacterial mats are found in a broad range of environments, some of which can be considered extreme, such as hypersaline ponds and lakes, thermal springs, dry and hot deserts and the cold environment of polar regions (Stal, 2000). In the Arctic and Antarctic regions, cyanobacterial mats are widespread in freshwater environments and they

often dominate the total biomass and productivity (Vincent, 2000).

The ubiquitous presence of cyanobacterial mats as well as their taxonomic composition and physiological activities in streams, ponds, lakes and melt-waters of different places in continental Antarctica are well documented (Howard-Williams & Vincent, 1989; Howard-Williams *et al.*, 1989; Broady & Kibblewhite, 1991; Vincent *et al.*, 1993a, b; Ellis-Evans, 1996; Fernández-Valiente *et al.*, 2001; De los Ríos *et al.*, 2004; Sabbe *et al.*, 2004). However, in the area of the maritime Antarctica these aspects are less well defined. This region is characterized by a less extreme climatic regime than other Antarctic areas, with higher mean temperatures and precipitation (Camacho, 2006). These climatic conditions as well as the complex geology lead to a great variety of water bodies and to a high number of ice-free freshwater ecosystems during summer months (Ellis-Evans, 1996). Taxonomical studies of microbial mats in Antarctic Peninsula (Vinocur & Pizarro, 1995) and King George Island (Vinocur & Pizarro, 2000) showed a richer species composition than in continental Antarctica, reflecting the broad

range of physical and chemical conditions of the studied lakes and ponds.

Physiological studies on carbon and nitrogen dynamics in two microbial mats in Livingston Island (Davey, 1993a, b) suggested that they were not limited by nutrient availability, whereas nitrogen fixation in these mats accounted just for 0.1–0.3% of the nitrogen accumulation by the mat. This situation contrasts with that of ponds of the McMurdo Ice Shelf in continental Antarctica, which are usually considered as N limited (Hawes *et al.*, 1993) and where nitrogen fixation could satisfy at least one-third of the N requirements of microbial mats (Fernández-Valiente *et al.*, 2001). However, carbon fixation showed similar rates both in maritime and continental Antarctic microbial mats (Davey, 1993a, b).

This research is part of an interdisciplinary project aimed at investigating the biological and ecological characteristics of aquatic ecosystems from Byers Peninsula in Livingston Island (South Shetland Islands) and to evaluate their sensitivity to potential climate change. Byers Peninsula is one of the most important limnological areas in maritime Antarctica (Ellis-Evans, 1996). It is an ice-free area with a high concentration of non-perennial ice-covered lakes and streams that has been designated as an Antarctic Specially Protected Area (ASP No. 126) through several recommendations under the Antarctic Protected Areas System because of its geological, archaeological and biological values. The present study was undertaken to examine in detail the community specific dominance, biomass composition, pigment content, photosynthesis, combined N assimilation and N₂ fixation of three representative microbial mats from Byers Peninsula.

Materials and methods

Study site

Byers Peninsula is located at the western end of Livingston Island. The peninsula is a 60.6-km² area mostly free of ice and snow cover during part of the Antarctic summer. The flat surfaces in Byers and the presence of over-deepened areas by glacial erosion have favoured water retention in more than 110 lakes and ponds of variable sizes, covering a total surface area of about 1.5% of Byers Peninsula (López-Martínez *et al.*, 1996). One of the studied microbial mats (stream mat) was located in the water-flooded shore of a small stream at South Beaches. The microbial mat was permanently covered by water, although the overlying water flux was low as most water circulated through the central part of the stream channel in which the mat did not appear. The second studied microbial mat (soil mat) was located in the central south-western plateau of the Peninsula at about 65 m a.s.l. on the moist soil of the north face of the catchment area of the Limnopolar Lake. The mat was not covered by water from the lake but remained damp through-

out because of heavy rainfall and runoff. The third studied mat (pond mat) was located in a small ephemeral pond about 500 m away from the second one. The pond was fed by snowmelt from a small catchment area but remained without any surface flow from mid summer (first week of January), so the mat was exposed to air from mid January, although it remained damp because of heavy rainfall, at least until sampling was done. Field work was conducted from 18 to 28 January 2002; the daily mean air temperature during this time ranged from 0.8 to 2.2 °C, relative humidity was very stable with values of about 90%, and monthly total rainfall ranged from 47 to 72 mm m⁻² (Bañón, 2004).

Water analysis

Mat-overlying water samples for nutrient (inorganic nitrogen and phosphorus) analysis were collected in acid-washed polyethylene bottles. All samples were filtered *in situ* using Whatman glass-fibre filters (grade GF/F) and the filtrate stored frozen until being analyzed in Spain. Filtrates were analyzed following standard analytical methods (APHA, 1992). Nitrate plus nitrite was measured after reduction of nitrate to nitrite and then by determination of nitrite with sulphonylamide and *N*-(1-naphthyl)ethylenediamine. Ammonia was analyzed using the phenol-hypochlorite method. Soluble reactive phosphorus was determined by the phosphomolybdic acid-ascorbic method. All these determinations were made spectrophotometrically using a Beckman DU-7 spectrophotometer.

Community analyses

Samples were taken with a metal corer (13–15 mm inner diameter) from areas of the microbial mats with homogeneous colour, texture and thickness. The cores were carefully cleaned of any soil or sediment particles and stored frozen (–20 °C) until being analyzed in Spain by light and epifluorescence microscopy. The classification system of Anagnostidis and Komarek (1988) and Komarek and Anagnostidis (1989) and the morphotype analysis of Broady & Kibblewhite (1991) were used for taxonomic identification of cyanobacteria. Diatoms were classified following Germain (1981) and Krammer & Large-Bertalot (1986, 1988, 1991). Dry weight was determined on samples kept frozen after melting and drying at 103 °C for 6 h. The weight of organic matter was determined after ignition of dry samples at 460 °C for 24 h, and the weight loss then subtracted from the previous weight (APHA, 1992). Total C and N contents of subsamples of dried, weighed cores were determined using an elemental analyzer (Perkin-Elmer 2400CHN) with a thermal conductivity detector. Total P was determined after persulphatic-acid hydrolysis of the mat at 135 °C for 2 h to hydrolyze any P-form to orthophosphate, and then orthophosphate was measured using the phosphomolybdic

acid-ascorbic method after pH-neutralization. Natural abundance of ^{13}C and ^{15}N was analyzed with an IRMS Micromass-Isochrom mass spectrometer.

Pigment determination

After sonication, photosynthetic pigments were extracted from the frozen cores with 90% acetone (chromatography grade) at 4 °C, concentrated by evaporation and resuspended in a smaller volume of acetone. Separation was performed with a Waters 2690 HPLC system equipped with a photo-diode array detector (Waters 996) on a Spherisorb S5 ODS2 chromatography column following the procedure described by Vincent *et al.* (1993a). Peaks were identified according to their absorption spectra and pigment concentrations were estimated using standards prepared with commercially available purified pigments (DHI, Denmark). Analysis of the UV-photoprotective sheath pigment scytonemin was performed following the procedures described by García-Pichel *et al.* (1992).

Extracellular polymeric substances

Total extracellular polymeric substances (EPS) were extracted with 2% EDTA in weight-known fractions from previously lyophilized samples. The carbohydrate content of the extracts was measured using the phenol-sulphuric acid method (Herbert *et al.*, 1971) with glucose as standard. The protein amount in the EPS was estimated according to Bradford (1976) taking bovine serum albumin (BSA) as standard.

Photosynthetic activity

Photosynthesis was measured by the stable isotope technique (^{13}C uptake) as described in Ariosa *et al.* (2006). To set up the assay, 10% of the water dissolved inorganic carbon concentration was added as $\text{NaH}^{13}\text{CO}_3$ (99% of ^{13}C atoms) (Cambridge Isotope Laboratories, MA). Isotopic enrichment was measured with an IRMS Micromass-Isochrom mass spectrometer. Dissolved inorganic carbon content in the water was calculated from alkalinity, determined by acid titration, by considering pH and temperature.

Light-dependent C-uptake

Experiments to study the light dependence of carbon assimilation were performed using the stable isotope technique (^{13}C uptake) as described above. An irradiance gradient was obtained with a log series of plastic neutral density screens. Light transmissions were measured with a Datalogger Licor LI-1000 equipped with a 2π quantum sensor. Data were fitted to the model of Platt *et al.* (1980), defining P_{max} as the maximum photosynthetic activity rate, α as the initial slope of the photosynthetic curve, and E_k

as the irradiance at which photosynthesis was saturated, defined as $P_{\text{max}}\alpha^{-1}$.

Microelectrode studies on light-saturated photosynthesis

Gross oxygenic photosynthesis was estimated at depth intervals of 200 μm through the vertical profile of each microbial mat core by measuring the decline in oxygen concentration (the slope of the curve) after shifting the samples from light to dark conditions for 4 s (Revsbech & Jørgensen, 1983). Oxygen concentrations were determined with a polarographic Clark-type oxygen microelectrode (diameter, 50 μm) (Diamond General, Ann Arbor, MI) while mat samples were exposed to constant saturating illumination of 750 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from a halogen lamp directed through glass fibre. The mean temperature of overlying waters during measurements was 10.6 °C. Photosynthetic rates were corrected for sediment porosity and integrated over the sediment column to calculate areal rates. Two profiles of photosynthetic activity were made at different spots in each of the three replicates per mat.

Uptake of ammonium and nitrate

The uptake of N from ammonium and nitrate was measured using the stable isotope technique (^{15}N uptake) as in Ariosa *et al.* (2006). To set up the assay, 10% of the water concentration of N-NH_4^+ or N-NO_3^- was added as $(^{15}\text{NH}_4)_2\text{SO}_4$ (98% of ^{15}N atoms) or as K^{15}NO_3 (99.9% of ^{15}N atoms) (Cambridge Isotope Laboratories, MA) respectively. Isotopic enrichment was measured with an IRMS Micromass-Isochrom mass spectrometer.

Nitrogenase activity

Nitrogenase activity was measured by the acetylene reduction technique as described in Fernández-Valiente *et al.* (2001). The ethylene concentration was determined using a gas chromatograph (Shimadzu model GC-8A) equipped with a flame ionization detector and Porapak N 80/100 column.

Statistical analyses

ANOVA or *t*-tests were used to compare replicated measurements. The SIGMASTAT program V2.03 was used for all statistical procedures.

Results

Phototrophic community structure and mat macroscopic features

The three mat types differed in colour, thickness and structure. The mat at the edge of the stream at South

Beaches was 2–3 mm thick and showed a pale orange colour, a smooth surface and was not very cohesive. The mat on moist soil near Limnopolar Lake was 3–4 mm thick and showed a dark purple colour, a wrinkled surface and a rigid, cohesive texture; it was firmly attached to small stones and grains of soil. The mat on the base of the ephemeral pond was about 4–5 mm thick, was dark orange-brown in colour, and had a smooth surface and cohesive texture, with small mineral particles intercalated within the mat matrix. All three mats showed some degree of vertical zonation, with a blue-green layer towards the bottom part of the mat.

Optical microscopic observations confirmed the differences in composition among the studied mats. The stream mat harboured a high density of diatoms which accounted for up to 70% of the biomass of phototrophs. Diatoms were particularly abundant in the upper layer of the mat, the predominant genera being *Navicula*, *Fragilaria*, *Stauroneis*, *Nitzschia*, *Gomphonema* and *Pinnularia*. Filamentous cyanobacteria of different filament width, members of the *Oscillatoriales*, were subdominant in this mat, and were preferentially located at the bottom layer. Most of the cyanobacterial biomass in this mat consisted of a thin (1–1.5 µm in diameter) cyanobacterium of the morphotype I class similar to that described in the analysis of Broady & Kibblewhite (1991) about oscillatorian diversity in the Ross Island and southern Victoria Land in continental Antarctica. Thicker cyanobacteria from morphotypes J (2–2.5 µm in diameter) and C (5.5–6 µm in diameter) were also present. According to Anagnostidis & Komarek (1988) all these morphotypes could be assigned to different species of the genus *Phormidium* (Broady & Kibblewhite, 1991). Filaments 2–2.5 µm in diameter with a clear partition assigned to the genus *Pseudanabaena* were also present. No heterocystous cyanobacteria were observed in this mat.

In contrast with the stream mat, diatoms were scarce in the soil mat. The matrix of this mat was formed by very thin filamentous cyanobacteria belonging to the genus *Leptolyngbya* (diameter 0.7–1 µm), which accounted for most of the biomass. In addition, a wide diversity of *Oscillatoriales* was found in this mat with diameters ranging from 2 to 8 µm and belonging to morphotypes C (diameter, 5.5–6), E (diameter, 8–11 µm), J (diameter, 2–3.5 µm), K (diameter, 4–6 µm) and M (diameter, 8–10 µm) (Broady & Kibblewhite, 1991). A filamentous cyanobacterium with a dark brown thick sheath (18 µm in diameter) could be seen profusely at the surface layer of the mat. Cyanobacterial cells 5.5 µm in diameter could sometimes be observed emerging from broken sheaths. This cyanobacterium has not been conclusively identified as yet, but it most probably belongs to the family *Phormidiaceae* genus *Porphyrosiphon* because of its thick, lamellated and coloured sheath. A large number of green and brown microcolonies of heterocystous cyano-

Table 1. Fresh weight (FW), dry weight (DW), ash-free dry weight (AFDW) and exopolysaccharide (EPS) content in three microbial mats in Byers Peninsula, Livingston Island (Antarctica). Data are means ± SD of three replicates

Parameter	Stream	Soil	Pond
FW (mg cm ⁻²)	272 ± 99	144 ± 41	338 ± 79
DW (mg cm ⁻²)	112 ± 45	33 ± 7	123 ± 32
AFDW (mg cm ⁻²)	27 ± 11	20 ± 4	44 ± 11
Water content (%)	59 ± 2	77 ± 2	64 ± 5
EPS (mg g ⁻¹ dry wt ⁻¹)	27 ± 3	60 ± 6	34 ± 6

Table 2. Carbon, nitrogen and phosphorus contents and molar ratios and natural abundances of ¹³C and ¹⁵N in three microbial mats in Byers Peninsula, Livingston Island (Antarctica). Data are means ± SD of three replicates

Parameter	Stream	Soil	Pond
Carbon (mg g ⁻¹ dry wt ⁻¹)	89.7 ± 21.5	262.7 ± 16.7	129.4 ± 13.3
Nitrogen (mg g ⁻¹ dry wt ⁻¹)	11.2 ± 2.6	16.1 ± 0.5	14.6 ± 2.1
Phosphorus (mg g ⁻¹ dry wt ⁻¹)	2.7 ± 0.8	0.54 ± 0.2	1.8 ± 0.2
C/N	8.0 ± 0.2	16.3 ± 1.0	8.9 ± 0.5
C/P	32.7 ± 7.8	486.4 ± 38.9	71.1 ± 7.3
N/P	4.1 ± 0.9	29.8 ± 2.7	8.0 ± 0.1
δ ¹³ C (‰)	-16.4 ± 0.6	-13.8 ± 0.4	-13.9 ± 1.1
δ ¹⁵ N (‰)	15.9 ± 2.5	3.9 ± 1.5	4.2 ± 1.0

bacteria from genus *Nostoc* could be observed in the bottom layer of the mat.

The pond mat showed a matrix formed by two types of filamentous cyanobacteria with diameters of 1 and 3 µm, respectively, which could be assigned to morphotypes I (1 µm in diameter) and J (3 µm in diameter). There was also an abundance of unicellular cyanobacteria (1.5 µm in diameter) intermixed with the filaments. Diatoms were also present but in a low density. As in the soil mat, the filamentous cyanobacterium with a dark brown thick sheath was also present in the surface layer of the mat. Different *Phormidium* species (morphotypes B and K, 4–5 µm in diameter, and morphotype E, 11 µm in diameter) and abundant microcolonies of *Nostoc* appeared in the deepest layer.

Biomass and elemental composition

Differences between fresh and dry weight indicated that all three mats had a high water content (Table 1). The highest water content was shown by the soil mat, which also showed the highest amounts of exopolysaccharide (Table 1) and the lowest biomass. Differences with the other two mats were statistically significant (ANOVA: $P=0.024$ for fresh weight; $P=0.019$ for dry weight; $P=0.017$ for ash-free dry weight; $P=0.036$ for water content and $P=0.002$ for EPS content).

The soil mat showed the highest C and N content per unit biomass (Table 2). Differences with the other mats were statistically significant (ANOVA: $P=0.002$ for C and $P=0.001$

for N). Phosphorus content was higher in the stream mat; the differences with the soil mat were significant ($P=0.004$), but not with the pond mat (ANOVA and Tukey test). Differences in C, N and P contents among the three mats are reflected in the C:N:P molar ratios (Table 2). For the soil mat, all molar ratios of carbon with respect to N and P, as well as the N/P ratio, were significantly higher than in the other two mats. The natural abundance of ^{13}C and ^{15}N of the stream mat was significantly lower and higher, respectively, than in the other two mats (ANOVA; $P=0.011$ for C and $P < 0.001$ for N) (Table 2).

The chlorophyll *a* content of the stream mat was higher than in the other mats (Table 3). The pond mat had a high pheophytin content (three times its chlorophyll *a* concentration), indicating a high degree of pigment degradation. In agreement with the dominance of diatoms, fucoxanthin was the main xanthophyll in the stream mat (Table 3), whereas myxoxanthophyll, a characteristic carotenoid of cyanobacteria, was the dominant xanthophyll in the pond and in the soil mats. There were lower levels of both xanthophylls than β carotene in the soil mat. Lutein, the characteristic carotenoid of chlorophytes, was only found in the stream mat, although at very low concentrations compared with the other carotenoids. The sheath pigment scytonemin, which confers UV radiation protection, was only present in soil and pond mats, but not in the stream mat (Table 3).

Table 3. Chlorophyll *a* and derivatives (pheophytin), main carotenoids and photoprotective sheath pigment contents in three microbial mats from Byers Peninsula, Livingston Island (Antarctica). Data are means \pm SD of three replicates

Parameter	Stream ($\mu\text{g cm}^{-2}$)	Soil ($\mu\text{g cm}^{-2}$)	Pond ($\mu\text{g cm}^{-2}$)
Chlorophyll <i>a</i>	61.8 \pm 15.4	8.8 \pm 2.4	29.0 \pm 33.9
Pheophytin <i>a</i>	3.3 \pm 1.7	0.5 \pm 0.3	87.0 \pm 93.8
Fucoxanthin	10.6 \pm 3.4	0.8 \pm 0.3	1.5 \pm 1.7
Myxoxanthophyll	4.1 \pm 0.8	1.1 \pm 0.4	19.3 \pm 0.6
β -carotene	6.7 \pm 2.0	1.7 \pm 0.3	6.7 \pm 1.7
Lutein	0.4 \pm 0.1	ND	ND
Scytonemin	ND	36.9 \pm 11.4	123.8 \pm 161.7

ND, not detected.

Table 4. Inorganic carbon (^{13}C) incorporation in three microbial mats from Byers Peninsula, Livingston Island (Antarctica). Data are means \pm SD of three replicates. Light refers to the photosynthetic activity measured under natural irradiance conditions, ranging from 340 to 860 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Dark refers to the photosynthetic activity measured in parallel to the light incubation but covered with aluminium foil. Formalin refers to the C incorporation into the sample measured under natural irradiance but with cells previously treated with 4% formaldehyde

Parameter of reference	Assay conditions	Stream	Soil	Pond
Chlorophyll ($\mu\text{g C mg}^{-1} \text{chlorophyll}^{-1} \text{h}^{-1}$)	Light	67.9 \pm 4.9	273.9 \pm 109.5	50.9 \pm 7.0
	Dark	2.2 \pm 0.5	2.0 \pm 3.5	8.4 \pm 0.1
	Formalin	0.04 \pm 0.01	1.4 \pm 2.5	6.1 \pm 5.2
Surface ($\mu\text{g C cm}^{-2} \text{h}^{-1}$)	Light	4.2 \pm 0.3	2.4 \pm 1.0	2.7 \pm 0.4
	Dark	0.1 \pm 0.1	0.02 \pm 0.03	0.4 \pm 0.1
	Formalin	0.002 \pm 0.001	0.01 \pm 0.02	0.3 \pm 0.3

Photosynthesis

Specific photosynthetic activity (photosynthesis per unit chlorophyll *a*) in the soil mat was 4–5 times higher than in the other two mats (Table 4), and this difference was significant (ANOVA, $P=0.009$). However, when data were expressed per unit area this soil mat activity was the lowest because of its lower chlorophyll content (see Table 3); in this case, differences with respect to the stream mat were significant (ANOVA and Tukey test; $P=0.029$), but not with respect to the pond mat. Differences between the incorporation of ^{13}C in cells incubated in the dark and in cells treated with formalin (dead cells) were only significant in the stream mat, indicating the possibility of some chemoautotrophic C assimilation in this mat but not in the other two mats.

Vertical profiles of O_2 evolution showed a typical pattern of two depth areas with peaks of oxygen evolution (Fig. 1). In the stream mat the shallower peak of oxygen production was found at a depth of 800 μm , corresponding to the layer where diatoms were more abundant, whereas the deeper peak was found at a depth of 2000 μm , showing lower photosynthetic activity and corresponding to the blue-green layer with the higher density of cyanobacteria. The vertical profile of the

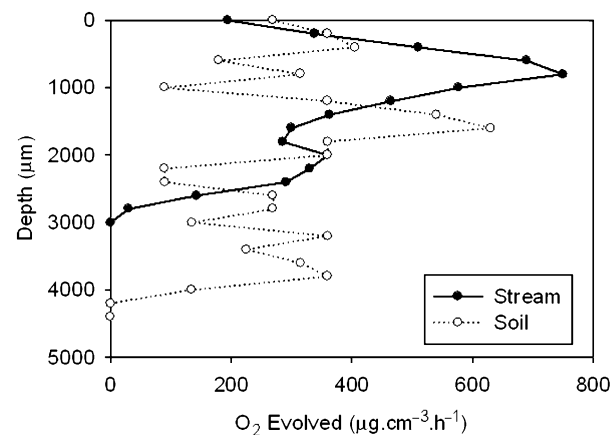
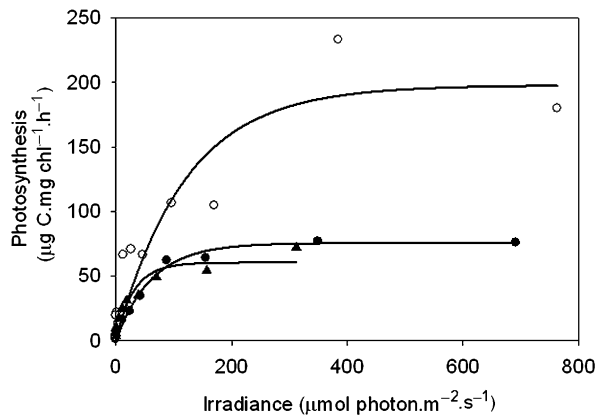


Fig. 1. Vertical profiles of oxygen evolution in two microbial mats in Byers Peninsula: (●), stream mat; (○), soil mat. Data are means of three replicates.



Parameters of equations

Parameter	Stream	Soil	Pond
P_{max} ($\mu\text{g C mg chl}^{-1}\text{h}^{-1}$)	75.5±4.7	197.7±47.6	60.5±12.9
α ($\mu\text{g C mg chl}^{-1}\text{h}^{-1}$) ($\mu\text{mol photon m}^{-2}\text{s}^{-1}$) ⁻¹	1.2±0.2	1.3±0.5	1.5±0.7
E_k ($\mu\text{mol photon m}^{-2}\text{s}^{-1}$)	62.9±12.9	152.0±71.7	40.3±17.6

Fig. 2. Photosynthesis vs. irradiance curves in three microbial mats in Byers Peninsula: (●), stream mat; (○), soil mat; (▲), pond mat. Data are means of three replicates. Mean irradiance during the assays were: stream mat, 691 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$; soil mat, 953 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$; pond mat, 390 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$.

Table 5. Nitrogen fixation in three microbial mats in Byers Peninsula, Livingston Island (Antarctica). Data are means ± SD of three replicates

Mat	N_2 fixation ($\text{nmol ethylene cm}^{-2}\text{h}^{-1}$)	N_2 fixation* ($\mu\text{g N cm}^{-2}\text{h}^{-1}$)
Stream	0.4 ± 0.2	0.002
Soil	7.1 ± 0.2	0.049
Pond	2.9 ± 1.1	0.020

*Calculated assuming an ethylene : N_2 conversion factor of 4 (Fernández-Valiente *et al.*, 2001).

soil mat showed the shallower peak of activity at a depth of 1600 μm and another area of high O_2 evolution between 3000 and 4000 μm . It was not possible to measure vertical profiles in the pond mat because the small mineral particles intermixed in the mat easily broke the glass microelectrode.

In agreement with the first set of experiments (Table 4) photosynthesis vs. irradiance curves showed significant differences in maximal photosynthetic activity per unit of chlorophyll between the soil mat and the other two mats (Fig. 2). The P_{max} of the soil mat was about three times higher than in the stream mat and pond mat. Differences in E_k and in the initial slope (α) were not significant, indicating relatively similar photosynthetic light efficiency at low irradiances.

Nitrogen assimilation

Rates of N_2 fixation in the stream mat (Table 5) were near the limit of detection of the method (0.2 $\text{nmol ethylene cm}^{-2}\text{h}^{-1}$), so the values must be interpreted with caution. However, N_2 fixation was well above our detection

Table 6. Nitrate and ammonium uptake expressed per surface and per chlorophyll in three microbial mats in Byers Peninsula, Livingston Island (Antarctica). Data are means ± SD of three replicates

Referenced to	Parameter	Stream	Soil	Pond
Chlorophyll ($\mu\text{g N mg}^{-1}$ chlorophyll ⁻¹ h ⁻¹)	NO_3^- uptake	37.6 ± 2.7	68.5 ± 8.9	28.5 ± 8.8
	NH_4^+ uptake	82.0 ± 6.1	135.9 ± 15.0	67.1 ± 19.8
Surface ($\mu\text{g N cm}^{-2}\text{h}^{-1}$)	NO_3^- uptake	0.23 ± 0.02	0.06 ± 0.008	0.08 ± 0.03
	NH_4^+ uptake	0.51 ± 0.04	0.12 ± 0.01	0.19 ± 0.06

limit in soil and pond mats, and differences between these mats were significant (*t*-test; $P < 0.001$) with higher rates for the soil mat regardless of the parameter of reference. Transformation of the data of N_2 fixation from acetylene reduction to N_2 fixed, assuming a conversion factor of 4 (Fernández-Valiente *et al.*, 2001), allows the calculation of the contribution of N_2 fixation to total N incorporation by the mats (Table 5). N_2 fixation represents 21.4% of total N incorporated by the soil mat and 6.4% in the case of the pond mat. The contribution of N_2 fixation in the stream mat was negligible.

Ammonium uptake was significantly higher than nitrate uptake in the three mats (Table 6) (paired *t*-test; $P = 0.002$, $n = 9$). The stream mat showed significantly higher values of both nitrate (ANOVA; $P < 0.001$) and ammonium uptake (ANOVA; $P < 0.001$) than the other two mats when referenced to surface area units (Table 6). However, when the data are expressed per chlorophyll, the soil mat showed the highest values (Table 6). Differences between the soil mat and the other two mats were significant for both ions (ANOVA; $P = 0.001$ and $P = 0.003$ for nitrate and ammonium uptake, respectively).

Discussion

Byers Peninsula can be classified into two main limnological areas: a central plateau of gentle undulating relief around 105 m a.s.l., where water retention in land depressions has produced many lakes and ponds with small watersheds and ultra-oligotrophic conditions, and the coastal area comprising the North, South and Western Beaches, where the streams and water bodies showed higher salt and nutrient concentrations due to their proximity to the sea and to the breeding colonies of southern elephant seals (*Mirounga leonina*), penguins and other sea birds (Toro *et al.* submitted). Microbial mats are extremely abundant in Byers Peninsula, particularly in the puddle soils of the catchment areas and at the bottom of small lakes and ponds of the central plateau, where they formed large expanses up to several hundred square metres in extent. In the coastal areas, microbial mats are usually restricted to the shore and bottom of the streams as the flat lowlands are covered by

extensive carpets of mosses and in some areas by the vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunt) Bartl.

In contrast to other studies which covered partial aspects of Antarctic microbial mats, such as taxonomy (Broady & Kibblewhite, 1991; Vinocur & Pizarro, 2000), microscopy (de los Ríos *et al.*, 2004), pigment organization (Vincent *et al.*, 1993a); photosynthesis (Vincent *et al.*, 1993b) and nitrogen fixation (Fernández-Valiente *et al.*, 2001), we have made an overall study of three representative microbial mats of Byers Peninsula. Two of the mats (soil and pond mats) were located in the central plateau, whereas the third (stream mat) was located in the coastal area at the South Beaches. This approach allows a better understanding of the differences observed in the composition, structure and physiological activities of these mats.

Overall, the results show striking differences between the stream mat and the other two mats. Our taxonomic analysis by light microscopy revealed that the stream mat was dominated by diatoms, whereas soil and pond mats were dominated by cyanobacteria. In agreement with these observations, the pigment analysis showed that fucoxanthin was the main carotenoid in the diatom-dominated stream mat, whereas myxoxanthophyll was the main carotenoid in the soil and pond mats, which were dominated by cyanobacteria.

The stream mat showed significantly lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ values than the other two mats. A natural abundance of ^{13}C can be influenced by a great number of environmental and physiological conditions, so there are many possible explanations for the differences in $\delta^{13}\text{C}$ between the stream mat and the other two mats. However, the most likely explanation could be the different species composition, which may produce a different isotopic signature. A natural abundance of ^{15}N has been used to identify N_2 -fixing organisms and to estimate the fractional contribution of atmospheric N_2 to N_2 -fixing organisms in terrestrial and aquatic systems (Gu & Alexander, 1993). The high $\delta^{15}\text{N}$ of the stream mat indicated, in agreement with the low rates of N_2 fixation and with the absence of heterocystous cyanobacteria, a low contribution of atmospheric-derived nitrogen to nitrogen content in this mat. Water chemistry studies of streams and ponds of Byers Peninsula indicated that the concentrations of combined N and soluble P are not limiting for the productivity of algal mats (Davey, 1993a, b; Toro *et al.*, submitted) especially because the pore water within the mats is usually much richer in N and P compounds than that of the overlying waters (Vincent *et al.*, 1993b), likely resulting from higher rates of nutrient recycling within the mat matrix. In the studied stream the concentrations of nitrate, ammonium and soluble reactive phosphorus were respectively $1.75 \pm 0.08 \mu\text{M}$, $1.46 \pm 0.06 \mu\text{M}$ and $0.49 \pm 0.01 \mu\text{M}$, which give an N/P ratio ranging from 2.88 to 6.58, close to the N/P ratio of the mat.

In a study of another stream mat in Byers Peninsula large amounts of diatoms, an absence of heterocystous cyanobacteria and low rates of nitrogen fixation (Davey, 1993a) were also reported. It is possible that abundant diatoms and the absence of heterocystous cyanobacteria could be characteristic of stream mats in Byers Peninsula, and may be related to the relatively high concentrations of combined N compounds supplied by the streams (Toro *et al.*, submitted). Microbial mats in maritime Antarctica are often overgrown by tufts of filamentous chlorophytes (Hawes, 1989; Davey, 1993a, b; Vinocur & Pizarro, 2000). Microscopical observations did not show the presence of chlorophytes in the studied mats, although their presence in very low abundance could not be discounted in the stream mat as traces of lutein were recorded in this mat. In fact, the central part of the riverbed of other streams located close to the studied stream mat, showing higher water velocities, was colonized by formations of hair-shaped colonies of chlorophytes, whereas the shores showing very slow water velocities were usually colonized by photosynthetic microbial biofilms such as that described. The adherence capacity of the different phototrophic microorganisms would probably determine their capacity for the occupation of stream zones where water traction would differ due to the water speed, thus shaping the microbial communities colonizing each part of the stream, as shown by such kind of environments at lower latitudes (Camacho *et al.*, 2005).

The soil and pond mats shared many characteristics, such as dominance of cyanobacteria, myxoxanthophyll as dominant carotenoid, presence of the sheath pigment scytonemin, similar isotopic signature and a high water content, even higher than in the permanently water-covered stream mat. The high water content of these mats was probably due to the frequent rainfall in the area, which provided a persistently wet environment, and especially to their higher content of EPS, which has a high capacity for water retention and provides protection against desiccation (Potts, 1996).

The soil and pond mats did differ, however, in physiological activity. The pond mat showed an extremely high amount of pheophytin, suggesting a high degree of degradation. This mat also showed the highest carotenoids/chlorophyll *a* ratio (0.94) and the highest amount of the sheath pigment scytonemin. These data and the low physiological activities of this mat suggest that this is an old mat in a stage of decay. Probably the high amount of scytonemin reflected a low rate of degradation of this pigment instead of a high rate of synthesis, particularly given the low specific photosynthetic activity of this mat; three times lower than that of soil mat. The soil mat also showed the maximal rates of N_2 fixation and the maximal rates of incorporation of combined N expressed per chlorophyll *a*, indicating that this mat was physiologically more active than the others.

The photosynthetic rates per unit of chlorophyll *a* of the three mats were low compared with other cyanobacterial

communities of more temperate environments (Ariosa *et al.*, 2006), but are in the range of other Antarctic microbial mats (Howard-Williams & Vincent, 1989; Howard-Williams *et al.*, 1989; Davey, 1993a, b; Vincent *et al.*, 1993b). It is possible that the depth layers where photosynthesis reached its maximal values and the overcast weather conditions of this region are conducive to a low P_{\max} and also affect E_k and α , which were lower and higher, respectively, than in other cyanobacterial communities (Ariosa *et al.*, 2006). Low P_{\max} , low E_k and high α values are characteristics of photosynthesis in a shaded environment (Boston & Hill, 1991).

Microbial mats are thought to be responsible for much of the primary production in extreme polar environments (Vincent, 2000). In Byers Peninsula, microbial mats support associated invertebrate populations including several species of *Nematoda*, *Tardigrada*, *Rotifera* and *Protozoa*, and in the stream mats of South Beaches can be also found larvae of two species of chironomids: *Parochlus steinenii* and *Belgica antarctica* (Toro *et al.*, submitted). Recent studies in the Arctic indicate that microbial mats in lakes and ponds may also provide a food source to certain zooplankton species (Rautio and Vincent, 2006). In addition to sustaining microinvertebrates, benthic microbial mats are an important autochthonous source of nutrients for the lakes. Such communities are often characterized by enriched nutrient conditions relative to the overlying phytoplankton (Bonilla *et al.*, 2006). Soil mats located in the catchment area of oligotrophic lakes of the central plateau are also an important allochthonous source of nutrients for the lakes via runoff (Toro *et al.*, submitted) and in this way may contribute to lacustrine foodwebs. In fact, the planktonic heterotrophic biomass of an extensively studied ultra-oligotrophic lake from Byers Peninsula is likely higher than that expected to be supported by autochthonous planktonic primary production (A. Camacho *et al.*, in preparation), and most likely can be sustained, in addition to planktonic primary production, by the nutrient inputs generated in benthic environments from both the lake and its catchment area (Camacho, 2006). The soil mats are also likely to be important in the successional development of the catchment areas as they contribute to the establishment of mosses, which appear as small colonies intermixed with microbial mats in some areas of the watersheds in the central plateau.

In summary, all these observations underscore the ecological importance and the taxonomic and physiological diversity of microbial mat communities in the maritime Antarctic region.

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