Tree Physiology 40, 259–271
doi:10.1093/treephys/tpz132

Research paper

Non-structural carbohydrate pools not linked to hydraulic strategies or carbon supply in tree saplings during severe drought and subsequent recovery

Steven A. Kannenberg[1,2,3] and Richard P. Phillips[2]

[1]School of Biological Sciences, University of Utah, 257 1400 East, Salt Lake City, UT 84112, USA; [2]Department of Biology, Indiana University, 1001 East 3rd Street, Bloomington, IN 47405, USA; [3]Corresponding author (s.kannenberg@utah.edu)

Received August 8, 2019; accepted November 16, 2019; handling Editor Michael Ryan

Non-structural carbohydrate (NSC) pools fluctuate based on the interplay between photosynthesis, demand from various carbon (C) sinks and tree hydraulic status. Thus, it has been hypothesized that tree species with isohydric stomatal control (i.e., trees that close stomata rapidly in response to drought) rely heavily on NSC pools to sustain metabolism, which can lead to negative physiological consequences such as C depletion. Here, we seek to use a species' degree of isohydry or anisohydry as a conceptual framework for understanding the interrelations between photosynthetic C supply, hydraulic damage and fluctuations in NSC pools. We conducted a 6-week experimental drought, followed by a 6-week recovery period, in a greenhouse on seven tree species that span the spectrum from isohydric to anisohydric. Throughout the experiment, we measured photosynthesis, hydraulic damage and NSC pools. Non-structural carbohydrate pools were remarkably stable across species and tissues—even highly isohydric species that drastically reduced C assimilation were able to maintain stored C. Despite these static NSC pools, we still inferred an important role for stored C during drought, as most species converted starches into sugars during water stress (and back again post-drought). Finally, we did not observe any linkages between C supply, hydraulic damage and NSC pools, indicating that NSC was maintained independent of variation in photosynthesis and hydraulic function. Our results advance the idea that C depletion is a rare phenomenon due to either active maintenance of NSC pools or sink limitation, and thus question the hypothesis that reductions in C assimilation necessarily lead to C depletion.

Keywords: anisohydric, carbon balance, carbon depletion, hydraulic damage, isohydric.

Introduction

Non-structural carbohydrates (NSCs) represent a major carbon (C) pool within woody plants, with many mature trees containing sufficient NSC stores to rebuild their canopy four times over (Hoch et al. 2003). Non-structural carbohydrates, consisting primarily of soluble sugars and starches, are also crucial metabolic substrates for myriad aspects of tree functioning such as leaf out, reproduction, defense, transport and storage (Chapin et al. 1990, Dietze et al. 2014, Hartmann and Trumbore 2016). Non-structural carbohydrates become especially important in the context of environmental stressors such as drought, when the mobilization of stored resources can become imperative for survival. During drought, trees are hypothesized to rely heavily on stored NSC due to reduced C supply through photosynthesis and a continued need for C to sustain metabolism (McDowell et al. 2008, McDowell 2011). While drought-induced declines in NSCs may not necessarily kill trees (Sala et al. 2010), changes in their mobilization do reflect numerous aspects of tree physiological function (O'Brien et al. 2014, Hartmann and Trumbore 2016). For example, trees can use NSCs to alleviate drought stress by increasing belowground allocation (Brunner et al. 2015, Karst et al. 2017), raising leaf water potential ($\sim_L$, O'Brien et al. 2014), osmoregulating cells (Dietze et al. 2014, Hartmann and Trumbore 2016) and repairing hydraulically damaged vessels (Klein et al. 2018, Trifilò et al. 2019). While the full spectrum of the roles NSCs play is yet to be resolved, NSC dynamics during water stress have provided a useful test bed for understanding basic patterns of tree C allocation.

Despite the fundamental role of NSC in many plant physiological processes, investigations of NSC dynamics during drought have found wildly contrasting results. For example, NSCs have been shown to decrease (Galiano et al. 2011, Woodruff 2014), not change (Anderegg et al. 2012, Anderegg and Anderegg 2013, Rosas et al. 2013) and even increase (Galvez et al. 2011, O'Brien et al. 2015, Piper and Fajardo 2016, Kannenberg and Phillips 2017) in response to water stress. While a non-declining NSC pool may seem counterintuitive in the face of reduced C supply, lessened demand in competing C sinks (such as growth) relative to photosynthesis or active allocation of limited photosynthate to NSC pools can both serve as mechanisms to preserve or increase NSC (Sala et al. 2012, Wiley and Helliker 2012, Dietze et al. 2014). These disparate results may reflect the fact that NSCs are not one homogenous pool and the functional role of NSC can be highly dependent on both chemical composition and location within a tree. For example, soluble sugars act as osmoregulatory compounds, while non-soluble carbohydrates such as starches serve as a longer-term storage pool and can be mobilized to support tree function years in the future (Richardson et al. 2013, Dietze et al. 2014, Muhr et al. 2016). Thus, fluctuations in sugar versus starch pools can be used to infer the functional demands of trees during environmental stress. In addition, while NSCs in more metabolically active tissues such as leaves and fine roots likely reflect recent C assimilation, respiratory demand or osmoregulatory needs, observations that tissues such as stems and coarse roots contain the largest and least temporally variable NSC pools suggest they function primarily as longer-term C stores (Dietze et al. 2014, Hartmann and Trumbore 2016). Moreover, the degree to which C storage is an actively regulated process, or merely represents a surplus of C due to sink limitation, is still unresolved (Sala et al. 2012, Wiley and Helliker 2012, Dietze et al. 2014, Körner 2015). Thus, there exists substantial complexity in how whole–tree NSC pools fluctuate in response to drought, introducing uncertainty in our general understanding of tree C dynamics.

The physiological changes trees undergo during drought stress can also have consequences for post-drought recovery and lagged tree mortality (Anderegg et al. 2013, Trugman et al. 2018, Kannenberg et al. 2019). However, the role NSCs play in promoting tree recovery is highly uncertain. Following a severe drought, stored C is important to facilitate recovery from drought-induced loss of leaf area, regrow roots and repair hydraulic damage (Doughty et al. 2014, Hagedorn et al. 2016, Klein et al. 2018). The ability of trees to replenish NSC—and thus recover—is likely contingent on two factors: (i) the cumulative C loss during drought and (ii) the use of NSC for facilitating repair processes. Unfortunately, the importance of post-drought NSC dynamics for tree function has been rarely investigated (though see Galiano et al. 2017, Tomasella et al. 2017 and Zeppel et al. 2019).

In an attempt to explain variation in NSC dynamics during drought, a popular framework has linked tree hydraulic strategies (i.e., isohydry versus anisohydry) with NSC pools (McDowell et al. 2008, McDowell 2011, Mencuccini et al. 2015). This framework posits that the ways various tree species regulate $\sim_L$ during drought has important consequences for stored C. According to this framework (hereafter referred to as the isohydry–anisohydry spectrum), isohydric trees close stomata to maintain 'safer' $\sim_L$ at the first signs of water stress, whereas anisohydric trees keep stomata open and lower their $\sim_L$ to maintain gas exchange (Tardieu and Simonneau 1998). Consequently, isohydric species are believed to more readily utilize NSCs during drought due to reductions in photosynthesis. Anisohydric trees, which may incur hydraulic damage by keeping stomata open during drought, likely experience little change in NSC pools. However, species with this strategy may need to mobilize NSCs post-drought in order to repair damaged vessels (Zeppel et al. 2019).

Most research to date on NSCs and the isohydry–anisohydry spectrum has been done in piñon–juniper woodlands, a model ecosystem for tree drought physiology that experiences frequent droughts and is adapted to low water availability. However, the utility of the isohydry–anisohydry spectrum for predicting NSC dynamics across a wider range of species is unresolved, as is the role of hydraulic strategies in mediating a plant's C storage post-drought. In this study, we sought to build on previous work that found anisohydric species experience greater hydraulic damage during drought than isohydric species and that hydraulic damage and C limitation can persist for weeks post-drought (Kannenberg et al. 2019). Here, we seek to test the hypotheses that:

(i) Isohydric species reduce NSC during drought and will convert NSC from starches to sugars in order to support metabolic function. Non-structural carbohydrate pools will recover post-drought due to a recovery of photosynthesis.
(ii) Anisohydric species, in contrast, will not reduce NSC during drought but will rely on sugars post-drought to repair drought-induced damage.

Thus, we seek to investigate the utility of the isohydry–anisohydry spectrum framework in predicting NSC dynamics and shed light on the mechanisms that control NSC pool fluctuations in drought-stressed trees.

Materials and methods

Experimental design

In a greenhouse, we exposed seven North American tree sapling species (*Acer saccharum Marshall*, *Carya ovata Mill.*, *Juniperus virginiana L.*, *Liriodendron tulipifera L.*, *Quercus alba L.*, *Quercus velutina Lam.* and *Sassafras albidum Nutt.*) to a 12-week drying–rewetting cycle. These species were chosen as they co-occur in Midwestern forests and are known to span a spectrum from highly isohydric to strictly anisohydric (Brzostek et al. 2014, Roman et al. 2015, Kannenberg et al. 2019). *Acer saccharum*, *J. virginiana*, *L. tulipifera* and *S. albidum* saplings were purchased from Cold Stream Farm (Free Soil, MI, USA), while *C. ovata*, *Q. alba* and *Q. velutina* saplings were purchased from Vallonia Tree Nursery (Indiana DNR, Vallonia, IN, USA). Trees at both nurseries (3–4 years old) were grown from local seed without transplanting, experienced similar growing season climate and were purchased as bare root saplings (39.175525, −86.505437, 0.5–2 m tall, depending on species). Within 2 weeks of their purchase in late winter, saplings were transplanted into 20 × 46 cm pots filled with a local mesic Typic Paleudalf soil that was cut with 50% coarse sand to facilitate drainage. Following planting, saplings were grown for 3 months (to ensure full leaf expansion) in a greenhouse where photoperiod was kept constant with grow lights, temperatures were maintained between 20 and 30 °C and relative humidity averaged 50%. Mean leaf, stem and root dry biomass for each species at the start of the experimental period, along with mean stem diameter, are available in Table S1 available as Supplementary Data at *Tree Physiology* Online.

Once trees were established and determined to be healthy, all trees were randomly assigned into treatment groups. Ten trees per species remained well-watered throughout the experiment as controls (volumetric water content (VWC) between 10 and 20%), while 20 trees per species were subjected to a drought experiment (Figure 1a). In the drought experiment, watering was reduced so that VWC ranged from 3 to 6% in most pots by week 4. Following this drought phase, all trees were watered similarly to controls for another 6-week period (i.e., the ‘recovery phase’). The length of the drought period was chosen so as to stress the trees nearly to mortality, but still provide the opportunity to recover. Thus, the drought phase lasted until some individuals of each species exhibited leaf browning, loss of turgor and were starting to die. These individuals were excluded from analysis but were used as an indicator to start the recovery phase.

Tree harvesting

Trees were randomly selected from each treatment and destructively harvested at four time points through the experiment. Control trees ($n = 5$) were harvested during the first week of the drought experiment, while drought-stressed trees ($n = 5$) were harvested three times during the experiment (week 6 of the drought phase and weeks 3 and 6 of the recovery phase). Following removal from pots and washing of roots, harvested trees were separated into leaves, stems, and roots. These samples were then dried in a 60 °C oven for 48 h and weighed. While tissue samples were still wet, a <5 g subsample was removed for NSC analysis, weighed, immediately placed in dry ice, frozen at −80 °C within 4 h and freeze-dried within a week to prevent degradation of organic compounds. These freeze-dried samples were then weighed again to create a wet-to-dry conversion factor for all tissues across all species. Freeze-dried subsamples were then ground to a fine powder using a Wiley mill. Whole-tree dry tissue biomass measurements

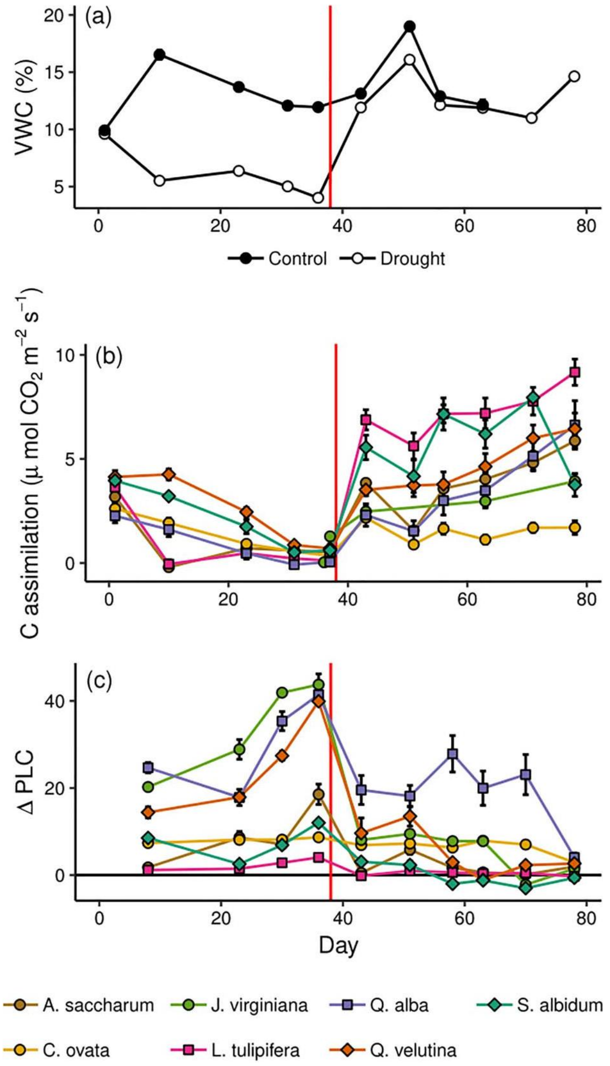

Figure 1. Time series of VWC (a), C assimilation (b) and ΔPLC (c) over the length of the experiment. The vertical red line represents the transition between the drought phase and the recovery phase. Error bars represent ± standard error. Carbon assimilation measurements in week 3 were cut to match our integrated C assimilation calculations (see Materials and methods).

were adjusted for the NSC subsample based on the wet-to-dry conversion factor.

Soil water content and C assimilation

Carbon assimilation (*A*) and VWC were measured on all trees weekly, with the exception of *J. virginiana* gas exchange, which was obtained for week 6 of the drought phase and weeks 1, 4 and 6 of the recovery phase. Measurements of *A* were obtained using a LI-6400 XT (LiCor Inc., Lincoln, NE, USA) with chamber conditions set to: 400 p.p.m. CO_2, 1500 μmol m^{-2} s^{-1} photosynthetically active radiation, 25 °C leaf temperature and a flow rate of 500 μmol s^{-1}. The VWC was measured at the same time as gas exchange with a HydroSense II moisture probe (Campbell Scientific, Logan, UT, USA). All measurements were performed between 10 a.m. and 2 p.m. on sunny days.

In order to understand potential source-driven changes in NSC pools, we quantified integrated C assimilation (in mol CO_2 m^{-2} day^{-1}) that occurred in between our NSC samplings. To do this, we cut an outlier in our *A* time series data (week 3) due to an accidental watering, though the inclusion of this outlier did not affect the ranking of our species' integrated C assimilation, nor did it qualitatively alter our results. We then integrated the area under the remaining *A* time series curve for each individual tree using the *AUC* function in the *DescTools* R package (Signorell et al. 2019). This function connects each time series measurement in a stepwise fashion and calculates the area under the corresponding curve. We performed this integration on specific time spans that matched our NSC samplings, under the assumption that variation in NSC will most closely track variation in C assimilation during the entire experiment prior to sampling. Therefore, our integrated C assimilation measurements were calculated for weeks 0–6 of the experiment (to match the drought sampling), weeks 0–9 (to match the 3 week recovery sampling) and weeks 0–12 (to match the 6 week recovery sampling). *Juniperus virginiana* was only sampled once during the drought phase and three times during the recovery phase. Thus, we could not obtain estimates of integrated C assimilation for this species that would be comparable to all other species. Therefore, we excluded this species for the integrated C assimilation analysis. It is important to note that our metric of integrated C assimilation is meant to encompass temporal variation in C source activity and thus is still a rate of C assimilation (mol CO_2 m^{-2} day^{-1}), rather than a total amount of fixed C.

Hydraulic traits and hydraulic damage

On most weeks between 10 a.m. and 2 p.m. on a sunny day, we measured $_{\sim L}$ on a subset of four randomly chosen trees in each treatment using a Model 600 Scholander pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Prior to measuring $_{\sim L}$, we equilibrated leaf water potential with stem water potential by covering each leaf with a plastic bag and aluminum foil for 15 min. We did this so as to make our water potential measurements more representative of our vulnerability curves (described below).

We derived soil water potential $(_{\sim soil})$ from the weekly VWC measurements using a soil water retention curve created on our soil. This curve was developed by co-locating a CS650 soil water content reflectometer and two 229 heat dissipation matric potential sensors (Campbell Scientific) in a pot of our soil and exposing the soil to four wetting/drying cycles that fluctuated VWC between 3 and 22%. Data were compiled into daily averages and fit to the van Genuchten (1980) model via the online *SWRC* tool (Seki 2007). We quantified a species' degree of anisohydry (σ) as the slope between mid-day $_{\sim L}$ and $_{\sim soil}$ (Martínez-Vilalta et al. 2014, Kannenberg et al. 2019). Only data collected during the drought phase were used for this metric so as to capture tree hydraulic responses when soil moisture was most limiting.

In order to calculate a species' embolism resistance and model drought-induced hydraulic damage, we created species-specific percent loss of hydraulic conductivity (PLC) curves using stem samples (0.3–1 m in length) from non-droughted individuals of each species during the last month of the experiment. These stem samples were immediately cut, placed in cold water, and re-hydrated with degassed water in a vacuum desiccator for 24 h prior to measurement. The PLC curves were developed using an air seeding method modified from Cochard et al. (1992), by which the fully hydrated stem segments were exposed to increasingly high pressure from 0 to 90 bar, while flow rate through the stem segment was measured with a XYL'EM apparatus (Bronkhorst, Ruurlo, The Netherlands). Three to five curves were created per species and a re-parameterized Weibull model was fit to the aggregated species data (Ogle et al. 2009) using the *fitPLC* R package (Duursma and Choat 2017). From these curves, we were able to derive the water potential at 50% of maximum conductivity (P_{50}) and the P_{50} safety margin (the difference between P_{50} and the minimum day-averaged stem water potential for a species during the drought phase). Further, we modeled drought-induced hydraulic damage (○PLC) by estimating PLC at each time point using $_{\sim L}$ measurements and our PLC curves, then subtracting each drought-stressed tree's PLC from the mean PLC in the control treatment for that species. Hydraulic traits and σ for all species are detailed in Table 1.

Non-structural carbohydrate analysis

Soluble sugar and starch concentrations in ground tissues were measured using a sequential extraction protocol adapted from Chow and Landhäussser (2004) and previously reported on by Kannenberg et al. (2018). Briefly, soluble sugars were extracted using a methanol:chloroform:water liquid phase extraction and starches were depolymerized in weak sulfuric acid at 90 °C for 30 min. Both extracted soluble sugars and depolymerized

Table 1. Water potential at which 50% of stem hydraulic conductivity has been lost (P_{50}), the P_{50} safety margin and the degree of anisohydry (σ) for all species. Uncertainty estimates for σ represent the 95% confidence interval.

Species	P_{50}	σ	P_{50} safety margin
A. saccharum	−6.26	0.261 ƒ 0.134	3.29
C. ovata	−0.56	0.235 ƒ 0.588	−3.32
J. virginiana	−4.81	0.739 ƒ 0.238	−0.87
L. tulipifera	−7.85	0.374 ƒ 0.200	5.83
Q. alba	−3.99	1.673 ƒ 1.09	−0.54
Q. velutina	−2.53	1.336 ƒ 0.434	−1.97
S. albidum	−1.77	0.443 ƒ 0.273	−0.26

starch solutions were exposed to concentrated sulfuric acid and phenol, followed by a 10-min color development phase and a 30-min room temperature water bath phase. NSC concentrations were determined colorimetrically on a Shimadzu UV-1700 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) set to 490 nm. Spectrophotometric data were converted into NSC concentrations using a standard curve created from a 1 mg/ml 1:1:1 glucose:fructose:galactose solution. NSC concentrations were scaled to mass using the dry biomass for each tissue, then NSC pool sizes (as a percent of total tree dry biomass) were calculated as the mass of NSC in each tissue divided by whole-tree NSC mass.

Statistical analysis

To assess temporal differences in NSC pool within a species, we used Tukey's HSD pairwise tests. Linear models were used to assess relationships between NSC pools, integrated C assimilation and hydraulic damage. All statistical analyses were conducted in R 3.5.1.

Results

Species' hydraulic traits and physiology

While anisohydric species lowered $\sim_{L}$ during drought, these species were not able to sustain carbon assimilation to a greater degree than more isohydric species. In response to experimental soil moisture reduction, all species significantly reduced *A* in the first or second week of the experiment (Figure 1b). *A* remained low for the rest of the drought phase, reaching between 0 and 14% of controls by the end of the drought treatment. Towards the end of the drought, these reductions in *A* were fairly similar in magnitude between species—only *Q. alba* and *A. saccharum* had higher *A* in week 4, *Q. alba* reduced *A* to a greater degree than other species in week 5 and *A* was higher in *J. virginiana* in week 6. These temporal divergences in species' *A* resulted in differences in the total amount of C supplied by photosynthesis, integrated over the length of time prior to sampling NSCs (Table 2). However, integrated C assimilation was not related to a species' degree of anisohydry or any other hydraulic traits.

Non-structural carbohydrate dynamics

Across tissues (leaves, stems, roots) and carbohydrate types (soluble sugars and starches), NSC pools remained mostly static during drought stress and subsequent recovery, as only *J. virginiana* decreased whole–tree NSC by the end of the drought treatment (48% reduction relative to controls, Figure 2). This reduction in whole–tree NSC was primarily driven by reductions in leaf sugars and starches, although root starch pools also decreased during drought for this species (Figures 3 and 4). Likewise, only one species altered post-drought NSC pools, as *L. tulipifera* elevated whole–tree NSC pools by 83% in week 3 and 100% in week 6 (Figure 2) due to increases in leaf and stem sugars (Figure 3). More nuanced shifts in NSC pools were observed across NSC types, such as drought-induced increases in *A. saccharum* stem sugars that persisted into recovery, post-drought decreases in *Q. velutina* leaf and stem starches and post-drought reductions in *S. albidum* root sugars (Figures 3 and 4). However, these shifts in NSC pool size, while statistically significant, were relatively minor and did not scale to affect whole–tree NSC pools.

In general, most tree species increased the proportion of total NSC that was soluble sugars, though this increase was only statistically significant (via pairwise comparison among all time points) for *A. saccharum*, *Q. alba* and *Q. velutina* (Figure 5). These trends in whole–tree NSC were underlain by tissue-specific differences, as increases in soluble sugars were largely driven by increases in leaf sugar, though *A. saccharum* drastically increased soluble sugar pools in the stem and *Q. velutina* increased soluble sugars in roots. Following drought, all species except *A. saccharum* decreased soluble sugar pools back to control levels with two exceptions, *Q. velutina* and *S. albidum*, which had reduced post-drought stem and root sugars (respectively) relative to controls.

Despite changes in total NSC pool size across species during drought stress and recovery, the partitioning of NSC among tissues (i.e., the proportion of total NSC stored in leaves or stems or roots) was fairly static (Figure 6). Only two species, *A. saccharum* and *Q. alba*, altered NSC pools among tissues, though this shift only occurred 6 weeks after the drought treatment ceased.

Linkages between C assimilation, NSC pools and hydraulic damage

In order to link observed changes in NSC pools with drought-induced photosynthetic and hydraulic limitations, we performed a series of linear models between NSC in various tissues, OPLC and integrated C assimilation over the course of the experiment. We found that C assimilation, integrated over time, was not related to OPLC at the end of the drought treatment

Table 2. C assimilation integrated over the time points at which NSCs were sampled: 'drought' (weeks 0–6), '3 weeks post-watering' (weeks 0–9) and '6 weeks post-watering' (weeks 0–12). *Juniperus virginiana* data are not included due to a limited number of sampling points (see Materials and methods). Uncertainty estimates represent standard error.

	Integrated carbon assimilation (mol CO_2 m^{-2} day^{-1})		
Species	Drought	3 weeks post-watering	6 weeks post-watering
A. saccharum	3.06 ± 0.35	6.13 ± 0.60	8.88 ± 1.37
C. ovata	5.11 ± 0.52	7.25 ± 0.62	8.42 ± 0.86
L. tulipifera	3.21 ± 0.41	11.37 ± 1.02	18.32 ± 2.55
Q. alba	3.85 ± 0.49	5.38 ± 0.71	7.63 ± 1.62
Q. velutina	10.14 ± 0.46	12.04 ± 0.91	13.96 ± 1.67
S. albidum	8.15 ± 0.57	11.85 ± 1.61	15.53 ± 3.23

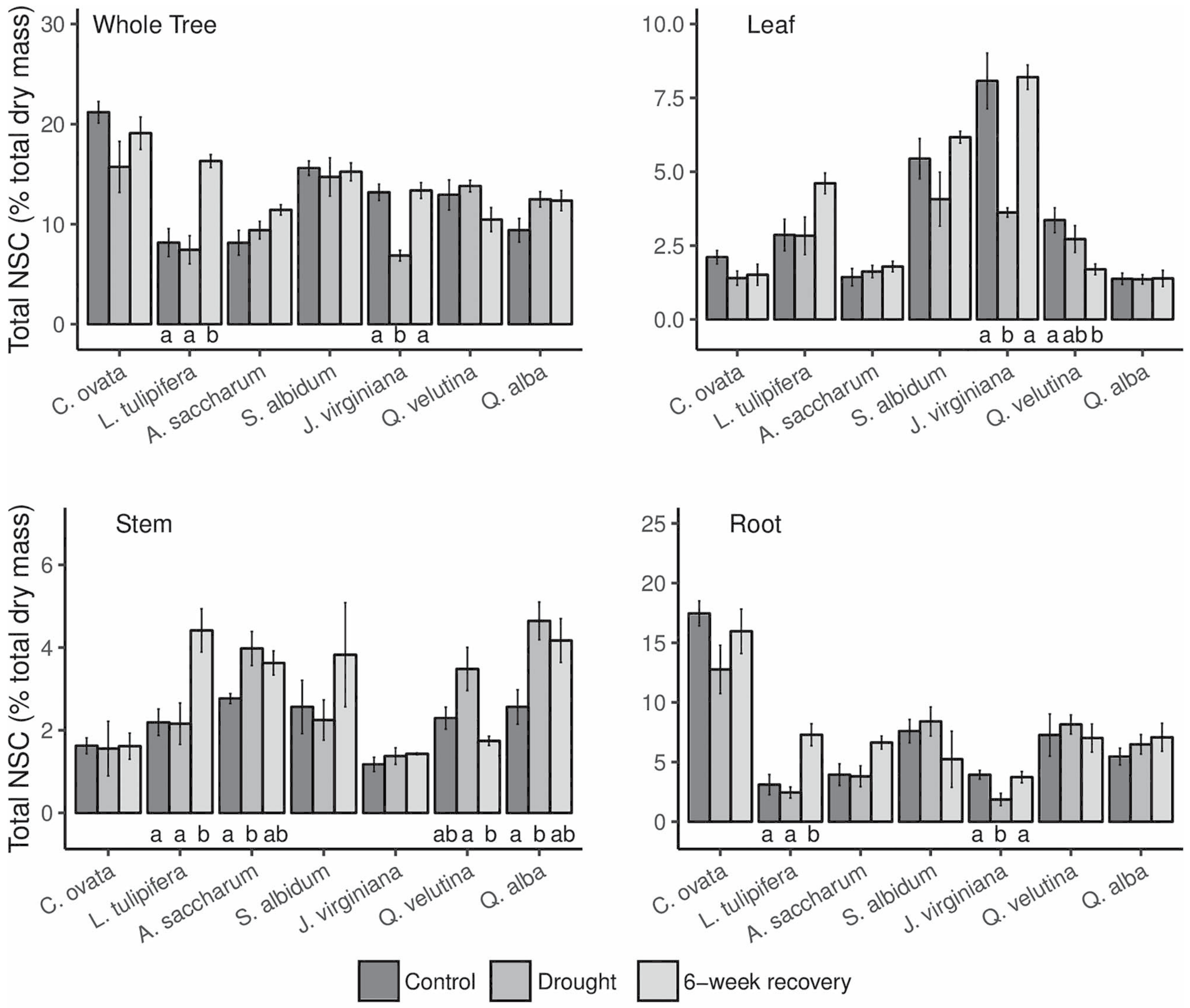

Figure 2. Total NSC (soluble sugar + starch mass as a percent of total tree dry biomass) for whole-tree, leaf, stem and root pools at each sampling point throughout the experiment. The *x*-axis is ordered according to anisohydricity, with low σ species on the left and high σ species on the right. Error bars represent ± standard error. Letter notation indicates within-species statistical differences via pairwise comparison and is only present when significant within-species differences occur.

or after a 3-week recovery. Integrated C assimilation was, however, related to OPLC at the end of the 6 week recovery ($P = 0.02$, $r^2 = 0.21$), but the slope of this relationship was extremely shallow. Moreover, variation in OPLC at this time point was negligible, as only 2 of 26 trees had OPLC >11%, indicating that this relationship—while statistically

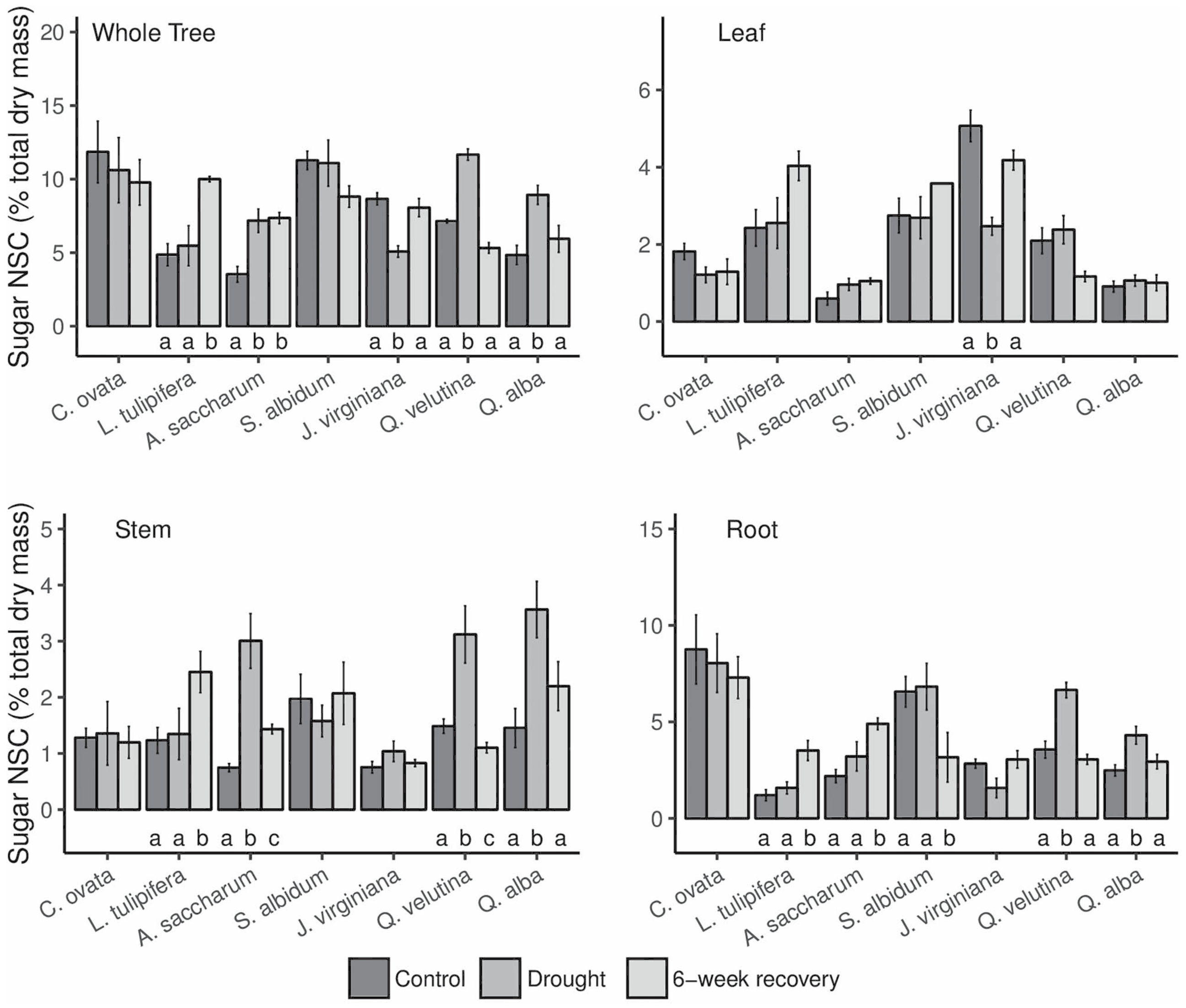

Figure 3. Sugar NSC (soluble sugar mass as a percent of total tree dry biomass) for whole-tree, leaf, stem and root pools at each sampling point throughout the experiment. The x-axis is ordered according to anisohydricity, with low σ species on the left and high σ species on the right. Error bars represent ± standard error. Letter notation indicates within-species statistical differences via pairwise comparison and is only present when significant within-species differences occur.

significant—was not very physiologically meaningful. Additionally, integrated C assimilation was not linked with whole-tree NSC pools at any time point. Due to a temporal mismatch between when trees were sampled for $\sim_{\mathrm{L}}$ and when trees were sampled for NSCs, we were unable to relate OPLC to NSC on an individual tree level. However, we detected no significant relationships at any time point between OPLC and NSC pools at the species level.

Discussion

The way tree species regulate the trade-off between depleting C reserves versus sustaining hydraulic damage has important implications for their ability to tolerate, and recover from, drought stress. In an attempt to test the trade-offs inherent in the isohydry–anisohydry spectrum framework, we sought to understand the interrelationships between C source activity (i.e., integrated C assimilation), NSCs and hydraulic damage. We found NSC depletion to be rare, even in extremely isohydric tree saplings. In the one species that reduced stored C during drought, NSCs recovered quickly and actually surpassed the size of the NSC pool in control trees. Despite reduction in NSCs being rare, even in the face of C limitation (Garcia-Forner et al. 2017, Weber et al. 2019), we observed evidence for an important role of NSCs during drought, as starches were frequently converted to soluble sugars during drought and subsequently polymerized back into starch post-drought. Finally, we found evidence that NSC pools during drought were not linked to either net C assimilation or hydraulic impairment. Our results add to existing evidence that NSC depletion is a rare phenomenon in response

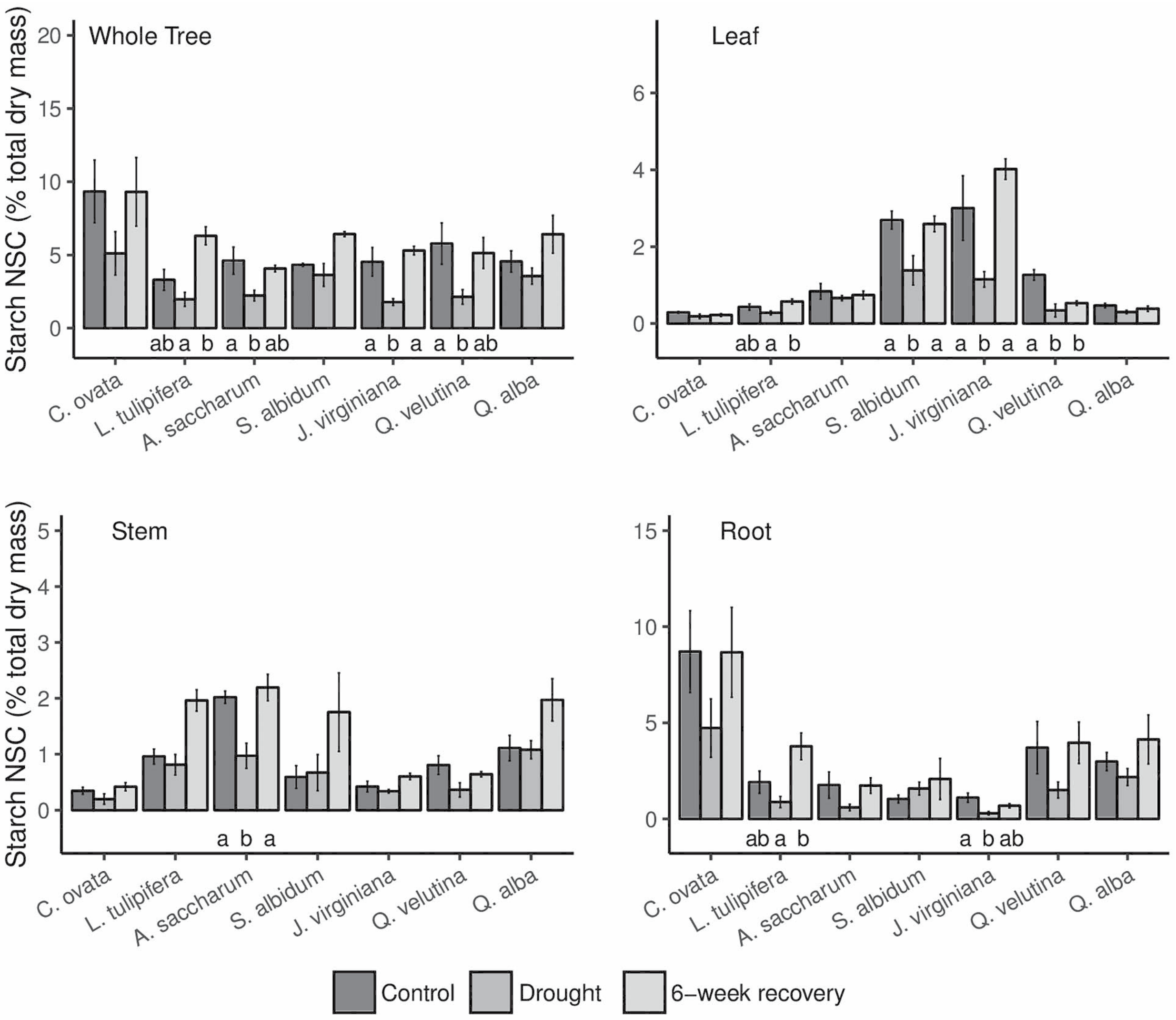

Figure 4. Starch NSC (starch mass as a percent of total tree dry biomass) for whole-tree, leaf, stem and root pools at each sampling point throughout the experiment. The *x*-axis is ordered according to anisohydricity, with low σ species on the left and high σ species on the right. Error bars represent ± standard error. Letter notation indicates within-species statistical differences via pairwise comparison and is only present when significant within-species differences occur.

to drought stress (Hartmann 2015, Dickman et al. 2019) and promote the idea that NSC pool fluctuations can be decoupled from C assimilation and plant hydraulic status.

We predicted that isohydric tree saplings would experience greater reductions in photosynthesis during drought than anisohydric saplings (owing to the rapid onset of stomatal closure), leading to decreases in NSC pools. However, we found that all species, not just isohydric ones, reduced photosynthesis in response to water stress and that whole-tree NSC pools were remarkably static in most species throughout the entire experiment. Out of our seven species, only one (*J. virginiana*) reduced whole-tree NSC after the 6-week drought period. It is notable that this species was our only gymnosperm, a taxa known to decrease NSC during drought more readily than angiosperms (Adams et al. 2017). While NSC depletion should theoretically occur during drought stress due to reductions in C assimilation, static NSC pools during drought are not an uncommon result (Anderegg et al. 2012, Anderegg and Anderegg 2013, Tomasella et al. 2017, Dickman et al. 2019, Jin et al. 2018, Kannenberg et al. 2018, Dietrich et al. 2018). Thus, in addition to being unable to link NSC pool fluctuations to hydraulic strategies (i.e., isohydry or anisohydry), we were also unable to use reductions in C assimilation as a predictor of NSC depletion (Garcia-Forner et al. 2017). Our results provide robust evidence that NSC pools can be maintained in the face of highly isohydric behavior. While the utility of the isohydry–anisohydry framework for predicting NSC dynamics has previously been questioned, our study is the first to observe a disconnect between C depletion and isohydric behavior across a large number of species while also

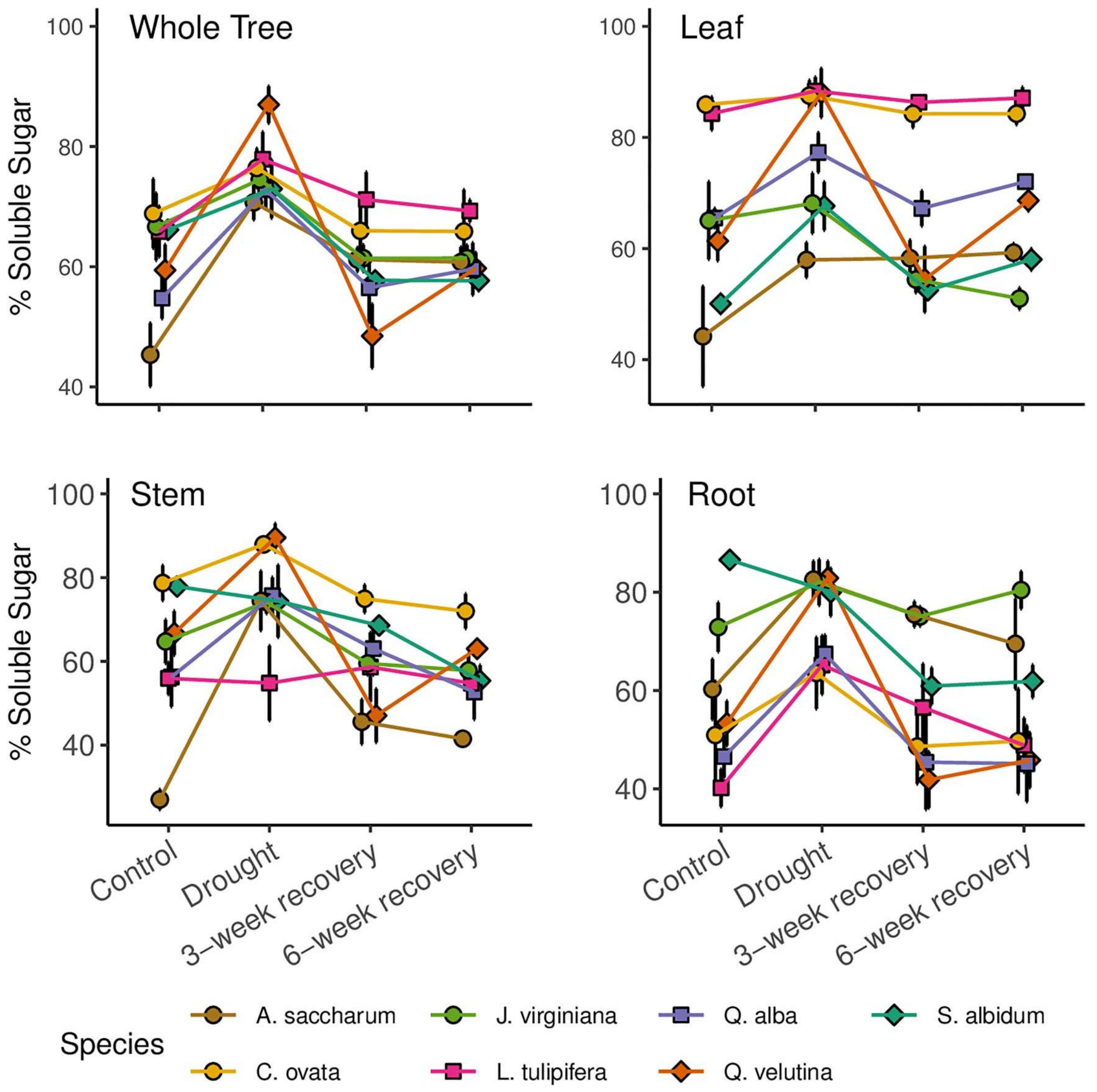

Figure 5. Percentage of the total NSC pool that were soluble sugars at each sampling point for each species. Error bars represent ± standard error.

quantifying each species' degree of anisohydry and investigating the consequences of isohydric and anisohydric behavior post-drought.

Our observation that anisohydric saplings were not able to maintain C assimilation to a greater degree than isohydric saplings runs counter to the classic predictions of the isohydry-anisohydry spectrum. A possible explanation for this result is that photosynthetic responses to drought were decoupled from hydraulic behavior (Garcia-Forner et al. 2016, Martínez-Vilalta and Garcia-Forner 2016). This decoupling could occur due to environmental conditions (e.g., differences in drought severity) or contextual factors (e.g., differences in rooting depth or tree age) that differ between a greenhouse and mature forest. In support of this hypothesis, previous work has shown that the photosynthetic responses of our study species tend to be similar irrespective of their degree of anisohydry in greenhouse studies (Kannenberg and Phillips 2017, Kannenberg et al. 2018), while mature trees of these species showed markedly different C assimilation rates during a natural drought (Brzostek et al. 2014, Roman et al. 2015). Additional manipulative experiments and observational studies across ecological gradients will be needed to uncover the factors that can cause these stark deviations between tree hydraulics and gas exchange. It is also important to note that our experiment was conducted on young trees in a manner that would constitute a fairly rapid and severe drought in a mature forest. For example, if our drought experiment was longer and less severe (potentially inducing less sink limitation), we may have observed that isohydric behavior caused C depletion. While static NSC pools despite 6 weeks of drastic C limitation is still a surprising result, the extent to which these results are generalizable to mature forests is unknown.

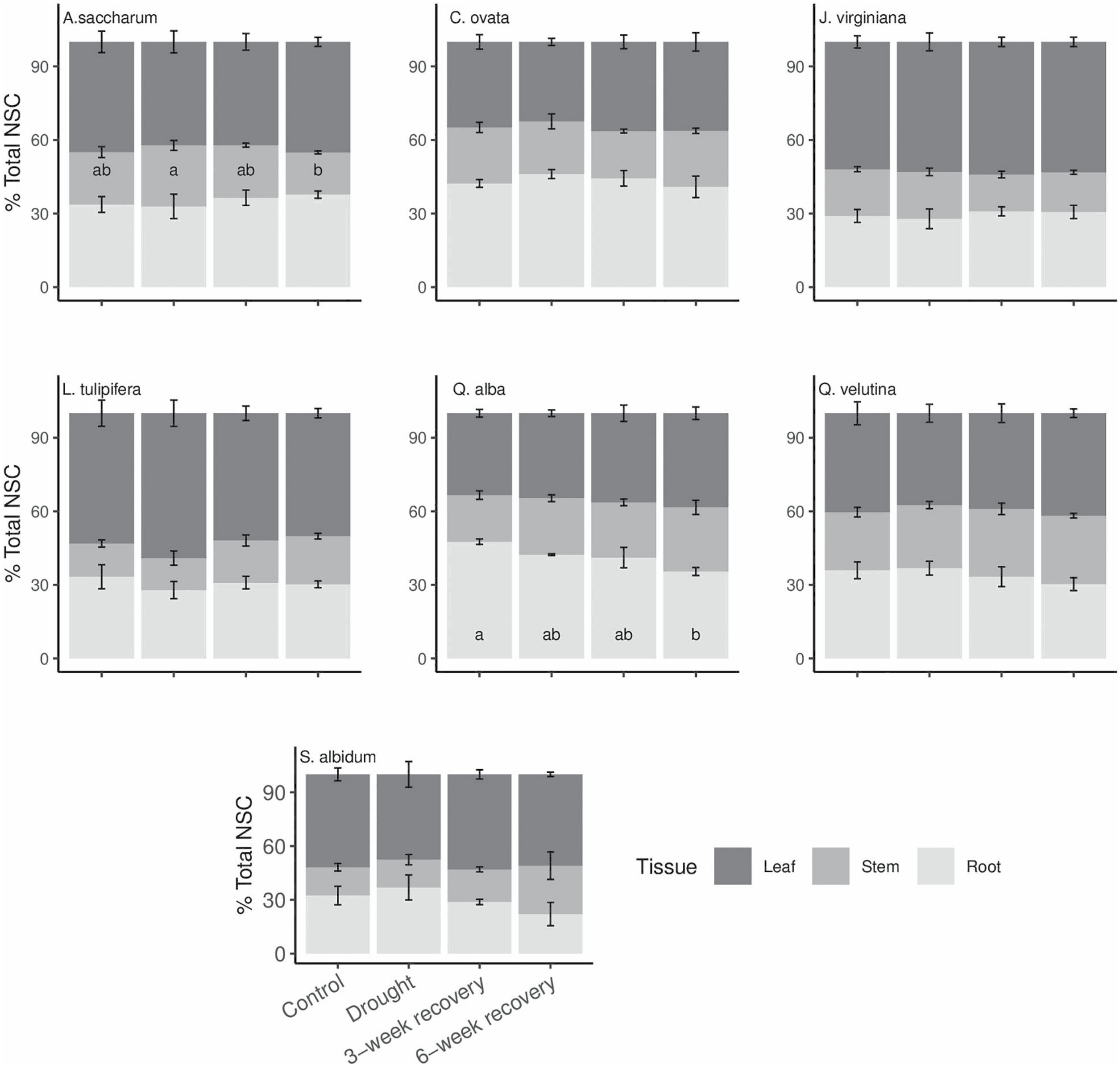

Figure 6. Partitioning of total NSC pool (soluble sugars + starch) across various tissues. Error bars represent ± standard error. Letter notation indicates statistical differences across time points via pairwise comparison within a species and tissue and is only present when significant differences occur.

There are a number of reasons why NSC pools may become decoupled from photosynthetic declines. First, our results could be the result of phloem transport limitation (McDowell 2011, Savage et al. 2016, Salmon et al. 2019), which would impair NSC mobilization and lead to the observed static NSC pools. While the highly negative water potentials and large amounts of hydraulic damage observed in our experiment indicate this could be a possibility, a recent modeling study claims that phloem transport limitation should be highly species specific and occur largely in trees with photosynthetic rates that are insensitive to water stress (Mencuccini et al. 2015), which we did not observe in our experiment. Further, if phloem loading was impaired, we would most likely observe a positive relationship between C assimilation, hydraulic damage and NSC pools in leaves (Salmon et al. 2019), since translocation of newly fixed C out of the leaf would be limited. However, we did not observe (i) any increases in the proportion of NSC stored in leaves during drought, (ii) a relationship between integrated C assimilation and leaf NSC during drought or (iii) hydraulic damage to be linked to the size of the NSC pool—in leaves or any other tissue.

Second, reductions in the strength of other C sinks such as growth or respiration could result in static NSC pools, especially during severe drought when growth may become limited by turgor (Körner 2003, 2015, Muller et al. 2011, Hoch 2015). While our drought phase was designed to avoid loss of turgor, a discrepancy between C sinks and sources could still occur

(Mitchell et al. 2014, O'Brien et al. 2015, Galiano et al. 2017, Huang et al. 2019). We lack the necessary data on C sink strength to test this mechanism, but a similar experiment on a subset of our species found that drought lessened respiratory C costs and decreased growth rates in one species (Kannenberg et al. 2018), proving a potential explanation for unchanging NSC pools. However, declines in C assimilation in our experiment were rapid and severe in all species. Therefore, decreases in growth or respiration not accompanied by reduced C supply were likely transient.

The maintenance of NSC pools may also be a consequence of our drought duration and intensity. Non-structural carbohydrate depletion has been hypothesized to occur more frequently in response to long and less severe droughts, where hydraulic damage is predicted to predominate during short and severe periods of water stress (McDowell et al. 2008, McDowell 2011). While our results (in conjunction with those of Kannenberg et al. 2019) do support this idea, NSC pools were maintained (i) despite weeks of near-zero C supply and (ii) even in species experiencing little hydraulic damage, indicating the interactions between photosynthesis, hydraulic damage and C storage are complex and perhaps not amenable to a simple C supply-based framework.

Finally, our saplings may have used their limited stored C for osmoregulatory purposes (Sala et al. 2012, Dietze et al. 2014). Water stress is known to increase the importance of soluble sugars to maintain turgor and the conversion of starch to soluble sugars is a fairly common response to drought (Klein et al. 2014, Mitchell et al. 2014, Woodruff 2014). These accumulated solutes could be used to lower turgor loss point (Bartlett et al. 2012) and thus better maintain physiological function at low water potentials. Our results support this hypothesis, as we found evidence that soluble sugars pools (especially in leaves) were prioritized during drought, indicating that interconversion between starches and sugars is an important process for maintaining physiological function in water-stressed trees. However, increases in sugars were not linked to a species' degree of anisohydry during—or after—drought, refuting our hypothesis that anisohydric tree saplings would have greater need of soluble sugars for embolism repair.

Ongoing debates in plant C relations center around the degree to which stored C fluctuates due to source or sink limitation (Körner 2003, 2015, Hartmann and Trumbore 2016), whether allocation to NSC represents an active versus passive process (Sala et al. 2012, Wiley and Hellier 2012, Dietze et al. 2014) and the degree to which NSC pool fluctuations are coupled to changes in plant hydraulic status (O'Brien et al. 2014, Sevanto et al. 2014, Adams et al. 2017). We failed to observe any linkages between NSC pools, time-integrated C assimilation and hydraulic damage in drought-stressed trees. While we do not have the detailed C allocation data necessary to infer active versus passive allocation or source versus sink limitations, our results indicate that some physiological process occurred that allowed for maintenance of NSC pools in the face of severe C limitation and that NSC pools remained static over a wide range of hydraulic strategies. By theorizing that NSC depletion occurs primarily in species that close stomata in response to water stress, the isohydry–anisohydry spectrum framework assumes C storage is a mostly passive process that reflects recent photosynthetic rates. However, if NSC pools also fluctuate based on sink limitation or active allocation to storage, C depletion is not necessarily a consequence of isohydric behavior. An improved understanding of the physiological mechanisms that can serve to maintain NSC during drought should enhance our ability to predict when, where and why trees may reduce stored C.

Conclusions

Many challenges currently exist in understanding the dynamics of NSC in water-stressed trees. Active versus passive allocation processes, the role that hydraulic status and phloem function play in C allocation dynamics, and differences in NSC use that are contingent on tissue and chemical composition are all factors that hinder our capacity to accurately predict C stores in water-stressed trees. We sought to leverage the isohydry–anisohydry spectrum framework to understand the interactions between tree hydraulics and NSC dynamics during drought. In accordance with theory, we found that anisohydric tree saplings drastically lowered water potentials during drought and consequently experienced large amounts of hydraulic damage that persisted for weeks and hindered recovery (Kannenberg et al. 2019). Thus, we confirmed that hydraulic damage is a core consequence of anisohydric behavior. However, we show here that the hypothesized consequence of isohydric behavior (C depletion) is rare, even in the face of near zero C assimilation. Our results, in combination with the numerous studies that have failed to observe decreases in NSC in both experimental and observational contexts, call for a conceptual rethinking regarding the consequences of isohydric behavior and of drought-induced NSC depletion in general. Non-structural carbohydrate depletion thus may be highly context-specific, only occurring in response to mild, extended droughts where water stress would reduce photosynthesis but not be so severe as to impose limits on phloem transport or competing C sinks such as growth. Experimental manipulations of drought severity and length should provide a fruitful avenue for understanding the context-specificity and prevalence of NSC depletion.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

Acknowledgments

We would like to extend a special thanks to Andrew Reese for extensive help with laboratory analysis. We also thank Lynda Delph for lending us equipment, Charlotte Bradley for laboratory and greenhouse assistance and the Indiana University greenhouse staff (especially Mike O'Connor).

Conflict of interest

None declared.

Authors' contributions

S.A.K. designed and implemented the experiment, with input from R.P.P. S.A.K. conducted data analysis and drafted the manuscript, with R.P.P. providing input on subsequent drafts.

Funding

Funding was provided by the Department of Energy, through the Ameriflux Management Project administered by Lawrence Berkeley National Laboratory, and the United States Department of Agriculture, Agriculture and Food Research Initiative, Physiology of Agricultural Plants Program (project no. INDW-2016-10549).

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