Do elevation of CO2 concentration and nitrogen fertilization alter storage and remobilization of carbon and nitrogen in pedunculate oak saplings ?

Séraphine Vizoso, Dominique Gerant, Jean-Marc J.-M. Guehl, Richard Joffre, Michel M. Chalot, Patrick Gross, Pascale Maillard

Tree Physiology 28, 1729–1739

Do elevation of CO_2 concentration and nitrogen fertilization alter storage and remobilization of carbon and nitrogen in pedunculate oak saplings?

SERAPHINE VIZOSO,[1] DOMINIQUE GERANT,[1] JEAN MARC GUEHL,[1] RICHARD JOFFRE,[2] MICHEL CHALOT,[1] PATRICK GROSS[1] and PASCALE MAILLARD[1,3]

[1] *IFR 110, "Génomique Ecophysiologie et Ecologie fonctionnelles," INRA-UHP Nancy 1, Faculté des Sciences et Techniques, 54506 Vandoeuvre, France*

[2] *Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, Dream Unit, 1919 Route de Mende, 34293 Montpellier Cedex 01, France*

[3] *Corresponding author (maillard@nancy.inra.fr)*

Received March 25, 2008; accepted June 27, 2008; published online September 2, 2008

Summary Soil nitrogen can alter storage and remobilization of carbon and nitrogen in forest trees and affect growth responses to elevated carbon dioxide concentration ($[CO_2]$). We investigated these effects in oak saplings (*Quercus robur* L.) exposed for two years to ambient or twice ambient $[CO_2]$ in combination with low- (LN, 0.6 mmol N l^{-1}) or high-nitrogen (HN, 6.1 mmol N l^{-1}) fertilization. Autumn N retranslocation efficiency from senescing leaves was less in HN saplings than in LN saplings, but about 15% of sapling N was lost to the litter. During the dormant season, nonstructural carbohydrates made up 20 to 30% of the dry mass of perennial organs. Starch was stored mainly in large roots where it represented 35–46% of dry mass. Accumulation of starch increased in large roots in response to LN but was unaffected by elevated $[CO_2]$. The HN treatment resulted in high concentrations of N-soluble compounds, and this effect was reduced by elevated $[CO_2]$, which decreased soluble protein N (–17%) and amino acid N (–37%) concentrations in the HN saplings. Carbon and N reserves were labeled with ^{13}C and ^{15}N, respectively, at the end of the first year. In the second year, about 20% of labeled C and 50% of labeled N was remobilized for spring growth in all treatments. At the end of leaf expansion, 50–60% of C in HN saplings originated from assimilation versus only 10–20% in LN saplings. In HN saplings only, N uptake occurred, and some newly assimilated N was allocated to new shoots. Through effects on the C and N content of perennial organs, elevated $[CO_2]$ and HN increased remobilization capacity, thereby supporting multiple shoot flushes, which increased leaf area and subsequent C acquisition in a positive feedback loop.

Keywords: ^{13}C, ^{15}N, carbon remobilization, carbon storage, elevated CO_2, nitrogen remobilization, nitrogen storage, Quercus robur.

Introduction

Elevation of atmospheric carbon dioxide concentration ($[CO_2]$) can enhance plant growth, thereby increasing the demand for mineral nutrients (Ceulemans and Mousseau 1994, Stitt and Krapp 1999). Responses to elevated $[CO_2]$ vary depending on nutrient availability and species. Given the importance of nitrogen limitation on the growth responses of temperate forests and the increase in N deposition (Bauer et al. 2001), the effect of soil N availability on tree responses to increasing atmospheric $[CO_2]$ is of particular interest. Positive effects of elevated $[CO_2]$ on carbon assimilation and plant growth are often limited on N-poor soils, as shown in *Quercus virginiana* Mill., *Quercus ilex* L. and *Quercus robur* L. (Tognetti and Johnson 1999*a*, 1999*b*, Maillard et al. 2001). In contrast, increasing N supplies can significantly enhance growth responses of trees to elevated $[CO_2]$ and modify C and N dynamics (Bauer et al. 2001, Maillard et al. 2001, Dyckmans and Flessa 2002).

Unlike annual plants, which respond to a CO_2-enriched atmosphere without carry-over effects of an earlier lower $[CO_2]$, except as mediated by an effect on seeds, perennial organs of trees may show cumulative effects of elevated $[CO_2]$ (Körner 2006). Internal reserves of stems and roots play an essential role during winter and early spring by supplying C and N for both maintenance metabolism and new growth, and their seasonal dynamics are central to the responses of trees to elevated $[CO_2]$.

Nitrogen availability during growth has marked effects on development and N storage capacity of temperate trees (Stephen et al. 2001). Although the effects of elevated $[CO_2]$ on soil-N uptake during the growing season have been documented, there have been few studies on N remobilization in trees growing in an elevated $[CO_2]$ atmosphere. Increased growth and storage sink capacity in response to elevated $[CO_2]$ may increase the extent of N storage and remobilization in several tree species (Temperton et al. 2003, Dyckmans and Flessa 2005). Changes in soil fertility may also alter the seasonal dynamics of storage and remobilization of C and N in forest trees and play an important role in the long-term responses to ele-

vated [CO_2].

Seasonal dynamics of C (Barbaroux et al. 2003) and N (Cheng and Fuchigami 2002, Gomez and Faurobert 2002) reserves have usually been studied separately, although C and N have interactive effects on vegetative development (Sauter and van Cleve 1994, Bollmark et al. 1999, Dyckmans et Flessa 2001, Cerasoli et al. 2004). Furthermore, there have been few studies on the dynamics of C and N reserves in forest trees in response to climate change.

Our aim was to examine the effects of N availability on storage and remobilization of C and N in *Quercus robur* saplings grown for 2 years in elevated [CO_2]. Saplings were subjected to long-term dual labeling with ^{13}C and ^{15}N isotopes at the end of the first growing season, allowing quantification of the effects of N availability and elevated [CO_2] on the accumulation of C and N compounds during the autumn, and their remobilization in spring.

Materials and methods

Plant material and cultivation

Two hundred acorns (*Quercus robur* L., Heuilly sur Saône, NE France) were sown in 5-l plastic containers filled with vermiculite. Half of the containers were placed in a 50-µm-thick transparent polypropylene tunnel (5 × 3 × 2.3 m) and exposed continuously to ambient atmospheric [CO_2] (A, 390 ± 30 µmol mol^{-1}). The other containers were placed in an identical tunnel, but exposed to elevated atmospheric [CO_2] (E, 700 ± 50 µmol mol^{-1}). The tunnels (A, E) were located in a greenhouse at INRA-Nancy, France. Seedlings in the A and E treatments were subdivided into two groups that were watered to field capacity twice daily with a complete nutrient solution (Le Blevennec 1986) containing either 6.1 (High N: HN) or 0.61 mmol N l^{-1} (Low N: LN). The seedlings were assigned to the N treatments in a full factorial design (A LN, A HN, E LN, E HN). To reduce the effect of environmental heterogeneity, plants were randomized within the tunnels throughout the experiment. Atmospheric [CO_2] in each tunnel was continuously monitored by infrared gas analyzers (ADC 225 MK3, U.K.) and controlled by an automated regulation system (Vivin et al. 1995, Maillard et al. 2001). Photosynthetic photon flux in the tunnels was about 60% of full sunlight and never exceeded 1400 µmol m^{-2} s^{-1}. Air temperature ranged from 15 to 25 °C and was maintained above 8 °C during cold periods. Vapor pressure deficit ranged from 1.01 to 2.02 kPa.

^{13}C and ^{15}N labeling

Ten saplings were randomly selected from each treatment and labeled during three 2-day cycles over one month [from October to November (Year 1)] with $^{13}CO_2$ and $^{15}NH_4NO_3$. The selected saplings were placed in a VTPH 5/1000 controlled environment chamber (Vötsch Industrietechnik GmbH, Reiskirchen-Lindenstruth, Germany) operated as a semi-closed system. The following day, each plant container was supplied with 20 ml of 10 mM $^{15}NH_4NO_3$ solution (Eurisotop, Gif sur Yvette, France). Saplings were exposed for two consecutive days to $^{13}CO_2$-enriched air (4.4 atom% ^{13}C) with a [CO_2] of either 390 or 700 µmol mol^{-1}. Chamber temperature was 20 ± 1 °C and relative humidity was 97%. Three high-pressure sodium vapor discharge lamps (SONT, Philips Electronics N.V., Amsterdam) provided a photosynthetic photon flux of 350 µmol m^{-2} s^{-1} at plant level. After each labeling cycle, labeled plants were returned to their respective tunnel.

At the end of the labeling period, roots of labeled saplings were washed to eliminate unabsorbed ^{15}N. Roots of unlabeled saplings were washed in the same way. All saplings were repotted before winter (Year 1) in 7-l plastic containers to prevent root confinement, and returned to their respective tunnel.

Sampling and growth measurements

For each treatment, half of the labeled saplings were harvested during dormancy (January of Year 2) and the remaining saplings were harvested in April of Year 2 when leaves of the new shoots had reached maturity. On each sampling date, three unlabeled saplings were harvested to assess baseline abundance of ^{13}C and ^{15}N. Harvested saplings were separated into old stem, large (diameter > 2 mm) and small (diameter < 2 mm) roots, and in April only, new leaves and new stems. Roots were washed to remove vermiculite and nutrient solution. In April, numbers and lengths of new shoots were measured. Leaf area was measured with a planimeter (Delta-T Devices, Cambridge, U.K.). To assess leaf N retranslocation efficiency, mature leaves and litter of eight unlabeled saplings were harvested in November (Year 1) and in January (Year 2), respectively. Plant material was frozen in liquid nitrogen, freeze-dried, weighed and ground to a fine powder with a laboratory mill (Tecator, Cyclotec 1093, Höganäs, Sweden) for C and N analyses.

Isotopic analyses

Total C and N contents and concentrations, and $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios of plant tissues were measured with an elemental analyzer (NA 1500 NCS, Carlo Erba, Milan, Italy) coupled to a Delta-S isotopic ratio mass spectrometer (Finnigan–Mat, Thermoquest Corp., San Jose, CA).

Isotopic abundance (atom% or *A*%) for C and N was computed as:

$$A_C = \frac{^{13}C}{^{12}C + ^{13}C} 100$$

$$A_N = \frac{^{15}N}{^{14}N + ^{15}N} 100$$

The contributions of C and N assimilated during spring growth (Year 2) and C and N remobilized from perennial organs were calculated with equations of isotopic dilution (Deléens et al. 1994). For simplicity, only variables related to C are shown. The corresponding variables for N can be defined by substituting ^{13}C and ^{12}C with ^{15}N and ^{14}N, respectively. Atom excess % is defined as the difference between the isotopic abundance of a given plant organ following administration of the ^{13}C or ^{15}N tracers ($A_{C,lab}$%), and the ^{13}C or ^{15}N natural

abundance measured for the same component of an unlabeled plant ($A_{C,unlab}$%).

$$^{13}C_{excess}\% = 100(A_{C,lab}\% - A_{C,unlab}\%)$$

Isotopic abundance (A%) of C in each plant organ between January (dormancy period, Year 2) to April (maturity of new spring leaves, Year 2) depended on both the relative contribution of C derived from labeled reserves (X_C, old C) of dormant plants and C assimilated during new leaf expansion (Y_C, new C) with $X_C + Y_C = 1$:

$$A_{C,lab,org}\%(X_C + Y_C) = X_C(A_{C,lab,pl}\%|_{Jan}) + Y_C(A_{C,unlab,org}\%)$$

where X_C, the proportion of old C, was estimated for a given plant as:

$$X_C = \frac{A_{C,lab,org}\%|_{Apr} - A_{C,unlab,org}\%}{A_{C,lab,pl}\%|_{Jan} - A_{C,unlab,org}\%}$$

Isotopic abundance for ^{13}C or in labeled plants ($A_{C,lab,pl}$) in January is assumed to correspond to the amount of C available for remobilization. We did not assess such values but assumed that they were equal to those of bulk plant material (Pellicer et al. 2000, Maillard et al. 2004).

Total C content (C_{tot}) comprised old C (C_{old}, incorporated before January) and new C (C_{new}, incorporated between January and April). Old C was incorporated in perennial organs of the labeled plant, and C_{new} was incorporated in newly formed leaves of the labeled plant. Thus, $C_{tot} = C_{old} + C_{new}$, where $C_{old} = X_C C_{tot}$ and $C_{new} = (1 - X_C)C_{tot}$.

Retranslocation efficiency from senescing leaves

Autumnal N retranslocation efficiency (RE_N, %) from senescing leaves was calculated according to Wang et al. (2003):

$$RE_N = \frac{N_{old}|_{Nov} - N_{litter}|_{Jan}}{N_{old}|_{Nov}} 100$$

where N_{old} is N content in old leaves, and N_{litter} is N content in litter.

Carbon and nitrogen remobilization

In perennial organs, the difference between total C (or N) content (C_{org}) in January and old C (or N) content ($C_{old,org}$) in April indicated the amount of stored C (or N) mobilized between January and April. Remobilization of C (RM_C, %) and N (RM_N, %) were calculated as:

$$RM_{C(or\ N)} = \frac{C(or\ N)_{org}|_{Jan} - C(or\ N)_{old,org}|_{Apr}}{C(or\ N)_{org}|_{Jan}} 100$$

Near-infrared spectroscopic measurements

Concentrations of soluble sugars, starch, total free amino acids, total soluble proteins, C and N of all samples of organs with a total dry mass of more than 1 g, and for each date, were determined by near-infrared spectroscopy (NIRSystems 6500, Foss NIRSystems, Raamsdonksveer, The Netherlands) as described by Joffre et al. (1992). Each sample was milled to a fine powder (Cyclotec 1093 Sample Mill, Tecator, Höganäs, Sweden) and scanned by NIRS.

Three distinct populations of NIR spectra, corresponding to leaves, stems and roots, were considered. Independent calibration equations between spectral and chemical data were built for each population with a partial least squares (PLS) algorithm (Martens and Naes 1989, Shenk and Westerhaus 1991*a*). About 60 samples were selected per population, based on Mahalanobis distances between all pairs of spectra (Select procedure of ISI software, see Shenk and Westerhaus 1991*b*), and analyzed for soluble sugars, starch, total free amino acids, total soluble proteins, carbon and nitrogen as described below. Chemical concentrations of all samples were obtained with standard errors for leaves, stems and roots, respectively, of 0.59, 0.45 and 0.75% for soluble sugars; 0.61, 0.70 and 0.70% for starch; 0.32, 1.07 and 1.07% for total free amino acids; 1.68, 1.12 and 1.12% for total soluble proteins; 0.41, 0.72 and 0.66% for carbon; and 0.10, 0.05 and 0.04% for nitrogen.

Carbohydrate analysis for NIRS calibration

Soluble sugars were extracted twice from 5–10 mg of ground sample by incubating in 80% boiling ethanol (1 ml) for 30 min and centrifuging for 10 min at 12,620 *g*. The pellet was retained, and the supernatants were combined and dried overnight in a vacuum-evaporator to eliminate ethanol. Dried extracts were solubilized in 0.5 ml of 0.02 *N* NaOH, and the fructose and sucrose converted to glucose equivalents (Boehringer 1984). Total glucose was quantified at 340 nm by an enzymatic method (hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49; Boehringer 1984). Starch was extracted from the pellet after extraction of soluble sugars by boiling for 1 h in 0.02 N NaOH, and hydrolyzed to glucose with α-amyloglucosidase (EC 3.2.1.3, Boehringer Manheim Biochemicals, Mannheim, Germany) in 0.32 M citrate buffer, pH 4.2 at 48 °C for 30 min. Glucose was assessed as described by Boehringer (1984).

Total soluble protein analysis for NIRS calibration

Total soluble proteins were extracted from 30 mg of ground tissue of each organ at 4 °C in 4 ml of 0.1 M phosphate buffer (pH 7.38) containing 60% (w/w) polyethylene glycol (PEG 20,000), polyvinyl-pyrrolidone (PVP) and 5 mM dithiothreitol. Concentrations of PVP varied with plant organ: 7% (w/w) PVP 40,000 for stems and large and small roots, and 3.75% (w/w) PVP 40,000 for leaves. Samples were sonicated (Bransonic 2510, Branson Ultrasonic Corporation, USA) for 30 min and centrifuged for 15 min at 12,620 *g*. Each supernatant was kept and the extraction was repeated twice on the pellet. The three supernatants were combined and total soluble protein concentration assessed colorimetrically at 595 nm according to Bradford (1976), using Coomassie Blue G 250

(Bio-Rad, 500-0006), with bovine serum albumin as the standard. Assuming that proteins contain about 22.6% N (Yeoh and Wee 1994), results were expressed as mg soluble protein N $(100\ g_{DM})^{-1}$.

Total amino acid analysis for NIRS calibration

Free amino acids were extracted from 10 mg of ground tissue for 10 min at 4 °C in 650 µl of 7:3 (v/v) methanol:water (MW). The mixture was centrifuged for 5 min at 12,620 *g*. The supernatant was retained, and the pellet was rinsed twice with 2 ml of MW and collected each time by centrifuging at 12,620 *g* for 15 min. The supernatants were combined and the free amino acid concentration was determined colorimetrically at 570 nm by the ninhydrin method (Yemm and Cocking 1955), with leucine as the standard. Assuming that mean N concentration of free amino acids is 13%, results were expressed as mg amino acid N $(100\ g_{DM})^{-1}$.

Statistical analysis

The experimental design was a full factorial with two factors: $[CO_2]$ (ambient and elevated) and N fertilization (LN and HN). Treatment effects were assessed by a two-way analysis of variance (ANOVA) followed by Tukey's test.

Results

Nitrogen mobilization at leaf fall

By November of Year 1, HN increased total leaf N content 400%, whereas $[CO_2]$ had no effect on leaf N content (Figure 1a). Leaf N concentration was reduced by both elevated $[CO_2]$ and LN (Figure 1b), but there was no $[CO_2] \times N$ interaction. Leaf N represented about 30% of sapling N in all treatments (Figure 1c).

In January, litter N content, representing N lost during leaf fall, was higher in the HN treatment than in the LN treatment. Nitrogen retranslocation efficiency from senescing leaves was higher in LN saplings (60%) than in HN saplings (35%) (Figure 1a). However, in all treatments, N lost in litter equaled about 15% of sapling N (Figure 1c).

Carbon and nitrogen storage and remobilization

Accumulations of C and N were strongly enhanced by HN, with elevated $[CO_2]$ having an additive effect (Figures 2a and 3a) in all perennial organs (Figures 2b, 2d, 2f, 3b, 3d and 3f). Total C and N contents of saplings (Figures 2a and 3a) and total C content of perennial organs did not change significantly from January to April (Figures 2b, 2d, and 2e). In contrast, total N content of old stems and large roots decreased between January and April (Figures 3b and 3d) but remained constant in fine roots (Figure 3f). In April, about 15% of total C and 40% of total N were allocated to new leaves (Figures 2e and 3e).

Old C was mobilized rapidly between January and April in perennial organs, especially of E HN saplings (Figure 2b and 2d). In old stems, remobilization accounted for 50–58% of C reserves, and up to 75% in old stems of E HN saplings. In large roots of HN saplings, 55–68% of the old C was mobilized. Old N was mobilized strongly in perennial organs and at a similar rate in all treatments (Figures 3b and 3d). Nitrogen remobilization accounted for 65–79% in old stems, and for 56–69% in large roots.

Perennial organs of HN saplings had high N concentrations (Table 1). Nitrogen concentrations of old stems and large roots decreased in response to elevated $[CO_2]$ with a $[CO_2] \times N$ interaction for large roots (Table 1). Nitrogen concentrations of old stems and large roots declined sharply from January to April (Table 1). Elevated $[CO_2]$ significantly reduced N concentrations in leaves and new stems of LN saplings (Table 1).

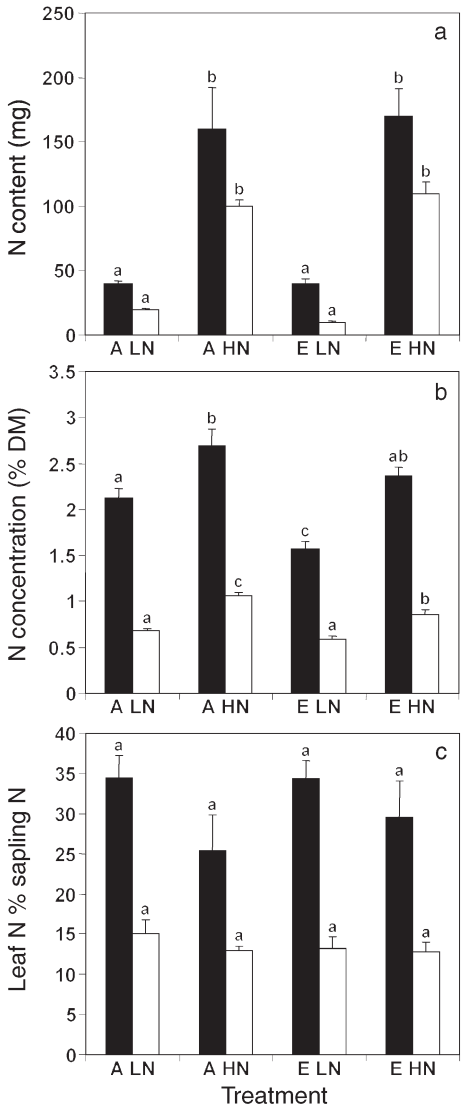

Figure 1. Nitrogen budget of old leaves (sampled in November, Year 1, filled bars) and litter (sampled after leaf fall in January, Year 2, open bars) of *Quercus robur* saplings grown in ambient (A) or elevated (E) $[CO_2]$ with low (LN) or high (HN) nitrogen fertilization. Significant treatment effects and their interactions are indicated by different letters (two-way ANOVA followed by Tukey's test, $P < 0.05$). Values are means ± SE ($n = 8$).

[CO_2] and N supply and starch and soluble sugar storage and remobilization

In January, starch concentration was greatest in large roots (35–50% DM), followed by small roots (10–20%) and old stem (10%) (Table 2). Elevated [CO_2] had no effect on starch concentrations of perennial organs, whereas LN increased the starch concentration in large roots (A: +17%, E: +31%), decreased it in small roots (A: –35%, E: –43%) and had no effect on starch concentration in old stems (Table 2). Soluble sugar concentrations were similar in all treatments, except in old stems of E LN saplings which had increased soluble sugar concentrations (Table 2).

From January to April, starch concentrations decreased in large and small roots in all treatments, and in old stems of HN saplings (Table 2). Soluble sugar concentrations decreased in old stems of LN saplings and strongly increased in large roots of HN saplings (Table 2). In April, starch concentration of new leaves was markedly increased by both elevated [CO_2] and LN (Table 2), and a similar but less pronounced effect was observed in new stems. The HN treatment increased the proportion of soluble sugars in new organs with no [CO_2] effect (Table 2).

[CO_2] and N supply and N storage and remobilization

Soluble protein and amino acid N concentrations represented less than 0.25% of organ dry mass and about 20 and 13% of their total N, respectively (Table 1). In January, HN greatly increased soluble protein and amino acid N concentrations. Elevated [CO_2] decreased soluble protein N (on average 17%) and amino acid N (37%) concentrations in HN saplings but had no effect in LN saplings (Table 1).

From January to April, soluble protein N concentrations decreased significantly in old stems in all treatments. Amino acid N concentrations were decreased in both old stems and large roots in all treatments (Table 1). In April, soluble protein N concentration in new leaves was higher than in perennial organs. The HN treatments greatly increased soluble protein and amino acid N concentrations in new organs, and this effect was lessened by elevated [CO_2] (Table 1).

Carbon dioxide concentration, nitrogen supply and spring growth

Spring growth was markedly increased by HN leading to large changes in development and organogenesis of axillary organs (Table 3). Lengths of new axes and numbers of branches and leaves were significantly increased in HN saplings compared with LN saplings (Table 3). In contrast, the N treatments had no effect on specific leaf area (SLA). Sapling morphology was modified by elevated [CO_2] (Table 3): branch length, main leaf area and SLA were markedly decreased, whereas numbers of

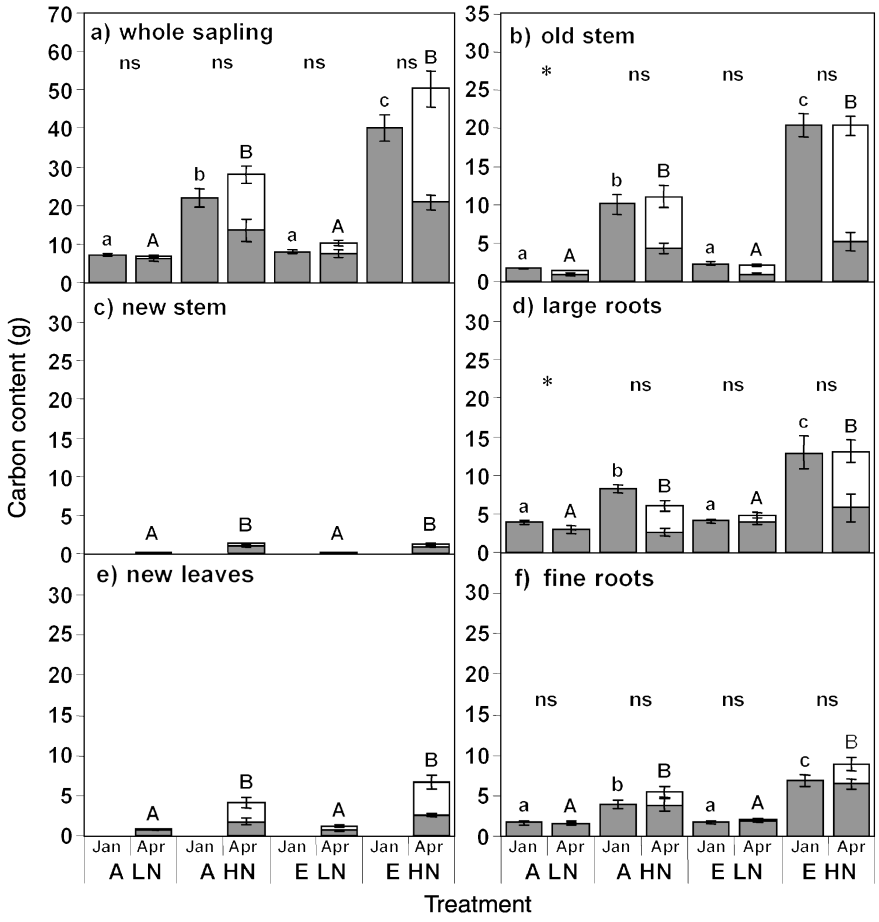

Figure 2. Old (filled bars) and new (open bars) C contents in whole saplings and organs of *Quercus robur* grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization, sampled in winter (January, Year 2) and after spring shoot flush (April, Year 2). Means ± SE ($n = 8$). For each organ, the significance of treatment effects and their interaction on total C are indicated by different letters (lowercase for January; uppercase for April) (two-way ANOVA followed by the Tukey test, $P < 0.05$). For whole saplings (a) and perennial organs (b, d, f), significant differences in total C content between January and April are indicated at the top of each figure (ns, non significant; *, $P < 0.05$).

branches and axillary leaves were strongly increased. The root:shoot ratio was strongly increased by LN, particularly in the ambient [CO_2] treatment (Table 3).

[CO_2] and N supply and partitioning of newly assimilated C and N between new and old organs

In April, new C and N uptake in whole saplings depended more on N availability than on [CO_2] (Figures 2a and 3a). At the end of leaf expansion, 50–60% of the C in HN saplings originated from assimilation compared with only 10–20% in LN saplings (Figure 2a). New N was incorporated in HN saplings only and represented less than 20% of total N (Figure 3a). New leaves contained 50–60% of new C, except in A LN saplings, where new C represented only 15% of total C (Figure 2e). The fraction of new C in new stems was low and never exceeded 20% of total C (Figure 2c) in HN saplings. New organs were built mostly with old N (75–95% of total N) (Figures 3c and 3e). New C and N were found in perennial organs (Figures 2b, 2d, 2f, 3b, 3d and 3f), with the largest accumulation occurring in old stems with about 45% of total new C and 33–49% of new N (Figures 2b and 3b).

Discussion

Elevated [CO_2] and N fertilization increased second-year growth of *Quercus robur* saplings, with N treatment having the greatest effect. This finding is in agreement with earlier results for *Quercus virginiana*, *Quercus ilex* (Tognetti and Johnson 1999*a*, 1999*b*) and *Quercus robur* (Maillard et al. 2001). Elevated [CO_2] induced similar changes in several other tree species (Saxe et al. 1998), but the physiological mechanisms controlling these changes remain poorly understood. Cell division and cell expansion may be affected, driven mainly by increased substrate availability and by differential expression of genes involved in cell cycling or cell expansion mediated by plant hormones (Pritchard et al. 1999). Low N supply typically induces a higher root:shoot ratio, an adjustment in biomass allocation in response to an imbalance of exogenous resources that tends to increase the supply of the limiting resource (Marschner 1995, Hermans et al. 2006).

Although elevated [CO_2] and HN stimulated growth of new shoots (Table 3) at the sapling level (Figures 2a and 3a), we observed no differences in total C and N contents between winter and spring, indicating that new C assimilation and N uptake were not achieved or were compensated by respiratory losses, and that initial new growth measured in April depended on C and N stored in perennial organs. We hypothesized that elevated [CO_2] and N fertilization increased the storage of both C and N compounds during the previous year and their remobilization at the beginning of the following spring.

In January, all perennial organs, especially those of HN saplings, had accumulated more C (Figures 2b, 2d and 2e) and more N (Figures 3b, 3d and 3e) in the elevated [CO_2] treatment than in the ambient [CO_2] treatment. The higher C:N ratio of saplings in the elevated [CO_2] treatment than in the ambient

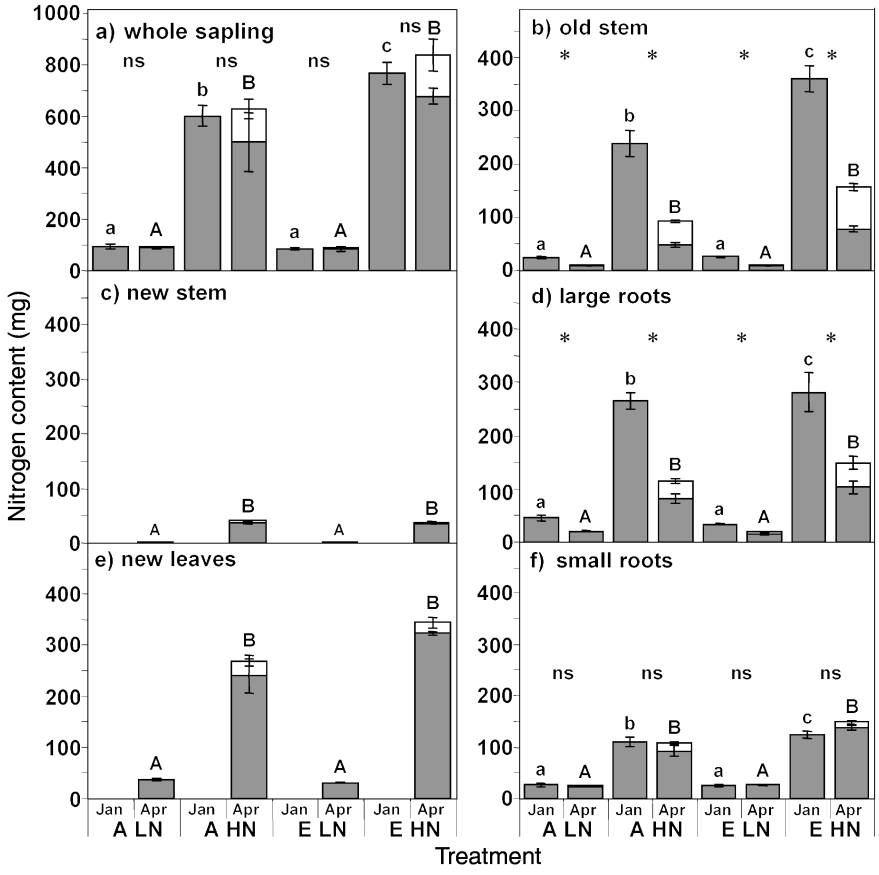

Figure 3. Old (filled bars) and new (open bars) nitrogen contents in whole saplings and individual organs of *Quercus robur* grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization, sampled in winter (January, Year 2) and after the new spring flush was expanded (April, Year 2). Means ± SE ($n = 8$). For each organ, the significance of treatment effects and their interaction on total N are indicated by different letters (lowercase for January, uppercase for April) (two-way ANOVA followed by the Tukey test, $P < 0.05$). For whole saplings (a) and perennial organs (b, d, f), significant differences in total N content between January and April are indicated at the top of each figure (ns, non significant; *, $P < 0.05$).

$[CO_2]$ treatment indicated that the additional increase in C was higher than the increase in N. Maillard et al. (2001) reported that, in 1-year-old oak saplings, elevated $[CO_2]$ and HN increased net CO_2 assimilation more than ^{15}N uptake, creating a C:N ratio imbalance in summer that was maintained after leaf shedding. Moreover, during leaf shedding the previous year, HN saplings recovered N from senescing leaves less efficiently than LN saplings (Figure 1a). Similar behavior was observed in N-deficient *Malus domestica* Borkh. and *Fagus sylvatica* L. (Millard and Thomson 1989, Dyckmans and Flessa 2001). These findings led us to hypothesize that N recovery from old leaves is more essential for survival and spring growth in N-deficient trees than in well-fertilized trees.

In January, oak saplings stored C as starch mainly in large roots, and starch concentrations were higher in LN saplings than in HN saplings with no effect of elevated $[CO_2]$ (Table 2). Nitrate limitation typically leads to large increases in starch concentration in plants (Stitt and Krapp 1999, Cheng and Fuchigami 2002). It has been assumed that starch accumulation is a passive response to decreased rates of growth. Conversely, in well-fertilized oak saplings, lower starch concentrations, even under elevated $[CO_2]$ conditions, imply that structural compounds account for a larger proportion of total biomass. Reduced starch accumulation could be related both to increased carbon requirements for the assimilation of inorganic N and to rapid consumption for the synthesis of structural compounds that occurred during the first year, as also shown in *Pinus nigra* L. (Luo et al. 2006).

In January, elevated $[CO_2]$ decreased N concentrations in all perennial organs only in the HN saplings (Table 1). This effect has been interpreted in different ways: higher nitrogen-use efficiency, higher growth under an elevated $[CO_2]$, accelerated senescence, and inadequate N fertilization or N uptake (Stitt and Krapp 1999). Nitrogen was stored in both soluble proteins and amino acids in all perennial organs. The HN treatment typically increased soluble protein and amino acid N storage in perennial organs (Table 1), but this effect was reduced by elevated $[CO_2]$. The HN-induced increase in amino acid N concentration was greater than the increase in soluble protein N concentration with the result that the ratio of soluble protein N to amino acid N was reduced by fertilization. Preferential storage of additional N in free amino acids rather than proteins in

Table 1. Nitrogen (N), soluble protein and amino acid N concentrations (g (100 g_{DM})$^{-1}$) in organs of *Quercus robur* saplings grown in ambient (A) or elevated (E) $[CO_2]$ with low (LN) or high (HN) nitrogen fertiliation. Saplings ($n = 8$) were sampled in winter (January, Year 2) and after the new spring flush had expanded (April, Year 2). Significance of treatment effects and their interaction are indicated by asterisks: ns, non significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Within a row, different lowercase letters indicate significant differences between treatments (two-way ANOVA, $P < 0.05$). Different uppercase letters indicate significant differences between January and April within a treatment.

Organ	Month	Treatment				Significance of treatment effects		
		A LN	A HN	E LN	E HN	$[CO_2]$	N	$[CO_2] \times N$
Nitrogen concentration (% DM)								
Old stem	January	0.68 ab B	1.12 c B	0.56 a B	0.82 b B	***	***	ns
	April	0.38 b A	0.48 c A	0.27 a A	0.39 b A	***	***	ns
Large roots	January	0.50 a B	1.40 c B	0.38 a B	0.98 b B	***	***	*
	April	0.32 ab A	0.99 c A	0.21 a A	0.60 b A	**	***	ns
Small roots	January	0.78 a A	1.31 b A	0.74 a A	0.86 a A	ns	***	ns
	April	0.75 a A	1.11 b A	0.66 a A	0.86 a A	**	***	ns
New leaves	April	2.08 b	3.17 d	1.24 a	2.49 c	***	***	ns
New stems	April	0.86 b	1.41 c	0.63 a	1.21 c	**	***	ns
Soluble protein nitrogen concentration (% DM)								
Old stem	January	0.16 a B	0.20 b B	0.14 a B	0.17 a B	**	***	ns
	April	0.10 a A	0.12 b A	0.08 a A	0.10 a A	***	***	ns
Large roots	January	0.11 a A	0.21 c B	0.09 a B	0.16 b A	***	***	*
	April	0.09 ab A	0.17 c A	0.05 a A	0.12 b A	***	***	ns
Small roots	January	0.18 a A	0.26 c A	0.18 a A	0.22 b A	**	***	*
	April	0.17 a A	0.25 b A	0.17 a A	0.20 a A	*	***	**
New leaves	April	0.36 b	0.55 d	0.28 a	0.46 c	***	***	ns
New stems	April	0.18 a	0.28 b	0.16 a	0.27 b	ns	***	ns
Amino acid nitrogen concentration (% DM)								
Old stem	January	0.07 a B	0.15 c B	0.06 a B	0.11 b B	**	***	*
	April	0.02 a A	0.04 b A	0.01 a A	0.02 a A	**	***	ns
Large roots	January	0.10 a B	0.33 c B	0.08 a B	0.23 b B	**	***	*
	April	0.05 ab A	0.21 c A	0.01 a A	0.13 b A	**	***	ns
Small roots	January	0.04 a A	0.15 b B	0.04 a A	0.07 a A	***	***	***
	April	0.05 ab B	0.08 b A	0.02 a A	0.05 ab A	**	**	ns
New leaves	April	0.03 b	0.04 c	0.02 a	0.03 b	***	***	ns
New stems	April	0.03 a	0.12 b	0.02 a	0.09 b	*	***	ns

perennial organs was also shown in apple trees (Cheng et al. 2004). This mechanism provides saplings the advantage that amino acids are readily available when growth resumes in spring (Cheng et al. 2004). Elevated [CO_2] decreased both soluble N concentration and total N concentration in fertilized oak saplings.

By using ^{15}N- and ^{13}C-labeling of reserves before winter, we demonstrated that spring growth of *Quercus robur* was strongly controlled by the availability of internal resources. About 20% of old C (Figures 2c and 2e) and 50% of old N

Table 2. Starch and soluble sugar concentrations (g 100 g_{DM})$^{-1}$) in organs of *Quercus robur* saplings grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization. Saplings ($n = 8$) were sampled in winter (January; Year 2) and after the new spring flush had expanded. (April; Year 2). Significance of treatment effects and their interaction are indicated by asterisks: ns, non significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Within a row, different lowercase letters indicate significant differences between treatments (two-way ANOVA, $P < 0.05$). Different uppercase letters indicate significant differences between January and April within a treatment.

Organ	Month	Treatment				Significance of treatment effects		
		A LN	A HN	E LN	E HN	[CO_2]	N	[CO_2] × N
Starch concentration (% DM)								
Old stem	January	8.10 a A	8.21 a B	8.22 a A	7.31 a B	ns	ns	ns
	April	8.86 b A	1.96 a A	9.87 b A	2.09 a A	ns	***	ns
Large roots	January	46.29 b B	39.59 a B	46.32 b B	35.19 a B	ns	***	ns
	April	29.19 b A	8.70 a A	37.92 b A	15.13 a A	**	***	ns
Small roots	January	12.42 a B	18.92 b B	11.68 a B	20.36 b B	ns	***	ns
	April	4.97 ab A	1.94 a A	5.10 ab A	6.00 b A	*	ns	*
New leaves	April	5.92 b	1.23 a	15.84 c	5.01 ab	***	***	*
New stems	April	4.75 b	1.97 a	8.27 c	1.88 a	*	***	*
Soluble sugar concentration (% DM)								
Old stem	January	3.33 ab B	3.14 a A	3.87 b B	3.29 ab A	ns	*	ns
	April	1.93 a A	4.06 b A	1.97 a A	3.79 b A	ns	***	ns
Large roots	January	3.48 a A	3.07 a A	3.82 a A	3.03 a A	ns	ns	ns
	April	6.22 b A	9.01 c B	4.54 a A	7.08 b B	***	***	ns
Small roots	January	2.88 a A	3.17 a A	3.13 a A	3.36 a A	ns	ns	ns
	April	2.48 a A	4.91 b B	2.64 a A	4.00 b A	ns	***	ns
New leaves	April	3.48 a	7.71 b	3.91 a	7.29 b	ns	***	ns
New stems	April	2.23 a	5.60 b	2.36 a	5.87 b	ns	***	ns

Table 3. Growth variables of new shoots and biomass root: shoot ratio of *Quercus robur* saplings grown in ambient (A) or elevated (E) [CO_2] with low (LN) or high (HN) nitrogen fertilization. Saplings ($n = 8$) were sampled after the new spring flush had expanded (April, Year 2). Significance of treatment effects and their interaction are indicated by asterisks: ns, non significant; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Within a row, different letters indicate significant differences between treatments (two-way ANOVA followed by the Tukey test, $P < 0.05$).

Organ	Treatment				Significance of treatment effects		
	A LN	A HN	E LN	E HN	[CO_2]	N	[CO_2] × N
Length (cm)							
Main stem	11.90 a	23.40 b	10.00 a	14.90 ab	ns	**	ns
Branches	8.10 a	13.10 b	6.20 a	9.90 ab	*	**	ns
Number							
Branches	5.60 a	21.70 b	8.10 a	32.80 c	**	***	ns
Main leaves	9.10 a	12.80 a	8.90 a	11.90 a	ns	*	ns
Axillary leaves	19.50 a	200.20 b	42.40 a	354.10 c	**	***	*
Area (dm^2)							
Main leaves	3.91 ab	5.08 b	2.02 a	2.32 ab	**	ns	ns
Axillary leaves	2.72 a	34.82 b	4.19 a	32.55 b	ns	***	ns
Specific leaf area ($dm^2\ g^{-1}$)							
Main leaves	2.63 b	2.41 b	1.98 a	2.22 ab	***	ns	*
Axillary leaves	3.16 c	2.73 b	2.16 a	2.49 ab	***	ns	***
Root:shoot ratio	3.87 c	1.31 a	2.91 b	1.12 a	***	***	*

(Figures 3c and 3e) were used in oak saplings for shoot growth in spring.

Carbon of new stems originated almost entirely from internal C resources (Figure 2c). In contrast, new leaves comprised 60% new C (Figure 2e), this percentage being smaller in LN saplings than in HN saplings. Conversely, Dyckmans and Flessa (2001) reported that, 2 weeks after bud burst, 38.9 and 52.7% of leaf C in HN- and LN-pretreated 3-year-old beech trees was derived from new C. However, in our experiment, the total amount of new C in beech leaves was 43% lower in LN trees compared with HN trees, 12 weeks after bud burst. Elevated [CO_2] significantly modified branching architecture (i.e., leaf and branch numbers, branch length) of HN saplings (Table 3), resulting in increased leaf area and subsequent C acquisition in an expected positive feedback loop. In fully expanded leaves of E HN saplings, the large amount of new C, which was unaccompanied by starch accumulation (Table 2), may indicate efficient utilization of new C for structural syntheses as reported in leaves of northern red oak seedlings (Dickson et al. 2000).

Remobilization of internal C from perennial organs was increased by HN, and additional remobilization from old stems was observed in response to elevated [CO_2] (Figure 2). For HN saplings, not all old C mobilized from the perennial organs was allocated to new shoots (Figure 2), reflecting respiratory C losses during the winter (maintenance) and early spring (growth). Respiratory C loss was compensated by new assimilation because the total C content did not change between January and April (Figures 2b, 2d and 2f). Thus, HN saplings renewed 50–60% of their total C during April (Figure 2a).

Because allocation of new C occurred in perennial organs and we did not assess ^{13}C labeling of starch, we were unable to calculate remobilization (old C) and renewal (new C) in the total starch pool. However, differences in starch concentrations were observed between January and April, suggesting that starch remobilization occurred in most perennial organs (Table 2). Spring remobilization of starch often results in its depletion in perennial organs, and the lowest starch concentrations have been observed just before bud break in some deciduous trees (Witt and Sauter 1994, Piispanen and Saranpää 2001, Barbaroux et al. 2003). The extent of starch remobilization was higher in HN saplings than in LN saplings (Table 2). The decrease in starch concentration in HN saplings was associated with an accumulation of soluble sugars in large roots and no change in old stems. We hypothesized that, in old stems of HN saplings, a significant portion of the soluble carbohydrates formed during starch hydrolysis is promptly converted to structural carbon compounds or exported. In contrast, in the old stems of LN saplings, no starch remobilization occurred in April, resulting in greater starch accumulation compared with HN saplings, which was not observed in January.

Our labeling experiment confirmed that, until April, no major N absorption occurred to supply N for new spring growth (Figure 3). Nitrogen uptake occurred only in HN saplings, and some new N was allocated to new shoots and new leaves (15–20% of total new N). During this period, internal N stores were used for new growth has been shown in *Fagus sylvatica* L. (Dyckmans and Flessa 2002) and *Alnus glutinosa* (L.) Gaertn., despite the latter species being an N_2-fixer (Temperton et al. 2003). In oak saplings, the extent of N remobilization was unaffected by elevated [CO_2] (Figure 3) (cf. Maillard et al. 2001). In contrast, Temperton et al. (2003) reported that elevated [CO_2] increased N remobilization for leaf growth in *A. glutinosa* but not in *Pinus sylvestris* L. When leaves had just completed expansion, N concentrations of all organs were lower in E HN saplings than in A HN saplings (Table 1). This difference may be partly linked to a delay between new CO_2 assimilation and new N uptake, because the new C:new N ratio was higher in E HN saplings than in A HN saplings (Figure 3). Nitrogen remobilization from perennial organs was not compensated by new N accumulation, leading to decreased N content over time (Figures 3b and 3d). Several studies have reported that stored N is mobilized and used to support early shoot growth leading to a depletion of stored N in source organs such as branches (Millard 1996, Sauter and Wellenkamp 1998). Under natural conditions, replenishment of N reserves in perennial organs mainly occurs at the beginning of leaf yellowing during late summer and early fall (Sauter and Witt 1997), so that until this time, N uptake is necessary for further N reserve replenishment to occur. Even if soluble proteins were significantly mobilized to support spring growth (Table 1), our results show that amino acids were the most heavily mobilized N fraction, as observed in *Prunus persica* L. by Gomez and Faurobert (2002) who hypothesized that the role of storage proteins is linked to growth initiation in spring.

In conclusion, through primary effects on C and N storage compounds, elevated [CO_2] and N fertilization have indirect consequences on remobilization the following spring, which supports new growth of oak saplings. Our results demonstrated that initial spring growth was strongly controlled by the availability of internal N. The contrasting N storage capacities imposed during the first year affected both C and N remobilization and uptake the following year. New spring shoot architecture was modified by elevated [CO_2] and N fertilization, leading rapidly to more efficient C acquisition and N uptake. However, immediately following full leaf expansion, the delay between new CO_2 assimilation and N uptake resulted in a decrease in N concentrations in E HN saplings. During this period, internal cycling of N in the tree might help overcome N deficiency under elevated [CO_2] conditions. Our data demonstrate the importance of N storage and remobilization for the initial growth of oak saplings.

Acknowledgments

This work was supported financially by NETCARB (Network for Ecophysiology in Closing Terrestrial CARbon Budget, EU project) coordinated by J. Ghashghaie (UMR 8079, CNRS-Université Paris-Sud, Orsay, France). The authors thank J. Marchand and C. Bréchet (Stable Isotope Laboratory, UMR 1137, INRA Nancy, France) for elementary and mass spectrometry analyses. Prof. S. Thornton (Nancy Université) and Dr. E. Dreyer (INRA, Nancy) are acknowledged for helpful discussions and critical reading of the manuscript.

References

Barbaroux, C., N. Bréda and E. Dufrêne. 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*). New Phytol. 157:605–615.

Barnes, R.J., M.S. Dhanoa and S.J. Lister. 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. Appl. Spectrosc. 43:772–777.

Bauer, G.A., G.M. Berntson and F.A. Bazzaz. 2001. Regenerating temperate forests under elevated CO_2 and nitrogen deposition: comparing biochemical and stomatal limitation of photosynthesis. New Phytol. 152:249–266.

Boehringer, S.A. 1984. Methods of enzymatic food analysis using single reagents. Boehringer Mannheim GmbH, Mannheim, Germany, 79 p.

Bollmark, L., L. Sennerby-Forsse and T. Ericsson. 1999. Seasonal dynamics and effects of nitrogen supply rate on nitrogen and carbohydrate reserves in cutting-derived *Salix viminalis* plants. Can. J. For. Res. 29:85–94.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Cerasoli, S., P. Maillard, A. Scartazza, E. Brugnoli, M.M. Chaves and J.S. Pereira. 2004. Carbon and nitrogen winter storage and remobilisation during seasonal flush growth in two-year-old cork oak (*Quercus suber* L.) saplings. Ann. For. Sci. 61:721–729.

Ceulemans, R. and M. Mousseau. 1994. Effects of elevated atmospheric CO_2 on woody plants. Tansley Review No. 71. New Phytol. 127:425–446.

Cheng, L. and L.H. Fuchigami. 2002. Growth of young apple trees in relation to reserve nitrogen and carbohydrates. Tree Physiol. 22: 1297–1303.

Cheng, L., F. Ma and D. Ranwala. 2004. Nitrogen storage and its interaction with carbohydrates of young apple trees in response to nitrogen supply. Tree Physiol. 24:91–98.

Damesin, C., S. Rambal and R. Joffre 1997. Between-tree variations in leaf $\delta^{13}C$ of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability. Oecologia 111: 26–35.

Deléens, E., J.B. Cliquet and J.L. Prioul. 1994. Using ^{13}C and ^{15}N label near natural abundance for monitoring carbon and nitrogen partitioning. Aust. J. Plant Physiol. 21:133–146.

Dickson, R.E., P.T. Tomlinson and J.G. Isebrands. 2000. Partitioning of current photosynthate to different chemical fractions in leaves, stems, and roots of northern red oak seedlings during episodic growth. Can. J. For. Res. 30:1308–1317.

Dyckmans, J. and H. Flessa. 2001. Influence of tree internal N status on uptake and translocation of C and N in beech: a dual ^{13}C and ^{15}N labeling approach. Tree Physiol. 21:395–401.

Dyckmans, J. and H. Flessa. 2002. Influence of tree internal nitrogen reserves on the response of beech (*Fagus sylvatica*) trees to elevated atmospheric carbon dioxide concentration. Tree Physiol. 22:41–49.

Dyckmans, J. and H. Flessa. 2005. Partitioning of remobilised N in young beech (*Fagus sylvatica* L.) is not affected by elevated [CO_2]. Ann. For. Sci. 62:285–288.

Gillon, D., R. Joffre and A. Ibrahima. 1999. Can litter decomposability be predicted by near infrared reflectance spectroscopy? Ecology 80:175–186.

Gomez, L. and M. Faurobert. 2002. Contribution of vegetative storage proteins to seasonal nitrogen variations in the young shoots of peach trees. J. Exp. Bot. 53:2431–2439.

Hermans, C., J.P. Hammond, P.J. White and N. Verbruggen. 2006. How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. 11:613–617.

Joffre, R., D. Gillon, P. Dardenne, R. Agneessens and R. Biston. 1992. The use of near-infrared reflectance spectroscopy in litter decomposition studies. Ann. For. Sci. 49:481–488.

Körner, C. 2006. Plant CO_2 responses: an issue of definition, time and resource supply. New Phytol. 172:393–411.

Le Blevennec, L. 1986. Mise au point d'une solution nutritive pour les cultures annuelles et pérennes. Cahiers Sci. Techniq. INRA 14: 29–32.

Luo, Z.B., C. Calfapietra, M. Liberloo, G. Scarascia-Mugnozza and A. Polle. 2006. Carbon partitioning to mobile and structural fractions in poplar wood under elevated CO_2 (EUROFACE) and N fertilization. Global Change Biol. 12:272–283.

Maillard, P., J.M. Guehl, J.F. Muller and P. Gross. 2001. Interactive effects of elevated CO_2 concentration and nitrogen supply on partitioning of newly fixed ^{13}C and ^{15}N between shoot and roots of pedunculate oak seedlings (*Quercus robur*). Tree Physiol. 21: 163–172.

Maillard, P., D. Garriou, E. Deléens, P. Gross and J.M. Guehl. 2004. The effects of lifting on mobilisation and new assimilation of C and N during regrowth of transplanted Corsican pine seedlings. A dual ^{13}C and ^{15}N labelling approach. Ann. For. Sci. 61:795–805.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, San Diego, 889 p.

Martens, H. and T. Naes. 1989. Multivariate calibration. John Wiley & Sons, Chichester, U.K., 419 p.

Millard, P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. J. Plant Nutr. Soil Sci. 159:1–10.

Millard, P. and C.M. Thomson. 1989. The effect of the autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple trees. J. Exp. Bot. 40:1285–1289.

Pellicer, V., J.M. Guehl, F.A. Daudet, M. Cazet, L.M. Rivière and P. Maillard. 2000. Carbon and nitrogen mobilization in *Larix* × *eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labelling: relationships with rooting. Tree Physiol. 20:807–814.

Piispanen, R. and P. Saranpää. 2001. Variation of non-structural carbohydrates in silver birch (*Betula pendula* Roth) wood. Trees 15:444–451.

Pritchard, S.S., H.H. Rogers, S.A. Prior and C.M. Peterson. 1999. Elevated CO_2 and plant structure: a review. Global Change Biol. 5:807–837.

Sauter, J.J. and B. van Cleve. 1994. Storage, mobilization and interrelations of starch, sugars, protein and fat in the ray storage tissue of poplar trees. Trees 8:297–304.

Sauter, J.J. and S. Wellenkamp. 1998. Seasonal changes in content of starch, protein and sugars in the twig wood of *Salix caprea* L. Holzforschung 52:255–262.

Sauter, J.J. and W. Witt. 1997. Structure and function of rays: storage, mobilisation, transport. *In* Trees—Contribution to Modern Tree Physiology. Eds. W. Eschrich and H. Ziegler. Bachuys Publishers, Leiden, The Netherlands, pp 177–195.

Saxe, H., D. Ellsworth and J. Heath. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. Tansley Review No. 98. New Phytol. 139:395–436.

Schimann, H., R. Joffre, J.C. Roggy and R. Lensi. 2007. Evaluation of the recovery of microbial functions during soil restoration using near-infrared spectroscopy. Appl. Soil Ecol. 37:223–232.

Shenk, J.S. and M.O. Westerhaus. 1991*a*. Population definition, sample selection, and calibration procedures for near-infrared reflectance spectroscopy. Crop Sci. 31:469–474.

Shenk, J.S. and M.O. Westerhaus. 1991*b*. Population structuring of near-infrared spectra and modified partial least-squares regression. Crop Sci. 31:1548–1555.

Stephen, D.W., P. Millard, M.H. Turnbull and D. Whitehead. 2001. The influence of nitrogen supply on growth and internal recycling of nitrogen in young *Nothofagus fusca* trees. Aust. J. Plant Physiol. 28:249–255.

Stitt, M. and A. Krapp. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ. 22:583–621.

Temperton, V.M., P. Millard and P.G. Jarvis. 2003. Does elevated atmospheric carbon dioxide affect internal nitrogen allocation in the temperate trees *Alnus glutinosa* and *Pinus sylvestris*? Global Change Biol. 9:286–294.

Tognetti, R. and J.D. Johnson. 1999*a*. The effect of elevated atmospheric CO_2 concentration and nutrient supply on gas exchange, carbohydrates and foliar phenolic concentration in live oak (*Quercus virginiana* Mill.) seedlings. Ann. For. Sci. 56:379–389.

Tognetti, R. and J.D. Johnson. 1999*b*. Responses to elevated atmospheric CO_2 concentration and nitrogen supply of *Quercus ilex* L. seedlings from a coppice stand growing at a natural CO_2 spring. Ann. For. Sci. 56:549–561.

Vivin, P., P. Gross, G. Aussenac and J.M. Guehl. 1995. Whole-plant CO_2 exchange, carbon partitioning and growth in *Quercus robur* seedlings exposed to elevated CO_2. Plant Physiol. Biochem. 33: 201–211.

Wang, W.Q., M. Wang and P. Lin. 2003. Seasonal changes in element contents in mangrove element retranslocation during leaf senescence. Plant Soil 252:187–193.

Witt, W. and J.J. Sauter. 1994. Starch metabolism in poplar wood ray cells during spring mobilization and summer deposition. Physiol. Plant. 92:9–16.

Yemm, E.W. and E.C. Cocking. 1955. The determination of aminoacids with ninhydrin. Analyst 80:209–213.

Yeoh, H.H. and Y.C. Wee. 1994. Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant species. Food Chem. 49:245–250.