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Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*)

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Summary

• The exhaustive distribution of total carbohydrate reserves was investigated in oak and beech trees that were approx. 40 yr old and felled at two dates (October 1999 and June 2000) to estimate variations in reserve amounts at the tree level.

• The total nonstructural carbohydrate (TNC) content was highest in the twigs and coarse roots, reaching 10 g 100 g^{-1} dry matter and 12 g 100 g^{-1} dry matter for beech and oak twigs, and 13 g 100 g^{-1} dry matter and 16 g 100 g^{-1} dry matter for beech and oak roots, respectively. Similar distribution in tree carbohydrates was observed for both species and date, but with contrasting starch/sugar sharing.

• Scaling-up to reserve amounts at tree level was performed with extensive organ biomass measurements. Based on the respective biomass of the organs, stem and roots contained the highest quantity of reserves. Between October (before leaf fall) and June (after bud-burst and leaf area index expansion) oaks used double the reserves of beeches.

• These differences in the allocation of carbohydrate reserves could arise from differential needs for spring growth and winter maintenance respiration between the two species.

Key words: *Fagus sylvatica* (beech), *Quercus petraea* (sessile oak), carbohydrate reserves, reserve utilization, tree scaling, interspecific comparison.

Introduction

Trees store considerable amounts of carbohydrates, fat and nitrogen compounds in the parenchymatous cells of their wood and bark (Kramer & Kozlowski, 1979). Reserve contribution to annual tree carbon balance is one of the keys to understanding tree survival. Storage pool is used during periods of low photosynthesis to fuel maintenance respiration (especially carbohydrates; Ögren, 2000), to cope with water stress and to build leaves in spring (especially in broad-leaved species). Seasonal reserve mobilization has been described primarily for young trees (Sauter & van Cleve, 1994; Witt & Sauter, 1994; Höll, 1997) and less is known about mature trees. Nevertheless, several studies have shown age-related changes in carbon allocation between tree organs. For example, in trees less than 20 yr old, highest biomass is allocated to roots (especially fine roots) unlike in older trees for which stem is the main biomass sink (Bartelink, 1998; Grulke & Retzlaff, 2001). This suggests that reserve dynamics in young trees cannot be extended to adult trees without (1) total carbohydrate content measurement in all tree compartments and (2) accurate estimates of their biomass.

In this study, carbohydrate management was compared in adult trees from two temperate deciduous species: sessile oak (*Quercus petraea*) and common beech (*Fagus sylvatica*). These two species differ in wood anatomy (Gasson, 1987) and exhibit contrasting growth patterns. In oak, a ring-porous species, trees achieve a large part of the annual radial stem

growth (including the entire early-wood with large vessels) before leaf expansion in spring (Dougherty *et al.*, 1979; Hinckley & Lassoie, 1981; Bréda & Granier, 1996). For beech, a diffuse-porous species, stem growth begins after leaf expansion. Another difference between ring-porous and diffuse-porous species is the radial distribution of living tissues, which contain reserve compounds (Höll, 1997; Saranpää & Höll, 1989). Ring-porous trees have clear delimited sapwood, unlike diffuse-porous species where living cells are diffusely distributed throughout the wood. Spring reserve mobilisation could be different depending on differences in spring carbon demand between diffuse and ring-porous species. In a previous detailed study, the seasonal dynamic of carbohydrate concentrations was monitored by monthly coring in the stem of adult oak and beech trees. The results showed that minimum reserve concentrations occurred in June and maximum reserve storage in October for both species, but with contrasting reserve concentrations and magnitudes (Barbaroux & Bréda, 2002). Another experiment investigates the exhaustive distribution of storage pool at tree level in order to quantify differences in carbon storage among organs and between the two species. The next step in constructing a whole-tree carbon balance is to scale up from reserve concentrations to reserve amounts at the tree level, using data from all organs. This scaling exercise from concentration to amount is especially complex for mature trees because the living biomass of each organ has to be estimated either by direct assessment on cut trees or by using allometric relationships. In this context, our objectives were: (1) to analyse reserve distribution in adult oak and beech trees at the periods of maximum and minimum reserve concentrations; (2) to quantify carbohydrate reserve amounts at the tree level; and (3) to compare reserve utilization between the two contrasting broad-leaved species.

Materials and Methods

Site and stand descriptions

The study was carried out on two separate stands, each composed principally of just one type of tree. We observed sessile oak (*Q. petraea* (Matt.) Liebl.) and common beech (*F. sylvatica* L.) in the state forests of Champenoux, France (48°44′ N, 6°14′ E, elevation 237 m) and Hesse, France (48°40′ N, 7°05′ E, elevation 300 m), respectively. The two sites are 60 km apart since we intended to compare the species in their optimal station rather than in a mixed stand, to avoid any interspecific competition effect. The oak trees were 45 yr old and the beech trees 35 yr old in 1999. The least age difference between the two stands is insignificant as they are the result of several years of natural regeneration and both are young high-forests. The oak stand is growing on a luvisol, developed over a deep loam (for further details, see Bréda *et al.*, 1995). In 1999, the stand density was 2553 stems ha^{-1}, basal area 26.7 m^2 ha^{-1} and dominant height 17 m. The beech soil type is intermediate between a luvisol and a stagnic luvisol (for further details, see Granier *et al.* (2000b). Stand density was 3800 stems ha^{-1}, basal area 19.6 m^2 ha^{-1}, and dominant height 13 m in 1999.

Plant material

Six dominant trees per species (see Table 1) were harvested in autumn during leaf fall (October 10–13, 1999 for oaks and November 2–4, 1999 for beeches) and the following spring after the leaves had fully expanded (June 1–7, 2000). These dates correspond to maximum and minimum reserve concentrations, as determined in a previous nondestructive experiment in the same stands by Barbaroux & Bréda (2002). Tree samples were taken from different organs (stem, branches, twigs, coarse and fine roots) and at different positions in each organ. Disk-like samples (1–2 cm longitudinal thickness) were taken from stem heights of 0, 1.3, 3, 6, 9, 12 and 15 m. Additional samples were taken from six lateral branches at different heights in the crown. Three segments from each branch were analysed: near the insertion to the stem, at the extremity of the branches (twigs from the two last growing seasons) and midway between these two points. Furthermore, samples were collected from two lateral roots from each of the three diameters classes: fine roots (diameter < 2 mm), medium roots (diameter = 2–5 mm) and coarse roots (diameter > 5 mm). For coarse roots, sample discs (5–10 cm longitudinal thickness) were spread over three distances from the stump: 15–30 cm, 70–100 cm and 150–250 cm.

Tissue sections were weighed immediately after cutting (i.e. f. wt), frozen and stored at −20°C, until freeze-dried. Dry

Table 1. Characteristics of the six 45-yr-old sessile oaks and six 35-yr-old beeches felled in October 1999 and June 2000

	October 1999				June 2000			
Species	Tree	C130 (mm)	Total height (m)	Crown length (m)	Tree	C130 (mm)	Total height (m)	Crown length (m)
Oak	1	522	16.5	4.8	4	534	17.3	5.3
	2	585	17.6	5.0	5	605	17.5	4.9
	3	571	18.1	5.0	6	546	17.5	4.5
Beech	1	456	14.7	6.2	4	461	14.6	6.9
	2	455	14.8	7.2	5	516	16.6	6.6
	3	534	15.4	7.6	6	530	16.8	8.4

weight was measured after freeze-drying for 1 week and relative water content determined. For oaks, heartwood was removed from stem sections with a saw. Wood and bark were analysed together, except for one tree of each species in October, for which the bark (phloem plus periderm tissues including cambium) was separated from the inner wood for all the organs. Entire samples were cut in small pieces with a saw and ground twice with a Cyclotec 1093 Sample Mill (Tecator, Höganäs, Sweden).

Carbohydrate analysis

Soluble sugars were extracted twice from 5–10 mg of powder with 80% boiling ethanol (1 ml), for 30 min. The extracts were centrifuged 10 min at 12 620 ***g***; combined and dried overnight with a vacuum-evaporator (Maxi-Dry Plus; Heto-model DW1, 0-110, Heto-Holten A/S Allerod, Denmark) to eliminate ethanol. The dried extracts were rehydrated in 0.5 ml of 0.02 N NaOH solution, and soluble sugars (glucose, fructose and sucrose) were measured using the colorimetric technique at 340 nm (spectrophotometer model 6100, Jenway Ltd., Essex, UK) (Boehringer, 1984). Starch was extracted from the remaining dried matter in a boiling solution of 0.02 N NaOH for 1 h. Starch was hydrolysed to glucose with α-amyloglucosidase (EC 3.2.1.3, Boehringer Mannheim Biochemicals, Mannheim, Germany) in a 0.32 M citrate buffer (pH 4.2) at 48°C for 30 min. The resulting glucose was assessed as described above (Boehringer, 1984). Starch was quantified as glucose equivalents. Total nonstructural carbohydrates (TNC) were calculated as the sum of total soluble sugars and starch. Carbohydrate concentrations were expressed as grams per 100 g of dry mass of the sample (g 100 g^{-1} DM).

Biomass estimation

The stem biomass was estimated as the product of volume by wood density (i.e. d. wt divided by the fresh volume). Branch and root biomass were estimated using allometric relationships depending on circumference at breast height (C130). Ottorini & Le Goff (1998) developed these relations for beech (on trees from our same stand) and by Drexhage *et al.* (1999) for oak roots without distinction between coarse and medium roots. The allometric relationship for branches in oak was established in this study.

For stems, a second disk-like sample was collected at each height along the stem and their fresh volumes were measured (for four thickness and two diameter measurements). These samples were dried at 105°C and weighed to determine wood density. Stem volume was calculated from circumferences and length measurements, with each segment modelled as a truncated cone. For oaks, sapwood volume was estimated from sapwood width measurements for each stem section. By contrast, for beech, we assumed that all the wood was sapwood even if diffuse cell mortality was observed close to the pith (Ceschia, 2001). Finally, stem biomass was calculated as the product of stem volume by stem wood density.

The six sampled branches of oaks were weighed in the field (Sartorius I 12 000 S, Göttingen, Germany, 0.1 g precision). Additional samples were collected for these branches, and f. and d. wts were measured for dry matter conversion:

$$\text{Branch dry. wt (g)} = 0.618 \times \text{branch f. wt (g)} - 0.277 \quad (R^2 = 0.998,\ n = 70) \qquad \text{Eqn 1}$$

Branch biomass was calculated as a function of basal branch diameter:

$$B_{branch}\ (\text{g dry wt}) = 0.026 \times D_{branch}\ (\text{mm})^{3.026} \quad (R^2 = 0.975,\ n = 50) \qquad \text{Eqn 2}$$

(B_{branch} = branch biomass; D_{branch} = branch base diameter).

Basal diameter was measured for each branch and total branch biomass was estimated for each oak from Eqn 2. In addition, all oak leaves were collected, dried at 80°C and weighed to obtain total leaf biomass. For beech, leaf biomass was calculated with allometric relationships depending on circumference at 1.30 m height (Ottorini & Le Goff, 1998).

The biomass of the different levels of the branches and roots were only measured for a subsample. Then, organ reserve concentrations for each tree were averaged, weighted by the biomass of the corresponding organ level. Organ reserve conversion amounts were calculated through the product of the total organ biomass and by the corresponding reserve concentration mean. Total reserve amount per tree is the sum of reserve amounts for all of the organs.

Finally, to allow direct comparison between carbon mass and total carbohydrate amount expressed as grams of carbon, the carbon content of each organ was measured for both species in the stem, roots, branches and bark, using an elemental analyser (NCS 2500, ThermoQuest, Milano, Italy). Total nonstructural carbohydrate concentrations (including soluble sugars and starch) were expressed in glucose equivalents and converted to carbon mass (gC) by applying a 40% carbon content in glucose.

Additional measurements

In October and June 1998, reserve concentrations were analysed on three oaks and three beeches from the same stand and with similar crown position within the canopy (dominant or codominant) (for further details on tree characteristics, see Barbaroux & Bréda, 2002). The samples considered were one stem core (5 mm diameter and 10 cm long) at 1.30 m, two coarse root cores and two twigs taken down with a gun. Organ biomass was estimated with allometric relationships depending on C130 for beech (Ottorini & Le Goff, 1998) and oak (Drexhage *et al.*, 1999; Barbaroux, 2002).

To test this alternative nondestructive sampling technique of tree reserves assessment, we applied it to each of the felled trees for which the exhaustive quantification was also performed. Note that for this partial subsampling method, sample reserve concentrations were extended to organ biomass estimation of each tree.

Statistics

Data were analysed by the GLM procedure (SAS software package, version 6.12; SAS, Cary, NC, USA). For each organ, distribution of reserve concentrations was studied with several sources of variation: species (including site), date, tree nested in species, organ position nested in tree (only for branches and roots) and sample position in the organ. In addition, the interaction between intraorgan level and species was tested. Note that random and fixed effects were not separated in this model because the experiment was aimed at extensively describing the TNC distribution in trees; we consider that our sampling scheme was not suitable to elaborate a general model of TNC variation. Statistically significant differences between different intraorgan levels were tested using the Student–Newman–Keuls multiple range test at $P = 0.05$. The sampling test was conducted using paired *t*-test comparisons between tree reserve amounts estimated from subsampled cores and exhaustive sampling, both from the felled trees.

Results

Reserve concentrations according to organs, date and species

Similar tree reserve distribution among organs was observed in both species and on the two sampling dates, but differences in TNC concentrations were observed between dates and species (Fig. 1). The TNC concentrations were lower in June compared with October, whatever the organ and the biochemical component (except roots for sugar) (Fig. 1 and Table 2). In October, oaks exhibited higher concentrations than beeches in all organs. Roots and branches had the highest reserve concentrations. In branches, TNC significantly increased with intrabranch position from the base to top (5.7–9.4 g 100 g^{-1} DM and 7.7–12.6 g 100 g^{-1} DM for beech and oak, respectively, in October) (Table 2). Conversely, branch position in the crown had no significant effect on carbohydrate concentrations (Table 2). For roots, TNC concentrations reached a maximum in the middle position along coarse roots (13.3 g 100 g^{-1} DM for beech and 16.2 g 100 g^{-1} DM for oak in October) and then decreased in medium and fine roots. Reserve concentrations in stem increased significantly from the base to top (2.5–4.6 g 100 g^{-1} DM and 4.1–8.8 g 100 g^{-1} DM for beech and oak, respectively, in October). The highest concentration of stem section was located in the crown and approached concentrations

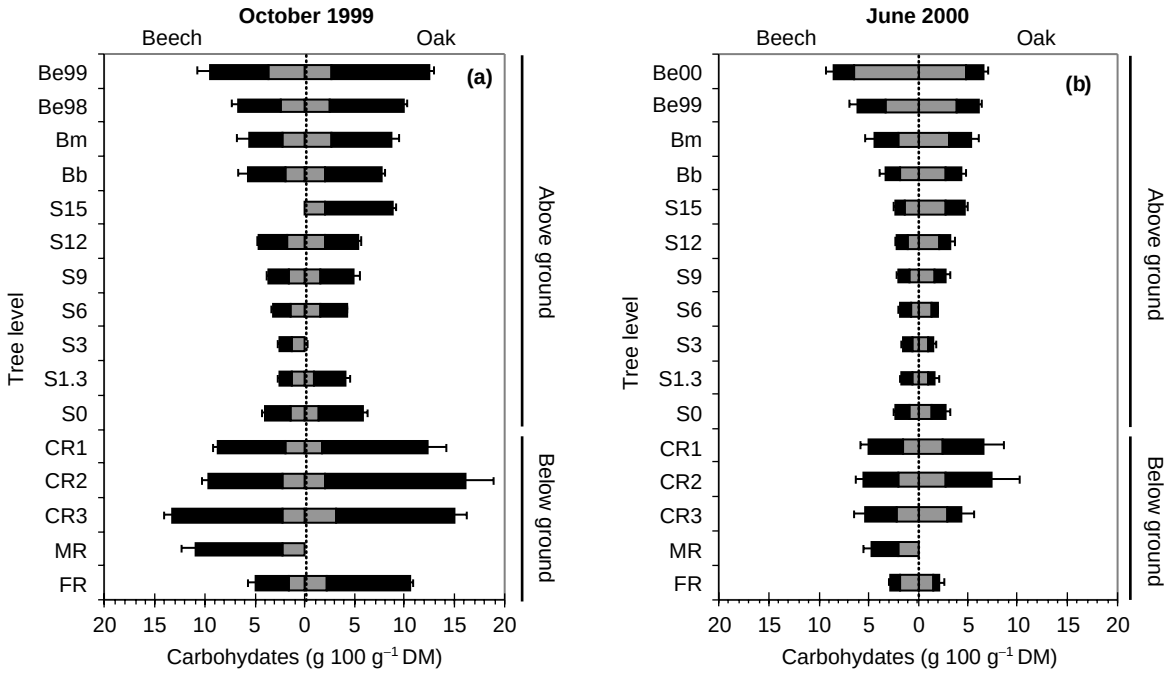

Fig. 1 Variations of total nonstructural carbohydrate (TNC) concentrations among organs into three each of oak and beech trees in October 1999 (a) and in June 2000 (b). Sugar (including glucose, fructose and sucrose) (grey bars) and starch concentrations (black bars) are represented. Vertical bars indicate SE on TNC ($n = 3$ trees per species). FR, fine roots; MR, medium roots; CR, coarse roots (for 15–30 cm length from the stump (CR1), for 70–100 cm length from the stump (CR2) and for 150–250 cm length from the stump (CR3); S, stem (for 0–15 m height); Bb, branch base; Bm, branch middle; Be, twigs; growth units of 1998, 1999 or 2000.

Table 2 Analysis of variance of the distribution of reserve concentration in branches, roots and stem of oak (*Quercus petraea*) and beech (*Fagus sylvatica*)

Variables	df	TNC	Starch	Sugar
Branches				
Species (+ site)	1	66.76***	66.16***	0.69
Date	1	235.53***	497.64***	135.25***
Tree (species)	9	10.00***	11.69***	2.24*
Position (tree)	30	0.96	0.52	0.83
Level	3	97.04***	20.58***	121.37***
Species × level	3	2.34(*)	2.27(*)	31.03***
Roots				
Species (+ site)	1	26.34***	20.05***	16.14***
Date	1	193.33***	208.97***	0.02
Tree (species)	9	5.64***	6.11***	2.24*
Position (tree)	6	0.99	0.94	1.11
Level	4	14.23***	11.81***	10.18***
Species × level	3	2.21(*)	3.19*	2.01
Stem				
Species (+ site)	1	90.57***	42.75***	63.03***
Date	1	228.15***	166.55***	34.54***
Tree (species)	9	5.24***	8.34***	4.51***
Level	6	23.88***	10.88***	23.98***
Species × level	6	5.74***	3.29**	5.31***

The effects of species (including site), date (October vs June), tree nested in species, organ position nested in tree and intraorgan level are shown. Sample numbers per organ were 275 for branches, 107 for roots and 84 for stem. *F*-value was reported and probability value was indicated: (*), $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. R^2 model ranged from 0.74 to 0.91. TNC, total nonstructural carbohydrate.

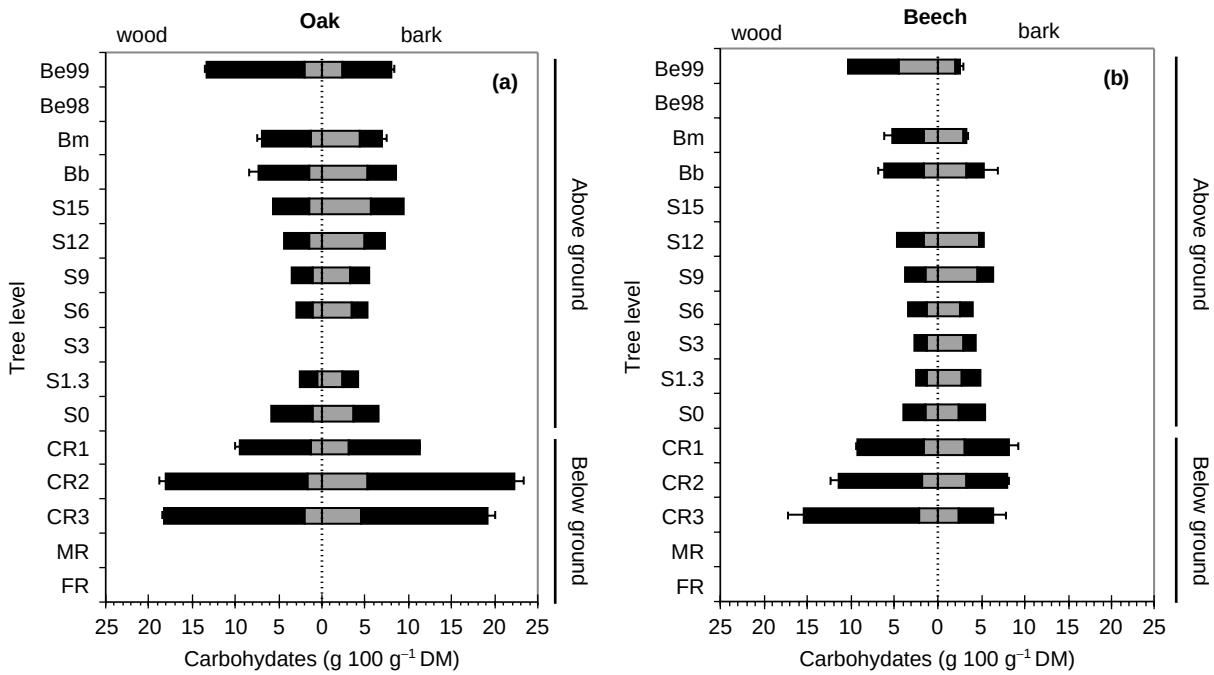

Fig. 2 Distribution of carbohydrate reserve concentrations between bark and wood in October 1999 for each organ of oak (a) and beech (b). Legend was the same as in Fig. 1.

observed at the base of the branches. Differences in TNC concentrations were mainly due to starch variations regardless of the source of variability (species, date or tree compartment). In June, a significant increase in sugar concentrations was observed in branches of both species compared with October (Table 2).

Results of the wood-bark separation in TNC storage are presented in Fig. 2. Bark reserves were generally two times

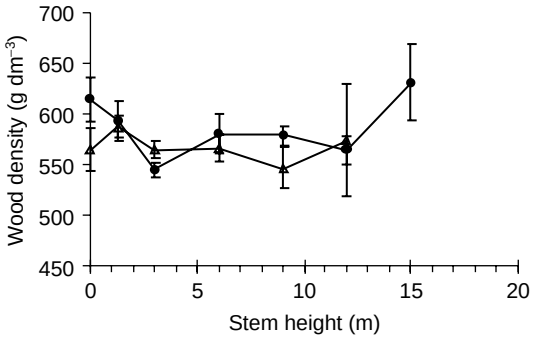

Fig. 3 Wood density evolution along stem height of oak (*Quercus petraea*, circles) and beech (*Fagus sylvatica*, triangles) trees. Vertical bars indicated standard errors (n = 6 trees per species, both October 1999 and June 2000 combined).

more concentrated than wood in stem and branches compared with bark reserves in roots and twigs. The TNC bark concentrations in October ranged from 4.1 to 22.5 g 100 g^{-1} DM for stem and roots of oak and from 4 to 8.3 g 100 g^{-1} DM for stem and roots of beech. Sugars were more concentrated in the bark than in the wood and represented a maximum of 66% and 88% of carbohydrates in above-ground part of oak and beech bark, respectively. Sucrose was the carbohydrate form most represented in bark (data not shown).

Organ biomass estimation

Stem wood density ranged between 550 g dm^{-3} and 600 g dm^{-3} all along the middle part of the stem (Fig. 3) regardless of the species. Wood density increased to 650 g dm^{-3} in the crown of the two species, and oak exhibited a higher density than beech at the base of the stem.

Biomass distribution was reported in Table 3 and showed differences between organs and species. Root biomass represented 20% and 19% of total wood biomass for oak and beech, respectively. Branch biomass represented 11% and 14% of total wood biomass for oak and beech, respectively. Finally, bark biomass was estimated at 16% and 6% of oak and beech stem biomass, 16% and 10% of oak and beech root biomass and 24% and 19% of oak and beech branches biomass, respectively. Even if bark reserve concentrations were higher than in wood, this organ contributed weakly to total reserve amounts.

Table 3 Average organ biomass (bark included) of felled oak (*Quercus petraea*) and beech (*Fagus sylvatica*) trees

Biomass (kg DM)	Oak	Beech
Stem	108 ± 10.6	84 ± 7.34
Stem sapwood	81 ± 5.3	84 ± 7.34
Branches	17 ± 2.08	18 ± 1.95
Coarse roots	30 ± 1.47	21 ± 1.77
Medium roots		1.34 ± 0.09
Fine roots	1.5 ± 0.07	1.17 ± 0.08
Leaves	3.8 ± 0.4	3.26 ± 0.23
Total sapwood (without leaves)	156.5	125.5

Data are means ± SE of six trees per species.

Carbon content in each organ showed no significant differences between organs for the two species (47% on average; see Table 4).

Reserve quantification at tree level

Organ TNC content exhibited large differences between organs depending on the unit used (concentrations (g 100 g^{-1} DM) or amounts (gC) expression (Fig. 4)). The stem was the most important carbohydrate storage compartment (mean 44%) for the two species and dates, followed by roots (40%) and then branches (16%). Net reserve utilization occurred in all organs between October and June, with 48% of mobilized reserves coming from the stem, 37% from coarse roots and 11% from branches of the two species. The mean TNC amounts mobilized reached 1985 gC for oak and 660 gC for beech, which corresponded to 2.8% and 1.2% of total carbon biomass for oak and beech, respectively. Note that this estimation of carbohydrate utilization is not suitable for separating potential fluxes between current photosynthate and storage pool during leaf-out.

Results of the subsampling test with different sample combinations are given in Table 5. The least significant change was obtained with stem–branch–root combined sampling, regardless of the species. Error sampling between the two methods was estimated up to 17% for oak and 5% for beech. This subsampling was a reasonable alternative method for a nondestructive estimation of TNC content in adult trees. For this reason, the three complementary trees per

Table 4 Mean carbon and nitrogen contents in different organs of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) 35- and 45-yr-old trees (Hesse and Champenoux sites)

	Carbon (%)		Nitrogen (%)	
Organs	Oak	Beech	Oak	Beech
Roots	46 ± 0.6 (8)	48 ± 0.4 (8)	0.42 ± 0.04	0.42 ± 0.10
Stem sapwood	48 ± 0.1 (4)	46 ± 0.8 (8)	0.11 ± 0.02	0.15 ± 0.01
Branches	46 ± 0.2 (6)	47 ± 0.1 (7)	0.60 ± 0.12	0.26 ± 0.04
Bark	48 ± 0.5 (9)	48 ± 0.3 (5)	0.72 ± 0.10	0.79 ± 0.08

Data are means ± SE. Samples number is indicated in parenthesis.

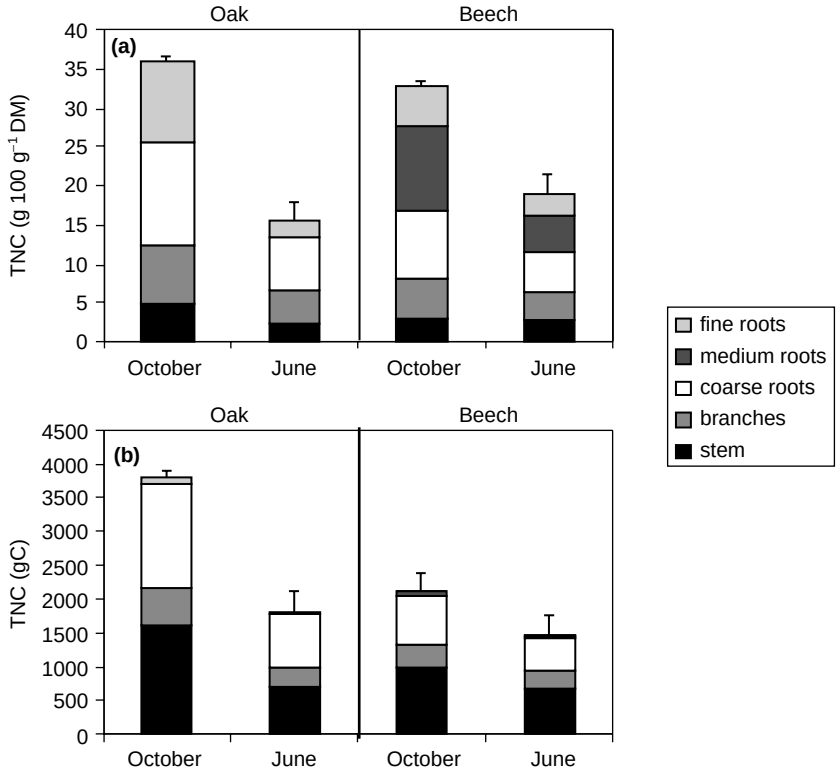

Fig. 4 Organ reserve distribution for oak and beech trees in October 1999 and June 2000. Results are expressed in mean concentrations (a) and in amounts of carbon (b). Vertical bars indicate standard errors at tree level (n = 3). TNC, total nonstructural carbohydrate.

Table 5 Statistical analyses of paired comparisons using a t-test between tree reserve amounts estimated from exhaustive sampling and from subsampled cores for oak (*Quercus petraea*), beech (*Fagus sylvatica*) and the two species combined (total)

	SBR	SR	SB	BR	S	B	R
Oak	1.09	–0.49	–1.38	2.92*	–3.79*	4.99**	2.45(*)
Beech	0.19	–2.40(*)	–1.02	2.47(*)	–3.79*	9.84***	1.37
Total	1.02	–1.78	–1.78	3.56**	–5.60***	9.51***	2.65*

Different subsamplings were tested for stem (S), roots (R), twigs (B) and various combinations of these three organs (represented by combined letters). Paired t-test values are presented and probability values were indicated: (*), $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

species (sampled with cores) were added in the estimation of total reserve amounts, taking into account the most accurate estimates (root–stem–branch). The mean reserves used between October and June (calculated from the six trees per species) reached 880 gC for beech and 2400 gC for oak, which represented 50% and 40% of their reserves, respectively.

At the tree level, reserve concentrations (on a relative tree biomass basis) appeared to have a greater variation in oak than beech for a similar dominant position within the stand (Fig. 5). The TNC reached an average of 6.8 ± 0.8 g 100 g^{-1} DM and 4.3 ± 0.3 g 100 g^{-1} DM for oak and beech, respectively, in October. In June, tree carbohydrate concentrations decreased to 3.4 ± 0.9 g 100 g^{-1} DM and 2.5 ± 0.5 g 100 g^{-1} DM for oak and beech, respectively. Interspecific differences of tree reserve content occurred mainly in October when oaks stored 36% reserves more than beeches, considering an absolute use of TNC. However, in relative terms, both species had a 40–50% reduction in their respective TNC pool from October to June Moreover, beech TNC concentrations showed no correlation with tree biomass, unlike oak, where TNC decreased slowly (not significant with slope test) as tree biomass increased in October However, the decrease in oak TNC concentrations with higher sapwood biomass in October was overcompensated by the biomass increase when TNC were expressed in amounts (data not shown).

Discussion

Reserve distribution between organs and species

No species differences were observed in the distribution of reserves in the various tree compartments. Coarse roots and twigs had the highest carbohydrate concentrations. The younger parts of the branch and stem wood (which are also closer to the leaves) stored much more TNC than the older ones. This agrees with results obtained for carbohydrate

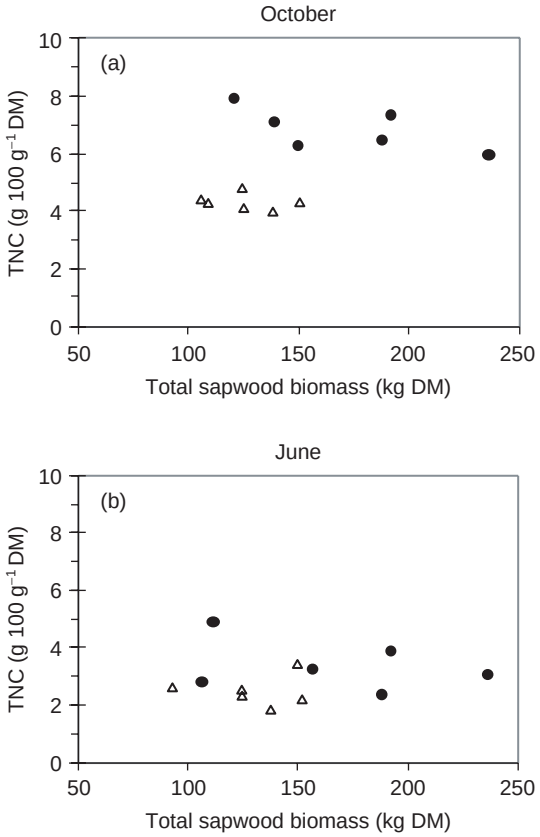

Fig. 5 Variability of tree reserve concentrations according to tree sapwood biomass for oak (*Quercus petraea*, circles) and beech (*Fagus sylvatica*, triangles) in October (a) and June (b) (n = 6 trees per species). TNC, total nonstructural carbohydrate.

reserve distributions (Kramer & Kozlowski, 1979; Samuelson & Kelly, 1996; Bollmark *et al.*, 1999) and protein reserve distributions in trees (Sauter *et al.*, 1989). Two hypotheses could explain these results: storage tissue distribution and sink distance. Indeed, wood anatomy changed between organs, and with the age of the organs. Cambial ageing results in an increase of vessel formation to the detriment of parenchyma cells, as demonstrated in the beech species (Vollenweider *et al.*, 1994) and in the oak species (Helinska-Raczkowska, 1994). For roots and bark, anatomy present an increase of the number of parenchyma cells compared with stem wood in beech and fruit trees (Gäumann, 1935; Tromp, 1983). Moreover, Lacointe *et al.* (1993) using carbon labelling experiments, found that young tissues have a higher priority than other tissues for using the reserves. The storage pool probably increased near carbon sinks for fast reserve utilization. Intra-organ reserve distribution showed larger variation along the organ rather than with organ position, as was reported by Wargo (1976) in oak roots.

Oak exhibited larger carbohydrate concentrations than beech for all organs regardless of the date, confirming the previously reported differences in the tree stems (Barbaroux & Bréda, 2002). For both species, net reserve utilization from each organ occurred between October and June. These differences in reserve levels were primarily due to changes in starch concentrations (Sauter & van Cleve, 1994; Witt & Sauter, 1994). By contrast, utilization of TNC occurred in all the organs proportional to the available amount of TNC so that the reserve distribution pattern was unchanged within the trees between the two dates.

The reserve storage contribution of each organ to the entire tree appears to be primarily determined by organ biomass. In mature trees, above-ground biomass contained most of the reserve amounts even if reserves were more concentrated in the below-ground parts (Murneek, 1942; Cranswick *et al.*, 1987; Kozlowski, 1992).

At tree level, beech TNC concentrations remained constant between trees for the two dates, suggesting only a biomass effect in the estimation of TNC amounts. Conversely, oak TNC concentrations seemed to be influenced by tree biomass, especially during reserve storage in autumn. However, these results could change depending on the changing biomass of trees as they age.

Inter-specific comparison in reserve amounts

The total amount of carbohydrate reserves used from leaf fall to spring leafing was two times higher in oak than in beech, once differences in biomass were taken into account. Mooney & Hays (1973) discussed the carbon resource requirement during leaf expansion as the reason why broad-leaved species mobilized a larger amount of reserves than evergreen species. The same argument could hold for oak and beech physiological functioning. For oak, earlywood construction cost potentially added to leaf formation in spring due to restoration of sap transport, unlike in beech where earlywood construction began after leaf expansion, as illustrated by seasonal pattern in phenology (Barbaroux & Bréda, 2002). Low reserve requirements for spring leafing has already been mentioned for diffuse porous trees (Höll, 1997). In beech, wood construction costs could be entirely supplied by new assimilate. Moreover, leaf construction itself and more generally carbon requirement do not depend entirely on reserve utilization. Autotrophic carbon exportation from leaves began 10 d after photosynthesis started and full autotrophy occurred 20 d later (Maillard *et al.*, 1994) (the leaf expansion period lasted about 30 d; Barbaroux & Bréda, 2002). Reserve utilization stopped when leaves became autotrophic, suggesting priority utilization of new assimilates. This has recently been confirmed by Dyckmans *et al.* (2000) who showed that only 44% of carbon in leaves came from carbon reserves in beech trees.

Moreover, previous measurements of the seasonal dynamics of TNC concentrations have shown that oaks also need more TNC during winter, possibly because of higher maintenance respiration in this ring-porous species (Barbaroux & Bréda, 2002). Indeed, the basal respiration rate of sapwood

Table 6 Carbon costs estimation during winter and spring (between October and June) and literature source for the nonmeasured process in our experiment

Variable (gC)	Beech	Oak	Function	Reference	Species
Maintenance respiration	584	780	Q_{10}, R_{15}, T	Damesin *et al*. (2002) Edwards & Hanson (1996)	*Fagus sylvatica* *Quercus* sp.
Leaf biomass	1490	1740	Measured	This study	
Reserve needs for leaf biomass	655	765	44%	Dyckmans *et al*. (2000)	*F. sylvatica*
Leaf growth respiration	387	452	0.26 gC gC^{-1}	Merino *et al*. (1984)	Deciduous
Reserve needs for leaf growth respiration	171	200	44%	Our hypothesis	
Reserves needs for leaf construction	826	965	44%	Dyckmans *et al*. (2000)	*F. sylvatica*
Earlywood biomass	–	680	Measured	This study	
Earlywood growth respiration	–	197	0.29 gC gC^{-1}	Damesin *et al*. (2002)	*F. sylvatica*
Total carbon costs	2461	3849			
Carbon reserve needs	1410	2622			
Our measurements	880	2400			
Difference	38%	8%			

Q_{10}, respiration rate. R_{15}, basal respiration rate for 15°C. T, temperature.

tissues and Q10 values were higher in oak stem (Edwards & Hanson, 1996) than beech stem (Damesin *et al*., 2002). Also, Edwards & Hanson (1996) showed lower maintenance respiration in diffuse porous trees (*Acer rubrum*) than ring porous trees (*Quercus* sp.). In the same way, chemical wood composition presented in Table 4 shows twice the amount of nitrogen content in oak branches than in beech branches, which could contribute to higher maintenance respiration in oak than beech (Maier *et al*., 1998).

Carbon partitioning at tree level

This scaling exercise of reserve estimation at tree level allowed us to discuss tree carbon balance. For this purpose, we used two methods to estimate annual carbon distribution from literature in order to compare with our carbon reserve estimation. Our first approach was to determine reserve carbon allocation during the vegetative season and the second approach was to evaluate carbon needs during winter and leaf formation. First, net carbon assimilation (removing total above- and below-ground respiration costs during the vegetative season) was estimated to be 6400 gC by Lebaube *et al*. (2000) for a dominant beech in our stand in the Hesse forest. These authors estimated an annual tree wood biomass increment of 4400 gC per tree and a leaf biomass of 1500 gC. Based on their results, the carbon pool for reserve storage and fine root growth could be estimated as net carbon assimilation minus leaf and wood biomass increment. However, making the assumption that 56% of leaf biomass came from new assimilate (Dyckmans *et al*., 2000), the estimation of carbon reserve storage and fine root growth approached 1160 gC per tree between June and September of 1997. In the Hesse forest, fine root growth was estimated to 60 gC m^{-2} yr^{-1} in 1997 (Epron *et al*., 1999), which corresponded to 682 gC per tree. This method of reserve storage estimation in beech reached 478 gC, which represented half of our estimation (880 gC). One possible reason of this difference could be the soil water deficit in 1997 (Granier *et al*., 2000a), leading to a reduced wood increment compared with the wettest year (1999). As a consequence, a longer period of carbon allocation to TNC storage probably occurred in 1997 between growth cessation and leaf fall, as the seasonal dynamics of TNC replenishment has shown (Barbaroux & Bréda, 2002).

Our second approach was to estimate carbon cost during winter and spring, which allowed us to estimate the carbon reserves needed between October and June. These calculations, as well as the literature sources, are detailed step by step on Table 6. For this period, above and below-ground maintenance respiration was calculated using a Q10 function and values of basal respiration calibrated by Damesin *et al*. (2002) in the same beech stand, and by Edwards & Hanson (1996) in a *Quercus* sp. stand, using our tree's volume and 1999 hourly temperatures. Winter maintenance respiration was equal to *c*. 584 gC per beech and *c*. 780 gC per oak. In June, our measure of leaf biomass was 1490 gC for beeches and 1740 gC for oaks, accounting for 45.7% of carbon content in leaf biomass (Santa Regina *et al*., 1997). Growth respiration of leaves was estimated from a carbon construction cost of 0.26 gC gC^{-1} (calculated from Merino *et al*., 1984). According to Dyckmans *et al*. (2000) only 44% of the leaf biomass came from carbon reserves (i.e. 655 gC and 765 gC for beeches and oaks, respectively). The same reserve contribution in the leaf biomass was also attributed to leaf growth respiration, so that reserves used for leaf growth respiration reached 171 gC and 200 gC for beeches and oaks, respectively. Reserves needed for leaf construction were estimated to 826 gC and 965 gC for beech and oak, respectively. Fine root growth occurred at the end of May in the beech forest (Farque, 1997) and was not accounted for in our carbon need estimation. Finally, carbon reserve needs for winter maintenance respiration and leaf construction totalled 1410 gC for beech and 1750 gC for oak. These estimations were above the

amounts of reserves used when we measured for beech in this study (880 gC) and below those of oak (2400 gC). For oak, deficit of carbon (650 gC) could be attributed to earlywood formation during leaf set-up. This earlywood construction cost approached our estimation of earlywood biomass (680 gC at tree level using allometric relationships) and earlywood growth respiration (197 gC according to carbon construction cost of 0.29 gC gC^{-1}; Damesin *et al.*, 2002). Our measurements of the carbon reserves used were almost the same as the carbon sink evaluations for oak trees (a difference of 8%) and were 38% less than expected for beech trees.

Whichever method of carbon cost estimation used, the results were generally higher in the carbon sink evaluation than in our measurements, especially for the beech trees. This suggests that there are remaining problems of scaling to estimate carbon tree balance on mature trees, with uncertainties on all carbon sink calculations. Moreover, carbon cost could be overestimated for carbon economy hypotheses such as energy recovering from photosynthesis for leaf respiration process in light (Villar *et al.*, 1995) or leaves preformed during the growing season (Fontaine *et al.*, 1999). These could reduce carbon needs during leaf formation. Reduced carbon needs could occur during winter and spring periods because of bark and stem photosynthesis (Pfanz & Aschan, 2001). Finally, one cannot exclude the possibility that other reserve materials (storage proteins, glycerol forms, free fatty or amino acids) might also be involved to refill the carbon sinks (Stepien *et al.*, 1994) especially for beech. Indeed, Höll (1997) has suggested that diffuse-porous trees such as beech (named 'fat trees') might preferably use reserve compounds other than TNC.

Conclusion

A similar intratree reserve distribution pattern was found in the two broad-leaved species. Oak exhibited higher starch concentrations than beech in all organs. Moreover, this study showed that oaks use twice the amount of carbon reserves as beeches, even if they are similar in relative TNC utilization and relative change among organs. Differential needs in maintenance respiration and spring growth (including leaf and initial wood) possibly explain these differences. More information is now needed on inter-tree reserve variability (especially between trees with different crown position within the canopy) to improve scaling-up to the stand level for modelling annual carbon balance in forest ecosystems.

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