Global J. Environ. Sci. Manage., 1 (1): 15-26, Winter 2015
DOI: 10.7508/gjesm.2015.01.002

# Particle-size fractions-dependent extracellular enzyme activity in sediments and implications for resource allocation in a subtropical mangrove ecosystem

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Received 6 October 2014; revised 2 November 2014; accepted 20 November 2014; available online 1 December 2014

**ABSTRACT:** The distribution of extracellular enzyme activities in particle-size fractions of sediments was investigated in a subtropical mangrove ecosystem. Five enzymes involved in carbon (C), nitrogen (N), and phosphorus (P) cycling were analyzed in the sand, silt, and clay of sediments. Among these fractions, the highest activities of phenol oxidase (PHO), β-D-glucosidase (GLU), and N-acetyl-glucosiminidase (NAG) were found in sand, and greater than bulk sediments of both intertidal zone (IZ) and mangrove forest (MG). This result implied that sand fractions might protect selective enzymes through the adsorption without affecting their activities. Additionally, the enzyme-based resource allocation in various particle-size fractions demonstrated that nutrients availability varied with different particle-size fractions and only sand fraction of MG with highest total C showed high N and P availability among fractions. Besides, the analysis between elemental contents and enzyme activities in particle size fractions suggested that enzymes could monitor the changes of nutrients availability and be good indicators of ecosystem responses to environmental changes. Thus, these results provided a means to assess the availability of different nutrients (C, N, and P) during decomposition of sediment organic matter (SOM), and thus helping to better manage the subtropical mangrove ecosystems to sequester C into SOM.

**Keywords***: Extracellular enzymes, Resource allocation, Particle-size fraction, Mangrove ecosystem*

## INTRODUCTION

Mangrove ecosystems, connecting land and ocean along tropical and subtropical zone, rank as one of the most productive biomes due to net primary production of approximately 218±72 Tg C yearly (Alongi, 2011; Tue *et al.*, 2012). Mangrove ecosystem is regarded as a source and sink of atmospheric $CO_2$ and organic carbon, thereby playing an important role in global carbon cycle (Tue *et al.*, 2012; Zhang *et al.*, 2012). Mangrove sediments receive high inputs of organic matter from leaf litter or dissolved organic carbon, and are responsible for approximate 10% of the global organic carbon burial in the coastal ocean (Bouillon *et al.*, 2007; Bouillon *et al.*, 2008). Therefore, this ecosystem can be considered as a potentially efficient pool for long-term sequestration of organic carbon.

All mangrove organic matter entering the sediment is degraded or chemically modified by microorganisms (Kristensen *et al.*, 2008). Microbial activity largely affects sediment carbon stabilization and nutrients cycling through producing extracellular enzymes to obtain nutrients for growth and enzyme synthesis (Allison and Jastrow, 2006; Nie *et al.*, 2014). Extracellular enzymes play important roles in decomposition of sediment organic matter, and are often used as indices of microbial activity and nutrients availability by affecting energy transfer, environmental quality and crop productivity (Allison *et al.*, 2007; Manju *et al.*, 2012; Mohebbi Nozar *et al.*, 2013; Nadeau *et al.*, 2007; Salazar *et al.*, 2013).

In ecosystems, soil and sediment could stabilize C mainly through the mechanism of mineral sorption and soil/sediment aggregation (Allison and Jastrow, 2006). Soil structure is consisted of mineral particles (sand, silt and clay) and organic compounds forming

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aggregates of different sizes and stability (Stemmer *et al*., 1998). Recent studies demonstrated that turnover time of soil organic matter varied with different particle-size fractions, and turnover rates generally decreased as sand < silt < clay (Bol *et al*., 2009; Yang *et al*., 2012). Due to the linkage between turnover rates of organic matter and the particle-size fractions, these fractions were proposed to play a key role in C sequestration (Six *et al*., 2002).

Soil organic matter (SOM) is important for a variety of biological, chemical and physical properties of soils (Bol *et al*., 2009). The different sequestration form of SOM could change soil organic carbon for storage and turnover (Cheng *et al*., 2010). Many studies pointed that most SOM transformations were catalyzed by intracellular and extracellular enzymes, releasing from bacteria, fungi, and plants (Allison and Jastrow, 2006; Salazar *et al*., 2013; Sinsabaugh, 2010; Stemmer *et al*., 1998). After enzymes excreted from microorganisms or plants into environment, they can be rapidly sorped, denatured, degraded or irreversibly inhibited. However, enzymes could also be persisted in soil through sorption on soil minerals or incorporation into humic material (Marx *et al*., 2005). In addition, some studies fractionated soils into different particle sizes and then determined the activities of enzymes in each fraction. They found that the distribution of enzymes among different fractions depended on the enzymes and their role in carbon (C), nitrogen (N), phosphorus (P) cycling. Moreover, the location of enzymes was also related to SOM quality and turnover in fractions (Allison and Jastrow, 2006; Kandeler *et al*., 1999; Marhan *et al*., 2007; Marx *et al*., 2005; Nie *et al*., 2014; Rao *et al*., 2000; Stemmer *et al*., 1998). Therefore, enzyme analysis in differently particle-size fractions can be considered as a key attribute to understand mechanisms driving C- and N-turnover, and also provide fundamental information about changes in the distribution of SOM. In mangrove ecosystem, there are at least two major types of sediment, locating in mangrove forest and intertidal zone. The SOM in mangrove forest might be significantly affected by the existence of trees, while the SOM in intertidal zone is possibly influenced by the settlement of particulate organic matter from tidal water (Bouillon *et al*., 2007; Bouillon *et al*., 2008; Kristensen *et al*., 2008; Rezende *et al*., 1990). In our previous study, it was found that the changes of SOM were different between mangrove forest and intertidal zone. Extracellular enzyme activity (EEA) is often used as indicator of SOM quality and transformation, and it has been conducted in bulk sediments of mangrove ecosystem. However, the information relating EEA of differently particle-size fractions of sediment to organic C, N and P pools in mangrove ecosystems are scarce. In this study, we chose wet sieving and centrifugation procedure to separate bulk sediments, both in mangrove forest and intertidal zone, to three fractions (sand, silt, and clay). Then, enzymes involved in C (phenol oxidase, peroxidase and β-D-glucosidase), N (N-acetyl-glucosiminidase), and P (acid phosphatase) cycling were analyzed in differently particle-size fractions. Furthermore, enzymatic resource allocation and the decomposition of SOM among C, N, and P availability in each fraction were studied by Microbial Allocation of Resource among Community Indicator Enzymes (MARCIE) (Sinsabaugh *et al*., 2002).

## MATERIALS AND METHODS

### *Sample collection*

Samples were collected from Mai Po Nature Reserve of Hong Kong (22°29'N to 22°31'N and 113°59'E to 114°03'E) in an intertidal estuary of the Pearl River Delta, China. Due to the different properties of mangrove forest and intertidal zone, two types of sediment were collected (Cao *et al*., 2011; Li *et al*., 2011). In this study, a total of four samples were collected in March 2012. Two samples from intertidal zone without mangroves were recorded as IZ1 (N22° 29' 40.7", E114° 01' 42.1'') and IZ2 (N22° 29' 56.8", E114°01'39.0'') and another two samples were taken from mangrove forest, recorded as MG1 (N22°29'39.8", E114°01'43.6'') and MG2 (N22°29'55.7", E114°01'41.2''). All samples were collected from surface sediments (0-2 cm) of multiple locations representing the type of the ecosystem. After sampling, the sediment samples were mixed to form a composite and were then sieved through a mesh size of 2 mm, and then stored -20 ºC (Toberman *et al*., 2008).

### *Fractionation procedures*

Wet sieving and centrifugation procedure according to Stemmer et al. (1998) was used to separate sediments into three size fractions, i.e., sand (63-2000 µm), silt (2-63 µm), and clay (<2 µm). Briefly, wet sediment (35 g equivalent dry weight) was placed into 150 mL beaker and dispersed in 100 mL of distilled water (10 ºC) using a probe type ultrasonic disaggregator with 50 J/s output energy for 120 s. Sand and silt were separated by wet

sieving manually using 400 mL of cooled distilled water. During this procedure, particles remaining on the sieves were collected as sand, and particles passing through the sieves were silt and clay. To separate silt-sized particles from clay, the remaining suspension were put into four 250 mL centrifuge bottles and centrifuged at approximately 150× *g* for 2 minutes at 15 ºC. After centrifugation, the pellets were re-suspended by cooled distilled water, and then re-centrifuged at 150× *g* for another 2 minutes at 15 ºC. This procedure was repeated twice, and all the supernatants were collected to obtain clay fraction. The supernatants were distributed into six 250 mL centrifuge bottles and centrifuged at 3900× *g* for 30 minutes at 15 ºC, and the pellets after the centrifugation were clay-sized fraction. All fractions were stored at 4 ºC, and enzyme activities in each fraction were determined as soon as possible.

*Enzyme assay*

Enzyme activity was measured according to methods described recently (Dick, 2011). Activities of phenol oxidase (PHO), peroxidase (POD), β-D-glucosidase (GLU), N-acetyl-glucosiminidase (NAG), and acid phosphotase (ACP) were analyzed in both bulk sediment (non-fractionated) and the differently particle-size fractions by using L-3,4-dihydroxy phenylalanine (for PHO and POD), *p*-Nitrophenyl-β-D-glucoside, *p*-nitrophenyl-N-acetyl-β-D-glucopyanoside, and *p*-nitrophenyl phosphate as substrate, respectively. Enzyme assays were carried out three times for each category of analysis and the mean from them was used in presentation of the results.

*MARCIE*

MARCIE uses enzyme activity to decomposition rate and to access relevant N and P availability. This model could facilitate estimation of decomposition rates in the field and improve ecological forecasting (Penton and Newman, 2007, 2008; Sinsabaugh *et al*., 2002; Sinsabaugh and Moorhead, 1994). In this model, extracellular enzymes were divided into four categories: Ec (GLU), En (NAG), Ep (ACP) and Eox (mean (PHO and POD)), reflecting those involved in C, N and P mineralization and lignin degradation, respectively. To eliminate the weighting effects of the more active enzymes, enzyme activities were normalized on a scale 0-1 by dividing each enzyme by the highest activity in that category. As for the value of Eox, the standardized values of both PHO and POD were averaged. Three additional ratios were formulated: Ec/En, Ec/Ep, and Ec/Eox. Ec/En reflects apparent N control over C mineralization, Ec/Ep is relative measure indicating P control over C mineralization, and Ec/Eox reflects potential lignin control over C mineralization (Penton and Newman, 2007, 2008; Sinsabaugh *et al*., 2002; Sinsabaugh and Moorhead, 1994).

*Total C, N and P*

Total C and N were determined by elemental analyzer (Eurovector EA3028, UK). Approximately 10 to 20 mg of dry sediment was used for each bulk sediment and particle-size fraction. Replicate analysis was carried out to obtain the average of the value. Total P was determined according to an analytical protocol developed by the Standards Measurements and Testing Program of the European Commission (SMT protocol) (Ruban *et al.*, 1999).

*Data Analysis*

Origin 8.0 was used to analyze data obtained from this study. Canoco 4.5 software was applied to conduct redundancy discriminate analysis (RDA) for ordination to analyze the relationship between elemental contents and enzyme activities. Furthermore, SPSS 21.0 was performed to examine the correlation between elemental ratios and enzymatic ratios by Pearson's coefficient.

## RESULTS AND DISCUSSION

*Particle-size fractions*

Table 1 shows the particle-size distribution of the four sediments from mangrove ecosystem. The distribution of the different fractions had no unifying trend for each sample. The results showed that both IZ1 and IZ2 had highest contents of silt, and followed by clay, and then the lowest, sand. For the sediments from mangrove forest, silt was also the highest in each sample, but the sand was higher than clay in MG1 while

Table 1: Particle size distribution of sediments in mangrove forest and intertidal zone at Mai Po Nature Reserve in Hong Kong

| Samples | Composition (%) | | |
|---|---|---|---|
| | Sand (>200 μm) | Silt (63-2 μm) | Clay (<2 μm) |
| IZ1 | 20.11 | 44.43 | 35.46 |
| IZ2 | 12.95 | 60.28 | 26.77 |
| MG1 | 34.24 | 41.35 | 24.41 |
| MG2 | 21.22 | 50.43 | 28.34 |

MG2 had higher clay than sand.

The total C, N and P in different particle size fractions differed distinctly (Table 2). Except IZ2, the C contents decreased in the order of sand > clay > silt. For IZ sediments, the N content increased with decrease of particle sizes (clay > silt > sand). However, the situation in MG sediments seems to be different, the N content decreased in the order of sand > clay > silt. Additionally, almost all the samples showed the order of clay > sand> bulk > silt for content of total P. Compared to the bulk sediment, 70%-85% and 78-92% of total C and total N, respectively were found in the particle size fractions smaller than 63 μm in IZ sediments. In MG sediments, only 34-52% and 51-72% of the total C and total N, respectively were located in fractions less than 63μm. This means sand fraction in MG possessed 48-66% total C and 30%-49% total N, whereas that in IZ had only 15%- 30% and 8%-22% of the total C total N, respectively. Among all samples, 65% of total P was found in the particle size fractions smaller than 63 μm.

*Enzyme activity*

PHO activities (Fig. 1a) in different particle-size fractions and bulk sediment demonstrated that there was no significant difference among different fractions of IZ1, but the rest 3 samples showed significant difference among them. Interestingly, PHO activity in sand fraction was the highest among fractions and bulk sediments except MG1, which had extremely high PHO activity in clay-size fraction. The PHO activities of sand-size fraction in IZ samples were quite close to the bulk sediments, while sand fraction of MG1 and MG2 had significantly higher activity of PHO than the bulk sediment by 1.63-fold and 2.78-fold, respectively.

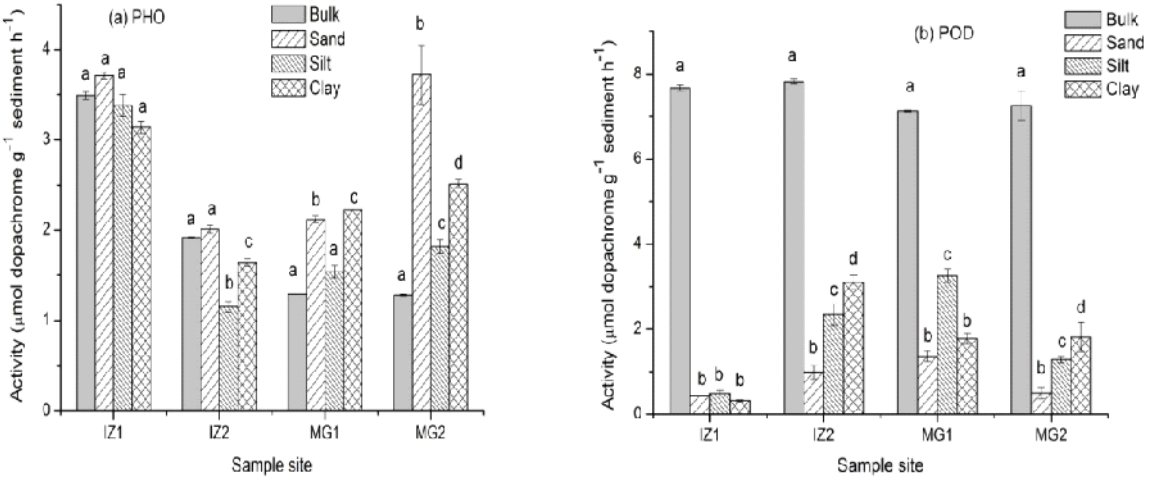


Fig. 1: Activity of oxidative enzymes in particle-size fractions in intertidal zone and mangrove forest in Mai Po Nature Reserve, Hong Kong. Different letter means there was a significant difference at $p<0.05$ level.

Table 2: Total carbon and nitrogen in the bulk sediments and the different particle-size fractions of sediments in mangrove forest and intertidal zone

| | TC(%) | | | | TN(%) | | | | TP(%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bulk | Sand | Silt | Clay | Bulk | Sand | Silt | Clay | Bulk | Sand | Silt | Clay |
| IZ1 | 1.59 | 1.64 | 1.37 | 1.39 | 0.17 | 0.19 | 0.14 | 0.20 | 0.14 | 0.17 | 0.15 | 0.18 |
| IZ2 | 1.47 | 1.53 | 1.25 | 1.83 | 0.15 | 0.16 | 0.13 | 0.22 | 0.13 | 0.16 | 0.09 | 0.20 |
| MG1 | 4.85 | 5.37 | 2.52 | 2.61 | 0.30 | 0.36 | 0.20 | 0.29 | 0.19 | 0.23 | 0.15 | 0.25 |
| MG2 | 3.35 | 5.82 | 2.11 | 2.39 | 0.24 | 0.37 | 0.19 | 0.28 | 0.19 | 0.25 | 0.17 | 0.24 |

As for the distribution of POD (Fig. 1b), it is obvious that bulk sediments had extremely high activity than the sand, silt and clay fractions in all samples. This implied that the POD might be lost during the procedure of fraction. Among these particle-size fractions, two distribution trends were observed. In IZ1 and MG1 sediments, the highest activity of POD was in silt-size fraction (5.9% and 46.7% of bulk sediment, respectively), while clay-size fraction had the highest activity of POD in IZ2 and MG2 samples (38.2% and 21.2% of bulk sediment, respectively). Moreover, the POD activities of sand-size fraction were almost the lowest among all samples, except IZ1. The lowest activity of POD approximately ranged from 2.7% to 19.6% of the bulk sediment.

The distribution of GLU (Fig. 2a) and NAG (Fig. 2b) were quite similar, and their activities in sand-size fractions were much higher than the bulk, silt, and clay fractions in all samples. GLU activities in silt- and clay-size fractions were close to the bulk sediment, and no significant differences were found among them. The distribution of NAG in silt- and clay-size fractions and bulk sediment were close between MG1 and MG2, while the activities of IZ1 and IZ2 decreased in the order: bulk > clay > silt. Compared to the bulk sediments, ACP activity (Fig. 2c) in each particle-size fraction was lower except for IZ1, which showed the highest activity in clay-size fraction. The activity of ACP decreased in the order of sand > clay > silt in MG1 and MG2, while

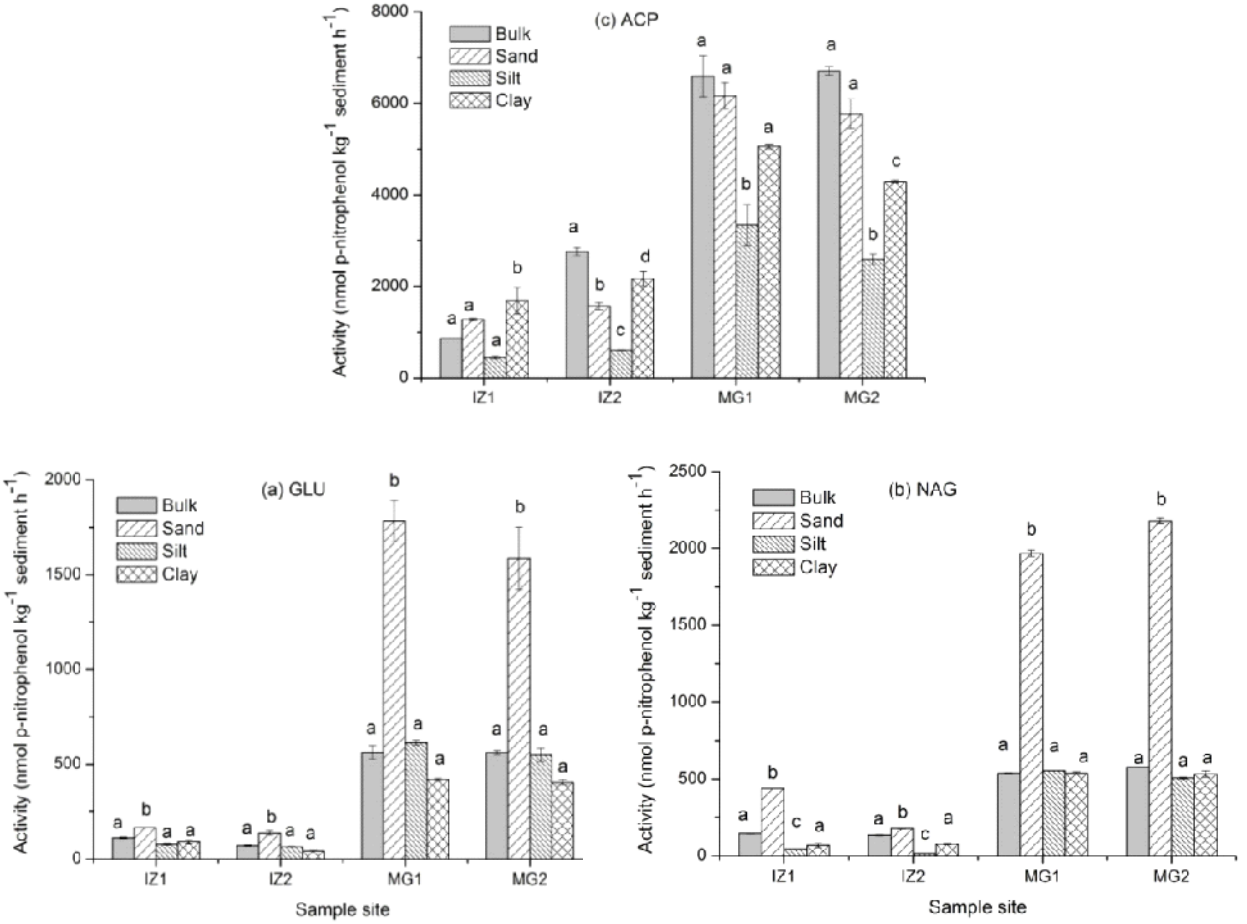


Fig. 2: Activity of hydrolytic enzymes in particle-size fractions in intertidal zone and mangrove forest in Mai Po Nature Reserve, Hong Kong. Different letter means there was a significant difference at $p<0.05$ level.

ACP activity decreased as clay > sand > silt in both IZ1 and IZ2.

In order to estimate the loss of enzyme through fractionation, the recovery of each assayed enzyme was calculated as the weighted sum of enzyme activity in each particle-size fraction dividing by the bulk sediment activity. The results revealed that POD activity decreased at least 70% in all samples, and ACP activity declined approximately more than 40% except IZ1 during the washing procedure of fractionation (Fig. 3). For PHO, GLU and NAG, the recoveries were >90% in all samples besides PHO (70.4%) and NAG (40.5%) in IZ2. Furthermore, recoveries of PHO, GLU and NAG in both MG1 and MG2 were higher than 100%, and the summed activities were approximately 1.37 fold to 1.91 fold of the untreated bulk sediments. However, recoveries of enzymes in IZ samples did not exceed 100%.

*Distribution of enzyme*

Free enzymes are unstable in soil once they release from their producers, and can be distributed in different particle-size fractions. Moreover, soil enzymes in differently particle-size fractions are proposed to be more or less sensitive to environmental changes and long-term management of ecosystems (Allison and Jastrow, 2006; Cheng *et al*., 2010; Kandeler *et al*., 1999; Marx *et al*., 2005; Nie *et al*., 2014).

*Oxidative enzymes*

In our study, the distribution of PHO in particle-size fractions varied with the review of Sinsabausgh (2010), which reported PHO tended to increase as particle-size decreased in aquatic ecosystem, and mean values for particle < 1 mm were close to the bulk soils. And findings of Grandy *et al.*, (2008) in two different ecosystems also supported that PHO activity decreased with increasing particle size of soil. PHO was preferentially stabilized on silt or clay surfaces through sorption due to the large surface areas of silt and clay fractions. Therefore, most PHO activity was assumed to be in the silt and clay-size fractions (Allison and Jastrow, 2006; Zavarzina, 2011). The reason why our results were inconsistent with the above-mentioned findings might be the different distribution of C in fractions and separation procedures used. According to Lagomarsino *et al*., (2012), enzyme activity was largely influenced by the organic C content and the quality of these C. Furthermore, the different physical protection mechanisms were also proposed to play important roles in the distribution of enzymes (Allison and Jastrow, 2006; Lagomarsino *et al*., 2012).

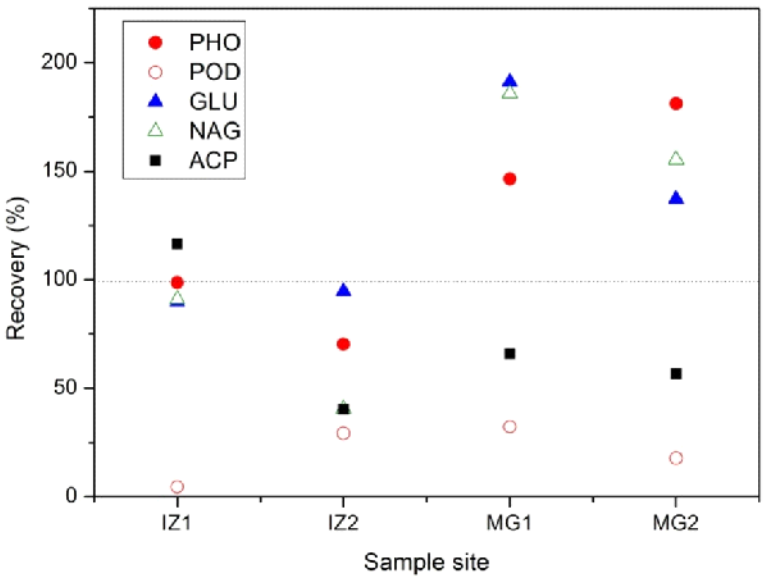


Fig. 3: Mean recoveries for assayed enzymes, calculated as the weighted sum of enzyme activity in the sand-, silt- and clay-size fractions, divided by the bulk sediment activity.

Obviously, our results almost followed this general trend showing the highest PHO activities in sand fraction due to the highest C contents in sand-size fraction. PHO, a mainly extracellular enzyme, is involved in the degradation of recalcitrant organic matter (Sinsabaugh, 2010). Therefore, the high activity of PHO in sand might be caused by the adsorption of PHO to particulate organic matter or litter debris, existing in sand fraction (Stemmer *et al*., 1998). Besides, the recovery of PHO (Fig. 3) indicated that more PHO activities were measured in both MG1 and MG2. These two sediments contained more litter compared to IZ sediments. Thus, this supported that the occluded or adsorbed PHO within sand fractions might be released or isolated by the ultra-sonication during the separation procedure.

Sinsabaugh and Findlay (1995) studied the distribution of POD in surface sediments of the Hudson River estuary. They found that POD activity varied with the sample sites and dates. The activity of POD in sediments from Kingston Shoal declined with particle-size fractions decreasing, but increased with particle-size fraction decreasing in Tivoli North Bay sediments. As for the rest sediments, it seemed that there was no obvious trend for distribution of POD in particle-size fractions, and the POD activity was even undetected when the particle-size was < 63 μm (silt and clay).

In our study, we also found the distribution of enzymes varied with the sample sites. However, the distribution pattern was nearly identical when the sample sites were close to each other. For example, IZ1 and MG1, located near next to each other, showed almost the same trend on distribution of POD in particle-size fractions (silt > clay > sand) as well as of IZ2 and MG2 (clay > silt > sand). The C content and C quality were assumed to act as important regulators for POD distribution (Sinsabaugh, 2010; Sinsabaugh and Findlay, 1995). Furthermore, POD inactivation was observed by cellulose, silica sand, and lignin (Qing *et al.*, 2004). Therefore, it can be concluded that the distribution pattern of POD was determined by the combined effects of C content, C quality and inactivation mechanisms of particle-size fractions. Except the chemical properties of fractions, the physical separation was also considered to be a key factor to influence the distribution pattern of POD due to its low recovery in each sample (Fig. 3). It indicated that washing and ultra-sonication procedures could loss or inactivate POD (Allison and Jastrow, 2006; Marx *et al*., 2005; Stemmer *et al*., 1998).

*Hydrolytic enzymes*

The distribution patterns of GLU and NAG (Figs. 2a and 2b) were reported to be variably in that both GLU and NAG activity increased with decrease of particle-sizes (Grandy *et al*., 2008). However, our results were quite similar with that of others, which showed the absolute activity of GLU and NAG generally decreased from coarse sand to the silt-size fractions (Marx *et al*., 2005).

In the current study, the decrease of C/N ratio supported a trend of GLU distribution in the order of sand > silt > clay. For NAG, the lowest activity was found in the silt and this might be caused by the lowest N content in the silt fraction (Nie *et al*., 2014). It was assumed that sand fraction, containing lots of polymeric materials, could account for the occurrence of high carbohydrase activity (e.g., GLU) in the fraction (Marx *et al*., 2005). Combined with C contents in particle-size fractions, it can be concluded that the highest activity of GLU and NAG in sand was probably caused by active microbes using abundant C to produce enzymes, as well as by providing more sites for enzymes to adsorb onto the sand fraction. The explanation was also applied to activities of NAG in fractions of restorated grassland soils (Allison and Jastrow, 2006). Furthermore, the lower potential activities of GLU and NAG in silt or clay-size fractions were presumably resulted from increasing complexity of the remaining organic materials and losing sites of adsorption for enzymes during degradation of SOM (Marx *et al*., 2005). It has to point out that interactions between enzymes and particle surfaces play an fundamental role in the enzyme activities observed and the adsorption by sand and smaller size fractions including silt and clay, and result in very different enzyme immobilization and inactivation, especially on clays due to stabilization of enzymes.

Besides, the higher recoveries of GLU and NAG (Fig. 3) with more than 100% in MG samples suggested that some protected GLU and NAG were released again due to the separation procedure. However, the recovery rates of these two enzymes in IZ sediments were less than 100%. This means the separation procedure could cause loss of some enzymes, and the amount of loss may depend on the type of sediments with different C and N contents. Sediments in mangrove forest could

receive more plant materials than intertidal zone, and thus causing significantly higher C content in each fraction of MG corresponding to IZ. Consequently, the higher C content in each fraction of MG samples indicated that GLU and NAG might be protected by the adsorption or stabilization mechanisms to particle-size fractions through physical and chemical processes (Stemmer *et al.*, 1998).

For ACP, there were two different distribution patterns in our study (Fig. 2c). The distribution of ACP in IZ sediments (clay > sand > silt) was in agreement in that phosphatase was predominant in the clay-size fractions (Marx *et al.*, 2005). While the trends of MG sediments (sand > clay > silt) showed a similar pattern with that of Rojo *et al*., (1990), which reported that phosphatase activity was concentrated in larger size soil fractions (2000-100 μm, i.e. approximately sand fraction). Clay-size fractions were proven to have higher amounts of P-substrates and microorganisms, and this might be the reason why clay showed highest activity in IZ sediments (Kandeler *et al*., 1999; Marx *et al*., 2005). For MG sediments, the sand fraction had highest C contents, as well as highest values of C/N ratio, implying the turnover rate of sand fraction in MG sediments was relatively fast and more phosphatase might be produced by microorganisms to meet the demand of P resource (Allison, 2006; Allison and Jastrow, 2006; Rojo *et al*., 1990). Consequently, it can be referred that the different distribution trends of ACP in IZ and MG sediments were probably caused by the type of sediments and requirements of microorganisms.

In addition, the recoveries of ACP in all samples were determined by the type of enzyme and fractionation. Recoveries of ACP in IZ2, MG1 and MG2 indicated that the separation procedures could contribute to loss of ACP through washing and ultra-sonication procedures (Marx *et al*., 2005; Stemmer *et al*., 1998). However, the higher recovery of IZ1 might be due to the predominant role of clay on adsorption of ACP (Rao *et al.*, 2000).

*Resource allocation to enzyme production*

Extracellular enzyme activity, influenced by biogeochemical conditions, was proposed to control microbial nutrient acquisition (Penton and Newman, 2007, 2008; Sinsabaugh and Moorhead, 1994). The values of Ec/En, Ec/Ep and Ec/Eox, relating to nutrient acquisition, were applied to estimate N, P and potential lignin controls over C mineralization (Fig. 4).

Silt size fraction in all samples showed the highest Ec/En value, exceeding 1.0, indicating silt size fraction

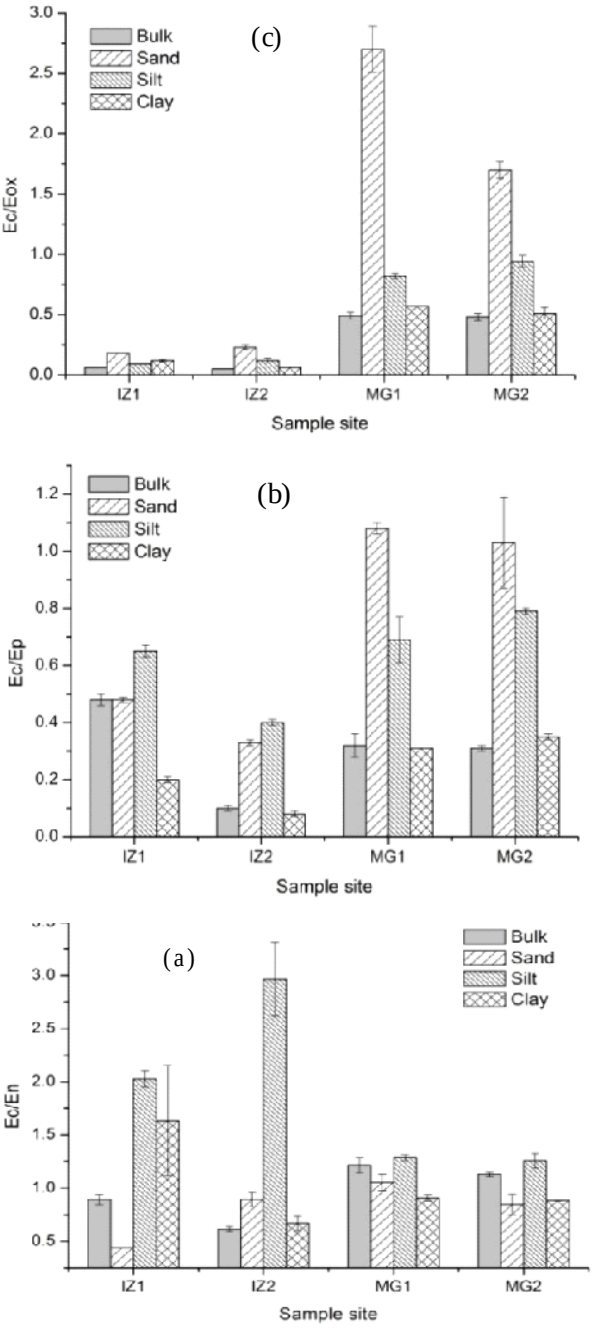


Fig. 4: Ec/En, Ec/Ep, and Ec/Eox values with standard error in different particle-size fractions.

was high N availability and supported the findings of the lowest NAG activity in all silt size fractions (Fig. 2b). All the fractions showed lower Ec/Ep than 1.0, except sand of MG1. Thus, it assumed that almost all fractions might be P-limited. The lowest activity of ACP combined with highest Ec/Ep value in IZ silt, as well as the highest activity of ACP with lowest Ec/Ep value in IZ clay, seemed to favor this assumption (Fig. 2c). For MG fractions, the lowest activity of ACP in MG silt did not show highest value of Ec/Ep among fractions. The reason might be the loss of ACP activity during the fractionation procedure (Fig. 3).

Among the different fractions, the order of Ec/Eox almost decreased as: sand > silt > clay. The highest Ec/Eox in sand size fraction indicates an apparent decrease in lignin control on C mineralization (Penton and Newman, 2007). The Ec/Eox value was proposed to correlate with the quality of substrates, and the fractions might be from ligninfied to humified along with decreasing particle-size (Ayuso *et al*., 2011; Penton and Newman, 2007; Sinsabaugh *et al*., 2002). According to Bol *et al*., (2009), lignin contents in different particle size fractions declined in the order of sand > silt > clay (nearly absent). Grandy *et al*. (2008) also observed that the lignin content was highest in the fractions of >63 µm (i.e. sand fraction). Therefore, it can assume that lignins play important roles in the C mineralization process, especially in sand fraction, which contains greater lignins.

By combining Ec/Ep and Ec/En for each particle size fraction, the influences of P and N on decomposition were constructed for all samples (Fig. 5). It is clear that the nutrients availability differed in particle-size fractions. Besides, it can be seen that all silt fractions and bulk sediments of MG were P-limited, and most of the clay fractions as well as sand fractions and bulk sediments of IZ were both N and P-limited. Among all fractions, only sand of MG1showed high N and P availability, which indicated more favorable decomposition conditions (Penton and Newman, 2008). Moreover, the higher Ec/Eox of sand in MG1 also predicted the higher decomposition rates (Penton and Newman, 2008). Additionally, the highest activities of hydrolases (GLU, NAG and ACP) in sand of MG1 (Fig. 2) among particle-size fractions also partially supported the prediction of faster decomposition of sand fraction in MG1.

*Relationship between elemental ratios and enzymatic ratios*

The relationship between elemental contents and enzyme activities were explored in this study, and the results are showed in Fig. 6 and Table 3. It is interesting

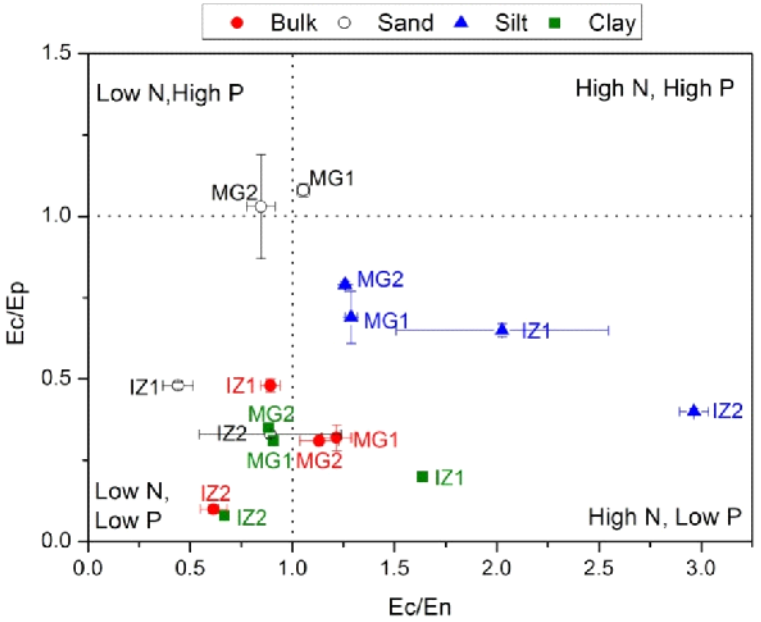


Fig. 5: N and P availability in different particle-size fractions. Each point represents the mean of one sample with standard error.

Table 3: Pearson correlation analysis of elemental and enzymatic parameters. * represents a significant correlation at $p < 0.05$ level; ** means a significant correlation at $p < 0.01$ level; *** at $p < 0.001$.

| | TC | TN | TP | C/N | N/P | C/P |
|---|---|---|---|---|---|---|
| PHO | -0.040 | 0.064 | 0.206 | -0.246 | -0.311 | -0.257 |
| POD | 0.040 | -0.112 | -0.285 | 0.256 | 0.255 | 0.249 |
| GLU | 0.908*** | 0.839*** | 0.600* | 0.775*** | 0.691** | 0.800*** |
| NAG | 0.893*** | 0.851*** | 0.643** | 0.718** | 0.641** | 0.745*** |
| ACP | 0.858*** | 0.852*** | 0.700** | 0.757*** | 0.624** | 0.759*** |
| Ec/En | -0.218 | -0.358 | -0.506* | -0.046 | 0.158 | 0.008 |
| En/Ep | 0.282 | 0.293 | 0.267 | 0.271 | 0.150 | 0.231 |
| Ec/Ep | 0.585* | 0.450 | 0.260 | 0.574* | 0.414 | 0.548* |
| Ec/Eox | -0.374 | -0.326 | -0.343 | -0.344 | 0.040 | -0.243 |

that all the hydrolytic enzymes positively correlated with TC, TN and TP, and also related to C/N, N/P and C/N ratios (at least $p < 0.05$), but no significance was found between them and oxidative enzymes. This result demonstrated that hydrolytic enzymes were more sensitive to resource availability than oxidative enzymes, which was consistent with the review of Sinsabaugh (2010). In several studies, enzymes were proposed to monitor the changes of nutrients availability and to be indicators of ecosystems responses to environmental changes (Hu *et al*., 2010; Penton and Newman, 2007, 2008; Sinsabaugh and Moorhead, 1994). However, no significance was found between enzymatic ratios and elemental ratios, except Ec/Ep and Ec/En. Negative correlation between Ec/En and TP, as well as positive relationship between Ec/Ep and TC, C/N and C/P, indicated that a proper ratio of C:N:P should be maintained by adjusting the dynamics of relative enzyme according to the limited factor (Sinsabaugh *et al*., 2009; Waring *et al*., 2014).

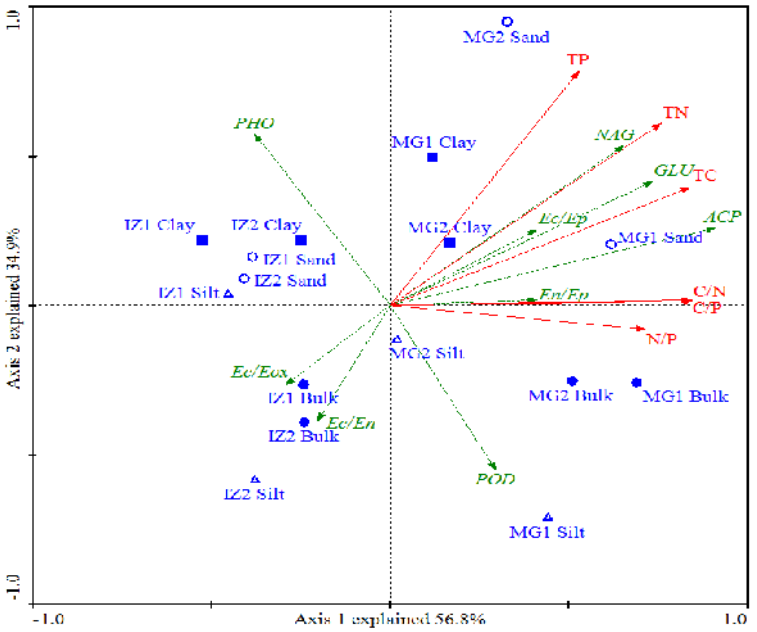


Fig. 6: Redundancy discriminate analysis (RDA) between elemental contents and enzyme activities in different particle-size fraction.

By comparison, it seems that hydrolytic enzymes played more important roles in decomposition of SOM in MG fractions, while oxidative enzymes (especially PHO) were more important for IZ fractions (Fig. 6). This result indirectly revealed that the quality of SOM varied widely between IZ and MG, and MG might be more decomposable than IZ revealed by higher Ec/Eox in Fig. 4c.

## CONCLUSION

In summary, this study revealed that the distribution of enzyme activities in different particle-size fractions varied with the types of sediment and enzymes in mangrove ecosystem. It also provided evidence to support that the enzymes (especially PHO, GLU and NAG) might be stabilized onto sand fractions by adsorption and aggregation. Furthermore, the enzyme-based resource allocation in various particle size fractions showed that sand of MG was high N and P availability, and possessed a higher value of Ec/Eox, indicating fast decomposition of SOM. For the rest fractions, all silt fractions and bulk sediments of MG were P-limited, and most of the clay fractions as well as sand fractions and bulk sediments of IZ were both N and P-limited. Besides, the analysis between elemental contents and enzyme activities in particle size fractions suggested that enzymes could monitor the changes of nutrients availability and be good indicators of ecosystem responses to environmental changes. Thus, these results provided a means to assess the availability of different nutrients (C, N, and P) during decomposition of SOM, and thus helping to better manage the subtropical mangrove ecosystems to sequester C into SOM.

## ACKNOWLEDGMENTS

This research project was supported by a Ph.D. studentship from the Graduate School, The University of Hong Kong (LL) and financial support of Environmental Toxicology Education and Research Fund of this laboratory.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**How to cite this article: (Harvard style)**

*Luo, L.; Gu, J.-D., (2015). Particle-size fractions-dependent extracellular enzyme activity in sediments and implications for resource allocation in a subtropical mangrove ecosystem, Global J. Environ. Sci. Manage., 1 (1): 15-26.*