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Detours on the phloem sugar highway: stem carbon storage and remobilization

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For trees to survive, they must allocate resources between sources and sinks to maintain proper function. The vertical transport pathway in tree stems is essential for carbohydrates and other solutes to move between the canopy and the root system. To date, research and models emphasize the role of tree stems as 'express' sugar highways. However, recent investigations using isotopic markers suggest that there is considerable storage and exchange of phloem-transported sugars with older carbon (C) reserves within the stem. Thus, we suggest that stems play an important role not only in long-distance transport, but also in the regulation of the tree's overall C balance. A quantitative partitioning of stem C inputs among storage and sinks, including tissue growth, respiration, and export to roots, is still lacking. Combining methods to better quantify the dynamics and controls of C storage and remobilization in the stem will help to resolve central questions of allocation and C balance in trees.

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Introduction

Trees are organisms that can live for decades, centuries, and even millennia. This long lifespan increases a tree's risk of encountering stressful conditions where normal functioning is disturbed and metabolism has to rely on stored resources [1]. Because trees are autotrophic organisms, storage of primary metabolites like carbohydrates is particularly important for survival during harsh environmental conditions and stress. Carbohydrates are allocated to various organs and processes operating on timescales ranging from hours to decades, each contributing to the tree's overall carbon (C) balance.

Tree stems are predominantly viewed as the infrastructure that provides mechanical support for the canopy and facilities transport between sources and sinks, but they also store ~40% of a tree's total **nonstructural carbohydrates** (NSCs; Box 1) [2•]. During C transport, there is substantial exchange of C between phloem and parenchyma of the stem [3], but a quantitative understanding of C partitioning between storage, tissue growth, and respiration as well as the underlying regulation of C partitioning is still lacking. Here we emphasize that the stem is itself a major C sink and an important organ for the regulation of the tree's C balance, not merely an 'express' highway for long-distance C transport. We stress the need for process-oriented research that will provide quantitative insights into dynamics and controls of C storage and remobilization in tree stems and along the transport pathway. Without such information, the stem will remain a missing link in whole-tree C balance.

Non-structural carbohydrates in the stem

NSCs exist in basically all components of living vegetative tissues of the plant; they can be found in vacuoles, plastids, and the cytosol of cells, as well as in the apoplast [4•]. Supply and demand of NSCs are often asynchronous and surplus sugars produced in leaves during daytime are stored as starch granules in chloroplasts. During the night, these starch granules are then hydrolyzed to glucose to fuel growth and respiration or exported as sucrose to other plant organs via the phloem [5].

Large amounts of NSCs are stored long-term in amyloplasts of **ray and axial parenchyma** cells. Because secondary growth in woody plants produces new cell layers interspersed with living parenchyma cells every year, the resulting tissues provide storage capacities for different temporal horizons: small branches and fine roots serve as seasonal storage, while large branches, coarse roots, and tree stems are used for decadal storage [6•]. Only the heartwood, comprising the inner part of tree branches and stems, does not contain living cells and therefore cannot remobilize remaining NSCs.

In the stem, NSC concentrations usually decrease across the sapwood towards the sapwood-heartwood transition zone and then remain constant (often at zero concentration in older trees) throughout the heartwood to the pith [7]. However, some studies have observed high levels of

Box 1 Glossary of key terms. Terms are bolded within text upon first mention

Term	Definition
Nonstructural carbohydrates (NSC)	Mainly sugars and starch, the major substrates for primary and secondary plant metabolism; 5-C sugars (i.e. glucose, fructose) function as metabolites and osmoregulators, while disaccharides and oligosaccharides (i.e. sucrose, raffinose) function as transport sugars
Ray and axial parenchyma	Living cells of the secondary xylem and phloem that function in many metabolic processes including NSC storage
Collection phloem	Sieve element-companion cell complex of the minor leaf vein that sucrose is loaded into after its production in the leaf mesophyll
Transport phloem	sieve element-companion cell complex in the major veins, petioles, branches, stems, and roots that transports and redistributes NSCs and other molecules to sinks along the vertical pathway
Release phloem	Sieve element-companion cell complex that unloads sucrose and other molecules into sink cells
Leakage-retrieval mechanism	Process by which NSCs traveling along the leaky transport phloem passively diffuse out and are actively loaded back into companion cells
Radiocarbon signatures	Well-documented changes in the radiocarbon signatures atmospheric carbon dioxide since the testing of atmospheric nuclear weapons in the 1960s provide an estimate for the mean time elapsed since C in NSC pools or plant tissues were fixed from the atmosphere
Lateral storage and remobilization	Lateral flow and accumulation of NSCs in sinks (i.e. parenchyma cells of stem xylem) and subsequent release of stored reserves back into the transport phloem
Apoplastic sensing	Proposed mechanism by which lateral flows are regulated through sensing of apoplastic sucrose concentrations; low apoplastic sucrose concentrations will reduce phloem loading

sugars [8••] and/or starch [9] deep in the xylem. NSC concentrations vary seasonally in the stems of several tree species [10], reflecting the accumulation, utilization, and redistribution of NSCs to buffer changing supply and demand throughout the year.

Whole-tree phloem transport

The Münch theory posits that the flux of carbohydrates from sources to sinks is driven by a hydrostatic pressure gradient in the phloem that causes mass flow of phloem sap along the sieve tubes [11,12•,13•]. The hydrostatic pressure is created by loading of sucrose into companion cells followed by diffusion into sieve tubes of the **collection phloem**. The increase in solute concentration in the sieve tubes decreases phloem water potential and causes inflow of water from surrounding tissues, mainly the xylem. Increasing turgor in the collection phloem of source tissues due to this inflow along with unloading of sucrose and ensuing decreases in turgor in the **release phloem** of sink tissues creates the gradient of hydrostatic pressure that drives phloem flow [14•].

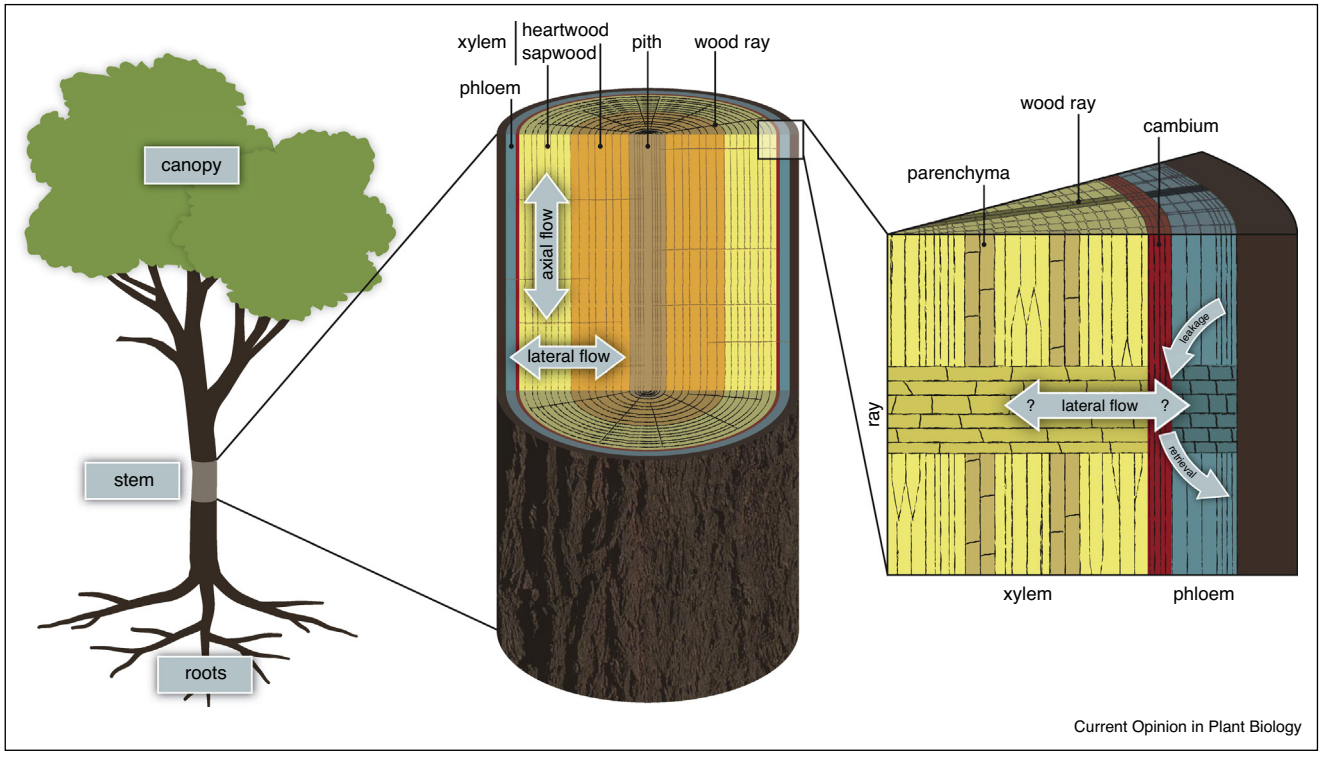
Stems are the linkage between the tree's main photosynthetic source, the canopy, and one of its major heterotrophic sinks, the root system. Secondary growth in stems produces layers of cells each growing season, divided into xylem, which transports mainly water and nutrients from the soil to the canopy and **transport phloem**, which redistributes carbohydrates and other organic and inorganic molecules across tree organs. Xylem water transport is much faster than phloem sap flow and can reach maximum peak velocities ranging from 16 to 45 m hour^{-1} depending on vessel size [15]. However, phloem velocities average between 22 cm hour^{-1} in gymnosperm trees and 56 cm hour^{-1} for angiosperms [16•], making tree stems large highways for both water and sugar transport.

Tree stems are more than just unidirectional 'express' highways

Viewing transport phloem in the tree stem as a simple sugar highway is too simplistic (Figure 1). During the last decades, evidence has accumulated that the transport phloem is not an 'express' highway, but rather a leaky pipe where carbohydrates passively diffuse out and are actively loaded back into companion cells during transport [17]; this has been termed the **leakage-retrieval mechanism**. In bean plants, about 6% of sugars are lost and 3.4% retrieved per centimeter of phloem length [18]. Thus, leakage and retrieval may provide resources locally for maintenance and growth of axial sinks like stem cambium [19].

The inverse flows involved in leakage and retrieval are thought to serve as short term buffers for imbalances between sources and sinks, but can also facilitate exchange of NSCs between the phloem and stem parenchyma [20,21]. Ray cells extend radially throughout the xylem and connect to the phloem, allowing for both the **lateral storage and remobilization** of NSCs into and out of the stem [22], which in turn supports the leakage and retrieval of solutes along the pathway [17].

Figure 1



Tree stems and phloem transport. Tree stems contain phloem, the main transport system connecting the major carbon source in the canopy to a large carbon sink in the roots. While phloem transport is often considered to be an express highway between canopy and roots, it is in fact more like a winding road interrupted by detours. Carbohydrates leak out of and are loaded back into the sieve elements of the phloem to provide energy and carbon skeletons for respiration, growth, and storage in living cells of the phloem, the cambium, and the xylem. Questions that need answering include: How much of the transported carbohydrates end up in parenchyma cells of wood rays in the xylem? and How are stored carbohydrates remobilized when source activity in the canopy is lower than overall carbon demand?

Recently, **radiocarbon signatures** of sugars and starch have demonstrated lateral storage by net inward mixing of younger NSCs into the stem in many temperate tree species [2•,23•]. The use of these mixed younger and older NSCs for respiration or growth of stem resprouts [24,25] support the idea of remobilization. While we have a basic understanding of the spatial and temporal distributions of NSCs in the stem, processes regulating the exchange of NSCs along the transport pathway — how younger C is mixed inward, and older C is used — remain poorly understood. In particular, the degree to which timescales of NSC storage result from physical transport and isolation (e.g. flow rates into and out of rays) versus active regulation requires further investigation [6•].

When the tree canopy is not the major source of NSCs (i.e. when deciduous trees lack leaves, or during extended drought), storage and remobilization of reserves in the stem may be particularly important. In fact, the transport pathway runs in reverse, supplying stored NSCs to grow buds and new leaves until they are able to fix excess C supply on their own. In addition to flowing in reverse, our understanding of phloem–xylem function has also been challenged. Recent work shows that long distance transport for springtime growth in young walnut trees is accomplished by NSC transport in the xylem, which is maintained with the recirculation of water by phloem Münch flow [26•].

Unknown role of tree stems in C balance

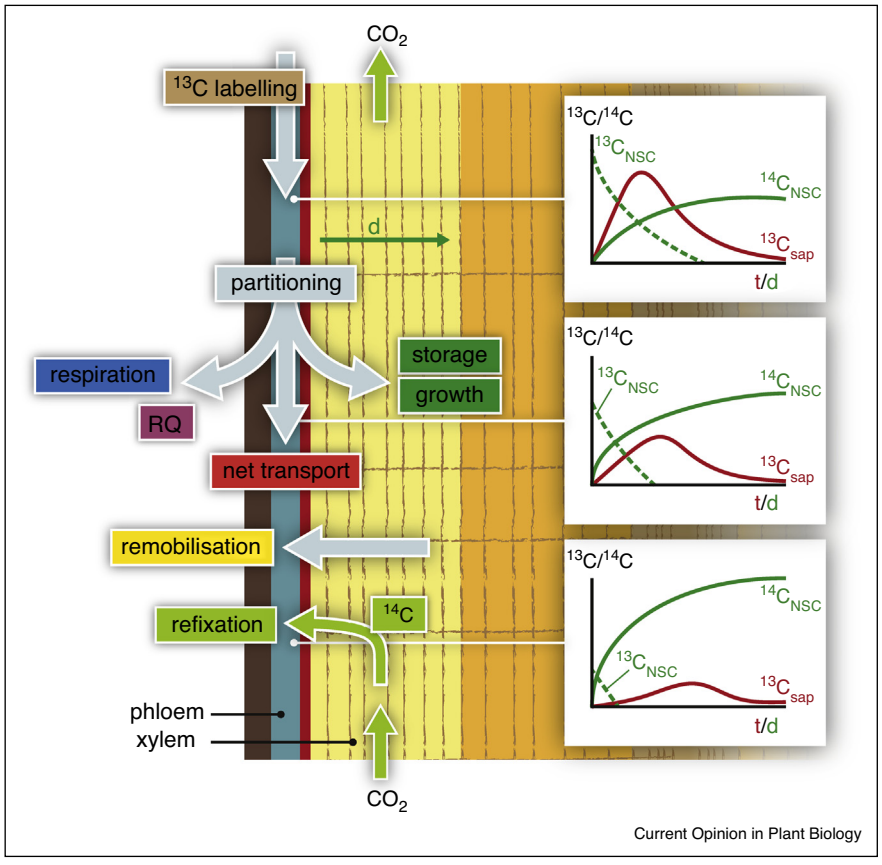
Isotopic tools have provided evidence for exchange dynamics along the leaky highway, particularly ^{13}C tracer studies that have monitored a label fixed in the canopy and respired from different organs over time. However, mass balance of the fate of the tracer in such studies is notoriously difficult. Strong evidence for phloem leakage and recovery comes from the observed ~10 fold reduction in the ^{13}C label associated with canopy-derived NSCs as it is transported between the top and bottom of the stem [27••]. Other canopy labeling studies tracing the ^{13}C distribution down the stem have quantified the label in CO_2 emitted from the stem, indicating not just transport, but also metabolism of canopy-derived sugar (reviewed in [28•]). Such studies have demonstrated

arrival of the tracer with downward transport rates of 0.2–1.2 m per hour (i.e. in the same range as water flow in phloem).

Studies tracking canopy-derived C also indicate the presence of additional mechanisms that allow the label to be detected in stem CO_2 efflux over subsequent weeks to months. This indicates that there is a second, slower process at work, as might be expected from phloem leakage and recovery. However, xylem-transported CO_2 can also influence signals in stem CO_2 efflux based on an estimated 50% of root respired CO_2 being retained and transpired upwards [29•]. The signal of respiration of ^{13}C-labeled photosynthetic products can therefore be diluted both via mixing of labeled and unlabeled NSCs before respiration, or by diffusion of unlabeled CO_2 from stem interiors. Teasing these signals apart requires using O_2 uptake rather than CO_2 release as a measure of local respiration rates [30••], as O_2 is far less soluble in stem water, or using the higher radiocarbon (^{14}C) content of in-stem CO_2 [25] to estimate the contribution from lateral diffusion.

In some cases, the ^{13}C label can be stored even longer, and is detectable in respiration [31] or new wood growth in subsequent years [32]. Thus, mixing and retrieval mechanisms have the potential to contribute to even long-term NSC stores in stems. Such studies are in accord

Figure 2



Partitioning of phloem transport. On its way from source to sink, fractions of phloem sap leaked out of sieve cells can be allocated to metabolic activity (e.g. respiration, cambial and wood growth) or stored within parenchyma cells of the xylem. These fractions are currently unknown and dedicated experimental approaches are required to provide information on these processes (see also Table 1). Labeling of phloem sap with stable carbon isotopes (^{13}C) via photosynthesis will yield information on the fraction of carbohydrates that remain in the sap on its way down (red lines in inlet panels). The ^{13}C signal decreases with distance (*d*) from the source (indicated by position on stem, distance increasing downwards) and over time (^{13}C signal of sap being diluted with 'fresh' photosynthates, *t* of *x*-axis on inlet panels is time). Storage of carbohydrates is indicated by ^{13}C signal of stem NSCs, which are likely to decline with distance from source and with distance from phloem (green dashed lines, *d* on *x*-axis of inlet panels is distance from phloem). The fraction of 'older' carbohydrates in stored NSCs consequently increases with distance from source and from phloem (^{14}C signal, green solid lines on inlet panels) as less 'fresh' photosynthates are stored. Partitioning of each fraction can be derived from comparing ^{13}C signals of stored NSCs, wood tissues, phloem sap, and respired CO_2 whereas the fraction of CO_2 transported in the xylem and contributing to stem emissions can be estimated by RQ (respiratory quotient, the amount of CO_2 evolved over the amount of oxygen used during respiration; the RQ can provide information on the substrates used in respiration, like carbohydrates or lipids, or indicate the fraction of root-derived CO_2 transported in the xylem and emitted by the stem) measurements.

Table 1

Future directions and tools for assessing whole-tree NSC transport

Question	Current understanding	New experiments/observations
How are NSCs that are loaded into the phloem in the canopy partitioned (Figure 2) as they move down the stem?	Label signal from ^{13}C fixed in the canopy is diluted in phloem sugars and stem CO_2 efflux as the distance from the canopy increases [27••]	Combine canopy ^{13}C labeling with natural radiocarbon measurements in phloem sap, wood and xylem NSCs and CO_2 efflux at different stem heights (panels in Figure 2). Use O_2 uptake (RQ; Figure 2) and C isotopes to differentiate local respiration from transported CO_2
What fraction of the C respired by roots originated in the canopy in the last few days?	Transport from canopy labeling combined with radiocarbon signature of root respiration indicates a mix of multi-year storage reserves and label C [31]	Perform more combined label/radiocarbon experiments. Add compound specific isotope measurements (e.g. sucrose, amino acids, organic acids) to understand changing sources of these compounds in phloem and stem CO_2
What is the role of CO_2 fixation and internal recycling in tree C balance?	A portion of ^{13}C-CO_2 label applied at stem base is transported to the canopy and fixed by photosynthetic tissues [29•] Low respiration quotients [30••] suggest CO_2 is also fixed within stems to produce organic acids	Labeling of in-stem CO_2 and tracking the label into tissues and organic compounds in xylem and phloem. Simultaneous monitoring of isotopes in CO_2 efflux and O_2 uptake in extracted tissues as well as stem fluxes with distance from the label injection
Does the remobilization of older reserves increase when canopy C sources decline (dormant season or extended drought)?	Radiocarbon signatures of NSC and in-stem CO_2 indicate storage reserves that are decades old contribute to respiration [2•,23•,24,25]	Use stem girdling to disrupt phloem transport and track O_2 uptake (RQ) as well as the ^{14}C of respired CO_2 with time (in extracted cores and stem chambers)

with the inferred use of storage reserves from either long-term labeling experiments [33] or the bomb ^{14}C signature. Krepowski *et al.* [32] showed large differences in the timescales of label persistence between deciduous and evergreen broadleaf trees; Epron *et al.* [28•] showed faster transport of label downward in gymnosperms compared to angiosperms. Thus, the mechanisms at work and how NSCs are allocated among them differ with the life strategy of the tree, a fact that must be kept in mind when comparing studies.

Regulation of exchange processes

While there is evidence that exchange of NSCs along the transport pathway is occurring, the regulation of these processes is not well understood. We know that they are controlled partly by the distribution of and coordination between sources and sinks. Further, it has been proposed that NSC storage and remobilization in ray and axial parenchyma cells of the stem involves both plasmodesmatal and transporter-mediated routes for exchange [3], with active uptake that is likely controlled by the concentration of sugars in the apoplastic space, at least for short term buffering [34].

But, it is unclear if **apoplastic sensing** plays a role in longer term NSC storage. The phloem is, however, not limited to the long distance transport of sugars as it also carries nutrients and signaling molecules like hormones, RNA, and proteins [35,36]. These signaling molecules may induce changes to the abundance and distribution of transporters and enzymes that then have the potential to modify the partitioning and allocation of stem reserves [37]. Understanding both the relative importance of various signaling molecules for exchange dynamics and how they exert control on processes like lateral storage and remobilization will help shed light on the mechanisms at play along the stem during long distance transport.

Tools and approaches for understanding stem storage and remobilization of whole-tree NSC transport

Future work should aim to combine the measurement of NSCs with isotope studies to piece together a detailed picture of storage and allocation processes in the stem, which will in turn inform our understanding of whole-tree transport. Historically, storage in the stem has been estimated by measuring NSCs from sapwood tissue collected at breast height, but it is clear that concentrations may vary with distance from the source and should be quantified both radially and axially at different stem heights [7,38]. Concurrent application and monitoring of isotopic signals like ^{13}C and ^{14}C along the stem will allow us to partition C to different sinks and tease apart the importance of and mechanisms behind different processes like lateral storage and remobilization and CO_2 fixation (Figure 2 and Table 1). Additional manipulation of source–sink relationships through girdling and defoliation may help to further resolve stem dynamics.

There are many complex questions that these methods will help address (see also Table 1): How much of the timescale involved in storage is related to physical transport (i.e. mixing going down the stem) versus actual chemical transformations (i.e. sugar to starch and back)?

How important is internal recycling/refixation of CO_2 in the stem xylem to the whole-tree C budget? Like short term buffering, does apoplastic sensing control the long term storage and remobilization of reserves and how do wood anatomy and leaf habit influence such processes? Answering these and similar questions will highlight the stem's role not only in C transport, but also in regulating the whole-tree C balance.

Summary and conclusion

The statement by Minchin *et al.* [39••] "There has been very little work to determine whether axial sinks alter the 'classical' model of source to sink transport, probably because of the experimental difficulties . . . " is still valid. We currently do not understand the dynamics and regulation of storage and exchange processes that occur along the transport pathway, and the stem remains a black box for our understanding of whole-tree C balance. Recent applications of X-ray micro-computed tomography imaging combined with machine learning have opened new avenues for *in vivo* observations of stem storage processes [40•]. In addition, further research using isotopic tracers and clever experimental designs (Figure 2) is required to produce quantitative evidence for C partitioning in tree stems and will show that tree stems are not just transport highways, but also play an important role in the C metabolism of trees.

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