Marine Pollution Bulletin xxx (2017) xxx–xxx

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 xxxx

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

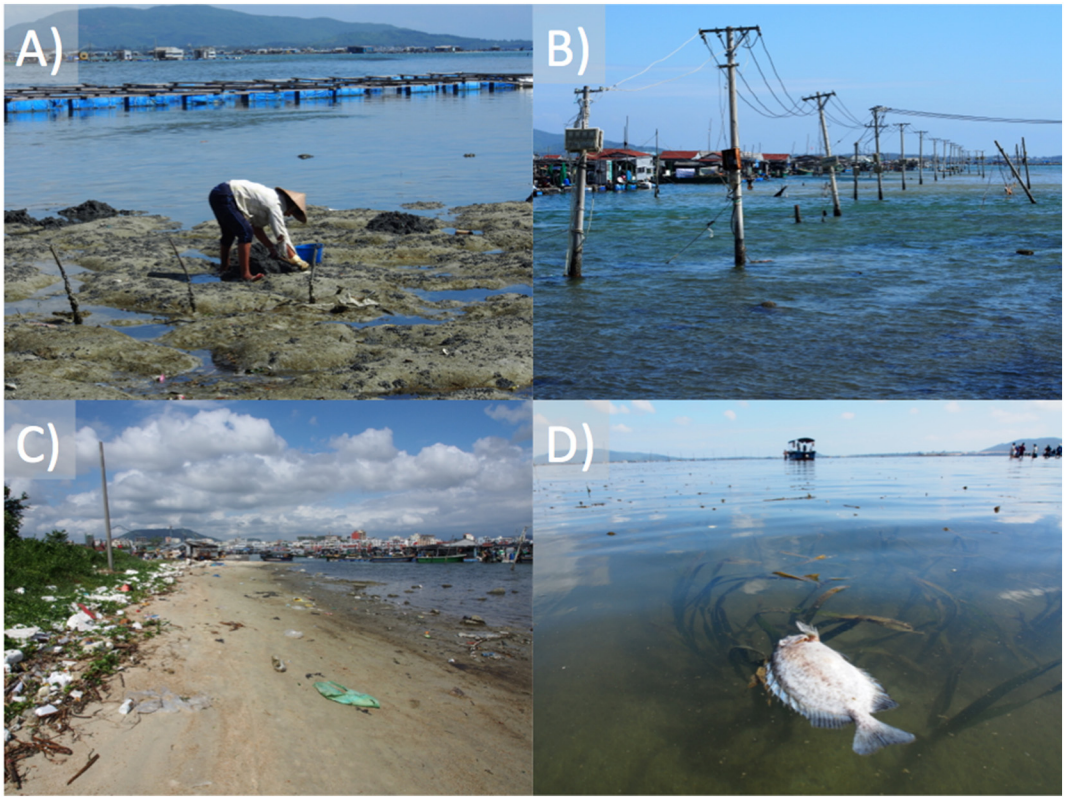

Fig. 1. Xincun Bay (Hainan Island, South China Sea) has a long history of anthropogenic impact, including: fish farming and aquaculture (A), seafloor modification for to supply energy demands of households and local industry (B), and pollution (C); all of which have resulted in poor water quality and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

Please cite this article as: Liu, S., et al., Effects of nutrient load on microbial activities within a seagrass-dominated ecosystem: Implications of changes in seagrass blue c..., Marine Pollution Bulletin (2017), http://dx.doi.org/10.1016/j.marpolbul.2017.01.056