Smith, Karon L. and Lake, Janice A. and Steven, Michael D. and Lomax, Barry H. (2016) Effects of elevated soil CO2 concentration on growth and competition in a grass-clover mix. International Journal of Greenhouse Gas Control . ISSN 1750-5836

Effects of elevated soil CO₂ concentration on growth

and competition in a grass-clover mix.

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Abstract

22 To investigate potential environmental affects in the context of carbon dioxide (CO_2) leakage

from Carbon Capture and Storage (CCS) schemes. The ASGARD (Artificial Soil Gassing

24 and Response Detection) facility was established, where CO_2 can be injected into the soil in

replicated open-air field plots. Eight plots were sown with a grass-clover mix, with four

26 selected for CO_2 treatment while four were left as controls. Observations of sward

productivity throughout the study allowed three effects to be distinguished: a direct stress

28 response to soil gassing, limiting productivity in both species but with a greater effect on the

clover; competition between the grass and clover affected by their differential stress

30 responses; and an overall temporal trend from dominance by clover to dominance by grass in

CO_2 treatments. The direct effect of soil CO_2 (or associated oxygen (O_2) deprivation due to

32 the high levels of CO_2 in the soil) gave estimated reductions in productivity of 42% and 41%

in grass, compared to 66% and 32% for clover in the high and low CO_2 gassed zones

34 respectively. Canopy CO_2 increased by 70 parts per million (ppm) for every 1% increase in

soil CO_2 and a significant positive response of stomatal conductance in clover was observed;

36 although carbon acquisition by the plants should not therefore be impeded, the reduction in

productivity of the gassed plants is indicative of carbon-based metabolic costs probably

38 related to soil CO_2 affecting root physiology. Biomass measurements made after gassing has

ceased indicated that recovery of vegetation was close to complete after 12 months.

1. Introduction

Carbon Capture and Storage (CCS) has been advocated as a means of reducing rising levels of atmospheric carbon dioxide (CO_2) to help mitigate climate change. Captured CO_2 is compressed and transported via pipeline to storage sites in deep geological reservoirs (depleted oil or gas reservoirs or deep saline aquifers). Geological evidence from oil and gas fields indicate that gases can remain trapped in suitable formations for millions of years. Although the risks of leakage from well-chosen sites are regarded as extremely small and protocols for leak detection have been developed (Leuning et al., 2008; Jenkins et al., 2016), it is nevertheless a regulatory requirement to demonstrate that the impacts of any possible leaks from CCS infrastructure, (including transportation pipelines) have been investigated and understood. In the unlikely event of captured CO_2 reaching the surface, CO_2 in the soil would rise, possibly to values approaching 100%; diffusion from the soil would lead to increased atmospheric CO_2, but to a much lesser extent due to rapid air mixing. CO_2 may also dissolve in soil water leading to changes in the pH level and possible uptake by plants in the transpiration stream (Steven et al. 2010). Atmospheric CO_2 may stimulate plant photosynthesis, but high soil concentrations are usually detrimental (IPCC, 2005). While much research in the context of global environmental change has been carried out to determine the effects of elevated atmospheric CO_2 on vegetation (Kimball et al., 1993; Van Noordwijk et al., 1998; Ghannoum et al., 2000; Moscatelli et al., 2001), much less is known about the potential effects of elevated soil CO_2.

Previous laboratory studies have reported significant plant stress responses to soil CO_2, with some suggestion of greater sensitivity in dicotyledons compared to monocotyledons (Noyes,

1914; Stolwijk and Thimann, 1957; Williamson, 1968; Glinski and Stepniewski, 1985;

66   Bunnell et al., 2002; Rodriguez et al., 2005). However, many of these studies were at

relatively low CO_2 (ca. 2 to 6%) concentrations, similar to background soil CO_2 in

68   agricultural systems (0.15 and 2.5% in the surface layers; Stolwijk and Thimann 1957;

Russell 1973), with occasional large excursions in soil CO_2 being recorded (10 and 12%

70   recorded (Chang and Loomis 1945; Stolwijk and Thimann 1957; Russell 1973; Glinski and

Stepniewski 1985). Natural CO_2 vents have been proposed as CCS leakage analogues, for

72   example at Stavešinci, Slovenia, where plant height corresponded inversely with soil CO_2

(Vodnik et al. 2006) and Latera, Italy, where Beaubien *et al*., (2008) found an ecological

74   gradient, with acid-tolerant grasses outcompeting clover near a CO_2 vent, consistent with the

suggestion of differential sensitivities of plants. However, these seeps have been leaking CO_2

76   for extended periods so that the vegetation growing in the vicinity may have become adapted

to the high soil CO_2 conditions. Moreover, at natural analogue sites, smaller concentrations of

78   methane and trace amounts of more toxic gases, such as hydrogen sulphide (H_2S) or sulphur

dioxide (SO_2) may also be present (Pfanz et al., 2004) making it difficult to attribute direct

80   CO_2 effects.

82   Assessment of the potential impact posed in the unlikely event of leakage of CO_2 from CCS

pipelines and storage infrastructure requires the application of realistic environmental

84   scenarios (West et al 2015). Here we describe a fully-replicated experimental open-air facility

where pure CO_2 gas was injected into previously undisturbed soil to determine specific

86   effects on the growth and health of vegetation. Within this experimental framework a mixture

of pasture grass and clover were sown to investigate the effects of differential sensitivities on

88   interspecies competition.

2. Methods

2.1 Experimental plots

The ASGARD (Artificial Soil Gassing And Response Detection) facility was located in a

field of permanent pasture at the Sutton Bonington campus of the University of Nottingham,

UK (N 52.8°, W 1.2°). CO_2 was injected into the soil in 16 field plots (each 2.5 m × 2.5 m)

via 20 mm (Inside Diameter (ID)) medium density polyethylene (MDPE) gas pipes. The

pipes were inserted into the ground at an angle of 45° to the vertical and the CO_2 was

delivered into the soil at a depth of 500 - 600 mm below the centre of each CO_2 gassed plot

via perforations in the end of the pipes. This depth was chosen to limit lateral gas migration

across the site. Food-grade, liquid CO_2 was stored in two 200 L cryogenic vessels (BOC,

Derby, UK), the liquid CO_2 was converted to gaseous phase CO_2 and regulated down to a

pressure of ~22psi (152 kPa) before being delivered via a single inlet mass flow sensor

(Alicat, Tucson, USA) to 16 individual mass flow controllers (Alicat, 0.1-10 L min^{-1}). CO_2

was delivered at a flow rate of 1 L min^{-1} to each experimental gassed plot. The mass flow

controllers were operated, and the system data logged, by a PC-based control system (TVC,

Great Yarmouth, UK). For a full site description and characterisation see Smith *et al.* (2013).

For this study eight experimental plots were used, each separated by a 1 m border. Four

randomly selected plots were injected with CO_2 gas and four acted as untreated controls.

Each experimental plot had a 0.25 m buffer zone around the edge, with the remaining area

sub-divided into sixteen 0.5 x 0.5 m sampling sub-plots, (Fig. 1). Above-ground biomass and

plant physiological measurements were measured in two transects running East-West

(subplots A_1-A_4) and North-South (A_3-D_3) crossing the zone of highest soil gas

concentration; a single transect running East-West (A_1-A_4) was used in the control plots.

Plots were hand dug and sown on 19th April 2010 with 'POCHON' Persistent Long Term

114   Grazing Ley, (Cotswold Seeds, Gloucestershire, UK), a mixture of 87.5% perennial rye grass

(*Lolium perenne*) and 12.5% white clover (*Trifolium repens*), at a rate of 3 g m^{-2}. The plots

116   were left to establish and weeded by hand throughout 2010 to ensure that only grass and

clover remained. CO_2 was delivered to the centres of the four plots from 21st March 2011 to

118   15th June 2012.

120   *2.2 Gas measurement*

CO_2 in the soil was monitored by means of permanently-installed vertical tubes located 0.15

122   and 0.7 m from the centre of each gassed plot at a depth of 0.3 m. Holes made in the end of

the tubes allowed air in the tube to equilibrate with the surrounding soil atmosphere. CO_2 and

124   oxygen (O_2) were measured two to three times per week using a GA5000 landfill gas

analyser (Geotech, Warwickshire, UK). Additional measurements to map soil gas

126   concentrations at 0.3 m depth were taken on three occasions – 27th June 2011, 19th October

2011 and 14th June 2012 by bar-holing on a grid at 0.5 m intervals across each plot (Fig. 1),

128   as described in Smith *et al.* (2005), giving a good overview of the horizontal distribution of

CO_2 within the soil. However it is intrinsically prone to some underestimation of CO_2

130   concentration because of the possibility of air mixing with the sample.

132   The seasonal average of CO_2 measured in the permanently installed tube at 0.15 m from the

centre of the plot, for the three months preceding each bar-holing measurement, was

134   compared with the bar-hole estimate for the same location by averaging the values for the

four closest bar-holes, inversely weighted according to their distance from the 0.15 m tube.

136   A similar calculation was made for the tube permanently installed at 0.7 m from the centre.

The CO_2 concentrations obtained by bar-holing were then scaled using the mean of the two

138   ratios of permanent tube to bar-hole CO_2. This method assumes that any effects of air mixing

6

are the same across the plot and that the spatial pattern represented by the bar-hole data is

140   consistent throughout the season, even though individual values may vary. The scaled CO_2

gas distribution within the plot was mapped using Surfer 7 (Golden Software Inc., Golden,

142   Co, USA) and used to divide the plots into high and low gas zones, corresponding

respectively to mean soil CO_2 concentrations above and below 10%. The average scaled CO_2

144   concentration in the high gas (sampling squares A_2, A_3, B_3 and C_3) and low gas (sampling

squares A_1, A_4 and D_3) zones were 19 and 5% respectively (Fig. 1).

146

Above-ground within-canopy CO_2 concentrations were measured in each of the sampling

148   sub-plots using an infra-red gas analyser (IRGA, Licor 6400XT, Licor Inc. Lincoln, USA) at

a height of 50 mm above the finished level of the soil on the plot in May and September

150   2011. One measurement for each sub-plot was taken.

152   *2.3 Biomass*

Biomass samples were collected at approximately 6 weekly intervals between April and

154   October 2011 and then from April to June 2012. Combined samples of grass/clover were

taken from subplots A_1-A_4, B_3, C_3 and D_3 in the gassed plots and subplots A_1-A_4 in the

156   control plots. A 0.2×0.2 m wooden frame was placed within each square sub-plot and all

biomass was scissor cut to the height of the frame (about 20 mm above the soil). The samples

158   were stored in plastic bags in a cool-box until analysis. Following collection of the

grass/clover samples, the plots were mown to a uniform height, as closely as possible to the

160   same height as the scissor cut. In the laboratory, the samples were separated by species,

weighed, dried at 85°C for 48 hours and then reweighed to determine the dry weight.

162

7

CO_2 delivery to the plots was switched off on 15th June 2012, and the vegetation was left to

164 recover. Additional samples were collected on 18th October 2012, 16th April 2013 and 10th

July 2013 following 4, 10 and 13 months of recovery respectively. For the October and July

166 measurements, the plots were mown 5 to 6 weeks earlier to provide a baseline for the

estimate of productivity; the April measurement represents the mean over-winter productivity

168 since the previous mowing in October.

2.4 Plant physiological measurements

170

Clover gas exchange parameters, photosynthetic rate and stomatal conductance, were

172 measured using an IRGA (Licor 6400XT, Licor Inc. Lincoln, USA) in the sample sub-plots

on three occasions during the 2011 growing season (during May, August and September)

174 seven to three days prior to harvest. It was not possible to perform gas exchange

measurements on the grass, as the leaves were too small following continual harvesting. One

176 plant per sub-plot was measured. A total of seven and four plants were measured in each CO_2

gassed and control plot respectively for each time point.

178

2.5 Meteorological conditions

Total daily rainfall and seasonal mean temperature were recorded at the University of

180

Nottingham meteorological station, located ~150 m from the ASGARD site. Seasonal

182 temperatures were generally in line with the 30 year mean (between 1981 and 2010).

Precipitation in 2011 was ~54% of the long-term average (between 1981 and 2010), whilst in

184 2012 precipitation was ~64% higher (see supplementary information on Table S1 for

meteorological data).

186

3. Results

3.1 Soil CO_2 and O_2 concentrations

Mean soil CO_2 was higher closer to the plot centre and also generally higher in 2012 when

190   compared to 2011. This may be due in part to bedding in of the pipework in the plots, or

possibly to the greater rainfall in 2012 (Supplementary Fig. S1). In 2011 the mean CO_2 in the

192   access tube 150 mm from the centre of the plot was 19.3% (±0.7), increasing to 37.5% (±1.9)

in 2012. At 0.7 m from the centre, mean CO_2 was 9.4% (±0.5) in 2011, increasing to 22.2%

194   (±1.7) in 2012. Peak CO_2 concentrations of 45.1, 75.2 and 90.6% in three of the four plots

were recorded on 20th April 2012, which coincided with the peak rainfall of 17.6 mm

196   recorded on 18th April 2012, suggesting that high soil moisture may have reduced the

diffusion rates of CO_2 and caused a build-up of injected CO_2 within the soil. In the control

198   plots, mean CO_2 was 0.7 % (±0.06) in 2011 and 1.0 % (±0.05) in 2012. Bar-holing data

indicated that the soil CO_2 was generally highest to the North of the plot centre and decreased

200   radially such that the CO_2 at the edge of the plot was similar to that seen in the controls.

Importantly the injected CO_2 (in plots 1, 3, 6 and 7) did not bleed into the adjacent control

202   (i.e. plots 2, 4, 5 and 8) (Fig. 2). There was a negative linear relationship (R^2=0.95) between

soil CO_2 and O_2 measured in the permanently installed access tubes, consistent with simple

204   displacement of normal soil air by the injected CO_2 as found at other artificially induced CO_2

sites e.g. ZERT site (Zero Emissions Research and Technology), USA (Zhou *et al.* 2013).

206

3.2 Plant Canopy CO_2

208   Within-canopy CO_2 concentrations at a 50 mm height showed a highly significant positive

correlation with soil CO_2 (Fig. 3). Mean data for the CO_2-gassed plots measured in May 2011

9

210   showed that the canopy CO_2 increases by about 70 parts per million (ppm) for every 1%

increase in the soil, to levels between 550 and 3,400 ppm, which are physiologically relevant

212   concentrations for plants (Brouder and Volenec 2008).

214   *3.3 Visual symptoms*

Stress symptoms were observed in both the grass and clover within 10 days of CO_2 delivery.

216   Although the stress was highly localised with the grass turning yellow and the clover leaves

turning purple in the highest CO_2 concentration areas (Supplementary Fig. S2). As the season

218   progressed, vegetation died back in the area of highest CO_2. However, where vegetation

survived there was an obvious change in the proportions of grass and clover. In the control

220   plots the clover was dominant whereas in the gassed plots, grass was dominant. This was

especially noticeable during the dry weather when the control plots remained green while the

222   gassed plots were visibly stressed.

224   *3.4 Clover gas exchange measurements*

Both mean photosynthetic rate (*A*) and mean stomatal conductance (g_s) measured during the

226   2011 growing season showed no significant difference between CO_2 gassed and non-gassed

control plants (Fig. 4). The high degree of variation in these measurements, in the range of

228   2.9 to 17.0 μmol m^{-2} s^{-1} for *A* and 0.04 and 0.08 mol m^{-2} s^{-1} for g_s is indicative of variation

under field conditions. Correlations of these parameters with accumulated biomass (in g m^{-2}

230   day^{-1}) show different responses between the CO_2 gassing and control plants (Fig. 5); control

plants exhibit a weak non-significant correlation between *A* and biomass, with greater

232   variation in measurements, whereas CO_2 gassed plants exhibit a stronger significant

correlation. A similar result is seen for g_s, but with a stronger correlation for non-gassed

234   control plants compared to gassed plants. Both parameters impact on accumulated biomass.

Soil and canopy CO_2 show no correlation with A, but a strongly significant and weak non-

236   significant correlation with g_s respectively (Fig. 5, see supplementary information Table S2

for statistical analysis).

238

3.5 Biomass

240   Trends in productivity (Fig. 6A) of the combined grass-clover system, as determined from

biomass indicate a treatment effect which is superimposed on seasonal variations. Data also

242   indicates some evidence of recovery after the CO_2 treatment was terminated (June 2012). The

relative dominance (D) of each component of the grass sward was expressed as the ratio of

244   the biomass (or productivity) of that component relative to the total, i.e.

246

$$D_{clover} = \frac{P(clover, g, t)}{P(clover, g, t) + P(grass, g, t)}$$

248   for the clover component, where P is the measured productivity as a function of gas treatment

g and time t. As there were only two species in the sward, the grass and clover dominance

250   values are strictly complementary, adding to 1. Clover was strongly dominant in the control

plots in the early stages, with the effect of soil CO_2 reducing dominance, averaged over the

252   gassing period, to 77% in the low CO_2 treatment and 62% of the control values in the high

CO_2 treatment. The relative dominance of clover declined in both gassed and control plots

254   throughout the period of study, including the recovery period (Fig. 6B). These data indicate a

severe fall in clover dominance with CO_2 gassing, so that while grass may also have been

256   stressed, it benefitted from the preferential decline in clover.

258   In the control plots, the system as a whole should have been operating to its maximum

        potential. Estimates of the productivities of the components relative to this local potential

260   were calculated by expressing the individual productivities for each treatment as a fraction of

        the combined (grass + clover) productivity in the control plots. We denote this parameter as

262   *performance, F*:

264   for clover:

$$F_{clover} = \frac{P(clover, g, t)}{P(clover, control, t) + P(grass, control, t)}$$

266   and for grass:

$$F_{grass} = \frac{P(grass, g, t)}{P(clover, control, t) + P(grass, control, t)}$$

268

        As both the individual productivities and the local potential are comparably affected by

270   temperature, rainfall and intrinsic soil factors, *performance* should provide a measure of the

        effects of gas treatment and competition (Fig. 7). The *F* curves for the control plots show

272   performance in the absence of stress by soil CO_2, while the corresponding low and high gas

        curves show the interaction of stress and competition. The data indicates that while clover

274   *performance* is consistently reduced by ~50% in the CO_2 plots, grass *performance* is more

        resilient, indicating lower sensitivity to soil CO_2; much of the time, grass performance in the

276   gassed regions even exceeds performance in the controls.

278   The complexity of the productivity responses to soil CO_2 suggests an interaction between

stress induced by CO_2 and competition. To disentangle these effects, the performance F of

280   each canopy component was plotted against its dominance D (Fig. 8). The predicted

maximum performance F_{max} when dominance equals 1 then corresponds to the case of zero

282   competition and provides an estimate of the basic response to gassing (see supplementary

information Table S3 for full statistical analysis). On this basis, high (19%) soil CO_2, causes a

284   42% decrease in grass and 66% decrease in clover performance.

3.6 Recovery

Following shut-down of CO_2 delivery (15th June 2012), further samples of the plots were

288   taken to assess the long-term implications of CO_2 release and to monitor post-CO_2 recovery.

In October 2012, measurements of biomass showed that the grass had recovered such that

290   there was no significant difference (measured using Student's T-test, $P=0.49$) between the

grass collected from the high gas zone and the controls. There was a decrease in the clover in

292   all of the plots including the controls but the amount of clover in the CO_2 plots remained

significantly lower ($P=0.003$) than in the controls. A second sampling of biomass, 10 months

294   after termination of the CO_2 treatment showed no significant difference (measured using

Student's T-test $P=0.60$) between the grass in the control and treatment, although there was a

296   significant difference between the clover collected from the gassed and control plots

(measured using Student's T-test $P=0.09$). However the amount of harvested clover biomass

298   in both experimental and control plots was greatly reduced. In June 2013 the plots were

mowed and 5 weeks later, in July, a further biomass sample was taken. For both species more

300   biomass was collected in July than April, as expected with the warmer weather, but grass

biomass was significantly lower ($P=0.03$) in previously gassed areas, as shown in the

302   performance data. By this time very little clover was present in any plot (control or treatment)

and differences between control and treatment were not significant.

304

4. Discussion

306   The aim of this study was to investigate the effects of elevated soil CO_2 as a result of

hypothetical CCS pipeline leakage on competition between species. The results show that

308   significant responses to soil CO_2 occur, with greater sensitivity seen in the clover than in

grass. A number of previous studies, discussed earlier, have found differences in the

310   sensitivity to soil CO_2 between species, with some suggestion that dicotyledons are more

sensitive than monocotyledons. However, most of these studies have been confined to

312   monocultures rather than interacting systems and the effects on competition have not

previously been subject to systematic study.

314

Although soil CO_2 was variable in our study, being particularly high after rainfall as noted in

316   earlier studies (Hinkle, 1994; Smith *et al.,* 2005; Patil *et al.* 2010; Al-Traboulsi *et al.* 2012a),

elevated concentrations of CO_2 in the soil automatically led to displacement of O_2 with a

318   strong negative correlation between the CO_2 and the O_2. The strength of this correlation

means that it is effectively impossible in this study to distinguish physiological responses to

320   elevated CO_2 from responses to low O_2. The difference between direct responses to CO_2 and

indirect responses to depletion of O_2 is not critical to the risk assessment for CO_2 leakage

322   from CCS, where the same linkage would apply, but an understanding of the mechanisms

may be important in wider applications (see Lake et al. 2013; Lake et al this issue).

324

Stress in the leaves of the grass and clover was observed within ten days, with yellowing of

326   the grass leaves and reddening of the clover leaves in the areas of high gas concentration

before foliage die back, with stress and die back being localised around the area of CO_2

328   injection. While yellowing is a common response to many forms of stress, in appropriate

circumstances yellowing and reddening of leaves are symptoms of nitrogen and phosphorus

330   deficiencies respectively (Lake et al. 2013; Bloem et al. 2003; Rosolem and Tavares 2006)

and the different colour responses shown by grass and clover could indicate differences in

332   physiological response to low O_2 and/or high CO_2. Placing these stress data into a broader

CCS framework and looking at the impact of small scale persistent CO_2 leakage, using the

334   ASGARD facility we have observed that CO_2-induced stress is highly localised across a

variety of crop plants. For example in plots sown with spring oilseed rape and autumn barley

336   stress, the subsequent yield decline was confined to ~0.5 m^2 within the 6.25 m^2 experimental

plots (Lake et al 2013). In the context of a large arable field under the conditions and CO_2 gas

338   flow rates utilised in these field trials, the damage would be minimal, representing less than

0.00006% of a hectare and yield loss would be equally small. This compares to UK yield

340   losses from unconstrained factors, for example, between 10 to 30% in sugar beet under

drought conditions (Ober et al. 2004); and up to 29% and 25 - 50% for oilseed rape and

342   potato, respectively (Zhou et al. 2001) due to disease.

344   The positive relationship between both *A* and *g$_s$* with accumulated biomass in clover holds for

both gassed and non-gassed plants; however, lower values and tighter correlations suggest

346   that both of these physiological traits are constrained under soil gassed conditions. However,

there is an impact on stomatal function causing an inability to close at high soil CO_2,

348   manifested as higher *g$_s$* when compared to non-gassed plants. Carbon acquisition (*A*) should

not, therefore be impeded. The reduction in biomass of gassed plants over time is indicative

350   of carbon-based metabolic costs associated with potential stresses. Regression analyses of

differential impacts of soil and canopy CO_2 concentrations suggest that the effect on stomatal

352   conductance is mainly due to soil CO_2 affecting root physiology. High canopy CO_2, is

documented as causing a reduction in g_s with an increase in carbon gain (Brouder and

354   Volenec 2008, Bunce 1995, Hill et al. 2007, Long et al. 2006, Soussana and Luscher 2007)

does not alleviate the loss of biomass in this study. This specific stomatal response requires

356   further study. The overall effect is seen in reduced total productivity for the clover in the

2011 season (Fig. 6).

358

Smith et al. (2013) reported changes in root structure within the same system. Numbers of

360   roots in the surface soil horizon of the high gas zone increased compared to the numbers in

the control plots. In contrast, in the deep soil horizon there was no difference between the

362   numbers of roots in the high gas zone and the control, but there was an increase in root

numbers in the low gas zone. It should be noted that due to the tendency for CO_2 to sink,

364   together with the effects of proximity to the surface, the deeper horizon tends to have greater

CO_2 concentrations. Evans (1977) found that root hairs of grasses, including *Lolium perenne,*

366   were longer and more frequent than for clovers, which gives the grasses a strong competitive

advantage in water and nutrient uptake. Evans (1978) also showed that although roots of

368   perennial rye-grass and white clover extended to the same depth in soil, there was almost four

times the amount of root length of rye-grass in the top 200 mm of soil when compared to

370   white clover, and almost three times as much root at 0.6-0.8 m depth. Mengel and Steffens

(1985) found that in low potassium soils rye-grass was more able to take up potassium than

372   clover due to its greater root length, four to six times that of red clover. These features

suggest that under stress conditions rye-grass will be a particularly strong competitor to

374   clover and this may explain the greater resistance of grasses to high soil CO_2 concentration.

Unfortunately, it was not possible to distinguish between grass and clover roots (Smith et al.

376   2013), and the results for total root numbers did not indicate whether increases include both

species, or whether one predominates over the other.

378

Our experimental findings are similar to those of Beaubien et al. (2008) who worked on

380   natural CO_2 vents in Latera, Italy and found that grasses were more dominant and that clover

and other non-grass species could not establish if soil CO_2 at 200 mm depth was greater than

382   20%. These results are also in line with above ground CO_2 enrichment experiments

performed at the Giessen FACE (Free Air Carbon dioxide Enrichment) facility, which have

384   reported an increase in grass biomass when compared to clover (Kammann et al 2005).

Previous soil CO_2 gassing studies have also suggested that dicotyledonous species may be

386   more sensitive to high soil CO_2 than monocotyledons (Noyes 1914; Stolwijk and Thimann

1957; Williamson 1968; Glinski and Stepniewski 1985; Bunnell et al. 2002; Rodriguez et al.

388   2005, Al-Traboulsi et al 2012b, Al-Traboulsi 2013) as seen here. Alternatively, the greater

sensitivity of clover found here may be due to the known requirement of symbiotic legume

390   root nodules for copious oxygen (Pugh et al., 1995).

392   *5. Conclusions*

Studies of soil CO_2 injection in a grass-clover sward have distinguished three effects: a direct

394   stress response to elevated soil CO_2, limiting productivity in both species; competition

between the grass and clover affected by differential stress responses to soil CO_2; and an

396   overall temporal trend from dominance by clover to dominance by grass in all treatments.

The direct effect of soil CO_2 (or the associated O_2 deprivation) was a reduction of

398   performance (or productivity relative to potential). The smaller effect on grass allowed it to

compete more effectively with clover in the gassed treatments. At one point in the study, the

400   effect of reduced competition led to an apparent positive response of grass to soil CO_2.

Canopy CO_2 was increased in areas of high soil CO_2 and positive responses may have been

402   expected. However, that is not the case here; increases in canopy CO_2 had little effect on

stomatal conductance. Reduction in biomass over the time course of the study suggests high

404   metabolic costs associated with the stress effects of soil CO_2. Superimposed on the stress and

competition effects was an overall decline in clover dominance, which was independent of

406   CO_2 treatment.

408   Regardless of the specific mechanism, it is clear that differential sensitivities to soil CO_2 exist

and that they result in large effects on interspecies competition. More complex polycultures

410   or non-leguminous dicotyledons may respond differently. In the context of addressing the

potential effects of CCS, further studies of these issues are needed, particularly in non-

412   cultivated ecosystems.

414   Importantly in assessing the risks created in the unlikely event of a potential leak of CO_2 from

a CCS transportation network or storage site, the following conclusions can be drawn.

416   Significant responses to soil CO_2 clearly occur, with greater sensitivity seen in clover than in

grass. However, the responses to soil CO_2 were localised, being confined to the experimental

418   plots in line with previous work. Although differential effects persisted to some extent after

gassing ceased, recovery close to the potential levels of the original level of productivity was

420   complete within a few months. Our results suggest that persistent effects would be small and

that ecological impacts in the unlikely event of a leak would be manageable without high

422   levels of intervention.

434

Figure Legends

436 **Fig. 1.** Schematic showing plot layout, gas measurement, bar-holing points and sampling

transects (A_1-A_4 and B_3-D_3) used in this study. Red squares mark areas of high soil CO_2 and

438 blue low soil CO_2.

440 **Fig. 2.** Representative contour map of seasonally adjusted soil CO_2 concentration (%)

measured on 12th June 2012, measured at 0.3 m depth. X and Y axes units are in cm. Z axis is

442 % CO_2. Plot boundaries are marked by the thick dotted line.

444 **Fig. 3.** Positive relationship between mean canopy CO_2 (ppm) and mean soil CO_2 (%) in

gassed plots measured in May 2011, ($R^2 = 0.9604$, p <0.0001).

446

Fig. 4. Clover mean photosynthetic rate (A) and stomatal conductance (B) during the

448   growing season 2011 of CO_2-gassed and non-gassed control plants. (Not statistically

significantly different (Student's t-test), n = 12 , bar = SE_{mean})

450

Fig. 5. Relationship of accumulated clover biomass with mean photosynthetic rate (A) and

452   with stomatal conductance (B) over the growing season May to October 2011. (Regression

analysis, (A) gassed plants $R^2 = 0.69$, $P = <0.005$, control plants $R^2 = 0.32$, $P = 0.07$; (B)

454   gassed plants $R^2 = 0.6$, $P = 0.001$, control $R^2 = 0.78$, $P = <0.0001$, n = 12)

456   **Fig. 6**. Combined productivity (A) and dominance (B) of grass and clover throughout the

period of study in the control plots, low (<10%), and high (>10%) CO_2 concentration zones

458   of the gassed plots. Hatched box corresponds to period of CO_2 treatment.

460   **Fig. 7**. Performance of clover (A) and grass (B) throughout the period of study in the control

plots, low (<10%), and high (>10%) CO_2 concentration zones of the gassed plots. Hatched

462   box corresponds to period of CO_2 treatment.

464   **Fig. 8.** Performance of clover (A) and grass (B) as a function of dominance for low (dashed

line grey symbols) and high gas (solid line open symbols).

466

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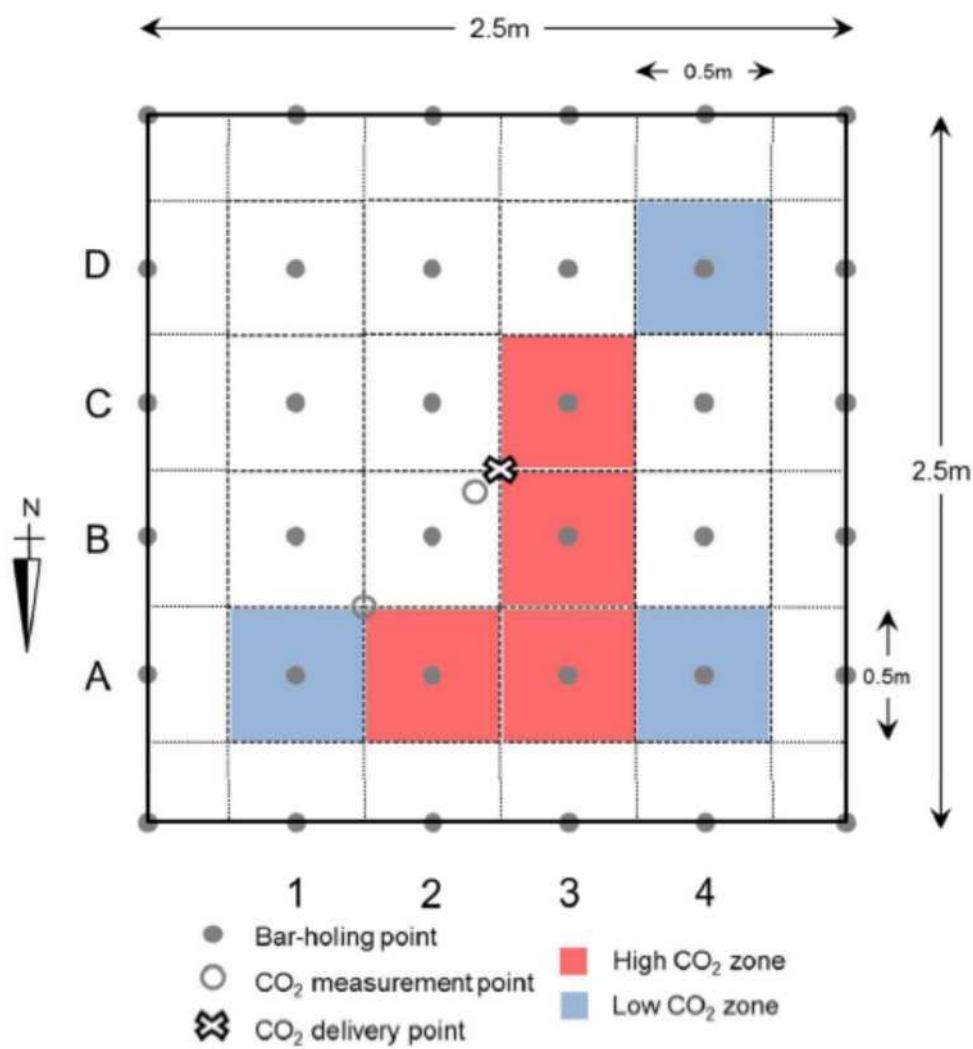(Brassica napus) in Southern England. Plant Pathol. **49,** 487−497.

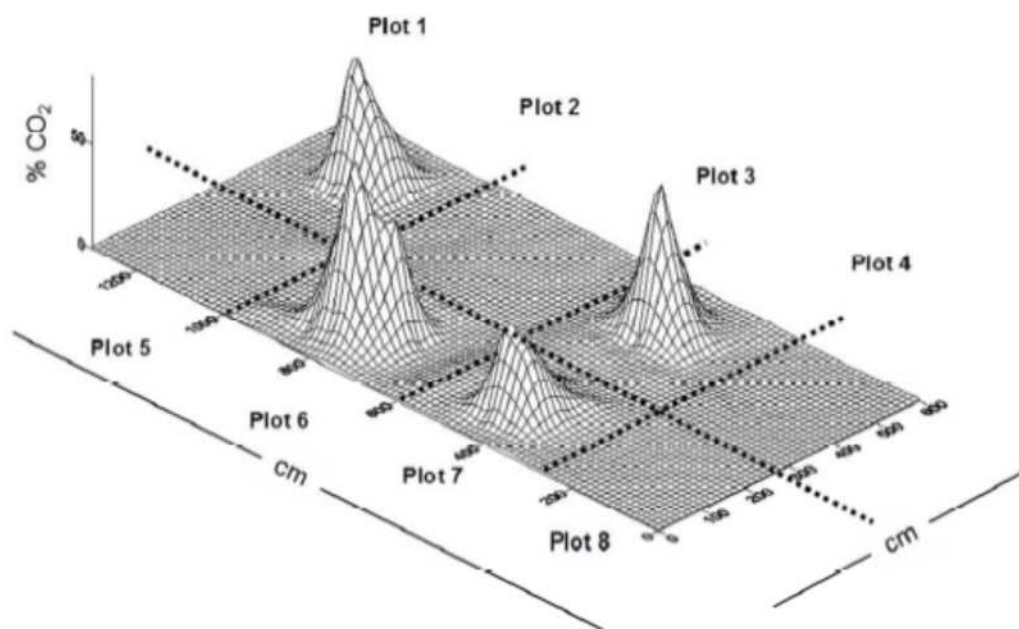626

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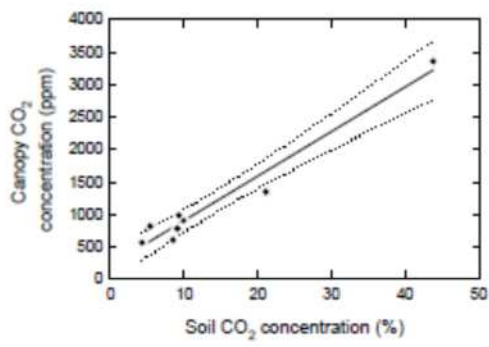
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Fig. 2

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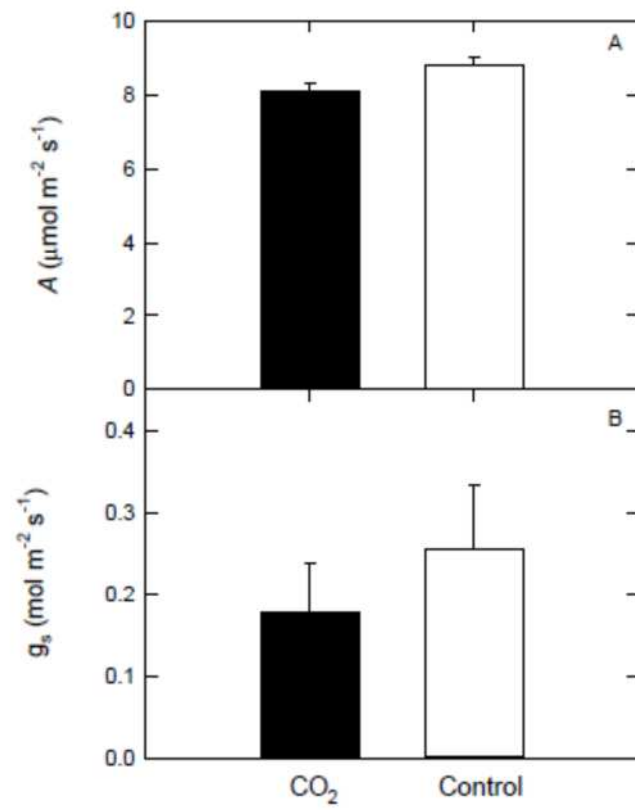
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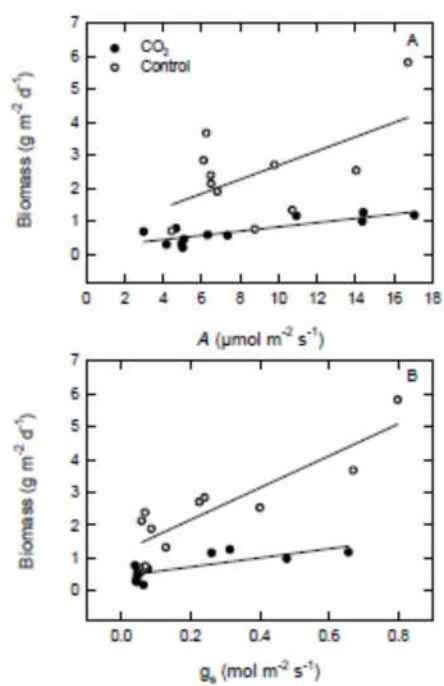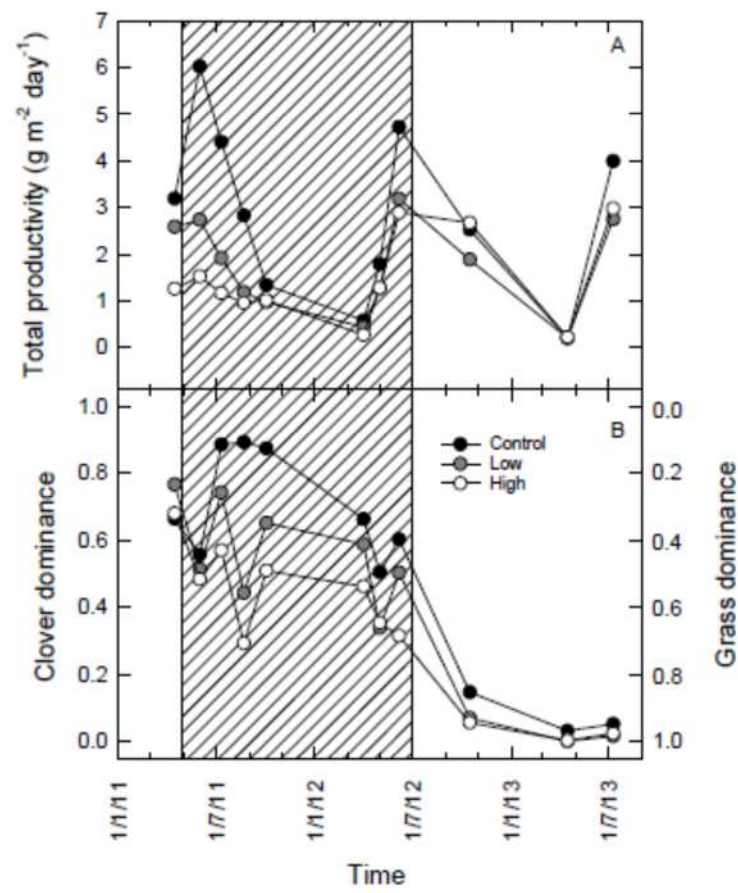
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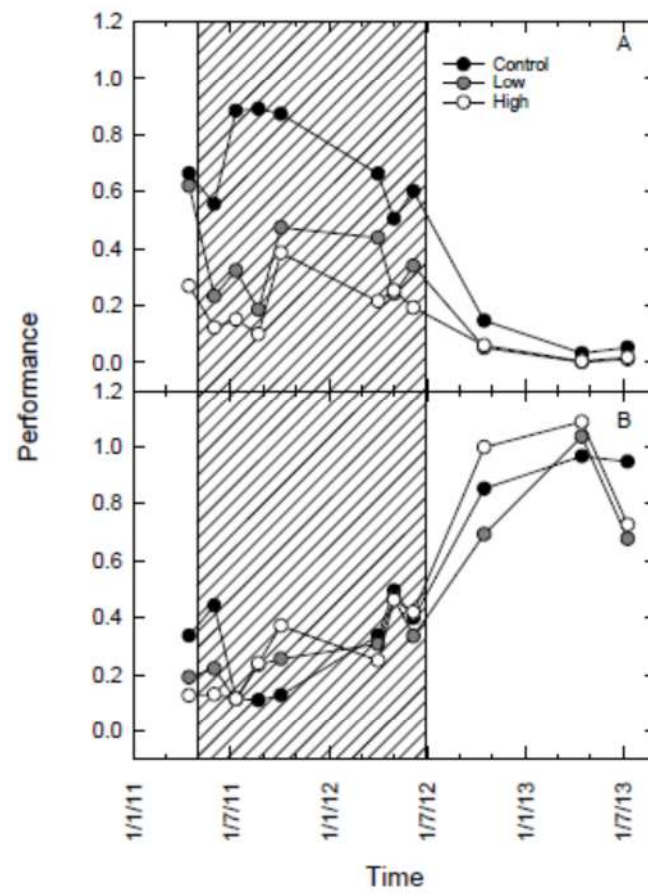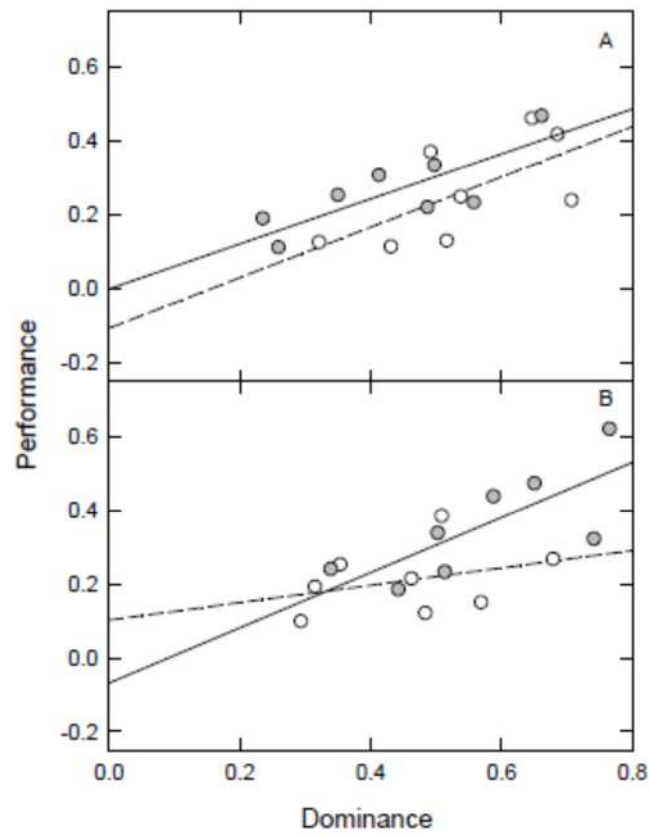654

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