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## Phytoplankton community and the purification effect of mangrove in the mangrove plantation-aquaculture coupling systems in the Pearl River Estuary

Qixin Huang<sup>a</sup>, Yu Liu<sup>a\*</sup>, Xingwen Zheng<sup>a</sup>, Guanqiu Chen<sup>a</sup><sup>a</sup>*School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou 510275, PR China*

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### Abstract

Shore water pollution has increased prominently as the rapid development of agriculture and industry. Nine ponds in the mangrove plantation-aquaculture A, B and C systems were established. The results showed that algal abundance ranged from  $10.5 \times 10^5$  to  $3744.3 \times 10^5$  ind./L and had positive correlations with Chl *a*, DO, COD and TP ( $r=0.697$ ,  $0.302$ ,  $0.350$ ,  $0.276$ , respectively;  $p<0.01$ ). Removal rates in mangrove *Bruguiera gymnorhiza* and *Rhizophora stylosa* for  $\text{NH}_3\text{-N}$ ,  $\text{NO}_3\text{-N}$ , TN,  $\text{PO}_4\text{-P}$  were 41.74%-88.43%.  $\text{NH}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  were lowered by 55.91%-75.31% in *Aegiceras corniculatum*. The system stability was enhanced after mangrove plantation by the indication of algal abundance and species composition.

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*Keywords:* phytoplankton; water quality; mangrove; purification; plantation-aquaculture coupling; the Pearl River Estuary

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### 1. Introduction

With the intensive marine aquaculture process, shore water pollution has increased prominently. In intensive marine shrimp culture, only 22%-24% of the nitrogen and 13% of the phosphorus of the feeding input was incorporated into the shrimp harvest, while 35%-57% was exported to the surrounding

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\* Corresponding author. Tel.: +86-20-84034271; fax: 86-(0)20-84037243.  
E-mail address: [eeslyu@mail.sysu.edu.cn](mailto:eeslyu@mail.sysu.edu.cn)

waters [1,2,3]. Nutrient enrichment in aquatic ecosystems can cause an increase in algae biomass and loss of ecosystem function [4]. As a primary producer, algae provide carbohydrates and oxygen by photosynthesis for trophic chains. Yet some algal blooms can cause harm to marine life [5] and could directly affect the harvest quantity and quality.

Mangroves are unique wetland systems along the coastline of tropical and subtropical regions. There are 26 true mangrove species and 11 semi-mangrove species in China, and 20 species (including both true mangrove and semi-mangrove species) grow in Guangdong Province [6]. Generally, mangrove species have a huge demand for nutrients because of their high primary productivity and rapid metabolism and turnover [7]. Over the recent few decades, mangrove wetlands have been utilized as sewage treatment system [8,9] for removing or retaining N, P [7,9,10,11] through the processes of sedimentation, filtration, microbial activity, plant absorption, etc. And it is reported that sewage discharges had no adverse effects on mangrove community structures [8].

Since Robertson and Phillip proposed the concept of “mangroves as filters of shrimp pond” in 1995 [12], people paid great interest in mangrove and aquaculture coupling systems [13,14]. There was a strong linkage between mangrove presence and fish yield [13,15,16]. The major reason for this was that mangrove ecosystem could provide abundant food inputs and nursery habitats for aquaculture [17,18].

Researches on constructed mangrove aquaculture systems have been started since 2002 [19]. Peng et al. (2009) studied three mangrove monoculture systems of *Sonneratia caseolaris*, *Aegiceras corniculatum* and *Kandelia obovata* and found that only *A. corniculatum* and *K. obovata* were efficient in removing DIN and PO<sub>4</sub>-P. However very limited information is available on dynamics of phytoplankton in these systems [20] and also few works have been done on other mangrove species.

*Kandelia obovata*, *Aegiceras corniculatum*, *Bruguiera gynorrhiza* and *Rhizophora stylosa* are four dominant mangrove species in the southern China. Three mangrove plantation-aquaculture systems in the Pearl River Estuary were constructed by using these four species. In this paper, the in-situ treatment efficiency of the mangrove was assessed based on the water quality dynamics and phytoplankton community and the inner mechanisms were analyzed.

## 2. Material and Methods

### 2.1. Experimental design

Nine mangrove plantation-aquaculture ponds were constructed in the eastern shore of the Pearl River Estuary (22°43.4'N- 22°43.9'N, 113°45.7E-113°46.3E)(Fig.1). They belong to three coupling systems (Fig. 1; Table 1).

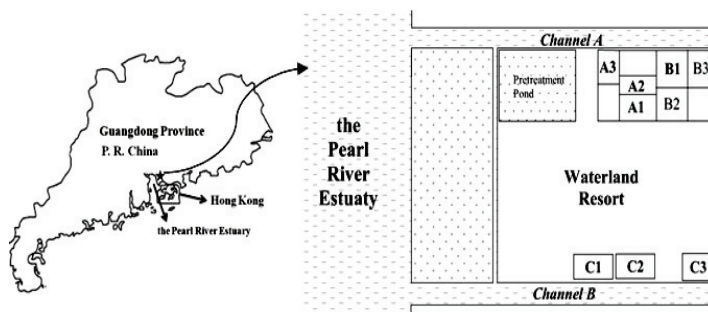


Fig. 1. The sketch map of the plantation-aquaculture ponds of three mangrove systems.

Table 1. Construction of the three mangrove treatment systems.

System	Pond Symbol	Plantation type	Pond area(ha)
A (ex-situ)	A1	<i>Kandelia obvata</i>	1.33
	A2	<i>Aegiceras corniculatum</i>	0.67
	A3	control	1.33
B (in-situ)	B1	<i>Kandelia obvata</i>	1.60
	B2	<i>Aegiceras corniculatum</i>	1.60
	B3	control	2.00
C (in-situ)	C1	<i>Bruguiera gynorrhiza</i>	1.47
	C2	<i>Rhizophora stylosa</i>	1.67
	C3	control	0.87

## 2.2. Field sampling and laboratory methods

All samples were collected every two months from September 2008 to March 2010 at three fixed sites in each pond. Water samples for oxygen (DO, COD<sub>Mn</sub>) and nutrient factors (NH<sub>3</sub>-N, NO<sub>3</sub>-N, TN, PO<sub>4</sub>-P and TP) were collected and analyzed according to the standards of marine investigation specification of China. The samples for nutrient factors analysis were filtered with 0.45µm GF/C fiberglass filters and stored at -20°C.

Water samples from the same three sites were mixed up for Chl *a* and phytoplankton analysis. Chlorophyll samples (250 mL) were stored at 4°C in the dark and filtered on 0.45 µm GF/C fiberglass filters. Concentrations were detected after overnight extraction in 90% acetone, using spectrophotometer (SOA, 2007, c). Phytoplankton samples (800 mL) were immediately preserved in Lugol's iodine solution (15% V/V) and the preserved samples were condensed two times using a siphon to 50 mL in laboratory later. The 50 mL samples were homogenized by gently inverting before algae identification and counting in Olympus CX21.

## 2.3. Purification efficiency assessment

Two trophic state indexes were calculated, the TSI of Carlson [21] and the TRIX proposed by Vollenweider et al.[23].

The trophic state index (TSI) uses algal biomass as the basis for trophic state classification. In our study TSI was calculated just with chlorophyll (µg/L) using the following equations [21]:

$$TSI(Chl) = 10 \times \left( 6 - \frac{\ln 2}{2.04 - 0.68 \ln Chl} \right) \quad (1)$$

The range of the index is from approximately 0 to 100. TSI (Chl) scales and descriptors for water quality are as follows: ≥70 hypereutrophic; 60-70 eutrophic to hypereutrophic; 50-60 eutrophic; 40-50 mesotrophic; 30-40 oligomesotrophic; ≤30 oligotrophic [22].

The trophic index (TRIX) [23, 24] is a combination of four state variables that express productivity directly. They are chlorophyll *a* (µg/L), oxygen as absolute deviation from saturation (%), nutritional

factors available, dissolved inorganic nitrogen ( $\mu\text{g/L}$ ) and total phosphorus ( $\mu\text{g/L}$ ). It was calculated as follows:

$$\text{TRIX} = [\text{Log}(\text{Chl } a \times \text{aD}\% \times \text{N} \times \text{P}) + 1.5] / 1.2 \quad (2)$$

where Chl *a* = chlorophyll *a* ( $\mu\text{g/L}$ ); aD%O = Oxygen as absolute % deviation from saturation; N =  $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$  ( $\mu\text{g/L}$ ); P = total phosphorus ( $\mu\text{g/L}$ ) [24].

The index is scaled from 0 to 10, covering a wide range of trophic conditions from oligotrophy to eutrophy. TRIx scales and descriptors for water quality are as follows:  $\leq 4$  high water quality; 4-5 good water quality; 5-6 mediocre water quality; 6-8 poor water quality [22,25].

The removal rate of mangrove on nutrients was determined by the following equation:

$$\text{Removal rate (\%)} = (\text{MC} - \text{ME}) / \text{MC} \times 100\% \quad (3)$$

where ME=mean value in experimental pond; MC=mean value in control pond.

#### 2.4. Statistical analysis

One way anova was used to compare the means of observations at the  $p=0.05$  level. Correlation coefficients were calculated to study the relationship among the factors ( $p<0.01$  and  $p<0.05$ ). All statistical analyses were carried out by the SPSS software (Version 17.0, SPSS Inc).

### 3. Results

#### 3.1. Physiochemical factors

Range and mean values of some physiochemical and biological factors in the aquaculture ponds are showed in Table 2. Dissolved oxygen fluctuated randomly within the wide range of 0.91-19.35 mg/L. Whereas  $\text{COD}_{\text{Mn}}$  ranged from 2.8-7.5 mg/L. Mean  $\text{COD}_{\text{Mn}}$  values in C3 was higher than that in C1 and C2 by 0.8 and 1.0 mg/L ( $p<0.05$ ), but no significant differences were found among ponds in System A and B.

Concentrations of  $\text{NH}_3\text{-N}$ , TN presented similar patterns. In months of 2008  $\text{NH}_3\text{-N}$ , TN in all ponds increased gradually and decreased in 2009, yet in B3 and C3 they went up again in late 2009 and early 2010. Mean value of  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  were below 0.56 mg/L and 0.06 mg/L in ponds except C3;  $\text{NO}_3\text{-N}$  in C3 was higher than all those in other eight ponds ( $p<0.05$ ) while  $\text{PO}_4\text{-P}$  in C3 was higher than those in seven ponds except B3 ( $p<0.05$ ). Mean concentration of TP in C3 was also 0.16 mg/L higher than that in C2.

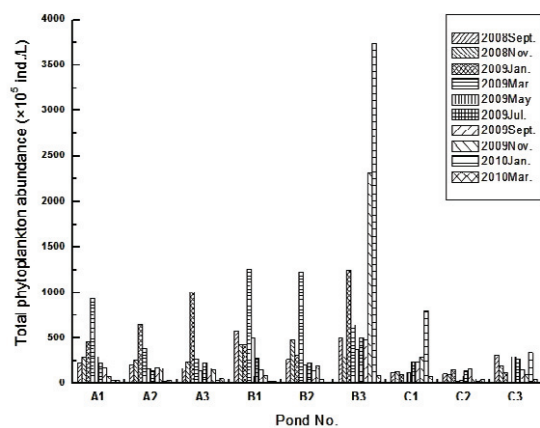
Concentration of Chl *a* spanned a broad range from 7.42-601.03 mg/L. C2 had the lowest Chl *a* in the whole study period. Chl *a* in B3 exceeded 150 mg/L except one month in March 2010.

#### 3.2. Phytoplankton composition and abundance

Over 165 phytoplankton species belonging to seven taxonomic groups were identified in the waters. Among them highest species number was appeared in Chlorophyta followed by Euglenophyta. Frequent dominant algae were *Dactylocopsis*, *Merismopedia*, *Oscillatoria* and *Synechystis* belonging to Cyanophyta; *Cyclotella*, *Chaeteros* and *Nitzschia* belonging to Bacillariophyta; *Euglena* belonging to

Table 2. Range and mean values of some physiochemical and biological factors in three mangrove systems (n=9).

	DO(mg/L)	COD <sub>Mn</sub> (mg/L)	NH <sub>4</sub> -N(mg/L)	NO <sub>3</sub> -N(mg/L)	TN(mg/L)	PO <sub>4</sub> -P(mg/L)	TP(mg/L)	Chl <i>a</i> ( $\mu$ g/L)
	min-max (mean)	min-max (mean)	min-max (mean)	min-max (mean)	min-max (mean)	min-max (mean)	min-max (mean)	min-max (mean)
A1	3.24-17.40 (8.84)	3.7-6.7 (5.22)	0.007-0.768 (0.53)	0.057-0.557 (0.26)	0.539-10.916 (4.63)	0.004-0.056 (0.04)	0.102-0.635 (0.32)	14.87-217.66 (128.47)
A2	3.20-18.72 (8.44)	4.3-6.5 (4.96)	0.062-1.784 (0.25)	0.029-0.663 (0.30)	0.510-13.319 (4.12)	0.004-0.092 (0.03)	0.096-0.675 (0.28)	32.24-368.78 (95.23)
A3	0.91-18.53 (7.91)	4.1-6.9 (4.94)	0.053-1.727 (0.52)	0.053-0.618 (0.25)	1.176-12.656 (4.71)	0.003-0.143 (0.03)	0.083-0.650 (0.30)	28.39-601.03 (140.81)
B1	2.90-16.26 (8.56)	3.7-7.1 (5.22)	0.004-1.859 (0.51)	0.028-0.611 (0.26)	0.804-13.610 (4.86)	0.003-0.066 (0.02)	0.081-0.413 (0.24)	14.61-278.16 (114.75)
B2	3.41-15.50 (8.49)	4.1-6.6 (5.25)	0.026-0.964 (0.24)	0.038-0.422 (0.25)	0.388-12.416 (4.33)	0.005-0.028 (0.01)	0.052-0.517 (0.23)	12.52-265.67 (105.11)
B3	2.36-19.35 (10.39)	4.5-7.0 (5.75)	0.020-1.548 (0.54)	0.093-0.576 (0.35)	1.227-10.272 (5.51)	0.011-0.180 (0.06)	0.065-0.598 (0.34)	62.49-481.82 (245.86)
C1	2.48-15.50 (10.55)	2.8-6.2 (4.86)	0.003-2.521 (0.56)	0.039-0.947 (0.50)	1.026-15.844 (5.02)	0.005-0.081 (0.03)	0.115-0.596 (0.29)	21.81-359.76 (128.49)
C2	4.16-12.17 (7.86)	3.9-5.8 (4.65)	0.004-0.550 (0.22)	0.037-0.695 (0.38)	0.238-9.377 (4.07)	0.003-0.021 (0.01)	0.072-0.773 (0.19)	7.42-57.04 (32.25)
C3	4.82-16.52 (9.69)	4.0-7.5 (5.69)	0.012-4.887 (1.47)	0.041-2.500 (0.92)	1.165-19.143 (8.62)	0.002-0.365 (0.10)	0.143-0.715 (0.36)	35.84-256.23 (123.97)

Fig. 2. Seasonal variations of the total phytoplankton abundance ( $\times 10^5$  ind./L) in the aquaculture ponds

Euglenophyta; *Chlorella* and *Selenastrum* belonging to Chlorophyta; *Chroomonas*, *Cryptomonas* and *Rhodomonas* belonging to Cryptophyta and *Chroomonas* belonging to Chrysophyta.

Algae abundance ranged from  $10.5 \times 10^5$  ind./L to  $3744.3 \times 10^5$  ind./L (Fig. 2). Similar with Chl *a*, algal abundances in System C were much lower and more stable than those in System A and B. It varied mostly within  $400 \times 10^5$  ind./L, but no significant difference were observed between plantation ponds and control pond.

In System A and B the algae abundance increased gradually before March 2009 (Phase I), this was exactly the adaptive phase of the mangroves. In Phase II algae abundance didn't exceeded  $300 \times 10^5$  ind./L and decreased with time except that in B3. There were two sudden increase of a tiny *Cyclotella* (which was recognized as *Cyclotella* blooms) in B3 in November 2009 and January 2010, while no similar records in plantation ponds and in-situ control pond (A1).

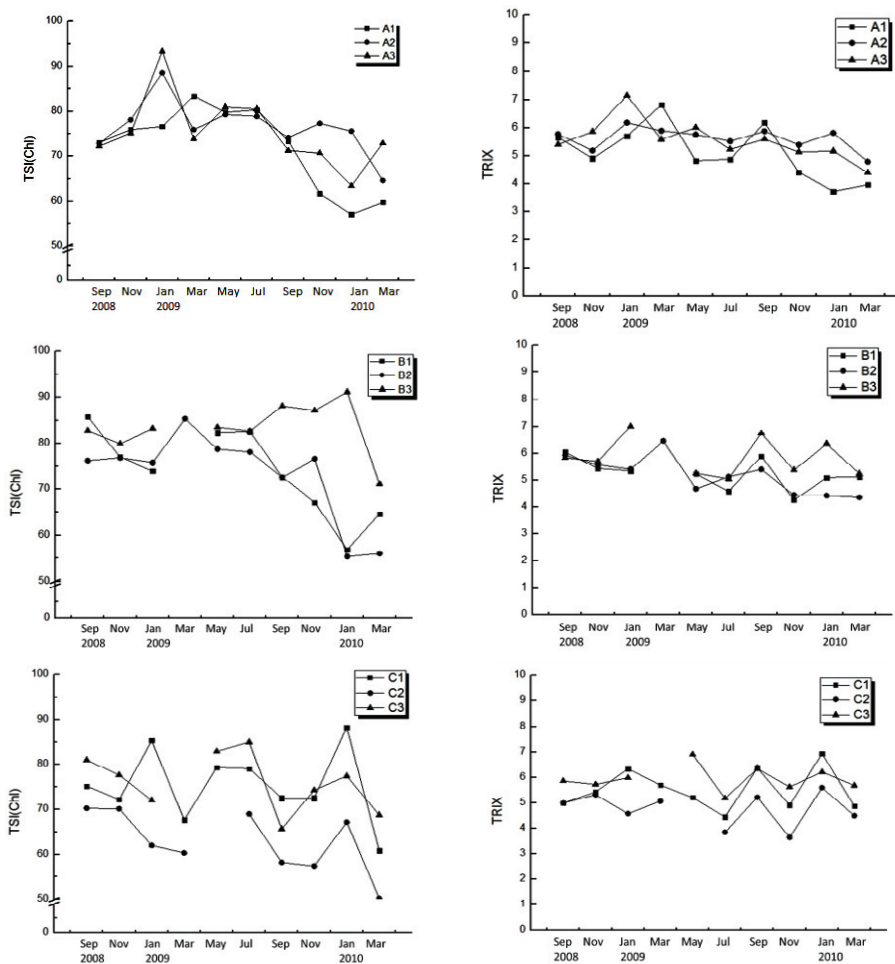


Fig. 3. Trophic State Index (TSI) with Chl *a* and TRIX index in the three mangrove systems in the study period.

### 3.3 Trophic status

The trophic state index of Carlson calculated by Chl *a* ranged from 50.2(C2, Mar. 2010) to 93.3(A3, Jan. 2009) which suggests eutrophic to hypereutrophic conditions (Fig. 3). In System A, TSI (Chl) were almost in the same condition of hypereutrophic before March 2009 (Phase I), in Phase II trophic status turned to eutrophic in A1. In System B, TSI (Chl) was quite stable in hypereutrophic level before July 2009. TSI (Chl) in B1 and B2 began going down to eutrophic from September 2009. In System C, TSI (Chl) in C2 was in eutrophic to hypereutrophic and lower than that in C1 and C2.

The trophic state index (TRIX) ranged from 3.62 (C2, Nov. 2009) to 7.41(A3, Jan. 2009). Minimum and maximum values of TRIX corresponded well with TSI (Chl). System A had a mediocre to good water quality and A1 had high water quality at the end of Phase II. Most of the time, A2 and A3 had a mediocre water quality and finally had a good water quality in March 2010. In System B, water quality in plantation ponds were always better than control pond. 70% of the time B1 and B2 had a mediocre water quality; B2 had good water quality at the end of Phase II; whereas 60% of the time B3 had in a poor water quality. In System C, C2 had a high to mediocre water quality whereas C1 and C3 had mediocre to poor water quality.

Both the TSI (Chl) and TRIX indicated that C2 had the best water quality, B1 and B2 were better than B3, A1 are better than A3.

### 3.4 Correlation relationships of significant variables and phytoplankton

Phytoplankton abundance was positively correlated with Chl *a*, DO, COD<sub>Mn</sub> and TP which are shown in Table 3, the correlation coefficient was 0.697, 0.302, 0.350, 0.276 ( $p < 0.01$ ) respectively. There was strong relationship between Chl *a* and PO<sub>4</sub>-P ( $r = 0.411$ ,  $p < 0.01$ ). Yet NH<sub>4</sub>-N, NO<sub>3</sub>-N and TN didn't show strong relationship with algae and Chl *a*.

Table 3. Correlation matrix of significant variables with phytoplankton abundance (n=65-90)

	PA <sup>a</sup>	DO	COD <sub>Mn</sub>	NH <sub>4</sub> - N	NO <sub>3</sub> -N	TN	PO <sub>4</sub> -P	TP	Chl <i>a</i>
PA	1.000	<b>0.302**</b>	<b>0.350**</b>	-0.017	-0.010	0.178	0.072	<b>0.276**</b>	<b>0.697**</b>
DO		1.000	<b>0.568**</b>	0.040	<b>0.334**</b>	<b>0.349**</b>	<b>0.321**</b>	<b>0.409**</b>	<b>0.351**</b>
COD <sub>Mn</sub>			1.000	-0.071	0.185	0.131	0.225	<b>0.389**</b>	<b>0.510**</b>
NH <sub>4</sub> -N				1.000	<b>0.261*</b>	<b>0.496**</b>	0.135	0.047	-0.020
NO <sub>3</sub> -N					1.000	<b>0.334**</b>	<b>0.554**</b>	-0.037	0.014
TN						1.000	<b>0.370**</b>	<b>0.254*</b>	0.059
PO <sub>4</sub> -P							1.000	<b>0.360**</b>	0.199
TP								1.000	<b>0.411**</b>
Chl <i>a</i>									1.000

a: PA- phytoplankton abundance.

\*  $p < 0.05$ . \*\*  $p < 0.01$ . Significant correlations are in bold.

## 4. Discussion

High phytoplankton biomass is a very common phenomenon within intensive aquaculture systems [26]. In our study total algal abundance exceeded 10<sup>6</sup> ind./L and the aquaculture waters were all in eutrophic conditions. But it is a necessity to have dense algae to sustain intensive aquaculture and

maintain a high productive aquatic ecosystem [26, 27]. The aim of mangrove plantation-aquaculture coupling systems was to control algal abundance within a desired and stable concentration.

Nutrient factors are the major indicators for the assessment of the mangrove purifying capacity. System A was filled up with water from Channel A that had been pretreated in the pretreatment pond, while System B directly from Channel A. The original waters were cleaner in System A, so the removal rates of System A were low. In System B, *A. corniculatum* was more efficient in removing nutrients than *K. obovata*. This result agreed with previous findings [19]. *R. stylosa* was more efficient in removing nutrients, average concentrations of NH<sub>3</sub>-N, NO<sub>3</sub>-N, TN, PO<sub>4</sub>-P were 45.71%-88.43% lower than those in the control pond. *B. gynorrhiza* also performed well with NH<sub>3</sub>-N, NO<sub>3</sub>-N, TN and PO<sub>4</sub>-P lower by 41.74%-72.64%. Considering phytoplankton abundance and trophic status determined by TSI (Chl) and TRIX, *R. stylosa* also had the best purifying capacity. The results indicate that *R. stylosa* and *B. gynorrhiza* were even better than *A. corniculatum* in removing N and P from aquaculture ponds.

In System B and C, significant differences in nutrient removal, algal abundance and trophic conditions were found between planted and unplanted systems, indicating that the presence of mangrove plants was important. Mangrove trees can export oxygen from their shoots to roots, some of this oxygen is used for root aerobic metabolism and excessive oxygen may diffuse into the rhizosphere [28,29]. Besides, their roots could provide a large surface area for microbial growth. It is obvious that the mangrove plants not only absorb N and P for their growth, they also enhance the efficiency of microorganism activities (Table 4).

In the mangrove system, the mechanism for the reduction of nitrogen may have been brought about by a combination of several processes: a. On the planting island organic N in soil is mineralized to NH<sub>4</sub>-N [30]; b. With an oxygen supply from the mangrove roots, nitrification of NH<sub>4</sub>-N to NO<sub>3</sub>-N and NO<sub>2</sub>-N at aerobic plant roots is activated, then it is finally derived from the system by subsequent rapid denitrification in the anaerobic parts of the substrate resulting in N<sub>2</sub> and N<sub>2</sub>O being released to the atmosphere [30,31,32]. c. NH<sub>4</sub>-N and NO<sub>3</sub>-N are taken up by plants and algae. d. NH<sub>4</sub>-N is also lost to the atmosphere via volatilization [30]. Whereas, in terms of P reduction, mangrove root filtration, sedimentation and soil adsorption were thought to be the main mechanism. Plant uptake or changes in soil properties around the rhizosphere zone may also play some roles in the P removal [9]. In conclusion, it is the co-effects of the "Planting Matrix-Mangrove-Phytoplankton-Microorganisms" in the coupling systems that reduce the nutrition load physically, chemically and biologically.

Mangroves are ligneous plants and not necessary to harvest or replace them regularly. The purifying capacity of mangrove was also found to be correlated to plant growth [8]. Thus we would expect that a mature mangrove system still has the same or even better function on controlling nutrient loads and algae and which could be used in coastal pollution controlling.

Table 4. Ponds Nutrients removal rates (%) in the aquaculture ponds

	NH <sub>3</sub> -N	NO <sub>3</sub> -N	TN	PO <sub>4</sub> -P	TP
A1	-2.34	-5.58	1.53	-8.25	-5.80
A2	51.00	-20.19	12.36	15.35	8.33
B1	5.96	24.98	11.70	63.57	30.74
B2	55.91	27.95	21.35	75.31	34.46
C1	62.03	45.96	41.74	72.64	19.29
C2	85.20	58.71	52.82	88.43	45.71



## 5. Conclusion

This two-year study demonstrated the feasibility of using constructed mangrove wetlands as biological treatment for aquaculture water purification. The co-effects of the “Planting Island Matrix-Mangrove-Phytoplankton-Microorganisms” are the main mechanisms in removing nitrogen and phosphorus and controlling algal growth. Nitrogen and phosphorus removal rates depended on mangrove species: *Rhizophora stylosa* had the highest removal rate which was 45.71%-88.43%, followed by *Bruguiera gynorrhiza* and *Aegiceras corniculatum*. Pond planted with *Rhizophora stylosa* also had better water quality based on results of  $\text{COD}_{\text{Mn}}$  and Chl *a*. Total abundance of phytoplankton ranged from  $10.5 \times 10^5$  ind./L to  $3744.3 \times 10^5$  ind./L in nine ponds within the study period and the aquaculture waters were all in eutrophic conditions. But the system stability was enhanced after mangrove plantation. An interesting finding is that aquaculture waters that have been pretreated in mangrove wetlands also have the capacity of maintaining stable algal abundance.

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