Trees (2015) 29:1633–1647
DOI 10.1007/s00468-015-1241-0

ORIGINAL PAPER

Photosynthetic carbon and nitrogen metabolism and the relationship between their metabolites and lipid peroxidation in dwarf bamboo (*Fargesia rufa* Yi) during drought and subsequent recovery

Chenggang Liu[1] · Yanjie Wang[1,2] · Kaiwen Pan[1] · Yanqiang Jin[3] · Jin Liang[1] · Wei Li[1] · Lin Zhang[1]

Received: 3 October 2014 / Revised: 14 May 2015 / Accepted: 3 June 2015 / Published online: 30 June 2015

Abstract

Key message **Differential regulations of C and N metabolism in dwarf bamboo improve the capacity of osmotic adjustment, and also their metabolites may play an important role for protection against membrane lipid peroxidation under drought, thus accelerating recovery after rewatering.**

Abstract Dwarf bamboo (*Fargesia rufa* Yi), is a staple food for the endangered giant panda, but little is known about the impact of drought on bamboo species and its recovery mechanism. This study investigated the response of carbon (C) and nitrogen (N) metabolism to drought and subsequent recovery, and the relationship of their metabolites with lipid peroxidation. Photochemistry was reversibly down-regulated after drought, but a longer recovery time is needed. The accelerated degradation of starch due to a rapid increase in amylase activity resulted in higher soluble sugar only in severe drought-stressed plants after 30 days of drought. Sucrose content was not affected by drought because of the relative increases in activities of invertase, sucrose synthase, and sucrose phosphate synthase. As nitrate concentration increased in parallel with nitrate reductase activity, ammonium (NH_4^+) production was enhanced by drought. Also, activated glutamine synthetase/glutamate synthase cycle stimulated NH_4^+ assimilation, while hydrolysis of soluble proteins was accelerated, resulting in accumulation of amino acids. After rewatering, re-balancing of C metabolism has gradually begun, but a stronger N metabolism was still observed. The notable positive correlations between MDA and the contents of starch and proline after 15 days of drought as well as between MDA and the contents of soluble sugar, NSC and proline after 30 days of drought were displayed. We conclude that dwarf bamboo may not only differently regulate its C and N metabolism to improve the capacity of osmotic adjustment but also employ its different metabolites protect against membrane

Communicated by M. Adams.

✉ Yanjie Wang
wyjilwm2015@163.com

✉ Kaiwen Pan
pankw@cib.ac.cn

Chenggang Liu
chenggangliu@hotmail.com

Yanqiang Jin
jinyq@live.com

Jin Liang
Liangjin@cib.ac.cn

Wei Li
liwei08@cib.ac.cn

Lin Zhang
zhanglin@cib.ac.cn

[1] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization and Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, No. 9, Section 4, Renmin South Road, Chengdu 610041, Sichuan, China

[2] College of Life Science, Sichuan Normal University, Chengdu 610101, China

[3] Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China

lipid peroxidation under different intensities and duration of drought, thus accelerating its recovery after rewatering.

Keywords Carbohydrates · Amino acids · Proline · Reactive oxygen species · Osmotic adjustment · Dwarf bamboo

Abbreviations

AMY	Amylase
C	Carbon
GDH	Glutamate dehydrogenase
GOGAT	Glutamate synthase
GOT	Glutamic-oxaloacetic transaminase
GPT	Glutamic-pyruvic transaminase
GS	Glutamine synthetase
INV	Invertase
LCP	Light compensation point
LSP	Light saturation point
N	Nitrogen
NH_4^+	Ammonium
NiR	Nitrite reductase
NO_3^-	Nitrate
NR	Nitrate reductase
NSC	Non-structural carbohydrate
P_{max}	Maximum net photosynthetic rate
ROS	Reactive oxygen species
SPS	Sucrose phosphate synthase
SS	Sucrose synthase
Φ	Apparent quantum yield

Introduction

Drought is the most common adverse environmental stress, and will become more severe and more frequent in the coming decades (IPCC 2012). Usually, drought can directly cause osmotic stress, resulting in dehydration and even death of plant cells (Basu et al. 2007). To cope with osmotic stress effectively, plants have evolved a great capacity to synthesize and accumulate nontoxic and compatible solutes that are termed as osmoprotectants (Patakas et al. 2002). These compatible solutes may stabilize proteins and cellular structures and/or maintain cell turgor by osmotic adjustment to improve drought adaptation of plants (Upadhyaya et al. 2013). Most of compatible solutes, such as carbohydrates and amino acids, have been proven to be closely associated with carbon (C) and nitrogen (N) metabolism (Basu et al. 2007; Sánchez-Rodríguez et al. 2011). Consequently, timely changes in C and N metabolism will be an important determinant in the ability to sustain osmotic adjustment for plants during a drought period.

Drought often inhibits photosynthesis and growth, which is associated with alterations in C and N metabolism (Raven et al. 2004). Many studies have showed that C and N metabolism are very sensitive to drought (Sánchez-Rodríguez et al. 2011; Liu et al. 2014). Under drought conditions, a decline in water availability for transport-associated processes results in changes in the concentrations of compatible solutes. For example, drought can cause an increase in the synthesis of amino acids, especially proline (Gupta et al. 2014). In contrast, drought leads to a decrease in the contents of soluble sugar and leaf N (Sinclair et al. 2000; Llorens et al. 2003; Gauthier et al. 2014). Meanwhile, the key enzyme activities involved in C and N metabolism also can be altered by drought. Drought can reduce or increase sucrose phosphate synthase (SPS) activity (Praxedes et al. 2006; Basu et al. 2007). Moreover, a decline in the activities of nitrate reductase (NR) and glutamine synthetase (GS) was observed under drought (Xu and Zhou 2006). Although recent studies refer to a more detailed characterization of C and N metabolism (Aranjuelo et al. 2011; Kang et al. 2011), changes in key enzyme activities and metabolites involved in C and N metabolism induced by drought are still being debated due to plant species (Sánchez-Rodríguez et al. 2011), stress intensity, and duration (Xu and Zhou 2006, 2007). Many metabolites are believed simply to accumulate under drought conditions (Muller et al. 2011), and metabolite patterns remain unclear. Therefore, a better understanding on these metabolic effects of drought would require an integrated investigation of metabolites and enzymatic activities.

On the other hand, drought also increases production of reactive oxygen species (ROS), such as superoxide anion, hydroxyl, and hydrogen peroxide (Upadhyaya et al. 2013). Unfortunately, overproduction of ROS is known to cause membrane lipid peroxidation and seriously damage plants (Upadhyaya et al. 2008). Meanwhile, besides enzymatic and non-enzymatic antioxidative defense systems, many osmoprotectants involved in C and N metabolism are important in scavenging or detoxifying ROS and reducing lipid peroxidation (Couée et al. 2006; Dhont et al. 2011; Upadhyaya et al. 2013). Recently, sugars have been proposed as emerging antioxidants in plants and might scavenge hydroxyl and superoxide radicals (Couée et al. 2006). Moreover, proline can act as a non-enzymatic antioxidant in plant cells under abiotic stress, scavenging ROS and preserving the intracellular glutathione pool (Reddy et al. 2004). Therefore, sugars and proline play a role in protection of membranes from lipid peroxidation. However, relatively little is known about the relationship between

these key metabolites in C and N metabolism and lipid peroxidation during drought.

Rewatering might alleviate the adverse effects of drought on plants, which has been documented mainly in herbaceous and woody plants (Cai et al. 2005; Xu and Zhou 2007; Upadhyaya et al. 2008). There are many indications that the velocity of recovery after relief from drought and the turning point between fast and slow recovery depend on plant species as well as stress intensity and duration (Flexas et al. 2006; Gallé and Feller 2007; Robredo et al. 2011). However, very little research is done in physiological and biochemical recovery of semi-woody plants after drought. Dwarf bamboo (*Fargesia rufa* Yi), a kind of rhizomatous, semi-woody and perennial evergreen plant, is one of the staple food for the endangered giant panda and is an abundant understory species growing in evergreen-deciduous broadleaf forest and coniferous-broadleaf mixed forest in subalpine zone, China (Li et al. 2013). Maintaining a high productivity of dwarf bamboo plays an essential role in giant panda's survival and conservation. However, dwarf bamboo is highly susceptible to drought due to its shallower roots. Hence, drought seriously affects its productivity (Liu et al. 2014). To date, much less attention has been paid to the response of dwarf bamboo to unfavorable drought conditions and the recovery after drought relief.

Therefore, the aims of our research were to (1) investigate the recovery capacities of dwarf bamboo after drought; (2) study how the metabolites and enzymes activities in C and N metabolism of dwarf bamboo were changed with different intensities and duration of drought, and (3) examine the relationship between these metabolites and lipid peroxidation during drought. To achieve these aims, we assessed plant water status, photosynthetic parameters, and key metabolites contents and enzymes activities involved in C and N metabolism. This study intends to provide a wider basis for understanding the response of semi-woody plant to environmental stress.

Materials and methods

Plant material and experimental design

The uniform and healthy dwarf bamboo plants were obtained from the nursery at Wanglang National Nature Reserve on March 2011, and then transplanted into 50 L plastic pots filled with 25 kg of homogenized topsoil from the field on the experimental site. Dwarf bamboo is a typical clonal species, so a standard plant consists of one clump (4–5 ramets) per pot. All plants were grown in a naturally lit growth room at Maoxian Mountain Ecosystem Research Station (103°53′E, 31°41′N, 1826 m) in southwestern China, under semi-controlled conditions with relative humidity 40–80 %, at 9–32 °C, and kept there for 16 months. During the period, bamboo shoots produced and developed into 1-year-old plants.

Prior to the beginning of the experiment, all plants were watered every 3 days with nearby stream water. Relative soil water content (RSWC) was determined as RSWC = 100 × [(SFW − SDW)/(STW − SDW)], where SFW is the soil fresh weight, SDW is the soil dry weight oven-dried for 72 h at 105 °C, and STW is the soil turgid weight after soaking in water for 24 h at room temperature. The water treatment was initiated in July 1, 2012. Plants were divided into three groups, one group was kept well-watered (control, 80 % RSWC) during the whole experiment, and the other two groups were subjected to moderate drought (MD, 50 % RSWC), and severe drought (SD, 30 % RSWC) for 30 days by withholding water using the weight method (Xiao et al. 2009; Liu et al. 2014). During the experiment, the pots were weighed every other day and then rewatered to 80, 50, and 30 % RSWC by replacing the amount of transpired water. Evaporation from the soil surface was prevented by enclosing the soil with plastic bags which were tied at the base of the stem of each seedling. In addition, this was carried out by using a watering system that allowed all pots to reach the designated soil water content at the same time. Afterwards, drought-stressed plants were rewatered for 15 days. The fully expanded source leaves of 1-year-old plants at similar developmental stages were sampled on days 0, 15, 30, and 45 of three treatments, rapidly frozen in liquid N_2, and stored at −80 °C until analysis. Each treatment had three replications with six standard plants per replication, totaling 18 standard plants.

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 80 °C for 24 h, and TW is the turgid weight after soaking in deionized water for 12 h at room temperature.

Light response curves

We randomly selected six plants from each treatment for the measurement of the photosynthetic light response. Light response curves were generated using a portable photosynthesis system (LI-6400, LI-COR Inc., USA). Leaves were placed in the LI-6400 chamber, which was adjusted to provide 380 μmol CO_2 mol^{-1}, 22 °C block temperature, and a relative humidity of 60 % in order to minimize the heterogeneity of the stomata. After 10 min of acclimation to

these conditions, LI-6400 was adjusted to vary the active photosynthetic photon flux intensity (PPFD) in the following order: 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, and 0 μmol photons m^{-2} s^{-1}. For each measurement, leaf dark respiration rate (R_d, μmol CO_2 - m^{-2} s^{-1}), light compensation point (LCP, μmol CO_2 - m^{-2} s^{-1}), and apparent quantum yield (Φ, mol CO_2 mol^{-1} photons, the slope) were obtained by linear regression using data obtained at PPFD of 0–200 μmol CO_2 m^{-2} s^{-1} (Hikosaka et al. 2004). These curves were fitted using the non-rectangular hyperbola model (Hikosaka et al. 2004).

$$P_n = \frac{\Phi I + P_{\max} - \sqrt{(\Phi I + P_{\max})^2 - 4\theta\Phi I P_{\max}}}{2\theta} - R_d,$$

where P_n is observed leaf net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), I is the incident PPFD (μmol photons m^{-2} s^{-1}), $P_{\max}$ is the maximum photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), and θ is the curvature, and Φ and R_d are defined above. Radiation at 90 % of the $P_{\max}$ (LSP, μmol CO_2 m^{-2} s^{-1}) was estimated utilizing the equation described (Prado and de Moraes 1997).

Biochemical analysis

Photosynthetic pigments (Chl *a*, Chl *b*, and Car) were extracted in dark from 0.2 g of frozen leaves using 5 mL 100 % acetone for 36 h at room temperature, measured at 662, 645, and 470 nm and their contents were calculated according to Liu et al. (2014).

Dry leaves (0.1 g) were extracted three times with 18 mL of 80 % ethanol at 80 °C for 30 min, and centrifuged at 3000*g* for 10 min. The resulting supernatant was used for determination of soluble sugar using anthrone method and sucrose using 3,5-dinitrosalicylic acid method (Zhang and Qu 2003). The ethanol-insoluble residue was used for starch determination using anthrone-H_2SO_4 method (Liu et al. 2014). Non-structural carbohydrate (NSC) was assumed to represent the sum of soluble sugar and starch.

NO_3^- was analyzed from an aqueous extraction of 0.2 g frozen leaves in 5 mL of deionized water for 10 min in a boiling water bath and measured at 410 nm (Li 2000). NH_4^+ was assayed by homogenization of 0.2 g frozen leaves in 2 mL 10 % HCl and determined by colorimetric method (Tang 1999). Reduced N was determined by micro-Kjeldahl method after digestion in H_2SO_4–H_2O_2 (Li 2000). Total N was defined here as the sum of NO_3^- and reduced N (Sánchez-Rodríguez et al. 2011).

Amino acids and proline were analyzed by homogenization of 0.2 g frozen leaves in 2 mL of 10 % acetic acid and 5 mL of 3 % sulfosalicylic acid, respectively, as previously described (Sánchez-Rodríguez et al. 2011). Soluble proteins were measured by homogenization of 0.2 g frozen leaves in 2 mL of 50 mM PBS (pH 7.8) containing 0.2 mM EDTA and 2 % (w/v) polyvinylpyrrolidone, and centrifuged at 12,000*g* for 20 min. The supernatant was determined with Bradford G-250 reagent, using bovine serum albumin (BSA) as a calibration standard (Bradford 1976).

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content. MDA was extracted with 50 mM phosphate-buffered saline (PBS, pH 7.8) and measured at 532 and 600 nm. Its content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Bian and Jiang 2009).

Enzyme extractions and assays

Invertase (INV, EC 3.2.1.26) and amylase (AMY, EC 3.2.1.1–2) catalyze the hydrolysis of sucrose and starch, respectively. Frozen leaves (0.2 g) were homogenized in pre-chilled water and extracted for 3 h in refrigerator. After centrifugation at 12,000*g* for 20 min, the supernatant was taken for determinations of INV and AMY by colorimetric method (Yang et al. 2004; Zhang and Qu 2003).

Sucrose synthase (SS, EC 2.4.1.13) reversibly catalyzes sucrose synthesis and hydrolysis, and sucrose phosphate synthase (SPS, EC 2.4.1.14) catalyzes a pivotal step of sucrose synthesis. The activities of SS and SPS were assayed as described by Zhang and Qu (2003). Frozen leaves (0.2 g) were ground in 2 mL chilled extraction buffer (50 mM HEPES–NaOH buffer (pH 7.5), 50 mM $MgCl_2$, 2 mM EDTA, 0.2 % BSA and 2 % polyvinylpyrrolidone). The homogenate was centrifuged at 12,000*g* for 10 min at 4 °C. The reaction mixture of SS, consisting of 50 mM HEPES–NaOH (pH 7.5), 50 mM $MgCl_2$, 100 mM uridine diphosphoglucose, 100 mM fructose, and enzyme extract, was pre-incubated for 30 min at 30 °C. The reaction was terminated by adding 2 M NaOH and boiled for 10 min. Afterward, it was mixed with 30 % HCl and 0.1 % *m*-dihydroxybenzene and retained in water bath at 80 °C for 10 min. Once the mixture cooled, its absorbance was measured at 480 nm. SPS activity was measured in the same way as SS activity. Fructose-6-phosphate was substituted for fructose.

Nitrate reductase (NR, EC 1.6.6.1) catalyzes the reduction of nitrate to nitrite. NR activity was measured following the method of Sánchez-Rodríguez et al. (2011). 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. The homogenate was centrifuged at 12,000*g* for 15 min at 4 °C. The reaction mixture containing 100 mM KNO_3, 2 % NADH and enzyme extract, was pre-incubated for 30 min at 25 °C. A control without NADH was run simultaneously. The

mixture was stopped by the addition of 1 % sulfanilamide (prepared in 3 M HCl) and 0.02 % *N*-(1-naphthyl) ethylene diamine dihydrochloride, and measured at 540 nm after color reflection for 15 min.

Frozen leaves (0.2 g) were homogenized in 2 mL of 50 mM Tris–HCl buffer (pH 7.8), consisting of 1 mM EDTA, 15 % glycerol, 14 mM 2-mercaptoethanol, and 0.1 % Triton-X-100. The homogenate was centrifuged twice at 10,000*g* for 10 min at 4 °C. The supernatant was used to measure the following six enzymes activities (Lillo 1984).

Nitrite reductase (NiR, EC 1.7.7.1) catalyzes the reduction of nitrite to ammonium. NiR activity was assayed by monitoring the reduction of NO_2^- using Griess reagent method (Lillo 1984). The reaction mixture contained 100 mM PBS (pH 6.5), 100 mM NaCl, 100 mM $NaNO_2$, 100 mM methyl viologen, 100 mM $Na_2S_2O_4$, and enzyme extract. After incubation for 30 min at 25 °C, NO_2^- content was colorimetrically measured at 520 nm.

Glutamine synthetase (GS, EC 6.3.1.2) catalyzes glutamine synthesis from glutamate and ammonia. GS activity was determined by estimating the formation of glutamylhydroxamate at 540 nm after complexing with acidified ferric chloride (Kaiser and Lewis 1984). The reaction mixture contained 100 mM Tris–HCl buffer (pH 7.4) (consisting of 80 mM hydroxylamine, 20 mM glutamate, 80 mM $MgSO_4$, and 2 mM EDTA-2Na), 10 mM ATP and enzyme extract. Two controls, one without hydroxylamine and the other glutamate were prepared.

Glutamate synthase (GOGAT, EC 1.4.7.1) catalyzes the transfer of the amide group from glutamine to α-oxoglutarate, and its activity was assayed as described by Tang (1999). The reaction mixture, consisting of 20 mM α-oxoglutarate, 10 mM KCl, 2 mM NADH, 25 mM Tris–HCl buffer (pH 7.8), and enzyme extract, was pre-incubated for 15 min at 30 °C. The reaction was started by adding 20 mM glutamine and determined by monitoring the oxidation of NADH at 340 nm. Two controls, one without glutamine and the other without α-oxoglutarate, were run simultaneously.

Glutamate dehydrogenase (GDH, EC 1.4.1.2) catalyzes the reversible amination of α-oxoglutarate to glutamate. GDH activity was measured by monitoring the oxidation of NADH at 340 nm as described by Tang (1999). The reaction mixture, containing 20 mM α-oxoglutarate, 1 M NH_4Cl and 25 mM Tris–HCl buffer (pH 7.8), was pre-incubated for 15 min at 30 °C. Subsequently, the reaction was started by adding 2 mM NADH and enzyme extract. Two controls, one without α-oxoglutarate and the other without NH_4Cl were prepared.

Glutamic-oxaloacetic transaminase (GOT, EC 2.6.1.1) catalyzes the transfer of the amino group of aspartate to α-oxoglutarate. GOT activity was defined by monitoring the decrease of NADH at 340 nm as performed by Tang (1999). The reaction mixture contained 1 mM NADH, 100 mM aspartate, 50 units malate dehydrogenase, 333 mM Tris–HCl buffer (pH 7.8), and enzyme extract. The reaction was started by the addition of 20 mM α-oxoglutarate. Two controls were run simultaneously, one without α-oxoglutarate and the other without aspartate.

Glutamic-pyruvic transaminase (GPT, EC 2.6.1.2) catalyzes the transfer of the amino group of alanine to α-oxoglutarate. GPT activity was determined in the same way as GOT activity. L-Alanine was substituted for aspartate and lactate dehydrogenase for malate dehydrogenase (Tang 1999).

Statistical analysis

All statistical analysis was performed using SAS 9.1 for windows (SAS Institute, Cary, NC). Differences in all dependent variables were tested using ANOVA at 0.05 level followed by Duncan's multiple range test. Pearson's correlation analysis was used to examine the relationship between key metabolites involved in C and N metabolism and MDA at different stages of treatment.

Results

Plant water status

LRWC decreased progressively with drought time, dropping by 7.2 % in MD plants and by 14.3 % in SD plants after 30 days of drought (Fig. 1). After 15 days of rewatering, LRWC returned back to the control level (Fig. 1).

Photosynthetic light response curve parameters

Photosynthetic light response curve parameters of dwarf bamboo during drought and subsequent rewatering were summarized in Table 1. After 30 days of drought, although Φ, P_{max} and LSP were significantly decreased, LCP was obviously increased (Table 1). After 15 days of rewatering, Φ, P_{max}, and LSP in drought-stressed plants did not restore to their control levels except LCP, whereas each of these parameters under MD had no significant difference from that under SD (Table 1).

Photosynthetic pigments

Chl *a* and Chl *b* significantly decreased and Chl *a*/Chl *b* increased after 30 days of drought, whereas Car significantly declined after 15 days of drought (Table 2). After 15 days of rewatering, pigments in MD plants returned to their control levels, and they in SD plants were still lower

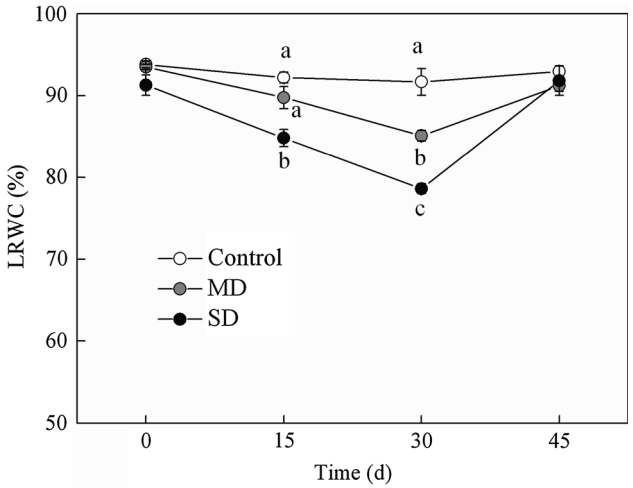

Fig. 1 Change in leaf relative water content (LRWC) of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open circles*), moderate drought (MD, *gray circles*), and severe drought (SD, *closed circles*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

than those of control (Table 2). There were no significant differences in the ratio of Chl *a*/Chl *b* among treatments after rewatering.

Carbohydrates and related enzymes

The contents of starch, sucrose, soluble sugar and NSC generally increased with drought time (Fig. 2). Starch content in MD and SD plants reached a peak after 15 days of drought, which was respectively 46.7 and 137.8 % higher than that of control (Fig. 2a). There were no significant differences in sucrose or soluble sugar content among treatments during the drought period, except that soluble sugar in SD plants showed a 37.2 % increase after 30 days of drought with respect to control (Fig. 2b, c). NSC content in SD plants increased by 23.9 % after 15 of days drought and by 42.4 % after 30 of days, respectively, compared with control, whereas no significant difference was observed between the control and MD (Fig. 2d). After 15 days of rewatering, the contents of different carbohydrates returned back to their control levels (Fig. 2).

AMY activity decreased slightly and then increased sharply with drought time (Fig. 3a). Its activity in MD and SD plants increased by 48.1 and 60.8 % after 30 days of drought, respectively, compared with control. The activities of INV, SS and SPS in all treatments generally increased (Fig. 3b, c, d). No significant differences in INV activity were observed among treatments during the drought period (Fig. 3b). SS activity increased by 19.2 % in MD plants and 31.7 % in SD plants after 15 days of drought, respectively, compared with control; whereas an obvious increase only existed in SD plants after 30 days of drought (Fig. 3c). SPS activity in MD and SD plants was higher by 23.8 and 64.2 % than that of control after 30 days of drought, respectively (Fig. 3d). After 15 days of rewatering, the activities of these four enzymes were still greater than their control levels except INV (Fig. 3).

Reduction of NO_3^- and NH_4^+ production

The concentration of NO_3^- generally increased with drought time (Table 3). Its concentration in MD and SD plants increased by 28.6 and 32.0 % after 30 days of drought, respectively, compared with control. NR activity displayed a similar pattern to the change of NO_3^- (Table 3). NiR activity, NH_4^+ concentration and the ratio of NH_4^+/NO_3^- firstly increased and then decreased with drought time (Table 3). There were almost no differences in NiR activity or the ratio of NH_4^+/NO_3^- among treatments, whereas NH_4^+ concentration was always higher than that of control after drought. After 15 days of rewatering, NO_3^- concentration and NiR activity returned back to their control levels, but NR activity, NH_4^+ concentration and the ratio of NH_4^+/NO_3^- in drought-stressed plants were still larger than those of control (Table 3).

Table 1 The photosynthetic light response curve parameters of dwarf bamboo after drought (30 days) and subsequent rewatering (15 days)

Time	Treatment	P_{max} (μmol CO_2 m^{-2} s^{-1})	Φ (mol CO_2 mol^{-1} photons)	LCP (μmol CO_2 m^{-2} s^{-1})	LSP (μmol CO_2 m^{-2} s^{-1})
Drought (30 days)	Control	9.07 ± 0.28a	0.029 ± 0.001a	19.6 ± 4.6c	772.2 ± 46.3a
	MD	6.47 ± 0.56b	0.022 ± 0.001b	31.8 ± 3.0b	558.1 ± 36.7b
	SD	4.78 ± 0.10c	0.015 ± 0.001c	68.6 ± 9.5a	312.8 ± 13.9c
Recovery (15 days)	Control	8.57 ± 0.22a	0.024 ± 0.002a	19.4 ± 6.4a	786.8 ± 41.4a
	MD	6.71 ± 0.11b	0.020 ± 0.001b	21.9 ± 0.5a	639.4 ± 4.6b
	SD	6.31 ± 0.11b	0.020 ± 0.001b	27.2 ± 6.0a	510.4 ± 56.3b

Well-watered (control), moderate drought (MD), and severe drought (SD). Values are mean ± SE ($n = 6$). Means followed by different letters are significantly different at $P < 0.05$ using Duncan's test

Table 2 Changes in photosynthetic pigments of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days)

Parameters	Treatment	Time (days)			
		0	15	30	45
Chl *a* (mg g^{-1} FW)	Control	1.527 ± 0.018a	1.531 ± 0.025a	1.550 ± 0.018a	1.548 ± 0.011a
	MD	1.523 ± 0.027a	1.504 ± 0.020a	1.464 ± 0.002b	1.500 ± 0.012a
	SD	1.518 ± 0.026a	1.469 ± 0.017a	1.378 ± 0.014c	1.419 ± 0.036b
Chl *b* (mg g^{-1} FW)	Control	0.808 ± 0.015a	0.831 ± 0.009a	0.865 ± 0.033a	0.867 ± 0.015a
	MD	0.798 ± 0.008a	0.740 ± 0.044a	0.702 ± 0.027b	0.785 ± 0.037ab
	SD	0.791 ± 0.025a	0.708 ± 0.044a	0.666 ± 0.009b	0.722 ± 0.020b
Car (mg g^{-1} FW)	Control	0.633 ± 0.003a	0.642 ± 0.005a	0.641 ± 0.006a	0.649 ± 0.011a
	MD	0.632 ± 0.003a	0.592 ± 0.011b	0.571 ± 0.002b	0.625 ± 0.011ab
	SD	0.627 ± 0.004a	0.572 ± 0.011b	0.554 ± 0.002c	0.612 ± 0.009b
Chl *a*/Chl *b*	Control	1.889 ± 0.020a	1.843 ± 0.042a	1.797 ± 0.060b	1.787 ± 0.043a
	MD	1.910 ± 0.053a	2.050 ± 0.147a	2.091 ± 0.083a	1.919 ± 0.104a
	SD	1.920 ± 0.027a	2.094 ± 0.144a	2.068 ± 0.009a	1.966 ± 0.041a

Well-watered (Control), moderate drought (MD), and severe drought (SD). Values are mean ± SE ($n = 3$). Means followed by different letters are significantly different between water treatments within a measurement period at $P < 0.05$ using Duncan's test

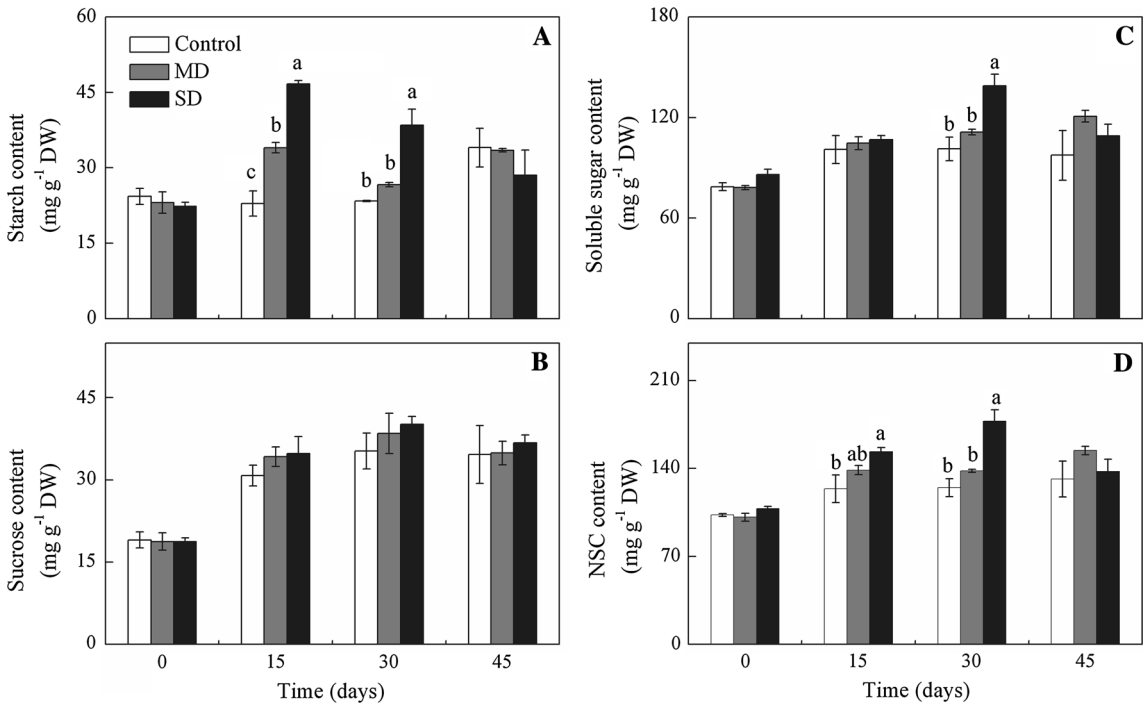

Fig. 2 Changes in the contents of starch (**a**), sucrose (**b**), soluble sugar (**c**) and NSC (**d**) of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open bars*), moderate drought (MD, *gray bars*) and severe drought (SD, *closed bars*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

Incorporation of NH_4^+ and assimilation products

The enzymes activities of the GS/GOGAT cycle first declined and then increased with drought time (Fig. 4a, b). Especially after 15 days of drought, GS/GOGAT activities in both MD and SD plants were lower by 23.6 and 43.7 % (Fig. 4a) as well as 18.6 and 32.4 % (Fig. 4b), respectively, compared with control. GDH activity increased gradually with drought time, whereas no significant difference was observed among treatments (Fig. 4c). After 15 days of rewatering, GS/GOGAT activities in drought-stressed plants were still higher than

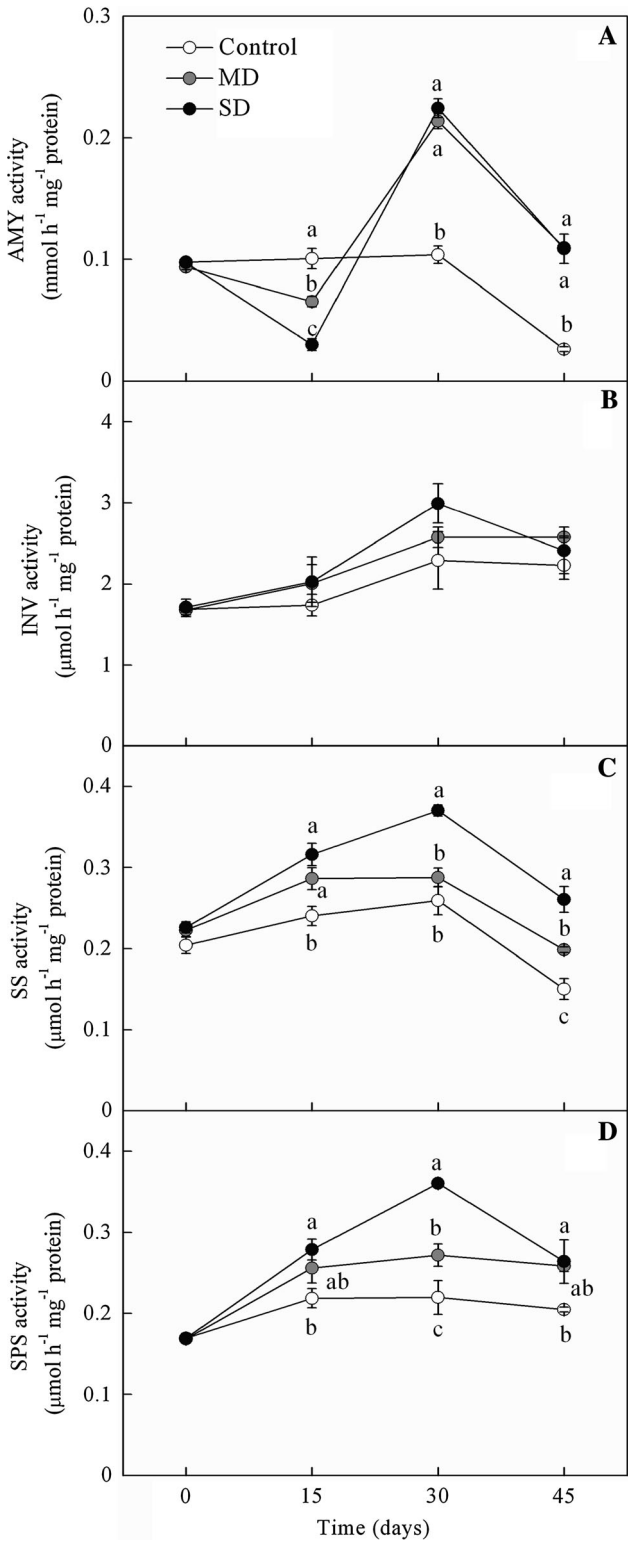

Fig. 3 Changes in the activities of AMY (**a**), INV (**b**), SS (**c**) and SPS (**d**) of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open circles*), moderate drought (MD, *gray circles*) and severe drought (SD, *closed circles*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

those of control, while GDH activity kept the similar level to the control (Fig. 4).

The activities of GOT and GPT declined initially and then increased rapidly with drought time (Fig. 5). Their activities in MD plants were always higher than those of control during the drought period, e.g., a 15.1 % increase in GOT and 22.2 % increase in GPT were observed after 30 days of drought, respectively, compared with control (Fig. 5). However, their activities in SD plants displayed an opposite pattern (Fig. 5). After 15 days of rewatering, the activities of GOT and GPT returned back to their levels except GPT in MD plants (Fig. 5).

Amino acids content first increased and then displayed a slight decrease with drought time, and its content in drought-stressed plants was always higher than that of control (Fig. 6a). Soluble proteins declined gradually with drought time, and especially after 30 days of drought, its content in MD and SD plants decreased by 8.7 and 25.9 %, respectively, compared with control (Fig. 6b). The contents of reduced N and total N generally increased with drought time (Fig. 6c, d). Compared with control, their contents in drought-stressed plants were always higher than that of control except reduced N during the whole of SD and total N after 30 days of SD. Proline content in drought-stressed plants was always higher than that of control (Fig. 7). After 15 days of rewatering, significant differences in these assimilation products except proline were mainly observed between the control and drought-stressed plants (Figs. 6, 7).

Lipid peroxidation

MDA content in drought-stressed plants was always higher than that of control, and especially after 30 days of drought, its content in MD and SD plants increased by 71.1 and 122.3 %, respectively, compared with control (Fig. 8). After 15 days of rewatering, its content only in MD plants restored to the control level (Fig. 8).

Relations of MDA and key compounds involved in C and N metabolism

During the drought period, MDA content was significantly positively correlated ($P < 0.05$) with: (1) the contents of starch and proline after 15 days of drought; (2) the contents of soluble sugar, NSC, and proline after 30 days of drought, and was significantly negatively correlated with soluble proteins ($P < 0.05$) (Table 4). After 15 days of rewatering, a significant negative correlation was observed between MDA content and nitrogenous metabolites contents except amino acids and proline ($P < 0.05$), whereas no significant correlation was found between MDA and different carbohydrates ($P > 0.05$) (Table 4).

Table 3 Changes in NO_3^- reduction and NH_4^+ production of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days)

Parameters	Treatment	Time (days)			
		0	15	30	45
NO_3^- (mg g^{-1} FW)	Control	2.470 ± 0.116a	2.702 ± 0.186b	3.850 ± 0.143b	3.757 ± 0.209a
	MD	2.331 ± 0.306a	3.341 ± 0.121b	4.952 ± 0.260a	3.970 ± 0.079a
	SD	2.369 ± 0.037a	5.415 ± 0.531a	5.081 ± 0.161a	4.165 ± 0.179a
NR (μmol h^{-1} mg^{-1} protein)	Control	0.020 ± 0.001a	0.021 ± 0.001b	0.028 ± 0.002b	0.028 ± 0.002b
	MD	0.021 ± 0.001a	0.027 ± 0.002b	0.037 ± 0.001a	0.032 ± 0.000ab
	SD	0.020 ± 0.001a	0.041 ± 0.004a	0.040 ± 0.001a	0.037 ± 0.002a
NiR (μmol h^{-1} mg^{-1} protein)	Control	0.172 ± 0.001a	0.145 ± 0.008a	0.210 ± 0.013a	0.186 ± 0.022a
	MD	0.173 ± 0.015a	0.153 ± 0.013a	0.211 ± 0.019a	0.201 ± 0.019a
	SD	0.161 ± 0.010a	0.148 ± 0.003a	0.232 ± 0.015a	0.203 ± 0.022a
NH_4^+ (mg g^{-1} FW)	Control	1.032 ± 0.010a	0.772 ± 0.006c	1.106 ± 0.009c	0.784 ± 0.004c
	MD	1.074 ± 0.029a	0.840 ± 0.005b	1.306 ± 0.007b	1.201 ± 0.009a
	SD	1.115 ± 0.025a	0.898 ± 0.007a	1.487 ± 0.015a	1.122 ± 0.012b
NH_4^+/NO_3^-	Control	0.420 ± 0.170a	0.289 ± 0.022a	0.288 ± 0.009a	0.210 ± 0.012b
	MD	0.474 ± 0.053a	0.252 ± 0.010a	0.265 ± 0.013a	0.303 ± 0.006a
	SD	0.471 ± 0.009a	0.169 ± 0.018b	0.293 ± 0.007a	0.270 ± 0.011a

Well-watered (Control), moderate drought (MD) and severe drought (SD). Values are mean ± SE ($n = 3$). Means followed by different letters are significantly different between water treatments within a measurement period at $P < 0.05$ using Duncan's test

Discussion

Changes in plant water status during drought and recovery

LRWC is considered a reliable indicator of water status that reflects the capacity of plants to re-establish their water balance after undergoing water deficit, and of their capacity to adapt or tolerate this stress (Basu et al. 2007). Some researchers have demonstrated that drought declines LRWC in several species of plants, including wheat and turfgrass (Selote and Khanna-Chopra 2006; DaCosta and Huang 2007). We also found a rapid decrease in LRWC of dwarf bamboo with the increasing intensity and duration of drought (Fig. 1), suggesting water imbalance of the cell in dwarf bamboo. However, LRWC of dwarf bamboo returned back to the control level after 15 days of rewatering (Fig. 1), which was consistent with previously published studies in other plant species (Cai et al. 2005; Miyashita et al. 2005; Gallé and Feller 2007; Bian and Jiang 2009). This indicates that dwarf bamboo seems to be capable of withstanding and surviving extreme drought events.

Changes in carbon metabolism during drought and recovery

Drought-induced decline in photosynthetic CO_2 assimilation is extensively considered to be the result of reduced LRWC (Cai et al. 2005; Gallé et al. 2007), and P_{max} may be considered as a measure of the photosynthetic capacity of the leaf (Murchie et al. 2002). Lawlor and Cornic (2002) indicated that the responses of photosynthetic physiology of plants to LRWC can be divided into two types. In type I: P_{max} is unaffected until a decrease in LRWC of more than 25 %, while LCP declines, which results from stomatal limitation. In type II: P_{max} decreases linearly with loss of LRWC, while LCP increases, which is attributed to non-stomatal limitation. The nonstomatal limitation is often implicitly assumed as a metabolic constraint, such as degradation of photosynthetic pigments from impaired chloroplast structure (Reddy et al. 2004). The limitation of photosynthesis under drought through metabolic impairment is a more complex phenomenon than stomatal limitation. In our study, the similar result to type II was found (Table 1), and a strong linear correlation between P_{max} and LRWC ($r = 0.80$, $P < 0.001$) was displayed. Thus, metabolic impairment seems to account mainly for the inhibition of P_{max} in dwarf bamboo under long-term (30 days) drought, as can also be confirmed by the drought-induced degradation of pigments (Table 2). Resultantly, after 15 days of rewatering, most of light response curve parameters and pigments in dwarf bamboo under long-term drought did not restore to their control levels (Table 1).

The accumulation of carbohydrates plays an essential role in protection of drought-stressed plants from osmotic stress (Patakas et al. 2002). It can not only maintain cellular growth and expansion of plants, but also keep stomata open for CO_2 assimilation at a lower water potential (Lawlor and Cornic 2002). Starch and sucrose are the predominant

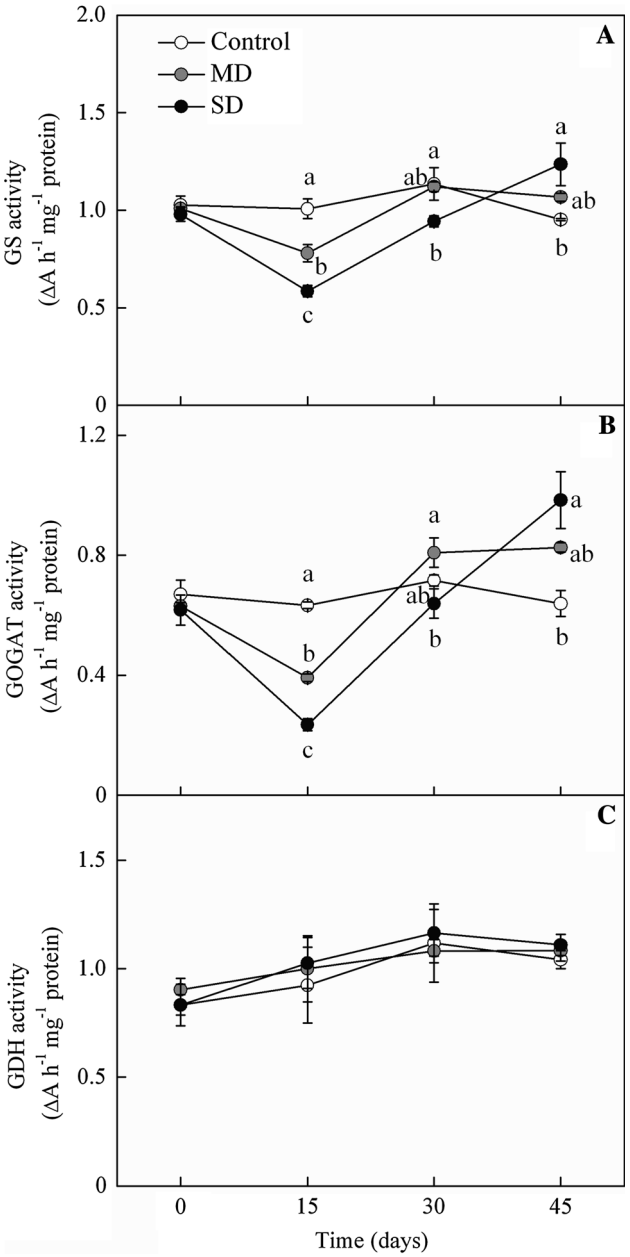

Fig. 4 Changes in the activities of GS (**a**), GOGAT (**b**), and GDH (**c**) of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open circles*), moderate drought (MD, *gray circles*), and severe drought (SD, *closed circles*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

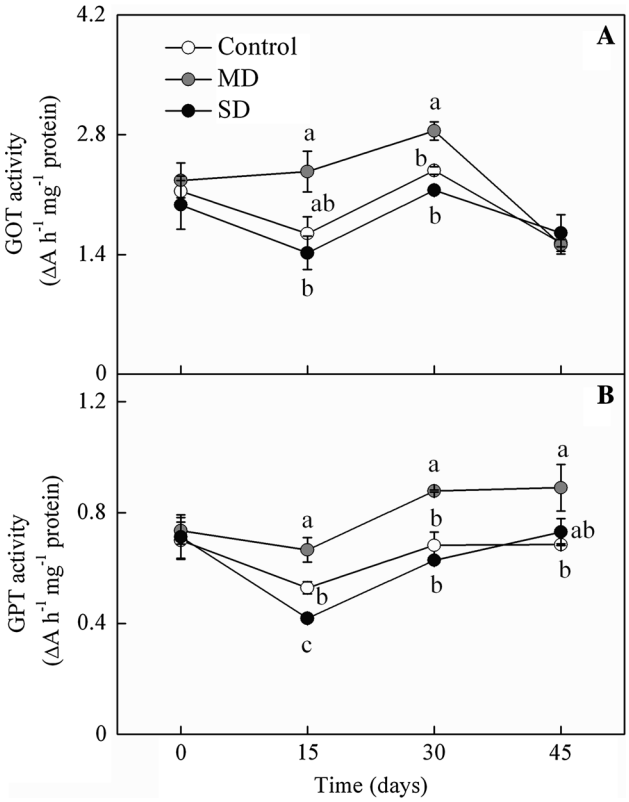

Fig. 5 Changes in the activities of GOT (**a**) and GPT (**b**) of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open circles*), moderate drought (MD, *gray circles*), and severe drought (SD, *closed circles*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

photosynthetic end-products. Thereto, the change of starch content under drought depends on photosynthetic efficiency and the enzymes involved in starch degradation, especially amylase (AMY). Our result showed that the starch content in dwarf bamboo was increased significantly after short-term (15 days) drought (Fig. 2a), possibly attributing to a sharp drop in AMY activity at that time (Fig. 3a). After long-term drought, however, enhanced AMY activity accelerated the rate of starch hydrolysis (Figs. 2a, 3a), leading to a higher soluble sugar content (Fig. 2c). These results suggest that accumulated soluble sugar in dwarf bamboo under long-term severe drought is mainly due to the hydrolysis of previously stored starch rather than to de novo synthesis. Usually, drought can induce transformation of photosynthetic products by modulating the activities of key enzymes related to C metabolism (Yang et al. 2004; Basu et al. 2007). However, in our study, sucrose and soluble sugar in MD plants almost remained unchanged due to the relative changes in the activities of INV, SS, and SPS during the drought period (Figs. 2b, c, 3b, d), which is similar to a recent study by Gallé et al. (2007). These results indicate that dwarf bamboo may vary the metabolites contents and enzymes activities of its C metabolism with different intensities and duration of drought to adaption to such stress. Abiotic stress such as drought causes disturbances in the cellular homeostasis. Therefore, re-adjustment of metabolic balance plays a central role in stress adaptation (Schlüter et al.

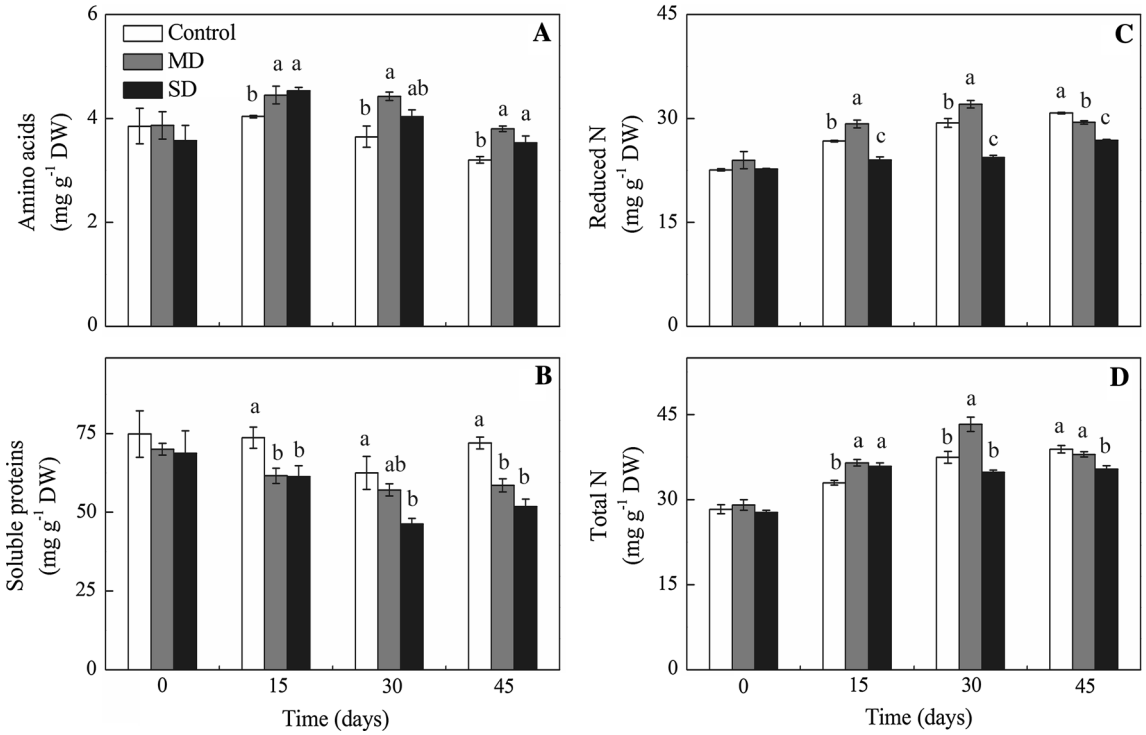

Fig. 6 Changes in the contents of amino acids (**a**), soluble proteins (**b**), reduced N (**c**), and total N (**d**) of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open bars*), moderate drought (MD, *gray bars*), and severe drought (SD, *closed bars*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

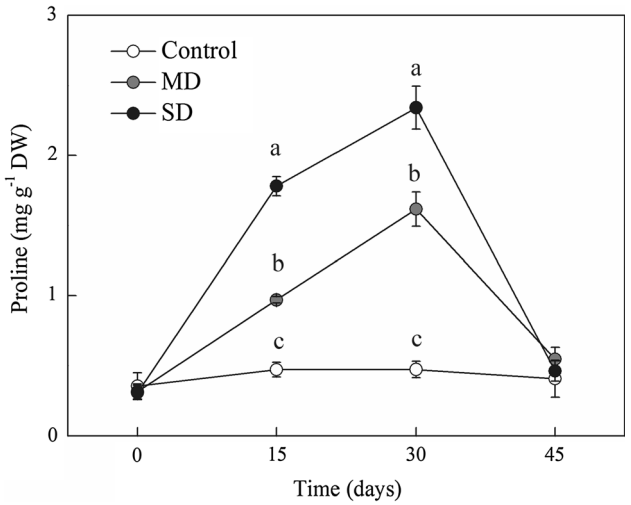

Fig. 7 Changes in proline content of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open circles*), moderate drought (MD, *gray circles*), and severe drought (SD, *closed circles*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

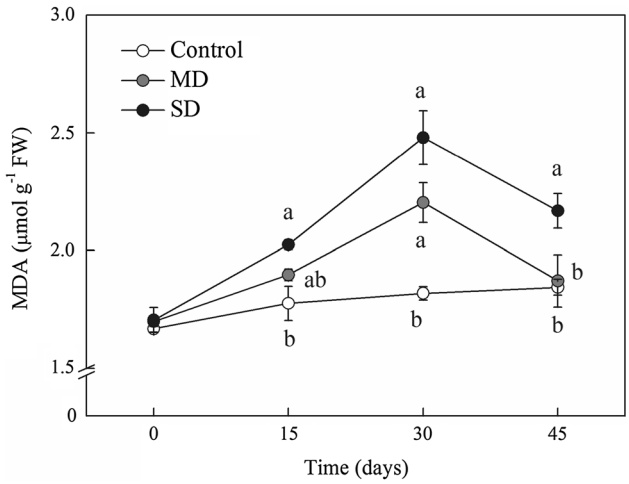

Fig. 8 Changes in malondialdehyde (MDA) content of dwarf bamboo during drought (30 days) and subsequent rewatering (15 days). Well-watered (Control, *open circles*), moderate drought (MD, *gray circles*), and severe drought (SD, *closed circles*). *Vertical bars* show ± SE of the mean ($n = 3$). *Different letters* indicate significant differences between water treatments within a measurement period at $P < 0.05$ using Duncan's test

2013). After rewatering, although these enzymes activities of C metabolism in the drought-stressed dwarf bamboo did not restore to their control levels, its metabolites returned to their control levels, (Figs. 2, 3), suggesting that re-balancing of its C metabolism has gradually begun. Thus, dwarf bamboo could adapt to drought of certain intensity and duration.

Table 4 Relationships between key metabolites involved in C and N metabolism and malondialdehyde (MDA) content during drought (30 days) and subsequent rewatering (15 days)

Parameters	Time (days)			
	0	15	30	45
Starch to MDA	−0.163	**0.714***	0.666	−0.388
Sucrose to MDA	−0.376	0.410	0.318	0.178
Soluble sugar to MDA	−0.075	−0.077	**0.680***	−0.001
NSC to MDA	−0.032	0.418	**0.693***	−0.130
Amino acids to MDA	0.042	0.595	0.304	0.226
Soluble proteins to MDA	0.216	**−0.720***	**−0.836****	**−0.755***
Reduced N to MDA	0.351	−0.429	−0.526	**−0.800****
Total N to MDA	0.332	0.623	−0.275	**−0.690***
Proline to MDA	0.437	**0.836****	**0.876****	0.094

Significant levels in bold: ** $P < 0.01$; * $P < 0.05$

Changes in nitrogen metabolism during drought and recovery

The regulation of N metabolism is of pivotal importance for drought tolerance, and interference between drought and N metabolism is a very complex network that affects a vast array of physiological and biochemical processes (Lawlor 2002). Drought generally reduces the uptake of available N and inhibits N-related enzymes activities in plants (Xu and Zhou 2006; Robredo et al. 2011). However, long-term drought enhanced NO_3^- concentration in dwarf bamboo, being associated in turn with a increased NR activity in the present study (Table 3), which has been documented in drought-tolerant tomato cultivar (Zarina) (Sánchez-Rodríguez et al. 2011). In addition, our result showed that the drought increased significantly the NH_4^+ concentration in dwarf bamboo (Table 3). NH_4^+, a major N source, is produced not only by the reduction of NO_3^- but also by the oxidation of glycine in an activated photorespiration process after water stress. Hence, the increase of NH_4^+ concentration in drought-stressed dwarf bamboo may be associated with its high activity of NiR (Table 3) and/or the oxidation of glycine in an activated photorespiration process after drought.

Overproduction of NH_4^+ will seriously damage plant cells (Thomas and Hilker 2000), thus it needs to be assimilated immediately through the GS/GOGAT cycle and/or the GDH pathway (Suzuki and Knaff 2005; Robredo et al. 2011). However, short-term drought depressed GS/GOGAT activities in dwarf bamboo (Fig. 4a, b) in spite of its increased NH_4^+ concentration (Table 3), which was similar to the result in leaves of *Medicago sativa* (Naya et al. 2007). This indicates that dwarf bamboo may tolerate temporary NH_4^+ accumulation. As drought time prolonged, GS/GOGAT activities were rapidly activated (Fig. 4a, b), which could accelerate NH_4^+ assimilation and enhance drought tolerance. This result is supported by Sánchez-Rodríguez et al. (2011). GDH is a key enzyme linking glutamate metabolism with the Krebs cycle and catalyzes the reversible conversion of glutamate to α-oxoglutarate. Drought can increase GDH activity to produce more amino acids for improving osmotic potential in plants (Sánchez-Rodríguez et al. 2011), or diminish its activity due to lack of α-oxoglutarate (Xu and Zhou 2006). Differently, GDH activity in dwarf bamboo was not affected by drought stress (Fig. 4c), as previously observed by Suzuki and Knaff (2005). Meanwhile, this also indicates that incorporation of NH_4^+ by GDH in dwarf bamboo is not a principal route for enhanced drought tolerance. GOT and GPT catalyze the process that glutamate is converted to alanine or aspartate and to α-oxoglutarate via reversible amino group transfer, resulting in consuming excessive NH_4^+ (Liu et al. 2014). In our study, MD obviously increased the activities of GOT and GPT, on the contrary, SD reduced their activities (Fig. 5a, b). Only under MD, therefore, the two enzymes in dwarf bamboo play an important role in reducing a toxicity caused by overproduction of NH_4^+.

During environmental stresses, numerous nitrogenous metabolites are critically regulated for stress responses at the biochemical level. Drought normally presents differential changes in nitrogenous compounds (Oliveira Neto et al. 2009). Amino acids contain several transfer compounds involved in N metabolism and important osmotic substances (Sibout and Guerrier 1998). We found that drought caused an obvious increase of amino acids in dwarf bamboo (Fig. 6a), which was due to a significant decline of soluble proteins (Fig. 6b) and activated GS/GOGAT cycle (Fig. 4a, b), indicating that drought strengthens the N transfer function and osmotic adjustment to a certain degree. Reduced N is mainly formed by proteins and amino acids (Sánchez-Rodríguez et al. 2011). Our result found that reduced N in dwarf bamboo was remarkably enhanced by MD, but was dramatically reduced by SD (Fig. 6c), which could result from the corresponding changes of its amino acids and soluble

proteins with different degrees of drought (Fig. 6a, b). Previous studies have showed that drought can reduce leaf N (Sinclair et al. 2000). In our study, however, drought significantly increased total N in dwarf bamboo (Fig. 6d). Total N is a critical indicator to determine the nutritional state of plants. Hence, the higher total N level could ensure the growth of drought-stressed plant. Proline plays a key role in not only osmotic protection but also protection of membranes from lipid peroxidation. Generally, drought can cause proline accumulation that is independent of changes in other amino acids (Sicher et al. 2012). A similar result was also found in our study (Fig. 7), which could improve the drought tolerance of dwarf bamboo.

The stronger rates of NO_3^- reduction and NH_4^+ assimilation were observed after rewatering, as indicated by increases in NR activity, NH_4^+ concentration, and GS/GOGAT activities (Table 3; Fig. 4a, b). However, Robredo et al. (2011) reported that drought decreases NO_3^- reduction and NH_4^+ assimilation of *Hordeum vulgare* through reduction in NR and GS activities, and they nearly restore to the control level after 3 days of rewatering. Additionally, though the two key enzymes in conversion pathway of amino acids nearly recovered to the control level after rewatering (Fig. 5a, b), amino acids content still displayed a higher level, may be relating to a lower contents of proteins (Fig. 6b). These results indicate that re-balancing of N metabolism in dwarf bamboo would require longer time.

Relationship of lipid peroxidation and C and N metabolism during drought and recovery

Overproduction of ROS by drought is known to membrane lipid peroxidation and seriously damage plants (Upadhyaya et al. 2008). At present, such destruction can be characterized by lipid peroxidation (MDA) that is a prevalent indicator of oxidative stress (Xu and Zhou 2006). The constant MDA content is found in *Poa pratensis* subjected to mild or short-term severe drought (Bian and Jiang 2009). Whereas, MDA content in dwarf bamboo appeared constant only under short-term mild drought and was remarkably enhanced under long-term moderate and severe drought (Fig. 8), which suggests that dwarf bamboo could withstand the drought of a certain intensity and duration. Furthermore, some studies showed that many osmoprotectants involved in C and N metabolism play a key role in scavenging or detoxifying ROS and reducing membrane lipid peroxidation (Couée et al. 2006; Dhont et al. 2011; Upadhyaya et al. 2013). Our research found that a notable positive correlation between MDA and the contents of starch and proline after 15 days drought, and the contents of soluble sugar, NSC as well as proline after 30 days drought ($P < 0.05$) (Table 4), inferring that dwarf bamboo could employ different metabolites involved in C and N metabolism with different duration of drought to protect against membrane lipid peroxidation.

Conclusion

This study provides new evidence on C and N metabolism against the negative impact of drought on bamboo species and subsequent recovery. It can be concluded that dwarf bamboo may not only differently regulate its C and N metabolism to improve the capacity of osmotic adjustment but also employ its different metabolites protect against membrane lipid peroxidation under different intensities and duration of drought for adapting such damage. First, photochemistry is depressed mainly by metabolic impairment under long-term drought and needs longer time to restore after rewatering. Second, an increase in soluble sugar caused by stored starch hydrolysis only plays a protective role in osmoregulation under long-term severe drought, and re-balancing of C metabolism has gradually begun after rewatering. Third, enhancement in N metabolism induced by drought can produce more amino acids and total N to counter osmotic damage and then to improve plant growth throughout the entire experimental period, and re-balancing of N metabolism would require longer time. Fourth, dwarf bamboo could employ different metabolites involved in C and N metabolism with different duration of drought to protect against membrane lipid peroxidation. In fact, bamboo roots should be considered in future researches to have a better comprehension on protection of metabolism regulation at the whole plant level in different water stresses.

Author contribution statement C. G. Liu and Y. J. Wang conceived and designed the experiments; C. G. Liu, Y. Q. Jin, and J. Liang performed the experiments; C. G. Liu, K. W. Pan, and Y. Q. Jin analyzed the data; W. Li and L. Zhang contributed reagents, materials, and analysis tools; and C. G. Liu and Y. J. Wang wrote the paper.

Acknowledgments We thank Zongping Tang and Lingling Duan for help in the field work, and Tingting Zhu for assistance with lab analysis. This work was funded by the National Natural Science Foundation of China (31470621; 31000293) and the National Natural Science Foundation of Shanghai, China (12ZR1427600).

Conflict of interest The authors declare that they have no conflict of interest.

References

Aranjuelo I, Molero G, Erice G, Avice JC, Nogués S (2011) Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). J Exp Bot 62:111–123. doi:10.1093/jxb/erq249

Basu PS, Ali M, Chaturvedi SK (2007) Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought. Indian J Exp Biol 45:261–267

Bian SM, Jiang YW (2009) Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Sci Hortic 120:264–270. doi:10.1016/j.scienta.2008.10.014

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Cai ZQ, Chen YJ, Guo YH, Cao KF (2005) Responses of two field-grown coffee species to drought and re-hydration. Photosynthetica 43:187–193. doi:10.1007/s11099-005-0032-z

Couée I, Sulmon C, Gouesbet G, El-Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J Exp Bot 57:449–459. doi:10.1016/j.mib.2006.01.001

DaCosta M, Huang B (2007) Changes in antioxidant enzyme activities and lipid peroxidation for bentgrass species in response to drought stress. J Am Soc Hortic Sci 132:319–326

Dhont C, Bertrand A, Castonguay Y, Isabel N (2011) Changes of carbon and nitrogen metabolites in white spruce (*Picea glauca* [Moench] Voss) of contrasted growth phenotypes. Trees Struct Funct 25:711–723. doi:10.1007/s00468-011-0549-7

Flexas J, Bota J, Galmés J, Medrano H, Ribas-Carbó M (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiol Plant 127:343–352. doi:10.1111/j.1399-3054.2005.00621.x

Gallé A, Feller U (2007) Changes of photosynthetic traits in beech saplings (*Fagus sylvatica*) under severe drought stress and during recovery. Physiol Plant 131:412–421. doi:10.1111/j.1399-3054.2007.00972.x

Gallé A, Haldimann P, Feller U (2007) Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. New Phytol 174:799–810. doi:10.1111/j.1469-8137.2007.02047.x

Gauthier PPG, Crous KY, Ayub G, Duan HL, Weerasinghe LK, Ellsworth DS, Tjoelker MG, Evans JR, Tissue DT, Atkin OK (2014) Drought increases heat tolerance of leaf respiration in *Eucalyptus globulus* saplings grown under both ambient and elevated atmospheric [CO_2] and temperature. J Exp Bot. doi:10.1093/jxb/eru367

Gupta N, Thind SK, Bains NS (2014) Glycine betaine application modifies biochemical attributes of osmotic adjustment in drought stressed wheat. Plant Growth Regul 72:221–228. doi:10.1007/s10725-013-9853-0

Hikosaka K, Kato MC, Hirose T (2004) Photosynthetic rates and partitioning of absorbed light energy in photoinhibited leaves. Physiol Plant 121:699–708. doi:10.1111/j.1399-3054.2004.00364.x

IPCC (2012) Managing the risks of extreme events and disasters to advance climate change adaptation. A special report of working groups I and II of the intergovernmental panel on climate change [Field CB, Barros V, Stocker TF, Qin D, Dokken DJ, Ebi KL, Mastrandrea MD, Mach KJ, Plattner GK, Allen SK, Tignor M, Midgley PM (eds)]. Cambridge University Press, Cambridge, UK, and New York, NY, USA, pp 582

Kaiser J, Lewis OAM (1984) Nitrate reductase and glutamine synthetase activity in leaves and roots of nitrate-fed *Helianthus annuus* L. Plant Soil 77:127–130. doi:10.1007/BF02182818

Kang Y, Han YH, Torres-Jerez I, Wang MY, Tang YH, Monteros M, Udvardi M (2011) System responses to long-term drought and re-watering of two contrasting alfalfa varieties. Plant J 68:871–889. doi:10.1111/j.1365-313X.2011.04738.x

Lawlor DW (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. J Exp Bot 53:773–787. doi:10.1093/jexbot/53.370.773

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ 25:275–294. doi:10.1046/j.0016-8025.2001.00814.x

Li HS (2000) The experimental principle and technology of plant physiology and biochemistry. Higher Education Press, Beijing

Li YP, Zhang YB, Zhang XL, Korpelainen H, Berninger F, Li CY (2013) Effects of elevated CO_2 and temperature on photosynthesis and leaf traits of an understory dwarf bamboo in subalpine forest zone, China. Physiol Plant 148:261–272. doi:10.1111/j.1399-3054.2012.01705.x

Lillo C (1984) Diurnal variations of nitrite reductase, glutamine synthetase, glutamate synthase, alanine aminotransferase and aspartate aminotransferase in barley leaves. Physiol Plant 61:214–218. doi:10.1111/j.1399-3054.1984.tb05899.x

Liu CG, Wang YJ, Pan KW, Zhu TT, Li W, Zhang L (2014) Carbon and nitrogen metabolism in leaves and roots of dwarf bamboo (*Fargesia denudata* Yi) subjected to drought for two consecutive years during sprouting period. J Plant Growth Regul 33:243–255. doi:10.1007/s00344-013-9367-z

Llorens L, Peñelas J, Estiarte M (2003) Ecophysiological responses of two Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*, to experimentally drier and warmer conditions. Physiol Plant 119:231–243. doi:10.1034/j.1399-3054.2003.00174.x

Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005) Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ Exp Bot 53:205–214. doi:10.1016/j.envexpbot.2004.03.015

Muller B, Pantin F, Génard M, Turc O, Freixes S, Piques M, Gibon Y (2011) Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. J Exp Bot 62:1715–1729. doi:10.1093/jxb/erq438

Murchie EH, Hubbart S, Chen YZ, Peng SB, Horton P (2002) Acclimation of rice photosynthesis to irradiance under field conditions. Plant Physiol 130:1999–2010. doi:10.1104/pp.011098

Naya L, Ladrera R, Ramos J, González EM, Arrese-Igor C, Minchin FR, Becana M (2007) The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. Plant Physiol 144:1104–1114. doi:10.1104/pp.107.099648

Oliveira Neto CF, Lobato AKS, Costa RCL, Maia WJMS, Santos Filho BG, Alves GAR, Brinez B, Neves HKB, Lopes MJS, Cruz FJR (2009) Nitrogen compounds and enzyme activities in sorghum induced to water deficit during three stages. Plant Soil Environ 55:238–244

Patakas A, Nikolaou N, Zioziou E, Radoglou K, Noitsakis B (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Sci 163:361–367. doi:10.1016/S0168-9452(02)00140-1

Prado CHBA, de Moraes JAPV (1997) Photosynthetic capacity and specific leaf mass in twenty woody species of Cerrado vegetation under field conditions. Photosynthetica 33:103–112. doi:10.1023/A:1022183423630

Praxedes SC, DaMatta FM, Loureiro MEG, Ferrão MA, Cordeiro AT (2006) Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. kouillou) leaves. Environ Exp Bot 56:263–273. doi:10.1016/j.envexpbot.2005.02.008

Raven JA, Handley LL, Andrews M (2004) Global aspects of C/N interactions determining plant-environment interactions. J Exp Bot 55:11–25. doi:10.1093/jxb/erh011

Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189–1202. doi:10.1016/j.jplph.2004.01.013

Robredo A, Pérez-López U, Miranda-Apodaca J, Lacuesta M, Mena-Petite A, Muñoz-Rueda A (2011) Elevated CO_2 reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. Environ Exp Bot 71:399–408. doi:10.1016/j.envexpbot.2011.02.011

Sánchez-Rodríguez E, Rubio-Wilhelmi MM, Rios JJ, Blasco B, Rosales MA, Melgarejo R, Romero L, Ruiz JM (2011) Ammonia production and assimilation: its importance as a tolerance mechanism during moderate water deficit in tomato plants. J Plant Physiol 168:816–823. doi:10.1016/j.jplph.2010.11.018

Schlüter U, Colmsee C, Scholz U, Bräutigam A, Weber AP, Zellerhoff N, Bucher M, Fahnenstich H, Sonnewald U (2013) Adaptation of maize source leaf metabolism to stress related disturbances in carbon, nitrogen and phosphorus balance. BMC Genom 14:442. doi:10.1186/1471-2164-14-442

Selote DS, Khanna-Chopra R (2006) Drought acclimation confers oxidative stress tolerance by inducing co-ordinated antioxidant defense at cellular and subcellular level in leaves of wheat seedlings. Physiol Plant 127:494–506. doi:10.1111/j.1399-3054.2006.00678.x

Sibout R, Guerrier G (1998) Solute incompatibility with glutamine synthetase in water-stressed (*Populus nigra*). Environ Exp Bot 40:173–178. doi:10.1016/S0098-8472(98)00032-X

Sicher RC, Timlin D, Bailey B (2012) Responses of growth and primary metabolism of water-stressed barley roots to rehydration. J Plant Physiol 169:686–695. doi:10.1016/j.jplph.2012.01.002

Sinclair TR, Pinter PJ, Kimball BA, Adamsen FJ, LaMorte RL, Wall GW, Hunsaker DJ, Adam N, Brook TJ, Garcia RL, Thompson T, Leavitt S, Mattias A (2000) Leaf nitrogen concentration of wheat subjected to elevated [CO_2] and either water or N deficits. Agric Ecosyst Environ 79:53–60. doi:10.1016/S0167-8809(99)00146-2

Suzuki A, Knaff DB (2005) Glutamate synthase: structural, mechanistic and regulatory properties, and role in the amino acid metabolism. Photosynth Res 83:191–217. doi:10.1007/s11120-004-3478-0

Tang ZC (1999) Experimental guide of modern plant physiology. Science Press, Shanghai

Thomas FM, Hilker C (2000) Nitrate reduction in leaves and roots of young pedunculate oaks (*Quercus robur*) growing on different nitrate concentrations. Environ Exp Bot 43:19–32. doi:10.1016/S0098-8472(99)00040-4

Upadhyaya H, Panda SK, Dutta BK (2008) Variation of physiological and antioxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. Acta Physiol Plant 30:457–468. doi:10.1007/s11738-008-0143-9

Upadhyaya H, Sahoo L, Panda SK (2013) Molecular physiology of osmotic stress in plants. In: Rout GR, Das AB (ed) Molecular stress physiology of plants. Springer, New York, pp 179–192. doi:10.1007/978-81-322-0807-5_7

Xiao XW, Yang F, Zhang S, Korpelainen H, Li CY (2009) Physiological and proteomic responses of two contrasting *Populus cathayana* populations to drought stress. Physiol Plant 136:150–168. doi:10.1111/j.1399-3054.2009.01222.x

Xu ZZ, Zhou GS (2006) Nitrogen metabolism and photosynthesis in *Leymus chinensis* in response to long-term soil drought. J Plant Growth Regul 25:252–266. doi:10.1007/s00344-006-0043-4

Xu ZZ, Zhou GS (2007) Photosynthetic recovery of a perennial grass *Leymus chinensis* after different periods of soil drought. Plant Prod Sci 10:277–285. doi:10.1626/pps.10.277

Yang JC, Zhang JH, Wang ZQ, Xu GW, Zhu QS (2004) Activities of key enzymes in sucrose-to-starch conversion in wheat grains subjected to water deficit during grain filling. Plant Physiol 135:1621–1629. doi:10.1104/pp.104.041038

Zhang ZL, Qu WJ (2003) The experimental guidance of plant physiology. Higher Education Press, Beijing