Marine Pollution Bulletin 117 (2017) 214–221

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Effects of nutrient load on microbial activities within a seagrass-dominated ecosystem: Implications of changes in seagrass blue carbon

Songlin Liu [a,b], Zhijian Jiang [a], Yunchao Wu [a,b], Jingping Zhang [a], Iman Arbi [a,b], Feng Ye [c], Xiaoping Huang [a,b,*], Peter Ian Macreadie [d]

[a] Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Key Laboratory of Marginal Sea Geology, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
[d] Blue Carbon Lab, Centre for Integrative Ecology, School of Life and Environmental Sciences, Faculty of Science Engineering & Built Environment, Burwood, Deakin University, Victoria 3125, Australia

ARTICLE INFO

Article history:
Received 13 December 2016
Received in revised form 22 January 2017
Accepted 25 January 2017
Available online 5 February 2017

Keywords:
Seagrass bed
Sediment organic carbon
Extracellular enzymes activities
PLFA
Microbial community structure
$\delta^{13}C$

ABSTRACT

Nutrient loading is a leading cause of global seagrass decline, triggering shifts from seagrass- to macroalgal-dominance. Within seagrass meadows of Xincun Bay (South China Sea), we found that nutrient loading (due to fish farming) increased sediment microbial biomass and extracellular enzyme activity associated with carbon cycling (polyphenol oxidase, invertase and cellulase), with a corresponding decrease in percent sediment organic carbon (SOC), suggesting that nutrients primed microorganism and stimulated SOC remineralization. Surpisingly, however, the relative contribution of seagrass-derived carbon to bacteria ($\delta^{13}C_{bacteria}$) increased with nutrient loading, despite popular theory being that microbes switch to consuming macroalgae which are assumed to provide a more labile carbon source. Organic carbon sources of fungi were unaffected by nutrient loading. Overall, this study suggests that nutrient loading changes the relative contribution of seagrass and algal sources to SOC pools, boosting sediment microbial biomass and extracellular enzyme activity, thereby possibly changing seagrass blue carbon.

1. Introduction

Seagrass ecosystems are globally-significant hotspots for organic carbon (OC, 'blue carbon') sequestration and storage, estimated to store up to 19.9 Pt C in their sediments - an amount equivalent to 10–times that stored in the earth's terrestrial soils (Fourqurean et al., 2012; Duarte et al., 2013; Greiner et al., 2013; Macreadie et al., 2014). Sediment microorganisms have a prominent contribution in determining the balance between sediment organic carbon (SOC) storage and remineralization processes within seagrass meadows (Sparling, 1992; Chambers et al., 2016), and can also contribute to a significant proportion of the seagrass SOC (30% of living C and over 8% of total OC for surface sediments; Danovaro et al., 1994). The relative use of the different sources of OC (seagrass, macroalgae, epiphytes, microphytobenthos, terrestrial organic matter) by microbes living within seagrass sediment is thought to depend primarily on the lability of OC (Jones et al., 2003; Holmer et al., 2004). Microbes primarily stimulate important OC transformation through the release of carbon-cycling extracellular enzymes, which play an important role in all biogeochemical cycles as proximate agents in crucial processes such as OC decomposition and energy transfer (Karaca et al., 2011; Shao et al., 2015).

Seagrass beds have been declining rapidly at a rate of 7% per year (Waycott et al., 2009), mainly due to nutrient pollution (Green and Short, 2003; Green et al., 2015). Increased nutrient loads to coastal areas can trigger the overgrowth of algae, most commonly in the form of epiphytes and macroalgae within seagrass beds (Hauxwell and Valiela, 2004; Burkholder et al., 2007), causing increases in the relative contribution of algae to the SOC pool within seagrass meadows (Volkman et al., 2008; Macreadie et al., 2012). It is thought that bacteria switch from seagrass to algal OC sources due to algal OC generally being more labile (Holmer et al., 2004), and, consequently, algae materials have relatively lower carbon burial efficiencies than seagrasses (Cebrian, 1999; Banta et al., 2004). Indeed, López et al. (1998) found that the addition of nutrient to seagrass sediment significantly increased ammonification rates, microbial *exo*-enzymatic activities and enhanced decomposition of SOC. However, empirical evidence of distinct shifts in sediment microbial communities, enzyme production and microbial organic carbon sources within seagrass meadows in response to nutrient loading is otherwise rare.

In this study, we investigated how microbial processes influence SOC transformation in response to nutrient enrichment of a seagrass meadow. Our study site was a ~200 ha mixed seagrass meadow (dominated by

* Corresponding author at: South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China.
E-mail address: xphuang@scsio.ac.cn (X. Huang).

http://dx.doi.org/10.1016/j.marpolbul.2017.01.056
0025-326X/

Thalassia hemprichii) in the southern shallow waters of the Xincun Bay, South China Sea (Huang et al., 2006). Xincun Bay has a long-history of urbanization and industrial development (Fig. 1), which has put pressure on the seagrass meadow, including high nutrient loading originated from fish farm expansion (Zhang et al., 2014; Liu et al., 2016). Liu et al. (2016) reported that increased nutrient enrichment could enhance the relative contribution of seagrass and also macroalgae and epiphyte to SOC, causing elevation of SOC levels and microbial biomass in Xincun Bay. What we still don't know, however, is how microbial activities changes in response to shifts in SOC sources and composition induced by nutrient loading.

Here we assessed phospholipid fatty acid (PLFA) profiles (Bossio and Scow, 1998; Li et al., 2015; Chambers et al., 2016) and compound-specific stable carbon isotope of PLFA (Boschker et al., 2000; Abraham and Hesse, 2003; Jones et al., 2003; Holmer et al., 2004; Bouillon and Boschker, 2006; Kohl et al., 2015) to determine how microbial communities and the microbial OC sources change in response to nutrient enrichment. In addition, we investigated how the extracellular enzyme activities, including polyphenol oxidase, peroxidase, invertase and cellulase, influence OC decomposition (Waldrop et al., 2004; Yin et al., 2014; Li et al., 2015; Shao et al., 2015). Our goal was to generate empirical data that could help understand how nutrient loading affects the carbon-sink capacity of Xincun Bay, thereby aiding resource managers to better manage anthropogenic stressors that affect this nearly-closed bay.

2. Materials and methods

2.1. Study site

Xincun Bay (18°24′34″N–18°24′42″N, 109°57′42″E–109°57′58″E) has only one narrow channel connecting to the South China Sea in the southwest (Fig. 2). *T. hemprichii* grows on sediment consisting of sand and terrigenous mud (Huang et al., 2006). In recent years, cage aquaculture has developed rapidly, and the nutrient concentration is more than twice higher at the seagrass bed nearest the fish farming area than at the farthest meadow, thereby providing a nutrient gradient along the seagrass meadow (Zhang et al., 2014).

2.2. Sampling and sample preparation

Three stations (1, 2, and 3) were selected at varying distances from the fish cage culture area (Fig. 2), representing a nutrient gradient from high (Station 1) to low (Station 3). Station 1 was located near the bay's entrance and at a distance of about 800 m from the fish cage culture systems, while station 3 was located far from (about 3 km) the fish cage culture systems. Station 2 was between them. The distance between two stations was about 1 km. Seawater was collected in December 2012, August 2013, December 2013, and August 2014 at each station. An organic glass hydrophore (KC Denmark A/S. Co., Denmark) was applied to collect the surface-water samples (below surface 50 cm) during high tide periods (water depths about 1.0–1.5 m). In August 2014, triple surface-sediment (5 cm inner diameter) samples were also collected of the top 3 cm at low tide at each station where *T. hemprichii* grows. All the samples were stored in an ice chest immediately after sampling until being transported to the laboratory within a few hours.

The seawater was filtered by low vacuum filtration onto pre-combusted GF/F filters (Whatman, 450 °C, 3 h). The filtrate was kept in polyethylene bottles and stored at −20 °C for nutrient analysis. In addition, each sediment sample was divided into two subsamples. One subsample was stored at 4 °C, while the other was stored in −20 °C for sediment parameter analysis.

2.3. Laboratory analysis

The stored seawater was analyzed for dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium) and dissolved inorganic phosphate (DIP) using an AQ-2 Automated Discrete Analyzer.

The sediment sample stored at 4 °C was used for grain size, pH, electrical conductivity (EC), enzyme activities (including polyphenol

Fig. 1. Xincun Bay (Hainan Island, South China Sea) has a long history of anthropogenic impact, including: fish farming and aquaculture (A), seafloor modification for to supply energy demands of households and local industry (B), and pollution (C); all of which have resulted in poor water quality and fish kills (D).

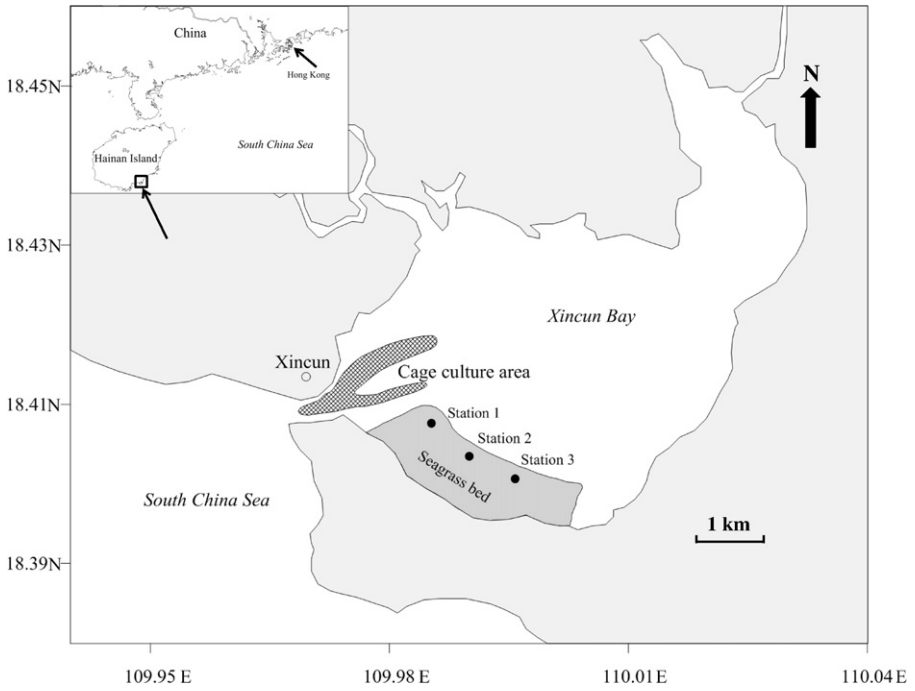

Fig. 2. Sampling sites in Xincun Bay, Hainan Island, South China Sea.

oxidase, peroxidase, invertase and cellulase) and microbial biomass carbon (MBC) analyses. Sediment grain size was analyzed using a laser particle size analyzer of Mastersize 2000. Sediment pH was measured in 1:2.5 sediment:water suspension and EC in 1:5 sediment: water suspension using a Seven 2Go™ pH Meter and YSI-85 Conductivity Meter, respectively (Lu, 1999). Polyphenol oxidase and peroxidase were determined by the spectrophotometry of pyrogallic acid (Guan, 1986; Yin et al., 2014). Invertase and cellulase activities were measured by the spectrophotometry of 3,5-dinitrosalicylic acid colorimetry (Yin et al., 2014; Guan, 1986; Shao et al., 2015). MBC was determined by the fumigation-extraction method (Vance et al., 1987; Yang et al., 2013). The fumigated and non-fumigated moist sediments were extracted with 0.5 M K_2SO_4 by shaking for 30 min, and then the extract was vacuum filtered through pre-combusted GF/F filters (Whatman, 450 °C, 3 h) into a pre-combusted apragaz bottle (450 °C, 3 h) for total OC analysis. OC content in the extracts was analyzed by TOC-Vcph (Shimadzu, Japan). Sediment MBC was calculated according to the following equation: $MBC = (C_{fumigated} - C_{non\text{-}fumigated}) / 0.38$.

The frozen sediment sample was freeze-dried, ground and homogenized using a mortar and pestle. Sediment was acidified (1 N HCl) overnight at room temperature to remove carbonate, followed by washing with distilled water and drying at 40 °C in an oven. Sediment $\delta^{13}C$ was analyzed on an isotope ratio mass spectrometer (Thermo Scientific MAT 253). The isotopic data were expressed in the conventional delta notation (‰): $\delta^{13}C_{sample} = (R_{sample} / R_{reference} - 1) \times 1000$ where $R = {}^{13}C / {}^{12}C$. The reference standard was Peedee Belemnite and the analysis uncertainty was ≤0.2‰. In addition, organic carbon (OC) and total nitrogen (TN) of sediment were determined using an elemental analyzer (Vario EL).

The remaining freeze-dried sediment was used for phospholipid derived fatty acids (PLFA) extraction and analysis. PLFA was extracted according to the Ester-Linked method (Schutter and Dick, 2000), which uses a mild alkaline transesterification methodology. The resulting fatty acid methyl esters (FAME) were detected on a gas chromatograph (Agilent HP 6890GC) equipped with a flame ionization detector and a capillary column (Aligent DB-5MS 30 m × 0.25 mm; film thickness 0.25 μm). The GC temperature ramping increased at a rate of 5 °C per minute from 80 to 240 °C, and final isotherm at 240 °C for 6 min. Individual fatty acid was identified with a MIDI Sherlocks microbial identification system (Microbial ID, Newark, DE). Concentration of each PLFA was calculated based on the 19:0 standard concentrations. Fatty acids with <0.5% of the total relative abundance were not included in the data set. For each sample, the abundance of individual FAME was expressed as nmol PLFA/g dry weight sediment. The PLFAs i14:0, 15:0, i15:0, a15:0, i16:0, 16:1ω7c, 17:0, a17:0, i17:0, cy17:0, 18:1ω7 and cy19:0 were chosen to represent the bacterial biomass (Zelles, 1999), while the polyenoic, unsaturated PLFA 18:2ω6, 9c was used as a signature for fungi, as it is suggested to be the fungi origin in soil (Bååth, 2003). The fungal biomass to the bacterial biomass ratio (F/B) was calculated using their molar concentration.

$\delta^{13}C$ of each individual FAME was determined using a gas-chromatograph-combustion-interface isotope-ratio mass spectrometer (GC-c-IRMS); a Thermo Scientific Trace GC Ultra GC connected to Delta V Advantage IRMS via a type III combustion interface from Thermo Finnigan. Stable carbon-isotope ratios for individual PLFA were calculated from FAME data by correcting for the one carbon atom in the methyl group that was added during derivatization. The weight-averaged isotopic ratios of i15:0 and a15:0 (i + a 15:0) were used to indicate bacterial $\delta^{13}C$ ratios after correction for isotopic fractionation in fatty acids (5.6‰) (Boschker et al., 1999). The isotopic ratios of 18:2ω6, 9c was employed to indicate fungal $\delta^{13}C$ ratios (Abraham and Hesse, 2003; Kohl et al., 2015), however, there was no information about the isotopic fractionation of the 18:2ω6, 9c in seagrass meadows. Stable carbon-isotopes are expressed in the delta notation relative to Vienna PDB as same as described above.

2.4. Statistical analysis

Data were tested for normality and log transformed to meet the assumptions for statistical analysis. One-way analysis of variance (ANOVA) was used to determine the statistically significant differences in seawater inorganic nutrient, sediment grain size, pH, salinity, SOC, TN, ratios of SOC to sediment TN (C/N), MBC, ratios of MBC to SOC (MBC/SOC), polyphenol oxidase, peroxidase, invertase, cellulase, total PLFAs, bacterial PLFAs, fungal PLFA, F/B, and the $\delta^{13}C$ values of SOC, i + a 15:0 and 18:2ω6, 9c among stations. Isotopic mixing models,

including a Bayesian approach, were applied with the software SIAR (Parnell et al., 2010) to estimate the proportional contribution of sources to the SOC, bacterial organic carbon (BOC) and fungal organic carbon (FOC). The $\delta^{13}C$ values of possible SOC, BOC and FOC sources were taken from Liu et al. (2016). Statistical analysis was performed with IBM SPSS Statistics 19.0 software.

3. Results

3.1. Seawater nutrient and sediment variables

The average concentrations of DIN and DIP in the seawater were 4.81 ± 2.18 μmol and 0.55 ± 0.38 μmol in the four times sampling, respectively (the first three seasons nutrient data come from Liu et al., 2016). Variations of DIN and DIP among the stations were shown in Fig. 3. There was significant difference in DIN concentrations among the three stations ($p < 0.05$), but not for DIP ($p > 0.05$). The DIN and DIP concentrations both showed a decreasing trend with increasing distance to the fish farming area.

Sand content of the sediment at each station was >90%, indicating that sand was the dominant sediment type (Table 1). The electrical conductivity, SOC, sediment TN, C/N ratios, MBC and MBC/SOC were found to be significantly higher at station 1 than at station 3. Moreover, the electrical conductivity, SOC and sediment TN presented a decreasing trend from station 1 to station 3, but pH values and C/N ratios were higher at station 3 than the other stations (Table 1).

3.2. Sediment oxidoreductase and hydrolase activities

The content ranges of polyphenol oxidase, peroxidase, invertase and cellulase activities were 0.21–0.41 mg/g, 0.59–0.79 mg/g, 0.33–0.92 mg/g and 0.13–0.97 mg/g, respectively, with the average activities as 0.28 mg/g, 0.71 mg/g, 0.56 mg/g and 0.42 mg/g, respectively. The polyphenol oxidase activity was significantly higher in station 1 than the other stations ($p < 0.05$, Fig. 4 I). The peroxidase activity showed the highest value in station 1, but was not pronounced among the stations (Fig. 4 II). The invertase and cellulose activities presented the highest in station 1, which increased the invertase and cellulase activities by 1.20–1.22 and 2.52–4.66 fold compared to the other two stations, respectively ($p < 0.05$, Fig. 4 III and IV). Moreover, polyphenol oxidase, peroxidase, invertase, and cellulose activities exhibited the decreasing trend from station 1 to 3 (Fig. 4).

3.3. Microbial community compositions

Analyses of total PLFAs, bacterial PLFAs and fungal PLFA, are widely used to estimate the total microbial biomass, bacterial biomass and fungal biomass, respectively (Frostegård and Bååth, 1996; Huang et al., 2014). The total PLFAs, bacterial PLFAs, fungal PLFA and F/B ratio varied significantly among stations (Fig. 5). Total PLFAs were significantly higher in station 1 in contrast to other stations, with a ~50% decrease in total PLFAs ($p < 0.05$, Fig. 5 I). Bacterial PLFAs and fungal PLFA accounted for about 40% and 7% of total PLFAs in the present study. Significantly higher bacterial PLFAs and fungal PLFA were also observed at the sites closer to fish farms than at sites distant from fish farming area ($p < 0.05$, Fig. 5 I, II). However, the F/B ratios were markedly lower in station 1 than station 3 ($p < 0.05$, Fig. 5 IV). Furthermore, bacterial PLFAs showed a trend of station 1 > station 2 > station 3, while the F/B ratios presented the opposite trend.

Table 1
Variation of sediment variables among the three stations (mean ± S.D.). The different capital letters (A, B) indicate significant differences among the three stations (S-N-K test, $p < 0.05$).

Variables	Station 1	Station 2	Station 3
Sand (%)	90.33 ± 9.69^{A}	93.00 ± 7.39^{A}	95.30 ± 6.38^{A}
pH	8.12 ± 0.04^{B}	8.24 ± 0.09^{AB}	8.24 ± 0.02^{A}
Electrical conductivity (dS/m)	4.00 ± 0.06^{A}	3.33 ± 0.20^{B}	3.32 ± 0.10^{B}
SOC (%)	0.33 ± 0.10^{A}	0.17 ± 0.03^{B}	0.14 ± 0.01^{B}
Sediment TN (%)	0.04 ± 0.01^{A}	0.018 ± 0.003^{B}	0.013 ± 0.003^{B}
C/N (mol/mol)	9.61 ± 0.69^{B}	11.18 ± 3.98^{AB}	12.84 ± 1.71^{A}
MBC (mg/kg)	300.54 ± 69.98^{A}	74.77 ± 37.63^{B}	61.11 ± 13.54^{B}
MBC/SOC (%)	9.23 ± 1.51^{A}	4.34 ± 1.37^{B}	4.42 ± 0.48^{B}

3.4. $\delta^{13}C$ of sediment organic carbon, i + a 15:0 and 18:2ω6, 9c, and their sources

$\delta^{13}C$ of SOC ($\delta^{13}C_{SOC}$) presented significant differences among the stations ($p < 0.05$, Fig. 6). $\delta^{13}C_{SOC}$ showed a trend of station 1 > station 2 > station 3 with the average $\delta^{13}C_{SOC}$ as −13.44‰, −15.28‰ and −15.47‰, respectively (Fig. 6). The average i + a 15:0 $\delta^{13}C$ ($\delta^{13}C_{bacteria}$) was −10.73‰, with more enriched values than the average $\delta^{13}C_{SOC}$ (−14.73‰) (Fig. 6). $\delta^{13}C_{bacteria}$ ranged from −8.72‰ to −12.01‰, which $\delta^{13}C_{bacteria}$ in station 1 was observed higher than other stations. $\delta^{13}C$ of PLFA 18:2ω6, 9c ($\delta^{13}C_{fungi}$) ranged between −25.24‰ and −27.08‰ (without isotopic fractionation), and the variation of $\delta^{13}C_{fungi}$ was not significant among the stations ($p > 0.05$, Fig. 6).

According to the $\delta^{13}C$ data of primary producers in Xincun Bay (Liu et al., 2016), the average $\delta^{13}C$ of seagrass, macroalgae and epiphyte, and suspended particulate organic matter (SPOM) were −8.99‰, −13.61‰ and −19.08‰, respectively (Fig. 6). The average relative contributions of primary producers to SOC and BOC were shown in Table 2. SOC sources were mainly composed of macroalgae and epiphyte as well as SPOM. The relative contribution of seagrass, and macroalgae and epiphyte to SOC increased from station 3 to 1, but SPOM showed the opposite trend. BOC mainly originated from seagrass, and the relative contribution of seagrass to BOC in station 1 was about 1.6 times compared with other two stations. In addition, the relative contribution

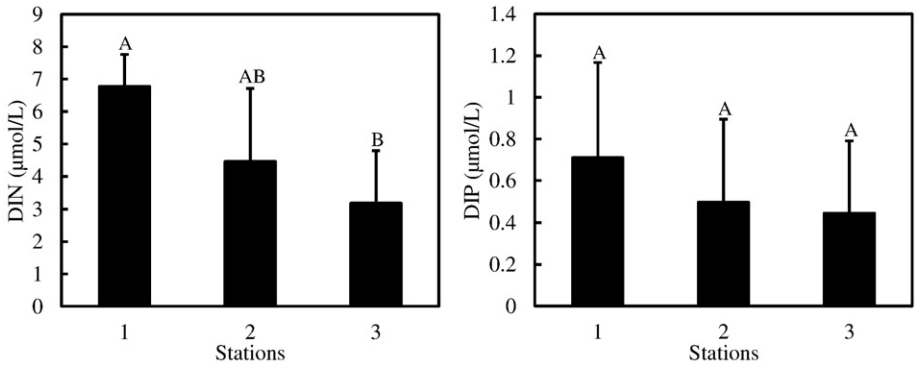

Fig. 3. Station variations of DIN and DIP (mean ± S.D.; the first three seasons nutrient data come from Liu et al., 2016). Different capital letters (A, B) over the bars indicate statistical significances among the stations (S-N-K test, $p < 0.05$).

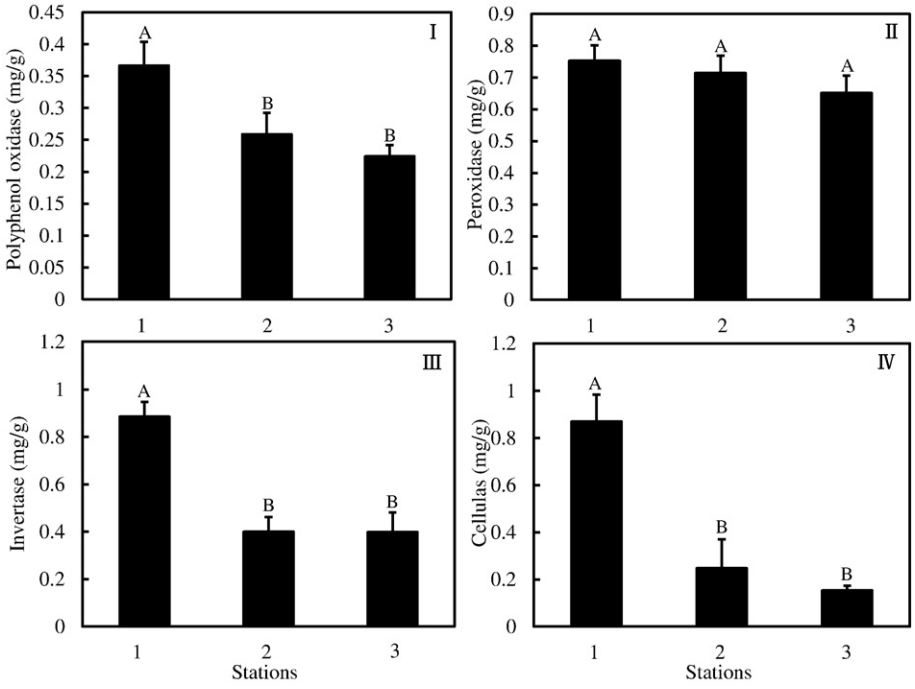

Fig. 4. Station variations of polyphenol oxidase (I), peroxidase (II), invertase (III) and cellulas (IV) activities (mean ± S.D.). Different capital letters (A, B) over the bars indicate statistically significances among the stations (S-N-K test, $p < 0.05$).

of macroalgae and epiphyte tended to be similar (34%), while that of SPOM was about 16% lower than the other two stations.

4. Discussion

4.1. Changes of organic carbon sources in sediment

The nutrient concentrations were generally higher in Xincun Bay than other seagrass beds reported in the literature (the DIN ranged from <0.37 to 3.17 μM, and the DIP ranged from 0.06 to 0.64 μM; Ziegler and Benner, 1999; Ziegler et al., 2004; Kiswara et al., 2009; Apostolaki et al., 2010), indicative of high nutrient loading within the seagrass bed. Moreover, significantly higher nutrient concentrations of *T. hemprichii* tissue P contents and root N contents (Zhang et al., 2014), epiphytic algae and macroalgae biomass (Liu et al., 2016) were observed near the fish farms, implying that the *T. hemprichii* seagrass bed was experiencing higher nutrient loading stress in the area closest to the nutrient sources.

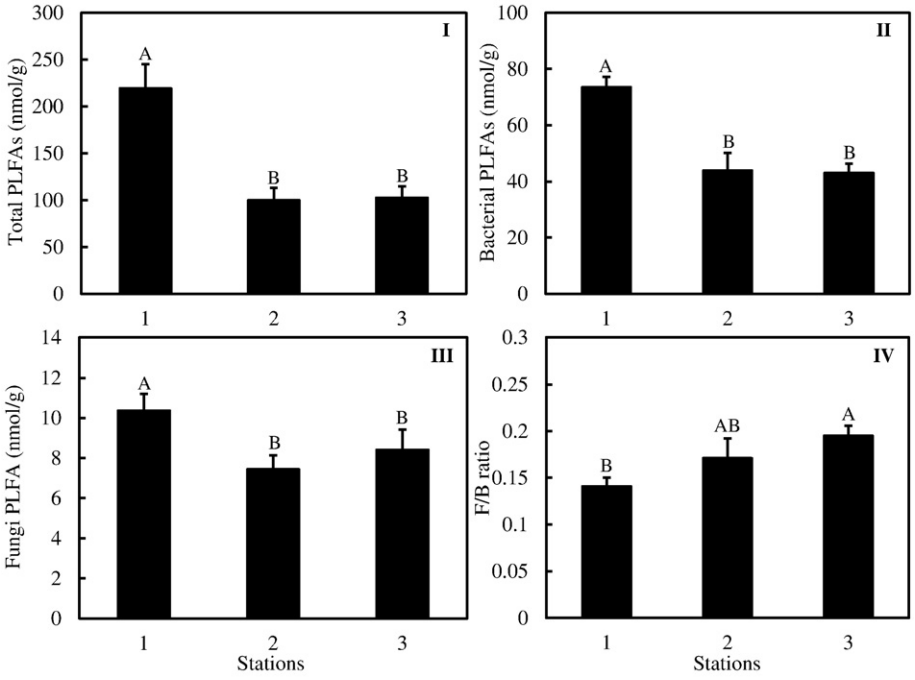

Fig. 5. Station variations of total PLFAs (I), bacterial PLFAs (II), fungi PLFA (III) and F/B ratio (IV) (mean ± S.D.). Different capital letters (A, B) over the bars indicate statistically significances among the stations (S-N-K test, $p < 0.05$).

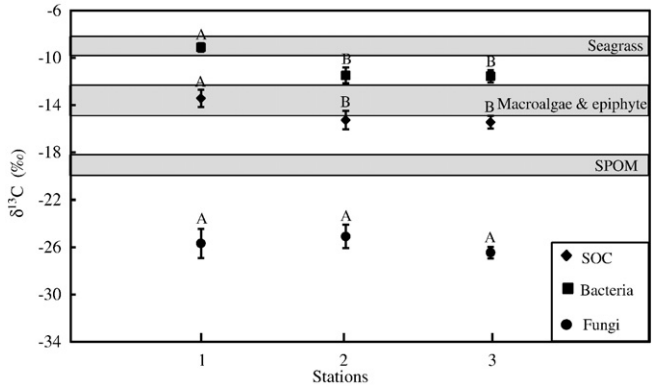

Fig. 6. $\delta^{13}C$ of SOC, bacterial biomarker i + a 15:0 PLFA and fungal biomarker 18:2ω6, 9c among the stations (mean ± S.D.). Light grey stripes represented the SOC possible sources $\delta^{13}C$ values in Xincun Bay from Liu et al. (2016) (mean ± S.D.). Different capital letters (A, B) over the bars indicate statistically significances among the stations (S-N-K test, $p < 0.05$).

The relative contribution of seagrass, and macroalgae and epiphyte to SOC gradually increased with decreasing distance from fish farms. This was mostly caused by high nutrient enrichment, which in turn enhanced the contribution of macroalgae and epiphyte as well as seagrass leaf litter to SOC. This finding is in agreement with a previous study in Xincun Bay (Liu et al., 2016).

Despite a variety of SOC sources available to bacteria (incl. seagrass, macroalgae and epiphyte, and SPOM), the $\delta^{13}C_{bacteria}$ values indicated that the primary BOC source likely originated from local seagrass production (i.e. autochthonous carbon). That is, the relative contribution of macroalgae and epiphytes to bacteria tended to be similar, though the contribution of macroalgae and epiphytes to SOC pools increased with elevated nutrient load. This finding challenges the notion that algal carbon is preferentially metabolized over seagrass detritus due to seagrass being more refractory and therefore less palatable (Hill et al., 2015; Trevathan-Tackett et al., 2015). It also contrasts the findings of Boschker et al. (2000) and Holmer et al. (2004) who reported a shift in the relative contribution to BOC from seagrass detritus to easily decomposed external sources (phytoplankton, macroalgae, seston) under organic and nutrient enriched conditions. The question is: why did bacteria in this study maintain their fidelity to seagrass as a primary carbon source for their metabolism despite increases in increased availability of (presumably) more labile forms of macroalgal and epiphytic forms of carbon?

To explain this finding, we suggest that seagrass OC at this particular site is more bioavailable or provides a more sustaining energy source than macroalgal and epiphytic carbon at the site. Holmer et al. (2001) found that in *T. hemprichii* sediment $\delta^{13}C_{bacteria}$ values resembled that of the seagrass (about −12‰), though $\delta^{13}C_{SOC}$ values were around −22‰. In addition, Chiu et al. (2013) reported that 55% of *T. hemprichii* leaf litter would be decomposed but only 5% could be stored in seagrass beds, indicating that *T. hemprichii* seagrass leaf litter has a high bioavailability for bacteria. Since Xincun Bay is a nearly closed bay, the stimulated *T. hemprichii* leaf litter production due to the high nutrient would have been mostly retained in situ (Liu et al., 2016). The *T. hemprichii* leaf detritus is likely to be a continuously-supplied source of bioavailable dissolved organic carbon to the SOC pool (Lavery et al., 2013), thereby elevating the relative contribution of seagrass to bacteria in the high nutrient area.

Table 2
Isotopic mixing models results based on $\delta^{13}C$ (%) values, the estimated 95% confidence intervals with mean in bracket are given for each possible source.

Type	Station	Seagrass	Macroalgae & epiphyte	SPOM
SOC	1	1%–43% (23%)	0%–65% (34%)	19%–65% (43%)
	2	0%–26% (11%)	0%–56% (29%)	35%–85% (60%)
	3	0%–25% (10%)	0%–54% (28%)	37%–87% (62%)
BOC	1	32%–88% (60%)	0%–59% (30%)	0%–23% (10%)
	2	13%–62% (39%)	0%–68% (35%)	4%–46% (26%)
	3	13%–62% (38%)	0%–67% (35%)	5%–47% (27%)

The $\delta^{13}C_{fungi}$ values observed in the current study were depleted by 10–12‰ relative to $\delta^{13}C_{SOC}$, which was higher than that of Kohl et al. (2015) who reported 2–4‰ depletion relative to bulk biomass in fungal cultures. Indeed, the PLFA of 18:2ω6, 9 is generally enriched in ^{13}C relative to fungal biomass in culture (−0.3–+2.5‰) (Abraham and Hesse, 2003). Since these available data all come from terrestrial ecosystems or laboratory cultures, there was no information about the isotopic fractionation of fungal PLFA within seagrass beds. Thus we cannot detect the relative contribution of primary producers to FOC. Nonetheless, the similar $\delta^{13}C_{fungi}$ among the stations also indicated similar relative contribution of each possible source to FOC.

4.2. Changes of sediment microbial community and enzyme activities

In Xincun Bay, nutrient load induced amounts of plant-derived OC input enhanced the SOC and also the labile organic carbon incoming (Liu et al., 2016), and consequently elevated the microbial biomass, including both the bacterial and fungal biomass, and the MBC composition in SOC (Bååth, 2003; Bossio and Scow, 1998). Therefore, significant stimulations of polyphenol oxidase, invertase and cellulase activities were observed neighboring to the cage culture area, which should be ascribed to abundant microorganisms excrete (Zhang et al., 2011; Shao et al., 2015). Each enzyme has its own substrate and ability to catalyze specific biochemical reactions (Song et al., 2012). Polyphenol oxidase could promote lignin degradation (Deforest et al., 2004; Waldrop et al., 2004), which directly reduce the refractory organic carbon in seagrass litter (Mateo et al., 2006). Cellulases are enzyme systems that degrade cellulose and release reducing sugars as the end products through providing more labile OC substrate for sediment heterotrophic microorganisms (Alvarenga et al., 2008; Zhang et al., 2011), while invertase catalyzes hydrolysis of sucrose releasing glucose and fructose as highly active metabolic compounds (Stemmer et al., 1998). Furthermore, polyphenol oxidase is also associated with eliminating phenolic compound, which could increase the activity of hydrolase enzymes and thus stimulate OC decomposition (Freeman et al., 2001).

Therefore, the enhanced seagrass as well as macroalgae and epiphyte derived OC in high nutrient load area, containing abundant lignin, cellulose, starch, sucrose and others (Mateo et al., 2006; Carlsson et al., 2007; Touchette and Burkholder, 2007; Lee et al., 2011), should have higher decomposition rates due to the elevated extracellular enzyme activities. This supports the above idea of higher transformation of seagrass to bacteria under higher nutrient loads. Whereas, peroxidase tended to be similar under different nutrient levels, even though there was significantly higher fungal biomass under high nutrient load. This might be due to high N availability blocking the expression of lignin-degrading peroxidases in some fungal taxa (Hammel, 1997).

In contrast to microbial, bacterial and fungal biomass, the F/B ratios were observed to be markedly higher in lower nutrient content area, indicating changes of microbial community structure. As a result of higher growth yield efficiency of fungi compared to bacteria, the higher F/B ratios implied higher OC storage potential and slower SOC turnover rate at sites distant from nutrient sources (Six et al., 2006; Malik et al., 2016). However, the SOC content was observed higher in the elevated level nutrient region, which seemed to be contradictory to the above results. SOC storage is a complex process controlled by OC input and output (Cheng et al., 2010; Yang et al., 2013), and thus the details of sediment core OC characteristics require further research.

5. Conclusion

We found that higher nutrient loads to a seagrass meadow elevated the meadow's sediment microbial biomass, extracellular enzyme

activities and the relative contribution of seagrass to bacteria, but considerably decreased F/B ratios compared to the low nutrient condition. These findings imply that high nutrient enrichment can enhance production of plant-derived OC to sediment, and thus increased microbes as well as its SOC transformation efficiency, which will presumably change seagrass blue carbon sequestration and storage capacity. What is unknown, however, is how the net carbon budget changes in response to nutrient loading, since, on the one hand, increases in plant-derived OC could enhance SOC content, yet, on the other hand, increased microbial transformation of OC could trigger net losses of OC (Sayer et al., 2011). Further work is needed to develop greenhouse gas budgets and core sediment OC characteristics across the nutrient gradient, as well a trace the fate of the different sources of carbon within the seagrass meadows.

Acknowledgments

This research was supported by the National Basic Research Program of China (2015CB452905, 2015CB452902), the National Natural Science Foundation of China (nos. 41306108, 41406128), the National Specialized Project of Science and Technology (2015FY110600). PM was supported by an Australian Research Council DECRA Fellowship (DE130101084) and a Linkage Project (LP160100242).

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