



Invited review: Part of an invited issue on carbon allocation

Carbon dynamics in trees: feast or famine?

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Research on the degree to which carbon (C) availability limits growth in trees, as well as recent trends in climate change and concurrent increases in drought-related tree mortality, have led to a renewed focus on the physiological mechanisms associated with tree growth responses to current and future climate. This has led to some dispute over the role of stored non-structural C compounds as indicators of a tree's current demands for photosynthate. Much of the uncertainty surrounding this issue could be resolved by developing a better understanding of the potential functions of non-structural C stored within trees. In addition to functioning as a buffer to reconcile temporal asynchrony between C demand and supply, the storage of non-structural C compounds may be under greater regulation than commonly recognized. We propose that in the face of environmental stochasticity, large, long-lived trees may require larger C investments in storage pools as safety margins than previously recognized, and that an important function of these pools may be to maintain hydraulic transport, particularly during episodes of severe stress. If so, survival and long-term growth in trees remain a function of C availability. Given that drought, freeze–thaw events and increasing tree height all impose additional constraints on vascular transport, the common trend of an increase in non-structural carbohydrate concentrations with tree size, drought or cold is consistent with our hypothesis. If the regulated maintenance of relatively large constitutive stored C pools in trees serves to maintain hydraulic integrity, then the minimum thresholds are expected to vary depending on the specific tissues, species, environment, growth form and habit. Much research is needed to elucidate the extent to which allocation of C to storage in trees is a passive vs. an active process, the specific functions of stored C pools, and the factors that drive active C allocation to storage.

Keywords: carbohydrates, carbon allocation, embolism, hydraulic safety, regulated storage, tree non-structural carbon storage.

Introduction

Carbohydrate metabolism is central to plant life: for plants to survive in the long term they necessarily need to assimilate sufficient carbon (C) to allow the buildup of structural biomass and to fuel the metabolism associated with the construction and maintenance of such biomass, including reproductive structures. Because plants lack the ability to move and seek shelter upon predation or environmental stress, a pool of non-structural C stored in the form of starch, lipids or fructans (strictly storage compounds, with no function other than storage) is also needed to buffer periods of stress (Chapin

et al. 1990, Kozlowski and Pallardy 2002). This is particularly important in long-lived plants where a single severe stress episode may compromise a lifetime investment for future reproduction.

Fluctuations of stored non-structural C compounds over time often arise because C supply by photosynthesis is not necessarily synchronized with C demand for functions such as growth and respiration. The controls over C storage in wild plants and the extent to which it is a passive consequence of imbalances between C supply and demand vs. an active process remain to be elucidated (Chapin et al. 1990). The term active in this case refers to a process that involves specific

genetic controls and which may take precedence over allocating fixed C to other processes when resources are limited. Although the distinction between active vs. passive in this context is critical, it is common to view stored C reserves as a purely passive pool. That is, when the supply of assimilated C does not meet C demand, reserves serve as a repository to meet demand and decrease (Figure 1a). Conversely, when C supply exceeds C demand, reserves serve to capture overflow and increase (i.e., accumulation reserve *sensu* Chapin et al. 1990; see Figure 1b). According to this perspective, the size of stored C pools can be used as an indicator of the C balance of the plant: positive when there is overflow and negative when reserves serve as a repository. Therefore, large constitutive C storage pools would be indicators of long-term positive C balance and, consequently, of the fact that C availability does not limit tree growth and survival. If, on the contrary, reserve formation is an actively regulated sink that competes for C with

growth, then the size of the stored C pool can no longer be used as an indicator of the C balance (i.e., if storage is a higher priority than growth, growth could still be limited by C availability). In this case, long-term tree growth and survival may still ultimately be a function of C availability. Whether, and the extent to which, tree growth and survival are a function of C availability in the long term has important implications for the understanding and prediction of tree and forest responses to environmental change (e.g., increasing frequency and intensity of drought and increasing atmospheric CO_2). If stored reserves reflect a surplus of C, increases of atmospheric CO_2 are not expected to stimulate growth in trees and forests in the long term (Körner 2003, Kirschbaum 2011). Similarly, the negative effects of drought on the tree C balance would not be realized until stored C pools begin to decline.

In this paper, we provide some examples illustrating that stored C reserves are not always a purely passive consequence of the balance between C supply via photosynthesis and C demands for growth and metabolism. We follow by briefly re-examining the significance of stored C pools in trees and their implications concerning the role of C availability as a driver of tree growth and survival. We then re-examine the notion that relatively large stored C pools in trees are indicators of surplus C and therefore that C is not limiting to tree growth in the long term. We suggest that the question of whether C limits growth in trees is perhaps too broad to be useful, because the answer depends on the time (short vs. long term) and spatial scale (specific tissues vs. whole plant) at which it is asked. With a special emphasis on tree responses to drought, we propose that keeping large, long-lived trees alive in the face of environmental stochasticity may entail larger C investments in storage pools as safety margins than previously recognized, regardless of whether growth at any given point in time is or is not limited by C supply. While such pools may serve as overflow/repository reservoirs depending on developmental stage and environmental conditions, we suggest that constitutive stored C pools in trees may be necessary to maintain vascular integrity, particularly during episodes of severe stress. If so, survival and long-term growth in trees remain a function of C availability, independent of spatial (throughout the tree) and temporal fluctuation in C supply.

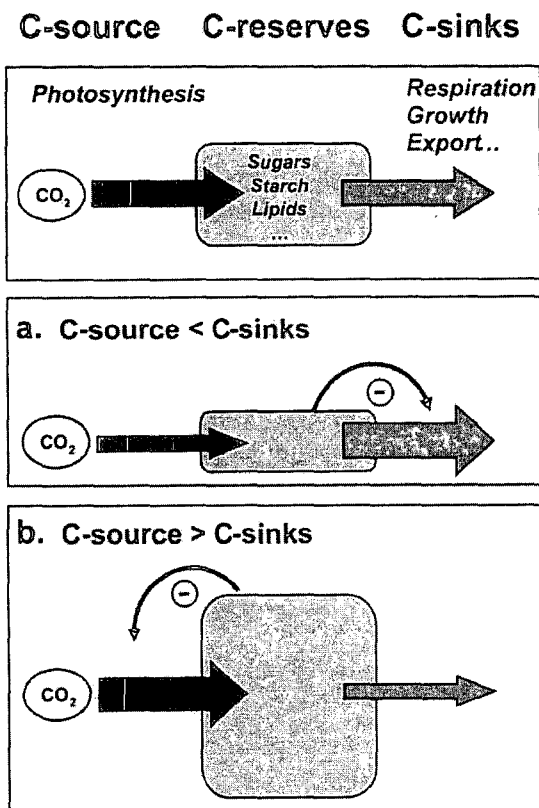


Figure 1. Schematic illustration of the reasoning to use the mobile C reserve concentration as indicators of C balance between sources and sinks. The thickness of the arrows is proportional to the amount of activity. Potential negative feedback and feedforward mechanisms are indicated by the circled minus sign. When sink activity is greater than source activity, the concentration of stored C pools is expected to decrease. Eventually, such decrease may limit sink activity and C demand (a). Conversely, when source activity is greater than sink activity, the concentration of stored C pools is expected to increase, which could down-regulate source activity (b). Adapted from: Sala et al. (2011) © Springer Science+ Business Media B.V. 2011.

Storage is not always a passive overflow/repository

That storage is not always a purely passive consequence of the balance between C supply via photosynthesis and overall C demands has been repeatedly recognized in the literature (Chapin et al. 1990, Cannell and Dewar 1994, Lacombe 2000, Le Roux et al. 2001). An obvious example is the active storage of C compounds in specialized storage organs. On a diurnal basis, both herbaceous (Smith and Stitt 2007) and woody

plants (Taylor et al. 2003, Walter et al. 2005; see also Webb and Kilpatrick 1993) also actively accumulate starch during the day to meet growth and metabolic demands at night regardless of competing growth demands. Note, however, that in woody plants diel fluctuations of starch vary depending on the specific organ (Bucci et al. 2003). Under optimal conditions, leaf starch accumulation concurrent with growth reflects that storage is not a purely passive overflow and that it is highly regulated. Similar to day/night cycles, on a seasonal basis, C supply and C demand are not necessarily synchronous (periods of maximum photosynthesis do not necessarily coincide with periods of maximum C demand) and C storage is also needed to cope with this asynchrony (Fischer and Höll 1992, Newell et al. 2002). However, very little is known about the fraction of seasonal storage that results from imbalances between C supply and demand (i.e., overflow/repository function) vs. that which results from actively regulated storage. Data showing that trees have relatively high constitutive C storage pools regardless of seasonal fluctuations (Hoch et al.

2003, Figure 2) suggest that a significant fraction of stored C in trees is not necessarily a result of the seasonal asynchrony between C supply and C demand. The question is the extent to which this fraction reflects a surplus of C in the long term (positive C balance), or whether it is the result of regulated storage that serves a function in addition to a C repository for growth and respiration. Recent research in trees suggests that, at least in some cases, storage of C compounds is an actively regulated process. For instance, the tapping of rubber trees for latex production (which involves a large C demand) was associated with a dramatic increase in non-structural carbohydrate (NSC) concentration, and differences in NSC concentrations between control and tapped trees were highest when latex production was highest (Silpi et al. 2007). In olive trees, Bustan et al. (2011) also showed no carbohydrate depletion even during years of high demand for fruit and oil production, leading to the suggestion that stored C reserves are an active sink. These examples illustrate that during periods of high C demand, stored C reserves are not depleted as might be

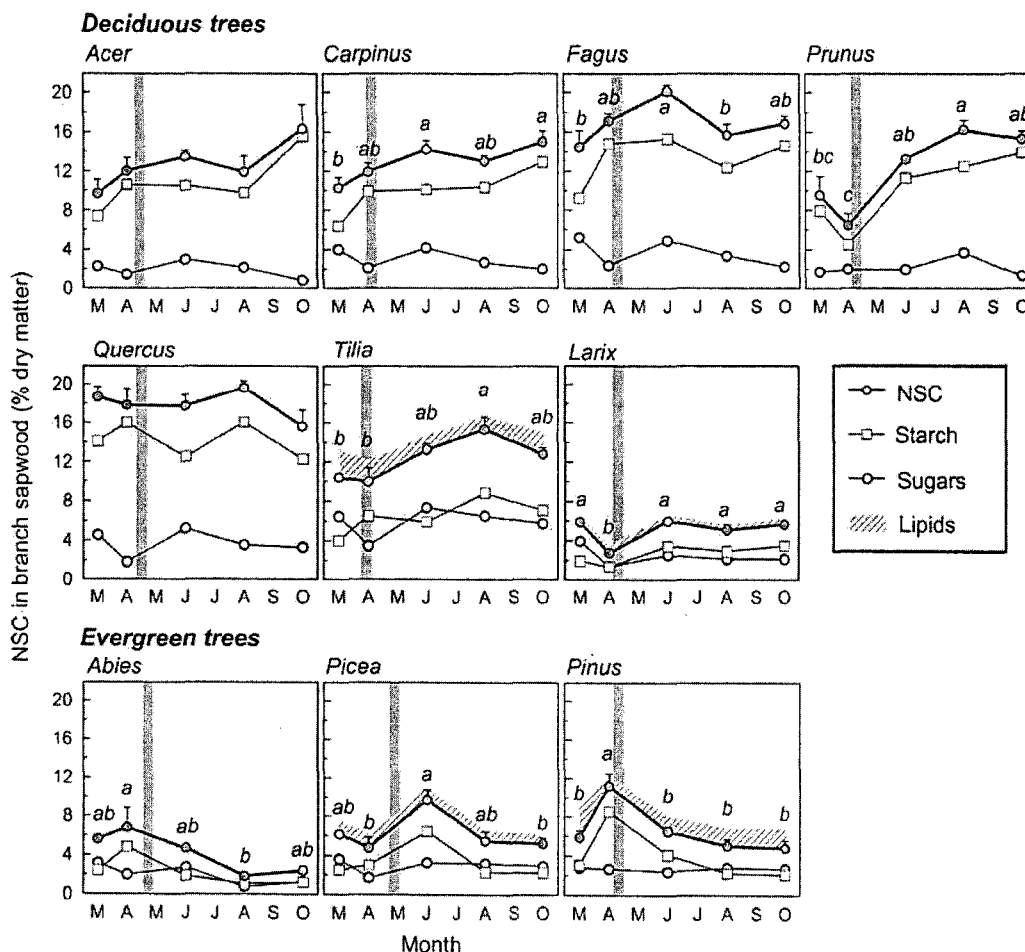


Figure 2. Seasonal variations of low-molecular-weight carbohydrates (glucose, fructose and sucrose), starch and NSC (sugars + starch) concentrations in branch sapwood of 10 mature tree species. For those species where lipids could be detected, the concentrations of lipids are indicated by the shaded area on top of the NSC curve. Values are means of two to six trees per species. Note that minimum seasonal concentrations generally remain high suggesting that trees have more stored C than needed to meet the seasonal asynchrony between C supply and C demand. Adapted from: Hoch et al. (2003).

expected (or even increase), which suggests that they serve some other function in addition to a repository of C.

Recent work in herbaceous plant model systems on the dynamics of starch storage and its relationship with growth offers important insights and may serve as a basis to predict potential similarities and differences with woody plants. In these model systems, storage is actively regulated via a very tight and complex genetic and metabolic coordination between C supply via photosynthesis, growth and storage (Smith and Stitt 2007). This coordination entails rapid short-term changes in transcript levels of important genes related to C metabolism as well as longer-term changes in enzyme activity (Smith and Stitt 2007). The metabolic regulation at the gene level suggests that similar molecular regulatory mechanisms may operate in trees. However, it is entirely unknown how this regulation is coordinated at the whole plant level in long-lived, large plants (e.g., trees) where C supply and demand is often not synchronized. Recent work in *Arabidopsis* has also shown that starch content in leaves at the end of the day is an integrator of overall plant metabolic activity and it is negatively correlated with biomass accumulation (Sulpice et al. 2009). The simplest explanation would be that plants maximize growth at the expense of their C reserves. Instead, results indicated that limited supply of available C at night (limited carbohydrate pool) led to an *increase* in storage during the day as an adjustment to *optimize* growth in the long term. This is because C starvation at night causes a strong growth inhibition, which, if persistent, could become chronic and decrease long-term growth (Smith and Stitt 2007). Short-term growth inhibition prevented this by allowing C supplied by photosynthesis to be allocated to storage (i.e., 'regulated storage') and ensure sufficient C supply at night (Smith and Stitt 2007). This work provides an important message that may be relevant for the interpretation of storage dynamics in woody plants: plants with limited carbohydrate supply inhibit growth in the short term in favor of starch storage as a strategy to *optimize* growth in the long term. These results, combined with the examples above in trees indicating that storage may be an active sink, suggest that trees also actively regulate storage at the expense of short-term growth to optimize growth and survival in the long term.

Why do trees have relatively large stored C pools?

The significance of stored C pools as reservoirs to buffer imbalances between C supply and demand has been repeatedly recognized in the literature (Ziegler 1964, Iwasa and Kubo 1997, Kozlowski and Pallardy 2002, Barbaroux et al. 2003, López et al. 2009, Genet et al. 2010). However, year-round high non-structural C reserve pools have been shown for mature trees in temperate (Hoch et al. 2003) and tropical (Würth et al. 2005) climates, regardless of seasonal fluctuations, habitat and

climate (Körner 2003). Such large pools suggest that trees store more C than needed to meet typical demands over time (see below). Estimates of how long stored C pools could sustain growth in the absence of photosynthesis (assuming that these pools remain available) are quite variable (Ludovici et al. 2002, Hoch et al. 2003, Würth et al., 2005), but the contribution could be very large. For instance, stored C pools in the aboveground wood of mature temperate deciduous trees were estimated to be sufficient to replace the entire leaf canopy four times (Hoch et al. 2003). Why do trees maintain such large pools of stored C? It is possible that the overabundance (i.e., C in excess of that needed for growth and respiration) of stored C in contemporary trees reflects higher atmospheric CO₂ concentrations relative to the past when CO₂ concentrations were much lower (which was the case during the last glacial period). That is, non-structural C storage may reflect inherent traits selected for when atmospheric C availability was more limiting (Körner 2003). If so, current tree growth has little potential to respond to elevated atmospheric CO₂ concentrations in the long term (Körner 2003). Storage of excess C may provide a number of derived benefits such as the maintenance of large labile nitrogen (N) pools as Rubisco and the production of root exudates for microbial-mediated nutrient cycling (Millard et al. 2007).

It has also been suggested that a considerable fraction of the starch pool stored in the xylem of trees becomes sequestered rather than stored (Millard et al. 2007; see also Millard and Grelet 2010 for a review), in which case it no longer serves as reserves. Sequestration of stored C pools could occur as a result of reduced enzymatic access to the inner nuclei of starch granules (Srichuwong and Jane 2007) or the compartmentalization away from sites of phloem loading (Quick et al. 1992). This could happen at sites where parenchymatic tissues become hydraulically isolated due to irreversible embolism of adjacent xylem. The possible sequestration of stored C pools is consistent with evidence that plants often fail to use all carbohydrate reserves following herbivory, shade or drought (Chapin et al. 1990, Millard and Grelet 2010, Sala et al. 2010, and references therein; Piper 2011). Similarly, in most forest ecosystems under normal circumstances, a large fraction of new root growth occurs at the expense of recently stored C rather than older stored C (Gaudinski et al. 2009). Millard et al. (2007) also noted that NSC pools in trees are rarely fully depleted to the extent that N storage pools are, and that even girdling the phloem does not deplete NSC in roots completely (Jordan and Habib 1996, Bhupinderpal-Singh et al. 2003).

The large pools of stored C reserves in mature trees led Körner (2003), Millard et al. (2007) and Millard and Grelet (2010) to suggest that they reflect an overall sufficient C supply at ambient atmospheric CO₂ concentrations and that C supply via photosynthesis does not limit tree growth. However, if a significant fraction of the stored C pool is sequestered and

not available, then the overabundance of C is only apparent (i.e., C supply could still limit growth). Further, in light of results showing that storage can be an actively regulated sink that competes with other functions, it is worthwhile to raise the question: are the relatively large constitutive C pools in trees a purely passive consequence of a positive C balance, or do trees actively allocate to storage to maintain certain minimum reserves? If the latter, why do these reserves exist? What is their function? How do life history and environment determine the minimum size of stored C pools? The answers to these questions are important because if stored C pools become sequestered and/or trees actively allocate C to storage in the long term, then tree growth may still be fundamentally dependent on C availability. This potential dependency has important implications for our understanding of tree responses to elevated CO₂ and climate change (Körner 2003, Kirschbaum 2011), including increasing drought frequency and intensity (IPCC 2007).

Clearly, the size of the stored C pool increases with plant size: small plants with lower biomass require smaller buffers to cope with periodic and episodic stresses relative to large plants. What may be relevant is the size of the total non-structural C pool (kg) relative to total living plant biomass (i.e., an average concentration or amount of stored C per unit living biomass). Is this ratio constant among plants, or do certain life forms require higher stored C per unit living biomass? To our knowledge, this type of analysis has not been conducted, probably because data in the literature are very limited due to the difficulty of quantifying total pools of stored C and total living biomass in large plants. It could be that trees have higher ratios of stored C per unit of living biomass, and that proportionally higher C allocation to storage does not necessarily reflect an overabundance, but that it also serves a function yet to be determined. In the past, however, we have interpreted the relatively large stored non-structural C pools in trees as well as the tendency for increasing pools with tree size and environmental stress (Hoch et al. 2002, Hoch and Körner 2003, Würth et al. 2005, Sala and Hoch 2009, Woodruff and Meinzer 2011a) as indicators of 'excess' C. This may be the case at certain times, but not always. The extent to which stored C pools reflect a positive C balance (excess C) is relevant in the context of the contrast between the traditional C-centric approach to tree growth, where C availability is the major driver of tree growth, and the relatively recent perspective that tree growth is often not C-limited.

Growth and C storage in trees: from C limitation to sink limitation

The forestry and forest physiology and modeling literature has been traditionally dominated by a carbon-centric perspective. Net primary productivity (NPP) is the difference between total

carbon assimilation (gross primary productivity, GPP), minus respiration (*R*, including export from roots). This means that NPP is solely a function of GPP and *R* and that as long as there is carbon, trees will grow. Trees will cease to grow only when *R* is greater than GPP.

The carbon-centric perspective is at the core of most process-based models of forest and tree productivity and has several important implicit assumptions. With one notable exception that we are aware of (Davi et al. 2009), storage is assumed to be a passive sink: available carbon is diverted to growth and metabolism, but not actively to storage. Changes in storage occur only as a consequence of temporary imbalances between C input via photosynthesis and C demand for respiration, both of which are controlled by environmental factors and nutrient content. As mentioned above, the passive overflow/repository paradigm of storage is not always correct. Second, in most models, the storage C pool does not change in the long term and all C assimilated is eventually available for growth and metabolism. This is not consistent with recent results showing increases in the concentration of non-structural C compounds (on a mass basis) with tree age and size (Sala and Hoch 2009, Piper and Fajardo 2011, Woodruff and Meinzer 2011a) in spite of well-documented age-related growth declines in trees (Ryan and Waring 1992, Ryan et al. 1997). Although the fraction of C allocated to storage relative to structural growth and metabolism may be minute on an annual basis, over time this fraction accumulates and storage pools in mature trees become large and could potentially furnish a large fraction of C needed for growth in a given season. Why then do large trees fail to use their storage pool for growth? Several non-mutually exclusive factors may explain this. First, growth may be limited by factors other than the supply of C by photosynthesis. For instance, it has been known for decades that growth-related processes such as cell expansion, cell wall synthesis and protein synthesis are substantially more sensitive to water stress than to photosynthesis (Hsiao 1973, Hsiao et al. 1976). This illustrates a direct effect of water availability on growth, which is also ignored in most tree and forest process-based models where water availability limits growth only indirectly via its effects on C assimilation. Second, if stored C pools in trees become sequestered, they are no longer available and cannot be used. Third, trees may actively maintain relatively large thresholds of stored C pools for a reason yet to be deciphered, a topic on which we elaborate below. In summary, increasing evidence indicates that key assumptions in most process-based models are not correct. This presents an important challenge for modeling tree and forest responses under climate change and increased drought. If models are correct but for the wrong reasons, our ability to predict tree and forest responses to future climate may not be on a sound footing. The challenge is to elucidate the temporal and spatial scales at which these assumptions become critical.

Another area of study that has focused on C as a primary driver of productivity is the research that has been done on the mechanisms underlying the well-known age- and size-related growth declines in trees and forests (Ryan et al. 1997). The two classical groups of hypotheses to explain size/age-related declines of growth rates in trees and stands focused on the extent to which growth declines occurred as a result of reduced C supply via photosynthesis (assimilation hypotheses) or an increase of C demand due to increases of the respiratory load (respiration hypotheses; see Sala et al. 2011 for a discussion). However, both of these groups of hypotheses rest on the same assumption: that growth declines are due to limiting C availability. This perspective is now questioned based on current research indicating that alternative factors (e.g., turgor, long-distance transport of assimilates, nutrients) contribute to growth limitations in tall trees (Woodruff et al. 2004, Sala et al. 2011, Woodruff and Meinzer 2011b).

Research on the extent to which drought may cause trees to die due to C starvation (McDowell et al. 2008) has also emphasized the role of C availability. For instance, Adams et al. (2009) attributed the earlier death of trees subjected to drought and high temperature (which imposed an increased respiratory demand) relative to trees subjected to drought only to a depletion of stored reserves. Although this could have been the case, drought is known to impact the mobilization and long-distance transport of stored reserves, and the case has been made that trees could die (perhaps due to lack of C in localized tissues) well before they entirely consume their stored C pool (Sala 2009, Sala et al. 2010). This distinction is important because tree survival may depend not on the overall size of stored C pools, but rather on the accessibility and transport of these pools under drought.

In summary, the C-centric paradigm of tree growth and productivity does not appear to adequately capture underlying physiological complexities in most situations, but particularly under drought. A number of critical questions remain to be resolved. (i) To what extent and under what conditions is C actively allocated to storage at the expense of growth? (ii) Do stored C pools serve additional specific functions necessary for tree survival, other than the overflow/repository function to match C supply with C demand? (iii) If so, do woody plants exhibit thresholds of stored C pools below which they cannot survive? (iv) Do these thresholds vary depending on the tissue, species and environment? (v) How does the environment affect the mobility and transport of stored C (e.g., does water stress decrease the accessibility and transport of stored C)? (vi) Do stored C pools eventually become sequestered and unavailable (i.e., no longer storage)? (vii) To what extent is growth under natural conditions limited by C availability relative to the direct effect (not mediated via photosynthesis) of other factors (e.g., water, temperature, nutrients)?

The first challenge to the C-centric perspective came from the work of Körner and collaborators (summarized in Körner 2003). Based on data for mature trees in four climatic zones (high-elevation treeline, temperate lowland forests, Mediterranean sclerophyllous woodland and semi-deciduous tropical forests), reduced or zero growth was most often associated with maximum C loading of trees, suggesting that C assimilation exceeds C demand. These data also showed that NSC pools are generally high throughout the year, even after intensive reproductive events. The overall conclusion from these studies was that trees exhibit an overabundance of stored C and that growth is not limited by C supply. Instead, sink activity (i.e., growth) and its direct control by the environment or developmental constraints restrict biomass production of trees under current ambient CO₂ concentrations.

The sink limitation hypothesis suggests that in cases of drought, reductions in turgor-driven cell expansion have a greater and more immediate impact on tree growth than reductions in photosynthesis. This hypothesis is consistent with research indicating that trends in stem elongation and foliar morphological characteristics occur largely due to the influence of water stress on cell turgor and associated limitations on the sink strength of growing tissues (Marshall and Monserud 2003, Woodruff et al. 2004, Ishii et al. 2008, Meinzer et al. 2008). The sink limitation hypothesis is also consistent with the fact that growth is determined by many factors, several of which are more sensitive to drought than C assimilation (Hsiao 1973, Hsiao et al. 1976, Walter et al. 2009). Therefore, growth declines do not necessarily indicate C limitation, as traditionally assumed. Accordingly, most research in herbaceous and woody plants shows that stored NSC increases rather than decreases under moderate drought (Sala and Hoch 2009, Galvez et al. 2011, Muller et al. 2011, Woodruff and Meinzer 2011a). Sala and Hoch (2009) were the first to investigate stored NSC concentrations in trees of different heights as a means of investigating mechanisms for size-related reductions in productivity in trees and stands following canopy closure. Their research showed that the concentration of NSCs in branches of ponderosa pine increased with tree height at drier and moister sites (despite hydraulic constraints associated with taller trees), and that of lipids increased with tree height at drier, but not at moister sites (Figure 3). Woodruff and Meinzer (2011a) analyzed NSC in Douglas-fir trees of different height classes over a 17-month period and found that NSC concentrations were positively correlated with tree height and inversely correlated with leaf water potential and osmotic potential (Figure 4). This study also showed that although height- and water-stress-related trends in storage of NSC varied dramatically with seasonal changes in phenology, the larger, more water-stressed and slower-growing trees maintained a considerable amount of stored NSC year round (Figure 5). As in earlier work leading to the sink limitation hypothesis and assuming

that storage reflects the end balance between C supply and C demand, we interpreted these results (see also Sala et al. 2011) to indicate that water, and not C, limits growth under moderate drought and that the traditional C-centric perspective is not correct.

The notion that tree growth may not be limited by C supply is a clear (and needed) departure from the traditional C-centric perspective whereby growth is fundamentally dependent on C availability. However, both perspectives are based on the same assumption: that C storage is not an active sink and that either all C is eventually available for growth or that if C accumulates in storage pools it reflects an overabundance of C.

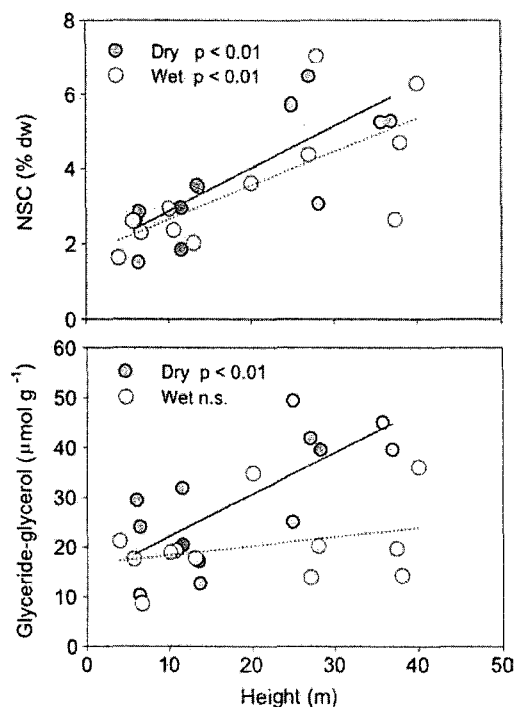


Figure 3. Concentration of NSCs (i.e., sum of free sugars and starch, top panel) and lipids (measured as glyceride–glycerols, bottom panel) in branch sapwood of ponderosa pines as a function of tree height. Data are for a dry (solid symbols) and a nearby moister site (open symbols). Adapted from: Sala and Hoch (2009).

Therefore, the same critical questions remain unanswered: to what extent is storage in trees an active sink that competes with other functions? If so, do stored C pools serve other functions in addition to buffers of C to match supply and demand by specific sinks?

Growth and C storage in trees: from C limitation to sink limitation ... and half way back

As noted above, C storage serves as a reservoir to buffer imbalances between C supply and demand, and conditions when growth is constrained by factors other than C supply often lead to an increase in C storage. Further, some of the stored C may eventually become sequestered and is no longer available as a reserve pool. Here, we suggest a possible additional explanation: as long-lived, perennial organisms, trees actively maintain a minimum threshold pool of stored C, whose osmotically active, soluble fraction is not only important to maintain cell turgor but may also be critical to maintain vascular integrity (xylem and phloem) in the face of fluctuating, and often unpredictable, environmental conditions. Phloem and xylem functions are intimately related (Thorpe and Minchin 1996). Long-distance phloem transport occurs by bulk flow following osmotically generated pressure gradients between source (where loading of sugars into phloem elements is followed by an influx of water) and sink organs (where sugar unloading is followed by water efflux; see Münch 1927). Thus, osmotically driven water supply, which ultimately comes from the xylem, is fundamental for phloem transport.

The importance of vascular integrity in plants cannot be overestimated. Trees (and plants in general) are modular organisms, and as opposed to animals, the integration of modules relies entirely on the delivery of water, nutrients and metabolites via the vascular system. For instance, under drought, a number of hydraulic and chemical signals are delivered throughout the tree via the vascular system including increased abscisic acid delivery from roots (Davies and Zhang 1991, Liang et al. 1996, but see Perks et al. 2002).

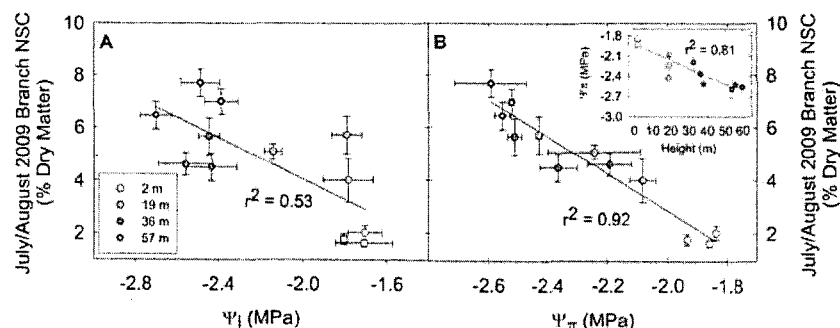


Figure 4. Percent dry matter of shoot NSCs plotted against leaf water potential (Ψ_l) (A) and shoot osmotic potential at full turgor (Ψ_π) (B) for four Douglas-fir height classes. Ψ_π plotted against height (inset, b). Error bars represent standard errors of three branches per tree. Adapted from: Woodruff and Meinzer (2011a).

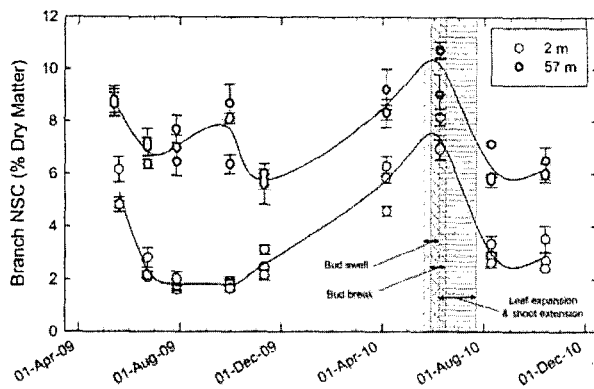


Figure 5. Percent dry matter of branch NSCs in 2 and 57 m Douglas-fir height classes plotted over a 17-month sampling period. Shaded areas indicate periods of time during which key phenological events occurred. Adapted from: Woodruff and Meinzer (2011a).

Signal delivery, in turn, causes changes in hormonal interactions (Wilkinson and Davies 2010) which ultimately result in a suite of responses in different parts of the plant. In addition to regular (e.g., seasonal) stressful conditions, long-lived plants are likely to experience periods of extreme stress during their lifetime. Therefore, natural selection should favor a coordination of responses that maintain the integrity of the vascular system, thereby enhancing stress resistance in the long term. If the xylem of a droughted plant experiences sufficiently high levels of irreversible embolism, death by desiccation will ensue and a lifetime investment in future reproduction is lost.

The increasing size and complexity of the vascular system of trees during ontogeny imposes the additional challenge of maintaining vascular transport: water, nutrients and assimilated substances are transported through larger distances (and therefore greater resistances) and against gravity. For instance, it is well known that size of trees imposes significant constraints on water transport that exacerbate the effects of drought (Ryan and Yoder 1997, Ryan et al. 2006). This is consistent with reports of greater drought-induced canopy dieback and mortality in large-sized trees (Nepstad et al. 2007, Floyd et al. 2009), although mortality in smaller trees with limited access to deeper water sources may also occur (Elliott and Swank 1994).

Research has shown that plant xylem can experience daily and seasonal cycles of embolism and refilling even under relatively benign, non-extreme conditions. Leaves (Zwieniecki et al. 2000, Nardini et al., 2001, 2003, Bucci et al. 2003, LoGullo et al. 2005, Johnson et al. 2009, 2011) and roots (McCully et al. 1998, Domec et al. 2006) seem particularly prone to losing and regaining a substantial fraction of their xylem hydraulic conductivity diurnally, but stems also exhibit this behavior (Salleo et al. 1996, Zwieniecki and Holbrook 1998, Melcher et al. 2001, Brodersen et al. 2010). There is increasing evidence that NSCs, particularly sugars, may be involved in the sensing (Secchi and Zwieniecki 2011) and

reversal (Bucci et al. 2003, Nardini et al. 2011) of embolism. For example, in leaf petioles of two tropical savanna tree species, Bucci et al. (2003) observed embolism-induced loss of xylem hydraulic conductivity in the morning followed by recovery in the afternoon, while the water in the remaining functional xylem conduits was still under considerable tension. This diurnal pattern was associated with the disappearance of starch grains and an increase in the sugar content in petioles from morning to afternoon. In addition to tension-induced embolism, trees growing in temperate regions also normally experience and recover from freeze-thaw-induced xylem embolism (Sperry and Sullivan 1992, Sperry et al. 1994, Mayr et al. 2003, 2006, McCulloh et al. 2011). In some species, the magnitude of winter embolism can exceed that induced by summer drought (McCulloh et al. 2011). It is not known whether carbohydrate transformations play a role in reversing freeze-thaw-induced embolism, but NSC content may be near its annual minimum value during the winter months in some conifers that experience substantial freeze-thaw-induced embolism (Woodruff and Meinzer 2011a). These and other observations imply that if carbohydrate transformations are integral to the mechanism of embolism repair, species-specific minimum pool sizes of stored C may be necessary to avoid catastrophic xylem failure. Thus C starvation and hydraulic failure may be inextricably linked as causes of drought-induced plant mortality rather than being alternative mechanisms (McDowell et al. 2011).

The dynamics of starch deposition and utilization follows the 'onion model', whereby starch is deposited in successive layers to form a starch granule, and degradation upon demand proceeds from outer to inner layers (Tester et al. 2004). If the constitutive C storage pools in trees serve as long-term buffers to maintain hydraulic integrity under severe episodic stresses, the onion model supports the hypothesis that older starch layers are only used very occasionally during periods of extreme stress when C demands greatly exceed supply, but not under typical conditions. Limited evidence is consistent with this interpretation. For instance, Vargas et al. (2009) monitored the age of C used to form new roots in tropical tree species prior to and after a hurricane that caused severe disturbance and loss of root mass. Prior to the hurricane, root growth was supported by C assimilated relatively recently. However, after the damage to crowns and roots from the hurricane, new root production relied on C assimilated as long ago as 11 years previously. This suggests that older C pools are only used under extreme conditions. Galiano et al. (2011) reported one of the very few examples showing an almost complete depletion of stored C reserves under drought (although they only measured stored C reserves in one tissue). In their study, total stored NSC in the bole sapwood of Scots pine (*Pinus sylvestris* L.) was almost completely depleted prior to mortality after a period of several years of extreme drought. Whether this reflected a depletion of

stored C pools throughout the tree or was just localized is not known. Even when it was only localized in the main trunk, the maintenance of hydraulic function under extreme drought might no longer have been possible, in which case whole-tree water transport and tree survival might have been compromised.

Responses of stored C reserves to drought are complex and appear to vary depending on the timing, species and the organ in question. For instance, Gruber et al. (2011) showed no decreases of stored carbohydrates in Scots pine during a year of severe drought. Galvez et al. (2011) showed a large increase of total NSCs in roots of aspen seedlings (*Populus tremuloides* Michx.) subjected to severe drought for 3 months. Although this study did not last long enough to allow the seedlings to die, increases of root NSC in aspen under drought have also been reported in the field (Anderegg et al. 2012) in stands affected by drought-induced sudden aspen decline, a condition responsible for widespread mortality in western North America. Aspen mortality, therefore, does not appear to be mediated via a depletion of root carbohydrates, but rather via loss of root biomass. As opposed to Scots pine, aspen is a resprouting, clonal species where resilience to severe drought may be a function of new root growth, rather than the repair of existing damage.

Conclusions

Research on the degree to which C availability limits growth in trees, as well as recent trends in climate change and concurrent increases in drought-related tree mortality on a global scale, have led to a renewed focus on the physiological mechanisms associated with tree responses to current and future climate. For instance, there has been renewed focus on C starvation as a process directly leading to tree mortality under drought. This has led to many questions and some dispute over the role of stored carbohydrates as an indicator of overall tree vigor, and more specifically, over its suitability as a reliable indicator of a tree's current demands for photosynthate. A first step towards resolving much of the uncertainty surrounding these issues could be achieved by developing a greater understanding of the different functions that may be performed by NSC that is stored within trees. In addition to functioning as a passive overflow/repository pool, the storage of non-structural C compounds may be under greater regulation than commonly recognized. We propose that, in addition to meeting C demands for growth, respiration, defense and export, an important function of stored C may be to maintain hydraulic transport. Given that drought, freeze-thaw events and increasing tree height all impose additional constraints on vascular transport, the general trend of an increase in NSC concentrations with tree size, drought or cold is consistent with (although it does not prove) this scenario. If the maintenance of relatively large constitutive stored C pools in trees

serves to maintain hydraulic integrity, then the minimum thresholds are expected to vary depending on the species, environment, growth form and habit. It may also vary depending on the specific tissues and their hydraulic characteristics. Much research is needed to elucidate the extent to which allocation of C to storage in trees is a passive vs. an active process, the specific functions of stored C pools and the factors that drive active C allocation to storage. Unequivocal tests of the extent to which storage is an active sink in trees will be challenging due to the lack of the types of starch mutants used in herbaceous plant model systems. Therefore, accurate direct measurements of all the components of the tree C balance concurrent with changes in C pools under varying environmental conditions will be necessary in order to address outstanding questions.

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