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# Nitrogen removal from Lake Caohai, a typical ultra-eutrophic lake in China with large scale confined growth of *Eichhornia crassipes*



CHEMOSPHI

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# HIGHLIGHTS

• We evaluated N removal from Lake Caohai by large-scale application of Eichhornia crassipes.

- The TN concentration in the water obviously decreased after E. crassipes was planted.
- N assimilation by *E. crassipes* was the main pathway of N removal from the lake.
- Large scale utilization of *E. crassipes* for N removal from the lake is practicable.

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# ABSTRACT

An ecological engineering project, with large-scale utilization of *Eichhornia crassipes* (coverage area  $\sim$ 4.3 km<sup>2</sup>) for pollution control in an open ultra-eutrophic lake, Lake Caohai, was first implemented in 2011. In this study, the efficiency of N removal using *E. crassipes* in the lake was evaluated. After *E. crassipes* was planted in May, the concentrations of TN and NH<sup>4</sup><sub>4</sub> in Waicaohai, the main part of Lake Caohai, were significantly decreased within a month, and then, remained stable from June to November, 2011, although the lake had received waste water continuously from river inlets. The average concentrations of TN, NH<sup>4</sup><sub>4</sub>–N and NO<sup>3</sup><sub>3</sub>–N in water of Xi Yuan Channel (outlet) were reduced to 3.3, 0.02 and 0.8 mg L<sup>-1</sup> from 13.8, 4.7 and 5.8 mg L<sup>-1</sup> in river inlets, respectively. The DO levels in 2011 were not decreased, but concentrations of TN and NH<sup>4</sup><sub>4</sub> were significantly reduced when compared with the historical data from 2007 in the lake. Assimilation by *E. crassipes* was the main pathway to remove N in Lake Caohai, accounted for 52% of the total N influent (936 t), or 64% of the removed N (761 t). These results indicated that large scale utilization of *E. crassipes* for removal of N in the eutrophic lake is practicable.

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## 1. Introduction

Accelerated eutrophication has led to many problems of the freshwater bodies in the world (Babourina and Rengel, 2011). Of lakes, about 54% in Asia, 53% in Europe, 28% in Africa, 48% in North America and 41% in South America are eutrophic (Chorus and Bartram, 1999). In China, Lake Dianchi is in a very severe state of eutrophication, which not only caused cyanobacterial (blue-green algae) bloom, decreased biodiversity and degraded ecosystem services, but also impacted the availability of drinking water resources (Wang et al., 2009). Diverse studies have shown that N and P are mainly responsible for eutrophication in the aquatic environment (Smith, 2003; Xu et al., 2010). Both of these elements

are considered to be the control targets for the restoration of aquatic ecosystems (Huett et al., 2005; Elser et al., 2007; Li et al., 2009).

To remove N and P in eutrophic lakes, various strategies have been applied, such as sediment dredging and sediment curing, and macrophyte planting (Murphy et al., 1999; Desprez, 2000; Lauridsen et al., 2003). Macrophyte planting is an attractive phytoremediation approach because it is a low-cost, easily managed and is considered a desired technology (Sun et al., 2009), especially for large water volume. However, in large lakes, there were few successful examples of submerged or emerging macrophyte restoration. This might be due to: (1) growth of submerged or emerging macrophytes is affected by the depth and transparency of the water; and (2) submerged or emerging macrophytes might assimilate nutrients from sediment instead of from the water. Macrophyte biomass, if not harvested, decomposes in lakes, and as a result, the assimilated nutrients are released into the aquatic ecosystems.



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Utilization of floating plants, if harvested, may be a valuable strategy to remove nutrients in lakes because several floating plants have a high ability to assimilate nutrients and are not restricted by the water depth and the transparency of the lakes (Mench et al., 2009). *Eichhornia crassipes* is a common free floating aquatic plant native to South America and is now widespread in all tropical and sub-tropical climate areas. It has a high capability to grow in heavily polluted water to assimilate nutrients, metal ions and reduce organic accumulations (Malik, 2007; Mishra and Tripathi, 2009; Polomski et al., 2009).

Lake Caohai, an internal lake of Lake Dianchi, is located in the southwest city of Kunming, and received large quantities of sewage from the city through the influx from rivers. The lake is the most polluted part in Lake Dianchi (Wang et al., 2009). During the past decade, many projects had been executed to improve the treatment of municipal and industrial sewages to control external nutrient loads. Sediment dredging, submerged and emerging macrophyte restoration had all been practiced in the lake. However, the polluted levels (especially TN) had not significantly decreased. In 2011, an experimental plan focused on large-scale growth of *E. crassipes* (ultimately covering  $\sim$ 4.3 km<sup>2</sup>) to remove the pollutants from the lake was executed in Lake Caohai.

Previous studies about pollutant removal using *E. crassipes* usually were conducted in laboratories, or in small water bodies, such as rivers, ponds (Polprasert and Khatiwada, 1998; Rommens et al., 2003; Jayaweera and Kasturiarachchi, 2004; Zimmels et al., 2006; Polomski et al., 2009). There were few applications of *E. crassipes* for pollution control in large water bodies. Due to the different hydrologic and limnological conditions, the results obtained from small water bodies and laboratories were not directly applicable to large-scale utilization of *E. crassipes* in lakes. There were also few researchers focused on the pollutant removal process by *E. crassipes* from large water bodies. Large-scale research using *E. crassipes* for eutrophication control is unprecedented, so that it is important to investigate the true effects of nutrient removal by *E. crassipes* in an ultra-eutrophic lake.

N is one of the main pollutants in the eutrophic lakes. Compared with the removal of P from wastewater, N removal process is more complex. Our previously research showed that E. crassipes could effectively remove N by assimilation and nitrification/denitrification in the simulated eutrophic water (Gao et al., 2012a,b). In this study, we hypothesis that large-scare confined growth of E. crassipes could also effectively reduce the N concentration in an open ultra-eutrophic lake, Lake Caohai, and the N removal was mainly caused by the E. crassipes. The main objective of this study was to assess the N removal efficiencies and characteristics of largescale utilization of E. crassipes in Lake Caohai. The main contents of this paper are: (1) the assessment of spatial-temporal variations of N concentrations in the water associated with the confined growth of E. crassipes in 2011; (2) evaluation of the changes of N concentrations from 2007 to 2011; and (3) the effects of N mass balance in Lake Caohai influenced by the confined growth of E. crassipes.

# 2. Materials and methods

### 2.1. Study areas

Lake Caohai, located in the northern part of Lake Dianchi, has a total area of about  $10.5 \ {\rm km^2}$ , consists of Dongfengba (area

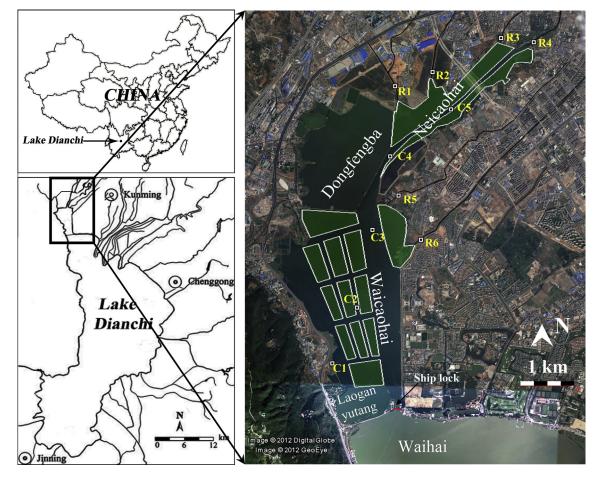


Fig. 1. The location of Lake Caohai and sampling sites in the lake.

~2.4 km<sup>2</sup>), Neicaohai (area ~1.8 km<sup>2</sup>), Waicaohai (area ~5.8 km<sup>2</sup>) and Laoganyutang (area ~0.5 km<sup>2</sup>), within which Dongfengba and Laoganyutang were separated from Neicaohai and Waicaohai by dams (Fig. 1). The average water depth is about 2.5 m. There are six rivers which flow through the Kunming connected to Neicaohai and Waicaohai. Xi Yuan channel (C1) is the only water outlet channel in the lake (Fig. 1). Lake Caohai, occupying about 3% of total Dianchi Lake, has received about 45% of the area's treated/untreated wastewaters flowing into the lake. From 2007 to 2009, the concentrations of TN and TP were 12–20 and 1.2–1.6 mg L<sup>-1</sup>, respectively. The total amounts of N and P influx into the lake were estimated to be about 2000 and 200 t year<sup>-1</sup>, respectively.

#### 2.2. Ecological engineering using E. crassipes in the lake

The ecological engineering in Lake Caohai consisted of three steps: firstly, in early May of 2011, seedlings of *E. crassipes* were planted in man-made enclosures, which were made of plastic foam and mesh (Fig. 2, confined growth of *E. crassipes* in Lake Caohai); secondly, the macrophytes were harvested using specially designed ships in mid November of 2011 till late January of 2012; and thirdly, the harvested biomass of the macrophytes were processed to produce biogas and organic fertilizer. The coverage of *E. crassipes* in the lake was about 0.3 km<sup>2</sup> (~9 kt) at the start and by November 2011, the coverage had reached ~4.3 km<sup>2</sup> (~211 kt), which did not include the coverage in Dongfengba and Laoganyutang.

# 2.3. Sampling and analytical methods

1 L mixed water sample was collected from three depths (0–0.5, 1-1.5, and 0.5 m above the bottom) of the water column at each sampling site (Fig. 1, R1–R6 and C1–C5), using a cylinder sampler. The water samples were preserved with 0.5 mL of chloroform, and then kept in a refrigerator until chemical analysis. During the growth season of E. crassipes and before harvest (early May to later November 2011), water samples were collected at one or halfmonthly intervals. Water temperature and DO were measured in situ by portable meter at the depth of  $\sim$ 0.5 m (YSI Pro Plus, USA). TN, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were determined according to the Standard Methods (APHA, 1998). The coverage area was determined by GPS in the situ and calculated using Google Earth Map. The biomass was measured in 9 quadrates of 1 m<sup>2</sup> by electronic scale. The total biomass of E. crassipes was calculated by the average weight (fw) per unit area multiplied coverage area. Plant samples were also collected before harvest to measure the content of TN in the plant tissues according to Bao (2000). All measurements were determined in triplicate.

#### 2.4. Data acquisition

Data before May 2011 were provided by Environment Monitoring Test Center of Kunming. Data for effluent from the lake were provided by Management Center of Xi Yuan Channel of Lake Caohai.

# 2.5. Statistics analysis

All statistical analyses were conducted using SPSS software (SPSS 16.0, Chicago, USA). Data were presented as mean  $\pm$  standard deviation. Normality and homogeneity of variance among groups were checked by the Kolmogorov–Smirnov one-sample test and Levene's test, respectively. When necessary, data were transformed (taking reciprocal or logarithm) for normalization to acquire homogeneity of variance. Intergroup differences were assessed by ANOVA with least significant difference (LSD) test. Analysis of temporal variances, multivariate analysis of variance (MANOVA-repeated measure) was also performed. The significance levels were set at p < 0.05.

#### 3. Results

## 3.1. Changes of water chemistry since year 2007

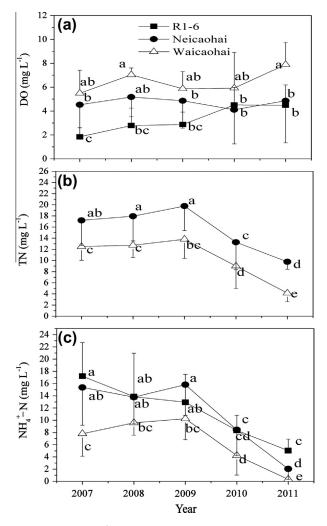
The average content of DO was  $1.8 \pm 0.8 \text{ mg L}^{-1}$  in 2007 and gradually increased to  $4.5 \pm 1.6 \text{ mg L}^{-1}$  in 2011 at the river inlet at Northern side of Neicaohai (Fig. 3a, label R1–6). Away from the river inlet, the contents of DO in water at Neicaohai were stable from  $4.1 \pm 2.9$  to  $5.2 \pm 1.7 \text{ mg L}^{-1}$  (p > 0.05, Fig. 3a, label Neicaohai). During the same period, the contents of DO at Waicaohai changed significantly from  $5.5 \pm 1.9$  to  $7.9 \pm 1.9 \text{ mg L}^{-1}$  (p < 0.05, Fig. 3a, label Waicaohai), especially in the year with cultivation of *E. crassipes*, it increased significantly over 33% compared to that in 2010. The results showed that, in the year (2011) with cultivation of *E. crassipes*, the contents of DO ( $4.9 \pm 3.5 \text{ mg L}^{-1}$  at Neicaohai) did not decrease significantly (p > 0.05).

The TN concentrations in water were not significantly (p > 0.05) different between years from 2007 to 2009 both at Neicaohai  $(18 \pm 4.4 - 20 \pm 4.4 \text{ mg L}^{-1})$  and at Waicaohai  $(13 \pm 2.5 - 14 \pm 3.5 \text{ mg L}^{-1})$ . The TN concentrations at Neicaohai  $(13 \pm 4.9 \text{ mg L}^{-1})$  and at Waicaohai  $(9.0 \pm 4.0 \text{ mg L}^{-1})$  in 2010 were significantly lower (p < 0.01) than those in 2009. In the year of 2011, The TN concentrations at Neicaohai  $(9.9 \pm 1.3 \text{ mg L}^{-1})$  and at Waicaohai  $(4.1 \pm 1.5 \text{ mg L}^{-1})$  were further significantly lower (p < 0.01) than those in the year of 2010 (Fig. 3b).

The concentrations of  $NH_4^+$ –N showed a similar pattern as that of TN (Fig. 3c). At the river inlet, the average concentrations of  $NH_4^+$ –N were not significantly different (13 ± 4.5–17 ± 5.5 mg L<sup>-1</sup>,



Fig. 2. The planted Eichhornia crassipes in the encloses of Lake Caohai (August-13, 2011).



**Fig. 3.** DO (a), TN (b),  $NH_4^+$ –N (c) of the inlets, Neicaohai and Waicaohai of Lake Caohai from 2007 to 2011. Historical data of TN in R1–6 were not obtained. Different characters in the figures represent the significant differences (p < 0.05).

p > 0.05) from 2007 to 2009, and then, significantly (p < 0.01) decreased to  $8.3 \pm 2.5 \text{ mg L}^{-1}$  in 2010, and further decreased to  $5.2 \pm 1.9 \text{ mg L}^{-1}$  in 2011 (Fig. 3c, label R1–6). Away from the river inlet, the concentrations of NH<sub>4</sub><sup>+</sup>-N in water at Neicaohai showed a similar pattern as the concentrations at the river inlet, being stable at  $14 \pm 4.4 - 16 \pm 5.0 \text{ mg L}^{-1}$  (*p* > 0.05, Fig. 3c, label Neicaohai) from 2007 to 2009, then significantly (p < 0.01) decreased to  $8.4 \pm 4.2 \text{ mg L}^{-1}$  in 2010 and further decreased (p < 0.01) to  $2.0\pm1.7\ \mbox{mg}\ \mbox{L}^{-1}$  in 2011. During the same period, the concentrations of NH<sub>4</sub><sup>+</sup>-N at Waicaohai also showed a similar pattern as that at Neicaohai, i.e. not significantly different (8.3 ± 3.9- $10 \pm 3.4 \text{ mg L}^{-1}$ , p > 0.05) from 2007 to 2009, then remarkably decreased (p < 0.01) to 4.2 ± 3.2 mg L<sup>-1</sup> in 2010 and further decreased (p < 0.01) to  $0.3 \pm 0.3$  mg L<sup>-1</sup> in 2011 (Fig. 3c, label Waicaohai). In the year of 2011, with the cultivation of *E. crassipes*, the average concentration of NH<sub>4</sub><sup>+</sup>-N in water decreased over 40% at the river inlet, over 67% at Neicaohai and over 89% at Waicaohai compared to that in 2010.

# 3.2. Effects of E. crassipes on nitrogen distribution

The results showed a general and clear pattern (p < 0.05) of nitrogen distribution in Lake Caohai. The concentrations of TN, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> reduced spatially following the direction of water flow, i.e. from river inlet (R1–6) to Neicaohai (C5 and C4) to Waicaohai (C3 and C2) to Xi Yuan Channel (water outflow, C1)

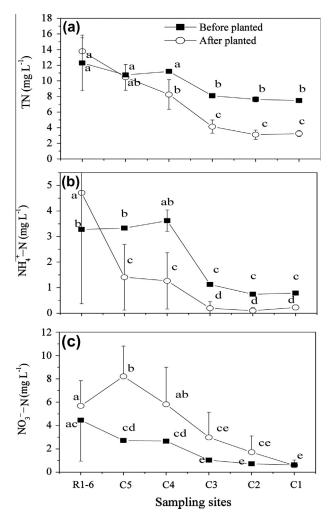
(Fig. 4a-c). The concentration of the TN had a 39% reduction (p < 0.05) from river inlet (12.3 mg L<sup>-1</sup>) to Xi Yuan channel (7.5 mg L<sup>-1</sup>) in 2011 before the growth of *E. crassipes* (May, 2011). After the confined cultivation of *E. Crassipes*, it had a greater reduction of 76% (p < 0.05) from river inlet (14 mg L<sup>-1</sup>) to the Xi Yuan channel (3.3 ± 0.9 mg L<sup>-1</sup>) (Fig. 4a) (June–November, 2011).

The concentration of  $NH_4^+$ –N had a 76% reduction (p < 0.05) from river inlet (3.3 mg L<sup>-1</sup>) to Xi Yuan channel (0.8 mg L<sup>-1</sup>) in 2011 before the growth of *E. crassipes*. After the confined cultivation of *E. Crassipes*, it had a greater reduction of 96% (p < 0.05) from river inlet (4.7 mg L<sup>-1</sup>) to the Xi Yuan channel (0.02 ± 0.01 mg L<sup>-1</sup>) (Fig. 4b).

The concentration of  $NO_3^-$ –N had a 82% reduction (p < 0.05) from river inlet (4.5 mg L<sup>-1</sup>) to Xi Yuan channel (0.8 mg L<sup>-1</sup>) in 2011 before the growth of *E. crassipes*. After the confined cultivation of *E. crassipes*, it had only a reduction of 86% (p < 0.05) from river inlet (5.8 mg L<sup>-1</sup>) to the Xi Yuan channel (0.8 ± 0.2 mg L<sup>-1</sup>) (Fig. 4c). After the confined cultivation of *E. crassipes*, the concentrations of  $NO_3^-$  increased for all sampling sites except Xi Yuan channel.

# 3.3. Changes of nitrogen concentration during the growing season of *E.* crassipes

In Lake Caohai, *E. crassipes* grows well from early May to early November each year. The 2011 results showed that, over the grow-

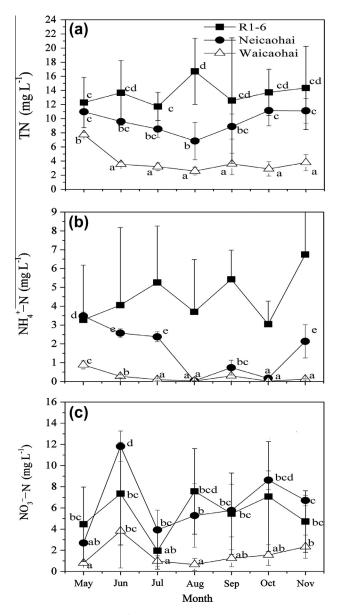


**Fig. 4.** TN (a),  $NH_4^+$ -N (b) and  $NO_3^-$ -N (c) of the sampling sites before and after E. crassipes planted in 2011 (different characters in the figures represent the significant differences, p < 0.05).

ing season of *E. crassipes*, the concentrations of TN did not change significantly (p > 0.05) at the river inlet (11.7–14.3 mg L<sup>-1</sup>), except for a sudden increase (p < 0.05) in August. At Neicaohai and Waicaohai, the concentrations of TN were decreased (p < 0.05) starting from May, and then, were increased gradually from August (p < 0.05) at Neicaohai, and kept stable (p > 0.05) at Waicaohai (Fig. 5a). Statistics also revealed that the significant (p < 0.01) changes of TN concentration followed the water inflow and effluence direction over the locations, i.e. the concentrations were lower at Waicaohai than that at Neicaohai and the river inlet.

The concentrations of  $NH_4^+$  changed significantly (p < 0.05) based on the location and followed the same pattern as that of TN in general. The results showed that the concentrations of  $NH_4^+$  fluctuated (p < 0.05) at river inlet, decreased more rapidly in the first few months both at Neicaohai and Waicaohai, then increased (p < 0.05) in November at Neicaohai, and kept at very low level at Waicaohai (Fig. 5b).

The changing pattern of NO<sub>3</sub><sup>-</sup> was different from those of TN and NH<sub>4</sub><sup>+</sup>. The results indicated an increase (p < 0.05) from May to June,



**Fig. 5.** Changes of TN (a),  $NH_4^+$ –N (b) and  $NO_3^-$ –N (c) in the sampling sites after confined growth of *E. crassipes* in Lake Caohai (different characters in the figures represent the significant differences, *p* < 0.05).

a decrease in July, and then a gradual increase from August to November. When comparing locations, the concentration was the lowest (p < 0.05) at Waicaohai and there was no significant difference between Neicaohai and river inlet (Fig. 5c).

## 3.4. Effects of E. crassipes on nitrogen removal in 2011

Lake Caohai had a water volume of 18.1 million  $m^3$  in 2011. The lake received more than 70 million  $m^3$  of water from May to November in 2011 (Table 1). The biomass of *E. crassipes* reached 211 kt in November 2011. After the harvest, the removed N in the biomass of the macrophyte was about 486 t and accounted for 64% of TN influent (Table 2). The mass balance suggested that a total of 761 t of N (including the N assimilated) was removed (Table 2), which suggested that nitrification–denitrification and other biological processes took place in the Lake Caohai.

# 4. Discussions

The results showed that the eco-engineering of a large application of E. crassipes for removal of nutrients in the eutrophic lake had positive effects. The concentrations of TN and NH<sup>+</sup> were significantly decreased in 2010 compared to the levels before 2009, which resulted from about 2 km<sup>2</sup> of naturally grown *E. crassipes* (~100 kt) in Lake Caohai. In 2011, the concentrations of TN and  $NH_4^+$  were further decreased significantly mainly due to the larger scale growth of *E. crassipes* ( $\sim$ 4.3 km<sup>2</sup>, 211 kt). Due to the lake is an open lake, the N inputs into the lake were fluctuant and complex, the relationship between growth of E. crassipes and concentrations of N in the water during the period of plant growth was not obvious. Although it is difficult to assess the contribution to N removal made by E. crassipes in relation to this background of falling N inputs directly (Fig. 3c), the decline concentrations of  $NH_{4}^{+}$  from 2010 to 2011 could indicate this point to some extend. When compared to 2010, the concentration of  $NH_4^+$  in water decreased over 40% at the river inlet, but over 67% at Neicaohai and over 89% at Waicaohai (Fig. 3c). N distribution pattern in Lake Caohai also showed that E. crassipes could significantly improve the water quality in the lake despite with continuous influence of external pollutants.

Decreased DO by E. crassipes had been previously documented (Rommens et al., 2003; Villamagna and Murphy, 2010) beneath the mats of growing macrophytes. They prevent the transfer of oxygen from the air to the water surface and block light necessary for photosynthesis by algae and submerged macrophytes. However, according to the historical data, it was found that the DO concentrations were not decreased in 2011 when E. crassipes was planted, especially in Waicaohai where the average DO concentrations reached to 7.8 mg  $L^{-1}$  (Fig 3a). This may be due to a number of factors: (1) Lake Caohai was serious polluted by organic pollutants, the background value of DO in the lake was low  $(4-6 \text{ mg L}^{-1})$ from 2007 to 2010. Confined growth of E. crassipes can accelerate the removal of pollutants by microorganism in rhizosphere of the roots of E. crassipes (Chaudhry et al., 2005), hence reducing the DO consumption in the process of decomposing organic pollutants in the water; (2) a certain amount of cyanobacterial occurrence, and consequential photosynthesis could release a large amount of oxygen, and then the cyanobacteria itself could be confined and filtered by the rhizosphere of the macrophytes; (3) DO concentrations in the water of river inlets were increased after 2009 (Fig. 3a) by river remediation; (4) the block design of the enclosures may have increased overall photosynthesis via increased transparency and light penetration between the blocks; and (5) The continuous flow of the water may also help the distribution of oxygen in the water.

The concentrations of TN and  $NH_4^+$  were quickly decreased and maintained at lower levels after *E. crassipes* was planted in 2011,

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#### Table 1

Nitrogen fluxes carried by water flow in Lake Caohai during May–November in 2011.

Month	Water effluent at Xi Yuan (×10 <sup>6</sup> t)	N concentration of effluent waters $(mg L^{-1})$	N flux out from lake (t)	Averaged balance of evaporation and precipitation ( $\times 10^6 t$ )	Water inflow from rivers (×10 <sup>6</sup> t)	N concentration of inflow waters (mg $L^{-1}$ )	N flux into lake (t)
5	12.104	3.48	42.1	0.315	12.419	12.3	152.6
6	14.018	5.2	72.9	0.315	14.333	13.7	195.8
7	11.135	3.3	36.7	0.315	11.450	11.7	134.2
8	5.987	2.94	17.6	0.315	6.302	16.7	105.2
9	14.168	1.99	28.2	0.315	14.483	12.6	182.1
10	6.071	2.22	13.5	0.315	6.386	13.7	87.5
11	5.192	2.6	13.5	0.315	5.507	14.3	79.0
Total	70.789		224.5	2.205	70.880		936.4

Table 2

Nitrogen assimilated by macrophyte (*E. crassipes*) and nitrogen removal in Lake Caohai from May to November in 2011.

Nitrogen storage in water in May <sup>a</sup> (t)	Nitrogen storage in water in November <sup>b</sup> (t)	Total nitrogen removed <sup>c</sup> (t)	Macrophyte assimilated and removed by $harvest^{d}\left(t ight)$	Macrophyte assimilated percentage (%)
157.6	108.2	761.3	485.6	63.8%

 $^{
m a}$  Nitrogen storage in water in May = TN concentration in water in May imes Water volume in the lake in May.

<sup>b</sup> Nitrogen storage in water in November = TN concentration in water in November × Water volume in the lake in November.

<sup>c</sup> Total nitrogen removed = N flux into lake (Table 1) – N flux out from lake (Table 1) + (Nitrogen storage in water in May – Nitrogen storage in water in November). <sup>d</sup> Macrophyte assimilated and removed by harvest = TN concentration in organism at harvest time × macrophytes biomass by harvest. From our determination, the average

TN concentration in organism at harvest time was 4.1% dw, the macrophytes biomass by harvest was 21.1 kt fw, and the moisture content of marophytes was 94.4%.

although the lake received a large amount of continuous influent external pollutants (Figs. 5a and b). In November of 2011, the concentrations of TN and NH<sup>+</sup><sub>4</sub> increased slightly, probably because the E. crassipes had stopped growing and began to decompose. Therefore, the suitable harvesting period of E. crassipes should start before November and be coordinated with the growth of the macrophytes, i.e. keeping a suitable level of coverage and not a maximum coverage. In the present study, it was found that the efficiencies of  $NH_4^+$  removal were higher than that of TN and  $NO_3^-$ (Figs. 4 and 5). This phenomenon may be due to the following two reasons. Firstly, E. crassipes may enhance the process of nitrification in the water via increased microorganism activities, which could convert NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>. The increased concentration of NO<sub>3</sub><sup>-</sup> at C5 compared to that at river inlets (R1-6) (Fig. 4c) seems to support this reason. Moorhead and Reddy (1988) reported that E. crassipes could release oxygen and provide aerobic microsites on the roots, which might benefit for nitrification. Other studies showed that a large number of nitrifying bacteria attached to the roots of this macrophyte enhanced nitrifying activities in the water (Gao et al., 2012a). Secondly, E. crassipes may have evolved a selective absorption of nutrients. Previously, Romments et al. (2003) found that *E. crassipes* had a preference for assimilating NH<sup>+</sup><sub>4</sub> over  $NO_3^-$ .

In Lake Caohai, the influent channels of N came from six inflowing rivers, and the effluent pathway was the Xi Yuan Channel (C1). The N fluxes to escape the aquatic ecosystem were mainly the N harvested via the biomass of *E. crassipes*, and of denitrification by microorganisms. Although the N flux over the sediment/water interface was not well understood, the removal, by *E. crassipes* assimilation, accounted for a 64% reduction of TN loading from the lake and improved the water quality, indicated by low concentrations of  $NH_4^+$ –N (0.2 mg L<sup>-1</sup>) and  $NO_3^-$ –N (0.8 mg L<sup>-1</sup>). During the same seasons in 2007–2009, about 27% of TN was eliminated in the lake via natural processes, which did not involve the *E. crassipes* (unpublished data 2012). The N mass balance suggested about 36% of TN removal via the processes of nitrification/denitrification in 2011, which may be enhanced by *E. crassipes* (Zhang, 2009; Gao et al., 2012b).

Although the results suggested the tremendous potential to use E. crassipes to control eutrophication, application of this macrophyte may impose challenges to ecological safety, harvesting and post processing. In terms of ecological safety, E. crassipes is one of the world's most prevalent invasive aquatic plants and can have adverse ecological effects when engineered for utilization in aquatic ecosystems. In the present study, we have not discussed the ecological effects of E. crassipes in the lake because the aquatic ecosystems of Lake Caohai had been serious affected by industrial wastewater and domestic sewage during the past 30 year. Previous investigations showed that the submerged plants were almost absent and only two contamination resistance species of zoobenthos (Chironomus and Limnodrilus hoffmeisteri) existed in Lake Caohai (unpublished data 2012). In fact, depending on the ecosystem evolution status, bioremediation using E. crassipes could result in positive and safe bio-manipulation for purifying polluted water in lakes (Chen et al., 2012; Wang et al., 2012). In terms of harvesting and post processing, there were three major challenges. One of the challenges is the large amount of this invasive macrophyte (Malik, 2007). The second challenge is of the economical evaluation to harvest the huge amount of its biomass from lakes (could reach up to 600 t ha<sup>-1</sup>). The third challenge is related to water content, within the macrophytes' biomass, which is about 94-95% water. The high could cause the post processing problems such as easy rotting, liquefaction and low economical value. In this project, the floating block design for enclosures and high capacity (15 t) harvest vessel provided the answer to the first challenge (unpublished data 2012). A dehydrator offered a partial answer to the second and third challenges (unpublished data 2012). The engineering project using E. crassipes for water purification involved comprehensive technologies such as: designing block enclosures, confining growth, using large scale and efficient harvesting methods and post processing a huge volume of biomass with high water content. The harvested biomasses were processed to make organic fertilizer and biogas.

Lakes exist in two alternative stable states: oligotrophy (clear with abundant submerged macrophytes) and eutrophy (turbid with abundant algae) (Scheffer et al., 1993). The main purpose of

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this eco-restoration was to bring the eutrophy state back to oligotrophy state in the lake. Therefore, reducing the pollutants (especially nutrient) levels were the first and most important step for eco-restoration. When the external inputs were greatly reduced, the utilization of *E. crassipes* to remove nutrients could be the important and first step of ultra-eutrophic lake management, followed by restoration of the aquatic ecosystem with their expected structure and function.

# 5. Conclusion

In the open ultra-eutrophic lake, Lake Caohai, *E. crassipes* showed a significant positive effect by removing N. It could significantly decrease the concentrations of TN and  $NH_4^+$ . Assimilation by *E. crassipes* was the main pathway to remove N in Lake Caohai. Moreover, *E. crassipes* may improve the nitrification/denitrification in the water, which can enhance the removal of N. These results indicated large scale utilization of *E. crassipes* for removal of nutrients in eutrophic lakes was practicable.

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