



Effects of two estuarine intertidal polychaetes on infaunal assemblages and organic matter under contrasting crab bioturbation activity

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ARTICLE INFO

Keywords:
Interactions
Bioturbation
Polychaetes
Mudflats
Infaunal assemblages
Organic matter

ABSTRACT

Polychaetes and burrowing crabs are widely distributed in intertidal soft bottom environments, playing an important role in structuring infaunal assemblages through trophic and non-trophic interactions such as bioturbation. In southwestern Atlantic (SWA; 37°40'S, 57°23'W) intertidal mudflats, the polychaetes *Laeonereis acuta* and *Neanthes succinea* coexist with the burrowing crab *Neohelice (Chasmagnathus) granulata*. *N. granulata* and *L. acuta* strongly modify the sediment characteristics at different spatial scales, changing the infaunal assemblages and probably affecting feeding mode of *N. succinea*, which could prey upon *L. acuta*. Here, we experimentally evaluated the effects of constant densities of *L. acuta* and *N. succinea* on the benthic species assemblages and sediment organic matter (OM) content in two contrasting scenarios of crab bioturbation: inside and outside crab beds. We found that (1) both polychaetes did not affect the mean density of other macrofaunal organisms neither the meiofauna in general, but *L. acuta* modifies the abundance of specific groups such as foraminiferans; (2) polychaetes produced changes in meiofaunal spatial distribution probably by adding habitat heterogeneity; and (3) no evidence of predation of *N. succinea* on *L. acuta* were observed. Additionally, the variable effects of polychaetes on chlorophyll and OM content showed that they were species-specific and also modified by crab bioturbation. Moreover, effects of crab bioturbation on primary producers, quality and content of OM, and on some macro and meiofaunal organisms were found. Our results suggest that the effects of *L. acuta* and *N. succinea* on benthic species and OM content are mostly species-specific and, with regard to food sources (OM content and microphytobenthic biomass), strongly modified by larger scale crab bioturbation.

1. Introduction

Intertidal soft bottom systems are world widely distributed and are recognized as essential sites that provide unique ecosystem services (Elliott and Whitfield, 2011), such as flood and storm protection or cycling of nutrients (Atkins et al., 2011). They are characterized by low species diversity with high abundance and biomass (Elliott and Whitfield, 2011) and sediments with large amounts of organic matter (Spohn et al., 2013). These ecosystems may account for the 20% of the global marine primary production (Pedersen et al., 2004), are important stop over sites for several species of shorebirds (e.g. Morrison and Ross, 1989) and feeding sites for fishes (e.g. Green et al., 2009). Therefore, these are key systems in coastal food webs (Zedler and Callaway, 2001).

Interactions between organisms and environmental factors are structuring forces inside communities (Pennings and Bertness, 2001; Widdows and Brinsley, 2002). These interactions determine species abundance and distribution through competition (e.g. Connell, 1961), predation (e.g. Paine, 1966) and/or ecosystem engineering (e.g. Jones et al., 1997). In soft sediments, predation among infaunal organisms modify preys assemblages (e.g. polychaetes: Caron et al., 2004; nemertines: Thiel and Reise, 1993), generating multiple trophic levels (Thrush, 1999 and references therein) and linking meiofauna with higher-level predators (fishes or birds; Ambrose Jr., 1984). However, the effects of infaunal predation are linked to the quantity and quality of organic matter (OM, Venturini et al., 2011) because only a small portion is available for organisms (e.g. Dell'Anno et al., 2000; Fanjul

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et al., 2015). Therefore, predation and other physical factors, determine the relationships between species and consumption rates (Snelgrove and Butman, 1994; Papaspyrou et al., 2010).

Bioturbation is a common process, exerted by different organisms at different intensities and different time and spatial scales (Murray et al., 2002; Citadin et al., 2016). In soft bottom intertidals, burrowing species are relevant because through bioturbation they modify physical (e.g. Natálio et al., 2017), chemical (e.g. Chapman and Tolhurst, 2007) and biological (e.g. Papaspyrou et al., 2006) characteristics of sediments. These organisms generate high levels of spatial heterogeneity that favor the development of different infaunal species assemblages (e.g. Escapa et al., 2004; Papaspyrou et al., 2006) with consequences in the food webs and species interactions (e.g. Alvarez et al., 2015). Polychaetes and crabs are important bioturbator organisms, abundant and widely distributed in intertidal soft bottom environments (Wang et al., 2010; Venturini et al., 2011). In habitats where they coexist, the effects of polychaetes (which are relatively small-scale bioturbators) could be affected by the intensity of crab burrowing (which are relatively large-scale bioturbators).

In the intertidal soft bottoms of the Southwestern Atlantic (SWA; 37°40'S, 57°23'W) the burrowing crab *Neohelice (Chasmagnathus) granulata* and the polychaetes *Laeonereis acuta* and *Neanthes succinea* coexist (Iribarne et al., 1997; Palomo et al., 2003, 2004). *N. granulata* (up to 8 cm carapace width; Luppi et al., 2002) generates large areas with high density of burrows (i.e. crab beds, > 100 burrows m⁻²) which may reach up to 20 cm in surface opening diameter and 40 cm depth in mudflats (Iribarne et al., 1997; Alberti et al., 2015). The burrows increase habitat complexity and bioturbation affects the abundance and distribution of other species (e.g. fishes: Martinetto et al., 2005; polychaetes: Palomo et al., 2003; meiofaunal organisms: Escapa et al., 2004) and the sediment organic matter (OM) bioavailability and spatial distribution (Fanjul et al., 2015). The polychaete *L. acuta* is up to 6 cm length (Palomo and Iribarne, 2000), reaching densities up to 7400 ind m⁻² (Botto and Iribarne, 1999) depending on sites, seasons and years (e.g. from 500 ind m⁻² Palomo et al., 2003). In particular, the abundance of *L. acuta* is at least 2.5 times higher inside crab beds compared with similar areas outside crab beds (Botto and Iribarne, 2000). Bioturbation by *L. acuta* produces sediment mounds, which contain more OM than the surrounding sediments (Palomo and Iribarne, 2000).

On the other hand, the biology and ecology of the polychaete *N. succinea* is less known. In SWA mudflats, *N. succinea* can reach up to 8 cm length (Elías, 2002) and has densities between 47 ind m⁻² (Botto and Iribarne, 1999) and 255 ind m⁻² (Martinetto et al., 2005, 2011). Despite *N. succinea* inhabits brackish-water areas building galleries in intertidal sediments (Rioja, 1946), its bioturbation effects on OM are unknown. This species is a typical surface deposit-feeder (Fauchald and Jumars, 1979) but, depending on the habitat, it can be carnivorous (Pardo and Dauer, 2003) controlling the abundances of other polychaetes and possibly feeding on *L. acuta* (Gutiérrez et al., 2000). In fact, inside crab beds, this polychaete changes its trophic positions showing a

diet enriched ¹³C (Botto et al., 2005). Together, *L. acuta* and *N. succinea* constitute the main prey items for higher trophic level organisms in these areas (e.g. silverside: Martinetto et al., 2005 and birds: Botto et al., 1998) and are the principal constitutive taxon of macrofauna.

In intertidal soft bottoms of the SWA, the crab *N. granulata*, as mentioned above, by increasing OM total content and OM bioavailability and spatial distribution, increases abundances and change feeding behaviour of polychaetes inside crab beds. Thus, the objectives of this work were to evaluate the effects of the polychaetes *L. acuta* and *N. succinea* on the benthic species assemblage and the quality and content of OM in two contrasting scenarios: with and without crab bioturbation. We hypothesize that (a) *L. acuta* and *N. succinea* bioturbation have negative effects on meiofaunal densities, OM quality and content and microphytobenthic biomass (estimated on chlorophyll content); (b) *N. succinea* reduce *L. acuta* density, and this effect is exacerbated outside crab beds where OM is lower than inside crab beds; and (c) the outcome of these interactions (polychaetes-meiofauna-primary producers) is different inside than outside crab beds.

2. Materials and methods

2.1. Study site

The study was performed in a tidal flat area at Mar Chiquita Coastal Lagoon (37° 40' S, 57° 23' W; Argentina), a Man and the Biosphere Reserve from UNESCO, during November–December 2008 and January–February 2009. The coastal lagoon is a body of brackish water (46 km²) with low tidal amplitude (≤ 1 m) permanently connected to the sea (Reta et al., 2001) with a wide salinity range (2 to 35; Spivak et al., 1994). The intertidal zone is characterized by mudflats surrounded by a large *Spartina densiflora* salt marsh area (Isacch et al., 2006). The present study was made in open intertidal mudflats, which are the only habitats flooded daily by tides. In the same intertidal level (15 cm over mean lower low water, MLLW), we identified two sites inhabited by high densities of crab burrows and thus strongly bioturbated (hereafter “CB+”) and two sites not bioturbated by crabs, but with some occasionally isolated crab burrows during the warm season (hereafter “CB-” Fig. 1). The two sites of each type were similar in terms of hydrodynamic conditions and general characteristics, and also in the effects generated by active crab bioturbation on sedimentary characteristics in the case of CB+ (Iribarne et al., 1997; Botto and Iribarne, 2000).

2.2. Effects of *Laeonereis acuta* and *Neanthes succinea* on benthic assemblages and OM content

To evaluate the effects of the polychaetes *Laeonereis acuta* and *Neanthes succinea* on the benthic assemblage (i.e., macrofauna, meiofauna, and microphytobenthos), sediment organic matter quality (labile organic carbon, “LOC”) and sediment organic matter (“OM”) content, in two contrasting scenarios of crab bioturbation, two field experiments

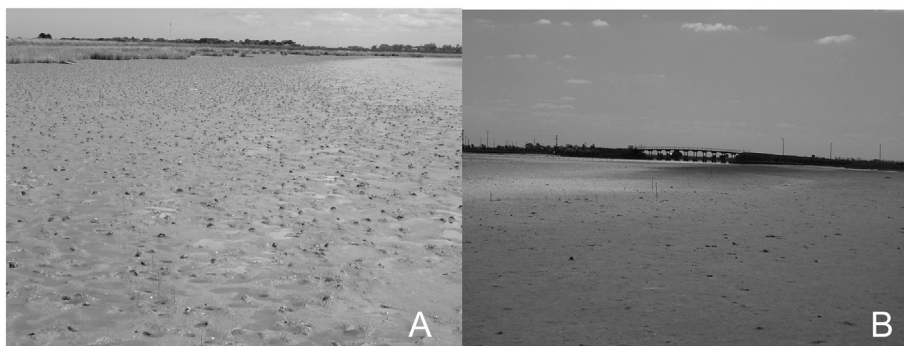


Fig. 1. Sites where experiments were deployed: (A) crab beds sites is shown; the caves are conspicuous and cover the whole intertidal area between the cordgrass and the sub tidal line. Next to the caves, there are mounds of reworked sediment. (B) outside crab beds site is shown, caves are scarce and scattered in the intertidal. Photo credit: (A) Paulina Martinetto and (B) M. Fernanda Alvarez.

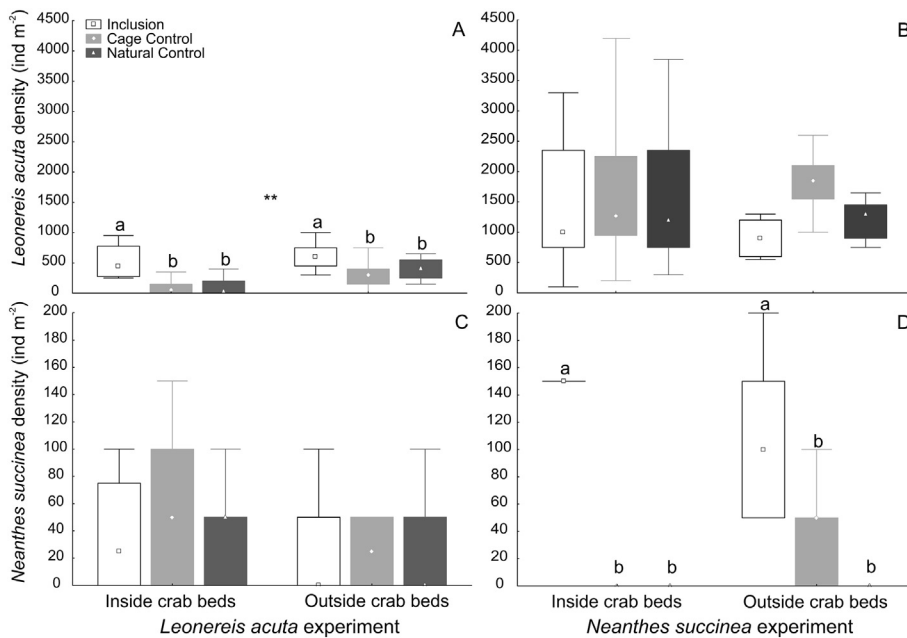


Fig. 2. Density of *Leonereis acuta* (A, B) and *Neanthes succinea* (C, D) polychaetes in the different treatments (inclusion, cage control and natural control) and in both sites (inside and outside crab beds) for both experiments: *L. acuta* inclusion experiment (A, C) and *N. succinea* inclusion experiment (B, D). Symbols within boxes denote the median and boxes denote 25 and 75 percentiles (50% of data). All data are presented prior to transformations. Different lower-case letters illustrate differences between treatments in descending order. Asterisks (**) illustrate differences between sites: inside crab beds and outside crab beds. Different capital letters illustrate differences with interactions between treatments and sites. Lack of letters indicates no differences.

were performed inside (CB+) and outside crab beds (CB-, $n = 2$ per site). For each experiment, 30 experimental units (16 cm diameter buried PVC pipes) were delimited in the mudflat, perpendicular to the shoreline and covering an area of 40 m. Ten of them were randomly designated to each of 3 treatments: (1) "Inclusion" (of *L. acuta* in the first experiment and *N. succinea* in the second experiment) (2) "cage control" (without polychaetes inclusion) and (3) "natural control" (natural tagged areas without manipulation). In the inclusion treatment, density was checked weekly (using an extra set of experimental pipes) and polychaetes were added if necessary to be maintained in 1000 ind m^{-2} for *L. acuta* and 150 ind m^{-2} for *N. succinea* throughout the experiment (4 weeks, see Supplementary information for further details). At the end of the experiment sediment samples for meiofauna (cores of 2 cm in diameter, 2 cm in depth), microphytobenthos (2 cm in diameter, 2 cm in depth), OM content and LOC (10 cm in diameter, 1 cm in depth) were taken from each experimental unit and the remaining sediment of each experimental plot was sieved ($250 \mu\text{m}$) to obtain the macrofauna. Macrofaunal and meiofaunal organisms (this latest sieved through $62 \mu\text{m}$ mesh) were preserved in 5% formalin until their identification and counting under binocular microscope. Meiofaunal samples were also stained with Bengal rose to facilitate their visualization (Higgins and Thiel, 1988). Microphytobenthic biomass was estimated spectrophotometrically as total, a, b and c chlorophyll concentration following the trichromatic methods (Jeffrey and Humphrey, 1975). OM content was determined by weight loss upon ignition and LOC was estimated by measuring carbohydrate, proteins and lipids content in sediment subsamples of near 40 g, following Fanjul et al., 2015, (see Supplementary information for major details).

In *N. succinea* inclusion, the *L. acuta* individual's size (total length, mm) was also measured at the end of the experiment, since *N. succinea* predatory behaviour could affect prey sizes distribution. When individuals were broken (about of 30% of total), the length was estimated using the relationship between the jaw size (J, measured under binocular microscope $40\times$, 0.001 mm precision) and the total length (TL) following Escapa et al. (2004) as follows:

Outside crab bed: $TL = - 8.37 (\pm 2.06) + 92.76 (\pm 4.15) * J$
 $r^2 = 0.87$.

Inside crab bed: $TL = - 14.95 (\pm 2.09) + 109.22 (\pm 4.1) * J$
 $r^2 = 0.90$

2.3. Statistical analysis

The null hypothesis of no differences in mean density of macro and meiofaunal organisms (ind m^{-2}), in mean chlorophyll concentrations ($\mu\text{g cm}^{-2}$), mean OM content (%) and in the ratio for labile organic carbon and total organic carbon (LOC:TOC ratio) among treatments (inclusions, cage control and natural controls) and sites (CB+, CB-) were analyzed with two-way ANOVA (factors: treatments and sites), applying Type III sum of squares for unbalanced data (Underwood, 1997), for each experiment separately (see Supplementary information for further details). Assumptions for general linear models were tested using the Shapiro-Wilk test for normality and the Cochran test for homoscedasticity; also, these tests were used to discuss other sources of variations (Underwood, 1997). Unequal N HSD post hoc was applied when corresponds (Zar, 1999). In *Neanthes succinea* inclusion, the size frequency distribution (SFD) of *L. acuta* prey was compared between treatments by Kolmogorov-Smirnov tests for each site independently (CB+, CB-). To evaluate if the SFD of *L. acuta* is naturally different between sites, it was compared between CB+ and CB- using natural control treatment plots and by Kolmogorov-Smirnov tests.

3. Results

3.1. Effects of *Laeonereis acuta* on benthic assemblages and OM content

Laeonereis acuta was found in all treatments in both areas with higher density in CB- and in the inclusions (Fig. 2A), showing that the experiment worked properly. Also, there were no differences between natural controls and cage controls indicating that any further effect found on infauna or food supply (OM content and microphytobenthic biomass) would be due to polychaete inclusion itself (Table 1). There were no differences in mean abundances of other macrofaunal species (i.e. the polychaetes *Neanthes succinea*, *Heteromastus similis* and *Nephtys fluviatilis*) between sites or treatments. Regarding meiofauna, the abundance of foraminiferans was higher in *L. acuta* inclusions in CB+ than in cage control in CB+ (Fig. 3A). Mean abundances of ostracods and nematodes showed no differences both, between treatments and sites; however, variance was higher in inclusions for both groups

Table 1

ANOVAs results on (1) macro and meiofauna, and (2) food sources (OM content and microphytobenthic) for inclusion experiments of (A) *Laeonereis acuta* and (B) *Neanthes succinea*.

Inclusion experiments	A) <i>Laeonereis acuta</i>						B) <i>Neanthes succinea</i>					
	Sites		Treatments		Treat × sites		Sites		Treatments		Treat × sites	
	F	df	F	Df	F	Df	F	df	F	df	F	df
1) Macro and Meiofauna												
<i>Laeonereis acuta</i>	12.00 **	1;47	11.69 **	2;47	0.97 ns	2;47	1.08 ns	1;49	1.84 ns	2;49	0.66 ns	2;49
Small <i>L. acuta</i>	–	–	–	–	–	–	1.35 ns	1;46	0.37 ns	2;46	0.17 ns	2;46
<i>Neanthes succinea</i>	1.94 ns	1;47	0.02 ns	2;47	0.24 ns	2;47	0.02 ns	1;49	35.62 **	2;49	1.78 ns	2;49
<i>Heteromastus similis</i>	0.77 ns	1;47	2.25 ns	2;47	0.22 ns	2;47	1.07 ns	1;49	0.51 ns	2;49	0.08 ns	2;49
<i>Nephtys fluviatilis</i>	0.002 ns	1;47	0.68 ns	2;47	0.61 ns	2;47	1.43 ns	1;49	1.54 ns	2;49	0.41 ns	2;49
Ostracods	2.32 ns	1;44	0.13 ns	2;44	1.65 ns	2;44	1.94 ns	1;46	1.1 ns	2;46	0.02 ns	2;46
Nematodes (b)	0.18 ns	1;44	1.96 ns	2;44	0.7 ns	2;44	6.54 *	1;46	0.1 ns	2;46	0.92 ns	2;46
Foraminiferans (a)	0.06 ns	1;44	8.95 **	2;44	6.34 **	2;44	2.01 ns	1;46	1.09 ns	2;46	0.63 ns	2;46
Copepods (b)	–	–	–	–	–	–	1.17 ns	1;46	0.3 ns	2;46	0.58 ns	2;46
2) Food sources												
Chl a	0.21 ns	1;47	3.77 *	2;47	0.98 ns	2;47	0.01 ns	1;49	4.3 *	2;49	4.29 *	2;49
Chl b	11.66 **	1;47	2.49 ns	2;47	1.24 ns	2;47	0.08 ns	1;49	4.93 *	2;49	0.57 ns	2;49
Chl c	0.04 ns	1;47	2.57 ns	2;47	0.66 ns	2;47	2.05 ns	1;48	3.38 *	2;48	0.49 ns	2;48
Chl total	0.23 ns	1;47	3.79 *	2;47	0.98 ns	2;47	0.01 ns	1;49	4.31 *	2;49	4.29 *	2;49
(%) OM	85.34 **	1;47	0.5 ns	2;47	3.36 *	2;47	42.19 **	1;48	5.14 **	2;48	0.94 ns	2;48
LOC	69.69 **	1;45	0.07 ns	2;45	1.86 ns	2;45	28.8 **	1;42	0.42 ns	2;42	0.49 ns	2;42
LOC:TOC	0.007 ns	1;45	1.02 ns	2;45	3.04 ns	2;45	6.58 *	1;42	1.73 ns	2;42	0.23 ns	2;42

Sites: inside and outside crab-bed. Treatments: polychaete inclusion, control cage and natural control. ns: $p > .05$. *: $p < .05$. **: $p < .01$. (a): indicate square root transformed data in *L. acuta* experiment; (b): indicate square root transformed data in *N. succinea* experiment.

(Cochran test $p < .05$, Fig. 3C and E) which would indicate effects on spatial distribution of these organisms (see Discussion). Copepods and small polychaetes (Ctenodrilidae family and juvenile of *L. acuta*) were

found only occasionally and in low densities thus no statistical analysis was done (see Table 1).

Concentrations of total chlorophyll and chlorophyll *a* were higher in

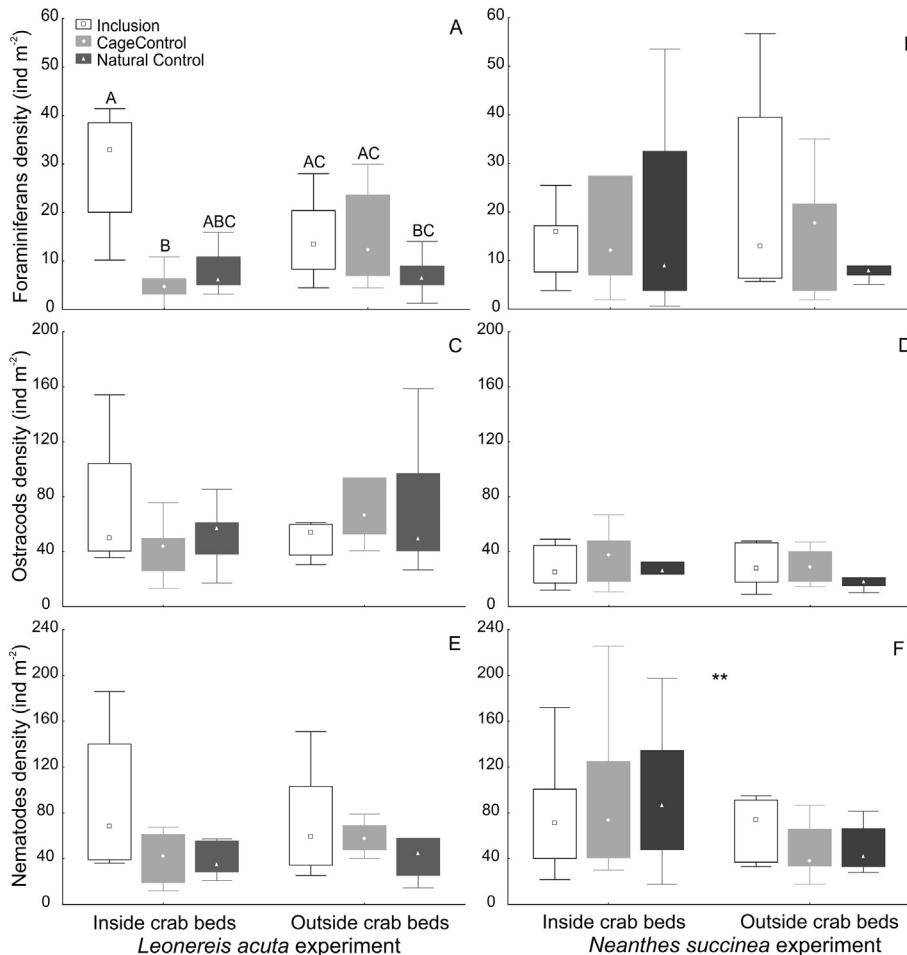


Fig. 3. Density of meiofaunal groups: foraminiferans (A, B), ostracods (C, D), and nematodes (E, F) in the different treatments (inclusion, cage control and natural control) and in both sites (inside and outside crab beds) in both experiments; *Laeonereis acuta* inclusion experiment (A, C, E) and *Neanthes succinea* inclusion experiment (B, D, F). Symbols within boxes denote the median and boxes denote 25 and 75 percentiles (50% of data). All data are presented prior to transformations. Different lower-case letters illustrate differences between treatments in descendant order. Asterisks (**) illustrate differences between sites: inside crab beds and outside crab beds. Different capital letters illustrate differences with interactions between treatments and sites. Lack of letters indicates no differences.

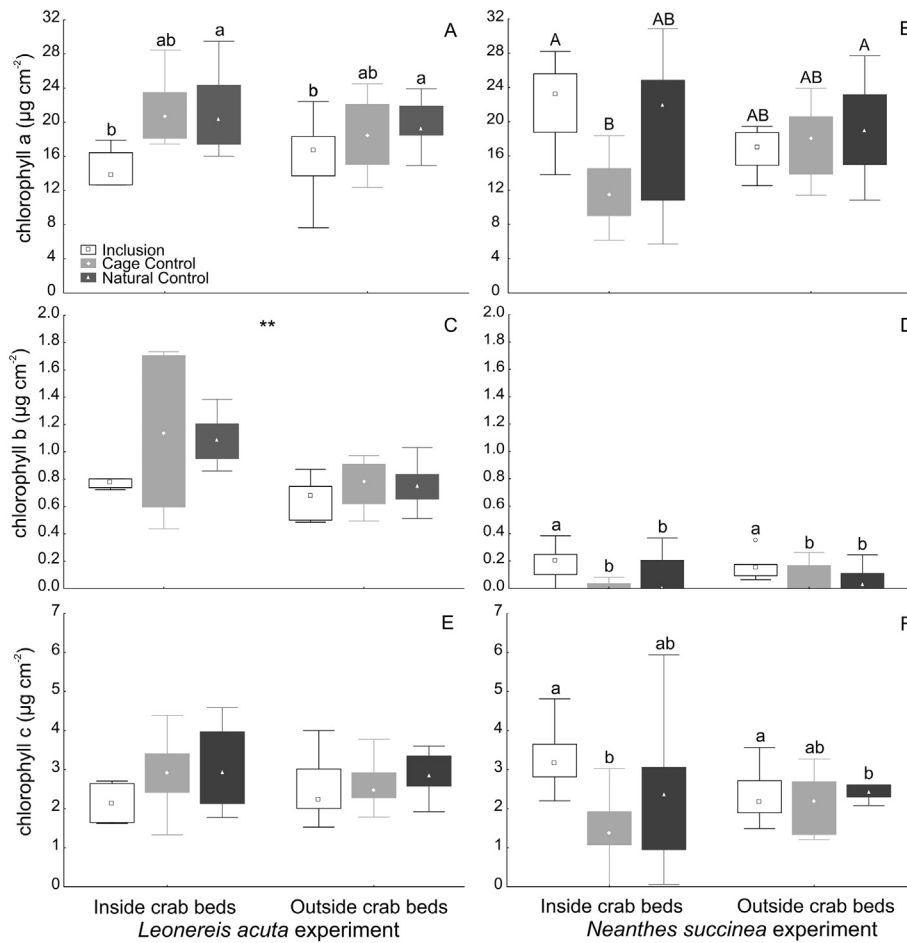


Fig. 4. Concentration of chlorophyll “a” (A, B), “b” (C, D) and “c” (D, E) in different treatments (inclusion, cage control and natural control) and in both sites (inside and outside crab beds); for both experiments: *Leonoreis acuta* inclusion experiment (A, C, E) and *Neanthes succinea* inclusion experiment (B, D, F). Symbols within boxes denote the median and boxes denote 25 and 75 percentiles (50% of data). All data are presented prior to transformations. Different lower-case letters illustrate differences between treatments in descendant order. Asterisks (**) illustrate differences between sites: inside crab beds and outside crab beds. Different capital letters illustrate differences with interactions between treatments and sites. Lack of letters indicates no differences.

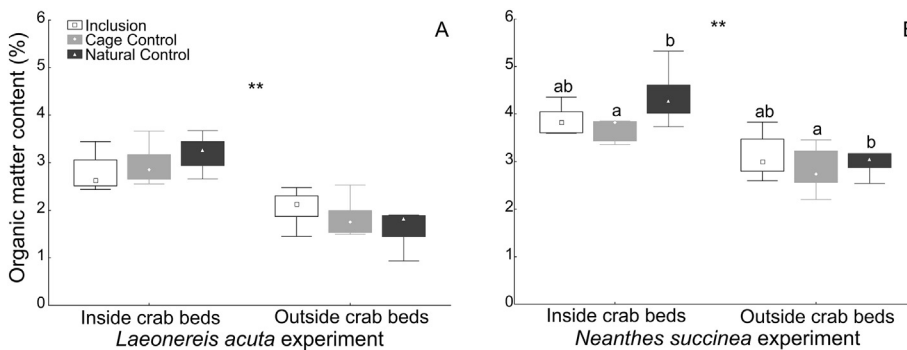


Fig. 5. Organic matter content (%) in the different treatments (inclusion, cage control and natural control) and in both sites (inside and outside crab beds) for both experiments: *Leonoreis acuta* inclusion experiment and (B) *Neanthes succinea* inclusion experiment. Different lower-case letters illustrate differences between treatments in descendant order. Asterisks (**) illustrate differences between sites: inside crab beds and outside crab beds. Different capital letters illustrate differences between treatments and sites.

the natural controls than in inclusion treatment (Fig. 4A). Chlorophyll b concentration was higher in CB+ than in CB- areas (all treatments, Fig. 4C). No differences were found for chlorophyll c (Fig. 4E). OM content was higher in inclusions treatments in CB+ and also in natural controls in CB+ than natural controls in CB- (Table 1; Fig. 5A). LOC content was higher in CB+ than in CB- for all treatments (Tables 1 and 2).

3.2. Effects of *Neanthes succinea* on benthic assemblages and OM content

Neanthes succinea was found in all treatments with higher abundance in inclusions, showing that the experiment functioned correctly (Fig. 2D). Also, there were no differences between natural controls and cage controls indicating that any further effect found on infauna or food supply would be due to polychaete inclusion itself. For other macrofaunal organisms, abundances showed no differences among treatments

as well as between sites (*N. fluviatilis*, *L. acuta* and *H. similis* polychaetes). With regard to meiofauna, there were no differences in mean abundance for ostracods, copepods, foraminiferans, and juvenile of *L. acuta* among treatments as well as between sites; however the abundance of foraminiferans presented higher variance in inclusion treatment (Cochran test $p < .05$, Fig. 3B). Nematodes density was higher in CB+ than in CB- (Fig. 3F). Since amphipods and polychaetes of the Ctenidrilidae family were only occasionally found, they were not included in the statistical analysis (see Table 1).

Total chlorophyll and chlorophyll a concentrations were higher in inclusion treatment in CB+; and in natural controls in CB- (Fig. 4B). Chlorophylls b and c concentrations were higher in inclusions in both areas (Fig. 4D and F). OM content was higher in natural control treatment than in cage control; in spite of this, given that inclusion treatment was not different to the other treatments, this artifact effect can be dismissed. Also, OM content was higher in CB+ than CB- (Fig. 5B).

Table 2

Mean (SD) values for labile organic carbon (LOC: mg C-LOC g dry sed⁻¹) and to ratio between labile organic carbon and total organic carbon (LOC/TOC) in both experiments (*Laeonereis acuta* and *Neanthes succinea*) in both sites: CB + = inside crab beds and CB – = outside crab beds and different treatments: IN = inclusion, CC = cage control and NC = natural control.

		CB +			CB –		
		IN	CC	NC	IN	CC	NC
<i>L. acuta</i> experiment	LOC	0.98 (0.2)	1.08 (0.22)	0.96 (0.24)	0.57 (0.11)	0.52 (0.11)	0.63 (0.12)
	LOC/TOC	7.39 (0.93)	8.53 (1.42)	7.25 (2.27)	6.81 (2.23)	7.16 (1.86)	9.37 (3.28)
<i>N. succinea</i> experiment	LOC	1.04 (0.16)	1.13 (0.41)	1.22 (0.31)	0.66 (0.13)	0.74 (0.26)	0.66 (0.18)
	LOC/TOC	6.36 (0.87)	7.09 (2.44)	6.55 (1.1)	5.02 (1.38)	6.4 (1.83)	4.93 (1.08)

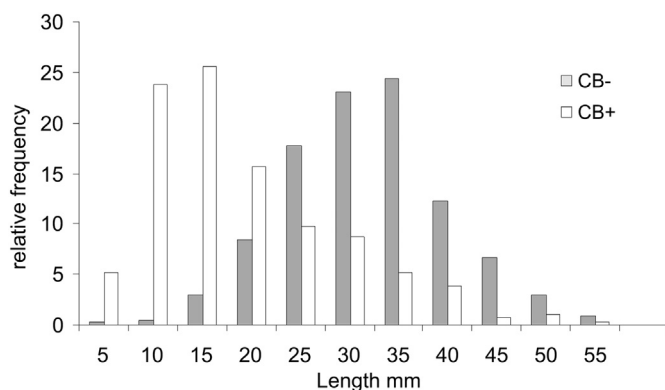


Fig. 6. Size frequency distribution of *Laeonereis acuta* inside (CB+) and outside (CB–) crab beds for the *Neanthes succinea* experiment (data from Natural control treatment).

See Table 1B for statistical details. LOC and LOC:TOC ratio was higher in CB+ than CB– (Table 2).

No differences in the SFD of *L. acuta* were found between treatments within each site (CB+ and CB–, K-S test, $p > .05$). The SFD was different between sites (K-S test, $p < .01$) since the relative frequency of small polychaetes (median size = 16.56 mm) was higher in CB+ (Fig. 6).

4. Discussion

Our experiments show that both polychaetes species do not affect abundance of other polychaetes or most meiofaunal organisms, except to increment abundance of specific groups such as foraminiferans. The other meiofaunal groups analyzed (such as for ostracods and nematodes), showed an increase in abundance variance. Moreover, contrary to our hypothesis, *N. succinea* would not shift to carnivorous feeding even in areas with poor OM content (i.e. sites without crab bioturbation). However, polychaetes affect the chlorophyll and OM content, but did it in different ways depending on the polychaetes species considered, and these interactions are modified by a larger scale bioturbation generated by crabs (see Fig. 7 as a summary of results). Differences between the crab and no crab bioturbation scenarios are evident in primary producers biomass, in OM content and quality (LOC), in abundance of macro and meiofaunal organisms (e.g., nematodes) and in the size frequency distribution of *L. acuta*.

Disruption of the sediment by activities as feeding or bioturbation strongly alters the sedimentary environment (Kristensen et al., 2014) and the abundances of infaunal organisms (Pillay et al., 2007). The net effect of disruption in sediment depends on the size of organisms and

the frequency of disturbance (Posey, 1987), having different consequences according with the species involved. The lack of negative effect of polychaetes on mean infaunal organisms density differed of previous work (Tita et al., 2000) and would be in relation with the small size of polychaetes (e.g. Natálio et al., 2017) or the different habit mode of species under study. Nonetheless, we observed that polychaetes were associated with an increase in abundance variability of some meiofaunal groups, such as foraminiferans. High polychaetes densities imply high bioturbation levels (Palomo and Iribarne, 2000), and it is known that they increase oxygen penetration (Meysman et al., 2005), stimulate microbial activity (Vasquez-Cardenas et al., 2016) and the benthic metabolism (up to 179%; Kristensen, 2001), promoting positive effects as a consequence of bioirrigation caused by its burrowing activity (Tita et al., 2000) and creating micro habitats for other small organisms as meiofauna (e.g. Ólafsson, 2003). Therefore, changes in variances of meiofaunal abundances related to variations in polychaetes densities could also be suggesting a change in meiofauna spatial distribution (see Alvarez et al., 2015) associated with sediment characteristics (i.e. for example more oxygen) or higher food supply (see below). Thus, although this hypothesis was not analyzed in the present study, since specifically micro-niches were not studied, this positive interaction is likely to happen.

Regarding food supply (OM content and microphytobenthic biomass), we observed that the effects of polychaetes are highly variable, depending on the type of chlorophyll analyzed and the presence of bioturbation by crabs. For example, the presence of *N. succinea* inside crab-beds increased concentration of chl a, while the presence of *L. acuta* increased OM content inside crab-beds. Overall, these results contradict our hypothesis, which inferred a negative effect of polychaetes on these variables. There are evidences of polychaetes species with a positive effect on microphytobenthos biomass (e.g. Magni and Montani, 2006; Dyson et al., 2007) by stimulation of microbial growth (Papasprou et al., 2006; Vasquez-Cardenas et al., 2016), and it was reported that these effects are species-specific (Papasprou et al., 2006; Vasquez-Cardenas et al., 2016). Thus, the different effects due to the presence of *N. succinea*, and *L. acuta* on microphytobenthos and OM content respectively may be due to different feeding modes or irrigation activities of each species (Kristensen and Kostka, 2005). The fact that these effects were observed inside crab-beds, suggest that the interaction between specific primary and secondary producers is affected by macro-scale bioturbation, which is in accordance with our hypothesis concerning the different outcome of the interactions inside or outside crabs beds.

Organic matter content and quality were in all cases higher inside crab beds. Other variables such as the concentration of chlorophyll “a”, “b” and “c”, the abundance of some groups of infaunal organisms and the frequency of the small individuals of the *L. acuta* polychaete, were also higher inside crab beds; although some of them in *L. acuta* inclusion and some others in *N. succinea* inclusions (see Fig. 7). In habitats where heterogeneity is scarce, burrowing organisms affect the 3-dimensionality of space, modifying the parameters and resources of the habitat. The burrows itself provide refuge against predation (Moksnes, 2002), work as OM traps and modify the particle size and water content of sediment (Botto and Iribarne, 2000). In turn, the bioturbation and burrow structures together modify the flux of nutrients (Fanjul et al., 2011) and bioturbation activities affects the spatial distribution of sediment OM content and its availability for other benthic organisms (Fanjul et al., 2015). Higher OMC and LOC could explain the differences in SFD of small *L. acuta* polychaete inside crab beds, because larvae choose organic-rich sediments for recruitment (Thiyagarajan et al., 2005) and, on the other hand, in this sites, increase consumption pressure by crabs, fishes and birds (e.g. Valiñas et al., 2010; Alvarez et al., 2013 and references therein). Here, despite the observed small-scale effects of bioturbation by polychaetes, we found, in a concordance with previous studies (see Alvarez et al., 2015), that burrowing by crabs itself affect the OM quality and content, modulating assemblages of benthic species.

	Inclusion of <i>L. acuta</i>	Inclusion of <i>N. succinea</i>
Inside crab beds	<ul style="list-style-type: none"> Without effect on macrofauna abundance Decrease concentration of chlorophyll (Chl) total and a 	<ul style="list-style-type: none"> Without effect on macro and meiofauna abundances
Outside crab beds	<ul style="list-style-type: none"> Higher concentration of Chl b (LA) Higher abundance of nematodes (LA) Higher OMC and LOC during both experiments Higher abundance of small <i>L. acuta</i> polychaetes (NE) 	<ul style="list-style-type: none"> Higher concentration of Chl total, a, b and c
	<ul style="list-style-type: none"> Increase abundance of foraminiferans Increase OMC Higher variance for ostracods and nematodes abundances 	<ul style="list-style-type: none"> Higher variance for foraminiferans abundance Higher concentration of Chl b and c
	<ul style="list-style-type: none"> Higher variance for nematodes abundance 	

Fig. 7. Summary of results of both inclusion experiments. LA (*Laeonereis acuta*) and NE (*Neanthes succinea*). The results as “increases or decreases” are comparative between different sites (inside and outside crab beds), but always within the same inclusion experiment.

In conclusion, the experimental maintenance of both polychaetes abundance did not modify mean densities of macro and meiofaunal species (except to foraminiferans). Nevertheless, it would be modifying spatial distribution of main meiofaunal groups, suggesting an increment of habitat heterogeneity for these organisms. Moreover, the interaction between polychaetes and primary producers suggests that these species-specific processes may be affected by crab bioturbation; thus, highlighting the importance of taking into account the species-specific character of infaunal interactions and the relevance of 3-dimensional spatial heterogeneity.

Acknowledgements

We thank Diana Montemayor and Fausto Firstater for their help in the field. We are also grateful to Fuerza Aérea Argentina of Mar del Plata for allowed us to work in C.E.L.P.A. This project was supported by Universidad Nacional de Mar del Plata (EXA421/08), ANPCyT (PICT 2007-01272), and CONICET (PIP 5669). M.F. Alvarez, M.C. Bazterrica, E. Fanjul, M. Addino and M. Valiñas were supported by doctoral scholarships from CONICET and Universidad Nacional de Mar del Plata. This is part of the Doctoral thesis of M.F. Alvarez.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seares.2018.06.008>.

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