Effect of phosphorus deficiency on photosynthetic inorganic carbon assimilation of three climber plant species

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Abstract

Background: P deficiency in karst areas significantly influenced leaf photosynthesis and carbon metabolisms in plants which were bad for plant growth. Meanwhile, fertilizer application would cause lots of environmental problems. Therefore planning and developing P deficiency-resistant plants in karst areas are important to prevent shortage of P resources and reduce the environmental impacts of P supplementation.

Results: This study examined the photosynthetic response of three climber plant species, namely, *Pharbitis nil* (Linn.) Choisy, *Lonicera pampaninii* Levl, and *Parthenocissus tricuspidata* (Sieb.et Zucc.) Planch to phosphorus (P) deficiency stress. The plants were exposed to P deficiency stress at three treatments of 0.125 mM, 0.031 mM, and 0 mM for 30 d; 0.250 mM P was used as the control. Photosynthetic responses were determined by measurement of leaf photosynthesis, chlorophyll fluorescence, carbonic anhydrase activity, and stable carbon isotope ratios. *Pharbitis nil* showed high CA activity, more negative δ¹³C values and could maintain long-term stable photosynthetic capacity. *Lonicera pampaninii* also showed high CA activity but positive δ¹³C values compared to *Pharbitis nil*, and its photosynthetic capacity decreased as P deficiency stress increased. *Parthenocissus tricuspidata* had a low photosynthesis and positive δ¹³C values compared to *Pharbitis nil*, it could grow normally even under 0 mM P.

Conclusions: *Pharbitis nil* was tolerant to long-term, severe P deficiency stress, a finding that is attributed to its stable PSII and regulation of carbonic anhydrase. *Lonicera pampaninii* showed a poor adaptability to short-term P deficiency, but exhibited long-term tolerance under 0.125 mM P concentration. *Parthenocissus tricuspidata* was tolerant to long-term P deficiency stress, may exhibit a stomatal limitation. Besides, P deficiency stress had little effect on the way of inorganic carbon utilization of the three climber plants. Different adaptation mechanisms to P deficiency stress should be considered for the selection of species when developing P deficiency-resistant plants.

Keywords: Adaptation mechanism; Carbonic anhydrase; Chlorophyll fluorescence; Composition of the stable carbon isotope; Photosynthesis

Background

Phosphorus (P) is an essential macronutrient for plant growth and development. It is a component of several cellular molecules, such as ATP, nucleic acids, phospholipids, and phosphorylated sugars, and thus plays a crucial role in carbon metabolism (Huang et al., 2008). However, inorganic P is one of the least available nutrients in the soils of several terrestrial ecosystems (Vance et al., 2003), which occasionally leads to P deficiency, especially in karst areas. P deficiency in modern agricultural systems can be alleviated by fertilizer application, but fertilizer costs are variable and concerns have been raised about their potential environmental impacts (Zhang et al., 2009). The continuing demand for P could deplete global P reserves by the end of the century (Byrne et al., 2011). Thus, improvements in P acquisition and P use efficiency are becoming increasingly important to prevent shortage of P resources and reduce the environmental impacts of P supplementation.

P deficiency induces a wide array of metabolic effects that limit plant growth. Hög-Hansen et al. have reported...
that a low P status induces changes in the relative growth of the roots and shoots rather than changes in the carbon uptake rates per unit mass or area of these organs (Hogh-Jensen et al., 2002). Usuda and Shimogawara (1995) showed that the soluble and insoluble protein contents of phosphorus-deficient maize decreased compared with that of the control plants. Other researchers reported that P deficiency significantly influences leaf photosynthesis and carbon metabolisms in plants (Rao, 1996; Foyer and Spencer, 1986; Fredeen et al., 1989; Rao and Terry, 1995). Inhibition of photosynthesis caused by P deficiency is mainly due to the decrease in the ribulose-1,5-bisphosphate (RuBP) pool size (Jacob and Lawlor, 1992; Pieters et al., 2001). But under stress conditions, carbonic anhydrase (CA) could always provide carbon and water sources for the photosynthesis process, and CA is involved in diverse physiological processes, such as ion exchange, acid–base balance, CO2 transfer, respiration, biosynthesis, and photosynthetic CO2 fixation (Badger and Price, 1994; Sasaki et al., 1998). Increased CA activity of Chlorella vulgaris under P deficiency facilitated the cellular mechanism of dissolved inorganic carbon (DIC) concentration and enhanced the CO2 influx to the site of Rubisco (Kozlowska-Szerenos et al., 2000). Chlorophyll a fluorescence (ChlF) may assess the integrity and efficiency of the photosynthetic apparatus and the overall health of the plant tissue (Roháček and Barták, 1999). Changes in ChlF emissions, arising mainly from PSII, provide information on almost all aspects of photosynthetic activity. Therefore, ChlF had also widely been used to probe photosynthetic function in higher plants and exhibit plant tolerance to environmental stresses (Gray et al., 2006; Guo et al., 2005; Panda et al., 2008).

The stable isotope technique is an important tool to identify the source of an element. The ratios of stable carbon isotopes δ13C have been successfully used to study photosynthesis (Motomura et al., 2008; Schwender et al., 2004; Tcherkez et al., 2009). The ratios of stable carbon isotopes δ13C in plants change when the carbon metabolic pathways and the sources of inorganic carbon consumed for photosynthesis are changed. The labeling of the stable carbon isotopes in exogenous bicarbonate can trace whether plants obtain CO2 from the conversion of bicarbonate through the action of CA or not. Use of bicarbonate labeled with stable carbon isotopes and the determination of stable isotopes may yield the bicarbonate utilization proportion in Broussonetia papyrifera (L.) Vent. under treatment with high concentrations (10 mM) of bicarbonate (Wu and Xing, 2012). Therefore, δ13C were measured to trace the metabolic route of the inorganic carbon source during photosynthesis (Motomura et al., 2008).

The Japanese morning glory (Ipomoea nil (L.) Roth. or Pharbitis nil (L.) Choisy) was first introduced to Japan from China over 1000 years ago as a medicinal herb (Kajita and Nishino, 2009). P. nil is a short-day plant requiring a single long dark period for floral induction. It is used as a model plant for photoperiodic flower induction studies (Nishino, 1976; Reese and Erwin, 1997). Dried buds of several species of the genus Lonicera (Caprifoliaceae) are commonly used in traditional Chinese medicine for latent-heat-clearing, antipyretic, detoxicant, and anti-inflammatory properties. Several reports have shown that Flos Lonicerae possess effective antioxidant properties (Ku et al., 2009; Xiang and Ning, 2008). Parthenocissus tricuspidata (Sieb. et Zucc.) Planch is a vertical virescent medicinal plant of the Vitaceae family that can climb to heights of 20 m or higher through attachment of its adhesive tendrils to supports (Kim et al., 2005; Wang et al., 2010). Several reports about these three climber plant species are available, Lonicera panpaninii is a pioneer species in the karst mountain areas where the vegetations were generally at P-limited stress (Du et al., 2011), P. nil and P. tricuspidata can also grow in the karst areas, however, distribution of the three climber plant species are different, so it is necessary to study their photosynthetic responses to P deficiency stress or their photosynthetic adaptation mechanisms in response to P deficiency stress.

Therefore, the aim of this study was to understand the photosynthetic inorganic carbon assimilation capacity of these three C3 plant species under P deficiency stress, and photosynthetic characteristics, ChlF parameters, CA activity under P deficiency stress were determined. Besides, the utilization of inorganic carbon sources was studied by comparing the differences between these three climber plants in terms of the foliar composition of the stable carbon isotope. The different adaptation mechanisms discovered could provide a general consideration for the planning and development of P deficiency-resistant plants.

Methods

Plant growth and P deficiency stress treatment

The experiment was conducted in a growth chamber at the Institute of Geochemistry, Chinese Academy of Sciences, Guizhou Province, China (26.35°N, 106.42°E). Seedlings of Pharbitis nil (Linn.) Choisy, Lonicera pampaninii Levil, and Parthenocissus tricuspidata (Sieb.et Zucc.) Planch were germinated and cultivated in 12-hole trays with quartz sand under a 12 h photoperiod (200 μmol m⁻² s⁻¹ PPFD), a day/night temperature cycle of 28°C /20°C, and 60% relative humidity. Plants were irrigated daily with 1/4-strength Hoagland solution (Hoagland and Arnon, 1950). After 75 d of growth, the nutrient solution was replaced by a modified Hoagland solution containing 6 mM KNO₃, 4 mM Ca(NO₃)₂, 2 mM MgSO₄, 2 mM Fe(Na)EDTA, 2 μM KCl, 50 μM
H$_3$BO$_3$, 4 μM MnSO$_4$, 4 μM ZnSO$_4$, 0.2 μM CuSO$_4$, and 0.2 μM (NH$_4$)$_6$Mo$_7$O$_24$ at pH 8.1 ± 0.5. The solution was supplemented with 10 mM NaHCO$_3$ which δ$^{13}$C was −17.23‰. Three plants from each treatment group were used for the measurement every 10 d after the onset of P deficiency. The fourth youngest fully expanded leaf from the top was selected for the reaction centers before measurement. The fourth youngest to the dark for 30 min to ensure complete relaxation of all photosynthesis measurement system. Leaves were adapted to the photosynthetic active radiation (PAR), temperature, and CO$_2$ concentration during the measurements were 300 μmol m$^{-2}$ s$^{-1}$, 30°C, and 400 μmol mol$^{-1}$, respectively.

Fluorescence measurements
Chlorophyll a was measured using a portable LI-6400XT photosynthesis measurement system. Leaves were adapted to the dark for 30 min in order to ensure complete relaxation of all reaction centers before measurement. The fourth youngest fully expanded leaf from the top was selected for the measurement every 10 d after the onset of P deficiency. 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 (Fm) was recorded after exposure to a 0.8 s saturating light pulse (6000 μmol m$^{-2}$ s$^{-1}$). Then plant leaves were light-induced by 1000 μmol m$^{-2}$ s$^{-1}$ radiation intensity light for 30 min. Actinic light (300 μmol m$^{-2}$ s$^{-1}$) was then applied for 1 min to drive photosynthesis. The maximum fluorescence in the light-saturated stage (F′m), basic fluorescence after induction (F′$_0$), and fluorescence yield in the steady state (F$_s$) were determined. The maximum quantum yield of PSII (Fv/Fm) was calculated as (Fm − F$_0$)/Fm, and the effective quantum yield of PSII (Φ$_{PSII}$) was calculated as ΔF/F′m = (F′m − F$_s$)/F′m.

Carbonic anhydrase activity
The fourth and fifth youngest fully expanded leaves from the top were chosen for CA activity measurement every 10 d after the onset of P deficiency. Three plants from each treatment group were used for the measurement. Leaf tissues (0.1 g to 0.2 g) were quickly frozen in liquid nitrogen and ground with 3 ml extraction buffer (0.01 M barbitone sodium with 0.05 M mercaptoethanol, pH 8.3). The homogenate was centrifuged at 10000 × g and 0°C for 5 min and then placed on ice for 20 min. The supernatant was used to determine 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°C to 2°C in a mixture containing 4.5 mL of 0.02 M barbitone buffer (5, 5-diethylbarbituric acid; pH 8.3), 0.4 mL of the sample, and 3 mL of CO$_2$-saturated H$_2$O. CA activity was expressed in Wilbur and Anderson (WA) units as WA = (t$_0$/t)−1, where t$_0$ and t are the time(s) measured for the pH change (8.2 to 7.2), with buffer alone (t$_0$) and with sample (t).

Stable carbon isotope ratios measurements
The stable carbon isotope ratio (δ$^{13}$C) was determined from the first youngest fully expanded leaf from the top using gas isotope ratio mass spectrometry (Mat-252, Finnigan MAT, Germany). Four expanded leaves at each stress treatment in each species of climber plant were randomly detached from the 24 seedlings every 10 d after the onset of P deficiency.

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

Results
Net CO$_2$ assimilation rate
Figure 1 shows the An of the three climber species; An varied with plant species, P deficiency stress, and stress duration. The An of P. nil was higher than those of the two other species. P. tricuspidata showed the lowest An, and no significant change of An with decreasing P concentration and increasing stress duration for P. tricuspidata was observed over the entire duration of P deficiency stress. On day 10, the An of P. nil under the 0.125 mM P treatment was higher than those in the other treatments, which showed no significant change. The An of L. pampalinii under the 0 mM P treatment was 35.62% that under the control; its An under the 0.031 mM P treatment was also lower than those under the control and 0.125 mM P treatments (Figure 1A). On day 20, the An of P. nil under the 0.031 mM P treatment was lower than those under other treatments. The An of L. pampalinii under the
control treatment was higher than those in other treatments, which showed no significant change (Figure 1B). On day 30, the An of *P. nil* under the 0.125 mM P treatment was higher than those under other treatments, which showed no significant change. The An of *L. pampaninii* under the 0 mM P treatment was 24.36% of the value under the control; its An under the 0.031 mM P treatment was also lower than those under the control and 0.125 mM P treatments (Figure 1C).

**Chlorophyll fluorescence**

Table 1 shows the maximal PSII photochemical efficiency (Fv/Fm) of the three species. The Fv/Fm values of *P. nil* and *P. tricuspidata* were not changed markedly with increasing P deficiency stress over the entire P deficiency stress duration, whereas the Fv/Fm of *L. pampaninii* showed a lower value under the 0 mM P treatment.

Table 2 shows the effective quantum yield of PSII (Φ<sub>PSII</sub>) of the two species. On days 10 and 20, the Φ<sub>PSII</sub> of *P. nil* and *P. tricuspidata* did not change markedly with increasing P deficiency stress; however, the Φ<sub>PSII</sub> of *L. pampaninii* showed a low value under the 0 mM P treatment. On day 30, the Φ<sub>PSII</sub> of *P. nil* showed a high value under the 0 mM P treatment, the Φ<sub>PSII</sub> of *P. tricuspidata* did not change markedly with increasing P deficiency stress, and the Φ<sub>PSII</sub> of *L. pampaninii* showed a low value under the 0 mM P treatment.

**Carbonic anhydrase activity**

CA activity varied with plant species, P deficiency stress level, and durations. CA activity was higher in *P. nil* and *L. pampaninii* than in *P. tricuspidata*, for which CA activity could hardly be determined and remained consistently low (Figure 2). On day 10, among the treatments, *L. pampaninii* showed the highest CA activity under 0.031 mM P treatment and the lowest value under the 0.125 mM P treatment, which was 49.25% of the value under the 0.031 mM P treatment. The CA activity of *P. nil* under 0.031 or 0 mM P treatment was higher than those under other treatments. Moreover, the CA activity of *P. nil* was always lower than that of *L. pampaninii* at all treatments (Figure 2A). On day 20, the CA activity of *P. nil* under the 0.031 mM P treatment was higher than

![Figure 1](http://www.as-botanicalstudies.com/content/55/1/60)

*Figure 1* Effects of P deficiency stress on the An of the three climber plant species (A. day 10, B. day 20, C. day 30). Mean ± SE (n = 5) followed by different letters in the same species and in the same treatment period indicate significant difference at P ≤ 0.05, according to one-way ANOVA and t-test.

| Stage  | Material      | P concentration (mM) | 0.250   | 0.125   | 0.031   | 0       |
|--------|---------------|----------------------|---------|---------|---------|---------|
| 10th day| *P. nil*      |                      | 0.77 ± 0.016ab| 0.80 ± 0.006a| 0.79 ± 0.007a| 0.80 ± 0.001a|
|        | *L. pampaninii*|                      | 0.79 ± 0.002a| 0.79 ± 0.010a| 0.77 ± 0.011a| 0.74 ± 0.027b|
|        | *P. tricuspidata*|               | 0.79 ± 0.005a| 0.78 ± 0.012a| 0.76 ± 0.006ab| 0.78 ± 0.005a|
| 20th day| *P. nil*      |                      | 0.80 ± 0.008a| 0.80 ± 0.002a| 0.77 ± 0.028ab| 0.80 ± 0.002a|
|        | *L. pampaninii*|                      | 0.78 ± 0.011a| 0.78 ± 0.001a| 0.77 ± 0.004ab| 0.73 ± 0.038b|
|        | *P. tricuspidata*|               | 0.78 ± 0.009a| 0.77 ± 0.014ab| 0.78 ± 0.002a| 0.77 ± 0.014ab|
| 30th day| *P. nil*      |                      | 0.75 ± 0.034a| 0.75 ± 0.013a| 0.75 ± 0.018a| 0.78 ± 0.009a|
|        | *L. pampaninii*|                      | 0.79 ± 0.005a| 0.79 ± 0.002a| 0.78 ± 0.005a| 0.74 ± 0.010b|
|        | *P. tricuspidata*|               | 0.76 ± 0.002a| 0.77 ± 0.009a| 0.78 ± 0.002a| 0.78 ± 0.002a|

Mean ± SE (n = 5) followed by different letters in the same species and in the same treatment period indicate significant difference at P ≤ 0.05, according to one-way ANOVA and t-test.
those under other treatments. The CA activity of *L. pampaninii* under 0.125 mM P treatment or control was higher than those under other treatments. The CA activity of *L. pampaninii* under the 0 mM P treatment showed the lowest value at only 25.34% of the value under the 0.125 mM P treatment (Figure 2B). On day 30, the CA activity of *P. nil* under the 0.125 and 0.031 mM P treatments was higher than those under the 0 mM P concentration or control treatments; the value under the 0.031 mM P treatment was the highest. *L. pampaninii* also showed the highest CA activity under the 0.031 mM P treatment; this value was the highest among all the species studied. The CA activity of *L. pampaninii* under the 0.125 mM P treatment was lower than those under other treatments (Figure 2C). In addition, the CA activity of *L. pampaninii* under the 0 mM P treatment on day 30 was higher than that on day 20.

**Carbon stable isotope ratios**

The $\delta^{13}$C value was significantly lower in *P. nil* than in the two other species. The $\delta^{13}$C values for *P. nil* were all lower than $-38\%$, whereas those of *L. pampaninii* and *P. tricuspidata* were around $-34\%$ and higher. On day 10, the $\delta^{13}$C values of *P. nil* and *L. pampaninii* under the 0.031 mM and 0 mM P treatments were a little higher than those under control or 0.125 mM P treatments. The $\delta^{13}$C value of *P. tricuspidata* under the 0 mM P treatment was more positive than those under the control, 0.125 mM, and 0.031 mM P treatments (Figure 3A). On day 20, the $\delta^{13}$C values of *P. nil* showed no significant change with increasing P deficiency stress, whereas those of *L. pampaninii* and *P. tricuspidata* showed more positive $\delta^{13}$C values under the 0.125 mM P treatment. In addition, the $\delta^{13}$C value of *P. tricuspidata* under the 0.031 mM P treatment was more negative than those of *P. tricuspidata* under the three treatments (Figure 3B). On day 30, the $\delta^{13}$C values of *P. nil* showed the highest $\delta^{13}$C value under the 0.125 mM P treatment.
P treatment, and its \( \delta^{13}C \) values under the 0.031 mM and 0 mM P treatments were slightly lower than those under the control or 0.125 mM P treatments (Figure 3C).

**Discussion**

*Pharbitis nil* (Linn.) Choisy

The photosynthetic rate of plants could be affected by a non-stomatal factor that primarily depends on the activity of intrinsic enzymes, photosynthetic apparatus, and their regulation mechanisms (Li et al., 2006). P deficiency could result in smaller in size of stomatal opening (Sarker et al., 2010), atmospheric CO\(_2\) became hard to entry into cell of plant, but through catalysis of CA in *P. nil*, which showed high activity, another carbon source could be supplied for photosynthesis of *P. nil* by the transformation from HCO\(_3^-\) to CO\(_2\) under P deficiency stress. Photosynthesis efficiency can be described by the maximal PSII photochemical efficiency (Fv/Fm) and actual photochemical quantum efficiency of open PSII (\( \Phi_{PSII} \)). The response of Fv/Fm and \( \Phi_{PSII} \) in *P. nil* to increased P deficiency indicated that *P. nil* was tolerant to 0 mM P concentration. *P. nil* maintained higher quantum efficiencies in the primary reaction center of the open PSII with long-term P deficiency stress durations, especially under 0 mM P. In other words, *P. nil* showed long-term tolerance to P deficiency stress. *P. nil* could maintain long-term high and stable photosynthetic inorganic carbon assimilation ability even under 0 mM P concentration.

Photosynthesis of plants is the most important process in carbon isotope fractionation in the nature, the more negative the \( \delta^{13}C \) value in carbon source of plants photosynthesis and the higher the photosynthetic rate were, the more negative the \( \delta^{13}C \) value in plants leaves was. In fact, after dissolved in the nutrient solution, \( \delta^{13}C \) value of atmospheric CO\(_2\) was about –11‰ (Clark and Fritz 1997), \( \delta^{13}C \) values of naturally grown C3 plants ranged from –22‰ to –34‰ (mean –27‰) (Chen et al., 2007), while \( \delta^{13}C \) values of *P. nil* plants grown in the modified Hoagland solution with 10 mM NaHCO\(_3\) which \( \delta^{13}C \) was –17.23‰, were very negative, the \( \delta^{13}C \) values of *P. nil* were all lower than –38‰. This observation suggest that inorganic carbon sources for photosynthesis do not come entirely from the atmospheric CO\(_2\), the carbon source for photosynthesis of *P. nil* also came from the CO\(_2\) supplied by the transformation from HCO\(_3^-\) through CA, and with higher photosynthetic inorganic carbon assimilation efficiency than the other two species, \( \delta^{13}C \) values of *P. nil* were more negative than those of the other two plants, *P. nil* absorbed and assimilated more CO\(_2\) translated from HCO\(_3^-\). But with increasing P deficiency stress and treatment duration, there was no significant change in \( \delta^{13}C \) values, indicated that long-term P deficiency stress had little effect on the way of inorganic carbon utilization.

*Lonicera pampaninii* Levl

Even though CA activity of *L. pampaninii* was also very high, however, when *L. pampaninii* was under P deficiency stress, especially under 0 mM P concentration, its photosynthetic inorganic carbon assimilation efficiency still decreased compared with those of the control. Therefore, *L. pampaninii* cannot adapt to P deficiency only by CA regulation. The response of Fv/Fm and \( \Phi_{PSII} \) in *L. pampaninii* to increased P deficiency indicated that *L. pampaninii* exhibited a poor tolerance under 0 mM P concentration as P deficiency stress increased. The quantum efficiencies in the primary reaction center of the open PSII decreased under 0 mM P concentration as P deficiency stress increased. The quantum efficiencies in the primary reaction center of the open PSII decreased under 0 mM P concentration as P deficiency stress increased. *L. pampaninii* showed no good adaptability to the short-term P deficiency stress, while *L. pampaninii* exhibited a long-term tolerance under 0.125 mM P concentration, its photosynthetic inorganic carbon assimilation ability was inhibited when it was under P deficiency stress especially under 0 mM P concentration.

With the regulation of high CA activity, *L. pampaninii* could assimilate CO\(_2\) which was transformed from HCO\(_3^-\), since the \( \delta^{13}C \) value of CO\(_2\) which was transformed from HCO\(_3^-\) was more negative, \( \delta^{13}C \) in *L. pampaninii* was around –34‰ which was more negative.
than the average (−27‰) of C3 plants. But less CO₂ which was transformed from HCO₃⁻ was assimilated by the photosynthesis process of L. pampaninii than P. nil, so the δ¹³C of L. pampaninii appeared more positive than that of P. nil. In addition, with increasing P deficiency stress and treatment duration, there was no significant change in δ¹³C values, indicated that the stomatal conductance of L. pampaninii remained constant or the way of inorganic carbon utilization in L. pampaninii did not change markedly under P deficiency stress.

**Parthenocissus tricuspidata** (Sieb.et Zucc.) Planch.

The CA activity of *P. tricuspidata* was too low to provide enough carbon through HCO₃⁻ to CO₂ transformation. *P. tricuspidata* had a low net CO₂ assimilation rate and could grow normally with little carbon and P, even under 0 mM P. The response of Fv/Fm and Φ₂₅₂₅ in *P. tricuspidata* to increased P deficiency indicated that *P. tricuspidata* exhibited a long-term tolerance even under 0 mM P concentration. *P. tricuspidata* maintained higher quantum efficiencies in the primary reaction center of the open PSII with longer P deficiency stress durations, especially under 0 mM P. In fact, *P. tricuspidata* grew slowly, its requirement of P was very low, the reaction center of the open PSII with longer P deficiency indicated that *P. tricuspidata* was tolerant to long-term P deficiency stress.

The photosynthetic inorganic carbonic ability of *P. tricuspidata* was low, so the carbon available for its photosynthesis was limited. The carbon assimilation ability was not significantly inhibited by P deficiency stress and treatment duration, there was no significant change in δ¹³C values, indicated that the stomatal conductance of *L. pampaninii* remained constant or the way of inorganic carbon utilization in *L. pampaninii* did not change markedly under P deficiency stress.

**Competing interests**
The authors declare that they have no competing interests.

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**Abbreviations**
ATP: Adenosine triphosphate; CA: Carbonic anhydrase; ChlF: Chlorophyll fluorescence; DIC: Dissolved inorganic carbon; DW: Dry weight; Φ₂₅₂₅: effective quantum yield of PSII; L. pampaninii: LonicerapampaniniiLev.; Fv/Fm: Maximum quantum yield of PSII; An: Net CO₂ assimilation rate; Planch: (P. tricuspidata); Parthenocissus tricuspidata (Sieb. Zucc.); Choisy (P. nil; Pharbitis nil (Linn.); P: Phosphorus; PAR: Photosynthetic active radiation; PSII: Photosystem II; RuBP: Ribulose-1,5-bisphosphate; δ¹³C: Stable carbon isotope ratio.
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