Distribution of carbon over plant and soil compartments during the growth of perennial plants

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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 those pools may occur. Post *et al.* (1990) estimated the global carbon reservoirs as shown in Table 1.

Table 1 Global carbon pools (Pg)

Pool	Total 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).
The uptake 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 extend 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 short-term and long-term changes in carbon flows in a plant/soil system in dependance of nitrogen with emphasis on perennial plant species *i.e.* grasses and trees, 2) to study decomposition of roots cultivated at different atmospheric CO_2 concentrations by following the respiration of soils amended with root material and by subsequent fractionation of soil organic material and 3) calibrating a simulation model with the obtained results in order to simulate changes in soil organic matter on the long term (about 50 year), exploring different climate change scenarios. The effects of CO_2 were studied in combination with other factors such as soil type, plant species, nitrogen level, temperature and soil moisture.
In Chapter 2, carbon allocation and water use measurements in juvenile grasses, which were exposed to elevated CO_2 for three weeks, are discussed. Chapter 3 describes the effects of elevated CO_2 on carbon distribution and water use in mature grasses which were cultivated for about 14 months at 350 or 700 $\mu l \cdot l^{-1}$ CO_2 and at two different nitrogen levels. In Chapter 4 an even longer CO_2 treatment is presented in which plants were exposed for 27 months to elevated CO_2 and two nitrogen levels. At the time of ^{14}C-labelling, a temperature increase and a lowered soil moisture content were added as additional treatment factors. In Chapters 5 and 6 the effects of elevated CO_2 on net carbon uptake, carbon allocation, and water use in Douglas-fir are discussed. The Douglas-firs had also been exposed for a long period (14 months) to 350 and 700 $\mu l \cdot l^{-1}$ CO_2 and were subsequently labelled with $^{14}CO_2$ for one month. Decomposition of soil organic matter and homogeneously ^{14}C-labelled grass roots cultivated at two CO_2 concentrations and two nitrogen levels is described in Chapter 7. In Chapter 8 decomposition of grass shoots is followed and results are presented on fractionation of the decomposing organic material in time. Finally, a modelling exercise was made to investigate the possible changes in soil organic matter dynamics on the long term in Chapter 9, simulating different scenarios of climate change.

2. Carbon allocation and water use in juvenile grasses under elevated CO_2

Abstract

In a preliminary experiment, the effect of elevated CO_2 on carbon allocation and water use of juvenile plants of *Lolium perenne* L. and *Festuca arundinacea* Schreber grown on two soil types (a loamy sand and a loam) was investigated. The plants were exposed to ambient and elevated CO_2 for three weeks in an atmosphere continuously labelled with $^{14}CO_2$. The mean transpiration of the plants tended to decrease by 18 % at the high CO_2 level and total yield of the species showed a nonsignificant increase of 11 %. A clear interaction was observed between plant species and soil type. *L. perenne* had 39 % more yield on loamy sand than on loam, whereas total yield of *F. arundinacea* on loam was 29 % higher than on loamy sand. Elevated CO_2 significantly stimulated root dry weight of *L. perenne* in loamy sand. This was confirmed by the percentage distribution of ^{14}C, which was strongly increased. Overall, elevated CO_2 decreased the percentage ^{14}C retained in the shoots, whereas a higher percentage was recovered in the roots. This increase in ^{14}C percentage in the roots was observed in all species/soil type combinations, except for *L. perenne* on loam, and indicates that at the time of labelling root growth was favoured more than shoot growth at elevated CO_2 compared with ambient CO_2. The results showed interactions between soil type, plant species and/or CO_2 treatment. Soil type and plant species are probably important co-factors with regard to the question whether soils can function as a storage medium for atmospheric carbon. In other words, the combination of plant species and soil type may strongly determine the ultimate capacity of ecosystems for sequestration of atmospheric carbon.

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. Hence, comparison of species responses should be an important issue in studies on the effects of elevated CO_2. The same applies to different soil types, since an increase in carbon allocation to the roots and soil and the subsequent decomposition of root-derived material is strongly dependent on soil type (Merckx *et al.*, 1985). Some soils have higher protective properties for organic matter than other soils, depending on the clay content (Hassink *et al.*, 1993). This may have implications for the storage capacities of different soil types, *e.g.* clay soils will possess higher potentials for carbon storage than sandy soils.

The objectives of this preliminary experiment were:

i) to determine the effects of elevated CO_2 on carbon allocation patterns and water use in two juvenile grasses (*L. perenne* and *F. arundinacea*), and

ii) to study the effect of soil type on carbon allocation patterns at different CO_2 levels. $^{14}CO_2$ was used to trace the carbon flows and the distribution in the plant/soil compartments.

2.2. Materials and methods

CO_2 treatments

Grass seeds (*Lolium perenne* L. cv 'Barlet' and *Festuca arundinacea* Schreber cv 'Barcel') were germinated in April on wet tissue paper in petridishes and after 10 days transferred to 3.7-l perspex columns (length 60 cm; diameter 9 cm), one plant per column. The columns were filled with a loamy sand (Ede, NL) or a loam (Maastricht, NL) with a bulk density of 1.3 $g \cdot ml^{-1}$ on dry weight basis. Particle size distribution (< 2 µm, 2-50 µm, 50-2000 µm) in the loamy sand was 3, 12, and 85 %, respectively, and in the loam 13, 71, and 16 %. Soil moisture content was adjusted to about 60 % of WHC (14 % w/w for the loamy sand and 17.5 % w/w for the loam soil) and the initial weight of the planted columns was determined subsequently. No additional fertilizer was applied to the plants. After four weeks, the column lids were sealed airtight with a silicon rubber (Q3-3481, Dow Chemical) to prevent exchange of $^{14}CO_2$ between growth chamber and columns. The plants were exposed to 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for three weeks in ESPAS growth chambers (a modernized version of the Experimental Soil Plant Atmosphere System, described by Merckx *et al.* (1986)) in an atmosphere with a constant specific activity. The preset atmospheric CO_2 levels were maintained either by injecting CO_2 or by removing it by carbosorb filters (Sodasorb, Grace). Both CO_2 an $^{14}CO_2$ were supplied from gas cylinders (100 % CO_2) and the inflows were controlled automatically by means of mass flow controllers (Brooks). CO_2 levels were measured by an URAS 10E infrared analyzer

(Hartmann & Braun). The specific activity was measured three times a week by sampling 150 ml of air in a glass bulb and injecting 5 ml 0.5 *M* NaOH. After 24 h the specific activity was obtained from analysis of a 1 ml aliquot by liquid scintillation counting (Tri-Carb 4530; Packard), using Ultima Gold (Packard).
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.
During the 3-week experiment, soil columns were flushed every 6 hours with CO_2-free air at a flow rate of about 40 $l \cdot h^{-1}$ 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. Transpiration of the plants was determined by weighing the columns and adjustment to the initial weight with de-ionized water.

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 *M* HCl. $^{14}CO_2$ was determined in a subsample by liquid scintillation counting 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 70°C for 24 hours. Dried plant material was ground and homogenized and a wet combustion procedure was used to determine total C and ^{14}C (Dalal, 1979). 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
Eleven *L. perenne* (six in loamy sand and five in loam soil) and eight *F. arundinacea* (four in loamy sand and four in loam soil) plants were exposed to 350 $\mu l \cdot l^{-1}$ CO_2 or 700 $\mu l \cdot l^{-1}$ CO_2, respectively. The experimental factors were two CO_2 treatments, two plant species and two soil types. The results were analyzed with ANOVA. Differences are considered to be significant when P-values were lower than 0.05.

2.3. Results

At elevated CO_2 plants tended to decrease transpiration by about 18 % compared with the controls (P=0.15) (Table 2). Loam soil had a strong negative effect on plant transpiration, in loamy sand transpiration was about 65 % higher than in loam soil. The CO_2 treatment resulted in a non-significant increase of total yield by 11 % compared with the control. This trend was more clear in the roots (+23 %; P=0.16) than in the shoots (+6 %; P=0.55). An interaction was observed between CO_2, plant species and soil type on root growth. Root growth of *L. perenne* was significantly stimulated in loamy sand and but tended to decrease in loam soil.
F. arundinacea reacted indifferently, no effects were observed. Total average yield was strongly dependent on plant species and soil type. *L. perenne* had 39 % more yield on loamy sand than

Table 2 Yield (g), total net uptake (kBq), percent distribution of ^{14}C (% of total net uptake) among different plant/soil compartments and total water use after treatment of juvenile *L. perenne* and *F. arundinacea* plants grown on two soils with 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for three weeks

	Loamy sand				Loam			
	L. perenne		*F. arundinacea*		*L. perenne*		*F. arundinacea*	
	350 (n=3)	700 (n=3)	350 (n=2)	700 (n=2)	350 (n=3)	700 (n=2)	350 (n=2)	700 (n=2)
Yield (g)								
Leaves	2.6	3.2	1.5	1.3	2.0	1.9	1.8	1.9
Roots	0.8	1.8	0.7	0.7	1.2	0.8	0.9	1.0
Total net uptake (kBq)	438	575	281	240	374	267	304	302
% distribution of total net uptake								
Leaves	72.2	65.1	62.9	55.9	60.8	59.6	68.8	62.1
Roots	18.0	29.6	29.7	32.8	26.0	23.8	21.9	25.6
Root/soil respiration	7.6	3.0	5.5	7.0	6.6	10.8	4.2	8.7
Soil	2.2	2.3	1.9	4.3	6.6	5.8	5.1	3.6
Total water use (ml)	320	290	305	290	175	150	250	150

Statistics[1]	S	P	T	interactions
Yield				
Leaves	<0.01	ns	ns	S*P
Roots	0.03	ns	ns	S*P*T
Total net uptake	ns	0.01	ns	S*P
% distribution				
Leaves	ns	ns	<0.05	S*P
Roots	ns	ns	0.06	
Root/soil respiration	ns	ns	ns	S*T
Soil	<0.001	ns	ns	
Total water use	<0.001	ns	0.15	

[1] S=Soil type; P=Plant species; T=Treatment; ns=not significant

in loam soil, whereas *F. arundinacea* had 29 % more yield on loam soil than on loamy sand. The mean dry weight of *L. perenne* was about 50 % more than *F. arundinacea*.
Total net ^{14}C-uptake was not clearly affected by the CO_2 treatment. It was affected by plant species again in dependence on soil type, showing the same interaction as for total yield. The distribution pattern of ^{14}C among the plant/soil compartments was slightly changed by the CO_2 treatment. At elevated CO_2 about 61 % was retained in the shoots compared with 66 % at ambient CO_2 (P=0.04). This was mainly at the favour of the root system in which the percentages were 28 % and 24 %, respectively. Soil type had a clear effect on the residue percentage which was more than twice as high in the loam soil than in the loamy sand.

2.4. Discussion

The observed decrease in transpiration is within the range that could be expected from earlier reports (Rogers *et al.*, 1994). Earlier reports indicate that carbon allocation to the roots is often stimulated under higher CO_2, although this effect is not always very clear. An increased allocation to the roots may result from stimulated photosynthesis by which a total available carbon amount in plant increases, but also from changes in carbon distribution patterns. Although this was only a small preliminary experiment, the results on *L. perenne* growing on loamy sand nicely illustrates the possible consequences. Total net uptake tended to increase from 438 to 575 kBq (+31 %), the percentage ^{14}C recovered in the roots increased from 18 to 29.6 % (+64 %) and as a result, the total increase of ^{14}C-carbon recovered in the roots increased from 79 to 170 kBq (+115 %). The same results were observed in another experiment with juvenile plants of *L. perenne* (Van Ginkel *et al.*, in prep). The mean ^{14}C-percentage in the roots in both soil types increased by 11 % at elevated CO_2 ($P=0.06$) at the expense of the shoots. Both plant species retained significantly less carbon in the leaves at elevated CO_2 compared with ambient CO_2 ($P<0.05$). This result would imply that the shoot/root ratio would decrease. Stulen and Den Hartog (1993) criticized the determination of changes in shoot/root ratio because of experimental errors due to, amongst others, problems on quantitatively recovering of (fine) roots and accounting for root decomposition. However, using ^{14}C as a tracer for carbon, an accurate measurement of the carbon amount allocated to the roots and soil is possible. In this experiment the total percentage of ^{14}C recovered in the root/soil compartment increased from 33.6 % to 39.0 % at elevated CO_2 compared with ambient ($P<0.05$).

Soil type significantly affected the soil residual ^{14}C. In the loam soil the higher residue may have resulted from protective properties of this finer textured soil in which organic matter is protected by clay particles from decomposition by soil microorganisms (Merckx *et al.*, 1985; Hassink *et al.*, 1993). Such mechanisms may have important consequences for the soil types which may be most suitable for the purpose of carbon storage. Although far-reaching conclusions can not be drawn from this preliminary experiment, it is clear that soil type significantly affected most of the measured parameters. The same applies for the type of plant species which may differ in their response to elevated CO_2 whether or not in dependence of additional factors such as soil type. In this study, *L. perenne* had a larger yield on loamy sand than on loam soil, whereas for *F. arundinacea* the opposite was found. The potential to accumulate carbon is apparently dependent on species/soil type interactions which means that some combinations of a plant/ soil system have more potential for carbon storage than other combinations. However, this conclusion must be taken with care since many other factors will be involved such as seasonal influences, plant age, species competition and nutrient availability.

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.

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. 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 $\mu l \cdot l^{-1}$) 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 $N \cdot ha^{-1} \cdot yr^{-1}$ and the higher treatment 300 mg N per pot corresponding with 400 kg $N \cdot ha^{-1} \cdot yr^{-1}$. 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 *L. perenne* plants and 24 *F. 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)).

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

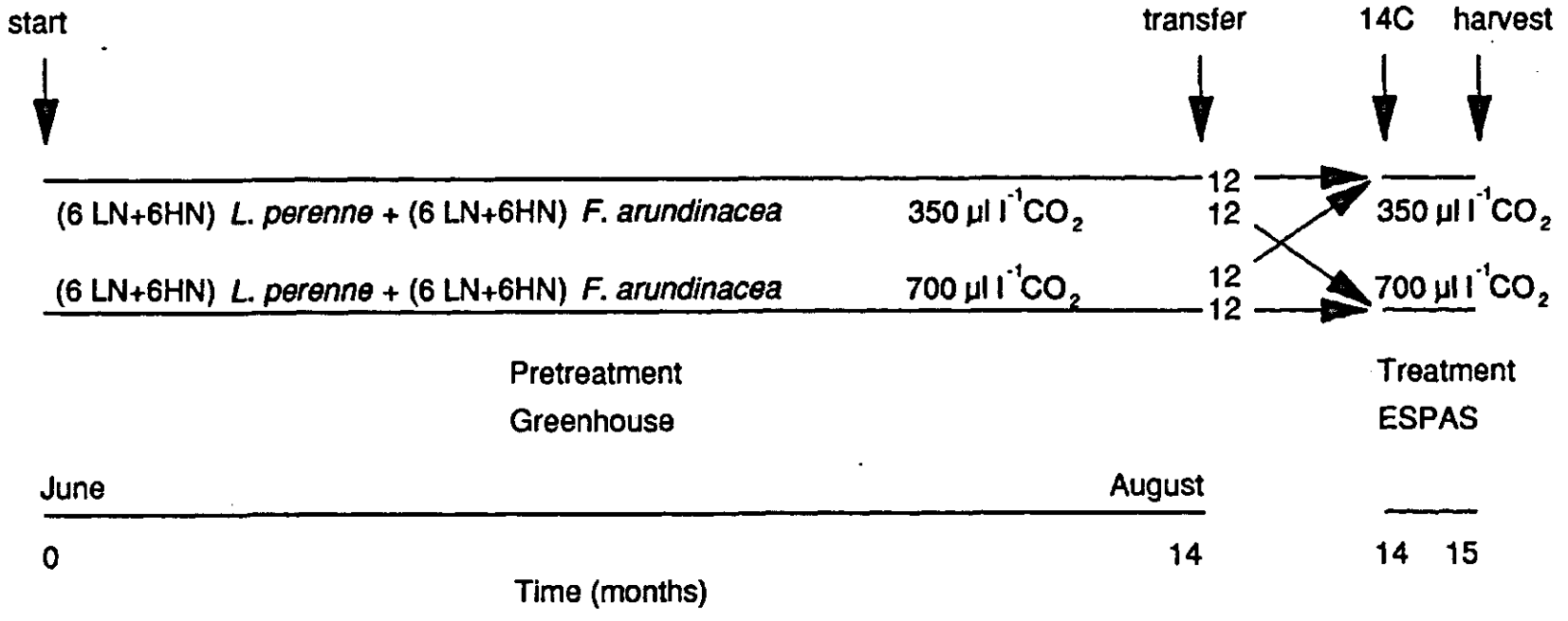


Figure 1 *Schematic diagram of the experimental design*

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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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.

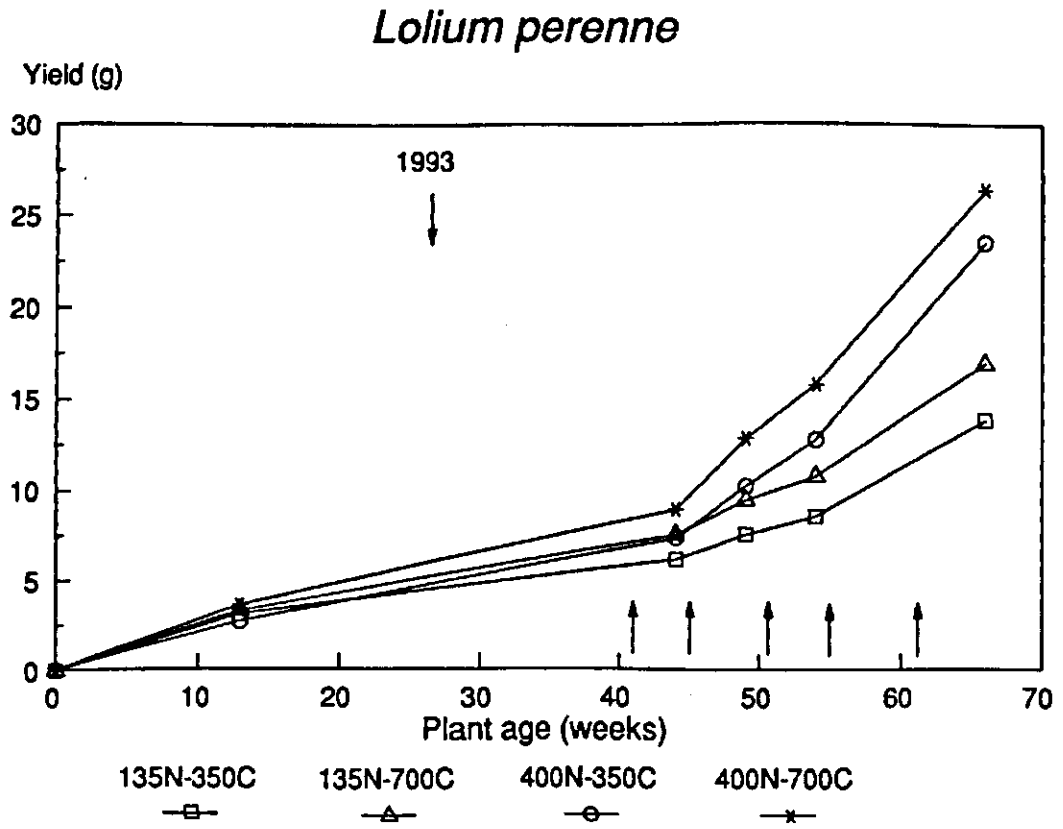
Prior to CO_2 treatment, the column lids were sealed with a silicon rubber (Q3-3481, Dow Chemical) to prevent exchange of $^{14}CO_2$ between growth chamber and columns. During the 3-week experiment, soil columns were flushed every 6 hours with CO_2-free air at a flow rate of about 40 $l \cdot h^{-1}$ 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 *M* 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 *L. perenne* and 24 *F. 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.



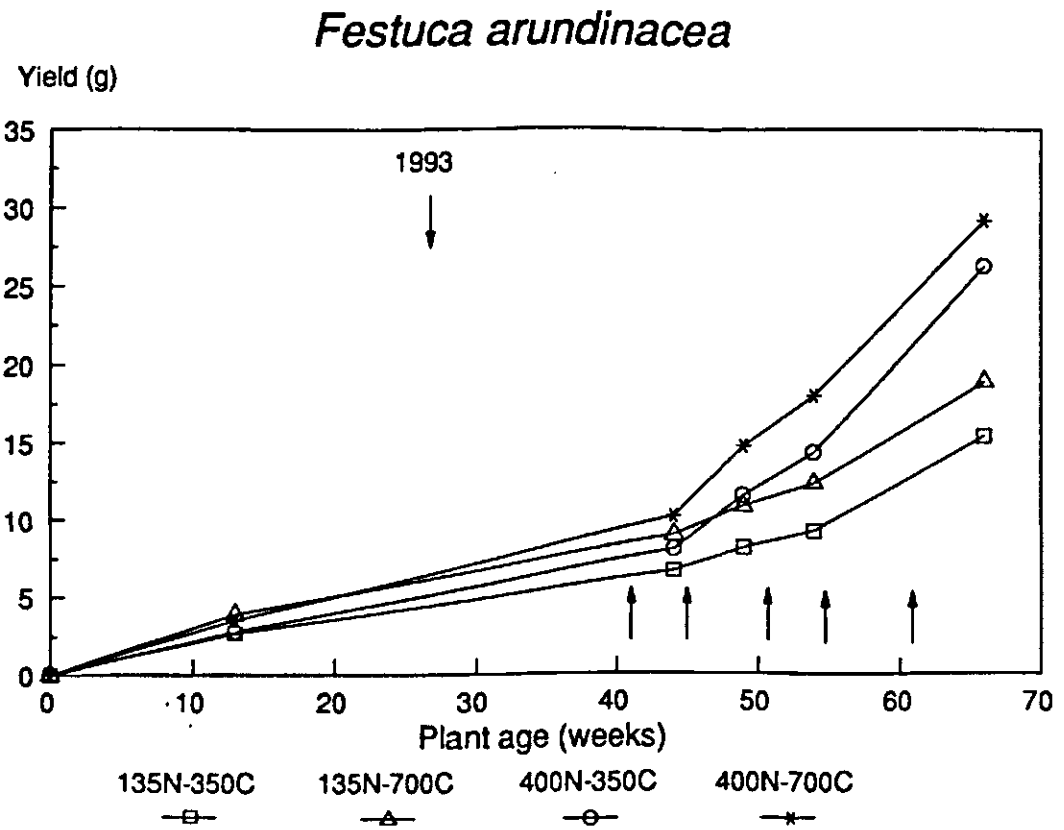


Figure 2 Yield of *L. perenne* and *F. arundinacea* during the pretreatment at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 at two nitrogen levels for 14 months. Nitrogen application indicated by arrows

3.3. Results

Figure 2 shows the cumulative shoot yields of *L. perenne* and *F. 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.

Table 3 Plant biomass (g), microbial biomass (µg C·g^{-1} soil), water use (ml) and shoot/root ratio after pretreatment of *L. perenne* and *F. 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).

Nitrogen level	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
L. 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
F. 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 *L. perenne* and *F. arundinacea* with two nitrogen levels (135 and 400 kg $N \cdot ha^{-1} \cdot yr^{-1}$) 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 1 (n=3).

Nitrogen level	135				400			
Pretreatment	350		700		350		700	
Treatment	350	700	350	700	350	700	350	700
L. 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
F. 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

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 (Chapter 5).

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 in Chapter 5. 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 $N \cdot ha^{-1} \cdot yr^{-1}$) 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 (*L. perenne* and *F. 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 loam soil with a bulk density of 1.2 $kg \cdot l^{-1}$ (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 $\mu l \cdot l^{-1}$) and elevated (700 $\mu l \cdot l^{-1}$) 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 $N \cdot ha^{-1} \cdot yr^{-1}$ and the higher treatment 300 mg N per pot corresponding with 400 kg $N \cdot ha^{-1} \cdot yr^{-1}$. 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 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 *L. perenne* plants and 54 *F. 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 $\mu l l^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 *M* HCl. $^{14}CO_2$ was determined in a subsample by liquid scintillation counting (Tri-Carb 4530; Packard) using Ultima Gold (Packard).

Statistics

Fifty-three *L. perenne* and 54 *F. 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.

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.

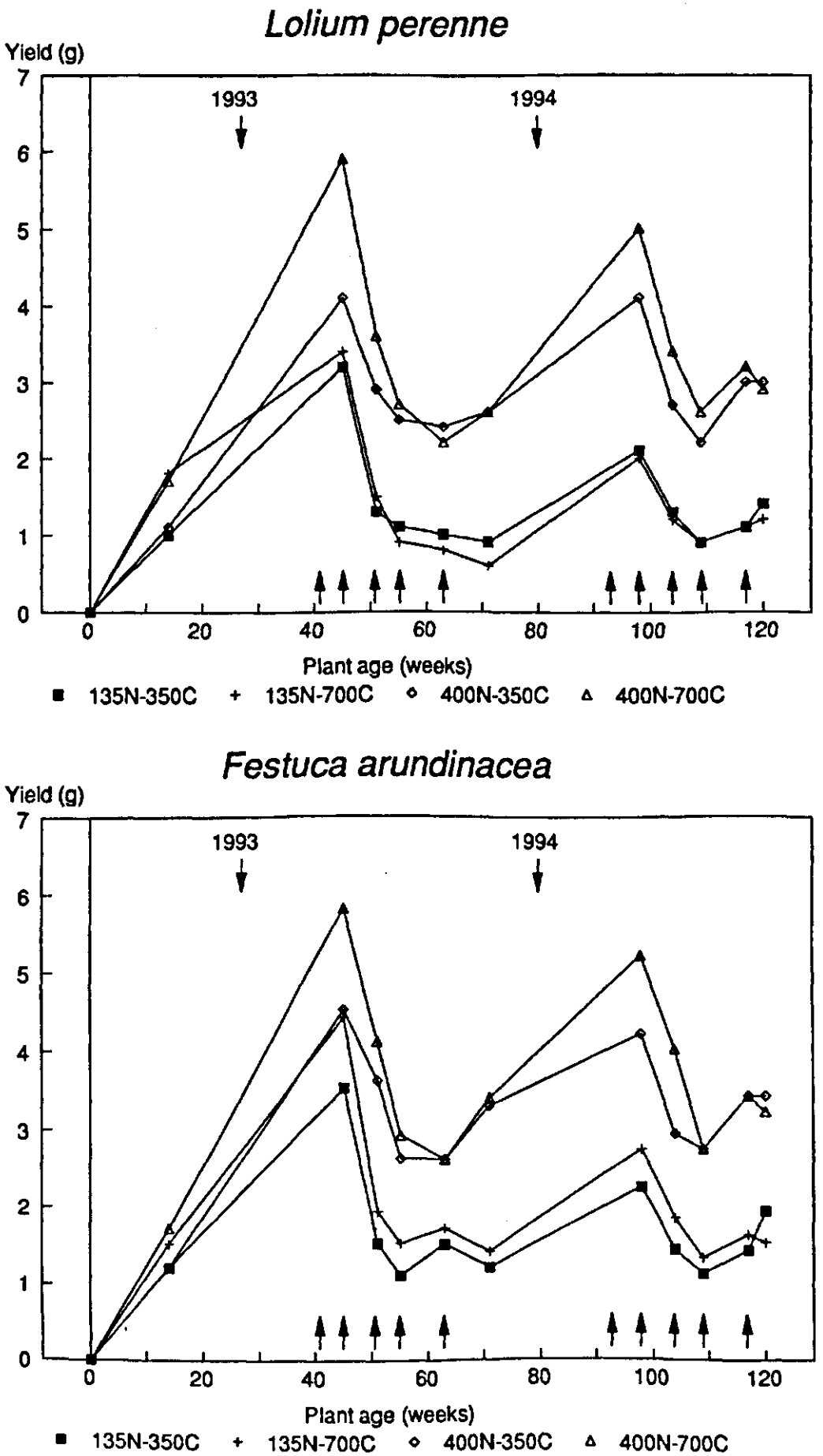


Figure 3 Yield of *L. perenne* and *F. arundinacea* during pretreatment at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 at two nitrogen levels for 27 months. Nitrogen application indicated by arrows.

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	LN	HN	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				HN			
	LT		HT		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 also observed for Douglas-fir (Chapter 5) 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 loam soil. 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. Carbon allocation and water use in juvenile Douglas-fir under elevated CO_2[1]

Abstract

In this study, the effect of an elevated CO_2 level on allocation of assimilates and water use efficiency of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) was investigated. Juvenile Douglas-firs were exposed to a long-term treatment at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for 14 months and subsequently crosswise transferred to phytotrons for a short-term treatment with 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for four weeks in an atmosphere continuously labelled with $^{14}CO_2$. No interactive effects on total net uptake of $^{14}CO_2$ between long-term treatment and short-term treatment were observed. The short-term treatment with 700 $\mu l \cdot l^{-1}$ CO_2 increased the total net uptake of $^{14}CO_2$ by 22 %, compared with the 350 $\mu l \cdot l^{-1}$ CO_2 treatment. The long-term pretreatment did not affect the total net uptake, suggesting that photosynthetic acclimation had not occurred. However, expressed per unit of needle mass a 14 % reduction was observed in the trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2. This was not due to a reduced sink strength of the root system. This reduced uptake per unit of needle mass after long-term treatment may have implications for carbon storage in forest ecosystems. The results showed that an initial growth stimulation can eventually be annulled by developing physiological or morphological adaptations. $^{14}CO_2$ in the root/soil respiration increased in the short-term treatment with 700 $\mu l \cdot l^{-1}$ CO_2, indicating a stimulated use of current carbon compounds either by roots or microorganisms. The water use efficiency during the short-term treatment with 700 $\mu l \cdot l^{-1}$ CO_2 increased by 32 %, but was not affected by the long-term pretreatment. Water use per unit needle mass during the short-term treatment was decreased both by the short-term treatment and by the long-term pretreatment by about 15 %. Some of the observed effects appeared to be persistent, such as total net $^{14}CO_2$ uptake and water use per unit needle mass, whereas others, total net $^{14}CO_2$ uptake and water use efficiency, were transient.

[1] A. Gorissen, P.J. Kuikman & H. van de Beek. 1995. *New Phytologist* 129: 275-282

5.1. Introduction

The carbon dioxide concentration in the atmosphere is increasing due to fossil fuel combustion and deforestation (Woodwell *et al.*, 1983; Musselman & Fox, 1991). However, the observed increase is less than expected on the basis of calculated emissions. Tans *et al.* (1990) argued that terrestrial ecosystems in the northern hemisphere must be responsible for sequestering substantial amounts of carbon dioxide. For this reason it is important to gain a better insight into the dynamics of carbon dioxide uptake by plants and translocation of carbon to the root system in perennial plant/soil systems.

A doubling of the CO_2 concentration will lead to an average increase of more than 30 % in crop yield under optimum conditions (Kimball, 1983; Cure & Acock, 1986). 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). Bazzaz (1990) and Bowes (1991) described that some species decrease their photosynthetic activity at higher CO_2 levels due to a variety of reasons *e.g.*, decreased activity of Rubisco, suppression of sucrose synthesis, and reduced specific leaf area.

Water use by plants is another issue that has received much attention, since water use efficiency (WUE; biomass accumulated per unit water used) can be increased by 30-60 % under elevated CO_2 levels (Rogers *et al.*, 1983; Norby *et al.*, 1986; Chaudhuri *et al.*, 1990). Bazzaz (1990) outlined that this increase results from decreased stomatal conductance. The impact of a reduced stomatal density (Woodward & Bazzaz, 1988) on WUE is uncertain. Where some evidence exists on adaptation of photosynthetic rates to elevated CO_2 levels, it is unknown whether or not increased water use efficiency is a transient phenomenon or a persistent adaptation of plant species.

The objectives of this study were:

i) to determine the effects of elevated CO_2 on biomass accumulation, carbon allocation patterns, and water use efficiency in juvenile Douglas-firs, and

ii) to study the effect of a long-term pretreatment at ambient and elevated CO_2 levels versus a short-term treatment. $^{14}CO_2$ was used to trace the carbon flows and the distribution in the plant/soil compartments. Before exposure to $^{14}CO_2$, the trees were pretreated in greenhouse compartments for more than one year at two CO_2 levels to enable us to distinguish between short-term (month) and long-term (year) effects.

5.2. Materials and methods

CO_2 treatments

Two-year-old and 3-year-old nursery-grown Douglas-firs (*Pseudotsuga menziesii* [Mirb.] Franco) were potted in 3.7-l perspex columns (length 60 cm; diameter 9 cm), one tree per column. The columns were filled with a sandy soil ($A_{1/2}$-horizon), collected from a Douglas-fir stand in forestry Kootwijk, The Netherlands (52°11' N, 5°46' E). By gentle vibration, in order to optimize the contact between roots and soil, the bulk density was adjusted to 1.2 g·ml^{-1}. Soil moisture content was adjusted to 60 % of WHC (about 14 % w/w) and the initial weight of the planted columns was determined subsequently. Figure 4 shows a schematic diagram of the experimental design. The trees were grown in two adjacent greenhouse compartments with ambient (about 350 µl·l^{-1}) and elevated (700 µl·l^{-1}) CO_2 levels for 14 months without additional light. During this long-term treatment, in the following referred to as pretreatment, the CO_2 levels were measured by an Ari-P analyzer (Siemens). Mean air temperature during this period

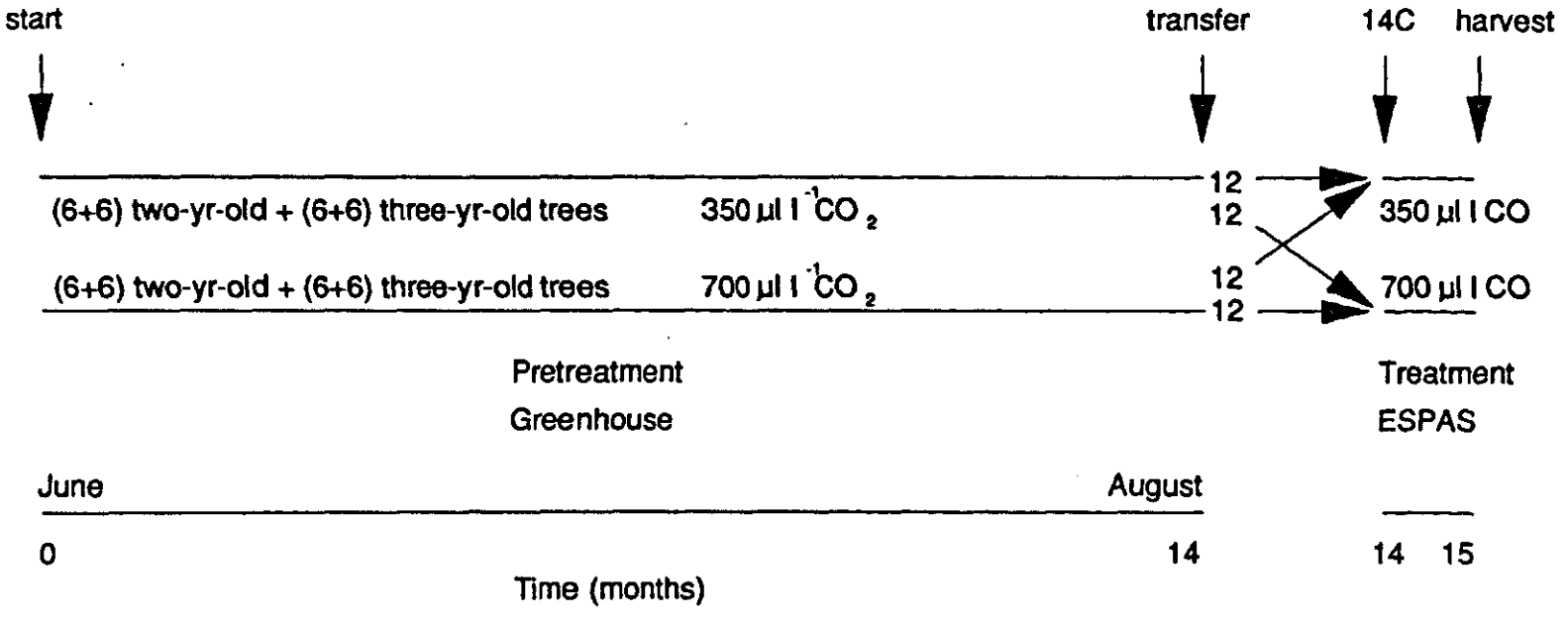


Figure 4 *Schematic diagram of the experimental design*

was 20.7°C (± 2.2) and 20.1°C (± 2.4), respectively, except during the winter months (December-March) when temperatures were 17.0°C (± 0.3) and 16.7°C (± 0.4). Relative humidities during the whole period were 80 % (± 3) and 84 % (± 3), respectively, measured by an Asman psychrometer. The trees were weekly moved around in the compartments and PAR levels were regularly measured in order to assure equal conditions for the trees. The columns were weighed weekly and readjusted to their initial weight with de-ionized water. No fertilizer was applied to the trees. Since none of the age classes contained 48 trees, we randomly took 24 trees from each age class and transferred them in September, after the pretreatment, to the ESPAS growth chambers (a modernized version of the Experimental Soil Plant Atmosphere System, described by Merckx *et al.* (1986)). The trees were exposed to 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for four weeks in an atmosphere with a constant specific activity (1.5 $kBq \cdot mg^{-1}$ C). Half of the trees 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 month of the experiment. The preset atmospheric CO_2 levels were maintained either by injecting CO_2 or by removing it by carbosorb filters (Sodasorb, Grace). Both CO_2 an $^{14}CO_2$ were 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). The specific activity was measured three times a week by sampling 150 ml of air in a glass bulb and injecting 5 ml 0.5 *M* NaOH. After 24 h the specific activity was obtained from analysis of a 1 ml aliquot by liquid scintillation counting (Tri-Carb 4530; Packard), using Ultima Gold (Packard).
Temperatures in the growth chambers (shoot 18°C at day; 14°C at night; roots 14°C at day; 12°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 (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 airtight with a silicon rubber (Q3-3481, Dow Chemical) to prevent exchange of $^{14}CO_2$ between growth chamber and columns. Airtightness was checked with 0.15 m H_2O overpressure. During the 4-week experiment, soil columns were flushed every 6 hours with CO_2-free air at a flow rate of about 40 $l \cdot h^{-1}$ 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. During the CO_2 treatment the trees were weighed twice a week and readjusted to their initial weight with de-ionized water.

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 *M* HCl. $^{14}CO_2$ was determined in a subsample by liquid scintillation counting using Ultima Gold (Packard).
The trees were harvested after 29 days in the ESPAS growth chambers. Dry weights of needles, branches/stem, and roots were determined after drying at 80°C for 24 hours. Dried plant material was ground and homogenized and a wet combustion procedure was used to determine total C and ^{14}C (Dalal, 1979). 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.
The amount of newly-formed biomass was calculated from the net uptake of ^{14}C and the specific activity of the atmosphere. This newly-formed biomass evidently not only consisted of structural material, but also of non-structural compounds. Old biomass was calculated by

subtracting the newly-formed biomass from the total biomass as measured at harvest time. During the treatment period (one month), old non-structural biomass will partly be respired and replaced by new ^{14}C-labelled compounds. We assumed that the loss of old biomass can be neglected compared with the amount formed during the previous three years.

Statistics

Twenty-four 2-year-old and 24 3-year-old Douglas-firs were exposed to a long-term treatment 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 trees pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 were transferred to one ESPAS growth chamber, the other half to a second chamber for a short-term treatment at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2. The experimental factors were four CO_2 treatments and two age classes, always six replications. The results were analyzed with ANOVA. Significant differences are reported when P-values were lower than 0.05.

5.3. Results

Although the trees were cultivated on a poor sandy soil and no fertilizer was applied, no visible symptoms of nutrient deficiencies were observed after the treatments. At the end of the pretreatment no visual differences between set bud of the trees could be observed. The pretreatment at 700 $\mu l \cdot l^{-1}$ CO_2 caused an overall increase in the mass of needles and roots ('old biomass') of 12 % (P=0.09) and 16 % (P=0.05), respectively (Table 7). The shoot/root ratio (S/R ratio) was not significantly affected by the pretreatment.

Interactive effects between pretreatment and treatment on total net $^{14}CO_2$ uptake and newly-formed biomass were not observed. The treatment at 700 $\mu l \cdot l^{-1}$ CO_2 in the ESPAS chambers increased new biomass in needles, branches and roots by 20 %, 15 %, and 28 %, respectively, but only significantly for the roots (Table 8). The overall net CO_2 uptake (defined as total ^{14}C recovered in trees, soil and root/soil respiration) by trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2 was about 9 % less compared with the pretreatment at 350 $\mu l \cdot l^{-1}$ CO_2 although this was not significant (P=0.28) (Table 8). This decrease becomes more prominent by the significant reduction of 14 % in total net uptake per unit of needle mass (P=0.03). The treatment in the ESPAS growth chambers caused a significant average increase of total net uptake by 22 % at 700 $\mu l \cdot l^{-1}$ CO_2 compared with 350 $\mu l \cdot l^{-1}$ CO_2. Per unit of needle mass the 700 $\mu l \cdot l^{-1}$ CO_2 treatment caused a significant increase of 16 % in total net uptake. The reduced total net uptake by the trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2 became also apparent in significant differences in the newly-formed biomass expressed as a percentage of total biomass: 15 %, 12 %, and 15 % in needles, branches, and roots, respectively, whereas the trees pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 incorporated 17 %, 16 %, and 18 %, respectively, in the same fractions (Table 9). The treatment effects showed the opposite results: the increased uptake at 700 $\mu l \cdot l^{-1}$ CO_2 resulted in 17 %, 15 %, and 18 % increase of new biomass in needles, branches and roots, respectively, compared with 15 %, 13 %, and 15 % in the 350 $\mu l \cdot l^{-1}$ CO_2 treatment.

The only interactive effects were observed between pretreatment and age for newly-formed root biomass and the shoot/root ratio. The 3-year-old trees showed an increase in newly-formed root biomass during the treatment after both pretreatments, whereas the older trees pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 showed a strong increase in newly-formed root biomass during the treatment, in contrast to the older trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2. A consequence of more root production in the 4-year-old trees pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 is a decreased shoot/root ratio.

The average water use in the ESPAS growth chambers was not significantly affected by pretreatment or by treatment, although it decreased at 700 $\mu l \cdot l^{-1}$ CO_2 during the treatment by

7 % (P=0.17) (Table 9). The trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2 used 16 % less water per unit needle mass than the trees pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 (P = 0.03) and the trees treated at 700 $\mu l \cdot l^{-1}$ CO_2 used 14 % less than the trees at 350 $\mu l \cdot l^{-1}$ CO_2 (P = 0.06). In addition, the water use efficiency was strongly affected by the treatment (P<0.001), but not by the pretreatment (P=0.84). The trees treated at 700 $\mu l \cdot l^{-1}$ CO_2 needed 32 % less water for the newly-formed biomass than the trees treated at 350 $\mu l \cdot l^{-1}$ CO_2.
The trees treated at 700 $\mu l \cdot l^{-1}$ CO_2 released, on average, 24 % more $^{14}CO_2$ in the root/soil respiration than the trees at 350 $\mu l \cdot l^{-1}$ CO_2 (P=0.04) (Table 9). The pretreatment did not affect $^{14}CO_2$ respiration in the root/soil compartment.
Table 10 shows the relative differences between the treatment combinations expressed as a percentage of the control combination (pretreatment 350 - treatment 350). Total net CO_2 uptake and uptake per unit needle mass was most stimulated in the 350-700 combination and was decreased in the 700-350 combination. Also expressed as % newly-formed biomass, the 350-700 combination resulted in the strongest increase. The percentage decrease in water use was strongest in the 700-700 combination. An effect on WUE was only observed during treatment with 700 $\mu l \cdot l^{-1}$ CO_2, independent of the pretreatment.

Table 7 Old biomass (g)[1] of Douglas-firs at the start of the treatment in the ESPAS growth chambers after pretreatment with 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for 14 months (n=12). See Figure 4 for information on experimental design.

	Pretreatment				Statistics[2]			
	3-year-old		4-year-old					
	350	700	350	700	A	P	A*P	LSD
Needles	8.0	9.9	18.8	20.4	0.001	0.08	ns	1.9
Branches/stem	10.8	14.2	39.4	38.9	0.001	ns	ns	4.3
Roots	12.2	18.4	38.0	39.7	0.001	0.06	ns	4.2
S/R ratio	1.6	1.4	1.6	1.5	ns	ns	ns	0.2

1 Old biomass was calculated by subtracting the newly-formed biomass from total dry weight. Newly-formed biomass was calculated as
[total kBq]/[specific activity (kBq.mg^{-1} C) of atmospheric CO_2]*[%carbon]/100.

2 LSD values for P ≤ 0.05. A=Age; P=Pretreatment.

5.4. Discussion

The increase in tree production during the long-term treatment was expected (Tinus, 1972; Sionit *et al.*, 1985; Kaushal *et al.*, 1989). The strong increase in root growth during the short-term treatment period (autumn) agrees well with earlier reports on carbon allocation in trees (Webb, 1977; Gorissen *et al.*, 1991). In autumn, the root system is apparently a strong sink for current assimilates. The relative growth stimulation found for old and newly-formed biomass agree with other observations (Kimball, 1983; Lord *et al.*, 1993). This study aimed to determine whether growth will be still stimulated after a long exposure period to elevated CO_2 as was questioned by Bazzaz (1990). Whereas needle mass of the trees exposed to the long-term treatment at 700 $\mu l \cdot l^{-1}$ CO_2 was 12 % higher at the start of the short-term CO_2 treatment total net $^{14}CO_2$ uptake in the ESPAS growth chambers decreased by 9 % (P=0.28) and therefore total net uptake per unit needle mass was reduced by 14 % (P=0.03). Apparently, the needles

Table 8 Total net uptake (kBq), uptake per unit needle mass, and newly-formed biomass (g)[1] of Douglas-fir after treatment with 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for four weeks in an atmosphere with a specific activity of 1.5 (±0.1) $kBq \cdot mg^{-1}$ C and 1.5 (±0.2) $kBq \cdot mg^{-1}$ C, respectively (n=6). See Figure 4 for information on experimental design.

Age	3-year-old				4-year-old				Statistics[1]			
Petreatment	350		700		350		700					
Treatment	350	700	350	700	350	700	350	700	A	P	T	LSD
carbon dioxide uptake												
Total net uptake	5490	7010	5410	7420	11220	13740	9650	11220	0.001	ns	0.02	1620
$kBq\ g^{-1}$ needle	623	671	479	661	529	574	335	486	0.006	0.02	0.03	75
newly-formed biomass												
Needles	1.5	2.1	1.6	1.9	3.1	4.2	3.6	3.5	0.001	ns	ns	0.6
Branches	2.5	3.2	2.0	2.6	5.5	5.3	3.7	5.1	0.001	ns	ns	1.1
Roots	3.0	3.7	3.7	4.7	4.7	7.6	4.5	4.9	0.001	ns	0.006	0.8
S/R ratio	1.4	1.6	1.1	1.1	1.9	1.3	1.9	1.9	0.001	ns	ns	0.2

1 Newly-formed biomass was calculated as [total kBq]/[specific activity ($kBq \cdot mg^{-1}$ C) of atmospheric CO_2]*[%carbon]/100.

2 LSD values for $P \leq 0.05$. A=Age; P=Pretreatment; T=Treatment; significant interactions between treatments, when observed, are mentioned in the text.

Table 9 New biomass (formed during treatment in ESPAS) as a percentage of total, water use, water use efficiency (WUE)[1], and root/soil respiration during treatment of Douglas-firs with 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for four weeks in an atmosphere with a specific activity of 1.5 (±0.1) $kBq \cdot mg^{-1}$ C and 1.5 (±0.2) $kBq \cdot mg^{-1}$ C, respectively (n=6). See Figure 4 for information on experimental design.

Age	3-year-old				4-year-old				Statistics[1]			
Petreatment	350		700		350		700					
Treatment	350	700	350	700	350	700	350	700	A	P	T	LSD
% newly-formed biomass												
Needles	15.5	18.9	13.8	16.4	14.9	17.2	14.8	14.7	ns	ns	0.06	2.2
Branches	19.5	21.3	10.7	17.3	12.3	11.2	8.8	11.0	0.001	0.001	0.02	1.9
Roots	20.7	22.2	16.9	22.3	11.7	15.8	9.9	11.4	0.001	0.05	0.01	2.4
water use												
Water use (ml)	1750	1710	1740	1720	2340	2180	2150	1870	0.001	ns	ns	220
Water use (g^{-1} needle)	211	168	154	151	112	91	93	85	0.001	0.02	0.09	21
WUE	4.0	5.2	4.3	5.4	5.5	7.9	5.7	7.2	0.001	ns	0.001	0.8
root/soil respiration												
$^{14}CO_2$ (kBq)	850	1130	860	1490	2190	2310	1830	2110	0.001	ns	0.05	330
SA ($kBq.mg^{-1}$ C)	0.74	0.75	0.75	0.83	0.77	0.87	0.77	0.86	ns	ns	0.03	0.07

1 WUE expressed as amount of newly formed biomass per liter water ($g \cdot l^{-1}$)

2 LSD values for $P \leq 0.05$. A=Age; P=Pretreatment; T=Treatment; significant interactions between treatments, when observed, are mentioned in the text.

Table 10 Total net uptake (kBq), uptake per unit needle mass, newly-formed biomass, water use, water use efficiency (WUE)[1], and root/soil respiration (as percentage of the control *viz.* pretreatment 350 $\mu l \cdot l^{-1}$ CO_2 - treatment 350 $\mu l \cdot l^{-1}$ CO_2) in the different treatment combinations (n=12). See Figure 4 for information on experimental design.

	Treatment combination (pretreatment-treatment)			
	350 - 350	350 - 700	700 - 350	700 - 700
		carbon dioxide uptake		
Total net uptake	0	24	-7	12
$kBq \cdot g^{-1}$ needle	0	8	-21	-1
		% newly-formed biomass		
Needles	0	38	13	17
Branches	0	6	-24	-4
Roots	0	45	11	24
		water use		
Water use (ml)	0	-5	-3	-12
Water use (g^{-1} needle)	0	-20	-21	-27
WUE	0	36	2	31
		root/soil respiration		
$^{14}CO_2$ (kBq)	0	13	-13	18
SA ($kBq \cdot mg^{-1}$ C)	0	7	-1	12

[1] WUE expressed as amount of newly-formed biomass per liter water ($g \cdot l^{-1}$)

were less efficient at any CO_2 concentration after the long-term treatment with 700 $\mu l \cdot l^{-1}$ CO_2. This resulted in a reduced relative growth rate. Our results clearly show that an initial growth stimulation can eventually be annulled by physiological or morphological adaptations. Cure & Acock (1986) argued that a strong sink strength is required for maintaining high photosynthetic activity at elevated CO_2 and Thomas & Strain (1991) showed that a reduced photosynthetic capacity can be associated with a small rooting volume. Arp (1991) analyzed 14 observations and showed that a small rooting volume (pot size < 3.5 l) was probably responsible for photosynthetic acclimation under high CO_2. Hence, the trees exposed to the long-term treatment at 700 $\mu l \cdot l^{-1}$ CO_2 possibly exhibited this acclimation response during the short-term treatment in the ESPAS growth chambers due to their more extended root systems. The relationship between total net uptake of $^{14}CO_2$ and dry root weight is shown in Figure 5. Regression analysis revealed a strong correlation between the variables ($P<0.001$). This correlation indicates that acclimation due to a limited pot size did not occur in this study. Although sink activity apparently plays a role in acclimation, the effect of CO_2 on photosynthetic capacity is still uncertain (Rogers *et al.*, 1994). Bazzaz (1990) suggested that a reduced specific leaf area (SLA), possibly associated with starch accumulation, could be responsible for the regularly observed disappearance of growth stimulation with time. Tolley & Strain (1984) observed such a reduced SLA in *Pinus taeda* seedlings after three months exposure to elevated CO_2 levels. Norby *et al.* (1992) also reported a reduced SLA in *Liriodendron tulipifera* after three growing seasons with exposure to ambient and elevated CO_2. A decreased SLA could explain the reduced net uptake per unit needle mass found in this study.

Even though the shoot/root ratio of the old biomass was rather insensitive to the long-term treatment, younger trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2 decreased the shoot/root ratio of new

biomass during the short-term treatment, whereas the older trees showed an overall increase. Other studies have reported both no effects on shoot/root ratio (Campagna & Margolis (1989) and Tinus (1972), and increased shoot/root ratios (Tolley & Strain, 1984; Norby *et al.*, 1986). Tolley & Strain (1984) found that the effect of elevated CO_2 on shoot/root ratio differed for two species and depended on irradiance levels, and our results suggest that the effect might depend on age as well.

The data of the root/soil respiration already showed in an early stage that the use of current photosynthates increased in the high CO_2 treatment. The increased respiration of $^{14}CO_2$ could not only be attributed to a stimulated photosynthesis, but also to a shift in the allocation pattern within the tree. The answer to this question could be given after harvest. Total net uptake showed an average increase of about 22 %, which is of the same order as reported for arable crops (Kimball, 1983; Cure & Acock, 1986). The distribution patterns among tree and soil compartments were not clearly affected by the CO_2 levels. Only the distribution within the two age classes were significantly different. The youngest trees retained less carbon in the needles and translocated relatively more carbon to the root system. On average, the shoot/root ratio was not affected. Hence, the observed increased $^{14}CO_2$ root/soil respiration at 700 $\mu l \cdot l^{-1}$ CO_2 must be due to increased net uptake of $^{14}CO_2$ and an unchanged distribution pattern among tree compartments.

Morison (1993) summarized that, especially on short time scales, elevated CO_2 usually increases the WUE. In spite of an increased WUE, Norby *et al.* (1986) observed that total water use by *Quercus alba* seedlings was not affected by elevated CO_2 and concluded that an increased WUE is not necessarily associated with a decreased total water use by a vegetation. Our results confirm this conclusion. We also observed an increased WUE and an unaffected total water use under elevated CO_2. However, we found that long-term exposure to 700 $\mu l \cdot l^{-1}$ CO_2 reduced the net uptake of $^{14}CO_2$, possibly induced by a reduced SLA. If growth stimulation by CO_2 does indeed disappear with time, then an increased WUE will result in less water use by a vegetation under higher CO_2 levels. Bazzaz (1990) argued that an increased WUE is primarily caused by a decreased stomatal conductance, although a decrease in stomatal density has been reported for several species (Woodward & Bazzaz, 1988). Our results support more or less both possibilities. Supposed that stomatal density was strongly reduced during the 700 $\mu l \cdot l^{-1}$ CO_2 long-term treatment, then the water use of these plants would also be expected to be reduced during the short-term treatment. This phenomenon was not observed: plants pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 or 700 $\mu l \cdot l^{-1}$ CO_2 transpired the same amount of water. Water use per unit needle mass was decreased by 18 % by the pretreatment at 700 $\mu l \cdot l^{-1}$ CO_2. However, this effect was almost compensated for by the increased needle mass, resulting in the same amount of total water use. Theoretically, the reduced water use per unit needle mass, and even the net uptake of $^{14}CO_2$ per gram needle, could result from a decreased stomatal density, since the reaction of stomatal conductance to elevated CO_2 will be rather instantaneous when it responds to internal CO_2 concentrations (Mott, 1988). This transient response is likely to disappear after the cause, elevated CO_2, has been removed. So, reduced stomatal conductance during the long-term treatment at 700 $\mu l \cdot l^{-1}$ CO_2 is probably not the primary cause for the reduced water uptake per unit needle mass during the short-term treatment.

The increased WUE during the short-term treatment at 700 $\mu l \cdot l^{-1}$ CO_2, however, can not result from changes in stomatal density, since needle growth did not occur at the time of the treatment. Also the treatment period was too short for establishing differences in stomatal densities. Consequently, the increased WUE at the 700 $\mu l \cdot l^{-1}$ CO_2 short-term treatment was caused by a faster response, probably stomatal conductance. Morison (1993) mentioned our decreasing knowledge on WUE when longer time scales are considered. Our results also underline the need for more detailed comparisons of instantaneous and long-term effects of elevated CO_2 on physiological, anatomical and morphological aspects.

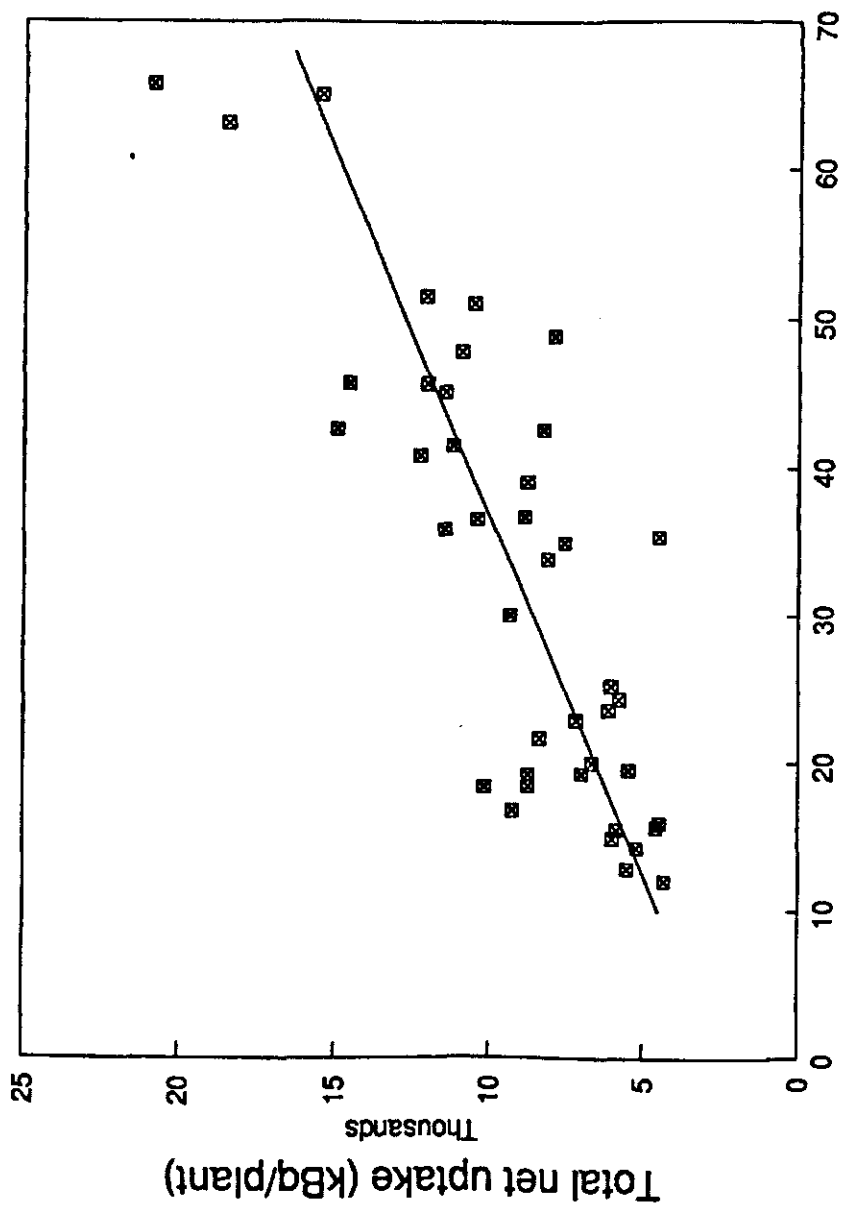


Figure 5 *Relationship between total net uptake of $^{14}CO_2$ and dry root weight*

From the present results it is concluded that the total net uptake of CO_2 by juvenile Douglas-firs is stimulated by elevated CO_2 levels, whereas the distribution pattern may not be affected. This stimulation disappeared with time and even resulted in a decreased uptake. This may have implications for the potential role of forest ecosystems since it lessens the possible sink function for atmospheric CO_2. Total water use was not affected, but water use per unit needle mass decreased. This effect persisted after the cause, elevated CO_2, was removed. Water use efficiency increased with the CO_2 level. However, this effect was transient: it disappeared after the treatment had stopped. The share of current assimilates in the root/soil respiration increased under elevated CO_2.

6. Below-ground carbon dynamics in juvenile Douglas-fir under elevated CO_2[2]

Abstract

In this study the effect of elevated CO_2 on below-ground carbon dynamics in Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) was investigated. Juvenile Douglas-firs were exposed to a long-term treatment at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for 14 months and subsequently crosswise transferred to phytotrons for a short-term treatment with 350 and 700 $\mu l \cdot l^{-1}$ CO_2 for four weeks in an atmosphere continuously labelled with $^{14}CO_2$. The total amount of ^{14}C-carbon allocated to the soil compartment increased by 28 % in the elevated CO_2 treatment, compared with ambient, which is comparable with the increase in total net uptake. The increase was found in all soil compartments: roots, root/soil respiration, microbial biomass and soil residue. Total (^{14}C-labelled + non-labelled) root/soil respiration was not significantly affected by treatment or pretreatment. The short-term CO_2 treatment increased $^{14}CO_2$ in the root/soil respiration at 700 $\mu l \cdot l^{-1}$ CO_2 by 23 % compared with 350 $\mu l \cdot l^{-1}$. This was accompanied by an increased specific activity of the root/soil respiration, which indicates that current (labelled) assimilates were preferentially used by the root system or the microbial biomass in the highest CO_2 treatment. The size of the soil microbial biomass was increased by 59 % at elevated CO_2. The calculated share of the microbial biomass in the total root/soil respiration increased in the 3-year-old trees at elevated CO_2, but decreased in the 4-year-old roots. The long-term pretreatment caused an increase of total amount of ^{14}C-carbon in the roots of the 3-year-old trees, but a decrease in the 4-year-old trees, possibly affected by a reduced sink strength of the older root system.
The results show that an increased stimulation of net CO_2 uptake at elevated CO_2, also affects carbon flows in the soil. However, interactions with the age of the trees and adaptation of the trees to long-term CO_2 treatment highly complicates the interpretation of the results.

[2] A. Gorissen, J.H. van Ginkel & H. van de Beek (in prep)

6.1. Introduction

Elevated CO_2 often stimulates root growth more than growth of other plant organs (Rogers *et al.*, 1994). Norby *et al.* (1992) showed that photosynthesis responded positively throughout the three-year exposure period although plant biomass was not increased. They attributed this observation to an increased fine root production and CO_2-efflux from the soil. This CO_2-efflux may be additionally increased since root respiration per gram dry weight is greater than shoot respiration (Farrar, 1981). A higher production of fine roots, which have a rapid turnover time, will probably stimulate the size and activity of the soil microbial biomass. Changes in root growth and turnover, microbial biomass, and root/soil respiration play an important role in the sequestering capacity of soils with regard to atmospheric carbon and a profound knowledge of the sizes and fluxes of soil carbon pools is important. Below-ground carbon cycles may play a major role in either positive or negative feedback mechanisms to the atmospheric CO_2 concentration, since the amount of carbon stored in soil equals twice the amount present in the atmosphere (Post *et al.*, 1990). Dixon and Turner (1991) summarized the potential changes in terrestrial below-ground carbon dynamics as predicted by several simulation models and observed significant differences between the model outputs varying from positive to negative net carbon fluxes from the atmosphere to the soil. Unfortunately, the effects of increased allocation of carbon to the roots and soil and/or a changed plant quality on soil carbon dynamics were not taken into account. An increased supply of carbohydrates by the root system may induce a priming effect on decomposition of soil organic matter (Jenkinson, 1991), thus exerting a positive feedback on the rising atmospheric CO_2 level. Körner and Arnone (1992) described how soil CO_2 production in an artificial tropical ecosystem was stimulated by elevated CO_2 levels. They attributed their observation to a stimulated breakdown of soil organic matter induced by an increased turnover of fresh root-derived material. On the other hand, Lekkerkerk *et al.* (1990) reported a decreased decomposition of soil organic matter when wheat was grown at 700 $\mu l \cdot l^{-1}$ CO_2. They suggested that extra input of easily decomposable root-derived material from wheat plants at the higher CO_2 level was preferentially used by the soil microbial biomass. In addition, Diaz *et al.* (1993) observed that mineral nitrogen was immobilized at higher CO_2 concentrations and they attributed this to an increased release of carbon by roots at elevated CO_2. They concluded that this feedback mechanism would reduce the plant growth stimulation. Phenomena such as a diminishing stimulation of plant growth, changes in the quality of roots and root-derived material, and changes in soil organic carbon dynamics are of utmost importance and needs full attention with respect to simulation models describing the future role of terrestrial ecosystems in feedback mechanisms towards the atmospheric CO_2 concentration (Rogers *et al.*, 1994).

The objectives of this study were:

i) to determine total carbon allocation to the soil compartment, including roots, root/soil respiration, microbial biomass and soil residues, in Douglas-firs exposed to elevated CO_2 for a long period (14 months), and

ii) to study carbon dynamics in the soil compartment. ^{14}C-carbon was used as a sensitive tracer for carbon flows in the system. The effects of elevated CO_2 on tree physiological aspects are described in Chapter 5.

6.2. Materials and methods

For a description of the experimental design and details on the measuring methods we refer to Chapter 5. Total soil nitrogen was determined by digestion (Van Ginkel and Sinnaeve, 1980). The percentage of the root/soil respiration that could be accounted to the roots was calculated from the specific activities as follows. Suppose that after three weeks in the growth chamber the root respiration has a specific activity equal to that of the atmosphere (SA_a), that is the highest value the root respiration can theoretically reach. Due to the fast turnover times of the root carbohydrate pool (see Farrar, 1981), the unlabelled carbohydrate pool will be completely replaced by current assimilates. The specific activity of the respiration of the microbial biomass is assumed to equal the specific activity of the microbial biomass (SA_mb) itself owing to the rapid turnover. Than the *minimal* share of the root respiration (x_rr) and the *maximum* share of microbial respiration (x_mb) in the total respiration during the fourth week can be calculated from the following formula with SA_rs as the specific activity of the root/soil respiration in the fourth week:

$$[x\_rr * SA\_a + x\_mb * SA\_mb] = SA\_rs \quad (1)$$

x_mb can be substituted since x_rr and x_mb add up to 1:

$$[x\_rr * SA\_a + (1-x\_rr) * SA\_mb] = SA\_rs \quad (2)$$

From (2) we can derive the minimal share of the roots:

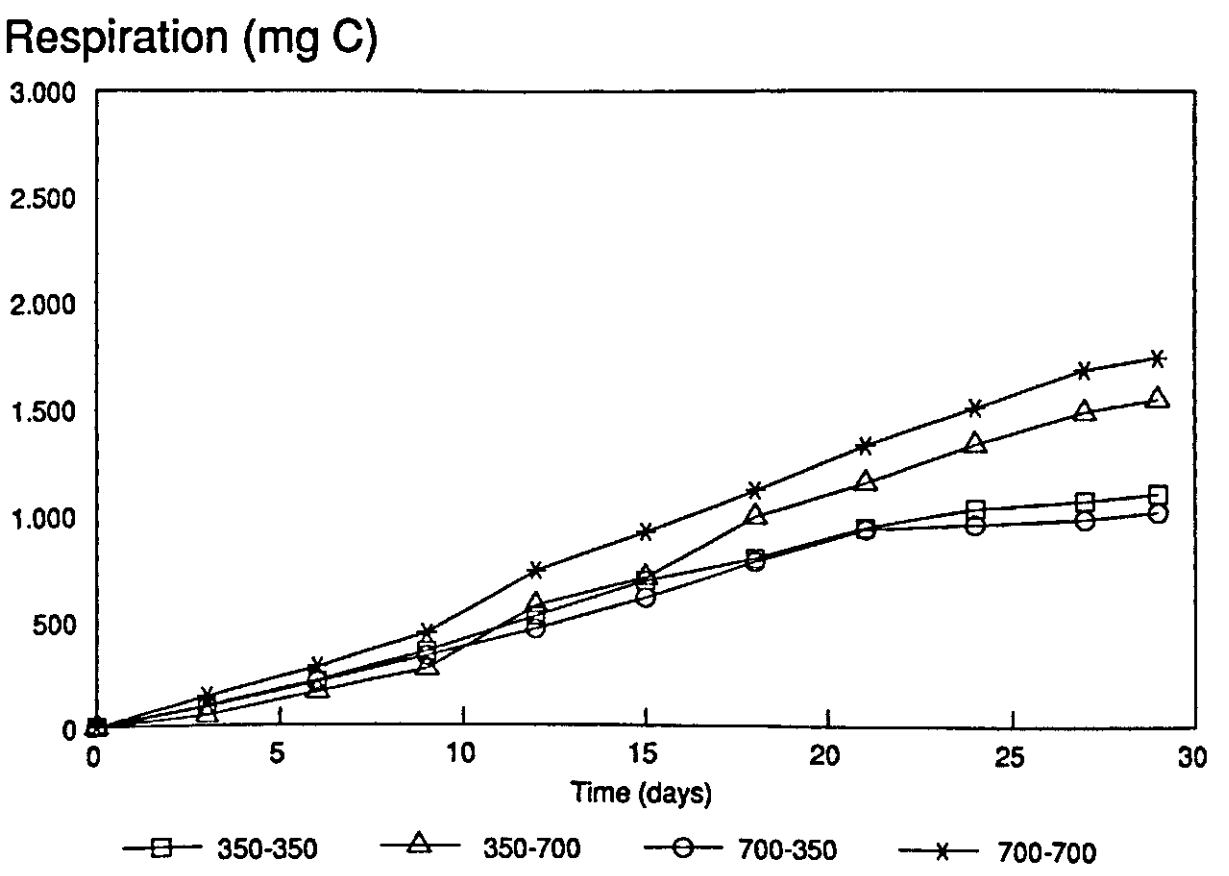
$$x\_rr\_min = [SA\_rs - SA\text{-}mb] / [SA\_a - SA\_mb] \quad (3)$$

If the assumption that the specific activity of the root respiration equals the specific activity of the atmosphere is not valid and thus lower, the share of the root respiration would increase.

6.3. Results

The total respiration (^{14}C-labelled + non-labelled) in the root/soil compartment (Figs 6a & 6b) was only affected by the age of the trees. At the end of the experiment the 4-year-old trees trees had respired 84 % more than the 3-year-old trees ($P < 0.001$). The total respiration of current assimilates, labelled with ^{14}C (Figs 7a & 7b), was affected both by age and treatment: the 4-year-old trees respired 95 % more ^{14}C-carbon than the 3-year-old trees ($P < 0.001$) and the 700 treatment caused an increase of 23 % compared with the 350 treatment ($P = 0.05$). The resulting specific activity of the root/soil respiration (Figs 8a and 8b), expressed as $kBq \cdot mg^{-1}$ C, was only affected by the treatment. The 700 treatment had a 10 % higher specific activity than the 350 treatment ($P = 0.03$). The pretreatment did not significantly affect any of these measurements.

Table 11 shows that the total net uptake, expressed as total ^{14}C recovered in the plant/soil system, was increased in the 700 treatment by 22 % compared with the 350 treatment ($P = 0.02$). The cumulated amount of current assimilates allocated to the root/soil compartment increased by 28 % in the 700 treatment ($P < 0.01$). This increased transport of current assimilates to the root and soil resulted in an increase of all compartments: the amounts of ^{14}C



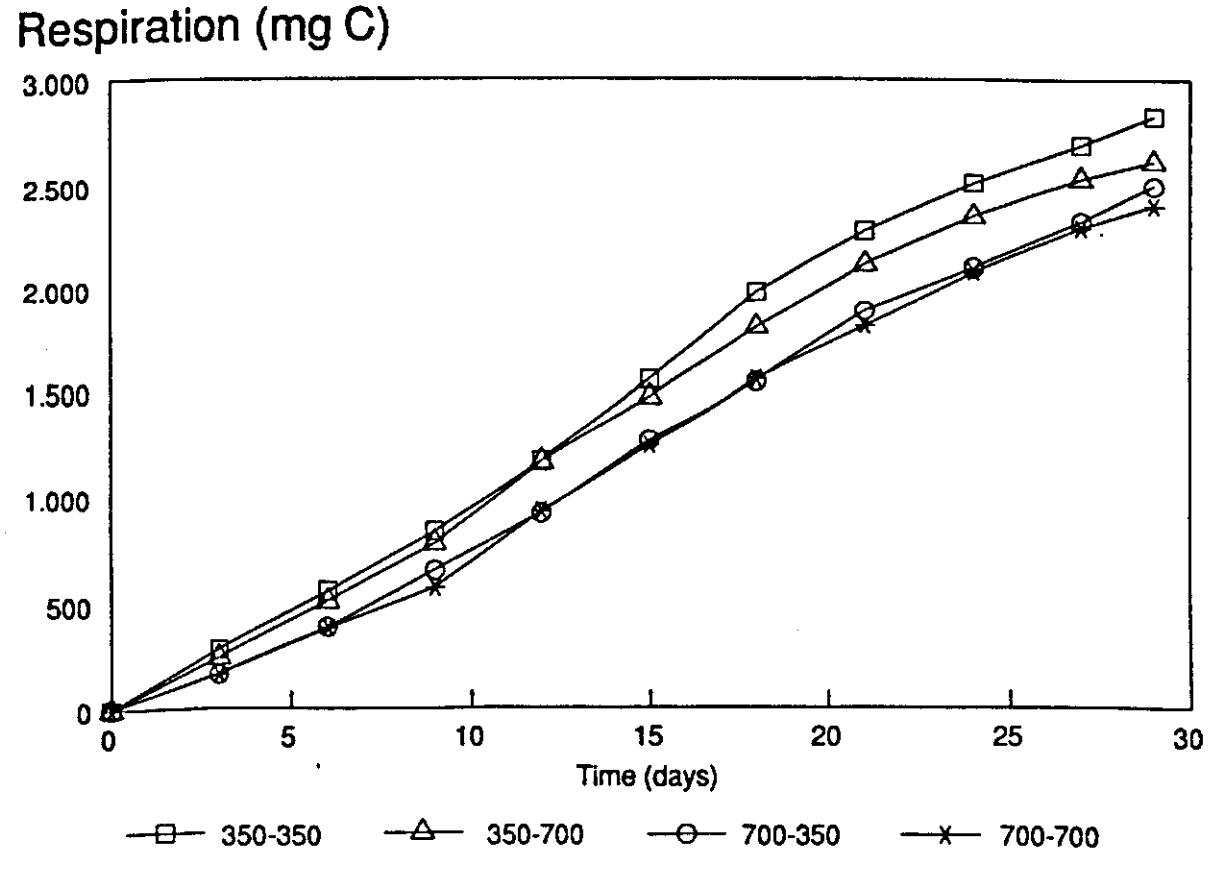
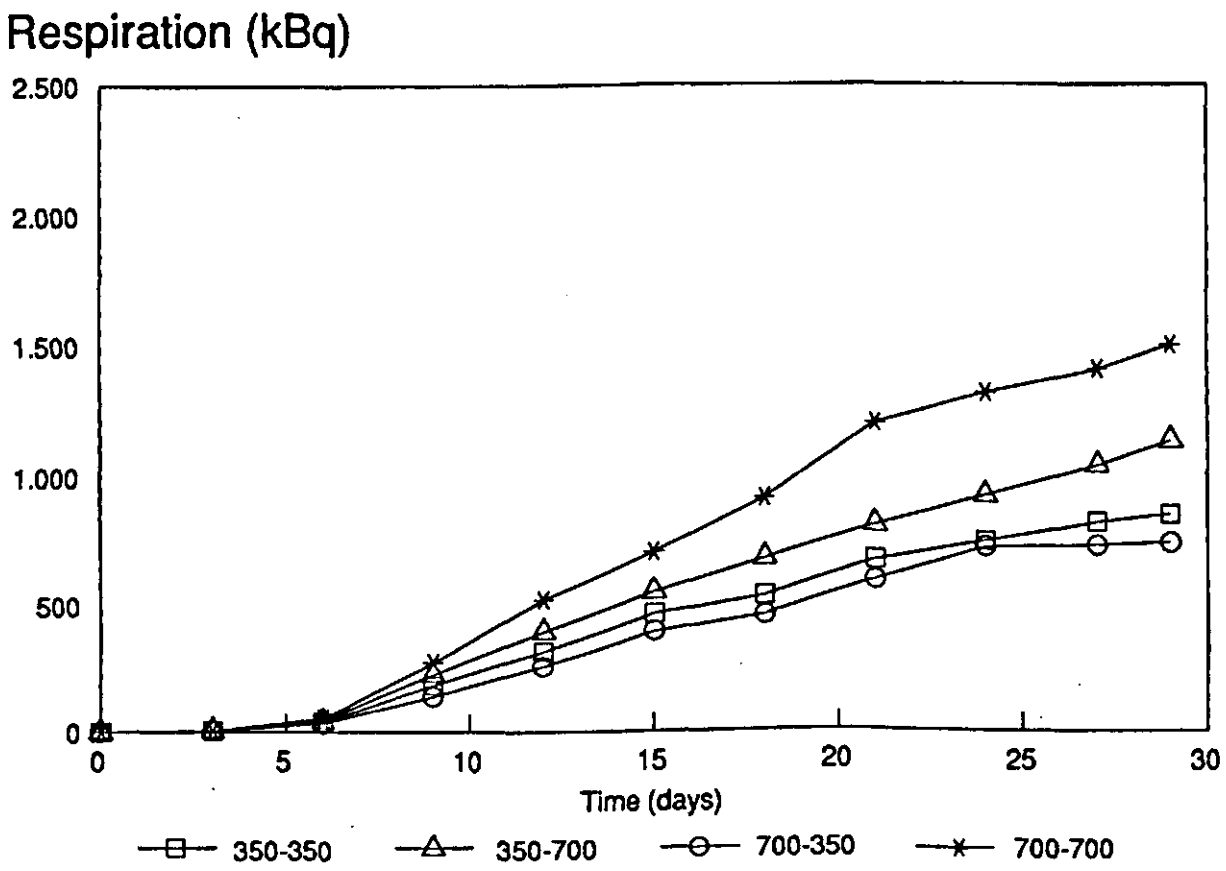


Figure 6 Total root/soil respiration of 3-year-old (above) and 4-year-old (below) Douglas-firs during the short-term CO_2 treatment



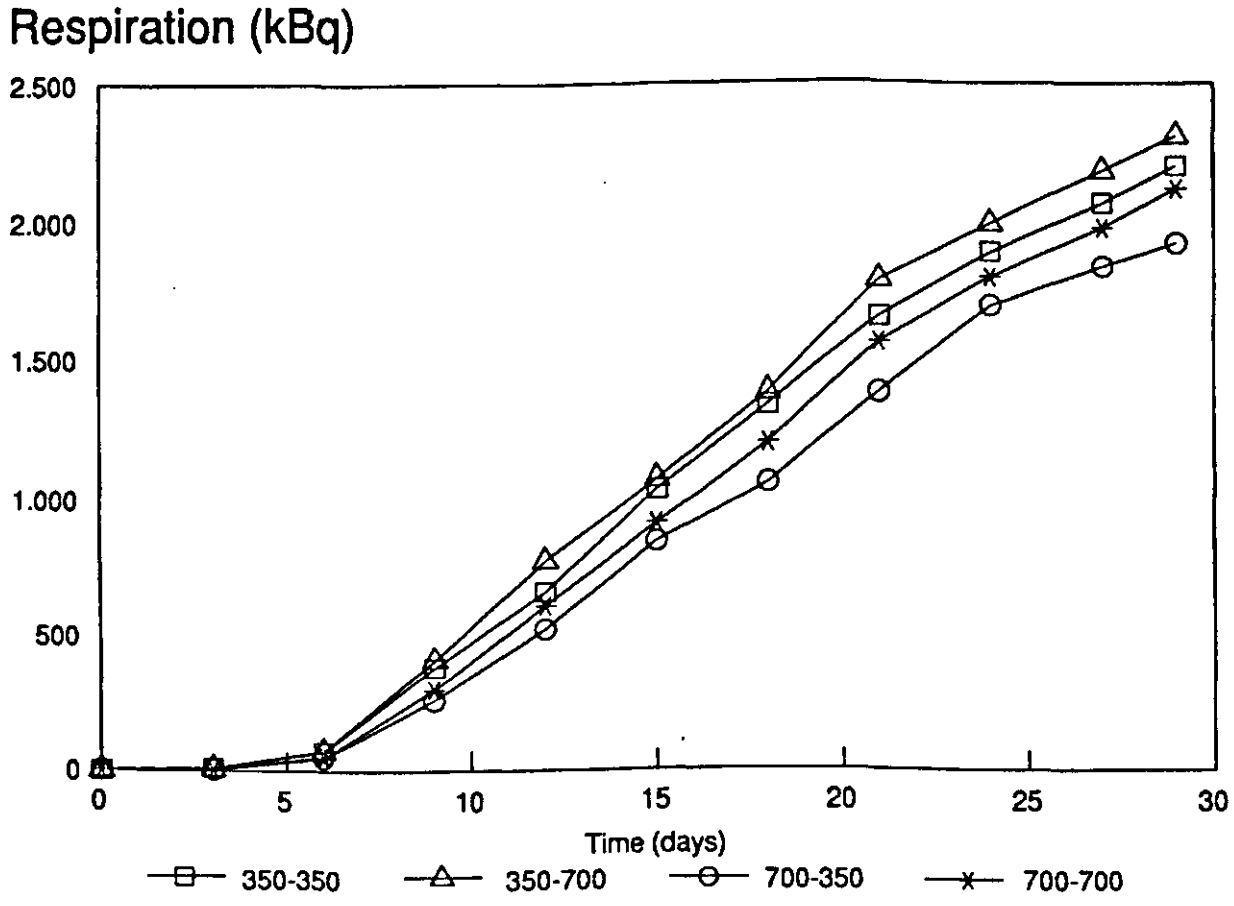
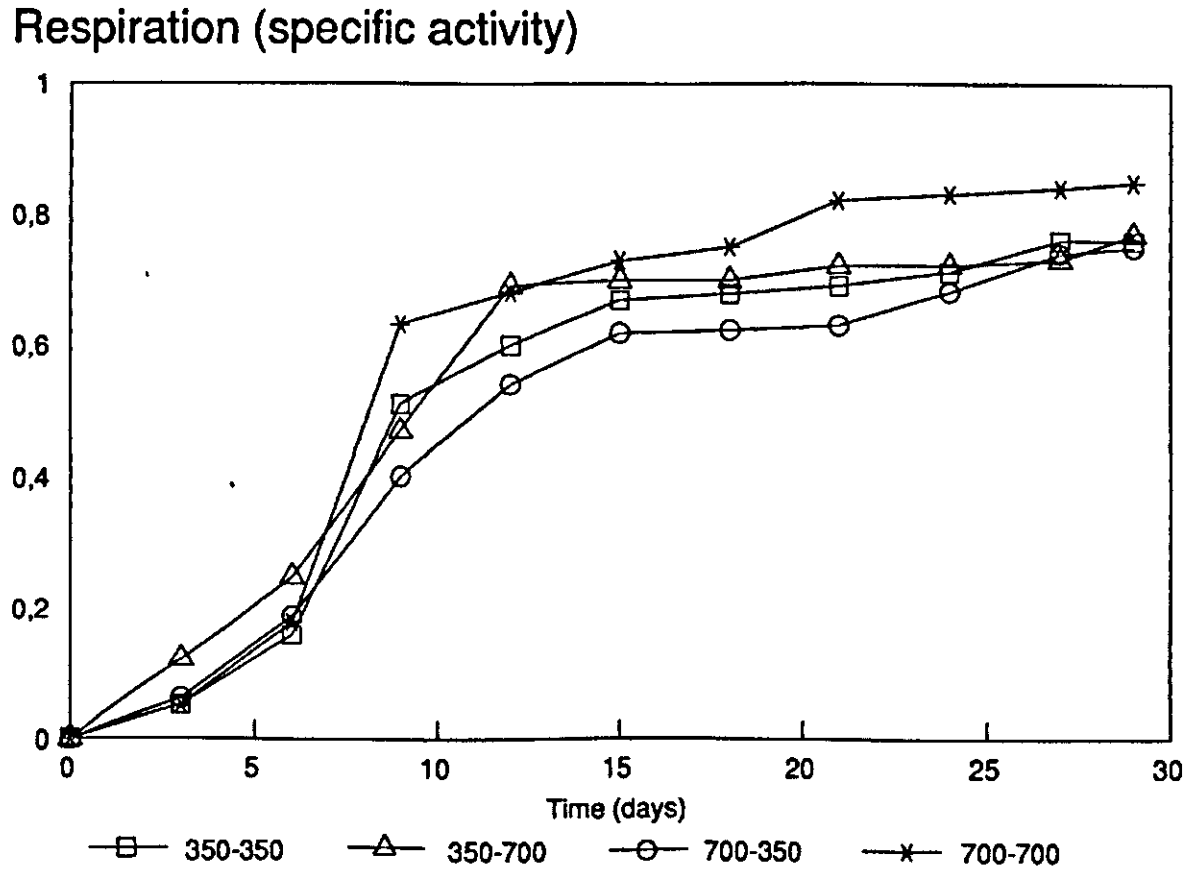


Figure 7 Total ^{14}C-root/soil respiration of 3-year-old (above) and 4-year-old (below) Douglas-firs during the short-term CO_2 treatment



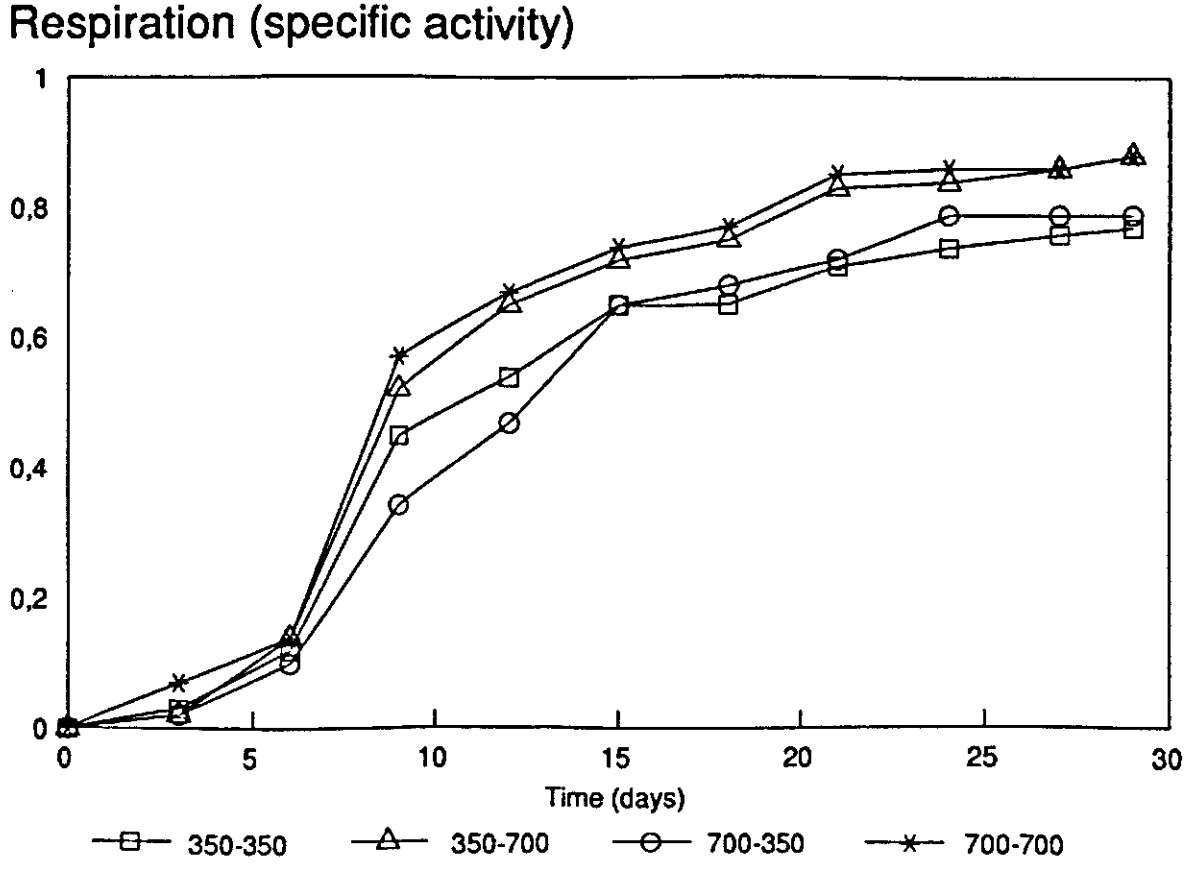


Figure 8 Specific activity (kBq·mg^{-1} C) of the root/soil respiration of 3-year-old (above) and 4-year-old (below) Douglas-firs during the short-term CO_2 treatment

Table 11 Dry weight of roots, total net uptake (kBq) and distribution of current assimilates (kBq) among different soil compartments after treatment of Douglas-fir with 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 for four weeks in an atmosphere with a specific activity of 1.5 (±0.1) $kBq \cdot mg^{-1}$ C and 1.5 (±0.2) $kBq \cdot mg^{-1}$ C, respectively (n=6).

Age	3-year-old				4-year-old				Statistics[1]		
Pretreatment	350		700		350		700				
Treatment	350	700	350	700	350	700	350	700	A	P	T
uptake and distribution											
Total uptake	5490	7010	5410	7420	11220	13740	9650	11220	0.001	ns	0.02
Roots	1903	2163	2135	2834	3064	4780	2721	2940	0.001	ns	0.007
Microbial biomass	46	88	60	87	111	118	116	161	0.001	ns	0.009
Root/soil respiration	845	1128	863	1493	2194	2305	1826	2110	0.001	ns	0.05
Soil residue	79	33	46	114	146	186	138	343	0.001	0.08	0.02
Total soil	2874	3412	3103	4528	5514	7389	4800	5554	0.001	ns	0.004

[1] A=Age; P=Pretreatment; T=Treatment; significant interactions between treatments, when observed, are mentioned in the text

Table 12 Specific activity (kBq·mg^{-1} C), total microbial biomass (µg g^{-1} soil), and contribution of roots and microbial biomass to the root/soil respiration after treatment of Douglas-fir with 350 µl·l^{-1} CO_2 and 700 µl·l^{-1} CO_2 for four weeks in an atmosphere with a specific activity of 1.5 (±0.1) kBq·mg^{-1} C and 1.5 (±0.2) kBq·mg^{-1} C, respectively (n=6).

Age	3-year-old				4-year-old				Statistics[1]		
Pretreatment	350		700		350		700				
Treatment	350	700	350	700	350	700	350	700	A	P	T
specific activity											
Roots	0.35	0.35	0.27	0.35	0.20	0.26	0.16	0.18	0.001	0.03	0.05
Microbial biomass	0.11	0.11	0.14	0.12	0.20	0.15	0.23	0.22	0.001	0.04	ns
Root/soil respiration	0.76	0.77	0.76	0.85	0.77	0.88	0.79	0.88	ns	ns	0.03
microbial biomass											
Microbial biomass	112	194	108	187	140	207	138	203	ns	ns	0.001
%contribution to root/soil respiration											
Maximal % microbial biomass	29	43	29	33	35	24	41	30	ns	ns	ns
Minimal % roots	71	57	71	67	65	76	59	70	ns	ns	ns
Ratio mb/r	0.41	0.75	0.41	0.49	0.54	0.32	0.69	0.43	ns	ns	ns
%N	0.64	0.70	0.49	0.55	0.38	0.42	0.45	0.44	0.001	0.02	ns

[1] A=Age; P=Pretreatment; T=Treatment; significant interactions between treatments, when observed, are mentioned in the text.

recovered in the roots, microbial biomass, root/soil respiration and soil residue increased by 29 % ($P < 0.01$), 37 % ($P < 0.01$), 23 % ($P = 0.05$) and 66 % ($P = 0.02$), respectively. The amount of current assimilates in the root system showed a significant interaction between age and pretreatment ($P < 0.01$); the 3-year-old trees of the 700 pretreatment showed a 22 % increase compared with the 350 pretreatment, whereas the 4-year-old trees showed a 28 % decrease. The overall total microbial biomass increased in the 700 treatment by 59 % ($P < 0.001$).
The specific activity of the root system was decreased by the pretreatment and increased by the treatment by 17 % and 17 %, respectively (Table 12). The specific activity of the microbial biomass was increased by the pretreatment by 25 % ($P = 0.04$). The maximum relative contribution of the microbial biomass to the root/soil respiration was affected by an interaction between age and the short-term CO_2 treatment ($P=0.03$) (Table 12). In the 3-year-old trees the minimal share of the roots was decreased from 71 % to 62 % by elevated CO_2, whereas in the 4-year-old trees it increased from 62 % to 73 %.
The N-concentration in the roots was affected by an interactive effect between age and pretreatment ($P<0.001$). The N-content was lowest in the older trees and for both pretreatments, whereas the younger trees had higher N-concentrations dependent of the pretreatment. The 3-year-old trees pretreated at 350 $\mu l \cdot l^{-1}$ CO_2 contained higher N-concentrations than the matching trees cultivated at 700 $\mu l \cdot l^{-1}$ CO_2.

6.4. Discussion

The additional amount of carbon allocated to the soil in the 700 CO_2 treatment compared with ambient CO_2 was evenly distributed among the different compartments in soil compared with the control treatment. Both age classes showed an increased specific activity of the respiration at the high CO_2 treatment. This means that the contribution of current assimilates in the root/soil respiration was relatively higher than of the older carbon pools. An explanation for this observation may be furnished by a preferential use of current assimilates by the root system itself or the soil microbial biomass as formulated by Lekkerkerk *et al.* (1990). Since the specific activity of the root respiration should meanwhile equal the specific activity of current assimilates due to the fast turnover times of the non-structural carbon pools (Farrar, 1981), the higher specific activity in the total root/soil respiration in the 700 CO_2 treatment was probably caused by a preferential use of current assimilates by the soil microbial biomass.
The increased supply of carbon compounds to the root system seems to be preferred above older non-labelled organic material, resulting in an increased specific activity of the root/soil respiration. Norby *et al.* (1986) found increased levels of sugars at in leaves of *Quercus alba* seedlings after exposure to 690 $\mu l \cdot l^{-1}$ CO_2. Supposed that these increased amounts of sugars are evenly distributed among shoot and root, and the increased allocation of total ^{14}C to the roots support this assumption, it is clear that the rhizosphere microflora can, potentially, benefit from the increased supply of substrate. The enhanced root growth and root exudation in soil will stimulate the size and activity of the microbial biomass (Merckx *et al.*, 1986). Our results show that the size of the microbial biomass was increased by the 700 CO_2 treatment, which is in accordance with the results presented by Zak *et al.* (1993). However, the 700 CO_2 pretreatment did not increase the size of the microbial biomass. In Chapter 5 was reported that the old root dry weight of the 4-year-old trees hardly increased at elevated CO_2. Although the total amounts of roots was equal after pretreatment with 350 or 700 $\mu l \cdot l^{-1}$ CO_2, the total amount of current assimilates allocated to the soil decreased by 22 % in the 4-year-old trees. This observation may be related to adaptations such as the decrease in total net CO_2 uptake per unit needle mass after the long-term pretreatment as was described in Chapter 5.

However, this reduction in total net uptake was found in both the 3-year-old and 4-year-old trees. The decrease in total carbon allocation to the soil was observed only in the older trees. In the 3-year-old trees still 33 % more ^{14}C-carbon was allocated to the soil after pretreatment at 700 $\mu l \cdot l^{-1}$ CO_2, compared with 350 $\mu l \cdot l^{-1}$ CO_2. This result may point to a possible reduced sink strength of the roots of the older trees. Arp (1991) showed that a limited pot size probably reduced the sink strength of the root system, resulting in a decreased stimulation of photosynthesis. In our experiment no relation was found between the root system and total net ^{14}C-uptake (Fig. 5), and we concluded that photosynthetic acclimation did not occur in this experiment. However, although this photosynthetic acclimation was not observed, the reduced carbon allocation in the older trees possibly does result from a reduced sink strength of the roots.

The age of the trees did also not affect the size of the microbial biomass despite a 2.5 times greater root system in the 4-year-old trees compared with the 3-year-old trees, the former allocating about 67 % more current assimilates to the soil compartment. Although this higher amount hardly stimulated the size of the microbial biomass, the specific activity was increased by 66 %. So, the share of current assimilates in the microbial biomass in the 4-year-old trees was larger than in the 3-year-old trees despite the bigger root systems. The activity of the microbial biomass was apparently stimulated by a bigger root size (higher specific activity), whereas the size of the microbial biomass was not. In contrast, the short-term CO_2 treatment did not change the specific activity of the microbial biomass, but did increase it's size. To date, the relationship between activity and size of the microbial biomass on the one hand and the size of the root system and supply of substrate on the other hand is still unclear. Possibly, soil nutrients strongly affects this relationship.

The ratio of the root respiration and the microbial respiration has always been a challenging question for soil microbiologists. The calculated minimal share of the roots was on average 67 % in this study, which is in accordance with Johansson (1992), who calculated that root respiration constituted 68 % of rhizosphere respiration. These findings are in contrast to values presented by Helal and Sauerbeck (1991), who found a ratio of about 4:1 for microbial *vs* root respiration. Whether or not the absolute percentages were valid, they were affected by an interaction between age and the short-term CO_2 treatment. In the 3-year-old trees the share of the microbial biomass increased at elevated CO_2, whereas it decreased in the 4-year-old trees. The underlying mechanisms are yet not understood, but the reported change in quality of plant material, in terms of changes in carbon to nitrogen ratio or lignin to nitrogen ratio (Melillo *et al.*, 1982), and subsequent effects on decomposability of root-released material (*e.g.* exudates), and the availability of mineral nitrogen, are probably involved in these findings. Also, a reduced sink strength of the root system as described above, is possibly related to these differences.

Changes in the soil compartment are often difficult to measure and in that sense, the soil compartment still remains a black box. However, for simulation models predicting the future role of terrestrial ecosystems with respect to carbon dynamics in vegetations and soils (Jenkinson *et al.*, 1991), it is essential to include changes in quantitative and qualitative carbon allocation to the roots and soil and changes in the activity and size of the soil microbial biomass, probably in dependance of many factors such as species, soil type, temperature, moisture and nutrient availability.

7. Decomposition of grass roots cultivated at two atmospheric CO_2 levels as influenced by temperature and soil moisture[3]

Abstract

Decomposition of grass roots (*Lolium perenne* L.) cultivated at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 and soil organic matter was followed during two months at two temperatures (14°C and 20°C) and at two soil moisture levels. The increased temperature stimulated decomposition of both roots and soil organic matter by 26 % and 30 %, respectively. A small change in soil moisture content had no effect on decomposition of roots or soil organic matter. After two months the roots cultivated at 700 $\mu l \cdot l^{-1}$ CO_2 showed a 24 % decrease in decomposition compared with roots cultivated at 350 $\mu l \cdot l^{-1}$ CO_2. Decomposition of roots cultivated under 700 $\mu l \cdot l^{-1}$ CO_2 at 20 °C equalled that of roots cultivated under 350 $\mu l \cdot l^{-1}$ CO_2 indicating that changes in decomposition of roots can potentially annul the additional CO_2 release under an increased temperature. Although the observations need validation on a longer term than covered in this study under field conditions, they show that it may be important to implement changes in plant quality into simulation models describing carbon dynamics in a changing climate.

[3] A. Gorissen, J.H. van Ginkel, J.J.B. Keurentjes & J.A. van Veen. 1994. Soil Biology & Biochemistry 27: 117-120.

7.1. Introduction

Elevated atmospheric CO_2 concentrations may lead to an array of changes in the global environment, including an expected increase in plant growth by about 30 % at doubled CO_2 concentrations (Kimball, 1983). The increased CO_2 level not only causes quantitative changes in plant biomass, but also the quality of plant tissue may change in terms of C/N ratio or lignin content (Eamus and Jarvis, 1989; Coûteaux *et al.*, 1991). The enhanced fixation of CO_2 is potentially a relevant process with regard to the global C-cycle since it is estimated that the total amount of carbon present in the atmosphere, *viz* 750 Pg C (Post *et al.*, 1990), passes through the terrestrial biomass within about 6-7 yr (Johnson and Kern, 1991). Whether this results in a substantial influence on the global carbon cycle depends on the fate of the fixed carbon in terrestrial ecosystems. A few studies have investigated decomposition of leaf litter with different qualities cultivated at elevated CO_2 in litter bags (Coûteaux *et al.*, 1991; Cotrufo *et al.*, 1994; Kemp *et al.*, 1994). However, decomposition of roots has so far been neglected. This signifies an important omission, since 10-40 % of assimilated carbon during the growth of plants is transported to the roots and rhizosphere (Van Veen *et al.*, 1989). Exudates, sloughed-off cells and dead roots will be subject to decomposition by soil biological activity. This root-derived material goes through various stages of decay ultimately to become part of soil organic matter. Factors influencing decay of root material and soil organic matter which are related to climate change involve soil temperature, soil moisture content, and the quality of plant residues to be decomposed.

We have tried to elucidate how decomposition of soil organic matter derived from roots is affected by these factors using homogeneously ^{14}C-labelled grass roots as a sensitive tool to distinguish between decomposition of the root material and native soil organic matter.

A higher temperature increased decomposition of plant residues but this increase was strongly counteracted by differences in plant quality caused by elevated CO_2.

7.2. Materials and methods

Rye grass plants (*L. perenne* L. cv 'Barlet') were cultivated under two atmospheric CO_2 concentrations, 350 and 700 $\mu l \cdot l^{-1}$, in ESPAS growth chambers (Merckx *et al.*, 1986). Plants were grown from seed on a nutrient solution (Steiner, 1961) (18°C (day, 16 h); 14°C (night, 8 h); relative humidity 70 %; light intensity 80 $W \cdot m^{-2}$) and continuously labelled with $^{14}CO_2$, supplied from cylinders, in an atmosphere with a constant specific activity. After 4 weeks, the plants were harvested and divided into shoots and roots, dried for 48 h at 80°C, weighed and roots were ground (1 mm). After wet combustion (Dalal, 1979), total carbon was determined by titration and ^{14}C-carbon by liquid scintillation counting (Merckx *et al.*, 1986). Total N was determined by digestion (Van Ginkel and Sinnaeve, 1980). C/N ratios were 18 and 32 for the roots grown at 350 and 700 $\mu l \cdot l^{-1}$ CO_2, respectively. Pots were filled with 30 g loamy sand soil (dry weight; density 1.3 $kg \cdot l^{-1}$) and two soil moisture contents were established (water potentials 9.8 kPa and 61.8 kPa). The soils were amended with 1 mg root material g^{-1} dry soil and were placed at one of two temperatures (14°C and 20°C) in closed 1.5-l containers with a NaOH solution in a small vessel to trap $^{(14)}CO_2$. After 1, 2, 4, 8, 16, 32 and 64 d the solutions were changed and analyzed on total C and ^{14}C (Merckx *et al.*, 1986). Measurements were done in 8-fold at days 1, 2 and 4, in 4-fold at days 8 and 16, and in 2-fold at day 32 and 64. Results were analyzed by ANOVA.

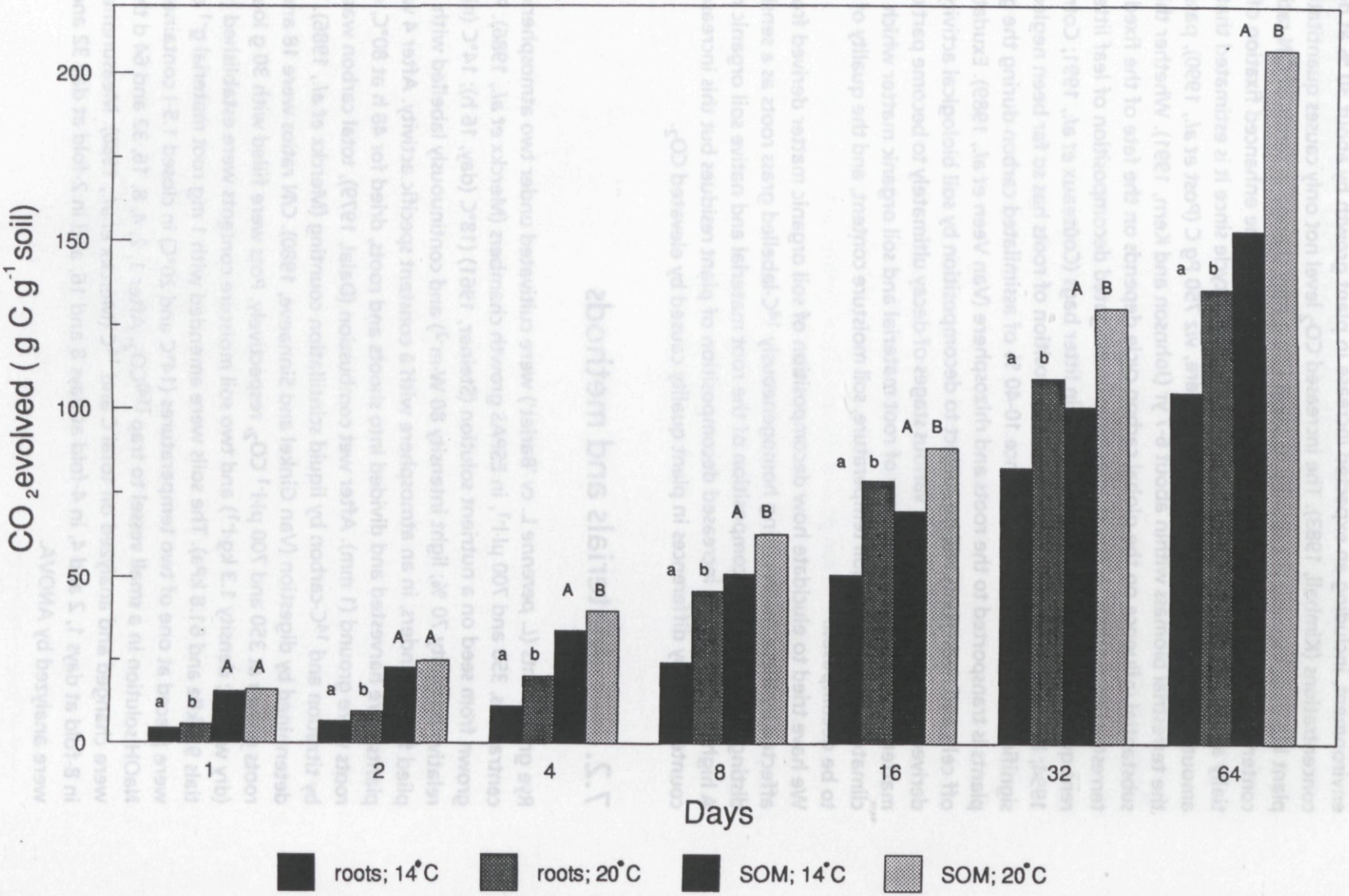


Figure 9 **Effect of temperature on decomposition of grass roots and native soil organic matter. Means with a different letter differ significantly (P<0.005). Note that decomposition of roots is not compared with decomposition of SOM.**

7.3. Results and discussion

Root-derived CO_2 at 20°C consistently exceeded root-derived CO_2 at 14°C ending up in a 26 % increased decomposition of grass roots after 64 d (Fig. 9; $P<0.001$). Total soil organic matter-derived CO_2 showed the same pattern: at 20°C decomposition was increased by 30 % compared with 14°C ($P<0.001$). Global warming will undoubtedly accelerate the decomposition of soil organic matter (SOM) and plant residues and thus promote the increase in atmospheric CO_2 concentrations (Jenkinson *et al.*, 1991). Model estimates by Jenkinson *et al.* (1991) predicted a strong increase in CO_2 release from SOM when global temperature increases. However, these predictions were based on the assumption that global plant production remained constant and they ignored possible changes in the ratios between decomposable and resistant plant residues, which may result in a negative feedback mechanism.
The effect of climate change on soil moisture contents is not well documented and even the estimations of future moisture regimes are not very accurate. Global Circulation Models predict differences in precipitation patterns over the world, but how soil moisture contents will be affected is not clear. An overall increase of rainfall by 5-10 % is predicted, but also reductions in summer rainfall are expected (Bultot *et al.*, 1988; Armstrong and Castle, 1992). It appears from our study that small changes in soil moisture content are of minor importance with regard to decomposition processes. The increase in the water potential from 9.8 kPa to 61.8 kPa did not affect the decay of either roots or SOM (Fig. 10). Whether this also applies to situations with rapidly-changing soil water potentials remains questionable since drying-rewetting regimes certainly stimulate breakdown of SOM (Kieft *et al.*, 1987; Van Gestel *et al.*, 1992).
Decomposition of roots cultivated at 700 $\mu l \cdot l^{-1}$ CO_2 showed an initial increase in decomposability during the first 2 days compared with roots cultivated at 350 $\mu l \cdot l^{-1}$ CO_2 ($P<0.001$) (Fig. 11). Increased amounts of easily-decomposable carbon compounds in the roots, *e.g.* sucrose, under elevated CO_2 (Rowland-Bamford *et al.*, 1990) might be responsible for this observation. The available nitrogen was apparently not limiting during this period. After 8 days decomposition of the roots grown at 700 $\mu l \cdot l^{-1}$ CO_2 was markedly decreased and at the end of the incubation the decrease in decomposition mounted to 24 % compared with the roots cultivated at 350 $\mu l \cdot l^{-1}$ CO_2 ($P<0.001$). This decrease is possibly due to a change in root quality such as a limited nitrogen supply by the root material, caused by an increased C/N ratio from 18 to 32 in the roots grown at 350 and 700 $\mu l \cdot l^{-1}$ CO_2, or by a changed lignin content (Melillo *et al.*, 1982). Figure 12 shows that decomposition of roots cultivated under 700 $\mu l \cdot l^{-1}$ CO_2 at 20°C equaled that of roots cultivated under 350 $\mu l \cdot l^{-1}$ CO_2 and decomposed at 14°C. The changed decomposition rate of root material did not affect the decomposition rate of SOM, which was only stimulated by a higher temperature. The extra N released by decomposition of root material with a low C/N ratio was probably used to further decompose root material rather than SOM. Our results indicate that a decreased decomposability of roots grown under elevated CO_2 concentrations can potentially annul the additional CO_2 release from SOM as estimated by Jenkinson *et al.* (1991), who assumed an increase in temperature of 1.2 to 3.0°C over the next 60 years.
Jenkinson *et al.* (1991) concluded that their model should be coupled to models predicting changes in global plant production, but our results indicate that it may be just as important to implement changes in plant quality into simulation models describing carbon dynamics in a changing climate, since the effects are likely to be substantial in counteracting the stimulated decay of plant debris and soil organic matter under increasing temperature.

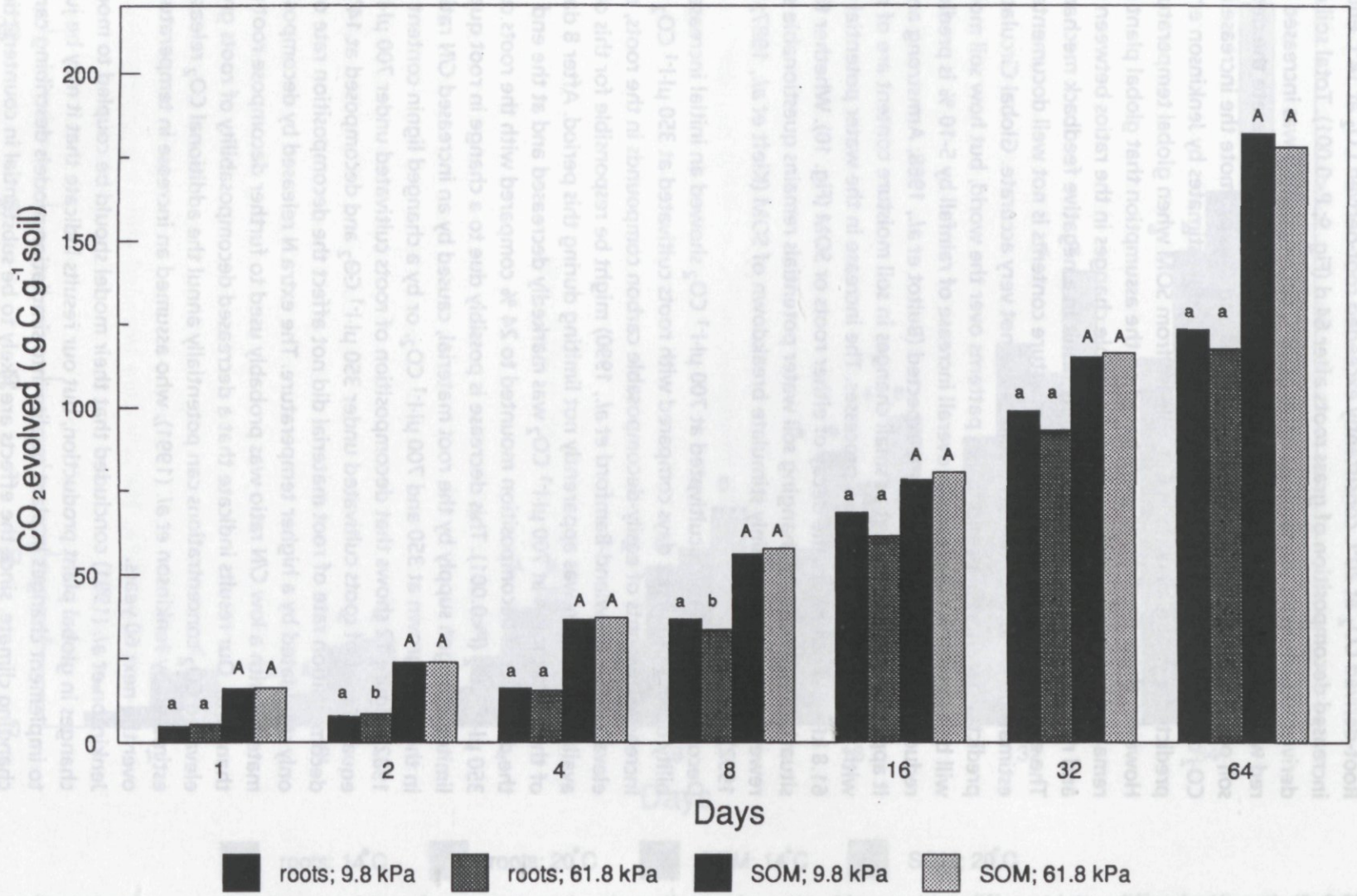


Figure 10 **Effect of soil moisture on decomposition of grass roots and native soil organic matter. Means with a different letter differ significantly (P<0.005). Note that decomposition of roots is not compared with decomposition of SOM**

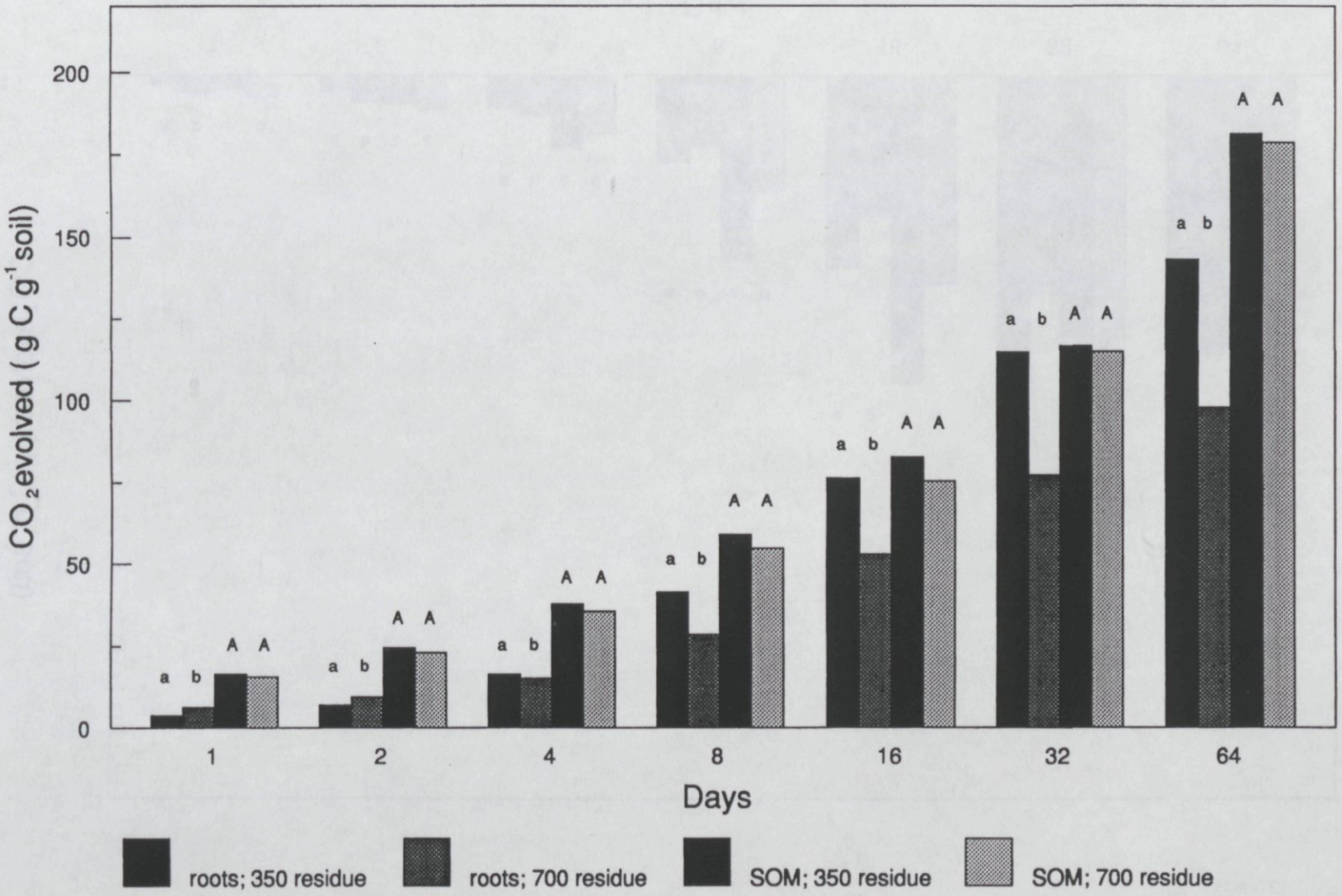


Figure 11 Effect of plant quality on decomposition of grass roots and native soil organic matter. Means with a different letter differ significantly ($P<0.005$). Note that decomposition of roots is not compared with decomposition of SOM

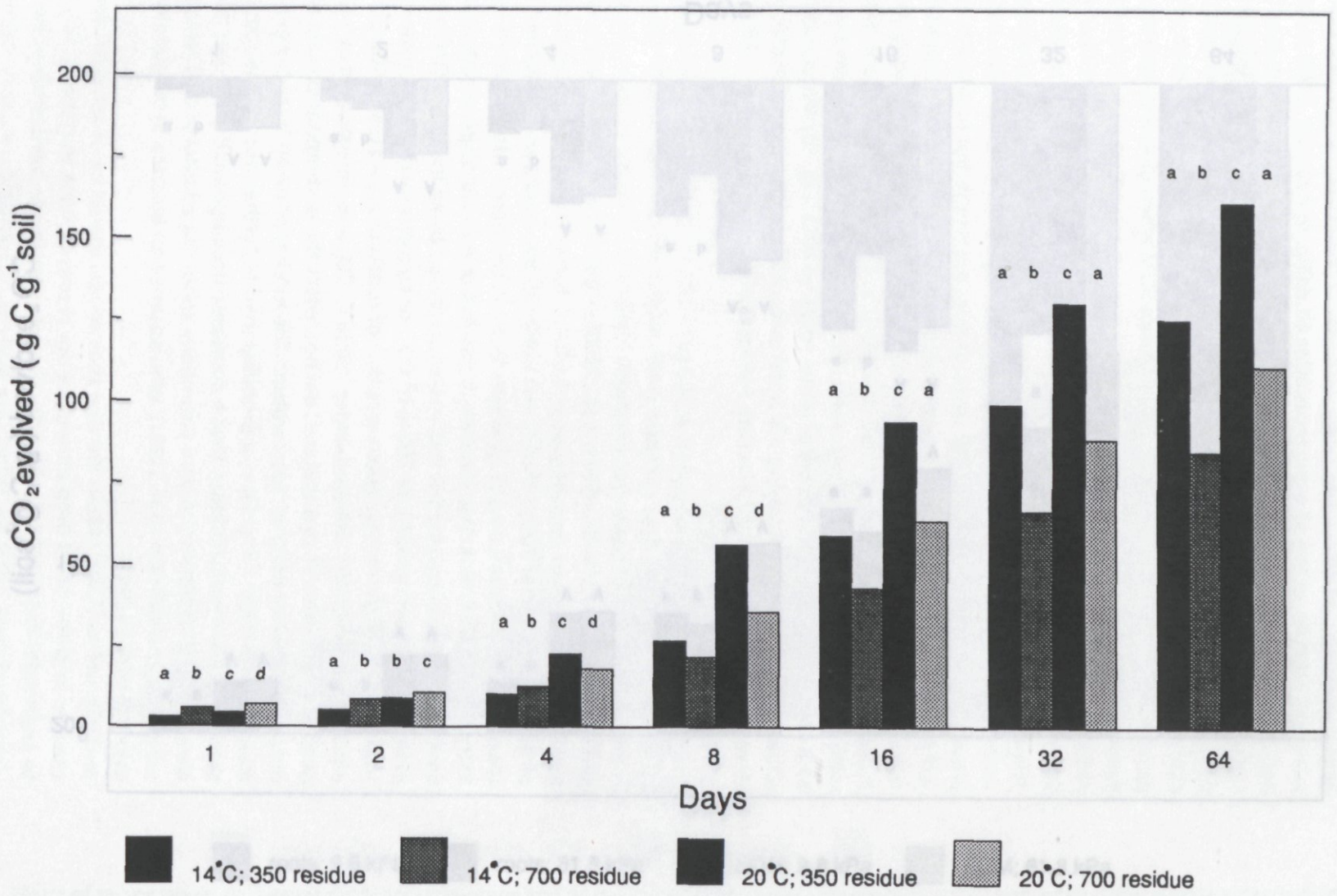


Figure 12 *Overall effect of temperature and quality of root tissue on decomposition of grass roots. Means with a different letter differ significantly (P<0.005)*

8. Fractionation of plant material cultivated at two atmospheric CO_2 levels at different stages during decomposition in soil[4]

Abstract

In order to improve the understanding of decomposition of plant residues and of nutrient cycling in soil under elevated CO_2 there is a need for development of methods to quantify biologically-meaningful fractions of soil organic matter which turn over in the short or medium-term. Homogeneously ^{14}C-labelled shoots from ryegrass grown at ambient (350 $\mu l \cdot l^{-1}$) or elevated (700 $\mu l \cdot l^{-1}$) CO_2 concentrations were added to a loamy sand and incubated for a period up to 200 days. Three size-density methods were used in order to elucidate the decomposition of the plant material. One approach involved density separation in Ludox TM40 but only included soil materials >150 µm. The other two approaches in which sodium polytungstate was used as a density agent included all solid and soluble soil material. One of these involved a size separation (at 100 µm) prior to density separation, while the other was performed on whole soil.

Differences in the decomposition patterns of *Lolium perenne* shoot material grown at ambient and elevated CO_2 concentrations were measured by CO_2 respiration after 10 days and were observed within the large (>150 µm) light Ludox fractions. At the end of the experiment no differences between shoot material grown at ambient and elevated CO_2 concentrations were detected in either the CO_2 evolution or in the different soil organic matter fractions. This indicates the importance of long-term incubation experiments in which the decomposition of rather easily decomposable shoots and more recalcitrant root material are studied.

Density fractionation in a centrifuge (10000*g*) without initial size-separation substantially reduced the recovery of freshly-added plant material in the light fraction. We assume that this was partly due to the loss of air entrapped in intact tissue during centrifugation, and partly due to interactions between small heavy particles and the large light plant material. Fractionation by size and density thus seems a more powerful approach for separating soil organic matter fractions than fractionation based on density alone. Separation of finer textured materials (<100 µm) by density resulted in fractions with similar specific activity, indicating that they did not differ greatly in their turnover rates. The changes with time in the specific activity of the fine fractions indicated that they acted as a sink for microbial products, and only contributed slightly to the mineralization of the freshly added C.

The soluble carbon was consistently the most ^{14}C-enriched fraction and contained a substantial amount of ^{14}C throughout the incubation period. The large, light fractions consisted of identifiable plant residues and were enriched in ^{14}C during the 200 day incubation. Subdivision of the large fraction by density resulted in fractions with considerably different initial enrichment, presumably due to greater airfilled porosity in less decomposed or frayed materials. Losses of 'native' soil carbon were small, compared with the analytical uncertainties, and thus the identification of active 'native' soil fractions was hampered.

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[4] J. Magid, A. Gorissen & K.E. Giller. Soil Biology & Biochemistry (submitted)

8.1. Introduction

For prediction of the impact of elevated CO_2 on soil organic matter dynamics on the long-term simulation models have to be adapted and further developed. Predictive models of organic matter (OM) decomposition in soil invariably identify several soil organic matter (SOM) pools with different rates of turnover (Jenkinson *et al.*, 1987; Parton *et al.*, 1987; Verberne *et al.*, 1990; Hansen *et al.*, 1991). These SOM pools are often defined in an arbitrary conceptual way to enable functioning of the models. Most simulation models of organic matter decomposition in soil operate with an 'active' pool which drives the turnover processes in the short term, for instance over a single growing season. An increased understanding and verification of this concept is important for a better prediction of the consequences of changes in quantity or quality of carbon input into soil. Refinement and improvement of the models necessitates the separation of quantifiable, biologically meaningful fractions with measurable and distinct turnover rates. In a review on physical fractionation of soil and organic matter Christensen (1992) concluded that while density fractionation of soil has shown some promise in this respect, there is a considerable need for further testing of methods in this area.
Earlier work with density fractionation has focused on differences between soils (Turcheneck and Oades, 1979; Sollins *et al.*, 1984; Zhang *et al.*, 1988), or the study of long-term changes within a specific soil, such as transition from virgin to cultivated soil (Tiessen and Stewart, 1983; Dalal and Mayer, 1986). Organic solvents have been commonly used as density agents (Greenland and Ford, 1964; Turcheneck and Oades, 1979; Dalal and Mayer, 1986), but their toxicity and the potential for carbon adsorption to soil materials has justified a search for inorganic density reagents. Of these sodium polytungstate ($Na_6[H_2W_{12}O_{40}]$) has been described as a non-toxic reagent (Elliot *et al.*, 1991). Recently, Ludox TM40 (a colloidal silica suspension) was tested for the separation of live and dead roots from mangrove forests (Robertson and Dixon, 1993), and Meijboom *et al.* (in press) have used Ludox for separation of soil macroorganic matter, concluding that it is easy to use and cheap, and does not alter the characteristics of the organic matter fractions. We decided to use Ludox together with two other approaches with sodium polytungstate to study decomposition of plant materials cultivated at different CO_2 levels.
Melillo *et al.* (1982) showed that the chemical composition or 'quality' of plant residues strongly affected the dynamics of decomposition, and that the lignin to nitrogen ratio was the parameter which best predicted the decomposition rates. One possible effect of an elevated CO_2 concentration in the atmosphere is that changes in plant residue composition occur.
In Chapter 7 it was shown that the rate of decomposition of ryegrass (*L. perenne* L.) roots was reduced possibly due to the higher C/N ratios found when the plants were cultivated at elevated CO_2.

The main objectives of this study were:

i) to compare the decomposition of plant materials which had been grown at an ambient or an elevated CO_2 concentration;
ii) to trace the fate of the carbon in homogeneously ^{14}C-labelled plant materials in soil; and
iii) to elucidate the resolutive potentials of three different methods for fractionation of the soil organic matter based on size and density.

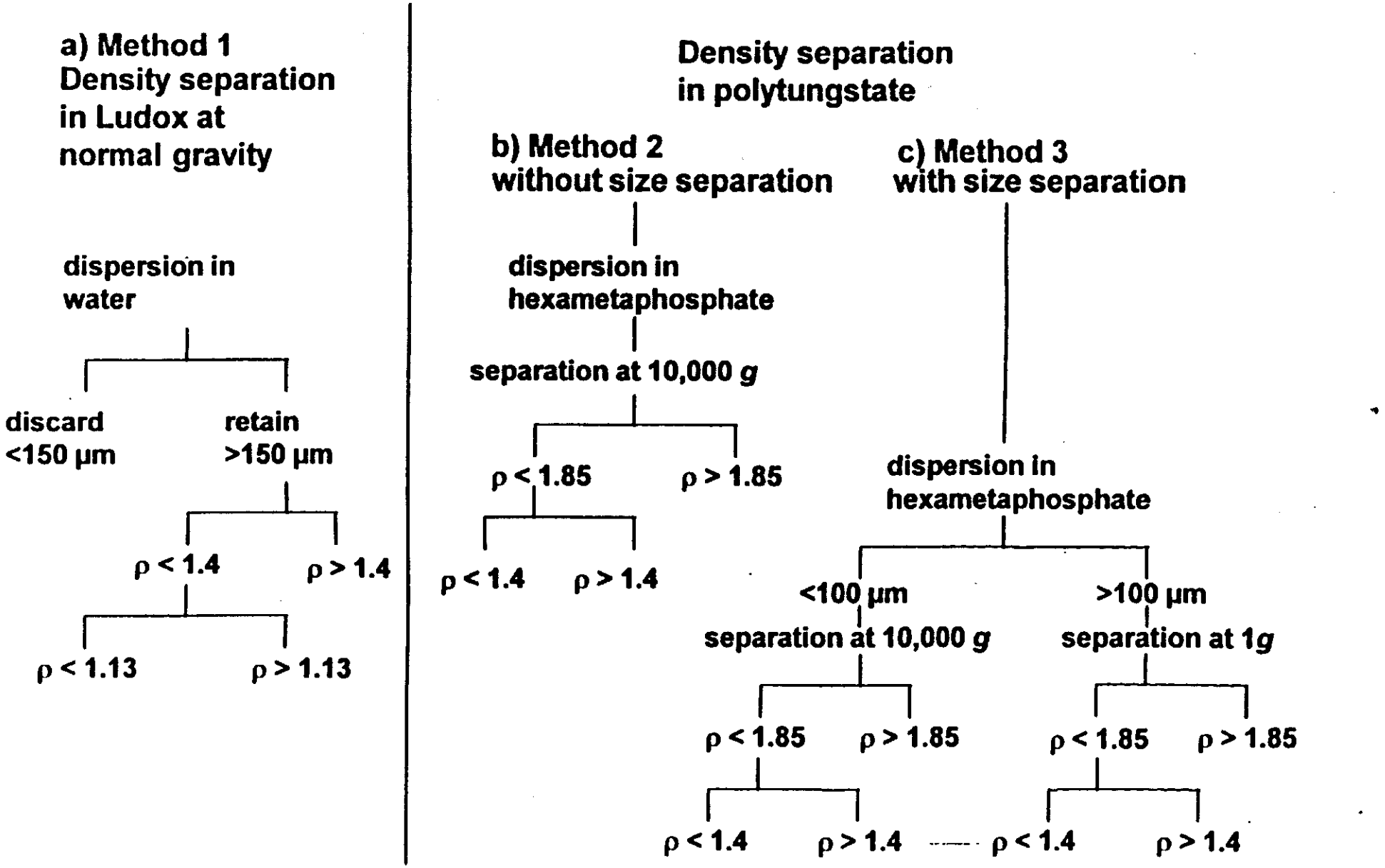


Figure 13 Procedures used for density fractionation of soil in a) Ludox (Method 1) or in b) sodium polytungstate without size separation (Method 2) or c) with size separation (Method 3)

8.2. Materials and methods

Incubation

A loamy sand (Ede soil, 3 % clay, 12.5 % silt, 81 % sand, 3.5 % OM, pH[KCl] 6.4) was amended with homogeneously ^{14}C-labelled shoot material of *L. perenne* L. cv. 'Barlet' (Table 13). The plant materials had been harvested from plants grown from small, 14-day-old seedlings which were exposed at ambient (350 $\mu l \cdot l^{-1}$) and elevated (700 $\mu l \cdot l^{-1}$) CO_2 concentrations for 60 days in a modernized version of the phytotrons described by Merckx *et al.* (1986). The growth chambers allow continuous $^{14}CO_2$-labelling of the atmosphere and growth conditions were light 16h each day, PAR 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature day/night 18/14°C and relative humidity 70 %. Plant material was added to moist (pF 2.8) soil (1.2 mg plant material per g dry soil) and the plant material was thoroughly mixed with the soil. Portions (150 g) of the amended moist soil (equivalent to 130 g dry soil) and unamended control soil were weighed into 150 ml polyethylene beakers. The soils were then compressed to 100 ml to give a bulk density of 1.3 $g \cdot ml^{-1}$ on a dry soil basis. Each beaker of soil was placed in a 1-l jar containing a CO_2 trap (10 ml 1 *M* NaOH) and water was added to the base of the jar to maintain a water-saturated atmosphere. The soils were then incubated in the dark at 14°C and destructively harvested at several sampling dates over a 200-day period. Six jars containing only the CO_2 traps and water at the base were sampled at each harvesting date and served as controls. Soil respiration was monitored by changing NaOH in the incubation jars at 1, 2, 4, 10, 25, 40, 100, 200 days and measuring the trapped CO_2.

Fractionation

Over the 200-day incubation period the soils were fractionated at different times using three size-density approaches. Due to the extensive manipulation involved, fractionation of the soil using three methods at all sampling dates was not feasible. All three approaches were used at day 0 and day 100 for comparative purposes. Three replicates of each treatment (350 $\mu l \cdot l^{-1}$ CO_2 material, 700 $\mu l \cdot l^{-1}$ CO_2 material and unamended soil) were harvested at each sampling date.

Method 1. Fractionation of macro organic matter using Ludox TM40 (Fig. 13a).

This method has been described by Meijboom *et al.* (in press). Sixty g soil subsamples were pushed through a 250 µm sieve, subsequently washed with tap water on a 150 µm sieve, and thus particles smaller than 150 µm were discarded. Particles larger than 150 µm were decanted in water into a fraction containing mainly sand, and a fraction enriched in organic matter. The organic fraction was transferred into a silica suspension (Ludox TM40, ρ 1.39), dispersed with a plastic rod and allowed to settle for 5 minutes. Material from the top 2 cm of the suspension was skimmed with a sieve and transferred to a silica suspension (ρ 1.13). The material remaining in the high density suspension was isolated by sieving, and constituted the heavy Ludox fraction. Similarly the skimmed material was allowed 5 minutes settling in the less dense suspension, and separated into medium (ρ >1.13) and light (ρ <1.13) fractions. Total C and ^{14}C were determined as described below.

Methods 2 and 3. Fractionation of whole soil using sodium polytungstate.

In an attempt to obtain a more complete picture of the ^{14}C distribution in the soil two other approaches retaining *all* particulate and soluble material were tested, using sodium polytungstate ($Na_6[H_2W_{12}O_{40}]$) as a low viscosity density agent. The physical properties of sodium polytungstate as a density agent have been described in detail by Plewinsky and Kamps (1984). To disperse the soil materials we used a dilute sodium hexametaphosphate (SHMP) solution (5‰)

Table 13 Characteristics of the shoot material cultivated at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2

Shoot material	N %	Lignin %	C/N	Lignin/N	Cellulose %	WSC %	SA ($Bq \cdot mg^{-1}C$)
350	2.2	0.6	18	0.3	9.4	33	650
700	0.9	0.7	37	0.8	8.7	41	570

Table 14 Recoveries of total added C (%) in fractions from shoot material grown at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 (standard errors in square brackets) separated using Method 1

Shoot material	Time (days)	Light >150µm ρ <1.13	Medium >150µm ρ 1.13-1.40	Heavy >150µm ρ >1.4	CO_2 respired	Recovery
350	0	30.6 [3.7]	10.1 [1.4]	24.7 [3.8]	0.0 [0.0]	65.4
350	10	4.9 [1.1]	2.2 [0.5]	8.8 [0.2]	35.1 [0.2]	51.0
350	100	0.7 [0.1]	0.9 [0.2]	4.8 [0.7]	53.9 [0.2]	60.3
700	0	26.4 [2.0]	15.9 [1.2]	12.7 [1.4]	0.0 [0.0]	55.0
700	10	7.8 [1.4]	5.9 [0.9]	14.2 [0.2]	27.4 [0.3]	55.3
700	100	0.7 [0.2]	0.7 [0.3]	7.6 [0.0]	53.4 [0.2]	62.4

Table 15 Recoveries of total added C (%) in fractions from shoot material grown at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 (standard errors in square brackets) separated using Method 2

Shoot material	Time (days)	Light ρ <1.40	Medium ρ 1.40-1.85	Heavy ρ >1.85	Dispersion agent soluble	CO_2 respired	Recovery
350	0	5.6 [0.3]	75.8 [7.1]	2.3 [0.5]	12.1 [2.4]	0.0 [0.0]	95.7
350	10	2.9 [0.4]	40.8 [0.6]	9.1 [1.2]	5.1 [0.1]	35.1 [0.2]	93.0
350	40	1.5 [0.1]	29.3 [0.5]	13.1 [0.8]	2.5 [0.1]	46.8 [0.4]	93.1
350	100	2.5 [0.1]	26.9 [1.0]	9.0 [0.3]	2.5 [0.1]	53.9 [0.2]	94.7
700	0	1.9 [0.5]	66.3 [3.2]	2.2 [0.2]	13.0 [0.6]	0.0 [0.0]	83.3
700	10	1.4 [0.1]	45.8 [1.2]	9.2 [2.1]	3.2 [0.1]	27.4 [0.3]	86.9
700	40	1.4 [0.1]	30.1 [1.5]	14.4 [0.9]	1.9 [0.1]	44.7 [0.3]	92.6
700	100	1.9 [0.0]	28.6 [0.9]	7.9 [0.6]	1.6 [0.1]	53.4 [0.2]	92.4

because, apart from facilitating dispersion, it also removes some complexed calcium ions that otherwise would form insoluble calcium polytungstate salts during the density separation.

Method 2. Fractionation without size-separation (Fig. 13b).
Five grams soil subsamples were weighed into 40 ml Nalge PP centrifuge tubes, and 20 ml 5 ‰ sodium hexametaphosphate solution was added, before shaking end over end for 45

minutes. Soil particles were recovered by centrifugation for 1 h at 10000*g*, and the supernatant was analyzed for dissolved organic carbon and ^{14}C. The pellet was redispersed in a 15 ml 53 % sodium polytungstate solution (ρ 1.85), and then separated by density at 10000*g* for 1 h into a distinct dense pellet (ρ >1.85) and less dense floating material. It should be noted that it is necessary to take the remaining dispersing agent into account when establishing the initial density separation conditions of 53 % sodium polytungstate. The supernatant was transferred to a clean centrifuge tube and diluted with water to a density of 1.4, and separated at 10000*g* for 1h. The supernatant was filtered through a Whatman GFF (binder free glass fibre) filter, the filtrate was subsampled and the ^{14}C dissolved in the density agent was determined. The pellets were washed in water and freeze-dried. Total C and ^{14}C on the filters and in the pellets was determined by titration and scintillation counting, after combustion of the materials as described below.

Method 3. Fractionation with size separation (Fig. 13c).
Fifteen gram soil subsamples were weighed into 40 ml Nalge PP centrifuge tubes, and 20 ml 5 ‰ sodium hexametaphosphate solution was added, before shaking end over end for 45 minutes. Dispersed samples were washed with water on a nylon sieve (100 µm), and the washing water was decanted into 250 ml Nalge centrifuge tubes. The washed particles remaining on the sieve (>100 µm) were separated at normal gravity during 5 minutes in sodium polytungstate at a density of 1.85. Subsequently, the floating fraction was further separated at a density of 1.4. Thus, the density separation of large particles (>100 µm) yielded three density fractions: ρ >1.85, ρ 1.85-1.4 and ρ <1.4. The fine fractions were centrifuged at 10000*g* for 1h, and the pellet transferred to 40 ml Nalge PP centrifuge tubes. The subsequent density fractionation and analysis of fine fractions was performed according to the scheme described for Method 2.

Chemical analysis
The total N content of the plant materials was determined using a Carlo-Erba automatic CHN analyzer. The cellulose content was determined as acid-detergent fibre and the lignin as acid-detergent lignin using the method of Goering and van Soest (1970). The bicarbonate and carbonate ions in the NaOH solutions were precipitated with excess $BaCl_2$. Total CO_2 was determined by titrating the remaining NaOH with 0.05 *M* HCl. Total ^{14}C in 0.5 ml NaOH samples was determined by liquid scintillation counting (Tri-Carb 4530; Packard) in 3 ml of Ultima Gold (Packard). A modified combustion procedure (Dalal, 1979; Merckx *et al.*, 1985) was used to determine total C and ^{14}C in the soil. One gram of soil or a sufficient amount of a soil fraction was digested in 5 ml of a solution of 2.0 g $K_2Cr_2O_7$ in 25 ml of concentrated H_2SO_4 and H_3PO_4 (3/2 v/v) at 160°C for 2h. Evolved CO_2 was trapped in 5 ml of 1 *M* NaOH and estimated as described above.

Statistical analysis
The treatment means were compared using a Student's t-test and considered to be significantly different when $P<0.05$.

8.3. Results

CO_2 evolution
CO_2 evolution was initially rapid and with linear respiration rates over the first 10 days with both plant materials (Fig. 14). Between 10 and 100 days the respiration rates slowed progres-

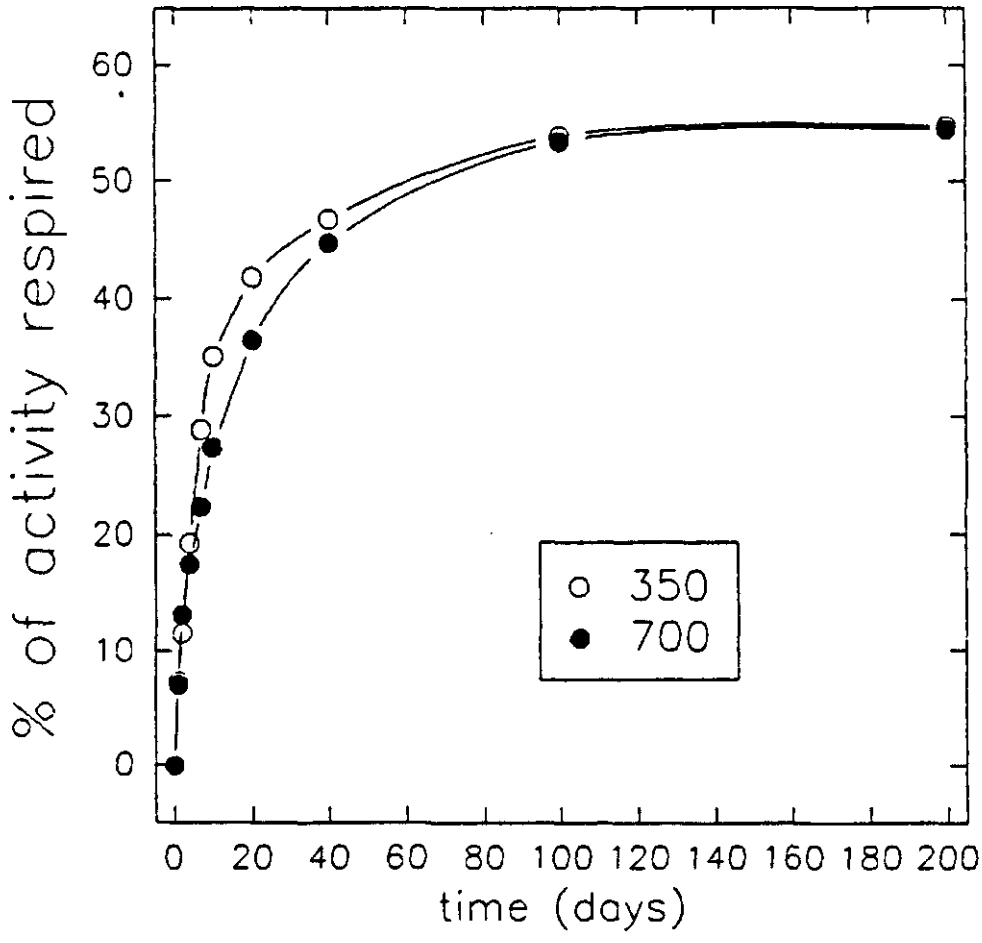


Figure 14 Respiration from soil of added C during incubation of homogeneously ^{14}C-labelled from *L. perenne* grown at ambient (350 $\mu l \cdot l^{-1}$) or elevated (700 $\mu l \cdot l^{-1}$) CO_2 concentrations

sively and there was virtually no respiration from the added plant material after 100 days. There was significantly less evolution of CO_2 from the plant material grown at elevated CO_2 concentrations from the first harvest at 4 days until 40 days, but this difference was minimal from 100 days onwards.

Distribution of added C in different size-density soil organic matter fractions
The distribution of ^{14}C and the specific activities of the fractions were similar with both plant materials.

Method 1. Ludox fractions.
Immediately after addition of the plant residues grown at the ambient CO_2 concentration only 65 % of the C was recovered in the three soil organic matter fractions (Table 14). Substantial amounts of the added C were present in each of the three fractions, with most in the heavy and light fractions. After 10 and 100 days of incubation recoveries of added C were less than 17 %, and 7 %, respectively. For the shoot material grown at 700 $\mu l \cdot l^{-1}$ CO_2 the corresponding figures for recovery of added C were 55 %, 28 % and 9 % respectively. The light and medium fractions were depleted most rapidly with both plant residues, indicating that the material contained in the heavy fraction was either initially more stable, or that lighter fractions became heavier during their decomposition. A larger proportion of the added C was recovered initially in the heavy fraction with the plant material grown at 350 $\mu l \cdot l^{-1}$ CO_2, but this declined very rapidly compared with the corresponding fraction with the plant material grown at 700 $\mu l \cdot l^{-1}$ CO_2.
As all of the soluble material and particles < 150 µm was discarded, 35-50 % of the added C was not accounted for (Table 14). When the total amount of the added C which was recovered in the three Ludox fractions (i.e. all particulate material >150 µm) is considered, differences were observed between the two plant materials (Fig. 15). Initially a larger relative amount of the added C from plants grown at ambient CO_2 concentrations was recovered as large particles compared with the plant material which had been grown at elevated CO_2 concentrations, but it decomposed more rapidly.

Method 2. Tungstate fractions without initial size fractionation
A different picture was observed when the whole soil was included in the fractionation. Without any size fractionation (Method 2) 83 % of the added C from the plant material grown with 350 $\mu l \cdot l^{-1}$ CO_2 was recovered in particulate fractions and roughly 12 % in the dispersing agent at day 0 (Table 15). This indicates that about half of the C unaccounted for in the Ludox fractions at day 0 was lost as fine solid particles, or was adsorbed to fine particles. At day 10 and day 100, 60 % and 45 % respectively, of the C added originally was recovered in particulate fractions, and most of the remainder had been respired as CO_2 (Fig. 14).
The heaviest fraction (ρ >1.85) increased until day 40, but was then smaller at day 100.
Total recoveries of C added in the plant material grown at 700 $\mu l \cdot l^{-1}$ CO_2 were less than with the material grown at 350 $\mu l \cdot l^{-1}$ at days 0 and 10 (Table 3) but the reasons for this are not clear. Most of the added C respired from the soil during the incubation was derived from the intermediate fraction, although the amount of soluble C also clearly diminished with time (Table 15). The amount of the added C recovered in the lightest fraction ρ <1.4 was unexpectedly small compared with both Ludox and tungstate fractions from size separated whole soil (Method 3, Table 16).

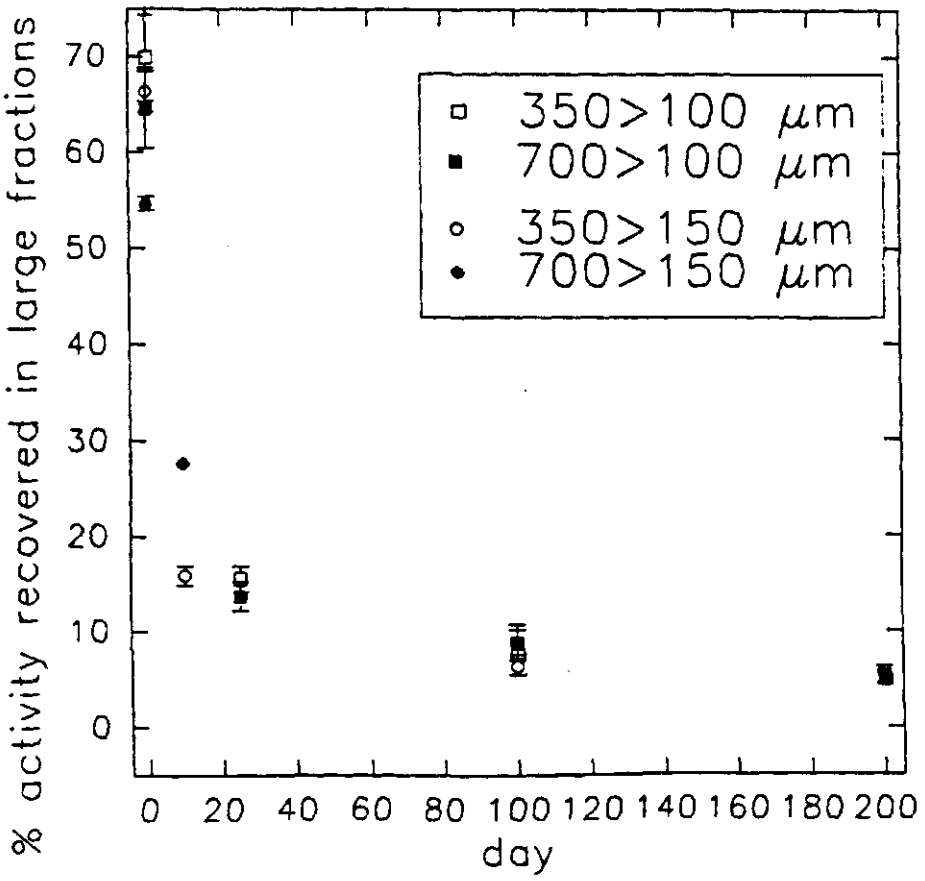


Figure 15 Recovery (%) of C added as homogeneously ^{14}C-labelled *L. perenne* shoot material grown at ambient (350 $\mu l \cdot l^{-1}$) or elevated (700 $\mu l \cdot l^{-1}$) CO_2 concentrations in soil fractions >100 µm (Method 1) or >150 µm (Method 3) over time

Table 16 Recoveries of total added C (%) in fractions from shoot material grown at 350 $\mu l \cdot l^{-1}$ CO_2 and 700 $\mu l \cdot l^{-1}$ CO_2 (standard errors in square brackets) separated using Method 3.

Shoot material	Time (days)	Large light >100µm ρ <1.40	Large medium >100µm ρ 1.40-1.85	Large heavy >100µm ρ >1.85	Fine light <100µm ρ <1.40	Fine medium <100µm ρ 1.40-185	Fine heavy <100µm ρ >1.85	Dispersion agent soluble	Density agent soluble	CO_2 respired	Recovery
350	0	44.7 [4.4]	22.4 [0.8]	2.8 [1.0]	0.6 [0.0]	8.8 [0.6]	2.0 [0.0]	10.4 [2.3]	0.4 [0.02]	0.0 [0.0]	91.9
350	25	7.3 [1.2]	6.3 [0.2]	2.0 [0.3]	1.4 [0.1]	17.3 [0.8]	8.6 [0.9]	4.0 [0.3]	1.7 [0.03]	44.2 [0.2]	92.7
350	100	3.7 [0.4]	1.9 [0.5]	2.0 [0.2]	1.4 [0.1]	22.9 [1.1]	5.7 [0.6]	2.1 [0.2]	0.7 [0.05]	53.5 [0.2]	93.8
350	200	2.1 [0.5]	1.6 [0.3]	1.3 [0.1]	0.9 [0.0]	19.5 [0.6]	9.8 [0.8]	1.7 [0.1]	0.9 [0.00]	54.6 [0.3]	92.4
700	0	32.8 [2.9]	30.3 [4.0]	1.5 [0.2]	0.7 [0.0]	8.5 [0.9]	1.9 [0.4]	13.5 [4.2]	0.3 [0.04]	0.0 [0.0]	89.3
700	25	5.0 [1.1]	7.2 [0.9]	1.6 [0.2]	1.1 [0.2]	15.9 [0.4]	12.4 [0.2]	2.5 [0.2]	1.5 [0.04]	39.1 [0.3]	86.3
700	100	5.6 [1.7]	2.0 [0.3]	1.3 [0.1]	0.8 [0.1]	20.3 [0.3]	5.6 [0.3]	1.4 [0.0]	0.6 [0.02]	53.3 [0.2]	90.9
700	200	1.2 [0.4]	2.7 [0.7]	1.5 [0.1]	0.9 [0.1]	16.5 [0.8]	10.3 [0.7]	1.7 [0.1]	0.9 [0.08]	54.4 [0.3]	90.0

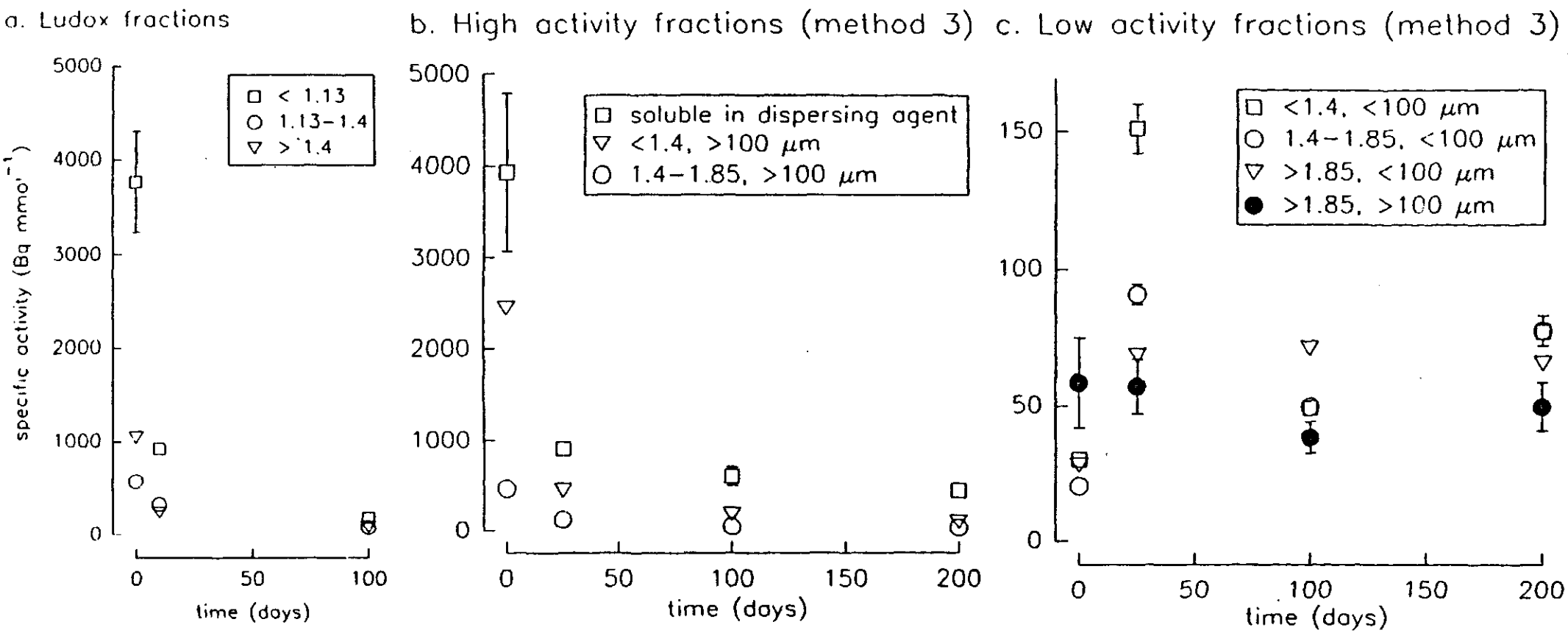


Figure 16 Specific activity of C recovered in size-density fractions obtained by a) density fractionation in Ludox (Method 1) and density fractionation in sodium polytungstate following size fractionation (Method 3); b) fractions with high specific activity and c) fractions with low specific activity

Method 3. Tungstate fractions after initial size separation
Total recoveries of the added carbon were between 90 and 94 %. More than 60 % of the C added in both plant materials was recovered in the large/light and large/medium fractions (that is all material >100 µm, ρ <1.85; Table 16). The soluble added C, the large/light and the large/medium fractions, but not the large/heavy fraction, decomposed rapidly. Hardly any ^{14}C was found in the fine/light (ρ <1.4) material at any time indicating this fraction to be of minor importance. The fine/medium (ρ 1.4-1.85) and fine/heavy (ρ >1.85) fractions increased in ^{14}C content during the course of the experiment.
Considering the large particulate material together (i.e. all material >100 µm), more was recovered initially from the soil amended with the plant material grown under elevated CO_2 as was found with the fraction >150 µm in the Ludox method (Fig. 15). In contrast to the results found with the Ludox method there were minimal differences between the two plant material treatments.

Specific activity of the different size-density soil organic matter fractions

Method 1. Ludox fractions
The specific activity of all Ludox fractions showed a rapid decrease with time (Fig. 16a), indicating that the C added in the plant material was considerably more labile than the 'native' soil C present in the fractions. The specific activity of the light and medium fractions decreased particularly rapidly in time. At 100 days the specific activity of all fractions were comparable.

Method 3. Tungstate fractions after initial size separation
The specific activities of large light and large medium fractions separated in tungstate were distinct until day 200, but the most enriched fraction throughout the incubation was the C dissolved in the dispersing agent (Fig. 16b). All these fractions showed a rapid decay in specific activity, in contrast with all the fine or the large heavy fractions (Fig. 16c). The specific activity in the large heavy fraction was constant, while the fine fractions increased their specific activity from a low level at day 0. The fine light fraction was distinct at day 25, but otherwise there were no considerable or systematic differences in specific activities.

8.4. Discussion

Limitations of the different fractionation methods
Before a detailed consideration of the decomposition of the added shoot material is possible, a discussion of the limitations of the density fractionation methods employed and the differences the between the methods is necessary.
The Ludox method (Method 1; Meijboom *et al.*, in press) and the methods we examined using sodium polytungstate differ in two main ways. First, the large particles of organic matter are separated using a sieve of 150 µm in the method of Meijboom *et al.* (in press), justified by the fact that the sieve became blocked if smaller sieve sizes were used. Second all soluble C and solid material which will pass through a 150 µm mesh is discarded. The inclusion of all fine and soluble material in the methods we employed using sodium polytungstate thus resulted in much greater overall recoveries (83-95 %; Tables 15 and 16) than with the Ludox method (51-65 %; Table 14).
In undecomposed plant tissues a considerable volume of air will be present in intercellular spaces. Furthermore, as the tissues die intracellular spaces, especially in xylem and phloem tissue, will become airfilled. We hypothesize that the airfilled porosity is an important feature

in determining the densities of larger organic particles, and further that the least decomposed tissues will retain the largest amount of air when subjected to density agents, at least under normal gravity.

In Method 2, we used sodium polytungstate to separate fractions of different density of the whole soil without size separation. The amount of the added C recovered in the ρ <1.4 fraction at time 0 was reduced by a factor of 10 compared with the two other methods (Tables 14-16). In the high gravity field created during centrifugation air entrapped in plant material may become liberated, and so the density of the plant material may have increased. Furthermore Golchin *et al.* (1994) noted that the recovery of organic matter particles was improved when density suspensions were allowed to stand for 30 minutes before centrifugation. Indeed Greenland and Ford (1964) found that mechanical occlusion of light fraction particles during centrifugation led to reduced recoveries of soil organic matter in the light fraction. As we were unaware of this effect at the outset no such resting period was specified in our procedure, and the centrifugation was started a few minutes after the end of the dispersion step. The extent to which such occlusion can occur even after 30 minutes of rest should perhaps be examined as this is clearly a drawback in such methods.

For Method 3, we chose a size of 100 µm for separation of large particles because, from calculations using Stoke's Law, this was the smallest size which would settle within a reasonable waiting time at normal gravity. The finer material was then separated using sodium polytungstate as a density agent using centrifugation at 10000*g*.

We suspect that the density separation of fine fractions may be especially difficult to perform reproducibly, and that the variation of the fine medium and fine heavy fractions (Table 16) may have been influenced by small differences in actual density of the sodium polytungstate solution. Plewinsky and Kamps (1984) demonstrated the formation of linear density gradients during high speed centrifugation of sodium polytungstate solutions. Richter *et al.* (1975) found that the proportion of soil carbon recovered in the lighter fraction more than doubled when the density of bromoform/ethanol mixtures was raised from 1.9 to 2.0. Due to this difficulty and the additional complications that may be expected with dispersion of material from fine-textured soils, results from density fractionation of finer particles must be viewed with some caution. This applies particularly to density agents with high viscosities. Because we used translucent containers we observed that there was no clear separation of particles in the heavy Ludox TM40 (ρ 1.39) solution with Method 1. After skimming off the top layer the remaining material was found to be mostly suspended in the Ludox, and only to a small degree had it settled on the bottom of the containers. Furthermore some of the material skimmed from the top had the same appearance as the remaining material. According to the specifications provided by the manufacturer, Ludox is at least 50 times more viscous than water (at ρ 1.38: 37.0 cP at 25°C) and 25 times more viscous than sodium polytungstate solution at the same density (Plewinsky and Kamps, 1984). This suggests that density separation of the smaller particles in the heavy Ludox was incomplete due to the effects on the velocity of small particles in this highly viscous solution. Indeed more than twice as much of the added C was found with both plant materials at day 100 in the large/light fraction (ρ <1.4; Table 16) of the sodium polytungstate (Method 3) compared with the sum of the light and medium fractions (ρ <1.4; Table 14) of the Ludox method. This may possibly be due to the enhanced resolution of small particles in the tungstate solution, as small ^{14}C-labelled particles were presumably more predominant at this late stage of decomposition.

Dynamics of plant residues in large (> 100 µm) size-density fractions

Despite the limitations of the size/density fractionation methods described above, the methods separated fractions which turned over at different rates. The large, particulate organic matter fractions and the soluble ^{14}C-labelled C recovered in the dispersing agent with Methods 2 and

3 decreased with time at rates consistent with the breakdown of the added plant materials monitored as soil respiration (Tables 14-16; Fig. 14). Decay of the residues was rapid with roughly half of the C respired from the added material in the first 10 days, presumably due to the large proportion of water-soluble C in the residues and their small lignin contents (Table 13). There were clearly two phases of decomposition which were demonstrated both by the respiration data and by the recoveries of added C in the different large fractions (Fig. 14 and 18). This implies that the decomposition of added (^{14}C-labelled) C recovered in each fraction cannot be described using first order decay rates as is usually assumed in simulation models. The fact that the specific activities of the rapidly degrading (the most 'active') fractions also decreased rapidly with time (Fig. 16) indicates that the native SOM present in each of these fractions was much more recalcitrant than the added materials. Thus although there were clearly differences between the solid fractions in their overall decomposition it appears that none of the fractions represented a distinct homogeneous pool with a uniform rate of decay.

Initially the added C was distributed, albeit unevenly, among all fractions for at least two reasons. Firstly, the labelled plant materials contained 30-40 % water soluble C, which may have been redistributed and subsequently adhered to particles of all size fractions during dispersion. It is unlikely that much of this soluble C was immobilized in the microbial biomass as the soils were transferred into the dispersing agent directly after the ^{14}C-labelled plant material had been mixed in. As the incubation proceeded the amount of the added C recovered in the fine fractions increased (Tables 15 and 16) and much of this is likely to be in microorganisms or in microbial debris. Secondly the fact that the plant material was added as coarsely-ground particles may have reduced the resolution of the methods. As discussed above, the air-filled porosity of the plant material is probably an important feature determining its specific density, and this will change during decomposition. Experimental constraints dictated that we could not add large, structurally intact, ^{14}C-labelled plant residues to soil, and this may have decreased the resolutive capability of the methods and as well have influenced the outcome of the incubation experiment, as discussed by Jensen (1994).

Dynamics of plant residues in fine (< 100 µm) size-density fractions

Little of the added plant material was in the fine fractions isolated in Method 3 initially (<12 %), but this more than doubled at subsequent harvests (Table 16). Most of the fine material was recovered in the medium density fraction but some of the apparent changes in the distribution of the added C between the fine fractions, especially between the medium and heavy fractions, may be due to slight differences in the density of the solutions used as discussed above. The specific activities of the three fine fractions all increased from the very small initial values, and this was particularly pronounced in the fine/light fraction (Fig. 16c). Overall the amount of the added C present in the fine fraction increased throughout the 200 day incubation period (Table 16), indicating that the fine fractions represented a sink for the products of the decomposition process. The specific activity of the fine/light fraction at 100 days was only a third of that found at 10 days indicating some turnover in this fraction (Fig. 16c). Apart from this there was little evidence for turnover of C in the fine fractions suggesting that these fractions were of relatively little importance as a source of energy for the microbial biomass, at least during the period of this incubation.

Dynamics of plant residues with different qualities

The ryegrass shoots grown at either 350 or 700 µl·l^{-1} CO_2 contained a large amount of water-soluble C and had lignin contents less than 1 % (Table 13) which led to rapid decomposition (Fig. 14). The short initial stimulation, also observed in Chapter 7 with roots, was possibly due to the greater amount of soluble carbon present in the material grown at elevated CO_2.

Decomposition of the plant material grown at elevated CO_2 was significantly retarded between day 4 and day 45 compared with the material grown at ambient CO_2. This was presumably related to the differences in C/N ratio, which were 18 and 37 for the materials grown at 350 and 700 $\mu l \cdot l^{-1}$ respectively, as the two materials did not differ substantially in lignin or cellulose (Table 13). The slower decomposition of the material grown at elevated CO_2 was apparent in the fractions larger than 150 µm at day 10, but not in the fractions >100 µm at 25 days (Fig. 15). Density separation of the large particles did not, however, add significant information on these differences as this difference was detectable in all Ludox fractions (Table 14).

From 100 days the total respiration from both materials was similar which indicates the importance of prolonged incubation periods in order to detect relevant differences in decomposition patterns. The decomposition of these shoot materials reported here was much faster than decomposition of the root material from the same plant species reported in Chapter 7; after 40 days 46 % of the shoots was respired as CO_2 whereas after 64 days only 40 % of the roots was decomposed at 14°C. These differences in decomposition rate between shoots and roots may affect the results of long-term experiments, since Van Ginkel *et al.* (in prep) observed a reduced decomposition of root material cultivated at 700 $\mu l \cdot l^{-1}$ CO_2, compared with ambient CO_2, even after two growing seasons under field conditions.

9. Simulation of decomposition of roots and soil organic matter under different scenarios of climate change

Abstract

Soil organic matter dynamics will be affected by elevated CO_2 due to changes in the amount and quality of plant debris and changes in temperature. To predict these changes on the long-term the simulation model by Verberne *et al.* (1990) was adapted and calibrated using data from the decomposition experiments described in Chapter 7. Distinction was made between decomposition of root residues and soil organic matter. The calibrated version of the model simulated the measured decomposition of roots and soil organic matter satisfactory. The adapted parameter values were subsequently used in the model for long-term simulations of soil organic matter. The simulations were done for a soil with an initial carbon content of 1.8 %, corresponding with about 70000 kg $C{\cdot}ha^{-1}{\cdot}yr^{-1}$.

Simulations were performed for reference conditions with or without a carbon input of 1000 or 2500 kg $C{\cdot}ha^{-1}{\cdot}yr^{-1}$ and conditions in which the carbon input into soil was increased by 15 % to 1150 and 2875 kg $C{\cdot}ha^{-1}{\cdot}yr^{-1}$ with an accompanying increase in temperature of 1.5 to 3.0°C. To leave the soil fallow would result in a loss of 10300 kg C per hectare (-14.7 %) at a varying temperature from 0°C in winter until 17°C in summer. Increasing the temperature with 3°C would yield in a loss of 12900 kg C per hectare (-18.4 %). To sustain the carbon content in soil organic matter a constant input of plant debris is necessary. With annual inputs of 1000 and 2500 kg $C{\cdot}ha^{-1}$ and a naturally varying temperature the changes in soil carbon content would be -7.7 % and +2.5 %, respectively. This would imply that the carbon content in this soil can only be sustained by a highly productive vegetation. When a changed climate is simulated by introducing a 15 %-increase in carbon input and a 3°C-increase in temperature it is clear that the temperature effect overrules the effect of the extra carbon input during the first 50 years. When the increase in temperature is 1.5°C, a value which is most likely to occur according the IPCC, the increased decomposition of organic matter is fully compensated by 15 % extra input of carbon.

For a soil with a lower initial carbon content of 1 %, the outcome is different. An annual input of 1000 kg C per hectare would suffice to sustain the soil carbon content, it would steadily increase by 16.7 % (6500 kg $C{\cdot}ha^{-1}$) after 50 years with an input of 2500 kg. Simulated climate change would reduce this increase 14.8 % (5800 kg $C{\cdot}ha^{-1}$). Sequestering capacities of soils in a changing climate (*i.e.* increased carbon input into soils and increased temperatures) will strongly depend on initial carbon content, productivity of the vegetations and the potential of soils to protect organic matter. External factors which will affect carbon dynamics in an ecosystem are inputof nitrogen (atmospheric deposition or fertilization) and management measurements such as set-aside policy. When highly productive vegetations are replaced by less productive vegetations carbon will inevitably be lost from soil organic matter.

9.1. Introduction

Effects of elevated CO_2 on soil organic matter dynamics will mainly be exerted via the supply of plant residues or via increased temperatures. Total allocation of carbon to roots and the supply of root residues to soils will probably increase (Norby, 1987; Lekkerkerk *et al.*, 1990) and consequently the amount of substrate available for the soil microbial biomass. This increase in quantity may be accompanied by changes in quality of the substrate in terms of an increased C/N ratio or lignin content (Cotrufo *et al.*, 1994). An increase in C/N ratio would probably strongly effect the mineral N pool in the soil which is in particular necessary for decomposition of structural materials with a low nitrogen content. Although Melillo *et al.* (1982) showed that decomposition of plant debris was related to lignin/nitrogen ratio, changes in these properties are nevertheless still unclear (Fog, 1988) and therefore poorly implemented in simulation models. Besides the amount and quality of plant production, elevated CO_2 may also affect environmental factors such as temperature and moisture. An increased temperature will stimulate the activity of the soil microbial biomass and thus the decomposition of plant residues and soil organic matter. According to the IPCC the temperature is expected to increase by about 1.5°C in the middle of next century with a maximum of about 3°C (Watson *et al.*, 1990). Such an increase would undoubtedly stimulate decomposition processes in the soil, although in dependence of the present temperature. An increase from 15°C to 18°C would result in a much stronger stimulation in absolute amounts than an increase from 5°C to 8°C. Jenkinson *et al.* (1991) reported model results that predicted an additional release from soil organic matter mounting to about 19 % of the CO_2 released by combustion of fossil fuels when the temperature increases by 1.8°C during the next 60 years.

Changes in soil carbon content are difficult to measure due to the vast amounts of carbon present in soil organic matter and the slow processes involved in decomposition. Extrapolations of these slow changes over decades necessitates the use of simulation models. Simulation models describing soil organic matter dynamics has been under investigation for several decades (Parnas, 1975; Van Veen *et al.*, 1984; Verberne *et al.*, 1990). Usually, these models distinguish several soil organic matter pools with different rates of decomposition, depending on the very nature of the pool and environmental conditions, such as temperature, available nitrogen, soil type, and soil moisture. The first division in pools is usually made between recently added plant residues and native soil organic matter, both to be further subdivided in smaller pools, with different turnover rates.

Decomposition of organic material is mainly governed by the soil microbial biomass. This biomass only represents a small pool of organic material in soils, but its activity is of utmost importance for almost all organic matter turnover. Thus, the soil microbial biomass is a most important organic matter pool. In most models soil organic matter is differentiated into an active pool, with high decomposition rates and stable, rather inert organic matter consisting of very resistant compounds such as fulvic and humic acids. Verberne *et al.* (1990) based her model on the concept of physical protection of part of the active soil organic matter. This concept of physical protection of the microbial biomass and the active pool of soil organic matter is thought to depend on the clay and silt content of the soil. Such protection quality of soils could play a role in its sequestering potentials. The higher the physical protection of organic matter, the slower the decomposition of these materials.

The objectives of this modelling study were:

i) to calibrate the model of Verberne *et al.* (1990) and Verberne (1992) for separate simulation of root material and soil organic matter and

ii) to simulate soil carbon dynamics for a period of 50 years in a system with annual input of root material in which the total amount of substrate, *i.e.* root residues, is increased by about 15 % and the temperature is increased by 3°C.

9.2. Materials and methods

Description of the model Verberne

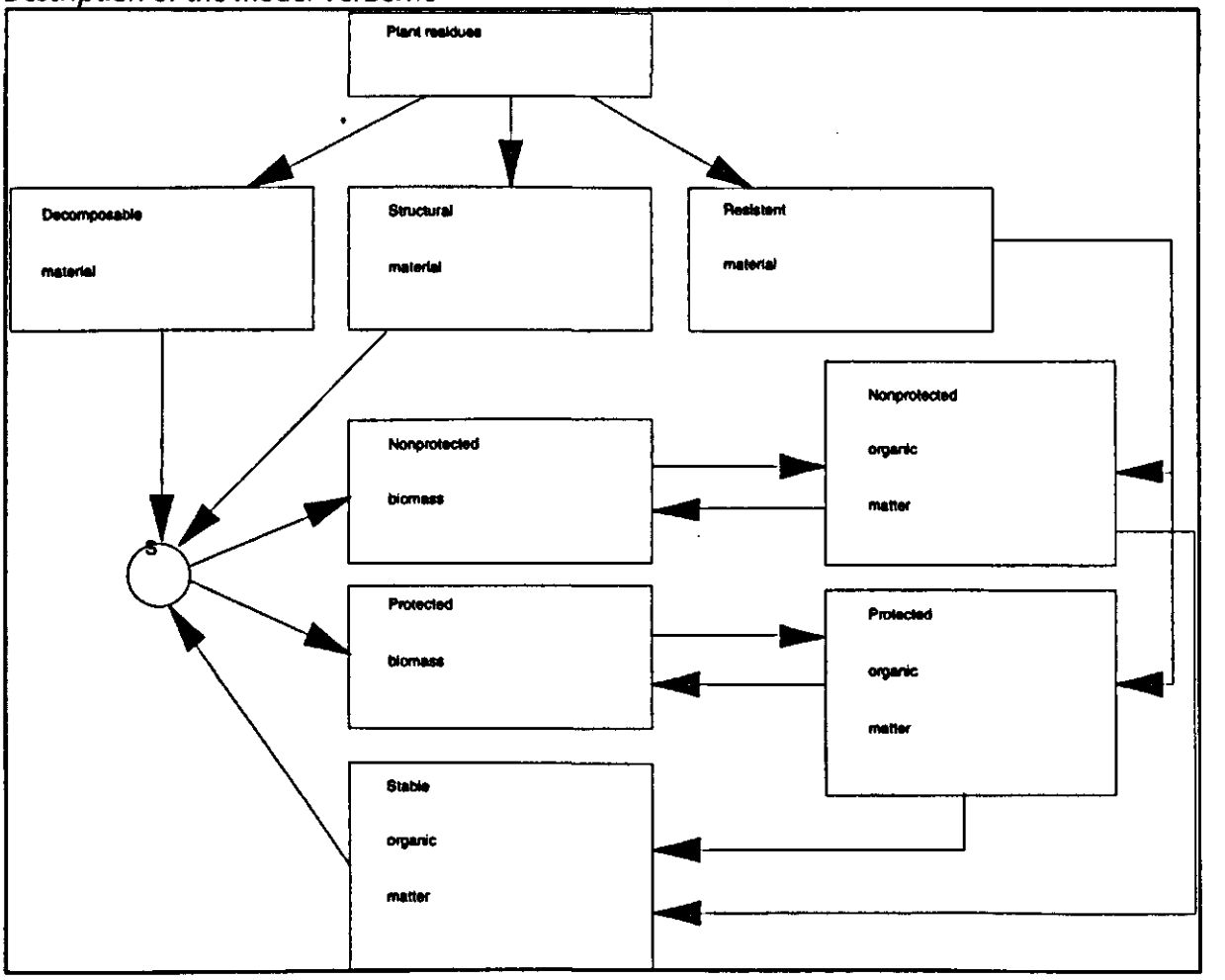


Figure 17 Carbon flows of the model

Figure 17 shows the carbon pools in the model, which distinguishes plant residues and soil organic matter pools. The plant residues entering the soil compartment are divided into three fractions with different decomposition rates: easily decomposable material (DPM; carbohydrates, proteins, etc), structural material (SPM; cellulose, hemicellulose, etc) and resistant material (RPM; lignified material). Each fraction has its own C/N ratio, which can be altered in the model. Decomposable and structural material are assumed to be decomposed by the microbial biomass, whereas the resistant fraction directly enters the active soil organic matter pool (protected + nonprotected organic matter). The soil organic matter consists of microbial biomass (BIOMP + BIOMN; protected + nonprotected), active soil organic matter (POM + NOM; protected + nonprotected) and stable soil organic matter (SOM). The distribution of the

decomposable and structural material among the protected and nonprotected biomass is governed by a soil-type specific parameter S.

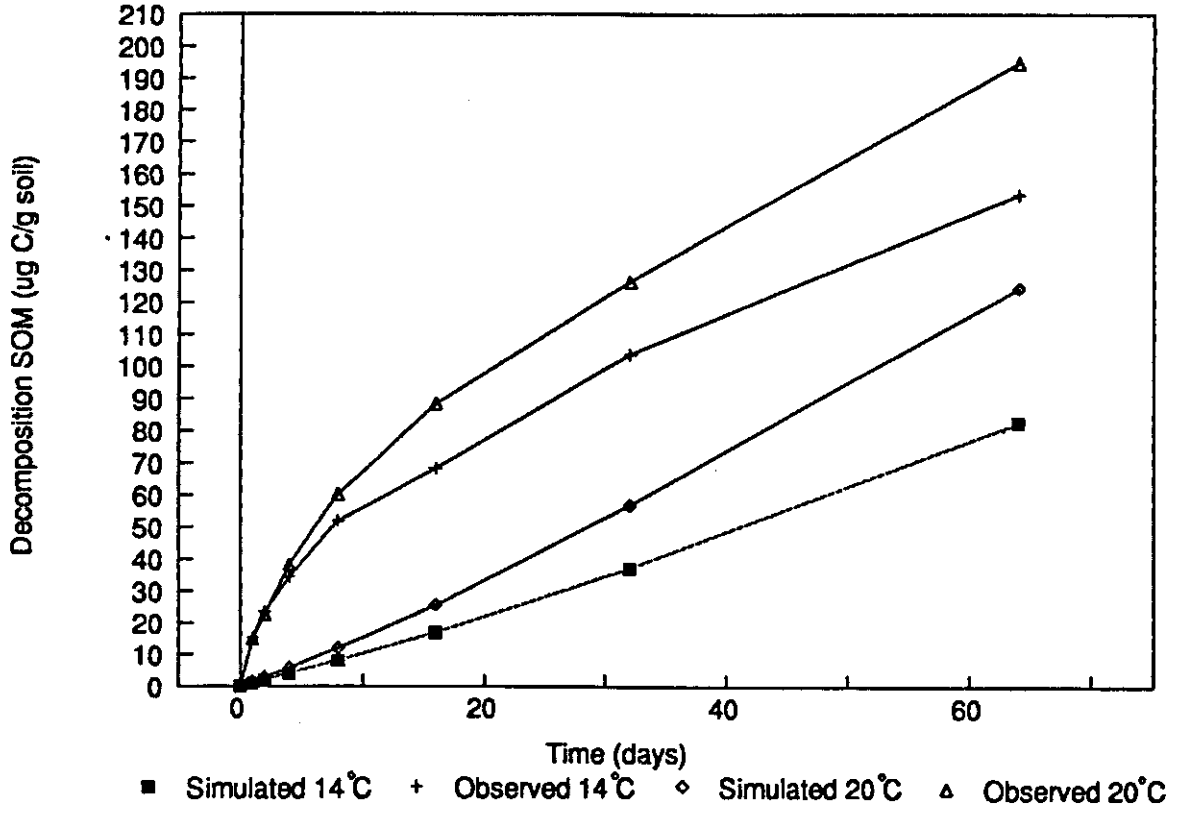
Parameter values

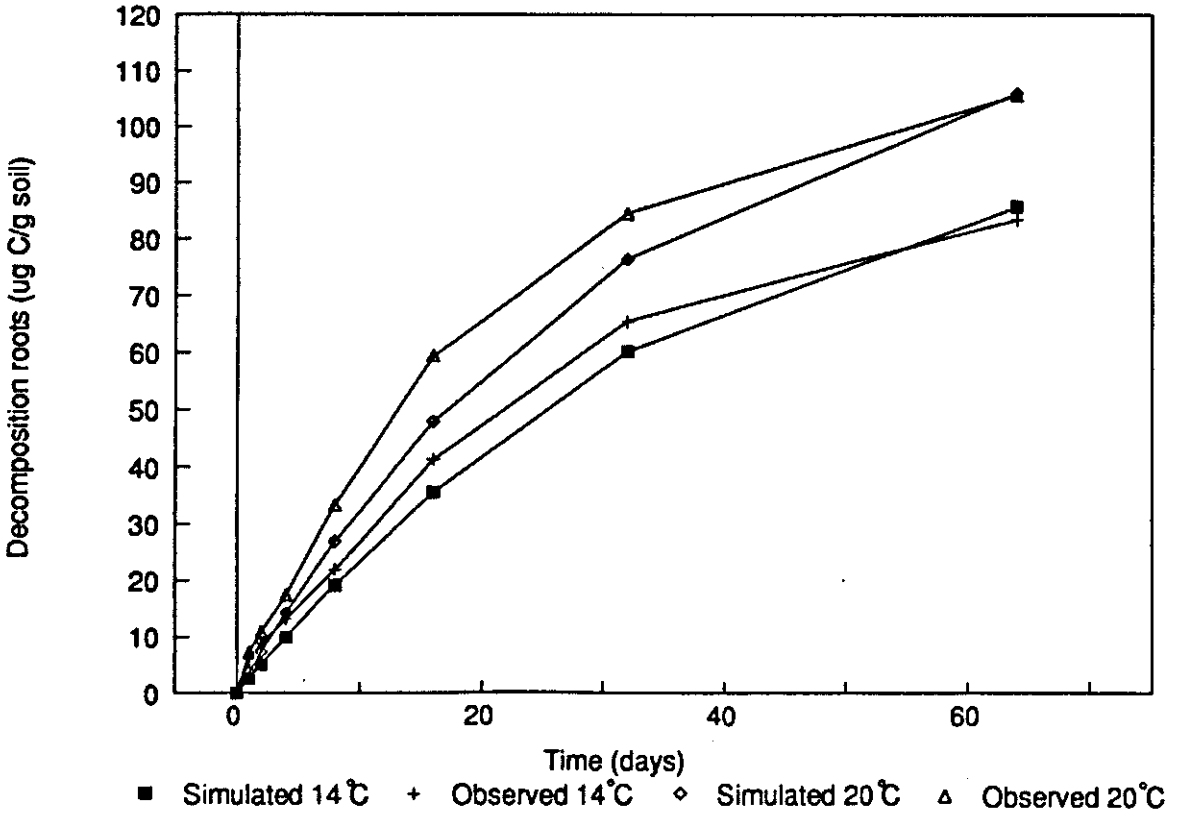
The initial parameter values used in the simulations were obtained from Verberne *et al.* (1990) and Verberne (1992) and the model was calibrated by using the data on decomposition of the roots with a C/N ratio of 32 and soil organic matter (Chapter 7). Figures 18 and 19 show the simulated and observed decomposition rates of SOM and roots. The parameter values which best predicted the final decomposition rates of SOM during the last 48 days of the experiment were chosen as standard values for the model. During the first weeks of the experiments the observed decomposition of SOM was much higher than the simulated decomposition due to disruption of the soil when the roots were added. Table 17 shows the standard values for the parameters used in the model.

Table 17 Standard parameter values

State variables	C/N ratio	Transformation		Decomposition rates (d^{-1})		Efficiency
C_{dpm}	6	DPM	-> BIOM	k_1	0.05	0.4
C_{spm}	150	SPM	-> BIOM	k_{2m}	0.002	0.3
C_{rpm}	100	RPM	-> NOM	k_{3m}	0.001	1.0
C_B	8	BIOM	-> NOM	k_{4n}	0.05	1.0
		BIOM	-> POM	k_{4p}	0.005	1.0
C_N	15	NOM	-> BIOM	k_5	0.01	0.25
		NOM	-> SOM	k_8	1.0E10-6	1.0
C_P	10	POM	-> BIOM	k_6	1.5E10-4	0.2
		POM	-> SOM	k_9	1.0E10-6	1.0
C_S	10	SOM	-> BIOM	k_7	4.0E10-7	0.2

Simulations were carried out for the Ede loamy sand used in the decomposition experiment described in Chapter 7. This soil contained about 1.8 % soil organic carbon, corresponding with an amount of about 70000 kg C·ha^{-1} when the thickness of the organic layer is estimated at 0.3 m with a density of 1.3 (kg·l^{-1}). The annualamounts of carbon allocated to the soil were taken as 1000 and 2500 kg·ha^{-1} representing low and high productive systems (Van Veen *et al.* 1989). The input of plant material was assumed to consist of 46 % DPM, 50 % SPM and 4 % RPM, with an average C/N ratio of 32. The quality of the carbon input (C/N ratio, lignin content, phenolic compounds etc.) was not included in the simulations since no reliable relationship with decomposition rates exist to date (Fog, 1988). The parameter S was fixed at a value 0.3. The fraction of protected active organic matter was set to 0.31, of non-protected organic matter to 0.011, of non-protected microbial biomass to 0.015, and protected microbial biomass to 0.003. The calculated total microbial biomass is about 320 µg C·g^{-1} soil which is of the same order as the measured levels in the Ede loamy sand as reported in Chapter 3. An increase of 15 % in carbon input was assumed to occur when the CO_2 concentration increases from 350 to 700 µl·l^{-1}, comparable with the mean growth stimulation of shoots of *Lolium perenne* and *Festuca arundinacea* during two seasons as reported in Chapter 3 and Chapter 4, and an increase in temperature of 3°C. This increase in carbon input may be relatively low, since root yield was found to increase more than shoot yield under elevated CO_2.





Figures 18 & 19 Observed and simulated decomposition of SOM and roots at 14 and 20°C during 64 days

The following simulations were carried out for a period of 50 years:

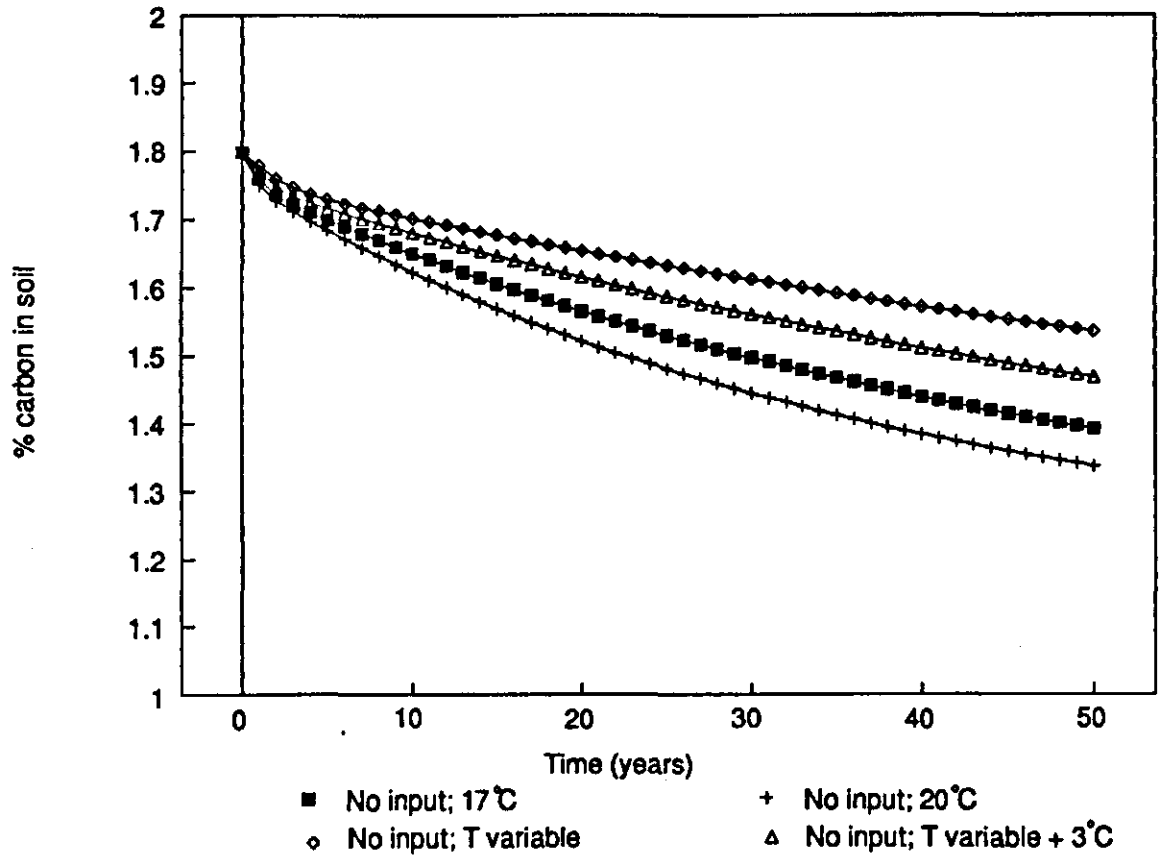
i) a fallow soil with a continuous temperature of 17°C,
ii) a fallow soil with an increased temperature of 3°C,
iii) a soil with a low carbon input (1000 kg $C \cdot ha^{-1} \cdot yr^{-1}$) during the growing season,
iv) a soil with a high carbon input (2500 kg $\cdot ha^{-1} \cdot yr^{-1}$),
v) iii and iv re-simulated with a 15 % increase in carbon input into soil corresponding with 1150 and 2875 kg $\cdot ha^{-1} \cdot yr^{-1}$ and a temperature increase of 3°C, and
vi) the simulations of iii, iv, and v recalculated with a more natural occurring temperature regime varying from 0°C in the winter to 17°C in the summer. The carbon percentage in soil was calculated as output variable.

9.3. Results

To leave the soil fallow at a temperature of 17 °C would result in a net carbon loss of 22.4 % after 50 years (Fig. 20). The total soil carbon content has then decreased from 1.8 to 1.4 %. A temperature increase of 3°C stimulates this carbon loss to 25.4 %. When no carbon inputs occur the soil carbon content will steadily decrease, although the rate will slow down.
An annual carbon input of 1000 up to 2500 kg C per hectare was not sufficient to sustain the initial carbon contents in this soil (Fig. 21). After 50 years this content decreased to 1.5 % and 1.65 %, respectively. When the effects of elevated CO_2 (an 15 %-increased carbon input and a temperature increase of 3°C) were included, the decrease was enlarged to 1.45 % and 1.6 %, respectively.
Figure 22 shows the results when the temperature varied during the season. It is clear that an annual input of 1000 kg C per hectare would be still insufficient to keep the carbon content at the initial level, but 2500 kg C would satisfy. With an input of 1000 kg C the net soil carbon loss would be 7.7 %, whereas with an input of 2500 kg C the soil carbon content would increase by 2.5 %. It is also shown that a 15 %-increase in carbon input can not level out the increased loss of carbon due to a higher temperature, although at an annual input of 2875 kg C per hectare and a 3°C higher temperature the soil carbon content equals the initial content after 50 years.
The influence of the initial carbon content of soils is shown in Figure 23. In this more hypothetical soil (all values were the same as for the Ede loamy sand, except the carbon content) with an initial carbon content of 1 % the highest loss after 50 years was 5 % with a annual carbon input of 1150 kg per hectare and an increased temperature. When the carbon input was increased to 2500 or 2875 kg per hectare the final soil carbon content increased by about 15 %.

9.4. Discussion

During the next decades the atmospheric CO_2 concentration will continue to increase and, as a consequence, the temperature will also increase by about 1.5 to 3.0°C until the middle of the next century. The model results by Jenkinson (1991) predicted a positive feedback of an increasing temperature on the atmospheric CO_2 concentration by a stimulated decomposition of soil organic matter, thus creating an extra source for CO_2. The potential role of soils to function as a sink or a source for atmospheric carbon dioxide is still unclear because negative feedback mechanisms such as an increased carbon input into the soil or reduced decomposition rates of plant residues were not implemented in their model. The annual input of plant debris was held at a constant value. In this chapter we simulated both the effects of an increased



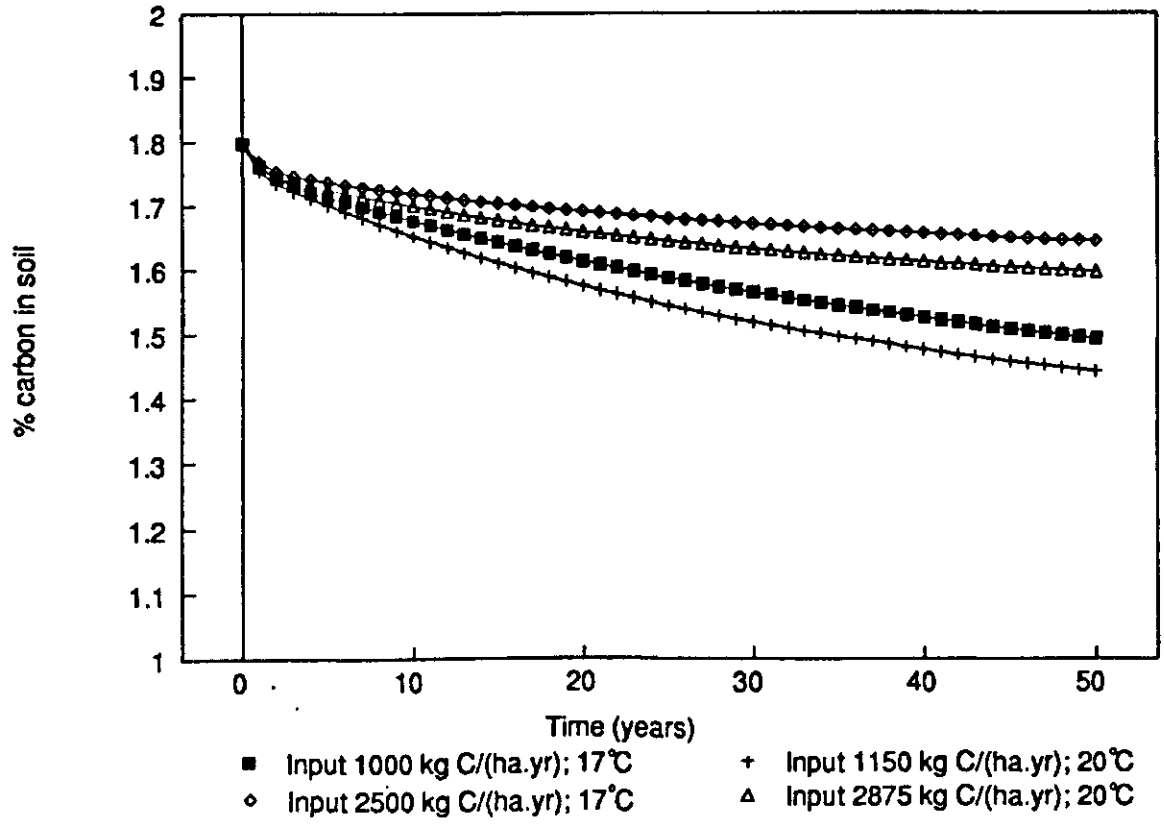
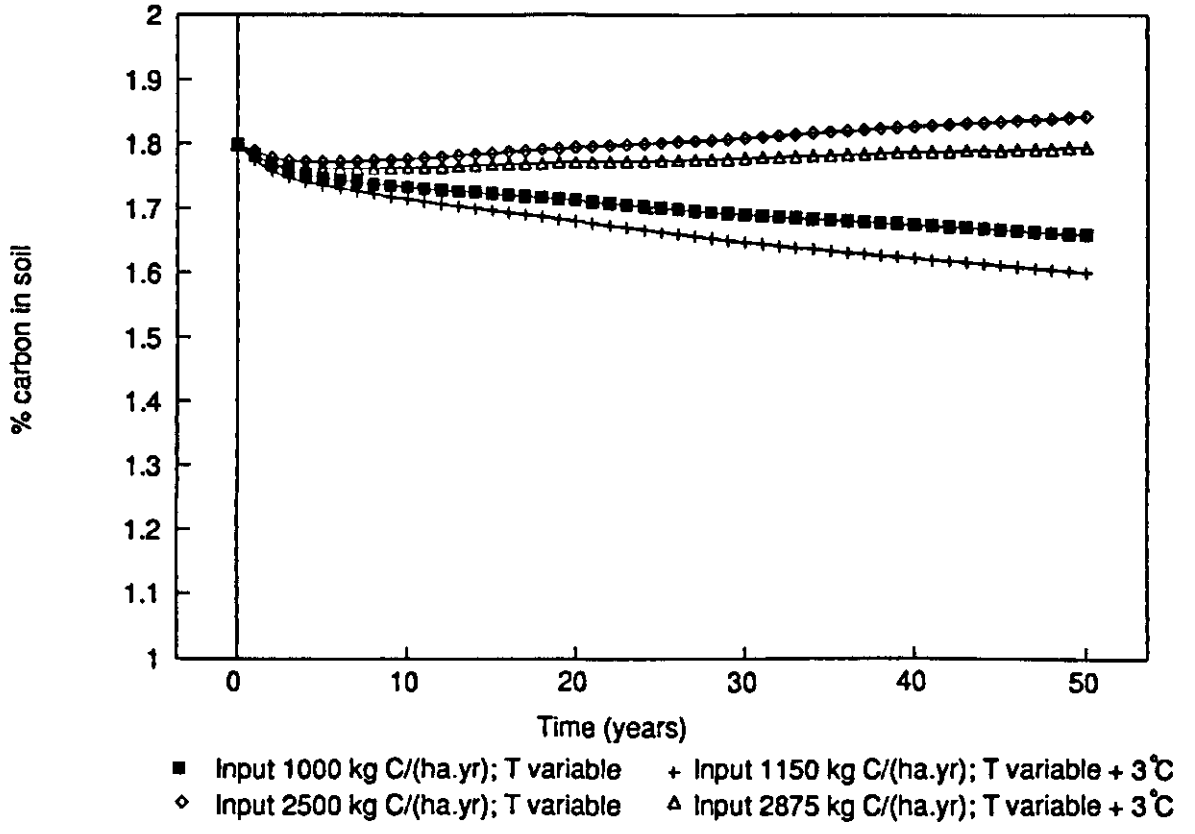
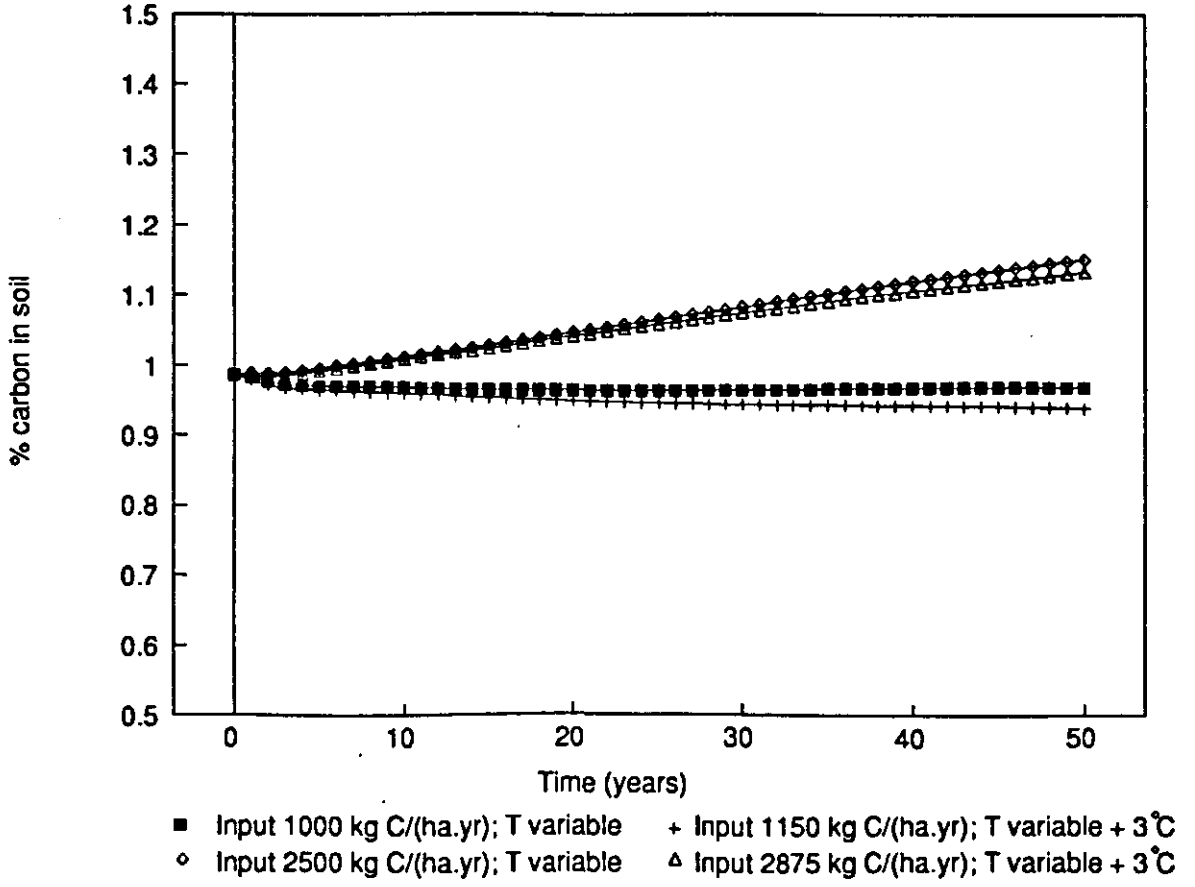


Figure 20 Decomposition of SOM in a fallow soil at 17 and 20 °C

Figure 21 Decomposition of SOM in a soil with standard carbon inputs (1000 and 2500 kg $C \cdot ha^{-1} \cdot yr^{-1}$) at 17°C and 15 %-increased carbon inputs at 20°C





Figures 22 & 23 Decomposition of SOM in a soil with standard carbon inputs (1000 and 2500 kg C $ha^{-1} \cdot yr^{-1}$) at a variable temperature and 15 %-increased carbon inputs at a variable temperature +3°C. Initial carbon contents 1 8 % and 1.0 %, respectively

carbon input into soils and an increased temperature. We first calibrated a simulation model (Verberne *et al.*, 1990; Verberne, 1992) using the data obtained in Chapter 7. These data provided the possibility to clearly distinguish between CO_2 originating from soil organic matter and CO_2 originating from added roots. The parameter values used for the simulation of decomposing roots were lower than the values used by Verberne *et al.* (1990). This may be caused by the nature of the decomposing material *i.e.* roots compared with shoots which decompose faster as was already observed in Chapter 8.
Fallow soils will undoubtedly loose carbon from soil organic matter. Figure 20 shows that this loss may amount to more than 20 % in 50 years, although the rate of CO_2 release slows down in time. This is due to a constant flow of carbon from the active carbon pools to the stable carbon pool with a turnover time of more than 1000 years (Verberne *et al.*, 1990). The total carbon loss per hectare will be about 15700 kg at a constant temperature of 17°C and about 17800 kg at 20°C. These differences are leveled out a little bit when a natural temperature regime during the year is used in the simulation. This reduction is mainly caused by the fact that during the winter period with temperatures near the freezing-point decomposition processes are almost idle. The annual loss will be 10300 kg C (-14.7 %) at the reference regime and 12900 kg (-18.4 %) at an increased temperature.
As could be expected, fallow soils will loose carbon and this phenomenon has undoubtedly repercussions for set-aside policies of agricultural soils. Without an ensured regular input of plant residues, either leaf litter or root-derived material, these soils will function as a source for carbon dioxide. To sustain the carbon content in soils, input via plant residues is necessary. When annual carbon inputs of 1000 to 2500 kg C per hectare occur, the decrease in soil organic matter is strongly reduced to -16.8 % and -8.5 %, respectively (Fig. 21) and when the temperature used in the model is following a more natural course the carbon loss is further reduced to -7.7 % at an input of 1000 kg C and the carbon content is even increased by 2.5 % with an annual input of 2500 kg C. This would imply that the carbon content of this soil can only increase in a highly productive system and otherwise it will be doomed to decrease. When a changed climate is simulated by increasing the annual carbon inputs by 15 % to 1150 and 2875 kg C per hectare, respectively, it is clear that in all cases the stimulated decomposition of soil organic matter by the increase in temperature will overrule the extra 15 % carbon input into soil (Figs 21 and 22). The net result is a decrease in soil carbon content compared with the control. The observed 2.5 % increase at an input of 2500 kg C is reduced to 0 % at an input of 2875 kg C and +3°C. However, the carbon content will increase on a longer term than 50 years since the slopes of the lines are still positive, in contrast to the input of 1000 and 1150 kg C where negative slopes are observed after 50 years.
Apparently, soils with a high organic matter content need a high carbon input via plant residues to maintain the initial carbon content. For this soil this required annual carbon input would be in between 1000 and 2500 kg C per hectare. Soils with a lower organic matter content would probably require a lower carbon input to sustain their organic matter content. This is shown in Figure 23 where the initial carbon content in soil is reduced to 1 %. The simulation is only performed for a varying temperature during the season. In this case an annual input of 1000 kg C per hectare would be almost sufficient to sustain the initial carbon content, since after 50 years only 2 % is lost. An increase in temperature and carbon input results in a net loss of 5 % after 50 years. A higher productive vegetation on this soil would steadily increase the soil carbon content as can be observed at an input of 2500 kg C. The scenario with an increased carbon input and an increased temperature only reduces the increase of 16.7 % to 14.8 % after 50 years, corresponding with an increase of 6500 kg C and 5800 kg C per hectare.
In Figure 24 the effect of a 1.5°C temperature increase on decomposition of soil organic matter is shown compared with no increase and 3°C increase, in combination with a 15 % increase in carbon input. The increase of 1.5°C is considered as most likely to occur within 50 years by the

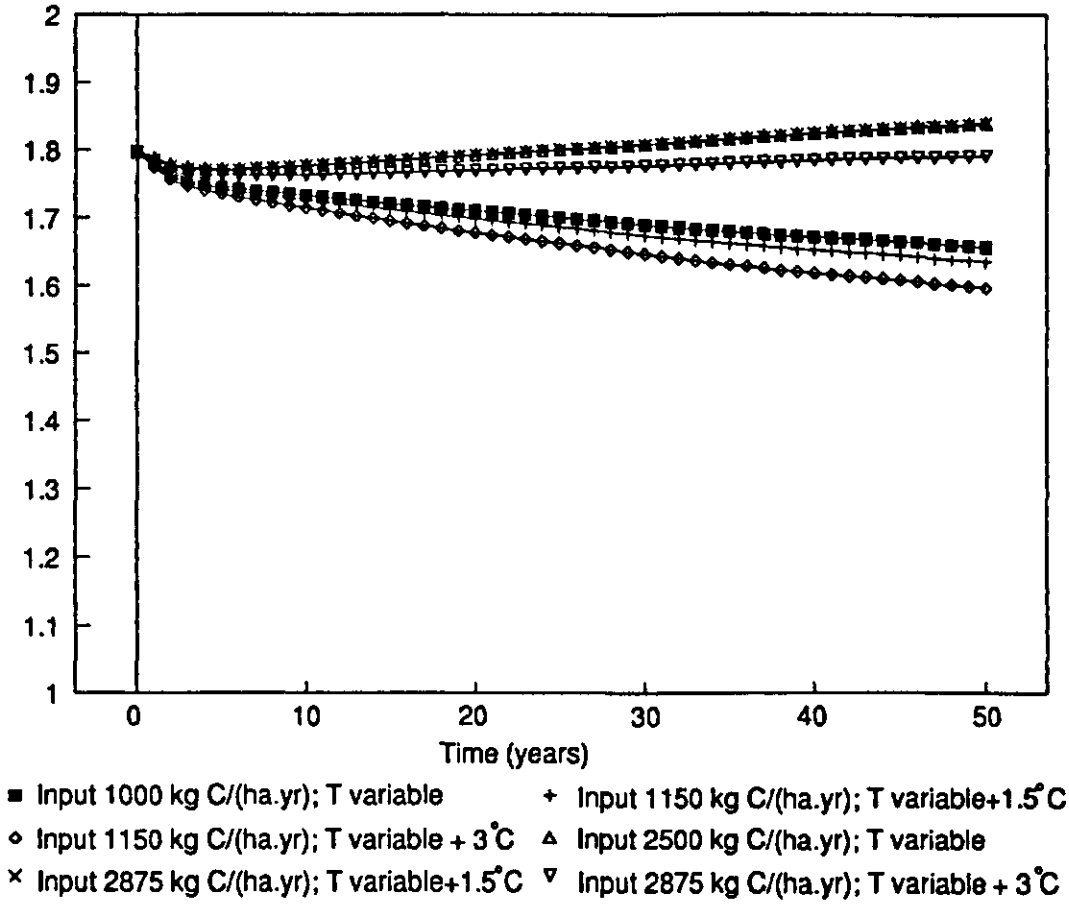


Figure 24 Decomposition of SOM in a soil with standard carbon inputs (1000 and 2500 kg $C \cdot ha^{-1} \cdot yr^{-1}$) at a variable temperature and 15 %-increased carbon inputs at variable temperatures + 1.5°C and +3°C

IPCC. At the higher carbon input level the increase in decomposition of organic matter due to 1.5°C increase in temperature, is fully compensated by the 15 % extra input of carbon. This model output clearly shows that soil organic matter dynamics is balancing between the effect of increased temperature and carbon input via plant debris. The higher decomposition of soil organic matter at higher temperatures can be levelled out by extra carbon input due to increased carbon allocation to the roots and soil. Whether changes in temperature and carbon input will result in a sink or a source function of the soil strongly depends on the extend of temperature increase which will be realized, the extra carbon allocation to the roots and soil under elevated CO_2 and on the soil at issue. The lower the initial carbon content of a particular soil, the bigger the possibilities for sequestering carbon; the higher the initial content, the bigger the difficulties to compensate the increased decomposition under elevated CO_2. Also the capacity of soils to protect organic matter (Hassink *et al.*, 1993) will affect the carbon sequestering capacity of that soil.

Concerning carbon sequestering in soils, several questions still exist which need our full attention, especially in longer term experiments under field conditions:

i) How will carbon distribution to the roots and soil (shoot/root ratio, root turnover, production of exudates) be affected under field conditions and will plant yield still be stimulated under elevated CO_2 on the long-term and under which conditions with regard to nutrient supply? In Chapter 2 was shown that the stimulation of the net $^{14}CO_2$ uptake in Douglas-fir was disappearing after 14 months of exposure to elevated CO_2. Oechel *et al.* (1994) also found some evidence that CO_2 fertilization in arctic tundra had a transient nature. The implications for simulation studies are clear: when the response of plants, crops or vegetations to elevated CO_2 has a transient nature, extrapolations over time by simulation models will be over-estimated.

ii) How will the quality of plant residues change in terms of C/N ratio, lignin content etc. and will this indeed lead to decreased decomposition rates (Chapter 7) on the long-term. The implementation of these changes in modelling studies is essential, but to date impossible due to the fact that a change in quality is also related to growth stimulation (see i) and the fact that the relation between quality and decomposability is still unclear (Fog, 1988).

iii) How will competition between plants (to sustain stimulated growth under elevated CO_2) and microbial biomass (for decomposition of nitrogen poor plant residues) affect the availability of mineral nitrogen.

iv) How will other factors influence soil organic matter dynamics such as air pollutants (atmospheric nitrogen deposition) or management measurements (set-aside policy). Atmospheric nitrogen may strongly influence carbon sequestering in soils, since it may help to sustain plant growth in time and because significant amounts of nitrogen will be stored together with carbon. On the other hand, deposition of nitrogen probably stimulates decomposition of plant debris or soil organic matter, although contrary results have been reported in literature (Fog, 1988). Set-aside policies will reduce carbon inputs into soil which will result in a net loss of soil organic carbon, although the response will strongly depend on whether these soils are kept fallow or replanted with perennial vegetations such as grasslands or forests.

10. Summary

The increase in 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 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 atmospheric CO_2 concentration. A secondary effect, due to stimulated plant growth, may be exhaustion of soil nutrients. Also a rise in temperature, one of the most important co-effects, will exert important changes such as extending the growing season, altering the availability of soil moisture and increasing the mineralization rate. In arable crops some of these effects may be compensated for by means of management practices such as manuring or sprinkling. In contrast to agricultural ecosystems, mostly consisting of annual crop species, natural ecosystems with perennial species will be exposed for a much longer time to the environmental changes and therefor be more capable of adapting to these changes or, for the same reasons, be more vulnerable due to nutrient deficiency or changes in soil moisture availability. Differential species responses with respect to carbon fixation, exploration of the soil in search of nutrients and water etc. could result in a shift in species composition. A second important item is whether ecosystems could function as a sink for atmospheric carbon, especially soil organic matter is mentioned for this purpose. With regard to the soil carbon balance several annotations can be made. Firstly, carbon transport within plants to the roots and soil is a most important process for carbon input into soil. The absolute carbon amounts allocated to the soil depend on i) the total net CO_2 fixation and ii) the carbon distribution among the shoot, root and root-released materials. Secondly, the decomposition rate of the carbon compounds entering the soil determines the residence time of carbon in the soil. Decomposition of these carbon compounds by the microbial biomass is strongly affected by their quality and by environmental factors such as available nutrients, soil moisture and temperature. The latter factor, temperature, strongly counteracts the effect of an increased carbon allocation to the soil, but may also, confusing this issue, increase the mineralization of nutrients from soil organic matter which is required to sustain increased plant growth under elevated CO_2. Hence, the balance between the effect of increased temperature (mineralization of both nutrients and CO_2) and the quality (C/N ratio, lignin content etc.) and quantity (growth stimulation on the long term) of carbon input via plant debris strongly determines the outcome whether terrestrial ecosystems will function as a sink or a source for atmospheric CO_2.

In this study, the effects of an elevated atmospheric CO_2 concentration on total net CO_2 fixation, carbon distribution among shoot, root and soil, and water use were investigated. Decomposition rate of grasses cultivated at different CO_2 concentrations was followed at different temperatures and soil water contents. Below, the main results of this study will be discussed separately.

Effects on plant growth and yield

These effects were studied in different species (*Pseudotsuga menziesii, Lolium perenne* and *F. arundinacea*), different soil types (loamy sand and loam soil), and at two nitrogen addition rates (135 and 400 kg $N \cdot ha^{-1} \cdot yr^{-1}$). In Chapter 2, results are described of experiments in which juvenile grasses were exposed to 350 and 700 $\mu l \cdot l^{-1}$ CO_2 during three weeks. Total shoot yield showed a clear interaction between plant species and soil type. *L. perenne* obtained a 39 % higher yield on loamy sand than on loam soil, whereas *F. arundinacea* had a 29 % higher yield

on loam soil. The total average yield of *L. perenne* was about 50 % higher than of *F. arundinacea*. Shoot yield was not increased in this preliminary experiment. In Chapter 3 it was shown that during a long-term experiment (14 months) 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 %.
So, at the end of the growing season it seemed that growth stimulation had disappeared. It was remarkable that early in the next growing season shoot yield was increased again at elevated CO_2, but, again, the stimulation had disappeared at the end of the growing season (Chapter 4). After this second season the cumulative 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 were limiting the growth stimulation at elevated CO_2, because nitrogen stimulated plant growth to a much higher degree than CO_2 until the end of the experiments. Whether or not the same periodicity occurred in Douglas-fir is, of course, not known, but dry weight of the needles was about 12 % higher after 14 months exposure to elevated CO_2, compared with ambient (Chapter 5).
The short-term treatment with 700 $\mu l \cdot l^{-1}$ CO_2 (1 month) increased the total net uptake of $^{14}CO_2$ in Douglas-fir by 22 %, compared with the 350 $\mu l \cdot l^{-1}$ CO_2 treatment (Chapter 5). The pretreatment did not affect the total net uptake, suggesting that photosynthetic acclimation had not occurred. However, expressed per unit of needle mass a 14 % reduction was observed in the trees pretreated at 700 $\mu l \cdot l^{-1}$ CO_2. This was not due to a reduced sink strength of the root system. This reduced uptake per unit of needle mass after long-term treatment may have implications for carbon storage in forest ecosystems. The results showed that an initial growth stimulation may eventually diminish. In grasses a similar phenomenon was observed: 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). After a long-term CO_2 treatment all species in this study tended to decrease their response to elevated CO_2.
Short-term (3 weeks) exposure to elevated CO_2 significantly stimulated root dry weight of juvenile *L. perenne* plants in loamy sand, but not in loam soil, and root dry weight of *F. arundinacea* was not clearly affected in both soil types (Chapter 2). However, on the long term it became evident that root dry weight of *L. perenne* and *F. arundinacea* was significantly increased by elevated CO_2 in loamy sand and loam soil. 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 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. At the low nitrogen supply elevated CO_2 did not always increase root dry weight. However, it should be noted that in Douglas-fir, grown on a poor sandy soil, root dry weight had increased by 16 % after 14 months growth at elevated CO_2, without additional nitrogen supply (Chapter 5). It is clear that complicated interactions exist between CO_2, species, and soil type 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 the absolute amounts will be smaller.

Effects on carbon distribution

The most relevant observation from the juvenile grass plants was that about 61 % of the ^{14}C was recovered in the shoots after exposure to elevated CO_2, compared with 66 % at ambient CO_2. The difference was mainly at the favour of the root system in which 28 % and 24 % was recovered, respectively. This observation in most of the plant/soil type combinations shows that root growth was favoured more than shoot growth at the time of labelling. On average, elevated CO_2 increased the total amount of ^{14}C allocated to the soil from 33.6 % to 39.0 % of the total net uptake compared with ambient 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 had resulted in a decreased shoot/root ratio, although at the time of labelling no effects on the ^{14}C-carbon distribution pattern was 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 period in which the labelling was performed. The increase in root weight demonstrates that, assuming that elevated CO_2 did not stimulate root turnover, an increased allocation of carbon 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.

Also in Douglas-firs, which were labelled in the same period as the grasses, no significant changes in carbon distribution pattern were observed. The increased carbon uptake at elevated CO_2 was evenly distributed among the different plant/soil compartments at the time of labelling. The total amount of ^{14}C-carbon allocated to the soil compartment increased by 28 % in the elevated CO_2 treatment, compared with ambient, which was comparable with the increase in total net uptake. The increase was found in all soil compartments: roots, root/soil respiration, microbial biomass and soil residue.

Effects on water use

In the juvenile grasses elevated CO_2 decreased the mean transpiration by about 18 % (Chapter 2). In the 14-month-old grasses the short-term CO_2-treatment caused an average decrease of 34 %, although depending on 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). In Douglas-fir total transpiration decreased only by 7 % and expressed per gram needle tissue transpiration decreased by 17 % (Chapter 5). The water use efficiency increased by 32 %. The response of *F. arundinacea* towards water use seems more pronounced than the responses of *L. perenne* and *P. menziesii* and may imply that the capacity of these species to adapt to drier conditions at elevated CO_2 decreases in the same order. These were direct responses to the short-term CO_2 treatment, probably caused by stomatal closure. The long-term CO_2 pretreatment, which had actually stopped at the time of the short-term CO_2 treatment (see *e.g.* Figs 1 & 4), caused a 25 % decrease in water use per gram shoot tissue in grasses, and a 16 % decrease in Douglas-fir. This could not be due to a transient response to elevated CO_2, such as effects on stomatal aperture, but must be caused by more persistent effects such as changes in specific leaf area or stomatal density.

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, although depending on species and nitrogen supply (Chapter 4), disappearance of growth stimulation on the long-term seemed still possible, since the increase in accumulated shoot yield at elevated CO_2 tended to be reduced. Hence, the sink function of soils for atmospheric carbon would then not be realized through extra carbon input on the longer-term. However, the total carbon input into soils is not the only relevant process for the soil carbon balance, also the total CO_2 output due to decomposition of

plant debris and soil organic matter, which is determined by the activity of the microbial biomass, has to be considered. In Chapter 6 we found that the short-term CO_2 treatment (1 month) increased $^{14}CO_2$ in the root/soil respiration of *P. menziesii* at 700 $\mu l \cdot l^{-1}$ CO_2 by 23 % compared with 350 $\mu l \cdot l^{-1}$ CO_2. This was accompanied by an increased specific activity of the root/soil respiration, which indicates that current (labelled) assimilates were preferentially used by the root system or the microbial biomass in the highest CO_2 treatment. The total size of the soil microbial biomass was increased by 59 % at elevated CO_2, probably caused by an increased input of fresh substrate by Douglas-fir roots.
In Chapter 7 we followed the decomposition rate of homogeneously ^{14}C-labelled roots which were cultivated at 350 and 700 $\mu l \cdot l^{-1}$ CO_2 in a ^{14}C-labelled atmosphere. Two temperatures (14°C and 20°C) and two soil moisture levels (10 kPa and 62 kPa) were included as additional treatments. Small differences in soil moisture content appeared to be not important with regard to decomposition of the plant residues in the range studied. However, much drier or wetter conditions would inevitable slow down decomposition processes. A temperature increase of 6°C strongly stimulated the decay of the root material and soil organic matter by 26 % and 30 %, respectively, over a two-months period. Global warming will undoubtedly accelerate the decomposition rate of plant residues and soil organic matter, thus having a positive feedback on the atmospheric CO_2 concentration. After two months the total decomposition of roots cultivated at 700 $\mu l \cdot l^{-1}$ CO_2 was about 24 % lower than roots cultivated at 350 $\mu l \cdot l^{-1}$ CO_2. This decrease was probably caused by differences in C/N ratio which were 18 and 32, respectively, for the root materials cultivated at 350 and 700 $\mu l \cdot l^{-1}$ CO_2, although other factors determining 'plant quality' are probably also involved. The results indicate that the decreased decomposability of root material can potentially annul the additional CO_2 release from soil organic matter caused by an increase of 1.2 to 3.0°C over the next 60 years and urge the need not only to implement enhanced decomposition of soil organic matter due to an increased temperature and changes in total carbon input in soils into simulation models describing global carbon dynamics under climate change. Also changes in plant quality and subsequent effects on decomposition rate, in dependence of available nitrogen, should be included after validation of the observations in longer-term experiments under field conditions. In these experiments both decomposition of roots and shoots should be studied since in Chapter 8 it appeared that the decomposition rate of shoot material was faster than root material. The decomposition rate of shoots cultivated at elevated CO_2 was retarded after 10 days. This was also observed in the Ludox fractions, where more ^{14}C was recovered in the large light fraction, which is thought to consist of plant material that has not yet been decomposed. Despite the differences in C/N ratio no differences in the decomposition rate of this shoot material were observed after 200 days. More insight into the decomposition processes is necessary for the development of models describing soil carbon dynamics. Especially knowledge about the input of carbon into the active pools in soil and the decomposition rates of these pools is important for describing carbon fluxes in soil. For this purpose fractionation techniques need to be further developed for an adequate separation of biologically meaningful fractions.
In Chapter 9 a simulation model was calibrated using data obtained in Chapter 7 to predict long-term (50 years) changes in soil organic matter due to an increased input of carbon into the soil under elevated CO_2 and an increased temperature. It was shown that fallow soils will loose carbon, a consequence that is often overlooked in set-aside policies. To sustain the carbon content in soils, regular input via plant residues is necessary. In soils at equilibrium, carbon input will balance the carbon output. Climate change will affect this equilibrium by the input of extra carbon due to stimulated growth, assumed that this will occur on the long term, and an increased decomposition rate of plant debris and soil organic matter at higher temperatures. The model output clearly showed that soil organic matter dynamics is balancing be-

tween the effect of increased temperature on decomposition rates and carbon input via plant debris. Whether changes in temperature and carbon input will result in a sink or a source function of the soil strongly depends on the extent of temperature increase which will be realized, the extra carbon allocation to the roots and soil under elevated CO_2 (and the persistency on the long-term!) and on the soil at issue.

The above-mentioned effects were obtained on a relatively short-time scale. Some major questions still need to be answered, especially in long-term experiments under field conditions:

i) How will carbon distribution to the roots and soil (shoot/root ratio, root turnover, production of exudates) be affected under field conditions and will plant yield still be stimulated under elevated CO_2 on the long-term and under which conditions with regard to nutrient supply and soil type? In Chapter 2 was shown that the stimulation of the net $^{14}CO_2$ uptake in Douglas-fir was disappearing after 14 months of exposure to elevated CO_2. The implications for simulation studies are clear: when the response of plants, crops or vegetations to elevated CO_2 has a transient nature, extrapolation over time by simulation models will over-estimate the carbon allocation to the soil.

ii) How will the quality of plant residues change in terms of C/N ratio, lignin content etc. and will this indeed lead to decreased decomposition rates (Chapter 7) on the long-term. The implementation of these changes in modelling studies is essential, but to date impossible due to the fact that a change in quality is also related to growth stimulation (see i) and the fact that the relation between quality and decomposability is still unclear (Fog, 1988).

iii) How can we appreciate the role of higher a temperature, which will increase the mineralization rate of plant residues and soil organic matter? This process will create an extra source of CO_2, but also warrants a sufficient nutrient supply required to sustain increased plant growth.

iv) How will competition between plants (to sustain stimulated growth under elevated CO_2) and microbial biomass (for decomposition of nitrogen poor plant residues) affect the availability of nutrients.

v) How will other factors influence soil organic matter dynamics such as air pollutants (atmospheric nitrogen deposition) or management measurements (set-aside policy). Atmospheric nitrogen may strongly influence carbon sequestering in soils, since it may help to sustain plant growth in time and because significant amounts of nitrogen will be stored together with carbon. Set-aside policies will reduce carbon inputs into soil which will result in a net loss of soil organic carbon, although the response will strongly depend on whether these soils are kept fallow or replanted with perennial vegetations such as grasslands or forests.

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STELLINGEN[1]

1. De opslagmogelijkheden voor koolstof in de bodem bij een verhoogd atmosferisch CO_2 gehalte en een verhoogde temperatuur worden in sterke mate bepaald door bodem-eigenschappen zoals organische stof gehalte en textuur
   Hoofdstukken 2 en 10

2. Reductie van de stikstofdepositie kan de opslagcapaciteit voor koolstof van ecosystemen beïnvloeden door effecten op plantengroei en decompositie van plantenresiduen
   Hoofdstukken 3, 4 en 7

3. De groeistimulatie en de waterhuishouding bij een verhoogd CO_2-gehalte zijn soort-specifiek en veranderen de concurrentieverhouding tussen plantensoorten
   Hoofdstukken 3, 4 en 5

4. Onderzoek is nodig om *lange termijn* veranderingen in stimulatie van plantengroei en decompositie van plantenresiduen te kunnen bepalen
   Hoofdstukken 4, 5, 7 en 8

5. De interactieve effecten op langere termijn van temperatuur (verhoogde mineralisatie van koolstof en nutriënten) en een verhoogd CO_2-gehalte dienen onder (semi) veld-omstandigheden vastgesteld te worden in (mini-)FACE-experimenten met een verhoogde temperatuur.
   Hoofdstuk 10

6. *Set-aside* policy versterkt het broeikaseffect
   Hoofdstuk 10

7. Opdrachtgevers willen altijd op zo kort mogelijke termijn uitspraken over een zo lang mogelijke termijn.

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1 Stellingen behorend bij het NOP-verslag 'Distribution of carbon over plant and soil compartments during the growth of perennial plants', project 850029.