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**Effects of elevated soil CO₂ concentration on growth
and competition in a grass-clover mix.**

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CCS, roots

Abstract

22 To investigate potential environmental affects in the context of carbon dioxide (CO₂) leakage
from Carbon Capture and Storage (CCS) schemes. The ASGARD (Artificial Soil Gassing
24 and Response Detection) facility was established, where CO₂ 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 CO₂ 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
CO₂ treatments. The direct effect of soil CO₂ (or associated oxygen (O₂) deprivation due to
32 the high levels of CO₂ 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 CO₂ gassed zones
34 respectively. Canopy CO₂ increased by 70 parts per million (ppm) for every 1% increase in
soil CO₂ 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 CO₂ affecting root physiology. Biomass measurements made after gassing has
ceased indicated that recovery of vegetation was close to complete after 12 months.

40



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1. Introduction

42

Carbon Capture and Storage (CCS) has been advocated as a means of reducing rising levels
44 of atmospheric carbon dioxide (CO₂) to help mitigate climate change. Captured CO₂ is
compressed and transported via pipeline to storage sites in deep geological reservoirs
46 (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.
48 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),
50 it is nevertheless a regulatory requirement to demonstrate that the impacts of any possible
leaks from CCS infrastructure, (including transportation pipelines) have been investigated
52 and understood. In the unlikely event of captured CO₂ reaching the surface, CO₂ in the soil
would rise, possibly to values approaching 100%; diffusion from the soil would lead to
54 increased atmospheric CO₂, but to a much lesser extent due to rapid air mixing. CO₂ may also
dissolve in soil water leading to changes in the pH level and possible uptake by plants in the
56 transpiration stream (Steven et al. 2010). Atmospheric CO₂ may stimulate plant
photosynthesis, but high soil concentrations are usually detrimental (IPCC, 2005). While
58 much research in the context of global environmental change has been carried out to
determine the effects of elevated atmospheric CO₂ on vegetation (Kimball et al., 1993; Van
60 Noordwijk et al., 1998; Ghannoum et al., 2000; Moscatelli et al., 2001), much less is known
about the potential effects of elevated soil CO₂.

62

Previous laboratory studies have reported significant plant stress responses to soil CO₂, with
64 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 CO₂ (ca. 2 to 6%) concentrations, similar to background soil CO₂ 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 CO₂ being recorded (10 and 12%
70 recorded (Chang and Loomis 1945; Stolwijk and Thimann 1957; Russell 1973; Glinski and
Stepniewski 1985). Natural CO₂ vents have been proposed as CCS leakage analogues, for
72 example at Stavešinci, Slovenia, where plant height corresponded inversely with soil CO₂
(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 CO₂ vent, consistent with the
suggestion of differential sensitivities of plants. However, these seeps have been leaking CO₂
76 for extended periods so that the vegetation growing in the vicinity may have become adapted
to the high soil CO₂ conditions. Moreover, at natural analogue sites, smaller concentrations of
78 methane and trace amounts of more toxic gases, such as hydrogen sulphide (H₂S) or sulphur
dioxide (SO₂) may also be present (Pfanzen et al., 2004) making it difficult to attribute direct
80 CO₂ effects.

82 Assessment of the potential impact posed in the unlikely event of leakage of CO₂ 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 CO₂ 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

90 2.1 Experimental plots

The ASGARD (Artificial Soil Gassing And Response Detection) facility was located in a
92 field of permanent pasture at the Sutton Bonington campus of the University of Nottingham,
UK (N 52.8°, W 1.2°). CO₂ was injected into the soil in 16 field plots (each 2.5 m × 2.5 m)
94 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 CO₂ was
96 delivered into the soil at a depth of 500 - 600 mm below the centre of each CO₂ gassed plot
via perforations in the end of the pipes. This depth was chosen to limit lateral gas migration
98 across the site. Food-grade, liquid CO₂ was stored in two 200 L cryogenic vessels (BOC,
Derby, UK), the liquid CO₂ was converted to gaseous phase CO₂ and regulated down to a
100 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). CO₂
102 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,
104 Great Yarmouth, UK). For a full site description and characterisation see Smith *et al.* (2013).

106 For this study eight experimental plots were used, each separated by a 1 m border. Four
randomly selected plots were injected with CO₂ gas and four acted as untreated controls.
108 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
110 plant physiological measurements were measured in two transects running East-West
(subplots A₁-A₄) and North-South (A₃-D₃) crossing the zone of highest soil gas
112 concentration; a single transect running East-West (A₁-A₄) 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. CO₂ was delivered to the centres of the four plots from 21st March 2011 to
118 15th June 2012.

120 2.2 Gas measurement

CO₂ 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. CO₂ and
124 oxygen (O₂) 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
CO₂ within the soil. However it is intrinsically prone to some underestimation of CO₂
130 concentration because of the possibility of air mixing with the sample.

132 The seasonal average of CO₂ 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 CO₂ concentrations obtained by bar-holing were then scaled using the mean of the two
138 ratios of permanent tube to bar-hole CO₂. This method assumes that any effects of air mixing

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 CO₂
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 CO₂ concentrations above and below 10%. The average scaled CO₂
144 concentration in the high gas (sampling squares A₂, A₃, B₃ and C₃) and low gas (sampling
squares A₁, A₄ and D₃) zones were 19 and 5% respectively (Fig. 1).

146

Above-ground within-canopy CO₂ 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 A₁-A₄, B₃, C₃ and D₃ in the gassed plots and subplots A₁-A₄ 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

CO₂ 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.

170 *2.4 Plant physiological measurements*

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 CO₂
gassed and control plot respectively for each time point.

178

2.5 Meteorological conditions

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

188 3.1 Soil CO₂ and O₂ concentrations

Mean soil CO₂ 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 CO₂ 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 CO₂ was 9.4% (± 0.5) in 2011, increasing to 22.2%
194 (± 1.7) in 2012. Peak CO₂ 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 CO₂ and caused a build-up of injected CO₂ within the soil. In the control
198 plots, mean CO₂ was 0.7 % (± 0.06) in 2011 and 1.0 % (± 0.05) in 2012. Bar-holing data
indicated that the soil CO₂ was generally highest to the North of the plot centre and decreased
200 radially such that the CO₂ at the edge of the plot was similar to that seen in the controls.

Importantly the injected CO₂ (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 CO₂ and O₂ measured in the permanently installed access tubes, consistent with simple
204 displacement of normal soil air by the injected CO₂ as found at other artificially induced CO₂
sites e.g. ZERT site (Zero Emissions Research and Technology), USA (Zhou *et al.* 2013).

206

3.2 Plant Canopy CO₂

208 Within-canopy CO₂ concentrations at a 50 mm height showed a highly significant positive
correlation with soil CO₂ (Fig. 3). Mean data for the CO₂-gassed plots measured in May 2011

210 showed that the canopy CO₂ 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 CO₂ delivery.
216 Although the stress was highly localised with the grass turning yellow and the clover leaves
turning purple in the highest CO₂ concentration areas (Supplementary Fig. S2). As the season
218 progressed, vegetation died back in the area of highest CO₂. 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 CO₂ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for A and 0.04 and 0.08 $\text{mol m}^{-2} \text{s}^{-1}$ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ reducing dominance, averaged over the
252 gassing period, to 77% in the low CO₂ treatment and 62% of the control values in the high
CO₂ 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 CO₂ 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 CO₂, 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 CO₂ plots, grass *performance* is more
resilient, indicating lower sensitivity to soil CO₂; 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 CO₂ suggests an interaction between
stress induced by CO₂ 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 CO₂, causes a
284 42% decrease in grass and 66% decrease in clover performance.

286 3.6 Recovery

Following shut-down of CO₂ delivery (15th June 2012), further samples of the plots were
288 taken to assess the long-term implications of CO₂ release and to monitor post-CO₂ 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 CO₂ plots remained
significantly lower ($P=0.003$) than in the controls. A second sampling of biomass, 10 months
294 after termination of the CO₂ 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 CO₂ as a result of
hypothetical CCS pipeline leakage on competition between species. The results show that
308 significant responses to soil CO₂ 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 CO₂ 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 CO₂ 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 CO₂ in the soil automatically led to displacement of O₂ with a
318 strong negative correlation between the CO₂ and the O₂. The strength of this correlation
means that it is effectively impossible in this study to distinguish physiological responses to
320 elevated CO₂ from responses to low O₂. The difference between direct responses to CO₂ and
indirect responses to depletion of O₂ is not critical to the risk assessment for CO₂ 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 CO₂
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 O₂ and/or high CO₂. Placing these stress data into a broader
CCS framework and looking at the impact of small scale persistent CO₂ leakage, using the
334 ASGARD facility we have observed that CO₂-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 m² within the 6.25 m² experimental
plots (Lake et al 2013). In the context of a large arable field under the conditions and CO₂ 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 CO₂,
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 CO₂ concentrations suggest that the effect on stomatal
352 conductance is mainly due to soil CO₂ affecting root physiology. High canopy CO₂, 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 CO₂ to sink,
364 together with the effects of proximity to the surface, the deeper horizon tends to have greater
CO₂ 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 CO₂ concentration.

Unfortunatly, 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 CO₂ vents in Latera, Italy and found that grasses were more dominant and that clover
and other non-grass species could not establish if soil CO₂ at 200 mm depth was greater than
382 20%. These results are also in line with above ground CO₂ 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 CO₂ gassing studies have also suggested that dicotyledonous species may be
386 more sensitive to high soil CO₂ 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 CO₂ injection in a grass-clover sward have distinguished three effects: a direct
394 stress response to elevated soil CO₂, limiting productivity in both species; competition
between the grass and clover affected by differential stress responses to soil CO₂; and an
396 overall temporal trend from dominance by clover to dominance by grass in all treatments.

The direct effect of soil CO₂ (or the associated O₂ deprivation) was a reduction of
398 performance (or productivity relative to potential). The smaller effect on grass allowed it to

400 compete more effectively with clover in the gassed treatments. At one point in the study, the effect of reduced competition led to an apparent positive response of grass to soil CO₂.

402 Canopy CO₂ was increased in areas of high soil CO₂ and positive responses may have been expected. However, that is not the case here; increases in canopy CO₂ had little effect on stomatal conductance. Reduction in biomass over the time course of the study suggests high metabolic costs associated with the stress effects of soil CO₂. Superimposed on the stress and competition effects was an overall decline in clover dominance, which was independent of CO₂ treatment.

408 Regardless of the specific mechanism, it is clear that differential sensitivities to soil CO₂ exist and that they result in large effects on interspecies competition. More complex polycultures 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-cultivated ecosystems.

414 Importantly in assessing the risks created in the unlikely event of a potential leak of CO₂ from a CCS transportation network or storage site, the following conclusions can be drawn.

416 Significant responses to soil CO₂ clearly occur, with greater sensitivity seen in clover than in grass. However, the responses to soil CO₂ were localised, being confined to the experimental 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 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 levels of intervention.

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434

Figure Legends

436 **Fig. 1.** Schematic showing plot layout, gas measurement, bar-holing points and sampling
transects (A₁-A₄ and B₃-D₃) used in this study. Red squares mark areas of high soil CO₂ and
438 blue low soil CO₂.

440 **Fig. 2.** Representative contour map of seasonally adjusted soil CO₂ concentration (%)
measured on 12th June 2012, measured at 0.3 m depth. X and Y axes units are in cm. Z axis is
442 % CO₂. Plot boundaries are marked by the thick dotted line.

444 **Fig. 3.** Positive relationship between mean canopy CO₂ (ppm) and mean soil CO₂ (%) 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 CO₂-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%) CO₂ concentration zones
458 of the gassed plots. Hatched box corresponds to period of CO₂ 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%) CO₂ concentration zones of the gassed plots. Hatched
462 box corresponds to period of CO₂ 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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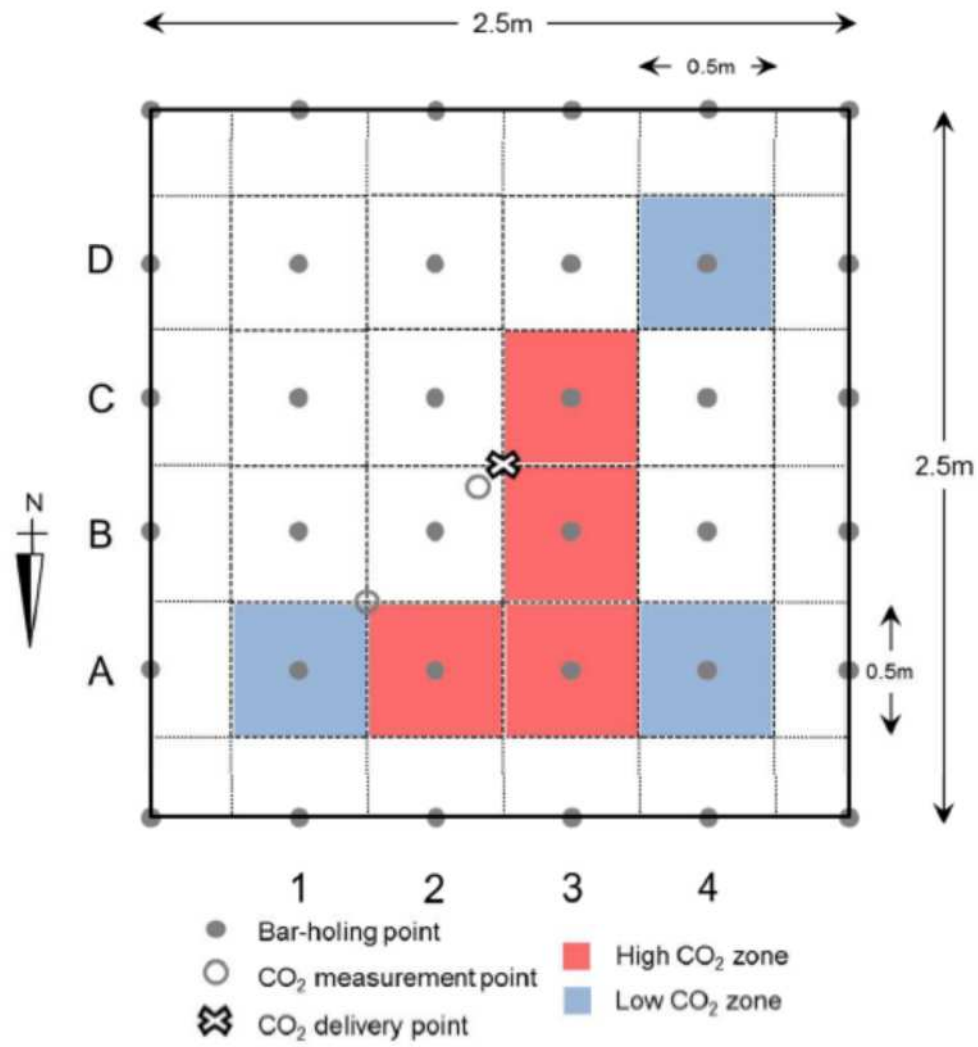


Fig. 1
Smith et al 2016

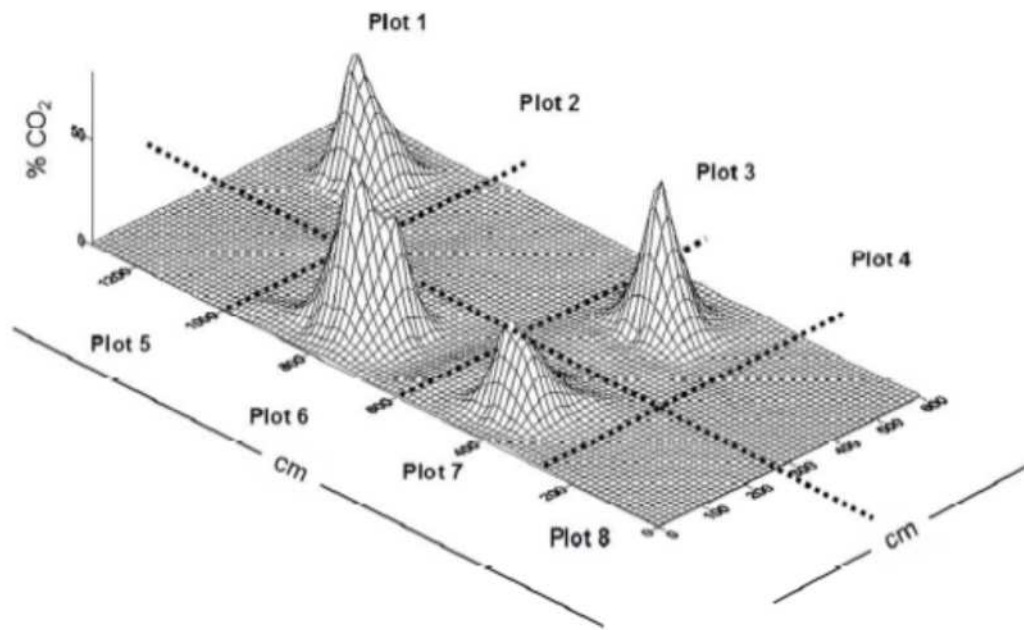


Fig. 2
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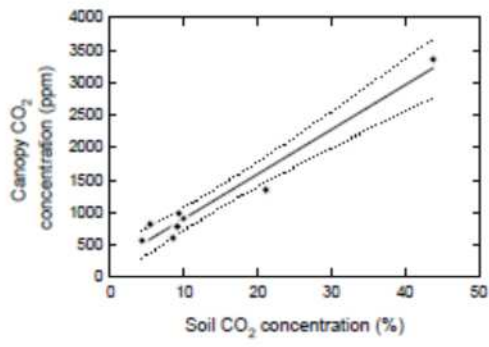


Fig. 3
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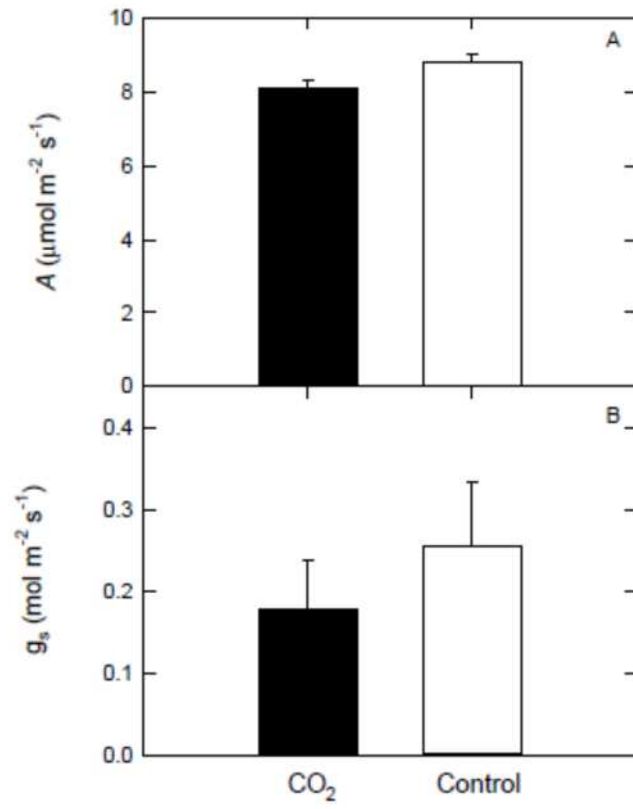


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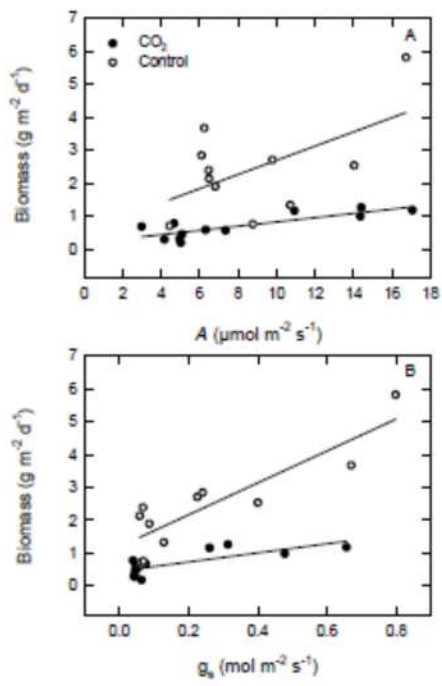


Fig. 5
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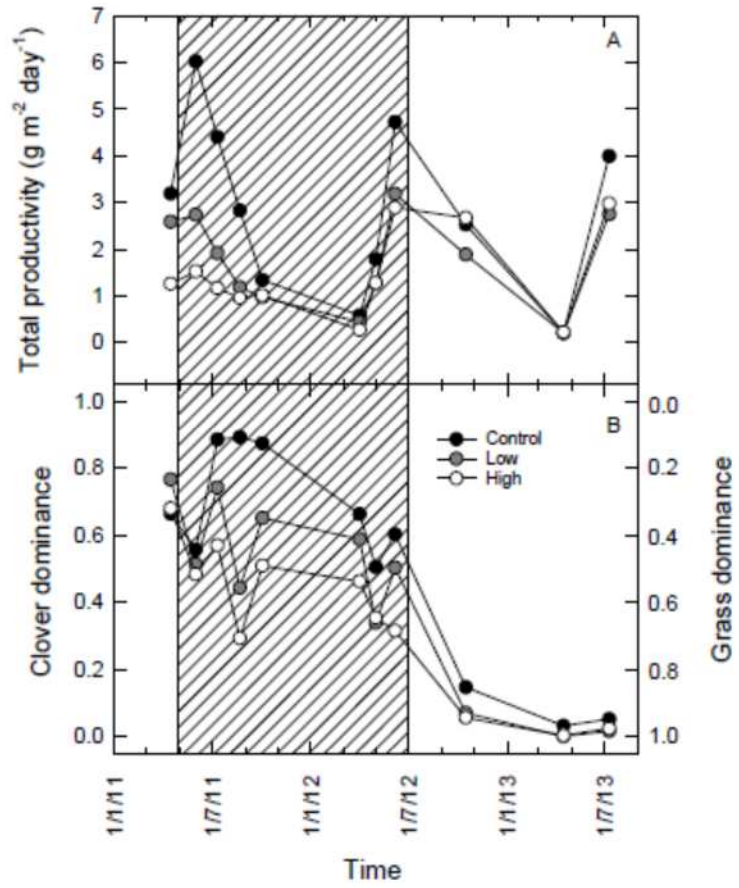


Fig. 6
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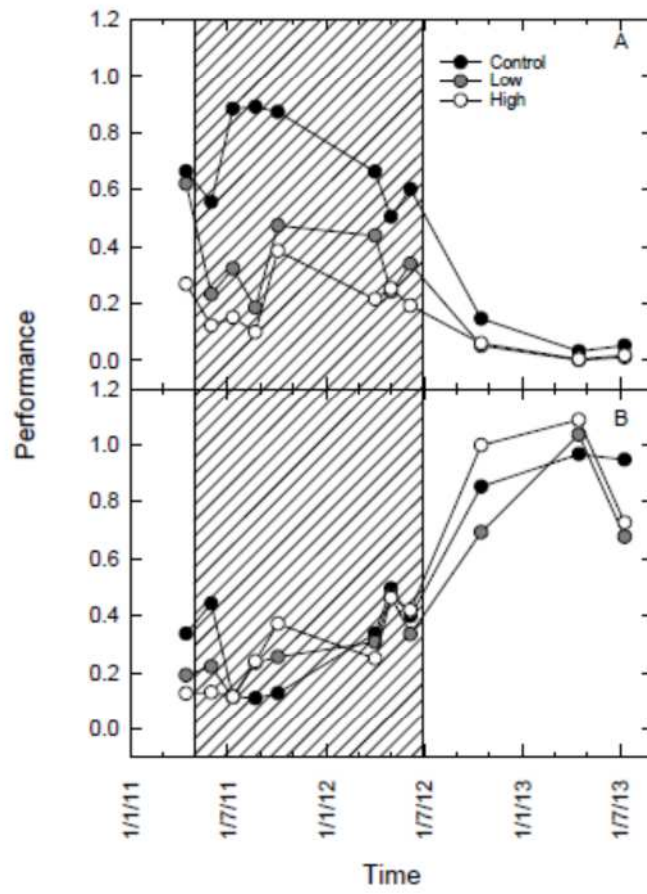


Fig. 7
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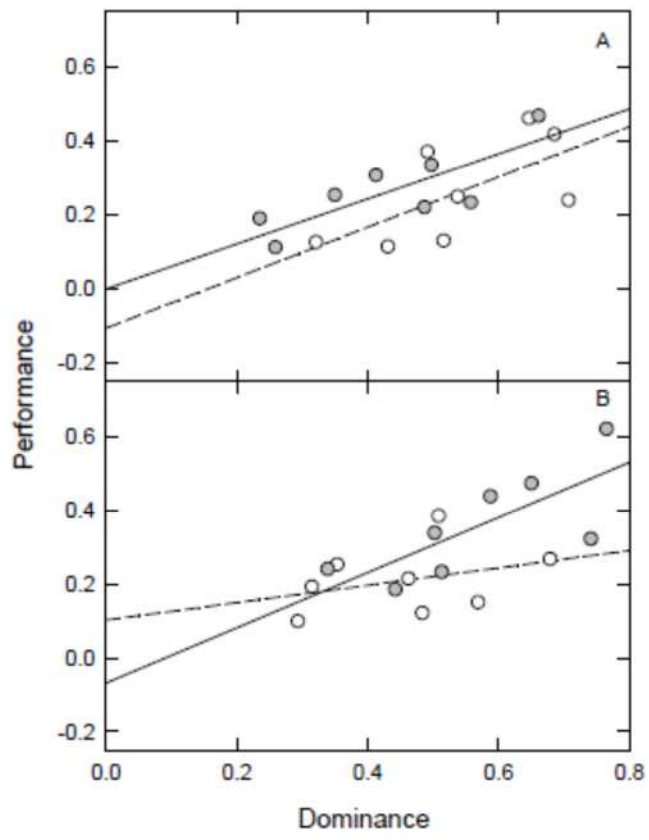


Fig. 8
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