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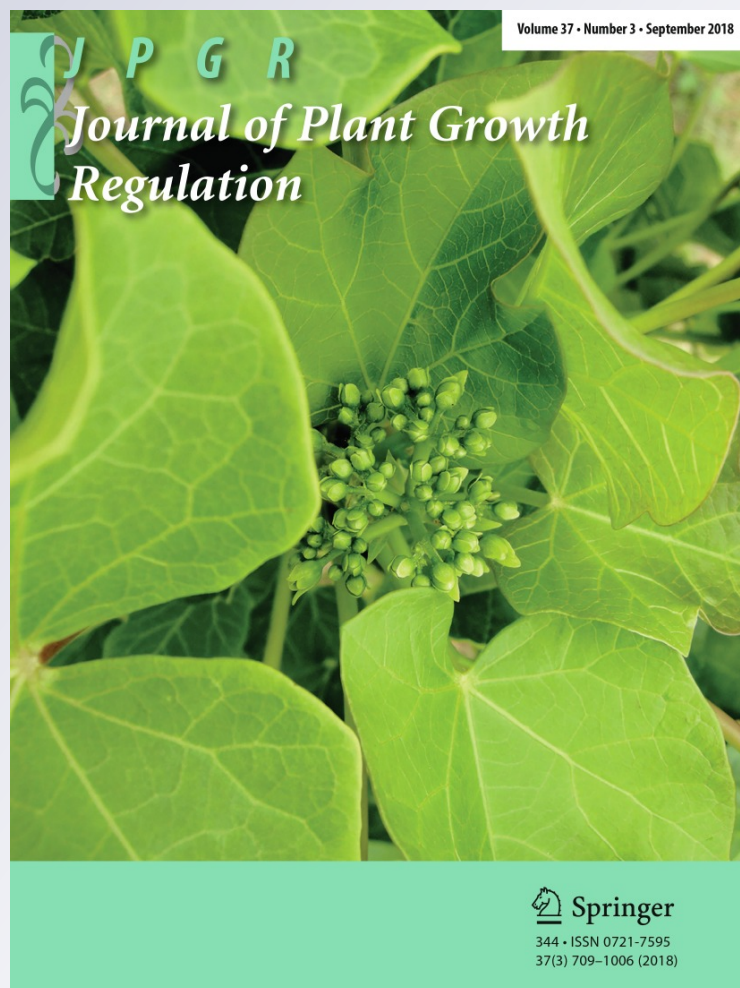
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Unfolding Non-structural Carbohydrates from Sapling to Dying Black Locust on China's Loess Plateau

Qingyin Zhang¹ · Xiaoxu Jia^{2,3,4} · Ming'an Shao^{1,2,3} · Changkun Ma¹

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Abstract

Non-structural carbohydrates (NSC) play important roles in metabolic processes of plants and represent important functional traits in plant adaptation to the external environment. However, there are few reports concerning intra-annual NSC distribution in temperate deciduous forests, especially for the purpose of comparison among different stand ages on China's Loess Plateau. Here, NSC allocation dynamics with age ring-porous black locust tree was determined for the growing (May) and dormant (November) seasons over the period from sapling to dying trees—defined as a completely defoliated tree with dried branches in the growing season. It was noted that regardless of tree age, NSC concentration was highest in coarse roots [16.4 g per 100 g of dry mass (16.4% DM)] and stems (15.1% DM). At the tree level, NSC concentration was highest (14.3% DM) in a 30-year-old stand in November and lowest (4.1% DM) in dying stands in May. The pool of NSC at tree level was highest (25.2 kg DM per tree) in 30-year-old stands in November and lowest (0.13 kg DM per tree) in sapling stands in May. The concentration of NSC was significantly lower in May than in November for all tree ages, organs, and biochemical components. The results underscored the importance of NSC in plant growth on China's Loess Plateau. It also provided a useful insight into the dynamics of NSC from sapling to dying broadleaved tree species.

Keywords Black locust · Non-structural carbohydrate · Dynamics · Concentration · Biomass

Introduction

Carbohydrate reserves are mobilized in trees to fuel maintenance and respiration during winter (Ögren 2000), cope with soil water deficit and support new growth in spring (Dunn and others 1990; Canham and others 1999). Carbohydrates

in plants are generally classified as structural (SC) and non-structural (NSC) carbohydrates (Li and others 2002, 2008). Mainly as soluble sugars and starch, NSCs are products of photosynthesis, which also provide substrates for growth and metabolism (Woodruff and Meinzer 2011). The contents of NSCs and their composition (ratio of glucose to starch) reflect the balance between carbon sources and sinks in plants (Chapin and others 1990; Hoch and others 2003; Würth and others 2005). Studies on intra-annual dynamics of NSC are needed to understand the response of tree growth to climate change (Adams and others 2013; Hartmann and others 2013; Andrew and others 2013; O'Brien and others 2014). Although the NSC pool is depleted if demand exceeds supply (for example, when metabolic and growth requirements are high), it is recharged if supply exceeds demand (for example, when environmental conditions permit high rates of photosynthesis or when metabolic and growth requirements are low) (Chapin and others 1990; Grulke and Retzlaff 2001).

The mobilization of seasonal reserves is fully documented for matured trees (Galiano and others 2011; Adams and others 2013) and saplings (Anderegg and Anderegg 2013;

✉ Xiaoxu Jia
jiaxx@igsnr.ac.cn

✉ Ming'an Shao
shaoma@igsnr.ac.cn

¹ College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, China

² Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

³ College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100190, China

⁴ State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, China

O'Brien and others 2014), but less known for dying trees. Several studies have demonstrated changes in the allocation of carbon between tree organs associated with age. For example, carbohydrates are mostly allocated to roots (especially fine roots) in trees less than 20 years old, but to stems in older trees (Bartelink 1998; Grulke and Retzlaff 2001). This suggests that carbohydrate reserve dynamics in young trees cannot be extrapolated for adult trees without measuring carbohydrate contents in all the component organs of the tree (Barbaroux and others 2002). In addition, reductions in productivity associated with tree age and size in forest stands and individual trees following canopy closure are well documented (Ryan and Waring 1992; Ryan and others 1997).

The content and allocation ratios of NSC in roots, stems, and twigs can vary during growing and dormant seasons in response to the use or storage of NSC (Salomón and others 2016). This suggests that enhanced NSC storage and consumption in the root system could reflect a trade-off between clonal vegetative resilience and aboveground performance. For black locust (*Robinia pseudoacacia* L.), there are studies on the effects of soil water content on carbohydrate partitioning in various organs of saplings (Ji and others 2009; Wang and others 2015). However, studies on the intra-annual dynamics of NSC from saplings to dying trees are scarce for deciduous temperate forest trees on China's Loess Plateau.

China's Loess Plateau is famous for its deep loess, poor land-use management, and high soil erosion. To improve the fragile ecosystem on the Loess Plateau, extensive vegetation restoration and environmental protection programs have been implemented in the past decades (IPCC 2014). In addition, there has been a widespread use of black locust in the afforestation, the so-called 'Grain for Green' program (GFGP), of the naturally fragile ecosystem (Lü and others 2012). The focus of this program has been on ecological benefits such as increased soil carbon sequestration (Deng and others 2014), water loss reduction, soil erosion control (Brown and others 2007), and desertification control and biodiversity conservation (Porto and others 2009). However, the arising concern related with the current restoration program is the degradation of vegetation, including accelerated tree death and "small-aged-tree" phenomena where matured trees develop abnormally short trunks (Chen and others 2010).

Because NSC dynamics are critical for the growth and response of black locust to environmental disturbances, it was hypothesized that NSC concentration or pool reflects black locust growth. With the widespread implementation of GFGP, it was next hypothesized that NSC concentration and pool increase with increasing age of black locust tree, although a dying black locust tree depletes NSC concentrations or pools at the tree scale. In this study, NSC concentration was measured for saplings to dying black locust trees under growing and dormant seasons on China's Loess

Plateau. Thus, the objectives of the study are (1) to compare NSC concentrations in roots, stems, branches, seeds, and leaves of black locust trees from sapling to dying; and (2) to quantify NSC concentrations and pools at the tree scale.

Materials and Methods

Study Sites

This study was conducted at the Yehe National Forestry Center, a managed forest extending over 10,996 ha in the Qishui watershed on the Loess Plateau of China (34.55°N, 107.90°E; 1080 m a.s.l.). The area has a homogeneous temperate continental climate with a mean annual temperature of 11.5 °C, ranging from −2 °C in January to 26 °C in July. The mean annual regional precipitation is 592 mm, with 70% occurring in the growing season from June to September. Only a few species of the forest trees, including *Robinia pseudoacacia* (L.), *Pinus tabulaeformis* (Carr.) and *Platycladus orientalis* (L.) Franco, support abundant undergrowth vegetation. The forest is on a conservation easement and is relatively managed as open stands. The soil is more of clay, with sand and loam mixtures at different depths.

Plant Material

Following a feasibility study in March 2015, five pure stands were selected from saplings to dying trees (of 4-, 10-, 20-, and 30-year-old trees) over the space of 3 km (Table 1). In the study, a dying tree was defined as a completely defoliated tree with dried branches in the growing season. It is worth noting that dying trees covered an area of up to 5 ha⁻¹ in the 30-year-old stands in the east of the Loess Plateau. Most of the dying trees were 28 years old and were not influenced by biotic and fire agents. All five stands were on sunny slopes with a slope gradient of less than 20°, largely eliminating the effect of topography.

Early wood, late wood, and total wood ring widths were measured in May 2015 for 10 randomly selected trees in each stand using a 4.3-mm-diameter standard hand increment borer (Häglof Company Group, Långsele, Sweden). The diameter at breast height (DBH) of the trees was measured in the six stands in May 2015. The leaf area index (LAI) was calculated for July 2015 by processing digital hemispherical photographs using CAN-EYE (Demarez and others 2008).

In this study, plant saplings were collected in the growing season (May 2015 with bud burst on April 25) and in the dormant season (November 2015 with leaf fall on October 28). The dates respectively corresponded to the minimum and maximum pools of carbohydrates in black locust (Su 2016). The two key dates were also in sync with the

Table 1 Mean stand characteristics, including coordinates and elevations of the sites, tree density, stand age, mean diameter at breast height (DBH), mean tree height, and leaf area index (LAI) in July 2015

Stand	Latitude/longitude	Elev. (m)	TD (ha ⁻¹)	SA	DBH (cm)	TH (m)	BA (m ⁻² ha ⁻¹)	LAI
4-year	34°33'42"N, 107°54'49"E	1085	1000	3	2.2±0.4	2.6±1.1	0.4	1.41
10-year	34°33'42"N, 107°54'37"E	1080	2500	10	11.2±1.2	9.1±0.9	24.6	3.45
20-year	34°33'30"N, 107°54'41"E	1068	2000	22	14.1±0.8	13.6±1.6	31.2	2.48
30-year	34°33'43"N, 107°54'58"E	1119	1000	31	21.8±1.3	17.8±1.3	37.3	1.74
Dying	34°33'45"N, 107°55'13"E	1127	500	28	17.6±2.3	10.6±1.8	12.2	NA

Elev elevation, *TD* tree density, *SA* stand age, *DBH* diameter at breast height, *TH* tree height, *BA* basal area, *LAI* leaf area index

conceptual model of the stand which stated that the NSC pool is depleted when demand exceeds supply in spring and the pool recharged when supply exceeds demand in autumn (Chapin and others 1990; Grulke and Retzlaff 2001). Then NSC concentration was compared between the two key periods.

Plant saplings were collected from various plant organs (> 5 mm coarse root, stem, bark, branch, and leaf in May, and then seed in November) of five randomly selected trees per stand in May and November of 2015. The coarse roots were collected in May from five selected trees per stand. Also the stems were collected at breast height on the south side of the trunk. As the sapwood consisted of NSC almost from the plant stem (Barbaroux and others 2002; Andrew and others 2013), the analysis was conducted only on the outer 2 cm (sapwood) of each increment core from the selected trees per stand (except for the 4-year-old stand) using a 4.3-mm-diameter standard hand increment borer (Häglof Company Group, Långsele, Sweden). Because of the small stem diameter of the 4-year-old stand, samplings were collected from the stems of felled trees. Sun-exposed terminal branches were collected from the upper 1/3 of the canopy of the selected trees per stand in May. Stem bark (both outer and inner) was collected at breast height of the trees in May. The sampling methods of leaves and seeds were the same as those for branches. All the samples were collected late in the morning and sealed in plastic bags, labeled and stored in a cooler with ice within 1 h after collection. In November, a second sample was collected using the same methods. Each sample was collected in three replicates.

Carbohydrate Analyses

The analyses for NSC determination followed those described by Andrew and others (2013). Leaves, stems, branches, bark, and seeds were finely sliced with a razor blade and ground with a ball mill (FOSS CT410, Sweden). About 0.5 g of each plant powder organ was vacuum-infiltrated with 80% ethanol for 15 min and then boiled. Macerated samples were extracted three times with 7.5 ml of fresh 80% ethanol for 15 min and centrifuged at 7000 rpm (Zhang

and others 2013). Supernatants of each sample were combined, filtered through a 0.45- μ m syringe filter and analyzed for soluble sugars. The ethanol-insoluble pellets were used to determine starch content.

The ethanol-soluble fractions were analyzed for sucrose, glucose, fructose, and sorbitol using a Waters (Milford, MA, USA) Alliance high-pressure liquid chromatographic (HPLC) system with a Waters Sugar-pak and solvent (Andrew and others 2013). Sugars were detected with a Waters 1525 refractive-index detector and Waters PC-based Breeze 2 software. The separated soluble sugars were identified and quantified with known standards and converted to gram sugar per 100 g of dry mass of the sample.

Starch was extracted from the remaining dry matter in a boiling solution of 0.02 NaOH for 1 h, followed by hydrolysis to glucose with α -amylglucosidase (EC 3.2.1.3, Boehringer Mannheim Biochemicals, Mannheim, Germany) in a 0.32 M citrate buffer (pH 4.2) at 48 °C for 30 min (Barbaroux and others 2002). Glucose formed by hydrolysis was measured colorimetrically at 340 nm (spectrophotometer model 2550, Shimadzu, Japan). Starch concentrations were calculated from standard curves and expressed as gram starch per 100 g of dry mass of the sample. Total concentration of NSC was calculated as the sum of concentrations of soluble sugars and starch for each organ.

Biomass Estimation

The leaf, stem, branch, and root biomasses were estimated using allometric relationships based on diameter at breast height (D) and height (H) of the tree (Bi and others 1993). The allometric relationships for black locust seed biomass were established by Wang and others (2005). The bark biomass was estimated by Shen and others (2015) for black locust on the Loess Plateau. To estimate the biomass of dying trees, a systematic search for black locust biomass estimation was done using Google Scholar Search. Different combinations of key words (“black locust,” “*Robinia pseudoacacia*,” “tree,” “forest,” “biomass,” and “productivity”) were used in the search. However, there was no empirical formula for the estimation of the biomass of black locust.

Moreover, Chi and others (2016) noted through a destructive method that the average biomass of dead black locust is 35 kg per tree, which is far below the estimated value in this study (83.9 kg per tree, Table 2). This discrepancy can be explained in terms of the finer DBH of dead black locust in the former study. In this study therefore, biomass was calculated and compared at the tree scale between different stands using allometric relationships as explained above. An allometric relationship was also used to estimate biomass of dying stands despite the fact that it over- or under-estimated biomass due to withered organs.

The biomass of different organs was estimated for each stand and carbohydrate concentrations in each organ averaged and weighted for each stand. Organ pools and concentrations were calculated using the product of the total biomass of the respective organ. Then the total NSC pool per tree was obtained by summing up the pools for all of the organs.

Statistical Analyses

For each tree, species and concentration distribution was analyzed by the use of the various sources of variation—organ, sampling date, age class, and so on. A two-way analysis of variance (ANOVA) was used to test the effects of the differences among sampling date and age class for each organ. In addition, the interaction between sampling date and age class for each organ was tested. Statistically significant differences at the organ scale were determined using the Student–Newman–Keuls multiple-range test at $p < 0.05$. All data were checked to conform with the assumptions of analysis of variance. All the statistical analyses were done in SPSS 18.0 (SPSS for Windows, Chicago, USA).

Results

Biomass Estimation

The biomass distribution in Table 2 showed differences between organs and stand ages. Seed biomass was ignored because it formed a very small fraction of the total biomass. Stem biomass represented 20, 40, 44, and 49 of total wood

biomass, respectively, for the 4-, 10-, 20-, and 30-year-old stands and 45% of the dying stands, contributing significantly to the respective total pools. Leaf biomass accounted for the lowest proportion of the total biomass, except for seed biomass.

Partitioning NSC Among Tree Organs and Age Class

The partitioning of NSC concentrations differed significantly among the tree organs (Fig. 1; Table 3). The concentrations were higher in coarse roots (16.4% DM) and stems (15.1% DM), and lower in leaves (5.1% DM), branches (2.5% DM), and bark (2.0% DM).

The partitioning of NSC concentrations and pools differed significantly among tree age classes (Figs. 1, 2; Table 3). The concentrations were higher in the 30-year-old stand for roots, stems, leaves, branches, but not for bark than in other stand ages. The concentrations of NSC at the tree scale were highest (14.3% DM) in the 30-year-old stand and lowest (4.1% DM) in dying stands. Also, NSC pools at the tree scale were highest (25.2 kg DM per tree) in the 30-year-old stand and lowest (0.13 kg DM per tree) in the 4-year-old stand.

NSC in Growing and Dormant Seasons

The concentrations and pools of NSC were significantly lower in May than in November for all tree ages, organs, and biochemical components (Figs. 1, 3). The concentrations of NSC during the growing season (May) for all tree ages were 3/4 of those during the dormant season (November). Organ NSC concentrations increased seasonally, particularly in coarse roots (53%) and stems (50%) in the 10-year-old stand, but the increase was lower in tree bark for all tree ages, especially in the 4-year-old stand. The concentrations of NSC at the tree scale increased significantly by 21, 45, 28, and 24% for the period from May to November, respectively, in 4-, 10-, 20-, 30-year-old stands and by 30% in dying stands. The pool of NSC at the tree scale was highest (25.2 kg DM per tree) in the 30-year-old stand in November and lowest (0.13 kg DM per tree) in the 4-year-old stand in May.

The data obtained in this study showed dynamic changes between growing and dormant seasons in terms

Table 2 Averages of organ biomass of black locust tree (*Robinia pseudoacacia*) from saplings to dying stands

Biomass (kg MD)	4 years	10 years	20 years	30 years	Dying tree
Stem	0.8 ± 0.11	15.1 ± 2.1	31.8 ± 4.32	85.6 ± 2.29	37.7 ± 4.66
Branch	0.3 ± 0.02	9.5 ± 0.86	18.7 ± 2.67	46.1 ± 3.86	21.8 ± 2.62
Leaf	0.2 ± 0.01	1.4 ± 0.13	2.0 ± 0.22	3.4 ± 0.73	NA
Root	0.6 ± 0.07	9.8 ± 1.17	16.9 ± 1.98	34.9 ± 2.99	19.2 ± 1.93
Bark	0.2 ± 0.02	1.8 ± 0.17	2.3 ± 0.13	3.6 ± 0.47	2.9 ± 0.17
Total biomass	2.1 ± 0.12	37.5 ± 3.72	71.9 ± 5.56	173.7 ± 13.94	83.8 ± 9.59

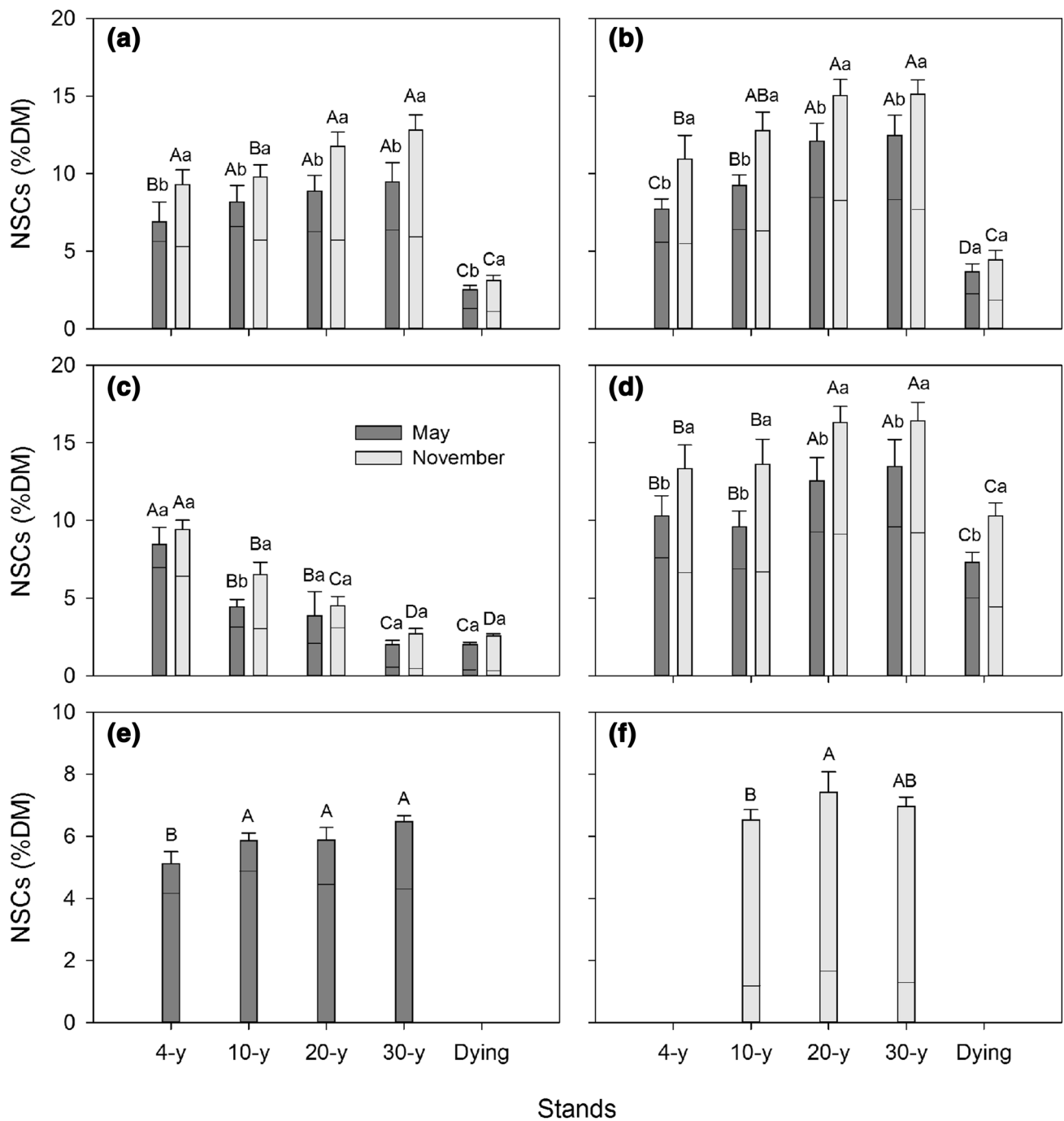


Fig. 1 Non-structural carbohydrate concentrations (starch + sugar) in each black locust plant organ from saplings to dying trees in May and November 2015. Plot **a** is for branch, **b** stem, **c** bark, **d** root, **e** leaf, and **f** seed. Error bars indicate standard deviations ($n=5$ trees per

treatment). Different lowercase letters denote significant differences between sampling dates within a stand age and different capital letters denote significant differences between stand ages with the same sampling date ($p < 0.05$)

of the partitioning of sugar and starch (Fig. 3). Sugar concentrations remained unchanged for all organs of all tree ages between May and November, but starch concentrations were approximately two-fold higher during the dormant (November) than the growing (May) season for some

organs. The starch-to-sugar ratio in all the organs and across the tree ages surprisingly had a clear seasonal trend (Fig. 4). Sugar concentrations accounted for a much higher proportion of total NSC concentration in May (62–77%) than in November (39–54%) for all tree ages.

Table 3 Summary of two-way ANOVA for the effects of age (from saplings to dying stand) and sampling date on the distribution of carbohydrates in branches, stems, bark, and coarse roots of black locust trees (*Robinia pseudoacacia*)

Variable	df	Sugar		Starch		Total NSC	
		F	P	F	P	F	P
Organ	3	158.2	0.000	35.9	0.000	186.5	0.000
Date	1	5.4	0.023	315.1	0.000	143.2	0.000
Age	5	58.4	0.000	11.2	0.000	59.1	0.583
Organ × date	3	0.3	0.861	6.3	0.001	3.2	0.026
Organ × age	15	14.6	0.000	1.7	0.056	10.6	0.000
Date × age	5	0.3	0.900	0.7	0.653	0.9	0.514
Organ × date × age	15	0.2	1.000	0.4	0.990	0.4	0.961

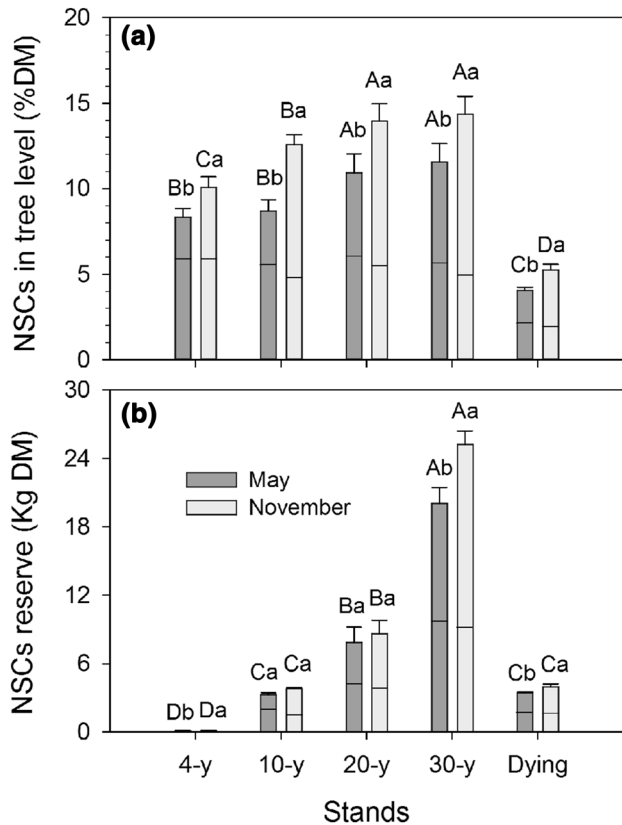


Fig. 2 Plots of NSC concentrations (a) and pools (b) at the tree scale for black locust (*Robinia pseudoacacia*) in May and November 2015. Leaf, stem, branch, and root biomass were estimated and extrapolated at the tree scale using allometric relationships based on stem diameter at breast height and the whole tree height. Note that missing leaf NSC data for November were neglected. Error bars indicate standard deviations ($n=5$ trees per treatment). Different lowercase letters denote significant differences between sampling dates within a stand age and different capital letters denote significant differences between stand ages with the same sampling date ($p < 0.05$)

Discussions

NSC Distribution for Age Class and Plant Organ

Despite similar climatic and edaphic conditions, the concentrations and pools of NCS differed at the tree scale from saplings to dying trees, which indicated that the partitioning of NSC varied with stand type. Consistent with other studies (Niinemets 1997; Sala and Hoch 2009), the concentration of NCS increased with increasing tree age, except for dying trees (Fig. 2), probably due to the large difference in photosynthesis among the tree stands (Körner 2003; Sala and Hoch 2009). Higher NSC concentrations in larger trees could also be partly associated with the higher light availability to taller trees (Niinemets 2010). Tolerance to low light conditions decreased with increasing tree size due to lower photosynthetic rates and a higher fraction of non-photosynthetic biomass (Valladares and Niinemets 2008). Saplings, however, allocated less carbon to storage than mature trees, even if grown under more light and optimal conditions (Niinemets 2010).

The estimated concentration of NCS in this study was higher for coarse roots and stems and lower for leaves (Fig. 1), which was in agreement with other studies on the distribution of NSC in deciduous plants (Samuelson and Kelly 1996; Bollmark and others 1999; Zhang and others 2015). The higher NSC concentration in roots and stems than in other parts of the black locust plant can be attributed to the high metabolic activity of sapwood and the long transport function of carbohydrates (Yang and others 2016). In addition, roots and stems form an important NSC storage reserve, reflecting the buffering capacity of trees against stress (Galiano and others 2011). In this study, NSC concentration was lowest for leaves; generally 2/3 of the concentration in stems or roots for black locust (Zhang and others 2015) and also for other tree species (Barbaroux and others 2002). This could be related to the characteristics of carbon allocation and transport in tree plants (Yang and others 2016). Furthermore, big trees generally have a higher NSC concentration and pool than small trees (Figs. 1, 2). Again this could be explained by developed root systems

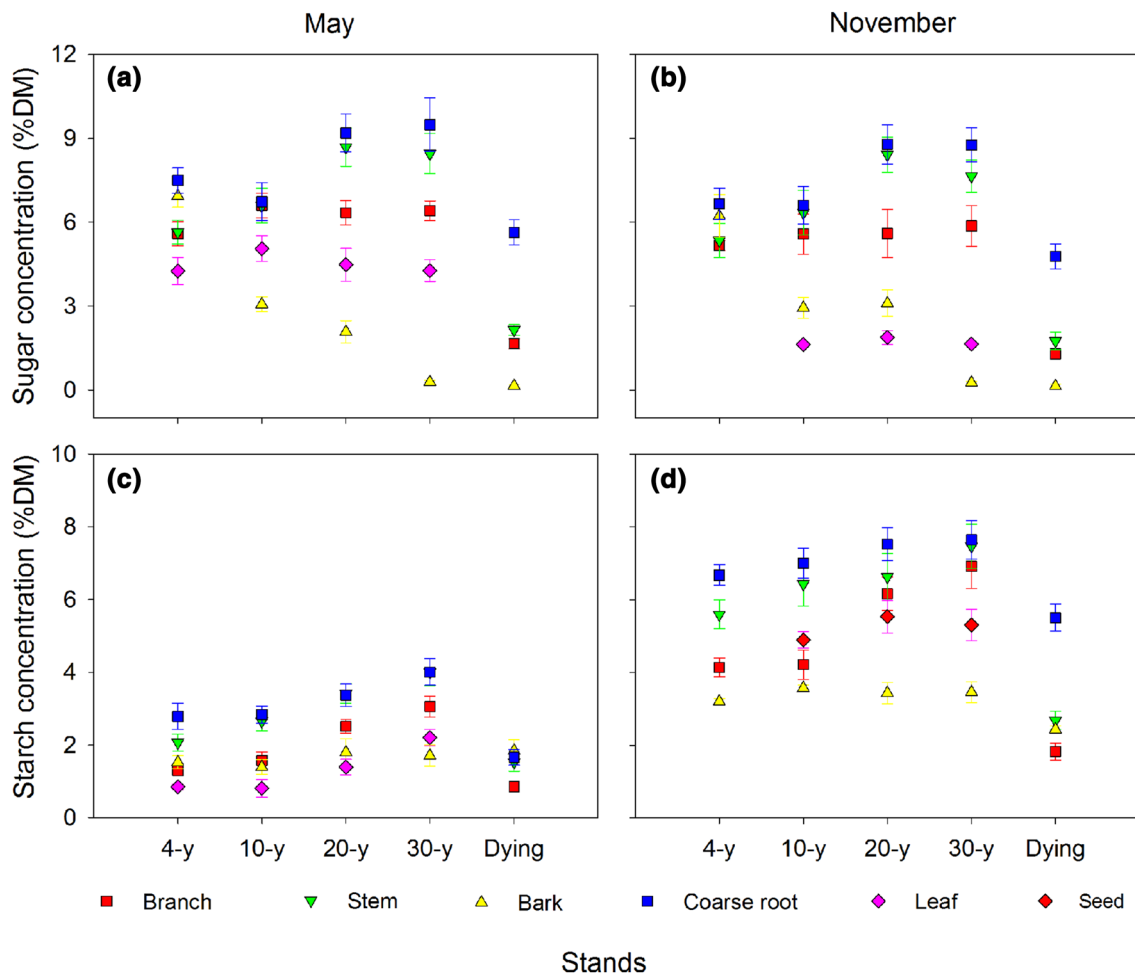


Fig. 3 Plots of sugar (glucose, sucrose, and fructose) (a, b) and starch (c, d) concentrations in black locust plant organs from saplings to dying trees in May and November 2015. Error bars indicate standard

errors ($n=5$ trees per treatment). Leaf and seed samples were collected in May and November 2015, respectively

considered to be large carbon sinks. This sink is often at the end of a long carbon transport chain which prevents the complete replenishment of NSC in roots (Minchin 2007). The tree-scale sampling for NSC concentration in this study represented the NSC budget for a whole tree because coarse roots, stems, and branches contribute substantially to the NSC pool (Würth and others 2005).

It is interesting to note that in this study, dying trees showed NSC pool depletion (Fig. 2). Firstly, for dying trees, photosynthetic capacity was not sufficient to provide the required energy to support plant respiration, thus the non-recharge of the NSC pool (Ni and others 2017). Secondly, decreasing biomass of dying trees resulted in the lowering of the NSCs pool. Generally, the degradation of vegetation could be explained by soil moisture decline. In arid and semi-arid regions, water is a key consideration for afforestation efforts (Cao and others 2009; Jin and others 2011; Deng and others 2016). However, the widespread implementation

of GFP has intensified the depletion of soil moisture (Jia and others 2017). In addition, forests are clearly not a suitable choice of vegetation in areas with mean annual precipitation of less than 600 mm (Deng and others 2016). Therefore, determining the correct relationship between soil water availability and the NSC pool from saplings to dying trees can strengthen the existing understanding of the processes of growth and deterioration of forestry ecosystems.

NSCs in Growing and Dormant Seasons

Although the variations in NSC were very similar among all the tree ages, there were differences in NSC between growing and dormant seasons. For example, total NSC and starch concentrations increased from May to November (Figs. 2, 3). The trends could be explained by the replenishment of NSC concentration during the summer growing season (for example, NSC increased from May to August, see Andrew

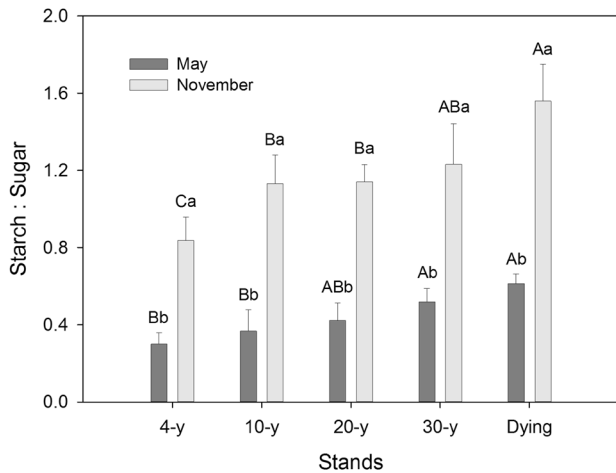


Fig. 4 Plot of starch-to-sugar ratios at the tree scale from saplings to dying trees in May and November 2015. Error bars indicate standard deviations ($n=5$ trees per treatment). Different lowercase letters denote significant differences between sampling dates within a stand age and different capital letters denote significant differences between stand ages with the same sampling date ($p < 0.05$)

and others 2013), when photosynthesis exceeded metabolic demand for carbon. However, more sampling frequency is necessary for accurate determination of the dynamics of NSC concentrations. The conversion of (readily transported and metabolically active) sugar to (immobile and metabolically inert) starch (Andrew and others 2013; Woodruff and Meinzer 2011) varies with season (Fig. 3). For example, the sugar concentration in May accounted for a much higher proportion (61–77%) of NSC concentrations for all tree ages, whereas the starch concentration in November was higher than that of sugar for all tree ages. The increase in starch concentration from May to November was due to the larger increase in total NSC concentration over the same period. This conformed well with the standard conceptual model, for which the NSC pool is depleted when demand exceeds supply over the course of the summer growing season (for example, May), and the pool recharges when supply exceeds demand over the course of the winter dormant season (for example, November) (Chapin and others 1990; Grulke and Retzlaff 2001).

Clearly, the sampling for plant NSC in this study failed to capture the complete picture of the NSC budget at the tree scale. Long-term sampling and parallel data (for example, radial stem growth) are needed for a full understanding of carbohydrate demand and supply, together with the conversion of sugar to starch and vice versa, in different stand ages (Andrew and others 2013). It will also deepen the existing insight into the processes of mobilization of reserves (as storage in leaves, roots, stems and branches) and the influences of the overall dynamics of the NSC budget at the tree scale.

Conclusions

A unique short-term study was conducted to quantify the seasonal variability of NSC concentrations using the dominant forest tree species on the Loess Plateau. The study showed that NSC concentrations at the tree scale generally increased with tree age. Intra-tree patterns of NSC distribution from saplings to dying trees were similar. NSC concentrations and pools showed a highly seasonal dynamic at the tree scale. In the month of November, starch concentrations were higher than sugar concentrations for all tree ages. Irrespective of the above findings, an accurate construction of the NSC budget requires a long sampling period and parallel data (stem growth) for measuring NSC concentrations in all plant organs, including fine roots and twigs. This would be critical for scaling up measured concentrations to plantation, regional, and global scales.

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