Regenerative bioturbation by intertidal burrowing crabs modifies microphytobenthic composition and enhances primary production in SW Atlantic mudflats

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ABSTRACT: In coastal systems, benthic microalgae are important primary producers that significantly contribute to global primary production. Microphytobenthic (MPB) community structure and the ecosystem functions that it mediates are modulated by biotic and abiotic factors. Through burrowing activity, different types of bioturbators can impact MPB in different ways. Through directed sampling and field experiments performed at the Mar Chiquita coastal lagoon (Argentina), we evaluated the effect of regenerative bioturbation (continuous digging and maintenance of burrows, with sediment transfer from depth to surface) by the intertidal burrowing crab *Neohelice* (*Chasmagnatus*) *granulata* on MPB. We compared sediment properties and MPB attributes between natural burrowed and non-burrowed areas. Moreover, we experimentally manipulated regenerative bioturbation to evaluate if MPB composition and primary production are altered by crab burrowing activity. Field sampling showed that MPB and sediment properties differed between natural burrowed and non-burrowed areas. Experimental results indicated that regenerative bioturbation by *N. granulata* increased sediment oxygen concentration at each measured depth and also changed MPB composition (due to the reduced total abundance of cyanobacteria), but nearly doubled primary production in comparison with plots without bioturbation. Thus, the net effects of regenerative bioturbation were positive for MPB productivity, reinforcing the idea that this kind of bioturbation is an important biological force that enhances primary production in intertidal systems.

KEY WORDS: Regenerative bioturbation · Microphytobenthos · Burrowing crab · *Neohelice* (*Chasmagnatus*) *granulata* · Intertidal sediments

1. INTRODUCTION

Microphytobenthic (MPB) communities consist of photoautotrophic microorganisms, mainly diatoms, cyanobacteria, flagellates and green algae, that live in and on subtidal and intertidal sediments where light reaches the sediment surface (Underwood 2001).

These communities play an important role in key ecosystem functions, such as primary production, sediment stabilization and biogeochemical cycling (Middelburg et al. 2000). MPB cover large areas and have high areal productivity rates (Heip et al. 1995, MacIntyre et al. 1996). Indeed, at the top 1–2 mm of the sediment, benthic microalgae can achieve similar

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areal productivity rates as phytoplankton in the overlying water column (Underwood & Kromkamp 1999), and may contribute up to 50% of estuarine primary production (e.g. northern Adriatic coasts, Blackford 2002; Colne estuary, UK, Underwood et al. 2005). MPB stabilize the sediment surface due to the extrusion of extracellular polymeric substances (Underwood et al. 2005). These molecules form a network that traps and binds sediment particles, increasing sediment cohesion and stability (de Brouwer et al. 2002, Stal 2010). MPB can also modulate benthic biogeochemical processes, such as denitrification, nitrification and N_2 fixation (Sundbäck et al. 2004, Stutes et al. 2006). These processes are fundamental for intertidal system functioning, and in tidal flats their dynamics are regulated by different attributes of MPB such as composition, functional diversity, species richness, abundance and evenness (Thrush et al. 2017).

The composition of phototrophic communities is determinant for coastal ecosystem dynamics (Duarte 1995). MPB productivity is the consequence of the different responses to irradiance of its constituent taxa (Underwood et al. 2005). The exposure to high irradiance levels can limit MPB productivity (Serôdio et al. 2008). MPB dominated by diatoms are tolerant to high light intensities due to physiological strategies (developed by epipsammic and epipelic diatoms) and behavioral strategies (developed only by epipelic diatoms), so photoinhibition is apparently absent when the biofilm as a whole is measured (Serôdio 2004), thus ensuring high and constant levels of production (Mouget et al. 2008). As for many kinds of communities, composition also influences sediment biogeochemical cycles via species traits (Tilman 1999). MPB dominated by cyanobacteria increase sulfide oxidation and nitrogen fixation in the surrounding environment (e.g. Janousek 2009), while those dominated by diatoms usually show high rates of inorganic nitrogen consumption (i.e. ammonia and nitrate) and oxygen production (Nilsson & Sundbäck 1996). MPB structure is also influenced by abiotic and biotic interactions (Kristensen 2007). For example, the microscale distribution of sediment particles of varying sizes can directly influence diversity (Paterson & Hagerthey 2001), and factors like temperature, desiccation and salinity also determine composition, as they differently affect the success of each taxonomic group (Underwood & Provot 2000). Herbivory and bioturbation are important biotic factors that influence MPB growth and development (Admiraal 1984, Needham et al. 2011). Benthic macrofauna present a wide range of strategies related to their movement and feeding behavior, so

their impact on MPB is very variable (Waldbusser et al. 2004). While some studies have shown that deposit feeding organisms may decrease MPB abundance due to grazing, others have shown that sediment reworking during feeding activities has a positive effect on MPB (e.g. Armitage et al. 2009, Sandwell et al. 2009).

Bioturbation by benthic macrofauna is widely recognized as a key ecological process due to its effect on ecosystem functioning in coastal and marine habitats (Teal et al. 2013). It is defined as all types of transport processes carried out by organisms that directly or indirectly affect sediment matrices, including sediment reworking and burrow ventilation (Kristensen et al. 2012). Bioturbation affects sediment properties such as sedimentary oxygen concentration, pH and redox gradients (Daleo et al. 2007, Queirós et al. 2015), permeability, organic matter subduction (Lohrer et al. 2004, Fanjul et al. 2015), NH_4^+ release (Emmerson et al. 2001, Biles et al. 2002) and grain size distribution (Escapa et al. 2007). Moreover, bioturbation can affect macrofaunal community structure (Volkenborn et al. 2007, Alvarez et al. 2013), as well as bacterial abundance, composition and activity (Bertics & Ziebis 2009, Gilbertson et al. 2012). Bioturbators also regulate carbon (Kristensen 2001) and nitrogen cycles (Bertics et al. 2010, Fanjul et al. 2011) due to the alteration of the physico–chemical environment that affects bacterial assemblages (Laverock et al. 2011). MPB primary production is favored by the presence of diverse and abundant macrobenthic bioturbators, indicating complex feedbacks and interactions (Lohrer et al. 2004, 2010).

Although much information is available about the role of some bioturbation modes, such as the upward/downward conveyors and biodiffusers, the role of regenerative bioturbation remains relatively unexplored (but see Escapa et al. 2008, Wang et al. 2010, Needham et al. 2011, 2013, Alberti et al. 2015, Fanjul et al. 2015). Regenerative bioturbators dig and continuously maintain burrows, thereby transferring large amounts of deep sediment to the surface. Their burrows also enhance sediment trapping (Botto et al. 2006, Escapa et al. 2008) and are passively irrigated, which contrasts with the active burrow irrigation that characterizes other bioturbation modes (Kristensen et al. 2012). Regenerative bioturbation is typically exerted by burrowing crustaceans such as burrowing crabs that inhabit saltmarsh and mangrove sediments (Kristensen et al. 2012). Their burrowing activities increase the potential for biological and chemical exchange by increasing the area of sediment–water/air interface between 150 and 380% (Kristensen

2008). New Zealand estuaries, for example, are inhabited by the regenerative bioturbator crab *Austrohelice crassa*, a key species in the regulation of ecosystem functioning (Needham et al. 2013). In Chinese salt marshes, the burrowing crabs *Helice tridens tientsinensis*, *Sesarma dehaani*, *S. plicata* and *Uca arcuata* dominate the macrofauna, constructing burrows with high morphological variation that strongly affect the flow of energy and materials (Wang et al. 2010). Mangrove sediments also show high rates of regenerative bioturbation, mainly by sesarmid crabs (e.g. *Neoepisesarma versicolor*, *S. messa*) and fiddler crabs (e.g. *U. pugilator*, *U. pugnax*, *U. vocans*, *U. annulipes*), which affect sediment biogeochemistry and microbial activity (Kristensen 2008).

The intertidal burrowing crab *Neohelice* (*Chasmagnathus*) *granulata* (up to 40 mm carapace width) is one of the most abundant benthic macroinvertebrates in southwest Atlantic (SWA) protected soft-bottom intertidal systems (Iribarne et al. 1997). This crab has long been considered to be an allogenic ecosystem engineer (sensu Jones et al. 1996) because it modifies the physical and chemical sedimentary environment, thus affecting resource availability to co-inhabitant species (e.g. polychaetes: Escapa et al. 2004; fishes: Martinetto et al. 2005; birds: Iribarne et al. 2005; plants: Daleo & Iribarne 2009; snails: Alvarez et al. 2013). This species can remove up to 2.4 kg m^{-2} d^{-1} of salt marsh sediment and up to 6 kg m^{-2} d^{-1} in mudflats (Iribarne et al. 1997, Botto & Iribarne 2000) as a result of burrow excavation and maintenance, which includes sediment removal and transport from depth to the surface and also passive trapping of sediment into open burrows (Botto & Iribarne 2000, Escapa et al. 2008). This crab needs to remove large amounts of sediment in order to maintain large burrows (up to 17 cm width and 1 m depth), thus promoting high rates of regenerative bioturbation (sensu Kristensen et al. 2012). This activity modifies the sedimentary matrix through geochemical alterations and also changes grain size distribution (Escapa et al. 2007), sediment water content (Bortolus & Iribarne 1999), organic matter content and inorganic nutrient availability (Fanjul et al. 2007, 2011). Given that these factors are known to affect MPB (Underwood 2001), we expect an indirect impact of regenerative bioturbation on MPB as a result of modifying the sedimentary environment.

In this study, we investigated the effects of regenerative bioturbation by *N. granulata* on MPB abundance/diversity/composition and the possible impact on intertidal MPB-mediated ecosystem functions, such as primary production. Given the high bioturba-tion intensity of *N. granulata*, we expected that it would affect MPB.

2. MATERIALS AND METHODS

2.1. Study site

The study was carried out between February 2014 and February 2015 at intertidal mudflats in the Mar Chiquita coastal lagoon (Argentina, 37° 32′ S, 57° 19′ W). This site is the southernmost in a series of temperate coastal lagoons along the SWA coast (Perillo 1995) and is dominated by a semidiurnal microtidal regime (tidal amplitude <1 m), with low current velocities (mean: 5.4 cm s^{-1}; Iribarne & Botto 1998) and highly variable salinity (from 0.5 to 34; Reta et al. 2001). In this coastal lagoon, the high intertidal zones are characterized by the presence of salt marshes vegetated by *Sporobolus densiflorus* (*Spartina densiflora*), while bare mudflats are found in the lower intertidal zones (Iribarne et al. 1997), and its sediments are mostly composed of fine sands, silts and clays.

Within these sediments, MPB is mainly composed of diatoms and cyanobacteria, while euglenophytes and chlorophytes are present in lower proportions (Alvarez et al. 2013, Alberti et al. 2017). These intertidal flats (i.e. saltmarshes and mudflats) are also inhabited by the burrowing crab *Neohelice granulata* (Iribarne et al. 1997, Luppi et al. 2002). This species is active year round (primarily from early spring to late autumn) and can excavate substrates over a wide range of environmental conditions (e.g. from hypersaline to brackish water conditions) and sedimentary contexts (from muddy sediments dominated by silts and clay to sandy and even gravelly sandy sediments). Thus, *N. granulata* can withstand a high range of environmental variability through physiological adaptations and without affecting its burrowing activity (Halperin et al. 2000, Luppi et al. 2013). Locally, the burrows can be found from the lowest areas of mudflats to the highest areas of salt marshes, but crab burrowing activity is concentrated at marsh–mudflat edges where higher burrow densities are generally registered (Spivak et al. 1994, Iribarne et al. 2005, Mendez Casariego et al. 2011). When crabs inhabit salt marshes, they behave as herbivores, feeding mainly on *Sporobolus* spp., and their burrowing activity is facilitated by marsh plants (Bortolus et al. 2002), thus generating areas of high burrow density below plant canopies. However, crabs inhabiting mudflats change their trophic mode to

deposit feeding (Botto et al. 2005, Alberti et al. 2011), and their burrowing activity is concentrated in patches that exhibit high variation both in areal extent and temporal stability (Botto & Iribarne 1999, Iribarne et al. 2005). This is partially explained by the high mobility rate among patches in short periods (Luppi et al. 2013) and also by the low site fidelity that this species shows (Nuñez et al. 2018).

2.2. Sediment characterization and MPB abundance/diversity/composition in natural bioturbated and non-bioturbated patches

Directed field sampling was conducted in summer 2014 in order to characterize sediment properties and MPB abundance/diversity/composition in naturally occurring bioturbated and non-bioturbated patches in Mar Chiquita mudflats. Along these mudflats are highly bioturbated patches that are interspersed with few or no bioturbated patches, and this feature has allowed assessing a broad range of ecological patterns (Botto & Iribarne 1999, Escapa et al. 2004, Iribarne et al. 2005, Fanjul et al. 2007). To include environmental variability, we distributed our sampling effort in 3 different mudflat areas within the coastal lagoon, each area consisting of 2 paired patch types: high bioturbation intensity patches (crab sites) and nearby patches with low or no bioturbation (no-crab sites). In these patches, we characterized sediment physical properties (grain size distribution, water content and salinity) and MPB abundance/diversity/ composition and biomass.

In each patch (crab and no-crab sites), we randomly collected sediment samples during low tides, using corers of different diameter. A first set of samples (2 cm diameter and 1 cm depth; n = 10 at each site) was used for determination of sediment physical parameters. Water content was obtained as the difference between wet and dry weight (after drying at 60°C to a constant weight). Sediment salt content was measured in a 1:2 dry-sediment/distilled water extract. Sediment salinity was then estimated from salt sediment content and water sediment content data. Grain size distribution was measured by wet sieving, using standard mesh size sieves (500, 250, 125, 62 μm). Each retained fraction, as well as the <62 μm fraction, was dried (60°C to constant weight) and weighed.

To estimate MPB biomass, a second set of sediment samples was collected (2.4 cm diameter and 1 cm depth, sample size based on previous studies by Pan et al. 2013 and Alvarez et al. 2013

conducted in this region) and maintained in darkness and frozen until analysis. Biomass was estimated as total chlorophyll *a* (chl *a*) content (Brotas et al. 1995). Pigments were extracted from sediment using 90% acetone (Lorenzen 1967). Chl *a* and phaeophytin content were determined using spectrophotometric analysis (Jeffrey & Humphrey 1975) before and after sample acidification.

A last set of sediment samples (1 cm diameter and 1 cm depth; n = 10 at each site) was used for MPB parameters (species richness, evenness, total abundance, diversity). Samples were collected and immediately preserved in a mixture of distilled water, alcohol and formalin solution (6:3:1; Prescott 1951). To characterize MPB composition, the abundance of different groups included in MPB (cyanobacteria, chlorophytes, euglenophytes and diatoms) was quantified. MPB was identified to the lowest possible taxonomic level, following commonly used keys (i.e. cyanobacteria, Guarrera et al. 1972, Komárek & Anagnostidis 1985, 1989, 1999, 2005; chlorophytes, Hindák 1990; euglenophytes, Tell & Conforti 1986; and diatoms, Cox 1996, Prygiel & Coste 2000). MPB counts were performed in a 0.5 ml Sedgwick–Rafter chamber under an optical microscope (McAlice 1971). Species abundances were expressed as the number of individuals cm^{-2} of sediment, considering only those with chloroplast remains.

2.3. Effects of regenerative bioturbation by *N. granulata* on MPB

In order to evaluate the effect of *N. granulata* bioturbation activity on MPB, a field experiment was conducted. The experimental site was near sampled area 2 in a patch with low/no bioturbation. It consisted of 2 experimental treatments (n = 10 each): (1) 'crab exclusion' and (2) 'crab inclusion.' The experimental plots of each treatment (randomly assigned) were surrounded with plastic mesh (1 cm mesh size) boxes (all sides 0.6 m long, 0.5 m tall). Crab exclusions were achieved with a mesh box closed with a mesh lid, and additional smaller mesh wall (0.1 m tall and 0.1 cm mesh size) attached to the bottom to prevent the entry of small crabs. Crab inclusions consisted of mesh boxes (similar those used for crab exclusion) with a half lid (to allow free access of crabs and to replicate the possible shading effect present in crab exclusions), and with smaller mesh added in 2 of the walls (to replicate the possible artifact effect on water flow dynamics). Crab density in crab-inclusion plots was maintained throughout the experiment, simulat-

ing the natural densities in the study area (~60 crabs m^{-2}, Iribarne et al. 1997, Escapa et al. 2004).

Several previous studies have shown success of crab-inclusion experiments based on low or no mortality and the normal crab behavior evidenced by bioturbation rates similar to those at natural crab sites (e.g. Fanjul et al. 2007, 2008, 2011, Alberti et al. 2011). This kind of box design does not significantly affect flow dynamics or light incidence (Hillebrand & Kahlert 2002, Armitage & Fong 2006, Cheverie et al. 2014) and thus these boxes do not affect MPB dynamics (Alvarez et al. 2013). Once installed, the experiment ran for 60 d (summer 2015), and the experimental plots were periodically inspected to guarantee the absence of crabs in crab exclusions; small crabs were manually removed when necessary. Crab-inclusion plots were monitored to ensure that crabs were actively constructing and maintaining their burrows.

After 60 d, sediment samples were collected from each plot. A set of samples (2 cm diameter and 1 cm depth) was used for determination of physico–chemical parameters (water content, salinity, organic matter content). Water content and salinity were measured as detailed in Section 2.2. Organic matter (OM) content was determined by weight loss upon ignition (5 h at 550°C). pH was measured *in situ* in the top centimeter of sediment using a hand-held pH meter (FC200, Hanna Instruments). Oxygen profiles were assessed *in situ* by measuring oxygen concentration every 100 µm in the top millimeter; and every 500 µm between the first and third millimeter of depth. We constructed a device that consisted of a micromanipulator (MM2, Unisense AS) screwed to an iron stick. In each experimental plot, we first inserted the device into the sediment and then mounted the oxygen microsensor (Clark type glass microsensor; 50 µm tip, fast response; Unisense) to the micromanipulator. Sensor current was measured with a picoammeter (PA2000; Unisense AS) that was placed outside the experimental boxes. The microelectrode was calibrated with both air-saturated and oxygen-free N$_2$-saturated water at the same temperature and salinity as pore water. To avoid pseudoreplication, we measured oxygen concentration within each box at one randomly selected point, avoiding crab burrows, mounds and other biogenic structures. Grain size distribution was not measured in this experiment because over short time periods crab bioturbation does not affect mean particle size (e.g. Needham et al. 2011).

Another set of samples (2.4 cm diameter and 1 cm depth) was collected for determination of chl *a* (details in Section 2.2). The last set of sediment samples (1 cm diameter and 1 cm depth) was collected for determi-

nation of MPB parameters. Composition was determined as detailed in Section 2.2. Richness, abundance, diversity and evenness were calculated using these data. Gross primary production (GPP) was estimated by the light–dark shift method inside experimental plots using the oxygen microsensors described above, following Revsbech & Jorgensen (1983). Measurements were taken every 100 µm until readings stabilized, up to depths where values were equal to 0. Dark periods were generated abruptly, covering the whole equipment with an opaque cover; meanwhile, the data were being transmitted to a computer in real time through an A/D C216 USB converter (SensorTrace suite version 1.0; Unisense). GPP was then estimated by integrating data obtained at each depth following Glud et al. (1992). Values obtained in mmol O$_2$ d^{-1} m^{-2} were then converted to g C d^{-1} m^{-2} using the photosynthetic quotient (Strickland & Parsons 1972).

2.4. Data analysis

Differences in physico–chemical parameters (i.e. sediment salinity, water content, pH, OM), MPB characteristics (i.e. chl *a*, richness, Shannon index and evenness) between crab and no-crab sites for the field sampling data, and also differences in GPP between crab-inclusion and crab-exclusion treatments for the field experiment data, were tested using *t*-tests (Zar 1999). Parametric tests were performed after confirming normality and homoscedasticity; otherwise, we used a Wilcoxon test (Conover 1999). Differences in the proportion of grain size fractions were analyzed with permutational ANOVA (PERMANOVA) based on the Euclidean dissimilarity index (Anderson 2001). Differences in MPB composition between crab and no-crab sites and between crab-inclusion and -exclusion treatments were tested using PERMANOVA, based on the Bray-Curtis dissimilarity index with 9999 permutations (Anderson 2001). PERMANOVA was used to compare sediment oxygen profiles between treatments based on the Euclidean dissimilarity index (Anderson 2001).

3. RESULTS

3.1. Sediment characterization and MPB abundance/diversity/composition in natural bioturbated and non-bioturbated patches

Sediment salinity was higher at crab sites, with an average value of 18 (Table 1). Sediment water content did not differ among sites, presenting an

Table 1. Summary of statistical tests performed to compare mean (SD) values of measured variables at crab and no-crab sites. T_{obs}: observed t-statistic value; *significant at p < 0.05

Variable	Crab sites	No-crab sites	df	T_{obs}	p	Test
Water content[a] (%)	37.76 (8.07)	38.90 (6.85)	58	−0.79	0.43	t-test
Salinity	18.01 (7.16)	7.03 (3.49)	58	856	<0.0001*	Wilcoxon test
Chlorophyll a (µg cm^{-2})	3.47 (0.83)	4.5 (0.84)	58	−4.76	<0.0001*	t-test

[a]ln(x)-transformed data

average of 38% (Table 1). Grain size distribution was different among crab and no-crab sites in areas 1 and 3 ($F_{5,24}$ = 30.08; p = 0.01 and 0.02, respectively), but there were no differences in area 2 (p = 0.18). No-crab sites had higher proportions of fine sand (125 µm) than crab sites, and both had a lower proportion of medium and coarse sands than of fine sand (Table 2).

In terms of MPB, biomass (estimated as chl a content) presented the highest values at no-crab sites (Table 1). MPB was composed of a total of 36 taxa (i.e. each identified species, genus or group listed in Table 3). At crab sites, a total of 14, 15 and 20 taxa were identified in areas 1, 2 and 3, respectively, and 19 taxa were identified at no-crab sites (in each of 3 areas; Table 3). These taxa belonged to 4 MPB groups (i.e. diatoms, cyanobacteria, euglenophytes and chlorophytes). At crab sites, we found the highest abundance, where the mean value reached up to 3.20×10^6 ind. cm^{-2} (SD: 1.02×10^6; Fig. 1A; observed t-statistic value [T_{obs}] = 3.14; p < 0.05). Richness and Shannon index were higher at no-crab sites (Fig. 1B; Wilcoxon test: T = 193.5; p < 0.05 & Fig. 1D; T_{obs} = −2.65; p < 0.05), with no differences in evenness

between sites (Fig. 1C; T_{obs} = 0.06; p > 0.05). Diatoms and cyanobacteria were the most abundant groups, while euglenophytes and chlorophytes presented lower abundances (Table 3). Epipelic diatoms (motile taxa) represented 75% of diatom richness. Diatoms did not change in relative abundance between crab and no-crab sites, representing approximately 50% of the total abundance (Fig. 2B). Relative abundance of cyanobacteria was 10% higher at crab sites, and chlorophytes were more abundant at no-crab sites (Fig. 2B). These differences in relative abundance of different taxa between sites supported the PERMANOVA results, which indicated that MPB composition differed between sites (Fig. 2A; $F_{1,58}$ = 28.32; p < 0.05).

3.2. Effects of regenerative bioturbation by *Neohelice granulata* on MPB

Sediment salinity was similar between treatments (Table 4). Water content was higher and pH was lower in crab-exclusion plots (Table 4). OM content did not differ between treatments, presenting a mean value of 4.6% (Table 4). Oxygen profiles were different between treatments (Fig. 3; $F_{1,12}$ = 6.29; p < 0.05): up to the top millimeter, oxygen concentration was higher in crab-inclusion plots. The highest oxygen concentration was found at 0.2 mm depth in crab-inclusion plots and gradually descended to ~0 µM at 1 mm depth. In crab-exclusion plots, the highest value was registered at 0.1 mm, and oxygen concentration descended steeply from a depth of 0.3 mm to 0 µM at 1 mm depth.

Table 2. Sediment fraction characterization at crab sites and no-crab sites with total averages. Values correspond to the median (SD) fraction proportion

Sediment fraction (%)	Crab site 1	Crab site 2	Crab site 3	Average crab sites	No-crab site 1	No-crab site 2	No-crab site 3	Average No-crab sites
Mud (<62 µ)	20.37 (3.02)	19.27 (1.82)	35.24 (4.11)	24.96 (8.54)	15.66 (1.67)	16.47 (2.65)	18.01 (1.78)	16.71 (2.38)
Very fine sand (62–125 µ)	13.62 (2.69)	25.28 (3.29)	43.32 (3.55)	27.41 (11.98)	20.23 (7.21)	16.90 (4.80)	25.21 (4.41)	20.78 (6.17)
Fine sand (125–250 µ)	32.34 (3.08)	29.48 (2.05)	15.49 (5.07)	25.77 (7.73)	58.84 (8.00)	41.45 (8.77)	38.33 (4.49)	46.21 (10.68)
Medium sand (250–500 µ)	17.25 (7.19)	13.31 (3.84)	2.72 (0.92)	11.09 (8.87)	1.21 (0.33)	8.63 (4.44)	7.53 (0.31)	5.79 (4.21)
Coarse sand (>500 µ)	12.78 (1.52)	12.19 (2.94)	2.36 (1.58)	9.11 (5.22)	4.88 (1.02)	10.28 (2.31)	6.86 (2.68)	7.34 (3.49)

Table 3. Abundance of microphytobenthic taxa found at sites with and without crabs. Values correspond to mean (SD) ind. $\times 10^4$ cm^{-2} of sediment. Dashes indicate taxa not found at the corresponding site

Identified taxa	Crab sites			No-crab sites		
	1	2	3	1	2	3
Cyanobacteria						
Chroococcus sp.	2.49 (2.23)	1.64 (0.71)	2.4 (2.02)	2.15 (1.48)	1.36 (1.7)	0.84 (0.54)
Chroococcal spp.	9.03 (5.29)	4.88 (1.87)	0.81 (0.82)	2.98 (2.5)	3.33 (3.07)	1 (0.75)
Spirulina sp.	1.37 (0.85)	0.42 (0.353)	4.87 (3.69)	4.3 (1.41)	1 (0.53)	2.69 (1.85)
Filamentous cyanobacteria[a]	152 (81)	148 (49.51)	52.20 (26)	108 (34.40)	53.30 (16.50)	23.30 (5.62)
Chlorophyta						
Schroederia nitzschioides	–	–	10.30 (8.32)	2.22 (0.78)	0.85 (1.01)	21.6 (11.30)
S. setigera	–	–	3.63 (3.27)	13.30 (6.27)	2.12 (1.46)	3.36 (1.38)
Ulothrix sp.	–	–	0.03 (0.06)	0.72 (0.72)	0.25 (0.24)	0.07 (0.08)
Unidentified Clorophyte	1.69 (1.43)	3.19 (1.32)	0.58 (1.51)	0.09 (0.09)	0.70 (0.69)	–
Euglenophyta						
Euglena sp.	0.81 (0.41)	3.90 (2.55)	2.31 (1.40)	2.55 (0.49)	0.95 (0.32)	0.51 (0.36)
Bacillariophyta						
Asterionellopsis sp.	0.08 (0.11)	0.03 (0.09)	0.60 (0.46)	0.02 (0.04)	–	1.36 (0.86)
Entomoneis alata	6.01 (2.45)	5.15 (2.16)	6.50 (3.40)	5.76 (1.2)	7.37 (7.28)	4.39 (2.3)
Melosira moniliformis	1.68 (1.12)	0.32 (0.39)	1.57 (0.97)	0.40 (0.39)	0.13 (0.18)	1.03 (0.85)
Nitzschia filiformis	–	0.27 (0.22)	0.06 (0.08)	0.26 (0.83)	0.05 (0.10)	–
N. longissima var. *closterium*	0.24 (0.25)	0.08 (0.08)	0.28 (0.16)	0.11 (0.09)	0.12 (0.20)	1.57 (0.96)
Nitzschia sp 1.	–	–	0.15 (0.49)	–	0.19 (0.38)	0.02 (0.05)
Nitzschia sp 2.	–	–	–	–	–	0.59 (0.42)
Odontella aurita	0.28 (0.18)	0.27 (0.27)	1.58 (0.52)	0.65 (0.81)	0.10 (0.10)	0.56 (0.34)
Surirella spp.	13.20 (5.84)	14.20 (3.94)	10 (3.48)	17.70 (5.84)	10.70 (6.82)	3.80 (1.79)
Trybionella compressa	–	–	2.35 (1.31)	3.79 (1.42)	2.34 (1.02)	1.76 (1)
Other pennate diatoms[b]	171 (34.60)	178 (36.9)	133 (31.70)	154 (34.40)	150 (31.80)	105 (40.30)
Other centric diatoms[c]	1.33 (0.71)	0.87 (0.56)	3.95 (0.66)	1.54 (0.31)	0.46 (0.21)	3.31 (1.50)

[a]Includes *Leiblenia epiphytica*, *Lyngbya aestuarii*, *Lyngbya* sp., *Oscillatoria formosa* and *Oscillatoria* sp.; [b]Length <40 μm. Includes *Amphora* sp., *Cymbella* sp., *Navicula criptocephala*, *Navicula* sp., *Eunotia* sp., and *Martyana martyi*; [c]Includes *Coscinodiscus* sp., *Cyclotella meneghineana*, *Actinoptychus splendens* and *Thalassiosira decipiens*
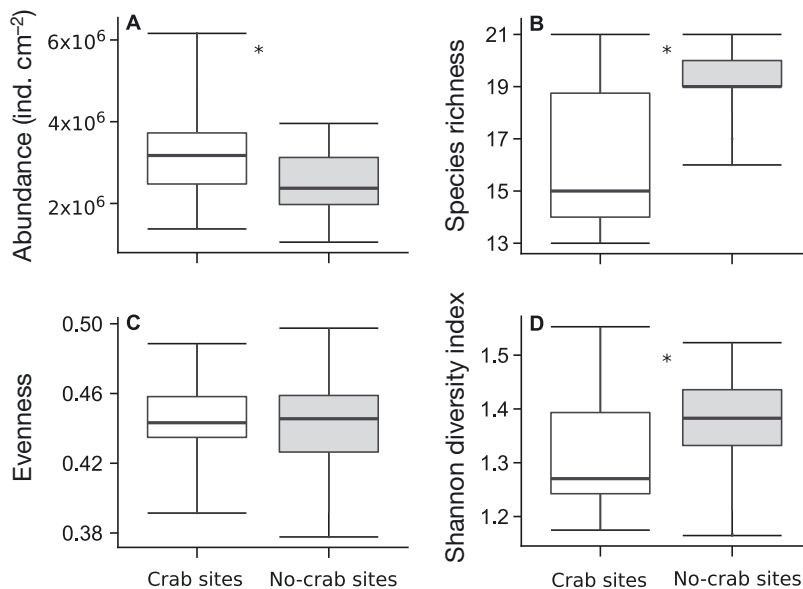
Fig. 1. Total microphytobenthos (A) abundance, (B) richness, (C) evenness and (D) Shannon diversity index at crab sites and no-crab sites. Here, and in other figures, the box represents 25th and 75th quartiles, the median is the line within the box, and bars are the maximum and minimum values. *p < 0.05

MPB biomass (estimated as chl *a* content) did not differ between treatments (Fig. 4A; $T_{obs} = -1.17$; p > 0.05), and 14 taxa were identified in the experimental plots. Total abundance was higher in crab-exclusion plots (Fig. 4B; $T_{obs} = -4.53$; p < 0.05), and no differences were found for diversity parameters between treatments (richness: Fig. 4C; Wilcoxon test: T = 51.5; p > 0.05; evenness: Fig. 4D; $T_{obs} = -0.55$; p > 0.05 and Shannon index: Fig. 4E; $T_{obs} = -0.6$; p > 0.05). Diatoms, composed of 85% epipelic taxa, presented the highest abundance, followed by cyanobacteria, chlorophytes and euglenophytes (Table 5). Analysis of composition using PERMANOVA showed that communities were different between treatments (Fig. 5; $F_{1,18} = 6.26$; p < 0.05), possibly related to lower diatom relative abundance and
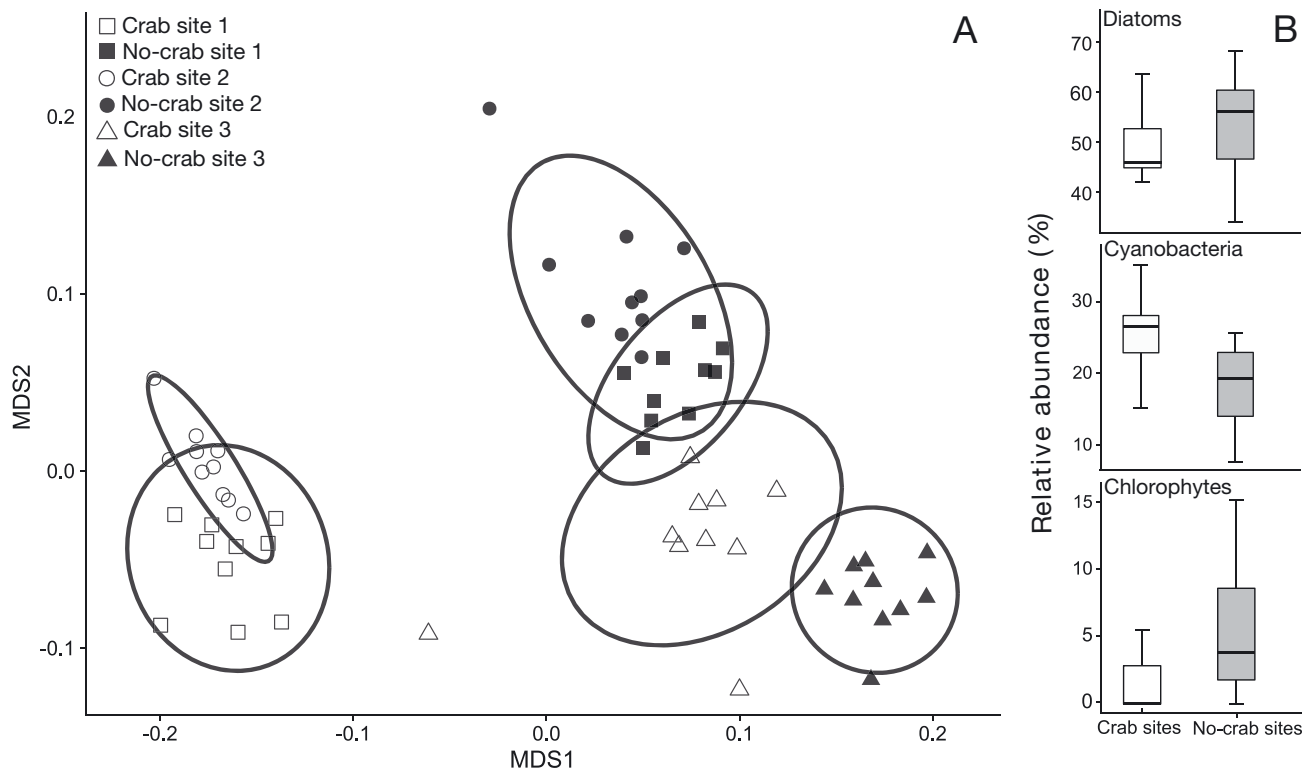
Fig. 2. (A) Multi-dimensional scaling plot based on total abundance for each identified microphytobenthic (MPB) taxon at crab sites (n = 3) and no-crab sites (n = 3). Lines represent the confidence ellipse at the 0.95 level. Stress: 0.09. (B) Relative abundance of main MPB groups at crab and no-crab sites

higher cyanobacteria relative abundance in crab-exclusion treatments (Fig. 5B). GPP was higher in crab-inclusion plots (Fig. 4F; $T_{obs} = -2.39$; p < 0.05), with a mean value of 657.29 mmol O_2 d^{-1} m^{-2}, approximately double the production observed in crab-exclusion plots.

4. DISCUSSION

4.1. Regenerative bioturbation effects on MPB

In our study site, MPB were dominated by diatoms (63%) and cyanobacteria (30%), and also contained euglenophytes and chlorophytes (7%). Diatoms showed the highest richness (20 taxa), while the other groups were less well represented (cyanobacteria = 8; euglenophytes = 1; chlorohytes = 4 taxa). Grain size distribution is a key factor for classification of benthic environments, and our data from the 3 areas showed different grain size distribution between crab and no-crab sites in 2 of these areas. The differ-

ences in grain size between crab and no-crab sites could be related to the changes in MPB composition that we found between sites. It has been proposed that cyanobacteria prefer sandy sediments for colonization (Paterson & Hagerthey 2001). Our results showed that the relative abundance of cyanobacteria was almost 10% higher at crab sites, where the proportion of medium and coarse sands (250–500 and >500 μm) is higher than at no-crab sites, which matches with what was expected. Benthic diatoms have been conventionally divided into 2 groups with regard to their structure: epipelon, the free-living diatoms that usually dominate mud and very fine sand environments; and epipsammon, the diatoms

Table 4. Summary of statistical tests performed to compare mean values of measured variables in crab-inclusion and crab-exclusion treatments. Values correspond to means (SD). T_{obs}: observed t-statistic value; OM: organic matter; *significant at p < 0.05

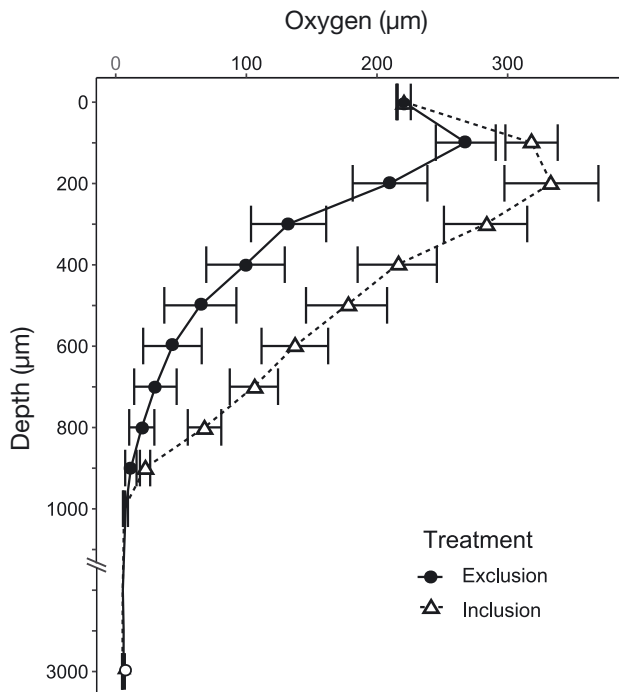| Variable	Inclusion	Exclusion	df	T_{obs}	p	Test
Salinity	12.60 (3.72)	9.20 (4.83)	18	−1.76	0.09	t-test
Water content (%)	40 (9.71)	52.90 (13.44)	18	2.46	0.02*	t-test
OM (%)	4.30 (1.07)	4.84 (0.91)	18	1.20	0.24	t-test
pH	7.16 (0.19)	6.60 (0.17)	18	1	<0.01*	Wilcoxon test

Fig. 3. Oxygen concentration profile in crab-exclusion and crab-inclusion treatments from the field experiment. Values are means ± SE

attached to sand grains that are commonly found in sandflats (>500 μm) (e.g. Round 1965, Admiraal 1984). There were no differences when comparing the relative abundance of diatoms between crab and no-crab sites; however, if epipelic–epipsammic classification is considered, we found that some of the epipelic taxa presented almost the double relative abundance in no-crab than in crab sites, where the proportion of fine sands is higher than in crab sites.

Exhaustive analysis of MPB composition showed that the dissimilarity between crab and no-crab sites was greater in 2 of the 3 areas that we sampled. There is an increasing consensus that the effects of different kinds of bioturbation (like other ecological processes) depend on the environmental and biological context (Needham et al. 2011). The same type of bioturbation at the same intensity may produce different effects on biogeochemical processes depending on sedimentary context (e.g. cohesive vs. non-cohesive sediments; Mermillod-Blondin & Rosenberg 2006). Particularly, the effect of crab bioturbation on ecosystem processes is highly context dependent (Fanjul et al. 2011, Needham et al. 2011).

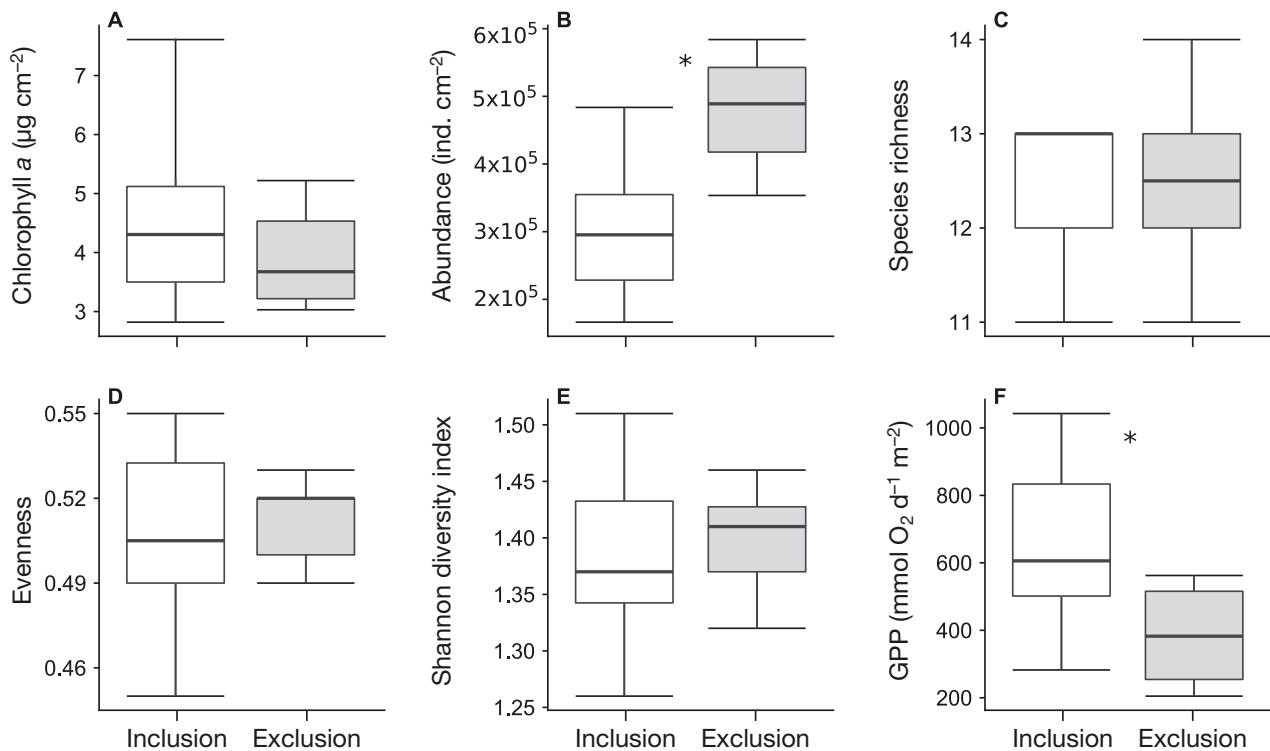When we manipulated regenerative bioturbation intensity in experimental plots, we found that crabs

Fig. 4. (A) Biomass (measured as chlorophyll a concentration), (B) abundance, (C) richness, (D) evenness, (E) Shannon diversity index and (F) gross primary production (GPP) of microphytobenthos in crab-exclusion and crab-inclusion treatments from the field experiment. *$p < 0.05$

Table 5. Abundance of microphytobenthic taxa found in crab-inclusion and crab-exclusion treatments. Values correspond to mean (SD) ind. × 10^4 cm^{-2} of sediment

Identified taxa	Inclusion	Exclusion
Cyanobacteria		
Chroococcal spp.	0.52 (0.35)	0.58 (0.37)
Myxosarcina sp.	0.60 (0.43)	0.57 (0.19)
Filamentous Cyanobacteria[a]	4.87 (2.7)	17.6 (7.00)
Chlorophyta		
Schroederia setigera	3.47 (1.90)	9.28 (4.62)
Euglenophyta		
Euglena sp.	0.36 (0.19)	0.51 (0.36)
Bacillariophyta		
Craticula pampeana	0.51 (0.17)	1 (0.47)
Melosira moniliformis	0.14 (0.23)	0.08 (0.07)
Nitzschia longissima var. *closterium*	0.12 (0.14)	0.25 (0.24)
Nitzschia sp 1.	0.11 (0.20)	0.02 (0.05)
Rhopalodia sp.	0.15 (0.16)	0.28 (0.12)
Surirella brebisonii	0.21 (0.07)	0.32 (0.13)
Trybionella compressa	0.28 (0.16)	0.14 (0.15)
Other pennate diatoms[b]	16.10 (6.74)	16.90 (3.31)
Other centric diatoms[c]	0.43 (0.13)	0.57 (0.19)

[a]Includes *Leiblenia epiphytica*, *Lyngbya aestuarii*, *Lyngbya* sp., *Oscillatoria formosa* and *Oscillatoria* sp.
[b]Length: <40 µm. Includes *Amphora* sp., *Cymbella* sp., *Navicula criptocephala*, *Navicula* sp., *Eunotia* sp., and *Martyana martyi*.
[c]Includes *Coscinodiscus* sp., *Cyclotella meneghineana*, *Actinoptychus splendens* and *Thalassiosira decipiens*.

affected MPB composition. These differences in MPB composition could be attributed to a 50% reduction in the relative abundance of filamentous cyanobacteria in crab-inclusion plots (Fig. 5B). Other taxa like *Schroederia setigera* (Chlorophytes) and large diatoms like *Craticula pampeana* and *Rhopalodia* sp. also showed a 50% reduction in abundance. The bio-

turbation process could impact MPB composition by different mechanisms. Filamentous cyanobacteria form a dense network of entangled trichomes (Stal 1994), which could result in a physically stable sediment surface. Regenerative bioturbation by *Neohelice granulata* generates the rupture of the sedimentary matrix as a result of its burrowing activity; this destabilization could break the cyanobacterial network and prevent new establishment of another cyanobacterial network (Barth 2003). This makes cyanobacteria susceptible to be more easily removed (i.e. resuspended or transported) from bioturbated than non-bioturbated areas. Thus, the reduction in cyanobacterial abundance found in this work could be related to this physical disturbance. Moreover, the limited mobility of this group makes it more vulnerable to resuspension by bioturbation (Armitage & Fong 2004) in comparison with epipelic diatoms, which move through the sediment, possibly giving them the ability to counteract sediment destabilization (Consalvey et al. 2004). In the same way, it is important to note that regenerative bioturbation by *N. granulata* includes the deposition of large amounts of sediment near burrow entrances. This causes the burial of MPB near crab burrows, which could differently impact microalgal groups with different levels of mobility (Jesus et al. 2005), or groups that react to a stimulus or disturbance at different times (e.g. changes in radiation incidence; Mouget et al. 2008, Wulff et al. 2008). Finally, at our study site, the disturbance produced by *N. granulata* reduces herbivory pressure by hydrobioid snails on MPB and also negatively affects other infaunal organisms that graze on MPB (Escapa et al. 2004, Alvarez et al. 2013). Thus, the process of regenerative bioturbation could impact MPB not only directly through physical destabilization and burial, but also through indirect interactions with other macrofaunal organisms that graze on MPB.

4.2. Bioturbation impact on MPB primary production

Although MPB primary production of intertidal systems is widely recognized by its high contribution to total primary production (e.g. Underwood et al. 2005), there have been no previously reported measurements of intertidal MPB primary production on the SWA temperate coasts. Thus, the MPB primary production data presented in
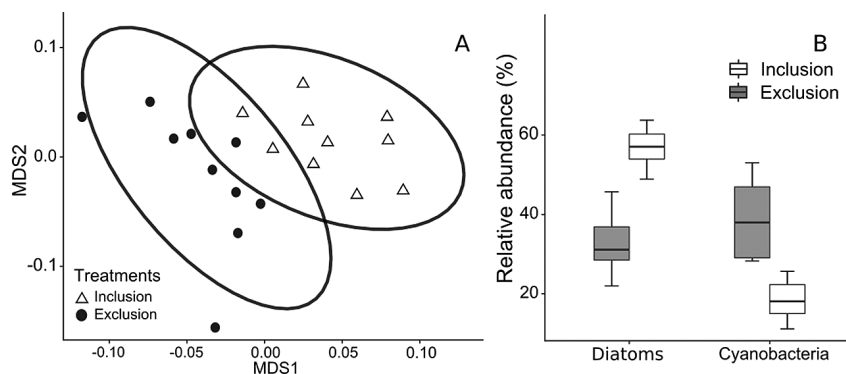

Fig. 5. (A) Multi-dimensional scaling plot based on total abundance for each identified microphytobenthic (MPB) taxon in crab-exclusion and crab-inclusion treatments from the field experiment. Lines represent the confidence ellipse at the 0.95 level. Stress: 0.15. (B) Relative abundance of dominant MPB groups in crab-exclusion and crab-inclusion treatments

this study are the first for this region; however, these are micro-scale estimates and should be interpreted with caution since they do not include processes that operate on a larger scale (e.g. macrofaunal community respiration or large-scale environmental heterogeneity). Our results show high MPB productivity, presenting values near the highest registered for similar systems (median 0.5 g C m^{-2} d^{-1}; Cebrian 1999). MPB primary production rates are as high as those for the dominant intertidal vascular plants of this region. Primary production of *Sporobolus densiflorus* in our study region is on average 2.46 g C m^{-2} d^{-1} (González Trilla et al. 2010), which is similar to the value that we registered for MPB without crabs (2.38 g C m^{-2} d^{-1}) and half of the value registered with crabs (4.07 g C m^{-2} d^{-1}). Since almost half of total areal extension corresponding to mudflats at our study site is highly bioturbated by crabs (Iribarne et al. 2005), MPB makes a significant contribution to primary production in this particular zone (i.e. soft-bottom tidal flats). In addition, the presence of MPB in marsh and shallow sub-tidal sediments could also significantly contribute to global productivity (Hughes 1999). In this sense, MPB contribution to global primary production in our study site is underestimated because we did not take into account the high MPB abundance in salt marshes (M. Giorgini unpubl. data).

The importance of bioturbation effects in the functioning of intertidal systems (Teal et al. 2013) and on MPB productivity (Lohrer et al. 2004) is widely recognized, but there is scarce evidence regarding the role of regenerative bioturbation on MPB productivity (but see Needham et a. 2011). Here, we have demonstrated that the burrowing crab *N. granulata* enhances MPB primary production to levels almost twice as high as those in areas without bioturbation, while at the same time reducing MPB total abundance, thus evidencing an increase of biomass-specific productivity rates. Chl *a* concentration is often used as a proxy of MPB biomass. In this sense, we expected a positive correlation between chl *a* and abundance and also between chl *a* and MPB primary production. In our experiment, we found the same concentration of chl *a* between crab-exclusion and crab-inclusion treatments. In contrast, we found higher MPB abundance but lower MPB primary production in crab-exclusion plots. This may be due to the changes observed in MPB composition between treatments, which could imply shifts for taxa with different pigment production, resulting in a non-linear relationship between productivity and pigment concentration (Falkowski & Kiefer 1985). These results of primary production enhancement by bioturbation

and uncorrelated chl *a* and productivity are similar to results reported for the regenerative bioturbator crab *Austrohelice crassa* (Needham et al. 2011), and surficial biodiffuser urchins of the genus *Echinocardium* (Lohrer et al. 2004), which enhance MPB production up to 35 %.

The enhancement of primary production could be explained by several factors. First, regenerative bioturbation reduces MPB abundance, which could benefit productivity by alleviating competition (Morrisey 1988). Particularly, the reduction in total abundance of microalgae in our system could be attributed to the decline in the abundance of cyanobacteria, a group characterized by a great functional diversity but a low photosynthetic rate (e.g. Glud et al. 1992). Second, in our experiment, oxygen concentration was higher in bioturbated than in non-bioturbated sediment, so this kind of reworking is likely to increase the supply of resources and oxic layers, probably stimulating aerobic microbial metabolism (e.g. Fanjul et al. 2015). The increase in sediment oxygen concentration is usually related to changes in grain size distribution, but over a short period, grain size is not affected by regenerative bioturbation (Needham et al. 2011). Thus, changes in sediment oxygen concentration may be due to an indirect effect of the higher GPP generated by regenerative bioturbation or by a direct effect of bioturbation. Since we did not test these hypotheses, we could not attribute the increase in oxygen concentration to either of these factors. Finally, it is known that regenerative bioturbation can improve ammonia availability in the surface sediment layers (e.g. Fanjul et al. 2011, Needham et al. 2011) where MPB live, and as a consequence MPB primary productivity increases (Lohrer et al. 2004, Sandwell et al. 2009). Thus, throughout the enhancement of benthic metabolism and ammonia availability, regenerative bioturbators could be stimulating MPB primary production. At bioturbated sediments, we found lower water content, which is commonly expected to cause negative effects on MPB primary production (Coelho et al. 2009). However, the combined increase in oxygen concentration and ammonia availability could result in increased MPB productivity (Needham et al. 2011), prevailing over the possible negative effects of low water content. MPB may also be negatively affected by other kinds of bioturbation (e.g. epifaunal biodiffusers; Webb & Eyre 2004) due to different effects on physico–chemical sedimentary environment and also because bioturbators often graze on MPB. Surficial biodiffusers, for example, can mix the surficial sediment as they move, and they have little if any effect upon the increase in

ammonia release (e.g. sea cucumber *Australostichopus mollis,* sand dollars), and the net result is a negative effect on primary producer biomass (MacTavish et al. 2012, Li et al. 2013). Contrary to these kinds of bioturbation, net effects of regenerative bioturbation by *N. granulata* are positive for MPB productivity, so based on our results we suggest that this crab constitutes an important biological force that enhances primary production at SWA mudflats.

4.3. Conclusions

Our results show that the burrowing crab *N. granulata* affects the abundance, composition and the main ecosystem functions mediated by MPB at the SWA mudflats. The decrease in MPB abundance could be explained by a reduction in cyanobacterial abundance and, as a consequence, MPB composition is affected. The modification of MPB composition together with the increased availability of ammonia could explain the increase in MPB primary production. *N. granulata* enhances the productivity of marsh plants (*Sporobolus* spp.; Daleo et al. 2007), and similarly, regenerative bioturbation by *N. granulata* increases MPB primary production. To our knowledge, this is the first study where the primary production of MPB was quantified in this region. The production rate is high when compared with similar systems and almost equal to the production of marsh plants that occur in the upper intertidal levels.

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