Fluxes of Organic Carbon Settled in the Seagrass Area at Khung Kraben Bay, Chanthaburi Province, Thailand

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ABSTRACT

Organic carbon flux plays an important role in the functioning of marine ecosystems. However, organic carbon buried in sediments of tropical seagrass meadows remains incompletely characterized. This research was conducted to investigate the fluxes of organic carbon in sediments settled in three densities of seagrass cover (> 75 %, < 25 %, and no seagrass) at Kung Kraben Bay, Chanthaburi Province during summer (March) and rainy season (June) in 2020. The results showed that organic carbon fluxes in the rainy season differed from those in the summer. The average organic carbon flux in the rainy season (10.50±5.54 g $C{\cdot}m^{-2}{\cdot}d^{-1}$) was higher than in the summer (1.00±0.55 g $C{\cdot}m^{-2}{\cdot}d^{-1}$). Organic carbon in the sediment was highest in areas with > 75 % seagrass cover, followed by < 25 % cover, and no seagrass. The organic carbon flux in seagrass-rich areas (> 75 % cover) was on average 3.63 times higher than in areas without seagrass in the summer, and 2.33 times higher in the rainy season. Stable carbon isotopes were used to identify the sources of organic matter transported into the seagrass areas. The results showed that the organic carbon in trapped sediment samples was sourced from algae, phytoplankton, shrimp feed, and mangrove leaves. Their temporal and spatial variation depended on the distance from the sources and the influences of environmental conditions such as runoff and sediment resuspension.

Keywords: Carbon isotope, Fluxes of organic carbon, Fluxes of sediment, Khung Kraben Bay

INTRODUCTION

Global warming from the greenhouse effect is now a threat to ecosystems worldwide. It is caused by emissions of greenhouse gases into the atmosphere from human activities; the most abundant of these gases is carbon dioxide (Pearson *et al*., 2000), Atmospheric carbon dioxide concentrations reached 379 ppm in 2021, an increase of 5.3 % compared to 2020, with an overall increase of about 13 % between 1990 and 2021 (Crippa *et al*., 2022; United Nations Environment Programme, 2022). However, there is a process commonly known as a carbon sink, which is the absorption of atmospheric carbon back into ecosystems that can help keep the climate in balance. Carbon storage in the ocean (called "Blue Carbon") is considered a very important element of the Earth's carbon cycle. Marine habitats including seagrass areas, saltwater swamp forests, and mangrove forests can account for up to 55 % of the total carbon storage; this represents the densest carbon deposit on Earth (Duarte *et al*., 2005; Macreadie *et al*., 2014). Despite the relatively small area of these marine habitats, carbon storage is 35 times greater than that of terrestrial forests (Macreadie *et al*., 2014).

Seagrass is a salinity-adapted monocotyledon plant that grows well in coastal areas (Waycott *et al*., 2009). It is one of the most productive ecosystems in the world (Morgan and Kitting, 1984; Fortes, 1995; Terrados *et al*., 1998; Hemminga and

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Duarate, 2000) and is important to the larger marine ecosystem as a food source, a spawning ground, and a hiding place for aquatic animals. Seagrass can fix carbon dioxide from the atmosphere, store it in above ground and underground stems, and transfer it to sediments in the seagrass beds. Through the slow process of mineralization, this organic matter will be converted to inorganic substances, which will then be passed on to other consumers in the food chain (Waycott *et al*., 2009; Mcleod *et al*., 2011; Serrano *et al*., 2013; Macreadie *et al*., 2015).

The process and scale of carbon sequestration in seagrass beds are important to the global environment. Carbon dioxide is extracted from the atmosphere and transformed, with water, into energy and structures of the seagrass through photosynthesis. The carbon is then fixed and stored in underground structures (Valentine and Heck, 1999). The carbon deposited in sediment through this process is equal to 0.16×10^{15} g C·year^{-1}, or about 15 % of the total carbon accumulated in marine ecosystems (Duarte and Chiscano, 1999). Seagrass beds are also considered to be a very important carbon sink, capable of storing carbon for thousands of years (Macreadie *et al*., 2014). The organic carbon sequestration in seagrass beds is estimated to be about 83 g C·m^{-2}·year^{-1} (Duarte *et al*., 2005), accounting for 10-18 % of ocean carbon sequestration. The global carbon deposition rate in seagrass beds is approximately 27-44 Tg C·year^{-1} (Kennedy and Björk, 2009).

Khung Kraben Bay is located in Khlong Khut Subdistrict, Tha Mai District, Chanthaburi Province, on the eastern coast of the Gulf of Thailand. The bay has been classified as an important national wetland with an area of approximately 640 hectares (4,000 rai), shaped like a stingray (Figure 1). The estuary opens to the sea at only through a narrow strait (about 600 m wide) located on the western side. The average depths within the bay and at the bay mouth are 0.7-3.0 m and 0.6-1.5 m, respectively, and a diurnal tide is dominant (Sasaki and Inoue, 1985). Seven natural canals flow into the bay, and mangrove forests line the perimeter. Beyond the mangrove zone are coastal aquaculture areas such as shrimp ponds and caged fish ponds. In addition to the mangrove ecosystem, Khung Kraben Bay also has fertile seagrass beds covering an area of approximately 200 ha (1,245 Rai) (Department of Marine and Coastal Resources, 2015).

Five species of seagrasses are found in Kung Kraben Bay including *Enhalus acoroides*, *Halodule pinifolia*, *H. uninervis*, *Halophila decipiens*, and *H. minor* (Aryuthaka *et al*., 1992), but *E. acoroides* and *H. pinifolia* are dominant. Large seagrass areas line the central and the inner regions, close to the coastline and adjacent to the mangrove areas around the bay, and these beds are relatively stable (Vichkovitten, 1998; Suriyaphant and Ketma, 2016). The seagrasses are a source of food, refuge, spawning, and habitat for aquatic animals such as snapper shrimp, cockle, blue crab, hermit crab, damselfish, squid, and seahorse (Department of Marine and Coastal Resources, 2018). It is also a source of food for dugongs that often come to eat seagrass in this area (Department of Marine and Coastal Resources, 2012).

The knowledge of carbon sequestration by seagrass beds in Thailand is still limited. There is an urgent need for studies here to quantify the storage of carbon by seagrass beds. This research focuses on studying the fluxes of sediment and organic carbon settling in seagrasses in areas with different amounts of seagrass cover and in different seasons. The stable isotopes of carbon in the trapped sediment are also analyzed to investigate the sources of organic carbon. The data obtained from the study will be useful in understanding the holistic carbon sequestration in the seagrass beds of Kung Kraben Bay, Chanthaburi Province, and other tropical coastal seas.

MATERIALS AND METHODS

The study area was in Khung Kraben Bay, Khlong Khut Sub-district, Tha Mai District, Chanthaburi Province, located between latitude 12º 33'-12º 36' N and longitude 101º 52'-101º 54' E (Figure 1). Field data and samples were collected twice in 2020: the dry season in March and the wet season in June.

Sediment traps were placed in the tidal zone during low tide at three replicate sampling stations for each level of seagrass cover: 0 % cover or unavailable seagrass (US), seagrass cover < 25 % or degraded seagrass (DS), and seagrass cover > 75 % or natural seagrass (NS) (English *et al*., 1997; McKenzie *et al*., 2001). A quadrat of 50×50 cm was used to assess the percent of seagrass cover at each station. The distance between each replication with the same seagrass cover was 100 m. Sampling areas without seagrasses (US stations) were located at least 500 m from any seagrass beds, and only where large seagrass debris was not found in soil sediment within a depth of 30 cm. The sampling method was adapted from Stankovic *et al*. (2017) and Thorhaug *et al*. (2017). It should be noted that the characteristics of the cover of the two dominant seagrasses are different; *Halodule pinifolia* has a markedly lower land cover density than *Enhalus acoroides*. Therefore, only *H. pinifolia* was found in the sampling area with < 25 % seagrass cover and only *E. acoroides* was in the area with > 75 % seagrass cover.

The sediment trap consisted of a glass bottle of 6.5 cm diameter and 10.5 cm height. Wire mesh with a 1-cm mesh size was installed over the mouth of the bottle to prevent interference from living organisms. The bottle was attached to a meter-long iron rod which was drilled into the seafloor to hold the bottle just above the seafloor. The sediment traps were placed according to the three seagrass cover densities (> 75 % (NS), < 25 % (DS), and 0 % (US)) in three replications (at least 100 m apart) for a total of nine samples each season. Sediment, collected seven days after the traps were placed, was used to assess fluxes and sources of organic carbon settled into the study site (Ricart *et al*., 2015). The dry weight of all sediment samples was over 200 g, and therefore sufficient for the analysis of sediment and organic carbon fluxes, and stable carbon isotopes.

Organic matter in the sediment was analyzed by using the loss on ignition method (LOI) modified from Strickland (1965). A portion of each sediment sample was weighed and dried

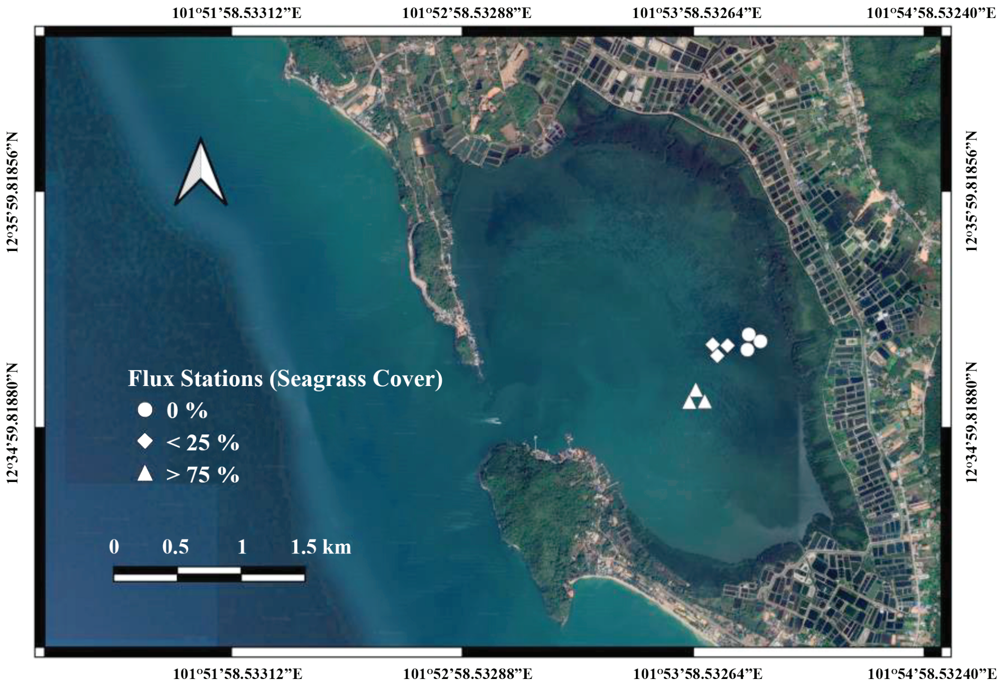

Figure 1. Map of the three types of seagrass sampling stations based on seagrass cover, namely US (0 %), DS (< 25 %), and NS (> 75 %) at Khung Kraben Bay, Chantaburi Province, Thailand.

at 60-70 ºC in an oven for 48 h or until the weight was stable. The sediment was then baked at 550 ºC for 3 h, left to cool in a desiccator, and weighed. The weight lost after baking was used to calculate LOI (%) as shown in Equation 1.

$$LOI = \frac{W_B - W_A}{W_B} \times 100 \quad (1)$$

Here, W_B and W_A are sediment weight (g) before and after being baked at 550 ºC, respectively.

Another sub-sample from the same sediment trap was analyzed for organic carbon and its stable isotopes by an Elemental Analyzer combined with a continuous flow Isotope Ratio Mass Spectrometer (EA-IRMS, EA1112-Delta Plus, Thermo Fisher Scientific). We first sieved (0.4 mm) the sample to remove large particles including shell fragments. Approximately 2-5 g of the sample was transferred to a 20 mL tube and 2N HCl solution was dripped into the sample in a hood until no bubbles were generated; this removed inorganic carbon. After removing as much of the liquid fraction as possible with a Pasteur pipette, the HCl was removed by drying at 40 °C for 48 h and vacuum drying for 24 h. The sample of 6-30 mg was pelletized with a tin disk and was introduced into the EA-IRMS to determine mass fractions of organic carbon and its $^{13}C/^{12}C$ in the pellet.

The $^{13}C/^{12}C$ of each sample is conventionally reported as the relative per mil (‰) difference between the sample $^{13}C/^{12}C$ ratio (Rsam) and the ratio of a standard (Rstd) as follows:

$$\delta\, ^{13}C = \frac{R_{sam} - R_{std}}{R_{std}} \times 1{,}000 \quad (2)$$

The precision (1 standard deviation) of organic carbon mass fraction analyses was less than 5 %. The $\delta\, ^{13}C$ measurements were calibrated against IAEA-CH-3, with a precision of less than 0.2 ‰ estimated from repeated measurements of laboratory standards (Amino Standard, SI Science).

The mass fluxes of sediment (Equation 3) and organic carbon (Equation 4) in seagrass areas were calculated using a modified method from Wahyudi *et al*. (2016).

$$S_{flux} = \frac{S_{dry}}{TA} \times 24 \quad (3)$$

$$C_{flux} = R_{org}\, S_{flux} \quad (4)$$

$$R_{org} = \frac{C_{org}}{S_{dry}} \times 100 \quad (5)$$

where S_{flux} is the sediment flux ($g \cdot m^{-2} \cdot d^{-1}$), S_{dry} is the dry weight of the sediment in the trap (g), T is the sediment capture period (h), and A is the internal area of the sediment trap (m^2). C_{flux} is the organic carbon flux ($g\ C \cdot m^{-2} \cdot d^{-1}$), and R_{org} is the organic carbon fraction in the dry-weight sediment sample (Equation 5).

Analysis of Variance (ANOVA) at a 95 % confidence level was used to analyze the spatial and temporal differences in sediment weight, the organic carbon in sediment, the fluxes of sediment and organic carbon, and the carbon isotope ratio. In case of statistically significant differences, the differences between groups were tested with Tukey's test.

RESULTS AND DISCUSSION

The average LOI of sediment dry weight for samples of trapped sediment from the three levels of seagrass cover during the summer was 8.33± 2.45 %, 6.88±0.86 %, and 6.71±0.57 % for NS (> 75 %), DS (< 25 %), and US (0 %), respectively. During the rainy season, the LOI was 1.69±0.72 %, 2.25±0.80 %, and 1.02±0.17 % for NS, DS, and US, respectively, lower than in the summer (Figure 2). The statistical analysis revealed that the means were seasonally different ($p<0.05$), but were not different among the three seagrass covers. Low LOI during the rainy season was expected because of high runoff, inferred from high precipitation (300-400 mm) in June, compared to that in March

(30-90 mm) (Amatayakul and Chomtha, 2016). The runoff likely carried eroded sediment from land and waterways to settle in the study area. Thus, higher content of inorganic than organic matter in the transported sediments causes lower LOI in the rainy season than in the summer due to the higher runoff.

The average mass of sediment fluxes settled in the seagrass areas during summer was 127.69±32.82 $g \cdot m^{-2} \cdot d^{-1}$, 59.85±9.57 $g \cdot m^{-2} \cdot d^{-1}$, and 12.60±2.87 $g \cdot m^{-2} \cdot d^{-1}$ for NS, DS, and US, respectively (Figure 3). The average fluxes during the rainy season were 1,424.22±108.17 g·m-2·d-1, 969.72± 26.08 $g \cdot m^{-2} \cdot d^{-1}$, and 576.74±113.20 $g \cdot m^{-2} \cdot d^{-1}$ for NS, DS, and US, respectively. Sediment fluxes during the rainy season were much higher than those in the summer resulting from high water runoff. The statistical analysis also confirmed the difference between the seasonal values ($p<0.05$). There were also differences among the three types of seagrass cover, with highest sediment mass collected in the

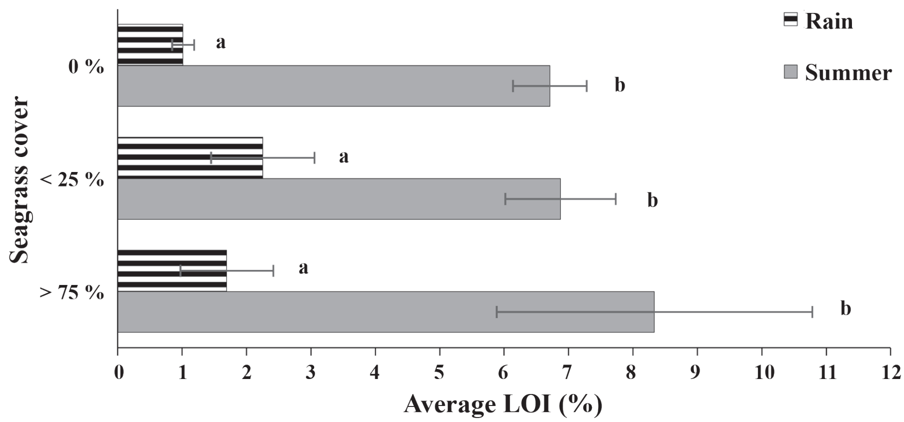

Figure 2. Average LOI (% dry weight) in trapped sediment in three levels of seagrass cover, namely US (0 %), DS (< 25 %), and NS (> 75 %) in the summer and the rainy season. Different letters to the right of error bars (±SE) indicate significant differences ($p<0.05$) between groups.

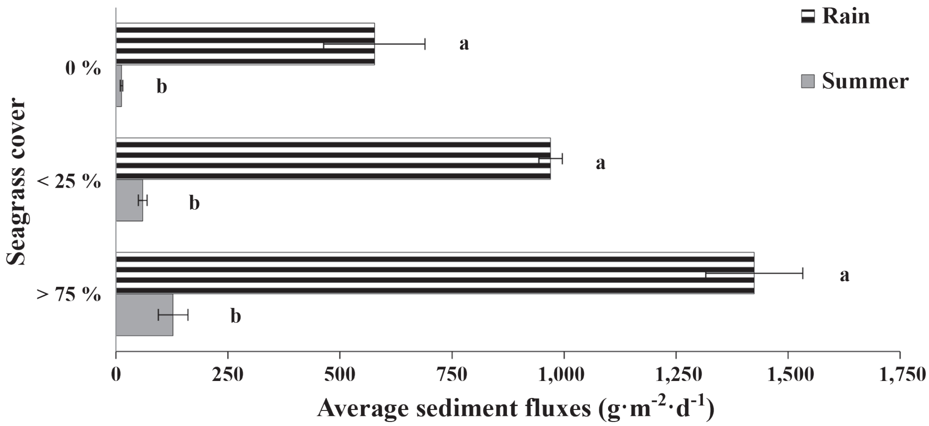

Figure 3. Average sediment fluxes (dry weight) collected in sediment traps in three levels of seagrass cover, namely US (0 %), DS (< 25 %), and NS (> 75 %) in the summer and the rainy season. Different letters to the right of error bars (±SE) indicate significant differences ($p<0.05$) between groups.

seagrass cover > 75 % (NS), and the lowest in 0 % seagrass cover (US). Most seagrass-free areas are closer to the coast than the heavy seagrass areas (Figure 1). Although the coast is a source of sediment from runoff, the sediment fluxes were highest in dense seagrass-covered areas and lowest in seagrass-free areas. The results show the effectiveness of seagrass in trapping sediment; on average, this was 10.13 times higher than in the seagrass-free area during the summer and 2.47 times higher in the rainy season. More sedimentation in seagrass areas than in seagrass-free areas can be attributed to several factors, such as the nature of the seagrass leaf cover that facilitates sediment trapping, the water currents that differ by area and season (Sirimungkara *et al*., 2016), and differences in depth and distance from the coastline. Detailed studies of the influence of each of these factors on sedimentation in the Kung Kraben Bay area would improve our understanding of sediment flux dynamics.

The percentage of organic carbon in trapped sediment in US and DS was higher in the summer (2.98±0.41 % and 2.04±0.05 %, respectively) than in the rainy season (1.21±0.35 % and 0.86±0.62 %, respectively). In contrast, a higher value was recorded for NS in the rainy season (1.14±0.19 %) than in the summer (1.07±0.15 %), although the difference was small (Figure 4). The statistical analyses using one-way ANOVA and Tukey's test indicated that in the rainy season, the means for all three levels of seagrass cover were similar. Values for US and DS in the summer were significantly higher than in the rainy season, and also higher than NS in the summer. The seasonal averages show that organic carbon content during the summer is generally greater than during the rainy season.

The organic carbon content in sediment was reported for seagrass ecosystems in Chillika and Palk Bay located along the eastern coast of the Indian Gulf (Ganguly *et al*., 2017a). The organic carbon content in the soil sediments in summer (0.98±0.33 % and 1.01±0.33 %, respectively) was higher than in the rainy season (0.69±0.22 % and 0.97±0.19 %, respectively), but without statistical differences. This is probably a response to the temporal variation in net seagrass community production in the area. The seagrass production is higher in the summer than in the rainy season due to higher photosynthetically active radiation, water temperature, salinity and lower dissolved nutrients. In contrast, water column productivity dominated by a fast-growing phytoplankton community shows a reverse seasonal trend, with higher values during the rainy season than in the summer (Ganguly *et al*., 2017b).

From our study, we cannot specify exactly what factors control the variation in organic fraction in the settled sediment. In terms of spatial variation, high organic carbon in the summer in nearshore areas may be due to the influence of mangroves and shrimp aquaculture activities located along the coastline within the bay (Figure 1). Thus, a higher percentage of organic carbon was found in the nearshore stations than those offshore. The organic carbon portion in the sediment was low in the rainy season probably because of high erosion, which caused more inorganic sediment to be transported to the area, where it then precipitated.

The amount of organic carbon in settling flux (Figure 5) showed a trend in line with that of the sediment flux (Figure 3). This similarity occurred even though the percentage of organic carbon in the sediment was similar among the sites during the rainy season and was greatest in the area without seagrass cover in the summer (Figure 4). The highest average carbon flux was recorded in the rainy season (16.15±2.57 g $C{\cdot}m^{-2}{\cdot}d^{-1}$) in NS and the lowest was in the summer in US (0.38±0.13 g $C{\cdot}m^{-2}{\cdot}d^{-1}$). The statistical analysis revealed that the organic carbon flux was significantly different ($p<0.05$) between seasons. An analysis by clustering with Turkey's test revealed that all levels of seagrass cover in summer were similar, but all seagrass levels were different ($p<0.05$) during the rainy season.

The seasonal variation in the organic carbon flux is a result of environmental factors, such as the loading of organic matter from the land via runoff, that cause the organic carbon flux in the rainy season to be greater than in the summer. Spatial differences, categorized by seagrass cover, were also evident, whereby seagrass-covered areas had significantly greater fluxes of organic carbon falling and being trapped on the seafloor than those without seagrass. The margin of seagrass meadows has been determined to be a boundary that impedes water flow, increasing turbulence at the edge of the canopy (Granata *et al*., 2001) and enhancing the accumulation of particles inside the seagrass meadows (Macreadie *et al*., 2010). The study of variability in sedimentary organic carbon in patchy seagrass landscapes found that the amount of organic carbon accumulated in areas without seagrass (1.5 % C_{org}DW) was less than in areas with seagrass (1.75 % C_{org}DW) (Ricart *et al*., 2015). Our present study found that the organic carbon flux in the densest (> 75 %) seagrass cover was on average 3.63 times higher than in seagrass-free areas in the summer and 2.33 times higher in the rainy season.

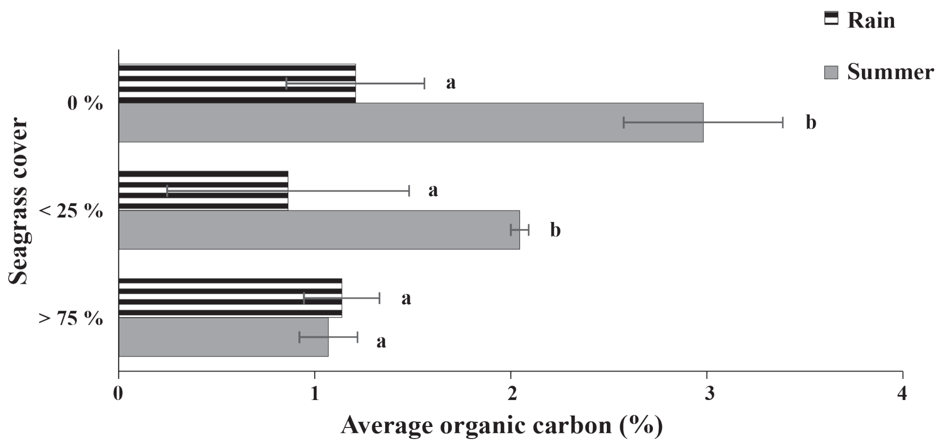

Figure 4. Average organic carbon fraction (% of dry weight sediment) in trapped sediment in three levels of seagrass cover, namely US (0 %), DS (<25 %), and NS (>75 %) in the summer and the rainy season. Different letters to the right of error bars (±SE) indicate significant differences (p<0.05) between groups.

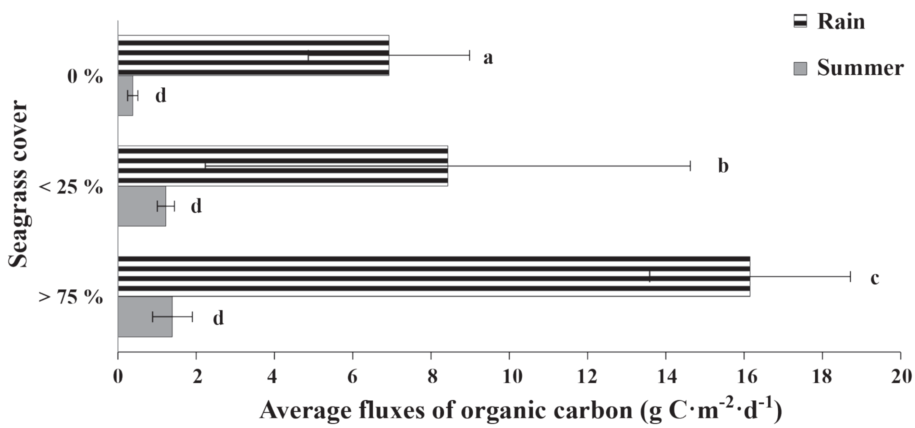

Figure 5. Average fluxes of organic carbon collected in sediment traps in three levels of seagrass cover, namely US (0 %), DS (< 25 %), and NS (>75 %) in the summer and the rainy season. Different letters to the right of error bars (±SE) indicate significant differences (p<0.05) between groups.

In the summer, average carbon isotopic ratios ($\delta^{13}C$) for organic matter in the trapped sediment (Figure 6) were -20.1±0.3 ‰, -23.3±0.1 ‰, and -24.4 ±0.1 ‰ for NS, DS, and US, respectively. During the rainy season, average $\delta^{13}C$ values were -22.2±0.0 ‰, -22.8±0.1 ‰, and -24.4±0.1 ‰ for NS, DS, and US, respectively. The values in all stations in both seasons were in the range of -25.2 to -19.7 ‰. The mean $\delta^{13}C$ in the captured sediment between seasons was not statistically different ($p>0.05$), but there was a difference among levels of seagrass cover. The isotopic ratios in summer in areas with > 75 % seagrass cover were significantly different from other sampling sites. In the rainy season, the average $\delta^{13}C$ in 0 % seagrass cover area was significantly different from > 75 % and < 25 % sites.

The measured $\delta^{13}C$ in this study is similar to the results from other studies of seagrass areas, such as Hemminga and Mateo (1996). To investigate the sources of organic carbon precipitated in the seagrass areas, the results of this study were compared with those of Thimdee *et al*. (2003), who examined the sources and movement of organic matter in Khung Kraben Bay based on $\delta^{13}C$ and the carbon to nitrogen ratio (C/N ratio). In their study, five major sources were identified that contribute to the organic matter in water and sediment, namely mangrove leaves, seagrass, algae, phytoplankton, and shrimp feed. The $\delta^{13}C$ and C/N ratio, respectively, of mangrove leaves were -29 ‰ and 40-105, seagrass -11 ‰ and 19, algae -16 ‰ and 18, phytoplankton -21 ‰ and 5-6, and shrimp feed -23 ‰ and 7. Seagrass had the highest $\delta^{13}C$ value, followed by algae, phytoplankton, shrimp feed, and mangrove leaves. We can use these reference values to compare with the results of the present study to identify the sources of organic sediments settled in various seagrass sites. During the summer, seagrass-free areas had values of $\delta^{13}C$ around -24.4 ‰, which is close to the $\delta^{13}C$ value for shrimp feed and phytoplankton. In the sites with seagrass cover < 25 %, the average value (-23.3 ‰) was almost the same as that of shrimp feed, and in sites with seagrass cover > 75 %, the average value (-20.1 ‰) was similar to those reported for phytoplankton and algae deposition. During the rainy season, in areas without seagrass, the average $\delta^{13}C$ of -24.4 ‰ implies a

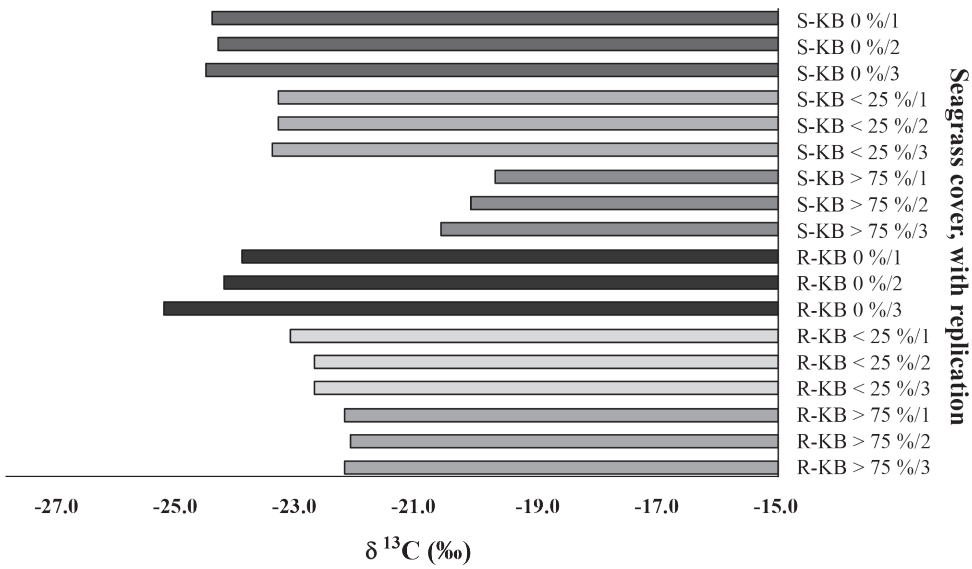

Figure 6. Carbon isotopic ratio ($\delta^{13}C$) of organic matter in trapped sediment in three levels of seagrass cover, namely US (0 %), DS (< 25 %), and NS (> 75 %) in the summer (S) and the rainy (R) season at Khung Kraben Bay (KB). The number following a slash represents the replication.

contribution of organic carbon from shrimp feed and phytoplankton, which is as significant as in summer. In the areas with seagrass cover < 25 % (-22.8 ‰) and > 75 % (-22.2 ‰), δ^{13}C was similar to values for shrimp feed and phytoplankton. The isotope results showed relatively low δ^{13}C in the rainy season (-22 ‰, for NS), which may suggest a small contribution from algae and seagrass deposition. Delta δ^{13}C values from our samples may not be close to that of seagrass deposition (-11 ‰) because the organic matter falling from seagrass is small compared with other sources such as mangrove leaves, shrimp feed, and phytoplankton. Confirming this requires further investigation. However, as in summer, the higher sediment δ^{13}C in the higher seagrass coverage areas may suggest that the seagrass itself (i.e., autochthonous source) contributed some of the organic material. Although the limited analysis of the δ^{13}C signal alone precludes further source identification in the present study, further analysis with additional data (e.g., C/N) would allow a more detailed examination of the source composition distribution and its relationship to seasonal runoff across the study area.

CONCLUSION

The study of organic carbon fluxes in the seagrass area of Khung Kraben Bay, Chanthaburi Province revealed that the seasonal variation in the carbon flux was due to runoff from the land (as inferred from precipitation data), that produces more fluxes during the rainy season than in the summer. The spatial differences, categorized by seagrass cover, were clear; areas with denser seagrass cover had significantly higher organic carbon flux. The organic carbon flux in seagrass-rich areas (cover > 75 %) was on average 3.63 times higher than in areas without seagrass in the summer and 2.33 times higher in the rainy season. The carbon isotopic ratio can be used to identify the sources of organic matter falling into the seagrass area. The results suggest that the organic carbon settled in the area was sourced from algae, phytoplankton, shrimp feed, and mangrove leaves. Their temporal and spatial variation depend on the distance from the sources and the influences of environmental conditions such as runoff and sediment resuspension.

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