BioOne COMPLETE

Seasonal patterns of nonstructural carbohydrate reserves in four woody boreal species1

Author: Morgan E. Furze

Seasonal patterns of nonstructural carbohydrate reserves in four woody boreal species[1]

Morgan E. Furze[2]

Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138

Anna M. Jensen

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Jeffrey M. Warren

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

Andrew D. Richardson

Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011
School of Informatics, Computing, and Cyber Systems, Northern Arizona University, Flagstaff, AZ 86011

Abstract. Plants store nonstructural carbohydrates (NSCs), such as sugars and starch, to use as carbon and energy sources for daily maintenance and growth needs as well as during times of stress. Allocation of NSCs to storage provides an important physiological strategy associated with future growth and survival, and thus understanding the seasonal patterns of NSC reserves provides insight into how species with different traits (*e.g.,* growth form, leaf habit, wood anatomy) may respond to stress. We characterized the seasonal patterns of NSCs in four woody boreal plant species in Minnesota, USA. Sugar and starch concentrations were measured across the year in the roots and branches of two conifer trees, black spruce (*Picea mariana* (Mill.) B.S.P.) and eastern tamarack (*Larix laricina* (Du Roi) K. Koch), as well as in the leaves and branches of two evergreen broadleaf shrubs, bog Labrador tea (*Rhododendron groenlandicum* (Oeder) Kron & Judd) and leatherleaf (*Chamaedaphne calyculata* (L.) Moench). In general, seasonal variation was dominated by changes in starch across all organs and species. While similar seasonal patterns of NSCs were observed in the shrubs, different seasonal patterns were observed between the trees, particularly in the roots. Our results suggest that species-specific traits likely have consequences for organ-level storage dynamics, which may influence whole-plant growth and survival under global change.

Key words: boreal, carbohydrates, carbon allocation, NSC, SPRUCE

Woody plants can allocate products of photosynthesis to storage in the form of nonstructural carbohydrates (NSCs) (Chapin *et al*. 1990, Hartmann and Trumbore 2016). NSCs, consisting primarily of sugars and starch, are stored throughout the plant, for example, in the ray parenchyma cells of branches, stems, and roots. These reserves can then serve as carbon sources for metabolic processes, such as growth and respiration, as well as for times of stress, such as drought (Sevanto *et al*. 2014) and disturbance (Carbone *et al*. 2013). Thus, an understanding of the seasonal changes in the amount of stored NSCs and how these reserves are distributed throughout the plant may provide insight into species' responses to stress associated with global change.

In boreal ecosystems, long-lived woody plants often accumulate NSCs during the summer to support maintenance processes during the winter. However, the demands of several months can be

satisfied by a single day's carbon gain in some cold-dwelling species (Körner 1998). Our current knowledge of allocation and storage processes in the boreal zone comes largely from shrub species (Reader 1978, Landhäusser and Lieffers 1997, Zasada *et al*. 1994), with less work focusing on mature trees (Landhäusser and Lieffers 2003; Klõšeiko *et al*. 2006). Findings from boreal plant species (Whitney 1982, Zasada *et al*. 1994, Landhäusser and Lieffers 1997) indicate dynamic root reserves, with concentrations often peaking between late summer and the dormant season. But we still lack an understanding of how storage differs throughout the year between boreal species and whether the same seasonal patterns hold in both below- and aboveground organs.

To establish seasonal patterns of NSCs and improve our understanding of storage processes in woody boreal species, we studied the mature conifer trees, the evergreen *Picea mariana* (Mill.) B.S.P. (black spruce) and the deciduous *Larix laricina* (Du Roi) K. Koch (eastern tamarack), as well as the evergreen broadleaf shrubs *Rhododendron groenlandicum* (Oeder) Kron & Judd (bog Labrador tea) and *Chamaedaphne calyculata* (L.) Moench (leatherleaf) in the Marcell Experimental Forest (Minnesota, USA). We selected samples that were collected at four time points in 2013 and then measured the concentrations of sugars and starch in roots and branches in the tree species and leaves and branches in the shrub species.

We addressed two questions. First, do NSC concentrations change throughout the year? Second, are the seasonal patterns different for each species? We hypothesized that (a) total NSCs will vary throughout the year with different seasonal patterns for sugars and starch; (b) seasonal dynamics of NSCs will be similar between the two evergreen shrub species but dissimilar between the two trees, which have different leaf habits; and (c) maximum total NSC accumulation will occur in the early summer for the evergreen species and in the fall for the deciduous species. Our results provide insight into how boreal species distribute NSCs to various organs throughout the year, which may have implications for whole-plant growth and survival in the context of global change.

Materials and Methods. STUDY SITE AND SPECIES. We obtained samples from an 8.1-ha *Picea-Sphagnum* ombrotrophic bog (S1 bog) (47°30.5′N, 93°27.2′W) within the Marcell Experimental Forest about 40 km north of Grand Rapids, MN, USA. The mean annual temperature is 3.3 °C, and mean annual precipitation is 780 mm, with two-thirds falling as rain in the summer and one-third falling as snow in the winter. The S1 bog is an acidic and nutrient-deficient peatland. Its surface has a hummock-hollow microtopography with a layer of various *Sphagnum* moss species (Kolka *et al*. 2011). Following silvicultural strip cuts in 1969 and 1974, the S1 bog naturally regenerated a mixed-age forest dominated by black spruce, eastern tamarack, bog Labrador tea, and leatherleaf. Both black spruce and eastern tamarack are conifers; the former is evergreen, and the latter is deciduous. While the shrubs are evergreen, leaves persist for only two growing seasons; they usually last throughout the first winter but drop during the summer and autumn of the second year (Lems 1956, Tendland *et al*. 2012).

FIELD COLLECTION. For black spruce and eastern tamarack, coarse roots with developed secondary xylem as well as multiyear (1–2 yr) woody branch samples were collected from mature trees approximately 5–8 m tall. For bog Labrador tea and leatherleaf, current-year leaves (2013) and multiyear (1–2 yr) woody branch samples were collected. Branch samples included phloem for NSC analyses. We selected our samples from organs that were previously collected for a different purpose during 2013 field campaigns at the Marcell Experimental Forest. Thus, the individual plants used in this study may vary from month to month for each species (Table S1).

LABORATORY PREPARATIONS AND NONSTRUCTURAL CARBOHYDRATE ANALYSIS. Samples were microwaved for 90 sec (full effect) to stop enzymatic activity, oven-dried at 70 °C, and homogenized to a fine powder (SPEX SamplePrep 1600 MiniG, Metuchen, NJ). To measure sugar and starch concentrations (adapted from Chow and Landhäusser 2004), 10 mg of freeze-dried tissue (FreeZone 2.5, Labconco, Kansas City, MO; Hybrid Vacuum Pump, Vacuubrand, Wertheim, Germany) was extracted with 80% ethanol followed by colorimetric analysis with phenol-sulfuric acid to determine bulk sugar concentrations. The resulting bulk sugar extract was read at 490 nm with a microplate reader (Epoch Microplate Spectrophotometer, Bio-Tek Instruments, Winooski, VT). Bulk sugar concentrations (ex-
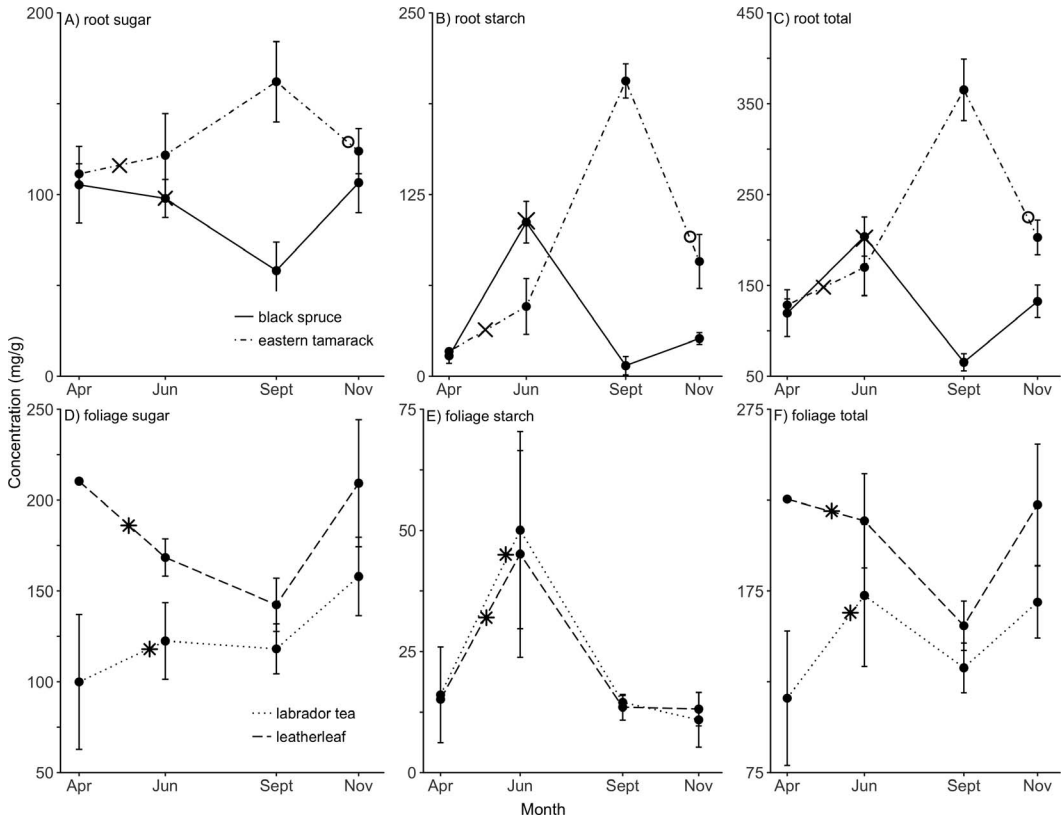
FIG. 1. Seasonal dynamics of sugar (left column), starch (center column), and total NSC (right column) concentrations in roots from black spruce and eastern tamarack (top row, A–C) and in leaves from bog Labrador tea and leatherleaf (bottom row, D–F) sampled at the Marcell Experimental Forest in 2013. Symbols mark the start of the following phenological events: leaf out (X), flowering (asterisk), leaf fall (open circle). Error bars denote ± 1 SE of the mean. In some cases, the error is smaller than the size of the symbol.

pressed as mg sugar per g dry wood) were calculated from a 1:1:1 glucose:fructose:galactose (Sigma Chemicals, St. Louis, MO) standard curve.

The remaining tissue residue was solubilized and then digested with an α-amylase/amyloglucosidase digestive enzyme solution to determine starch concentrations. Glucose hydrolysate was determined using a PGO-color reagent solution (Sigma Chemicals) and read at 525 nm with the microplate reader. Starch concentrations (expressed as mg starch per g dry wood) were calculated based on a glucose (Sigma Chemicals) standard curve. Uncertainty of NSC measurements is addressed in Methods S1.

Data from this project are available for download and public use (Furze et al. 2018). In Figs. 1 and 2, sampling months April, June, September, and November correspond to day of year (DOY) 103, 169, 254, and 317, respectively. Phenological events in 2013 were also recorded, and the onset of each event is denoted by a unique symbol in Figs. 1 and 2 to show their timing in relation to observed NSC dynamics: black spruce leaf out DOY 169–176; eastern tamarack leaf out DOY 134, leaf off DOY 309; bog Labrador tea flowering DOY 157; and leatherleaf flowering DOY 141–148.

STATISTICAL ANALYSIS. To compare NSC storage between the two tree species, we used a two-way analysis of variance (ANOVA) to individually analyze sugar, starch, and total NSC (sum of sugars and starch) concentrations in roots among sampling months and species. Root diameter was not included as a covariate in our analyses based on its weak association with NSC concentrations (Fig. S1). For the two shrub species whose extremely fine root systems are unlikely to provide much NSC storage, the same analyses were
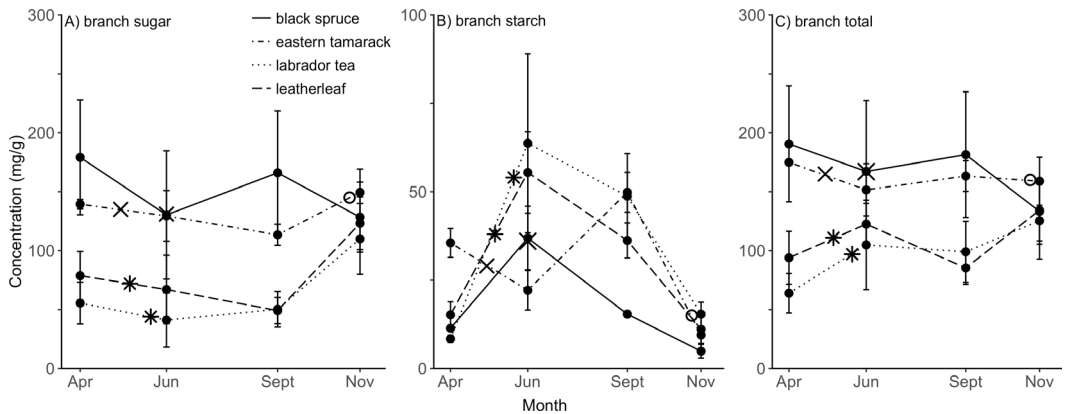
FIG. 2.   Seasonal dynamics of (A) sugar, (B) starch, and (C) total NSC concentrations in branches from black spruce, eastern tamarack, bog Labrador tea, and leatherleaf sampled at the Marcell Experimental Forest in 2013. Symbols mark the start of the following phenological events: leaf out (X), flowering (asterisk), leaf fall (open circle). Error bars denote ± 1 SE of the mean. In some cases, the error is smaller than the size of the symbol.

conducted but using the NSC concentrations in leaves instead of roots.

To compare NSC storage between the four species, we used a two-way ANOVA to individually analyze sugar, starch, and total NSC concentrations in branches among sampling months and species. Woody branch samples were the common organ collected across both trees and shrubs. This study precludes a repeated measures approach, and ANOVA results are presented in Table 1. For significant ANOVAs, differences between pairs of means were evaluated using Tukey's HSD, $\alpha = 0.05$. For significant interaction effects (month × species), concentrations in individual organs were assessed across sampling months for each species (Table S2).

Results. Overall, sugar and total NSC concentrations did not significantly vary throughout the year in tree roots, shrub leaves, or the branches of trees and shrubs, whereas starch concentrations were seasonally dynamic for all.

SEASONAL PATTERNS OF NSC RESERVES IN TREES: BLACK SPRUCE AND EASTERN TAMARACK. Starch but not sugar concentrations in the roots significantly varied throughout the year and differed between black spruce and eastern tamarack (Fig. 1A, B). Variation in root sugar concentrations was not well explained by either factor ($F_{7,57} = 0.78$, $R^2 = 0.09$, $P = 0.61$; month, $P = 0.99$; species, $P = 0.09$; Table 1A). Sampling month explained much of the variation in root starch concentrations ($F_{7,57} = 6.48$, $R^2 = 0.44$, $P < 0.0001$; month, $P < 0.01$; species, $P = 0.049$; Table 1A). However, the effect of sampling month on starch concentrations depended on species (interaction $P < 0.0001$; Table 1A). In general, concentrations of starch in the roots were higher in eastern tamarack than black spruce and peaked later in September as opposed to June for each species, respectively (Table S2). When eastern tamarack's root starch concentrations peaked in September, they were 28 times greater than black spruce's at the same time, as well as two times greater than black spruce's

Table 1.   Results of analysis of variance testing for main effects of sampling month, species, and their interaction on sugar, starch, and total concentrations for roots from two tree species, leaves from two shrub species, and branches from all four species. *P* value is the level of statistical significance based on an *F* test, with bold type indicating results significant at $P \leq 0.05$.

	Tree roots			Shrub leaves			Tree and shrub branches		
	Sugar	Starch	Total	Sugar	Starch	Total	Sugar	Starch	Total
Month	0.99	**< 0.01**	0.20	0.21	**0.01**	0.30	0.34	**< 0.0001**	0.96
Species	0.09	**0.049**	**0.01**	**0.02**	0.80	**0.03**	**< 0.001**	**0.048**	**< 0.01**
Interaction	0.50	**< 0.0001**	**< 0.001**	0.80	0.99	0.83	0.71	**< 0.01**	0.75

peak in June (Fig. 1B). Additionally, total NSC concentrations in the roots differed between tree species ($P = 0.01$) but were not seasonally dynamic ($P = 0.20$; Table 1A).

Seasonal Patterns of NSC Reserves in Shrubs: Bog Labrador Tea and Leatherleaf. Starch but not sugar concentrations in the leaves varied throughout the year, and the seasonal dynamics were similar for bog Labrador tea and leatherleaf (Fig. 1D, E). Sampling month and species explained some of the variation in sugar ($F_{7,31} =$ 1.76, $R^2 = 0.28$, $P = 0.13$; month, $P = 0.21$; species, $P = 0.02$) and starch concentrations ($F_{7,31} = 1.88$, $R^2 = 0.30$, $P = 0.11$; month, $P = 0.01$; species, $P = 0.80$; Table 1B). There was a general trend of increasing sugar concentrations from September to November (Fig. 1D), while starch concentrations peaked in June and declined onward (Tukey's HSD, September–June $P = 0.05$, November–June $P = 0.01$; Fig. 1E). Total concentrations in the leaves were higher in leatherleaf than bog Labrador tea (Tukey's HSD, $P = 0.03$), and, much like the roots of our tree species, total NSC concentrations were not seasonally dynamic ($P = 0.30$; Table 1B).

Seasonal Patterns of NSC Reserves in Trees and Shrubs. Starch but not sugar concentrations in branches varied throughout the year in both trees and shrubs. Sampling month and species explained most of the variation in branch sugar ($F_{15,35} = 2.36$, $R^2 = 0.50$, $P = 0.02$; month, $P = 0.34$; species, $P < 0.001$) and starch concentrations ($F_{15,35} = 5.40$, $R^2 = 0.70$, $P < 0.0001$; month, $P < 0.0001$; species, $P = 0.048$; Table 1C). However, the effect of sampling month on starch concentrations depended on species (interaction $P < 0.01$; Table 1C). Branch sugar concentrations did not vary throughout the year and did not differ between black spruce and eastern tamarack (Tukey's HSD, $P = 0.80$) or between bog Labrador tea and leatherleaf (Tukey's HSD, $P = 0.88$) but did differ between all shrub-tree species combinations (Tukey's HSD, bog Labrador tea-black spruce, $P < 0.001$; leatherleaf-black spruce, $P < 0.01$; bog Labrador tea-eastern tamarack, $P < 0.01$, leatherleaf-eastern tamarack, $P = 0.04$; Fig. 2A). In contrast, branch starch concentrations generally varied throughout the year with evergreen species peaking in June and deciduous eastern tamarack peaking in September (Fig. 2B; Table S2).

We did not find dynamic changes in total NSC concentrations in the branches of our four species. While some variation in total NSCs existed, it cannot be fully explained by sampling month and species ($F_{15,35} = 1.51$, $R^2 = 0.40$, $P = 0.15$; month, $P = 0.96$; species, $P < 0.01$; Table 1C). Similar to branch sugar concentrations, total NSC concentrations did not differ between either tree species (Tukey's HSD, $P = 0.98$) or between either shrub species (Tukey's HSD, $P = 0.97$) but did differ between all shrub-tree species combinations (Tukey's HSD, bog Labrador tea-black spruce, $P = 0.02$; leatherleaf-black spruce, $P = 0.04$; bog Labrador tea-eastern tamarack, $P = 0.03$, leatherleaf-eastern tamarack, $P = 0.07$; Fig. 2C).

Discussion. Seasonal Patterns of NSC Reserves in Trees: Black Spruce and Eastern Tamarack. Whereas in deciduous tree species new growth in the spring is supported by NSCs in overwintering organs, evergreen species can begin photosynthesizing immediately using the previous year's foliage; the ability to photosynthesize immediately diminishes the need to draw on stored reserves and influences NSC dynamics (Wyka and Oleksyn 2014, Jensen et al. 2015). The observed seasonal patterns of starch in the two trees, black spruce and eastern tamarack, were dynamic in the roots and were unique for each species, possibly reflecting differences in leaf habit as outlined above, as well as contributions by other seasonally dynamic processes, such as secondary root or fine root production (Iversen et al. 2018). While future root sampling should include larger sample sizes to better represent the population and resolve dynamics, the data we present are still valuable, as seasonal NSC dynamics for roots are less reported.

At the start of the growing season, we observed differences in storage dynamics in the roots between the trees. As starch concentrations rapidly increased and peaked in June for black spruce, an evergreen conifer, concentrations gradually increased and did not peak until the autumn for eastern tamarack, a deciduous conifer. The large and relatively quick increase in starch reserves for black spruce may be attributed to its ability to photosynthesize immediately with older needles before new shoot, stem, and root growth begins, whereas starch reserves gradually increased over the growing season in eastern tamarack as demands slow.

Toward the end of the growing season, total NSC concentrations of roots in September were nearly six times larger in eastern tamarack than in black spruce. This is in agreement with higher storage requirements for deciduous species compared to evergreen species prior to the dormant season due to their annual leaf drop and regrowth (Dickson 1989). After this point, starch root concentrations declined in eastern tamarack; this decline prior to the dormant season is likely associated with root growth. As a future warmer climate may threaten to lower the water table and stress the root system in these environments, it will be important to link root phenology with NSC dynamics going forward in order to better predict species' resiliency in bog ecosystems.

SEASONAL PATTERNS OF NSC RESERVES IN SHRUBS: BOG LABRADOR TEA AND LEATHERLEAF. Bog Labrador tea and leatherleaf have the same growth form (shrub) and leaf habit (evergreen). Thus, similar seasonal patterns may be expected in both species if such traits are drivers of allocation. In both species, leaf starch concentrations peaked in June and declined onward. Previous studies have shown a buildup of starch in foliage and branches before bud burst (Oleksyn *et al*. 2000, Bansal and Germino 2009), and remobilization of these reserves to build new tissues (Cerasoli *et al*. 2004). Declines in both species occurred post-flowering, a similarly expensive phenological stage that has been followed by degradation of starch in the leaves and/or branches for several months in other plant species (Zieslin *et al*. 1975, Sanz *et al*. 1987, Hagidimitriou and Roper 1994, Monerri *et al*. 2011).

Furthermore, the highest sugar concentrations for both shrub species were measured in the leaves. This finding is in line with previous studies that examined storage in boreal species (compiled in Martínez-Vilalta *et al*. 2016) as well as with the role that sugars play in osmoregulation and phloem loading (Chaves *et al*. 2003, Sala *et al*. 2012, Savage *et al*. 2016). Additionally, sugar and total NSC concentrations were highest in November for both species. Accumulation of NSCs in evergreen shrub leaves prior to the dormant season may be particularly important for frost tolerance and springtime growth of species in environments that experience extreme winter temperatures (Ino *et al*. 2003, Palacio *et al*. 2007).

SEASONAL PATTERNS OF NSC RESERVES IN TREES AND SHRUBS. Branches were the common organ sampled across the four species and can provide insight into storage differences between trees and shrubs. Just like we observed for starch in the tree roots and shrub leaves, starch in the branches was seasonally dynamic, generally peaking earlier in June for evergreen species compared to September for deciduous eastern tamarack. While branch sugar and total concentrations did not significantly vary throughout the year, there were storage differences between trees and shrubs. Concentrations of sugars and total NSCs in the branches were higher in each tree species than each shrub species but similar among themselves.

These findings suggest that growth form (*i.e.,* tree *vs*. shrub) may influence NSC storage, at least in the branches. However, this does not hold true when looking across multiple organs. For example, while leaf NSC concentrations were often similar among the shrubs, root NSC concentrations were not similar among the trees. Thus, organ level NSC storage is likely influenced by many potentially confounding factors, including growth form, leaf habit, and wood anatomy, to name a few. Including a deciduous shrub species in future comparisons may help to tease apart their contributions.

Additionally, the timing of maximum accumulation of total NSCs was inconsistent between organs and species. For instance, we hypothesized that maximum total NSC accumulation for the deciduous conifer would occur in the fall; this held true in the roots but not in the branches of eastern tamarack. We also hypothesized that maximum total NSC accumulation for the three evergreen species would occur in the early summer; this was observed only in the roots of black spruce. The different maximum total NSC storage times observed for individual organs imply a role for within-plant allocation of reserves throughout the year, and species-specific traits, again, influence these dynamics.

Further, previous studies have identified total NSC concentrations that are only weakly seasonal in several tree species (Hoch *et al*. 2003, Würth *et al*. 2005, Richardson *et al*. 2013), implying that the trees examined were fully charged with carbon and that carbon was not a limiting resource for growth under natural conditions (Hoch 2015, Körner 2015). However, more pronounced seasonal variation, particularly driven by starch, may be expected in environments with greater asynchro-

nies in supply and demand, as in the boreal zone (Martínez-Vilalta *et al*. 2016). Overall, we observed total concentrations in individual organs of both trees and shrubs that were only weakly seasonal, with contributions from a highly dynamic starch pool as expected. We suggest that increasing sample sizes, sampling multiple organs including stemwood (Furze *et al*. 2018), and examining whole-plant total NSC pools by summing individual organ-level pools together would help to further resolve seasonal patterns of total NSCs and produce the predicted patterns of maximum total NSC storage.

To better understand (a) NSC dynamics in the context of source-sink relationships and (b) the responses of plants (*e.g.,* growth) to global change, studies should focus on integrating the study of NSCs with measurements of physiology and phenology under both natural and experimental conditions. We conducted this study on vegetation that is directly adjacent to the Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) experiment, in which open-top chambers receive various levels of warming and elevated carbon dioxide (Hanson *et al*. 2017). Future work at SPRUCE will combine the aforementioned measurements to explore whether these tree and shrub species are carbon limited.

Conclusions. In this study, we quantified the seasonal patterns of NSC reserves for four woody boreal species in the upper midwestern USA. We measured concentrations of sugars and starch in branches and roots of black spruce, an evergreen conifer, and eastern tamarack, a deciduous conifer, as well as branches and leaves of bog Labrador tea and leatherleaf, both evergreen broadleaf shrubs. In general, seasonal dynamics were dominated by changes in starch in both below- and aboveground organs, with species-specific traits contributing to storage differences.

Our data support the idea that species-specific traits influence NSC dynamics, highlighting the need to gain a comprehensive understanding of storage and allocation processes in various organs of diverse species across time and space to better predict the responses of woody boreal species to global change.

Literature Cited

BANSAL, S. AND M. J. GERMINO. 2009. Temporal variation of nonstructural carbohydrates in montane conifers: Similarities and differences among developmental stages, species and environmental conditions. Tree Physiol. 29: 559–568.

CARBONE, M. S., C. I. CZIMCZIK, T. F. KEENAN, P. F. MURAKAMI, N. PEDERSON, P. G. SCHABERG, X. XU, AND A. D. RICHARDSON. 2013. Age, allocation and availability of nonstructural carbon in mature red maple trees. New Phytol. 200: 1145–1155.

CERASOLI, S., P. MAILLARD, A. SCARTAZZA, E. BRUGNOLI, M. M. CHAVES, AND J. S. PEREIRA. 2004. Carbon and nitrogen winter storage and remobilisation during seasonal flush growth in two-year-old cork oak (*Quercus suber* L.) saplings. Ann. For. Sci. 61: 721–729.

CHAPIN, F. S., E. D. SCHULZE, AND H. A. MOONEY. 1990. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21: 423–447.

CHAVES, M. M., J. P. MAROCO, AND J. S. PEREIRA. 2003. Understanding plant responses to drought—From genes to the whole plant. Funct. Plant Biol. 30: 239–264.

CHOW, P. S. AND S. M. LANDHÄUSSER. 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol. 24: 1129–1136.

DICKSON, R. E. 1989. Carbon and nitrogen allocation in trees. Ann. For. Sci. 46: 631s–647s.

FURZE, M. E., S. TRUMBORE, AND H. HARTMANN. 2018. Detours on the phloem sugar highway: Stem carbon storage and remobilization. Curr. Opin. Plant Biol. 43: 89–95.

FURZE, M. E., A. M. JENSEN, J. M. WARREN, AND A. D. RICHARDSON. 2018. SPRUCE S1 Bog Seasonal Patterns of Nonstructural Carbohydrates in Larix, Picea, Rhododendron, and Chamaedaphne, 2013. Oak Ridge National Laboratory, TES SFA, US Department of Energy, Oak Ridge, TN. <https://doi.org/10.25581/spruce.037/1473917>.

HAGIDIMITRIOU, M. AND T. R. ROPER. 1994. Seasonal changes in nonstructural carbohydrates in cranberry. J. Am. Soc. Hortic. Sci. 119: 1029–1033.

HANSON, P. J., J. S. RIGGS, W. R. NETTLES, J. R. PHILLIPS, M. B. KRASSOVSKI, L. A. HOOK, L. GU, A. D. RICHARDSON, D. M. AUBRECHT, D. M. RICCIUTO, J. M. WARREN, AND C. BARBIER. 2017. Attaining whole-ecosystem warming using air and deep-soil heating methods with an elevated CO_2 atmosphere. Biogeosciences 14: 861–883.

HARTMANN, H. AND S. TRUMBORE. 2016. Understanding the roles of nonstructural carbohydrates in forest trees—From what we can measure to what we want to know. New Phytol. 211: 386–403.

HOCH, G. 2015. Carbon reserves as indicators for carbon limitation in trees, pp. 321–346. *In* U. Luttge and W. Beyschlag [eds.], Progress in Botany. Springer International Publishing, Cham, Switzerland.

HOCH, G., A. RICHTER, AND C. KÖRNER. 2003. Nonstructural carbon compounds in temperate forest trees. Plant Cell Environ. 26: 1067–1081.

INO, Y., T. MAEKAWA, T. SHIBAYAMA, AND Y. SAKAMAKI. 2003. Two types of matter economy for the wintering of evergreen shrubs in regions of heavy snowfall. J. Plant Res. 116: 327–330.

IVERSEN, C. M., J. CHILDS, R. J. NORBY, T. A. ONTL, R. K. KOLKA, D. J. BRICE, K. J. MCFARLANE, AND P. J.

HANSON. 2018. Fine-root growth in a forested bog is seasonally dynamic, but shallowly distributed in nutrient-poor peat. Plant Soil. 424: 123–143.

JENSEN, A. M., J. M. WARREN, P. J. HANSON, J. CHILDS, AND S. D. WULLSCHLEGER. 2015. Needle age and season influence photosynthetic temperature response and total annual carbon uptake in mature *Picea mariana* trees. Ann. Bot. 116: 821–832.

KLÕŠEIKO, J., M. MANDRE, AND R. KORSJUKOV. 2006. Needle carbohydrate concentrations in Norway spruce as affected by wood ash application to soil. Proc. Est. Acad. Sci. Biol. Ecol. 55: 123–136.

KOLKA, R. K., S. D. SEBESTYEN, E. S. VERRY, AND K. N. BROOKS, eds. 2011. Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, FL.

KÖRNER, C. 1998. A re-assessment of high elevation treeline positions and their explanation. Oecologia 115: 445–459.

KÖRNER, C. 2015. Paradigm shift in plant growth control. Curr. Opin. Plant Biol. 25: 107–114.

LANDHÄUSSER, S. M. AND V. J. LIEFFERS. 1997. Seasonal changes in carbohydrate storage and regrowth in rhizomes and stems of four boreal forest shrubs: Applications in *Picea glauca* understorey regeneration. Scand. J. For. Res. 12: 27–32.

LANDHÄUSSER, S. M., AND V. J. LIEFFERS. 2003. Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees Struct. Funct. 17: 471–476.

LEMS, K. 1956. Ecological study of the peat bogs of eastern North America: Notes on the behavior of *Chamaedaphne calyculata*. Can. J. Bot. 34: 197–207.

MARTÍNEZ-VILALTA, J., A. SALA, D. ASENSIO, L. GALIANO, G. HOCH, S. PALACIO, F. I. PIPER, AND F. LLORET. 2016. Dynamics of non-structural carbohydrates in terrestrial plants: A global synthesis. Ecol. Monogr. 86: 495–516.

MONERRI, C., A. FORTUNATO-ALMEIDA, R. V. MOLINA, S. G. NEBAUER, A. GARCÍA-LUIS, AND J. L. GUARDIOLA. 2011. Relation of carbohydrate reserves with the forthcoming crop, flower formation and photosynthetic rate, in the alternate bearing "Salustiana" sweet orange (*Citrus sinensis* L.). Sci. Hortic. (Amsterdam) 129: 71–78.

OLEKSYN, J., R. ZYTKOWIAK, P. KAROLEWSKI, P. B. REICH, AND M. G. TJOELKER. 2000. Genetic and environmental control of seasonal carbohydrate dynamics in trees of diverse *Pinus sylvestris* populations. Tree Physiol. 20: 837–847.

PALACIO, S., P. MILLARD, M. MAESTRO, AND G. MONTSERRAT-MARTÍ. 2007. Non-structural carbohydrates and nitrogen dynamics in Mediterranean sub-shrubs: An analysis of the functional role of overwintering leaves. Plant Biol. 9: 49–58.

READER, R. J. 1978. Contribution of overwintering leaves to the growth of three broad-leaved, evergreen shrubs belonging to the Ericaceae family. Can. J. Bot. 56: 1248–1261.

RICHARDSON, A. D., M. S. CARBONE, T. F. KEENAN, C. I. CZIMCZIK, D. Y. HOLLINGER, P. MURAKAMI, P. G. SCHABERG, AND X. XU. 2013. Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. New Phytol. 197: 850–861.

SALA, A., D. R. WOODRUFF, AND F. C. MEINZER. 2012. Carbon dynamics in trees: Feast or famine? Tree Physiol. 32: 764–75.

SANZ, A., C. MONERRI, J. GONZALEZ-FERRER, AND J. L. GUARDIOLA. 1987. Changes in carbohydrates and mineral elements in *Citrus* leaves during flowering and fruit set. Physiol. Plant. 69: 93–98.

SAVAGE, J. A., M. J. CLEARWATER, D. F. HAINES, T. KLEIN, M. MENCUCCINI, S. SEVANTO, R. TURGEON, AND C. ZHANG. 2016. Allocation, stress tolerance and carbon transport in plants: How does phloem physiology affect plant ecology? Plant Cell Environ. 39: 709–725.

SEVANTO, S., N. G. MCDOWELL, L. T. DICKMAN, R. PANGLE, AND W. T. POCKMAN. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant Cell Environ. 37: 153–161.

TENDLAND, Y., S. PELLERIN, P. S. HADDAD, AND A. CUERRIER. 2012. Impacts of experimental leaf harvesting on a North American medicinal shrub, *Rhododendron groenlandicum*. NRC Res. Press 251: 247–251.

WHITNEY, G. G. 1982. The productivity and carbohydrate economy of a developing stand of *Rubus idaeus*. Can. J. Bot. 60: 2697–2703.

WÜRTH, M. K. R., S. PELÁEZ-RIEDL, S. J. WRIGHT, AND C. KÖRNER. 2005. Non-structural carbohydrate pools in a tropical forest. Oecologia 143: 11–24.

WYKA, T. P. AND J. OLEKSYN. 2014. Photosynthetic ecophysiology of evergreen leaves in the woody angiosperms—A review. Dendrobiology 72: 3–27.

ZASADA, J. C., J. C. TAPPEINER III, B. D. MAXWELL, AND M. A. RADWAN. 1994. Seasonal changes in shoot and root production and in carbohydrate content of salmonberry (*Rubus spectabilis*) rhizome segments from the central Oregon Coast Ranges. Can. J. For. Res. 24: 272–277.

ZIESLIN, N., A. HURWITZ, AND A. H. HALEVY. 1975. Flower production and the accumulation and distribution of carbohydrates in different parts of Baccara rose plants as influenced by various pruning and pinching treatments. J. Hortic. Sci. 50: 339–348.