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Nutrient limitation status in a subtropical mangrove ecosystem revealed by analysis of enzymatic stoichiometry and microbial abundance for sediment carbon cycling

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ABSTRACT

Mangrove ecosystem plays an important role in global cycling of nutrients. According to nutrient availability, microorganisms can optimize the allocation of C-, N- and P-degrading enzymes. Thus, in this study, enzymatic stoichiometry involved in C, N and P acquisition, and the microbial abundance were analysed in coastal mangrove sediments in two different seasons. Activities of β-1,4-glucosidase (GLU), *N*-acetyl-β-glucosaminidase (NAG), acid phosphotase (ACP) and phenol oxidase (PHO) were determined, and then applied to calculate the enzymatic C:N (GLU:NAG), C:P (GLU:ACP) and N:P (NAG:ACP) acquisition, respectively. Besides, GLU:PHO was applied as relative recalcitrance of sediment organic matter (SOM). The results showed the enzymatic stoichiometry and microbial abundance had strongly seasonal variations. Compared to other ecosystems, GLU:ACP and NAG:ACP ratios were largely lower, suggesting that sediments in this ecosystem might be microbial P-limitation. Moreover, the higher GLU:PHO ratio implied that SOM in this ecosystem might be more decomposable. Furthermore, the imbalance of nutrients for microbial abundance was revealed by the strong correlations relating sediment N:P to GLU:NAG, GLU:ACP, and GLU:PHO. On the other hand, microbial P limitation in this ecosystem may enhance C storage and sequestration because the higher C contents in sediments of March than November occurred with lower GLU:ACP and NAG:ACP ratios as well as bacterial abundance.

1. Introduction

Mangrove ecosystem, along coastline of tropical and subtropical region, is one of the most productive wetlands in the world, and is characterized by efficient cycling of nutrients and active exchange between terrestrial and marine ecosystem (Tam and Yao, 2002; Koné and Borges, 2008; Tue et al., 2012). It is capable of producing approximately 218 ± 72 Tg carbon (C) and sequester C in the order of 23 Tg C a^{-1}, indicating importance of mangrove ecosystem in C cycling (Bouillon et al., 2008; Koné and Borges, 2008). Also, some researchers studied the whole-island C stocks in the tropical pacific, and reported that mangrove ecosystem accounted for 24–34% total island C stock with a coverage of only 12–13% of the land (Donato et al., 2012). On the other hand, due to the inland freshwater (including excessive nutrients) and tidal flush by human activities, it is assumed that this ecosystem should be rich in nutrients such as nitrogen (N) and phosphorus (P) (Tam and Yao, 2002; Wu et al., 2008; Zhou et al., 2008; Cao et al., 2011). However, divergent result has been showed by Kristensen et al. (2000) who argued that the sediment in mangrove ecosystem was generally nutrients-limited due to the high contents of organic matter and the very low levels of dissolved and particulate nutrients. Therefore, the problem whether the mangrove ecosystem is rich or lack of nutrients, is unclear and needs to be explored from different perspective.

Well known, the cycling of nutrients in soil/sediment is closely related to microbial communities inhabiting in. Since the environmental resources usually are not able to meet the demands of microbial growth, microorganisms have to produce the relative extracellular enzymes to obtain C and other nutrients though catalysing decomposition of SOM (Lucas et al., 2007; Zhou et al., 2008; Geisseler and Horwath, 2009; Cao et al., 2011; Waring et al., 2014). Accordingly, extracellular enzyme activities (EEA) are often used as indicators of nutritional status and microbial activities in different ecosystems (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012; Sinsabaugh et al., 2012; Weintraub et al., 2013). For example,

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β-1,4-glucosidase (GLU), hydrolysing glucose from cellulose and celloligosaccharides, is the most commonly measured indicator for C acquisition (Knight and Dick, 2004; Moscatelli et al., 2012). *N*-acetyl-β-glucosaminidase (NAG) catalyses the terminal reaction in chitin degradation, is one of the N-targeting hydrolytic enzymes (Sistla and Schimel, 2013; Stone et al., 2014). Acid phosphotase (ACP) plays an important role in hydrolysing the phosphomonoesters and in some cases phosphodiesters and releasing assimilable inorganic P for microbes and plants (Sinsabaugh et al., 2008; Huang et al., 2011; Hou et al., 2015). Additionally, phenol oxidase (PHO), one of the enzymes that can attack phenolics, has been proposed as an enzymic 'latch' of C storage as well as the cycling of other nutrients in terrestrial ecosystems through regulating GLU, NAG and ACP activities (Freeman et al., 2001, 2004; Fenner et al., 2005b; Kandeler et al., 2006; Toberman et al., 2008). Taken all together, studying enzyme activities in natural ecosystems can provide valuable information for better understanding and then managing ecosystems.

So far, plenty of studies have been focused on soil enzymes in different terrestrial ecosystems, and some new insights have started to emerge. For instance, except exploring variations of EEA in different ecosystems, few recent studies have applied the relative abundance of EEA involved in cycling of C and other nutrients to understand the relationship between microbial biomass stoichiometry and the elemental composition of available organic matter. A term called enzymatic stoichiometry, i.e., ratio of commonly measured enzyme potentials, has been introduced in this new study area. (Sinsabaugh et al., 2012; Waring, 2013; Arnosti et al., 2014; Waring et al., 2014; Tapia-Torres et al., 2015). In principle, ecoenzymatic stoichiometry can be used as indicators of microbial resource allocation to the acquisition of C and other nutrients, and also reflect the relative recalcitrance of organic matter (Sinsabaugh et al., 2009; Hill et al., 2012; Sinsabaugh and Follstad Shah, 2012; Sinsabaugh et al., 2012; Hill et al., 2014; Waring et al., 2014). For example, the ratios of GLU:NAG, GLU:ACP and NAG:ACP activities were measured as enzymatic C:N, C:P and N:P acquisition activity, respectively (Sinsabaugh et al., 2009). The lower GLU:NAG indicates lower N availability, while the lower GLU:ACP and NAG:ACP values suggest lower P availability (Waring et al., 2014). Moreover, the ratio of GLU:PHO was applied as a relative measure of organic matter recalcitrance. The more recalcitrance is represented by lower GLU:PHO value (Sinsabaugh and Follstad Shah, 2012).

Among ecosystems, the enzymatic stoichiometry often varies widely due to different environmental resources and microbes (Sinsabaugh and Follstad Shah, 2012; Waring et al., 2014). Environmental factors, such as pH, temperature and moisture content, have also been reported to act as important roles in enzyme potential activities in terrestrial and freshwater systems (Kandeler et al., 2006; Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012). However, until now, there is no relevant information about microbial nutrient acquisition revealed by enzymatic stoichiometry and the interrelationship among enzymatic stoichiometry, microbial abundance and environmental factor in coastal mangrove ecosystems. Concerning the importance of mangroves in C cycling, therefore, it is necessary to understand microbial nutrient availability and then better manage C storage in this ecosystem. Thus, in this study, we chose a coastal mangrove ecosystem to investigate the ratios of C-, N- and P-acquiring enzymes to represent microbial nutrient acquisition. On the other hand, we determined the abundance of bacteria and fungi (consisting of approximately 91% total microbial biomass) in order to find the relationship between microbial abundance and enzymatic stoichiometry. Additionally, the correlations relating environmental factors to enzymatic stoichiometry as well as microbial abundance were explored to find out the environmental constraints on microbial EEA processes.

2. Materials and methods

2.1. Sample collection

Mai Po Nature Reserve (22°29′N to 22°31′N and 113°59′E to 114°03′E), an intertidal estuary of the Pearl River Delta (China), is the largest coastal wetland in Hong Kong and located at the northwestern corner of the New Territories of Hong Kong (Li et al., 2011b; Luo and Gu, 2015). This nature reserve consists of subtropical mangroves, intertidal mudflats, and large quantities of wastewater including domestic sewage and industrial wastewater discharged by Shenzhen River and inland river of Hong Kong. In this reserve, the dominated mangrove trees are *Kandelia obovota* (L.) Drue and the monitored physicochemical characteristics of sediments vary greatly between intertidal zone and mangrove forest (Li et al., 2011a, 2011b; Shen et al., 2012; Luo and Gu, 2015). Therefore, the sediments located at intertidal mudflat and mangrove forest are assumed as two different ecological niches. In the present study, four sites in Mai Po Nature Reserve of Hong Kong (Fig. 1) were chosen to collect sediment samples. Two samples collected from intertidal mudflat zone were recorded as intertidal site 1 (IZ1) and intertidal site 2 (IZ2), severally. The other two samples from mangrove forest were recorded as mangrove site 1 (MG1) and mangrove site 2 (MG2), respectively. Each sample was taken from surface layer (0–2 cm) in triplicate. After sampling, the sediment was sieved through a mesh of 2 mm, and then stored at −20 °C (Toberman et al., 2008). Due to the varied properties of sediments in different seasons in this wetland, samples were collected on both dry (March) and wet seasons (November) in 2012, respectively.

2.2. Sediment physichochemical properties

The temperature and pH of sediments were measured *in situ* using an IQ180G Bluetooth Multi-Parameter System (Hach Company, Loveland, USA). The moisture content of sediments was determined with the oven drying method (105 °C for 48 h) (Qian et al., 2011). Approximately 10–20 mg dry sediment was used to determine the total C (TC) and N content (TN) by elemental analyzer (Eurovector EA3028, UK). Total phosphorus (TP) was estimated according to an analytical protocol developed by the Standards Measurements and Testing Program of the European Commission (SMT protocol) (Ruban et al., 1999). In addition, the soluble phenolics, inhibitors of microbes and hydrolases activities, were determined by Folin Ciocalteu Method (Toberman et al., 2008).

2.3. Enzyme assays

The enzyme activity of sediment was assayed within 7 days after sampling. The procedures of assaying GLU, NAG and ACP were the same except the buffer and substrate used (Table 1). Briefly, 1 g sediment was weighed into a 50 mL Erlenmeyer flask, and then added 0.2 mL of toluene, 4 mL of buffer and 1 mL of substrate. The flask was sealed with stopper, mixed thoroughly, and incubated at 37 °C. After 1 h of incubation, the stopper was removed, and 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M tris (hydroxymethyl) aminomethane (THAM) buffer (100 mM, pH 12) were added to flask. Then, the sediment suspension was filtered through Whatman No.2 filter paper, and the filtrate was measured at 405 nm. A control was prepared for each assay in similar manner as described above, but the substrate was added after termination of the reaction using THAM buffer. There was a difference in assaying of ACP, in which 0.5 M NaOH was used to replace THAM buffer after incubation. The

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