Research article

Effects of phosphorus application on photosynthetic carbon and nitrogen metabolism, water use efficiency and growth of dwarf bamboo (Fargesia rufa) subjected to water deficit

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A B S T R A C T

Dwarf bamboo (Fargesia rufa Yi), one of the staple foods for the endangered giant pandas, is highly susceptible to water deficit due to its shallow roots. In the face of climate change, maintenance and improvement in its productivity is very necessary for the management of the giant pandas’ habitats. However, the regulatory mechanisms underlying plant responses to water deficit are poorly known. To investigate the effects of P application on photosynthetic C and N metabolism, water use efficiency (WUE) and growth of dwarf bamboo under water deficit, a completely randomized design with two factors of two watering (well-watered and water-stressed) and two P regimes (with and without P fertilization) was arranged. P application hardly changed growth, net CO2 assimilation rate (Pn) and WUE in well-watered plants but significantly increased relative growth rate (RGR) and Pn in water-stressed plants. The effect of P application on RGR under water stress was mostly associated with physiological adjustments rather than with differences in biomass allocation. P application maintained the balance of C metabolism in well-watered plants, but altered the proportion of nitrogenous compounds in N metabolism. By contrast, P application remarkably increased sucrose-metabolizing enzymes activities with an obvious decrease in sucrose content in water-stressed plants, suggesting an accelerated sucrose metabolism. Activation of nitrogen-metabolizing enzymes in water-stressed plants was attenuated after P application, thus slowing nitrate reduction and ammonium assimilation. P application hardly enlarged the phenotypic plasticity of dwarf bamboo in response to water in the short term. Generally, these examined traits of dwarf bamboo displayed weak or negligible responses to water-P interaction. In conclusion, P application could accelerate Pn and sucrose metabolism and slow N metabolism in water-stressed dwarf bamboo, and as a result improved RGR and alleviated damage from soil water deficit.

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1. Introduction

Due to the potential effects of climate change on precipitation and temperature patterns, plants are often subjected to temporary periods of soil water deficit during their life cycle. Soil water deficit is the most common environmental stress factor limiting the growth, development, productivity and regeneration of plants worldwide (Liu et al., 2014). Within a certain threshold of soil water stress, most plants are able to express several stress-related genes, which lead to a series of remarkable changes in morphological and physio-biochemical traits to resist or adapt to adverse environments (Lawlor and Cornic, 2002; Sato et al., 2010). However, these changes may vary greatly depending on the plant species, stress...
duration and intensity, as well as soil conditions (Reddy et al., 2004).

Phosphorus is one of 17 essential nutrients required for plant growth, accounting for about 0.2% of a plant's dry weight. In general, more than 80% of the total amount of P in the soil is immobile and unavailable (e.g., Ca-P, Fe-P, Mg-P, Al-P and organic-P) (Suriyagoda et al., 2011). P can be readily absorbed by plants in the orthophosphate (P_i) forms (HPO_4^{2-} and H_2PO_4^-), which occur in soil solutions at very low concentrations (<10 μM). Most soils, even fertile types, are deficient in P, as its uptake via root absorption is faster than its replenishment in soil solutions (Suriyagoda et al., 2011). Recent studies have demonstrated that P deficiency inhibits processes related to energy metabolism and biochemical synthesis, such as photosynthesis, respiration (especially the glycolytic pathway) and N fixation; P deficiency also reduces the activities of key enzymes involved in C and N metabolism (Burman et al., 2004; dos Santos et al., 2004; Burman et al., 2009). Moreover, P deficiency causes aberrant root architecture in terms of number and density of lateral roots, and thus affects P_i transport, which serves as a long-distance signal (Lin et al., 2014). Consequently, P is considered the most important element limiting plant growth.

Increasing water deficit significantly decreases P and water availability in soils (Suriyagoda et al., 2014), which further aggravates the difficulty of their utilization. This situation inevitably induces metabolic disorders and eventually results in serious reduction in productivity (Singh et al., 2006a). P fertilization not only alleviates the scarcity of available P in the soil but also improves plant stress tolerance (Cortina et al., 2013). A number of studies have shown that P-fertilized plants under water stress conditions exhibit enhanced root growth potential, xylem hydraulic conductivity and water use efficiency (WUE) (Jones et al., 2005). Other studies have also indicated that P application can affect dry matter partitioning because plants invest relatively less C and N into roots and more into leaves, which can increase the rates of leaf relative growth and CO_2 assimilation per unit of leaf area, as well as carbohydrate contents (e.g., sucrose, soluble sugar and starch) (dos Santos et al., 2004; Burman et al., 2009). In addition, P fertilization can accelerate nitrate (NO_3^-) reduction and ammonium (NH_4^+) assimilation and produce more nitrogenous compounds in water-stressed plants by promoting NO_3^- absorption and activation of enzymes (e.g., nitrate reductase, NR; glutamine synthetase, GS and glutamate synthase, GOGAT) (Burman et al., 2004; Garg et al., 2004). Finally, the adaptive changes of C and N metabolism induced by P fertilization are beneficial for plant survival and growth in drought-prone environments (Burman et al., 2004).

Several studies have analyzed possible regulatory mechanisms behind the effect of P application on drought response in plants. However, no comprehensive understanding exists from the viewpoint of C and N metabolism. Meanwhile, these studies are concentrated in a number of herbaceous and woody species, but did not focus on bamboo species, a semi-woody plant that is widespread all around the world. Weih (2001) showed evidence that fast-growing plant species are more sensitive to water and nutrient stress than slow-growing ones. Dwarf bamboo (Fargesia rufa Yi), one of the staple foods of the endangered giant pandas, is a prominent understory under a mixed canopy of the evergreen Abies fargesii var. faxoniana and deciduous Betula utilis in subalpine zone, China. However, dwarf bamboo is highly susceptible to water stress due to its shallow roots (Liu et al., 2014). Given its fast-growing trait, dwarf bamboo makes soil P-deficient, which adversely affects subsequent seedling regeneration after mass flowering. Therefore, water and nutrient are the two determining factors for dwarf bamboo growth and yield. Maintenance and improvement in the productivity of dwarf bamboo will be an essential issue for the giant panda's survival and conservation in water deficit environments.

This study was designed to investigate how P application regulates photosynthetic C and N metabolism and WUE of dwarf bamboo under different water conditions to improve its growth, and whether the morphological and physio-biochemical traits are affected by the combined factors. To answer these questions, we investigated plant growth, water status, gas exchange and chlorophyll fluorescence parameters, levels of key compounds and enzymes activities involved in C and N metabolism. Also, we assessed phenotypic plasticity by using the above morphological and physio-biochemical traits.

2. Materials and methods

2.1. Plant material, growth conditions and experimental design

The experiment was carried out at Maoxian Mountain Ecosystem Research Station (103° 53'E, 31° 41'N, 1826 m), Chinese Academy of Sciences in southwestern China. On March 2013, the uniform and healthy plants (2 years old) of dwarf bamboo were obtained from the seedling nursery at Wanglang National Nature Reserve (103° 55' E, 32° 49' N, 2300 m) and then transplanted into 50 L plastic pots filled with 25 kg of homogenized topsoil from the experimental site. Each pot had one standard plant including 4–5 ramets. All plants were grown in a naturally lit greenhouse under a semi-controlled environment with a temperature range of 18–32 °C and relative humidity of 50%–85%, and irrigated regularly with water from a nearby stream. Prior to the treatments, total P (0.67 g kg^-1) and available P (5.1 mg kg^-1) in soils were first determined. About 4 months after the transplanting, the treatments, a completely randomized design with two factors of two water regimes and two P fertilization level, were applied for 45 days. Sixty standard plants were randomly allocated to all 4 combinations of water and P in the trial. The pots were weighed every other day and rewatered to 80%–85% (well-watered) and 30%–35% (water-stressed) relative soil water content by replacing the amount of transpired water. The two P fertilization treatments were without fertilization (no fertilizer supplied to plants) and with fertilization (6 g of calcium superphosphate containing 16% P_2O_5 supplied to each plant every 15 days). The amount of P application was determined by the highest concentration of available P loss from bamboo soil (Chen et al., 2011). To avoid systematic error caused by the possible differences in fluctuating environmental condition, all pots were rotated every five days during the experiment. In each treatment, three replicates, each including five plants, were used. Plant samples were collected at the end of the experiment.

2.2. Growth analysis

All plants were individually harvested and separated into leaves, stems and roots at the end of the experiment. The plant parts were washed, oven-dried at 70 °C for 72 h, and weighed. On the basis of these data, leaf mass ratios (LMR), stem mass ratios (SMR), root mass ratios (RMR) and aboveground mass ratios (AGMR) were obtained. The relative growth rate in terms of aboveground biomass (RGR) for each standard plant was calculated as (ln W_2−ln W_1)/(t_2−t_1), where W_2 is the final biomass, W_1 is the initial biomass, and t_2−t_1 is the time interval (days), respectively.

2.3. Leaf relative water content

Leaf relative water content (LRWC) was calculated according to the following formula: LRWC (%) = [(FW−DW)/(TW−DW)] × 100. Here, FW is the fresh weight, DW is the dry weight after drying at...
70 °C for 48 h, and TW is the turgid weight after soaking in deionized water for 12 h at room temperature.

2.4. Carbon isotope composition

The leaves, oven-dried at 70 °C for 48 h, were ground to a fine powder in a ball mill and then analyzed for δ13C by isotope ratio mass spectrometry (IRMS, GVI-Isoprim, Elementar, Hanau, Germany). The overall precision of the δ values was better than 0.1‰, as determined from repeated samples.

2.5. Gas exchange and chlorophyll fluorescence measurements

The net CO2 assimilation rate (Pn), stomatal conductance (Gs), and intercellular CO2 concentration (Ci) were measured on fully expanded leaves at similar development stages with a portable open-flow gas exchange system (LI-6400, LI-COR Inc., USA) during the late morning (9:00–11:00 h). For all cases, the air relative humidity, CO2 concentration and photon flux density were maintained 60%–70%, 380 μmol mol−1 and 800 μmol m−2 s−1, respectively. Intrinsic water use efficiency (WUEint) was calculated by dividing the instantaneous values of Pn by Gs. The maximum quantum efficiency of photosystem II (Fv/Fm) was measured on the same leaves as above with a portable pulse amplitude modulated fluorometer (PAM-2100, Walz, Effeltrich, Germany). The leaves were dark-adapted with clips for 40 min. The minimal fluorescence (F0) was measured under a weak pulse of modulating light over 0.8 s, and maximal fluorescence (Fm) was induced by a saturating pulse of light (8000 μmol m−2 s−1) applied over 0.8 s. Then, Fv/Fm was calculated, where Fv was the difference between Fm and F0.

2.6. Carbohydrates determination

Leaves (0.2 g DW) were extracted three times with 6 mL of 80% ethanol at 80 °C for 30 min. The resulting supernatant was analyzed for soluble sugar by the anthrone method and sucrose by the 3,5-dinitrosalicylic acid method (Zhang and Qu, 2003). The ethanol-insoluble residue was extracted for starch and measured by the anthrone–H2SO4 method (Liu et al., 2014). Non-structural carbohydrate (NSC) is defined here as the sum of soluble sugar and starch.

2.7. Analysis of N forms, soluble protein, and free amino acid

For NO3 and nitrite (NO2) concentrations, aqueous extract from 0.2 g of frozen leaves in 5 mL of deionized water was used. For NH4 concentration, 0.2 g of frozen leaves homogenized in 2 mL of 10% HCl was used. The resulting supernatants were analyzed using quantitative colorimetric method as described by Tang (1999). Reduced N content was determined using micro-Kjeldahl method after digestion in H2SO4/H2O2 (Liu et al., 2014). Total N content is assumed to represent the sum of NO3 and reduced N (Sánchez-Rodríguez et al., 2011). Free amino acids and proline were extracted with 2 mL of 10% acetic acid and 5 mL of 3% sulfosalicylic acid, respectively. The resulting supernatants were analyzed according to the method of Liu et al. (2014). Soluble proteins were determined using Bradford G-250 reagent.

2.8. Enzyme extractions and assays

Frozen leaves (0.2 g) were homogenized in pre-chilled water, extracted for 3 h in refrigerator. After centrifugation at 12,000 × g for 20 min, the supernatant was taken for determinations of invertase (INV, EC 3.2.1.26) and amylase (AMY, EC 3.2.1.1–2) by colorimetric method (Zhang and Qu, 2003).

Frozen leaves (0.2 g) were ground in pre-chilled extraction buffer (50 mM HEPES–NaOH buffer pH 7.5, 50 mM MgCl2, 2 mM EDTA, 0.2% BSA and 2% polyvinylpyrrolidone) and centrifuged. The resulting supernatant was used for determining sucrose synthase (SS, EC 2.4.1.13) and sucrose phosphate synthase (SPS, EC 2.4.1.14) activities as described by Liu et al. (2014). Frozen leaves (0.2 g) were homogenized in 2 mL of 25 mM phosphate buffer saline (PBS, pH 8.7) containing 10 mM cysteine and 1 mM EDTA and centrifuged. The resulting supernatant was taken for assays nitrate reductase (NR, EC 1.6.6.1) activity by the diazocoupling method using Griess reagent (Sánchez-Rodríguez et al., 2011).

Frozen leaves (0.2 g) were homogenized in 2 mL of 50 mM Tris-HCl buffer (pH 7.8), containing 1 mM of EDTA, 15% glycerol, 14 mM of 2-mercaptoethanol, and 0.1% Triton-X-100. The homogenate was centrifuged twice at 10,000 × g for 10 min at 4 °C. The resulting supernatant was used for measuring the activities of nitrite reductase (NR, EC 1.7.7.1), GS (EC 6.3.1.2), GOGAT (EC 1.4.7.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), glutamic-oxaloacetic transaminase (GOT, EC 2.6.1.1), and glutamic-pyruvic transaminase (GPT, EC 2.6.1.2) (Lillo, 1984). NR activity was defined by the disappearance of NO2 by using Griess reagent method at 520 nm (Lillo, 1984). GS activity was measured by estimating the formation of glutamylhydroxamate at 540 nm after reacting with acidified ferric chloride (Liu et al., 2014). GOGAT, GDH, GOT, and GPT activities were assayed by monitoring the oxidation of NADH at 340 nm as described by Tang (1999).

2.9. Statistical analysis

The data were analyzed with ANOVA, and comparisons between treatment means were carried out, using the F test at 5% probability. Two-way ANOVA was used to evaluate the effects of water availability, P application, and their interaction on all dependent variables. The phenotypic plasticity index (PI), ranging from 0 to 1, was calculated for each variable as the difference between the maximum and the minimum mean value divided by the maximum mean value (per trait per treatment combination). Pearson’s correlation analysis was used to examine the relationships between specific variables. All statistical analyses were performed using statistical software package SAS 9.1 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Plant growth

RGR was lower in water-stressed plants than in well-watered plants. P application significantly increased RGR by 17.1% in water-stressed plants. No significant differences were noted for LMR, RMR and AGMR regardless of water availability or P application (Table 1).

3.2. Leaf relative water content, photosynthetic and fluorescence parameters and water use efficiency

Irrespective of P application, LRWC, Pn, Gs, Ci, Fv/Fm were always lower in water-stressed plants than in well-watered plants, but water use efficiency (WUEint and δ13C) presented an opposite trend (Table 2). P application did not change the above parameters of well-watered plants, whereas P application caused significant increases in Pn (49.7%), Gs (87.6%) and Fv/Fm (4.9%) in water-stressed plants (Table 2).
3.3. Carbohydrates and related enzymes

Lower starch content, as well as higher soluble sugar content, NSC content and soluble sugar/starch ratios were obtained in water-stressed plants compared with their counterparts, regardless of P application (Table 3). Sucrose content significantly increased in water-stressed treatment for non-fertilized plants, whereas in fertilized plants, sucrose content decreased with water stress (Table 3). P application had no effects on the parameters related to carbohydrates of well-watered plants (Table 3). P application also did not alter the contents of soluble sugar and NSC as well as soluble sugar/starch ratios of water-stressed plants, but increased starch content to a certain extent (31.1%) and significantly decreased sucrose content (62.5%) (Table 3). Regardless of P application, water stress significantly increased AMY activity (Fig. 1A). P application did not alter AMY activity in well-watered plants, but decreased its activity by 26.8% in water-stressed plants (Fig. 1A). Water stress did not affect the activities of INV, SS and SPS for non-fertilized plants, whereas in fertilized plants, their activities were enhanced by water stress (Fig. 1B–D). In well-watered plants, no changes in the activities of INV, SS and SPS were observed after P supply, whereas P application caused significant increases in INV (76.9%), SS (47.4%) and SPS (33.3%) in water-stressed plants (Fig. 1B–D).

3.4. Nitrate reduction and ammonium production

Water-stressed plants generally showed higher NO₃⁻ and NH₄⁺ concentrations as well as NR and NiR activities compared with well-watered plants under two P-treated conditions, although NO₃⁻ concentration and NH₄⁺/NO₃⁻ ratios were not affected by water stress (Table 4). P application hardly altered the above parameters of well-watered plants except NiR activity, whereas in water-stressed plants, P application caused significant reductions in NO₃⁻ (21.6%), NR (22.5%), NO₂⁻ (15.1%) and NH₂⁺ (13.3%), and increase in NH₄⁺/NO₃⁻ ratios (9.6%) (Table 4).

3.5. Incorporation of ammonium and assimilation products

Irrespective of P supply, the activities of GS, GOGAT and GDH were higher in water-stressed than in well-watered plants (Fig. 2). P application significantly decreased the activities of GS and GOGAT, except GOGAT activity in well-watered plants, but did not change GDH activity regardless of water availability (Fig. 2).

The GPT activity was higher in non-fertilized plants, whereas GOT activity was higher in fertilized plants under water-stressed treatment (Fig. 3). P application strongly increased and decreased GPT activity in well-watered and water-stressed plants respectively; in terms of GOT activity, only significant decrease was observed with P application in water-stressed plants (Fig. 3).

To a certain extent, water stress enhanced the levels of nitrogenuous compounds under two P-treated conditions, but this did not apply to levels of reduced N, total N and soluble proteins (Table 5). However, in water-stressed plants, significant decrease in the levels of reduced N (8.0%), total N (10.5%), amino acids (10.0%) and proline (31.9%) with unchanged soluble proteins contents was observed (Table 5).

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Table 1
Growth status of dwarf bamboo for non-fertilized (−P) and fertilized (+P) treatments with and without water stress. Asterisk represents significant difference between P levels within the same water supply (F test, P < 0.05). Values are means ± S.E.

| Traits       | Well-watered | Water-stressed |
|--------------|--------------|----------------|
|              | −P           | +P             | −P            | +P            |
| RGR (mg g⁻¹ d⁻¹) | 54.6 ± 1.5 a  | 56.2 ± 1.3 a   | 40.4 ± 1.5 b  | 47.3 ± 1.8 b* |
| LMR (g g⁻¹)   | 0.279 ± 0.003 a | 0.285 ± 0.018 a | 0.272 ± 0.032 a | 0.287 ± 0.009 a |
| RMR (g g⁻¹)   | 0.377 ± 0.016 a | 0.353 ± 0.020 a | 0.384 ± 0.023 a | 0.353 ± 0.014 a |
| AGMR (g g⁻¹)  | 0.023 ± 0.016 a | 0.064 ± 0.020 a | 0.016 ± 0.023 a | 0.064 ± 0.014 a |

Table 2
Leaf relative water content, photosynthetic and chlorophyll fluorescence parameters, water use efficiency in dwarf bamboo for non-fertilized (−P) and fertilized (+P) treatments with and without water stress. Statistics as in Table 1.

| Traits       | Well-watered | Water-stressed |
|--------------|--------------|----------------|
|              | −P           | +P             | −P            | +P            |
| LRWC (%)     | 92.9 ± 1.2 a  | 91.0 ± 0.8 a   | 72.3 ± 1.6 b  | 74.4 ± 1.7 b  |
| PN (µmol m⁻² s⁻¹) | 4.77 ± 0.16 a | 4.94 ± 0.05 a  | 0.86 ± 0.09 b | 1.24 ± 0.10 b*|
| GC (mmol m⁻² s⁻¹) | 136 ± 1.1 a | 140 ± 8 a      | 1.2 ± 1 b    | 21 ± 1 b*     |
| C (µmol mol⁻¹) | 244 ± 14 a   | 264 ± 7 a      | 1.64 ± 9 b   | 179 ± 21 b    |
| Fv/Fm         | 0.79 ± 0.01 a | 0.79 ± 0.02 a  | 0.69 ± 0.01 b| 0.73 ± 0.01 b*|
| WUEmax (µmol mol⁻¹) | 38 ± 4 b  | 36 ± 2 b       | 74 ± 8 a    | 60 ± 7 a      |
| δ¹³C (‰)     | −32.37 ± 0.33 b | −31.45 ± 0.06 b | −28.35 ± 0.81 a | −29.55 ± 0.30 a |

Table 3
Changes in the contents of carbohydrates and the ratio of soluble sugar/starch of dwarf bamboo for non-fertilized (−P) and fertilized (+P) treatments with and without water stress. Statistics as in Table 1.

| Traits         | Well-watered | Water-stressed |
|----------------|--------------|----------------|
|                | −P           | +P             | −P            | +P            |
| Starch (mg g⁻¹ DW) | 28.72 ± 2.66 a | 27.63 ± 1.86 a | 13.16 ± 1.85 b | 17.25 ± 5.70 a |
| Sucrose (mg g⁻¹ DW) | 17.30 ± 1.96 b | 19.68 ± 1.32 a | 22.70 ± 0.33 a | 8.51 ± 0.49 b* |
| Soluble sugar (mg g⁻¹ DW) | 54.34 ± 1.04 b | 52.02 ± 1.54 b | 77.00 ± 0.50 a | 71.87 ± 2.28 a |
| NSC (mg g⁻¹ DW)   | 83.07 ± 3.23 a | 79.65 ± 0.91 b | 90.21 ± 2.32 a | 89.12 ± 3.50 a |
| Soluble sugar/starch | 1.92 ± 0.16 b  | 1.89 ± 0.08 b  | 6.06 ± 0.72 a  | 5.59 ± 2.24 a  |
3.6. Proportion of explained variance and phenotypic plasticity

Among all of the variables analyzed, few (including LMR, RMR and AGMR) were not significantly affected by water availability and P application (Table 6). All variables (except sucrose) displayed weak or negligible responses to the interaction of water and P treatments, which explains less than 10% of the total data variation (Table 6).

### Table 4

| Traits          | Well-watered | Water-stressed |
|-----------------|--------------|----------------|
|                 | _P_          | _+P_           | _P_           | _+P_           |
| _NO_3 (mg g⁻¹ DW) | 5.137 ± 0.403 b | 4.426 ± 0.110 b | 6.288 ± 0.057 a | 4.932 ± 0.135 a* |
| NR (µmol h⁻¹ mg⁻¹ protein) | 0.029 ± 0.001 b | 0.026 ± 0.002 b | 0.050 ± 0.002 a | 0.038 ± 0.002 a* |
| _NO_2 (mg g⁻¹ DW) | 0.044 ± 0.006 a | 0.041 ± 0.004 a | 0.053 ± 0.006 a | 0.045 ± 0.001 a* |
| NR (µmol h⁻¹ mg⁻¹ protein) | 0.294 ± 0.002 a | 0.161 ± 0.014 b* | 0.359 ± 0.035 a | 0.344 ± 0.012 a |
| _NH_4 (mg g⁻¹ DW) | 1.563 ± 0.025 b | 1.514 ± 0.044 b | 1.763 ± 0.025 a | 1.529 ± 0.029 a* |
| _NH_4/NO_3 | 0.307 ± 0.019 a | 0.343 ± 0.018 a | 0.280 ± 0.002 a | 0.310 ± 0.006 a* |

Fig. 1. Changes in the activities of amylase (AMY, A), invertase (INV, B), sucrose synthase (SS, C) and sucrose phosphate synthase (SPS, D) of dwarf bamboo for non-fertilized (_P_) and fertilized (_+P_) treatments with and without water stress. Means followed by same letter do not differ significantly between water treatments within the same P level. Asterisk represents significant difference between P levels within the same water supply (F test, _P_ < 0.05). Vertical bars show ± S.E.

Fig. 2. Changes in the activities of glutamine synthetase (GS, A), glutamate synthase (GOGAT, B) and glutamate dehydrogenase (GDH, C) of dwarf bamboo for non-fertilized (_P_) and fertilized (_+P_) treatments with and without water stress. Statistics as in Fig. 1.

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3.6. Proportion of explained variance and phenotypic plasticity

Among all of the variables analyzed, few (including LMR, RMR and AGMR) were not significantly affected by water availability and P application (Table 6). All variables (except sucrose) displayed weak or negligible responses to the interaction of water and P treatments, which explains less than 10% of the total data variation (Table 6).
The average PI relative to the P factor (PI\textsubscript{Phosphorus}) for all variables was 30.0% lower than that relative to the water factor (PI\textsubscript{water}) (PI\textsubscript{Phosphorus} = 0.28; PI\textsubscript{water} = 0.40). Average PI for all trait groups (growth, photosynthesis and fluorescence, C, N and water) relative to the P factor was much lower than that relative to the water factor (PI\textsubscript{Phosphorus}: 0.20–0.35; PI\textsubscript{water}: 0.21–0.60) (Table 6). Moreover, some variables (photosynthesis and fluorescence, C, N and water) responded strongly to water availability and minimally to P application (e.g. PI\textsubscript{Phosphorus} = 0.38; PI\textsubscript{water} = 0.85). By contrast, growth showed similar responses to respective treatments. However, plants generally revealed greater responses to water availability than to P application (Table 6, Fig. 4).

4. Discussion

4.1. Growth and water status

Accumulation, allocation and transportation of dry matter in different plant organs are strongly influenced by water and nutrient availability in soils (Sun et al., 2013). Under moist conditions, fertilization significantly affects plant growth in terms of biomass increase in roots, stems, and leaves (Graciano et al., 2005; Jones et al., 2005). Differently, P application had no effects on RGR, LMR, RMR and AGMR of dwarf bamboo under well-watered treatment (Table 1). As soil moisture content decreases, the effect of fertilization on plant growth gradually attenuates (Saneoka et al., 1990) and even disappears under severe water-deficit conditions (30% field capacity) (Yin et al., 2012). Similarly, our results showed that P application had no effects on LMR, RMR and AGMR of dwarf bamboo under water-stressed treatment (Table 1). However, its RGR under water-stressed treatment was significantly affected by P application (Table 1), similar to the results reported by Dias et al. (2007), which is mostly associated with physiological adjustments (i.e. \( P_{\text{r}} \), \( P < 0.001 \)) rather than with morphological adjustments (i.e. RMR and LMR, \( P > 0.05 \)). Unfortunately, the results do not support the hypothesis on the correlation of high RGR to differences in biomass allocation (Poorter and Nagel, 2000).

Water relations of plants are essential in the maintenance and/or regulation of C and N metabolism of environmentally stressed plants. Being the external morphology of plants, leaves are most sensitive to water stress. Therefore, LRWC are believed to be effective and reliable indicators of hydration capacity and tolerance of plants (Basu et al., 2007). In our study, the curling of dwarf bamboo leaves was observed when water stress decreased the LRWC. Several studies have shown that P fertilization can directly enhance LRWC, as well as free and bound water components in water-stressed plants (Singh et al., 2006a; Sato et al., 2010). However, P application had minimal effect on LRWC of dwarf bamboo, regardless of water availability (Table 2), as also shown in previous studies of other crop species (Garg et al., 2004; Singh et al., 2006b; Burman et al., 2009). Moreover, the LRWC of dwarf bamboo was influenced by water-P interaction (Table 6), which is due to interactive effects on leaf area (Burman et al., 2004; Garg et al., 2004).

4.2. Photosynthetic carbon metabolism and water use efficiency

The rate of CO\textsubscript{2} assimilation gradually declines with decreasing LRWC (Lawlor and Cornic, 2002). However, a considerable debate exists on whether water stress mainly inhibits photosynthesis through stomatal closure or through metabolic impairment (Reddy et al., 2004). Lawlor (2002) reviewed published studies and found the following: i) \( P_{\text{n}} \) decreases mainly as a consequence of \( C_{\text{i}} \) and \( G_{\text{s}} \) synchronous reduction in plants subjected to mild or rapid water stress; ii) metabolic constraint is the major determinant of \( P_{\text{n}} \) inhibition under severe or slow water stress, wherein asynchronous changes in \( C_{\text{i}} \) and \( G_{\text{s}} \), as well as other vulnerable physiological processes, are observed. In this study, \( P_{\text{n}} \) limitation of dwarf bamboo after water stress was affected not only by stomatal limitation but also by impaired photosynthetic apparatus, especially photosystem II, as manifested by corresponding changes in gas exchange and chlorophyll fluorescence parameters (Table 2). Kleiner et al. (1992) reported that high nutrient availability does not improve the physiological performance of two oak species (Quercus rubra and Q. prinus). Nevertheless, P application improved \( G_{\text{s}} \) and \( F_{\text{v}}/F_{\text{m}} \) of water-stressed dwarf bamboo, thus alleviating the negative impact of water stress on \( P_{\text{n}} \) (Table 2), which has been

### Table 5

| Traits                  | P-watered         | P-stressed       | Water-stressed |
|-------------------------|-------------------|------------------|---------------|
|                         | ±P                | ±P               | ±P            |
| Reduced N (mg g\textsuperscript{-1} DW) | 25.51 ± 0.16 a | 23.95 ± 0.32 a* | 26.95 ± 0.53 a | 24.81 ± 0.56 a* |
| Total N (mg g\textsuperscript{-1} DW)   | 30.64 ± 0.34 b | 28.39 ± 0.27 b* | 33.24 ± 0.51 a | 29.74 ± 0.54 b* |
| Soluble proteins (mg g\textsuperscript{-1} DW) | 53.06 ± 0.31 a | 45.76 ± 1.54 a* | 48.01 ± 2.07 a | 49.27 ± 1.56 a |
| Amino acids (mg g\textsuperscript{-1} DW)  | 2.72 ± 0.08 b | 2.63 ± 0.06 b | 3.00 ± 0.10 a | 2.70 ± 0.04 a* |
| Proline (mg g\textsuperscript{-1} DW)     | 0.41 ± 0.13 b | 0.40 ± 0.10 b | 1.88 ± 0.17 a | 1.28 ± 0.10 a* |
Table 6  
Proportion of explained variance by water and phosphorus effects and by their interactions (two-way ANOVA), and the index of phenotypic plasticity of morphological and physio-biochemical traits of dwarf bamboo. Significant levels: *** P < 0.001; ** P < 0.01; * P < 0.05; ns non-significant (P > 0.05). The underlined bold values represent the mean plasticity index (PI) for each trait classification.

| Traits                | Effects | $R^2$ Water | $R^2$ Phosphorus | Plasticity index (PI) Water | Plasticity index (PI) Phosphorus |
|-----------------------|---------|-------------|------------------|-----------------------------|--------------------------------|
| **Growth**            | RGR     | 54.9***     | 7.3*             | 2.9**                       | 89.1                           | 0.30                           | 0.18                           |
|                       | LMR     | <0.1**      | 0.3**            | <0.1**                      | 4.3                            | 0.27                           | 0.27                           |
|                       | RMR     | <0.1**      | 2.1**            | <0.1**                      | 22.0                           | 0.17                           | 0.20                           |
|                       | AGRMR   | <0.1**      | 2.2**            | <0.1**                      | 22.0                           | 0.11                           | 0.13                           |
| **Photosynthesis and fluorescence** | $P_n$    | 1472.6***   | 7.9*             | 1.1*                        | 98.9                           | 0.85                           | 0.38                           |
|                       | $G_i$   | 91.9***     | 0.3**            | <0.1**                      | 85.2                           | 0.92                           | 0.59                           |
|                       | $C_i$   | 31.6**      | 1.1**            | 0.1**                       | 67.2                           | 0.52                           | 0.36                           |
|                       | $R_e/F_m$ | 520.6***   | 21.3**           | 25.0**                      | 98.6                           | 0.12                           | 0.04                           |
| **Carbon**            | Starch  | 14.5**      | 0.2**            | 0.6**                       | 65.6                           | 0.73                           | 0.51                           |
|                       | Sucrose | 10.4**      | 43.6**           | 85.8**                      | 94.6                           | 0.48                           | 0.47                           |
|                       | Soluble sugars | 201.3***   | 6.3**            | 0.9**                       | 96.3                           | 0.34                           | 0.12                           |
|                       | NSC     | 9.6*        | 0.7**            | 0.2**                       | 56.6                           | 0.17                           | 0.13                           |
|                       | Sugar/starch | 11.0**     | <0.1**          | <0.1**                      | 58.2                           | 0.80                           | 0.48                           |
|                       | AMY     | 50.5***     | 5.5**            | 4.5**                       | 88.3                           | 0.58                           | 0.37                           |
|                       | INV     | <0.1**      | 9.1**            | 8.1**                       | 68.3                           | 0.43                           | 0.49                           |
|                       | SS      | 41.2***     | 29.7**           | 9.1**                       | 90.9                           | 0.37                           | 0.32                           |
|                       | SPS     | 2.5**       | 14.0**           | 7.1**                       | 74.6                           | 0.23                           | 0.26                           |
| **Nitrogen**          | NO$_3^-$ | 14.0**      | 21.7**           | 2.1**                       | 82.5                           | 0.24                           | 0.26                           |
|                       | NR      | 43.2***     | 8.9**            | 3.0**                       | 87.6                           | 0.51                           | 0.35                           |
|                       | NO$_2^-$ | 1.9**       | 1.3**            | 0.1**                       | 29.3                           | 0.36                           | 0.36                           |
|                       | NR      | 38.6***     | 13.8**           | 8.6**                       | 88.4                           | 0.46                           | 0.42                           |
|                       | NH$_3$  | 11.4**      | 19.6**           | 8.4**                       | 83.1                           | 0.12                           | 0.14                           |
|                       | NH$_3$/NO$_3^-$ | 4.7**     | 5.7**         | <0.1**                      | 56.7                           | 0.20                           | 0.21                           |
|                       | GS      | 53.5***     | 21.9**           | 1.8**                       | 90.6                           | 0.33                           | 0.26                           |
|                       | GOGAT   | 110.6***    | 42.2**           | 15.1**                      | 95.5                           | 0.60                           | 0.47                           |
|                       | GDH     | 97.4***     | 27.8**           | 5.4**                       | 92.9                           | 0.73                           | 0.37                           |
|                       | GPT     | 65.5***     | 10.1**           | 59.7**                      | 94.4                           | 0.52                           | 0.57                           |
|                       | GOT     | 15.4**      | 0.4**            | 0.3**                       | 66.8                           | 0.28                           | 0.19                           |
|                       | Reduced N | 7.3**       | 18.9**           | 0.5**                       | 76.9                           | 0.10                           | 0.12                           |
|                       | Total N | 21.0**      | 44.7**           | 2.1**                       | 89.4                           | 0.10                           | 0.13                           |
|                       | Amino acids | 6.0*        | 6.8**            | 2.0**                       | 64.8                           | 0.13                           | 0.14                           |
|                       | Proteins | 0.2*        | 4.0**            | 7.9**                       | 60.3                           | 0.17                           | 0.17                           |
|                       | Proline | 85.3***     | 5.6**            | 5.3**                       | 92.3                           | 0.85                           | 0.59                           |
| **Water**             | LRWC    | 562.2***    | <0.1**          | 6.4**                       | 98.6                           | 0.23                           | 0.06                           |
|                       | WUE$_{intr}$ | 27.3***   | 1.8**            | 0.9**                       | 65.5                           | 0.68                           | 0.55                           |
|                       | $\delta^{13}$C | 40.8***  | <0.1**          | 5.2**                       | 85.2                           | 0.16                           | 0.08                           |

Fig. 4. Responses to phosphorus and water availability, calculated by the plasticity index of morphological and physio-biochemical variables of dwarf bamboo. Growth (open diamonds), photosynthesis and fluorescence (open circles), carbon (open triangles), nitrogen (closed diamonds) and water (closed circles).

confirmed in several plants (dos Santos et al., 2004; Garg et al., 2004; Burman et al., 2009; Sato et al., 2010). Meanwhile, some other reasons may have contributed to the improvement of $P_n$ after P application, including increased ATP and NADPH productions, activated enzyme activities in Calvin cycle, enhanced regeneration of sugar phosphate, higher output rate of photosynthates and cell proliferation (Rao and Terry, 1995).

Furthermore, WUE is considered as an important component of adaptation to water stress. WUE$_{intr}$, the ratio of $P_n$ to $G_s$ to water, is often used as an index for plant water use (Gilbert et al., 2011). $\delta^{13}$C is a proxy for the integrated response of $P_n$ and $G_s$, and can be used to infer average plant WUE$_{intr}$ over the time when the plant organic matter is formed (Battipaglia et al., 2013). In this study, P application did not affect WUE$_{intr}$ and $\delta^{13}$C of dwarf bamboo under both water-treated conditions (Table 2). Also, a significant positive correlation between WUE$_{intr}$ and $\delta^{13}$C ($r = 0.81$, $P < 0.01$) and negative correlations between WUE$_{intr}$ and $P_n$ ($r = -0.76$, $P < 0.01$) as well as $\delta^{13}$C and $P_n$ ($r = -0.87$, $P < 0.01$) were observed. These results indicate that water is possibly preferred for non-photosynthetic tissues (e.g. vascular and supporting) in this case. Furthermore, no significant effects of water-P interaction on WUE$_{intr}$ and $\delta^{13}$C were detected (Table 6), as has also been shown in other plants of previous reports (Jones et al., 2005).

Water deficit can put plants on the path to carbon starvation as a threat to their survival (McDowell, 2011). However, P fertilization can regulate carbohydrate contents adaptively by altering
correlative enzyme activities in carbohydrate metabolism under water deficit conditions (Rao and Terry, 1995). After P application, similar results existed in water-stressed dwarf bamboo although carbohydrates and related enzymes activities in well-watered plants displayed no changes (Table 3, Fig. 1). Starch, a carbon reserve, is accumulated during the day and consumed at night, providing reduced carbon for plants (Pokhilo et al., 2014). In our study, P application, to a certain extent, increased starch content of water-stressed dwarf bamboo (Table 3), possibly due to higher $P_n$ and/or an obvious decline in AMY activity. Similar findings have been described previously in Vigna aconitifolia (Garg et al., 2004) and Cyamopsis tetragonoloba (Burman et al., 2009). Therefore, P application could tightly regulate starch synthesis and degradation under water stress to avoid periods of starvation. Sucrose metabolism not only provides energy and carbon skeletons for growth and development of plant but also plays an important role in adjusting their response to abiotic stresses through offering hexoses as essential metabolites and signaling molecules (Ruan et al., 2012). Our results showed that P application significantly increased the activities of INV, SS and SPS in water-stressed dwarf bamboo (Fig. 1), resulting in the reduction of sucrose content, suggesting that sucrose metabolism is accelerated (Table 3). However, P application unchanged soluble sugar with a decline soluble sugar/starch ratio and unchanged NSC in water-stressed dwarf bamboo (Table 3), indicating that more soluble sugars could be transported to non-photosynthetic tissues to regulate NSC balance in the entire plant. These situations might not only provide more carbon and energy for water-stressed dwarf bamboo to avoid carbon starvation and maintain RGR (Table 1), but also alleviate the damage of water stress to plants through facilitating biosynthesis of heat shock proteins (Hsps) and non-enzymic antioxidants (e.g. glutathione and ascorbic acid) (Liu et al., 2013). In parallel, sucrose metabolites (e.g. glucose and fructose), also could exert signaling roles in regulating the response of dwarf bamboo to water stress (Liu et al., 2013).

4.3. Nitrogen metabolism

Nitrogen is not only an important nutrient that plants require in great quantities but also an essential building block of chlorophyll, amino acids, proteins and nucleic acids. Plants readily take up and directly utilize two forms of soil N ($NO_3^-$ and $NH_4^+$) for growth. Generally, water stress can reduce available N uptake and NR activity, resulting in a decrease in $NH_4^+$ production (Lawlor, 2002). Conversely, $NO_3^-$ reduction in dwarf bamboo was stimulated by water stress in this study (Table 4), as is similar to the result in Zarina, a stress-tolerant tomato cultivar and translates as higher concentrations of N as well as amino acids and proteins (Sánchez-Rodríguez et al., 2011). In addition, similar results have been also observed in Fargesia demudata Yi (Liu et al., 2014). Some studies have indicated that P application adaptively regulates the level of N metabolism by changing its correlative enzymes activities under water stress (Burman et al., 2004, 2009; Garg et al., 2004). Our studies found that P application exhibited minimal effects on $NO_3^-$ reduction and $NH_4^+$ assimilation in well-watered dwarf bamboo (Table 4, Fig. 2). This observation could be associated with the growth period of dwarf bamboo, because available P in the soil can now sustain the functional requirement of the seedlings. By contrast, P application decreased $NO_3^-$ concentration and NR activity as well as the activities of GS/GOGAT involved in $NH_4^+$ assimilation in water-stressed dwarf bamboo, thus considerably reducing the amount of $NH_4^+$ (Table 4), suggesting that $NO_3^-$ reduction and $NH_4^+$ assimilation are slowed. $NH_4^+$, a major N source, is not only essential for living cells but also a ubiquitous intermediate in plant metabolism, however, it is notorious for its toxic effects in most higher plants while presenting in excess (Li et al., 2010). Hence, decreasing the amount of $NH_4^+$ after P application in water-stressed dwarf bamboo might reduce or avoid toxic effects caused by the excessive accumulation of $NH_4^+$.

On the other hand, the result of incorporation of $NH_4^+$ can be quantitatively analyzed by reduced N that is mainly formed by proteins and amino acids (Sánchez-Rodríguez et al., 2011). Thereinto, glutamate can be converted into other forms, particularly $a$-ketoglutaric acid, for consuming excessive $NH_4^+$ through deamination reactions catalyzed by relevant enzymes, such as GPT and GOT (Liu et al., 2014). In this study, P application increased and reduced GPT activity in well-watered and water-stressed dwarf bamboo, respectively (Fig. 3), indicating that GPT is relatively more sensitive to P application than GOT in both water treatments. The metabolism of nitrogenous compounds is essential to living processes, and during environmental stresses a number of nitrogenous metabolites are critically regulated for stress responses at the biochemical level. Water deficit normally presents differential changes in nitrogenous compounds (Liu et al., 2014). In our study, P application obviously decreased levels of nitrogenous compounds in water-stressed dwarf bamboo (Table 5), mainly because of low rates of $NO_3^-$ reduction, $NH_4^+$ assimilation and transamination reaction which is contrary to other studies (Garg et al., 2004; Burman et al., 2009). Thus, decreased levels of nitrogenous compounds induced by P application in water-stressed dwarf bamboo might provide carbon skeletons ($a$-ketoglutaric acid) and energy to improve its RGR (Table 1).

4.4. Phenotypic plasticity

Phenotypic plasticity that has been defined as a change in the phenotype expressed by a single genotype in different environments is important to identify plant functional traits in which plasticity may play a determinant role in plant response to global change (Gratani, 2014). In our study, the ability of dwarf bamboo to change its morphological and physio-biochemical traits in response to variations in water and P application may be translated into a high phenotypic plasticity, particularly in response to water, but the responses of these traits to water-P interaction were weak or negligible (Table 6, Fig. 4). Although some studies have indicated that P fertilization positively affects the morphological and physio-biochemical traits of water-stressed plants (dos Santos et al., 2006; Burman et al., 2009); however, P application did not substantially enlarge the phenotypic plasticity of dwarf bamboo in response to water in the short term (Table 6). Therefore, the extent to which phenotypic plasticity may facilitate survival under changing environmental conditions still remains largely unknown because the findings are sometimes controversial (Gratani, 2014). Given that short-term studies on plant phenotypic plasticity are unable to provide its comprehensive information due to the complexity of its theme; in the near future, it will be important to increase long-term studies on natural populations in order to understand their plastic responses to rapid climate change and then predict as well as manage the effects of climate change on native species and crop plants.

5. Conclusions

Phosphorus application only altered the proportion of nitrogenous compounds in N metabolism and almost had no effects on other morphological and physio-biochemical traits in well-watered dwarf bamboo. However, P application accelerated $P_n$ and sucrose metabolism and slowed N metabolism in water-stressed dwarf bamboo, resultantly improving RGR and reducing damage from soil water deficit. Although the morphological and physio-biochemical...
traits of dwarf bamboo were independently affected by water availability and P application, these traits generally displayed weak or negligible responses to water-P interaction. Moreover, P application hardly enlarged the phenotypic plasticity of dwarf bamboo in response to water in the short term. Future studies should therefore focus more on the impact of long-term P application on plant phenotypic plasticity in response to water.

Authors’ contributions

C.L. and Y.W. designed the practical part of the study, carried out the physiologic studies, analyzed the data, and drafted the manuscript. K.P. and Y.J. helped to revise the manuscript. W.L. and L.Z. contributed reagents/materials/analysis tools. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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