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JERÓNIMO PAN, CONSTANZA N. BOURNOD, NATALIA V. PIZANI, DIANA G. CUADRADO, AND NOELIA B. CARMONA

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Characterization of Microbial Mats from a Siliciclastic Tidal Flat (Bahía Blanca Estuary, Argentina)

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Biofilms and microbial mats cover the tidal flats of the central zone of the Bahía Blanca estuary (Argentina), creating extensive layers. The objective of this study was to characterize the microphytobenthic communities in these biofilms and mats, from sediment cores taken in March, June, September and December 2010. Microorganisms were identified and enumerated by microscopy, and their biomass (chlorophyll *a*, biovolume) quantified at two different stations in the lower supratidal zone, located ~210 m apart from each other (namely S1 and S2). Additionally, the colloidal carbohydrates produced by these microbial communities were quantified, together with physical parameters such as temperature, granulometry, moisture and organic matter content of the sediment layers that comprise a typical epibenthic mat. On the other hand, changes in biomass and colloidal carbohydrate content were studied through a half-tidal cycle (7 h). There were significant seasonal differences in microphytobenthic biovolume ($P < 0.001$) with a considerably lower biomass in summer, but no significant differences in microalgal biovolume between stations ($P = 0.454$). Cyanobacterial biomass (largely composed of the filamentous *Microcoleus chthonoplastes*) was dominant on all dates at both stations, followed by pennate diatoms. Chlorophyll *a* and colloidal carbohydrate contents in sediment presented a similar pattern to that of microalgal biovolume; with a 5-fold variation in chlorophyll *a* for S1 between consecutive sampling events on September and December. There were significant differences between sampling dates in colloidal carbohydrates ($P < 0.001$) with the lowest values recorded during fall and winter; conversely there were no significant differences between stations ($P = 0.324$). Silt was the dominant sediment fraction at S1 while sand dominated throughout the uppermost 20 mm at S2. Chlorophyll *a* contents did not show significant differences throughout a half-tidal cycle, likely the product of vertical migration along the section sampled. Conversely, the content of colloidal carbohydrates varied 5-fold, showing a significant ($P < 0.001$) and steady increase with time of exposure to air and pointing to the rapid metabolic rates of the community. In conclusion, the microphytobenthic community of the Bahía Blanca estuary presented marked seasonality in its biological parameters and overall physiognomy, also showing elevated metabolic rates when subject to tidal fluctuations.

Keywords: biofilms, cyanobacteria, diatoms, microbial mats, microphytobenthos, tidal flat

Introduction

Complex interactions and feedbacks between physical, sedimentary, biological and chemical processes take place in tidal flats, which call for a multidisciplinary approach to their study (de Brouwer et al. 2000, Stal 2010). It is well known that the upper several millimeters of illuminated sediments is a zone of intense microbial and geochemical activity (MacIntyre et al. 1996) and due to its influence in sediment stabilization, the microphytobenthos of this zone constitutes an important biogeomorphological force (Stal 2010).

The community structure of microphytobenthos is determined by seasonality of physical (e.g., temperature, light, resuspension) and chemical parameters (e.g., pore-water nutrient and oxygen concentration, pH and redox potential E_h) (Jesus et al. 2009, and references therein). In response, microorganisms secrete large amounts of extracellular polymeric substances (EPS), which are complex molecules consisting mostly of carbohydrates, although they may also contain various other components such as proteins, lipids and lipopolysaccharides (Stal 2010).

Colloidal carbohydrate concentrations in sediments are directly related to epipelic diatom biomass (Madsen et al. 1993), but this predictable relationship is not apparent in mixed

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assemblages such as mats of cyanobacteria and diatoms of the supratidal zone (Underwood 1997; Underwood and Smith 1998). Underwood et al. (1995) stated that the measurement of the colloidal carbohydrate fraction is a useful operational proxy for microbial EPS. EPS exudation in benthic diatoms occurs mostly during daytime and emersion of the sediment (Stal and de Brouwer 2005), and is either a result of their migration influenced by the inundation, or unbalanced growth (Stal 2010; Stal and de Brouwer 2003).

Cyanobacteria prefer fine sandy sediment as substrate for the formation of microbial mats (Watermann et al. 1999). On the other hand, sediments with very fine silt and mud with adsorbed nutrients are colonized by diatoms (Stal 2010), which present elevated growth rates when nutrients are high, and outcompete cyanobacteria, generally adapted to low nutrient regimes. Additionally, an inverse relationship has been observed between EPS content and sediment grain size (de Brouwer et al. 2003), i.e., fine-grained sediments present high amounts of EPS.

From a geobiologic point of view (Nealson and Ghiorse 2001), the geomicrobial activity taking place in present-day primary sedimentary structures allows the inference of those biogeochemical and early diagenetic processes which might have taken place in analogous sedimentary structures preserved in the fossil record. In that sense, Noffke et al. (2001) introduced the term MISS (Microbially Induced Sedimentary Structures) to define primary sedimentary structures that arise syndepositionally from the interaction of biofilms and microbial mats with the physical sediment dynamics in siliciclastic aquatic environments. Also, a set of criteria for biogenicity of MISS was established to identify biogenic structures (Noffke 2009).

The aim of this study was to make an exploratory analysis of microbial mats and biofilms in a siliciclastic tidal flat in the Bahía Blanca estuary throughout a year, attending to the seasonality. We provide a first-hand biological characterization of the autotrophic organisms forming the microbial mat and their pattern of carbohydrate secretion, and contrast it with studies in other estuaries. Also, the changes in biomass and colloidal carbohydrates were quantified through a tidal cycle.

To our knowledge, this study is the first of its kind in Argentina, and one of the very few done in South America. Demergasso et al. (2003) carried out a seasonal study of microbial mats from a high-altitude hypersaline Andean lagoon; on the other hand, our study focuses on an estuarine environment, in which sedimentary processes are of paramount importance.

Materials and Methods

Study Site

Puerto Rosales (38° 55′ S; 62° 03′ W) is located on the northern margin of the central zone of the Bahía Blanca estuary, Buenos Aires Province, Argentina (Figure 1). The estuary is categorized as mesotidal (Hayes 1979); semidiurnal tides predominate and the average tidal amplitude is 2.5–3.4 m during neap and spring tides, respectively. A dry temperate climate is characteristic for the area, with a mean annual air temperature of 15.6°C (mean temperatures range from 22.7°C in January to 8.1°C in July). Surface seawater mean annual temperature at Puerto Rosales is 14.1°C. Mean precipitation is low (460.5 mm) and evaporation rates are high (Piccolo and Diez 2004). On average, cumulative solar radiation in a cloudless

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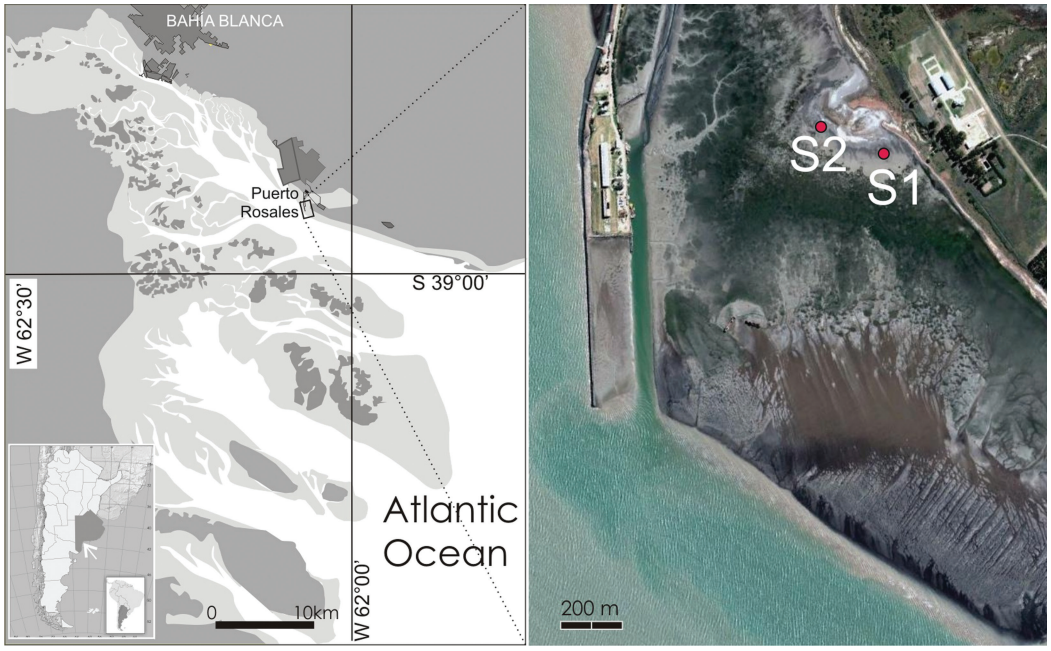

Fig. 1. Study area, Puerto Rosales (38° 55′ S; 62° 03′ W), located in the Bahía Blanca estuary, Argentina. Inset shows sampling stations S1 and S2 (color figure available online).

day is 28 MJ m^{-2} in summer and 11 MJ m^{-2} in winter (Beigt 2006).

In Puerto Rosales, extensive tidal flats (~1000 m wide) with low slopes (~0.4° gradient), are composed of siliciclastic sediments ranging from fine sand to mud. Siliciclastic grains are predominantly composed of quartz with minor amounts of feldspars. The supratidal area is flooded by seawater, reaching ~10 cm depths during spring high tides. Local winds from SW to NE (NW direction) generate waves with short wavelengths and < 6 s periods. The significant wave height in Puerto Rosales is 0.3 m (Nedeco-Arconsult 1983). Wind velocity presents maximum values in summer and minimum velocities in fall and winter; the monthly mean velocity ranges between 15.9 km h^{-1} and 32 km h^{-1} (Piccolo and Diez 2004).

Two sampling sites (S1 and S2, Figure 1), located ~210 m apart from each other, were established in the lower supratidal area at Puerto Rosales, on the basis of sedimentologic characteristics and preliminary field observations. S1 was characterized by patches bioturbated by the burrowing crab *Neohelice* (= *Chasmagnatus*) *granulata* and finer-grain sediments with respect to S2. On the other hand, the coarser sediments on S2 were undisturbed by macrozoobenthos at the time of this study.

Sampling of Biofilms and Microbial Mats

Four sampling events were carried out in the tidal flats at Puerto Rosales, on 3/10, 6/28, 9/13 and 12/7/2010, roughly corresponding to the fall, winter, spring and summer seasons in the Southern Hemisphere, respectively. Sampling was consistently done at daytime hours and during low tide.

Fresh, undisturbed mat and sediment samples were collected in plastic Petri-dishes to qualitatively analyze their composition and structure under a stereoscopic microscope (Nikon SMZ 1500), and an epifluorescence microscope (Nikon Eclipse 80i) for a reliable identification of cyanobacteria on the basis of phycoerythrin autofluorescence after excitation with a Nikon G-2A filter combination (510–560 nm). Additionally, a discrete number of microbial mat and surface sediment samples were taken for analysis under Scanning Electron Microscopy (SEM; JEOL35 CF 8, Tokyo). SEM samples were fixed in a mixture of 2.5% glutaraldehyde in Sorensen phosphate buffer, washed in the same buffer, dehydrated in an acetone series (10 to 80%), and eventually dried by critical point and coated with gold.

For compositional quantification and enumeration of microorganisms, sediment samples (n = 3) were taken with a plastic cylindrical corer (inner diameter = 10 mm, height = 5 mm) and preserved in 10 ml of 4% formalin (stock solution prepared with filtered natural seawater). In order to disassemble the biofilm matrix and enhance contact of the preservative with the microorganisms, the samples were homogenized in a shaker (Bandelin Sonorex Tk52). The 1-ml aliquots of the suspension were taken and diluted by addition of 9 ml of 0.45-μm filtered seawater. An aliquot of this final suspension was mounted onto a Neubauer chamber and counted in a Nikon Eclipse (DIC 600x) inverted microscope (adapted from LeGresley and McDermott 2010). Taxa were classified into the following taxonomic groups: filamentous cyanobacteria and centric and pennate diatoms (the latter, discriminated into < 9 μm, 10–19 μm, 20–39 μm, 40–100 μm, and > 100 μm size ranges). Additionally, standard measurements of cell linear dimensions were performed on 30 specimens of each category, for biovolume estimations (Hillebrand et al. 1999; Sun and Liu 2003).

Chlorophyll *a* concentration in sediments (expressed as μg Chl *a* cm^{-2}) was estimated from cylindrical sediment cores (n = 3; inner diameter = 10 mm, height = 5 mm), which were frozen and kept at −20°C upon arrival to the lab. Chlorophyll *a* extraction was made in 90% acetone, and measured spectrophotometrically (model Beckman BU530) after Lorenzen (1967) and Moed and Hallegraeff (1978).

Colloidal carbohydrates were extracted incubating sediment samples (cylindrical cores; n = 3; diameter = 10 mm, height = 5 mm) in 5 ml saline solution (2.5%) for 15 minutes at 20°C, then centrifuged for 15 min; a 1-ml aliquot of the supernatant was taken, 1 ml of 5% (w/v) aquose phenol solution, and 5 ml of concentrated sulfuric acid were added (Underwood et al. 1995). Colloidal carbohydrates were then quantified following the colorimetric method in Dubois et al. (1956). Absorbance was measured spectrophotometrically at 485 nm, after stabilizing the color of the solution for 1 h. Glucose was used for the standard curve, hence carbohydrate content is expressed as μg glucose equivalents cm^{-2} (Underwood et al. 1995).

Surface (0–2 mm) and sub-surface (3–8 mm) sediment temperature was measured *in situ* at Puerto Rosales with a Hanna Instruments probe (model HI991003) (n = 6–18). On the fall (March 2010) and summer (December 2010), duplicate or triplicate sediment samples were collected using sawn-off 50 ml medical syringes to obtain small cores of sediment and separated into three or four layers (layer heights ranging from 4–10 mm), according to the layered structure visible under a stereoscopic microscope. For each layer, sediment grain size, moisture retention, and organic matter content were determined.

Sediment grain size was determined by laser diffraction using a Malvern-Mastersizer-2000 particle analyzer, for particles ranging between 0.2–2,000 μm (i.e., colloids to sand). Moisture content was calculated from weight differences before and after drying samples at 60°C to a constant weight for 4 days (Christie et al. 2000). Total organic carbon content (TOC) was calculated from weight loss on ignition (LOI) after drying samples and ashing/combusting at 500°C for 4 h in a muffle furnace (Blakemore et al. 1987).

Sampling Throughout a Half-tidal Cycle

Microbial mats and biofilms at S1 were sampled periodically every hour during part of an ebb period and part of a flood period in order to study if there were physiological or behavioral variations within the community, which would translate into microalgal biomass and carbohydrate content variations. Surface sediment samples (cylindrical cores; n = 3; inner diameter = 10 mm, height = 5 mm) were taken at S1 on March 19, 2010, spanning a 7-h time frame, from the moment in

which the site became exposed (i.e., 11:15 to 18:15). Chlorophyll *a* and colloidal carbohydrate content were determined as described here.

Results

General Tidal Flat Characteristics

Most of the sedimentary surface at both stations is covered by tissue-like mats with a planar, table-like morphology that preserved a formerly physically shaped tidal surface from wave, tide and wind erosion (Figures 2A, 2B). Also, a suite of MISS are developed throughout the year (Cuadrado et al. 2011; Cuadrado and Pizani 2007).

The main hydrodynamic processes in this siliciclastic setting are wave action and bottom currents that cause erosion, deposition and reworking of sediments, especially in winter when storms are more severe. Under these circumstances, the coherent microbial mat layers are torn and ripped-up, becoming locally detached from the substrate and generating "flipped-over mat" structures (Figure 2C) and other erosional features like patchy break-ups (Figure 2D), which may evolve into 'erosional pockets' (Noffke 1999). On the other hand, microbial mats and biofilms also experience periodical desiccation, especially in summer, creating characteristic sedimentary structures such as "shrinkage cracks" and "polygonal oscillation cracks," usually covered by a thin layer of salts (Figures 2E, 2F).

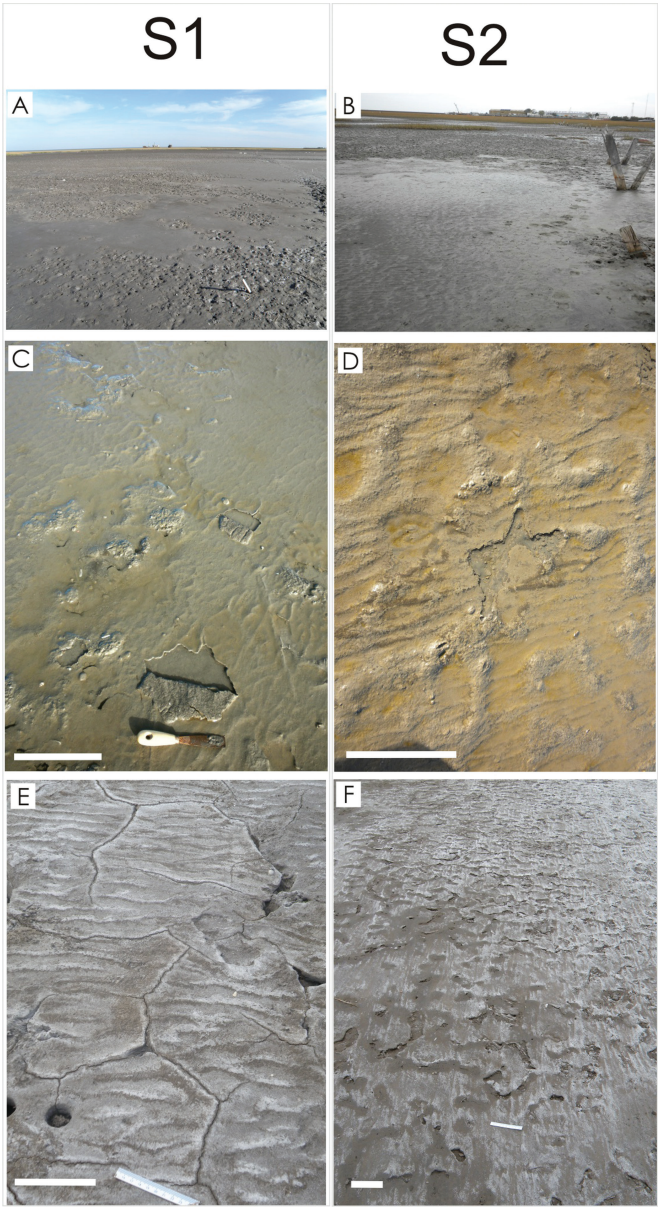

Fig. 2. Sampling stations S1 and S2 in Puerto Rosales. (A) and (B) General view. (C) and (D) Destruction of microbial mat due to wave erosional forces during winter (scale bar = 15 cm). (E) and (F) Tidal flat covered by evaporites during summer (scale bars = 10 cm) (color figure available online).

Qualitative Analysis of Microphytobenthic Community

The topmost (2 mm in S1) of tidal sediments at Puerto Rosales presented a mucilaginous matrix, part of the natural sediment biofilm composed of microbial communities embedded in EPS. The sediment particles presented no grain-to-grain contact, as they were surrounded by EPS in a three-dimensional network. The microphytobenthos community integrating the surface biofilm and microbial mats consisted in unicellular (epipelic diatoms) and filamentous algae (cyanobacteria) (Figure 3).

The smaller pennate diatoms ($< 40\ \mu$m) included the genera *Diploneis*, *Nitzschia* and *Navicula*, while the larger-sized representatives included species of the latter two genera, and also species of the genera *Gyrosigma*, *Cylindrotheca* and *Pleurosigma*. Centric diatom representatives included the marine genera *Thalassiosira* and *Coscinodiscus*, and also *Cyclotella meneghiniana* and *Paralia sulcata*. All cyanobacteria found in the sediments were non-heterocystous filamentous species, with *Microcoleus chthonoplastes* being the dominant species, and the genera *Oscillatoria* and *Arthrospira* being also present. A few samples taken for zoobenthos characterization, indicated that meio- and macrofauna were absent at both stations, with the exception of the above-mentioned *Neohelice granulata*, gastropods and undermat miners (Carmona et al. 2011).

Quantitative Analysis of the Microphytobenthic Community

There were significant differences in microphytobenthic biomass (expressed as microalgal cell biovolume) along the year [two-way ANOVA, $F(3,16) = 20.92$; $P < 0.001$] (Figure 4). Both stations showed a fluctuating fall-spring microphytobenthic biomass pattern, with a significantly lower biomass in the Austral summer (December). S1 showed a peak in biomass in June (Figure 4A) due to an increased biovolume of cyanobacteria and $> 40\ \mu$m pennate diatoms. Similarly, S2 had a peak in cyanobacteria biovolume in September.

Conversely, there were no significant differences in microalgal biovolume between sampling stations [$F(1,16) = 0.59$; $P = 0.454$]. With the exception of S2 in June, where the biovolume

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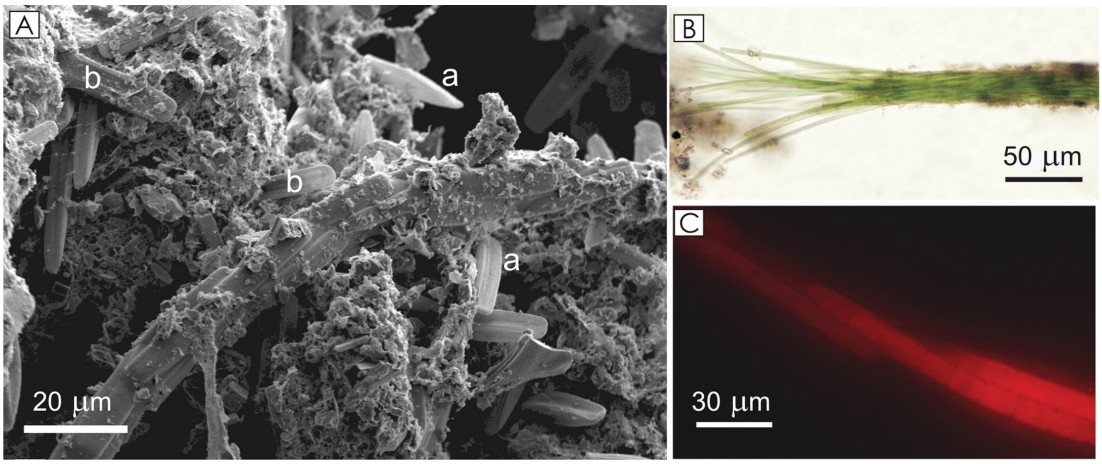

Fig. 3. Representative microphytobenthos from Puerto Rosales tidal flats (A) Scanning electron micrograph of the biofilm at the emersion period, showing the diatom-dominated assemblage (a: *Navicula phyllepta*, b: *Plagiotropis* sp.) surrounded by an EPS matrix. (B) Light- and (C) epifluorescence microscope micrographs of the trichomes of *Microcoleus chthonoplastes*, the dominant cyanobacteria in microbial mats. Arrow in (B) indicates sediment particles attached to cyanobacterium sheath. Micrograph in (C) shows phycoerythrin autofluorescence after excitation with a Nikon G-2A filter combination (510–560 nm) (color figure available online).

of cyanobacteria only amounted to 30% of the total microphytobenthos biovolume (Figure 4B), cyanobacterial biomass was dominant on all dates at both stations sampled, reaching up to 82% of the biovolume at S1 in September. Pennates in the 40–100 μm size range (e.g., *Nitzschia* spp., *Diploneis* spp., *Amphora* spp.) were the most abundant diatoms at both stations, while >100 μm pennates (e.g., *Pleurosigma* spp., *Gyrosigma* spp.) were less frequent. At both stations, centric diatoms were the least abundant group.

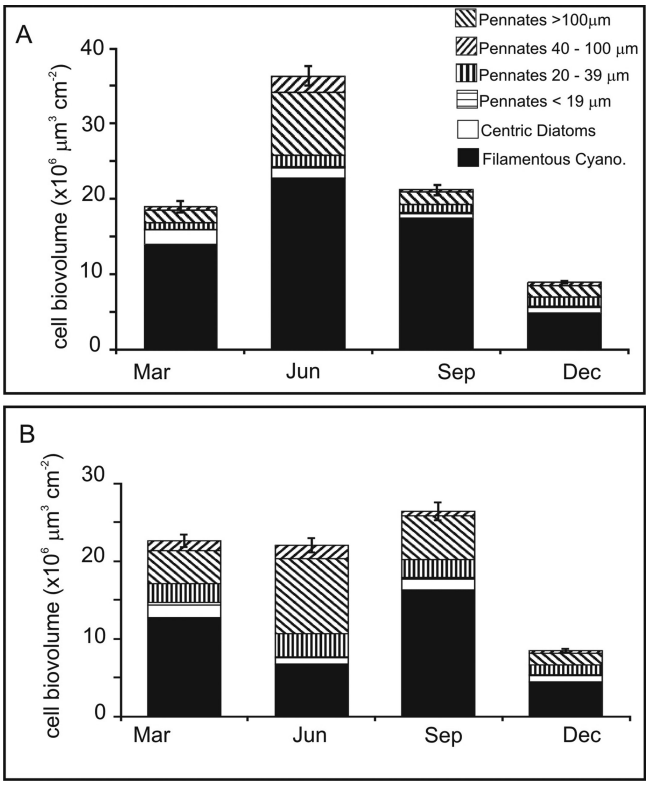

Fig. 4. Seasonal variation in microphytobenthos biovolume at stations S1 (A) and S2 (B), in Puerto Rosales. Stacked bars are means (n = 3) for each taxonomic and size-fraction component, SE for the total microphytobenthos biovolume.

Chlorophyll *a* and colloidal carbohydrate contents in sediment presented a similar pattern of variation (Figures 5A, 5B). There were significant differences in chlorophyll *a* content between stations [two-way ANOVA, $F(1,23) = 6.36$; $P = 0.023$] and sampling seasons [$F(3,23) = 35.56$; $P < 0.001$]. The highest chlorophyll *a* concentration (mean ± SE) was registered in September for both stations (13.15 ± 0.97 and 10.13 ± 0.34 μg Chl *a* cm^{-2}, for S1 and S2, respectively). There was a 5-fold variation in chlorophyll *a* content for S1 between the consecutive sampling dates of September and December, when the lowest concentration (2.62 ± 0.56 μg Chl *a* cm^{-2}) was recorded. Similarly, there was a 3-fold variation in chlorophyll *a* for S2, between September and March, when the lowest concentration (3.29 ± 0.14 μg Chl *a* cm^{-2}) was recorded.

There were significant seasonal differences in colloidal carbohydrates [two-way ANOVA, $F(3,23) = 14.91$; $P < 0.001$], but conversely there were no significant differences between stations [$F(1,23) = 1.03$; $P = 0.324$]. Colloidal carbohydrates had a ~5-fold content variation, with higher values (mean ± SE) in September for S1 (253.42 ± 31.27 μg eq glu cm^{-2}), and in December for S2 (170.54 ± 14.3 μg eq glu cm^{-2}). The lowest values for both stations were recorded during fall and winter.

No significant differences were found between surface and sub-surface sediment temperatures [two-way ANOVA, $F(1,143) = 0.001$; $P = 0.976$]. However, there were significant seasonal differences in sediment temperature [$F(3,143) = 101.93$; $P < 0.001$]. Averaged surface and sub-surface temperature closely followed seasonal fluctuations in air temperature, with values of (mean ± SE) 24.63 ± 0.82°C in March; 11.27

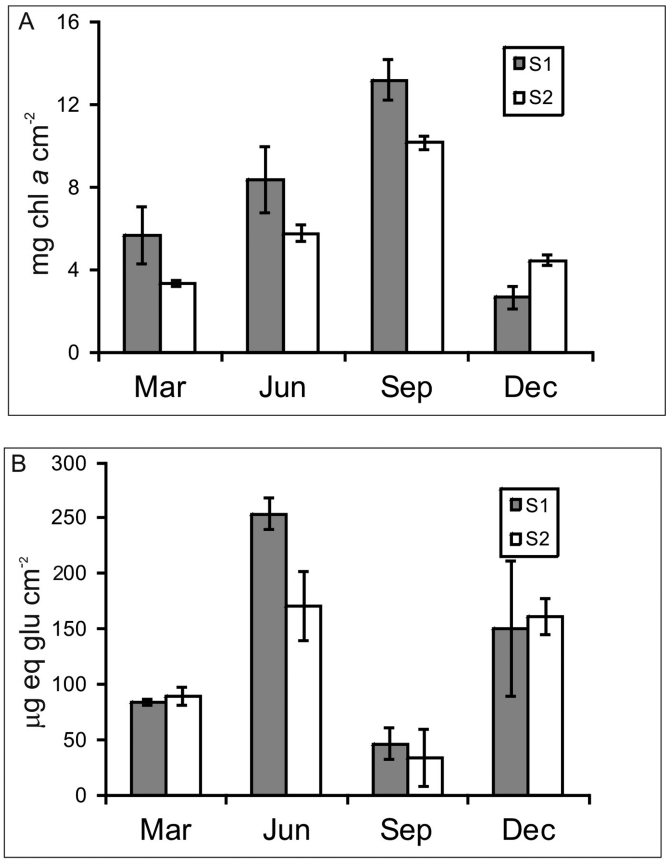

Fig. 5. Seasonal variation in chlorophyll *a* (A) and colloidal carbohydrates (B) contents in sediment at stations S1 and S2, in Puerto Rosales. Bars are means (n = 3) ± SE.

± 0.09°C in June; 18.16 ± 0.12°C in September; and 23.75 ± 0.11°C in December 2010. There was a ∼2 fold decrease in sediment temperature from early fall to the Austral winter.

S1 presented a ∼20-mm-thick microbial mat, including an uppermost thin biofilm (Figure 6). The granulometric analysis evidenced that silt was the dominant sediment fraction in early fall in the upper layers (12 mm deep), while coarser grain sediments (i.e., fine sand) were dominant in deeper layers (> 12 mm). Interestingly, in early summer, there was a thin layer of sand (2 mm) in the surface, probably the result deposition by winter/spring storm events or eolic transport from the upper supratidal; the underlying sediments were mostly dominated by silt (8–23 mm), with sand underneath.

On the other hand, the pattern for S2 did not show any layer or seasonal changes in sediment grain size, being dominated by sand throughout the uppermost 20 mm. S2 presented a thinner microbial mat, in comparison to S1. Clay was scarce in all layers of both stations and for both seasons. Moisture retention by the different sediment layers was related to sediment granulometry. All layers at S1, where silt was the most abundant fraction, comparatively retained a higher proportion of moisture than those at S2 (Figure 7).

Organic matter content did not show marked seasonal differences at S1, nor did it present differences between the upper (0–8 mm) and subjacent (8–13 mm) layers. Values ranged from 3.5% wt to 7.2%wt, the difference between layers never being larger than 1.6%wt. On the other hand, S2 presented a ∼2-fold increase in organic matter in September compared to the previous sampling event in June, reaching up to 10.7%wt in the 0–8.5-mm upper layer. This increase might probably relate to an increase in microalgal biomass, which also peaked in September at S2 (Figure 5A). After this increase in organic matter content, values decreased 2-fold in December. The subjacent 8.5–18.5 mm layer consistently presented lower organic matter content, ranging from 1.9 to 4.7%wt.

Biomass and Carbohydrate Changes Throughout a Half-Tidal Cycle

Chlorophyll *a* contents in sediment did not show a particular pattern of variation throughout the 7 h of the survey, albeit presenting a 1.7-fold variation with mean ± SE values ranging between 7.76 ± 2.06 and 13.19 ± 0.88 μg Chl *a* cm^{-2}. A one-way ANOVA did not show significant differences in chlorophyll *a* content [$F(6,20) = 1.33$; $P = 0.306$] throughout the sampling period. On the other hand, the content of colloidal carbohydrates varied 5-fold, showing a significant and steady increase with time of exposure to air [one-way ANOVA, $F(6,20) = 24.42$; $P < 0.001$], and reaching up to 1322.34 ± 45.42 μg eq glu cm^{-2} (Figure 8A).

Discussion

Filamentous cyanobacteria dominated in abundance the microphytobenthos at Puerto Rosales on most dates, constituting up to 82% of the photoautotroph biovolume during Austral spring (Figure 4). In turn, the most abundant cyanobacterium was *Microcoleus chthonoplastes*, which typically has many trichomes within a common sheath threaded into a spiral arrangement (Figure 3C). The resulting mesh of interweaving cyanobacterial filaments together with the microbially secreted EPS, entangles sand grains more efficiently than a diatom biofilm (de Winder et al. 1999) and contributes significantly to an increment in the cohesiveness of sediments, while being firmly attached to the substratum. Stal et al. (1985) studied cyanobacterial succession during mat development and concluded that in well-established mats, the dominant organism was *M. chthonoplaste*s, to which they attributed the formation of a tough coherent mat.

In that sense, the dominance in biomass of *M. chthonoplastes* at both stations is indicative of well-developed microbial mats presenting an elevated resistance to erosion, and a protective cover to the underlying sediments. Microbial mats in which this cyanobacterium is dominant are termed "epibenthic mats" by Noffke (2010), and are typically found in the supratidal zone. The biomass enrichment and traping and binding of mineral particles by filamentous cyanobacteria (i.e., levelling, *sensu* Noffke and Krumbein 1999) determines the occurrence of an advanced morphological stage of the tidal flat known as "planar surface" (Figure 2).

The seasonal differences in microphytobenthos biovolume (Figure 4) and chlorophyll *a* and colloidal EPS contents in

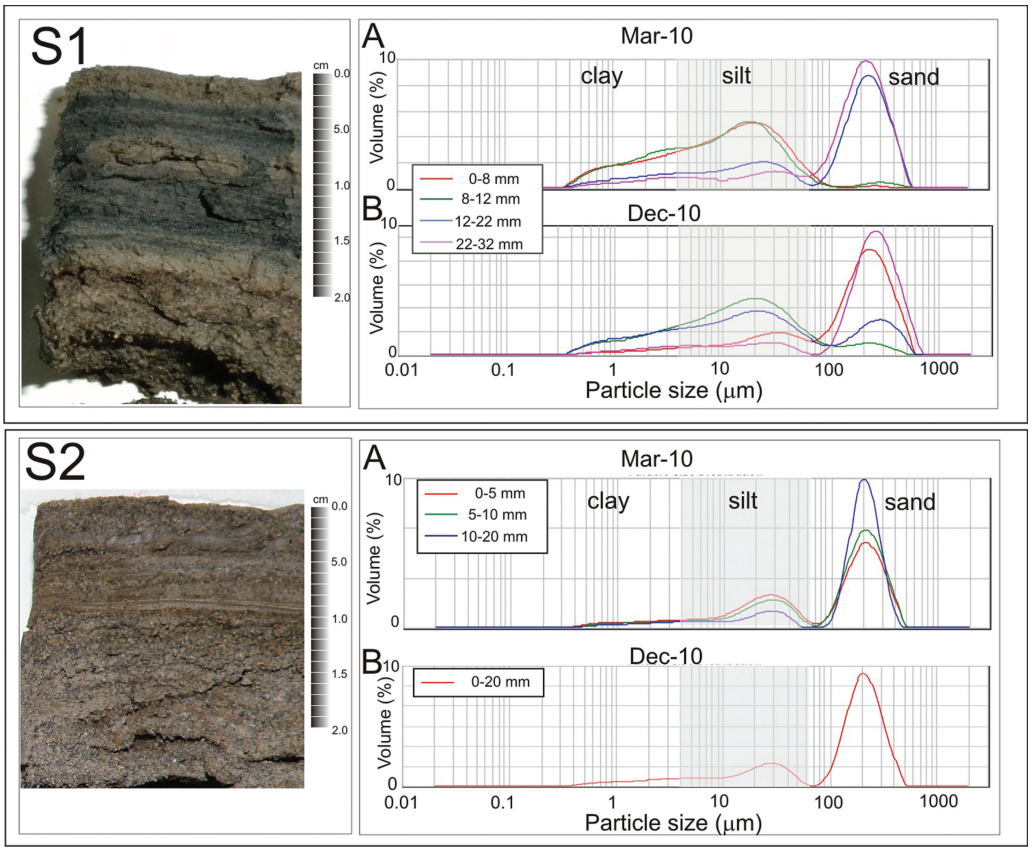

Fig. 6. Photographs of characteristic layering of surface sediments in Puerto Rosales, and granulometric analysis for stations S1 and S2, from samples collected on (A) March and (B) December 2010. Note the reducing layers in cross-sections of S1, and the microsequences of alternating light and dark layers in S2 (color figure available online).

sediment (Figure 5) might, in the absence of benthic predators, be related to external physical forcings. The significantly lower biomass in summer (Figures 4, 5A), is probably related to an increase in evaporation/desiccation rates in the tidal flat, itself the product of increased radiation. Average total radiation for June 2010 was 172 W m^{-2} (EMAC weather station, IADO; data not shown), while average total radiation for September 2010 was 389 W m^{-2}, representing a 2.25-fold increase since June. In December 2010 average total radiation was 468 W m^{-2}, almost three times higher than the radiation received by the exposed sediments during winter. Although benthic biofilm communities from temperate environments have shown resistance and resilience to desiccation, desiccated sediments usually show a lower chlorophyll *a* fluorescence signal and benthic diatoms cease their migration to surface layers (McKew et al. 2011).

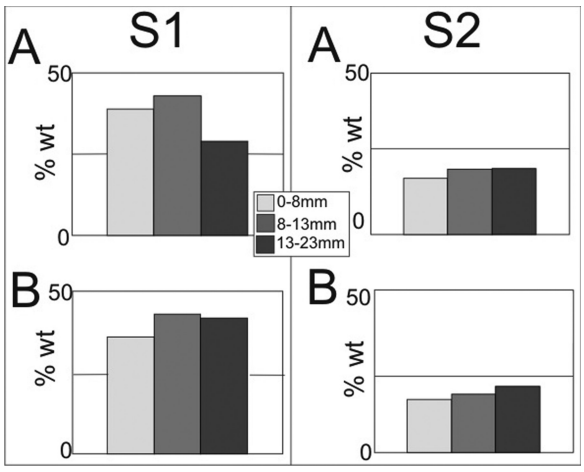

Fig. 7. Moisture retention plots by the different layers from samples collected on (A) March and (B) December 2010 for stations S1 and S2 in Puerto Rosales.

Distinct seasonal cycles in microphytobenthic biomass determined by physical parameters have been previously described in analogous systems to the one in this study. In that sense, the peak in sediment chlorophyll *a* content in spring registered at both stations (Figure 5A) is similar to that reported by Fidalgo e Costa et al. (2002) for intertidal estuarine and lagoon environments, and by Jesus et al. (2009) for the Tagus estuary in Portugal. Similarly, Aberle and Wiltshire (2006) found a distinct seasonality with shifts in community composition and succession patterns in the microphytobenthos of lakes.

The intensely studied tidal flats at Mellum Island (southern North Sea; Gerdes et al. 1985; Noffke et al. 1997; Noffke 1998, 1999; Noffke and Krumbein 1999), share the characteristic of being mesotidal with the Bahía Blanca estuary. The

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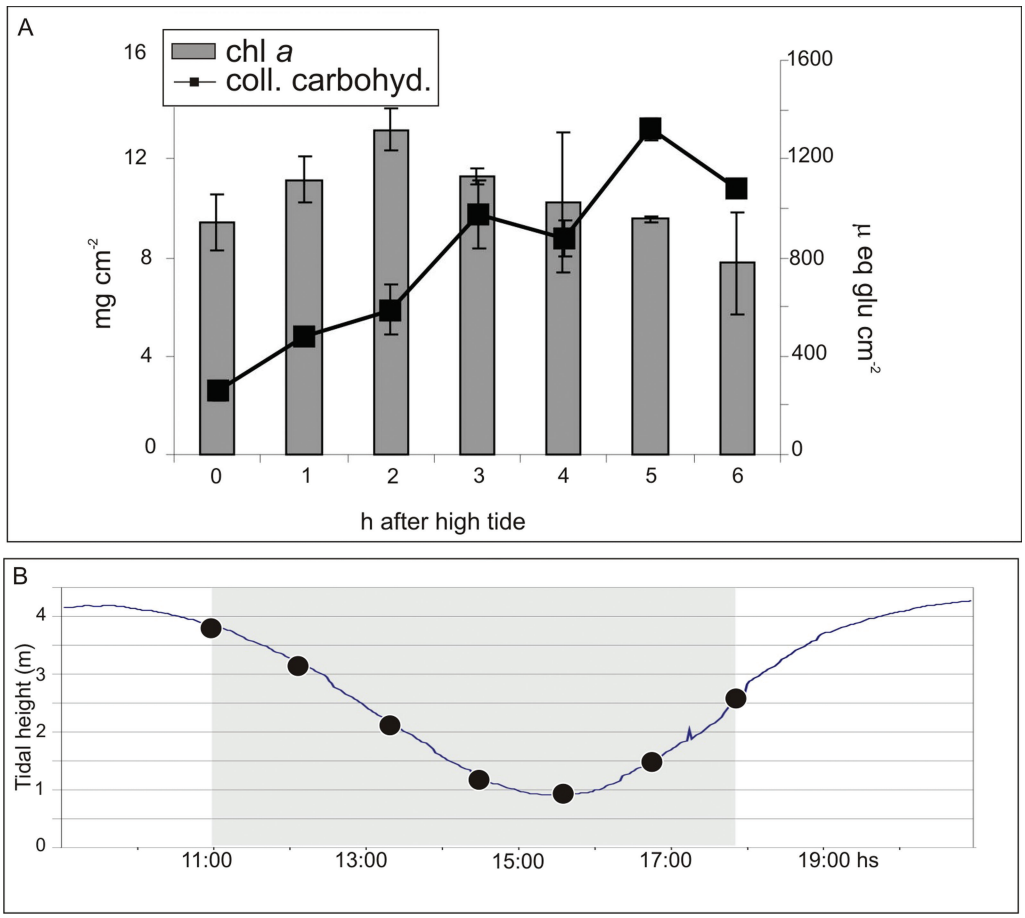

Fig. 8. Variation in (A) chlorophyll *a* and colloidal carbohydrates following an ebbing tide cycle (6 h, 3/19/2010) (B) at S1 in Puerto Rosales. Bars and squares are means (n = 3) ± SE (color figure available online).

microphytobenthic community is similar in being dominated by the cyanobaterium *M. chthonoplastes*, and other filamentous species such as *Oscillatoria*. In this study, the epibenthic mat remained clearly visible during winter, congruently to the reports of Noffke and Krumbein (1999) that mats in the lower supratidal zone at Mellum Island were able to withstand strong winter hydrodynamic impacts (Figures 2C, 2D). Moreover, in the Bahía Blanca estuary, the epibenthic mat showed peak biomass values in winter, while the lowest biomass was registered in summer (Figure 4). Conversely, the pattern reported for Mellum Island (Noffke and Krumbein 1999) indicates low biomass in winter and an annual maximum in summer.

In general, the content of chlorophyll *a* and colloidal carbohydrates in sediments were correlated, except for spring, when colloidal carbohydrate content was significantly lower than for the other seasons (Figure 5B). However, de Winder et al. (1999) demonstrated that colloidal carbohydrates and microphytobenthic biomass do not necessarily have to be correlated parameters. These authors quantified the water-soluble (colloidal) and EDTA-extractable (capsular) fractions of carbohydrates in a cyanobacterial mat (*M. chthonoplastes*) and a diatom biofilm and concluded that the chlorophyll-specific carbohydrate content of the two communities was very different. The diatom biofilm contained up to 100 times more colloidal carbohydrate than the cyanobacterial mat.

In turn, the concentrations of colloidal carbohydrates in the diatom biofilm correlated with chlorophyll *a* biomass, but this was not the case with the carbohydrate in the EDTA extract, while on the other hand, neither colloidal nor EDTA-extractable carbohydrate in the cyanobacterial mat correlated with chlorophyll *a*. Moreover these authors concluded that colloidal carbohydrate production by diatoms was enhanced by light, while no light-dependent increase in carbohydrate concentration was found for cyanobacteria (de Winder et al. 1999). This would explain why in this study, when the microbial mat formed by *M. chthonoplastes* made up the bulk biomass at both stations in spring (in terms of cell biovolume, Figure 4; and presumably in terms of chlorophyll *a*, Figure 5A), colloidal carbohydrate contents were lowest (Figure 5B), while biomass was maximum.

Epipelic microalgae are known to migrate to sediment surfaces when the tidal flat is exposed at low tide and to descend before it is flooded (reviewed by McIntyre et al. 1996; Stal 2010), what is evidenced to the naked eye by a coloration change in the sediment to a brownish-green. Following an ebb tidal stage, no significant differences in chlorophyll *a* content were found for the uppermost 5 mm sampled (Figure 8), which might represent the approximate scale of vertical migration. On the other hand, the significant differences found in colloidal carbohydrates suggest rapid metabolic rates by the

microalgal community; in a 7-hour-interval, there was a 5-fold change in colloidal carbohydrate content, showing a significant and steady increase with time of air exposure (Figure 8).

The concentration of colloidal carbohydrates in sediments is regulated by a balance between EPS production by photoautotrophs (a protective mechanism against desiccation; Decho 1990), and heterotrophic bacterial production coupled with β-glucosidase activity (van Duyl et al. 2000). Microorganisms may produce EPS for various reasons; while cyanobacteria produce EPS as a structural cell component (i.e. the sheath that encases trichomes), EPS secretion by epipelic diatoms is associated with their displacement in sediments (Hoagland et al. 1993), and as a means of structural protection from predation and/or potentially toxic contaminants (Davies et al. 1998; Lawrence et al. 1995, 1998; Neu and Lawrence 1997). Similarly to the present study, Perkins et al. (2003) found that EPS showed opposite patterns to chlorophyll *a* over tidal emersion in the Eden estuary, with concentration showing a negative correlation with water content. This was attributed to EPS being a highly hydrated, soluble substance, whereas the content of chlorophyll *a* is independent of the aqueous phase.

The exudation of EPS is one of the mechanisms by which microbial mats and biofilms render stability to the sediments (Stal 2010). Additionally, there are architectural features to be considered, such as the entanglement of cyanobacteria filaments which contribute physically to the stabilization of tidal flats (Margulis et al. 1980). The biostabilization generated by mats in the Bahía Blanca estuary can be classified as Type I (BSI, *sensu* Noffke 2010). The effect of baffling and trapping (Noffke 1998) from cyanobacteria and the sticky EPS results in the accumulation of smaller particles that alternate with sandy layers in millimeter-scale sequences (e.g., cross-section in S2, Figure 6). Such laminations are termed "biolaminites" by Gerdes et al. (1991) and are one of the textures that can be found in both modern and fossil MISS. The sediment-EPS interaction has important sedimentological implications not just because of the role of EPS in sediment stabilization, but also in the precipitation of authigenic minerals (reviewed by Decho 2000, 2010; Stal 2010; Sutherland 2001). This is a crucial first step of early diagenesis, leading to the preservation of primary sedimentary structures in the geological record (Winsborough 2000).

Conclusions

The microphytobenthic communities in the central zone of the Bahía Blanca estuary are subject to large yearly variations in biomass (dictated primarily by physical forces), yielding a significantly lower biomass during the summer. Colloidal carbohydrate contents fluctuated throughout the year, accordingly with the microphytobenthic biomass. However, colloidal carbohydrates seemed to have responded only to diatom biomass in spring, as evidenced by the minimum colloidal carbohydrate that was registered when cyanobacteria biomass was maximum. Additionally, the large differences in colloidal carbohydrate content (5-fold) registered in the sediment throughout a 7-h air exposure coinciding with a half-tidal cycle, point to the elevated metabolic rates with which the microbial community responds to physical forcings.

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