



Effect of crab bioturbation on organic matter processing in South West Atlantic intertidal sediments



Eugenia Fanjul ^{a,*}, Mauricio Escapa ^a, Diana Montemayor ^a, Mariana Addino ^a, María Fernanda Alvarez ^a,
María A. Grela ^{b,c}, Oscar Iribarne ^a

^a Laboratorio de Ecología, Instituto de Investigaciones Marinas y Costeras (IIMyC; Consejo Nacional de Investigaciones Científicas y Técnicas - Universidad Nacional de Mar del Plata), CC 573 Correo Central, B7600WAG Mar del Plata, Argentina

^b Departamento de Química, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350 Mar del Plata, Argentina

^c Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, C1033AAJ Buenos Aires, Argentina

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ABSTRACT

Organic matter (OM) remineralization plays a key role in controlling the biogeochemistry of marine sediments. Through their burrowing activities, bioturbating macrofauna not only induces physical, chemical and biological modifications, which can affect microbial communities responsible for organic matter remineralization, but it could also directly affect the distribution and bioavailability of sedimentary organic matter. Through in situ experiments manipulating crab and burrow density in intertidal soft-bottoms, we assessed if crab-bioturbation affects benthic metabolism, and the amount, distribution, and bioavailability of sedimentary OM. Crab-bioturbation enhanced overall benthic metabolism and benthic flux of dissolved OM toward the water column at both mudflat and saltmarsh zones. Moreover, our results revealed that bioturbation also changes the quality, bioavailability and distribution of sedimentary OM in mudflats and saltmarshes. Overall, bioturbation enhanced the proportion of labile organic carbon of bioturbated sediments and homogenized the sediment column in terms of their proportion of labile organic carbon. However, crabs also generated biogenic structures (e.g., mounds) that could promote spatial heterogeneity of high nutritional-value OM. Bioturbation-induced changes on benthic metabolism and on OM availability would result in a reduction of the storage capacity of carbon in our intertidal systems. Previous works indicated that crab-burrows trap detritus and OM-rich sediments. Our results suggest that detritus are efficiently remineralized at bioturbated sediment, and finally they are quickly exported to the water column as CO₂ and DOC. Thus, crabs are modifying the OM processing at intertidal soft bottoms, and the ways in which carbon is exported to coastal waters.

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1. Introduction

Shallow coastal ecosystems (including marshes and mangroves, intertidal and subtidal sediments, tidal plains and seagrass beds) are distributed worldwide and though they represent less than 2% of the oceanic surface, they account for about 20% of the global marine primary production (Charpy-Roubaud and Sournia, 1990; Pedersen et al., 2004). Although some of the carbon fixed by plants is consumed by herbivores, most of the fixed carbon enters benthic detrital trophic pathway (e.g., Cebrian and Lartigue, 2004; Mann, 1988; Schlesinger, 1997). The carbon input into sediments can be as detrital particulate organic matter (OM) from plant dead tissues, but also as soluble organic secretions from primary producers (i.e., from roots of vascular plants, Hines et al., 1989; from exopolymeric secretions of microphytobenthos, Smith and Underwood, 2000). As a result, organic matter in marine sediments is composed of a mixture of labile and refractory organic compounds

(e.g., Burdige, 2006; Canfield et al., 2005). This sedimentary OM is converted back to inorganic carbon by hydrolysis/fermentation (involving dissolved organic carbon formation, DOC) and subsequent mineralization to CO₂ by heterotrophic microorganisms (Canfield, 1993; Kristensen and Holmer, 2001). Moreover, labile organic carbon (LOC), often measured as the sum of protein, carbohydrate and lipid carbon, has been reported as the fraction of organic carbon potentially available to benthic consumers (Fabiano et al., 1995). Thus, the geochemistry of marine sediments is controlled not only by the composition of the material initially deposited in the sediments (e.g., Fenchel et al., 1998), but also by the chemical, biological, and physical processes that affect this material after its deposition (processes commonly referred to as “early diagenesis”; sensu Berner, 1980; Burdige, 2006).

Benthic macrofauna affects microbial processes in sediments by their burrowing and feeding activities (e.g., Kristensen and Kostka, 2005; Pappaspyrou et al., 2006, 2010), and directly participates in sedimentary processes through OM metabolism coupled with aerobic respiration and metabolic excretions (Furukawa, 2005). By means of active or passive irrigation and sediment reworking, bioturbation injects

* Corresponding author. Tel.: +54 223 4753554.
E-mail address: mefanjul@gmail.com (E. Fanjul).

oxygen and other electron acceptors into anoxic sediment (e.g., Daleo et al., 2008; Fanjul et al., 2007; Gribsholt et al., 2003; Kostka et al., 2002), and it rapidly transports organic substances (i.e., dissolved, particulate, and also compounds adsorbed into mineral particles) between oxic and anoxic surfaces (Aller, 1994; Fanjul et al., 2007). Organic matter at oxic surfaces are thus displaced toward deep sediment dominated by anaerobic bacteria, and in the opposite direction, relatively refractory substances in anoxic deep sediments are transported to aerobic microorganisms capable to degrade it (Kristensen and Holmer, 2001; Kristensen et al., 2012; Montague, 1982), usually increasing overall benthic community metabolism (Andersen et al., 1992; Fanjul et al., 2011; Kristensen, 1985). Moreover, since sorption of dissolved organic substances is a redox-dependent process (Chin et al., 1998; Komada et al., 2004), bioturbators could also impact on DOC accumulation and benthic flux by creating a redox-oscillating environment (Aller, 1994). Indeed there are evidences of differences in DOC flux between bioturbated and non-bioturbated sediments (e.g., Burdige and Homstead, 1994; Michaud et al., 2006). Thus, to identify the factors affecting transport processes, composition, reactivity, and distribution of sedimentary OM, it is critical to understand which processes are controlling mineralization, benthic metabolism and DOC export toward coastal waters.

South West (SW) Atlantic intertidal sediments are mostly pristine systems, which are intensely bioturbated by the burrowing crab *Neohelice (Chasmagnathus) granulata*. Bioturbation coefficients in these sediments are among the highest reported worldwide, with values around $250 \text{ cm}^{-2} \text{ yr}^{-1}$ (Fanjul et al., 2007) and sediment reworking rates of up to $5.9 \text{ kg dry sediment m}^{-2} \text{ d}^{-1}$ (Iribarne et al., 1997), values that are similar to those found for the ghost shrimp *Callinectes kraussi* (up to $12 \text{ kg wet sediment m}^{-2} \text{ d}^{-1}$ Branch and Pringle, 1987; Kristensen et al., 2012). This burrowing crab, according to the reworking mode, is a “regenerative” bioturbator (sensu Solan and Wigham, 2005; Kristensen et al., 2012), and affects the whole intertidal ecosystem functioning. For example, crab-bioturbation favors plant growth (Daleo et al., 2007), modifies benthic community structure (Escapa et al., 2004), mediates predator–prey interactions (Escapa et al., 2004; Martinetto et al., 2005), and affects microalgal assemblages (Alvarez et al., 2013). Crab-bioturbation also affects carbon burial (Gutiérrez et al., 2006), enhances sediment oxygenation (Daleo et al., 2007), benthic metabolism (Fanjul et al., 2011) and nutrient benthic flux (Fanjul et al., 2011). Oxygen uptake increases from $200 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ in non-bioturbated zones up to $6000 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ in bioturbated zones (Fanjul et al., 2011). In marshes with low content of dissolved inorganic nitrogen ($\sim 18 \mu\text{M}$), crabs favor dissolved inorganic nitrogen flux toward the sediment, increasing from $-100 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at non-bioturbated sediments to $-700 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ at bioturbated sediments (Fanjul et al., 2011).

Recent studies suggested that the extensive crab-bed zones affect the particle movement and near-bottom fluid dynamic (Escapa et al., 2008), and burrows could locally increase the sedimentary OM content of such zones by passively trapping detritus and OM-rich sediments (Botto et al., 2006; Escapa et al., 2008; Montemayor et al., 2011). Indeed, crab activity produces important zones of macro-detritus retention that can enhance the amount of OM that is stored in saltmarshes (e.g., Botto et al., 2006; Gutiérrez et al., 2006). This could be particularly important in systems with low OM content, such as in many SW Atlantic intertidal soft bottoms (e.g., Mar Chiquita, $37^\circ 45\text{S}$: 3.8%; Bahía San Antonio, $40^\circ 46\text{S}$: 1.8%; Luppi et al., 2013). Although it is well known that saltmarsh sediments store large amounts of OM, little is known about OM quality in saltmarsh soils (Spohn et al., 2013), and about the spatial variability of this OM. We hypothesize that bioturbation by *N. granulata* impacts on OM processing affecting its availability (e.g., by enhancing the proportion of readily labile compounds to total organic matter) and also stimulating their remineralization rate (by enhancing OM and solute transport processes, and creating a redox-oscillating environment). Thus, the aim of this work is to evaluate the role of the burrowing crab *N. granulata* on the overall benthic metabolism and

benthic flux of dissolved organic substances (i.e., DOM, dissolved carbohydrates and proteins flux across the water-sediment interface), and on the amount, distribution, composition and reactivity of sedimentary OM at intertidal soft bottoms. For this purpose, we conducted field experiments manipulating bioturbation intensity.

2. Material and methods

2.1. Study area

The study was conducted between September 2008 and March 2009, in the southern part of the Samborombom bay ($36^\circ 22' \text{ S}$, $56^\circ 45' \text{ W}$, southern mouth of La Plata River, Argentina), a natural protected area affected by low amplitude ($< 1.4 \text{ m}$) semidiurnal tides and characterized by large intertidal areas inhabited by burrowing crabs (Daleo et al., 2007; Luppi et al., 2013). *N. granulata*, a large-sized burrowing crab (up to 40 mm carapace width; Spivak et al., 1994) occurs in the whole intertidal (Iribarne et al., 1997), from low mudflats to high saltmarshes composed of *Spartina alterniflora* at the low zone, and *Spartina densiflora* at the high zone (Isacch et al., 2006). Crabs are mostly deposit feeders in mudflats, and herbivorous–detritivorous in saltmarshes (Bortolus and Iribarne, 1999; Botto et al., 2005; Iribarne et al., 1997). *N. granulata* excavates semipermanent burrows generating extensive burrowing beds commonly with up to 60 burrows m^{-2} (Botto and Iribarne, 2000; Iribarne et al., 1997), which cover up to 80% of the intertidal areas of SW Atlantic estuaries and bays (Botto et al., 2006; Iribarne et al., 2005). This crab is active over a wide range of environmental conditions (Luppi et al., 2013). Crabs leave burrows but stay near burrow entrances during low-tide, and show more intense activity during high tide periods (Luppi et al., 2013). This natural system allows us to evaluate the biogeochemical functioning in areas where crab densities are among the few remaining examples of present densities closely similar to those of the ancient population baselines (Fanjul et al., 2007), that have now largely been reduced in other systems (e.g., Wang et al., 2010).

2.2. Experimental design

To evaluate the effect of *N. granulata* on benthic metabolism and benthic flux of dissolved organic substances (i.e., flux of dissolved substances across the water-sediment interface), and on sedimentary OM quality and bioavailability, we conducted a series of field experiments manipulating bioturbation intensity in a *S. alterniflora* saltmarsh and bare intertidal mudflat (see Fig. 1). Each experiment had three treatments (6 replicates each one): (1) unoccupied burrows (thereafter B treatment); (2) inclusion of adult crabs *N. granulata* in burrows (thereafter B + C treatment); and (3) exclusion of crabs and burrows used as control (thereafter NBC). Plastic mesh boxes (0.75 cm mesh opening; 0.4 m height, 0.5 m diameter; Fig. 1a and b) with a cover of the same mesh (treatment NBC) or without cover (treatments B and B + C) were used to delimit each experimental unit (see the use of similar boxes in Fanjul et al., 2007; Thomas and Blum, 2010; Needham et al., 2011). Experimental units were deployed in natural sediments without crab bioturbation. Cylindrical holes (3.5 cm in diameter and 40 cm in depth) were excavated in soil at B and B + C treatments in order to reach 30 holes m^{-2} , which was a density similar to natural burrow densities (i.e., mean natural density: 29.6 burrows m^{-2} ; SD: 6.1; Fanjul et al., 2011). Then, one adult crab was added to each hole in B and B + C treatments, and in a few days the crabs colonized, maintained and often expanded the holes transforming it into burrows (see Fanjul et al., 2007; Iribarne et al., 2005; Thomas and Blum, 2010). The experimental units were periodically monitored to check that crabs did not colonize NBC treatments (i.e., closed boxes, see Fig. 1). After 2 months of their inclusion, the crabs inhabiting boxes corresponding to treatment B were removed by hand, and the plastic mesh covers were attached to those boxes to inhibit further crab colonization. By this

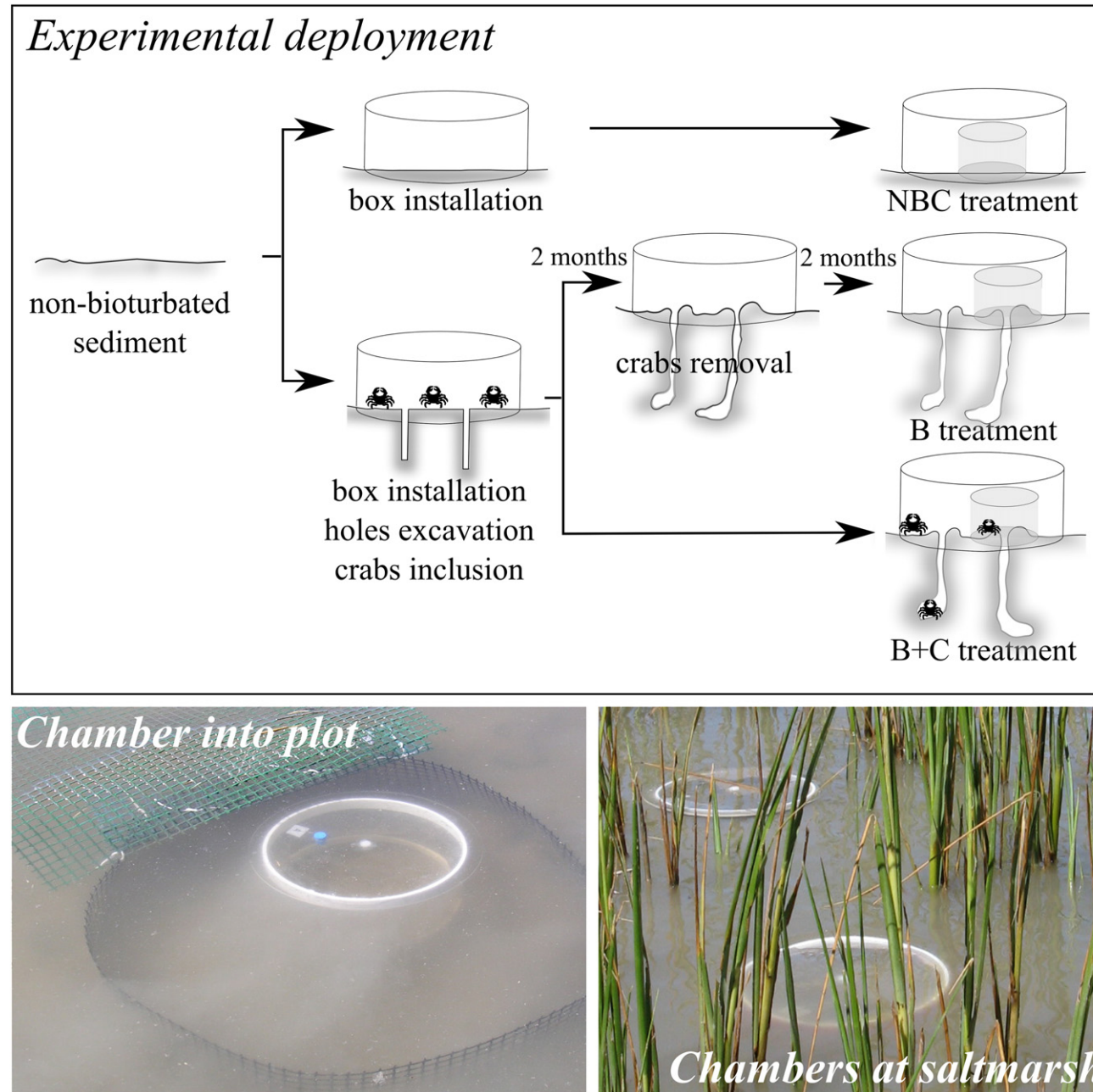


Fig. 1. Brief scheme of the different steps during the experimental setup, including boxes installation, excavation of holes, inclusion/exclusion of crabs, and chamber insertion (upper panel). Bottom panels show a close view photographs of benthic chambers into the experimental plots during incubation in NBC treatment at mudflat (left) and in B + C treatments at saltmarsh (right) during flooded tide. Notice sampling ports located in chamber lids. Photo credits: E. Fanjul (left-bottom panel); M. Escapa (right-bottom panel).

procedure, we achieved the configuration for treatment B which consisted of unoccupied crab burrows of nearly identical characteristics (size, architecture, and wall) than natural burrows.

2.2.1. Organic matter quality

To test the effects of crabs on the quality and content of OM, sediment samples were collected from treatments B + C and NBC at both mudflat and saltmarsh experiments. Sediment from fresh mounds (see Escapa et al., 2008), from burrow wall, from surface (0.5 cm in depth), and deep sediment (10–11 cm in depth) were collected from B + C treatment, and only the surface and deep sediments were collected from NBC treatment ($n = 6$ each sediment type). After homogenization each sample was examined to determine water content, total organic matter, total carbohydrates, proteins, and lipids (LIP). Water content (%) was calculated as the difference between wet and dry weight (oven dried at 60 °C until constant weight). Total organic matter was determined by weight loss upon ignition for 8 h at 500 °C. Proteins were extracted (0.1 N NaOH during 2 h at 60 °C) and measured photometrically by the Coomassie Blue reaction following Mayer et al. (1986), and were expressed as bovine serum albumin equivalents. Total carbohydrates were extracted and determined as glucose equivalents by phenol–sulfuric acid method (Underwood et al., 1995). Total extractable lipid content was analyzed gravimetrically in methanol–chloroform extracts as described by Rontani and Volkman (2005), weighing the dry residue after complete solvent evaporation. Carbohydrate, protein, and lipid concentrations were expressed as carbon equivalents using a conversion factor of 0.45, 0.49, and 0.75, respectively (Fabiano et al., 1995). The sum of carbohydrate, protein, and lipid carbon was reported as the labile carbon fraction (LOC), assumed to be the fraction of total OM potentially available to consumers (Danovaro, 1996; Fabiano and Danovaro, 1994; Fabiano et al., 1995). The ratio of each carbon pool (i.e., PRT, CHO, and LIP) to total labile organic carbon was calculated for each sediment type. Sediments treated in a muffle furnace (550 °C, 6 h) were used as blanks for each chemical analysis.

2.2.2. Organic matter bioavailability

Potentially bioavailable carbon was assayed in aerobic long-term laboratory incubations. To assess the bioavailability of bulk sedimentary OM, approximately 15 g of sediment from each type used for quality assessment was submerged 30 s into liquid-N₂ and transferred into acid cleaned (10% HCl) 100 ml glass incubation flasks. Incubations were initiated by adding inoculates composed of an assemblage of natural microorganisms (i.e., ~100 µL of 1:1 mix of fresh-untreated sediment and sterile artificial sea water). Samples were incubated in the dark at room temperature (22 °C) and monitored for CO₂ production over a 250-day period. At each sampling time, flasks were vented with humidified air and then gastight recapped. After a 5 h period, each flask headspace was sampled for CO₂ concentration, and immediately measured by gas chromatography (thermal conductivity detection, Porapak Q column at 35 °C, He UHP as carrier gas). The CO₂ produced over incubation interval was used as an index of the amount of carbon available to microorganisms. Reactive, intermediate and refractory carbon pools were estimated by following the formulae reported by Robertson et al. (1999).

2.2.3. Benthic flux across the water-sediment interface

After 2 months of having the experiment running (i.e., 2 months after treatment B was achieved), in situ measurements of benthic metabolism and benthic flux were done by installing a flexible benthic chamber at each experimental plot. Chambers were constructed according to Asmus et al. (1998) and following the modification by Fanjul et al. (2011). Briefly, a flexible transparent PVC film (10 cm in height) was mounted on the top of a PVC tube (16 cm in diameter, 12 cm in height). The lid (mounted on a PVC ring of 16 cm in diameter and 1.5 cm in height) was attached to the top of the flexible film (see bottom panels in Fig. 1). The lid had two small septa that allowed to sample water

inside the chambers using syringes, and also to take direct measurements with needle sensors. Chambers ($n = 18$, for each mudflat or saltmarsh zone) were inserted ~12 cm into the sediment during flooding tide such that 10 cm of the chamber was above the sediment surface. Incubations were performed during the flooded periods in mudflats and saltmarshes (water level was between 30 and 100 cm above the chamber lids). Incubations were conducted in dark conditions ($n = 6$ each treatment), by wrapping chambers with opaque aluminum foil. At the end of the incubation, the volume of water inside each chamber was determined by measuring chamber height (i.e., water height) with a ruler. Chambers in treatments B and B + C were placed in such a way that one burrow was included into each chamber (burrow diameter: ~3.6 cm; burrow depth >40 cm). Given the chamber area (0.0201 m²), this was a burrow density of ~50 burrows m⁻². This density was higher than the mean burrow density but remained within the range reported for SW Atlantic soft-bottoms (often >60 burrows m⁻², Botto and Iribarne, 2000; Gutiérrez et al., 2006; Iribarne et al., 1997). For these chambers, burrow volume was measured at low tide by adding a measured quantity of water into each burrow, and this volume was added to each chamber volume.

Samples of chamber-enclosed water (50 ml for carbohydrates, protein and dissolved organic carbon analysis; 20 ml for total inorganic carbon analysis) were taken by inserting a needle attached to a syringe through the septa located in the chamber lid, 10 min and 3 h after the start of incubation in chambers in both mudflat and saltmarsh experimental units. Additionally, near-chamber water temperature, and dissolved O₂ concentration in chamber-enclosed water were monitored during each sampling period. Water temperature was measured with a hand thermometer, and dissolved O₂ was measured in situ by inserting needle sensors (Unisense A/S) through the septa located in the chamber lid. Oxygen was measured to corroborate that its concentration did not decay more than 20% during incubation (data not shown). If this happened, incubation was excluded from the experiment. Water samples designated for carbohydrates (HCO), protein (PRT) and dissolved organic carbon (DOC) analysis were in situ filtered through 0.7 µm glass fiber-filter (GF/F Whatman, pre-combusted at 500 °C for 2 h), and kept at -20 °C in amber glass vials until analysis. All labware to be in contact with these samples were previously washed in 10% HCl, rinsed with deionized water, and dried at 60 °C in a conventional lab oven; and the glassware was also baked clean of any residual organic matter in a muffle furnace at 550 °C (Burdige and Homstead, 1994). Carbohydrate concentration was determined spectrophotometrically according to Dubois et al. (1956), with glucose as a reference. Concentration of proteins was determined photometrically by the Coomassie Blue and expressed as albumin equivalents, following Mayer et al. (1986). Dissolved organic carbon was measured by wet oxidation with potassium persulfate in sealed ampoules following the Menzel and Vaccaro (1964) procedure, after a complete removal of inorganic carbon. Following the oxidation step, inorganic carbon derived from DOC was measured by gas chromatography as described above. Samples for total inorganic carbon analysis (TCO₂) measurements were in situ transferred to gastight vials previously purged with UHP Helium, and analyzed for CO₂ headspace content by gas chromatography after vial acidification with 100 µL 1 N phosphoric acid. Flux rates at each chamber were calculated from the differences in solute concentration between time-sequential samples and referred to the enclosed water volume, the surface area of the sediment enclosed, and the duration of the incubation period.

2.3. Statistical analysis

The null hypothesis of no crab effect on carbon as carbohydrates (C-CHO), proteins (C-PRT), lipids (C-LIP), LOC content, on the ratio of each carbon pool to LOC, on OM bioavailability in sediments, and on the benthic flux of each substance (i.e., DOC, dissolved carbohydrates and dissolved proteins) were evaluated independently for each parameter

and intertidal site (i.e., mudflat and saltmarsh) using one way ANOVAs (Zar, 1999). We used a posteriori multiple comparison tests to evaluate statistical differences among groups when detecting significant differences with ANOVA (Underwood, 1997). Normality and homoscedasticity of data were evaluated by using the Shapiro–Wilk and Levene tests, respectively (Zar, 1999) and when assumption could not be met even when using common monotonic data transformations, non-parametric tests were used instead of ANOVA (i.e., Kruskal–Wallis test; Underwood, 1997).

3. Results

3.1. Organic matter quality

Results showed that bioturbation affects the organic matter quality and spatial distribution at both intertidal zones (i.e., mudflat and saltmarsh). At mudflat sediments, the highest carbohydrate content was registered for superficial non-bioturbated sediment, and the lowest content occurred in crab-mound sediment (Table 1, Fig. 2a). The highest protein content was observed in mound sediments, but burrow walls also had high protein content. The lowest protein content occurred in deep sediment at B + C treatment (Table 1, Fig. 2b). Lipid and TOC content was higher in non-bioturbated superficial sediments than the other sampled-sediments (Table 1, Fig. 2c and d).

At saltmarshes, the highest carbohydrate contents were obtained in mounds, burrow walls, and in both superficial sediments from B + C and NBC treatments (Table 1, Fig. 2e). The highest protein content occurred in deep bioturbated sediment, whereas the deep non-bioturbated sediment showed the lowest protein content (Table 1, Fig. 2f). Lipid content was higher in superficial bioturbated sediment than in other sediment types (Table 1, Fig. 2g). In turn, mounds showed the highest TOC content, while deep non-bioturbated sediments showed the lowest contents (Table 1, Fig. 2h).

Also, when analyzing the content of labile organic carbon, differences were found among sediment types at mudflats (Table 1, Fig. 3a), with the highest content in superficial non-bioturbated sediment (16.44 mg C g dry sediment⁻¹), and the lowest at mounds and deep non-bioturbated sediments (1.32 and 2.96 mg C g dry sediment⁻¹, respectively). However, the analysis of the labile fraction of total organic carbon (i.e., % LOC), showed that the highest % LOC occurred in non-

bioturbated superficial sediment (Table 1, Fig. 3b). In turn, at saltmarsh LOC content was highest in both superficial sediments (i.e., from NBC and B + C treatments), whereas the lowest content was found in non-bioturbated deep sediment (Table 1, Fig. 3c). The highest % LOC was also found in both superficial sediments (Table 1, Fig. 3b and d).

At mudflat, mounds had low contribution of carbon as carbohydrates to LOC fraction (ANOVA: $F_{5,24} = 2.64$, $p < 0.05$; Table 2), and high contribution of C as proteins to LOC (ANOVA: $F_{5,24} = 7.53$, $p < 0.05$; Table 2). However, at saltmarsh the highest C-CHO contribution was found in mound sediments (ANOVA: $F_{5,24} = 3.27$, $p < 0.05$; Table 2); and the highest proportion of C-PRT was found in bioturbated deep-sediment (ANOVA: $F_{5,24} = 5.50$, $p < 0.05$; Table 2).

3.2. Organic matter bioavailability

As occurred with OM quality, habitats (i.e., mudflat and saltmarsh) and bioturbation also affected the reactive, intermediate and refractory carbon pools (assayed from long-term incubations). At mudflat, reactive C pool was lower at crab mounds than the other sediment types, and superficial bioturbated and non-bioturbated sediments had high content of reactive C (Table 3, Fig. 4a). Intermediate reactivity pool was higher in superficial non-bioturbated sediments than in any other sediment (Table 3, Fig. 4a); and refractory C pool was higher in both (deep and superficial) non-bioturbated sediments (Table 3, Fig. 4a). These patterns differed for saltmarsh sediments, where the lowest reactive C pool was found in both deep-sediments, and the highest reactive pool occurred in superficial bioturbated sediment (Table 3, Fig. 4b). Both superficial and deep non-bioturbated sediments had the highest intermediate C pool (Kruskal–Wallis test: $H_{5,30} = 11.18$, $p < 0.05$; Fig. 4b). Refractory C pool was higher in bioturbated than in non-bioturbated sediments (Table 3, Fig. 4b).

When analyzing the ratio of reactive C pool to total carbon, it was found that at mudflats the highest ratio of reactive C was found in superficial bioturbated sediment (23.5%), followed by superficial non-bioturbated sediment (22.0%), while mounds (10.2%) and deep non-bioturbated sediments (8.7%) had the smallest proportions (Table 3). These patterns were similar in saltmarshes, where burrow walls, superficial bioturbated, and superficial non-bioturbated sediments had the highest proportions of reactive carbon (16.6, 28.1 and 26.3% respectively; Table 3). Lowest contributions were obtained in both deep bioturbated and non-bioturbated sediments (5.2 and 6.0% respectively).

3.3. Benthic flux across the water-sediment interface

At mudflats, temperature of near-chamber water remained with no change during incubation (mean: 19.4 °C; SD: 1.3 °C). Mudflat analysis revealed a clear bioturbation effect on CO₂ benthic fluxes, with bioturbated B + C treatment showing a net CO₂ release from sediment, whereas NBC and B treatments showed a net CO₂ flux toward sediment (ANOVA: $F_{2,15} = 14.05$, $p < 0.05$; Fig. 5a). Carbohydrate flux was also affected by bioturbation, showing a release from the sediment for B + C treatment, and a net flux toward the sediments in NBC treatment, while B treatment showed an intermediate value (ANOVA: $F_{2,15} = 151.00$, $p < 0.05$; Fig. 5b). Non-bioturbated sediment (i.e., NBC treatment) showed a net protein flux from water column toward sediment, while at both bioturbated sediments (i.e., B and B + C) there was no difference between treatments (ANOVA: $F_{2,15} = 21.92$, $p < 0.05$; Fig. 5c). Dissolved organic carbon (DOC) flux at non-bioturbated treatment showed a net DOC uptake, whereas a DOC release from sediments was observed in bioturbated treatments, with higher values for B + C than for B treatment (ANOVA: $F_{2,15} = 19.81$, $p < 0.05$; Fig. 5d).

At saltmarsh, temperature did not change significantly during the incubations (mean: 18.6 °C; SD: 0.9 °C). In this zone, bioturbation enhanced the CO₂ flux (ANOVA: $F_{2,15} = 80.48$, $p < 0.05$; Fig. 5e), whereas there was no bioturbation effect on benthic fluxes of both carbohydrates and proteins (Fig. 5f and g). DOC flux was toward the

Table 1
Statistical summary of ANOVAs from the effect of bioturbation on organic matter quality at mudflats and marshes.

Source	df	Mudflat		Saltmarsh	
		MS	F	MS	F
Carbohydrates (C-CHO) ^{a,c}					
Treatment	5	0.896	12.2*	0.116	19.2*
Error	24	0.074		0.006	
Proteins (C-PRT) ^{a,c}					
Treatment	5	0.244	4.0*	0.192	3.1*
Error	24	0.060		0.061	
Lipids (C-LIP)					
Treatment	5	0.149	11.7*	0.842	5.4*
Error	24	0.013		0.157	
Total organic C (TOC)					
Treatment	5	485.581	197.3*	43.340	11.7*
Error	24	2.461		3.710	
Labile organic C (LOC) ^b					
Treatment	5	5.022	24.9*	8.5234	20.4*
Error	24	0.206		0.417	
LOC to TOC ratio (% LOC)					
Treatment	5	791.650	8.5*	168.190	16.0*
Error	24	93.250		10.500	

^a Log-transformed data (mudflat).

^b Square root transformed data (mudflat).

^c Log-transformed data (marsh).

* Indicates $p < 0.05$.

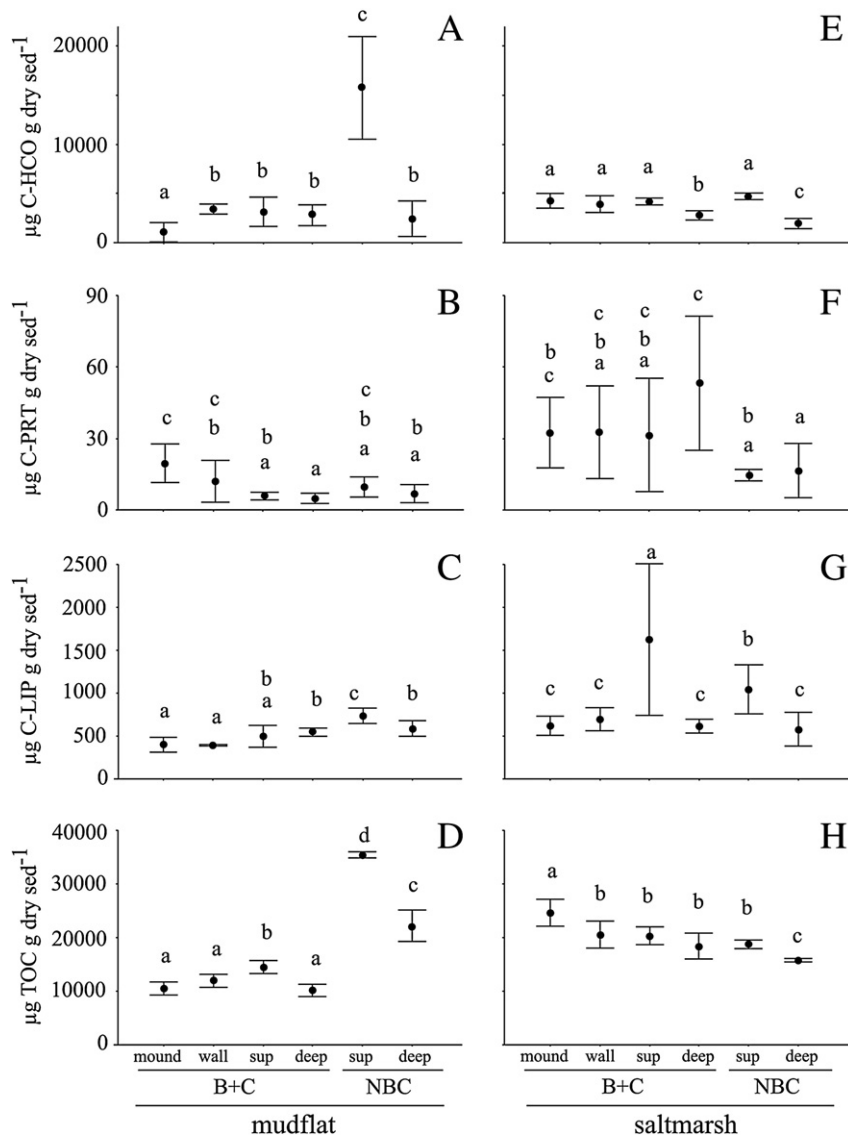


Fig. 2. Content of carbon as carbohydrates (A and E), proteins (B and F), lipids (C and G), and total organic carbon (D and H) at the different sediment types from experimental treatments in the mudflat and the saltmarsh. Different letters indicate significant differences from ANOVAs results ($n = 6$ each sediment type; LSD test, $\alpha = 0.05$).

sediment at non-bioturbated treatment, but it was toward the water column at both bioturbated treatments (i.e., B and B + C; Fig. 5h).

4. Discussion

Sedimentary OM is a mixture of plant and animal reactive detritus together with poorly reactive polymeric organic carbon (Canfield et al., 2005). Microorganisms often use first the labile organic carbon in such a way that poorly reactive C tends to accumulate into sediments (Burdige, 2006). Our results showed that LOC was between 15.6 and 30.0% of total organic carbon at the saltmarsh, and between 12.1 and 46.5% at mudflat sediments. Also, reactive C pool showed values between 5.2 and 28.1% in the saltmarsh, and between 8.7 and 23.5% of total carbon in the mudflat, and these values are high when compared to the reported worldwide values (5–15% Fabiano et al., 1995; Yingst, 1976; but see Papaspyrou et al., 2005). The results presented here showed that bioturbation affected the quality, bioavailability and the spatial distribution of sedimentary OM in mudflats and saltmarshes. In contrast to what was found in other systems where bioturbated sediments had higher % LOC than non-bioturbated sediments (e.g., Aller and Aller, 1986; Papaspyrou et al., 2005), our results showed the highest LOC contents in non-bioturbated superficial sediment. The % LOC was

mostly similar among bioturbated sediment types. This seems to be because the sediment reworking exerted by crabs (e.g., Bertness, 1985; Fanjul et al., 2007; Kristensen, 1988; Needham et al., 2010) transports and homogenizes the OM through the sediment column (Andersen and Kristensen, 1988; Burdige, 2006; Gutiérrez et al., 2006). As in other saltmarsh crabs (e.g., *Austrohelice crassa*, *Sesarma reticulatum*, *Uca* spp.), *N. granulata* does not secrete mucus to lining burrow walls (Kristensen, 2008; Needham et al., 2010), which may constitute an important fraction of the LOC pool in many bioturbated sediments (see Kristensen, 1985; Kristensen and Kostka, 2005; Papaspyrou et al., 2005).

Organic nitrogen content (i.e., proteins) is considered as a limiting factor for deposit feeder organisms (Pusceddu et al., 1999); and protein to carbohydrate ratio (PRT:CHO) is a parameter commonly used to estimate the degradation state of LOC (Danovaro et al., 1993). The low PRT:CHO ratio found in sediments analyzed here (between 0.035 and 0.0007) suggests a clear role of proteins as limiting factor for consumers (Fabiano et al., 1995). The highest PRT:CHO ratio at the mudflat was found in crab-mounds, and the smallest in non-bioturbated superficial sediment. Thus, although non-bioturbated surface sediment presented high contents of TOC, this C has a relatively low nutritional quality in terms of N contribution (Pusceddu et al., 1999). However, in the saltmarsh the highest PRT:HCO ratio was found in bioturbated deep-

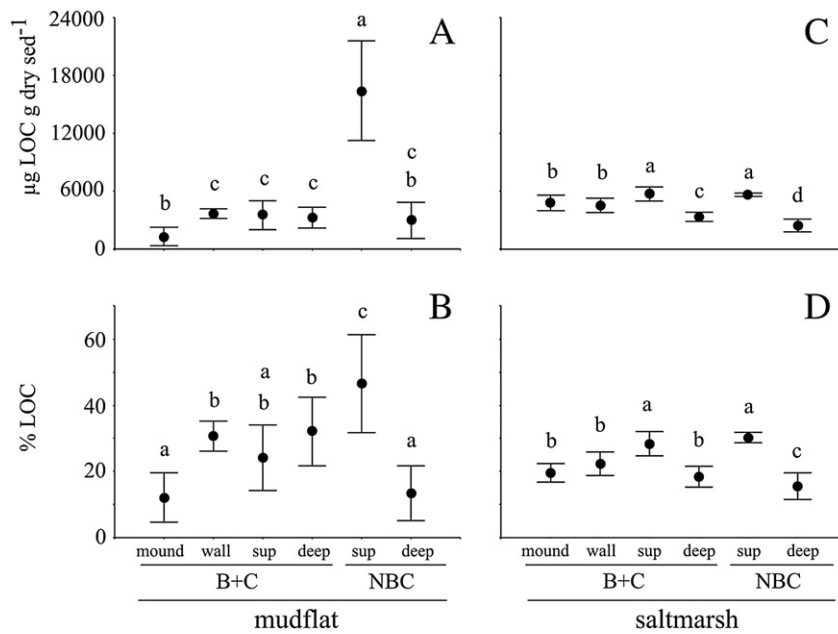


Fig. 3. Content of labile organic carbon (A and C), and contribution of labile organic carbon to total organic carbon (% LOC, B and D) at the different sediment types ($n = 6$ each sediment type) in experimental treatments in the mudflat and the saltmarsh.

sediment, and although this sediment had a low TOC content, it may be important in nutritional terms. The construction and maintenance of burrows by *N. granulata* contributes to the homogenization of the sediment column in terms of their LOC proportion, but their activity also generates mounds, which although containing low net amounts of OM and low % LOC (this study; Gutiérrez et al., 2006) have great nutritional value due to their relatively high content of organic nitrogen.

Overall labile organic carbon content in bioturbated and non-bioturbated areas in mudflats and saltmarshes could be estimated considering burrow density (roughly 30 burrows m^{-2}), burrow size (cylindrical burrows; 3.6 cm in diameter, 100 cm in depth for saltmarsh; and 4 cm in diameter, 40 cm depth for mudflat; see Botto and Iribarne, 2000; Iribarne et al., 1997), and the area covered by mounds (1 mound per burrow, 49 cm^2 mound $^{-1}$ at saltmarsh; 28 cm^2 mound $^{-1}$ at mudflat; personal observation). This estimation showed that there was no difference in overall TOC content between bioturbated and

non-bioturbated sediments at saltmarsh and mudflat, but the whole LOC fraction was higher for bioturbated (saltmarsh: 19.2%, mudflat: 31.1%) than for non-bioturbated zones (saltmarsh: 16.4%, mudflat: 16.1%). These LOC fractions were higher than reported for most eutrophic estuarine sediments (~3% LOC; Fabiano and Danovaro, 1994), and were among LOC values reported for oligotrophic marine and estuarine systems (e.g., ~20% LOC Fichez, 1991; 23.1% Danovaro et al., 1993). When a system becomes eutrophic, their OM quality decreases (Fabiano et al., 1995). In this sense, our results showed that bioturbation by *N. granulata* increases the nutritional quality of sedimentary OM (i.e., increase the fraction of LOC) both in mudflats and saltmarshes, probably contributing to maintain oligotrophic state in our systems (see also Pusceddu et al., 2003). This agrees with the previously reported role of *N. granulata* on increasing sediment oxygenation (Daleo et al., 2007; Fanjul et al., 2007), dissolved nutrient export rate (Fanjul et al., 2008, 2011), and denitrification rates (Fanjul et al., 2007, 2011).

Detritus from leaves and stems of *Spartina* spp. at different decomposition stages constitute the major source of OM in intertidals from SW Atlantic bays and estuaries (Botto et al., 2006; Montemayor et al., 2011). *Spartina* spp. roots, in addition to their secretion of LOC that

Table 2

Percentages of labile organic carbon as carbohydrates (C-HCO), proteins (C-PRT), and lipids (C-LIP) for different sediment types in experimental treatments in the mudflat and saltmarsh (mean and standard deviation in brackets). Different letters indicate significant differences from ANOVAs ($n = 6$ each sediment type; LSD test, $\alpha = 0.05$).

	C-HCO (%)		C-PRT (%)		C-LIP (%)	
Mudflat						
B + C						
Mound	71.0	(26.2) ^b	2.0	(1.4) ^a	27.0	(25.7) ^a
Burrow wall	93.5	(0.9) ^a	0.3	(0.3) ^b	6.1	(0.7) ^{b,c}
Superficial	88.0	(7.3) ^a	0.2	(0.1) ^b	11.4	(7.2) ^{a,b}
Deep	84.9	(7.3) ^{a,b}	0.2	(0.09) ^b	15.0	(2.7) ^a
NBC						
Superficial	95.1	(2.7) ^a	0.1	(0.04) ^b	4.8	(2.7) ^c
Deep	79.1	(11.8) ^{a,b}	0.3	(0.3) ^b	20.6	(11.6) ^a
Saltmarsh						
B + C						
Mound	86.4	(1.7) ^a	0.7	(0.2) ^a	12.9	(1.7) ^c
Burrow wall	83.4	(5.3) ^{a,b}	0.7	(0.3) ^a	15.9	(5.4) ^{b,c}
Superficial	72.4	(12.2) ^c	0.5	(0.3) ^a	27.1	(12.2) ^a
Deep	79.9	(2.9) ^{a,b}	1.6	(0.9) ^b	18.4	(3.0) ^{b,c}
NBC						
Superficial	81.3	(5.3) ^{a,b}	0.2	(0.04) ^a	18.4	(5.0) ^{b,c}
Deep	76.1	(4.4) ^{b,c}	0.6	(0.2) ^a	23.3	(4.5) ^{a,b}

Table 3

Statistical summary of ANOVAs for the effects of bioturbation on organic matter bioavailability at mudflats and marshes.

Source	df	mudflat		saltmarsh	
		MS	F	MS	F
Reactive C pool					
Treatment	5	28,398,129	9.38*	18,164,980	5.17*
Error	24	3,027,660		3,513,484	
Intermediate C pool ^a					
Treatment	5	30,406,450	5.20*		
Error	24	5,850,066			
Refractory C pool					
Treatment	5	1.42E+08	15.82*	6.80E+07	5.04*
Error	24	8.96E+06		1.35E+07	
Reactive C pool to TOC ratio					
Treatment	5	217.253	3.34*	0.486	15.78*
Error	24	65.138		0.031	

^a Kruskal–Wallis test, see Section 3.

* Indicates $p < 0.05$.

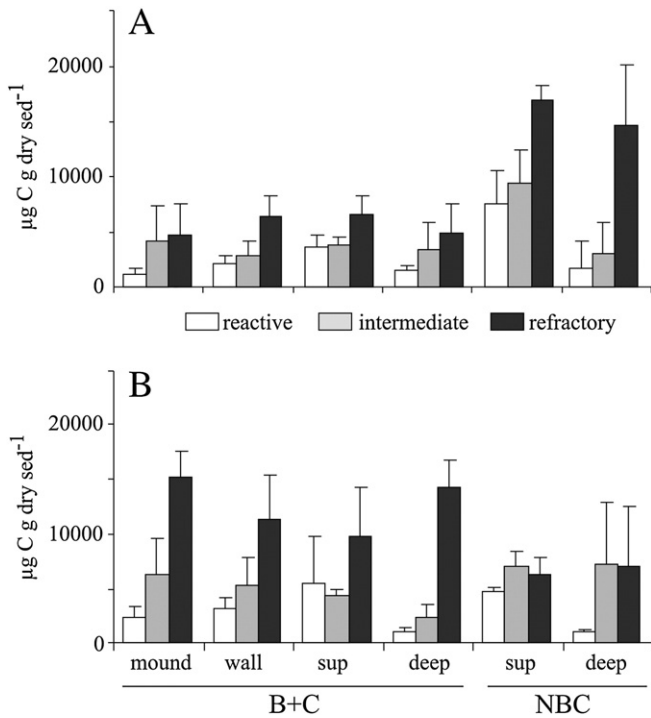


Fig. 4. Content of reactive, intermediate, and refractory carbon pools according to bioavailability assays at the different sediment types in experimental treatments in the mudflat (A) and the saltmarsh (B).

fuel microbial activity (Hines et al., 1989), efficiently transport oxygen to anoxic sediments, having a great impact on sediment diagenesis (Holmer et al., 2002; Sundby et al., 2003). Thus, the interaction among macrofauna, macrophytes and microbial activities could have a significant effect on the OM preservation in marine and coastal sediments (e.g., Burdige, 2006; Woulds et al., 2007). The structure of roots, in turn, stabilize the substrate and reduce the maintenance of burrows, consequently reducing reworking rates (Bertness, 1985) and probably decreasing the vertical turnover rate of materials during crab burrowing (Wang et al., 2010). Although we did not directly assess the effects of plants on OM processing, our results showed higher rates of net sediment metabolism (i.e., CO₂ production) in saltmarsh sediments than in mudflat, suggesting an impact of vegetation on microbial activity in both bioturbated and non-bioturbated sediments (see also Wang et al., 2010). Also, our results showed that bioturbation enhanced the whole benthic metabolism about 8000% in the saltmarsh, but only about 200% in the mudflat. This is a somewhat surprising result, since TOC content was similar at both zones and reworking rates are slow in the saltmarsh (e.g., Iribarne et al., 1997), so we would expect a smaller bioturbation effect in this intertidal zone (see Wang et al., 2010). However, refractory C content was higher in the saltmarsh than in the mudflat, and it is known that this C only could be quickly decomposed in a redox oscillating environment as those generated by bioturbation (see Aller, 1994). Moreover, saltmarsh plants continuously provide new detritus material (e.g., dead roots and rhizomes, dead aerial tissues) to the sediment, which also could be quickly decomposed in a bioturbated system, contributing largely to enhance benthic metabolism in marsh-bioturbated sediments. Also, the interaction between crab bioturbation and saltmarsh plants could be another plausible explanation for this result, since *N. granulata* stimulates primary production of *Spartina* spp. (Daleo et al., 2007), and it is known that the photosynthetic activity is directly related to the secretion of LOC by roots (Hines et al., 1989) that fuel microbial metabolism. Although there is plenty of information on the effect of saltmarsh plants (e.g., Andersen and Kristensen, 1988; Gribsholt et al., 2003; Pedersen et al., 2004; Sundby et al., 2003)

and crab bioturbation (e.g., Fanjul et al., 2011; Gribsholt et al., 2003; Kostka et al., 2002; Needham et al., 2011) on sediment biogeochemistry, the net effect of the interaction between plants and crab bioturbation on benthic biogeochemistry remains largely unexplored.

The effect of *N. granulata* bioturbation on benthic metabolism was higher than those reported for other widely distributed bioturbators such as fiddler crabs (*Uca* spp.: between 200 and 300% Nielsen et al., 2003) and polychaetes (*Nereis virens*: up to 75% Papaspyrou et al., 2010; *Arenicola marina*: no effect Papaspyrou et al., 2007). The increase in CO₂ production in bioturbated sediments was a combination of the direct metabolic contribution of *N. granulata* and benthic macrofauna, but also it could be a result of the stimulation of the heterotrophic microbial activity by bioturbation. The stimulation of microbial metabolism induced by this crab seems to be due to O₂ injection and the enhanced solute transport into sediment (Fanjul et al., 2007, 2011), but also due to the increased bioavailability of the sedimentary OM (this study). Fanjul et al. (2011) showed that *N. granulata* enhance O₂ uptake about 250% in the mudflat, and about 1600% in the saltmarsh; our results showed a 200% increment of CO₂ flux in bioturbated mudflat and an increment of 8000% in the saltmarsh area. Given that oxygen is utilized both in aerobic metabolism and for chemical re-oxidation of reduced metabolites (e.g., sulfide and iron (II)), the oxygen uptake is not a direct measurement of aerobic metabolism. These results suggest that at mudflats, bioturbation enhances oxygen-consuming processes (aerobic respiration and re-oxidation), but in saltmarshes strongly enhances both aerobic and anaerobic metabolism (see also Aller, 1994; Gribsholt et al., 2003).

Bioturbation may affect benthic DOC fluxes in several ways (e.g., Aller, 1994; Burdige and Homstead, 1994; Michaud et al., 2006). Bioturbation could enhance DOC oxidation (by microbial assemblage and benthic organisms), which leads to higher CO₂ production and to smaller DOC fluxes (Burdige and Homstead, 1994). Redox oscillation produced by bioturbation could also affect benthic DOC fluxes, by releasing sorbed organic carbon and then enhancing DOC oxidation. However, sediment reworking during burrow construction and maintenance, and the increase in exchange area produced by burrows, could result in an enhancement of benthic DOC fluxes. Indeed, our results showed that *N. granulata* shifts the magnitude and direction of DOC benthic flux, which is toward sediment in non-bioturbated sites but is released to water column when *N. granulata* is present in both mudflat and saltmarsh zones. This stimulation effect was higher in mudflats than in saltmarshes, where possibly was highly stimulated DOC oxidation by heterotrophic bacteria in the rhizosphere (see discussion in Fanjul et al., 2011). This leads to a smaller enhancement of benthic DOC fluxes in spite of the effect of sediment reworking, and to a higher effect of stimulation of CO₂ fluxes, which is consistently with our results.

Bioturbation-induced changes on benthic metabolism and on OM availability would result in a reduction of the storage capacity of carbon in our intertidal systems (see Aller, 1994; Burdige, 2006; Gutiérrez et al., 2006; Wang et al., 2010). However, several studies performed in our coasts suggest that *N. granulata* burrows act as passive traps for organic matter, increasing the storage capacity of the system, and probably decreasing the export of organic material (Botto et al., 2006; Escapa et al., 2008; Montemayor et al., 2011). Despite this apparent contradiction, our results along with results of previous works (Botto et al., 2006; Escapa et al., 2008; Montemayor et al., 2011), suggest that crab burrows trap organic carbon (i.e., detritus and OM rich sediment), which is efficiently degraded and remineralized at bioturbated sediment, and finally it is quickly exported to the water column as CO₂ and DOC. Considering that one of the most important sources of nutrients for estuarine and coastal waters comes from estuarine organic matter, and that only a small fraction of it is exported as macrodetritus (Nixon, 1980), this bioturbation-induced increase in benthic flux of dissolved organic matter toward water column could be an important contribution to sustain coastal food webs.

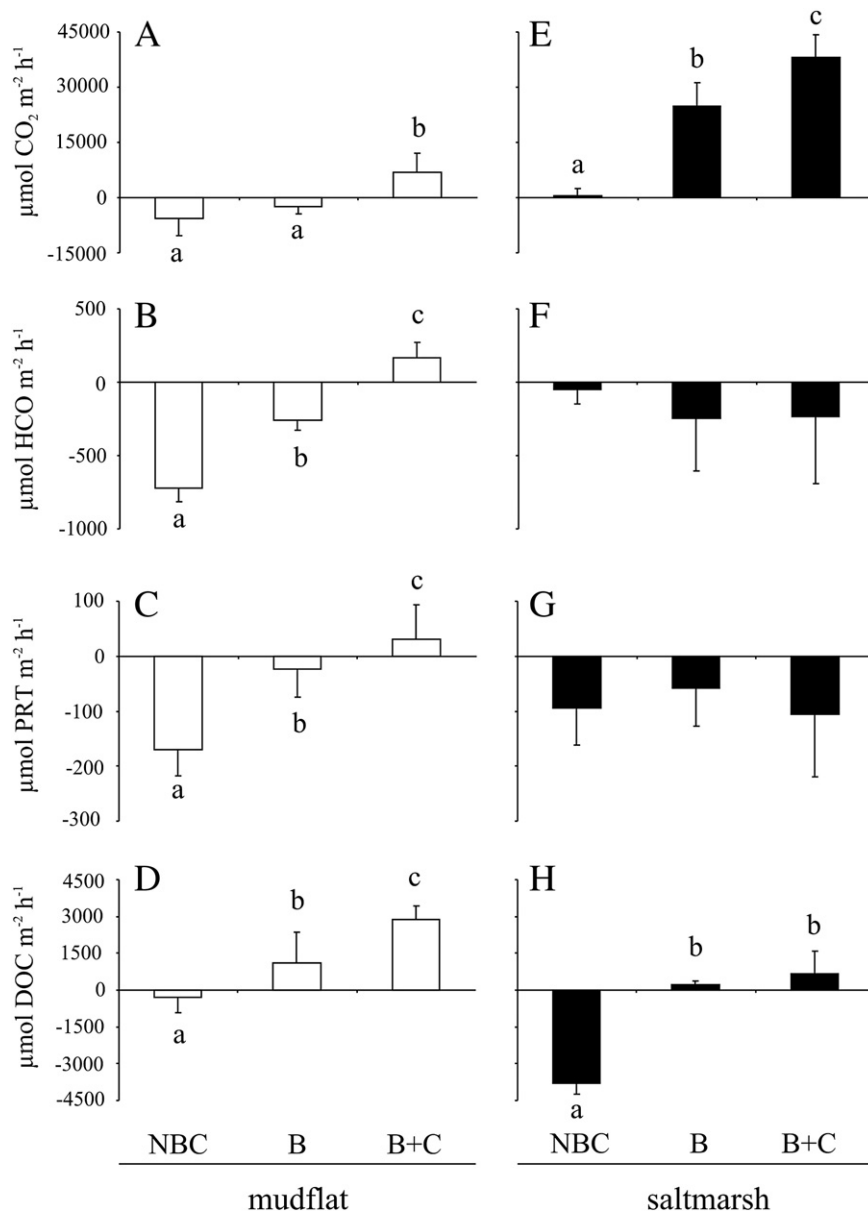


Fig. 5. Benthic metabolism (A and E), and benthic flux of dissolved carbohydrates (B and F), proteins (C and G), and dissolved organic carbon (D and H) for the experimental treatments NBC, B and B + C in the mudflat and saltmarsh. Negative values indicate flux from water column toward sediment. Different letters indicate significant differences from ANOVA results ($n = 6$ each treatment; LSD test, $\alpha = 0.05$).

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