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Research

Respiration and photosynthesis characteristics of current-year stems of *Fagus sylvatica*: from the seasonal pattern to an annual balance

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Summary

- Temperature and light responses of CO_2 efflux of *Fagus sylvatica* (beech) current-year stems were measured for 1 yr to estimate their annual carbon balance.
- Gas exchanges were determined using infrared gas analysis. Seasonal patterns of a fluorescence parameter ($(F_v/F_m)_{max}$), nitrogen and chlorophyll contents were also assessed in stems and leaves, using standard techniques.
- Basal respiration rates at 20°C (R^{20}) were very high during the growing season, reaching a maximum of 17 170 μmol m^{-3} s^{-1}. Light-saturated assimilation followed the same seasonal pattern as R^{20}. During the winter, chlorophyll content was undiminished compared with the summer, N content was slightly increased, and despite low $(F_v/F_m)_{max}$ values, instantaneous maximum assimilation could account for 80–110% of the respiration.
- For an average-size stem (4 mm diameter), the estimated annual respiration was 0.5 g carbon with 55% of this amount attributed to maintenance respiration. The potential assimilation contributed 0.2 g carbon and approximately compensated for the growth respiration. Information on older branches and trunks is now needed for estimations at the tree and stand levels.

Key words: beech, chlorophyll, fluorescence, nitrogen, leaf, stem, respiration, assimilation.

Introduction

To determine whether forests are sinks or sources of carbon, the flux of CO_2 uptake and release must be quantified accurately. Characterization of leaf photosynthesis and its temporal and spatial variation in the canopy are well known compared with the knowledge on functioning of the woody tissues. Nevertheless, interest in stem respiration is increasing as many quantitative estimates have shown that it is a large component of the annual carbon balance of forest ecosystems. Total autotrophic respiration can consume more than 50% of the carbon fixed by leaves in forests (Ryan, 1991). In temperate deciduous forests, the trunk and branch respiration represents 33% to 37% of the total carbon loss by ecosystem respiration (Granier *et al.*, 2000; Janssens *et al.*, 2001), and up to 50% of the above-ground autotrophic respiration (Edwards *et al.*, 1981). Branch respiration could account for half of the total above-ground woody respiration (Damesin *et al.*, 2002), so both trunk and branch components should be considered in estimating the respiratory component at the stand level. So far, attention has been given primarily to trunks, and more information is needed on branch respiration characteristics, especially in young branches.

Respiration strongly depends on temperature. The relationship between air and tissue temperature and respiration is well described by an exponential equation in terms of Q_{10} (the change in respiration with a 10°C change in air temperature) and basal respiration at a given temperature. Generally, Q_{10} values are close to 2 and basal respiration shows large seasonal variations (Linder & Troeng, 1981; Damesin *et al.*, 2002; Edwards *et al.*, 2002). In some studies, Q_{10} clearly varied among months (Paembonan *et al.*, 1991; Lavigne, 1996). An increase in Q_{10} with decreasing stem diameter, and higher respiration rates for branches than for trunks (Möller *et al.*,

1954; Sprugel, 1990; Damesin *et al.*, 2002), may generally be explained by a higher proportion of living cells vs dead cells in smaller stems (Kramer & Kozlowski, 1979; Ryan, 1990; Stockfors, 2000). Given that small stems typically are nitrogen-enriched relative to larger stems, and that nitrogen content strongly affects maintenance respiration rates (Ryan, 1991; Maier *et al.*, 1998; Ceschia *et al.* 2002), high CO_2 efflux is expected in young stems even during the dormant season.

Under light, the stem CO_2 efflux could be diminished. It is already known that stems of woody species can assimilate CO_2. Pearson & Lawrence (1958) verified that pigments in *Populus tremuloides* stem tissue were indeed chlorophylls. Several studies conducted in the 1970s proved the existence of stem photosynthesis and quantified rates of carbon assimilation in a variety of species (Perry, 1971; Keller, 1973; Wiebe, 1975; Foote & Shaedle, 1976a). More recently, in the 1990s, efforts have mostly focused on furthering our understanding of stem photosynthesis from an ecophysiological point of view, and on considering its significance for the tree carbon gain (see Pfanz & Aschan, 2001). In general, young stems appear green during their first several weeks, with a thin bark which may transmit a large part of the incident light. These structural characteristics suggest that young stems may have a significant assimilation rate relative to the respiration rate. This assimilation has several advantages over leaf photosynthesis in that (1) there is very little associated water loss, and (2) it proceeds at high CO_2 concentrations and, consequently, is likely to promote little photorespiration. Thus, stem photosynthesis, also called 'refixation' for woody stems (i.e. stems without stomata), may be a means for improving the carbon balance of stems.

Beech is one of the main European temperate species and beech forests were among sites chosen for the European Research Programmes EUROFLUX and CARBOEUROFLUX. Carbon balance at the ecosystem level was established from eddy flux measurements associated with measurements at the organ level (Valentini *et al.*, 1996; Granier *et al.*, 2000). Nevertheless, to my knowledge, nothing was done on physiological performance of very young stems from adult trees. Hence, the objectives of the present study were:

1 To track the response of current-year stem respiration to air temperature throughout one year. This should allow us to determine whether it changes over the seasons, especially between the growth and nongrowth periods.

2 To determine the photosynthetic performance of young stems. The goal was to quantify assimilation rates and assess whether stem nitrogen content and photosystem II (PSII) functioning could explain seasonal variation.

3 To estimate the annual carbon flux of a current-year stem. In particular, I was interested in determining at the annual scale whether the CO_2 assimilation would compensate for the respiration loss.

To answer these questions, gas exchange, chlorophyll and nitrogen contents, and fluorescence parameters were measured in leafy stems of adult trees over 1 yr.

Materials and Methods

Site description

The experiment was conducted in a temperate climate on three isolated, adult *Fagus sylvatica* trees. The trees were growing on the campus of the University of Paris XI (48°50′ N, 02°10′ E), 25 km south-east of Paris, France, at an elevation of 65 m. The mean annual precipitation is 685 mm. The mean annual minimum and maximum temperatures were 7°C and 16°C, respectively (15-yr averages). More information on meteorological conditions for the year 2000–01 is presented in Damesin & Lelarge (2003). The trees were 20 yr old. Their mean height (± SD) was 6.7 m (± 0.3) and their mean circumference at 1.30 m was 65 cm (± 5).

All parameters were recorded on leaves and stems of young branches from the outer crown (at about 2 m height) through at least 1 yr. Short mature growth units were not considered. Almost all measurements were recorded from spring 2000 to spring or summer 2001 on stems produced in spring 2000. Fluorescence measurements were done on stems produced in spring 2001 for the time period from spring 2001 to summer 2002. For each recording date and each tree, leafy stem samples were taken from three aspects: south, east and west.

Gas exchange measurements

Gas exchanges were measured in the laboratory with a LI-6400 portable photosynthesis system and a 6400-05 conifer chamber (Li-Cor, Inc., Lincoln, NE, USA). This is a transparent, cylindrical chamber (7.5 cm diameter, 5 cm long) wherein temperature and CO_2 are controlled by the LI-6400 Portable Photosynthesis System. Once the sample was collected, the stem was immediately recut under distilled water in the section of tissue produced the previous year. After removal of the leaves, the current-year part of the stem was put in the chamber and the cut end was kept submerged in water during measurements. When the stem was longer than the chamber length, the second and the third internodes were put in the chamber.

Light and temperature responses were made at sufficient water supply and ambient CO_2 concentration (360 ppm). Dark respiration rates were determined by wrapping the chamber, and the section of stem outside the chamber, in a black cloth. The temperature was increased from 18 to 20°C to 30–32°C with four intermediate temperatures. The light response was performed at 25°C by increasing light intensities from 0 to approximately 500–700 μmol m^{-2} s^{-1}. A gallium arsenide phosphide PAR (photosynthetically active radiation) sensor, which was intercalibrated with the LiCor PAR sensor, was put in the conifer chamber near the sample. The stem was illuminated with a 90° angle of incidence to the top surface. Light was produced by an artificial source (Halogen Photo Optic Lamp Xenophot; Osram, München, Germany). The different

intensities were obtained using filters which blocked infrared radiation. The stem was allowed 15–20 min to acclimatize at each temperature or light condition. At each condition, CO_2 efflux was measured four times at 30-s to 1-min intervals. Gas exchange measurements under the various conditions described above were generally taken on three to six different stems (i.e. one or two stems per tree) over the course of several days. The mean date was used as the date for the graphs. The samples were measured in diameter and length, then oven-dried at 60°C for 48 h to express the CO_2 efflux in terms of the total dry mass, volume and area units (the total area was considered even if half of the stem was illuminated). Gas exchange measurements were done from April 2000 to June 2001.

Data analysis

The respiration rates were adjusted for temperature variation using a simple exponential equation (Eqn 1). Respiration rates (i.e. CO_2 efflux, R) were expressed in terms of Q_{10}, the change in rate with a 10°C change in temperature, as follows:

$$R = R_{Tb} Q_{10}^{(T-T_b)/10} \quad \text{Eqn 1}$$

where T is air temperature in Celsius, and R_{Tb} is the basal respiration rate. A basal temperature (T_b) of 20°C ($R_{Tb} = R_{20}$) was chosen. Thermocouple probes were used to check that there were no large differences (maximum 0.6°C) between the stem temperature, which is the more biologically relevant reference, and air temperature; R was the CO_2 efflux measured and R_{20} and Q_{10} values were calculated for each stem sample.

To separate maintenance and growth respiration, I defined the stem growth period as the time when biomass increase occurred; this was from the end of April to September, and corresponded to the period found for trunks (Lebaube *et al.*, 2000). Respiration measurements taken from November 2000 to March 2001 were considered to be outside the growth period. I determined Q_{10} and R_{20} monthly for growth respiration in May, June, July, August and September. I assumed that maintenance respiration and its response to temperature during the dormant season were also representative of the growing season, bearing in mind that a down-acclimatization of maintenance respiration is possible during the warmer period (Stockfors & Linder, 1998) and that positive relationships between maintenance respiration and growth rate may occur (Amthor, 1989; Lavigne & Ryan, 1997). I calculated maintenance respiration using average Q_{10} and R_{20} values for the nongrowth period. For each sample, I calculated the growth respiration by subtracting the estimated maintenance respiration (adjusted for temperature) from the total CO_2 efflux measured, as in Damesin *et al.* (2002).

The photosynthetic rate was calculated by subtracting the mean CO_2 efflux rate in the light from the mean dark CO_2 efflux, assuming that dark respiration does not change under light. The response of CO_2 efflux to light was adjusted using a nonhyperbolic rectangular hyperbola (see Johnson & Thornley, 1985):

$$A = 1/(2\theta)(\alpha \text{ PAR} + A_{max} - ((\alpha \text{ PAR} + A_{max})^2 - 4\theta\alpha \text{ PAR } A_{max})0.5) \quad \text{Eqn 2}$$

(PAR is photosynthetically active radiation; A is the assimilation rate; α is the apparent photosynthetic efficiency; A_{max} is the light saturated assimilation rate; and θ is a dimensionless parameter ($0 \le \theta \le 1$)). Refixation was estimated from mass-based CO_2 efflux in the dark (R_d) and under light saturation (R_l) as follows:

$$\% \text{ refixation} = (R_d - R_l)/(R_d)100 \quad \text{Eqn 3}$$

All equations were fitted using SAS statistical software, version 8.02 (SAS Institute 1999). All nonlinear regressions were performed with the NLIN procedure. For fits with Eqn 2, values of A_{max} were fixed for each sample as the measured value at PAR saturation. Parameter estimates (Q_{10} and R_{20}, θ and α) were accepted when $P < 0.01$.

Chlorophyll content

Periodically (about each month, more frequently during the beginning of the growth period), three leafy stems were collected from each tree. They were frozen immediately in liquid nitrogen and stored at −80°C until analysis. Chlorophyll content was extracted with 80% acetone. For each leafy stem, six discs were sampled randomly on four to six leaves, as well as the proximal half of the second internode of the stem. Leaf discs were ground in a mortar placed on ice. An aliquot of the stems over 3 weeks old was cut finely with a pair of pruning shears, then ground for 15 s in a grinder (Type MM200; Retsch, Haan, Germany) and finally in a mortar. Following centrifugation of the crude extract, the absorbance of the clear supernatant was measured at 663.6 nm and 646.6 nm using a UV-VIS spectrometer (Lambda 18; Perkin Elmer, Shelton, CT, USA). All the samples were measured in duplicate. The amount of chlorophyll a and b was calculated according to Porra *et al.* (1989). Six other foliar discs, and the part of the stem not used for chlorophyll measurements, were oven-dried at 60°C for 48 h to determine dry weight.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured *in situ* on attached and intact stems and leaves with a portable pulse-modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany). Maximal optimal photochemical efficiency of PSII ($(F_v/F_m)_{max}$) was determined with the ratio of variable to maximal fluorescence (i.e. $F_v/F_m = (F_m - F_o)/F_m$, where F_o and F_m are initial and maximal fluorescence of dark-adapted leaves. F_o was determined

with a modulated measuring light from a light-emitting diode (< 0.5 μmol m^{-2} s^{-1}, 655 nm) at a frequency of 600 Hz. The maximal fluorescence (F_m) was obtained with a brief saturation light pulse (0.8 s, > 5000 μmol m^{-2} s^{-1}). Measurements were conducted before sunrise (i.e. after complete relaxation of the PSII) at ambient conditions on three to five current-year stems per tree, with one measurement per leaf on four or five leaves per stem and three or four recordings along the stem. Fluorescence was measured on the upper surface of the leaves and stems.

Nitrogen content and stem growth

Each month, three leafy stems were clipped from each tree (generally in mid-morning around 10:00 hours), immediately immersed in liquid nitrogen, and stored at −80°C until lyophilization. An initial sampling was done on the buds on April 13. For every sampling (including that of buds), leaf and stem parts were separated and each tissue was weighed, finely ground (Type MM200; Retsch) and homogenized. Stem samples were cut manually into small pieces with pruning shears before grinding. Nitrogen content was determined on aliquots of the homogenized powder in an elemental analyser (Model NA-1500; Carlo Erba, Milan, Italy). All stems sampled for nitrogen and gas exchange measurements were also measured for length and basal diameter, and weighed for dry mass after lyophilization. For each stem, the area and weight of its leaves, after oven-drying at 60°C, were measured to calculate the leaf mass per area (LMA).

Estimation of annual carbon flux

Current-year-stem respiration throughout 1 yr was estimated using monthly derived Q_{10} and R_{20} values, hourly air temperature data, and stem biomass. The hourly air temperature recorded at the experimental site from March 2000 to April 2001 was used. Hourly PAR was reconstructed from the records of daily radiation at the meteorological site of Fontainebleau (approx. 60 km distant). The annual maintenance and growth components were calculated separately using Q_{10} and R_{20} obtained from maintenance and growth respiration, respectively. The maintenance component was calculated over the entire year, and the growth one from April to September. The time between two measurement dates was separated at mid-point and the corresponding Q_{10} and R_{20} were applied to each period. The same calculation protocol was used with monthly θ and α to calculate assimilation rate (A). I assumed that θ and α were temperature independent. I also assumed that the stem is sun-exposed and horizontal. To take into account the temperature effect on stem assimilation (Brayman & Shaedle, 1982), I used the linear relationship of Johnson & Thornley (1984):

$$A(T) = A(T_r)((T - T^*)/(T_r - T^*)) \quad \text{Eqn 4}$$

(T_r is a reference temperature (here, 25°C) and T^* is the temperature at which photosynthetic activity ceases). I set $T^* = -3$°C, which is the limit temperature for stem photosynthesis in *Populus* spp. (Foote & Shaedle, 1976a,b). The dry mass unit was used to scale up to the year because its measurement is more accurate than the calculation of stem area and volume. The stem dry matter increase, measured on samples during the experiment, was used as a representative pattern of the growth of an average stem. I assumed that the increase between two successive measurement periods was linear.

Results

Phenology

Stem tissue underwent dry matter incrementation from budburst (25 April, 2000, on average) until the end of the summer (Fig. 1a). Until the end of May, both stem length and diameter increased (Fig. 1b). They continued to increase progressively throughout the summer to reach average values in the winter around 18 cm and 3.8 mm for length and diameter, respectively. The LMA stabilized more rapidly than stem biomass (Fig. 1a). The maximum value was reached by mid-June (about 100 g m^{-2}).

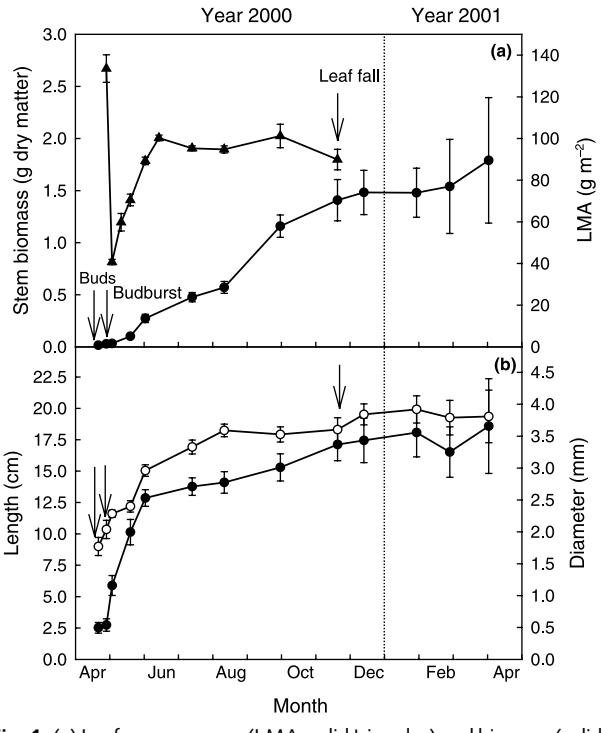

Fig. 1 (a) Leaf mass per area (LMA, solid triangles) and biomass (solid circles) and (b) length (solid circles) and diameter (open circles) of current-year stems from *Fagus sylvatica*. Points are means (± 1 SE, $n > 9$).

Seasonal respiration characteristics

The relationship between air temperature and respiration was well-described by an exponential equation. The coefficient of determination (r^2) was generally higher than 0.8. The monthly mean Q_{10} showed seasonal variations (Fig. 2a). From budburst to early July, mean Q_{10} was quite stable and ranged between 1.7 and 1.8. The Q_{10} peaked (2.4) in late September. During winter the values progressively decreased to reach the lowest value (1.4) by mid-February. In May and June 2001, mean Q_{10} reached similar values to those in May and June 2000.

The basal respiration at 20°C (R_{20}) also varied seasonally, although not in the same way as Q_{10}. The seasonal variation of R_{20} was consistent among the area-, volume-, and dry matter-bases presented (Fig. 2b,c,d) except for the first two points of dry-matter-based R_{20}. Very high values were obtained at budburst (7.2 μmol m^{-2} s^{-1} or 17 170 μmol m^{-3} s^{-1} or 45 μmol g^{-1} dry matter s^{-1}). The values strongly decreased until June. The lowest R_{20} occurred during winter with a minimum in December (0.5 μmol m^{-2} CO_2 s^{-1} or 736 μmol m^{-3} s^{-1} or 0.8 μmol g^{-1} dry matter s^{-1}). A slight increase occurred in April 2001. The Q_{10} and R_{20} values obtained for the growth respiration are shown in Table 1.

Assimilation gas exchange measurements

The CO_2 efflux from stems decreased when light increased (see examples of light response curves in Fig. 3). Generally, when PAR was greater than 500 μmol m^{-2} s^{-1} the values

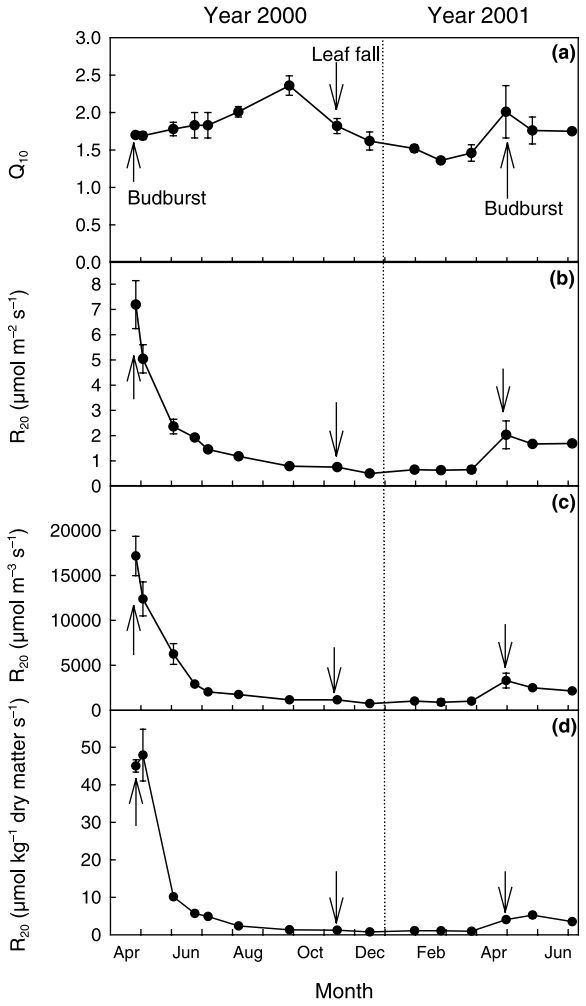

Fig. 2 Seasonal variation of (a) Q_{10} (the change in respiration with a 10°C change in air temperature); and of (b) area-based, (c) volume-based and (d) dry matter-based R_{20} (respiration normalized at 20°C), obtained from total respiration measurements recorded on stems of *Fagus sylvatica* grown in 2000. Points are means (± 1 SE) of values obtained from at least three stems. Note: small error bars may be hidden by symbols for the means.

Table 1 The Q_{10} and R_{20} of the growth respiration for current-year stems of *Fagus sylvatica* grown in spring 2000

Date	Q_{10}	R_{20} (μmol CO_2 kg^{-1} dry matter s^{-1})
April 25	1.7 (± 0.03)	44.0 (± 1.64)
May 2	1.7 (± 0.05)	46.9 (± 6.9)
June 1	1.8 (± 0.09)	9.1 (± 0.72)
June 22	1.8 (± 0.17)	4.7 (± 0.13)
July 5	1.6 (± 0.02)	3.8 (± 0.41)
August 4	2.4 (± 0.14)	1.4 (± 0.17)
September 23	2.7	0.5

Values are means ± 1 SE. The number of replicates was at least three, except in September when there was one. The date indicated is the mean day of year for the replicated measurements.

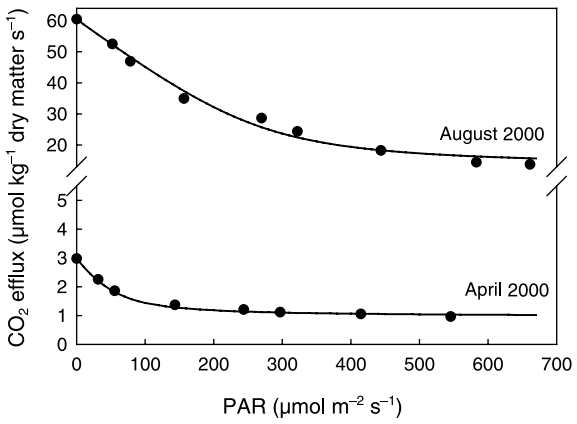

Fig. 3 Light response of CO_2 efflux measured on current-year stems of *Fagus sylvatica* just after budburst (in April 2000) and in summer (August 2000). The points represent means of at least three measurements on the same stem. Error bars are hidden by symbols; PAR is photosynthetically active radiation. Lines were fitted using PAR values and Eqn 2 (see the Materials and Methods section); θ = 0.91 in April; and θ = 0.72, in August.

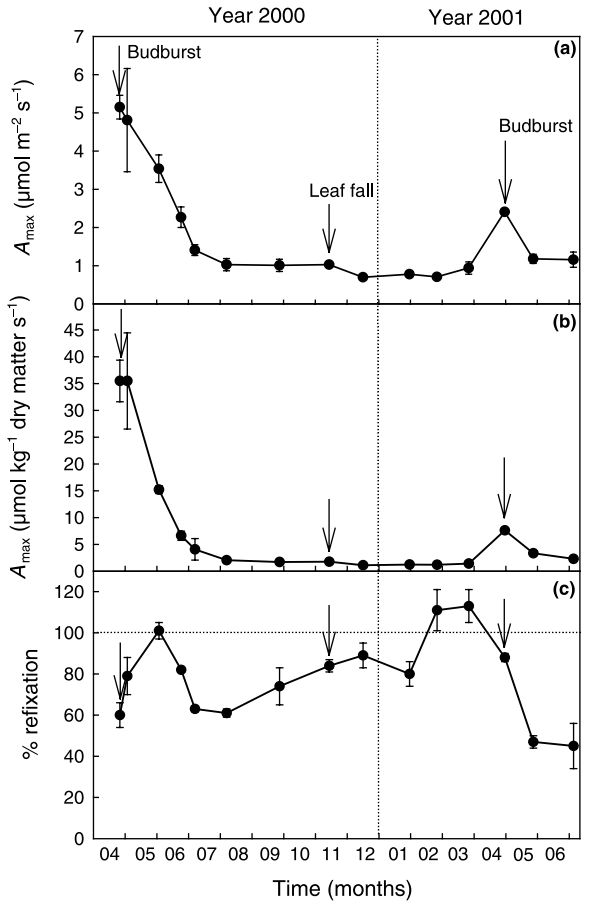

Fig. 4 Seasonal variation of (a) area-based and (b) dry-matter-based light-saturated *Fagus sylvatica* stem assimilation (A_{max}); and (c) refixation under light saturation as a percentage of dark respiration calculated from Eqn 3 (see the Materials and Methods section). All measurements were made at 25°C. Points are means of values obtained from at least three stems (except in April 2001 when there was one sample).

remained quite stable. The maximum assimilation rate changed strongly with seasons and showed the same pattern as that of R_{20} (Fig. 4a,b). Nevertheless, from April 2000 to April 2001, the ratio of light-saturated assimilation to respiration varied and ranged from 60% to 110% (Fig. 4c). The minima occurred just after budburst and in summer, the maxima occurred at the end of the winter period when the stem was almost 1 yr old. The monthly values of θ and α obtained using Eqn 2 (see examples in Fig. 3) are shown in Table 2; θ was 0.88, on average, over the year. The apparent photosynthetic efficiency (α) showed high values from April to June. From the summer, α was always < 0.01 μmol CO_2 μmol^{-1} PAR.

Fluorescence measurements

Stems and leaves showed a similar seasonal pattern to the predawn maximum F_v/F_m (Fig. 5a). Throughout the months, the values of stems were always slightly lower than leaves. Just after budburst, the values were about 0.65 and reached their maximum at the beginning of July with 0.78 for stems and 0.81 for leaves. For both tissues, a significant decrease occurred at the leaf-fall period. For stems, the values still decreased during winter and reached their lowest value of 0.25 at the end of March 2002; this corresponded to low ambient temperature (Fig. 5b). Thereafter, the values progressively increased to reach similar values to the previous year at the same period.

Chlorophyll and nitrogen contents

At budburst, the chlorophyll content, expressed on an area basis, was similar between stems and leaves, whereas it was three times lower for stems than for leaves if expressed on a dry matter basis (Fig. 6a,b). Thereafter, regardless of the unit basis, the values were clearly higher in leaves except at the

Date	*n*	θ	α (μmol CO_2 μmol^{-1} photons)
April 25, 2000	4	0.93 (± 0.02)	0.017 (0.003)
May 2, 2000	2	0.94 (± 0.01)	0.019 (0.001)
June 22, 2000	3	0.86 (± 0.05)	0.015 (0.004)
July 5, 2000	2	0.96 (± 0.01)	0.002 (0.001)
August 4, 2000	6	0.81 (± 0.05)	0.008 (0.002)
September 23, 2000	6	0.75 (± 0.05)	0.009 (0.002)
November 9, 2000	8	0.86 (± 0.02)	0.006 (0.001)
December 11, 2000	3	0.85 (± 0.07)	0.004 (0.001)
January 24, 2001	3	0.81 (± 0.07)	0.006 (0.002)
February 19, 2001	3	0.89 (± 0.05)	0.002 (0.000)
March 21, 2001	4	0.65 (± 0.04)	0.007 (0.001)
April 24, 2001	1	0.99	0.005
May 21, 2001	3	0.96 (± 0.03)	0.003 (0.001)
June 28, 2001	1	1	0.004

The mean date of the measurements, the number of replicates (*n*), and the parameter means (± 1 SE) are listed. $P < 0.01$.

Table 2 Parameters characterizing the response of *Fagus sylvatica* stem assimilation to PAR (from Eqn 2; see the materials and Methods section): θ and the apparent photosynthetic efficiency based on incident PAR, α

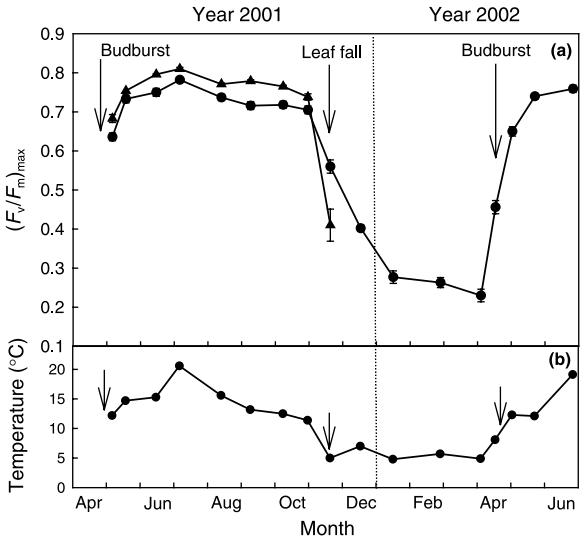

Fig. 5 Seasonal patterns of (a) dark-adapted photosystem II (PSII) photochemical efficiency ($(F_v/F_m)_{max}$) of *Fagus sylvatica* stems (solid circles) and leaves (solid triangles) grown in 2000; and (b) air temperature at each measurement period. Fluorescence measurements were conducted before sunrise at ambient conditions on three to five leafy stems per tree. Note: small error bars may be hidden by symbols for the means.

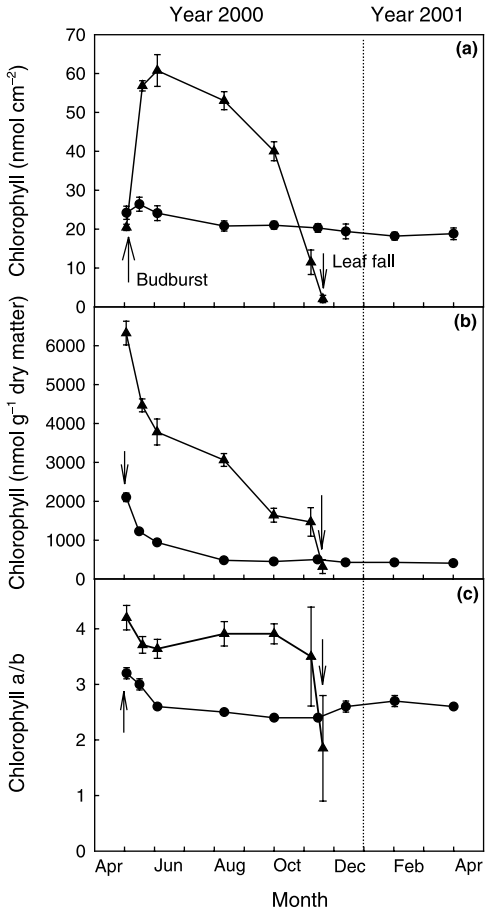

Fig. 6 Seasonal variation of (a) area-based, and (b) dry-matter-based chlorophyll content; and of (c) chlorophyll a / b ratios of *Fagus sylvatica* stems (solid circles) and leaves (solid triangles) grown in 2000. Points are means (± 1 SE, $n = 9$). Note: small error bars may be hidden by symbols for the means.

senescence period. Unlike leaves, for which there was a decrease, the stems had constant values during the end of the summer of approximately 20 nmol cm^{-2} and 470 nmol g^{-1} dry matter. The values remained stable during the winter. At the end of the leaf-fall period (mid-November), the leaf values were lower than those found for stems. Chlorophyll a/b values were always lower in stems than in leaves except at the leaf-fall period. The maximum values were found at budburst. In August, values were 2.5 and 3.9 for stems and leaves, respectively.

The nitrogen content was the same in stems and leaves in the buds, with a value of 3% (Fig. 7). For both tissues, the content increased and thereafter showed a clear decrease during the first 10 d of May (day of year 121–130). The decrease continued less sharply to reach a value of 0.6% for stems and a value 3.4-times higher for leaves in August. During the leaf-fall period, the leaf nitrogen content dropped and the stem content slightly increased to reach and maintain values near 1% throughout the winter.

Annual carbon balance

Estimates of monthly stem respiration are shown in Fig. 8. The maximum values occurred during May and June (i.e. the early growth period). During autumn and winter, the values (between 0.02 and 0.03 g C month^{-1}) were 2.7–3.7 times lower than in June. The percentage of growth respiration to monthly respiration depended on the month: it ranged from 98% (in May) to 36% (in September). Growth respiration

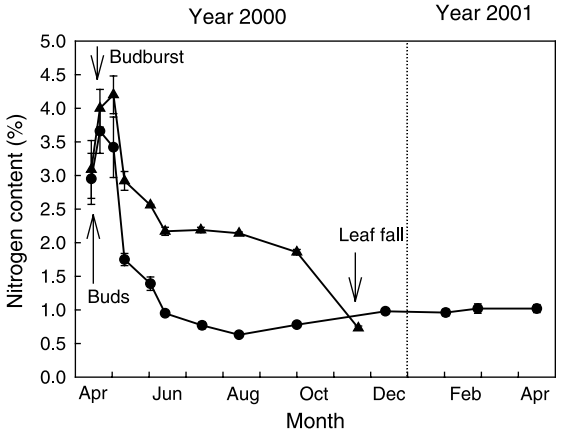

Fig. 7 Seasonal variation of nitrogen content of *Fagus sylvatica* stems (solid circles) and leaves (solid triangles) grown in 2000. Points are means (± 1 SE, $n = 9$). Note: small error bars may be hidden by symbols for the means.

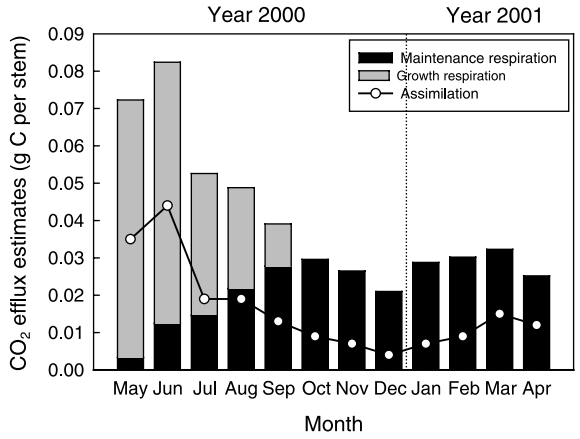

Fig. 8 Monthly variation of respiration (histogram) and assimilation (points and lines) estimates of an average-sized stem of *Fagus sylvatica* grown in 2000. Growth (tinted columns) and maintenance (closed columns) respiration are indicated. Estimates were calculated using monthly dry-matter-based Q_{10} (the change in respiration with a 10°C change in air temperature) and R_{20} (respiration normalized at 20°C) derived from measurements on stems, and hourly measurements of air temperature and radiation recorded near the experimental site. Assimilation is estimated using monthly θ and α from Eqn 2 (see the Materials and Methods section). To simplify the figure, the data for April 2000 (for only 5 d after budburst) were combined with those of May 2000.

accounted for 45% of the total annual respiration. The monthly assimilation was high at the beginning of the growth period and accounted for more than 50% of the monthly respiration. During winter, this proportion decreased and ranged between 20% and 30% with an increase in March 2001. At the annual scale, an average stem respired 0.48 g C and assimilated 0.19 g C.

Discussion

In this study I observed monthly variation of Q_{10} with no clear seasonal trend. The measurement-temperature interval was constant, so this variation cannot be attributed to the temperature dependence of Q_{10} highlighted by Tjoelker *et al.* (2001). Several authors have also reported strong Q_{10} differences between dates within a year, especially in conifer species (Paembonan *et al.*, 1991; Lavigne, 1996; Carey *et al.*, 1997; Stockfors & Linder, 1998), but these differences are still unexplained. The maximum basal respiration values we measured (17 700 μmol m^{-3} s^{-1}) are higher than those found on current-year peach stems (Grossman & Dejong, 1994) and much higher than those found on older stems. In comparison, Damesin *et al.* (2002) found, during the growth period, maximum values of approximately 165 and 4700 μmol m^{-3} s^{-1} for beech trunks and small branches, respectively (values adjusted to 20°C). In this period (end of April–May), high instantaneous respiration compensated for the low biomass of current-year stems, and 15% of the total annual respiration occurred in 1 month. Assuming 49% carbon in the stem biomass (Matthews, 1993), I calculated the construction cost of the current-year stems by dividing the biomass at the end of the growth period by the total annual growth respiration. I obtained a construction cost of 0.31 g C g^{-1} C, which is within the range of values found in the literature, which shows high interspecies variability (Lavigne & Ryan, 1997). This cost is close to that found for *Fagus sylvatica* trunks (Damesin *et al.*, 2002) and higher than estimates obtained for conifer species (Carey *et al.*, 1996, 1997; Stockfors & Linder, 1998). For the dormant season, the respiration rates are a lot higher than those found on older parts of the woody tissue, especially trunks, of the same (Ceschia *et al.* 2002; Damesin *et al.*, 2002) or different species (Ryan *et al.*, 1994).

For the photosynthesis characteristics, the apparent quantum efficiencies for stems at the very young stage (April–June) were close to those found generally for leaves (Vera *et al.*, 1999; Lessmann *et al.*, 2001). Later in the year the lower values were higher than the value (0.0014 μmol CO_2 μmol photons) found for *Pinus monticola* branches (Cernusak & Marshall, 2000). During summer, the efficiency of photosystem II in stem tissue was nearly that of leaves. The parallel increase of F_v/F_m for both tissues during growth reveals a similar pattern of maturation for the photochemical efficiency of PSII. The saturated light intensity observed in the current study is lower than the high value (2000 μmol PAR m^{-2} s^{-1}) found by Cernusak & Marshall (2000) but similar to values generally found to fall between 300 and 600 μmol m^{-2} s^{-1} (Foote & Shaedle, 1976b; Pilarski, 1989; Pfanz *et al.*, 2000; Wittmann *et al.*, 2001). If incident light is corrected to the percentage of transmitted light through the external bark (20% for 1-yr-old stems (data not shown); same value as that found for young branches by Pfanz & Aschan, 2001), then the actual *in situ* light saturation would be approximately 100 μmol m^{-2} s^{-1}, which is comparable to that utilized by shade-adapted chloroplasts.

The low chlorophyll a/b ratio of stems relative to leaves confirmed the stem tissue acclimatizes to shade conditions, as a decrease in irradiance is commonly observed to result in a decrease of the chlorophyll a/b in leaves (Murchie & Horton, 1997). The chlorophyll a/b ratios we observed are similar to those found for the upper branches of a *P. monticola* canopy (Cernusak & Marshall, 2000) and for sun-exposed branches (Pearson & Lawrence, 1958) or trunks (Kharouk *et al.*, 1995) of *P. tremuloides*. By red-light fluorescence, we observed that the chlorophyll in current-year stems of beech exists in some cells of the corticular parenchyma, but mostly in the pith and in the medullary rays (data not shown), as in *Vitis vinifera* (Kriedemann & Buttrose, 1971). The location of chlorophyll in the rays (far from the incident light) is not yet fully understood (Pfanz & Aschan, 2001) but may be explained by proximity to the surrounding vascular system and its supply of carbon for photosynthesis, as was recently shown in herbaceous plants by Hibberd & Quick (2002). From an evolutionary

point of view, one might question whether the presence of all material needed for photosynthesis, especially chlorophyll, is simply a remnant from ancestral green stems, or whether it was selected for by improving the carbon balance of the individual.

The parallel trends of maximum dark respiration and photosynthesis rates is striking and suggests the existence of an adjustment between anabolism and catabolism. Foote & Shaedle (1976a) found, as we did, that photosynthetic activity closely paralleled respiration over the course of an entire year in a young *P. tremuloides*. Moreover, the correlation was also reported for a given time among branches of *P. monticola* (Cernusak & Marshall, 2000) and *Populus tremula* (Aschan *et al.*, 2001). These authors showed that refixation was independent of the internal stem CO_2 concentration. In my study, the decrease of nitrogen and chlorophyll contents between budburst and June could explain the decrease of stem assimilation during growth. Contrary to what was found for developing leaves (Reich *et al.*, 1991), stems very early in the growing season showed high photosynthetic rates per unit of stem nitrogen (mass-based unit) compared with summer values. This suggests that, as in mature leaves (Evans, 1989), the nitrogen content of the current-year growing stems reflects quite well the concentrations of nitrogenous compounds that directly control maximum photosynthetic rates. One may question whether the adjustment between respiration and assimilation rates could be unbalanced under a change in environmental variables, such as CO_2 enrichment wherein respiration may be increased (Edwards *et al.*, 2002) and photosynthesis not increased as it proceeds at CO_2 saturation. At the maximum, the stem CO_2 assimilation rate is approximately four times lower than the value measured at saturated CO_2 on beech leaves (Epron *et al.*, 1995). Nevertheless, if the carbon assimilated by growing stems is used to construct their organic matter, this could be an additional factor determining the carbon isotopic composition ($\delta^{13}C$) of the whole woody tissue (Damesin & Lelarge, 2003). Indeed, it has been shown recently that stem assimilation discriminates against ^{13}C and should be considered when interpreting the $\delta^{13}C$ signature of photosynthetic woody tissue (Cernusak *et al.*, 2001).

One may expect that stem photosynthesis of a deciduous species is important when leaves are absent. During winter, the chlorophyll contents of our beech stems in the current study were undiminished by low temperature, as demonstrated also for *V. vinifera* (Kriedemann & Buttrose, 1971). The increase in nitrogen content is caused by translocation of nitrogen during leaf fall. The difference between the lowest summer stem value and the highest winter values represents 16% of the nitrogen present in mature leaves. This percentage is the same as the contribution of previous-year nitrogen to leaf nitrogen determined on the same species by labelling experiments (Dyckmans & Flessa, 2001). Nevertheless, the absolute assimilation rates in winter were very low. This was explained by the strong decrease in the maximal optimal photochemical efficiency of PSII. From this point of view, the beech stems seem to be more sensitive to low-temperature constraint than the leaves of evergreens in winter (Skillman *et al.*, 1996; Lundmark *et al.*, 1998; Oliveira & Penuelas, 2002). The *in situ* results presented herein are consistent with those of Larcher & Nagele (1992) who found, using cut small branches of the same species, a strong winter inhibition of the F_v/F_m requiring 20 d of reactivation. I checked for this phenomenon on my samples and found that even after 1 d at 20°C the F_v/F_m did not increase (data not shown). Nevertheless, the photosynthesis rate could exceed that of respiration. A percentage of refixation around 100% was also found by Foote & Shaedle (1976a) and Cernusak & Marshall (2000).

To scale up to the tree level, I calculated, using the allometric relationship established by Ottorini & Le Goff (1998), that the current-year stem (diameter < 4 mm) biomass of a tree with a circumference at breast height of 45 cm is 0.84 kg. Consequently, I calculated that current-year stems of a tree respired 468 g C over 1 yr. During the vegetative season (May to September), these stems respired around 150 g C. This value represents approximately 8% and 1.4% of the above-ground respiration and carbon assimilation, respectively, estimated by Lebaube *et al.* (2000) for the same period and a similar-sized tree. Eight per cent is a high value given that the current-year stems represent barely 1% of the whole tree above-ground woody tissue biomass (Ottorini & Le Goff, 1998). Moreover, the winter assimilation (approx. 63 g C only for the current-year stems) could partly diminish the carbohydrate reserve cost of respiration during the leafless period (Barbaroux *et al.*, 2003).

In conclusion, this study showed that respiration of current-year stems is not a negligible component of the tree carbon loss, despite their low biomass. Compared with leaves, the structural (area–volume ratio) and biochemical (nitrogen, chlorophyll contents) parameters of stems are less advantageous (except just after budburst) for photosynthesis, and the instantaneous net CO_2 exchange is rarely positive. Nevertheless, their photosynthetic capacity allows them to partly compensate for their carbon loss, and refixation may equal the growth respiration on a yearly basis. It would be interesting for comparison to conduct a study similar to this one but with the gas exchange measurements taken *in situ* under natural field conditions. At the very least, more information is now needed, especially concerning branch and trunk assimilation, to quantify how stem photosynthesis may influence tree and stand productivity. The major difficulties will involve taking into account the variability among branches, which may be important (Cernusak & Marshall, 2000), and estimating the amount of light intercepted by branches and trunks throughout days and seasons.

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