International Biodeterioration & Biodegradation 132 (2018) 10–17

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

# Influence of mangrove roots on microbial abundance and ecoenzyme activity in sediments of a subtropical coastal mangrove ecosystem

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ARTICLE INFO

*Keywords:*
Rhizosphere
Phenol oxidase
Hydrolase
Enzymatic stoichiometry
Soluble phenolics

ABSTRACT

Although the rhizosphere effect has been widely studied, the relevant information about how mangrove roots affect microbial and enzymatic activity was still lacking due to the particularity of mangroves and intertidal zone. In order to understand the effects of mangrove roots on microbial growth and the indicators of nutrient cycling, microbial abundance as well as extracellular enzyme activities were investigated in both rhizosphere and non-rhizosphere sediments of a coastal mangrove wetland ecosystem. Comparing to non-rhizosphere sediments, bacterial abundance was slightly lower, but fungal abundance was notably higher in the rhizosphere sediments. Moreover, β-glucosidase (GLU), N-acetyl-glucosaminidase (NAG) and acid phosphatase (ACP) activities were enhanced clearly, whereas phenol oxidase (PHO) activity was significantly reduced in the rhizosphere sediments. Interestingly, it was found that soluble phenolics were closely related to fungal abundance and PHO activity ($p < 0.05$). Both fungal abundance and PHO activity were also significantly correlated with each other ($p < 0.05$). Therefore, the strong correlation between fungal abundance and both phenolics and PHO activity might partially suggest that fungi might contribute to the reduction of soluble phenolics.

## 1. Introduction

Mangrove ecosystem represents a complex ecotone between terrestrial and marine environments along tropical and subtropical coastlines (between 35°N and 35°S latitude) (Bouillon et al., 2008; Wu et al., 2008; Pires et al., 2012). These intertidal forests are ecologically and economically important, because they contribute to coastline protection, produce detritus to sustain an extensive food web, act as nutrient filters between land and ocean ecosystems, and so on (Holguin et al., 1992; Flores-Mireles et al., 2007; Bouillon et al., 2008; Pires et al., 2012). Moreover, mangrove ecosystems are considered as carbon (C) sink and secondary treatment system for wastewater due to the high productivity and ability to thrive against different environmental stresses (Kathiresan and Bingham, 2001; Bouillon et al., 2008; Wu et al., 2008; Saxena et al., 2013). Mangroves can withstand the harsh conditions such as high levels of salinity because mangrove root membranes are unique and can prevent salt entering while allowing water to pass through. On the other hand, mangrove can tolerate the anaerobic environments since mangrove roots are well-developed aerial to allow oxygen moving to underground roots (Kathiresan and Bingham, 2001; Saxena et al., 2013).

The importance of roots has been widely emphasized in lots of studies. The soil adhering to the root surface, no more than 4 mm, is defined as rhizosphere. In fact, most soils are rhizosphere soils at one time or another since plant roots can influence majority of the upper layer of the earth (Olson et al., 2004; Rengel and Marschner, 2005; Manoharachary and Mukerji, 2006; Egamberdieva et al., 2011; Pires et al., 2012; Brzostek et al., 2013; DeAngelis, 2013). Mostly, the rhizosphere is a special niche and has various physico-chemical characteristics compared to bulk soils due to the direct or indirect influences by root exudates (Manoharachary and Mukerji, 2006; Nannipieri et al., 2008; Pathan et al., 2014). Root exudates (e.g., organic acids and inorganic compounds) are frequently reported to stimulate the microbial activity, diversity as well as enzyme activity through altering soil physicochemical properties and substrates for microorganisms. Traditionally, this phenomenon is termed as “rhizosphere effect” (Reddy et al., 1987; Priha et al., 1999; Manoharachary and Mukerji, 2006; Nannipieri et al., 2008; Brzostek et al., 2013; Zhang et al., 2014), and

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https://doi.org/10.1016/j.ibiod.2018.05.002
Received 19 March 2018; Received in revised form 17 April 2018; Accepted 3 May 2018

this effect depends on the host plants, soil types and environmental variables (e.g., pH, moisture, and nutrients) (Kandeler et al., 2002; Rengel and Marschner, 2005; Nannipieri et al., 2008).

In the rhizosphere, soil microbes and enzymes are important for nutrients cycling between soils and plants (DeAngelis, 2013; Bell et al., 2014a). Abundant and diverse microorganisms in soils are essential agents in nutrient cycling and soil organic matter (SOM) turnover. Soil enzymes responsible for C and nutrient cycling, are usually produced by microorganisms to generate foods when them feel starving (Vázquez et al., 2000; Haase et al., 2008; Pivničková et al., 2010; Brzostek et al., 2013; Zhang et al., 2014). From this aspect, soil microbial activity and enzymatic activity are often used to indicate the quality of SOM and nutrient acquisition under different environmental conditions. Plenty of studies have shown that both microbial and enzymatic activities are higher in the rhizosphere soils than bulk soils, which are also called "rhizosphere effect". In general, the "rhizosphere effect" varied with the plant species, soil types as well as the climatic conditions (Reddy et al., 1987; Vázquez et al., 2000; Tam et al., 2001; Kandeler et al., 2002; Nannipieri et al., 2008; Egamberdieva et al., 2011). However, the "rhizosphere effect" seemed not always be stimulatory due to the strong competition of nutrients between plants and microbes, especially under harsh and nutrient deficiency environments (Priha et al., 1999; DeAngelis, 2013; San-An et al., 2014).

To our knowledge, mangrove ecosystems are largely different from the typical terrestrial ecosystems in many ways due to the regular tidal cycles and sometimes freshwater flooding (Wu et al., 2008; Pires et al., 2012). Although the microbial and enzymatic activities in rhizospheric soils are well studied in many terrestrial ecosystems, the disparity between rhizosphere and non-rhizosphere sediments remains poorly documented in mangrove ecosystems. In previous study, Luo et al. (2016) reported that the abundance of bacterial laccase-like genes (relating to phenolics degradation) in mangrove forest was higher than intertidal zone without mangrove, suggesting mangrove roots might play an important in regulating several enzymes involving in SOM degradation. Therefore, to help understanding the effect of mangrove roots in microorganisms and enzymes, this study investigated the abundance of bacteria and fungi, extracellular enzyme activities involved in SOM degradation and nutrient cycling. Furthermore, the interrelationships among microbial abundance, enzyme activity and environmental variables were explored with the aim of providing more information for future studies.

## 2. Materials and methods

### 2.1. Study site

Mai Po Nature Reserve (between 22°29′N and 22°31′N and between 113°59′E and 114°04′E) is located in the North West New Territories of Hong Kong. Mai Po was chose to conduct this study because it is considered as 'Wetland of International Importance' under the prestigious Ramsar Convention in 1995. It comprises of drainage channels, fishponds, intertidal mudflats, and sub-tropical mangroves dominated by *Kandelia obovata* (Cao et al., 2011; Li et al., 2011; Shen et al., 2012). This ecosystem is a shallow estuary with a maximum tidal range of 2.8 m, and frequently contaminated by human activities since large amounts of domestic sewage and industrial wastewater are input (Cao et al., 2011).

### 2.2. Sediment sampling and characterization

Four sites (recorded as S1, S2, S3 and S4, respectively), along from closing to the intertidal zone to deep in the mangrove forests, were chose to collect both rhizosphere and non-rhizosphere sediments in January of 2014 (Fig. S1). Sediments adhering to mangrove roots no more than 4 mm were taken as rhizosphere sediments, while non-rhizosphere sediments were collected as least 4 mm away from any roots (Alguacil et al., 2005; DeAngelis et al., 2008; Nannipieri et al., 2008; Brzostek et al., 2013). For each sample, three subsamples were mixed to compose one sample. The sediments were homogenized and sieved through a mesh size of 2 mm for removing stone or roots, and then transported to lab immediately for further analyses. Sediments were stored at 4 °C for determining the physicochemical characteristics and enzyme activities. For DNA analyses, sediments were stored at −20 °C before DNA extraction.

The pH of wet sediments was measured by using an IQ180G Bluetooth Multi-Parameter System (Hach Company, Loveland, CO, USA). Wet sediments were applied to determine the concentration of soluble phenolics by Folin-Ciocalteau's method proposed by Toberman et al. (2008) (described in Supporting Information (SI)). Dry sediments were used to determine the contents of total C (TC) and N (TN) by Elemental analyzer (Eurovector EA3028, UK). Moreover, total P (TP) of dry sediment was measured by SMT protocol of the European Commission (Ruban et al., 1999).

### 2.3. Microbial abundance by qPCR

Whole community DNA was extracted by MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) and the extracted DNA was stored at −20 °C. The concentration of DNA in all samples ranged from 15.5 to 36.0 ng/μL. Quantitative polymerase chain reaction (qPCR) assays were used to determine the abundance of bacteria and fungi. The PCR primers Eub338/Eub518 were used for bacterial abundance and ITS1-F/5.8S were applied for fungal abundance (Rousk et al., 2010). The detailed procedure of qPCR was described in Supporting Information (SI).

### 2.4. Enzyme assays

A series of extracellular enzymes were assayed, including both oxidase (phenol oxidase (PHO)) and hydrolases (β-glucosidase (GLU), *N*-acetyl-glucosaminidase (NAG) and acid phosphatase (ACP)) involved in C, N and P cycling. PHO is proposed as a regulator of hydrolases and also an enzymic latch of C store in peatlands due to its role in degrading phenolics (inhibitor of hydrolases) (Freeman et al., 2001; Fenner et al., 2005; Toberman et al., 2008). GLU is the most commonly measured indicator of C dynamics because of its importance in hydrolysing cellulose and polymeric saccharides to glucose. NAG and ACP are usually applied to reveal N and P acquisition, respectively (Moorhead et al., 2012, 2013). The enzyme activity of sediment was assayed within 7 days after sampling. The methods used to measure enzyme activity were based on Methods of Soil Enzymology (Dick, 2011). The procedures of assaying PHO activity were different with assaying activity of GLU, NAG and ACP. The substrates and buffers used in this study were listed in Table S1. The activity of each enzyme was expressed as μmol product $g^{-1}$ sediment $h^{-1}$. The details of enzyme assay were described in Supporting Information (SI).

In order to characterize enzyme allocation patterns, ecoenzymatic stoichiometry was applied to estimate microbial N and P limitation relative to C. The enzymatic GLU:NAG and GLU:ACP were calculated, and used as the microbial N and P limitation relative to C (Moorhead et al., 2012, 2013). The ratio of GLU:PHO was calculated as a relative measure of organic matter recalcitrance (Sinsabaugh and Follstad Shah, 2012).

### 2.5. Data analyses

One-way analysis of variance (ANOVA) conducted by Origin 8.0 was applied to analyse significant difference of enzyme activity and microbial abundance between rhizosphere and non-rhizosphere sediments. Statistical significance was accepted at $p < 0.05$. In order to test the interrelationships among microbial abundance, enzyme activity and environmental variables, Canoco 4.5 and SPSS 21.0 were used to perform redundancy analysis (RDA) and Pearson correlation analysis.