

Fiddling with the carbon budget: fiddler crab burrowing activity increases wetland's carbon flux

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Abstract

Coastal wetlands store significant amounts of carbon through sequestration. Salt marshes are also known to harbour high densities of crabs, which increase the sediment-atmosphere exchange interface through their burrowing behaviour. We hypothesized that this additional and reactive interface area could mediate gas exchange and, ultimately, could influence carbon sequestration. CO₂ fluxes were measured over patches characterized by different densities of fiddler crab, *Minuca pugnax*, burrows within a natural salt marsh located on the coast of Massachusetts (USA). Even accounting for the importance of ecological factors such as differences in organic matter content of the soil and presence of *Spartina alterniflora*, we demonstrated that CO₂ release increased if local crab burrow density is considered. The increase in vertical CO₂ fluxes linked to burrow density was higher for the non-vegetated areas with respect to *S. alterniflora* patches. By means of burrow casting and morphological analyses of the burrows, we could relate this difference in CO₂ fluxes to structural differences of the burrows themselves, which were larger and deeper in the non-vegetated areas. Our results strongly emphasize the importance of including the faunal component, and specifically the dominant burrowing species, in carbon budget assessments for vegetated coastal habitats. This study also emphasizes the critical role of community-scale factors within the salt marsh, which are often overlooked, for large scale carbon budget assessments.

KEYWORDS

Coastal wetlands, carbon budget, *Minuca pugnax*, bioturbation, CO₂ fluxes, burrow casts, climate change

1. INTRODUCTION

Vegetated coastal ecosystems, such as salt marshes, mangroves and seagrass beds, have been increasingly recognized for their importance in storing carbon (Donato et al., 2011; Tomohiro & Masakazu, 2019a). Their capacity for carbon sequestration and their potential for long-term carbon, or ‘blue carbon’, storage render these shallow water habitats valuable assets to mitigating climate change (Howard et al., 2017). It has been recently estimated that, although they only occupy 0.2 % of the total ocean surface, they are responsible for 50% of the total carbon storage in marine environments, with salt marshes accounting for 46.9% of such total carbon burial (Duarte, Losada, Hendriks, Mazarrasa, & Marbà, 2013). Estimates of the amounts, fluxes and dynamics of blue carbon storage per year, however, are still poorly constrained, due to the relatively limited amount of field measurements and modelled data (Hayes et al., 2018). One of the main uncertainties related to blue carbon estimates is indeed the lack of information on the role of biotic and abiotic factors in carbon cycling of coastal systems, an issue highlighted in the Second State of the Carbon Cycle Report, which called for novel knowledge regarding “the uncertain role of tidal wetlands, estuaries, and the coastal ocean in the continental budget”(Hayes et al., 2018). As an example, the crucial bioturbation activities of crabs, polychaetes and molluscs populations dominant in coastal wetlands are often neglected in studies addressing carbon dynamics (Huhta, 2007; Kristensen et al., 2012).

Due to their abundance, their feeding behaviour and bioturbation activities, brachyuran crabs are known to be a critical biotic factor affecting the carbon cycles of vegetated coastal marsh community (Kristensen, Bouillon, Dittmar, & Marchand, 2008; Martinetto, Montemayor, Alberti, Costa, & Iribarne, 2016). Brachyuran crabs are important ecosystem engineers that significantly modify their habitat, as they directly or indirectly affect soil biogeochemical processes through their surface feeding behaviour, food-storing behaviour and the construction and maintenance of burrows (Alberti et al., 2015; Andreetta et al., 2014;

Aschenbroich et al., 2016; Kristensen & Alongi, 2006; Robertson, 1986). Their burrows are known to allow oxygen to penetrate deep sediment layers, otherwise hypo- or anoxic, and create an extension of costal marshes sediment-air interface (Kristensen, 2008; Smith III, Boto, Frusher, & Giddins, 1991). From such newly created surface, carbon dioxide (CO₂) can be released to the atmosphere at low tide, by the decomposition of the soil organic matter as well as from faunal, root and culm respiration (Howes, Dacey, & Teal, 1985; Martinetto et al., 2016; Morris & Whiting, 1986; Pölmann, Diele, Mehlig, & Nordhaus, 2014; Raich & Potter, 1995; Tomohiro & Masakazu, 2019a). Ultimately, the previously buried and anoxic organic matter is oxygenated, promoting microbial decomposition and increasing the release of CO₂ by up to 70% (Gribsholt, Kostka, & Kristensen, 2003a; Otani et al., 2010).

The significant role of the crab *Neohelice granulata* for soil carbon dynamics has been extensively studied in South West Atlantic salt marshes (Alberti et al., 2015; Martinetto et al., 2016). The bioturbation activities of this dominant crab proved to increase the export of CO₂ and dissolved organic carbon to the water column (Fanjul et al., 2015; Fanjul, Grela, Canepuccia, & Iribarne, 2008), but no information on their effect on the release of CO₂ to the atmosphere is available. Moreover, brachyuran crab influence on biogeochemical fluxes of North West Atlantic (NWA) marshes have been mostly overlooked. In North American coastal wetlands, bioturbation by brachyurans, such as various species of fiddler crabs (family Ocypodidae) and the purple marsh crab (*Sesarma reticulatum*), is one of the most prominent biotic factors affecting the community. Fiddler crabs of the genera *Minuca* and *Leptuca* often outnumber *S. reticulatum* in NWA marshes (Raposa et al., 2018; Szura et al., 2017), with burrow densities ranging from approximately 20 to 350 burrows m⁻² (Gribsholt, Kostka, & Kristensen, 2003b; Michaels & Zieman, 2013). Due to these densities, the extended surface area they create may exert a considerable effect on the vertical CO₂ fluxes, but to date very few

studies have estimated these effects in NWA marshes and none of them was performed *in situ* (Gribsholt et al., 2003b; Gribsholt & Kristensen, 2002; Taylor & Allanson, 1993).

To close this knowledge gap, the present study was designed to assess the impact of burrowing behaviour of the dominant fiddler crab, *Minuca pugnax*, on CO₂ fluxes within the salt marshes of Cape Cod, Massachusetts, USA. CO₂ fluxes were measured *in situ* in the summer of 2017 in areas characterized by different burrow densities, including no-burrow areas, using a portable gas analyser. To account for the role exerted by other abiotic and biotic factors on CO₂ fluxes, measurements were taken across patches of bare soil vs areas colonized by the marsh grass *Spartina alterniflora* and across areas characterized by high and low organic matter content in the sediment. We hypothesized that: 1) the CO₂ flux would be higher in patches where the soil is rich in organic matter, because it provides more reactant to the oxidation reaction (Howes et al., 1985); 2) the presence of *S. alterniflora* would increase the CO₂ flux, because of root and rhizome respiration (Howes et al., 1985; Morris & Whiting, 1986; Raich & Potter, 1995; Wigand, Brennan, Stolt, Holt, & Ryba, 2009), and 3) a high density of crabs burrows would increase the CO₂ flux, due to the increase in sediment-air interface. We also hypothesized an additive effect of the above three factors, since the increase in sediment-air interface due to the presence of crab burrows will further promote microbial decomposition, both under high organic matter and in the presence of *S. alterniflora* (Howes et al., 1985; Martinetto et al., 2016; Morris & Whiting, 1986; Raich & Potter, 1995; Tomohiro & Masakazu, 2019b), and, ultimately, intensify the release of CO₂.

2. METHODS

2.1 Study site

The study was performed within a natural salt marsh located at the outlet of the Herring River in Wellfleet, Cape Cod, Massachusetts, US (41°55'53.5"N 70°03'58.8"W) (Figure 1). In

121 this area, most of the salt marshes have been restricted by dikes hampering seawater flow inland
122 (Portnoy & Giblin, 1997). The study site is cut off from the Herring river estuary by the
123 Chequessett Neck dike and remains one of the few natural marshes along the estuary. These
124 marshes are colonized by the native marsh grass *Spartina alterniflora* and host a variety of
125 burrowing crabs. The dominant burrowing species found are two fiddler crabs: *Minuca pugnax*
126 and *Leptuca pugilator* (Ocypodidae) along with the purple march crab *Sesarma reticulatum*
127 (Sesarmidae) (Bertness, 1985). The two areas chosen to conduct the study, only housed *M.*
128 *pugnax* and, to a lesser extent, *S. reticulatum* (determined *a priori*). Burrows of both species
129 can be easily distinguished from one another (Bertness & Miller, 1984). *M. pugnax* burrows
130 typically have a circular opening and house one individual per burrow. Contrarily, the *S.*
131 *reticulatum* burrow openings are larger, not circular, generally show an inclination with respect
132 to the sediment surface and the burrow shape is shallower with multiple surface openings
133 (Bertness, Brisson, Bevil, & Crotty, 2014). *M. pugnax* burrows were chosen as the focus of this
134 study due to their high density throughout the marsh and appropriate burrow opening size to
135 fit within the available measuring equipment.



FIGURE 1. Location of the salt marsh sampling site (indicated with the red triangle) within the outlet of the Herring River in Wellfleet, Cape Cod, Massachusetts, US (41°55'53.5"N 70°03'58.8"W).

2.2 Experimental design and preliminary analyses

A 3-way full factorial design was developed to assess the effect of *M. pugnax* burrow density (fixed and orthogonal), *S. alterniflora* presence (fixed and orthogonal) and high vs low organic matter content (fixed and orthogonal) on CO₂ flux rates.

Two areas with different organic matter concentrations (high and low) were determined by loss on ignition method (Heiri, Lotter, & Lemcke, 2001). Sediment was dried at 65°C and incinerated at 550°C. Loss upon ignition was calculated based on dry- and ash-weight.

After we identified high and low organic matter areas, a non-vegetated patch and a *S. alterniflora* vegetated patch were selected within those areas. Densities of *S. alterniflora* shoots, *M. pugnax* and *S. reticulatum* burrows were assessed across each area and patch along 2

transects (15.24 m), parallel to the bank of the bay. Along each transect five quadrats (24.5×24.5 cm) were randomly selected and all burrows and shoots were counted. *S. alterniflora* plants heights and diameters were also measured using a measuring tape.

2.3 Burrow casting

Burrow casts were performed by pouring a polyester resin (Polymer Planet, marine grade DCPD type resin and methyl ethyl ketone peroxide, MEKP, hardener) within *M. pugnax* burrow openings. PVC collars were set-up along two 7.62 m transects within each patch of each area. Burrows used for burrow casting were not used for flux measurements due to time constraint of performing measurements and casting during the same day. For each cast, the following parameters were measured in the lab: burrow depth, burrow wall surface area and volume. Burrow depth was measured as the orthogonal distance from the burrow opening to the deepest part of the cast using a measuring tape. Burrow cast surface area was determined by wrapping a tape with known width around the cast, so that it was completely covered, making sure the tape did not overlap itself. The cast surface area was hence assessed by calculating the surface area of the tape based on its length and diameter (Bartolini et al., 2011). Burrow cast volume was estimated from the height of water displacement when placing the cast within a cylinder with known diameter. Burrow cast opening diameter of each retrieved cast was measured by using a Vernier calliper.

2.4 Flux rate measurements

To account for spatial heterogeneity and internal variability, two replicate transects, 7.62 m long, were selected within each patch in each area and two replicate measurements of CO₂ fluxes were performed along each transect, for each burrow crab density level defined in the design. The crab burrow density treatments were chosen based on the different densities

recorded in the study areas and on minimum and maximum amount of active crab burrows that fit within the measuring PVC collars. Thus, burrow density levels were no burrows, 1 burrow and three burrows per collar, corresponding to 16.66 burrows m⁻² and 49.98 burrows m⁻², respectively. Active burrows were identified based on the presence of an individual inside and well-maintained burrow openings. A minimum distance of 30 cm was kept from *S. reticulatum* burrows to assure no interference with the measurements. Replicate measurements along each transect were placed at an average of 116 cm from each other, and treatments did not cross one another. Two replicates for each crab burrow density were measured every week for four consecutive weeks within each area (high or low organic matter) for each patch (vegetated vs non-vegetated) along each transect (within each patch). A total of 12 replicates for each crab burrow treatment were measured with 144 measurements taken in total. For each flux measurement that included burrows, the burrows opening surface area was assessed. The maximum and minimum burrow opening diameter of the elliptical opening was measured prior to flux measurements within each chamber that contained a burrow. Additionally, the height and diameter of any *S. alterniflora* present was measured. All measurements were done using a measuring tape.

PVC collars (10.16 cm in diameter) were slightly pushed into the sediment so that the collar and chamber were completely sealed off when measuring gas flux. Extra attention was made when placing the collars to ensure burrow entrances were not damaged or the burrow was not cut off from the opening. Measurements were made by connecting a 6.3 cm tall × 11.4 cm diameter non-transparent chamber, with a fan installed inside to keep air circulation to an LGR ultraportable CO₂, CH₄ and H₂O Analyzer (LGR Corp, CA, USA). The chamber was placed on top of the PVC collars. Pressure equilibration occurred through a 6.51 m long and 0.4572 cm inner diameter plastic tubing connected the chamber and in contact with the atmosphere for an average period of 5 minutes before starting a new measurement. Carbon dioxide flux

measurements lasted 5 minutes per treatment (with approximately 10 second sampling intervals), based on observed periods for linear rates of gas concentration change and to avoid excessive chamber warming (Brannon et al., 2016; Martin & Moseman-Valtierra, 2015). The gas flux was determined by the following formula:

$$F=(dc/dt) \times (1/V_0) \times (P/P_0) \times (T_0/T) \times (V/S) \quad (1)$$

where F is the flux rate, dc/dt is the slope of the CO₂ concentration vs. time, V₀ is the CO₂ molar volume under standard conditions (i.e., 22.4 L mol⁻¹), P is the air pressure at our study site, P₀ is the standard air pressure, T is the air temperature during each measurement, T₀ is the standard temperature, and V is the head space volume of chamber, including the tubing volume and average volume of burrows determined in a separate assessment, S is the soil surface area and inner burrow wall surface area within the collar. Only flux data with coefficients of determination (r²) of the linear regression higher than 0.95 were used. The calculation was conducted in Matlab (MATLAB and Statistics Toolbox Release 2016a, The MathWorks, Inc., Natick, Massachusetts, United States), using a code developed by Eckhardt and Kutzbach (2016). Measurements were made an hour after the collars were placed (Pülmanns et al., 2014) and plants were cut off at their base in order to avoid CO₂ release due to the set-up.

The additional volume created by crab burrows within each measurement was accounted for during gas flux calculations. The frequency distributions of the minimum and maximum burrow diameter of the casts sampled within the non-vegetated and vegetated patches, respectively, overlapped with the maximum burrow diameter of burrows used during CO₂ measurements. Thus, our casts were an accurate representation of the burrows used within the CO₂ flux measurements and their approximated volume was calculated based on the average

volume of the collected casts. Average volumes were calculated separately for non-vegetated and vegetated patches, due to very different burrow cast parameters.

Following environmental factors were monitored after each single gas flux measurement: Soil temperature and soil water-filled pore space (ProCheck soil moisture meter, Decagon Devices, Inc. Pullman WA, USA) and pH and redox (Spectrum FieldScout SoilStik pH meter, and electrode meter, Spectrum Inc. Aurora IL, USA).

2.5 Calculations

The total burrow wall surface area per m² was calculated within each area and patch by multiplying the average burrow wall surface area of casts within either non-vegetated and vegetated patches with the average burrow density measured for each area and patch.

The burrow opening surface area taken from flux measurements was used to calculate the total surface area of burrow opening per m² across each area and patch. This provided a larger dataset than using burrow cast opening measurements. Calculation were made by multiplying the average burrow opening surface area with the average burrow density measured for each area and patch (area × patch).

Within each area and patch, the CO₂ flux increase due to burrow presence was calculated as follows:

$$\text{CO}_2 \text{ flux increase due to crab presence} = (\text{Av. CO}_2 \text{ flux}_{\text{burrow}}) \times \text{Burrow Density}_{\text{area} \times \text{patch}} \quad (2)$$

While the CO₂ flux measured over plane sediment, therefore disregarding burrow presence was calculated as follows:

$$\text{CO}_2 \text{ flux disregarding burrow presence} = \text{Av. CO}_2 \text{ flux plane sediment}_{\text{area} \times \text{patch}} \quad (3)$$

2.6 Statistical analysis

Two-way full factorial Permutational analysis of variance (PERMANOVA, Anderson, 2001) designs, all based on Euclidian distances of Log ($X + 1$) transformed data, were performed to test the null hypothesis of no differences between areas and patches in (1) organic matter content, (2) *M. pugnax* burrow density, (3) *S. alterniflora* shoot density, (4) measured burrow opening surface area and (5) calculated burrow opening surface area.

Three-way full factorial PERMANOVA designs, based on Euclidian distances, were performed to test the null hypothesis of no differences in (1) the recorded environmental variables, (2) in surface area of burrow openings and (3) calculated and measured CO₂ fluxes across high vs low organic matter content areas, non-vegetated and vegetated patches and burrow densities (three levels). Environmental parameters were normalised prior the analysis, while all other considered variables were log transformed. Regression analysis was carried out to determine the effect of burrow surface area opening on the carbon flux. Diagnostic Plots for Linear Regression Analysis helped to identify any influential outliers based on Cook's distance, which were subsequently removed. All PERMANOVA tests were performed using PRIMER 7 and PERMANOVA+ routine, were based on 9999 permutations and the PERMDISP test was used to test the homogeneity of multivariate dispersions. Graphs were constructed in R (R Core Team 2017).

3. RESULTS

3.1 Preliminary analyses

Preliminary surveys and analyses confirmed a strong difference in organic matter content between the patches we selected as representative of rich vs poor organic matter content areas ($F = 400.59$, $df = 1$, $P < 0.001$, ANOVA test), regardless of the presence of *S. alterniflora*. On the other hand, there was no difference in terms of *S. alterniflora* shoot density between the

two groups of vegetated patches we selected in high vs low organic matter content areas ($F = 1.34$, $df = 1$, $P = \text{NS}$, ANOVA test). Densities of purple marsh crab, *S. reticulatum*, burrow did not differ between rich vs low organic matter content patches and vegetated vs non-vegetated areas ($F = 0.21$, $df = 1$, $P = 0.65$ and $F = 0.83$, $df = 1$, $P = 0.37$, ANOVA test for rich vs low organic matter and vegetated vs non-vegetated patches, respectively). Thus, we could proceed to apply a full-factorial design to analyse the impact of different burrow densities across such environmental factors.

The density of *M. pugnax* burrows was neither statistically different between areas characterized by high vs low organic matter content ($F = 0.99$, $df = 1$, $P = \text{NS}$, ANOVA test) nor between vegetated vs non-vegetated patches ($F = 1.98$, $df = 1$, $P = \text{NS}$, ANOVA test). Within those areas we observed a large variability among patches, with burrow densities comprised between $33.32 \pm 26.04 \text{ m}^{-2}$ and $54.98 \pm 30.47 \text{ m}^{-2}$. At all sites, the burrows of *M. pugnax* were from a maximum of five times to minimum of two times more abundant than *S. reticulatum*, showing to be the dominant species in these marshes.

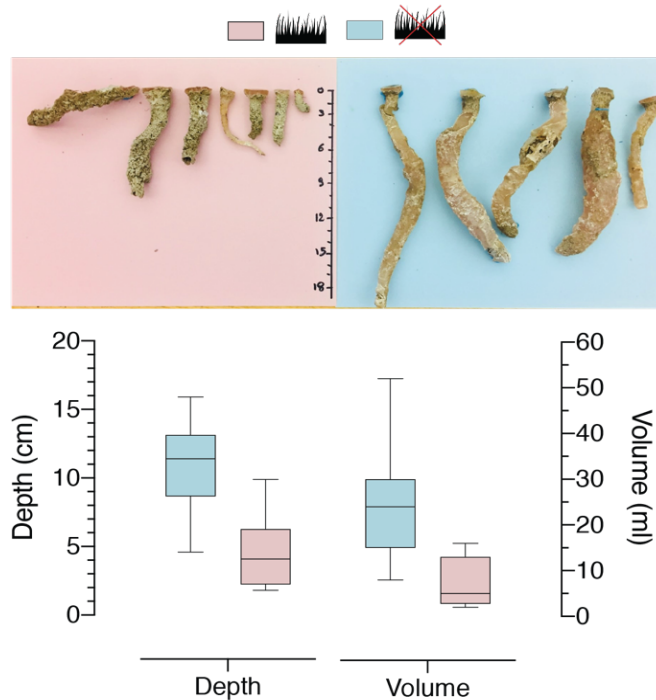


FIGURE 2. Impact of *Spartina alterniflora* on crab burrow shape and size. Bar plot showing the depth (left side) and volume (right side) of *M. pugnax* burrow casts. The presence of *S. alterniflora* is illustrated by the pink color while its absence is represented by the light blue color.

3.2 Burrow casts

The structure of *M. pugnax* burrows significantly varied between vegetated vs non-vegetated areas (Figure 2). In particular, the main shaft of the casts obtained in the non-vegetated patches was on average longer, resulting in burrows that were significantly deeper ($t = 5.26$; $P < 0.001$, t-test) and larger in volume ($t = 4.87$; $P < 0.001$, t-test) than the ones recorded within vegetated patches. This difference in architecture ultimately affected the bioturbation capacity of *M. pugnax* populations at the different areas. We calculated that, on average, the burrows of this species extended the sediment surface area by 34% and 9% in non-vegetated and vegetated plots, respectively.

TABLE 1. Mean vertical CO₂ flux expressed as $\mu\text{m m}^{-2} \text{s}^{-1}$ measured using a non-transparent chamber above marsh sediment containing 3, 1 - equivalent to 49.98 burrows m⁻², 16.66 burrows m⁻², respectively - and no burrows. These different burrow densities were selected across high and low organic matter areas within non-vegetated and *S. alterniflora* vegetated patches.

	CO ₂ flux ($\mu\text{m m}^{-2} \text{s}^{-1}$)	n
High organic matter	7.51 ± 0.64	72
Bare sediment	6.15 ± 1.03	36
3 burrows	10.23 ± 2.67	12
1 burrow	4.79 ± 0.44	12
No burrows	3.45 ± 0.62	12
<i>S. alterniflora</i>	8.87 ± 0.69	36
3 burrows	10.22 ± 1.40	12
1 burrow	7.97 ± 0.96	12
No burrows	8.42 ± 1.19	12
Low organic matter	2.75 ± 0.21	72
Bare sediment	1.61 ± 0.15	36
3 burrows	1.99 ± 0.32	12
1 burrow	1.61 ± 0.26	12
No burrows	1.22 ± 0.18	12
<i>S. alterniflora</i>	3.90 ± 0.28	36
3 burrows	4.20 ± 0.63	12
1 burrow	3.66 ± 0.44	12
No burrows	3.85 ± 0.42	12

3.3 Flux rates

To account for the assessed variability in burrow densities, we recorded the CO₂ fluxes from experimental chambers covering no-burrows, low burrow density (16.66 burrows m⁻²) and high burrow density (49.98 burrows m⁻²) patches. The recorded CO₂ fluxes were significantly higher in the patches characterized by higher organic matter ($F = 133.58$, $df = 1$, $P < 0.001$, ANOVA test, Table 1 and Figure 3), regardless of the presence of *S. alterniflora*. We also verified a

significant influence of the presence of *S. alterniflora* on such fluxes ($F = 61.78$, $df = 1$, $P < 0.001$, ANOVA test, Table 1 and Figure 3). Carbon dioxide fluxes measured within vegetated patches ($6.39 \pm 0.47 \mu\text{m m}^{-2} \text{s}^{-1}$) was 1.64 times higher than fluxes recorded in non-vegetated ones ($3.88 \pm 0.58 \mu\text{m m}^{-2} \text{s}^{-1}$) (Table 1 and Figure 3).

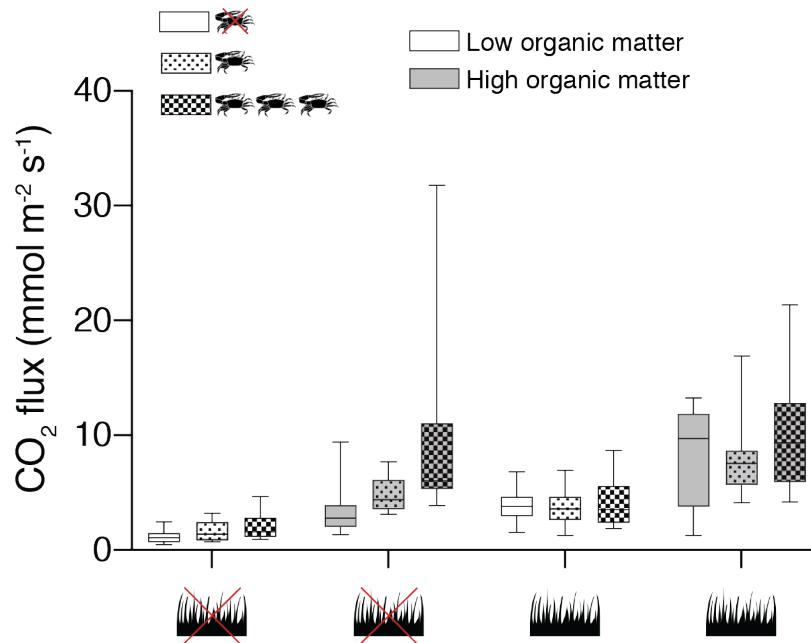


FIGURE 3. Measurement of carbon dioxide flux under four experimental setting and three levels of burrow crab density. Measurement represented by bars with white (grey) background where conducted over low (high) organic matter soil. The presence of *Spartina alterniflora* is illustrated by grass icon while the absence of *Spartina alterniflora* is represented by the red-crossed grass icon. The density of crab burrows is represented by the size of the checked pattern. The different levels correspond to no-burrows, 16.66 burrows m^{-2} (low density) and 49.98 burrows m^{-2} (high density) patches.

We consistently observed a significant difference in CO_2 fluxes coming from patches with different burrow densities, regardless of the presence of *S. alterniflora* and the amount of organic matter present ($F = 8.58$, $df = 1$, $P < 0.01$, ANOVA test, Table 1 and Figure 3). Statistically higher CO_2 releases were recorded from the high burrow density patches with

respect to both the low density and no-burrow patches, showing a strong influence of such biotic factor on total CO₂ fluxes. There was, however, a statistical difference in fluxes between the high burrow density chambers tested on non-vegetated patches and the ones measured in the vegetated ones ($F = 3.12$, $df = 2$, $P < 0.05$, interaction factor organic matter content \times vegetated plots, ANOVA test, Table 1 and Figure 3). We also recorded higher CO₂ fluxes from the areas rich in organic matter (Table 1 and Figure 3).

The regression analyses between the recorded CO₂ fluxes and the surface area of burrow openings observed in each patch show significant positive slopes only for non-vegetated patches in high organic matter areas (Figure 4).

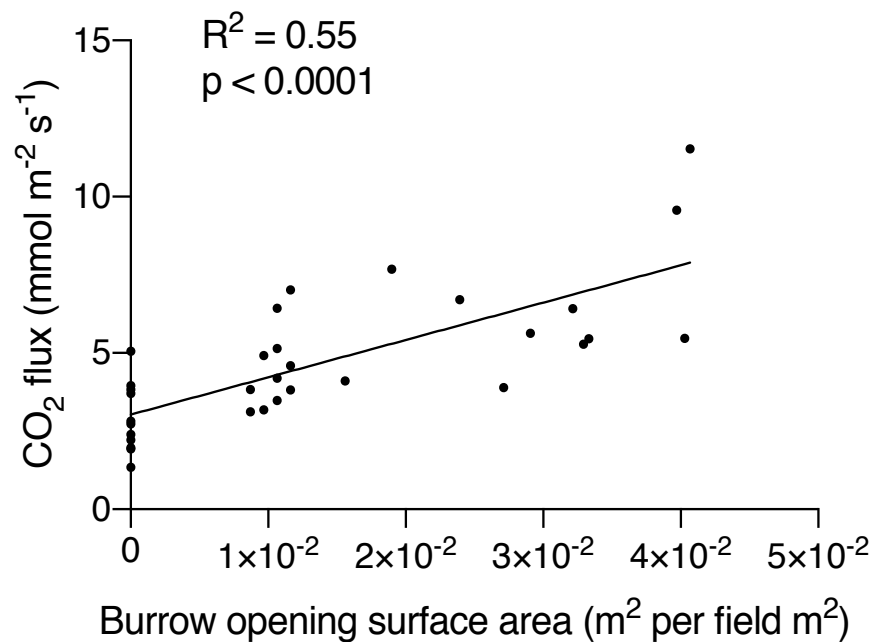


FIGURE 4. Relationship between crab burrows and carbon dioxide flux at non-vegetated patches within high organic matter areas.

4. DISCUSSION

This study shows that the presence and density of fiddler crab burrows are crucial to significantly increase the overall CO₂ fluxes of these New England salt marshes (Table 1). In

particular, our results show that the bioturbation activity of the dense populations of fiddler crabs is an important, but often neglected, factors that interplays with other biotic and abiotic characteristics of these habitats, such as organic content of the soil and the presence of vegetation (Figure 3), to ultimately shape the carbon budget of these ecosystems.

In line with our hypotheses, we recorded significantly higher CO₂ fluxes within the areas characterized by higher organic matter. This is coherent with previous studies, which showed how the CO₂ released by sediments depends, in part, on the cycling of organic matter and its microbial oxidation (Alongi, Sasekumar, Tirendi, & Dixon, 1998; Alongi, Tirendi, Trott, & Xuan, 2000; Howes et al., 1985; Raich & Potter, 1995). The presence of *S. alterniflora* also had a significant influence on such fluxes, as suggested by previous work showing that culms, root and rhizome respiration of this marsh grass can sustain high CO₂ fluxes (Howes et al., 1985; Morris & Whiting, 1986; Raich & Potter, 1995; Wigand et al., 2009). Even accounting for the importance of the above factors, and net of an obvious and uncontrolled variability at micro-scale, our data clearly show that CO₂ release from vegetated coastal environment increases if local crab burrow density is taken into account and ultimately suggest potential large-scale impacts of crab burrows on local and global salt marsh carbon budget assessment.

Across the high burrow density patches, we recorded, however, higher CO₂ fluxes from the areas rich in organic matter (Figure 3). We hypothesise that these lower CO₂ fluxes recorded for the same burrow density within the low organic matter are due to the limited amount of organic matter that is to oxidize by sediment microbial communities, even when exposed to additional oxygenation through burrows. Indeed, carbon dioxide emission due to respiration of *M. pugnax* individuals contributed only 7 to 9% and 16 to 37% to the total CO₂ flux recorded from its burrow in high organic matter and in low organic matter areas, respectively. Since our measures were taken at least 30 cm away from the burrows of other crabs species, in particular from the sympatric purple marsh crab *S. reticulatum*, we conclude that the flux rates we

measured for burrows primarily reflect enhanced microbial respiration, as found in other habitats (Booth, Fusi, Marasco, Mbobo, & Daffonchio, 2019; Fanjul et al., 2015; Otani et al., 2010).

There was also a significant difference in fluxes between high burrow density trials carried out within non-vegetated vs *S. alterniflora* patches (Figure 3). A sound explanation for these differences comes from our study on the burrow architecture at the different sites. We found that *M. pugnax* burrows were significantly larger and deeper in the non-vegetated areas and this larger net increase of the sediment-air interface was mirrored by their higher contribution to CO₂ fluxes in those quadrats. The shape and size of fiddler crab burrows are known to show intraspecific differences related to various sediment characteristics, such as the particle size and moisture and oxygen concentration (Bartolini et al., 2011; Chen, Hwang, Mayfield, Chen, & Lin, 2017), and biotic factors, such as the presence of roots (Lim & Rosiah, 2007; Wang, Bertness, Li, Chen, & Lü, 2015). We can only hypothesize that *M. pugnax* can adapt the shape of their burrows to the complex structure of *S. alterniflora* roots and rhizomes, changing their overall bioturbation potential and, ultimately, their impact on CO₂ fluxes.

The regression analyses between the recorded CO₂ fluxes and the surface area of burrow openings observed in each patch show significant positive slopes only for non-vegetated patches in high organic matter areas (Figure 4). This result confirms our hypotheses that only the large and deep burrows excavated in the non-vegetated areas are significantly increasing the air-sediment interface and that this increase enhanced microbial respiration, which will be higher in areas with high organic matter. The relatively low R-squared of the linear model could suggest that the increase in burrow opening surface area per m², which is related to an increase in burrow wall surface area, might not be the only environmental parameter driving the CO₂ flux increase. However, it should be noted that there is a lot of variability among the patches with no burrows, which lower the predictive power of the regression tests. Moreover,

if data are averaged for each replicated treatment (i.e., 0, 16.66 burrows m⁻² and 49.98 burrows m⁻²) we obviously observe a drop in the p-value because of the low replication but a very high R-squared ($R^2 = 0.96$). This highlights the need for more extensive studies with a larger range in burrow densities to fully document the role of burrowing on CO₂ release in salt marshes and wetlands.

Our results strongly emphasize the importance of including the faunal component, and specifically the dominant burrowing species, in carbon budget assessments for vegetated coastal habitats. If future surveys and field studies will not consider faunal bioturbation as a critical factor, they will underestimate vertical CO₂ fluxes and, ultimately, overestimate carbon retention rates and net carbon storage. The increase of vertical CO₂ flux related to *M. pugnax* burrow density was particularly strong within non-vegetated patches rich in organic matter. All along the East coast of North America, such bare areas are frequent within salt marshes (Adam, 2002; Gedan, Silliman, & Bertness, 2009) and could extensively contribute to their overall carbon budget. These results also highlight the high variability in CO₂ fluxes at micro- to mesoscale within salt marshes and, ultimately, the importance of accounting for site specific biotic and abiotic characteristics at such short scale when aiming to assess true carbon budgets. As complex ecotones between the land and the sea, vegetated coastal ecosystems represent a patchwork of microhabitats in which environmental and biological factors, including species interaction within the community, should be studied at local scale to design reliable and sound large-scale carbon budget assessments. This may prove important because ongoing human driven degradation of salt marshes can lead to an increase in the number of bare patches (Gedan et al., 2009) and to a consequent shift in the architecture and density of crab burrow density and architecture (Lim & Rosiah, 2007; Wang et al., 2015). Ultimately, these structural modifications could lead to an increased release of buried carbon stocks through oxidation and hence add to current climate crisis. Further investigations should focus on CO₂ flux

429 measurements using a larger range of crab burrow densities and environmental factors to more
430 accurately quantify how CO₂ flux responds to the natural variances within these important blue
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432

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