Change in the community structure and organic carbon content of meio- and macrobenthos between tidal flat and salt marsh areas colonized by *Spartina alterniflora* in the Bahía Blanca estuary (SW Atlantic)

Leandro J. Reyna Gandini [1], Flavia A. Funk [2,3], Paula D. Pratolongo [2,3]

[1] Laboratorio de Ecología, Instituto de Investigaciones Marinas y Costeras (IIMyC), CONICET-UNMdP, Mar del Plata, Argentina.
(LJRG) E-mail: leandroreynagandini@gmail.com. ORCID iD: https://orcid.org/0000-0002-0102-8158
[2] Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), CONICET-UNS, Bahía Blanca, Argentina.
[3] Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina.
(FAF) (Corresponding author) E-mail: ffunk@criba.edu.ar. ORCID iD: https://orcid.org/0000-0003-4694-3701
(PDP) E-mail: paulapra@criba.edu.ar. ORCID iD: https://orcid.org/0000-0001-8271-7942

Summary: Salt marshes are regarded as among the most productive coastal ecosystems, important "blue carbon" sinks and a support for benthic communities with large abundances, whose structure may be strongly influenced by salt marsh vegetation. During the last few decades, *Spartina alterniflora* has been colonizing bare mudflats in the Bahía Blanca estuary, and a large increase in the area covered by salt marshes has been reported. This colonization can strongly influence the structure of benthic fauna and its role in the carbon cycle. The hypothesis of this study was that the community structure and the organic carbon contained in the meio- and macrobenthos change between tidal flats and salt marshes recently colonized by *S. alterniflora*. Response variables studied to compare the tidal flat and salt marsh were density, biomass and production to biomass (P/B) ratio of macro- and meiobenthos. Density and biomass of Gastropoda and P/B ratio of Nematoda were higher on the salt marsh than on the tidal flat. By contrast, density and biomass of Polychaeta were higher on the tidal flat. These results suggest that the expansion of *S. alterniflora* marshes on tidal flats produces changes in the structure of the macro- and meiobenthos community (taxonomic composition and biomass) that have an influence on carbon cycling.

Keywords: meiobenthos; macrobenthos; biomass; P/B ratio; carbon; Bahía Blanca estuary.

Cambios en la estructura de la comunidad y contenido de carbono orgánico del meio- y macrobentos entre las áreas de planicies de marea y marismas colonizadas por *Spartina alterniflora* en el Estuario de Bahía Blanca (Atlántico SO)

Resumen: Las marismas son consideradas uno de los ecosistemas costeros más productivos, importantes sumideros de "carbono azul" y soporte para comunidades bentónicas con grandes abundancias, cuya estructura puede estar fuertemente influenciada por la vegetación de las marismas. Durante las últimas décadas, *Spartina alterniflora* ha estado colonizando las planicies de mareas sin vegetación en el estuario de Bahía Blanca, reportándose un gran incremento del área cubierta por las marismas. Esta colonización puede influir fuertemente en la estructura de la fauna bentónica y en su rol en el ciclo de carbono. La hipótesis de este estudio fue que la estructura de la comunidad y el carbono orgánico contenido en el meiobentos y macrobentos cambian entre las planicies de marea y las marismas recientemente colonizadas por *S. alterniflora*. Las variables de respuesta estudiadas para comparar la planicie de marea y la marisma fueron la densidad, biomasa y la relación producción/biomasa (P/B) del macrobentos y meiobentos. La densidad y biomasa de Gastropoda y la relación P/B de Nematoda fueron mayores en la marisma que en la planicie de marea. Por el contrario, la densidad y biomasa de Polychaeta fueron mayores en la planicie de marea. Estos resultados sugieren que la expansión de las marismas de *S. alterniflora* sobre las planicies de marea genera cambios en la estructura de la comunidad del macrobentos y meiobentos (composición taxonómica y biomasa) que influyen en el ciclo del carbono.

Palabras clave: meiobentos; macrobentos; biomasa; relación P/B; carbono; estuario de Bahía Blanca.

INTRODUCTION

Carbon dioxide (CO_2) represents about 74% of the global emissions of greenhouse gases and its atmospheric concentration has increased by 1.1% to 1.6% per year in the last three decades as a result of anthropic activities (Olivier et al. 2017). A fraction of this CO_2 is sequestered and stored by mangroves, tidal salt marshes and seagrasses within the sediments, the biomass living aboveground and belowground, and detritus, which contribute to the blue carbon pool. Carbon sequestration is among the most important ecosystem services provided by salt marshes, with an estimated global average accumulation rate in sediments of 245 ± 26 gC m^{-2} year^{-1} (Chastain et al. 2018). However, factors such as climatic conditions, tidal range, vegetation type, maturity of the salt marsh, sediment type and benthos composition can modify the ecosystem functioning, and strongly affect the carbon storage capacity of a salt marsh, which may even change from sink to source (Mann 2009).

Salt marshes are among the most productive ecosystems worldwide and often have large abundances of benthic fauna (Levin and Talley 2002). On salt marshes, a low percentage of the plant primary productivity is consumed by herbivores, and most plant biomass is transformed into detritus and incorporated into the sedimentary matrix (Bergamino and Richoux 2015). Detritus is susceptible to microbial decomposition, and the associated carbon is partially incorporated into bacterial biomass, entering the benthic food web mostly through macrobenthos and meiobenthos consumption (Danovaro and Gambi 2002, Mann 2009). Salt marshes are often characterized by detritus-based food webs, in which benthic fauna is a link between detritus, decomposers, and higher trophic levels (Danovaro and Gambi 2002). Thus, benthic invertebrates are fundamental to the regulation of the ecological processes and functions of these ecosystems (Chen et al. 2009), especially those related to the transfer of carbon. Despite their importance, the macrobenthos and meiobenthos have a differential contribution to the carbon cycle and the flow of energy. A primary difference arises from the unequal amounts of carbon that these fractions consume and contain, but although the meiobenthos usually has a lower stock biomass than the macrobenthos, its higher dynamics and shorter turnover generates a high production that frequently exceeds that of the macrobenthos (Giere 2008)

Spartina alterniflora (Loisel.) is a common dominant species on salt marshes of the southwest Atlantic (Isacch et al. 2006). In the Bahía Blanca estuary, this species has increased its cover during the last few decades, forming new marshes by colonizing bare tidal flats (Pratolongo et al. 2013). The rate of expansion of *S. alterniflora* marshes in the Bahía Blanca estuary is similar to that found in areas where this species has been introduced as exotic (Pratolongo et al. 2013), and it has been proposed that this species is not native to South America but was introduced from North America or Europe in the early 19th century (Bortolus et al. 2015). As an ecosystem engineer, *S. alterniflora* is able to modify key properties of the physical and chemical environment, such as hydrology, particle movements and habitat characteristics (Levin and Talley 2002). In locations where *S. alterniflora* is considered an introduced species, the colonization of unvegetated tidal flats has been shown to strongly influence the structure and function of benthic fauna (Neira et al. 2005). Changes in the abiotic conditions, such as the sediment organic matter or grain size, have a major influence on the composition and distribution of invertebrates in these environments (Santos et al. 2020). In the Jiangsu coastland (Chine), Zhou et al. 2009 reported that the expansion of *S. alterniflora* decreased the macrobenthos diversity of the tidal flats, and forced the niche of native species composing the macrobenthos on the tidal flats to move seaward.

The effect of *S. alterniflora* colonization on benthic organism communities has not been studied in the study area. Therefore, the main hypothesis of this study was that the community structure and the organic carbon contained in the benthic meio- and macrobenthos change between tidal flats and salt marshes recently colonized by *S. alterniflora*. Accordingly, the objectives of this study were to compare tidal flats and *S. alterniflora* salt marshes in terms of (1) the community structure of macrobenthic and meiobenthic communities; and (2) the organic carbon contained in these fractions of the benthic fauna.

MATERIALS AND METHODS

Study area

The Bahía Blanca estuary (Fig. 1) covers an area of 2290 km^2 characterized by a series of tidal channels and islands surrounded by extensive intertidal areas arranged in a complex mosaic of salt marshes (296 km^2) and tidal flats (578 km^2) (Isacch et al. 2006, Pratolongo et al. 2013). The estuary occupies a transition zone between humid subtropical and cold semiarid climates, with a mean annual temperature of 15.5°C and a widely variable mean annual precipitation that ranges from 350 mm up to 1080 mm, with an average value of 550 mm (Celleri et al. 2018). Freshwater inputs to the estuary are scarce. The two major tributaries are the Sauce Chico and Naposta Rivers, with mean annual runoff flows of 1.5-1.9 and 0.5-0.9 m^3 s^{-1}, respectively (Perillo et al. 2001). Freshwater inputs from other smaller tributaries into the estuary are intermittent and only significant during periods of high local precipitation. The water column is characterized by high turbidity and high levels of particulate organic matter (between 300 and 1000 mgC m^{-3}), so it is considered a moderately to highly eutrophic estuary (Fernández Severini et al. 2011). The tidal regime is mesotidal (tidal range: 1.4-3.8 m) and semi-diurnal (Perillo et al. 2001).

The study was conducted on Villa del Mar salt marsh, located in the middle zone of the Bahía Blanca estuary. The intertidal covers about 6 km^2 and extends for more than 1 km across the tidal gradient. The intertidal is characterized by a gentle slope and an absence
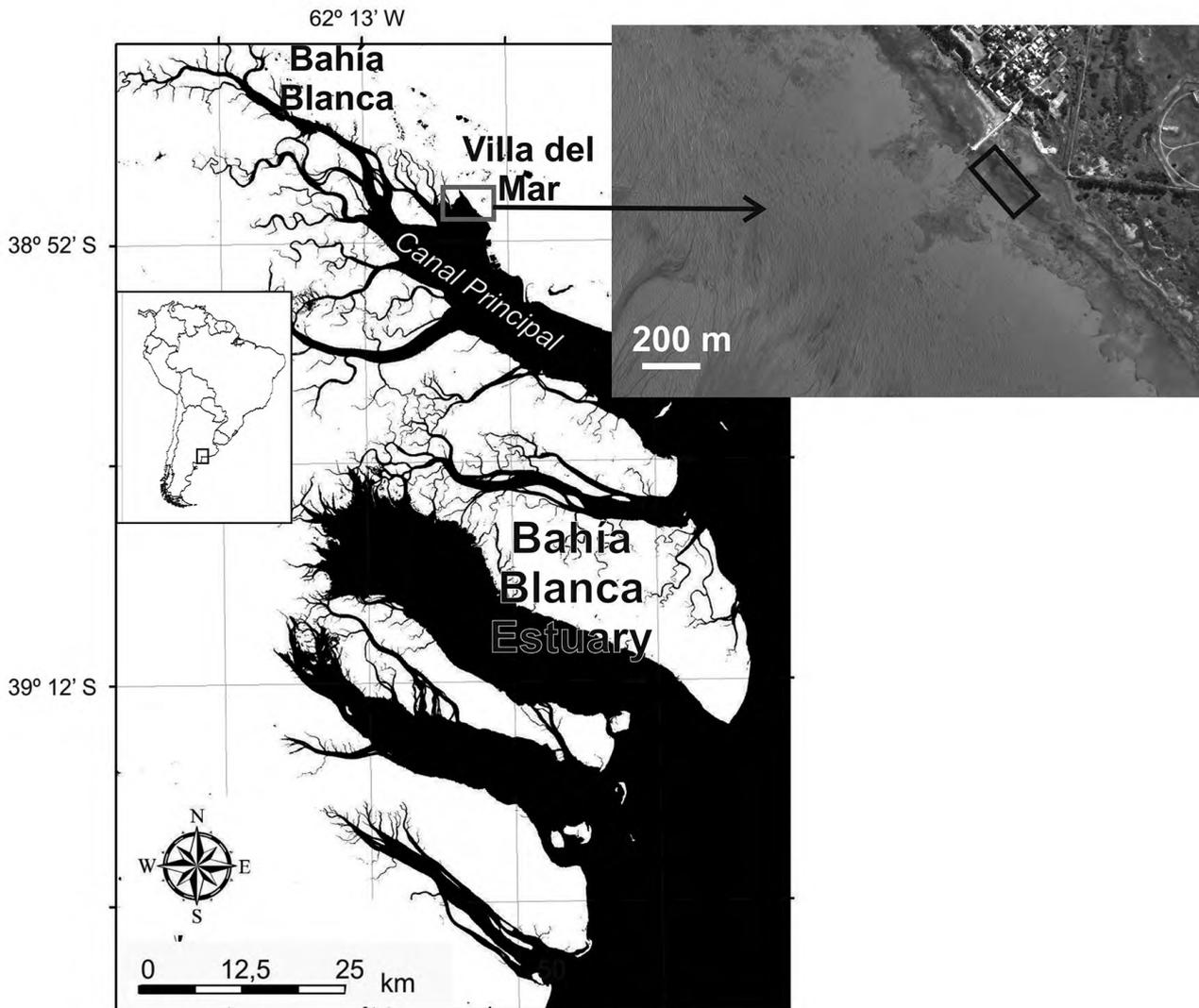
Fig. 1. – Location of the study area in the Bahía Blanca estuary, Argentina. Rectangle indicates sampling sites for salt marsh of *S. alterniflora* and tidal flat.

of tidal creeks and channels, and the sediments are dominated by fine mud (87% mud and 13% sand) (Calvo-Marcilese and Pratolongo 2009, Pratolongo et al. 2010). The monospecific salt marsh of *S. alterniflora* forms a strip approximately 150 m wide that occupies the low intertidal areas (being daily affected by tides), while salt marsh of *Sarcocornia ambigua* is dominant in the upper intertidal zone (Pratolongo et al. 2010). In the study site, the annual net aerial primary productivity of *S. alterniflora* ranges from about 482 to 936 g $m^{-2} yr^{-1}$ (Trilla et al. 2009). Also, Bortolus et al. (2015) found records that the first *S. alterniflora* specimens in Argentina were collected in 1902 at Punta Alta (located approximately 3 km from Villa del Mar).

Sampling

Field sampling was carried out in November 2018 on a salt marsh of *S. alterniflora* and tidal flats located at similar elevations within the intertidal fringe.

Sediment samples for macrobenthos analysis (five samples per habitat) were extracted with PVC tubes (10 cm diameter and 15 cm depth). Each sample was sieved wet through a 500 µm mesh. The retained material was fixed in 10% formalin and preserved in 70% ethanol. Sediment samples for meiobenthos analysis (five samples per habitat) were obtained with PVC tubes (2.5 cm diameter and 6 cm depth; because of the high density of the meiobenthos, it is advisable to extract smaller samples than for the macrobenthos). To avoid subsampling errors, these samples were collected independently and adjacent to each of the macrobenthos samples. Samples were fixed in 4% formaldehyde with 7% $MgCl_2$ and seawater, and then each sample was sieved through 500 and 45 µm meshes. The resulting material was centrifuged with a Ludox® solution (Eleftheriou 2013). The samples were preserved in 70% ethanol and stained with Bengal Rose solution; we analysed three aliquots (1.5 mL) from each sample.

Macrobenthos and meiobenthos organisms were counted to estimate faunal density and classified into taxonomic groups on the basis of stereomicroscopic observations. All macrobenthic organisms were categorized into Gastropoda, Decapoda, Polychaeta, Bivalvia, Amphipoda and Priapulida; meiobenthic organisms were in turn classified into Ciliophora, Nematoda, Foraminifera, Crustacea and Turbellaria.

Biomass and production to biomass ratio

The small size of some benthos taxa does not allow direct biomass measurements of individuals. Thus, estimates of individual biomass were not obtained from direct weight but from volume (biovolume) estimates. The biovolume estimates were made from measurements of the body length and maximum width of individual specimens using photographs (Eleftheriou 2013). Biomass of the meiobenthos and some taxa of the macrobenthos (Gastropoda, Polychaeta, Priapulida and Amphipoda) was estimated through the body volume using photographs of each individual, then the biovolume was converted to carbon content (CC=dry weight×0.363) (Eleftheriou 2013). The biomass (wet weight) of the largest benthos (Decapoda and Bivalvia) was obtained directly by weighing individuals (±0.01 mg). Body volume of Gastropoda was calculated following McClain and Nekola (2008), whereas for Pri-apulida and Polychaeta the formula from Eleftheriou (2013) was applied (Table 1). The samples with the largest number of individuals (between 5 and 60) of these three taxa were weighed and the total biovolume was calculated. Using these pairs of estimates, a regression equation was constructed to allow the wet weight (WW) of each individual to be estimated from its biovolume. The WW was converted to dry weight (DW=WW×0.234) and then to carbon content (Eleftheriou 2013). Body volumes of Nematoda, Turbellaria and Crustacea were calculated using the formula from Eleftheriou (2013). For Ciliophora and Foraminifera the formulas of body volume from Putt and Stoecker (1989) and Gerlach et al. (1985) were applied, respectively (Table 1). Body volume was converted to WW using the specific gravity (1.13 g cm^{-3}), and the carbon content was calculated using the formula CC=WW×0.116 (except for Nematoda CC=WWx0.124) (Eleftheriou 2013).

The production to biomass (P/B) ratio of macrobenthos was estimated using an Excel application template freely provided by Brey (2001). The conversion factors of Brey (2001) were used to perform the transformations from mass to energy (from mgC to J assuming 1 mgC = 45.7 J). Estimates of the P/B ratio of each meiobenthos group were obtained using the formula from Vranken and Heip (1986) log P/B=−1.288−0.44 log W (where W is the mean individual weight in kcal). The individual

Table 1. – Formula for calculating body volumes of different benthos taxa. L, length; w, maximum width; D, diameter; CL, cephalic length; V, volume; mm³, cubic millimetres; nl, nanoliters; mg, milligrams.

	Formula	Units	Reference
Macrobenthos			
Gastropoda	$V = \frac{1}{3} \times \pi \times \left(\frac{w}{2}\right)^2 \times L$	mm³	McClain and Nekola 2008
Polychaeta	$V = L \times w^2 \times 530$	nl	Eleftheriou 2013
Anphipoda	$DW = 1.592924 \times CL^{3.94344}$	mg	Martins et al. 2002
Priapulida	$V = L \times w^2 \times 530$	nl	Eleftheriou 2013
Meiobenthos			
Ciliophora	$V = \frac{4}{3} \times \pi \times \left(\frac{D}{2}\right)^3$	mm³	Putt and Stoecker 1989
Nematoda	$V = L \times w^2 \times 530$	nl	Eleftheriou 2013
Turbellaria	$V = L \times w^2 \times 550$	nl	Eleftheriou 2013
Crustacea	$V = L \times w^2 \times 260$	nl	Eleftheriou 2013
Foraminifera	$V = \frac{4}{3} \times \pi \times \left(\frac{D}{2}\right)^3$	mm³	Gerlach et al. 1985

weight was converted from μg DW to kcal assuming 1 g DW=5.3 kcal (Peters and Peters 1986).

Statistical analyses

To analyse the differences between the salt marsh and tidal flat, we compared the density, biomass and P/B ratio data at both habitats using Student t tests. Prior to analysis, some data were transformed (logarithm for the biomass and density of Polychaeta, total of macrobenthos biomass) to fulfil the requirements of normality. The data of Ciliophora density and density and biomass of Decapoda, Priapulida and Bivalvia (whether transformed or untransformed) did not follow a normal distribution (Shapiro-Wilks normality test), so the nonparametric Wilcoxon test was applied. Only P/B ratio data of Nematoda, Ciliophora, Gastropoda and Polychaeta were analysed because they were the most representative taxa and they were present in both habitats, thus allowing comparisons to be made.

To identify possible sample groupings among habitats, non-metric multidimensional scaling ordination (nMDS) was used to contrast benthos abundances, based on Bray-Curtis similarity indices. Ordination goodness of fit was evaluated using the stress value; the interpretation of stress was as follows: stress values of <0.1 provide good fit and stress values of <0.2 a useful fit (Clarke

1993). The data were square-root transformed to reduce impacts of extremely high counts of individual taxa. The significant differences between groups were analysed with one-way analysis of similarity (ANOSIM). Similarity percentage analyses (SIMPER) were used to determine the percentage of similarity of groups and the particular taxa responsible for differences between groups (Clarke 1993). Statistical analyses of density, biomass and P/B ratio data were carried out using InfoStat software (Di Rienzo et al. 2018), while nMDS, ANOSIM and SIMPER were carried out using the PRIMER 7 software package (Clarke and Gorley 2015). The results presented are based on untransformed data.

RESULTS

Density and structure of benthic invertebrate assemblages

A total of six macrobenthos taxa were collected from a tidal flat and salt marsh of *S. alterniflora*. The most common taxonomic groups were Polychaeta (53±4% and 6±0.8% of the total density) and Gastropoda (43±4% and 88±1% of the total density) on the tidal flat and salt marsh, respectively. The density of Polychaeta was significantly higher on the tidal flat ($p<0.001$), while the density of Gastropoda was
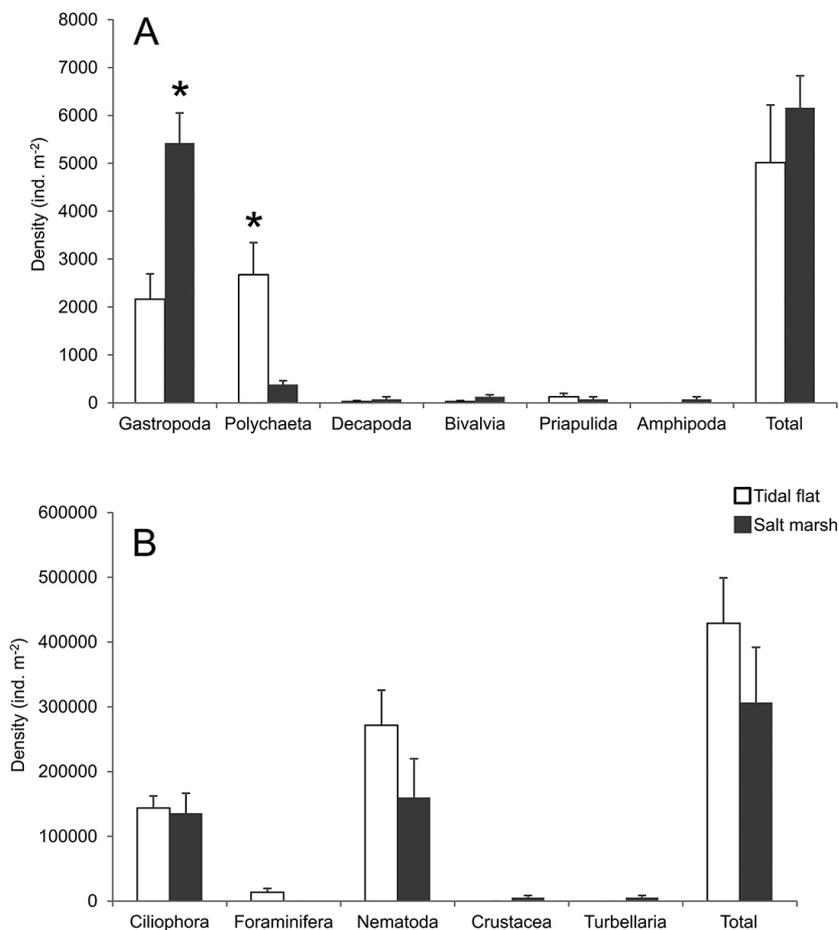

Fig. 2. – Density (individual m^{-2}) of benthic organisms at each sampling site. Values are mean ± standard error. A, density of macrobenthos; B, density of meiobenthos. Asterisk indicates significant differences between habitats ($p<0.05$).

Table 2. – Summary of the results of Student *t* (t) and Wilcoxon (W) test for comparisons between the salt marsh and the tidal flat. We compared the density, biomass and P/B ratio data of macrobenthos and meiobenthos in both habitats. The degrees of freedom for all comparisons were df=8. Bold numbers correspond to a significant statistical difference.

|	Density		Biomass		P/B ratio	
	Statistic	*p*-value	Statistic	*p*-value	Statistic	*p*-value
Macrobenthos						
Gastropoda	t=3.97	**<0.01**	t=3.74	**<0.01**	t=1.76	0.12
Polychaeta	t=5.30	**<0.001**	t=4.04	**<0.01**	t=1.47	0.18
Decapoda	W=30.50	0.72	W=30	0.72	----	----
Bivalvia	W=35.5	0.17	W=35	0.13	----	----
Priapulida	W=25	0.68	W=26	0.84	----	----
Total	t=0.83	0.43	t=2.63	**<0.05**	----	----
Meiobenthos						
Nematoda	t=1.38	0.2	t=1.99	0.08	t=3.03	**<0.05**
Ciliophora	W=21.5	0.24	t=0.60	0.56	t=0.26	0.79
Total	t=1.11	0.29	t=1.83	0.10	----	----
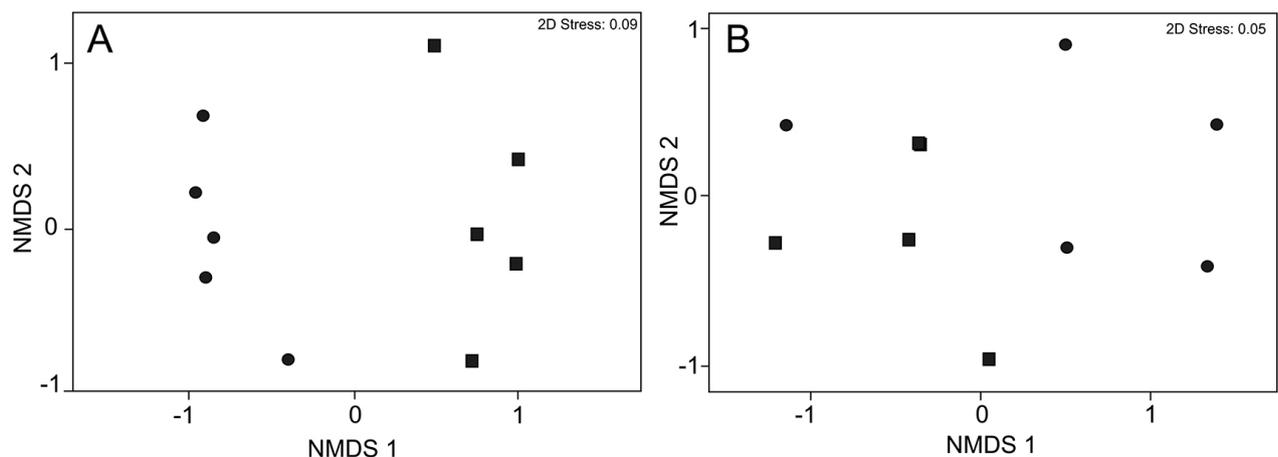
Fig. 3. – Non-metric multidimensional scaling (nMDS) ordination of benthic organism abundance between the salt marsh of *S. alterniflora* (circle) and the tidal flat (squares). A, nMDS plot of macrobenthos; B, nMDS plot of meiobenthos. Each point represents the community composition of benthic organisms in a sample.

significantly higher on the salt marsh (*p*<0.01) (Table 2, Fig. 2A). Amphipoda were present only on the salt marsh of *S. alterniflora*. Total macrobenthos density did not differ between the two habitats (Table 2). A total of five meiobenthos taxa were collected from the tidal flat and salt marsh. The dominant taxonomic groups were Ciliophora (35±4% and 48±6% of the total density) and Nematoda (61±6% and 47±7% of the total density) on the tidal flat and salt marsh, respectively. However, the Ciliophora, Nematoda and total meiobenthos densities did not differ between the two habitats (Table 2, Fig. 2B). Crustacea and Turbellaria were present only on the salt marsh of *S. alterniflora*, while Foraminifera were only found on the tidal flat.

The sampling sites were split into two categories based on macrobenthos abundance according to the results of nMDS analysis (stress value = 0.09), one for the salt marsh of S. *alterniflora* and one for the tidal flat (Fig. 3A). Furthermore, the results of ANOSIM showed that significant differences existed between these two groups (R=0.84, *p*<0.01). SIMPER analysis showed that the group average similarity of the salt marsh was 73.9%, with Gastropoda representing its characteristic taxon (contribution rate of 46.7%). The group average similarity of the tidal flat was 78.7%, with Polychaeta as its characteristic taxon (contribution rate of 46.2%). The average dissimilarity between the habitats was 29.7%, Bivalvia and Priapulida were the taxa that most contributed to the observed differ-
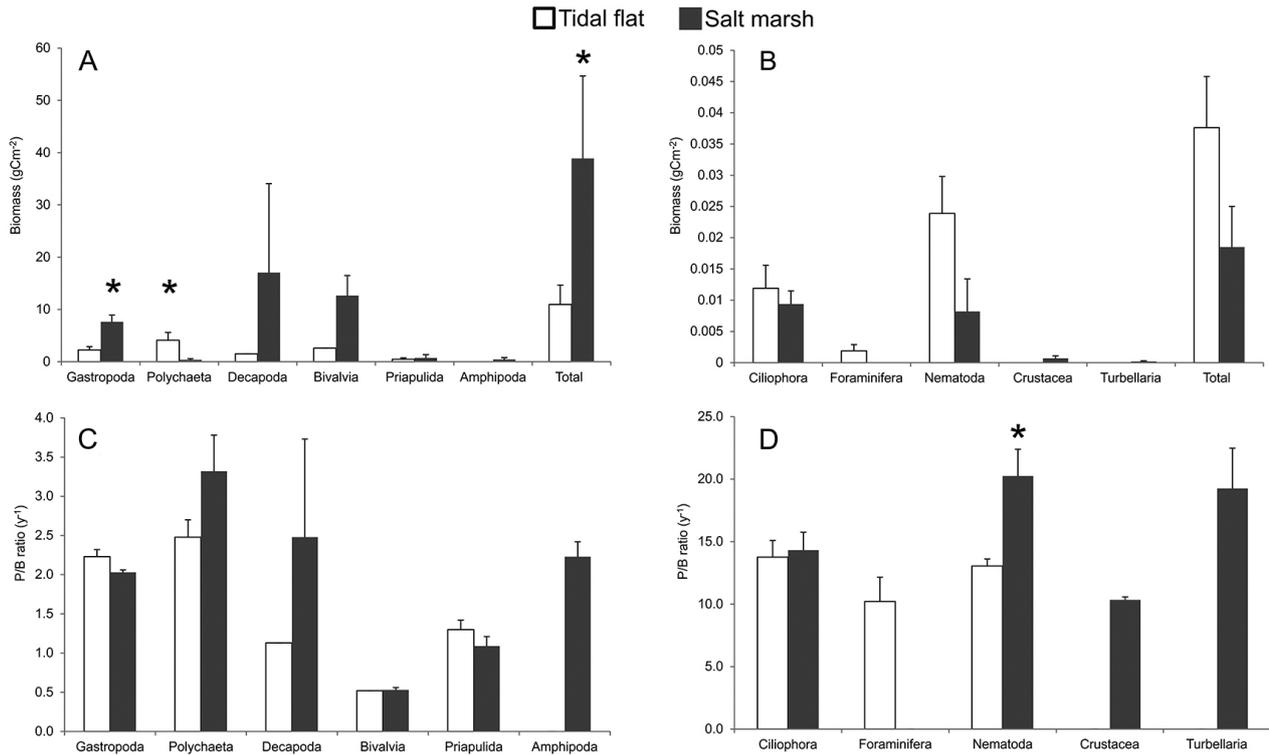
Fig. 4. – Biomass (gC m^{-2}) and P/B ratios (y^{-1}) of benthic organisms at each sampling site. Values are mean ± standard error. Biomass (A) and P/B ratios (C) of macrobenthos. Biomass (B) and P/B ratios (D) of meiobenthos. Asterisk indicates significant differences between habitats ($p < 0.05$).

ences between habitats (accumulated contribution rate of 46.8%). On the other hand, meiobenthos community assemblages showed no significant differences between the salt marsh and tidal flat (ANOSIM, R=0.3, p=0.06). Also, the results of nMDS analysis (stress value = 0.05) showed no clear separation between the sampling sites (Fig. 3 B).

Biomass and P/B ratio

The total macrobenthos biomass was 38.89±15.78 and 10.93±3.69 gC m^{-2} on the salt marsh and tidal flat, respectively. The biomass of Polychaeta was significantly higher on the tidal flat than on the salt marsh (p<0.01) (Table 2, Fig. 4A). In contrast, the biomass of Gastropoda and total macrobenthos biomass were significantly higher on the salt marsh than on the tidal flat (p<0.01 and p<0.05, respectively) (Table 2, Fig. 4A). The Polychaeta and Gastropoda P/B ratios showed no difference between habitats (Table 2, Fig. 4C).

The total meiobenthos biomass was 18.46 and 37.62 mgC m^{-2} on the salt marsh and tidal flat, respectively. There were no significant differences in total meiobenthos biomass and biomass of the groups between habitats (p>0.05) (Table 2, Fig. 4B). The P/B ratio of Nematoda was significantly higher on the salt marsh than on the tidal flat (p<0.05) (Table 2, Fig. 4D). For the P/B ratio of Ciliophora no significant differences were observed between habitats (Table 2, Fig. 4D).

DISCUSSION

Our study demonstrated that the expansion of *S. alterniflora* and the replacement of bare tidal flats with salt marshes have a significant effect on the macrobenthos and meiobenthos communities. The changes involve their structure, in terms of taxonomic composition, and the amount of organic carbon contained in the benthic organisms. This finding is in agreement with several studies that have reported changes in the macrobenthos community after colonization of natural habitats by *S. alterniflora* (Brusati and Grosholz 2006, Su et al. 2020, Lu et al. 2022).

Regarding the macrobenthos, the presence and density of the dominant taxonomic groups in our study area were similar to those reported elsewhere on salt marshes (Chen et al. 2009, Santos et al. 2020). The density and biomass of Gastropoda were higher on the salt marsh than on the tidal flat, and Amphipoda were present only on the marsh. Gastropoda (represented by a single epifaunal species, *Heleobia australis*) was the characteristic taxon and accounted for 88% of the total macrobenthos on the salt marsh. Carcedo and Fiori (2011) also found a higher density of *H. australis* on salt marshes of *S. alterniflora* than on adjacent tidal flats in Bahía Blanca estuary. Molluscs were also found to be the dominant group on *S. alterniflora* marshes from a wide variety of geographical locations (e.g. Jiangsu coast, Chine, Ge et al. 2020; Paranaguá bay, Brazil, Netto al. 2018). Several studies (e.g. Grudemo and Bohlin 2000; De Francesco and Isla 2003) have

demonstrated that the distribution of *H. australis* is constrained by variables of the physico-chemical (salinity, temperature and type of sediment) and biological environment (competition, parasitism and predation). The aboveground biomass of *S. alterniflora* may provide refuge and/or trophic support for benthic organisms (Brusati and Grosholz 2006). Canepuccia et al. (2007) suggested that temperature and dehydration may be key factors that regulate *H. australis* distribution, so the presence of vegetation would reduce stress, resulting in higher densities and biomass. Furthermore, the presence of macrophytes with a complex architecture increases the spatial heterogeneity, protecting the macrobenthos from predation, especially epibenthic organisms whose visibility would be reduced (Lewis and Eby 2002). Thus, the increase in the colonization of *S. alterniflora* on tidal flats can enhance the density and biomass of *H. australis* in the study area.

The density and biomass of Polychaeta were higher on the tidal flat, where it was the characteristic taxon (representing 53% of all individuals). While the aerial structures of *S. alterniflora* plants provide epibenthic organisms with refuge from predation and ameliorate physical conditions leading to desiccation stress, roots and rhizomes reduce habitat suitability for some infaunal taxa (Brusati and Grosholz 2006). For instance, it has been reported that dense root mats may reduce the density and biomass of infaunal invertebrates by decreasing the space available for burrowing (Neira et al. 2005, Brusati and Grosholz 2006). On the tidal flat, *Laeonereis culveri* was the most abundant species within the Polychaeta. This is the common dominant deposit-feeder of intertidal macrobenthic communities in estuaries of the Buenos Aires Province, Argentina (Martin and Bastida 2006). The organic matter in the sediment plays an important role as a food source of this polychaete and its biomass and secondary production could be dependent on the amount, quality and availability of organic matter in the sediment (Martin and Bastida 2006). Differences in the species composition are accompanied by differences in diet and feeding modes. The most abundant organism on tidal flats (Polychaeta) are mainly consumers of detritus, while most organisms on salt marshes (Gastropoda) consume primarily periphyton (Albertoni et al. 2001).

It is recognized that the colonization of bare tidal flats with *S. alterniflora* can positively or negatively alter the species richness and biomass of the meiobenthos (Wang et al. 2010, Lin et al. 2015). *S. alterniflora* may lead to severe physico-chemical and biological alterations of key sediment properties, such as grain size composition, benthic microalgal biomass and water and organic matter content, thus affecting the meiobenthos biodiversity (Neira et al. 2005, Lin et al. 2015). Meiobenthic organisms are especially sensitive to anoxic conditions, so they commonly inhabit the first few millimetres close to the sediment surface. However, the radial oxygen loss from *S. alterniflora* roots creates an oxygenated micro-environment at greater depths, favouring larger meiobenthos densities and a higher diversity in association with the rhizosphere (Wardle et al. 2001, Lin et al. 2015). In our study, meiobenthos density and total biomass were simi-

lar on the salt marsh and tidal flat, and values were within the ranges reported elsewhere on salt marshes (e. g. Gulf of Mexico, southern Brazilian coast) (Wardle et al. 2001, Netto et al. 2018). However, when separate taxa were considered, Crustacea, Turbellaria and Foraminifera showed significant differences between habitats. Crustacea and Turbellaria were only present on the salt marsh, possibly responding to the forementioned physico-chemical changes in the sediment that occurred after plant colonization.

Foraminifera, by contrast were only present on the tidal flat. The duration and frequency of tidal inundation have been pointed as the most important variables controlling the distribution of Foraminifera within the intertidal zone (Edwards et al. 2004). The close association between foraminiferal species and inundation levels has been widely used to define foraminiferal zones that provide accurate indicators of former sea levels in intertidal deposits (e.g. Horton et al. 1999). It should be noted that plant colonization may also introduce changes in the microtopography (a few centimetres) at the same elevation within the intertidal that have not been considered in this work. In this same location (Villa del Mar), Calvo-Marcilese and Pratolongo (2009) found large differences in the abundance and number of foraminiferal species at different elevations within the same salt marsh. In that study, a hundredfold increase in the abundance of Foraminifera was reported for samples collected in the lower limit of the salt marsh compared with samples obtained at mid-marsh elevations. Therefore, the differences observed between tidal flats and salt marshes may be due to local changes in the microtopography and the associated environmental variables (de Rijk and Troelstra 1997).

According to our results, the carbon stock contained in the macrobenthos (total macrobenthos biomass) was approximately 11 gC m^{-2} on the tidal flat and 39 gC m^{-2} on the salt marsh. The meiobenthos, in turn, represented only 0.02 and 0.04 gC m^{-2} on the tidal flat and salt marsh, respectively (0.34% and 0.05% of the carbon pool associated with the macrobenthos). Despite their considerably smaller biomass, meiobenthic organisms have a higher turnover rate (P/B ratio), so they play a disproportionally large role in the trophic transfer of carbon and may be responsible for a large proportion of the secondary production (Sellanes et al. 2003). That is, there is an inverse relation between weight and production in the meiobenthos and macrobenthos. The production and energy flow are considerably higher in meiobenthos than in macrobenthos due to the more effective use of food. The mass-specific metabolic rate of meiobenthos is about five times greater than that of macrobenthos, so meiobenthos consumes five times more food (i.e. carbon) than macrobenthos per unit of biomass (Giere 2008). This is why the meiobenthos, together with the bacteria, uses the greatest portion of the energy consumed by the bottom ecosystem and forms a "small food web". However, there is a complex interaction within this "small food web" where the meiobenthos can affect microbial communities through grazing, while spatial-temporal fluctuations of bacteria affect the distribution and abundance of meiobenthos (Leguerrier et al. 2003, Giere 2008).

Also, the meiobenthos is considered to be primarily linked to the detritus/bacteria-based food chain and the energy fixed by the meiobenthos is often transmitted to higher trophic levels, such as the macrobenthos. Thus, small macrofauna and nekton (usually juvenile stages of larger fishes or crustaceans) can use meiobenthos as a food source (Heymans and Baird 1995). According to the different roles that these groups play in the energy balance and the carbon cycle, the expansion of salt marshes over tidal flats in the study area may involve a faster flow of energy and carbon transfer by the meiobenthos, along with a greater increase in food web complexity (Giere 2008).

Our results suggest that the expansion of *S. alterniflora* produces changes in the macrobenthic and meiobenthic communities that have an influence on the carbon cycling. This influence on the C cycle is due to the differences in the biomass of the taxa present in the two habitats, levels in the trophic web, feeding guilds (deposit feeders and herbivores), modes of life (infaunal or epifaunal) and activities such as bioturbation. It is largely recognized that the impact of plant colonization on C sequestration and storage is conditioned by plant biomass production and changes in sedimentation rates (Chastain et al. 2018), but it also depends on the indirect effects of benthic fauna. Changes in faunal composition may alter not only the amount of C that is temporarily stored in faunal biomass, but also the amount of CO_2 released by respiration and the quality of the organic matter delivered to the system through excretions. Along with changes in the species composition, there might be changes in bioturbation rates that may also alter the ventilation and irrigation of sediments. Burrowing animals, through mixing and disturbing the sediment layers, play a significant role in the release of carbon that is already sequestered in the sediment matrix. For instance, their activity increases the availability of oxygen, accelerating organic matter decomposition, and through biodeposition and excretion they enhance the localized accumulation of organic matter and promote hotspots of microbial activity (Thomson et al. 2019). Over time the expansion of *S. alterniflora* and the loss of bare tidal flats are expected to produce the changes in the macro- and meiobenthic assemblages described in this work. According to our results, a decreasing biomass of deposit feeders would augment the carbon sequestration capacity recognized worldwide for salt marshes, but it may also have major consequences for the trophic transfer of organic carbon to higher levels in the food webs (for example, commercially important fish). Moreover, external forces such as climate change, especially rising sea level, along with anthropic activity, can interact with marsh development to produce highly different patterns (Levin et al. 2006). Thus, more research is necessary to obtain a better understanding of the changes that the expansion of *S. alterniflora* introduces to the carbon cycle within the estuary.

ACKNOWLEDGEMENTS

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