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Large seasonal fluctuations in whole-tree carbohydrate reserves: is storage more dynamic in boreal ecosystems?

C. Fermaniuk[1,*], K. G. Fleurial[1], E. Wiley[2] and S. M. Landhäusser[1]

[1]*Department of Renewable Resources, University of Alberta, Edmonton, AB, T6G 2R3, Canada and* [2]*Department of Biology, University of Central Arkansas, Conway, AR, 72035, USA*
** For correspondence. E-mail cfermani@ualberta.ca*

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• **Background and Aims** Carbon reserves are a critical source of energy and substrates that allow trees to cope with periods of minimal carbon gain and/or high carbon demands, conditions which are prevalent in high-latitude forests. However, we have a poor understanding of carbon reserve dynamics at the whole-tree level in mature boreal trees. We therefore sought to quantify the seasonal changes in whole-tree and organ-level carbon reserve pools in mature boreal *Betula papyrifera.*
• **Methods** Non-structural carbohydrate (NSC; soluble sugars and starch) tissue concentrations were measured at key phenological stages throughout a calendar year in the roots, stem (inner bark and xylem), branches and leaves, and scaled up to estimate changes in organ and whole-tree NSC pool sizes. Fine root and stem growth were also measured to compare the timing of growth processes with changes in NSC pools.
• **Key Results** The whole-tree NSC pool increased from its spring minimum to its maximum at bud set, producing an average seasonal fluctuation of 0.96 kg per tree. This fluctuation represents a 72 % change in the whole-tree NSC pool, which greatly exceeds the relative change reported for more temperate conspecifics. At the organ level, branches accounted for roughly 48–60 % of the whole-tree NSC pool throughout the year, and their seasonal fluctuation was four to eight times greater than that observed in the stemwood, coarse roots and inner bark.
• **Conclusions** Branches in boreal *B. papyrifera* were the largest and most dynamic storage pool, suggesting that storage changes at the branch level largely drive whole-tree storage dynamics in these trees. The greater whole-tree seasonal NSC fluctuation in boreal vs. temperate *B. papyrifera* may result from (1) higher soluble sugar concentration requirements in branches for frost protection, and/or (2) a larger reliance on reserves to fuel new leaf and shoot growth in the spring.

Key words: Non-structural carbohydrates, whole-tree NSC storage, *Betula papyrifera*, seasonal dynamics, boreal forest.

INTRODUCTION

Non-structural carbohydrates (NSCs) in trees, primarily in the form of soluble sugars and insoluble starch, act as metabolic substrates and can be stored as reserves. NSCs can be remobilized from storage to support growth and other metabolic functions when a tree is in carbon deficit, such as during regular seasonal growth or during more unpredictable events like drought or defoliation. As such, NSC dynamics have been tightly linked to growth and mortality processes (Landhäusser *et al.*, 2012; Adams *et al.*, 2013; Sevanto *et al.*, 2014), and thus have received significant attention. Nonetheless, it is still poorly understood how NSC storage dynamics, at the whole-tree and organ level, interact with both seasonal growth processes and responses to stress (Sala *et al.*, 2012; Dietze *et al.*, 2014).

The seasonal dynamics of NSC concentrations at the organ and whole-tree level are thought to be driven by phenological changes, which vary among climates and plant functional types and traits (Martínez-Vilalta *et al.*, 2016). In general, reserve fluctuations throughout the year are believed to be a function of the balance between the supply of incoming carbon (source) and the demand for growth, respiration or other physiological processes (sinks) (Chapin *et al.*, 1990) – with larger fluctuations expected to occur in more seasonal climates with greater source–sink asynchronies. Although both temperate and boreal climates are considered to be highly seasonal, the lower winter temperatures and shorter growing season characteristic of the boreal climate might yield a potential for larger seasonal oscillations of NSCs in this region. This pattern, however, has yet to be confirmed (Martínez-Vilalta *et al.*, 2016).

Regardless of climate, NSC accumulation at the whole-tree level occurs when source activity exceeds sink activity, and remobilization occurs under the opposite conditions (Mooney, 1972; Chapin *et al.*, 1990); in deciduous species, this would imply a buildup of reserves as growth slows towards the end of the growing season, and a decline throughout the non-photosynthetic period to support maintenance respiration and early spring growth (Kozlowski, 1992; Furze *et al.*, 2019). At the organ level, however, seasonal storage patterns may contrast in part due to differences in the timing and duration of organ growth processes (Delpierre *et al.*, 2016),

as well as the possible interplay of a 'sink hierarchy' where a prioritized allocation of photosynthates to sinks in closer proximity to source organs may occur (Wright, 1989; Wardlaw, 1990; Minchin *et al.*, 1993; Landhäusser and Lieffers, 2012; Hartmann *et al.*, 2018).

In mature trees, the seasonality of carbon storage at the whole-tree level is commonly inferred by observing changes in the NSC concentrations of individual tissues or organs over time. Though these smaller scale measurements are vital when considering such functions as osmoregulation and frost tolerance, inferring whole-tree storage dynamics from these concentrations alone can be problematic. These measures provide little estimation of the storage at the whole-tree level, as organs or tissues may display contrasting seasonal NSC dynamics (Delpierre *et al.*, 2016), nor do they account for the relative mass of the organs storing the NSCs, making comparisons difficult between individuals or species that have different organ biomass proportions or sizes. A more suitable way to make these comparisons may instead be to estimate carbon pool sizes and their seasonal changes through the use of allometric scaling of whole-tree carbon dynamics (Hartmann *et al.*, 2018). Despite the notion that some carbon in the reserve pool may be permanently sequestered and thus unavailable for metabolism (Millard *et al.*, 2007; Wiley *et al.*, 2016), scaling seasonal NSC dynamics with tree biomass could establish estimates of the total amount of reserves available to a tree and their fluctuation in time, which coupled with NSC concentrations can provide a more complete approach for comparing carbon dynamics across individuals and species (Barbaroux *et al.*, 2003; Furze *et al.*, 2019; Schoonmaker *et al.*, 2021). However, few studies have taken this approach, as whole-tree NSC estimates are often difficult to obtain, particularly for large trees, and thus there is a significant need to establish how reserve patterns at the whole-tree level relate to concentration patterns at the organ level. While such data are critical for the development and evaluation of ecosystem-level models used to predict forest responses to global change (Dietze *et al.*, 2014), very few studies have used this approach with high temporal and spatial resolutions (Gough *et al.*, 2009; Furze *et al.*, 2019; Schoonmaker *et al.*, 2021).

Paper birch (*Betula papyrifera*) is a fast growing, relatively shade-intolerant species, which extends trans-continentally across large climatic and edaphic gradients in North America from the north-west Alaskan treeline to the more temperate forests of the north-eastern USA (Safford *et al.*, 1990). The aim of this study was to characterize the seasonal dynamics of tissue and organ NSC concentrations, as well as estimates of whole-tree and organ-level NSC pool sizes, in relation to defined phenological stages and growth of boreal *B. papyrifera* and draw parallels to data collected from *B. papyrifera* trees at a more temperate climate. Specifically, we sought to: (1) investigate seasonal shifts in carbohydrate concentrations (NSC, soluble sugar and starch) in key tissues and organs in response to growth processes; (2) estimate, based on allometric estimates, the size of the whole-tree NSC pool and the relative contribution of different organs to that pool; and (3) explore reserve storage and remobilization at the whole-tree and organ level, by examining seasonal changes and determine the magnitude of these changes.

METHODS

Study site description and sample collection

In late May 2018, ten healthy co-dominant *B. papyrifera* trees were selected in a large mature monospecific and even-aged birch stand located at the southern edge of the boreal dry mixedwood forest region in Alberta (54°17′13.59″N, 112°46′27.74″W). Trees in the stand showed evidence of having been cut back to their base ~30 years ago, thus regenerating from basal sprouts and developing a closed canopy with very little understorey vegetation. The surface soil and main rooting space in this site is dominated by an 18-cm-deep organic horizon that consisted of well-decomposed material. Overall, the site was considered rich, with a mesic moisture regime and moderately to well-drained soils.

To allow for the repeated sampling of individual trees without severely impacting an individual stem, we selected multi-stemmed individuals (hereafter called 'trees') for this study. Selected trees had three to five stems that were all co-dominant (i.e. upper crown had access to full light conditions) and were between 9 and 13 m tall. Trees were a minimum of 20 m apart from each other. To determine the seasonal fluctuations of NSCs in *B. papyrifera*, the following seven tissues (organs) were sampled in each tree throughout the course of a year: (1) fine roots – < 2 mm in diameter, (2) coarse roots – between 10 and 15 mmin diameter, (3) current (1-year-old) twigs – < 3 mm in diameter, (4) large diameter branch – between 4 and 7 mm in diameter, (5) fully expanded leaves (when available) and springtime buds, (6) stemwood – water-conducting xylem (sapwood), and (7) inner bark – including the phloem and excluding the outer bark. Xylem sap was also collected during the period between soil-thaw and bud-break by drilling a hole at breast height (0.19 mm diameter and 2.5 cm deep), connecting a spile, hose and collection tube. Root samples were carefully collected by following roots from the base of each tree using hand pruners. Crown samples (shoot and leaf material) were collected by cutting a branch from the outer portion of the crown fully exposed to light using a tall ladder (5 m) and a pole pruner (5 m). The two stem tissues samples (6) and (7) were collected by chiselling a 1.9-cm-wide by 1.9-cm-long by 1.5-cm-deep sample at breast height. To minimize the impact of sampling on individual stems, samples were obtained from different dominant stems at each sampling date.

Sampling dates aimed to capture the main phenological stages of *B. papyrifera* during a calendar year. Thus, samples were collected when more than half of the trees were determined to have reached a particular phenological stage. The eight sampling dates were as follows: (1) early shoot expansion – shoot expansion only with preformed leaves < 5 cm, 24 May 2018; (2) long-shoot expansion – long-shoot growth (shoots were > 10 cm with neo-formed leaves), 19 June 2018; (3) late growing season – long-shoot growth terminated (bud set), but leaves green, 7 August 2018; (4) leaf abscission – leaves yellow, 3 October 2018; (5) autumn dormancy – soil temperatures below 3 °C, 15 November 2018, (6) late winter dormancy – soil still frozen but xylem sap flows, 8 April 2019; (7) early spring and bud-break – bud swell and leaf tips emerging, upper 10 cm of soil thawed, 8 May 2019; and to close the seasonal cycle (8)

long-shoot expansion (2019) – long-shoot growth (shoots were < 10 cm with neo-formed leaves); 11 June 2019.

To quantify the seasonal production of fine roots, root ingrowth cores were established in six areas that were spaced at a minimum of 20–30 m apart and within 5–10 m of the measured *B. papyrifera* trees. Prior to establishing root ingrowth cores, the sparse herbaceous vegetation was uprooted and removed. In each area, five circular holes (15 cm in diameter) were then cored to a depth of 15 cm in the late spring (24 May 2018) and the soil was discarded. The holes were then filled with clean peat material containing no roots. One core of peat material per area was removed at each of the following five phenological stages: (3) late growing season, (4) leaf abscission, (5) autumn dormancy, (7) bud-break and (8) long-shoot expansion. *Betula papyrifera* roots were identified and extracted from the cores, oven dried at 70 °C, and weighed to determine total root biomass.

To determine the seasonality of radial growth in the xylem tissue, 5-cm cores were extracted at all phenological stages with an increment borer adjacent to the location of the stem NSC sample collection. The cores were then mounted onto boards, sanded to 400 grit, and scanned at a resolution of 1000 dpi. The width of the most recent xylem ring was then measured using ImageJ software (Schneider *et al.*, 2012).

Soil moisture and climate data

To monitor soil moisture and temperature, five soil moisture/temperature sensors (5TM VWC + Temp, Decagon Devices Inc., Pullman, WA, USA) were installed at 15 cm depth in each of the six areas previously described to examine seasonal fine root production. Sensors were set to log data at hourly intervals, and daily averages were calculated across all blocks. In general, soil moisture levels suggested that the study site had received sufficient rainfall throughout the experimental period and trees did not experience water stress. Climate data were obtained from the Abee, Alberta, weather station (54.28°N,112.97°W, 12 km from the study site), provided by Alberta Agriculture and Forestry, Alberta Climate Information Service (ACIS, 2019).

Carbohydrate analysis

Tissue samples were oven dried (at 100 °C for 1 h and then at 70 °C for 72 h), ground using a ball mill (TissueLyser II; Qiagen, Hilden, Germany), and then stored in airtight containers until analysis. Following the protocols described by Landhäusser *et al.* (2018), soluble sugar concentrations were determined after a hot ethanol (80 %) extraction followed by a colorimetric quantification using phenol–sulphuric acid. The phenol–sulphuric acid method was also used to determine sugar concentration of the xylem sap following a 100× dilution with deionized water. Starch was hydrolysed to glucose using α-amylase from *Bacillus licheniformis* (Sigma-Aldrich A3403) and amyloglucosidase from *Aspergillus niger* (Sigma-Aldrige A1602). Glucose concentration was quantified colorimetrically using glucose oxidase/peroxidase-*O*-dianisidine solution and converted to starch equivalent. All analyses included a lab standard for *Populus tremuloides* root tissue ($n = 70$) of 10.04 ± 0.46 % dry weight sugar, and 7.86 ± 0.34 dry weight starch. We report all carbohydrate concentrations as a percentage of sample dry weight.

Allometric scaling from multi-stem NSC concentrations to single stem whole-organ and whole-tree pools

We used the NSC concentration measurements of the multi-stemmed trees described above to estimate seasonal whole-tree and organ-level NSC pool sizes for single-stemmed birch trees, using allometric equations specific to *B. papyrifera* with a diameter at breast height (DBH) > 2.5 cm (Jenkins *et al.*, 2004; Chojnacky *et al.*, 2014). This approach assumes that that multi- and single-stemmed birch trees have similar NSC concentrations throughout the year. To test this assumption, we compared NSC concentrations of the multi-stemmed trees with that of nine single-stemmed co-dominant birch trees growing in the same stand at three phenological stages during the study. These single-stemmed trees were of the same age and DBH range as our multi-stemmed trees. NSC concentrations and seasonal dynamics were similar between the two tree types (Supplementary Data Table S1), supporting the validity of our approach.

First, to estimate the above-ground biomass (kg), *bm*, for each above-ground component (branches, stemwood and inner bark) of the nine single-stemmed trees, we used the following formula based on Jenkins *et al.* (2004):

$$bm = B{*}f \tag{1}$$

For these above-ground components, B was estimated as a function of DBH (cm), using the equation:

$$B = \exp\left(\beta_0 + \beta_1 \ln \mathrm{DBH}\right) \tag{2}$$

where $\beta_0 = -2.2271$ and $\beta_1 = 2.4513$ (Chojnacky *et al.*, 2014). Above-ground B includes foliage, branches, stemwood and bark. The fractions (f) of foliage, stemwood and bark were calculated based on the α_0 and α_1 coefficients from table 1 in Jenkins *et al.* (2004), as:

$$f = \exp(\alpha_0 + (\alpha_1/\mathrm{DBH})) \tag{3}$$

The branch fraction was estimated by subtracting the foliage, stemwood and bark fractions:

$$\mathrm{f_{Branch}} = 1 - \mathrm{f_{Foliage}} - \mathrm{f_{Stemwood}} - \mathrm{f_{Bark}}$$

For the below-ground component (i.e. coarse roots), the same equation (2) was applied to calculate below-ground B, using $\beta_0 = -1.4485$ and $\beta_1 = -0.03476$ (Chojnacky *et al.*, 2014). We also included an estimate of B for the fine roots using $\beta_0 = -1.8629$ and $\beta_1 = -0.77534$ (Chojnacky *et al.*, 2014). The biomass (kg) for each below-ground component was then determined by multiplying the total above-ground biomass (i.e. the sum of bm_{Foliage}, bm_{Branch}, bm_{Stemwood} and bm_{Bark}) by its respective B. For each major component (i.e. branches, stemwood, bark and coarse roots), the average biomass of all nine single-stemmed trees was then calculated and is provided in Supplementary Data Table S2.

Second, to obtain NSC pool size estimates of each major component for single-stemmed trees, the organ-level NSC

TABLE 1. *Changes in sugar and starch concentrations of tissues in mature* Betula papyrifera *between subsequent sampling dates*

	Change in sugar and starch concentrations (Δ%)											
	May/18 – Jun/18		Jun/18 – Aug/18		Aug/18 – Oct/18		Oct/18 – Nov/18		Nov/18 – Apr/19		Apr/19 – May/19	
	Sugar	Starch	Sugar	Starch	Sugar	Starch	Sugar	Starch	Sugar	Starch	Sugar	Starch
Fine roots	−0.70	−0.19	−0.21	1.29	1.39	2.09	1.85	−2.42	−0.13	−0.28	−1.71	−0.03
	(0.19)**	(0.16) ^{NS}	(0.13) ^{NS}	(0.34)**	(0.42)**	(0.68)*	(0.70)*	(0.62)**	(0.58) ^{NS}	(0.28) ^{NS}	(0.43)**	(0.26) ^{NS}
Coarse roots	−1.17	0.29	0.27	1.40	0.87	0.25	0.71	−2.43	−0.58	−0.03	−0.36	0.33
	(0.17)***	(0.30) ^{NS}	(0.24) ^{NS}	(0.47)*	(0.27)*	(0.56) ^{NS}	(0.38) ^{NS}	(0.41)**	(0.37) ^{NS}	(0.13) ^{NS}	(0.28) ^{NS}	(0.20) ^{NS}
Inner bark	0.22	0.84	−1.09	0.60	2.38	−1.37	0.01	−0.30	−1.12	0.37	−1.71	0.63
	(0.37) ^{NS}	(0.25)**	(0.37)*	(0.29)(*)	(0.37)***	(0.19)***	(0.37) ^{NS}	(0.05)***	(0.37)*	(0.11)**	(0.37)***	(0.18)**
Stemwood	−0.10	0.05	0.12	0.74	0.45	−0.28	0.20	−0.50	−0.27	0.19	−0.03	−0.03
	(0.09) ^{NS}	(0.08) ^{NS}	(0.09) ^{NS}	(0.08)***	(0.09)***	(0.08)**	(0.09) ^{NS}	(0.08)***	(0.09)*	(0.08)(*)	(0.09) ^{NS}	(0.08) ^{NS}
Branches	0.20	1.49	0.31	2.96	2.25	−3.58	1.27	−0.76	−1.26	0.64	−1.25	1.15
	(0.22) ^{NS}	(0.27)***	(0.13)(*)	(0.39)***	(0.43)***	(0.33)***	(0.50)*	(0.16)***	(0.39)**	(0.09)***	(0.38)**	(0.24)***
Twigs	0.85	1.24	−0.42	2.90	1.34	−3.47	1.09	−0.64	−0.31	0.61	−1.96	1.16
	(0.30)*	(0.33)**	(0.21) ^{NS}	(0.51)***	(0.64) ^{NS}	(0.42)***	(0.73) ^{NS}	(0.16)***	(0.42) ^{NS}	(0.10)***	(0.24)***	(0.24)***
Leaves & buds	−0.38	1.28	0.38	0.23	4.94	−1.42	NA	NA	NA	NA	−0.52	−0.23
	(0.64) ^{NS}	(0.47)*	(0.54) ^{NS}	(0.65) ^{NS}	(0.72)***	(0.48)*					(0.36)(*)	(0.10)(*)

Changes in concentrations are the differences in the estimated marginal means for each specified contrast. *P*-values for each comparison were adjusted using the Behjamini–Yekutieli method, where: NS = not significant, (*) $P \leq 0.1$, * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

concentrations from each of the ten multi-stemmed trees were multiplied by the average biomass of each respective component derived for the single-stemmed trees. Since our trees were small in diameter and we did not collect the whole depth of the xylem, we acknowledge that scaling up NSC concentrations based on the outer 1.5 cm could have yielded a potential overestimation of the stemwood NSC pool size by not accounting for (1) the biomass of inactive stem tissue (i.e. heartwood), and (2) a potential decrease of NSC concentrations and seasonal fluctuation with increased sapwood depth (Hoch *et al.*, 2003). However, we believe that this overestimation is minimal in our trees, as these trees had very little heartwood, and the radial decline of NSC concentrations with sapwood depth was found to be small in diffuse-porous species, such as *B. papyrifera* (Hoch *et al.*, 2003; Furze *et al.*, 2020). For the branches, the NSC pool was calculated using the average NSC concentrations of branch and twig samples. The pool for each component (organ) was summed together to yield a whole-tree NSC pool size. The maximum difference (greatest seasonal change) of the NSC pool size in the different organs and in the whole tree were determined by subtracting the minimum annual estimate from the maximum annual estimate of the respective pools for each individual tree and organ.

For the whole-tree NSC pool estimates, we used the estimates for coarse roots, stemwood, inner bark and branches, but did not include fine roots and foliage, as their contribution to the overall pool size was very low. For our nine single-stemmed trees, the average $bm_{\text{Fine root}}$ and bm_{Foliage} were 0.9 and 1.09 kg, respectively, which together represent 4.4 % of the total biomass. Adding their mass produced only small differences (increases) in the whole-tree NSC pool (the maximum being a difference of 11 % of the total) (Supplementary Data Table S3).

Statistical analysis

All statistical analyses were performed using R v.3.5.1 (R Core Team, 2018); linear mixed-effect (LME) models were fit by maximum likelihood using the NLME package (Pinheiro *et al.*, 2021). A repeated-measures approach was incorporated to account for the lack of independence among observations across sampling dates. To assess whether NSC (combined sugar and starch), sugar and starch concentrations for each organ, whole-tree NSC pool size, fine root biomass, and xylem radial growth varied across sampling dates, we used LME models with sampling date as a fixed effect and tree or block (for fine root biomass data) as a random effect. To assess if NSC pool size and the maximum seasonal change differed between organs, we used LME models with sampling date and organ as fixed effects and tree as a random effect. In most models (stemwood sugar, stemwood starch, inner bark sugar and fine root biomass excepted), a by stratum variance structure was incorporated to account for heteroscedasticity in model residuals. Fixed effects were tested using analyses of variance (ANOVAs), and with the exception of NSC pool size models, significant ANOVAs were further evaluated with planned contrasts using the Behjamini–Yekutieli method of *P*-value adjustment. Since all pairwise comparisons were of interest for NSC pool size estimates, differences among organs within a sampling date, and differences

among all sampling dates within an organ and at the whole-tree level were evaluated with Tukey's honest significant difference (HSD; $\alpha = 0.05$) test. Two-tailed paired *t*-tests ($\alpha = 0.05$) were applied to compare concentrations of NSCs, sugar and starch of tissues between the start and end of the seasonal cycle. Normality of the paired differences and the presence of outliers were assessed through visual inspection of histograms and boxplots; when strong evidence of non-normality was present, the Wilcoxon signed-ranks test was used.

RESULTS

Overview

NSC concentrations and NSC pools, as well as the individual fractions of soluble sugars and starch, varied seasonally at the different phenological stages for all tissues examined ($P \leq 0.001$ for all tests; Supplementary Data Table S4 and S5). However, the seasonal dynamics in concentration and pool size were dependent on the organ examined (all interactions $P \leq 0.001$; Tables S4 and S5). In addition, fine root biomass and xylem radial growth differed across phenological stages (both $P \leq 0.001$). Post-hoc test results are provided throughout the following subsections, which are organized chronologically by phenological stage; each subsection presents the comparisons made between the title phenological stage and previous phenological stage. Pool size comparisons are detailed separately in the last subsection.

We attempted to sample the first (2018) and last (2019) collection of NSCs at a similar phenological stage (i.e. the early shoot expansion stage when only preformed leaves were present); however, we were only able to complete the last collection at a slightly later stage when some neo-formed leaves were already being produced. When compared between the first and last collection, total NSC concentrations were significantly higher in the inner bark, twigs, branches and leaves (all $P \leq 0.05$; Fig. 1A, B) at the last collection – driven predominantly by higher starch concentrations (Fig. 2A, B; Supplementary Data Table S6). Total NSC concentrations in the stemwood and coarse roots were similar between the two collections (Fig. 1B, C), while NSC concentrations were marginally lower ($P = 0.063$) in the 2019 fine roots (Fig. 1C). Widths of the newly produced xylem at both times did not differ between these two collections ($P = 1.00$; Fig. 3B). Overall, these relatively slight year-to-year differences in NSC concentrations were probably not associated with differing soil or climate conditions (Fig. 3C, D), but rather due to samples being collected somewhat later in 2019.

Shoot expansion period

Between early shoot expansion and the long shoot expansion stage where shoots produced neo-formed leaves, NSC concentrations increased significantly in the large branches and twigs (both $P \leq 0.001$; Fig. 1A). These increases were primarily associated with increases in starch concentrations (Table 1; Fig. 2A). Starch concentration also increased in the inner bark at this time ($P \leq 0.01$; Fig. 2B), though NSC concentrations were not affected ($P = 0.12$; Fig. 1B). At this same time, sugar and starch concentrations remained unchanged in the stemwood (both $P > 0.76$; Fig. 2B), although radial growth of the xylem had been initiated (0.46 mm of growth, Fig. 3B). Below-ground NSC concentrations decreased from 3.06 to 2.18 % in the fine roots ($P \leq 0.001$; Fig. 1C), which was driven by a reduction in sugar concentrations ($P \leq 0.01$; Fig. 2C). A similar reduction in sugar concentration was also found in the coarse roots ($P \leq 0.001$; Fig. 2C), though this reduction did not significantly affect overall NSC concentrations ($P = 0.19$; Fig. 1C).

Late growing season period

Between the period of long shoot expansion and the time when shoot expansion ceased and terminal bud set had occurred, above-ground NSC concentrations increased significantly in the twigs, branches and stemwood (Table 2; Fig. 1A, B). Much like during the shoot expansion period, this increase was largely attributed to an increase in starch concentrations (Table 1; Fig. 2A, B). However, no changes in NSC concentrations were observed in the inner bark ($P = 0.96$; Fig. 1B) and leaves ($P = 0.97$; Fig. 1A) at this time, though sugar concentrations decreased from 5.5 to 4.4 % in the inner bark ($P \leq 0.05$; Fig. 2B). During this same time, xylem ring width had increased by an additional 0.54 mm ($P \leq 0.05$; Fig. 3B). Below-ground NSC concentrations also increased in both the fine and coarse roots (Table 2; Fig. 1C), and like above-ground tissues, this increase was driven by increased starch concentrations (Table 1; Fig. 2C). Lastly, root mass in the ingrowth soil cores had accumulated to 56 g m^{-3} soil ($P \leq 0.05$; Fig. 3A).

Leaf abscission period

During leaf abscission, average daily air temperatures dropped below 0 °C, and daily soil temperatures were near 5 °C (Fig. 3C). In comparison to the late growing season, we observed higher sugar concentrations and lower starch concentrations in all above-ground organs (Table 1; Fig. 2A, B), but the increase of sugar concentrations was not significant in the twigs ($P = 0.16$). In these above-ground organs, total NSC concentrations were higher in the leaves ($P \leq 0.01$), lower in the twigs ($P \leq 0.05$), and remained unchanged in the stemwood, inner bark and large branches (all $P > 0.13$; Fig. 1A, B). No further growth in the xylem ring was observed ($P = 1.00$; Fig. 3B). Below ground, root NSC concentrations peaked at this time with 6.7 % in the fine roots ($P \leq 0.01$; Fig. 1C) and 5.7 % in the coarse roots ($P = 0.19$; Fig. 1C). The higher values were driven by increases in both sugar and starch concentrations (both $P \leq 0.05$) in the fine roots (Fig. 2C), and by sugar concentrations only in the coarse roots ($P \leq 0.05$; Fig. 2C). During this time, fine root biomass in ingrowth soil cores had increased by an additional 65.8 g to a total of 121.8 g m^{-3} soil ($P \leq 0.01$; Fig. 3A).

Autumn dormancy period

When average daily soil temperatures dropped to near 3 °C and daytime air temperatures remained below freezing (Fig. 3C), NSC concentrations remained unchanged in all above-ground tissues compared to the leaf abscission period in early October (Table 2; Fig. 1A, B). In these same tissues, significant

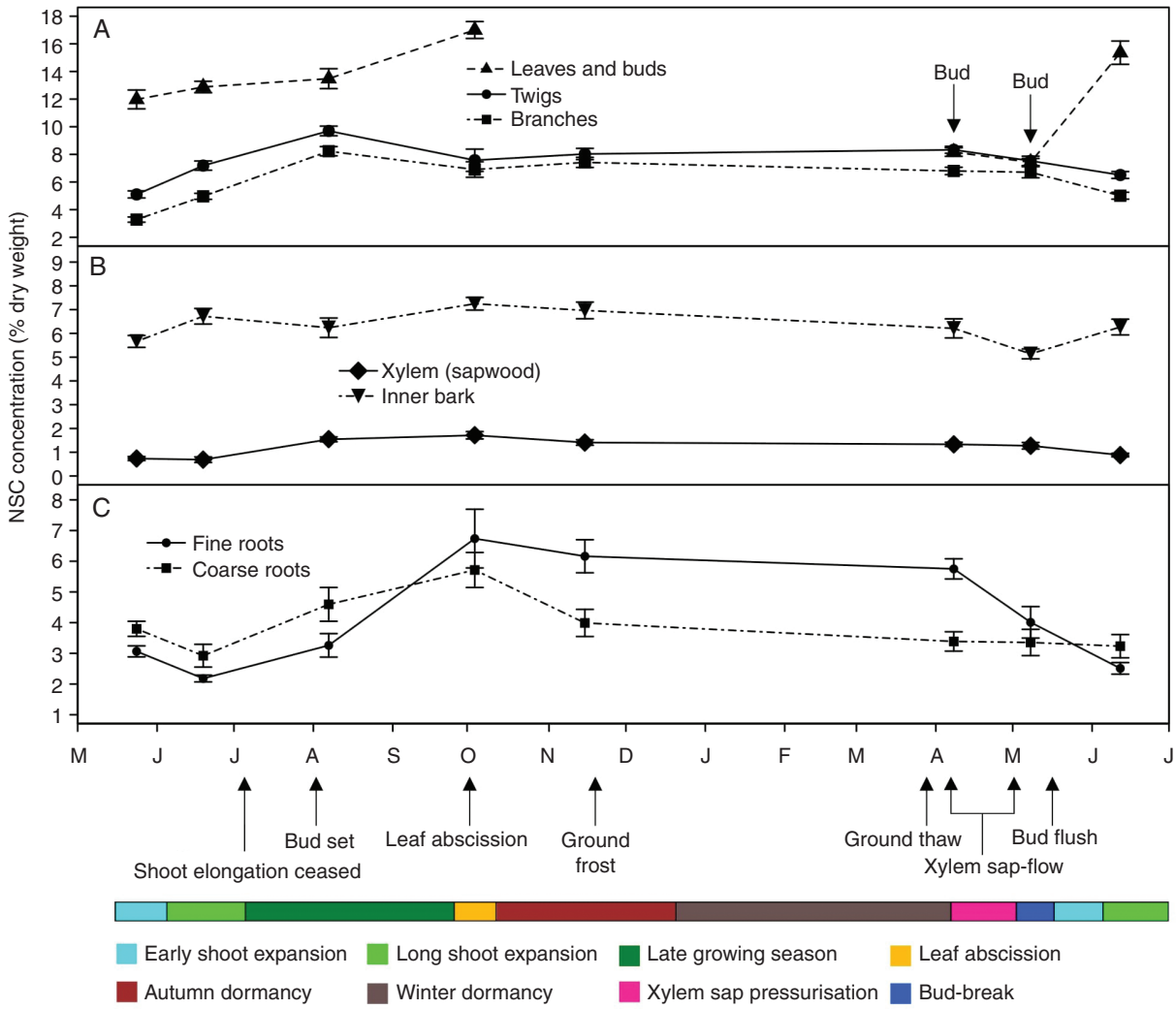

FIG. 1. Average (± s.e.) seasonal non-structural carbohydrate (NSC: combined sugar and starch) concentrations in tissues of ten *Betula papyrifera* trees. (A) Leaves and buds, current (1-year-old) twigs, and branches, (B) stem xylem and inner bark, and (C) coarse and fine roots ($n = 10$).

reductions of starch were observed (Table 1), but only the branches experienced a significant increase in sugar concentrations ($P \leq 0.05$; Fig. 2A, B). No further xylem growth was observed ($P = 1.00$; Fig. 3B). Below-ground NSC concentrations were reduced from 5.7 to 4.0 % in the coarse roots ($P \leq 0.01$) but remained unchanged in the fine roots ($P = 1.00$; Fig. 1C) compared to the leaf abscission period. The reduction of NSCs in the coarse roots was solely driven by reduced starch concentrations ($P \leq 0.01$; Fig. 2C); although starch concentrations were reduced in the fine roots ($P \leq 0.01$), this tissue also experienced a significant increase in sugar concentrations during this time ($P \leq 0.05$; Fig. 2C). Lastly, fine root biomass had not increased since leaf abscission ($P = 1.00$; Fig. 3A).

Late winter dormancy period

Soil temperatures remained near 0 °C throughout the entire winter season (mid-November to early April; Fig. 3C). Monthly average air temperatures were −7 °C throughout December and January, dropped to −21 °C in February, and rose to −4 °C by March (Fig. 3C). Between the early and later dormancy stage, no changes were observed in NSC concentrations in any above-ground tissue (Table 2; Fig. 1A, B). However, the branch, inner bark and stemwood tissues exhibited significant reductions in sugar concentrations towards the late dormant season that corresponded to equal increases in starch concentrations (Table 1; Fig. 2A, B). Starch concentration in the twigs increased from 0.27 to 0.88 % ($P \leq 0.001$; Fig. 2A) during this time, but a reduction in sugar concentrations was not detectable ($P = 1.00$; Fig. 2A). Unlike the above-ground tissues, NSC, sugar and starch concentrations remained constant in both the fine and coarse roots during this time (Tables 1 and 2; Figs 1C and 2C). Lastly, no xylem or fine root growth occurred between mid-November and early April (both $P > 0.5$; Fig. 3A, B).

Early spring and bud-break period

When average daily air temperatures rose above 0 °C in early spring, positive sap pressure began to develop in the stem and overwintering buds began to swell in preparation for flush

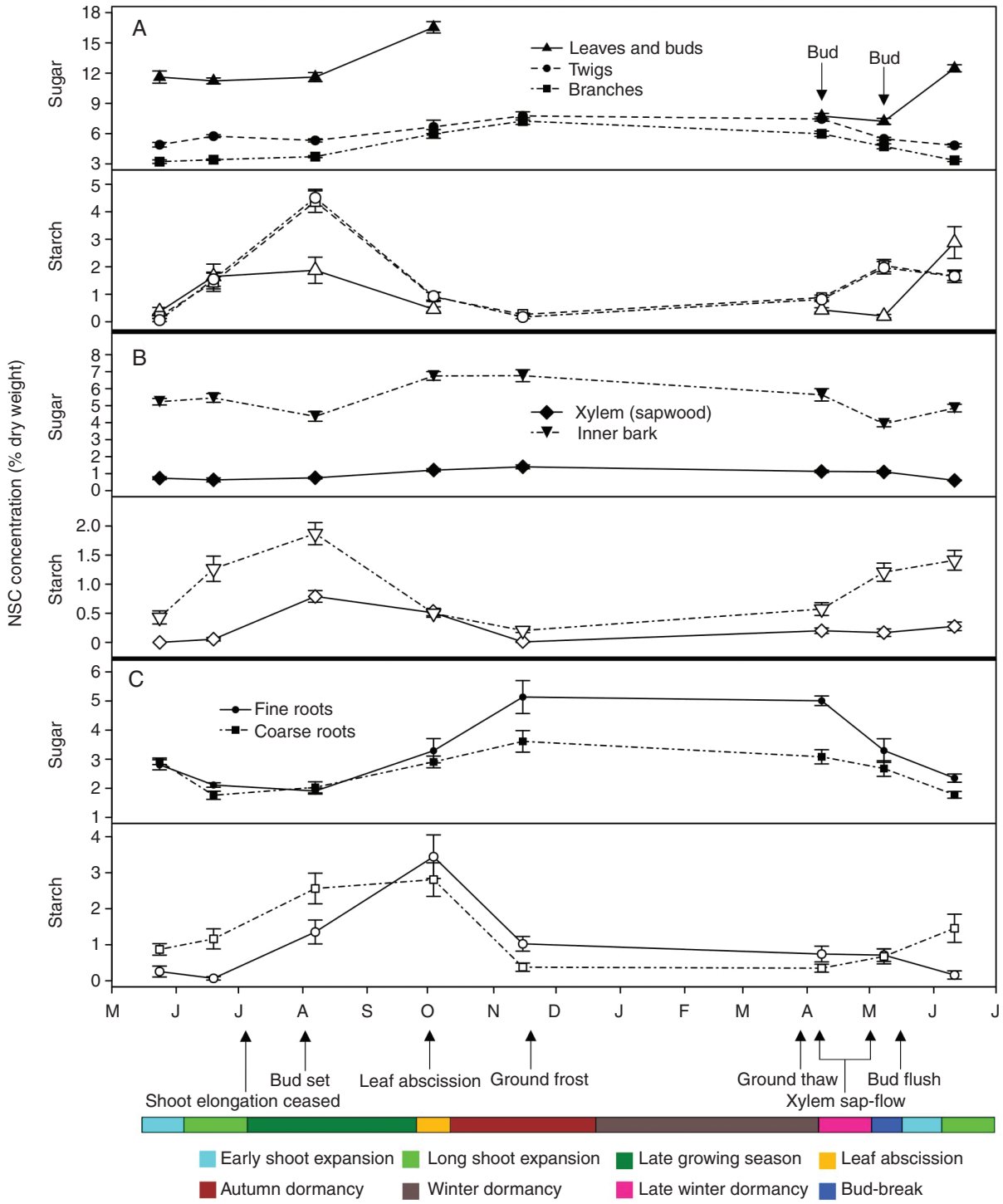

FIG. 2. Average (± s.e.) seasonal sugar and starch concentrations in tissues of ten *Betula papyrifera* trees. (A) Leaves and buds, current (1-year-old) twigs, and branches, (B) stem xylem and inner bark, and (C) coarse and fine roots (n = 10).

(Fig. 3C). In comparison to the late dormancy stage, we observed significantly reduced sugar concentrations that coincided with increased starch concentrations in the twigs, branches and inner bark (Table 1; Fig. 2A, B), but total NSCs remained unchanged in these tissues (all $P > 0.13$; Fig. 1A, B). NSC concentrations did not change in the swelling buds during this time ($P = 0.27$; Fig. 1A), but sugar and starch concentrations were marginally reduced (both $P \leq 0.08$; Fig. 2A). Sugar, starch and overall NSC concentrations did not change in the stemwood during this time (all $P > 0.11$; Figs 1B and 2B), and no significant change in xylem ring width was detected ($P = 1.00$; Fig. 3B). Additionally, sugar concentrations in the xylem sap collected on 17 and 26 April did not differ ($P = 0.51$), and together averaged 6.57 ± 2.09 mg mL^{-1} (± s.d.) (data not shown). By the

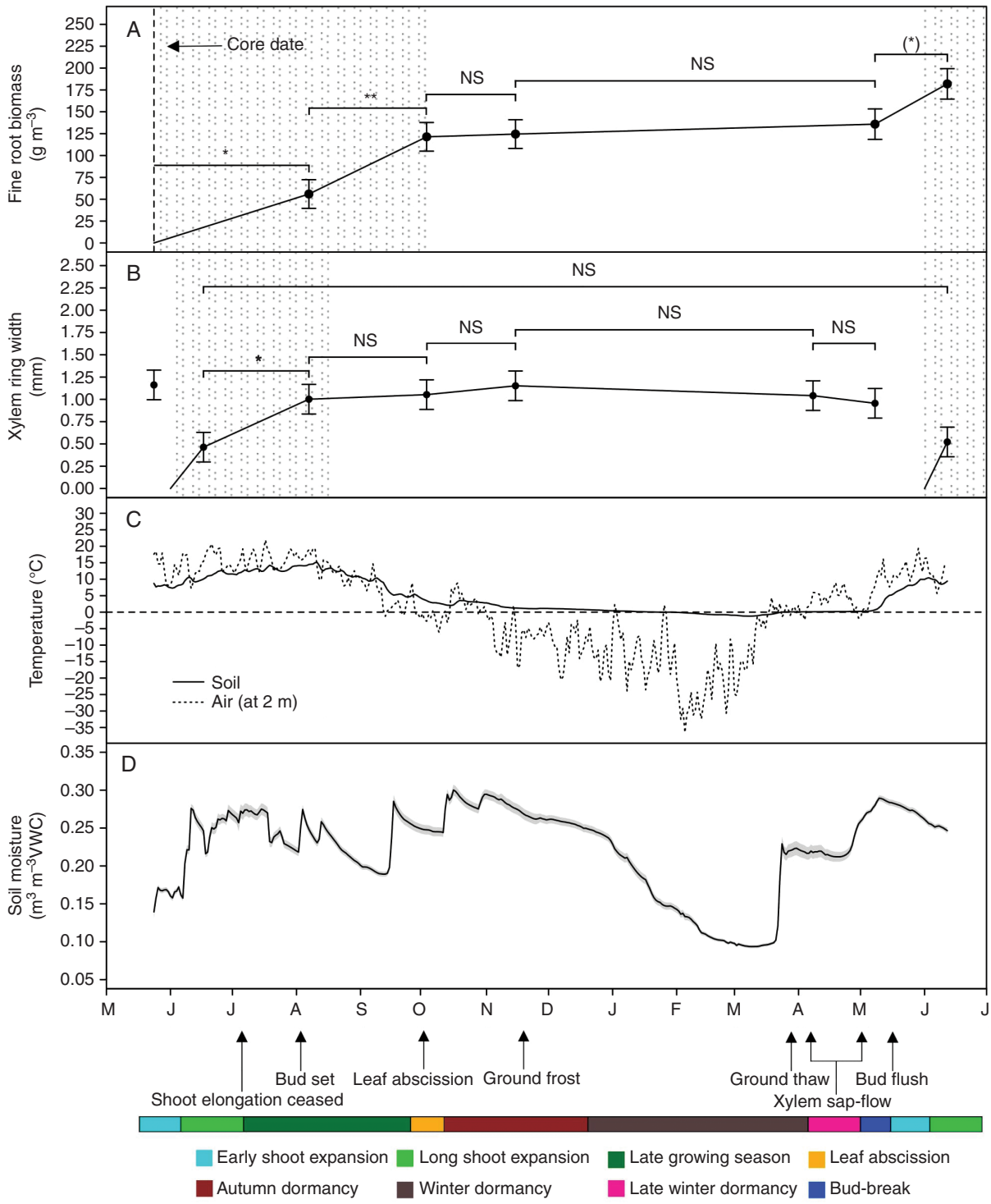

FIG. 3. Seasonal dynamics of: (A) fine root biomass of *Betula papyrifera* (g m^{-3} soil); (B) secondary xylem production in *Betula papyrifera*, presented as the width of the most recent xylem ring; (C) average daily soil temperature at 15 cm depth, and daily air temperature from a nearby weather station in Abee, Alberta; and (D) average daily soil moisture at 15 cm depth. Data points in the upper two panels represent the estimated marginal means (± s.e.) of repeated-measures linear mixed-effects models, and brackets with asterisks denote Behjamini–Yekutieli significant differences between planned comparisons, where: NS = not significant, (*) $P \leq 0.1$, *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$. The grey stippled areas represent the approximate periods of (A) fine root and (B) radial xylem growth. Shaded grey areas in the lower two panels denote ± s.e.

time of bud-break, soil temperature remained near 0 °C (Fig. 3C) and NSC concentrations were reduced in the fine roots ($P \leq 0.05$) but not in the coarse roots ($P = 1.00$; Fig. 1C), in comparison to late dormancy measurements. This change in the fine roots was driven solely by reduced sugar concentrations ($P \leq 0.01$; Fig. 2C) and did not coincide with increased fine root

Table 2. *Changes in total non-structural carbohydrates (NSC) concentrations of tissues in mature* Betula papyrifera *between subsequent sampling dates*

	Change in NSC concentration (Δ%)					
	May/18 – June/18	Jun/18 – Aug/18	Aug/18 – Oct/18	Oct/18 – Nov/18	Nov/18 – Apr/19	Apr/19 – May/19
Fine roots	−0.88 (0.18)***	1.08 (0.39)*	3.48 (1.02)**	−0.58 (1.08)^{NS}	−0.41 (0.60)^{NS}	−1.74 (0.58)*
Coarse roots	−0.88 (0.45)^{NS}	1.67 (0.61)$^{(*)}$	1.12 (0.60)^{NS}	−1.73 (0.47)**	−0.60 (0.30)^{NS}	−0.03 (0.33)^{NS}
Inner bark	1.06 (0.39)^{NS}	−0.48 (0.49)^{NS}	1.01 (0.45)^{NS}	−0.28 (0.40)^{NS}	−0.76 (0.54)^{NS}	−1.07 (0.47)^{NS}
Stemwood	−0.05 (0.14)^{NS}	0.86 (0.13)***	0.17 (0.14)^{NS}	−0.3 (0.12)^{NS}	−0.08 (0.06)^{NS}	−0.06 (0.10)^{NS}
Branches	1.68 (0.22)***	3.27 (0.31)***	−1.33 (0.65)^{NS}	0.52 (0.72)^{NS}	−0.62 (0.48)^{NS}	−0.09 (0.44)^{NS}
Twigs	2.09 (0.46)***	2.51 (0.50)***	−2.12 (0.79)*	0.46 (0.77)^{NS}	0.30 (0.41)^{NS}	−0.80 (0.38)^{NS}
Leaves & buds	0.90 (0.79)^{NS}	0.60 (0.82)^{NS}	3.50 (0.93)**	NA	NA	−0.75 (0.39)^{NS}

Changes in concentrations are the differences in the estimated marginal means from the linear mixed-effects models for each specified contrast. *P*-values for each comparison of sampling dates were adjusted using the Behjamini–Yekutieli method and indicate significant changes in NSC concentrations accordingly: NS = not significant, (*) $P \leq 0.1$, *$P \leq 0.05$, **$P \leq 0.01$ and *** $P \leq 0.001$.

biomass in the ingrowth cores ($P = 1.00$; Fig. 3A). Only once long shoot expansion had commenced did we detect a marginal increase in root mass (46 g m^{-3} soil, $P \leq 0.1$; Fig. 3A), which was similar to the amount that had accumulated in the first part of the 2018 growing season when ingrowth soil cores were installed ($P = 0.61$).

Seasonal changes in whole-tree and organ-level NSC pool sizes

Throughout the growing season, the estimated whole-tree NSC pool increased from its minimum immediately after spring flush to its maximum at bud set with a small decline through the leaf abscission and dormant period (Fig. 4A). Between minimum and maximum NSC storage, the average change in the whole-tree NSC pool was 0.96 kg per tree, which represents a 72 % increase of the whole-tree NSC pool over this period (Fig. 4A).

Although stemwood represented the largest fraction of tree biomass (Supplementary Data Table S2), the NSCs stored in the branches made up the majority of the whole-tree NSC pool throughout the year (~48–60 % of the whole-tree NSC pool; Fig. 4A, B; Table 3). The coarse root pool exhibited significantly greater NSC mass in comparison to the stemwood pool at all sampling dates except for late winter dormancy and bud-break (both $P = 0.41$; Fig. 4C, D; Table 3). However, the mass stored in the coarse root pool was only greater than the inner bark pool during the times of early shoot expansion and leaf abscission (both $P \leq 0.024$; Fig. 4C, D; Table 3). When comparing the stemwood and inner bark pools, NSC mass was significantly greater in the inner bark pool throughout all shoot expansion stages, as well as during autumn dormancy (all $P \leq 0.017$; Fig. 4C; Table 3).

The smallest (minimum) NSC pool size was observed during the early shoot expansion stage in the branches but was delayed into the long-shoot expansion stage for the stemwood and coarse roots (Fig. 4B–D). In the inner bark, this minimum NSC pool size was not reached until bud-break (Fig. 4C). The largest (maximum) NSC pool size was observed at bud set in the branches but was delayed into the leaf abscission period for the stemwood, inner bark and coarse roots (Fig. 4B–D). The largest seasonal fluctuation in NSC pool size occurred in the branches, which exhibited a change in NSC (~0.73 kg) nearly four times greater than that of the coarse roots (~0.23 kg; $P \leq 0.001$) and stemwood (0.17 kg; $P \leq 0.001$), and nearly eight times greater than the fluctuation observed in the inner bark (~0.09 kg; $P \leq 0.001$; Fig. 4C, D). No significant difference in maximum seasonal fluctuation was observed between the coarse roots and stemwood pools ($P = 0.252$), but both organs exhibited stronger fluctuations than the inner bark pool (both $P \leq 0.004$; Fig. 4C, D).

DISCUSSION

In this comprehensive seasonal NSC study, we found that the seasonal dynamics of whole-tree NSC pools of boreal *B. papyrifera* were largely in agreement with the general understanding of the dynamics of storage of carbohydrate reserves in cold-temperate deciduous trees (Mooney, 1972; Chapin *et al.*, 1990; Hoch *et al.*, 2003). Nevertheless, the magnitude of the fluctuation in NSC pool size over the growing season was truly remarkable. From their minima, the mass of NSCs in the study trees increased by over 72 % throughout the growing season, greatly exceeding the seasonal NSC fluctuation observed in *B. papyrifera* from a temperate environment (~28 % increase; Furze *et al.*, 2019). It should be noted that the seasonal fluctuation of NSCs in temperate birch did not include estimates for the inner bark NSC pool. However, if we were to exclude this component from our calculations for boreal birch, the seasonal fluctuation of the whole-tree NSC pool would be even greater (~87 % increase; Supplementary Data Table S3). Although the inner bark pool did not exhibit extensive NSC fluctuations throughout the year (Fig. 4C), the inclusion of this pool increased our estimates of whole-tree NSC storage by 14–23 % throughout the year (Table S3). In addition, the absolute size of the inner bark NSC pool was significantly greater than the stemwood pool at times, despite its lower biomass (Table 3; Supplementary Data Table S2). Thus, the inner bark contains a substantial proportion of the whole-tree NSC pool and should be included when estimating carbon storage in trees – an aspect which has often been overlooked in previous studies (Wiley *et al.*, 2019).

In our trees, the whole-tree NSC pool built up from its minimum of 1.33 kg shortly after spring flush to its maximum of 2.29 kg late in the growing season, declining only slightly during leaf abscission and the dormant period (Fig. 4A). In birch growing in a more temperate climate, this seasonal increase

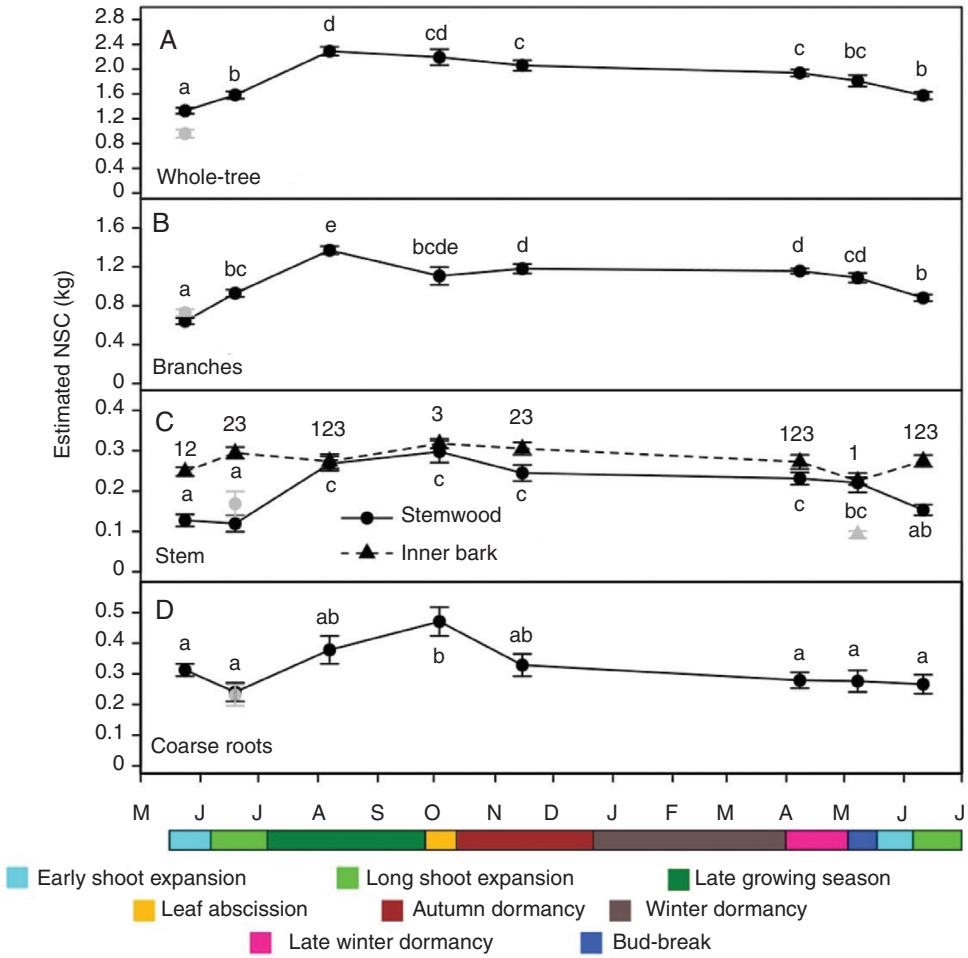

FIG. 4. Mean pool size (black points) and maximum seasonal change (grey points) of non-structural carbohydrates (NSC, kg) in the (A) whole-tree, (B) branches, (C) stemwood and inner bark, and (D) coarse roots of ten mature *Betula papyrifera* trees over a calendar year. Error bars represent s.e. The maximum seasonal change was taken as the difference between the maximum NSC (out of eight possible sampling dates) and NSC at the time of minimum pool size. Letters and numbers denote significant differences (Tukey's HSD, $\alpha = 0.05$) in NSC pool size among sampling dates for each organ and at the whole-tree level separately.

was driven solely by NSC accumulation in the branches (supporting information table S7 in Furze *et al.*, 2019), whereas we observed accumulation not only in the branches, but in the stemwood and coarse root pools as well (Fig. 4B–D). Differences in ontogenetic stage and size of the individuals between the two studies (with the temperate trees being larger and older) could at least partially explain why such a difference in the magnitude of seasonal NSC fluctuation was observed (Hartmann *et al.*, 2018). For instance, the hydraulic constraints imposed with increasing tree height may require taller trees to maintain more NSCs for hydraulic function, which in turn may lead to reduced fluctuations in NSC mass (Sala *et al.*, 2012). In addition, we acknowledge that sampling and scaling up the most dynamic outer rings of stemwood could have resulted in a slight overestimation of NSC fluctuation in the stemwood pool and thus the whole tree, although recent research has suggested that the degree of seasonal fluctuation may remain relatively high across sapwood depth in species with diffuse-porous wood anatomies, such as *B. papyrifera* (Furze *et al.*, 2020).

Despite tree size and methodological differences between the two studies, we believe that the observed disparity in seasonal fluctuations is unlikely to be explained by these factors alone. We hypothesize that these differences might represent a response to a more northern climate, where trees experience shorter growing seasons and greater climatic (e.g. temperature) variability and may thus require a stronger drawdown of reserves in the spring to initiate new growth, as well as prioritize reserve storage across the whole tree once refilling begins (Wiley and Helliker 2012; Martínez-Vilalta *et al.*, 2016). Apart from the contrasting climates that could drive these phenological differences through acclimation, one can also speculate that genetic divergences between these widely spaced populations could also play a role, with populations being exposed to differing selection pressures in their respective environments (Blumstein *et al.*, 2020). Evidence of substantial heritable genetic variation associated with climate and significant genotype × environment interactions for the NSC concentrations in *Populus trichocarpa* suggests that adaptive differences in storage between populations may have arisen in response to climatic differences (Blumstein and Hopkins, 2021). Alternatively, adaptive differences in storage patterns could also stem from differences in the disturbance regime between the two regions. For example,

TABLE 3. *Estimated marginal means, standard error (s.e.), and Tukey's HSD (α = 0.05) comparisons for a repeated-measures linear mixed-effects model testing for the effect of organ and sampling date on organ-level NSC pool size (kg)*

Organ	Mean NSC (kg)	s.e.	1. Branches	2. Stemwood	3. Inner bark
24 May 2018					
1. Branches	0.640	0.032	–		
2. Stemwood	0.127	0.017	−0.513***	–	
3. Inner bark	0.248	0.016	−0.392***	0.121***	–
4. Coarse roots	0.312	0.019	−0.328***	0.185***	0.064*
17 June 2018					
1. Branches	0.928	0.038	–		
2. Stemwood	0.119	0.020	−0.809***	–	
3. Inner bark	0.295	0.017	−0.633***	0.175***	–
4. Coarse roots	0.240	0.031	−0.689***	0.121**	−0.054^{NS}
7 August 2018					
1. Branches	1.370	0.040	–		
2. Stemwood	0.268	0.019	−1.101***	–	
3. Inner bark	0.274	0.019	−1.096***	0.005 ^{NS}	–
4. Coarse roots	0.378	0.045	−0.991***	0.110 (*)	0.105 ^{NS}
3 October 2018					
1. Branches	1.106	0.090	–		
2. Stemwood	0.298	0.022	−0.808***	–	
3. Inner bark	0.318	0.011	−0.788***	0.020 ^{NS}	–
4. Coarse roots	0.471	0.043	−0.635***	0.172**	0.152**
15 November 2018					
1. Branches	1.180	0.047	–		
2. Stemwood	0.245	0.018	−0.935***	–	
3. Inner bark	0.306	0.016	−0.875***	0.061*	–
4. Coarse roots	0.327	0.033	−0.852***	0.084$^{(*)}$	0.023 ^{NS}
8 April 2019					
1. Branches	1.156	0.030	–		
2. Stemwood	0.231	0.013	−0.924***	–	
3. Inner bark	0.272	0.020	−0.883***	0.041 ^{NS}	–
4. Coarse roots	0.279	0.025	−0.877***	0.047 ^{NS}	0.006 ^{NS}
8 May 2019					
1. Branches	1.087	0.044	–		
2. Stemwood	0.221	0.021	−0.866***	–	
3. Inner bark	0.225	0.011	−0.862***	0.005 ^{NS}	–
4. Coarse roots	0.276	0.031	−0.811***	0.055 ^{NS}	0.051^{NS}
11 June 2019					
1. Branches	0.880	0.033	–		
2. Stemwood	0.153	0.013	−0.727***	–	
3. Inner bark	0.275	0.020	−0.605***	0.122***	–
4. Coarse roots	0.266	0.029	−0.614***	0.113**	−0.009 ^{NS}

Comparisons are presented as the difference in means between row organ and column organ for each sampling date, where: NS = not significant, (*) $P \le 0.1$, *$P \le 0.05$, **$P \le 0.01$, and ***$P \le 0.001$.

differences in constitutive defence in *B. papyrifera* was higher in more northern boreal populations of birch that are more prone to fire and browsing pressure from disturbance associated with hare populations (Bryant *et al.* 2009). This could suggest a potentially greater need for reserve pools in the shoots of boreal birch populations for shoot regeneration and the production of epidermal resin glands that exude defence compounds onto the bark of the new shoots (Bryant *et al.*, 2009). If true, our finding may suggest that despite the seasonal similarities between boreal and temperate environments, the differences in climate or disturbances regimes that do exist may drive selection of trees in the boreal region to exhibit stronger seasonal fluctuations in NSC reserves, particularly so in the branches.

The greatest seasonal fluctuation of NSC mass we observed was in the branches of our birch trees – nearly four-fold greater than in the stemwood and coarse root pools, and eight-fold greater than the inner bark pool (Fig. 4B–D) – indicating that the branch storage pools were the most dynamic and probably the main source of NSC remobilized to support processes relying on storage. Notably, the seasonal trend of NSCs in the branch pool mirrored that of the whole tree (Fig. 4A, B), thus suggesting that whole-tree storage dynamics were largely

driven by storage changes at the branch level in these trees. Considering that the minimum branch NSC pool size occurred shortly after leaf-out, we can, at least in part, attribute this fluctuation in NSC reserves to be associated with the remobilization of branch reserves to fuel the flushing and expansion of new leaves and shoots. In comparing branch carbohydrate concentrations between the pre-leaf-out period at late dormancy and post-leaf period prior to substantial shoot expansion, the difference in concentration was roughly 3.5 %, which is much greater than what has been observed in temperate birch and boreal aspen (*Populus tremuloides*) during leaf flush (Landhäusser, 2011; Furze *et al.*, 2019). This might also indicate that boreal birch shoots become carbon autonomous later in development compared to shoots of temperate birch or even boreal aspen (Landhäusser, 2011). The reliance of new leaf and shoot expansion on proximal NSC pools (i.e. in the twigs and branches) has been well documented in deciduous trees (e.g. Landhäusser and Lieffers, 2003; Landhäusser, 2011; Klein *et al.*, 2016), but the role of more distal pools such as those in the stem and roots is less clear during this phenological stage (Landhäusser and Lieffers, 2003; Landhäusser and Lieffers, 2012). Despite the overall cost of this process to the whole-tree carbon pool, reserves appeared to recover in the branches soon after initial shoot and leaf expansion – roughly 30 d after bud-break (Fig. 4B), which is in line with past estimates for *Carpinus betulus*, *Fagus sylvatica* and *P. tremuloides* (Schädel *et al.*, 2009; Landhäusser, 2011).

Unlike the branches, reserve accumulation in roots was delayed into the late growing season after bud set, with the seasonal maximum being further delayed into the leaf abscission period (Fig. 4D), probably due to competing growth sinks in the crown and stem (Landhäusser and Lieffers, 2012). Although shoot growth was mostly terminated by late June, the duration of stem radial growth extended from approximately early June to late July (Fig. 3B). At no point throughout this period did we detect a reduction in the stemwood NSC pool (Fig. 4C), which suggests that this growth was fuelled primarily by current photoassimilates, reducing NSCs available for transport below ground. The resumption of fine root growth appeared to coincide with the onset of stem radial growth by early June, but extended much later in the season, only ceasing around leaf abscission (Fig. 3A). The significant reduction in fine root NSC concentrations in early June (Fig. 1C) coupled with the slight reduction in the coarse root NSC pool (Fig. 4D) suggest that root reserves, either alone or with current photoassimilates, were remobilized to initially fuel root growth. Interestingly, the fine root mass present in August 2018 ingrowth cores (56 g m^{-3} soil) was similar to the mass produced by June in the 2019 season (46 g m^{-3} soil) (Fig. 3A), seemingly indicating a burst of fine root growth early in the growing season, followed by a period of minimal growth throughout July which is well aligned with the period of stem radial growth. This apparent cessation of root growth during the period of active radial stem growth provides additional evidence supporting the concept of a sink-hierarchy in large trees (Wright, 1989; Wardlaw, 1990). Specifically, this finding supports the notion that the stem represents a higher priority sink due to its proximity to photosynthetic organs during the growing season and that the more distal root system will only start filling NSC reserves once this sink is mostly sated (Minchin *et al.*, 1993; Landhäusser and Lieffers, 2012). It is also possible that this apparent shift in allocation priority from above- to below-ground organs may, at least in part, be regulated by changes in temperature and day length or the senescence of leaves and the breakdown of the photosynthetic system in early autumn.

Between the late growing season and leaf abscission stage, we observed a sharp increase in the NSC concentration in leaves during the abscission period, which may have consequences for nutrient availability at this site (Fig. 1A). Despite excluding foliar NSC in our calculation of whole-tree pool size, if we were to consider the October pool size both with and without foliage, the difference equates to a loss of roughly 0.19 kg (or 8.7 %) of NSCs in the abscised leaves, most of which were soluble sugars. Such a buildup of soluble sugars in the foliage may be a mechanism to protect the senescing leaves from early frost damage, which may otherwise prevent the complete remobilization and resorption of leaf nutrients into overwintering tissues. This buildup could also be associated with the breakdown of phloem transport between the leaves and neighbouring tissues (i.e. twigs) at the abscission zone, as has been similarly found in the senescing leaves of *Ricinus communis* (Jongebloed *et al.*, 2004). Although this may be viewed as a waste of carbohydrate at the tree level, this loss of carbon to the leaf litter may benefit decomposers as an easily accessible energy source that supports rapid decomposition of leaf material and release of nutrients, facilitating greater rates of nutrient uptake for the tree (Sayer *et al.*, 2006). Indeed, Zukswert and Prescott (2017) observed very rapid decomposition of *B. papyrifera* litter over a 93-d period, and interestingly, the percentage of litter mass lost during this time was considerably greater than that observed for *P. tremuloides* – another fast-growing species common to the study region. Though other physical and chemical leaf/litter characteristics play a role in the decomposability of leaf material (Zukswert and Prescott, 2017), we suspect one reason for this difference is lower leaf sugar concentrations present in *P. tremuloides* at the time of abscission (~14 % dry weight; Landhäusser and Lieffers, 2003).

The observed reduction of fine root sugars during early spring was probably linked to the development of root pressures in association with the characteristic period of sap exudation or 'bleeding' of excised or injured *B. papyrifera* stems (Fig. 2C). During this period of bleeding, it is thought that positive xylem pressures help refill embolisms that have accumulated in the xylem conduits over the winter (Essiamah and Eschrich, 1985; Sauter & Ambrosius, 1986; Milburn and Kallarackal, 1991; Westhoff *et al.*, 2008; Hölttä *et al.*, 2018). Unlike the genus *Acer* where the refilling is primarily due to a local pressurization in the stem (Tyree, 1983), positive pressures are present throughout the whole xylem system in *Betula* – with the development of root pressure playing an important role in refilling conduits from the bottom up (Westhoff *et al.*, 2008). Though the concept of root pressure has yet to be thoroughly explained, it is generally agreed that the active uptake of mineral solutes allows for a pressure buildup as a result of an osmotic influx of soil water into the root. The expenditure of energy for this uptake would explain the loss of sugar observed in the fine roots during this time. While root reserve translocation or growth expenditure in the fine roots could also reduce

root NSC concentrations, there is little evidence for fine root growth (Fig. 3A) or changes in other organ NSC pool sizes (Fig. 4B–D) at this time.

As lower temperatures commenced in autumn, we observed a significant starch–sugar conversion in all above-ground tissues (Fig. 2A, B), which we attribute to the development of cold hardiness (Charrier *et al.*, 2013; Furze *et al.*, 2019; Schoonmaker *et al.*, 2021). In perennial species, this conversion into soluble sugars is a common frost protection strategy which prevents intracellular ice formation and stabilizes cell membranes and proteins during dehydration caused by extracellular ice formation (Welling and Palva, 2006; Kasuga *et al.*, 2007; Morin *et al.*, 2007; Tarkowski and Van den Ende, 2015). In contrast to the birch trees in our study, which retained very little starch in the above-ground tissues at the onset of dormancy (~2.7 % of above-ground NSCs; Fig. 2A, B), starch remained a relatively important component of above-ground storage in temperate *B. papyrifera* at the height of winter (~10 % of above-ground NSCs; Furze *et al.*, 2019), which may reflect a diminished need for frost protection due to milder winters. At the time of starch conversion to soluble sugars in our trees, the branches still retained over half the whole-tree NSCs, which potentially indicates a heightened need for frost protection in crown tissue where there is a greater proportion of living cells in branch phloem and overwintering buds, less insulation with depth (thinner bark), and greater convective heat loss due to wind exposure. This idea is further supported by higher sugar concentrations in the branches and twigs of our trees at the beginning of dormancy (~7.5 % dry weight; Fig. 2A) compared to temperate birch at the height of winter (~6.4 % dry weight; estimated using pool size values (Fig. 4) and organ biomass estimates (supporting information table S2 in Furze *et al.*, 2019). Considering that the amount of NSCs accumulated in late autumn can be positively correlated with frost tolerance (Charrier *et al.*, 2013), we can speculate that any interruption of starch accumulation throughout the growing season (i.e. drought or herbivory events) could greatly reduce the extent of frost protection in these tissues at dormancy, potentially resulting in extensive tissue mortality over winter (e.g. Galvez *et al.*, 2013). Frost damage to branch xylem and/or phloem due to reduced growing season starch accumulation could be a simple mechanism underlying the widespread branch dieback observed in North American birch stands. Extensive branch winter dieback was also found to be associated with reduced stemwood starch concentrations in European *Quercus* spp. following a severe drought (Bréda *et al.*, 2006), and in *Quercus velutina* following complete defoliation (Wiley *et al.*, 2017).

In the roots, this starch–sugar conversion did not occur in October when soil temperatures neared 5 °C, but instead was delayed into mid-November when root temperatures reached 3 °C. Landhäusser *et al.* (1996) found that *B. papyrifera* seedlings collected at the arctic tree-line exhibited fine root growth when kept at a root zone temperature of 3 °C; although we did not detect fine root growth at this temperature, we did observe a large, though non-significant, decline of NSCs in the coarse root pool between October and November when soil temperatures ranged between 5 and 3 °C (Figs 1C and 3C). Although respiration associated with maintenance and cold acclimation probably contributed to this decline to a certain extent, we suspect that a portion of this decline also reflects a growth expenditure, potentially supporting late season root elongation and diameter growth (Desrochers *et al.*, 2002; Landhäusser and Lieffers, 2003) as *B. papyrifera* root growth appears less sensitive to low soil temperatures (i.e. 3 °C) than other coexisting boreal deciduous species such as *Populus balsamifera* and *P. tremuloides* (Landhäusser *et al.*, 1996; Landhäusser and Lieffers, 1998; Wan *et al.*, 2001; Landhäusser, 2003), probably an appropriate adaptation for a broadleaf species that forms the northern limit of deciduous trees in North America (Landhäusser and Wein, 1993).

CONCLUSION

To the best of our knowledge, this is the first study that has comprehensively estimated whole-tree and organ-level NSC pool size dynamics in relation to the phenology of mature boreal *B. papyrifera*. The branches represented more than half the total NSCs stored in the whole tree throughout the year, and thus was probably the most important source of remobilized NSCs for early shoot/leaf expansion and possibly stem radial growth in spring. Root reserves appeared to fuel the flushing of fine root growth in the spring and possibly coarse root growth in late autumn as well, but stemwood reserves did not appear to fuel stem diameter growth. Lastly, the seasonality of whole-tree NSC pools agreed with expected storage patterns for deciduous species, but the seasonal fluctuation between the annual maxima and minima in this northern climate represented a much larger proportion of whole-tree NSCs in comparison to values reported for its temperate conspecific. Although this may be in part due to differences in size of the trees, we suspect that these differences may also result from higher NSC requirements (soluble sugars concentrations) of boreal trees for frost protection coupled with a stronger drawdown of reserves in the spring to initiate new growth.

SUPPLEMENTARY DATA

Supplementary data are available online at *Annals of Botany* online and consist of the following. Table S1. Comparison of mean NSCs and standard error of organs over time for multi-stemmed and single-stemmed *Betula papyrifera* trees from a monospecific birch stand located at the southern edge of the boreal dry mixedwood forest region in Alberta. Table S2. Diameter at breast height of the nine single-stemmed trees used to calculate the average biomass of the coarse root, stemwood, inner bark and branch fractions of *Betula papyrifera* trees at the study site. Table S3. Average whole-tree NSC pools of *Betula papyrifera* throughout a calendar year with all organ pools considered, coarse root, stemwood, inner bark and branch pools considered, and only the coarse root, stemwood and branch pools considered. Table S4. Analysis of variance results for repeated-measures linear mixed-effects models testing for the effect of sampling date on the estimated whole-tree NSC pool size, and the effect of sampling date, organ and their interaction on the estimated organ-level NSC pool size and seasonal change of organ NSC pool size of ten mature *Betula papyrifera* trees. Table S5. One-way analysis of variance results for repeated measures linear mixed-effects models testing the effect of sampling date on total non-structural

carbohydrate, sugar and starch concentrations in root, stem and crown tissues of ten mature *Betula papyrifera* trees. Table S6. Two-tailed paired *t*-test and Wilcoxon signed-ranks test results comparing tissue NSC, sugar and starch concentrations between the first and last sampling dates of the seasonal cycle in mature *Betula papyrifera*.

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