The Contribution of Crab Burrow Excavation to Carbon Availability in Surficial Salt-marsh Sediments

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ABSTRACT

Geomorphology, vegetation and tidal fluxes are usually identified as the factors introducing variation in the flushing of particulate organic matter (POM) from tidal marshes to adjacent waters. Such variables may, however, be insufficient to explain export characteristics in marshes inhabited by ecosystem engineers that can alter the quantity and quality of POM on the marsh surface that is subject to tidal flushing. In this study we evaluated the balance between transfer of buried sedimentary organic carbon (C) to the marsh surface due to crab excavation (measured from the mounds of sediment excavated from burrows) and outputs of C from the surface due to sediment deposition within crab burrows (estimated from sediment deposited within PVC burrow mimics), in a Southwestern Atlantic salt marsh supporting dense (approximately 70 ind m^{-2}) populations of the crab *Chasmagnathus granulatus*. C excavation by crabs was much greater than deposition of C within crab burrow mimics. Per area unit estimates of the balance between these two processes indicated that crabs excavated 5.98 g m^{-2} d^{-1} and 4.80 mg m^{-2} d^{-1} of total and readily (10 d) labile C, respectively. However, sediments excavated by crabs showed a significantly lower content of both total and readily-labile C than sediment collected in burrow mimics. This indicates that ecosystem engineering by burrowing crabs causes a net decrease in the concentration of C in the superficial sediment layers and, thus, an overall decrease in the amount of C that can be washed out of the marsh by tidal action. Incorporating the in situ activities of ecosystem engineers in models of marsh export should enhance understanding of the function of marshes in estuarine ecosystems.

Key words: burrows; carbon; crab; ecosystem engineer; excavation; export; salt marsh; sediment.

INTRODUCTION

Salt marshes are highly productive ecosystems that are intimately linked to adjacent estuaries by fluxes of particulate and dissolved organic matter (Adam 1990; Mitsch and Gosselink 1993; Childers and others 2000; Odum 2000; Teal and Howes 2000).

The degree to which salt marshes contribute to estuarine productivity and functioning has long been of interest (Teal 1962; Odum and De la Cruz 1967; Valiela and others 2000), and it is well recognized that this contribution depends on the degree to which organic matter is processed within the marsh prior to export (Adam 1990). For a given level of marsh productivity, the quantity and quality of exported particulate organic matter (POM) is a function of retention time and decay rates, which

are known to be highly variable from one system to another (Gallagher and others 1980; Findlay and others 1990; Bouchard and Lefeuvre 2000). Geomorphology, dominant vegetation and the physical dynamics of tidal flushing are usually identified as the most important factors causing variation in POM export characteristics (Odum and others 1979; Nixon 1980; Gallagher and others 1980; Findlay and others 1990; Dame and Allen 1996; Childers and others 2000). However, these variables may be insufficient to explain POM export characteristics in marshes inhabited by organisms that physically modify the structure of the environment (*ecosystem engineers*; Jones and others 1994, 1997) in ways that significantly affect POM retention, decay or both.

Many salt marshes support high densities of crabs that excavate burrows over extensive areas, profoundly modifying the physical structure of the environment (for example, Bertness 1985; Takeda and Kurihara 1987; Taylor and Allanson 1993; Iribarne and others 1997). The excavation activities of crabs and the resulting burrows may well have important consequences for the retention of POM in salt marshes. However, crab excavation may have different effects on POM retention from that of the resulting crab burrows. Excavation turns over marsh sediments (Katz 1980; Montague 1982; McCraith and others 2003) bringing buried material to the marsh surface, exposing the sediments to oxygen and increasing the amount of material that can be exported by tidal flushing. In contrast, crab burrows can function as traps for particles that are transported by the overlying water (DePatra y Levin 1989; Botto and Iribarne 2000) and sinks for materials arising from crab activities (for example, feces, previously excavated sediments), potentially contributing to POM burial within the marsh. Given these different processes, the quantity of organic matter at the marsh surface available for transport to the estuary during tidal inundation would be the balance between the deposition of POM into crab burrows and the excavation of POM by burrowing crabs.

Data on the net effect of crab excavation *versus* burrow trapping on the quantity of POM on the surface of salt marshes are sparse. Montague (1982) estimated that fiddler crabs, *Uca pugnax*, excavated a total amount of carbon (C) equivalent to 20% of annual belowground production of smooth cordgrass (*Spartina alterniflora*) in Sapelo Island (Georgia, USA). The quantity of C made available for tidal flushing via excavation was much greater than the amount of C collected in burrows. Such a finding is likely to be general to other crab species and marshes, because burrow construction and maintenance requires the removal of sediments from

burrows, and this likely occurs at higher rates than passive sediment trapping in the burrows. However, such a net effect on the quantity of C resulting from higher rates of excavation than burrow trapping does not take into account the quality of the C excavated by crabs relative to the quality of the C collected in burrows. Several lines of evidence suggest that excavation by burrowing crabs results in non-selective removal of sediments from the deposits to the surface (see Botto and Iribarne 2000; McCraith and others 2003). In contrast, the deposition of sediment within crab burrows may well be enhanced in crab feces—likely higher in organic matter content relative to surrounding sediments (for example, Kraeuter 1976)—and enriched in low-density organic particles suspended by tidal currents. Differences in the processes involved in the excavation of POM and their deposition within burrows could result in shifts in the lability of the C available at the marsh surface. For example, if C deposited in burrows is relatively more labile than that excavated by crabs, the net effect of crab excavation would be a decrease in the lability of the C available at the marsh surface. Such a decrease in the average lability of the C available at the marsh surface would imply a longer-term exposure of surficial C to tidal flows and, concomitantly, an increased probability of C export out of the marsh. Although the opposite effect is also possible (that is, C excavated by crabs is more labile than C deposited in burrows leading to increased lability of C at the marsh surface and decreased probability of C export), in either case it is clear that changes in the relative lability of C at the marsh surface due to crab burrowing may have important consequences for the quantity and quality of C that can be flushed from the marsh surface by tidal action.

In this study we investigated the balance between the excavation of sedimentary organic C by crabs and the deposition of sedimentary organic C in crab burrows in a Southwestern Atlantic salt marsh inhabited by the burrowing crab, *Chasmagnathus granulatus*. *C. granulatus* is the dominant macroinvertebrate in salt marshes from Southern Brazil to Northern Patagonia (Argentina; Boschi 1964), markedly modifying the physical structure of these environments (Iribarne and others 1997; Bortolus and Iribarne 1999). This species (up to 4 cm carapace width) commonly occurs at densities of more than 60 crabs m^{-2}, constructs large burrows (up to 1 L volume and 1 m depth), and excavates sediments at high rates compared to other marine benthic invertebrates (Iribarne and others 1997). To understand the consequences of *C. granulatus* burrowing on C quantity and quality

at the marsh surface, we compared total C and readily labile C (that is, the amount of C mineralized by microorganisms during 10 d laboratory incubations) in sediments excavated from crab burrows and sediments deposited in crab burrow mimics of different sizes. Using randomization and resampling techniques, we combined the data on C excavation, C deposition into burrows, burrow density and size frequency distribution to estimate the net transport of both total and readily labile C to the surface due to crab activity.

METHODS

Study Site

The study was conducted at the Mar Chiquita coastal lagoon (Argentina, 37°45′S), a 46 km^2 body of brackish water with semidiurnal low amplitude (< 1 m) tides (Fasano and others 1982). High intertidal elevations in this lagoon have large marshes that are almost monospecific stands of the dense-flowered cordgrass, *Spartina densiflora* (Iribarne and others 1997). Sampling was conducted in the summer of 2003 in a salt marsh located at the eastern margin of Cangrejito creek—a small tidal creek draining an approximately 1,500 ha basin that flows into the lagoon about 1,000 m from the lagoon inlet. In the absence of rain events that can flood the marsh for several weeks, marsh inundation is restricted to spring tide periods (10–12 days a month). The proportion of marsh flooded by tides largely depends on rainfall events and wind intensity and direction.

The study location covered a rectangular area of approximately 100 × 50 m aligned parallel to the shoreline. This area encompassed the lower elevations of the marsh (approximately between 0 and 30 cm above the mean high tide level) and is flooded during spring tides even in the absence of strong tidal-forcing by wind or high precipitation. Measurements were carried out during 5 summer days of spring tide because crabs are more active in summer concentrating their burrowing activities when sediments are moist and soft due to concurrent or recent tidal inundation (J. Gutiérrez, personal observation). Crab excavation decreases as the neap-tide periods advances and the sediments become progressively dryer and harder due to long exposure to air.

Crab Excavation

Sediment excavated and deposited on the marsh surface by burrowing crabs during a 5-d spring-tide period (January 20–24, 2003) was collected after removal of pre-existing surficial sediment mounds associated with 72 previously tagged burrows of known burrow entrance diameter (measured with a precision of 1 mm). Twelve randomly selected burrows were sampled in each of the following burrow entrance diameter ranges: 10–19, 20–29, 30–39, 40–49, 50–59, and 60–69 mm. Sampling encompassed burrows from 10–69 mm diameter because 1 cm is the smallest entrance diameter observed in the field—recently settled crabs occur in association with adult burrows—and burrows larger than 7 cm are less than 0.5% of the total. The adjacent sediment mounds that were subsequently formed by crab excavation in these burrows were collected daily (that is, after two tidal cycles) during the whole 5-d period. Samples were stored cold (0–2°C) until analysis of total and readily labile C. Because samples were stored for less than 3 weeks, no significant effects of storage on C decomposition would be expected (see Stenberg and others 1998).

Sediment Deposition into Burrows

Direct measurement of the rates of sediment deposition within burrows is not possible because sediments deposited during the sampling period cannot be distinguished from preexisting sediments. Consequently, the amount of sediment deposited daily into crab burrows over the same time interval was estimated by deploying PVC pipes buried in the sediment with the opening flush with the sediment surface. *C. granulatus* burrows in salt marshes are usually vertical cylinders of variable opening diameter (up to 7 cm) and depth (40–100 cm) (Iribarne and others 1997), and PVC pipes are reasonable mimics (see Appendix). We used pipes of different diameters (10, 20, 30, 40, 50, and 60 mm) that span the range of natural burrow openings observed in the field. These pipes were capped at the bottom end. Although *C. granulatus* burrows are of variable depth, we used a constant pipe depth of 40 cm. The empirical relationship between particle collection efficiency and aspect ratio (depth/diameter ratio) of cylindrical sediment traps indicate that the use of a constant depth of 40 cm would not bias the results for any of the range of pipe diameters used in the study. A depth of 40 cm (the minimum observed for natural *C. granulatus* burrows in Mar Chiquita; see Iribarne and others 1997) gives an aspect ratio greater than 5 for all the diameter classes of burrow mimics. Particle trapping efficiency of cylindrical traps is positively related to aspect ratio but is asymptotic at an aspect ratio of 5 (Emerson 1991). Therefore, we would expect no variation in particle deposition into

burrow mimics or natural crab burrows provided the depth exceeds five diameters.

Crabs had free access to burrow mimics to allow transport of C in and out of burrows due to crabs. Twelve mimics of each diameter class were used. As with the sediment excavation study, material deposited into burrow mimics was sampled daily (that is, after two tidal cycles) during the whole 5-d period. At each sampling date, all the water and sediments retained in each mimic were collected and gravitationally filtered on filter paper. The retained sediments were stored for less than 3 weeks at 0–2°C and, then, analyzed for total and readily labile C. C losses due to filtration are not expected to bias our estimate of C deposition within burrows because similar losses are expected to occur in real burrows via water drainage.

Total and Labile Carbon

Daily samples of: (1) the sediments deposited within six burrow mimics of each diameter class and, (2) the sediments excavated from six burrows of each diameter class (that is, 36 from a total of 72 samples in both cases), were dried at 50°C for 48 h, weighed, and used for the measurement of total C (*hereafter* TOTAL C). The remaining samples were used to measure short-term respiration potential as an indicator of readily (10 d) labile C (*hereafter* LABILE C). Each sample was initially mixed and sieved through a 2 mm mesh.

TOTAL C was estimated from a combination of loss-on-ignition (LOI) and dry combustion (DC). Such a combination of methods was deemed necessary to circumvent the limitations of both methods. LOI (that is, mass loss following combustion at approximately 500°C) measures volatilization of all material—including water retained in clay structures after oven drying—and, thus, can overestimate total C. The DC method avoids any gravimetrical effect of the evaporation of water retained in clays because total C is measured from the evolution of CO_2 and CO during combustion (Sollins and others 1999). However, because DC temperatures ($> 1,000$°C) are higher than the temperature of $CaCO_3$ decomposition (825°C), total organic C can be overestimated if the sediment samples contain a considerable amount of $CaCO_3$. Based on these considerations, we conducted the following analyses:

1. Subsamples (10 ± 1 g) of all the samples destined for the analysis of total C were incinerated in a furnace (450°C during 6 h) and LOI was calculated as the difference in dry weight of the sample before and after incineration.

2. Subsamples (20 µg) from 12 samples (six of sediments excavated by crabs and six of sediments deposited in burrow mimics) were also analyzed for total C by DC at 1,020°C in a Carlo Erba 1500 C-N Analyzer (Sollins and others 1999).

3. Subsamples (1.2–1.5 g) from 12 samples (six of sediments excavated by crabs and six of sediments deposited in burrow mimics) were analyzed for carbonate content by sequential loss-on-ignituion (SLOI; Heiri and others 2001). Subsamples were first incinerated in a furnace at 450°C during 6 h to remove organic matter. Then, these subsamples were weighed, incinerated in a furnace at 950°C for 2 h to remove carbonates, and re-weighed. Carbonate content was estimated as the weight loss at 950°C multiplied by 1.36, which should theoretically equal the weight of the carbonate in the original sample if we assume a weight of 44 g mol^{-1} for CO_2 and 60 g mol^{-1} for CO_3^{2-}.

TOTAL C was estimated from LOI values according to the following equation:

$$\text{TOTAL C} = \text{LOI}[(\text{DC} - CO_3^{2-})/\text{LOI}].$$

First, measures of C obtained by DC were corrected for CO_3^{2-} decomposition using the CO_3^{2-} estimates obtained after SLOI. Then, the CO_3^{2-}-corrected DC values were used to correct the LOI values for volatiles other than C lost during LOI. Uncorrected DC measures were, on average, 56% of LOI values for sediments excavated by crabs and those collected within burrow mimics, which implies that volatiles other than C comprise the 44% of weight loss after LOI. CO_3^{2-} content of the samples was, on average, 15 and 12% for sediments excavated by crabs and collected within burrow mimics, respectively. Correction of DC values for CO_3^{2-} losses gave estimates of TOTAL C equal to 44 and 41% of LOI for sediments excavated by crabs and collected within burrow mimics, respectively.

Respiration potential was measured by incubating sediments at 25°C for 10 d and measuring the CO_2 evolved during this period (Robertson and others 1999). It was necessary to pool the sediments collected from each burrow mimic on the different sampling dates because daily sediment deposition in some burrow mimics often resulted in insufficient material for incubation. Although crabs daily excavated an amount of sediment that was large enough for conducting incubations, materials excavated from individual burrows on the different sampling dates were also pooled to allow a valid comparison with the materials collected in burrow

mimics. Assays used 5 ± 0.5 g (wet) of the pooled sediment deposited in each burrow mimic or excavated from each burrow during the 5-d period. Samples were standardized to 100% moisture by adding water to the incubation jars until a thin layer lay over the sediment. The remaining portion of each sediment sample was weighed before and after drying at 50°C for 48 h to determine water content, and these data were used to estimate the dry weight of the corresponding incubated sediment sample. Gas samples (9 ml) were taken at the end of the incubation period and the concentration of C dioxide measured by thermal conductivity gas chromatography (Robertson and others 1999).

Burrow Density and Size Frequency Distribution

The number of burrows in 36 sampling plots (35 × 35 cm) was determined on a single day in January 2003. Because burrow entrances are more or less oval in shape when viewed from overhead (minor/major diameter ratio > 0.62), the major and minor diameter of each burrow was measured with calipers to the nearest millimeter and the average of both diameters was used as an indication of burrow entrance size. Entrance diameters were measured at the level of the surface plane because burrows intersect it forming quite definite angles (see Iribarne and others 1997). The location of each sampling plot was established using a 35 × 35 cm wooden frame placed randomly in areas within the selected range of marsh elevation.

Data Analysis

Comparisons of Excavation and Burial. *t*-tests adjusted for inequality of variances (Welch's approximate *t*; Zar 1984) were used to evaluate: (1) whether the amount of sediment (dry weight), TOTAL C, and LABILE C excavated by crabs differed from that deposited in burrow mimics; and (2) whether the percentage of TOTAL C and LABILE C differed between sediments excavated by crabs and deposited in burrow mimics. This test was chosen because it is robust to heterogeneity of variances—which were consistently observed in the data after *F*-tests (Zar 1984)—and non-normality (Zar 1984). Correlation analysis (Zar 1984) was carried out to evaluate functional relationships between: (1) the amount of sediment, TOTAL C, and LABILE C excavated by crabs or deposited into burrows, and burrow or burrow mimic diameter; (2) the percentage of TOTAL C and LABILE C in sediments and burrow or burrow mimic diameter; and (3) the percentage of TOTAL C and LABILE C

and the amount of sediment (dry weight) excavated by crabs or deposited into burrows. Normality of the errors was evaluated using the Shapiro–Wilk test (Zar 1984). The assumptions of linearity and homoscedasticity were graphically evaluated. Transformation [$y = \log(x + 1)$] was only necessary to linearize the relationship between TOTAL C and the amount of sediment excavated by crabs.

Areal Estimates of Excavation, Burial and Net Transport to the Surface. Randomization and resampling—a computer-based method that obtains estimates of the mean and standard deviation of particular variables using random combinations of relevant field measures (Manly 1998)—was used to obtain per unit area estimates of (1) the amount of sediment, TOTAL C, and LABILE C excavated by crabs or deposited into crab burrows and, (2) the net transport of sediment, TOTAL C, and LABILE C to the marsh surface due to burrowing crabs (10,000 iterations in all cases).

Per unit area estimates of the amount of sediment, TOTAL C, and LABILE C excavated by crabs or deposited into crab burrows were obtained by randomization and resampling procedures combining: (a) data on the amount of material excavated from individual burrows (and deposited in burrow mimics) of different size classes (that is, 1–6 cm entrance diameter); and (b) data on the density of burrows of each size class in the field sampling plots. Estimates of total excavation by crabs (and deposition within burrows) at each sampling plot were obtained by assigning values of crab excavation (and deposition within burrow mimics) to each of the burrows observed in the sampling plot. Such an assignment was carried out by randomizing and resampling measures of excavation by crabs (and deposition within burrow mimics) observed in burrows (and mimics) pertaining to the same size class as the burrow in question.

Per area unit estimates of the net transport of sediment, TOTAL C, and LABILE C to the marsh surface due to burrowing crabs were obtained by first estimating the balance between excavation and deposition in individual burrows. Values for excavation and deposition obtained from burrows and mimics of the same size class (that is, 1–6 cm entrance diameter) were randomly combined and the difference was calculated. We then assigned these estimates to each of the burrows in the field sampling plots. Such an assignment was carried out by randomizing and resampling estimates of the difference between excavation and deposition corresponding to burrows of the same size class for each of the burrows in the sampling plots.
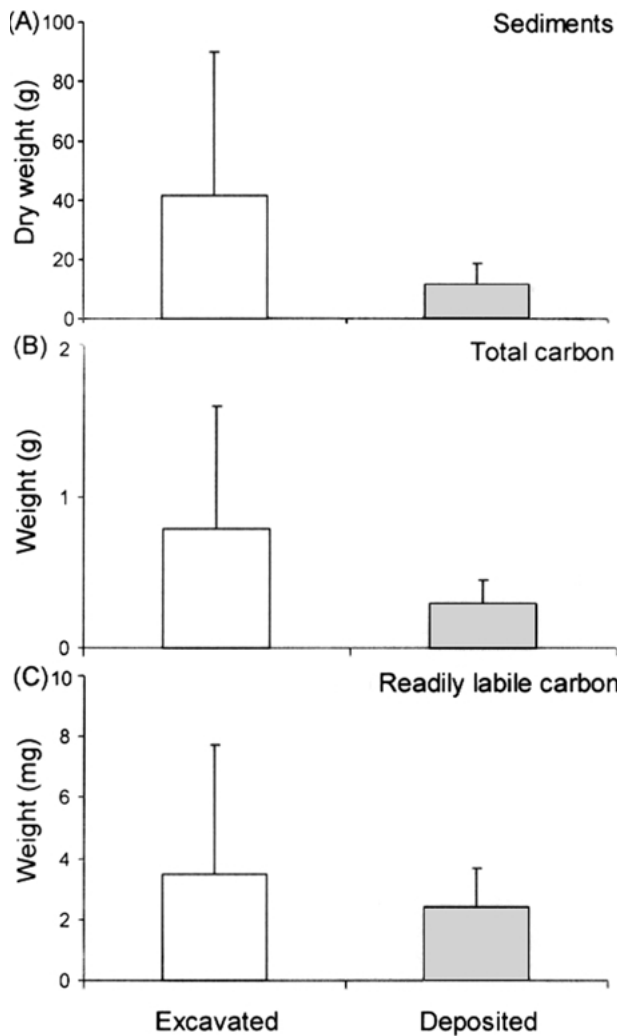
Figure 1. Mean (± SD) amount of **A** sediment, **B** total carbon, and **C** readily (10 d) labile carbon excavated by crabs and deposited in burrow mimics during the 5-d study period. $P < 0.05$ in all cases (t-tests adjusted for unequal variances).

RESULTS

The amount of sediment (dry weight) excavated from individual crab burrows ranged between 0 and 90.77 g d^{-1} (Mean = 9.02, SD = 16.09), whereas the amount of sediment deposited in individual burrow mimics ranged between 0 and 17.6 g d^{-1} (Mean = 3.87, SD = 5.14). The percentage of burrows where sediment excavation was observed at each sampling date ranged between 30.55 and 75.0% (Mean = 50.55, SD = 15.88). The amount of sediment excavated by crabs was significantly greater than the amount of sediment deposited into crab burrows during the 5-d measurement period (t-test: $t = 3.76$, $df = 70$, $P < 0.01$; Figure 1A).
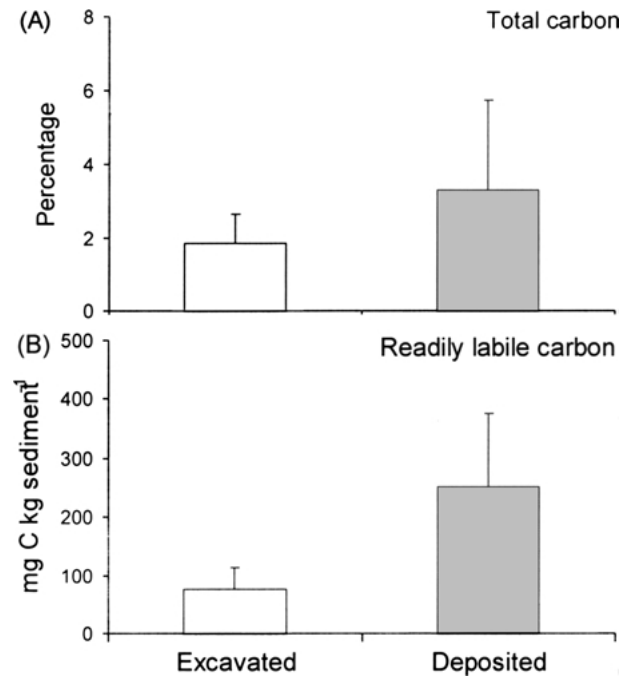

Figure 2. Mean (± SD) proportion of **A** total carbon, and **B** readily labile carbon in the sediments excavated by crabs and deposited in burrow mimics during the 5-d study period. $P < 0.01$ in both cases (t-tests adjusted for unequal variances).

Sediments deposited into burrows had a significantly higher C content than sediment excavated by crabs, for both TOTAL C (t-test: $t = 3.44$, $df = 41$, $P < 0.01$; Figure 2A) and LABILE C (t-test: $t = 8.67$, $df = 40$, $P < 0.01$; Figure 2B). However, the amount of C excavated by crabs was significantly greater than the amount of C deposited into their burrows during the 5-d period, for both TOTAL C (t-test: $t = 3.65$; $df = 38$, $P < 0.01$; Figure 1B) and LABILE C (t-test: $t = 1.39$; $df = 41$, $P < 0.05$; Figure 1C).

The amount of sediment (dry weight), TOTAL C, and LABILE C excavated by crabs during the 5-d study period, as well as the percentage of TOTAL C and LABILE C in these sediments, were not correlated with burrow diameter ($r^2 < 0.05$, $df = 35$, $P > 0.05$, all cases). Similarly, the amount of sediment (dry weight), TOTAL C, and LABILE C deposited in burrow mimics during the 5-d period, and the percentage of TOTAL C and LABILE C in these sediments were not correlated with the diameter of burrow mimics ($r^2 < 0.05$, $df = 35$, $P > 0.05$, all cases). There was no correlation between the amounts of sediment excavated from burrows and its C content (both TOTAL C and LABILE C; $r^2 < 0.01$, $df = 35$, $P > 0.05$ in both cases). However, there was a negative relationship between the amount of sediment collected within
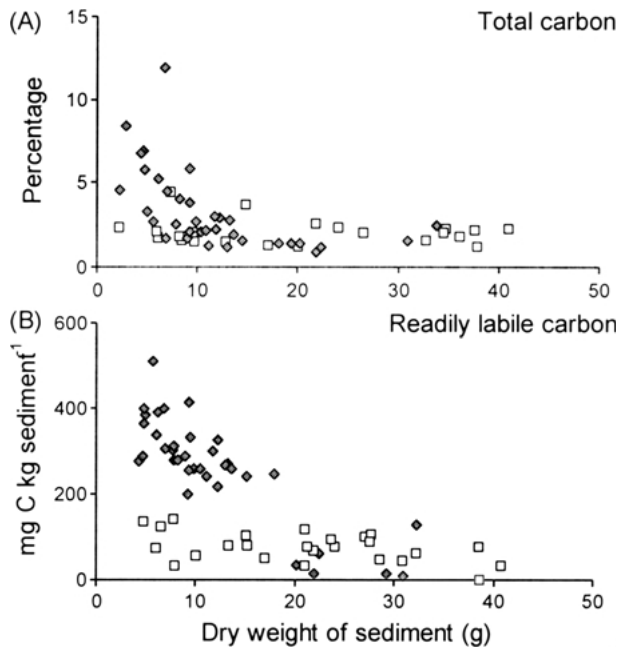
Figure 3. **A** Total carbon (%), and **B** readily (10 d) labile carbon (mg C kg sediment^{-1}) as a function of the dry weight of sediments excavated by crabs (empty squares) or deposited into burrow mimics (gray diamonds) during the 5-d study period. For clarity, points corresponding to values of excavation exceeding 50 g were not plotted, although they were considered in the correlation analysis.

Figure 4. Frequency distribution of crab burrow sizes pertaining to the size classes considered in this study ($n = 308$). Size classes are defined as the average of the maximum and minimum burrow entrance diameters rounded down to the nearest centimeter. Sampling encompassed only burrows from 10 to 69 mm diameter because 1 cm was the smaller entrance diameter observed in the field—recently settled crabs occur in association with adult burrows—and burrows larger than 7 cm were less than 0.5% of the total.

burrow mimics and its percentage of C for both TOTAL C ($r^2 = 0.40$, $df = 35$, $P < 0.01$) and LABILE C ($r^2 = 0.70$, $df = 35$, $P < 0.01$). The relationship between percent TOTAL C in sediments and the amount of sediments collected from burrow mimics was best described by a logarithmic function ($y = -5.58 \ln x + 20.25$; Figure 3A), whereas the relationship between percent LABILE C in sediments and the amount of sediments collected from burrow mimics best fit a linear model ($y = -13.43 x + 423.78$; Figure 3B).

Based on the observed burrow density (69.84 burrows m^{-2}; SD = 35.57) and size frequency distribution (Figure 4) we estimated that, on an areal basis, crab burrowing led to net transport of sediment, TOTAL C, and LABILE C to the marsh surface (Table 1). The net transport of sediment and TOTAL C to the surface (that is, excavation minus deposition into burrows) represented 53 and 44% of the total sediment and carbon translocated (that is, deposited plus excavated) by crabs, respectively. On the other hand, the net transport of LABILE C to the surface was estimated to be only 6% of the labile C translocated by crabs (Figure 5).
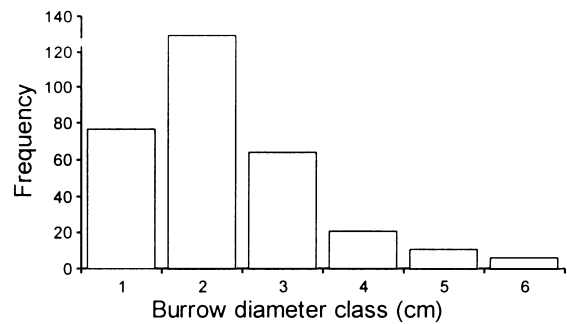
DISCUSSION

The results of this study lead to the following conclusions:

1. Crab excavation and particle deposition within crab burrows collectively leads to net excavation of both TOTAL C and LABILE C.
2. Crab activities are expected to decrease the concentration of TOTAL C and LABILE C at the marsh surface because of the lower content of TOTAL C and LABILE C in excavated sediments relative to those deposited in crab burrows.
3. Crab burrows and burrowing collectively lead to a decrease in the ratio of LABILE C to TOTAL C on the marsh surface because of a larger proportion of LABILE C to TOTAL C in sediments deposited into burrows relative to excavated sediments.

The latter, however, is likely of little significance for the overall decomposability of sedimentary organic carbon at the marsh surface and, thus, for its probability of tidal export, because LABILE C constitutes less than 1% TOTAL C both in sediments excavated by crabs and deposited in burrow mimics. Consequently, the subsequent discussion focuses on the first two conclusions and their implications.

Net C Excavation by Crabs

The amount of TOTAL C and LABILE C excavated by crabs was larger than the quantity collected in

Table 1. Excavation, Deposition into Burrows and Net Transport to the Surface of Sediments and C due to the Burrowing Activity of the Crab *Chasmagnathus granulatus*

	This study[a]	Montague (1982)
Crab density (burrows m^{-2})	69.84 (35.57)	88 (5)[b]
Sediment excavated (g m^{-2} d^{-1})	547.08 (11.53)	6.07 (0.17)
Sediment deposited into burrows (g m^{-2} d^{-1})	172.79 (3.01)	–
Net sediment transport to the surface (g m^{-2} d^{-1})	380.79 (10.13)	–
C (total) excavated (g m^{-2} d^{-1})	10.28 (0.06)	0.65 (0.02)
C (total) deposited into burrows (g m^{-2} d^{-1})	4.15 (0.02)	0.08 (0.01)
Net C (total) transport to the surface (g m^{-2} d^{-1})	5.98 (0.15)	0.57[b]
C (readily labile) excavated (mg m^{-2} d^{-1})	42.57 (0.82)	–
C (readily labile) deposited into burrows (mg m^{-2} d^{-1})	37.24 (0.66)	–
Net C (readily labile) transport to the surface (mg m^{-2} d^{-1})	4.80 (0.18)	–

Net transport estimates are from randomization and resampling (see Methods). Results from a previous study (Montague 1982) on the effects of the burrowing activity of fiddler crabs Uca pugnax in Airport Marsh (Sapelo Island, GA) are included for comparative purposes.
[a]*Net transport estimates are not exactly the difference between the values of excavation and deposition into burrows because estimates of the rates of each variable per area unit were obtained from different resampling trials.*
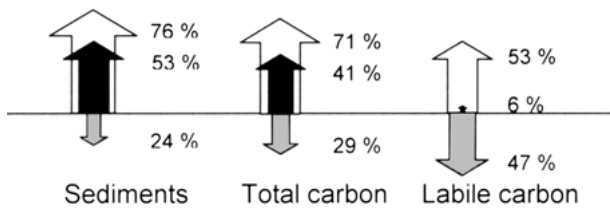[b]*Burrows larger than 10 mm diameter only.*

Figure 5. Sediment, total carbon, and readily (10 d) labile carbon excavated by crabs (*empty arrows*), deposited in burrows (*gray arrows*), and the balance between both processes (*black arrows*). Data are the mean values shown in Table 1, but for comparative purposes they are expressed as a percentage of the mean amount deposited in burrows plus the mean amount excavated by crabs (that is, total sediment translocated). *Arrows* pointing upwards indicate removal from burrows, whereas *arrows* pointing downwards indicate addition into burrows. Net transport percentages are not exactly the difference between the values of excavation by crabs and deposition in burrows because excavation, deposition and net transport per area unit were estimated in separate randomization and resampling trials.

burrow mimics (Figure 1). This suggests that burrowing crabs can affect the function of salt marshes and the C balance of estuarine ecosystems by releasing C that otherwise could remain more deeply stored in the marsh sediments. In fact, given the anaerobic conditions that usually characterize subsurface salt-marsh sediments (Adam 1990) and the limited efficiency of anaerobic microorganisms in the hydrolysis of structurally complex macromolecules (Young and Frazer 1987; Kristensen and others 1995), it is often hypothesized that salt marshes may function as long-term sinks for sedimentary organic C derived from the belowground

plant biomass or contributed by sedimentation and vertical marsh accretion (for example, DeLaune and others 1981; Good and others 1982). Excavation by crabs, however, is likely an efficient mechanism for the transport of C to surface sediment layers, where opportunities for aerobic decomposition and/or tidal transport are higher (see also De la Cruz and Hackney 1977; Montague 1982).

To what degree is net C excavation by crabs likely a typical feature of the marsh? On a *per burrow* basis, the magnitude and direction of the balance between C excavation by crabs and C deposition within burrows is expected to depend primarily on tidal regime and seasonal crab activity. This study was conducted during a period of spring tides with daily flooding of the low marsh. C deposition within burrows by tides would be expected to be negligible during periods of neap tide (that is, no tidal flooding and no sediment transport), or higher than that observed in this study during periods of precipitation runoff (that is, higher sediment transport and higher particle deposition into burrows). Crab excavation also decreases as the neap-tide periods advances and the sediments become progressively dryer and harder (J. Gutiérrez, personal observation). This study was conducted during summer, when crabs are the most active. However—as we earlier mentioned—crabs are less active during the cold, winter months, mostly remaining within their burrows (see Delhey and others 2001). Because the burrows of *C. granulatus* remain open during the winter months, the deposition of C within burrows would likely exceed C excavation during this period. Data on both tidal regime and temperature-driven vari-

ation in crab activity would therefore be needed to estimate annual C excavation. These same variables would also be needed to estimate the role of *C. granulatus* in controlling C availability in salt marshes of different elevations, and along its distributional range (subtropical microtidal Southern Brazil to cold-steppe macrotidal estuaries of Northern Patagonia, Argentina; Boschi 1964).

Net excavation of C by *C. granulatus* is consistent with the findings of Montague (1982) for *Uca pugnax* in a Georgia (USA) salt marsh. However, the net amount of excavated sediments and C was two orders of magnitude greater in our study system (Table 1). The large differences in net sediment and C excavation between the two study systems cannot be explained by differences in burrow density [70 burrows m^{-2} (this study) versus 88 burrows m^{-2} (Montague 1982); see Table 1]. The differences can however be explained from the *per capita* excavation rates of *C. granulatus* versus *U. pugnax*. *C. granulatus* excavated a mean of 9.02 g dry sediment burrow^{-1} d^{-1}, almost two orders of magnitude larger than the value observed by Montague (1982) for *U. pugnax* (0.13 g burrow^{-1} d^{-1}). This is not surprising given that *C. granulatus* (4 cm maximum carapace width; Boschi 1964) is larger than *U. pugnax* (2.3 cm maximum carapace width; Williams 1984) and constructs larger burrows [*C. granulatus*: up to 7 cm diameter, 1 m depth and 1 L volume (Iribarne and others 1997); *U. pugnax*: up to 3 cm diameter, 0.25 m depth and 0.25 L volume (Bertness and Miller 1984; Bertness 1985)]. Nevertheless, it is important to note that the values of density and *per capita* excavation rates reported by Montague (1982) for *U. pugnax* correspond only to large, adult crabs (that is, burrows larger than 10 mm diameter), while juveniles of this species can occur at densities as high as 500 ind m^{-2} (see Montague 1980 for a review). It is uncertain, however, whether juveniles could substantially contribute to sediment excavation in this species. It is also known that the youngest crab stages in *U. pugnax* (that is, less than 3 mm carapace width) do not construct burrows and that subsequent juvenile stages construct only shallow burrows (Montague 1980); all of which suggests that their *per capita* contribution to excavation will be smaller than that of adults.

Decrease in C Availability at the Marsh Surface Due to Crab Activities

The sediment excavated by crabs had a lower percentage of TOTAL C and LABILE C than the sediment collected by burrow mimics (Figure 2). Therefore, crab effects on C availability at the marsh surface can be viewed as a combination of inputs of sediments of low C content and outputs of sediments of larger C content. This is expected to result in an overall decrease in the concentration of C at the superficial sediment layers and, thus, in the amount of C that can be washed out from the marsh by tidal action. In such a manner, burrowing crabs would affect the function of salt marshes as donors of sedimentary organic C to adjacent waters.

Carbon excavation by crabs is likely to largely determine the C content of surface marsh sediments. Considering the mean area covered by individual mounds of sediment excavated by crabs during 24 h (that is, 110 cm^{-2}; J. Gutiérrez, personal observation), the mean density of burrows (that is, 70 burrows m^{-2}; this study), and the proportion of burrows where excavation occurs during a period of 24 h (that is, 30–75%; this study), we estimate that crabs can cover between 23 and 58% of the marsh surface with the sediments they excavate in just 1 day (that is, assuming no areal overlap between excavated sediment mounds). This suggests that the marsh surface would be completely covered by sediments recently excavated by crabs and that new inputs to the marsh via sedimentation—usually less than 1.5 cm y^{-1} and often much less (Mitsch and Gooselink 1993)—would be rapidly mixed and diluted by the large amounts of sediments excavated by crabs (that is, 547.08 g m^{-2} d^{-1} in this study). Based on a bulk density of 0.3–0.6 g cm^{-3} (J. Gutiérrez, personal observation) this would result in an equivalent deposition rate of 0.18–0.36 cm d^{-1}, substantially large relative to inputs via sedimentation. Accordingly, the value of mean TOTAL C obtained in this study for the sediments excavated by crabs (that is, 4.53%) was similar to that of surface (0–3 cm depth) sediments during the same period (that is, 4.38%; J. Gutiérrez, personal observation).

In the above context, the fact that sediments deposited in burrow mimics have higher C content than those that crabs excavate and deposit on the marsh surface suggests that surface sediments are not being unselectively transported into the burrows as bedload tidal transport but that mechanisms favoring the deposition of C into burrows might be operating. Deposition of C-enriched sediments within burrows would be expected as a consequence of selective transport of low-density organic particles at the substrate surface by the gentle flow conditions that characterized the marsh during the study period (that is, no storm or rainwater runoff). Alternatively, such C could derive from *C. granulatus* feces. Individual crabs kept unfed

in aquaria during 12 h produce between 0 and 3.7 g (dry weight) feces (Mean = 1.02, SD = 0.98; F. Botto, unpublished data). Considering that crabs remain in their burrows when the marsh is uncovered at low tide, a significant fraction of fecal output could be released inside burrows. Irrespective of mechanism, it seems unlikely that C-enriched sediment deposits into burrows in a quantity similar to the amount of C excavated by crabs. The negative relationship between the amounts of sediment collected within burrow mimics and its percentage of both TOTAL C and LABILE C (Figure 3) indicates that C-rich sediment inputs to burrows are relatively small and subject to dilution by larger inputs of C-poor sediments.

C Excavation by Crabs and C Deposition within Burrows Compared with Plant Production

The importance of crab burrows and burrowing to the redistribution of C in marsh sediments can be better appreciated when estimates of C excavation by crabs and C deposition within burrows are juxtaposed with available data on production by marsh plants. Our estimates of the daily amount of sedimentary organic C collected in crab burrow mimics (approximately 10 g m^{-2} d^{-1}; Table 1) are larger than estimates of litter production by *Spartina densiflora* (2–6 g m^{-2} d^{-1} at the Otamendi Natural Reserve, Argentina, 32°10′S; Vicari and others 2002). This suggests that crab burrows might be major (although possibly short-term) sinks for C derived from aboveground plant tissues. Similarly, the daily amount of C excavated by crabs (approximately 10 g m^{-2} d^{-1}; Table 1) represents between 0.2 and 5% of annual estimates of belowground production for *Spartina* species, which range between 0.2 and 6.2 kg m^{-2} y^{-1} (Good and others 1982; unfortunately, there are no estimates of belowground production by *S. densiflora* that would allow for a more specific comparison). At this stage, however, we refrain from attempting a quantitative estimation of the proportion of belowground production that is annually excavated by crabs because we cannot provide good estimates of seasonal- and tide-related weekly variation in crab activity and excavation rates, as noted above (see *Net C excavation by crabs*).

Potential Control of C Export from Marshes by Ecosystem Engineers

The role of burrowing crabs as controllers of C availability in surface salt marsh sediments indicates that marsh organisms could significantly influence the export of C from salt marshes via mechanisms other than POM production or consumption. Both the deposition of C in crab burrows and the excavation of C by burrowing crabs are good examples of how ecosystem engineering by organisms could affect the export of materials from salt marshes. There are other engineering activities that likely affect the function of salt marshes as sources or sinks of C in estuarine ecosystems. In the particular case of burrowing crabs, other engineering mechanisms that are potentially relevant to export include the diffusion of oxygen through burrow walls into anaerobic marsh sediments and its concomitant enhancement of in situ microbial decomposition (Taylor and Allanson 1993; Gribsholt and others 2003), the burial of plant litter under the sediments excavated from burrows (Takeda and Kurihara 1987) and—possibly—the deposition of macrodetritus into crab burrows due to active transport by crabs, which is known as an important mechanism controlling the export of organic matter from mangrove ecosystems (for example, Robertson 1986; Twilley and others 1997). In addition, organisms other than burrowing crabs may well have important effects on the export of C from salt marshes. For example, foraging geese can bury significant amounts of litter by trampling, reducing its exposure to tidal flows (for example, Zacheis and others 2001). Marsh plants oxidize the sediments in which they grow through both active metabolic processes and passive oxygen release by their roots (Armstrong 1979) and—as with crab burrows—would be expected to have an important impact on the rates of in situ decomposition. Furthermore, marsh plants retain an important amount of detached plant material in their canopies (that is, preventing its contact with marsh sediments; Newell and others 1998), which may also have important consequences in terms of their retention times and decay rates. All these examples illustrate potentially important contributions of ecosystem engineers to C export from salt marshes. Given the likely ubiquity of marsh ecosystem engineering, incorporating the activities of ecosystem engineers in current models of marsh export might lead to a more complete understanding of the function of marshes in the fluxes of materials in estuarine ecosystems.

APPENDIX: VALIDATION OF BURROW MIMICS

The validity of using bottom-capped PVC pipes as mimics of crab burrows depends on: (1) whether pipes are colonized by crabs in the same proportion as crabs occupying burrows; (2) whether crabs are able to go in and out from mimics as they do in burrows; and (3) whether crabs remain in mimics as often and as long as they do in burrows. These factors were evaluated as follows:

(1) We introduced 36 PVC burrow mimics (1–6 cm diameter; 6 replicates each) and we made 36 holes in the sediment of the same size and shape as the mimics. Concurrently, we sampled presence/absence of crabs in 50 burrows. After 24 h, we found crabs in 30 out of 36 mimics (83.33%) and 31 out of 36 holes (86.11%; Chi-square test: $\chi^2 = 0.23$, $P > 0.05$). This was consistent with the percentage of natural burrows occupied by crabs in the field (that is, 43 out of 50; 86%).

(2) Crabs were introduced into 12 PVC burrow mimics (2 and 4 cm diameter, 6 replicates each) and 12 holes of the same size and shape as mimics made in the sediments. The water at the bottom of the mimics and holes was agitated with an L-shaped wire stake and the escape response of crabs was recorded. Crabs were equally able to climb out from burrow mimics and holes in response to agitation. In both treatments, all crabs reached the substrate surface in less than 10 s from the start of agitation.

(3) We deployed twelve burrow mimics (2 and 4 cm diameter, 6 replicates each) and we made 12 holes of the same size and shape as the mimics. Twenty-four crabs were labeled on the carapace with an indelible marker and one crab was introduced into each of the mimics and holes. After 24 h we found the same number of crabs remaining in mimics as in holes (Chi-square test: $\chi^2 = 0.50$, $P > 0.05$). Two out of 12 of the marked crabs were recovered in both PVC mimics and holes. Of the remaining mimics and holes we found either other crabs (Mimics: 5 out of 12, Holes: 6 out of 12) or no crabs (Mimics: 5 out of 12, Holes: 4 out of 12).

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