Carbon sequestration processes in tropical seagrass beds

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I would like to dedicate this thesis to my wife Elizabeth Kissanga and my daughters Evaresta Lyimo and Glory Lyimo. It is they, day after day, that are the most important thing to me. I would also like to dedicate this thesis to all my family and friends, who give me so much. I especially want to dedicate this thesis to my father Dominick Lyimo and to my mother Evaresta Manyanga.
This thesis is composed of five manuscripts that will be referred to by their roman numerals

I. Gullström M, Lyimo LD, Dahl M, Samuelsson GS, Eggertsen M, Anderberg E, Rasmusson LM, Linderholm HW, Knudby A, Bandeira S, Nordlund LM, Björk M. Blue carbon storage in tropical seagrass meadows is influenced by plant structure, sediment composition and landscape configuration (manuscript)

II. Lyimo LD, Gullström M, Hamisi, MI, Lyimo TJ, Björk M. Emission of nitrous oxide and methane from tropical seagrass meadows: effects of gas transport through seagrass plants and eutrophication (manuscript)


IV. Deyanova D, Gullström M, Lyimo LD, Dahl M, Hamisi MI, Mtolera MSP, Björk M Prolonged shading and simulated grazing negatively affects the productivity of a tropical seagrass meadow

V. Lyimo LD, Gullström M, Lyimo TJ, Deyanova D, Dahl M, Hamisi MI, Björk M. Shading and simulated grazing of seagrass leaves increases sulphide production and methane emission in a tropical seagrass meadow (manuscript)
My contribution to the papers

Paper I: Participating in the planning of the study and writing with co-authors, participating in the performance of all sampling except at Inhaca Island in Mozambique.
Paper II: Planning the study, performing experiments, data analysis and writing with co-authors.
Paper III: Participating in the planning, performing experiments, and writing with co-authors.
Paper IV: Participating in the planning, performing experiments and writing with co-authors.
Paper V: Planning the study, performing experiments, data analysis and writing with co-authors.
Abbreviations

C                        Carbon
C: N                       Ratio of Carbon and Nitrogen
C_T                      Total carbon
HC                       High clipping
HS                       High shading
IPCC                     Intergovernmental Panel for Climate Change
LC                       Low clipping
LS                       Low shading
NCP                      Net Community productivity
N_T                      Total Nitrogen
THAA                     Total hydrolysable amino acid
WIO                      Western Indian Ocean
1. Introduction

1.1 Seagrass and climate change mitigation

Carbon dioxide levels in the atmosphere have never been higher in the past 700000 years (http://en.wikipedia.org/wiki/Image:CO2-temperature-plot.png) and the global average concentration reached 401.62 parts per million (ppm) in December 2015 (www.esrl.noaa.gov/gmd/ccgg/trends/). The increase in CO$_2$ is coinciding with industrialisation, post 1750, and has thus been attributed to human use of fossil fuels (Nellemann and Corcoran 2009). Changes in land use pattern, livestock keeping, vegetation clearing and burning of fossil fuel are other examples of major anthropogenic activities also responsible for observed increase in greenhouse gas emission into the atmosphere (Solomon 2007; Nellemann and Corcoran 2009; Donato et al. 2011). Fourqurean et al. (2012) and Pendleton et al. (2012) argued that the deterioration of natural marine ecosystems (such as salt marshes, mangrove and seagrass beds) that serve as carbon sinks may contribute to climate change through re-emissions of locked carbon dioxide and other greenhouse gases. Trumper (2009) suggest that a reduction in greenhouse gases emission by 85% by the year 2050 (from levels seen in 2000) is needed if the 2°C goal is to be kept. However, this strategy will not be possible if the restoration and conservation of natural carbon sinks are ignored. Fourqurean et al. (2012) and Pendleton et al. (2012) suggested combining both reductions in emission of anthropogenic greenhouse gases and restoration and conservation of natural ecosystem that have high carbon sequestration and long-term storage. Coastal marine ecosystems including seagrass meadows saltmarshes and mangroves are responsible for ~70% of the organic carbon absorption in marine environments (Kennedy and Björk 2009; Nellemann and Corcoran 2009; Mcleod et al. 2011; Fourqurean et al. 2012; Macreadie et al. 2013), making them “hot spot” blue carbon sinks (Howard et al. 2016). Mcleod et al. (2011) showed that seagrass habitats could bury organic carbon at a rate that is 35 times faster than that of tropical rainforests. Seagrass meadows also trap organic carbon from external sources, (Kennedy et al. 2010) and approximately 50% of the organic carbon locked in the sediment is not of seagrass origin, creating a need to find out other contributors to C$_{org}$ in seagrass sediments. An understanding of the processes and environmental factors that drive carbon trapping in seagrass meadows is therefore of crucial importance.
There is, however, large gaps and great uncertainties regarding estimations of organic carbon storage capacity and burial rates in seagrass meadows, particularly in the Western Indian Ocean (WIO) region.

1.2 Methane and nitrous oxide emission in seagrass meadows

Alongside CO₂, nitrous oxide (N₂O) and methane (CH₄) are among the most important greenhouse gases in the atmosphere (Kreuzwieser et al. 2003), accounting for about 25% of global mean radiative forcing (IPCC 2007). The atmospheric concentrations of CH₄ and N₂O are increasing at rates of 0.7% yr⁻¹ (Khalil and Rasmussen 1994) and 0.31% yr⁻¹ (Yang et al. 2009; Stocker et al. 2013), respectively, an increase attributed to human activity. This contributes approximately 20% and 6% to the global warming effect, respectively (IPCC 2007). It is estimated that about one-third of the global CH₄ comes from natural sources (Lelieveld 2006; Forster et al. 2007), and of these emissions, the largest quantity originates from wetland methanogens that produce CH₄ while decomposing organic material (Bousquet et al. 2006; Lelieveld 2006).

1.3 Seagrasses: overview and distribution

The seagrasses constitute a heterogeneous group of flowering plants (angiosperms), adapted to grow and reproduce submerged in seawater, usually in shallow marine waters down to a depth where about 11% of surface light reaches the bottom (Duarte 1991; Orth et al. 2006; Duarte and Gattuso 2008). They have reproductive organs (flowers and seeds) (Figure 1), roots, rhizomes, stems and leaves (Figure 5) as most terrestrial plants (Bjork et al. 2008), and can be found on the coasts of every continent except Antarctica (Short et al. 2007). The biodiversity of seagrasses, as well as their abundance and biomass increases towards the tropics (Short et al. 2007), particularly in the Indo-pacific region including the East African coast and islands of the WIO, where 14 seagrass species have been reported (Gullström et al. 2002; Ochieng and Erftemeijer 2003; Gullström et al. 2006; Duarte et al. 2012).
1.4 Importance of seagrass ecosystems

Seagrass occupy a relatively small area of the coastal oceans (~ 0.1-0.2%) (Duarte 2002; Kennedy et al. 2010; Fourquean et al. 2012) and yet, they play a large role in the coastal zone by providing several important ecosystem goods and services (Bjork et al. 2008; Barbier et al. 2011). Seagrass meadows supply food to mega-herbivores such as dugongs, sea turtles, sea urchins (see Figure 2), manatees, water birds and herbivorous fish (Barbier et al. 2011). They provide breeding nurseries and feeding grounds to migratory and stationary fish and to other invertebrate organisms (Jackson et al. 2001; Unsworth and Cullen 2010). Seagrasses are also ecosystem engineers, in the sense that they significantly modify the abiotic environment of their ecosystem (Jones et al. 1996). For instance, seagrass leaves reduce hydrodynamic stress by attenuating currents and waves (van der Heide et al. 2012), improve light conditions by trapping suspended sediment and nutrients (van der Heide et al. 2007), and increases pH by absorbing CO₂ (Hendriks et al. 2014) creating favourable habitat to carbonate-associated organisms.
1.4.1 Seagrass carbon sequestration and storage

Like other plants, seagrasses utilize light energy and carbon dioxide to synthesize their own food, which allows them to grow and reproduce. Carbon fixation in seagrass leaves usually exceeds their immediate metabolic needs (Duarte and Cebrian 1996), hence a large proportion of excess carbohydrates organic carbon is transported to the roots and rhizomes where it is stored (Figure 3), and eventually exuded in the sediment, forming anaerobic organic-rich soil (autochthonous). In addition, seagrass stems and leaves can trap and settle down suspended organic carbon from outside the meadows (allochthonous) from the water column (Mateo et al. 2006; Fourquarean et al. 2012; Howard et al. 2016). This type of blue carbon can persist for millennia and can thus make seagrass meadows highly efficient in long-term carbon storage (Fourquarean et al. 2012; Macreadie et al. 2014; Howard et al. 2016). The $C_{org}$ accumulation in seagrass meadows is estimated to be 83 g C m$^{-2}$ yr$^{-1}$ (Duarte et al. 2005). Its global carbon burial rates of 27–44 Tg C yr$^{-1}$ comprise 10–18% of the total carbon storage in the ocean (Kennedy and Björk 2009; Kennedy et al. 2010). However, the estimations of seagrass carbon budgets are highly variable as they depend on the species considered, the local environment and time of the year, as well as the associated epiphytes (Macreadie et al. 2014). Current reports show that, *Posidonia oceanica* found in the Mediterranean Sea is thought to be the most effective species in terms
of long-term carbon storage, sustaining carbon burial rates of 17-191 g C m\(^{-2}\) per year, forming layers of dead organic matter that can persist thousands of years (Fourqurean et al. 2012). Even though much information on seagrass carbon sequestration is present for temperate and Mediterranean species (Fourqurean et al. 2012; Lavery et al. 2013), information on carbon storage in tropical seagrass meadows is scarce, particularly from the WIO region.

![Figure 3. Seagrass T. hemprichii with exposed roots and rhizomes at Bwejuu Tanzania (Photos by Mats Björk)](image)

1.5 Anthropogenic threats to seagrasses and its possible effect on global warming

Seagrasses inhabit coastal environments and are therefore subjected to numerous anthropogenic activities such as sewage disposal, mariculture, propeller boating activities, destructive fishing, construction works and dredging, threatening them to extinction (Short and Wylie-Echeverria 1996; Duarte 2002; Roca et al. 2016). Increased eutrophication has the most severe negative impacts on the productivity and hence the distribution of seagrasses (Hemminga 1998; Cardoso et al. 2004; Ralph et al. 2007). Surface runoff and nutrient fluxes reduce water clarity and attenuate light (Hemminga 1998), encouraging massive growth of opportunistic macroalgae (see Figure 4) and epiphytes (Cambridge and McComb 1984; Cambridge et al. 1986; Fitzpatrick and Kirkman 1995; Hemminga 1998; Duarte 2002), which may outcompete seagrass for light and nutrients. Prolonged light reductions below the
minimum light requirement of the species may lead to seagrass loss and ultimately affecting the composition of the associated biological community (Cardoso et al. 2004). It is believed that about a third to half of the world’s seagrasses have been lost since 1879 (Waycott et al. 2009) and the continuing rate of disappearance is estimated to ca 110 km² per year (Waycott et al. 2009; Mcleod et al. 2011). The C sink capacity of seagrass habitats can be affected by anthropogenic disturbances in different ways: i) by reducing the filtering capacity of seagrasses, the suspended allochthonous C that contributes to the below-ground sediment C stock will be reduced (Burden et al. 2013). ii), loss of the photosynthetic capacity by e.g. grazing or shading (Dahl et al. 2016) may reduce the total amount of inorganic carbon fixed by seagrass. iii) severely disturbed seagrasses could cause loss of C stored in the plant material itself (structural C) due to plant die off, which could be exported and lost from the seagrass ecosystem, if not buried into the seagrass sediment C stock (Macreadie et al. 2014). iv) disturbed seagrasses could result in the release of buried ancient sedimentary C via erosion (Dahl et al. 2016), leaching, and microbial mineralization (Lee and Dunton 2000; Macreadie et al. 2014), potentially resulting in the release of other greenhouse gases such as methane and nitrous oxide (Kreuzwieser et al. 2003).

Figure 4. Seagrass T. hemprichii covered by algae (Ulva sp) (attenuating light and compete for nutrients) at Ocean Road Tanzania (Photos by Liberatus Lyimo)
1.5.1 The effect of light limitation on the resource allocation in seagrass

Light quality and quantity reaching the leaves are the main determinant of seagrass growth and distribution as it directly affect the oxygen production and total carbohydrate accumulation in plant (Ralph et al. 2007). Orth et al. (2006) argued that light attenuation or change in optical properties of water inhibit seagrass growth by lowering its photosynthetic efficiency, and subsequently affects the carbon fixation and allocation within the plant, which might in turn reduce the autochthonous C in seagrass meadows (see also review by Ralph et al. 2007 and reference there in). In extreme light reduction, the reserve organic C in the seagrass biomass is mobilized to optimize carbon balance during early stages of disturbances (Alcoverro et al. 2001). However, with time, rhizomes can be a respiratory burden (Hemminga 1998), if its demand for C is higher than the supply from the above-ground biomass (Ralph et al. 2007).

1.5.2 Production and release of sulphide in seagrass sediment

Seagrasses often grow in oligotrophic coastal waters (Holmer and Bondgaard 2001; Borum et al. 2007). Where they, in order to oxidize toxic metabolites in the rhizosphere, solely depend on photosynthetic oxygen and passive diffusion oxygen from the water column down into the sediment (Pedersen and Borum 1998). Sand-Jensen et al. (1982) reported that oxygen produced by seagrass plants during photosynthesis during the day is partially lost to the water column, and partly transported to below-ground tissues via the lacunae system (see also Borum et al. 2007). It has been assumed that the photosynthetic oxygen generated during daylight covers dark period (night-time) respiratory demands of underground tissues (Smith et al. 1984; Pedersen and Borum 1998). However, this photosynthetic oxygen pool is rapidly diminished in the dark, and the oxic conditions in below-ground tissues is partially maintained by oxygen diffusing from the water column to roots and rhizomes via air-filled lacunae system (Greve et al. 2003; Borum et al. 2005). This mechanism is, however, insufficient to maintain oxic conditions if the water column oxygen concentration is low or if the oxygen demand of plant tissues or the sediment is remarkably high. Poor oxygen supply might also occur during daylight if plants exhibit severe physiological stress (Koch and Erskine 2001; Greve et al. 2003). Under hard stress (from e.g. light limitation or overgrazing events) the oxygen content of below-ground tissues will be too
low to maintain the oxic-microshield around the rhizosphere allowing sulphide invasion (Pedersen et al. 2004). Sulphide diffuses via root/rhizome tissues to the photosynthesizing tissues (van der Heide et al. 2012), where it inhibits photosystem II (Dooley et al. 2015) as well as the activity of cytochrome c oxidase in mitochondria (Koch et al. 1990; Lamers et al. 2013), leading to a subsequent blocking of energy production and negative impacts on a range of other metal containing enzymes (Dooley et al. 2015). High sulphide concentration in plants have shown to impair also the uptake of nitrogen, phosphorus and iron (Koch et al. 1990, van der Heide et al. 2012). Studies by Lamers et al. (2013), Calleja et al. (2007), and Holmer et al. (2006), showed that tropical seagrasses are highly susceptible to sulphide toxicity due to prevailing warmer conditions and high carbonate sediments, characterized with less iron contents, which is responsible for sulphide detoxification through the formation of pyrite. Sulphide toxicity concentrations vary greatly among different seagrass beds and tolerance to its accumulation vary among seagrass species (Calleja et al. 2007). To be able to assess the different effects of sulphide toxicity in submerged plants to the meadow scale, a deeper understanding of biogeochemical, physiological and ecological processes is of high importance (Lamers et al. 2013).

1.6 Greenhouse gas transport within marine plants

Most submerged plants possess aerenchyma tissue as a morphological adaptation to flooding conditions. The aerenchyma (air-filled) tissue is required to aerate the roots and rhizoplane of wetland plants and constitutes the major means for the flux of O₂ through plants (Smith et al. 1984; Justin and Armstrong 1987; Pedersen and Borum 1998). This channel is also a means for gases to escape from the root zone to the atmosphere via the shoots. Gases that have been shown to escape into the atmosphere via aerenchyma of wetland plants are: (1) N₂O (Kreuzwieser et al. 2003), (2) CH₄ (Rusch and Rennenberg 1998), and (3) CO₂ (Thomas et al. 1996). Although, the role of wetland plants as conduits for CH₄ and N₂O has been investigated for numerous plants such as mangrove (Kreuzwieser et al. 2003), black alder (Alnus glutinosa, L.) Gaertn., (Rusch and Rennenberg 1998) and agricultural plants such as rice (Schütz et al. 1991), still there is a clear paucity of information on whether seagrass plants are capable of mediating greenhouse gases to the atmosphere.
1.7 Objectives

The overall objective of this thesis has been to quantify the amount of organic carbon stored in tropical seagrasses and elucidate the effects of seagrass disturbances on sequestration and greenhouse gases emission. The overall interest has been to study the contribution of seagrass in climate change mitigation.

The specific objectives in this work were:

i. To quantify organic carbon storage in tropical seagrass meadows
ii. To assess the effects of nutrient loading on the emission of CH₄ and N₂O gases from tropical seagrass meadows
iii. To examine the effects of shading and simulated grazing on seagrass biomass and sediment organic carbon storage
iv. To examine the effects of stress (shading and simulated grazing) on the productivity and resource allocation in tropical seagrass meadows
v. To examine the effects of shading and simulated grazing on accumulation of sulphide in sediment pore water and the emission of CH₄ from seagrass sediments
2. Methods used

2.1 Seagrass species

All the work in this thesis have focused on upper intertidal seagrass meadows that cope with intricate combinations of stresses, such as light, temperature, desiccation, and coastal anthropogenic activities. Four seagrass species were studied; *Thalassodendron ciliatum*, (Forsk.) den Hartog; *Thalassia hemprichii*, (Ehrenb.) Asche; *Cymodocea serrulata* (R. Br.) Asch & Magnus and *Enhalus acoroides*. (L.f) Royle. The appearance of these seagrasses are depicted in Figure 5. The selection of these four seagrass species was because they are important meadow-building habitats and the most common species across the East African coast, including the selected study sites.

*Figure 5. Seagrass species used in this study A) E. acoroides, B) C. serrulata, C) T. ciliatum and D) T. hemprichii, with their approximate leaf length. Illustrations are modified from Richmond (2002).*
All study sites were located in the coastal areas of the WIO, more specifically at Zanzibar, the Tanzanian mainland and Inhaca Island in the southern part of Mozambique (Figure 6). In Paper III, IV and V, we manipulated seagrass plots by shading and seagrass clearing. Light reduction through shading is clearly associated with increased nutrients and sediment load, which is considered the most acute threat to seagrass ecosystems globally (Grech et al. 2012). Seagrass clearing was performed to simulate overgrazing events commonly induced by overfishing of predators, where continuous removal of plant material is exerted by grazing organisms (e.g. sea urchins and herbivorous fish) in high densities (Heck and Valentine 1995; Rose et al. 1999). The experimental set-up comprised of five different treatments including two levels of shading i.e. low shading (LS; ~64 % light reduction: with an average of 163µmol quanta m⁻² s⁻²) and high shading (HS~75 % light reduction: with an average of 114µmol quanta m⁻² s⁻²), two levels of clipping i.e. low clipping (LC; ~50 % half of the photosynthetic tissue removed) and high clipping (HC~100 % removal of above-ground seagrass tissue) and undisturbed seagrass in control (C: with an average of 470µmol quanta m⁻² s⁻²) plots (Figure. 7A &B). Each plot covered 10 m² and was randomly distributed within a 40 m x 40 m area in a homogenous seagrass meadow, where plots had similar sediment characteristics and water conditions. Light reduction was measured (micromoles/m²/s) underneath the shading cloths using Odyssey light loggers (Odyssey, New Zealand) (see Paper IV). Leaf clipping was done at 3-5 days interval and the shading cloths were cleaned daily by removing fouling organisms and debris carried with the tide. This study was done to extrapolate on the effect of these stresses on methane emission, sulphide toxicity concentration, and sediment organic C storage as well as resource allocation in seagrass parts.
Figure 6. Map of coastal Tanzania with the study sites indicated by numbers of the corresponding papers in which these were examined.
Figure 7. (A) Explanations to symbols for control and treatments. Control (C) low shading (LS); high shading (HS); low clipping (LC) and high clipping (HC). (B) The experimental setting with randomized complete block design (n=4), with filling patterns corresponding to treatments described in (A) and (C) bare sand (BS) not shown in the diagram. Modified from Paper IV.

Figure 8. Shading experiment in T. hemprichii meadow conducted for 150d at Chwaka bay Zanzibar (Photos by Martin Gullström)
2.3 Total carbon (Cₜ), organic carbon (Cₜₒᵣₑₙ), total nitrogen (Nₜ), and total inorganic carbon (TIC) determination

2.3.1 Sediment and biomass sample collection

In paper I, a transect was made (Figure 9A) to estimate a seagrass area of approximately 200 m x 200 m. The total area was again subdivided into six sub-areas (66.7m x 66.7m). All the sediment cores in this study were collected using Perspex core (0.5m length and 0.08m) (Figure 9B). The sediments in the core were extruded and sectioned into 3 sections i.e. 0-5 cm (upper), 5-25 cm (middle) and 25-50 cm (lower) for paper I, while for paper II the cores were sectioned into 5 sections (0-2.5, 2.5-5, 5-10, 10-15, and 20-25 cm). Iron framed quadrats (Figure 9C) with the dimension of 0.25m x 0.25m were randomly thrown within the meadow to collect plant biomass, in each meadow 3 replicates of above and below-ground biomass were collected.

Figure 9: A) Transect to estimate the area for sample collection B) core with sediment samples taken during sampling C) quadrat place in seagrass biomass to estimate coverage and density (Photos A & B by Lina Rasmusson and C by Liberatus Lyimo).

Paper I, II and III are based on marine sediment carbon determination. A subsample of 20 ml sediment was ground and homogenized into a fine powder with a mixing mill (Retsch 400 mm). The Cₜ, organic carbon (Cₜₒᵣₑₙ) and Nₜ were determined using an organic elemental analyser (Flash 2000, Thermo Fischer scientific, Waltham, MA,USA) (Verardo et al. 1990; Dahl et al. 2016). Samples were compressed in a sample container and then introduced into a combustion column reactor (Kristensen and Andersen 1987; Verardo et
al. 1990). Sediment carbonate was eliminated by acidifying the samples with 0.2M HCl to determine total inorganic carbon present in the samples. According to Kristensen and Andersen (1987) and Verardo et al. (1990), this procedure provides rapid and routine analyses of marine sediments, yielding a relative precision of + 0.3% of the measured value for organic carbon and + 1.6% for nitrogen. An alternative method which could have been used is the “ash combustion method” (Gibbs 1977; Heath et al. 1977) that has been used for the separation of organic from inorganic forms of carbon. The major drawback of the technique is that it is exclusive for carbon determination. In addition, its accuracy is temperature dependent, and incomplete carbon separation may result, if the combustion temperature falls below 1050°C (Gibbs 1977). The organic carbon elemental analyser used in this study was chosen, as it is a more reliable and practical way for separation of organic and inorganic carbon and total N in marine sediments. One of the drawbacks of this method is that very small amount of samples are used (7-8 mg) which, if not well homogenized will limit the representation of the total sample collected.

2.4 Determination of total carbohydrates and of starch

The plant samples for total carbohydrate determination (Paper IV) were collected at the end of experiment i.e. after 150 d. All the samples were collected between 1:00-2:00pm to avoid variation in carbohydrate, which might be caused by different sampling time. The collected samples were dried at 60°C for 48 h and ground to fine powder. In this study, we used the anthrone colorimetric method to determine total carbohydrate. Anthrone reagent was prepared as described by (Hedge et al. 1962; Pearson 1976; Rose et al. 1991) by dissolving 200 mg of anthrone in 100 ml of ice cold 95% H₂SO₄, made by adding 500 ml of concentrated acid to 200 ml of water. The reaction was carried out under conditions similar to those used by Hedge et al.(1962a). This method has been extensively used as a convenient and specific reagent for estimation of a variety of carbohydrates in plant parts as well as in seawater and sediments (Yemm and Willis 1954; Strickland and Parsons 1972). The method is quantitatively used in the determination of starch, glucose, fructose, and other naturally occurring carbohydrates based on their molar absorption coefficient (Handa 1966). An alternative method that could have been used instead of anthrone is the phenol-sulfuric acid method (Dubois et al. 1956). The method is suitable for total carbohydrate analysis because its molar absorption coefficient for most sugars fall within a short range compared to the anthrone method. However, the technique has several limitations. Firstly, the colouring agent phenol is a neurotoxin (Albalasmeh et al. 2013) and repeated inhalation may cause serious problems. Secondly, it is suitable only
for carbohydrates which are polymers of simple glucose, since it presents the results in term of glucose-equivalent concentrations (Mecozzi 2005). The anthrone method was chosen as more reliable and practical way of carbohydrate determination in the laboratory. One of the drawbacks is that it is time consuming and need experienced personnel to handle and run the extraction process.

2.5 Sediment pore water sulphide estimations

In Paper V in this thesis, sulphide and CH$_4$ emission was measured in disturbed seagrass *T. hemprichii* meadows. A spectrophotometric technique was used for the determination of sulphide by its incorporation into methylene blue, in the presence of acidic N,N-dimethylp-phenylenediamine and ferric ammonium sulphate (Lawrence et al. 2000). The stability of the colour and the sensitivity and the specificity of the reaction for sulphide makes this method superior to other techniques. Alternative technique could have been used to measure sulphide concentrations by electrochemical detection (Lawrence et al. 2000). The advantageous of this technique is that it is easy and quick to use in the field (Jeroschewski et al. 1996). However, it has several drawbacks including long response times and poisoning of the reference electrode component by sulphide.

2.6 Determination of N$_2$O emissions

In paper II, N$_2$O emission rates were measured during low tide (at approximately 10-20 cm. depth) N$_2$O was collected by means of a static chamber technique (0.5 m long and 0.08 m ID; area 0.05275m$^2$ and volume 0.854 L). The chamber was tightly closed with rubber stopper and wrapped with black plastic sheet to avoid photosynthetic activity. Two chambers were deployed in each meadow, one enclosed seagrass plants, while the other was placed in-between seagrass within the same meadow. The aim was to explore if seagrasses are capable of mediating transport of these gases from the sediment to the atmosphere. The chambers were gently pushed into the sediment to a depth of approximately 0.33 m to ensure a seal against the ambient atmosphere and incubated for 3 h. Twelve gas subsamples were taken from each chamber using a 1-mL gas-tight syringe (BRAUN Omnifix®-F solo German). The syringe with the sample was inserted into a rubber bung and stored at room temperature for 2-3 days prior to analysis. In the laboratory we used the GC-ECD technique to quantify the amounts of N$_2$O emitted (Wang...
et al. 2010). Alternative approaches could have been used to quantify N₂O emission rates is by the acetylene inhibition technique (Seitzinger et al. 1993). The method is comparatively easy and fast and has been one of the most widely used technique for measuring denitrification in aquatic sediments (Christensen and Sørensen 1986; Revsbech et al. 1988). The reason why we didn’t chose this technique is because of its inhibition effects, for example, acetylene inhibits methanogenesis (Oremland and Taylor 1975) and sulphate reducing bacteria (Payne and Grant 1982). According to Wang et al. (2010) the GC-ECD technique is more stable and has high level of precision.

2.7 Determination of CH₄ emissions

In paper II and V, we also determined CH₄ emissions rates in a seagrass meadow. The gas was collected in the same way as described in section 2.6 above. In the laboratory, we used the GC-FID technique to quantify the amounts of CH₄ emitted. The alternative technique that could have been used to measure CH₄ emission rates is by non-dispersive infrared CH₄ analyser (Bartlett et al. 1987; Crosson 2008). The major drawback of this technique is the low sensitivity necessary to measure concentrations of weaker absorbers such methane (Crosson 2008), therefore the technique could not fit into our measurements.

2.8 Determination of total hydrolysable amino acids (THAA)

In paper III, we also quantified the amount of total hydrolysable amino acids in order to assess the status of organic carbon degradation in disturbed seagrass plots. 5 ml of subsample was collected from each depth segment in collected cores. The sample were freeze-dried at -60 °C prior to the analysis, 50 mg subsamples were ground and homogenized. For a detailed description of the THAA analysis, see Paper III:
3. Key findings

3.1 Carbon sequestration

In paper I, we conducted a comprehensive field survey to quantify the amount of organic carbon stored in tropical seagrass meadow types across the coast of eastern Africa, dominated by either *T. hemprichii*, *C. serrulata*, *E. acoroides* or *T. ciliatum*, as well as in unvegetated areas. We assessed the strength of the relationship to environmental attributes at a hierarchy of scales. The findings revealed that sedimentary organic carbon differed greatly among seagrass habitats as well as across sites and was clearly higher in seagrass sediment compared to levels of nearby unvegetated sediments. The variations in organic carbon stock of seagrass sediment were highly related to density of sediment, below-ground seagrass biomass and landscape configuration. In specific, *T. ciliatum* showed a high organic C sequestration capacity, and the highly seagrass-dominated Chwaka Bay had a very high organic carbon storage in all meadow types (Paper I). In addition, the level of eutrophication appeared to influence the organic carbon stocks in the sediment, as within the mainland sites studied in paper II, the most nutrient exposed sites also had the highest organic carbon content in the sediments (Paper II).

3.2 Emission of nitrous oxide and methane from tropical seagrass meadows at different eutrophication levels

In Paper II, we assessed the emission of N$_2$O and CH$_4$ in polluted and relatively pristine *T. hemprichii* meadows. The aim of this paper was, first to assess the effects of eutrophication on organic matter deposition, second to determine its effects on the emissions of N$_2$O and CH$_4$ in pollution impacted seagrass meadow and third to explore if seagrass plant can act as conduit of greenhouse gases from the sediments to the atmosphere via aerenchyma tissue.

The results clearly showed that carbon and total nitrogen are high in nutrient-rich sites (Paper II: Figure 3 &4), concurring with Hendriks et al. (2008) that seagrass meadows have high ability to trap and settle down suspended organic matter thereby increasing allochthonous C. There was a relatively low but significant emission of both N$_2$O and CH$_4$ in all seagrass meadows (Paper II Figure 2a & b), and the emissions increased considerably at the sites with high
nutrient load and high below-ground biomass as previously described by Kreuzwieser et al. (2003) (Paper II: Table 1). The proportion of carbon did not differ in above-ground biomass between all sites and the proportion of total nitrogen in the above-ground biomass were significant lower at Mbweni site compared to other sites (Paper II: Table 2). The C:N ratio in both above-and below-ground biomass was significantly lower at Ocean Road compared to the other three sites. The high proportion of below-ground to above-ground biomass found here (in line with what have been found in other studies, e.g. (Brouns 1985) together with a high root production and turnover rate (Duarte et al. 1998) can have caused anaerobic conditions and stimulated anaerobic microbes such denitrifying bacteria and methanogens, which re-mineralized stored nutrients and released it as N₂O and CH₄ gas from the sediment.

3.3 Effects of gas transport through seagrass plants

Some plants thriving in highly reduced sediment like rice (Oryza sativa), Spartina and mangroves, which all seem to mediate the transport of greenhouse gases from the sediment to the atmosphere via their lacunae system (Yu et al. 1997; Rusch and Rennenberg 1998; Kreuzwieser et al. 2003). However, in this study, no significant increases in gas emission were measured over seagrass plants compared to when measured on a sediment surface without seagrass except for the most highly eutrophicated site (Paper II: Figure 2). This indicates that seagrass normally do not facilitate the transport of greenhouse gases from the sediments to the atmosphere.

3.4 Effects of shading and simulated grazing on carbon sequestration

In Paper III, we manipulated seagrass plots in a T. hemprichii meadow to assess impacts on the carbon sequestration process and sedimentary carbon storage by (i) shading at two different intensity levels (low shading, LS, and high shading, HS) causing light reductions comparable to defined levels of eutrophication or sedimentation, and (ii) clipping of above-ground biomass to simulate two levels of grazing pressure (Low clipping, 50%, LC, and high clipping, 100% HC). We hypothesized that: (1) shading would cause a lower community productivity and a reduced plant biomass, and (2) clipping of above-ground biomass would lead to a reduction of below-ground biomass and intensified erosion of the upper sediment layer. The findings revealed significantly decreased net community productivity (NCP) (Paper III: Figure 2a), and carbon content in the below-ground biomass in HS and HC treatments, as well as in the unvegetated area compared to the seagrass
controls. The results showed no significant effects in organic carbon, total nitrogen, C:N ratio and total hydrolysable amino acids (THAA) in surface sediments (Paper III: Figure 4a & b). The proportion of carbon in rhizomes was significantly lower in both HC and HS compared to seagrass control, while no disturbance effects were revealed in proportion of carbon in roots or shoots (Paper III: Figure 3). In terms of carbon per area (g m$^{-2}$), HC and HS showed significantly lower levels in both rhizomes and roots compared to the seagrass control, whereas for shoots the HC treatment showed significantly lower carbon level compared to the control (Paper III: Figure 3). The proportion of total nitrogen in the rhizomes was higher in the HS and LS treatments compared to the seagrass control plots. No effects of disturbance were revealed in the proportion of nitrogen in roots. The proportion of total nitrogen was affected by HS in shoots as compared to the seagrass control. The C:N ratio in rhizomes was significantly affected by both HS and HC compared to the seagrass control (Paper III). The C:N ratio in shoot was negatively affected by high intensity disturbances compare to the control. While the C:N ratio in roots did not differ between any disturbance treatment and seagrass control. The observed effects of disturbances could be due to a decreased photosynthetic efficiency of the seagrass community following a decrease in above-ground biomass due to stresses imposed. Similarly, Alcoverro et al. (1999) reported reduced carbon biomass when seagrass *Zostera marina* was exposed to low light irradiance.

3.5 Effects of prolonged shading and simulated grazing on productivity and resource allocation

In Paper IV, we used the same experimental settings as in Paper III in order to assess the effects of shading and simulated grazing on seagrass productivity and resource allocation. The results showed that all disturbances (HC, HS, LC and LS) significantly reduced the above-and below-ground biomass (Paper IV: Figure 2A). This was likely due long-term shading and leaf clipping which forced the plant to respond by increasing “resource use efficiency” by reducing below-ground growth and leaf shedding. In addition, the proportion of carbohydrate in plant storage organs (rhizome and roots) was severely reduced by disturbances and more importantly, the organic carbon storage level diminished with level of disturbance (Paper IV: Figure 7A&B). In this study, the rhizome was revealed to be the main storage organ of total carbohydrate in *T. hemprichii* as it was previously described by Pirc (1989) and Jiang et al. (2013) in Mediterranean and tropical seagrass, respectively. From this findings, HS and HC significantly reduced both total carbohydrates and starch, which was likely due to the reduced above-ground biomass that is
responsible for conversion of inorganic carbon to organic carbon (total carbohydrates, among them starch). Limited photosynthetic rates in seagrasses due environmental stress has significant effects in the main storage organs of the plant (rhizome). Silva et al. (2013) also reported similar observations in disturbed seagrass *Zostera marina* and *Cymodocea nodosa* in Ria Formosa coastal lagoon Southern Portugal. Light attenuation (Alcoverro et al. 1999; Jiang et al. 2013; Silva et al. 2013) and reduction in leaf properties such as leaf area index and number of shoots (Paper IV: Figure 2E & F) significantly reduced the carbon fixation rates and hence also the carbon biomass accumulation (Paper III). If such stress prolongs, the storage organs (rhizomes and roots) tend to mobilize its stored foods (starch and other carbohydrates) to support newly growing leaves. Alcoverro et al. (1999) and Lee and Dunton (1997) also reported diminished proportion of carbohydrates when seagrass *Zostera marina* and *Thalassia testudinum* were stressed by light reduction, respectively.

3.6 Sulphide production and methane emission in a disturbed tropical seagrass meadow

In Paper V, we used the same experimental setup as described in Papers III and IV to assess the effects of disturbances on the production of sulphide as well as the emission of CH₄ gas. High sulphide production was measured in highly disturbed treatments (Figure 10a &b). Such pronounced effects from disturbance on sulphide production was probably due to the low above-ground biomass observed (Paper IV). Physiologically, reduced above-ground biomass affects photosynthetic rates of the seagrass and subsequently less photosynthetic-oxygen is produced and transported to the underground tissues. Less photosynthetic oxygen in the underground tissue stimulates anaerobic microbial processes such sulphate-reducing bacteria that uses seawater column sulphate as their main terminal electron acceptor. An increased sulphide concentration in the sediment might have negative effects on seagrass plants as it is an effective phytotoxin (Borum et al. 2005; Calleja et al. 2007). Less sulphide concentration in the top 9 cm observed in this study could probably be due to high aeration from photosynthetic oxygen and from the water column. A decrease in the pore water sulphide concentration at the bottom (21 cm) of some of the sediment samples might indicate the end of the sulphate-reducing zone in the seagrass *Thermophic*. 
Parallel to sulphide levels, we assessed the emission of CH$_4$ in disturbed seagrass meadows. High CH$_4$ emission emerged in highly disturbed seagrass treatments (Figure 11). This is likely due to a decrease of photosynthetically derived oxygen transported to below-ground tissues and an increased degradation of below-ground tissues (Paper V). The non-structural carbohydrates of the rhizomes decompose very rapidly (Paper IV) and might contribute as a potential substrate for marine anaerobes. In the present findings, the co-existence of sulphate reducing bacteria (SRB) and methanogens in high disturbed (HC, LC and HC) was probably due excess availability of substrates from decomposing below-ground biomass and or the presence of non-competitive substrates (e.g. trimethylamine *not measured in this study*) that allows the existence of methanogens and sulphate-reducing bacteria.

Figure 10. Average porewater sulphide concentration in (a) shading and (b) leaf clipping treatments at Chwaka bay after 150d of disturbances. Data points are mean ± SE (n=4). For abbreviations see figure 7
Figure 11. Methane emission rate measured after 150d of continuous shading and leaf clipping. Letters above bars indicate significant differences between treatments (One-way ANOVA; P < 0.01). Error bars show mean ±SE (n = 4).
4. Discussion

This work surveyed the amount of organic carbon stored in different tropical and subtropical coastal seagrass meadows along the eastern African coast. The assessment included the major habitat-building seagrass species in the region (Paper I). The findings generally revealed a relatively high organic carbon storage, ranging from 1684 to 10819 g m$^{-2}$ across sites. This suggests that tropical seagrass meadows are good climate change mitigation agents. Significant variations in carbon stock were found across sites as well as between the same species in different localities (Paper I). For example, in Chwaka Bay at the east coast of Unguja, Zanzibar, the organic carbon storage was clearly higher in both seagrass meadows and unvegetated areas compared to other sites (Paper I). This brought us to the conclusion that carbon stocks in seagrass meadows are not only influenced by seagrass meadow productivity and filtering capacity but also by other environmental factors such as marine ecosystems configuration, sediment characteristics (Paper I) and eutrophication (Paper II). Tropical subtidal seagrass sediments of high organic content influenced by high temperature and low oxygen supply can support anaerobic microbial activities such as methanogenesis and denitrification in the seagrass rhizosphere. Consequently, our observations of N$_2$O and CH$_4$ emissions in all studied sites can be explained by such prevailing conditions and that emissions increased in nutrient-rich sites (Paper II). In specific, the emission rates of both N$_2$O and CH$_4$ showed positive correlations with below-ground seagrass biomass (Paper II), potentially being a result of high turnover rate of roots (Duarte et al. 1998). Thus, anthropogenic disturbances like eutrophication might in these coastal waters change the function of the seagrass meadows from being sinks to become sources of greenhouse gases (Paper II and V).

Seagrass meadows are well known for their high productivity and high carbon sequestration capacity (Mcleod et al. 2011). However, what will happen if seagrass meadows are disturbed? We experimentally manipulated a seagrass (Thalassia hemprichii) meadow in Chwaka Bay to understand effects of disturbance on productivity (from plant to meadow scale), carbon sequestration, CH$_4$ gas emission and sulphide production (Papers III-V). After five months of shading and simulated grazing, we found significant decreases in above- and below-ground biomass in all disturbed seagrass plots (Paper IV). In addition, the findings revealed that disturbances had negative effects on both seagrass plant photosynthesis and the net community productivity.
(Papers III and IV). Disturbances (in high-intensity plots) caused a loss of carbon biomass and reduced the carbon sequestration capacity of the meadow (Paper III). However, no effects were seen in the sedimentary carbon storage during the course of the experiment (Paper III).

The two types of disturbances also affected sediment processes (Paper V), as pronounced effects on CH$_4$ gas emission and sulphide production were found. This was probably a result of reduced photosynthetic rates (due to shading) and a loss of above- and below-ground seagrass biomass (due to both shading and simulated grazing). This might in turn cause an increased decomposition (consuming oxygen) in the sediment and reduced transport of oxygen to underground tissues, resulting in oxygen deficiency in the sediment. Such conditions stimulate anaerobic microbes such as sulphate reducing bacteria (Pedersen et al., 2004) and methanogens (archaea). An increased sulphide concentration in the sediment might have contributed to the negative effects we observed on seagrass plants as it is an effective phytotoxin (Borum et al. 2005; Calleja et al. 2007). Holmer et al. (2003) argued that even low concentrations (20-30 µM) of sulphide in sediment porewater might cause a significant decrease in seagrass cover of Mediterranean *Posidonia oceanica*. However, we did not observe any major seagrass die-off even at as high concentration as 550 µM sulphide in the highly disturbed *T. hemprichii* plots (Paper V). The emission rates of CH$_4$ in the Chwaka Bay experiment (Paper V) were generally low from a global scale perspective (Table 2 in Paper V), but high compared to the surveyed sites at the mainland (Paper II). However, there was a several-fold increase in the CH$_4$ emission in the disturbed seagrass plots (Paper V).

Despite the decisive role seagrass plays in atmospheric carbon reduction, they are the most threatened marine vegetation (Duarte 2002) in the world. Seagrasses are very sensitive to disturbances and a small change in their natural environments may severely affect their productivity (Hemminga 1998; Ralph et al. 2007). Industrialization and coast development are major stressors that have increased pollution and physical disturbance on marine vegetation in Tanzania. Ecosystem services such as carbon sequestration is thereby highly threatened. This study gives an early warning to policy makers and other climate change stakeholders on the importance and anthropogenic threats that might accelerate climate change effects.
5. Concluding remarks

In this thesis, the major focus has been to explore the processes and capacity of carbon storage in tropical seagrass ecosystems. Several specific questions were addressed and the data generated allowed the following more general conclusions to be drawn:

- WIO seagrass meadows are important blue carbon stocks as they store substantial amounts of organic carbon in their tissues and underlying sediments (Paper I)
- Organic carbon storage in seagrass meadows is not only a function of species composition, plant morphology and abundance but is also influenced by landscape configuration (Paper I) and nutrient flux (Paper II)
- Disturbances with damaging effects of carbon fixation can negatively affect the carbohydrate and starch reserved in rhizomes (Paper IV)
- High-intensity shading and simulated grazing negatively affected seagrass biomass, reduced the photosynthetic efficiency and depleted organic carbon in the biomass (Papers III and IV)
- High-intensity shading and simulated grazing significantly contributed to methane emission and production of sulphide in the studied seagrass meadow (Paper V)
- Excess nutrient fluxes in coastal vegetation contribute to the emission of N₂O gas into the atmosphere (Paper II)
6. Future research

The findings of this work give a deeper insight into the importance of different seagrass species in climate change mitigation through carbon sequestration and storage. Still, however, many questions remain unclear such as:

What amount of organic C is fixed by the seagrass itself and what amounts originate from other sources (for example from the terrestrial) in seagrass meadows of eastern Africa? Answers could be achieved by stable isotope signature and mass spectrometry, to determine the proportion of organic C originated from other sources.

Which are the key environmental factors that influence organic C deposition in tropical seagrass meadows? Temperature, morphology, sediment depth, sediment texture and/or other factors?

In the present study, high emissions of CH₄ and sulphide production were revealed in high-intensity disturbance treatments, indicating the co-existence of methanogens and sulphate reducing bacteria. Future challenges could be to understand what makes them to co-exist in disturbed seagrass. Is it an unlimiting substrate availability and/or other factors? Studies focusing on the amount and types of substrates (e.g. trimethylamine, methylamine and acetate) as well as production of methanogens are needed.

Additional studies should also integrate more extensive sampling strategies to include other seagrass species to get a more general picture on carbon sequestration and storage in tropical seagrass meadows.
7. Sammanfattning

kustmiljön kan också bidra till att koldioxid och andra växthusgaser såsom metan (CH₄) börjar läcka ut ur sedimenten och bidra till klimatförändringar.

Denna avhandling handlar om hur olika faktorer, både naturligt förekommande men också olika typer av klimatrelaterade stressmoment orsakade av mänskliga aktiviteter, påverkar kollagringens kapacitet hos tropiska sjögräsarter vid västra Indiska Oceanens kuster i östra Afrika. Samspelet mellan sjögräsängarna och viktiga växthusgaser har också undersöks. Den första studien klargjorde hur effektivt olika arter binder kol i sedimenten i förhållande till region och också med avstånd till olika kolkällor på land. Studiens resultat fastställde att det inte alltid var vilken art som dominerade i sjögräsängen som spelade den största rollen utan att också avståndet till andra ekosystem, såsom mangroveområden, och partikelstorleken i sedimenten som påverkade halten av organiskt kol i sedimentet. De efterföljande studierna gick mer detaljerat in på hur olika typer av störningar påverkar sjögräsens förmåga att binda kol och andra växthusgaser. I en större studie där olika störningar simuleras, såsom effekten av skuggning samt betning (genom klippning) av sjögräs, sågs många negativa effekter. Produktiviteten, och därmed kolinslagringen, minskade drastiskt. Detta kan kopplas till förändringar i syrgastransporten ner till sedimenten där man såg att sulfitid började anrikas i den syrefattiga miljön och metan började läcka ut tillbaka till havsvattnet. I ett annat försök där effekten av övergödning undersöktes såg man även här att sjögräsen påverkades negativt och halten av metan men även lustgas (N₂O) som läckte ut från sjögräsängarna till vattnet och atmosfären ökade kraftigt.

Sammanfattningsvis visar denna avhandling att sjögräsängar i östafrikanska kustzoner fungerar som effektiva naturliga kolsänkor där koldioxid kan bindas under långa perioder och i och med det inte bidra till ökade klimatförändringar. Dessutom visas också att flera av de störningar som utgör hot mot sjögräsen, det vill säga minskad ljustillgång, övergödning och förhöjt betningstryck, kan negativt påverka sjögräsängarnas funktion från att vara kolsänkor till att istället bli källor till koldioxidutsläpp samt flera andra växthusgaser då dessa läcker ut ur systemet. Värdet i att bevara och skydda dessa viktiga marina ekosystem blir på detta sätt ännu mer klart.
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