

# Adaptive variation and plasticity in nonstructural carbohydrate storage in a temperate tree species

Meghan Blumstein<sup>1</sup> and Robin Hopkins<sup>2</sup>

<sup>1</sup>Massachusetts Institute of Technology

<sup>2</sup>Harvard University Faculty of Arts and Sciences

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## Abstract

Trees' total amount of nonstructural carbohydrate (NSC) stores and the proportion of these stores residing as insoluble starch are vital traits for individuals living in variable environments. However, our understanding of how stores vary in response to environmental stress is poorly understood as the genetic component of storage is rarely accounted for in studies. Here, we quantified variation in NSC traits in branch samples taken from over 600 clonally transplanted black cottonwood (*Populus trichocarpa*) trees grown in two common gardens. We found heritable variation in both total NSC stores and the proportion of stores in starch ( $H^2_{TNC} = 0.19$ ,  $H^2_{PropStarch} = 0.31$ ), indicating a substantial genetic component of variation. In addition, we found high amounts of plasticity in both traits in response to cold temperatures and significant genotype-by-environment (GxE) interactions in the total amount of NSC stored (54% of P is GxE). This finding of high GxE indicates extensive variation across trees in their response to environment, which may explain why previous studies of carbohydrate stores' responses to stress have failed to converge on a consistent pattern. Overall, we found high amounts of environmental and genetic variation in NSC storage concentrations, which may bolster species against future climate change.

## Keywords:

Nonstructural carbohydrates, climate change, plasticity, adaptation, temperate forests, ecophysiology, *Populus trichocarpa* (black cottonwood)

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## Introduction

Climate is changing and extreme temperature and precipitation events are expected to intensify over the coming century (IPCC, 2013a), presenting immense challenges for immobile, long-lived organisms such as trees. Rates of forest tree mortality are already increasing worldwide as a result of shifting drought regimes, extreme temperatures, and pest outbreaks associated with global change (Allen et al., 2010; Anderegg et al., 2015; Anderegg et al., 2016; McDowell et al., 2018). In response to change, individual plants may physiologically adjust by plastically altering traits in the short term (Anderson, Inouye, McKinney, Colautti, & Mitchell-Olds, 2012; Des Marais, Hernandez, & Juenger, 2013; Pritzkow, Williamson, Szota, Trouve, & Arndt, 2020; Valladares et al., 2014; Valladares, Sanchez-Gomez, & Zavala, 2006; Wortemann et al., 2011), while populations may undergo local adaptive evolution through shifts in their genetic composition over longer time scales (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008; Davis, Shaw, & Etterson, 2005). Thus, understanding the extent to which trees are able to alter their traits in response to climatic change over short (plastic) and long (evolutionary) timescales is critical to predicting species' survival.

One key trait that enables plants to tolerate stochastic environments is the storage of nonstructural carbohydrates (NSCs). NSCs are the sugars and starches produced via photosynthesis and stored in the parenchyma cells of plants' woody tissues for later use (Chapin III, Schulze, & Mooney, 1990; Plavcová & Jansen, 2015). NSCs fuel photosynthesis at night, leaf-out in spring, and are also thought to serve as a back-up osmolytic or energetic reserve during periods of environmental extremes, like drought or freezing temperatures (Adams et al., 2017; Chapin III et al., 1990; H. Hartmann & Trumbore, 2016; McDowell et al., 2008; Sevanto, McDowell, Dickman, Pangle, & Pockman, 2014). Total NSC storage concentrations are known to vary seasonally (eg. Furze et al., 2019), across species (eg. Martínez-Vilalta et al., 2016), and within species (eg. Blumstein et al., 2020) and this variation in NSC storage concentrations has been linked to prolong survival under drought (O'Brien, Reynolds, Ong, & Hector, 2017). However, what controls variation in total NSC stores and how sensitive it is to environment is still poorly understood. Thus, we are limited in our ability to predict whether forest trees will be able to vary NSC stores in order to tolerate or adaptively evolve in response to climate change.

Total NSC stores can be broken down into two categories: soluble sugars and insoluble starches (Kaplan & Guy, 2004; Krasensky & Jonak, 2012; Thalmann & Santelia, 2017). Sugars affect cells' osmotic balances and are readily accessible for metabolism as they are dissolved in solution, while starches are considered the longer-term storage molecule as they are insoluble and thus must be broken back down into sugar to be used by the cell as an osmolyte or metabolite. Sugars can hydrolyze into and out of starch form via a number of enzymatic pathways (Thalmann & Santelia, 2017). The rate of starch transition varies daily (A. Tixier, Orozco, Roxas, Earles, & Zwieniecki, 2018), seasonally (Furze et al., 2019; Martínez-Vilalta et al., 2016; Richardson et al., 2013; Wurth, Pelaez-Riedl, Wright, & Korner, 2005), by plant tissue (Furze et al., 2019; Martínez-Vilalta et al., 2016), and in response to stress, such as water deficit (Adams et al., 2017; Landhäusser & Lieffers, 2011; Mitchell et al., 2013; Sevanto et al., 2014), high salinity (Chen, Chen, & Wang, 2007; Goyal, 2007; Kanai et al., 2007; Kempa, Krasensky, Dal Santo, Kopka, & Jonak, 2008; Ma et al., 2013), or extreme temperatures (Hoermiller et al., 2017; Kaplan & Guy, 2005; Nagao, Minami, Arakawa, Fujikawa, & Takezawa, 2005; Vasseur, Pantin, & Vile, 2011). Under such environmental stressors, starch is degraded, leading to a subsequent rise in soluble sugars in stressed tissues (Thalmann & Santelia, 2017) and an increased ability to withstand the applied stress. Thus, a plant's ability to convert NSC stores from starch to sugar, and back again, is an important consideration for survival under climate change. While variation in starch degradation rates in response to environment is well document, it is not yet understood if individuals exhibit differential sensitivities to environment, indicating a potential for adaptive evolution in response to increased stress.

Given that both the *total* amount of NSC stores a plant holds and the *proportion* of those stores reserved in starch can play crucial roles in woody plant survival under environmentally induced stress, measuring the degree of heritable genetic and plastic variation in these traits will be critical for predicting tree species' persistence under climate change. Transplant experiments across multiple sites with replicate clones or related individuals can be used to disentangle sources of variation (Josephs, Berg, Ross-Ibarra, & Coop, 2019; Nuismer & Gandon, 2008). Genetic variation in these designs is measured within an environment across unique genotypes, while plastic variation is measured across environments within unique genotypes. In practice, because measuring plastic variation results in the additional capture of genetic variation by virtue of gathering data from multiple common environments, it is often broken down into three components; variation *within* a garden attributed to genetic differences (G), variation *between* gardens attributed to environmental plasticity (E), and the interaction between genotype and environment (GxE) (ie. some genotypes can be more plastic than others). While there is strong evidence that heritable genetic variation is responsible for some of the variation in NSCs in some plants such as *Populus trichocarpa* (Blumstein et al., 2020) and *Pinus sylvestris* (Oleksyn, Zytowskiak, Karolewski, Reich, & Tjoelker, 1999), to our knowledge, no study to date has quantified the extent of plastic variation in NSCs. Furthermore, no study has examined genetic or plastic variation in the proportion of NSC that is kept in starch versus sugar, a potentially critical aspect of plant response to stress.

By partitioning the variation in NSCs we can not only predict the potential for tress to respond to increased

prevalence of stress, we can also begin to understand if trees are already locally adapted to variation in environmental stress across their ranges. If greater total NSC storage and more rapid transition between starch and sugar storage can increase survival in stressful events, we predict genetic variation in these traits to reflect geographic variation in stress. By controlling for neutral population genetic variation across the range of a tree species (*e.g.* Fst) we can determine the extent to which traits show genetic differentiation (*e.g.* Qst) reflective of local adaption.

Here, we measure genetic and plastic variation in *total* NSC storage and the *proportion* of NSC stores held in insoluble starch versus soluble sugars. To do so, we utilized two Department of Energy (DOE) common gardens of black cottonwood (*Populus trichocarpa*) growing in the western United States. Each garden contains clonally replicated genotypes from multiple populations across the species range. We extracted sugars and starches from branch woody tissue of trees grown in two common gardens located at similar latitudes, but spanning a continental to coastal environmental cline (Figures 1 & 2A). Our objective is to understand the acclimatory and evolutionary potential of black cottonwood trees under future climate change. We accomplish this by parsing the amount of phenotypic variation attributable to genetic variation (G), environmental plasticity (E), and genotype-by-environment interactions (GxE) for both the total concentration of NSC stores, as well as the proportion of total stores in insoluble starch. In addition, we search for signatures of current local adaptation in both traits across the environmental gradient of source populations.

## Materials and Methods

### *Sample collection*

We collected branch samples from two Department of Energy (DOE) common gardens in Oregon, USA in January of 2017. The gardens are located near Clatskanie, Oregon (46.12°N, 123.27°W; MAT = 10.4°C, MAP = 1,545 mm) and Corvallis, Oregon (44.56°N, 123.26°W; MAT = 11.2°C, MAP = 1,030 mm) respectively, which represent contrasting environments with similar daylength patterns (Figure 2A). Each garden contains the same 1,060 unique genotypes from 19 populations, which were replicated clonally three times each and planted out in 2009. The collection of each accession is described in Slavov et al. (2012). However, we only sampled genotypes from 17 of the populations, as some populations had high mortality, and from the 917 of 1,060 which have full genomic information available (Evans et al., 2014) (Figure 1). In Corvallis, all three replicates were coppiced (aboveground biomass harvested) in the winter of 2013-2014, while only one replicate was coppiced in Clatskanie during the same period (Figure 1). Thus, we only sampled branches from coppiced replicates for this study. Non-coppiced trees in Clatskanie had an average diameter at breast height (DBH) in 2016 of  $155.2 \pm 46.9$  mm, while coppiced trees averaged  $84.0 \pm 28.0$  mm. DBH in Corvallis across all coppiced replicates in 2016 was  $44.4 \pm 14.9$  mm.

We collected all samples between January 6<sup>th</sup> and January 11<sup>th</sup> 2017, between 7 A.M. and 4 P.M. using pruning shears for branch sampling. We took all branch samples as 2cm segments from the previous growing season's growth segment (2015) of coppiced replicates as it was accessible across all individuals. All samples were kept on dry ice during field collection, then shipped overnight to Cambridge, MA and stored at -80°C. In total, we collected and processed 616 branch samples (Figure 1). We collected additional stem and root tissue during this time period and included aspects of this work in the discussion for comparison (Blumstein et al., 2020)(Table S1).

### *NSC Laboratory Preparation*

We initially freeze-dried samples for 24-hours (FreeZone 2.5; Labconco, Kansas City, MO, and Hybrid Vacuum Pump, Vacuubrand, Wertheim, Germany), then ground them to a fine powder (mesh 10, Thomas Scientific Wiley Mill, Swedesboro, NJ, USA; SPEX SamplePrep 1600; MiniG, Metuchen, NJ) and stored them in sealed glass vials. Sugar and starch extraction protocols were adapted from Chow & Landhäusser (2004).

We extracted sugar from 20 mg of dried, ground tissue using 80% hot ethanol, followed by a colorimetric assay with phenol and sulfuric acid, and read using a spectrophotometer at 490nm (Thermo Fisher Scientific GENESYS 10S UV-Vis, Waltham, MA). We calculated sugar concentrations of mg sugar per g of dry wood

using a 1:1:1 glucose-fructose-galactose standard curve (Sigma Chemicals, St. Louis, MO). We extracted starch using the tissue remaining after sugar extraction. We solubilized tissue in NaOH, then incubated it for 24-hours with alpha-amylase and amyloglucosidase digestive enzymes, which digested starch into glucose. We then assayed the solutions using a PGO-color reagent solution (Sigma chemicals) and read them on the spectrophotometer at 525nm. Starch concentrations of mg glucose-starch-equivalent per g dry wood were calculated based on a glucose standard curve (Sigma Chemicals).

For all lab analyses, we included at least two internal laboratory standards (*Quercus rubra* stemwood from Harvard Forest, MA;  $42.01 \pm 5.13$  mg\*g-1 Sugar,  $30.17 \pm 4.23$  mg\*g-1 starch). This acid methodology extracts all fructose, glucose, sucrose, and starch, as well as other oligosaccharides and other glucans (Landhauser et al., 2018). We then report these metrics as *sugar* and *starch* concentrations (mg\*g-1) in the supplement, as well as the *total nonstructural carbohydrates (TNC)* concentration (sugar + starch) and the *proportion* of starch to total (starch / (sugar + starch)) in the main text. All statistical analyses were performed in Rv.3.5.1(R. C. D. Team, 2018).

#### *Determining environmental conditions of population origins and gardens*

We accessed Daymet daily meteorological data for the past 38 years (1980-2018) at each site via the *daymetr* package in R(Hufkens, Basler, Milliman, Melaas, & Richardson, 2018). Daymet data are 1km<sup>2</sup> gridded estimates of daily weather variables, interpolated from weather station data(Thornton et al., 2018). Using these data, we calculated common descriptive climate variables that represent the temperature and aridity of our genotype provenances and 2 common garden sites. All climatic variables we calculated were highly colinear, thus we used a PCA analysis to describe the major axes of variation via the *vegan* v.2.5-3 package in R (Oksanen et al., 2018) (Figure 2A).

#### *Control for spatial autocorrelation*

In common garden studies the spatial autocorrelation, or the probability that individuals growing closer together are more similar, of samples must be taken into account (Stopher et al., 2012). To account for geographic patterns within each of our gardens (Clatskanie and Corvallis), we used a thin-plate spline method (Blumstein et al., 2020; Evans et al., 2014) via the *fields* (9.6) (Nychka, Furrer, Paige, & Sain, 2017) package in R. This method fits an interpolated surface to the garden, which uncovers regions of each site that significantly differ from the mean. To correct these patterns of spatial concordance, we take the residuals from the thin plate spline and add them back to the model intercept, thus removing spatial trends and placing sample values back on a biologically meaningful scale. We did this for each of our metrics independently; sugar concentration, starch concentration, total nonstructural carbohydrate (TNC) concentration, the proportion of starch (starch / TNC), and diameter at breast height (DBH).

#### *Calculating the genetic contribution via heritable variation*

We estimated components of variation across all the data from both gardens to calculate broad-sense heritability and  $Q_{st}$ . The spatial autocorrelation corrected data were used to parse variation in our nested hierarchical structure of population, genotype, and environment via the following equations:

$$(1) Y_{Gip} = ss_G + \alpha_G + \alpha_{Gp} + \alpha_{Gpg} + \varepsilon_{Gipg}$$

Our goal is to quantify within-garden genetic variation, we therefore parsed variation in branches grown in two different gardens (G) using the fixed effect  $ss_G$ . The parameters  $p$  for population (i.e. provenance of genotype),  $g$  for genotype, and  $i$  for the  $i^{th}$  individual tree sampled are all random effects. All branch data were modeled as gamma distributions using Bayesian mixed regression models via the package *rstan* v.2.18.2 in (T. S. D. Team, 2014). The random effects outcomes ( $\alpha$ 's) and fixed effect ( $ss_G$ ) of equation (1) were estimated as the mean of 6,000 random draws from the posterior distribution (Table 1 & S3).

The resultant fixed and random effect estimates ( $ss_G + \alpha_G + \alpha_{Gp} + \alpha_{Gpg}$ ) from equation 1 were then used as our genetic estimate for each genotype and are displayed in all graphical analyses (often referred to as a

Best Linear Unbiased Prediction or BLUP). The variation parameters estimated from equation 1 were used to calculate broad-sense heritability,  $H^2$ , and  $Q_{st}$  of each of the traits.  $H^2$  was calculated for all traits in stems, branches, and roots, using the random effects variances from equation (1) as:

$$(2) (\sigma_{\text{Genotype}}^2 + \sigma_{\text{Population}}^2) / (\sigma_{\text{Population}}^2 + \sigma_{\text{Genotype}}^2 + \sigma_{\text{Microenvironment}}^2)$$

Population variance was included with Genotype as it is also representative of genetic differences between individuals. Genotype variance was taken as the variance among replicates and microenvironmental variance was taken as the residual variance of the model.  $Q_{st}$  was calculated via the formula (Spitze, 1993; Whitlock & Gilbert, 2012):

$$(3) \sigma_{\text{Population}}^2 / (2\sigma_{\text{Genotype}}^2 + \sigma_{\text{Population}}^2)$$

$F_{st}$  was taken from a previous publication using the same genotypes and calculated in 1-kb windows as  $(\pi_T - \pi_S) / \pi_T$ ; where  $\pi_T$  is SNP diversity across all individuals and  $\pi_S$  is weighted within-population SNP diversity (Evans et al., 2014).

#### *Calculating plasticity: environmental and genotype-by-environment contribution*

Plastic variation is defined by Scheiner and Goodnight as the variation due to environment (E) and genotype-by-environment (GxE) interactions (Scheiner & Goodnight, 1984). To calculate each, we used a Bayesian mixed model regression analysis in R using the *rstanarm* v. 2.19.2 package (Goodrich, Gabry, Ali, & Brilleman, 2019) via the following equation.

$$(4) Y_{igG} = \alpha_G + \alpha_g + \alpha_{G:g} + \varepsilon_{igG}$$

The model calculates the variation within the random effects of Environment (G or Garden), Genotype (g), and GxE (G:g, or Garden:genotype) (Table S3). We then use these variances, estimated as the mean of 6,000 random draws from the posterior distribution of equation (4), to calculate the contribution of an individuals' phenotypic due to plasticity, also known as the  $S$  index (Scheiner & Lyman, 1989).

$$(5) S = (\sigma_E^2 + \sigma_{\text{GxE}}^2) / (\sigma_G^2 + \sigma_{\text{GxE}}^2 + \sigma_E^2 + \sigma_e^2)$$

We then used these properties to calculate the proportion of plasticity due to environment versus genotype-by-environment interactions (Table 2).

$$(6) \sigma_{\text{Plasticity}}^2 = (\sigma_E^2 + \sigma_{\text{GxE}}^2)$$

We build this model separately from our heritability model because of the way  $H^2$  and  $S$  are defined in the literature. Plasticity ( $S$ ) estimates require us to separate variation due to GxE interactions from genetic variation. However, GxE interactions would be partially captured under the umbrella of genetic variation in our heritability model. Conversely, our heritability model also examines the variation due to population in order to calculate  $Q_{st}$ , which is partially captured by the G and GxE random effect terms from our plasticity model. In order to accurately parse the subtle differences in how heritability and plasticity define genetic variation, we run two separate models.

We also used Relative Distance Plasticity Index (RDPI) as a measure of genotypic plasticity, which is a more general way of calculating plasticity that doesn't rely on assumptions of the underlying distribution of the data (Valladares et al., 2006).

$$(7) RDPI = \sum \frac{|X_{\text{Clatskanie}} - X_{\text{Corvallis}}|}{\max(X_{\text{Clatskanie}}, X_{\text{Corvallis}})} / N$$

RDPI measures the absolute difference in genetic trait values between genotypes grown in two different environments, then normalizes that measure by the maximum of the two values. All of these measures are then summed and divided by the number of samples to get the final average RDPI metric.

## Results

### *Environmental conditions of population origins and gardens*

Our genotypes originated from a steep climatic gradient of temperature (mean annual temperature: 10–17°C), aridity (mean annual precipitation: 609mm–2,705mm), and continentality (temperature difference between hottest and coldest month: 14 – 26°C). When summarized via a Principle Components Analysis (PCA), the first major axis of variation amongst climate variables across sites (PC1) describes an axis of cold and wet sites to hot and dry sites (Figure 2A). The second axis of variation represents continentality, ranging from sites with consistently cold winters and high amounts of snowfall to sites that are generally warmer and do not experience as extreme lows (Figure 2A). In comparison to Clatskanie, Corvallis is hotter, drier, and has more continentality, meaning colder, wetter winters and hotter, dryer summers.

While these are the average site conditions, at the time of sampling, both Clatskanie and Corvallis experienced the lowest temperatures recorded for those dates over the past 38 years (Figure 2 B-E, solid line). Minimum temperatures ranged from -10°C to 0°C and maximum temperatures never reached higher than 3–5°C. This is a key detail as starch synthesis and degradation enzymes cease to perform under 3–5°C (Pollock & Lloyd, 1987), thus these short-term temperature drops may have influenced our measures (Figure 2 B-E).

### *Total phenotypic variation*

Across all tissues measured, NSC concentrations were highest in branches (Clatskanie:  $30.8 \pm 8.3 \text{ mg} \cdot \text{g}^{-1}$ , Corvallis:  $30.3 \pm 6.2 \text{ mg} \cdot \text{g}^{-1}$ ) and lowest in stems (Clatskanie:  $15.6 \pm 6.0 \text{ mg} \cdot \text{g}^{-1}$ ) (Table 1 & S1, Figure S1). The proportion of total NSCs in starch ranged from 0.10  $\pm$  0.08 in branches in Corvallis to 0.28  $\pm$  0.18 in roots in Clatskanie (Table 1 & S1, Figure S1).

### *The genetic contribution (G)*

We found heritable variation in branches for both total NSCs and proportion of NSCs in starch (Table 1, Branch  $H^2_{\text{TNC}} = 0.19$ , Branch  $H^2_{\text{PropStarch}} = 0.31$ ). As previously reported, this is similar heritability as total NSC storage in *P. trichocarpa* stems and roots (Table S1; Blumstein et al., 2020). Here we found that the proportion of starch to total stores is also heritable in roots (Root  $H^2_{\text{PropStarch}} = 0.37$ ), but not stems (Stem  $H^2_{\text{PropStarch}} = 0.01$ ).

Branch genetic variation does not exhibit high degrees of across population variation in either total NSCs or proportion of stores in starch ( $Q_{\text{st TNC}} = 0.07$ ,  $Q_{\text{st Proportion}} = 0.04$ ; Table 1). This stands in contrast to stems and roots, which demonstrate extensive differentiation across populations in total stores ( $Q_{\text{st Roots}} = 0.30$ ,  $Q_{\text{st Stems}} = 0.31$ ) and stem partitioning between sugar and starch ( $Q_{\text{st Stems}} = 0.66$ ) (Table S1). However, the finding of high  $Q_{\text{st}}$  value for stems in the partitioning between sugar and starch is unlikely biologically meaningful.  $H^2$  for stems is only 0.01 for this trait, thus the high  $Q_{\text{st}}$  value is calculated using just 1% of the phenotypic variation measured.

### *Plasticity: Environmental Contribution (E)*

The proportion of NSC that trees put into starch vs. sugar is a plastic trait by both metrics of plasticity, RDPI and  $S$  (Table 2,  $\text{RDPI}_{\text{proportion}} = 0.50$ ,  $S_{\text{proportion}} = 0.74$ ). This plastic variation is largely attributable to the effect of garden, which explains 97% of total plasticity (equation 7; Table 2, Figure 3). On average, Corvallis has higher sugar concentrations and lower starch concentrations, while Clatskanie has higher starch concentrations and lower sugar concentrations (Figures 3B & S2, Table 1). This pattern is reflected in the proportion of NSC stores in starch, where the proportion of starch to total stores in Corvallis is 50% more than in Clatskanie (Figure 3B). Thus, there is a clear difference in the degree to which total stores are partitioned between sugar and starch within each garden.

In contrast, total NSC stores are only considered environmentally plastic by one metric ( $RDPI_{TNC} = 0.03$ ,  $STNC = 0.43$ ). Only 54% of the plastic variation ( $S$ ) measured is explained by environment, while the rest is attributed to GxE effects. Thus, we find high genetic variation in TNC across the gardens, but low plasticity and almost no difference between the two gardens in average total NSC concentrations ( $\Delta_{\text{garden}} = 0.5 \text{ mg} \cdot \text{g}^{-1}$ , Table 1, Figure 3A).

### *Plasticity: Genotype-by-Environment Contribution (GxE)*

Genotype-by-environment interactions explain almost 50% of the variation between gardens in total NSC stores (Table 12 Figure 3A), but only 3% of the variation between gardens in the proportion of NSC in starch (Table 2, Figure 3B). Thus, genetic differences explain most of the variation in total NSC stores, while plasticity explains most of the variation in the proportion of starch vs. sugar.

In addition, some genotypes are more plastic than others, particularly in the amount of NSC they allocate to starch (Table 2, Figure 4). This pattern of increasing plasticity follows a latitudinal trend, where northern populations are more plastic in each trait measured than southern populations (Figure 4). However, while data trend this way, they are not significant at the 0.05 level (Figure 4).

## **Discussion**

### *Genetic (G) variation in both total stores and partitioning between sugar and starch*

Genetic variation in traits act as the raw material with which populations can adapt to novel stress. Here, we found heritable genetic variation in total NSC concentrations (sugar + starch) in the branches of black cottonwood. Our results reveal that these trees could potentially evolve greater NSC storage in response to increased stress. An increase in the concentration of NSC a tree stores may confer enhanced resilience during times of photosynthetically limiting stress by serving as a fuel source or osmotic reservoir (Dietze et al., 2014; Henrik Hartmann et al., 2018; H. Hartmann & Trumbore, 2016; O'Brien et al., 2017; Wiley & Helliker, 2012). Experimental and observational studies have demonstrated that trees will draw down NSC stores when experiencing photosynthetically limiting environmental conditions (Adams et al., 2013; Adams et al., 2017; Henrik Hartmann, Ziegler, Trumbore, & Knapp, 2013; Landhäusser & Lieffers, 2011; Sevanto et al., 2014), such as drought, suggesting that NSCs may be serving a critical metabolic function. In addition, seedlings with higher NSC stores live longer through drought, indicating that higher NSC concentrations may enable trees to live longer under environmental stress (O'Brien et al., 2017). Given that climate forecasts generally predict more extreme weather events (IPCC, 2013b), the existence of heritable variation in total NSC stores may be crucial for tree populations to evolve in response to climate-driven selection in the future.

In addition to total storage, the proportion of stores individuals put into starch versus sugar at any given time may be crucial for withstanding future climate-driven selection. We also found heritable variation in the proportion of these total stores residing in insoluble starch for branches, stems, and roots (Table 1, Table S1). Across most winter deciduous species, the proportion of total stores in starch is at its lowest in the winter, particularly in January, the month we sampled in (Furze et al., 2019; Martínez-Vilalta et al., 2016). This is thought to be because the rate of starch degradation and synthesis is controlled in part by enzymes with different temperature sensitivities. Starch degradation enzymes are less sensitive to low temperatures than starch synthesis enzymes (Pollock & Lloyd, 1987), although below 3-5°C both degradation and synthesis enzymes cease to function. Thus, at low temperatures (but still above 3-5°C) starch is degraded, leading to a corresponding increase of sugar in cells (Zwieniecki, Tixier, & Sperling, 2015). This newly available pool of sugar can be used for maintenance respiration and may confer an increased cold tolerance to individuals or even signal when to break dormancy in spring (Aude Tixier, Gambetta, Godfrey, Orozco, & Zwieniecki, 2019). Thus, being sensitive to changes in temperature and shifting stores between starch and sugar may be critical for tree survival under future, more chaotic climate regimes. Our results indicate that there is genetic variation in if or how trees shift stores between starch and sugar. This opens the possibility that trees can adapt their allocation strategy to better fit a changing, more stressful climate.

Although we found heritable variation in both branch total NSC stores and proportion of stores in starch,

we did not find evidence that this variation was currently locally adapted. Differentiation across populations in genetic variation of these traits is minimal. The lack of adaptive signatures in branch total NSC stores stands in contrast to adaptive differentiation previously reported for stems and roots. One reason for this divergence may be that branches are the most proximal of the three tissues to carbon sources (leaves) and some sinks (buds and flowers). Over the course of a year, branch NSC stores fluctuate much more than roots or stems as they are the first storage sink to fill with new photosynthates and the first to be drawn down in spring (Furze et al., 2019). This pattern could also be caused by the fact that black cottonwoods have photosynthetic bark. Branches are exposed to more sunlight than stems and roots and thus may see a higher degree of variation in NSC produced via opportunistic bark photosynthesis. Together, these sources of variation may have a genetic signal, but the other sources of fluctuations may mask the genetic variation in storage preventing selection from effectively causing genetic differentiation.

### *Environmental (E) plasticity in partitioning between sugar and starch*

Immediate response to stress depends on an organism’s ability to plastically adjust trait values to accommodate changing environments. The degree to which an organism can plastically respond will, in many causes, determine its ability to survive stress. Here, we found extensive plastic variation due to environmental response in the proportion of NSC stores in starch versus sugar (Table 2, Figure 3, solid black lines). In Clatskanie an average of 20% of total NSC stores were found in starch, while only half that amount was found in Corvallis (Figure 3). Clatskanie has a coastal climate with rainfall spread throughout the year and small temperature differences between winter and summer (Figure 2). In contrast, Corvallis has a continental climate which regularly experiences extreme temperatures and long periods without rainfall (Figure 2). Thus, these results may reflect the differential enzymatic sensitivities of starch degradation and synthesis to average climatic conditions at these two sites (Pollock & Lloyd, 1987; Aude Tixier et al., 2019). The warmer average temperatures in Clatskanie could have led to a higher proportion of NSC being left in starch while the colder, more stressful conditions of Corvallis resulted in more sugar storage.

The observed plasticity in proportion of starch storage could also be driven by the weather patterns on the dates of sampling. We happened to collect woody tissues on the coldest days recorded over the past 38 years in Clatskanie and extremely cold days in Corvallis (Figure 3). Low temperatures in Clatskanie fell below -10°C some days and highs never went above 4°C, within the minimum temperature range that starch synthesis and degradation enzymes can work (Pollock & Lloyd, 1987). The sharp drop in temperatures due to the polar vortex may have halted enzymatic activity entirely. Thus, instead of starch steadily degrading to sugar as temperatures drop, the quick temperature change may have prevented starch from degrading further. Conversely, we travelled to Corvallis after sampling in Clatskanie, where the temperature reached just above 5°C on our sample dates; just above the minimum temperature range for enzymatic activity. Thus, starch may have degraded into sugar in trees at this site. Such a quick change could be possible given that starch synthesis and degradation have been observed on diurnal scales (A. Tixier et al., 2018).

It is difficult to pinpoint whether the plasticity in the proportion of NSC in starch between the two gardens was attributable to the average climate of the two sites, or the weather at the time of sampling. However, there is mounting evidence that this plasticity in the synthesis and degradation of starch in plants is critical for seasonal signaling in plants (Gibon et al., 2009; Palacio, Gunter, Sala, Korner, & Millard, 2014; Aude Tixier et al., 2019). Branch NSC stores begin to synthesize from sugar into starch as temperatures rise in spring (Furze et al., 2019; Martínez-Vilalta et al., 2016), and this process likely occurs faster in branches than in roots because branches are exposed to air and not insulated in the soil. Thus, temperature gradients across the plant may drive the movement of carbohydrates upward in spring to support leaf flush and stem growth (Sperling, Silva, Tixier, Theroux-Rancourt, & Zwieniecki, 2017). This synthesis of starch or movement of carbohydrates could be the signal plants sense in spring to break dormancy or initiate leafout. Thus, it may be this plasticity in the conversion of sugar to starch that is in part driving observed plasticity in phenological timing, a key trait for future tree adaptation to climate change (Alberto et al., 2011; Hall et al., 2007; Keller et al., 2011).

### *Environmental plasticity in total NSC stores unveiled when tree size is considered*



While partitioning of stores between sugar and starch is plastic, we found total NSC stores to be the same, on average, between the two gardens. This is particularly interesting as the timing of leaf out and growth rates of trees significantly differ between the two sites. In Clatskanie, leaves come out earlier than Corvallis, potentially giving the Clatskanie trees a greater opportunity to produce carbon compared to the Corvallis trees (Evans et al., 2014). This carbon advantage is reflected in the diameters of trees at both sites, where trees in Clatskanie are almost double the size of trees in Corvallis (Figure 5). However, both sites average the same total NSC storage concentrations. Without the growth data, total NSC stores appear to have no environmentally driven plasticity, however with the growth data, the lack of difference between the two sites could actually be indicative of environmental plasticity in a tradeoff between the growth and storage. Trees growing in more variable or extreme conditions may “bet-hedge” by storing more NSC at the expense of other uses, such as growth (Sala, Woodruff, & Meinzer, 2012; Wiley & Helliker, 2012). This particular pattern has been shown in *Arabidopsis*, where plants favor storage over growth when photosynthetic productivity declines (Gibon et al., 2009; Smith & Stitt, 2007). Our results here suggest that this may be similar in the case in black cottonwoods, where genotypes grown in the continental garden (Corvallis), with larger temperature and precipitation extremes, maintain the same concentration of stores, but down-regulate growth to compensate. Thus, proportionally more energy is allocated to storage at the expense of growth in the more stressful environment.

### *Genotype-by-environment (GxE) plasticity in total stores, but not in proportion of stores in starch*

Genetic variation in environmental response could provide a key mechanism through which populations can evolve a more adaptive response to future environmental stress. Here, we found that plastic variation in total storage is almost entirely comprised of genotype-by-environment interactions. The average total NSC storage concentrations between the two gardens only differ by 0.5 mg\*g<sup>-1</sup>, but individual genotypes differ by -3.4 to 3.3 mg\*g<sup>-1</sup> between the two gardens. This flexibility amongst genotypes may be the result of intrinsic differences in response to environment (Des Marais et al., 2013) and could explain why previous studies have failed to converge on a consistent response to stress to date (Adams et al., 2017; Thalmann & Santelia, 2017). These works have found that NSC concentrations may decrease, increase, or remain stable in response to stressors such as drought and extreme temperature. Thus, our work underscores the importance of controlling for genetics when conducting any NSC manipulations, as the high heritability and GxE of the trait can confound interpretation of results.

In contrast, plastic variation in the proportion of NSC stores in starch is almost entirely attributed to differences in environment and not GxE interactions. This finding further supports a model where by the amount of NSC residing in starch is driven by intrinsic enzymatic environmental limits. While GxE for this trait is lower than the amount of GxE for total NSC stores, there is a clear latitudinal pattern in the proportion of starch GxE variation (Figure S3). The trending correlation between latitude and the proportion of starch RDPI suggests that some genotypes may be more plastic than others in their ability to move between sugar and starch (Figure 5). In particular, genotypes from northern populations appear to have more flexibility between the two gardens. Put another way, northern genotypes are more responsive to environmental differences between the two sites. One possible explanation for this trend is that their starch degradation enzymes may be more sensitive to temperature fluctuations or are able to continue to act at slightly lower temperatures than those from southern populations. This may be beneficial to trees in northern latitudes experiencing extreme temperature lows and large temperature swings throughout the day. Although the amount of variation explained by GxE interactions may be small, the importance for immediate environmental response may be very important and is worth further investigation.

## **Conclusion**

Our study is the first of its kind to study heritable variation and plasticity in branch nonstructural carbohydrate storage. NSC stores have been demonstrated over the past decade to confer resilience to climate stress in woody species and are likely critical for plant response to future climate. In nearly all previous experiments on NSC variation, it was simply assumed that the variation observed in the experiment is due to predictable responses to environmental variation or perturbation (E). We demonstrate that, in fact, a

significant amount of biologically meaningful variation in NSCs is due to genetic variation between trees. This means that there are genetic mechanisms that regulate differences in NSC storage, even between trees grown in the same environment. More importantly, we find extensive variation across trees in their response to environmental variation. Nearly all previous studies have ignored this source of variation and assumed that trees of a given species will show a predictable and uniform response to their environment. Overall, we demonstrate that the concentration of total NSCs that trees store as well as the degree to which they partition these stores between soluble sugars and starch have both adaptive genetic variation and plasticity, potentially bolstering forest tree species against climate change in the short-and-long term.

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## Figures and Tables

Table 1. Phenotypic means and standard deviations, variance component estimates from equation (1), and parameters for total nonstructural carbohydrate (TNC) storage concentrations and the proportion of total stores in starch of branch tissues of black cottonwood trees.

	Clatskanie	Clatskanie	Corvallis	Corvallis							
Branch Trait	$\mu$	$\sigma$	$\mu$	$\sigma$	$(\sigma^2_{Gipg})$	$(\sigma^2_{Gpg})$	$(\sigma^2_{Gp})$	$(\sigma^2_G)$	$(\beta_G)$	$H^2$	$Q$
TNC	30.8	8.3	30.3	6.2	31.39	6.567	1.004	222.759	-0.099	0.19	0.0
Proportion	0.2	0.07	0.1	0.08	0.002	0.001	0	0.003	-0.102	0.31	0.0

Table 2. Variance component estimates from equation (4) and parameters for total nonstructural carbohydrate (TNC) storage concentrations and the proportion of total stores in starch of branch tissues of black cottonwood trees.

Branch Trait	$\sigma^2_g$	$\sigma^2_G$	$\sigma^2_{G:g}$	RDPI	S	(%) of S that is GxE
TNC	2.4	3.4	3.2	0.03	0.43	46
Proportion	0.025	0.091	0.017	0.5	0.75	3

Figure 1. The transplant design. Genotypes were sourced from 17 populations from across western North America and replicated three times each in two common gardens located in Clatskanie and Corvallis. In the winter of 2013-2014, one replicate in Clatskanie and all three replicates in Corvallis were coppiced aboveground and allowed to regrow (shown as small trees). Sample tissues collected in January 2017 are reflected by the purple ovals with the subsequent number of samples taken of each tissue from each garden indicated.

Figure 2. (A) A principle components analysis (PCA) of the climate over the past 38 years at each of the genotype's source locations and the two common gardens. Dots represent each genotype's source location in climate space, colored by latitude, and the two black triangles represent the common gardens in the climate space. (B-E) The average minimum and maximum temperatures over the past 38 years in Clatskanie and Corvallis (boxplots) as compared to the minimum and maximum temperatures in the year we collected samples (2017, black lines). Samples were collected in (B,D) Clatskanie on January 5<sup>th</sup> – January 9<sup>th</sup> and in (C,E) Corvallis on January 10<sup>th</sup>– January 11<sup>th</sup>. Data is sourced from Daymet (Thornton et al., 2018).

Figure 3. Plasticity between the two common gardens in the two traits measured, (A) total NSC stores (TNC) and (B) the proportion of TNC in starch. Black lines represent the average value across all genotypes, with error bars indicating one standard deviation from the mean. The other lines are colored by latitude and each line represents a genotype, where genotype values were estimated using equation (1).

Figure 4. The relative distance plasticity index (RDPI) of the (A) proportion of starch to total and (B) total amount of NSC stores plotted by the latitude of each genotype's source location. Black dots represent each genotype's RDPI and the line fits are shaded gray for one standard deviation. A larger RDPI indicates that there is a larger percent change in genotypic trait values between gardens.

Figure 5. (A) Genetic variation in diameter at breast height (DBH) as compared to (B) genetic variation in total nonstructural carbohydrates as measured at each common garden.







