

ASSESSMENT OF TROPICAL BLUE CARBON  
RESERVES IN THAILAND

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## **CERTIFICATE OF AUTHORSHIP / ORIGINALITY**

I certify that the work presented in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree, except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Pemika Apichanangkool

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### **Chapter 2:**

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## ABBREVIATION

C <sub>org</sub>	Organic carbon
df	Degrees of freedom
DO	Dissolved oxygen
GPP	Gross primary production
MS	Mean square
NCP	Net community production
POC	Particulate organic carbon
R	Respiration
SPM	Suspended particulate matter

## ABSTRACT

Carbon dioxide (CO<sub>2</sub>) emission through human activities is one of the most critical issues affecting the entire globe. Among the solutions, carbon sequestration is an important way to reduce atmospheric CO<sub>2</sub>. Vegetated coastal habitats – seagrasses, saltmarshes, and mangroves – are among the most effective carbon sinks of the world. These habitats capture and store (sequester) large quantities of organic carbon (C<sub>org</sub>), termed ‘blue carbon’. The rapid decline of seagrass in many areas around the world, especially in Southeast Asia has motivated us to study the carbon-sink capacity of tropical Blue Carbon habitats, as well as the impact of the loss of seagrass. This study comprised of three major aims: 1) to investigate the impact of seagrass loss on blue carbon sink capacity; 2) to investigate the influence of seagrass species-specific canopy structure on blue carbon sink capacity; and 3) to investigate the feasibility of using artificial seagrass for blue carbon restoration.

Seagrass meadows at Haad Chao Mai National Park, Trang, Thailand trap allochthonous (externally-produced) carbon into sediment reaching up to 90% of C<sub>org</sub> stored. At a pristine meadow, seagrass densities play a major role in determining the sediment C<sub>org</sub> stock. Seagrass canopy height was found to be not important when comparing C<sub>org</sub> sink capacity between *Thalassia hemprichii* (medium-sized species) and *Enhalus acoroides* (large-sized species) in this study. On the other hand, seagrass densities influenced the trapping capacity of allochthonous carbon. The sediment



organic carbon sources of *T. hemprichii* and *E. acoroides* beds for all densities tested were similar (dominated by suspended particulate matter and mangrove for the top 15 cm of sediment). High shoot densities of seagrass could promote the settlement of suspended particles by increasing the chance of particle to contact directly with leaf blade. Seagrass biomass influenced the community metabolism. The Net Community Production (NCP) of seagrass meadows was higher with increased above-ground biomass. NCP measured in meadows with 75% cover of *T. hemprichii* ( $104.59 \pm 21.72$  mmol C m<sup>-2</sup> d<sup>-1</sup>) and *E. acoroides* ( $166.92 \pm 12.32$  mmol C m<sup>-2</sup> d<sup>-1</sup>) were higher than these of NCP measured in meadows with 12% cover of *T. hemprichii* ( $63.54 \pm 5.53$  mmol C m<sup>-2</sup> d<sup>-1</sup>), *E. acoroides* ( $78.09 \pm 4.63$  mmol C m<sup>-2</sup> d<sup>-1</sup>) and unvegetated sediment ( $53.36 \pm 4.11$  mmol C m<sup>-2</sup> d<sup>-1</sup>). Seagrass loss following elevated sedimentation and increasing water turbidity lead to the loss of 89% of sediment organic carbon (C<sub>org</sub>) stock. Loss of seagrass resulted in the loss of allochthonous carbon trapped by the seagrass canopy. Loss of seagrass also altered the sediment grain size distribution. Elevation of coarse grains was found in a denuded site compared to a pristine meadow. About 50% of sediment grain size from the pristine meadow consisted of fine sand (0.125 – 0.25 mm), while 50% of sediment from the pristine meadow consisted of very fine sand (0.0625 – 0.125 mm). The evidence of a weakened blue carbon sink due to seagrass loss was also found as a reduction of carbon sequestration. The level of Net Community Production (NCP) at a denuded site ( $21.13 \pm 8.30$  mmol C m<sup>-2</sup> d<sup>-1</sup>) was lower than the NCP measured at a pristine meadow ( $53.36 \pm 4.11$  mmol C m<sup>-2</sup> d<sup>-1</sup>).

While the negative impact of seagrass loss on blue carbon sink capacity was evaluated, artificial seagrass was shown to be an innovative technique to enhance particle- and organic carbon deposition. The particle deposition measured at the denuded site with artificial seagrass was 3-times higher than the particle deposition rate measured at the

denuded site without artificial seagrass. The organic carbon trapped by artificial seagrass was 12-times higher than occurred at these denuded sites without artificial seagrass. There was no significant difference in the particle deposition rate and organic carbon deposition rate between an artificial seagrass experiment and the natural pristine seagrass meadows. Thus, artificial seagrass is an effective tool to recover blue carbon sink capacity where the allochthonous carbon is a major carbon source, artificial seagrass is an effective tool in the recovery of blue carbon sink capacity – it enables a more rapid recovery and requires less effort than other restoration techniques. For better estimates of blue carbon sink capacity, seagrass abundance was recommended as an appropriate monitoring indicator because it influences the sediment  $C_{org}$  stock, while species-specific canopy height did not play an important role determining sediment  $C_{org}$  stock in this particular study.

## CHAPTER 1

### LITERATURE REVIEW

The carbon cycle is a constant process of carbon dioxide (CO<sub>2</sub>) exchange between the atmosphere, ocean and land. CO<sub>2</sub> is produced and absorbed by many microorganisms, plants, and animals (Post et al., 1990). However, industrialisation and rapid population growth have caused a rapid increase in greenhouse gases and other atmospheric aerosol emissions over the 20<sup>th</sup> century (Solomon et al., 2009). Consequently, the balance of CO<sub>2</sub> in the carbon cycle has changed because the CO<sub>2</sub> emitted by mankind is much higher than the amount of CO<sub>2</sub> absorbed by existing natural sinks. The release of greenhouse gases is the major cause of global climate change (IPCC, 2001). Atmospheric carbon dioxide (CO<sub>2</sub>) is the primary greenhouse gas released due to human activities such as deforestation, the combustion of fossil fuels (coal, natural gas, and oil) and the production of livestock (Beauchemin et al., 2010, Duxbury, 1994). Earth's atmospheric CO<sub>2</sub> level increased to 400.23 parts per million (ppm) in February 2015 (Dlugokencky and Tans, 2015). This increasing atmospheric CO<sub>2</sub> level is having a severe impact on the world, causing changing weather patterns, ecosystem destruction and the loss of livelihoods (Rozenzweig and Parry, 1994, Guan, 2009). To prevent an increase of 2°C in the global mean temperature by the year 2050, global CO<sub>2</sub> emissions must be reduced by 85% (IPCC, 2007).

Recent research has highlighted the valuable role of natural carbon sinks to sequester carbon and mitigate climate change (McLeod et al., 2011, Nellemann et al., 2009, Duarte et al., 2013b). However, a significantly large proportion of natural ecosystems are rapidly declining (Orth et al., 2006, Waycott et al., 2009). Consequently, the ability to absorb and reduce CO<sub>2</sub> via the natural ecosystem sequestration is reduced.

Understanding the significant role these natural ecosystems play in absorbing and storing carbon is a crucial issue, and it is important to find an effective approach to restore these degraded carbon sinks.

In this chapter, the big picture of global climate change is described – its cause, impact and ways to mitigate it. The four carbon colors (black, brown, green and blue) and their impact on the global carbon cycle are introduced, followed by the role of the ocean in the carbon cycle. The significant roles of vegetated coastal ecosystems as blue carbon sinks, and seagrass meadows as blue carbon hot spots, are highlighted. The loss of seagrass, which impacts long-term carbon sink capacity, and the possible ways to restore blue carbon sinks, are also reviewed. At the end of the chapter, the knowledge gaps in seagrass research are stated.

## **1.1 BLACK AND BROWN CARBON: THEIR IMPACT ON GLOBAL CLIMATE CHANGE**

In this section, the different forms of carbon was described. Each form of carbon has different source and impact on a global ecosystem. The particulate matter in the atmosphere – atmospheric aerosol – consists of solid or liquid particles suspended as a gas. The diameter of atmospheric aerosol particles can range from nanometers (nm) to tens of micrometers ( $\mu\text{m}$ ). They are produced by many sources including natural and anthropogenic sources (Ramanathan and Carmichael, 2008). Natural sources of atmospheric aerosol includes: wind-borne dust, sea spray, volcanic activity and biomass burning (Rose, 1977, Mbourou et al., 1997, Andreae and Merlet, 2001). A number of human activities increase atmospheric aerosols, including agriculture, heavy industry, and the transportation sector (Sharma et al., 2010, Sheesley et al., 2010). Aerosols in the atmosphere (atmospheric particles) are major contributors to climate change as they

play an important role in the global radiative balance (Charlson et al., 1992). The absorption of solar radiation by aerosols could significantly impact atmospheric energy distribution and hydrologic processes (Menon et al., 2002). Black and brown carbon are two very important light absorbing substances of atmospheric aerosols, as both are a major cause of global warming as they absorb heat in the atmosphere and reduce albedo (Menon et al., 2002). They are also a major cause of severe health problems in humans (Feng et al., 2013). The light absorbed by the black and brown carbon could lead to a warming effect, rivaling that of anthropogenic carbon dioxide (Andreae, 1995).

### **1.1.1 Black carbon**

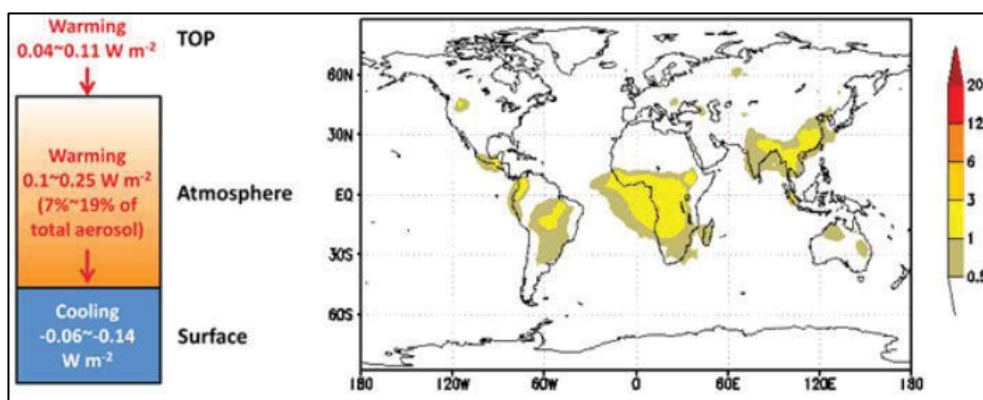
Black carbon is a component of soot formed by the incomplete combustion of fossil fuel, biofuels and biomass. The main source of black carbon emission is due to biomass burning (Feng et al., 2013). Black carbon is the strongest light absorbing component of particulate matter contributing towards 72% of global atmospheric absorption (Feng et al., 2013). The high light absorption capacity of black carbon could impact atmospheric energy distribution and hydrologic processes (Barnett et al., 2005). Black carbon is linked to a range of climate impacts including increasing temperatures, accelerating ice and snow melting, and disrupting precipitation patterns (Menon et al., 2002, Ramanathan and Carmichael, 2008). Absorption of the solar radiation before it reaches the Earth's surface, atmospheric heating, and surface cooling lead to a reduction in cloud cover through evaporation and the suppression of upward motion for cloud formation (Koch and Genio, 2010). Globally, the strong absorption of solar radiation could impact negatively on evaporation and rainfall (Ramanathan and Carmichael, 2008). The deposition of black carbon on snow and ice leads to darkening of the surface of the ice, thus decreasing its reflectivity, thereby accelerating snow and ice melting

rates (Flanner et al., 2007). The warming and melting of snow and ice could have a severe impact on sensitive regions such as the Arctic and the Himalayas (McConnell et al., 2007). Black carbon was reported to be a main contributing factor towards the increasing melting rates of some glaciers and snowpack in parts of the Hindu Kush-Himalayan-Tibetan region (Bonasoni et al., 2012). It is also reportedly responsible for a rise in global temperatures of up to 0.3°C or 0.4°C (Chung and Seinfeld, 2005). Thus, reducing black carbon could be an effective strategy to mitigate global warming (Hansen et al., 2000).

### **1.1.2 Brown carbon**

Brown carbon is light-absorbing organic carbon. Brown carbon originates from the combustion of coal, smoldering fires (tar materials), biomass burning (pyrolysis products), soil emissions, and biogenic emissions (humic-like substances) (Alexander et al., 2008, Bond, 2001, Mukai and Ambe, 1986). As opposed to black carbon, brown carbon has a wide range of absorptive variables. Brown carbon can strongly absorb ultraviolet wavelengths (Yang et al., 2009). The absorption efficiency and spectral dependence of brown carbon depends on its origins (Feng et al., 2013). Recently, brown carbon has been gaining attention as a potential cause of climate change. Brown carbon emissions from biomass burning are associated mostly with organic compounds of extremely low volatility, which indicates that brown carbon from biomass burning can be an important factor in aerosol radiative forcing (Lack et al., 2012). Feng et al. (2013) reported that the strongly absorptive brown carbon could be responsible for up to 19% of the absorption by anthropogenic aerosols, whilst 72% is attributed to black carbon, and 9% is due to sulphate and non-absorbing organic aerosols coated with black carbon. As with black carbon, the result of brown carbon absorption could warm the top of the

atmosphere and cool the Earth's surface. A significant amount of brown carbon (10–35 mg m<sup>-2</sup>) has been reported in four main global regions: Africa, South America, South Asia and East Asia (Arola et al., 2011). Atmospheric warming and surface cooling (in W m<sup>-2</sup>) due to light absorption by organic brown carbon aerosol is shown in Figure 1.1.



**Figure 1.1** Atmospheric warming and surface cooling (in W m<sup>-2</sup>) due to light absorption by organic brown carbon aerosol (Feng et al., 2013).

## 1.2 GREEN AND BLUE CARBON: CARBON CAPTURED THROUGH BIOSEQUESTRATION

Biosequestration is the process to remove atmospheric carbon by primary producers and microbial organisms. Green and blue carbon is a term used to describe the carbon sequestered by terrestrial forest and coastal ecosystems, respectively.

### 1.2.1 Green carbon

Green carbon is the atmospheric carbon captured by terrestrial plants through photosynthesis and subsequently stored in plant biomass and soil. The forest can store ~45% of terrestrial carbon at a rate ranging from 0.3 to 5.0 Pg C yr<sup>-1</sup> (Nellemann et al., 2009). These large quantities of CO<sub>2</sub> can be stored in plant biomass and soil for several decades. However, the green carbon sink's potential is limited, as the carbon is returned

to the atmosphere as CO<sub>2</sub> or methane when biomass is broken down (IPCC, 2007). The soil carbon storage of terrestrial ecosystem becomes saturated when the net carbon fixed annually reaches zero (Rabenhorst, 1995). Moreover, the longevity of the above- and below-ground carbon sink is uncertain (Schlesinger and Lichter 2001). Whilst the capacity of terrestrial ecosystems as a carbon-binding habitat has gained worldwide attention, there are also the “hidden gems” of coastal and ocean ecosystems called “blue carbon” sinks. The role these ecosystems play in sequestering carbon needs to be further explored.

### **1.2.2 Blue carbon**

Blue carbon is the carbon stored in the coastal and oceanic ecosystems, initially, via photosynthesis. Although, blue carbon sinks cover only 0.2% of the seafloor; they contribute about 50% of total C<sub>org</sub> burial in ocean sediments (Nellemann et al., 2009). Unlike green carbon, however, the carbon stored in the coastal and oceanic ecosystems does not remain for decades or centuries, but rather for millennia in a particular species (Mcleod et al., 2011). Large quantities of blue carbon can be sequestered and stored in coastal ecosystems – mangroves, tidal marshes, and seagrasses (Mcleod et al., 2011). A significant amount of carbon is stored in plant biomass (above-ground and below-ground biomass) and sediment (Nellemann et al., 2009). Thus, these coastal ecosystems are now recognised for their role in mitigating climate change.

## **1.3 THE OCEAN CARBON CYCLE**

The ocean is an important part of the global carbon cycle. Atmospheric CO<sub>2</sub> diffuses into the seawater’s surface via the “solubility pump” and becomes dissolved inorganic carbon (DIC) (Post et al., 1990). A number of factors influence the quantity of CO<sub>2</sub> that

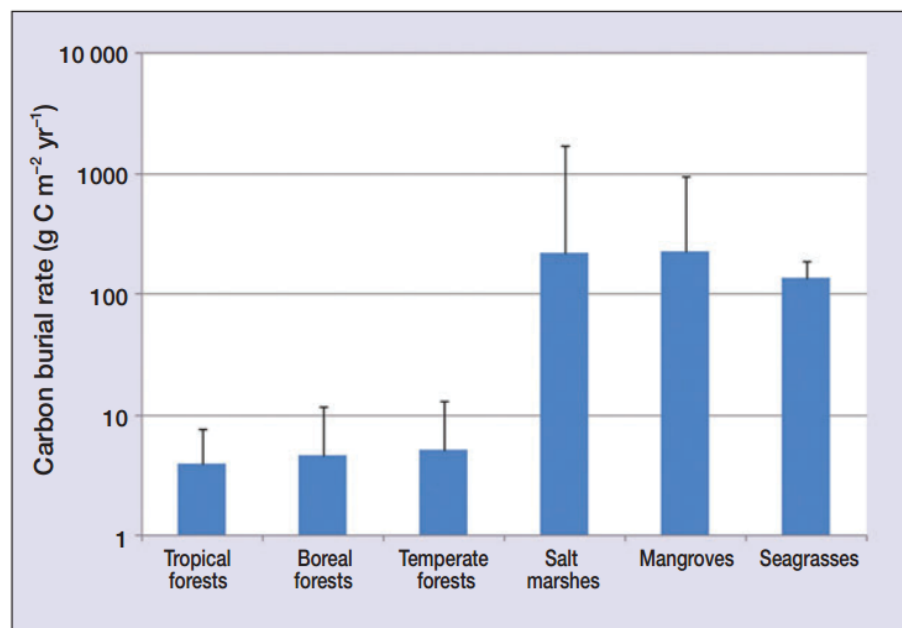


diffuses and dissolves in surface seawater, including water temperature, salinity, total alkalinity and the atmospheric concentration of CO<sub>2</sub> (Takahashi et al., 1993, Goyet et al., 1999, Weiss et al., 1982). Marine organisms use carbon through biological and chemical processes. In some organisms, carbon also gets incorporated into marine organisms as organic matter or structural calcium carbonate (Morse et al., 2007). The dissolved CO<sub>2</sub> in the surface of the seawater turns into organic matter through the process of photosynthesis by primary producers (e.g. phytoplankton) (Post et al., 1990). The process of carbon being moved in one direction by these organisms is called the “biological pump” (Post et al., 1990). The organic matter produced by phytoplankton enters the marine food web and a proportion of the carbon exported from surface waters can sink and slowly accumulate on the seafloor (Longhurst and Harrison, 1989). To build the materials of shells and skeletons, carbon is also used for creating calcium carbonate by shell-building (calcifying) organisms (such as coral) and plankton (like coccolithophores and foraminifera) (Iglesias-Rodriguez et al., 2008). The formation of calcium carbonate in surface waters causes a net evolution of CO<sub>2</sub> back to the atmosphere, but the dead cells (fossilised carbonate organisms) that sink deep into the water form a carbon-rich sediment (Ekdale and Berger, 1978). The carbon cycle in the ocean can lock up carbon for millions of years (Post et al., 1990).

### **1.3.1 Ocean vegetated “blue carbon” habitats**

The carbon captured by the ocean’s vegetation is called “blue carbon”, and this accounts for 50–71% of the atmospheric carbon captured by living organisms (Nellemann et al., 2009). The carbon sequestration of blue carbon habitats is greater than other habitats based on the unit area. Blue carbon vegetated habitats include mangroves, salt marshes and seagrasses. Carbon captured by the vegetated blue carbon habitat can be stored in

the sediment, in living biomass (above ground and below ground biomass), and in non-living biomass (such as litter and dead wood). Although vegetated blue carbon habitats are smaller in area than terrestrial forests, the long-term carbon sequestration of these vegetated blue carbon habitats are much greater than that of terrestrial forests (Mcleod et al., 2011) (Fig. 1.2). The sediment carbon burial rate in vegetated blue carbon habitats is relatively high. The organic carbon ( $C_{org}$ ) burial rate measured in saltmarshes and mangroves ( $1.51 \text{ Mg ha yr}^{-1}$  and  $1.39 \text{ Mg ha yr}^{-1}$ , respectively) for example, was found to be 10- and 6-times higher than the burial rate measured in the undisturbed Amazonian forest (Grace et al., 1995, Nellemann et al., 2009).



**Figure 1.2** Mean long-term rates of C sequestration ( $\text{g C m}^{-2} \text{ yr}^{-1}$ ) in soils in terrestrial forests and sediments in vegetated blue carbon habitats (saltmarsh, mangrove and seagrass). Error bars indicate maximum rates of accumulation (Mcleod et al., 2011).

Unlike terrestrial ecosystems, anoxic sediment in coastal ecosystems leads to the constant preservation of carbon (Henrichs and Reeburgh, 1987). In addition, the vertical accretion of sediment in response to rising sea levels enhances the health of these ecosystems (McKee et al., 2007). Thus, the size of the sediment  $C_{org}$  pool can increase as long as sea level rises and accumulation of soil by the coastal ecosystem remains able to keep pace (Chmura et al., 2003). The sediment  $C_{org}$  pool of saltmarshes, mangroves and seagrasses is not only derived from their ecosystem, called autochthonous carbon, but it is also derived from external sources, known as allochthonous carbon (Saintilan et al., 2013). The complexity of a plant's physical structure (dense vegetation, leaf canopy and root system) enhances the trapping capacity and promotes the allochthonous input into the sediment (Agawin and Duarte, 2002, Dittmar et al., 2001), thereby allowing the amount of sediment  $C_{org}$  stock to increase.

Unlike wetland freshwater where the  $CO_2$  is a terminal electron acceptor, vegetated coastal ecosystems accumulate sediment  $C_{org}$  without the emission of  $CH_4$  (Bridgham et al., 2006). The oxidation of organic matter in vegetated coastal ecosystems occurs through the reduction of sulphate (Isaksen and Finster, 1996). At high concentrations of sulphate, such as found in seawater, the terminal electron acceptor inhibits methanogenesis (Isa et al., 1986). Therefore, the capacity of vegetated coastal ecosystems to sequester  $C_{org}$  is often greater than the capacity of freshwater wetlands.

Among the vegetated blue carbon habitats, seagrass meadows are a vital part of the climate change solution as they are uniquely able to store carbon in their sediment for

thousands of years (Nellemann et al., 2009). Although, seagrass meadows account for a small percentage of global coastal areas, they provide a huge blue carbon sink in the world's oceans (Mcleod et al., 2011).

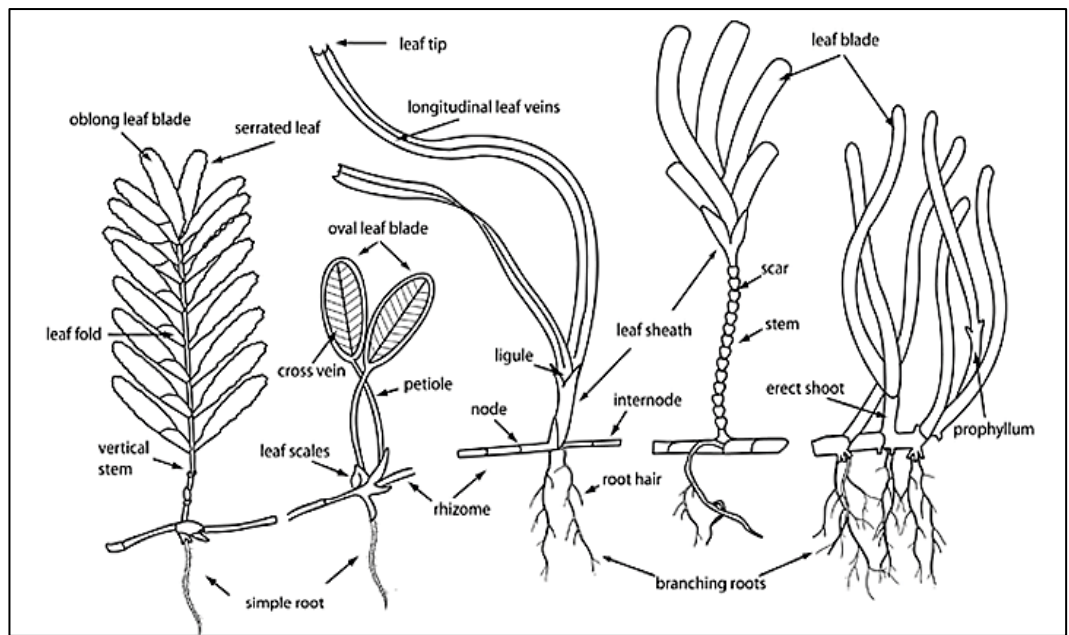
#### **1.4 SEAGRASS MEADOWS: VITAL CARBON SINKS**

Seagrasses are regarded as one of the most important blue carbon ecosystems – they only cover approximately 0.1% of the coastal ocean, yet they store as much as 15% of the total carbon that accumulates in marine sediments (Kennedy et al., 2010). However, this blue carbon reservoir has largely been ignored in carbon accounting (Macreadie et al., 2014). The following topics introduce the basic information of seagrass, including taxonomic information, morphological features and the ecology of seagrass. The key functions of seagrass habitats are presented, including their ecological function, and their critical role as blue carbon sinks, focusing on the tropical seagrass ecosystem. Loss of seagrass and the resulting impact on the blue carbon sink capacity is also addressed.

##### **1.4.1 Introduction**

Seagrass are marine flowering plants (Division Angiospermae) from four plant families: *Posidoniaceae*; *Zosteraceae*; *Hydrocharitaceae*; *Cymodoceaceae*. They are all in the order Alismatales (in the class of monocotyledons). Seagrasses are not categorised into a taxonomic group, but they are an ecological group of plants which grow fully submerged in seawater, pollinate under water (except for *Enhalus* which emerges to the water surface for reproduction) and have underground organs including complex root systems (root and rhizome) (Hartog and Kuo, 2006). Twelve major divisions of approximately sixty seagrass species have been classified (Kuo and Hartog, 2001). As the reproductive organs of seagrasses are rarely found in some species, the

morphological features have been used for the identification of seagrass species including blade width, blade tips, root and rhizome, as well as anatomical features such as epidermal cell and fiber distribution (Kuo and Hartog, 2006). Figure 1.3 illustrates the main morphological features of seagrasses to distinguish between the different taxonomic groups.



**Figure 1.3** The morphological features of seagrass (McKenzie, 2008).

Seagrasses have a broad global distribution in both temperate and tropical regions. A similar number of seagrass species in temperate and tropical regions has been reported, with some overlapping species between these two regions (Short et al., 2007). Most of the tropical seagrass species were found in shallow water (< 10 m water depth). Their distribution is from the upper intertidal zone to the great depths of the subtidal zone. Despite this, a number of environmental factors constrain the distribution of tropical seagrass, such as light level, temperature, as well as water current, depth and nutrient levels (Dennison, 1987, Duarte, 1991, Vermaat and Verhagen, 1996).

Tropical seagrasses can be found in estuaries, shallow coastal habitats, and coral reef habitats (Fig. 1.4). The linkage between seagrass and the adjacent ecosystem, especially coral reef and mangrove, provides both physical and biological support to these ecosystems. This includes the effect of seagrasses on buffering and stabilising sediment, as well as nutrient loading, which benefits the coral reef ecosystem (Boer, 2007). The habitat connectivity between seagrass, mangrove and coral reef ecosystems provides a benefit for marine organisms as sources of shelter and food. Habitat migration during life stages has been reported in some marine vertebrate species. For example, a number of fish species were observed in all of the different life stages of the seagrass, mangrove and coral reefs (Morinière et al., 2002). Because seagrass meadows are both a high food abundance and low predation habitat, seagrass meadows have been reported to be nurseries for coral reef fish populations (Nagelkerken et al., 2002).

Seagrasses provide a number of ecosystem services to the marine ecosystem. As mentioned above, seagrasses function as a nursery in addition to providing shelter for marine life (Orth et al., 2006). Seagrasses also play an important role in maintaining populations of commercially important species of fish and some invertebrates. For example, for some marine organisms such as sea cucumbers (*Holothuria scabra*), seagrass meadows are the permanent habitat for their full life cycle (Wolkenhauer et al., 2010).

High biodiversity of marine organisms is often found in seagrass meadows. For example, a positive, significant relationship has been observed between the above ground biomass of *Thalassia testudinum* Banks ex König, and mobile macroinvertebrates (Heck and Crowder, 1991). Macrofaunal abundance, biomass and production have been observed to be much greater in seagrass meadows than in

unvegetated habitats (Edgar, 1990), and fish assemblages in seagrass meadows are different from those of bare sands (Guidetti, 2000). The megaherbivores such as dugongs (*Dugong dugon*), green sea turtles (*Chelonia mydas*) and manatees (*Trichechus manatus*) graze directly on seagrass (Aragones and Marsh, 2000). Seagrasses also play a role in the detrital food chain, and in nutrient cycling (Ziegler and Benner, 1999b). Seagrass canopies are effective filters of nutrient inputs to the coastal ocean (Agawin and Duarte, 2002) because they can modify the water flow, and thereby enhance sedimentation and prevent erosion (Fonseca, 1989). The complex root system stabilises the sediment and forms a large organic matter pool (Gacia et al., 2002). Exported seagrass detritus can become an organic matter source for food-limited environments such as the deep sea (Suchanek et al., 1985). The organic carbon ( $C_{org}$ ) produced by seagrass is buried in the sediment supporting seagrass meadows, making it a blue carbon hot spot.



**Figure 1.4** Seagrass meadow on Green Island reef flat, offshore Cairns, North Queensland, Australia represents tropical seagrass associated with coral reef ecosystem (Apichanangkool, 2012).

#### 1.4.2 Seagrass meadows are a key blue carbon sink

Recently, the role of seagrass meadows as a key blue carbon sink has been reported with the global estimation of  $C_{org}$  stored in seagrass meadows as 19.9 Pg (Fourqurean et al., 2012). Although seagrass meadows only cover 0.1% of the world ocean's area, the carbon stored in seagrass meadows accounts for 10–18% of the total oceanic carbon burial, with an accumulation rate from 48–112 Tg C yr<sup>-1</sup> (McLeod et al., 2011).

Seagrass ecosystems are one of the most productive ecosystems on Earth with the average production rate of around 2.7 g DW m<sup>-2</sup> d<sup>-1</sup> (Duarte and Chiscano, 1999). Seagrass meadows tend to support autotrophic communities, as the rate of gross primary production (GPP) is usually higher than the respiration rate (Duarte et al., 2010, Barrón et al., 2004). This indicates the amount of photosynthetic carbon captured by seagrass is higher than the carbon released by metabolic respiration (Ziegler and Benner, 1999a). In addition, most of what seagrass produces is not consumed by heterotrophs, further supporting seagrass meadows as net autotrophic communities.

The capacity of seagrass to accumulate organic carbon in sediment depends on the fate of seagrass production, which can be described by the following mass-balance equation (Mateo et al., 2006):

$$\Delta B_t = NPP - H - DP$$

$$\Delta DM_t = DP + I - D - E - RA$$



Where:

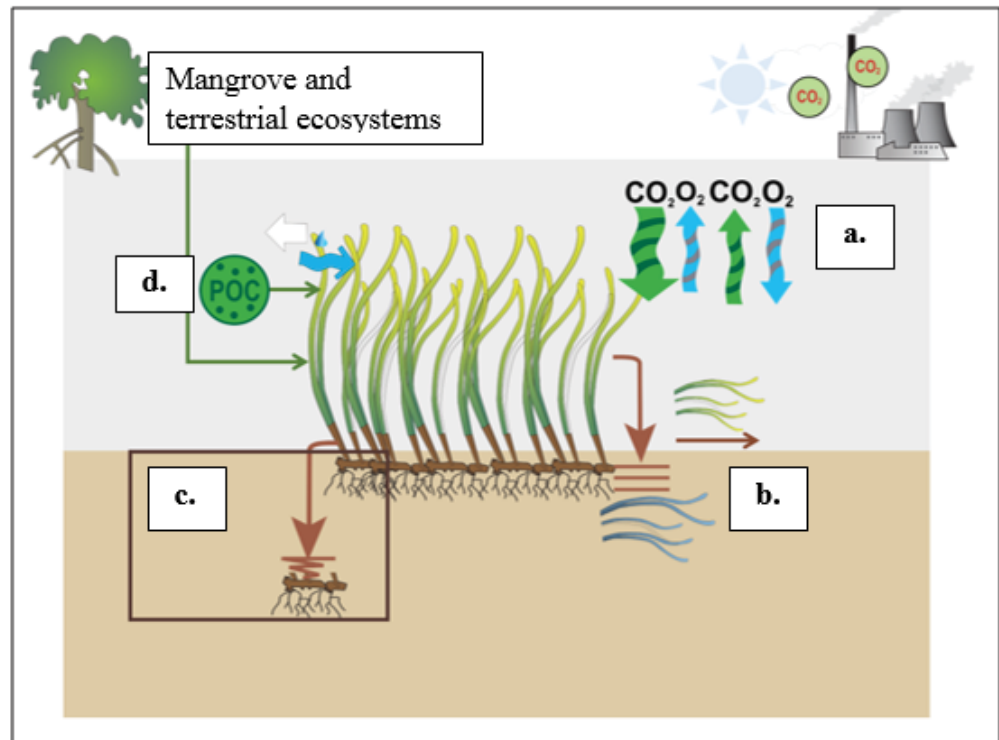
- $\Delta Bt$  (the living compartment) is the change in seagrass biomass per unit time
- $\Delta DMt$  (the detrital compartment) is the change in seagrass degradable detrital mass per unit time
- NPP is the temporal rate of net primary production
- H is herbivory, DP is detrital production, I is import (allochthonous)
- D is decomposition
- E is export (leakage)
- RA is refractory accumulation.

Seagrass species have a wide variation in their rates of primary production (Duarte and Chiscano, 1999), which may ultimately affect the amount of carbon stored in the sediment underlying seagrass meadows (Duarte et al., 2010). The community metabolism measured in seagrass meadows are different between community types and species (Duarte et al., 2010). Tropical seagrass meadows tend to support higher metabolic rates than temperate seagrass meadows (Duarte et al., 2010). The net community production (NCP) of seagrass ecosystems reported from worldwide temperate and tropical seagrass meadows has a broad range, from -477 to 531 mmol O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> (Duarte et al., 2010). However, not all NCP is buried and remaining within seagrass meadows. A previous report estimated that about 30% to 50% of NCP is buried, while the rest could be consumed and/or exported to nearby ecosystems (Kennedy et al., 2010, Duarte et al., 2010).

Carbon stored in seagrass sediment is derived from two major sources: 1) autochthonous carbon; and 2) allochthonous carbon (Boschker et al., 2000, Kennedy et al., 2010, Agawin and Duarte, 2002). The overall  $C_{org}$  stocks and fluxes in seagrass meadows are shown in Figure 1.5. Autochthonous carbon source is derived from seagrass itself, in the leaf litter and the below-ground tissue. To produce organic carbon, seagrass uses bicarbonate ( $HCO_3^-$ ) (followed by  $CO_2$  uptake) from the water column as an inorganic carbon source for photosynthesis (Beer et al., 2002). The accumulated  $C_{org}$  in seagrass meadows can be derived from carbon fixation and the direct accumulation of seagrass materials. Senescing leaves or broken seagrass leaves can be either accumulated on the top of the sediment surface or exported to nearby ecosystems. Seagrass litter can be broken down by the process of microbial decomposition (Mateo et al., 2006). The anoxic sediments underneath seagrass meadows, however, slows the decomposition process because the supply of oxygen, which is an electron acceptor for microbes in the sediment, is insufficient (Pollard and Moriarty, 1991). The slow microbial decomposition rate is one of the factors promoting the long-term storage of carbon in seagrass meadows (Holmer et al., 2004).

Seagrass litter can also contribute to the  $C_{org}$  sediment pool directly through leaching, bioturbation and burial (Mateo et al., 2006). The below-ground seagrass tissues (root and rhizome) are a major component of the autochthonous sediment  $C_{org}$ . The sediment  $C_{org}$  can be derived from  $C_{org}$  exudation, as well as the root and rhizome materials, which can all be consumed by microbes (Moriarty et al., 1986). Seagrass roots and rhizomes were reported as major contributors to sediment  $C_{org}$  stock in the temperate seagrass meadow containing *P. oceanica*, where a thick fibrous mat had been formed and remained intact for thousands of years (Mateo and Romero, 1997, Fourqurean et al., 2012, Mateo et al., 2006).

In order to better understand carbon fluxes and stocks, a study of sediment  $C_{org}$  origin is necessary. To identify the origin of sediment  $C_{org}$ , we require data showing clear isotopic distinction among the different carbon “fingerprints” for each carbon source (Macreadie et al., 2014).



**Figure 1.5** The conceptual diagram of  $C_{org}$  stocks and fluxes in seagrass meadows. a) Seagrass meadows tend to be autotrophic communities as they uptake carbon more than they produce carbon from respiration. b) Leaf litter is a component of sediment  $C_{org}$  and is exported to the nearby ecosystem. c) The autochthonous carbon (source) is derived from sources within seagrass meadows such as leaf litter and below-ground tissue. d) The seagrass canopy can trap and filter allochthonous  $C_{org}$  from mangrove materials and suspended organic carbon. The diagram was produced using the Integration and Application Network (IAN), University of Maryland Center for Environmental Science, Cambridge, Maryland.

### 1.4.3 Isotopic composition: the blue carbon “fingerprint”

Identification of the origin of sediment  $C_{org}$  is important to better understand the blue carbon sink capacity in seagrass ecosystems (Macreadie et al., 2014). The sediment  $C_{org}$  pool in seagrass meadows can be derived from a variety of sources, such as seagrass, mangrove and suspended particulate matter (SPM) (Kennedy et al., 2010). There are a number of methods to determine the sources of sediment  $C_{org}$ , such as using biomarkers and stable isotope analysis to compare the C:N ratios of the possible source and the sediment (Boschker et al., 1999, Vizzinia et al., 2002, Thornton and McManus, 1994). The stable carbon isotope  $\delta^{13}C$ , is most widely used to evaluate the source of sediment  $C_{org}$  in coastal ecosystems (Rodelli et al., 1984, Cifuentes et al., 1996, Wang et al., 2003, Kennedy et al., 2010, Kennedy et al., 2004).

There are two natural stable isotopes of carbon:  $^{12}C$  (98.9%) and  $^{13}C$  (1.1%). A delta value ( $\delta$ ) is calculated from the ratio of heavy ( $^{13}C$ ) to light stable ( $^{12}C$ ) isotopes in samples ( $R_{sample}$ ) relative to the ratio of the standard ( $R_{standard}$ , from Vienna Pee Dee Belemnite) using the following the equation (Smith and Epstein, 1971):

$$\delta X = 1000[(R_{sample}/R_{standard}) - 1], \text{ where } R = ^{13}C/^{12}C$$

If  $\delta X$  shows a positive result, it means the sample contains more of the heavier isotope than the standard.

The chemical, biological and physical process produce the distinct isotopic signatures of the biological material (Gannes et al., 1998). In plant tissues, the carbon-fixation

process is a primary cause of variation in  $^{13}\text{C}/^{12}\text{C}$  ratios (O'Leary, 1981). The mass difference between the isotopic form of an element leads to differences in the physical processes and chemical reaction of the isotope. For example,  $^{12}\text{C}$  is lighter than  $^{13}\text{C}$ , so the reaction in photosynthesis of  $^{12}\text{C}$  is faster than  $^{13}\text{C}$  (Smith and Epstein, 1971). Generally, the lighter isotope tends to form a weaker covalent bond, consequently reacting faster than the heavier isotope. The differences in the bond energy of the isotope and the reaction rate are a result of the variation in isotopic abundance between chemical species. The change of isotopic abundance between chemical species due to the physical or chemical processes is called "fractionation" (O'Leary, 1981). Isotopic fractionation can be expressed by the fractionation factor,  $\alpha$  (Hoefs, 1980). The fractionation could lead to deplete of isotopic signatures, for example  $^{13}\text{C}$  of the organic carbon ( $\text{C}_{\text{org}}$ ) is depleted due to the fractionation during photosynthesis (Farquhar et al., 1989, Cuy et al., 1993).

Carbon fractionation in seagrass ( $\text{C}_3$  plant) occurs in two photosynthetic processes: 1) diffusion/dissolution – the diffusivities of  $\text{CO}_2$  contains  $^{13}\text{C}$  and  $^{12}\text{C}$  which is controlled by the concentration of  $\text{CO}_2$  between inside and outside of a leaf or cell; and 2) carboxylation – fractionation by the enzyme ribulose-1, 5- bisphosphate carboxylate (RUBISCO) (Benedict et al., 1980, Farquhar et al., 1989, Hemminga and Mateo, 1996).  $^{13}\text{C}$  of seagrass is less depleted compared with other aquatic primary producers such as mangrove, phytoplankton and macroalgae (Lepoint et al., 2004). A recent study reported that the variability of  $\delta^{13}\text{C}$  from 216 seagrass samples collected globally ranged from -19.6‰ to -4.8‰ with a median value of -10.3‰ (Kennedy et al., 2010).

There are three environmental factors which influence these variations in seagrass  $\delta^{13}\text{C}$ , namely source of carbon, irradiance, and temperature (Hemminga and Mateo, 1996). In

addition,  $\delta^{13}\text{C}$  could be different within plant organs and during post-depositional decomposition (Papadimitriou et al., 2005, Fourqurean and Schrlau, 2003). Although various isotopic compositions can be found in organs of seagrass, most of the  $\delta^{13}\text{C}$  measurements assumed that seagrass leaf material is a representative tracer of seagrass-derived carbon (Kennedy et al., 2010). Leaf material has been used to determine the origin of sediment  $\text{C}_{\text{org}}$  because the leaf production rate of seagrass is around 5-times higher than that of the rhizome production rate (Bittaker and Iverson, 1976). There is little information on the variation in the amount of  $\delta^{13}\text{C}$  during the decomposition stage (Kennedy et al., 2010). In South Florida, only a small variation in the  $\delta^{13}\text{C}$  was found in the decomposition stage of seagrass (Zieman et al., 1984), whilst the  $\delta^{13}\text{C}$  in *Thalassia testudinum* depleted by 2‰ during decomposition (Fourqurean and Schrlau, 2003). However, the variance of  $\delta^{13}\text{C}$  due to the difference of plant organs, as well as the post-depositional and seasonal changes, is small when compared to inter-source differences as it does not affect the overall outcome.

A recent study reported that the  $\delta^{13}\text{C}$  of sediment was lighter than the  $\delta^{13}\text{C}$  of seagrass, indicating significant input of allochthonous  $\text{C}_{\text{org}}$  into seagrass meadows (Kennedy et al., 2010). Using the mixing model calculation, Kennedy et al (2010) found that 50% of the sediment  $\text{C}_{\text{org}}$  from 207 sites (temperate and tropical seagrass meadows) around the world is derived from allochthonous sources (dominated by phytoplankton and mangrove). Variability of  $\delta^{13}\text{C}$  in sediment was also found among seagrass species. The sediment  $\delta^{13}\text{C}$  ranged from -7.3‰ to -26.4‰ in 20 seagrass species (Kennedy et al., 2010). This variability of sediment  $\text{C}_{\text{org}}$  sources could be determined by two main factors: 1) the supply rate of the sources; and 2) the species-specific characteristics of seagrass (Kennedy et al., 2010). High levels of allochthonous carbon trapped by seagrass meadows was found in tropical seagrass. In South East Asia, 70% of sediment

$C_{org}$  is derived from allochthonous sources, and 95% of sediment  $C_{org}$  in the western Pacific is derived from terrestrial sources (Kennedy et al., 2010). The large amount of allochthonous C in the sediment could indicate the effective trapping capacity of seagrass – one of its unique characteristics compared to others ecosystems – and its enhanced blue carbon sink capacity (Kennedy et al., 2010, Macreadie et al., 2010).

The study of isotopic compositions as a blue carbon fingerprint could provide additional supportive evidence that seagrass meadows are a significant blue carbon sink as they do not only store the autochthonous  $C_{org}$ , but they also import the allochthonous  $C_{org}$  from other sources (Macreadie et al., 2014). However, seagrass meadows have been threatened by anthropogenic activities worldwide, raising concern for the loss of its blue carbon sink capacity.

## **1.5 LOSS OF BLUE CARBON SINK CAPACITY**

### **1.5.1 Seagrass degradation**

Seagrass loss is accelerating worldwide, with the annual rate of loss reaching 7% since 1990 (Waycott et al., 2009). Seagrasses are sensitive to changing of environmental conditions, such as water quality, light intensity, temperature, and nutrient. Anthropogenic stress is a major cause of this rapid loss of seagrass, on a scale of up to hundreds of square kilometers (Hastings et al., 1995, Waycott et al., 2009, Orth et al., 2006). In South East Asia and the Caribbean, the loss of seagrass is expected to accelerate due to growing human pressure on coastal zones (Duarte, 2002). Increasing human populations, followed by agricultural and coastline development, is resulting in increasing pollution, changing coastline conditions, loading of estuaries with sediment and nutrients, the introduction of non-native species, damage from boat moorings, and

an overall increase in water turbidity (Duarte, 2002, Occhipinti-Ambrogi and Savini, 2003, Delgado et al., 1999, Hastings et al., 1995, Ruiz and Romero, 2003). For example, the large damage of seagrass at Khao Bae Na, Haad Chao Mai National Park, Thailand is the impact of high water turbidity resulting from sediment loading. In the global scale, seagrass ecosystem is potentially affected from climate change.

Seagrass loss impacts directly on associated marine organisms. Loss of seagrass leads to the subsequent loss of habitat and food sources for many marine species (Duarte, 2002, Worm et al., 2006). Seagrass loss also impacts on humans and the global economy because seagrass meadows are a source of many fishes for coastal fisheries (Torre-Castro and Rönnbäck, 2004).

#### 1.5.2 Blue carbon coastal ecosystem degradation

Recent studies have provided evidence of blue carbon coastal ecosystem degradation (Pendleton et al., 2012, Fourqurean et al., 2012). Pendleton et al (2012) reported that seagrass loss could potentially lead to significant carbon emissions, converting ocean ecosystems from sinks to sources. Assuming that 100% of the  $C_{org}$  in the top meter of the sediment would be released following seagrass loss, the carbon released into the atmosphere could reach up to 299 Tg C (Fourqurean et al., 2012). Pendleton et al (2012) estimated that carbon released globally as a consequence of seagrass loss from the upper 1 m of sediment is  $\sim 5 \times 10^6 - 3.3 \times 10^7$  Mg  $CO_2$ , which is equivalent to 1%–6% of carbon released from global deforestation. The release of  $CO_2$  from degraded seagrass habitats could cost the global economy US\$1.9–13.7 billion annually (Pendleton et al., 2012).



There is an urgent need to understand the level of carbon loss occurring at sites that have been affected by different types of degradation, such as sediment loading, contaminant draining, oil spills, and mooring. Thus, in order to mitigate climate change, it is of utmost importance to find a feasible way to recover elements of the degraded carbon sink, such as lost seagrass meadows.

## **1.6 RECOVERING AND IMPROVING COASTAL BLUE CARBON SINK CAPACITY**

The imbalance between seagrass loss and its potential to recover is a significant issue in many areas around the world. Seagrass loss is accelerating, and natural recovery of seagrass would take a long time, ranging from decades to centuries (Bryars and Neverauskas, 2004, Hastings et al., 1995, Kirkman and Kuo, 1990). To accelerate the recovery of seagrass, restoration techniques have been developed since the 1970s (Wear, 2006). Restoration is usually described as returning an ecosystem to a close approximation of its condition prior to disturbance (Gordon, 1996). Techniques that have been used for restoration include seagrass transplantation, planting seedlings, and the facilitation of natural recovery (Wear, 2006). Although a large number of seagrass restoration projects have been conducted around the world, the number of successful seagrass restorations has varied due to a number of factors such as habitat selection, environmental condition, and plant donor population (Calumpong and Fonseca, 2001, Katwijk et al., 2009).

The original drivers of seagrass loss such as increased water turbidity, increased loading of terrestrial sediment, and increased wave energy, limit the chances of recruitment and survival of naturally restored seagrass (Ruiz and Romero, 2003, Badalamenti et al., 2006). In Australia, high wave energy limits the feasibility of the introduction of

seagrass seedlings (Seddon et al., 2005). In addition, low light condition was reported as a limited factor leading to the failure of seagrass restoration in the Chesapeake Bay where there is only a 2% recovery of seagrass (Moore et al., 2012).

Seagrass restoration also requires considerable effort and funding. The estimated minimum number of staff required for a restoration project, for example is 7 to 9, depending on the complexity of the restoration project (e.g. bare-root planting or stapling) and the habitat type (e.g. intertidal habitat or subtidal) (Calumpong and Fonseca, 2001). The cost of seagrass restoration is dependant on the method used (Calumpong and Fonseca, 2001). In 1998 for example, the cost of seagrass restoration using the transplantation method was US\$ 37,000 per hectare (Fonseca et al., 1998). The full cost of seagrass restoration in the Federal Court system was reported as much as US\$ 630,000 per hectare (Spurgeon, 1998). Moreover, there are a number of additional factors which contribute to the excessive cost of seagrass transplantation (Calumpong and Fonseca, 2001), including:

- 1) Poor site selection
- 2) Lack of experience by staff who conducted the project
- 3) Frequent disturbances (e.g. bioturbation, storms) requiring re-transplanting
- 4) Water depths that require use of scuba divers
- 5) Low water velocity
- 6) Soft sediment
- 7) Planting in cold water
- 8) Capitalisation (e.g. purchasing equipment)
- 9) Extra site preparation (e.g. creation of subtidal dikes)
- 10) Excessively frequent monitoring

- 11) Laborious monitoring (blade width, length, and animal count)

Thus, a more pragmatic way to increase successful seagrass restorations, while also reducing the effort and cost involved, is needed.

### **1.6.1 The potential role of seagrass restoration to enhance blue carbon sink capacity**

Recent studies have begun to investigate the recovery of carbon sequestration and carbon stocks after seagrass restoration (Greiner et al., 2013). Two recent research studies have conducted the monitoring of seagrass restoration on sediment organic carbon level of *Zostera marina* (Greiner et al., 2013) and *Posidonia australis* (Marbà et al., 2015). The results of blue carbon sink recovery following seagrass restoration is still limited and may not be generalisable to all seagrass species. It was found that a timeframe of more than 10 years was needed to recover the stored carbon to the same level as that of natural healthy seagrass meadows (Greiner et al., 2013, Marbà et al., 2015). The carbon accumulation rate measured at the restored seagrass meadows – using seedling techniques – was reported to increase and be comparable to that of natural seagrass meadows within 12 years of restoration (Greiner et al., 2013). With seagrass planted over 18 years ago, the carbon burial rate increased to a similar level as that measured in a pristine seagrass meadows ( $26.4 \pm 0.8 \text{ gC}_{\text{org}} \text{ m}^{-2} \text{ yr}^{-1}$ ) (Marbà et al., 2015). The result of blue carbon sink recovery following seagrass restoration is still limited and may not be generalizable to all seagrass species. The research that investigate the potential of seagrass restoration in blue carbon sink recovery is still needed. As the loss of blue carbon sink capacity following habitat degradation is an

urgent issue, we need to find a less time consuming, and more cost effective way to recover the blue carbon sink capacity before it shifts from a sink to a source.

### **1.6.2 Artificial seagrass: an alternative way to restore a seagrass habitat**

Artificial seagrass has been widely used for field and laboratory experiments (Shahbudin et al., 2011). Artificial seagrass is a replication of natural seagrass using a various kind of materials, such as plastic ribbon. The ability of artificial seagrass to attract marine fish and invertebrates is well known (Bell, 1985, Macreadie et al., 2010, Sogard, 1989). Artificial seagrass has been reported to provide ecosystem services comparable to natural seagrass meadows – they behave as nurseries for juvenile fish and habitats for diverse fish species (Macreadie et al., 2010, Virnstein and Curran, 1986). Artificial seagrass have been shown to stabilise the sediment composition within the transplanted area (Campbell and Paling, 2003). They facilitate natural processes to promote seagrass recruitment into previously damaged areas. However, there are potential limitations in the use of artificial seagrass that need to be considered. For example, the possibility that artificial seagrass might cause health problem in marine organisms as a result of the breakdown of the material. If the surrounding environment became contaminated, the material might be consumed by living organisms. Thus, it is unclear if artificial seagrass can play a role in ecological services and climate change mitigation in conjunction with natural seagrass.

## **1.7 KNOWLEDGE GAPS AND RESEARCH PRIORITIES**

### **1.7.1 Estimates of carbon loss following different levels of habitat degradation**

While the fate of lost carbon is still debatable, a recent study has estimated the extent of carbon released from habitat degradation by assuming that all carbon stocks are simply

remineralised (Fourqurean et al., 2012). Despite this, there is insufficient data to estimate the degree of carbon loss at sites that have been affected by different types of habitat degradation. There are a number of habitat degradation. Some processes of degradation might cause the remineralisation of sediment organic carbon, or they might lead to the export of sediment organic carbon to other ecosystems. Similarly, some processes, such as sediment loading, might potentially lock the organic carbon underneath the new sediment layer. In fact, it has been suggested that even a slight disturbance to seagrass meadows can result in 25% – 100% carbon loss (Pendleton et al., 2012). To quantify the possible carbon released from seagrass carbon stores after habitat degradation, it is necessary to know how much carbon is leaked following seagrass loss as a result of the different types of habitat disruptions, such as the loading of anthropogenic sediment, and increasing water turbidity. Without seagrass to stabilise the sediment and prevent erosion, the physical forces of waves and currents disturb the surface sediments by removing the  $C_{org}$  from sediment (Boer, 2007). Another factor to consider is that there may be a varied level of disruption in sedimentation at different depths along the vertical sediment depth gradient (Macreadie et al., 2014). The sediment depth gradient could reflect the net accumulation of sediment at a particulate time. For example, the low level of organic carbon at a certain depth level of particulate sediment could indicate the low input of organic carbon or high export of organic carbon at that time over time. Thus, information about the influence of seagrass loss at different sediment depths needs to be further considered. Furthermore, the carbon stored at a particular sediment depth could reflect the net carbon accumulation including both the loss and accumulation of these stores.

### **1.7.2 Species-specific canopy structure and the blue carbon capacity**

Estimates of coastal carbon stocks are limited because of a poor understanding of the underlying mechanisms that control carbon sequestration in these ecosystems (Macreadie et al., 2014). The morphological variability in seagrasses has been reported in both tropical and temperate zones (Apichanangkool and Prathep, 2014, Vermaat and Verhagen, 1996). Seagrass canopy structure varies among seagrass species. This species-specific canopy structure of seagrass controls the trapping capacity which potentially influences the blue carbon sink capacity of that seagrass (Lemmens et al., 1996). Whilst there are more than 60 seagrass species, the data set of seagrass sediment  $C_{org}$  stocks is limited as most of the studies have focused on the temperate seagrass species, *P. oceanica*. The highest sediment  $C_{org}$  stocks were reported for *P. oceanica* meadows with the sediment  $C_{org}$  stock up to 10,500–40,000 g  $C_{org}$  m<sup>-2</sup>. However, the corresponding measurements of carbon storage for other seagrass species, especially for the tropical seagrass species, are not known.

### **1.7.3 The effective way to recover blue carbon sink capacity**

Restoration efforts to mitigate climate change have only recently been addressed (Greiner et al., 2013, Marbà et al., 2015). Seagrass restoration using the seedling technique has been conducted to enhance blue carbon sequestration in degraded seagrass (Greiner et al., 2013). However, this technique is time-consuming and limited to areas where seagrass meadows have been degraded by anthropogenic activities (Katwijk et al., 2009). Although the natural recovery of seagrass at degraded habitats is possible, a more effective method to restore seagrass and recover their blue carbon sink capacity is required.

The body of this thesis is comprised of three chapters. The first relates to the initial investigation of the impact of seagrass loss. The second chapter examines how to

approach the recovery of lost carbon, including the characters (size and density) that most effectively enhance the blue carbon sink capacity. The third and last chapter applies the results presented in Chapters 2 and 3 to hypothesise a method by which the creation of artificial seagrass can contribute to the recovery of blue carbon capacity in areas where allochthonous carbon is a major source.

**CHAPTER 2**  
**THE IMPACTS OF TROPICAL SEAGRASS LOSS ON BLUE CARBON SINK**  
**CAPACITY**

**2.1 INTRODUCTION**

The critical role of some terrestrial ecosystems as both a source and a sink of organic carbon is well-known, whereas the role of vegetated coastal ecosystems in sequestering and storing carbon – “blue carbon ecosystems” – has only recently gained attention (Nellemann et al., 2009). Seagrasses are regarded as one of the most important blue carbon ecosystems – covering approximately only 0.1% of the coastal ocean yet storing as much as 15% of the total carbon that accumulates in marine sediments (Kennedy et al., 2010).

Seagrass meadows tend to support autotrophic communities. The rate of Gross Primary Production (GPP) is usually higher than the respiration rate (Duarte et al., 2010), which indicates that photosynthetic carbon captured by seagrass is higher than the carbon released by metabolic respiration (Ziegler and Benner, 1999a). In addition, the organic carbon ( $C_{org}$ ) can be preserved for millennia beneath seagrass meadows – below-ground (roots and rhizomes) production is buried in the sediment and accumulates over a long time as ‘refractory carbon’ (Mateo et al., 2006). The accumulation and preservation of organic matter in seagrass meadows is not only derived from autochthonous sources, but also from allochthonous, terrestrial and pelagic sources (Kennedy et al., 2010). Seagrass canopies promote the capture of allochthonous carbon into the seagrass associated sediment by filtering and trapping particles from the water column (Agawin



and Duarte, 2002). This could increase the levels of blue carbon in the sediment associated with seagrass.

Large areas of seagrass meadows and their associated ecosystems are under threat by anthropogenic activities. To date, about one-third of global seagrass meadows have been destroyed and seagrass loss is accelerating: in 1970, it was reported that seagrass meadows were disappearing at a rate of 0.9% per year, whereas in 2000 reported losses were estimated at 7% per year (Waycott et al., 2009). Recent studies have begun to provide evidence that habitat degradation of blue carbon coastal ecosystems has the potential to lead to significant carbon emissions, hence converting ocean ecosystems from sinks to sources (Fourqurean et al., 2012). Pendleton et al (2012) estimated that carbon released as a consequence of seagrass loss over the upper 1 m of sediment is  $\sim 5 \times 10^6 - 3.3 \times 10^7$  Mg CO<sub>2</sub>, which is equivalent to 1% – 6% of carbon released from deforestation globally. Releasing of CO<sub>2</sub> from degraded seagrass habitats could result in economic cost of 1.9 – 13.7 Billion US dollars annually (Pendleton et al., 2012).

Although the extent of carbon released from habitat degradation has been estimated by assuming that all carbon stocks are remineralised (Pendleton et al., 2012), there is insufficient data to estimate the level of carbon loss at sites that have been affected by different types of degradation. In fact, it has been suggested that even a slight disturbance to a seagrass meadow can result in 25% – 100% of carbon loss (Pendleton et al., 2012). Sediment that is retained, buried or redistributed would lead to a low carbon emission. To quantify carbon loss in a coastal ecosystem, we need to estimate carbon loss as a proportion of degradation because different types of habitat degradation could lead to different levels of carbon loss. Carbon loss following habitat degradation

could occur in terms of both physical loss from the meadow, such as through erosion, and remineralization to the atmosphere.

Seagrass meadow degradation and loss as a result of increased sediment loading have been described for several seagrass species worldwide (Fourqurean and Rutten, 2004, Gacia et al., 2003, Cabaço et al., 2008). For example, over a 10-year period in the Khao Bae Na, Haad Chao Mai National Park, Thailand, poor land practices, increasing in sediment loads and water turbidity led to the almost complete destruction of its seagrass meadows and in particular four dominant species: *Enhalus acoroides* (L.f.) Royle, *Cymodocea rotundata* Asch. & Schweinf., *Halophila ovalis* (R. Brown) Hooker f. and *Thalassia hemprichii* (Ehrenberg) Ascherson (Mukai et al., 1999). The seagrass at Khao Bae Na died, apparently due to light deprivation caused by sediment loading. Unfortunately, there is no monitoring report to confirm the precise time when seagrass completely disappeared from the denuded meadow. Based on interviews with researchers, the ranger at Haad Chao Mai National Park and the local fisheries, we found that seagrass gradually disappeared over the last 10 years.

In this study, we have investigated the impact of seagrass loss through comparisons between a pristine meadow, a denuded site and in unvegetated sediment on: 1) sediment organic carbon stock; 2) sediment organic carbon sources; 3) sediment grain size distribution; and 4) community metabolism as a measure of carbon sequestration. The study was undertaken at Haad Chao Mai National Park, Trang Province, Thailand in the Andaman Sea situated near the Kuan Tung Ku Estuary and 1 km from a mangrove forest. Three different meadows within Haad Chao Mai National Park were chosen that had been affected by anthropogenic impacts. The meadows comprised: 1) a pristine meadow vegetated with *Enhalus acoroides*; 2) a denuded site where seagrass had

previously grown 10 years prior but was no longer present (Supanwanid and Lewmanomont, 2003, Mukai et al., 1999); and 3) unvegetated sediment where there was no evidence that seagrass had previously been present. We hypothesised that the: 1) stock of carbon stored in the sediment at a denuded site would be similar to that in unvegetated sediment and lower than in a pristine meadow; 2) a lower stock at a denuded site would not be due to recently deposited sediment from land as a consequence of changing land management; 3) the changed water profile and flow dynamics at a denuded site would alter the grain size distribution at a denuded site compared with a pristine meadow and result in a distribution more like that in unvegetated sediment; and 4) a denuded site and unvegetated sediment would support lower community metabolism and would be net heterotrophic, as opposed to net autotrophic as would be found in a pristine meadow. Sediment carbon stocks, sediment grain size distribution, and community metabolism were compared among a pristine meadow, a denuded site and in unvegetated sediment at three different depths from the sediment surface (0–3, 3–10, and 10–15 cm).

## **2.2 MATERIALS AND METHODS**

### **2.2.1 Study site**

This investigation was conducted at Haad Chao Mai National Park, Trang Province, southwest Thailand, (7° 22' 21"N and 99° 20' 27"E) in the Andaman Sea. To investigate the impact of seagrass loss on the blue carbon sink capacity of this site, we compared carbon stocks and fluxes from denuded site, pristine meadow and unvegetated sediment. The previous reports and literature showed that the denuded site was once seagrass meadows, whereas the unvegetated sediment was not once seagrass meadow. The distance between the denuded and a pristine meadow was approximately 1 km.

Information pertaining to the history of the denuded site, including the causes of seagrass loss, and the species composition before loss was determined from published literature, interviewing local residents, and a site survey. The distance between the unvegetated sediment site and the denuded site was approximately 600 m, and the distance between the unvegetated sediment site and the pristine meadow was 400 m. Examination of sediment showed that there was no seagrass material present in the top 15 cm of the unvegetated sediment site, indicating no colonisation by seagrass in the recent period (data not provided).

All sampling sites were located in the same general area, but under two different sedimentary settings. All sites were adjacent to mangrove stands (approximately 1 km far from all of three study areas). The unvegetated and pristine sites were separated from the denuded site by a large tidal channel that linked an enclosed bay with the outer ocean. In addition, the unvegetated and pristine sites were mostly influenced by ocean waters and were protected from the tidal channel by a large sand bar that ran along the southern bank of the channel.

The pristine meadow chosen was a seagrass meadow that covered 18 km<sup>2</sup>. Nine of the twelve seagrass species reported in Thailand are found at this site. Seagrass grows from the upper intertidal zone where it is exposed during a low tide (approx. 4–5 hours in the dry season and 0.5–1 hour in the wet season) to the sub-tidal zone where it is continuously submerged at a depth greater than 4 m. There is a semi-diurnal tide at this site (Poovachinranon and Chansang, 1994). In this area, seagrass is found as both mono-specific meadows and multi-specific meadows. The dominant seagrass species is *Enhalus acoroides* and an area of a healthy meadow that was monospecific for *E. acoroides* was chosen to be a pristine site for this study. *E. acoroides* are widely

distributed in the lower intertidal to subtidal zone having a high abundance (75% covers).

### **2.2.2 Sample collection**

Sampling was conducted to examine: 1) the differences in sediment organic carbon stocks ( $C_{org}$ ) and sediment grain sizes between a pristine meadow, a denuded site and in unvegetated sediment; and, 2) the differences in sediment  $C_{org}$  stock and grain size at different sediment depths. To analyse the sediment  $C_{org}$  stock and grain size, ten sediment cores, 15 cm in depth, were collected using a PVC corer (30 cm depth, 10 cm inner diameter) at the pristine meadow, the denuded site and in unvegetated sediment. Although the sediment depths for core compression effects was not measured, the level of core compression was indicated by measuring of sediment dry bulk density. Sediment cores were stored vertically and transported to a temporary work-station that was equipped with tools for sectioning and preserving sediment cores. The sediment cores were sliced into 3 sections representing 3 known volumes of wet sediment, 3, 7 and 5 cm depth, respectively cm before transferring to a plastic zip lock bag. My decision to look at the three different depth layers stems from my consideration of the depth at which the sediment properties would be influenced by any new environmental influences. If only the top 3 cm were affected and the first section being studied was 0-15 cm, any change in the top 3 cm would be masked by the underlying 12 cm of material (unchanged). The result from preliminary study showed that the sediment

could be separated into three sections based on colour and seagrass material (root and rhizome). The sections were 0-3 cm, 3-10 cm – where I found the below-ground materials, and more than 10 cm – where the colour of sediment was very light and no below-ground material of seagrass was present.

Samples were preserved in the dark at 4°C and then dried at 50°C for 48 hours in the laboratory. For sediment C<sub>org</sub> analysis, dried samples were sieved (2 mm mesh) to separate the large particles of shell, wood and rock, and then ground into a fine powder (<40 µm) with a ball grinder (Planetary Micro Mill Pulverisette7, Fritsch GmbH).

### **2.2.3 Bulk density**

The dry weight of the sediment was obtained and used with the volume of wet sediment collected in each section of the cores to calculate the bulk density according to the following equation:

$$\text{Dry Bulk Density (g sediment dry weight)} = \frac{\text{Mass dried sediment (g)}}{\text{Volume sample (ml)}}$$

### **2.2.4 Organic carbon analysis**

Total carbon and nitrogen content was analysed using a Truspec Carbon Nitrogen Analyser (LecoTruSpec Micro, TCN Analyser, LECO Corp., St. Joseph, MI). To determine the total organic carbon (TOC), direct acidification was used to remove calcium carbonate from the sediment sample. Dried sample (200 mg) was resuspended in MilliQ water and 1 M HCl was added until the effervescence ceased. Acidified samples were agitated using an ultrasonic bath before centrifugation at 1,000 g for 10 minutes. The acidified samples were rinsed using MilliQ water until the pH was >5. The samples were transferred to an aluminium cup and dried at 60°C before analysis in a

TCN analyser. The percentage of organic carbon content (%C<sub>org</sub>) was recorded. Sediment organic carbon (C<sub>org</sub>) stock (Mg C<sub>org</sub> ha<sup>-1</sup>) was calculated by multiplying C<sub>org</sub> content (mgC<sub>org</sub> in 100 mg Sediment) with the DBD (g Sediment cm<sup>-3</sup>) and sediment depth (cm).

### 2.2.5 Isotopic analysis

The reference samples (the potential sources of organic carbon stored in sediment of pristine meadow, denuded site and unvegetated sediment) including seagrass leaves, mangrove leaves, and suspended particulate matter (SPM) were collected. The same leaf age and size of *R. apiculata*, the dominated species of mangrove forest nearby the study site, were collected. Five leaf samples from five mangrove trees were pooled to be one replicate, with three replicates in total (n = 3). The SPM were collected from the water column (n = 5) at the intertidal zone and subtidal zone. 1000 ml of seawater was collected using plastic bottles. The water samples were filtered through pre-combusted (Whatman GF/F) glass fiber filters.

Acidification was used to remove calcium carbonate from sediment samples before placing samples into tin capsules (12 x 6 mm) and weighing with an analytical semi-micro balance (0.01 mg precision); the required mass was 5–20 mg. The stable isotopic composition of samples was analysed using mass spectrometry (Europa Scientific 20-20 Isotope Ratio Mass Spectrometry linked to an Automated Nitrogen Carbon Analyser-Solids and liquids). Preparation unit  $\delta$  was calculated from the ratio of heavy (<sup>13</sup>C) to

light stable ( $^{12}\text{C}$ ) isotopes in the samples ( $R_{\text{sample}}$ ) relative to the ratio of the standard ( $R_{\text{standard}}$ , from Vienna Pee Dee Belemnite) following the equation:

$$\delta_{\text{sample}} = 1000 \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right], \text{ where } R = {}^{13}\text{C}/{}^{12}\text{C}$$

To determine the main  $\text{C}_{\text{org}}$  source, N:C was plotted against  $\delta^{13}\text{C}$  in the ternary diagram. N:C was used instead of C:N ratios because the larger number in the denominator of N:C ratios are more statistically robust (Goni et al., 2003). The ternary diagram has been used to calculate organic matter sources in seagrass ecosystems (Dittmar et al., 2001, Goni et al., 2003, Gonnea et al., 2004). A triangle was created with three end members (the three potential sources including seagrass, mangrove and SPM) that is expanded according to the standard deviations of the end members to account for natural variability and analytical error. Samples that fell within the ternary diagram were considered to be a mixture of these three sources. Samples that fell outside the diagram indicated the presence of a  $\text{C}_{\text{org}}$  source which deviated from the others. Samples that fell between two end members of the diagram indicated a mixture of  $\text{C}_{\text{org}}$  sources.

### **2.2.6 Grain size analysis**

Approximately 1 g of each sediment sample was mixed with 0.5% calgon and 800 mL of deionised water. The sediment grain size distribution was analysed using a particle size analyser (Malvern Mastersizer 2000, Malvern, Britain) with a pump speed of 2,200 rpm without sonication. The sediment particle size was analysed between 0.02–2000  $\mu\text{m}$ . The relative distribution of the particle volume was measured.

### **2.2.7 Measurement of benthic net community production**



To compare benthic community production between a pristine meadow, a denuded site and in unvegetated sediment, benthic chamber incubations were performed. Benthic net community metabolism was estimated by measuring changes in dissolved oxygen (DO) in the incubation chamber. The water depth during the experiment was approximately 0.5–1 m. The incubation chamber (a plastic cylinder, 12 cm diameter and 20 cm high) was installed during low tide when the water depth was about 0.5 m. The chamber was inserted into the sediment into the sediment and contained approximately 1.5 L of water. The chamber was simply pushed in. The depth of insertion was standardized at 5 cm. The mean depth of the rhizomes of *E. acoroides* and *T. hemprichii* was 7.7 cm and 6.5 cm respectively. Thus, the chamber insertion did not damage the rhizome. Water volume was estimated as the chamber volume minus the volume of chamber inserted into the sediment. The chamber was closed with a rubber grommet. Rubber grommets were used to seal the rubber tubes' points of entry into the chambers. Plastic stoppers was used to close the rubber tubes during incubation. All of the incubation chambers were placed during high tide by snorkelling. Samples were incubated under dark (black painted chamber) and light (clear chamber) conditions for 2 hours (10.00 to 12.00), with a total of 12 incubation chambers each, for both light and dark conditions. A rubber tube (50 mm diameter) was inserted for sampling purposes. Water samples were collected before closing the chamber and after incubation using a 25 mL syringe. The water samples were collected under water by opening the valves of the plastic stoppers and directly inserting the syringes. Thus, the water was collected directly from the chamber without being exposed to oxygen. The water samples were immediately analysed for oxygen concentration ( $\text{mg L}^{-1}$ ) using a dissolved oxygen probe (LDO probe, Model HQ40d, Hach company, Loveland, CO, USA).

Community respiration (R) was estimated using the change detected in Dissolved Oxygen (DO) concentration in the dark incubation chamber, while net community production (NCP) was estimated using the change detected in the DO concentration in the clear incubation chamber. Gross Primary Production (GPP) was calculated using the following equation:  $GPP = R + NCP$ .

NCP ( $\text{mmolC m}^{-2} \text{d}^{-1}$ ) and R ( $\text{mmolC m}^{-2} \text{d}^{-1}$ ) was calculated following the equations:

$$NCP = \left[ \left( \frac{V \times C}{A} \right) \times \left( \frac{FC - IC}{T} \right) \right] / 12$$

$$R = \left[ \left( \frac{V \times C}{A} \right) \times \left( \frac{FD - ID}{T} \right) \right] / 12$$

where V is volume of water enclosed above the sediment (L); C is the conversion factor of O<sub>2</sub> values to C values ( $\text{mg L}^{-1}$ ) which is equivalent to  $0.344 \text{ mg C mgO}_2^{-1}$  based on a photosynthetic quotient and respiration quotient of 1.2; A is the area of sediment enclosed by bottle ( $\text{m}^2$ ); FC and FD are the final oxygen concentrations ( $\text{mg L}^{-1}$ ) in the clear and dark chambers respectively; and, IC and ID are the mean initial oxygen concentrations ( $\text{mg L}^{-1}$ ) in the clear and dark chambers respectively. 12 is the conversion factor of gC to molC.

### 2.2.8 Statistical analysis

A power analysis was conducted to determine the sample sizes required to detect a change in sediment carbon stocks in each meadow (pristine meadow, denuded site and unvegetated sediment). To reach the power (probability of not making a Type II error), 10 samples (*n*) were required.

The mean value of sediment organic carbon stock was used in a repeated-measures Analysis of Variance (ANOVA), where there were three meadows (pristine meadow, denuded site and unvegetated sediment) and three sediment depths (0–3, 3–10, and 10–15 cm). We used a repeated-measures ANOVA because the sediment  $C_{\text{org}}$  stocks were measured from the same sediment core at different depths (sediment depth is the repeated variable). The mean value of community metabolism (GPP, NCP and R) was used in a one-way Analysis of Variance (ANOVA). When the ANOVA resulted in a significant variation among treatments, post hoc comparisons were tested using Tukey's method (95% confidence level). All data were transformed as needed (square root or log-transform) to meet the analysis assumption including homogeneity of variance and normal distribution.

## **2.3 RESULTS**

### **2.3.1 Sediment Dry Bulk Density (DBD) and sediment organic carbon ( $C_{\text{org}}$ ) content**

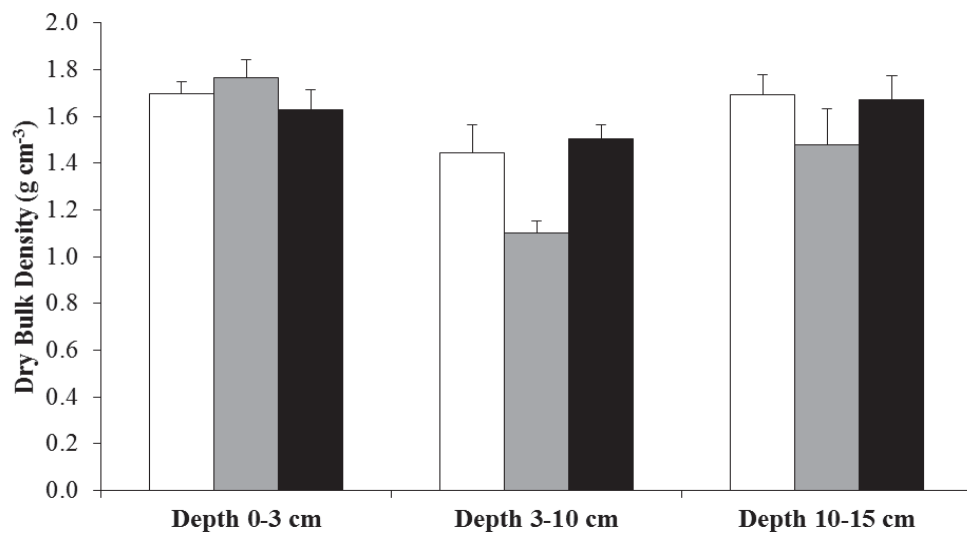
There was no significant difference in sediment DBD measured at three sediment depths (0–3, 3–10, and 10–15 cm) ( $P = 0.323$ ). The sediment DBD at three sediment depths (0–3, 3–10, and 10–15 cm) ranged from  $1.70 \pm 0.05$  to  $1.44 \pm 0.12 \text{ g cm}^{-3}$  at the pristine meadow,  $1.77 \pm 0.08$  to  $1.10 \pm 0.05 \text{ g cm}^{-3}$  at the denuded site and  $1.67 \pm 0.10$  to  $1.50 \pm 0.06 \text{ g cm}^{-3}$  in an unvegetated sediment (Fig. 2.1a). The sediment  $C_{\text{org}}$  content was measured as a percentage of carbon in dried sediment (mg C in 100 mg sediment). The sediment  $C_{\text{org}}$  varied from  $1.52 \pm 0.27\%$  measured at the pristine meadow to  $0.11 \pm 0.01\%$  measured at the denuded site (Fig. 2.1b).

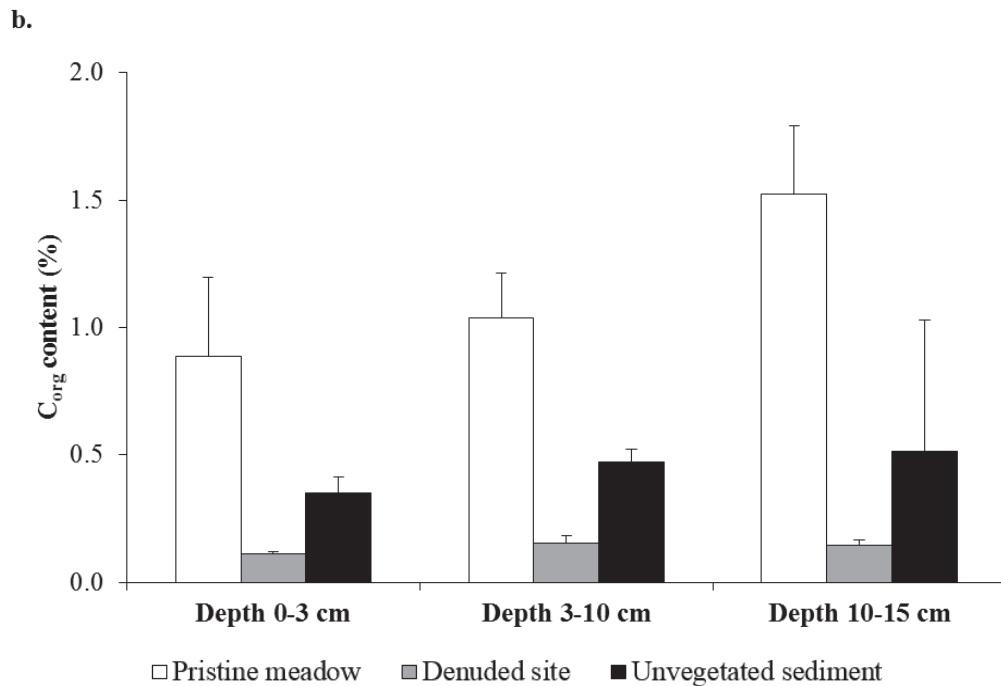
### 2.3.2 Impact of seagrass loss on sediment organic carbon ( $C_{org}$ ) stocks in the upper 15 cm of sediment

The impact of seagrass loss on sediment  $C_{org}$  stocks was examined by comparing sediment  $C_{org}$  among three sites at Haad Chao Mai National Park. A significant difference ( $P = 0.000$ ) in sediment  $C_{org}$  stock among the three sites was observed (Table 2.1). Comparing of the sediment  $C_{org}$  stock among three sites (denuded site, pristine site, and unvegetated sediment) was shown in Fig. 2.2. A post hoc comparison showed that the sediment  $C_{org}$  stock at the denuded site ( $0.19 \pm 0.02 \text{ Mg } C_{org} \text{ ha}^{-1}$ ) was significantly lower than the  $C_{org}$  stock at the pristine site ( $1.71 \pm 0.22 \text{ Mg } C_{org} \text{ ha}^{-1}$ ) or in unvegetated sediment ( $0.72 \pm 0.06 \text{ Mg } C_{org} \text{ ha}^{-1}$ ) (Fig. 2.2).

Comparing of the sediment  $C_{org}$  stock among three sediment depth (0-3, 3-10, and 10-15 cm) was shown in Fig. 2.3. A post hoc comparison showed that sediment  $C_{org}$  stock at the top surface (0–3 cm) of the pristine meadow ( $1.09 \pm 0.18 \text{ Mg } C_{org} \text{ ha}^{-1}$ ) was lower than the  $C_{org}$  stock at a sediment depth of 3–10 cm ( $1.47 \pm 0.25 \text{ Mg } C_{org} \text{ ha}^{-1}$ ) and at 10–15 cm ( $2.56 \pm 0.48 \text{ Mg } C_{org} \text{ ha}^{-1}$ ). Sediment  $C_{org}$  stock levels were similar for all three depths (0–3, 3–10 and 10–15 cm) in unvegetated sediment ( $0.57 \pm 0.11$ ,  $0.72 \pm 0.09$  and  $0.86 \pm 0.09 \text{ Mg } C_{org} \text{ ha}^{-1}$  respectively) and at the denuded site ( $0.2 \pm 0.02$ ,  $0.17 \pm 0.03$  and  $0.21 \pm 0.03 \text{ Mg } C_{org} \text{ ha}^{-1}$  respectively) (Fig. 2.3).

a.





**Figure 2.1** Sediment Dry Bulk Density (DBD) and sediment organic carbon ( $C_{org}$ ) content. Sediment DBD (a) and sediment organic carbon (b) content measured at three different depths (0–3, 3–10 and 10–15 cm) at a pristine meadow, a denuded site and in an unvegetated sediment in the Haad Chao Mai National Park, Trang Province, Thailand. Mean  $\pm$  S.E.M, n=10.

**Table 2.1** Repeated-measures ANOVA of sediment  $C_{org}$  stocks in a pristine meadow, a denuded site and in unvegetated sediment in the Haad Chao Mai National Park, Trang Province, Thailand at three sediment depths (0–3 cm, 3–10 cm, and 10–15 cm). n = 10.

Source	SS	df	MS	F	p> F
<b>A. Between-subjects</b>					
Meadows	12.615	2	6.307	123.174	0.000*

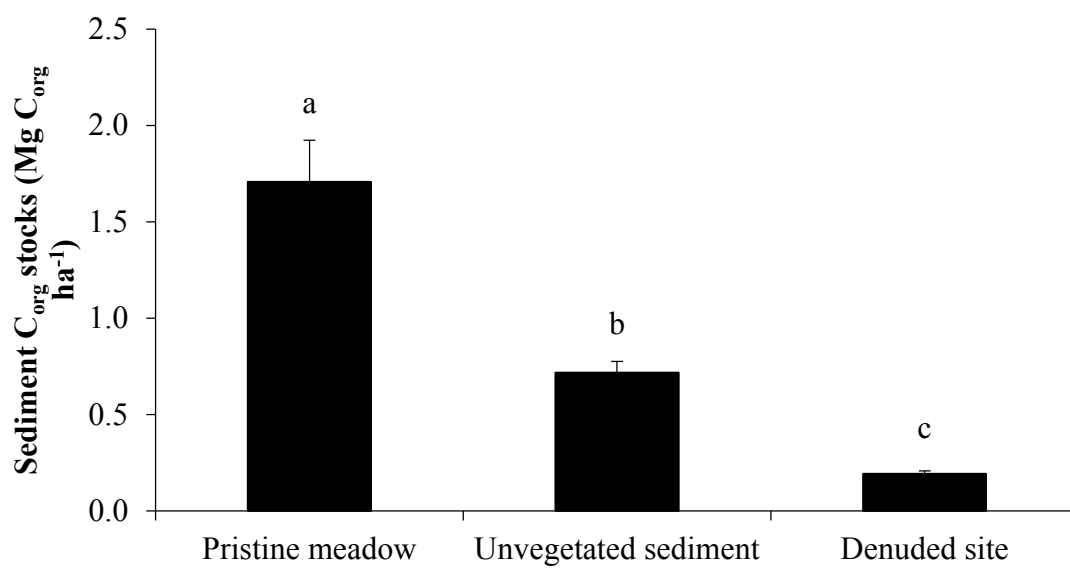
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Error	1.383	27	0.051		
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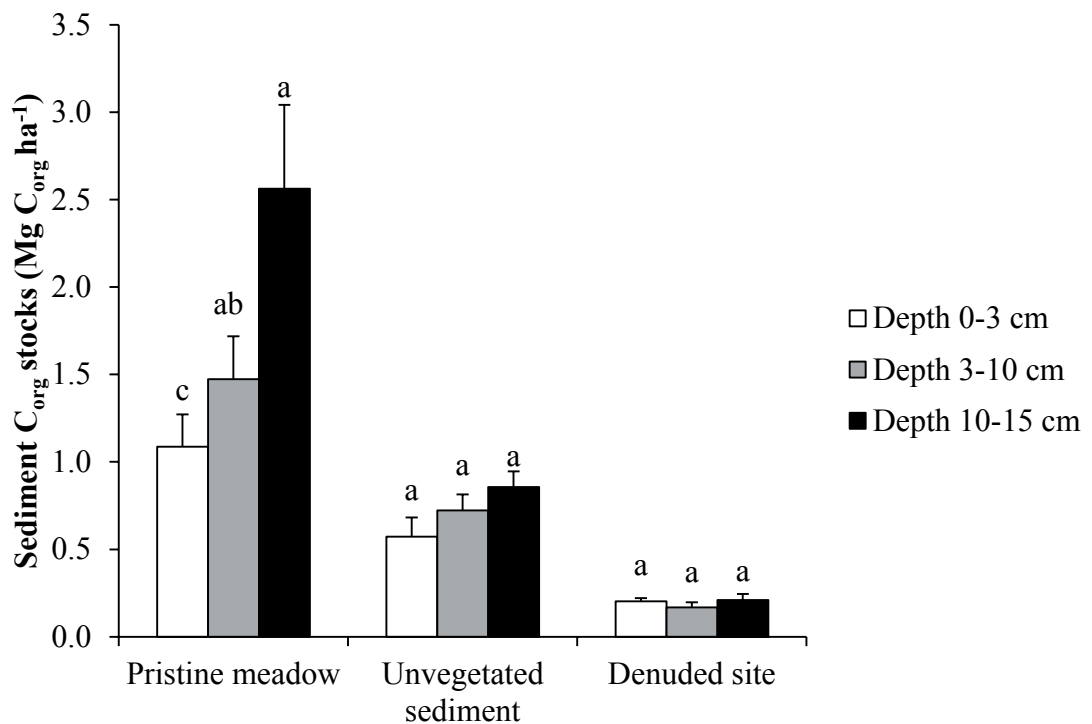
**B. Within-subjects**

Depths	0.568	2	0.284	4.559	0.015*
Depths x meadows	0.476	4	0.119	1.912	0.122
Error (Depths)	3.362	54	0.062		

---



**Figure 2.2** Comparison of sediment  $C_{org}$  stock in the upper 15 cm of sediment. Sediment  $C_{org}$  stocks were measured in the upper 15 cm of sediment in a pristine meadow, a denuded site and in unvegetated sediment in the Haad Chao Mai National Park, Trang Province, Thailand. The upper plot shows the average stock over 1 cm using data from the whole core. The letters, a, b and c indicate significant differences between conditions as determined by the Tukey post hoc test (95% confidence level). The same letters given for different conditions indicates that no significant difference (95% confidence level) was detected. Mean  $\pm$  S.E.M, n=10.



**Figure 2.3** Comparison of sediment carbon stock between three sediment depths (0–3, 3–10, and 10–15 cm) in a pristine meadow, denuded site and in unvegetated sediment. Sediment  $C_{org}$  stocks were measured at three depths in the upper surface of sediment (0–3, 3–10, and 10–15 cm) in a pristine meadow, a denuded site and in



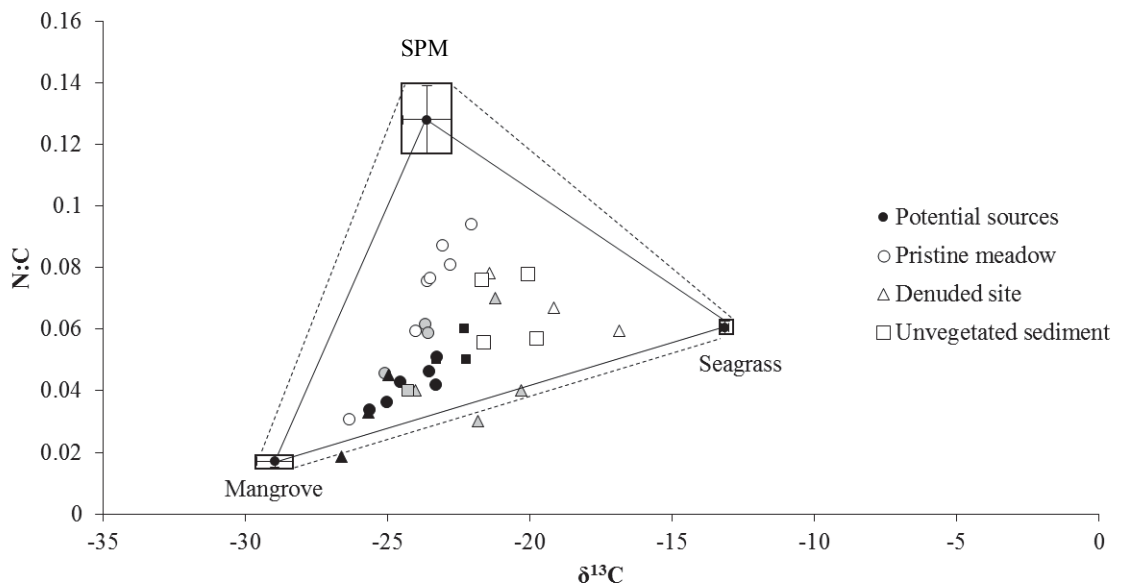
unvegetated sediment in the Haad Chao Mai National Park, Trang Province, Thailand. The letters, a, b and c indicate significant differences between conditions as determined by the Tukey post hoc test (95% confidence level). The same letters given for different conditions indicates that no significant difference (95% confidence level) was detected. Mean  $\pm$  S.E.M, n=10.

### 2.3.3 Sediment organic carbon ( $C_{org}$ ) sources

$\delta^{13}C$  varied among the five  $C_{org}$  sources analysed: seagrass leaves ( $-13.15 \pm 0.06\text{‰}$ ); mangrove (*R. apiculata*) leaves ( $-28.96 \pm 0.28\text{‰}$ ); epiphyte ( $-11.18 \pm 0.49\text{‰}$ ); intertidal SPM ( $-23.42 \pm 0.28\text{‰}$ ); and subtidal SPM ( $-23.81 \pm 0.47\text{‰}$ ). C:N ratios also varied among  $C_{org}$  sources including  $16.53 \pm 0.26$  for seagrass leaves,  $58.94 \pm 3.41$  for mangrove leaves,  $15.41 \pm 1.70$  for epiphytes,  $7.82 \pm 0.26$  for intertidal SPM, and  $8.12 \pm 0.36$  for subtidal SPM.

The potential  $C_{org}$  sources of sediments at a pristine meadow, denuded site and an unvegetated sediment are plotted in the ternary diagram (Fig. 2.4). Because the ternary diagram can only have three end members of the potential sources, the three most likely end members including mangrove, seagrass and SPM (combination of intertidal and subtidal SPM) were chosen.

Most of the sediment samples taken from the pristine meadow, denuded site and the unvegetated site fell within the ternary diagram (Fig. 2.4). The majority of sediment samples taken from the surface of the pristine meadow fell near the SPM and mangrove end members. Most of the sediment samples taken from the denuded site fell along the tie lines connecting seagrass and mangrove end members. Sediment samples taken from the unvegetated site fell close to the tie lines connecting seagrass and mangrove end members.



**Figure 2.4** N/C vs.  $\delta^{13}\text{C}$  in ternary mixing diagrams of the potential sediment  $\text{C}_{\text{org}}$  sources including seagrass, mangrove and SPM. The potential sources are expanded according to the standard deviations of the end members to account for natural

variability and analytical error. Samples that fall within the ternary diagram were collected from a pristine meadow, a denuded site and in unvegetated sediment at three depths from the sediment surface: 0–3 (white), 3–10 (grey), and 10–15 cm (black).

#### **2.3.4 Sediment grain size distribution**

Larger grain sizes were found in the sediment taken from the denuded site, compared with the pristine meadow. At pristine meadow, the volume of the very fine sand sediment was varied from 60.08, 59.09, and 59.36 % at sediment depth 0-3, 3-10, and 10-15 cm, respectively, while the percent of the very fine sand measured at unvegetated sediment was 3.55, 4.82, and 5.08 % at sediment depth 0-3, 3-10, and 10-15 cm, respectively. The very fine sand proportion measured at denuded site was 36.35, 41.60, and 33.67 % at sediment depth, respectively. More than 50% of the volume of the sediment from the pristine meadow consisted of very fine sand (0.0625–0.125 mm), whereas more than 50% of the volume of sediment from the unvegetated and denuded sites consisted of fine sand (0.125–0.25 mm) (Table 2.2).

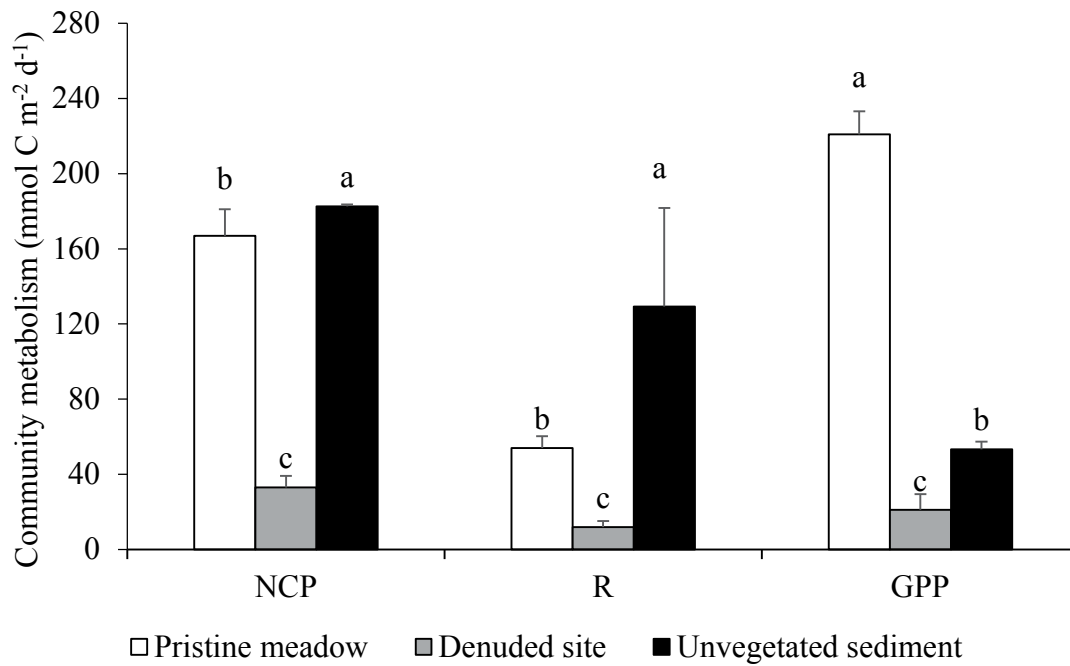
### 2.3.5 Impact of seagrass loss on benthic community metabolism

Benthic community production – net community production, respiration and gross primary production – were compared among a pristine meadow, a denuded site and in unvegetated sediment in the Haad Chao Mai National Park, Trang Province, Thailand (Fig. 2.5).

To assess the carbon sequestration capacity of the pristine meadow, the denuded site and in unvegetated sediment, we estimated benthic community metabolism. Gross Primary Productivity (GPP) was greater than community respiration (R) for the pristine meadow ( $220.89 \pm 17.82 \text{ mmol C m}^{-2} \text{ d}^{-1}$  and  $53.97 \pm 6.28 \text{ mmol C m}^{-2} \text{ d}^{-1}$  for GPP and R, respectively), the denuded site ( $32.99 \pm 3.29 \text{ mmol C m}^{-2} \text{ d}^{-1}$  and  $11.86 \pm 5.34 \text{ mmol C m}^{-2} \text{ d}^{-1}$  for GPP and R, respectively) and in unvegetated sediment ( $182.58 \pm 52.45 \text{ mmol C m}^{-2} \text{ d}^{-1}$  and  $129.22 \pm 56.95 \text{ mmol C m}^{-2} \text{ d}^{-1}$  for GPP and R, respectively). Photosynthesis/respiration ratios (P/R) were above 1 for the pristine meadow ( $3.07 \pm 0.63$ ), the denuded site ( $8.06 \pm 6.11$ ) and for the unvegetated sediment ( $2.33 \pm 1.06$ ). Net community production (NCP) was measured in the pristine meadow ( $166.92 \pm 12.32 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) was significantly higher than in the denuded site ( $21.13 \pm 8.30 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) and in unvegetated sediment ( $53.36 \pm 4.11 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) (Fig. 2.5).

**Table 2.2** The percentage volume of sediment grain sizes (mm) in a pristine meadow, a denuded site and in unvegetated sediment at three depths (0–3 cm, 3–10 cm, and 10–15 cm) in the Haad Chao Mai National Park, Trang Province, Thailand. Mean  $\pm$  S.E.M., n =10.

Sediment grain size (mm)	Sediment type	% volume								
		Pristine meadow			Unvegetated sediment			Denuded site		
		0–3 cm	3–10 cm	10–15 cm	0–3 cm	3–10 cm	10–15 cm	0–3 cm	3–10 cm	10–15 cm
0.0313–0.0625	Coarse silt	0.15 $\pm$ 0.01	00.11 $\pm$ 0.03	00.30 $\pm$ 0.03	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	04.06 $\pm$ 1.40	03.0 $\pm$ 0.12	00.91 $\pm$ 0.30
0.0625–0.1250	Very fine sand	60.08 $\pm$ 0.72	59.09 $\pm$ 1.02	59.36 $\pm$ 0.65	03.55 $\pm$ 0.34	04.82 $\pm$ 0.19	05.08 $\pm$ 0.05	36.35 $\pm$ 6.57	41.60 $\pm$ 1.03	33.67 $\pm$ 4.70
0.125–0.25	Fine sand	33.90 $\pm$ 0.82	22.50 $\pm$ 3.58	31.12 $\pm$ 0.75	64.85 $\pm$ 2.45	68.73 $\pm$ 1.79	71.52 $\pm$ 0.33	56.99 $\pm$ 6.29	54.10 $\pm$ 1.14	56.40 $\pm$ 3.20
0.25–0.5	Medium sand	05.87 $\pm$ 0.73	16.87 $\pm$ 4.30	08.34 $\pm$ 0.66	29.01 $\pm$ 0.01	25.40 $\pm$ 0.31	23.36 $\pm$ 0.37	02.6 $\pm$ 0.81	01.29 $\pm$ 0.3	08.78 $\pm$ 1.87
0.5-1	Coarse sand	00.00 $\pm$ 0.00	00.84 $\pm$ 0.28	00.00 $\pm$ 0.00	01.62 $\pm$ 0.51	00.74 $\pm$ 0.52	00.04 $\pm$ 0.01	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.14 $\pm$ 0.14
1-2	Very coarse sand	0.00 $\pm$ 0.00	00.51 $\pm$ 0.31	00.00 $\pm$ 0.00	00.97 $\pm$ 0.44	00.31 $\pm$ 0.23	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00



**Figure 2.5** Benthic community production. Benthic community production – net community production, respiration and gross primary production – were compared among a pristine meadow, a denuded site and in unvegetated sediment in the Haad Chao Mai National Park, Trang Province, Thailand. The letters, a, b and c indicate significant differences between conditions as determined by the Tukey post hoc test (95% confidence level). The same letters given for different conditions indicates that no significant difference (95% confidence level) was detected. Mean  $\pm$  S.E.M, n=3.

## 2.4 DISCUSSION

At Khao Bae Na, Haad Chao Mai National Park, Trang, Thailand, coastal development, specifically from the development of a port, local urban population growth within the catchment, rubber plantation runoff and road building, has likely led to a loss of seagrass in this area. Ten years after seagrass gradually disappeared, our analysis showed that the sediment in this location stored less organic carbon in the top 15 cm of sediment compared to a nearby area that still supported a healthy meadow. The carbon storage in the upper 15 cm at a denuded site was ~89% lower compared to a pristine meadow. The  $C_{org}$  stock (average reported over 1 cm depth) at each of 3 depths within the sediment profiles across the examined meadow was similar at a denuded site and in unvegetated sediment. This observation suggested two possible mechanisms for the lower level of carbon stock measured at a denuded site. Firstly, this location could have been buried in 15 cm of sediment transported from other locations containing a lower organic carbon content leading to a lower carbon stock in the upper 15 cm sediment profile. Secondly, the re-suspension and export of fine, organic rich sediment, oxidation or lack of trapping capacity and filtration of the sediment organic matter after seagrass loss could have led to a decrease of  $C_{org}$  stored in the upper sediment profile.

The distinct stable isotopic composition ( $\delta^{13}C$ ) of sediment has been used to ‘fingerprint’ the sources of  $C_{org}$  that accumulate in seagrass meadows (Macreadie et al., 2013). Our analysis showed that all three sources including seagrass, mangrove and suspended particulate matter (SPM) contribute to sediment  $C_{org}$  deposited throughout a pristine meadow, a denuded site and in unvegetated sediment. We found that allochthonous carbon is the major contributor to blue carbon stock of tropical seagrasses at Haad Chao Mai National Park (Thailand). One of the dominant sources of sediment

$C_{org}$  for a pristine meadow sediment was mangrove and SPM. The relative supply rates of particular organic matter sources often relate to the variability of sediment  $C_{org}$  sources (Kennedy et al., 2010). Tropical seagrass meadows are often adjacent to mangrove forests and the spatial linkage between seagrass and mangrove could exchange the  $C_{org}$  among these ecosystems (Hemmingal et al., 1994). A previous study reported that 70% of sediment  $C_{org}$  stored in South East Asian seagrass meadows was derived from allochthonous sources (as opposed to autochthonous carbon) and 95% of the sediment in Western Pacific seagrass meadows was derived from terrestrial sources rather than seagrass (Kennedy et al., 2010). In our study, all three study areas (pristine meadow, denuded and unvegetated sediment) were located close to mangroves (within 1 km); consequently, the dominant sediment  $C_{org}$  at all of three study areas was derived from mangrove.

Sediment  $C_{org}$  from the denuded site are dominated by seagrass and mangrove source. Plantations of rubber and palm trees are also common around the study areas. A similar proportion of mangrove/ terrestrial derived sediment  $C_{org}$  sources between a denuded site and a pristine meadow, indicating the similar origin of sediment at these two sites. Thus, loss of  $C_{org}$  from the original seagrass sediment would be a cause of sediment organic carbon poor at a denuded site rather than the deposition of land  $C_{org}$  sediment. The difference in carbon stock between a denuded and a pristine meadow would suggest erosion, oxidation and lack of trapping capacity and filtration of the sediment organic matter after seagrass loss that cause of change rather than accelerated sediment deposition.

Sediment  $C_{org}$  source measured at a pristine meadow was also dominated by SPM indicating the more effective trapping capacity of the seagrass canopy at a pristine



meadow, compared with a denuded site and unvegetated sediment. Seagrass canopy structure has been reported to be the main factor that influences its trapping capacity via the alteration of water flow and direct attachment of SPM from the water column (Agawin and Duarte, 2002, Komatsu et al., 2004). Thus, loss of seagrass at a denuded site could lead to the loss of trapping capacity, consequently decreasing the chance to input allochthonous carbon into the sediment.

Alteration of water flow due to loss of seagrass canopy could also change the sediment grain size distribution. A comparison of relative grain size (<0.5 mm) at each of the three sites indicated that the proportion of finer sand fractions measured from a pristine meadow was higher than the proportion of finer fractions measured from a denuded site and unvegetated sediment (Fig. 2.5). Loss of above-ground seagrass structure is likely to cause a reduction in the percentage of finer sand fractions. Settlement and retention of silt and clay require a slow water velocity, which does not occur here. At faster water velocities sand or coarse sand are the major particle sizes to be deposited and retained (Alther, 1981, Hjulström, 1955). Seagrass canopy structure reduces the flow and turbulence of water (Keulen and Borowitzka, 2002, Gambi et al., 1990) leading to the retention of finer particles. In addition, the roots and rhizomes more effectively trap sediment leading to a lower re-suspension rate. In the Mediterranean, *Posidonia oceanica* has been found to reduce sediment re-suspension more than 3-fold compared to unvegetated sediment (Gacia and Duarte, 2001). The grain size distribution in an unvegetated sediment still has a higher proportion of coarser sand and it may be that the grain size distribution at a denuded site will eventually more closely mirror that of an unvegetated sediment.

Given that grain size distribution is different at a denuded site compared to a pristine meadow and unvegetated sediment, one may ask if this change in grain size affects the sediment carbon stock. The relationship between sediment grain size and sediment carbon content has been widely reported in the transitional water habitat (i.e. lagoons and estuaries). However, there is little information that directly indicates the relationship between sediment grain size and organic carbon in the seagrass ecosystem. Kennedy et al. (2010) reported that on a global average, the grain size fractions of silts and clays (<0.063 mm, coarse silt) contain the highest organic matter content. A comparison of carbon stocks of the sediment in a denuded site, pristine meadow and unvegetated sediment (Fig. 2.2) showed that a denuded site actually had the lowest carbon stock, whereas based simply on grain size analysis, it would have been predicted that unvegetated sediment would have the lowest carbon stock. The higher sediment  $C_{org}$  stocks at the unvegetated sediment compared with those at the denuded site could be explained by the location of the denuded site. The pristine meadow and unvegetated sediment have been equally exposed to deposition and erosion as the denuded site. Although the large sand bar did not protect the pristine meadow, unvegetated sediment, or denuded site from ocean waters, the location of the denuded site may have been the main obstruction to the import of seagrass materials from the pristine meadow. An analysis of sediment isotopic composition indicated that seagrass was a major source of sediment organic carbon in the unvegetated sediment. This result could be explained by the distance between the unvegetated sediment and the pristine meadow, which is less than the distance between the denuded site and the pristine meadow. Moreover, the slower water current in the river channel between the pristine meadow and the denuded site might have caused seagrass material to sink before reaching the denuded site.

The higher sediment  $C_{org}$  stocks at the unvegetated site compared with those at the denuded site could also be explained by sediment grain size. A large proportion of medium sized sand particles in the unvegetated sediment could enhance benthic microalgae production. Therefore, higher carbon biomass could be stored in unvegetated sediment in comparison to the denuded site. At a denuded site and in unvegetated sediment, the main primary producer should be the benthic microalgae (MacIntyre et al., 1996). The relationship between benthic microalgae communities and sediment grain size has been examined, where higher biomass of the benthic microalgae has been found as the percentage of fine particles decreases (Cahoon et al., 1999). For this reason, it might be expected that a large proportion of medium sized sand particles in unvegetated sediment could enhance the benthic microalgae production. Therefore, higher carbon biomass could be stored in in unvegetated sediment compared with a denuded site.

The negative interaction between sediment grain size and benthic microalgae could be supported with evidence of benthic community metabolism. Measurements of benthic community metabolism in a pristine meadow, denuded site and in unvegetated sediment showed a higher GPP than R value, indicating that all of the three communities tended to be autotrophic. Relatively low respiration rate at a denuded site indicated that there is a completed decomposition and remineralisation process at a denuded site after 10 years of seagrass loss.

NCP at a denuded site was ~5-times lower than in a pristine meadow, and even lower in unvegetated sediment, implying that the higher NCP in unvegetated sediment was derived from higher benthic microalgae biomass. NCP in a pristine meadow dominated by *E. acoroides* ( $458.10 \pm 10.17 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) was relatively 1.6-times higher than

the local NCP that was reported previously (Duarte et al., 2013b). This result was similar to that reported by Duarte et al. (2010) for *E. acoroides*. The NCP for *E. acoroides* is about 5-times higher than the mean of values taken from 155 different sites of temperate and tropical seagrass meadows (Duarte et al., 2010), suggesting a very high carbon sink capacity at the meadow of this tropical seagrass species. However, the calculation of  $C_{org}$  burial based on the carbon accumulation rate of a previous study (Kennedy et al., 2010) (the accumulation of seagrass organic matter is between 41 and 66  $gC\ m^{-2}\ yr^{-1}$ ) showed only 6% to 9% of NCP could be buried in a pristine meadow. Around 91% to 94% of seagrass NCP could be exported to nearby areas or ecosystems, remineralised by bacterial activity and/or consumed by herbivores.

In a pristine meadow, the carbon stock was varied along the sediment depth profile. The highest sediment  $C_{org}$  stock (average reported over 1 cm depth) was found over the sediment depth of 10–15 cm below the sediment surface. This change in stock with depth was similar to the previous report where sediment  $C_{org}$  stock over the top 25 cm from tropical seagrass meadows were highly variable, including increases, decreases and no change. (Lavery et al., 2013). This observation could be due to the following: 1) Under a constant rate of remineralisation, the  $C_{org}$  accumulation rate (for a time period equivalent to 0–10 cm below the sediment surface) was lower than the  $C_{org}$  accumulation rate of the lower layer (10–15 cm below the sediment surface); and 2) Under a constant rate of sediment  $C_{org}$  accumulation, the remineralisation rate would increase. Sediment dating could indicate an outline history of sediment. However, it is unlikely that there will be a constant accumulation rate and change in sediment age over a short time scale (Serrano et al., 2012).

The results showed that seagrass loss at Khao Bae Na, Trang, Thailand resulted in a lower carbon stock in the sediment. While there was no difference in hydrodynamic conditions, the sediment  $C_{org}$  stocks at the denuded site was significant lower than those at the pristine meadow and in unvegetated sediment. The denuded site was formerly seagrass meadow, dominated by *E. acoroides*. After the seagrass died (apparently due to light deprivation caused by sediment loading) a combination of several factors – the re-suspension and export of fine, rich organic sediment, oxidation or lack of trapping capacity, and filtration of the sediment organic matter – could have led to a decrease of  $C_{org}$  stored in the upper sediment profile.

While, there is a lack of sediment  $C_{org}$  stocks particularly in the Indo-Pacific bioregion (Duarte et al., 2013a), our research suggested that seagrass meadows in this region are one of the most critical blue carbon sinks in the world. Activities similar to those described in the Khao Bae Na, Haad Chao Mai National Park occur in many areas along the Gulf of Thailand and the Andaman Sea, Thailand, resulting in lower water clarity and increased sediment deposition. These activities could be one of the major causes of vulnerability of blue carbon sinks in Thailand and the tropical Indo-Pacific Bioregion, especially when they occur close to areas supporting seagrass species that are less able to cope with enhanced sediment loads and reduced water clarity. The change in carbon stock in seagrass ecosystems is likely to be accelerated by increasing population growth and poor land use practice (Waycott et al., 2009). Thus, it is important to monitor the long-term change in carbon stocks and carbon budget, including the fate of existing sediment carbon stocks at degrading meadows. There is also a need to understand if seagrass restoration can play a potential role in the recovery of carbon stock which has been lost to seagrass degradation (Greiner et al., 2013, Duarte et al., 2013c)



## CHAPTER 3

### THE INFLUENCES OF SPECIES-SPECIFIC SEAGRASS CANOPY

#### STRUCTURE ON BLUE CARBON SINK CAPACITY

##### 3.1 INTRODUCTION

Seagrass meadows have been reported to store 19.9 Pg of organic carbon globally and between 4.2 to 8.4 Pg C<sub>org</sub> on a areal basis in just the top 1 m of sediment (Fourqurean et al., 2012). Thus, seagrass meadows are seen to be high value sinks for organic carbon, commonly termed as having a high blue carbon sink capacity (Nellemann et al., 2009). The results of Fourqurean et al. (2012) extended our knowledge of the global extent of organic carbon stored in these sediments and the site-specific variation in carbon stored amongst seagrass beds. However, the processes that contribute to these differences have not yet been fully investigated. Processes likely to affect the amount of organic carbon stored in the sediment are both site- and species-specific differences (Lavery et al., 2013).

The seagrass canopy affects water flow and turbulence, enhancing sedimentation, reducing re-suspension of particles and hence aiding in the retention of smaller organic rich particles (Gacia et al., 1999). Seagrass meadows dynamically interact with a range of physical processes, for example local hydrodynamic forces which vary due to differences in the complexity of structure, size of leaf, and shoot density of the meadow (Sand-jensen, 1998). For seagrass species with leaf clusters and erect canopies, such as *Amphibolis griffithii* (Black) den Hartog, an abrupt change in water velocity was observed within the canopy, whereas in seagrass species with uniform leaf shape, such as *Posidonia sinuosa* Cambridge & Kuo, water flow appeared to be smoothly directed

over the canopy, and water velocity gradually decreased down through the canopy (Keulen and Borowitzka, 2002). Keulen & Borowitzka (2002) found that different patterns of water flow through the seagrass canopy could influence the stability of sediment in seagrass meadows. Lower velocities of water flow underneath the canopy of *A. griffithii* compared with *P. sinuosa* for example, was sufficient to reduce re-suspension of sediment.

The influence of canopy height (leaf size) on water velocities is also important. Reduced water flow inside the canopy has been reported in *Enhalus acoroides* (L.f.) Royle, which has large leaf sizes. In contrast, much larger water velocities have been measured in the canopies of *Thalassia hemprichii* (Ehrenberg) Ascherson and *Halophila ovalis* (R. Brown) Hooker f., which have small leaf size (Komatsu et al., 2004). Consequently, the flow pattern within the large leaf size species, such as *E. acoroides*, promoted more sedimentation that was enriched with finer particles compared to the smaller leaf size species (Komatsu et al., 2004). Similar interactions between seagrass leaf size and water flow was reported in the temperate seagrass species, *Posidonea oceanica*, where the total quantity of particles trapped increased with an increase in leaf surface area (Gacia et al., 1999). Furthermore, in a previous study, clear differences between water velocity profiles were observed in seagrass meadows of different densities (Peterson et al., 2004). Greater flow and wave energy reduction was also found inside the canopy of meadows with higher leaf density (Peterson et al., 2004, Fonseca and Cahalan, 1992). Maximum water movement was measured in the highest density of *Zostera marina* (1200 shoot m<sup>-2</sup>) (Eckman, 1987). Clearly, water velocity varies among seagrass species and abundance. However, there is currently no research that directly examines whether seagrass species (canopy height), abundance and other morphometric characteristics affect sediment organic carbon (C<sub>org</sub>) storage beneath their meadows.



Organic carbon sequestration can be promoted by direct trapping by the seagrass canopy. Seagrass leaves are a substrate for a number of epiphytes, which can physically bind sestonic particles with adhesive polysaccharide substances (Agawin and Duarte, 2002). Agawin and Duarte (2002) suggested that particle ingestion by epiphytes appear to be the dominant particle trapping mechanism of tropical seagrass meadows. A number of abiotic factors such as light, temperature, nutrients, and water motion determine the structure of epiphytic community, as well as biotic factors such as grazing pressure by herbivores and the suitability of the leaf substrate (Borowitzka et al., 1990, Castejon-Silvo and Terrados, 2012). The latter factor varies among seagrass species, morphology and density. Variability in seagrass shoot density leads to different levels of light penetration, thus epiphytic biomass is modified (Castejon-Silvo and Terrados, 2012). Large amounts of leaf-associated epifaunal species were reported to be much less abundant on seagrasses after seagrass density was reduced by a half (Edgar and Robertson, 1992). Moreover, the available surface area for epiphyte settlement is limited by leaf size, leaf age and stem ages (Borowitzka et al., 1990). Mature and diversified epiphyte communities were found on long-lived species, such as *P. oceanica*, while the fast-growing species of epiphyte was found on short-lived species such as, *Cymodocea nudosa*. Tomasko and Lapointe (1991) reported that the size of seagrass leaf correlated with the trapping capacity, such that a larger leaf surface area promotes direct particle trapping from the water column through the provision of suitable substrates for epibiont colonisation and epiphyte biomass development. While there is evidence to indicate that seagrass provide both indirect and direct trapping mechanisms, the variability of seagrass (complexity of structure, size of leaf, and shoot density) still needs to be taken into account to improve global estimates of carbon stocks.

Efficient particle trapping by the seagrass canopy also results in the deposition of mineral particles and organic matter from other sources, leading to increased allochthonous inputs into seagrass meadows. Variability in the proportional contribution of diverse sources into the sediment organic carbon pool of seagrass meadows occurs, but, on average, 50% of the organic carbon stored in the sediment has been reported to be derived from sources outside the seagrass meadow (Kennedy et al., 2010). Again site and species-specific differences in the proportion of allochthonous carbon have been reported. For example, in South East Asia, 70% of organic carbon stored in seagrass meadows is derived from allochthonous sources (Kennedy et al., 2010).

The rates of primary production (autochthonous) which may ultimately affect the amount of carbon stored in the sediment underlying seagrass meadows varies widely between seagrass species (Duarte et al., 2010). Above-ground production ranges from 0.003 to 15 gDW m<sup>-2</sup> d<sup>-1</sup> (Dawes, 1998) and the below-ground production ranges from 0.001 to 20 gDW m<sup>-2</sup> d<sup>-1</sup> (Duarte and Chiscano, 1999, Duarte et al., 1998). Duarte et al. (2010) reported that species-specific differences in primary production, measured as Gross Primary Production (GPP), were related to variations in biomass. The measurement of Net Community Production (NCP) and its variation amongst seagrass species is relevant to the assessment of blue carbon sink capacity, and in the same study Duarte et al., (2000) found the highest median of NCP in *E. acoroides*, *Ruppia maritima* L. and *Syringodium filiforme* Kützing (Duarte et al., 2010).

Here, we investigated the influence of 1) species-specific seagrass canopy height (measured as leaf size), canopy structure (measured as biomass) and abundance (measured as percent cover), and 2) community metabolism (NCP, R and GPP) on the capacity of meadows to store organic carbon (C<sub>org</sub>) in the sediment. This research was

conducted on *T. hemprichii* and *E. acoroides* meadows in Laem Yong Lam, Haad Chao Mai National Park, Trang Province, Thailand in the Andaman Sea. These are the largest, most diverse, and healthiest seagrass beds in Thailand. The impact of seasonal variation on seagrass production for these two tropical species is similar (Rattanachot and Prathep, 2011). They have the same pattern, but different rates, of above- to below-ground biomass production (Prathep et al., 2010). They have different leaf lengths, which defines canopy height; *T. hemprichii* has a medium canopy height, 10-40 cm leaf blade length, whereas *E. acoroides* has a larger canopy height, 30-150 cm leaf blade length (Kuo and Hartog, 2001, Short and Duarte, 2001). We hypothesised that 1) species-specific canopy height, canopy structure and abundance would impact on blue carbon sink capacity such that the large leaf-sized species (*E. acoroides*) at high abundance would sustain a larger organic carbon stock than the medium leaf-sized species (*T. hemprichii*) with low abundance. Both seagrass species would sustain larger organic carbon stocks than unvegetated sediment; 2) a higher proportion of allochthonous carbon would be trapped by the large leaf-sized species at higher abundance than by the medium-sized species at lower abundance or unvegetated sediment; 3) higher Net Community Production (NCP) would occur in meadows with higher seagrass biomass than in meadows with lower seagrass biomass. To investigate the influence of seagrass species-specific canopy height, canopy structure, and abundance on blue carbon sink capacity, sediment was sampled at sites with two different seagrass species at two different percentage covers and at an unvegetated sediment site.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Study site

Seagrass meadow at Haad Chao Mai National Park, Trang Province, southwest Thailand (7° 22' 21" N and 99° 20' 27" E) in the Andaman Sea is the largest seagrass meadow in Thailand (covers 18 km<sup>2</sup>). This seagrass meadow is situated 1 km from a mangrove forest. Nine of the twelve seagrass species reported in Thailand are found at this site. The lower intertidal zone of this site is dominated by two seagrass species that vary in their morphological characteristics: *T. hemprichii* is a medium leaf-sized species and *E. acoroides* is a large leaf-sized species.

To examine the influence of seagrass abundance on blue carbon sink capacity, we selected two common percentage covers for *T. hemprichii* and *E. acoroides* at this site (75% cover was considered as high coverage with seagrass leaves; 12% cover was considered as low coverage with seagrass leaves) and adjacent unvegetated sediment. In total there were 5 treatments: 1) 75% cover of *T. hemprichii*; 2) 75% cover of *E. acoroides*; 3) 12% cover of *T. hemprichii*; 4) 12% cover of *E. acoroides*; and 5) an unvegetated sediment.

**3.2.2 Sampling** To determine where to collect the cores for each treatment, quadrats (0.25 m<sup>2</sup>) were positioned to estimate seagrass abundance. To avoid any impact from edge effects, the influence from patch contraction, habitat loss, or meadow expansion, the distance between quadrats was more than 2 m. To avoid the adjacent meadows of different species having any effect on each other, the distance between (the patches of the two seagrass species (*T. hemprichii* and *E. acoroides*)) was more than 30 meters.

The shoot density (i.e. number of shoots m<sup>-2</sup>) was estimated by counting the number of shoots per quadrat. The longest seagrass leaf samples were collected within quadrats to determine the leaf width and leaf length (n = 10).

Sediment samples were collected using PVC cores (30 cm deep, 10 cm inner diameter) (n = 10). The sediment cores were sliced into 3 sections representing 3 known volumes of wet sediment, 0–3, 3–10, and 10–15 cm. Samples were stored in the dark at 4°C and then dried at 50°C for 48 hours in the laboratory. Large particles were removed from the sediment samples using a 2 mm sieve. Sediment dry bulk density (DBD) was calculated as a dried mass per unit volume of sediment according to the following equation:

$$\text{Dry Bulk Density (g sediment dry weight)} = \frac{\text{Mass dried sediment (g)}}{\text{Volume sample (ml)}}$$

For stable isotopic analysis, the three potential sources of organic carbon that could enter the sediment profile were collected including seagrass leaves, mangrove leaves, and suspended particulate matter (SPM). Samples of *Rhizophora apiculata* Bl. leaves of the same age and size were collected from the nearby mangrove forest. Five leaf samples from five mangrove trees were pooled to become one replicate, with three replicates in total (n = 3). The SPM were collected from the water column (n = 5) at the intertidal zone and subtidal zone. The water samples were filtered through pre-combusted glass fiber (Whatman GF/F) filters. Samples were acidified to remove calcium carbonate from sediment before placing samples into tin capsules (12 x 6 mm) and weighing with an analytical semi-micro balance (0.01 mg precision); the required mass was 5–20 mg.

### **3.2.3 Sediment organic carbon content**

The dried sediment and seagrass samples were prepared for analysis of organic carbon content ( $C_{org}$ ) by grinding into a fine powder ( $< 40 \mu m$ ) using a ball grinder (Planetary Micro Mill PULVERISETTE7, Fritsch GmbH) before acidifying with 1 M HCl. The dried acidified sediment sample was transferred to an aluminium cup for analysis of  $C_{org}$  content ( $\%C_{org}$ ) using Carbon Nitrogen Analyser (LecoTruSpec Micro, TCN Analyser, LECO Corp., St. Joseph, MI). The percentage of organic carbon content ( $\% C_{org}$ ) was recorded. Sediment organic carbon ( $C_{org}$ ) stock ( $Mg C_{org} ha^{-1}$ ) was calculated by multiplying  $C_{org}$  content (mg  $C_{org}$  in 100 mg sediment) with the DBD ( $g \text{ sediment cm}^{-3}$ ) and sediment depth (cm).

### 3.2.4 Isotopic analysis

The stable isotopic composition of samples was analysed using mass spectrometry (Europa Scientific 20-20 Isotope Ratio Mass Spectrometry linked to an Automated Nitrogen Carbon Analyser-Solids and liquids). The stable isotopic composition ( $\delta$ ) was calculated from the ratio of heavy ( $^{13}C$ ) to light stable ( $^{12}C$ ) isotopes in the samples ( $R_{sample}$ ) relative to the ratio of the standard ( $R_{standard}$ , from Vienna Pee Dee Belemnite) following the equation:

$$\delta_{sample} = 1000 \left[ \left( \frac{R_{sample}}{R_{standard}} \right) - 1 \right]$$

The potential  $C_{org}$  sources contributing to the sediment samples were quantified using the stable isotope mixing models (IsoSource software version 1.3), the Visual Basic<sup>®</sup> program was used to calculate the contribution of four potential sources to the carbon trapped during each experiment. The range of feasibility contributions was calculated using a 1% increment and a tolerance of 0.1(24).

### 3.2.5 Measurement of benthic community metabolism

Benthic community metabolism (measured as NCP, R, and GPP) was compared among the five treatments using incubation chambers. The incubation chambers (plastic cylinders with sampling ports) covered 0.6 m<sup>2</sup> of sediment surface and contained ~1.5 L of headspace (estimation of water volume from the chamber volume minus volume of sediment chamber inserted into sediment to a standardised depth of 5 cm). Samples were incubated in darkness (black painted chamber) and under light (clear chamber) conditions for 2 hours (10:00 to 12:00), with a total of 12 incubation chambers for each treatment. Water samples were collected before closing the chamber and after incubation using a 25 mL syringe. Change in dissolved oxygen (DO, mg L<sup>-1</sup>) within the incubation chamber was measured using a Dissolved Oxygen Probe (LDO probe, Model HQ40d, Hach Company, Loveland, CO, USA).

Community respiration (R) was estimated using the change detected in DO concentration in the dark incubation chamber, while the Net Community Production (NCP) was estimated using the change detected in the DO concentration in the clear incubation chamber. Gross Primary Production (GPP) was calculated using the equation:

$$GPP = R + NCP$$

### 3.2.6 Statistical analysis

A power analysis was conducted to determine the sample size required to detect change in sediment carbon stocks for each of the 5 treatments: 1) 75% *T. hemprichii* cover; 2) 75% *E. acoroides* cover; 3) 12% *T. hemprichii* cover; 4) 12% cover *E. acoroides*; and 5) unvegetated sediment. The mean value of seagrass leaf width and length were tested

using a t-test to identify differences between *T. hemprichii* and *E. acoroides*. The differences in above- and below-ground biomass among the 4 treatments (excluding unvegetated sediment) were tested using one-way Analysis of Variance (ANOVA). The mean value of the sediment organic carbon stock was used in a repeated-measures ANOVA, where there were five treatments and three sediment depths (0-3, 3-10, and 10-15 cm). To compare community metabolism (NCP, R, and GPP) for each of the 5 treatments ( $n = 3$  for the dark incubation chamber and  $n = 3$  for the light incubation chamber), data was analysed using the one-way ANOVA.

When the ANOVA resulted in a significant variation among treatments, post hoc comparisons were tested using the Tukey's method (95% confidence level). All data were transformed as needed (usually square root or  $\log_{10}$ ) to meet the analysis assumption including homogeneity of variance and normal distribution.

### **3.3 RESULTS**

#### **3.3.1 Seagrass species-specific canopy height and abundance**

There were significant differences in leaf length ( $p = 0.001$ ) and leaf width ( $p < 0.001$ ) between *T. hemprichii* and *E. acoroides*. There was no significant difference in the leaf length between meadows with a 75% cover and 12% cover of *T. hemprichii* and *E. acoroides*. *T. hemprichii* had a curved leaf that was  $14.49 \pm 0.35$  cm in length and  $1.53 \pm 0.02$  cm in width. The tips were rounded and smooth, and shoots with 2-6 leaves emerged from thick rhizomes. *E. acoroides* had ribbon-like leaves that were  $108 \pm 11.54$  cm in length and  $1.8 \pm 0.09$  cm in width. Leaves of *E. acoroides* had smooth rounded tips and the edges of the leaves were slightly rolled.



Seagrass abundance (75% cover and 12% cover) measured as shoot density, varied from  $28.40 \pm 4.02$  shoots  $m^{-2}$  (*E. acoroides* 12% cover) to  $333.20 \pm 15.04$  shoots  $m^{-2}$  (*T. hemprichii* 75% cover, Fig. 3.1). The shoot density was higher in *T. hemprichii* compared to *E. acoroides* at both 75% and 12% covers.

### **3.3.2 Canopy structure in low and high density of *T. hemprichii* and *E. acoroides***

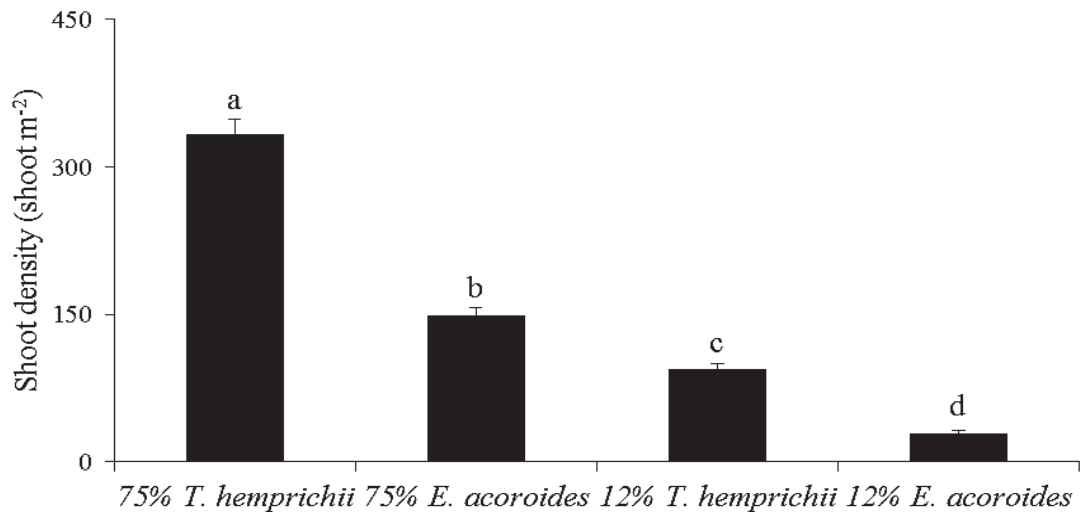
There were significant differences in the above- and below-ground biomass ( $p < 0.001$ ) among the four treatments. At the 75% cover, the average above-ground biomass was significantly higher in *E. acoroides* than in *T. hemprichii*, while there was no significant difference in the above-ground biomass between *T. hemprichii* and *E. acoroides* measured at a 12% cover. There was a significant difference in the below-ground biomass between *T. hemprichii* and *E. acoroides* at both levels of leaf abundance.

The average above-ground biomass was significantly higher for the 75% cover than in the 12% cover for both *T. hemprichii* and *E. acoroides*. There were significant differences of the below-ground biomass between the 75% cover and the 12% cover of *T. hemprichii*, while there was no significant difference in the below-ground biomass between the 75% cover and the 12% cover for *E. acoroides* (Fig. 3.2).

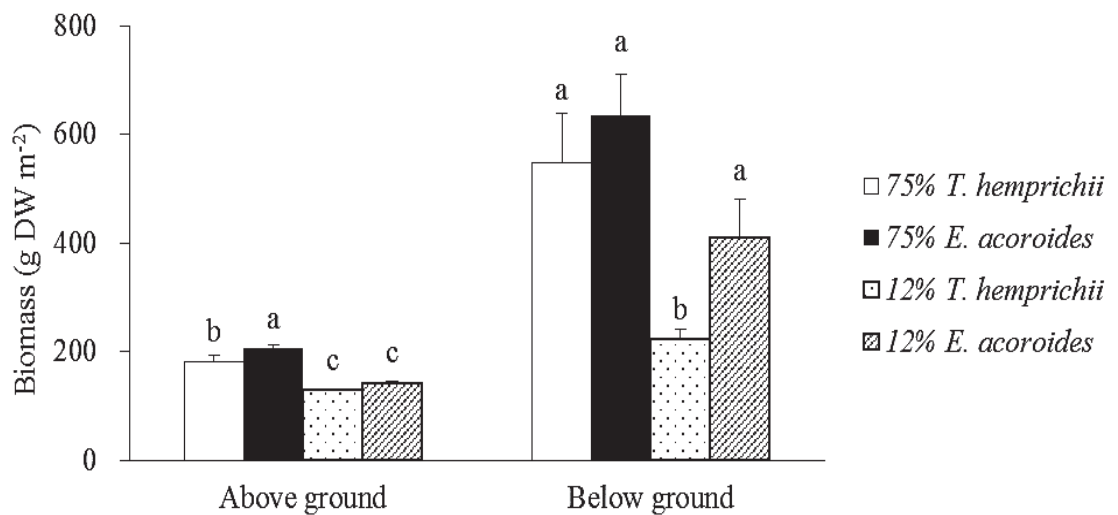
### **3.3.3 The influence of seagrass species-specific canopy height, structure, and abundance on sediment organic carbon ( $C_{org}$ ) stock**

Dry Bulk Density (DBD) (mean  $\pm$  S.E.M) of sediment in depth sections 0–3, 3–10, and 10–15 cm, collected from five treatments varied from  $1.26 \pm 0.13$  to  $1.85 \pm 0.01$  g sediment DW  $cm^{-3}$  (Table 3.1). The sediment  $C_{org}$  content was measured as a percentage of carbon in dried sediment (mg C in 100 mg sediment). The highest sediment organic carbon content was found under the 75% cover with *E. acoroides* at 10–15 cm depth

below the sediment surface ( $1.52 \pm 0.27 \%$ ), whereas the lowest sediment  $C_{org}$  content was found under the 12% cover of *T. hemprichii* at a 0–3 cm depth ( $0.11 \pm 0.03\%$ ). Sediment  $C_{org}$  stocks for each section and the 15 cm depth, were calculated using the DBD and  $C_{org}$  data (Table 3.1)



**Figure 3.1** Comparison of seagrass shoot densities in seagrass meadows with 75% and 12% covers of *T. hemprichii* and *E. acoroides*. The letters, a, b and c, indicate significant differences between treatments as determined by Tukey post hoc tests (95% confidence level). The same letters given for different treatments indicates that there was no significant difference detected (95% confidence level). Mean  $\pm$  S.E.M, n=10.

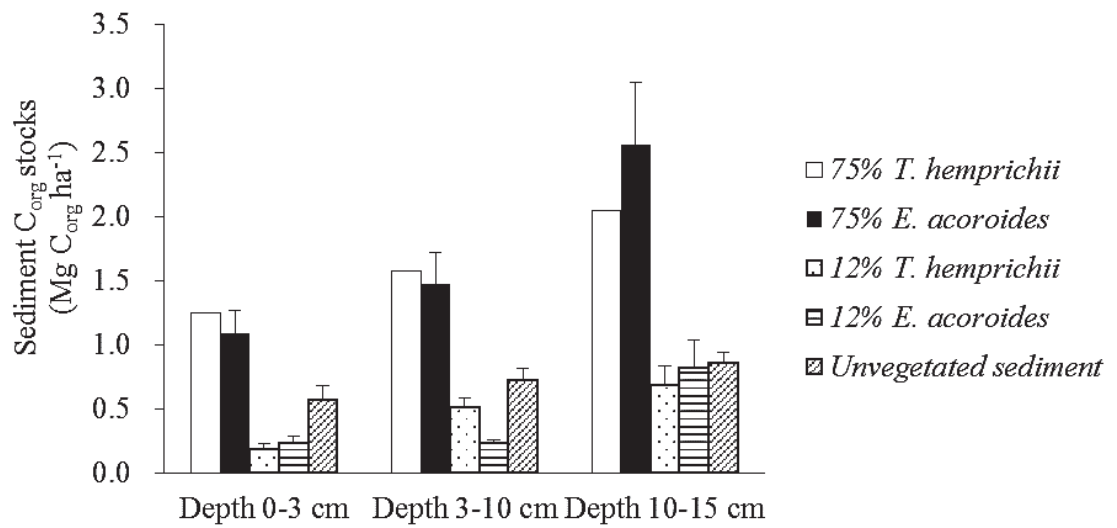


**Figure 3.2** Comparison of seagrass above- and below-ground biomass in seagrass meadows with 75% and 12% covers of *T. hemprichii* and *E. acoroides*. The letters, a, b and c, indicate significant differences among treatments as determined by Tukey post hoc tests (95% confidence level). The same letters given for different treatments indicates that no significant difference (95% confidence level) was detected. Mean  $\pm$  S.E.M, n=10.

**Table 3.1** Comparison of sediment dry-bulk density (DBD) and organic carbon ( $C_{org}$ ) content among two seagrass species (*T. hemprichii* and *E. acoroides*) at 75% and 12% covers and unvegetated sediment at 3 depths (0–3 cm, 3–10 cm, and 10–15 cm) below the sediment surface (Mean  $\pm$  S.E.M; n=10).

Species	Percent cover (%)	Depth (cm)	DBD (g DW cm <sup>-3</sup> )	$C_{org}$ content (%)	Stock at each depth section (Mg $C_{org}$ ha <sup>-1</sup> )
<i>T. hemprichii</i>	75	0-3	1.55 $\pm$ 0.05	0.79 $\pm$ 0.05	3.74 $\pm$ 0.80
		3-10	1.26 $\pm$ 0.13	1.21 $\pm$ 0.37	11.04 $\pm$ 3.97
		10-15	1.43 $\pm$ 0.13	1.40 $\pm$ 0.17	10.23 $\pm$ 1.67
				<b>Total</b>	25.02 $\pm$ 4.05
	12	0-3	1.72 $\pm$ 0.04	0.11 $\pm$ 0.03	0.56 $\pm$ 0.13
		3-10	1.70 $\pm$ 0.04	0.30 $\pm$ 0.04	3.60 $\pm$ 0.52
		10-15	1.79 $\pm$ 0.11	0.39 $\pm$ 0.08	3.41 $\pm$ 0.78
				<b>Total</b>	7.58 $\pm$ 0.78
	<i>E. acoroides</i>	75	0-3	1.70 $\pm$ 0.05	0.63 $\pm$ 0.09
3-10			1.44 $\pm$ 0.12	1.04 $\pm$ 0.18	10.31 $\pm$ 1.72
10-15			1.69 $\pm$ 0.08	1.52 $\pm$ 0.27	12.82 $\pm$ 2.40
				<b>Total</b>	26.39 $\pm$ 2.95
12		0-3	1.68 $\pm$ 0.06	0.14 $\pm$ 0.03	0.69 $\pm$ 0.17
		3-10	1.85 $\pm$ 0.10	0.13 $\pm$ 0.01	1.66 $\pm$ 0.15
		10-15	1.57 $\pm$ 0.07	0.52 $\pm$ 0.13	4.12 $\pm$ 1.06
				<b>Total</b>	6.47 $\pm$ 1.01
Unvegetated sediment		N/A	0-3	1.63 $\pm$ 0.00	0.35 $\pm$ 0.06
	3-10		1.50 $\pm$ 0.06	0.47 $\pm$ 0.05	5.06 $\pm$ 0.64
	10-15		1.67 $\pm$ 0.10	0.51 $\pm$ 0.05	4.40 $\pm$ 0.45
				<b>Total</b>	11.18 $\pm$ 0.93

There were significant differences in  $C_{\text{org}}$  stocks between meadows with 75% and 12% covers for *T. hemprichii*, *E. acoroides* and unvegetated sediment (average reported over 1 cm depth) ( $p < 0.001$ ) (Table 3.2). Higher  $C_{\text{org}}$  stocks were found in meadows with a 75% cover of *T. hemprichii* ( $1.25 \pm 0.27$ ,  $1.58 \pm 0.57$  and  $2.05 \pm 0.33$  Mg  $C_{\text{org}}$  ha<sup>-1</sup> at sediment depths 0–3, 3–10 and 10–15 cm, respectively) and *E. acoroides* ( $1.09 \pm 0.18$ ,  $1.47 \pm 0.25$  and  $2.56 \pm 0.48$  Mg  $C_{\text{org}}$  ha<sup>-1</sup> at sediment depths 0–3, 3–10 and 10–15 cm, respectively) compared to meadows with a 12% cover of *T. hemprichii* ( $0.19 \pm 0.04$ ,  $0.51 \pm 0.07$  and  $0.68 \pm 0.16$  Mg  $C_{\text{org}}$  ha<sup>-1</sup> at sediment depths 0–3, 3–10 and 10–15 cm, respectively), *E. acoroides* ( $0.23 \pm 0.06$ ,  $0.24 \pm 0.02$  and  $0.82 \pm 0.21$  Mg  $C_{\text{org}}$  ha<sup>-1</sup> at sediment depths 0–3, 3–10, and 10–15 cm, respectively) and unvegetated sediment ( $0.57 \pm 0.11$ ,  $0.72 \pm 0.09$  and  $0.86 \pm 0.09$  Mg  $C_{\text{org}}$  ha<sup>-1</sup> at sediment depths 0–3, 3–10, and 10–15 cm, respectively) (Fig. 3.3). There was significant differences in  $C_{\text{org}}$  stocks among the three sediment depths (0–3, 3–10, and 10–15 cm) for all of the species and covers ( $p < 0.001$ ) (Table 3.2).



**Figure 3.3** Comparison of sediment C<sub>org</sub> stock in seagrass meadows with 75% and 12% covers of *T. hemprichii* and *E. acoroides*, and unvegetated sediment across three sediment depths (0–3, 3–10, and 10–15 cm). \* indicate significant difference between seagrass species and between percent cover (95% confidence level). The letters a, b and c indicate significant differences among 5 meadows as determined by the Tukey post hoc tests (95% confidence level). Mean ± S.E.M, n=10.

### 3.3.4 Sediment organic carbon ( $C_{org}$ ) sources

The  $\delta^{13}C$  varied among five potential  $C_{org}$  sources including seagrass leaves ( $-13.15 \pm 0.06\text{‰}$ ), mangrove (*R. apiculata*) leaves ( $-28.96 \pm 0.28\text{‰}$ ), intertidal SPM ( $-23.42 \pm 0.28\text{‰}$ ) and subtidal SPM ( $-23.81 \pm 0.47\text{‰}$ ). C:N ratios also varied among the potential  $C_{org}$  sources;  $16.5 \pm 0.3$  for seagrass leaves,  $58.9 \pm 3.4$  for mangrove leaves,  $7.8 \pm 0.3$  for intertidal SPM, and  $8.1 \pm 0.4$  for subtidal SPM.

Using the stable isotope mixing model (IsoSource software package) and the  $\delta^{13}C$  of potential sources, the range of their possible contributions to the sediment samples were quantified (Table 3.3). At 75% and 12% covers for *T. hemprichii* and *E. acoroides*, the average proportional contribution of seagrass to the sediment organic carbon pool varied between 3–30%. The average proportional contribution of seagrass to the sediment organic carbon pool of an unvegetated sediment varied between 14–46%.

Using the mean of the source contribution (%) and the total amount of  $C_{org}$  content in sediment, the  $C_{org}$  content from each source (seagrass, mangrove and SPM) in the sediment (mixture) was shown in Fig. 3.4.

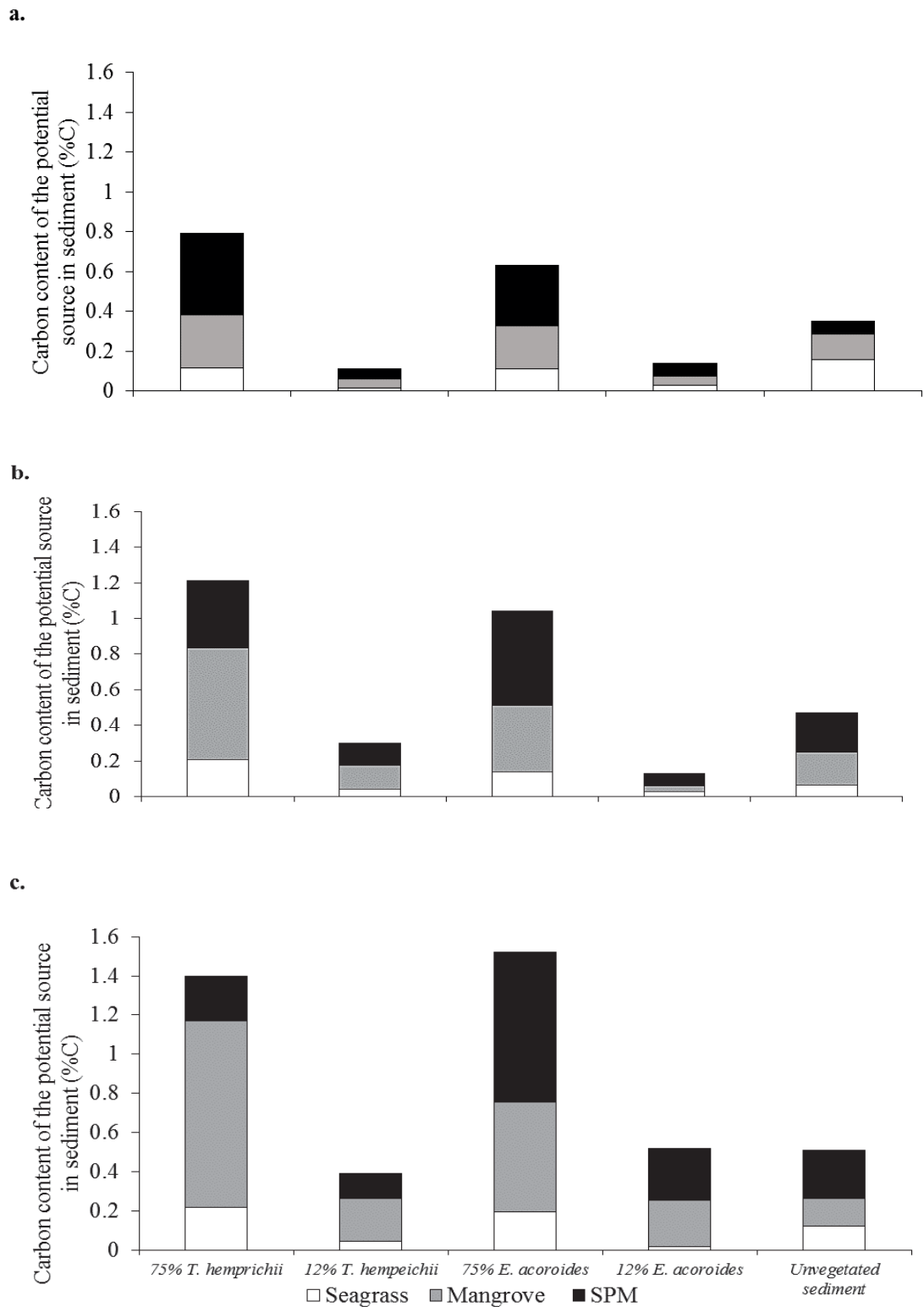
**Table 3.2** Summary of repeated-measures ANOVA of sediment organic carbon ( $C_{org}$ ) stocks comparing seagrass meadows with a 75% cover of *T. hemprichii*, 75% cover of *E. acoroides*, 12% cover of *T. hemprichii* and 12% cover of *E. acoroides* and unvegetated sediment at three depths, 0–3 cm, 3–10 cm, and 10–15 cm; \* indicates a significant difference among treatments (95% confidence level) (n = 10).

Effect	Source	df	MS	F	P
Between-Subjects	Treatments	4	0.061	3.491	0.000*
	Error	45	0.017		
Within-Subjects	Depth	2	0.355	18.784	0.000*
	Depth x treatments	8	0.020	1.054	0.402
	Error	90	0.019		



**Table 3.3** Mean, minimum and maximum source contribution (%) (Statistic output from IsoSource) of carbon at sediment depths of 0–3, 3–10, and 10–15 cm at 75% and 12% covers of *T. hemprichii* and *E. acoroides* and in unvegetated sediment.

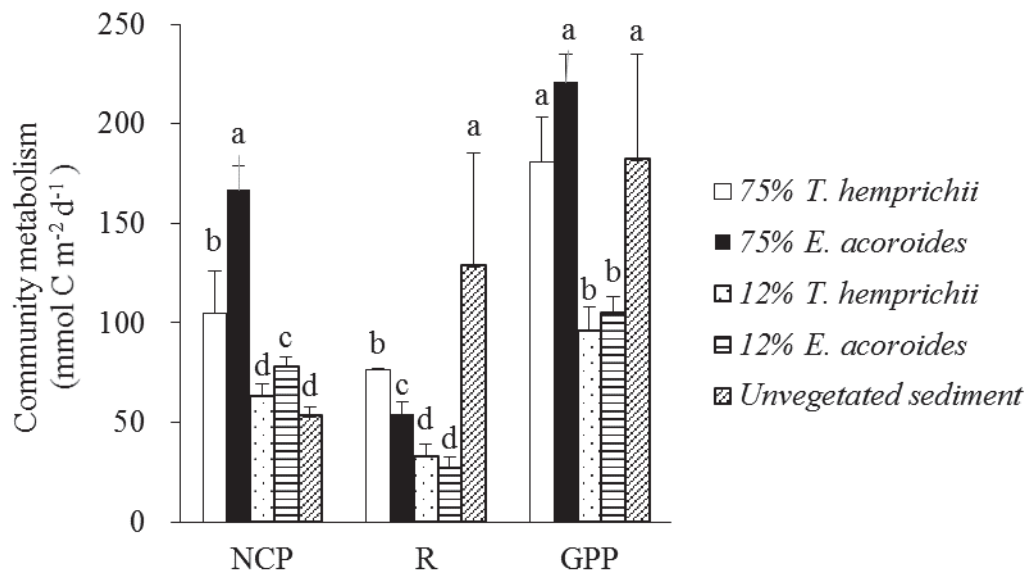
Species	Percent cover (%)	Depth (cm)	Seagrass			Mangrove			SPM		
			Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
<i>T. hemprichii</i>	75	0-3	9	14.9	32	22	33.7	67	1	51.4	69
		3-10	0	17.3	23	18	51.8	63	14	30.9	82
		10-15	12	15.5	19	61	68	75	6	16.5	27
<i>T. hemprichii</i>	12	0-3	3	14.5	30	17	39.6	70	0	45.9	80
		3-10	10	13.5	17	38	45	52	31	41.5	52
		10-15	7	11	15	48	56	64	21	36	45
<i>E. acoroides</i>	75	0-3	7	17.5	33	14	34.7	65	2	47.8	79
		3-10	6	13.5	30	21	35.9	68	2	50.5	73
		10-15	7	12.9	30	25	36.7	70	0	50.4	68
<i>E. acoroides</i>	12	0-3	7	20.6	36	5	31.8	62	2	47.6	88
		3-10	18	22	26	17	25	33	41	53	65
		10-15	0	3	6	40	46	52	42	51	60
Unvegetated sediment	N/A	0-3	42	45.5	49	29	36	43	8	18.5	29
		3-10	2	13.8	29	16	39.2	69	2	47.1	82
		10-15	15	24	40	10	27.8	59	1	48.3	75



**Figure 3.4** Carbon content of each potential source (seagrass, mangrove and SPM) in sediment depth 0–3 (a), 3–10 (b), and 10–15 cm (c) from seagrass meadows with 75% and 12% covers of seagrass species *T. hemprichii* and *E. acoroides* and in unvegetated sediment.

### 3.3.5 Benthic community metabolism

There were significant differences in community metabolism (NCP, R and GPP) among meadows with 75% and 12% covers of *T. hemprichii*, *E. acoroides* and in unvegetated sediment ( $p < 0.001$ ) (Table 3.4). Higher NCP was measured in meadows with 75% cover of *T. hemprichii* ( $104.59 \pm 21.72 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) and *E. acoroides* ( $166.92 \pm 12.32 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) compared those with a 12% cover of *T. hemprichii* ( $63.54 \pm 5.53 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ), *E. acoroides* ( $78.09 \pm 4.63 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) and unvegetated sediment ( $53.36 \pm 4.11 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) (Fig. 3.5). Higher respiration (R) was measured in unvegetated sediment ( $129.22 \pm 56.45 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) compared to meadows with a 75% cover of *T. hemprichii* ( $76.31 \pm 1.06 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) or *E. acoroides* ( $53.97 \pm 6.28 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) and a 12% cover of *T. hemprichii* ( $32.79 \pm 6.08 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) or *E. acoroides* ( $27.23 \pm 5.58 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ). Higher GPP was measured in meadows with a 75% cover of *T. hemprichii* ( $180.90 \pm 22.39 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) or *E. acoroides* ( $220.89 \pm 14.17 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) and in unvegetated sediment ( $182.58 \pm 52.45 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) compared with meadows with a 12% cover of *T. hemprichii* ( $180.90 \pm 22.39 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ) or *E. acoroides* ( $220.89 \pm 14.17 \text{ mmol C m}^{-2} \text{ d}^{-1}$ ). All treatments showed that GPP was greater than R for all conditions – i.e. P/R ratios were above 1.



**Figure 3.5** Comparison of benthic community production (NCP, R and GPP) among 75% and 12% cover of seagrass species; *T. hemprichii* and *E. acoroides* meadow and an unvegetated sediment. The letters, a, b and c, indicate significant differences among treatments as determined by the Tukey post hoc tests (95% confidence level).

**Table 3.4** Comparison of community metabolism (NCP, R and GPP) between two seagrass species (*T. hemprichii* and *E. acoroides*) at 75% and 12% coverage and in unvegetated sediment. Data was analysed using the one-way ANOVA of NCP, R, and GPP; \* indicates a significant difference among treatments (95% confidence level) (n=3).

	<b>Source</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P</b>
NCP					
	Between Groups	4	0.113	94.880	0.000*
	Within Groups	10	0.001		
	Total	14			
R					
	Between Groups	4	5123.726	62.646	0.000*
	Within Groups	10	84.788		
	Total	14			
GPP					
	Between Groups	4	8743.22	15.853	0.000*
	Within Groups	10	551.501		
	Total	14			

### 3.4 DISCUSSION

*Thalassia hemprichii* (Ehrenberg) Ascherson and *Enhalus acoroides* (L.f.) Royle are the dominant seagrass species at Laem Yong Lam, Haad Chao Mai National Park, Trang Province, Thailand in the Andaman Sea. These two seagrass species are different in their morphological characteristics, especially canopy height (leaf size) and leaf shape. Leaves of *E. acoroides* were larger than the leaves of *T. hemprichii* (leaf length of *E. acoroides* was, on average  $108 \pm 11.54$  cm, 7.5-times longer and 1.2-times wider than that of *T. hemprichii*). At a particular percent cover, higher shoot density was found in *T. hemprichii* than with *E. acoroides*. At a 75% cover, lower amounts of above-ground biomass were found in *T. hemprichii* than in *E. acoroides*, while the below-ground biomass was similar between these two seagrass species.

Differences in seagrass species have been reported to be a major factor affecting surrounding water velocity (Komatsu et al., 2004). Water flow inside beds of *E. acoroides* has been shown to be much lower than inside beds of *T. hemprichii* (Komatsu et al., 2004); however that report did not consider leaf abundance. Moreover, the specific character of *E. acoroides* such as the mucus leaf would increase the leaf trapping capacity of this species. Thus, a higher proportion of finer grained, organic rich particles might be hypothesised to have been stored within the sediment of the large-sized seagrass species, *E. acoroides*, compared to the medium-sized species, *T. hemprichii*. Surprisingly, our analysis showed that at a particular percent cover, there were similar levels of sediment  $C_{org}$  stock in both *T. hemprichii* and *E. acoroides* seagrass meadows (Fig. 3.3). The similarity in the sediment  $C_{org}$  stock for both species at 75% cover and at 12% cover was more than there was for either species at 75% cover and 12% cover. While there is no doubt that the large canopy size (long and wide leaf

blades) of *E. acoroides* could support enhanced blue carbon sink capacity, by reducing the water flow profile to promote the settlement of suspended organic carbon and increasing the trapping capacity of leaf canopy, one may ask what is the specific characteristics underlying the medium-sized species, *T. hemprichii* to store the same level of  $C_{org}$  in the sediment as the large-sized species, *E. acoroides*. This question could be explained by the result of sediment  $C_{org}$  from the different seagrass abundances.

Seagrass abundance is likely to be an important factor enhancing  $C_{org}$  level in *T. hemprichii* beds. Although, our results showed that the above-ground biomass of *T. hemprichii* was around 1.3-times lower than that of *E. acoroides*, the shoot densities of *T. hemprichii* were around 3-times higher than those of *E. acoroides* in both the 12% and 75% covers. The sediment  $C_{org}$  stocks measured at high shoot density of *T. hemprichii* and *E. acoroides* were ~4-times higher than sediment  $C_{org}$  stocks measured at the low shoot density of *T. hemprichii* and *E. acoroides*. High shoot densities could play a major role in enhancing the organic carbon stores within the sediment to increase blue carbon sink capacity by enhancing the direct contact of suspended particles with their leaf blades. Consequently they would promote the settlement of suspended particles from the water column (Agawin and Duarte, 2002). In addition, high seagrass densities enhanced the suspended particle deposition into the meadows by reducing water flow and increasing the chance of particles binding to seagrass leaves directly (Peralta et al., 2008).

The sediment organic carbon sources of *T. hemprichii* and *E. acoroides* beds for all percent covers were similar (dominated by SPM and mangrove for the top 15 cm of sediment.) Both seagrass species have a similar capacity to trap and store organic carbon, especially allochthonous carbon. However, the amount of allochthonous carbon

trapped by seagrass meadows with 12% covers of either seagrass species was lower than for the 75% cover. Seagrass meadows with a 75% cover could not only promote higher autochthonous carbon in the sediment  $C_{org}$  pool, but they also promoted higher allochthonous carbon compared with seagrass meadows at a 12% cover. This result indicated that seagrass abundance plays a more important role in determining the level of allochthonous carbon input in the sediment, than seagrass canopy height.

Seagrass biomass directly affects community metabolism where Net Community Production (NCP) was found to be higher at sites where seagrass biomass was highest for both species. Comparing sediment  $C_{org}$  stocks between vegetated treatments (12% and 75% covers of *T. hemprichii* and *E. acoroides*) with unvegetated sediment, showed a higher sediment  $C_{org}$  in the 75% cover for either seagrass species compared to unvegetated sediment. As well as the sediment  $C_{org}$  stock, NCP in 75% covers of either seagrass species was higher than these in unvegetated sediment. Interestingly, similar levels of NCP were found in the 12% cover of *T. hemprichii* and in unvegetated sediment. Moreover, the sediment  $C_{org}$  stock in the upper 15 cm of the sediment was similar between unvegetated sediment and meadows with a 12% cover of *T. hemprichii* and *E. acoroides*. Our analysis indicated that low seagrass abundance could result in low levels of blue carbon sink capacity comparable to unvegetated sediment. Thus, declining seagrass abundance (percent cover and density) seems to be a major factor constraining the carbon sink capacity of seagrass ecosystems.

The variability of seagrass abundance (percent covers and densities) have been reported worldwide (Harrison, 1982, Prathep et al., 2010). For example, minimum abundance of tropical intertidal seagrass meadows was often observed in the dry season in North Queensland, Australia, with around 10% cover and subsequent recovery of seagrass



during the wet season with more than 40% cover (Lanyon and Marsh, 1995). The variation of seagrass between months was reported from Koh Tha Rai, Thailand. The highest percent cover (around 40% cover) of *E. acoroides* was found during September, while the lowest percent cover (around 2% cover) was found during June (Prathep et al., 2010). If low seagrass abundance has a negative effect on sediment  $C_{org}$  stock, there would be an overestimation of global  $C_{org}$  stock if spatial variation of seagrass abundance (i.e. percent cover) has not been taken into account for the estimation of global carbon stock.

In this particular study, we found that the species-specific canopy height is not a good indicator of blue carbon sink capacity. Seagrass canopy structure (above-ground biomass) and abundance (percent cover and shoot density) are better indicators of the amount of  $C_{org}$  stored. However, this may not be the case for comparisons with other species, as other characteristics may be more important such as nutrient content, decomposition rate or chemical composition in seagrass materials. Variation in the decomposition rates have been reported for both tropical and temperate regions (Fourqurean and Schlau, 2003, Kenworthy and Thayer, 1984). The decomposition rate is determined by the chemical composition and anatomy of seagrasses (Fourqurean and Schlau, 2003). For example, the same level of C:N:P in rhizomes and roots, similar fibre content and lipid concentration has been reported for both *T. hemprichii* and *E. acoroides* in Thai water (Yamamuro and Chirapart, 2005). However, it is noted that these similarities do not extend to all seagrass species as demonstrated by the variation in C:N:P among 27 seagrass species at 30 locations worldwide (Duarte, 1990).

Sediment  $C_{org}$  stock measured over 15 cm of sediment at our study site was around 50% lower than the sediment  $C_{org}$  stock over 15 cm (calculation based on the mean  $C_{org}$  stock

over 1 m) for the global data of 219 cores from both tropical and temperate zones (Fourqurean et al., 2012). However, we found that sediment  $C_{org}$  stock increased with increasing sediment depth, as sediment  $C_{org}$  stock measured at a sediment depth of 10–15 cm was 2-times higher than those of the sediment depth 0–3 cm. The change in sediment  $C_{org}$  stock with depth could be a result of the variability in DBD and  $C_{org}$  content, which means that in previous years (deeper sediment) our study site might have contained a  $C_{org}$  stock more in line with the global data reported.

Recently, a model organism that most previous research on blue carbon has investigated for its blue carbon sink potential – *P. oceanica* could store carbon in the deep mat of roots and rhizomes (autochthonous source) (Mateo et al. 1997), while our results provide evidence that a large proportion of blue carbon stored in tropical seagrass meadows is derived from allochthonous sources, especially mangrove and suspended particulate matter (SPM). The percent of allochthonous carbon in our study (~90 %) was around 1.8-times higher than the global data estimation (~50 %) (Kennedy et al., 2010). This could indicate that although tropical seagrass-derived carbon in the sediment is likely to be less than that of temperate seagrass meadows possibly due to their lower net community production (Duarte et al., 2010). Conversely, the effective trapping capacity of these tropical seagrass meadows could promote these sites to be blue carbon hot spots by accumulating allochthonous carbon, which seems to be the major  $C_{org}$  sources contributing to tropical blue carbon sink.

Both *T. hemprichii* and *E. acoroides* tended to be autotrophic communities based on the community metabolism result (GPP being higher than R), indicating both seagrass meadows tended to act as blue carbon sinks. The respiration rates derived for both *T. hemprichii* and *E. acoroides* were ~2-times lower than the previous report from 403

individual estimates of 155 different sites in both tropical and temperate regions (Duarte et al., 2010). Furthermore, the NCP data from both *T. hemprichii* and *E. acoroides* was higher than the mean reported in the global data set for tropical seagrass meadows (23.73 mmol O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup>) (Duarte et al., 2010). Most seagrass meadows in South East Asia, as well as the dominant seagrass species in this region, have been poorly examined (Duarte et al., 2010);. Our analysis of community metabolism showed that the tropical seagrass meadows dominated by *T. hemprichii* and *E. acoroides*, the dominant seagrass species in SE Asia are among the most highly productive ecosystems and act as strong blue carbon sinks.

In conclusion, the results of this study indicate that medium size canopy species, such as *T. hemprichii*, as well as large size canopy species, such as *E. acoroides*, are important carbon sinks that enhance carbon accumulation in the sediment in comparison to unvegetated sediment. Shoot density seems to be one of the most important factors controlling the carbon sink capacity of seagrass. High shoot density promotes high blue carbon sink capacity, whereas low shoot density showed the same level of sediment C<sub>org</sub> stock as unvegetated sediment. Declining seagrass abundance seems to be a major factor constraining the carbon sink capacity of seagrass ecosystems. Seagrass biomass also impacts on community metabolism. Lower NCP was found in seagrass with lower biomass. Both *T. hemprichii* and *E. acoroides* meadows act as a carbon sink with high production and low metabolic respiration. The major carbon source for these sediments was allochthonous, indicating the importance of enhanced trapping ability of seagrasses in promoting blue carbon sink capacity.

## CHAPTER 4

### RECOVERING LOCAL BLUE CARBON STOCKS WITH ARTIFICIAL SEAGRASS

#### 4.1 INTRODUCTION

Climate change as a consequence of excess carbon in the Earth's atmosphere caused by human activities, has gained attention as an issue of global concern (Parmesan and Yohe, 2003, Walther et al., 2002, Solomon et al., 2009). To help mitigate climate change, reducing carbon emission is a necessary action (Lal, 2004, Canadell and Raupach, 2008). Natural carbon capture and storage by photosynthesis organisms – called biosequestration – can contribute to the mitigation of atmospheric CO<sub>2</sub> levels (Macreadie et al., 2014, Mcleod et al., 2011). Biosequestration has been focused mostly on terrestrial ecosystems as they can store CO<sub>2</sub> in biomass and soil for several decades (Nellemann et al., 2009). However, unlike carbon stored in terrestrial ecosystems, the carbon stored in the coastal and oceanic ecosystems – blue carbon – does not remain for decades or centuries, but rather for millennia (Mcleod et al., 2011).

Blue carbon is the carbon captured by vegetated coastal habitats (i.e. seagrass, mangrove, and saltmarsh). Among a variety of vegetated coastal habitat types, seagrass meadows are one of the most important blue carbon ecosystems, as they can sequester carbon up to 35-times faster than terrestrial forests (Mcleod et al., 2011). On a global scale, seagrass meadows have the capacity to store 4.2 to 8.4 Pg C<sub>org</sub> in the top meter of sediment (Fourqurean et al., 2012). Furthermore, the blue carbon capacity of seagrass is

not only enhanced by their high primary production, but it is also supported by the trapping capacity of seagrass canopies (Kennedy et al., 2010).

Seagrass canopies play an important role as particle filters, reducing water flow and therefore trapping suspended particulate matter (SPM) from the water column, transferring allochthonous carbon into seagrass meadows (Agawin and Duarte, 2002, Hendriks et al., 2008). On average, more than 50% of  $C_{org}$  stored in seagrass meadow sediment is allochthonous carbon, indicating that allochthonous carbon is the major contributor to sediment  $C_{org}$  stock (Kennedy et al., 2010). Enhancing the allochthonous carbon storage capacity of seagrass canopies could promote a more effective blue carbon ecosystem. However, the reality is that the blue carbon sink capacity of seagrass ecosystems has been depleted (Waycott et al., 2009). The loss of seagrass is a global crisis that needs to be urgently considered as it could potentially lead to the global loss of a significant blue carbon sink (Macreadie et al., 2014, Marbà et al., 2015).

As much as 29% of the world's seagrass areas have been destroyed (Waycott et al., 2009). The loss of seagrass meadows could potentially lead to the release of carbon back into the atmosphere (Pendleton et al., 2012). The release of carbon from the degraded seagrass meadow is up to  $3.3 \times 10^7$  Mg  $CO_2$ ; this could change seagrass meadows from being a blue carbon sink to an actual source of carbon (Pendleton et al., 2012). As the loss of seagrass is accelerating, it would take a long time – from decades to centuries – to recover the degraded seagrass meadows (Calumpong and Fonseca, 2001). As the carbon emissions from seagrass degradation are equivalent to 3–19% of that of global deforestation (Pendleton et al., 2012), the on-going loss of this blue carbon sink is an important issue. Thus, it is necessary to find an effective mitigating approach that involves the conservation and restoration of seagrass meadows.

The restoration of seagrass meadows has been attempted since the 1970s by way of a number of techniques, such as seagrass transplantation, seedling or ramet planting, passive fertilisation and recruitment facilitation (Wear, 2006). However, restoration is not always successful due to a number of factors such as, lack of experience, the limitations of selected techniques and poor habitat selection. In the latter case, high wave energy, low light conditions, and catchment runoff have been suggested to be major factors in determining the lack of success of seagrass restoration (Calumpong and Fonseca, 2001). For example, only 0–2% of seagrass survived after the transplantation of *Enhalus acoroides* to a high wave exposed area (Lawrence et al., 2007). Seagrass restoration is also challenging because it is expensive and laborious. Around 7 to 9 people are the minimum number required in restoration projects, depending on the technique used and the habitat type (Calumpong and Fonseca, 2001). The full cost of seagrass restoration by the U.S. Federal Court system was reported to be as much as US\$630,000 per hectare (Spurgeon, 1998).

Two studies have recently investigated the potential of seagrass restoration to recover seagrass carbon sequestration (Marbà et al., 2015, Greiner et al., 2013). After 12 years of seedling restoration and water quality improvement, blue carbon sequestration in a previously degraded seagrass meadow was recovered (Greiner et al., 2013). Another study reported that carbon burial rates in a degraded seagrass area equalled that of a pristine meadow after 18 years of replanting (Marbà et al., 2015). Obviously, performing restoration to recover seagrass carbon sequestration would be time-consuming. Furthermore, seagrasses are sensitive to either environmental factors (such as light intensity, desiccation, and temperature) or anthropogenic pollutants (such as industrial waste and agriculture runoff) (Dennison, 1987, Delgado et al., 1999, Shafer et al., 2007). Thus, techniques that could help restore blue carbon sink capacity during the

early stages of natural seagrass recovery, and also support the success of natural seagrass restoration, are needed.

Artificial seagrasses are replicated from natural seagrass characteristics. It is often made from a plastic ribbon. Artificial seagrass have been used as an experimental system in a number of studies, such as investigating the critical relationship of seagrass canopies to associated marine organisms and the surrounding environment (Lee et al., 2001). Artificial seagrasses are also used in “eco-engineering” for coastal protection and erosion control. Sediment in coastal habitats can be retained by artificial seagrass because the artificial seagrasses reproduce the natural hydrodynamic process by minimising water velocity. Almasi et al. (1987) reported that higher sedimentation rates were found in artificial seagrass than in the adjacent bare sand. In another experiment, artificial seagrass was shown to be environmentally friendly, as it could be colonised by epiphytes, which subsequently attracted invertebrates and fish (Bell et al., 1985, Bologna and Heck, 2000, Lee et al., 2001, Sogard, 1989, Macreadie et al., 2010).

Although using artificial seagrass in scientific experiments and coastal engineering is not a new trend, there has been no research investigating the potential of artificial seagrass to enhance particle trapping capacity, which is one aspect of blue carbon function. Using artificial seagrass as a pre-filter in areas of high water turbidity would be an ideal solution for improving the degraded areas and allowing the natural seagrass to recover. Consequently, successful recovery of the natural seagrass could potentially lead to full function as a blue carbon habitat, allowing particle retention in the sediment, sediment stabilisation, and the enhancement of sediment accumulation rates, leading to a set of biogeochemical conditions in the sediment.

In this study, we aimed to investigate the potential role of artificial seagrass in facilitating the trapping of carbon into the sediment surface by measuring the particle and carbon deposition rate, and the source of particle deposition. To address these issues, the artificial seagrass was placed in a denuded seagrass meadow, within a pristine seagrass meadow, and in unvegetated sediment. Sediment traps were installed to measure particulate deposition. We hypothesised that these artificial seagrasses behaved to some extent like pristine seagrass meadow (such as *E. acoroides*) by trapping allochthonous carbon to recover the carbon deposition rate. Consequently, seagrass blue carbon sink capacity would be enhanced.

## **4.2 MATERIALS AND METHODS**

### **4.2.1 Study site**

The study was conducted in the intertidal zone, Laem Yong Lam, Haad Chao Mai National Park, Trang Province, southwest Thailand, (7° 22' 21" N and 99° 20' 27" E) in the Andaman Sea (Fig. 4.1). There are three different locations within the study site: 1) a “pristine meadow”, covered with a large, healthy seagrass area; 2) a “denuded site”, where the seagrass meadow had been lost due to anthropogenic impacts; and 3) “unvegetated sediment”, adjacent bare sand. All three sample sites were located in the same general area of the intertidal flat where there was a semi-diurnal tide, and the site was often exposed during low tide for approx. 4–5 hours in the dry season and 0.5–1 hour in the wet season.

At the denuded site, increasing sediment loads and water turbidity have led to the complete destruction of seagrass meadows where four species used to exist: *Enhalus acoroides* (L.f.) Royle, *Cymodocea rotundata* Asch. & Schweinf., *Halophila ovalis* (R.



Brown) Hooker f. and *Thalassia hemprichii* (Ehrenberg) Aschers. There has been no report or observation of seagrass recovery at this site, during a past 10-year period of progressive seagrass loss. Recently, the construction surrounding the site has been stopped. The level of sediment loading has not reoccurred.

All three sampling sites were located approximately 1 km from the mangrove forest (dominated by *Rhizophora apiculate* Bl.). A large tidal channel linked the estuary and the open sea, which also separated a pristine meadow and a region of unvegetated sediment from the denuded site. The distance between the unvegetated sediment site and the denuded site was approximately 600 m, and the distance between the unvegetated sediment and the pristine meadow was 400 m.

#### **4.2.2 Experimental design**

*Enhalus acoroides*, the dominant seagrass species in the pristine meadow was chosen to be a model (canopy height and shoot density) for an artificial seagrass experimental unit. The artificial seagrass leaf (40 cm long and 2 cm width of plastic ribbon), with five leaves per shoot was tied to 1 x 1 m square meshes. Artificial seagrass was placed randomly within an area of  $2.5 \times 10^5 \text{ m}^2$  in the pristine meadow, denuded site and in a region of unvegetated sediment ( $n = 4$ ). In each location the artificial seagrass was “planted” with 150 shoots (per square meter)  $\text{m}^2$ . At a pristine meadow, an artificial seagrass unit was placed in a gap between seagrass patches where the shoot density of the pristine seagrass meadow was similar to that of the artificial seagrass.

To investigate the particle deposition rate, particle traps were installed following the method described in Gacia et al. (1999). To minimise interference between water flow and the particle trap, as well as to prevent internal re-suspension, 20 mL cylindrical

glass tubes with 5:1 ratios of length and diameter were used (25 mm diameter). Four tubes were attached to a 30 cm wooden pole at one particular water depth ( $n = 4$ ). The particle trap was positioned in the centre of an artificial seagrass unit. There were six treatments: 1) denuded with artificial seagrass; 2) denuded site; 3) pristine meadow with artificial seagrass; 4) pristine meadow; 5) unvegetated sediment with artificial seagrass; and 6) unvegetated sediment. The artificial seagrass was placed at the denuded site, pristine meadow and unvegetated sediment to investigate whether artificial seagrass could facilitate particle trapping to the sediment surface. The particle deposition rate under natural conditions—where there was no artificial seagrass—was also measured in these three study areas as a control treatment. Unfortunately, the particle traps at the unvegetated sediment site disappeared before sampling could take place. The number of replicates in each experiment was changed due to loss of some of the sediment traps. Replication numbers ( $n$ ) were 3, 2, 4, 3, and 4 for the denuded with artificial seagrass site, denuded site, pristine meadow with artificial seagrass, pristine meadow, and unvegetated sediment with artificial seagrass, respectively.

#### **4.2.3 Sample analysis**

Trapped particles were collected after 90 days. The dry weight (g DW) of the particle was assessed after being dried for 48 hours at 60°C. The sample was ground before being analysed for  $C_{org}$  content and isotopic composition.  $C_{org}$  content (% C) of particle deposition was analysed using a high-temperature combustion method on an elemental analyser (ECS 4010 CHNSO, Costech, US).  $C_{org}$  content was determined by the difference of the total carbon content and inorganic carbon content.

The source of  $C_{org}$  was determined by analysing isotopic composition using an isotope ratio mass spectrometry technique (Delta V<sup>TM</sup> Isotope Ratio Mass Spectrometer,

Thermo Scientific, USA). The ratio of heavy ( $^{13}\text{C}$ ) to light stable ( $^{12}\text{C}$ ) isotopes in the samples ( $R_{\text{sample}}$ ) relative to the ratio of the standard ( $R_{\text{standard}}$ , from Vienna Pee Dee Belemnite) was calculated following the equation:

$$\delta_{\text{sample}} = 1000 \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \text{ where } R = \frac{^{13}\text{C}}{^{12}\text{C}}$$

For the isotope mixing model (IsoSource software version 1.3), the Visual Basic<sup>®</sup> program was used to calculate the contribution of three potential sources (seagrass, mangrove and suspended particulate matter) to the carbon trapped in each experiment. Relative contributions of each source were examined at a 1% increment and 0.1 resolution.

#### **4.2.4 Statistical analysis**

The mean value of particle deposition rates and  $C_{\text{org}}$  deposition rates was used in a one-way ANOVA, where there were five treatments. When the ANOVA resulted in a significant variation among treatments, post hoc comparisons were tested using Tukey's test (95% confidence level). All data was transformed as needed (usually square root or  $\log_{10}$ ) to meet the analysis assumption including homogeneity of variance and normal distribution.

### **4.3 RESULT**

#### **4.3.1 Particle deposition**

The potential of artificial seagrass to restore blue carbon sink capacity was investigated by comparing particle deposition rates and  $C_{\text{org}}$  deposition rates among five treatments.

Particle deposition rates differed significantly between treatments ( $p = 0.126$ , Table 4.1a). A post hoc comparison showed that the particle deposition rate at the denuded site ( $165 \pm 118.77 \text{ g DW m}^{-2} \text{ day}^{-1}$ ) was significantly lower than at the denuded site with artificial seagrass ( $498.37 \pm 86.41 \text{ g DW m}^{-2} \text{ day}^{-1}$ ). The particle deposition rates at the pristine meadow ( $428.80 \pm 44.45 \text{ g DW m}^{-2} \text{ day}^{-1}$ ), the pristine meadow with artificial seagrass ( $418.37 \pm 78.63 \text{ g DW m}^{-2} \text{ day}^{-1}$ ) and the unvegetated sediment site with artificial seagrass ( $303.67 \pm 71.69 \text{ g DW m}^{-2} \text{ day}^{-1}$ ) were intermediate between the particle deposition rate at the denuded and at the denuded site with artificial seagrass (Fig. 4.1a.).

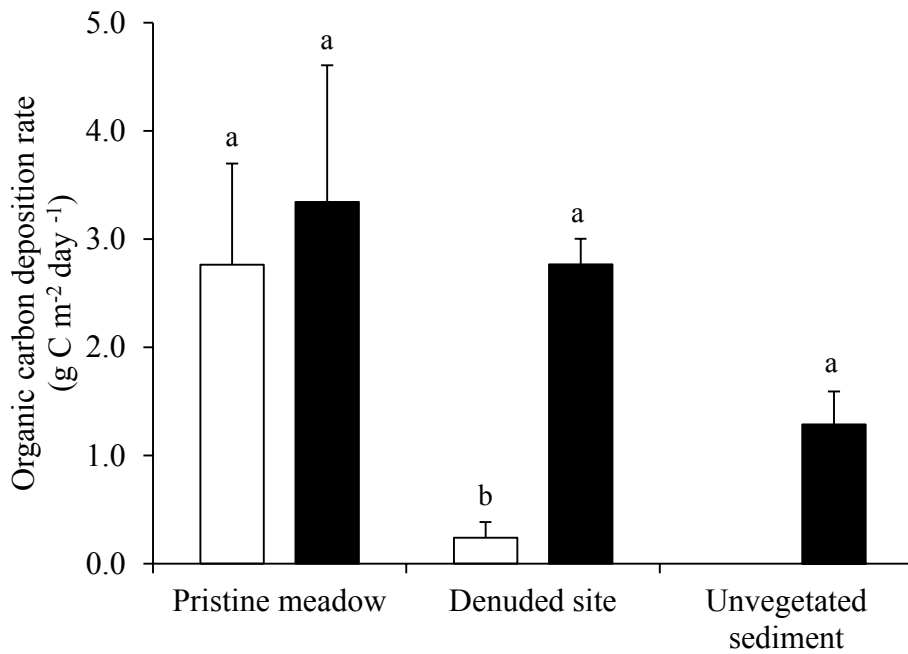
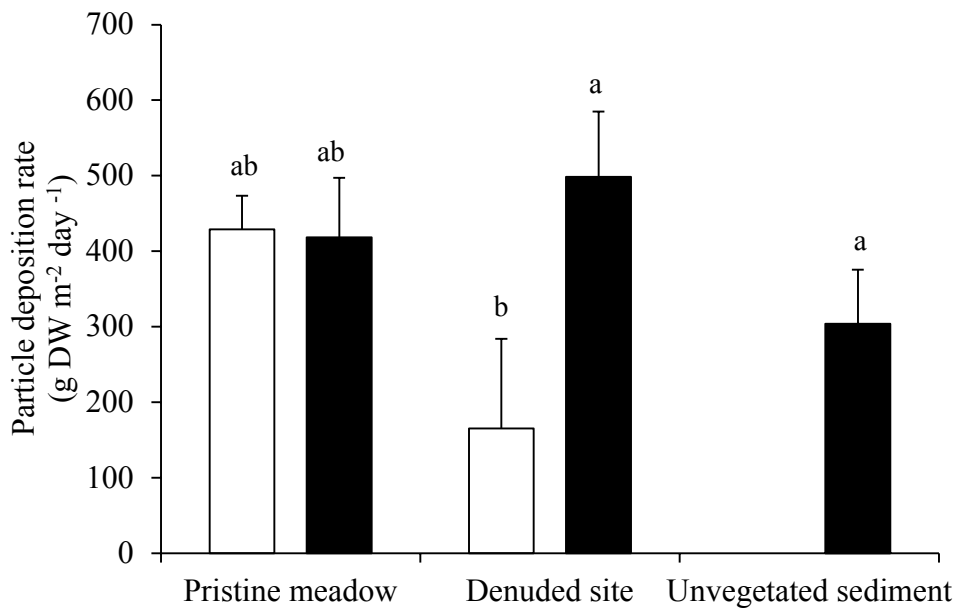
#### **4.3.2 Organic carbon ( $C_{\text{org}}$ ) deposition**

$C_{\text{org}}$  content (DW%) of particle deposition ranged from  $0.14 \pm 0.07$  to  $1.01 \pm 0.42 \text{ g C m}^{-2} \text{ day}^{-1}$  and was particularly low in the denuded site, and high in the pristine meadow with artificial seagrass treatment.

$C_{\text{org}}$  deposition rates also differed significantly between treatments ( $p = 0.181$ , Table 4.1b.).  $C_{\text{org}}$  deposition rate in the denuded site ( $0.24 \pm 0.15 \text{ g C m}^{-2} \text{ day}^{-1}$ ) was significantly lower than  $C_{\text{org}}$  deposition rate in the denuded site with artificial seagrass ( $2.77 \pm 0.24 \text{ g C m}^{-2} \text{ day}^{-1}$ ).  $C_{\text{org}}$  deposition rate in the denuded site with artificial seagrass was not significantly different from  $C_{\text{org}}$  deposition rate in the pristine meadow ( $2.76 \pm 0.93 \text{ g C m}^{-2} \text{ day}^{-1}$ ) and in the pristine meadow with artificial seagrass ( $3.34 \pm 1.26 \text{ g C m}^{-2} \text{ day}^{-1}$ ).  $C_{\text{org}}$  deposition rate at in the unvegetated sediment site with artificial seagrass ( $1.29 \pm 0.21 \text{ g DW m}^{-2} \text{ day}^{-1}$ ) was not significantly different from other artificial seagrass experiments carried out the denuded site and pristine meadow (Fig. 4.1b.).

**Table 4.1** Comparison of particle deposition rate and C<sub>org</sub> deposition rate among five experiments (denuded with artificial seagrass, denuded site, pristine with artificial seagrass, pristine meadow, and unvegetated sediment with artificial seagrass). Data was analysed using the one-way ANOVA of particle deposition rate and C<sub>org</sub> deposition rate; \* indicates a significant difference among five treatments (95% confidence level).

	Source	SS	df	MS	F	Sig
<b>a. Particle deposition rate</b>	Between groups	170328.898	4	42528.224	2.282	0.126
	Within groups	205284.702	11	18662.246		
	Total	375613.600	15			
<b>b. C<sub>org</sub> deposition rate</b>	Between groups	17.942	4	4.485	1.897	0.181
	Within group	26.008	11	2.364		
	Total	43.950	15			



□ Treatments without artificial seagrass ■ Treatments with artificial seagrass

**Figure 4.1** Comparison of deposition of a) particle and b)  $C_{org}$  among 1) denuded with artificial seagrass and 2) denuded site at Khao Bae Na and 3) pristine with artificial seagrass; 4) pristine meadow, and 5) unvegetated sediment with artificial seagrass. The letters, a, b and c, indicate significant differences among treatments as determined by the Tukey post hoc tests (95% confidence level). Mean  $\pm$  S.E.M.

### **4.3.3 Source of $C_{org}$ of particle deposition**

The source of the  $C_{org}$  from the particle deposition traps was examined using the stable isotope mixing model (IsoSource software package). The average proportional contribution of seagrass to  $C_{org}$  of particle deposition was highest in the pristine meadow (19%) and lowest in the unvegetated sediment site with artificial seagrass (7%). The range of contributions to  $C_{org}$  of particle deposition was shown in Table 4.2. Allochthonous sources (mangrove and SPM) accounted for more than 80% of  $C_{org}$  of particle deposition.

**Table 4.2** Mean, minimum and maximum of the source contribution (%) (Statistic output from IsoSource) of carbon deposited at the denuded with artificial seagrass site, the denuded site, the pristine meadow with artificial seagrass, the pristine meadow, and in unvegetated sediment with artificial seagrass.

Experiment	Seagrass			Mangrove			Suspended particulate matter		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Denuded with artificial seagrass	1	<b>16</b>	31	9	<b>39</b>	68	1	<b>46</b>	9
Denuded site	0	<b>11</b>	26	22	<b>43</b>	73	1	<b>47</b>	78
Pristine with artificial seagrass	12	<b>16</b>	19	38	<b>45</b>	52	29	<b>40</b>	50
Pristine meadow	4	<b>19</b>	32	0	<b>30</b>	55	13	<b>50</b>	96
Unvegetated with artificial seagrass	3	<b>7</b>	1	69	<b>76</b>	83	7	<b>18</b>	28

#### 4.4 DISCUSSION



The significant volume of carbon trapped by artificial seagrass in this experiment indicates the capacity of artificial seagrass to trap the particles from the water column. This result is consistent with our previous study (Apichanangkool et al under review) which showed that around 90% of  $C_{org}$  trapped in sediments was derived from allochthonous sources. A similar result was reported by Kennedy et al. (2010), where allochthonous carbon comprised 70% of  $C_{org}$  in Southeast Asian meadows. Furthermore, artificial seagrass supports particle deposition rates equal to and above the range observed in the natural Southeast Asian seagrass meadows ( $18.8 \pm 2.0$  to  $681.1 \pm 102$  g DW  $m^{-2}$   $day^{-1}$ ) (Gacia et al., 2003). However, our data was collected over a short timescale, and would not be representative of the average annual deposition because our experiments were not conducted over a range of weather conditions (such as storms and heavy rain) or different air exposure periods within seasons, which may either enhance or diminish deposition rates. Thus, the data represented in this study is the average deposition for a particular time of the year (January to April 2014).

In a pristine seagrass meadow, shoot density seems to be the major factor in determining the level of  $C_{org}$  deposition (Greiner et al., 2013). The shoot density generated by some natural seagrass restoration techniques, such as seedling restoration, is often low at the early stage of restoration. Because shoot density slowly increases over time, a restored seagrass meadow could take more than ten years to reach the same density that it originally had. Thus, the age of a restored seagrass meadow impacts directly on the recovery of its blue carbon capacity (Marbà et al., 2015). While the complete recovery of restored seagrass meadows and their blue carbon sink capacity would not be fully completed at the early stage of restoration, the trapping capacity of artificial seagrass might provide a useful supplement to seagrass restoration during their early stages. In addition, artificial seagrass would buffer environmental conditions, such

as slowing down the water velocity, stabilising the sediment, and reducing water turbidity; therefore potentially accelerating the full restoration of a meadow. Furthermore, restoration of blue carbon sink capacity using artificial seagrass requires comparatively little expense and effort, as only 2–3 people are required to install artificial seagrass in the field (Calumpong and Fonseca, 2001) and the estimated cost for artificial seagrass is around US\$ 100,000 per hectare. However, artificial seagrass is not an effective long-term solution, because it is a depreciating asset that requires maintenance, and it lacks the ability to adapt to changing environmental conditions. The success of using artificial seagrass to promote allochthonous carbon trapping and restore blue carbon sink capacity would vary due to both physical and biological processes at the particular site. The trapping of allochthonous carbon by artificial seagrass would be more effective in areas of high particle loading such as those surrounded by an allochthonous carbon source, e.g. mangrove forests, like in Southeast Asian seagrass meadows. However, artificial seagrass may be less relevant in some areas where autochthonous materials are the major  $C_{org}$  source, such as *Posidonia* ecosystems in the temperate region (Kennedy 2010).

However, artificial seagrass is not an effective long-term solution, because it is a depreciating asset that requires maintenance, and it lacks the ability to adapt to changing environmental conditions. The success of using artificial seagrass to promote allochthonous carbon trapping and restore blue carbon sink capacity would vary due to both physical and biological processes at the particular site. The trapping of allochthonous carbon by artificial seagrass would be more effective in areas of high particle loading such as those surrounded by an allochthonous carbon source, e.g.

mangrove forests, like in Southeast Asian seagrass meadows. However, artificial seagrass may be less relevant in some areas where autochthonous materials are the major  $C_{org}$  source, such as *Posidonia* ecosystems in the temperate region (Kennedy 2010). We suggest that the most useful scenario for deploying artificial seagrass for blue carbon gains may be in a denuded site, where the water quality is very poor and seagrass transplants would not survive. While the ability of artificial seagrass to trap the allochthonous carbon into sediment surface has been found, the subsequent fate of that carbon has not been determined. Another environmentally beneficial aspect surrounding the use of artificial seagrass is its potential role as a habitat for a range of aquatic lifeforms. A number of epiphytes and some invertebrates have been found colonizing on and within artificial seagrass units. The role of artificial seagrass leaves as a substrate for epiphytes and as an attractive habitat for invertebrates is another issue worthy of further investigation.

Although the result of this research study showed that artificial seagrass significantly support particle deposition, only one particular type of artificial seagrass (plastic ribbon) was used. Different types of artificial seagrass might demonstrate different effects in their trapping capacity. In addition to the types of artificial seagrass, the role of artificial seagrass to preserve the carbon is still arguable as the ability of seagrasses to sequester carbon is due to the sediment stability and the anoxic sedimentary conditions they create.

In conclusion, artificial seagrass facilitated carbon deposition at a denuded site to the same level as at a pristine meadow, indicating that the trapping capacity of artificial seagrass is similar to that of a natural seagrass canopy. This is the first study introducing artificial seagrass as a useful supplement for restoring blue carbon sink capacity.

Trapping of allochthonous carbon is a unique strategy for seagrass meadows to enhance their blue carbon sink capacity. While the loss of sediment  $C_{org}$  is increasing rapidly as a result of the decline in seagrass meadow areal extent, the trapping capacity of artificial seagrass could increase the deposition rate of sediment  $C_{org}$  and reduce the exposure of the underlying sediment to further loss. The trapping capacity of artificial seagrass would also provide benefits to enhance natural seagrass restoration.

## CHAPTER 5

### CONCLUSIONS AND FUTURE WORK

#### 5.1 CONCLUSION

Among the solutions to climate change, the trapping and storage of carbon by primary producers and microbial organisms living in sediment – biosequestration – is one of the most effective carbon sinks (Nellemann et al., 2009). While research on biosequestration has mostly focussed on terrestrial forests, coastal ecosystems are the hidden gem of the world’s carbon stores, so-called blue carbon sinks (Nellemann et al., 2009). Seagrass meadows are one of the most significant blue carbon sinks as they can store carbon for millennia (Mateo and Romero, 1997). However, the long-term prospects of seagrass carbon sinks have been threatened by a number of anthropogenic activities (Orth et al., 2006, Waycott et al., 2009). Different types of habitat degradation have lead to different levels of carbon loss (Pendleton et al., 2012). In order to develop global seagrass carbon budgets, we need to assess global stocks of different seagrass species, as well as understand the risks of losing these stocks (Macreadie et al., 2014). Furthermore, finding an effective way to recover blue carbon sink capacity is an issue of global significance as the loss of seagrass could trigger marine sediment to shift from being carbon sinks to carbon sources; consequently, carbon emissions would be increased (Marbà et al., 2015). The studies presented in this thesis were focused on the studies presented in this thesis were focused on 1) quantifying the blue carbon capacity following habitat degradation and in different seagrass species-specific canopy structures (Chapters 1 and 2), and 2) managing the blue carbon sink through restoration

of seagrass (Chapter 3). Sediment samples were collected using coring techniques – the best method to collect sediment for analysis of  $C_{org}$  stock (Macreadie et al., 2014). This study suggested that seagrass loss leads to a significantly weakened global carbon sink capacity (loss of  $C_{org}$  from pre-existing sediment). The study also illustrates the ecological consequences of seagrass loss due to anthropogenic activities, especially land development in Thailand – a tropical Indo-Pacific bioregion where the loss of seagrass is widespread. This study has also significantly progressed our understanding of seagrass species-specific canopy structures and their role in controlling storing blue carbon. An innovative method of the restoration of seagrass blue carbon sink capacity has also been described.

#### **5.1.1 Loss of sediment $C_{org}$ stock following seagrass degradation**

This thesis highlights the importance of tropical seagrass meadows as one of the globe's blue carbon sinks with high capacity to trap allochthonous carbon (Chapters 2 and 3). Tropical seagrass meadows have accumulated more than 90% of allochthonous carbon (mangrove and suspended particulated matter) (Chapters 2 and 3). Measuring community metabolism also confirmed that seagrass meadows tend to be autotrophic communities ( $P > R$ ) and act as autochthonous blue carbon sinks because carbon captured by seagrass was greater than carbon released from respiratory metabolism (Chapter 3). As few research studies have been conducted in tropical seagrass meadows, especially in South East Asia, the result of this thesis could result in a contribution to understanding the role that seagrass meadows play in South East Asia. We found that seagrass promotes up to 90% of allochthonous contribution to the meadow's sediment, as well as maintaining high primary production and low metabolic rate (Chapters 2 and 3).

Compare with the tropical forest, the sediment  $C_{org}$  stocks measured at the tropical pristine seagrass meadows, Haad Chao Mai Natiol Park, Trang, Thailand was 12.9% lower than soil carbon measured at the primary forest in Bukit Timah Nature Reserve, Singapore (Ngo et al., 2013). In comparison with marsh and mangrove, the sediment  $C_{org}$  stocks at pristine seagrass meadows was around 21.6% and 80.81% lower than the sediment  $C_{org}$  stocks measured at marsh and mangrove within the Karstic Landscape of the Mexican Caribbean (Adame et al., 2013).

The occurrence of seagrass loss is widespread, especially in South East Asia. The anthropogenic activities (i.e. the poor land practice) following rapid urban growth increased sediment loads and water turbidity into coastal areas (Chapter 2). Over the last 10 years, seagrass has gradually disappeared from the meadow at the Khao Bae Na, Haad Chao Mai National Park, Thailand (Chapter 2). Negative impacts of seagrass loss on sediment  $C_{org}$  were described in this study (Chapter 2). Seagrass loss resulting from increased sediment loads and water turbidity could lead to the loss of 89% of carbon from the original sediment (Chapter 2).

The top 10 cm of sediment is the most vulnerable pool of  $C_{org}$  to become lost during seagrass meadow degradation (Chapter 2). Loss of sediment  $C_{org}$  stock would result from the re-suspension and export of fine, organic rich sediment, microbial remineralisation and oxidation of the sediment organic matter (Macreadie et al., 2014). Furthermore, in a denuded site, the lack of trapping capacity could lead to a reduction in more than 90% of  $C_{org}$  stored in the sediment.

The loss of allochthonous carbon resulting from the absence of seagrass trapping capacity seems to be the major cause of loss of sediment  $C_{org}$  stock in this particular study (Chapter 2). Changing water velocity and lack of trapping capacity due to the

absence of seagrass canopy also leads to a reduction in the percentage of finer sand fraction settlement (Chapter 2 and Chapter 3). It was apparent this had occurred in sediment at the denuded site, which contained more of the smaller grain size, than unvegetated sediment, and larger grain size than in a pristine meadow. A positive relationship was found between sediment grain size and sediment  $C_{org}$  content as the finer grain size ( $<0.063$  mm) contains the highest organic matter content. (Kennedy et al., 2010). Conversely, there is an advantage to unvegetated sediment's larger sediment grains as they can provide a suitable habitat for benthic microalgae (Cahoon et al., 1999). An increase of large sediment grain size settlement in a denuded site might lead to benthic microalgae becoming the primary producer. The low respiration rate at a denuded site (5 times lower than at a pristine meadow) indicates that 10 years of seagrass loss is long enough to lead to decomposition and remineralisation of most of the carbon from the top 15 cm of sediment.

Sediment poor in  $C_{org}$  was not only found in denuded sites – it was also found in seagrass meadows with low abundance (12% cover with around 93 shoots per  $m^2$ ) (Chapter 3). The same sediment  $C_{org}$  stock found over 5 cm depth at low seagrass abundance tended to be similar to the level of sediment  $C_{org}$  stock of in unvegetated sediment. The results reported in Chapter 3 suggest that seagrass abundance is an important factor in determining the variability of sediment  $C_{org}$  stock. So when calculating the global blue carbon estimate, factors such as lack of spatial variation in seagrass abundance should also be carefully considered to avoid an over- or under-estimation of carbon stock which could result in gross errors.

### **5.1.2 Spatial variability of sediment $C_{org}$ stock in seagrass ecosystems**



A direct influence of seagrass above-ground biomass on NCP was found (Chapter 3). There was only 6% to 9% of NCP buried in seagrass meadows; therefore, seagrass NCP was not a significant component of the sediment  $C_{org}$  pool in this particular seagrass meadow (Chapter 2). The major source contributor to sediment  $C_{org}$  stock was allochthonous carbon (Chapters 2 and 3). Thus, trapping ability of seagrass canopies facilitated the blue carbon sink, rather than NCP at this particular site. The role of the seagrass canopy to enhance sedimentation and reduce resuspension of particles is well known in both direct (epiphytic ingestion and canopy direct binding) and indirect (interaction with hydrodynamic forces) mechanisms (Agawin and Duarte, 2002, Hendriks et al., 2008, Keulen, 1998, Komatsu et al., 2004, Peterson et al., 2004, Sand-jensen, 1998). Seagrass species with larger canopy height (leaf size) were reported to promote a higher rate of sedimentation (Komatsu et al., 2004), and hence they were expected to have larger sediment  $C_{org}$  stocks than seagrass species with smaller canopy heights. In contrast to this general expectation, medium-sized canopy species, such as *T. hemprichii*, maintain a similar blue carbon stock to that of large-sized canopy species, such as *E. acoroides* (Chapter 3). Thus, canopy height (leaf size) is not a species-specific trait relevant to carbon capture found in this study (Chapter 3). In Chapter 3, we found a strong indication that seagrass abundance is an effective indicator of blue carbon stock at local scales. The shoot density of the medium-sized canopy, *T. hemprichii*, seemed to be a major strategy in promoting the input of  $C_{org}$ , and hence the sediment  $C_{org}$  stock in the meadows of *T. hemprichii* was similar to *E. acoroides*, which has a large canopy size species, but lower shoot density.

### **5.1.3 Blue carbon restoration using artificial seagrass**

This thesis is the first to discover that an artificial seagrass could potentially trap as much organic carbon as natural seagrass. The result from Chapter 4 also suggested an alternative way to apply artificial seagrass as a pre-filter to buffer environmental conditions for the successful recovery of natural seagrass. In Chapter 2, the significant role of the allochthonous carbon as a major contributor to sediment  $C_{org}$  stock was demonstrated. Thus, enhancing the allochthonous trapping capacity seems to be the most effective way to improve water quality and subsequently to restore the blue carbon sink capacity of seagrass (Chapters 2 and 4). We developed artificial seagrass by replicating the most important physical characteristics (abundance and canopy height) for elevating blue carbon sink capacity (Chapters 2 and 3). Like the natural seagrass meadows, artificial seagrass performed a significant role by increasing  $C_{org}$  particle deposition into a denuded site (Chapter 4). Artificial seagrass canopies increased the chance of allochthonous carbon input to sediment by up to 90% (Chapters 2 and 4). While common seagrass restoration techniques, such as seedling technique, need more than 10 years to fully recover blue carbon sink capacity (Greiner et al., 2013, Marbà et al., 2015), artificial seagrass could promote the  $C_{org}$  deposition in the early stages of the natural seagrass restoration (Chapter 4).

With ever increasing amounts of atmospheric carbon emissions, the need to employ powerful methods to reduce carbon is now an urgent issue (Pendleton et al., 2012, Sheesley et al., 2010, Solomon et al., 2009). The loss of seagrass areas has also been rapidly increasing worldwide (Waycott et al., 2009). If this continues, the consequence will be the natural rate of carbon sequestration dropping below that of carbon emission. Furthermore, habitat degradation could change the role of blue carbon sinks to become a carbon source (Pendleton et al., 2012). In the event of a severe loss of blue carbon sink

capacity (Chapter 2), this study suggests using artificial seagrass to assist in the fast recovery of seagrass blue carbon sink capacity.

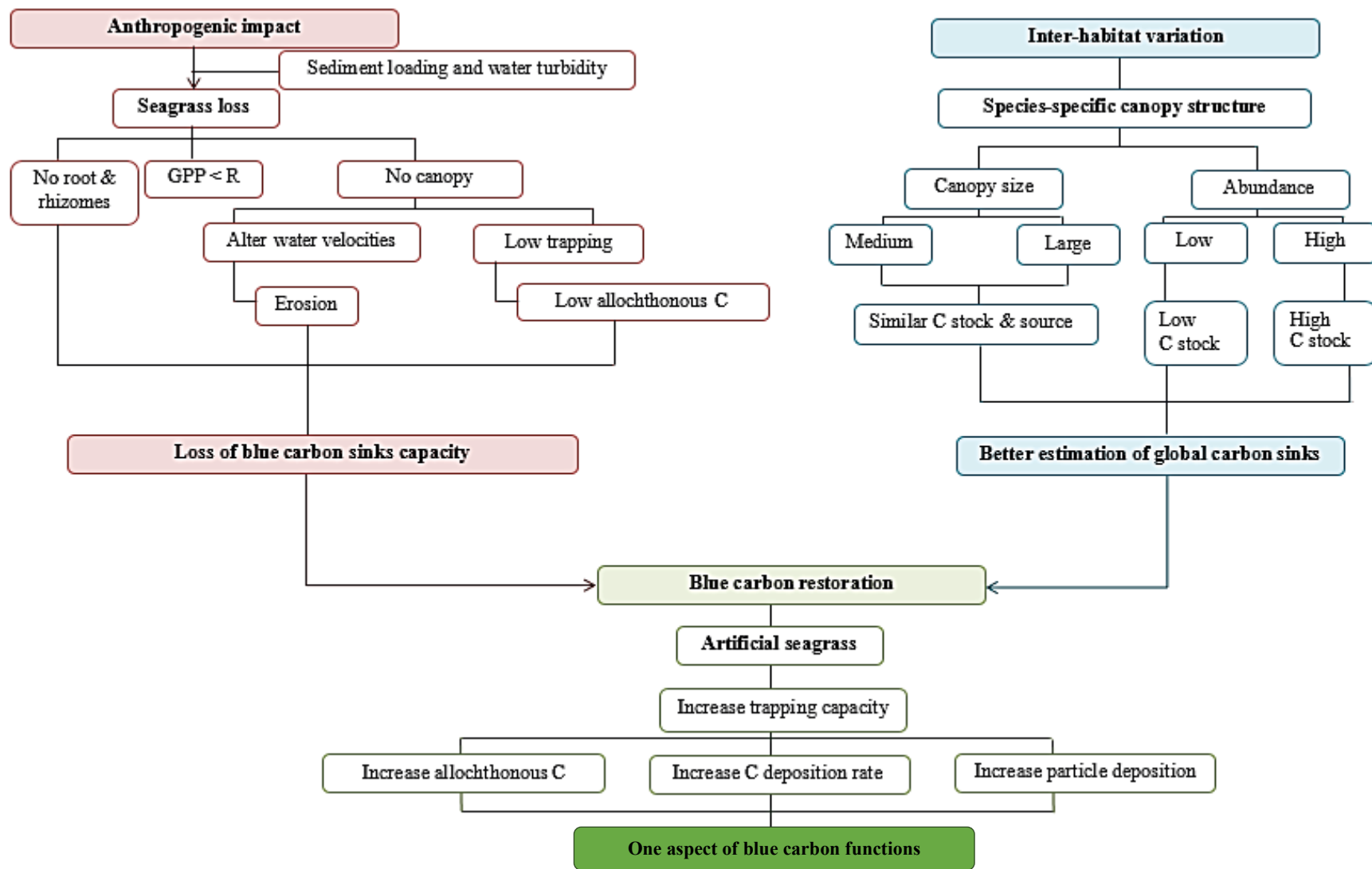
## **5.2 SUMMARY OF RESEARCH FINDINGS: KEY FINDINGS FOR TROPICAL BLUE CARBON SINK CAPACITY**

The overall findings of this research (Chapters 2–4) are shown in Figure 5.1.

Major findings from this study:

- There are negative impacts of tropical seagrass loss on blue carbon sink capacity. Seagrass loss results in a weakened blue carbon sink.
- Over the last 10 years of seagrass loss, the sediment  $C_{org}$  stock measured at a denuded site was around 89% lower than at a pristine meadow and in unvegetated sediment.
- There are similar levels of mangrove/terrestrial organic matter deposition between a denuded site and pristine meadow, indicating the cause of sediment  $C_{org}$  loss at a denuded site is the loss of  $C_{org}$  from the original seagrass sediment.
- Seagrass loss leads to an increase in coarse-grained sediment settlement.
- Seagrass loss reduces the potential to sequester carbon.
- Both medium sized and large sized seagrasses are important carbon sinks that enhance carbon accumulation in the sediment in comparison to bare sediment.

- Seagrass abundance influences blue carbon stock. Higher sediment C<sub>org</sub> stock was found with high seagrass abundance, compared with those of low seagrass abundance.
- Low seagrass shoot density showed the same level of sediment C<sub>org</sub> stock as bare sediment.
- A scarcity of seagrass impacts negatively on the community's metabolism.
- Seagrass meadows of *Enhalus acoroides* and *Thalassia hemprichii* are tropical blue carbon hotspots as they act carbon sinks with high production and low metabolic respiration.
- More than 90% of sediment C<sub>org</sub> stock measured in pristine seagrass meadows is derived from allochthonous carbon sources (mangrove and suspended particulate organic matter).
- The trapping capacity of seagrass species is a major factor in determining seagrass blue carbon sink capacity.



**Figure 5.1** The summary of key findings for tropical blue carbon reserves.

### 5.3 IMPLICATIONS FOR FUTURE RESEARCH

Most coastal habitats in Southeast Asia have been converted to aquaculture and/or urban settlements (Kuramoto and Minagawa, 2001, Supanwanid and Lewmanomont, 2003). Unfortunately, the monitoring of tropical seagrass distribution, especially seagrass in Southeast Asia, is very poor (Short, Coles & Pergent-Martini 2001). Only one seagrass bed, Kao Bae Na in the Andaman Sea, Thailand, has been classified as a degraded site. While data is limited in relation to the degree of habitat replication, clear evidence of the negative impact of seagrass degradation on blue carbon sink capacity has been shown in this study. The severe loss of sediment  $C_{org}$  stock reported in this study should encourage the conservation and restoration of Southeast Asian seagrass meadows.

This study confirms that the degree of carbon loss varies due to the type of degradation; for example, the loading of sediment and increasing of water turbidity from the land could potentially lead to an 89% loss of sediment  $C_{org}$  stock from pre-existing seagrass meadows (Chapter 2). While a number of different types of habitat degradation have been reported worldwide (Waycott et al. 2009), the degree of carbon that would be vulnerable to loss from each type of habitat should be studied. In addition, the blue carbon sink capacity of low-abundance seagrass meadows tends to be as vulnerable as denuded sites (Chapters 2 and 3). Thus, not only does habitat loss negatively impact the blue carbon sink capacity, but low-abundance seagrass meadows could also be a source of carbon rather than a sink. While a degraded area leaking sediment  $C_{org}$  stock was found, the fate of the carbon released following the seagrass loss, which may include re-deposition, exportation, and/or atmospheric emission, is still unresolved.

Further research is required to investigate whether the loss of seagrass could change the benthic community structure. While the physical conditions (i.e. sediment grain size

distribution) of a denuded site was predicted to change to become similar to the conditions of an unvegetated sediment site, the long term monitoring of changing biological conditions, which could enhance or protect the underlying sediment  $C_{org}$  stock, such as monitoring whether a denuded site could be replaced by the benthic microalgae, is suggested. Finally, some additional parameters, such as sedimentation rate and dynamics would be useful evidence to understand changing in blue carbon sink capacity.

The findings of this study provide new knowledge that can be directly applied to improve degraded blue carbon areas. Artificial seagrass has emerged as a very useful tool in blue carbon sink capacity restoration. It would be an effective tool to adjust physical conditions, such as slowing down water currents and trapping suspended particles from the water column. Consequently, the surrounding water would be cleared and light penetration into the water column would increase. Change in environmental conditions (such as light intensity, water velocity, and water turbidity) should be monitored, and pioneer seagrass such as *Halophila ovalis* and *Cymodocea rotundata* should be established, within the artificial seagrass area. Chapter 3 described that seagrass abundance (percent cover and density) is a major factor promoting settlement of allochthonous carbon. Thus, using high density artificial seagrass is recommended as the most effective trapping method. However, further research should be conducted to confirm the role of artificial seagrass in the preservation of carbon in the sediments. For large scale conservation, setting up artificial seagrass as a pre-filter in areas of high water turbidity is an idea for creating improved environmental conditions for the generation of natural seagrass.

While the seagrass above-ground biomass has not been considered part of sediment  $C_{org}$  stock, as it is only a minor component of the carbon storage facility within the meadows, as well as its carbon storage capacity only being short-term, its canopy plays a significant role in trapping a large amount of allochthonous carbon (Chapters 2–4). Thus, the importance of allochthonous carbon should be included when calculating the amount of carbon stored, as well as the export of carbon from seagrass meadows to other ecosystems. The clear evidence that seagrass abundance influence the blue carbon sink capacity suggests that better estimates of seagrass areas such as seagrass percent cover, and biomass, is required to avoid underestimating or overestimating the carbon stock. While seagrass abundance is recognised as an indicator of blue carbon sink capacity, the variations in seagrass abundance (seasonal, temporal and/or spatial variation) should be considered as factors that would impact carbon flux (accumulation, stabilisation, or decline). The sediment  $C_{org}$  stocks measured in this study were two times lower than previously published estimates for seagrass from 946 distinct seagrass meadows (Fourqurean et al., 2012). However, it was found that the sediment  $C_{org}$  stock tends to rise with increasing sediment depth. As the organic-rich sediment might be within the deeper sediment (> 15 cm of sediment depth), a deeper core for blue carbon research is recommended.

A number of future research directions were highlighted during this research study, especially the severe loss of sediment carbon storage, and effective ways to restore this lost carbon. This study has also significantly progressed the understanding of how underlying seagrass canopy structures drive the variability of seagrass blue carbon sink capacity, consequently raising the need for further robust estimates of global carbon storage. The work contained in this thesis has: developed an understanding of the impact of seagrass loss on blue carbon sinks, identified a significant feature of seagrass



canopy which influences the blue carbon sink, and provided an alternative method to recover the degraded carbon pool. According to the aims of this study, it can be concluded that: seagrass loss could lead to a loss of carbon storage in sediment (mostly allochthonous carbon) and then lead to a weakening of carbon sequestration capacity, seagrass abundance has strongly influenced blue carbon sink capacity and is more important in promoting sediment  $C_{org}$  pool than seagrass canopy height (leaf size), and artificial seagrass is a short term method of restoring sediment  $C_{org}$  stock by enhancing allochthonous carbon input.

## **BIBLIOGRAPHY**

- ADAME, M. F., KAUFFMAN, J. B., MEDINA, I., GAMBOA, J. N., TORRES, O., CAAMAL, J. P., REZA, M. & HERRERA-SILVEIRA, J. A. 2013. Carbon stocks of tropical coastal wetlands within the Karstic landscape of the Mexican caribbean. *PlosOne*.
- AGAWIN, N. S. R. & DUARTE, C. M. 2002. Evidence of Direct Particle Trapping by a Tropical Seagrass Meadow. *Estuaries*, 25, 1205-1209.
- ALEXANDER, D. T. L., CROZIER, P. A. & ANDERSON, J. R. 2008. Brown carbon spheres in East Asian outflow and their optical properties. *Science*, 321, 833-836.
- ALTHER, G. R. 1981. Transport of degraded sediment after disposal operation in Lake Erie *Ohio Academic Sciences*, 81, 2-8
- ANDREAE, M. O. 1995. Climatic effects of changing atmospheric aerosol levels. *World Survey of Climatology*, 16, 347-398.
- ANDREAE, M. O. & MERLET, P. 2001. Emission of trace gases and aerosols from biomass burning. *Global Biogeochemical Cycles*, 15, 955-966.
- APICHANANGKOOL, P. 2012. Seagrass meadow on Green Island reef flat.
- APICHANANGKOOL, P. & PRATHEP, A. 2014. Changes in seagrass leaf reddening and morphology in response to emersion. *Botanica Marina*, 57, 433-440.
- ARAGONES, L. & MARSH, H. 2000. Impact of dugong grazing and turtle cropping on tropical seagrass communities. *Pacific Conservation Biology* 5, 277-288.

- AROLA, A., SCHUSTER, G., MYHRE, G., KAZADZIS, S., DEY, S. & TRIPATHI, S. N. 2011. Inferring absorbing organic carbon content from AERONET data. *Atmospheric chemistry and physics*, 11, 215-225.
- BADALAMENTI, F., CARLO, G. D., D'ANNA, G., GRISTINA, M. & TOCCACELI, M. 2006. Effects of dredging activities on population dynamics of *Posidonia oceanica* (L.) Delile in the Mediterranean sea: the case study of Capo Feto (SW Sicily, Italy). *Hydrobiologia*, 555, 253-261.
- BARNETT, T. P., ADAM, J. C. & LETTENMAIER, D. P. 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature*, 438, 303-309.
- BARRÓN, C., MARBÀ, N., TERRADOS, J., KENNEDY, H. & DUARTE, C. M. 2004. Community metabolism and carbon budget along a gradient of seagrass (*Cymodocea nodosa*) colonization. *Limnology and Oceanography*, 49, 1642-1651.
- BEAUCHEMIN, K. A., JANZEN, H. H., LITTLE, S. M., MCALLISTER, T. A. & MCGINN, S. M. 2010. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. *Agricultural Systems*, 103, 371-379
- BEER, S., BJÖRK, M., HELLBLÖM, F. & AXELSSON, L. 2002. Inorganic carbon utilization in marine angiosperms (seagrasses). *Functional Plant Biology*, 29, 349 - 354.
- BELL, J. D. 1985. Artificial seagrass: How useful is it for field experiments on fish and macroinvertebrates? *Journal of Experimental Marine Biology and Ecology*, 90, 171-177.

- BELL, J. D., STEFFE, A. S. & WESTOBY, M. 1985. Artificial seagrass: How useful is it for field experiments on fish and macroinvertebrates? *Journal of Experimental Marine Biology and Ecology*, 90, 171-177.
- BENEDICT, C. R., WONG, W. W. L. & WONG, J. H. H. 1980. Fractionation of the Stable Isotopes of Inorganic Carbon by Seagrasses. *Plant Physiology*, 65, 512-517.
- BITTAKER, H. F. & IVERSON, R. L. 1976. *Thalassia testudinum* productivity: A field comparison of measurement methods. *Marine Biology (Berlin)*, 77, 19-26.
- BOER, W. F. D. 2007. Seagrass–sediment interactions, positive feedbacks and critical thresholds for occurrence: a review. *Hydrobiologia*, 591, 5-24.
- BOLOGNA, P. A. X. & HECK, K. L. 2000. Impacts of seagrass habitat architecture on bivalve settlement. *Estuaries*, 23, 449-457.
- BONASONI, P., CRISTOFANELLI, P., MARINONI, A., VUILLERMOZ, E. & ADHIKARY, B. 2012. Atmospheric pollution in the Hindu Kush–Himalaya region. *Mountain research and development* 32, 468-479.
- BOND, T. C. 2001. Spectral dependence of visible light absorption by carbonaceous particles emitted from coal combustion. *Geophysical research letters*, 28, 4075-4078.
- BOROWITZKA, M. A., LETHBRIDGE, R. C. & CHARLTON, L. 1990. Species richness, spatial distribution and colonisation pattern of algal and invertebrate epiphytes on the seagrass *Amphibolis griffithii* *Marine Ecology Progress Series*, 64, 281-291.
- BOSCHKER, H. T. S., BROUWER, J. F. C. D. & CAPPENBERG, T. E. 1999. The contribution of macrophyte-derived organic matter to microbial biomass in salt-

marsh sediments: Stable carbon isotope analysis of microbial biomarkers.

*Limnology and Oceanography*, 44, 309-319.

BOSCHKER, H. T. S., WIELEMAKER, A., SCHAUB, B. E. M. & HOLMER, M.

2000. Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera* spp. meadows. *Marine Ecology Progress Series*, 203, 181-189.

BRIDGHAM, S. D., MEGONIGA, J. P., KELLER, J. K., BLISS, N. B. & TRETIN, C.

2006. The carbon balance of North American wetlands. *Wetlands*, 26, 889-916.

BRYARS, S. & NEVERAUSKAS, V. 2004. Natural recolonization of seagrasses at a

disused sewage sludge outfall. *Aquatic Botany*, 80, 283-289.

CABAÇO, S., SANTOS, R. & DUARTE, C. M. 2008. The impact of sediment burial

and erosion on seagrasses: A review. *Estuarine, Coastal and Shelf Science*, 79, 354-366.

CAHOON, L. B., NEARHOOF, J. E. & TILTON, C. L. 1999. Sediment grain size

effect on benthic microalgal biomass in shallow aquatic ecosystems. *Estuaries*, 22, 735-741.

CALUMPONG, H. P. & FONSECA, M. S. 2001. Seagrass transplantation and the other

seagrass restoration methods. In: SHORT, F. T. & COLES, R. G. (eds.) *Global seagrass research methods*. Elsevier.

CAMPBELL, M. L. & PALING, E. I. 2003. Evaluating vegetative transplant success in

*Posidonia australis*: a field trial with habitat enhancement. *Marine Pollution Bulletin*, 46, 828-834.

CANADELL, J. G. & RAUPACH, M. R. 2008. Managing forests for climate change

mitigation. *Science*, 320, 1456-1457

- CASTEJON-SILVO, I. & TERRADOS, J. 2012. Patterns of spatial variation of nutrient content, epiphyte load and shoot size of *Posidonia oceanica* seagrass meadows (Mediterranean Sea). *Marine Ecology*, 33, 165-175
- CHARLSON, R. J., SCHWARTZ, S. E., HALES, J. M., CESS, R. D., JR., J. A. C., HANSEN, J. E. & HOFMANN, D. J. 1992. Climate forcing by anthropogenic aerosols. *Science*, 255, 423-430.
- CHMURA, G. L., ANISFELD, S. C., CAHOON, D. R. & LYNCH, J. C. 2003. Global carbon sequestration in tidal, saline wetland soils. *Global Biogeochemical Cycles*, 17.
- CHUNG, S. H. & SEINFELD, J. H. 2005. Climate response of direct radiative forcing of anthropogenic black carbon. *Journal of Geophysical Research: Atmospheres*, 110.
- CIFUENTES, L. A., COFFIN, R. B., SOLORZANO, L., CARDENAS, W., ESPINOZA, J. & TWILLEY, R. R. 1996. Isotopic and elemental variations of carbon and nitrogen in a mangrove estuary. *Estuarine, Coastal and Shelf Science*, 43, 781-800.
- CUY, R. D., FOGEL, M. L. & BERRY, J. A. 1993. Photosynthetic fractionation of the stable isotopes of oxygen and carbon. *Plant Physiology*, 101, 37-47.
- DAWES, C. J. 1998. *Marine Botany*, New York, John Wiley & Sons, Inc.
- DELGADO, O., RUIZ, J., PEREZ, M., ROMERO, J. & BALLESTEROS, E. 1999. Effects of fish farming on seagrass (*Posidonia oceanica*) in a Mediterranean bay: seagrass decline after organic loading cessation *Oceanologica Acta* 22.
- DENNISON, W. C. 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Botany*, 27, 15-26.

- DITTMAR, T., LARA, R. J. & KATTNER, G. 2001. River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. *Marine Chemistry*, 73, 253-271.
- DLUGOKENCKY, E. & TANS, P. 2015. *Recent Global CO<sub>2</sub>* [Online]. NOAA/ESRL. Available: [www.esrl.noaa.gov/gmd/ccgg/trends/](http://www.esrl.noaa.gov/gmd/ccgg/trends/) [Accessed 23/4/2015 2015].
- DUARTE, C. M. 1990. Seagrass nutrient content *Marine Ecology Progress Series*, 67, 201-207.
- DUARTE, C. M. 1991. Seagrass depth limits. *Aquatic Botany*, 40, 363-377.
- DUARTE, C. M. 2002. The future of seagrass meadows. *Environmental Conservation*, 29, 192-206.
- DUARTE, C. M. & CHISCANO, C. L. 1999. Seagrass biomass and production: A reassessment. *Aquatic Botany*, 65, 159-174.
- DUARTE, C. M., KENNEDY, H., MARBÀ, N. & HENDRIKS, I. 2013a. Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean & Coastal Management*, 83, 32-38.
- DUARTE, C. M., LOSADA, I. J., HENDRIKS, I. E., MAZARRASA, I. & MARBÀ, N. 2013b. The role of coastal plant communities for climate change mitigation and adaptation. *Nature Climate Change*, 3, 961-968.
- DUARTE, C. M., MERINO, M., AGAWIN, N. S. R., URI, J., FORTES, M. D., GALLEGOS, M. E., MARBÀ, N. & HEMMINGA, M. A. 1998. Root production and below-ground seagrass biomass. *Marine Ecology Progress Series*, 717, 97-108.
- DUARTE, C. M., NÚRIA, M., GACIA, E., FOURQUREAN, J. W., BEGGINS, J., BARRON, C. & APOSTOLAKI, E. T. 2010. Seagrass community metabolism:

- assessing the carbon sink capacity of seagrass meadows. *Global Biogeochem Cycles*, 24, 1-8.
- DUARTE, C. M., SINTES, T. & MARBÀ, N. 2013c. Assessing the CO<sub>2</sub> capture potential of seagrass restoration projects. *Journal of Applied Ecology*, 50, 1341-1349.
- DUXBURY, J. M. 1994. The significance of agricultural sources of greenhouse gases. *Fertilizer research*, 38, 151-163
- ECKMAN, J. E. 1987. The role of hydrodynamics in recruitment, growth and survival of *Argopecten irradians* (L.) and *Anomia simplex* (D'Orbligny) within eelgrass meadow. *Journal of Experimental Marine Biology and Ecology*, 106, 165-191.
- EDGAR, G. J. 1990. The influence of plant structure on the species richness, biomass and secondary production of macrofaunal assemblages associated with Western Australian seagrass beds. *Journal of Experimental Marine Biology and Ecology*, 137, 215-240.
- EDGAR, G. J. & ROBERTSON, A. I. 1992. The influence of seagrass structure on the distribution and abundance of mobile epifauna: pattern and process in a Western Australian Amphibolis bed. *Journal of Experimental Marine Biology and Ecology*, 160, 13-31.
- EKDALE, A. A. & BERGER, W. H. 1978. Deep-sea ichnofacies: modern organism traces on and in pelagic carbonates of the western equatorial pacific. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 23, 263-278.
- FARQUHAR, G. D., EHLERINGER, J. R. & HUBICK, K. T. 1989. Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40, 503-537.



- FENG, Y., RAMANATHAN, V. & KOTAMARTHI, V. R. 2013. Brown carbon: a significant atmospheric absorber of solar radiation? *Atmospheric chemistry and physics*, 13, 8607–8621.
- FLANNER, M. G., ZENDER, C. S., RANDERSON, J. T. & RASCH, P. J. 2007. Present-day climate forcing and response from black carbon in snow. *Journal of geophysical research: Atmospheres* 112.
- FONSECA, M. S. 1989. Sediment stabilization by *Halophila decipiens* in comparison to other seagrasses. *Estuarine, Coastal and Shelf Science*, 29, 501-507.
- FONSECA, M. S. & CAHALAN, J. A. 1992. A preliminary evaluation of wave attenuation by four species of seagrass *Estuarine Coastal and Shelf Science*, 35, 565-576.
- FONSECA, M. S., KENWORTHY, W. J. & THAYER, G. W. 1998. Guidelines for the conservation and restoration of seagrass in Unites States and adjacent waters. *In*: 12, D. A. S. N. (ed.) *NOAA'S coastal ocean program* NOAA Coastal Ocean Office.
- FOURQUREAN, J. W., DUARTE, C. M., KENNEDY, H., MARBÁ, N., HOLMER, M., MATEO, M. A., APOSTOLAKI, E. T., KENDRICK, G. A., KRAUSE-JENSEN, D., MCGLATHERY, K. J. & SERRANO, O. 2012. Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, 5, 505-509.
- FOURQUREAN, J. W. & RUTTEN, L. M. 2004. The impact of hurricane Georges on soft bottom, back reef communities: site- and species-specific effectes on south Florida seagrass beds. *Bulletin of Marine Science*, 75, 239-257.
- FOURQUREAN, J. W. & SCHRLAU, J. E. 2003. Changes in nutrient content and stable isotope ratios of C and N during decomposition of seagrasses and

- mangrove leaves along a nutrient availability gradient in Florida Bay, USA. *Chemistry and Ecology*, 19, 373-390.
- GACIA, E. & DUARTE, C. M. 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow: The balance between deposition and resuspension. *Estuarine, Coastal and Shelf Science*, 52, 505-514.
- GACIA, E., DUARTE, C. M., MARBÀ, N., TERRADOS, J., KENNEDY, H., FORTES, M. D. & TRI, N. H. 2003. Sediment deposition and production in SE-Asia seagrass meadows. *Estuarine Coastal and Shelf Science*, 56, 909-919.
- GACIA, E., DUARTE, C. M. & MIDDELBURG, J. J. 2002. Carbon and nutrient deposition in the Mediterranean seagrass (*Posidonia oceanica*). *Limnology Oceanography*, 47.
- GACIA, E., GRANATA, T. C. & DUARTE, C. M. 1999. An approach to measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. *Aquatic Botany*, 65, 255-268.
- GAMBI, M. C., NOWELL, A. R. M. & JUMARS, P. A. 1990. Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. *Marine Ecology Progress Series*, 6, 159-169.
- GANNES, L. Z., RIO, C. M. N. D. & KOCH, P. 1998. Natural Abundance Variations in Stable Isotopes and their Potential Uses in Animal Physiological Ecology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 119, 725-737.
- GONI, M. A., TEIXEIRA, M. J. & PERKEY, D. W. 2003. Sources and distribution of organic matter in a river-dominated estuary (Winyah Bay, SC, USA). *Estuarine Coastal and Shelf Science*, 57, 1023-1048.

- GONNEEA, M. E., PAYTAN, A. & HERRERA-SILVEIRA, J. A. 2004. Tracing organic matter sources and carbon burial in mangrove sediments over the past 160 years. *Estuarine Coastal and Shelf Science*, 61, 211-227.
- GORDON, D. R. 1996. Experimental translocation of the endangered shrub, *Apalachicola rosemary* *Conradina glabra*. *Biological Conservation*, 77, 19-26.
- GOYET, C., COATANOAN, C., EISCHEID, G., AMAOKA, T., OKUDA, K., HEALY, R. & TSUNOGAI, S. 1999. Spatial variation of total CO<sub>2</sub> and total alkalinity in the northern Indian Ocean: A novel approach for the quantification of anthropogenic CO<sub>2</sub> in seawater. *Journal of Marine Research*, 57, 135-163.
- GRACE, J., LLOYD, J., MCINTYRE, J., MIRANDA, A. C., MEIR, P., MIRANDA, H. S., NOBRE, C., MONCRIEFF, J., MASSHEDER, J., MALHI, Y., WRIGHT, I. & GASH, J. 1995. Carbon dioxide uptake by an undisturbed tropical rain forest in southwest Amazonia, 1992 to 1993. *Science*, 270, 778-780.
- GREINER, J. T., MCGLATHERY, K. J., GUNNELL, J. & MCKEE, B. A. 2013. Seagrass Restoration Enhances “Blue Carbon” Sequestration in Coastal Waters. *PLoS One*, 8, e72469.
- GUAN, L. 2009. Preparation of future weather data to study the impact of climate change on buildings. *Building and Environment*, 44, 793-800.
- GUIDETTI, P. 2000. Differences among fish assemblages associated with nearshore *Posidonia oceanica* seagrass beds, rocky–algal reefs and unvegetated sand habitats in the Adriatic sea. *Estuarine, Coastal and Shelf Science*, 50, 515-529.
- HANSEN, J., SATO, M., RUEDY, R., LACIS, A. & OINAS, V. 2000. Global warming in the twenty-first century: An alternative scenario. *PNAS*, 97, 9875-9880.

- HARRISON, P. G. 1982. Spatial and temporal patterns in abundance of two intertidal seagrasses, *Zostera americana* Den Hartog and *Zostera marina* L. *Aquatic Botany*, 12, 305-320.
- HARTOG, C. D. & KUO, J. 2006. Taxonomy and biogeography of seagrasses. In: LARKUM, A. W. D., ORTH, R. J. & DUARTE, C. M. (eds.) *Seagrasses: biology, ecology and conservation* Springer Netherlands.
- HASTINGS, K., HESP, P. & KENDRICK, G. A. 1995. Seagrass loss associated with boat moorings at Rottneest Island, Western Australia. *Ocean & coastal management*, 26, 226-246.
- HECK, K. L. & CROWDER, L. B. 1991. Habitat structure and predator-prey interactions in vegetated aquatic systems. In: BELL, S. S., MCCOY, E. D. & MUSHINSKY, H. (eds.) *Habitat structure: the physical arrangement of objects in space*. New York: Chapman and Hall.
- HEMMINGA, M. A. & MATEO, M. A. 1996. Stable carbon isotopes in seagrasses: Variability in ratios and use in ecological studies. *Marine Ecology Progress Series*, 140, 285-289.
- HEMMINGA, M. A., SLIM, F. J., KAZUNGU, J., GANSSSEN, G. M., NIEUWENHUIZEL, J. & KRUYT, N. M. 1994. Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). *Marine Ecology Progress Series*, 106, 291-301.
- HENDRIKS, I. E., SINTES, T., BOUMA, T. J. & DUARTE, C. M. 2008. Experimental assessment and modeling evaluation of the effects of the seagrass *Posidonia oceanica* on flow and particle trapping. *Marine Ecology Progress Series*, 356, 163-173.

- HENRICHS, S. M. & REEBURGH, W. S. 1987. Anaerobic mineralization of marine sediment organic matter: Rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiology Journal*, 5, 191-237.
- HJULSTRÖM, F. 1955. Transportation of detritus by moving water. *In*: TRASK, P. O. (ed.) *Recent marine sediments*. American Association of Petroleum Geology.
- HOEFS, J. 1980. *Stable Isotope Geochemistry*, Berlin, Springer-Verlag.
- HOLMER, M., DUARTE, C. M., BOSCHKER, H. T. S. & BARRÓN, C. 2004. Carbon cycling and bacterial carbon sources in pristine and impacted Mediterranean seagrass sediments. *Aquatic Microbial Ecology*, 36, 227-237.
- IGLESIAS-RODRIGUEZ, M. D., HALLORAN, P. R., RICKABY, R. E. M., HALL, I. R., COLMENERO-HIDALGO, E., GITTINS, J. R., GREEN, D. R. H., TYRRELL, T., GIBBS, S. J., DASSOW, P. V., REHM, E., ARMBRUST, E. V. & BOESSENKOOL, K. P. 2008. Phytoplankton Calcification in a High-CO<sub>2</sub> World. *Science*, 320, 336-340.
- IPCC 2001. *Climate change 2001: The scientific basis. contribution of working group I to the third assess-ment report of the intergovernmental panel on climate change*, New York, Cambridge University Press.
- IPCC 2007. *Climate change 2007: The physical science basis*, Cambridge, U. K, Cambridge University Press.
- ISA, Z., GRUSENMEYER, S. & VERSTRAETE, W. 1986. Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technical aspects. *Applied and Environmental Microbiology*, 51, 572-579.
- ISAKSEN, M. F. & FINSTER, K. 1996. Sulphate reduction in the root zone of the seagrass *Zostera noltii* on the intertidal flats of a coastal lagoon (Arcachon, France) *Marine Ecology Progress Series*, 137, 187-194.

- KATWIJK, M. M. V., BOS, A. R., JONGE, V. N. D., HANSEN, L. S. A. M.,  
HERMUS, D. C. R. & JONG, D. J. D. 2009. Guidelines for seagrass restoration:  
Importance of habitat selection and donor population, spreading of risks, and  
ecosystem engineering effects. *Marine Pollution Bulletin*, 58, 179-188.
- KENNEDY, H., BEGGINS, J., DUARTE, C. M., FOURQUREAN, J. W., HOLMER,  
M., MARBÀ, N. & MIDDELBURG, J. J. 2010. Seagrass sediments as a global  
carbon sink: Isotopic constraints. *Global Biogeochem Cycles*, 24, 1-8.
- KENNEDY, H., GACIA, E., KENNEDY, D. P., PAPADIMITRIOU, S. & DUARTE,  
C. M. 2004. Organic carbon sources to SE Asia coastal sediments. *Estuarine  
and Coastal Shelf Science*, 60, 59-68.
- KENWORTHY, W. J. & THAYER, G. W. 1984. Production and decomposition of the  
roots and rhizomes of seagrasses, *Zostera marina* and *Thalassia testudinum*, in  
temperate and subtropical marine ecosystems. *Bulletin of Marine Science*, 3,  
364-379.
- KEULEN, M. V. 1998. *Water flow in seagrass ecosystem*. Doctor of Philosophy,  
Murdoch university.
- KEULEN, M. V. & BOROWITZKA, M. A. 2002. Comparison of water velocity  
profiles through morphologically dissimilar seagrasses measured with a simple  
and inexpensive current meter. *Bulletin of Marine Science* 71, 1257-1267.
- KIRKMAN, H. & KUO, J. 1990. Pattern and process in southern Western Australia  
seagrasses. *Aquatic Botany*, 37, 367-382.
- KOCH, D. & GENIO, A. D. D. 2010. Black carbon semi-direct effects on cloud cover:  
review and synthesis. *Atmospheric Chemistry and Physics*, 10, 7685–7696.
- KOMATSU, T., UNEZAWA, Y., NAKAOKA, M., SUPANWANID, C. &  
KANAMOTO, Z. 2004. Water flow and sediment in *Enhalus acoroides* and other

- seagrass beds in the Andaman Sea, off Khao Bae Na, Thailand. *Coastal Marine Science*, 29, 63-68.
- KUO, J. & HARTOG, C. D. 2001. Seagrass taxonomy and identification key. *In*:  
SHORT, F. T. & COLES, R. G. (eds.) *Global Seagrass Research Methods*.  
Elsevier Science B.V.
- KUO, J. & HARTOG, C. D. 2006. Seagrass morphology, anatomy, and ultrastructure.  
*In*: LARKUM, A. W. D., ORTH, R. J. & DUARTE, C. M. (eds.) *Seagrass:  
biology, ecology and conservation*. Springer Netherlands.
- KURAMOTO, T. & MINAGAWA, M. 2001. Stable Carbon and Nitrogen Isotopic  
Characterization of Organic Matter in a Mangrove Ecosystem on the  
Southwestern Coast of Thailand. *Journal of Oceanography*, 57, 421-431.
- LACK, D. A., LANGRIDGE, J. M., BAHREINI, R., CAPP, C. D.,  
MIDDLEBROOK, A. M. & SCHWARZ, J. P. 2012. Brown carbon and internal  
mixing in biomass burning particles. *PNAS*, 109, 14802–14807.
- LAL, R. 2004. Soil carbon sequestration to mitigate climate change. *Geoderma*, 123, 1-  
22.
- LANYON, J. M. & MARSH, H. 1995. Temporal changes in the abundance of some  
tropical intertidal seagrasses in North Queensland. . *Aquatic Botany*, 49, 217-  
237.
- LAVERY, P. S., MATEO, M. Á., SERRANO, O. & ROZAIMI, M. 2013. Variability in  
the carbon storage of seagrass habitats and its implications for global estimates  
of Blue Carbon ecosystem service. *PLoS One*, 8, e73748.
- LAWRENCE, K., PONGPIRIYAKIT, T., JIAWKOK, S., MEECHUKAN, A. &  
MADADAM, N. 2007. Seagrass replanting at Trang. Thailand.

- LEE, S. Y., FONG, C. W. & WU, R. S. S. 2001. The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. *Journal of Experimental Marine Biology and Ecology*, 259, 23-50.
- LEMMENS, J. W. T. J., CLAPIN, G., LAVERY, P. & CARY, J. 1996. Filtering capacity of seagrass meadows and other habitats of Cockburn Sound, Western Australia. *Marine Ecology Progress Series*, 143, 187-200.
- LEPOINT, G., DAUBY, P. & GOBERT, S. 2004. Applications of C and N stable isotopes to ecological and environmental studies in seagrass ecosystems. *Marine Pollution Bulletin*, 49, 887-891.
- LONGHURST, A. R. & HARRISON, W. G. 1989. The biological pump: Profiles of plankton production and consumption in the upper ocean. *Progress in Oceanography*, 22, 47-123.
- MACINTYRE, H. L., GEIDER, R. J. & MILLER, D. C. 1996. Microphytobenthos: The ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. *Estuaries*, 19, 186-201.
- MACREADIE, P., HINDELL, J., KEOUGH, M., JENKINS, G. & CONNOLLY, R. 2010. Resource distribution influences positive edge effects in a seagrass fish. *Ecology*, 91, 2013-2021.
- MACREADIE, P. I., BAIRD, M. E., TREVATHAN-TACKETT, S. M., LARKUM, A. W. D. & RALPH, P. J. 2014. Quantifying and modelling the carbon sequestration capacity of seagrass meadows – A critical assessment. *Marine Pollution Bulletin*, 83, 430-439.



- MACCREADIE, P. I., HUGES, A. R. & KIMBRO, D. L. 2013. Loss of 'Blue Carbon' from coastal salt marshes following habitat disturbance. *PLos One*, 8, e69244.
- MARBÀ, N., ARIAS-ORTIZ, A., MASQUÉ, P., KENDRICK, G. A., MAZARRASA, I., BASTYAN, G. R., GARCIA-ORELLANA, J. & DUARTE, C. M. 2015. Impact of seagrass loss and subsequent revegetation on carbon sequestration and stocks. *Journal of Ecology*, 103, 296-302.
- MATEO, M. A., CEBRIÁN, J., DUNTON, K. & MUTCHLER, T. 2006. Carbon flux in seagrass ecosystems. In: WDA, L., RJ, O. & CM, D. (eds.) *Seagrasses: Biology, Ecology and Conservation*. Dordrecht, The Netherlands: Springer.
- MATEO, M. A. & ROMERO, J. 1997. Detritus dynamics in the seagrass *Posidonia oceanica*: elements for an ecosystem carbon and nutrient budget. *Marine Ecology Progress Series*, 151, 43-53.
- MBOUROU, G. N. T., BERTRAND, J. J. & NICHOLSON, S. E. 1997. The diurnal and seasonal cycles of wind-borne dust over Africa north of the equator. *Journal of Climate and Applied Meteorology*, 36, 868-882.
- MCCONNELL, J. R., EDWARDS, R., KOK, G. L., FLANNER, M. G., ZENDER, C. S., SALTZMAN, E. S., BANTA, R., PASTERIS, D. R., CARTER, M. M. & KAHL, J. D. W. 2007. 20th-century industrial black carbon emissions altered arctic climate forcing. *Science*, 317, 1381-1384.
- MCKEE, K. L., CAHOON, D. R. & FELLER, I. C. 2007. Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Global Ecology and Biogeography*, 16, 545-556.
- MCKENZIE, L. 2008. *Seagrass educators handbook*, Seagrass-watch.
- MCLEOD, E., CHMURA, G. L., BJÖRK, M., BOUILLON, S., DUARTE, C. M., LOVELOCK, C., SALM, R., SCHLESINGER, W. & SILLIMAN, B. 2011. *A*

*blueprint for Blue carbon: towards an improved understanding of the role of vegetated coastal habitats in sequestering CO<sub>2</sub>*, *Frontiers in Ecology and the Environment*.

MENON, S., HANSEN, J., NAZARENKO, L. & LUO, Y. 2002. Climate effects of black carbon aerosols in China and India. *Science*, 297.

MOORE, K. A., SHIELDS, E. C., PARRISH, D. B. & ORTH, R. J. 2012. Eelgrass survival in two contrasting systems: role of turbidity and summer water temperatures. *Marine Ecology Progress Series*, 448, 247-258.

MORIARTY, D. J. W., IVERSON, R. L. & POLLARD, P. C. 1986. Exudation of organic carbon by the seagrass *Halodule wrightii* Aschers. and its effect on bacterial growth in the sediment. *Journal of Experimental Marine Biology and Ecology*, 96, 115-126.

MORINIÈRE, E. C. D. L., POLLUX, B. J. A., NAGELKERKEN, I. & VELDE, G. V. D. 2002. Post-settlement life cycle migration patterns and habitat preference of coral reef fish that use seagrass and mangrove habitats as nurseries. *Estuarine, Coastal and Shelf Science*, 55, 309-321.

MORSE, J. W., ARVIDSON, R. S. & LÜTTGE, A. 2007. Calcium carbonate formation and dissolution. *Chemical Reviews*, 107, 342-381.

MUKAI, H., AIOI, K., LEWMANOMONT, K., MATSUMASA, M., NAKAOKA, M., NOJIMA, S., C, S., T., S. & TOYOHARA, T. 1999. Dugong grazing on *Halophila* beds in Haad Chao Mai National Park, Trang Province, Thailand- How many dugongs can survive? In: KOIKE, I. (ed.) *Effects of Grazing and Disturbance by Dugongs and Turtles on Tropical Seagrass Ecosystems*. Japan: Ocean Research Institute, University of Tokyo.

- MUKAI, H. & AMBE, Y. 1986. Characterization of a humic acid-like brown substance in airborne particulate matter and tentative identification of its origin. *Atmospheric environment*, 20, 813-819.
- NAGELKERKEN, I., ROBERTS, C. M., VELDE, G. V. D., DORENBOSCH, M., RIEL, M. C. V., MORINIÈRE, E. C. D. L. & NIENHUIS, P. H. 2002. How important are mangroves and seagrass beds for coral-reef fish? The nursery hypothesis tested on an island scale. *Marine Ecology Progress Series*, 244, 299-305.
- NELLEMANN, C., CORCORAN, E., DUARTE, C. M., VALDES, L., DEYOUNG, C., FONSECA, L. & GRIMSDITCH, G. 2009. *Blue carbon. A rapid response assessment*, Norway, United Nations Environment Programme, GRID-Arendal.
- NGO, K. M., TURNER, B. L., MULLER-LANDAU, H. C., DAVIES, S. J., LARJAVAARA, M., HASSAN, N. F. B. N. & LUM, S. 2013. Carbon stocks in primary and secondary tropical forests in Singapore. *Forest ecology and management*, 296, 81-89.
- O'LEARY, M. H. 1981. Carbon isotope fractionation in plants. *Phytochemistry*, 20, 553-567.
- OCCHIPINTI-AMBROGI, A. & SAVINI, D. 2003. Biological invasions as a component of global change in stressed marine ecosystems. *Marine Pollution Bulletin*, 46, 542-551.
- ORTH, R. J., CARRUTHERS, T. J. B., DENNISON, W. C., DUARTE, C. M., FOURQUREAN, J. W., JR., K. L. H., HUGHES, A. R., KENDRICK, G. A., KENWORTHY, W. J., OLYARNIK, S., SHORT, F. T., WAYCOTT, M. & WILLIAMS, S. L. 2006. A global crisis for seagrass ecosystems. *BioScience*, 56, 987-996.

- PAPADIMITRIOU, S., KENNEDY, H., KENNEDY, D. P., DUARTE, C. M. & MARBÁ, N. 2005. Sources of organic matter in seagrass-colonized sediments: A stable isotope study of the silt and clay fraction from *Posidonia oceanica* meadows in the western Mediterranean. *Organic Geochemistry*, 36, 949–961.
- PARMESAN, C. & YOHE, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37-42.
- PENDLETON, L., DONATO, D. C., MURRAY, B. C., CROOKS, S., JENKINS, W. A., SIFLEET, S., CRAFT, C., FOURQUREAN, J. W., KAUFFMAN, J. B., MARBÀ, N., MEGONIGAL, P., PIDGEON, E., HERR, D., GORDON, D. & A.BALDERA 2012. Estimating global “Blue Carbon” emissions from conversion and degradation of vegetated coastal ecosystems. *PLos One*, 7, e43542.
- PERALTA, G., DUREN, L. A. V., MORRIS, E. P. & BOUMA, T. J. 2008. Consequences of shoot density and stiffness for ecosystem engineering by benthic macrophytes in flow dominated areas: a hydrodynamic flume study. *Marine Ecology Progress Series*, 368, 103-105.
- PETERSON, C. H., LUETTICH, R. A., MICHELI, F. & SKILLETER, G. A. 2004. Attenuation of water flow inside seagrass canopies of differing structure. *Marine Ecology Progress Series*, 268, 81-92.
- POLLARD, P. C. & MORIARTY, D. J. W. 1991. Organic carbon decomposition, primary and bacterial productivity, and sulphate reduction, in tropical seagrass beds of the Gulf of Carpentaria, Australia *Marine Ecology Progress Series*, 69, 149-159.

- POOVACHINRANON, S. & CHANSANG, H. 1994. Community structure and biomass of seagrass beds in the Andaman Sea. I. Mangrove-associated seagrass beds. *Phuket Marine Biology Center Research Bulletin*, 59, 53-64.
- POST, W. M., PENG, T. H., EMANUEL, W. R., KING, A. W., DALE, V. H. & DE AUGELIS, D. L. 1990. The global carbon cycle. *American Scientist*, 78, 310-326.
- PRATHEP, A., RATTANACHOT, E. & TUNTIPRAPAS, P. 2010. Seasonal variations in seagrass percentage cover and biomass at Koh Tha Rai, Nakhon Si Thammarat Province, Gulf of Thailand. *Warasan Songkhla Nakharin*, 32, 497-204.
- RABENHORST, M. C. 1995. Carbon storage in tidal marsh soils. In: LAL, R., KIMBLE, J., LEVINE, E. & STEWART, B. A. (eds.) *Soils and global change. Advances in soil science*. CRC Lewis.
- RAMANATHAN, V. & CARMICHAEL, G. 2008. Global and regional climate changes due to black carbon. *Nature geoscience* 1, 221-227.
- RATTANACHOT, E. & PRATHEP, A. 2011. Temporal variation in growth and reproduction of *Enhalus acoroides* (L.f.) Royle in a monospecific meadow in Haad Chao Mai National Park, Trang Province, Thailand. *Botanica Marina*, 54, 201-207.
- RODELLI, M. R., GEARING, J. N., GEARING, P. J., MARSHALL, N. & SASEKUMAR, A. 1984. Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. *Oecologia*, 61, 326-333.
- ROSE, W. I. 1977. Scavenging of volcanic aerosol by ash: Atmospheric and volcanologic implications. *Geology*, 5, 621-624.

- ROZENZWEIG, C. & PARRY, M. L. 1994. Potential impact of climate change on world food supply. *Nature*, 367.
- RUIZ, J. M. & ROMERO, J. 2003. Effects of disturbances caused by coastal constructions on spatial structure, growth dynamics and photosynthesis of the seagrass *Posidonia oceanica*. *Marine Pollution Bulletin*, 46, 1523-1533.
- SAINTILAN, N., ROGERS, K., MAZUMDER, D. & WOODROFFE, C. 2013. Allochthonous and autochthonous contributions to carbon accumulation and carbon store in southeastern Australian coastal wetlands. *Estuarine, Coastal and Shelf Science*, 128.
- SAND-JENSEN, K. 1998. Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology*, 39, 663-679.
- SEDDON, S., WEAR, R. J., VENEMA, S. & MILLER, D. 2005. Seagrass rehabilitation in Adelaide metropolitan coastal waters II. Development of donor bed independent methods using *Posidonia* seedlings. . In: REPORT TO THE COASTAL PROTECTION BRANCH, D. F. E. A. H. (ed.). Aquatic sciences Adelaide.
- SERRANO, O., MATEO, M. A., RENOM, P. & JULIÀ, R. 2012. Characterization of soils beneath a *Posidonia oceanica* meadow. *Geoderma*, 185-186, 26-36.
- SHAFER, D. J., SHERMAN, T. D. & WYLLIE-ECHEVERRIA, S. 2007. Do desiccation tolerances control the vertical distribution of intertidal seagrasses? *Aquatic Botany*, 87, 161-166.
- SHAHBUDIN, S., JALAL, K. C. A., KAMARUZZAMAN, Y., NOOR, N. M.-., DAH, T. C. & JOHN, B. A. 2011. Artificial Seagrass: A Habitat for Marine Fishes. *Journal of Fisheries and Aquatic Science*, 6, 85-92.

- SHARMA, A. R., KHAROL, S. K., BADARINATH, K. V. S. & SINGH, D. 2010. Impact of agriculture crop residue burning on atmospheric aerosol loading – a study over Punjab State, India. *Annales Geophysicae*, 28, 367-379.
- SHEESLEY, R. J., SCHAUER, J. J. & ORF, M. L. 2010. Assessing the impact of industrial source emissions on atmospheric carbonaceous aerosol concentrations using routine monitoring networks. *Journal of the air & waste management association*, 60, 149-155.
- SHORT, F., CARRUTHERS, T., DENNISON, W. & WAYCOTT, M. 2007. Global seagrass distribution and diversity: A bioregional model. *Journal of Experimental Marine Biology and Ecology*, 350, 3-20.
- SHORT, F. T. & DUARTE, C. M. 2001. Methods for the measurement of seagrass growth and production. In: SHORT, F. T. & COLES, R. G. (eds.) *Global seagrass research method*. Elsevier Science B.V.
- SMITH, B. N. & EPSTEIN, S. 1971. Two categories of  $^{13}\text{C}/^{12}\text{C}$  Ratios for Higher Plants. *American Society of Plant Biologists*, 47, 380-384.
- SOGARD, S. M. 1989. Colonization of artificial seagrass by fishes and decapod crustaceans: importance of proximity to natural eelgrass. *Journal Experimental Marine Biology and Ecology*, 133, 15-37.
- SOLOMON, S., PLATTNER, G.-K., KNUTTI, R. & FRIEDLINGSTEIN, P. 2009. Irreversible climate change due to carbon dioxide emissions. *PNAS*, 106, 1704-1709.
- SPURGEON, J. 1998. The socio-economic costs and benefits of coastal habitat rehabilitation and creation. *Marine pollution bulletin*, 37, 373-382.

- SUCHANEK, T. H., WILLIAMS, S. W., OGDEN, J. C., HUBBARD, D. K. & GILL, I. P. 1985. Utilization of shallow-water seagrass detritus by Caribbean deep-sea macrofauna:  $\delta^{13}\text{C}$  evidence. *Deep Sea Research*, 32, 2201–2214.
- SUPANWANID, C. & LEWMANOMONT, K. 2003. The seagrass of Thailand. In: GREEN, E. P. & SHORT, F. T. (eds.) *World Atlas of Seagrasses*. Berkeley, CA: University of California Press.
- TAKAHASHI, T., OLAFSSON, J., GODDARD, J. G., CHIPMAN, D. W. & SUTHERLAND, S. C. 1993. Seasonal variation of  $\text{CO}_2$  and nutrients in the high-latitude surface oceans: A comparative study. *Global Biogeochemical Cycles*, 7, 843–878.
- THORNTON, S. F. & MCMANUS, J. 1994. Application of Organic Carbon and Nitrogen Stable Isotope and C/N Ratios as Source Indicators of Organic Matter Provenance in Estuarine Systems: Evidence from the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science*, 38, 219-233.
- TORRE-CASTRO, M. D. L. & RÖNNBÄCK, P. 2004. Links between humans and seagrasses – An example from tropical East Africa. *Ocean & Coastal Management* 47, 361-387.
- VERMAAT, J. E. & VERHAGEN, F. C. A. 1996. Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: coupling demographic and physiological patterns. *Aquatic Botany*, 52, 259-281.
- VIRNSTEIN, R. W. & CURRAN, M. C. 1986. Colonization of artificial seagrass versus time and distance from source. *Marine Ecology Progress Series*, 29, 279-288.
- VIZZINIA, S., SARÀA, G., MICHENER, R. H. & MAZZOLA, A. 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for



secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta Oecologica*, 23, 277–285.

- WALTHER, G.-R., POST, E., CONVEY, P., MENZEL, A., PARMESAN, C., BEEBEE, T. J. C., FROMENTIN, J.-M., HOEGH-GULDBERG, O. & BAIRLEIN, F. 2002. Ecological responses to recent climate change. *Nature*, 416, 389-395.
- WANG, X.-C., CHEN, R. F. & BERRY, A. 2003. Sources and preservation of organic matter in Plum Island salt marsh sediments (MA, USA): long-chain n-alkanes and stable carbon isotope compositions. *Estuarine, Coastal and Shelf Science*, 58, 917-928.
- WAYCOTT, M., DUARTE, C. M., CARRUTHERS, T. J. B., ORTH, R. J., DENNISON, W. C., OLYARNIK, S., CALLADINE, A., FOURQUREAN, J. W., HECK, K. L., HUGHES, A. R., KENDRICK, G. A., KENWORTHY, W. J., SHORT, F. T. & WILLIAMS, S. L. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *PNAS*, 106, 12377–12381.
- WEAR, R. 2006. Recent advances in research into seagrass restoration. *SARDI research report series*. Coastal protection branch, Department of environment and heritage.
- WEISS, R. F., JAHNKE, R. A. & KEELING, C. D. 1982. Seasonal effects of temperature and salinity on the partial pressure of CO<sub>2</sub> in seawater. *Nature*, 300, 511-513.
- WOLKENHAUER, S.-M., UTHICKE, S., BURRIDGE, C., SKEWES, T. & PITCHE, R. 2010. The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds. *Journal of the Marine Biological Association of the United Kingdom*, 90, 215-223.

- WORM, B., BARBIER, E. B., BEAUMONT, N., DUFFY, J. E., FOLKE, C., HALPERN, B. S., JACKSON, J. B. C., LOTZE, H. K., MICHELI, F., PALUMBI, S. R., SALA, E., SELKOE, K. A., STACHOWICZ, J. J. & WATSON, R. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* 314, 787-790.
- YAMAMURO, M. & CHIRAPART, A. 2005. Quality of the Seagrass *Halophila ovalis* on a Thai Intertidal Flat as Food for the Dugong. *Journal of Oceanography*, 61, 183-186.
- YANG, M., HOWELL, S. G., ZHUANG, J. & HUEBERT, B. J. 2009. Attribution of aerosol light absorption to black carbon, brown carbon, and dust in China – interpretations of atmospheric measurements during EAST-AIRE. *Atmospheric chemistry and physics*, 9, 2035-2050.
- ZIEGLER, S. & BENNER, R. 1999a. Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. *Marine Ecology Progress Series*, 180, 149-160.
- ZIEGLER, S. & BENNER, R. 1999b. Nutrient cycling in the water column of a subtropical seagrass meadow. *Marine Ecology Progress Series*, 188, 51-62.
- ZIEMAN, J. C., MACKO, S. A. & MILLS, A. L. 1984. Role of seagrasses and mangroves in estuarine food webs: Temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bulletin of marine science*, 35, 380-392.

## APPENDIX

### APPENDIX 1 SEAGRASS DEGRADATION AT KHAO BAE NA, HAAD CHAO MAI NATIONAL PARK, TRANG, THAILAND



Seagrass loss at Koa Bae Na, Haad Chao Mai, Trang province, Thailand from 1999–2012. (A) Large meadow of seagrasses at the intertidal zone with four dominant species namely *Halophila ovalis*, *Cymodocea rotundata*, *Thalassia hemprichii* and *Enhalus acoroides*. Photo was shown in the report of Dugong grazing study by Mukai et al. (1999)(Mukai et al., 1999). (B) Seagrass was completely lost from the intertidal zone. Photo was taken in 2012 by Apichanangkool P.

## APPENDIX 2 SEMINARS AND PRESENTATION FROM THIS DISSERTATION

Poster presentation: Sedimentary organic carbon pools in *Zostera muelleri* subsp. *capricornii* aschers. Meadows at the Pontal da Ferradura Convention Centre in Búzios, Rio de Janeiro, Brazil, 10<sup>th</sup> International Seagrass Biology Workshop. 25<sup>th</sup> November to 30<sup>th</sup> November, 2012

Oral presentation: Assessment of tropical Blue Carbon reserves at University of Technology, Sydney, Australia, International Blue Carbon Scientific Working Group. 15<sup>th</sup> to 17<sup>th</sup> May 2013.

Oral presentation, Assessment of tropical Blue Carbon reserves, Faculty of Science, University of Technology, Sydney, Australia, Three minutes thesis competition. 30<sup>th</sup> August 2014.