Chapter 11
Carbon Storage in Trees: Does Relative Carbon Supply Decrease with Tree Size?

Anna Sala, Willa Fouts, and Günter Hoch

Abstract Until very recently, age- and size-related declines in productivity of individual trees and stands have been attributed to reductions of carbon availability due either to progressive increases in carbon sinks other than growth (Respiration hypothesis) or to decreases carbon sources (Assimilation hypothesis). Although the validity of these hypotheses is now questioned and new alternative explanations have been proposed, actual data on size-dependent changes of stored mobile carbon in mature trees (used as indicators of carbon balance between source and sink activities) are surprisingly limited. Based on available data for mature trees and consistent with evidence that mature trees under current atmospheric CO_2 concentrations are not carbon limited, the relative carbon supply in trees does not become increasingly limited as they grow large. In spite of many uncertainties, research to date points to the need to question the historically carbon-centric mechanisms proposed to explain age-related growth declines in trees and forests. Future research should focus on whether and when alternative growth limiting factors (e.g. turgor, long distance transport of assimilates, nutrients) may contribute to growth limitations in tall trees.

1 Introduction

Height growth poses significant challenges in trees including increases in biomechanical risks associated with heavier loads (Niklas 2007; King 2011), increase of the proportion of woody, non-photosynthetic tissues (Kira and Shidei 1967;

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Whittaker and Woodwell 1967; Peet 1980; Kozlowski et al. 1991), increase of distance over which vascular tissues need to move water, nutrients and assimilated substances throughout the tree (Mencuccini et al. 2007; Mencuccini and Hölttä 2010), and an increase of the gravitational pull against the ascent of water (Koch et al. 2004; Woodruff and Meinzer 2011). Because trees (like any other plant) must ensure favorable water and carbon balance over time, it is not surprising that they undergo a variety of biochemical, physiological, anatomical and structural adjustments that minimize the negative effects of height growth, thereby sustaining them as biomechanically, biophysically and physiologically viable organisms (Ishii 2011; Scholz et al. 2011; Steppe et al. 2011; Woodruff and Meinzer 2011).

A very well-known and common phenomenon occurring as individual trees or mono-specific stands age and increase in size is a decline of growth rates and net primary productivity (Ryan et al. 1997). However, the underlying mechanisms for this general pattern are still under debate and remain unresolved. Several explanations have been proposed over the years to explain the so called age-related decrease of tree and forest productivity, including increased relative respiration losses, nutrient limitations and decreases of carbon assimilation, which in turn, are caused by a variety of factors such as hydraulic constraints, reductions of leaf area, and genetic factors (Gower et al. 1996; Ryan et al. 1997, 2004; Day and Greenwood 2011; Steppe et al. 2011). These classical explanations can be grouped into two main classes of competing hypotheses: 'Respiration hypotheses' (age-related declines in productivity are due to proportional increases of respiratory demand as trees increase in size) and 'Assimilation or Gross Primary Productivity hypotheses' (reduced growth rates result from declining carbon assimilation in response to a variety of causes). A common feature of these two competing groups of hypotheses is that they both ultimately predict that age-related declines in growth rates are due to changes in C-balance with tree size and age. In other words, they both predict reductions of carbon available for growth as trees age and grow tall. Although the validity of these hypotheses has now been questioned and alternative hypotheses have been proposed (see below), the carbon-centric view embodied by these two classes of hypotheses has never been properly tested. Surprisingly little research has examined patterns of stored non-structural carbon as trees age and grow large, which is the main focus of this chapter. We ask the following two questions: (a) Does the concentration of stored non-structural carbon compounds change as trees increase in size or age? (b) If so, do these changes suggest that trees become carbon limited as they increase in size? We first provide a very brief outline of the historical development of the two classical hypotheses proposed to explain height/age-related growth declines and outline current alternative hypotheses. We then examine the use of stored non-structural carbon compounds in trees as indicators of carbon balance and relative carbon source-sink activities, and briefly review evidence to date on whether mature trees under ambient CO_2 levels are carbon limited. We follow by presenting the limited available data on changes in stored carbon pools with tree height and their implications for the mechanisms responsible for the height/age-related decline of productivity in trees and forests. Finally, we provide suggestions and methodological considerations for future research.

2 Mechanisms for the Age-Related Decline in Productivity

Until recently, the mechanisms underlying the age-related decline in above ground net primary productivity of forests (Ryan et al. 1997) have been embodied in two main categories of competing hypotheses (Gower et al. 1996; Ryan et al. 2004). The ‘Respiration Hypothesis’, initially proposed by Kira and Shidei (1967), predicted that age/size related declines in growth rates result from a progressive imbalance between carbon assimilation and respiration as trees age and grow tall due to the increased respiratory load imposed by the greater proportion of woody, non-photosynthetic tissues. However, neither initial field observations and modeling approaches (Ryan and Waring 1992), nor recent experimental approaches (Ryan et al. 2004) provide support for the respiration hypothesis. The alternative “Carbon Assimilation hypothesis” in contrast, predicted that age/size-related decreases in carbon assimilation, rather than increases in respiration, were responsible for the age-related declines in growth rates. While a number of factors have been invoked to explain such age-related declines in gross primary production (Ryan et al. 1997), intense recent research has focused on the negative effect of tree size on water relations and gas exchange (Ryan et al. 2006). This is because as trees grow tall, the increase in gravitational pull against the ascent of water combined with the increase of the overall xylem hydraulic resistance from roots to leaves result in greater xylem tensions and a potentially higher risk of cavitation and hydraulic failure (Zimmermann 1983; Tyree and Sperry 1989). Because stomatal closure is the most effective mechanism to reduce water loss and minimize the risk of hydraulic failure, tall trees are expected to close stomata and reduce carbon assimilation to a greater extent than shorter trees (Friend 1993). Indeed, this prediction was first corroborated by Yoder et al. (1994) in the field. Subsequently, Ryan and Yoder (1997) proposed the Hydraulic Limitation Hypothesis (HLH) as a possible mechanism to explain the age-related decline of productivity in individual trees and stands. The HLH as initially stated proposed that hydraulic constraints on gas exchange as trees grow and increase in height were responsible for the age-related decline of productivity in trees. The HLH was extraordinarily well received because of the initial lack of support for the respiration hypothesis (Ryan and Waring 1992) and because compensatory mechanisms to minimize height-related hydraulic constraints (e.g. Coyea and Margolis 1992; Goldstein et al. 1998; Barnard and Ryan 2003; Zhang et al. 2009; Woodruff and Meinzer 2011) are generally not enough to offset the negative effect of height on gas exchange (Ryan et al. 2006).

About a decade after the formal publication of the HLH, a review of the literature by Ryan et al. (2006) concluded that in spite of compensatory mechanisms to offset path length effects and to cope with the negative effects of gravity, hydraulic constraints on gas exchange are very common in tall trees. Importantly, however, they found no evidence supporting that reductions in carbon assimilation in tall trees are responsible for growth reductions. In other words, hydraulic limitations on gas exchange and on growth are very common in trees, but the first is not the cause for the latter. This left the mechanisms responsible for the age-related decline in tree and forest productivity unresolved.

It is noteworthy that both the respiration and the carbon assimilation hypothesis ultimately predicted that trees become carbon limited as they grow tall and mature. Such a prediction is in stark contrast with increasing evidence that mature trees growing under ambient atmospheric CO_2 concentrations are not carbon limited (Körner 2003, see below). Surprisingly, up to very recently, this evidence was almost entirely ignored in the debate, perhaps because the implicit prediction that reduced available carbon would lead to C-limitation for growth was assumed to hold true.

There is now evidence that reduced turgor during cell expansion at the top of tall trees is responsible, at least in part, for reduced cell extension and growth (Koch et al. 2004; Woodruff et al. 2004; Ishii et al. 2008; Meinzer et al. 2008; Woodruff and Meinzer 2011). This and the lack of support for the respiration and the hydraulic limitation hypotheses as initially stated led to questioning of the critical assumption that growth in tall trees is limited by carbon availability (Ryan et al. 2006) and to suggestions that factors limiting cell growth and carbon demand may be ultimately responsible for the reduced growth rates in tall trees.

Although support for the carbon assimilation and respiration hypotheses is lacking and current research invokes mechanisms independent of carbon availability to explain the age- and size-related decrease in tree productivity, the notion that carbon availability limits growth in tall or old trees remains common. This is due in part to the scarcity of actual data on changes of non-structural carbon availability as trees increase in size and age.

3 Mobile C-Reserves in Trees

About half of all dry plant biomass consists of C, rendering C the most abundant plant nutrient. In addition to current photosynthesis, plants temporarily rely on stored C-reserves in order to maintain their metabolism. During periods of high photosynthetic activity, a fraction of recently assimilated C is generally directly fed into C storage pools. This process might be an active formation of C-stores, that otherwise would be used for C-sink activities (e.g. respiration, growth), or a passive accumulation of surplus photoassimilates during periods when photosynthate production exceeds the total C requirements of a plant (Chapin et al. 1990). Hereafter, we refer to all compounds that are orderly degraded and re-synthesized in response to changes in C source-sink-balances as ‘mobile C compounds’. In principle, this definition includes all compounds that serve any cell function (in addition to storage), but which can be converted (recycled) to provide additional C-sources. However, the most important and ubiquitous mobile C compound is starch, which is exclusively synthesized for storage. Besides starch, other strict storage compounds are fructans and neutral lipids. While fructans are used alternatively to starch in some species, neutral lipids are considered to serve mainly as long-term C-stores, due to the relatively high energy demand for re-mobilization of fatty acids (Hoch et al. 2002). Low molecular weight sugars are highly mobile (readily ‘available’ for C-sink processes) and therefore are considered important C-reserves in plants.

However, their function as metabolic intermediates and osmotically active compounds (e.g. Arndt et al. 2008) clearly restricts a complete exhaustion of free sugars in living tissue. The frequency with which C-reserves are used and re-synthesized varies from diel (day-night-cycle of leaf-starch) to seasonal (e.g. winter dormancy in temperate climates) to episodic (e.g. re-flushing after leaf-loss to herbivores) cycles. Finally, the extent to which plants depend on mobile C compounds differs among life forms (e.g. annual vs. perennial plants) and environmental conditions (e.g. seasonal vs. non-seasonal climates).

Trees are distinguished from other functional plant types by a high proportion of heterotrophic tissue (i.e. wood), leading to higher C-demand per leaf area basis compared to other plant types (Stevens and Fox 1991), although a significant fraction of the wood consists of dead, non-respiring tissue (heartwood, Larcher 2003). In addition, hydraulic constraints in tall trees reduce leaf gas exchange and net-photosynthesis at the leaf level (see Ryan et al. 2006). Therefore, trees are generally considered to be more susceptible to C-limitation than most other plant types (Stevens and Fox 1991; Kozlowski and Pallardy 1997), emphasizing the importance of C-storage in this plant functional group, especially in mature, tall individuals. In seasonal climates where trees are periodically dormant (e.g. during winter or the dry season), stored C-reserves are needed to maintain cell function. Leaf flushing in deciduous trees depends on C stored during the previous season because of the lack of photosynthetically active tissue at bud break (Landhäusser and Lieffers 2003; Schädel et al. 2009). In evergreen trees of temperate climates, shoot growth is also partially supplied from C-reserves, which accumulate in mature leaves prior to bud break (Fischer and Höll 1991). Fruiting and especially mast fruiting has been traditionally thought to resemble a major sink for stored C-reserves in trees (Kozlowski and Pallardy 1997). However, current research suggests that the formation of fruits is largely supplied by current photoassimilates and thus independent of the import of stored C, even in masting tree species (Hoch 2005; Hoch and Keel 2006, and unpublished data by A. Sala and G. Hoch).

To the extent that mobile C-compounds resemble a longer term ‘buffer’ between C-source and -sink activities, their tissue concentrations mirror (at least to a certain degree) the net-balance between C-acquisition by photosynthesis and the sum of C used for biosynthesis and respiration (Fig. 11.1). Thus, deliberate comparative analyses of mobile carbon concentrations may be used as a proxy for the C-supply status of a plant. Clearly, the C-source and -sink activities are not independent processes, but are mutually influenced by complex and still not completely clarified feed-back and feed-forward mechanisms (Smith and Stitt 2007). Progressively decreasing C-reserve concentrations will ultimately induce negative effects on C-sink activities (i.e. C-limitation), while a sustained C surplus will increase the tissue concentration of mobile C compounds to a threshold at which negative feed-back mechanisms will lead to a down-regulation of photosynthesis (Fig. 11.1). Therefore, a plant’s mobile C pool will neither be completely exhausted at C limitation, nor increase infinitely in situations of C oversupply, but its size will respond within certain limits to changing C source-sink relations. Evidence of the responsiveness of mobile C-stores to C source-sink imbalances suggests the validity of this approach.

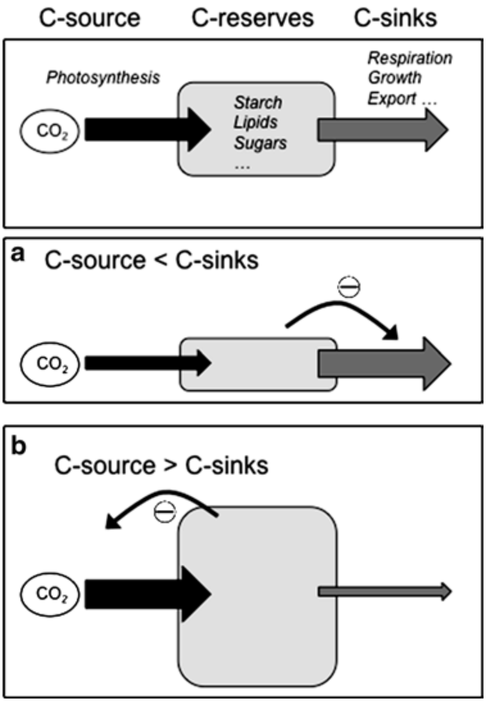

Fig. 11.1 Schematic illustration of the potential use of mobile carbon reserve concentration as indicators of carbon balance between sources and sinks. The thickness of the *arrows* is proportional to the amount of activity. Potential negative feedback and feedforward mechanisms are indicated by the *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 carbon demand (*panel 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 (*panel b*)

For example, tissue concentrations of starch and low molecular weight carbohydrates generally increase in plants exposed to elevated atmospheric CO_2 (e.g. Teng et al. 2006) and decrease in plants growing at low CO_2 concentrations (e.g. Agüera et al. 2006). Similarly, scion-trunk girdling of 3-year-old *Prunus* trees induced higher concentrations of starch in leaves and shoots and decreased starch concentrations in roots (Jordan and Habib 1996). Finally, non-structural carbohydrate concentrations have been shown to decrease in plant tissues after experimental defoliation (Canham et al. 1999; Li et al. 2002) and shading (Piper et al. 2009).

So far, comparative analyses of C-reserve concentrations have been mainly used to investigate the extent to which insufficient photosynthetic C-supply explains the limits of tree growth in cold climates. A series of studies along altitudinal gradients from lower elevations to the alpine treeline found no indication of decreasing tissue concentrations of mobile C-reserves in treeline-forming trees (e.g. Hoch and Körner 2003, 2005; Hoch et al. 2002; Piper et al. 2006; Shi et al. 2006, 2008). Rather, there is a common trend of increasing concentrations of C-stores with elevation, which suggests C-sink limitation, rather than a limitation of C-source activities, as the physiological mechanism behind cold climate treeline formation. Increased concentrations of mobile C reserves at low, growth-limiting temperatures were also found in tree seedlings under controlled greenhouse-conditions (Domisch et al. 2001; Solfjeld and Johnsen 2006; Hoch and Körner 2009), in conifer seedlings transplanted to different altitudes across the treeline ecotone (Bansal and Germino 2008), as well as in dwarfed spruce trees growing on permafrost amidst tall forest trees (Hoch 2008). Similar to the above described studies along altitudinal gradients, comparative analyses of tissue concentrations of mobile C compounds in trees of different size classes can be employed to estimate the C supply status of trees and to elucidate the question whether insufficient C-balance explains the decreasing productivity of trees approaching their maximum height (Sala and Hoch 2009).

4 Existing Evidence for Sufficient C-Supply at Current Atmospheric CO_2 Concentrations

Independent of the comparative approach mentioned above, there is existing evidence for sufficient C supply of mature trees at current atmospheric CO_2 concentrations. For example, year-round high concentrations of mobile C reserve pools were described for mature trees in temperate (Hoch et al. 2003) and tropical (Würth et al. 2005) climates. Hoch et al. (2003) calculated that the mobile C reserves stored as starch and low molecular weight sugars in the aboveground wood of mature deciduous trees at a temperate mixed forest, would be sufficient to re-place the entire leaf canopy four times (disregarding the high degree of C-autonomy of developing leaves, which strongly decreases the effective need for stored C). Unlike mobile N- or P-compounds, mobile C-compounds are often not recovered from senescent leaves prior to leaf fall (but see Newell et al. 2002), and a considerable fraction of the starch pool stored in wood is likely never re-mobilized in trees, suggesting that parts of the starch synthesized by trees is sequestered rather than stored (Millard et al. 2007). Consequently, Körner (2003) and Millard et al. (2007) argued that the large pools of stored C-reserves in mature trees reflect an overall sufficient C-supply at ambient atmospheric CO_2 concentrations (which have increased by over 30% since the beginning of the industrial revolution in the middle of the eighteenth century, from c. 280 ppm CO_2 to over 385 ppm CO_2 in the present). If we consider the changes in atmospheric CO_2 over relatively recent geological times, the presumed overabundant C-supply of trees at present times seems logically consistent. For instance, Antarctic ice core analyses by Petit et al. (1999) and Siegenthaler et al. (2005) revealed that during the last 650 000 years the CO_2 concentration in the earth's atmosphere has never significantly exceeded 300 ppm (with minima as low as c. 180 ppm during the peaks of the ice ages). Further, the present CO_2 concentrations are likely the highest over the past 20–25 Ma (Pearson and Palmer 2000; Pagani et al. 2005). Thus, most modern plant species evolved at CO_2 concentrations markedly lower than today, and may have evolved to maximize the uptake and use of this ‘scarce’ nutrient (Körner 2006). Millard et al. (2007) even suggested that if CO_2 was limiting growth during plant evolution, the relatively inefficient carboxylation function of Rubisco would have been improved, which has not been the case. Instead, they suggested that Rubisco may serve as a form of nitrogen storage, which may lead to Rubisco concentrations in leaves even higher than actually needed to sustain a favorable carbon balance. In conclusion, the large C-reserve pools found in mature trees might reflect the current overabundance of atmospheric CO_2. In turn, this could also mitigate potential increases of carbon demands as trees grow old and tall.

5 Does Carbon Supply Become Limiting as Trees Increase in Size?

The approach used to asses C-balance at treeline mentioned above, was recently used by Sala and Hoch (2009) in ponderosa pine (*Pinus ponderosa*) to examine whether carbon supply limits growth as mature trees increase in size. To our knowledge, this and the recent work of Genet et al. (2010) remain the only published data to date on changes in stored C with size in mature trees. In contrast to the predictions of the respiration and carbon assimilation hypotheses, but consistent with the argument that mature trees are not carbon limited, Sala and Hoch (2009) found that size/height-related growth reductions were associated with significant increases of non-structural carbohydrates (NSC) in needles (not shown), branches and bole sapwood (Fig. 11.2) as well as of lipids in branch sapwood (Fig. 11.3) once active growth had ceased (mid-August). Although lipids in the bole decreased significantly with height and size, this did not necessarily indicate insufficient C-supply of tall trees (cf. Sala and Hoch 2009). These results not only suggest that carbon supply does not limit growth in ponderosa pine as trees increase in size, but that net C-supply increases with tree height. Further, we also found that increases of the concentration of mobile carbon compounds with tree height were stronger in a drier site relative to a moister site (Figs. 11.2 and 11.3), indicating that water availability might negatively impact C-sink activities (e.g. growth) more than net-photosynthesis.

In a study of relative carbon allocation to growth, storage and reproduction in European beech (*Fagus sylvatica*) and sessile oak (*Quercus petraea*) Genet et al. (2010) found that NSC concentrations in stems did not change in trees from stands between 50 and ca. 150–200 years old. In contrast, concentrations in beech roots decreased as stand age increased from 50 to 200 years old. Overall, however, estimated whole tree NSC concentrations and carbon allocation to storage was constant (oak) or slightly increased (in beech) with stand age. These results led the authors to interpret that carbon investment in storage with stand age was the cause for an observed decrease of carbon allocation to growth. However, we suggest (see above) that the age-related growth decline could result from hydraulic constraints, independently of carbon availability. If so, carbon investment into storage could be a consequence, rather than a cause of limited growth. Ultimately, both studies (Sala and Hoch 2009; Genet et al. 2010) show no age- or size-related declines of mobile C

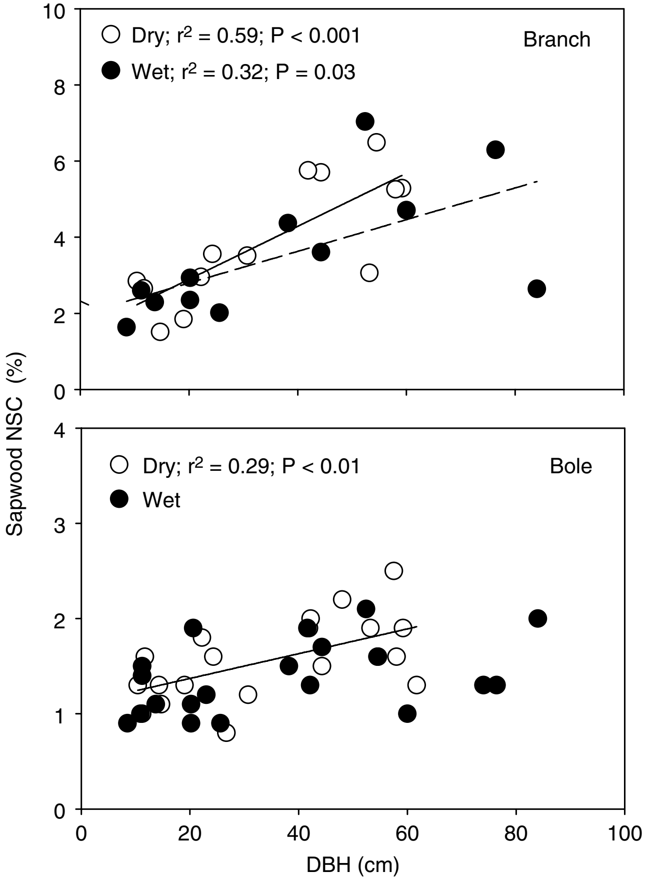

Fig. 11.2 Sapwood non structural carbohydrate (*NSC*) concentration in terminal, upper canopy branches (*upper panel*) and in the main trunk (bole; *lower panel*) of *Pinus ponderosa* as a function of tree diameter at breast height (*DBH*). Only the outer ten rings of sapwood were analyzed in the bole samples. Data were collected in August 2007 in a relatively moister site (wet, *black circles*) and drier site (dry; *open circles*) in Western Montana, USA (E-L Ranch site). *Lines* denote significant ($P<0.05$) linear relationships within each site (Redrawn from Sala and Hoch 2009)

reserves, which we interpret as evidence that carbon availability does not decrease with tree size or age.

In mature coast redwoods (*Sequoia sempervirens*; among the tallest trees on earth) Bentrup (2009) found increases, no change, or decreases of NSC with tree height depending on the tissue analyzed and the season of the year, with a tendency for an increase in NSC concentrations with tree height at the end of the season. Such mixed results led Bentrup (2009) to tentatively conclude that the tallest trees may temporarily be carbon limited early in the growing season. However, as we discuss below, seasonal trends of NSC concentrations need to be interpreted carefully due to potential confounding effects of phenology.

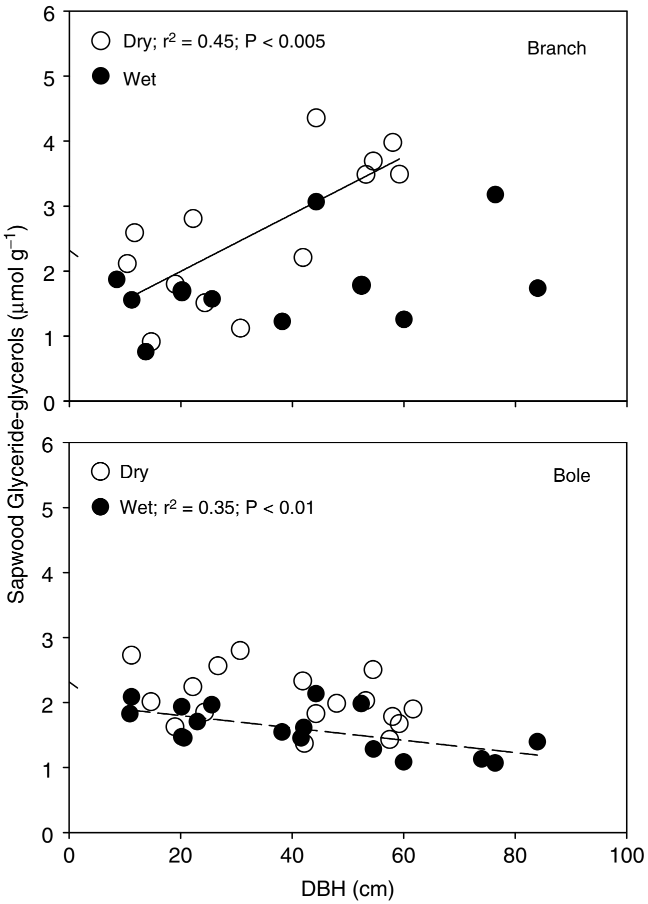

Fig. 11.3 Sapwood lipid concentration (measured as glyceride-glycerols) in terminal, upper canopy branches (*upper panel*) and in the main trunk (bole; *lower panel*) of *Pinus ponderosa* as a function of tree diameter at breast height (*DBH*). Only the outer ten rings of sapwood were analyzed in the bole samples. Data were collected in August 2007 in a relatively moister site (wet, *black circles*) and drier site (dry; *open circles*) in Western Montana, USA (E-L Ranch site). *Lines* denote significant ($P<0.05$) linear relationships within each site (Redrawn from Sala and Hoch 2009)

In addition to the published studies outlined above, here we present preliminary data for mature trees of two additional species: Douglas-fir (*Pseudotsuga menziesii*), a common and wide-spread species in western North America, and whitebark pine (*Pinus albicaulis*) a high elevation, five needle pine in the Rocky and Cascade Mountains and the Sierra Nevada. Along with Douglas-fir, we also collected supplementary data for ponderosa pine. Data for Douglas-fir and ponderosa pine were collected during the summer (mid-August) of 2009 on a dry, steep South-facing slope in the Rattlesnake Valley of western Montana (46.6°N and 113.2°W; 1,180 m elevation). The site has sparse trees which allowed us to sample isolated trees of

Table 11.1 Species sampled at each site, number of individuals sampled, DBH (cm), height (m) and age (yr) ranges

Species	Site	Number of trees	DBH range	Height range	Age range
Ponderosa pine	EL Ranch				
	Moist	12–20	8.5–84	3.9–40	15–126
	Dry	12–20	10.4–61.7	5.7–37.2	18–110
	Rattlesnake	10	10.8–67.2	5.5–32.7	n.m.
Douglas-fir	Rattlesnake	7	11.7–69	6.1–22.1	n.m.
Whitebark pine	Flint Creek	9	19–53.5	9.5–17.2	63–157

Tissues sampled were terminal branch sapwood (1- and 2-yr-old segments) at EL Ranch, bole sapwood at DBH (ten most recent rings) at EL Ranch and Rattlesnake, and 1-yr-old needles, terminal branch sapwood (6–8-yr-old segments) and bole sapwood at DBH (all sapwood; 9–90 rings) at Flint Creek. Data for Ponderosa pine at the EL Ranch site are from Sala and Hoch (2009) and are shown here for comparative purposes. n.m. = not measured

different sizes (Table 11.1). Mean annual air temperature in Missoula, Montana (ca. 5 km from the site) during the last 10 years is 8.1°C, with mean January and July temperatures of −2.4°C and 21.1°C, respectively. Mean annual precipitation is 417 mm.

Data for whitebark pine were collected at the end of the growing season (end of September) in 2007 at the Flint Creek range of western Montana (46.2°N and 113.3°W; 2,300 m elevation) in a mixed stand dominated by whitebark pine and subalpine fir (*Abies lasiocarpa*). Mean annual air temperature at the closest weather station (Anaconda, Montana, ca. 1,700 m elevation) is 6.1°C, with mean January and July temperatures of −4.5°C and 17.6°C, respectively. Mean total annual precipitation is 350 mm. Although the Flint Creek area is generally dry, the specific site is located on a local plateau, where the sampled stand surrounds a relatively moist meadow with an ephemeral spring dissecting it. For simplicity, we only show the data from samples collected in 2007, a low cone year, although data for other years shows similar patterns. At this site, we sampled nine trees with no or minimal shading effects by neighboring trees (Table 11.1).

Details on tree size, age and tissues sampled at each site are given in Table 11.1. All samples were immediately stored in a cooler with ice. After return to the laboratory (on the same day of sampling), all samples were microwaved (i.e. shock-heated) to interrupt all enzymatic activity. The bark and phloem was removed from bole and branch samples. For bole sapwood, the ten most recent rings were analyzed in all cases, except for whitebark pine, where all sapwood was analyzed (Table 11.1). All samples were oven dried for 2 days at 65°C, ground to a fine powder using a Wiley mill, and analyzed for NSC. For whitebark pine, samples were also analyzed for total acylglycerols (lipids). Chemical analyses for NSC and lipids followed the procedures described in Sala and Hoch (2009). In whitebark pine, which often has a unique growth form with multiple trunk individuals, DBH is a good indicator of overall tree size (biomass; Callaway et al. 2000). For instance, at our site trees are relatively short with rounded canopies and height is less meaningful than DBH. Because of this, and because DBH is correlated with age and height in ponderosa

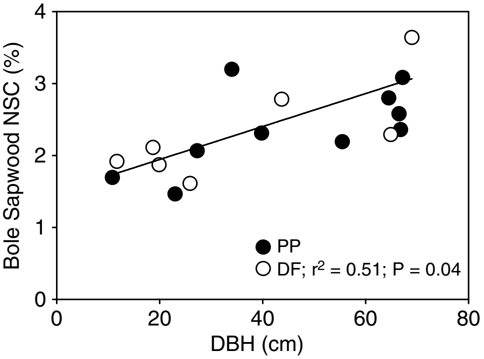

Fig. 11.4 Bole sapwood NSC concentrations in *P. ponderosa* (PP) and *Pseudotsuga menziesii* (DF) as a function of tree diameter at breast height (*DBH*). NSC concentrations were measured in the outer ten rings of sapwood. Trees were sampled in mid-August 2009 in the Rattlesnake Valley of western Montana. *Lines* denote significant ($P < 0.05$) linear relationships within a species

pine and Douglas-fir, we opted to present all data as a function of DBH. However, results did not change when expressed as a function of height (not shown).

In ponderosa pine and Douglas-fir NSC concentrations in the outer sapwood tended to increase with DBH (Fig. 11.4), which corroborates our previous conclusion (Sala and Hoch 2009). However, in contrast to the drier site (E-L Ranch) sampled by Sala and Hoch (2009) during an exceptionally hot and dry year, the increase of NSC with tree size in ponderosa pine at the Rattlesnake site was not statistically significant (precipitation during the 2009 summer was above average). Apparently, both short- and long-term differences in water availability influence NSC concentrations and changes with tree size. In no case, however, did NSC decrease with tree size as expected based on predictions derived form the original hydraulic limitation hypothesis.

In whitebark pine neither NSC in bole and branch sapwood and in needles, nor lipids in branch and bole sapwood (Fig. 11.5) decreased with increasing tree diameter. On the contrary, there was a statistically significant increase of lipids in branch sapwood with tree age ($r^2 = 0.52$ $P = 0.04$; not shown). Note, however, that these results are for the entire sapwood and we have not systematically measured changes of NSC concentrations with sapwood depth. However, bole sapwood NSC in our data did not change significantly as a function of sapwood depth or number of rings (not shown), suggesting little change in NSC concentrations with sapwood depth. Overall, results for whitebark pine are consistent with our previous conclusion that carbon supply does not become increasingly limited as trees increase in size.

Besides mature trees, changes in NSC concentrations with plant size have also been examined in saplings of *Abies balsamea* (a shade tolerant tree), *Acer rubrum* (intermediate shade tolerance) and *Pinus strobus* (a pioneer, shade-intolerant tree) in a moderately deeply shaded environment (up to 13% full sunlight) in Minnesota (Machado and Reich 2006). Saplings in this study were small (up to 1.3 m in height) and ranged from 6–8 to 21–24 years of age, depending on the species. The small

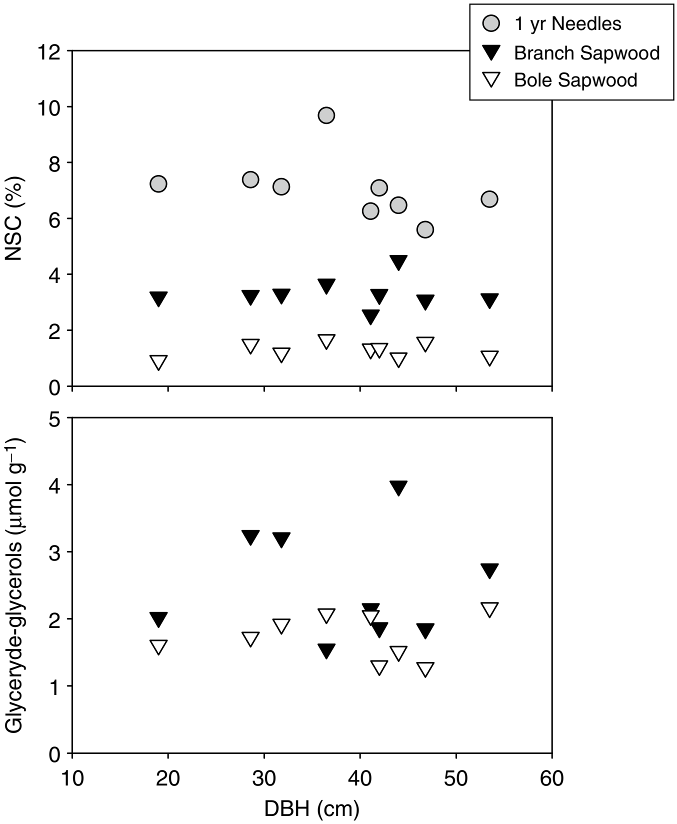

Fig. 11.5 NSC concentrations (*upper panel*) and total lipid concentration (measured as glyceryl-glycerols; *lower panel*) in whitebark pine (*Pinus albicaulis*) as a function of diameter at breast height (*DBH*). *Gray circles*: 1 year old needles; *black triangles*: terminal branch sapwood; *white triangles*: bole sapwood. All sapwood was analyzed in bole samples (see text). Lipids were not analyzed in needles. Trees were sampled in late September 2007 at the Flint Creek range of western Montana

stature of the saplings combined with the moist, shaded environment, suggests that hydraulic constraints on gas exchange were not an issue. However, carbon acquisition was likely constrained by low light, and changes in NSC with sapling size provide insight into size-related carbon source-sink dynamics. NSC concentrations in this study were measured in leaves, stems and roots at the end of the summer, which allowed estimation of whole plant NSC concentration as a function of plant size. While patterns of NSC concentration with sapling size within a single species were not necessarily consistent among different tissues, whole plant NSC concentrations did not change significantly with sapling size in *Abies* or *Acer* (with high and intermediate shade tolerance, respectively), but it significantly decreased in *Pinus* (shade-intolerant). To our knowledge this is the only published convincing evidence of increasingly limited carbon supply via photosynthesis with increases in plant size.

Such carbon limitation, however, is to be expected in this particular case involving a pioneer, light-demanding species growing in a deeply shaded environment. Notably, no such pattern was found in the more shade tolerant species equipped to function under low light environments. It remains to be seen whether the size-dependent decreases of NSC concentrations observed in *Pinus* saplings also occur once trees reach the canopy and the upper foliage becomes exposed to full light. Our previous (Sala and Hoch 2009) and current results in mostly isolated, mature trees suggest these patterns disappear or even reverse.

6 Methodological Considerations and Future Research Questions

Assuming that the concentration of stored carbon compounds at the end of the season reflects the end balance between carbon demand for earlier growth, and carbon supply by photosynthesis, data available so far suggest that the relative carbon supply in mature trees does not become increasingly limited as they grow larger. However, the limited available data preclude broad generalizations and highlight the need for further research to address pending questions. First, additional research is needed to elucidate the mechanisms responsible for the decline in their growth rates as trees become larger and older, including additional work on the role of turgor in cell expansion (Woodruff and Meinzer 2011), genetic and ageing factors (Day and Greenwood 2011), nutrient limitation, and long distance transport of assimilates. The latter is a major void in our understanding of tree physiology under environmental stress (Sala et al. 2010) and may be involved in growth declines as trees grow old and large (Woodruff and Meinzer 2011). With respect to tree carbon dynamics, there are also many open questions. For instance: (1) in what tissues and at what times during the year is the concentration of mobile carbon compounds most likely to reflect actual imbalance between sources and sinks (see Bansal and Germino 2008)?; (2) are height-related changes in stored carbon pools species-specific?; (3) how does environmental stress influence relative source and sink activities?; (4) when and to what extent does carbon allocation to reserve and other functions compete against growth as a carbon sink?; (5) does stored mobile carbon in trees become inaccessible? The latter question is very relevant as there is some evidence that plants fail to use stored C pools even under stress (Chapin et al. 1990; Li et al. 2002; Piper et al. 2009, but see Vargas et al. 2009), which suggests that these pools become unavailable over time (Chapin et al. 1990; Millard et al. 2007; Srichuwong and Jane 2007), and thus, by definition, are no longer mobile carbon pools. The question remains whether such inaccessibility varies over time and depends on stress (e.g. Vargas et al. 2009). If carbon reserve compounds become sequestered over time and there is a temporal mismatch between carbon supply by photosynthesis and carbon demand (i.e. times of maximum photosynthesis do not coincide with times of maximum carbon demand), then the issue of carbon limitation for certain functions still remains on the table. Therefore, it is crucial to understand the relative stress-sensitivity of carbon demand functions vs. carbon supply from photosynthesis and/or storage. However, current experimental evidence that changes in tissue concentrations of mobile carbon compounds reflect relative source-sink activities (see above) suggest that only surplus carbon is sequestered. Clearly, much research is needed to fully understand how tree size, environment, stress, and species-specific life history and ecology influence the balance between instantaneous carbon demand and carbon supply, active and passive allocation to storage, and accessibility of storage pools.

The concentration and seasonal dynamics of mobile carbon compounds varies depending on the organ and the species-specific phenological development (e.g. Barbaroux and Bréda 2002; Newell et al. 2002; Hoch et al. 2003; Sayer and Haywood 2006). Such variation suggest that an accurate picture of source-sink dynamics in trees requires detailed seasonal measurements of phenology in major organs (leaves, branches, roots and stems) and their corresponding mobile carbon compound concentrations. When such sampling intensity is unfeasible, the decision on when and what to sample becomes important. If access to stored carbon pools to meet instantaneous carbon demands is not an issue, once seasonal growth is completed, the residual mobile carbon compound concentration should reflect the end balance (deficit, surplus, or no change; see above). In this case, if we are interested in evaluating whether carbon supply becomes increasingly limiting for growth as trees increase in size, sampling tissues that significantly contribute to total stored carbon pools at the end of the active growing season is best. Further, because the concentrations of mobile C-reserves in plants are the net-outcome of multiple active (e.g. reserve formation and use) and passive (e.g. accumulation of overabundant photoassimilates) processes, patterns of change in NSC concentration with size are very sensitive to timing and phenology and need to be interpreted with caution. For instance, in deciduous and evergreen temperate tree species, transient, fast changes of C-reserve concentration have been shown to occur just prior and after bud break (Barbaroux and Bréda 2002; Hoch et al. 2003; Schädel et al. 2009). Therefore, comparative studies on changes of NSC concentrations in response to factors other than phenology need to rule out potential confounding effects due to phenology. Otherwise, even slight temporal phenology shifts (such as the time of leaf flushing from saplings to mature trees; Mediavilla and Escudero 2009), could induce differences in mobile C reserve concentrations which are unrelated to the integrated net C-balance.

In deciduous species, roots and main stems generally contain the largest fraction of the total pool of stored carbon (Kozlowski and Keller 1966; Barbaroux et al. 2003). In contrast, needles usually represent the largest C-storage pool in conifers (Hoch et al. 2002; Li et al. 2002), although roots and stems also contribute significantly. However, the strong and rapid fluctuations of NSC concentrations in needles of conifers over short-time scales, particularly around bud break (see above) may complicate the interpretation of age-related changes in the concentration of stored carbon pools. Despite the fact that trunks and main stems may constitute a very large fraction of the total stored carbon pools due to their large biomass, in most trees NSC concentrations in trunks and main stems are lower relative to branches, fine roots and foliage, and tend to exhibit the lowest seasonal variation (Hoch et al. 2003). This suggests that trunks and main stems are less dynamic storage organs, whose NSC concentration reflects longer term carbon source-sink balance. This and the fact that they represent a significant fraction of the total stored C pool, makes them good candidates to evaluate tree size effects on carbon source-sink dynamics. A complication when using stems, however, is that concentration of mobile carbon compounds often varies from the outer to the inner sapwood, and accessibility to these different compartments may vary. Therefore, the specific sapwood fraction sampled is also an important consideration for the interpretation of results, particularly in species with large amounts of sapwood. While in many species NSC concentration is highest in the outer sapwood and decreases with distance from the bark (Barbaroux and Bréda 2002; Hoch et al. 2003), in other species the reverse is true (Newell et al. 2002; Pruyn et al. 2005). In pines, lipid concentrations have also been show to increase from the outer to the inner sapwood (Saranpää and Nyberg 1987). In species with large amounts of sapwood, larger trees may have much more sapwood than smaller trees. In this case, if the concentration of NSC is greatest in the outer sapwood and declines appreciably as a function of distance from the bark, then the larger amount of sapwood in larger trees may cause a dilution effect leading to an apparent decrease of NSC with tree size. Yet results from the outer sapwood may not show any change or may even show an increase with tree size. Because of this, it may be best to standardize analysis to specific radial portions of the sapwood (i.e. a specific number of tree rings from the cambium) where accessibility is highest (e.g. next to the cambium), particularly in species with strong radial changes in sapwood NSC or lipid concentration and with large sapwood.

7 Conclusions

The two classical hypotheses to explain size/age- related declines of growth rates in trees and stands predicted that carbon availability for growth becomes increasingly limiting as trees increase in size (due either to progressive increases in carbon sinks other than growth or to decreases carbon sources, respectively). Although the validity of these hypotheses is now questioned and new alternative explanations have been proposed, actual data on size-dependent changes of stored mobile carbon in mature trees as indicators of carbon balance between source and sink activities is surprisingly limited. This has contributed to a common default assumption that carbon availability limits tree growth in large, old trees. This assumption conflicts with increasing evidence that mature trees under current atmospheric CO_2 concentrations have substantial amounts of stored carbon and are not carbon limited. To date, convincing evidence showing a decrease of the relative carbon supply with increasing tree size exists only for small saplings of shade-intolerant species in deeply shaded environments. In contrast, and assuming that concentration of stored carbon compounds at the end of the season reflects the end balance between carbon demand for earlier growth and carbon supply by photosynthesis, available data to date for four different evergreen temperate conifers (*Pinus ponderosa*, *P. albicaulis*, *Pseudotsuga menziesii* and *Sequoia sempervirens*) and two broadleaved trees (*Fagus sylvatica* and *Quercus petraea*) suggest that the relative carbon supply in trees does not become increasingly limited as they grow larger. On the contrary, in some cases, the data indicate an increasing oversupply of carbon with increasing size. Clearly, much research is still needed to understand how tree size, environment, stress, and species-specific life history and ecology influence the balance between instantaneous carbon demand and carbon supply, the allocation to storage, and the accessibility of storage pools.

In spite of many uncertainties, research to date points to the need to question the historically carbon-centric mechanisms proposed to explain age-related growth declines in trees and forests. Future research should focus on whether and when alternative growth limiting factors (e.g. turgor, long distance transport of assimilates, nutrients) may contribute to growth limitations in tall trees.

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