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Research

Plant carbohydrate storage: intra- and inter-specific trade-offs reveal a major life history trait

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Summary

- Trade-offs among carbon sinks constrain how trees physiologically, ecologically, and evolutionarily respond to their environments. These trade-offs typically fall along a productive growth to conservative, bet-hedging continuum. How nonstructural carbohydrates (NSCs) stored in living tree cells (known as carbon stores) fit in this trade-off framework is not well understood.
- We examined relationships between growth and storage using both within species genetic variation from a common garden, and across species phenotypic variation from a global database.
- We demonstrate that storage is actively accumulated, as part of a conservative, bet-hedging life history strategy. Storage accumulates at the expense of growth both within and across species. Within the species *Populus trichocarpa*, genetic trade-offs show that for each additional unit of wood area growth (in $cm^2\ yr^{-1}$) that genotypes invest in, they lose 1.2 to 1.7 units ($mg\ g^{-1}$ NSC) of storage. Across species, for each additional unit of area growth (in $cm^2\ yr^{-1}$), trees, on average, reduce their storage by 9.5% in stems and 10.4% in roots.
- Our findings impact our understanding of basic plant biology, fit storage into a widely used growth-survival trade-off spectrum describing life history strategy, and challenges the assumptions of passive storage made in ecosystem models today.

Introduction

Trade-offs define the limits of life (Futuyma & Moreno, 1988; Agrawal *et al.*, 2010) and maintain biological diversity at multiple scales of organization (e.g. Vellend & Geber, 2005). Trade-offs commonly form when natural selection acts simultaneously on multiple traits that share finite energetic resources (Mooney, 1972; Agrawal, 2020). For organisms with limited means of increasing their resource acquisition, particularly sessile tree species, determining how they allocate resources is critical for understanding basic physiological processes, determining functional roles in ecological communities, and predicting success under current and future climates (e.g. Herms & Mattson, 1992; Dietze *et al.*, 2014). The importance of trade-offs has thus led to decades of research on how woody plants partition their limited supply of carbon amongst the competing demands of metabolism, growth, reproduction, and defense – referred to as carbon allocation (Mooney, 1972; Hartmann *et al.*, 2020). Patterns of carbon allocation trade-offs are thought to underlie variation in plant survival strategies, which generally follow an axis of investment in productivity and competition (represented by growth), vs longevity and persistence (Wright *et al.*, 2004; Agrawal *et al.*, 2010). The trade-offs along this major axis underpin our understanding of basic plant biology and community dynamics, and are now being used to reduce the complexity of plant systems to better parametrize ecosystem models, a key tool for predicting global climate (Wright *et al.*, 2004; Dietze *et al.*, 2014; Jones *et al.*, 2020).

How storage, or the labile, nonstructural carbohydrates (NSCs) residing in woody tissues, is related to growth and thus factors into trees' carbon budgets, has been long debated (Chapin *et al.*, 1990; Kozlowski, 1992; Korner, 2003; Sala *et al.*, 2012; Dietze *et al.*, 2014). One hypothesis, passive accumulation, posits that storage is the lowest priority sink and accumulates as the by-product of photosynthesis after other sink demands are filled. According to this hypothesis, storage is a plastic trait that increases only if there is a surplus of supply after growth, defense, reproduction, and all other carbon demands are met. Although evidence against this hypothesis is increasing (Sala *et al.*, 2012), storage pools are generally represented as passive in most plant models, where carbohydrates accumulate only after other needs are met (Le Roux *et al.*, 2001; Dietze *et al.*, 2014). The alternative hypothesis, active accumulation, is that storage is a competing sink in itself and has evolved to accumulate at the expense of growth as part of a growth–survival trade-off (e.g. Wright *et al.*, 2004; Reich & Cornelissen, 2014). Active accumulation could be achieved by an upregulation of carbohydrate storage in cells or a downregulation of growth or other carbon sinks, sometimes referred to as 'quasi-active storage' (Sala *et al.*, 2012; Dietze *et al.*, 2014). To evolve, storage must be a heritable trait (i.e. variation is genetic and can be passed onto offspring) that can be selected to increase at the expense of growth (Chapin *et al.*, 1990). Such a genetic trade-off indicates a relationship formed by either adaptive selection or a genomic constraint. In either case, the relationship is the result of a genetic trade-off between two traits that is passed onto offspring (high storers and low growers will beget higher storing and lower growing offspring and vice versa). Given the active storage hypothesis, we predict the investment in storage to fall along a competition vs longevity spectrum of plant life-history traits, where an abundant investment in storage as part of a conservative, bet-hedging strategy provides a fitness benefit in more stochastic or extreme environments.

To date, progress has been made toward understanding the role of carbohydrate stores in plant response to environmental extremes (i.e. storage plasticity). Storage is generally thought to serve as a safeguard for plants by providing carbon for both predictable and unpredictable carbon supply fluctuations (e.g. night, dormant season, and environmental disturbance) (Chapin *et al.*, 1990; Hartmann & Trumbore, 2016). Of the components of NSCs, only starch has no other function than storage. Soluble sugars serve as substrates for respiration and other metabolic functions (Hartmann & Trumbore, 2016). However, because starch is a source of soluble sugars and soluble sugars are used to synthesize starch, the sum of the two interchangeable pools (NSCs) is often used as a measure of storage, even if at any given time only a fraction is long-term storage. Otherwise, measures of starch alone may underestimate storage, particularly as sugars are known to flux in and out of starch on seasonal to hourly timescales (e.g. Martínez-Vilalta *et al.*, 2016; Tixier *et al.*, 2018).

Indeed, having higher NSC stores at the onset of stress has been shown to prolong life under drought and shade conditions in tropical seedlings (Poorter & Kitajima, 2007; O'Brien *et al.*, 2014). These results suggest that there may indeed be a selective benefit to higher stores. Similarly, studies of plant responses to environmental disturbance, such as defoliation (Piper *et al.*, 2015; Wiley *et al.*, 2017; Miranda *et al.*, 2020), 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; Adams *et al.*, 2017; O'Brien *et al.*, 2017), and even CO_2 stress (Huang *et al.*, 2019), have demonstrated that NSC stores can be maintained or enhanced following disturbance, often at the expense of growth. While these studies suggest a temporary fitness benefit to investing in storage over other uses, they report plastic responses to the environment and not necessarily evolvable life history strategies. Is there also genetic variation that causes some individual trees to store more carbon and other individuals to store less carbon? And if so, is this variation associated with genetic variation in growth? To truly understand if carbon storage is a priority sink that is actively allocated to at the expense of growth, we must answer these questions and demonstrate, at the genetic level, that variation in storage trades off with growth and can evolve in response to selection.

Demonstrating a trade-off between allocation traits is challenging as both plastic and genetic variation in sources and sinks can mask underlying patterns (see Fig. 1). Thus, apparent relationships measured between two traits could reflect any number of processes, such as genetic variation in carbon supply (Fig. 1ii,iii), rather than a true trade-off. This phenomenon is best summarized by the analogy of the trade-off between buying a car and a house. We know intuitively that individuals must choose how much wealth to allocate to each, and thus a trade-off exists. However, when examined across all individuals, there is often a positive relationship between investment in cars and houses, because people with more money can afford to spend more on both (from Agrawal, 2020; Fig. 1). Just as individuals vary in their wealth, trees can vary in their capacity to generate a carbon supply, due to both plastic variation in response to growing conditions and genetic controls on supply-related traits, like leaf-out timing. As carbon allocation trade-offs are inherently formed by competition for the limited resource of carbon supply (Mooney, 1972; Hartmann *et al.*, 2020) and the traits that contribute to plant carbon supply are heritable to differing degrees (e.g. McKown *et al.*, 2014; see 'Carbon supply' in the Materials and Methods section), we must account for heritable differences in carbon supply when evaluating trade-offs amongst carbon sinks.

Here, we investigate a growth–storage genetic trade-off using a common garden of black cottonwood (*Populus trichocarpa*). Common gardens take individuals from populations across their range and grow them in the same location. Thus, differences observed amongst individuals are presumed to be genetic and not plastic. We hypothesize that a trade-off between growth and storage exists, reflecting a conservative-to-productive spectrum of life history strategies. We next examine growth and storage trade-offs across species to see if patterns uncovered within *Populus trichocarpa* are recapitulated at higher taxonomic scales. Genetic intra-specific trade-offs reveal the evolutionary potential and limitations of species, while inter-specific trade-offs inform our understanding of the ecology and life history strategies that differentiate species (Tilman, 1990; Wright *et al.*, 2004). Furthermore, patterns of trade-offs across species of plants form the foundation of many predictive models of plant success across different environments and communities (Wright *et al.*, 2004); none of which incorporate strategies of carbon allocation to storage. We predict that if a heritable, evolvable growth–storage trade-off exists within species, that it will also be seen across species following a similar pattern of conservative to productive life history strategies.

Materials and Methods

Intra-specific variation

Field collection We utilized data collected from the Department of Energy black cottonwood (*Populus trichocarpa*; Torr. & Gray) common garden located near Clatskanie, Oregon (46.12°N, 123.27°W). The garden contains three randomized blocks, each containing clonal replicates of *c.* 1100 unique genotypes for a total of *c.* 3300 individuals in the garden. All trees

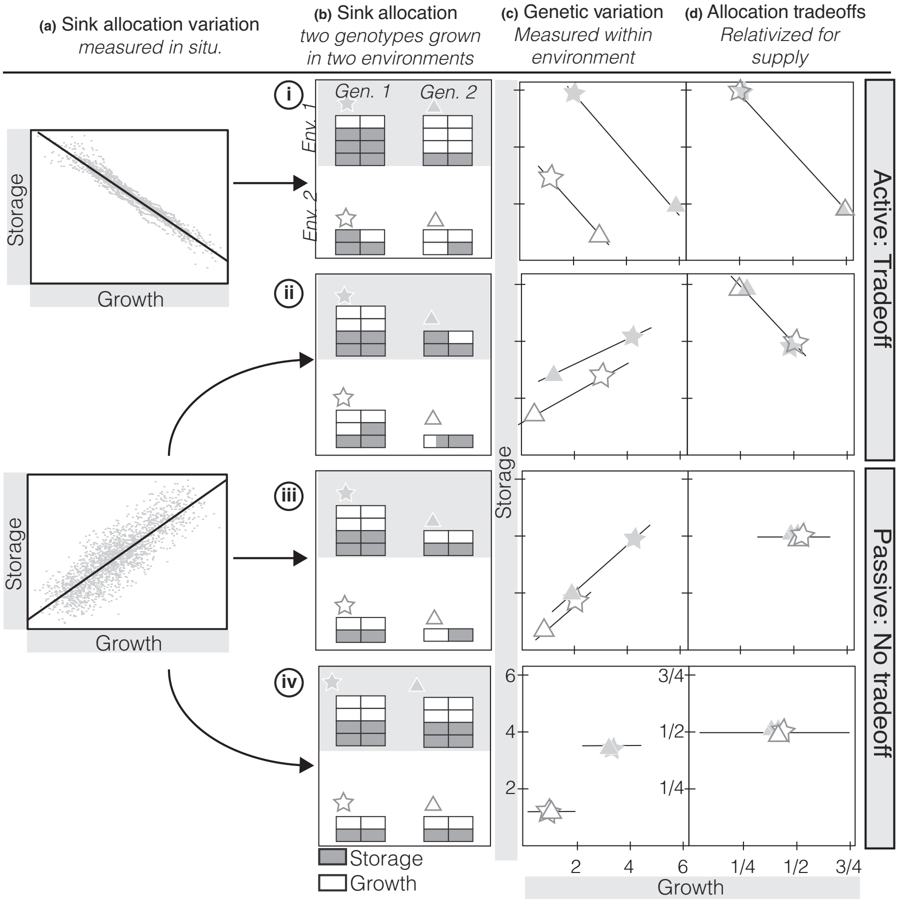

Fig. 1 For storage reserve formation to be considered an active process, nonstructural carbohydrate (NSC) storage accumulation must exhibit a heritable trade-off with growth. Here we demonstrate how (a) relationships measured in the field can be deceiving when (b) genetic variation in sinks and supply are not controlled for. For example, a positive relationship between growth and carbon supply can actually be the result of (iv) plasticity in carbon supply or (iii) plasticity and genetic variation in supply, both indicating there is no real relationship between growth and supply. Conversely, a (ii) positive relationship between growth and storage may actually mask a trade-off when genetic variation in carbon supply is high. This also demonstrates (i) why finding a within species trade-off between two traits in the field is actually very difficult, as this assumes there is no genetic variation in carbon supply. Thus, for NSC storage to be active, it must (c) have genetic variation underlying storage and (d) that variation must trade-off with growth.

originate from 19 different provenances (referred to here as populations) (Supporting Information Fig. S1). Given high mortality of trees from three of the populations (most northern and two most southerly), we ultimately only consider individuals from 16 populations from a range of 44.75°N to 52.8°N latitude. For our study we ensured traits were collected on at least five genotypes from each population, but often more depending on the trait (more later). Collection of each accession and population assignments determined by sequence data are from previously published analyses (Slavov *et al.*, 2012; Evans *et al.*, 2014).

All individuals were planted in 2009 and one replicate was coppiced in December of 2014. Bud burst/bud set and our 2014 growth observations were made prior to this coppicing event, while NSC storage and our 2016 growth observations were measured after. Thus, we used data from three replicates of uncoppiced trees for phenology and 2014 growth observations (Evans *et al.*, 2014), and two replicates for 2016 growth and NSC storage (Blumstein *et al.*, 2020), in our analyses. We chose to include multiple years of growth and phenology data in our analysis to control for potential interannual variability and ontogenetic effects. Given the high time and monetary cost of processing NSC storage, we were only able to include one year of measurements. However, to avoid other sources of variability, we made sure to sample at the onset of the dormant season when NSC stores are at their peak and when there is no input of soluble sugars from photosynthesis. Notably, no individual in the garden had yet reached reproductive age (i.e. not yet produced seed or pollen), thus individuals were not yet allocating carbon to reproduction, which reduces variability.

Carbon supply A plant's total carbon supply is a function of its (1) growing season length, (2) total leaf area of canopy, and (3) photosynthetic efficiency (i.e. $A_{\max}$). We approximated carbon supply by including both the length of the growing season (data from Evans *et al.*, 2014) and leaf area index (LAI) of each genotype in our principal component analysis (PCA) (more later).

Growing season length. The genotypes growing in the common garden come from a steep latitudinal gradient of provenance sites (46°N–53°N) and thus have a high degree of variation in the length of their growing season. Phenological timing is well known to be locally adapted in temperate forests; with early bud flush constrained by selection of frost damage to sensitive leaf tissues, while longer growing seasons are selectively advantageous due to greater resource acquisition and competitive ability in warmer populations (Richardson *et al.*, 2018). We used bud flush and bud set score data that were collected during the 2010 growing season to estimate growing season length for ease of interpretation (Methods S1; Tables S1,S2; Figs S2–S5).

Leaf area index. *Populus* is a well-studied genus due to its potential human-use applications (e.g. biofuel) and have many allometric scaling equations established for genotypes growing in a common garden or plantation setting. In *Populus,* maximum LAI scales exponentially with tree volume (Swamy *et al.*, 2006). We used tree volume estimates (in cm^3) taken after the 2013 growing season, when genotypes were around 4 yr old and an exponential scaling equation taken from the literature to predict LAI (Swamy *et al.*, 2006).

$$\text{LAI} = 0.008\ \text{Volume}^{0.47} \qquad \text{Eqn 1}$$

Photosynthetic efficiency (A_{max}). We do have LI-COR measurements of photosynthetic rate measured in a glasshouse for a subset of genotypes found in the garden. We chose to exclude these measurements in our estimates of carbon supply variation because they were not taken in the same environment as our carbon sink measurements and because they do not have a strong heritable basis ($H^2 \sim 0.1$) (McKown *et al.*, 2014). The low heritability means variation in photosynthetic rate between trees was almost entirely due to microenvironmental variation or measurement error and not genetic differences between individuals. In practice, this means that our estimate of A_{max} was nearly the same across all genotypes because most variation was excluded as it was not genetic. In our supplementary methods we demonstrate that including these A_{max} estimates in our model does not significantly alter our results (described later) and that most within site variation in carbon supply was driven by LAI and growing season length.

Nonstructural carbohydrate storage We utilized NSC storage measurements from a previous publication (Blumstein *et al.*, 2020), which quantified carbon storage by measuring NSC concentrations extracted from stems and roots collected from 6 to 10 January 2017, from 07:00 h and 16:00 h ($N_{\text{stems}} = 242$, $N_{\text{roots}} = 241$), shipped overnight on dry ice and kept in a −80°C freezer until freeze-dried. We measured the outer 2 cm of stem and 1.5 cm of coarse root cores, taken at breast height (1.4 m) and 30 cm along the root from the base of the tree respectively and report values as total NSC concentrations (fructose + glucose + sucrose + starch) (Chow & Landhausser, 2004). Briefly, sugars were extracted in 80% ethanol, read using a colorimetric phenol assay. Starches were then digested using amyloglucosidase and alpha-amylase overnight, then read with a colorimetric assay. More details in Blumstein *et al.* (2020). Only xylem tissue was used, excluding outer bark, cortex, and phloem.

We report these carbohydrate concentrations as one combined metric of NSCs, representing sugar and starch concentrations together (see Introduction section). We recognize that sampling a single time-point has its limitations given that NSC stores can fluctuate over the course of the year (Furze *et al.*, 2019). However, because we are not interested in seasonal or short-term environmentally induced fluctuations in storage, we chose to sample during the dormant season when there is no supply from photosynthesis and the phloem is largely shut-down, and when relative difference amongst genotypes, our focus, are minimized. Studies have found that seasonal variation in stems and roots are small relative to those in leaves and terminal branches (Martínez-Vilalta *et al.*, 2016; Furze *et al.*, 2019).

Size and growth We used two estimates of diameter growth to calculate basal area increment; one taken in November of 2014 and the second in July of 2016. The reason we used both measures is that (1) growth rates may change with ontogeny (e.g. Mencuccini *et al.*, 2007) and (2) we had three replicates of growth measures in 2014, while only two in 2016. Thus, we wanted to ensure we were accurately capturing growth variation, although both years of observations ultimately were highly correlated (Fig. S5). In both years, diameter at breast height (DBH) was measured in millimeters, *c.* 1.5 m from the ground using DBH tape. We then converted DBH to annual basal area increment according to the following equation:

$$\text{BAI} = \frac{\pi\left(\frac{\text{DBH}}{2}\right)^2}{\text{Age}} \qquad \text{Eqn 2}$$

Since all trees were planted in 2009, they are presumed to be the same age at any given time (5 yr old in 2014 and 7 yr old in 2016).

Calculation of genetic variation and heritability In a common garden design, we assume that any differences measured between individuals are due to genetic differentiation because environmental variation is largely minimized across the single growing site. However, within a garden there may still be microenvironmental variation which causes samples to be spatially autocorrelated (i.e. plants growing closer together may be more similar due to a similar microenvironment). We corrected for spatial autorelationship and measured replicate genotypes across randomized blocks to ensure we estimated genetic and not environmentally dependent variation. We conducted these statistical analyses in R v.3.5.1 (R Core Team, 2021).

To control for spatial autocorrelations in the common garden, we used a thin-plate spline method (Evans *et al.*, 2014) via the FIELDS (9.6) package in R to fit a 3-d surface over values in the garden. We then took the residuals from this surface and used them as our phenotypic estimates.

Using our spatially-corrected phenotypic estimates, we parsed genetic variation from the total phenotypic variation measured across replicates. The model followed the formulation:

$$Y_{ig} = \alpha_g + \epsilon_{ig} \qquad \text{Eqn 3}$$

where α is the random effect of each g genotype and ε is the residual error of the i^{th} individual of genotype g. In the case of our common garden design, the residual error ε represents the deviation of replicate i from α_g, where α_g is the model fit intercept for genotype g; and Y_{ig} is the phenotypic value measured for replicate i of genotype g. The α_g estimates for each trait represents the genetic contribution to phenotypic variation and is roughly equivalent to the average of the replicates by genotype. When we refer to genetic variation in the article, we are referring to these α_g

estimates. For the genotypes that are missing a complete set of three replicates due to coppicing, variation estimates will be pulled toward the grand mean of the model as they have lower power.

To run the model, we used a Bayesian hierarchical model framework, via the RSTAN v.2.18.2 package. All growth data was normally distributed and modeled as such. Nonstructural carbohydrates data were right-skewed and thus modeled as a gamma distribution with log-link (see Blumstein *et al.*, 2020, for further details). The random effect of each genotype (α_g) of Eqn 1 was estimated using 6000 random draws from the posterior distribution of the model. We then used the mean value of the draws as our parameter estimate for each trait and genotype. These estimates for the genetic variation in each trait are also known as the best linear unbiased predictions (BLUPs).

Heritability was calculated using the variation parsed from Eqn 1, following the equation:

$$H^2 = \frac{\sigma^2_{\mathrm{Genotype}}}{\left(\sigma^2_{\mathrm{Genotype}} + \sigma^2_{\mathrm{Microenvironment}}\right)} \qquad \text{Eqn 4}$$

where $\sigma^2_{\mathrm{Genotype}}$ is the variation due to a tree's genotype and $\sigma^2_{\mathrm{Microenvironment}}$ is the residual variation of the model, presumed to be the microenvironmental and measurement error differences among replicates within a genotype. In the context of our study, $\sigma^2_{\mathrm{Genotype}}$ is the variance among α_g values.

Detecting NSC storage trade-offs To compare across our many traits visually and in a PCA, we *z*-scored all genetic trait variation using the 'scale' function in R (center and scale set to true). We then fit standardized major axis (SMA) slopes and confidence intervals (CIs) (mode of type II linear regression) to pairwise combinations of our data, using the package LMODEL2 v.1.7-3 in R (Fig. S5; Wright *et al.*, 2004; Warton *et al.*, 2006). In type II regressions, both X and Y are dependent variables, thus both are measured and presumed to include some error and the result tells us how they co-vary, rather than how Y depends on X. In the case of an SMA type II regression, it is not possible to calculate a P-value, but we can report the 95% CIs around our slope parameter (Warton *et al.*, 2006). If they do not cross zero, this is a good indication that the relationship is true.

We then used the VEGAN 2.5-5 package in R to examine all traits together in a PCA (Figs 2, S4). While we had growing season length and growth measurements for almost every genotype in the garden, we had fewer NSC measures due to the difficult nature and expense of extracting NSCs. Thus, in total we had 104 genotypes that had observations for all traits examined. These spanned the entire sampling range with at least four genotypes and their corresponding replicates from each population (Table S3).

To demonstrate whether trade-offs arise once variation in carbon supply is accounted for, we regressed genetic variation in carbon supply proxies (scaled data) with sink data (unscaled) and compared the residuals of these models. To do so, we defined variation in carbon supply as the genotypic values of principal component one (PC1) from our previous analysis (see justification of PC1 as supply earlier). Finally, we compared the residuals of these models against each other via type II regression, standard major axis method, to understand how trait relationships changed once differences in productivity were removed (Warton *et al.*, 2006).

Testing for signatures of local adaptation We tested whether our trade-off axes exhibited signatures of local adaptation using $Q_{\mathrm{st}}/F_{\mathrm{st}}$ comparisons. To calculate Q_{st} values for our principal components (PCs), we determined the degree to which phenotypic variation could be explained by among-population differences vs within-population differences. If populations are locally adapted and a trait is differentially selected across an environmental gradient, then phenotypic variation should be large between populations and small within. To get between and within population variation, we again parsed spatially corrected phenotypic variation using a Bayesian hierarchical model in the package RSTANARM v.2.18.2. Like the model for heritability, the formula includes an extra random effect term for population (α_p):

$$Y_{igp} = \alpha_p + \alpha_{gp} + \epsilon_{igp} \qquad \text{Eqn 5}$$

We then used the variation due to population (α_p) and the variation due to genotype (α_{gp}) to calculate Q_{st}, or the degree of divergence in a trait's variation among populations vs within.

$$Q_{\mathrm{st}} = \sigma^2_{\mathrm{Population}} / \left(2\sigma^2_{\mathrm{Genotype}} + \sigma^2_{\mathrm{Population}}\right) \qquad \text{Eqn 6}$$

We use the equation from Whitlock & Gilbert (2012), which includes a 2 in the denominator due to the additional nested variable in the model. If variation among populations is higher than variation within populations, our results are consistent with directional selection across populations. We can further compare whether the value of Q_{st} is representative of selection or random processes by comparing it to F_{st}, or the variation at neutral genomic loci between and within populations. Traits varying due to selection across the environment are expected to have Q_{st} exceeding neutral variation in F_{st}. All genotypes sampled in the garden had full genome sequences at 18× median depth available (Evans *et al.*, 2014). The F_{st} estimates were taken from previous work (Evans *et al.*, 2014), where F_{st} was calculated in 1-kb windows using all genotypes available in the mapping population as $(\pi_T - \pi_S)/\pi_T$; where π_T is single nucleotide polymorphism (SNP) diversity across all individuals and π_S is weighted within-population SNP diversity (Evans *et al.*, 2014).

Inter-specific variation

We updated an existing database of seasonal studies of NSC storage concentrations (Martínez-Vilalta *et al.*, 2016) to include additional studies from May 2012 up to March 2020. We followed the selection protocols outlined in Martínez-Vilalta *et al.* (2016), which restricted the inclusion of studies to (1) those that are run for 4 months or more, (2) the same individuals or populations were measured as least three times during the duration of the study, (3) plants were mature (no seedlings or saplings included), and (4) tissue sampled was not bark, phloem or cortex. We also excluded any experimental groups, natural treatments (e.g. trees under drought conditions), or agricultural studies, but kept corresponding controls where present. Finally, we only examined samples taken from stem cores or coarse roots, for comparison to the intra-specific patterns in *Populus trichocarpa* and because NSC concentrations vary substantially across organs (e.g. Martínez-Vilalta *et al.*, 2016; Furze *et al.*, 2019). We were interested in total storage (see Introduction section), thus we examined only total NSC storage (not sugars or starch separately). At the end of this process, we had 71 species from 46 studies that met all our criteria; representing Boreal to Tropical Biomes.

Once our data were filtered by our criteria, we summarized the data to species by biome, meaning that if the same species was sampled growing in multiple different biomes, we separated estimates by biome. Inter-specific variation often reflects, in part, the environment that species are grown, and we wanted to capture some of that variation to help us better understand potential relationships between traits. Within each study in the database ($N_{\text{study}} = 46$, representing 2524 observations), we first averaged across years, so that we had one estimate for each month of study (i.e. average across interannual variation). Note stems and roots were summarized separately ($N_{\text{studies\_with\_stems}} = 41$, $N_{\text{studies\_with\_roots}} = 21$ (some studies had both)). We then took the maximum NSC value for each species within each study. We chose to take the maximum rather than the mean as storage is well known to be dynamic throughout the year (e.g. Martínez-Vilalta *et al.*, 2016; Furze *et al.*, 2019), hitting a high point each year when stores are recharged. Thus, the maximum most likely represents the high point of storage for a species and helps to resolve the fact that each study samples at different times and a different number of times throughout the year, making comparisons of averages challenging. Finally, we took the average maximal NSC value for species (averaging across studies) by biome. For example, if we had two studies of *Pinus sylvestris* in temperate forests and two in boreal, we would have four observations of maximal NSCs, then average the two temperate observations and two boreal observations respectively, finishing with two final observations of maximum NSCs for *Pinus sylvestris* in a temperate biome and maximum NSCs in a boreal biome. We then calculated the annual basal area increment (radial growth rate) for each study (Eqn 2).

Finally, we determined the wood density of each species using the TRY global plant trait database (Trait Name: 'Stem specific density (SSD) or wood density (stem dry mass per stem fresh volume'), averaging by species if multiple entries existed (Kattge *et al.*, 2020)). The data were previously standardized to g cm^{-3}.

Trait–trait comparisons were performed via type II linear regressions, the standardized major axis method, and were performed in R using the LMODEL2 v.1.7-3 package (Wright *et al.*, 2004; Warton *et al.*, 2006; Legendre, 2018). Nonstructural carbohydrate concentration data had a large range in observations, thus we used the natural log of NSC (base *e*) for the inter-specific trait–trait comparisons described earlier.

Results

Intra-specific trade-offs

We quantified significant heritable genetic variation in carbon storage, growth, and traits underlying carbon supply in a common garden of black cottonwood (*Populus trichocarpa*) ($H^2 = 0.32$–0.79; Table S4). We found positive pairwise relationships between carbon storage, growth, growing season length, and LAI; revealing individuals with a longer growing season and larger canopy growth and storage (Fig. S5).

To further test whether this trend was due to higher variation in supply (Fig. 1ii–iv), we used a PCA to collapse the major explanatory axes of trait variation (Fig. 2). PC1 explains 53% of the variation in our data and reflects a gradient of carbon supply; highly productive genotypes with large canopies and long growing seasons from populations in southern latitudes to less productive genotypes sourced from northern latitudes (Fig. 2a,c). PC1 also significantly correlates with variation in modeled carbon supply for these genotypes and latitude, indicating that it accurately captures variation in supply among genotypes in the garden (Fig. S6). In contrast, PC2, which is formed from the residuals of PC1 and accounts for 23% of the total variation in our data, reflects a trade-off between growth and storage, indicating that individuals with high stores have low growth, and vice versa (Fig. 2a,d). When we control for variation in carbon supply, approximated by PC1, in our genetic estimates of growth and storage, we found a significant negative relationship between residual genetic variation in carbon storage traits and growth as predicted by an active allocation trade-off (Fig. 3).

Finally, we found extensive variation in allocation strategy within each population of black cottonwood, but little divergence between populations, lending little support to hypotheses of local adaptation in this trade-off across populations ($Q_{\text{st}} = 0.11 \pm 0.06$, $F_{\text{st}} = 0.17 \pm 0.09$, Fig. S7).

Inter-specific trade-offs

We found negative relationships between annual growth rate and the log of carbon storage across species (type II linear regression, SMA method; Fig. 4a,b; Tables S5,S6). Data inputs met test assumptions of normality (Fig. S8). We document negative relationships between growth (BAI) and storage (logNSC concentrations) across species, for both roots (*m*(95% CI): −0.11 (−0.18 to −0.07), R^2: 0.27) and stems (*m*(95% CI): −0.1 (−0.15 to −0.07), R^2: 0.13). We found positive relationships between log storage and wood density (Fig. 4c,d) for both roots (*m*(95% CI): 5.5 (3.6–78.4), R^2: 0.17) and stems (*m*(95% CI): 5.7 (4.3–7.3), R^2: 0.14).

Discussion

Growth–storage trade-offs exist within species

We first established that variation in carbon sinks (including storage) is heritable. As heritability is a relative metric within a

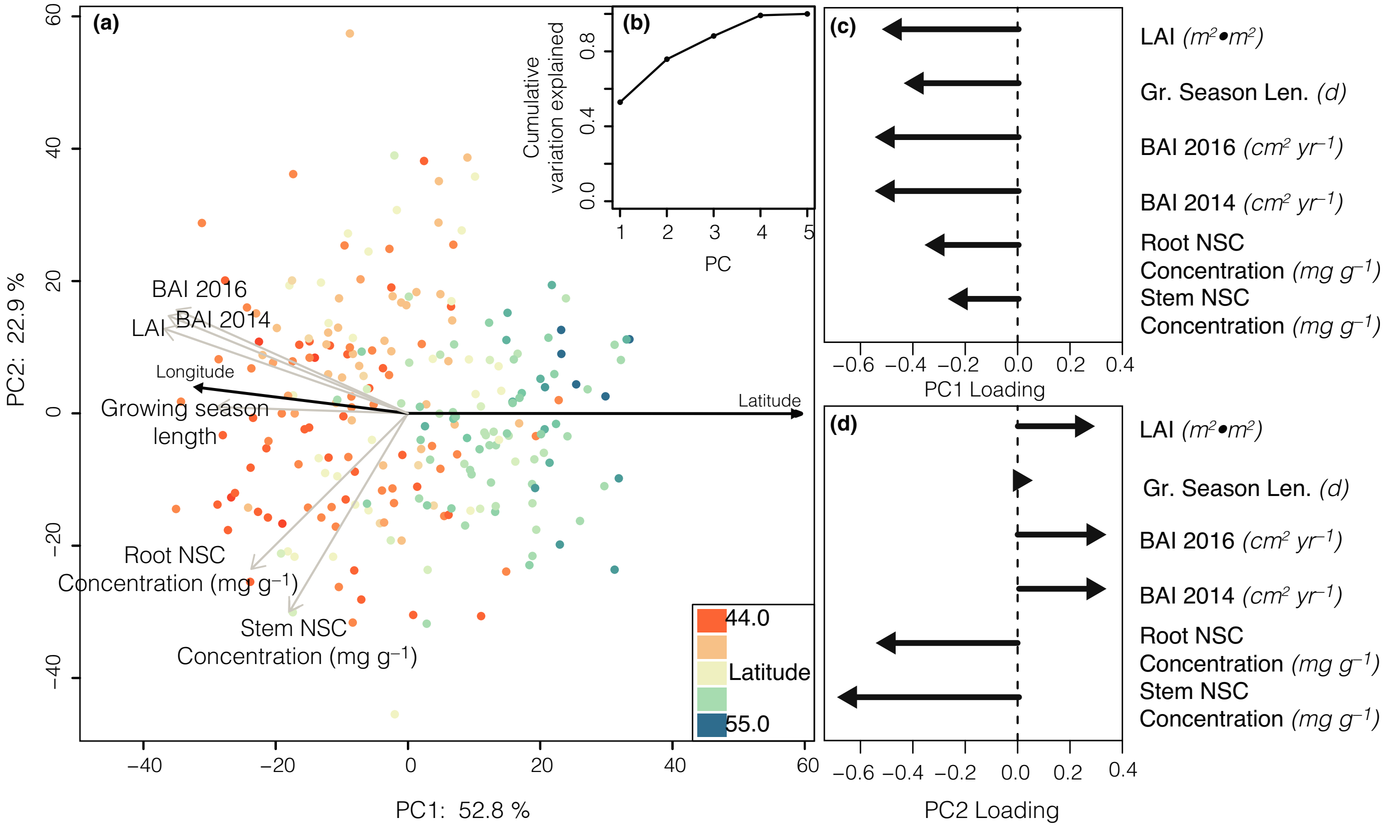

Fig. 2 A principal components analysis (PCA) of supply, growth, and storage traits measured in *Populus trichocarpa* (n = 104 genotypes with all measurements). (a) The PCA results, where each dot is a genotype in the common garden, colored by the latitude of its provenance, and arrows indicate increasing values of each trait. (b) The cumulative variation explained by each principal component (PC). Each PC is then decomposed in (c) and (d), further highlighting synergies and trade-offs amongst traits on each PC axis. Latitude and longitude of provenance is superimposed using env fit in the VEGAN package in R. Note, all data were z-scored for the analysis, the units in (c) and (d) reflect the original units of measurements.

common garden, it is important to note that our measures were similar to those for growth, phenology (Evans *et al.*, 2014; McKown *et al.*, 2014), and carbon storage (branches, Blumstein & Hopkins, 2021) in studies done in different environments, using the same genotypes. Our finding of heritability in all allocation traits is crucial because genetic variation is passed onto offspring and can evolve in response to selection to generate allocation trade-offs (Reich *et al.*, 2003; Donovan *et al.*, 2011). To next demonstrate that carbon storage is actively allocated by trees, we searched for a negative relationship, or trade-off, between the genetic variation we quantified in storage and in growth. However, when considering variation in carbon sinks, we must also consider variation in carbon supply. For highly productive species like black cottonwood, variation in carbon supply can swamp the signal of a trade-off in carbon sinks (Fig. 1) (van Noordwijk & de Jong, 1986; Osnas *et al.*, 2018; Agrawal, 2020). When we controlled for variation in carbon supply as approximated by PC1 of our traits, we found that growth and storage flipped from a positive association to a negative one (Fig. 3). We are confident that PC1 accurately captures variation in supply, as it is tightly correlated with modeled carbon supply using measurements taken on the same genotypes, growing season length, and latitude, which are all known proxies of annual photosynthetic uptake (Fig. S6). The trade-off we uncovered has substantial amounts of underlying heritable variation. For each unit of growth that genotypes invest in, they forgo 1.2 to 1.7 units of storage; or for each unit of storage that genotypes invest in, they forgo 0.6–0.8 units of growth (Fig. 3). Thus, a small increase in storage investment may result in a large cost to competitive growth over the lifetime of the tree.

Interestingly, our estimate of variation in carbon supply, as represented by PC1, includes high loadings for growth and storage too. Statistically this makes sense, as we initially found a positive relationship between growth, storage, and supply before comparing the residuals of growth/storage and supply. This could also have biological meaning. While some plants invest a relatively higher proportion of carbon in storage than growth, they may still have a higher overall supply than other plants. Thus, these plants may have an additional passive reservoir of stores and relatively higher growth rates as a result of their excess supply. Indeed, the idea that plants can passively accumulate stores when supply is in excess, while simultaneously maintaining a reserve pool, is generally accepted (Chapin *et al.*, 1990; Dietze *et al.*, 2014).

While we considered growth and storage in this study to establish storage as a priority sink, more attention needs to now be given to how storage varies with other sinks such as defense, reproduction, and metabolism. Defense has been demonstrated

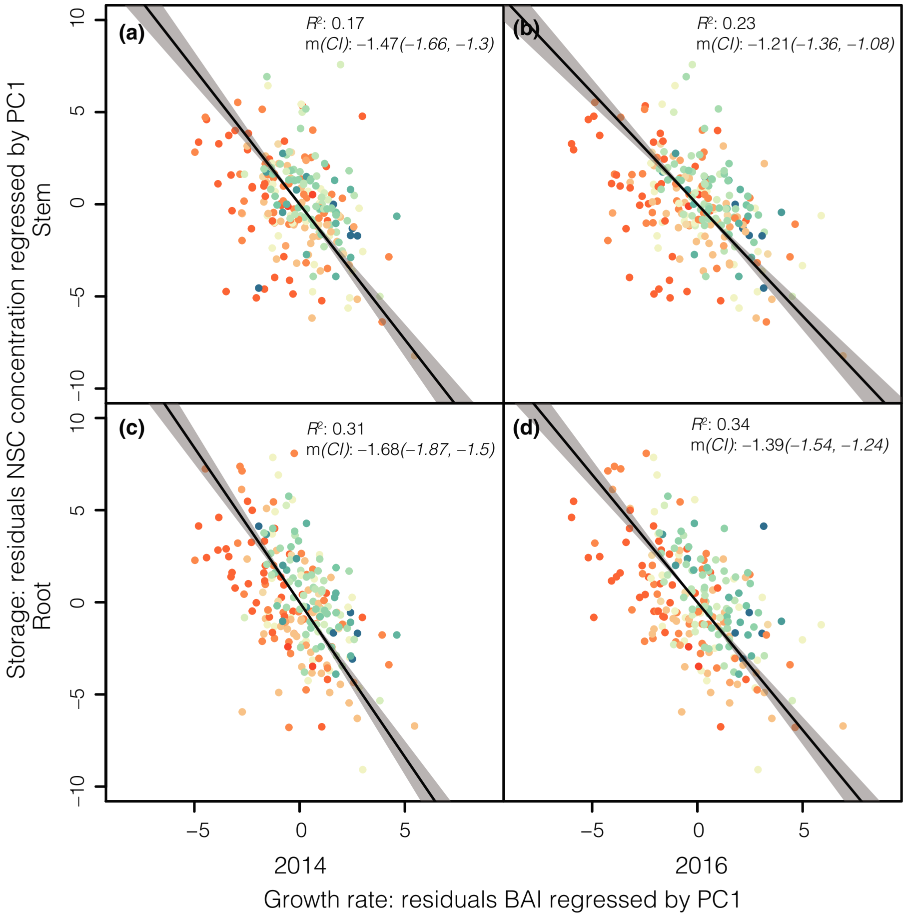

Fig. 3 Intra-specific relationships between storage and growth. Plots show comparisons of stem nonstructural carbohydrate (NSC) storage vs (a) growth rate (BAI) as measured in 2014 and (b) 2016; as well as root NSC storage vs (c) 2014 and (d) 2016 growth rates. Residuals from linear regressions (type II) between genetic variation in growth and storage (unscaled data) and principal component one (PC1) (scaled), which represents differences in carbon supply between individuals (Supporting Information Fig. S9). With variation due to PC1 removed, the pairwise relationships between carbon sink traits flip from positive (Fig. S5), to negative relationships, indicating a trade-off hidden by high variation in tree carbon supply acquisition. Each point represents a genotype in the graph (n = 104 genotypes), colored by provenance, where warm colors are southern latitudes to cool colors in northern latitudes. Standardized major axis slopes and 95% confidence intervals (CIs) are given in the upper right, along with the coefficients of determination (R^2).

to sometimes trade-off with growth within species (Herms & Mattson, 1992; Agrawal, 2020). Thus, if growth and defense covary, so may storage and defense. In addition, stored carbohydrates may provide the substrate or energy for the manufacturing of some defense compounds (Hartmann & Trumbore, 2016), further complicating the relationships between the three. The relationship between reproduction and storage is also critical to understand, as selection acts on genetic variation to increase fitness, and fitness is defined as reproductive success. Understanding how storage relates to reproduction may illuminate how storage has been shaped by evolution and thus what drives variation in storage across the landscape and through time. Finally, we examined only secondary growth in this study (diameter expansion). Additional consideration to primary growth could be informative in future studies, although DBH is known to correlated well with biomass (Jenkins *et al.*, 2003).

Our finding here of active accumulation is mechanism-agnostic, meaning increased storage could be achieved by an upregulation of carbohydrate accumulation in cells or a downregulation of growth or other carbon sinks, sometimes referred to as 'quasi-active storage' (Sala *et al.*, 2012; Dietze *et al.*, 2014). In either case, there is evidence that having higher stores may adaptively benefit individuals under certain environmental conditions (Poorter & Kitajima, 2007; O'Brien *et al.*, 2014). Thus, we might expect that higher stores would confer a fitness benefit under certain environmental conditions, which could lead to patterns of local adaptation (i.e. high stores are selected for in some environments). However, we found little evidence for local adaptation in trade-off strategies; instead we find high amounts of within population variation. This variation may be critical in enabling trees, which are long-lived, sessile organisms, to persist in temporally or microenvironmentally heterogenous environments (de la Mata *et al.*, 2017). Given that evolutionary response to selection requires genetic variation, the abundant genetic variation we observe within populations of black cottonwood in a growth–storage trade-off is promising for evolutionary responses to future environmental change.

In sum, our results demonstrate a clear genetic trade-off between growth and storage in *Populus trichocarpa*, indicating that storage can actively compete for carbon. However, we did not find evidence of local adaptation in this trade-off at present. Studies of what conditions benefit high storers vs fast-growers are needed to further understand how variation in this trade-off is maintained across space and time. Furthermore, the establishment of carbon storage as a competitive sink necessitates new research into the relationships between carbon storage and other carbon sinks, such as disease resistance, metabolism, and reproduction (Huang *et al.*, 2019).

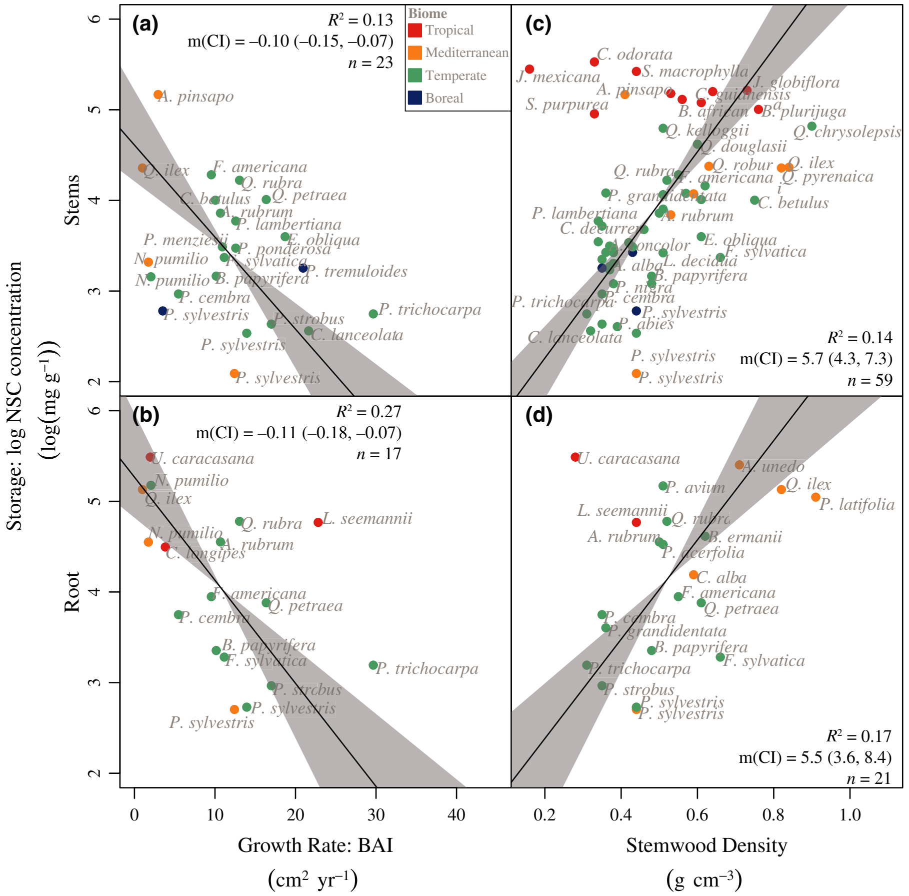

Fig. 4 Inter-specific relationships between storage and growth. Carbon storage in (a) stems and (b) roots and growth rate and (c, d) wood density respectively. Points represent the maximal nonstructural carbohydrate (NSC) measurements (average of top 25th quantile) for each species/biome combination. For example, if there are three studies of *Pinus sylvestris* conducted in temperate forests and three conducted in Mediterranean forests, we would average the top quantile of values for each biome and plot the two resultant values (*Pinus sylvestris*: Mediterranean, *Pinus sylvestris* Temperate). Growth rates are reported in the NSC database for each study where it is available, density measurements fare from the try database (Kattge *et al*., 2020). Points are colored by biome. Model fits are linear regressions (type II), standard major axis (SMA) method. The model slopes and 95% confidence intervals (CIs), coefficients of determination (R^2), and number of observations in the database for each respectively are in text.

Inter-specific trade-offs between storage and growth

Our findings in black cottonwood establish that a growth–storage trade-off exists and can evolve. To further determine if our finding of active allocation of carbon represents a fundamental aspect of plant life-history strategy beyond black cottonwood, we analyzed negative relationships between growth and storage across tree species. We found negative relationships between annual growth rate and carbon storage across species utilizing a global database of carbon storage measurements, supporting a trade-off between the two (Martínez-Vilalta *et al.*, 2016; Fig. 4). For each additional 1 cm^2 yr^{-1} of area growth, trees reduce their storage by 9.5% in stems and 10.4% in roots (Fig. 4). The trade-off between growth and storage follows the established conservative-to-competitive spectrum, with conservative slow-growing species storing more and competitive fast-growing species storing less (Wright *et al.*, 2004). This conservative-competitive trade-off is further underscored by the positive relationship between log storage and wood density (Fig. 4c,d). For each additional 0.1 g cm^{-3} in wood density, carbon storage concurrently increased by 76.8% in stems and 73.3% in roots respectively. These relationships suggest that an investment in high wood density may represent an evolved conservative, 'safe' growth-strategy at the expense of efficient, fast growth (Maherali *et al.*, 2004).

We deliberately chose to examine total NSCs as our metric for storage rather than starch alone, which is strictly the storage molecule (Hartmann & Trumbore, 2016). However, we sampled early in the dormant season for our intra-specific work to ensure we were capturing the maximum NSCs set aside. Nonstructural carbohydrates play a variety of roles in parenchyma cells of plants, including substrate for respiration and osmoregulation (Sapes & Sala, 2021). Thus, variation we see may in part be the result of an active allocation to metabolic needs. Intuitively we would expect higher allocation to metabolic respiration in trees with higher growth rates and a higher proportion of metabolically active biomass (e.g. Reich *et al.*, 2006). If so, variation due to active allocation to metabolic demands would weaken, rather than strengthen the trade-offs we uncovered. Finally, sugars are converted in and out of starch on seasonal, daily and even hourly timescales in all tissues, whereas total NSCs are more stable (e.g. Tixier *et al.*, 2018). This back-and-forth between sugar and starch has been linked to the sensitivity of starch hydrolysis enzymes to environment (Thalmann & Santelia, 2017) and suggests that examining starch alone may be misleading.

Notably here we did not control for variation in carbon supply across species, but still saw a trade-off. This suggests that variation in carbon supply is smaller than variation in carbon sink allocation across this range of species (Fig. 1i). The relative amount of supply vs sink variation differing within vs across species may, in part, explain why relationships between traits can break down or reverse when examined across different scales. For example, a recent study highlighted that the relationship between

N_{mass} and other leaf economics spectrum (LES) traits is maintained across taxonomic scales, while the relationship between leaf lifespan and leaf mass per area (LMA) is negative within species, but positive at higher levels of organization (Anderegg *et al.*, 2018). This is likely because leaf lifespan, when examined across species, follows a gradient from deciduous angiosperm species with cheap leaves to conifers with long-lived needles that they invest a lot of resources in. However, when examined within species, leaf lifespan is more a reflection of growing season length, or proximally carbon supply, in deciduous species. Relatedly, our lack of samples from tropical biomes may have also allowed us to see a trade-off without controlling for supply. Tropical species are highly productive and thus if more were included, supply variation may have exceeded sink variation across species. This pattern can be seen in Fig. 4, where tropical species move toward high growth/high storage or high storage/low density quadrants respectively. In sum, while carbon supply can confound patterns, it may only be a problem at smaller taxonomic scales of analysis or when larger ranges of productivity are considered.

While our database is the most complete to date and offers the first evidence of an inter-specific growth–storage trade-off, the data are still imperfect and thus come with a few caveats and calls for future study. First, we only examined bulk NSCs (glucose, fructose, sucrose, and starch) in stem and coarse root storage. Other organs, such as branches, and compounds, like sugar alcohols and lipids, may also contribute to storage budgets. Although organs tend to be correlated in their storage amounts (Hartmann & Trumbore, 2016; Martínez-Vilalta *et al.*, 2016; Furze *et al.*, 2019) and bulk NSCs in general captures *c.* 85–95% of carbon reserves (Hoch *et al.*, 2003), future studies should examine the extent to which other compounds and organs contribute to whole-tree storage. Second, cross-species trade-offs do not inherently represent genotypic differences, but can reflect plastic response to environment, or essentially the geographic distribution of species (Reich *et al.*, 2003; Fig. 1). However, our finding of a heritable trade-off combined with prior evidence that LES traits are likely shaped by local adaptive evolution give us higher confidence that the across-species trade-off we have uncovered is also reflective of an evolved response (Donovan *et al.*, 2011). Regardless, further study is necessary to test if inherited high stores are adaptive in certain environments, and demonstrate that storage gradients are the result of adaptive evolution. Third, as with the intra-specific analysis, we did not consider other carbon sinks. Variation in how other carbon sinks relate to storage may in part explain the low R^2 of our models and deserves further study (Fig. 4a,b). Finally, our criteria cut-off was for studies that took measurements over 4 months or more, indicating that we may have not captured the seasonal maxima in all cases. However, we summarized to species to increase the number of measurements under consideration and further examined our data for biases. Two-thirds of our studies were conducted over the course of a year or more, and of the remaining one-third that were shorter, all had measurements somewhere in September–February, the seasonal maxima for many biomes (Martínez-Vilalta *et al.*, 2016), suggesting that we likely captured the maxima or near maxima for the species under consideration.

Conclusion

Our findings establish storage for the first time as a priority allocation sink. The universality of the growth–storage trade-off across levels of biological organization has been difficult to demonstrate, in part due to the obfuscation of patterns by other sources of variation as discussed earlier (van Noordwijk & de Jong, 1986; Osnas *et al.*, 2018; Agrawal, 2020). The relationship between storage and growth necessitates a rethinking of how we structure and parameterize plant productivity models. At present, most models treat storage as a black-box that represents the difference between carbon supply and the demands of growth, respiration, and reproduction (Le Roux *et al.*, 2001; Dietze *et al.*, 2014). While some advancements have been made to split storage into ‘fast’ and ‘slow’ carbon pools, reflecting the scales at which they are used, no models have as yet allowed NSC pools to compete directly with other sinks for resources (Le Roux *et al.*, 2001; Richardson *et al.*, 2013; Dietze *et al.*, 2014). This result has far reaching-implications, as understanding carbon allocation trade-offs are critical for our ability to predict plant success under current and future environments (Chapin *et al.*, 1990; Herms & Mattson, 1992; Hartmann *et al.*, 2020; Jones *et al.*, 2020).

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Author contributions

Conceptualization: MB, RH; methodology: MB, AS; funding acquisition: MB, DJW, AS; visualization: MB, RH, NMH, AS; writing – original draft: MB, RH; writing – review and editing: MB, NMH, DJW, RH, AS.

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Data availability

All data are available in the main text or the supplementary materials. *Populus trichocarpa* common garden data and relevant code are housed on github at https://github.com/blumsteinm/Ptri_Clatskanie.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Map of genotype provenances and common garden setup.

Fig. S2 Probability of budburst based on genotype and growing degree days.

Fig. S3 Bud set and leaf senescence stage relationship.

Fig. S4 Principal component analysis utilizing budset and bud flush in lieu of calculated growing season length.

Fig. S5 A pairwise comparison of genetic variation in all *Populus trichocarpa* traits measured.

Fig. S6 The relationship between modeled carbon assimilation and provenance, growing season length, and principal component one from Fig. 2.

Fig. S7 Q_{st} vs F_{st} of the first two principal components in Fig. 2.

Fig. S8 Distribution of inter-specific data inputs.

Fig. S9 Genetic variation in 2014 and 2016 BAI and stem and root nonstructural carbohydrate concentrations vs principal component one.

Methods S1 Calculating growing season length.

Table S1 Bud flush scoring rubric.

Table S2 Bud set scoring rubric.

Table S3 Population sampling information.

Table S4 *Populus trichocarpa* trait averages and heritabilities.

Table S5 Type II regression outputs from Fig. 4.

Table S6 Inter-specific nonstructural carbohydrate data by species and organ.