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Age, allocation and availability of nonstructural carbon in mature red maple trees

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Summary

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- The allocation of nonstructural carbon (NSC) to growth, metabolism and storage remains poorly understood, but is critical for the prediction of stress tolerance and mortality.
- We used the radiocarbon (¹⁴C) 'bomb spike' as a tracer of substrate and age of carbon in stemwood NSC, CO₂ emitted by stems, tree ring cellulose and stump sprouts regenerated following harvesting in mature red maple trees. We addressed the following questions: which factors influence the age of stemwood NSC?; to what extent is stored vs new NSC used for metabolism and growth?; and, is older, stored NSC available for use?
- The mean age of extracted stemwood NSC was 10 yr. More vigorous trees had both larger and younger stemwood NSC pools. NSC used to support metabolism (stem CO₂) was 1–2 yr old in spring before leaves emerged, but reflected current-year photosynthetic products in late summer. The tree ring cellulose ¹⁴C age was 0.9 yr older than direct ring counts. Stump sprouts were formed from NSC up to 17 yr old.
- Thus, younger NSC is preferentially used for growth and day-to-day metabolic demands. More recently stored NSC contributes to annual ring growth and metabolism in the dormant season, yet decade-old and older NSC is accessible for regrowth.

Introduction

Nonstructural carbon (NSC) compounds are the primary products of photosynthesis, consisting mainly of mobile sugars (e.g. sucrose, glucose, fructose) and immobile starch and lipids. Once assimilated, NSC may be allocated to metabolism or new tissue growth, exported to the soil through roots, used to produce protective compounds or stored for future use (Chapin *et al.*, 1990; Herms & Mattson, 1992). Stored NSC provides an essential carbon (C) buffer to plants when demand (growth, protection and metabolism) exceeds supply (photosynthesis), such as during the night, the dormant season or in response to environmental stress or disturbance. However, allocation to and from storage, and the accessibility and function of stored NSC, still remain poorly understood within long-lived plants (Sala *et al.*, 2012).

Mature trees contain large amounts of NSC (Körner, 2003), several times that needed to regrow the canopy foliage (Hoch *et al.*, 2003; Würth *et al.*, 2005). Allocation of NSC to storage has traditionally been thought of as a passive process, in which NSC is allocated to the storage pool only after all other demands for C have been met (Chapin *et al.*, 1990). However, NSC storage may also be an active process, in which NSC is allocated at

the expense of growth (Wiley & Helliker, 2012), presumably as an evolutionary adaptation to cope with stress factors, such as variable climate, pests and disease, or disturbance. In addition to serving as a large buffer pool of C, NSC may also support important reserves of mobile nitrogen (Millard & Grelet, 2010) and may help to maintain hydraulic conductivity during drought and freeze–thaw events (Sala *et al.*, 2012). Thus, an understanding of NSC dynamics is important for the prediction of tree stress responses, mortality and resilience to global change.

Previous work has indicated that NSC allocated to metabolism and growth can come from newer (recently photosynthesized) or older (stored) sources. Ecosystem-scale pulse-labeling studies have shown that the majority of new NSC is respired in days (Carbone *et al.*, 2007; Höglberg *et al.*, 2008; see review by Epron *et al.*, 2012 and references therein), demonstrating a fast link between assimilation, allocation and metabolism, with rapid use of new NSC even in belowground processes. NSC allocated to foliage and fruits also appears to be from newly assimilated NSC (Keel *et al.*, 2006; Hoch *et al.*, 2013). However, there is also evidence that NSC allocated to root (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006; Carbone *et al.*, 2011) and stem (Muhr *et al.*, 2013) respiration can be several years old, reflecting a significant

contribution of stored NSC. New roots, leaf buds and flowers have also been shown to grow from stored NSC up to 2 yr old (Gaudinski *et al.*, 2009; Ichie *et al.* 2013). In tree rings, stored NSC up to 3 or 4 yr old has been incorporated into tree ring cellulose growth (Keel *et al.*, 2006; Krepkowski *et al.*, 2013).

More recently, evidence has been obtained that much older NSC is present in mature trees. Richardson *et al.* (2013) found that the NSC extracted from the stemwood of temperate forest trees was 7–14 yr old. Yet, the availability of this decade-old NSC to support the growth and metabolism of these trees is not known (Rocha, 2013). NSC accumulated in woody tissues may become sequestered, and therefore not available to the tree for future use (Millard *et al.*, 2007). However, Vargas *et al.* (2009) demonstrated that NSC as old as 10 yr was allocated to new root growth following a major hurricane disturbance in a dry tropical forest, proving that some older NSC may still be accessible.

Collectively, these results suggest that trees have both fast and slower cycling NSC pools (Carbone & Trumbore, 2007; Kuptz *et al.*, 2011; Richardson *et al.*, 2013). There is also evidence for mixing between these components (Keel *et al.*, 2006, 2007), and fast and slow pools probably have different ecological and C-cycling roles that have not been characterized fully. Hence, the identification of the factors controlling the amount and residence time of NSC within trees, the allocation of new vs stored NSC to different processes and the accessibility of stored NSC is needed to improve storage and allocation representations in current C-cycling models.

To better understand these aspects of NSC allocation and storage, we studied mature red maple (*Acer rubrum*) trees in two New England, USA temperate forests. Applying the radiocarbon (^{14}C) 'bomb spike' approach (Trumbore, 2006), we estimated the age (a proxy for the mean residence time, MRT) of C in stemwood NSC, stem CO_2 emissions, tree ring cellulose and stump sprouts regenerated following harvesting. The main goal of our research was to use these isotopic measurements, together with stemwood NSC concentrations, to compare the NSC used for day-to-day metabolism, annual wood growth and regrowth to the NSC actually present in the stemwood. Specifically, we addressed three questions. First, how does the age of stored NSC vary with tree size, tree age, and growth and respiration rates? Second, what is the age of NSC allocated to stem respiration and wood growth? Finally, is decade-old NSC accessible to trees?

Materials and Methods

The work was conducted between 2011 and 2012 in two New England, USA mixed hardwood forests. Harvard Forest (42.53°N, 72.17°W, 340 m above sea level) is located in central Massachusetts and has a mean annual temperature of 8.2°C and a mean annual precipitation of 1270 mm. Approximately 250 km to the northeast, Bartlett Experimental Forest (44.06°N, 71.29°W, 270 m above sea level) is located in the White Mountain National Forest, New Hampshire. Bartlett Experimental Forest is slightly cooler, with a mean annual temperature of 7.3°C, but, at 1270 mm, the mean annual precipitation is the same as at Harvard Forest. Red maple (*Acer rubrum* L.) is a

dominant tree species at both sites. It is considered as a generalist species (Abrams, 1998) that grows throughout the eastern USA from Maine to Florida.

Stem CO_2 emissions

At each site, we selected five red maple trees, ranging in size from 9 to 34 cm diameter at breast height (DBH; 1.4 m above ground level; Table 1). Static chambers (volume, 2.93 l) were installed to measure the stem CO_2 flux and to collect CO_2 for ^{14}C analyses. Each chamber was constructed from a plastic polypropylene pipe T-fitting permanently capped on the top and bottom ends. For opening and closing the chamber, a threaded cap with an O-ring seal was screwed on to the outer side of the T. On the inner side, there was a 256-cm² rectangular opening in direct contact with the tree stem. Chambers were attached to the stem at breast height using a hot glue gun, and then sealed to the stem surface with caulking (Nautiflex; OASE GmbH, Oerel-Barchel, Germany). While not in use, a cap with a fine wire mesh screen was screwed on to the T, so that chambers remained open to the ambient atmosphere, but were closed to insects and birds.

Before sampling for ^{14}C , stem CO_2 flux rates were measured on each chamber. The chamber was closed with a threaded screw cap containing gas inlet and outlet ports, as well as a probe for measuring air temperature inside the chamber. Air was circulated with a pump at 0.5 l min⁻¹ within the chamber headspace through an infrared gas analyzer (IRGA, LI-820; Licor, Lincoln, NE, USA) for 5 min. We recorded the change in CO_2 concentration every 1 s, and then fitted a linear model using least-squares regression to determine the rate of CO_2 accumulation. The slope of the fitted equation, the chamber volume and the tree stem surface area covered by the chamber were used to calculate the stem CO_2 flux rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Molar fluxes were based on concentrations adjusted for temperature and atmospheric pressure.

To collect samples for the ^{14}C content of stem CO_2 emissions, the chambers were closed using a threaded screw cap containing gas inlet and outlet ports, and the chambers were left to accumulate CO_2 for 7 d. At this time, CO_2 concentrations in each chamber were measured with the IRGA, and samples were extracted through the outlet port with pre-evacuated air canisters (X3 series; LabCommerce Inc., San Jose, CA, USA). To keep pressure at ambient levels, a soda lime column was attached to the inlet port of each chamber, allowing CO_2 -free air to pass into the chamber whilst the samples were extracted. At each site, an atmospheric air sample was taken at chamber height with a pre-evacuated 6-l canister that filled slowly over 2 h. These air samples were used to correct the reported $^{14}\text{CO}_2$ emitted from the stem for the air remaining in the chambers when the CO_2 accumulation began using a two-end-member mixing model. Stem CO_2 flux measurements and sampling for $^{14}\text{CO}_2$ analysis were conducted in the field on two different occasions in 2012: early spring before leaf-out (mid-April) and late summer (beginning of September). We interpret these measurements as a proxy for stem respiration, even though CO_2 emitted from the stem may

integrate both root and stem respiration sources (Bloemen *et al.*, 2013; Trumbore *et al.*, 2013).

Stemwood NSC and tree ring cellulose

Two shallow stemwood cores were collected from each tree with a standard 4.3-mm increment borer (Häglof Company Group, Långsele, Sweden) at the same height as the chambers in September 2011. Cores were placed in clean plastic drinking straws and immediately frozen in the field on dry ice. On return to the laboratory, the cores were dried and the outer 2 cm of each core were isolated. One core was used for total NSC concentration analyses and the other for ^{14}C analyses.

The NSC concentration analysis was conducted at the USDA Forest Service in Burlington, VT, USA following the methods of Wong *et al.* (2003). Briefly, ethanol-soluble fractions were analyzed for sucrose, glucose, fructose, raffinose and stachyose using a Waters Alliance HPLC system (Milford, MA, USA). Sugars were detected with a Waters 2414 refractive index detector and Waters PC-based Empower software. The separated soluble sugars were identified and quantified with known standards and converted to mg sugar g^{-1} oven-dry wood. The ethanol-insoluble pellets were used to determine starch content after gelatinization with 0.1 M KOH and neutralization with acetic acid by hydrolysis with amyloglucosidase (Wargo *et al.*, 2002). Glucose formed by hydrolysis was determined with a glucose hexokinase kit (Pointe Scientific, Canton, MI, USA) at 340 nm with a Bio-Tek Instruments Elx800 UV microplate reader (Winooski, VT, USA). Concentrations of starches were calculated from glucose standard curves and expressed as mg starch g^{-1} oven-dry wood.

For ^{14}C analyses, soluble NSC (total sugars) was extracted from each core sample by hot water extraction, as described in Richardson *et al.* (2013). Other existing extraction methods using ethanol or methanol, such as developed by Wong *et al.* (2003), cannot be applied to isolate NSC for ^{14}C analysis because they introduce too much extraneous C that alters the ^{14}C content of the NSC pool. Previous work established very good agreement between NSC concentrations and the yield of extracted sugars for the ^{14}C protocol (Richardson *et al.*, 2013). In addition, only sugar and not starch was extracted for ^{14}C analysis because of the previously established correspondence between ^{14}C of starch and sugar pools (Richardson *et al.*, 2013) and the time and expense associated with ^{14}C measurements.

The innermost ring (i.e. oldest) in each 2-cm stemwood core was isolated for ^{14}C analyses. Holocellulose was extracted using a traditional batch processing cellulose extraction method, based on the work of Leavitt & Danzer (1993). Samples sealed in AnkomTM (Macedon, NY, USA) F57 Dacron filter bags were placed in a 50-mm-ID (200 ml) Soxhlet apparatus and extracted with 600 ml of a 2 : 1 mixture of toluene and ethanol, and then with 600 ml ethanol, each for *c.* 24 h. After the solvent extraction, samples were boiled gently for 2 h in Milli-Q water and then placed in a bleaching solution (4 g of sodium chlorite and 2 ml of glacial acetic acid added to 600 ml of water) at 70°C. An additional 4 g of chlorite and 2 ml of acetic acid were added after

3–4 h, after which the samples were washed several times with Milli-Q water over 3–4 h and dried at 60°C overnight.

Tree ages and growth rates

Trees were also cored to the pith in September 2011 with an increment borer at the same height as the shallower NSC cores to determine tree age and growth rates. These cores were mounted in grooved wooden blocks and sanded. Annual xylem increments were microscopically measured to a resolution of 0.001 mm using a Velmex sliding stage unit (Velmex Inc., Bloomfield, NY, USA) with MeasureJ2X software (VoorTech Consulting, Holderness, NH, USA) according to the methods of Stokes & Smiley (1968). The computer program COFECHA was used to detect potential cross-dating errors in ring series (Holmes, 1983). We converted ring width data to basal area increments ($\text{cm}^2 \text{yr}^{-1}$) assuming a circular outline of stem cross-sections. In addition to the total number of rings in the core, we counted the number of rings in the outer 2 cm of stemwood, corresponding to the tissue used for NSC and sugar ^{14}C analyses.

Stump sprouts

After timber harvesting, red maple is characteristically a prolific stump sprouter. To obtain stump sprouts, which are formed from NSC stored in the still-living root system, we took advantage of harvest activities that occurred at each site during the late autumn and winter of 2010–2011. Shortly after snowmelt in 2011, we identified *c.* 15 red maple stumps at each site. Stumps ranged greatly in size from 3.7 to 66 cm in basal diameter. Each stump was partially covered with thick black plastic to completely block photosynthetically active radiation. Thus, when the sprouts emerged beneath the plastic in the spring, they could not incorporate any new C through photosynthesis. Between April and May, each stump was checked every 2–7 d for sprouts underneath the plastic. Any sprouts that were present were collected and immediately air dried. Sprouts ranged in size from 1 to 3 cm in length. To determine the age of the tree from which each sprout was collected, we cut disks from each stump with a chain saw. These were air dried and then planed smooth with an electric hand planer before counting rings under a dissecting microscope.

Isotope analyses

For isotopic analysis of the C in stump sprouts, tree ring cellulose, annual plants (to be described) and extracted sugars, these samples were first combusted to CO_2 . The combusted samples, as well as the samples already in the gas phase (ambient air and stem CO_2 emissions), were then purified on a vacuum line and converted to graphite (Xu *et al.*, 2007). Aliquots of CO_2 were extracted from each sample for $\delta^{13}\text{C}$ measurement on a Thermo Electron Gas Bench II coupled with a Delta Plus Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany). Graphite was analyzed for its ^{14}C content at the W.M. Keck Carbon Cycle Accelerator Mass Spectrometry facility at UC Irvine (KCCAMS; Southon *et al.*, 2004). Data are reported in Fraction

Modern ($F^{14}C$), the ratio of $^{14}C/^{12}C$ in a sample divided by that of a standard of fixed isotopic composition (0.95 times the $^{14}C/^{12}C$ of oxalic acid I standard from the year 1950; Reimer *et al.*, 2004). Measurements have been corrected for the effects of mass-dependent isotope fractionation by normalizing to a common $\delta^{13}C$ value (-25‰) and assuming that ^{14}C is fractionated twice as much as ^{13}C (Stuiver & Polach, 1977). The measurement precision ($\pm 1SD$) for all $F^{14}C$ is ≤ 0.0024 and for $\delta^{13}C$ values is $\leq 0.15\text{‰}$.

Determination of ^{14}C -based ages

The mean age of C in the sugars, stump sprouts and CO_2 emitted by stems was determined using the ^{14}C ‘bomb spike’ approach, with ages estimated by direct comparison with the northern hemisphere atmospheric $^{14}CO_2$ record (Levin *et al.*, 2008; X. Xu, unpublished). Using this approach, the ^{14}C age is an accurate estimate of the MRT of C pools 20 yr old and younger (assuming they are well mixed; Richardson *et al.*, 2013). We monitored for local offsets in this record with air and annual plant samples (grown in non-forested areas: jewelweed, *Impatiens capensis* Meerb.; ragweed, *Ambrosia artemisiifolia* L.; Japanese stilt grass, *Microstegium vimineum* Trin.) that were collected at each site (and processed for ^{14}C as described previously) to quantify the site-specific background atmospheric $^{14}CO_2$, and used as the signature of photosynthetic products at the time of sampling. For 2011, the background values were 1.0444 and 1.0488 at Harvard Forest and Bartlett Experimental Forest, respectively, and, in 2012, they were 1.0372 and 1.0403, respectively. Because samples were collected over 2 yr (atmospheric $^{14}CO_2$ is declining with time), when plotting multi-year data together, we report the difference between the sample $F^{14}C$ and the atmosphere $F^{14}C$ at the time each sample was collected (Fig. 1). Finally, tree one at Harvard Forest was eliminated from the final ^{14}C results and analyses because the tree ring cellulose $F^{14}C$ indicated the presence of pre-bomb C (i.e. 1954 or earlier), and therefore it was not possible to determine a ^{14}C -based age.

Data analyses

We used principal component analysis (PCA) to identify the major axes of variation in our multivariate dataset (tree age, DBH, mean basal area increment, total NSC concentration, stem CO_2 flux rate and sugar age). PCA is a widely used data reduction technique in which new composite variables (components) are constructed as linear combinations of the original independent variables. It is particularly appropriate when there is a high degree of correlation among the independent variables. The first few components can typically explain much of the variability in the original dataset. Our PCA was conducted in MATLAB (function `pca`; MathWorks, Natick, MA, USA) on the correlation matrix, because units differed among variables (McGarigal *et al.*, 2000).

To investigate how patterns of tree growth were related to NSC concentration and age, we combined data from this study with data from an additional 27 red maple trees sampled in 2010 (nine each at Harvard Forest and Bartlett Experimental Forest,

and an additional nine at Howland Forest, Maine) and reported in Richardson *et al.* (2013). We used an autocorrelation analysis (function `autocorr`; MATLAB) of the last 50 yr of ring width chronology for each tree to quantify the inherent ‘memory’ in the annual growth increment – that is, how well was growth in year t correlated with lagged growth in years $t-1$, $t-2$, $t-3$...? For each tree, we identified the maximum lag (in years) that was significant (at 95% confidence) in the ring width autocorrelation function. We conducted this analysis on residuals of raw ring width detrended with an inflexible spline (1–2 piece, second- and third-order polynomial, robust smoothing; Garcia, 2010) to remove tree size and age-related trends associated with tree growth (Fritts, 1976).

Results

Stemwood NSC and tree ring cellulose

Across all trees, the concentrations of total stemwood NSC averaged $18.6 \pm 4.7 \text{ mg g}^{-1}$ oven-dry wood (total = starch + sugars, mean $\pm 1SD$; Table 1). Sugars averaged $4.5 \pm 1.2 \text{ mg g}^{-1}$, contributing 11–32% of the extracted total stemwood NSC across the different trees, with a mean of *c.* 24%. Tree DBH and height were positively correlated with total stemwood NSC concentration ($r=0.71$, $P=0.02$ and $r=0.67$, $P=0.03$, respectively; Table 1) and sugar $\delta^{13}C$ ($r=0.70$, $P=0.03$ and $r=0.61$, $P=0.06$, respectively; Table 2). Sugar $F^{14}C$ was similar at Harvard Forest and Bartlett Experimental Forest, 1.0851 ± 0.0182 and 1.0900 ± 0.0297 , corresponding to ^{14}C ages of 9.3 ± 3.5 and 10.3 ± 5.9 yr, respectively (Fig. 1; Table 2). Sugar ^{14}C age was positively correlated with the number of rings in the 2-cm core from which the NSC was extracted ($r=0.84$, $P<0.01$; Fig. 2a). Using the relationship between sugar ^{14}C age and starch ^{14}C age established in Richardson *et al.* (2013) for red maple, the estimated concentration-weighted mean ^{14}C age of total NSC across all trees was 9.1 ± 7.8 yr.

The age of the cellulose in the innermost ring (oldest) of the 2-cm cores, determined by ^{14}C analyses, and the age of the cellulose determined by visually counting the number of rings in the 2-cm cores were extremely similar (Fig. 2b). For three of the trees, the difference between the two measurements fell within the uncertainty of the tree ring cellulose ^{14}C measurement error (± 1 yr). Across all cores, the tree ring cellulose ^{14}C age was 0.9 ± 1.8 yr older (mean $\pm 1SD$) than the number of rings in the 2-cm core. Interestingly, the difference between the two measurements was positively related to the extracted sugar ^{14}C age from the stemwood ($r=0.60$, $P=0.08$), indicating that the age of NSC allocated to ring growth reflects that of the stemwood NSC pool to some degree.

Stem CO_2 emissions

In early spring (April), before leaf-out, the average stem CO_2 flux rate across all trees was $0.44 \pm 0.28 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ (mean $\pm 1SD$) at a mean chamber air temperature of $10^\circ C$ (Table 1). In the late summer (September), the average rate was

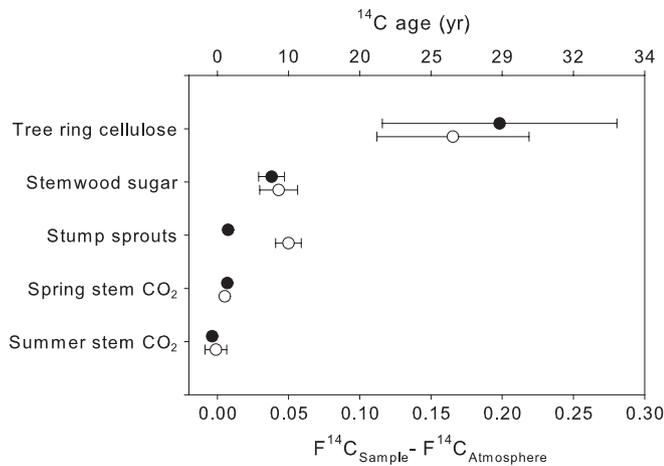


Fig. 1 Difference between the ^{14}C of samples and ^{14}C of the atmosphere at the time at which each sample was collected (in Fraction Modern) on the bottom x axis, for cellulose in the innermost (oldest) ring of the 2-cm-deep stemwood core, extracted stemwood sugars, stump sprouts and stem CO_2 emitted in spring and summer. Corresponding ^{14}C age (yr) on the top x axis. Symbols and error bars represent the mean \pm 1SD of ($n = 4$) red maple (*Acer rubrum*) trees at Harvard Forest (closed circles) and ($n = 5$) trees at Bartlett Experimental Forest (open circles), both sites located in New England, USA. In some cases, the error is smaller than the size of the symbol.

$0.94 \pm 0.44 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 22°C . This corresponds to a seasonal Q_{10} temperature sensitivity of $c. 2$. Spring stem $\text{F}^{14}\text{C}\text{CO}_2$ was higher ($P = 0.06$ by paired t -test) than that of late summer, 1.0447 ± 0.0042 and 1.0369 ± 0.0122 , respectively (Fig. 1, Table 2). This corresponded to a ^{14}C age of 1.5 ± 1.0 yr in spring and, in summer, it reflected the current-year photosynthetic products with the exception of one tree (Bartlett-5). This tree emitted 3.4-yr-old C in spring and 7.3-yr-old C in summer. In addition, stem-emitted $\delta^{13}\text{CO}_2$ was more negative ($P = 0.07$ by paired t -test) in spring than in late summer, $-25.2 \pm 1.8\text{‰}$ and $-24.1 \pm 1.3\text{‰}$, respectively (Table 2). In both spring ($r = 0.77$, $P = 0.01$) and late summer ($r = 0.65$, $P = 0.05$), stem-emitted $\delta^{13}\text{CO}_2$ was positively correlated with the stemwood sugar $\delta^{13}\text{C}$ extracted in late summer of the previous year. By contrast, there was no significant correlation (both $P > 0.1$) between stemwood sugar F^{14}C and stem-emitted F^{14}CO_2 in either spring or summer.

Tree characteristics and NSC

The PCA shows how tree characteristics, such as size, age and growth rate, co-vary with stemwood NSC concentration, stemwood sugar age and stem CO_2 flux (Fig. 3). The dataset passed the Kaiser–Meyer–Olkin sampling adequacy test (0.73), as well as Bartlett’s test of sphericity ($\chi^2 = 35.0$, $P < 0.01$). Component 1 explained 70% of the total variance in the dataset, and is interpreted as a general indicator of tree vigor. Trees with positive scores on component 1 were faster growing and larger in size, and had higher stemwood NSC concentrations, greater stem CO_2 flux rates and younger stemwood sugars (6 ± 4 yr for trees with component 1 > 0 , compared with 14 ± 4 yr for trees with component 1 < 0). Tree age was not a major factor on

Table 1 Diameter at breast height (DBH, cm), height (m), age (yr), nonstructural carbohydrate (the sum of sugars + starch) concentrations extracted from 2-cm stemwood cores in late summer (mg g^{-1} dry wood), and spring and summer stem CO_2 flux rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for red maple (*Acer rubrum*) trees at Harvard Forest ($n = 5$) and Bartlett Experimental Forest ($n = 5$)

Sample ID	DBH (cm)	Height (m)	Age (yr)	Sucrose (mg g^{-1})	Glucose (mg g^{-1})	Fructose (mg g^{-1})	Raffinose (mg g^{-1})	Stachyose (mg g^{-1})	Total sugar (mg g^{-1})	Starch (mg g^{-1})	Total nonstructural carbohydrates (mg g^{-1})	Spring stem CO_2 ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	Summer stem CO_2 ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)
Harvard-1	9	11.0	65	2.30	0.13	0.10	0.25	0.00	2.80	8.62	11.42	0.14	0.23
Harvard-2	18	23.0	75	2.43	0.10	0.07	0.48	0.05	3.15	12.09	15.24	0.94	0.61
Harvard-3	21	20.3	78	6.18	0.12	0.07	0.50	0.12	7.01	14.76	21.77	0.29	1.49
Harvard-4	26	26.2	84	4.64	0.36	0.23	0.22	0.01	5.48	18.29	23.77	0.33	1.16
Harvard-5	34	26.3	84	4.75	0.15	0.14	0.43	0.07	5.56	18.54	24.10	0.41	1.21
Harvard mean	21.6 (9.3)	21.4 (6.3)	77.2 (7.9)	4.06 (1.66)	0.17 (0.11)	0.12 (0.07)	0.38 (0.13)	0.05 (0.05)	4.8 (1.78)	14.46 (4.22)	19.26 (5.65)	0.42 (0.31)	0.94 (0.51)
Bartlett-1	10	9.7	58	1.70	0.09	0.07	0.10	0.02	2.00	10.65	17.65	0.27	0.51
Bartlett-2	16	16.8	60	3.96	0.11	0.06	0.05	0.04	4.25	15.67	14.92	0.29	1.33
Bartlett-3	21	20.4	69	3.48	0.28	0.21	0.41	0.05	4.45	17.69	22.14	0.49	1.34
Bartlett-4	26	19.3	113	2.59	0.16	0.12	0.29	0.03	3.21	10.15	13.36	0.31	0.45
Bartlett-5	34	24.0	115	5.25	0.54	0.45	0.47	0.06	6.80	14.97	21.77	0.96	1.02
Bartlett mean	21.4 (9.2)	18.0 (5.3)	83.0 (28.6)	3.40 (1.35)	0.24 (0.19)	0.18 (0.16)	0.26 (0.19)	0.04 (0.02)	4.14 (1.78)	13.83 (3.28)	17.97 (3.95)	0.44 (0.29)	0.93 (0.43)
Mean	21.5 (8.7)	19.7 (5.8)	80.1 (20.0)	3.73 (1.47)	0.20 (0.15)	0.15 (0.12)	0.32 (0.16)	0.05 (0.03)	4.47 (1.17)	14.14 (3.58)	18.61 (4.65)	0.44 (0.28)	0.94 (0.44)

Both sites located in New England, USA. For site and global means, values in parentheses indicate \pm 1SD.

Table 2 The $F^{14}C$ (in Fraction Modern) and $\delta^{13}C$ (‰) of cellulose extracted from the innermost (oldest) ring in the 2-cm stemwood core, extracted stemwood sugars, and stem CO_2 emitted in spring and summer for red maple (*Acer rubrum*) trees at Harvard Forest ($n = 4$) and Bartlett Experimental Forest ($n = 5$)

Sample ID	Tree ring cellulose		Stemwood sugar		Spring stem CO_2		Summer stem CO_2	
	$F^{14}C$	$\delta^{13}C$ (‰)	$F^{14}C$	$\delta^{13}C$ (‰)	$F^{14}C$	$\delta^{13}C$ (‰)	$F^{14}C$	$\delta^{13}C$ (‰)
Harvard-2	1.4614	-22.8	1.0993	-29.1	1.0470	-24.9	1.0300	-24.0
Harvard-3	1.2830	-25.6	1.0880	-28.9	1.0486	-26.3	1.0386	-23.0
Harvard-4	1.1405	-27.4	1.0944	-29.5	1.0470	-25.4	1.0325	-25.8
Harvard-5	1.0959	-24.3	1.0587	-27.7	1.0401	-22.9	1.0404	-22.4
Harvard mean	1.2452 (0.1647)	-25.0 (2.0)	1.0851 (0.0182)	-28.8 (0.8)	1.0457 (0.0038)	-24.9 (1.4)	1.0354 (0.0049)	-23.8 (1.5)
Bartlett-1	1.3092	-27.7	1.1226	-30.6	1.0450	-24.9	1.0300	-24.0
Bartlett-2	1.1702	-28.6	1.0888	-29.6	1.0392	-26.3	1.0386	-23.0
Bartlett-3	1.0959	-25.7	1.0495	-26.9	1.0424	-25.4	1.0325	-25.8
Bartlett-4	1.3666	-24.6	1.1145	-28.2	1.0412	-22.9	1.0404	-22.4
Bartlett-5	1.1199	-24.8	1.0747	-28.0	1.0515	-24.9	1.0300	-24.0
Bartlett mean	1.2123 (0.1194)	-26.3 (1.8)	1.0900 (0.0297)	-28.7 (1.4)	1.0439 (0.0048)	-25.5 (2.2)	1.0381 (0.0166)	-24.4 (1.2)
Mean	1.2270 (0.1327)	-25.7 (1.9)	1.0878 (0.0239)	-28.7 (1.1)	1.0447 (0.0042)	-25.2 (1.8)	1.0369 (0.0121)	-24.1 (1.3)

The measurement precision ($\pm 1SD$) for all $F^{14}C$ is ≤ 0.0024 and for $\delta^{13}C$ values is $\leq 0.15\text{‰}$. For site and global means, values in parentheses indicate $\pm 1SD$. Both sites located in New England, USA.

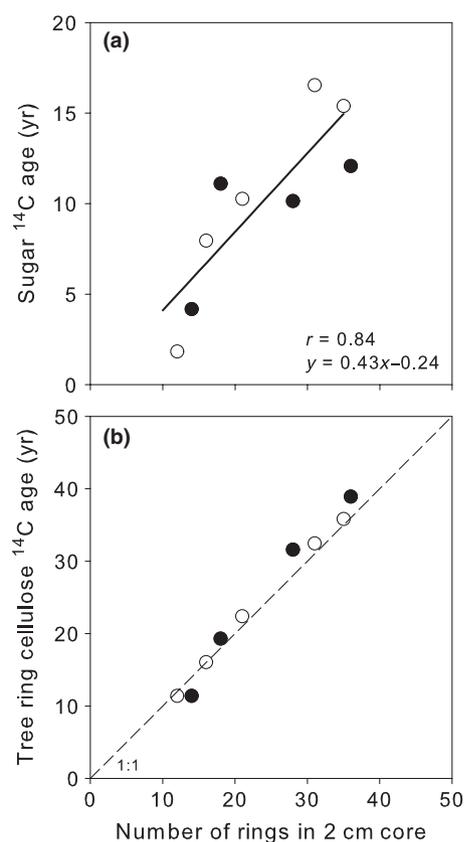


Fig. 2 (a) The linear relationship between the number of rings in the 2-cm stemwood core and the ^{14}C age (yr) of the sugar extracted from the same core for ($n = 4$) red maple (*Acer rubrum*) trees at Harvard Forest (closed circles) and ($n = 5$) trees at Bartlett Experimental Forest (open circles), both sites located in New England, USA. (b) The relationship between the number of rings in the 2-cm stemwood core and the ^{14}C age (yr) of the cellulose from the innermost ring (oldest) in the same core for ($n = 4$) red maple trees at Harvard Forest (closed circles) and ($n = 5$) trees at Bartlett Experimental Forest (open circles). The dashed line is the one to one line.

component 1. Component 2 explained an additional 20% of the total variance in the dataset, and distinguished between the oldest trees (> 100 yr) with lower metabolism and younger trees ($c. 65$ yr) with higher metabolism. In addition to being older, trees with positive scores on component 2 had lower stemwood NSC concentrations, lower stem CO_2 flux rates and older stemwood sugars relative to trees with negative scores on this component.

The ring width autocorrelation analysis indicated substantial carry-over or memory effects from year to year, with significant correlation in lagged ring widths of up to 3 yr for the mean ring width of all 36 trees (Fig. 4a). Furthermore, trees with longer growth memory (i.e. an autocorrelation function with longer statistically significant lags) tended to have smaller and older NSC. Thus, the maximum significant lag (x axis) was negatively correlated with the NSC concentration (y axis, Fig. 4b; $r = -0.27$, $P = 0.10$, slope = -3.21 ± 0.52 , intercept = 32.36 ± 2.24 ; coefficient estimates $\pm 1SE$ via reduced major axis regression and 1000 bootstraps) and positively correlated with sugar ^{14}C age (y axis, Fig. 4c; $r = 0.33$, $P = 0.04$, slope = 2.54 ± 0.40 , intercept = 1.52 ± 1.74 ; coefficient estimates $\pm 1SE$ via reduced major axis regression and 1000 bootstraps). Together, these relationships were significant with a multiple linear regression ($r = 0.40$, $P = 0.05$, maximum significant lag = $3.78 \pm 2.81 + 0.12 \pm 0.13 \times \text{sugar } ^{14}C \text{ age} - 0.07 \pm 0.01 \times \text{NSC concentration}$). Comparable results were obtained when the ring widths were detrended with more flexible splines and not detrended at all. More flexible splines resulted in shorter maximum significant lags in the autocorrelation function. The individual tree correlations with maximum significant lag and NSC age and concentration were in the same direction, but differed in significance (Supporting Information Table S1).

Stump sprouts

The $F^{14}C$ of newly emerged stump sprouts was 1.0507 ± 0.0044 ($n = 10$) at Harvard Forest and 1.0931 ± 0.0238 ($n = 7$) at

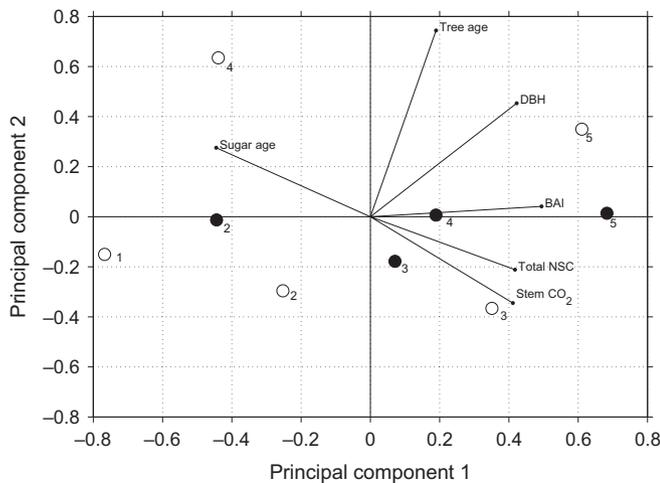


Fig. 3 Biplot of the two principal axes of the principal component analysis performed on tree characteristics for ($n = 4$) red maple (*Acer rubrum*) trees at Harvard Forest (closed circles) and ($n = 5$) trees at Bartlett Experimental Forest (open circles), both sites located in New England, USA. Vectors indicate the contribution of each variable (tree age, diameter at breast height (DBH), mean basal area increment (BAI), total nonstructural carbon (NSC) concentration, stem CO_2 flux rate and sugar age) to the principal components 1 and 2. Numbers identify individual trees and correspond to sample IDs in Tables 1 and 2.

Bartlett Experimental Forest, corresponding to ^{14}C ages of 2.0 ± 1 yr and 11.1 ± 4 yr, respectively (mean \pm 1SD, Fig. 1, Table 3). Trees from which sprouts were collected were much younger at Harvard Forest (46 ± 17 yr) than at Bartlett Experimental Forest (81 ± 32 yr). Individual sprout NSC ages ranged from 1 to 17 yr, and were strongly related to the age of the tree from which the sprout grew ($x = \text{tree age}$, $y = \text{sprout age}$, slope = 0.18 ± 0.03 , intercept = -5.52 ± 2.29 ; coefficient estimates \pm 1SE via reduced major axis regression and 1000 bootstraps; $r = 0.72$, $P < 0.01$). Sprout biomass $\delta^{13}\text{C}$ ranged from -22.0‰ to -28.5‰ , and was related to both the stump diameter ($r = 0.78$, $P < 0.001$) and tree age ($r = 0.74$, $P < 0.001$), consistent with the observed relationship between stemwood sugar $\delta^{13}\text{C}$ and tree DBH.

Discussion

Implications for NSC model representations

Collectively, our results support model representations of NSC storage and allocation that incorporate the following characteristics. The NSC storage pool accumulates with time as the tree becomes larger and older. The NSC storage pool includes both fast (younger) and slow (older) cycling subpools, with exchange between the two. In terms of allocation, there is preferential use of newer NSC (fast pool) for day-to-day metabolism and growth. However, the older NSC (slow pool) remains accessible to the tree given other physiological constraints.

Our previous model of NSC (Richardson *et al.*, 2013) distinguished between fast and slow cycling NSC, with the fast pool supporting metabolism and growth, and transfer from the fast to slow pools occurring in proportion to the gradient between the

two pools. The fast pool varied in size on seasonal time scales in response to the balance between supply and demand. At the ecosystem scale, the total NSC pool was constrained to have an MRT of 10 yr. The fast pool was estimated to have an MRT of < 1 yr, meaning that the slow pool had an MRT > 20 yr. Our new data are consistent with this structure, which we apply here to represent the C dynamics of individual trees in a conceptual model (Fig. 5). In this framework, vigorous trees (Fig. 5a) have a larger, but very dynamic, fast pool, with substantial inputs from new photosynthates, and (essentially) unidirectional transfer from the fast to the slow pool. The age of NSC in the fast pool is < 1 yr because it is flushed out regularly: large inputs and outputs, and thus fast turnover. The age of the NSC in the slow pool increases only slowly over time, because the addition of new photosynthates to this pool keeps it comparatively young. By contrast, trees of low vigor (Fig. 5b) have a smaller fast pool, fewer new inputs from photosynthates and bidirectional transfers between the slow and fast pool. The age of NSC in the fast pool is > 1 yr, because new inputs are minimal, and there is contribution from the slow pool. The age of NSC in the slow pool of low vigor trees increases over time, because of minimal new inputs and slow draw-down of the total NSC pool. In the discussion below, we illustrate how our results are consistent with these concepts.

Stemwood NSC pool

Extracted stemwood sugars were, on average, a decade old (Richardson *et al.*, 2013). The PCA indicated that, among trees, the larger and more vigorous red maples had higher stemwood NSC concentrations and comparatively younger stemwood sugars than smaller and less vigorous trees. This suggests that the more vigorous trees add more new photosynthates to the existing NSC pool each year and rely less on stored NSC for growth and metabolism (i.e. large, fast pool), consistent with the results of ring autocorrelation which show smaller memory effects and our conceptual model (Fig. 5). By contrast, trees with lower vigor add less new photosynthates each year (smaller fast pool) and draw to a larger degree on stored (on average, older, slow pool) NSC for growth and metabolism.

Allocation to metabolism

Overall, NSC used for metabolism was much younger than the extracted stemwood sugars. The CO_2 emitted by the stems was, on average, 1–2 yr old in the spring and < 1 yr old in the summer, whereas stemwood sugars were 10 yr old. This suggests a seasonal shift in the balance of new and old NSC being used for respiratory substrate. However, different combinations of substrate ages could result in the observed ages. For example, the NSC used in spring may be the products of photosynthesis from 1–3 yr previously, a mixture of mostly last year's photosynthesis and a small amount of decade-old NSC, or some other combination. Nevertheless, the change in the $\delta^{13}\text{C}$ emitted by the stem provides further evidence of a seasonal shift in respiratory substrate, with greater contribution of more negative $\delta^{13}\text{C}$ substrates in spring before leaf-out and less negative

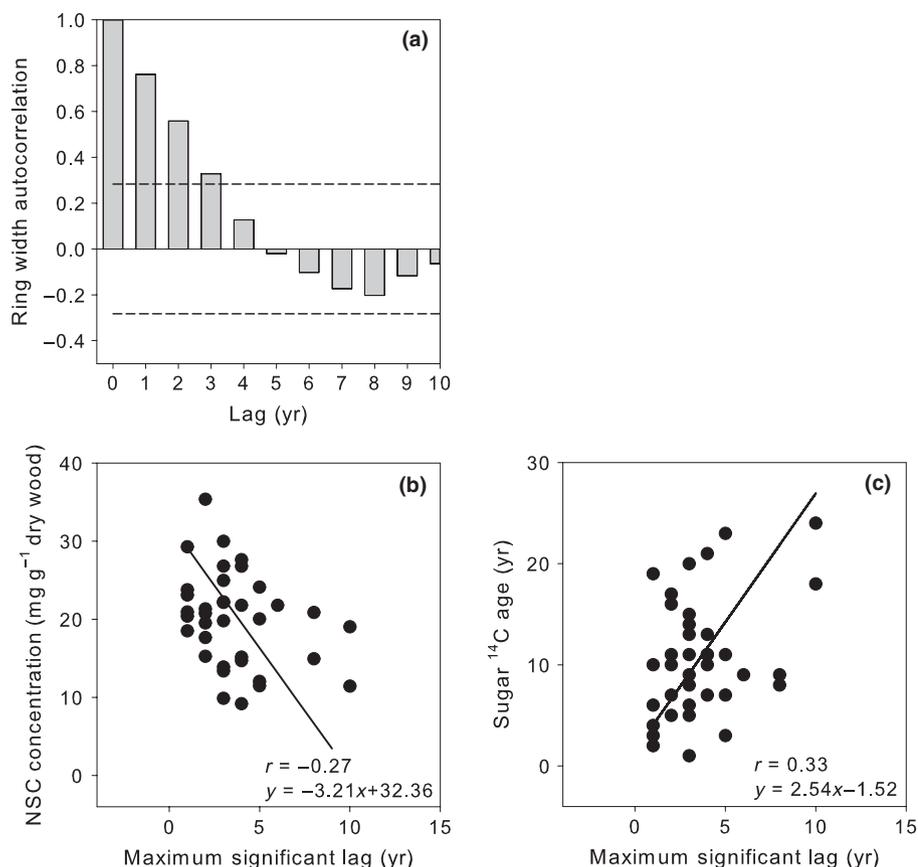


Fig. 4 Autocorrelation function for detrended mean ring width (a), with the mean ring width calculated across ($n = 36$) red maple (*Acer rubrum*) trees. The dashed lines show the cutoff for coefficients significantly different from zero at 95% confidence. Relationship between the maximum significant lag time (yr) for each tree from the autocorrelation analysis of ring width with nonstructural carbon (NSC) concentration (mg g^{-1} dry wood) (b) and sugar ^{14}C age (yr) extracted from that tree's stemwood (c).

Table 3 Tree stump diameter (cm), tree stump age (yr), sprout biomass $F^{14}\text{C}$ (in Fraction Modern) and sprout biomass $\delta^{13}\text{C}$ (‰) for red maple (*Acer rubrum*) at Harvard Forest ($n = 10$) and Bartlett Experimental Forest ($n = 7$)

Site	Stump diameter (cm)	Tree stump age (yr)	Sprout $F^{14}\text{C}$	Sprout $\delta^{13}\text{C}$ (‰)
Harvard	3.7	35	1.0475	-27.9
Harvard	10.0	33	1.0472	-28.5
Harvard	15.0	37	1.0453	-27.9
Harvard	15.4	33	1.0493	-25.2
Harvard	18.0	45	1.0601	-27.4
Harvard	18.5	52	1.0516	-24.8
Harvard	18.7	35	1.0481	-26.7
Harvard	27.0	42	1.0498	-25.9
Harvard	33.8	56	1.0549	-26.9
Harvard	66.0	88	1.0536	-24.8
Harvard mean	22.6 (17.3)	45.6 (16.9)	1.0507 (0.0044)	-26.6 (1.4)
Bartlett	6.8	23	1.0687	-27.8
Bartlett	7.5	-	1.0987	-27.2
Bartlett	36.0	70	1.0655	-23.7
Bartlett	36.0	93	1.1193	-23.8
Bartlett	39.0	115	1.0773	-22.7
Bartlett	46.0	90	1.1257	-24.2
Bartlett	64.0	97	1.0968	-22.0
Bartlett mean	33.6 (20.5)	81.3 (32.0)	1.0931 (0.0238)	-24.5 (2.2)
Mean	27.1 (18.9)	59.0 (28.9)	1.0682 (0.0262)	-25.7 (2.0)

The measurement precision ($\pm 1\text{SD}$) for all $F^{14}\text{C}$ is ≤ 0.0022 and for $\delta^{13}\text{C}$ values is $\leq 0.15\text{‰}$. For site and global means, values in parentheses indicate $\pm 1\text{SD}$. Both sites located in New England, USA.

$\delta^{13}\text{C}$ in the late growing season. Both of these results are consistent with 'last in, first out' dynamics proposed by Laointe *et al.* (1993), in which the most recent NSC assimilated is the most

accessible, and thus the first to be used. They are also consistent with the two-pool structure described in Richardson *et al.* (2013).

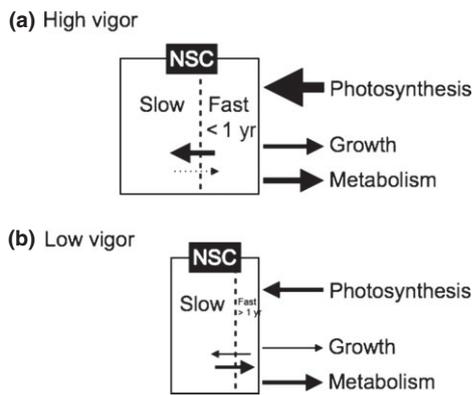


Fig. 5 Conceptual model of nonstructural carbon (NSC) pool dynamics in individual red maple (*Acer rubrum*) trees of high vigor (a) and low vigor (b). Box and arrow sizes are roughly proportional to the size of the pools and fluxes, respectively. In the vigorous trees, the fast pool is larger, and has a mean age of < 1 yr as a result of greater new photosynthetic inputs. In the low vigor trees, the fast pool is small, and has a mean age of > 1 yr, because of minimal new inputs, and transfer from the slow pool to the fast pool.

Allocation to growth

Similar to the NSC allocated to metabolism, the NSC allocated to ring growth was also much younger than the extracted stemwood sugars. The NSC used to make tree ring cellulose was, on average, < 1 yr old, supporting previous work which indicated that stored NSC from one growing season is allocated to wood growth in the following year (Kagawa *et al.*, 2006; Richardson *et al.*, 2013). At the same time, the persistent autocorrelation of tree ring widths indicates strong control of growth processes by internal C dynamics. The relationships between the maximum significant lag in the ring width autocorrelation function and the size of the NSC pools and ^{14}C age of stemwood sugars are exciting, and novel evidence for the important role of stored NSC in mediating ecological memory (i.e. carryover effects in tree growth; Rocha *et al.*, 2006). However, it is clear that, even in trees with the longest memory, rings are still being formed from comparatively recent, and not decade-old, stored NSC. Our results show that trees with larger and younger NSC pools have less carryover (more recently stored NSC contribution to tree ring cellulose growth) and weaker (or shorter) ring width autocorrelation functions (i.e. vigorous trees with large fast pools; Fig. 5a). These relationships between growth and NSC may also be influenced by different factors, such as wood anatomy (diffuse vs ring porous), plant functional type (deciduous vs conifer), plant strategies (fast or slow growing), ecological history and/or climate, and would be exciting to test in other tree species or across environmental gradients.

Finally, autocorrelation in tree ring records has been acknowledged previously as a major challenge to the derivation of accurate climate proxies (Hughes *et al.*, 1982); the results presented here suggest that finer temporal resolution proxies might be obtained from individuals or species with comparatively younger NSC pools (or larger fast NSC pools). Vigorous trees with larger/younger NSC pools should be more tightly regulated by climate (e.g. temperature extremes, soil moisture or drought, growing

season length, etc.) relative to low vigor trees, for which growth is restricted by other persistent limitations (e.g. suppression by competition or nutrient deficiencies, etc.).

Availability of stored NSC

The NSC remobilized from the root systems to grow stump sprouts proves that decade-old (and older) NSC is available to the tree for new growth when necessary. We did not measure the age of extracted NSC in the roots of these trees, and therefore do not know how it compares (younger, older or similar) with the NSC remobilized to form the sprouts. However, when standardizing the measurements for tree age (using the regression between tree age and sprout age), the age of NSC used to make sprouts is the same as that of the extracted stemwood sugars, suggesting that 10 yr is a reasonable estimate of the mean age of accessible NSC in red maple. Understanding how the concentration and age of NSC varies across the different components (i.e. roots, stems, branches) of the tree and within these components (i.e. radially across xylem rings and coarse roots) would be of great value. This information would improve whole-tree NSC size and age budgets, and help to elucidate how much, and how quickly, the mixing of new and stored NSC occurs within the tree (both vertically and radially across rings). Such measurements might clarify why the extracted stemwood sugar ^{14}C age is related to, but substantially younger than, the number of rings over which we sampled (Fig. 2a).

It is not clear what factors may influence and trigger the use of older stored NSC pools. The trees from which the stump sprouts were collected may not have had any physiological impediment to the remobilization of stored NSC, which may not always be the case. For example, in a previous study, we found that paper birch (*Betula papyrifera* Marsh.) trees gradually declining in growth over 10 yr retained substantial unused NSC (starch) in the outer stemwood even after they died (Richardson *et al.*, 2013). This is in contrast with a more severe and abrupt trigger, such as complete stem harvest (this study) or hurricane disturbance (Vargas *et al.*, 2009), in which older NSC was found to be accessible. Both hydraulic failure (McDowell *et al.*, 2008) and stress-related impairment of NSC remobilization and long-distance transport (Sala *et al.*, 2010) have been identified as mechanisms that could limit the accessibility of stored NSC to specific tissues. However, the nature and magnitude of the physiological challenge required to access older stored NSC are unknown, and information on this will help us to understand and model tree responses to a range of stresses and ecological conditions, such as release from suppression, wound response, disease, pests and climate change.

Tree size and the NSC pool

We observed two important relationships between tree size and NSC. First, NSC concentrations were greater in larger trees, supporting previous work by Woodruff & Meinzer (2011) and the hypothesis that stored NSC plays an important role in maintaining hydraulic conductivity as trees become taller (Sala *et al.*, 2012). These forests have not experienced major drought recently (approximately the last 40 yr), but there is evidence of regular

severe droughts in the last 500 yr (Pederson *et al.*, 2013). Moreover, there are regular summer droughts and freeze–thaw cycles in these ecosystems. The use of sugar solutes to create positive pressures in stems to overcome xylem embolism created by drought and freeze–thaw cycles may be one function of (and reason for) the presence of decade-old NSC in these trees, justifying the cost to maintain NSC within the tree for so long. However, other stress factors, such as late spring frosts (Hufkens *et al.*, 2012) and ice storms (Halman *et al.*, 2011), may also explain the increasing NSC concentrations with height, as large canopy trees are more susceptible to severe damage from such events, and it would be advantageous to maintain a large C buffer pool for recovery.

The second relationship with tree size observed in this study was with stemwood $\delta^{13}\text{C}$ -NSC (also observed between tree size and remobilized NSC in stump sprout biomass $\delta^{13}\text{C}$). This positive relationship can be explained by the fact that, in larger trees, there is a greater proportion of assimilation from sun-lit leaves which discriminate less than shade leaves (Francey *et al.*, 1985; Ehleringer *et al.*, 1986). This $\delta^{13}\text{C}$ -NSC pattern could be enhanced further because, near the forest floor, the CO_2 concentrations are higher and contain less ^{13}C (more negative $\delta^{13}\text{C}$) because of the influence of respiration sources (McDowell *et al.*, 2011). However, we do not believe that this was a major factor in our data, given the well-mixed atmospheric CO_2 concentrations at the foliage height of even our smallest trees (<http://atmos.seas.harvard.edu/lab/data/nigec-data.html>). These results suggest challenges for studies using ^{13}C to partition ecosystem-level autotrophic and heterotrophic respiration sources, as the autotrophic respiration component will reflect these large $\delta^{13}\text{C}$ -NSC substrate variations between trees of different sizes.

Conclusions

This study uniquely compares the NSC used for stem respiration, annual wood growth and regrowth from stump sprouts with the NSC present in the stemwood of red maple, a dominant tree species across most of the eastern USA. Using the ^{14}C ‘bomb spike’ as a tracer of substrate and age, our data provide new insights into the allocation and storage processes of NSC in mature trees, and how NSC pools and processes should be represented in ecosystem C models. We build on previous work by Richardson *et al.* (2013), confirming that decade-old NSC pools are present within red maple stemwood. As trees become larger and older, the stemwood NSC pool becomes older as NSC accumulates over time. However, more vigorous trees have, on average, younger NSC pools, reflecting greater new photosynthetic inputs. NSC from previous years buffers ring growth and dormant season stem respiration; however, younger stored NSC sources and new NSC sources, when available, are preferentially used. The degree of memory in tree ring growth is related to both the size and age of stemwood NSC, suggesting that NSC pool dynamics may influence inter-annual tree growth patterns over several years. Finally, the NSC remobilized for the growth of stump sprouts unequivocally proves that these decade-old and older NSC pools can be accessible to trees. We conclude that NSC plays an ecologically

important role in tree growth and metabolic processes over years to decades, and thus is essential for the understanding and prediction of terrestrial C-cycle responses to global change.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 The sensitivity of the autocorrelation analyses of ($n = 36$) trees at Harvard Forest, Bartlett Experimental Forest and Howland Forest to different tree ring width detrending approaches

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