Adaptation of arable crops and perennial vegetations to a changing climate (CROPCHANGE)

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A. Gorissen & J.H. van Ginkel

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Addresses

Location Wageningen:
P.O. Box 14, 6700 AA Wageningen
The Netherlands
phone (+) 31 8370 75700
fax (+) 31 8370 23110
e-mail postmaster@ab.agro.nl

Location Haren:
P.O. Box 129, 9750 AC Haren
The Netherlands
phone (+) 31 50 337777
fax (+) 31 50 337291
e-mail postmaster@ab.agro.nl

Table of Contents

		page
1.	Introduction	1
1.1.	General scope	1
1.2.	Elevated CO2 and carbon allocation in a plant/soil system	2
1.3.	Elevated CO2 and carbon dynamics in the soil	2
1.4.	Objectives	3
2.	Carbon allocation in juvenile grasses under elevated CO2 at two nitrogen levels	5
2.1.	Introduction	5
2.2.	Materials and methods	6
	The Espas growth chambers	6
2.3.	Results	7
2.4.	Discussion	8
3.	Carbon allocation and water use in mature grasses under elevated CO2 at two soil nitrogen levels	11
3.1.	Introduction	13
3.2.	Materials and methods	13
3.3.	Results	18
3.4.	Discussion	19
4.	Carbon allocation in mature grasses under elevated CO2 at two nitrogen levels, two temperature levels and two soil moisture levels	21
4.1.	Introduction	22
4.2.	Materials and methods	22
4.3.	Results	25
4.4.	Discussion	28
5.	Decomposition of grass roots cultivated at two CO2-levels and two nitrogen levels as influenced by growing plants in wind tunnels	31
5.1.	Introduction	32
5.2.	Materials and methods	32
5.3.	Results	34
5.4.	Discussion	37
6.	Summary	41
	Literature	45

1. Introduction

1.1. General scope

The global carbon cycle plays a key role in climate change since CO_2 and CH_4 are both important greenhouse gasses. According to Cornway *et al.* (1988), the atmospheric CO_2 level will steadily rise with a rate of *ca.* 1.2 $\mu l \cdot l^{-1}$ per year. The increase in greenhouse gasses in the atmosphere is expected to cause a warming of the surface of the earth by about 1.5 - 4.5 °C during the next century (Mitchell *et al.*, 1990). This climate change, which also includes changes in precipitation patterns, will undoubtedly affect active carbon pools and fluxes between these pools. On the long term, large shifts between these pools may occur. Post *et al.* (1990) estimated the global carbon reservoirs as shown in Table 1.

Table 1. Global carbon pools (Pg)

Carbon pool	Total amount of C (pg)
Atmosphere	750
Vegetation	550
Soils	1500
Oceans	38000
Recoverable fossil fuels	4000

After Post *et al.* (1990).

Carbon exchange between these compartments may be very large. Because 100 to 120 Pg carbon is yearly fixated by photosynthesis, the whole carbon content in the atmosphere passes through the vegetation every 5 to 7 years (Johnson and Kern, 1991). About 50 % of this amount is almost directly released by plant respiration and another 50 % is released by decomposition of plant residues and soil organic matter.
However, the global carbon cycle is not fully understood, since some carbon is missing in this cycle. Tans *et al.* (1990) calculated that the increase in the atmospheric CO_2 concentration was less than expected from known carbon emissions and uptake processes. They argued that natural ecosystems must be responsible for the unbalanced calculations. Recently, Fisher *et al.* (1994) claimed that they found a substantial missing link in the carbon cycle, the South American savannas. Although an increase in carbon uptake by terrestrial ecosystems may play an important role in feedback mechanisms with regard to increasing atmospheric CO_2 levels, a debate is afoot about the persistency of this mechanism. Increases in carbon uptake may be reduced due to adaptation, nutrient limitations, or genetic limitations, especially in natural ecosystems with perennial species. Oechel *et al.* (1994) showed that reactions of natural ecosystems in the Arctic tundra may not always be positive on the long term.

1.2. Elevated CO_2 and carbon allocation in a plant/soil system

An increasing CO_2 concentration in the atmosphere will lead to an array of changes in the environment. With regard to plant growth a mean increase of about 30 % has been observed in most arable crops (Kimball, 1983). Apart from quantitative changes in biomass production, qualitative changes may also occur, such as changes in starch content, lignin content or the carbon/nitrogen ratio (C/N ratio) (Eamus and Jarvis, 1989; Coûteaux *et al*, 1991).
Fixation of CO_2 by terrestrial ecosystems is an important process in the global carbon cycle since the total CO_2 content of the atmosphere passes through the plant biomass in about 6-7 years (Post *et al*, 1990; Johnson & Kern, 1991). Whether or not the increased CO_2 uptake under conditions of an elevated atmospheric CO_2 concentration will exert a substantial influence on the carbon cycle depends on the residence time of carbon compounds in the terrestrial ecosystem, especially that of carbon in the soil. This residence time is determined to a great degree by the microbial activity in the soil.
In contrast to the effects of CO_2 on photosynthesis and growth of above-ground plant parts, the effects of carbon allocation to the roots and soil have received little attention (Norby, 1994). In those cases where research on roots has been performed, an increase in root mass was usually found (Rogers *et al.*, 1994). In view of the fact that up to 40 % of assimilated carbon is allocated to the root system and the rhizosphere during the season (Van Veen *et al*, 1989), the soil compartment forms a potential storage medium for carbon.
The supply of carbon compounds from the roots to the soil (the rhizodeposition) consists of root exudates, sloughed-off cells, mucilage and dead roots. This rhizodeposition forms an important source for substrate for soil microorganisms. Decomposition of these carbon compounds will result in the formation of soil organic matter. An elevated atmospheric CO_2 concentration seems favourable for the microbial biomass, since the supply of substrate via the roots is stimulated (Lekkerkerk *et al.*, 1990). This increased input may have disadvantageous effects on plant growth, even at high nutrient levels. Diaz *et al.* (1993) described that mineral nutrients were immobilized by the increased activity of the microbial biomass, resulting in a nutrient limitation for plant growth. The activity and size of the soil microbial biomass does not solely depend on the quantity of substrate that is supplied, but also to a large extent on the quality of the substrate. This quality, *e.g.* expressed as C/N ratio or lignin content, determines the degree of difficulty with which rhizodeposition can be decomposed. Besides quantity and quality of the substrate, environmental factors such as temperature, humidity and available mineral nitrogen also play an important role in decomposition processes.

1.3. Elevated CO_2 and carbon dynamics in the soil

Mineral nitrogen is usually essential for the decomposition of rhizodeposition. This nitrogen may originate from atmospheric deposition or mineralization of (nitrogen-rich) old organic material. Native organic matter can be an important nitrogen source due to the relatively large amounts of nitrogen that are stored in this material, but for microorganisms it has the disadvantage that the nitrogen is fixed in resistant compounds which are very difficult to decompose.
An increased allocation of carbon compounds to the roots and soil can in theory lead to two scenarios with regard to soil organic matter dynamics under an elevated atmospheric CO_2-

concentration. Should an increased supply of easily decomposable plant material (sugars, amino acids etc.) lead to an increased decomposition of old organic matter in order to meet the nitrogen requirement, then this will lead to a positive feedback on the atmospheric CO_2 concentration. This scenario will amplify the stimulating effect of an increasing temperature on decomposition processes. If such a reaction does not occur, and the quality of plant material changes in such way that it is decomposed less rapidly, as was reported by Cotrufo *et al.* (1994) for tree litter, then this will result in a negative feedback: the residence time of carbon compounds in the soil will increase. The soil will function as a storage medium for atmospheric carbon. The extent of this storage capacity on the long-term will depend on factors such as (external and internal) nitrogen sources, plant biomass production on the long term, adaptation processes of the microbial biomass etc.
Apart from carbon and nitrogen dynamics, two other factors which are involved in climate change, play a crucial role: temperature and soil moisture. The temperature will increase in all scenarios and will positively affect decomposition of plant residues and soil organic matter, since the activity of the soil microbial biomass will increase, partly depending on the soil moisture content. Accordingly, simulation models predict that soil organic matter will start to function as a source of atmospheric CO_2 (Jenkinson *et al.*, 1991). However, these models seldom take into account possible negative feedback mechanisms as described above.

1.4. Objectives

The objectives of this research were 1) to quantify changes in carbon flows in a plant/soil system with perennial plant species *i.e.* grasses, 2) to study decomposition processes by long-term incubation of root residues under semi-natural conditions. The effects of elevated CO_2 were studied in combination with other factors such as plant species, nitrogen level, soil type and temperature.
In Chapter 2 the results are presented on juvenile grasses which were treated for twelve weeks at 350 or 700 $\mu l \cdot l^{-1}$ CO_2 at two nitrogen levels. In Chapter 3 mature grasses (*Lolium perenne*) were treated for 14 months with 350 or 700 ppm CO_2 at two nitrogen levels. After the pretreatment they were pulse-labelled with ^{14}C. In Chapter 4 an experiment is described in which both species were exposed to 350 or 700 $\mu l \cdot l^{-1}$ CO_2 at two nitrogen levels for 27 months and subsequently labelled with ^{14}C at two temperatures and two soil moisture levels. Decomposition of grass roots was studied in windtunnels during two growing seasons (Chapter 5). The roots had been cultivated at two CO_2 concentrations and two nitrogen levels and were homogeneously labelled with ^{14}C. Finally, a summary is given in Chapter 6.

4

2. Carbon allocation in juvenile grasses under elevated CO_2 at two nitrogen levels

Abstract

In a preliminary short-term experiment, the effect of elevated CO_2 on carbon allocation in juvenile plants of *Lolium perenne* L. grown at two nitrogen levels, was investigated. The plants were exposed to ambient (350 $\mu l \cdot l^{-1}$ CO_2) and elevated CO_2 (700 $\mu l \cdot l^{-1}$ CO_2) for twelve weeks in an atmosphere continuously labelled with $^{14}CO_2$. At harvest, total shoot and root yield, and the distribution of ^{14}C among shoot, root, root/soil respiration, and soil residue were determined. No interactions between CO_2 and nitrogen were observed in this short-term experiment. At the high nitrogen treatment, shoot and root yield increased by 120 % and 28 % compared with the low nitrogen treatment. The shoot/root ratio at high nitrogen was 58 % higher than at low nitrogen. At high nitrogen 57 % was recovered in the shoots compared with 46 % at low nitrogen.
Dry weight of the shoots tended to increase by 6 % at elevated CO_2 and dry weight of the roots significantly increased by 61 %. As a result the shoot/root ratio decreased by 34 % at elevated CO_2. The distribution of ^{14}C among the different plant/soil compartments was affected by the CO_2 treatment. At elevated CO_2 46 % of the total net $^{14}CO_2$ uptake was recovered in the shoots compared with 56 % at ambient CO_2. The percentages recovered in the root and root/soil respiration were accordingly higher at 700 $\mu l \cdot l^{-1}$ CO_2. Due to the increased uptake and the shift in carbon distribution, the absolute amount of ^{14}C-translocated to the soil increased by 64 % at elevated CO_2.

2.1. Introduction

A doubled CO_2 concentration often increases photosynthesis in rye grass plants (Nijs *et al.*, 1988; Ryle *et al.*, 1992), which may lead to an average increase of more than 30 % in crop yield under optimum conditions (Kimball, 1983; Cure & Acock, 1986). Especially in C3 plants, photosynthesis is more stimulated than in C4 plants (Poorter, 1993). In general, elevated CO_2 levels result in plant biomass accumulation (Canham & McCavish; 1981; Sionit *et al.*, 1985; Kaushal
et al., 1989; Lord *et al.*, 1993) and increased allocation of assimilates to the root system was observed several times (Norby & O'Neill, 1991; Körner & Arnone; 1992). This increase not only comprises an absolute increase, but also a relative increase in carbon allocation to the roots. Differential responses of species could change competitive relationships (Bazzaz, 1990), especially in nutrient-limited ecosystems. A higher investment in the rooting system could virtually increase the search capacity for nutrients and water and also the potential capacity to store atmospheric carbon below-ground. Also in view of storage of atmospheric carbon in soils an increased allocation to the roots and soil is a favourable effect of increased plant growth.

The objective of this preliminary experiment was to determine the effects of elevated CO_2 on carbon fixation and carbon allocation patterns in juvenile grasses (*Lolium perenne*) at two

levels of soil mineral nitrogen. $^{14}CO_2$ was used to trace the carbon flows and the distribution in the plant/soil compartments.

2.2. Materials and methods

The Espas growth chambers

Two identical Espas (Experimental Soil Plant Atmosphere System) growth chambers were used, permitting working in an airtight system with the possibility to provide continuously CO_2, $^{13}CO_2$ or $^{14}CO_2$. The Espas growth chambers consist of separate root and shoot compartments, allowing temperature controls for aerial and underground parts. The preset CO_2 level can be maintained automatically using mass-flow controllers (Brooks) by either injection of CO_2 from a pressurized cylinder or by removal of CO_2 from the air by a solid carbosorb filter. The shoot compartment has a total volume of 2.25 m^3 and temperature, relative humidity, light intensity, wind speed and CO_2 concentration (Uras 10E; Hartmann and Braun) are controlled and corrected every five minutes automatically and data are stored for further analyses. The base plate, separating the root and shoot compartment, allows working with 24 soil cylinders in the Espas growth chambers.

Plant and soil

The grass species and the soil used throughout the whole experiment were *Lolium perenne* L. cv 'Barlet' and the top layer (20 cm) of an arable sandy loam (clay 3 %, carbon 2.1 %, pH 5.0) originating from Horst (NL). The transparent perspex soil columns of (length 200 mm; diameter 90 mm) were filled with 1800 g moistened soil until a final density of approximately 1.3 $g.cm^{-3}$ was achieved by gentle vibration. Two nitrogen levels were applied to obtain four types of grass, hereafter referred to as 350LN, 350HN, 700LN and 700HN; half of the plants did not receive additional nitrogen (mineral N was 37.4 $\mu g \cdot g^{-1}$ dry soil based on soluble total N in the soil solution) and to the other half 52 $\mu g \cdot g^{-1}$ N urea (135 $kg \cdot ha^{-1}$) was added. The plants were cultivated in two Espas growth chambers under the following conditions: light 16h, P.A.R.
300 $\mu mol.m^{-2} \cdot s^{-1}$, temperature shoot compartment 18/16 °C (day/night), root compartment 16/ 14 °C (day/night), air humidity 70 %-80 % (day/night) and a CO_2 concentration or 350 and 700 $\mu l \cdot l^{-1}$. All environmental variables were checked with a third independent meter to assure identical conditions. The water content was kept at about 13.4 % w/w (60-70 % of field capacity). The twelve 350LN and 350HN planted columns were placed in the Espas growth chamber for 12 weeks with a CO_2-concentration of 350 $\mu l \cdot l^{-1}$ and were continuously labelled with $^{14}CO_2$ provided from a pressurized cylinder with a specific activity of 0.84 $\pm$0.07 Bq $^{14}C \cdot \mu g \cdot C^{-1}$. The treatment with the twelve 700LN and the 700HN columns was performed in the second Espas growth chamber with a CO_2-concentration of 700 $\mu l \cdot l^{-1}$. The columns were airtight sealed with silicon rubber (Q3-3481; Dow Chemical) to trap the underground CO_2 and $^{14}CO_2$ respiration. Soil columns were flushed every 6 hours with CO_2-free air at a flow rate of about 25 $l \cdot h^{-1}$ for 15 minutes, to prevent O_2 deficiency and to remove CO_2 from the soil.
The CO_2 was conducted through a bottle with 300 ml 2 *M* NaOH. At harvest the shoots were cut at soil level, and roots and soil were separated by gently shaking the soil root core. The remaining roots were removed from the collected soil by handpicking and, subsequently, the

root material was washed with tap water to remove adhering soil particles. Shoots, roots and soil were dried (70 °C) and analyzed on dry weight content, total carbon and ^{14}C-carbon.

Analyses
The total carbon content and the ^{14}C-carbon content of the soil, roots and shoots were determined using a modified wet combustion method (Dalal, 1979). Dried (70°C) and ground roots (30 mg) and soil (1 g) were digested in 5 ml of a 10 % (w/v) solution of $K_2Cr_2O_7$ in a mixture of concentrated H_2SO_4 and H_3PO_4 (3:2 v/v) on a heating block at 160°C. The CO_2 evolved was trapped in 10 ml of a 0.5 *M* NaOH solution and the absorbed CO_2 was determined by titration with HCl of the excess NaOH after precipitation of HCO_3^-/CO_3^{2-}species by $BaCl_2$. $^{14}CO_2$ was determined by liquid scintillation counting; 0.5 ml of the 0.5 *M* NaOH solution was mixed with 3 ml of Ultima Gold (Packard) and counted on a liquid scintillation counter (Tri-Carb 4530; Packard) with a counting efficiency of 91 %. The NaOH solution of the soil/root respiration was analyzed on carbon and ^{14}C-carbon. Carbon and ^{14}C-carbon were analyzed as described above.

Statistics
Twelve plants were exposed to 350 $\mu l \cdot l^{-1}$ CO_2 and 12 plants to 700 $\mu l \cdot l^{-1}$ CO_2 half of the plants were grown at low nitrogen and half of the plants at high nitrogen. After twelve weeks the plants were harvested. The experimental factors were two CO_2 treatments, two nitrogen levels, in six replicates. The results were analyzed by ANOVA (Genstat 5; release 3.1) and differences are called significant when P-values were lower than 0.05.

2.3. Results

CO_2
Since no interactions were observed between the CO_2 and the nitrogen treatment we will restrict the discussion of the results to the main effects. After twelve weeks of growth, the dry weight of the shoots (Table 2) grown at an elevated CO_2 concentration of 700 $\mu l \cdot l^{-1}$, (700 plants) was 2.33g carbon. This was 6 % higher than the 2.19g carbon of the plants grown at an ambient CO_2 concentration of 350 $\mu l \cdot l^{-1}$, (350 plants), although this difference was not significant. In contrast the dry weight of the roots of the 700 plants (1.53g carbon) was 61 % higher (P=0.002) than the 350 roots (0.95g carbon). As a result the shoot to root ratio was decreased by 34 % in the 700 plants (P=0.01). There was no effect on the decomposition of the native soil organic matter (calculated as total root/soil respiration - CO_2 respiration originating from root-derived material) by the CO_2 treatment. The total net uptake of $^{14}CO_2$ by the 700 plants (shoot, root, respiration and soil residue) was 31 % (P<0.001) higher than by the 350 plants. The distribution of ^{14}C within the plants and soil was significantly affected in the 700 shoots 46 % of the total net uptake was recovered compared with 56 % for the 350 plants (P=0.02). The percentage in the 700 roots (31 %) was 18 % higher than the share of the 350 plants (26 %), although this effect was not significant. The respiration of the 700 plants was 14.6 % of the total net ^{14}C uptake and this was 62 % higher compared with the 9.0 % of the 350 plants (P<0.001). The ^{14}C-percentage of the soil residue remained unaffected and was about 9 % for both the 350 and the 700 plants. The total amount of the ^{14}C-translocation to below-ground (roots, soil residue and respiration) for the 700 plants (2213 Bq) was 64 % higher (P<0.001) compared with the 350 plants (1349 Bq).

Nitrogen

The shoot yield after twelve weeks showed a considerable increase of 120 % ($P<0.001$) in the High Nitrogen (HN) treatment (3.11g carbon) compared with the Low Nitrogen (LN) treatment (1.41g carbon). The HN roots (1.76g carbon) yielded 28 % more than the LN roots (1.37g carbon) but the difference was not significant. As a result of the enormous increase in the HN shoot dry weight the shoot/root ration of the HN plants (1.90) increased by 58 % ($P=0.004$) compared with the LN plants (1.20). As for the CO_2 treatment, the nitrogen treatment did also not effect there was decomposition of the native organic matter. The total net uptake of $^{14}CO_2$ by the HN plants (shoot, root, respiration and soil residue) was 68 % ($p<0.001$) higher than by the LN plants. From the total amount of fixed ^{14}C 46 % was recovered in the 350 shoots and amounted to 57 % in the 700 plants ($P<0.01$). The LN roots represented 31 % of the total net ^{14}C uptake against 25 % for the HN roots ($P=0.03$). The percentage in the root/soil respiration in the LN plants was 12.6 % towards 11.0 % for the HN plants ($P<0.01$). Finally, the soil residue in the LN plants contained 10.4 % and for the HN plants this percentage was 6.9 % ($P=0.02$). The total amount of the translocated ^{14}C to below-ground (roots, soil residue and respiration) in the 700 plants (2035 Bq) was 33 % higher ($P=0.02$) compared with the 350 plants (1528 Bq).

Table 2 Dry weight (g C) of shoots and roots, shoot/root ratio (S/R ratio), total amount of ^{14}C recovered in different compartments (kBq) and %-distribution of ^{14}C in *Lolium perenne* at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for twelve weeks at two nitrogen levels (n=6)

	CO_2		Nitrogen		Statistics
	350	700	Low	High	$LSD_{0.05}$
DW shoot	2.19	2.33	1.41	3.11	0.32
DW root	0.95	1.53	1.05	1.43	0.35
S/R ratio	2.31	1.52	1.34	2.17	0.62
kBq shoot	1838	1947	1159	2474	287
kBq root	795	1283	851	1161	287
Kbq soil	274	333	278	307	108
kBq resp	280	597	352	502	67
% shoot	55.9	46.4	45.5	56.8	7.9
% root	26.1	30.7	31.5	25.3	5.6
% soil	9.0	8.2	10.4	6.9	2.8
% resp	9.0	14.6	12.6	11.0	1.0

2.4. Discussion

The progressive increase in the atmospheric CO_2 concentration will probably lead to significant effects on terrestrial ecosystems. In view of these changes, the aim of this research was to investigate if, and to what extent, grass growth is altered in an elevated CO_2 atmosphere and what the consequences are for the distribution of carbon within the plants and the soil. The results confirm other observations (Hocking *et al.*, 1992; Norby *et al.*, 1992) that considerably more root material is formed in an elevated CO_2 atmosphere. A consequence of a larger

root system was an increased respiration rate by probably both the root system and the soil microbial biomass. Due to the elevated CO_2 level growth of the shoots was stimulated by only 6 %, whereas root growth was much more stimulated by 61 %. Under elevated CO_2 total net CO_2 uptake increased by 31 %, but due to a shift in carbon distribution (53 % was allocated to the soil compartment at elevated CO_2 vs 42 % at ambient CO_2), the absolute amounts of carbon translocated below-ground (roots, respiration and soil residue) increased by 64 % compared with ambient CO_2. The resulting 113 % higher respiration will be partly caused by the increased root respiration and partly by the fact that more carbon was released in the rhizosphere as substrate for the soil microbial biomass. Whipps (1985) found that the ratio of water-soluble to insoluble extracts in the rhizosphere of maize was higher in an elevated CO_2 than in ambient air, which indicated that more organic compounds were released from the roots to the rhizosphere. An increased use of current assimilates at elevated CO_2 was also found in Douglas-fir (Gorissen *et al.*, 1995a). In other experiments it was demonstrated that the decomposition of leaf litter (Cotrufo *et al.*, 1994; Kemp *et al.*,1994) and grass roots (Gorissen *et al.*, 1995b) grown at elevated CO_2 was retarded. Both a greater allocation of carbon to the soil and a retarded decomposition of the (root) material are important aspects with regard to global change. It is not inconceivable that soils can function as a sink for the raise in atmospheric CO_2, although much work still has to be done to translate short-term experiments to long-term responses under field circumstances.

It is known that a change in nitrogen supply alters the distribution within the plant (Brouwer, 1966; Brouwer *et al.*, 1969). At low nitrogen levels root growth is more stimulated than shoot growth resulting in a decrease in the S/R (shoot/root) ratio of the plant. As clearly shown in this study, the dry matter partitioning of plants grown at elevated CO_2 showed an increase in carbon allocation to the soil. Doubling the mineral nitrogen concentration caused the complete opposite and showed a decrease of this percentage. On the other hand, the absolute amounts of carbon transported to the soil followed exactly the same pattern at an increased nitrogen and an increased CO_2 concentration. Doubling the mineral nitrogen content in the soil raised the carbon amount below-ground to the same extent as doubling the CO_2 concentration. In other words in this experiment fertilizing with carbon at low nitrogen showed up the same result as fertilizing with nitrogen at low CO_2 concentration (350 $\mu l \cdot l^{-1}$). The absence of interactions between CO_2 and nitrogen is possibly due to the fact that nitrogen was not limiting at the low nitrogen level, although even under nutrient limitation, positive effects of CO_2 have been reported.

3. Carbon allocation and water use in mature grasses under elevated CO_2 at two soil nitrogen levels

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 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* L. and *Festuca arundinacea* Schreber was investigated. Plants were *pretreated* at 350 and 700 $\mu l \cdot l^{-1}$ at two nitrogen levels (135 and 400 kg $N \cdot ha^{-1} \cdot yr^{-1}$) for 14 months and subsequently crosswise transferred to ESPAS-phytotrons for a short-term *treatment* at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for three weeks.

The pretreatment stimulated cumulative total shoot yield until the end of the experiment by about 16 %, although no CO_2 pretreatment effects were observed in the yields of the last cutting. 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 growth stimulation by elevated CO_2 was not primarily caused by exhaustion of other nutrients in soil. The CO_2 pretreatment effect on root growth showed an interaction with the nitrogen treatment. At the lower nitrogen level root dry weight was not increased at 700 $\mu l \cdot l^{-1}$ 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. The distribution of ^{14}C among above- and below-ground compartments was not affected by the CO_2 treatments. This apparent contradiction with the increased root dry weight may be explained by the observation that at the time of labelling, no effect on shoot yield of the last cutting was found. The effect of CO_2 seemed to have disappeared at the end of the season.

Total water use was interactively affected by CO_2 pretreatment, CO_2 treatment, nitrogen and plant species. In general, the elevated CO_2 treatments caused a substantial reduction in water use up to 38 % compared with ambient CO_2. The reduction in total water use of *F. arundinacea* was much higher than in *L. perenne*. This may imply that *F. arundinacea* is more capable to adapt to an elevated CO_2 level and drier conditions than *L. perenne* and urges the need for more extensive studies on differential species responses to elevated CO_2.

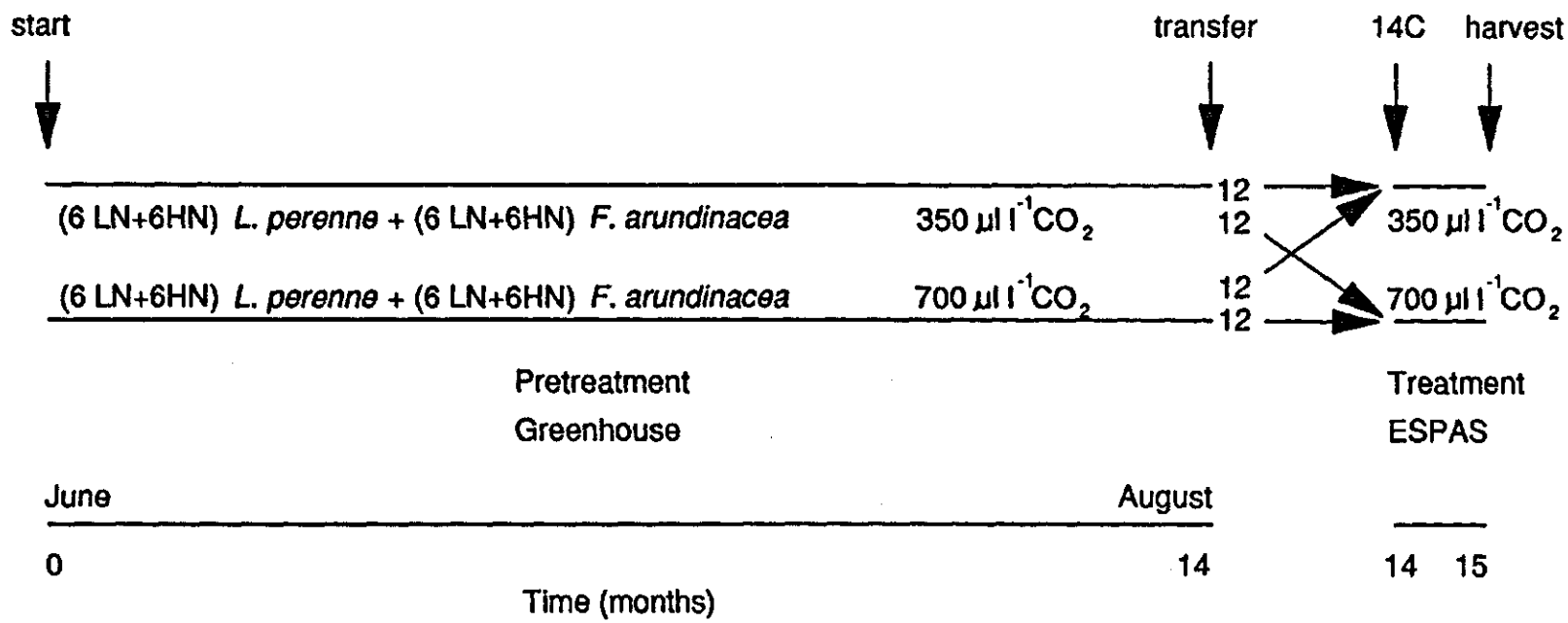

Figure 1 Schematic diagram of the experimental design

3.1. Introduction

The atmospheric CO_2 concentration has steadily increased from 270 $\mu l \cdot l^{-1}$ to the present value of about 355 $\mu l \cdot l^{-1}$ since the Industrial Revolution. This increase will result in stimulation of photosynthesis of most crops, especially C3 crops. 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 about 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. Gorissen *et al.* (1995a) found that total net CO_2 uptake in Douglas-fir was stimulated by 22 % at 700 $\mu l \cdot l^{-1}$ CO_2 compared with ambient CO_2 with the strongest growth stimulation found in the root system. However, the stimulation of total net uptake had disappeared after 14 months growth under elevated CO_2. Reduced nitrogen concentrations in tallgrass prairie ecosystems were reported by Owensby *et al.* (1993), but also an increased nitrogen use efficiency and they concluded that a substantial increase in production of natural nutrient-limited ecosystems is not impossible. Although numerous studies have investigated the effects of elevated CO_2 on plants, relatively few have focussed on roots (Rogers *et al.*, 1994). They reviewed 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 questions and uncertainties still have to be solved or cleared up.

The objectives of this study were

i) to study the effect of elevated CO_2 on biomass production, carbon allocation and water use in 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, and
iii) to study how carbon allocation patterns are affected in two different grass species.

3.2. Materials and methods

CO_2 treatments

Lolium perenne L. cv 'Barlet' plants and *Festuca arundinacea* Schreber cv 'Barcel' plants were cultivated from seed in 2-L columns. The columns were filled with loamy sand (Ede, NL) with a bulk density of 1.2 $kg \cdot l^{-1}$ (dry weight) and soil moisture was adjusted to about 14 % (w/w; about 70 % of water holding capacity). 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 $\mu l \cdot l^{-1}$) and elevated (700 $\mu ll \cdot^{-1}$) CO_2 levels for 14 months (June 1992 - September 1993) without additional.light. 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. Figure 1 shows a schematic diagram of the experimental design. In spring 1993, the nitrogen treatment was started, the lower nitrogen treatment receiving KNO_3 in an amount of 100 mg N per pot corresponding with 135 kg N $ha^{-1} \cdot yr^{-1}$ and the higher treatment 300 mg N per pot corresponding with 400 kg N $ha^{-1} \cdot yr^{-1}$. The first supply consisted of 1/3 of the total annual supply followed by four portions of 1/6 during the course of the growing season. After the pretreatment 24 *Lolium perenne* plants and 24 *Festuca arundinacea* plants were randomly selected and transferred to the ESPAS growth chambers (a modernized version of the Experimental Soil Plant Atmosphere System, described by Merckx *et al.* (1986)).

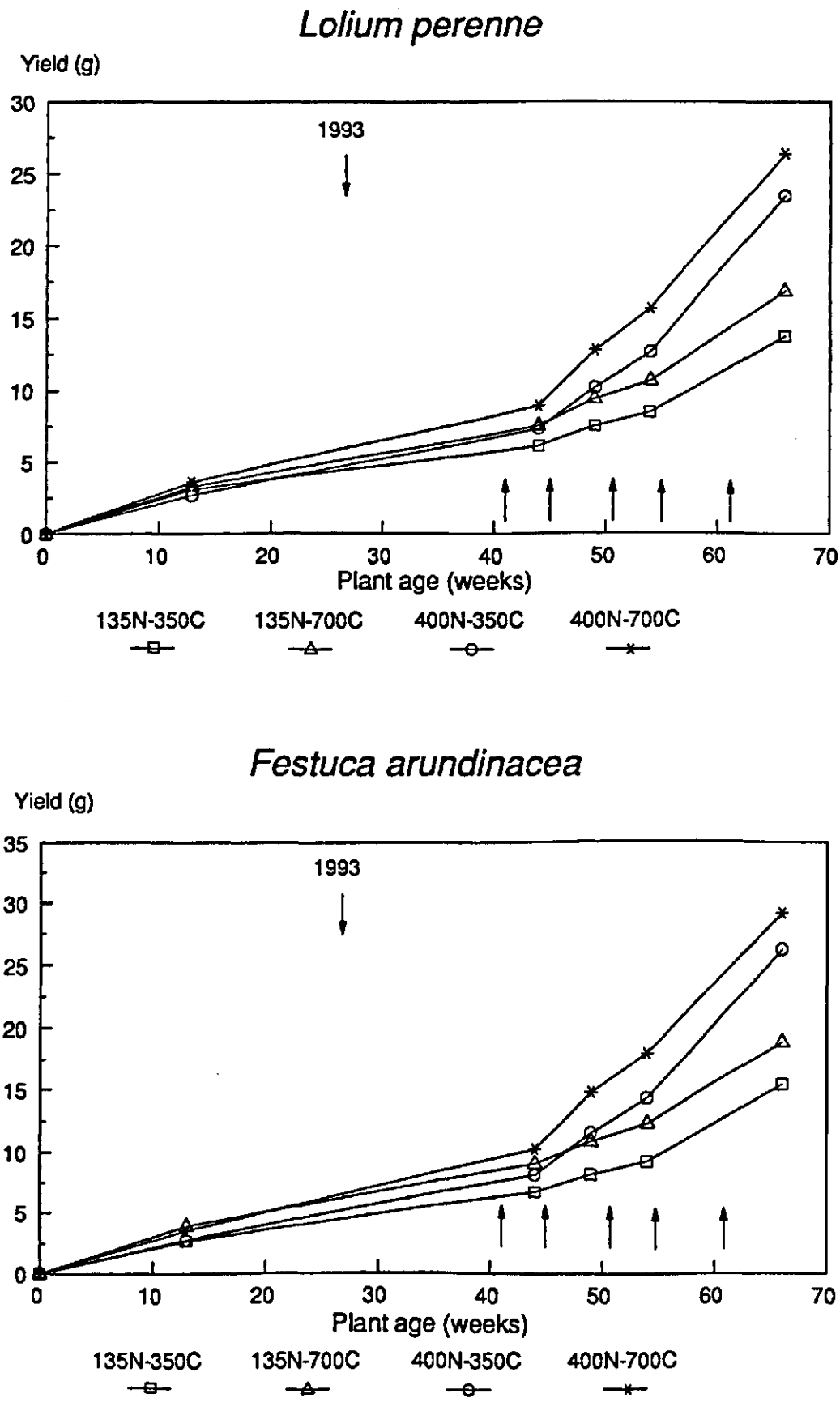

Figure 2 Yield of *Lolium perenne* and *Festuca arundinacea* during the pretreatment at 350 $\mu l.l^{-1}$ CO_2 and 700 $\mu l.l^{-1}$ CO_2 at two nitrogen levels for 14 months. Nitrogen application indicated by arrows.

Half of the plants from each greenhouse compartment were placed in one ESPAS chamber and *vice versa* to distinguish between pretreatment and treatment effects. *Pretreatment* is explicitly used for the first 14 months, whereas *treatment* is used for the last three weeks of the experiment. After three days of acclimatation, the plants were pulse-labelled for one day with $^{14}CO_2$ supplied from a cylinder. The exact absolute amounts of $^{14}CO_2$ injected into the chambers are not known. For this reason, the effects of the CO_2 treatment on changes in carbon allocation patterns could be measured, but not the effect on the total net $^{14}CO_2$ uptake. Species effect, nitrogen effect and CO_2 pretreatment effect on total net $^{14}CO_2$ uptake were not affected by this unknown amount, 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 $\mu l \cdot l^{-1}$ 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 mass 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 (250 µmol $m^{-2} \cdot s^{-1}$ at plant level) by means of a PAR-meter. Wind velocity was set at 0.1 $m \cdot s^{-1}$. 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 columns. During the 3-week experiment, soil 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 *M* NaOH solution. The soil microbial biomass was measured using the Fumigation-Centrifugation method (Van Ginkel *et al.*, 1994).

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 (Tri-Carb 4530; Packard) using Ultima Gold (Packard). The plants were harvested after 21 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 modified wet combustion procedure was used to determine total C and ^{14}C (Dalal, 1979; Merckx *et al.*, 1985). 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 *M* NaOH, and processed as described above.

Statistics

Twenty-four *Lolium perenne* and 24 *Festuca arundinacea* plants were pretreated in greenhouse compartments at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2, respectively. After 14 months, half of the plants pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 was transferred to one ESPAS growth chamber, the other half to a second chamber (Fig. 1). The experimental factors were four CO_2 treatments, two nitrogen levels and two species, always three replicates. The results were analyzed with ANOVA. Differences are called significant when P-values were lower than 0.05.

Table 3 Plant biomass (g), microbial biomass (µg C·g^{-1} soil), water use (ml) and shoot/root ratio after pretreatment of *Lolium perenne* and *Festuca arundinacea* with two nitrogen levels (135 and 400 kg N ha^{-1}·yr^{-1}) with 350 µl·l^{-1} CO_2 and 700 µl·l^{-1} CO_2 for three weeks at 350 µl·l^{-1} CO_2 and 700 µl·l^{-1} CO_2 for 14 months, followed by a treatment (n=3).

	135 kg N·ha^{-1}·yr^{-1}				400 kg N·ha^{-1}·yr^{-1}			
Pretreatment	350		700		350		700	
Treatment	350	700	350	700	350	700	350	700
Lolium perenne								
	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
Microbial biomass	263	268	279	279	283	295	309	249
	Water use							
Water use (ml)	658	483	360	277	927	708	1083	867
Water use (ml·g^{-1} leaf)	122	98	53	53	90	63	108	79
Festuca arundinacea								
	Biomass							
Leaves	5.7	6.8	6.7	6.6	10.1	14.0	10.9	11.6
Roots	6.3	8.5	5.6	8.3	11.2	15.6	15.6	14.5
Shoot/root ratio	0.9	0.8	1.2	0.8	0.9	0.9	0.7	0.8
Microbial biomass	248	270	230	271	293	251	257	283
	Water use							
Water use (ml)	875	650	602	418	1560	785	1275	683
Water use (ml·g^{-1} leaf)	158	97	89	66	156	57	119	60
Statistics[1]		N	P	T	S		interactions	
Leaves		<0.001	ns	ns	0.09			
Roots		<0.001	0.02	ns	ns		N*P;N*S	
Shoot/root ratio		0.05	ns	ns	ns		N*P	
Microbial biomass		0.09	ns	ns	0.07			
Water use (ml)		<0.001	<0.01	<0.001	0.04		N*T;N*P;T*S	
Water use (ml·g^{-1} leaf)		ns	<0.001	<0.001	0.02		N*P;T*S	

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

Table 4 Distribution of ^{14}C (% of net total uptake) among different plant/soil compartments after treatment of *Lolium perenne* and *Festuca arundinacea* with two nitrogen levels (135 and 400 kg N $ha^{-1} \cdot yr^{-1}$) and with 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for three weeks after applying a ^{14}C-pulse-label at day (n=3).

Nitrogen level	135				400			
Pretreatment	350		700		350		700	
Treatment	350	700	350	700	350	700	350	700
Lolium perenne			*% 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
Festuca arundinacea			*% distribution*					
Leaves	50.0	46.7	56.7	47.5	45.0	55.0	50.7	45.7
Roots	20.3	16.5	16.0	20.3	36.6	30.1	27.7	27.0
Root/soil respiration	25.5	32.2	21.6	22.5	16.3	10.7	19.3	25.1
Microbial biomass	1.7	1.3	1.7	2.1	1.0	0.8	1.3	1.1
Soil residue	2.5	3.4	4.1	7.6	1.1	3.3	1.0	1.1
Shoot/root ratio ^{14}C	2.7	2.5	3.6	2.4	1.3	1.8	2.1	1.7
Total net uptake (kBq)	134	95	122	69	373	227	322	172
Statistics[1]		N	P	T	S		interactions	
Leaves		ns	ns	ns	ns			
Roots		<0.001	ns	ns	ns			
Root/soil respiration		<0.001	ns	ns	ns			
Microbial biomass		<0.001	0.02	ns	<0.01			
Soil residue		<0.01	ns	0.02	ns			
^{14}C shoot/root ratio		0.07	ns	ns	ns			
Total net uptake (kBq)		<0.001	ns	nr	0.04		N*T	

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

3.3. Results

Figure 2 shows the cumulative shoot yields of *Lolium perenne* and *Festuca arundinacea* during the pretreatment. During the first season, no differences in shoot yield between the two species were observed, but in the second season the yield of *F. arundinacea* was about 12 % higher than the yield of *L. perenne* ($P<0.001$). The first cutting after sowing showed that the CO_2 pretreatment had increased shoot growth by about 25 % ($P<0.001$). 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 cumulative yield reduced to about 16 % ($P<0.001$). From the very start of the nitrogen application in the second season, the 400 N treatment increased the shoot yield of the first cutting by about 18 % ($P<0.001$), independent of the CO_2 pretreatment. The average increase amounted to 63 % ($P<0.001$) at the end of the experiment.

The results after the CO_2 treatment in the ESPAS growth chambers are shown in Table 3. During this 3-weeks treatment the 400 N treatment increased shoot yield by about 85 % compared with the 135 N treatment. The CO_2 treatment showed a weak interaction with nitrogen ($P=0.08$): at 135 N the 700 CO_2 treatment did not affect shoot yield, whereas it was still 14 % increased at 400 N. Root dry weight at the end of the experiment was strongly increased by the CO_2 pretreatment, although in dependence of the nitrogen level ($P<0.02$). At 135 N no increase in root dry weight was observed at 700 CO_2, whereas at 400 N the root weight was 46 % higher. This interaction between CO_2 pretreatment and nitrogen ($P=0.02$) was also observed for the mean shoot/root ratio which decreased from about 1.0 to 0.7 in the 700 CO_2/400 N treatment. The shoot/root ratio was different for the two species, *L. perenne* had a s/r ratio of 1.1 and *F. arundinacea* of 0.9 ($P=0.03$).

Interactive effects between nitrogen and CO_2 treatment, nitrogen and CO_2 pretreatment, and CO_2 treatment and species were observed for total water use (Table 3). The reduction in total water use in the 700 CO_2 treatment was 26 % at 135 N, compared with the 350 CO_2 treatment and 37 % at 400 N ($P<0.01$). The 700 CO_2 pretreatment reduced the total water use at 135 N by 38 % and only by 2 % at 400 N compared with the 350 CO_2 pretreatment ($P<0.001$). The 700 CO_2 treatment reduced total water use in *L. perenne* by 23 % but in *F. arundinacea* by 41 % ($P<0.001$). Water use per gram dry shoot tissue showed the same tendencies, except for nitrogen. The mean total water use at 400 N increased by 83 %, compared with 135 N, whereas the water use per gram tissue appeared to be equal at both nitrogen levels.

The main effect of nitrogen on total net $^{14}CO_2$ uptake showed a 150 % increase at 400 N compared with 135 N. The CO_2 pretreatment and the species had no effect on total net uptake (Table 4). The percent distribution of ^{14}C among the different compartments is shown in Table 4. The percentage recovered in the shoots was not affected by any of the CO_2 treatments. The overall percentage recovered in the roots was about 23 % and was unaffected by the CO_2 pretreatment and the CO_2 treatment. In the 400 N treatment this percentage was increased from 19 % to 28 % ($P<0.001$), although this was weakly depending on the species involved ($P=0.09$): in *L. perenne* the percentage was increased by 22 % and in *F. arundinacea* by 62 %. The percentage in the root/soil respiration decreased in the 400 N treatment by 29 % compared with the 135 N treatment ($P<0.001$), 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 ($P<0.001$). In the 700 CO_2

pretreatment this percentage increased by 20 % (P=0.02), compared with the 350 CO_2 pretreatment. The residual ^{14}C in soil was also decreased in the 400 N treatment compared to the 135 N treatment (P<0.01), whereas it increased by 95 % in the 700 CO_2 treatment compared with the 350 CO_2 treatment (P=0.02).

3.4. Discussion

Stimulating effects of CO_2 have often been reported, but doubts have been raised about the persistency on the long term (Bazzaz, 1990). Adaptation of the plants or exhaustion of soil nutrients, such as nitrogen, could eventually reduce the initial stimulation (Goudriaan and De Ruiter, 1983; Cure *et al.*, 1988; Oechel *et al.*, 1994). In this study, some evidence was found that an initial stimulation of shoot growth was disappearing during the second season. After 66 weeks the cumulative shoot yield was still increased by 16 % after an initial increase of 25 %. 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 final slopes in Figure 2 support this conclusion since the rates of shoot biomass production were almost equal at the end of the experiment. Both grass species reacted in the same way, although the yield of *F. arundinacea* was 12 % higher. Exhaustion of soil nutrients, morphological/physiological adaptations or genetic limitations of the plants are possible explanations (Oechel *et al.*, 1994). Nitrogen may play some role in the reduction of growth stimulation as indicated by the weak interaction between the short-term CO_2 treatment and nitrogen resulting in equal shoot yields at 350 and 700 CO_2 at 135 N and a 14 % increase at 700 CO_2 and 400 N. However, the absence of interaction between the long-term CO_2 pretreatment and nitrogen on shoot yield of the last cut before the short-term ESPAS treatment indicates that nitrogen was not a limiting factor.

The 400 N treatment increased shoot yield throughout the pretreatment and treatment by 63 % and 85 %, respectively. The fact that nitrogen stimulated plant growth until the end of the experiment to a much higher degree than CO_2 (63 % *vs* 16 %), shows that serious exhaustion of other nutrients did not occur. This implies that exhaustion of soil nutrients is not likely to be responsible for the disappearance of the growth stimulation by elevated CO_2. Maybe similar mechanisms play a role as suggested for Douglas-fir under elevated CO_2 (Gorissen *et al.*, 1995a).

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 strongly stimulated by elevated CO_2. This was also expressed in the *s/r* ratio which was significantly lower in the 400 N/700 CO_2 pretreatment: 0.7 compared with 1.0 in the three other combinations. However, no differences could be detected at the ^{14}C shoot/root ratio, which indicates that the bigger root system in the 700 CO_2 pretreatment was probably formed in an earlier stage when growth stimulation was still present. The ^{14}C results indicate that root growth was not stimulated anymore at the time of labelling. This conclusion must be drawn with care since root growth is strongly influenced by seasonal fluctuations. The interactions with the CO_2 treatments show that nutrients *e.g.* nitrogen, 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. One would

expect that the increased root growth in the 400 N/ 700 CO_2 pretreatment could also be observed in the percentage distribution of ^{14}C. However, no interaction occurred, only a main effect of nitrogen was found: in the 400 N treatment an increased transport (both in absolute and relative terms) of carbon to the root system occurred, although this was different for both species involved. Especially in *F. arundinacea* a strong increase of 62 % was observed. Remarkable is the observation that increased ^{14}C-carbon allocation to the roots at high N was accompanied by a reduced amount in the root/soil respiration, soil residue and soil microbial biomass. In contrast to results presented by Liljeroth *et al.* (1990) root losses are apparently more restricted at a higher soil nitrogen level compared with a low level.

In the 700 CO_2 pretreatment, the total soil microbial biomass was not affected although a higher percentage ^{14}C was found in the microbial biomass. The first observation would be in agreement with the observation that growth stimulation was small at that time so that the release of root-derived materials in soil was equal for both CO_2 pretreatment levels. The slightly increased percentage ^{14}C in the microbial biomass may have resulted from changes in the quality of root-derived products. More readily available substrates would increase its utilization rate.

Water use during the treatment was affected by the pretreatment, which actually had stopped at the time of the treatment since the CO_2 treatments were sequential. The mean reduction in total water use by 16 % must be caused by a prolonged, persistent effect, possibly caused by a reduced specific leaf area as argued by Gorissen *et al.* (1995a). Also the water use per unit leaf mass was decreased by the CO_2 pretreatment by 25 %, 9 % more than the reduction which was found in Douglas-fir (*Pseudotsuga menziesii*) after a similar treatment. The CO_2 treatment also reduced total water use and water use per unit leaf mass by 34 % and 36 %, respectively, but this may be caused by a fast response, probably the transient effect of CO_2 on stomatal conductance. *F. arundinacea* seemed more sensitive to this effect than *L. perenne* and *P. menziesii* according the difference in decrease in water use per unit leaf mass of 47 %, 21 %, and 14 % respectively. One may hypothesize that *F. arundinacea* is more capable to adapt to an elevated CO_2 level and drier conditions than *L. perenne* and *P. menziesii*. Total water use was increased by 83 % in the 400 N treatment compared with the 135 N treatment, but this was in accordance with the increased shoot mass in the 400 N treatment. This effect was not observed when water use was expressed per unit leaf mass.

4. Carbon allocation in mature grasses under elevated CO_2 at two nitrogen levels, two temperature levels and two soil moisture levels

Abstract

Doubts have often been raised about the duration of generally observed stimulation of plant growth under elevated CO_2. Nutrient availability is often mentioned as one of the limiting factors in natural ecosystems. In this chapter, a study was performed on long-term effects of elevated CO_2 on *Lolium perenne* L. and *Festuca arundinacea* Schreber. Plants were exposed to 350 and 700 $\mu l \cdot l^{-1}$ CO_2 at two nitrogen levels (135 and 400 kg N $ha^{-1} \cdot yr^{-1}$) during 27 months. After this pretreatment, the plants were transferred to ESPAS-phytotrons for two sequential short-term experiments with two temperatures and two soil moisture levels. Half of the plants pretreated with 350 $\mu l \cdot l^{-1}$ CO_2 were exposed to 350 $\mu l \cdot l^{-1}$ CO_2 and half to 700 $\mu l \cdot l^{-1}$ CO_2. All the plants of the 700 $\mu l \cdot l^{-1}$ CO_2 pretreatment were exposed to 700 $\mu l \cdot l^{-1}$ CO_2 in the ESPAS-phytotrons.

Elevated CO_2 increased the cumulative shoot yield on average by 14 % during the pretreatment. In *L. perenne* the increase was only observed at the high nitrogen level, whereas *F. arundinacea* showed an increase at both nitrogen levels. During the pretreatment a yearly pattern was observed in the stimulation of shoot growth. At the first two cuttings of the first growing season an increase in yield was found, which had disappeared in the last cuttings of the season. This pattern was repeated in the second season. The high nitrogen level caused an overall yield increase of 114 % in *L. perenne* and 91 % in *F. arundinacea*. The overall yield of *F. arundinacea* was 18 % higher than the yield of *L. perenne*. Root yield was increased by 22 % under elevated CO_2, although in dependance of species and nitrogen level. At high nitrogen root dry weight of both species was increased by about 33 % at elevated CO_2, whereas at low nitrogen only *L. perenne* showed an increased root dry weight. Thus, on average, the dry weight of the root system (+22 %) was more increased than the shoot (+14 %). This resulted in a decreased shoot/root ratio. At elevated CO_2, a more than proportional amount of carbon seems to be allocated to the soil compartment, although for accurate estimates effects of elevated CO_2 on root death (root turnover) will have to be included. *F. arundinacea* had a much higher root dry weight than *L. perenne*, 10.1 g *vs* 4.8 g. This difference clearly shows that some plant species are probably more capable to allocate high amounts of carbon to the roots and soil than others.

Net uptake and distribution pattern of $^{14}CO_2$ among plant and soil were not affected by the CO_2 treatment nor by an increased temperature. This might be related with the disappearance of growth stimulation at the end of the season. A lower soil moisture level decreased the net $^{14}CO_2$ uptake, but did not affect the distribution pattern. The results stress the importance of long-term experiments and measurements of carbon allocation patterns during the season.

4.1. Introduction

A stimulation of plant growth as a direct effect of elevated CO_2 is reported in most studies, but often the results were obtained using annual species or in short-term experiments (Rogers *et al.*, 1994). Norby (1994) pointed out the relevance of studying root responses under elevated CO_2 in order 'to understand the critical feedbacks and adjustments that occur within a plant and between plant and soil'. An increased root growth and a decreased shoot/root ratio may play an important role with regard to 'the missing carbon' in the global calculations by Tans
et al. (1990). Recently, evidence was obtained that at least part of this missing carbon can be found in roots and soil of natural ecosystems (Fisher *et al.*, 1994). Many uncertainties still exist on long-term reactions of plants, responses of ecosystems, and with regard to interactive effects with environmental factors such as temperature, nutrient availability, soil moisture and soil type. Also differential responses of plant species may have important implications for ecosystem responses. Differences in response between C3 and C4 plants are well-documented (Rogers *et al.*, 1994), but also differences between species of the same family may occur (Bazzaz *et al*, 1993). Oechel *et al.* (1994) observed that the response of an arctic tundra ecosystem was disappearing with time and imputed this to factors such as limited nutrient supply or genetic limitations of the plant community. If this is a wide-spread phenomenon, it is important to elicit the mechanisms and factors that are involved in order to be able to answer questions concerning carbon storage in vegetations and ecosystems.

The objectives of this experiment were

i) to determine long-term effects of elevated CO_2 and nitrogen supply on growth and carbon allocation patterns in two mature grasses (*Lolium perenne* and *Festuca arundinacea*) as an extension of the studies presented in the Chapters 2 and 3, and
ii) to study short-term effects of temperature and soil water content on carbon allocation patterns at different CO_2 and nitrogen levels.
$^{14}CO_2$ was used to trace the carbon flows and the distribution in the plant/soil compartments.

4.2. Materials and methods

CO_2 treatments
Lolium perenne L. cv 'Barlet' plants and *Festuca arundinacea* Schreber cv 'Barcel' plants were cultivated from seed in 2-L columns. The columns were filled with a löss soil with a bulk density of 1.2 kg·l^{-1} (dry weight) and soil moisture was adjusted to about 20 % (w/w; about 70 % of the water holding capacity). 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 27 months (June 1992 - October 1994) without additional light. 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 deionized water. In spring 1993 the nitrogen treatment was started, the lower nitrogen treatment receiving KNO_3 in an amount of 100 mg N per pot corresponding with 135 kg N ha^{-1}·yr^{-1} and the higher treatment 300 mg N per pot corresponding with 400 kg N ha^{-1}·yr^{-1}. The first supply consisted of 1/3 of the total annual supply followed by four portions of 1/6 during the course of the growing seasons. Before each nitrogen supply the shoots were cut, dried at

80°C and weighed. During the last 6 weeks of the pretreatment half of the plants was exposed to a drier soil moisture regime with a moisture content of about 12.5 % (w/w). *Pretreatment* is explicitly used for the first 27 months, whereas *treatment* is used for the last week of the experiment in which the ^{14}C-labelling took place. After the pretreatment 53 *Lolium perenne* plants and 54 *Festuca arundinacea* plants were transferred to the ESPAS growth chambers (a modernized version of the Experimental Soil Plant Atmosphere System, described by Merckx *et al.* (1986)) for two sequential short-term experiments with two different temperatures. One week before the transfer to the growth chambers the plants were cut. Half of the plants from 350 $\mu l \cdot l^{-1}$ pretreatment were placed in the 350 $\mu l \cdot l^{-1}$ ESPAS chamber and half in the 700 $\mu l \cdot l^{-1}$ chamber. *All* plants from the 700 $\mu l \cdot l^{-1}$ pretreatment were placed in the 700 $\mu l \cdot l^{-1}$ ESPAS chamber. In this way, three treatment combinations were obtained: 350 $\mu l \cdot l^{-1}$ pretreatment-350 μll^{-1} treatment, 350-700 and 700-700. The plants were continuously labelled for one week with $^{14}CO_2$ supplied from a cylinder. 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). In the first experiment the temperatures in the growth chambers were for the shoot 21°C at day; 18°C at night and for the root 18°C at day and night. In the second experiment the temperature was set at -3°C; the shoot temperature was set at 18°C at day and 15°C at night and the root temperature at 15°C at day and night. Temperatures were measured by a platinum resistance thermometer Pt_{100}, relative humidity (70 % at day; 80 % at night) using a capacitive humidity sensor and irradiation (250 $\mu mol\ m^{-2} \cdot s^{-1}$ at plant level) by means of a PAR-meter. Wind velocity was set at 0.1 $m \cdot s^{-1}$. All environmental variables were checked with a third independent meter to assure identical conditions. Day/night rhythm was 14/10 h.

Analyses

The plants were harvested after one week in the ESPAS growth chambers. The roots were separated from soil by sieving and subsequently washed with tap water. 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 modified wet combustion procedure was used to determine total C and ^{14}C (Dalal, 1979; Merckx *et al.*, 1985). 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 *M* NaOH, and measured 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 (Tri-Carb 4530; Packard) using Ultima Gold (Packard).

Statistics

Fifty-three *Lolium perenne* and 54 *Festuca arundinacea* plants were pretreated in greenhouse compartments at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2, respectively. After 27 months, half of the plants pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 were transferred to the 350 $\mu l \cdot l^{-1}$ ESPAS growth chamber, the other half to the 700 $\mu l \cdot l^{-1}$ chamber and all plants pretreated at 700 $\mu l \cdot l^{-1}$ CO_2 to the 700 $\mu l \cdot l^{-1}$.

In the ESPAS growth chambers additional treatments were included *i.e.* two temperatures and two soil moisture levels.

The experimental factors were four CO_2 treatments, two nitrogen levels, two species, two temperatures and two soil moisture levels. Differences are called significant when P-values were lower than 0.05.

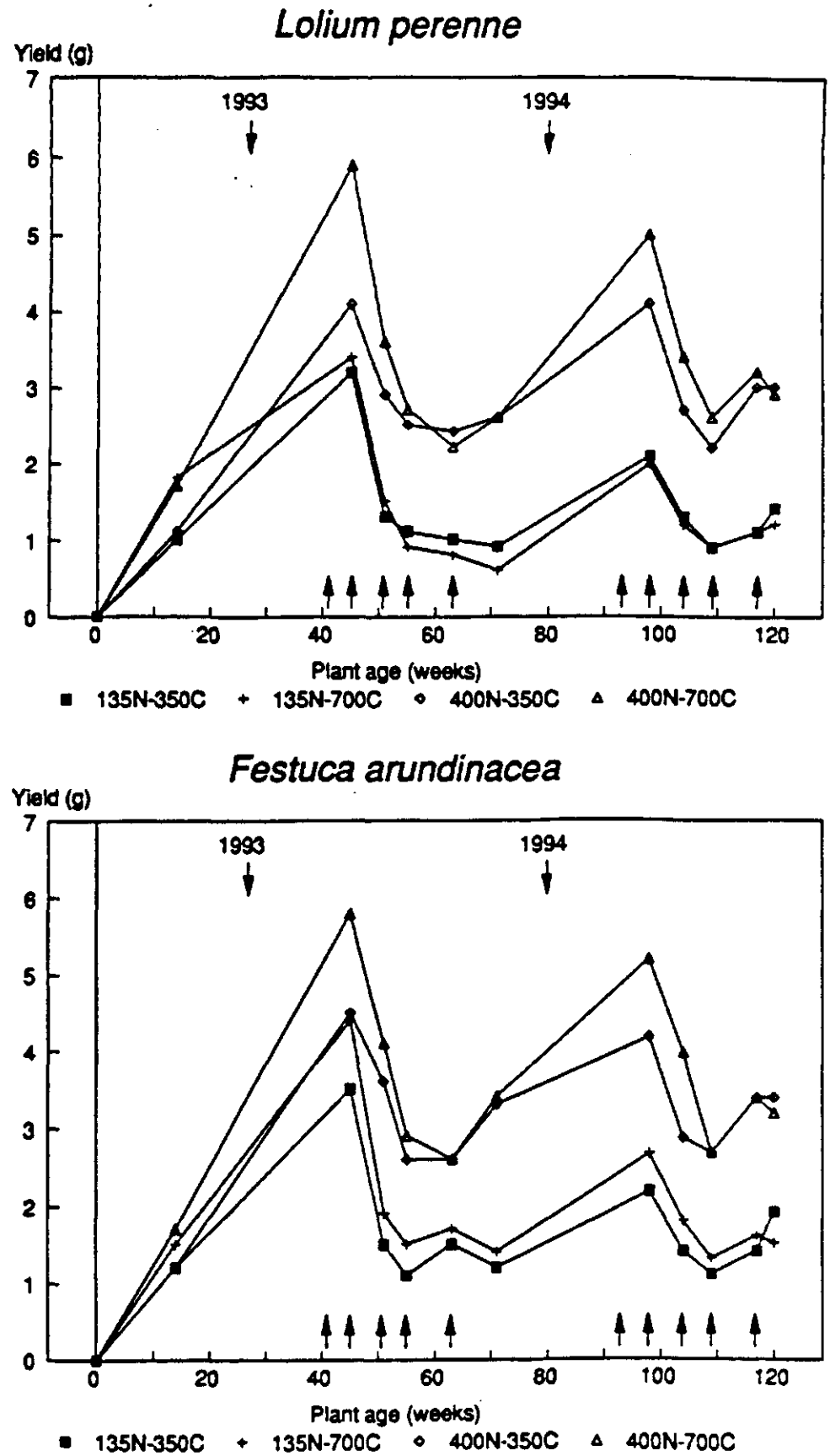

Figure 3 Yield of *Lolium perenne* and *Festuca arundinacea* during pretreatment at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 at two nitrogen levels for 27 months. Nitrogen application indicated by arrows.

4.3. Results

The shoot yields during the 27 months pretreatment are shown in Figure 3. In the first year a pattern was observed in which a stimulating effect of CO_2 was observed after the first two cuttings, especially at the high nitrogen level ($P<0.001$). Later on in the season the yield increase tended to disappear and at the last cutting no significant differences were found anymore. In the second year this pattern was repeated. At the low nitrogen level, differences between elevated and ambient CO_2 were less pronounced and varying between the species. In *F. arundinacea* elevated CO_2 increased shoot yield, but in *L. perenne* no stimulation was observed after the first cutting.

Table 5 shows the cumulative data over the two seasons. The total average increase in shoot yield at elevated CO_2 was 14 %. After the second full growing season all the proportional increases due to elevated CO_2 were lower than in after the first season: 0.7, 17.0, 18.3, and 13.4 % compared with 5.9, 19.9, 24.0, and 15.2 %. For *L. perenne* a strong significant interaction between CO_2 and nitrogen was observed ($P<0.001$). At the lower nitrogen level *L. perenne* was not able to conserve the initial growth stimulation and after two growing seasons the cumulated yield at ambient CO_2 equalled the yield at elevated CO_2. *F. arundinacea* was better capable of conserving the stimulating effect at both nitrogen levels. At the lower nitrogen level relatively even more than at the higher nitrogen level (18.3 % *vs* 13.4 %), although in absolute amounts the increase was 40 % higher at high nitrogen (3.3 g *vs* 4.6 g).

The overall effect of a treble nitrogen dose resulted in a 114 % increase in *L. perenne* and 91 % in *F. arundinacea*. The overall yield of *F. arundinacea* (28.1 g) was about 18 % higher than the yield of *L. perenne* (24.2 g).

The shoot dry weight after the ^{14}C-labelling for one week was only significantly affected ($P<0.001$) in both species by the nitrogen treatment (Table 6). At high nitrogen shoot dry weight increased from an average of 1.3 g to 3.0 g in *L. perenne* and from 1.7 g to 3.4 g in *F. arundinacea*. CO_2, temperature, and moisture did not affect shoot yield during this period. At elevated CO_2 the mean root dry weight was increased by 22 % although in dependance of nitrogen and species. Dry weight of *L. perenne* roots was significantly affected by the CO_2 treatments and nitrogen, without interaction. At low nitrogen the dry weights for the 350-350, 350-700, and 700-700 CO_2 combinations were 3.0, 3.0, and 3.8 g, respectively, and at high nitrogen 5.6, 5.0, and 7.1 g. In the 700-700 treatment a mean increase of 33 % was found compared with 350-350 ($P=0.02$). Dry root weight of *F. arundinacea* showed a significant interaction between CO_2 treatments and nitrogen ($P<0.001$). At the low nitrogen level dry weights were 7.0, 7.7, and 6.6 g for 350-350, 350-700, and 700-700, respectively, and for the high nitrogen level 11.6, 10.8, and 15.5 g. At high nitrogen the increase in the 700-700 treatment was about 34 % compared with the 350-350 treatment.

The overall shoot/root ratio at harvest was decreased by the CO_2 treatment ($P=0.02$) from 0.46 to 0.32 in the 350-350 and the 700-700 treatments. When the cumulative shoot yields were used for calculating the shoot/root ratio the same tendency was observed: 4.5 *vs* 4.0. The low nitrogen level tended to decrease the shoot/root ratio from 0.43 to 0.34 ($P=0.10$). In *F. arundinacea* a much lower shoot/root ratio was found than in *L. perenne*: 0.27 compared with 0.51 ($P<0.001$). This was also found when the cumulative shoot yields were used: 5.5 *vs* 3.0. *F. arundinacea* had an average root weight of 10.1 g whereas *L. perenne* only had 4.8 g.

Table 5 Cumulative shoot yield of *Lolium perenne* and *Festuca arundinacea* during the 27 months pretreatment at 350 and 700 $\mu l.l^{-1}$ CO_2 at two nitrogen levels (135 kg N $ha^{-1}.yr^{-1}$ = LN and 400 kg N $ha^{-1}.yr^{-1}$ = HN).

		Week	Cumulative yield				Percentage increase				Statistics[1]		
			350		700		LN		HN				
			LN	HN	350	700	350	700	350	700	P	N	P*N
Lolium perenne	Sowing	0	0	0	0	0	0	0	0	0			
	Cutting92-1	14	1.0	1.1	1.8	1.7	0	80.0	0	54.5	<0.001	nr	nr
	Cutting93-1	45	4.2	5.2	5.2	7.6	0	23.8	0	46.2	<0.001	<0.001	<0.001
	Cutting93-2	51	5.5	8.1	6.7	11.2	0	21.8	0	38.3	<0.001	<0.001	<0.007
	Cutting93-3	55	6.6	10.6	7.6	13.9	0	15.2	0	31.1	0.55	<0.001	0.19
	Cutting93-4	63	7.6	13.0	8.4	16.1	0	10.5	0	23.8	0.002	<0.001	0.63
	Cutting93-5	71	8.5	15.6	9.0	18.7	0	5.9	0	19.9	0.37	<0.001	0.20
	Cutting94-1	98	10.6	19.7	11.0	23.7	0	3.8	0	20.3	0.02	<0.001	<0.001
	Cutting94-2	104	11.9	22.4	12.2	27.1	0	2.5	0	21.0	0.03	<0.001	0.002
	Cutting94-3	109	12.8	24.6	13.1	29.7	0	2.3	0	20.7	0.001	<0.001	0.008
	Cutting94-4	117	13.9	27.6	14.2	32.9	0	2.2	0	19.2	0.52	<0.001	0.18
	Cutting94-5	120	15.3	30.6	15.4	35.8	0	0.7	0	17.0	0.50	<0.001	0.92
Festuca arundinacea	Sowing	0	0	0	0	0	0	0	0	0			
	Cutting92-1	14	1.2	1.2	1.5	1.7	0	25.0	0	41.7	0.002	nr	nr
	Cutting93-1	45	4.7	5.7	5.9	7.5	0	25.5	0	31.6	<0.001	<0.001	0.15
	Cutting93-2	51	6.2	9.3	7.8	11.6	0	25.8	0	24.7	0.01	<0.001	0.76
	Cutting93-3	55	7.3	11.9	9.3	14.5	0	27.4	0	21.8	<0.001	<0.001	0.53
	Cutting93-4	63	8.8	14.5	11.0	17.1	0	25.0	0	17.9	0.005	<0.001	0.39
	Cutting93-5	71	10.0	17.8	12.4	20.5	0	24.0	0	15.2	0.20	<0.001	0.62
	Cutting94-1	98	12.2	22.0	15.1	25.7	0	23.8	0	16.8	<0.001	<0.001	0.01
	Cutting94-2	104	13.6	24.9	16.9	29.7	0	24.3	0	19.3	0.001	<0.001	0.001
	Cutting94-3	109	14.7	27.6	18.2	32.4	0	23.8	0	17.4	0.36	<0.001	0.40
	Cutting94-4	117	16.1	31.0	19.8	35.8	0	23.0	0	15.5	0.61	<0.001	0.52
	Cutting94-5	120	18.0	34.4	21.3	39.0	0	18.3	0	13.4	0.27	<0.001	0.82

[1] P=Pretreatment; N=Nitrogen; nr=not relevant

Table 6 Biomass of shoot and root (g), shoot/root ratio (S/R), total net $^{14}CO_2$ uptake and distribution (% of total net uptake) among above- and below-ground plant/soil compartments of *Lolium perenne* and *Festuca arundinacea* after a pretreatment of 27 months at 350 and 700 $\mu l.l^{-1}$ CO_2 at two nitrogen (N) levels (135 and 400 kg $ha^{-1}.yr^{-1}$) and a short-term treatment at two moisture levels (M) in soil (12.5 % and 20 % w/w) and two temperatures (T) levels ('ambient' and + 3°C). The plants from the 350 pretreatment were divided among the 350 and 700 short-term treatments. Significant differences are mentioned in the text. N=Nitrogen; T=Temperature; M=Moisture; L=Low; H=High.

	CO_2-treatment combination ($\mu l.l^{-1}$)																			
	350-350								350-700								700-700			
	LN				HN				LN				HN				LN			
	LT		HT		LT		HT		LT		HT		LT		HT		LT		HT	
	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM	LM	HM
Lolium perenne																								
Shoot	1.8	1.5	1.4	1.4	2.8	3.1	3.6	3.3	0.3	1.7	0.7	1.8	2.7	4.0	2.3	2.3	1.5	0.9	1.1	1.5	2.7	3.4	2.4	3.1
Root	3.8	2.5	2.8	2.9	8.4	5.9	4.5	3.5	3.4	3.0	3.2	2.2	5.7	5.2	5.0	4.0	4.0	5.1	3.3	3.2	7.4	6.5	8.2	6.2
S/R	0.70	0.54	0.46	0.53	0.50	0.54	0.87	0.90	0.05	0.57	0.25	0.89	0.51	0.76	0.45	0.60	0.42	0.15	0.40	0.47	0.49	0.56	0.35	0.52
kBq	350	571	286	656	841	755	945	1160	104	762	441	688	1190	1610	927	1090	337	510	373	763	1010	1390	798	1540
%above	65.3	77.2	60.8	59.7	64.6	62.6	79.1	66.4	63.0	60.4	73.5	69.3	61.9	69.5	68.0	76.2	59.5	59.9	57.2	58.8	67.8	67.8	68.1	71.5
%below	34.7	22.8	39.2	40.3	35.4	37.4	20.9	33.6	37.0	39.6	26.5	30.7	38.2	30.5	32.0	23.8	40.5	40.1	42.8	41.2	32.2	32.3	31.9	28.5
Festuca arundinacea																								
Shoot	1.8	1.4	1.7	1.5	3.2	3.6	4.8	3.8	1.8	2.0	1.9	2.2	2.7	4.2	3.0	2.9	2.2	1.0	1.1	1.6	2.5	3.6	2.9	4.1
Root	8.1	5.9	6.7	7.3	14.3	11.3	9.6	11.6	8.8	8.1	6.4	7.8	12.1	11.0	8.5	11.8	5.9	7.3	5.1	8.4	15.9	16.9	12.2	17.4
S/R	0.22	0.22	0.25	0.22	0.23	0.33	0.51	0.33	0.20	0.26	0.30	0.30	0.23	0.39	0.39	0.26	0.36	0.16	0.24	0.19	0.17	0.24	0.26	0.24
kBq	216	370	257	349	552	999	654	1090	420	359	609	587	634	1193	632	1282	377	419	355	699	576	897	487	1161
%above	51.8	75.5	64.4	65.7	67.4	71.3	75.0	66.3	68.3	64.4	78.4	65.2	64.4	76.8	62.1	74.9	63.4	69.5	87.1	79.1	71.9	78.8	64.5	67.1
%below	48.2	24.5	35.6	34.3	32.6	28.7	25.0	33.7	31.7	35.6	21.6	34.8	35.6	23.2	37.9	25.1	36.6	30.5	22.9	20.9	28.1	21.2	35.5	32.9

The overall total net $^{14}CO_2$ uptake increased under elevated CO_2 independent of other treatment factors (P=0.02). In the 350-700 treatment the net uptake increased from 633 to 793 kBq (+25.3 %) and in the 700-700 treatment to 730 kBq (+15.3 %) compared with the control treatment 350-350. The effect of nitrogen on net $^{14}CO_2$ uptake was dependent on the species involved: in *L. perenne* the uptake increased from 492 to 1122 kBq (+128 %) and in *F. arundinacea* from 429 to 830 (+94 %) (P=0.01). A lower soil moisture level caused a strong reduction in total net uptake from 889 to 560 kBq (-37 %), but no interaction with CO_2 was found. No main or interactive effects were observed for temperature. At the high temperature level only a small, non-significant increase by about 9 % was found.

The distribution pattern of the current assimilates among above-ground (shoots) and below-ground (roots + soil) compartment was not clearly affected by any of the treatment factors.

4.4. Discussion

The mean increase in yield after the first growing season (±16 %) caused by elevated CO_2 was comparable with the results described in Chapter 3. The same yield pattern during the season was observed in this experiment. We concluded in Chapter 3 that growth stimulation was disappearing with time. However, this experiment showed that growth stimulation may be time dependent. After the winter period a new growing season possibly affects the vitality of plants and the possibility to respond positively to an increased CO_2 concentration. Thus, the conclusion of Chapter 3 must be adjusted in the sense that growth stimulation in grasses may be maintained during more than one growing season. The question arises whether growth stimulation under elevated CO_2 will indeed last 'for ever', when nutrients are not limiting. When the cumulative yields after the first and second growing season are compared, decreasing tendencies are found, which might indicate that growth stimulation still may disappear on the long term. This is not due to nitrogen limitation, because of high level of nitrogen addition.
It is also not plausible that other nutrients are limiting since the growth rates at high nitrogen still exceeded those at the low nitrogen level. Figure 2 in Chapter 3 clearly shows this difference in growth rate. It would be interesting to know whether a decreased net CO_2 uptake after long-term exposure to elevated CO_2 as earlier observed for Douglas-fir by Gorissen *et al.* (1995a) could have influenced the observed growth patterns during this experimental period. Also in these grasses we found a reduced stimulation of total net CO_2 uptake as a long-term response to elevated CO_2. The instantaneous stimulation (in the 350-700 treatment combination) amounted to 25.3 %, whereas stimulation on the long-term (in the 700-700 treatment combination) was 15.3 %, compared with the control plants (the 350-350 combination). These observations urge the need for real long-term experiments (*e.g.* 5-10 years) and should include measurements on photosynthesis, respiration, nutritional status of plant and soil, and more measurements on carbon allocation among plant and soil compartments *during* the season.

The root dry weights were strongly increased at elevated CO_2. It was surprising that *L. perenne* plants had lower root dry weights than the plants in Chapter 3, although they were one year older. *L. perenne* probably better prospers in a loamy sand (used in chapter 3) than in löss.
In *F. arundinacea* root dry weights were similar as in Chapter 3. This might imply that in general *F. arundinacea* will be more capable to explore the soil in search of nutrients with

possible implications for the persistency of growth stimulation at elevated CO_2 on the long term. Elevated CO_2 appeared to be more beneficial for root growth than for shoot growth. The shoot/root ratio decreased by 30 % at 700 $\mu l \cdot l^{-1}$ CO_2. This is in agreement with findings by Van Ginkel *et al.* (unpublished) and supports the theory that total carbon allocation to the roots and soil is more stimulated under elevated CO_2 than net CO_2 uptake.

As in Chapter 3, the distribution of ^{14}C among the above-ground and below-ground compartment was not affected by CO_2. Figure 3 shows that the effects of elevated CO_2 on shoot yield had disappeared at the time of ^{14}C-labelling and this might as well apply to the carbon distribution pattern. The relative increase in root weight must have resulted from changes in carbon distribution earlier in the season, unless root turnover was strongly reduced at elevated CO_2. This also urges the need for long-term experiments on carbon allocation and root turnover *during* the season.

Although Ryle and Powell (1992) did not observe interactive effects between CO_2 and temperature on white clover yield, an increased temperature reduced the stimulating effect of CO_2 on photosynthetic rate in a study of Ziska and Bunce (1994). In our experiment no significant effect of temperature on neither net CO_2 uptake nor the carbon distribution pattern was found. Water stress is often ameliorated by an increased CO_2 concentration (Rogers *et al.*, 1994), since CO_2 induces partial closure of stomata. In this experiment a lower soil moisture content decreased total net $^{14}CO_2$ uptake, probably due to closure of stomata, but no interactive effect with CO_2 was found.

5. Decomposition of grass roots cultivated at two CO_2-levels and two nitrogen levels as influenced by growing plants in wind tunnels

Abstract

Many studies on the effects of elevated CO_2 are observed from experiments with a duration of less than one year. It is often questioned whether these effects would also be found after a longer period. In this chapter an experiment is described in which plant growth and decomposition of root material is followed during two growing seasons under semi-natural conditions in wind-tunnels. *Lolium perenne* L. plants were homogeneously labelled with ^{14}C at 350 $\mu l \cdot l^{-1}$ CO_2 or 700 $\mu l \cdot l^{-1}$ and two nitrogen levels in ESPAS growth chambers in order to obtain four types of root material with different qualities. After harvest the roots were used in the wind-tunnel experiment. Root material was added to a loamy sand in 1.25 l-pots. Four tunnels were used, two with ambient air and two with ambient air + 350 $\mu l \cdot l^{-1}$ CO_2. Each tunnel contained 60 pots (15 of each treatment), several containers were planted immediately in spring with *L. perenne* plants, others were planted in the second year and the remaining containers stayed unplanted until the end of the experiment.
Elevated CO_2 tended to increase root dry weight more than shoot dry weight. Especially after the second growing season a significant difference was observed: shoot yield was increased by 13 %, whereas root yield showed an increase of 92 %. The decrease of the shoot/root ratio confirms the findings in the preceding chapters.

The overall decomposition of root residues was 54 % after the first growing season and increased to 67 % after the second season. A clear planting effect was observed in the decomposition rate. Unplanted pots showed a significant lower decomposition of ^{14}C-residues than the planted pots. Thus, plant growth stimulated decomposition, but the CO_2 concentration in the atmosphere in which the plants were growing did not show a clear effect.

The CO_2 level at which the root residues were cultivated significantly affected decomposition of the four types of root material. After the first growing season the mean decomposition of the residues cultivated at elevated CO_2 showed an overall decrease by 19 % compared with the residues cultivated at ambient air. After the second growing season the decomposition of the 700LN (cultivated at 700 $\mu l \cdot l^{-1}$ CO_2 and low nitrogen) residues in the unplanted pots was 13 % lower compared with the 350LN residues, while the 700HN residues did not differ significantly from the 350HN residues. These results show that a decrease in decomposition of root residues cultivated at elevated CO_2 can also be observed on the long-term, although in dependance of nitrogen availability.

5.1. Introduction

The increased atmospheric CO_2 level may lead to an array of changes. Not only plant biomass is stimulated (Kimball, 1983), but also the quality of plant tissues may change in terms of C/N ratio or lignin content (Eamus and Jarvis, 1989; Coûteaux *et al.*, 1991). These changes may lead to differences in decomposability of shoots (Cotrufo *et al.*, 1994) or roots (Gorissen *et al.*, 1995b). However, these results on decomposition were obtained from relatively short-term experiments with a duration of about 8 and 2 months, respectively. With regard to the question whether soils can function as a sink for atmospheric CO_2, two basic processes within ecosystems are relevant:

i) total carbon allocation to the roots and soil and
ii) decomposition rates of plant debris and soil organic matter.

It is necessary to study these processes on a term as long as possible, since short-term responses, which may appear transient on the longer-term, are probably not relevant for possibilities of carbon sequestering in soils.

The objectives of this experiment were

i) to determine long-term effects of elevated CO_2 on growth of *L. perenne* under semi-field conditions as an extension of the studies presented in the Chapters 2, 3, and 4 and
ii) to study long-term effects of elevated CO_2 and nitrogen levels on decomposition rates of root residues in dependance of plant growth. $^{14}CO_2$ was used as a tracer to determine carbon decomposition rates.

5.2. Materials and methods

Wind tunnels

For the decomposition experiments four identical wind tunnels were designed. The material of the wind tunnels was polycarbonate and the dimensions were 200*60*60 cm (l*w*h). Two wind tunnels had a constant flow (1 $m \cdot s^{-1}$) of ambient air and of two wind tunnels the ambient air was enriched with 350 $\mu l \cdot l^{-1}$ CO_2 (mass flow controller; Brooks) supplied from a pressurized cylinder. The wind tunnels were put in open air under normal natural circumstances without any disturbance of buildings or trees. A special developed computer program monitored every quarter of an hour the CO_2 concentration (Ari-P analyzer; Siemens) in each tunnel and data were stored for further analyses.

^{14}C labelling of roots

The grass species used throughout the whole experiment was *Lolium perenne* cv 'Barlet'. The grass roots needed for decomposition were cultivated in two identical Espas (Experimental Soil Plant Atmosphere System) growth chambers (light 16h day^{-1}, P.A.R. 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature day/night 18/14°C and relative humidity 70 %). The Espas growth chambers allow, throughout the whole growth period, a continuous $^{14}CO_2$-labelling the of atmosphere at 350 and
700 $\mu l \cdot l^{-1}$ CO_2 (specific activity: 0.84 $Bq \cdot \mu g\ C^{-1}$) needed for a homogeneously labelling of plant material. The polyethylene soil containers had a volume of 1l and contained 1300 g of dry soil with a density of 1.3 $g \cdot cm^{-1}$. During the experiment the water content of the soil was kept at about 14 % w/w (60-70 % of field capacity). Two nitrogen levels, Low Nitrogen and High Nitrogen, were applied to obtain four types of grass roots with different quality (here-

after referred to as 350LN, 350HN, 700LN and 700HN); half of the plants did not receive any additional nitrogen and to the other half 2g Sporumix PG kg^{-1} dry soil was added. The composition of Sporumix PG is: N total 14 %, P_2O_5 16 % K_2O 18 %, Cu 0.12 %, B 0.03 %, Mo 0.20 %, Mn 0.16 %, Zn 0.04 % and Fe 0.09 %.
The soil used both for the cultivation of the ^{14}C-labelled grass roots and for the decomposition experiments in the wind tunnels was a loamy sand (Clay 3 %, C 1.7 % and pH 6.2) of Ede-NL. The labelled roots were dried at 70°C and ground through a 1 mm sieve.

Decomposition experiment
The polyethylene soil containers in the wind tunnels had a volume of 1250 ml. All pots contained 1300 g of dry soil with a density of 1.3 $g.cm^{-3}$ and received 100 mg homogeneously labelled carbon kg^{-1} dry soil of one of the four different types of grass roots. Each tunnel contained 60 soil containers (15 of each treatment), several containers were planted immediately in spring with *Lolium perenne* plants, others were planted in the second year and the remaining containers stayed unplanted until the end of the experiment. The grasses were cut four times a year and at the same time an appropriate fertilizer amendment (diluted Steiner nutrient solution) was supplied (total 135 $kg{\cdot}ha^{-1}$ N $year^{-1}$). The soil containers were kept at about 14 % w/w (60-70 % of field capacity) and watering was performed by adjusting to a certain weight.

Harvest
The harvests took place in autumn according the schedule of Table 7. At soil level the shoots were cut, and roots and soil were separated by gently shaking the soil root core. The remaining roots were removed from the soil by handpicking and, subsequently, the root material was washed with tap water to remove adhering soil particles. Dry weights of the shoots and roots were determined after drying at 70°C.

Table 7 Harvest schedule for the decomposition experiment (soil containers are planted in spring (I) and harvested at the end of the growing season) and after the second growing season (II).

Planting	Growing season	
	I	II
Unplanted		
350LN	4*3	4*3
350HN	4*3	4*3
700LN	4*3	4*3
700HN	4*3	4*3
Planted at start		
350LN	4*3	4*3
350HN	4*3	4*3
700LN	4*3	4*3
700HN	4*3	4*3
Planted second year		
350LN		4*3
350HN		4*3
700LN		4*3
700HN		4*3

Analyses
The specific activity of the labelled root material, used for the decomposition experiment, was determined using a modified wet combustion method (Dalal 1979). Dried (70°C) and ground roots (30 mg) were digested in 5 ml of a 10 % (w/v) solution of $K_2Cr_2O_7$ in a mixture of concentrated H_2SO_4 and H_3PO_4 (3:2 v/v) on a heating block at 160°C. The CO_2 evolved was trapped in 10 ml of a 0.5 *M* NaOH solution and the absorbed CO_2 was determined by titration with 0.2 *M* HCl of the excess NaOH after precipitation of HCO_3^-/CO_3^{2-} species by $BaCl_2$. $^{14}CO_2$ was determined by liquid scintillation counting; 0.5 ml of the 0.5 *M* NaOH solution was mixed with 3 ml of Ultima Gold (Packard) and counted on a liquid-scintillation counter (Tri-Carb 4530; Packard) with a counting efficiency of 91 %. Prior to analysis the soil was sieved (2 mm) and the microbial biomass (^{14}C and C) was determined with the Fumigation Centrifugation method (Van Ginkel *et al.* 1994). After drying the soil at 70°C the total carbon and ^{14}C-carbon content of 1 g were determined as described above.

Statistics
The CO_2 treatment was performed in duplicate in four wind tunnels. The decomposition experiment had a completely balanced block design with 3 blocks of 20 experimental units (treatment combinations) in all the four wind tunnels. At harvest of each treatment one soil container was harvested from each block (triplicate). The results were analyzed by ANOVA (Genstat 5; release 3.1) and differences are called significant when P-values were lower than 0.05.

5.3. Results

Influence of CO_2 on shoot and root yield
The total yield of shoot and root accumulated over the first growing season showed no significant differences in dry weight (Table 8). After the second growing season no significant differences were observed between the shoots and the roots of the plants planted in spring of the second year (one year old plant). The shoots of the grass plants planted in spring of the first year (two year old plants) in the elevated CO_2 wind-tunnels showed an increase in dry weight of 13 % ($P<0.01$) compared with the shoot of the wind-tunnels with ambient air. The roots of the these two year old plants at elevated CO_2 level showed an enormous increase of 92 % ($P<0.001$) in dry weight compared with the roots in wind-tunnels at ambient air.

Influence of planting and year effect
After the first growing season the added ^{14}C-grass residues of the planted pots had a significant ($P<0.05$) higher decomposition rate (55.0 %) than the added ^{14}C-residues in the unplanted pots (52.6 %). After the second growing season this effect was even more pronounced with a significant ($P<0.01$) higher decomposition rate in the planted pots (68.0 %) compared with the unplanted pots (64.5 %). The ^{14}C-residues in the unplanted and the planted pots situated in the wind-tunnels with the elevated CO_2 concentration showed no significant difference with the planted and unplanted pots in the wind-tunnels with ambient air. The overall decomposition of the ^{14}C-residues after the first growing season was about 54 % and increase until 67 % after the second growing season. Also for the ^{14}C-soil microbial biomass there was no different development in the wind-tunnels with the elevated CO_2 concentration compared with the wind-tunnels with ambient air. After one growing season the ^{14}C-soil microbial biomass in the unplanted pots (8.2 μg $C \cdot g^{-1}$) was significant higher ($P<0.001$) than the ^{14}-SMB in the planted pots (6.9 μg $C \cdot g^{-1}$). After the second growing season the difference

between the unplanted pots (6.5 µg $C \cdot g^{-1}$) and the planted pots (5.0 µg $C \cdot g^{-1}$) was even higher (significance $P<0.001$).

Influence of root quality on decomposition

Comparing the wind-tunnels with elevated CO_2 with the wind-tunnels with ambient air there were no significant differences observed in decomposition between the four different types of added ^{14}C-grass roots (data not given). After one year the decomposition of the added ^{14}C-grass roots cultivated at 700 µl·l^{-1} CO_2 at low nitrogen level (700LN) in the unplanted pots was significantly ($P<0.001$) retarded by 28 % (Table 9) compared with the added ^{14}C-grass roots cultivated at 350 µl·l^{-1} CO_2 at low nitrogen level (350LN). The decomposition of the added
^{14}C-grass roots cultivated at 700 µl·l^{-1} CO_2 at a high nitrogen level (700HN) of the unplanted pots was also significantly ($P<0.05$) retarded compared with the added ^{14}C-grass roots cultivated at 350 µl·l^{-1} CO_2 at high nitrogen level (350HN) however to a much lower extend (-8 %). After one growing season the decomposition of the 700LN residues in the planted pots was significant ($P<0.001$) lower (19 %) compared with the planted pots with the 350LN residues. The decomposition of the 700HN residues of the planted pots were significant ($P<0.001$) lower (16 %) compared with the planted pots with the 350HN residues. After the first growing season the mean decomposition of the residues cultivated at elevated CO_2 showed an overall decrease by 19 % compared with the residues cultivated at ambient air. After the second growing season the decomposition of the 700LN residues in the unplanted pots was 13 % lower ($P<0.001$) compared with the 350LN residues, while the 700HN residues did not differ significantly from the 350HN residues. The decomposition of the 700LN residues of the planted pots (grass planted in spring of the second year - one year old) was retarded by 18 % ($P<0.001$) compared with the planted pots with the 350LN residues. There was no significant difference between the 700HN and 350HN residues in the planted pots. The decomposition of the 700LN residues in the planted pots (grass planted in spring of the first year - two years old) was retarded by 10 % ($P<0.001$) compared with the planted pots with the 350LN residues. Also with these two years old plants there was no significant difference between the decomposition of the 350HN and the 700HN grass roots. Comparing the decomposition of the residues planted with a one- or two-years-plant, only the decomposition of the residues of the 350LN with a one year old plant was 6 % higher ($P<0.05$) than the 350LN planted with a two years old plants, while the other treatments remained unaffected. After the second growing season the mean decomposition of the residues cultivated at elevated CO_2 showed an overall decrease by 7 % compared with the residues cultivated at ambient air.

Influence on the development of the ^{14}C soil microbial biomass

No significant differences were observed in the ^{14}C-content of the soil microbial biomass (SMB) between the wind-tunnels with ambient air and the wind-tunnels with an elevated CO_2 concentration. In none of the treatments were significant differences in ^{14}C-content of the SMB between the 350LN and the 350HN pots (Table 10). In the 700LN and the 700HN treatment only the one and the two years old planted pots after the second growing season were not significantly different from each other. After the first growing season the ^{14}C-SMB in the unplanted 700LN pots was 33 % higher than the unplanted 350LN pots, while the unplanted 700HN pots were 36 % higher than the unplanted 350HN pots ($P<0.001$). The ^{14}C-SMB in the planted pots with the 700LN residues was 34 % higher compared with the 350LN planted pots, while the ^{14}C-SMB in the 700HN planted pots was 52 % higher than the in the 350HN planted pots ($P<0.001$). After the second growing season the ^{14}C-SMB in the unplanted 700LN pots was still 28 % higher then the unplanted 350LN pots, while the ^{14}C-SMB of the unplanted 700HN pots was 47 % higher compared with the unplanted 350HN pots

($p<0.001$). Comparing the ^{14}C-SMB of all treatments planted with a-one or two-years-old plant there is no significant effect of planting year. The ^{14}C-SMB in the planted 700LN pots was 28 % higher than the planted 350LN pots, the planted 700HN pots are 27 % higher than the 350HN pots. Planting had a strong effect on the ^{14}C-SMB: after one growing season the ^{14}C-SMB in the planted pots was 16 % lower compared with the unplanted pots, after the second growing season this difference (23 %) was even higher.

Table 8 Total shoot and root dry weights (g) and S/R ratio of *Lolium perenne* after the first (I) and second (II) growing season at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 (n=12)

	Growing season			
	I		II	
Planting	350	700	350	700
Planted spring year I				
Shoot	4.01	4.14	8.69	9.81
Root	3.62	4.49	7.73	14.85
Planted spring year II				
Shoot		-	4.23	4.75
Root		-	7.25	8.94
			$P_{0.05}$ (shoot)	0.75
			$P_{0.01}$ (root)	3.02

Table 9 % decomposed after the first (I) and the second (II) growing season as influenced by planting and time (n=6)

Planting	Growing season	
	I	II
Unplanted		
350LN	64.7	70.5
700LN	46.7	61.0
350HN	51.6	64.6
700HN	47.3	62.1
Planted spring year I		
350LN	65.2	76.8
700LN	52.6	63.2
350HN	55.6	66.8
700HN	46.6	67.4
Planted spring year II		
350LN	-	72.3
700LN	-	65.3
350HN	-	64.4
700HN	-	68.4
		$LSD_{0.05}$ 4.4

Table 10 ^{14}C-soil microbial biomass (μg $^{14}C \cdot g^{-1}$ dry soil) after the first (I) and the second (II) growing season as influenced by planting and time (n=6)

Planting	Growing season	
	I	II
Unplanted		
350LN	6.9	5.5
700LN	9.2	6.9
350HN	6.5	5.5
700HN	10.1	8.1
Planted spring year I		
350LN	5.7	4.3
700LN	7.6	5.9
350HN	5.6	4.6
700HN	8.6	5.5
Planted spring year II		
350LN	-	4.4
700LN	-	5.2
350HN	-	4.3
700HN	-	5.7
		$LSD_{0.05}$ 0.8

Table 11 Initial chemical composition of the root material

Treatment	% lignin	% N	C/N	lignin/N
350LN	5.3	0.59	64	9.0
350HN	4.1	1.56	24	2.6
700LN	4.8	0.56	68	8.6
700HN	3.1	1.01	38	3.1

5.4. Discussion

The increase in shoot and root yield agrees well with the observations in the Chapters 2,3 and 4. Again root growth seems to more stimulated than shoot growth as indicated by the decreasing shoot/root ratio's in all observations. However our principal objective of the study was to investigate if there was a difference in decomposition of grass roots with a different origin (CO_2 and nitrogen) under semi-natural circumstances and if this decomposition would be influenced by planting with grass under different CO_2 concentrations (ambient air and elevated CO_2). Moreover to confirm that results obtained in short-term laboratory experiments have also significance on the long term. It was clear that the origin of the ^{14}C-residues had a significant effect on decomposition and so had planting, but not the CO_2 concentration at which plants were growing. Although the C/N and lignin content was the same within the low nitrogen ^{14}C-residues (Table 11) nevertheless the 350 ^{14}C-residues showed a significant

higher decomposition than the 700 ^{14}C-residues, even after two growing seasons. The high nitrogen ^{14}C-residues (not different in lignin content but significant different in C/N ratio) had only a significant difference in decomposition within the planted pots in the first growing season. After the first growing season the overall decomposition of the 700LN residues (planted and unplanted) was 24 % lower than the 350 residues, while the 700HN residues decomposed 12 % slower than the 350HN residues. After the second growing season the overall decomposition of the 700LN residues was 14 % lower than the 350LN residues while the high N treatment was not significant different anymore. This data are in good agreement with Gorissen *et al.* (1995b) who found in a short-term laboratory decomposition experiment with homogeneously labelled ^{14}C-grass roots, that 700 roots showed a decrease in decomposition of 24 % compared with the 350 roots after 64 days. Our results show that these differences are also observed after a long-term incubation during two growing seasons under more natural circumstances. In another microcosm experiment Cotrufo *et al.* (1994) also found a significant decrease in decomposition after 155 days of birch and spruce litter cultivated in an elevated CO_2 atmosphere.
In litterbag experiments Kemp *et al.* (1994) found a decrease in decomposition of the litter of the (C3) prairie gras *Poa pratensis*, cultivated in open top chambers with elevated CO_2. Evidence is accumulating that both leaves (Cotrufo *et al.*, 1994); Kemp *et al.*, 1994) and roots (Gorissen *et al.*, 1995b; this study) are slower decomposed when they were cultivated at elevated CO_2. Although it is difficult to compare microcosm and litterbag studies with studies where a root/soil system is involved, these studies clearly show that the quality of the material has been altered during its growth in an elevated CO_2 atmosphere and subsequently also its decomposition. The differences in decomposition of plant material grown in ambient air or at elevated CO_2 are considered by several workers (Cotrufo *et al.*, 1994; Kemp *et al.*, 1994; O'Neill 1994) to be evoked by a shift in the (chemical) quality of the plant material, lignin:N, lignin:
P, and/or a change in C/N ratio probably caused by a relative lower uptake of nutrients by plants in an elevated CO_2 atmosphere (Melillo *et al.* 1982; Overdieck *et al.*, 1986 and Conroy *et al.*, 1993). However, in our experiments there was hardly any difference between lignin content and C/N ratio of the low nitrogen root material, but nevertheless big differences in decomposition were observed between the 350LN and the 700LN root residues. Though the 350HN and the 700HN roots had a lower C/N ratio than the low nitrogen root residues (Table 11), the difference in decomposition was much less with compared with the low nitrogen roots. Also Cotrufo *et al.* (1994) did not find any relation between mass loss and litter quality and pointed out that differences in leaching of the decomposing litter from the different species could have caused the discrepancy between mass loss and litter quality. In our study, leaching was physically not possible but we also did not find any relation between root quality, C/N ratio and decomposition. It seems necessary to look more for a combination of other (soil?) parameters with quality and C/N ratio. More attention also has to be paid to the combination of plant quality, and the important role of available (or added Fog, 1988) nitrogen in the soil (solution) and other external factors, rather than only considering the internal quality of the plant material. Our higher decomposition results in the planted versus unplanted pots are in accordance with results obtained by Sallih *et al.* (1988) who also observed a stimulation effect in the presence of roots on decomposition of added plant residues. However they contradict the results of other workers (Reid *et al.*, 1982; Martin, 1987; Bek, 1994) who found that growing plants tended to slow down the respiration rate. Bek (1994) explained this phenomenon that plants successfully compete with the soil microbial biomass for the available mineral nitrogen. The difference with our study is that they directly added the total amount of nitrogen needed for the whole (short-term) laboratory experiment to the soil in contrast with our N-addition which was given at (4) regular intervals during the growing

season after every cut. The soil microbial biomass in the planted soil was already favored above the unplanted soil because of the input of fresh substrate from the plant. This activated soil microbial biomass of the planted soil could probably better profit from the new supplied nitrogen and was extra stimulated to decompose more of the added root material. This resulted in higher decomposition rates of the added ^{14}C-residues in the planted pots than the unplanted pots which did not receive additional nitrogen during the experiment. The higher decomposition rate in the planted pots compared with the unplanted pots resulted in a significant lower ^{14}C-soil microbial biomass (^{14}C-SMB). Also the higher decomposition rate of the 350 ^{14}C-residues compared with the 700 ^{14}C-residues, resulted in a significant lower ^{14}C-SMB. The same combination of higher decomposition rates towards reduced sizes of the labelled soil microbial biomass was found by Sallih *et al.* (1988) and Bottner *et al.* (1988). Bottner *et al.* (1988) pointed out that the presence of roots reduced the size of the labelled soil microbial biomass, but increased its activity. Hence the development of the ^{14}C-SMB in the soil was strongly negatively correlated ($P<0.001$) with the ^{14}C-root residues decomposed. It was clear that only the changes in root quality induced by CO_2, and not by nitrogen, caused this correlation between ^{14}C-SMB and decomposition, notwithstanding the fact that the quality of the added roots in terms of C/N or lignin was rather similar. Also planting, but not the CO_2 atmosphere in which the plants were growing, had a strong significant effect on the development of the ^{14}C-SMB. The overall mean ^{14}C-SMB after the first growing season (planted and unplanted) of the 700 ^{14}C-residues was 43 % higher compared with the 350 ^{14}C-residues and was after the second season still 29 % higher. Within the CO_2 treatment we observed hardly any difference between the decomposition of low and high nitrogen ^{14}C-residues. In summary, this study confirms earlier observations in the laboratory that decomposition of roots cultivated at elevated CO_2 is retarded compared with ambient-CO_2, even on the long-term under more natural circumstances outdoors as influenced by plant growth. This observation strongly urges the need to implement such changes in models describing organic matter dynamics in soils.

6. Summary

The increase of the atmospheric CO_2 level since the Industrial Revolution will strongly affect carbon dynamics in terrestrial ecosystems. Elevated CO_2 will stimulate plant growth, especially of C3 plants. This effect may be very beneficial with regard to the yields of arable crops and could, potentially, cause a new 'green revolution'. However, several draw-backs may arise due to accompanying effects of an increased CO_2 concentration. One of the most important coeffects will be a rise in temperature, which may extend the growth period of plants, but also affects *e.g.* winter hardiness and availability of soil moisture. A secondary effect may be exhaustion of soil nutrients due to stimulated plant growth. In arable crops some of these effects may be compensated by means of management practices such as manuring or sprinkling. In contrast to annual crop species, perennial species will be exposed for a much longer time to the environmental changes and therefor be more capable of adapting to these changes. On the other hand, species in natural ecosystems could also be more vulnerably to nutrient deficiencies or changes in soil moisture availability. Consequently, an important question is how species growing in terrestrial ecosystems react on the longer-term on changes in the atmospheric CO_2 concentration and accompanying changes in the environment. A second important question for policy makers is whether the soil could function as a sink for atmospheric carbon. With regard to this question several annotations can be made. Firstly, carbon transport in the plants to the soil and rhizosphere is the most important process for carbon input into the soil. The absolute amounts of carbon allocated to the soil compartment depends on the total net CO_2 fixation and the carbon distribution among the plant/soil compartments. Secondly, the fate of the carbon compounds entering into the soil must be considered. Decomposition of these carbon compounds by the microbial biomass is strongly affected by their quality and by environmental factors such as available nutrients, temperature and soil moisture.

In this study the effects of an elevated atmospheric CO_2 concentration on total net CO_2 fixation, carbon distribution among several plant/soil compartments and water use were investigated. Also decomposition of grasses cultivated at different CO_2 concentrations and nitrogen levels was followed at different temperatures and soil water contents.

Effects on plant growth and yield
These effects were studied in different species (*Lolium perenne* and *Festuca arundinacea*), different soil types (loamy sand and löss), and at two nitrogen addition rates (135 and 400 kg N $ha^{-1} \cdot yr^{-1}$). In Chapter 2 juvenile *L. perenne* plants were exposed to 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for twelve weeks at two nitrogen levels and continuously labelled with $^{14}CO_2$. In this short-term experiment no interaction between CO_2 and nitrogen were observed. At the high nitrogen treatment, shoot and root yield increased by 120 % and 28 % compared with the low nitrogen treatment. The shoot/root ratio at high nitrogen was 58 % higher than at low nitrogen. At high nitrogen 57 % was recovered in the shoots compared with 46 % at low nitrogen. Dry weight of the shoots tended to increase by 6 % at elevated CO_2 and dry weight of the roots significantly increased by 61 %. As a result the shoot/root ratio decreased by 34 % at elevated CO_2. The distribution of ^{14}C among the different plant/soil compartments was affected by the CO_2 treatment. At elevated CO_2 46 % of the total net $^{14}CO_2$ uptake was recovered in the shoots compared with 56 % at ambient CO_2. The percentages recovered in the root and root/soil respiration were accordingly higher at 700 $\mu l \cdot l^{-1}$ CO_2. Due to the increased uptake and the

shift in carbon distribution, the absolute amount of ^{14}C-translocated to the soil increased by 64 % at elevated CO_2. In the following experiments the persistency of these effects on the longer-term was investigated.

In Chapter 3 it was shown that during a long-term experiment elevated CO_2 increased shoot yield by 25 % at the start of the growing season. However, this stimulation disappeared during the growing season and no differences were observed in the last cutting and the initial stimulation 25 % in the cumulative yield was decreased to 16 %. It was remarkable that early in the next growing season shoot yield was increased again at elevated CO_2, but this stimulation also disappeared in the course of the season (Chapter 4). After the second season the increase in shoot yield was 14 %. The periodicity in growth stimulation was evident, but the underlying mechanisms are not understood. It is not likely that other nutrients than nitrogen are limiting the stimulation by elevated CO_2, because nitrogen stimulated plant growth to a much higher degree than CO_2 until the end of the experiments. In this experiment it was observed that the plants pretreated at 700 $\mu l \cdot l^{-1}$ CO_2 and subsequently treated with 700 $\mu l \cdot l^{-1}$ showed a stimulation in total net $^{14}CO_2$ uptake of 15 %, compared with the control (350-350), whereas the plants pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 and treated at 700 $\mu l \cdot l^{-1}$ CO_2 showed an increase of 25 % (Chapter 4). This was probably due to adaptation of the plants after a long-term exposure to elevated CO_2.

Elevated CO_2 significantly stimulated root dry weight of juvenile *L. perenne* plants in loamy sand (Chapter 2). Also on the long term it became evident that root dry weight of *L. perenne* and *F. arundinacea* was significantly increased by elevated CO_2. After 14 months exposure to 700 $\mu l \cdot l^{-1}$ CO_2 root dry weight was increased in both species by about 46 % at the high nitrogen addition rate (Chapter 3). Also after 27 months, root dry weight was still significantly increased by about 27 % in *L. perenne* and by 34 % in *F. arundinacea* at the high nitrogen supply (Chapter 4). At low nitrogen only *L. perenne* showed an increase in root dry weight.

Nitrogen played an obscure role in the increase in root dry weight induced by elevated CO_2. The absolute amounts in root dry weight increased with increasing nitrogen supply and a stimulating effect of elevated CO_2 was more or less general at the high nitrogen supply. Elevated CO_2 sometimes did increase root dry weight at the low nitrogen supply and sometimes no increase was observed. However, it is clear that complicated interactions existed between plant species, soil type, and CO_2 on the one hand and nitrogen on the other. In general, stimulation of root growth by elevated CO_2 seems to be more substantial when nitrogen is available in sufficient amounts. At lower nitrogen levels stimulation may also occur, but will be smaller in absolute amounts.

Effects on carbon distribution

The most relevant observation from the juvenile grass plants was that about 46 % of the ^{14}C was recovered in the shoots after exposure to elevated CO_2, compared with 56 % at ambient CO_2. The difference was mainly at the favour of the root system in which 31 % and 26 % was recovered, respectively. This observation shows that root growth was favoured more than shoot growth leading to a decreased shoot/root ratio at elevated CO_2. In the older grasses which were treated 14 months or 27 months at elevated CO_2 (Chapter 3 and Chapter 4) this increased carbon allocation to the roots also resulted in a decreased shoot/root ratio, although at the time of labelling no effects on the ^{14}C-carbon distribution pattern were observed. This was probably related to the fact that shoot growth stimulation by elevated CO_2 had disappeared at the end of the season. The results presented in Chapter 5 also confirmed the conclusion that root growth was favoured more than shoot growth at elevated

CO_2. The increase in root weight demonstrates that, assuming that elevated CO_2 did not stimulate root turnover, an increased carbon allocation to the roots must have occurred during some periods of the growing season. The question whether or not root turnover was affected by elevated CO_2 needs more attention in future research.

Effects on water use
The short-term CO_2 treatment caused a mean decrease of 34 % in the 14-month-old grasses, although depending on plant species. The same was found when transpiration per gram shoot tissue was calculated. *F. arundinacea* decreased transpiration at elevated CO_2 much more than *L. perenne*, 47 % *vs* 21 %, respectively (Chapter 3). The response of *F. arundinacea* towards water use seems more pronounced than the responses of *L. perenne* and may imply that the capacity of the species to adapt to drier conditions at elevated CO_2 decreases in the same order.

Effects on decomposition processes
Although the accumulated dry weight yield of shoots and roots of grasses was still increased after two growing seasons at elevated CO_2, depending on species and nitrogen supply (Chapter 4), disappearance of growth stimulation is not unimaginable. Hence, the possibilities that the soil will function as a carbon sink would then not be realized through extra carbon input on the longer-term. However, not only the input of carbon is relevant, also the output from plant residues and soil organic matter, which is determined by the activity of the microbial biomass, has to be considered.
In Chapter 5 we followed the decomposition of homogeneously ^{14}C-labelled roots which were cultivated at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 and two nitrogen levels in a ^{14}C-labelled atmosphere. The root residues were added to a loamy sand and part of the pots were planted with *L. perenne* plant. Decomposition was followed during two growing seasons. The overall decomposition of root residues amounted to 54 % after the first growing season and increased to 67 % after the second season. A clear planting effect on the decomposition rate was observed. Unplanted pots showed a significant lower decomposition of ^{14}C-residues than the planted pots, showing that plant growth stimulated decomposition. The CO_2 concentration in the atmosphere in which the plants were growing did not clearly affect the decomposition rate.

The CO_2 level at which the root residues were cultivated, clearly affected the decomposition rate. After the first growing season the mean decomposition of the residues cultivated at elevated CO_2 showed an overall decrease by 19 % compared with the residues cultivated at ambient CO_2. After the second growing season the decomposition of the residues cultivated at high CO_2 and a low nitrogen level in the unplanted pots was 13 % lower compared with the residues cultivated at ambient CO_2 and low nitrogen, while the residues cultivated at elevated CO_2 and high nitrogen did not differ significantly from the residues cultivated at ambient CO_2 and high nitrogen. The results confirm that a decrease in decomposition rate of root residues cultivated at elevated CO_2 can also be found after on a long-term, although in dependance of nitrogen availability. This decreased decomposability can possibly annul the additional CO_2 release from soil organic matter caused by an increase of temperature. Especially knowledge about the long-term effects on input of carbon into soil and the decomposition rates of this carbon is important for predicting the role that soil organic matter in terrestrial ecosystems may play in the question regarding sequestration of atmospheric carbon.

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