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# Potential impact of $CO_2$ leakage from carbon capture and storage (CCS) systems on growth and yield in spring field bean

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ABSTRACT

Anthropogenic carbon dioxide ($CO_2$) emissions are a major factor contributing to rising global temperatures and climate change. Carbon capture and storage (CCS) is a potential mitigating measure which would allow industrial emissions to be captured and stored within deep geological sites. However, in view of possible subsequent leakage to the biosphere, this study examined the impact of elevated soil $CO_2$ on root and shoot growth and crop yield in spring field bean (*Vicia faba* L.) under field conditions. The results revealed a strong inverse correlation between soil $CO_2$ and $O_2$ concentrations. An area containing plants exhibiting severe chlorosis, reduced growth and extensive mortality developed during the gassing period where soil $[CO_2]$ was greatest and $[O_2]$ was lowest. Root and shoot growth in surviving plants was significantly lower in gassed than in control plots when soil $[CO_2]$ exceeded 10%. Mean values for vegetative (shoot, stem and leaf dry weight plant$^{-1}$, leaf area plant$^{-1}$) and reproductive variables (pod and seed number plant$^{-1}$ and seed dry weight plant$^{-1}$ were reduced by 36–65% compared to control plants. The only variable which was positively affected by gassing was individual seed dry weight, which was increased by 18%. The results demonstrate the severity of damage to terrestrial vegetation that may be induced by $CO_2$ leakage from CCS transport or storage facilities.

## 1. Introduction

The increasing atmospheric concentration of greenhouse gases, particularly $CO_2$, has resulted in progressive warming of the Earth's climate in a phenomenon often referred to as global climate change. Atmospheric $CO_2$ concentration $[CO_2]$ has increased at a mean rate of 1.9% yr$^{-1}$ from 280 ppm before the industrial revolution to the current level of *c.* 387 ppm (IPCC, 2007). Carbon capture and storage (CCS) has been proposed as an effective option for reducing $CO_2$ emissions resulting from combustion of fossil fuels and would enable continued use of coal, oil and gas for the foreseeable future (Gough and Shackley, 2005). CCS technology can be used to capture $CO_2$ from major industrial sources before it is released to the atmosphere; this is then transported and injected into secure geological or oceanic reservoirs for underground storage as compressed gas or fluid. Although CCS technology has gained wider public acceptance than alternative energy sources such as nuclear power, $CO_2$ leakage from transport pipelines or storage sites (Klusman, 2003) may migrate to the Earth's surface where it adversely affects water and soil properties and plant growth (Steven and Smith, 2010). The ability to determine the integrity of prospective transport and storage facilities, a proper understanding of the pathways through which $CO_2$ migrates to the Earth's surface, and assurance that leakage from storage facilities is minimal are essential for effective sequestration (Amonette and Barr, 2010). However, as CCS technology has only been recently been implemented, plant responses to $CO_2$ leakage from CCS facilities have not been examined in detail.

As roots are important in determining plant growth, development and yield, roots and shoots must both be examined to obtain a full understanding of plant responses to elevated soil $[CO_2]$ (Upchurch and Ritchie, 1983). However, relatively little is known regarding the effects of elevated soil $[CO_2]$ on root growth and development as traditional methods for studying roots are both labour-intensive and destructive. Nevertheless, several studies have examined the adverse effect of elevated soil $[CO_2]$ on the growth of plants and their root systems at sites where substrate concentrations are high, including landfill sites where increased soil $[CO_2]$ was shown to influence root growth in green ash (*Fraxinus lanceolata* Borkh.) and hybrid poplar (Populus spp.; Flower et al., 1981). The impact of natural $CO_2$ springs on root function has been also examined and inhibition of root respiration was found in several grass species at Stavešinci in Slovenia (Maček et al., 2005). Similarly, injection of $CO_2$ into undisturbed soil profiles substantially reduced both above- and below-ground vegetative biomass in turf composed of *Lolium perenne* L., *Festuca rubra* L. and *Poa pratensis* L. (Pierce and Sjögersten, 2009) and significantly

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impaired root survival and growth in seedlings of *Vicia faba* L. under field conditions (Al-Traboulsi, 2011).

Hypoxia caused by depletion of $O_2$ is common in flooded or waterlogged soils and landfill sites and presents an unfavourable environment for most plant species (Drew, 1997; Parent et al., 2008) which may adversely affect their growth and productivity (Pociecha et al., 2008). Several studies have shown that a lack of sufficient $O_2$ to support respiration causes damage and root death in plants exposed to hypoxia (Kozlowski, 1984; Crawford, 1992; Henshaw et al., 2007; Shi et al., 2007; Horchani et al., 2009). The effect of flooding has been examined in *V. faba* major L. (Balakhnina and Bennicelli, 2010) and numerous other species including *Fraxinus pennsylvanica* (Sena Gomes and Kozlowski, 1980). Low soil $[O_2]$ associated with hypoxic conditions induced by flooding may also reduce root permeability (Clarkson et al., 2000) and leaf area, contributing to the inhibition of photosynthesis and assimilate production during the later stages of growth (Sena Gomes and Kozlowski, 1980).

It might be intuitive to assume that roots are the first organs to be damaged by hypoxic or waterlogged conditions as they are the initial point of contact with an adverse soil environment. However, information concerning the impact of elevated soil $[CO_2]$ and depleted $[O_2]$ on plant growth and productivity in the absence of waterlogging, such as might be induced by leaks from CCS systems, is extremely limited. The present study simulated the impact of the altered soil gaseous environment resulting from leakage of $CO_2$ from CCS transport pipelines or storage sites on the growth and productivity of spring field bean (*V. faba* L.), an important crop in temperate regions. The specific objective was to test the hypothesis that elevated soil $[CO_2]$ adversely affects root and shoot growth and crop performance due to the hypoxic conditions induced.

## 2. Materials and methods

### 2.1. Site description

The Artificial Soil Gassing and Response Detection (ASGARD) facility at the University of Nottingham Sutton Bonington Campus (52.8°N, 1.2°W) was used. ASGARD is located in a field previously under permanent pasture. The soil is a sandy loam to a depth of 60 cm, below which a 20 cm gravel layer overlies clay (Ford, 2006). ASGARD comprises 34 plots, each 2.5 m × 2.5 m in area, with 50 cm wide grass paths between them; eight plots were used in this study. $CO_2$ (British Oxygen Company, Windleham, UK) was injected into the soil at a rate of 1 L min$^{-1}$ through diffusers located 60 cm below the centre of four gassed plots to produce a gradient of soil $[CO_2]$; this was greatest close to the injector and decreased towards the edges. The remaining four plots were ungassed control plots. Computer software (TVC, Great Yarmouth, UK) controlled and recorded gas temperature, pressure and flow rate to each gassed plot. Grazing animals were excluded by an electric fence. The injection system was installed in 2006, two years before the experiment began.

### 2.2. Gas measurements

Two permanent plastic sampling tubes (100 cm deep × 19 mm internal diameter) were installed vertically to a depth of 30 cm at distances of 15 and 70 cm from the centre of each gassed plot (Fig. 1a). These were sealed at the bottom but holes near their lower end allowed gas exchange to maintain equilibrium with the surrounding soil atmosphere. A valve at the top of each sampling tube allowed soil $[CO_2]$ and $[O_2]$ to be measured using a GA2000 Landfill Gas Analyser (Geotechnical Instruments, Warwickshire, UK).

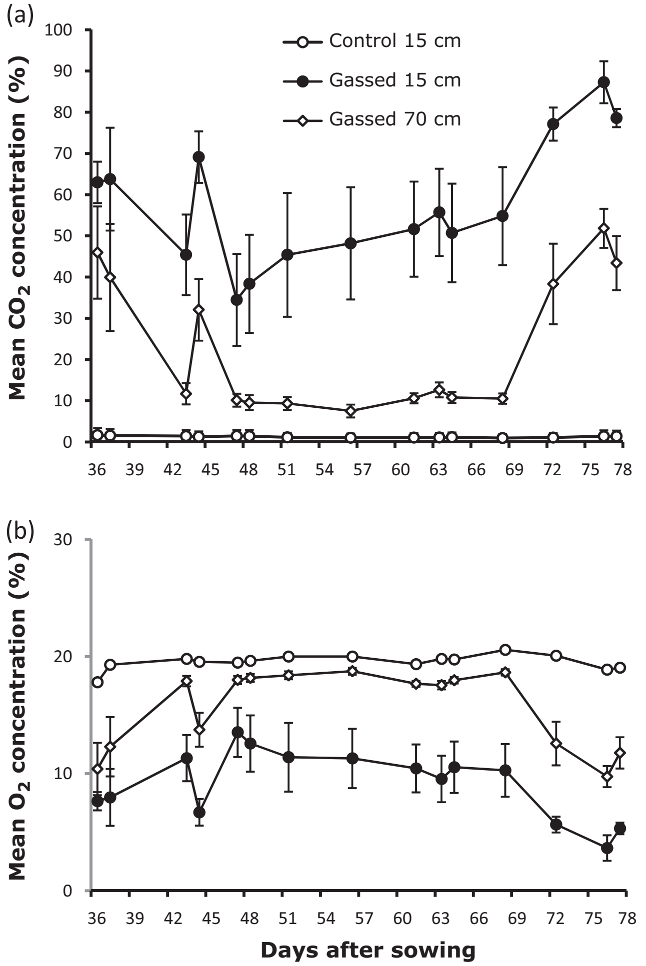

**Fig. 1.** Mean $CO_2$ (a) and $O_2$ concentrations (b) in control and gassed plots measured 15 or 70 cm from the plot centre between 10 July and 20 August 2008 (36-77 DAS). Double standard errors of the mean are shown ($n = 4$).

Barholing measurements were used to map the underground dispersion of $CO_2$ and $O_2$. All plots were nominally sub-divided into 25 squares, each 50 cm × 50 cm in area, and a purpose-designed barhole device (Peter Wood & Co Ltd, Sheffield, UK) was inserted to a depth of 30 cm at each corner of all squares. The GA2000 probe was inserted into the hole immediately after removing the barholing device to measure $[CO_2]$ and $[O_2]$. The maximum $[CO_2]$ value displayed over a 40 s sampling period was recorded as the concentration for that location and $[CO_2]$ values for all corners of each square were used to calculate the mean value. The mean values for each square in the four replicate gassed plots were displayed as contour maps using a grid-based graphics program, Surfer 07 (Golden Software Inc., Golden, Co, USA). Measurements at ASGARD in 2006 revealed a strong negative linear correlation between soil $[CO_2]$ and $[O_2]$ ($R^2 = 0.96$; Steven and Smith, 2010) whereby:

$$[O_2] = 20.078 - 0.2147[CO_2] \tag{1}$$

where [] denotes percentage gas concentration. This equation was used to estimate $[O_2]$ from the corresponding $[CO_2]$ values to construct $[O_2]$ contour maps.

### 2.3. Minirhizotron technology

Root distribution was examined using a minirhizotron system (Bartz Technology Inc., Santa Barbara, CA, USA). On 1 May 2008, two transparent tubes (125 cm long × 5 cm diameter) were installed at an angle of 45° to the soil surface to a vertical depth of 70 cm in each gassed plot; one tube was installed in each control plot. Tubes

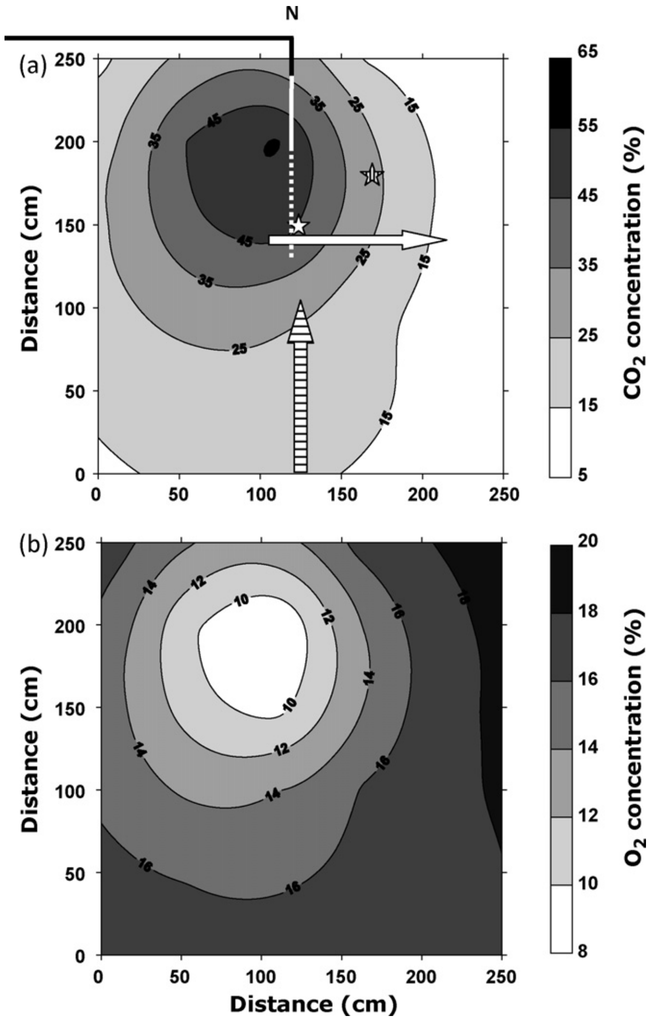

**Fig. 2.** Contour maps showing mean distribution of (a) $CO_2$ and (b) $O_2$ concentrations at 30 cm depth in the gassed plots. *X* and *Y* axes represent plot area (2.5 m × 2.5 m; *n* = 4). In (a) the horizontal black line and solid and dashed white line entering the plot from the North (N) represent the $CO_2$ supply line. The white and stippled stars represent the sampling points 15 and 70 cm from the plot centre; horizontal white and vertical stippled arrows represent orientations of the Bartz minirhizotron tubes.

in the gassed plots were inserted in different orientations i.e. tube $G_A$ entered the soil 100 cm from the plot centre, where soil [$CO_2$] was low, and sloped perpendicularly downwards across plant rows towards the plot centre (Fig. 2a). Tube $G_B$ entered the soil 15 cm from the plot centre, where soil [$CO_2$] was greatest, between two plant rows, and sloped parallel to the plant rows, towards the edge of the plot. Tube $G_A$ allowed the shallow roots of the potentially least damaged plants and the deeper roots of the most damaged plants near the plot centre to be examined. Conversely, tube $G_B$ examined the shallow roots of the most damaged plants near the plot centre and the deeper roots of the least damaged plants near the plot margins.

A BTC-2 microvideo camera system (Bartz Technology Inc., Santa Barbara, CA, USA) was used to record images of roots growing against the outer surface of the access tubes. The BTC I-CAP Image Capture System, a software and hardware package for digitising images, was used to collect and process root images. Images (12.5 mm vertical × 18 mm horizontal, area 2.43 $cm^2$) were recorded at 1 cm vertical depth intervals in each tube on seven sampling dates between July and August 2008. A total of 63 frames representing soil depths between 0 and 63 cm were analysed for each tube and sampling date, giving a total of 5292 images (63 depths × 12 tubes × 7 dates). The images were grouped within the depth ranges 0–10, 10–20, 20–30, 30–40, 40–50, 50–60 and 60–70 cm and analysed using RooTracker Version 2.0.3, a software package for root image analysis (Dave Tremmel, Duke University, Durham, NC, USA). The number of live main and lateral roots, root length and root diameter within each category were determined for each image.

### 2.4. Plant material and measurements

A dwarf field bean variety (*V. faba* L. *cv*. The Sutton; Thompson and Morgan Seeds, Suffolk, UK) was chosen to match the planned early summer sowing date, avoid lodging and facilitate measurements of root and shoot growth. This variety can be sown between March and July under field conditions in the UK and, although only 30 cm tall, produces numerous pods. As a short season crop which reaches maturity in 10–12 weeks, it was ideally suited for the present study. All plots were cultivated manually before hand-sowing seeds on 4 June 2008 at a depth of 5 cm in eight rows per plot. Spacing was 30 cm between rows and 23 cm within rows, giving a population of 80 plants $plot^{-1}$.

$CO_2$ injection began on 4 July 2008, 31 days after sowing (DAS), to allow the plants to establish fully prior to exposure as previous studies of winter-sown field bean showed that exposure to elevated soil [$CO_2$] from the time of sowing prevented germination and seedling establishment in the centre of gassed plots where [$CO_2$] was highest (Al-Traboulsi, 2011). Soil [$CO_2$] and [$O_2$] at a depth of 30 cm were measured daily 15 cm from the centre of the control plots and 15 and 70 cm from the centre of the gassed plots (Fig. 1a) between 10 July and 20 August 2008 (36-77 DAS). Chlorophyll content, plant height and leaf and pod number $plant^{-1}$ were determined non-destructively during the injection period for 10 randomly selected plants located 30, 60, 90 and 120 cm from the centre of each plot. The chlorophyll content of healthy leaves from the top, middle and bottom of each plant was determined using a portable chlorophyll meter (SPAD 502, Minolta, Chicago, USA); five SPAD readings for each leaf were averaged to provide mean values.

At harvest on 21 August 2008 (77 DAS), the gassed plots were sub-divided into 25 squares, each 50 cm × 50 cm in area, before recording plant number and harvesting plants from all squares. Six 50 cm × 50 cm squares were randomly selected for destructive harvest in each control plot. Height to the tip of the main stem was measured using a ruler and pod number was counted for all plants in each square. The plants were then divided into leaves, stems and pods; seed number $pod^{-1}$ and seed number $plant^{-1}$ were counted. Sub-samples (*c*. 20%) of leaves and stems from each square were weighed to determine fresh weight and leaf area was measured using a leaf area meter (Li-Cor Model 3100, Lincoln, NE, USA). All sub-samples were oven-dried at 85 °C for 48 h before determining dry weight. Mean monthly values for air temperature and relative humidity and total monthly rainfall and irradiance during the experimental period and the preceding 10 year period (1997–2007) were obtained from the Sutton Bonington meteorological centre.

### 2.5. Statistical analysis

The effects of treatment and location within the gassed plots on plant height, leaf and pod number $plant^{-1}$ and SPAD values were tested using repeated measures ANOVA. $CO_2$ treatment was the main effect (independent factor) with two levels, gassed and control; plant location (10, 30, 60, 90 and 120 cm from plot centre) and date were analysed as repeated measures. Interaction effects were also tested i.e. $CO_2$ × location, $CO_2$ × date, date × location, $CO_2$ × date × location. Effects of $CO_2$ enrichment and depth in the soil profile on root number, length and diameter were tested

**Table 1**
Mean daily air temperature, relative humidity, total rainfall and cumulative irradiance during the experimental period in 2008 and mean values for the preceding 10 year period (1997-2007). Standard errors of the mean are shown for mean values.

| | Mean air temperature (°C) | | Relative humidity (%) | | Total rainfall (mm) | | Total irradiance (MJ $m^{-2}$) | |
|---|---|---|---|---|---|---|---|---|
| Month | 1997/2007 | 2008 | 1997/2007 | 2008 | 1997/2007 | 2008 | 1997/2007 | 2008 |
| June | 15.2 ± 0.2 | 14.2 ± 0.3 | 79.0 ± 1.5 | 80.3 ± 1.5 | 56.3 ± 12.9 | 39.6 | 530 ± 10.4 | 564.2 |
| July | 16.6 ± 0.5 | 16.4 ± 0.4 | 80.1 ± 1.5 | 81.1 ± 1.4 | 57.6 ± 5.6 | 63.0 | 513 ± 21.6 | 527.0 |
| August | 16.7 ± 0.1 | 16.7 ± 0.2 | 80.5 ± 1.6 | 81.7 ± 1.3 | 63.2 ± 23.9 | 71.8 | 446 ± 8.1 | 391.9 |

using repeated measures ANOVA with [$CO_2$] as the main effect (independent factor) with two levels; the influence of depth (7 depths) and date (7 dates) were analysed as repeated measures. Interaction effects for $CO_2$ × depth, $CO_2$ × date, date × depth and $CO_2$ × date × depth were tested. One-way ANOVA was used to analyse leaf, stem and root fresh and dry weights, leaf area, pod number plant$^{-1}$ and seed number plant$^{-1}$ at harvest, with $CO_2$ as the main factor with two levels, control and gassed. All analyses were performed using SPSS 16.0 and significance was accepted at $P \leq 0.05$. Mean values for growth variables determined at harvest for all plants present within the 25 sub-plots in the gassed plots were displayed on contour maps using Surfer 07 (Golden Software, Inc., Colorado, USA). The correlations between shoot dry weight (g plant$^{-1}$) and soil [$CO_2$] and [$O_2$] were tested for significance and linear relationships fitted.

## 3. Results

### 3.1. Climatic conditions

Mean air temperature and relative humidity and monthly rainfall and irradiance during the experimental period were generally comparable to the corresponding values for the preceding 10 year period, although rainfall in June and August 2008 was respectively below and above the long-term average (Table 1). The differences in monthly rainfall were reflected by the above- and below-average cumulative irradiances in June and August.

### 3.2. Soil $CO_2$ and $O_2$ concentrations

Soil [$CO_2$] 15 cm from the centre of the gassed plots was much greater than at 70 cm from the centre ($P < 0.05$; Fig. 1a), and values for both locations were greater than in the control plots ($P < 0.05$). The increased [$CO_2$] in the gassed plots was reflected by a sharp decline in soil [$O_2$] relative to control plots (Fig. 1b), particularly near the plot centre. Mean soil [$CO_2$] was greatest near the injection point (56%; Fig. 2a), while [$O_2$] was at its lowest (5%; Fig. 2b). [$CO_2$] decreased towards the edges of the gassed plots, where a seasonal mean of 8.3% was recorded; seasonal mean [$O_2$] increased to 19.7% near the edges of the gassed plots, identical to the value for control plots (19.6%).

### 3.3. Growth parameters and chlorophyll content

The leaves of plants located near the $CO_2$ injection point became chlorotic 11 days after injection began (42 DAS), and this was followed by premature senescence, leaf abscission and plant death. An area of visible injury 100–120 cm in diameter developed close to the injection point, where [$CO_2$] ranged from 20 to 60% and [$O_2$] from 8 to 16%. Seeds and seedlings at the centre of this impact zone were killed, whereas seedlings further from the plot centre were chlorotic and stunted but survived. This effect intensified with time as plants near the injection point which experienced soil [$CO_2$] of 45–60% died before the experiment ended. The number of surviving plants 10 and 30 cm from the centre of gassed plots decreased by *c.* 50% between 43 and 48 DAS compared to distances of 90 and 120 cm from the centre and in the control treatment; the decrease in survival 60 cm from the centre of gassed plots was smaller (12.5%). At 69 DAS, survival 10, 30 and 60 cm from the plot centre was respectively 25, 50 and 75% compared to greater distances in the gassed plots and the control plots, in which no mortality occurred.

The $CO_2$ × date, $CO_2$ × location and $CO_2$ × date × location interactions were significant for plant height and leaf number plant$^{-1}$ (Table 2). The date × location interaction was significant for height, while the $CO_2$ × location interaction was significant for pod number plant$^{-1}$. Height increased with time in both treatments, but control plants reached a maximum of 55.1 ± 3.4 cm at 69 DAS compared to 35.3 ± 6.3 and 38.5 ± 4.9 cm at distances of 10 and 120 cm from the centre of gassed plots ($P < 0.001$). Leaf number plant$^{-1}$ increased with time in both treatments (Fig. 3a; $P < 0.05$), but was again greater in control plants ($P < 0.05$). Surviving plants 10 cm from the centre of gassed plots had fewer leaves than those further from the centre during the later stages of the experiment; at 36 DAS, leaf number plant$^{-1}$ 10 cm from plot centre was 10.5% greater in control plants (29 ± 2.8) than in gassed plants (26 ± 3.5), but this difference increased to 40.8% by 69 DAS. Mean pod number plant$^{-1}$ at 54 DAS for surviving plants 10 cm from the plot centre was 4-fold greater in control plants (6.6 ± 1.4) than in gassed plants (1.5 ± 0.3; Fig. 3b), but decreased by 28% in gassed plants between 61 and 69 DAS ($P < 0.05$); no significant change in pod number plant$^{-1}$ with

**Table 2**
Summary of two-way repeated measures ANOVA to determine the effect of elevated soil $CO_2$ on plant height, leaf number plant$^{-1}$, pod number plant$^{-1}$ and SPAD values ($n = 4$ comprising 40 gassed plants and 40 control plants).

| Variable | Source | *F* | *P* |
|---|---|---|---|
| Plant height (cm) | $CO_2$ | 8.7 | 0.025 |
| | Date | 41.2 | <0.001 |
| | Location | 2.1 | 0.119 |
| | $CO_2$ × date | 7.5 | 0.014 |
| | $CO_2$ × location | 6.6 | <0.001 |
| | Date × location | 5.8 | <0.001 |
| | $CO_2$ × date × location | 6.5 | <0.001 |
| Leaf number plant$^{-1}$ | $CO_2$ | 7.1 | 0.037 |
| | Date | 38.0 | <0.001 |
| | Location | 1.6 | 0.20 |
| | $CO_2$ × date | 7.6 | 0.016 |
| | $CO_2$ × location | 3.5 | 0.02 |
| | Date × location | 2.2 | 0.098 |
| | $CO_2$ × date × location | 3.2 | 0.027 |
| Pod number plant$^{-1}$ | $CO_2$ | 3.4 | 0.12 |
| | Date | 38.0 | 0.003 |
| | Location | 0.66 | 0.62 |
| | $CO_2$ × date | 0.87 | 0.43 |
| | $CO_2$ × location | 4.34 | 0.009 |
| | Date × location | 0.55 | 0.81 |
| | $CO_2$ × date × location | 1.04 | 0.42 |
| SPAD values | $CO_2$ | 10.1 | <0.01 |
| | Date | 22.2 | <0.001 |
| | Location | 5.72 | 0.022 |
| | $CO_2$ × date | 6.97 | 0.002 |
| | $CO_2$ × location | 7.71 | 0.009 |
| | Date × location | 3.27 | <0.001 |
| | $CO_2$ × date × location | 4.95 | <0.001 |

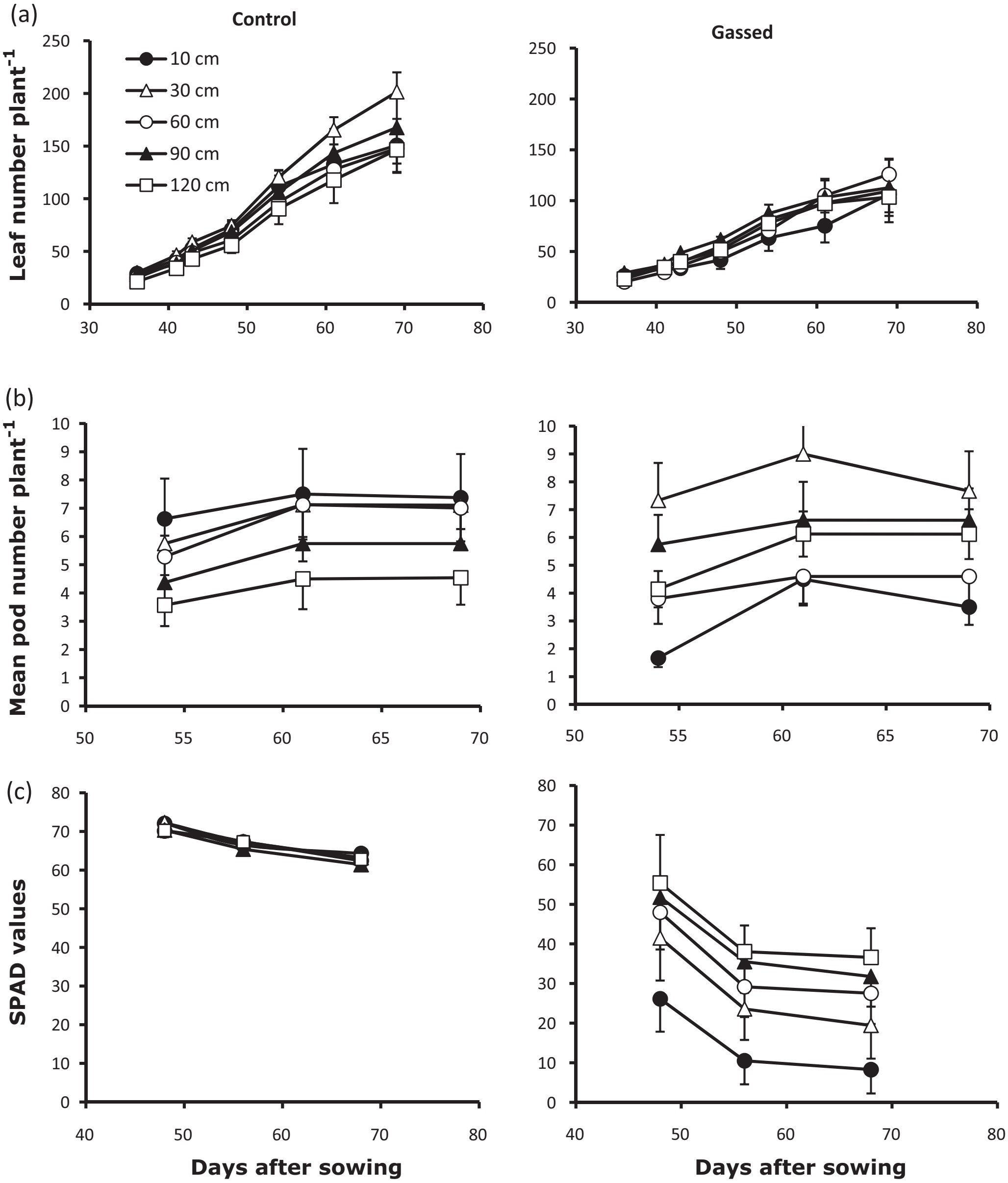

**Fig. 3.** (a) Leaf number plant$^{-1}$, (b) pod number plant$^{-1}$ and (c) SPAD values in control and gassed plots at distances of 10, 30, 60, 90 and 120 cm from the centre. Single standard errors of the mean are shown ($n = 4$ comprising 40 gassed and 40 control plants). Note differing $x$-axis scales.

time was apparent at greater distances from the centre of gassed plots.

The effect of $CO_2$, date, location and the $CO_2 \times$ date, $CO_2 \times$ location, date × location and $CO_2 \times$ date × location interactions for chlorophyll content were all significant ($P < 0.05$; Table 2). SPAD values increased greatly with distance from the centre of gassed plots but decreased with time at all locations ($P < 0.05$; Fig. 3c). At 48 DAS, mean SPAD values for plants located 10 and 120 cm from the centre of gassed plots were respectively 26 ± 8.2 and 56 ± 12, compared to 69 ± 1.7–72 ± 1.0 in control plants. The more rapid decline in SPAD values between 56 and 68 DAS in gassed plants indicates that chlorophyll degradation was accelerated.

### 3.4. Growth variables at harvest

Mean values for all growth variables except individual seed weight were greatly reduced in gassed plants at harvest (Table 3). Leaf and stem dry weight and leaf area plant$^{-1}$ were respectively 64% ($P < 0.001$), 53% ($P < 0.001$) and 65% ($P < 0.001$) lower in gassed plants than in control plants. Exposure to elevated soil $CO_2$ also reduced pod and seed number plant$^{-1}$ by 42 and 46% respectively ($P < 0.001$), but the effect on seed number pod$^{-1}$ was much smaller (11%). Individual seed weight was increased by 13.5% relative to control plants but this was insufficient to prevent a 36% reduction in seed weight plant$^{-1}$ due to the greatly reduced pod number plant$^{-1}$.

Fig. 4 illustrates the substantial spatial variation in leaf area and leaf, seed and shoot dry weight plant$^{-1}$ at harvest in the gassed plots. As shown by the non-destructive growth measurements for the same plants, values for all variables were greatest near the edges of the gassed plots and decreased sharply towards the injection point. The pattern of growth in the gassed plots reflected that for gas dispersion (Fig. 2) as plants exposed to lower [$CO_2$] than experienced at the plot centre survived, but showed more severe growth reductions than plants furthest from the injection point or in the

**Table 3**
Growth characteristics at harvest (mean ± SEM) for plants grown in control and $CO_2$-enriched soils ($n = 4$ comprising 400 gassed and 100 control plants). Percentage changes in vegetative and reproductive variables in gassed relative to control plants are shown; ↓ and ↑ denote increases and decreases.

| Parameter | Control | Gassed | $F$ | $P$ | (Change %) |
|---|---|---|---|---|---|
| Shoot dry weight (g $plant^{-1}$) | 29.2 ± 1.03 | 14.8 ± 0.9 | 102.7 | <0.001 | 49.3↓ |
| Stem dry weight (g $plant^{-1}$) | 9.05 ± 0.5 | 4.28 ± 0.2 | 60.7 | <0.001 | 52.7↓ |
| Leaf dry weight (g $plant^{-1}$) | 6.95 ± 0.4 | 2.54 ± 0.2 | 90.8 | <0.001 | 63.5↓ |
| Leaf area ($cm^2$ $plant^{-1}$) | 1224 ± 72.4 | 430 ± 37.3 | 65.5 | <0.001 | 64.9↓ |
| Pod number $plant^{-1}$ | 6.7 ± 0.4 | 3.9 ± 0.26 | 32.4 | <0.001 | 41.8↓ |
| Seed number $pod^{-1}$ | 3.04 ± 0.07 | 2.7 ± 0.12 | 4.3 | <0.05 | 11.1↓ |
| Seed number $plant^{-1}$ | 20.3 ± 1.2 | 11.0 ± 0.7 | 38.9 | <0.001 | 45.8↓ |
| Seed dry weight (g $plant^{-1}$) | 7.5 ± 0.40 | 4.8 ± 0.30 | 24.9 | <0.001 | 36.0↓ |
| Mean seed weight (g $seed^{-1}$) | 0.37 ± 0.01 | 0.42 ± 0.02 | 1.9 | ns | 13.5↑ |

control plots. Shoot dry weight $plant^{-1}$ was negatively correlated with [$CO_2$] ($P < 0.001$) and positively correlated with [$O_2$] ($P < 0.001$; Fig. 5); similar correlations were apparent for all growth variables examined.

### 3.5. Root growth

The effects of [$CO_2$] and soil depth on root number, length and diameter were significant ($P < 0.05$ to <0.001; Table 4), as was the $CO_2 \times$ date interaction for root number and length. The $CO_2 \times$ depth × date interaction for root number and length approached significance, suggesting that the pattern of root distribution with depth differed between treatments. The gas concentration records showed that roots in the $G_A$ orientation experienced a mean [$CO_2$] of 20% at 30 cm depth, whereas the corresponding value for the $G_B$ orientation was 42% due to their proximity to the centre of the gassed plots.

Main and lateral root numbers and length (Fig. 6a AND b) increased much more rapidly with time and roots reached greater maximum depths in the control plots than for both minirhiztron orientations in the gassed treatment. At 63 and 77 DAS, main and lateral root numbers and length were greater in the 10–20 cm horizon of the control treatment and the $G_A$ orientation in the gassed plots than for any other horizon. Main root number in the 10–20 cm horizon was respectively 68% and 87% greater in control plants than in the $G_A$ and $G_B$ orientations in the gassed treatment at 63 and 77 DAS. Main root number and length were consistently greater for the $G_A$ than for the $G_B$ orientation for all horizons and sampling dates ($P < 0.05$). The main roots penetrated to 70 cm by 77 DAS in the control plots (Fig. 6), but reached a maximum depth of only 40 cm

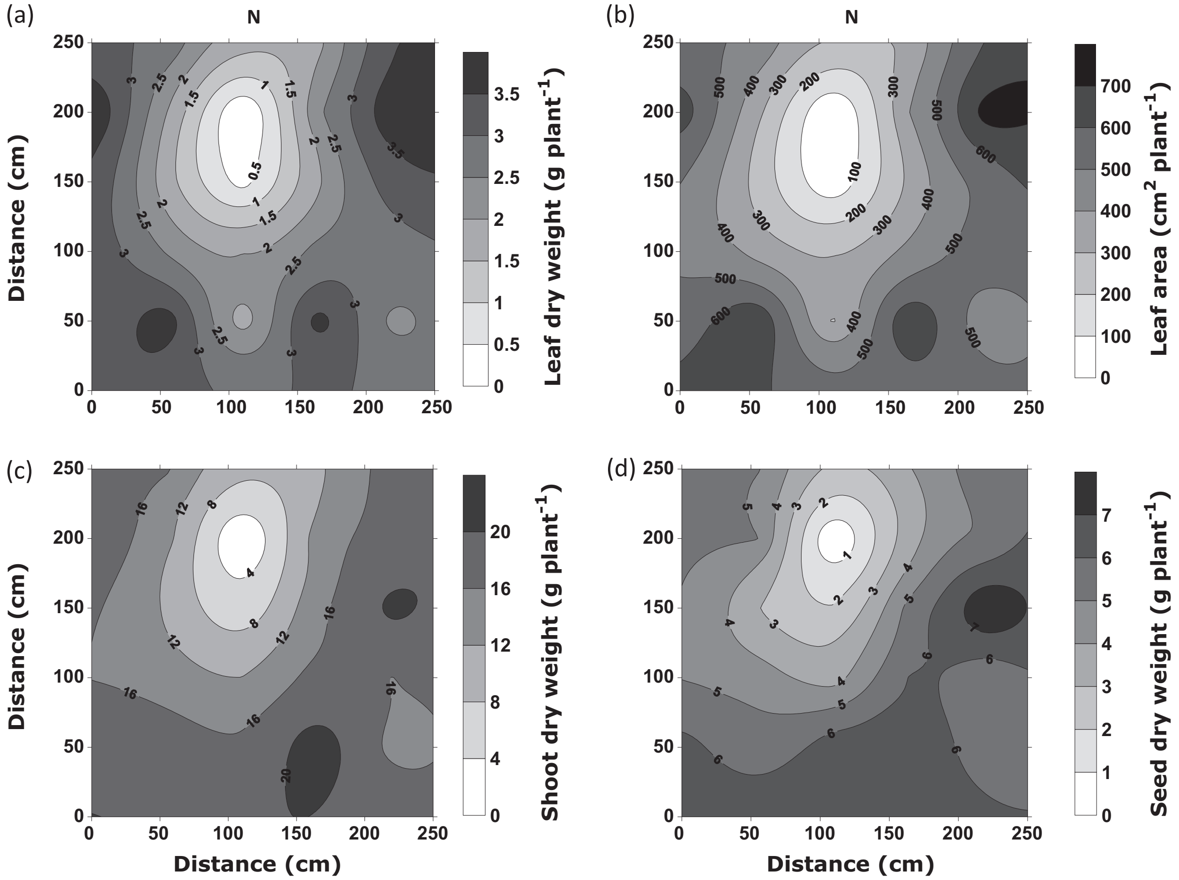

**Fig. 4.** Contour maps showing (a) leaf dry weight (b) leaf area, (c) shoot dry weight and (d) seed dry weight $plant^{-1}$ in the gassed plots at harvest. $X$ and $Y$ axes represent plot area (2.5 m × 2.5 m). Contour values ($Z$) represent mean values ($n = 4$ comprising 400 gassed plants). $N$ denotes North.

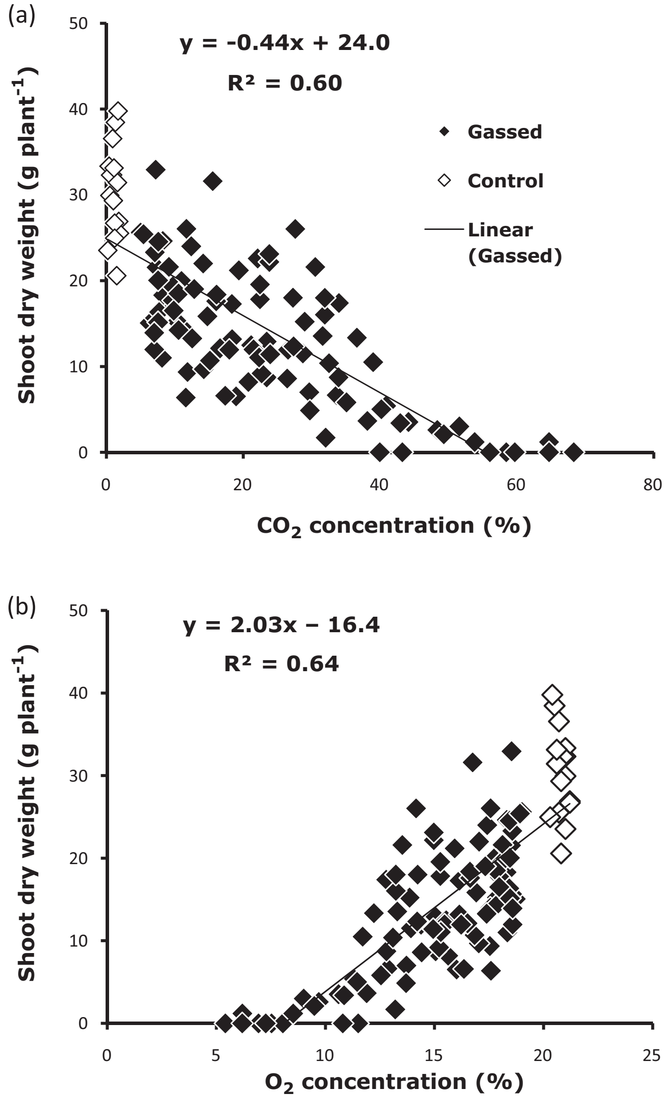

**Fig. 5.** Correlation between shoot dry weight at harvest and (a) mean soil $CO_2$ and (b) mean $O_2$ concentration. Regression equations are shown.

**Table 4**
ANOVA summary for the root characteristics of plants grown in control or $CO_2$-enriched soils measured at seven dates between July and August 2008 ($n = 4$).

| Variable | Source | *F* | *P* |
|---|---|---|---|
| Root number | $CO_2$ | 7.34 | 0.013 |
| | Date | 5.38 | 0.019 |
| | Location | 3.13 | 0.049 |
| | $CO_2$ × date | 9.42 | <0.001 |
| | $CO_2$ × location | 1.90 | 0.115 |
| | Date × location | 3.96 | 0.006 |
| | $CO_2$ × date × location | 1.90 | 0.077 |
| Root length (mm) | $CO_2$ | 6.74 | 0.016 |
| | Date | 1.59 | 0.195 |
| | Location | 4.23 | 0.001 |
| | $CO_2$ × date | 20.90 | <0.001 |
| | $CO_2$ × location | 0.88 | 0.550 |
| | Date × location | 1.56 | 0.067 |
| | $CO_2$ × date × location | 1.42 | 0.063 |
| Root diameter (mm) | $CO_2$ | 5.60 | 0.026 |
| | Date | 0.55 | 0.651 |
| | Location | 2.20 | 0.072 |
| | $CO_2$ × date | 13.80 | <0.001 |
| | $CO_2$ × location | 1.09 | 0.388 |
| | Date × location | 2.39 | 0.001 |
| | $CO_2$ × date × location | 1.12 | 0.304 |

at 63 DAS for both orientations in the gassed treatment ($P < 0.05$; Table 4). By 77 DAS, maximum rooting depth in the $G_B$ orientation had declined to 30 cm due to the disappearance of roots in the 30–40 cm horizon, whereas roots in the $G_A$ orientation were still present to 40 cm, although none penetrated to greater depths. Numerous lateral roots were produced in the 10–30 cm horizons of control plots, and mean lateral root numbers in the 10–20 cm horizon were respectively 2.6 ± 1.1 and 3.7 ± 2.0 at 63 and 77 DAS. By contrast, lateral root production was greatly reduced in the $G_A$ orientation (0.2 ± 0.2 and 0.4 ± 0.2 respectively at 63 and 77 DAS, and was almost entirely suppressed in the $G_B$ orientation (Fig. 6).

## 4. Discussion

### 4.1. Soil gaseous environment

Injection of $CO_2$ reduced soil $[O_2]$ to an extent related to $[CO_2]$ in the soil atmosphere (Fig. 2). $[O_2]$ was much lower throughout the gassed plots than in control plots (19.6%) and other well-aerated field soils, in which $[O_2]$ is typically *c.* 20% (Good and Patrick, 1987). The contour maps of gas dispersion (Fig. 2) suggest that $CO_2$ migrated isotropically from the injection point to the topsoil, with the result that $[CO_2]$ decreased towards the plot margins. The inverse correlation between soil $[CO_2]$ and $[O_2]$ suggests direct physical displacement of $O_2$ by injection of $CO_2$. Previous studies at the same site also showed a strong negative correlation between soil $[CO_2]$ and $[O_2]$ ($R^2 = 0.97$; Steven and Smith, 2010); these authors concluded that injected $CO_2$ displaced $O_2$ and no significant microbial or other effects were involved. Vodnik et al. (2006) also reported a negative correlation between $[O_2]$ and $[CO_2]$ at a site naturally enriched with $CO_2$ in Slovenia. However, in contrast to the conclusions of Steven and Smith (2010), hypoxia induced by elevated soil $[CO_2]$ affected the arbuscular mycorrhizal fungal communities associated with the roots of several species to an extent which depended on the severity of $CO_2$ exposure (Maček et al., 2011).

Interestingly, the area of highest $[CO_2]$ and lowest $[O_2]$ was displaced towards the northern edge of the gassed plots rather than being directly above the injection point (Fig. 2). We speculate that this occurred because the supply pipe sloped from the soil surface at the northern edge of the plot to the diffuser 60 cm below the plot centre. Some of the $CO_2$ released may have moved preferentially along the outer surface of the supply pipe rather than dispersing vertically upwards through the soil, causing the observed asymmetric gas dispersion. However, this does not invalidate the experimental objective of investigating the potential impact of $CO_2$ leakage from CCS systems as effects on crop performance were related the actual distribution of $[CO_2]$ and $[O_2]$ within the plots.

### 4.2. Shoot growth

The combination of elevated soil $[CO_2]$ and depleted $[O_2]$ greatly affected plant survival, growth and shoot biomass near the injection point, but these effects decreased with increasing distance (Figs. 3 and 4); all growth variables were negatively correlated with soil $[CO_2]$ and positively correlated with $[O_2]$ (Fig. 5). It is likely that plant mortality depended on a combination of elevated soil $[CO_2]$ and exposure duration as the proportion of dead plants increased with time in the most affected area of the gassed plots; thus, exposure to $[CO_2]$ of 25–60% was lethal, whereas concentrations of 5–25% allowed survival but greatly reduced growth relative to control plants. These findings substantiate those of Williamson (1968), who reported that soil $[CO_2]$ >6% was toxic to broad bean (*V. faba* L.). Negative correlations between plant growth and soil

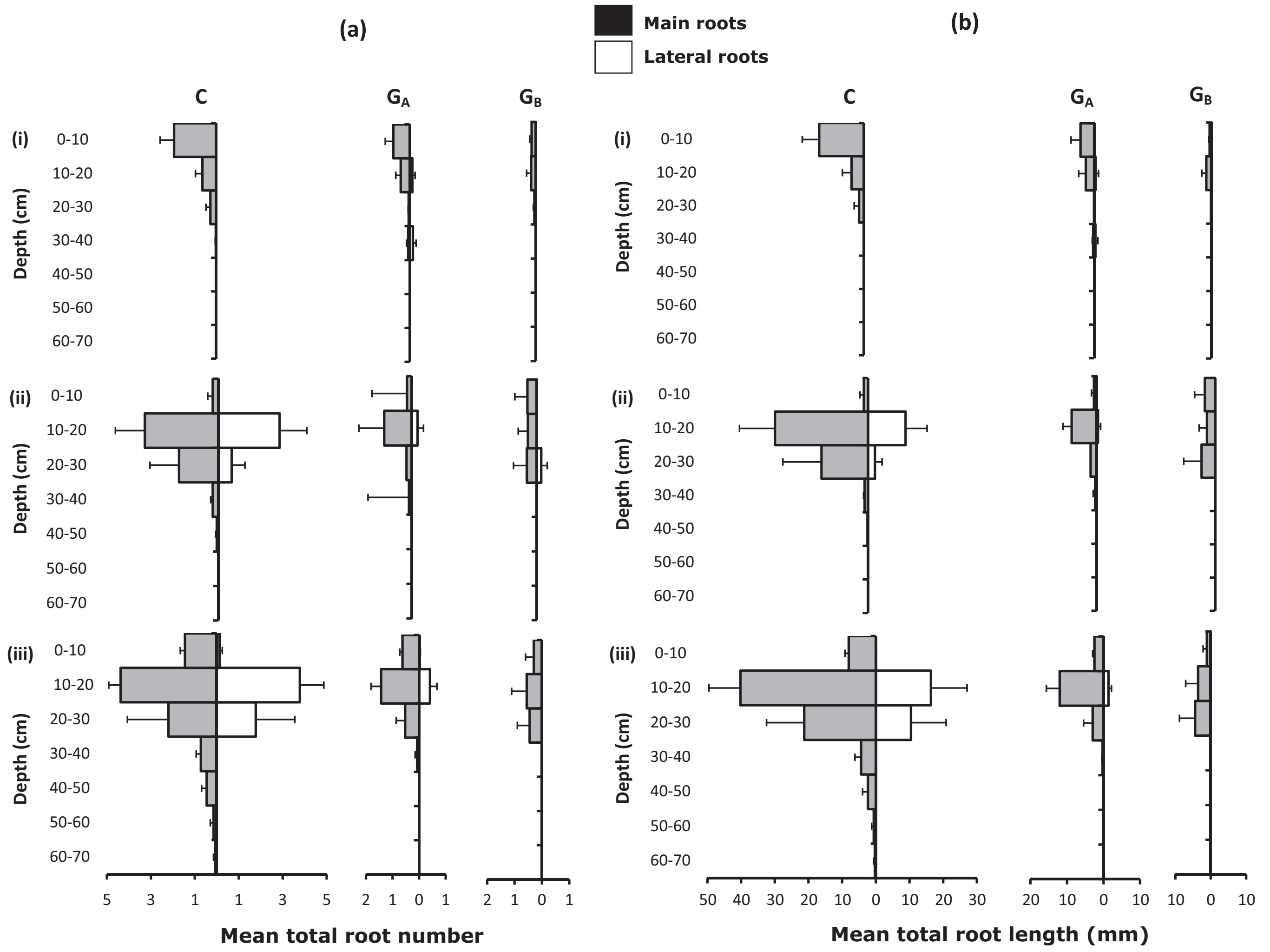

**Fig. 6.** (a) Main and lateral root numbers and (b) main and lateral root lengths in the control treatment (C) and $G_A$ and $G_B$ orientations in the gassed plots for the 0–10, 10–20, 20–30, 30–40, 40–50, 50–60 and 60–70 cm horizons at (i) 40, (ii) 63, and (iii) 77 DAS. Single standard errors of the mean are shown ($n = 4$).

[$CO_2$] were also found when maize (*Z. mays* L.) and various native species were grown at a site naturally enriched with $CO_2$ in Slovenia (Vodnik et al., 2005, 2006).

Elevated soil [$CO_2$] is likely to impact on plant survival and performance by depriving roots of $O_2$, whose concentration in the soil atmosphere was 16 and 8% respectively at $CO_2$ concentrations of 25 and 60%. This observation substantiates research at Mammoth Mountain, California, where high soil [$CO_2$] (20–95%) resulting from seepage from beneath a volcano caused the death of trees by depriving their roots of $O_2$ and interfering with nutrient uptake (McGee and Gerlach, 1998; Sorey et al., 2000). As in the present study, Pociecha et al. (2008) found that 8-week-old *V. faba* plants subjected to hypoxia induced by flooding for 7 d showed substantial reductions in leaf area, stem elongation and biomass production.

The combination of high soil [$CO_2$] and low soil [$O_2$] induced visible foliar injury in the area of greatest [$CO_2$] soon after gassing began. These symptoms began with yellowing of leaves caused by a rapid decline in chlorophyll content; thus, SPAD values were much lower in gassed than in control plants and declined more rapidly with time (Fig. 3c). Chlorosis occurred first in the older leaves and this was followed by premature senescence and abscission. These observations confirm evidence that reductions in chlorophyll a and b concentration in winter oilseed rape (*Brassica napus* L.) resulting from depletion of soil [$O_2$] by flooding were greatest in the oldest leaves (Zhou and Lin, 1995). Chlorosis was also observed when pea (*Pisum sativum* L.), maize (*Z. mays* L.) (Przywara and Stepniewski, 1999) and lucerne (*Medicago falcata* L.; Smethurst and Shabala, 2003) were grown on waterlogged soil. The rapid decline in photosynthetic rate when intolerant species encounter waterlogged or hypoxic soil conditions has often been attributed to $O_2$ deficiency and associated reductions in stomatal conductance (Huang et al., 1997; Malik et al., 2001). It is therefore suggested that hypoxic conditions induced by $CO_2$ injection into the soil in the present study rapidly induced chlorosis, reduced leaf area and induced premature senescence, ultimately causing plant death in the areas of lowest soil [$O_2$]. A rapid increase in the foliar production of ethylene is a general response when plants encounter abiotic stress factors (Abeles et al., 1992), and waterlogging has previously been shown to increase internal ethylene concentrations in various crop species (Visser and Pierik, 2007; Irfan et al., 2010) including *V. faba* (El-Beltagy and Hall, 1974); it is therefore likely that the hypoxic conditions in the gassed plots promoted synthesis of this important gaseous plant growth regulator. Many of the observed responses, including progressive leaf abscission from older to younger leaves, reduced shoot growth, increased leaf senescence and abscission (Jackson and Osborne, 1970; Abeles et al., 1992; Kieber, 1997; Pell et al., 1997; Vodnik et al., 2006) are typical those associated with stress-induced increases in endogenous ethylene levels.

Although the vegetative and reproductive parameters determined at maturity (Table 3; Fig. 4) were lowest in the area of greatest soil [$CO_2$], the less extreme gaseous environment near the plot margins was sufficient to reduce all growth variables relative to control plants. Comparable results were obtained when winter-sown field beans were exposed to a similar gaseous environment at the ASGARD site, as germination and seedling establishment were prevented near the $CO_2$ injection point; growth of the surviving seedlings again increased towards the plot margins, but was still lower than in control plants (Al-Traboulsi, 2011).

### 4.3. Root growth

The major impact of changes in the soil gaseous environment on root number, length and rooting depth substantiates reports that root elongation is sensitive to soil aeration and may be reduced by elevated [$CO_2$] and depleted soil [$O_2$] (Grable and Danielson, 1965; Bengough et al., 2006; Visser and Pierik, 2007). In the present study, elevated [$CO_2$] and reduced [$O_2$] suppressed lateral root production almost completely (Fig. 6). As the continued growth of lateral roots is vital to exploit the water and nutrient reserves present in previously untapped areas of soil, the sharp reduction in their production in the gassed plots is likely to have limited supplies of these essential resources, adversely affecting growth and survival.

Displacement of $O_2$ from $CO_2$-saturated soil pores in the gassed plots induced hypoxia rather than anoxia as the roots were not fully deprived of $O_2$ (minimum [$O_2$] of 8%). The impact of elevated $CO_2$, which was intended to be the dominant factor examined, was inextricably linked with $O_2$ deficiency and the consequent influence of hypoxic soil conditions. *V. faba* was clearly unable to survive the prolonged stress associated with reduced soil [$O_2$] during the 49 day injection period in areas of the gassed plots where [$CO_2$] was greatest. El-Beltagy and Hall (1974) concluded that, as a mesophyte, *V. faba* lacks the necessary anatomical, physiological and biochemical adaptations to cope with hypoxic conditions induced by waterlogging; these include the formation of aerenchyma in maize and rice (*Oryza sativum* L.) to facilitate the diffusion of oxygen from shoots to the roots (Atwell et al., 1988; He et al., 1994; Bailey-Serres and Voesenek, 2008; Perata et al., 2011). In this regard, Walter et al. (2004) reported that the leguminous species, French bean (*Phaseolus vulgaris* L.), is also highly susceptible to $O_2$-deficiency as no adventitious roots were produced when plants were exposed to hypoxic conditions induced by flooding. The hypoxic conditions imposed in the present study in the absence of flooding may also have increased competition between roots and soil microorganisms for $O_2$, to the point where this became a major limitation for root respiration and plant growth, development and yield (Jackson, 1985; Veen, 1988; Hinsinger et al., 2009).

The observed symptoms and growth effects are typical responses to hypoxia, suggesting that they resulted primarily from $O_2$ deficiency, with elevated [$CO_2$] enrichment being a secondary factor. It is unlikely that climatic conditions imposed any additional constraint on crop performance as they were comparable to the long-term means for the preceding 10 year period (Table 1). Smith et al. (2004) reported that simulated underground leaks of methane ($CH_4$) induced similar stress symptoms and growth responses in *V. faba*, suggesting that chlorosis and growth reductions may be generic responses to the displacement of soil $O_2$ by other gases; [$O_2$] was <10% in their study. However, in the previous study, stress symptoms developed only in seedlings with small root and shoot biomass, but not in well established plants, suggesting that the severity of the stress imposed was more extreme in the present study. It is likely that the impact of hypoxia was intensified by the additional damaging impact of high soil [$CO_2$], resulting in extensive mortality and severe growth and yield reductions.

### 4.4. Yield and yield components

The major impact of gassing on reproductive characteristics at harvest (Table 3) is unsurprising in view of the substantial effects on both root and shoot growth (Table 3; Figs. 3–6). The large reduction in seed number (46%) and dry weight plant$^{-1}$ (36%) in gassed plants was almost entirely attributable to the 42% reduction in pod number plant$^{-1}$, as the impact on seed number pod$^{-1}$ was small (11%). The only reproductive variable to show a positive response was individual seed weight, which was 13.5% greater than in control plants, although this was insufficient to maintain seed yield plant$^{-1}$ (Table 3).

Although prolonged exposure to abiotic stress often affects reproductive development and seed yield, the nature of these effects and the ability of plants to adopt effective compensatory responses vary greatly depending on extrinsic factors, such as the timing, duration and severity of stress and climatic conditions, and intrinsic variation in the sensitivity of individual species and genotypes (Black et al., 2000, 2007, 2010, 2012). Prolonged exposure to abiotic stresses such as atmospheric pollution is known to induce effects on reproductive development and yield similar to those reported here. For example, season-long exposure of mustard (*Brassica campestris* L.) to ambient [$O_3$] (42–54 ppb) at Varanasi, India reduced seed number and weight plant$^{-1}$ by 23% relative to plants grown in clean air (Singh et al., 2009). Similarly, filled grain number ear$^{-1}$ and yield plant$^{-1}$ were reduced when two Bangladeshi wheat varieties were exposed to 60 or 100 ppb $O_3$ for 7 h d$^{-1}$ for three months (Akhtar et al., 2010), while Sarkar and Agrawal (2010) reported that ambient [$O_3$] at Varanasi, India decreased above-ground biomass, seed number plant$^{-1}$ and grain yield in two Indian wheat varieties; grain yield was reduced by up to 46%. Such effects are widespread, as meta-analysis of 53 studies of wheat showed that elevated [$O_3$] (mean of 73 ppb) decreased grain yield by 29% compared to plants grown in clean air (Feng et al., 2008). Thus, similar effects on vegetative growth, reproductive development and yield may be induced by various stress factors in a range species, even though the initial contact point (roots or shoots) and mode of action (e.g. hypoxic soil conditions, gaseous pollutants) may differ greatly.

Stress-induced reductions in root and shoot growth in response to abiotic factors have been attributed to reductions in assimilate production or changes in partitioning (Wittig et al., 2009), perhaps resulting from callose deposition in the phloem (Matyssek et al., 2004). In support of this view, the major reductions in leaf number, leaf area and SPAD values and the extensive premature leaf senescence seen in gassed plants (Figs. 3 and 4) strongly suggest that assimilate supplies to support vegetative and reproductive growth were severely limited.

However, evidence that elevated endogenous ethylene concentrations suppress growth and induce premature senescence and abscission suggests an attractive alternative explanation (Gallie et al., 2009). Wilkinson and Davies, 2010 proposed that cross-talk between abscisic acid (ABA) and ethylene has a key role in mediating responses to various abiotic stress factors including waterlogging, which represents a milder stress than the severely depleted [$O_2$] and elevated [$CO_2$] soil atmosphere imposed in the present study. It is well documented that, when roots encounter adverse soil conditions such as compaction, ABA and 1-aminocyclopropane carboxylic acid (ACC), the precursor of ethylene, are transported through the xylem to the shoots where they induce various effects including reduced stomatal opening and growth (Roberts et al., 2002). Wilkinson and Davies (2010) suggested that any factor which increases the production or perception of ethylene is likely to block ABA-dependent responses and that increased ethylene biosynthesis is important in mediating reductions in root and shoot growth induced by abiotic stress factors. They proposed that cross-talk between ABA and ethylene is important in regulating plant responses to abiotic stresses, perhaps mediated by interacting nitrogen-activated protein kinase (MAPK) cascades (Baier et al., 2005). Further research is needed to elucidate the role of cross-talk between these plant growth regulators in mediating plant growth and yield responses to the elevated [$CO_2$] and severely depleted [$O_2$] soil environment which may be induced by leakage of $CO_2$ from CCS transport and storage facilities.

## 5. Conclusions

The extensive plant mortality and major reductions in root and shoot growth induced by the combined effect of elevated soil [$CO_2$] and the consequent hypoxic conditions show that *V. faba* lacks the physiological and anatomical adaptations to cope with such conditions. The progressive decline in [$CO_2$] and increase in [$CO_2$] towards the edges of the gassed plots enabled plants to survive and perform more effectively, but growth was still greatly reduced relative to control plants. These findings suggest that $CO_2$ leaking from CCS facilities may reach concentrations which are lethal for unadapted plant species, and might seriously impact on root and shoot growth and function, biomass production and yield at lower concentrations. Secure geological storage of $CO_2$ is therefore vital. As *V. faba* has proved to be highly sensitive to elevated soil $CO_2$ and depleted soil [$O_2$], further field studies involving species better adapted to such conditions are needed to improve our understanding of the impact of leakage from CCS systems on plant survival, growth and yield.

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