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Potential effect of fiddler crabs on organic matter distribution: A combined laboratory and field experimental approach

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ABSTRACT

Bioturbators play a key role in estuarine environments by modifying the availability of soil elements, which in turn may affect other organisms. Despite the importance of bioturbators, few studies have combined both field and laboratory experiments to explore the effects of bioturbators on estuarine soils. Herein, we assessed the bioturbation potential of fiddler crabs *Leptuca leptodactyla* and *Leptuca uruguayensis* in laboratory and field experiments, respectively. We evaluated whether the presence of fiddler crabs resulted in vertical transport of sediment, thereby altering organic matter (OM) distribution. Under laboratory conditions, the burrowing activity by *L. leptodactyla* increased the OM content in sediment surface. In the long-term field experiment with areas of inclusion and exclusion of *L. uruguayensis*, we did not observe influence of this fiddler crab in the vertical distribution of OM. Based on our results, we suggest that small fiddler crabs, such as the species used in these experiments, are potentially capable of alter their environment by transporting sediment and OM but such effects may be masked by environmental drivers and spatial heterogeneity under natural conditions. This phenomenon may be related to the small size of these species, which affects how much sediment is transported, along with the way OM interacts with biogeochemical and physical processes. Therefore, the net effect of these burrowing organisms is likely to be the result of a complex interaction with other environmental factors. In this sense, we highlight the importance of performing simultaneous field and laboratory experiments in order to better understanding the role of burrowing animals as bioturbators.

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1. Introduction

How organisms interact with their environment and how those interactions modify ecosystem functioning is a central theme in ecological studies. The important role of ecosystem engineers in modifying the local environment has long been recognized (Jones

et al., 1994). Studies have investigated how engineers change the environment and how the intensity of those changes can modify our understanding of ecosystem functioning (Wang et al., 2010; Richards et al., 2011; Schaller, 2014). Bioturbators, for example, have a significant impact on sediment biogeochemistry (Kristensen et al., 2012). The bioturbation alters sediment matrices and the distribution of its components (Meysman et al., 2006; Kristensen, 2008; Michaels and Ziemann, 2013). Sediment texture, influx of soil particles and interstitial transport of solutes in different ecosystems (Meysman et al., 2006) are modified by bioturbation, stimulating organic matter (OM) decomposition and remineralization (Kristensen, 2000; Lohrer et al., 2004; Martinez-Garcia et al.,

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2015) and enhancing nutrient flux throughout the ecosystem (Fanjul et al., 2015). Then, the ecological consequences of bioturbation have been extensively investigated in many groups of burrowing invertebrates, such as polychaetes (Giangrande et al., 2001; Volkenborn et al., 2007), bivalves (Kanaya, 2014; Harris et al., 2015), shrimps (Gagnon et al., 2013), crayfishes (Usio and Townsend, 2004), and crabs (Kristensen, 2008; Pülmanns et al., 2014).

Burrows and galleries constructed by some organisms, such as burrowing crabs, tend to expose deep soil areas, with higher OM contents (Michaels and Ziemann, 2013), acting on both carbon export via CO₂ release, increasing decomposition and facilitating aerobic degradation (Kristensen et al., 2008; Araújo et al., 2012; Pülmanns et al., 2014; Martinetto et al., 2016), as well as carbon storage, by increasing retention of nutrients in the environment (Martinetto et al., 2016). In turn, bioturbator crabs may promote changes in the estuarine carbon pathways, which are a relevant issue due to the importance of mangrove soils in stored carbon and its relation with climate change (Donato et al., 2011; Murdiyarso et al., 2015). Consequently, these organisms profoundly modify soil characteristics by changing biogeochemical processes (Araújo et al., 2012; Chatterjee, 2014). However, bioturbation effects on the carbon cycle caused by burrowing crabs are usually related to crab density (Kristensen et al., 2008; Andreetta et al., 2014) and size, with larger species presenting more significant effects (Araújo et al., 2012; Pülmanns et al., 2014).

Fiddler crabs are burrowing animals broadly distributed in tropical and subtropical estuaries (Crane, 1975). These bioturbators are classified as biodiffusers and regenerators, according to sediment transport they perform in coastal ecosystems (see categories in Kristensen et al., 2012). Therefore, they are key organisms in mangrove forests (Smith III et al., 1991). Fiddler crabs are often used as a model species to test the effects of bioturbation on the distribution of soil components (e.g. OM, metals; Nielsen et al., 2003; Kristensen and Alongi, 2006; Wang et al., 2010). High density and wide distribution in intertidal areas facilitate the use of fiddler crabs in laboratory experiments (Wolfrath, 1992; Thomas and Blum, 2010; Fanjul et al., 2015). However, there are few studies performing both laboratory and long-term field experiments with fiddler crabs. In this study, we investigated the bioturbation potential of *Leptuca leptodactyla* (previously *Uca leptodactyla*) and *Leptuca uruguayensis* (previously *Uca uruguayensis*) on mangrove soil at laboratory and field experiments, respectively. Experiments were performed to evaluate fiddler crab bioturbator activity using OM as a marker to trace sediment transport between vertical soil layers. We hypothesized that fiddler crabs promote vertical transport of sediment and OM by up-taking sediment from deeper layers to the soil surface through burrowing.

2. Materials and methods

2.1. Study area

For the laboratory experiment, crabs and sediment were collected at Portinho Mangrove, Praia Grande, São Paulo/Brazil (23°59'11.26"S; 46°24'11.83"W). Field experiment were performed in the Itapanhaú River mangrove (25°50'13.61"S; 46°09'09.44"W), Bertioga, São Paulo/Brazil. These mangroves are characterized by typical vegetation (*Rhizophora mangle*, *Avicennia shaueriana* and *Laguncularia racemosa*) and a semidiurnal tide flow (Eichler et al., 2006). Although different crab species were used to perform laboratory and field experiments, it is worth noticing that *L. leptodactyla* and *L. uruguayensis* belong to the genera *Leptuca* (Shih et al., 2016) and are similar regarding body size as well as the bulk of sediment they are able to transport during excavation

(Machado et al., 2013). In this sense, it could be expected that both fiddler crabs have comparable bioturbation effects in mangrove soils.

2.2. Laboratory experiment

The bioturbation potential of *L. leptodactyla* on mangrove soil was investigated by manipulating its presence in terrariums at laboratory. This species was chosen as a model of bioturbator because it is found at high density at Portinho mangrove (LF Natálio, pers. obs.). Our experimental design consisted of three treatments, two with crabs and one without them. Both treatments with crabs were identical except for the position at which sediment was sampled: inside the burrow or near the burrow. These treatments were defined to verify the effect of crab bioturbation on OM content resulting from the burrowing excavation (inside the burrow) and, consequently, from the excavation pellet deposition (near the burrow). Each treatment had eight replicates (39 cm × 22 cm × 24.5 cm terrariums), and in the treatments with crabs, eight *L. leptodactyla* adult females were added per terrarium (equivalent density reported in the field: 94 ind/m² - LF Natálio, pers. obs.).

In order to run the laboratory experiments, 128 *L. leptodactyla* adult females (carapace width larger than 4.1 mm; Cardoso and Negreiros-Fransozo, 2004) were collected from Portinho Mangrove. Only females were used to standardize crab effects, since bioturbation behavior (e.g. excavation and feeding) differs between sexes (Caravello and Cameron, 1987; Weis and Weis, 2004). In the laboratory, crabs were acclimated for 48 h at 25 °C in terrariums with sediment moistened with estuarine water.

Sediment of high (20.09 ± 1.34% (mean ± SE)) and low (1 ± 0.11%) OM content was collected from two different sites in Portinho mangrove. Ten sediment samples of each site were dried in a heater (60 °C, 72 h), and then a 10 g portion of dried sediment was burned in a muffle (500 °C, 4 h) to obtain OM content (%) by loss on ignition (Heiri et al., 2001). Sediment was boxed and transported to the laboratory, where it was carefully sieved in a 2 mm mesh to remove macrofauna and organic detritus that could overestimate organic content. Afterwards, the boxed sediment was kept for 48 h at 25 °C to remove the excess of surface water. Thus, we avoided the instability of high humidity sediment which may disturb the sediment superposition. After that, to each terrarium we added the sediment with high OM content (4 cm layer) in the bottom and then the sediment with low OM content above it (4 cm layer). Such vertical gradient in OM % was set up in order to test our hypothesis: if fiddler crabs transport OM through excavation by carrying sediment from bottom to surface, we would expect to find an increase in OM content on the top layer at the end of experiment. The 8 cm sediment layer was chosen based on the mean maximum depth of burrows reported for *L. leptodactyla* (7.3 ± 1.76 cm; Machado et al., 2013). Fiddler crabs were maintained for 72 h, a period we estimated necessary to construct and maintain burrows (based on our personal observations). Temperature (25 °C) and photoperiod (12 h of light and 12 h of darkness) were controlled.

Sediment sampling was performed twice: at the beginning (before introduction of crabs in the terrariums, *T_i*) and after 72 h (*T_f*) of experiment. In both moments we collected three sediment samples from the top layer (4 cm depth) in each terrarium using a corer (≈ 3 cm in diameter). Sediment analysis were performed as the methodology described above (Heiri et al., 2001).

2.3. Field experiment

The bioturbation potential of *L. uruguayensis* on mangrove soil was investigated by manipulating its presence at field. We used

L. uruguayensis as a model species due to its high abundance in Bertioga mangroves and because such areas are not exposed to or situated in tourist locations, which could lead to movement or destruction of the experimental cages. The experiment consisted of two treatments: inclusion and exclusion cages. For each cage, we evaluated the vertical distribution of OM content by sampling sediment from two depths, upper (0–4 cm) and lower (4–8 cm) layers. In inclusion cages, we added *L. uruguayensis* adult females (carapace larger than 5.9 mm; Hirose et al., 2013) proportionally to the surrounding mangrove area (92 ind/m²).

On February 2012, we placed twelve cages avoiding forest edges and mangrove trees (Fig. 1A). Cages were made out of polyvinyl chloride (PVC) pipes, with a 0.25 m² area (0.5 m × 0.5 m), and fitted with canvas (2 mm mesh, Fig. 1B) extending from the top (70 cm above soil surface) to the base (50 cm below soil surface). The top and the base of cages were covered with a 1 cm mesh canvas to prevent entry of animals and leaves, which could overestimate the OM content. We chose a mesh size that allowed water flow. To install the cages, sediment was removed and sieved to exclude all macrofauna (>2 mm). Then, the sieved sediment was added into cages. The experiment started seven months after to allow the reestablishment of the soil matrix. We randomly selected the treatments of inclusion (23 *L. uruguayensis* adult females per cage, n = 6 cages) and exclusion (n = 6 cages). The experiment lasted for 109 days (September 2012 to January 2013) and cages were checked biweekly for maintenance and removal of invading crabs.

During the experiment, we sampled sediment eight times and assessed redox potential and dissolved oxygen, all in periods of spring low tides. The first sampling was executed 9 days after introducing crabs into the cages, and those following were performed in 12–16-day intervals, except for the last, which was conducted 28 days after the previous sampling. In all samplings, we took one composite sample (hereafter “sample”) of each experimental cage and five samples in random locations of the mangrove to characterize the non-manipulated environment. Each sample was composed by three corer of sediment, which were homogenized for OM content analysis. Sediment was collected with a corer (≈3 cm in diameter) and immediately divided in two fractions: upper (0–4 cm) and lower (4–8 cm) layers. Sampling depth was chosen based on the mean maximum burrow depth reported for *L. uruguayensis* (6.12 ± 2.04 cm; Machado et al., 2013). Sediment samples were kept frozen for posterior OM content analysis, using the same procedure in the laboratory experiment (see above). Sampling of redox potential (pHmeter PG 1400 Gehaka® with ORP SRR03 electrode) and dissolved oxygen (SensoDirect OXI200 Lovibond®) were performed on soil surface water (water pools) in the cages and in the non-manipulated environment (n = 5). Measurements were not taken until the electrode value stabilized.

2.4. Data analysis

For laboratory and field experiments, we analyzed the ratio between final and initial OM in each experimental unit (terrarium or cage) as a response variable. This procedure was carried out to minimize any bias due to the different initial conditions among replicates. For the laboratory experiment, we applied a one-way ANOVA to evaluate the effect of treatments (three levels) on corrected OM content. Field data were randomized to assure independence of upper and lower layers; in turn, we used only one layer (upper or lower) of sediment sampled from each cage. Thus, we obtained three samples for each treatment and depth in each sampling date. We applied a two-way ANOVA to evaluate corrected OM content, considering treatment (two levels) and depth (two levels). We could not use this approach in the non-manipulated environment due to the collection of an odd number of samples; instead, we presented a description of temporal variation of OM content for each sediment depth.

For each abiotic factor (redox potential and dissolved oxygen), we applied a one-way ANOVA to compare levels between inclusion and exclusion cages and non-manipulated environment on the 91st day, near the end of the experiment. It was not possible to compare treatments and non-manipulated environment on the last day (109th day) due to the absence of surface water in some cages. When a factor presented an associated effect, we used Tukey's HSD test to identify differences between groups. Variance homogeneity was verified via Cochran's test.

3. Results

3.1. Laboratory experiment

Once experiments were finalized, we could notice visually the vertical transport of sediment by burrowing activity of *L. leptodactyla* through burrows at the terrariums' side (Fig. 2B), plus the presence of excavation pellets from the bottom layer (high OM content) (Fig. 2D). It was not observed in terrariums without crabs (Fig. 2A and C). Also, OM content was affected by crab activity since there was an increase in the OM content in the superior layers of sediment on treatment with crabs (one-way ANOVA, $F_{(2, 21)} = 21.576$, $p < 0.0001$; *a posteriori* Tukey's test, $p < 0.05$). In terrariums with crabs, regardless of the position of sampled sediment, OM content showed a 1.5-fold increase in the upper layer. Contrastingly, the control treatment did not differ in OM content throughout the experiment (Fig. 3A).

3.2. Field experiment

There was no effect of treatment and depth on OM content after

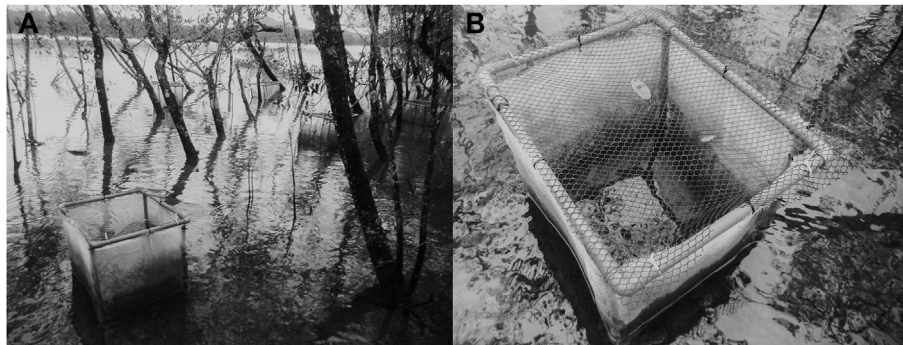


Fig. 1. Itapanhaú River mangrove, Bertioga, São Paulo, Brazil. (A) Mangrove overview with experimental cages during high tide. (B) Detail of experimental cage.

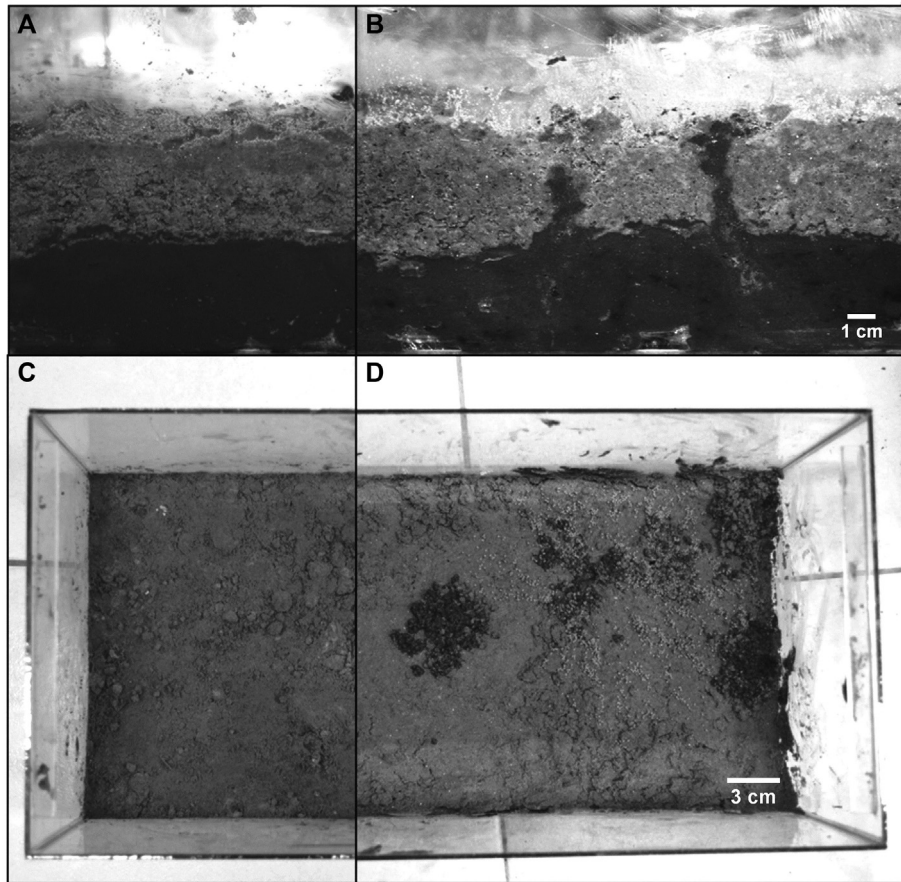


Fig. 2. Lateral and top views of the terrariums in the absence (A, C) and presence (B, D) of bioturbation activity by *Leptuca leptodactyla*. Burrowing pellets are the dark pellets in “D”.

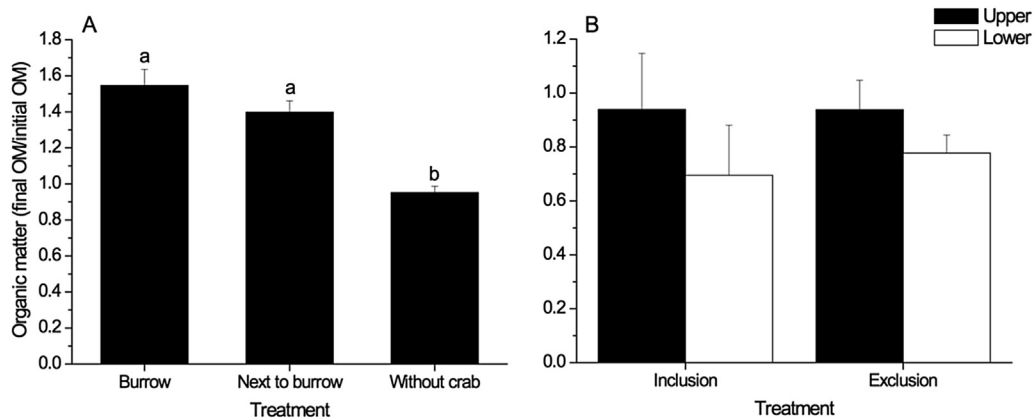


Fig. 3. Corrected (mean \pm SE) sedimentary organic matter content (A) in terrariums with and without *Leptuca leptodactyla* in the laboratory experiment ($n = 8$), and (B) in cages from the field experiment ($n = 3$). Different letters indicate statistical difference between treatments (Tukey’s test, $p < 0.05$).

the experimental period (Table 1, Figs. 3B, 4A and B). In the inclusion treatment, OM content varied from 3.21% to 6.66% in the upper layer and from 2.24% to 6.93% in the lower layer (Fig. 4A). As to the exclusion treatment, OM content varied from 3.22% to 6.37% and from 2.68% to 5.90% in the upper and lower layers, respectively (Fig. 4B). In the surrounding non-manipulated environment, OM content variation ranged from 2.70% to 27.21% in the upper layer and from 2.77% to 26.17% in the lower layer, with similar mean values in both layers. Also, we noticed a marked temporal variation in OM content, with a maximum on the 91st day (Fig. 4C).

Table 1

Two-way ANOVA used to evaluate the effects of treatment (inclusion and exclusion cages) and depth (upper and lower) on corrected organic matter content (final/initial OM ratio).

Source of variation	df	MS	F	p-value
Treatment (T)	1	0.005	0.07	0.796
Depth (D)	1	0.123	1.75	0.223
T \times D	1	0.005	0.07	0.793
Residue	8	0.070		

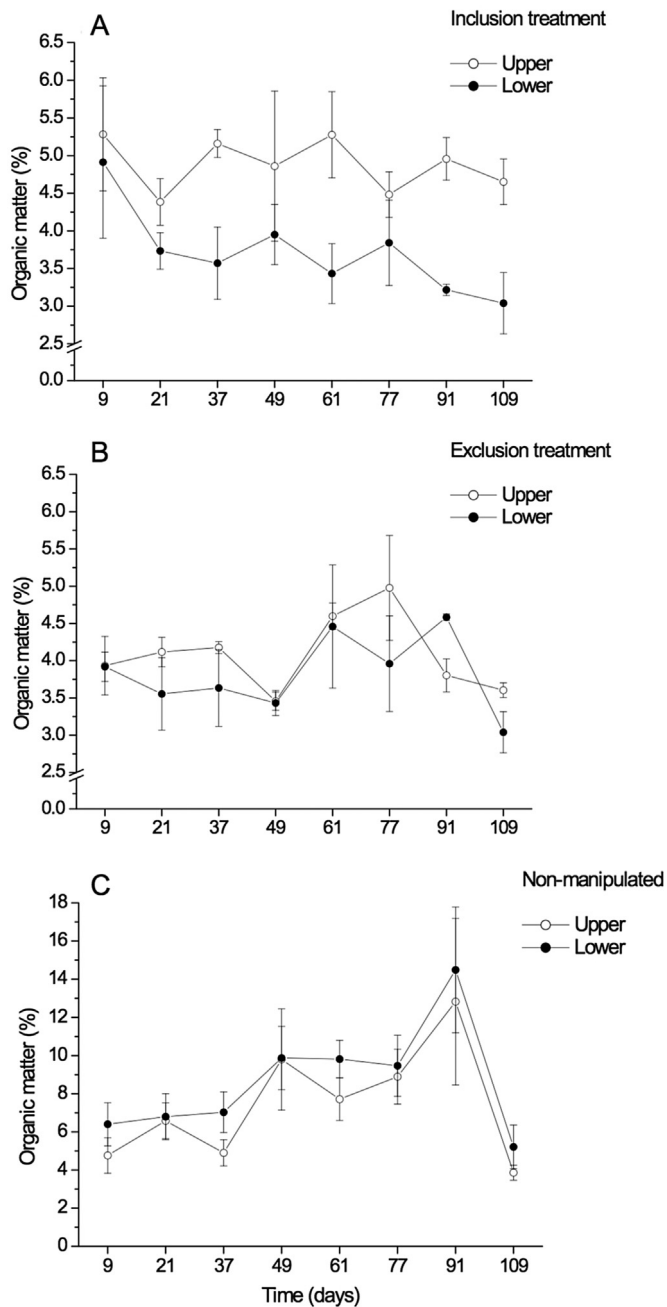


Fig. 4. Temporal variation of organic matter content (mean \pm SE) throughout the field experiment in the upper (0–4 cm) and lower (4–8 cm) soil layers of the *Leptuca uruguayensis* inclusion (A) and exclusion treatments (n = 3) (B), and non-manipulated environment (n = 5) (C).

Redox potential (Eh) and dissolved oxygen (DO) levels varied over time, but values were similar among inclusion and exclusion cages and non-manipulated environment along the experiment (Fig. 5), except for DO on day 49. Eh was always observed with positive mean values and was similar throughout the experiment for all treatments (~ 180 mV, Fig. 5A). In contrast, we observed a tendency to decreasing DO levels during the experiment inside the cages and in the non-manipulated environment (Fig. 5A). On the 91st day, both cage treatments and non-manipulated environment presented similar Eh (one-way ANOVA, $F_{(2,13)} = 0.323$, $p = 0.73$) and DO (one-way ANOVA, $F_{(2,13)} = 1.120$, $p = 0.356$) values.

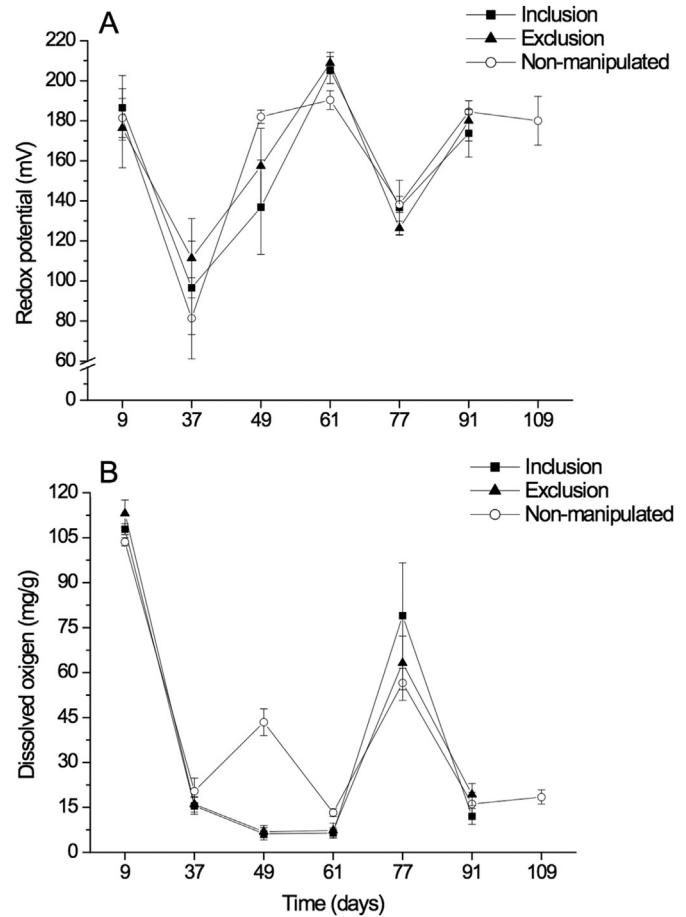


Fig. 5. Temporal variation of redox potential (A) and dissolved oxygen (B) levels (mean \pm SE) throughout the field experiment of the *Leptuca uruguayensis* inclusion and exclusion treatments and non-manipulated environment (n = 4–6).

4. Discussion

The experiment in laboratory showed clearly that the fiddler crab *L. leptodactyla* promotes vertical transport of sediment by reworking soil particles and organic matter (OM) along their burrow (sediment column) and in the surrounding area (sediment surface). Such result suggests these crabs are able to modify their own environment via bioturbation, being well-known ecosystem engineers (see Kristensen, 2008). However, from the field experiment, we observed that the activity of a closely related species *L. uruguayensis* may not be intense enough to affect OM distribution along vertical soil layers. In this sense, we partially confirm our hypothesis.

Due to the observed effects of burrowing found in the laboratory experiment with *L. leptodactyla*, we also expected to detect an effect of *L. uruguayensis* presence on the vertical distribution of OM in the field experiment. However, the homogeneous pattern of OM between upper and lower layers was maintained in both field inclusion and exclusion treatments, with a slight tendency to OM increase in the upper layer of the inclusion treatment. This suggests bioturbation promoted by *L. uruguayensis* may not alter the vertical distribution of sediment and associated elements, neither by extraction of surface OM for feeding (Reinsel, 2004; Sayão-Aguiar et al., 2012), trapped detritus in the burrow (Botto et al., 2006), nor by OM transportation from deeper layers. Moreover, bioturbator density and size correlate with the amount of burrow-excavated sediment (Lim and Diong, 2003; De Backer et al., 2011;

Pülmanns et al., 2014), which in turn alters the impact of bioturbation. Crab density is unlikely to explain the field results, mainly since it was higher than what has been reported in other studies of *L. uruguayensis* (Masunari, 2012; Machado et al., 2013). The small size of *L. uruguayensis* when compared to other burrowing estuarine crabs, such as other fiddler crabs, *Neohelice granulata* and *Ucides cordatus*, may explain the absence of an effect of this crab on OM distribution. Thus, burrowing by small crab species, be they of the same size or smaller than our model, may not be intense enough to modify OM distribution in the soil column of mangrove ecosystems or, at least, to generate remarkable alterations.

However, our lab results demonstrated that even small species of fiddler crabs (*L. leptodactyla*) have a great potential to transport sediment and OM to the soil surface by burrowing. Other studies have also reported bioturbation by fiddler crabs (mainly excavation and feeding behavior) modifying the distribution of nutrients and organic compounds (Botto and Iribarne, 2000; Kristensen and Alongi, 2006; Wang et al., 2010). Then, the OM transported by small crabs (e.g. *L. uruguayensis*) from lower layers to the soil surface may be carried and/or modified by biogeochemical and physical processes, masking the detection of a bioturbation effect. Bioturbation is already known for stimulating decomposition and remineralization of OM (Kristensen, 2000; Lohrer et al., 2004; Martinez-Garcia et al., 2015), which is essential to nutrient cycling (Ryther and Dunstan, 1971; Arndt et al., 2013), microbial activity (Canfield et al., 1993) and primary production (Howarth, 1988; Kreis et al., 2015). Also, crab burrowing behavior enhances nutrient flux in estuaries (Fanjul et al., 2011, 2015) and intensifies atmospheric release of CO₂ and H₂S (Pülmanns et al., 2014; Zhao et al., 2014). Physical processes may also be responsible for carrying dissolved organic carbon by leaching (Camilleri and Ribbi, 1986; Davis et al., 2006) and OM degradation (Arzayrus and Canuel, 2004). Organic matter may be dragged by tidal water (Tam and Wong, 1998; Tue et al., 2012; Cawley et al., 2014), since burrowing pellets of *L. uruguayensis* are completely disintegrated during high tides (Botto and Iribarne, 2000). Additionally, field data demonstrated maintenance of positive redox potential values and, in general, a decrease in dissolved oxygen values in puddles. A positive redox potential represents oxidative conditions and oxygen consumption, which in turn indicate remineralization processes (Aller, 1994; Kristensen and Holmer, 2001).

Unexpectedly, soil redox values did not differ between inclusion and exclusion treatments. Similarly, Mchenga et al. (2007) did not find differences in redox potential on soil surface with and without *Helice formosensis*, but observed variation of potential among layers of soil with burrows. Furthermore, due to the similarity of redox potential levels between cages and non-manipulated environment, we assure that the cage structure did not interfere in any natural processes that modify redox potential levels as response. On the other hand, the apparatus may have interfered on OM content inside the experimental cages. The non-manipulated environment exhibited a pattern of high values of OM in the lower layer, while we noticed accumulation of OM in the upper layer of sediment inside the cages. This may have been caused by the development of biofilm (LF Natalio, pers. obs.) and/or OM surface accumulation inside the cages, since the canvas and our monitoring prevented entering of grazing and deposit feeders. However, this pattern was detected in all cages, indicating a standardized influence in the inclusion and exclusion treatments.

Experimental studies in ecology are a useful way to test hypothesis and develop concepts (Underwood et al., 2000), as well as to provide an important tool for coastal management and ecosystem conservation (Castilla, 2000). Although the experimental apparatus may have biased some environmental

parameters in this study, empirical approaches in the field are a powerful tool to observe processes in natural conditions and are necessary to complement laboratory experiments. In this study, both field and laboratory experiment showed contrasting results and allowed us to reach a more complete and interesting insight regarding the role of fiddler crab as bioturbators. Therefore, we suggest further studies associating field and laboratory experiments in order to understand the role of bioturbators on ecosystem functioning and their contribution to ecosystem services.

5. Conclusion

The laboratory experiment with *L. leptodactyla* supported the hypothesis that fiddler crabs perform vertical transport of sediment and OM. Burrowing behavior continuously carries sediment and associated elements from lower to upper layers and potentially promotes OM accumulation on the soil surface. However, the field experiment with *L. uruguayensis* showed a different conclusion, suggesting other strong variables interacting with transported OM and preventing surface accumulation. Therefore, although the ability of fiddler crabs in transporting and redistributing sediment and OM by burrowing, the synergy between their bioturbation and other environmental factors may promote more complex effects in OM distribution than that expected with vertical transport caused by crabs solely.

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References

- Aller, R., 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. *Chem. Geol.* 114, 331–345. [http://dx.doi.org/10.1016/0009-2541\(94\)90062-0](http://dx.doi.org/10.1016/0009-2541(94)90062-0).
- 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. *J. Sea Res.* 85, 524–533. <http://dx.doi.org/10.1016/j.seares.2013.08.010>.
- Araújo Jr., J.M.C., Otero, X.L., Marques, A.G.B., Nóbrega, G.N., Silva, J.R.F., Ferreira, T.O., 2012. Selective geochemistry of iron in mangrove soils in a semiarid tropical climate: effects of the burrowing activity of the crabs *Ucides cordatus* and *Uca maracoani*. *Geo-Marine Lett.* 32, 289–330. <http://dx.doi.org/10.1007/s00367-011-0268-5>.
- Arndt, S., Jørgensen, B.B., LaRowe, D.E., Middelburg, J.J., Pancost, R.D., Regnier, P., 2013. Quantifying the degradation of organic matter in marine sediments: a review and synthesis. *Earth-Science Rev.* 123, 53–86. <http://dx.doi.org/10.1016/j.earscirev.2013.02.008>.
- Arzayrus, K.M., Canuel, E.A., 2004. Organic matter degradation in sediments of the York River estuary: effects of biological vs. physical mixing. *Geochimica Cosmochimica Acta* 69 (2), 455–463. <http://dx.doi.org/10.1016/j.gca.2004.06.029>.
- Botto, F., Iribarne, O., 2000. Contrasting effects of two burrowing crabs (*Chasmagnathus granulata* and *Uca uruguayensis*) on sediment composition and transport in estuarine environments. *Estuar. Coast. Shelf Sci.* 51, 141–151. <http://dx.doi.org/10.1006/ecss.2000.0642>.
- Botto, F., Iribarne, O., Gutierrez, J., Bava, J., Gagliardini, A., Valiela, I., 2006. Ecological

- importance of passive deposition of organic matter into burrows of the SW Atlantic crab *Chasmagnathus granulatus*. Mar. Ecol. Prog. Ser. 312, 201–210. <http://dx.doi.org/10.3354/meps312201>.
- Camilleri, J.C., Ribbi, G., 1986. Leaching of dissolved organic carbon (DOC) from dead leaves, formation of flakes from DOC, and feeding on flakes by crustaceans in mangroves. Mar. Biol. 91, 337–344. <http://dx.doi.org/10.1007/BF00428627>.
- Canfield, D.E., Thamdrup, B., Hansen, J.W., 1993. The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction. Geochimica Cosmochimica Acta 57 (16), 3867–3883. [http://dx.doi.org/10.1016/0016-7037\(93\)90340-3](http://dx.doi.org/10.1016/0016-7037(93)90340-3).
- Caravello, H.E., Cameron, G.N., 1987. The effects of sexual selection on the foraging behaviour of the Gulf Coast fiddler crab, *Uca panacea*. Anim. Behav. 35, 1864–1874. [http://dx.doi.org/10.1016/S0003-3472\(87\)80079-9](http://dx.doi.org/10.1016/S0003-3472(87)80079-9).
- Cardoso, R.C.F., Negreiros-Franzoso, M.L., 2004. A comparison of the allometric growth in *Uca leptodactyla* (Crustacea: Brachyura: ocypodidae) from two subtropical estuaries. J. Mar. Biol. Assoc. U. K. 84, 733–735. <http://dx.doi.org/10.1017/S0025315404009828h>.
- Castilla, J.C., 2000. Roles of experimental marine ecology in coastal management and conservation. J. Exp. Mar. Biol. Ecol. 250, 3–21. [http://dx.doi.org/10.1016/S0022-0981\(00\)00177-5](http://dx.doi.org/10.1016/S0022-0981(00)00177-5).
- Chatterjee, S., 2014. Reproductive biology and bioturbatory activities of two sympatric species of fiddler crab *Uca lactea annulipes* and *Uca triangularis* Bengali (Decapoda: ocypodidae) at the East Midnapore Coastal Belt of West Bengal, India. J. Biol. Life Sci. 2 (5), 106–127. <http://dx.doi.org/10.5296/jbls.v5i2.5809>.
- Cawley, K.M., Yamashita, Y., Maie, N., Jaffé, R., 2014. Using optical properties to quantify fringe mangrove inputs to the dissolved organic matter (DOM) pool in a subtropical estuary. Estuaries Coasts 37, 399–410. <http://dx.doi.org/10.1007/s12237-013-9681-5>.
- Crane, J.H., 1975. Fiddler Crabs of the World. Ocypodidae: Genus *Uca*. Princeton University Press, New Jersey, p. 635.
- Davis, S.E., Childers, D.L., Noe, G.B., 2006. The contribution of leaching to the rapid release of nutrients and carbon in the early decay of wetland vegetation. Hydrobiologia 569, 87–97. <http://dx.doi.org/10.1007/s10750-006-0124-1>.
- De Backer, A., Coillie, F.V., Montserrat, F., Provoost, P., Van Colen, C., Vincx, M., Degraer, S., 2011. Bioturbation effects of *Corophium volutator*: importance of density and behavioural activity. Estuar. Coast. Shelf Sci. 91, 306–313. <http://dx.doi.org/10.1016/j.ecss.2010.10.031>.
- Donato, D.C., Kauffman, J.B., Murdiyarsa, D., Kurnianto, S., Stidham, M., Kanninen, M., 2011. Mangroves among the most carbon-rich forests in the tropics. Nat. Geosci. 4, 293–297. <http://dx.doi.org/10.1038/ngeo1123>.
- Eichler, P.P.B., Eichler, B.B., David, C.J., de Miranda, L.B., Sousa, E.C.P.M., 2006. The Estuary Ecosystem of Bertioga, São Paulo, Brazil. J. Coast. Res. 1110–1113.
- Fanjul, E., Bazterrica, M.C., Escapa, M., Grela, M.A., Iribarne, O., 2011. Impact of crab bioturbation on benthic flux and nitrogen dynamics of Southwest Atlantic intertidal marshes and mudflats. Estuar. Coast. Shelf Sci. 92, 629–638. <http://dx.doi.org/10.1016/j.ecss.2011.03.002>.
- Fanjul, E., Escapa, M., Montemayor, D., Addino, M., Alvarez, M.F., Grela, M.A., Iribarne, O., 2015. Effect of crab bioturbation on organic matter processing in South West Atlantic intertidal sediments. J. Sea Res. 95, 206–216. <http://dx.doi.org/10.1016/j.seares.2014.05.005>.
- Gagnon, J.M., Beaudin, L., Silverberg, N., Mauviel, A., 2013. Mesocosm and in situ observations of the burrowing shrimp *Calocaris templemani* (Decapoda: thalassinidea) and its bioturbation activities in soft sediments of the Laurentian Trough. Mar. Biol. 160 (10), 2687–2697. <http://dx.doi.org/10.1007/s00227-013-2262-0>.
- Gianguarante, A., Licciano, M., Fanelli, G., 2001. Bioturbation behaviour in two Mediterranean polychaetes. J. Mar. Biol. Assoc. U. K. 81, 341–342. <http://dx.doi.org/10.1017/S0025315401003836>.
- Harris, R.J., Pilditch, C.A., Hewitt, J.E., Lohrer, A.M., Van Colen, C., Townsend, M., Thrush, S.F., 2015. Biotic interactions influence sediment erodibility on wave-exposed sandflats. Mar. Ecol. Prog. Ser. 523, 15–30. <http://dx.doi.org/10.3354/meps11164>.
- Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J. Paleolimnol. 25 (1), 101–110. <http://dx.doi.org/10.1023/A:1008119611481>.
- Hirose, G.L., Fransozo, V., Tropea, C., López-Greco, L.S., Negreiros-Franzoso, M.L., 2013. Comparison of body size, relative growth and size at onset sexual maturity of *Uca uruguayensis* (Crustacea: Decapoda: ocypodidae) from different latitudes in the south-western Atlantic. J. Mar. Biol. Assoc. U. K. 93, 781–788. <http://dx.doi.org/10.1017/S0025315412001038>.
- Howarth, R.W., 1988. Nutrient limitation of net primary production in marine ecosystems. Annu. Rev. Ecol. Syst. 19, 89–110. <http://dx.doi.org/10.1146/annurev.es.19.110188.000513>.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69 (3), 373–386. <http://dx.doi.org/10.2307/3545850>.
- Kanaya, G., 2014. Effects of infaunal bivalves on associated macrozoobenthic communities in estuarine soft-bottom habitats: a bivalve addition experiment in a brackish lagoon. J. Exp. Mar. Biol. Ecol. 457, 180–189. <http://dx.doi.org/10.1016/j.jembe.2014.03.022>.
- Kreus, M., Schartau, M., Engel, A., Nausch, M., Voss, M., 2015. Variations in the elemental ratio of organic matter in the central Baltic Sea: Part I - linking primary production to remineralization. Cont. Shelf Res. 100, 25–45. <http://dx.doi.org/10.1016/j.csr.2014.06.015>.
- Kristensen, E., 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426, 1–24. <http://dx.doi.org/10.1023/A:1003980226194>.
- Kristensen, E., 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. J. Sea Res. 59, 30–43. DOI: 0.1016/j.seares.2007.05.004.
- Kristensen, E., Alongi, D.M., 2006. Control by fiddler crabs (*Uca vocans*) and plant roots (*Avicennia marina*) on carbon, iron, and sulfur biogeochemistry in mangrove sediment. Limnol. Oceanogr. 51, 1557–1571. <http://dx.doi.org/10.4319/lo.2006.51.4.1557>.
- Kristensen, E., Flindt, M.R., Ulomi, S., Borges, A.V., Abril, G., Bouillon, S., 2008. Emission of CO₂ and CH₄ to the atmosphere by sediments and open waters in two Tanzanian mangrove forests. Mar. Ecol. Prog. Ser. 370, 53–67. <http://dx.doi.org/10.3354/meps07642>.
- Kristensen, E., Holmer, M., 2001. Decomposition of plant materials in marine sediment exposed to different electron acceptors (O₂, NO₃⁻, SO₄²⁻), with emphasis on substrate origin, degradation kinetics, and the role of bioturbation. Geochimica Cosmochimica Acta 65 (3), 419–433. [http://dx.doi.org/10.1016/S0016-7037\(00\)00532-9](http://dx.doi.org/10.1016/S0016-7037(00)00532-9).
- Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta, G.T., 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. 446, 285–302. <http://dx.doi.org/10.3354/meps09506>.
- Lim, S.S.L., Diong, C.H., 2003. Burrow-morphological characters of the fiddler crab, *Uca annulipes* (H. Milne Edwards, 1837) and ecological correlates in a lagoonal beach on Palau Hantu, Singapore. Crustaceana 76, 1055–1069. <http://dx.doi.org/10.1163/156854003322753411>.
- Lohrer, A.M., Thrush, S.M., Gibbs, M.M., 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431, 1092–1095. <http://dx.doi.org/10.1038/nature03042>.
- Machado, G.B.O., Gusmão Junior, J.B.L., Costa, T.M., 2013. Burrow morphology of *Uca uruguayensis nobilis*, 1901 and *Uca leptodactylus* Rathbun, 1898 (Decapoda: ocypodidae) from a subtropical mangrove forest in the western Atlantic. Integr. Zool. 8, 307–314. <http://dx.doi.org/10.1111/j.17494877.2012.0029-7.x>.
- Martinetto, P., Montemayor, D.I., Alberti, J., Costa, C.S.B., Iribarne, O., 2016. Crab bioturbation and herbivory may account for variability in carbon sequestration and stocks in South West Atlantic salt marshes. Front. Mar. Sci. 3, 1–12. <http://dx.doi.org/10.3389/fmars.2016.00122>.
- Martinez-Garcia, E., Carlsson, M.S., Sanchez-Jerez, P., Sanchez-Lizaso, J.L., Sanz-Lazaro, C., Holmer, M., 2015. Effect of sediment grain size and bioturbation on decomposition of organic matter from aquaculture. Biogeochemistry 125, 133–148. <http://dx.doi.org/10.1007/s10533-015-0119-y>.
- Masanari, S., 2012. Hood construction as an indication of the breeding period of the fiddler crab *Uca* (*Leptuca*) *leptodactyla* Rathbun, 1898 (Decapoda, Ocypodidae) from Guaratuba bay, Southern Brazil. Crustaceana 85, 1153–1169. <http://dx.doi.org/10.2307/41720763>.
- Mchenga, I.S.S., Mflilinge, P.L., Tsuchiya, M., 2007. Bioturbation activity by the grassid crab *Helice formosensis* and its effects on mangrove sedimentary organic matter. Estuar. Coast. Shelf Sci. 73, 316–324. <http://dx.doi.org/10.1016/j.ecss.2007.01.016>.
- Meyersman, F.J., Middelburg, J.J., Heip, C.H., 2006. Bioturbation: a fresh look at Darwin's last idea. Trends Ecol. Evol. 21, 688–695. <http://dx.doi.org/10.1016/j.tree.2006.08.002>.
- Michaels, R.E., Ziemann, J.C., 2013. Fiddler crab (*Uca* spp.) burrows have little effect on surrounding sediment oxygen concentrations. J. Exp. Mar. Biol. Ecol. 448, 104–113. <http://dx.doi.org/10.1016/j.jembe.2013.06.020>.
- Murdiyarsa, D., Purboposito, J., Kauffman, J.B., Warren, M.W., Sasmito, S.D., Donato, D.C., Manuri, S., Krisnawati, H., Taberima, S., Kurnianto, S., 2015. The potentials of Indonesian mangrove forests for global change mitigation. Nat. Clim. Change. <http://dx.doi.org/10.1038/nclimate2734>.
- Nielsen, O.L., Kristensen, E., Macintosh, D.J., 2003. Impact of fiddler crabs (*Uca* spp.) on rates and pathways of benthic mineralization in deposited mangrove shrimp pond waste. J. Exp. Mar. Biol. Ecol. 289, 59–81. [http://dx.doi.org/10.1016/S0022-0981\(03\)00041-8](http://dx.doi.org/10.1016/S0022-0981(03)00041-8).
- Pülmans, N., Diele, K., Mehlig, U., Nordhaus, I., 2014. Burrows of the semi-terrestrial crab *Ucides cordatus* enhance CO₂ release in a North Brazilian mangrove forest. Plos One 9, 1–13. <http://dx.doi.org/10.1371/journal.pone.0109532>.
- Reinsel, K.A., 2004. Impact of fiddler crab foraging and tidal inundation on an intertidal sandflat: season-dependent effects in one tidal cycle. J. Exp. Mar. Biol. Ecol. 313, 1–17. <http://dx.doi.org/10.1016/j.jembe.2004.06.003>.
- Richards, P.J., Hohenthal, J.M., Humphreys, G.S., 2011. Bioturbation on a south-east Australian hillslope: estimating contributions to soil flux. Earth Surf. Process. Landforms 36, 1240–1253. <http://dx.doi.org/10.1002/esp.2149>.
- Ryther, J.H., Dunstan, W.M., 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171, 1008–1013. <http://dx.doi.org/10.1126/science.171.3975.1008>.
- Sayão-Aguiar, B., Pinheiro, M.A.A., Colpo, K.D., 2012. Sediment bioturbation potential of *Uca rapax* and *Uca uruguayensis* as a result of their feeding activity. J. Crustacean Biol. 32, 223–229. <http://dx.doi.org/10.1163/193724011X615451>.
- Schaller, J., 2014. Bioturbation/bioirrigation by *Chironomus plumosus* as main factor controlling elemental remobilization from aquatic sediments? Chemosphere 107, 336–343. <http://dx.doi.org/10.1016/j.chemosphere.2013.12.086>.
- Shih, H.T., Ng, P.K.L., Davie, P.J.F., Schubart, C.D., Türkay, M., Naderloo, R., Jones, D., Liu, M.Y., 2016. Systematics of the family Ocypodidae Rafinesque, 1815 (Crustacea: Brachyura), based on phylogenetic relationships, with a reorganization of subfamily rankings and a review of the taxonomic status of *Uca* Leach, 1814,

- sensu lato and its subgenera. *Raffles Bull. Zoology* 64, 139–175. ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print).
- Smith III, T.J., Boto, K.G., Frusher, S.D., Giddins, R.L., 1991. Keystone species and mangrove forest dynamics: the influence of burrowing by crabs on soil nutrient status and forest productivity. *Estuar. Coast. Shelf Sci.* 33, 419–432. <http://dx.doi.org/10.1163/156854003322753411>.
- Tam, N.F.Y., Wong, Y.S., 1998. Variations of soil nutrient and organic matter content in a subtropical mangrove ecosystem. *Water, Air, Soil Pollut.* 103, 245–261. <http://dx.doi.org/10.1023/A:1004925700931>.
- Thomas, C., Blum, L.K., 2010. Importance of the fiddler crabs *Uca pugnax* to salt marsh soil organic matter accumulation. *Mar. Ecol. Prog. Ser.* 414, 167–177. <http://dx.doi.org/10.3354/meps08708>.
- Tue, N.T., Quy, T.D., Hamaoka, H., Nhuan, M.T., Omori, K., 2012. Sources and exchange of particulate organic matter in an estuarine mangrove ecosystem of Xuan Thuy National Park, Vietnam. *Estuaries coasts* 35, 1060–1068. <http://dx.doi.org/10.1007/s12237-012-9487-x>.
- Underwood, A.J., Chapman, M.G., Connell, S.D., 2000. Observations in ecology: you can't make progress on processes without understanding the patterns. *J. Exp. Mar. Biol. Ecol.* 250, 97–115. [http://dx.doi.org/10.1016/S0022-0981\(00\)00181-7](http://dx.doi.org/10.1016/S0022-0981(00)00181-7).
- Usio, N., Townsend, C.R., 2004. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. *Ecology* 85, 807–822. <http://dx.doi.org/10.1890/02-0618>.
- Volkenborn, N., Hedtkamp, S.I.C., Van Beusekom, J.E.E., Reise, K., 2007. Effects of bioturbation and bioirrigation by lugworms (*Arenicola marina*) on physical and chemical sediment properties and implications for intertidal habitat succession. *Estuar. Coast. Shelf Sci.* 74, 331–343. <http://dx.doi.org/10.1016/j.ecss.2007.05.001>.
- Wang, J.Q., Zhang, X.D., Jiang, L.F., Bertness, M.D., Fang, C.M., Chen, J.K., Hara, T., Li, B., 2010. Bioturbation of burrowing crabs promotes sediment turnover and carbon and nitrogen movements in an estuarine salt marsh. *Ecosystems* 13, 586–599. <http://dx.doi.org/10.1007/s10021-010-9342-5>.
- Weis, J.S., Weis, P., 2004. Behavior of four species of fiddler crabs, genus *Uca*, in southeast Sulawesi, Indonesia. *Hydrobiologia* 523, 47–58. <http://dx.doi.org/10.1023/B:HYDR.0000033093.84155.1d>.
- Wolfrath, B., 1992. Field experiments on feeding of European fiddler crab *Uca tan-geri*. *Mar. Ecol. Prog. Ser.* 90, 39–43.
- Zhao, H., Yang, W., Fang, C., Qiao, Y., Xiao, Y., Cheng, X., An, S., 2014. Effects of tidewater and crab burrowing on H₂S emission and sulfur storage in *Spartina alterniflora* marsh. *Clean. – Soil, Air, Water* 42 (9999), 1–7. <http://dx.doi.org/10.1002/clen.201300845>.