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RESPONSES TO NITROGEN AND PHOSPHATE OF PHENOTYPIC PLASTICITY OF *SAGITTARIA GRAMINEA*: AN EXOTIC SPECIES IN YALU RIVER, DANDONG, CHINA

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Abstract

The phenotypic plasticity of *Sagittaria graminea* to nitrogen and phosphate including morphology, growth and biomass allocation, and the impact of the biological invasion on biodiversity and grassland agroecosystem were investigated. The nitrogen was from NH_4NO_3 and the concentration was 0g (control), 0.4g/kg (LN),0.6g/kg (MN) and 0.8g/kg (HN) air-dried soil; the phosphate was from NaH_2PO_4 and the concentration was 0g (control), 0.2g/kg (LP), 0.4g/kg (MP) and 0.6g/kg (HP) air-dried soil. According to the characteristics of the flowering, seedling and clonal reproduction of the *Sagittaria graminea*, it was harvested in the blooming period, and the height of each plant, the number of leaves, and the female and male flowers were counted. The results showed that *Sagittaria graminea* had plasticity and adaptability to different nutriention. With the increase of nitrogen, the root biomass was decreased and total biomass, leaf biomass, flower biomass, relative growth rate, net assimilation rate, leaf number and plant height was increased; with the increase of phosphate, total biomass and leaf biomass increased and plant height, petiole biomass, root biomass, root/shoot, relative growth rate and net assimilation rate were not changed.

Key words: *Sagittaria graminea*, Biomass allocation; Biological invasion.

Introduction

Nowadays, the biological invasion is becoming more and more serious with globalization (Fleming & Dibble, 2015; Powell *et al.*, 2013), which is harmful to community biodiversity and ecosystem structure, causing great economic losses and ecological disaster (Chen *et al.*, 2015; Lowry *et al.*, 2013). The invasion usually depends on exotic invasiveness and community invisibility (Erfmeier & Bruelheide, 2010; Pyšek & Richardson, 2007). The phenotypic plasticity is a key factor in species invasion, which means the good adaptation of sunlight, nutrition, water and so on (Bruckman & Campbell, 2016; Li *et al.*, 2016; You *et al.*, 2014); meanwhile, the nutrition of environmental resource is another factor, especially nitrogen and phosphate, which would increase the invasion of exotic species (Bozzolo & Lipson, 2013). Since nitrogen and phosphorus are increased in aquatic ecosystem by eutrophication, it would be useful to find the mechanism of invasion based on the plasticity of exotic species.

Sagittaria graminea, member of family Alismaceae, is a perennial aquatic plant, naturalized in North America, which has become a naturalized species in the eastern part of Australia and is a B-class weed in the United States. It was first found in Yalu River Wetland National Nature Reserve (Dandong, Liaoning, China) and riverside wetlands along the coast across China and East Asia in 1995. It has formed a large area of single dominant community, with a typical invasion (Zhang *et al.*, 2011). At present, there have been few studies on the new invasive land of the *Sagittaria graminea* (Zhang *et al.*, 2014; Zhang *et al.*, 2009) and the potential ecological risk is unexpected. This study is to find how phenotypic plasticity and biomass change with different nitrogen and phosphate and whether the change would increase the invasion of *Sagittaria graminea* based on potted experiment, which would provide the theory evidence for prediction and prevention of the potential threat of *Sagittaria graminea*.

Materials and Methods

Experimental materials: *Sagittaria graminea* is a perennial clonal plant, and seed production is one way of reproduction, and carmus is another way to get new plants. In Yalu River estuary wetlands, from the end of May to early June every year, new *Sagittaria graminea* grows from the persistent green carmus. The plant height of *Sagittaria graminea* is about 40-100 cm, petiole (4); male flowers are born in the 4-8 (11) section of the racemes, all 3 rounds, flowering from July to August. Polymerization achenes are spherical and takes 8 to 9 months to mature. Leaf-bases are oval, cylindrical, hollow, monoecious. Female flowers are located at the lower 1-3(4) section of the racemeal, and male flower locates at the 4-8 (11) section of the racemeal. All flower are three in a round, and flowering from July to August. Aggregated achenes spherical and mature from August to September.

The 300 experimental samples of *Sagittaria graminea* carmus were collected from the Yalu River wetlands in autumn 2009 with at least 5 meters apart from each other and the size from 4 to 6 mm, which were sowed in plastic bowls and covered with 0.5 cm nutritive soil in greenhouse. At the beginning of May 2010, the carmus began to sprout, then compound fertilizer (made of urea, ammonium hydrogen phosphate, and potassium sulfate) was given at same concentration after euphylla. After 4 weeks, the seedlings of similar growth were included. The initial plant height was 15 ± 2.5 cm and the initial biomass was 0.14±0.05 g.

Experimental design and methods: In early June 2010, we chose *Sagittaria graminea* of diameter 30 cm and height 32 cm, and the substrate was composed of nutrient soil (collected from the soil 5-25 cm underground in the secondary forest near Liaodong College) and river sand by 1: 1, in which the available organic matter was 18.8g/kg, available nitrogen was 98mg/kg and available phosphorus was 12mg/kg, and pH 6.51. The substrate of each bowl was 10 kg, and 5 ramps per bowl. Before the experiment, running water was added to each bowl and the water level was 5cm lower than the surface. After 7 days in sunlight, the nutritional addition experiment began.

The nitrogen was from NH_4NO_3 and the concentration was 0g(control), 0.4g/kg(LN),0.6g/kg(MN) and 0.8g/kg(HN) air-dried soil; the phosphate was from NaH_2PO_4 and the concentration was 0g(control), 0.2g/kg(LP), 0.4g/kg(MP) and 0.6g/kg(HP) air-dried soil, during the experiment, NH_4NO_3 and NaH_2PO_4 aqueous solution was given to the experimental groups every 2 weeks for 4 times.

Determination of indicators: According to the characteristics of the flowering, seedling and clonal reproduction of the *Sagittaria graminea*, we harvested in the blooming period on July 28, 2010, and measured the height of each plant, the number of leaves, and the female and male flowers. The leaf area was measured with LI3000 leaf area meter. Then the plants were divided into roots, petiole, leaves, and flowers, placed in the oven at 105ºC for 30 min, and the dried at 80ºC to constant weight to record the total biomass, leaf biomass, petiole biomass, root biomass, and flower biomass.

The main parameters were as follows: Leaf biomass ratio (LBR)=leaf biomass/total biomass; Root biomass ratio(RBR)=root biomass/total biomass; Support biomass ratio (SBR)= support structural biomass/plant weight, and the petiole biomass was considered as support structure based on the biological characteristics of *Sagittaria graminea*; Flower biomass ratio (FBR)=flower biomass/ total biomass; Leaf area ratio (LAR)=total leaf area/ total plant weight; Relative growth rate (RGR)=biomass increment per unit time; net assimilation rate (NAR)= net photosynthetic product per unit area per unit time; Total number of flowers = female flowers + male flowers; sex ratio= male flowers/female flower.

Statistical analysis: The homogeneity of variance was tested by Levene's test, then one-way ANOVA was conducted with homogeneity, otherwise the variables were logarithmically transformed before the test. We transformed the dates of the leaf area, total number of flowers, female flowers and male flowers. If F value differed significantly ($p<0.05$) the Duncan test would be further conducted for multiple comparisons. The statistical analysis was executed by SPSS 13.0 software (SAS Institute, Cary, NC, USA) and data collation, calculation and mapping were carried out by Microsoft Excel 2003(Microsoft, Redmend, Washington, USA).

Results

Effects of nitrogen and phosphorus on plant morphological characteristics of *Sagittaria graminea*: Nitrogen application could increase the plant height, leaf number, leaf area, and total leaf area but had no significant effect on leaf area ratio (Table 1) and the leaf area, total leaf area and leaf area ratio were increased with the increase of nitrogen concentration, which were significantly higher than the control group ($p<0.05$). Especially in HN group, the plant height and leaf number were significantly higher than MN and LN ($p<0.05$). The effect of phosphorus on leaf area and total leaf area was similar to that of nitrogen ($p<0.05$), but not plant height. The number of leaves was significantly more in HP than that of MP and LP ($p<0.05$).

Effects of nitrogen and phosphorus on plant growth characteristics of *Sagittaria graminea*: The relative growth rate (RGR) and net assimilation rate (NAR) of *Sagittaria graminea* were increased first and then declined with the increase of nitrogen concentration, which was higher in experimental group than the control group, and reached a maximum in HN (Fig. 1). The RGR and NAR was increased with the phosphorus concentration, with significant differences in MP and HP when compared with the control group (Fig. 1, $p<0.05$).

Effects of nitrogen and phosphorus on sexual reproduction of *Sagittaria graminea*: The number of female flowers, male flowers and the total number of flowers of *Sagittaria graminea* were all increased with the increase of nitrogen concentration, and the total number of flowers and male flowers reached maximum in MN, while the number of female flowers reached the maximum in HN. All were significantly more than those of the control (Fig. 2). Nitrogen fertilization could increase the sex ratio of the leaves, which was significantly higher than the control ($p<0.05$), and the sex ratio reached maximum when the amount of nitrogen was $0.2g \cdot kg^{-1}$. The total number of flowers and the number of male flowers were significantly increased with the application of phosphate, but without significant difference ($p> 0.05$). The number of female flowers was not changed with phosphate, and the sex ratio was reduced without significant differences ($p>0.05$). The results indicated that nitrogen mainly increased the total flowers, male and female flowers, while phosphate mainly increased the total flowers and male flowers.

Effects of nitrogen and phosphorus on plant biomass allocation of *Sagittaria graminea*: The effects of nitrogen and phosphorus on the total biomass, LBR and FBR of *Sagittaria graminea* were the same, which meant that the total biomass and LBR increased with the concentration and reached the maximum in HN and HP, while FBR increased first and then decreased with increasing nutrient concentration, reaching the maximum in LN and LP (Figs. 3 and 4). SBR increased first and then decreased with the increase of nitrogen concentration and reached the minimum in HN, while decreased with the increase of nitrogen concentration, and reached the minimum value when the amount of nitrogen was $0.2\ g \cdot kg^{-1}$. The effect of phosphorus on SBR and RBR was consistent; both decreased with the increase of phosphorus concentration, and reached the minimum value in HP. It showed that nitrogen could increase the total biomass of the plant, and a certain amount of nitrogen could increase the leaf biomass, promote more biomass to aboveground part than underground part, and phosphorus would increase more of the total plant biomass, rhizomes and flowers than petiole and underground part.

Table 1. Effects of different nutrients on morphological characteristics in a clonal monoecious species, *Sagittaria graminea*.

Sources	Treatment	Plant height (cm)	Leaf numbers (n)	Leaf area (cm^2)	Total leaf area (cm^2)	Leaf area ratio
Nitrogen	Control	33.04 ± 1.08a	4.00 ± 0.25a	113.04 ± 2.52a	228.28 ± 4.52a	80.98 ± 1.05a
	LN	35.78 ± 1.81a	3.56 ± 0.34a	163.33 ± 3.89b	322.49 ± 8.86b	86.12 ± 1.44b
	MN	55.27 ± 2.59b	5.27 ± 0.36b	214.32 ± 7.03c	402.05 ± 4.79c	85.61 ± 0.64b
	HN	36.95 ± 2.69b	14.19 ± 0.62c	208.78 ± 4.52d	173.32 ± 8.14c	84.61 ± 1.51b
Phosphorus	Control	33.04 ± 1.08a	4.00 ± 0.25a	113.04 ± 2.52a	228.28 ± 4.52a	80.98 ± 1.05ab
	LP	33.42 ± 1.30a	4.15 ± 0.25a	116.34 ± 3.28a	246.92 ± 6.77b	84.23 ± 1.90a
	MP	33.90 ± 0.94a	3.67 ± 0.29a	147.23 ± 2.27b	281.15 ± 6.15c	75.03 ± 1.19c
	HP	33.50 ± 1.30a	5.00 ± 0.25b	171.79 ± 3.62c	325.40 ± 6.62d	78.84 ± 1.04bc

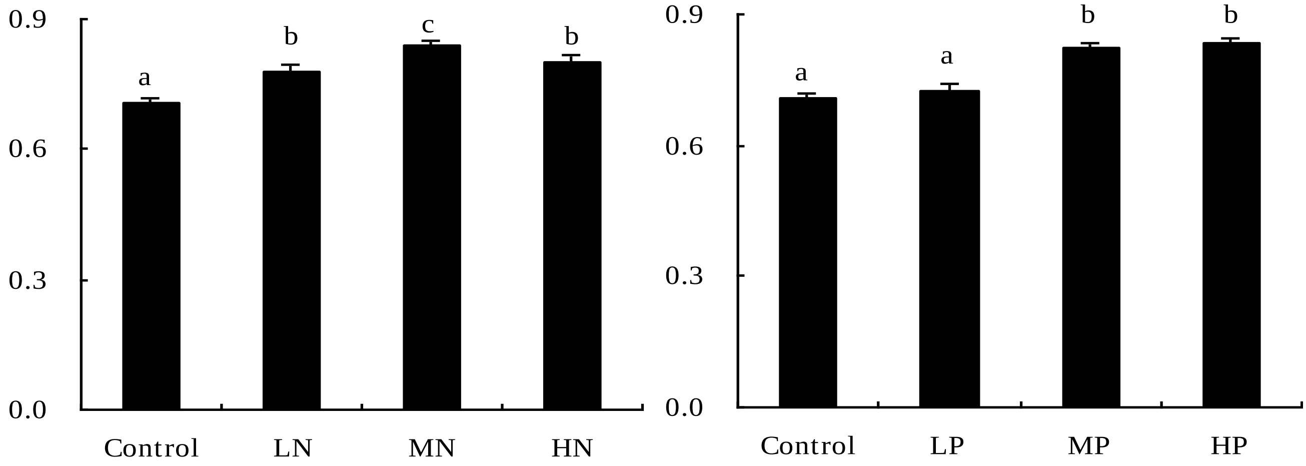

Fig. 1. Effects of different nutrients on Growth Characteristics in a clonal monoecious species, *Sagittaria graminea* (Mean ± SE).

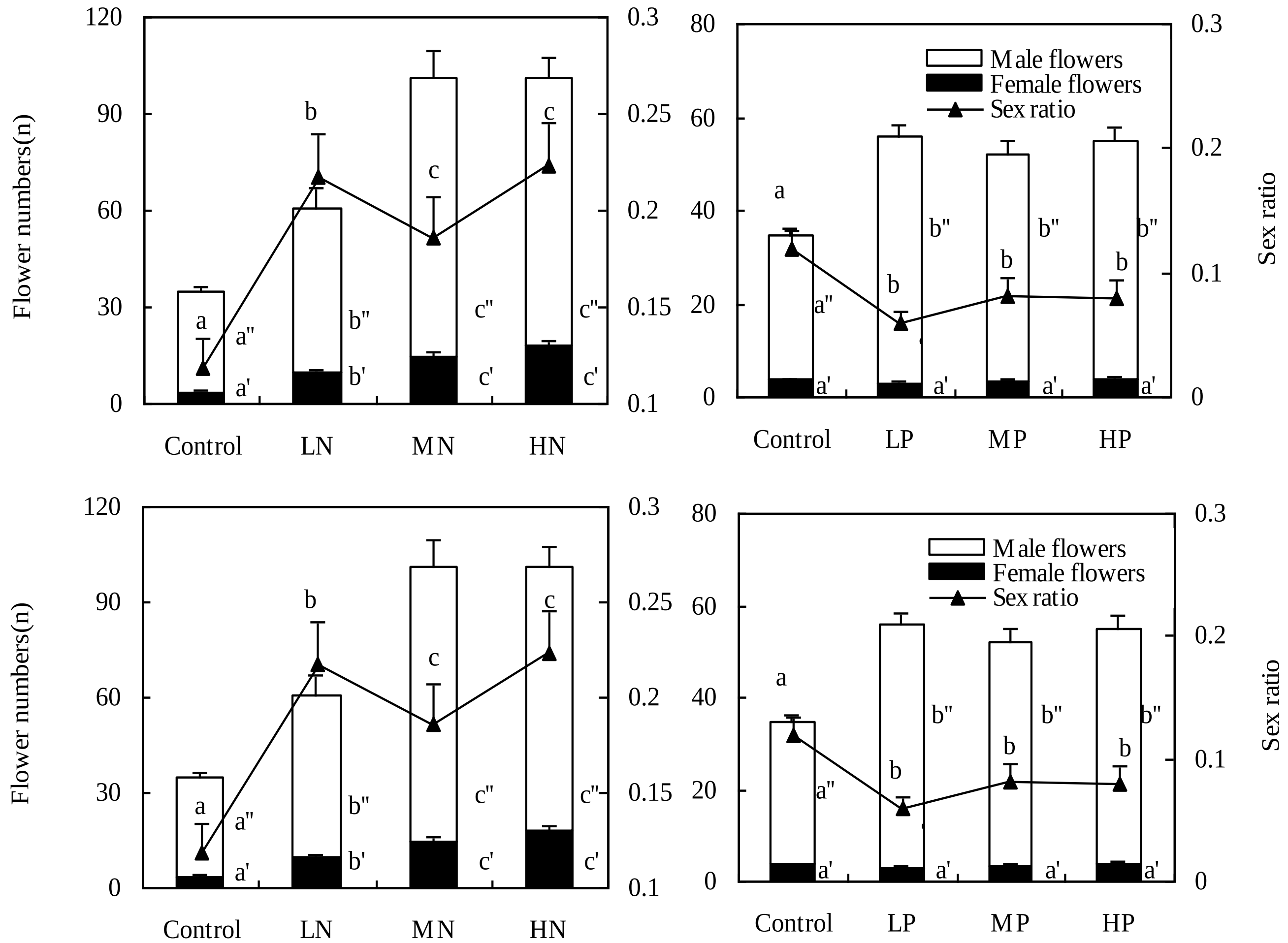

Fig. 2. Effects of different nutrients on sexual reproduction in a clonal monoecious species, *Sagittaria graminea* (Mean ± SE).

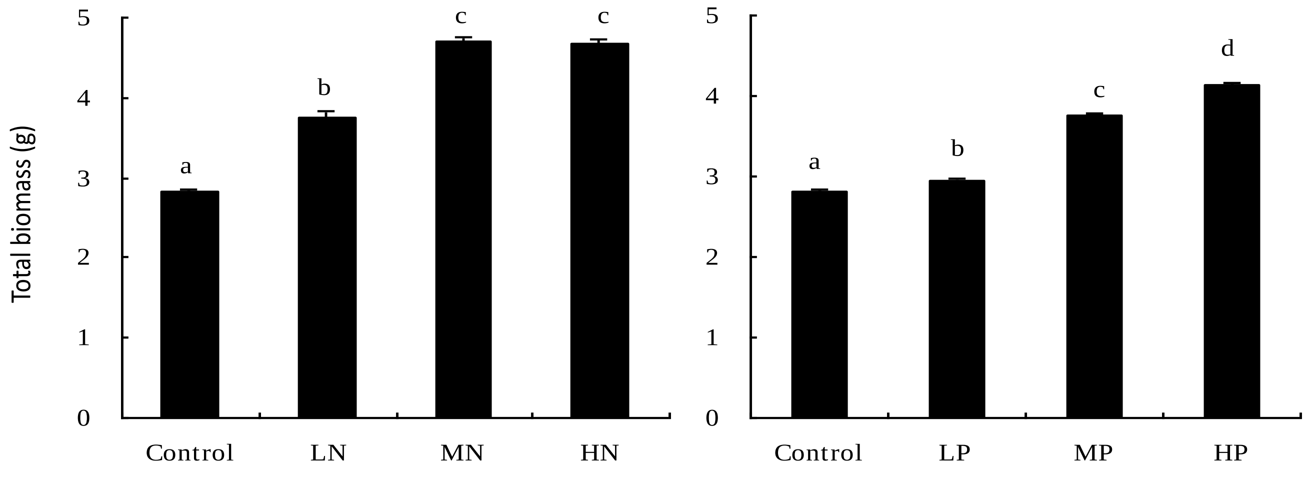

Fig. 3. Effects of different nutrients on total biomass in a clonal monoecious species, *Sagittaria graminea* (Mean ± SE).

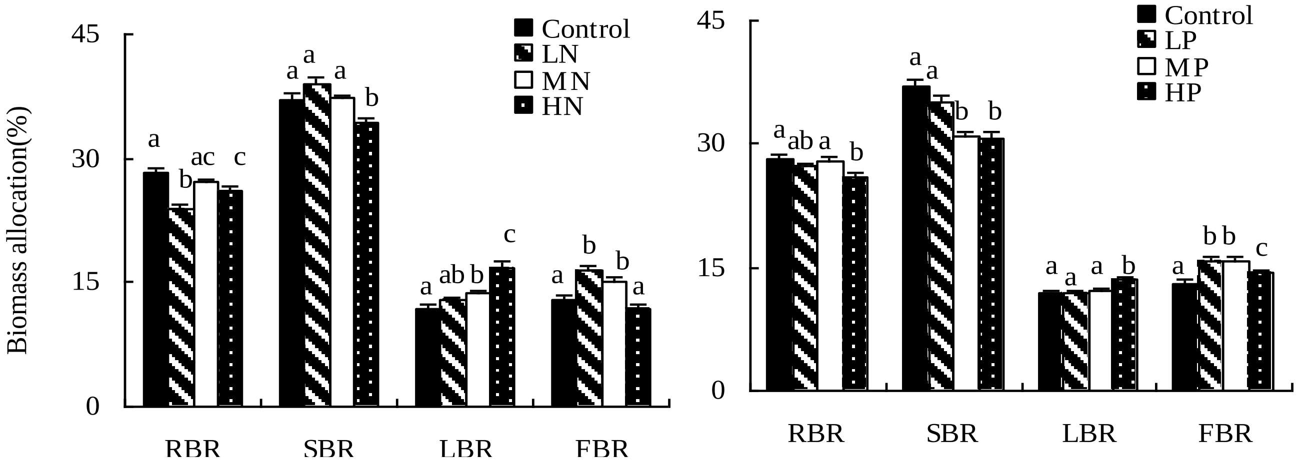

Fig. 4. Effects of different nutrients on biomass allocation in a clonal monoecious species, *Sagittaria graminea* (Mean ± SE); Root biomass ratio, RBR; Support biomass ratio, SBR; Leaf biomass ratio, LBR; Flower biomass ratio, FBR.

Discussion

Phenotypic plasticity would enhance the tolerance and adaptability of plant to heterogeneous habitats based on more resources obtained by altering their morphology, growth, biomass allocation and breeding strategies (Valladares *et al.,* 2014). The increase in nutrients can promote the invasion of exotic species (Heckman *et al.,* 2017). The results showed that with the increase of nitrogen and phosphorus, the leaf number, leaf area and the plant height of *Sagittaria graminea* increased, which meant in high nutrient condition, on the one hand, the *Sagittaria graminea* would expand their own canopy structures to produce large amounts of larger leaves to expand its own canopy structure, and to maximize the occupied resources space aboveground; on the other hand, by increasing the photosynthetic area, *Sagittaria graminea* would capture and absorption more sunlight and inhibit the growth and reproduction of other species by shading, and enhance its competitiveness to achieve its expansion in the heterogeneous habitat.

In the study, we found that with the increase of nitrogen and phosphorus, the total biomass, leaf biomass ratio and RGR of *Sagittaria graminea* were increased while the RBR was decreased, which indicated that the increase in soil nutrients could promote its invasion. In the nutrient-rich habitat, *Sagittaria graminea* is more likely to increase the biomass accumulation of plant vegetative organs to enhance its competitive advantage, especially the roots and leaves. The increasing leaf area and root absorption area will help to increase the absorption of nitrogen by the root and the leaves as well as its competitiveness. SBR increased first and then decreased with the increase of nitrogen and reached minimum in HN, which changed not too much. RBR decreases with increasing nitrogen and became minimal in 0.2 g/kg. The effect of phosphorus on SBR and RBR was consistent, which gradually decreased with the increasing of phosphorus and got minimal with nitrogen supplied was 0.6 g/kg. This indicated that with poor soil nutrients, in order to maximize the availability of absorbing nutrients in the environment, *Sagittaria graminea* allocated more resources to the underground absorption organs to maintain its life activities. The resource allocation strategy of the "growth maximization" when nutrient is rich and the "performance maintenance" when the nutrient is poor is the adaptation to the changing environment. This adaptation is beneficial for the biological species to broaden its niche and enhance its invasiveness, which is similar to the resource allocation strategy for invasive plants such as aircraft grass and silver gingko (Kapoor, 2011; Quan *et al.,* 2014).

The fertility of exotic plants is a key factor of successful invadication (Barrett, 1983). In general, plants with more male flowers will achieve male fit by attracting pollinators, while more female plants will achieve female fitness by producing more seeds (Reichard & Hamilton, 1997; Thomson & Goodell, 2001). This study showed that the increase in nutritions significantly increased the number of *Sagittaria graminea* flowers, which meant more nutrition was helpful for invasidation. With less nitrogen nutriention, there would be more female flowers in *Sagittaria graminea* to save more seed for desperse (Hwang & Lauenroth, 2010; Wang *et al.*, 2010). With more nitrogen nutriention, there would be more flowers in *Sagittaria graminea* to attract more pollinators and showed greater intrusiveness, which was similar the findings about aircraft grass and eupatorium adenophorum reported by Wang and Feng (2005). With less phophate nutrition, the *Sagittaria graminea* increased its male fitness by producing more male flowers to attract pollinators. It is generally believed that plants are easy to invade in an adequate nutrient supply environment, but the rational use of nutrition is the reason for the successful invasion of the *Sagittaria graminea* with less nitrogen and phosphorus.

Conclusions

By changing the supply of nitrogen and phosphorus, the growth of *Sagittaria graminea* was changed. In nutrient-rich environment, the *Sagittaria graminea* grows fast, the biomass is more, the foliage is lush, and the construction speed is fast and the competition is strong, which is beneficial to the population expansion. When the nutrient resources are poor, the number of females and seeds are large, conducive to its diffusion and spread. It is conducive to its spread.

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