CAN WATER HYACINTH (*EICHHORNIA CRASSIPES*) BE CONTROLLED BY REDUCING NITROGEN AND PHOSPHORUS POLLUTION OF WATER BODIES?

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Abstract. Increasing concentration of nutrients in water is regarded as one of the most important external factors responsible for the invasion of the common water hyacinth. In order to test whether nutrient availability limits the growth and spread of this species, we investigated the influence of water nitrogen (N) and phosphorus (P) concentrations on growth using both greenhouse and field experiments. We found that in the greenhouse experiment, only high P concentration (>1.25 mg l^{-1}) can significantly increase numbers of ramets and leaves, and N concentration exceeding 62.5 mg l^{-1} can greatly increase water hyacinth biomass in a tank. In the field experiment, the clonal growth of water hyacinth was not correlated with N and P concentrations in water bodies where the range of N and P concentrations was narrow compared with their range in the greenhouse. This suggests that controlling water hyacinth through minimizing sewage discharging is impractical, the importance of the ability of water hyacinth to grow clonally should be considered.
Keywords: *invasive species, eutrophication, aquatic plant, wetlands*

Introduction

Invasive plants are attracting increasing research attention, and the objective of most research on invasive species is how to control their spread. Compared to terrestrial invasive plants, the control of aquatic invasive plants is more difficult because flowing water can transport plants or their propagules, aiding their spread. Therefore, many control methods applied in the case of aquatic invasive plants are ineffective (Rinella et al., 2009; Kovalenko et al., 2010; Baars, 2011; Patel, 2012; Paynter et al., 2012).

Eichhornia crassipes (Mart.) Solms, commonly known as water hyacinth, is a widespread aquatic invasive plant. This plant continues to be perceived as amongst the worst of invading aquatic weeds, despite the effort and resources spent over many years to improve management strategies (Heard et al., 2014). It now occurs in at least 62 countries between 40°N and 45°S latitude, and interferes with fishing, transport, the use of water for drinking purposes, irrigation, electric power generation, and also affects biodiversity (Howard and Harley, 1997; de Groote et al., 2003). Much effort has been made to control it (Hill, 2014). For the management of water hyacinth, given current knowledge, the best we can hope for is to reduce its biomass and reproductive potential, and to reduce the surface area covered by the weed, thus reducing the cost and impact of infestations and slowing the rates of spread (Julien, 2008). To counter the threat of water hyacinth in ecosystems, different types of control methods, including chemical, mechanical, and biological control, have been developed. However, to date these methods either have negative side effects, or have not been effective (Cilliers, 1991).

Although intentional introductions and environmental change caused by human activities are among conditions that have facilitated water hyacinth invasions, the biological traits of water hyacinth, such as high population growth rate (Sale et al. 1985; Gopal, 1987; Santamaría, 2002), and broad tolerance to a range of pH and nutrient concentrations are regarded as the most important factors responsible for their rapid spread (Xie andYu, 2003; Xie et al., 2004). With an average annual productivity of 50 dry (ash-free) tonnes per hectare, the water hyacinth is one of the most productive plants in the world (Abbasi and Nipaney, 1986). Many studies have shown that the clonal growth of water hyacinth is correlated with the nutrient level of water bodies, especially with nitrogen (N) and phosphorus (P) (Xie and Yu, 2003; Bownes et al., 2013; Hill, 2014), and based on this methods for controlling water hyacinth through minimizing water pollution by N and P have been suggested (Zulu et al., 2000; Sinkala et al., 2002; Chamier et al., 20112). Although water hyacinth makes use of nutrients where available, it can also grow well in clear lakes with low nutrient availability. It has also been observed to grow in extremely polluted waters containing rich organic matter, minerals and heavy metals, as well as in waters with high acidity or alkalinity (Gopal, 1987). Is it possible to control water hyacinth through controlling nutrient concentrations in water? In other words, is nutrient availability in aquatic environments a factor that limits the growth of water hyacinth? If nutrient availability of an aquatic environment is not a limiting factor, any attempts to control the weed by reducing water pollution will be not effective or practical.

In this study, we conducted a greenhouse experiment in which water hyacinth was grown at experimentally manipulated levels of nutrients, and a field experiment in which water hyacinth was grown across a naturally varying range of nutrient availability. We also conducted a broad field observational study in which naturally occurring water hyacinth and associated nutrient availabilities were monitored. Our aims were to examine the relationship between water hyacinth growth and nutrient availability in water bodies, and to test whether nutrient availability in aquatic environments is a factor that limits the growth and spread of water hyacinth.

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Materials and Methods

Greenhouse experiment design

The greenhouse experiment was carried out in August 2004. The temperature range during the experiment was from 29°C to 36°C. The experiment was conducted under natural light. The experimental plants were cultured in plastic tanks, which were 70 cm long, 50 cm wide and 40 cm deep, each of which contained 100 liters of culture solution with varying N and P concentrations. Water hyacinth plants used in the experiment were collected from the river located in Shanghai suburbia. In order to ensure the consistency of plant material for the experiment, the collected water hyacinth plants were cultivated in a big concrete tank in an unheated greenhouse for several months before the experiment to produce enough ramets. Only newly produced ramets of similar size (bearing 5-6 leaves) were chosen for the experiment. Each tank contained only one healthy ramet.

The formulation of the culture solution consisted of a single concentration Hoagland's solution (Torrey and Machlis, 1956), with NH_4NO_3 and KH_2PO_4 as N and P sources, respectively. Studies have shown that water hyacinth can tolerate high concentrations of N (300 mg l^{-1}) (Fox et al., 2008) and P (40 mg l^{-1}) (Haller et al., 1974) in water. It has also been shown that water hyacinth can absorb and store N and P in excess of what it requires for growth (hyper-accumulation or luxury uptake) (Alves et al., 2003; Chen et al., 2010)—a reason for its use in numerous waste water treatment systems. Based on water hyacinth's wide range of nutrient tolerance, we set up a complete factorial experiment with five levels of concentration of both N and P, i.e., N0 (Hoagland's solution but without added N, as control), N1 (0.5 mg l^{-1}), N2 (2.5 mg l^{-1}), N3 (12.5 mg l^{-1}), N4 (62.5 mg l^{-1}), P0 (Hoagland's solution but without added P, as control), P1 (0.05 mg l^{-1}), P2 (0.25 mg l^{-1}), P3 (1.25 mg l^{-1}), P4 (6.25 mg l^{-1}). With the exception of N and P, other nutrient elements were in accordance with their concentration in Hoagland's solution. This made for a total of 25 nutrient treatments (5N×5P). Each set of 25 treatments was replicated three times for a total of 75 tanks. The depth of the culture solution was approximately 30 cm, which was maintained through adding culture solution (twice) during the experimental period.

Numbers of ramets and leaves were recorded once every two days from the second day of cultivation to harvest day. In previous experiments with water hyacinth cultivation, we found that a single ramet of water hyacinth could produce approximately 25-30 ramets and cover the surface of the experimental tanks within 30 days. Thus, we ran the experiment for only 30 days following which all ramets were collected and their biomass was obtained after oven drying at 80 °C for 24 hours.

Field experiment

To test whether water hyacinth responded to nutrient concentrations similarly in the field and in controlled environments, we grew it in water bodies at several locations. The

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field observations were performed in Liantang town, Xiqin town, Zhujiajiao town and Qingpu town in Shanghai in August 2004. All these towns are in Qingpu district, to the west of Shanghai.

At these four towns we randomly chose one water body (for a total of three rivers and one pond), and set up five plots in each. Each plot, was a netted square (1 m×1 m) enclosed by a wooden frame. A single ramet of water hyacinth was grown in each plot. We recorded the number of ramets 5 times in each plot during the 40-day cultivation period. In addition, we sampled water using a water sampler in each plot from the surface to 30cm depth, and analyzed these samples for N and P concentrations.

Field observations

Water hyacinth occurs in almost all the water bodies of Shanghai between June and October every year. The massive invasions of water hyacinth in Shanghai might be related to the nutrient concentrations in these water bodies. To examine the relationship between the abundance of water hyacinth and nutrient availability in water, we conducted field observations in Shanghai. Water hyacinth biomass per unit area was randomly sampled, and associated samples of water for nutrient analysis were obtained from nine water bodies of Shanghai suburbia. Water hyacinth was harvested from an area of 1 m^2, and weighed to estimate the biomass (fresh weight). We sampled water to a depth of 30 cm using a water sampler. Four samples were randomly taken from each water body, which were then mixed to make a single, composite sample. All these water samples were transported in cooler boxes to the laboratory at Institute of Soil Science, Chinese Academy of Sciences in Nanjing for analysis. Water samples were filtered through Whatman GF/F filters, and filtrates were analyzed for total organic carbon (TOC), N and P concentrations. The concentration of TOC was determined by potassium dichromate - concentrated sulphuric acid method, N was tested by Kjeldahl method, and P was tested by Mo-Sb colorimetric method; all these methods were according to standard protocols (Eaton et al. 1995).

Data analysis

We analyzed the effects of the N and P treatments on numbers of ramets and leaves in the greenhouse experiment using a repeated measure two-way ANOVA with a *post hoc* Tukey test. In two-way repeated measure ANOVA, Mauchly’s Test of Sphericity showed that numbers of ramets and leaves increased significantly with cultivation days ($p<0.001$), so the assumption of the Sphericity test was rejected, and Greenhouse Geisser test of within-subjects effects was conducted. Also, after harvesting all the water hyacinth in each tank, we analyzed the effects of the N and P treatments on dry mass of water hyacinth using two-way ANOVA with a *post hoc* Tukey test. Before the analysis, the data were square-root-transformed to improve normality.

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For the field experiment, the concentrations of N and P sampled from water bodies and the number of ramets recorded from the same sites were analyzed by one-way ANOVA to determine the significant differences among the 4 sites.

Pearson's linear simple correlation was used to determine whether N and P concentrations of water were related to number of ramets (in the field experiment), and to test the relationship between water hyacinth biomass and water nutrient concentration (TOC, N, P) (in the observational study). All analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL 60606).

Results

Table 1. *The effects of N and P treatments on ramet number, leaf number and biomass of water hyacinth, analyzed by two-way ANOVA, cultivation days were considered as repeated measure factors in using two-way ANOVA*

Items	Factors	df	F	p
Ramet No.	Days	5.20	1702.67	0.000
	Days*N	20.82	2.10	0.004
	Days*P	20.82	7.43	0.000
	Days*N*P	83.27	1.34	0.043
Leaf No.	Days	1.00	788.71	0.000
	Days*N	4.01	3.51	0.013
	Days*P	4.01	4.09	0.006
	Days*N*P	16.06	0.96	0.514
Biomass	N	4	5.93	0.001
	P	4	3.73	0.01
	N*P	16	2.34	0.011

The results of the greenhouse experiment showed that addition of N, P and their interaction all significantly affected ramet and leaf production over time, with a significant increase in biomass by the end of the 30-day period (*Table 1*) (*Appendix 1*). The post-hoc Tukey test showed that the number of ramets was significantly higher in P4 than in P0 and P2 treatments (p=0.001, 0.039), but there were no differences with other P treatments, or among N treatments (*Fig. 1*). In the case of number of leaves treatments N3 and P4 had significant higher numbers of leaves than the nutrient controls (p=0.045, 0.006), but there were no differences among the N- or P-addition treatments (*Fig. 2*). The dry mass of water hyacinth was significantly higher in N4 than in the other N-addition treatments ($p<0.01$); dry mass was significantly higher in P3 than in the P0, P1 and P2 treatments ($p< 0.05$) (*Fig. 3*). These results showed that within the range of N and P concentrations in this experiment, high concentrations of N (N4) or P (P3)

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increased water hyacinth biomass, and high P significantly increased the number of leaves and ramets.

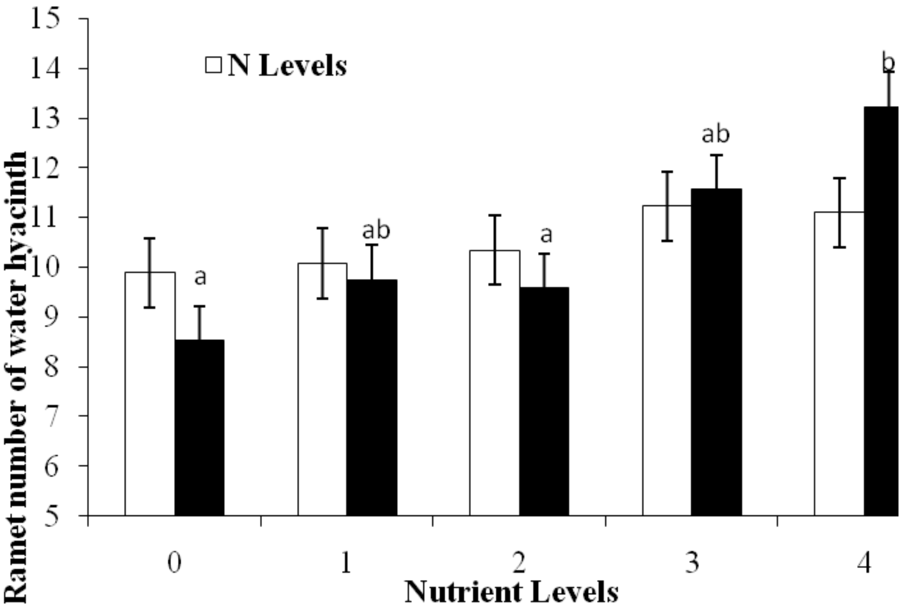

Figure 1. *The number of ramets of water hyacinth grown in the greenhouse at different N and P levels show marginal Mean±S.E., and different letters in capital and lowercase indicate statistically significant differences (p < 0.05) among N or P levels.*

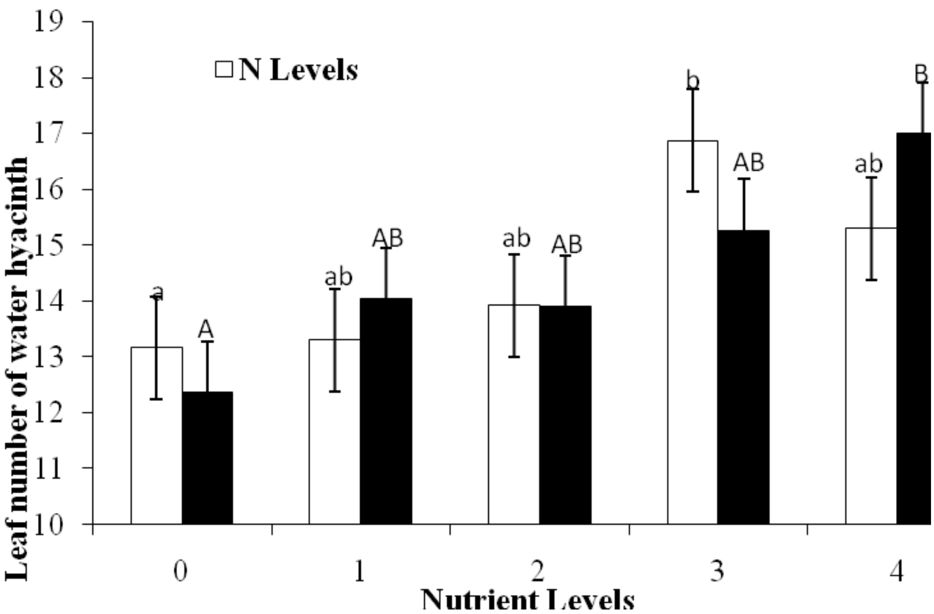

Figure 2. *The number of leaves of water hyacinth grown in the greenhouse at different N and P levels, show marginal Mean±S.E., and different letters in capital and lowercase indicate statistically significant differences (p <0.05) among N or P levels.*

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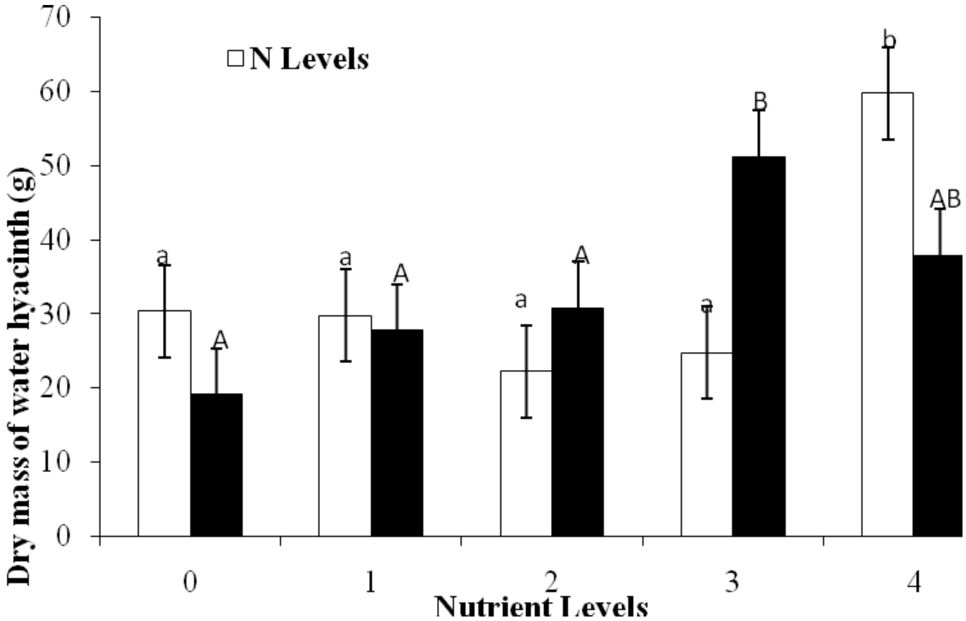

Figure 3. *The dry mass of water hyacinth grown in the greenhouse at different N and P levels, show marginal Mean±S.E., and different letters in capital and lowercase indicate statistically significant differences (p <0.05) among N or P levels.*

In the rivers and pond, water hyacinth grew very rapidly. The rate of ramet production in the field was greater than in the greenhouse. The mean ramet number of water hyacinth at the four sites all increased over time (*Fig. 4*), but there were no significant differences among the four water bodies (p=0.39 >0.05, *Table 2*), although the concentrations of both N (p< 0.001, *Table 2*) and P (p < 0.05, *Table 2*) differed significantly among the four sites. The concentration of N ranged from 1.68 ± 0.118 mg l^{-1} to 3.65 ± 0.217 mg l^{-1}, while the concentration of P ranged from 0.28 ± 0.03 mg l^{-1} to 0.43 ± 0.08 mg l^{-1}. However, there was no significant relationship between number of ramets at the time of harvest and N and P concentration of water bodies (with N: r=0.15, p=0.54, with P: r=0.19, p=0.42, *Fig. 5*).

Table 2. *The differences of N, P concentrations and ramets of water hyacinth cultivated in different water bodies, analyzed by one-way ANOVA, cultivation days were considered as repeated measure factors in using one-way ANOVA.*

Items	Factors	df	F	p
N	Site	3	29.51	0.000
P	Site	3	4.40	0.019
Ramets	days	2.28	511.75	0.000
	Days*Site	6.83	1.08	0.39

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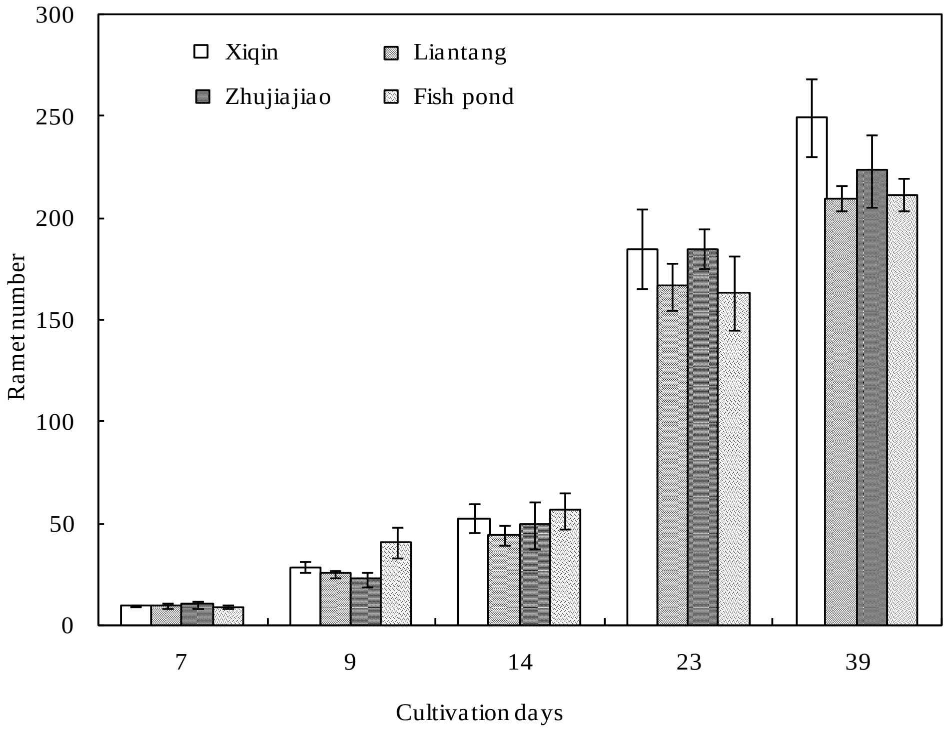

Figure 4. *The change in number of ramets (Mean± SE) produced by a single parent ramet in the field experiment conducted in four different aquatic bodies.*

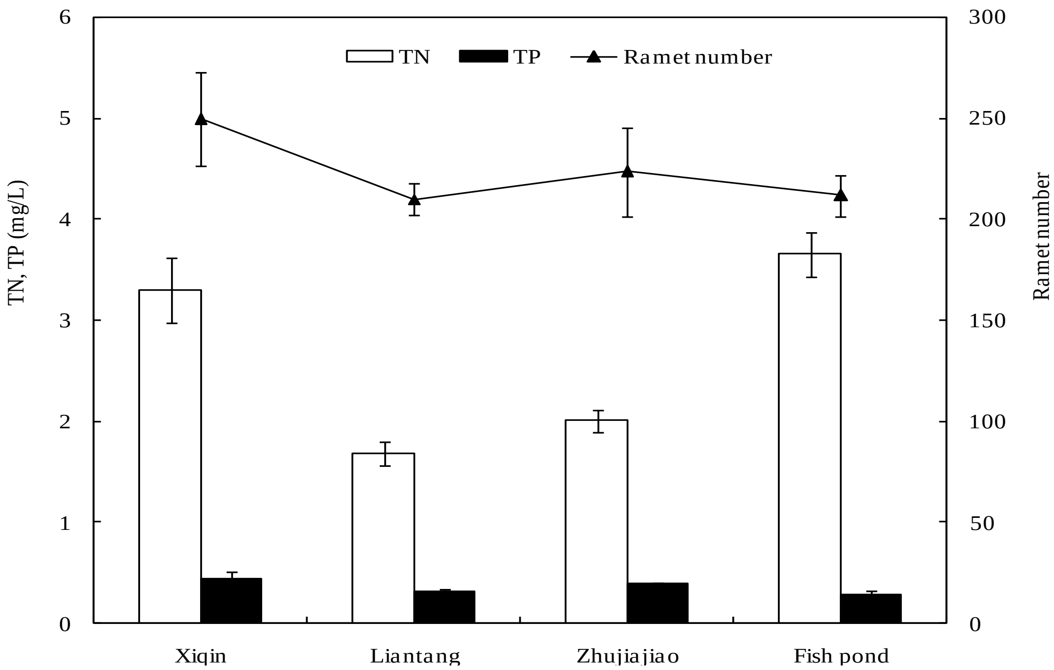

Figure 5. *The N, P concentration of different water bodies, together with the total number of ramets produced by parent ramets after 39 days of cultivation; data depicted are Mean ± SE.*

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In the observational study of nine water bodies, although the samples of TOC, N and P have big coefficient of variances (CV), they showed no correlations with water hyacinth biomass sampled in the same sites ($p > 0.05$) (*Table 3*).

Table 3. *Water hyacinth biomass (fresh weight) and concentrations of TOC, N and P sampled from different water bodies of Shanghai, and the correlations between biomass and TOC, N and P, analyzed by Pearson's linear correlation method.*

Sampling site	TOC (mgl^{-1})	N (mgl^{-1})	P (mgl^{-1})	Biomass (kg m^{-2})
Sitang, SJ	11.17	13.67	0.58	14.6
Yuhu, JS	6.55	2.97	0.06	19.6
Hongguang, JS	17.51	3.69	0.11	41.4
Baiyang, JS	22.10	3.80	0.06	18.0
Dongcun, JS	15.40	3.66	0.03	25.6
Mianzhang, FX	30.26	4.01	0.05	21.5
Caoyang, FX	7.60	5.40	0.08	20.6
Donghai, NH	24.42	5.03	0.19	35.7
Junken, NH	27.13	4.81	0.18	20.1
Mean±SE	18.02±2.85	5.23±1.09	0.15±0.05	24.1±2.9
CV	47.45%	62.35%	114.98%	36.46%
r (with biomass)	0.30	-0.233	-0.142	/
p (two-tailed)	0.433	0.546	0.715	/

Discussion

Numerous studies on the response of water hyacinth to nutrient concentrations in water, have suggested that water hyacinth is highly responsive to nutrients, with increased productivity correlated with increased N (Reddy et al., 1989, 1990; Heard and Winterton, 2000; Wilson et al., 2005; Coetzee et al., 2007) and P (Xie et al., 2004; Chen et al., 2010; You et al., 2014). However, this does not explain why water hyacinth is widely distributed and has strong invasive capacity since its wide distribution would suggest a wide tolerance for environmental conditions, including nutrient availability. Our greenhouse experiment supports this proposition. The experiments performed in the greenhouse showed that there was a significant increase in the production of ramets and leaves of water hyacinth and in water hyacinth biomass only at very high N ($N > 62.5$ mg l^{-1}) and P ($P > 1.25$ mg l^{-1}) concentrations. However, water hyacinth was able to grow in water with low concentrations of N or P – at lower nutrient concentrations the growth of water hyacinth was not significantly different from the controls. Studies have also shown that the overall nutrient requirement of water hyacinth is very low (Carignan et al., 1994; DeBusk et al., 2001; Xie and Yu, 2003; Xie et al., 2004). Therefore, whether water hyacinth can grow and whether water hyacinth can produce more leaves and

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ramets are different. The invasion and spread of water hyacinth, and even the control to water hyacinth are mainly related to whether water hyacinth can grow in water bodies.

From the results of field study, the concentrations of N and P in the field water were lower and quite narrow compared with those set in the greenhouse. Thus, in the field experiment the clonal growth of water hyacinth was not correlated with N and P concentrations of water bodies. The results of field observation were consistent with findings from the greenhouse experiment at nutrient levels that were comparable to those encountered in the field. Thus, typical ranges of variation in the concentration of N and P in the field water are unlikely to influence the growth and clonal reproduction of water hyacinth. The studies thought that the clonal growth of water hyacinth was correlated to the concentrations of N and P, only when the concentration of N or P was in relatively high situation, the growth of water hyacinth will increase significantly. But in water bodies of field, such high concentration of N and P was not common. And also, in these water bodies of field, water hyacinth can also grow and grow out daughter ramets in clonal way in these N and P concentration ranges. Besides, due to the floating characteristic of water hyacinth, and flowing of field water, the lack of nutrients can be compensated through the moving of water hyacinth or the flow of water. Therefore, in the field water bodies, the concentration of N and P nutrients will not be a limiting factor for the growth of water hyacinth.

In addition, from the water quality data of field observation (*Table 3*), it can be seen that the concentrations of N and P of water bodies (N: 1.68 ± 0.118 mg l^{-1}-3.65 ± 0.217 mg l^{-1}, P: 0.28 ± 0.03 mg l^{-1} -0.43 ± 0.08 mg l^{-1}) were also close to the low nutrient levels we set in greenhouse experiment. From the data of greenhouse and field experiments, we can see the growth and clonal action would not be affected in these nutrient ranges. N: 1.5mgL^{-1}, P: 0.3mgL^{-1}And also, the survey of Shanghai water quality conducted in 2001 indicated that more than 88% of water bodies were classified as Grade V(those used for agriculture, N: 1.5mgL^{-1}, P: 0.3mgL^{-1}) (Wang, 2001). It further showed that all most all the water bodies of Shanghai were suitable for the growth of water hyacinth. Therefore, although N and P concentrations might not be very high, the N and P pools in the urban water are large enough to support the growth of water hyacinth. So, under the current status of N and P distribution in Shanghai water bodies, N and P availability would not be the main or only factors that determine whether or not water hyacinth thrives and flourishes. In addition, the control of aquatic invasive plants can not only stay in control of its biomass growth, the important goal should be to control the coverage area of invasive plants on the water (Gao et al., 2013). The ramet number and leaf number of water hyacinth are involved to the coverage, so the biomass growth may not be the final control purpose for aquatic invasive plants.

Many researchers have suggested that an effective method for controlling water hyacinth growth should be to reduce the discharge of sewage with high N and P to water bodies (Howard and Harley, 1997; Zulu et al., 2000; Sinkala et al., 2002). However, in the case of Shanghai, the purification of its water bodies to the standards of drinking

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water resources (N: 0.2 mgl^{-1}, P: 0.02 mgl^{-1}) by reducing the discharge of pollutants may be difficult. Our greenhouse experiment showed that water hyacinth can still grow and propagate well even under low N and P availability. Therefore, under the current situation it may be impractical to control water hyacinth invasion by controlling sewage discharge. As one of the world's most widespread aquatic weeds, water hyacinth has a strong invasive capacity, and it has special adaptations which allow it to grow and spread rapidly in freshwater. It is also known that water hyacinth interferes with agricultural (irrigation) and urban waterways (Kathiresan, 2000). So, the underlying mechanisms for successful invasions of water hyacinth appear to be far more complicated than pollution of waterways.

The development of effective management strategies requires an understanding of the weed's biology, ecology and how these change in response to changing conditions and to control techniques. An important role for weed scientists and weed managers is to offer plausible, information based, strategies and thus assist the decision making process (Julien, 2008). Although improving aquatic environments may play an important role in controlling water hyacinth in the long term, across a wide range of concentrations N and P do not appear to limit water hyacinth spread. For these reasons, we should consider not only the invasibility of the environment, in this case, nutrient rich water bodies (Lonsadle, 1999; Davis et al., 2005), but also the invasive capacity of the invader (Lonsadle, 1999; Davis et al., 2005) before controlling water hyacinth, therefore, those control ways against the growth characteristic of water hyacinth should be recommended, such as biological control (Ding et al., 2006): introducing natural enemies (Hill and Cilliers, 1999; Coetzee et al., 2007; Coetzee et al., 2011) and allelopathic control (Kathiresan, 2000; Kathiresan and Dhavabharathi, 2008; Kong, 2010; Khaket et al., 2012; Chai et al., 2013). In addition, the biological control of water hyacinth has also been highly successful in a number of large tropical water bodies, mainly in Africa (Marlin et al., 2013; Venter et al., 2013), but also in Australia (Dhileepan et al., 2013).

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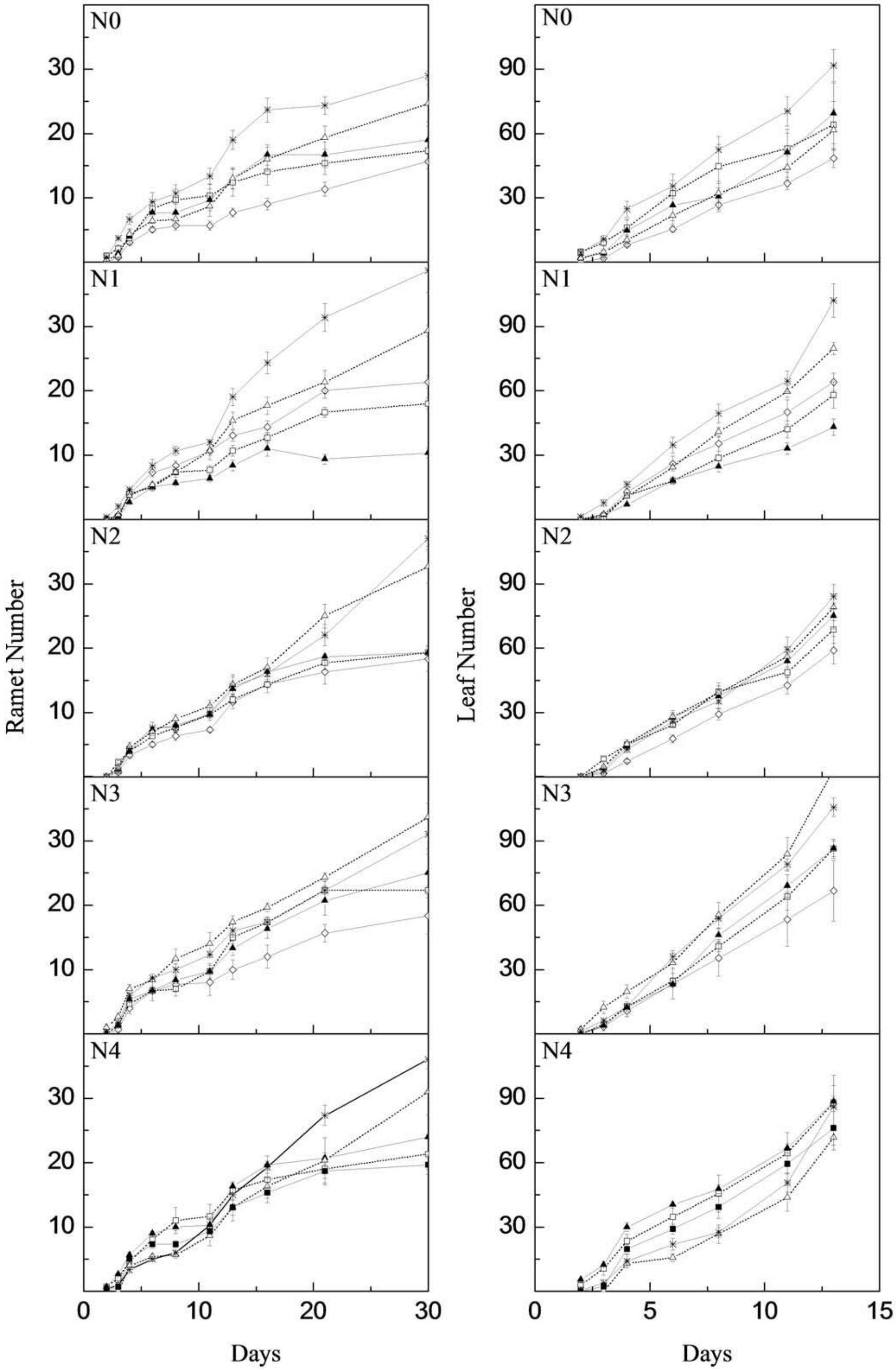
APPENDIX



Appendix 1. *At each N level in the greenhouse experiment, the changes in number of ramets (left) and number of leaves (right) in different P concentration with time. The data are mean±S.E.*

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