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ORIGINAL ARTICLE

Non-structural carbohydrate dynamics in *Robinia pseudoacacia* saplings under three levels of continuous drought stress

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Abstract

Key message **Only severe drought stress induced lower non-structural carbohydrate concentrations in *Robinia pseudoacacia* saplings, and non-structural carbohydrate reduction occurs solely in branches and stems at the end of the growing season.**

Abstract A better understanding of non-structural carbohydrate (NSC) dynamics in trees under drought stress is critical to elucidate the mechanisms underlying forest decline and tree mortality that result from extended periods of drought. We performed a continuous controlled drought pot experiment from May 14 to September 7, 2013 using *Robinia pseudoacacia* saplings under 14, 10, 8, and 6 % soil water content that represented the absence of drought (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress, respectively. Growth, leaf gas exchange, and NSC concentrations in different sapling tissues were measured once a month. The results showed that net photosynthetic rates and the relative increments of basal diameter and height decreased with the intensification of drought stress. Saplings showed lower starch and NSC concentrations and higher soluble sugar concentrations and ratios of sugars to starch only under SD and VSD. The concentrations of starch and NSC were significantly decreased under SD and VSD solely in branches and stems at the end of the growing season. Our results indicated that the timescale of physiological processes plays an important role in NSC dynamics, and that only severe drought stress significantly decreases NSC concentrations, and NSC reduction occurs solely in branches and stems at the end of the growing season.

Keywords Carbon starvation · Drought progression · Soluble sugars · Starch · Tree tissues

Introduction

Drought-induced forest decline occurs worldwide and is likely to be exacerbated by future climate change with far-reaching ecological consequences (Adams et al. 2009; Allen et al. 2010; Bréda et al. 2006). However, the mechanisms of drought-induced tree decline, even death remain unclear (McDowell et al. 2008; Sala et al. 2010). Parker and Patton (1975) suggested that trees are vulnerable to carbon starvation induced by drought stress mainly due to the reduction in photosynthesis, but with less decrease in respiration than photosynthesis, which leads to a severe and eventually lethal decrease in the reserves of non-structural carbohydrates (NSCs). This suggestion was also supported by other more recent studies (Adams et al. 2009; Bréda et al. 2006; McDowell et al. 2008; Sala et al. 2010) and used to explain their results. However, there is considerable variability and complexity in the response of NSCs to drought, due to differences in drought properties (i.e., intensity and progression) as well as in tree size, age, tissues, and species (Hartmann et al. 2013; Sala et al. 2011,

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2012). Consequently, the lack of agreement among studies on the effect of drought on NSC dynamics suggests that further investigation is needed to elucidate this hypothesis (McDowell et al. 2008; O'Grady et al. 2013).

Different levels of drought intensity induce different physiological processes (Ditmarová et al. 2010; Hsiao 1973), which may induce various NSC dynamics in trees (McDowell et al. 2008). It has been speculated that severe drought stress may not induce NSC depletion, as happens under mild or moderate drought stress, since trees may die of irreversible xylem cavitation (McDowell et al. 2008). However, the lack of studies focusing on the effect of drought intensity on NSC dynamics has impeded our progress in the exploration of the hypothesis that there is a drought intensity level that results in trees dying from NSC depletion (McDowell et al. 2008).

In addition, drought progression may also influence NSC dynamics (McDowell 2011). In the early phases of drought, NSC concentrations may increase because growth decreases before declines in the rates of photosynthesis occur (Hsiao 1973; Körner 2003). However, as drought conditions develop, photosynthesis may decline more than respiration, and as a result NSC concentration decreases with the increasing imbalance between carbohydrate production and use (Hsiao 1973; McDowell 2011). In the absence of firm evidence, it is assumed that only long-lasting drought stress will lead to NSC depletion (McDowell 2011). Most previous studies used short-term drought treatments and compared the initial and final NSC concentrations, neglecting the dynamic and interdependent changes over time (Anderegg and Anderegg 2013; Regier et al. 2010). As a result, it is necessary to explore how drought progression affects NSC dynamics (McDowell and Sevanto 2010; Sala et al. 2010).

It has been demonstrated that different tree tissues have distinct functions and store different amounts of carbohydrates (Hoch et al. 2003; Millard et al. 2007; Würth et al. 2005). Variations in NSC concentrations resulting from drought stress in different tree tissues may vary significantly. Drought-induced changes in carbon allocation, use, and transport have been observed to differ between above- and below-ground tree tissues. Hartmann et al. (2013) reported that under drought conditions, NSC concentrations were significantly reduced in roots, but not in leaves or branches. However, the measurement of NSC concentrations in different tree tissues is arduous and has only been carried out in a few studies (Dietze et al. 2013). Studies that have focused on one or a few tree tissues may have led to ambiguous conclusions (Ryan 2011); thus, it has been recommended that it is necessary to take into account the carbon metabolism in each tissue (Hoch et al. 2003; Ryan 2011; Würth et al. 2005). Overall, it is essential to compare the response of NSC concentration in different tree tissues to drought stress with different intensities and progression.

In this study, we investigated the effect of drought intensity and progression on NSC dynamics of different tissues in saplings of *Robinia pseudoacacia*, a species that was widely used for afforestation in the Loess Plateau of China due to its rapid growth, acclimation potential to drought and low soil fertility, and nitrogen fixation ability. In addition, we examined gas exchange and plant growth in order to interpret more comprehensively the mechanisms involved in NSC variations caused by drought stress. Specifically, we hypothesized that (1) severe, but not moderate, drought stress would influence NSC concentration significantly; (2) the decrease in NSC concentration would be observed after long-term drought stress, but not during the initial phases; and (3) the decrease in NSC concentration would only occur in some sapling tissues.

Materials and methods

Experimental site

A controlled pot experiment was conducted at the Institute of Soil and Water Conservation of the Chinese Academy of Sciences in Yangling, Shaanxi Province (34.2823°N; 108.0744°E), China. The climate of this region is warm continental monsoon with hot and wet summers and cold and dry winters. The study site has a mean annual rainfall of 632 mm, which mainly falls from July to September and a mean annual temperature of 12.9 °C. The main soil type is an Eum-Orthic Anthrosol according to the Food and Agricultural Organization soil classification system (FAO 1998).

Sapling preparation

Seventy-two 1-year-old *R. Pseudoacacia* saplings of similar size were obtained from a local tree nursery on April 8, 2013, when they started to bud. The roots were washed and each sapling was weighed before it was transplanted to a separate pot (30 cm in height, 27 cm in diameter). The bottom of each pot was covered with about 3 kg of stones (3 cm in diameter). A plastic tube (3 cm in diameter, 30 cm in length) used for irrigation was placed vertically just above the stones. Filter paper was then placed over the stones and around the plastic tube. Approximately, 14 kg of soil was added to the pot over the filter paper and around the sapling. This arrangement facilitated water uptake by the roots and prevented sealing of the soil surface during irrigation. The soil was collected from the 0–30 cm soil layer of a 5-year abandoned local grassland. The collected soil was first air dried and then sieved through a 10-mesh screen to remove any roots, coarse debris, and grit. The content of soil organic carbon (8.89 g kg^{-1}), total nitrogen (0.96 g kg^{-1}), available phosphorus (1.47×10^{-2} g kg^{-1}) and available potassium (0.15 g kg^{-1}) were measured using the potassium dichromate and sulfuric acid method, micro-Kjeldahl method, $NaHCO_3$ method, and NH_4OAc and flame photometer method (Du et al. 2007), respectively. Soil pH (7.71) was measured in 0.01 M $CaCl_2$ (1:5) according to method 4B2 of Rayment and Higginson (1992). After transplanting, the basal diameter and height of each sapling were measured using a vernier caliper with an accuracy of 0.01 mm and a tape measure with an accuracy of 0.1 cm, respectively.

At the experimental site, the pots were placed in rows with 50 cm apart from the neighbors under full sunlight. They were kept well watered prior to the application of drought treatments, and the gravimetric soil water content was initially maintained at field capacity (19.98 %). The soil surface was well ventilated throughout the experiment.

Application of drought treatments

On May 14, 36 days after transplanting, when all saplings had expanded leaves, four pots were randomly selected for measuring pre-treatment photosynthesis and sampling, while the remaining sixty-eight pots were numbered and randomly divided into four groups ($n = 17$ each group). Saplings were randomly reassigned until no significant differences in diameter and height were detected between groups by one-way analysis of variance (ANOVA) ($P = 0.947$ for basal diameter; $P = 0.286$ for height). Each group was randomly subjected to a different drought intensity treatment: absence of drought stress (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress (Li et al. 2008; Liu et al. 2013). The soil gravimetric water content in the four treatments was maintained at 14 % (AD), 10 % (MD), 8 % (SD), and 6 % (VSD), which were equivalent to 70, 50, 40, and 30 % of field capacity, respectively. The soil gravimetric water content of 14 % does not induce drought stress, since it is sufficient to satisfy the water requirements of *R. pseudoacacia* growing in this soil type, and also cannot prevent low oxygen concentrations or any other undesirable effects on nutrient availability (Liu et al. 2013).

Pots were weighed every 2 days using an industrial-grade balance with an accuracy of 5 g. Initially, the pots were allowed to dry until the intended water content was attained around May 17 for AD, May 22 for MD, May 30 for SD, and June 7 for VSD. Subsequently, each pot was irrigated to maintain the desired pot mass, and this watering regime was continued until September 7, when the last four living saplings under VSD group were sampled and there were four, three, two living saplings left in the AD, MD and SD sapling groups after sampling, respectively. During the experiment, a rainshed (3 m in height) was used when it rained to prevent the addition of water to the pots. Pot positions were changed regularly throughout the experiment to reduce any micro-environmental effects.

Measurements and sampling

Apart from four saplings on May 14, sixteen healthy-looking saplings, four from each group, were also randomly selected for measuring net photosynthesis between 09:00 and 11:00 h using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) on June 17, August 3, and September 7. Measurements were performed on the youngest fully expanded leaves of every selected sapling. The reference CO_2 concentration in the chamber was set at 385 ppm using a 12-g Li-Cor CO_2 cartridge as a CO_2 source. The light environment in the chamber was set at 1000 μmol m^{-2} s^{-1} using the 6400-2B red/blue LED light source.

On the same days, the basal diameter and height of the same saplings that were selected for photosynthesis measurements were also measured. The saplings were then harvested for NSC analysis. They were divided into leaves, branches, stems, coarse roots (>2 mm), and fine roots (<2 mm) and washed separately with distilled water. Any remaining surface water was then removed with blotting paper, and all sample tissues were weighed, packed into labeled envelopes, and placed in a portable cooler. Sampling was performed between 14:00 and 17:00 h, because NSC concentrations in the leaves fluctuate daily with photosynthetic activity and the export of starch from the leaves (Graham et al. 2003). Within 2 h of collection, the samples were microwaved for 90 s at 650 W to prevent enzymatic carbohydrate reactions (Hoch et al. 2002). Subsequently, sample tissues were dried at 80 °C until constant mass, and the dried material was weighed. For NSC measurements, all dried samples were ground using a Wiley Mill until they were sufficiently fine to pass through a 40-mesh screen.

NSC analysis

NSC concentration was defined as the sum of soluble sugar and starch concentrations that were measured using the anthrone method (Yemm and Willis 1954). Ground material (0.1000 g) was placed into a 10-ml centrifuge tube, and 2 ml of 80 % ethanol was then added. The mixture was incubated at 80 °C in a shaking water bath for 30 min and then centrifuged at $5000\times g$ for 5 min. A further two extractions from the pellets were carried out with 80 %

ethanol. The supernatant was retained, combined, and stored at −20 °C for soluble sugar determination.

Starch was extracted from the ethanol-insoluble pellet after ethanol was first removed by evaporation. The starch in the residue was then released by boiling in 2 ml distilled water for 15 min. After cooling to room temperature, 2 ml 9.2 M $HClO_4$ was added, and the mixture was shaken for 15 min. Four milliliters of distilled water was then added, and the mixture was centrifuged at $5000\times g$ for 5 min. A further extraction was carried out with 2 ml 4.6 M $HClO_4$. The supernatant was also retained, combined, and stored at −20 °C for starch determination.

Sugar and starch determination were performed based on the absorbance at 625 nm using the same anthrone reagent in a spectrophotometer (Yemm and Willis 1954). Sugar concentration was calculated from the regression equations based on glucose standard solutions and starch concentration by multiplying glucose concentration with a conversion factor of 0.9 (Osaki et al. 1991). Sugar and starch concentrations were expressed as a % of dry matter.

Calculations and statistical analysis

The relative increments of basal diameter and height of saplings were calculated as follows:

$$R_i = \frac{b_i - b_{i-1}}{b_{i-1}},$$

where R_i is the relative increment of basal diameter (mm) or height (cm) on the sampling date; b_i is the basal diameter or height on the sampling date; and b_{i-1} is the basal diameter or height on the previous sampling date.

The ratios between soluble sugar and starch concentrations were calculated for each tissue to evaluate drought-induced changes in mobilization-accumulation and osmotic adjustments (Piper 2011).

All statistical analyses were performed using SPSS v.17.0 (SPSS, Inc., Chicago, USA). Repeated-measures analysis of variance (ANOVA) was used to determine the effects of drought intensity (AD, MD, SD, and VSD) and progression (May 14, June 17, August 3 and September 7) on net photosynthetic rates, the relative increments of basal diameter and height, and NSC (sugar and starch) concentrations in each tissue. The assumption of sphericity was tested using Mauchly's sphericity test (Mauchly 1940). NSC concentrations in each tissue type were tested for normality. The Bonferroni test was used to identify significant differences ($P < 0.05$) in the mean net photosynthetic rates, relative increments of basal diameter and height, and NSC (sugar and starch) concentrations in each tissue. The homogeneity of variance was tested using Levene's test.

Results

Gas exchange and growth of saplings under different drought treatments

The effects of drought intensity and progression on the net photosynthetic rates and relative increments of basal diameter and height were all significant ($P < 0.01$; Fig. 1). The variation in the net photosynthetic rates of saplings under MD, SD, and VSD with drought progression was different from that under AD. The net photosynthetic rate of saplings under AD reached the maximum value on June 17 and significantly decreased thereafter, reaching the lowest value on September 7 ($P < 0.05$; Fig. 1), while those under MD, SD, and VSD fluctuated throughout the experiment, and the highest values were observed on May 14, before drought treatments were initiated. Thus, the net photosynthetic rates of saplings under MD, SD, and VSD were significantly lower than that under AD. These results showed that the net photosynthetic rates of saplings decreased with the intensification of drought stress (Fig. 1a).

The variation in the relative increments of basal diameter and height of saplings under drought stress was consistent with that under AD. The relative increments of basal diameter increased until they reached the highest value on August 3 and then decreased (Fig. 1b), while those of height decreased throughout the experiment (Fig. 1c). Moreover, the relative increments of basal diameter and height of saplings decreased with the intensification of drought stress.

Soluble sugar concentrations in different sapling tissues

Different sapling tissues (leaves, stems, branches, coarse roots, and fine roots) showed different sugar concentration responses to drought intensity and progression (Fig. 2). Under AD, sugar concentrations throughout the experiment increased in leaves but decreased in the other tissues ($P < 0.01$; Table 1; Fig. 2). The variation in sugar concentration in different sapling tissues under MD followed similar patterns to that under AD. As a result, sugar concentrations under MD did not show any significant differences from those under AD. However, sugar concentrations under SD and VSD exhibited evidently different variation patterns from those under AD and were significantly higher than those under AD. Sugar concentrations under SD and VSD were higher by 7.4 and 16.0 % in leaves, 13.5 and 19.7 % in branches, 25.7 and 76.3 % in stems, 24.6 and 67.0 % in coarse roots, and 26.8 and 28.1 % in fine roots, respectively, compared to those under AD.

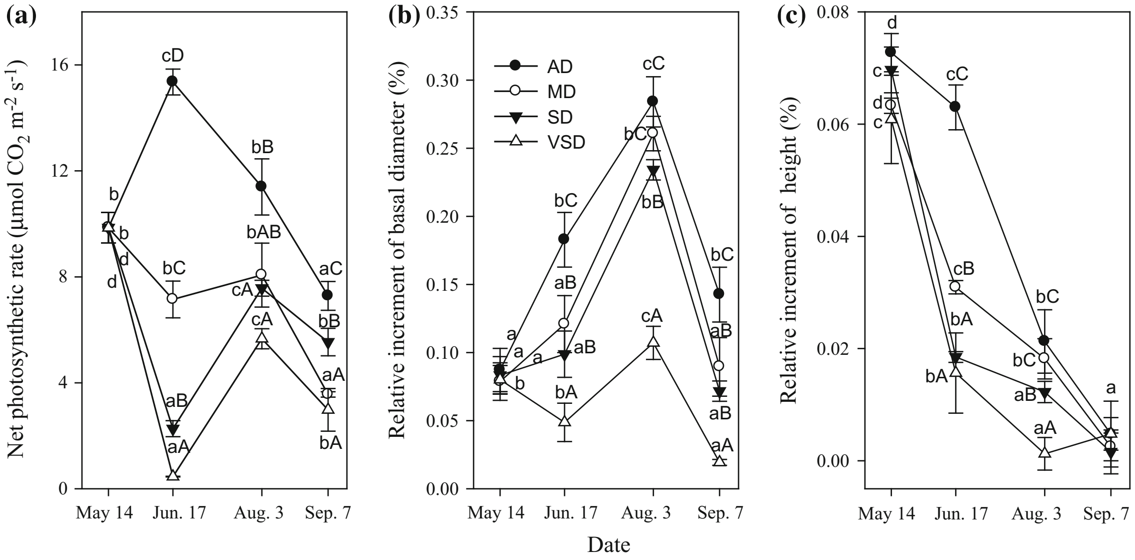

Fig. 1 Variation in **a** the net photosynthetic rates, and relative increments of **b** basal diameter, and **c** height of saplings under the absence of drought (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress on four different sampling dates in 2013. Net photosynthetic rate on May 14, before drought treatments were initiated, is the mean value of four random saplings. *Bars* indicate one standard error of the mean ($n = 4$). Different *small letters* indicate significant differences between sampling dates, while *capital letters* between different drought intensity levels (Bonferroni test; $P < 0.05$)

Starch concentrations in different sapling tissues

Starch concentrations decreased in leaves, but increased in the other tissues throughout the experiment (Fig. 3). In leaves, starch concentrations under MD, SD, and VSD were 15.8, 17.8, and 43.9 % lower, respectively, compared to those under AD ($P < 0.05$; Fig. 3a). In the other tissues, starch concentrations under AD and MD showed similar variation patterns with no significant differences ($P > 0.05$; Fig. 3b). However, starch concentrations under SD and VSD were significantly lower than those under AD. Starch concentrations under SD and VSD were lower by 27.5 and 11.2 % in branches, 3.7 and 7.6 % in stems, 4.6 and 27.5 % in coarse roots, and 1.9 and 5.4 % in fine roots, respectively, compared to those under AD.

During August 3 and September 7, starch concentrations in branches and stems under SD and VSD were significantly decreased, while those under AD were significantly increased ($P < 0.05$; Fig. 3b, c). Meanwhile, starch concentrations in coarse roots under VSD were significantly increased, while those under AD remained constant.

NSC concentrations in different sapling tissues

Both drought intensity and progression significantly influenced NSC concentrations in different sapling tissues ($P < 0.01$; Table 1). NSC concentrations decreased in leaves, but increased in the other tissues throughout the experiment (Fig. 4a–e). In leaves, NSC concentrations under MD, SD, and VSD were 9.4, 6.8, and 18.6 % lower, respectively, compared to those under AD ($P < 0.05$ Fig. 4a). In branches, stems, and coarse roots, the variation in NSC concentrations with drought progression under MD was similar to that under AD ($P > 0.05$), while NSC concentrations under SD and VSD were significantly lower than those under AD ($P < 0.05$; Fig. 4b–e). NSC concentrations under SD and VSD were lower by 19.7 and 5.3 % in branches, 1.0 and 1.9 % in stems, and 1.0 and 15.6 % in coarse roots, respectively, compared to those under AD. In fine roots, NSC concentrations under VSD were significantly lower on August 3, but significantly higher on September 7, compared to those under AD ($P < 0.05$; Fig. 4e).

In branches and stems, NSC concentrations under SD and VSD were significantly decreased from August 3 to September 7, while those under AD were significantly increased ($P < 0.05$; Fig. 4b, c). In coarse roots, NSC concentrations under VSD were significantly increased during the same period, while those under AD remain constant (Fig. 4d).

The ratios of soluble sugars to starch in different sapling tissues

The effects of drought intensity and progression on the ratios of soluble sugars to starch in each tissue were all

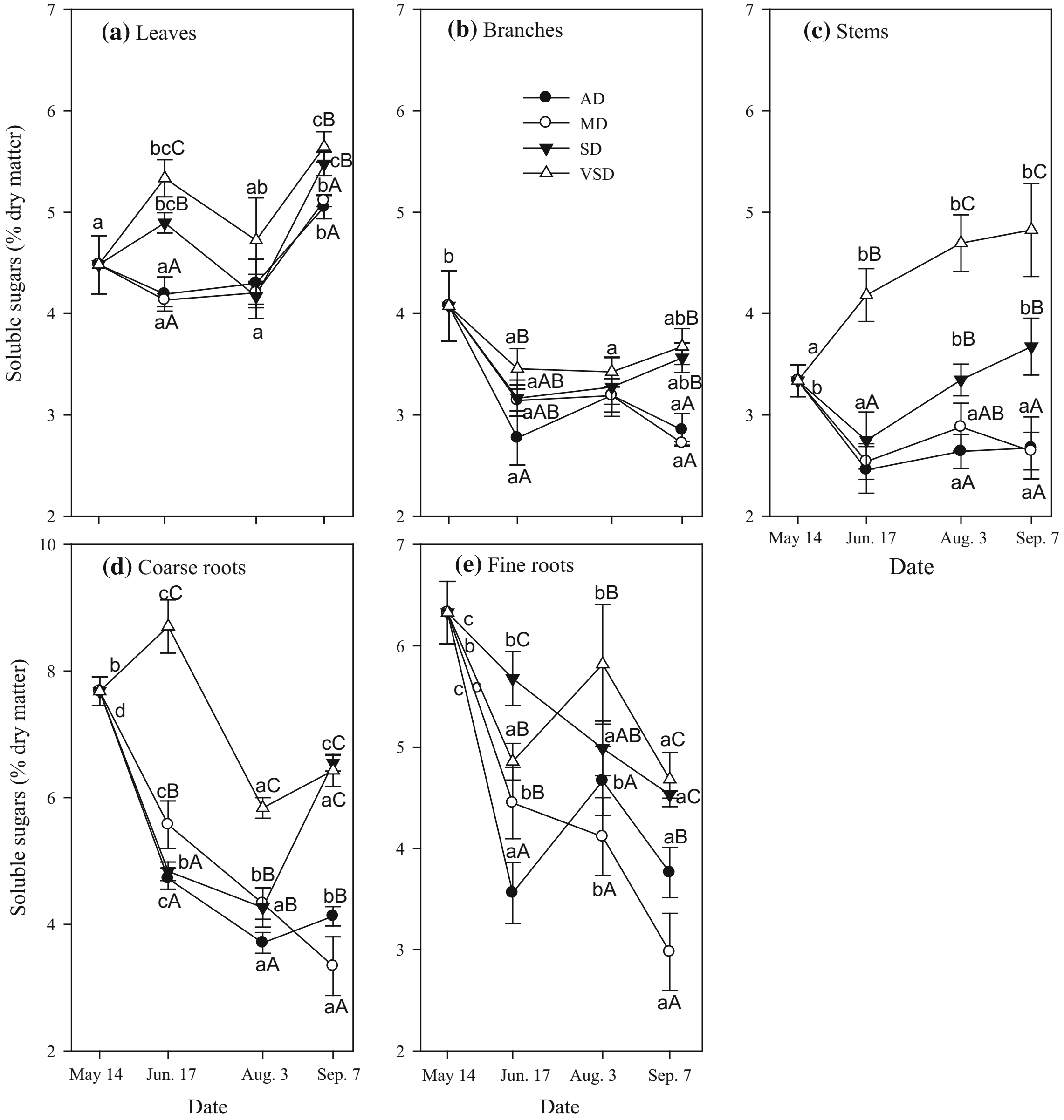

Fig. 2 Soluble sugar concentrations in **a** leaves, **b** branches, **c** stems, **d** coarse roots, and **e** fine roots under the absence of drought (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress on four different dates in 2013. Sugar concentration on May 14, before drought treatments were initiated, is the mean value of four random saplings. *Bars* indicate one standard error of the mean ($n = 4$). Different *small letters* indicate significant differences between sampling dates, while *capital letters* between different drought intensity levels (Bonferroni test; $P < 0.05$)

significant ($P < 0.01$; Table 1). Despite drought intensity, the ratios of soluble sugars to starch in leaves tended to increase throughout the experiment, while that in all other tissues tended to decrease ($P < 0.05$; Fig. 5). No significant differences were observed between the ratios under AD and MD in any tissue ($P > 0.05$), except for leaves, in which the ratios were significantly higher under MD than that under AD ($P < 0.05$). However, in each tissue, the ratios were significantly higher under SD and VSD than under AD ($P < 0.05$).

Discussion

In this study, both drought intensity and progression significantly affected net photosynthetic rates, the relative increments in basal diameter and height, and NSC dynamics in different sapling tissues. Although drought stress decreased the net photosynthetic rates and growth of saplings under MD, SD, and VSD, only SD and VSD significantly decreased NSC concentrations. Meanwhile, NSC concentration reduction was observed only in

Table 1 *F*-statistics and probabilities (*P*) from repeated-measures analysis of variance on soluble sugar, starch, non-structural carbohydrate (NSC) concentrations and the ratios of soluble sugars to starch (sugars: starch) in different sapling tissues

Variables	Tissues	Drought intensity		Drought progression		Drought intensity × progression	
		F	*P*	*F*	*P*	*F*	*P*
Sugars	Leaves	18.17	<0.01	45.19	<0.01	5.15	<0.01
	Branches	10.53	<0.01	53.30	<0.01	3.15	<0.01
	Stems	121.88	<0.01	12.85	<0.01	16.57	<0.01
	Coarse roots	190.39	<0.01	444.44	<0.01	61.00	<0.01
	Fine roots	35.71	<0.01	133.86	<0.01	10.44	<0.01
Starch	Leaves	46.47	<0.01	485.17	<0.01	15.76	<0.01
	Branches	122.72	<0.01	557.65	<0.01	69.15	<0.01
	Stems	20.98	<0.01	1399.78	<0.01	42.38	<0.01
	Coarse roots	56.99	<0.01	811.38	<0.01	14.60	<0.01
	Fine roots	25.13	<0.01	2413.43	<0.01	46.99	<0.01
NSC	Leaves	11.48	<0.01	149.56	<0.01	6.87	<0.01
	Branches	54.39	<0.01	225.78	<0.01	31.70	<0.01
	Stems	6.33	<0.01	1078.44	<0.01	32.43	<0.01
	Coarse roots	24.80	<0.01	584.24	<0.01	8.45	<0.01
	Fine roots	7.37	<0.01	1612.56	<0.01	46.82	<0.01
Sugars: starch	Leaves	145.01	<0.01	414.38	<0.01	27.00	<0.01
	Branches	60.81	<0.01	620.28	<0.01	26.66	<0.01
	Stems	100.52	<0.01	1500.93	<0.01	19.15	<0.01
	Coarse roots	79.28	<0.01	1590.94	<0.01	33.04	<0.01
	Fine roots	39.71	<0.01	1310.90	<0.01	20.19	<0.01

Columns present the *F* and *P* values of drought intensity, progression, and intensity–progression interaction (drought intensity × progression)

branches and stems at the end of the experiment. These results are consistent with all our initial hypotheses.

Effects of drought intensity on NSC dynamics

In this study, the effects of drought intensity on NSC dynamics in different sapling tissues were all significant. In different sapling tissues, NSC concentrations and their components under SD and VSD, but not under MD, showed significant differences compared to those under AD. Under SD and VSD, soluble sugar concentrations were higher, while starch concentrations were significantly lower than those under AD. Meanwhile, the increase in soluble sugars was lower than the decrease in starch; thus, NSC concentrations under SD and VSD were significantly lower than those under AD. These results are consistent with our first hypothesis that severe, but not moderate, drought stress would influence NSC concentration significantly.

Differences among the effects of drought intensity on NSC concentrations may result from the changes in carbon source or sink and the underlying regulatory mechanisms. Studies have found that, under carbohydrate deficiency, trees can increase NSC storage at the expense of growth (Pantin et al. 2013; Saffell et al. 2014; Wiley et al. 2013). Moreover, Wiley et al. (2013) found that trees under moderate carbohydrate deficiency maintained NSC levels of tress without carbohydrate deficiency, while trees under severe carbohydrate deficiency had significantly lower NSC. In our study, net photosynthetic rates decreased with the intensification of drought stress (Fig. 1a), which induced more severe carbohydrate deficiency under SD and VSD than under MD. As a result, NSC concentrations under MD were consistent with, while those under SD and VSD were significantly lower than, those under AD. However, NSC storage under drought stress is more complicated than that regulated only by carbohydrate source, as drought stress not only influences carbon source, but also carbon sink (Fatichi et al. 2014). However, carbon sink characteristics were not investigated in this study; thus, further studies coupling measurements of cell division and expansion with measurements of photosynthesis and NSC dynamics will aid in elucidating the mechanisms potentially underlying the effect of drought intensity on NSC (Epron et al. 2012).

Soluble sugars play an important role in osmotic adjustment, vascular transport, and embolism refilling

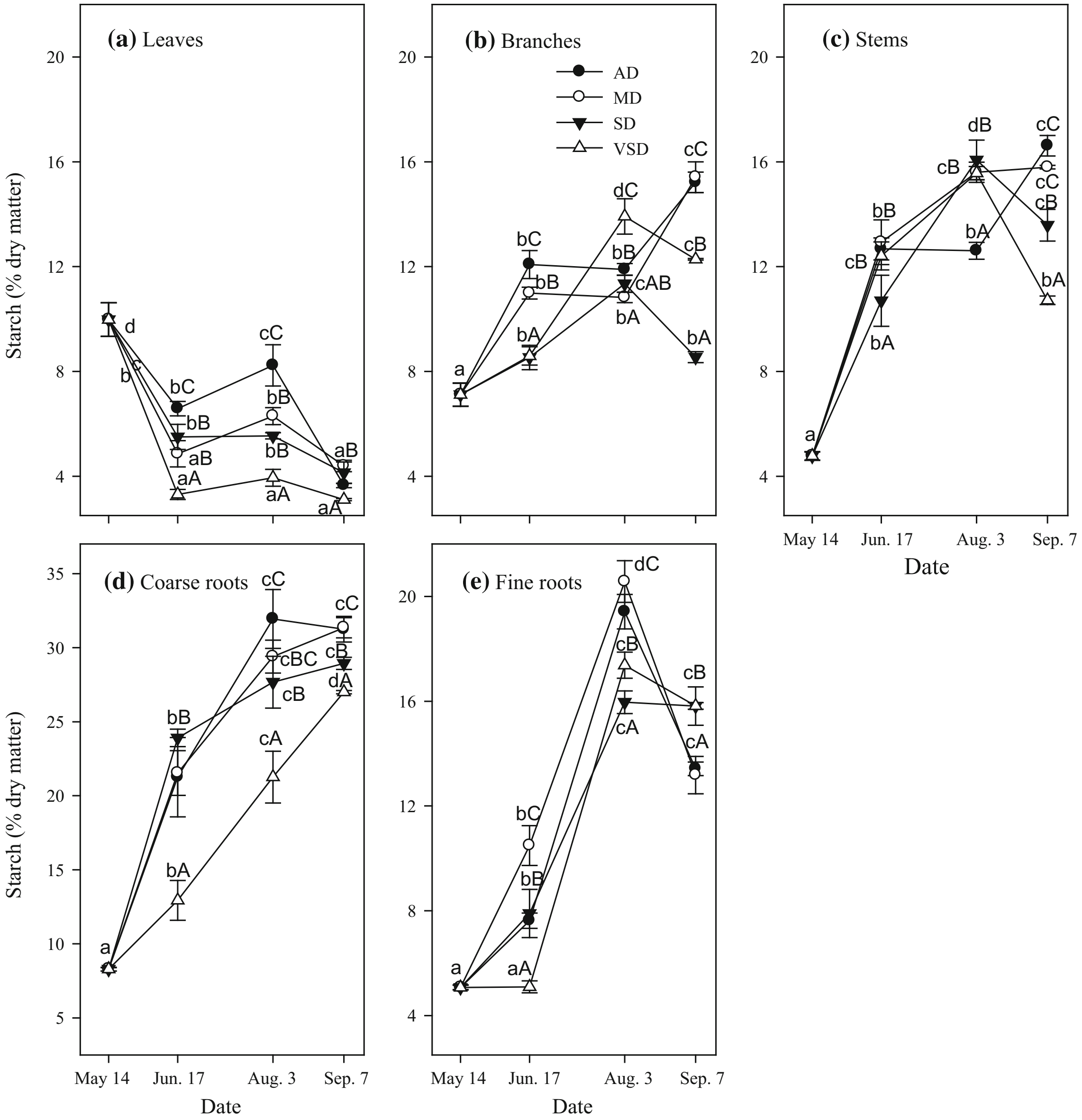

Fig. 3 Starch concentrations in **a** leaves, **b** branches, **c** stems, **d** coarse roots, and **e** fine roots under the absence of drought (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress on four different sampling dates. Starch concentration on May 14, before drought treatments were initiated, is the mean value of four random saplings. *Bars* indicate one standard error of the mean ($n = 4$). Different *small letters* indicate significant differences between sampling dates, while *capital letters* between different drought intensity levels (Bonferroni test; $P < 0.05$)

(Sala et al. 2012). The increase in soluble sugar concentrations has been reported in various studies (Latt et al. 2001; Silva et al. 2010). Increased sugar concentrations can decrease tree water potential, maintain cell turgor, and increase water absorption (Handa et al. 1983; Silva et al. 2010). Meanwhile, under drought stress, the synthesis of starch is inhibited, and the activity of hydrolases is accelerated, resulting in the increase in the ratio of soluble sugars to starch. Latt et al. (2001) reported the transformation of starch to sugars in the boles and structural roots of trees under drought stress. In our study, however, an increase in the concentration of soluble sugars and the ratios of soluble sugars to starch was only observed under SD and VSD. Silva et al. (2010) reported that K^+ and Na^+ play the most important roles in osmotic adjustment under higher water content conditions, while soluble sugars are more important under relatively lower water content conditions. These results may suggest that *R. pseudoacacia* saplings can regulate osmosis through other pathways under MD, while they have to increase sugar concentrations to cope with the increasing drought stress under SD and VSD.

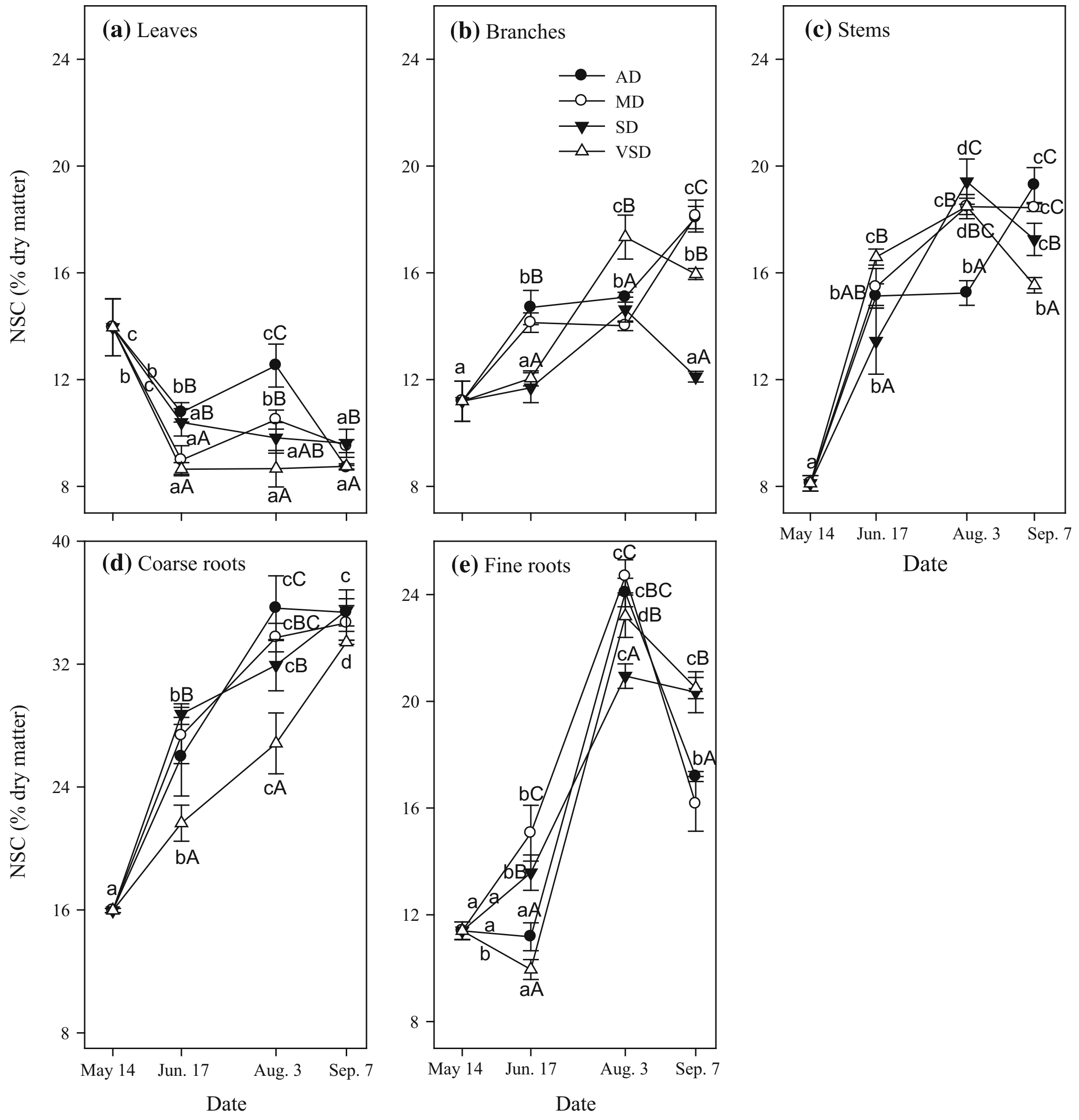

Fig. 4 Non-structural carbohydrate (NSC) concentrations in **a** leaves, **b** branches, **c** stems, **d** coarse roots, and **e** fine roots under the absence of drought (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress on four different sampling date. NSC concentration on May 14, before drought treatments were initiated, is the mean value of four random saplings. *Bars* indicate one standard error of the mean ($n = 4$). Different *small letters* indicate significant differences between sampling dates, while *capital letters* between different drought intensity levels (Bonferroni test; $P < 0.05$)

Effects of drought progression on NSC dynamics

NSC dynamics in different sapling tissues under drought treatments were generally consistent with those under AD from June 14 to August 3. However, NSC concentrations in branches and stems under SD and VSD decreased from August 3 to September 7, when those under AD significantly increased. Further, photosynthesis significantly decreased in all of the saplings (Figs. 1a, 4b, c) and, therefore, the carbon assimilates were reduced. These results are consistent with our second hypothesis that a decrease in NSCs would be observed when the drought period was long, but not during the initial drought phase.

Studies have indicated that the timescale of various physiological processes during different drought stages play an important role in NSC dynamics (Fatichi et al. 2014). In the short term, an acclimation lag of photosynthesis than growth may result in the accumulation of starch and NSCs (Hsiao 1973; Körner 2003). In the medium term, the decline in photosynthesis before respiration can reduce NSC storage, because of the utilization of NSCs for metabolism (Hsiao 1973; McDowell 2011). In the long term,

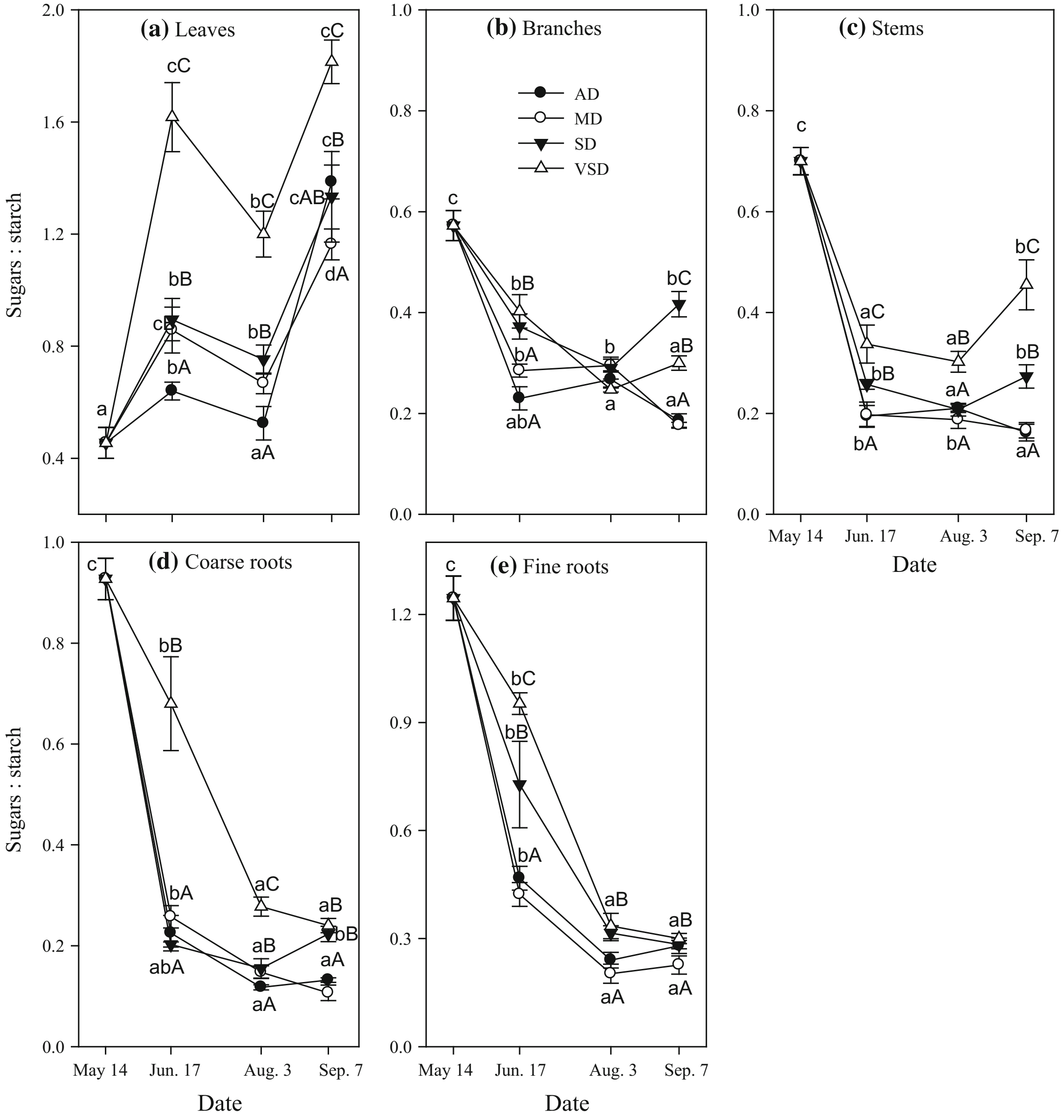

Fig. 5 The ratios of soluble sugars to starch (sugars: starch) in **a** leaves, **b** branches, **c** stems, **d** coarse roots, and **e** fine roots under the absence of drought (AD), moderate drought (MD), severe drought (SD), and very severe drought (VSD) stress on four different sampling dates in 2013. Sugars: starch on May 14, before drought treatments were initiated, is the mean value of four random saplings. *Bars* indicate one standard error of the mean ($n = 4$). Different *small letters* indicate significant differences between sampling dates, while *capital letters* between different drought intensity levels (Bonferroni test; $P < 0.05$)

sophisticated acclimation mechanisms are likely to fine-tune tree growth with photosynthesis to ensure NSC storage (Fatichi et al. 2014; Hoch et al. 2003; Kozlowski 1992). However, under conditions where trees cannot coordinate growth with photosynthesis, consumption of NSCs occurs (McDowell et al. 2008). In our study, we did not observe any higher NSC concentrations under drought stress compared to AD, which indicated that the time frame of 34 days (from the initiation of drought treatment to the first time sampling date) was too long for the down-regulation of photosynthesis. The lower, but continuous, increase in NSC concentrations under SD and VSD from May 14 to August 3 might be the result of the acclimation mechanisms that allowed saplings to adjust the balance of carbon sink and source, preventing NSC depletion and sustaining survival and reserve accumulation under drought stress (Scartazza et al. 2013). The significant decrease in NSC concentrations in branches and stems under SD and VSD at the end of the growing season may result from the maladjustment of growth, photosynthesis, and translocation of NSCs to metabolism. At the end of the growing season, as net photosynthetic rates under different drought treatments decreased significantly, saplings under AD and MD maintained relatively sufficient NSC supply by decreasing their growth, while saplings under SD and VSD, whose growth had decreased or even stopped prior to this period, consumed NSCs to maintain normal metabolism. In addition, we observed that NSC concentrations in roots under SD and VSD increased significantly concurrently with the NSC decrease in stems and branches, while those under AD and MD remained constant. Therefore, NSC increase in roots under SD and VSD might also result from the translocation of NSCs from the stems and branches to the roots.

Effects of drought stress on NSC dynamics in different sapling tissues

In this study, significant NSC decreases were observed in branches and stems under SD and VSD from August 3 to September 7, but not in any other tissues. This supported our third hypothesis that the decrease in NSCs would only occur in some tissue types. In contrast, Landhäusser and Lieffers (2012) found that NSCs in roots of mature *Populus tremuloides* Michx. under carbohydrate deficiency decreased earlier and more than that in branches, and after carbohydrate supply returned to normal, root NSCs recovered slower than branches. Further, Hartmann et al. (2013) found that NSCs were significantly decreased in roots, but not in leaves or branches, of *Picea abies* saplings under <3 % relative extractible soil water content. These differences may be related to the characteristics of carbon allocation and transport. Under optimum conditions, tissues near to the leaves, the major carbon source, appear to be favored in carbon allocation at the expense of other distant tissues (Jordan and Habib 1996), while under drought stress, trees tend to allocate more carbon to roots to facilitate water absorption and survive (Chapin et al. 1990). Large trees have root systems that are considered to be large carbon sinks, but these sinks are at the end of a long carbon transport chain, and the long-distance transport of carbon prevents the complete replenishment of NSCs in roots (Minchin 2007; Wardlaw 1990). However, in relatively small trees, the stem is short and forms a relatively small sink that can be fully replenished in time (Ludovici et al. 2002; Sanz-Pérez et al. 2009). Consequently, our result is contrary to that of Landhäusser and Lieffers (2012). In addition, severe drought can also impede carbon translocation from leaves, branches, and stems to roots (Hartmann et al. 2013; Sala et al. 2010). In our study, drought stress conditions were less severe than those used by Hartmann et al. (2013), and the different result between us may result from that carbon translocation from the canopy to the roots might be impeded by the severe drought stress in their study. Our results suggested that the effects of drought on NSC dynamics in different sapling tissues are distinctive, and that NSC reduction differs between trees of different age and size. Therefore, in future studies, the effects of drought on NSC dynamics are needed to be evaluated in different tissues from trees of different age and size, since focusing on a few specific tissues and ignoring the age and size of the tree could lead to erroneous conclusions (Sala et al. 2011).

NSCs play fundamental roles in plant germination, growth, reproduction, and defense, and may also be crucial to plant survival under drought stress (Kozlowski 1992). In particular, NSCs are mobilized in trees to maintain respiration during winter, build leaves and support new growth in spring, provide energy for any adaptive responses to soil water deficits, and provide resistance against attacks from pathogens and herbivores (Barbaroux and Bréda 2002; Kozlowski 1992). Furthermore, NSCs play a vital role in frost protection (Galvez et al. 2013; Hoch et al. 2003) and may be associated with winter embolism in large xylem vessels, which ensures that water flow pathways are restored in spring before the onset of transpiration (Bréda and Granier 1996; Sperry et al. 1994). *R. pseudoacacia* is a ring-porous species that achieves a large part of its annual stem growth and needs access to reserves for the new leaf flush before the leaf expansion in spring (Bréda and Granier 1996; Landhäusser 2011). Compared to diffuse-porous species, ring-porous species exhibit higher maintenance respiration (Barbaroux and Bréda 2002), higher basal respiration rates in sapwood tissues, and higher Q10 values (Edwards and Hanson 1996). As a result, *R. pseudoacacia* needs relatively large quantities of NSCs during the winter and spring. However, in our study, NSC concentrations decreased significantly in stems and branches under SD and VSD at the end of the growing season (Fig. 4b, c). Moreover, at the end of the growing season, since photosynthesis ceases but respiration continues, and there are increasing demands on NSCs in order to resist both frost and drought stress, NSC concentrations in the stems and branches would continue to decrease. Consequently, *R. pseudoacacia* saplings under SD and VSD may be vulnerable to frost and other stresses during the dormant season or may be unable to sprout in the following spring. Therefore, it is essential in future studies to explore the variation in NSCs and respiration during the dormant season to definitively reveal the full effect of drought on NSC dynamics (Galvez et al. 2013).

Conclusions

This study investigated the NSC dynamics in different tissues of *R. pseudoacacia* saplings subjected to different levels of drought stress. The results illustrated that both drought intensity and progression had significant effects on NSC dynamics of different sapling tissues. Only under severe drought stress were significantly lower NSC concentrations throughout the drought treatment observed and only towards the end of the growing season did significant decrease of NSCs prematurely occur. The significant reduction of NSC concentrations observed at the end of the growing season in branches and stems under severe drought stresses, may adversely affect their ability to protect against other stresses, such as frost during the dormant season, and impede sprouting in the following spring. This study showed that drought characteristics (intensity and progression) and tissue type are important for elucidate the relationship between NSC storage and drought stress. However, further study is needed to investigate NSC dynamics during the dormant season and sink characteristics under drought stress.

Author contribution statement Ting Zhang and Yang Cao designed and carried out the experiments. Ting Zhang wrote the manuscript with contributions from Yang Cao, Yunming Chen, and Guobin Liu.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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