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

Laura E Agusto[1,1], Benoit Thibodeau[2,2], Jianwu Tang[3,3], Faming Wang[4,4], and Stefano Cannicci[1,1]

[1]The University of Hong Kong
[2]University of Hong Kong
[3]Marine Biological Laboratory
[4]Chinese Academy of Sciences

November 30, 2022

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. CO fluxes were measured over patches characterized by different densities of fiddler crab, , 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 , we demonstrated that CO release increased if local crab burrow density is considered. The increase in vertical CO fluxes linked to burrow density was higher for the non-vegetated areas with respect to patches. By means of burrow casting and morphological analyses of the burrows, we could relate this difference in COfluxes 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.

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

Laura E Agusto[1], Benoit Thibodeau[2*], Jianwu Tang[3], Faming Wang[3,4], Stefano Cannicci[1*]

[1]The Swire institute of Marine Science and Division of Ecology and Biodiversity, The University of Hong Kong, Hong Kong, Hong Kong SAR

[2]Division of Earth and Planetary Science and the Swire Institute of Marine Science, The University of Hong Kong, Hong Kong, Hong Kong SAR

[3]The Ecosystems Center, Marine Biological Laboratory, The University of Chicago, Woods Hole, The United States of America

[4]Xiaoliang Research Station for Tropical Coastal Ecosystems, Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, and the CAS engineering Laboratory for Ecological Restoration of Island and Coastal Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, P.R. China

*Correspondence

Benoit Thibodeau, Division of Earth and Planetary Science, The University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong SAR. Email: bthib@hku.hk.

Stefano Cannicci, The Swire institute of Marine Science and Division of Ecology and Biodiversity, The University of Hong Kong, Hong Kong, Hong Kong SAR Email: cannicci@hku.hk

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. CO_2 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 CO_2 release increased if local crab burrow density is considered. The increase in vertical CO_2 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 CO_2 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.

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;

72 Aschenbroich et al., 2016; Kristensen & Alongi, 2006; Robertson, 1986). Their burrows are

73 known to allow oxygen to penetrate deep sediment layers, otherwise hypo- or anoxic, and

74 create an extension of costal marshes sediment-air interface (Kristensen, 2008; Smith III, Boto,

75 Frusher, & Giddins, 1991). From such newly created surface, carbon dioxide (CO_2) can be

76 released to the atmosphere at low tide, by the decomposition of the soil organic matter as well

77 as from faunal, root and culm respiration (Howes, Dacey, & Teal, 1985; Martinetto et al., 2016;

78 Morris & Whiting, 1986; Pülmanns, Diele, Mehlig, & Nordhaus, 2014; Raich & Potter, 1995;

79 Tomohiro & Masakazu, 2019a). Ultimately, the previously buried and anoxic organic matter

80 is oxygenated, promoting microbial decomposition and increasing the release of CO_2 by up to

81 70% (Gribsholt, Kostka, & Kristensen, 2003a; Otani et al., 2010).

82   The significant role of the crab *Neohelice granulata* for soil carbon dynamics has been

83 extensively studied in South West Atlantic salt marshes (Alberti et al., 2015; Martinetto et al.,

84 2016). The bioturbation activities of this dominant crab proved to increase the export of CO_2

85 and dissolved organic carbon to the water column (Fanjul et al., 2015; Fanjul, Grela,

86 Canepuccia, & Iribarne, 2008), but no information on their effect on the release of CO_2 to the

87 atmosphere is available. Moreover, brachyuran crab influence on biogeochemical fluxes of

88 North West Atlantic (NWA) marshes have been mostly overlooked. In North American coastal

89 wetlands, bioturbation by brachyurans, such as various species of fiddler crabs (family

90 Ocypodidae) and the purple marsh crab (*Sesarma reticulatum*), is one of the most prominent

91 biotic factors affecting the community. Fiddler crabs of the genera *Minuca* and *Leptuca* often

92 outnumber *S. reticulatum* in NWA marshes (Raposa et al., 2018; Szura et al., 2017) , with

93 burrow densities ranging from approximately 20 to 350 burrows m^{-2} (Gribsholt, Kostka, &

94 Kristensen, 2003b; Michaels & Zieman, 2013). Due to these densities, the extended surface

95 area they create may exert a considerable effect on the vertical CO_2 fluxes, but to date very few

96　studies have estimated these effects in NWA marshes and none of them was performed *in situ*

97　(Gribsholt et al., 2003b; Gribsholt & Kristensen, 2002; Taylor & Allanson, 1993).

98　　To close this knowledge gap, the present study was designed to assess the impact of

99　burrowing behaviour of the dominant fiddler crab, *Minuca pugnax*, on CO_2 fluxes within the

100　salt marshes of Cape Cod, Massachusetts, USA. CO_2 fluxes were measured *in situ* in the

101　summer of 2017 in areas characterized by different burrow densities, including no-burrow areas,

102　using a portable gas analyser. To account for the role exerted by other abiotic and biotic factors

103　on CO_2 fluxes, measurements were taken across patches of bare soil vs areas colonized by the

104　marsh grass *Spartina alterniflora* and across areas characterized by high and low organic matter

105　content in the sediment. We hypothesized that: 1) the CO_2 flux would be higher in patches

106　where the soil is rich in organic matter, because it provides more reactant to the oxidation

107　reaction (Howes et al., 1985); 2) the presence of *S. alterniflora* would increase the CO_2 flux,

108　because of root and rhizome respiration (Howes et al., 1985; Morris & Whiting, 1986; Raich

109　& Potter, 1995; Wigand, Brennan, Stolt, Holt, & Ryba, 2009), and 3) a high density of crabs

110　burrows would increase the CO_2 flux, due to the increase in sediment-air interface. We also

111　hypothesized an additive effect of the above three factors, since the increase in sediment-air

112　interface due to the presence of crab burrows will further promote microbial decomposition,

113　both under high organic matter and in the presence of *S. alterniflora* (Howes et al., 1985;

114　Martinetto et al., 2016; Morris & Whiting, 1986; Raich & Potter, 1995; Tomohiro & Masakazu,

115　2019b), and, ultimately, intensify the release of CO_2.

116

117　**2. METHODS**

118　**2.1　Study site**

119　　The study was performed within a natural salt marsh located at the outlet of the Herring

120　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 CO_2 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 is 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 CO_2

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

176   recorded in the study areas and on minimum and maximum amount of active crab burrows that

177   fit within the measuring PVC collars. Thus, burrow density levels were no burrows, 1 burrow

178   and three burrows per collar, corresponding to 16.66 burrows m^{-2} and 49.98 burrows m^{-2},

179   respectively. Active burrows were identified based on the presence of an individual inside and

180   well-maintained burrow openings. A minimum distance of 30 cm was kept from *S. reticulatum*

181   burrows to assure no interference with the measurements. Replicate measurements along each

182   transect were placed at an average of 116 cm from each other, and treatments did not cross one

183   another. Two replicates for each crab burrow density were measured every week for four

184   consecutive weeks within each area (high or low organic matter) for each patch (vegetated vs

185   non-vegetated) along each transect (within each patch). A total of 12 replicates for each crab

186   burrow treatment were measured with 144 measurements taken in total. For each flux

187   measurement that included burrows, the burrows opening surface area was assessed. The

188   maximum and minimum burrow opening diameter of the elliptical opening was measured prior

189   to flux measurements within each chamber that contained a burrow. Additionally, the height

190   and diameter of any *S. alterniflora* present was measured. All measurements were done using

191   a measuring tape.

192   PVC collars (10.16 cm in diameter) were slightly pushed into the sediment so that the collar

193   and chamber were completely sealed off when measuring gas flux. Extra attention was made

194   when placing the collars to ensure burrow entrances were not damaged or the burrow was not

195   cut off from the opening. Measurements were made by connecting a 6.3 cm tall × 11.4 cm

196   diameter non-transparent chamber, with a fan installed inside to keep air circulation to an LGR

197   ultraportable CO_2, CH_4 and H_2O Analyzer (LGR Corp, CA, USA). The chamber was placed

198   on top of the PVC collars. Pressure equilibration occurred through a 6.51 m long and 0.4572

199   cm inner diameter plastic tubing connected the chamber and in contact with the atmosphere for

200   an average period of 5 minutes before starting a new measurement. Carbon dioxide flux

201 measurements lasted 5 minutes per treatment (with approximately 10 second sampling

202 intervals), based on observed periods for linear rates of gas concentration change and to avoid

203 excessive chamber warming (Brannon et al., 2016; Martin & Moseman-Valtierra, 2015). The

204 gas flux was determined by the following formula:

205

206 $F = (dc/dt) \times (1/V0) \times (P/P0) \times (T0/T) \times (V/S)$ (1)

207

208 where F is the flux rate, dc/dt is the slope of the CO_2 concentration vs. time, V0 is the CO_2

209 molar volume under standard conditions (i.e., 22.4 L mol^{-1}), P is the air pressure at our study

210 site, P0 is the standard air pressure, T is the air temperature during each measurement, T0 is

211 the standard temperature, and V is the head space volume of chamber, including the tubing

212 volume and average volume of burrows determined in a separate assessment, S is the soil

213 surface area and inner burrow wall surface area within the collar. Only flux data with

214 coefficients of determination (r^2) of the linear regression higher than 0.95 were used. The

215 calculation was conducted in Matlab (MATLAB and Statistics Toolbox Release 2016a, The

216 MathWorks, Inc., Natick, Massachusetts, United States), using a code developed by Eckhardt

217 and Kutzbach (2016). Measurements were made an hour after the collars were placed

218 (Pülmanns et al., 2014) and plants were cut off at their base in order to avoid CO_2 release due

219 to the set-up.

220 The additional volume created by crab burrows within each measurement was accounted

221 for during gas flux calculations. The frequency distributions of the minimum and maximum

222 burrow diameter of the casts sampled within the non-vegetated and vegetated patches,

223 respectively, overlapped with the maximum burrow diameter of burrows used during CO_2

224 measurements. Thus, our casts were an accurate representation of the burrows used within the

225 CO_2 flux measurements and their approximated volume was calculated based on the average

226 volume of the collected casts. Average volumes were calculated separately for non-vegetated

227 and vegetated patches, due to very different burrow cast parameters.

228 Following environmental factors were monitored after each single gas flux measurement:

229 Soil temperature and soil water-filled pore space (ProCheck soil moisture meter, Decagon

230 Devices, Inc. Pullman WA, USA) and pH and redox (Spectrum FieldScout SoilStik pH meter,

231 and electrode meter, Spectrum Inc. Aurora IL, USA).

232

233 **2.5   Calculations**

234 The total burrow wall surface area per m^2 was calculated within each area and patch by

235 multiplying the average burrow wall surface area of casts within either non-vegetated and

236 vegetated patches with the average burrow density measured for each area and patch.

237 The burrow opening surface area taken from flux measurements was used to calculate the

238 total surface area of burrow opening per m^2 across each area and patch. This provided a larger

239 dataset than using burrow cast opening measurements. Calculation were made by multiplying

240 the average burrow opening surface area with the average burrow density measured for each

241 area and patch (area × patch).

242 Within each area and patch, the CO_2 flux increase due to burrow presence was calculated as

243 follows:

244 CO_2 flux increase due to crab presence = (Av. CO_2 flux $_{burrow}$) × Burrow Density$_{area \times patch}$ (2)

245 While the CO_2 flux measured over plane sediment, therefore disregarding burrow presence

246 was calculated as follows:

247 CO_2 flux disregarding burrow presence = Av. CO_2 flux plane sediment $_{area \times patch}$ (3)

248

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 CO_2 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

274   two groups of vegetated patches we selected in high vs low organic matter content areas ($F =$

275   1.34, df = 1, $P =$ NS, ANOVA test). Densities of purple marsh crab, *S. reticulatum*, burrow did

276   not did not differ between rich vs low organic matter content patches and vegetated vs non-

277   vegetated areas ($F = 0.21$, df = 1, $P = 0.65$ and $F = 0.83$, df = 1, $P = 0.37$, ANOVA test for rich

278   vs low organic matter and vegetated vs non-vegetated patches, respectively). Thus, we could

279   proceed to apply a full-factorial design to analyse the impact of different burrow densities

280   across such environmental factors.

281   The density of *M. pugnax* burrows was neither statistically different between areas

282   characterized by high vs low organic matter content ($F = 0.99$, df = 1, $P =$ NS, ANOVA test)

283   nor between vegetated vs non-vegetated patches ($F = 1.98$, df = 1, $P =$ NS, ANOVA test).

284   Within those areas we observed a large variability among patches, with burrow densities

285   comprised between 33.32 ± 26.04 m^{-2} and 54.98 ± 30.47 m^{-2}. At all sites, the burrows of *M.*

286   *pugnax* were from a maximum of five time to minimum of two times more abundant than *S.*

287   *reticulatum*, showing to be the dominant species in these marshes.

288

289
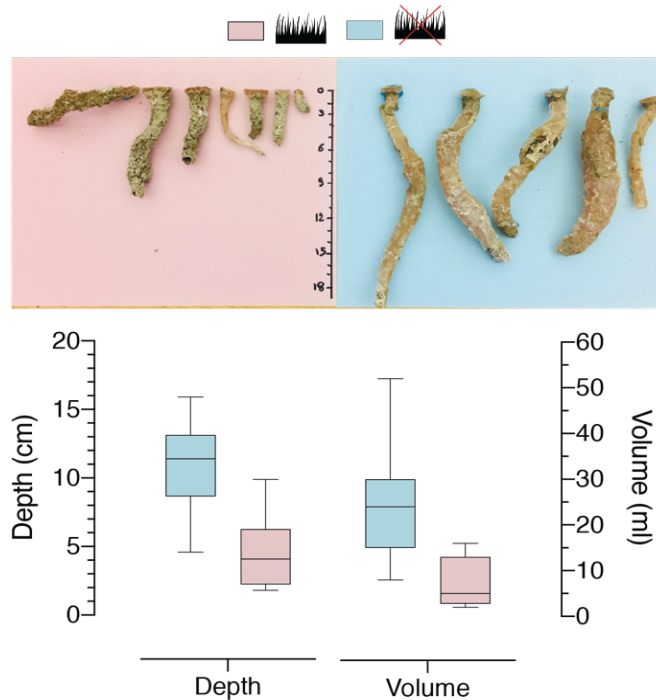
FIGURE 2. Impact of Spartina alterniflora on crab burrow shape and size. Bar plot showing the depth (left side) and volume (right side) of M. pugnax burrow casts. The presence of S. alterniflora is illustrated by the pink color while its absence is represented by the light blue color.

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) then the ones recorded within vegetated patches. This differences 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 CO_2 flux expressed as $\mu m\ m^{-2}\ s^{-1}$ 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 ($\mu m\ m^{-2}\ s^{-1}$)	n
High organic matter	**7.51 $\pm$ 0.64**	**72**
Bare sediment	6.15 $\pm$ 1.03	36
3 burrows	10.23 $\pm$ 2.67	12
1 burrow	4.79 $\pm$ 0.44	12
No burrows	3.45 $\pm$ 0.62	12
S. alterniflora	8.87 $\pm$ 0.69	36
3 burrows	10.22 $\pm$ 1.40	12
1 burrow	7.97 $\pm$ 0.96	12
No burrows	8.42 $\pm$ 1.19	12
Low organic matter	**2.75 $\pm$ 0.21**	**72**
Bare sediment	1.61 $\pm$ 0.15	36
3 burrows	1.99 $\pm$ 0.32	12
1 burrow	1.61 $\pm$ 0.26	12
No burrows	1.22 $\pm$ 0.18	12
S. alterniflora	3.90 $\pm$ 0.28	36
3 burrows	4.20 $\pm$ 0.63	12
1 burrow	3.66 $\pm$ 0.44	12
No burrows	3.85 $\pm$ 0.42	12

3.3 Flux rates

To account for the assessed variability in burrow densities, we recorded the CO_2 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 CO_2 fluxes we 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 ± 0.47 µm m^{-2} s^{-1}) was 1.64 times higher than fluxes recorded in non-vegetated ones (3.88 ± 0.58 µm m^{-2} s^{-1}) (Table 1 and Figure 3).
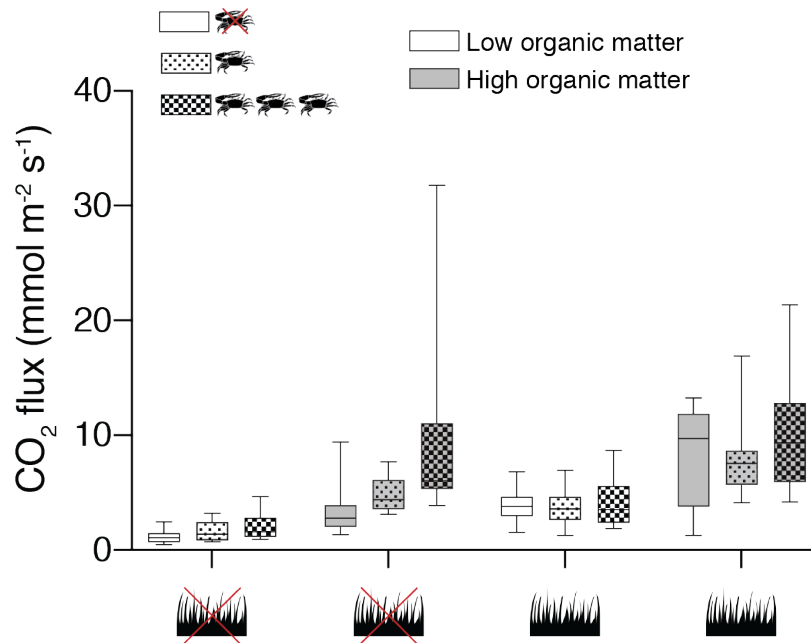

FIGURE 3. Measurement of carbon dioxide flux under four experimental setting and three levels of burrow crab density. Measurement represented by bars with white (grey) background where conducted over low (high) organic matter soil. The presence of *Spartina alterniflora* is illustrated by grass icon 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

337 respect to both the low density and no-burrow patches, showing a strong influence of such

338 biotic factor on total CO_2 fluxes. There was, however, a statistical difference in fluxes between

339 the high burrow density chambers tested on non-vegetated patches and the ones measured in

340 the vegetated ones ($F = 3.12$, df $= 2$, $P < 0.05$, interaction factor organic matter content $\times$

341 vegetated plots, ANOVA test, Table 1 and Figure 3). We also recorded higher CO_2 fluxes from

342 the areas rich in organic matter (Table 1 and Figure 3).

343   The regression analyses between the recorded CO_2 fluxes and the surface area of burrow

344 openings observed in each patch show significant positive slopes only for non-vegetated

345 patches in high organic matter areas (Figure 4).

346

347
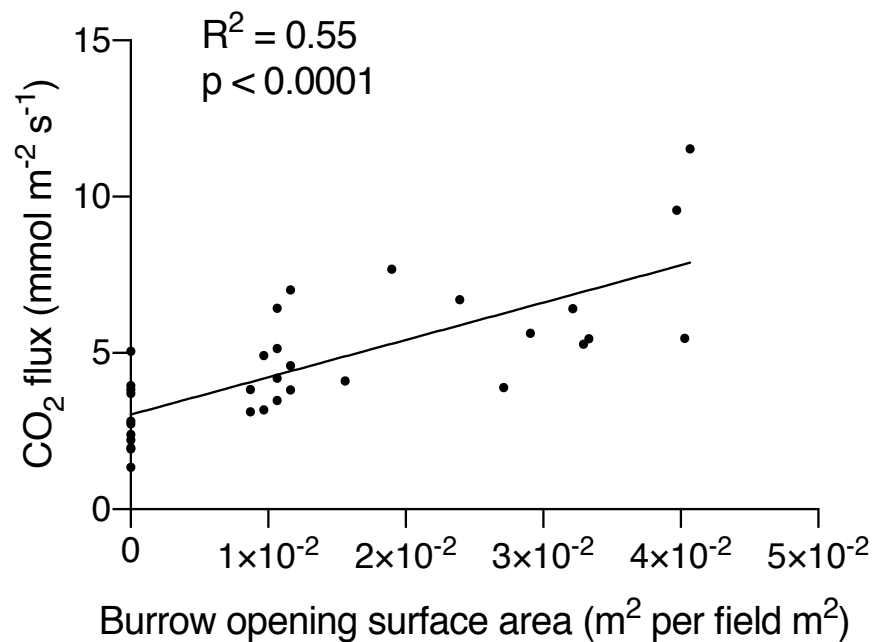
348 **FIGURE 4**. Relationship between crab burrows and carbon dioxide flux at non-vegetated
349 patches within high organic matter areas.

350

351   **4. DISCUSSION**

352   This study shows that the presence and density of fiddler crab burrows are crucial to

353 significantly increase the overall CO_2 fluxes of these New England salt marshes (Table 1). In

354 particular, our results show that the bioturbation activity of the dense populations of fiddler

355 crabs is an important, but often neglected, factors that interplays with other biotic and abiotic

356 characteristics of these habitats, such as organic content of the soil and the presence of

357 vegetation (Figure 3), to ultimately shape the carbon budget of these ecosystems.

358   In line with our hypotheses, we recorded significantly higher CO_2 fluxes within the areas

359 characterized by higher organic matter. This is coherent with previous studies, which showed

360 how the CO_2 released by sediments depends, in part, on the cycling of organic matter and its

361 microbial oxidation (Alongi, Sasekumar, Tirendi, & Dixon, 1998; Alongi, Tirendi, Trott, &

362 Xuan, 2000; Howes et al., 1985; Raich & Potter, 1995). The presence of *S. alterniflora* also

363 had a significant influence on such fluxes, as suggested by previous work showing that culms,

364 root and rhizome respiration of this marsh grass can sustain high CO_2 fluxes (Howes et al.,

365 1985; Morris & Whiting, 1986; Raich & Potter, 1995; Wigand et al., 2009). Even accounting

366 for the importance of the above factors, and net of an obvious and uncontrolled variability at

367 micro-scale, our data clearly show that CO_2 release from vegetated coastal environment

368 increases if local crab burrow density is taken into account and ultimately suggest potential

369 large-scale impacts of crab burrows on local and global salt marsh carbon budget assessment.

370   Across the high burrow density patches, we recorded, however, higher CO_2 fluxes from the

371 areas rich in organic matter (Figure 3). We hypothesise that these lower CO_2 fluxes recorded

372 for the same burrow density within the low organic matter are due to the limited amount of

373 organic matter that is to oxidize by sediment microbial communities, even when exposed to

374 additional oxygenation through burrows. Indeed, carbon dioxide emission due to respiration of

375 *M. pugnax* individuals contributed only 7 to 9% and 16 to 37% to the total CO_2 flux recorded

376 from its burrow in high organic matter and in low organic matter areas, respectively. Since our

377 measures were taken at least 30 cm away from the burrows of other crabs species, in particular

378 from the sympatric purple marsh crab *S. reticulatum*, we conclude that the flux rates we

379 measured for burrows primarily reflect enhanced microbial respiration, as found in other

380 habitats (Booth, Fusi, Marasco, Mbobo, & Daffonchio, 2019; Fanjul et al., 2015; Otani et al.,

381 2010).

382   There was also a significant difference in fluxes between high burrow density trials carried

383 out within non-vegetated vs *S. alterniflora* patches (Figure 3). A sound explanation for these

384 differences comes from our study on the burrow architecture at the different sites. We found

385 that *M. pugnax* burrows were significantly larger and deeper in the non-vegetated areas and

386 this larger net increase of the sediment-air interface was mirrored by their higher contribution

387 to CO_2 fluxes in those quadrats. The shape and size of fiddler crab burrows are known to show

388 intraspecific differences related to various sediment characteristics, such as the particle size

389 and moisture and oxygen concentration (Bartolini et al., 2011; Chen, Hwang, Mayfield, Chen,

390 & Lin, 2017), and biotic factors, such as the presence of roots (Lim & Rosiah, 2007; Wang,

391 Bertness, Li, Chen, & Lü, 2015). We can only hypothesize that *M. pugnax* can adapt the shape

392 of their burrows to the complex structure of *S. alterniflora* roots and rhizomes, changing their

393 overall bioturbation potential and, ultimately, their impact on CO_2 fluxes.

394   The regression analyses between the recorded CO_2 fluxes and the surface area of burrow

395 openings observed in each patch show significant positive slopes only for non-vegetated

396 patches in high organic matter areas (Figure 4). This result confirms our hypotheses that only

397 the large and deep burrows excavated in the non-vegetated areas are significantly increasing

398 the air-sediment interface and that this increase enhanced microbial respiration, which will be

399 higher in areas with high organic matter. The relatively low R-squared of the linear model

400 could suggests that the increase in burrow opening surface area per m^2, which is related to an

401 increase in burrow wall surface area, might not be the only environmental parameter driving

402 the CO_2 flux increase. However, it should be noted that there is a lot of variability among the

403 patches with no burrows, which lower the predictive power of the regression tests. Moreover,

404   if data are averaged for each replicated treatment (i.e., 0, 16.66 burrows m^{-2} and 49.98 burrows

405   m^{-2}) we obviously observe a drop in the p-value because of the low replication but a very high

406   R-squared ($R^2 = 0.96$). This highlight the needs for more extensive studies with a larger range

407   in burrow densities to fully document the role of burrowing on CO_2 release in salt marshes and

408   wetlands.

409   Our results strongly emphasize the importance of including the faunal component, and

410   specifically the dominant burrowing species, in carbon budget assessments for vegetated

411   coastal habitats. If future surveys and field studies will not consider faunal bioturbation as a

412   critical factor, they will underestimate vertical CO_2 fluxes and, ultimately, overestimate carbon

413   retention rates and net carbon storage. The increase of vertical CO_2 flux related to *M. pugnax*

414   burrow density was particularly strong within non-vegetated patches rich in organic matter. All

415   along the East coast of North America, such bare areas are frequent within salt marshes (Adam,

416   2002; Gedan, Silliman, & Bertness, 2009) and could extensively contribute to their overall

417   carbon budget. These results also highlight the high variability in CO_2 fluxes at micro- to

418   mesoscale within salt marshes and, ultimately, the importance of accounting for site specific

419   biotic and abiotic characterises at such short scale when aiming to assess true carbon budgets.

420   As complex ecotones between the land and the sea, vegetated coastal ecosystems represent a

421   patchwork of microhabitats in which environmental and biological factors, including species

422   interaction within the community, should be studied at local scale to design reliable and sound

423   large-scale carbon budget assessments. This may prove important because ongoing human

424   driven degradation of salt marshes can lead to an increase in the number of bare patches (Gedan

425   et al., 2009) and to a consequent shift in the architecture and density of crab burrow density

426   and architecture (Lim & Rosiah, 2007; Wang et al., 2015). Ultimately, these structural

427   modifications could lead to an increased release of buried carbon stocks through oxidation and

428   hence add to current climate crisis. Further investigations should focus on CO_2 flux

429   measurements using a larger range of crab burrow densities and environmental factors to more

430   accurately quantify how CO_2 flux responds to the natural variances within these important blue

431   carbon ecosystems.

432

433   **ACKNOWLEDGEMENTS**

REFERENCES

Adam, P. (2002). Saltmarshes in a time of change. *Environmental Conservation*, *29*(1), 39–61. https://doi.org/10.1017/S0376892902000048

Alberti, J., Daleo, P., Fanjul, E., Escapa, M., Botto, F., & Iribarne, O. O. (2015). Can a Single Species Challenge Paradigms of Salt Marsh Functioning? *Estuaries and Coasts*, *38*, 1178–1188. https://doi.org/10.1007/s12237-014-9836-z

Alongi, D. M., Sasekumar, A., Tirendi, F., & Dixon, P. (1998). The influence of stand age on benthic decomposition and recycling of organic matter in managed mangrove forests of Malaysia. *Journal of Experimental Marine Biology and Ecology*, *225*(2), 197–218.

Alongi, D. M., Tirendi, F., Trott, L. A., & Xuan, T. T. (2000). Benthic decomposition rates and pathways in plantations of the mangrove Rhizophora apiculata in the Mekong delta, Vietnam. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps194087

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*, 32–46.

Andreetta, A., Fusi, M., Cameldi, I., Cimò, F., Carnicelli, S., & Cannicci, S. (2014). Mangrove carbon sink. Do burrowing crabs contribute to sediment carbon storage? Evidence from a Kenyan mangrove system. *Journal of Sea Research*, *85*, 524–533. https://doi.org/10.1016/j.seares.2013.08.010

Aschenbroich, A., Michaud, E., Stieglitz, T., Fromard, F., Gardel, A., Tavares, M., & Thouzeau, G. (2016). Brachyuran crab community structure and associated sediment reworking activities in pioneer and young mangroves of French Guiana, South America. *Estuarine, Coastal and Shelf Science*, *182*, 60–71. https://doi.org/10.1016/j.ecss.2016.09.003

Bartolini, F., Cimo, F., Fusi, M., Dahdouh-Guebas, F., Penha-Lopes, G., & Cannicci, S. (2011). The effect of sewage discharge on the ecosystem engineering activities of two East African fiddler crab species: consequences for mangrove ecosystem functioning. *Marine Environmental*

463   *Research*, *71*(1), 53–61. https://doi.org/10.1016/j.marenvres.2010.10.002

464   Bertness, M. D. (1985). Fiddler crab regulation of Spartina alterniflora production on a New England

465   salt marsh. *Ecology*, *66*(3), 1042–1055. https://doi.org/10.2307/1940564

466   Bertness, M. D., Brisson, C. P., Bevil, M. C., & Crotty, S. M. (2014). Herbivory drives the spread of

467   salt marsh die-off. *PLoS ONE*, *9*(3). https://doi.org/10.1371/journal.pone.0092916

468   Bertness, M. D., & Miller, T. (1984). The distribution and dynamics of Uca pugnax (Smith) burrows in

469   a new England salt marsh. *Journal of Experimental Marine Biology and Ecology*, *83*(3), 211–237.

470   https://doi.org/10.1016/S0022-0981(84)80002-7

471   Booth, J. M., Fusi, M., Marasco, R., Mbobo, T., & Daffonchio, D. (2019). Fiddler crab bioturbation

472   determines consistent changes in bacterial communities across contrasting environmental

473   conditions. *Scientific Reports*, *9*(1), 3749. https://doi.org/10.1038/s41598-019-40315-0

474   Brannon, E. Q., Moseman-Valtierra, S. M., Rella, C. W., Martin, R. M., Chen, X., & Tang, J. (2016).

475   Evaluation of laser-based spectrometers for greenhouse gas flux measurements in coastal marshes.

476   *Limnology and Oceanography: Methods*, *14*(7), 466–476. https://doi.org/10.1002/lom3.10105

477   Chen, T. Y., Hwang, G. W., Mayfield, A. B., Chen, C. P., & Lin, H. J. (2017). The relationship between

478   intertidal soil composition and fiddler crab burrow depth. *Ecological Engineering*, *100*, 256–260.

479   https://doi.org/10.1016/j.ecoleng.2016.12.011

480   Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., & Kanninen, M. (2011).

481   Mangroves among the most carbon-rich forests in the tropics. *Nature Geoscience*, *4*(5), 293–297.

482   https://doi.org/10.1038/ngeo1123

483   Duarte, C. M., Losada, I. J., Hendriks, I. E., Mazarrasa, I., & Marbà, N. (2013). The role of coastal plant

484   communities for climate change mitigation and adaptation. *Nature Climate Change*, *3*(11), 961–

485   968. https://doi.org/10.1038/nclimate1970

486   Eckhardt, T., & Kutzbach, L. (2016). MATLAB code to calculate gas fluxes from chamber-based

487   methods. PANGAEA. https://doi.org/10.1594/PANGAEA.857799

488   Fanjul, E., Escapa, M., Montemayor, D., Addino, M., Alvarez, M. F., Grela, M. A., & Iribarne, O.

489   (2015). Effect of crab bioturbation on organic matter processing in South West Atlantic intertidal

490   sediments. *Journal of Sea Research*, *95*, 206–216. https://doi.org/10.1016/j.seares.2014.05.005

491   Fanjul, E., Grela, M. A., Canepuccia, A., & Iribarne, O. (2008). The Southwest Atlantic intertidal

492   burrowing crab Neohelice granulata modifies nutrient loads of phreatic waters entering coastal

493   area. *Estuarine, Coastal and Shelf Science*, *79*(2), 300–306.

494   https://doi.org/10.1016/j.ecss.2008.04.005

495   Gedan, K. B., Silliman, B. R., & Bertness, M. D. (2009). Centuries of Human-Driven Change in Salt

496   Marsh Ecosystems. *Annual Review of Marine Science*, *1*(1), 117–141.

497   https://doi.org/10.1146/annurev.marine.010908.163930

498   Gribsholt, B., Kostka, J. E., & Kristensen, E. (2003a). Impact of fiddler crabs and plant roots on

499   sediment biogeochemistry in a Georgia saltmarsh. *Marine Ecology Progress Series*, *259*, 237–

500   251. https://doi.org/10.3354/meps259237

501   Gribsholt, B., Kostka, J. E., & Kristensen, E. (2003b). Impact of fiddler crabs and plant roots on

502   sediment biogeochemistry in a Georgia saltmarsh. *Marine Ecology Progress Series*, *259*, 237–

503   251.

504   Gribsholt, B., & Kristensen, E. (2002). Effects of bioturbation and plant roots on salt marsh

505   biogeochemistry : a mesocosm study. *Marine Ecology Progress Series*, *241*, 71–87.

506   Hayes, D. J., Vargas, R., Alin, S., Conant, R. T., Hutyra, L. R., Jacobson, A. R., … Woodall, C. W.

507   (2018). Chapter 2: The North American Carbon Budget. Second State of the Carbon Cycle

508   Report(SOCCR2): A Sustained Assessment Report [Cavallaro, N., G. Shrestha, R. Birdsey, M. A.

509   Mayes, R. G. Najjar, S. C. Reed, P. Romero-Lankao, and Z. Zhu (eds.)]. U.S. Global C, 71–108.

510   https://doi.org/10.7930/SOCCR2.2018.Ch2

511   Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and

512   carbonate content in sediments: reproducibility and comparability of results. *Journal of*

513   *Paleolimnology* (Vol. 25).

514 Howard, J., Sutton-Grier, A., Herr, D., Kleypas, J., Landis, E., Mcleod, E., … Simpson, S. (2017).

515   Clarifying the role of coastal and marine systems in climate mitigation. *Frontiers in Ecology and*

516   *the Environment*, *15*(1), 42–50. https://doi.org/10.1002/fee.1451

517 Howes, B. L., Dacey, J. W. H., & Teal, J. M. (1985). Annual Carbon Mineralization and Belowground

518   Production of Spartina Alterniflora in a New England Salt Marsh. *Ecology*, *66*(2), 595–605.

519   https://doi.org/10.2307/1940408

520 Huhta, V. (2007). The role of soil fauna in ecosystems: A historical review. *Pedobiologia - International*

521   *Journal of Soil Biology*, *50*(6), 489–495.

522 Kristensen, E. (2008). Mangrove crabs as ecosystem engineers; with emphasis on sediment processes.

523   *Journal of Sea Research*, *59*(1–2), 30–43. https://doi.org/10.1016/j.seares.2007.05.004

524 Kristensen, E., & Alongi, D. M. (2006). Control by fiddler crabs (Uca vocans) and plant roots

525   (Avicennia marina) on carbon, iron, and sulfur biogeochemistry in mangrove sediment. *Limnology*

526   *and Oceanography*, *51*(4), 1557–1571.

527 Kristensen, E., Bouillon, S., Dittmar, T., & Marchand, C. (2008). Organic carbon dynamics in mangrove

528   ecosystems: A review. *Aquatic Botany*, *89*(2), 201–219.

529   https://doi.org/10.1016/j.aquabot.2007.12.005

530 Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C., & Banta, G. (2012).

531   What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine*

532   *Ecology Progress Series*, *446*, 285–302. https://doi.org/10.3354/meps09506

533 Lim, S. S. L., & Rosiah, A. (2007). Influence of pneumatophores on the burrow morphology of Uca

534   annulipes (H. Milne Edwards , 1837) (Brachyura, Ocypodidae) in the field and in simulated

535   mangrove micro-habitats. *Crustaceana*, *80*(11), 1327–1338.

536 Martin, R. M., & Moseman-Valtierra, S. (2015). Greenhouse gas fluxes vary between Phragmites

537   australis and native vegetation zones in coastal wetlands along a salinity gradient. *Wetlands*, *35*(6),

538   1021–1031. https://doi.org/10.1007/s13157-015-0690-y

539 Martinetto, P., Montemayor, D. I., Alberti, J., Costa, C. S. B., & Iribarne, O. (2016). Crab Bioturbation
540   and Herbivory May Account for Variability in Carbon Sequestration and Stocks in South West
541   Atlantic Salt Marshes. *Frontiers in Marine Science*, *3*(July), 1–12.
542   https://doi.org/10.3389/fmars.2016.00122

543 Michaels, R. E., & Zieman, J. C. (2013). Fiddler crab (Uca spp.) burrows have little effect on
544   surrounding sediment oxygen concentrations. *Journal of Experimental Marine Biology and*
545   *Ecology*, *448*, 104–113. https://doi.org/10.1016/j.jembe.2013.06.020

546 Morris, J. T., & Whiting, G. J. . (1986). Emission of Gaseous Carbon Dioxide from Salt-Marsh
547   Sediments and Its Relation to Other Carbon Losses. *Estuaries*, *9*(1), 9–19.

548 Otani, S., Kozuki, Y., Yamanaka, R., Sasaoka, H., Ishiyama, T., Okitsu, Y., … Fujiki, Y. (2010). The
549   role of crabs (Macrophthalmus japonicus) burrows on organic carbon cycle in estuarine tidal flat,
550   Japan. *Estuarine, Coastal and Shelf Science*, *86*(3), 434–440.
551   https://doi.org/10.1016/j.ecss.2009.07.033

552 Portnoy, J. W., & Giblin, A. E. (1997). Effects of historic tidal restrictions on salt marsh sediment
553   chemistry. *Biogeochemistry*, *36*(3), 275–303. https://doi.org/10.1023/A:1005715520988

554 Pülmanns, N., Diele, K., Mehlig, U., & Nordhaus, I. (2014). Burrows of the Semi-Terrestrial Crab
555   Ucides cordatus Enhance CO2 Release in a North Brazilian Mangrove Forest. *PLoS ONE*, *9*(10),
556   e109532. https://doi.org/10.1371/journal.pone.0109532

557 Raich, J. W., & Potter, C. S. (1995). Global patterns of carbon dioxide emissions from soils. *Global*
558   *Biogeochemical Cycles*, *9*(1), 23–36. https://doi.org/10.1029/94GB02723

559 Raposa, K. B., McKinney, R. A., Wigand, C., Hollister, J. W., Lovall, C., Szura, K., … Watson, E. B.
560   (2018). Top-down and bottom-up controls on southern New England salt marsh crab populations.
561   *PeerJ*, *2018*(5), 1–25. https://doi.org/10.7717/peerj.4876

562 Robertson, A. I. (1986). Leaf-burying crabs: Their influence on energy flow and export from mixed

563   mangrove forests(Rhizophora spp.) in northeastern Australia. *Journal of Experimental Marine*

564   *Biology and Ecology*, *102*(2), 237–248.

565   Smith III, T. J., Boto, K. G., Frusher, S. D., & Giddins, R. L. (1991). Keystone species and mangrove

566   forest dynamics: the influence of burrowing by crabs on soil nutrient status and forest productivity.

567   *Estuarine, Coastal and Shelf Science*, *33*(5), 419–432.

568   Szura, K., McKinney, R. A., Wigand, C., Oczkowski, A., Hanson, A., Gurak, J., & Gárate, M. (2017).

569   Burrowing and foraging activity of marsh crabs under different inundation regimes. *Journal of*

570   *Experimental   Marine   Biology   and   Ecology*,   *486*,   282–289.

571   https://doi.org/10.1016/j.jembe.2016.10.029

572   Taylor, D. I., & Allanson, B. R. (1993). Impacts of dense crab populations on carbon exchanges across

573   the surface of a salt marsh. *Marine Ecology Progress Series*, *101*(1–2), 119–130.

574   https://doi.org/10.3354/meps101119

575   Tomohiro, K., & Masakazu, H. (Eds.). (2019a). *Blue Carbon in Shallow Coastal Ecosystems: Carbon*

576   *Dynamics, Policy, and Implementation*. Singapore: Springer Singapore.

577   Tomohiro, K., & Masakazu, H. (2019b). *Blue Carbon in Shallow Coastal Ecosystems*. *Blue Carbon in*

578   *Shallow Coastal Ecosystems*. https://doi.org/10.1007/978-981-13-1295-3

579   Wang, J., Bertness, M. D., Li, B., Chen, J., & Lü, W. (2015). Plant effects on burrowing crab

580   morphology in a Chinese salt marsh: Native vs. exotic plants. *Ecological Engineering*, *74*, 376–

581   384.

582   Wigand, C., Brennan, P., Stolt, M., Holt, M., & Ryba, S. (2009). Soil respiration rates in coastal marshes

583   subject to increasing watershed nitrogen loads in southern New England, USA. *Wetlands*, *29*(3),

584   952–963. https://doi.org/10.1672/08-147.1

585