



Interannual dynamics of stemwood nonstructural carbohydrates in temperate forest trees surrounding drought

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Received: 23 June 2022 / Accepted: 14 October 2022 / Published online: 16 November 2022
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Abstract Interactions between water and carbon dynamics underlie drought-related tree mortality. While whole-tree water relations have been shown to play a key role in the response to and recovery from drought, the role of nonstructural carbohydrates (NSC) and how their storage and allocation changes surrounding drought events deserves further attention and is critical for understanding tree survival. Here, we quantified in situ NSC responses of temperate forest trees to the 2016 drought in the northeastern United States. Sugar and starch concentrations were measured in the stemwood of five tree species from 2014 to 2019, which allowed us to monitor NSCs in relation to climatic conditions before,

during, and after the natural drought. We found that immediately following the drought, measured stemwood NSC concentrations decreased. However, NSC concentrations rebounded quickly within three years. Notably, trees allocated proportionally more to starch than to sugars following the 2016 drought. In winter 2017, starch comprised 45% of total stemwood stores, whereas starch made up only 1–2% in other years. Further, we modeled and assessed the climatic drivers of total NSC concentrations in the stem. Variation in total NSC concentrations was significantly predicted by the previous year's temperature, precipitation, and standardized precipitation-evapotranspiration index (SPEI), with stemwood concentrations decreasing following hotter, drier periods and increasing following cooler, wetter periods. Overall, our work provides insight into the climatic drivers of NSC storage and highlights the important role that a tree's carbon economy may play in its response and recovery to environmental stress.

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Project funding: This work was supported by the National Science Foundation's Graduate Research Fellowship (Grant Nos. DGE-1144152 and DGE-1745303) and the Garden Club of New Jersey.

The online version is available at <http://www.springerlink.com>.

Guest editor: Yanbo Hu.

Corresponding editor: Yanbo Hu

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11676-022-01566-2>.

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Keywords Drought · Nonstructural carbohydrates · Starch metabolism · Forest trees

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Introduction

Widespread and large-scale tree mortality events have occurred across forested biomes over the past several decades (Breshears et al. 2005; Allen et al. 2010). In temperate forests of North America, these mortality events have often been associated with drought and resulted in rapid shifts in ecosystem structure and function (Shaw et al. 2005; Williams et al. 2010; van Mantgem et al. 2016). Drought poses a complex, multi-dimensional challenge for trees because it impacts the tightly interrelated systems of water and carbon (C). Hydraulic failure, the limitation of water transport due to xylem embolism, and C starvation, the impairment of C-dependent metabolic and hydraulic processes due to a limited nonstructural carbohydrate (NSC) supply, have been implicated as mechanisms of drought-related mortality (Sala et al. 2010; McDowell 2011). While hydraulic failure following drought is well studied, the role that NSCs serve during drought is still poorly understood.

NSCs, consisting mainly of soluble sugars and insoluble starch, are used to support C-demanding processes and can be allocated to storage in parenchyma cells for later use (Hartmann and Trumbore 2016; Hartmann et al. 2020). They are known to be used as metabolites at night and during the dormant season, and more recent evidence suggests that they may play a role in surviving environmental extremes (O'Brien et al. 2014; Blumstein et al. 2020). In the case of drought, the production of new NSCs is limited through stomatal closure, thus previously stored NSCs are thought to serve as a buffer by fueling cellular maintenance and osmoregulation to support hydraulic integrity (Sala et al. 2012; Brien et al. 2014; Sevanto et al. 2014; Secchi et al. 2017). Given their potential role as osmoregulators, changes in NSCs can negatively feedback on hydraulic function. For example, low NSC concentrations in the stem have been linked to declines in xylem resistance to embolism (Tomasella et al. 2020).

The link between stored NSCs and hydraulic functioning has prompted research into how NSCs vary before, during, and after drought and is critical for predicting the physiological responses of trees. Drought can alter a plant's C balance by decreasing photosynthesis (Martínez-Vilalta et al. 2002), reducing xylogenesis (Bréda et al. 2006; McDowell et al. 2010; Cailleret et al. 2017), and altering NSC concentrations (Anderegg et al. 2013; Hartmann et al. 2013; Adams et al. 2017). How NSC concentrations change in response to drought is still, however, unclear.

Some studies have recorded an increase in NSC stores following drought, others a decrease, while still others remained unchanged (reviewed in Adams et al. 2017). While informative, these prior studies have been limited in the length of time and frequency with which plants were measured prior to and following drought. Having a limited

window of observation may produce misleading results given that plant NSC stores are known to fluctuate seasonally (Martínez-Vilalta et al. 2016; Furze et al. 2018) and vary by plant life strategies (Furze et al. 2018). Furthermore, many previous studies have quantified NSC responses to experimental drought (Anderegg et al. 2012; Adams et al. 2013; Hartmann et al. 2013; Quirk et al. 2013; Sevanto et al. 2014; Dickman et al. 2015). The monitoring of mature trees under natural drought conditions in diverse ecosystems (Dietrich et al. 2018) is necessary to improve our understanding of basic tree physiology and predictions of forest ecosystem function. Moreover, resolving the degree of change and duration of recovery of NSCs in drought stressed trees will inform predictions of forest dieback.

To explore how NSCs respond to drought in eastern North American temperate forest trees, we took advantage of a natural drought that occurred in 2016 and measured sugar and starch concentrations in the stemwood of five tree species between 2014 and 2019. The interannual nature of our sampling and its concurrence with the 2016 drought provided a unique opportunity to examine NSCs in relation to climatic conditions before, during, and after natural drought conditions. In addition, using available data, we were able to parse seasonal cycling from climate response across the five species to understand how NSC concentrations co-vary with drought, time of year, and species.

1. We predict that NSC concentrations will be depleted following the 2016 drought, then recover slowly over the course of several growing seasons, as predicted by drought-legacy effects (Anderegg et al. 2013, 2015).
2. We predict that the climatic conditions of the prior growing season will drive inter-annual variation in NSC concentrations.

Materials and methods

Study site and sample collection

Samples were collected from 2014 to 2019 at Harvard Forest, a mixed temperate forest located in Petersham, MA, USA (42.53°N, 72.17°W). We sampled 24 mature trees: red oak (*Quercus rubra* L., n = 6), white pine (*Pinus strobus* L., n = 6), red maple (*Acer rubrum*, n = 6), paper birch (*Betula papyrifera*, n = 3), and white ash (*Fraxinus americana* L., n = 3). These trees were sampled in 2014–2015 for a different study, but the onset of the 2016 drought initiated the extension of our sample collection. The northeastern United States experienced one of the warmest and driest summers on record in 2016, following historically low winter snowfalls, which, coupled with secondary stressors such as insects (Barker Plotkin et al. 2021), led to widespread

forest and crop mortality events in the northeast (Sweet et al. 2017). The drought lasted through the growing season and put 2016 in the top three driest years since 1970 (Fig. 1).

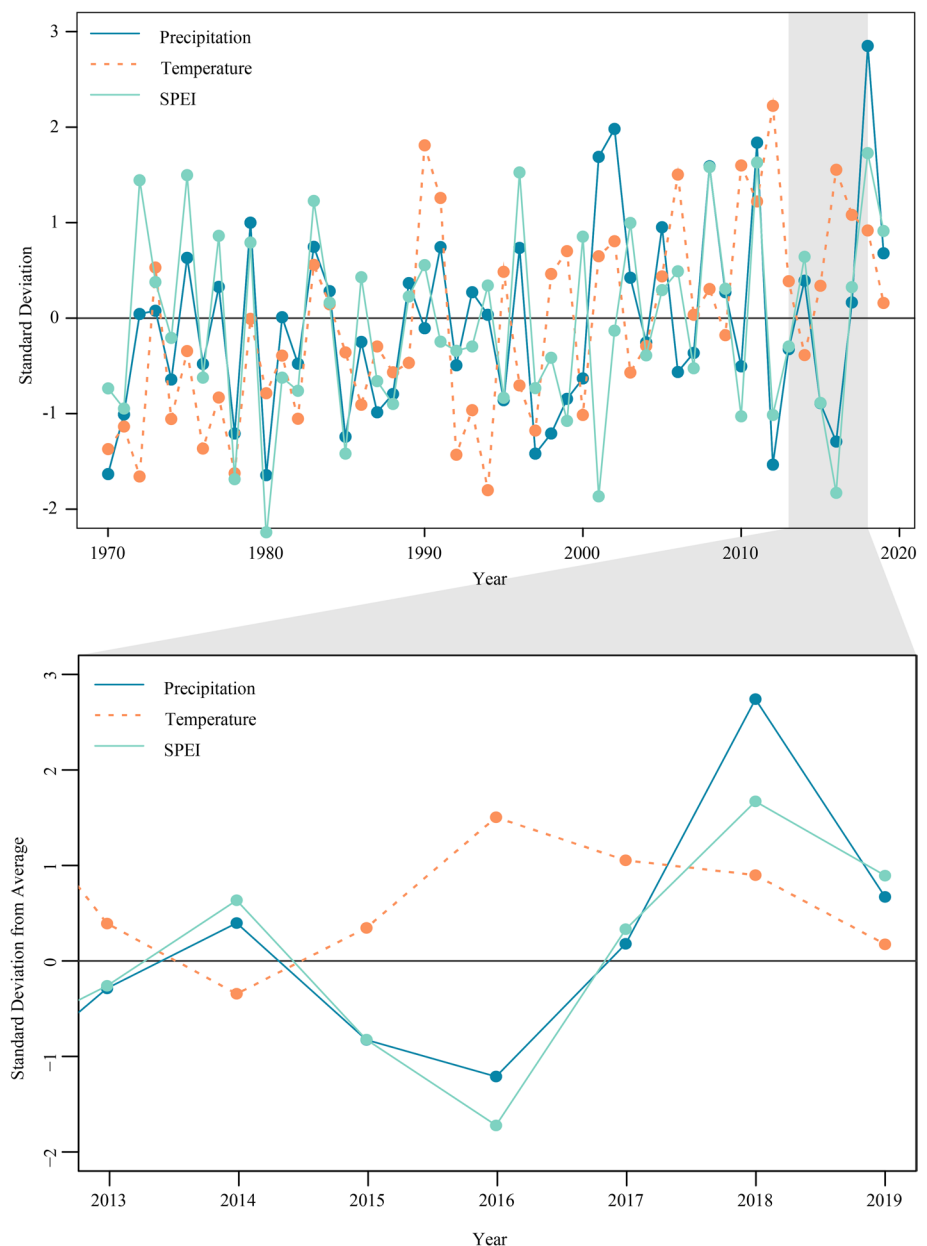
A stemwood core was collected using a standard 4.3-mm increment borer (Haglöf Company Group, Långsele, Sweden) in the winter of 2014–2019. The exact sampling times were February 2014, January 2015, February 2016, February 2017, and February 2019. The dormant season was chosen to minimize the effects of phloem activity and diurnal changes in NSC concentrations (Bowen and Hoad 1968; Aloni et al. 1991). Additionally, the stemwood was the largest biomass component of our study trees and, in general, has been previously shown to store about 40% of a tree's total NSCs (Richardson et al. 2015), making it an ideal organ

for monitoring NSC dynamics under stress since it captures the response of a large portion of a tree's reserves. Individual tree characteristics including diameter at breast height, height, age, approximate number of annual rings in the outer 3 cm of xylem of stemwood cores, and estimated sapwood-heartwood transition zones are provided in Table S1.

NSC concentrations

For NSC analysis (Chow and Landhäusser 2004; Landhäusser et al. 2018) of each stem core, the bark was removed and the outer 3 cm of the xylem was used. To determine sugar concentrations (mg g^{-1} dry wood), dried and ground tissue was extracted with hot ethanol followed by colorimetric

Fig. 1 Weather data from the Shaler Meteorological Station at the Harvard Forest (Peter-sham, MA, USA) from 1970 to 2019 (Boose and Gould 2019) and monthly SPEI data from the same period (Global SPEI Database). Annual precipitation, temperature, and SPEI data shown as the standard deviation from the average over this ~50-year period. The study period 2014–2019 is enlarged and indicated by the shaded gray area



analysis with phenol and sulfuric acid. The remaining tissue was solubilized and digested with α -amylase and amyloglucosidase, and subjected to a PGO-color reagent solution to obtain starch concentrations (mg g^{-1} dry wood). Extracts were read on a microplate reader (Epoch Microplate Spectrophotometer; Bio-Tek Instruments, Winooski, VT, USA) or a spectrophotometer (Thermo Fisher Scientific GENESYS 10S or 180 UV-Vis, Waltham, MA, USA). Total NSC concentrations were determined by summing sugar and starch concentrations for each sample. Additional methodological details are provided in Methods S1.

Meteorological data

To examine NSC dynamics from our study years 2014–2019 in the context of the 2016 drought and long-term climatic conditions, we obtained precipitation and temperature data from the Shaler Meteorological Station at Harvard Forest for the period 1970–2019 (Fig. 1; Boose and Gould 2019). In addition, we utilized the standardized precipitation-evapotranspiration index (SPEI; Global SPEI Database) from a 1° -gridded global map created using the Climatic Research Unit (CRU) data (Climatic Research Unit Database). We chose to include SPEI as it reflects the evaporative demand on plants and is thus a better measure of plant water stress than temperature or precipitation alone.

Given that NSC concentrations measured in January/February are in part the product of photosynthesis from the previous growing season, we took moving averages of the previous year's temperature, precipitation, and SPEI up to the month of sampling:

$$T_t = \sum (T_t : T_{(t-12)})/12, \quad (1)$$

where T represents the monthly average temperature and t is the time in months. These data were then used in model construction; see Statistical analyses for details.

Statistical analyses

All statistical analyses were performed in the programming language R v.4.0.0. To compare total NSC concentrations before, during, and after the 2016 drought, we first performed a one-way ANOVA using the base function “aov” to test if total NSC concentrations differed between years (Fig. 2). We then performed Tukey's HSD post-hoc testing to make pairwise comparisons between years (Table S2).

Next, we examined if and how the previous year's temperature, precipitation, and SPEI patterns drove interannual variation in total NSC concentrations across species. We first used a linear model to test how total NSC concentrations varied by these inputs using the base “lm” function in R (Fig. S1). To capture potential non-linearities in our

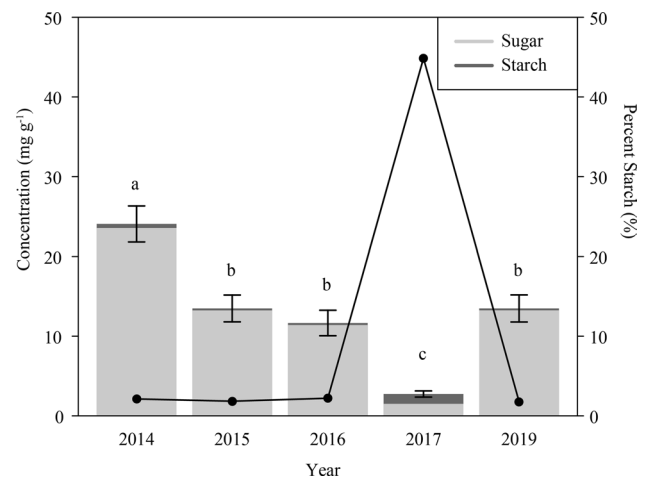


Fig. 2 NSC concentration (left axis) measured in the outer 3 cm of stemwood in early winter (January/February) between 2014 and 2019. Bars represent total NSC concentrations averaged across temperate tree species ($n=5$ species) in the study, with error bars indicating the standard error across trees ($n=24$ trees). Bars are further divided into sugar (light gray) and starch (dark gray). Letters represent significant differences between years ($\alpha=0.05$) based on Tukey's HSD post-hoc testing. Percent starch (right axis) from 2014 to 2019 is represented by the solid black points and line. The exact sampling times were February 2014, January 2015, February 2016, February 2017, and February 2019

relationship variables to NSC storage, we also used a random forest to fit the same model (total NSC versus weather variables) (Fig. S2). To do so, we used the randomForest package in Liaw and Wiener (2002).

However, NSCs naturally follow seasonal cycles in temperate deciduous forests, with NSCs generally building up over the growing season and declining over the dormant season (Richardson et al. 2013; Furze et al. 2018). Thus, to account for the cyclical nature of total NSC concentrations and to better visualize what may be happening over the time-series of our data, we also included data previously collected on the same individuals for each month of the year in 2014 (Furze et al. 2018; Furze 2020). This additional data allowed us to uniquely examine how climate drives NSC variation beyond seasonal cycles.

We constructed a linear model using the base “lm” function in R. Specifically, to account for the seasonal cycle of NSCs, we used a cosine function:

$$\cos(2\pi/Tx) \quad (2)$$

where T is the period of recurrence (every 12 months) and x is the month of analysis (month 1 through month 62).

We then successively compared how the cosine seasonal cycle function and (1) previous year's precipitation, (2) previous year's temperature, (3) previous year's SPEI and (4) all three together predicted total NSC concentrations across all

species. The best fit model was determined to be the cosine seasonal cycle function and all three variables combined (Table S3). We then added species as a factor to the model to explain interannual variation.

Finally, to better understand how well the cosine model performs when making predictions in-between our measured data, we used a jack-knifing approach. This analysis allows us to investigate the sensitivity of our model predictions to each date of data input and test how well the model can make out-of-bag estimates. To do so, we iteratively dropped each month of data from the training dataset and re-fit the model. We then predicted the missing month of data and compared these estimates to those using the full model (Fig. S3). Unsurprisingly, the more extreme NSC storage months have a higher leverage on model predictions (Fig. S3 A). Divergences in these months are disproportionately driven by the species with lower NSC levels on average, particularly white pine and paper birch (Fig. S3 B). However, divergences never exceeded 5 mg g^{-1} in NSC, which falls within the range of our measurement error from our assays (Methods S1).

Results

Total NSC concentrations and percent starch differ from year-to-year

Throughout the study period 2014–2019, total NSC concentrations significantly differed between years ($F_{(4,115)} = 21.18$, $P < 0.001$); Fig. 2; Table S2). Post-hoc testing demonstrated that total NSC concentrations in the winter of 2017 following the 2016 drought were significantly lower than concentrations in all other years (Fig. 2; Table S2).

Notably, the proportion of total NSC stores as starch in early winter remained relatively stable from year-to-year except in the winter following the 2016 drought. In the winter of 2017, 45% of all stores were found to be in starch, whereas starch comprised only 1%–2% of total stores in the other years (Fig. 2).

Total NSC concentrations are predicted by previous year's temperature, precipitation, and SPEI

NSC concentrations measured in January/February varied significantly with the previous year's average SPEI ($m = 65.0$, $P < 0.01$) and monthly precipitation ($m = -0.9$, $P < 0.001$), but not previous year's average temperature ($m = 4.5$, $P = 0.33$; Fig. S1). These variables were also found to be important in a random forest analysis (Fig. S2A), with this machine-learning method suggesting that relationships between variables may not be perfectly linear (Fig. S2 B–D). In particular, there may be a threshold response of

total NSC concentrations to the amount of precipitation in the previous year when average monthly precipitation falls below 90 mm (Fig. S2 D).

The base model we used to predict how monthly total NSC concentrations varied with a seasonal cycle was significant, but poorly captured interannual and interspecific variation ($P_{\text{model}} = 0.001$, $R^2 = 0.02$; Fig. 3; Table S3). Adding in the previous year's precipitation to the model also resulted in significant predictions, but poorly explained variation ($P_{\text{ppt}} = 0.001$, $R^2 = 0.03$; Fig. 3). While precipitation itself was a significant predictor in the model, it did not robustly predict interannual variation in total NSC concentrations ($m = 0.01$, $P = 0.04$). Our model formulation with seasonal cycle and SPEI performed better than the precipitation model ($P_{\text{model}} < 0.001$, $R^2 = 0.11$; Fig. 3; Table S3) and our model with seasonal cycle and prior temperature performed best of the three models with one climate variable included ($P_{\text{model}} < 0.001$, $R^2 = 0.22$; Table S3; Fig. 3).

When all climate parameters were included in the model with seasonal cycle, all parameters were found to be highly significant and the model explained the highest amount of variation of our tested set ($P_{\text{model}} < 0.001$, $R^2 = 0.25$; Table S3; Fig. 3). Finally, adding species as a factor to our model of seasonal cycle and all three climate parameters vastly improved the variance explained by the model ($P_{\text{model}} < 0.001$, $R^2 = 0.62$; Table S4; Fig. 4).

Discussion

NSC depletion and ultimately C starvation as a contributor to drought-related tree mortality is not universal (Adams et al. 2017). In this study, we found that total NSC and sugar concentrations decreased following the 2016 drought, but rebounded quickly to pre-drought levels in subsequent years (Fig. 2). Our long-term study offers insight into how NSCs are impacted by environmental variation and how climatic factors can drive their depletion and recovery.

The proportion of total NSC in starch increases following drought

While total NSC and sugar concentrations decreased following the 2016 drought, starch concentrations increased. Starch comprised 45% of total stores following the 2016 drought, a nearly 23-fold increase compared to other years. During drought stress, plants close their stomata to avoid xylem hydraulic failure, but this comes at the cost of reduced C gain and initiates the use of previously stored reserves to survive through the period of stress (McDowell et al. 2008; McDowell 2011). Thus, accumulating starch under drought stress might seem counterintuitive. However, starch is an important regulator of source-sink relationships and starch

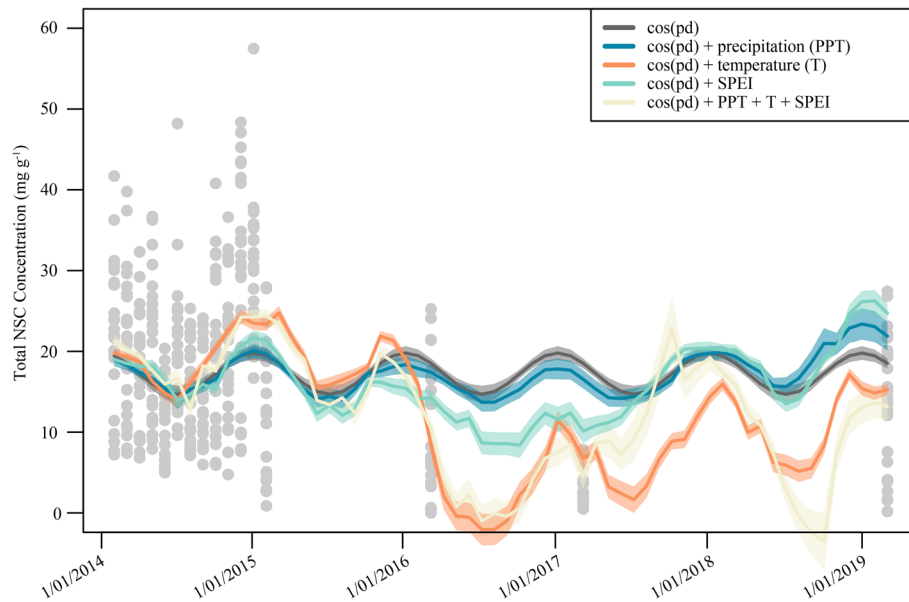
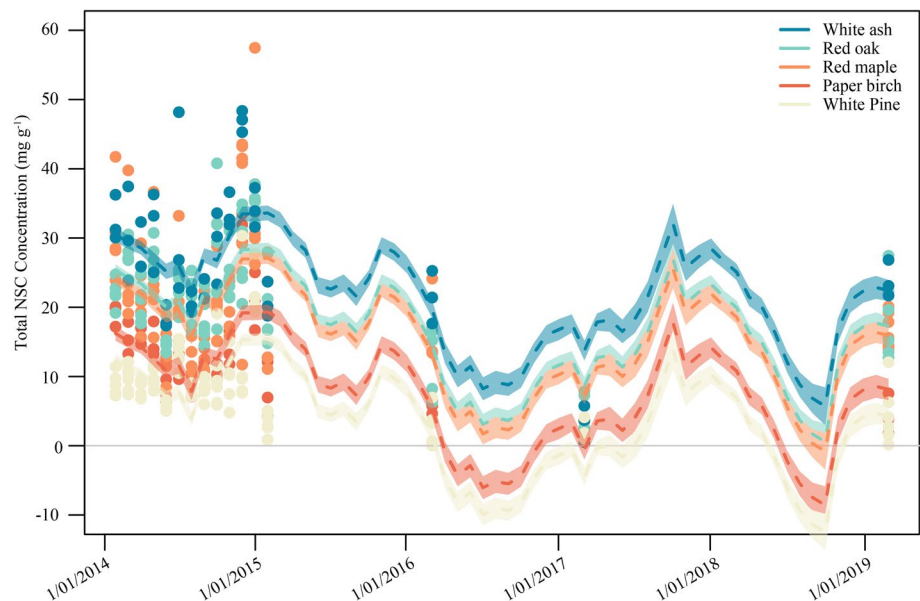


Fig. 3 A cosine model of total NSC concentration patterns in the stemwood over the 62 months of this study in relation to climate. Our annual measurements of variation from 2014 to 2019 are combined with seasonal variation measurements taken on the same individuals in 2014 (Furze et al. 2018) to demonstrate how the amplitude of annual NSC variation is predicted by previous year's precipitation

(blue line), previous year's temperature (orange line), previous year's SPEI (green line), and previous year's precipitation, temperature, and SPEI (yellow line). Field-based measurements are indicated by gray dots, while the model predictions are represented by solid lines and the standard deviation of model predictions are shaded around each line

Fig. 4 The seasonal model, with previous year's temperature, precipitation, and SPEI as predictors, broken down by species



accumulation has been demonstrated in plants under various environmental stressors (Dong and Beckles 2019).

For example, studies on wheat, rice, and *Arabidopsis* have shown that enzymes in the sucrose-to-starch pathway can be activated or deactivated under water deficit/drought, leading to increased starch accumulation (Yang et al. 2004; Valerio et al. 2011; Thitisaksakul et al. 2012; Zhang et al. 2012;

Prasch et al. 2015). One way in which the maintenance or biosynthesis of starch may lead to better stress outcomes, at least for plants experiencing salt stress, is by increasing sink strength which promotes sugar unloading and continued photosynthesis (Gao et al. 1998; Yin et al. 2010; Thitisaksakul et al. 2017a, b; Thitisaksakul et al. 2017a, b). These studies suggest that the accumulation of insoluble starch in

the stem may be an attempt to drive sugars toward that large, empty sink. However, although we quantified NSC responses to drought in the stem, a large C sink, we acknowledge that measuring only one organ may limit the extension of our findings since NSC dynamics may differ between organs. For example, drought led to a decline in NSCs in the roots, but not the canopy of Norway spruce trees (Hartmann et al. 2013).

NSCs were depleted following drought, but rebounded quickly

As predicted, total NSC concentrations dropped steeply following the 2016 drought. However, total NSC concentrations rebounded quickly following the drought year to the same level they were at the two years leading up to the drought, although they never fully reached 2014 levels. It is possible that concentrations began to rebound even sooner than this, but we were unable to collect samples in winter 2018. Regardless, we expected to see drought-legacy effects in our results. Since water uptake is tightly tied to photosynthesis and C acquisition, we predicted a slow rebound of NSC stores following drought, which would also presumably be accompanied by a slow rebound of growth (Vanderklein and Reich 1999; Deslauriers 2014). Growth is highly sensitive to decreased cell turgor and is inhibited by drought before C uptake even becomes limiting (Körner 2015).

Drought legacy effects can take many different forms, but are most often cited as multi-year growth reductions following drought (Anderegg et al. 2015) or increased vulnerability to cavitation (Hacke et al. 2001; Anderegg et al. 2012). For example, in the southwestern United States, previous work in trembling aspen showed that hydraulic damage persisted, and often accumulated, for multiple years following drought without any indication of repair and recovery (Anderegg et al. 2013). However, other studies have reported a lack of xylem embolism formation in temperate trees in response to drought (Dietrich et al. 2018). Given the opportunistic nature of our study, we were unable to measure the integrity of the hydraulic system in our study trees, thus it is possible that even though NSC concentrations began to replenish and the trees seemed to survive the drought, there may be lasting hydraulic dysfunction and a lag in mortality (Anderegg et al. 2013). However, this seems unlikely given the rapid nature of recovery in our species. An additional limitation of our study is that we did not measure associated changes in tree growth rates which would have improved our understanding of tradeoffs between growth and NSC storage as well as the physiological mechanisms underlying drought-related tree decline and recovery. It is possible that if growth was hampered following drought, that NSC stores would subsequently build up rapidly as other sinks were temporarily unavailable. Future field studies should consider NSC dynamics,

growth rates, and tree hydraulics in parallel during both drought and drought recovery. Specifically, monitoring NSC dynamics in multiple organs and on finer timescales (i.e. seasonally) surrounding drought will continue to improve our understanding of tree responses.

Interannual variation in total NSC concentrations is predicted by the prior year's temperature, precipitation, and SPEI

We found substantial year-to-year variation in total NSC concentrations measured in the early winter, ranging from an average of 5 to 25 mg g⁻¹ across study years and species. The low-point of 5 mg g⁻¹ clearly followed the 2016 drought and was significantly predicted by the prior year's temperature, precipitation, and SPEI (Figs. 2 & 3, Table S2). However, of the models that included each climate variable independently, prior year's temperature had the highest explanatory power, indicating it has an important role in driving variation. While temperature influences several physiological processes, its role as a key driver of both NSC variation and drought stress is likely due to its link to vapor pressure deficit (VPD). Increased VPD coupled with limited soil moisture increases the potential for hydraulic failure and leads to declines in belowground and stomatal conductance, decreased photosynthesis, and depletion of NSC stores as demands for allocation of NSCs to cellular maintenance and osmoregulation increase (Williams et al. 2013; McDowell et al. 2022). Higher temperatures in the summer may limit photosynthesis, leading to a decrease in C supply and thus a lower amount of NSC stores the following winter due to a lack of replenishing during the growing season.

The tight coupling of temperature and NSC stores also likely reflects the fact that fluctuations in temperature, both high and low, may impact NSC concentrations, while precipitation or SPEI may only impact storage in extreme situations. For example, a warmer than usual year may directly affect photosynthetic productivity, but a slightly wetter year likely doesn't boost productivity and ultimately affect NSC storage values. Thus, SPEI and precipitation may be less effective, particularly on shorter time scales. To improve the predictive power of prior precipitation and SPEI in the model, we would likely need a much longer time series of measures with multiple droughts in order to define the threshold at which water limitation impacts storage.

For every degree of additional warming in a year, our model including all species and climate parameters predicts that NSC stores will decrease by 3 mg g⁻¹ the following winter. If trends remain linear and average annual temperatures increase anywhere between the predicted 2–6 °C over the next 65 years, we would expect trees to be pushed toward their physiological limits in the near future (ClimateNorthAmerica; Wang et al. 2016). While further attention is

warranted to examine the mechanism behind these fluctuations and to get a better sense for how trees might respond to predicted change, our results do suggest that trees may become increasingly stressed in eastern temperate forests as temperatures rise.

There is some evidence that this may have already begun to happen at this study site Harvard Forest. Between 2007 and 2010, stemwood NSC concentrations were measured seasonally on mature red maple and red oak trees (Richardson et al. 2013). Dormant season measurements of total NSC concentrations (starch, sucrose, fructose, glucose) from this study ranged between 22 and 64 mg g⁻¹ across red oak and red maple as compared to our measurements from 2014 to 2019, which ranged between 2 and 30 mg g⁻¹ for the same species. While slightly different NSC quantification methods were used, making direct comparison of these data challenging (Quentin et al. 2015; Landhäusser et al. 2018), this decline suggests that tree stores may already be in decline.

Conclusion

By measuring in situ stemwood NSC concentrations in temperate tree species across multiple years, our work provides unique insight into the influence of interannual climatic variation and associated stressors like drought on NSC storage dynamics. Notably, we demonstrated that total NSC concentrations significantly decreased, while starch accumulated in response to drought. However, total NSC concentrations rebounded quickly in subsequent years, suggesting limited legacy effects to storage in eastern temperate forests. The decline and rebound of total NSC concentrations closely followed variation in previous year's temperature, precipitation, and SPEI, allowing us to build a predictive framework for NSC storage in response to climate. Our model suggests that NSC stores on average may be on the decline as temperatures continue to rise with climate change, calling into question how much longer NSCs may be able to serve as a buffer to change for temperate trees.

Acknowledgements We thank Harvard University and Harvard Forest for use of their facilities.

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