

Dynamics of nonstructural carbohydrates in seagrass *Thalassia hemprichii* and its response to shading

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Abstract

A field survey was performed to examine nonstructural carbohydrate (NSC) dynamics in seagrass *Thalassia hemprichii* at the Xincun Bay in southern China. An indoor experiment to investigate the response of NSC in *T. hemprichii* to shade was conducted. Belowground tissue of *T. hemprichii* was the dominant site of NSC reserves, and soluble sugar was the primary storage compound. The starch content of belowground tissue was lower in high intertidal areas than in low intertidal areas, indicating that the longer air exposure in high intertidal areas resulted in less NSC synthesis and less accumulation of NSC in *T. hemprichii*. The lowest level of soluble sugar and its proportion to NSC in belowground tissue were observed near the cage culture area, where the nutrient concentration in water and sediment was the highest; while the highest level of that was observed near the coastal shrimp farm, where salinity was the lowest. Soluble sugar in belowground tissue showed the following trend: summer>spring>winter>autumn. This corresponded to seasonal changes in the intensity of light. Leaf sugar accumulated during the autumn-winter period, providing a carbon and energy source for flower bud formation and seed germination. Short-term shading decreased NSC accumulation. Collectively, these results suggest that nutrient enrichment, freshwater discharge and exposure to air affect NSC dynamics in *T. hemprichii*. Light intensity, flower bud formation, and seed germination were all found to induce seasonal variations in NSC in *T. hemprichii*.

Key words: *Thalassia hemprichii*, nonstructural carbohydrates, Xincun Bay, dynamics, shade

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1 Introduction

Seagrass beds are important coastal ecosystems. They provide valuable ecological goods and services (Dennison, 2009). In recent years, about 2%–5% of seagrasses populations are lost each year (Duarte and Gattuso, 2010). This decline has been attributed to a wide variety of human and natural disturbances (Manzanera et al., 1998; Neckles et al., 2005; Burkholder et al., 2007; Waycott et al., 2007; Björk et al., 2008; Marbà and Duarte, 2010). The dynamics of nonstructural carbohydrate (NSC) reserves in particular play an important role in determining seagrass growth and its response to environmental disturbances (Touchette, 2007; Leoni et al., 2008; Nejrup and Pedersen, 2008; Biber et al., 2009).

Under excessive nitrogen conditions, carbon requirements for the synthesis of amino acids may exceed carbon fixation capacity, leading to a decrease in NSC concentration and affecting NSC reallocation (Invers et al., 2004; Brun et al., 2008; Leoni et al., 2008). While seagrasses are under hypo- and hypersaline conditions, modifications in carbohydrate catabolism and metabolism become necessary to allow for the accumulation or degradation of organic solutes (Chollett et al., 2007; Touchette, 2007). The rates of photosynthesis of intertidal sea-

grasses may decline when air exposure advances beyond a certain degree (Shafer et al., 2007). This reduces NSC synthesis. Many studies have examined the effects of nitrogen enrichment and hyposaline stress on NSC dynamics in seagrasses through laboratory and field experiments (Invers et al., 2004; Chollett et al., 2007; Brun et al., 2008). However, building up a relationship between environmental stress and NSC dynamics in seagrasses by field survey is hitherto not available. A mixed seagrass meadow with an area of about 200 ha can be found in the southern shallow waters of the Xincun Bay, Hainan Island, China. *Thalassia hemprichii* is the dominant seagrass species (Huang et al., 2006). This seagrass segment of the bay is subjected to a variety of environmental stresses, such as nutrient input from floating fish-cage mariculture systems, fresh water discharge from coastal shrimp farms, and air exposure. Whether these environmental stresses affect NSC dynamics in *T. hemprichii* at the Xincun Bay merits further research.

The seasonal dynamics of seagrass carbon resources are crucial to assessing the whole-plant responses to environmental stress (Serrano et al., 2011). Despite this, only a few seagrasses have been observed in studies that evaluated seasonal changes in NSC. They include *Zostera marina* (Burkholder et al.,

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1992; Burke et al., 1996; Touchette and Burkholder, 2002), *Zostera noltii* (Pirc, 1989; De Rosa et al., 1990), *Posidonia oceanica* (Pirc, 1985, 1989), *Cymodocea nodosa* (Pirc, 1989), *Thalassia testudinum* (Lee and Dunton, 1996), *Ruppia maritima* (Lazar and Dawes, 1991). Information on seasonal changes in NSC in *T. hemprichii* is not available, and which factors induce seasonal variations in NSC levels in *T. hemprichii* at Xincun Bay remains largely unknown. Light is the one of the most important abiotic factors that affects seagrass productivity and spatial distribution (Ralph et al., 2007). Seagrasses are highly sensitive to light limitation (Short et al., 2007). During periods of reduced photosynthesis attributable to light limitation, sucrose synthesis decreases and the stored NSC in belowground tissue can be reallocated to meet the carbon demand of the seagrasses (Olivé et al., 2007; Serrano et al., 2011). A few studies have been carried out on the effects of light reduction on NSC dynamics in seagrasses, such as *Halophila ovalis* (Eklöf et al., 2009), *Halodule pinifolia* (Longstaff and Dennison, 1999), *Posidonia sinuosa* (Collier et al., 2009), *P. oceanica* (Ruíz and Romero, 2001), *Z. noltii* (Peralta et al., 2002; Olivé et al., 2007; Brun et al., 2008), *Z. marina* (Alcoverro et al., 1999) and *Amphibolis griffithii* (Mackey et al., 2007). Although Uy (2001) assessed the long-term shade acclimation capacity of *T. hemprichii* with respect to carbon assimilation and subsequent translocation using the stable isotope ^{13}C , little information on the effects of shading on the soluble sugar and starch in *T. hemprichii* is available.

Consequently, focusing on tropical seagrass bed, the aims of this study were to examine: (1) NSC dynamics in *T. hemprichii* at Xincun Bay, South China; (2) the response of NSC in *T. hemprichii* to short-term reductions in light. The re-

sults may help to provide a rapid indicator for an early warning of potential seagrass death, and strengthen the understanding needed to improve management and protection of these environmentally important marine angiosperms.

2 Materials and methods

2.1 Sampling and sample preparation

The study was conducted at Xincun Bay ($18^{\circ}24'34''\text{N}$ – $18^{\circ}24'42''\text{N}$, $109^{\circ}57'42''\text{E}$ – $109^{\circ}57'58''\text{E}$), located in southeastern Hainan Island, South China Sea (Fig. 1). This bay is almost entirely closed with only one narrow channel connected to the open sea to the southwest. *T. hemprichii* meadows extend along a vertical gradient of about 1 m in intertidal areas. Three transects were selected, with two sampling positions in each transect: (1) high intertidal, with an emersion period of about 3 h in each tidal cycle (i.e., in each 12 h); (2) low intertidal, where *T. hemprichii* is exposed to the air during spring tides for only short periods of time (<1 h) (Fig. 1). At Transect 1, the *T. hemprichii* meadow was located near the bay's entrance and dominated by nutrient inputs from the fish cage culture systems. The *T. hemprichii* meadow in Transect 2 was located near a series of shrimp pools; and Transect 3 was located near Nanwan village, considered as control transect. *T. hemprichii* was sampled in July 2009 (summer), October 2009 (autumn), January 2010 (winter), and May 2010 (spring). At each sampling site, more than 20 individual plants of *T. hemprichii* were collected, and subsequently grouped to form a composite plant sample. Three plant samples were prepared at every sampling site.

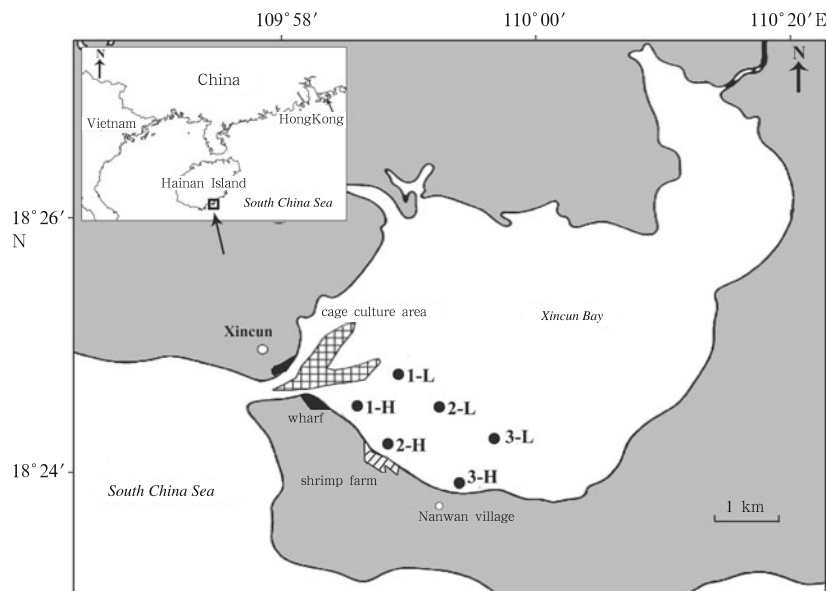


Fig.1. Sampling sites at the Xincun Bay, Hainan Island, South China Sea.

2.2 Laboratory methods

Plants were carefully retrieved and separated into roots, rhizomes and leaves. Rhizomes, roots and shoots were oven-dried (60°C) until constant weight. Subsamples of all fractions were powdered and stored for NSC content analysis. The samples were twice extracted in hot 80% ethanol. Soluble sugar content was determined using the anthrone-sulfuric acid method

(Yemm and Willis, 1954). Starch content of the remaining materials was also analyzed by anthrone assay, following gelatinization at 100°C for 15 min and solubilization in 70% perchloric acid (Quarmby and Allen, 1989).

Water samples were collected at 0.5 m depth in opaque bottles for dissolved inorganic nitrogen (DIN=nitrate+nitrite+ammonium) and dissolved inorganic phosphate (DIP). They

were transported on ice. In addition, sediment samples collected with an 8-cm-diameter corer driven about 13 cm into the sediments. Pore water from sediment samples was obtained by centrifugation (4 000 g for 15 min) and then diluted (1:5, v/v) with low nitrogen seawater (<0.1 $\mu\text{mol/L}$). After filtration (Whatman GF/C), the DIN and DIP contents of the samples were measured using standard colorimetric techniques with a CANY 722s spectrophotometer. Nitrate, nitrite, ammonium, and DIP were measured with a zinc-cadmium reduction method, hydrochloride naphthodiamide method, hypobromite oxidation method, and phosphorus molybdenum blue spectrophotometry, respectively (GB17378.4 2007). Salinity was evaluated using a dissolved oxygen/ conductivity meter (YSI, model 85, USA).

2.3 Laboratory experiment

About 120 individual shoots of *T. hemprichii* were collected from Xincun Bay. Plants were carefully removed by hand to ensure that belowground structures remained intact and then transported to the laboratory. They were then averagely held in four aquaria with 40 L seawater collected from the sampling site under saturated light conditions (ca. 250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) at 25°C until used in the experiments (7 d). Prior to be used in the experiments, transplant units were standardized so that shoots consisted of a single apical shoot with two rhizome internodes and associated roots. *T. hemprichii* was covered with a variable number of neutral density screens, establishing four groups: one control group (250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), and

three experimental groups (150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 75 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 25 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Each treatment group included three aquaria of 15 L, and ten individual shoots of *T. hemprichii* were randomly reallocated into each aquarium. Seawater was carefully renewed daily. Plants were maintained under these conditions for 14 d. At the end of the experiment, plants were carefully retrieved and separated into roots, rhizomes and leaves to determine their soluble sugar and starch concentrations.

2.4 Data analysis

Seasonal variations in NSC and the effects of light reduction on NSC were analyzed using one-way ANOVA. Treatment means were compared and separated by least significant difference (LSD) at $P < 0.05$.

3 Results

3.1 Variations in salinity and nutrients at seagrass bed of *Thalassia hemprichii*

Seasonal changes in salinity and nutrient concentrations in a seagrass bed of *T. hemprichii* were shown in Table 1. On average, seawater salinity was lowest in Transect 2, and the seawater salinity of Transect 1 was almost the same as that of Transect 3. DIN concentration in seawater and pore water showed the same trend, with Transect 1 > Transect 2 > Transect 3. Similarly, DIP concentration in pore water was highest in Transect 1, but DIP concentration in seawater showed no difference across the three transects.

Table 1. Variations in salinity and nutrients (DIN and DIP) in seagrass bed of the Xincun Bay, South China

| Season | Transect | Seawater DIN/ $\mu\text{mol}\cdot\text{L}^{-1}$ | Seawater DIP/ $\mu\text{mol}\cdot\text{L}^{-1}$ | Porewater DIN/ $\mu\text{mol}\cdot\text{L}^{-1}$ | Porewater DIP/ $\mu\text{mol}\cdot\text{L}^{-1}$ | Seawater salinity |
|---------|----------|--|--|---|---|-------------------|
| Spring | 1 | 5.23 | 0.54 | 57.76 | 2.41 | 31.2 |
| | 2 | 3.47 | 0.49 | 40.64 | 1.03 | 28.8 |
| | 3 | 2.00 | 0.49 | 37.91 | 0.70 | 31.0 |
| Summer | 1 | 8.07 | 0.71 | 45.29 | 2.62 | 32.8 |
| | 2 | 3.23 | 0.55 | 50.39 | 0.84 | 30.2 |
| | 3 | 3.20 | 0.58 | 38.10 | 1.40 | 33.6 |
| Autumn | 1 | 6.84 | 0.51 | 49.44 | 2.93 | 30.2 |
| | 2 | 4.05 | 0.55 | 39.96 | 0.67 | 28.5 |
| | 3 | 3.24 | 0.49 | 34.66 | 0.96 | 30.1 |
| Winter | 1 | 6.09 | 0.49 | 51.51 | 2.03 | 33.6 |
| | 2 | 3.31 | 0.31 | 42.04 | 0.64 | 30.2 |
| | 3 | 2.65 | 0.43 | 38.29 | 0.47 | 33.8 |
| Average | 1 | 6.56 | 0.56 | 51.00 | 2.50 | 32.0 |
| | 2 | 3.52 | 0.47 | 43.26 | 0.80 | 29.4 |
| | 3 | 2.77 | 0.50 | 37.24 | 0.88 | 32.1 |

3.2 Nonstructural carbohydrate dynamics in *Thalassia hemprichii*

In general, both soluble sugar and starch content were higher in belowground tissue than in shoots (Fig. 2). On average, the soluble sugar and starch had similar concentrations in aboveground parts. In belowground parts, the concentration of soluble sugar was slightly higher than the concentration of starch. For Transects 1 and 2, the proportion of soluble sugar to NSC in both tissues was greater in high intertidal areas than in low intertidal areas. For Transect 3, it showed a converse trend. Soluble sugar (61.4%) dominated over starch in aboveground tissue at the 3-L station. Among the three transects, the highest level of soluble sugar and its proportion to NSC in belowground tissue were observed in Transect 2, but they were both the lowest in Transect 1. The starch concentration of belowground tissue was higher in low intertidal areas than in high intertidal ar-

reas for all transects.

One-way ANOVA showed that the seasonal variation of soluble sugar in both tissues (aboveground: $F=3.014$, $P=0.054$; belowground: $F=1.459$, $P=0.256$) and starch in belowground tissue ($F=0.370$, $P=0.776$) were not significant, except starch in aboveground tissue ($F=7.176$, $P=0.002$). However, levels of soluble sugar in both tissues and of starch in belowground tissue still changed with the seasons. The soluble sugar content in aboveground tissue was higher during the autumn-winter period than during the spring-summer period, while soluble sugar in belowground tissue exhibited the opposite trend, peaking in summer and decreasing to a minimum in the autumn (Fig. 3). Starch concentration in aboveground tissue peaked in autumn and diminished to minimum levels in winter, while starch content in belowground tissue was higher during the summer-autumn period than during the spring-winter period. The propor-

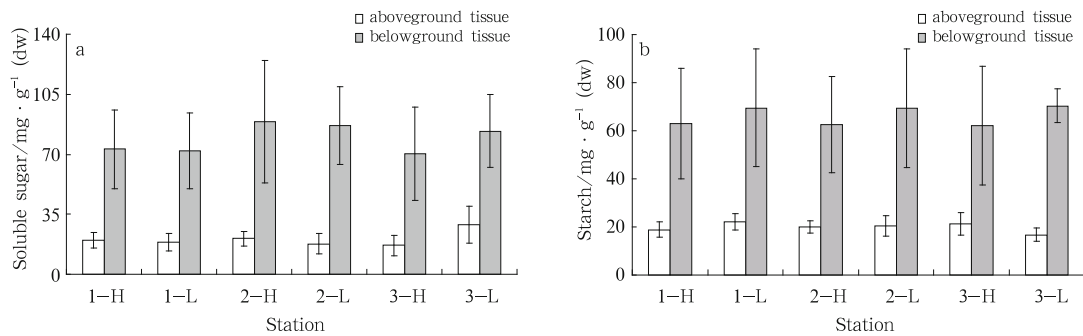


Fig. 2. Concentration of soluble sugar (a) and starch (b) in *T. hemprichii* among sampling sites at the Xincun Bay, South China.

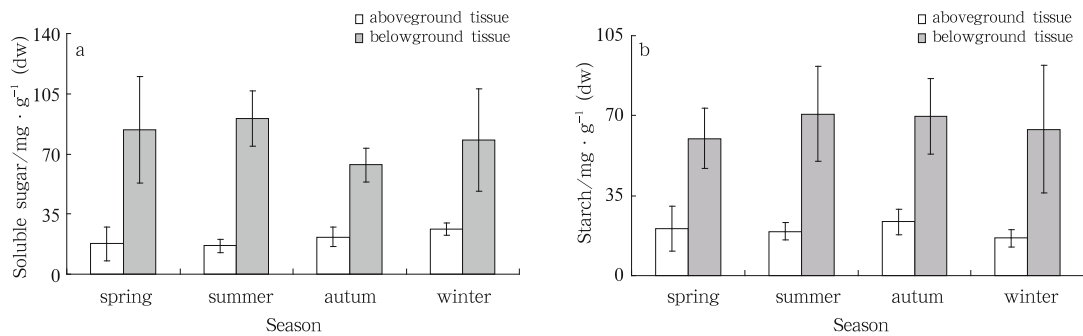


Fig. 3. Seasonal variation of soluble sugar (a) and starch (b) in *T. hemprichii* at the Xincun Bay, South China.

tion of soluble sugar to NSC in aboveground tissue was the highest during winter (61.4%), and the proportion of soluble sugar in belowground tissue was the lowest during autumn (48.2%).

3.3 Effect of shading on nonstructural carbohydrates in *Thalassia hemprichii*

The effect of shading on the NSC in *T. hemprichii* is shown in Fig. 4. There was a strong experimental effect. Shading considerably reduced soluble sugar (aboveground: $F=60.7$, $P<0.01$; belowground: $F=63.8$, $P<0.01$), starch (aboveground: $F=34.39$, $P<0.01$; belowground: $F=42.78$, $P<0.01$) in above-

ground tissue and belowground tissue. Soluble sugar content in both tissues decreased with increasing shading level. Relative to control treatment, both moderate and heavy shade level reduced starch concentration in aboveground and belowground tissue. However, light shading only lowered starch concentration in aboveground tissue, while starch level in belowground tissue was enhanced. Furthermore, the proportion of soluble sugar to NSC in both tissues was reduced by moderate and heavy shading, relative to that in the control treatment. For aboveground tissue, the proportion of soluble sugar to NSC was enhanced by light shading to 63.7%.

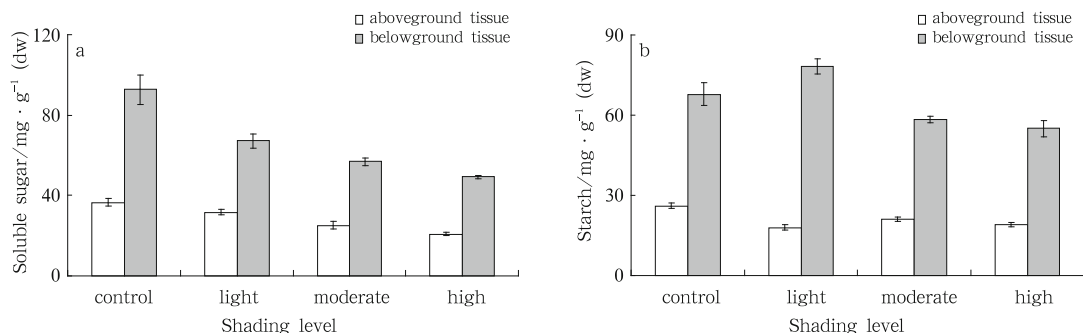


Fig. 4. Effects of shading on the contents of soluble sugar (a) and starch (b) in *T. hemprichii*.

4 Discussion

To the best of our knowledge, this is the first report to discuss the relationship between environmental stress (excessive nutrients, salinity reduction, air exposure, light) and NSC dynamics in seagrasses using field surveys. The present results showed that the dominant carbon storage tissue for *T.*

hemprichii was belowground tissue, with its primary storage compound as soluble sugar. Starch content in belowground tissue of *T. hemprichii* was higher in low intertidal areas than that in high intertidal areas. This indicated that factors other than light intensity impacted the distribution pattern of NSC in *T. hemprichii* along the intertidal gradient. The concen-

tration of soluble sugar in aboveground tissue of *T. hemprichii* was higher during the autumn-winter period than during the spring-summer period. Soluble sugar in belowground tissue showed the opposite trend. The seasonal dynamics of soluble sugar might play an important role in the growth and propagation of seagrass. We found that light shading level, close to the onset saturating light of *T. hemprichii*, did not decrease but rather enhanced starch content in belowground tissue.

4.1 Factors controlling nonstructural carbohydrates dynamics in *Thalassia hemprichii*

For all three transects, there was less starch in belowground tissues of plants from high intertidal areas than plants from low intertidal areas. Physiologically, this may mean that *T. hemprichii* grown in high intertidal area suffer longer durations of air exposure (3 h) than those in low intertidal areas (<1 h). When air exposure extends beyond a certain time, photosynthesis of *T. hemprichii* is weakened by high levels of irradiance, high temperatures, and desiccation (Jupp et al., 1996; Shafer et al., 2007). This decreases NSC synthesis and accumulation. According to our own observations, it took 40–50 min to reach critical threshold of relative water content (RWC_{cr}) of *T. hemprichii*. Before reaching RWC_{cr} , photosynthesis of *T. hemprichii* continuously increased since air exposure, but after the RWC_{cr} was reached, the rate of photosynthesis declined sharply¹⁾. Among all three transects, soluble sugar and its proportion to NSC in belowground tissue was highest in Transect 2, and lowest in Transect 1. Due to freshwater effluent from the shrimp pool, seawater of Transect 2 was reduced to lower salinity at low tide. This changed the osmotic pressure of *T. hemprichii*. Accumulated sugar is one of the main osmotics in seagrasses (Touchette, 2007). In this way, the increased level of soluble sugar in belowground tissue in response to lower salinity was observed in the *T. hemprichii* from Transect 2. Similarly, for *P. oceanica* (Ruíz et al., 2009) and *R. maritime* (Murphy et al., 2003), levels of soluble sugar were also found to increase for osmoregulatory adjustment as well. Excessive amounts of nutrients were produced by fish cage culture system in Transect 1. Nitrogen assimilation requires carbon skeletons for the respiratory pathway and reserves (Leoni et al., 2008). Under excessive nitrogen conditions, the carbohydrate reserves were reallocated to synthesizing amino acids, leading to lower levels of soluble sugar in belowground tissue of *T. hemprichii*. Invers et al. (2004) also found that increased nitrogen availability could affect *P. oceanica* survival through the decrease in their carbon reserves. In this way, the dynamics of NSC in *T. hemprichii* could be used as a bioindicator of human pressure at Xincun Bay (Romero et al., 2007).

Soluble sugar in belowground tissue of *T. hemprichii* showed the following trend: summer>spring>winter>autumn, corroborating seasonal change of light intensity at Xincun Bay [summer: 1 861 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; spring: 1 520 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; winter: 1 438 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; autumn: 1 415 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Xu et al., 2008)]. As shown, summer is the major period of NSC accumulation in belowground tissue of *T. hemprichii*. These reserves can be important for metabolic activity and growth during other seasons, allowing overwintering and re-growth under conditions of negative carbon balance (Alcoverro et al., 2001; Serrano et al., 2011). However, soluble sugar in aboveground tissue of *T. hemprichii* may

accumulate during the autumn-winter period. *T. hemprichii* in Xincun Bay began to flower in early November and the seedlings appeared in late February of the next year (Xu et al., 2008). Accumulation of soluble sugar and starch was more conducive to the differentiation of flower buds, flowering shoot production, and vegetative proliferation (Palacios and Zimmerman, 2007). This was further confirmed by the observation that the concentration of starch in the leaves of *T. hemprichii* was higher in autumn than during other seasons. Likewise, the concentration of soluble sugar in belowground tissue of *T. hemprichii* was lower during the autumn-winter period than during the spring-summer period, indicating an effective soluble sugar translocation from the belowground tissue to aboveground tissue during the autumn-winter period. Sucrose should also be considered to be one of the major carbohydrate reserves in ungerminated seeds, especially in shoots (Sugiura et al., 2009). During seed germination, the stored starch is hydrolyzed to soluble sugar, which serves as energy sources and raw materials for building the structural components of the plant (Koops and Groeneveld, 1990; Sugiura et al., 2009). Therefore, the accumulation of leaf sugar during the autumn-winter period provides a source of carbon and energy source for flower bud formation and seed germination of *T. hemprichii*. Alcoverro et al. (2001) also found that stored carbon resources of *P. oceanica* were used for the typical growth of new leaves in the autumn.

4.2 Response of nonstructural carbohydrates in *Thalassia hemprichii* to shading

The data obtained from the effect of shading on NSC of *T. hemprichii* suggested that *T. hemprichii* could not balance the plant's carbon budget. Shade caused photosynthetic adaptation to low light in *T. hemprichii*, including a reduced relative maximum electron transport rate and minimum saturating irradiance (data not shown). For *T. hemprichii*, the light shading level (150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) reduced levels of soluble sugar in both types of tissue and starch in aboveground tissue, but increased starch content in belowground tissue. Because light shading level was close to the onset saturating light of (182±23) $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Pollard and Greenway, 1993), the concentration of NSC in belowground tissue did not always change, but a decrease in the relative amount of soluble sugar to total NSC was observed. Furthermore, the soluble sugar content and its proportion to NSC in belowground tissue decreased as the amount of light decreased. There are two possible reasons for this: the first is lower levels of photosynthesis under shade conditions may decrease NSC synthesis and the export of incorporated carbon to the non-photosynthetic organs (Uy, 2001). The second is increased carbon demand for belowground tissue to respire anaerobically during low photosynthetic periods caused by low light (Lee and Dunton, 1997; Lee et al., 2007). Similarly, the changes in the carbohydrate content of *Syringodium isoetifolium* showed a promise of being as sub-lethal indicators of light reduction (Fokeera-Wahedally and Bhikajee, 2005).

The large mass of its belowground tissue gives *T. hemprichii* a large capacity to store carbohydrate reserves (Longstaff and Dennison, 1999; Alcoverro et al., 2001; Collier et al., 2009), and delays the need to make structural changes to balance metabolic processes under light deprivation (Collier et al., 2011). This allows *T. hemprichii* to tolerate reduced light conditions for considerably longer periods than species

¹⁾Jiang Zhijian, Huang Xiaoping, Zhang Jingping, et al. 2013. The effects of air exposure on the desiccation rate and photosynthetic activity of *Thalassia hemprichii* and *Enhalus acoroides*.

with less belowground biomass, such as *Zostera muelleri* and *Z. noltii*. A complete burial experiment performed on *Z. noltii* showed that the plants died between the first (7% mortality) and the second week (67% mortality) (Cabaco and Santos, 2007). This shows that the belowground tissue of *T. hemprichii* plays an important role in supplying carbohydrates during early periods of shading. Despite the benefits of the large belowground biomass of *T. hemprichii* for storage, it can also pose a considerable respiratory burden (Alcoverro et al., 2001; Ralph et al., 2007; Collier et al., 2009). If shading event occurs in summer, it can reduce carbon reserve accumulation in *T. hemprichii*. This may lead to inhibition of flower bud formation in autumn and seed germination in winter. Also, the time required to re-establish reserves can be an important determinant of a meadow's ability to withstand repeated shading events (Collier et al., 2009).

In summary, our results showed that nutrient enrichment, freshwater discharge, and air exposure all affected NSC dynamics in *Thalassia hemprichii* among transects at Xincun Bay. Light intensity, seagrass flower bud formation, and seed germination were all found to induce seasonal changes in NSC in *T. hemprichii*. Short-term light reduction resulted in decreased concentrations of NSC. The dynamics of NSC in *T. hemprichii* and its response to shading indicated that NSC, especially soluble sugar in belowground tissue, can provide a rapid and precise indicator of environmental stress.

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