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Changes in carbon uptake and allocation patterns in *Quercus robur* seedlings in response to elevated CO_2 and water stress: an evaluation with ^{13}C labelling

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Summary – A semi-closed $^{13}CO_2$ labelling system (1.5% ^{13}C) was used to assess both carbon uptake and allocation within pedunculate oak seedlings (*Quercus robur* L) grown under ambient (350 vpm) and elevated (700 vpm) atmospheric CO_2 concentration ($[CO_2]$) and in either well-watered or droughted conditions. Pulse-chase ^{13}C labelling data highlighted the direct positive effect of elevated CO_2 on photosynthetic carbon acquisition. Consequently, in well-watered conditions, CO_2-enriched plants produced 1.52 times more biomass (dry mass at harvest) and 1.33 times more dry root matter (coarse plus fine roots) over the 22-week growing period than plants grown under ambient $[CO_2]$. The root/shoot biomass ratio was decreased both by drought and $[CO_2]$, despite lower N concentrations in CO_2-enriched plants. However, both long-term and short-term C allocation to fine roots were not altered by CO_2, and relative specific allocation (RSA), a parameter expressing sink strength, was higher in all plant organs under 700 vpm compared to 350 vpm. Results showed that C availability for growth and metabolic processes was greater in fine roots of oaks grown under an elevated CO_2 atmosphere irrespective of soil water availability.

elevated CO_2 / drought / growth / ^{13}C labelling / carbon assimilation / carbon allocation

Résumé – Effets de l'augmentation de la concentration atmosphérique en CO_2 et de la sécheresse sur l'assimilation et la redistribution du carbone de plants de *Quercus robur* : une approche par marquage ^{13}C. Un système semi-fermé de marquage isotopique par $^{13}CO_2$ (1,5 % ^{13}C) a été utilisé pour évaluer l'assimilation et la répartition du carbone pour des plants de chêne (*Quercus robur* L) élevés sous une concentration atmosphérique en CO_2 ($[CO_2]$) ambiante (350 vpm) ou élévée (700 vpm) et en conditions d'alimentation hydrique optimale ou limitante. Les résultats obtenus à partir de cinétiques de charge-redistribution de ^{13}C montrent un effet direct de l'augmentation de $[CO_2]$ sur l'acquisition photosynthétique de carbone. En conditions d'alimentation hydrique optimale,

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la biomasse totale des plants croissant sous [CO_2] élevée a été multipliée par 1,52 (matière sèche à la fin de la période de croissance de 22 semaines) comparativement au plants croissant sous [CO_2] ambiante, cependant que la matière sèche des racines (racines fines et grosses) était multipliée par 1,33. Le rapport biomasse racinaire/biomasse aérienne des plants était diminué à la fois par la sécheresse et par l'augmentation de [CO_2], en dépit de concentrations tissulaires en N plus faibles dans les plants croissant en conditions de [CO_2] élevée. Toutefois, l'allocation de carbone aux fines racines (diamètre < 2 mm), considérée soit de façon intégrée dans le temps (accumulation de biomasse), soit à court terme (données issues des marquages isotopiques), n'était pas affectée par la [CO_2]. Le taux d'allocation spécifique de carbone (RSA), un paramètre exprimant la force des puits de carbone, était plus élevé à 700 vpm qu'à 350 vpm pour l'ensemble des compartiments des plants. Les résultats font ressortir une augmentation de la disponibilité en C pour la croissance et le métabolisme dans les fines racines en relation avec l'augmentation de [CO_2] et indépendamment des disponibilités hydriques dans le sol.

CO_2 élevé / sécheresse / croissance / marquages ^{13}C / assimilation du carbone / redistribution du carbone

INTRODUCTION

There is now good agreement among different climate models that accumulation of carbon dioxide and other greenhouse gases in the atmosphere linked to human activities could cause an increase in mean global temperature at the surface of the earth of at least 1 °C over the next 50 years and of about 2–4 °C before the end of the next century. Owing to both the increase in potential evapotranspiration linked to these changes and to concurrent changes in the precipitation regime at the European temperate, and namely Mediterranean, latitudes forecasted by General Circulation Models, plant communities will, in addition to enhanced temperature, have to face more severe drought conditions in the future, and will therefore be subjected, particularly in the case of long-living woody communities, to increasing risks of environmental inadaptation and dye-back (Beerling et al, 1996).

Atmospheric CO_2 concentration ([CO_2]) is presently 360 vpm, and could reach 530 vpm, ie, about twice the preindustrial level of last century, by the year 2050, and 700 vpm in 2100 (Post et al, 1990). In their recent evolutionary history, plants have never experienced such elevated CO_2 together with drought. There are several mechanisms by which atmospheric CO_2 may interfere with drought adaptation features of plants. Elevated atmospheric CO_2 is known to generally stimulate water-use efficiency in trees primarily as a result of lowered leaf stomatal conductance, enhanced photosynthesis or both factors in combination (Eamus, 1991; Guehl et al, 1994), allowing the maintenance of higher leaf water potentials at a given soil water content (Masle, 1992; Tyree and Alexander, 1993). However, drought resistance mechanisms could also be largely determined by processes occurring after carbon assimilation, ie, by the efficiency of C transfer to, and utilization by, the sink organs. Much fewer studies have focused on this latter aspect. An increased C-sink activity of the root system, promoted by the allocation of recently-fixed carbon, is often reported in CO_2-enriched trees (Stulen and Den Hertog, 1993; Norby, 1994; Rogers et al, 1994; Vivin et al, 1995), and may enhance the potential for water and nutrient acquisition through a greater absorptive root area and a higher specific root activity (Rogers et al, 1994; Morgan et al, 1994). Increased C availability in the different plant tissues is also likely to promote osmotic adjustment,

which leads to the maintenance of turgor potential and plant growth under drought (Morse et al, 1993; Tschaplinski et al, 1995b; Vivin et al, 1996; Picon et al, 1997).

The use of stable ^{13}C isotope as a tracer is a powerful approach to assess whole-tree C allocation (Deléens et al, 1995; Vivin et al, 1995). In the present study, we examined to what extent growth, carbon uptake and allocation to fine roots, coarse roots, stem and leaves of pedunculate oak (*Quercus robur* L) seedlings are changed by the interactive effects of atmospheric [CO_2] and soil water availability. *Q robur* is a deciduous drought-tolerant species with a deep rooting pattern, allowing efficient soil water extraction, and is of major area representativity in France (Vivin et al, 1993). We hypothesized that elevated CO_2 would stimulate plant growth and carbon uptake, even if soil water availability is limiting, and would increase both carbon allocation and C availability to the below-ground system. Such patterns may be a key in the extent to which elevated CO_2 may alleviate the effects of water stress in plants (Bazzaz, 1990; Morison, 1993).

MATERIALS AND METHODS

Plant material and experimental setup

Pedunculate oak acorns (*Q robur* L, provenance Manoncourt) were collected in Autumn 1993 in a parent stand close to Nancy (Lorraine, France), soaked in fungicide (Rhodiasan, Rhône Poulenc Paris, France) and stored at –1 °C in plastic bags over-winter. In March 1994, the acorns were peeled, soaked in water and sown in 5-L cylindrical plastic containers filled with a shagnum-peat and sand mixture (1/1, v/v). The substrate was fertilized with delayed Nutricote 100 (N, P, K 13–13–13 + trace elements) at the time of sowing, and the level of fertilizer supply (5 kg m^{-3}) was chosen to provide optimal plant nutrition conditions throughout the experimental period. Sixty pots were randomly assigned to two groups of 30 replicates, and placed inside two 50-µm - thick transparent polypropylene tunnels (5 × 3 × 2.3 m) located at Inra - Nancy. Seedlings were continuously exposed to either ambient (350 ± 30 vpm) or high (700 ± 50 vpm) atmospheric [CO_2]s, which were measured by means of two infrared gas analyzers (ADC 225 MK3, UK) and controlled by an automated regulation system (Guehl et al, 1994; Vivin et al, 1995). Air temperature inside the tunnels ranged from 11 °C (minimum night temperature) to 30 °C (maximum diurnal temperature) during the experimental period; maximum daily values of VPD ranged from 10.1 to 20.2 hPa. Plants were grown under natural photoperiod. Photosynthetic photon flux density (PPFD) was about 60% of the outside conditions and did not exceed 1 200 µmol m^{-2} s^{-1} at plant level, even in sunny conditions.

All plants were watered with deionized water twice a week to maintain soil water content to field capacity. Eighteen weeks after germination, 15 seedlings were randomly assigned in each tunnel, to well-watered or water-stressed treatments, and water supply was withheld in the latter treatment. Plant transpiration was assessed gravimetrically and direct evaporation from the containers was prevented by covering the substrate with white waxed cardboard disks. Leaf predawn water potential (Ψ_w) of mature leaves was measured with a Scholander pressure chamber simultaneously to plant sampling.

^{13}C labelling experiment

At the end of August (week 22 after sowing), eight plants from each CO_2 treatment were randomly selected from the set of 15 and placed in a controlled environment chamber for a short-term ^{13}C labelling experiment. The labelling system described in detail elsewhere (Vivin et al, 1995) was designed (i) to supply a constant ^{13}C-enriched CO_2 atmosphere to the shoots (1.5 atom%, or ca 0.4% over the ambient atmospheric level) and (ii) to monitor [CO_2] in both above- and below-ground compartments of the plant–soil system in accordance with plant growing [CO_2]. Air temperature within the above-ground compartment was 23 °C, relative humidity was up to 70% and PPFD was 350 µmol m^{-2} s^{-1} at leaf level, which was close to the mean photosynthetic photon flux density (PPFD) level received by the plants in the tunnels.

Three plants were harvested after the 12-h loading period; the five remaining plants were

harvested after a 60-h chase period (three nights and two days), simultaneously to five unlabelled plants (to measure baseline plant ^{13}C abundance). Plants were separated into leaves, stems, coarse roots (comprising mainly the tap root) and fine roots (< 2 mm diameter). The leaf area from the three aerial growth flushes produced (flush 1 denotes the oldest one) was measured using a planimeter (DeltaT Devices, UK). Roots were separated from soil by gently shaking and washed with deionized water. Plant components were dried at 65 °C for 48 h and finely ground to pass a 40-mesh screen. Powdered plant tissues were combusted at 1050 °C, and their C and N concentrations and the molar $^{13}C/^{12}C$ ratio were measured using an element analyser coupled with an isotope ratio mass spectrometer (Delta S, Finnigan-Mat, Bremen, Germany). Isotopic results were expressed in terms of the conventional $\delta^{13}C_{PDB}$ notation (Boutton, 1991). Distribution of newly incorporated ^{13}C atoms within a plant was expressed in two complementary ways as relative specific allocation (RSA) and partitioning (%P, see *Appendix 1* for expressions). RSA describes the proportion of newly incorporated atoms relative to total atoms in a given sample, and is also interpreted as an index of C turnover whereas %P describes the proportion of the labelled element in a given sample relative to the total labelled element in that plant (Deléens et al, 1995).

Simultaneously to the ^{13}C labelling experiment, biomass and allometric parameters were assessed by measuring plant leaf area, root/shoot (R/S, g g^{-1}) mass ratio, fine root mass ratio (fine root mass/plant mass, g g^{-1}), fine root density (fine root mass/plant leaf area, g dm^{-2}) and growth efficiency (annual stem mass per plant leaf area, g dm^{-2}). Biomass partitioning among the plant components was assessed by determining (1) the leaf mass ratio (LMR, leaf dry mass/whole plant dry mass, g g^{-1}), (2) the stem mass ratio (SMR, stem dry mass/whole plant dry mass, g g^{-1}), (3) the root mass ratio (RMR, root mass/whole plant mass, g g^{-1}).

Data analysis

Daily monitoring indicated that, with the exception of atmopheric $[CO_2]$, environmental conditions were similar between the two tunnels. In order to minimize possible tunnel effects, plants were rotated monthly between tunnels. The experiment was a two by two factorial to determine the effects of CO_2 and water on plant variables. The individual container was considered as the experimental unit. Data were analysed using a two-way ANOVA to test for significant ($P < 0.05$) treatment differences in plant variables.

RESULTS

Water relations

After the 22-week experimental period, well-watered plants had similar leaf predawn water potential values ($\Psi_w = -0.44$ MPa) in both $[CO_2]$s (table I). The drought treatment, which was started on week 18, significantly decreased Ψ_w in both CO_2 treatments, but this effect was slightly more pronounced under elevated $[CO_2]$ (–2.6 MPa) than under ambient $[CO_2]$ (–1.9 MPa). Plant transpiration measured from week 18

Table I. Average values of plant transpiration (integrated over weeks 18–22) and leaf predawn water potential (Ψ_w) measured at the end of the experiment (week 22). The significance of CO_2 and water supply regime effects is indicated for the different parameters; ns: non significant, * $P < 0.05$, ** $P < 0.01$, $n = 13$.

	Well-watered		*Droughted*		*ANOVA*		
Variables	*350*	*700*	*350*	*700*	CO_2	H_2O	$a \times b$
Plant transpiration (g day^{-1})	74.9	68.6	53.6	56.5	ns	**	ns
Leaf Ψ_w (MPa)	–0.44	–0.42	–1.89	–2.66	ns	**	ns

to the end of the experiment was unaffected by CO_2, but was decreased by drought (–29 and –18% under ambient and elevated [CO_2], respectively; table I).

Plant growth and biomass

Q robur plants generally produced three aerial growth flushes during the experimental period (table II). No significant CO_2

Table II. Average values of dimension and biomass characteristics of the plants at the end of the experiment (week 22).

	Well-watered		*Droughted*		*ANOVA*		
Variables	*350*	*700*	*350*	*700*	CO_2	H_2O	$a \times b$
Root collar diameter (mm)	9.3	10.6	7.3	8.5	**	**	ns
Stem length (cm)							
1st flush	13.9	14.9	12.7	13.8	ns	ns	ns
2nd flush	26.6	28.3	21.5	28.3	*	ns	ns
3rd flush	21.3	34.2	17.9	24.6	*	*	ns
total	61.9	77.5	52.1	66.8	**	*	ns
Leaf number							
1st flush	6.5	6.5	6.7	6.8	ns	ns	ns
2nd flush	13.4	16.3	10.8	15.3	*	ns	ns
3rd flush	14.1	19.1	10.5	14.2	*	*	ns
total	34.0	41.9	27.9	36.3	**	*	ns
Leaf area (dm^2 $plant^{-1}$)							
1st flush	2.1	2.0	2.0	2.2	ns	ns	ns
2nd flush	4.8	5.3	3.9	4.7	*	ns	ns
3rd flush	2.6	5.9	1.9	2.8	*	*	ns
total	9.4	13.1	7.8	9.7	**	**	ns
Dry mass (g)							
leaf	5.9	9.9	4.9	6.9	**	**	ns
stem	6.4	11.5	3.9	7.2	**	**	ns
coarse root	9.6	12.1	5.7	8.0	**	**	ns
fine root (< 2 mm)	4.9	7.2	2.6	3.7	**	**	ns
total	26.8	40.8	17.1	25.9	**	**	ns
Specific leaf area (m^2 kg^{-1})	16.1	13.2	15.9	13.8	*	ns	ns
Leaf area ratio (m^2 kg^{-1})	3.6	3.2	4.6	3.7	**	**	ns
Growth efficiency (g dm^{-2})	66.9	87.8	51.2	75.5	**	**	ns
Root/shoot ratio (g g^{-1})	1.25	0.96	0.97	0.86	*	*	ns
LMR (g g^{-1})	0.22	0.24	0.29	0.27	ns	**	ns
SMR (g g^{-1})	0.24	0.28	0.23	0.28	**	ns	ns
RMR (g g^{-1})	0.54	0.48	0.48	0.46	*	**	ns
Fine/coarse root ratio (g g^{-1})	0.51	0.58	0.47	0.47	ns	*	ns
Fine root density (g dm^{-2})	0.54	0.59	0.35	0.40	ns	**	ns

The significance of CO_2 and water supply regime effects is indicated for the different parameters; ns: non-significant, * $P < 0.05$, ** $P < 0.01$, $n = 13$.

effect on stem length was observed for the first flush, which probably reflects the predominant contribution of acorn reserve mobilization. For the second and third growth flush, a significant stimulation of stem length by elevated [CO_2] was observed in both well-watered and droughted plants (table II). At the end of the experiment in the well-watered plants, elevated [CO_2] significantly enhanced root collar diameter (+14%), total stem length (+25%), number of leaves per plant (+32%) as well as plant leaf area (+39%) (table II), but not single leaf area. Drought significantly decreased all growth variables in both CO_2 conditions, no CO_2 × water interactive effects were observed (table II).

Well-watered plants grown under elevated [CO_2] produced 1.52 times more total biomass and 1.33 times more dry root matter (coarse plus fine roots) over the 22-week growing period than plants grown under ambient [CO_2] (table II). The root systems of plants from both CO_2 treatments extended to the bottoms of the pots. Elevated [CO_2] had no effect on LMR, but significantly increased SMR and decreased RMR in both watering conditions (table II). Consequently, root/shoot ratio was 23% lower in well-watered plants grown under high [CO_2] than in ambient CO_2-treatment plants (table II).

Average plant specific leaf area (SLA) was significanly decreased by [CO_2], but was unaffected by drought (table II). In addition, elevated CO_2 promoted a significantly higher growth efficiency (+31% in well-watered conditions and +47% in droughted conditions), but slightly increased fine root density and the fine root/coarse root ratio (table II).

C–N concentrations and natural ^{13}C isotope composition

Elevated CO_2 slightly but significantly increased whole-plant, stem, root, but not leaf, C concentrations in both watering conditions (table III). Indeed plant N uptake was significantly increased in CO_2-enriched plants (+35%, in well-watered treatment) but not enough to compensate for plant C uptake (+52%). Plant N concentration and C/N ratio over the 22-week growing period

Table III. Carbon and nitrogen concentrations (10^{-2} g g^{-1}) in the different plant compartments at the end of the experiment (week 22).

		Well-watered		*Droughted*		*ANOVA*		
		350	*700*	*350*	*700*	CO_2	H_2O	$a \times b$
Leaf	C	45.39	44.54	45.09	44.65	**	ns	ns
	N	3.28	2.77	3.41	2.59	**	ns	ns
Stem	C	43.98	44.27	44.02	44.40	*	ns	ns
	N	0.98	0.92	1.10	0.98	**	**	ns
Coarse root	C	42.81	42.89	42.82	43.43	*	ns	ns
	N	1.63	1.41	1.86	1.59	**	**	ns
Fine root	C	40.29	42.42	41.60	41.35	*	ns	*
	N	1.75	1.68	1.94	1.61	**	ns	*
Plant	C	43.22	43.57	43.55	43.76	*	*	ns
	N	1.86	1.64	2.13	1.68	**	**	**

The significance of CO_2 and water supply regime effects is indicated for the different parameters; ns: non-significant, * $P < 0.05$, ** $P < 0.01$, $n = 13$.

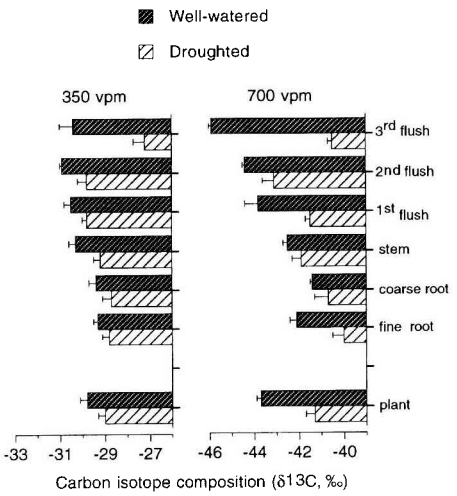

Fig 1. Carbon isotope composition values ($\delta^{13}C$, ‰) at the whole-plant level and in the different parts of *Quercus robur* plants grown under a two by two factorial combination of atmospheric $[CO_2]$s and watering regimes. (▨), Well-watered plants; (▨), droughted plants. $n = 5$.

were affected by the elevated $[CO_2]$, by −11 and +13%, respectively (table III). Drought increased plant C and N concentrations in both CO_2 treatments.

Carbon isotope composition of all plant components was on average 12‰ more negative in unlabelled plants in elevated CO_2 than in ambient CO_2 (fig 1). Such a large difference can only be accounted for by differences in source air isotopic composition (δ_a) between the two tunnels and not by differences in isotopic discrimination by the plants. The plants in ambient CO_2 exhibited δ values ranging between −27.7 and −30.3‰, which are consistent with a δ_a value equal to that of the outside atmosphere (ie, −8‰). The mean δ_a value in the elevated CO_2 tunnel was unknown but was obviously much less negative than −8‰, reflecting the combined influence of the CO_2 from the cylinder (typical values of about −35‰; Ehleringer, 1991) and from the greenhouse (about −8‰) with an additional (but probably small because of the continuous air extraction from the tunnel) effect due to carbon isotope discrimination by the plants within the tunnel.

There was a close correlation between the δ values of the different plant components at the individual plant level (data not shown). Roots exhibited δ values about 1.5‰ less negative (less discrimination) than stems and leaves. Similar results have been observed in other studies (Gebauer and Schulze, 1991; Guehl et al, 1994), but their interpretation remains unclear. For both $[CO_2]$, $\delta^{13}C$ increased with drought (fig 1), reflecting stomatal closure and decreased leaf intercellular $[CO_2]$ in the droughted conditions (Farquhar et al, 1989; Picon et al, 1997). It is noteworthy that this effect of drought was most pronounced in the most recently formed plant components, ie, in leaves of the third flush and in fine roots (elevated $[CO_2]$ only). This probably reflects the fact that these components were formed after the onset of drought, thus the isotopic signature of structural C was affected by drought.

^{13}C relative specific allocation and partitioning

Daily plant carbon assimilation rates, calculated from δ_p values of the labelled plants and expressed either on a plant basis (table IV) or on a plant leaf area basis (fig 2), were significantly higher in the elevated CO_2 treatment whatever the plant water status. Drought reduced daily plant carbon uptake per unit leaf area, but values remained higher under elevated $[CO_2]$ than ambient $[CO_2]$ despite lower leaf predawn water potential in CO_2-enriched plants (fig 2, table I).

In the well-watered treatments, relative specific allocation values (RSA), considered either immediately after the labelling, or after the 60-h chase phase, were signifi-

Table IV. Plant $^{13}C_{excess}$ (µg day^{-1} plant^{-1}) in the different treatments (see *Appendix 1*) and levels of significance of the different effects.

	Well-watered		*Droughted*	
	350	*700*	*350*	*700*
Plant $^{13}C_{excess}$ (µg day^{-1} plant^{-1})				
0 h	443.4	1571.9	186.6	466.0
60 h	356.0	1952.1	150.2	286.4

ANOVA: $CO_2 < 0.001$; $H_2O < 0.001$; $CO_2 \times H_2O < 0.001$; time: ns.

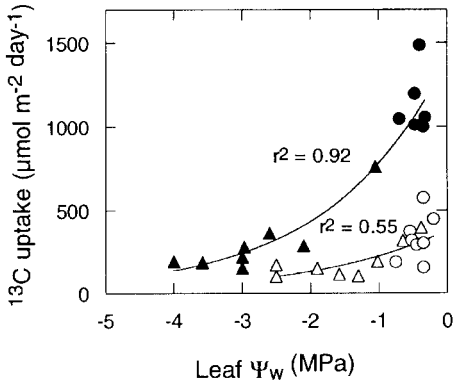

Fig 2. Relationship between leaf predawn water potential (Ψ_w, MPa) and daily ^{13}C excess uptake calculated from labelling experiment data and expressed on a plant leaf area basis (µmol ^{13}C m^{-2}). (Open symbols), 350 vpm; (dark symbols), 700 vpm; (circles), well-watered plants; (triangles), droughted plants.

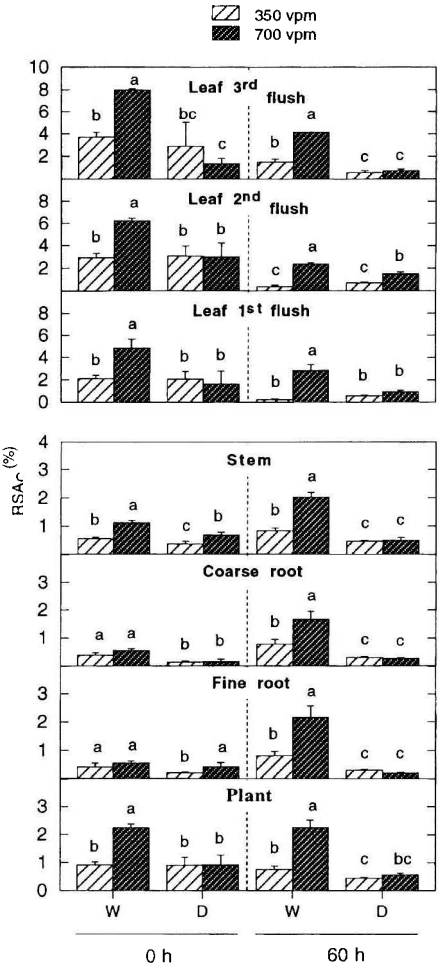

Fig 3. Carbon relative specific allocation mean values (RSA_C, %) at the whole-plant level and in the different parts of *Quercus robur* plants grown under a two by two factorial combination of atmospheric [CO_2]s and watering regimes. Labelling experiment was performed 22 weeks after sowing and plants were harvested immediately after the 12-h $^{13}CO_2$ pulse phase (0 h) and at the end of the ^{13}C chase phase (60 h). $n = 3$ to 5, mean values not sharing common letters indicate significant differences (ANOVA followed by Fisher's PLSD test, $P < 0.05$). (W), well-watered plants; (D), droughted plants.

cantly higher under 700 vpm than under 350 vpm [CO_2] at the whole-plant level and in all plant parts (fig 3). During the chase phase, the recently photoassimilated ^{13}C was translocated from the mature leaves, in which the proportion of new carbon decreased, to the expanding leaves (3rd flush), stem and total roots, in which this proportion increased (fig 3). However, similarly to long-term biomass C allocation, short-term ^{13}C partitioning to the below-ground parts was less pronounced under 700 vpm than under 350 vpm [CO_2] (fig 4). Elevated CO_2 did not affect C allocation to fine roots (fig 4), but RSA values were markedly higher in the fine roots under elevated CO_2 (2.2%) than under ambient CO_2 (0.8%) after the 60-h chase period (fig 3). In addition, under ambient [CO_2], the proportion of new ^{13}C was practically nil after the 60-h chase phase in the leaves of the first and second flushes, whereas the amount of new ^{13}C remained higher under elevated [CO_2] (fig 3). Under both ambient and elevated [CO_2], drought reduced both plant RSA (fig 3) and the relative proportion of C translocated to below-ground parts of the plants (fig 4). In contrast with the stimulation of C acquisition per unit leaf area (fig 2), elevated [CO_2] did not increase the plant RSA value (fig 3) under droughted conditions. This is to be related to the lower leaf area ratio values observed under elevated [CO_2].

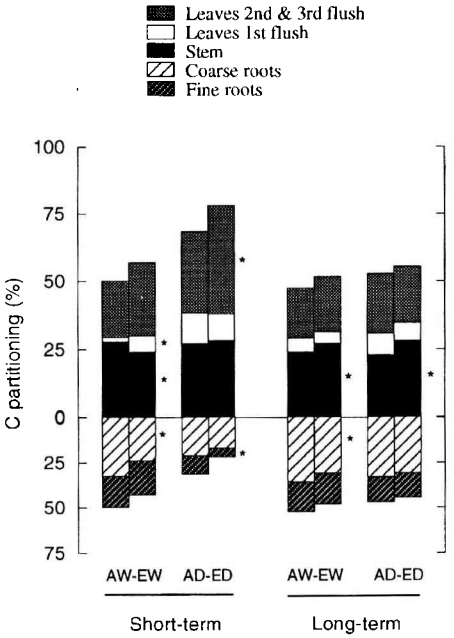

Fig 4. Short-term ^{13}C and long-term biomass C partitioning mean values (%) in the different parts of *Quercus robur* plants grown under a two by two factorial combination of atmospheric [CO_2]s and watering regimes. Labelling experiment was performed 22 weeks after sowing (see *Materials and methods*), and short-term C data represent plants harvested at the end of the ^{13}C chase phase (60 h). A, ambient [CO_2]; E, elevated [CO_2]; W, well-watered conditions; D, droughted conditions. Asterisks indicate statisticaly significant ($P < 0.05$, $n = 3$ to 5) differences between CO_2 treatments for the different plant parts (between brackets). (A), 350 vpm; (E), 700 vpm; (W), well-watered plants; (D), droughted plants.

DISCUSSION

Carbon uptake rates issued from the ^{13}C labelling data highlighted the direct positive effect of elevated CO_2 on C acquisition (fig 2, table IV), and are consistent with previous studies that have indicated a pronounced photosynthetic stimulation in *Q robur* in response to CO_2 enrichment (Vivin et al, 1995; Picon et al, 1996a, b). Consequently well-watered plants grown under elevated CO_2 produced 1.52 times more total biomass (dry mass at harvest) over the 22-weeks period than plants grown under ambient CO_2 (table II), as commonly reviewed in woody plants (Poorter, 1993; Ceulemans and Mousseau, 1994; Wullschleger et al, 1995) and namely in The *Quercus* genus (Norby, 1996). The relative magnitude of this response has often been positively related to water and/or nutrient availability in the growth medium (Conroy

et al, 1988; Johnsen, 1993; Samuelson and Seiler, 1994; Dixon et al, 1995, Hibbs et al, 1995; Picon et al, 1996a; Townend, 1995) and to the genetic capacity of plants to increase the size or the number of their C sink organs (Kaushal et al, 1989; Vivin et al, 1995). It is also noteworthy that our 1.32-fold increase in annual growth efficiency (table II) closely corresponds to the average response in many CO_2-enrichment experiments with woody plants (Wullschleger et al, 1994; Norby, 1996). In the present study, the growth and biomass of stem, which represents a major C sink specific to woody plants leading to the constitution of metabolically inactive C pools (namely lignin and cellulose), were particularly increased in response to elevated CO_2 (table II, fig 3). It has already been observed that elevated CO_2 leads to increased wood density and increased the thickness of the cell walls in coniferous species (Conroy et al, 1990); but there is no straightforward conclusion on how the fractions of lignin and cellulose are affected by rising atmospheric CO_2 in trees. Thus, whether the amounts of carbon fixed in such metabolically inert pools differ with the availability of carbohydrates and water remains an open question.

According to the theory of the balanced shoot and root activity in response to various resources (as atmospheric CO_2 concentration, nutrients, soil water) (Chapin, 1980), a stimulation of the specific activity of the shoot is expected to increase either the structure or the function of the root system in order to balance the internal resource demand. However, although plant N concentration was lower in CO_2-enriched well-watered plants (table III), the R/S ratio was slightly decreased by elevated CO_2 in this experiment. Such an observation has also been reported in a few other studies on woody species (Norby and O'Neill, 1989; Guehl et al, 1994; Tschaplinksi et al, 1995a; Vivin et al, 1995; Picon et al, 1996a) and is not consistent with the basic asumption that a larger proportion of the recently photoassimilated C is allocated to the below-ground structures when internal resources become limiting (Larigauderie et al, 1988, 1994; Sinclair, 1992; El Kohen et al, 1992). Furthermore, plants grown under limiting water supply generally allocate relatively more recently fixed C to the below-ground compartment (Wilson 1988, Geiger and Servaites, 1991); this effect would be advantageous for the acquisition of water under field conditions (Tyree and Alexander, 1993; Morison, 1993). But surprisingly in this experiment, not only the R/S ratio but also short-term (fig 4) or time-integrated (table II, fig 4) below-ground C allocation were reduced by drought in both CO_2 treatments. Similar obervations were made in *Alnus rubra* by Arnone and Gordon (1990) and by Hibbs et al (1995). In fact surveying the available data on the influence of elevated CO_2 on the distribution of dry matter between different plant organs measured as the R/S ratio, no general pattern emerges (Stulen and Den Hertog, 1993; Rogers et al, 1994; Norby, 1994). The average R/S ratio values compiled from 398 observations on 73 tree species grown in elevated CO_2 have been shown to remain constant over a broad spectrum of soil water or nutrient status (Wullschleger et al, 1995). Norby (1994) emphasized that ontogenic shifts, such as the change in allometric parameters between roots and shoots with age may have limited the relevance of R/S ratio results.

The analysis of metabolically-active plant compartments is more relevant than static measurements of R/S ratio (Norby, 1994). An increased total root biomass at elevated CO_2 (see reviews by Rogers et al, 1994; Norby et al, 1995) could be the result of increased root storage, in which case no improvement in water and resource acquisition might be expected (Larigauderie et al, 1994). In the present study, elevated CO_2 increased root biomass, and this effect was higher in fine roots (+47%) than in coarse roots (+26%). It is also noteworthy that both

our short-term ^{13}C labelling and biomass results pointed to a similarity in the patterns of C allocation to fine roots of *Quercus robur* in both CO_2 treatments. About 18% of the recently photoassimilated ^{13}C is allocated to fine roots after a 60-h chase period under non-limiting soil water availability at this point in plant development (fig 4). Similarly, no difference in ^{14}C distribution to metabolically-active roots were reported in the few CO_2 experiments on herbaceous species (Lekkerkerk et al, 1990; Billes et al, 1993; Paterson et al, 1996) and on woody plants (Rouhier et al, 1994), but Norby et al (1987) found that seedlings of *Pinus echinata* grown at 695 vpm [CO_2] allocated proportionately more ^{14}C-labelled photosynthates to fine roots than seedlings grown at 368 vpm CO_2.

The lack of response to [CO_2] in C allocation to fine roots does not mean that root activity has not been modified by CO_2 enrichment. Original insights into fine root activity in response to doubling [CO_2] were again provided by the ^{13}C labelling approach. RSA values, which constitute a measure of the short-term relative growth rate and sink strength, were 2.66 times higher in CO_2-enriched fine roots grown under non-limiting water availability (fig 3), suggesting a greater specific activation of the short-term component of photoassimilate allocation to this compartment by the CO_2 conditions (Thomas and Strain, 1991). In the droughted fine roots, this CO_2 effect was less apparent, although RSA values, after the ^{13}C pulse phase period were slighly higher in fine roots of CO_2-enriched plants (fig 3), as suggested by Masle (1992).

Our results confirmed that C availability for growth and metabolic processes will be greater in all plant parts and particularly in fine roots of *Quercus robur* plants grown under an elevated CO_2 atmosphere. Although no architectural and structural analyses of fine root system were performed in this study, we can assume from a previous experiment using minirhizotrons (Vivin, unpublished results) that CO_2-enriched plants would therefore exploit a greater soil volume and/or a given volume of soil more thoroughly. In addition, an increased supply of photosynthates to below-ground could also stimulate various metabolic or microbial activities in the fine roots and associated rhizosphere (Stulen and Den Hertog, 1993; Norby 1994). However, whether water and resource acquisition would be enhanced under elevated [CO_2] remains an open question in this experiment.

Because this experiment was conducted in controlled environments with containerized plants over a relatively short period of time the results of this study need to be taken as a potential indicator of what may happen in forests in the future. Further studies should be of a long enough duration to gain an understanding of seasonal and ontogenetic trends.

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APPENDIX 1

Expressions required to compute mg excess ^{13}C, relative specific allocation (RSA_C), and relative distribution of excess ^{13}C (P). R represents the absolute ratio ($^{13}C/^{12}C$) and F represents the fractional abundance [$^{13}C/(^{13}C+^{12}C)$] (adapted from Deléens et al, 1995). The absolute $^{13}C/^{12}C$ for R_{PDB} is 0.0112372.

a. $$\delta\,^{13}C_{PDB} = \left| \frac{R_{sample}}{R_{PDB}} - 1 \right| \times 1000$$

b. $$R_{sample} = \left| \frac{\delta\,^{13}C_{PDB}}{1000} + 1 \right| \times R_{PDB}$$