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Research paper

Coordination between growth, phenology and carbon storage in three coexisting deciduous tree species in a temperate forest

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In deciduous trees growing in temperate forests, bud break and growth in spring must rely on intrinsic carbon (C) reserves. Yet it is unclear whether growth and C storage occur simultaneously, and whether starch C in branches is sufficient for refoliation. To test in situ the relationships between growth, phenology and C utilization, we monitored stem growth, leaf phenology and stem and branch nonstructural carbohydrate (NSC) dynamics in three deciduous species: *Carpinus betulus* L., *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Liebl. To quantify the role of NSC in C investment into growth, a C balance approach was applied. Across the three species, >95% of branchlet starch was consumed during bud break, confirming the importance of C reserves for refoliation in spring. The C balance calculation showed that 90% of the C investment in foliage (7.0–10.5 kg tree^{-1} and 5–17 times the C needed for annual stem growth) was explained by simultaneous branchlet starch degradation. Carbon reserves were recovered sooner than expected, after leaf expansion, in parallel with stem growth. *Carpinus* had earlier leaf phenology (by ~25 days) but delayed cambial growth (by ~15 days) than *Fagus* and *Quercus*, the result of a competitive strategy to flush early, while having lower NSC levels.

Keywords: bud break, growth onset, mixed forest, starch degradation.

Introduction

Trees allocate resources to multiple processes, including growth, reproduction, storage and defense. Allocation of carbon (C) into storage is key for tree survival in unfavorable conditions such as shading by taller neighbors after canopy closure or for successful regrowth after strong damages such as wind break, frost damage or herbivory (Chapin et al. 1990). These C reserves, mostly in the form of nonstructural carbohydrates (NSC), and primarily starch, often change in a seasonal cycle. Hence, NSC can buffer times of zero or low C uptake in photosynthesis, for example, under very deep shade (Myers and Kitajima 2007), or due to stomatal closure during drought (Klein et al. 2014, Savage et al. 2015). Nonstructural carbohydrate formation, mostly in the form of starch, is considered an overflow response or a precaution measure during periods of abundant C uptake (Körner 2003) and also depends on the activity of living wood parenchyma cells (Da Silva et al. 2014), and hence can also occur at times of limited C supply (Hartmann et al. 2015). It has been further hypothesized that C reserve formation might even become enhanced during periods of C shortage (Sala et al. 2012, Wiley and Helliker 2012). In a study of drought-stressed pines in a semi-arid forest, branch C reserves were sequestered at the cost of growth cessation (Klein et al. 2014).

Deciduous trees growing in temperate regions must complete all their physiological activities during the growing season, before dormancy. Specifically, C reserves must be kept over the dormant period to ensure C supply for next year's bud break. In turn, branchlet starch concentration decreases immediately before bud break, as observed in branch sapwood of mature

Carpinus betulus L. and *Fagus sylvatica* L. growing at the Swiss canopy crane (SCC) temperate forest site (Schädel et al. 2010). The reliance of new shoot and leaf growth on proximal starch pools has been also known in fruit trees growing in orchards, such as avocado (Liu et al. 1999). Yet it is unclear whether these proximal starch pools are sufficient to supply C for the new tissues, or whether additional C needs to be imported from other tissues like the stem (Klein and Hoch 2015). In mature *F. sylvatica* and *Quercus petraea* (Matt.) Liebl., stem sapwood soluble sugars are gradually decreased and starch is increased before bud break (Barbaroux and Bréda 2002), in contrast to potential C export from stems to buds. Responses can also differ between species: *Q. petraea* generally relies more on C reserves than *F. sylvatica*, probably due to earlier stem growth onset and higher winter respiration levels (Barbaroux et al. 2003).

In the long term, stem growth onset needs to be synchronized with C supply from fresh assimilates, i.e., it should take place soon after bud break, when leaves are unfolded and photosynthesizing. Developing shoots of *Populus tremuloides* have been shown to become C autonomous only a few days after bud break (Landhäusser 2011). Similarly, young leaves of *F. sylvatica* and *Q. petraea* start to assimilate CO_2 when 10–50% expanded, as shown using $^{13}CO_2$ pulse labeling on trees at the aforementioned SCC forest site in central Europe (Keel and Schädel 2010). Bud break may occur on very different dates for different tree species: at the SCC site, the 2002–04 average bud break day of year (DOY) of *C. betulus* and *F. sylvatica* was 91 ± 1 and 114 ± 1, respectively (Asshoff et al. 2006). The temporal time lag between species' flushing time decreased when early spring was cooler than usual, preventing flushing despite stepwise dormancy release, for example, in 2013 (average bud break DOY of 104 ± 1 and 110 ± 1 for *C. betulus* and *F. sylvatica*; Vitasse and Basler 2014). In a 1-year-old poplar plantation, stem growth onset was timed together with leaf expansion (Deslauriers et al. 2009). Stem growth onset was synchronized with bud break in *F. sylvatica*, but not in *Q. petraea*, where stem growth started 10 days before bud break (Barbaroux and Bréda 2002). Oaks might be exceptional in that matter, due to earlywood vessel formation: stem growth onset in *Quercus robur* L. and *Quercus pyrenaica* Willd. took place 25–45 days before bud break (Pérez-de-Lis et al. 2016). In general, leaf and cambium growth phenologies are typically not simultaneous and may respond in a different way to environmental cues (Delpierre et al. 2016).

As generally assumed, recovery of C reserves should take place as soon as C investment into growing tissues is completed, for example, at the end of the growing season (Hoch et al. 2003, Richardson et al. 2013). Deslauriers et al. (2009) showed in planted poplars that tissue starch pools decline as stem growth progresses (C supply to growing meristems), but soluble sugars increase. A gradual increase in stem and branchlet starch concentrations is usually observed only after growth is completed (Liu et al. 1999). Similarly, in mature *F. sylvatica* and *Q. petraea* in the field, stem sapwood soluble sugars do not recover before autumn (Barbaroux and Bréda 2002). Therefore, in both species, the partitioning of NSC between starch and soluble sugars changes from 30 : 70 (starch : soluble sugars) in June to 70 : 30 in October (Barbaroux et al. 2003). The temporal decoupling between growth and C storage is sometimes driven by additional processes such as freezing tolerance, relying on conversion of stem wood starch to sugars before winter (Kandler et al. 1979, Fischer and Höll 1992, Piispanen and Saranpää 2001). Subtle changes still occur: for example, stem sapwood starch declines during the growing season in *Q. petraea* but increases in *F. sylvatica* (Barbaroux and Bréda 2002).

To test and quantify the role of C reserves in spring growth, and the coordination between C storage and growth during the growing season, we monitored stem growth, leaf phenology and NSC dynamics in mature deciduous trees in a temperate forest site at the foothill of the Jura Mountains in Switzerland. Then, we applied a C balance approach to quantify the C fluxes and pools at the tree and compartment scales.

Our research questions were as follows: (Q1) To what degree does C supply for bud break and leaf growth rely on shoot starch? (Q2) What is the level of synchronization (in number of days) between leaf unfolding and stem growth? (Q3) What is the time required (in number of weeks) for C reserves to recover after bud break and stem growth? For our study, we selected three tree species with contrasting spring phenology, wood anatomy and growth rate: *C. betulus*, an early flushing species with diffuse-porous wood; *F. sylvatica*, an intermediate flushing, fast growing species with diffuse-porous wood; and *Q. petraea*, a late flushing species in this site (based on the phenological monitoring of adult trees in 2012; Vitasse 2013) with ring-porous wood known to produce large vessels early in the growing season. Based on these interspecific differences, we expected differential NSC dynamics among the species, for example, that branchlet starch should decrease earlier in *Carpinus* than in *Fagus* and *Quercus*.

Materials and methods

Study site and study species

Our study was performed in a mixed forest site located 12 km southwest of Basel, Hofstetten, Switzerland (47°33′N, 7°36′E, 550 m above sea level). This is the SCC site, with a 45 m canopy crane permitting access to >100 tree canopies (Pepin and Körner 2002). The site is dominated by ~100- to 120-year-old deciduous trees (mostly *F. sylvatica*, *Q. petraea* and *C. betulus*) and coniferous trees (mostly *Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L. and *Abies alba* Mill.). Trees form a closed canopy with heights of 30–40 m and a leaf area index of ~5 (Leuzinger and Koerner 2007). The soil is shallow silty-loamy rendzina on calcareous bedrock. The climate is mild temperate, with mean January and July temperatures of 2.1 and

Table 1. Climatic conditions in 2014 at the SCC forest site (Hofstetten, SO, Switzerland). Temperatures are continuously recorded at the crane top ~40 m height. Temperatures are means of all days in a certain month (preferred position: L144).

Month	Mean T (°C)	Mean T_{max} (°C)	Mean T_{min} (°C)	Precipitation (mm)
January	5.1	7.5	2.9	78.8
February	5.9	9.2	3.4	90.4
March	9.0	12.3	5.7	15.4
April	11.7	15.2	8.6	55.0
May	12.9	16.6	9.7	89.4
June	18.5	22.5	14.3	51.8
July	18.5	21.9	15.4	214.2
August	16.9	19.9	14.4	92.2
September	16.0	19.4	13.1	29.9
October	13.9	16.4	11.4	81.1
November	8.3	10.7	6.3	70.1
December	4.5	6.4	3.1	42.3
Annual	11.8	14.8	9.0	910.6

19.1 °C and mean temperature during the growing season (May–September) of 14.7 °C. Mean annual precipitation is ~900 mm. Climatic conditions of 2014 are summarized in Table 1. We monitored stem growth, leaf phenology and stem and branch NSC dynamics in three to five trees from each of three deciduous species, namely *C. betulus*, *F. sylvatica* and *Q. petraea*. For clarity and brevity, hereafter, we will refer to each species by its genus. Stem diameter at breast height (DBH) was 37.5 ± 2.9, 61.1 ± 4.4 and 43.4 ± 2.2 cm for *Carpinus*, *Fagus* and *Quercus*, respectively.

Radial stem growth

Permanent tree girth tapes (UMS, München, Germany) were installed on all study trees in 2009. Stem diameter variations were monitored once a week (with the exception of a few occasions) during the growing season (April–October). Measurement resolution was 0.1 mm, and to minimize the potential effect of bark expansion (diurnal variations of up to 0.3 mm), measurements were performed consistently between 11:00 and 14:00 h. To estimate the stem growth onset date from the growth curves, a statistical approach was applied using Tukey tests to find the earliest date when stem diameter increments were significantly different from zero. However, due to the large variation in growth among individual trees of each species, the mean stem growth onset date could not be identified. Instead, a geometric approach was used, whereby growth rates (% increment per month) were calculated for each period between two observations. In turn, the average date between two observations with growth rate >0.02% per month was taken as the date of stem growth onset for each individual tree. Due to our weekly measurement time resolution, estimation error was hence 3 days at most.

Leaf phenology

The 'average' phenological stage of the selected trees was assessed using a scale from 0 to 4 according to Vitasse et al. 2014 (0, dormant bud; 1, bud swelling; 2, bud break; 3, leaf-out; 4, leaf unfolding). By average, we mean the phenological stage reached by the majority of the tree crown. Observations were made one to two times a week in spring 2012, 2013 and 2014, using one or two of three slightly different methods: (i) direct observation of tree crowns from the canopy crane gondola, (ii) analysis of images recorded by a phenology camera (Mobotix, Langmeil, Germany) installed at 30 m above ground on the crane tower and (iii) observation from the ground using binoculars (Canon 10 × 30 image stabilization binoculars; Tokyo, Japan). Leaf phenology in the low canopy (Method (iii)) was on average 1 day earlier than in the high canopy (Methods (i) and (ii)), but this small difference was not statistically significant. Note that in 2012 and 2013, phenology was monitored on the same or neighboring individuals as in 2014. Overall, and considering observations in additional individuals, our observations represented well the average phenological development of the three studied species in our forest site during 2012–14.

Nonstructural carbohydrates

Microcores taken from the east sides of all trees at breast height (1.3 m) and branchlets from the 2012 and 2013 growing seasons were used to determine NSC content. Samples were taken on eight sampling dates: biweekly between early March and early June 2014, and once more in early September 2014. All samples were subject to heat shock using a microwave on site immediately after harvest. In the laboratory, samples were dried at 75 °C to weight constancy in a drying oven and ground using a ball mill (Retsch, Hann, Germany) at a frequency of 25 tilts s^{-1} until tissues had turned into fine powder (~5 min). Nonstructural carbohydrate analyses followed the method by Wong (1990), modified as described in Hoch et al. (2002). Dried wood powder (8–12 mg) was extracted with 2 ml deionized water at 100 °C for 30 min. An aliquot of each sample extract was taken for the determination of low molecular weight carbohydrates using invertase (from baker's yeast; Sigma-Aldrich, Buchs, Switzerland) to break sucrose into glucose and fructose. Glucose and fructose were converted into gluconate-6-phosphate using glucose hexokinase (Sigma Diagnostics, St Louis, MO, USA) and phosphogluconate isomerase (from baker's yeast; Sigma-Aldrich). The total amount of gluconate-6-phosphate was determined as the increase in NADH + H^+ using a photometer (HR 700; Hamilton, Reno, NE, USA). For starch determination, the remaining extract was incubated at 40 °C for 15 h with amyloglucosidase (from *Aspergillus niger*; Sigma-Aldrich) to break starch into glucose. Nonstructural carbohydrate was determined as the total amount of glucose as described above. Starch content was calculated as total NSC minus free sugars. All concentrations were calculated on a % dry matter basis.

Upscaling of measurements into C pools

We followed the procedures of the recently developed C allocation dynamics model presented in Klein and Hoch (2015).

Compartment C pools were calculated using verified allometric relationships between stem DBH and each compartment biomass. These relationships were developed by tree harvest at forest stands in Germany (for *Q. petraea* and *C. betulus*; Suchomel et al. 2012) and the Netherlands (for *F. sylvatica*; Bartelink 1997), i.e., under lowland temperate conditions similar to our site. Carbon amounts scale with tree size, and hence, to allow for unbiased comparison between the species, all estimates were calculated based on DBH of 40.0 cm, the typical size of the *Carpinus* and *Quercus* study trees. Carbon investment in stem growth was calculated by applying our stem increment measurements (%) on the estimated stem C pools. Carbon investment in leaf growth required the conversion of phenological grading on specific days (see above, in a number scale) to leaf biomass development in grams. For this purpose, we employed leaf growth curves for *F. sylvatica* (Damesin and LeLarge 2003) and an average of two available curves for two *Quercus* species, namely *Quercus ilex* (Gratani and Bonito 2009) and *Quercus rotundifolia* (Mediavilla and Escudero 2003). The latter two curves came from evergreen, rather than deciduous, oak species as in our case, which had no leaf biomass development data available. Leaves from evergreen and deciduous oaks differ in phenology and specific leaf area, but not in the dynamics of single leaf growth. Therefore, these curves provided the best available estimate. The percent of final leaf biomass at each observation date was then adjusted to the total foliage C pool calculated above. *Carpinus* leaves were already emerging (phenological stages 2–3) at the time of our first observation (21 March) and hence were not included in this analysis.

Stem NSC concentrations measured at 1–3 cm depth below the cambium were upscaled to the total stem depth (typically 20 cm) according to their radial distribution (i.e., declining with depth). For each tree species, we calculated a depth-quenching factor, namely the ratio between the average NSC concentration along the entire radial depth and that at 1 cm depth. Data for the study trees were available from a former study on the radial distribution of NSC in stems assessed on the same trees (Hoch et al. 2003). The calculated depth-quenching factor was 0.64, 0.33 and 0.70 for *Fagus*, *Quercus* and *Carpinus*, attesting to the contrasting radial NSC distributions among the species. Branchlet NSC pools were calculated as the product of NSC concentrations measured in branchlets and the biomass of the two lowest branch size classes, i.e., twigs (diameter <4 cm) and branchlets (4 cm < diameter < 7 cm). For convenience, both classes together are termed here 'branchlets'. Although all our branchlet NSC measurements came from branchlets of diameter <4 cm, it is reasonable to assume similar NSC levels in the larger size class, as shown for adult trees of both *F. sylvatica* and *Q. petraea* growing in a forest in northeast France, ~150 km north from our site (Barbaroux et al. 2003). In that study, NSC levels were not statistically different across wood samples from 1-year-old twigs and from major branches.

Statistical analysis

The effect of time (date) on wood NSC concentration was tested by analysis of variance (ANOVA) after verifying that the residuals conform to the assumptions of the ANOVA. Species-specific NSC means on each sampling date were compared using Tukey–Kramer tests. All statistical analyses were performed in JMP Pro 11 (SAS, Cary, NC), with $\alpha = 0.05$.

Results

Leaf phenology and stem growth onset

Leaf phenology observations in 2012–14 in the study site confirmed a consistent sequence among the study species (as reported earlier in this site; Asshoff et al. 2006): bud break of *Carpinus* started the earliest in early April and was quickly followed by leaf-out, *Fagus* was second with bud break beginning around mid-late April with fast-developing leaves, and *Quercus* bud break also occurred around mid-late April with bud development typically lasting longer, i.e., leaf-out at the end of April or beginning of May (Table 2, Figure 1). Stem growth onset also showed a consistent, yet different, species sequence: *Fagus* and *Quercus* stem growth started at the end of April up to early May, whereas *Carpinus* did not grow before late May. At the species level, this meant that stem growth onset was well coordinated with leaf expansion in *Quercus* and *Fagus*, but lagged behind in *Carpinus*, where growth of the different compartments was separated by 6–7 weeks.

Stem and branchlet NSCs in relation to growth

Leaf and stem growth dynamics in 2014 followed the aforementioned long-term patterns observed in earlier years. During the more intensive 2014 campaign, starch and soluble sugar concentrations in branchlet and stem wood were measured at eight time-points along the growing season (Figure 1). Across the

Table 2. Day of year of leaf phenology stages and stem growth onset in Hofstetten forest in 2012–14. Values are means ± standard errors (n = 2–10; not available (NA) when based on camera observations, see Materials and methods) (preferred position: L252).

Year, species	Bud swelling	Bud break	Leaf-out	Leaf unfolding	Stem growth onset
2012					
F. sylvatica	110 ± 3	114 ± 3	117 ± 2	119 ± 2	NA
Q. petraea	NA	NA	123	123	NA
C. betulus	NA	NA	97	97	NA
2013					
F. sylvatica	108 ± 1	111 ± 1	114 ± 1	118 ± 1	139 ± 0
Q. petraea	NA	NA	119	NA	147 ± 15
C. betulus	76 ± 2	104 ± 1	109 ± 0	114 ± 1	181 ± 5
2014					
F. sylvatica	90 ± 2	95 ± 3	100 ± 2	104 ± 2	122 ± 4
Q. petraea	93 ± 0	100 ± 1	104 ± 1	109 ± 3	121 ± 3
C. betulus	NA	80 ± 0	83 ± 1	88 ± 3	183 ± 2

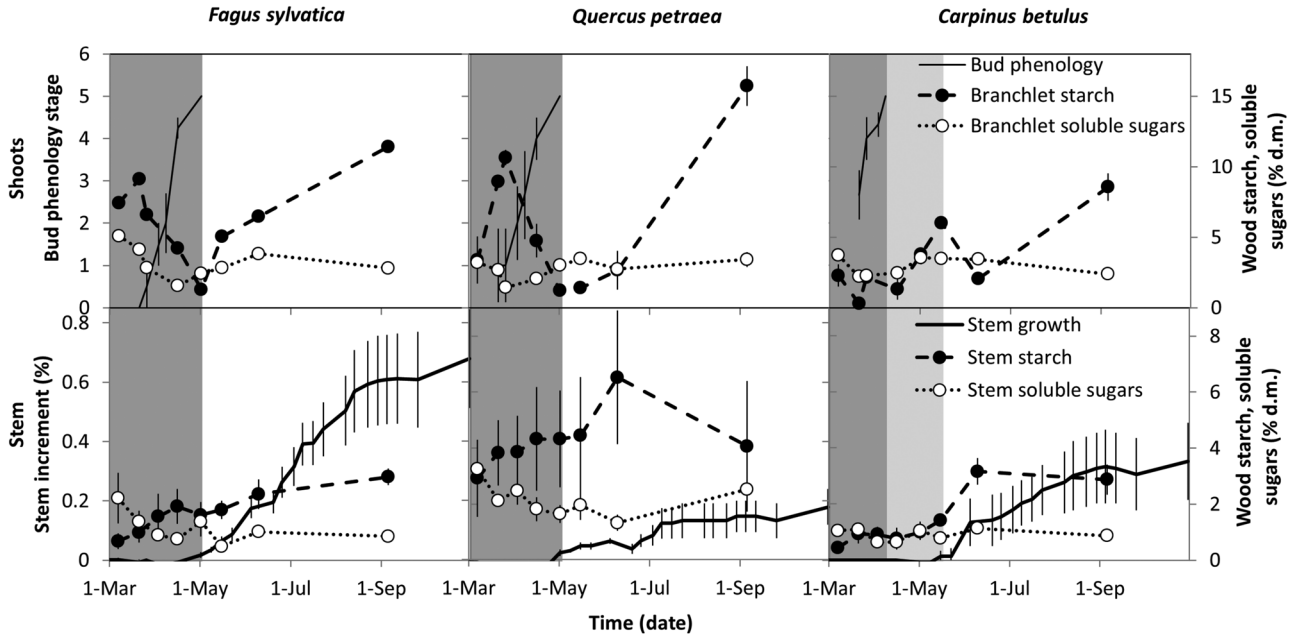

Figure 1. Dynamics of bud phenology and branchlet NSCs (upper panels) and stem growth and NSC (lower panels) at breast height during the 2014 growing season in three tree species growing at the SCC forest site. Shaded areas in the graphs denote the species-specific period before complete leaf expansion (dark gray) and the period between leaf expansion and stem growth onset (light gray; in *Carpinus* only). Values are means ± SE (n = 4–5 for *Fagus* and 3–4 for *Quercus* and *Carpinus*; error bars are not visible when too small).

Table 3. Statistical significance (P-value tested by ANOVA) of the effect of time (date) on wood NSC concentration of the three studied tree species at the SCC forest site. Significant effects at $\alpha = 0.05$ are in bold (preferred position: L267).

Tissue and compound	*Fagus sylvatica*	*Quercus petraea*	*Carpinus betulus*
Branchlet starch	**<0.0001**	**<0.0001**	**<0.0001**
Branchlet soluble sugars	**<0.0001**	0.224	**0.003**
Stem starch	0.105	0.970	**<0.001**
Stem soluble sugars	0.240	0.145	0.403

three species, branchlet starch dynamics were the most significant (Table 3), decreasing during leaf growth to a minimum concentration at leaf expansion (1.3 ± 0.0% dry mass (d.m.)), and increasing during summer, together with stem growth, to a maximum reached in September (11.9 ± 2.1% d.m.). *Carpinus* showed more complex dynamics, with two additional low points, one in mid-March and another in early June, well into the stem growth phase. Among species, mean branchlet starch concentrations were two times higher in *Fagus* and *Quercus* (6.5 ± 1.1 and 6.1 ± 1.8% d.m., respectively) than in *Carpinus* (3.3 ± 1.0% d.m.). Changes in soluble sugars were smaller but also tended to decrease during leaf growth and recover thereafter, though less so in *Quercus* (Table 3).

Stem NSC changes along the growing season were less pronounced than in branchlets (Figure 1; note the different y-axis scales). Stem starch tended to increase during the season, but this was significant in *Carpinus* only (Table 3). Variations in stem soluble sugars over the season were not significant in any of the species. The higher stem NSC in *Quercus* than the other two species (4.3 vs 1.4–1.7% d.m. on average) was partly related to differences in the radial distribution of NSC within stems of these species, underrepresented in our shallow sapwood sampling (see Materials and methods).

Large differences were observed in the relative annual stem growth increment, with *Fagus* growth doubling and quadrupling that of *Carpinus* and *Quercus*, respectively. Growth interruption in June (e.g., stem shrinkage in *Quercus*) correlated with hot and dry conditions during that month in 2014 (Table 1). Across the three species, branchlet and stem NSC concentrations in September were usually higher (in starch) or lower (in soluble sugars) than their early March values (Figure 1). This suggested either (i) major interannual differences in tissue NSC concentration or (ii) that further changes occurred at the end of the growing season (October) and potentially at any time before March of the following year.

Tree C pools and allocation to growing tissues

Nonstructural carbohydrate and growth measurements were upscaled into tree C pools and fluxes using a recently developed C allocation model (Klein and Hoch 2015) and integrating allometric equations developed in other lowland forest sites in Central Europe (Bartelink 1997, Suchomel et al. 2012). Differences among species in compartment C pools (Table 4) reflected substantial morphological and anatomical differences. For example, mean sapwood density in *Fagus* and *Quercus* trees in our site was ~0.70 and 0.66 g cm^{-3} (data not shown), in agreement with the 9% smaller stem C pool estimated for *Quercus* than for

Fagus. The lower stem C pool of *Carpinus* is related to its lower stature. Branchlet (of diameter <7 cm) and foliage C pools were only ~14 and 3% of the stem pool. Nonstructural carbohydrate pools were largest in *Quercus* and larger in stems than branchlets. But the smaller branchlets' biomass compared with that of the stem was partially made up for by the significantly higher maximum NSC concentrations (Figure 1).

Amounts of C used for annual stem increment were higher in *Fagus* than in the other two species (2.28 vs 0.55–0.74 kg; Table 4), mainly due to its stronger growth (Figure 1). These amounts were expectedly smaller than the existing, cumulative, C pools (222.8–335.7 kg). The stem starch C pool could theoretically (not accounting for maintenance respiration) supply the C required for stem growth for 7, 17 and 30 years (in *Fagus*, *Carpinus* and *Quercus*, respectively) based on the 2014 growth. Comparing these stem growth C investment amounts with those of the foliage growth (C allocation flux which, in deciduous trees, is also a compartment pool) indicated the much higher C sink strength in foliage vs stem in these trees (Table 4).

Table 4. Tree compartment C pools in individuals of 40 cm DBH of the three studied tree species and based on allometric equations developed at similar forest sites in Central Europe. Annual stem increment was calculated based on our observations in the SCC forest site (preferred position: L297).

	C pool (kg)		
	Fagus sylvatica	*Quercus petraea*	*Carpinus betulus*
Stem	335.7	307.2	222.8
Branchlets (<7 cm)	39.7	36.4	42.7
Foliage	10.5	9.6	7.0
Max. stem starch	16.1	16.3	12.3
Max. branchlet starch	11.4	14.3	9.1
Annual stem increment	2.28	0.55	0.74
Annual stem/foliage C investment ratio	0.22	0.06	0.11
Reference	Bartelink (1997)	Suchomel et al. (2012)	

To further explore the C investment in developing leaves, we followed the dynamics of the total foliage C pool and branchlet starch C pool in *Fagus* and *Quercus* (Figure 2). In both species, the foliage C pool increased with leaf development, however not monotonously, and the fastest C accumulation was in the first and second week of April for *Fagus* and *Quercus*, respectively. Branchlet C stored in starch simultaneously decreased in *Fagus* in a linear manner from 9.1 to 1.3 kg, and increased in *Quercus* from 3.1 to 9.7 kg before decreasing again in mid-April to 1.1 kg (Figure 2). The 7.8 kg product of branchlet C degradation in *Fagus* was without parallel increase in branchlet soluble sugar level (Figure 1), indicating the use of C for growth or respiration. This amount (7.8 kg) was comparable to but still lower than the 10.5 kg C required for the foliage (Table 4).

Discussion

Growth and C reserves

Across three major deciduous forest tree species, tissue starch concentration decreased in branchlets during bud break and leaf growth (Figure 1), resolving our first research question, Q1. In *Fagus* and *Quercus*, where data were available, we were able to quantitatively show that almost all the C required for new leaf growth came from breakdown of branchlet starch (Figure 2). There was no evidence of C import from the stem into growing shoots, e.g., a decrease in stem NSC before or during the time of bud break. In *Carpinus* branchlets, starch decreased from 2.3% d.m. to near zero between 7 and 21 March, but earlier observations are needed to test whether leaf growth also relied on branchlet starch in this species. The delayed decrease in *Quercus* branchlet starch may be explained by a relatively low leaf C demand during bud development and opening (March and

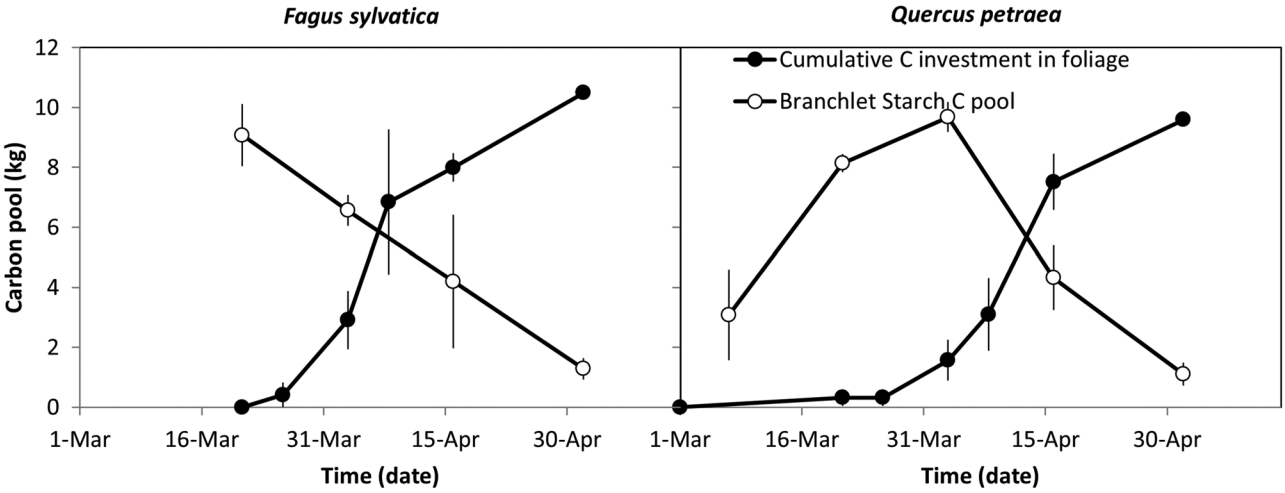

Figure 2. Changes in total foliage C pool and branchlet starch C pool during bud break and leaf growth in 2014 in two tree species growing at the SCC forest site. Values are means ± SE (n = 4–5 for *Fagus* and 3–4 for *Quercus*; error bars are not visible when too small).

early April), and overall the 8.6 kg C from this starch degradation was reasonably close to the total leaf C demand of 9.6 kg. But our estimates for new leaf C demand were probably excessive, considering that leaves in these trees become a C source already when 10–50% unfolded, and hence ~30% of their growth C demand relies on fresh photoassimilates (Keel and Schädel 2010), and rates of photosynthesis in leaves of our study trees are relatively high, up to 10–16 μmol m^{-2} s^{-1} at saturated light conditions (Bader et al. 2010). Assuming that leaves are C autonomous already at phenological stage 4 would mean considerably lower C demands of <8.0 and 7.5 kg in *Fagus* and *Quercus*, respectively, instead of 10.5 and 9.6 kg. On the other hand, with a lack of available data, our estimates precluded any growth-related respiration (the respiration cost of tissue formation), which could amount to up to 0.26 g C per any gram C invested directly in leaf biomass (Merino et al. 1984, Barbaroux et al. 2003), in turn increasing the leaf C demand by a ratio to match. In addition, we did not account for C investment in shoot elongation in our study trees, occurring at least partly simultaneously with leaf growth. The observed discrepancies between the functions of C loss (starch degradation) and gain (leaf growth) in our analysis (Figure 2), though not large, might be explained by this growth-related respiration and by the timing of leaf C autonomy. Finally, an additional C source for bud break and leaf development can come from woody tissue photosynthesis (Aschan et al. 2001, Aschan and Pfanz 2003, Vandegehuchte et al. 2015), which might be possible in the three studied species, as they have green growing shoots.

Regarding our second research question Q2, we found that stem growth onset was synchronized with C supply from fresh assimilates, taking place after leaves were unfolded and photosynthesizing (Figure 1, Table 2). Foliage and stem growth were decoupled from each other, with an intermission (in *Carpinus*) or without one (in *Fagus* and *Quercus*). The ~25 days earlier leaf phenology in *Carpinus* than in *Fagus* and *Quercus* in 2012 and 2014 (with up to 3 days estimation error; Table 2) is in agreement with previous observations in these trees (Asshoff et al. 2006). This is further evidence that the smaller interspecific differences observed in 2013 (Table 2; Vitasse 2013, Vitasse and Basler 2014) were an exception rather than the norm. As for our third research question Q3, a time lag between growth and C storage did not generally occur, as growth and C storage were mostly simultaneous, indicating high overabundance of C under these benign conditions. This is also in line with the relatively high photosynthesis rates in the leaves of our study trees (Bader et al. 2010). Among the three species, *Quercus* has higher maximum photosynthesis rates (16 μmol m^{-2} s^{-1} throughout summer) than the other two (10–11 and 8–9 μmol m^{-2} s^{-1} in July and September, respectively). Here, *Quercus* had higher starch levels during most of the growing season. The recovery of C reserves was not suspended until C investment into growing tissues was completed. Instead, C storage occurred together with growth pulses in different compartments. Notably, NSC dynamics were different between stem and branchlets (Figure 1), meaning that NSC levels in one compartment cannot be generalized to other compartments, let alone to the whole-tree scale. Three-compartment C allocation models (foliage, stem and roots, as in Klein and Hoch 2015) hence need to be extended. We also note that the end-season starch level was still higher than that at the season onset, reflecting interannual differences and/or winter NSC dynamics. For example, in mature conifers (*P. abies* and *L. decidua*) coexisting in an Alpine valley, tissue starch disappears in winter and is probably transformed into freeze-resistant NSC (Simard et al. 2013).

Divergence in species growth strategies

The three tree species studied here belong to two different families of the order Fagales, namely Fagaceae (*Quercus* and *Fagus*) and Betulaceae (*Carpinus*), the latter species clearly diverging from the other two in the studied responses. We still do not fully understand why *Carpinus* stem growth onset is substantially delayed, but it potentially relates to the third (May–June), larger decline in branchlet starch, from 6 to 2% d.m. (Figure 1). This starch degradation could not have occurred earlier, since the starch pool had to recover first after the flower and leaf production (the first virtually emptied it). Therefore, the stem growth delay is interpreted as a physiological cost of the early and abundant flowering and flushing in this species, which produces inflorescences before leaf unfolding (Schädel et al. 2010). This interpretation is in agreement with the observation that understory trees leaf-out earlier due to ontogenic changes, unrelated to microtemperature differences (a vertical thermal profile; Vitasse 2013). Our *Carpinus* trees were clearly shorter (~20–25 m) than their neighbors (*Quercus* and *Fagus* of ~30–40 m, and other coexisting species such as *P. abies* and *L. decidua* of 35–40 m) and as understory vegetation can certainly benefit from an early growing season onset, before being out-shaded by neighbors. It is also possible that shading impedes a direct NSC provision from leaves to growing woody tissues, thus selecting for starch accumulation in the canopy of *Carpinus*. On the other hand, *Carpinus* might be unique in that branchlet starch is not the only C reserve utilized during bud break. In a previous study of these specific trees, Schädel et al. (2010) showed that branch sapwood cell-wall hemicelluloses (the second most abundant polysaccharides in plants) were degraded during bud break in *Carpinus* but not in *Fagus*. But starch is still the major C reserve in *Carpinus* branchlets, and hence, the combination of relatively low NSC levels, early leaf-out and flowering could explain the delayed stem growth.

Despite their similarities in leaf and stem growth phenologies, *Fagus* and *Quercus* were different in their C management and might employ contrasting strategies. Carbon use was very much growth-oriented in *Fagus*, whereas *Quercus* grew little while maintaining a large stem starch pool (Figure 1, Table 4). Are these two species at the opposite sides of a safety–efficiency C

use continuum? These differences might relate to wood anatomy differences between the diffuse-porous *Fagus* and the ring-porous *Quercus*, the latter being at higher hydraulic risk (Klein 2014) and hence needing to maintain larger C reserves (e.g., for recovery of hydraulic conductivity), at the expense of C investment into new tissue growth. It has been previously noted that *Q. petraea* use more C reserves than *F. sylvatica* (Barbaroux et al. 2003) due to higher rates of respiration in winter and earlier stem growth onset, related to the formation of earlywood vessels (Barbaroux and Bréda 2002, Pérez-de-Lis et al. 2016). The latter cause is not valid for our study, since we did not observe earlier stem growth onset in *Quercus* than in *Fagus* in 2014, nor in previous years (T. Klein, unpublished data).

Conclusions

This study highlights the tight coordination between growth, phenology and C storage in deciduous tree species in a temperate forest. Specifically, we conclude that:

(i) New foliage growth is a major C sink in deciduous tree species, requiring 5–17 times the C needed for the annual stem growth.
(ii) Breakdown of branchlet starch is the main C source for bud break and leaf development, with >95% starch consumption, and accounting for ~90% of the C investment in foliage.
(iii) Carbon reserves recover 2–6 weeks after leaf expansion, in parallel with stem growth.
(iv) The late stem growth in *Carpinus* is probably the result of a competitive strategy to flush early, while having lower NSC levels than coexisting, taller, *Fagus* and *Quercus*.
(v) The ability to grow and store C simultaneously and the large amount of starch stored in the stem, which would be theoretically sufficient for stem growth for 7–30 years, indicate that healthy, mature deciduous trees are not C limited at present.

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Conflict of interest

None declared.

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