Carbon Transfer Processes of Food Web and Trophic Pathways in a Tropical Eutrophic Seagrass Meadow

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Seagrass meadows provide important habitats and rich organic carbon sources for consumers at different trophic levels but are threatened by accelerating eutrophication in coastal waters. Nevertheless, at present, carbon transfer processes throughout the food web and trophic pathways in eutrophic seagrass meadows are still poorly known. To resolve this issue, carbon sources of different trophic communities in a eutrophic tropical seagrass meadow [Xincun (XC) bay, South China Sea] under eutrophication were examined in summer and winter using dual stable isotopes. The $\delta^{13}C$ value of omnivores and carnivores overlapped more with that of herbivores and planktivores/filter feeders, which mainly overlapped with that of epiphytes in summer and macroalgae in winter. Meanwhile, epiphytes and macroalgae exhibited high biomass and corresponding highest contribution to herbivores, omnivores, and carnivores in summer and winter, respectively. These results suggest that the grazing food chain was the main trophic pathway in this eutrophic seagrass meadow, and that the transfer of carbon flow in the grazing food chain was mainly dominated by the proliferating epiphytes or macroalgae carbon. In contrast, the contribution of seagrass to detritivores in both seasons was higher than that of other food sources. Our findings suggest that in eutrophic tropical seagrass meadows, the proliferation of epiphytes or macroalgae induced by high nutrient loading, as well as their seasonal changes, has a greater impact on the transfer of carbon in the grazing food chain than that in the detritus food chain, and the seagrass fueled the food web mainly through the detritus food chain.

Keywords: seagrass meadow, eutrophication, carbon sources, carbon transfer process, grazing pathway, detritus pathway

INTRODUCTION

Seagrass meadows are highly complex and productive marine ecological systems that provide numerous ecological services. They not only provide spawning grounds (Jiang et al., 2020), nursery regions (Madi Moussa et al., 2020), habitat, and predation refuge areas for abundant fish, crabs, and other invertebrates (Barry et al., 2021; Tew et al., 2021), but they also provide nutrients and energy

sources for the food web of the ecosystem (Park et al., 2013). Seagrass is not the only carbon source of this food web, which may also include significant contributions from other carbon sources, such as macroalgae, epiphyte, phytoplankton, and sediment microphytobenthos (Ouisse et al., 2012). However, the fate of these organic carbon sources remains controversial.

Seagrass is generally considered to have poor nutrition and low palatability for herbivores (Vergés et al., 2011; Boudouresque et al., 2016). Therefore, it is generally considered to be rarely ingested by herbivores (Nakamoto et al., 2019). However, mild seasonal variations in tropical seagrass meadows generally allow seagrass to remain abundant throughout the year (Nakamoto et al., 2019), which may increase the likelihood and intensity of seagrass consumption by herbivores (Heck et al., 2020). For example, previous reports have suggested that living seagrass provided main carbon sources for numerous herbivores and invertebrates in non-eutrophic tropical seagrass meadows (Burkholder et al., 2012; Chiu et al., 2013; Villa et al., 2019).

However, coastal seagrass meadows are threatened by accelerating eutrophication due to rapid economic development (Jiménez-Ramos et al., 2019; Kermagoret et al., 2019; Li et al., 2019). A vast amount of nutrient loading may cause the proliferation of phytoplankton, epiphytes, and opportunistic algae, which results in shading seagrass, eventually leading to the migration of primary producer structures from seagrass to phytoplankton and opportunistic algae-dominated communities in seagrass meadows (France, 2015; Barnes, 2019; Robertson and Savage, 2020). These variations would undoubtedly affect the feeding choices of consumers and the transfer pathway of carbon flow throughout the food web of seagrass meadows, but little is known about them. Previous studies on seagrass meadows have explored the spatial effects of eutrophication on the structure of primary producers (Han et al., 2016), trophic relations (Baden et al., 2010; Schmidt et al., 2017), and food web structure (Tewfik et al., 2007; Coll et al., 2011) from a spatial perspective. However, seasonal changes may also influence the structure of primary producer communities (Riegl et al., 2005; Qiu et al., 2017), and there is still little evidence of how temporal changes affect carbon transfer processes and carbon flow pathways of the food web in tropic eutrophic seagrass meadows.

Xincun bay is a tropical, nearly closed bay located in the southeast of Hainan island, China, and has a typical seagrass meadow (Huang et al., 2019). This seagrass meadow distributes abundant and diverse seagrass throughout the year (Huang et al., 2006), and has a long history of fish cage culture, which results in substantial nutrient loading (Liu et al., 2017). Meanwhile, the abundance of macroalgae *Ulva pertusa* fluctuates seasonally, with the highest and lowest abundance during winter and summer, respectively (Xu et al., 2009; Huang et al., 2019). Therefore, this study provides us with a natural experimental site to explore the key carbon transfer processes of the food web and trophic pathways in eutrophic tropical seagrass meadows.

In this study, we collected the main organic carbon sources, namely, seagrass, epiphytes, macroalgae, particulate organic matter (POM; mainly including phytoplankton) and sediment organic matter (SOM), and consumers in the summer and winter of the XC seagrass meadow to (1) explore the main carbon flow

pathway of the food web in the eutrophic seagrass meadows, (2) study the influencing factors of carbon transfer in grazing and detritus food chain, and (3) assess the trophic role of seagrass in the food web of eutrophic seagrass meadows.

## MATERIALS AND METHODS

Study Area
This study was carried out in a tropical mixing seagrass meadow in XC (southeastern Hainan island, South China Sea, **Figure 1**), with *Enhalus acoroides* and *Thalassia hemprichii* as the dominant seagrass species (Huang et al., 2019). Seagrass mainly grows in a sandy substrate with an average water depth of <1.5 m from the southeast to the southwest of the bay (Huang et al., 2006).The total seagrass area is 175 ha (Huang et al., 2019). A vast amount of seagrass (*E. acoroides* and *T. hemprichii*) leaf detritus accumulated in the southern shallow waters of this bay.

Sample Collection, Processing, and Analysis
Samples were collected from four stations during low tide (**Figure 1**) in summer (August 2018) and winter (January 2019). One to three samples of each item were collected from each station. The POM samples were collected through filtering surface seawater samples onto pre-combusted Whatman GF/F filters (0.7 μm pore size, heated for 3 h at 450°C). The seawater nutrient samples were collected from previously filtered seawater and stored in bottles. The SOM was sampled by scraping the upper 1 cm layer using a spade. The samples of seagrass (*E. acoroides* and *T. hemprichii*) and macroalgae (*Ulva pertusa*) were collected and washed by hand. Meanwhile, in order to analyze the biomass of macroalgae, a 0.25-m² quadrat was used when the macroalgae samples were collected. The epiphyte samples were scraped from the surface of fresh seagrass leaves that had been measured in the area. The fish and crustacean samples were collected with a combination of trammel and fishing trap nets, while the other invertebrates were collected using a mesh screen (0.5 mm mesh). All the samples were stored at −20°C immediately after collection.

In the laboratory, the filters (POM samples) and epiphytes were acidified with 1 N HCl for 24 h to remove inorganic carbonates. The SOM samples were also acidified (1 N HCl) until the bubbling stopped to remove carbonates. All the acidified samples were washed with distilled water. The washed samples and seagrass, and macroalgae samples were dried at 60°C for 48 h. After weighing, the biomass of epiphytes and macroalgae was calculated and represented as the dry weight per square meter (g DW/m²). The dried samples, namely, seagrass, macroalgae, epiphyte, and SOM, were ground into a fine powder using a mortar and pestle. Muscle tissues were taken from the consumer samples and stored at −20°C after identification, weighing, and measurement. The frozen muscle sample was also ground into a fine powder using mortar and pestle after being dried. The POM and powdered samples were stored in a desiccator before analysis.

The dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) and dissolved inorganic phosphate (DIP) were measured following the methods developed by

FIGURE 1 | Sample sites in Xincun bay, Hainan island, South China Sea.
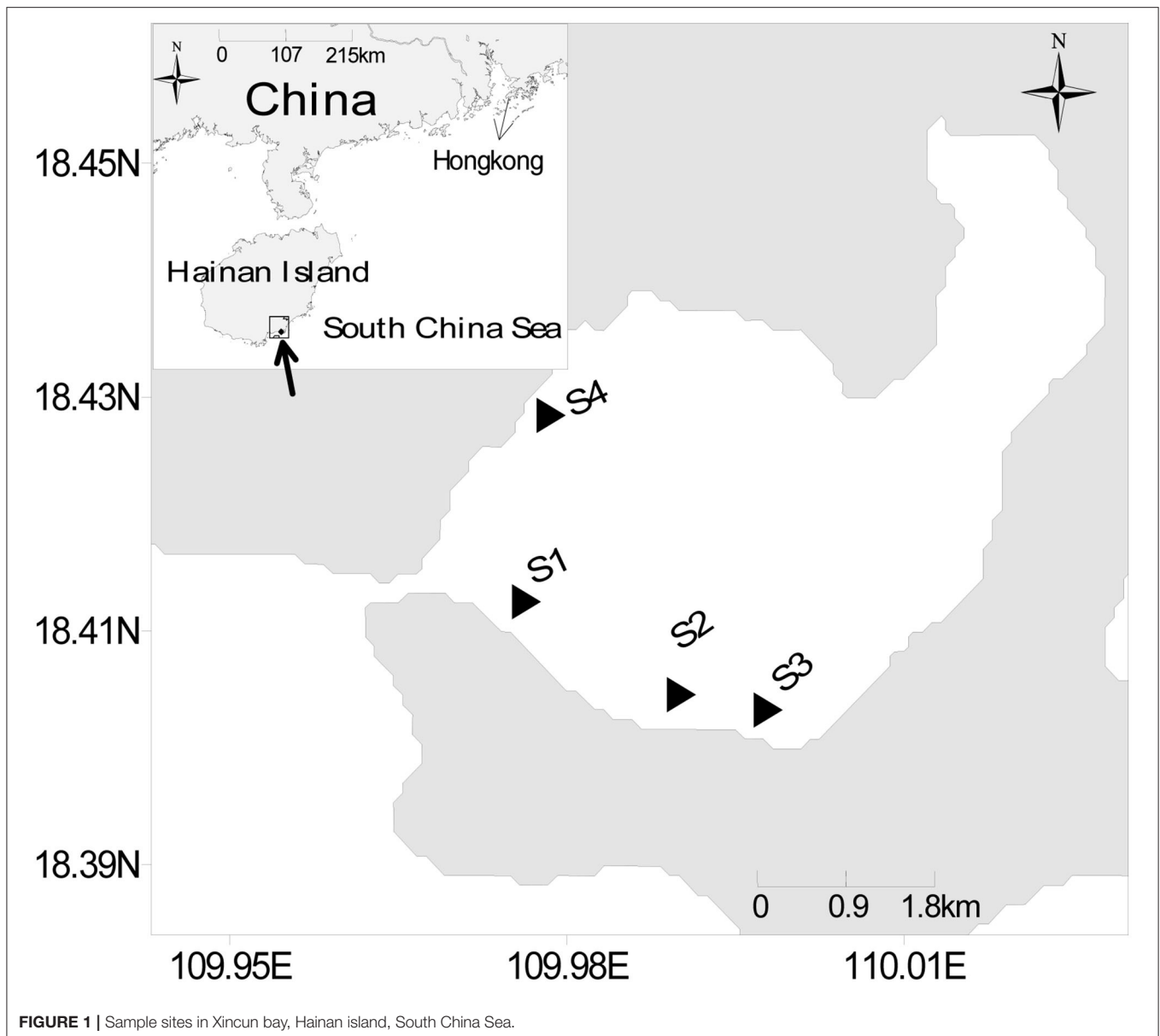
Grasshoff (2009) using a CANY 722s spectrophotometer. Stable isotope compositions of all the samples were analyzed using a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific, Waltham, MA, United States). Stable isotope ratios were calculated using the following formula:

$$\delta^{13}C \text{ or } \delta^{15}N \ (\%) = (R_{sample}/R_{standard} - 1) \times 1,000$$

where δ is the deviation from the standard reference material (Vienna Pee Dee Belemnite and atmospheric N_2) in parts per mil (%), and R represents the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. In sample analysis, protein B2115-114859 (Elemental Microanalysis Ltd.)

($\delta^{13}C = -26.98\%$, $\delta^{15}N = 5.94\%$) was used as a working standard with an analytical uncertainty of $<0.15\%$ for stable isotope values.

Data Analysis

There remains a controversy about classifying consumers into the specific trophic group since the same consumer may have different dietary characteristics in different geographical environments. However, a discriminant function analysis (DFA) has been successfully conducted to classify samples into groups (Jepsen and Winemiller, 2002; Burress et al., 2013; Yoon et al., 2015). $\delta^{13}C$ and $\delta^{15}N$ values were used as variables to examine whether samples could be classified into predefined trophic groups. To determine the main carbon flow pathway in the seagrass meadow and the role of seagrass in the food web, we

predefined trophic groups, based on the available literature, into herbivore, detritivore, planktivore/filter feeder, omnivore, and carnivore (**Supplementary Table 1**).

Additionally, Student's t-test was performed to test the seasonal differences in seawater nutrient, macroalgae, and epiphyte biomass, and the isotope ratios of food sources and consumers. Before this test, normality and homogeneity of the data were examined by the Shapiro–Wilk test and Levene's test, respectively. The non-parametric two-sample Mann–Whitney U-test was performed when the t-test assumptions failed. All the statistical analyses were performed using the IBM SPSS statistics 26 software, and a $p < 0.05$ indicated a statistically significant difference.

The contribution rate of potential food sources to consumers was estimated using the Bayesian model in the SIMMR package (Parnell and Inger, 2019), which was based on the SIAR package (Parnell et al., 2010), and implemented in the R 3.6.0 software (R Core Team, 2019). In order to reduce the number of food sources in the calculations, *E. acoroides* and *T. hemprichii*, and their epiphytes were considered as a single group called seagrass and epiphyte, respectively. Meanwhile, POM and SOM were not considered as potential food sources of herbivores based on the published literature (Du et al., 2019; Lin et al., 2021). In addition, considering that SOM is a mixture of other primary food sources (i.e., seagrass, epiphytes, macroalgae, and POM) (Carlier et al., 2009; Xu et al., 2018), we excluded it from the mixing model. Therefore, for herbivores, the potential food sources used in the SIMMR mixing model only included seagrass, epiphyte, and macroalgae. For the other trophic groups (i.e., planktivores, detritivores, omnivores, and carnivores), the potential food sources included seagrass, epiphytes, macroalgae, and POM. Except for the mean isotopic ratios (and SE) of each food source and the isotopic ratios of consumers, the SIMMR mixing model still required trophic enrichment factors (TEFs). Considering there are different trophic groups (see Section Data Analysis), we, therefore, used the widely accepted TEFs (**Table 1**) to estimate the direct contribution of food sources to herbivores, planktivores, detritivores, and omnivores, and the indirect contribution of food sources to carnivores. The SIMMR mixing model was not used to compute the contribution of low trophic level fauna (i.e., polychaetes, small crustaceans, and bivalves) to high trophic level consumers (i.e., omnivores and carnivores) because, in some cases, similar potential prey for high trophic level consumers had different isotope compositions. Therefore, such contributions were qualitatively determined by evaluating the isotope similarity between them and considering the TEFs (Abrantes and Sheaves, 2009). The outputs of the contribution rates in this model were expressed as 2., 25, 50, 75, and 97.5% credible interval (CI).

RESULTS

Seawater Nutrients and Biomass of Macroalgae and Epiphytes

The concentrations of DIN and DIP in the seawater of XC bay ranged from 5.5 to 22 and 0.2 to 2.9 µM, respectively.

The average concentrations of DIN and DIP were 9 and 5 µM in summer, and 16 and 1.6 µM in winter, respectively. A statistical analysis displayed significant differences in DIN and DIP between the seasons ($p < 0.05$), with higher concentrations in winter (**Figure 2**). Similarly, significant differences in the biomass of macroalgae and epiphytes were also observed between the seasons, with markedly higher and lower values in winter for macroalgae and epiphytes, respectively.

Stable Isotope Composite of Food Sources

The δ^{13}C values of each food source were similar between the two seasons, particularly for seagrass, epiphytes, and SOM, with annual average values of −9.7, −13.5, and −14.8%, respectively (**Table 2**). Significant differences were found in the δ^{13}C values of macroalgae and POM between the two seasons, which both had higher values in summer. Among all the food sources, POM showed the lowest δ^{13}C values in both summer (mean = −16%) and winter (mean = −19%), while the highest δ^{13}C values were from macroalgae (mean = −7.3%) and seagrass (mean = −10.1%) in summer and winter, respectively.

The δ^{15}N values of seagrass, epiphyte, macroalgae, POM, and SOM were also similar between summer and winter, with the corresponding annual average values of 5.6, 7.5, 9.6, 5.3, and 6.1%, respectively. In both seasons, POM and macroalgae had the minimum and maximum δ^{15}N values, respectively (**Table 2**).

Isotope Values of Trophic Groups

A total of 74 and 78 species of consumers were collected in summer and winter, respectively (**Figure 3**). Based on the isotope ratios of the consumers, the DFA revealed that 93.8 and 87.5% of consumers could be successfully classified into the predefined trophic groups in summer and winter, respectively.

The δ^{13}C values of consumers showed a significant difference between seasons ($U = 2,563.5$, $p = 0.01$), with values ranging from −18.6 ± 0.2 (mean ± SE for carnivorous fish *Abudefduf vaigiensis*) to −10% (detritivorous gastropod *Notosinistersubaura*) in summer and from −18.6 (carnivorous fish *Glossogobius aureus*) to −8.5 ± 3.1% (mean ± SE for detritivorous echinoderm *Archaster typicus*) in winter, respectively. Among the trophic groups, planktivores/filter feeders displayed the smallest δ^{13}C ranges in both summer (1.4%) and winter (1.4%), while the maximum δ^{13}C ranges were displayed by carnivores (8.5%) and herbivores (82%) in summer and winter, respectively. Meanwhile, the average δ^{13}C values of omnivores ($t_{50} = 2.577$, $p = 0.013$) and herbivores ($U = 15$, $p = 0.019$) varied significantly between seasons (**Figure 3**), with the values (mean ± SE, hereinafter the same) being −13.7 ± 1.2 and −12 ± 0.9%, respectively, in summer, and −14.8 ± 1.5 and −14.3 ± 2.2%, respectively, in winter.

Similar δ^{15}N range of consumers was identified in both seasons, with values ranging from 7.1 for detritivorous echinoderm *Hemicentrotus pulcherrimus* to 13.9 ± 0.1% for carnivorous fish *Scomberoides lysan* in summer and from 7.4 ± 0.3 for planktivorous bivalve *Trachycardium sp.* to 13.8 ± 0.8% for carnivorous fish *Lutjanus fulviflamma* in winter, respectively. Among the trophic groups, the δ^{15}N values of herbivores, detritivores, omnivores, and carnivores were all higher in winter

TABLE 1 | Mean ± standard deviation (SD) of trophic enrichment factors (TEFs) used to calculate the contribution of potential food sources to the different trophic groups.

Trophic groups	$\delta^{13}C$ (%)	Source	$\delta^{15}N$ (%)	Source
Detritivores	0.4 ± 1.3	Carvalho et al., 2020	3.2 ± 1.2	Riccialdelli et al., 2017
Planktivores	0.4 ± 1.3	Carvalho et al., 2020	3.2 ± 1.2	Riccialdelli et al., 2017
Herbivores	0.5 ± 1.2	Mascart et al., 2018	3.2 ± 1.2	Riccialdelli et al., 2017
Omnivores	0.6 ± 1.2	Beesley et al., 2020	3.7 ± 0.7	Dézerald et al., 2018
Carnivores	0.91 ± 1.04	Xu et al., 2018	4.6 ± 1.2	Kaymak et al., 2018
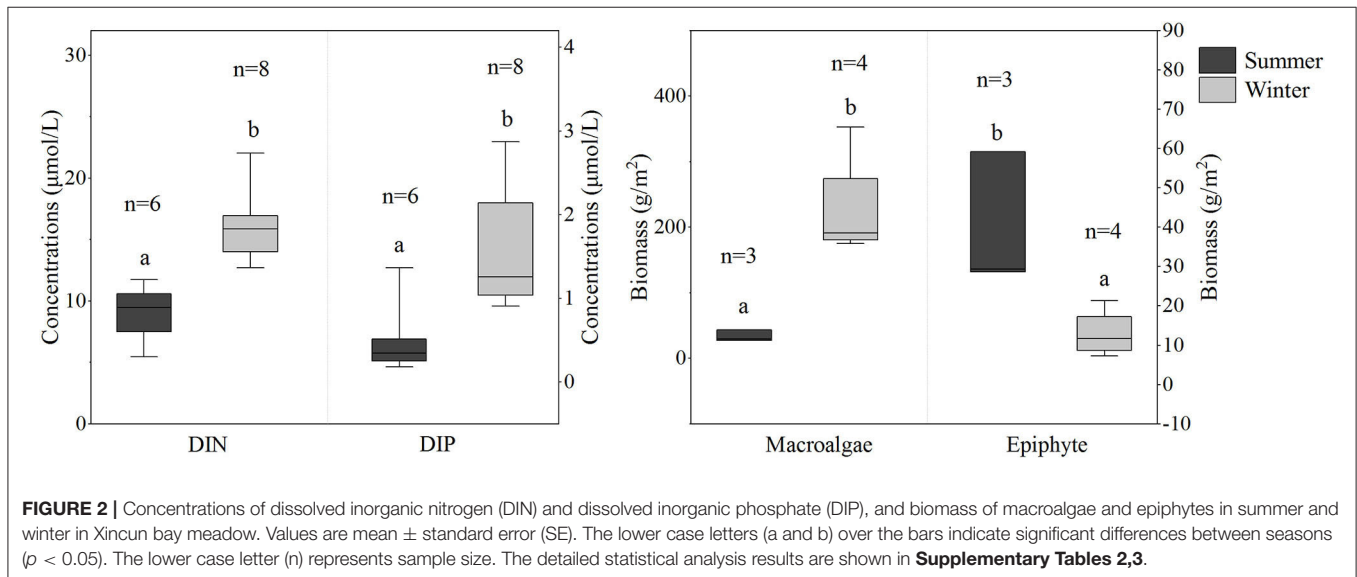
FIGURE 2 | Concentrations of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP), and biomass of macroalgae and epiphytes in summer and winter in Xincun bay meadow. Values are mean ± standard error (SE). The lower case letters (a and b) over the bars indicate significant differences between seasons ($p < 0.05$). The lower case letter (n) represents sample size. The detailed statistical analysis results are shown in **Supplementary Tables 2,3**.

TABLE 2 | The $\delta^{13}C$, $\delta^{15}N$ values and sample size (mean ± SD) of food sources in summer and winter in Xincun bay.

Food sources	$\delta^{13}C$ (%)		$\delta^{15}N$ (%)		Sample size (n)	
	Summer	Winter	Summer	Winter	Summer	Winter
Seagrass	−10.1 ± 0.8[a]	−9.5 ± 0.5[a]	5.4 ± 0.7[a]	5.9 ± 1.6[a]	12	14
Epiphyte	−13.5 ± 1.3[a]	−13.5 ± 1.3[a]	7.4 ± 0.8[a]	7.6 ± 0.4[a]	6	7
Macroalgae	−7.3 ± 1.1[b]	−15.0 ± 1.7[a]	9.4 ± 0.4[a]	9.9 ± 0.1[a]	4	4
POM	−16.0 ± 0.9[b]	−19.0 ± 0.5[a]	5.1 ± 1.1[a]	5.5 ± 0.2[a]	5	3
SOM	−14.8 ± 0.4[a]	−14.7 ± 1.6[a]	5.5 ± 0.5[a]	6.6 ± 0.5[a]	3	4

Different lower-case letters (a and b) indicate significant differences ($p < 0.05$) for $\delta^{13}C$ and $\delta^{15}N$ of food sources between summer and winter. Detailed statistical analysis results are in **Supplementary Table 4**.
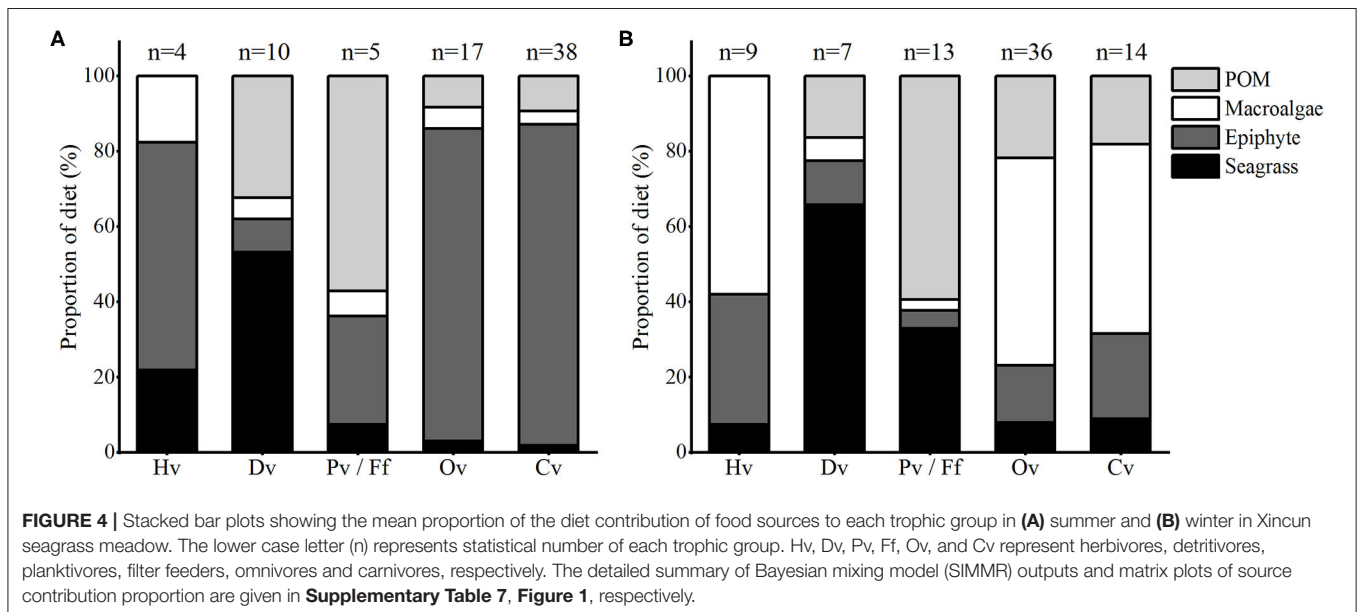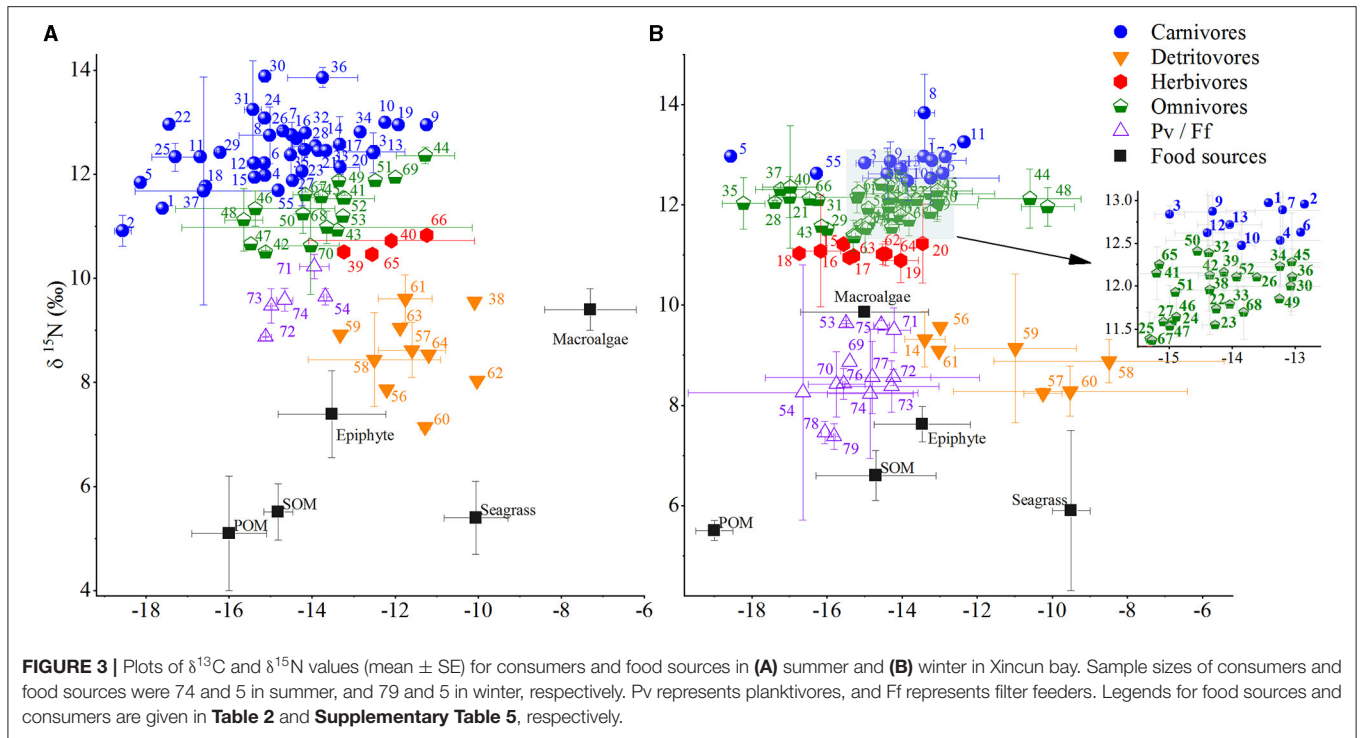
than in summer, but they were inverse for planktivores/filter feeders. Meanwhile, the $\delta^{15}N$ values of planktivores/filter feeders, omnivores, and carnivores varied significantly between seasons (**Supplementary Table 6**), with average values of 9.6, 11.3, and 12.5% in summer, and 8.5, 11.9, and 12.9% in winter, respectively.

Contribution of Food Sources to the Trophic Groups

The results of SIMMR mixing model suggested a clear seasonal distinction in the contribution of food sources to most consumers (**Figure 4**, **Supplementary Table 7**, **Figure 1**), especially for the contribution of food sources to omnivores and carnivores. In summer, epiphytes exhibited higher contribution to omnivores (83, 95% CI: 64–95%, **Supplementary Figure 1a**)

and carnivores (85, 95% CI: 73–95%, **Supplementary Figure 1b**) compared with other food sources, while in winter, macroalgae contributed more to omnivores (55, 95% CI: 46–62%, **Supplementary Figure 1c**) and carnivores (50, 95% CI: 37–63%, **Supplementary Figure 1d**). On the contrary, the contribution of food sources to detritivores showed clear seasonal stability. In both seasons, seagrass contributed most to detritivores (mean > 53%, **Supplementary Figures 1e,f**).

For herbivores, large uncertainties seemed to hamper accurate quantifications. While in summer, the posterior probability distribution for epiphytes shifted strongly toward higher values (**Supplementary Figure 1g**), and the contribution proportion of epiphytes (61, 51–73%) was significantly higher than that of other food sources within the CI of 50% (**Supplementary Table 7**).

FIGURE 3 | Plots of δ^{13}C and δ^{15}N values (mean ± SE) for consumers and food sources in (A) summer and (B) winter in Xincun bay. Sample sizes of consumers and food sources were 74 and 5 in summer, and 79 and 5 in winter, respectively. Pv represents planktivores, and Ff represents filter feeders. Legends for food sources and consumers are given in **Table 2** and **Supplementary Table 5**, respectively.

FIGURE 4 | Stacked bar plots showing the mean proportion of the diet contribution of food sources to each trophic group in (A) summer and (B) winter in Xincun seagrass meadow. The lower case letter (n) represents statistical number of each trophic group. Hv, Dv, Pv, Ff, Ov, and Cv represent herbivores, detritivores, planktivores, filter feeders, omnivores and carnivores, respectively. The detailed summary of Bayesian mixing model (SIMMR) outputs and matrix plots of source contribution proportion are given in **Supplementary Table 7**, **Figure 1**, respectively.

On the contrary, in winter, the contribution of macroalgae (58, 51–65%) was significantly higher than that of epiphytes (35, 27–43%) and seagrass (8, 4–10%) within the CI of 50%. For planktivores, in summer, the contribution proportions of seagrass (8%, 95% CI: 1–19%) and macroalgae (7%, 95% CI: 1–16%) were the lowest, although the contribution range of epiphytes (95% CI: 6–53%) and POM (95% CI: 36–77%) overlapped a lot, the posterior probability distribution for POM shifted strongly toward higher values, and the contribution of POM was significantly higher than that of epiphytes within the CI of 50% (**Supplementary Table 7**). However, in winter, the

contribution of POM was significantly higher than that of the other food sources (**Supplementary Table 7**).

DISCUSSION

Isotope Ratios Characteristics of Food Sources
The δ^{13}C and δ^{15}N values of seagrass, epiphytes, and SOM fell within the range of previously reported values of the seagrass meadow distributed in the Indo-Pacific region (Du et al., 2016) and this study area (Fan et al., 2011; Liu et al., 2017). Higher

$\delta^{15}N$ values were observed for the five food sources in winter than in summer, which might be attributed to anthropogenic nitrogen sources (Arbi et al., 2018; Becherucci et al., 2019). In this study, this was evidenced by significantly high seawater nutrient concentration in winter. In contrast, a significant seasonal difference in $\delta^{13}C$ values was only observed in POM and macroalgae, with higher values in summer. Similarly, substantial temporal variation in the stable isotopes of POM and macroalgae was also found in *Zostera marina* and *Cymodocea nodosa* seagrass meadows (Vizzini and Mazzola, 2003; Mittermayr et al., 2014), respectively. Such seasonal variations may be attributed to environmental factors and/or biotic physiological processes (Mittermayr et al., 2014; Jankowska et al., 2019; Nakamoto et al., 2019).

The existing literature shows that the decomposition of macroalgae is one of the main reasons for the increase in their $\delta^{13}C$ values (Corbisier et al., 2004). Although high temperatures in summer usually result in the death and decomposition of macroalgae (Guo et al., 2006), this might not be the main reason for the extremely high isotope values of macroalgae in summer, since macroalgae decomposition usually causes small $\delta^{13}C$ variations (within 1%) (Raven et al., 2002). Wang and Yeh (2003) suggested that organisms with $\delta^{13}C$ values >-10% must use bicarbonate (HCO_3^-). In this site, the pH in summer was usually higher than that in winter (http://hnsthb.hainan. gov.cn), indicating that CO_2 in the seawater might be limited in summer. A previous study has proved that macroalgae are capable of both C3 and C4 photosynthesis and can take up HCO_3^-, which has higher $\delta^{13}C$ values than CO_2 (Wang and Yeh, 2003; Xu et al., 2012; Valiela et al., 2018). The limited CO_2 concentration resulted in higher HCO_3^- absorption, which might cause macroalgae to have higher $\delta^{13}C$ values in summer. In addition, higher irradiance and growth rate can also affect the isotope composition of primary producers. It has been reported that macroalgae have higher $\delta^{13}C$ values with increased photon fluence rates and growth rate, where photon fluence rate acts as a major factor between the two, and 10 to 25% ^{13}C can accumulate in macroalgae (Wiencke and Fischer, 1990; Wang and Yeh, 2003). Therefore, the higher $\delta^{13}C$ values of macroalgae in summer might result from mixed factors, but this requires further research.

In contrast, $\delta^{13}C$ variations in POM are often attributed to differences in growth rate (Gu et al., 2006) and species composition (Sun et al., 2012). Previous studies have shown that a high growth rate of phytoplankton could lead to strong ^{13}C enrichment (Gu et al., 2006), and that there was no obvious change in the composition of species in this site (Wei et al., 2014). Therefore, the significantly high $\delta^{13}C$ of POM in summer may be mainly due to its high growth rate. This was further proved by the high summer biomass of POM in this site (Wei et al., 2014). In addition, decomposed macroalgae were observed in this site in summer, which might also result in higher $\delta^{13}C$ values for POM (Malet et al., 2008; Schaal et al., 2010). It is interesting that the $\delta^{13}C$ values for POM in this study were close to the values previously measured in the same site (Fan et al., 2011) and around the mid south coast of Korea (Kang et al., 2009), but higher than values dominated by marine phytoplankton (Schaal et al., 2010; Du et al., 2019). Such higher $\delta^{13}C$ values for

POM may be attributed to the specific taxonomic composition of phytoplankton in this site. The $\delta^{13}C$ values of the diatom-dominated coarse POM were generally higher than those of the nanoplankton-dominated POM (e.g., nano-flagellates) (Kang et al., 2009). A previous study has proved that diatoms are the dominant species of phytoplankton in present study area (Wei et al., 2014). Therefore, the high contribution of diatoms to the phytoplankton biomass might be the main reason for the higher $\delta^{13}C$ values of POM in this site.

Key Carbon Transfer Processes and Major Trophic Pathway in the Seagrass Meadow

This study showed that herbivores have a wide range of $\delta^{13}C$ values, which overlaps rarely with that of seagrass, but wider than that of epiphytes in summer and macroalgae in winter. This indicates that macroalgae or epiphytes would be preferentially fed upon. A previous study has suggested that herbivores prefer to consume high-protein primary producers with lower C/N ratios (Macreadie et al., 2017). *Enhalus acoroides* and *T. hemprichii*, which are dominated in the investigated area, have richer lignocellulose tissue and lower nitrogen content than macroalgae and epiphytes, making them less nutritious and energetically favorable for consumption by herbivores (Enríquez et al., 1993; Macreadie et al., 2017). Therefore, the seagrass meadow in XC bay might mainly provide a habitat and shelter for herbivores, while the food was mainly supplied by macroalgae and epiphytes. Meanwhile, a large number of epiphytes consumed by herbivores may also cause seagrass leaves to fall on the sediment floor, thus indirectly providing seagrass carbon sources for detritivores and decomposers (Hily et al., 2004; Ouisse et al., 2012).

The $\delta^{13}C$ values of detritivores in the XC meadow were close to those of epiphytes and seagrass. However, the more depleted $\delta^{15}N$ values in detritivores indicated that seagrass was their main food source, since the enrichment factors of detritivores that were the main benthic invertebrates in our study ranged from 2.7‰ to 3.6‰ (average 3.2‰) (Zanden and Rasmussen, 2001; Parker et al., 2008; Caut et al., 2009; Taylor et al., 2017). Detritivores are known to feed on decomposed plant materials rather than living plants (Platell et al., 2009; Lebreton et al., 2012; Ouisse et al., 2012), and previous studies in this study area (Fan et al., 2011) and other temperate seagrass meadows (Mascart et al., 2018) have shown that there was no obvious difference in the isotope composition between living seagrass leaves and its detritus. Therefore, seagrass carbon in the study area probably passes into the food web mainly through the detritus pathway. Similarly, seagrass was rarely consumed directly by herbivores and was passed into the food web more through detritivores feeding on dead seagrass laves, which was also found in temperate seagrass meadows (Boudouresque et al., 2016; François et al., 2018).

In addition, previous studies have shown that seagrass could also provide food sources for planktivores/filter feeders through small seagrass detrital particles (POM and SOM) formed by microbial degradation (Thresher et al., 1992; Vizzini et al., 2002). In this study, the food sources of planktivores/filter feeders were indeed POM and SOM, as evidenced by the more depleted

δ¹³C values for planktivores/filter feeders, POM, and SOM. However, the main component of POM was phytoplankton (see Isotope Ratios Characteristics of Food Sources), and macroalgae and epiphytes had more contribution than seagrass to SOM in this site (Liu et al., 2016a). Therefore, the carbon sources of planktivores/filter feeders might originate more from phytoplankton, macroalgae, and epiphytes. Seagrass might play a greater role in the collection of food for filter feeders and planktivores by decreasing water flow to enhance the sedimentation of suspended particulate matter (Larkum et al., 2006) and capturing particulates on its leaves by the seagrass canopy and plants (Kennedy et al., 2004), respectively.

The δ¹³C values of predators (omnivores and carnivores) exhibited a characteristic of overlapping more with those of epiphytes in summer, and macroalgae and epiphytes in winter. This indicated that the main trophic base of high trophic level consumers originated from macroalgae or epiphytes. This result is consistent with other studies on eutrophic temperate seagrass meadows with abundant macroalgae or epiphytes (Hyndes and Lavery, 2005). However, they are different from previous studies on non-eutrophic tropical seagrass meadows, where most high trophic level consumers were supported mainly by carbon that originated from seagrass and epiphytes (Connolly and Waltham, 2015; Du et al., 2016, 2019), which might be attributed to the lack of or low biomass of macroalgae there. Furthermore, the δ¹³C values of most predators were relatively concentrated and overlapped more with those of herbivores and planktivores/filter feeders rather than detritivores, suggesting that the main trophic pathway of the food web was the grazing pathway (involving a combination of phytoplankton, macroalgae, epiphytes, herbivores, planktivores/filter feeders, and their predators) in the XC seagrass meadow.

Flow of Seagrass Carbon in Food Web and Its Influencing Factors

In this study, the eutrophication-induced proliferation of macroalgae and epiphytes, as well as their seasonal changes, significantly affected their contribution to the diet of consumers and carbon transfer in the food chain, but this effect was observed only on specific species/groups. According to the SIMMR result (**Figure 4**, **Supplementary Table 7**), the transfer of seagrass carbon in the grazing food chain pathway was markedly limited by the abundance of epiphytes and macroalgae, at least compared with the situation of overgrazing by large grazers (Valentine and Duffy, 2006; Hyndes et al., 2014). In summer, epiphytes with higher biomass contributed significantly to the diets of herbivores and omnivores. In winter, epiphytes still contributed to the diet of herbivores to some extent, which might be attributed to the relatively conservative feeding habits of some herbivores (Bremm et al., 2016), while herbivores and omnivores mainly relied upon palatable and abundant macroalgae (see Contribution of Food Sources to the Trophic Groups). Meanwhile, the predators mainly preyed on herbivores rather than detritivores in both seasons (see Key Carbon Transfer Processes and Major Trophic Pathway in the Seagrass Meadow). These indicated that the carbon flow pathway of this seagrass meadow food web was affected by the seasonal abundance of epiphytes and macroalgae, and that epiphyte- and macroalgae-derived carbon was mainly transmitted through herbivores/omnivores to predators in summer and in winter.
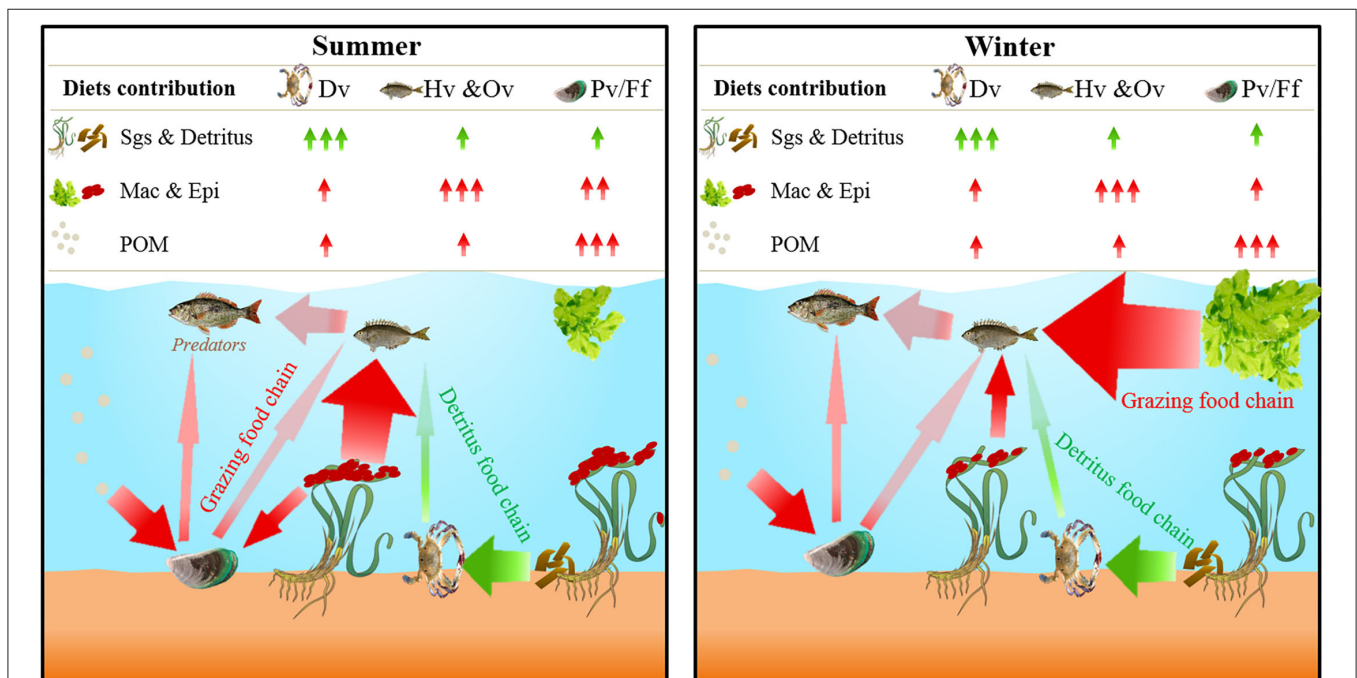

FIGURE 5 | Conceptual summary of findings for the carbon transfer process of food web and trophic pathway in a eutrophic tropical seagrass meadow.

These were also proved by the higher contribution of epiphytes in summer and macroalgae in winter to carnivores.

However, the transfer of seagrass carbon in the detrital food chain was still relatively stable, even in the presence of abundant macroalgae. The mixing model indicated that the contribution of seagrass to detritivores was higher than that of other food sources, with a high average value (>53%) in both summer and winter (**Figure 4**). This result is higher than that of Park et al. (2013) in temperate eutrophic *Z. marina* meadow, but similar to the results of Sepulveda-lozada et al. (2015) in tropical eutrophic seagrass meadow. It is possible, because the absolute decomposition of seagrass dead leaves takes longer compared with macroalgae (Lavery et al., 2013; Liu et al., 2016b; Trevathan-Tackett et al., 2020), which might accumulate adequate food for the diet of detritivores. Meanwhile, seagrass meadows in tropical regions may accumulate more seagrass debris than those in temperate regions as a result of relatively mild seasonal changes (Nakamoto et al., 2019). Therefore, adequate seagrass detritus throughout the year in tropical seagrass meadows might keep the transfer pathway of carbon derived from seagrass stable along the detritus food chain (**Figure 5**).

Overall, our study clarified the transfer process of each carbon source in the XC seagrass meadow food web by identifying the carbon sources of each trophic group, and identified its main trophic pathway (grazing food chain), influencing factors (seasonal variations), and the trophic role of seagrass (provide carbon source through the detritus food chain). However, our results explored the process of carbon transfer in a specific tropical seagrass meadow. In further studies, spatial comparisons are also necessary to enhance our understanding of the effect of different primary producer structures on the process of carbon transfer in seagrass meadows.

CONCLUSIONS

Our study demonstrated that grazing food chain was the main trophic pathway in eutrophic tropical seagrass meadows. Meanwhile, seasonal variations could result in the seasonal proliferation of macroalgae and epiphytes, which could obviously affect carbon transfer processes and trophic pathways in the food web. In summer, abundant epiphytes, rather than seagrass, chiefly fueled the food web through the grazing food chain. In winter, the food web is mainly fueled by prolific macroalgae through grazing food chain. In contrast, seagrass was relatively abundant throughout the year, and fueled the food web mainly through the detritus food chain in both seasons. In addition, although the proliferation of macroalgae and epiphytes seems to limit the transfer of seagrass carbon in the grazing food chain, seagrass trapped particles into sediment and on seagrass leaves by meadow architecture, which could also be used in the food web. Hence, seagrass could also play an important role in the carbon flow transfer of the food web in eutrophic tropical seagrass meadows.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by South China Sea Institute of Oceanology, Chinese Academy of Sciences.

AUTHOR CONTRIBUTIONS

LC: conceptualization, methodology, investigation, formal analysis, visualization, and writing—original draft. ZJ: conceptualization, investigation, and writing—review and editing. XH: supervision, conceptualization, writing—review and editing, and funding acquisition. YW: investigation and writing—review. SL, JL, QC, and JH: investigation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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