

# Effects of low nutrition on photosynthetic capacity and accumulation of total N and P in three climber plant species

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**Abstract** To study the effects of low nutrition on photosynthetic capacity and accumulation of total nitrogen (N) and phosphorus (P) in three climber plant species, *Pharbitis nil* (Linn.) Choisy, *Lonicera japonica* Thunb. and *Parthenocissus tricuspidata* (Sieb. et Zucc.) Planch, all climber plants were exposed to low nutrition at 6 levels (Hoagland solution as control, 1/2, 1/4, 1/8, 1/16 and 1/32-strength Hoagland solution) for 30 days. Photosynthetic capacity was determined by measuring leaf chlorophyll fluorescence, chlorophyll content, carbonic anhydrases activity and growth. Accumulation of total N and P was studied by measuring N and P content in plant tissues. Low nutrition decreased the photosynthetic capacity of *P. nil*, while *L. japonica* maintained high photosynthetic capacity under low nutrition. Photosynthetic apparatus of *P. tricuspidata* suffered no damage when exposed to low nutrition. *L. japonica* and *P. tricuspidata* had better adaptability to low nutrition than *P. nil*. With a faster growth rate, *P. nil* consumed more nutrition (N and P), and its growth was mainly affected by P deficiency under low nutrition. Although *L. japonica* suffered damage from N and P deficiency simultaneously, but the nutrient deficiency was not serious except for 1/32-strength Hoagland solution. *P. tricuspidata* grew slowly, so its requirement of N and P

were the least, even if it was mainly affected by the P deficiency, it could still grow well under low nutrition. With the consideration of fertilizing N and P fertilizers in karst areas which were with lower N and P contents, plant species, N/P ratio threshold and low nutrition level should be taken into account synchronously. This study could provide a general consideration for the planning and developing low nutrition resistant plants and fertilizing the three climber plant species in the low nutrition environment.

**Keywords** Carbonic anhydrase · Chlorophyll fluorescence · N, P accumulation · Karst environment · Plant growth

## Abbreviations

CA	Carbonic anhydrase
ChlF	Chlorophyll fluorescence
N	Nitrogen
P	Phosphorus
<i>P. nil</i>	<i>Pharbitis nil</i> (Linn.) Choisy
<i>L. japonica</i>	<i>Lonicera japonica</i> Thunb
<i>P. tricuspidata</i>	<i>Parthenocissus tricuspidata</i> (Sieb. et Zucc.) Planch
PSII	Photosystem II
WA	Wilbur and Anderson
Fv/Fm	Maximum quantum yield of PSII

## 1 Introduction

Karst terrain covers a vast 0.34 million km<sup>2</sup> land in southwestern China (Yuan 2001), which gives rise to a fragile environment and low productive economy. In fact,

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the lack of mineral elements is an inherent factor leading to the fragile agro-ecological environment in the karst areas. Available element contents in cultivated soil delivered from carbonate rocks are merely 31.1 % of those from non-carbonate rocks (Chen and Bi 2011). Nutrient (nitrogen and phosphorus) limitation has strongly affected competition between plant species, as species vary in their ability to cope with low nutrient resources (Koerselman and Meulerman 1996). Nitrogen (N) and phosphorus (P) are essential macronutrients for plant growth and development. However, inorganic P is one of the least available nutrients in the soils of several terrestrial ecosystems (Vance et al. 2003; Qiu and Lian 2012), causing problems that could lead P deficiency, especially in the karst areas where dissolved P concentration was no more than 0.33 mmol/L (Alloush et al. 2003). According to those researchers, P deficiency significantly influenced leaf photosynthesis and carbon metabolisms in plants (Rao 1996; Foyer and Spencer 1986; Fredeen et al. 1989). Besides, the karst vegetations were also generally at N- and P- co-limited stresses, N also played an important role in the formation of chloroplast structure and hence to the photosynthetic intensity and productivity of plants (Du et al. 2011; Doncheva et al. 2001; Liu et al. 2013). But the question was how to select appropriate kinds of plants for the ecological restoration or how to apply N and P fertilizers in karst areas which were with lower N and P contents.

Lianas contributed substantially to the diversity of the forest and were widely used for producing medicine. They also played a substantial role in forest regeneration, so they had a special superiority in ecological restoration (Bongers et al. 2002). Both *Pharbitis nil* (Linn.) Choisy and *Lonicera japonica* Thunb. were excellent medicinal plants and afforestation plants widely cultivated in China (Jung et al. 2008; Stanturf et al. 2004; Kumar et al. 2005). *Parthenocissus tricuspidata* (Sieb. et Zucc.) Planch was a vertical virescence medicinal plant of the Vitaceae family that might climb 20 m or higher by adhesive tendrils attaching to supports (Wang et al. 2009; Kim et al. 2005). They were more superior in growth, breeding, adaptability and competition than woody and herbage plants. They had the developed root system, higher biomass, good for soil fixation and planting (Schnitzer and Bongers 2002), under drought stress conditions, these three climber plants were all with good adaptability and different mechanisms of photosynthetic response and adaptability respectively (Xing and Wu 2012). There was no research on the comparative studies of different adaptability in these three climber plants to low nutrition till now.

According to those studies, photosynthetic activities could be considered to represent the growth potential of a plant (Mooney 1972; Walters et al. 1993). Changes in Chlorophyll fluorescence (ChlF) emission, arising mainly

from photosystem II (PSII), provided information regarding almost all aspects of photosynthetic activity and therefore, reflected plant tolerance to environmental stresses (Panda et al. 2008). Photosynthesis of plant was affected by its photosynthetic activity. Photosynthesis was the important energy source and material basis for plant growth. Chlorophyll was an important pigment which participated in the absorption, transmission and transformation of light energy for photosynthesis (Wu et al. 2008), its content was closely related with leaf photosynthetic rate and organic matter accumulation, growth and development, yield in plant. The higher the chlorophyll content in plant and higher the photosynthetic capacity (Gitelson et al. 2003). Meanwhile, Carbonic anhydrases (CA) were zinc-containing enzymes to transport carbon dioxide and protons across biological membranes and to retain inorganic carbon within the cell (Tavallali et al. 2009). It was involved in diverse physiological processes including photosynthetic carbon dioxide fixation (Moroney et al. 2001). CA played an important role in photosynthesis process. Higher CA activity was more in favor of plant photosynthesis (Wu and Xing 2012).

This study selected *P. nil*, *L. japonica* and *P. tricuspidata* as experiment materials, simulated low nutrition environment of soil in southwestern karst areas and cultivated these three climber plants in different concentration nutrition. Determined their CA activities, ChlF parameters, chlorophyll contents, growth and N, P accumulation and compared the growth quality between *P. nil*, *L. japonica* and *P. tricuspidata*. Discussed the different adaptability of these three climber plants to low nutrition of soil, and provided general consideration for improving and repairing fragile karst ecological environment by biological method.

## 2 Materials and methods

### 2.1 Plant growth and treatment

The experiment was conducted in an artificial climatic chamber at the Institute of Agricultural Engineering, Jiangsu University, Jiangsu Province, China (N 32°11' and E 119°27'). The seedlings of *P. nil*, *L. japonica* and *P. tricuspidata* were germinated and cultivated in 12-hole trays with quartz sand under a 12-h photoperiod ( $280 \pm 20 \mu\text{mol}/(\text{m}^2 \text{sec})$  PPFD), a day/night temperature cycle of 27/23 °C, and 70 % of relative humidity. Plants were irrigated daily with 1/4-strength Hoagland solution [6 mmol/L  $\text{KNO}_3$ , 4 mmol/L  $\text{Ca}(\text{NO}_3)_2$ , 2 mmol/L  $\text{MgSO}_4$ , 2 mmol/L  $\text{Fe}(\text{Na})\text{EDTA}$ , 1 mmol/L  $\text{NH}_4\text{H}_2\text{PO}_4$ , 2  $\mu\text{mol}/\text{L}$  KCl, 50  $\mu\text{mol}/\text{L}$   $\text{H}_3\text{BO}_3$ , 4  $\mu\text{mol}/\text{L}$   $\text{MnSO}_4$ , 4  $\mu\text{mol}/\text{L}$   $\text{ZnSO}_4$ , 0.2  $\mu\text{mol}/\text{L}$   $\text{CuSO}_4$ , and 0.2  $\mu\text{mol}/\text{L}$   $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}$ ] (Hoagland and Arnon 1950). After 2 months of growth, 5 different

nutrient concentration solutions (1/2, 1/4, 1/8, 1/16, 1/32-strength Hoagland solution) and original Hoagland solution as control were used to simulate six nutrition levels to seedlings that germinated healthily and uniformly. In this experiment, available N content in karst soil was equal to 1/2-strength Hoagland solution, available P content in karst soil was between 1/2 and 1/4-strength Hoagland solution, available potassium content in karst soil was between 1/4 and 1/8-strength Hoagland solution (Jiang 2000; Zhao et al. 2007). The experiment was arranged in a completely randomized design, 12 healthy and uniform seedlings from each species of climber plants were used under each level. The treatment lasted for 30 days and then measurements were done.

## 2.2 Carbonic anhydrase activity measurement

The fourth and fifth youngest fully expanded leaves from the top were chosen for CA activity measurement. Three plants from each treatment group were used for the measurement. Leaf tissues (0.3–0.8 g) were quickly frozen in liquid N and ground with 3 mL extraction buffer (0.01 mol/L barbitone sodium with 0.05 mol/L mercaptoethanol, pH 8.3). The homogenate was centrifuged at 13,000 r/min and 0 °C for 5 min, and then placed on ice for 20 min. The supernatant was used to analyze the CA activity using the pH method described by Wilbur and Anderson (1948) with modifications (Wu et al. 2011). In brief, CA activity was assayed at 0–2 °C in a mixture containing 4.5 mL, 0.02 mol/L barbitone buffer (5, 5-diethylbarbituric acid; pH 8.3), 0.4 mL sample and 3 mL CO<sub>2</sub>-saturated water. CA activity was expressed in Wilbur and Anderson (WA) units as  $WA = (t_0/t) - 1$ , where  $t_0$  and  $t$  were the time (sec) measured for the pH change (8.2–7.2) with buffer alone ( $t_0$ ) and with sample ( $t$ ).

## 2.3 Determination of ChlF

ChlF was measured with IMAGING-PAM modulated ChlF imaging system (IMAGING-PAM, Heinz Walz GmbH, Germany). Leaves were dark adapted for 30 min to ensure complete relaxation of all reaction centers before the measurements. As mentioned above, the fourth youngest fully expanded leaf from the top was selected for the measurement. Three plants from each treatment group were used for the measurement. The minimum ChlF ( $F_0$ ) was determined using a measuring beam, whereas the maximum ChlF ( $F_m$ ) was recorded after a 0.8 s saturating light pulse [6,000  $\mu\text{mol}/(\text{m}^2 \text{ sec})$ ]. Maximal PSII photochemical efficiency ( $F_v/F_m$ ) was calculated as  $(F_m - F_0)/F_m$ .

## 2.4 Determination of chlorophyll content

Chlorophyll content was measured with SPAD-502 chlorophyll content measuring apparatus (SPAD-502, Konica

Minolta, Tokyo). The fourth youngest fully expanded leaf from the top was selected for the measurement and three plants from each treatment group were used for the measurement.

## 2.5 Determination of growth status

Three plants from each treatment group were used for the measurement of stem diameter, leaf area and height respectively. Stem diameter was determined by vernier caliper (0–150, Chengliang, Chengdu), leaf area was measured with a portable leaf area measurement instrument (AM-200, ADC, UK). Height was measured with a ruler.

## 2.6 Determination of N and P content

Three plants from each treatment group were selected and dried in oven under 80 °C, then the plant dry weight was weighed by BT125D electronic balance (BT125D, Sartorius, Germany). 0.2–0.4 g drying of plant tissue were used for digesting with H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion method (Xu 2000), and N, P concentrations (mg/L) in the digestion solution were determined with AA3 continuous-flow analyzers (AA3, Seal Analytical, Germany). N and P % were calculated as follows:

$$N\% = (C_1 \times n_1 \times V)/M; P\% = (C_2 \times n_2 \times V)/M,$$

where  $C_1$  and  $C_2$  were the concentrations of N and P in the digestion solution,  $n_1$  and  $n_2$  were the dilution factor,  $V$  was the volume of the digestion solution,  $M$  was the quality of drying of plant tissue.

## 2.7 Statistical analysis

All measurements were subjected to analysis of variance (ANOVA) to discriminate significant differences (defined as  $P \leq 0.05$ ) between group means. Data were shown as the mean  $\pm$  standard error (SE). These mean data were analyzed statistically using a factorial design by SPSS software (version 13.0, SPSS Inc), and mean results were compared by LSD post hoc test at 5 % significance level ( $p < 0.05$ ).

# 3 Results

## 3.1 Carbonic anhydrase activity

CA activities varied with plant species and low nutrition levels. CA activity of *P. nil* was higher than the other two species. CA activity of *P. tricuspidata* was the lowest and nearly undetectable, *P. tricuspidata* had no CA activity (Table 1). CA activities of *P. nil* under 1/2 and 1/4-strength

**Table 1** Effects of low nutrition on carbonic anhydrase (CA) activity (WAU/gFW) of three climber plant species (samples  $n = 5$ )

Material	Nutrient concentration					
	Control	1/2	1/4	1/8	1/16	1/32
<i>P. nil</i>	1,397 ± 364 <sup>a</sup>	1,469 ± 157 <sup>a</sup>	1,647 ± 42 <sup>a</sup>	1,231 ± 125 <sup>a,b</sup>	903 ± 95 <sup>b</sup>	717 ± 247 <sup>b</sup>
<i>L. japonica</i>	132 ± 18 <sup>c</sup>	145 ± 10 <sup>c</sup>	1,000 ± 202 <sup>a</sup>	408 ± 45 <sup>bc</sup>	345 ± 125 <sup>bc</sup>	501 ± 63 <sup>b</sup>
<i>P. tricuspidata</i>	92 ± 52 <sup>a</sup>	52 ± 14 <sup>ab</sup>	5 ± 3 <sup>b</sup>	5 ± 2 <sup>b</sup>	9 ± 2 <sup>b</sup>	14 ± 11 <sup>b</sup>

The mean ± SE followed by *different letters* in the same row differ significantly at  $P \leq 0.05$ , according to one-way ANOVA and  $t$  test

**Table 2** Effects of low nutrition on maximal PSII photochemical efficiency (Fv/Fm) of three climber plant species (samples  $n = 5$ )

Material	Nutrient concentration					
	Control	1/2	1/4	1/8	1/16	1/32
<i>P. nil</i>	0.75 ± 0.006 <sup>a</sup>	0.74 ± 0.006 <sup>a</sup>	0.73 ± 0.006 <sup>ab</sup>	0.63 ± 0.028 <sup>b</sup>	0.67 ± 0.028 <sup>ab</sup>	0.53 ± 0.042 <sup>b</sup>
<i>L. japonica</i>	0.55 ± 0.038 <sup>ab</sup>	0.53 ± 0.039 <sup>ab</sup>	0.69 ± 0.011 <sup>a</sup>	0.70 ± 0.009 <sup>a</sup>	0.66 ± 0.005 <sup>a</sup>	0.42 ± 0.064 <sup>b</sup>
<i>P. tricuspidata</i>	0.57 ± 0.038 <sup>a</sup>	0.63 ± 0.015 <sup>a</sup>	0.64 ± 0.030 <sup>a</sup>	0.60 ± 0.021 <sup>a</sup>	0.64 ± 0.017 <sup>a</sup>	0.58 ± 0.017 <sup>a</sup>

The mean ± SE followed by *different letters* in the same row differ significantly at  $P \leq 0.05$ , according to one-way ANOVA and  $t$  test

**Table 3** Effects of low nutrition on chlorophyll content (SPAD/%) of three climber plant species (samples  $n = 5$ )

Material	Nutrient concentration					
	Control	1/2	1/4	1/8	1/16	1/32
<i>P. nil</i>	33.83 ± 0.33 <sup>a</sup>	34.17 ± 1.04 <sup>a</sup>	33.60 ± 0.32 <sup>a</sup>	27.57 ± 0.77 <sup>b</sup>	21.27 ± 1.05 <sup>c</sup>	16.67 ± 1.36 <sup>d</sup>
<i>L. japonica</i>	23.37 ± 3.78 <sup>b</sup>	27.97 ± 3.10 <sup>b</sup>	34.77 ± 2.94 <sup>ab</sup>	36.27 ± 2.04 <sup>a</sup>	32.37 ± 0.45 <sup>ab</sup>	34.00 ± 1.61 <sup>ab</sup>
<i>P. tricuspidata</i>	29.90 ± 1.84 <sup>a</sup>	28.90 ± 1.15 <sup>a</sup>	25.73 ± 2.15 <sup>ab</sup>	29.17 ± 1.10 <sup>a</sup>	23.53 ± 1.64 <sup>b</sup>	25.70 ± 0.47 <sup>ab</sup>

The mean ± SE followed by *different letters* in the same row differ significantly at  $P \leq 0.05$ , according to one-way ANOVA and  $t$  test

Hoagland solution were a little higher than that under control, while the values under 1/16 and 1/32-strength Hoagland solution were lower compared to control. CA activities of *L. japonica* under 1/4, 1/8, 1/16 and 1/32-strength Hoagland solution were higher than that under control or 1/2-strength Hoagland solution, the value under 1/4-strength Hoagland solution was the highest.

### 3.2 Chlorophyll fluorescence

Maximal PSII photochemical efficiency (Fv/Fm) of *P. nil* and *L. japonica* had the lowest value under 1/32-strength Hoagland solution, Fv/Fm value of *P. nil* under 1/8-strength Hoagland solution was also lower than that under control or 1/2-strength Hoagland solution (Table 2). Fv/Fm of *P. tricuspidata* did not change markedly with increasing low nutrition.

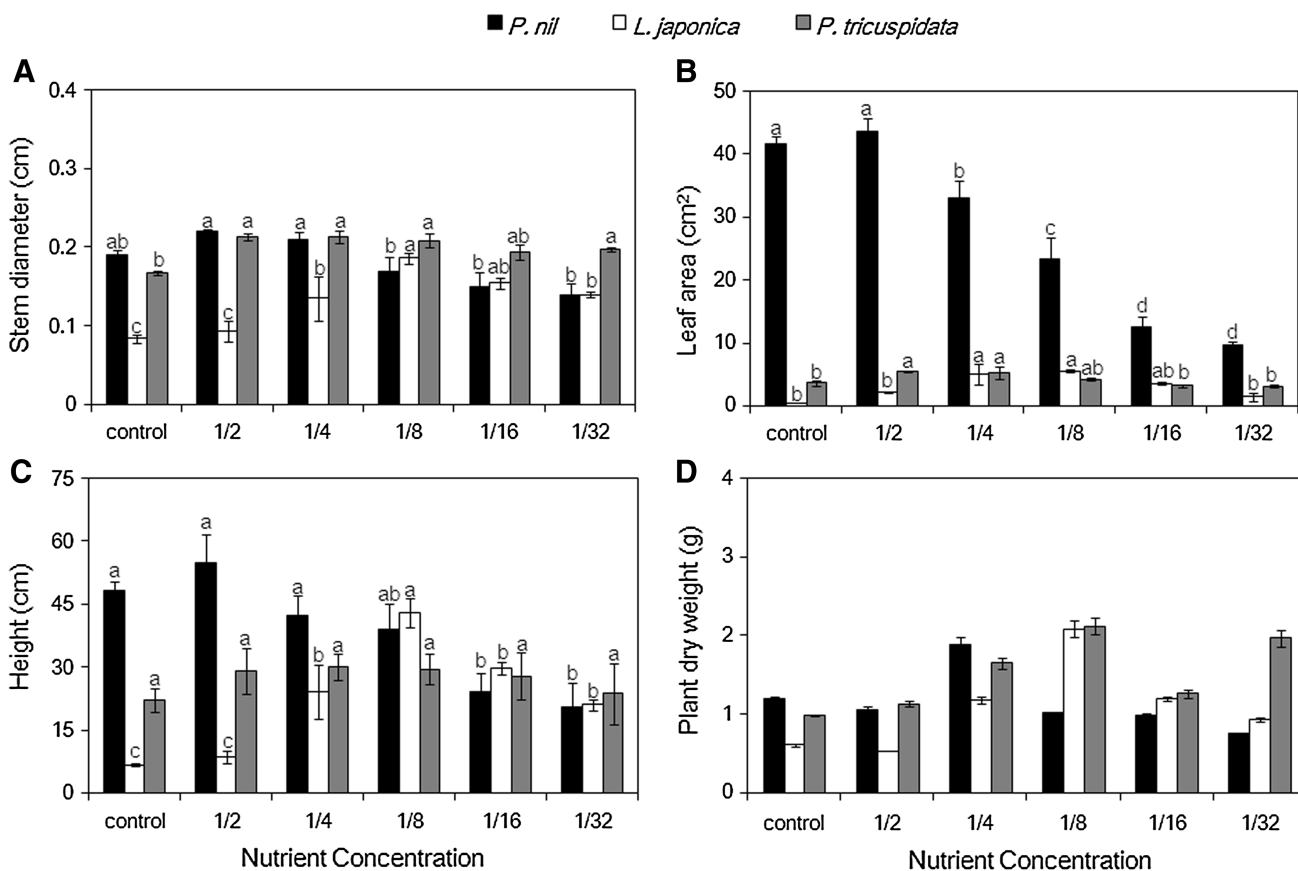
### 3.3 Chlorophyll content

Chlorophyll contents (SPAD/%) of *P. nil* under 1/8, 1/16, 1/32-strength Hoagland solution were lower than that under 1/2, 1/4-strength Hoagland solution and control (Table 3),

chlorophyll content of *P. nil* under 1/32-strength Hoagland solution was only 48.8 % of that under the 1/2-strength Hoagland solution. Whereas chlorophyll contents of *L. japonica* under 1/4, 1/8, 1/16 and 1/32-strength Hoagland solution were higher than that under control or 1/2 and chlorophyll content of *L. japonica* under 1/8 was 1.6 times of that under control. Chlorophyll content of *P. tricuspidata* had a lower value under 1/16-strength Hoagland solution compared to other levels.

### 3.4 Plant growth

Low nutrition (1/8, 1/16, 1/32-strength Hoagland solution) was associated with a lower stem diameter of *P. nil*, whereas stem diameters of *L. japonica* and *P. tricuspidata* were higher than that under control (Fig. 1a). Leaf area of *P. nil* decreased significantly under low nutrition (1/4, 1/8, 1/16, 1/32-strength Hoagland solution), and the value under 1/32 was only 23.1 % of that under control (Fig. 1b). Leaf areas of *L. japonica* and *P. tricuspidata* had higher value under 1/4 or 1/8-strength Hoagland solution compared to other levels and leaf areas of *L. japonica* and *P. tricuspidata* under low nutrition were all not lower than that under



**Fig. 1** Effects of low nutrition on stem diameter, leaf area, height and plant dry weight in three climber plant species. **a** Stem diameter, **b** leaf area, **c** height, **d** plant dry weight. The mean  $\pm$  SE followed by different letters in the same species significantly at  $P \leq 0.05$ , according to one-way ANOVA and *t* test

control respectively. Heights of *P. nil* under 1/16 and 1/32-strength Hoagland solution decreased compared to other levels (Fig. 1c). Low nutrition was associated with a higher height of *L. japonica* and value of *L. japonica* under 1/8 was the highest. Height of *P. tricuspidata* appeared independent of low nutrition. Plant dry weight of *P. nil* under 1/4 was higher than that under other levels, but the value under 1/32 became lower than that under control (Fig. 1d). Low nutrition (1/4, 1/8, 1/16, 1/32-strength Hoagland solution) was also associated with higher plant dry weight of *L. japonica*, and the value of *L. japonica* under 1/8 was also the highest. Plant dry weight of *P. tricuspidata* increased under 1/4, 1/8 and 1/32-strength Hoagland solution compared to other levels.

### 3.5 Accumulation of total N and P

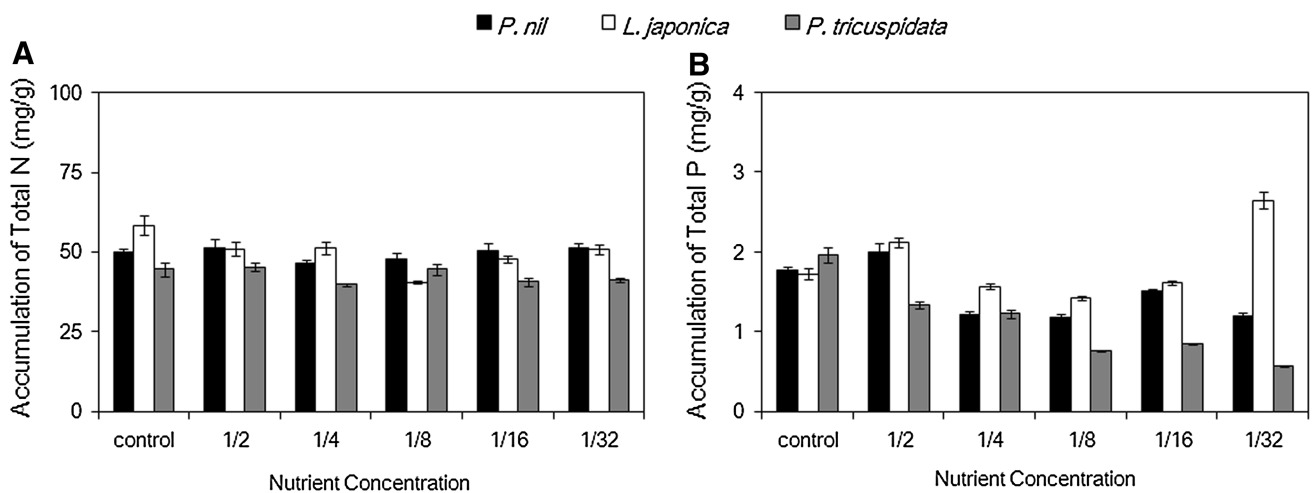
N/P ratio of *P. nil* and *P. tricuspidata* under low nutrition (1/4, 1/8, 1/16, 1/32-strength Hoagland solution) was higher than that under control or 1/2-strength Hoagland solution (Table 4). N/P ratio of *P. nil* under 1/8 or 1/32 was higher than that under 1/2, 1/4 and 1/16-strength Hoagland

solution. N/P ratio of *L. japonica* under control was higher than that under 1/2 and 1/32. Value under 1/32 was the lowest and value under 1/8 was the highest. N/P ratio of *P. tricuspidata* had the highest value under 1/32-strength Hoagland solution, N/P ratio of *P. tricuspidata* under 1/8-strength Hoagland solution was higher than that under control, 1/2, 1/4, and 1/16-strength Hoagland solution.

Figure 2a showed the total N accumulation of three species. Total N accumulation of *P. nil* and *P. tricuspidata* appeared independent of low nutrition. Low nutrition was associated with a little lower total N accumulation of

**Table 4** Effects of low nutrition on N/P (mg/mg) ratio of three climber plant species

Material	Nutrient concentration					
	Control	1/2	1/4	1/8	1/16	1/32
<i>P. nil</i>	28.28	23.63	34.33	40.69	30.07	40.10
<i>L. japonica</i>	28.21	24.05	32.15	33.41	28.95	16.31
<i>P. tricuspidata</i>	25.03	25.00	39.38	61.96	48.73	87.99



**Fig. 2** Effects of low nutrition on accumulation of total nitrogen (N), phosphorus (P) in three climber plant species. **a.** accumulation of total N, **b** accumulation of total P

*L. japonica* compared to control, value under 1/8-strength Hoagland solution was the lowest.

Figure 2b showed the total P accumulation of three species. Low nutrition (1/4, 1/8, 1/16, 1/32-strength Hoagland solution) was associated with a lower total P accumulation of *P. nil* and *P. tricuspidata* compared to control or 1/2-strength Hoagland solution. Total P accumulation of *P. nil* under 1/16 was higher than that under 1/4, 1/8 and 1/32-strength Hoagland solution. Whereas total P accumulation of *L. japonica* decreased under 1/4, 1/8 and 1/16-strength Hoagland solution compared to control, 1/2 or 1/32-strength Hoagland solution, and total P accumulation of *L. japonica* under 1/32 became the highest. Total P accumulations of *P. tricuspidata* under 1/8 and 1/16 were higher than that under 1/32 but lower than that under 1/2 or 1/4-strength Hoagland solution.

## 4 Discussion

### 4.1 Photosynthetic activity

Changes in ChlF emission, arising mainly from PSII, provided information regarding almost all aspects of photosynthetic activity which could represent the plant growth potential (Panda et al. 2008). In this study, the response of Fv/Fm of *P. nil* to increased low nutrition indicated that the reaction center of PSII could have been damaged under 1/8, 1/16 and 1/32-strength Hoagland solution. Reaction center of PSII of *L. japonica* was damaged under 1/32-strength Hoagland solution. Effect of low nutrition on the photosynthesis of *P. tricuspidata* did not involve any damage to the PSII reaction centers. Under low nutrition conditions, CA played an important role in the

photosynthesis process. CA was involved in diverse physiological processes (Badger and Price 1994; Sasaki et al. 1998). Higher CA activity was in favor of plant photosynthesis. Activated by low nutrition, CA activities of *P. nil* under 1/2 and 1/4-strength Hoagland solution had some increase, which could prevent the reaction center of *P. nil* from damage. Lower CA activity of *P. nil* under 1/8, 1/16 and 1/32-strength Hoagland solution showed bad flexibility and worse regulatory ability. A substantial increase of CA activity of *L. japonica* occurred under 1/4-strength Hoagland solution, indicating that high CA activity was activated to respond low nutrition, and a stable photosynthetic activity could be maintained. Although CA activity of *L. japonica* under 1/32 had some increase compared to 1/16-strength Hoagland solution, regulation of CA could not prevent reaction center of *L. japonica* from damage. Different increase of CA activity between *L. japonica* and *P. nil* under 1/4 compared to 1/2-strength Hoagland solution indicated that CA of *L. japonica* was more sensitive to low nutrition and more flexibility than *P. nil*. CA activity of *P. tricuspidata* was the lowest and nearly undetectable, but *P. tricuspidata* could also maintain a stable photosynthetic activity under low nutrition.

### 4.2 Plant growth

When plant was under adversity stress conditions, its various physiological processes would be affected, and thus affected the chlorophyll content in plant directly or indirectly (Gitelson et al. 2003). Leaf photosynthesis could be estimated from measurements of chlorophyll content. The decrease of chlorophyll content of *P. nil* under 1/8, 1/16 and 1/32-strength Hoagland solution indicated that the photosynthesis of *P. nil* would decrease. Whereas the

increase of chlorophyll content of *L. japonica* under 1/4, 1/8, 1/16 and 1/32-strength Hoagland solution was good for the photosynthesis of *L. japonica*. Chlorophyll content of *P. tricuspidata* was not affected by low nutrition expected for 1/16-strength Hoagland solution, but it could maintain a stable photosynthetic activity.

Growth analysis indicated that *P. nil* grew fast and could accumulate more dry matter under 1/4-strength Hoagland solution but it could not grow well under 1/8, 1/16 and 1/32-strength Hoagland solution. Whereas *L. japonica* demonstrated the best adaptability to 1/8-strength Hoagland solution, *L. japonica* could grow well under 1/4, 1/8 and 1/16-strength Hoagland solution. *P. tricuspidata* grew slowly, low nutrition had less influence on its growth, but it accumulated less dry matter under 1/16-strength Hoagland solution compared to 1/4, 1/8 and 1/32-strength Hoagland solution.

#### 4.3 Accumulation of total N and P

The ‘N/P tool’ could predict the limiting nutrient for plant growth on a community level conceptually simple and accurately. It could be used in restoration projects aiming at a improving of site fertility (Koerselman and Meulerman 1996). Moreover, there were different N/P ratio thresholds for N or P limitation among species in a given area (Li et al. 2011). In this study, the N/P ratio of three climber species under control was taken as threshold respectively. N/P ratio of *P. nil* or *P. tricuspidata* under low nutrition (1/4, 1/8, 1/16 and 1/32-strength Hoagland solution) was higher than that under control or 1/2-strength Hoagland solution respectively. The accumulation of total P in *P. nil* or *P. tricuspidata* under low nutrition (1/4, 1/8, 1/16 and 1/32-strength Hoagland solution) was lower than that under control or 1/2-strength Hoagland solution, but the accumulation of total N in *P. nil* or *P. tricuspidata* under low nutrition (1/4, 1/8, 1/16 and 1/32-strength Hoagland solution) was not significantly lower than that under control or 1/2-strength Hoagland solution respectively, so the nutrient limitation for growth of *P. nil* or *P. tricuspidata* under 1/4, 1/8, 1/16 and 1/32-strength Hoagland solution was P rather than N. N/P ratio of *P. nil*, *L. japonica* or *P. tricuspidata* under 1/8-strength Hoagland solution was higher than that under 1/4 and 1/16-strength Hoagland solution, which indicated that nutrient limitation for growth of *P. nil*, *L. japonica* or *P. tricuspidata* under 1/8-strength Hoagland solution was P rather than N compared to 1/4 and 1/16-strength Hoagland solution. While nutrient limitation for growth of *P. nil* or *P. tricuspidata* under 1/16-strength Hoagland solution was N rather than P compared to 1/8 and 1/32-strength Hoagland solution. N/P ratio of *L. japonica* under 1/32 was lower than that under other levels and

accumulation of total P in *L. japonica* under 1/32 was higher compared to other levels, so the nutrient limitation for growth of *L. japonica* under 1/32-strength Hoagland solution was N rather than P. N/P ratio of *L. japonica* under 1/8 was higher than that under other levels, nutrient limitation for growth of *L. japonica* under 1/8-strength Hoagland solution was P rather than N. But compared to 1/4, 1/8, 1/16-strength Hoagland solution and control, nutrient limitation for growth of *L. japonica* under 1/2-strength Hoagland solution was N rather than P.

Therefore nutrient limitation for growth varied with low nutrition level and plant species. When N/P ratio threshold for N or P limitation changed, nutrient limitation for growth of plants changed too. With the consideration of fertilizing N and P fertilizers in karst areas which were with lower N and P contents, plant species, N/P ratio threshold and low nutrition level should be taken into account synchronously.

## 5 Conclusions

We can conclude from discussions above, CA activity, Fv/Fm, chlorophyll content and growth of *P. nil* decreased with decreasing nutrition concentrations. *P. nil* could not grow well when exposed to low nutrition conditions (1/8, 1/16 and 1/32-strength Hoagland solution) and *P. nil* was not adapt to low nutrition (1/8, 1/16 and 1/32-strength Hoagland solution). Under low nutrition conditions (1/4, 1/8, 1/16 and 1/32-strength Hoagland solution) especially 1/8-strength Hoagland solution, *L. japonica* had a higher value of chlorophyll content and better photosynthetic efficiency, it could grow well and accumulate more organic matter, it had a good adaptability to low nutrition (1/4, 1/8 and 1/16-strength Hoagland solution); For *P. tricuspidata*, its growth was not affected significantly. The nutrient elements in soil of karst areas were very low and not good for growth of many plants. On the other hand *L. japonica* and *P. tricuspidata* had good adaptability to low nutrition environments, *P. tricuspidata* could even grow on the wall. With a faster growth rate, *P. nil* consumed more nutrition (N and P), and its growth was mainly affected by P deficiency under low nutrition. Although *L. japonica* suffered damage from N and P deficiency simultaneously, but the nutrient deficiency was not serious except for 1/32-strength Hoagland solution. *P. tricuspidata* grew slowly, so its requirement of N and P was the least. Even if it was mainly affected by the P deficiency, it could still grow well under low nutrition. With the consideration of fertilizing N and P fertilizers in karst areas which were with lower N and P contents, plant species, N/P ratio threshold and low nutrition level should be taken into account synchronously. This study could provide a general consideration for planning

and developing low nutrition resistant plants, and fertilizing the three climber plant species in the low nutrition environment.

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