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Nonstructural Carbon in Woody Plants

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Abstract

Nonstructural carbon (NSC) provides the carbon and energy for plant growth and survival. In woody plants, fundamental questions about NSC remain unresolved: Is NSC storage an active or passive process? Do older NSC reserves remain accessible to the plant? How is NSC depletion related to mortality risk? Herein we review conceptual and mathematical models of NSC dynamics, recent observations and experiments at the organismal scale, and advances in plant physiology that have provided a better understanding of the dynamics of woody plant NSC. Plants preferentially use new carbon but can access decade-old carbon when the plant is stressed or physically damaged. In addition to serving as a carbon and energy source, NSC plays important roles in phloem transport, osmoregulation, and cold tolerance, but how plants regulate these competing roles and NSC depletion remains elusive. Moving forward requires greater synthesis of models and data and integration across scales from -omics to ecology.

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INTRODUCTION

Carbon is the building block of all life. How plants store and allocate carbon to grow and survive has long been of central interest to plant physiologists, ecosystem ecologists, and biogeochemists. Growing concerns over climate change have pushed the importance of the terrestrial carbon cycle into the minds of policy makers, land managers, and the general public. Woody plants are of particular interest because they constitute the largest pool of aboveground biomass, and their diverse strategies for carbon use and allocation are deeply connected to ecological and evolutionary theories of trade-offs and niche partitioning. Woody plant survival requires active tolerance to a wide range of stresses and disturbances, including drought, flooding, freezing, and pollution. The need to balance competition for external resources with tolerance for damage and disturbance, such as herbivory, fire, wind, and ice, causes woody plants to invest large amounts of carbon in lignified support tissues, defense, and storage. These complex metabolic and structural demands require plants to have evolutionary strategies to coordinate the uses of a limited supply of mobile, nonstructural carbon (NSC). Despite substantial effort by plant physiologists and ecologists to study the end result of these adaptations and associated trade-offs, as manifest in plant growth, respiration, reproduction, and turnover, there remain large uncertainties, many unanswered questions, and ongoing debates about the underlying dynamics of NSC.

Like many scientific phenomena, our paradigm of what NSC is and how it functions is tied in large part to what we can measure. Therefore, the scientific knowledge on NSC is based predominantly on measurements of concentrations of specific compounds that are assayed from

destructive samples. Overall, this has led to a somewhat circular logic, in which NSC is defined as the plant's reserves and then that function is ascribed to what we know how to measure. Research on NSC compounds has historically focused on carbohydrates, in particular simple sugars and starch, though there is evidence that oligosaccharides, polysaccharides, lipids, and amino acids function as sources of stored energy and carbon for biosynthesis (48). NSC pool sizes are typically estimated allometrically, and dynamics are inferred from the net changes in concentration or pool size (69). Carbon isotopes have also been used to date different NSC pools or to provide a more dynamic picture of how recent photosynthate is allocated (13, 106).

Despite the complexity of NSC regulation (17), the textbook view of the role of NSC in woody plants remains that derived from Kozlowski's encyclopedic 1992 review of carbohydrates in woody plants (63). Newly assimilated carbon in the chloroplast is exported to the cytosol as triose-phosphate, from which sucrose is synthesized. Some sucrose is converted to starch in the chloroplast and used during the night to support growth and metabolism when no new assimilates are being synthesized. Additionally, sucrose or other oligosaccharides may be loaded into the phloem, where they are translocated by mass flow to different components of the plant. Kozlowski presented a conceptual model of how woody plants store and allocate carbon in which NSC is taken up by different tissues in response to their intrinsic sink strengths. This source–sink paradigm is focused predominantly on the metabolic functions of NSC and in particular on plant growth.

Even in Kozlowski's review, however, there was ample evidence that the story is not this simple. For example, studies demonstrated that carbon transport is often compartmentalized by localized phloem connections (133), meaning phloem is not a single pool for which sinks compete, but even now it is unclear whether, and under what conditions, such fine-scale detail is important for predicting plant responses. Effects of nutrients and hormonal signaling on NSC dynamics, along with possible roles of NSC in vascular transport, cold tolerance, and osmotic regulation, are mentioned, but they are viewed as secondary to the idea of different plant tissues competing for carbon. This model predicts a seasonal cycle in NSC pools, which decline rapidly during times of leaf-out, rapid growth, or reproduction; rise late in the growing season as growth slows and NSC stores are replenished; and decline slowly through the dormant season to meet respiratory demands. This cycle is thought to be most dramatic in temperate deciduous species, which store larger amounts of NSC and accumulate them earlier in the year than evergreens.

The source–sink model does not make predictions about the forms NSC can take. However, sugars (especially sucrose) and sugar alcohols are the dominant NSCs exported from leaves into the phloem. Because sugars are used directly for cellular metabolism, conventional wisdom holds that sugars reflect a short-term pool and starch a more recalcitrant storage. However, Kozlowski also reviewed evidence for seasonal cycles in the balance between sugars and starch, which suggests that sugar is directly involved in cold tolerance. Starch accumulated late in the growing season is converted to sugar during dormancy but then resynthesized to starch as dormancy ends. Overall, this suggests that the specific compounds making up NSC matter and may have individual functional roles.

The two decades since Kozlowski's seminal review have seen several advances that are of direct relevance to how we view NSC. There has been a shift in research emphasis from growth to carbon fluxes (3). There has also been considerable research on the constraints of nutrients on carbon flows as well as on ecological stoichiometry, which considers how organisms and their interactions in ecosystems are affected by their relative elemental requirements (124). The rise of -omics research and improvements in quantitative physiology have opened the window to a better understanding of how plants regulate their internal metabolism and the genes involved. This understanding is more nuanced than simply viewing hormones as turning processes on and off (e.g., reproduction) and raises the possibility that plants are adjusting source and sink

Passive   **Quasi-active**   **Active**
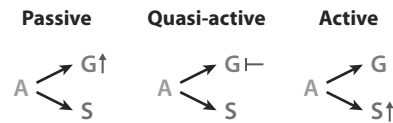
Figure 1

Alternative models for allocation of new assimilate (A) to storage (S). In all cases, we assume that growth (G) < A. Passive storage occurs when G is upregulated but limited by factors other than carbon. Quasi-active storage occurs when G is inhibited by the plant to divert carbon to S. Active storage occurs when S is upregulated and thus competes with G.

strengths to control allocation, rather than allocation being controlled by source and sink strengths. Other work has demonstrated plant thermal acclimation in both photosynthetic and respiratory processes (123), suggesting that both sources and sinks adjust to climate. Further evidence suggests that this acclimation allows plants to balance gross primary production and net primary production (79). Others have put forth provocative ideas suggesting that plant growth is limited not by photosynthesis (carbon source) but rather by sinks, owing in part to thermal limits on the rate of biosynthesis, and therefore NSC dynamics are merely a passive overflow (61). There has also been increasing discussion about the nonmetabolic roles of NSC, for example, in osmotic regulation, vascular transport, and cold tolerance (113).

In recent years there has been a vigorous debate on how NSC pools build up over time (47, 48, 113, 145). At the core of this debate is the extent to which temporal dynamics of NSC reflect, at least in part, active storage as opposed to a purely passive process driven by the balance between carbon supply via photosynthesis and demand for growth and respiration (63) (**Figure 1**). By "active," we mean the upregulation of storage at the expense of growth, even when conditions are favorable for growth. Closely related is what we term quasi-active storage, which is when storage occurs owing to the downregulation of growth. Both active and quasi-active storage imply a gene-regulated trade-off between storage and growth. The potential significance of active storage was highlighted in a seminal paper by Chapin et al. (17). They defined three main processes by which NSC builds up in plants: (*a*) accumulation, a purely passive process that occurs when supply exceeds demand; (*b*) reserve formation, an active process by which resources are stored at the expense of other processes; and (*c*) recycling, an active process by which compounds initially invested in growth or defense are broken down and reused to support future growth. Despite relatively abundant data consistent with reserve formation in woody plants, they concluded that unequivocal empirical evidence of a growth–storage trade-off was limited and well documented only for herbaceous plants. They also concluded that recycling is important for mineral nutrients but not NSC and that accumulation is most pronounced in species with inherently slow growth rates.

Despite immense methodological progress in recent decades and repeated recognition in the literature that NSC storage in woody plants may be in part an active process, the extent to which the temporal dynamics of NSC storage result from passive versus active processes remains unresolved (11, 17, 69, 74, 113, 145). This slow progress highlights that experimental tests to disentangle passive from active storage in woody plants are difficult; growth and photosynthesis are often not synchronized, and overall, whole-plant carbon balance (including growth, metabolism, and storage) is challenging to measure (109). Further, long life spans, large size, and relatively abundant NSC pools (48, 111) with slow turnover rates in woody plants make experimental manipulations and the interpretation of results exceedingly complicated. As a consequence, the assumption that NSC storage is a purely passive process continues to dominate (74).

The remainder of this review is divided into four sections. First, we review how NSC pools and fluxes are represented in models, with particular focus on Earth system models and the

terrestrial carbon cycle. In essence, we ask how (and whether) conceptual models of NSC have been translated into mathematical models, wherein differences among models often highlight unresolved questions. Second, we review recent ecological and ecophysiological field observations and experiments measuring NSC dynamics to identify whether recent data support or refute the passive paradigm. Third, we review recent physiological experiments to evaluate our current understanding of the mechanisms and processes responsible for NSC regulation. Fourth, we ask whether a new conceptual model of NSC dynamics has emerged from the past 20 years of research and identify key questions and uncertainties to be addressed.

MODELING NONSTRUCTURAL CARBON ALLOCATION AND STORAGE

The conversion of conceptual models to numerical models often highlights key uncertainties and allows for more quantitative tests of theories. The importance of NSC in woody plants is demonstrated by the observation that in numerical simulation models operating at a wide range of scales—from individual trees to forest stands to whole ecosystems—some representation of NSC is needed for biological realism. However, our current understanding of carbon allocation and storage processes is limited and impairs model development (2, 36, 113). Indeed, model representations of NSC tend to take a black-box approach, and the dynamics of the associated carbon pools are rarely evaluated or tested against field data (74). In this section, we review the ways in which allocation to and from NSC is treated in different models and then discuss the role of NSC in affecting the responses of the modeled system.

The Functional Role of Nonstructural Carbon

Even models of individual plant growth rarely make a formal distinction between structural carbon (e.g., cellulose and lignin) and NSC pools (74). Here, we focus on those models that explicitly model NSC as a separate pool. We note that in every model of which we are aware, the functional role of NSC is to buffer the plant's capacity for maintenance and growth against deficits (on a timescale of hours to months) in current assimilates (114). There is usually no metabolic cost assigned to storage in these models, and stored carbon is generally treated as completely available for future use—i.e., it does not become sequestered or otherwise unavailable over time (cf. 113). Other roles of NSC (e.g., osmotic roles and cold tolerance) are generally not considered. However, some models also use NSC mass balance as a mechanistic trigger for plant mortality (26, 35, 95); this is an obvious improvement over previous statistical approaches (28, 53).

Allocation and Transport of Nonstructural Carbon

The mass balance in a typical model assumes that NSC represents photosynthetic production minus respiratory processes and allocation to new tissue growth (20, 114). Thus, the net change in NSC represents the relatively small balance of several much larger fluxes (20), which makes it a major challenge to get NSC dynamics even approximately correct. Although there are numerous possible variations, most models work more or less as follows. At each time step, new photosynthetic products are transferred to one or more (e.g., separate root, trunk, and crown) storage pools. The respiratory costs of maintenance and new growth (131) may be satisfied either before or after this transfer and mixing takes place. Allocation to growth (potentially limited by environmental conditions, phenology, or substrate availability) then occurs from the storage pool(s), with most of the substrate for growth being provided by the nearest storage pool. In most models with multiple storage pools, transfer among storage pools is possible.

Models without a storage pool typically allocate current assimilates to the production of new tissues, assuming that allocation fractions are fixed in time. By comparison, most models that incorporate storage adopt some version of the dynamic-allocation transport-resistance approach to partitioning (130), which is a mathematical formalization of the source–sink model (63), but this approach is not universal (20). Here, NSC flows from source leaves to sinks (i.e., storage and/or growth) according to basic flux equations (analogous to Ohm's law) in proportion to a gradient in NSC concentration multiplied by a conductance (2, 7, 26, 76). Conductance (1/resistance) is defined in terms of conducting tissue biomass or path length and is commonly modified by environmental scaling factors [e.g., conductance is reduced under suboptimal temperature or moisture conditions (116)]. Storage then supplies the substrate for new tissue growth, which is controlled by supply and/or demand (26, 94). Some models consider feedbacks between photosynthesis and NSC in foliage; thus, when NSC builds up, photosynthesis is downregulated (2). In some models, storage actively competes with other sinks (2, 73), whereas in others, assimilates are passively allocated to storage only after respiration and growth have been fully satisfied (143).

What Does Modeled Nonstructural Carbon Represent?

Biochemically, models vary in what the storage pool corresponds to. Sugars, which are soluble and important for osmotic regulation, and starch, which is insoluble, have functionally different roles in transport and storage. However, many models do not distinguish between sugars and starch (20, 59, 95, 105), whereas in other models there is a distinction between labile carbon and starch (25, 26, 76) in which starch must be converted to a labile form (i.e., sugars) before it can be transported or metabolized. A small minority of models attempt to explicitly represent interconversions between sugars and starch (40). There are also models where storage is considered to be starch, and conversion of sugars to starch (and vice versa) is implicit (73, 116).

Similarly, models vary in the number of different storage pools. Several models include just a single NSC pool (20, 59, 94, 116). Other models distinguish between "storage" pools and "transient" pools (95, 106) or further subdivide these pools into various above- and belowground biomass components (2, 7, 73, 143). In this structure, the transient pool provides the substrate for new tissue growth and respiration and represents the sum of NSC that is being mobilized from storage as well as recent assimilates (30, 67). Other models distinguish between a "fast" pool (with a mean residence time of <1 year) and a "slow" pool (with a mean residence time of ∼20 years), with transfers from slow to fast occurring if the fast pool is sufficiently depleted (106). These approaches are consistent with observational evidence (16) for NSC pools with distinctly different availabilities and mean residence times (e.g., long-term storage versus ready-to-use reserves).

The Importance of Modeling Nonstructural Carbon: Model Performance and Process Insights

Including NSC in models of plant growth is important for a variety of reasons. First, it enhances the physiological realism of the model. For example, storage is necessary if plants are to maintain metabolic function through carbon deficit periods. By including a storage pool, comparatively high rates of tree growth can be sustained even when rates of photosynthesis are lower than respiratory demands (114). The storage pool also allows dormant-season carryover of assimilates, which can then be used to support new tissue growth (in excess of current photosynthetic products) during the following growing season.

Second, modeling can be used to examine the consequences of storage. For example, an analysis of the opportunity costs of investing in storage highlighted the ecological role that NSC plays

in trade-offs between seedling growth and survivorship rates (59). Similarly, a study where plant functional types were specified according to their propensity to store NSC suggested that the fitness cost of low rates of storage was minimal because the reserve pool was rarely depleted (35). From a more physiological point of view, model-based analyses have demonstrated the role of NSC in stress tolerance to ozone (105) and how NSC is critical in mediating the divergent short- and long-term responses of the respiration:photosynthesis ratio to increases in temperature (25).

Model–Data Evaluations

In studies that have modeled the seasonal accumulation of NSC, there is substantial divergence among models in the magnitude of the seasonal amplitude (calculated as the ratio of the size of the pool at its annual maximum to that at its annual minimum). In some models, spring leaf-out and wood growth draw down the storage reserves almost to zero, and the size of the NSC pool varies by a factor of two or more over the course of the year (20, 94, 114). In other models, there are substantial reserves remaining even when NSC is at its seasonal minimum, and the seasonal maximum size of the NSC pool is no more than 25% larger than the seasonal minimum (106, 116). However, relatively few modeling studies have rigorously evaluated model predictions of NSC dynamics against scaled-up measurements of tissue NSC concentrations. The few exceptions range from an excellent agreement between model and data (20) to instances where the modeled NSC was seasonally more dynamic than the scaled-up field measurements (114). Without a doubt, there is a pressing need for better data sets against which models can be evaluated and improved.

In the absence of data to evaluate modeled NSC dynamics, some studies have used other types of data to provide indirect support for the model. For example, a model with one NSC pool was able to simulate the seasonally varying isotopic signature (^{13}C and ^{18}O) of xylem cellulose (94). Another study used radiocarbon-based estimates of NSC age to directly constrain the size-weighted mean residence time of the total (fast + slow) NSC pool (106). This study also tested different competing representations of NSC reserves (no pool, one pool, two pools) against ecosystem-level carbon-dioxide (CO_2) flux and annual woody biomass increment. Agreement was best with the two-pool model, but modeled seasonal NSC was inconsistent with stem-wood NSC concentration data. These model–data comparisons of seasonal dynamics represent just the beginning in terms of the range of experimental and observational studies potentially available to test both mathematical and conceptual models of NSC dynamics.

OBSERVATIONS AND EXPERIMENTS

Newly Assimilated Carbon

The majority of our knowledge of new assimilates in woody plants comes from isotopic work, primarily pulse and continuous labeling (with ^{13}C or ^{14}C). These studies revealed how much and on what timescales new assimilates are allocated to growth, respiration, defense, storage, and exudates by following the fate of individual atoms as they move through plants.

New assimilates can be incorporated into plant tissues within minutes, but most are either exported from the leaves to other plant tissues or used by the plant's metabolism in respiratory processes within one day of assimilation (83). New assimilates can also be translocated to the roots and exported to symbiotic and free-living microbes within days (31, 50). Mixing of new assimilates with existing NSC within the tree occurs rapidly (54, 55). Mean residence times for new assimilates used for metabolism range from hours to days in leaves and branches up to several

days to weeks in stems and roots; however, there is also evidence for multiple pools with different mean residence times (15, 50, 142).

The time required for translocation depends on the distance over which assimilates must travel and is generally longer in larger plants (85). For example, the time for newly assimilated carbon to be transported and respired by roots varies from <1 day in ~1-m-tall woody shrubs (15) to >10 days in >25-m-tall trees (67), with the majority of studies finding time lags of 4–5 days for intermediate-size trees (68). Measurements of photosynthesis and soil respiration have shown that translocation times vary from hours to weeks within the same vegetation type (137). This suggests that multiple factors influence the translocation times, including environmental drivers (e.g., slower with colder temperatures and drier soils), stem diameter, viscosity, and turgor pressure (23, 107). Finally, phloem anatomy plays a role in translocation rates, with angiosperms generally being faster (0.2–6.0 m/h) than gymnosperms (0.1–0.4 m/h) (30).

Observed Nonstructural Carbon Variability

Only a handful of studies have scaled NSC concentration measurements to mass estimates at the whole-tree or forest-stand level. At the forest-stand level, NSC pool estimates range between 230 and 1,600 g C/m^2, enough to completely regrow foliage one to four times (42, 43, 48, 149). At the whole-tree level, the distribution of NSC among plant components varies widely. However, in most studies the largest pools are in stem wood or coarse roots, though foliage may represent a large pool in conifers (48). If we focus specifically on these pools, NSC concentrations are highest near the cambium or in young sapwood and are higher in temperate deciduous species than in evergreen species (48, 149). With increasing tree age and/or height, NSC concentrations in stem wood generally remain stable or increase (41, 98, 111, 128). In mature deciduous trees, NSC amounts, the NSC fraction allocated to starch versus sugar, and their seasonal fluctuations are higher in ring-porous trees (e.g., *Quercus*). In these trees, NSC is mostly restricted to the sapwood, compared with diffuse-porous (e.g., *Fagus*) trees, where NSC is found throughout the entire stem (4, 48, 98). Thus, the total NSC pool in ring-porous trees is positively correlated with the sapwood area. Furthermore, the relative allocation to storage and reproduction increases with age in diffuse-porous species but not in ring-porous species (41).

Seasonal NSC patterns reach a maximum just before leaf fall; in diffuse-porous trees, they reach a minimum during bud break and the onset of shoot expansion, and in ring-porous trees, they reach a minimum during early wood formation, which occurs prior to leaf-out (4, 98). Similar seasonal trends have been observed in drought-deciduous trees in semiarid climates, where stem-wood NSC concentrations decrease during leaf expansion and reproduction and are higher during the dry season than during the wet season (92, 149). In evergreen conifers, NSC declines sharply from the sapwood to hardwood, concentrations of sugars are higher during winter than during summer, and starch peaks at the beginning of the growing season (34, 128). In deciduous conifers, seasonal variations are smaller and similar to those in diffuse-porous trees (48). Overall, seasonal fluctuations of stored NSC rarely fall below certain relatively high minima (48, 149).

Direct measurements of NSC age suggest that younger NSC is preferentially used for growth and metabolism. New leaves, sapwood, and fine roots are predominantly made from current-year assimilates, though almost half can originate from NSC that is several years old (32, 54, 84). Trees utilize recent NSC (current and previous year) for reproductive masting—years of large, synchronized seed production among conspecific individuals—regardless of the interval between mast years (52). New roots, leaf buds, and flowers grow from stored NSC up to two years old (52, 139), and stored NSC up to three or four years old has been incorporated into tree ring cellulose growth (54, 65). NSC used for root (14, 22, 51) and stem (12, 87) respiration can be several years old.

However, other studies reveal that decade-old NSC can be accessible to the plants. Using the ^{14}C "bomb spike" approach, Richardson et al. (106) found NSC from mature temperate tree stem wood to be 7–14 years old. The ages of sugar and starch were similar, implying regular interconversion between these compounds. Similar results were found in *Acer rubrum* L., where NSC age increased with the age of the tree; however, comparatively younger NSC was found in more vigorous trees (12). Ten-year-old NSC was allocated to new root growth following a major hurricane disturbance in a dry tropical forest (139), red maple stump sprouts were grown from remobilized NSC in roots as old as 17 years (12), and new roots in desert palms were produced from remobilized NSC as old as >60 years (R. Vargas, C. Czimczik, S. Bullock, X. Xu & S. Djuricin, unpublished data). These results demonstrate that older NSC remains accessible to plants, but the mechanisms (at the physiological and molecular levels) that trigger the storage of NSC and the use of older pools remain unclear.

Experimental Manipulations

Experimental manipulations push systems out of their normal conditions and often help reveal key features about underlying dynamics. In this section, we briefly review NSC responses to experimental manipulations of CO_2, flooding, ozone, acid deposition, and defoliation.

For plants grown under elevated CO_2 concentrations, a short-term increase in gross primary production and water-use efficiency in response to elevated CO_2 is all but unavoidable (21), even under low-light conditions, though over the longer term there are many ways that gross primary production may be actively or passively downregulated. In response to this increase in gross primary production, the initial response of almost any plant grown under elevated CO_2 is to increase growth (1). Although elevated CO_2 experiments are not equitably distributed across biomes or stand ages (75), this nonetheless suggests that carbon limitation is widespread. Leaf NSC concentrations show large increases (46, 62), which are due primarily to starch rather than sugar accumulation (80). Fewer data are available for longer-term storage, but NSC concentrations generally increase in plants exposed to elevated atmospheric CO_2 and decrease in plants growing at low CO_2 concentrations (18, 61).

Flooding experiments represent a unique NSC perturbation because anoxia shuts down plant respiration, shifting metabolism instead to a far less efficient and somewhat toxic alcohol fermentation pathway. Soil inundation isolates the impacts on roots from stems and leaves and has little direct impact on the photosynthetic machinery. Roots are hypothesized to compensate by decreasing metabolic demand, reducing growth and nutrient uptake, or consuming more carbohydrates to compensate for inefficiency (66). There is a general pattern of increased leaf sugar and starch across a wide range of biomes and flood tolerances (66, 121). NSC buildup coincides with a large reduction in photosynthesis and transpiration resulting from reductions in stomatal conductance (121). Stomatal closure is thought to occur because of either chemical signaling (29) or increased leaf NSC via phloem limitation. Phloem limitation suggests that roots are probably not consuming more carbohydrates to make up for inefficiencies, which also makes sense because of ethanol toxicity.

Tropospheric ozone is a potent oxidizing agent and also a greenhouse gas. Background atmospheric ozone concentrations in northern midlatitudes have increased substantially in recent decades (19, 141), and ozone concentration is correlated with increased tree mortality (28). The main negative effect of ozone on plants is the inhibition of carbon assimilation via damage to the photosynthetic apparatus (103), reducing carbon assimilation in plants (146, 147). Ozone damage typically reduces leaf sucrose and has little effect on leaf starch (147). There is no clear effect of ozone on stem NSC, with some studies showing increases and others decreases, but most found reductions in phloem sugars or translocation (71, 81). In the roots there was a general trend of

reduced starch concentrations, but impacts on root sugars are mixed (81, 115, 129). Overall, most carbon pools show neutral or negative responses, consistent with an overall reduction of photosynthesis. The reduced root:shoot ratio, phloem transport, and root starch are also consistent with the need to repair foliar damage.

Acid deposition acts through changes in soil cations, causing a transient fertilization and eventual deficiency, and through foliar damage, in particular a reduction in cold tolerance that is mediated in part by foliar sugar concentrations (132). Simulated acid fog experiments isolate the latter effect and have generally reduced foliar sugar and starch or had a nonsignificant effect (97, 120). There are limited data on the effects of acid on other pools, but available data suggest that stem starch decreases (119) and root starch increases (9), though the latter result is from a natural soil pH gradient rather than a controlled experiment.

Multiple disturbances can cause a loss of leaves and/or fine roots, which limits the capacity to photosynthesize and take up nutrients and water and which may further reduce ecosystem gross primary production following disturbances (77, 138). Defoliation studies have shown that NSC stores often decrease soon after defoliation (78, 135). Notably, after defoliation, woody plants have the capacity to remobilize old carbon reserves (>11 years old) (139). A few studies have investigated how repeated defoliation or disturbance affects NSC. For several months after a defoliation event, young *Eucalyptus* plants did not experience carbon limitation (5), and repeated defoliation events (spaced by several years of canopy recovery) led to an accumulation of NSC in *Pinus nigra* Arnold (96). In contrast, there is evidence of carbon limitation in root systems of mature *Populus* after repeated defoliation (70), but much more research should be done in terms of disturbance intensity/severity and legacy effects on carbon storage and remobilization of NSC pools in woody plants (56, 136).

Additional Constraints

The dynamics of NSC interact with several other ecophysiological processes that constrain how NSC is stored and allocated, including stoichiometric and thermal constraints on growth and translocation. Storage is mediated by nutrient availability, with larger allocation to storage when growth is limited by nutrients (58). However, whether this response is purely stoichiometric is unclear, as an active stress response would also favor storage over growth (60), and, ironically, preferential allocation to fine roots over stems further exacerbates carbon:nitrogen imbalances.

Across a wide range of growing-season lengths, NSC concentration in all tree organs increases with elevation from closed forest stands toward altitudinal tree lines, when sampling takes into account differences in phenology among elevations (47, 48). This pattern is interpreted to reflect a thermal growth limitation (61), though others argue that the tree line is a reflection of disturbance and recruitment limitation, not growth (127).

Relationships to Demographic Processes

NSC provides energy and carbon for respiration, growth, defense, reproduction, resprouting, and survival (7, 17, 63). In this section, we focus on the NSC relationships with mortality and resprouting. The importance of NSC storage for survival, particularly when forests experience a stress event such as severe drought, has been a topic of much debate in recent years (82, 113, 145). Relatively few studies have directly addressed the question of whether a larger NSC allocation confers a survival advantage. Experimental defoliation of several temperate species demonstrated that saplings with larger initial NSC reserves were more likely to survive (10). This agrees with observational evidence from neotropical and temperate seedlings showing that seedlings with larger

NSC reserves are more likely to survive (59, 89) and observation of depleted NSC in stem wood of *Pinus sylvestris* L. following a drought episode (38). Results of drought experiments on seedlings and saplings have been more complex. A recent drought experiment using one conservative-growing species (*Pinus radiata* D. Don) and two fast-growing *Eucalyptus* species showed depleted NSC for *Pinus* but not *Eucalyptus* (86). This response conforms to the suggestion that allocation to growth versus allocation to storage and defense underlies the trade-off between growth and survival (33, 101). The relationship between NSC depletion and mortality risk, and how this varies among different groups, is poorly understood (112).

Stores of NSC play a critical role in resprouting following disturbance (8). Resprouting in trees after low but pervasive levels of disturbance is supported by both below- and aboveground reserves, whereas resprouting in shrubs and trees subject to frequent, severe disturbance is supported by belowground reserves (93). Mediterranean woody shrubs in fire-prone heathlands allocate and accumulate more NSC belowground than sympatric, obligate seeders do (6, 58, 140). Here, starch reserves have been identified as critical for resprouting and are stored in xylem parenchyma ray tissue of woody underground organs, such as burls, lignotubers, and roots (6, 140). Similarly, savanna trees, which persist through repeated top kill by resprouting, also store large amounts of NSC (e.g., sugars plus starch = 28–30% mass) belowground in specialized organs (i.e., lignotubers) (49, 144). Observations of $\delta^{13}C$ patterns, ^{14}C labeling studies, and defoliation experiments demonstrate that these belowground NSC reserves support coppicing after fire damage as well as bolting of the main stem to escape the fire kill zone generated by the grass layer (117, 144). Finally, lignotuber NSC reserves are recharged by the initial coppice shoots over the following growing season and after bolting, and thus increase with plant age. Belowground NSC also supports the production of fine roots in systems with severe and frequent fire disturbances (72). Given that resprouting is a common strategy that can be more successful than seed regeneration even outside of fire-prone systems (27) and that resprouting appears to be the ancestral trait among angiosperm trees (24), we should expect selective pressure along a growth–storage trade-off. Furthermore, we would expect a different trade-off among conifers, where resprouting appears to be a derived state.

PHYSIOLOGICAL PROCESSES AND MECHANISMS

Regulation of Storage

How do woody plants gauge when and how much carbon should be partitioned to storage? Storage pools ultimately depend on the relative rates of synthesis and degradation of storage compounds. The metabolism of starch and storage lipids (the latter mostly in oilseeds) has been under intense study (122, 125, 150), but it is not the focus here. To date, our understanding of the regulation of storage is based mostly on diurnal starch dynamics in leaves of *Arabidopsis* and other herbaceous model systems, where starch accumulation occurs during the day to support growth and respiration at night. A main conclusion from these studies is that the synthesis and degradation of starch on a diurnal basis are controlled by interdependent regulatory networks that ultimately allow plants to balance carbon supply via photosynthesis with carbon use for growth and other activities (44, 125, 126). Overall, regulation at the gene and enzyme levels occurs in response to sugars and redox state (mediated by sugar and/or light). These in turn depend on the interaction of endogenous clocks and environmental signals (e.g., light, water, and temperature) and their subsequent effect on the hormonal regulation of growth. These signals interact with multiple specialized isoforms of the enzymes involved in NSC synthesis and degradation. Each enzyme isoform has different (and often contrasting) regulatory mechanisms and varies in abundance depending on the specific storage organ. This results in an extraordinarily complex regulatory system. An important result in

Arabidopsis is that starch is the most reliable indicator of metabolic integration (126). Furthermore, plants that are carbon starved under prolonged nights and subsequently subjected to light and favorable conditions suppress growth for a few hours and partition carbon to storage (44, 126). This short-term growth reduction provides a clear example of quasi-active storage and was interpreted as a strategy to prevent chronic carbon starvation and optimize growth in the long term.

The enormous complexity of regulatory mechanisms documented in leaves of herbaceous species highlights that elucidating the regulatory networks that integrate diurnal and seasonal storage in woody plants (including nonphotosynthetic tissues) will be a daunting challenge. Woody plants are often large, modular, and long lived, which results in multiple, conflicting, and lagged signals over time that travel through the vascular system from different organs. How these signals are integrated in woody plants and their consequences for the whole-plant carbon balance are exceedingly difficult to demonstrate in mature trees under natural conditions.

Preliminary results from a recent meta-analysis based on approximately 200 perennial species from different functional types and biomes (A. Sala & J. Martínez-Vilalta, unpublished data) suggest that plants often accumulate starch during the growing season when soluble sugars are close to or at their seasonal minima and when NSC (the sum of starch and soluble sugars) declines. This suggests that, analogous to the diurnal fluctuation in herbaceous plant leaves, plants store starch during the growing season even if supply does not meet demand. These data are correlative, and much research will be required to elucidate whether long-term storage in woody plants (including nonphotosynthetic tissues) is analogous to the diurnal regulation observed in *Arabidopsis* leaves and, if so, by which mechanisms this occurs. It is encouraging, however, that reserve deposition on a seasonal basis in woody tissues of trees has regulatory mechanisms similar to those described in *Arabidopsis* (125). Advances in -omics and systems biology will be critical to unravel the complexity of storage regulation in trees, for example, by identifying when genes controlling storage are turned on (active storage) or when those controlling growth are turned off (quasi-active storage).

Functions of Nonstructural Carbon

Traditionally, NSC storage has been viewed as a reservoir pool to supply carbon for growth and respiration. However, in plants, water movement is driven purely by gradients of water potential. Based on accumulating evidence, the osmotic function hypothesis (113) proposed that, in addition to sources of carbon for structural growth and energy, NSC storage could serve a critical osmotic function to maintain turgor and long-distance vascular integrity in xylem and phloem, the only means by which plants integrate their overall function.

It is now known that the xylem in plants often experiences daily and seasonal cycles of embolism and refilling (90) and that NSC, particularly sugars, may be involved in embolism sensing and reversal (113). Sugars are also involved in phloem transport, which is intimately related to xylem transport. Sugars accumulate in the phloem to be transported but also to generate osmotically driven pressure gradients in order to drive long-distance phloem transport (88) and trigger water influx from the xylem. The specific mechanisms of long-distance phloem transport in trees remain a mystery (134). Despite their size, phloem pressure in trees is often lower than that in herbaceous plants, and unknown mechanisms must drive long-distance transport, perhaps involving a relay system of continuous solute exchange (134). Interestingly, phloem loading in the leaves of trees is passive and driven via gradients of soluble sugars from high concentrations in mesophyll source cells to low concentrations in the phloem (104). Although highly speculative at this point, it could be that high NSC inventories in trees serve multiple osmotic purposes. For instance, they may allow trees to overcome hydraulic constraints due to height (110, 148) while supplying soluble sugars for phloem loading and long-distance transport (37).

The potential osmotic function of NSC is consistent with results showing that drought led to an increase of NSC concentrations in drought-resistant plants or genotypes but not in drought-intolerant plants (99, 102). Hydraulic failure in *Pinus* seedlings subjected to drought may also be associated with a loss of adequate tissue carbohydrate content required for osmoregulation (118). The strong seasonal increase of soluble sugars during winter months in woody plants that experience chilling or freezing temperatures (63) also supports the osmotic function of storage (64, 108). A similar pattern occurs in drought-deciduous species during the dry season (92, 149). Accumulation of soluble sugars in cells slows down water movement from the cell to the apoplast, where extracellular freezing lowers the water potential. In addition, sugars have been involved in several cryoprotective roles, including membrane stabilization and protein protection. Finally, sugars have some reactive oxygen species–scavenging properties, thus preventing oxidation damage under cold temperatures.

In summary, in addition to acting as a carbon reservoir for growth and respiration, stored NSC appears to serve a critical osmotic function (via continuous exchange of the soluble, osmotically active fraction) to maintain turgor and vascular transport. This raises the possibility that what has been interpreted as sequestered, inaccessible NSC (17, 100) may actually reflect minimum pools that are maintained for osmotic functions. This, in turn, would partially explain (*a*) why woody plants often fail to use NSC reserves as expected (112), except when nearing death (38, 39, 45); (*b*) why it is often difficult to deplete the NSC storage pool in trees; and (*c*) why under limiting carbon supply some plants increase rather than decrease storage.

CONCLUSIONS

Active, Quasi-Active, and Passive Storage

The primary debate about the role and dynamics of NSC revolves around whether allocation to storage is active, quasi-active, or passive (**Figure 1**). A closely related issue is that the role of carbon starvation in tree mortality is critical yet unresolved. Likewise, numerous questions remain regarding the details of NSC in mathematical models, such as what physiological mechanisms control how deeply plants deplete their NSC pools under different conditions, which have potentially large implications for forecasting plant responses to climate change, disturbance, and stress.

In resolving these and other standing questions, it is important to begin with unavoidable constraints, such as the conservation of mass and energy. Common statements about storage occurring when assimilation exceeds growth are simply statements of mass balance and not evidence for one theory over another (e.g., active versus passive storage). Such statements do not identify when sink strength has been downregulated to promote storage, when storage is an actively competing sink, or when sink strength has been upregulated but supply exceeds demand (passive accumulation) owing to other constraints, such as thermal reaction kinetics, stoichiometry, or physical limits. Furthermore, the conversion of sugar to starch (and back again) is not a spontaneous reaction at ambient temperatures, but instead involves biochemical pathways that are clearly regulated by the plant. Under both the passive-transport model and Fick's law, any such conversion induces a concentration gradient and thus flux into or out of storage. Therefore, the ubiquitous presence of starch demonstrates that, on some level, active storage is clearly occurring, though it does not indicate which biophysical processes are regulating such storage.

It is also clear, both from first principles and from observations, that NSC is being used as a carbon and energy source when leaves are absent, such as during dormancy or defoliation, and when demand exceeds supply, such as when light levels are below the carbon compensation point.

Similarly, resprouting requires the use of belowground NSC to rebuild both stem and foliage, and observations suggest that plants are able to draw upon much older NSC reserves to do so than they commonly use for annual growth and maintenance (12). Observations suggest that obligate resprouters are actively storing NSC. Furthermore, we should expect resprouting to provide a selective pressure for active storage.

Although passive accumulation clearly can and does occur over the short term, over the long term one must ask why plants would not downregulate photosynthesis to shift limited resources, such as water and nutrients, elsewhere. For example, over the short term, flood-induced anoxia induces a strong sink limitation on root metabolism, which causes stomatal closure. More generally, long-term downregulation may be an acclimatory response, reducing photosynthetic capacity or increasing the root:shoot ratio, or an evolutionary response, selecting for different allocation patterns. Thus, passive storage at the tree line (61) may simply reflect the translation of growth and photosynthesis regulation strategies to a different environment than the one the species is adapted to. Although this argument does set a high bar against dismissing the idea of active storage, it is far from constituting proof. Nonetheless, it reminds us that the timescale of NSC response is an important part of the active/passive debate that is not often stated explicitly and is likely confounding the current debate.

The emerging overall conceptual model suggests that active, quasi-active, and passive storage are not mutually exclusive and that all three processes occur. In keeping with classic concepts like Liebig's law of the minimum, sometimes plants are limited by carbon, but sometimes they are limited by other constraints. There is a need to better clarify which physiological and ecosystem responses are a result of active, quasi-active, and passive processes. Doing so will require more focused efforts to bring together mathematical models and data, as this will help identify how often and under what conditions different processes dominate. Model tests against experimental data and under extreme conditions (e.g., droughts, floods, fires) will be particularly important for identifying where different assumptions break down. Similarly, there is a need to go beyond NSC concentration measurements and to push genomic and biochemical tools out of the lab and into woody ecosystems in order to relate regulatory mechanisms to actual pool sizes and whole-plant carbon balance. To refine our conceptual models, we need to better understand what internal and external factors control the genes for growth versus active storage and when they are being turned on. This mechanistic understanding of what drives storage will then need to be distilled to simple mathematical models to provide a predictive capacity. This work will also need to be done in an evolutionary context to resolve questions about ecological and evolutionary trade-offs.

Modeling Nonstructural Carbon: Pools, Compounds, Processes, and Future Directions

The partitioning of NSC between sugars and starch has traditionally been viewed primarily in terms of short- and long-term storage. There is growing evidence that this partitioning involves additional physiological processes related to osmotic regulation, both for turgor and transport, and a seasonal cycling between sugar and starch for cold tolerance. However, beyond a coarse starch-versus-sugar distinction, there is no theory that explains the diversity of NSC compounds. Some evidence suggests consistent differences in where and when different compounds are used [e.g., sugars of the raffinose family predominate during dormancy, whereas sugar alcohols tend to be found during growth (57, 91)]. An important question is, to what extent do different NSCs serve different functional roles in the plant? There is need for a better understanding of what regulates the intertransformations of different NSC compounds [e.g., what factors regulate the active transformation of starch into sugar (122, 150)] and what their different roles are.

In addition to the questions that arise from competing conceptual models, important questions often arise from the process of translating conceptual models to mathematical models. For example, the above discussion on NSC compounds is related to the question of how many pools are required to model NSC dynamics and suggests that at least two pools are required (e.g., fast/slow or sugar/starch). Models also currently assume that there is no metabolic cost to storage, but physiological estimates are available for the ATP costs of starch synthesis and hydrolysis, although other costs are less well constrained. Models also have considerable uncertainty related to the upper and lower bounds on NSC pool sizes and the relationships between NSC and demographic rates. On the lower bound, there is evidence that not all NSC is available, so models that utilize 100% of NSC for bud burst and spring growth are in error. However, there is not a simple distinction between available and unavailable NSC, as the NSC used for resprouting is older than that used for bud burst, which highlights the open question of how plants regulate how deeply to deplete their NSC pools under different conditions. This question is tightly linked to demographic and allocation issues, as there is a direct mass-balance constraint between NSC availability and demographic processes such as growth, reproduction, and resprouting. By contrast, the relationship between NSC and mortality is almost completely unconstrained by data, though there are strong reasons to expect that the risk of mortality is more complicated than the simple assumption that mortality occurs when NSC stores are completely depleted (i.e., pure carbon starvation).

In addition to questions about the lower limits of NSC, there are open questions about the anatomical and biochemical upper limits on the NSC capacity of woody plants and how those relate to biological and physical traits and feedbacks, such as foliar starch accumulation. Models also vary considerably in how they account for phloem conductance. On the one hand, there is the question of whether the complexity of transport is even necessary to accurately describe NSC source–sink dynamics; on the other hand, these processes may be key to understanding the osmotic functions of NSC and setting the lower bound on NSC depletion.

Finally, as with the conceptual models, there is the important and unresolved question of whether storage competes with other sinks, occurs passively, or is quasi-active owing to the regulation of sources and sinks. As stated above, all three processes appear to be occurring, and further model–data synthesis is required to determine their relative importance. Resolving these questions is integral to improving our capacity to anticipate how woody plants will respond to changes in CO_2, climate, stress, and disturbance.

DISCLOSURE STATEMENT

ACKNOWLEDGMENTS

LITERATURE CITED

1. Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytol.* 163:351–72

2. Allen MT, Prusinkiewicz P, DeJong TM. 2005. Using L-systems for modeling source-sink interactions, architecture and physiology of growing trees: the L-PEACH model. *New Phytol.* 166:869–80

3. Baldocchi D. 2008. Breathing of the terrestrial biosphere: lessons learned from a global network of carbon dioxide flux measurement systems. *Aust. J. Bot.* 56:1–26

4. Barbaroux C, Bréda N. 2002. Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiol.* 22:1201–10

5. Barry KM, Quentin A, Eyles A, Pinkard EA. 2012. Consequences of resource limitation for recovery from repeated defoliation in *Eucalyptus globulus* Labilladière. *Tree Physiol.* 32:24–35

6. Bell TL, Ojeda F. 1999. Underground starch storage in *Erica* species of the Cape Floristic region—differences between seeders and resprouters. *New Phytol.* 144:143–52

7. Berninger F, Nikinmaa E, Sievanen R, Nygren P. 2000. Modelling of reserve carbohydrate dynamics, regrowth and nodulation in a N_2-fixing tree managed by periodic prunings. *Plant Cell Environ.* 23:1025–40

8. Bond WJ, Midgley JJ. 2001. Ecology of sprouting in woody plants: the persistence niche. *Trends Ecol. Evol.* 16:45–51

9. Brunner I, Brodbeck S, Walthert L. 2002. Fine root chemistry, starch concentration, and "vitality" of subalpine conifer forests in relation to soil pH. *For. Ecol. Manag.* 165:75–84

10. Canham CD, Kobe RK, Latty EF, Chazdon RL. 1999. Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia* 121:1–11

11. Cannell M, Dewar R. 1994. Carbon allocation in trees: a review of concepts for modelling. *Adv. Ecol. Res.* 25:59–104

12. Carbone MS, Czimczik CI, Keenan TF, Murakami PF, Pederson N, et al. 2014. Age, allocation and availability of nonstructural carbon in mature red maple trees. *New Phytol.* 200:1145–55

13. Carbone MS, Czimczik CI, McDuffee KE, Trumbore SE. 2007. Allocation and residence time of photosynthetic products in a boreal forest using a low-level ^{14}C pulse-chase labeling technique. *Glob. Change Biol.* 13:466–77

14. Carbone MS, Still CJ, Ambrose AR, Dawson TE, Williams AP, et al. 2011. Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration. *Oecologia* 167:265–78

15. Carbone MS, Trumbore SE. 2007. Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. *New Phytol.* 176:124–35

16. Chantuma P, Lacointe A, Kasemsap P, Thanisawanyangkura S, Gohet E, et al. 2009. Carbohydrate storage in wood and bark of rubber trees submitted to different level of C demand induced by latex tapping. *Tree Physiol.* 29:1021–31

17. Chapin FS, Schulze E, Mooney HA. 1990. The ecology and economics of storage in plants. *Annu. Rev. Ecol. Syst.* 21:423–47

18. Coley PD, Massa M, Lovelock CE, Winter K. 2002. Effects of elevated CO_2 on foliar chemistry of saplings of nine species of tropical tree. *Oecologia* 133:62–69

19. Cooper OR, Parrish DD, Stohl A, Trainer M, Nédélec P, et al. 2010. Increasing springtime ozone mixing ratios in the free troposphere over western North America. *Nature* 463:344–48

20. Cropper WP, Gholz HL. 1993. Simulation of the carbon dynamics of a Florida slash pine plantation. *Ecol. Model.* 66:231–49

21. Curtis PS. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environ.* 19:127–37

22. Czimczik CI, Trumbore SE, Carbone MS, Winston GC. 2006. Changing sources of soil respiration with time since fire in a boreal forest. *Glob. Change Biol.* 12:957–71

23. Dannoura M, Maillard P, Fresneau C, Plain C, Berveiller D, et al. 2011. In situ assessment of the velocity of carbon transfer by tracing ^{13}C in trunk CO_2 efflux after pulse labelling: variations among tree species and seasons. *New Phytol.* 190:181–92

24. Del Tredici P. 2001. Sprouting in temperate trees: a morphological and ecological review. *Bot. Rev.* 67:121–40

25. Dewar RC, Medlyn BE, McMurtrie RE. 1999. Acclimation of the respiration photosynthesis ratio to temperature: insights from a model. *Glob. Change Biol.* 5:615–22

26. Dick JM, Dewar RC. 1992. A mechanistic model of carbohydrate dynamics during adventitious root development in leafy cuttings. *Ann. Bot.* 70:371–77

27. Dietze MC, Clark JS. 2008. Changing the gap dynamics paradigm: vegetative regeneration control on forest response to disturbance. *Ecol. Monogr.* 78:331–47

28. Dietze MC, Moorcroft PR. 2011. Tree mortality in the eastern and central United States: patterns and drivers. *Glob. Change Biol.* 17:3312–26

29. Else MA, Tiekstra AE, Croker SJ, Davies WJ, Jackson MB. 1996. Stomatal closure in flooded tomato plants involves abscisic acid and a chemically unidentified anti-transpirant in xylem sap. *Plant Physiol.* 112:239–47

30. Epron D, Bahn M, Derrien D, Lattanzi FA, Pumpanen J, et al. 2012. Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. *Tree Physiol.* 32:776–98

31. Epron D, Ngao J, Dannoura M, Bakker MR, Zeller B, et al. 2011. Seasonal variations of belowground carbon transfer assessed by in situ $^{13}CO_2$ pulse labelling of trees. *Biogeosciences* 8:1153–68

32. Felten S von, Hättenschwiler S, Saurer M, Siegwolf R. 2007. Carbon allocation in shoots of alpine treeline conifers in a CO_2 enriched environment. *Trees* 21:283–94

33. Fine PVA, Mesones I, Coley PD. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305:663–65

34. Fischer C, Höll W. 1991. Food reserves of Scots pine (*Pinus sylvestris* L.). *Trees* 5:187–95

35. Fisher R, McDowell N, Purves D, Moorcroft P, Sitch S, et al. 2010. Assessing uncertainties in a second-generation dynamic vegetation model caused by ecological scale limitations. *New Phytol.* 187:666–81

36. Friedlingstein P, Joel G, Field CB, Fung IY. 1999. Toward an allocation scheme for global terrestrial carbon models. *Glob. Change Biol.* 5:755–70

37. Fu QS, Cheng LL, Guo YD, Turgeon R. 2011. Phloem loading strategies and water relations in trees and herbaceous plants. *Plant Physiol.* 157:1518–27

38. Galiano L, Martínez-Vilalta J, Lloret F. 2011. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytol.* 190:750–59

39. Galvez DA, Landhausser SM, Tyree MT. 2013. Low root reserve accumulation during drought may lead to winter mortality in poplar seedlings. *New Phytol.* 198:139–48

40. Génard M, Dauzat J, Franck N, Lescourret F, Moitrier N, et al. 2008. Carbon allocation in fruit trees: from theory to modelling. *Trees* 22:269–82

41. Genet H, Bréda N, Dufrêne E. 2010. Age-related variation in carbon allocation at tree and stand scales in beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) using a chronosequence approach. *Tree Physiol.* 30:177–92

42. Gholz H, Cropper W. 1991. Carbohydrate dynamics in mature *Pinus elliottii* var. *elliottii* trees. *Can. J. For. Res.* 21:1742–47

43. Gough CM, Flower CE, Vogel CS, Dragoni D, Curtis PS. 2009. Whole-ecosystem labile carbon production in a north temperate deciduous forest. *Agric. For. Meteorol.* 149:1531–40

44. Graf A, Smith AM. 2011. Starch and the clock: the dark side of plant productivity. *Trends Plant Sci.* 16:169–75

45. Hartmann H, Ziegler W, Trumbore S. 2013. Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy. *Funct. Ecol.* 27:413–27

46. Hättenschwiler S, Handa I, Egli L. 2002. Atmospheric CO_2 enrichment of alpine treeline conifers. *New Phytol.* 156:363–75

47. Hoch G, Körner C. 2012. Global patterns of mobile carbon stores in trees at the high-elevation tree line. *Glob. Ecol. Biogeogr.* 21:861–71

48. Hoch G, Richter A, Körner C. 2003. Non-structural carbon compounds in temperate forest trees. *Plant Cell Environ.* 26:1067–81

49. Hoffmann WA, Orthen B, Franco AC. 2004. Constraints to seedling success of savanna and forest trees across the savanna-forest boundary. *Oecologia* 140:252–60

50. Högberg P, Högberg MN, Göttlicher SG, Betson NR, Keel SG, et al. 2008. High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. *New Phytol.* 177:220–28

51. Hopkins F, Gonzalez-Meler MA, Flower CE, Lynch DJ, Czimczik C, et al. 2013. Ecosystem-level controls on root-rhizosphere respiration. *New Phytol.* 199:339–51

52. Ichie T, Igarashi S, Yoshida S, Kenzo T, Masaki T, Tayasu I. 2013. Are stored carbohydrates necessary for seed production in temperate deciduous trees? *J. Ecol.* 101:525–31

53. Keane RE, Austin M, Field C, Huth A, Lexer MJ, et al. 2001. Tree mortality in gap models: application to climate change. *Clim. Change* 51:509–40

54. Keel SG, Seidwolf RTW, Körner C. 2006. Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. *New Phytol.* 172:319–29

55. Keel SG, Siegwolf RTW, Jaggi M, Körner C. 2007. Rapid mixing between old and new C pools in the canopy of mature forest trees. *Plant Cell Environ.* 30:963–72

56. Keeley J. 2004. Impact of antecedent climate on fire regimes in coastal California. *Int. J. Wildland Fire* 13:173–82

57. Keller JD, Loescher WH. 1989. Nonstructural carbohydrate partitioning in perennial parts of sweet cherry. *J. Am. Soc. Hortic. Sci.* 114:969–75

58. Knox KJE, Clarke PJ. 2005. Nutrient availability induces contrasting allocation and starch formation in resprouting and obligate seeding shrubs. *Funct. Ecol.* 19:690–98

59. Kobe RK. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos* 80:226–33

60. Kobe RK, Iyer M, Walters MB. 2010. Optimal partitioning theory revisited: nonstructural carbohydrates dominate root mass responses to nitrogen. *Ecology* 91:166–79

61. Körner C. 2003. Carbon limitation in trees. *J. Ecol.* 91:4–17

62. Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, et al. 2005. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. *Science* 309:1360–62

63. Kozlowski TT. 1992. Carbohydrate sources and sinks in woody plants. *Bot. Rev.* 58:107–222

64. Kozlowski TT, Pallardy S. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. *Bot. Rev.* 68:279–334

65. Krepkowski J, Gebrekirstos A, Shibistova O, Bräuning A. 2013. Stable carbon isotope labeling reveals different carry-over effects between functional types of tropical trees in an Ethiopian mountain forest. *New Phytol.* 199:441–51

66. Kreuzwieser J, Papadopoulou E, Rennenberg H. 2004. Interaction of flooding with carbon metabolism of forest trees. *Plant Biol.* 6:299–306

67. Kuptz D, Fleischmann F, Matyssek R, Grams TEE. 2011. Seasonal patterns of carbon allocation to respiratory pools in 60-yr-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling. *New Phytol.* 191:160–72

68. Kuzyakov Y, Gavrichkova O. 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. *Glob. Change Biol.* 16:3386–406

69. Lacointe A. 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. *Ann. For. Sci.* 57:521–33

70. Landhäusser SM, Lieffers VJ. 2012. Defoliation increases risk of carbon starvation in root systems of mature aspen. *Trees* 26:653–61

71. Landolt W, Günthardt-Goerg M, Pfenninger I, Scheidegger C. 1994. Ozone-induced microscopical changes and quantitative carbohydrate contents of hybrid poplar (*Populus × euramericana*). *Trees* 8:183–90

72. Langley J, Drake B, Hungate BA. 2002. Extensive belowground carbon storage supports roots and mycorrhizae in regenerating scrub oaks. *Oecologia* 131:542–48

73. Le Dizès S, Cruiziat P, Lacointe A, Sinoquet H, Le Roux X, et al. 1997. A model for simulating structure-function relationships in walnut tree growth processes. *Silva Fenn.* 31:313–28

74. Le Roux X, Lacointe A, Escobar-Gutiérrez A, Le Dizès S. 2001. Carbon-based models of individual tree growth: a critical appraisal. *Ann. For. Sci.* 58:469–506

75. Leakey ADB, Bishop KA, Ainsworth EA. 2012. A multi-biome gap in understanding of crop and ecosystem responses to elevated CO_2. *Curr. Opin. Plant Biol.* 15:228–36

76. Levy PE, Lucas ME, McKay HM, Escobar-Gutierrez AJ, Rey A. 2000. Testing a process-based model of tree seedling growth by manipulating [CO_2] and nutrient uptake. *Tree Physiol.* 20:993–1005

77. Li J, Powell TL, Seiler TJ, Johnson DP, Anderson HP, et al. 2007. Impacts of Hurricane Frances on Florida scrub-oak ecosystem processes: defoliation, net CO_2 exchange and interactions with elevated CO_2. *Glob. Change Biol.* 13:1101–13

78. Li M, Hoch G, Körner C. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees* 16:331–37

79. Litton CM, Raich JW, Ryan MG. 2007. Carbon allocation in forest ecosystems. *Glob. Change Biol.* 13:2089–109

80. Luo Z-B, Calfapietra C, Liberloo M, Scarascia-Mugnozza G, Polle A. 2006. Carbon partitioning to mobile and structural fractions in poplar wood under elevated CO_2 (EUROFACE) and N fertilization. *Glob. Change Biol.* 12:272–83

81. Lux D, Leonardi S, Muller J, Wiemken A, Fluckiger W. 1997. Effects of ambient ozone concentrations on contents of non-structural carbohydrates in young *Picea abies* and *Fagus sylvatica*. *New Phytol.* 137:399–409

82. McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, et al. 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytol.* 178:719–39

83. McLaughlin SB, McConathy RK, Beste B. 1979. Seasonal changes in within-canopy allocation of ^{14}C-photosynthate by white oak. *For. Sci.* 25:361–70

84. McNeely R. 1994. Long-term environmental monitoring of ^{14}C levels in the Ottawa region. *Environ. Int.* 20:675–79

85. Mencuccini M, Hölttä T. 2010. The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytol.* 185:189–203

86. Mitchell PJ, O'Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA. 2013. Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytol.* 197:862–72

87. Muhr J, Angert A, Juárez RN, Muñoz WA, Kraemer G, et al. 2013. Carbon dioxide emitted from live stems of tropical trees is several years old. *Tree Physiol.* 33:743–52

88. Munch E. 1927. Dynamik der Saftstromungen. *Ber. Dtsch. Bot. Ges.* 44:69–71

89. Myers JA, Kitajima K. 2007. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *J. Ecol.* 95:383–95

90. Nardini A, Lo Gullo MA, Salleo S. 2011. Refilling embolized xylem conduits: Is it a matter of phloem unloading? *Plant Sci.* 180:604–11

91. Nelson E, Dickson R. 1981. Accumulation of food reserves in cottonwood stems during dormancy induction. *Can. J. For. Res.* 11:145–54

92. Newell EA, Mulkey SS, Wright SJ. 2002. Seasonal patterns of carbohydrate storage in four tropical tree species. *Oecologia* 131:333–42

93. Nzunda EF, Griffiths ME, Lawes MJ. 2008. Sprouting by remobilization of above-ground resources ensures persistence after disturbance of coastal dune forest trees. *Funct. Ecol.* 22:577–82

94. Ogee J, Barbour MM, Wingate L, Bert D, Bosc A, et al. 2009. A single-substrate model to interpret intra-annual stable isotope signals in tree-ring cellulose. *Plant Cell Environ.* 32:1071–90

95. Ogle K, Pacala SW. 2009. A modeling framework for inferring tree growth and allocation from physiological, morphological and allometric traits. *Tree Physiol.* 29:587–605

96. Palacio S, Hernández R, Maestro-Martínez M, Camarero JJ. 2012. Fast replenishment of initial carbon stores after defoliation by the pine processionary moth and its relationship to the re-growth ability of trees. *Trees* 26:1627–40

97. Paynter VA, Reardon JC, Schelburne VB. 1992. Changing carbohydrate profiles in shortleaf pine (*Pinus echinata*) after prolonged exposure to acid rain and ozone. *Can. J. For. Res.* 22:1556–61

98. Piispanen R, Saranpää P. 2001. Variation of non-structural carbohydrates in silver birch (*Betula rendula* Roth) wood. *Trees* 15:444–51

99. Piper FI. 2011. Drought induces opposite changes in the concentration of non-structural carbohydrates of two evergreen nothofagus species of differential drought resistance. *Ann. For. Sci.* 68:415–24

100. Piper FI, Reyes-Díaz M, Corcuera LJ, Lusk CH. 2009. Carbohydrate storage, survival, and growth of two evergreen nothofagus species in two contrasting light environments. *Ecol. Res.* 24:1233–41

101. Poorter L, Kitajima K. 2007. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology* 88:1000–11
102. Regier N, Streb S, Cocozza C, Schaub M, Cherubini P, et al. 2009. Drought tolerance of two black poplar (*Populus nigra* L.) clones: contribution of carbohydrates and oxidative stress defence. *Plant Cell Environ.* 32:1724–36
103. Reich PB. 1987. Quantifying plant response to ozone: a unifying theory. *Tree Physiol.* 3:63–91
104. Rennie EA, Turgeon R. 2009. A comprehensive picture of phloem loading strategies. *Proc. Natl. Acad. Sci. USA* 106:14162–67
105. Retzlaff WA, Weinstein DA, Laurence JA, Gollands B. 1996. Simulated root dynamics of a 160-year-old sugar maple (*Acer saccharum* Marsh) tree with and without ozone exposure using the TREGRO model. *Tree Physiol.* 16:915–21
106. Richardson AD, Carbone MS, Keenan TF, Czimczik CI, Hollinger DY, et al. 2013. Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytol.* 197:850–61
107. Ruehr NK, Offermann CA, Gessler A, Winkler JB, Ferrio JP, et al. 2009. Drought effects on allocation of recent carbon: from beech leaves to soil CO_2 efflux. *New Phytol.* 184:950–61
108. Ruelland E, Vaultier MN, Zachowski A, Hurry V. 2009. Cold signalling and cold acclimation in plants. In *Advances in Botanical Research*, Vol. 49, ed. J-C Kader, M Delseny, pp. 35–150. San Diego, CA: Academic
109. Ryan MG. 2011. Tree responses to drought. *Tree Physiol.* 31:237–39
110. Ryan MG, Phillips N, Bond BJ. 2006. The hydraulic limitation hypothesis revisited. *Plant Cell Environ.* 29:367–81
111. Sala A, Hoch G. 2009. Height-related growth declines in ponderosa pine are not due to carbon limitation. *Plant Cell Environ.* 32:22–30
112. Sala A, Piper FI, Hoch G. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytol.* 186:274–81
113. Sala A, Woodruff DR, Meinzer FC. 2012. Carbon dynamics in trees: feast or famine? *Tree Physiol.* 32:764–75
114. Sampson DA, Johnsen KH, Ludovici KH, Albaugh TJ, Maier CA. 2001. Stand-scale correspondence in empirical and simulated labile carbohydrates in loblolly pine. *For. Sci.* 47:60–68
115. Samuelson LJ, Kelly JM. 1996. Carbon partitioning and allocation in northern red oak seedlings and mature trees in response to ozone. *Tree Physiol.* 16:853–58
116. Schaefer K, Collatz GJ, Tans P, Denning AS, Baker I, et al. 2008. Combined Simple Biosphere/Carnegie-Ames-Stanford Approach terrestrial carbon cycle model. *J. Geophys. Res.* 113:G03034
117. Schutz AEN, Bond WJ, Cramer MD. 2009. Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. *Oecologia* 160:235–46
118. Sevanto S, McDowell NG, Dickman LT, Pangle R, Pockman WT. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant Cell Environ.* 37:153–61
119. Shigihara A, Matsumoto K, Sakurai N, Igawa M. 2008. Growth and physiological responses of beech seedlings to long-term exposure of acid fog. *Sci. Total Environ.* 391:124–31
120. Shumejko P, Ossipov V, Neuvonen S. 1996. The effect of simulated acid rain on the biochemical composition of Scots pine (*Pinus sylvestris* L.) needles. *Environ. Pollut.* 92:315–21
121. Sloan JL, Jacobs DF. 2012. Leaf physiology and sugar concentrations of transplanted *Quercus rubra* seedlings in relation to nutrient and water availability. *New For.* 43:779–90
122. Smith AM, Zeeman SC, Smith SM. 2005. Starch degradation. *Annu. Rev. Plant Biol.* 56:73–98
123. Smith NG, Dukes JS. 2012. Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO_2. *Glob. Change Biol.* 19:45–63
124. Sterner RW, Elser JJ. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton, NJ: Princeton Univ. Press
125. Stitt M, Zeeman SC. 2012. Starch turnover: pathways, regulation and role in growth. *Curr. Opin. Plant Biol.* 15:282–92
126. Sulpice R, Pyl ET, Ishihara H, Trenkamp S, Steinfath M, et al. 2009. Starch as a major integrator in the regulation of plant growth. *Proc. Natl. Acad. Sci. USA* 106:10348–53
127. Sveinbjörnsson B. 2000. North American and European treelines: external forces and internal processes controlling position. *Ambio* 29:388–95

128. Terziev N, Boutelje J, Larsson K. 1997. Seasonal fluctuations of low-molecular-weight sugars, starch and nitrogen in sapwood of *Pinus sylvestris* L. *Scand. J. For. Res.* 12:216–24

129. Thomas VFD, Braun S, Flückiger W. 2005. Effects of simultaneous ozone exposure and nitrogen loads on carbohydrate concentrations, biomass, and growth of young spruce trees (*Picea abies*). *Environ. Pollut.* 137:507–16

130. Thornley JHM. 1991. A transport-resistance model of forest growth and partitioning. *Ann. Bot.* 68:211–26

131. Thornley JHM, Cannell MGR. 2000. Modelling the components of plant respiration: representation and realism. *Ann. Bot.* 85:55–67

132. Tomlinson GH. 2003. Acidic deposition, nutrient leaching and forest growth. *Biogeochemistry* 65:51–81

133. Turgeon R. 2006. Phloem loading: how leaves gain their independence. *BioScience* 56:15–24

134. Turgeon R. 2010. The puzzle of phloem pressure. *Plant Physiol.* 154:578–81

135. Vanderklein D, Reich P. 1999. The effect of defoliation intensity and history on photosynthesis, growth and carbon reserves of two conifers with contrasting leaf lifespans and growth habits. *New Phytol.* 144:121–32

136. Vargas R. 2012. How a hurricane disturbance influences extreme CO_2 fluxes and variance in a tropical forest. *Environ. Res. Lett.* 7:035704

137. Vargas R, Baldocchi DD, Bahn M, Hanson PJ, Hosman KP, et al. 2011. On the multi-temporal correlation between photosynthesis and soil CO_2 efflux: reconciling lags and observations. *New Phytol.* 191:1006–17

138. Vargas R, Hasselquist N, Allen EB, Allen MF. 2010. Effects of a hurricane disturbance on aboveground forest structure, arbuscular mycorrhizae and belowground carbon in a restored tropical forest. *Ecosystems* 13:118–28

139. Vargas R, Trumbore SE, Allen MF. 2009. Evidence of old carbon used to grow new fine roots in a tropical forest. *New Phytol.* 182:710–18

140. Verdaguer D, Ojeda F. 2002. Root starch storage and allocation patterns in seeder and resprouter seedlings of two Cape Erica (Ericaceae) species. *Am. J. Bot.* 89:1189–96

141. Vingarzan R. 2004. A review of surface ozone background levels and trends. *Atmos. Environ.* 38:3431–42

142. Warren JM, Iversen CM, Garten CT, Norby RJ, Childs J, et al. 2012. Timing and magnitude of C partitioning through a young loblolly pine (*Pinus taeda* L.) stand using ^{13}C labeling and shade treatments. *Tree Physiol.* 32:799–813

143. Weinstein DA, Beloin RM, Yanai RD. 1991. Modeling changes in red spruce carbon balance and allocation in response to interacting ozone and nutrient stresses. *Tree Physiol.* 9:127–46

144. Wigley BJ, Cramer MD, Bond WJ. 2009. Sapling survival in a frequently burnt savanna: mobilisation of carbon reserves in *Acacia karroo*. *Plant Ecol.* 203:1–11

145. Wiley E, Helliker B. 2012. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytol.* 195:285–89

146. Wittig VE, Ainsworth EA, Long SP. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments. *Plant Cell Environ.* 30:1150–62

147. Wittig VE, Ainsworth EA, Naidu SL, Karnosky DF, Long SP. 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. *Glob. Change Biol.* 15:396–424

148. Woodruff DR, Meinzer FC. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. *Plant Cell Environ.* 34:1920–30

149. Würth M, Pelaez-Riedl S, Wright S, Körner C. 2005. Non-structural carbohydrate pools in a tropical forest. *Oecologia* 143:11–24

150. Zeeman SC, Kossmann J, Smith AM. 2010. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu. Rev. Plant Biol.* 61:209–34

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