Climate Change Research: Evaluation and Policy Implications
S. Zwerver, R.S.A.R. van Rompaey, M.T.J. Kok and M.M. Berk, (Eds.)

Carbon allocation in mature grass (*Lolium perenne*) under elevated CO_2 at two soil nitrogen levels.

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Abstract

The uptake of atmospheric carbon by terrestrial ecosystems may play an important role in the global carbon cycle since every 6-7 years the whole atmospheric carbon content passes through the plant biomass. Major uncertainties in this area concern the persistency of growth stimulation by elevated CO_2 and effects on carbon allocation to the soil compartment. In this study the effect of elevated CO_2 on growth and carbon allocation of *Lolium perenne* was investigated. Plants were *pretreated* at 350 and 700 $\mu L\ L^{-1}$ at two nitrogen levels (135 and 400 kg N $ha^{-1}\ yr^{-1}$) for 14 months and subsequently crosswise transferred to ESPAS-phytotrons for a short-term *treatment* at 350 and 700 $\mu L\ L^{-1}$ CO_2 for three weeks.

The pretreatment stimulated total shoot growth until the end of the experiment, although no CO_2 pretreatment effects were observed in the yields of the last cut. The higher nitrogen level almost doubled shoot yield throughout the experiment. The fact that nitrogen stimulated shoot growth until the end of the experiment suggests that the disappearance of the growth stimulation of shoots by elevated CO_2 was not primarily caused by exhaustion of nitrogen or other nutrients in soil. The nitrogen effect on root growth showed an interaction with the CO_2 pretreatment. At the lower nitrogen level root dry weight was not increased at 700 $\mu L\ L^{-1}$ CO_2, whereas at the higher nitrogen level a strong increase was observed. This interaction indicates that nitrogen may have important implications for stimulating effects of elevated CO_2 on root growth on the long-term and thus on carbon allocation to the soil.

1. INTRODUCTION

The concentration of CO_2 has steadily increased since the Industrial Revolution from 270 $\mu L\ L^{-1}$ to the present value of about 355 $\mu L\ L^{-1}$. The effects on crop yield have been surveyed by Kimball (1983), Cure & Acock (1986) and several others, who concluded that a doubling of the CO_2 concentration will probably result in an average increase of 30% in crop yield under optimal conditions. Bazzaz (1990) questioned whether such a stimulation would be prolonged in time, since plants adapt to changing circumstances and soil nutrients may be exhausted on the long-term. Although numerous studies have investigated the effects of elevated CO_2 on plants, only few have also focussed on roots (Rogers *et al.*, 1994). They described plant reactions on elevated CO_2 with emphasis on the soil compartment and concluded that exploration of CO_2 effects on roots and soil needed strong attention since many uncertainties still have to be solved. The objectives of this study were i) to study the effect of elevated CO_2 on biomass production and carbon allocation in a perennial species exposed to elevated CO_2 for a long period, ii) to determine how the effects of elevated CO_2 are affected by different nitrogen application rates.

2. MATERIALS AND METHODS

2.1. CO_2 treatments

Lolium perenne cv 'Barlet' plants were cultivated from seed in 2-L columns. The columns were filled with Ede loamy sand with a bulk density of 1.2 kg l^{-1} (dry weight) and soil moisture was adjusted to about 14% (w/w). All columns received 100 mg N (KNO_3) at the start of the experiment. The plants were grown in two adjacent greenhouse compartments with ambient (about 350 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 levels for 14 months (June 1992 - September 1993) without additional light. During this pre-incubation period, in the following referred to as pretreatment, the CO_2 levels were measured by an Ari-P analyzer (Siemens). The columns were watered weekly and at several dates readjusted to their initial weight with de-ionized water. In spring 1993 the nitrogen treatment was started, the lower nitrogen treatment receiving an amount of nitrogen corresponding with 135 kg N ha^{-1} yr^{-1} and the higher treatment corresponding with 400 kg N ha^{-1} yr^{-1}. After the pretreatment 24 *Lolium perenne* plants were randomly selected and transferred to the ESPAS growth chambers (Experimental Soil Plant Atmosphere System. Half of the plants from each greenhouse compartment were placed in one ESPAS chamber and *vice versa* to distinguish between *pretreatment* and *treatment* effects. After three days of acclimatization, the plants were pulse-labelled for one day with $^{14}CO_2$. Due to a technical flaw the specific activities in the growth chambers are unknown. For this reason, the effects of the CO_2 treatment on the total net CO_2 uptake could not be measured. Nitrogen effect and CO_2 pretreatment effect on total net uptake were not affected by this flaw, since these treatments were equally distributed among the systems. After the 1-day pulse-labelling the growth cabinets were flushed with fresh air and the plants were further treated with 350 or 700 μL L^{-1} CO_2 for three weeks. The preset atmospheric CO_2 levels were maintained either by injecting CO_2 or by removing it by carbosorb filters (Sodasorb, Grace). CO_2 was supplied from gas cylinders (100% CO_2) and the inflows were controlled automatically by means of Brooks flow controllers. CO_2 levels were measured by an URAS 10E infrared analyzer (Hartmann & Braun). Temperatures in the growth chambers (shoot 20 °C at day; 15 °C at night; roots 16 °C at day; 11 °C at night) were measured by a platinum resistance thermometer Pt_{100}, relative humidity (70% at day; 80% at night) using a capacitive humidity sensor and irradiation (300 μmol m^{-2} s^{-1} at plant level) by means of a PAR-meter. Wind velocity was set at 0.1 m s^{-1}. All environmental variables were checked with a third independent meter to assure identical conditions. Day/night rhythm was 16/8 h.

Prior to CO_2 treatment, the column lids were sealed with a silicon rubber (Q3-3481, Dow Chemical) to prevent exchange of $^{14}CO_2$ between growth chamber and soil columns. During the experiment, columns were flushed every 6 hours with CO_2-free air at a flow rate of about 40 L h^{-1} for 15 minutes, to prevent O_2 deficiency and to remove CO_2 from the soil. Root/soil-respired CO_2 was trapped by conducting the air through a 300 mL 2 N NaOH solution.

2.2. Analyses

Root/soil respiration was measured every third day by taking an aliquot of 1 mL of the NaOH solution and precipitating the HCO_3^- and CO_3^{2-} ions with excess $BaCl_2$. Total CO_2 was determined by titrating the remaining NaOH with 0.2 N HCl. $^{14}CO_2$ was determined in a subsample by liquid scintillation counting using Ultima Gold (Packard).

The plants were harvested after 21 growth days in the ESPAS growth chambers. Dry weights of shoots and roots were determined after drying at 80°C for 24 hours. Dried plant material was ground and homogenized and a wet combustion procedure was used to determine total C and ^{14}C. Plant material (30 mg) and soil (1 g) were digested with a 5 ml solution of 2.0 g $K_2Cr_2O_7$ in 25 ml H_2SO_4 and H_3PO_4 (3/2 v/v) at 160°C for 2 hours. Released CO_2 was trapped in 10 ml 1.0 N NaOH, and processed as described above.

3. RESULTS

Figure 1 shows the cumulative shoot yields of *Lolium perenne* during the pretreatment. The first cut after sowing showed that the CO_2 pretreatment had increased shoot growth by about 20% ($P<0.04$). In the second season, after the N treatment started, this CO_2 pretreatment effect could be observed until the end of the experiment, although the increase in total yield reduced to about 16% ($P<0.01$). The last cut before the treatment in the ESPAS growth chambers revealed no differences in yield anymore. From the very start of the nitrogen application in the second season, the 400 N treatment increased the shoot yield of the first cut by about 18% ($P<0.01$), independent of the CO_2 pretreatment. The average increase amounted to about 65% ($P<0.001$) at the end of the experiment.

The results after the CO_2 treatment in the ESPAS growth chambers are shown in table 1. During this treatment the 400 N treatment increased shoot yield by about 91% compared with the 135 N treatment. The CO_2 pretreatment strongly increased dry root weight at the end of

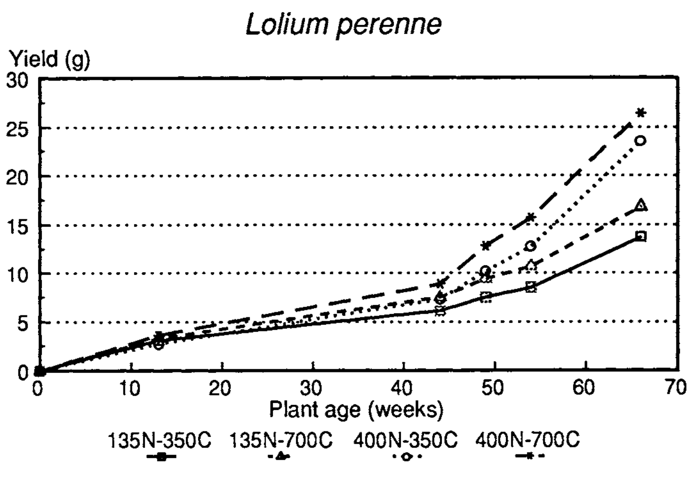

Figure 1. Yield of *Lolium perenne* during the pretreatment at 350 and 700 $\mu L\ L^{-1}$ CO_2 at two nitrogen levels (135 kg N ha^{-1} yr^{-1}).

Table 1
Biomass (g) and shoot/root ratio after treatment of *Lolium perenne* with two nitrogen levels (135 and 400 kg N ha^{-1} yr^{-1}) and with 350 μL L^{-1} CO_2 and 700 μL L^{-1} CO_2 for three weeks after applying a ^{14}C-pulse-label at day 1 (n=3).

	Nitrogen level							
	135				400			
	Pretreatment							
	350		700		350		700	
	Treatment							
	350	700	350	700	350	700	350	700
	Plant biomass							
Leaves	5.4	4.9	6.8	5.5	10.5	11.2	10.4	10.9
Roots	4.5	4.5	4.5	4.6	7.5	10.2	14.9	18.2
Shoot/root ratio	1.2	1.1	1.5	1.2	1.4	1.1	0.7	0.6

Statistics[1]	N	P	T	interactions
Leaves	<0.001	ns	ns	
Roots	<0.001	0.02	ns	N*P
Shoot/root ratio	ns	ns	ns	

[1] N=Nitrogen; P=Pretreatment; T=Treatment; ns=not significant.

the experiment, dependent on the nitrogen level. At 135 N no increase was observed at 700 CO_2, whereas at 400 N dry root weight was strongly increased.

The percent distribution of ^{14}C among the different compartments is shown in table 2. The percentage recovered in the shoots and roots was not affected by any of the treatments. The percentage in the root/soil respiration decreased in the 400 N treatment by 29% compared with the 135 N treatment, but was unaffected by the other treatments. Also the percentage ^{14}C recovered in the microbial biomass decreased from about 2% to 1% in the 400 N treatment compared with the 135 N treatment. The residual ^{14}C in soil was also decreased in the 400 N treatment compared to the 135 N treatment, whereas it was increased by 95% in the 700 CO_2 treatment compared with the 350 CO_2 treatment.

Although the main effect of the CO_2 treatment can not be estimated (see above), the main effect of nitrogen showed a 150% increase at 400 N compared with 135 N. The CO_2 pretreatment had no effect on total net CO_2 uptake.

Table 2
Distribution of ^{14}C (% of net total uptake) among different plant/soil compartments after treatment of *Lolium perenne* with two nitrogen levels (135 and 400 kg N ha^{-1} yr^{-1}) and with 350 μL L^{-1} CO_2 and 700 μL L^{-1} CO_2 for three weeks after applying a ^{14}C-pulse-label at day 1 (n=3).

	Nitrogen level							
	135				400			
	Pretreatment							
	350		700		350		700	
	Treatment							
	350	700	350	700	350	700	350	700
	% distribution							
Leaves	47.0	47.1	48.6	48.4	56.1	54.0	55.7	48.6
Roots	27.8	21.5	18.4	12.9	24.4	20.2	22.0	31.1
Root/soil respiration	21.5	24.8	29.0	33.6	16.9	22.2	20.1	18.2
Microbial biomass	1.9	2.1	2.3	2.7	1.2	1.2	1.5	0.5
Soil residue	1.8	4.5	1.7	2.5	1.4	2.4	0.7	1.5
Shoot/root ratio ^{14}C	1.9	2.4	2.7	3.7	2.5	3.0	3.2	1.6
Total net uptake (kBq)	178	94	160	92	435	171	362	327

Statistics[1]	N	P	T	interactions
Leaves	ns	ns	ns	
Roots	ns	ns	ns	
Root/soil respiration	0.03	ns	ns	
Microbial biomass	<0.001	ns	ns	
Soil residue	ns	ns	ns	
^{14}C shoot/root ratio	0.07	ns	ns	
Total net uptake (kBq)	<0.001	ns	nr	

[1] N=Nitrogen; P=Pretreatment; T=Treatment; ns=not significant; nr=not relevant

4. DISCUSSION

Stimulating effects of CO_2 have often been reported, but frequently doubts have been raised about the persistency on the long-term. Adaptation of the plants or exhaustion of soil nutrients could eventually reduce the initial stimulation. In this study some evidence was found that an initial stimulation of shoot growth was reduced during the second season. After 66 weeks the cumulative shoot yield was still increased by 16% after an initial increase of about 20%. However, the last cut before the treatment in the ESPAS growth chambers revealed no differences in yield indicating that the growth stimulation, due to the pretreatment, had disappeared. The slopes in figure 1 support this conclusion since the rates of shoot biomass production are almost equal at the end of the experiment. It seems not plausible that nitrogen plays an important role in this reduction in growth stimulation, since it occurred at both nitrogen levels almost at the same time. Exhaustion of other soil nutrients or morphological/physiological adaptations of the plants are more likely explanations.

The 400 N treatment stimulated shoot growth throughout the pretreatment and treatment. Although the amount applied was three times higher than in the 135 N treatment, the yield was almost doubled. The fact that nitrogen stimulated plant growth until the end of the experiment suggests that serious exhaustion of other nutrients did not occur. This implies that, with regard to the disappearance of the growth stimulation by elevated CO_2, probably other mechanisms play a role than exhaustion of soil nutrients.

In contrast to shoot yield, a strong interaction between nitrogen and CO_2 pretreatment was observed for root dry weights. In the 400 N treatment root dry weight strongly increased by 46% at 700 CO_2, whereas no increase was found at 135 N. Summarizing, at the low nitrogen level growth stimulation by CO_2 disappeared both in shoots and roots, whereas at the high nitrogen level only the root growth was still stimulated by elevated CO_2. This was also expressed in the s/r ratio (Table 1) which was lowest in the 400 N/700 CO_2 pretreatment.

The interactions with the CO_2 treatments indicate that nutrients such as nitrogen, apart from acclimation, may have important implications for stimulating effects of CO_2 on the longer term especially with regard to the carbon storage capacity of terrestrial ecosystems. Also with regard to a possible decrease in decomposition rates of litter (Coûteaux *et al.*, 1991; Cotrufo *et al.*, 1994) and roots (Gorissen *et al.*, 1994) the availability of nitrogen may play a key role in the sequestration capacities of natural ecosystems.

5. REFERENCES

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