

Non-structural carbon compounds in temperate forest trees

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ABSTRACT

The current carbon supply status of temperate forest trees was assessed by analysing the seasonal variation of non-structural carbohydrate (NSC) concentrations in leaves, branch wood and stem sapwood of 10 tree species (six deciduous broad-leaved, one deciduous conifer and three evergreen conifer trees) in a temperate forest that is approximately 100 years old. In addition, all woody tissue was analysed for lipids (acylglycerols). The major NSC fractions were starch, sucrose, glucose and fructose, with other carbohydrates (e.g. raffinose and stachyose) and sugar alcohols (cyclitols and sorbitol) playing only a minor quantitative role. The radial distribution of NSC within entire stem cores, assessed here for the first time in a direct interspecific comparison, revealed large differences in the size of the active sapwood fraction among the species, reflecting the specific wood anatomy (ring-porous versus diffuse-porous xylem). The mean minimum NSC concentrations in branch wood during the growing season was 55% of maximum, and even high NSC concentrations were maintained during times of extensive fruit production in masting *Fagus sylvestris*. The NSC in stem sapwood varied very little throughout the season (cross species mean never below 67% of maximum), and the small reductions observed were not significant for any of the investigated species. Although some species contained substantial quantities of lipids in woody tissues ('fat trees'; *Tilia*, *Pinus*, *Picea*, *Larix*), the lipid pools did not vary significantly across the growing season in any species. On average, the carbon stores of deciduous trees would permit to replace the whole leaf canopy four times. These data imply that there is not a lot of leeway for a further stimulation of growth by ongoing atmospheric CO₂ enrichment. The classical view that deciduous trees rely more on C-reserves than evergreen trees, seems unwarranted or has lost its justification due to the greater than 30% increase in atmospheric CO₂ concentrations over the last 150 years.

Key-words: carbohydrates; C-balance; deciduous; evergreen; lipids; starch; sugars.

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INTRODUCTION

We are entering a carbon-rich world. Whether or not tree growth will respond to the continuing atmospheric CO₂-enrichment depends on the current carbon supply status of trees. In contrast to mineral nutrients, it is largely unknown whether carbon is still a limiting resource for tree growth outside plantation forests at current ambient CO₂ concentrations. A rapid and straightforward approach to assess the carbon supply status of a plant is to measure the size of its overall non-structural carbon pool across periods of varying demand (Körner 2003). Non-structural carbon compounds either accumulate or decrease depending on the carbon source–sink balance (photosynthesis versus respiration and growth; Mooney 1972; Chapin, Schulze & Mooney 1990). In temperate climates, non-structural carbon compounds may become depleted during the dormant season, when photosynthetic tissues are either shed (deciduous species) or inactive (evergreen tree species). Another period of potential C-shortage is the phase of maximum growth in spring and early summer, when carbon demand may exceed carbon supply by photosynthesis.

Non-structural carbon compounds (mainly free carbohydrates and starch) in temperate forest trees have been analysed in several species during the last 100 years or so (for some early works see, e.g. Leclerc du Sablon 1904; Sinnott 1918; Gäumann 1927, 1935; Wight 1933). However, these studies largely focused on a single species and often used very young individuals (≤ 5 years, e.g. Bonicel, Haddad & Gagnaire 1987; Gansert & Sprick 1998; Bollmark, Sennerby-Forsse & Ericsson 1999; Schaberg *et al.* 2000). When mature trees were studied, the analysis was commonly restricted to either branch or stem wood, but not both, although the non-structural carbon dynamics in the branch wood of trees do not necessarily correspond to those in the stem (Newell, Mulkey & Wright 2002). Another problem occurs with the incomplete sampling of stem sapwood. For example, most studies restricted sampling to the youngest tree rings (e.g. Höll 1985, first 5–7 rings in *Picea abies*; Fischer & Höll 1992, first 10 rings; and Terziev, Boutelje & Larsson 1997, first 10 mm from phloem in *Pinus sylvestris*), whereas the concentrations over the entire active sapwood compartment are hardly ever reported, although species differ with respect to their sapwood/heartwood ratio (Magel *et al.* 1997). Thus, our present knowledge of the non-structural carbon dynamics of trees in temperate climates is in fact rather patchy and not as clear and conclu-

sive as has often been assumed (e.g. Kramer & Kozłowski 1979).

Here we present a comparative study of the dynamics of non-structural carbon compounds in leaves and branches as well as stem sapwood in mature trees of 10 different species, growing under the same climatic conditions. The inclusion of conifers allows us to test whether non-structural carbon compounds show a more pronounced seasonal variation in deciduous than in evergreen trees, as was suggested by Kramer & Kozłowski (1979) and Piispanen & Saranpää (2001), because deciduous trees, unlike evergreens, are assumed to store high amounts of carbon reserves over winter in order to support the leaf flush and initial tree ring formation (ring-porous trees) in spring.

The non-structural carbon pool of plants comprises several compound classes (Chapin *et al.* 1990). Quantitatively, the most important carbon compounds for storage commonly are non-structural carbohydrates (NSC). In all trees, low molecular weight sugars (glucose, fructose, sucrose, etc.) and starch are abundant. In many tree families oligosaccharides of the raffinose series and/or fructans can also be important storage compounds (Fischer & Höll 1991, 1992; Eissenstat & Duncan 1992). Some tree genera (e.g. *Pinus*, *Tilia*) are additionally able to accumulate considerable amounts of neutral lipids in their woody tissue, with concentrations even exceeding those in NSC (Hoch, Popp & Körner 2002). Sinnott (1918) classified these genera as 'fat-trees', to separate them from those, in which NSC serves as the main carbon storage form ('starch-trees'). Hemicellulose, which can make up more than 35% dry matter (DM) in the secondary xylem of some hardwood species (Garrote, Dominguez & Parajo 1999), is also likely to contribute to the carbon reserve pool (Brinson & Dey 1985), but their role as carbon storage compounds has not yet been demonstrated clearly (Chapin *et al.* 1990). Given their ubiquitous occurrence and quantitative significance, we primarily analysed the seasonal variations of glucose, fructose, sucrose and starch (NSC) in leaves and above-ground woody tissues, but we also quantified the contribution of other low molecular weight sugars and sugar alcohols. All woody tree organs were also examined for lipids (acylglycerols).

MATERIAL AND METHODS

Study site

Trees were sampled in a mature, mixed forest stand (approximately 100 years old and mean canopy height approximately 30 m) using the Swiss Canopy Crane (SCC; Pepin & Körner 2002) near the village of Hofstetten (47°33' N, 7°36' E, 500 m a.s.l.), located 12 km south-west of Basel, Switzerland. The forest has a comparatively high growth rate, a basal area of approximately 40 m² ha⁻¹, and is well supplied with mineral nutrients based on standard forestry criteria (S. Braun, personal comm.). It stocks on shallow soils of the rendzina type on Jurassic calcareous bedrock, with ground cover dominated by herbaceous plants (e.g.

Helleborus foetidus, *Mercurialis perennis* and *Paris quatri-fovia*) and understorey shrubs (e.g. *Daphne mezereum* and *Lonicera xylosteum*). The climate is oceanic with an annual precipitation of around 990 mm and usually only a few weeks of slight snow cover during mid-winter. The mean air-temperature over the approximately 6 month growing season from April to October is 15.4 °C, with a mean temperature for the warmest month (August) of 19.2 °C (1989–2000 11 years mean, weather station Metzleren, C. Trefzger and J. Paulsen, personal comm.). The winters are mild (dormant season mean: 4.5 °C) with the mean temperature of the coldest month (January) being around 2.1 °C. In all tree species leaf-bud break occurs within 4 weeks between mid-April and mid-May. In deciduous trees leaf-yellowing starts by the end of September, but some leaves can be photosynthetically active until end of October.

Tree species and tissue sampling

Ten different tree species (Table 1) were sampled at the following dates: 22–24 June 1999, 17–19 August 1999, 1–2 October 1999, 13–14 April 2000 and 1 March 2002. In the 1999 growing season, when sampling took place from June to October, *Fagus* had a masting year with massive fruit production. At each sampling date (except for March 2002, where only branch wood and evergreen conifer needles were taken) the following tissues were sampled between 1000 and 1500 h: (1) leaves (or buds on 13 April 2000) from deciduous trees or 1 year old needles in the case of evergreen trees; (2) xylem of branches with bark removed and diameters between 0.5 and 1 cm; and (3) stem xylem cored to the centre at breast height (using a 0.5-cm stem corer; Suunto, Vantaa, Finland). Leaves (needles) and branches were sampled from a crane gondola at the sunlit part of the individual crowns, and the replication for the tree species varied from three to six trees, depending on the availability of individuals reaching the top of the forest canopy (Table 1). Two to four stem cores per species were taken from similar-sized trees just outside the reach of the crane, not to risk any damage to the experimental trees in the crane area. For separate NSC analyses, the fresh cores were immediately fractionated into 2 cm sections, starting from the cambium. Immediately after collection all samples were heated in a microwave oven at 600 W for 90 s in a field laboratory at the crane site to denature enzymes (Popp *et al.* 1996). After returning to the laboratory in Basel, the material was dried to weight-constancy at 75 °C, ground to fine powder and stored at 4 °C, well sealed over silica gel until analysis.

Chemical analyses

Gas-chromatographic analyses

To estimate the contribution of all low molecular weight carbohydrates (and sugar alcohols) others than those covered by the NSC method (see below), we scanned branch wood material sampled in March and June, as well as leaves

Table 1. Sampled species, replications (n) per sampling date, mean stem radius, SLA^a of leaves at the upper part of the crowns and values of sapwood density^b

Species	n for leaves and branches	n for stem wood	Mean stem diameter (cm)	SLA ± SE (cm ² g ⁻¹)	Sapwood density (g cm ⁻³)
Deciduous broad-leaved					
<i>Acer campestre</i>	4	2	32	96.3 ± 2.9	0.63
<i>Carpinus betulus</i>	5	2	22	119 ± 2.1	0.71
<i>Fagus sylvatica</i>	6	3	32	97.7 ± 2.9	0.67
<i>Prunus avium</i>	3	2	20	101 ± 2.7	0.57
<i>Quercus petraea</i>	4	2	34	86.8 ± 1.3	0.67
<i>Tilia platyphyllos</i>	3	2	30	144 ± 9.1	0.49
Deciduous conifer					
<i>Larix decidua</i>	5	3	40	98.1 ± 2.0	0.54
Evergreen conifers					
<i>Abies alba</i>	2	2	32	37 ± 5	0.43
<i>Picea abies</i>	4	2	30	26.1 ± 1.7	0.47
<i>Pinus sylvestris</i>	5	4	40	30 ± 2.7	0.52

^aSLA values are means of two measurements in June and in August 1999. ^bValues are means of two sapwood stem cores.

and stem wood (first 2 cm section) sampled in June for all free carbohydrates by gas chromatography. The samples were extracted in methanol : chloroform : water (12 : 5 : 3) at 60 °C for 30 min. After phase separation by addition of water and chloroform, aliquots of the aqueous phases were vacuum dried, silylized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethyl-chlorosilane (TMCS) (both Pierce Chemical Comp., Rockford, IL, USA) and analysed on a capillary column (HP1; 30 m × 0.20 mm i.d., 0.2 µm film thickness) on a HP 8690 gas chromatograph (Agilent, Palo Alto, CA, USA; detector: FID; analysis software: HP ChemStation). Phenyl-β-glucopyranosid was used as internal standard.

Non-structural carbohydrates

The NSC are defined here as free, low molecular weight sugars (glucose, fructose and sucrose) plus starch. They were analysed as described in Wong (1990) and Hoch *et al.* (2002). Ground plant material was extracted for 30 min in distilled water and starch and sucrose were broken down to glucose and glucose and fructose, respectively, with a dialysed crude enzyme ('Clarase' from *Aspergillus oryzae*, Enzyme Solutions Pty Ltd, Crydon South, Victoria, Australia) by incubation at 40 °C for 15 h. After treatment of the solution with phosphoglucose-isomerase, the total amount of glucose (corresponding to NSC) was determined photometrically in a microplate photometer (HR 7000; Hamilton, Reno, NE, USA) after conversion of glucose to gluconate-6-phosphate (hexokinase; Sigma Diagnostics, St. Louis, MO, USA). An aliquot of the original extract was treated with invertase and phosphoglucose-isomerase (both Sigma Diagnostics) to determine the amount of glucose, fructose and sucrose via the glucose test (see above). Starch was calculated as NSC minus free sugars. Pure starch and glucose, fructose and sucrose solutions were used as standards and standard plant powder (Orchard leaves; Leco, St.

Joseph, MI, USA) was included to control reproducibility of the extractions. The NSC concentrations are expressed on a percentage dry matter basis.

Lipids

In woody tissues lipid (acylglycerol) concentrations were determined using the method of Eggstein & Kuhlmann (1974) as described in detail in Hoch *et al.* (2002). Following a saponification of lipids by extracting ground wood powder in aqueous NaOH for 30 min, the amount of glycerol was determined following an enzymatic conversion of glycerol to glycerol-3-phosphate in a 96-well microplate reader. Pure glycerol was used as standard. All enzymes were purchased from Roche Diagnostics, Rotkreuz, Switzerland. The results were calculated as percentage dry matter.

Calculations and statistics

Variations in tissue density, due to species specific differences in cell wall composition (cellulose to lignin ratio) and cell wall thickness, may affect the ranking of species with respect to concentrations of non-structural carbon compounds. Therefore, we assessed wood densities in all species (Table 1) and calculated concentrations of non-structural carbon compounds also on a volume basis (mg cm⁻³). Although these calculations led to higher volume concentrations in species with denser tissue, they had no influence on statistics and the overall outcome of this study. Therefore we have refrained from presenting such volume-based data in addition to dry matter-based data, but the presented density data (Table 1) permit a conversion.

A *post hoc* analysis of the stem core fractions revealed that all species except *Larix* showed decreasing NSC concentrations from the outermost tree rings towards the pith, with no clear end. Generally, the species revealed remarkably high NSC concentrations even at the innermost zones

of the stem. Nevertheless, the sapwood zone showed a decrease of NSC in the direction to the pith, whereas there was virtually no change in NSC concentrations within the heartwood zone. Thus, the zone, which showed a clear radial decrease of NSC, was considered active sapwood. The NSC concentrations found in the various 2 cm stem sections of the sapwood as defined before were averaged.

The seasonal amplitude of NSC was expressed as relative variation ($\text{Diff \%} = \% \text{ variation from the seasonal maximum}$), and as Diff C, the absolute change of non-structural carbon in $\text{mg C g}^{-1} \text{ DM}$, assuming a C-fraction of 0.41 g C g^{-1} low molecular weight sugar (mean of glucose, fructose and sucrose) and 0.44 g C g^{-1} starch.

Within species, differences of NSC concentrations among the sampling dates were analysed for significance by Tukey–Kramer honestly significant difference (HSD) test. Significant differences in the seasonal dynamics of NSC between deciduous and evergreen trees were determined by repeated measures analysis of variance (ANOVA). For this purpose, three trees per species were used for leaves and branch wood and two trees per species for stem sapwood. Trees were the subjects and the sampling dates the repeated measures. For those species in which four, five or six tree canopies were sampled, the replication was reduced to three by randomly selecting pairs or triplets of trees, which were averaged before the analysis, so that the contribution of each species to the functional type (deciduous or evergreen) mean was evenly balanced. All concentrations were transformed with $\arcsin \times [\text{square root}(y)]$ prior to statistical analyses to meet the requirement of normal distribution and equal variance. JMP 3.2.2 (SAS Institute, Cary, NC, USA) was used for all statistical tests.

RESULTS

Gas-chromatographic screening of non-structural carbohydrates and sugar alcohols

The contribution of non-structural carbohydrates (including sugar alcohols) other than those covered by the photometric ‘NSC-method’ to the whole non-structural carbohydrate pool was generally low. The gas-chromatographic analysis of branch wood sampled in March 2002, revealed that ‘NSC-carbohydrates’ (starch, sucrose, glucose and fructose) made up at least 80% of all non-structural carbohydrates in all species (in most of the cases more than 85%, Fig. 1). In addition to ‘NSC-carbohydrates’ only raffinose and stachyose were detectable in all 10 species. Sugar alcohols were present in all samples and characteristic for the different species (Fig. 1). Within the hexitols only sorbitol in *Prunus* branch wood exhibited quantitatively significant concentrations ($4.2 \pm 0.5 \text{ mg g}^{-1} \text{ DM}$, i.e. 4% of all non-structural carbohydrates). With the exception of *Fagus* and *Prunus*, cyclitols other than the ubiquitous myo-inositol, could be detected in all species in various concentrations (Fig. 1). The most important cyclitols were pinitol ($2.4 \pm 0.1 \text{ mg g}^{-1} \text{ DM}$ in *Abies*, 2.8 ± 0.1 in *Larix*, 3.0 ± 0.1 in *Picea* and 10.4 ± 0.1 in *Pinus*), quercitol ($5.2 \pm < 0.1 \text{ mg}$

$\text{g}^{-1} \text{ DM}$ in *Quercus* and $4.0 \pm < 0.1$ in *Tilia*) and quebrachitol ($9.4 \pm 0.1 \text{ mg g}^{-1} \text{ DM}$ in *Acer* and $1.3 \pm < 0.1$ in *Carpinus*). A previous gas chromatographic analysis in leaves, branch sapwood and stem sapwood sampled in June 1999 (with only one replicate per species and tissue type, data not shown), indicated that the fractions of ‘NSC-carbohydrates’ within the whole non-structural carbohydrate pool in leaves and stem sapwood was similar to that in branch sapwood sampled in March. Thus, the analysis of carbohydrates by the NSC-method should permit an adequate assessment of the overall carbohydrate supply status of all investigated tree species.

Seasonal dynamics of non-structural carbohydrates

Leaves

By mid-April, all species except *Tilia* were still in the prior to leaf-bud break stage (i.e. prior to the onset of the growing season defined by phenology, Fig. 2). From June to October, the concentrations and variations of NSC were very similar among all species, and the starch and free-sugar concentrations decreased continuously over this period from 15–20% to 7–10% DM in all 10 species, irrespective of leaf functional type (Fig. 2). Although the NSC concentrations in leaves were similar across species, the proportion between starch and sugars differed among the broad-leaved species. For example, *Carpinus*, *Quercus* and *Tilia* had higher free sugar than starch concentrations throughout the growing season. Generally, the seasonal decrease of sugar concentrations was more pronounced in deciduous than in evergreen species (Fig. 2). The NSC in last year needles of evergreen conifers increased prior to leaf-bud break in spring, presumably because of photosynthetic activity during March and early April, with maximum starch concentrations between 17 and 20% DM in mid-April, just before bud break (Fig. 2).

Branch sapwood

The seasonal variation as well as the concentrations of NSC in branch sapwood was not as uniform among the species as they were in leaves (Fig. 3). With the exception of *Tilia*, the starch concentrations in broad-leaved trees were always significantly higher than the concentrations of free sugars. In all deciduous trees, except *Quercus*, starch increased from March to October. The sugar concentrations in branches did not change significantly over the season, with only a slight decrease during leaf-bud break in most of the species. *Prunus* was the only species in which branch sapwood showed a significant decrease of starch in April (just prior to leaf-bud break), most likely associated with enhanced carbon demand for massive flowering during the first half of April. In all other species, flowering started after or approximately at the time of leafing and was not reflected by decreasing NSC concentrations. *Fagus* was the only deciduous tree species showing a significant decrease

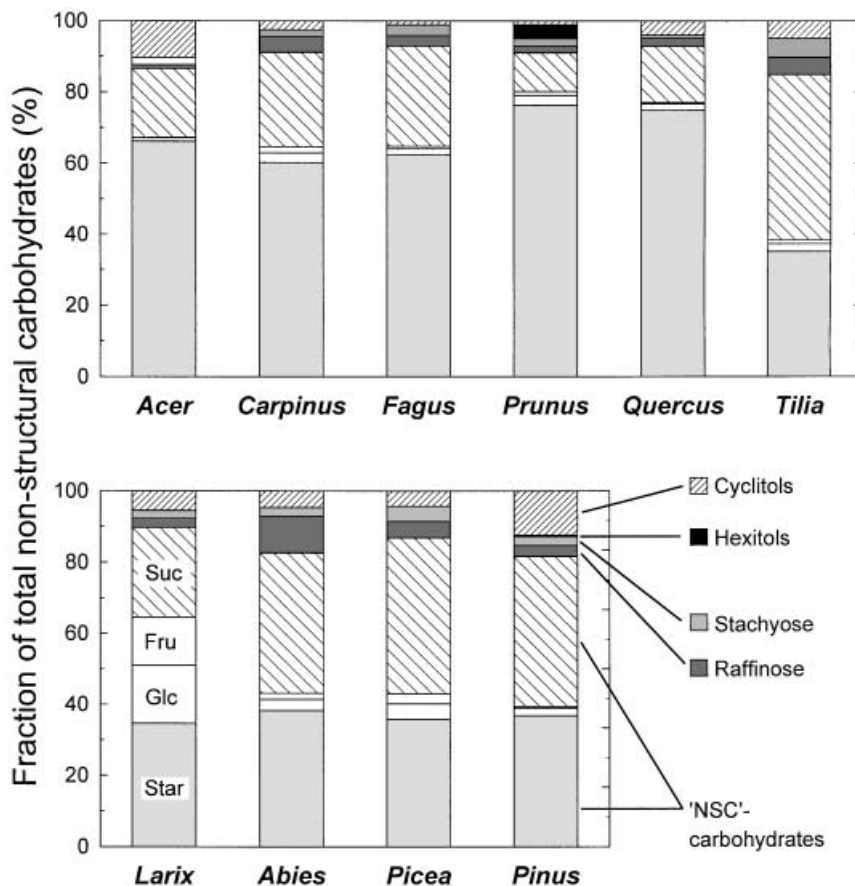


Figure 1. Fractions of single non-structural carbohydrates (NSC) in branch sapwood of 10 mature tree species within the whole non-structural carbohydrate pools. Samples were collected on 1 March 2002, $n = 3$ per tree species. 'NSC-carbohydrates': Star (starch), Glc (glucose), Fru (fructose) and Suc (sucrose). All sugar alcohols are cyclitols, except for *Prunus* where the major compound is sorbitol. The quantitatively most important cyclitols are quebrachitol in *Acer* and *Carpinus*, quercitol in *Quercus* and *Tilia*, pinitol in all four conifers.

of NSC concentrations in branch sapwood from June to August (Fig. 3). This reduction of NSC was most likely due to the fact that *Fagus* had a masting year in 1999. Nevertheless, from August to October, the NSC concentrations in *Fagus* branch wood increased again to a similar level as in June. The three evergreen species studied exhibited branch wood NSC-dynamics that were different from the deciduous trees. In *Pinus* and *Abies* starch increased prior to leaf-bud break and then decreased towards the end of the season, whereas in *Picea* the maximum starch concentration was reached in June (Fig. 3).

Among the branch wood samples taken from all 10 species, detectable quantities ($> 0.5\%$ DM) of lipids were only found in *Tilia*, *Larix*, *Picea* and *Pinus*, but the concentrations were generally low and did not change significantly during the growing season (Fig. 3). Therefore, the inclusion of lipids did not change the seasonal dynamics as described for non-structural carbohydrates alone (Fig. 3).

Stem sapwood

The distribution of NSC from the cambium towards the pith differed strongly from species to species (Fig. 4). NSC

was detectable even at the innermost core-sections (approximately 80–100 years old) in all trees. Nevertheless, there were big species-specific differences in the radial decrease of NSC as well as in its seasonal amplitude (maximum versus minimum concentrations). *Quercus*, as the only ring-porous tree sampled, showed the strongest decrease of NSC and almost no seasonal variation in the wood cores deeper than 6 cm towards the pith, while there was almost no centripetal reduction of NSC concentrations in the diffuse-porous *Carpinus* and *Tilia* and thus the whole stem wood was considered sapwood in these species (Fig. 4). Most interestingly, *Prunus*, which has a semiring-porous wood anatomy, exhibited a radial stem NSC gradient somewhere between *Quercus* and *Carpinus*. The unorthodox NSC distribution in *Larix*, with maximum concentrations between 4 and 6 cm depth (Fig. 4), was due to very high and seasonal stable concentrations of free sugars in this stem area, whereas starch decreased constantly with the age of the wood section.

In contrast to branches, the seasonal differences of NSC in stems were not significant for any of the investigated species (Fig. 5). Nevertheless, some species (*Acer*, *Prunus*, *Pinus*, *Quercus*) tended to exhibit the lowest NSC concen-

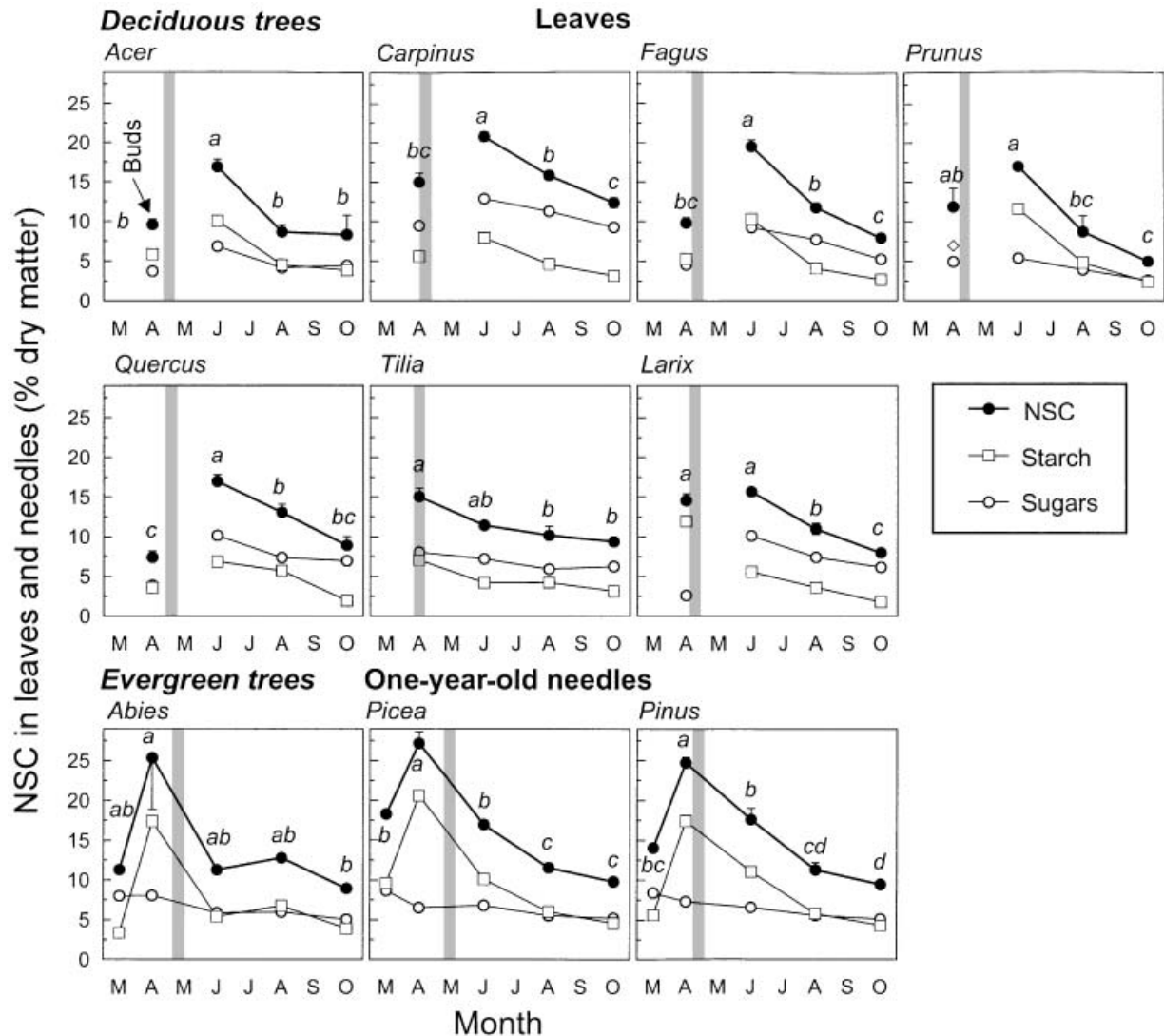


Figure 2. Seasonal variations of low molecular weight carbohydrates (glucose, fructose and sucrose), starch and NSC (sugars + starch) concentrations in leaves or 1-year-old needles of 10 mature tree species. Values are means of two to six trees per species. For clarity, standard errors are indicated for NSC only. The average period of bud break is indicated by a grey area for each species. Notice that all symbols left from grey bars are for buds in all deciduous species. Different letters indicate significantly different NSC concentrations among sampling dates at $P < 0.05$ by Tukey-Kramer HSD test.

trations during mid-season (June) when stem growth was strongest, whereas others (*Carpinus*, *Fagus*, *Abies*, *Picea*) had slightly higher NSC concentrations during the very same period (Fig. 5). With the exception of *Larix* and *Pinus*, the concentrations of starch were significantly higher than the concentrations of free sugars, and the seasonal course of NSC generally followed the dynamics of starch. Free-sugar concentrations in stem sapwood were lowest during summer in most species, and tended to increase towards winter especially in broad-leaved trees.

As for the branch sapwood, lipids were detected in higher concentrations only in *Tilia*, *Larix*, *Picea* and *Pinus*. In contrast to the branch data, the lipid concentrations in the stem sapwood were almost as high as the NSC concentrations in *Tilia* and *Pinus* (Fig. 5), but again, the seasonal

change in lipid concentrations was negligible in all four species.

Deciduous versus evergreen trees

For the comparison between deciduous and evergreen trees, the NSC concentrations at each sampling date were averaged for each of the two functional types (leaf-phenotypes). The rapid development of deciduous leaves during spring is accompanied by an increasing toughness of the tissue and decreasing specific leaf area (SLA), changing the dry matter reference over time. This makes comparisons of compound concentrations between dates and species more complex during this period, and we therefore restricted the comparison of leaves to the sampling dates from June to October,

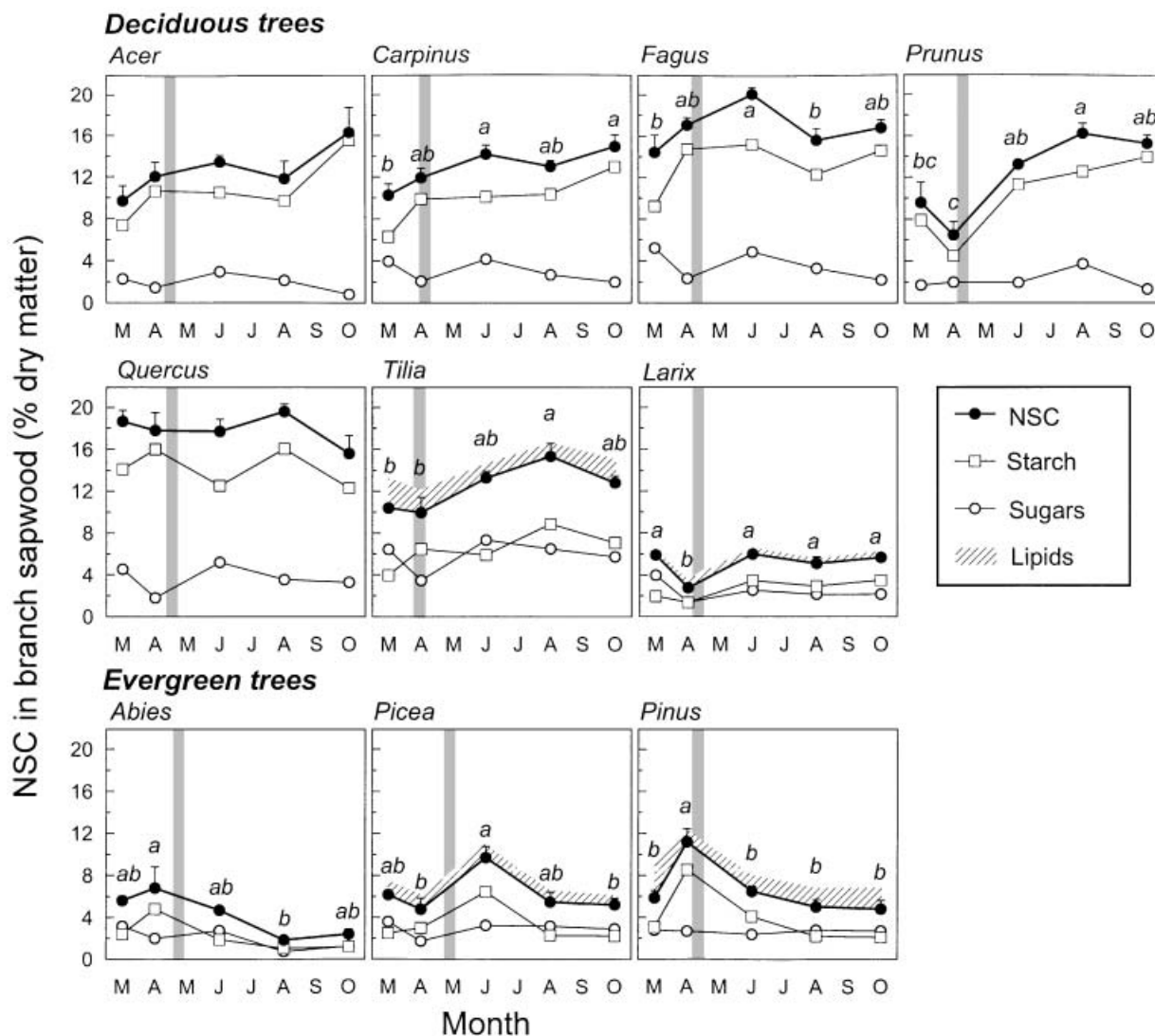


Figure 3. Seasonal variations of low molecular weight carbohydrates (glucose, fructose and sucrose), starch and NSC (sugars + starch) concentrations in branch sapwood of 10 mature tree species. For those species where lipids could be detected, the concentrations of lipids are indicated by the shaded area on top of the NSC curve. Values are means of two to six trees per species. For further details see Fig. 2.

when leaves had matured and SLA stayed almost constant over time (the decrease of SLA from June to August 1999 was not significant across species; <5%).

From mid-season towards the end of the growing season, both functional types showed a strong reduction of NSC in leaves, and the concentrations were very similar between both groups (Fig. 6). Thus, a repeated measures analysis detected no significant difference between functional types, but a highly significant change over time, irrespective of the functional type (Table 2). Due to the parallel decrease in both groups, the functional type \times time interaction was also not significant.

In branch sapwood, there was a highly significant difference between functional types, because of the lower NSC concentrations in evergreens (cross growing season mean NSC concentrations for deciduous trees = $12.8 \pm 0.6\%$ DM

and for evergreen trees = $5.8 \pm 0.6\%$ DM, Fig. 6). The variation of NSC in branch sapwood over time irrespective of functional type was also significant, as well as the interaction between functional type and time. The latter was due to the opposite direction of the seasonal NSC trends (Fig. 6, Table 2). On average, the branch wood of deciduous trees exhibited a slight increase of NSC concentrations over the course of the growing season from April to October (and consequently a slight decrease over winter), whereas in conifers NSC in branches increased from March to April and then decreased towards October (with no change of concentration over winter, Fig. 6).

Due to the weak seasonal change of NSC in the stem sapwood of deciduous and evergreen trees (Fig. 6), there was no significant time, or functional type \times time effect in the repeated measures analysis (Table 2). However, the

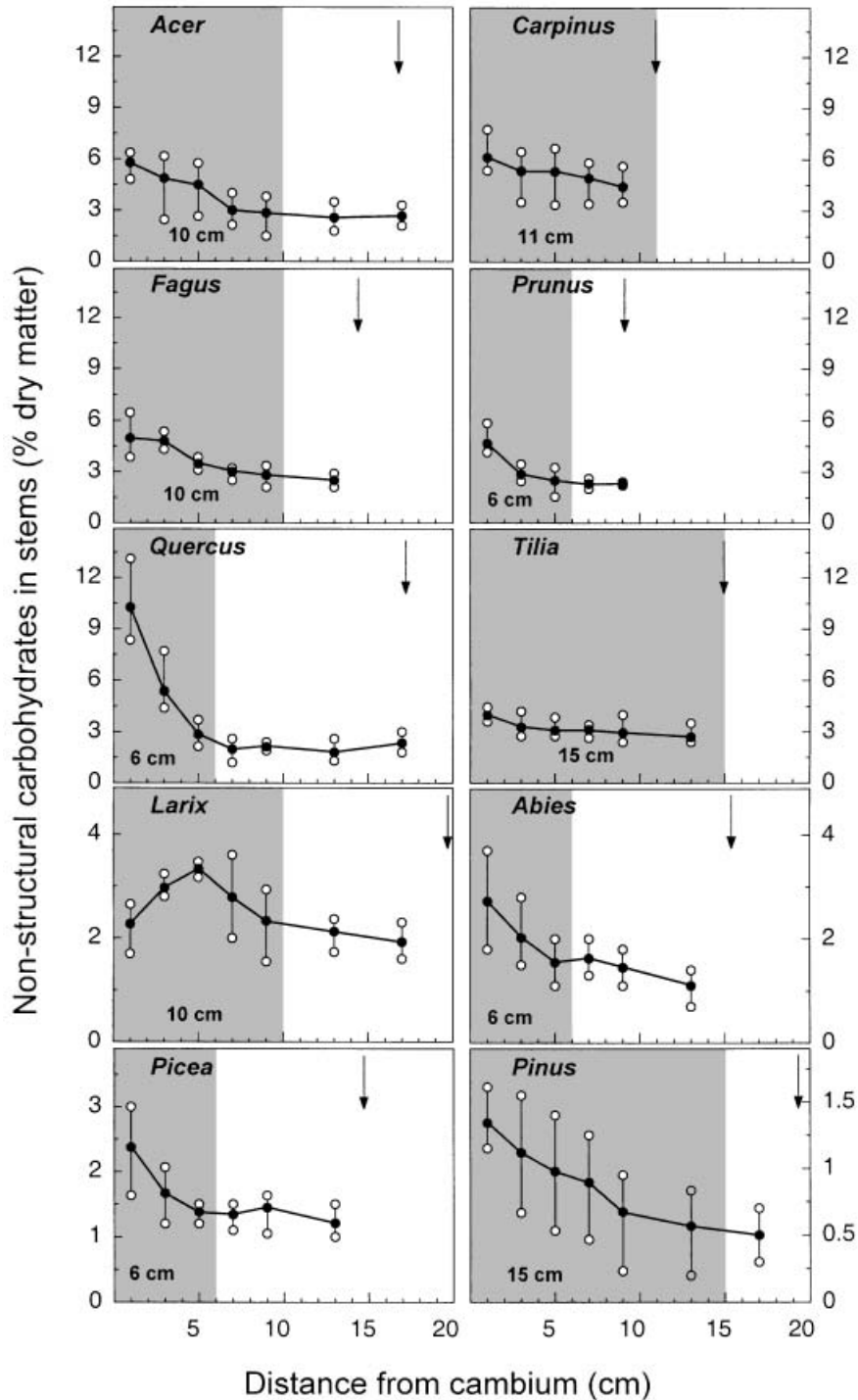


Figure 4. Radial distribution of NSC (free sugars + starch) in stem cores of 10 tree species. The black circles are cross-seasonal means (four values per stem section) and the white circles are the maximum and minimum values during the growing season. The grey area indicates the estimated sapwood fraction, and the average position of the pith is marked by an arrow for each species. Note the different scale of the y-axis for conifers.

seasonal mean concentrations of NSC were 2.6 times higher in deciduous stem sapwood than in evergreen sapwood (deciduous trees = $4.7 \pm 0.1\%$ DM and for evergreen trees = $1.8 \pm 0.1\%$ DM), which translated into a significant difference between the two functional types (Table 2). Since

some of the deciduous species have higher wood densities than the evergreen conifers (Table 1) this difference gets even more pronounced if the NSC concentrations are expressed on a volume basis (mg cm^{-3}). If any, the seasonal differences in NSC concentrations between the two func-

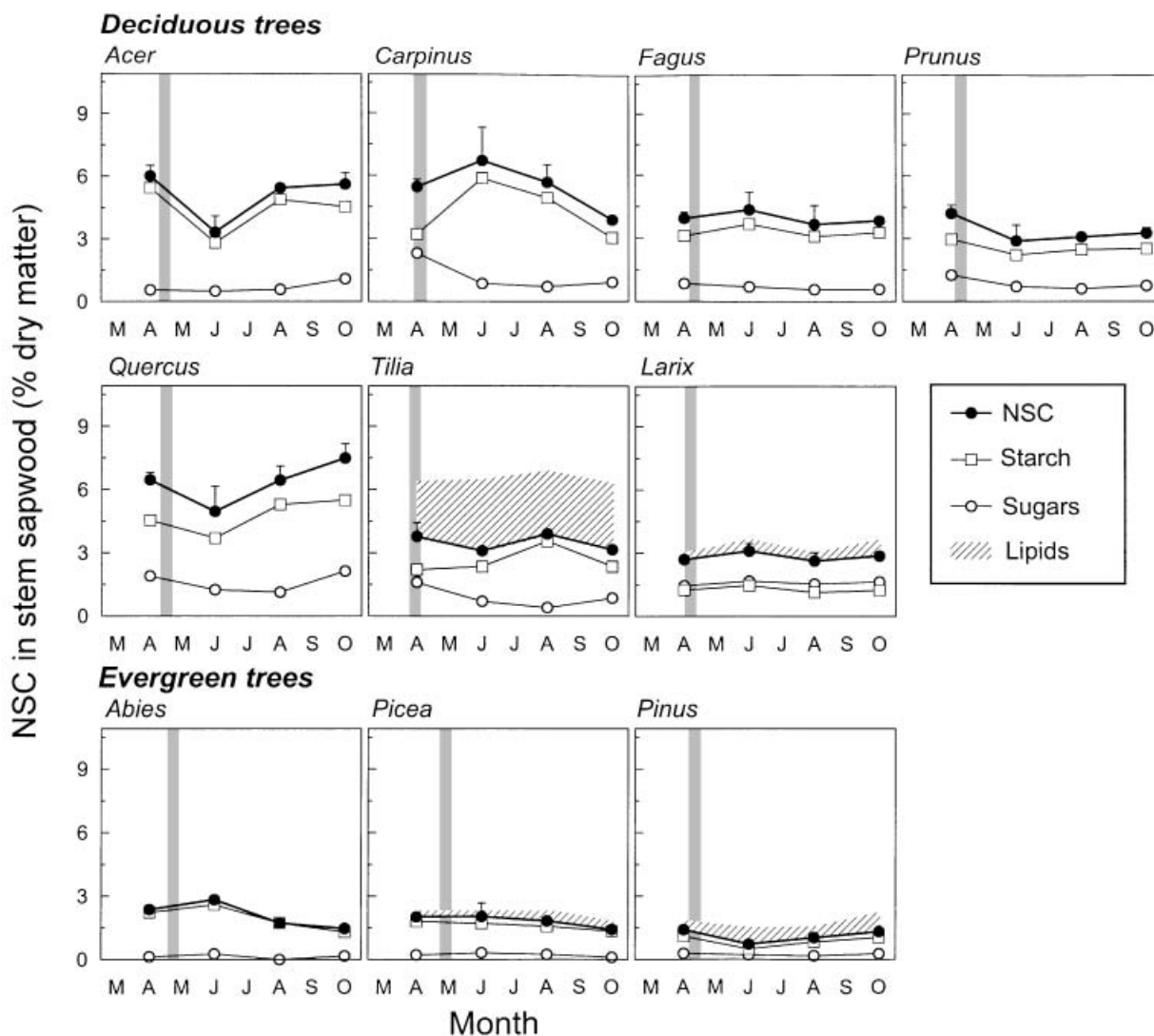


Figure 5. Seasonal variations of low molecular weight carbohydrates (glucose, fructose and sucrose), starch and NSC (sugars + starch) concentrations in stem sapwood of 10 mature tree species. For those species where lipids could be detected, the concentrations of lipids are indicated by the shaded area on top of the NSC curve. Values are means of two to four trees per species. For further details see Fig. 2.

Table 2. Repeated measures analyses for effects of functional type (deciduous versus evergreen) and time of year on the NSC concentrations

	Leaves ^a		Branch sapwood		Stem sapwood	
	F	P	F	P	F	P
Between subjects						
Functional type	0.2	0.637	26.9	<0.001	18.9	<0.001
Within subjects						
Time	39.5	<0.001	4.5	0.007	2.9	0.067
Funct. type × Time	2.2	0.126	6.3	0.001	0.7	0.592

^aValues between June and October only, i.e. after leaves had matured. *P*-values smaller than 0.05 are in bold.

tional types were due to the different starch dynamics. Thus, the sugar concentrations of the two groups correlated very well between the same tissues and dates, whereas the starch concentrations of the same samples differed substantially between deciduous and evergreen trees (Fig. 7).

Amplitude of seasonal NSC variations

Since the seasonal amplitude of NSC is the key towards identifying periodic source–sink disparities, we calculated the relative variation of NSC pools (Diff %) and the difference between highest and lowest concentrations (Diff C in mg C g⁻¹ DM), for all 10 species and separately for deciduous and evergreen trees. NSC concentrations of leaves were comparable among the different species in the second half of the growing season only (see above), thus, we restricted the comparison to woody tissues (branches and stems).

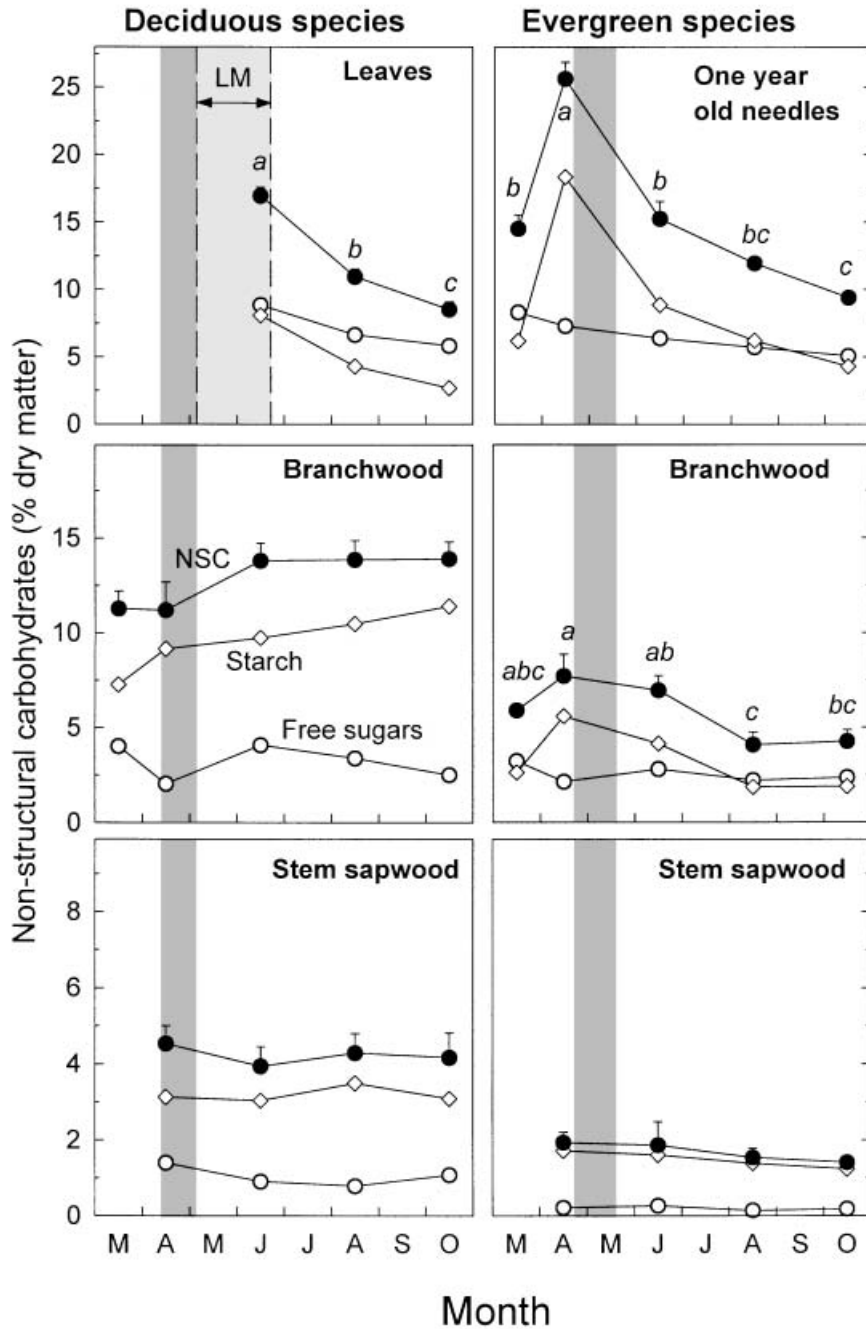


Figure 6. Average seasonal variations of non-structural carbohydrate concentrations (in percentage dry matter) in leaves, branch sapwood and stem sapwood of deciduous and evergreen tree species. Leaves and branch wood: $n = 21$ for deciduous and $n = 9$ for evergreen trees; stem sapwood: $n = 14$ for deciduous and $n = 6$ for evergreen trees. For clarity, standard errors are indicated for NSC only. The average period of bud break is indicated by the dark grey area for each species, the approximate duration of leaf-maturation (LM) of deciduous leaves is indicated by the light grey area. Different letters indicate significantly different NSC concentrations among sampling dates at $P < 0.05$ by Tukey–Kramer HSD test.

The relative variation of NSC was very different among the species for branch sapwood (20% in *Quercus* to 73% in *Abies*) and for the stem sapwood (15% in *Larix* to 50% in *Pinus*, Fig. 8). On average, the evergreen conifers showed higher relative variations in sapwood NSC than deciduous trees, particularly in branch sapwood (evergreens $60 \pm 5\%$, deciduous $38 \pm 6\%$, Fig. 8). However, since

the absolute NSC concentrations (% DM) varied greatly between the species (Figs 4 & 5), those species with the highest Diff % were not implicitly those with the highest Diff C. If, for example, one account for the lower NSC concentrations in conifer branch wood, the difference between the Diff % of non-structural carbohydrate compounds in evergreen and deciduous trees disappeared when

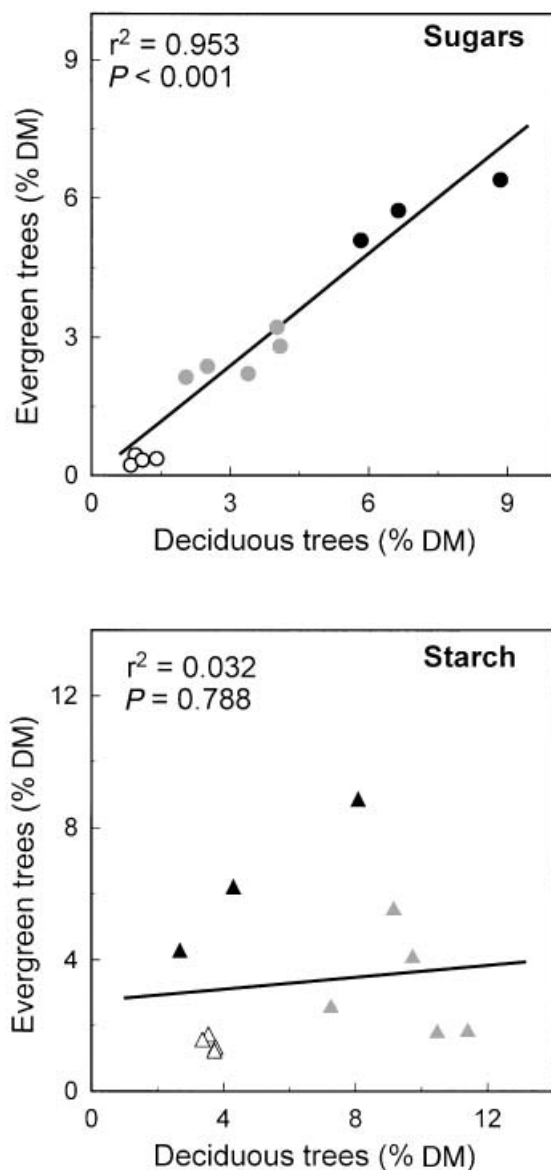


Figure 7. Paired correlation of non-structural carbohydrate concentrations (% dry matter) between deciduous and evergreen tree tissues (leaves, branch sapwood and stem sapwood). Each point represents one tissue and date. Open symbols represent stems, grey symbols branches and black symbols leaves. For leaves only measurements from June to October were used.

expressed as Diff C (Fig. 8). Diff C-values were two to more than three times higher in branch sapwood than in stem sapwood for both functional types (Fig. 8).

DISCUSSION

To our knowledge the current study is the first attempt to describe the seasonal dynamics of non-structural carbon compounds simultaneously in a representative sample of temperate forest tree species. Given the same soil and climatic conditions, the comparison reflects true species and

functional type effects. Furthermore, the trees (except for *Larix*) were not planted, hence their presence reflects natural recruitment and selection. We may, thus, expect representative tree responses.

Seasonal dynamics of NSC

The increasing NSC concentrations in branch sapwood of all deciduous species during the time of leaf-bud break and strongest shoot growth, is in contrast to the widespread assumption that in deciduous trees the high carbon demand for leafing and growth during the early growing season is facilitated mainly by old C-reserves (e.g. Kramer & Kozłowski 1979). Although some of the tree's potential carbon storage pools (e.g. roots) were not accounted for in this study, a strong dependency of bud break on stored carbon reserves should, by any means, be indicated by decreasing NSC pools in branches, which are closest to the carbon sinks (buds). Zimmermann (1974) had already suggested that the problem 'is not a simple one', and that 'some shoot systems seem to draw primarily on current photosynthates'. Our data indeed suggest that newly formed leaves (and the growing shoots) of deciduous trees become autonomous from stored C reserves at a very early developmental stage, and thus do not drain heavily on stored carbohydrates. The only exception seems to be *Larix*, where NSC concentrations were significantly lower in April, due to low sugar concentrations. However, *Larix* is not native to such low elevations and mild winter climates. A constant increase of starch in branch wood of deciduous trees across the growing season was formerly also found in *Populus × canadensis* trees (Sauter & van Cleve 1994) and *Betula pubescens* (Mäenpää *et al.* 2001), but Bonicel *et al.* (1987) described a decreasing trend during the growing season in *Populus trichocarpa × Populus deltoides* branches. The seasonal dynamics of NSC in the branch wood of evergreen conifers, found in the current study, especially the early season starch increase, seem to be typical for evergreen trees, and underpin recent findings in sub-alpine *Pinus cembra* trees (Hoch *et al.* 2002) and in Mediterranean evergreen broad-leaved trees (Körner 2003).

In contrast to bud break and shoot growth, preleafing flowering (*Prunus*) or heavy fruiting (masting in *Fagus*) have been associated with a decrease of NSC concentrations in the branch sapwood of those species. Gäumann (1935) described a very strong reduction of non-structural carbon reserves in *Fagus sylvatica* during masting years (approximately -70%, i.e. more than twice as high as for non-masting individuals). Nevertheless, the NSC decrease in *Fagus* found in the current study, showed a very moderate reduction of the trees' NSC-pools (-22% NSC reduction in branch sapwood from June to August, Fig. 4), indicating that even the extensive production of fruit in one year, is only moderately dependent on stored carbon in this species.

Surprisingly, the NSC dynamics in stem sapwood did not correspond to that in branch sapwood in most of the trees

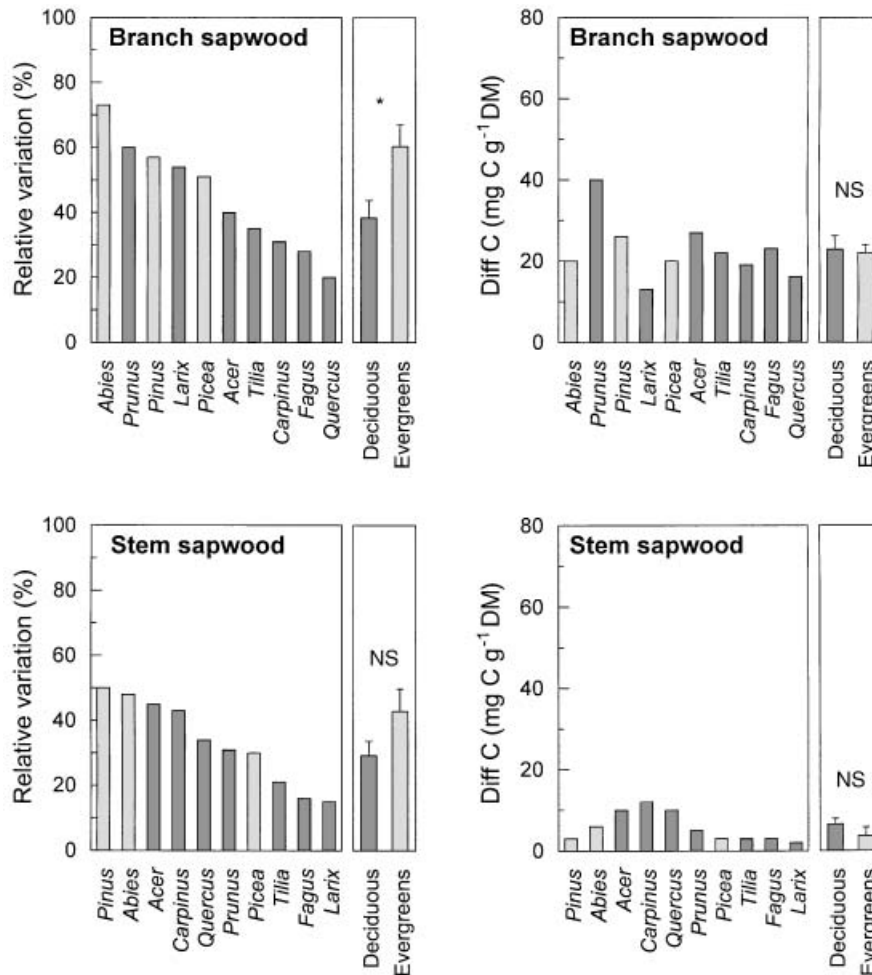


Figure 8. Relative variation of NSC (Diff %) and difference between minimum and maximum NSC concentrations (Diff C; mg C g⁻¹ dry matter) during the growing season in branch and stem sapwood of 10 tree species and the mean values for deciduous ($n = 7$) and evergreen trees ($n = 3$) + SE. For all calculations see Material and methods. (*) indicates significant differences between functional type-means at $P < 0.05$ by Student t -test. NS, not significant.

and were rather small and not uniform among the species. Even the masting event in *Fagus* had no significant effect on the stem NSC concentrations. *Quercus*, the single ring-porous tree sampled, was the species exhibiting the most pronounced (albeit not significant) decrease of NSC concentrations during the early season. In contrast to diffuse-porous trees, ring-porous trees have to produce new water-conducting tissue before leaf-bud break, because they have to replace the xylem of the previous year, which had been ceasing function over winter (Zimmermann 1974). The differences in wood anatomy between ring- and diffuse-porous xylem are also reflected in the fraction of sapwood within the whole stem (Fig. 4).

We found no recent literature on the seasonal variation of NSC in stems of mature deciduous trees, but Gäumann (1935) also found no significant variation of NSC in stem sapwood of *Fagus sylvatica* that was approximately 110 years old. Similar to the deciduous species, the stem wood of evergreen conifers showed very weak seasonal

NSC dynamics in the current study. For *Pinus sylvestris* this finding confirms earlier observations by Fischer & Höll (1992) and Terziev *et al.* (1997). In both reports the sum of soluble sugars and starch stayed fairly constant over the growing season in stem sapwood of 30 and 65-year-old-trees. For *Picea abies* stems, Höll (1985) reported a winter maximum of NSC, but little variation during the growing season, again this was similar to our findings.

Despite their different leaf phenology, the concentrations as well as the slopes of the decreasing leaf-NSC were surprisingly similar between the deciduous and the evergreen species from June to October. In deciduous trees the structural development of leaves was commonly completed by the end of June, and thus the dry matter reference stayed more or less the same during the second half of the growing season (constant SLA). Therefore, the observed decrease of NSC concentrations (on a percentage dry matter basis) in leaves of deciduous trees after the end of June is not biased by an increase in leaf-toughness and reflects a real

reduction of non-structural carbohydrates in this tissue type.

Although leaves were already yellowing by the beginning of October, NSC concentrations (especially of free sugars) were still quite high (approximately 10% DM) in all deciduous species. Bonicel *et al.* (1987) reported that leaves of young *Populus trichocarpa* × *Populus deltoïdes* trees from Grenoble, France, still contained approximately 6.5% DM free sugars and more than 1% DM starch at the beginning of leaf-yellowing. In their review on carbon storage, Chapin *et al.* (1990) found that non-nitrogenous organic compounds are often not recycled from senescing leaves. However, since leaves sampled in October were not fully senescent, the remaining NSC could have been recovered from the leaves before shedding. Mäenpää *et al.* (2001), for example, found almost no NSC in completely yellow leaves of *Betula pubescens* in Northern Sweden.

Mature (1 year old) needles of evergreen conifers exhibited the typical increase of starch immediately prior to leaf-bud break (e.g. Fischer & Höll 1991; Schaberg *et al.* 2000). Hansen & Beck (1994) demonstrated that nearly all the carbon needed for flushing in *Pinus sylvestris* is supplied from this early season starch pool in mature needles.

Amplitude of seasonal NSC dynamics and functional type-specific differences

The magnitude of relative NSC variation (Diff %) in the woody tissues of the trees (branches and stem) was very different among the species (Fig. 8). The mean relative NSC variation across all species was 33% for stem sapwood and 45% for branch wood. This amplitude of the NSC variation seems to be in a similar range in other regions with a comparable growing season length (approximately 6 month). Schaberg *et al.* (2000) found a seasonal NSC variation of approximate 40% in stem sapwood of *Picea rubens* growing in Vermont, USA, and Gäumann (1935) calculated that *Fagus sylvatica* uses up 25–30% of its complete NSC and hemicellulose pool during a non-masting season in a Swiss lowland forest.

In the current study, the largest NSC fraction within the non-structural carbon pool was starch throughout the growing season in most species. Starch, different to free sugars, is osmotically inactive and accumulates as a storage compound during times of surplus carbon supply (Chapin *et al.* 1990). The persistent abundance of relatively high starch concentrations in woody tissues throughout the year suggests that carbon is not a particularly limiting resource for these mature trees under current ambient CO₂ concentrations. The seasonally constant lipid concentrations in all four 'fat-trees', also points at a rather stable and high carbon charging of the trees (Hoch *et al.* 2002).

By the transformation of the relative variation of NSC into an absolute change of carbon (Diff C), we were able to compare the seasonal NSC variation among the species quantitatively. The variation in NSC confined mainly to small branches, which thus appear as the major buffer for C surplus or shortage in mature trees. Stem reserves are

hardly involved under 'normal' operation (no disturbance, insect outbreaks, etc.). The repeated assessments of NSC over the season may suggest that there is no shortage in C compounds, which would cause plants to draw on stem reserves more heavily.

The great differences found in the relative variations of NSC among the species nearly disappeared for Diff C, because trees with higher NSC concentrations tended to have lower relative variations of NSC, and vice versa. Consequently, a small relative variation of a big NSC pool resembles the same Diff C, as a pronounced relative variation of a small NSC pool. Whereas the evergreen species exhibited higher relative variations of NSC, especially in the branch sapwood, Diff C was equal between the two functional types. Thus, the contribution of carbon reserves in woody organs for growth and respiration is likely to be very similar in deciduous and evergreen trees.

This result is quite unexpected, given the general belief that evergreen species supply growth mainly 'online' by carbon fixed concurrently in older needles, whereas deciduous trees generally rely on non-structural carbon compounds accumulated during the previous growing season (Kramer & Kozlowski 1979; Höll 1997; Piispanen & Saranpää 2001). Our comparative assessment of a suite of species belonging to both functional types illustrates no such difference in the magnitude of the NSC variations. Yet, the NSC trends during the season differ in their direction. Deciduous trees accumulate NSC during the season and use some of it during the dormant season and bud break. In evergreen trees the winter loss of NSC is nearly zero, most likely due to some photosynthetic activity (Hansen, Vogg & Beck 1996), causing stocks to peak at bud break (Hansen *et al.* 1997).

Based on measurements of stem diameter, tree height and sapwood density, we estimated the approximate biomass of the above-ground woody tissue for each of the 10 species. By using estimates for the total forest basal area and its increment, we then calculated the mean non-structural C pool (incl. lipids) per hectare of a (hypothetical) deciduous and an evergreen forest, composed by such species as studied here (Table 3). In both 'forest types' the seasonal reduction of C stored in non-structural carbon compounds (Diff C) resembles only a part (60–70%) of the estimated amount of C needed for annual xylem growth (Table 3). If the metabolic C costs for the formation of tissue are added, the seasonal reduction of stored carbon may be half the amount of C bound in newly formed xylem (Mooney 1972; Chapin 1989). In deciduous trees, the absolute amount of C in non-structural compounds is approximately three times higher than that needed for the annual above-ground xylem increment, but this amount is only 1.2 times higher in evergreen species. Since deciduous trees with their soft leaves are more often defoliated by outbreaks of herbivores than evergreen trees, they may rely more on such carbon reserves for an additional leaf flushing during the growing season (Kramer & Kozlowski 1979). Without accounting for the metabolic costs, the amount of C stored in stems and branches of the deciduous trees stud-

Table 3. Estimates of mean above-ground xylem (stem and branch wood) dry biomass, its non-structural carbon (ns-C) pool and Diff C, calculated for a hectare of deciduous ($n = 7$) and evergreen ($n = 3$) forest, in relation to the estimated amount of C for annual above-ground wood-increment and the amount of C in total leaf mass

	Deciduous species	Evergreen species
Above-ground xylem (t ha ⁻¹)	242	187
ns-C pool in above-ground xylem (t C ha ⁻¹)	6.0	2.0
Diff C (t C ha ⁻¹ a ⁻¹)	1.3	0.8
Annual above-ground increment (t C ha ⁻¹ a ⁻¹) ^a	1.8	1.5
Total leaf mass (t C ha ⁻¹) ^b	1.5	3.5

^aCalculated from girth tape measurements by R. Asshoff (unpubl. data). ^bDeciduous forest: LAI = 5, SLA = 17.7 m² kg⁻¹; Evergreen forest: LAI = 3, SLA = 4.0 m² kg⁻¹.

ied here would be sufficient to rebuild the whole leaf canopy more than four times (Table 3). In evergreen trees, which maintain several needle generations, the amount of C in needle tissue is approximately 1.8 times that contained in the above-ground non-structural carbon pool in woody tissue (Table 3).

In summary, the current study revealed no particular shortage in non-structural carbohydrates in any of the 10 mature temperate forest tree species throughout the growing season – even including periods of extensive fruit production (in masting *Fagus*). The inclusion of the lipid pool does not change the picture, although some trees, such as *Pinus* and *Tilia*, store quantitatively significant amounts of lipids in their woody tissues. A recent detailed analysis of lipids in different species of pines confirmed that this pool is far more stable over the season than is the NSC pool (Hoch *et al.* 2002; Hoch & Körner 2003). Thus, based on their current carbon charging, these trees seem well supplied with carbon and there may not be much of a leeway (if any) for further increases in carbon assimilation by elevated atmospheric CO₂ concentrations, as might be assumed based on photosynthetic characteristics alone. This hypothesis, is now under exploration at this forest site, using the new web-FACE technique (Pepin & Körner 2002).

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