Soil Biology & Biochemistry 41 (2009) 2422–2429

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# Repeated $^{14}CO_2$ pulse-labelling reveals an additional net gain of soil carbon during growth of spring wheat under free air carbon dioxide enrichment (FACE)

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ARTICLE INFO

*Article history:*
Received 17 March 2009
Received in revised form
19 August 2009
Accepted 27 August 2009
Available online 12 September 2009

*Keywords:*
FACE
$^{14}CO_2$ pulse-labelling
Spring wheat
Carbon sequestration
Soil mineral fractions
Priming effect
Microbial biomass

ABSTRACT

Rising levels of atmospheric $CO_2$ have often been found to increase above and belowground biomass production of C3 plants. The additional translocation of organic matter into soils by increased root mass and exudates are supposed to possibly increase C pools in terrestrial ecosystems. Corresponding investigations were mostly conducted under more or less artificial indoor conditions with disturbed soils. To overcome these limitations, we conducted a $^{14}CO_2$ pulse-labelling experiment within the German FACE project to elucidate the role of an arable crop system in carbon sequestration under elevated $CO_2$. We cultivated spring wheat cv. "Minaret" with usual fertilisation and ample water supply in stainless steel cylinders forced into the soil of a control and a FACE plot. Between stem elongation and beginning of ripening the plants were repeatedly pulse-labelled with $^{14}CO_2$ in the field. Soil born total $CO_2$ and $^{14}CO_2$ was monitored daily till harvest. Thereafter, the distribution of $^{14}C$ was analysed in all plant parts, soil, soil mineral fractions and soil microbial biomass. Due to the small number of grown wheat plants (40) in each ring and the inherent low statistical power, no significant above and belowground growth effect of elevated $CO_2$ was detected at harvest. But in comparison to ambient conditions, 28% more $^{14}CO_2$ and 12% more total $CO_2$ was evolved from soil under elevated $CO_2$ (550 μmol $CO_2$ $mol^{-1}$). In the root-free soil 27% more residual $^{14}C$ was found in the FACE soil than in the soil from the ambient ring. In soil samples from both treatments about 80% of residual $^{14}C$ was found in the clay fraction and 7% in the silt fraction. Very low $^{14}C$ contents in the CFE extracts of microbial biomass in the soil from both $CO_2$ treatments did not allow assessing their influence on this parameter. Since the calculated specific radioactivity of soil born $^{14}CO_2$ gave no indication of an accelerated priming effect in the FACE soil, we conclude that wheat plants grown under elevated $CO_2$ can contribute to an additional net carbon gain in soils.



## 1. Introduction

The increasing concern about the rising $CO_2$ concentration in the atmosphere and the associated predicted climatic changes have raised solicitous questions about the impact of a $CO_2$ enriched world on the functioning of both natural and agro-ecosystems. In particular, plant physiology, soil carbon, soil nutrient cycles and soil population structures and functions will probably be affected with large-scale, unpredictable economic repercussions (Hendry, 1992). Therefore, numerous investigations have been conducted in the past 20 years to elucidate the reactions predominantly of C3 plants and the linked soil carbon cycle to the expected, changed atmospheric $CO_2$ content. Many of these studies were carried out in controlled environments (e.g. closed chambers) and are of limited value to describe the situation under real field conditions. A much better tool to investigate the different aspects of plant physiology under elevated $CO_2$ is the application of the Free Air Carbon Dioxide Enrichment (FACE) facilities (Hendry, 1992; Lewin et al., 1992) which avoid the known side effects of enclosure chambers.

The generally higher above and belowground productivity of C3 plants under elevated $CO_2$ leads to the conclusion that more rhizodepositions (roots and exudates) are transferred into soils, potentially increasing soil carbon content. However, most FACE and outdoor chamber studies have failed to detect significant changes of soil organic carbon (SOC), mainly due to the already existing large and often spatially heterogeneous pool of SOC (Jastrow et al., 2005). Only by meta-analysis techniques Jastrow et al. (2005) and De Graff et al. (2006) could calculate an increase in soil carbon content under elevated $CO_2$. But this result was only valid when N availability was high (De Graff et al., 2006). Since special ecosystem types are over-represented in these meta-analyses and individual C3 plant species differ considerably in their reaction on elevated $CO_2$ (Ainsworth and Long, 2005), no general conclusions can be drawn from these calculations for a particular plant-soil system.

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doi:10.1016/j.soilbio.2009.08.018

A better tool to assess additional carbon input into soils by plants under elevated $CO_2$ than conventional carbon analyses is the application of carbon isotope techniques ($^{13}C$, $^{14}C$; Pendall, 2002). A common way in FACE experiments is the use of $CO_2$ which is produced from fossil fuel and is thus depleted in $^{13}C$. This allows a more precise quantification of carbon sequestration under FACE, but a concomitant $^{13}C$ labelling of the ambient air treatment would require a controlled addition of pure $^{13}CO_2$ or $^{13}C$ depleted $^{12}CO_2$ which makes this approach prohibitively expensive (Pendall, 2002).

A more suitable way to apply $^{13}C$ as a tracer to both, elevated and ambient $CO_2$ plots is to use soils with a long-term cultivation of C4 plants. These produce soils with SOC which is less depleted in $^{13}C$ than that generated by C3 plants. But the availability of C4 soils is rather limited and in previous experiments these soils were transferred from their original locations to different kinds of containers to cultivate C3 plants (Ineson et al., 1996; Leavitt et al., 2001; Cardon et al., 2001; Hoosbeck et al., 2004). However, the disturbance of soil structure and the transfer in a new plant-climate system can intensify SOC loss (Leavitt et al., 2001) and confound the results.

A further approach to follow carbon sequestration under different $CO_2$ atmospheres is the pulse-labelling with $^{13}CO_2$ or $^{14}CO_2$. This technique has advantages and disadvantages which are discussed by Voroney et al. (1991) and Meharg (1994). The main disadvantage of the pulse-labelling approach can largely be overcome by a frequently repeated pulse-labelling to consider the changing C translocation within the plants during growth. To our knowledge, up to day only Leavitt et al. (2001) applied this technique with $^{13}CO_2$ for crops under field conditions within the Maricopa (Arizona, USA) FACE experiment. But due to a heterogeneous isotopic $^{13}C$ distribution in plants and in soil organic matter the $^{13}C$ analyses did not indicate a distinguishable difference of carbon sequestration by plants from ambient and elevated $CO_2$ regimes.

This very limited and uncertain data base about carbon sequestration by C3 plants grown under elevated $CO_2$ requires further attempts to elucidate this question. Especially, more extensive FACE experimentations with the major crops and within the major growing zones are an obvious need (Ainsworth and Long, 2005). Due to the above described problems with $^{13}CO_2$ pulse-labelling, we conducted a $^{14}CO_2$ pulse-labelling experiment within the German FACE experiment in Braunschweig, Lower Saxony. Since wheat is currently the most important crop, we selected a spring wheat cultivar which was cultivated in field plots under ambient and elevated $CO_2$ atmospheres till maturity and thereby repeatedly treated with $^{14}CO_2$. The main objective of this investigation was to follow the above and belowground distribution of $^{14}C$ assimilated during $^{14}CO_2$ pulse-labelling by the plants. More specifically, we analysed the soil born $^{14}CO_2$ during plant growth and determined the concomitant liberation of total $CO_2$ for a calculation of its specific activity. This should allow a comparison of possible 'priming effects' under ambient and FACE growth conditions. After harvest the amount and $^{14}C$-activity of the root material and the microbial biomass C were determined. The residual $^{14}C$ in soil and its distribution within the mineral fractions was analysed.

## 2. Materials and methods

### 2.1. Site and FACE description

The German FACE experiment as part of the "Braunschweig Carbon Project" (Weigel and Dämmgen, 2000) was established in 1999 in an agricultural field at the experimental farm of the Federal Research Institute (vTI) in Braunschweig, Lower Saxony, Germany (52°18′N, 10°26′E, 76 m above sea-level). The site is in the cool temperate region (mean daily air temperature 8.8 °C; mean annual rainfall: 618 mm) on a Cambisol. The SOC content in the selected experimental plot with ambient $CO_2$ (1.14 ± 0.04%) was almost identical to that of the FACE plot (1.12 ± 0.07%). Also no significant differences were found for the distributions of the mineral particle size fractions between the treatment plots. Corresponding to the classification of the ISSS the soil is composed of 5% clay, 11% silt and 84% sand and has a pH of 6.3–6.5.

The research site has been used for agricultural crop cultivation (only C3 plants) for more than 30 years. Corresponding to the usual practice of this region winter barley, sugar beet and winter wheat was cultivated in a crop rotation. At the time of the present investigation (2005) winter wheat was grown.

The design of the FACE facility was furnished by the Brookhaven National Laboratory according to Hendry (1993). It consisted of four rings (20 m diameter), two were flushed with ambient air, serving as controls, and two were fumigated at daylight with approximately 550 μmol $CO_2$ $mol^{-1}$, 180 μmol $mol^{-1}$ above ambient. The concentration of elevated $CO_2$ was regulated by considering wind speed, wind direction and current $CO_2$ concentration. No $CO_2$ enrichment took place at wind speeds >6 m $s^{-1}$. The four rings were 100 m apart from each other to avoid a contamination of control rings with additional $CO_2$ from $CO_2$-enriched rings.

### 2.2. Experimental design of the $^{14}CO_2$-labelling experiment

For the $^{14}C$-labelling experiment four stainless steel cylinders (21.3 cm inner diameter, 74 cm height) were forced into an unplanted soil plot within one of the $CO_2$-enriched and one of the control ring, leaving a 4 cm rim above the soil surface. The columns, arranged in a quadratic layout, were placed near the centre of the rings. After one week germination in the greenhouse 10 spring wheat plants (*Triticum aestivum* L., cv. Minaret) were planted in late April to each of the columns. This plant density corresponded to 280 plants $m^{-2}$. The soil removed to implant the wheat seedlings was used to estimate microbial biomass C ($C_{mic}$) by a modification of the fumigation–extraction method (Vance et al., 1987; Mueller et al., 1992; see below). To track volumetric water content a TDR (Time Domain Reflectometry) probe (IMKO, Ettlingen, Germany) was buried in the centre of one column in each ring. The measurements in this column were assumed to be representative for the other three columns in each ring.

The measurement of $^{12/14}CO_2$ evolution from soil required an airtight separation of the canopy air and the soil atmosphere. This was realized by the end of May, when plants had developed their tillers and were on the stage of stem elongation (growth stage 26–29, Tottman and Broad, 1987). Each column was covered with a round PVC plate (diameter 23.0 cm) containing 16 holes. In 10 of these, PVC tubes (3.2 cm inner diameter, 4.0 cm length) had been glued and served as exits for the wheat plants through the PVC plate. After the wheat plants had been cautiously passed through the PVC-tubes acid washed gravel was poured around each plant up to a height of 1 cm in the PVC tubes. A warm (35 °C) liquid mixture (7:12 w/w) of Vaseline, a silicone paste (NG 3170, Thauer & Co, Dresden, Germany) and a catalyst was poured around the plants forming within 1 min a 2 cm high, soft, airtight sealing. In the same way the exit for the cable of the TDR probe was sealed. The remaining upper 1 cm in each PVC tube was filled with water to verify the integrity of the seal around the plants before and during the whole experiment. After the leak test, the rim of the PVC lid was glued (silicon) with the rim of the columns.

Around the columns in both $CO_2$ treatments a metallic frame with a U-shaped cross-section was dug into the soil surface, enclosing an area of 70 × 70 cm. For $^{14}CO_2$-labelling quadratic

Plexiglas chambers (100 cm high, 70 cm side length) were placed into the trenches of the frames, filled with acidified water (pH 4). For the production of labelled $^{14}CO_2$ a 1.5 M $Na_2^{14}CO_3$ solution (spec. activity: 1.8 kBq mg $C^{-1}$) was pipetted first in two 1200 ml and later in 2300 ml glass bottles each provided with two glass stop cocks on top. The amount of the $Na_2^{14}CO_3$ solution was calculated to fill the whole volume of the bottles with $^{14}CO_2$ after evacuation of the bottles and acidification of the $Na_2^{14}CO_3$ solution with a 5% $H_2SO_4$. For the $^{14}C$-labelling procedure the glass bottles filled with the $^{14}CO_2$ were placed on each chamber and connected via PVC tubings with the atmospheres of the chambers directly above the ventilators affixed inside on the top plate of the chambers. A second tubing connected the $^{14}CO_2$-bottle with a glass vessel, which was installed 1 m above the chamber and contained 5% $H_2SO_4$. In-between this connection, an electric valve was inserted. Fig. 1 illustrates the experimental design.

During the $^{14}C$-labelling procedure the atmospheres of the chambers were continuously pumped through IR-detectors (Uras 10, Hartmann and Braun, Frankfurt/Main, Germany) to monitor the $CO_2$ concentrations in the chambers and to regulate the addition of the $^{14}CO_2$. In the ambient ring the electric valve was opened at 340 μmol $mol^{-1}$ $CO_2$ and in the $CO_2$-enriched ring at 530 μmol $mol^{-1}$ allowing the 5% $H_2SO_4$ to flow slowly into the $^{14}CO_2$-bottles and so to transfer the corresponding amount of $^{14}CO_2$ into the chambers. When the $CO_2$ concentration reached the upper limits of 380 and 570 μmol $mol^{-1}$, respectively, the valves were closed. The $^{14}C$-labelling was started on the 6th of June and was repeated 12 times till to the beginning of ripening at mid-July. Each labelling was finished when in one of the two $^{14}CO_2$-bottles the space was completely filled with the 5% $H_2SO_4$. At the same time the labelling in the other treatment was stopped and the amount of the diluted acid in the $^{14}CO_2$-bottle was recorded. The Plexiglas chambers were removed and till to the next labelling the wheat plants grew under the conditions of the ambient and FACE ring.

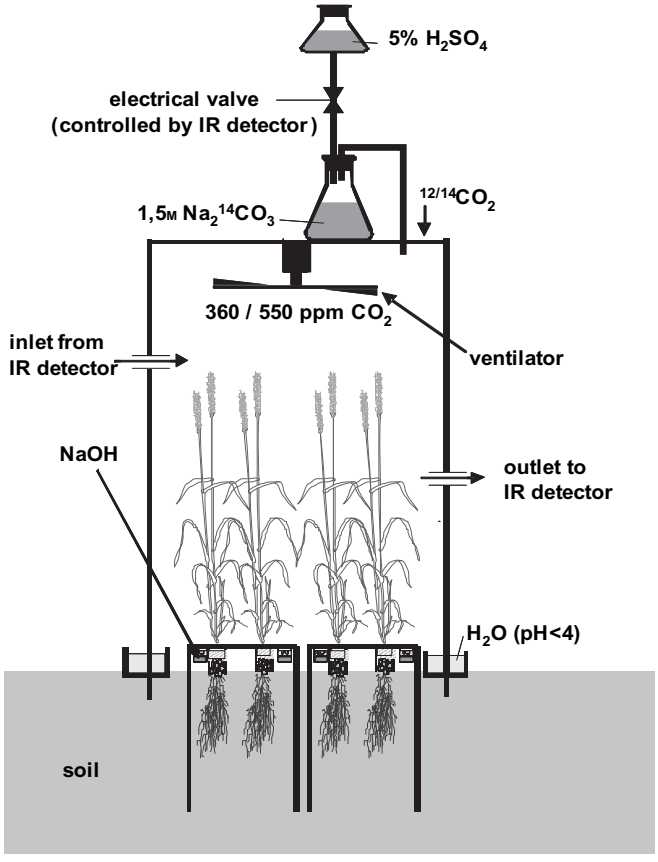


**Fig. 1.** Experimental design for $^{14}CO_2$ pulse-labelling of spring wheat plants grown in soil columns under ambient and FACE conditions.

During the $^{14}C$-labelling procedure the photosynthetically active radiation (PAR) and the temperatures inside and outside the chambers were recorded. During the growth period the plants were fertilized several times with a liquid NPK fertilizer amounting to a calculated total addition of 220 kg N $ha^{-1}$. The volumetric water content of the soil in the columns under both $CO_2$ regimes was measured daily and was maintained at a value of 20–30%. The $^{14}C$-labelling was performed during the late morning and lasted 1–2 h, depending on the weather conditions. During this time the temperature inside the chambers could rise, but was limited to a maximal value of 4 °C above ambient. A further increase of temperature was avoided by protecting the whole chamber from direct sun light with a sunshade and/or by placing buckets with ice inside the chamber. Just before the start of the first $^{14}C$-labelling procedure 6 plastic beakers with 12 ml of a 30% carbonate-free NaOH were placed through the remaining still open wholes of the PVC plates, covering each column. The wholes above the alkaline were closed with rubber bungs. The alkaline was exchanged each day till to the end of the experiment at the 2nd of August. The unified contents of the beakers from each column sampled per day were brought to a defined volume (500 ml) and aliquots were used to determine their $^{14}C$ content by liquid scintillation counting (LSC) and their total $CO_2$ content by gas chromatography (Martens, 1987).

### 2.3. Soil and plant analyses after harvest

After termination of the experiment the plants were harvested two days later by cutting at ground level and subsequent drying at 80 °C for 48 h. The plant biomass was separated into stems, leaves, glumes and grain. These were milled and 5 replicates of 25 mg were combusted in an oxidizer (Biological Oxidizer, OX-300, Zinsser Analytic, Frankfurt/Main, Germany) and measurement of absorbed $^{14}CO_2$ by LSC.

After removal of the above ground plant material soil samples from the upper 30 cm were taken from each column and visible stones and roots were removed. In these field fresh samples microbial biomass $^{12}C$ and $^{14}C$ was estimated by a modification of the fumigation-extraction method (CFE, Vance et al., 1987) as proposed by Mueller et al. (1992). This technique takes into account that fresh root material can considerably influence the quantification of microbial biomass C (Sparling et al., 1985; Mueller et al., 1992). The method started with a 30 min pre-extraction by shaking two 20 g (dry weight) soil samples with 80 ml 0.05 M $K_2SO_4$ each on a rotary shaker. The soil suspensions were passed through a 2 mm sieve which collected the remaining roots and the sieved soil suspensions were centrifuged. After decantation of the clear supernatants few drops of $CHCl_3$ were added to one soil sample and this was fumigated as usual (Vance et al., 1987). After fumigation and removal of the $CHCl_3$ by repeated evacuation the fumigated and non-fumigated soil sample were extracted for 30 min with 80 ml 0.5 M $K_2SO_4$ on a rotary shaker and filtered through a filter paper. Total organic carbon in the extracts was determined in an automatically operating instrument with a u.v.-persulfate oxidation and quantification of liberated $CO_2$ by an IR detector (Liqui-TOC, Elementar Analysentechnik, Hamburg, Germany). Aliquots of the extracts were taken to quantify the $^{14}C$ content by LSC. Microbial biomass C and $^{14}C$ was calculated by the application of a $k_{EC}$ value of 0.35, which had been established in a previous experiment by a calibration of the applied CFE technique against the fumigation-incubation method (CFI, Jenkinson and Powlson, 1976). The estimation of microbial biomass C and $^{14}C$ was carried out in quadruplicates.

For further analyses, the columns were excavated and their soil was removed corresponding to the three optically different layers of 0–30 cm, 30–45 cm and 45–70 cm. For sieving (<2 mm) the different soil layers of each column were slightly dried and the visible roots were collected, dried and weighed. Due to minor amounts of roots in the two lower layers, the residual $^{14}C$ content of each column was estimated only in the soil of the 0–30 cm layer. After mixing of this soil layer in a cement mixer, ten random samples of about 50 g were taken and dried (105 °C). Since the root material was expected to contain a much higher $^{14}C$ signature in comparison to the soil, special efforts were made to remove all root residues inevitably remaining in soil samples after a 2 mm sieving (Kuzyakov et al., 2001). For an efficient removal of root residues we applied the technique of soil sample dispersion in a heavy halogenated hydrocarbon (Ford et al., 1969). Fifty milliliter chloroform were added to each dried soil sample and the suspension was vigorously stirred for 30 s. Thereafter, the soil sedimented within a few seconds and the fine roots and other residual light plant material accumulated at the surface and could be separated from soil on a 250 μm sieve. This procedure was repeated once. After evaporation of the $CHCl_3$ the soil was ground and 5 replicates of 2 g of each pulverised soil sample were combusted in the above mentioned $^{14}C$-carbon oxidizer and measurement of absorbed $^{14}CO_2$ by LSC, giving a total of 50 estimates of $^{14}C$ for each soil column.

These root-free soil samples were used to analyse the distribution of soil-bound residual $^{14}C$ within soil particle size fractions. The applied fractionation procedure allowed to separate the fractions >63 μm, 63–20 μm, 20–2 μm and <2 μm (North, 1976; Christensen, 1992; Amelung and Zech, 1999). The principal steps are a physical dispersion of soil with ultrasound and a separation of particles by sieving and sedimentation. 20 g soil (dwt.) was suspended in 150 ml distilled water. The suspension was treated with 60 J $ml^{-1}$ ultrasound and sieved through a 63 μm sieve. For a disintegration of the remaining meso- and micro-aggregates in the filtrate the suspension was treated with 440 J $ml^{-1}$ ultrasound. Thereafter, eight repeated centrifugations at 400 rev $min^{-1}$ (24 *g*) and re-suspension separated the silt fraction (63–2 μm) as pellet and the clay fraction (<2 μm) in the combined supernatants. By the addition of 50 ml of a 10% solution of $MgCl_2$ the clay fraction was precipitated and isolated by centrifugation at 3200 rev $min^{-1}$. The pellet of the silt fraction was re-suspended and sieved through a 20 μm sieve. After washing with distilled water, the sieve contained the 63–20 μm fraction. In the filtrate the 20–2 μm particles were allowed to settle and were isolated by decantation of the supernatant. The isolated fractions were dried at 70 °C and their weight estimated. The $^{14}C$ contents in these fractions were estimated by dry combustion in the $^{14}C$-carbon oxidizer.

### 2.4. Statistical analyses

Each of the soil columns was considered as a replicate within one $CO_2$ treatment ($n = 4$). Results were given as mean of the four replicates and their corresponding standard deviation. The significance ($P < 0.05$) of the effects of elevated $CO_2$ was tested using a paired Student's *t*-test as calculated by the Sigma-Plot software (version 8.0).

## 3. Results

### 3.1. Plant growth

The wheat plants showed no detectable plant disease. Each plant developed on average 3.4 ears under both treatments. The 10 plants in each column produced a mean above ground biomass of 137.4 ± 8.8 g (dry weight) in the ambient $CO_2$ ring and 136.7 ± 10.7 g in the $CO_2$-elevated ring (Table 1). From each column the average total amount of roots collected from the upper 45 cm was 11.4 ± 1.2 g (dry weight) in the ambient $CO_2$ ring and 11.4 ± 0.8 g in the elevated $CO_2$ ring (Table 1). Below 45 cm soil depth only minor root material was detected. From these results no significant influence of elevated $CO_2$ on above and belowground plant growth of spring wheat could be derived. The known reduced transpiration of C3 plants under FACE was also observed in this experiment. Under the elevated $CO_2$ conditions 32% less water was necessary to keep the soil water content at the same level as in the ambient $CO_2$ ring (data not shown).

### 3.2. Uptake and distribution of $^{14}C$ during wheat growth

The plants under both $CO_2$ regimes were $^{14}CO_2$-labelled in total for 18 h. During this time the uptake of $^{14}CO_2$ indicated at all dates a higher assimilation of $^{14}CO_2$ under elevated $CO_2$ than under ambient $CO_2$ concentration. This was especially pronounced at days with a high photosynthetically active radiation (Fig. 2). During the 13 $^{14}CO_2$ pulse-labelling events the plants under ambient $CO_2$ assimilated a total of 22.74 l $^{14}CO_2$, while those under FACE used 25.76 l $^{14}CO_2$, corresponding to an additional $^{14}CO_2$ uptake of 13.3%.

Corresponding to this difference the above ground plant material grown in each column contained under ambient $CO_2$ an average of 2920 ± 256 kBq $^{14}C$ after harvest and under elevated $CO_2$ 3257 ± 262 kBq $^{14}C$. The corresponding values for the root material were 77.0 ± 5.5 kBq $^{14}C$ and 81.6 ± 12.4 kBq $^{14}C$, respectively. However, due to the variability of plant growth these differences were not significant.

Total $^{14}C$-activity recovered from above and belowground plant material developed under FACE was higher by 11.4% than that of plant material grown under ambient conditions. The additional uptake of $^{14}CO_2$ under elevated $CO_2$ was also reflected in the amount of $^{14}CO_2$ liberated from soil in the columns and absorbed in the NaOH. Root respiration and microbial mineralization of rhizodeposits evolved during the whole experimental time in total 274 ± 18 kBq $^{14}CO_2$ under ambient $CO_2$ and 350 ± 18 kBq $^{14}CO_2$ under FACE, corresponding to an increase of 27.7% (Fig. 3).

The higher carbon input by roots and its mineralization by micro-organisms under FACE was confirmed by the analysis of total $CO_2$ absorbed in the NaOH (Fig. 4). 11.2 ± 0.12 g $CO_2$–C were

**Table 1**
Mean (g dry weight $column^{-1}$) and total (g dry weight 4 $columns^{-1}$) above and belowground plant biomass of ripe spring wheat plants, grown under ambient or FACE conditions in undisturbed soil columns.

| | Above ground ambient | | | Above ground FACE | | | Below ground ambient | Below ground FACE |
|---|---|---|---|---|---|---|---|---|
| | Stems, leaves, glumes | Grain | Total | Stems, leaves, glumes | Grain | Total | Roots | Roots |
| Mean ± SD[a] | 69.7 ± 5.0 | 67.7 ± 3.9 | 137.4 ± 8.8 | 69.9 ± 5.0 | 66.9 ± 5.7 | 136.7 ± 10.7 | 11.4 ± 1.2 | 11.4 ± 0.8 |
| Total | 278.9 | 270.8 | 549.7 | 279.4 | 267.5 | 546.9 | 45.7 | 45.4 |
| HI[b] | | | 0.49 | | | 0.51 | | |

[a] SD = Standard deviation ($n = 4$).
[b] HI = Harvest index.

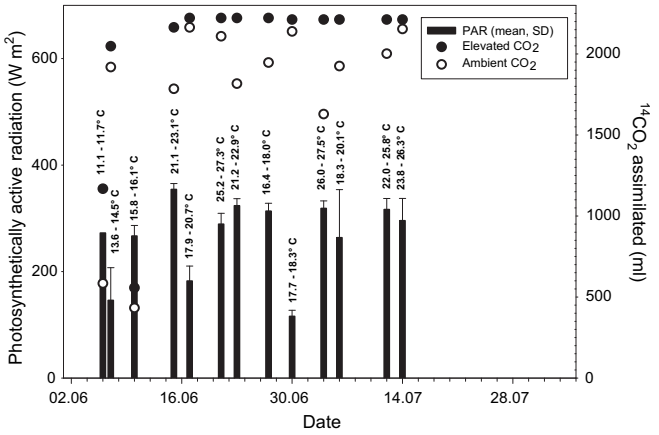


**Fig. 2.** Photosynthetically active radiation (PAR), air temperature and amount of assimilated $^{14}CO_2$ during pulse-labelling of spring wheat grown in soil columns under ambient and FACE conditions.

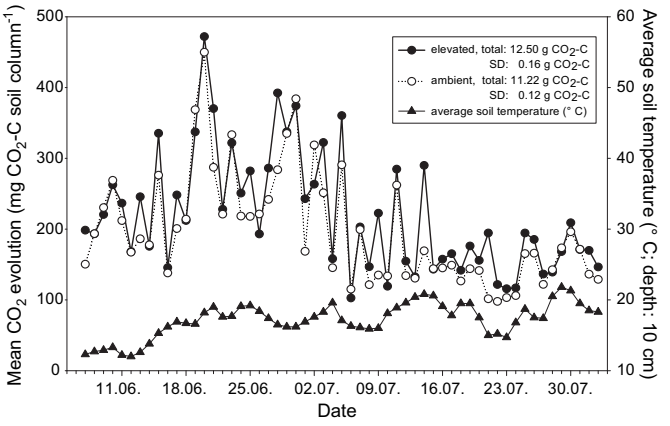


**Fig. 4.** Daily evolution of total $CO_2$ from soil columns and associated soil temperature during growth of $^{14}CO_2$ pulse-labelled spring wheat cultivated under ambient and FACE conditions.

liberated under ambient conditions and 12.5 ± 0.16 g $CO_2$–C were found under elevated $CO_2$, an increase of 11.6%.

The parallel determination of $^{14}CO_2$ and total $CO_2$ allowed the calculation of the specific $^{14}C$-activity of evolved $^{14}CO_2$ and hence to assess a possible difference in mineralization of pre-existing SOC (priming effect). This approach requires the same specific $^{14}C$-activity of plant derived $^{14}CO_2$ liberated from soil under both $CO_2$ regimes. This can be assumed for the present experiment since the wheat roots grown under ambient and FACE conditions had the same specific $^{14}C$-activity at harvest (20.6 Bq ± 1.7 mg $C^{-1}$ and 20.7 ± 0.8 Bq mg $C^{-1}$, respectively; data not shown). Fig. 5 illustrates the specific $^{14}C$-activity of $^{14}CO_2$ evolved from the soil under ambient and elevated $CO_2$, calculated for seven selected time intervals between successive $^{14}C$-labellings. The same parameter was also calculated for the whole growth period between the first $^{14}CO_2$-labelling and harvest. The first two calculations indicated a slightly higher mineralization of SOC under FACE but later this trend was reversed. Also the calculation for the whole period indicated a slight, but significantly higher mineralization of SOC under ambient $CO_2$.

The higher translocation of carbon belowground under FACE resulted also in a higher amount of $^{14}C$ retained after harvest in soil. Under this condition each soil column contained a mean $^{14}C$-activity of 54.3 ± 5.6 kBq in the upper 30 cm and was higher by 27.2% as found for a soil column under ambient $CO_2$ (Table 2). Since

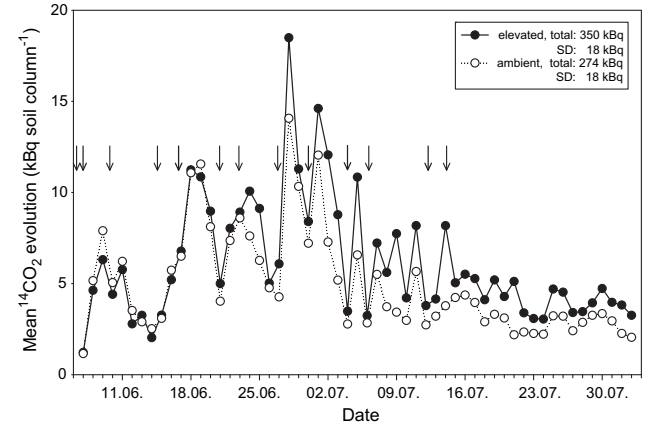


**Fig. 3.** Daily evolution of $^{14}CO_2$ from soil columns during growth of $^{14}CO_2$ pulse-labelled spring wheat cultivated under ambient and FACE conditions. Arrows indicate day of $^{14}CO_2$ pulse-labelling.

the variability (coefficient of variation) of determined $^{14}C$-activity in replicate sub-samples of one soil column were only 3–6%, we assume that the root residues with their high specific activity were successfully removed and did not influence the results of residual $^{14}C$ content in soil.

During soil fractionation 98% of original soil weight was recovered as particle sizes fractions. These fractions contained 83–97% of total residual $^{14}C$-activity of each root-free soil sample. The percentage distribution of this recovered $^{14}C$-activity in the particle size fractions was very similar in the soil samples from the ambient and FACE ring. More than three-quarter of total labelled residues (76 and 80%, respectively) were found in the stable pool of the clay bound organic matter and only 7% were analysed for the two silt fractions (63–2 μm) in both soil variants. The sand fraction (>63 μm) contained 17% (ambient) or 13% (FACE) of totally recovered $^{14}C$-activity. Fig. 6 illustrates that the difference found for total

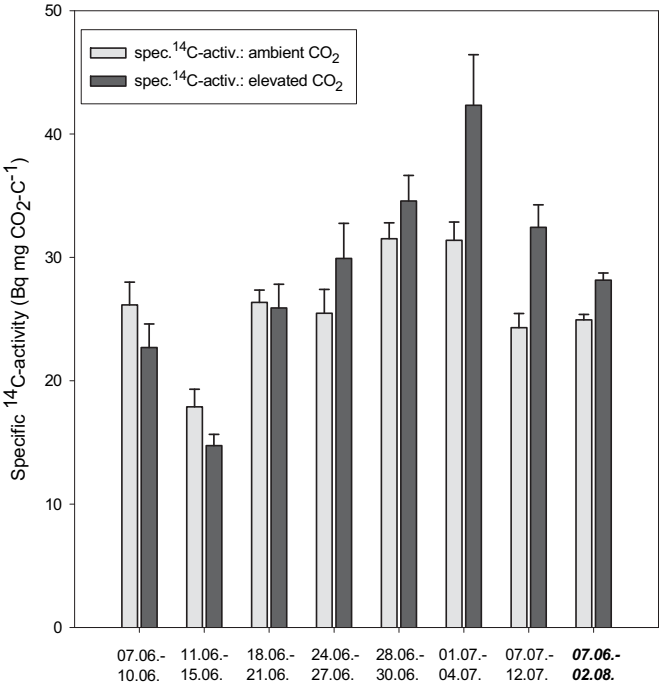


**Fig. 5.** Specific activity of soil born $^{14}CO_2$ calculated for selected periods between successive $^{14}CO_2$ pulse-labellings and for the whole experimental time of spring wheat cultivation under ambient and FACE conditions.

**Table 2**
Distribution of $^{14}C$ (kBq) after harvest of 10 spring wheat plants grown in soil columns under ambient or FACE conditions and a concomitant repeated pulse labelling with $^{14}CO_2$.

| | Above ground plant material | | Roots | | Total $^{14}CO_2$ evolved from soil till harvest | | Residual $^{14}C$ in soil | |
|---|---|---|---|---|---|---|---|---|
| | Ambient | FACE | Ambient | FACE | Ambient | FACE | Ambient | FACE |
| Mean | 2920 | 3257 | 77.0 | 81.6 | 274 | 350 | 42.7 | 54.3 |
| ±SD[a] | ±256 | ±262 | ±5.5 | ±12.4 | ±17.5 | ±18.0 | ±5.6[b] | ±5.6[b] |
| Total | 11 680 | 13 028 | 308 | 326 | 1096 | 1400 | 171 | 217 |
| Significance of difference | $P = 0.115$ | | $P = 0.522$ | | $P = 0.001$ | | $P = 0.029$ | |

[a] SD = Standard deviation ($n = 4$).
[b] = Standard deviation ($n = 50$).

residual $^{14}C$-between soil samples from the two $CO_2$ treatments was mainly due to the different amounts of $^{14}C$ in the clay fraction.

The percentage distribution of totally recovered $^{14}C$-activity after harvest was also very similar under both $CO_2$ regimes: under ambient conditions 90.2% were fixed in the above ground biomass, 8.3% were liberated from soil as $^{14}CO_2$, 2.3% were found as root material and 1.3% remained as residual $^{14}C$-activity in soil, the corresponding data for the FACE variant were 87.0%, 9.3%, 2.2% and 1.5%, respectively.

### 3.3. Soil microbial biomass C and $^{14}C$

Microbial biomass C estimated in soil samples from the columns under ambient and elevated $CO_2$ conditions before wheat growth showed no significant difference. Mean values of 185.5 ± 17.4 and 177.8 ± 7.2 μgC $g^{-1}$ soil, respectively, were found. In spite of the rhizodepositions of wheat plants supplied to the soils during plant growth, no increase of microbial biomass C after harvest in August was measured. A mean of 180.4 ± 5.0 μg $C_{mic}$ $g^{-1}$ soil was found in the soil columns under ambient conditions and 181.5 ± 12.0 μg $C_{mic}$ $g^{-1}$ soil under FACE.

A reliable assessment of microbial biomass $^{14}C$ contents failed due to very low $^{14}C$ activities in the extracts of the applied CFE method. There was in fact a small difference of $^{14}C$ contents in the $K_2SO_4$ extracts of the controls and the fumigated samples indicating the formation of $^{14}C$-labelled biomass. However, this difference of about 10–20 counts $min^{-1}$ (dpm) did not allow a reasonable calculation of microbial biomass $^{14}C$ and to evaluate a possible difference in microbial biomass $^{14}C$ contents in the soil from the ambient and the FACE plot.

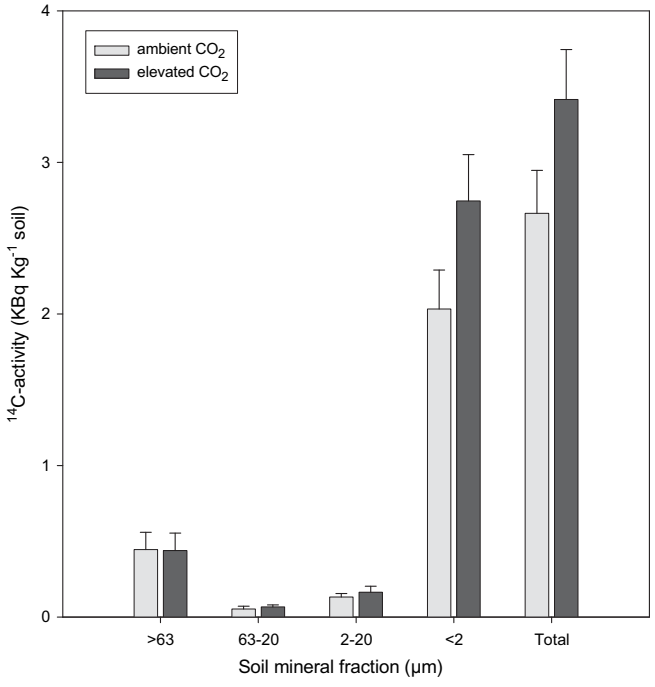


**Fig. 6.** Distribution of $^{14}C$-activity within the different soil mineral fractions after repeated $^{14}CO_2$ pulse-labelling of spring wheat grown under ambient and FACE conditions.

## 4. Discussion

### 4.1. Plant growth

The aim of this investigation was to elucidate the possible effect of elevated atmospheric $CO_2$ on the belowground carbon input during the growth of the spring wheat cultivar "Minaret". Since the amount of rhizodepositions is related to the photosynthetic capacity (Dijkstra et al., 2006), the extent of the $CO_2$ effect will generally depend on the development of plant biomass. But this can vary considerably, even when the same cultivar was grown. This was demonstrated by the unchambered field trials within the European Stress Physiology and Climate Experiment (ESPACE; Jäger et al., 1999; Bender et al., 1999).

The almost identical amounts of above and belowground plant biomass including grain yield (Table 1) under ambient and elevated $CO_2$ conditions in the present experiment contradicts the general experience of increasing plant growth of C3 plants with increasing $CO_2$ content of the atmosphere, although this growth stimulation has been found to be rather small for wheat (Amthor, 2001; Weigel et al., 2005; Ainsworth and Long, 2005). The missing effect of elevated $CO_2$ on plant biomass in the present investigation may be attributed to the small numbers of plants which already caused a coefficient of variation of 6% (control) and 8% (FACE), respectively, and so did not allow detecting the expected differences.

### 4.2. Distribution of $^{14}C$-activity after wheat harvest

In the present experiment the fixation of labelled $^{14}CO_2$ per unit time was clearly enhanced under elevated $CO_2$. This was reflected in the higher total $^{14}C$-activity in the shoots and the roots at harvest, but due to the mentioned variability in plant biomass production these differences were also not significant (Table 2). However, a highly significant difference was calculated for $^{14}CO_2$ liberated from the soil columns under FACE (+27.7%) and those under ambient conditions. The evolution of total $CO_2$ (Fig. 3) was also significantly higher under elevated $CO_2$ (+11.7% for FACE) but less pronounced than that of $^{14}CO_2$. This different effect of FACE conditions on soil born $CO_2$ and $^{14}CO_2$ may be due to the selection of days with a high photosynthetically active radiation for $^{14}CO_2$-labelling and a concomitant higher difference in $^{14}CO_2$ fixation. Only three of thirteen labellings took place on cloudy days (Fig. 2). In the only similar investigation monitoring total soil $CO_2$

respiration under wheat, Pendall et al. (2001) measured a substantial increase of total soil respiration by 70% and 40% under FACE in two successive years during day 50 and 100 of spring wheat growth. However, this FACE experiment at Maricopa (Arizona) was run in a desert with high radiation and soil temperatures between 30 °C and 35 °C, about 10–15 °C higher than those of our experiment (Fig. 3). A much lower stimulation of soil respiration under elevated $CO_2$ (+19%) was reported by Billes et al. (1993) in a growth chamber experiment with spring wheat during the first 4 weeks of growth.

In the present experiment we could directly show that during the growth of a crop plant under FACE more carbon was sequestrated into an undisturbed soil than under ambient $CO_2$. The percentage increase of residual soil-$^{14}C$ under FACE (+27.2%) corresponded to the measured increase of liberated $^{14}CO_2$ (+27.7%). Using this analogy we expect that the additional total $CO_2$ respired from the soil columns under FACE (+11.6%) indicates approximately the same percentage of additional total carbon sequestrated under FACE. This assumption requires that the distribution of assimilated $^{14}C$ in shoots, roots, soil respiration and soil organic carbon represent also the corresponding distribution of total carbon. This also implies that the carbon allocation pattern did not change considerably during a day and the days between two labellings. Swinnen et al. (1994) pulse-labelled the spring wheat cultivar "Minaret" in the field at different times of the day and found that the timing influenced the gross net carbon assimilation, but had no significant effect on percentage of carbon distribution. Carbon allocation by cereals changes during growth with a high translocation of photosynthates belowground by young plants and a continuously decline of this fraction during further development (Keith et al., 1986; Gregory and Atwell, 1991; Jensen, 1993). During the period of pronounced belowground carbon translocation the time between $^{14}CO_2$ pulse-labellings were 1–4 days with one exception of 5 days (Fig. 2). For this short span of time we do not expect a considerable change of carbon distribution due to a change of plant development. Therefore, we believe that the application of the above described analogy between totally evolved $CO_2$ and additionally sequestrated carbon under FACE can be justified.

The extent of this additional sequestration of carbon under FACE will of course be determined by the reaction of wheat plants to elevated $CO_2$ relative to the control. Up to the growth phase of grain filling increasing temperature will enhance the $CO_2$ effect (Long, 1991; Manderscheid et al., 2003). The difference in wheat growth response is more pronounced under ample nitrogen and limited water availability (Kimball et al., 2002; De Graff et al., 2006). Latter effect is caused by the improved water-use efficiency of C3 and C4 plants under elevated $CO_2$. In addition, the particular wheat cultivar (Manderscheid and Weigel, 1999) and its growth conditions will also determine the reaction to elevated $CO_2$ (Bender et al., 1999). It can be assumed that these differences will also influence the difference of carbon transferred belowground.

### 4.3. Priming effect

The often found increased allocation of carbon belowground in elevated $CO_2$ atmospheres has raised the question of an additional mineralization of the pre-existing soil organic matter (positive 'priming effect'), which is supposed to be caused by the observed higher microbial activity under this condition. This effect could possibly offset an additional soil carbon gain under elevated $CO_2$ (Cheng, 1999; Cardon et al., 2001; Gill et al., 2002). Under this aspect, the quantification of plant root-induced priming effects and the underlying mechanisms have gained additional, actual importance. Since no (Haider et al., 1989), negative (Reid and Goss, 1983; Martin, 1987; Cheng, 1996) or positive (Helal and Sauerbeck, 1984; Cheng et al., 2003) priming effects were observed, different theories were offered to explain the corresponding results (Dormaar, 1990; Kuzyakov, 2002). Fu and Cheng (2002) concluded that obviously the particular plant species, the growth conditions, the development stage, the soil nutrient status and the soil type affect the direction and magnitude of the priming effects. This is also valid for possible accelerated priming under elevated atmospheric $CO_2$ (Cheng, 1999). The calculation of the specific $^{14}C$-activity of evolved $^{14}CO_2$ from soil columns under both $CO_2$ regimes (Fig. 5) observed in the present experiment indicates that the higher microbial activity under FACE did not lead inevitably to a higher mineralization of the pre-existing SOC, rather a slightly higher mineralization of SOC under ambient $CO_2$ can be derived. However, due to the above made assumptions as a base for these calculations we would not push this result too far. Therefore, we conclude that the additional residual $^{14}C$ and the missing additional priming effect analysed for the soil columns under FACE must have led to a net increase of carbon in comparison to the soil under ambient $CO_2$. The fixation of most of this additional carbon in the clay fraction suggests that under FACE conditions a considerable contribution was made to the long-term storage of soil carbon in this soil.

### 4.4. Soil microbial biomass C and $^{14}C$

Although agricultural plants release during their growth 1200–2900 kg C $ha^{-1}$ into soils (Whipps, 1990) no increase of microbial biomass C could be measured after growth of the spring wheat plants in this present investigation. In view of the large carbon input, this result seems unexpected, but in accordance with other investigations (Patra et al., 1990; Nieder et al., 2008). Referring to the investigations by Anderson and Domsch (1985) it can be concluded that most of the carbon released by plants into the soil is used for activation and maintenance of the initially dormant microbial population. These processes lead only to the release of $CO_2$ without cell growth. Only additional carbon sources, if available, can initiate a proliferation of microbial biomass. Obviously, the higher carbon input during wheat growth under elevated $CO_2$, did not change the availability of carbon in an extent which allowed a significant proliferation of the microbial biomass.

## 5. Conclusions

By a repeated $^{14}CO_2$ pulse-labelling between stem elongation and begin of ripening we could show for the first time that a crop plant (spring wheat cultivar) grown under FACE conditions deposited significantly more carbon to soil than those grown under ambient $CO_2$ in the field. This was indicated by a 28% higher liberation of soil born $^{14}CO_2$ and 12% higher evolution of total $CO_2$. The higher carbon input was also reflected in a 27% higher content of $^{14}C$-labelled residual soil carbon at harvest. Three-quarter of residual $^{14}C$ was integrated into the stable, clay bound soil organic matter pool. Using the analogy between soil born $^{14}CO_2$ and residual $^{14}C$ in soil which increased under elevated $CO_2$ by 27% and 28%, respectively, we assume that 12% more total $CO_2$ correspond to about the same percentage increase in total carbon under FACE. From a calculation of the specific activity of the $^{14}CO_2$ evolved from soil under both $CO_2$ conditions we conclude that the additional carbon input under elevated $CO_2$ did not induce an accelerated degradation of pre-existing soil organic matter (no positive priming effect). The experimental data of the present investigation allows deducing that the growth of wheat plants under FACE conditions leads to an additional net gain of soil organic carbon under the conditions of a temperate climate and an ample water and nutrient supply.

## Acknowledgements

We thank Gisela Niegisch, Ute Wildschütz, Carina Trenkler, Anke Mundt, for technical assistance in the field and laboratory. Technical assistance by the FAL experimental farm and the provision of weather data by the DWD is gratefully acknowledged. We are also grateful to George Hendrey, Keith Lewin and John Nagy (Brookhaven National Laboratory, Upton, New York, USA) for their help in establishing and operating the FACE system and to the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) for financing the FACE unit.

The study was partly supported by the German Research Foundation (DFG).

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