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Effects of Shading on the Growth and Carbon Storage of *Enhalus acoroides*

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Abstract: Light intensity is one of the main factors determining the growth and distribution of seagrasses, but seagrasses differ in their responses to changes in the light environment, resulting in inconsistent adaptation. To investigate the effect of light reduction on *Enhalus acoroides* (L. f.) Steud., we simulated different light intensities by setting up in situ shade shelters with three light environments: full light (CK), moderate shading (MS) and high shading (HS), and investigated the growth response and adaptation mechanism of *E. acoroides* to a low-light environment. The results showed that the leaf length and leaf width of *E. acoroides* decreased in the low-light environment. Plant density, biomass, and chlorophyll content (Chl) decreased significantly with the prolongation of shading. In addition, the sediment carbon content of seagrass beds was significantly reduced in the shading treatments compared to the full-light treatment. After the restoration of light, the chlorophyll content of *E. acoroides* increased compared to that in the shading period, but its leaf morphology, plant density and biomass did not return to the level of full light treatment. Our study highlights that long-term light reduction leads to a significant reduction in seagrass biomass and its sediment carbon content, which in turn, may reduce the carbon storage capacity of seagrass beds.

Keywords: *Enhalus acoroides*; shading; growth; carbon storage

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1. Introduction

As one of the typical blue carbon ecosystems, seagrass meadows contain an annual carbon stock of at least 27.4 Tg C worldwide, which is equivalent to 10~15% of the total global ocean carbon sequestration, and thus play an important role as the global carbon sinks [1]. In addition, seagrass meadows can provide habitat, breeding and feeding places for many marine organisms and have the functions of adsorbing suspended particulate matter, improving water quality, reducing wave energy and maintaining the coast [2]. However, large-scale degradation and the disappearance of seagrass meadows have occurred globally due to human activities and climate change [3]. Among them, the reduction in water transmittance caused by human activities is considered one of the main reasons for seagrass ecosystem degradation [4]. Light intensity plays a key role in the growth, survival and distribution of seagrasses [5]. In contrast to effects on terrestrial plants, solar energy is affected by many factors before reaching seagrass leaves, causing light energy loss, among which the air–water interface leads to a certain amount of light energy loss; meanwhile, the suspended particulate matter in the water and the increase in water depth cause the rapid attenuation of solar energy. Studies have shown that the seabed environment in which seagrasses live requires an average of 11% surface irradiance in order to maintain the normal growth and development of seagrasses [6].

The weakening of the light environment of water Is mainly caused directly or indirectly by human activities, mainly including dredging projects, the construction of coastal wharves, and the discharge of aquaculture sewage and domestic wastewater [7–9]. Light reduction decreases the photosynthetic rate of seagrasses, which in turn affects their

growth and physiological properties, mainly in terms of a reduced growth rate, altered leaf morphology, reduced biomass [10–12], and increased consumption of non-structural carbohydrates [13]. However, there is some variation in the response of different species to changes in the light environment due to factors such as the growth, habitat and life history of seagrasses [14].

Enhalus acoroides is a large, long-lived seagrass with the ability to reproduce both sexually and asexually. It is widely distributed in the Indo-West Pacific, and in China, it is found only on Hainan Island, mainly in Xincun Bay and Li'an Lagoon in Lingshui [15]. The fishery is well developed in Li'an Lagoon, and the discharge of aquaculture sewage and domestic wastewater, among other pollutants, has a significant impact on the health status of seagrass beds [16]. In the past 10–20 years, the distribution area and plant density of *E. acoroides* have decreased dramatically, and few studies have been reported regarding the effects of external environmental changes on *E. acoroides* [17]. In this study, we examined *E. acoroides*, simulated an environment reflecting the reduced light transmission of water by building a shade shelter in situ, explored differences in the response of *E. acoroides* to changes in the light environment, analyzed the degradation mechanism of *E. acoroides*, and provided a theoretical basis for determining a reasonable conservation strategy.

2. Materials and Methods

2.1. Study Area

Li'an Lagoon is located in the southeastern part of Lingshui County, with an area of approximately 9 km^2, and it is connected to the outer sea only by a tidal branch of approximately 60 m [16]. The distribution area of seagrasses in the lagoon is approximately 1.42 km^2, and the species include *E. acoroides*, *Thalassia hemprichii*, *Halophila ovalis* and *Cymodocea rotundata* [18]. However, due to long-term human interference, the seagrass meadow of Li'an Lagoon is gradually developing into an ecosystem, with *E. acoroides* as the single dominant species. Therefore, *E. acoroides* was selected as the research object in this experiment.

2.2. Experimental Design and Sample Collection

The experiment was divided into two phases. The first phase consisted of three and six months of shading, and the second phase consisted of three months of recovering light. *E. acoroides*, located in the intertidal zone with a patchy distribution and basically uniform growth (only *E. acoroides* was distributed in the area where shading was performed), were selected in the study area on December 2020, and the light environment with reduced water transmittance was simulated by building a shading shelter in situ. The size of the shading shed was 2 m × 2 m, and the height of the shed was slightly higher than the plant height of *E. acoroides* in a completely floating state. The shading material was a black shading net with uniform density, and the shading intensity was manipulated by changing the number of overlapped shading nets and measuring the light intensity directly below the shading net in situ using an underwater irradiance meter (ZDS-10W-2D). The light measurements were carried out in full sunlight at noon, and the results of the measurements are shown in Table 1.

Based on the light measurements, the shading rates of the two shading treatments were approximated as 60% (single shading) and 90% (double shading), which were labelled as moderate shading (MS) and high shading (HS), respectively, while the full-light treatment (no shading) was used as the control (CK). Three sample plots were selected in the study area, labeled ZG1, ZG2, ZG3. Three light treatments were set up in each sample plot, with three sets of replicates for each treatment, for a total of 27 sample squares (Figure 1). Due to tidal changes, the shading canopy is sometimes exposed to the water, and sometimes completely submerged. To reduce the experimental error caused by wave interference or the attachment of other marine organisms, we visited the sample site regularly every month to replace the shading net.

Table 1. The measurement of light intensity in situ.

Site	Control/lx	Single/lx	Intensity	Control/lx	Double/lx	Intensity
ZG1	76,000	28,300	62.76	78,400	6580	91.61
	78,700	27,800	64.68	76,900	7000	90.90
	75,300	29,400	60.96	76,200	7800	89.76
ZG2	56,500	18,500	67.26	51,800	2530	95.12
	59,200	20,500	65.37	52,400	3310	93.68
	58,300	22,700	61.06	55,600	2890	94.80
ZG3	36,900	14,500	60.70	25,800	2280	91.16
	28,500	12,400	56.49	26,900	2560	90.48
	35,200	16,700	52.56	26,200	1680	93.59
Average	——	——	61.32	——	——	92.34

Note: ZG1, ZG2, ZG3 are three sample plots in study area. Single, the light intensity of the single-layer shading net; Double, the light intensity of the double-layer shading net.

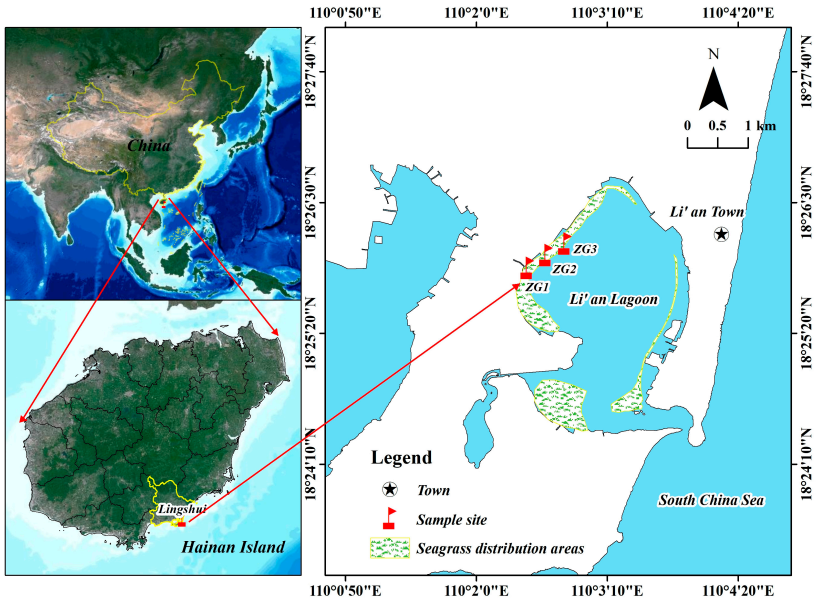

Figure 1. Study area.

Samples were collected at low tide when the *E. acoroides* was exposed to the surface. The seagrasses were collected in the test plots using 25 cm × 25 cm sampling frames directly below the shading shelters. The *E. acoroides* were dug out from the sampling frames together with the rhizomes, and the sediments were collected using PVC pipes at a depth of 30 cm with intervals of 0–5, 5–10, 10–20 and 20–30 cm.

2.3. Measurement of Indicators

The leaf length and leaf width of *E. acoroides* in all sampling frames were measured with a measuring tape, and the number of leaves was subsequently counted. Plant density was calculated by taking the number of *E. acoroides* in each sampling frame and dividing it by the area of the sampling frame (0.0625 m^2) to calculate the plant density of *E. acoroides* under different light treatments (plants/m^2). Seagrasses were carefully retrieved, and subsequently dried to constant weight at 60 °C for the determination of biomass [12].

Three *E. acoroides* were randomly selected from each sample frame as the sample plants to be tested, and the middle part of their leaves was taken for the determination of the chlorophyll content. The fresh leaves were cut into small pieces of about 0.20 cm, mixed well and weighed to 0.20 g. The leaves were then put into a stoppered graduated test tube with 80% acetone solution for chlorophyll extraction. When the leaf tissue had all turned white, it indicated that the chlorophyll had been extracted cleanly [19]. After that, the absorbance values of the extracts at the corresponding wavelengths were measured

using a UV spectrophotometer (UV-5200), and the content of chlorophyll and chlorophyll a/b value (Chl a/Chl b) were calculated accordingly [20].

Sediment samples were stored in desiccators prior to analysis. After being naturally air dried, the sediment samples were ground and homogenized with a mortar and pestle and subsequently passed through a 100-mesh sieve for the determination of the sediment organic carbon content. The sediment organic carbon content was determined via the potassium dichromate–sulfuric acid oxidation method. The organic carbon of the sediment was oxidized with a potassium dichromate–sulfuric acid solution under oil bath heating, and the remaining potassium dichromate was titrated with ferrous sulfate to calculate the organic carbon content from the amount of potassium dichromate consumed [21].

2.4. Statistical Analysis

Data were processed and analyzed using Excel 2019 and SPSS 26.0. The variability of the morphological characteristics, including the density, biomass and photosynthetic pigment content of the *E. acoroides,* and the sediment carbon content under different light treatments, were analyzed via one-way analysis of variance (ANOVA) and least significant difference (LSD) tests ($\alpha = 0.05$). The data in the graphs are the "mean ± standard error". Plots were generated using Origin 9.8.

3. Results

3.1. Morphology

When light was reduced, the leaf length and leaf width of *E. acoroides* decreased significantly ($p < 0.05$), and the decrease was higher in the 6-month shading treatment than in the 3-month shading treatment (Table 2). With the increase in shading intensity, the number of leaves of *E. acoroides* showed a decreasing trend. After the restoration of light, the leaf length of the plants in the shading treatment was still significantly smaller than that of the plants in the full-light treatment ($p < 0.05$), but the leaf width and leaf number of the plants in the moderate shading treatment increased after light restoration compared with the shading period; the leaf length and leaf width of the plants in the high shading treatment did not tend to increase throughout the restoration period, indicating that the greater the shading intensity was, the weaker the ability of *E. acoroides* to restore normal growth was, and the effect caused by high shading still occurred.

Table 2. Effects of different light treatments on morphological characteristics of *E. acoroides*.

Period	Treatments	Leaf Length/cm	Leaf Width/cm	Leaf Number
S3	CK	34.98 ± 0.96 a	1.65 ± 0.02 a	4.34 ± 0.09 a
	MS	29.78 ± 1.17 b	1.47 ± 0.02 b	3.60 ± 0.11 b
	HS	25.25 ± 1.16 c	1.38 ± 0.02 c	3.35 ± 0.11 b
S6	CK	54.98 ± 1.55 a	1.84 ± 0.02 a	6.63 ± 0.08 a
	MS	22.79 ± 1.80 b	1.49 ± 0.03 b	5.00 ± 0.12 b
	HS	21.47 ± 1.40 b	1.40 ± 0.02 c	4.84 ± 0.12 b
R3	CK	24.31 ± 1.26 a	1.75 ± 0.02 a	5.41 ± 0.14 a
	MS	19.12 ± 0.87 b	1.50 ± 0.03 b	4.23 ± 0.12 b
	HS	16.30 ± 0.91 b	1.35 ± 0.03 c	3.58 ± 0.12 c

Note: S3, shading for 3 months; S6, shading for 6 months; R3, recovery for 3 months. CK–Control; MS–Moderate shading; HS–High shading. Different letters indicate significant differences at the 0.05 level. The same below.

3.2. Density

When the shading period was 3 months, the shoot density of *E. acoroides* decreased but was not significantly different from that of the control ($p > 0.05$) (Figure 2). After 6 months of shading treatment, the shoot density in the moderate shading and high shading treatments was significantly lower than that in the full-light treatment ($p < 0.05$), decreasing by 55.36% and 59.82%, respectively, compared with that of the control. After the restoration of light, the shoot density increased in all shading treatments but did not return to the same level as the control.

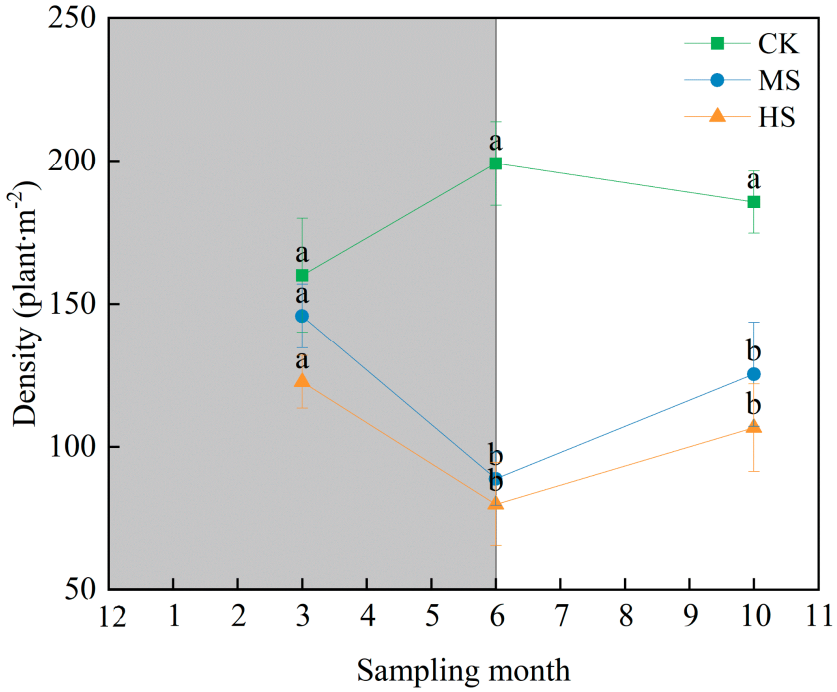

Figure 2. Effects of different light treatments on the density of *E. acoroides*. Note: The shaded part represents shading, and the blank part represents light recovery. Different letters in the same sampling period indicate significant differences at the 0.05 level.

3.3. Biomass

Three months of shading treatment caused a significant reduction in the biomass of *E. acoroides* compared to that in the full-light treatment ($p < 0.05$) (Figure 3). With an increasing shading treatment time, the biomass of the moderate shading and heavy shading groups decreased by 53.36% and 54.10%, respectively, compared to that of the full-light treatment group. The biomass remained lower during the period of light restoration.

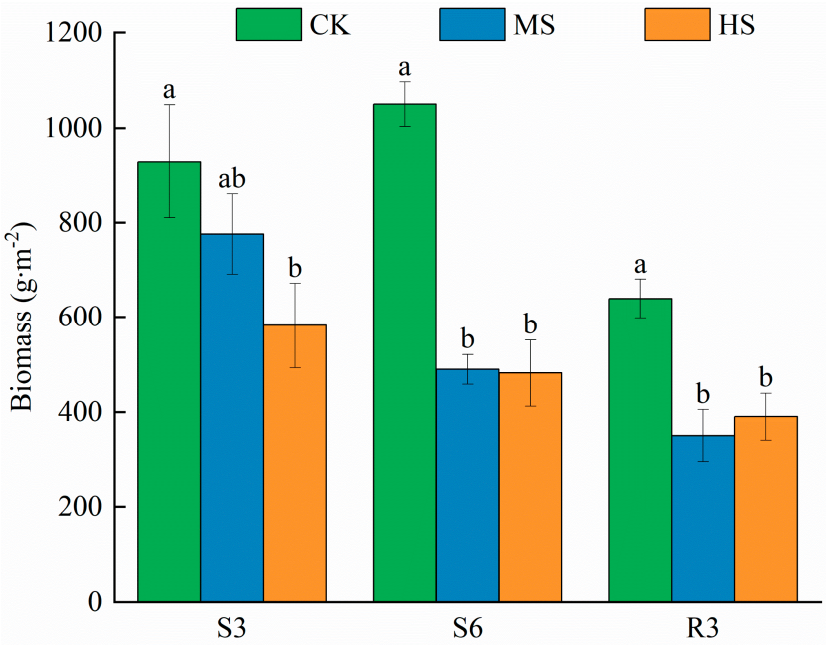

Figure 3. Effects of different light treatments on the biomass of *E. acoroides*. Note: Different letters in the same sampling period indicate significant differences at the 0.05 level.

3.4. Chlorophyll

The maximum chlorophyll a and chlorophyll b contents in *E. acoroides* occurred in the control group throughout the shading period. As the shading intensity increased, the chlorophyll a/b values showed a tendency to decrease (Table 3). When the shading duration reached 6 months, the chlorophyll content of the shading treatment was significantly lower than that of the control ($p < 0.05$). After the restoration of light, the total chlorophyll content increased significantly compared to that in the shading period ($p < 0.05$), and the chlorophyll a/b values did not differ significantly from those in the control ($p > 0.05$).

Table 3. Effects of different light treatments on the chlorophyll content of *E. acoroides*.

Period	Treatments	Photosynthetic Pigments (mg·g^{-1})			
		Chl a	Chl b	Total Chl	Chl a/Chl b
S3	CK	0.142 ± 0.016 a	0.062 ± 0.007 a	0.204 ± 0.022 a	2.419 ± 0.206 a
	MS	0.108 ± 0.010 a	0.056 ± 0.004 a	0.164 ± 0.014 a	1.899 ± 0.094 b
	HS	0.125 ± 0.014 a	0.049 ± 0.004 a	0.175 ± 0.018 a	2.495 ± 0.153 a
S6	CK	0.207 ± 0.012 a	0.084 ± 0.005 a	0.291 ± 0.018 a	2.484 ± 0.036 a
	MS	0.087 ± 0.008 b	0.041 ± 0.004 b	0.128 ± 0.011 b	2.222 ± 0.083 b
	HS	0.082 ± 0.007 b	0.039 ± 0.004 b	0.121 ± 0.011 b	2.196 ± 0.093 b
R3	CK	0.301 ± 0.024 a	0.142 ± 0.019 a	0.434 ± 0.032 a	2.156 ± 0.341 a
	MS	0.229 ± 0.019 b	0.102 ± 0.014 a	0.330 ± 0.022 b	2.233 ± 0.205 a
	HS	0.230 ± 0.018 b	0.100 ± 0.016 a	0.334 ± 0.018 b	2.539 ± 0.475 a

Note: Chl a—chlorophyll a content; Chl b—chlorophyll b content; Total Chl—Total chlorophyll content; Chl a/Chl b—chlorophyll a/b values. Different letters indicate significant differences at the 0.05 level.

3.5. Sediment Carbon

The sediment carbon content was higher in the control than in the shading treatment (Figure 4). There was no significant difference in the sediment carbon content at different depths in the control ($p > 0.05$). When the light was reduced, the sediment carbon content at 0–5 cm in the high shading treatment was significantly lower than that in the control at the same depth (34.36%). With increasing depth, the sediment carbon content under moderate shading decreased significantly ($p < 0.05$).

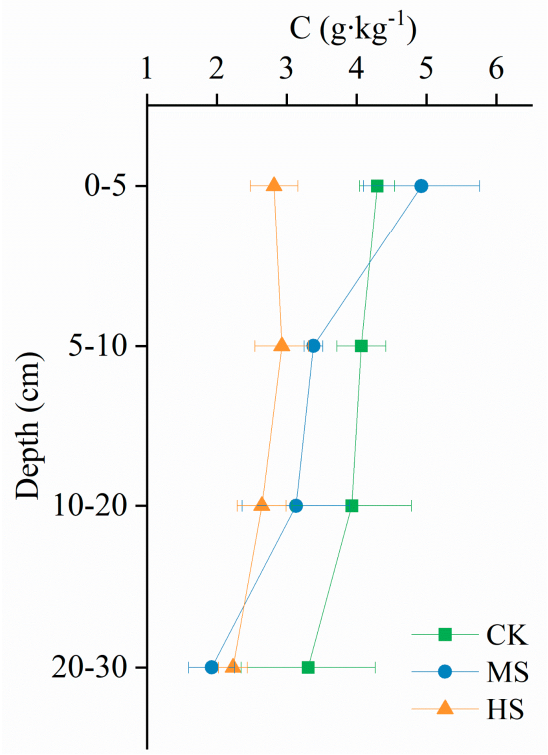

Figure 4. Effects of shading on sediment carbon content in seagrass beds.

4. Discussion

4.1. Response of Seagrass Growth to Shading

Changes in the leaf morphology of seagrass can be used to visualize its response to changes in the light environment [4]. Studies have shown that light reduction significantly reduces the leaf length and width of *Zostera marina* [22]. At 64% and 75% shading intensities, the number of leaves of *T. hemprichii* was significantly lower than that in the full-light treatment [23]. In the present study, the leaf length and width of *E. acoroides* decreased with increasing shading intensity, and the number of leaves also tended to decrease. When light is insufficient, seagrasses are able to persist in this stressful environment by altering their leaf morphology or maintaining a small number of leaves to effectively reduce their respiratory demand in the low-light environment. In contrast, an increase in the leaf length can enhance the capture of light by seagrasses in low-light environments [24]. The reason for this difference could be due to the higher shading intensity and longer shading period in this study. In addition, the shoot density of *E. acoroides* did not change significantly at 3 months of shading treatment, which may be related to the time of shading initiation. We started the shading treatment of *E. acoroides* in winter, when the growth rate of *E. acoroides* was low. Indeed, carbohydrates were accumulated by seagrasses in the spring and summer when the sunlight was sufficient to meet their carbon demand in the low-light environment in the early period. The shoot density of *E. acoroides* decreased significantly with the increasing duration of the shading treatment, which was in summer. Seagrasses exposed to high temperatures may require more light to maintain a carbon balance and are thus more susceptible to light reduction [25].

4.2. Response of Seagrass Chlorophyll Content to Shading

Photosynthetic pigments play an irreplaceable role in photosynthesis in plants [26]. Studies have shown that the regulation of the photosynthetic structure in response to low light usually includes an increase in the chlorophyll content and a decrease in the chlorophyll a/b ratio [27,28]. The increase in chlorophyll content can significantly enhance the absorption, transfer and conversion of energy from weak light in the optical system to improve the utilization efficiency of light energy [29]. Different results were obtained in our study. When the duration of the shading treatment was 3 months, the chlorophyll content of *E. acoroides* was not significantly different from that of the control, indicating that this seagrass was tolerant to short-term light stress; as the duration of shading increased, the chlorophyll content decreased sharply. This shows that a long-term light intensity that is too low is detrimental to chlorophyll synthesis, which is similar to observations made in previous studies [30]. After shading, the chlorophyll a/b values of *E. acoroides* were lower than those in the full-light treatment. Light reduction usually decreases the proportion of red light absorption in seagrass, and chlorophyll b is more capable of absorbing blue light than chlorophyll a. The reduction in chlorophyll a/b values in low-light environments indicates that the seagrass has an enhanced ability to utilize blue light, thus improving light capture in low-light environments [31].

4.3. Effect of Shading on the Carbon Storage of Seagrass Beds

Seagrass accumulates organic matter via photosynthesis, and light reduction decreases its photosynthetic rate [32], which in turn affects productivity [33] and even causes biomass loss [34]. In the present study, as the shading intensity increased, the amount of effective light that *E. acoroides* could receive decreased, thus inhibiting its growth and biomass accumulation. The loss of aboveground biomass reflects both the growth and development of seagrasses in low-light environments and suggests that seagrasses are able to avoid being shaded by their own leaves by reducing their leaf area [35]. The loss of leaves reduces photosynthesis in seagrasses to some extent, and the reduction in oxygen delivery to the rhizome may cause anaerobic conditions in the subsurface tissues, thus causing a reduction in the biomass of the subsurface [36].

The seagrass canopy capture of organic matter from the surrounding habitat is the main source of sediment carbon, and the underground biomass usually has a high lignin content and relatively low decomposition rate, so the reduction in the seagrass biomass disrupts its CO_2 uptake process [37]. There is a distinct lack of research on the effects of reduced light on the sediment carbon storage of seagrass beds, but existing studies suggest that the degradation and loss of seagrass beds may result in the loss and release of sediment carbon [38]. For example, the large-scale loss of *Posidonia oceanica* has been shown to lead to an 11–25% reduction in sediment carbon stocks [39]. In *Thalassia testudinum*, shading treatments have also been shown to result in a loss of up to 47% of sediment carbon stocks in the surface layer alone [40]. In the present study, the sediment carbon was lower in the shading treatment than in the control, and it decreased significantly with increasing depth. This may be due to the loss of aboveground biomass caused by reduced light, resulting in a decrease in its efficient trapping of allochthonous carbon and reducing the deposition of aboveground tissues to the detritus layer [37]. In addition, a reduction in root biomass would decrease the flux of the root exudation of the dissolved organic carbon into the sediment [41]. Therefore, biomass loss due to light reduction would further lead to a reduction in sediment carbon stocks. However, *Zostera nigricaulis* showed no significant change in the carbon content of sediment during up to two years of intense disturbance [42]. Possible reasons for this difference in results include the contribution of seagrass itself to the sediment carbon pool [43], the hydrological conditions of the study area [44], and the influence of the composition and activity of the microbial community on sediment mineralization [45].

4.4. Response of Seagrass to Light Restoration

After the restoration of light, the chlorophyll contents of *E. acoroides* increased compared to those in the shading period, providing a favorable material basis for its photosynthesis under suitable light conditions. However, the physiological recovery was not reflected by the leaf morphology, shoot density or biomass. During the period of light restoration, the leaf and biomass of *E. acoroides* remained reduced. Similar differences were observed in the growth and physiological responses of *Amphibolis griffithii* and *Z. marina* to restored light [13,46]. This may be related to the seasonal growth of seagrass. In addition, the growth of *E. acoroides* during the period of light restoration may be limited by typhoons. However, the more important reason is that long-term light stress may have a devastating effect on the growth of seagrass and that recovery would be difficult in the short term even when the stress is removed. Future increases in the photosynthetic rate may be effective in alleviating the damage caused by the pre-light-stressed environment, but long-term light reduction will affect photosynthesis and photomorphic construction, eventually leading to death. The sediment carbon of *Posidonia australis* also increased significantly after restoration compared to the disturbed period [47]. This shows that a suitable growth environment is conducive to promoting the accumulation of organic matter in seagrasses, thereby increasing their CO_2 sequestration capacity.

5. Conclusions

In this study, the effects of shading and the restoration of light on the growth and carbon storage capacity of *E. acoroides* were investigated through in situ experiments over a period of 10 months. The results show that the growth of *E. acoroides* was significantly negatively affected by the low-light environment, which also caused a significant loss of shoot density. Short-term low-light conditions had no significant effect on the chlorophyll content of *E. acoroides*, but a prolonged reduction in light was detrimental to its chlorophyll synthesis. In addition, long-term light reduction not only causes the loss of seagrass biomass, but also may lead to the carbon that is stored being unable to have a long-term stable existence in its sediment due to the loss of seagrass shelter. After the restoration of light, the chlorophyll content of *E. acoroides* increased compared to this content during the

shading period, but its leaf morphology, shoot density and biomass did not return to the level of the full-light treatment, and the effects caused by shading remained.

The degradation and disappearance of seagrass beds and the loss of their carbon storage capacity are issues of concern. Our study highlights that the carbon storage capacity of seagrass beds may eventually decrease in environments with reduced light exposure over time, at least at a 60–90% shading intensity. This implies that we need to promote the conservation and restoration of seagrass ecosystems through the enhanced management of water quality. To provide better management measures, we can learn as much as possible about the light compensation points of *E. acoroides* by conducting experiments with wider shading intensity ranges in the future.

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