Effects of shading and simulated grazing on carbon sequestration in a tropical seagrass meadow

Martin Dahl¹*, Diana Deyanova¹, Liberatus D. Lyimo¹,², Johan Näslund³, Göran S. Samuelsson¹, Matern S. P. Molera⁴, Mats Björk¹ and Martin Gullström¹

¹Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm SE-106 91, Sweden; ²School of Biological Science, University of Dodoma, Box 338, Dodoma, Tanzania; ³Aquabiota Water Research, Stockholm 115 50, Sweden; and ⁴Institute of Marine Sciences, University of Dar es Salaam, P.O. Box 668, Zanzibar, Tanzania

Summary
1. There is an ongoing world-wide decline of seagrass ecosystems, one of the world’s most efficient carbon sink habitats. In spite of this, there is a clear lack of studies experimentally testing the effects of anthropogenic disturbances on carbon sequestration of seagrass systems.
2. We assessed the effects of two disturbances of global concern on the carbon sink function in a five-month in situ experiment within a tropical seagrass (Thalassia hemprichii) meadow by testing the impacts of shading and simulated grazing at two levels of intensity using shading cloths and clipping of shoot tissue. We measured the effects of these disturbances on the carbon sequestration process by assessing the net community production (NCP), carbon and nitrogen content in tissue biomass, and organic matter and THAA (total hydrolysable amino acids) in the sediment down to 40 cm depth.
3. Treatments of high-intensity shading and high-intensity clipping were similarly impacted and showed a significantly lower NCP and carbon content in the below-ground biomass compared to the seagrass control. No significant effects were seen in organic carbon, total nitrogen, C:N ratio and THAA in the sediment for the seagrass treatments. However, both clipping treatments showed different depth profiles of carbon and THAA compared to the seagrass control, with lower organic carbon and THAA content in the surface sediment. This can be explained by the clipping of shoot tissue causing a less efficient trapping of allochthonous carbon and reduced input of shredded seagrass leaves to the detritus sediment layer. In the clipping plots, erosion of the surface sediment occurred, which was also most likely caused by the removal of above-ground plant biomass.
4. Synthesis. Our findings show that during the course of this experiment, there were no impacts on the sedimentary carbon while the high-intensity disturbances caused a clear depletion of carbon biomass and reduced the seagrass meadow’s capacity to sequester carbon. From a long-term perspective, the observed effect on the carbon biomass pool in the high-intensity treatments and the sediment erosion in the clipping plots may lead to loss in sedimentary carbon.

Key-words: aquatic plant ecology, blue carbon sequestration, changing climate, disturbances, ecosystem production, in situ experiment, marine vegetation, natural carbon sinks

Introduction
Coastal habitats function as sinks for atmospheric CO₂ (Tokoro et al. 2014) and carbon sequestration in vegetated coastal ecosystems (i.e. seagrass meadows, mangrove forest and saltmarshes) is more efficient than in terrestrial ecosystems (Mcleod et al. 2011). Together, the coastal vegetated habitats constitute about half of the ‘blue carbon’ burial of the marine environment (Duarte et al. 2013), where seagrasses alone are responsible for 10–15% (Duarte, Middelburg & Caraco 2005; Kennedy & Björk 2009). The understanding of impacts on carbon sequestration in seagrass habitats from anthropogenic disturbance is limited, although human-induced impacts have drastically increased in recent decades and almost a third of all seagrass areas have been lost in the last 140 years (Waycott et al. 2009), with a current loss rate of about 1.5% year⁻¹ (Pendleton et al. 2012). The degradation of seagrass areas does not only lead to a decline in seagrasses’ ability to act as sinks of atmospheric CO₂ but could also cause a release of greenhouse gases from carbon-rich sediments to the atmosphere.

*Correspondence author. E-mail: martin.dahl@su.se

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The carbon sequestration efficiency of seagrass meadows is related to a high net primary production, a low decomposition rate in the sediment and the ability to trap allochthonous organic matter in the canopy (Fonseca & Cahalan 1992; Agawin & Duarte 2002; Hendriks et al. 2008; Duarte et al. 2011). Most seagrass meadows are net autotrophic (Duarte et al. 2010) and a high rate of photosynthesis is needed to build up the carbon biomass pool. The standing plant biomass is thus a major source of sedimentary carbon and could be seen as the first of three interconnected phases of the carbon storage process, followed by the detritus and refractory phases (Cebrian 1999). These phases act on different time-scales where the biomass and detritus phases comprise short-term carbon storage pools. The detritus phase occurs in the upper layer of the sediment where a large part of the biomass litter is being decomposed (Duarte & Cebrian 1996). The belowground plant parts are more decay resistant (Kenworthy & Thayer 1984; Klap, Hemminga & Boon 2000), and a large below-ground biomass could therefore increase carbon sequestration capacity. Through sedimentation, a smaller portion of the organic matter will be buried and becomes part of the long-term storage pool, the third phase, where the carbon has been remineralized into decay-resistant refractory carbon.

Eutrophication and sedimentation, with light reduction as their main impact, are globally seen as the major anthropogenic disturbances to seagrass ecosystems (Orth et al. 2006; Björk et al. 2008) and is decreasing the carbon burial capacity (Schmidt et al. 2012). Light reduction and a decrease in photosynthesis affect both morphological and physiological processes of the seagrass, such as reduced growth rate, loss of biomass (Terrados et al. 1998; Ruiz & Romero 2003) and depletion of stored carbohydrates in the rhizomes (Silva et al. 2013). The photosynthetic seagrass leaf tissue is also subjected to various degrees of grazing, which at high intensities could cause substantial loss in above-ground biomass and reduce the photosynthetic capacity of the plant. Grazing is, however, crucial for the seagrass ecosystem as herbivores play an important role in suppressing fast-growing epiphytic algae, especially in eutrophicated areas where they otherwise threaten to overgrow the seagrass (Gacia, Littler & Littler 1999). Overfishing in (and/or nearby) seagrass ecosystems may cause sea urchin outbreaks and alternation of the foodweb structure (Rose et al. 1999; Moksnes et al. 2008; Baden et al. 2012). A loss of predatory fish (e.g. triggerfishes) can cause a rapid increase in numbers of sea urchins (Valentine & Heck 1991; Alcoverro & Mariani 2004), which at high abundances can consume large parts of the above-ground biomass, leaving bare patches (Camp, Cobb & van Breedveld 1973; Maciá 2000; Alcoverro & Mariani 2002). During such intense grazing events, the effect is long lasting with a continued loss of seagrass (Heck & Valentine 1995). Reduction in photosynthesis, a decrease in carbon content of the plant and the removal of biomass all affect the input of carbon to the biomass pool, disrupt the carbon sequestration process and impact the amount of carbon that can potentially be stored in the sediment. Besides photosynthesis, trapping of organic matter from surrounding habitats is the major source of carbon in tropical seagrass meadows (Kennedy et al. 2010) and therefore a loss of above-ground biomass will lead to less accumulation of allochthonous organic matter and further reduce the input of organic matter to the seagrass meadow.

Studies on the impacts of disturbances on carbon sequestration in seagrass meadows are clearly lacking (Duarte et al. 2011; Mcleod et al. 2011), with a single experimental study on small-scale disturbances detecting no effect on sedimentary carbon (Macreadie et al. 2014) and a field survey assessing effects of disturbance (caused by eutrophication and sedimentation) at a meadow scale showing reduction in sedimentary carbon content from increased erosion of the sediment surface (Marbà et al. 2015). Here, we manipulated seagrass plots in a Thalassia hemprichii meadow to assess impacts on the carbon sequestration process and sedimentary carbon storage by (i) shading at two different intensity levels (high and low) causing a light reduction comparable to defined levels of eutrophication or sedimentation, and (ii) clipping of above-ground biomass to simulate two levels of grazing pressure. We hypothesized that (i) shading would cause a lower community productivity and a reduced plant biomass, and (ii) clipping of above-ground biomass would lead to a reduction of below-ground biomass and intensified erosion of the upper sediment layer.

Materials and methods

STUDY SITE AND EXPERIMENTAL DESIGN

The experiment was performed in Chwaka Bay on the east coast of Zanzibar Island (Unguja), Tanzania. Chwaka Bay is a semi-enclosed embayment with a maximum average tide of 3.2 m at spring tide (Cederlöf et al. 1995). The bay is dominated by extensive seagrass meadows and encompasses up to 11 species of seagrass (Gullström et al. 2006, 2012). The selected experimental site (06°09'S 39°26'E) was placed in the middle of an intertidal seagrass meadow (with a water depth of approximately 10 cm during low tide) dominated by Thalassia hemprichii (Ehrenberg) Ascherson, a climax species forming extensive meadows in Chwaka Bay (Gullström et al. 2006) and which is spread widely across the Indo-Pacific region (Short et al. 2010). The experiment was performed for almost five months from November 2013 through March 2014, which is the period of the north-eastern monsoon characterized by low precipitation and high air temperature (Shaghude et al. 2012). The set-up comprised six treatments: low and high shading intensity to simulate light reduction from, for example eutrophication or sedimentation, low and high clipping to simulate different levels of grazing intensity, and untreated seagrass and unvegetated areas as controls (Fig. 1). The high clipping intensity was set to simulate a grazing pressure similar to that of an overgrazing event caused by, for example a sea urchin outbreak, while the low clipping treatment was set to cause a severe but non-lethal impact to the seagrass. Within the 40 × 40 m experimental site, plots of 10 m² were set up for each treatment in a random block design (n = 4) and all of the measurements took place in the centre of the plots with at least 1 m to the plot edge to avoid allocation of resources from surrounding habitats (Marbà et al. 2002). For the light reduction treatments, single- or double-plastic shading cloths were mounted approxi-
COMMUNITY PRODUCTION MEASUREMENTS

Oxygen measurements were performed five times during the two last weeks of the experiment using enclosed chambers (height: ~30 cm, bottom area: 0.18 m², volume: ~4.2 L) made from plastic bags attached to PVC pipes. The chambers were placed in the centre of the plots (to avoid edge effects and ensure continuous shading) and pressed 10 cm into the sediment. The non-rigid plastic bags were subjected to the tidal and wave action that allowed natural stirring of the water within the chambers. A 50 mL water sample was taken from each chamber at the beginning and at the end of the experiment, and the oxygen level was measured instantly during ~1 h in the morning (in the early period of the day, the production is high, while the accumulated level of oxygen in the water is still moderate; too high oxygen levels makes the oxygen measurement unreliable; Beer, Björk & Beardall 2014) and with the outgoing tide, using an oxygen electrode (Multi 340i, CellOx 325). The measurements in the different treatments were randomly conducted and the time of the incubation was set to 1 h as longer periods would negatively affect the photosynthetic conditions within the chambers, leading to an underestimation of net community production (NCP) (Olivé et al. 2016). No separate respiration measurements in darkened chambers were taken as this is not representative of the respiration taking place in the light, and also since respiration is not constant on a diel basis (Rasmusson & Björk 2014). The large tidal variation in Chwaka Bay did not allow us to measure a full diurnal cycle. NCP was calculated as the difference in dissolved oxygen concentration between the start and end values of each plot. Seagrass plant material in the chambers was then collected and separated into shoots, roots and rhizomes before being dried at 60 °C for ~48 h to obtain dry weight. The biomass of the different plant parts in the chambers was compared to that of the plots to get representative measures of the different treatments, and the values given in the result were calculated as Δ mmol O₂ h⁻¹ m⁻².

SEDIMENT CHLOROPHYLL A CONCENTRATIONS

Photosynthetic microphytobenthos in the sediment could potentially contribute to the NCP in the chambers. Therefore, chlorophyll a (Chl a) from the sediment surface was measured as a proxy for benthic photosynthetic activity. Prior to each NCP measurement, six sediment samples in the upper 1 cm sediment layer were taken in the vicinity of the chambers before attaching the community metabolism chamber to the sediment, using a cut-off 10-mL syringe. Each sediment sample was immediately placed in a 15-mL tube wrapped in aluminium foil and stored at −20 °C for 1–3 days before analysis. During analysis, 10 mL of 90% acetone was added to the samples and stored overnight at 5 °C. Each sample was centrifuged, and 5 mL of the acetone solution was extracted and analysed at 665 and 750 nm wavelengths in a spectrophotometer before and after the addition of 0.2 m HCl. Subsequently, the amount of Chl a (mg m⁻²) was calculated using the method described by Lorenzen (1967).

ANALYSES OF SEDIMENT CHARACTERISTICS AND CARBON AND NITROGEN CONTENT IN BIOMASS AND SEDIMENT

At the end of the experiment, three sediment cores were taken from each plot using a sediment corer (ø = 8 cm, h = 50 cm). The cores were divided into five depth segments (0–2.5 cm, 2.5–5 cm, 5–15 cm, 15–25 cm, 25–35 cm) and where possible, a sixth segment was obtained (35–40 cm). The maximum depth reached in this study

![Fig. 1. Experimental design showing the two different disturbances at two levels of intensity and the untreated seagrass and unvegetated areas used as controls. The treatment with single shading cloth (low intensity) reduced the light availability with 64% and the treatment with double shading cloths (high intensity) with 75%. The low clipping treatment reduced the shoot biomass with 50% and the high clipping treatment with 100% and simulates different grazing intensities.](https://example.com/image1.png)
was 37 cm. The sediment samples were weighed, cleaned of roots and plant biomass and homogenized before being dried at 60 °C until the weight was stabilized (~48 h). A subsample of 20 mL sediment was ground and further homogenized into a fine powder with a mixing mill (Retsch 400 mm). Using an organic elemental analyser (Flash 2000, Thermo Fisher scientific, Waltham, MA, USA), sediment samples were analysed for total carbon (C_T), organic carbon (C_OC) and total nitrogen (N_T) as a percentage, and by subtracting the C_OC from the C_T, a measure of inorganic carbon (C_I) was obtained. The sediment in Chwaka Bay is rich in carbonates as it is mostly comprised of coral sand and calcifying algae (*Halimeda spp.*) (Tobisson et al. 1998; Muzuka et al. 2001); therefore, to obtain the C_OC content, the sediment samples were pre-treated with 1 M and 3.5 M HCl before being analysed in the elemental analyser until the reaction of carbonate was completed and the effervescence had stopped. In order to detect whether erosion had occurred among the treatments, the relative height (mm) of the sediment in each plot was measured as the distance between the water and the sediment surface (*n* = 3) and the values were compared to reference markings on a pole, permanently driven into the bottom. For comparable reasons, the mean value of the seagrass control was used as a baseline and set to zero from which the seagrass treatment values were calculated. As variation in sediment surface height could be an effect of trampling when cutting in the plots, the porosity (%) and density (g DW mL⁻¹) of the sediment was estimated. The porosity was calculated from the water content of the samples (wet weight minus dry weight) divided by the volume of sediment in each sample, whereas density was estimated by dividing dry-weighed sediment by the volume of the sediment. Three biomass samples (0.25 × 0.25 m quadrants) were collected in each plot, cleaned of epiphytes and algae, and divided into shoots, roots and rhizomes before being dried at 60 °C until the weight was stabilized (~48 h). Plant parts were separately analysed in an organic elemental analyser to obtain carbon and nitrogen content (in percentage). The assessment of nitrogen in the sediment and the seagrass tissue gives an indication of growth status of the plants and the degradation rate within the sediment (Enriquez, Duarte & Sand-Jensen 1993; López et al. 1998).

**QUANTIFYING TOTAL HYDROLYSABLE AMINO ACIDS (THAA) IN THE SEDIMENT**

As with nitrogen, the concentration of total hydrolysable amino acids (THAA) in sediment is an indirect estimate on the lability of organic matter and the degradation efficiency (Henrichs 1992). Therefore, we quantified the amount of THAA for the different treatments in the sediment. From one sediment core taken in three seagrass plots, a subsample of ~5 mL sediment was collected from each depth segment and immediately frozen at −20 °C until a −60 °C freezer was available. The samples were freeze-dried (~60 °C) prior to analysis, and a subsample of 50 mg was ground and homogenized. Following the procedure by Dauwe & Middelburg (1998), the samples were hydrolysed in 6 M HCl, boiled at 110 °C for 24 h and centrifuged (14 000 G) before adding 6 M NaOH and boric acid solutions to the supernatant for neutralization. After reaction with OPA (o-phthalalddehyde), fluorescence at 455 nm wavelength was measured in the samples using a fluorometer (FluoStar Optima) with an excitation wavelength of 340 nm. OPA (o-phthalalddehyde) reagent was prepared 24 h prior to the fluorescence measurements. Standard solutions for amino acids were created by diluting amino acid standard (Sigma, AA-S-18, St Louis, MO, USA) in phosphate buffer. Fluorescence of standard solutions was used to create a standard curve to quantify the total hydrolysable amino acids (nmol mg⁻¹). As the OPA–amino acid reaction is time-dependent, OPA reagent was added using an automated pump, and fluorescence was measured at exactly the same time interval for all samples. All 96-well microplates used included standard solutions.

**STATISTICAL ANALYSIS**

Prior to all analyses, the assumptions of normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene’s or Bartlett’s tests) were checked, and when necessary, the data were log₁₀- or square-root (√)–transformed. To test for differences among treatments in net community production and Chl a concentrations, a linear mixed-effects model design with repeated measures was used, where the measurement occasions were set as a random factor. The C and N content in shoots, rhizomes and roots, and relative sediment surface height were all tested for differences among the treatments using one-way ANOVA. For the sediment characteristics (%C, %N, C:N, THAA, density and porosity), a nested ANOVA was applied, with sediment depth nested in treatment. Where there were significant differences, Tukey’s HSD post hoc test was used to determine which treatments were significantly different from each other.

**Results**

**NET COMMUNITY PRODUCTION AND CHLOROPHYLL A CONCENTRATION IN THE SEDIMENT**

The net community production (NCP) was significantly lower in the high shading and high clipping (Tukey’s HSD test at *P* < 0.05) treatments, as well as in the unvegetated area (Tukey’s HSD test at *P* < 0.001), compared to the seagrass control (linear mixed-effects model, d.f. = 5, *F* = 7.35, *P* < 0.001).
The low shading and low clipping treatments also showed lower NCP than the seagrass control, although no significant effects were shown (Fig. 2a; Table S1 in Supporting Information). The high clipping treatment clearly had a greater Chl a content than the other three disturbance treatments (Tukey’s HSD test at $P < 0.05$), but not compared to the seagrass control and unvegetated area (Linear mixed-effects model, d.f. = 5, $F = 3.73, P = 0.015$; Fig. 2b; Table S1).

**CARBON AND NITROGEN CONTENT IN SEAGRASS BIOMASS**

The proportion of carbon in rhizomes was lower in both high-intensity disturbances (high shading and high clipping) compared to the seagrass control (Tukey’s HSD test at $P < 0.01$; Fig. 3a), while no disturbance effect was observed in the proportion of carbon in roots or shoots (Table 1; Fig. 3b,c; Table S2). For the carbon (g m$^{-2}$) in the total below-ground biomass, both high-intensity disturbance treatments were significantly lower than the seagrass control (Tukey’s HSD test at $P < 0.01$) with 45% (high shading) and 77% (high clipping) lower carbon content, whereas low shading and low clipping were 14% and 24% lower (although not significantly) compared to the seagrass control, respectively (ANOVA, d.f. = 4, $F = 16.04, P < 0.001$). When separating the below-ground plant tissue into roots and rhizomes, only high clipping showed a significantly lower carbon (g m$^{-2}$) in the rhizomes compared to the control plots (Tukey’s HSD test at $P < 0.001$; Fig. 3d), and for roots, both high-disturbance treatments had lower root carbon (g m$^{-2}$) than the controls (Tukey’s HSD test at $P < 0.01$; Fig. 3e). The carbon (g m$^{-2}$) in shoots was lower in all disturbance treatments than in the seagrass control (Tukey’s HSD test at $P < 0.05$; Fig. 3f). The proportion of nitrogen in rhizomes was higher in the two shading treatments compared to the seagrass control (Tukey’s HSD test at $P < 0.01$), which did not differ from the two clipping treatments (Fig. 3g). No effects were found for roots (Table 1; Fig. 3h), and the proportion of nitrogen in shoots was affected only by the high shading treatment, where it was significantly greater than in the seagrass control (Tukey’s HSD test at $P < 0.001$; Fig. 3i). The C:N ratio in the rhizomes was lower in all disturbance treatments compared to the seagrass control (Tukey’s HSD test at $P < 0.05$) except low clipping, which was not affected (Fig. 3j). The C:N ratio in the roots did not differ between any disturbance treatment and the seagrass control (Fig. 3k). The C:N ratio in the shoots was lower in the high-intensity disturbances than in the seagrass control (Tukey’s HSD test at $P < 0.05$), which indicates a reduction in growth, while the low-intensity disturbance gave similar results to those of the seagrass control (Fig. 3l; Table S2).

![Fig. 3](image-url) Mean ± SE %C (a, b, c), C (g m$^{-2}$) (d, e, f), %N (g, h, i) and C:N (j, k, l) in rhizomes, roots and shoots for all seagrass treatments. Letters (A-D) indicate significant differences (ANOVA, $P < 0.05$) among treatments.

SEDIMENT CHARACTERISTICS AND SEDIMENTARY CARBON, NITROGEN AND THAA CONTENT

In terms of %Corg, %NT and C:N ratio (Corg:NT), the unvegetated area treatment showed lower values compared to all other treatments (Tukey’s HSD test at $P < 0.001$; Fig. 4a), whereas no disturbance effect was found when comparing the treated areas to the seagrass control (Table 2). As with %Corg, %NT and C:N, no significant differences in THAA were seen among treatments (Table 2; Fig. 4b; Table S3). The clipping treatments had the lowest %Corg and THAA concentration in the first depth segment (0–2.5 cm) among the seagrass treatments. The %Corg in the low shading, high clipping and seagrass control treatments decreased with depth ($P < 0.05$), while the other treatments did not show such a typical pattern (Fig. 4a). In the case of the high clipping treatment, there was a low %Corg in the first segment (0–2.5 cm) and in contrast to the low shading and seagrass control treatments, the decrease in %Corg started at the second depth segment (2.5–5 cm). Both clipping treatments had a different depth profile, with the highest %Corg and THAA concentration in the second segment, whereas all other seagrass treatments showed a peak concentration in the first segment (Fig. 4). In terms of THAA (Fig. 4b), %N and C:N ratio, no significant patterns with depth were observed in any treatment. The unvegetated

**Table 1.** Summary of one-way ANOVA models for impacts on seagrass biomass divided into rhizomes, roots and shoots and for the different response variables

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Rhizomes</th>
<th></th>
<th>Roots</th>
<th></th>
<th>Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>$F$</td>
<td>$P$</td>
<td>MS</td>
<td>$F$</td>
</tr>
<tr>
<td>C (%)</td>
<td>4</td>
<td>7.9395</td>
<td>17.97</td>
<td>$&lt;$ 0.001</td>
<td>2.865</td>
<td>2.10</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>0.4418</td>
<td>1.363</td>
<td>0.4436</td>
<td>1427.8</td>
<td>12.303</td>
</tr>
<tr>
<td>C (g m$^{-2}$)</td>
<td>4</td>
<td>839.61</td>
<td>11.37</td>
<td>$&lt;$ 0.001</td>
<td>116.05</td>
<td>0.0044</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>0.026</td>
<td>0.0152</td>
<td>3.45</td>
<td>0.0115</td>
<td>0.0152</td>
</tr>
<tr>
<td>N (%)</td>
<td>4</td>
<td>0.617</td>
<td>23.76</td>
<td>$&lt;$ 0.001</td>
<td>0.0152</td>
<td>3.45</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>0.026</td>
<td>0.0152</td>
<td>3.45</td>
<td>0.0115</td>
<td>0.0115</td>
</tr>
<tr>
<td>C:N</td>
<td>4</td>
<td>74.906</td>
<td>15.16</td>
<td>$&lt;$ 0.001</td>
<td>1.3136</td>
<td>3.15</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>4.9419</td>
<td>0.4173</td>
<td>0.3</td>
<td>0.4173</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Significant values ($P < 0.05$) are shown in bold.

**Fig. 4.** Mean ($\pm$ SD) %C$_{org}$ (a) and THAA (b) for the different treatments. Note that the deepest one or two sediment segments are given as mean depth ($\pm$ SD) as the end of the individual sediment cores varied slightly.

area treatment clearly had a lower mean \%C_{org}, \%N_T and C:N ratio than the disturbance treatments, and among the seagrass treatments, the seagrass control had the highest C:N ratio due to lower \%N_T, although this was not significant (Fig. 5; Table S3). The largest part of the \%C_T in the sediment for all treatments was made up of C_i (Fig. 6). The relative sediment surface was lower in the clipping treatments compared to the seagrass control (Tukey’s HSD test at \( P < 0.05 \); Table 2; Fig. 7), and no difference was seen in compactness as porosity and density did not differ among seagrass treatments (Table S4).

**Discussion**

In a five-month *in situ* experiment, we studied the impacts of shading and simulated grazing on carbon sequestration processes, with particular focus on community productivity, the short-term pool of biomass and sediment detritus layers, and thus also the long-term carbon storage in the sediment. The findings demonstrate clear negative effects on net community productivity in high-intensity disturbance treatments, which were also subjected to a major loss of below-ground biomass. Such effects of disturbance on the overall productivity and biomass lead to reduced flow of organic carbon sequestered in the sediment, which might with time negatively affect the long-term carbon storage (Fig. 8). In addition, a significant impact on the sediment was seen as erosion in both clipping treatments, although a reduction in sedimentary carbon was not observed during the course of the experiment and is expected to occur over a longer time-scale.

The observed effects of high disturbance on net community productivity and the carbon biomass were likely due to a decreased overall photosynthetic capacity of the seagrass community. Seagrass has relatively high light requirements to support a large amount of non-photosynthetic tissue and are therefore more sensitive to light reduction than other marine plants (Erftemeijer & Lewis 2006). We suggest that in the shading treatments, plants were adapting to the lower light regimes by reducing both their above- and below-ground

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**Table 2. Summary of nested ANOVA models for the sedimentary characteristics (%C_{org}, %N_T, C:N and THAA) and result of the one-way ANOVA for the relative sediment surface height**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corg (%)</td>
<td>5</td>
<td>2.1</td>
<td>66.50</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Depth (C_{org})</td>
<td>16</td>
<td>0.1</td>
<td>4.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>58</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT (%)</td>
<td>5</td>
<td>0.1</td>
<td>6.20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Depth (NT)</td>
<td>16</td>
<td>0.006</td>
<td>0.30</td>
<td>0.995</td>
</tr>
<tr>
<td>Residual</td>
<td>58</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C:N</td>
<td>5</td>
<td>9.9</td>
<td>8.70</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>58</td>
<td>0.8</td>
<td>0.68</td>
<td>0.798</td>
</tr>
<tr>
<td>THAA (nmol mg^{-1})</td>
<td>4</td>
<td>183.7</td>
<td>0.75</td>
<td>0.565</td>
</tr>
<tr>
<td>Depth (THAA)</td>
<td>10</td>
<td>341.8</td>
<td>1.40</td>
<td>0.232</td>
</tr>
<tr>
<td>Residual</td>
<td>28</td>
<td>244.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative sediment height (mm)</td>
<td>4</td>
<td>1746.7</td>
<td>6.73</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Residual</td>
<td>15</td>
<td>259.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant values (\( P < 0.05 \)) are shown in bold.

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biomass as being described previously (Fokeera-Wahedally & Bhikajee 2005; Collier et al. 2009; Collier, Waycott & Ospina 2012). In contrast to the high shading plots, a substantial part of the net community productivity in the high clipping treatment seems to be related to the prevalence of photosynthesizing micro-organisms (microphytobenthos) rather than the seagrass plants. This is supported by the increased Chl a concentration in the upper sediment layer in the high clipping plots (where shoots were removed) and is likely caused by a higher insolation to the sediment surface. A reduced above-ground production would decrease the production in the below-ground parts (Duarte & Chiscano 1999) as the root–rhizome system is dependent on photosynthetically derived carbohydrates and oxygen transported from the shoots. The lower carbon percentage in the rhizomes for both high-intensity disturbances is most likely due to a depletion of carbohydrates. The rhizomes are the main carbon storage unit of the plant (Burke, Dennison & Morre 1996) and the high-intensity disturbances could have depleting carbohydrate concentrations in the rhizomes, as the seagrass could no longer obtain enough energy through photosynthesis (Zimmerman, Kohrs & Alberte 1996; Collier et al. 2009). The allocation of resources from the shoots to roots and rhizomes is of critical importance for the carbon sequestration process in seagrass meadows, and a reduced build-up of below-ground biomass could render lower sedimentary carbon over time as the below-ground biomass is more decay resistant with a high lignin content (Klap, Hemminga & Boon 2000) and a generally slow turnover rate (Duarte et al. 1998). Disturbances like light reduction and intensified grazing are known to reduce below-ground seagrass biomass (Portig et al. 1994; van Katwijk et al. 2011; D. Deyanova, M. Gullström, L.D. Lyimo, M. Dahl, M.I. Hamisi, M.S.P. Mtlera, M. Björk, submitted), and in our study, we found that the total below-ground carbon (as g m⁻²) was highly impacted, with a reduction by up to 77% (in the high clipping treatment). The high loss of biomass carbon in the high clipping plots was due to an effect in both rhizomes and roots, while in the high shading treatment, a reduction in carbon was only seen in the roots (Fig. 8). This indicates that mechanical removal of above-ground biomass had a more direct and immediate impact on carbon storage than shading. The reduction in below-ground carbon was most likely due to degradation of seagrass tis-

Fig. 8. A conceptual diagram summarizing the main impacts on the various phases of the carbon storage process (NCP, biomass, detritus and refractory storage) for the different treatments. The phases in the carbon storage process act on different time-scales leading to a chain of events where NCP and biomass have the shortest response time to a disturbance (such as light reduction and simulated grazing) and will be the first to be impacted resulting in a reduced carbon input and lower build-up of biomass in the seagrass meadow, which disrupts the carbon storage process. This was seen in the high-intensity disturbances (shading and clipping), which both showed a lower NCP and a reduction in below-ground carbon. Both clipping treatments also had a lower relative sediment surface (erosion) caused by the removal of the above-ground biomass. With an ongoing disturbance over a longer time perspective, the reduced input of organic matter and erosion may lead to a loss of carbon in the uppermost sediment detritus layer and over time also reduces the long-term carbon storage deeper down in the sediment.
issue, which could increase decomposition as bacterial abundance is correlating with organic matter content (Dale 1974), but due to the high oxygen consumption by detritus organisms, the sediment becomes anoxic and the degradation process slows down (Benner, MacCubbín & Hodson 1984).

The lower C:N ratio of the rhizomes in the shading treatments was because of the increase in nitrogen tissue concentrations, where the high-intensity treatment had a stronger overall impact on the seagrass plant, with a lower C:N ratio in both rhizome and shoot tissue. Light reduction is known to decrease the growth rate as it limits seagrass photosynthesis (e.g. Ruiz & Romero 2003; Beer, Björk & Beardall 2014) and as nitrogen, which is usually a limited nutrient in the marine environment and rapidly utilized as it gets available, was not used as quickly as in the unshaded treatments this indicates that seagrass growth was impaired in the shading treatments (Peralta et al. 2002). Increased biomass nitrogen levels have been noted in areas exposed to eutrophication (Schmidt et al. 2012) and could increase the degradation (Enríquez, Duarte & Sand-Jensen 1993) as more nutrients becomes available for degrading organisms. In contrast, the lower C:N ratio in the rhizomes and shoots of the high clipping treatment was mainly due to a decrease in carbon rather than an increase in nitrogen. Therefore, the effect was most likely not because of decreased seagrass growth rate but a depletion of carbon.

All seagrass treatments had a higher organic carbon content in the sediment than the unvegetated area, while the seagrass control had a mean (for the whole depth profile, ± SD) of 1.4 ± 0.3%, which is similar to the reported global averages (Kennedy et al. 2010; Fourqurean et al. 2012) and is almost five times higher than the unvegetated area (0.3 ± 0.05), illustrating the ability of seagrass to sequestrate organic carbon. Furthermore, the main carbon in the sediment is not organically derived from the seagrass ecosystem but made up of inorganic carbon as the sediment in the studied bay is comprised of coral sand and the calcifying algae *Halimeda* spp. (Tobisson et al. 1998; Muzzuka et al. 2001). This high deposition of inorganic material has also been reported by Kennedy et al. (2004) in tropical meadows. The carbon content in seagrass meadows generally decreases with depth (Fourqurean et al. 2012) as the organic matter is being consumed by degrading organisms and is remineralized over time (Burdige 2007). In our experiment, this was only seen in the seagrass control and low shading treatment, where the organic carbon decreased from the sediment surface and downwards. A similar pattern of decrease was seen in the high clipping treatment, although this started at the second sediment segment (2.5–5 cm) because of a peak carbon concentration at that depth. However, organic carbon in the high shading and low clipping treatments did not significantly decrease with depth, potentially because of large variation in the upper depth segments (0–2.5 and 2.5–5 cm).

The depth profile of the two clipping treatments differed from the other treatments (although not significantly) since there was a shift in organic carbon and THAA content in the two upper sediment layers (0–2.5 and 2.5–5 cm), where the

surface layer showed lower levels and the second depth segment higher levels. This was most likely due to a removal of above-ground biomass leading to a less efficient trapping of allochthonous carbon and reduced deposition of above-ground tissue to the detritus layer. The clipping of shoot tissue also caused a relatively lower sediment surface in the clipping plots due to erosion, which could not be related to increased compactness of the sediment as there was no difference in either porosity or density. The ability of the canopy to reduce wave action and the roots and rhizomes to stabilize the sediment becomes impaired by the loss of biomass; this subsequently results in erosion, as well as a reduction in carbon input, with less biomass to trap organic matter and photosynthesis (Marbà et al. 2015). In our experiment, the main cause for the erosion was, however, the reduction of shoot height as both clipping treatments had a lower sediment surface despite the fact that the below-ground biomass in low clipping was not impacted (Fig. 8). In areas like Chwaka Bay, with flat and shallow meadows as well as a large diurnal tidal variation (Cederlöf et al. 1995), wave action is considerable (Fonseca & Cahalan 1992) and thus causing potentially more pronounced sediment erosion. We believe that erosion will over time lead to a lower organic carbon in the uppermost layer and that this would reduce the carbon in deeper segments as well because of a continuous erosion and degradation due to re-oxygenation of the sediment.

The accelerating loss of seagrass meadows (Waycott et al. 2009) with the consequences of increasing atmospheric greenhouse gas emissions through the remineralization of stored carbon as well as the loss in capacity to continuously capture and store carbon is of major concern. Human-induced disturbances decrease the ability of seagrass to act as carbon sinks (Macreadie et al. 2012) and protection and restoration of seagrasses is an important part in mitigating global warming (Nellemann et al. 2009). Our findings highlight the complex nature of the carbon sequestration process with the different phases responding to disturbances at distinguished temporal scales, where the short-term biomass carbon pool was clearly impacted within the five months of disturbance while possible effects on the sedimentary carbon would need longer time to be observed. During prolonged disturbances, as presently seen in many coastal waters, the carbon storage capacity of seagrass meadows may eventually be reduced, leading to a loss in natural carbon sinks and a lower ability of seagrass habitats to mitigate global warming.

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Data accessibility

The data sets used for analyses of net community production, carbon and nitrogen content in sediments and biomass, sediment characteristics and THAA concentrations are accessible as Supporting Information.

References


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Mean (± SE) net community production (NCP) and chlorophyll a (Chl a) concentration in the sediment for the different treatments.

**Table S2.** Mean (± SD) carbon and nitrogen content in seagrass biomass for the different treatments.

**Table S3.** Mean (± SD) sedimentary carbon, total nitrogen, C:N and THAA concentration for the different treatments for the whole depth profile (0–30 cm).

**Table S4.** Mean sediment porosity and density (± SD) for the whole depth profile (0–30 cm).