

Ecologically driven selection of nonstructural carbohydrate storage in oak trees

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Summary

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- Leaf habit is a major axis of plant diversity that has consequences for carbon balance since the leaf is the primary site of photosynthesis. Nonstructural carbohydrates (NSCs) produced by photosynthesis can be allocated to storage and serve as a resiliency mechanism to future abiotic and biotic stress. However, how leaf habit affects NSC storage in an evolutionary context has not been shown.
- Using a comparative physiological framework and an analysis of evolutionary model fitting, we examined if variation in NSC storage is explained by leaf habit. We measured sugar and starch concentrations in 51 oak species (*Quercus* spp.) growing in a common garden and representing multiple evolutions of three different leaf habits (deciduous, brevideciduous and evergreen).
- The best fitting evolutionary models indicated that deciduous oak species are evolving towards higher NSC concentrations than their brevideciduous and evergreen relatives. Notably, this was observed for starch (the primary storage molecule) in the stem (a long-term C storage organ).
- Overall, our work provides insight into the evolutionary drivers of NSC storage and suggests that a deciduous strategy may confer an advantage against stress associated with a changing world. Future work should examine additional clades to further corroborate this idea.

Introduction

Plants exhibit remarkable diversity in traits, from morphological to phenological and from developmental to physiological. One major axis of diversity involves leaves and their persistence in the face of changing environmental conditions throughout the year. In temperate trees, co-existing deciduous and evergreen leaf habits represent two endpoints of a spectrum that illustrate distinct differences in resource allocation and tolerance to seasonal temperature fluctuations (Chabot & Hicks, 1982; Givnish, 2002; Van Ommen Kloeke *et al.*, 2012). Leaf habits range from evergreen to deciduous, with intermediate strategies such as brevideciduous. While deciduous species lack leaves for a large portion of the year, evergreen species maintain leaves year-round, which extends the number of days that photosynthesis can occur, but also incurs significant respiratory and construction costs (Kikuzawa, 1991). Because leaves are the main site of photosynthesis, leaf habit should be closely connected to the tree's carbon (C) balance, but the influence of leaf habit on the allocation and storage of nonstructural carbohydrates (NSC) is not well understood.

NSCs, mainly soluble sugars and insoluble starch, are used to support proper tree function (Hartmann & Trumbore, 2016; Hartmann *et al.*, 2020). In addition to serving as substrates for growth and respiration, NSCs play a multitude of roles in transport, metabolism, osmoregulation and defence (Savage *et al.*, 2016; Huang *et al.*, 2019, 2020; Gersony *et al.*, 2020). Importantly, NSCs are allocated throughout trees to different organs and can be stored for later use in parenchyma cells, with starch functioning as the longer-term storage molecule and contributing to the formation of reserves. Stored reserves act as a buffer for springtime leaf out and wintertime respiration as well as during drought and other climate extremes. NSC storage at the expense of other functions, such as growth, is a conservative strategy that may ensure the survival of long-lived trees (Sala *et al.*, 2012; Wiley & Helliker, 2012). Several studies have demonstrated that, when a tree's ability to make new NSCs is impaired by abiotic and biotic stress, stored NSCs provide resilience (McDowell *et al.*, 2008; Carbone *et al.*, 2013; Hartmann *et al.*, 2013; Sevanto *et al.*, 2014; Piper & Paula, 2020).

Beyond the tree level, NSCs play an important role in ecosystem processes such as nutrient cycling, for example, when they

are exchanged with symbionts involved in nutrient acquisition and defence (Bais *et al.*, 2006; Smith & Smith, 2011; Cheeke *et al.*, 2017). Furthermore, NSCs may also explain patterns of ecosystem C cycling; lags between C uptake and structural growth may reflect NSC storage in a given year and its use for growth in subsequent years (Gough *et al.*, 2009; Richardson *et al.*, 2013). Therefore, identifying the drivers of NSC storage is not only key for understanding C dynamics in trees, but also has broader implications for ecosystem function and the prediction of forest responses to global change (Merganičová *et al.*, 2019).

The required C investment into storage is expected to differ between trees based on traits such as leaf habit. It has been suggested that deciduous species have larger C reserves (i.e. NSC concentrations/storage) than evergreen species due to their reliance on stored NSCs for building a new canopy and supporting growth in the spring (Kozłowski & Pallardy, 1996; Vanderklein & Reich, 1999). However, this pattern has primarily emerged from comparisons between deciduous angiosperms and evergreen conifers (Shi *et al.*, 2008; Richardson *et al.*, 2013, 2015; Furze *et al.*, 2018a), which intertwine relatedness and leaf habit, and therefore lack the comparative framework necessary to test if leaf habit is a driver of NSC storage. Furthermore, since variation in NSCs is correlated with environmental gradients in temperature, precipitation, and elevation in some tree species (Blumstein *et al.*, 2020; Godfrey *et al.*, 2020; Hao *et al.*, 2021), it is essential to compare the physiological responses in different leaf habits under the same environmental conditions.

Oaks (genus *Quercus*) are an important clade for examining the establishment and persistence of long-lived species facing variable environments. By *c.* 45 Ma, two major oak lineages colonised North America and diversified southward, radiating sympatrically and in parallel into an array of different ecological habitats (Cavender-Bares, 2019; Kremer & Hipp, 2020). Transitions between leaf habits have occurred multiple times in the oaks, and the plasticity and evolvability of associated leaf traits (i.e. specific leaf area, leaf abscission timing) may have been important for the adaptation and persistence of oaks in new and variable environments (Cavender-Bares, 2019). Deciduousness is thought to have evolved as a strategy to persist during environmental challenges such as drought and freezing (Ramírez-Valiente & Cavender-Bares, 2017; Hipp *et al.*, 2018). Alongside such leaf traits which likely gave the oaks fitness advantages, their ability to store NSCs may have contributed to their survival in variable environments and eventual ecological dominance in northern temperate forests (Cavender-Bares, 2016).

To examine the influence of leaf habit on NSC storage, we measured sugar and starch concentrations in the leaves and stems of 51 oak species growing in a common garden setting in northern California (USA). These oak species differed in leaf habit, with deciduous, brevideciduous and evergreen strategies. By combining NSC measurements with an existing time-calibrated oak phylogeny and evolutionary model fitting, we used a comparative physiological framework to test whether variation in NSC storage is explained by a major axis of diversity: leaf habit. We hypothesised that the best fit model would indicate that leaf habit influences NSCs, such that NSC concentrations have evolved to be

higher in deciduous species compared with their brevideciduous and evergreen relatives.

Materials and Methods

Field collection

Samples were collected from 51 oak species at the Peter J. Shields Oak Grove of the University of California Davis Arboretum and Public Garden (Davis, CA, USA) in September 2019. This sampling occurred during the late growing season when most leaves were fully mature and seasonal leaf drop had not yet begun in the deciduous species. We sampled three trees of each oak species ($n = 27$), except in cases in which less than three trees were present ($n = 24$). In two cases, we sampled resprouted individuals from trees that had been previously cut down. Trees were planted in the collection from 1963 to 2016 and collection and sampling information for each individual tree are provided (Supporting Information Table S1).

A stem core and fully expanded leaves were collected from each tree. To obtain a core, we chiselled away the outermost stem tissues to expose the xylem and then used a 2-mm diameter micro-coring instrument (Trepfor, 15 mm length, University of Padua Italy; Rossi *et al.*, 2006). In line with the Arboretum's policy, samples were not collected from the root system, cores were collected if the stem diameter was $c. \geq 5$ cm, and a micro-coring instrument was used rather than a standard 4.3-mm increment borer to minimise potential damage and preserve the health and aesthetic value of the oaks in the public collection. The micro-coring instrument and chisel were sterilised between samples with 70% ethanol. The stem diameter was measured at breast height (DBH) when possible. For co-dominant stems resulting from bifurcations, the DBH is provided for the individual stem from which the core was obtained and the DBH below the split(s) is also often noted (Table S1). Mid-to-upper canopy leaves ($n = 3$) were accessed with a pole pruner and combined into a single sample for NSC analysis. Stem cores and leaves were kept on dry ice in the field and then stored at -80°C . Samples were moved directly from -80°C to the drying oven before grinding and NSC analysis.

NSC analysis

We measured sugar and starch concentrations (mg per g dry tissue) for leaves and stem cores using a publicly available and standardised protocol (Landhäusser *et al.*, 2018). Samples were placed in a drying oven at 100°C for 1 h to deactivate starch degrading enzymes, and then at 70°C for 2–3 d to completely dry the samples. Dried samples were ground (mesh 20; Thomas Scientific Wiley Mill, Swedesboro, NJ, USA; SPEX SamplePrep 5100 Mixer Mill, Metuchen, NJ, USA) and at least 10 mg of each sample was weighed out for sugar and starch analyses.

For sugar analysis, samples were extracted with hot ethanol, and the resulting bulk sugar extracts were subjected to a phenol-sulfuric acid reaction and measured at 490 nm on a spectrophotometer (Spectronic 20 Genesys, 4001/4; Spectronic Instruments,

Rochester, NY, USA). Following sugar extraction, the remaining tissue was sequentially digested for starch analysis with α -amylase and amyloglucosidase to produce glucose hydrolysate. Digested samples were subjected to a peroxidase-glucose oxidase colour reagent and measured at 525 nm on the spectrophotometer. We expressed both sugars and starch on the same scale (i.e. glucose equivalents) and summed them together to obtain total NSCs. Sugar, starch and total NSC concentrations for individual trees were averaged together by species for evolutionary model fitting. Furthermore, since tree size may be a confounding factor, the relationship between stem diameter (a proxy for tree size) and sugar, starch and total NSC concentrations of leaves and stems were independently assessed and the strength of the association was evaluated with phylogenetic linear regression under a lambda model using the R package `PHYLOLM` (Tung Ho & Ané, 2014). Sugar and starch concentration data are provided in Table S2.

Leaf habit assignment

Leaf habit was obtained for 156 oak species from recent work conducted on the major oak clades (Hipp *et al.*, 2018), which included a large majority of our study species as well as trees from the same study site, and was guided by previous coding and research (Nixon, 1997; de Beaulieu & Lamant, 2010; Schmerler *et al.*, 2012). We used additional sources to assign and cross-validate the leaf habit assignment for the 51 oak species in our study as needed. Leaf habit assignments are provided in Table S3. Oak species were categorised as deciduous (synchronous drop of leaves and their absence remains for a significant portion of the year, $n = 23$), brevideciduous (leaves generally present year-round or with a brief absence of leaves, $n = 11$), or evergreen (leaves present year-round with life span > 1 yr, $n = 17$) (Hipp *et al.*, 2018).

Evolutionary models

We used the time-calibrated phylogeny of the world's oaks from Hipp *et al.* (2020) in our analyses. This phylogeny was constructed using fossil data and restriction-site associated DNA sequencing (RAD-seq) for nearly 250 oak species.

To examine the evolution of leaf habit across the phylogeny, we pruned the phylogenetic tree to include 156 oak species, including our 51 study species, for which we had reliable leaf habit information. Bayesian stochastic character mapping (Huelsenbeck *et al.*, 2003) was used to estimate leaf habit at each node. We used the `make.simmap` function in the R package `PHYTOOLS` (Revell, 2012; R Core Team, 2014) to generate 1000 stochastically mapped trees using an all rates different model, which was shown to be the best fit for these data.

To investigate the influence of leaf habit on NSCs across the phylogeny, we used an evolutionary model fitting approach. We further pruned the 1000 stochastically mapped trees to only include oak species that were sampled in our study. While leaves were sampled from all 51 species in our study, stem cores were only sampled from 47 species because some stems were too small for coring as described above. Therefore, we pruned the 1000

stochastically mapped trees independently for the analysis of leaf and stem NSC data. For both leaves and stems, we assessed the fit of four evolutionary models to sugar, starch, and total NSC concentrations using the R package `OUWIE` (Beaulieu *et al.*, 2012; Beaulieu & O'Meara, 2019). We also assessed the fit of four evolutionary models to the proportion of starch in the stem (starch concentration/total NSC concentration).

The four evolutionary models fit were single-rate Brownian motion (BM1), multiple-rate Brownian motion (BMS), single-optimum Ornstein–Uhlenbeck (OU1), and multiple-optimum Ornstein–Uhlenbeck (OUM). With these evolutionary models we were able to determine how the evolution of NSC physiology and leaf habit are related, and whether and how leaf habit influences the primary trait optimum/optima (θ), the rate of stochastic fluctuations of trait evolution (σ^2), and the selective pull towards the primary trait optimum/optima (α) (Hansen, 1997; Butler & King, 2004).

If the BM1 or OU1 model is the best fit for the empirical data set, this suggests that NSCs evolve independently of leaf habit. In BM1, NSC evolution is modelled as a random walk and the expected differences between species are proportional to time (Felsenstein, 1985); under this model we expect a single rate of random fluctuations across species (one σ^2). In OU1, all species are modelled as evolving towards the same primary trait optimum (one θ) at the same rate (one σ^2) and selective pull (α). By contrast, if the BMS or OUM model has the best fit for the empirical data set, this suggests that leaf habit influences NSC evolution. BMS is modelled as having different rates for each leaf habit (three σ^2 s), which means that leaf habit influences the rate of NSC evolution, but NSC evolution within a leaf habit is modelled as a random walk and we would expect that NSC concentrations would not differ between leaf habits. OUM is modelled as evolution towards a different primary trait optimum for each leaf habit (three θ s), but the expected rate of evolution and selective pull for each leaf habit is the same (one σ^2 , one α).

Evolutionary models were fitted over 1000 stochastically mapped trees to account for uncertainty in ancestral leaf habit regimes. For `OUWIE` analyses, we confirmed that the eigenvalues of the Hessian were positive, which indicated that the parameter estimates were reliable (Beaulieu *et al.*, 2012). In cases in which eigenvalues were negative, the results for that tree and model were removed. We then compared the AICc (Akaike information criterion with a correction for small sample size) scores to determine the best fit model for each model set (Hurvich & Tsai, 1989; Burnham & Anderson, 2002). Additionally, we calculated the model-averaged parameter estimates and their uncertainty using the formulas described by Burnham & Anderson (2002) as well as code available on GitHub at github.com/andrew-hipp/PCM-2018. In brief, we calculated a weighted average for each model parameter using AICc weights and model-fitted parameters. Missing parameter estimates for the BM1 and BMS models were assigned a value of zero in the model-averaging process.

`OUWIE` uses complex OU models that can be incorrectly favoured over BM models if statistical power is low. To determine whether we could accurately fit the complex OU models with our data set, we simulated 1000 data sets for each of the

seven traits (sugar, starch, and total NSC concentrations in leaves and stems, and the proportion of starch in the stem) using the Generalised Hansen model simulator OUWie.sim in the R package OUWIE and the parameter estimates from the best fit model for each trait in the empirical data set (Beaulieu *et al.*, 2012; Boettiger *et al.*, 2012). The simulated data and our 1000 stochastically mapped trees were then fitted to all four models in OUWIE to determine whether the best fit model could be accurately recovered with our sample size. Furthermore, to determine whether we could reliably estimate model parameters (θ , σ^2 , α) and to determine the uncertainty associated with the parameter estimates, we performed 1000 simulations under the best fit model for each trait and calculated 95% confidence intervals for the parameters using the parametric bootstrapping function OUwie.boot in the R package OUWIE.

In addition, the contMap function in the R package PHYTOOLS was used to map the evolution of each trait onto the phylogeny. This estimates states at internal nodes using maximum likelihood and interpolates the states along each node using eqn (2) of Felsenstein (1985).

Phylogenetic ANOVA

We also used phylogenetic ANOVA to test for differences between leaf habits in each of the seven traits (sugar, starch, and total NSC concentrations in leaves and stems, and the proportion of starch in the stem). The phylogenetic ANOVAs and post hoc comparisons were performed for 1000 simulations using the function phylANOVA in the R package PHYTOOLS (Garland *et al.*, 1993; Revell, 2012) and we visualised differences in the traits between leaf habits using boxplots.

Results

We measured NSC concentrations in 51 oak species with different leaf habits that evolved independently multiple times (Fig. 1). Our 1000 stochastically mapped trees showed 105 transitions between leaf habits on average (min 63, max 146). Transitions from evergreen or deciduous to brevideciduous leaf habits (or vice versa) represent *c.* 95% of transitions on average in our stochastically mapped trees. Transitions between evergreen and deciduous leaf habits were much less common, with transitions from deciduous to evergreen not occurring and transitions from evergreen to deciduous representing *c.* 5% of transitions.

Differences in NSC concentrations between leaf habits were most pronounced for sugars in the leaves and starch in the stem (Fig. 2). In both cases, leaf sugar and stem starch concentrations were highest in deciduous species, while brevideciduous species tended to have intermediate concentrations and evergreen species had the lowest concentrations (Figs 2, 3a,e; leaf sugar $F = 15.38$, $P < 0.01$, deciduous-brevideciduous $P = 0.06$, deciduous-evergreen $P < 0.01$; stem starch $F = 11.30$, $P < 0.01$, deciduous-brevideciduous $P = 0.04$, deciduous-evergreen $P = 0.02$). By contrast, leaf starch and stem sugar did not differ among leaf habits (Fig. 3b,d; leaf starch $F = 0.49$, $P = 0.78$; stem sugar $F = 1.41$, $P = 0.52$). Sugar, starch and total NSC concentrations

in the leaves and stems were not significantly correlated with stem diameter (all $P \geq 0.07$) (Fig. S3). Additional plots of the evolution of leaf starch, leaf total NSC, stem sugar, and stem total NSC concentrations as well as the proportion of starch in the stem are provided in Figs S1 and S2.

Table 1 summarises the results of the four evolutionary model fits for leaf and stem NSC concentrations and Table 2 provides the model-averaged parameter estimates (θ , σ^2 , α) for each trait and each leaf habit across the four evolutionary models. Parameter estimates for all evolutionary models are reported in Tables S4 and S5. To discern which model fit best for a given trait, we compared differences in AICc scores among models and used the common threshold of a difference of 4 AICc units between models to determine if a model was better supported (lower AICc scores indicate better fits).

In the leaves, the multiple-optimum OUM model was strongly supported ($\Delta\text{AICc score} = 0$) for both sugar and total NSC concentrations (Table 1). This strong support for the OUM model implies that leaf habit influences leaf sugar and total NSC concentrations, yielding different concentration optima for each leaf habit (model-averaged parameter estimate $\theta_D > \theta_B > \theta_E$; Table 2). For leaf starch, the multiple-rate BMS model was the best fit ($\Delta\text{AICc score} = 0.35$) and the next best fit model was over 7 AICc units away (Table 1), indicating that leaf starch evolutionary optima did not differ between species with different leaf habits. We observed higher variability in deciduous and evergreen leaf starch compared with brevideciduous (Fig. 3b), which likely contributed towards the BMS model fitting the best because it models a different σ^2 for each leaf habit.

In the stem, single-optimum and multiple-optimum OU models were favoured. For stem sugar concentrations, it was difficult to discern between OU1 and OUM as the best fit model since the $\Delta\text{AICc score}$ between these two models was less than 4 (OU1 $\Delta\text{AICc score} = 0.23$; OUM $\Delta\text{AICc score} = 3.77$; Table 1). However, we consider the OU1 model the best fit as it was favoured in the majority of replications and it is the simpler model, suggesting that stem sugar concentrations did not differ between leaf habits (model-averaged parameter estimate $\theta_D = \theta_B = \theta_E$; Table 2). For stem total NSC, there was also mixed support for the OU1 ($\Delta\text{AICc score} = 2.27$) and OUM models ($\Delta\text{AICc score} = 0.67$) (Table 1). By contrast, for both stem starch concentration and the proportion of starch, the multiple-optimum OUM model was the best fit ($\Delta\text{AICc score} = 0$ for each) and the next best fit model was over 10 AICc units away for each (Table 1). Therefore, we find strong support that leaf habit influences starch storage in the stem.

The results of our simulations showed that our data set had enough statistical power to differentiate between the four evolutionary models. The best fit model for each trait was recovered as the best fit model during each simulation (Tables S6, S7). As in the OUWIE results from the empirical data set for stem total NSC and sugar, it was difficult to distinguish between OU1 and OUM as the best fit model following simulation (Table S7). Furthermore, there was variance in the simulated parameter estimates of θ , σ^2 , and α for each trait as indicated by 95% confidence intervals (Table S8). The simulated parameter

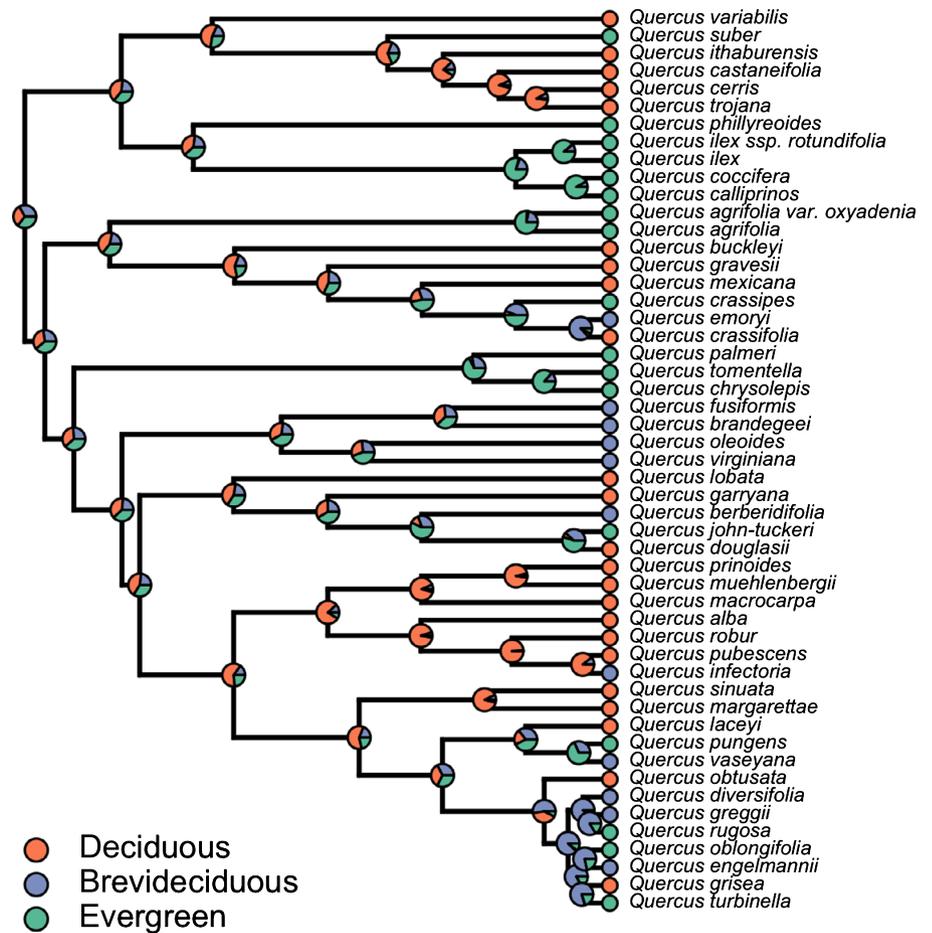


Fig. 1 Phylogeny of oak species sampled in this study (from the time-calibrated phylogeny of Hipp *et al.*, 2020) with summarised leaf habit estimates at the nodes based on 1000 stochastically mapped trees. Colour denotes the leaf habit for each species. Species and collection information is provided in Supporting Information Tables S1, S2. For *Quercus calliprinos* and *Q. infectoria*, we sampled *Q. coccifera* ssp. *calliprinos* and *Q. infectoria* ssp. *veneris*.

estimates aligned with the parameter estimates from the best fit model for each trait in the empirical data set, especially for the θ parameters (Figs S4, S5), however, the distributions of the σ^2 and α parameters indicated poor estimation power in some cases.

Discussion

Leaves play a key role in plant physiological processes as they produce the C currency of allocation, yet we have an incomplete picture of how major axes of diversity in leaves such as leaf habit may influence variation in NSC storage. Leaf habit has already been shown to influence other important aspects of tree physiology, including explaining variation in hydraulic properties such as vulnerability to freezing as well as leaf properties such as leaf mass per area and thickness between oak species (Cavender-Bares & Holbrook, 2001; Sancho-Knapik *et al.*, 2021). In this study, we presented leaf and stem NSC data for 51 oak species and identified evolutionary links between leaf habit and NSC concentrations. By fitting evolutionary models, our results showed that deciduous oak species are generally evolving towards higher NSC concentrations than their brevideciduous and evergreen relatives. For the majority of the traits measured, the best fit model was the multiple-optimum OUM model, indicating different optimal NSC concentrations for each leaf habit. However, there were some notable differences in the best fitting models between sugar

and starch, as well as leaves and stems, which highlights the unique role of each molecule and each organ in whole-plant C balance.

Previous work has demonstrated that NSC storage is primarily located in organs other than leaves (Würth *et al.*, 2005; Furze *et al.*, 2018a), and that the small pool of leaf NSCs, mostly as sugars, are quickly utilised locally or exported to other organs within a day (Blessing *et al.*, 2015; Furze *et al.*, 2019). Our results confirmed this by showing high leaf sugar concentrations and low leaf starch concentrations (Fig. 3a,b), and our model results indicated that leaf sugar concentrations, but not starch concentrations, differed between leaf habits based on the best fit multiple-optimum OUM model. We suggest that this is less a reflection of selection for leaf sugar storage and more a by-product of differences in photosynthetic rate among leaf habits.

In general, deciduous species tend to have higher photosynthetic rates during favourable conditions than evergreen species (Givnish, 2002; Wright *et al.*, 2004; Edwards *et al.*, 2017). This pattern has been documented in oaks in which gas exchange differed between oak species with different leaf habits and deciduous oak species had higher photosynthetic rates than evergreen oak species (Takashima *et al.*, 2004; Brüggemann *et al.*, 2009; Baldocchi *et al.*, 2010; Koller *et al.*, 2013). While we did not measure gas exchange on our study trees, previous work supports the data here showing higher leaf sugar concentrations in deciduous

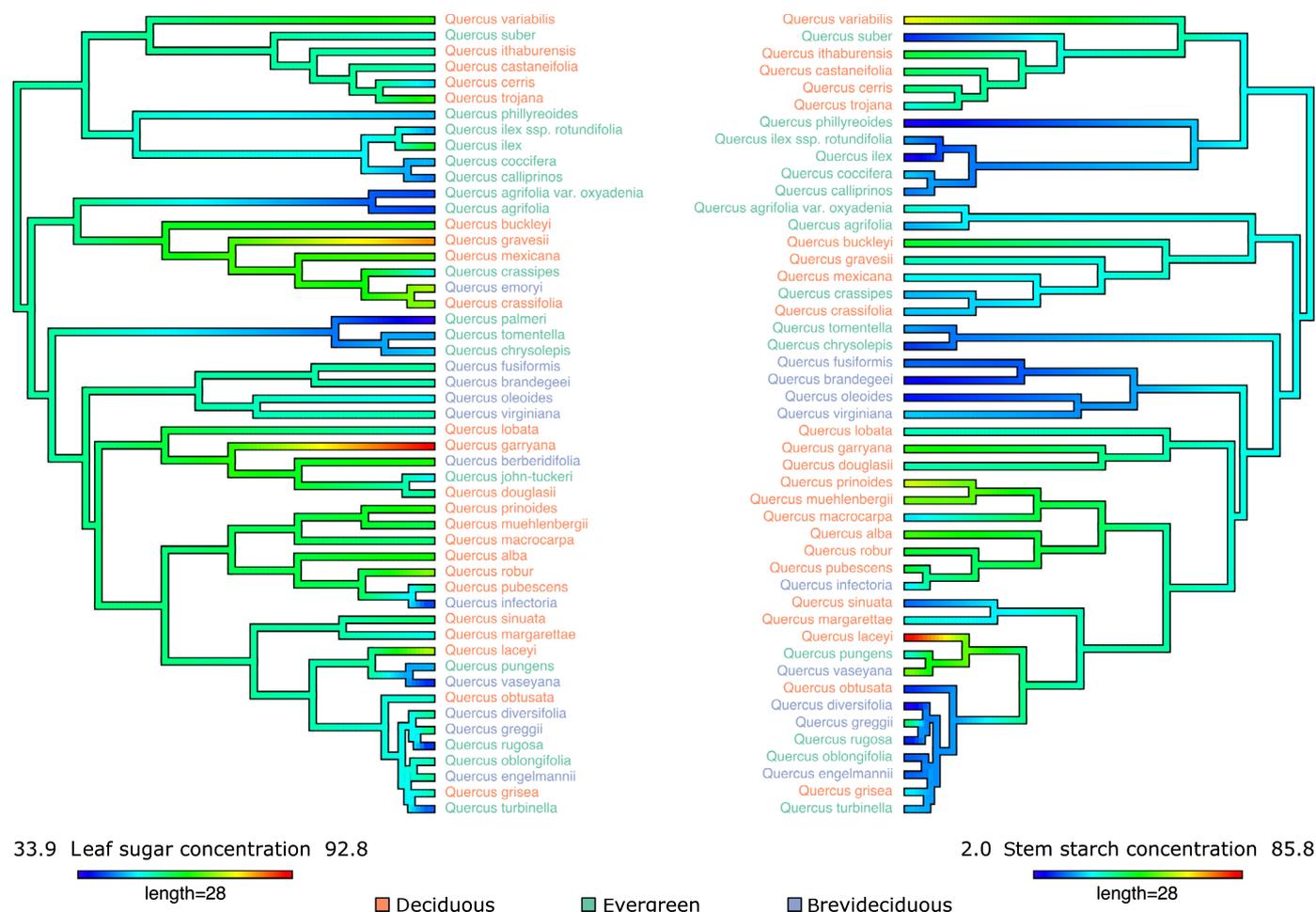


Fig. 2 Evolution of sugar concentrations in the leaves (left) and starch concentrations in the stem (right) mapped onto each phylogeny. Species names at the tips of each phylogeny are coloured by leaf habit. Additional plots of the evolution of leaf starch, leaf total nonstructural carbohydrate (NSC), stem sugar, stem total NSC and stem starch proportion are provided in Supporting Information Figs S1 and S2.

oak species. Furthermore, starch tended to be low or absent in the leaves of our study trees (Fig. 3b). Together, these results suggest that leaf NSCs are transient in nature and the leaf is not a major storage organ.

By contrast with the leaf, the stemwood represents a long-term C storage site in trees, and we expected that if leaf habit influences NSC storage then differences would be most apparent in the stem, particularly as insoluble starch due to its primary role as a storage molecule (Chapin *et al.*, 1990; Richardson *et al.*, 2015; Furze *et al.*, 2018a,b). In agreement with our expectation, our model results indicated that starch concentrations and the proportion of starch in the stem differed between leaf habits based on the best fit multiple-optimum OUM model. While there was mixed support for the single-optimum and multiple-optimum OU models as the best fit model for sugar concentrations, greater support for the simpler single-optimum OU1 model suggests that stem sugar concentrations did not differ between leaf habits. Similar stem sugar concentrations across species highlights the fact that soluble sugars serve nonstorage functions such as metabolism, osmoregulation, and transport, and are often maintained within an optimal range in plants (Hartmann & Trumbore, 2016; Dong & Beckles, 2019).

Our results provide the first phylogenetically informed support that ecologically driven evolution may be generating diversity in starch storage in trees, with deciduous oak species evolving towards higher starch concentrations and a greater proportion of starch in the stem than their relatives. This is further supported by recent work in *Populus trichocarpa* trees, which showed that NSC storage is a heritable trait that selection may act on (Blumstein *et al.*, 2020). Selection in deciduous tree species may favour higher starch concentrations and a greater proportion of starch in the stem to allow them to meet their overall higher C storage requirements compared with other leaf habits. Deciduous species rely on previously stored NSCs to support growth and leaf out and, more specifically, these species derive sucrose from stored starch reserves in the stemwood to support early season xylem production (Dougherty *et al.*, 1979; Kozłowski & Pallardy, 1997; Fajardo *et al.*, 2013). In addition to supporting springtime growth, stemwood starch reserves are involved in exchange processes that occur between the xylem and phloem and also buffer source–sink imbalances along the long-distance transport pathway from the canopy to the root system (Ziegler, 1964; De Schepper *et al.*, 2013; Minchin & Lacoite, 2017; Furze *et al.*, 2018b). These various roles of stem starch reserves may be

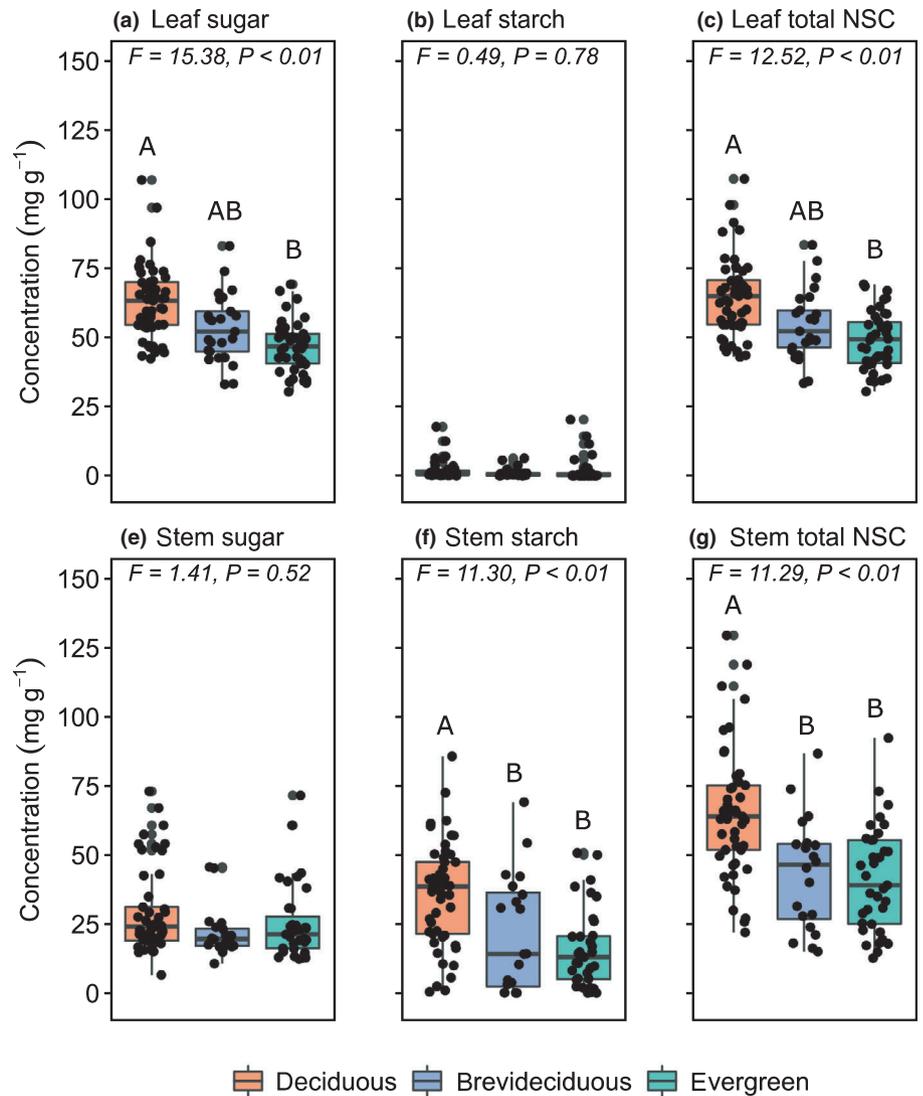


Fig. 3 Sugar, starch and total nonstructural carbohydrate (NSC) concentrations in the leaves (a–c) and stems (d–f) of individual oak trees by leaf habit. For each trait (a–f), the F -value and P -value are displayed from phylogenetic ANOVA testing and uppercase letters indicate the significance of differences between leaf habits based on post hoc testing. Proportion of starch in the stem is not displayed here, but phylogenetic ANOVA testing indicated that it differed between leaf habits ($F = 7.81, P = 0.02$). For each boxplot, the circles represent concentrations from each individual oak tree, the vertical size of each box is the interquartile range (IQR) with the bottom and top edges denoting the 25th and 75th percentile, respectively, and the whiskers extend to within $1.5 \times$ IQR from each edge.

particularly important for deciduous species when the canopy is bare and not the primary source of NSCs.

An interesting consideration is whether starch storage in deciduous trees might afford an advantage under global change; that is, what implications does selection for larger, and potentially excess, NSC reserves in deciduous species have for stress tolerance? We found that nearly 60% of the NSCs in the stemwood of deciduous oak species were stored as starch compared with *c.* 35% in brevideciduous and evergreen oak species. While quantifying reserves in relation to seasonal demands was beyond the scope of our study, previous studies indicate an excess of stemwood NSCs in temperate deciduous trees. For example, recent work quantifying whole-tree NSC storage showed that deciduous trees maintained large NSC reserves that were minimally depleted (*c.* 30%) by seasonal demands (Furze *et al.*, 2018a), and an earlier study found that NSC reserves in the branches and stemwood could rebuild the leaves over four times (Hoch *et al.*, 2003). However, whether NSC reserves in different tissues and at different depths (*i.e.* older reserves) are equally available for metabolism remains debated (Körner, 2003; Sala *et al.*, 2012; Furze *et al.*, 2020).

Nevertheless, evidence points towards stored starch as the main fuel source for survival during and recovery following stressors such as drought and disturbance, and we suggest that the higher starch concentrations associated with deciduousness may confer resilience for long-lived oak trees in a changing world (McDowell *et al.*, 2011; Adams *et al.*, 2017; Earles *et al.*, 2018; Smith *et al.*, 2018; Piper & Paula, 2020). How oak trees will respond to climate change is an important consideration for ecosystem structure and function given that the oaks contribute more biomass and diversity to temperate ecosystems of the Northern Hemisphere (United States and Mexico) than any other lineage (Cavender-Bares, 2016, 2019). If NSC storage influences resilience, then differences in storage patterns between trees (*i.e.* deciduous vs evergreen, angiosperm vs gymnosperm) have the potential to contribute to shifts in forest tree species composition in response to stress, which would have consequences for important ecological processes such as C and nutrient cycling at the ecosystem level.

The idea that deciduous oak trees may be more resilient is based on our results in aboveground organs and does not

Table 1 Summary of four evolutionary model fits for sugar, starch and total nonstructural carbohydrate (NSC) concentrations in the leaves and stems, as well as the proportion of starch in the stems of oaks.

Trait	Model	loglik	AICc	Δ AICc	AICc weight	Per cent
Leaf sugar	BM1	-217.72 \pm NA	439.68 \pm NA	56.5 \pm 0.06	0	0
	BMS	-205.02 \pm 0.06	418.92 \pm 0.11	35.73 \pm 0.12	0	0
	OU1	-197.25 \pm NA	401 \pm NA	17.82 \pm 0.06	0	0
	OUM	-185.93 \pm 0.03	383.2 \pm 0.06	0 \pm 0	1	100
Leaf starch	BM1	-127.06 \pm NA	258.38 \pm NA	27.82 \pm 0.16	0	0
	BMS	-111.02 \pm 0.09	230.91 \pm 0.18	0.35 \pm 0.04	0.966	87
	OU1	-115.75 \pm NA	238.02 \pm NA	7.45 \pm 0.16	0.028	10.1
	OUM	-114.78 \pm 0.04	240.88 \pm 0.07	10.32 \pm 0.18	0.007	2.9
Leaf total NSC	BM1	-219.73 \pm NA	443.71 \pm NA	50.84 \pm 0.06	0	0
	BMS	-207.55 \pm 0.05	423.97 \pm 0.10	31.09 \pm 0.10	0	0
	OU1	-199.57 \pm NA	405.65 \pm NA	12.77 \pm 0.06	0.002	0
	OUM	-190.78 \pm 0.03	392.9 \pm 0.06	0 \pm 0	0.998	100
Stem sugar	BM1	-191.65 \pm NA	387.58 \pm NA	27.92 \pm 0.03	0	0
	BMS	-178.74 \pm 0.06	366.44 \pm 0.13	6.78 \pm 0.12	0.031	8.2
	OU1	-176.67 \pm NA	359.89 \pm NA	0.23 \pm 0.03	0.827	88.3
	OUM	-175.98 \pm 0.02	363.42 \pm 0.05	3.77 \pm 0.05	0.141	3.5
Stem starch	BM1	-220.45 \pm NA	445.16 \pm NA	48.93 \pm 0.10	0	0
	BMS	-205.55 \pm 0.07	420.06 \pm 0.14	23.82 \pm 0.19	0	0
	OU1	-200.68 \pm NA	407.92 \pm NA	11.68 \pm 0.10	0.003	0.5
	OUM	-192.39 \pm 0.05	396.24 \pm 0.10	0 \pm 0.002	0.997	99.5
Stem total NSC	BM1	-221.29 \pm NA	446.84 \pm NA	28.09 \pm 0.08	0	0
	BMS	-209.09 \pm 0.04	427.12 \pm 0.09	8.36 \pm 0.11	0.015	1.9
	OU1	-207.24 \pm NA	421.03 \pm NA	2.27 \pm 0.08	0.305	31.5
	OUM	-203.98 \pm 0.05	419.43 \pm 0.10	0.67 \pm 0.04	0.681	66.6
Stem starch Proportion	BM1	-15.94 \pm NA	36.16 \pm NA	64.29 \pm 0.07	0	0
	BMS	-0.08 \pm 0.06	9.1 \pm 0.12	37.23 \pm 0.13	0	0
	OU1	11.89 \pm NA	-17.23 \pm NA	10.9 \pm 0.07	0.004	0
	OUM	19.8 \pm 0.04	-28.14 \pm 0.07	0 \pm 0	0.99	100

Evolutionary models were fit over 1000 stochastically mapped trees with a single topology. For each model, mean maximum log-likelihood (loglik) is the averaged loglik over the 1000 replications. Mean Akaike information criterion with a correction for small sample size (AICc) score is the averaged AICc score over 1000 replications. Mean Δ AICc (Δ AICc) is the AICc score of a model minus the minimum AICc score within the given set of models, averaged across 1000 replications. SE is provided for the mean loglik, AICc and Δ AICc for each model when applicable. AICc weight indicates the support for each model within a given set of models, ranging from 0 (no support) to 1 (full support). Per cent contains the % of 1000 replications in which each model was favoured (lowest AICc). Bold text indicates the best fit model within a given set of models based on the lowest AICc score. NA, not applicable.

Table 2 Model-averaged parameter estimates from the four evolutionary models for sugar, starch and total NSC concentrations in the leaves and stems, as well as the proportion of starch in the stems of oaks.

Trait	θ_D	θ_B	θ_E	σ^2_D	σ^2_B	σ^2_E	α
Leaf sugar	63.58 \pm 1.98	55.47 \pm 2.92	46.44 \pm 2.34	818.52 \pm 1.34	818.52 \pm 1.34	818.52 \pm 1.34	4.75 \pm 0.38
Leaf starch	0.08 \pm 0.25	0.07 \pm 0.39	0.08 \pm 0.31	0.25 \pm 0.32	0.13 \pm 370.56	0.51 \pm 4.24	0.0007 \pm 0.14
Leaf total NSC	65.66 \pm 2.21	56.72 \pm 3.34	48.71 \pm 2.63	934.94 \pm 5.21	934.94 \pm 5.21	934.94 \pm 5.21	4.49 \pm 0.40
Stem sugar	26.37 \pm 2.86	26.69 \pm 4.55	26.04 \pm 3.46	13.55 \pm 0.39	13.61 \pm 73.39	13.36 \pm 15.88	0.05 \pm 0.43
Stem starch	39.88 \pm 3.35	18.92 \pm 5.93	16.16 \pm 4.50	825.93 \pm 5.83	825.93 \pm 7.89	825.93 \pm 5.83	1.94 \pm 0.65
Stem total NSC	64.21 \pm 6.62	50.74 \pm 13.84	42.76 \pm 9.50	74.87 \pm 3.12	75.13 \pm 168.31	74.76 \pm 3.24	0.09 \pm 0.49
Stem starch proportion	0.57 \pm 0.04	0.34 \pm 0.06	0.36 \pm 0.05	0.10 \pm 50.84	0.10 \pm 50.84	0.10 \pm 50.84	1.97 \pm 50.84

θ is the primary nonstructural carbohydrate (NSC) concentration optimum, α is the strength of pull to the optimum, and σ^2 is the rate of stochastic fluctuations of trait evolution/stochastic motion. Parameter subscripts indicate leaf habit (D = deciduous, B = brevideciduous, and E = evergreen). Missing parameter estimates for the BM1 and BMS models were assigned a value of 0 in the model-averaging process. Model-averaged parameter estimates and uncertainty were calculated according to the formulas in Burnham & Anderson (2002). We calculated the among-model and within-model variances to estimate the contribution of among-model uncertainty and parameter uncertainty within models to total variance and then report it as SE. Parameter estimates for the evolutionary models are reported in Supporting Information Tables S4 and S5.

consider the belowground root system. Even though our previous work in temperate trees suggests that less than 25% of whole-tree NSC reserves are stored in the roots at peak storage (Furze *et al.*,

2018a), differences in root NSCs between leaf habits could play an important role in resilience and, therefore, the absence of root data for the oak trees in this study adds uncertainty to our

conclusion. In previous studies examining mature trees, the deciduous angiosperm *Q. rubra* had larger root NSCs than the evergreen gymnosperm *P. strobus* (Richardson *et al.*, 2015; Furze *et al.*, 2018a). While this comparison is problematic because phylogenetic differences confound the effects of differences in leaf habit, if the same pattern were to hold for our study system, then we would expect root NSC concentrations in deciduous oak species to be higher than in evergreen and brevideciduous oak species; therefore, providing additional support for our idea that a deciduous strategy may be advantageous. While there is a need for studies to measure root NSC concentrations across leaf habits within and between clades, there is one study that complements our findings. Wyka *et al.* (2016) selected three species pairs consisting of an evergreen and a related deciduous shrub species for Berberidaceae, Rosaceae, and Adoxaceae and showed that allocation to storage in seedlings was higher in the roots (and stems) of deciduous compared with evergreen species in terms of both NSC concentrations and whole-plant NSC pools.

Furthermore, our conclusions about leaf habit and NSC storage are based on NSC concentrations and do not take whole-plant NSC pools into consideration since biomass allocation could not be determined for our study trees. Broadly across plant species and biomes, angiosperms and deciduous species tend to have higher mean root/shoot biomass ratios than gymnosperms and evergreen species, respectively (Qi *et al.*, 2019). However, organ biomass ratios can vary within and between plant species and are influenced by factors such as developmental stage and growth environment (Poorter *et al.*, 2012). For example, previous studies on seedlings have reported that the root mass ratio tended to be larger in deciduous species than in evergreen (Antúnez *et al.*, 2001; Tomlinson *et al.*, 2013), whereas another study found no difference between leaf habits (Wyka *et al.*, 2016). Even if differences in biomass allocation to organs exist between leaf habits, biomass allocation may vary independently of NSC concentrations; therefore, future studies should consider how these patterns influence resilience between different leaf habits. Future studies should also further examine the evolutionary links between leaf habit and NSC storage across clades and seasons as well as in additional storage sites (i.e. roots). It is also of interest to determine additional factors that may drive selection for higher storage (i.e. environmental conditions, pathogens) and to explore starch metabolism in response to stress between leaf habits.

Conclusions

Here, we utilised a comparative physiological framework to explore NSC storage in trees. By measuring NSC concentrations among *Quercus* species, we examined if variation in NSC storage is explained by leaf habit, a major axis of diversity. Notably, we showed that deciduous oak species are evolving towards higher starch concentrations and a greater proportion of starch in the stemwood compared with their brevideciduous and evergreen relatives. In long-lived trees, selection for larger starch reserves may contribute to resiliency, suggesting a potential benefit to a deciduous strategy and highlighting the implications for the response and C storage of individual species and

forest ecosystems to global change. While we have explored the evolutionary links between leaf habit and NSC storage at the scale of the genus *Quercus*, future studies examining additional clades will provide insight into whether the patterns we show here are universal.

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Author contributions

MEF and DKW designed the research project. MEF and DKW completed sample collection with support from TK and AJM, MEF conducted laboratory analyses with support from BAH, MEF and DKW analysed the data. MEF interpreted the data with support from DKW and CRB, MEF wrote the manuscript with feedback and approval from co-authors.

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Data availability

The data that support the findings of this study are provided in the Supporting information of this article.

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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 Evolution of each trait for leaves mapped onto the phylogeny.

Fig. S2 Evolution of each trait for stems mapped onto the phylogeny.

Fig. S3 Relationship between stem diameter and NSC concentrations.

Fig. S4 Density plots of simulated parameter estimates for leaves.

Fig. S5 Density plots of simulated parameter estimates for stems.

Table S1 Collection information for individual trees.

Table S2 Sugar and starch concentration data.

Table S3 Leaf habit assignments.

Table S4 Parameter estimates from four evolutionary models for NSC concentrations in leaves.

Table S5 Parameter estimates from four evolutionary models for NSC concentrations in stems.

Table S6 AICc scores for four evolutionary models from the 1000 simulated data sets generated under the best fit model parameters for NSC concentrations in leaves.

Table S7 AICc scores for four evolutionary models from the 1000 simulated data sets generated under the best fit model parameters for NSC concentrations in stems.

Table S8 Mean and 95% confidence interval calculated across 1000 bootstraps for each parameter.

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