

Article

Phenological and Temperature Controls on the Temporal Non-Structural Carbohydrate Dynamics of *Populus grandidentata* and *Quercus rubra*

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Abstract: Temporal changes in plant tissue non-structural carbohydrates (NSC) may be sensitive to climate changes that alter forest phenology. We examined how temporal fluctuations in tissue NSC concentrations of *Populus grandidentata* and *Quercus rubra* relate to net and gross primary production (NPP, GPP) and their climatic drivers in a deciduous forest of Michigan, USA. Tissue NSC concentrations were coupled with NPP and GPP phenologies, declining from dormancy until GPP initiation and then increasing following NPP cessation. Warmer autumns extended the temporal gap between NPP and GPP cessation, prolonging the period of NSC accumulation. These results suggest that tissue NSC concentrations may increase with climate change.

Keywords: aspen; carbon; carbon cycle; non-structural carbohydrates; oak; photosynthesis; soluble sugars; starch

1. Introduction

Plant non-structural carbohydrates (NSC), comprised of starch and sugars, are primary intermediate products of carbon assimilation (C) that can be stored and used to meet future demands for growth and metabolism [1-3]. Stored NSC is fundamental to sustaining plant growth and metabolism during periods of high physiological stress and through dormancy, when photosynthetic C supply is low [2-7]. Because of a shifting balance between plant metabolic C demands and photosynthetic C supply, NSC concentrations in plant tissues can change on hourly to seasonal timescales [8-17].

Seasonal cycles of plant tissue NSC accumulation and depletion are strongly tied to the timing and magnitude of C assimilation and growth [18], indicators of C source and sink demand, respectively. In temperate deciduous trees, tissue NSC concentrations may decline during winter dormancy and early leaf expansion, when current C assimilation is insufficient to meet metabolic and early growth demands [1,2,12,16]. Non-structural carbohydrate concentrations in tissues may be replenished once leaf production has peaked and structural growth demands for current C assimilate decline [2,4-7]. The timing and magnitude of tissue NSC accrual and depletion is thus a function of two coupled, but sometimes temporally offset C cycling processes of growth and C assimilation.

Climate change may exert a substantial, indirect influence on seasonal cycles of tissue NSC by modifying the temporal relationship between growth and C assimilation. In particular, thermal regulation of growing season length and duration of the photosynthetic period may have important consequences for the periodicity of seasonal tissue NSC cycles in temperate deciduous forests. Numerous studies support a strong relationship between temperature and growing season duration, with warmer air or soil temperatures accelerating the initiation of spring net primary production (NPP) and delaying autumn senescence [19-23]. Warmer spring temperatures generally prompt an earlier onset of gross primary production (GPP), but temperature effects on the termination of GPP are less clear. If the temporal relationship between NPP and GPP is altered as temperatures rise, then shifts in C source-sink relationships may, in turn, affect tissue NSC concentrations, an outcome that could have broad ecological consequences for the resilience of forest growth following disturbance or climatic stress [3,24-31]. Forecasts of future plant tissue NSC have focused on the effects of rising atmospheric CO₂ [32]. Understanding how climate change will alter tissue NSC concentrations additionally requires investigation into how temperature constrains the timing of key regulatory ecosystem C supply and demand processes of C assimilation (*i.e.*, GPP) and growth (*i.e.*, NPP).

We examined how seasonal fluctuations in tissue NSC of *Populus grandidentata* and *Quercus rubra* relate to stand-scale C cycling processes of NPP and GPP and their climatic drivers, and we consider how future temperature rise may affect seasonal NSC cycles of depletion and accrual in a north temperate mixed deciduous forest of Michigan, USA. We focus our analysis on the phenological and environmental regulation of C cycling processes that constrain tissue NSC concentrations, and which may inform predictions of future forest C allocation to NSC. Using standard assays of tissue NSC concentration together with stand-scale assessments of GPP and NPP [18], we demonstrate how these coupled ecosystem C cycling processes interact to regulate highly dynamic, but generally predictable seasonal fluctuations in tissue NSC concentrations, and we consider how these labile C pools are likely to change as temperature increases.

2. Experimental Methods

2.1. Site Description

Our study was conducted at the University of Michigan Biological Station east of Pellston, MI, in northern lower Michigan, USA (45°35.5'N, 84°43'W). The study site lies on the northeastern side of an interlobate moraine, the slope of which gently decreases from SW to NE draining into nearby Douglas Lake. Soils are primarily composed of sand, excessively drained and of the Rubicon-East Lake series. Average annual (1942-2003) temperature is 5.5 °C and precipitation is 817 mm.

The ecosystem is a secondary successional mixed deciduous forest which regenerated following a clearcut and subsequent fire in the 1920s [33]. Forest canopy is approximately 22 m height. Forest composition is comprised primarily of *Populus grandidentata* Michx. (bigtooth aspen) and *P. tremuloides* Michx. (trembling aspen), which together comprise over 40% of the basal area, *Quercus rubra* L. (northern red oak), which comprises over 20% of the basal area, *Betula papyrifera* Marsh. (paper birch), *Fagus grandifolia* Ehrh. (American beech), *Acer saccharum* Marsh. (sugar maple), *Acer rubra* L. (red maple), and *Pinus strobus* L. (white pine). The understory consists mainly of *Pteridium aquilinum* (bracken fern) and seedlings of northern red oak, American beech, sugar and red maple, and white pine.

2.2. Plant tissue collection

P. grandidentata and *Q. rubra* individuals were selected for investigation of NSC because they are dominant canopy species at our site and exhibit contrasting xylem morphologies. *Q. rubra*, a ring-porous species, principally lays down vessel elements during early spring growth, while diffuse-porous *P. grandidentata* forms new vessel elements more uniformly throughout the year. Due to the destructive nature of tissue sampling, we extracted tissues over the course of the study from three different cohorts of dominant canopy aspen and oak trees located within the same stand. Sampling of three trees per species during 2005 was conducted four times on cohort 1, and during 2006 on six dates on cohorts 2 and 3. When transitioning from one cohort to the next, we conducted concurrent sampling of both cohorts on a common measurement date to examine variation between cohorts. To minimize diurnal variability in NSC, tissue samples were collected between 12:00 p.m. and 4:00 p.m.

We collected on every measurement date branch, bole, and coarse root (diameter > 5 cm) tissues for analysis of NSC. Leaf and branch tissues were excised from the mid-outer tree canopy using a shotgun. Bole and coarse root tissue (xylem only) were collected via a 5mm increment corer to a depth of 2 cm. Bark tissue was separated from wood prior to analysis. Bole samples were collected at ~1.37 m from the ground. Coarse roots were sampled 15–30 cm away from the base of the tree and at a soil depth of 10 cm. All tissue samples upon collection were placed on ice in 20 ml scintillation vials and later stored at -80 °C.

2.3. Starch and soluble sugar quantification

We quantified soluble sugars and starch in tissues using standard methods developed by Jones *et al.* [34]. Following freeze drying of tissues, soluble sugars (sucrose, glucose, and fructose) were extracted

from ~25 mg of tissue through 3–80% ethanol (5 ml) extractions at 80 °C for 5 min each. Extracts were centrifuged and the supernatants pooled, a 2 ml aliquot was removed and dried using a vacuum evaporator. Dried extract was resuspended with 3 ml deionized water and 40 mg polyvinylpolypyrrolidone (PVPP) and thoroughly vortexed. PVPP was spun down using a centrifuge and a 0.5 ml aliquot removed and assayed enzymatically according to a colorimetric assay adapted from Jones *et al.* [34] and modified for use on aspen tissues [35]. Soluble sugar recovery was >95%.

To quantify starch, tissue pellets already extracted for soluble sugars were resuspended using 1 ml of 0.2 N KOH and incubated at 80 °C for 25 min. KOH was neutralized by adding 0.2 ml of 1 N acetic acid. Starch was hydrolyzed to glucose with α -amylglucosidase solution (pH 7.05) at 55 °C for 1.5 hours and assayed according to Jones *et al.* [34]. Starch recovery was >95%. Total sample NSC was the sum of soluble sugars and starch averaged across xylem and phloem. Non-structural carbohydrate mass was converted to C mass using a fraction of 0.41 g C g⁻¹ dry weight for soluble sugars and 0.44 g C g⁻¹ dry weight for starch [2] and expressed as the concentration of dry tissue mass. Sugar and starch fractions were summed to determine dry mass percent NSC.

2.4. Gross and net primary production initiation and cessation

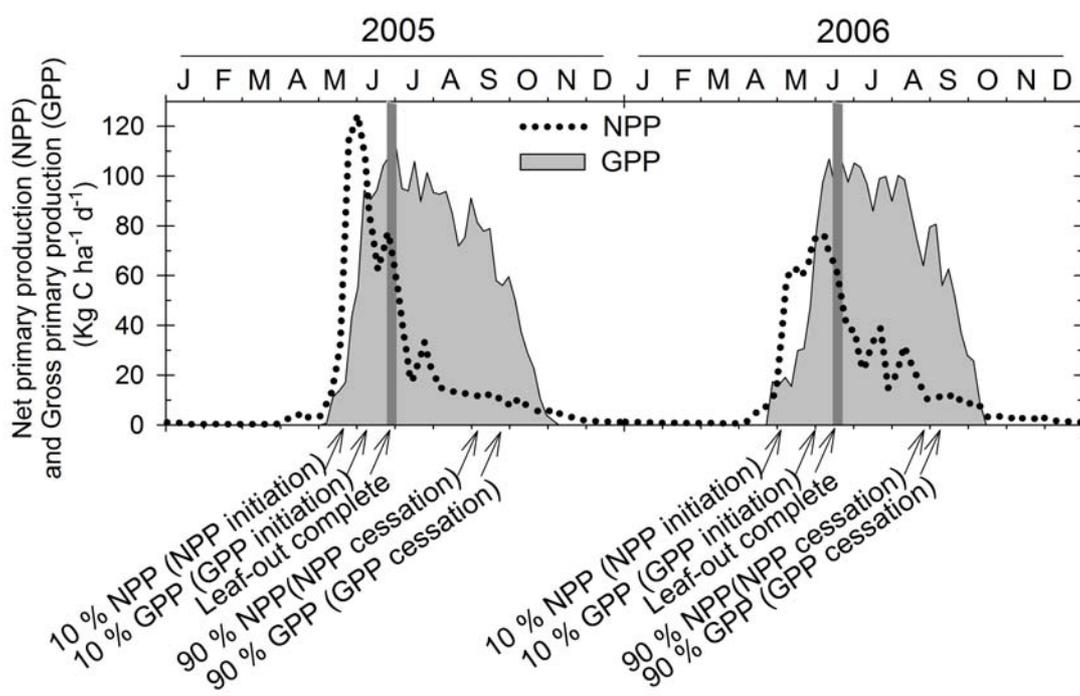
We used the methods of Gough *et al.* [18] to estimate the timing of NPP initiation and cessation from ecological inventories of daily net primary production of live wood, leaves, fruit, and branches, and fine roots for the entire forest from 2001–2003 and 2005–2006 (Figure 1). We did not calculate NPP in 2004 because high temporal resolution live wood dry mass production data were not available. Daily above- and belowground wood NPP was estimated from incremental changes in weekly to biweekly bole diameter (D) recorded for 190 trees with $D \geq 10$ cm using band dendrometers, and from allometric equations relating D to wood dry mass [36]. Daily leaf, fruit, and branch NPP was calculated by multiplying annual leaf, fruit, and fine branch dry mass production estimated from frequently sampled litter traps placed on the forest floor (area = 0.264 m², n = 20) by the interpolated daily fraction of annual vegetation area production calculated from twice weekly (during leaf expansion) to monthly measurements of vegetation area index. Daily fine root structural C production was the product of daily fine root turnover estimated from daily mean soil temperature (T_s , 7.5 cm) and standing fine root dry mass [36]. A site-specific C fraction of 0.49 (wood, leaves, fruit, fine branches) or 0.47 (fine roots) was used to convert dry mass to C mass [36]. Daily NPP was the sum of daily wood, leaf, fruit, fine branch, and fine root primary production. The times of initiation and cessation of NPP were defined as dates when 10% and 90% of total annual NPP, respectively, were achieved. Limitations and assumptions associated with this approach are detailed in [18].

We estimated the initiation and cessation of GPP from meteorological estimates of daily net ecosystem CO₂ exchange (NEE) between forest and atmosphere following Curtis *et al.* [37]. Detailed meteorological tower instrumentation, specifications, and data gap-filling procedures are described by Schmid *et al.* [38]. A 46 m tower equipped with eddy-covariance systems provided continuous measurements of 3-D turbulent velocity fluctuations and eddy-covariance fluxes of momentum (sonic anemometers; CSAT-3, Campbell Scientific, Inc.) and CO₂ fluxes as hourly averages (infrared gas analyzer; LI-6262 or LI-7000 from 2005; LI-COR Inc.; Lincoln, NE). CO₂ fluxes are subject to quality control, including outlier rejection, and a friction velocity $u^* \leq 0.35$ m s⁻¹ criterion to discard values

obtained under low turbulence conditions where the change of CO₂ storage in the canopy air space could be important. Daily GPP is the 24-h sum of daytime hourly NEE and ecosystem respiration, inferred from models relating nighttime net ecosystem CO₂ exchange to soil temperature (7.5 cm depth). Times of GPP initiation and cessation were the dates when 10% and 90% of total annual GPP, respectively, were attained.

We tracked leaf phenology with a LAI-2000 Plant Canopy Analyzer (LI-COR Inc.). Readings were taken every ~3 m along seven 60 m transects for an average of 120 samples on each of 12 sampling dates from May (leaf expansion) to November (leaf abscission) during 2005 and 2006.

Figure 1. Daily net primary production (NPP), gross primary production (GPP), and the timing of leaf-out completion, 2005-2006. The times of NPP and GPP initiation and cessation are the dates on which 10% and 90%, respectively, of total annual net primary production or gross primary production were achieved. Complete methods are described in [18].



2.5. Thermal degree days

We examined relationships between the dates of the initiation and cessation of NPP and GPP, and cumulative degree-days to evaluate how climate constrains the timing of ecosystem C cycling processes that putatively regulate tissue NSC depletion and accrual. Degree-days ($DD_{k,DOY_{i,n}}$) accumulate when mean air temperatures for day of year i (T_i) are greater or less than a base temperature (k) that, in principle, stimulates physiological and/or phenological activity [39]:

$$DD_{k,DOY_{i,n}} = \sum_{i=DOY_1}^{DOY_n} (T_i - k \text{ for } T_i > k, 0 \text{ for } T_i \leq k) \quad (1)$$

We used base temperatures of 4 °C and 20 °C to estimate cumulative heating (January - May) and cooling (June - September) degree-days, respectively, for years in which NPP data were available (2001-2003, 2004-2005; Gough *et al.* [18]). Base temperatures are published values optimized for a hardwood forest in New Hampshire [39].

2.6. Statistical analyses

We used ANOVA with repeated measures to analyze starch and soluble sugar concentrations among species, tissue types, and sampling dates. Species and tissue effects were analyzed using a split-block analysis since neither of these treatments could be randomized. Tissue NSC concentrations were square root transformed prior to statistical analyses to correct a non-normal distribution. Where sampling overlap occurred between aspen and oak cohorts, differences in NSC concentrations were tested using a two sample t-test ($\alpha = 0.05$); no significant difference between cohorts was found. Tissue starch and sugar concentrations were compared across dates using Tukey's HSD, $\alpha = 0.05$.

Levels of uncertainty associated with projections of tissue NSC concentrations (section 3.4) were estimated from the quadrature sum of the following component errors: interannual variability in rates of NSC accumulation between NPP and GPP cessation, and variation in the relationship between GPP and cooling degree days. For illustrative simplicity, mean 95% CIs were averaged across species. All statistical analyses were conducted using SAS statistical software (v. 9.1, SAS Institute, Cary, NC, USA).

3. Results

3.1. Sugar and starch concentrations by species, tissues, and over time

Non-structural carbohydrate concentrations varied significantly among tissues and between aspen and oak trees over time (Table 1, Figure 2). Among woody aspen tissues, NSC concentrations generally were greatest in branches, reaching 12% of dry mass (Figure 3a). Maximum aspen root NSC concentrations (4%) were greater than those of the stem (1.5%). Overall, oak tissue NSC concentrations were significantly greater than those of aspen. In oak, peak NSC concentrations in branches and roots reached 17% (Figure 2b). Maximum NSC concentrations were considerably lower (6.5%) in oak stems.

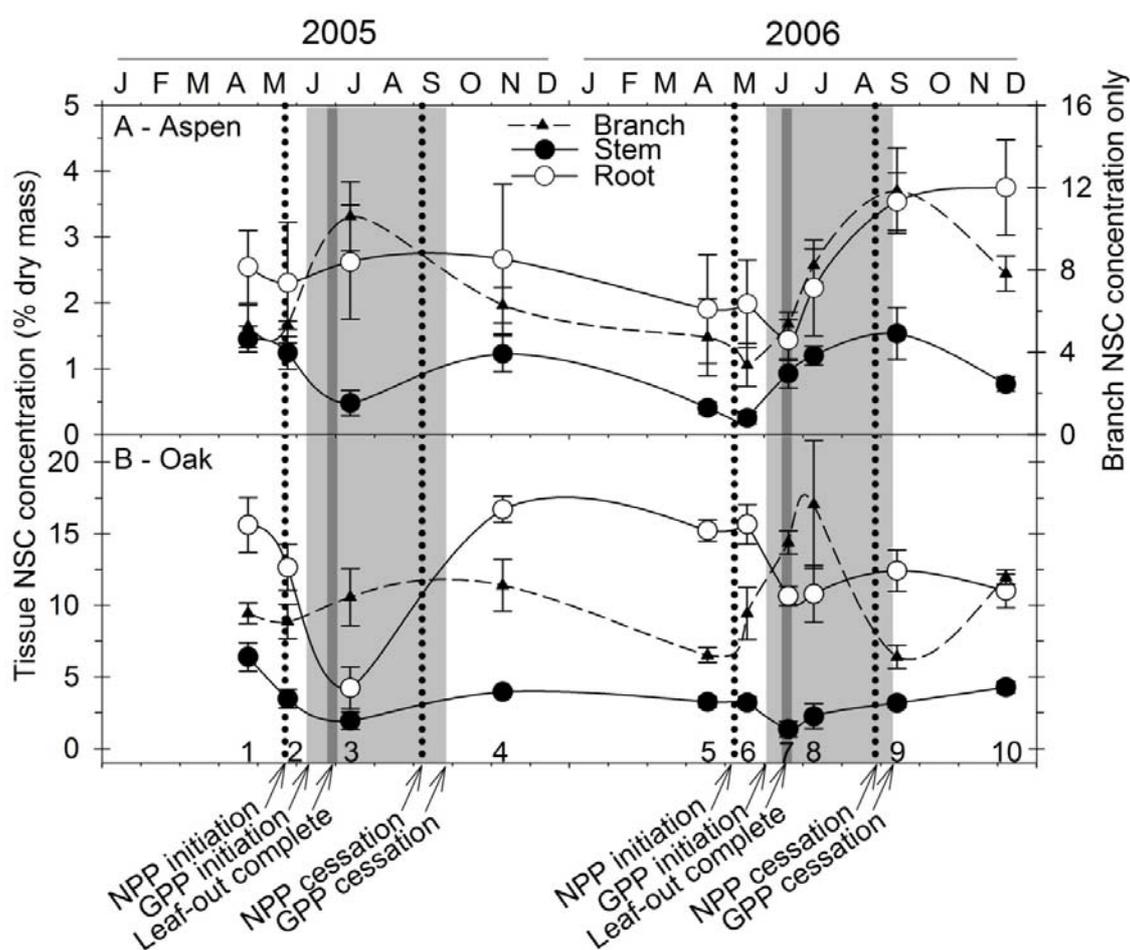
Tissue NSC concentrations fluctuated seasonally in aspen and oak tissues (Figure 2). Although substantial variation existed among tissues and species over time, NSC concentrations generally peaked during late summer or early dormancy. Aspen branches exhibited high seasonal amplitudes in starch concentrations, spanning 8% dry mass over time. Tissue NSC concentrations in aspen stems and roots varied seasonally to a lesser extent by up to 2% of dry mass. Seasonal changes in oak NSC were pronounced in all tissues, varying by 11% of dry mass in branches, 5% of dry mass in stems, and 11.5% of dry mass in roots.

3.2. Gross and net primary production, and seasonal non-structural carbohydrates dynamics

The timing of NSC accrual and depletion in aspen and oak roots and stems, primary NSC storage organs, often corresponded with forest phenological events that alter the balance between C

assimilation and growth (Figure 2). The initiation of NPP generally coincided with declining root and stem NSC concentrations in aspen and oak, with trends more pronounced in the latter. Aspen and oak root and stem NSC concentrations increased following leaf-out, with peak concentrations occurring after mid-summer NPP cessation and before GPP cessation. Branch NSC concentrations were more variable over time.

Figure 2. *Populus grandidentata* (aspen) and *Quercus rubra* (oak) non-structural carbohydrate (NSC) concentrations in branch, stem, and coarse root tissues, 2005–2006. Periods of NPP and GPP are bounded by black dashed vertical lines and the light grey-shaded area, respectively. The time of leaf-out completion is illustrated by the vertical dark-grey line. Note the different y-axis scales. Error bars represent 1 standard error.



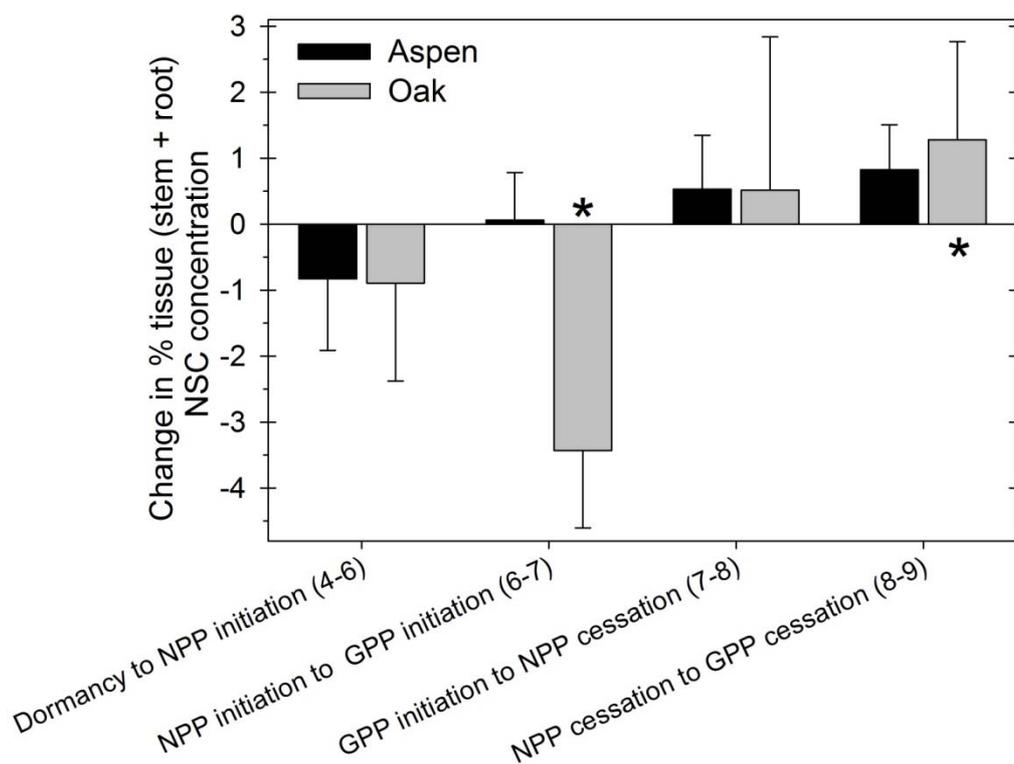
A focused examination of changes in root and stem tissue NSC concentrations between phenological boundaries during 2006, the year with the highest measurement frequency, further supports a coupling between the timing of NPP and GPP initiation and cessation, and seasonal trends in tissue NSC depletion or accrual (Figure 3). Significant declines in oak root and stem tissue NSC concentrations occurred following NPP initiation and prior to GPP initiation, when C demands for growth exceeded C supplied by current GPP. Significant increases in oak stem and root tissue NSC concentrations later occurred following NPP cessation and prior to the termination of GPP, suggesting that declining growth demands for recently assimilated C permitted the replenishment of NSC stores.

Changes in mean oak and aspen tissue NSC concentrations from dormancy until GPP initiation and between GPP initiation and NPP cessation were not significant.

Table 1. ANOVA statistics for treatment main effects and interactions on tissue NSC concentrations.

Parameter	d.f.	F	P
Replicate	2	2.41	0.0989
Species	1	383.31	0.0026
Tissue	3	215.48	<0.0001
Time	10	10.36	<0.0001
Species x Tissue	3	189.60	<0.0001
Species x Time	10	19.57	<0.0001
Tissue x Time	30	22.19	<0.0001
Species x Tissue x Time	28	6.60	<0.0001

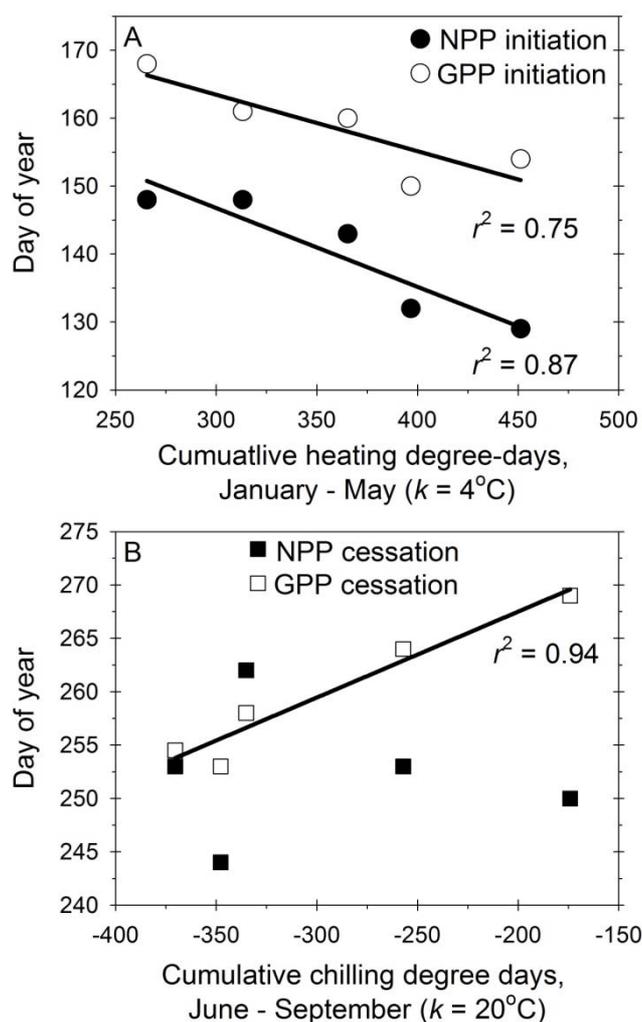
Figure 3. Changes between forest phenological boundaries in percent (%) tissue non-structural carbohydrate (NSC) concentrations averaged over stems and coarse roots, principal NSC storage organs. Numbers in parentheses indicate the contrasted measurement periods shown in Figure 2. An asterisk indicates a significant change between measurement dates (Tukey's HSD, $P < 0.05$).



3.3. Temperature and the timing of canopy carbon cycling processes

NPP and GPP phenologies, principal determinants of seasonal NSC cycles, closely corresponded with accumulated thermal degree-days. The initiation of both NPP and GPP in the spring was positively correlated with cumulative (January through May) heating degree-days, with NPP initiation averaging 19 days earlier than GPP initiation (Figure 4a). Among the 5 years examined, dates of NPP and GPP initiation spanned 19 and 18 days, respectively, depending on accumulated heating degree days from January through May. The cessation of the GPP occurred earlier in the year when more chilling degree-days accumulated from June through September, with timing varying by 16 days among 5 years (Figure 4b). The timing of NPP cessation was not correlated with cumulative (June through September) chilling degree-days.

Figure 4. The day of year of net primary production (NPP) and gross primary production (GPP) initiation in relation to cumulative heating degree-days from January through May (**A**) and the timing of NPP and GPP cessation as related to cumulative chilling degree days from June through September (**B**), 2001-2003, 2004, 2005. k is the baseline temperature used for the analysis. When trend line is shown, $P < 0.05$.



3.4. Simulated changes in tissue NSC with warming temperatures

We simulated the percent change in tissue NSC concentrations for oak and aspen boles and roots over a mean annual temperature rise of 1 to 7 °C, encompassing the range predicted for Michigan in 2095 (<http://www.ucsusa.org/greatlakes/glimpactmigrating.html>). This temperature span is concordant with the current rate of warming at our site of 1 °C over a quarter century [40]. We first estimated mean daily changes in tissue NSC concentrations between NPP and GPP cessation, a phase of NSC rise (Figures 2 and 3), by interpolating the daily incremental increase in tissue NSC between phenological periods. This phase of NSC accumulation was investigated because the timing of GPP cessation was temperature sensitive while NPP cessation was not; an indication that rising temperatures may extend C supply after growth demand has declined, thereby causing an increase in tissue NSC concentrations. Forecasts of tissue NSC depletion between NPP and GPP initiation were not examined because both phenologies responded in parallel to shifts in accumulation of degree days, with the slopes exhibiting overlapping 95% confidence intervals (Figure 4). This consistent offset between these processes suggests that warming temperatures would not affect early growing season NSC depletion. Next, we calculated changes in cooling degree days under rising temperature scenarios, which then were used to estimate changes in the timing of GPP cessation (Figure 4). Lastly, percent changes in tissue NSC concentration were estimated by multiplying mean daily changes in NSC concentrations between NPP and GPP cessation by the projected temporal offset (days) between these processes as temperatures increase.

Our modeling results suggest that percent changes in tissue NSC concentrations with rising temperatures of 1 to 7 °C are positively curvilinear, with similar trends across tissues and species (Figure 5). An approximate 1% increase in tissue NSC concentration occurred for every 1 °C rise in mean annual temperature. Very large 95% confidence intervals were associated with root projections because of high interannual variation in the accumulation rates of root NSC between NPP and GPP cessation. Uncertainty in projected bole NSC concentrations was much lower because of consistent interannual patterns of NSC accumulation (Figure 2). These results suggest that rising temperatures may exacerbate the temporal offset between the termination of late season growth (NPP) and canopy C assimilation (GPP), prompting an increase in the gross accumulation of late season tissue NSC.

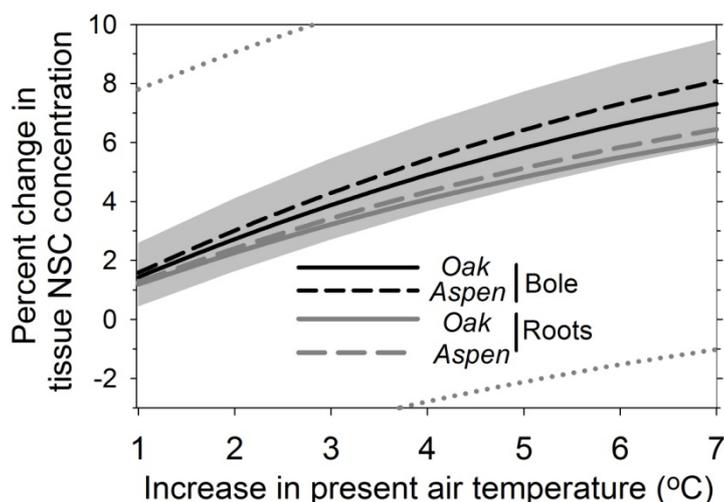
4. Discussion

We have shown that the timing of aspen and oak tissue NSC accrual and depletion was often coupled with the periodicity of NPP (forest growth) and GPP (gross C assimilation), phenologies that are commonly constrained by temperature in temperate forests [19-23]. Temporal shifts in tissue NSC have been broadly shown to correspond with tree phenological changes and with climatic events [8-17]. We are among the first to demonstrate the potential coupling of temperature regulated C cycling processes with temporal changes in tissue NSC concentrations [1-3], a result that suggests future warming may disrupt current cycles of NSC accumulation and depletion in our forest.

Seasonal cycles of tissue NSC accumulation and depletion at our site are consistent with those reported for other temperate, deciduous forests. We observed a general decline in NSC concentrations of major storage tissues (coarse roots and stems) from dormancy until GPP initiation and, subsequently, an accrual of tissue NSC after leaf-out and during the latter half of summer when NPP

had ceased and GPP was sustained. Seasonal trends that were especially pronounced in oak were more attenuated in aspen. Our findings reinforce prior results that tissue NSC of temperate deciduous trees accumulates when the supply of C assimilation exceeds current demands for NPP and, conversely, NSC may become depleted when current GPP is not sufficient to meet NPP demands [2,4,6,7].

Figure 5. Projected increases in tissue NSC concentrations for oak and aspen bole and roots, +1 to 7 °C mean annual temperature. Grey shading and dotted lines are 95% C.I. for bole and root NSC concentration projections, respectively.



Thermal regulation of NPP and GPP at our site suggests that seasonal NSC dynamics are partly constrained by temperature controls on the phenologies of C assimilation and allocation to growth. In our forest there was strong correspondence between cumulative heating degree days and the initiation of NPP and GPP, and between cumulative chilling degree days and the cessation of GPP in the autumn. Indirect thermal regulation of tissue NSC dynamics at our site may be caused by changes in the temporal relationship between the supply of recently assimilated C, and C demand for growth as temperatures rise. Numerous studies support a strong relationship between air temperatures and the duration of the growing season and canopy C assimilation or gross primary production [19-23], but none have related temperature to shifts in tissue NSC allocation.

Our results provide evidence that future warming could increase NSC accumulation in aspen and oak tissues. We predict an approximate 1% increase in tissue NSC concentrations for every 1 °C rise in mean annual temperature; the cause is the lengthening of the period between growth and C assimilation termination, a stage in which photosynthate allocation is redirected from growth to storage in NSC pools. If growth terminates during mid-summer independent of temperature as we observed, then extending C assimilation later into autumn might augment NSC accumulation by increasing the period in which assimilated C is allocated to storage NSC rather than to growth. We did not adjust our predictions of future late season NSC accumulation for a concurrent rise in plant respiration that might occur as temperatures increase [41-43]; however, we suggest that lengthening of the C assimilation period and, consequently, higher gross NSC production under warmer conditions may provide an important mechanism for offsetting rising metabolic costs, which have been

demonstrated to increase with temperature [5,44,45]. Interestingly, our findings suggest that rising air temperature may compound forecasted increases in tissue NSC caused by rising atmospheric CO₂ [32].

We acknowledge that our simulation, with its uncertainties, provides a preliminary forecast of temperature-related shifts in NSC for dominant species of a single ecosystem, and consider our analysis a basis for further hypothesis testing via empirical and modeling approaches. We note that high uncertainty in projections of root tissue NSC, caused by large apparent interannual variation in late-season NSC dynamics, may be the result of different sampling frequencies between years rather than biological variation. It is possible that in 2005, when late growing season tissue NSC was not sampled, our lower sampling frequency was not adequate to capture dynamic seasonal trends in carbohydrate concentrations observed during that same time period in 2006. Further, our NSC projections were inferred from correlative relationships between temperature driven phenology and carbohydrate dynamics, and could not be controlled for parallel changes in climate and physiology that may affect tissue NSC concentrations. We suggest that future studies of NSC-temperature interactions address these key uncertainties through high frequency NSC sampling conducted under experimentally controlled climate conditions.

A temperature induced rise in tissue NSC could have broad ecological implications, modifying forest biotic interactions while enhancing the resilience of forest growth to adverse climate change, disturbance, and rising metabolic demand. Stored NSC is mobilized to support growth and metabolism during periods of drought and low light availability when photosynthesis is depressed [3,24-26,46], conditions that may intensify regionally as climate changes (<http://www.ucsusa.org/greatlakes/glimpactmigrating.html>). Growth recovery following canopy defoliation also relies heavily on NSC stores, suggesting that climate change related increases in herbivory [47, 48] may be partially mitigated by a concurrent increasing supply of NSC. Higher tissue NSC concentrations also may affect the exchange of C and nutrients between plants and both herbivores and symbionts. Elevated CO₂ experiments show that rising NSC concentrations promote ectomycorrhizal fungi growth and colonization [49, 50], and the dilution of foliar nutrients available to herbivores [47,48,51,52]. A rise in tissue NSC also may positively or negatively change the concentration of secondary compounds known to thwart herbivores [47,48,53].

It is important to acknowledge that other climate parameters may affect the timing of NPP and GPP and thus alter the periodicity of tissue NSC accumulation and depletion. For example, photoperiod controls the timing of growth and C assimilation initiation and cessation in some woody species [54-56]. Additionally, water stress is implicated in early cessation of growth and C assimilation, the latter of which is prompted by accelerated leaf senescence [57]. A reduction in future precipitation could alter seasonal NSC dynamics by truncating the end of the growing season. Additional investigation is required to elucidate the suite of environmental controls on seasonal NSC dynamics, and to determine how future climate change will alter the allocation of assimilated C to growth or storage pools.

We note that tissue sugar and starch concentrations for oak and aspen at our site were comparable to those already published for these genera. Tissue NSC concentrations for oak coarse roots (4–17%), stems (1.5-6.5%), and branches (6.5-17%) were within the range for *Quercus* spp. [2,58,59]. Aspen stem (0.5-1.5%) and branch (3.5-12%) NSC concentrations were similar to those reported for *Populus* spp., but our coarse root tissue NSC concentrations (1.5–4%) were considerably lower than those

reported for mature *Populus tremuloides* by Landhausser and Lieffers [6]. Tissue NSC concentrations of aspen and oak at our site generally compare well to those of seven temperate deciduous species with the exception of our aspen stem NSC concentrations, which were somewhat lower [2].

The significant seasonal variation in NSC concentrations that we observed between aspen and oak is consistent with studies showing differences among plant functional groups in their NSC storage and remobilization patterns during periods of dormancy and growth [2,4,46]. Deviations in tissue NSC concentrations and seasonal amplitudes between aspen and oak at our site may be partly caused by differences in the timing of xylem production. Ring porous species such as oak concentrate their xylem production in the spring, while diffuse porous species distribute xylem production more evenly throughout the growing season. Greater spring demand for storage NSC in support of xylem construction may have prompted the higher oak tissue NSC concentrations and seasonal amplitudes that we observed relative to aspen. Similar disparities in tissue NSC concentrations and seasonal amplitudes among ring porous and diffuse porous species have been previously noted [2,58].

5. Conclusions

We conclude that rising temperatures may alter the timing of forest C source and sink relationships in a way that causes an increase in gross NSC concentrations of tissues. This enrichment of tissue NSC may be an important compensation mechanism for supporting growth as metabolic demands and disturbances increase with climate change. If rising CO₂ and temperature jointly contribute to enhanced NSC production, the ecological consequences could be broad and include changes in biotic interactions and in forest growth resiliency.

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