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ORIGINAL PAPER

Responses of nonstructural carbohydrates to shoot removal and soil moisture treatments in *Salix nigra*

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Abstract Aboveground disturbances are common in dynamic riparian environments, and *Salix nigra* is well adapted with a vigorous resprouting response. Soil moisture stresses are also common, and *S. nigra* is flood tolerant and drought sensitive. The objective of this study was to quantify nonstructural carbohydrate (NSC) reserves in *S. nigra* following shoot removal and soil moisture treatments. NSC reserves provide energy for regeneration of shoot tissue until new functional leaves are developed. Three soil moisture treatments: well-watered (W), periodic flooding (F) and drought (D); and three shoot removal treatments: no shoots removed (R0), partial shoot removal (R1), and complete shoot removal (R2) were applied. Plants were harvested when new shoot development was observed (day 13). Statistical significance in the 3 × 3-factorial design was determined in two-factor ANOVA at $P < 0.05$. Both roots and cuttings were important reservoirs for NSC during resprouting response, with decreases in root (31%) and cutting (14%) biomass in R2 compared to R0. Rapid recovery of photosynthetic surface area (from 15 to 37% of R0) was found in R1. A clear pattern of starch mobilization was found in roots in R0, R1 and R2, with lowest root starch concentration in W, F higher than W, and D higher than F. Shoot starch concentration was lower in F and D compared to W in R0, however, in R1 shoot starch was reduced in W compared to F and D, possibly indicating reduced rates of translocation during soil moisture stress. Evidence of osmotic adjustment was found in roots and shoots with higher total ethanol-soluble carbohydrates (TESC) during soil moisture stress in F and D treatments. Total plant NSC pool was greater in F and D treatments compared to W, and progressively reduced from R0 to R1 to R2. Results indicated negative effects of drought, and to a lesser extent periodic flooding on resprouting response in *S. nigra*, with implications for reduced survival when exposed to combined stresses of aboveground disturbance and soil moisture.

Keywords *Salix nigra* · Nonstructural carbohydrates · Flooding · Drought · Shoot removal

Introduction

Resprouting response of plants is an important evolutionary adaptation for survival in environments where aboveground disturbance causes damage to shoot tissue (Sakai et al. 1997; Sakai and Sakai 1998; Baker et al. 2005). *Salix nigra* Marshall is a vigorous resprouter with energy for resprouting response provided by mobilization of nonstructural carbohydrate reserves (NSC) which are end products of photosynthesis (Chapman 1992; Baker et al. 2005). Sprouters may store more NSC in belowground organs than nonsprouters, and some woody plants store enough NSC to meet the energy needs of more than one resprouting event following disturbance (Cruz and Moreno 2001; Wildy and Pate 2002; Schwilk and Ackerly 2005).

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The greatest demand on NSC following aboveground disturbance is prior to development of new photosynthetic tissues. Dependence on NSC reserves is brief and decreases rapidly as new leaves begin to produce photoassimilates (Tschaplinski and Blake 1995; Landhausser and Lieffers 2002; Wildy and Pate 2002). Reductions in root and stem NSC of *Populus maximowiczii* × *nigra* L. were observed 4 days after decaptitation treatment (Tschaplinski and Blake 1995). NSC reserves provided most of the growth increment in coppiced *Morus alba* L. saplings up to day 10, and 50% of the growth increment between days 10 and 16 as evidenced by C^{14} incorporation into new shoot growth (Satoh and Ohyama 1976).

In riparian environments, plants are also well adapted to a pulsing hydrological cycle with shallow water tables and spring/winter periodic floods (Junk et al. 1989; Mahoney and Rood 1992; Middleton 1999). When the soil water content is at or near field capacity, conditions are ideal for diffusion of oxygen and ions, and most nutrients are in a soluble form (Mitsch and Gosselink 1993). However, soil moisture stress as a result of periodic flooding and drought events is a common cause of mortality of willow cuttings at riparian restoration sites (McLeod and McPherson 1973; Mahoney and Rood 1992; Shields et al. 1995; Karrenberg et al. 2002; Martin et al. 2005; Pezeshki et al. 2007).

Drought stress, unlike other environmental stresses, develops gradually over time, slowly increasing in intensity as water potential drops. Plants utilize osmotic adjustment as a mechanism to maintain water balance during drought, and delay reduced-water potential by accumulating solutes including sugars in cells. Osmotic adjustment helps maintain root and leaf turgor that is important in root growth, water uptake and cytokinine synthesis, as well as, leaf growth, photosynthesis, and the production of photoassimilates (Fitter and Hay 2002; Larcher 2003). Plants respond to drought by closing their stomata to reduce transpirational water loss, causing a corresponding decrease in the rate of photosynthesis, and decreased production of photoassimilates. Other responses include reduced chlorophyll content, leaf size, and growth rate (Kozlowski and Pallardy 1997; Kozlowski 2002).

Flooding can lead to loss of oxygen in the soil (at a soil redox potential level of approximately +350 mV) and reduction of soil minerals over time, depending on temperature, availability of organic substances for microbial respiration, and chemical oxygen demand (DeLaune and Pezeshki 1991; Mitsch and Gosselink 1993). Lack of oxygen in the soil prevents aerobic root respiration and affects availability of plant nutrients and toxic materials in the soil (Pezeshki et al. 1999).

S. nigra is a wetland plant with adaptations for survival in flooded conditions including the development of lenticels, adventitious roots and aerenchyma tissue that facilitates transport of oxygen from aboveground shoots to belowground roots (Seago et al. 2005). However, oxygen is also leaked into the rhizosphere surrounding the roots which may help moderate the effects of toxic reduced ions (Godfrey and Wooten 1981; Pezeshki et al. 1998; Amlin and Rood 2001; Cronk and Fennessy 2001; Pezeshki 2001).

Plants respond to flooding with stomatal closure leading to reduced rates of photosynthesis and a corresponding decrease in the supply of photassimilates (Pezeshki et al. 1999; Pezeshki 2001). Rate of photoassimilate transport to roots can decrease during flooding resulting in accumulation of starch in leaves (Kogawara et al. 2006). Short periods of flooding followed by draining can result in a slow recovery of preflooded-photosynthetic rates as stomata reopen (Kozlowski and Pallardy 1997; Li et al. 2005). Mean photosynthetic rate in periodically flooded *S. nigra* was 5 μmol m^{-2} s^{-1} compared to 9 μmol m^{-2} s^{-1} in well-watered control with decreases during flooded phases and recovery during drained phases (Pezeshki et al. 1998).

Partitioning of carbohydrates in roots can be affected by flooding, with greater allocation to storage, and less allocation to biomass and metabolism (Kogawara et al. 2006). A 42% decline in NSC was found following early-fall floods of 3 to 27 days in American cranberry (*Vaccinium macrocarpon* Ait.; Botelho and van Heuvel 2006).

The effect of shoot removal on NSC dynamics in woody plants is poorly understood, with little information available concerning the combined effects of soil moisture stress and shoot removal. The objective of this study was to quantify the effects of shoot removal and soil moisture treatments on NSC reserves in *S. nigra*. We asked the following questions: (1) What is the effect of shoot removal on NSC reserves? (2) What is the effect of soil moisture stress on NSC reserves? and (3) What are the combined effects of shoot removal and soil moisture stresses on NSC reserves? We hypothesized that shoot removal would lead to decreases in NSC as plants use reserves to produce new shoot tissue, and this response would be reduced in plants exposed to both shoot removal and soil moisture stresses. We expected a greater tolerance of *S. nigra* to periodic flooded conditions compared to drought due to plant adaptations including aerenchyma tissue. We measured NSC reserves in plants treated with shoot removal at the peak of demand to support the resprouting response, just prior to new, mature leaf development on day 13 after treatment.

Materials and methods

Plant materials

Dormant black willow (*Salix nigra* Marshall) cuttings (20-cm long and a basal diameter of 7–11 mm) were collected from three donor trees in a small, localized population on the Loosahatchie River floodplain in western TN on 26 February 2006.

Greenhouse procedures

All branches were removed, and cuttings were soaked overnight prior to planting, one per pot, in gusseted poly-bags (0.076-mm thickness) 12 × 5 × 20 cm, with 4.2l volume, containing one part washed sand and one part commercial topsoil (v/v). Plants were maintained in a greenhouse with ambient photoperiod. Temperature ranged from 18 to 42°C and humidity ranged from 5 to 91%. Photosynthetic photon flux density (PPFD) averaged 1,200 μmol m^{-2} s^{-1} at the top of plant canopy at midday in sunny conditions. Plants were grown for 6 weeks prior to initiation of treatments and fertilized weekly with 25-ml Peter's Professional liquid fertilizer (20-20-20, NPK) at a rate of 1.0 g l^{-1} $week^{-1}$.

Subsampling

Ten plants were harvested on 6 April 2006, the day before pruning and soil moisture treatments were initiated. All shoots in subsampled plants were counted, shoot heights were measured, and leaf areas were calculated. Fresh shoot and root weights were measured followed by drying in an oven to determine fresh:dry weight conversions (Wildy and Pate 2002). Shoots removed in partial shoot removal treatment were compared to complete shoot removal treatment to determine percent of photosynthetic surface area removed in partial shoot removal treatment compared to intact control. Dry shoot weights were used to develop correlational relationships between shoot height and shoot dry weight, and also between leaf dry weight and leaf area (Tschaplinski and Blake 1995).

Shoot removal and soil moisture treatments

Following subsampling, 108 plants were randomly assigned to 9 combinations of shoot removal (3 levels) and soil moisture (3 levels) with 12 replicates per group. The shoot removal treatments were: (1) no shoots removed (R0), (2) partial shoot removal (R1), and (3) complete shoot removal. In shoot removal treatments, secondary shoots were removed at the point of attachment to the cutting. In R1 one "primary" shoot was left on each plant. R1 was designed for partial removal (approximately 86%) of photosynthetic surface area; whereas, R2 removed 100% of photosynthetic surface area. The three soil moisture treatment groups were: (1) well-watered and well-drained control (W), (2) periodic flooded treatment, with water level maintained 2.5 cm above the soil surface for 5 days followed by drained conditions for 5 days (F), and (3) drought, with water withheld until wilting was observed (D). Pots were arranged in a randomized block design to reduce effects of light gradients across the experimental table, and facilitate maintenance of soil moisture treatments. Each row of six pots consisted of one soil moisture treatment, selected randomly, with two replicates of each shoot removal treatment.

Soil measurements

Soil oxidation–reduction potential (E_h) was measured in nine replicates for both flooded and well-watered treatments at 10-cm depth to quantify oxidation–reduction in the soil during flooded and drained periods using platinum-tipped redox electrodes, a millivoltmeter (ORION, model 250A, Thermo Orion, Beverly, MA, USA) and a calomel reference electrode (Corning, model 476350) as described in Patrick and DeLaune (1977). Measurements were taken after redox electrodes had equilibrated in the soil for 2 h. $E_h = +350$ mV is the approximate level where oxygen begins to disappear in the soil (DeLaune and Pezeshki 1991). Soil water potential was measured in drought treatment (MPa, $n = 6$) with a dewpoint potentiometer (Decagon Devices Inc., model WP4, Pullman, WA, USA). Leaf chlorophyll content (LCC) in R0 and R1 ($n = 10$ leaves) was measured in chlorophyll content index units (cci) with a chlorophyll content meter (Opti-Sciences, model CCM-200, Tyngsboro, MA, USA).

Final harvest

Plants were harvested when new mature leaf development was observed, day 13 after treatment. Harvested plant material was immediately placed in a freezer to stop cellular respiration. Then, plants were dried in an oven at 100°C for 1 h, followed by 70°C for 48 h for shoots and roots, or 72 h for cuttings (Canham et al. 1999). Biomass was measured in roots, shoots and cuttings, and leaf area ratio (LAR) was calculated as leaf area (cm^2) divided by plant biomass of roots and shoots (excluding cutting). Then, a regression equation was developed to predict leaf area at final harvest; predicted leaf area $= -6.408 + 0.064 \times \text{shoot height} + 83.643 \times \text{shoot biomass}$, $r^2 = 0.89$. Dried plant material was ground to 1-mm particle size, prior to NSC (starch and TESC) analyses using a tabletop laboratory knife mill (Retsch, Grindomix model GM200, Glenn Mills Inc., Clifton, NJ).

NSC analyses

The procedures used in the NSC analyses are described in detail in Hall et al. (1999) and Hall (2000). Three replicate

Table 1 Summary of two factor ANOVAs for shoot removal, soil moisture, and the interaction of shoot removal and soil moisture (SR × SM) treatments performed on measured variables, including: starch and TESC concentration, starch, TESC, and NSC pool

Measured variable	Shoot removal (SR)			Soil moisture (SM)			SR × SM		
	df	*P*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Starch conc.									
Cutting	2	27.262	0.000	2	4.761	0.022	4	3.796	0.021
Root	2	10.082	0.001	2	203.545	0.000	4	5.061	0.007
Shoot	1	12.335	0.004	2	0.457	0.644	2	37.306	0.000
TESC conc.									
Cutting	2	0.759	0.479	2	2.175	0.136	4	1.152	0.358
Root	2	5.370	0.013	2	4.555	0.023	4	1.859	0.155
Shoot	1	29.469	0.000	2	7.586	0.007	2	8.253	0.006
Starch pool									
Cutting	2	42.160	0.000	2	2.442	0.115	4	4.798	0.008
Root	2	40.375	0.000	2	227.570	0.000	4	3.749	0.022
Shoot	1	150.335	0.000	2	3.622	0.059	2	21.879	0.000
TESC pool									
Cutting	2	1.845	0.181	2	2.265	0.127	4	1.235	0.324
Root	2	9.941	0.001	2	4.742	0.020	4	2.429	0.080
Shoot	1	2052.373	0.000	2	19.672	0.000	2	6.571	0.012
NSC pool									
Cutting	2	14.498	0.000	2	1.750	0.202	4	2.368	0.091
Root	2	13.648	0.000	2	15.340	0.000	4	2.468	0.082
Shoot	1	389.960	0.000	2	3.425	0.067	2	22.559	0.000
Total NSC	2	102.114	0.000	2	2.363	0.123	4	1.696	0.195

samples of cuttings, roots and shoots were analyzed for both TESC and starch in each of the nine treatment groups. Each sample consisted of three plants due to the small biomass of harvested plants. Extraction of low molecular weight carbohydrates was accomplished using 80% ethanol and constant mixing for 4 h at 17–24°C. A phenol-sulfuric acid assay was conducted to measure total 80% ethanol-soluble carbohydrates (TESC) using a sucrose standard with absorbance measured at 490 nm (Dubois et al. 1956). The procedure used for measurement of starch in plant tissue included gelatinization with heat-stable alpha amylase (Termamyl) in a 1-h 92–93°C water bath (Haissig and Dickson 1979). Gelatinization involves the breaking of hydrogen bonds among and within the starch molecules, which opens the granules to hydration and enzymatic hydrolysis. Hydrolysis to glucose was completed using alpha amyloglucosidase. This method is specific for starch because the enzymes hydrolyze only the bonds between glucose molecules within starch molecules (alpha 1-4 and alpha 1-6 linkages). Glucose was measured with glucose oxidase-peroxidase assay using a glucose standard. Absorbance was measured at 505 nm. Starch was calculated as glucose multiplied by 0.9 to account for the added weight of one molecule of water for every covalent bond hydrolyzed (Karkalas 1985). Total NSC was calculated as the sum of TESC and starch.

Data analysis

Two-factor ANOVAs were conducted to determine effects of shoot removal, soil moisture and the interaction of shoot removal and soil moisture on measured-response variables in this 3 × 3 factorial design (Table 1, SPSS 12.0, SPSS Inc., Chicago, IL). Simple effects analysis was conducted following a significant interaction between the two main factors, indicating that any differences in one treatment factor were dependent on the level of the second treatment factor, using one-way ANOVAs for each level of the independent variables and Tukey's post hoc tests to determine significant pair-wise differences between treatment groups for each measured variable ($P < 0.05$). LCC data were analyzed using a repeated measures procedure with two, between-subjects factors (shade and shoot removal) and one, within-subjects factor (sampling date).

Results

Soil moisture

Mean soil E_h on day 5 of first periodic flooded phase was +181 mV ($n = 9$). Mean E_h for drained phase on day 9 was +533 mV. During the second flooding phase, soil E_h

Table 2 Effects of soil moisture: well-watered (W), periodically flooded (F) and drought (D), and shoot removal: intact control (R0), partial shoot removal (R1) and complete shoot removal (R2) on measured variables at final harvest

Variable	Soil moisture						Shoot removal					
	W		F		D		R0		R1		R2	
Biomass												
Cutting	5.98	0.24	5.67	0.24	6.08	0.24	6.3a	0.24	6ab	0.24	5.42b	0.24
Shoot	*	*	*	*	*	*	*	*	*	*	N/A	N/A
LAR	59.2	1.4	64.5	1.52	61.3	1.4	80.9a	1.2	42.4b	1.2	N/A	N/A
LCC	*	*	*	*	*	*	*	*	*	*	*	*
Starch conc.												
Cutting	*	*	*	*	*	*	*	*	*	*	*	*
Root	*	*	*	*	*	*	*	*	*	*	*	*
Shoot	*	*	*	*	*	*	*	*	*	*	N/A	N/A
Starch pool												
Cutting	*	*	*	*	*	*	*	*	*	*	*	*
Root	*	*	*	*	*	*	*	*	*	*	*	*
Shoot	*	*	*	*	*	*	*	*	*	*	N/A	N/A
TESC conc.												
Cutting	2.6	0.5	3.8	0.4	4.0	0.4	3.9	0.5	3.2	0.4	3.2	0.4
Root	3.3b	0.7	4.5ab	0.6	6.2a	0.6	6.2a	0.6	4.8ab	0.6	3.1b	0.6
Shoot	*	*	*	*	*	*	*	*	*	*	N/A	N/A
TESC pool												
Cutting	15.79	3.05	21.55	2.91	24.67	2.91	25.24	3.05	19.36	2.91	17.42	2.91
Root	2.44	0.49	2.63	0.47	4.28	0.45	4.42	0.47	3.45	0.47	1.48	0.47
Shoot	*	*	*	*	*	*	*	*	*	*	N/A	N/A

Measured variables include: biomass (g), leaf area ratio (LAR), leaf chlorophyll content (LCC, cci units), starch concentration (%), starch pool (mg), total ethanol-soluble carbohydrates concentration (TESC, %), and TESC pool (mg). Letters indicate significant differences ($P < 0.05$) among means (with standard error shown to the right of each mean) for shoot removal and soil moisture treatments. * indicates interactions between shoot removal and soil moisture. N/A indicates not applicable

was +190 mV (day 13). Soil water potential in drought treatment was −1.0 MPa on day 4, gradually changing to −0.9 MPa on day 7, −0.9 MPa on day 11, and −0.8 MPa on day 12 ($n = 6$).

Biomass (g)

Shoot biomass removed in R1 was 44% of total biomass, and 85% of total shoot biomass. At final harvest, shoot biomass had recovered to 37% of control (R0; Table 2). Shoot biomass was greater in periodic flooded (1.39 g) compared to both drought (1.09 g) and well-watered (1.17 g) in R0 ($P < 0.01$; $F_{0.05,5,83} = 22.78$). Root biomass was significantly reduced (by 31%) in R2 compared to R1 and R0 ($P < 0.01$; $F_{0.05,8,95} = 6.97$). Cutting biomass was reduced in R2 (5.42 g) compared to R0 (6.30 g, $P = 0.03$).

Leaf area ratio (LAR) and leaf chlorophyll content (LCC)

LAR was reduced in R1 compared to R0 ($P < 0.01$; $F_{0.05,5,63} = 101.07$; Table 2). LCC was affected by a significant interaction between shoot removal and soil moisture in repeated-measures analysis using sample date as a within-subjects factor ($F_{0.05,5,64} = 1$; 158.14; $P < 0.01$). In periodic flooded treatment, LCC of R0 (2.59 cci) was greater than R1 (1.64 cci) on day 4 ($P = 0.02$). In R1, LCC of well-watered (3.03 cci) was greater than periodic flooded (1.86 cci) on day 12 ($P < 0.01$).

Starch concentration

Starch concentration (% of dry weight) in subsampled plants, prior to treatment initiation, was 2.3% in cuttings, 1.2% in roots and 17.8% in shoots. At final harvest, cutting starch concentration was reduced in R2 compared to both R0 and R1 in periodic flooded and drought conditions. In R2, cutting starch concentration was lower in drought compared to both periodic flooding and well-watered treatments ($F_{0.05,8,18} = 9.90$; $P = 0.02$; Fig. 1a).

In periodic flooded conditions root starch concentration in R2 (1.3%) was lower than in both R0 (2.9%) and R1 (2.5%). In well-watered conditions, root starch was greater in R0 (1.5%) than in R2 (0.5%). Within R0, R1 and R2

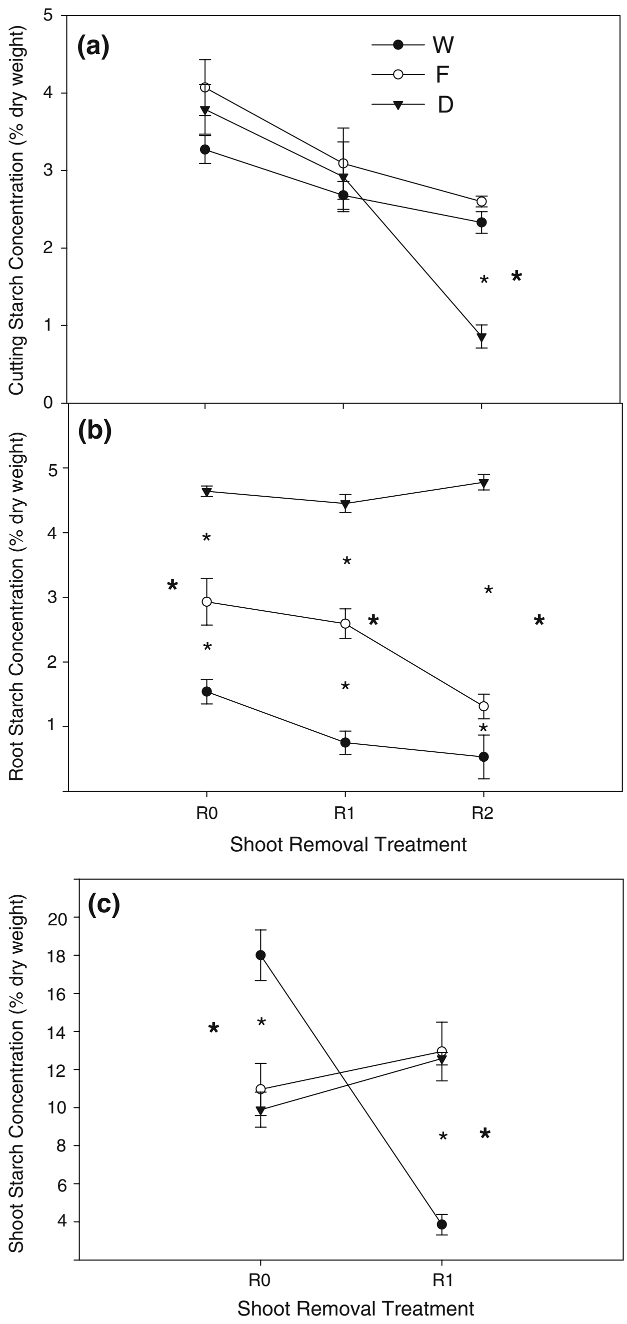

Fig. 1 Relationships among mean (±standard error) starch concentrations (% dry weight, $n = 3$) in **a** cutting, **b** root, and **c** shoot, in soil moisture treatments: well-watered (*W*), periodic flooded (*F*) and drought (*D*), at each level of shoot removal treatment: intact control (*R0*), partial shoot removal (*R1*), and complete shoot removal (*R2*). Significant differences ($P < 0.05$) between the inner pair of points are indicated with *small asterisks* (*), and *large asterisks* indicate significant differences between the outer pair of points

treatments, root starch concentrations in the three soil moisture treatments were significantly different from one another, with drought greater than periodic flooded, and periodic flooded greater than well-watered ($F_{0.05,8,18} = 55.93$; $P < 0.01$; Fig. 1b).

Shoot starch concentration in R0 (18.0%) was greater than R1 (3.8%) in well-watered conditions. In R0, greater shoot starch concentration was found in well-watered than in both drought and periodic flooded conditions. In R1, greater shoot starch concentration was found in both periodic flooded and drought compared to well-watered ($F_{0.05,5,12} = 17.57$; $P < 0.01$; Fig. 1c).

TESC concentration

TESC concentration in plants prior to initiation of shoot removal and soil moisture treatments was 2.2% in cuttings, 6.2% in roots, and 6.5% in shoots. At final harvest, no differences were found in cutting-TESC concentration (Table 2). Root TESC concentration was lower in well-watered (3.3%) compared to drought (6.2%, $P = 0.01$), and lower in R2 (3.1%) compared to R0 (6.2%; $P = 0.02$; $F_{0.05,8,21} = 3.52$; $P = 0.01$). Shoot TESC concentration was lower in periodic flooded compared to drought in R0, and reduced in well-watered compared to periodic flooded in R1. However, in periodic flooded conditions, shoot TESC concentration remained unchanged between R0 and R1. Shoot TESC concentration was greater in R0 than in R1 in well-watered and drought conditions ($F_{0.05,5,12} = 12.22$; $P < 0.01$; Fig. 2).

Starch pool

Cutting starch pool (measured in milligrams, mg) was greater in R0 and R1 compared to R2 in well-watered and drought conditions. In periodic flooded conditions, cutting starch pool was greater in R0 than in both R1 and R2 ($F_{0.05,8,18} = 13.55$; $P < 0.01$; Fig. 3a).

Root starch pool was reduced in R1 and R2 compared to R0 in well-watered conditions. In periodic flooded and drought conditions, root starch pool was reduced in R2 compared to R0 and R1. In both R0 and R1, root starch pool was greatest in D, with F lower than D, and W lower than F. In R2, root starch pool was higher in D than in both F and W ($F_{0.05,8,18} = 68.86$; $P < 0.01$; Fig. 3b).

In each of the three soil moisture treatments, shoot starch pool was greater in R0 than in R1. In R0, shoot starch pool was greatest in W, with F less than W, and D less than F. In R1, shoot starch pool was higher in D than in W ($F_{0.05,5,12} = 40.26$; $P < 0.01$; Fig. 3c).

TESC pool

No significant differences in cutting or root TESC pool were found. Shoot TESC pool was greater in R0 than in R1 in all three soil moisture treatments. In R0, shoot TESC pool was greater in periodic flooded than in well-watered.

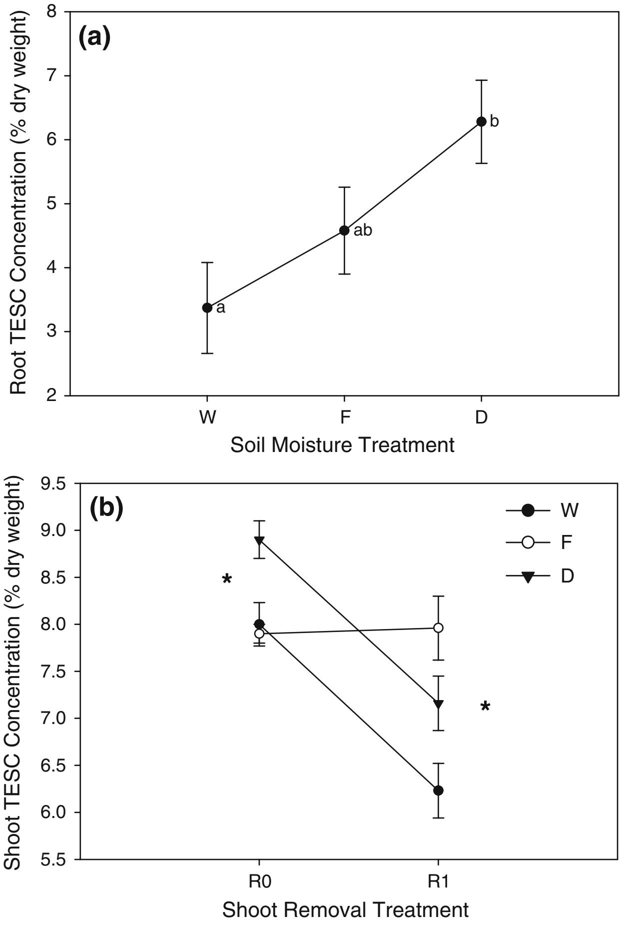

Fig. 2 Relationships among mean (±standard error) total ethanol-soluble carbohydrate concentrations (TESC, % dry weight, $n = 3$) in root and shoot, in soil moisture treatments: well-watered (*W*), periodic flooded (*F*) and drought (*D*), and in shoot removal treatments: intact control (*R0*), partial shoot removal (*R1*) and complete shoot removal (*R2*). Significant differences ($P < 0.05$) in root TESC concentration are indicated with small letters. Significant differences ($P < 0.05$) in shoot TESC concentration, between the inner pair of points are indicated with *small asterisks* (*), and between the outer pair of points are indicated with *large asterisks*. R2 is a complete shoot removal treatment, therefore, no data are available for shoots or leaves

In R1, shoot TESC pool was greater in drought than in well-watered ($F_{0.05,5,12} = 420.97$; $P < 0.01$; Fig. 4).

NSC pool

We found lowest total NSC pools in DR2, WR2 and FR2; and highest NSC pools in FR0, DR0 and WR0, with DR1, FR1 and WR1 intermediate to highest and lowest groups (Fig. 5a). Cutting NSC pool was lower in R2 (26.5 mg) compared to R0 (46.6 mg; $F_{0.05,8,18} = 5.85$; $P < 0.01$). Root NSC pool was reduced in both R1 and R2 compared to R0 in well-watered conditions. However, in drought conditions, root NSC pool was highest in R1, with R0 lower than R1, and R2 lower than R0 ($F_{0.05,8,18} = 26.58$; $P < 0.01$; Fig. 5b). Shoot NSC pool was greater in R0 than in R1 in all three soil moisture treatments. In R0, shoot

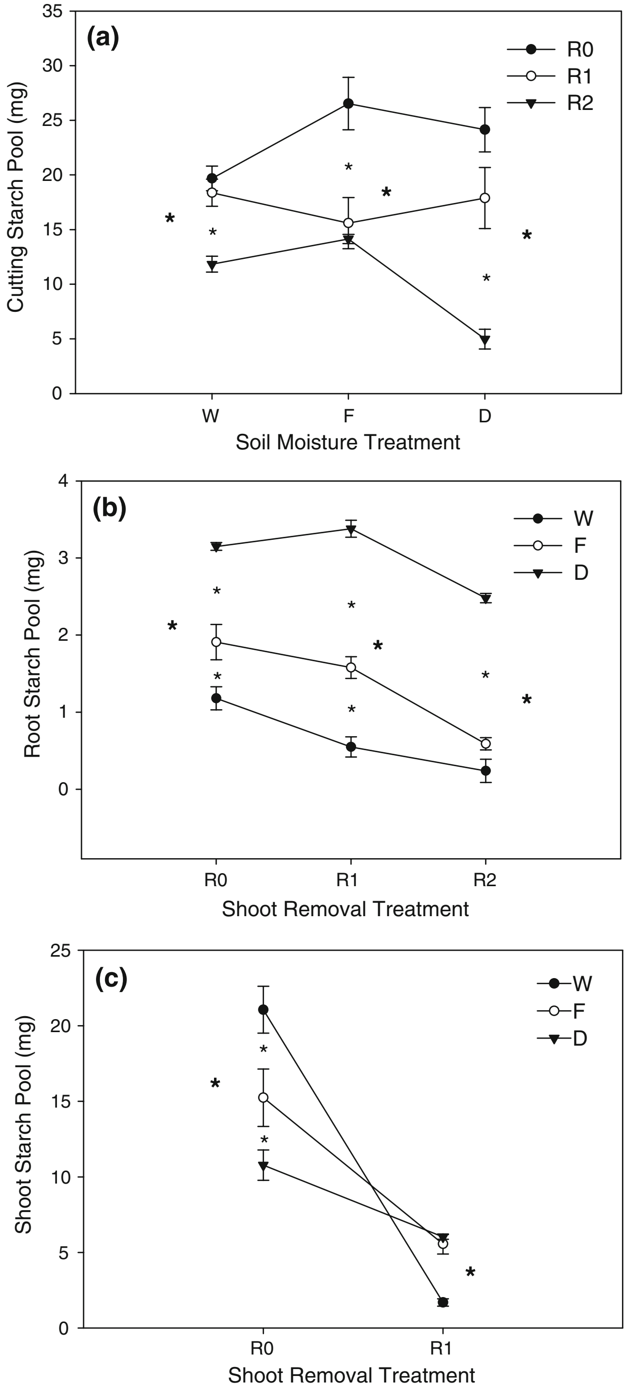

Fig. 3 Relationships among mean (±standard error) starch pools (mg, $n = 3$) in cuttings, roots, and shoots, in soil moisture treatments: well-watered (*W*), periodic flooded (*F*) and drought (*D*), and in shoot removal treatments: intact control (*R0*), partial shoot removal (*R1*) and complete shoot removal (*R2*). Significant differences ($P < 0.05$) between the inner pair of points are indicated with *small asterisks* (*), and between the outer pair of points are indicated with *large asterisks*

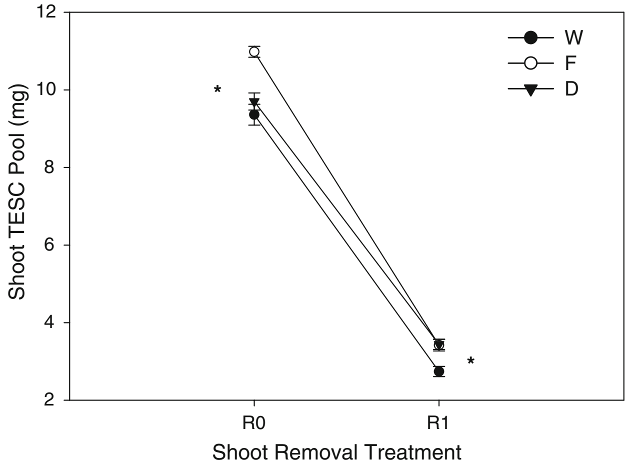

Fig. 4 Relationships among mean (±standard error) total ethanol-soluble carbohydrate pools (TESC, mg, $n = 3$) in shoots for each soil moisture treatment: well-watered (*W*), periodic flooded (*F*) and drought (*D*), at each level of shoot removal treatment: intact control (*R0*), and partial shoot removal (*R1*). Significant differences ($P < 0.05$) between the inner pair of points are indicated with *small asterisks* (*), and between the outer pair of points are indicated with *large asterisks*

NSC pool was reduced in drought (20.4 mg) compared to both well-watered (30.4 mg) and periodic flooded (26.2 mg) conditions. In R1, shoot NSC pool was reduced in well-watered (4.4 mg) compared to both periodic flooded (8.9 mg) and drought (9.4 mg; $F_{0.05,\ 5,12} = 88.38$; $P < 0.01$; Fig. 5c).

Discussion

Biomass

Complete removal of photosynthetic tissues led to a respiratory burden that was supported by NSC reserves during the 13-day experimental period which terminated when new shoot development was observed. As a result of this respiratory burden, root dry weight declined by 31.5% in R2 compared to both R1 and R0. Tschaplinski and Blake (1995) found a 19% reduction in root dry weight on day 44 after 78% removal of total leaf area in *Populus maximowiczii* Henry × *P. nigra* L. "MN9" 1-year-old trees. In another study, decapitation led to shedding of fine roots, but the structure of the root system remained intact in *Eucalyptus kochii* Maiden and Blakely subsp. *plenissima* Gardner (Brooker) with no further development of structural root mass until functional shoot to root ratio was recovered (Wildy and Pate 2002). Canham et al. (1999) also found reductions in biomass in response to defoliation in four tree species. In this study, complete shoot removal (R2) also resulted in a 14% reduction in cutting biomass

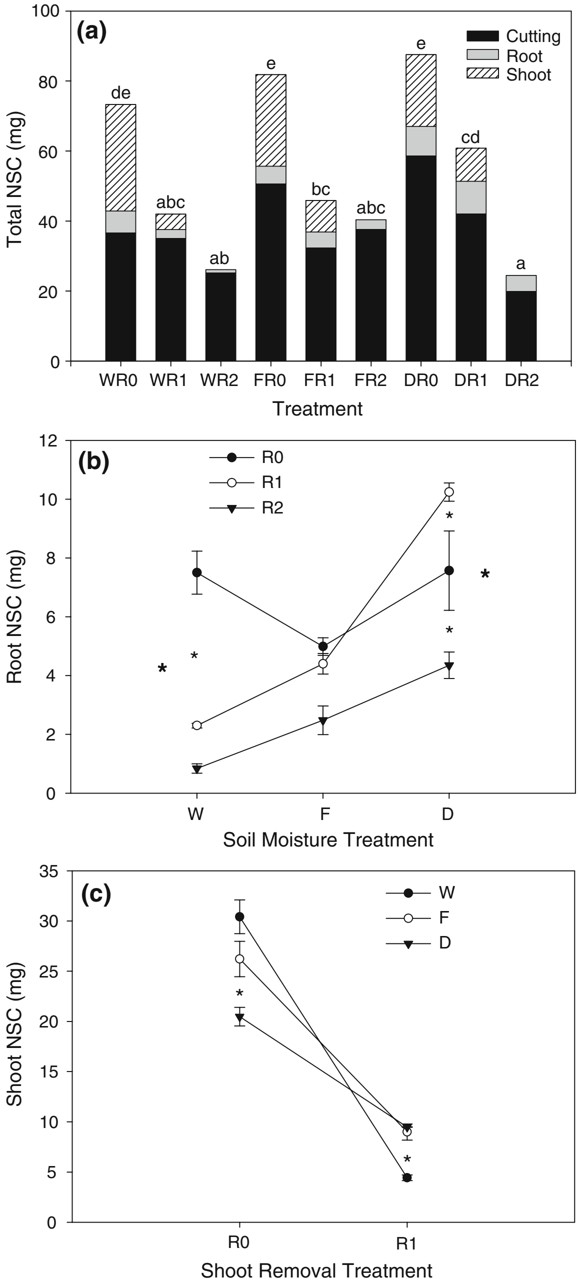

Fig. 5 Relationships among mean (±standard error) total nonstructural carbohydrate (NSC) pools (mg, $n = 3$) of different treatment groups: well-watered no shoot removal (*WR0*), well-watered partial shoot removal (*WR1*), well-watered complete shoot removal (*WR2*), periodic flooded no shoot removal (*FR0*), periodic flooded partial shoot removal (*FR1*), periodic flooded complete shoot removal (*FR2*), drought no shoot removal (*DR0*), drought partial shoot removal (*DR1*), and drought complete shoot removal (*DR2*). *Small letters* above each *bar* indicate significant differences ($P < 0.05$) among total NSC pools across all treatment groups. NSC pools of roots and shoots are compared in different soil moisture conditions: (*W*) well-watered, (*F*) periodically flooded, and (*D*), and in different shoot removal treatments; intact control (*R0*), partial shoot removal (*R1*) and complete shoot removal (*R2*). Significant differences ($P < 0.05$) between the inner pair of points are indicated with *small asterisks* (*), and between the outer pair of points are indicated with *large asterisks*

compared to R0. Both roots and cuttings were important reservoirs for NSC that were utilized during resprouting response which agrees with results from Chapman (1992) for *Salix purpurea* L. Shoot biomass in R0 was significantly increased in periodic flooded conditions compared to both drought (22%) and well-watered (16%) indicating greater carbon allocation to shoot growth. This finding is in agreement with other studies showing accelerated shoot growth in response to flooding (Kozlowski and Pallardy 1997).

LAR and LCC

At final harvest, LAR was lower in R1 compared to intact control, however, we found evidence of rapid recovery of photosynthetic surface area with shoot dry weight in R1 increasing from 15% of intact control on day 1 of treatment to 37% of intact control on day 13. Rapid leaf area recovery is important to maintain the high respiratory demands of the root system following aboveground disturbance until the root to leaf area imbalance can be corrected (Landhausser and Lieffers 2002). Chapman (1992) found convergence of shoot to root ratios in coppiced and intact *Salix purpurea* L. 2 months after treatment. Rapid resprouting response and replacement of damaged leaf area following aboveground disturbance is critical to the survival of *S. nigra* that is typically found in highly disturbed environments (Karrenberg et al. 2002).

LCC was negatively affected by shoot removal under periodic flooded conditions, reduced by 37% in R1 compared to R0. Soil moisture treatment also affected LCC in R1, with a reduction of 39% in periodic flooded compared to well-watered control. Lack of any detectable changes in LCC under drought treatment may have been the result of the short experimental period of 13 days. Drought stress develops gradually in intensity over time, unlike other environmental stresses, and it is possible that the 13-day experimental period was too short to see changes in LCC as a result of drought conditions (Fitter and Hay 2002; Larcher 2003). In this study, soil water potential measurements did indicate drought conditions, and the coarse sandy soil had a poor water holding capacity. Schaff et al. (2002) found a 28% reduction in LCC for *S. nigra* at high elevation plots with associated periodic drought conditions during the growing season in a field study. In a greenhouse study *S. nigra* exhibited significant negative effects of drought treatment including reductions in leaf area (18% of control), total biomass (15% of control) and net photosynthesis (33% of control) compared to less pronounced flooding effects on leaf area (99% of control), total biomass (100% of control), and net photosynthesis (56% of control; Pezeshki et al. 1998).

Starch concentration

NSC response of roots to shoot removal is rapid due to lack of downward translocaton of photoassimilates and continued root respiration (Kozlowski and Pallardy 1997). In *Populus maximowiczii* × *nigra* L., reduced root and stem NSC was found 4 days after decapitation (Tschaplinski and Blake 1995). Coppicing resulted in a decline in root starch concentration from 5 to 1% in *Betula pendula* Roth and from 3 to 1% in *Betula pubescens* Ehrh. after 3 weeks, compared to intact trees (Luostarinen and Kauppi 2005). In this study, a clear pattern of starch mobilization was found in roots across R0, R1, and R2, with significant differences between soil moisture treatments. Well-watered treatment exhibited greatest root starch mobilization with lower starch concentration than periodic flooded, and root starch concentration was lower in periodic flooded than in drought treatment. These results clearly indicate a strong influence of soil moisture treatment on ability to mobilize NSC in roots in both intact plants, and in plants treated with shoot removal. Starch mobilization in roots was strongly inhibited under drought conditions, and less affected in periodic flooded conditions. These results are consistent with the known morphological and physiological adaptations of *S. nigra* to flooding including development of porous aerenchyma tissue that is efficient in transporting oxygen from shoots to roots in flooded conditions (Pezeshki et al. 1998; Li et al. 2005). High root starch concentration and low leaf starch concentration in flood-tolerant *Nyssa aquatica* L. following a 32-day period of flooding, was reported to be important in maintaining plant functions in this species, in contrast to high leaf starch and reduced root starch in several flood-intolerant bottomland species (Gravatt and Kirby 1998). The reason/s for reduced starch mobilization under flooded conditions is unknown but could be due to several factors including a possible inhibition of starch degradation enzymes.

Although root starch was not mobilized in drought conditions, cutting starch was mobilized in R2 under drought conditions. This finding indicated that cutting starch was a more available reserve pool than root starch in drought conditions. Chapman (1992) found that coppiced *Salix purpurea* L. mobilized twice as much starch from cuttings and half as much from roots compared to intact plants.

In shoots, soil moisture conditions had a strong influence on both accumulation of starch reserves in intact plants (R0) and mobilization of starch reserves in plants treated with partial shoot removal (R1; Table 2). In well-watered conditions, shoot starch concentration was greater in R0 compared to R1. In R0, shoot starch concentrations were reduced in both periodic flooded and drought conditions compared to well-watered control, indicating that starch accumulation in leaves was inhibited perhaps due to a reduction in rate of photosynthesis, and corresponding decrease in photoassimilate production. Starch levels in stems of *Populus maximowiczii* × *nigra* L. dropped to 50% of intact plants by day 10 after 100% shoot removal, but no change in starch reserves was found in 50% defoliated plants indicating that plants with 50% defoliation treatment were able to compensate for removal of shoot tissue with increased photosynthetic rate in remaining leaves (Tschaplinski and Blake 1995).

In R1, shoot starch reserves were only mobilized in well-watered conditions, with starch concentration reduced to 21% of intact control. Reduced mobilization of shoot starch reserves in soil moisture stress may have been due to reduced translocation rate. Kogawara et al. (2006) reported reduced transport of photoassimilates and starch accumulation in leaves of *Eucalyptus camaldulensis* Dehnh 4 days after hypoxic treatment.

TESC concentration

Root TESC concentration in R1 remained high in both periodic flooded and drought conditions relative to well-watered control which may have indicated osmotic adjustment by accumulation of sugar in roots to maintain root turgor which is important in root function (Fitter and Hay 2002; Larcher 2003). Tschaplinski and Blake (1995) hypothesized that accumulation of solutes (sugars) in the roots was a mechanism for increasing water uptake from the soil, increasing root hydration and facilitating reestablishment of the water column in decapitated plants which is critical for transporting growth hormones produced in the root to stems and initiating new growth. In flood-tolerant *Melaleuca cajuputi* Powell seedlings, hypoxia induced a shift in partitioning of carbohydrates in roots, with an increase in water-soluble carbohydrates, and an associated decrease in starch, that was hypothesized to be important in maintaining energy production (Kogawara et al. 2006). Tschaplinski and Blake (1995) found an 80% reduction in root sucrose level in decapitated treatment with 2.5–4.5-fold increases in root monosaccharides (glucose and fructose) on day 4 after decapitation. However, Luostarinen and Kauppi (2005) found no difference in root sucrose in *Betula pendula* or *Betula pubescens* 3 weeks after coppicing treatment.

When complete shoot removal treatment was applied in this study, root TESC in periodic flooded conditions was only reduced by 5%, but in drought conditions root TESC was reduced by 47% (Table 2). This finding may indicate that *S. nigra* has the ability to maintain root function under combined stresses of flooding and complete shoot removal, but is more susceptible to drought injury in roots when the additional stress of complete shoot removal is applied. Quezada and Gianoli (2006) found that responses to drought stress were constrained in plants exposed to combined treatments of simulated herbivory and drought stress. They speculated that limited energy in plants with smaller photosynthetic surface area prevented normal phenotypic responses. However, loss of shoot tissue in drought conditions may also reduce soil moisture stress due to increase in root to shoot ratio, and decreased transpirational water loss (Kozlowski and Pallardy 1997).

Shoot TESC concentration was significantly affected by the interaction of shoot removal and soil moisture treatments. In R1, both well-watered and drought treatments had reduced shoot TESC concentration, 22 and 20% lower than R0. However, in periodic flooded conditions shoot TESC remained unchanged in R0 and R1 (Fig. 2.). Periodic flooded plants may have maintained greater TESC concentration in shoots to reduce negative effects from flooding through osmotic adjustment. In response to shoot decapitation, Tschaplinski and Blake (1995) found stem sugars dropped to 39–65% of control by day 4, with a 50% reduction in sucrose and galactose concentrations in 50% defoliation treatment.

Starch pool

Cutting, root and shoot starch pools responded to shoot removal with decreases in R1 and R2 compared to R0. Root starch pool was greater in drought than in periodic flooded, and greater in periodic flooded than in well-watered treatments in both R0 and R1. Also, in R2, root starch pool was greater in drought than in both periodic flooded and well-watered treatments. These results indicated less availability of starch reserves under soil moisture stress, with less mobilization of starch in drought compared to periodic flooded treatments. In R0, shoot starch pool was greater in well-watered than in periodic flooded, and greater in periodic flooded than in drought. Shoot starch pool was also greater in drought than in well-watered in R1. These results may have indicated reduced rates of net photosynthesis, and lower production of photoassimilates during soil moisture stresses, with more severe effects of drought compared to periodic flooding. These results are consistent with flood tolerant characteristics of *S. nigra*.

TESC pool

Shoot TESC pool responded to shoot removal with higher levels in R0 compared to R1 in well-watered, periodic flooded and drought conditions. Soil moisture affected shoot TESC pool with higher levels in periodic flooded compared to well-watered in R0, and higher levels in drought compared to well-watered in R1. This was another indication of osmotic adjustment during soil moisture stress.

Responses of starch reserves to shoot removal and soil moisture treatments were larger than TESC responses. Luostarinen and Kauppi (2005) also found more dramatic changes in starch than in soluble sugar reserves of *B. pendula* Roth and *B. pubescens* Ehrh. following coppicing. Less fluctuation in TESC concentration of roots and shoots under soil moisture stress may have resulted from osmotic adjustment, maintaining higher levels of TESC to reduce injury caused by drought and periodic flooding stresses.

NSC pool

Plant NSC pool decreased to 60% of intact control in R1 and 37% of control in R2 indicating a strong mobilization response to shoot removal. Highest levels of total NSC were found in groups with no shoot removal, and lowest levels in groups with complete shoot removal. However, soil moisture treatment significantly affected the responses of root and shoot NSC pools to shoot removal. In R1 and R2, root NSC pool was elevated in drought and periodic flooding compared to well-watered conditions. Following partial shoot removal, shoot NSC was greater in both drought and periodic flooding than in well-watered conditions. Higher TESC levels as a result of osmotic adjustment in roots and shoots contributed to greater NSC in periodic flooding and drought conditions. Although *S. nigra* is a vigorous resprouter following aboveground disturbance, the additional stress of soil moisture reduced mobilization of NSC reserves, perhaps indicating a less vigorous resprouting response, and having implications for increased mortality of *S. nigra* in field conditions.

Conclusions

Both roots and cuttings served as important reservoirs for NSC during resprouting response, and both showed decreases in biomass in R2 compared to R0. In R1 we found evidence of rapid recovery of photosynthetic surface area, indicating that the remaining shoots in R1 provided sufficient photoassimilates to maintain the existing root mass, while also rapidly producing new shoot tissue. Soil moisture treatments produced changes in carbon partitioning to growth with greater shoot biomass in periodic flooded conditions compared to both well-watered and drought in R0. Patterns of starch mobilization following shoot removal were strongly affected by soil moisture treatment with no detectable root starch mobilization in drought treatment and a significant reduction in root starch mobilization under periodic flooded conditions. However, plants exposed to complete shoot removal in drought conditions were able to utilize cutting starch reserves to a greater extent than in periodic flooded and well-watered conditions. Shoot starch reserves were only utilized in well-watered treatment following partial shoot removal. We found evidence of osmotic adjustment in roots and shoots in response to drought and periodic flooded treatments with higher TESC compared to well-watered control. However, combined drought and shoot removal stresses led to reduced root TESC that may indicate greater potential for plant injury (Table 2). Total plant NSC pool was reduced in response to complete shoot removal, but increased in response to periodic flooded and drought soil moisture treatments due to changes in carbon partitioning as indicated by increased TESC in roots and shoots. Results indicated negative effects of drought, and to a lesser extent periodic flooding, on starch mobilization to provide energy for resprouting response in *S. nigra*, with implications for reduced survival in the field when exposed to the combined stresses of aboveground disturbance and flooding or drought.

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