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Combined effect of burrowing mangrove crabs and tides on carbon fluxes

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ABSTRACT.—Sesarmid crabs act as mangrove ecosystem engineers due to their burrowing behavior in the sediment. The burial of leaves inside the sediment suggests a positive relationship between crab activity and carbon storage in mangrove forests. However, crab burrows increase the sediment-air interface and, thus, might amplify CO_2 fluxes from the sediment. Additionally, the tidal export of carbon from burrows acting as preferential flow paths may offset the enhancing effect of crab burrows on carbon storage. In this study, we investigated the interactive effect of burrowing crabs and tidal flows on mangrove carbon storage in a laboratory experiment. Significantly higher dissolved organic carbon (DOC) concentrations in the porewater were found in microtidal compared to mesotidal treatments, while the total amount of outflowing DOC was similar across tidal treatments. No significant effect of burrowing crabs on the DOC content of the porewater was found. Significantly lower CO_2 fluxes into the atmosphere were found in treatments with crabs present which is contrary to previous studies. We suggest that lower CO_2 flux values were a result of collapsed burrows that preserved the particulate organic carbon (POC) in deeper sediment layers. Previous studies, showing enhanced CO_2 fluxes from crab burrows, have been carried out in the field and did not take the potential of burrow collapse into account. We stress the importance of considering temporal variability in crab burrow stability and spatial variability in tidal dynamics when evaluating their interactive effect on carbon fluxes in mangrove forests.

Mangrove forests occupy the upper intertidal zone of soft sediment coasts at the land-sea interface of the (sub)tropics. They are characterized by harsh environmental conditions such as anoxic sediments, high temperatures and high salinity variations from daily tidal shifts.

Recognized as one of the most carbon-rich tropical ecosystems, mangrove forests store large amounts of carbon in a relatively small area (Matsui 1998, Fujimoto et al. 1999, Donato et al. 2011, Alongi 2014). They account for only 0.04% of the global ocean area but contribute 10%–15% of coastal organic carbon burial (Duarte et al. 2005, Giri et al. 2011, Alongi 2014, Collins et al. 2017). Besides below-ground root carbon, sediment organic matter (SOM) derived from within the forest (autochthonous) and adjacent terrestrial and marine sources (allochthonous) contribute to large belowground carbon stocks (Alongi 2014). The large belowground carbon stocks in mangrove forests can be retained for millennia due to the anoxic sediment conditions and the miniscule rate of microbial decay of organic matter (Atwood et al. 2017). The import, export and storage of organic carbon can vary greatly among locations, depending on sediment properties, temperature, precipitation, and geomorphological and hydrodynamical settings (Lee 1995, Bouillon et al. 2008, Alongi and Mukhopadhyay 2015, Bulmer et al. 2015, Rovai et al. 2018, Spivak et al. 2019). Additionally, the mangrove fauna has a notable impact on the carbon dynamics of these ecosystems.

Many detritivorous crabs (Crustacea: Decapoda), e.g. of the family Sesarmidae (Steinke et al. 1993, Dahdouh-Guebas et al. 1997, MacKenzie et al. 2020), bury and store detritus (such as autochthonous leaf litter or allochthonous seagrass blades or algal thalli) inside their burrows. They remove 28%–90% of the annual litter fall by consumption or burial (Robertson 1986, Robertson and Daniel 1989, Micheli 1993, Slim et al. 1997). Even though crabs consume a considerable amount of leaves immediately after pulling them into their burrows, about half of the leaf litter is stored for later ingestion or longer storage (Kristensen 2008; for discussion, *see* Forgeron et al. 2021). Burial and feeding of mangrove litter by detritivorous crabs limits the export of organic matter from mangroves, facilitates microalgal growth (Kristensen et al. 2008) and increases decomposition and turnover rates of organic matter (Kristensen and Pilgaard 2001). Generally, the bioturbating fauna in mangrove forests alters the sediment matrix and, thus, changes abiotic sediment-air and sediment-water interface interactions (Sarker et al. 2020). Bioturbation increases oxygen diffusion into the (upper layers of the) sediment and affects redox zonation and microbial communities and their contribution to organic matter decay (Kristensen and Holmer 2001, Kostka et al. 2002, Kristensen et al. 2008, Gillis et al. 2019).

As a result, the presence of burrowing crabs may contribute to carbon storage in mangrove sediments (Fig. 1). For instance, Andreetta et al. (2014) have shown a causal relationship between crabs and the sediment organic carbon (SOC) content in a Kenyan mangrove forest. They found that under certain hydrogeomorphological conditions burrowing crabs had an enhancing effect on the SOC content. On the other hand, the larger surface area of crab burrows, as compared to the burrow-free forest floor, might promote respiration rates of organic carbon and, therefore, increase the CO_2 exchange between the sediment and the atmosphere. Xiao et al. (2021) have found that the gas-phase concentrations of CO_2 in crab burrows in a salt marsh were six times greater than in ambient air. In addition, crab burrows have

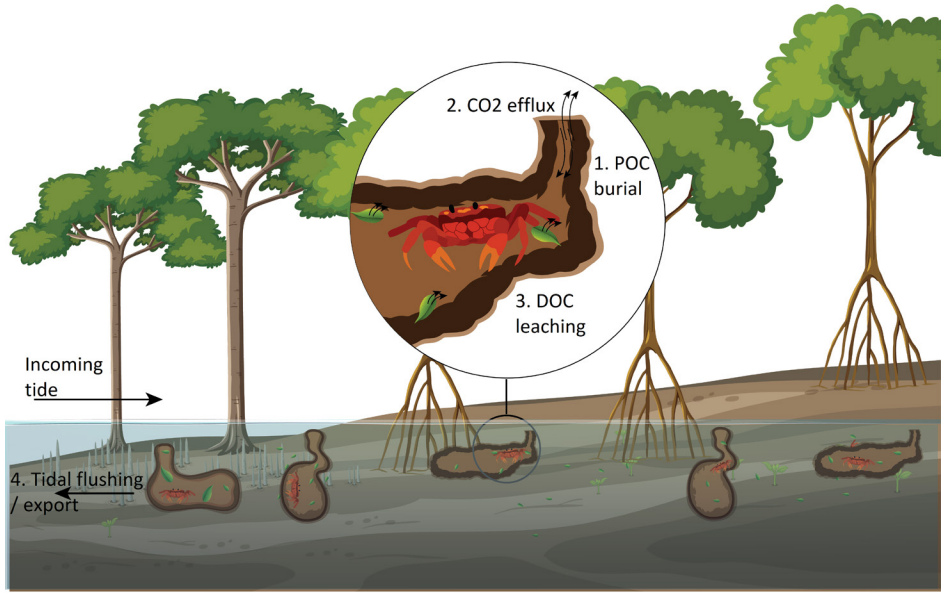

Figure 1. Schematic overview of potential impacts of crab burrows and tidal dynamics on carbon fluxes in mangrove forests, including (1) POC burial, (2) CO_2 efflux, (3) DOC leaching, and (4) tidal flushing/export.

a high permeability and can act as preferential flow paths in mangrove sediments upon tidal flushing (Tait et al. 2016) and, as such, facilitate organic carbon export from mangrove forests. For example, Stieglitz et al. (2013) estimated that the annual flushing of animal burrows in an Australian mangrove forest was equivalent to 20% of the annual river discharge in that region.

Given the uncertainties of the contribution of crabs and their burrows to carbon storage in mangrove forests, the primary objective of this study is to assess the combined impact of burrowing crabs and tidal flushing, using a microcosm experiment under controlled laboratory conditions.

Methods

The effects of crabs and tides on the dissolved organic carbon (DOC) and gaseous CO_2 flux were tested in a fully crossed experimental design. Crab treatments were defined by the presence or absence of crabs, whilst tides were defined by meso- and microtidal amplitudes.

Purchased specimens of *Neosarmatium africanum* (Sesarmidae; Ragionieri et al. 2012; formerly *Neosarmatium meinerti* De Man, 1887), a large semiterrestrial detritivorous and burrowing sesarmid crab, were used in the experiment. Crabs were of similar age, had a carapace size distribution between 19 and 55 mm and were equally distributed among experimental treatments. Including crabs of varying carapace sizes ensured a broad representation of different size classes, enhancing the generalizability of our findings across different crab sizes and preventing our results

from being limited to a specific size range. The experiment, using 12 replicates per treatment for a total of 48 microcosms, lasted for 10 consecutive days. Acrylic glass cylinders served as microcosms, arranged from bottom to top with layers of 5 cm gravel, 5 cm marbles, and 30 cm of sediment. Steel sieve cloths with a 1 mm mesh size were placed between the gravel and marble layers, and a finer 200 µm mesh separated the gravel from the sediment layer above. This layered arrangement was designed to prevent the loss of sediment from the system during tidal export. We used artificial sediment that was washed and homogenized before its introduction to the microcosms, aiming to reduce potential carbon contamination. The crabs were acclimated in aquaria under experimental conditions identical to those within the microcosms. In the experimental treatments with crabs present, crabs were starved for four days before entering the microcosms. Burrows with a diameter of approximately 4 cm and a depth of 15 cm were pre-dug into the sediment for treatments with crabs present.

Plugs that functioned as inlet and outlet valves for tidal manipulations were installed at the bottom of the microcosms. Tidal manipulations were realized using two peristaltic pumps (IPC-24 and IPC-N-24 microprocessor controlled multichannel dispensing pumps, Cole-Parmer, US, ISMATEC line, Germany). The pumps were programmed to cycle water in and out of the experimental units for a duration of 6 hours, respectively, which resulted in two complete tidal cycles per day (simulating natural semidiurnal tidal conditions). The water was pumped into the microcosms from a brackish water reservoir with a constant salinity of 17.5. The salinity was controlled several times during the experiment using a multiparameter meter (WTW 3420, Cole-Parmer, US), and adjusted if necessary. The water was pumped out of the microcosms into several collection tanks so that no organic carbon would reenter the system. In mesotidal treatments, the pumps were set to pump 300 ml into and out of the microcosms with a flow rate of 0.8334 ml min^{-1} per tidal cycle. For microtidal treatments, a flow rate of 0.2778 ml min^{-1} was utilized to pump 100 ml into and out of the microcosms per tidal cycle. Tides were adjusted so that for both meso- and microtidal treatments, the highest water level during high tide was 1 cm above the sediment. The tidal range was approximately 20 cm in mesotidal and 2 cm in microtidal treatments (note that in mesotidal treatments the water was flowing through more sediment with a higher porosity which explains the higher difference in tidal hub compared to differences in flow rates of meso- and microtidal treatments).

At the beginning of the experiment, four preweighed dried leaves of the mangrove species *Sonneratia alba* were placed into each microcosm as the primary source of organic carbon. Dried leaves had been collected from the same location and were at a similar degradation stage. After four days of the experiment, an additional preweighed leaf was added to all experimental units as some crabs had consumed nearly all leaves by then. After the experiment, the remaining leaves were sampled and dried for further analysis. The full experimental setup is visualized in Figure 2.

DOC Sampling and Analysis.—Porewater samples were collected three times throughout the experiment [at the start of the experiment (t_0), after 4 days (t_1), and after 10 days (t_2)]. For that, 10 ml of porewater was sampled directly from the outlet valves of the microcosms. Samples were filtered through 0.45 µm filters (Sartorius Stedim BioTech, Germany) and transferred into 24 ml glass vials. Samples were then

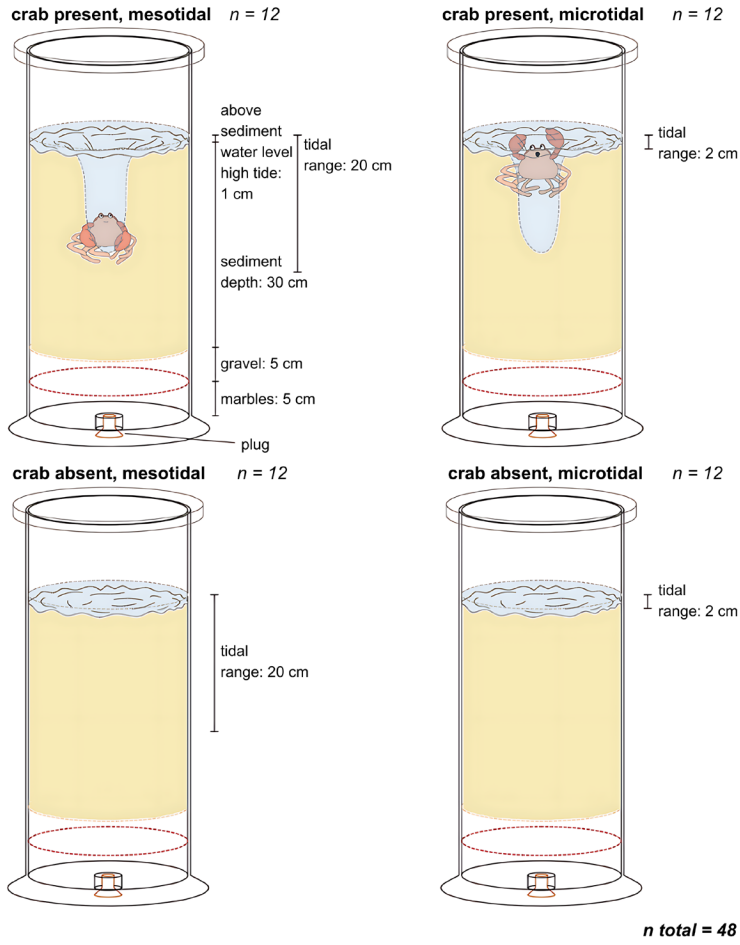

Figure 2. Experimental setup at the start of the experiment. The figure displays the crab and tidal treatments in the microcosms. From left to right: crab present, mesotidal; crab present, microtidal; crab absent, mesotidal; crab absent, microtidal.

immediately acidified with 125 µL of a about 2N HCl solution (32% HCl 1:5 dilution with MilliQ) and stored at 4 °C until the analysis was performed. Samples were analyzed for DOC content with a TOC analyzer (TOC-VCPH, Shimadzu, Japan) with high temperature combustion (720 °C) using established standard operation procedures (for a review on DOC analysis, *see* Halewood et al. 2022). For this, 75 µL of each sample was injected five times onto the catalyst bed of the analyzer where the sample was broken down into CO_2 and H_2O. CO_2 was then carried by ultrapure air to a nondispersive infrared (NDIR) sensor where CO_2 was detected. For the calibration, a standard solution of Potassium Hydrogen Phthalate (KHP, $C_8H_5KO_4$) was used. From the obtained DOC concentration values, we calculated the DOC concentration change (Equation 1) from t_0 to t_2.

$$\partial DOC_{\text{Conc}} = DOC_{\text{Conc},t_2} - DOC_{\text{Conc},t_0} \quad \text{(Eq. 1)}$$

Further, to account for the variance in outflowing water volumes across tidal treatments, we calculated the absolute DOC change (Equation 2), representing the total quantity of DOC exported from the system under each experimental condition.

$$\partial DOC_{\mathrm{Abs}} = \left(DOC_{\mathrm{Conc},t_2} \cdot V_{\mathrm{Out},t_2}\right) - \left(DOC_{\mathrm{Conc},t_0} \cdot V_{\mathrm{Out},t_0}\right) \quad \text{(Eq. 2)}$$

with $\partial DOC_{\mathrm{Conc}}$: changes of DOC concentration from t_0 to t_2 ($\frac{\mu\mathrm{mol}}{\mathrm{L}}$); $\partial DOC_{\mathrm{Abs}}$: changes of absolute DOC content from t_0 to t_2 (µmol); DOC_{Conc,t_0}: DOC concentration at t_0 ($\frac{\mu\mathrm{mol}}{\mathrm{L}}$); DOC_{Conc,t_2}: DOC concentration at t_2 ($\frac{\mu\mathrm{mol}}{\mathrm{L}}$); V_{Out,t_0}: total outflowing water volume at t_0 (meso- and microtidal: 0.01 L); V_{Out,t_2} = total outflowing water volume at t_2 (mesotidal: 6 L, microtidal: 2 L).

CO_2 Flux Sampling and Analysis.—Gaseous CO_2 fluxes were analyzed on days 8 and 9 of the experiment. Fluxes were measured in the dark using NDIR CO_2 sensors (K33-BLG, CO2Meter, US). For this, the microcosms were hermetically closed by fixing cups on the cylinders using vapor barrier tape. Measurements were conducted during low tide for both tidal treatments. Prior to the measurements, the crabs were removed from the microcosms. After installation, the sensors ran for 2 minutes to reach an equilibrium and steady air loop inside the chamber. Measurements were then taken every 30 seconds for 5 minutes. The CO_2 flux data was then processed using the software GasLab® (CO2Meter, US), and CO_2 fluxes were calculated according to Leopold et al. (2013) using the following equation:

$$F = \frac{\partial p\mathrm{CO}_2}{\partial t} \cdot \frac{V}{RTS} \quad \text{(Eq. 3)}$$

with F: CO_2 flux rate ($\frac{\mu mol\ \mathrm{CO}_2}{m^2 \cdot \mathrm{min}}$) ; ∂pCO_2: variation of CO_2 (ppm) ; ∂t: measurement time (min) ; V: volume of the measurement chamber (m^3); R: ideal gas constant (8,20528 * 10^{-05} $\frac{\mathrm{atm} \cdot m^3}{K \cdot \mathrm{mol}}$); T: absolute air temperature (K); S: surface area of the measurement chamber (m^2).

DOC Leaching Rate Analysis.—DOC leaching rates of the *S. alba* leaves were approximated in tidal treatments without crabs for sampling dates t_1 and t_2. Leaching rates were estimated based on the assumption that leaves were the only source of organic carbon in the microcosms, with the understanding that minor contributions from nonsterile conditions may have also been present. These values should therefore be considered as estimates (for a comprehensive review on leaching in mangroves, *see* Mamidala et al. 2023). Leaching rates were calculated using the following equation:

$$DOC_{\mathrm{le}} = \frac{\left(DOC_{t_0} \cdot V_{\mathrm{Out}} + \frac{\partial DOC \cdot V_{\mathrm{Cyl}}}{t_x}\right)}{m_{\mathrm{leave}}} \quad \text{(Eq. 4)}$$

with DOC_{le}: DOC leaching rate ($\frac{\mu mol}{g \cdot \mathrm{day}}$); DOC_{to}: DOC content at t_0 ($\frac{\mu\mathrm{mol}}{\mathrm{L}}$); ∂DOC: variation of DOC between t_0 and t_1 or t_2 ($\frac{\mu\mathrm{mol}}{\mathrm{L}}$) ; V_{Out}: daily tidal outflow (mesotidal: 0.6L, microtidal: 0.2L); V_{Cyl}: volume of the cylinder, calculated incorporating porosity values of the sediment, gravel and marble layers (2.57 L); t_x: days of t_1 (4) or t_2 (10); m_{leave}: initial dry mass of the leaves (g; note that for DOC_{le} calculations for t_2 the added leave weight at t_1 was incorporated).

Statistical Analysis.—All statistical tests and data visualization were carried out using R Studio, v4.0.5 (RStudio Team 2021). For data comparison, significance was accepted at a level of $\alpha = 0.05$ for all statistical tests conducted.

As whole leaves of *S. alba* were used in the experiment it was not possible to control for a similar weight of the leaves among all experimental units. Therefore, leaf weight (hereinafter referred to as POM) was used as a covariate in the statistical analysis. The effects of crabs and tides on DOC concentration changes and absolute DOC changes were tested using two-way Analyses of Covariance (ANCOVA) whilst correcting for POM. In our fully crossed design, the factor tide was defined by micro- vs mesotidal amplitudes and the factor crab was defined by presence vs. absence of the crabs. For the two-way ANCOVA models, the assumptions of normality of residuals, homogeneity of variances, linearity and homogeneity of regression slopes were tested by visual inspections of diagnostic plots and statistical validation. Our experimental design inherently supported the assumption of independence since the replicates were treated as separate experimental units that were randomly allocated several times throughout the experiment. The effects of crabs and tides on CO_2 flux were tested similarly using a two-way ANCOVA correcting for POM. CO_2 flux data was square root (sqrt) transformed to fulfill the assumption of normality of residuals (Shapiro–Wilks test: $W_{(40)} = 0.97$, $P = 0.21$). The effects of tides and time on leaching rates were tested using a two-way Analysis of Variance (ANOVA).

Results

DOC.—DOC concentrations in mesotidal and microtidal treatments, with and without crabs, showed an increase over time (Fig. 3A and B). Total outflowing DOC

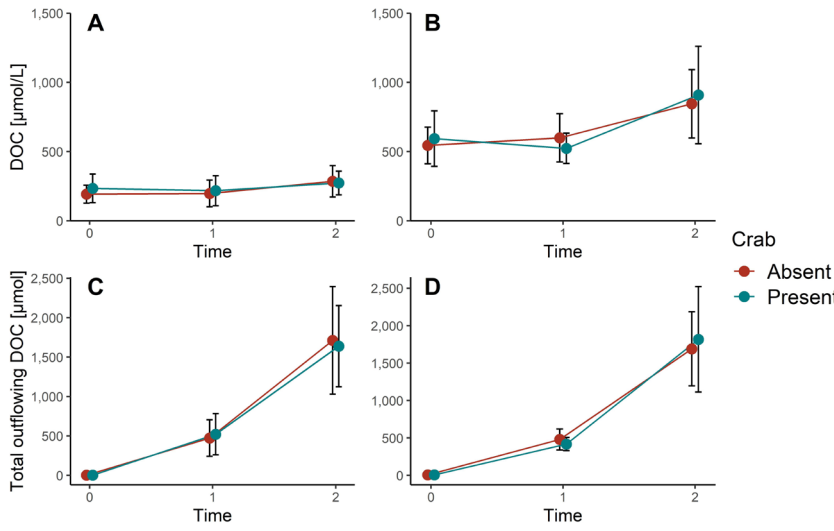

Figure 3. (A, B) DOC concentration and (C, D) outflow in different tidal and crab treatments for sampling times t_0, t_1, and t_2. A and C: mesotidal treatments; B and D: microtidal treatments. Data points and whiskers show the mean values and standard deviation ($n = 12$ for each treatment at each sampling time) and are connected by lines for visualization of trends.

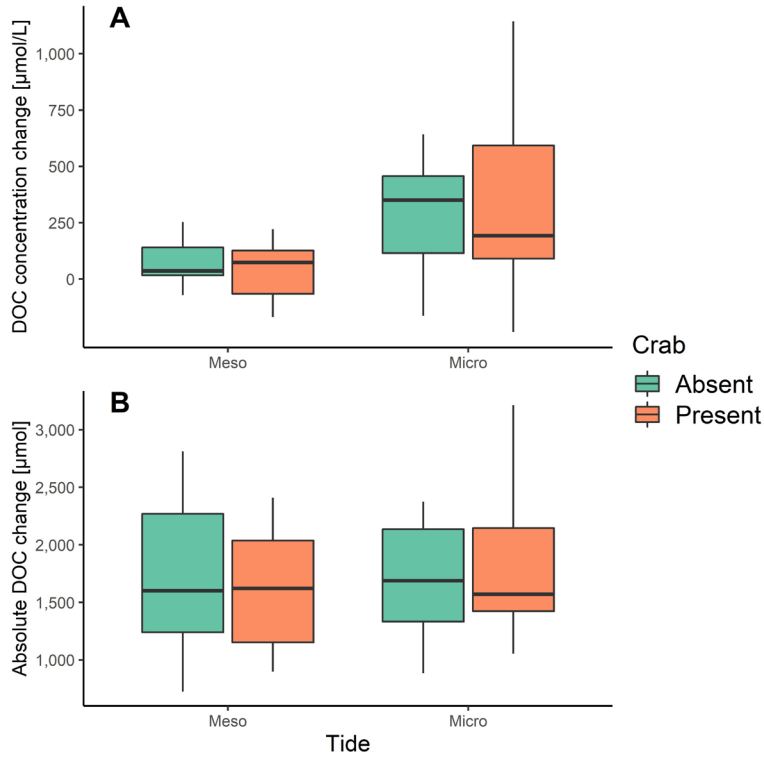

Figure 4. (A) DOC concentration change (µmol L^{-1}) and (B) absolute DOC change (µmol) in the experimental treatments between sampling dates t_1 and t_2. The tidal treatments are displayed on the *x*-axis and the crab treatments are visualized using colored boxplots.

increased similarly between meso- and microtidal treatments, with and without crabs (Fig. 3C and D).

The mean change in DOC concentration was significantly higher in microtidal (308 ± 342 µmol L^{-1}) compared to mesotidal treatments (66 ± 122 µmol L^{-1}; Fig. 4A; Table 1, factor "Tide": $F_{(1,40)} = 12.25$, $P < 0.01$). The presence of crabs did not affect the changes in DOC concentration in both tidal treatments, with mean values of 197 ± 224 µmol L^{-1} and 177 ± 334 µmol L^{-1} for crabs absent and present, respectively. The absolute DOC changes were neither affected by tidal nor crab treatments, with mean values

Table 1. Two-way ANCOVA of DOC. Sources of variation are the independent variables tides, crabs, the covariate POM and all their interaction terms. The response variables are DOC concentration change and absolute DOC change. The table displays the degrees of freedom (df), sum of squares (SS), *F*- and *P*-values (significance levels). Significant effects ($P < 0.05$) are highlighted with bold characters.

	DOC concentration change				Absolute DOC change			
Source of variation	df	SS	*F*	*P*	df	SS	*F*	*P*
Tide	1	701,075	12.2525	**0.0012**	1	68,142	0.1912	0.6643
Crab	1	4,622	0.0808	0.7778	1	8,404	0.0236	0.8787
POM	1	373,922	6.5349	**0.0145**	1	1,241,554	3.4833	0.0693
Tide × Crab	1	9,912	0.1732	0.6795	1	1,783	0.0050	0.9440
Tide × POM	1	141,397	2.4712	0.1238	1	382,051	1.0719	0.3067
Crab × POM	1	108,343	1.8935	0.1764	1	2,026	0.0057	0.9403
Tide × Crab × POM	1	109,889	1.9205	0.1735	1	407,412	1.1430	0.2914

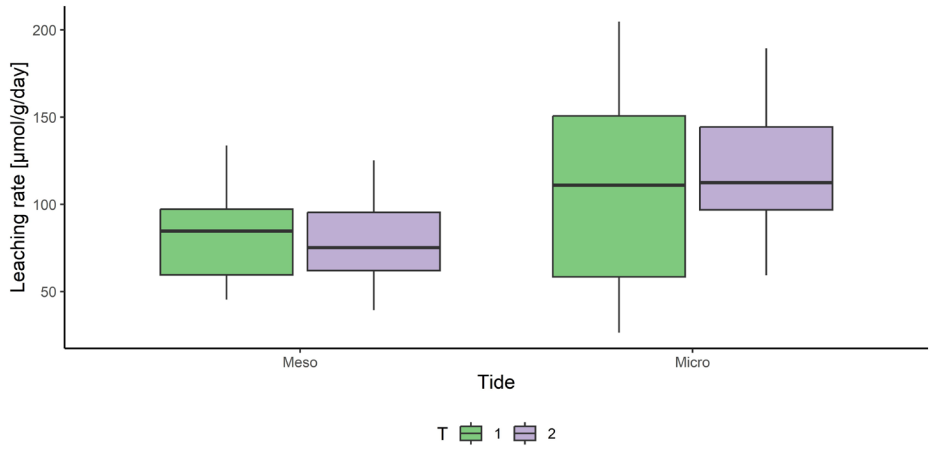

Figure 5. DOC leaching rates (µmol g^{-1} d^{-1}) in the tidal treatments with crabs absent at sampling times t_1 and t_2. The tidal treatments are displayed on the *x*-axis and the sampling date is visualized using colored boxplots (t_1 = green, t_2 = purple).

of 1748 ± 598 µmol and 1673 ± 592 µmol for microtidal and mesotidal treatments, respectively (Fig. 4B). The ANCOVA results indicate statistical significance of the covariate "POM" ($F_{(1,40)}$ = 6.53, P = 0.01) on DOC concentration changes. Neither DOC metrices were affected by any interaction terms (Table 1).

Leaching Rates.— DOC leaching rates ranged around similar values for t_1 and t_2 (Fig. 5). In mesotidal treatments, the leaching rate was 86 ± 36 µmol g^{-1} d^{-1} at t_1 and 80 ± 26 µmol g^{-1} d^{-1} at t_2. Leaching rates were significantly higher in microtidal treatments, reaching 109 ± 62 µmol g^{-1} d^{-1} at t_1 and 119 ± 38 µmol g^{-1} d^{-1} at t_2 (Table 2, factor "Tide": $F_{(1,21)}$ = 6.23, P < 0.02).

CO_2.—Gaseous CO_2 flux rates showed similar mean values between the tidal treatments (mesotidal: 113 ± 62, microtidal: 115 ± 60 mmol CO_2 m^{-2} d^{-1}, Fig. 6). For both tidal treatments, CO_2 flux rates were significantly higher with crabs absent (134 ± 72 mmol CO_2 m^{-2} d^{-1}) than with crabs present (94 ± 37 mmol CO_2 m^{-2} d^{-1}; Table 3, factor "Crab": $F_{(1,40)}$ = 4.00, P = 0.04). The factor "Tide" and the interaction term did not have a significant effect on the CO_2 flux rate.

Table 2. Two-way ANOVA of leaching rate. Sources of variation are the independent variables tides and sampling date *t* and their interaction term. The response variable is leaching rate. The table displays the degrees of freedom (df), sum of squares (SS), *F*- and *P*-values (significance levels). Significant effects (P < 0.05) are highlighted with bold characters.

Source of variation	df	SS	*F*	*P*
Tide	1	11398	6.2250	**0.0164**
t	1	34	0.0186	0.8922
Tide × *t*	1	831	0.4540	0.5040

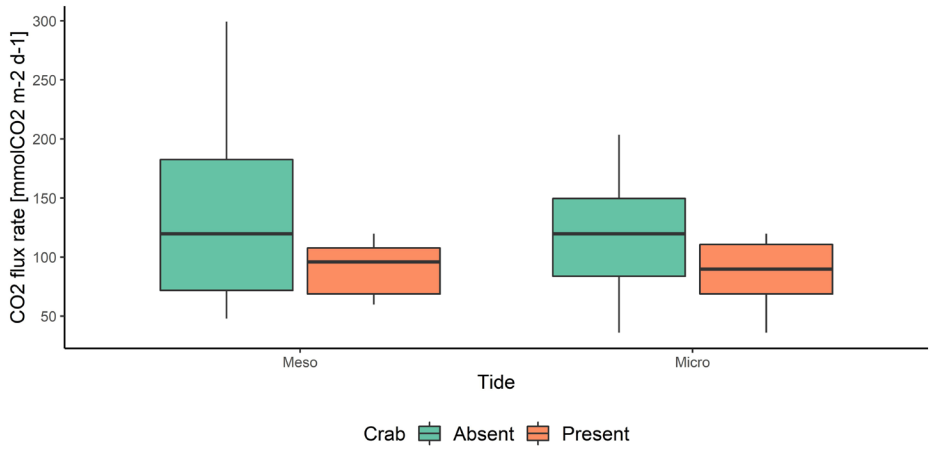

Figure 6. CO_2 flux rate (mmol CO_2 m^{-2} d^{-1}) in different experimental treatments. The tidal treatments are displayed on the *x*-axis and the crab treatments are visualized using colored boxplots (crab absent: blue, crab present: orange).

Table 3. Two-way ANCOVA of CO_2 flux. Sources of variation are the independent variables tides, crabs, the covariate POM and all their interaction terms. The response variable is CO_2 flux rate. CO_2 flux rate data was square root–transformed to obtain normality of residuals of the ANCOVA model. The table displays the degrees of freedom (df), sum of squares (SS), *F*- and *P*-values (significance levels). Significant effects ($P < 0.05$) are highlighted with bold characters.

Source of Variation	df	SS	*F*	*P*
Tide	1	0.0018	0.0003	0.9870
Crab	1	30.3716	4.0010	**0.0381**
POM	1	4.4545	0.6748	0.4163
Tide × Crab	1	5.0253	0.7613	0.3881
Tide × POM	1	10.5056	1.5914	0.2144
Crab × POM	1	0.2733	0.0414	0.8398
Crab × Tide × POM	1	4.1731	0.6322	0.4313

Discussion

No existing studies explicitly point out how burrowing crabs affect carbon storage, fluxes or dynamics in mangrove forests under different tidal conditions. Whereas a positive coupling between the presence and activity of burrowing crabs and carbon storage in mangrove forests has been suggested, there is prevailing consensus that crab burrows also enhance carbon losses from sediments by creating a larger sediment surface area, facilitating organic matter decay and decomposition through creating a larger volume of sediment with oxic conditions, and enhancing carbon export through tidal flushing. In our laboratory experiment under controlled conditions, the presence of burrowing crabs (*N. africanum*) did not have a significant impact on DOC concentrations of the porewater. Significantly higher DOC leaching rates were found in microtidal vs. mesotidal treatments, and DOC concentrations were significantly higher in microtidal than in mesotidal treatments. The total outflowing DOC was not significantly affected by tides or crabs. Gaseous CO_2 fluxes from the sediment into the atmosphere were significantly higher without crabs than when crabs were present, whereas tidal treatments did not affect CO_2 fluxes. We will discuss these findings in turn.

Crabs and Carbon Storage.—With increased outflowing DOC values over time in all treatments due to leaching from *S. alba* leaves, the presence of crabs did not significantly impact DOC concentrations in the porewater or the total amount of outflowing DOC. This might be due to several reasons. It was visually perceived throughout the experiment that crabs buried leaves into the sediment, which would result in a higher net increase in DOC contents compared to treatments with crabs absent. However, they also consumed most of the leaves (mesotidal: 60 ± 20%, microtidal 80 ± 20% of initial dry weight). Sesarmid crabs can have high assimilation efficiencies (up to 60% when feeding on decayed litter, Giddins et al. 1986). Consequently, despite burial and organic carbon possibly re-entering the system in form of feces, the crabs also might have assimilated large parts of the leaf material and counteracted the effect of buried leaves and feces on carbon contents in the sediment, at the same time fixing carbon in their biomass rather than releasing it into the porewater or the atmosphere.

On the other side, the experimental design did not allow for a horizontal export of the leaves. When not removed by consumption or burial (Forgeron et al. 2021), mangrove leaf litter may be exported with outgoing ebb tides in nature. Mangrove crabs can contribute to organic matter retention by consuming roughly 80% of the litterfall (Robertson and Daniel 1989, Nordhaus et al. 2006). Consequently, if horizontal export had not been suppressed in our experimental design, leaves might have been flushed out, resulting in lower DOC concentrations in treatments without crabs than with crabs present. Moreover, in this study, DOC was chosen to be the main response variable, as DOC is, besides dissolved inorganic carbon (DIC) and particular organic carbon (POC), the major driver of organic carbon export from mangrove forests (Twilley 1985, Bouillon et al. 2007, Kristensen et al. 2008, Ray et al. 2018). By using DOC as the response variable, the effect of leaching from the leaves was incorporated which can be crucial for comparing tidal effects on organic carbon storage in mangroves. However, POC might be as important to incorporate when investigating the effect of burrowing crabs on organic carbon storage in mangrove forests and should be considered in future studies. In contrast to all other previous studies (to the best of our knowledge) that have investigated the effects of crab burrows on gaseous CO_2 fluxes (e.g. Pülmanns et al. 2014, Tomotsune et al. 2020, Xiao et al. 2021), the present study showed significantly lower CO_2 fluxes when crabs were present. Higher CO_2 fluxes from crab burrows have been explained by an increased sediment-air interface and enhanced microbial decomposition of organic matter (Twilley and Rivera-Monroy 2009). For instance, Kristensen (2008) summarizes that sesarmid crab burrows can increase the sediment-air interface area by 150%–380%. However, our experimental sediment was very low in organic matter content. Hence, we might speculate that any increase in surface area, and thus in oxygenation and aerobic microbial activity, would not have resulted in an increased CO_2 release. Further, the above-mentioned transformation of POM into crab biomass might counteract CO_2 or DOC being released from the leaf litter or crab feces. Finally, crab burrows collapsed in all replicates with crabs present several times throughout the experiment. Therefore, CO_2 flux values were lower in treatments with crabs present, because the sediment-air interface was not substantially enhanced by crab activity over time. Further, as the leaves were then trapped inside the sediment, less CO_2 might have diffused to the sediment-air interface where measurements were made. As our understanding of the temporal dynamics of sesarmid crab burrows in the field

is in its infancy, we can hardly estimate how burying detritus in the sediment upon burrow collapse would affect carbon dynamics in the field. We hold, however, that this aspect must be considered in future studies (in the field). Micheli et al. (1991) found that *N. africanum* burrows collapse after about three weeks. If particulate organic matter (POM) is present in the burrows and trapped inside the sediment after collapsing, this may counteract the positive coupling of burrowing crabs and carbon storage through CO_2 release from mangrove sediments.

Combined Effects of Crabs and Tides on Carbon Storage.—Significantly higher DOC concentrations were found in microtidal treatments than under mesotidal conditions. We explain this mainly by a reduced amount of solvent (porewater) and longer retention time of the porewater in microtidal settings, resulting in higher concentrations of dissolved carbon. This is supported by the fact that there was a similar total DOC outflow across tidal treatments, indicating a similar amount of carbon leached between tidal treatments but different dilution scales due to the volume of porewater available.

Previous studies have identified the tidal amplitude to be a major driver of carbon export from mangrove forests (Twilley 1985, Taillardat et al. 2018). However, the present study suggests similar DOC export despite higher DOC concentrations in microtidal conditions due to higher tidal flushing in mesotidal conditions. This underscores the influence of tidal dynamics on solute concentration processes within mangrove ecosystems, highlighting how variations in tidal amplitude can affect the concentration of dissolved organic matter. This finding might be of importance when comparing spatial variabilities among mangrove forests, as higher DOC concentrations in the porewater can affect microbial biomass and carbon mineralization rates (Montaño et al. 2007).

Conclusions.—Despite the limitations of this laboratory study, such as the lack of horizontal tidal import and export, limited mixing of the water column during tidal flushing, limited vertical and horizontal movement of crabs, the present study can serve as a primer and pilot study for future experiments. We recommend future experiments to use larger experimental units such as mesocosms to mimic natural tide movements that allow for assessing the impact of horizontal tidal flows on carbon storage. Further, future experiments could benefit from adjusting the initial quantity of organic matter to extend the duration of the experiment, since we needed to add *S. alba* leaves partway through the experiment as a balance between maintaining active crab treatment conditions and the complexity of introducing additional organic matter. Together, we show that carbon dynamics in mangroves might be affected by differences in tidal amplitude, with higher DOC concentrations in microtidal environments but similar total DOC export across micro- and mesotidal conditions. Further, the effects of burrowing crabs on carbon storage in mangrove forests can be extensively affected by burrow stability and should be investigated in more detail both in field and laboratory studies.

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