Plant and Soil **157**: 215–225, 1993.

PLSO 9640

Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivum* L.) soil system in response to increased atmospheric CO_2 concentration

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Received 9 June 1992. Accepted in revised form 1 July 1993

Key words: carbon partitioning, CO_2 enrichment, nitrogen mineralization, nitrogen partitioning, rhizosphere, wheat

Abstract

The aim of this work was to examine the response of wheat plants to a doubling of the atmospheric CO_2 concentration on: (1) carbon and nitrogen partitioning in the plant; (2) carbon release by the roots; and (3) the subsequent N uptake by the plants. The experiment was performed in controlled laboratory conditions by exposing fast-growing spring wheat plants, during 28 days, to a $^{14}CO_2$ concentration of 350 or 700 $\mu L\ L^{-1}$ at two levels of soil nitrogen fertilization. Doubling CO_2 availability increased total plant production by 34% for both N treatment. In the N-fertilized soil, the CO_2 enrichment resulted in an increase in dry mass production of 41% in the shoots and 23% in the roots; without N fertilization this figure was 33% and 37%, respectively. In the N-fertilized soil, the CO_2 increase enhanced the total N uptake by 14% and lowered the N concentration in the shoots by 23%. The N concentration in the roots was unchanged. In the N-fertilized soil, doubling CO_2 availability increased N uptake by 32% but did not change the N concentrations, in either shoots or roots. The CO_2 enrichment increased total root-derived carbon by 12% with N fertilization, and by 24% without N fertilization. Between 85 and 90% of the total root derived-^{14}C came from respiration, leaving only 10 to 15% in the soil as organic ^{14}C. However, when total root-derived ^{14}C was expressed as a function of root dry weight, these differences were only slightly significant. Thus, it appears that the enhanced carbon release from the living roots in response to increased atmospheric CO_2, is not due to a modification of the activity of the roots, but is a result of the increased size of the root system. The increase of root dry mass also resulted in a stimulation of the soil N mineralization related to the doubling atmospheric CO_2 concentration. The discussion is focused on the interactions between the carbon and nitrogen allocation, especially to the root system, and the implications for the acquisition of nutrients by plants in response to CO_2 increase.

Abbreviations: −N-soil fertilization without nitrogen; +N-soil fertilization with nitrogen

Introduction

Large amounts of carbon are released from living roots into the soil, essentially as root and rhizosphere respiration but also, to a lesser extent, as rhizodeposited organic carbon which remains in the soil. It is estimated that the root-derived carbon accounts for between 10 and 40% of the total assimilated carbon (Van Veen et al., 1991). The large variation is principally related to plant species, their phenological stage and the environmental conditions for growth. The micro-

bial utilisation of root derived organic compounds for biosynthesis and energy production (Bottner et al., 1988; Merckx et al., 1985, 1987), is probably closely connected to a simultaneous transformation of native soil organic matter, leading to mobilization of nutrients, especially N mineralization from stable organic compounds (Billès et al., 1988; Clarholm, 1989; Texier and Billès, 1990). The mechanisms involved are microbial activity in the rhizosphere and competition for nutrients between plants and microorganisms. The plant and soil related controls of carbon and nitrogen interactions in the rhizosphere are still poorly understood and need to be clarified (Griffiths and Robinson, 1992).

Recently, the rhizosphere activity and the mobilization and turnover of nutrients have gained new focus in relation to the effect of increased atmospheric CO_2 concentration on the carbon and nitrogen interactions in the plant-soil system. Predicting the response of plants to higher levels of atmospheric CO_2 requires an understanding of the interactions between CO_2 and limiting environmental factors such as nutrient availability, especially nitrogen (Goudriaan and de Ruiter, 1983; Larigauderie et al., 1988; Mooney et al., 1991). According to the hypothesis of Luxmoore (1981), an increase in atmospheric CO_2 concentration, by stimulating plant production and increasing belowground carbon translocation may increase (1) the extent of the root system; and (2) the rhizospheric activity. This would result in increased nutrient mobilization due to N fixation, mycorrhizal activity and mineralization.

Materials and methods

Soil characteristics and fertilization

The soil for the culture experiment was sampled in autumn from the 15 cm Ap horizon of an acid brown soil, in a wheat cultivated field, in the 'Cevennes', France. The soil was sieved (4 mm), homogenized, air dried, and stored at room temperature. The physical and chemical characteristics of the soil are: sand = 73.6%; silt = 19.5%; clay = 6.9%; organic C = 0.74%; organic N = 0.065%; pH(H_2O) = 4.9; water holding capacity = 12.1% dry soil.

Two types of fertilization were applied: first, all experimental units received 4.6 mg P and 5.8 mg K, added as an aqueous solution of KH_2PO_4 (which also served to re-moisten the soil to 80% of its water holding capacity); then, during the experiment, half of the pots were N-fertilized with a NH_4NO_3 solution carried out by adding 8 mg N per pot every 7 days for 4 weeks (32 mg pot^{-1}).

Growth conditions and plant labelling

Atmospheric $^{14}CO_2$ application was performed over a period of 28 days from seed to ear formation stage, in a gas-tight Plexiglass chamber (length = 1.3 m; width = 1.0 m; height = 1.2 m) under controlled conditions. The two CO_2 experiments (350 and 700 μL L^{-1}) were carried out in the same cabin with the same experimental conditions. Diurnal temperatures in the chamber were 23/16 °C ± 1 °C (day/night) and relative air humidity was 70 to 80%. Light was provided by five Osram HQI-T 400 W lamps, hung above and outside the chamber. Photosynthetically Active Radiation, measured at 30 cm and 120 cm from the chamber base, was 500 and 1000 μmol m^{-2} s^{-1} respectively during the 16 h photoperiod. $^{14}CO_2$ was derived from a 0.5 *M* $Na^{14}CO_3$ solution (specific activity = 1.7 KBq mg^{-1} C) by automatic injection into 9 *M* sulphuric acid. Atmospheric CO_2 concentrations (350 and 700 μL L^{-1}) were continuously monitored by an infra-red gas analyser and CO_2 derived from shoot dark respiration was automatically eliminated up to the defined concentration.

Experimental conditions for wheat growth

One kilogram of the re-moistened soil was added into 28 (14 for controls and 14 for enriched treatment) black plastic pots (height = 18 cm, diameter = 9 cm, volume = 1L) (Fig. 1). The pots were fitted with 2 cm thick rubber plugs leaving a 2 cm gap between the base of the plug and the surface of the soil. A plastic perforated tube (2 cm diameter) inserted through the central aperture of the plugs down to the surface of the

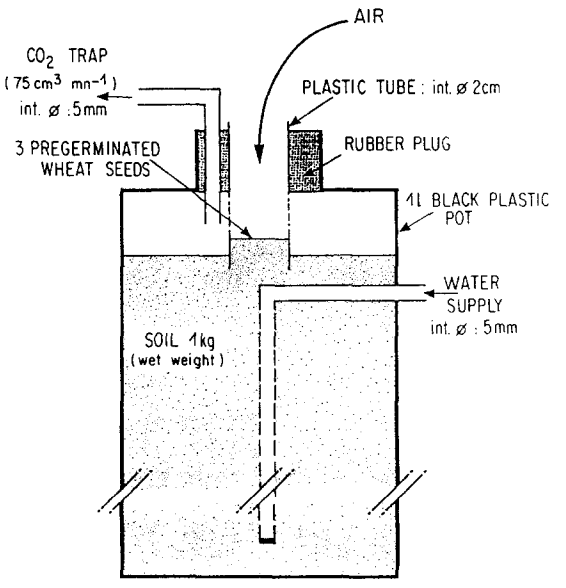

Fig. 1. Experimental design for continuous aeration and collection of CO_2.

soil allowed plant growth and air flow. A glass tube (0.5 cm diameter) inserted through a hole in the plug allowed air to be drawn from the surface of the soil and was connected to a trap for CO_2 measurements.

After two weeks of soil pre-incubation at room temperature three pre-germinated seeds of spring wheat (*Triticum aestivum* cv Florence Aurore) were planted in the soil through the central aperture. During the experiment the water loss from each pot was automatically monitored every hour by an electronic balance (sensitivity 1 g). As the weight decreased water was automatically added through a water-supplying pipe emerging at the centre of the soil volume (Fig. 1).

Eight pots of wheat were grown at each CO_2 concentration: 4 pots = SOIL(+N); 4 = SOIL(−N) and 6 pots were maintained without plants 3 = SOIL(+N); 3 = SOIL(−N).

Measurement of the CO_2 evolved from the soil

Sauerbeck and Johnen (1976) described a method which allows the measurement of CO_2 released from the surface of a soil separately from the atmospheric CO_2, without inhibiting the plant growth. In this experiment, a modification of this technique, which avoids physical separation between aerial and belowground parts, was tested. The technique prevents gas diffusion between the two compartments and avoids modifications of stem growth and root-shoot interactions. The air at the surface of the soil was drawn through the 0.5 cm tube (Fig. 1) by four Masterflex pumps, each one having four calibrated tubes. For each pump three of the tubes were connected to the pots and the fourth one was connected to the chamber and allowed the measurement of CO_2 concentration at four separated points within the chamber. The air from the soil surfaces contains the totality of the CO_2 released from root- rhizosphere- and soil-respiration, plus a fraction of CO_2 coming from the chamber through the central aperture. The CO_2 was trapped in columns containing glass beads and 50 mL of a solution of 1.0 *M* NaOH which was changed three times a week. The difference between the CO_2 drawn from the pots and the CO_2 drawn from the chamber gives the CO_2 evolved from roots and soil respiration. This kind of CO_2 calculation is valid if; (1) the CO_2 concentration inside the chamber is homogeneous; (2) the four tubes of each pump have the same delivery; and (3) the flow of the pumps is sufficient to prevent the soil CO_2 from escaping from the pot to the chamber through the central hole.

(1) The homogeneity of the CO_2 and specific activity (SA) inside the chamber were previously tested over 2 different periods of 24 h, from 4 different points. During the first 24 h the mean values were 25.99 ± 0.31 mg CO_2-C $24\ h^{-1}$ (coefficient of variation = 1.18%) and SA = 4.15 ± 0.04 KBq mg^{-1} CO_2-C (coef. var. = 0.90%). For the second 24 h period they were 26.24 ± 0.33 mg CO_2-C $24\ h^{-1}$ (coef. var. = 1.25%) and 4.06 ± 0.03 KBq mg^{-1} CO_2-C (coef. var. = 0.74%) respectively. The homogeneity inside the chamber was maintained by four ventilators.

(2) The flow homogeneity of the calibrated tubes was tested by an analysis of 10 series of CO_2 measurements lasting 2 h for each series, from 4 contiguous points with constant CO_2 concentration. The mean values for the 10 series of 4 points were 20.65 ± 0.38, 20.73 ± 0.33, 20.88 ± 0.41 and $20.58 \pm$

0.35 mg CO_2-C $24\,h^{-1}$. The mean coefficient of variation was 1.63%. There was no significant difference between the 4 mean values from the 10 series (two way ANOVA with cross classification).

(3) The CO_2 flow from the soil was negligible, compared to the flow of the pumps (75 cm^3 min^{-1}), allowing the replacement of the totality of the air above the soil surface in two minutes and giving negligible probabilities of a less of soil CO_2 through the 2 cm diameter central aperture.

The diffusion of labelled CO_2 from the atmosphere to the soil was not taken into account, since the level of atmospheric ^{14}C-CO_2 are probably negligible compared to the level of root derived ^{14}C-CO_2.

Sampling and analysis

Twenty eight days after sowing, i.e. roughly at the ear stage, the experiment was stopped. The shoots were cut off at the soil surface, the roots were separated from the soil by gentle shaking and the remaining small root fragments were removed by sieving (3 mm mesh) and with tweezers; finally the pooled root system was washed. Shoots and roots were dried at 80°C and weighed. The soil was air dried and sieved at 2 mm. The total C and ^{14}C contents of shoot, roots and soil were measured by dry combustion, using Carmograph 12A and liquid scintillation counting (Bottner and Warembourg, 1976). The total CO_2 trapped in the NaOH solution was measured colourimetrically by discolouration of a carbonate-bicarbonate buffer using a continuous flow analysis system (Chaussod et al., 1986).

Kjeldahl digestion and colourimetric analysis were used to determine the total nitrogen content of plant material and soil, using the continuous flow analysis system. The soil inorganic nitrogen was extracted from 50 g wet soil with 200 mL 0.5 *M* K_2SO_4. A 100 mL aliquot of the extract was steam distilled with the Dervarda mixture and the total inorganic nitrogen was determined colourimetrically following Berthelot's method using sodium phenate and hypochlorite reagents.

The specific activity (SA) of shoots were: 350 (−N) = 1.679; 350 (+N) = 1.649; 700 (−N) = 1.666 and 700 (+N) = 1.619 KBq mg^{-1} C. Lower SA in roots (of about 6%) compared to shoots is probably due to root pollution, with unlabelled soil native C, despite washing.

Statistical analysis

The data were analysed as a completely randomized experiment, with two CO_2 concentrations (350 and 700 $\mu L\ L^{-1}$ CO_2) and two N treatments (+N and −N), replicated four times. The significance of the two factors and their interaction was determined by a two way analysis of variance (ANOVA) and an F-test. Values in % (Table 2) were transformed in arcsine of the square root before variance analysis.

Results

Dry matter distribution

CO_2 enrichment significantly increased the total plant production by 34% in both the (+N) and the (−N) fertilized soil. Thus, the enhancing effect of increased CO_2 level was not altered by the N fertilization. Nevertheless, a significant interaction between CO_2 and N fertilizations was found when the distribution of dry matter between shoots and roots was considered (Table 1). When nitrogen was added (+N), CO_2 enrichment essentially stimulated shoot growth; total dry mass increased by 41% in the shoots and by 23% in the roots. Without N fertilization (−N) dry mass increase for shoots and roots was 33% and 37% respectively. The influence of CO_2 increase is obvious when shoot to root ratios are considered. The ratio increased significantly in response to the doubling of the CO_2 concentration in the (+N) fertilized soils ($p < 0.05$).

Distribution of assimilated carbon

Table 2 shows the amounts of radioactivity (KBq) and their relative distribution, after 28 days, in shoots, roots, root-derived CO_2 and root derived organic C remaining in the soil. The relative distribution is expressed as a percentage of the ^{14}C-labelled assimilated carbon:

Table 1. Effect of atmospheric CO_2 concentration and N supply on dry weight distribution, N concentration, N plant uptake and shoot to root ratio of wheat plants ($n = 4$) grown under $^{14}CO_2$ atmospheric for a 28 day period

	Whole plant Dry weight[a]	Shoots		Roots		N plant uptake[a]	Shoot/root ratio
		Dry weight[a]	N%	Dry weight[a]	N%		
350 μL CO_2 L^{-1}							
Soil (−N)	1869	1183	1.16	686	0.93	20.1	1.72
Soil (+N)	2188	1386	1.49	802	1.05	29.1	1.73
700 μL CO_2 L^{-1}							
Soil (−N)	2505	1567	0.90	939	0.94	22.9	1.67
Soil (+N)	2932	1950	1.42	982	1.09	38.4	1.99
Two way ANOVA:							
CO_2	57.1**	68.2**	19.0**	34.9**	3.1 ns	82.3**	8.52*
N-Fertil.	16.6**	26.2**	91.3**	4.7 ns	12.2*	270.8**	26.41**
$CO_2 \times N$	0.4 ns	2.5 ns	5.3 ns	1.0 ns	1.4 ns	30.3**	25.03**
Mean deviation	173.6	121.6	0.1	61.1	0.1	1.61	0.1

[a] dw and N plant uptake in mg pot^{-1}; ns = not significant, * and ** = significant at $p = 0.05$ and $p = 0.01$.

Table 2. Effect of atmospheric CO_2 concentration and N supply on the relative distribution of labelled carbon (KBq pot^{-1}) in wheat plants ($n = 4$) growing under $^{14}CO_2$ atmosphere for a 28 day period. Values in brackets are expressed as a percentage of total ^{14}C-labelled assimilated carbon

	Shoot (a)	Belowground translocated (b)	Root (c)	Root-derived carbon (d)	Respired CO_2 (e)	Soil (f)
350 μL CO_2 L^{-1}						
Soil (−N)	814 (46)	955 (54)	465 (26)	491 (28)	420 (24)	71 (4)
Soil (+N)	958 (47)	1073 (53)	518 (26)	556 (27)	472 (23)	84 (4)
700 μL CO_2 L^{-1}						
Soil (−N)	1081 (48)	1166 (52)	559 (25)	607 (27)	543 (24)	64 (3)
Soil (+N)	1331 (53)	1190 (47)	571 (23)	620 (25)	562 (22)	58 (2)
Two way ANOVA:						
CO_2	60.5**(**)	29.7**(**)	12.3**(**)	22.3**(ns)	36.7**(ns)	18.1**(**)
N-Fertil.	22.9**(*)	5.6*(*)	2.4 ns (ns)	4.0 ns (ns)	4.0 ns (ns)	0.8 ns (ns)
$CO_2 \times N$	1.7 ns (ns)	2.4 ns (ns)	1.0 ns (ns)	1.9 ns (ns)	0.9 ns (ns)	6.4*(ns)
Mean deviation	82.2 (2.1)	60.1 (2.1)	41.8 (1.4)	38.4 (1.7)	35.2 (1.5)	7.6 (0.4)

Percentage in brackets have been transformated in arcsine of the square root before variance analysis
(b) = (c) + (d); (d) = (e) + (f); (a) + (c) + (e) + (f) = Total assimilated C
ns = not significant, * and ** = significant at $p = 0.05$ and $p = 0.01$.

total assimilated-^{14}C = shoot-^{14}C (a) + belowground translocated-^{14}C (b),

belowground translocated-^{14}C = root-^{14}C (c) + root-derived-^{14}C (d),

root-derived-^{14}C = respired CO_2-^{14}C (e) + soil-^{14}C (f).

Significant differences were observed for the CO_2 factor, especially in the (+N) soil. The atmospheric CO_2 enrichment increased the proportion of carbon allocated to the shoots and decreased the proportion allocated below ground. The relative reduction of belowground allocation ($p < 0.01$) resulted in a both relative decrease in the root allocated-^{14}C and in the ^{14}C accumulated in the soil. The ^{14}C distributions in root-derived carbon and respired CO_2 were not significantly changed. At 350 μL L^{-1} CO_2 con-

centration the distribution pattern was unchanged by N fertilization, but at 700 μL L^{-1}, the modified distribution between shoot-^{14}C and belowground-^{14}C was slightly significant ($p < 0.05$).

Rhizospheric activity

The $^{14}CO_2$ drawn from the chamber and the $^{14}CO_2$ drawn from the soil surfaces are presented in Figure 2 on a daily basis. Differences between these two sources of CO_2 were significant ($p < 0.01$) from day 9 until the end of the experiment. Total release from the soil and root system was a function of the phenological stage of plants reaching maximum values 24 days after germination. That corresponded to the stage of stem elongation and ear filling and also to the maximum root development for the belowground system (Allard, 1980). The decrease observed towards the end of the 350 μL L^{-1} CO_2 experiment corresponded to a decline in the root activity (senescence). In contrast, at 700 μL L^{-1} CO_2, during the stem elongation stage, total respiration was maintained at significantly higher levels, or even continued to increase for the (+N) fertilized plants. The atmospheric CO_2 enrichment tended to prolong the high root and rhizosphere activity.

The total root-derived C (Table 2) was increased by the atmospheric CO_2 enrichment ($p < 0.01$) and not by the N fertilization. The CO_2 enrichment increased total root-derived carbon by 12% with (+N) soil, and by 24% with (−N) soil. The respired CO_2, accounted for 85 to 90% of the total root-derived C, leaving 10 to 15% as organic C remaining in the soil. This distribution was not modified by N fertilization. In response to the atmospheric CO_2 concentration, respired soil and root CO_2 was increased by 19% with (+N) treatment and by 30% with (−N) treatment, while soil-C was unchanged without N fertilization and decreased by 30% with N fertilization.

However, when the total root-derived ^{14}C was expressed as a function of the root mass the above mentioned differences were only slightly significant. The total rhizo-deposited carbon released from 100 mg of root (dry weight) was 72 KBq (−N) and 69 KBq (+N) for controls (350 μL L^{-1} CO_2) and 65 KBq (−N) and 63 KBq (+N) for enriched (700 μL L^{-1} CO_2) trees. It seems that the enhanced carbon release from the living roots in response to increased

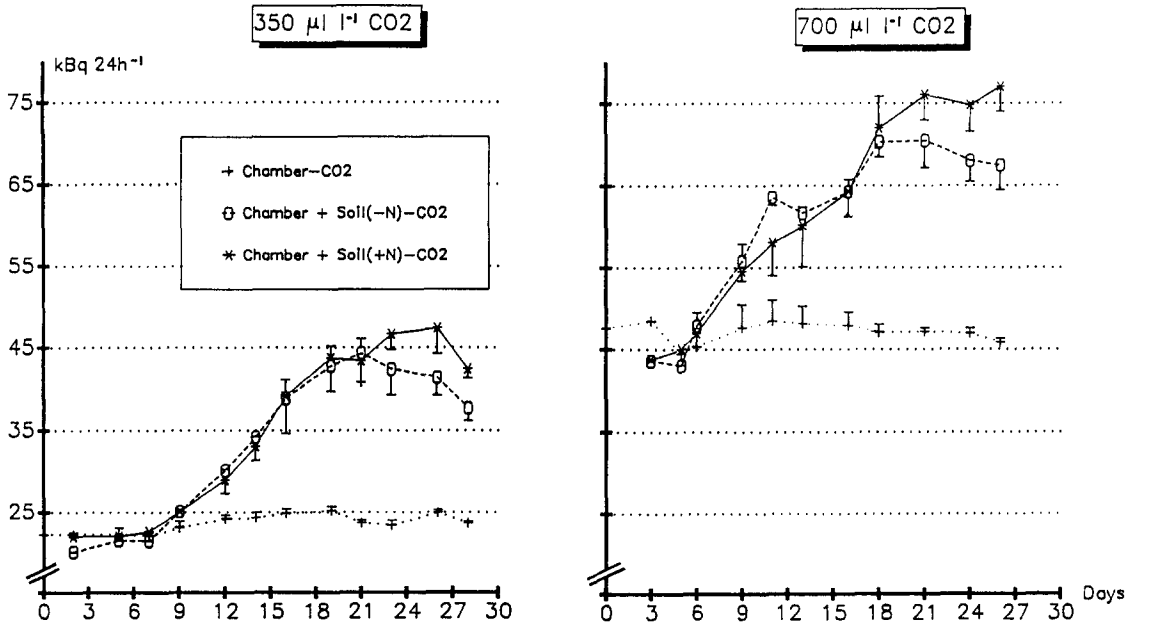

Fig. 2. Dynamics of root and soil derived ^{14}C-labelled CO_2 (KBq 24 h^{-1}) from wheat plants ($n = 4$) grown under 350 and 700 μL L^{-1} ^{14}C-labelled atmospheric CO_2 for a 28 day period.

atmospheric CO_2 concentration is not due to a modification of the activity of the roots, but is a result of the increased size of the root system (Gifford et al., 1985).

Nitrogen distribution

Doubling of the atmospheric CO_2 concentration modified the quality of the plant material. The carbon content of shoot and root remained constant (results not shown), but nitrogen concentration was modified (Table 1). For (−N) soils, the CO_2 enrichment reduced the N concentration in shoots by about 23% while the N concentration in roots was not modified. For (+N) soil, the CO_2 increase did not change the N concentrations in either the shoots or the roots. The additional N fertilization (32 mg N pot^{-1}) represent 100 and 75%, under 350 and 700 $\mu L\ L^{-1}$ CO_2 respectively, of the total N assimilation by plants in fertilized conditions. Therefore, it would seem that the plants were not N stressed.

The total N uptake for the whole plant increased by 14% in the (−N) treatment and by 32% in the (+N) treatment (Table 1). The CO_2 enrichment led to a modification of plant N concentration. For (+N) plants, increased N-uptake was equivalent too the biomass increment (34%), whereas for (−N) plants the same biomass increment was not accompanied by an identical increase of N-uptake (14%), this resulted in a decrease of N concentration only in the shoots of enriched plants.

The ratio of plant material to plant N represents the amount of dry matter produced per unit of total plant-N. Values of that ratio were: 350 (−N) = 10.75; 350 (+N) = 13.3; 700 (−N) = 9.14; 700 (+N) = 13.10 mg N g^{-1} of plant dry weight. They vary significantly in response to both the atmospheric CO_2 concentration and the N fertilization. CO_2 enrichment increased N use efficiency by 18% for (−N) treatment and was not changed for (+N) treatment. The N fertilization decreased, dry weight produced per mg of plant N content, by 19% for controls and 30% for enriched trees.

Net mineralization of soil nitrogen

The inorganic N amounts (Table 3) were measured at the end of the experiment (28 days) from the cultivated and uncultivated (control) pots, maintained in the same experimental conditions. For controls and (−N) treated soil the inorganic N production was low and accounted for only 21 and 23 $\mu g\ g^{-1}$ dry soil. The additional N fertilization (32 mg N pot^{-1}) probably led to a modification of the soil N net mineralization and accordingly influenced the inorganic N availability, since only 40 mg were found at the end of the experiment.

Table 3. Effect of atmospheric CO_2 concentration and N supply on the inorganic nitrogen ($\mu g\ g^{-1}$ dry soil) in uncropped and cropped soils with wheat plants ($n = 4$) for a 28 day period

	Uncropped soil Inorg. N	Cropped soil		Plant effect (%)
		Inorg. N	Inorg. + Plant N	
350 $\mu L\ CO_2\ L^{-1}$	(a)		(b)	(c)
Soil (−N)	21.0	4.5	26.5	+26.2 $p < 0.001$
Soil (+N −	39.0	2.9	34.7	−11.0 $p < 0.001$
700 $\mu L\ CO_2\ L^{-1}$				
Soil (−N)	23.0	7.0	32.0	+39.0 $p < 0.001$
Soil (+N)	42.0	5.4	47.4	+12.8 $p < 0.001$
Two way ANOVA:				
CO_2	5.9*	5.2 ns	135.0**	nd
N-Fertil.	325.0**	13.0*	227.0**	nd
$CO_2 \times N$	0.2 ns	4.3 ns	20.6**	nd
Mean deviation	1.8	1.4	1.6	nd

(c) = [(b) − (a)]% (a); ns = not significant, * and ** = significant at $p = 0.05$ and $p = 0.01$, nd = not determined.

The total net N mineralization in the cultivated pots was calculated (Table 3) as the total N taken up by plants plus the total inorganic N remaining in the soil at the end of the experiment. CO_2 enrichment had a significant effect on rhizospheric microbial activity with both factors and their interaction. A stimulation of N mineralization and N uptake in the cropped systems is reflected by the plant effect. In the (+N) soil and for normal conditions of CO_2 the effect is negative with an 11% decrease compared to the uncultivated control soil. Nitrogen loss by denitrification, stimulated in the rhizosphere, might account for this negative effect. For the other experimental conditions, the N mineralization was stimulated by the plants and their rhizosphere. The stimulation was lower in the (+N) soils than in (−N) soils but higher under 700 μL L^{-1} CO_2, reaching a maximum increase of nearly 40%.

Discussion

Variation of the shoot/root ratios

The theory of the balanced shoot and root activity in response to multiple resources, such as atmospheric CO_2 concentration and nutrient availability, has been summarised by Larigauderie et al. (1988). A stimulation of the specific activity of the shoot is expected to increase either the root size or the specific nutrient uptake rate in order to balance the internal resource demand. In conditions of low nutrient availability, the nutrient uptake is slowly influenced by the uptake capacity and the plant responds essentially by increasing the root mass (Chapin, 1980). In this experiment, the inorganic N production during 28 days was relatively low, accounting for 21 mg N kg^{-1} dry soil in the uncultivated control soil. The weekly N fertilization accounted for an additional amount of 32 mg inorganic N kg^{-1} soil. The increase of whole plant production by 34% in response to the CO_2 fertilization induced a modification of the C allocation, resulting in a slight decrease of the shoot to root ratio for the (−N) soil and an increase for the (+N) soil. These data agree with the results of Hocking and Meyer (1991) who observed that the shoot/root dry matter ratio of maize and wheat plants grown in a greenhouse decreased in response to elevated CO_2 concentration and increased with increasing N supply. Sionit et al. (1981), growing wheat at two CO_2 concentrations and four nutrient levels, observed an increase in the ratio with increased nutritional level for both 350 and 675 μL L^{-1} CO_2. In the same way, Whipps (1985) studying the effect of elevated CO_2 on young maize plants concluded that the CO_2 stimulation decreased the shoot/root ratio. Hunt et al. (1991), investigating the response of 27 herbaceous temperature species of widely different ecology to CO_2 enrichment, reported that for seven of them there was no response on either a shoot or a whole plant basis. In 14 species the shoot had a higher response than the whole plant, and in six species there was a lesser response in the shoot. So the problem of the interactive responses of plants to elevated CO_2 and N availability remains unsolved. According to Vessey et al. (1990) suggest that the response to elevated CO_2 concentration is an increase in carbon allocated to the belowground parts, essentially in nutrient (N) poor soils. The data resulted from the present experiment lend support to this conclusion.

Carbon loss by the roots

The purpose of this experiment was to measure the belowground translocation and especially the C loss through root respiration and rhizosphere activity in response to modifications of the atmospheric CO_2 concentration. Regardless of the soil-related controls of these processes such as soil texture and nutrient level (Helal and Sauerbeck, 1986; Merckx et al., 1985), one fundamental question was; how is the root derived carbon related to the atmospheric CO_2 concentration, i.e. is the carbon loss only controlled by modifications in the size of the living root system or is it controlled by a modification of the belowground carbon partitioning in the root-soil system? Since, in this experiment the root derived carbon has been found to be a constant fraction of the root carbon or biomass, the results suggest that the root respiration and the rhizosphere activity are essentially controlled by the modification of the size of the root system. This result

is consistent with the conclusions of Whipps (1985): for young maize plants exposed to $^{14}CO_2$, the percentage of carbon lost from the roots was similar at all times and at all CO_2 concentrations, as was the distribution of $^{14}CO_2$ between the fractions of plant, soil and respired CO_2. Thus, in maize and wheat plants, internal carbon loss seems to be controlled from the roots irrespective of the atmospheric CO_2 level. Whipps (1985) further concluded that the proportion of net fixed carbon translocated to the roots, which is subsequently lost, is similar for maize, wheat and barley, even when grown under different environmental conditions and even if wheat and barley translocated more carbon to the roots compared with maize.

Nevertheless, when the relative distribution of carbon in the plant-soil system is considered (Table 2) some variations are related to the CO_2 concentration increase. With fertilized plants, the belowground translocated C versus the shoot C is reduced in response to CO_2 increase. These observation must be compared to those of Poorter et al. (1988), who reported an increase of the root respiration related to the atmospheric CO_2 concentrations for young *Plantago major* plants but not for older ones. This was considered to be a consequence of a faster growth of roots during the initial growth stages. In our experiment, the difference was significant for the total root derived from biomass, and soil rhizodeposed carbon but not for respired CO_2. Nevertheless, these differences are generally low, even when they are significant, demonstrating that the variation of the root biomass in response to CO_2 enrichment remains the major factor controlling the belowground carbon loss.

N uptake and N partitioning

Besides the modifications in the C assimilation and allocation, another fundamental question was how does the CO_2 increase modify the N uptake of the plants and its partitioning. A general assumption is that, except in soils with high N availability, the increase of N uptake is lower in relation to the enhanced C assimilation resulting in N dilution and thus in higher C/N ratios of the whole plant material. Curtis et al. (1989), Israel et al. (1990), Larigauderie et al. (1988), Coleman et al. (1991) and Hocking and Meyer (1991) showed that CO_2-enriched plants accumulate more N than controls but the proportional increase in N is lower than the increase in dry matter. In other experiments, despite the increase in production, the total amount of N in the whole plant was not significantly changed by increasing CO_2 (Garbutt et al., 1990; Hocking and Meyer, 1985). In the present experiment the C assimilation was enhanced by 34% for both N treatments and the N uptake was increased by 14 and 32% in the (−N) and (+N) fertilized soils respectively. For the whole plant the N concentration shifted from 1.07 under 350 μL L^{-1} to 0.91 under 700 μL L^{-1} CO_2 without N fertilization, but values did not change with N fertilization (1.33 and 1.31). Thus, the nitrogen stress (−N) did not reduce the relative magnitude of the growth response, but decreased the N concentration of wheat plants in enriched atmospheric CO_2. The plant N concentration was not modified in the N fertilized soils. These results do not agree with the data of Goudriaan and de Ruiter (1983), who concluded that doubling atmospheric CO_2 had a larger effect on dry matter yield under good nutrient supply (about 1.5 times dry matter accumulation) and even under N shortage, part of the CO_2 effect was retained (1.2 times). In contrast, Hocking and Meyer (1985) found that a CO_2 level of 1500 μL L^{-1} increased dry matter production to about the same extent (18%) at all levels of supplied N.

In the (+N) fertilized soil the N partitioning in the plant organs was not modified by CO_2 enrichment, whereas in the (−N) soil, N partitioning was more affected than the carbon distribution, particularly reduced N in the shoots (Table 1). The N concentration was decreased by 25% in the shoots but not significantly changed in the roots. The shoot-to-root N ratio decreased from 2.2 to 1.6 for the (−N) soil and was not modified (2.5 and 2.6) in the (+N) soil.

These data suggest that for elevated CO_2 the stimulation of total production is not accompanied by a net increase in N availability. In N stressed soils, despite the enhanced development of the root system, plants were unable to gain enough nitrogen to maintain N concentrations

similar to those grown at ambient CO_2 concentration. Jansson and Persson (1982), Haider et al. (1987), Billès et al. (1988), Texier and Billès (1990), and many earlier publications observed that in cropped soils, net N mineralisation was generally stimulated by the root system. The stimulating effect was partly explained by the rhizosphere activity. Nevertheless, plants do not seem to use this as a strategy to improve the N availability, since the proportion of carbon exudated by the root is not increased and remains essentially related to the root biomass. Instead, there is a re-allocation of part of the N from the shoot to new growth and especially to the active root system, thus decreasing the N concentration in the shoots (Curtis et al., 1989) but not specifically stimulating the rhizosphere activity. Information about the behaviour of the plants and especially natural plants in response to elevated CO_2 in N stressed soils is inconclusive, so there is as yet no clear and generalised description of the internal N allocation.

Acknowledgements

Charles Breton provided valuable assistance. This research was jointly supported by the French Centre National de la Recherche Scientifique and the Ministère de la Recherche et de la Technologie.

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Section editor: H Lambers