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Carbon and Nitrogen Metabolism in Leaves and Roots of Dwarf Bamboo (*Fargesia denudata* Yi) Subjected to Drought for Two Consecutive Years During Sprouting Period

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Abstract Dwarf bamboo is an ecologically and economically important forest resource that is widespread in mountainous regions of eastern Asia and southern America. *Fargesia denudata*, one of the most important dwarf bamboos, is a staple food of the giant panda, but our knowledge about how *F. denudata* copes with drought stress is very limited. The objective of this study was to determine the responses of carbon (C) and nitrogen (N) metabolism to drought in leaves and roots of *F. denudata* plants. Plants were subjected to three water treatments, well-watered [WW, 85 % relative soil water content (RSWC)], moderate drought (MD, 50 % RSWC), and severe drought (SD, 30 % RSWC), for two consecutive years during the sprouting period. Plant growth parameters, levels of carbohydrates and N compounds, and activities of key enzymes involved in C and N metabolism were analyzed. In young leaves, C metabolism was in balance after drought stress, but nitrate (NO_3^-) reduction and ammonium (NH_4^+) assimilation were accelerated. In old leaves, drought stress decreased carbohydrate contents by spurring the activities of the main enzymes that participate in C metabolism, whereas N metabolism was enhanced only under SD. Roots showed unchanged C metabolism parameters under MD, together with stable NO_3^- reduction and the key enzymes related to NH_4^+ assimilation, whereas they were stimulated by SD. Hydrolysates of carbohydrates in old leaves could be transferred into roots, but only to meet MD. Meanwhile, roots could allocate more N nutrition to young leaves and less to old leaves. These changes regulated the overall metabolic balance of *F. denudata*. Consequently, the results indicate that different organs with various response strategies will be well adapted to different drought intensities for ensuring regular growth of *F. denudata* plants at the whole-plant level.

Keywords Dwarf bamboo · *Fargesia denudata* Yi · Drought stress · Carbon metabolism · Nitrogen metabolism · Sprouting period

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Introduction

The increasing frequency and intensity of drought caused by climate change have become a global issue in this century (McDowell 2011; Field and others 2012). Drought is one of the major abiotic stresses that adversely affect plant growth, development, and productivity (Sicher and others 2012). Several physiological and biochemical mechanisms such as photosynthesis and nutrient uptake and metabolism are involved in the adaptation of plants to drought stress (Robredo and others 2011). It is well known that drought can destroy the osmotic homeostasis of plant cells, lead to osmotic stress, and thus result in plant death for lack of adequate moisture ultimately. To cope with osmotic stress caused by drought, compatible solutes are synthesized and accumulated in several plants, promoting

additional moisture to be absorbed from the environment (Sibout and Guerrier 1998; Basu and others 2007). Most compatible solutes are carbon (C) -and nitrogen (N)-containing compounds, for example, carbohydrates, amino acids, and proteins (Wang and others 2012b). Accordingly, C and N metabolism is of pivotal importance for drought resistance (Commichau and others 2006; McDowell 2011; Sanchez-Rodriguez and others 2011).

Drought stress depresses photosynthesis, one of the key processes of C metabolism, which causes depletion of energy and sugar and decreases plant production (Cuellar-Ortiz and others 2008). Sucrose is a major product of photosynthesis that is transported from leaves to roots and is hydrolyzed by sucrose synthase (SS) and invertase (INV). Usually, SS and INV increase under drought conditions, which may account for the accumulation of hexoses (Praxedes and others 2006), but different responses of these enzymes to drought have been observed (González and others 1998; Praxedes and others 2006). Moreover, sucrose phosphate synthase (SPS), a key enzyme in sucrose synthesis, is reduced or increased by drought stress (Praxedes and others 2006; Basu and others 2007). Starch from photoassimilated carbon or formed by the uridine diphosphate glucose product of SS in the amyloplast may be hydrolyzed into glucose by increased amylase (AMY) under drought stress (Zeeman and others 2004). In any case, drought can lead to an accumulation of sugar through various higher enzymatic activities (Yang and others 2001) and eventually contribute to osmotic regulation.

Nitrogen is an essential inorganic nutrient that plants require in great quantities, and nitrate (NO_3^-) is one of most available N sources for plants (Lawlor 2002a). NO_3^-, taken up by plant roots from soil using a variety of transporters, is reduced to nitrite (NO_2^-) in the cytosol by nitrate reductase (NR), which is considered the rate-limiting step in N assimilation. In turn, the reduction of NO_2^- to ammonium (NH_4^+) is catalyzed by nitrite reductase (NiR) in the chloroplasts or plastids. Afterward, NH_4^+ from both NO_3^- reduction and soil is converted to glutamate by glutamine synthetase (GS) and the glutamate synthase (GOGAT) cycle or the alternative glutamate dehydrogenase (GDH) pathway (Thomas and Hilker 2000; Sanchez-Rodriguez and others 2011). Glutamate can be transaminated by glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) (González and others 1998; Hodges 2002). Under drought conditions, N content is usually reduced and enzyme activities involved in N metabolism are inhibited, resulting in changes in amino acid composition, such as proline accumulation, for drought tolerance (González and others 1998; Xu and Zhou 2006a, b; Robredo and others 2011).

Generally speaking, C metabolism provides available energy (ATP) and a C skeleton (α-oxoglutarate) for NO_3^- reduction and amino acid synthesis (Lawlor 2002a). However, it also competes against N metabolism for assimilatory power (NADH and NADPH) and for some intermediate products derived from the photoreaction stage, especially when the assimilatory power is inadequate (Lawlor 2002a; Sanchez-Rodriguez and others 2011). Furthermore, enhanced carbohydrate content can improve enzyme activities related to N metabolism to assist in NH_4^+ assimilation to a certain degree (Zhang and others 2011). Therefore, there are numerous points of reciprocal control between C and N metabolism on the gene expression level and enzyme activity (Ghisi and others 2002; Commichau and others 2006). In addition, their interaction is very sensitive to drought stress. For example, drought restricts the rate of photosynthetic carbon dioxide (CO_2) assimilation, leading to a lower rate of N assimilation and vice versa, which has been demonstrated by many studies (Foyer and others 1998; González and others 1998; Xu and Zhou 2006a, b). However, changes in key enzyme activities and metabolites involved in C and N metabolism induced by drought stress are still being debated, and discrepancies may result from plant species (Li and others 2009; Sanchez-Rodriguez and others 2011), stress intensity and duration (Xu and Zhou 2006a, b, 2007), leaf age (Pommel and others 2006; Xu and others 2008; Li and others 2009), and plant organs (Thomas and Hilker 2000; Gouia and others 2003).

Dwarf bamboo, which is a rhizomatous, semiwoody, and perennial evergreen plant, often dominates the main synusia in the understory of several subalpine and montane forests in eastern Asia and southern America (Saitoh and others 2002). It is well known, however, that drought will strongly affect the sprouting of dwarf bamboo and slow or cease their regeneration, thus decreasing the productivity and quality of the forest (Wang and Ma 1993). *Fargesia denudata*, one of the most important dwarf bamboos, is a staple food of the giant panda and is distributed only in the mountainous regions of the Sichuan and Gansu provinces in western China at 1,800–3,400 m a.s.l. (Wang and Ma 1993). Moreover, it is very susceptible to drought because it is a shallow rooted plant which requires higher water tables. Thus, drought strongly affects the growth and production of *F. denudata*; this may be attributed to altered C and N metabolism processes. In recent years, although a large number of studies have been conducted on biological and ecological characteristics, nutrient dynamics, and photosynthesis and respiration of *F. denudata* (Wang and Ma 1993), little information is available concerning its C and N metabolism under drought conditions, especially when drought occurs during sprouting period.

This study was designed to investigate whether drought for two consecutive years affects the C and N metabolism in the old and young leaves and roots of *F. denudata* differently during the sprouting period, and how different

organs regulate the coordination of C and N metabolism. Based on these two objectives, we analyzed the growth parameters, pigment contents, and the levels of components and the activities of important enzymes in C and N metabolism.

Materials and Methods

Plant Material and Treatments

Uniform and healthy *F. denudata* plants (2 years old) from the nursery at Wanglang National Nature Reserve were used in this research. Plants were transferred to a greenhouse at Maoxian Ecological Station, Chinese Academy of Sciences (103°53′E, 31°41′N, 1,860 m a.s.l.), with relative humidity between 55 and 80 % and a temperature range of 18–31 °C. Under these conditions, plants were grown separately in individual 50-L plastic pots filled with 25 kg of homogenized topsoil from a field on the experimental site. Plants were watered every other day using nearby stream water. After the plants had been growing for 3 months, three water treatments were applied twice: the first began in July 2011 and the second began in July 2012, and both were maintained for 45 days. The treatments were carried out by withholding water using the weight method as follows: well-watered [WW, 85 % relative soil water content (RSWC)], moderate drought (MD, 50 % RSWC), and severe drought (SD, 30 % RSWC). During treatment gaps, all plants were managed in the same way as before. Each treatment had three replications.

Plant Sampling

All plants were harvested after the second water treatment. Young (1-year-old) and old (3-year-old) leaves of *F. denudata* plants (excluding petioles) and their stalks were sampled separately, except roots and rhizomes. The plant materials were rinsed with distilled water and thoroughly blotted on filter paper. A part of the plant material was used for determining pigment, protein, amino acid contents, the concentrations of NO_3^-, NO_2^-, and NH_4^+, and enzymatic activities. The rest of the plant material was freshly weighed and then dried to constant weight for assaying biomass, carbohydrates, and reduced N.

Determination of Pigment Contents

Chlorophyll (Chl) was extracted in the dark from 0.2 g of frozen leaf tissue using 5 mL 100 % acetone for 36 h at room temperature (Xiong 2003). Pigment contents were estimated using the following equations: Chl $a = 11.75A662 - 2.35A645$, Chl $b = 18.61A645 - 3.96A662$, carotene (Car) = (1000A470 − 2.27 Chl a − 81.40 Chl b)/227 (Sükran and others 1998).

Determination of Carbohydrates

Leaves and roots (0.1 g dry weight) were extracted with 6 mL of 80 % (v/v) ethanol for 30 min in a water bath at 80 °C under agitation and centrifuged at 3,000×g for 10 min, and then the supernatant was collected. The process was repeated three times. The supernatant was combined, transferred into a beaker, evaporated to 3 mL, volumed to 50 mL, and then used for determination of soluble sugar by the anthrone method and sucrose by the 3,5-dinitrosalicylic acid (DNS) method (Zhang and Qu 2003). The ethanol-insoluble residue was extracted for starch and measured using the anthrone-H_2SO_4 method (Cuellar-Ortiz and others 2008).

Determination of N Forms, Soluble Protein, and Free Amino Acid

NO_3^- was analyzed from an aqueous extraction of 0.2 g frozen leaves and roots in 5 mL of deionized water for 10 min in a boiling water bath. The homogenate was centrifuged at 10,000×g for 10 min at room temperature and the supernatant was taken for NO_3^- determination. The reaction mixture [0.5 mL of the supernatant and 1 mL of 5 % (w/v) salicylic acid–sulfuric acid] was kept at room temperature for 20 min, completed with 2 mL of 8 % (w/v) NaOH, and determined at 410 nm (Li 2000).

NO_2^- was determined by homogenization of 0.2 g frozen leaves and roots in 2 mL of deionized water and centrifuged at 10,000×g for 10 min at room temperature. The assay mixture contained 1 mL of the supernatant, 2 mL of 1 % (w/v) sulfanilamide, and 2 mL of 0.02 % (w/v) of *N*-(1-naphthyl) ethylene diamine dihydrochloride, and then measured at 520 nm (Varner and others 1953).

NH_4^+ was assayed by homogenization of 0.2 g frozen leaves and roots in 2 mL of 10 % (v/v) HCl and centrifuged at 10,000×g for 10 min at 4 °C. The reaction mixture contained 0.5 mL of the supernatant, 1 mL of deionized water, 3 mL of triketohydrindene hydrate, and 0.1 mL of ascorbic acid, and then measured at 570 nm (Tang 1999).

Reduced N was determined by the micro-Kjeldahl method after digestion in H_2SO_4–H_2O_2 (Li 2000). Total N content was evaluated to represent the sum of NO_3^- and reduced N (Sanchez-Rodriguez and others 2011).

Free amino acid was assayed by homogenization of 0.2 g frozen leaves and roots in 2 mL of 10 % (v/v) acetic acid and centrifuged at 12,000×g for 10 min. The supernatant was used for the assay of free amino acid by the ninhydrin method (Xiong 2003).

Soluble protein was determined by homogenization of 0.2 g frozen leaves and roots in 2 mL of 50 mM cold sodium phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2 % (w/v) polyvinylpyrrolidone (PVP) and centrifuged at 12,000×*g* for 20 min. The supernatant was measured with Bradford G-250 reagent (Bradford 1976).

Enzyme Extraction and Assays

For determination of AMY and INV activities, 0.2 g frozen leaves and roots were ground in chilled distilled water, volumed, and kept at 4 °C for 3 h. The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C. INV activity was assayed by measuring reducing sugar content using the DNS method at 540 nm (Yang and others 2004). Boiled enzyme extraction as control was run simultaneously. AMY activity was determined by the DNS method at 520 nm (Zhang and Qu 2003).

SS and SPS activities were determined as described by Zhang and Qu (2003). Frozen leaves and roots (0.2 g) were homogenized in a chilled mortar in 50 mM HEPES–NaOH (pH 7.5) containing 50 mM $MgCl_2$, 2 mM ethylenediaminetetraacetic acid (EDTA), 0.2 % (w/v) bovine serum albumin (BSA), and 2 % (w/v) PVP. The homogenate was centrifuged at 12,000×*g* for 10 min at 4 °C. The assay mixture of SS contained 50 mM HEPES–NaOH (pH 7.5), 50 mM $MgCl_2$, 100 mM uridine diphosphoglucose (UDPG), 100 mM fructose, and enzyme extract. After incubation at 30 °C for 30 min, 2 M NaOH was added to the assay mixture to terminate the reaction, boiled for 10 min, and cooled. Afterward, 30 % (w/v) HCl and 0.1 % (w/v) *m*-dihydroxybenzene were added, the mixture shaken thoroughly, kept in a water bath for 10 min at 80 °C, and once the mixture cooled its absorbance was measured at 480 nm. SPS activity was determined in the same way as SS activity. Fructose-6-phosphate was substituted for fructose.

NR activity was determined as described by Xiong (2003). Frozen leaves and roots (0.2 g) were ground in a chilled mortar in 25 mM phosphate buffered 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 and the supernatant was used for NR assay. The reaction mixture [containing 100 mM KNO_3, 2 % (w/v) NADH, and enzyme extract] was incubated for 30 min at 25 °C. A control without NADH was run simultaneously. The reaction was terminated by adding 1 % (w/v) sulfanilamide (prepared in 3 M HCl) and 0.02 % (w/v) of *N*-(1-naphthyl)ethylene diamine dihydrochloride. After color reflection for 15 min, the assay mixture was centrifuged at 12,000×*g* for 5 min, and the absorbance was determined at 540 nm.

For analyses of NiR, GS, GOGAT, GDH, GPT and GOT activities, frozen leaves and roots (0.2 g) were extracted in a chilled mortar in 50 mM Tris–HCl (pH 7.8) consisting of 1 mM EDTA, 15 % (v/v) glycerol, 14 mM 2-mercaptoethanol, and 0.1 % (v/v) Triton X-100. The homogenate was centrifuged twice at 10,000×*g* for 10 min at 4 °C and the supernatant was removed and stored at 4 °C for the assay of five enzyme activities (Lillo 1984).

NiR activity was determined by following the decrease of NO_2^- at 520 nm using the modified 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.

GS activity was assayed by monitoring the formation of glutamyl hydroxamate at 540 nm after reacting with acidified ferric chloride (Kaiser and Lewis 1984). The reaction medium 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 blanks were also prepared, one without hydroxylamine and the other without glutamate.

GOGAT activity was measured by estimating the oxidation of NADH at 340 nm as described by Tang (1999). The reaction mixture contained 20 mM α-oxoglutarate, 10 mM KCl, 2 mM NADH, 25 mM Tris–HCl (pH 7.8), and enzyme extract. After incubation for 15 min at 30 °C, the reaction was started by adding 20 mM glutamine. Two controls, one without α-oxoglutarate and the other without glutamine, were run simultaneously.

GDH activity was determined as described by Tang (1999) with some modification. The reaction mixture containing 20 mM α-oxoglutarate, 1 M NH_4Cl, and 25 mM Tris–HCl (pH 7.8) was preincubated at 30 °C for 15 min. The reaction was excited by adding 2 mM NADH and enzyme extract and measured by monitoring oxidation of NADH at 340 nm. Two controls, one without α-oxoglutarate and the other without NH_4Cl, were run simultaneously.

GPT activity was determined by monitoring the reduction of NADH at 340 nm as described by Tang (1999). The reaction medium consisted of 1 mM NADH, 100 mM L-alanine, five units lactate dehydrogenase, 333 mM Tris–HCl (pH 7.8), and enzyme extract. The reaction was started by adding 20 mM α-oxoglutarate. Two blanks were also prepared, one without α-oxoglutarate and the other without L-alanine.

GOT activity was assayed in the same way as GPT activity. Malate dehydrogenase was substituted for lactate dehydrogenase and aspartate for L-alanine (Tang 1999).

Statistical Analysis

Data analyses were performed using SPSS 17.0 (SPSS, Inc., Chicago, IL). Two-way analysis of variance (ANOVA) was used to assess the effects of water

treatments, plant organs, and their interaction on all dependent variables ($P = 0.05$). Means among water treatments within the same plant organs were compared by the method of least significant differences (LSD) at the 0.05 level.

Results

Changes of Plant Growth

Drought stress significantly decreased the amount of sprouting and stem biomass of young *F. denudata* plants, but it did not affect those of old plants (Table 1). Generally, height, leaf number, and leaf biomass of young and old *F. denudata* plants were diminished by drought stress, whereas bamboo joint number was unchanged after drought stress. Root biomass and total underground biomass were remarkably reduced with increasing drought stress intensity (Fig. 1). Rhizome biomass was not affected by MD but showed a 40 % decrease under SD compared to WW.

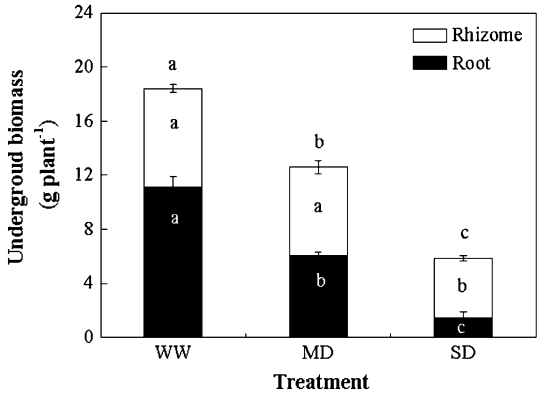

Fig. 1 Effects of drought stress on rhizome biomass (*white bars*) and root biomass (*black bars*) of *F. denudata* plants. *WW* well-watered treatment, *MD* moderate drought, *SD* severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

Photosynthetic Pigment Contents

With increasing drought stress intensity, the concentrations of Chl *a*, Chl *b*, and Car as well as the ratio of Chl *a* + *b*/Car in young leaves declined significantly, whereas the ratio of Chl *a*/*b* increased (Table 2). These photosynthetic parameters in old leaves were insignificant among the water treatments, except for Car.

Carbohydrates Contents

Starch content in all plant organs studied was not affected by drought stress (Fig. 2a). Sucrose content in young leaves was unchanged under drought stress, whereas it strongly decreased in old leaves with increasing drought stress intensity, and decreased in roots only under SD (Fig. 2b). The same pattern was observed in the case of soluble sugar (Fig. 2c). We also evaluated the effects across all treatments using a two-way ANOVA (Table 4). Plant organ and water levels also significantly affected sucrose and soluble sugar contents ($P < 0.01$), except for starch ($P > 0.05$), but their interaction had no significant effect on carbohydrates studied ($P > 0.05$, Table 4).

AMY, INV, SS, and SPS Activities

AMY activity in young leaves increased under drought stress, whereas its activity in old leaves and roots was unchanged (Fig. 3a). AMY activity in old leaves under SD was 29.9 % higher than that under MD. INV activity in all plant organs studied was not affected by drought stress, except in old leaves and roots under SD, where it rose significantly by 58.8 and 28.6 %, respectively, compared to WW (Fig. 3b). Moreover, its activity in roots under SD was 46.7 % greater than that under MD. SS activity in all organs

Table 1 Effects of drought stress on aboveground characteristics of *F. denudata* plants

Type/treatment	Amount of sprouting	Stem biomass (g plant^{-1})	Height (cm)	Leaf number	Leaf biomass (g plant^{-1})	Bamboo joint number
Young						
WW	5.67 ± 0.88^{a}	15.23 ± 1.20^{a}	108.00 ± 5.29^{a}	257.00 ± 18.77^{a}	12.20 ± 0.97^{a}	9.67 ± 0.33^{a}
MD	3.29 ± 0.81^{b}	6.92 ± 1.07^{b}	80.00 ± 5.66^{b}	121.67 ± 9.84^{b}	5.85 ± 0.17^{b}	10.00 ± 0.58^{a}
SD	2.75 ± 0.37^{b}	4.29 ± 0.64^{b}	68.88 ± 6.65^{b}	71.00 ± 3.51^{c}	4.29 ± 0.14^{b}	9.33 ± 0.33^{a}
Old						
WW	5.57 ± 0.25^{a}	15.25 ± 1.04^{a}	59.67 ± 2.78^{a}	174.67 ± 8.41^{a}	13.06 ± 0.20^{a}	6.67 ± 0.33^{a}
MD	6.17 ± 0.48^{a}	14.26 ± 1.23^{a}	51.00 ± 1.27^{b}	163.67 ± 12.41^{ab}	12.20 ± 0.64^{ab}	6.00 ± 0.58^{a}
SD	5.50 ± 0.22^{a}	13.80 ± 1.35^{a}	46.25 ± 2.12^{b}	140.33 ± 7.69^{b}	10.40 ± 0.76^{b}	6.00 ± 0.58^{a}

Values are mean ± SE. Different letters indicate significant differences ($P < 0.05$) between water treatments using the LSD test

WW well-watered, *MD* moderate drought, *SD* severe drought

Table 2 Effects of drought stress on photosynthetic pigment contents in *F. denudata* plants

Type/ treatment	Chl *a*	Chl *b*	Car	Chl *a*/*b*	Chl *a* + *b*/Car
Young leaves					
WW	1.98 ± 0.02[a]	1.20 ± 0.01[a]	0.66 ± 0.04[a]	1.65 ± 0.01[b]	4.80 ± 0.02[a]
MD	1.88 ± 0.01[b]	1.12 ± 0.05[b]	0.64 ± 0.03[b]	1.68 ± 0.01[b]	4.67 ± 0.03[b]
SD	1.73 ± 0.03[c]	0.97 ± 0.03[c]	0.62 ± 0.03[c]	1.77 ± 0.03[a]	4.30 ± 0.03[c]
Old leaves					
WW	1.93 ± 0.02[a]	1.33 ± 0.08[a]	0.65 ± 0.02[b]	1.46 ± 0.08[a]	4.97 ± 0.14[a]
MD	1.96 ± 0.03[a]	1.32 ± 0.02[a]	0.67 ± 0.01[a]	1.48 ± 0.02[a]	4.83 ± 0.02[a]
SD	1.96 ± 0.04[a]	1.30 ± 0.04[a]	0.68 ± 0.03[a]	1.50 ± 0.01[a]	4.84 ± 0.01[a]

Values are mean ± SE. Chl *a*, Chl *b*, and Car are expressed as mg g^{-1} FW. Different letters indicate significant differences ($P < 0.05$) between water treatments using the LSD test

WW well-watered, *MD* moderate drought, *SD* severe drought

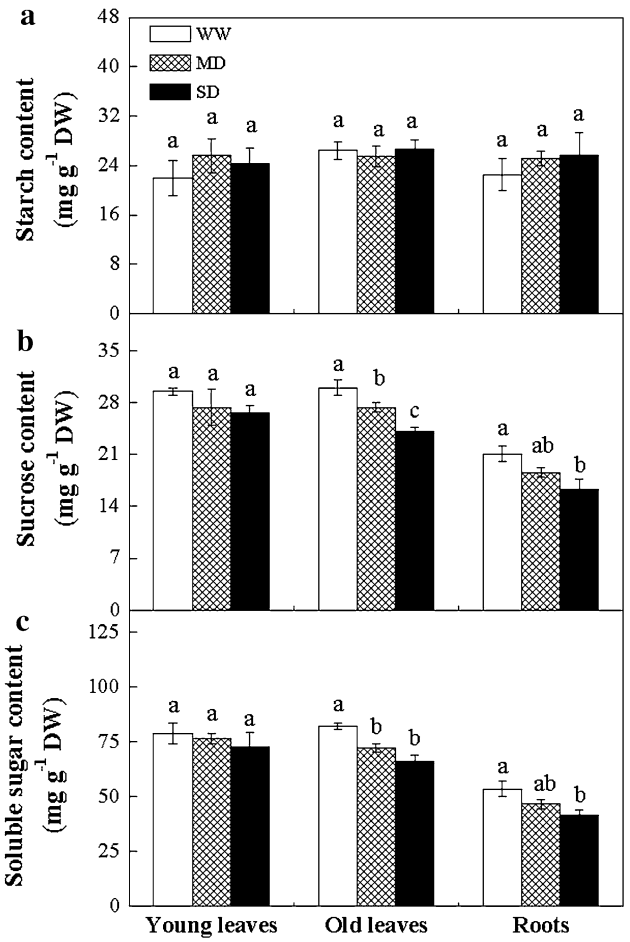

Fig. 2 Effects of drought stress on the contents of starch (**a**), sucrose (**b**), and soluble sugar **c** in young and old leaves and in roots of *F. denudata* plants. WW, well-watered treatment; MD, moderate drought; SD, severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

studied significantly increased with increasing drought stress intensity, except in roots under MD (Fig. 3c). SPS activity in young leaves was insignificant among water treatments (Fig. 3d). However, its activity in old leaves under MD and SD was reduced by 44.5 and 76.2 %,. respectively, compared to WW, and increased in roots only under SD. The organ and water levels and their interaction were significant for the activities of the four enzymes ($P < 0.05$), except the water level and interaction for AMY ($P > 0.05$) by the two-way ANOVA (Table 4).

Reduction of NO_3^- and NH_4^+ Production

NO_3^- concentration in all plant organs studied was not affected by drought stress, except in roots under SD, where it increased by 64.8 % with respect to that under WW (Table 3). NR activity in young leaves increased by 29.4 % under MD and was hardly affected by SD compared to WW. NR activity in old leaves and roots under SD was always higher than that under WW and MD, whereas its activity between WW and MD displayed no differences. NO_3^- concentration decreased or increased in all plant organs studied after drought stress. NiR activity under MD and SD increased by 27.9 and 33.7 % in young leaves and by 90.1 and 232.3 % in roots, respectively, compared to WW. A significant increase in NiR activity in old leaves was observed only under SD. NH_4^+ concentration in young leaves under MD and SD rose by 83.5 and 70.7 %, respectively, compared to WW. Its concentration increased only under SD in both old leaves and roots. The ratio of NH_4^+/NO_3^- in all plant organs studied presented different trends. The organ and water levels and their interaction significantly affected the above-mentioned parameters ($P < 0.01$), except the water level and organ-water level interaction for NO_3^- ($P > 0.05$) according to two-way ANOVA (Table 4).

Incorporation of NH_4^+ and Assimilation Products

GS and GOGAT activities in young leaves under MD and SD were enhanced significantly, by approximately 22.3 and 47.1 % and by 64.0 and 87.7 %, respectively, compared to

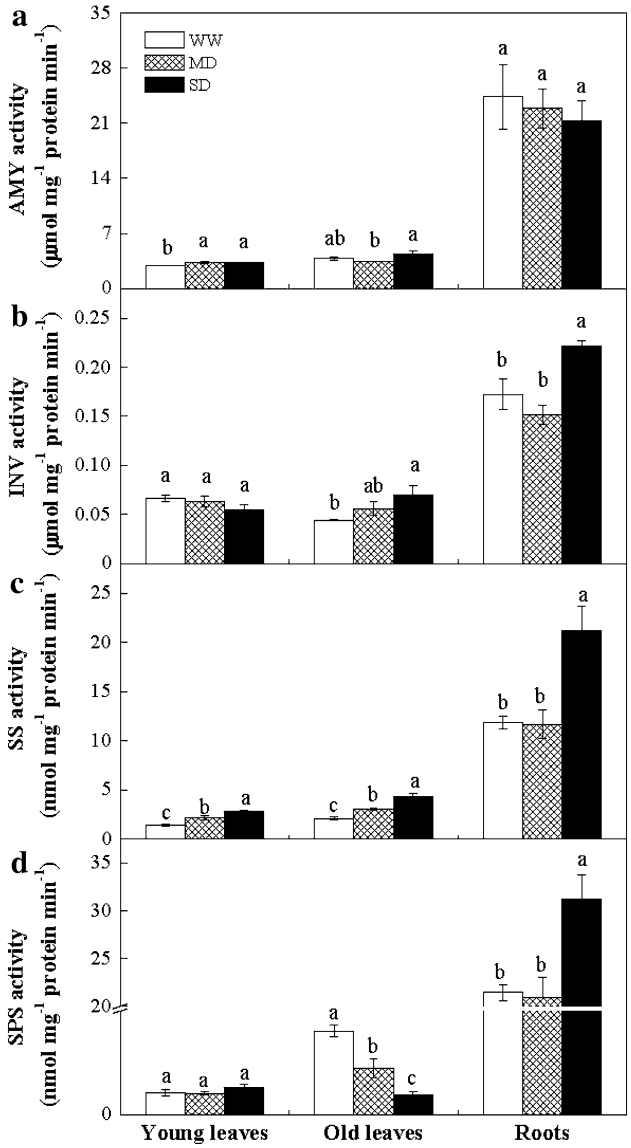

Fig. 3 Effects of drought stress on the activities of amylase (AMY, **a**), invertase (INV, **b**), sucrose synthase (SS, **c**), and sucrose phosphate synthase (SPS, **d**) in young and old leaves and in roots of *F. denudata* plants. *WW* well-watered treatment, *MD* moderate drought, *SD* severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

WW (Fig. 4). Drought stress had no effect on GS activity in old leaves, whereas its activity in old leaves under SD was 30.8 % higher than that under MD (Fig. 4a). GOGAT activity significantly increased after drought stress (Fig. 4b). Both enzymes' activities in roots were unchanged under MD but increased strongly under SD (Fig. 4). GDH activity was not affected in all plant organs studied under drought stress, but its activity in old leaves under SD was 34.6 % greater than that under MD (Fig. 5). Similarly, GOT activity was affected little by drought stress, except in roots under SD, where it showed a 41.3 % increase compared with that under WW (Fig. 6a). By contrast, GPT activity in young leaves under MD and SD rose by 16.2 and 21.6 %, respectively, whereas it increased only under SD in both old leaves and roots (Fig. 6b). The organ and water levels and their interaction significantly affected the above-mentioned enzyme activities ($P < 0.05$), except the organ-water level interaction for GS and GDH and the water level and organ-water level interaction for GOT ($P > 0.05$) by two-way ANOVA (Table 4).

Drought stress strongly decreased reduced N content in all plant organs studied, except for a 6.0 % increase in old leaves under SD compared with that under WW (Fig. 7a). Total N content in young and old leaves was not affected by drought stress, but it declined in roots (Fig. 7b). Moreover, its content in old leaves under SD was 13.1 % higher than that under MD. Amino acid content in young leaves was also unchanged after drought stress, whereas its content in old leaves and roots under SD increased by 10.2 and 29.5 % respectively, compared to that under WW (Fig. 7c). In general, soluble protein content declined after drought stress, except for a slight increase in old leaves under MD (Fig. 7d). The organ and water levels and their interaction significantly affected the four N components ($P < 0.05$), except water levels for total N using two-way ANOVA (Table 4).

Discussion

Plant Growth

Drought stress generally results in a strong reduction of the aerial parts of plants. For example, leaf morphological characteristics and quantity, stem height, and aboveground biomass of *Populus kangdingensis* and *Pinus halepensis* decreased significantly under drought conditions (Inclán and others 2005; Yin and others 2005). However, aboveground biomass was not affected by drought stress in barley (Sicher and others 2012), common bean (Cuellar-Ortiz and others 2008), and *Medicago sativa* (Erice and others 2010). In our study, aboveground growth parameters of young *F. denudata* plants were depressed by drought stress for two consecutive years during sprouting period, whereas those of old plants were hardly affected (Table 1). This result is attributed to the selected drought period which was carried out from July to August, which is the sprouting peak and fast growing period of *F. denudata*, where the growth in height is about 92 % of that for the whole year (Wang and Ma 1993). Moreover, the phenomenon also indicates that the sprouting period is a critical point when young *F. denudata* plants are sensitive to soil water and drought stress will seriously affect the quality of the young plants, whereas their tolerance against drought will be improved the following year.

Table 3 Effects of drought stress on NO_3^- reduction and NH_4^+ concentration as well as the ratio of NH_4^+/NO_3^- in *F. denudata* plants

Type/treatment	NO_3^-	NR	NO_2^-	NiR	NH_4^+	NH_4^+/NO_3^-
Young leaves						
WW	2.267 ± 0.370[a]	0.231 ± 0.009[b]	0.031 ± 0.000[b]	1.603 ± 0.051[b]	0.600 ± 0.011[c]	0.281 ± 0.051[b]
MD	2.785 ± 0.161[a]	0.299 ± 0.030[a]	0.028 ± 0.001[c]	2.050 ± 0.040[a]	1.024 ± 0.008[b]	0.370 ± 0.022[b]
SD	2.304 ± 0.435[a]	0.256 ± 0.010[ab]	0.033 ± 0.001[a]	2.144 ± 0.075[a]	1.101 ± 0.006[a]	0.524 ± 0.122[a]
Old leaves						
WW	2.600 ± 0.475[a]	0.349 ± 0.014[b]	0.034 ± 0.000[a]	3.329 ± 0.088[b]	0.691 ± 0.008[b]	0.285 ± 0.054[a]
MD	2.619 ± 0.076[a]	0.336 ± 0.006[b]	0.031 ± 0.000[b]	3.195 ± 0.173[b]	0.712 ± 0.005[b]	0.272 ± 0.006[a]
SD	2.869 ± 0.158[a]	0.455 ± 0.041[a]	0.021 ± 0.001[c]	4.339 ± 0.223[a]	0.870 ± 0.012[a]	0.305 ± 0.020[a]
Roots						
WW	1.257 ± 0.182[b]	0.806 ± 0.059[b]	0.009 ± 0.000[c]	1.925 ± 0.223[c]	0.654 ± 0.010[b]	0.543 ± 0.080[a]
MD	1.507 ± 0.122[b]	1.292 ± 0.178[b]	0.011 ± 0.001[b]	3.660 ± 0.044[b]	0.656 ± 0.004[b]	0.440 ± 0.034[ab]
SD	2.072 ± 0.112[a]	2.073 ± 0.165[a]	0.013 ± 0.002[a]	6.397 ± 0.013[a]	0.678 ± 0.004[a]	0.329 ± 0.018[b]

Values are mean ± SE. NO_3^-, NO_2^-, and NH_4^+ are expressed as mg g^{-1} FW; nitrate reductase (NR) and nitrite reductase (NiR) are expressed as nmol mg^{-1} protein min^{-1}. Different letters indicate significant differences ($P < 0.05$) between water treatments using the LSD test

WW well-watered, *MD* moderate drought, *SD* severe drought

Table 4 Tests for plant organ (O), water treatment (W), and their interactive effects with two-way ANOVA

Variables	Starch		Sucrose		Soluble sugar		AMY		INV		SS		SPS	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
For carbon metabolism parameters														
O	0.746	0.489	56.877	<0.001	64.968	<0.001	114.182	<0.001	242.114	<0.001	148.761	<0.001	377.932	<0.001
W	0.645	0.537	11.079	0.001	8.370	0.003	0.120	0.888	8.832	0.002	16.320	<0.001	5.676	0.012
O × W	0.290	0.880	0.508	0.731	0.565	0.691	0.324	0.846	7.833	0.001	7.200	0.001	10.264	<0.001

Variables	NO_3^-		NO_2^-		NH_4^+		NR		NiR		GS		GOGAT	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
For nitrogen metabolism parameters														
O	13.025	<0.001	1676.449	<0.001	736.718	<0.001	158.711	<0.001	218.745	<0.001	103.088	<0.001	467.789	<0.001
W	1.483	0.253	12.259	<0.001	675.004	<0.001	22.748	<0.001	188.887	<0.001	24.404	<0.001	89.778	<0.001
O × W	1.143	0.368	112.881	<0.001	321.143	<0.001	17.074	<0.001	73.465	<0.001	1.608	0.216	15.605	<0.001

Variables	GDH		GOT		GPT		Reduced nitrogen		Total nitrogen		Free amino acid		Soluble protein	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
For nitrogen metabolism parameters														
O	131.577	<0.001	289.962	<0.001	639.695	<0.001	3690.887	<0.001	796.910	<0.001	337.318	<0.001	769.531	<0.001
W	4.237	0.031	2.301	0.129	56.968	<0.001	16.452	<0.001	1.636	0.222	11.470	0.001	10.755	0.001
O × W	2.662	0.066	2.257	0.103	16.112	<0.001	32.607	<0.001	7.869	0.001	3.340	0.033	6.700	0.002

Underground parts of plants are also affected by drought stress. *F. denudata* has a complex underground system composed of a root system and rhizome system (Wang and Ma 1993). There was little difference in rhizome biomass under consecutive drought, whereas root and total underground biomass remarkably diminished with increasing drought stress intensity (Fig. 1). This result has been supported by the work of Yin and others (2005), who found a striking decrease in root dry matter of *P. kangdingensis*. Nevertheless, most recent studies have demonstrated that root growth is unaffected by drought stress (Inclán and others 2005; Erice and others 2010; Sicher and others 2012); this may be related to soil water potential. Both effects can lead to increases in the root weight ratio, root biomass/leaf area ratio, and root/shoot biomass ratio, as an adaptation trait to drought tolerance (Yin and others 2005; Zeid and Shedeed 2006; Erice and others 2010), because root growth is often less susceptible to drought than aerial growth and thus aerial parts contribute more to root to some extent (Zeid and Shedeed 2006). The morphological strategy that helps plants survive under drought conditions inevitably depends on the partitioning of C and N

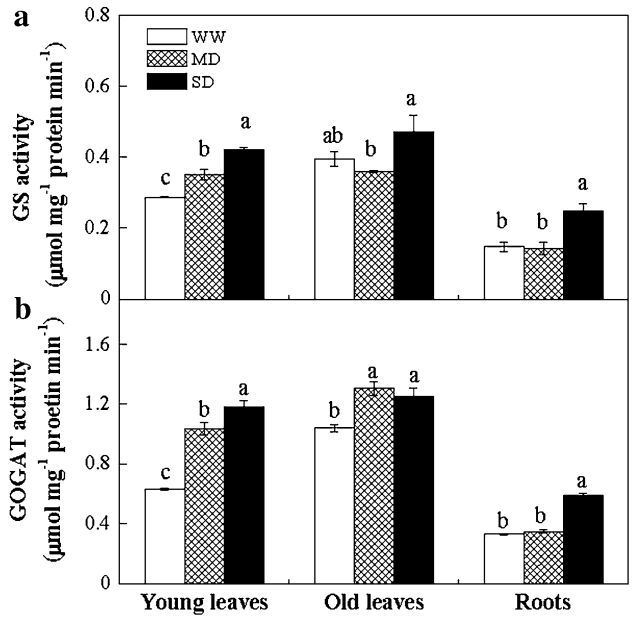

Fig. 4 Effects of drought stress on the activities of glutamine synthetase (GS, **a**) and glutamate synthase (GOGAT, **b**) in young and old leaves and in roots of *F. denudata* plants. *WW* well-watered treatment, *MD* moderate drought, *SD* severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

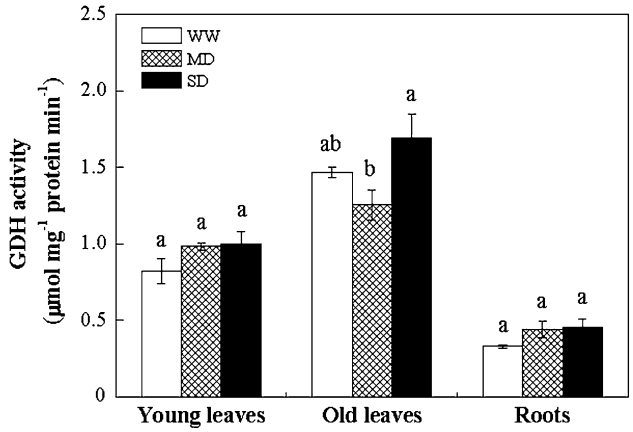

Fig. 5 Effects of drought stress on glutamate dehydrogenase (GDH) activity in young and old leaves and in roots of *F. denudata* plants. *WW* well-watered treatment, *MD* moderate drought, *SD* severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

compounds at the whole-plant level (Cuellar-Ortiz and others 2008; Erice and others 2010; Sanchez-Rodriguez and others 2011).

Carbon Metabolism

Carbon assimilation is an important part of the photosynthesis process and is strongly depressed by drought,

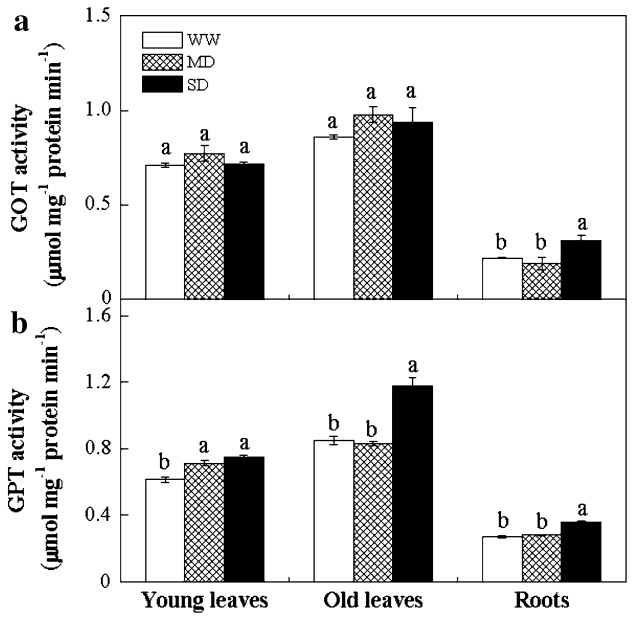

Fig. 6 Effects of drought stress on the activities of glutamic-oxaloacetic transaminase (GOT, **a**) and glutamic-pyruvic transaminase (GPT, **b**) in young and old leaves and in roots of *F. denudata* plants. *WW* well-watered treatment, *MD* moderate drought, *SD* severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

resulting in a decrease in plant primary production (Cuellar-Ortiz and others 2008). It is well known that a reduced CO_2 assimilation rate under drought conditions is associated with stomatal and nonstomatal limitations (Lawlor 2002b; Reddy and others 2004). Previous studies have indicated that when plants are subjected to rapid or mild drought stress, stomatal limitation is the major determinant of photosynthesis inhibition due to an ultimate decline in intercellular CO_2 concentration of leaves (Yin and others 2005). On the contrary, nonstomatal limitation normally accounts for a larger proportion of photosynthetic inhibition under slow, moderate, or severe drought stress, as reviewed by Lawlor (2002b). The nonstomatal limitation is often implicitly assumed as a metabolic constraint, such as degradation of photosynthetic pigments from impaired chloroplast structure (Reddy and others 2004). Photosynthetic pigments play an important role in the absorption and utilization of light energy and thus can be used as indicators of photosynthetic capacity (Johnston and others 1989; Yoo and others 2003). In our study, drought stress decreased Chl *a*, Chl *b*, and Car contents and the Chl *a* + *b*/Car ratio in young leaves of *F. denudata*, whereas it hardly affected them in old leaves (Table 2); this is consistent with the results found for the leaves of *Leymus chinensis* (Xu and Zhou 2007) and *M. sativa* (Zeid and Shedeed 2006). Besides, the changes in photosynthetic pigments during leaf ontogeny rely mainly on leaf age (Xu

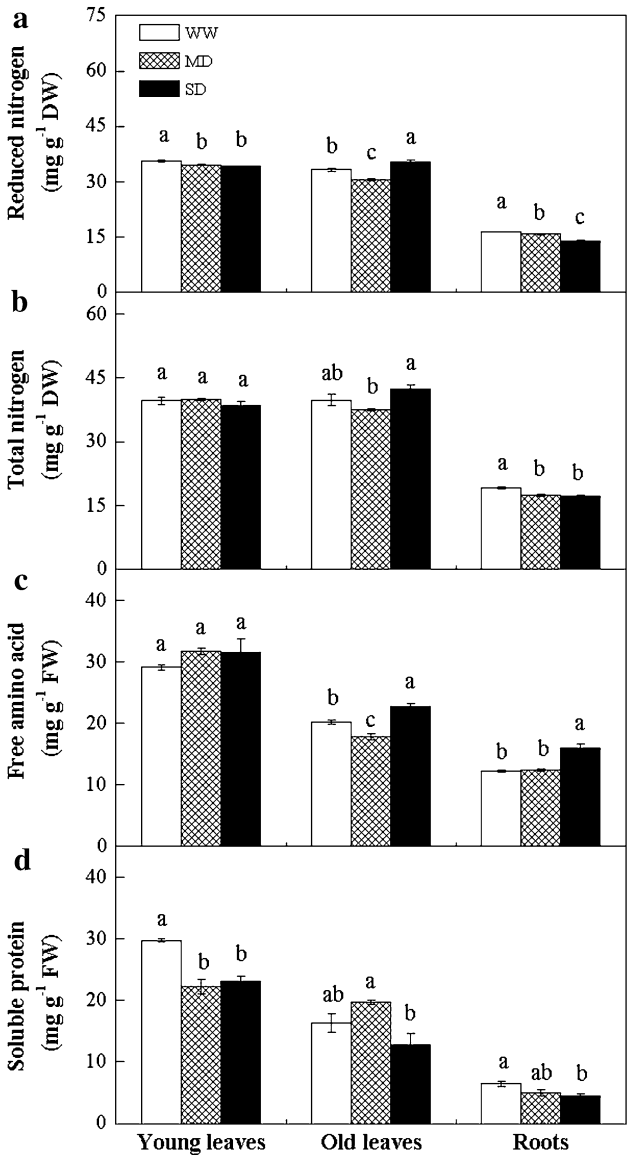

Fig. 7 Effects of drought stress on the concentrations of reduced nitrogen (**a**), total nitrogen (**b**), free amino acid (**c**), and soluble protein (**d**) in young and old leaves and in roots of *F. denudata* plants. *WW* well-watered treatment, *MD* moderate drought, *SD* severe drought treatment. *Different letters* indicate significant differences ($P < 0.05$) between water treatments using the LSD test. *Vertical bars* represent ± SE

and others 2008). Yoo and others (2003) found that mature green leaves could maintain stable levels of pigments for photosynthetic C assimilation. Therefore, we deduce that the old leaves of *F. denudata* may have a higher photosynthetic efficiency to be well adapted to consecutive drought.

In higher plants, starch is a predominant photosynthetic product that serves as a storage form of C in chloroplasts to support metabolism (Zeeman and others 2004). The change in starch content under drought conditions depends on photosynthetic efficiency and some enzymes involved in starch degradation, especially amylase (α- and β-amylase). Yang and others (2001) and Todaka and others (2000) reported that higher starch-hydrolyzing enzyme activity enhanced drought resistance of rice and cucumber, respectively, which could partly explain the decrease of starch with increasing drought intensity (Basu and others 2007). Our study showed that drought stress kept starch content unchanged, although AMY activity was stimulated in young leaves of *F. denudata* (Figs. 2a, 3a), suggesting that the demand for photosynthetic products may be reduced in young leaves subjected to drought stress. There are two reasons for the unaltered starch content in old leaves and roots under drought stress (Fig. 2a). One is that AMY activity remained relatively stable in both (Fig. 3a), and the other is that the resynthesis of starch related to sucrose degradation compensates for starch loss. Moreover, in common with the study on *M. sativa* leaves (Zeid and Shedeed 2006), a slight decrease or increase in AMY activity was observed under MD or SD, respectively (Fig. 3a). The result may be attributed to functional differences between α- and β-amylase under drought stress, because unlike β-amylase, which destroys intact starch granules, α-amylase mainly regulates starch decomposition (Todaka and others 2000; Yang and others 2001; Zeid and Shedeed 2006). Hence, we infer that synthesis and breakdown of starch are in dynamic balance at the whole-plant level in drought-stressed *F. denudata*.

Accumulation of soluble sugars, including sucrose, is an adaptive response to drought stress (Sicher and others 2012), and is closely associated with three key enzymes: INV, SS, and SPS. Drought stress can diminish the activities of SS and INV involved in sucrose-cleaving in the leaves of plants such as pea (González and others 1998) and *M. sativa* (Zeid and Shedeed 2006). Our study demonstrated that SS seemed to be more advantageous than INV in sucrose cleavage, although little change was detected in the conversion of sucrose and soluble sugar in young leaves of *F. denudata* (Fig. 2b, c), indicating that they can buffer fluctuations in sugar content for osmotic stress caused by drought. In contrast, SS and INV increased in old leaves after drought stress (Fig. 3b, c) with simultaneous lower sugars, which might account for accumulation of hexoses (Praxedes and others 2006; Basu and others 2007). Moreover, drought stress decreased sugar content in roots, especially under SD (Fig. 2b, c), resulting from a higher hydrolysis rate. SPS plays an important role in sucrose synthesis due to its high affinity to substrates. Haupt-Herting and Fock (2002) observed that the activity of SPS declined with reduced water potentials. However, different organs of *F. denudata* showed multiple changes in SPS activity under drought stress. This indicates that the partitioning of carbohydrates (soluble sugars, sucrose, and

starch) can regulate the water potential, which may be a response to this type of stress.

Nitrogen Metabolism

Nitrogen metabolism regulation is of key importance for drought tolerance, and interference between drought and N nutrition is a very complex network that affects almost all physiological processes in plants (Lawlor 2002a). Our results showed that drought stress hardly affected NO_3^- concentration in the leaves of *F. denudata*, although NR activity slightly increased (Table 3), which was attributed to the NO_3^- supply and transfer rate from the vacuole into cytoplasm. Similarly, Sanchez-Rodriguez and others (2011) reported that the higher NO_3^- and NR activity could improve the growth of tolerant tomato cultivars (cv. Zarina) under water deficit. However, several previous studies have reported that drought stress can inhibit the uptake of NO_3^-, with a resulting decrease in NR activity (Foyer and others 1998; Xu and Zhou 2006a; Robredo and others 2011). By contrast, NO_2^- concentration generally declined after drought stress and, in turn, NiR was stimulated positively, especially in young leaves (Table 3), perhaps because the degree of NiR expression was independent of the NO_2^- level (Heldt and Piechulla 2004). NH_4^+ is produced not only by the reduction of NO_3^- but also, more importantly, by the oxidation of glycine in an activated photorespiration process after water stress (Wang and others 2012a). Drought stress increased the NH_4^+ concentration in *F. denudata* leaves (Table 3), which agrees with the result obtained by Sanchez-Rodriguez and others (2011). In addition, the increment of NH_4^+ in young leaves was always greater than that in old leaves (Table 3). Our results also showed that MD had no effect on NO_3^- reduction and NH_4^+ production, whereas they were strongly enhanced under SD (Table 3). This indicates that roots subjected to different stress intensities probably have different NH_4^+ transmission mechanisms.

Excessive NH_4^+ from both NO_3^- reduction and photorespiration is toxic to plant cells, so it must be assimilated quickly by the GS/GOGAT cycle (Thomas and Hilker 2000). In general, drought stress leads to a decreased activity of this cycle, which has previously been documented for the leaves of *Populus nigra* (Sibout and Guerrier 1998) and *M. sativa* (Naya and others 2007). On the contrary, the activity of the GS/GOGAT cycle in *F. denudata* leaves increased under drought stress (Fig. 4a, b), especially in young leaves, due to a higher NH_4^+ concentration (Table 3). This result is supported by Sanchez-Rodriguez and others (2011), who noted that cv. Zarina displayed a significant increase in activity of this cycle for enhanced drought tolerance. Interestingly, the activity of the GS/GOGAT cycle in *F. denudata* roots was not affected by MD, whereas its activity significantly increased under SD (Fig. 4a, b), which coincided with the previous results (Xu and Zhou 2006a, b; Naya and others 2007). Thus, we confirmed that NH_4^+ assimilation of *F. denudata* is accelerated under drought stress at the whole-plant level, whereas the responses vary with plant organs and stress intensity.

On the other hand, although GDH has a lower affinity to NH_3, it is also an important pathway in fast NH_4^+ assimilation and alleviates the toxicity due to high NH_4^+ concentrations when the GS/GOGAT cycle is restrained (Cao and others 2007). Sanchez-Rodriguez and others (2011) demonstrated that GDH activity was significantly increased in sensitive tomato cultivars (Josefina and Salome) to eliminate NH_4^+ and generate more glutamate against osmotic damage caused by water stress. On the other hand, drought stress depressed GDH activity in *L. chinensis*, which might be related to decreased C skeleton (α-oxoglutarate), depending on photosynthetic capacity (Xu and Zhou 2006a). Differently, GDH activity in young and old leaves and in roots of *F. denudata* was not affected by drought stress (Fig. 5). Similar findings are observed in barley (Robredo and others 2011) and tolerant tomato cultivars (Zarina) (Sanchez-Rodriguez and others 2011) exposed to water stress. Consequently, we think that *F. denudata* has a strong ability to adapt to drought stress through NH_4^+ assimilation mainly via the GS/GOGAT cycle, not the GDH pathway. However, a few studies provide proof that GDH is not a representative route for NH_4^+ assimilation and glutamate formation in plants (Suzuki and Knaff 2005). Furthermore, the glutamate produced in the GS/GOGAT cycle or the GDH pathway can be converted to alanine or aspartate and to α-oxoglutarate via reversible amino group transfer. This process is catalyzed by GOT and GPT (González and others 1998). The two enzymes play an important role in the resynthesis of amino acids, with the availability of C skeletons from the Krebs cycle for transport to the shoot, and it is also believed that they control the redistribution of pools of C and N metabolism between plant cell cytoplasm and other compartments (Hodges 2002). Our results showed that GPT activity in *F. denudata* leaves was activated by drought stress, whereas GOT activity was not affected (Fig. 6). This suggests that the transamination function by GPT is the major pathway relative to GOT in our plants. However, both enzymes in *F. denudata* roots were significantly enhanced only under SD (Fig. 6), which might be associated with higher activity of the GS/GOGAT cycle in the roots (Fig. 4a, b).

The proportion of leaf N content is essential in estimating CO_2 assimilatory capacity, and especially in bamboo species, it is considered a critical factor in forage nutritional value (Wang and Ma 1993; Sinclair and others

2000). It has been reported that drought can depress plant N availability and reduce leaf N (Sinclair and others 2000). Reduced N, formed mainly by amino acids and protein, is usually used to quantitatively describe the result of the incorporation of NH_4^+ (Sanchez-Rodriguez and others 2011). In our study, reduced N and total N in *F. denudata* roots decreased after drought stress (Fig. 7a, b); this was attributed to an increased proportion of nitrogen reallocation in young leaves and/or proteolysis. Thus, although reduced N in young leaves declined due to protein degradation, total N remained stable (Fig. 7b). Both N forms in old leaves decreased under MD and increased under SD (Fig. 7a, b), a finding similar to that of previous studies on *L. chinensis* (Xu and Zhou 2006a). Moreover, free amino acid contains several transfer compounds involved in N metabolism and important osmotic substances (Sibout and Guerrier 1998). We observed that drought led to a slight accumulation of free amino acids in young leaves and roots (Fig. 7c), and, at the same time, soluble protein declined (Fig. 7d), indicating that drought strengthens the N transfer function and osmotic adjustment to a certain degree. However, our results also showed significant nonsynchronous changes in amino acids and proteins in old leaves (Fig. 7c, d), in agreement with the results of Sanchez-Rodriguez and others (2011). Our results also may explain the growth status of *F. denudata* plants.

Conclusions

The results presented in this article overall indicate that C and N metabolism in young and old leaves and in roots of *F. denudata* plants displays different responses to consecutive drought stress during the sprouting period. Drought stress scarcely affected carbohydrates contents in young leaves due to balanced regulation of related enzymes, whereas N metabolism was spurred by drought to relieve growth inhibition in young plants. On the other hand, drought stress accelerated hydrolysis of carbohydrates in old leaves, whereas only SD positively affected NO_3^- reduction and NH_4^+ assimilation. Unlike leaves, N metabolism in roots was steady under MD, while it was enhanced under SD, in parallel with C metabolism. Different organs of *F. denudata* plants with their various physiological strategies will ensure adaptation to different drought intensities for regular growth at the whole-plant level.

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