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Changes in dry weight and nitrogen partitioning induced by elevated CO_2 depend on soil nutrient availability in sweet chestnut (*Castanea sativa* Mill)

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Summary — The effect of 2 levels of atmospheric carbon dioxide (ambient, *ie* 350 ppm, and double, *ie* 700 ppm) and 2 contrasting levels of mineral nutrition on dry weight, nitrogen accumulation and partitioning were examined in 2-year-old chesnut seedlings (*Castanea sativa* Mill), grown in pots outdoors throughout the vegetative season. Fertilization had a pronounced effet on dry weight accumulation, tree height, leaf area, and plant nitrogen content. Carbon dioxide enrichment significantly increased total biomass by about 20%, both on fertilized and on unfertilized forest soil. However, the partitioning of biomass was very different: on the unfertilized soil, only the root biomass was increased, leading to an increase in the root: shoot ratio. Contrastingly, on fertilized soil only stem biomass and diameter but not height were increased. Carbon dioxide enrichment significantly reduced the nitrogen concentration in all organs, irrespective of the nutrient availability. However, the biomass increase made up for this reduction in such a way that the total nitrogen pool per tree remained unchanged.

elevated CO_2 / dry weight partitioning / nitrogen partitioning / *Castanea sativa* Mill

Résumé — Les effets d'un enrichissement en CO_2 sur la répartition de la matière sèche et de l'azote chez le châtaignier (*Castanea sativa* Mill) dépendent de la fertilité du sol. *On a étudié l'effet d'un doublement de la concentration en CO_2 de l'atmosphère (soit 350 vpm, teneur actuelle et 700 vpm) sur la répartition de la biomasse et du contenu en azote chez de jeunes plants de châtaigniers (*Castanea sativa *Mill). Les arbres, âgés de 2 ans, sont cultivés en pots à l'extérieur pendant toute une saison de végétation sous des tunnels ou miniserres recouvertes de propafilm et ventilées en permanence. Le doublement du CO_2 ambiant est obtenu par addition constante de CO_2 pur d'origine industrielle. Ces jeunes châtaigniers sont cultivés sous nutrition minérale contrastée (sol forestier auquel est ajouté ou non de l'engrais NPK en granulés).*

Une fertilisation du sol forestier d'origine augmente nettement la biomasse, la hauteur et la surface foliaire totale des arbres, ainsi que leur contenu en azote. L'augmentation de la biomasse due au doublement du CO_2 (de l'ordre de 20%) est la même quelle que soit la fertilité du sol. Par contre, la répartition de la matière sèche est très différente sur sol fertilisé ou non fertilisé. Sur sol pauvre, l'augmentation de biomasse est uniquement localisée dans les racines, d'où une augmentation du rapport parties souterraines/parties aériennes. Au contraire, sur sol fertilisé, l'augmentation de bio-

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masse concerne uniquement la partie aérienne, dont la tige grossit en diamètre et non pas en hauteur. L'enrichissement en CO_2 réduit de manière significative la concentration en azote de tous les organes, quel que soit le degré de disponibilité en azote du sol. Cependant, l'augmentation de biomasse des organes compense cette réduction de telle manière que le pool d'azote par arbre reste constant.

enrichissement en CO_2 / répartition de la matière sèche / distribution de l'azote /* Castanea sativa *Mill

INTRODUCTION

Among the effects of the increase in atmospheric CO_2, those concerning trees are particularly important because forest ecosystems are the major carbon store of the biosphere. Earlier work on the effect of elevated CO_2 on young trees (Eamus and Jarvis, 1989) has shown a general increase in total dry weight. Tree ring measurements over the past 100 years (Kienast and Luxmoore, 1988) have provided direct evidence of increase in tree growth, although this has not been directly related to elevated CO_2 alone. One may thus assume that an elevated CO_2 will induce an increase in the trees' carbon storage despite widespread tropical deforestation that is counteracting this effect (Houghton *et al*, 1991).

Generally, tree responses to CO_2 enrichment include an increase in net photosynthesis and thereby in growth and dry weight production (Jarvis, 1989). In most of the experiments reported in the literature, nutrients have been supplied in sufficient amounts. However, forests frequently grow on nutrient-poor soils and their productivity is strongly related to soil fertility. It has been demonstrated that a limitation in resources does not preclude plant growth response to CO_2 enrichment (Norby *et al*, 1986b). However, the limit in the CO_2 response may be connected with the total amount of nitrogen that could be obtained from a poor environment: growth stimulation will depend on the sink activity, which is itself stimulated by nutrient availability (Cromer and Jarvis, 1990).

Sweet chestnut, *Castanea sativa* Mill, is a relatively fast growing species, bearing large leaves with a relatively high photosynthetic capacity (Ceulemans and Saugier, 1991). Sweet chestnut is common in the French deciduous forest, being the third major genus following *Quercus* and *Fagus* in terms of area (one million hectares) and productivity. These specific features make *Castanea* a good model to investigate the effects of elevated CO_2 on temperate tree species.

The experiment reported here was designed to investigate the effect of elevated CO_2 in well-watered trees under full sunlight in 2 contrasting nutrient situations.

MATERIALS AND METHODS

Two-year-old bare-root chestnut seedlings were obtained from a forestry nursery (Bauchery et Fils, Crouy sur Cosson, La Ferté-St-Cyr, France). The seedlings were planted in cylindrical pots (25 cm diameter, 50 cm height) filled with 24 l of soil. The soil was taken from a nearby chestnut stand; it consisted of the upper 15 cm organic layer of forest soil sifted and homogenized after litter removal.

The main soil characteristics were as follows:

apparent density: 1.5 $g.cm^3$;

field capacity: 15% (weight fraction);

available water: 10% (weight fraction);

cation exchange capacity: 26 meq/1 000 g dry weight;

total nitrogen content: 0.37 g/1 000 g dry weight;

total organic matter: 10.6 g/1 000 g dry weight;

C/N: 16.5.

Fertilization of the soil was provided monthly with fertilizer granules spread over the pots' surface. These mineral granules (Engrais SECO, Ribécourt, France) contained 17% nitrogen (6.2 NO_3^- and 10.8 NH_4^+), 17% $P_2O_5^{--}$, (16% water-soluble) and 17% K_2O soluble in water. Forty granules were distributed monthly in each pot, providing 0.82 g N, 0.78 g P and 0.4 g K. These quantities were 3 times as high as the final mineral content of a tree at the end of 1 year's growth. These nutrients were progressively dissolved into the soil *via* an automatic drip system.

Twenty-four trees were planted in each mini-greenhouse. For various reasons (pests, breaks, etc), the number of trees analysed in each experimental situation varied between 16 and 20. This number is given in each specific table. *t*-Test was used for comparison of means and ANOVA to assess the interaction between CO_2 and fertilization treatments.

The pots were placed in trenches 2 m long and 1 m wide, covered with ventilated mini-greenhouses made of polypropylene films glued onto aluminium frames (1 m high). Air was blown continuously over the plants at a rate of 150 $l.s^{-1}$ which was sufficient to maintain the air temperature close to that of the outside air (+ 2 °C max). In half of these mini-greenhouses, a double CO_2 concentration (*ie*, 700 ppm) was maintained with pure industrial CO_2 introduced at a constant rate (120 $l.h^{-1}$) into the main air flow. The other half was ventilated with normal air.

The trees were watered daily with tap water in order to compensate for daily evapotranspiration (*ie* about 200 g water per pot).

Total leaf area per tree was computed by measurements of length (L) and width (W) of all leaves (S = L x W x 0.65). After leaf fall, all dead leaves were collected and weighed. Later, in January, the plants were dug up, roots were washed under water, and shoot and root dry weight were evaluated.

RESULTS

Dry weight partitioning

Figure 1 shows the effect of a double CO_2 on the dry weight partitioning between shoots and roots in the fertilized and unfertilized situation. In normal air, there was more than a doubling in dry biomass production of the seedling with the increase in nutrient availability. This confirms that trees' mineral nutrition was a strong growth limiting factor. It can also be noticed that fertilization enhanced the shoot (x 3) production more than the root (x 2) production. It followed that the root/shoot ratio decreased significantly, as previously described (Agren and Ingestad, 1987).

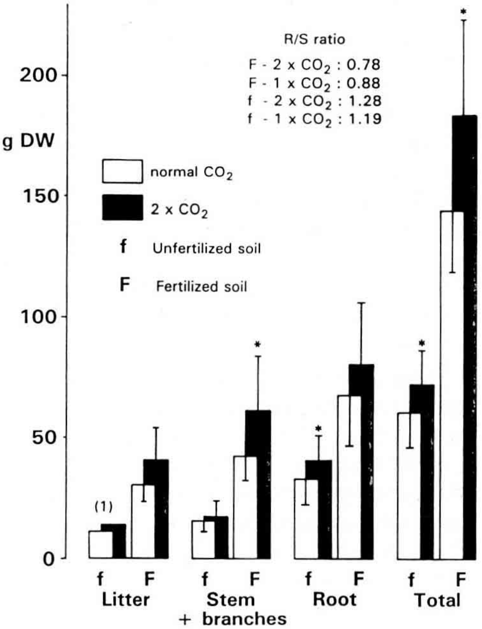

Fig 1. Dry weight of tree parts (g DW ± SD) under normal and elevated CO_2 in young chestnut seedlings cultivated on fertilized or unfertilized soil. R:S: Root to shoot ratio (root/stem + branches + leaves); * different from the control (normal CO_2) at 5%; (1): no SD for these data because the litter was collected as a whole.

The percentage of total dry weight increase due to CO_2 enrichment was equivalent in the unfertilized or fertilized situation: the doubling of atmospheric CO_2 was responsible for an increase of about 20% in total dry weight. However, CO_2 enrichment had a specific effect on dry weight partitioning to roots and shoots: on poor forest soil, the whole dry weight increase due to elevated CO_2 was allocated to the roots. The stem dry weight had been reported to be negatively affected by elevated CO_2 in this species (Mousseau and Enoch, 1989) which was not significant in the present experiment.

Contrastingly, on the fertilized soil, elevated CO_2 affected mostly stem + branches (+ 33%) and litter (+ 35%) dry weight accumulation (fig 1). A significant interaction between fertilization and CO_2 treatment was observed for these parameters (F = 5.06 and 5.39 respectively; df = 1.67). The corresponding increase in root dry weight, although noticeable in figure 1, was not significant at $P < 5\%$ and no interaction was noted.

In both fertilized and unfertilized situation, neither an increase in stem length nor any effect on branching due to the CO_2 treatment was noted (results not shown) although it has been reported in other species (Sionit *et al*, 1985). Therefore, when stem dry weight was increased (*ie* in the fertilized situation), this was mainly due to stem diameter increase (table I).

No effect of elevated CO_2 could be noted on leaf area development in unfertilized trees (table I) as reported earlier (Mousseau and Enoch, 1989). This was not the case with fertilized trees, for which leaf area per plant was significantly increased by the CO_2 treatment (table I).

Nitrogen distribution within the trees

Under both fertilization treatments, elevated CO_2 decreased nitrogen concentration in all organs. This decrease was especially significant in roots (table II). Litter (and not leaf) nitrogen content is mentioned in table II because the analyses were performed in winter, after leaf fall and nitrogen redistribution to other plant parts. The analysis made on a few green leaves at the end of the growing season (before yellowing: 1st September) showed a decrease in leaf nitrogen concentration in response to CO_2 enrichment similar to that found in other organs, irrespective of the fertilization treatment (table III).

Table I. Effect of CO_2 enrichment on the shoot growth characteristics of sweet chesnut grown at 2 levels of nutrient availability.

	Unfertilized		*Fertilized*	
	Normal CO_2	*2 x CO_2*	*Normal CO_2*	*2 x CO_2*
Stem + branch growth (cm)	76 ± 29	75 ± 29	178 ± 80	201 ± 60
Stem diameter (cm)	1.73 ± 0.02	1.79 ± 0.02	1.72 ± 0.03	2.03 * ± 0.03
Leaf area (dm^2)	18.9 ± 4	18.0 ± 8	30.2 ± 8	37.3 * ± 8

* Number different from control (normal CO_2) at $P < 5\%$ ± SD; $n = 16$ for the unfertilized condition, $n = 20$ for the fertilized condition.

Table II. Effect of nutrient availability on nitrogen partitioning in young chestnut trees *(Castanea sativa)* growing in normal or double atmospheric CO_2.

	Unfertilized		*Fertilized*	
	Normal CO_2	*2 x CO_2*	*Normal CO_2*	*2 x CO_2*
A. Percentage N concentration (±SD)				
Litter	0.88	0.98	0.63 ± 0.11	0.634 ± 0.09
Stems	0.75 ± 0.13	0.67* ± 0.13	0.734 ± 0.13	0.594* ± 0.09
Roots (coarse)	1.27 ± 0.23	1.01* ± 0.21	1.16 ± 0.9	0.793* ± 0.10
Roots (fine)	1.92 ± 0.22	1.62 ± 0.29	1.834 ± 0.16	1.312* ± 0.20
Total	1.06	0.92* ± 0.05	0.904 ± 0.12	0.70* ± 0.07
B. N pool size (g per organ (± SD)				
Litter	0.10	0.13	0.19 ± 0.04	0.26 ± 0.09
Stems	0.12 ± 0.03	0.12 ± 0.05	0.32 ± 0.05	0.36 ± 0.13
Roots (coarse)	0.38 ± 0.1	0.35 ± 0.09	0.69 ± 0.07	0.58 ± 0.12
Roots (fine)	0.04 ± 0.01	0.057* ± 0.01	0.072 ± 0.03	0.072 ± 0.03
Total	0.64 ± 0.1	0.66 ± 0.1	1.26 ± 0.15	1.28 ± 0.32

* Numbers different from control (normal CO2); $n = 17$ in the unfertilized condition and $n = 20$ in the fertilized condition.

More nitrogen in the soil increased the overall nitrogen concentration and content of the seedlings. The nutrient pool sizes were calculated by multiplying the mean nutrient concentration by the mean dry weight. In all cases, the increase in dry weight due to elevated CO_2 seemed to make up for the decrease in nitrogen concentration so that the total leaf nitrogen pool size remained similar. However, as more fine roots were produced in the unfertilized situation (results not shown) their N pool size was higher (table IIB). So, plants seem to invest a larger amount of their lower nitrogen concentration (table IIA) into their fine roots. This was not true in the fertilized situation as shown by the results from ANOVA analysis on fine roots.

The same conclusion may be drawn from table II for all organs and this resulted in a similar overall nitrogen content of the tree in normal and enriched CO_2.

DISCUSSION

The effect of elevated CO_2 on dry weight accumulation did not differ in the fertilized and unfertilized situation. This result is very similar to the study on yellow poplar *(Liriodendron tulipifera)* described by Norby and O'Neill (1991). However, these authors did not find any differences in dry weight partitioning of their trees. We may conclude, as did Idso *et al* (1991), that if there is no nutriment limitation, an increase in CO_2 will be of great benefit to tree growth.

Our results agree with the predicted general dependence of root/shoot ratios on internal nitrogen concentration (Thornley, 1972; Ågren and Ingestad, 1987).

In general, higher CO_2 concentrations produce tissues with lower nitrogen concentration (Williams *et al,* 1986; Brown, 1991). The comparison of chestnut behaviour in different nutritional conditions demonstrates that internal nitrogen concentration decreased both on fertile and unfertile soil under elevated CO_2. We may assume either: 1), a slower increase in nutrient uptake than in carbon assimilation; or 2), no increase in nitrogen uptake and a progressive dilution of this nitrogen into the plant: the second hypothesis is more probable in our case because the roots were limited in total nitrogen uptake by the size of the pots. This could suggest that even in the fertilized situation, the dry weight production could have been nutrient limited. This was not probable because the total nitrogen amount that was added to the pots was 3 times greater than the total plant nitrogen content at the end of the season. However, we cannot eliminate the hypothesis because a leaching of nitrogen with watering is always possible.

In forest ecosystems, these lower nitrogen concentrations could lead to nutrient deficiencies which would probably be compensated by an increase in the amount of fine roots and mycorrhiza (O'Neill *et al,* 1987) which would extract nutrients from a wider surrounding area.

In our experiment, after 1 year of CO_2 enrichment, the leaves that abscised from the enriched seelings contained a higher nitrogen level (table II) than the control leaves, although the reverse situation was found in green leaves (table III). It may be assumed that the amount of nitrogen compounds sent to the reserve organs in the

Table III. Effect of CO_2 enrichment on nitrogen concentration and pool size of chestnut leaves at the end of the growth period (1st September) under 2 fertilization treatments (no SD was calculated on these measurements because the few leaves sampled on all the plants were pooled to be analyzed).

	Unfertilized		*Fertilized*	
	Normal CO_2	*2 x CO_2*	*Normal CO_2*	*2 x CO_2*
Leaf N concentration				
% leaf area (mg/dm^2)	11.9	8.5	14.7	11.1
% DW (mg/100 mg)	1.65	0.98	2.42	1.8
Specific leaf dry weight (10^2 mg.dm^{-2})	0.139	0.115	0.164	0.162
Leaf dry weight (g per plant)	13.7	15.6	18.4	23
Leaf N content (g per plant)	0.22	0.15	0.44	0.41

fall was affected by the CO_2 treatment. Norby *et al* (1986a) also found that there was less nitrogen to translocate in elevated CO_2. However, Couteaux *et al* (1991) showed that, after a 2-year CO_2 enrichment, the results were different: the chestnut litter nitrogen content was significantly decreased by a double CO_2 concentration and the total amount of nitrogen which returned to the soil from litter decomposition was lowered, contributing to increase the deficit in soil nutriment. Overall, the fact that the totality of additional dry weight in seedlings grown in high CO_2 was allocated to the roots in low nutritional conditions might confer an advantage to tree survival capacity in a double-CO_2 world, particularly if the water stresses were expected to increase.

It is of interest to foresters that a tree is able to partition larger amounts of dry weight to the trunk. This was the case of the CO_2 enriched chestnut in a well fertilized soil: although trunk height was not changed, an increase in diameter led to a greater wood volume. Such an increase depends on cell division in the cambium which we may assume to be stimulated by high CO_2 levels. Moreover, in the case of *Pinus radiata*, an elevated CO_2 has been shown to also increase wood density (Conroy *et al*, 1990).

Lastly, our results emphasize the need for controlling, or at least measuring, the nutrient conditions of the experimental tree seedlings submitted to an increase in CO_2 before any conclusions about the latter effect can be made and extrapolated to forest ecosystems.

REFERENCES

Ågren GI, Ingestad T (1987) Root: shoot ratio as a balance between nitrogen productivity and photosynthesis. *Plant Cell Environ* 10, 579-586

Brown KR (1991) Carbon dioxide enrichment accelerates the decline in nutrient status and relative growth rate of *Populus tremuloides* Michx seedlings. *Tree Physiol* 8, 161-173

Brown K, Higginbotham KO (1986) Effects of carbon dioxide enrichment and nitrogen supply on growth of boreal tree seedlings. *Tree Physiol* 2, 223-231

Ceulemans R, Saugier B (1991) Photosynthesis. *In: Physiology of Tree* (Raghavendra AS, ed) J Wiley (in press)

Conroy JP, Milham PJ, Mazur M, Barlow EWR (1960) Growth dry weight partitionning and wood properties of *Pinus radiata* D Don after 2 years of CO_2 enrichment. *Plant Cell Environ* 13, 329-337

Couteaux MM, Mousseau M, Celerier ML, Bottner P (1991) Atmospheric CO_2 increase and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. *Oikos* 61, 54-64

Cromer RN, Jarvis PG (1990) Growth and biomass partitioning in *Eucalyptus grandis* seedlings in response to nitrogen supply. *Aust J Plant Physiol* 17, 503-515

Eamus D, Jarvis PG (1989) The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. *Adv Ecol Res* 19, 1-55

Houghton RA, Skole DL, Lefkowitz DS (1991) Changes in the landscape of Latin America between 1850 and 1985: net release of CO_2 to the atmosphere. *For Ecol Manage* 38, 173-199

Idso SB, Kimball BA, Allen SG (1991) CO_2 enrichment of sour orange trees: 2.5 years into a long-term experiment. *Plant Cell Environ* 14, 351-353

Jarvis PG (1989) Atmospheric carbon dioxide and forests. *Phil Trans R Soc Lond B* 324, 369-392

Kienast F, Luxmoore RL (1988) Tree ring analysis and conifer growth responses to increased atmospheric CO_2 levels. *Oecologia* 76, 487-495

Kramer PJ, Kozlowski TT (1979) *Physiology of Woody Plants.* Academic Press, NY

Mousseau M, Enoch ZH (1989) Carbon dioxide enrichment reduces shoot growth in sweet chestnut seedlings (*Castanea sativa* Mill). *Plant Cell Environ* 12, 927-934

Norby RJ, Pastor J, Melillo JM (1986a) Carbon-nitrogen interactions in CO_2-enriched white oak: physiological and long-term perspectives. *Tree Physiol* 2, 233-241

Norby RJ, O'Neill EG, Luxmoore RJ (1986b) Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient poor soil. *Plant Physiol* 82, 83-89

Norby RJ, O'Neill EG (1991) Leaf area compensation and nutrient interactions in CO_2 enriched seedlings of yellow poplar *(Liriodendron tulipifera* L). *New Phytol* 117, 515-528

O'Neill EG, Luxmoore RJ, Norby RJ (1987) Increases in mycorrhizal colonisation and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO_2 atmosphere. *Can J For Res* 17, 878-883

Sionit N, Strain BR, Riechers GH, Jaeger CH (1985) Long-term atmospheric CO_2 enrichment affects the growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can J For Res* 15, 468-471

Thornley JHM (1972) A balanced quantitative model for root: shoot ratios in vegetative plants. *Ann Bot* 36, 431-441

Williams WE, Garbutt K, Bazzaz FA, Vitousek PM (1986) The response of plants to elevated CO_2. IV. Two deciduous tree communities. *Oecologia* 69, 454-459