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3 **Plant responses to simulated carbon**  
**capture and transport leakage: the effect of impurities in the CO<sub>2</sub> gas**  
**stream.**

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15 **Running Title**

**Impurities in the CO<sub>2</sub> gas stream**

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Key Words: extreme CO<sub>2</sub>, soils, gas exchange, SO<sub>2</sub>, H<sub>2</sub>S, crops, soil pH, carbon capture and  
storage, CCS

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24 **Abstract** To deliver an effective transition from a carbon-based to a carbon-free energy  
market, bridging technologies are required. One such possibility is the use of carbon capture  
and storage, (CCS). However, before such innovations can be rolled out a key requirement is  
27 to understand the environmental impact of these technologies. Recent experimental work has  
demonstrated that small scale CO<sub>2</sub> leakage from CCS pipeline infrastructure has a localised  
and possibly transient impact. However, what remains unknown is the possibility of  
30 synergistic impact of impurities in the CO<sub>2</sub> gas stream. Here we report the impact of two  
impurities SO<sub>2</sub> (100 ppm SO<sub>2</sub> in pure CO<sub>2</sub>) and H<sub>2</sub>S (80ppm H<sub>2</sub>S in pure CO<sub>2</sub>) on the growth  
and performance of two crop species (spring wheat, *Triticum aestivum* and beetroot, *Beta*  
33 *vulgaris*) in fully replicated experiments. Our data show that when compared to CO<sub>2</sub>-only  
gassed controls, the impact of these impurities are minimal as there are no statistically  
significant differences between performance parameters (photosynthesis, stomatal  
36 conductance and transpiration) or biomass. These results signify that from a plant health  
perspective it may not be necessary to completely remove these specific impurities prior to  
CO<sub>2</sub> transportation.

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## Introduction

48 Many high CO<sub>2</sub> emitting industries (e.g. power stations) in the UK are distant from potential  
carbon storage sites (offshore geological reservoirs) and therefore an infra-structure of CO<sub>2</sub>  
transportation must be initiated to carry the CO<sub>2</sub> to safe storage. As such there is a need to  
51 understand the risks involved and mitigation of potential leaks associated with CCS and  
dense-phase CO<sub>2</sub> transportation networks into the environment. Recent experimental work  
has highlighted that the effects of CO<sub>2</sub> leakage on vegetation are highly localised (e.g. Zhou  
54 et al., 2013, Sharma et al., 2014, see Smith et al., 2016) and transient with recovery of  
vegetation close to complete after 12 months (Smith et al., 2016) and that stress is induced by  
direct CO<sub>2</sub> exposure in addition to a function of O<sub>2</sub> depletion (Lake et al., 2016).

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There are, however, two largely unresolved issues; firstly is the role played by both soil type  
and soil structure in mitigating and/ or enhancing observed plant stresses and secondly, the  
60 effects of impurities such as SO<sub>2</sub> and H<sub>2</sub>S that may be present in the CO<sub>2</sub> gas stream. Here we  
address the second issue namely that of impurities in the gas stream.

63 Impurities in the CO<sub>2</sub> gas stream are a consequence of the specific combusted fuels and  
capture technologies (Porter et al., 2015). Impurities not only act on the transport properties  
of the gas stream (Skaugen et al., 2016) but in the event of leakage into the soil environment,  
66 will impact on vegetation (including crop plants) growing above the pipeline. The range of  
impurities and potential concentrations within a pure CO<sub>2</sub> gas stream include both  
biologically toxic and non-toxic compounds all of which can impact on transportation  
69 processes. Non-toxic impurities include H<sub>2</sub>O and O<sub>2</sub> (Brown et al., 2014, Porter et al., 2015)

and are not detrimental to plants at normal levels in the soil. However, some are known to adversely affect vegetation e.g. SO<sub>x</sub> and NO<sub>x</sub> when present in atmospheric pollution.

72 Atmospheric loading of these gases reduces the ability of plants to tolerate other abiotic stress factors. For example, the freezing tolerance of heather (*Calluna vulgaris*) is adversely affected by long-term experimental fumigation of SO<sub>2</sub> (plus NO<sub>2</sub>) at a concentration of 40 nl  
75 l<sup>-1</sup> (40 ppb) (Caporn et al., 2000); and when *in situ* tolerant plants surrounding a lignite-based thermal power station in the Chennai region of India were monitored for chlorophyll, water content and pH of leaves under constant SO<sub>2</sub> values of 13 to 18 μg m<sup>-3</sup> (13 to 18 ppb)  
78 (Govindaraju et al., 2012), all three parameters were reduced suggesting that stress is experienced under constant air pollution associated with coal combustion. H<sub>2</sub>S has been studied more extensively and is now thought to be involved in biochemical signalling in  
81 plants, primarily by priming the biochemical defence responses to abiotic stress, comprehensively reviewed by Lisjak et al., (2013).

84 Studies specifically involving the soil or root environment are very few in this particular context, Christou et al. (2013) demonstrated the priming ability in strawberry to enhance tolerance to salt stress by subjecting roots to H<sub>2</sub>S treatment in hydroponic systems. They  
87 found no effect of H<sub>2</sub>S on chlorophyll fluorescence, stomatal conductance or water content of leaves compared to non-treated controls, while Cheng et al. (2013) found beneficial effects of H<sub>2</sub>S for root protection during extreme hypoxia events in *Pisum sativum*, again in hydroponic  
90 systems.

To date there have been no studies into the effects on vegetation of SO<sub>2</sub> and H<sub>2</sub>S as  
93 components in a CO<sub>2</sub> gas stream delivered directly into the soil environment. To address

these knowledge gaps we build on recent experimental protocols (Lake et al., 2016) to test for differences in plant stress as a function of impurities within a pure CO<sub>2</sub> stream.

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## **Materials and methods**

### *Experimental setup*

99 Soil chambers were constructed of acrylic plastic with pipe inlets to allow CO<sub>2</sub> gassing of the soil environment exclusively. The experimental system was housed in a controlled environment growth facility (UNIGRO, UK) to standardise the following environmental  
102 variables: irradiance was 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (at plant height), day/night as 12/12 hours; temperature 21/18°C; and relative humidity 60%. Gas was supplied from either an integral supply (pure CO<sub>2</sub>) or a gas cylinder and separated prior to entering each individual soil  
105 chamber by two flow rate step-down manifolds. Gas was delivered to each individual chamber at a rate of 30 ( $\pm 15$ ) mL min<sup>-1</sup> to maintain CO<sub>2</sub> at steady state. Gases were exhausted to the atmosphere via a separate manifold to prevent build up within the growth room. In all  
108 experiments gas concentrations (CO<sub>2</sub> and O<sub>2</sub>) were measured daily using the GEOTECH GA5000 gas analyser (Geotech, Warwickshire, UK).

### 111 *CO<sub>2</sub> impurities*

To examine the specific effects that impurities within the CO<sub>2</sub> stream may have on plant responses to simulated CCS leakage certified custom gas mixes were used (manufactured and  
114 supplied by BOC, UK). The effect of SO<sub>2</sub> was studied using a mix of 100 ppm SO<sub>2</sub> in pure CO<sub>2</sub> and H<sub>2</sub>S using 80ppm H<sub>2</sub>S in pure CO<sub>2</sub>. These values were derived as midrange values for these impurities present in the gas stream from different carbon capture technologies

117 (Table 1). To test for specific effects of the impurities, treatment plants (CO<sub>2</sub> + SO<sub>2</sub> or CO<sub>2</sub> +  
H<sub>2</sub>S) were compared to treatment CO<sub>2</sub>- only gassed control plants.

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### *Crop species*

Crop plants used were spring wheat (*Triticum aestivum* v Tybault - a monocotyledon, grass)  
123 and beetroot (*Beta vulgaris* v Pablo F1 - a dicotyledon, vegetable). Crops were sown and  
grown in Levington's no. 3 multipurpose compost within an environmental controlled growth  
room (details above) for 1 to 2 weeks before being transplanted into the soil chambers. They  
126 were then left to allow sufficient root growth before gassing commenced (approximately 2  
weeks later). The gassing period lasted for up to 5 days. After that time, plants become pot-  
bound which affects physiology and no longer reflects field conditions, hence the experiment  
129 was terminated. : Replication consisted of four control plants gassed with CO<sub>2</sub> and six plants  
gassed with CO<sub>2</sub> + H<sub>2</sub>S and six plants gassed with CO<sub>2</sub> + SO<sub>2</sub>.

### *Biomass (shoot)*

132 Plants were harvested between and at the end of each experiment. All shoots (leaves and  
stems) were taken from each plant, weighed, then dried at 80° C for 2 days and re-weighed.  
Biomass was measured as fresh and dry weight.

135

### *Plant gas exchange*

Gas exchange parameters (photosynthesis (*A*), stomatal conductance (*g<sub>s</sub>*) and evaporation (*E*)  
138 are a measure of plant performance under experimental conditions and determines both the  
ability of plants to acquire carbon and the rate of simultaneous water loss. Measurements

were made using a Li-Cor 6400x IRGA (Li-Cor Inc, Lincoln, Nebraska, USA) on each  
141 replicate plant prior to and then daily during gassing until harvest.

### *Soil pH*

144 Samples were dried at  $40 \pm 4^\circ\text{C}$  and pH determined following the method of Taylor et al.,  
(2005).

147 All statistical analyses were carried out using Minitab v 12 (USA). Student's t-tests of each  
treatment from each other (comparison of means).

## 150 **Results**

### *CO<sub>2</sub> concentrations*

Comparisons between impurity plus CO<sub>2</sub> experiments and pure CO<sub>2</sub> experiments indicate that  
153 levels of CO<sub>2</sub> and O<sub>2</sub> are similar across all experiments (Table 2).

### *Biomass*

156 Fig 1 shows the biomass measurements of wheat (A & B) and beetroot (C & D) when  
compared to CO<sub>2</sub> gassed control plants. In all measured parameters there is no statistically  
significant additional effect of added impurities compared to CO<sub>2</sub> gassed controls.

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### *Gas exchange*

Fig 2 shows gas exchange parameters ( $A$ ,  $g_s$  and  $E$ ) of wheat and Fig 3 of beetroot compared to  $\text{CO}_2$  gassed controls. All parameters are affected within the first day of gassing manifested as a dramatic reduction. Photosynthetic rate ( $A$ ) is less affected than both  $g_s$  and  $E$ . Species differences are apparent with  $\text{SO}_2$  causing greater reductions on  $A$  in wheat than  $\text{H}_2\text{S}$  while  $\text{H}_2\text{S}$  has a greater effect on  $A$  than  $\text{SO}_2$  on  $A$  in beetroot. Although there are no significant differences between  $\text{CO}_2$  gassed control plants and those with added impurities, Table 3 more clearly illustrates the differences in response of each species when the effect of impurities is calculated as a % of  $\text{CO}_2$ -gassed control plants. Both respond with a slight decrease in overall biomass with addition of  $\text{H}_2\text{S}$  (black outline), while plant performance parameters are differentially affected; wheat is adversely affected by  $\text{SO}_2$  (dashed outline) and beetroot by  $\text{H}_2\text{S}$  (black outline). Fig 4 shows the correlations of stomatal conductance ( $A$ ), transpiration rate ( $B$ ) and photosynthetic rate ( $C$ ) with  $\text{CO}_2$  concentrations during each experiment. There is a much stronger correlation with  $\text{CO}_2$  concentration and both  $g_s$  and  $E$  (water loss) than with  $A$  (carbon gain).

### *Soil pH*

Table 4 shows the pH of soil prior to growing plants and the experimental treatments along with post-gassing (experimental end). In all cases, the pre-gassed compost is significantly more acidic than with plants and gasses ( $p = <0.01$ , Student's t-test of means). Soil in the wheat experiment with  $\text{SO}_2$  added is significantly more acidic than with  $\text{CO}_2$  alone ( $p = 0.013$ , Student's t-test of means).

### **Discussion**

CO<sub>2</sub> concentrations (and O<sub>2</sub>-depletion) are comparable for both sets of experiments. As the impurities are mixed within the CO<sub>2</sub> gas stream, uniformity of impurity is delivered throughout. Biomass data is consistent with previous studies of CO<sub>2</sub> gassing alone (Lake et al., 2016a) and provides evidence that there is no additional effect on productivity when SO<sub>2</sub> or H<sub>2</sub>S are present within the CO<sub>2</sub> gas stream. Gas exchange data suggest the mechanism as a disruption to water relations measured as  $g_s$  and  $E$  as evidenced by much stronger correlations between CO<sub>2</sub> concentration and both  $g_s$  and  $E$  (water loss) than with  $A$  (carbon gain) (Fig 4). This is commensurate with previous studies using this system which demonstrated that the main effect of CO<sub>2</sub> gassing is to reduce stomatal conductance with consequent loss of stomatal control (Lake et al 2016b). However, again there is no additional effect from impurities added to the CO<sub>2</sub> gas stream. While all gas exchange parameters are considerably reduced under CO<sub>2</sub> gas alone compared to non-gassed plants (Lake et al 2016a), species responses to each impurity are evident. Table 3 shows the % change in plants under CO<sub>2</sub> + SO<sub>2</sub> and CO<sub>2</sub> + H<sub>2</sub>S from CO<sub>2</sub> gassed control plants. Although the changes are small, and not statistically significant, when calculated as % change SO<sub>2</sub> shows slight increases in biomass measurements, compared to H<sub>2</sub>S which shows slight decreases. Gas exchange parameters are reduced under SO<sub>2</sub> in wheat, whereas they are reduced under H<sub>2</sub>S in beetroot. This suggests that different stress mechanisms may be employed by different species in response to different impurities and importantly that all impurities cannot be assumed to produce the same results.

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Soil pH (Table 4) of the compost before adding the crop plant and prior to gassing is significantly lower than after the experiments illustrating the ability of plants to influence their soil environment and raise pH to a more favourable level. Plants achieve this by producing root exudates to counter or increase acidity dependent on soil conditions as well as

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influence interactions with other organisms (Wang et al., 2016, Sarker & Karmoker 2016,  
210 Bais et al., 2006). Only under CO<sub>2</sub> + SO<sub>2</sub> in wheat does the soil become significantly lower in  
pH than CO<sub>2</sub> gassing alone, however, this is still above the pH of pre-gassed compost, and  
did not translate into any additional impact on biomass.

## 213 **Conclusions**

For the first time our data demonstrate that trace amounts of impurities SO<sub>2</sub> and H<sub>2</sub>S in pure  
CO<sub>2</sub> that are likely to be entrained within a CCS CO<sub>2</sub> stream have a negligible impact on  
216 plant functional biology (at least under these experimental conditions) when compared to  
plants exposed to pure CO<sub>2</sub>. Therefore these data imply that from a plant health perspective it  
may not be necessary to completely remove these specific impurities at concentrations tested  
219 prior to transportation.

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228

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**Figure legends:**

**Figure 1.** Growth characteristics of wheat grown with CO<sub>2</sub> + SO<sub>2</sub> (A), CO<sub>2</sub> + H<sub>2</sub>S (B) and beetroot grown with CO<sub>2</sub> + SO<sub>2</sub> (C), CO<sub>2</sub> + H<sub>2</sub>S (D) compared to pure CO<sub>2</sub> control after 4 to 5 days treatment. Wheat: leaf height, leaf no., tiller no., fresh weight and dry weight of all top growth (leaf material) and % moisture of all top growth. Beet fresh weight and dry weight of all top growth (leaf material) and % moisture of all top growth [n= 4 to 6, bar = SEmean].

**Figure 2.** Comparison of time course gas exchange measurements for wheat treated with CO<sub>2</sub> (control) or CO<sub>2</sub> + impurity (SO<sub>2</sub> or H<sub>2</sub>S). Stomatal conductance (g<sub>s</sub>), transpiration rate (E) and photosynthetic rate (A) pre-gassing (day 0) and subsequent daily measurement during gassing. [n = 4 or 6, bar = SEmean].

**Figure 3.** Comparison of time course gas exchange measurements for beetroot treated with CO<sub>2</sub> (control) or CO<sub>2</sub> + impurity (SO<sub>2</sub> or H<sub>2</sub>S). Stomatal conductance (g<sub>s</sub>), transpiration rate

(E) and photosynthetic rate (A) pre-gassing (day 0) and subsequent daily measurement during  
315 gassing. [n = 4 or 6, bar = SEmean].

**Figure 4.** Correlations of gas exchange parameters with CO<sub>2</sub> concentration. All individual  
318 points inclusive of CO<sub>2</sub> control and CO<sub>2</sub> + impurities. (A) Stomatal conductance; R<sup>2</sup> = 0.79;  
(B) Transpiration rate; R<sup>2</sup> = 0.84; (C) photosynthetic rate R<sup>2</sup> = 0.38; (Solid line is the linear  
regression and the dotted line the 95% confidence intervals around the regression, n = 10)

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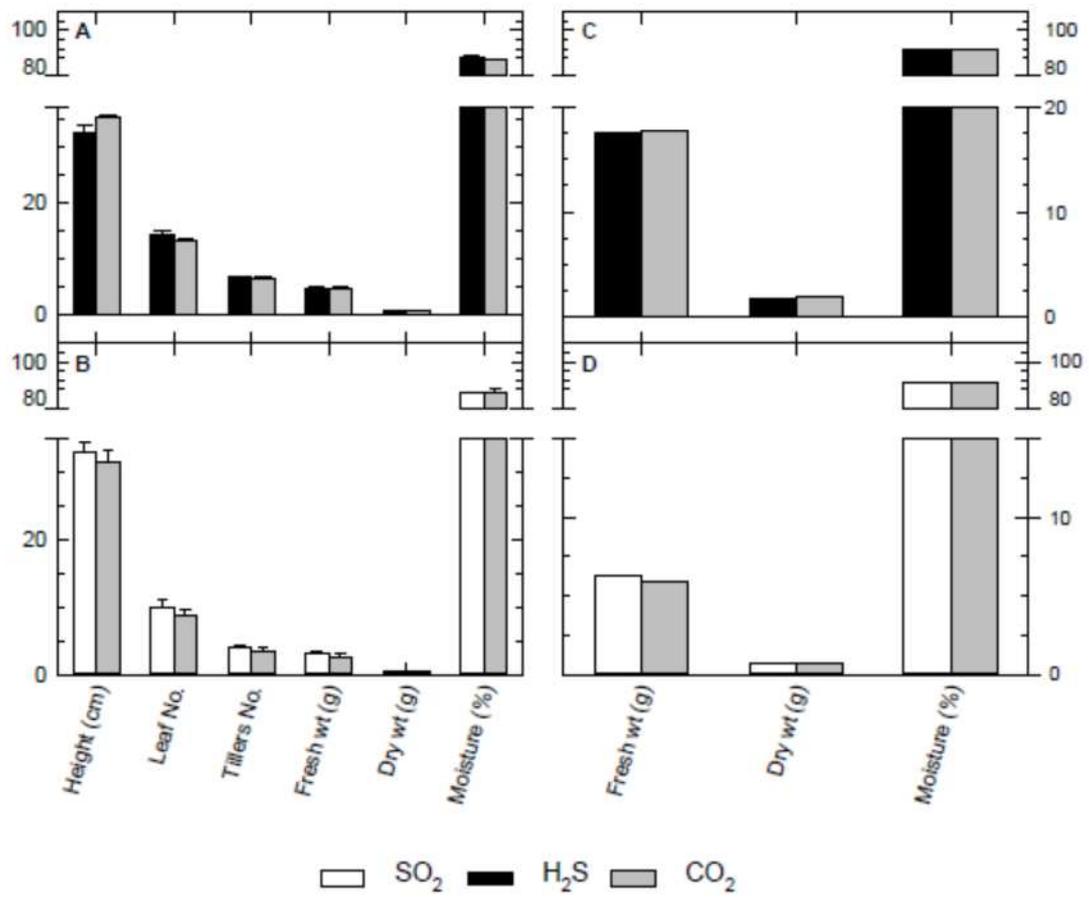


Figure 1

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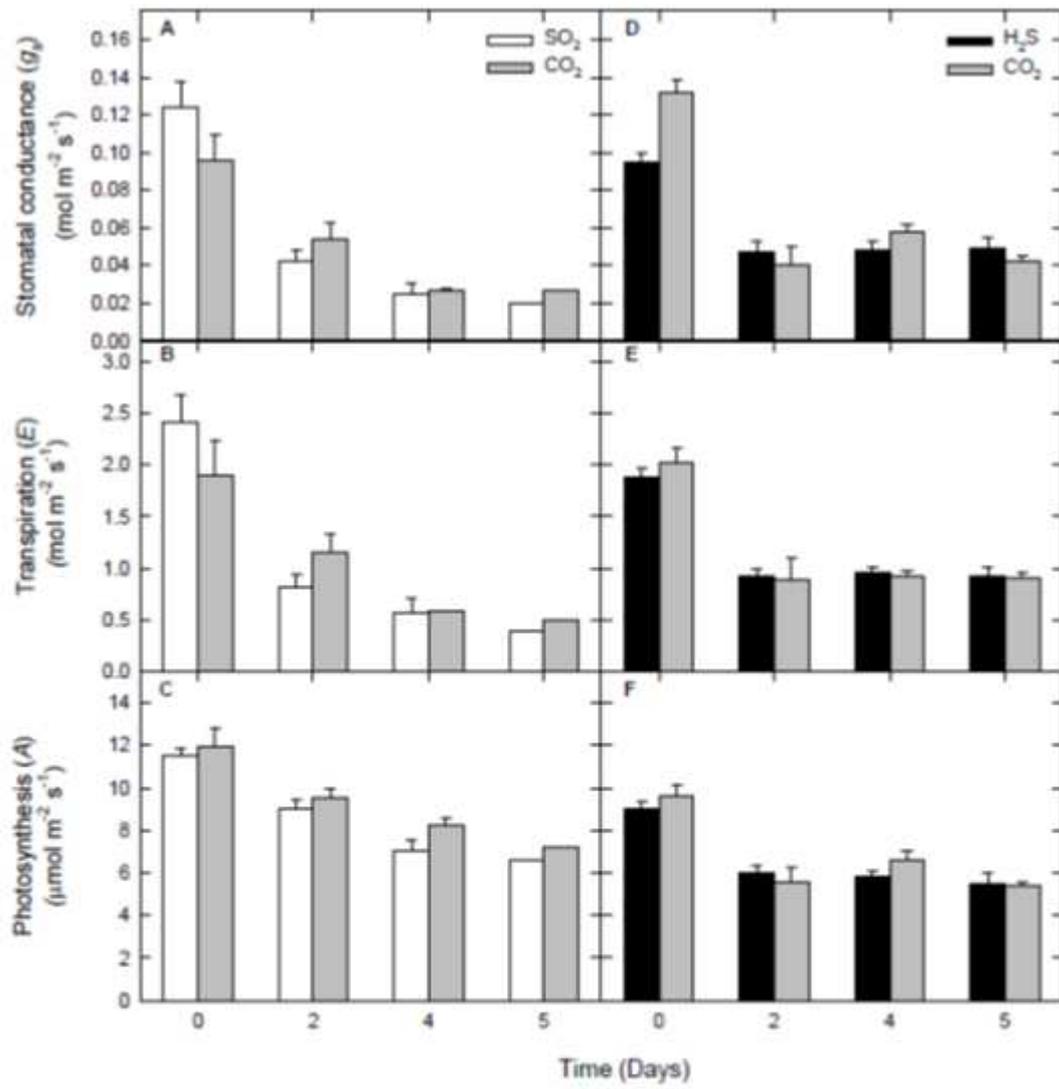


Figure 2

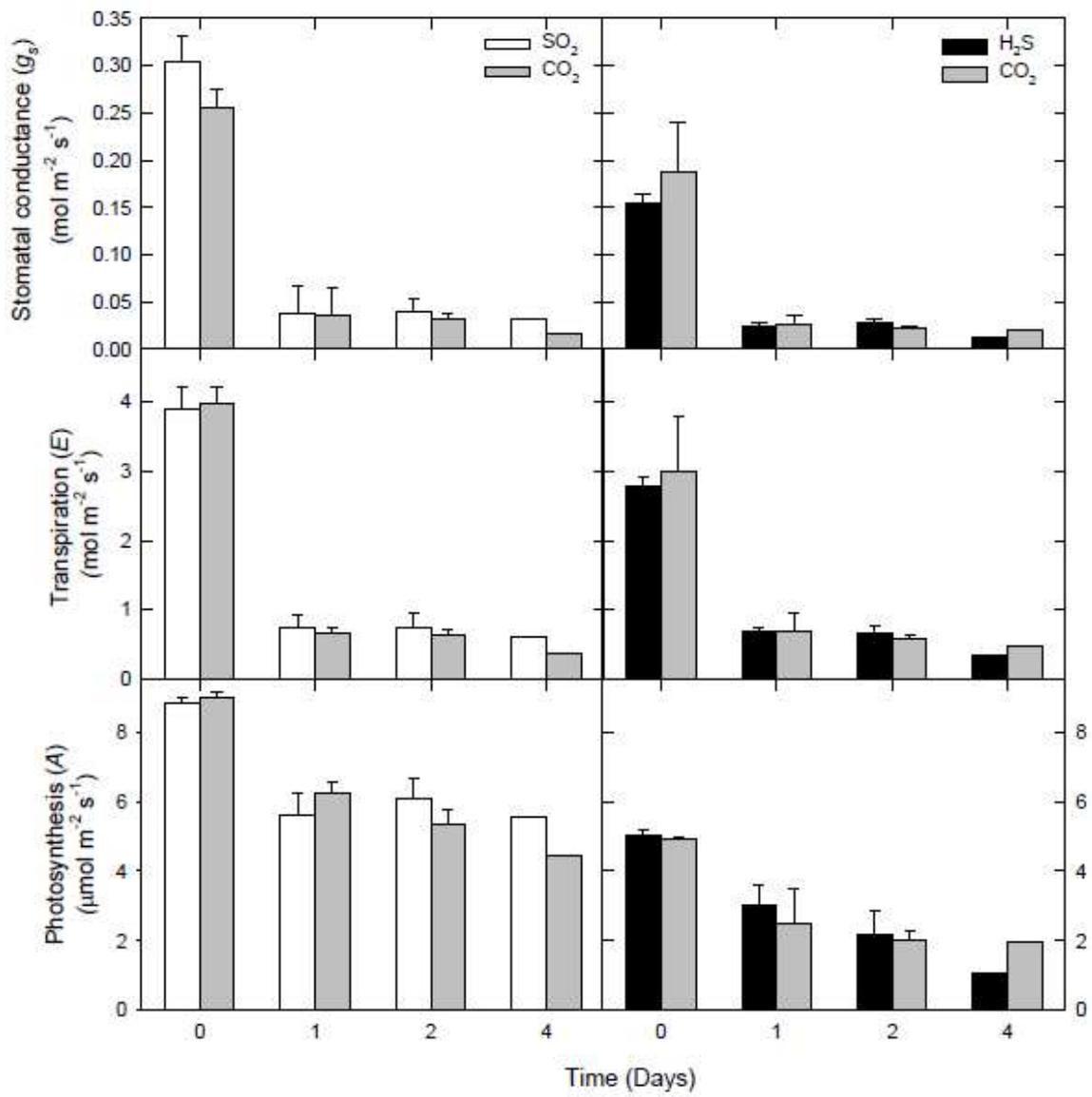


Figure 3

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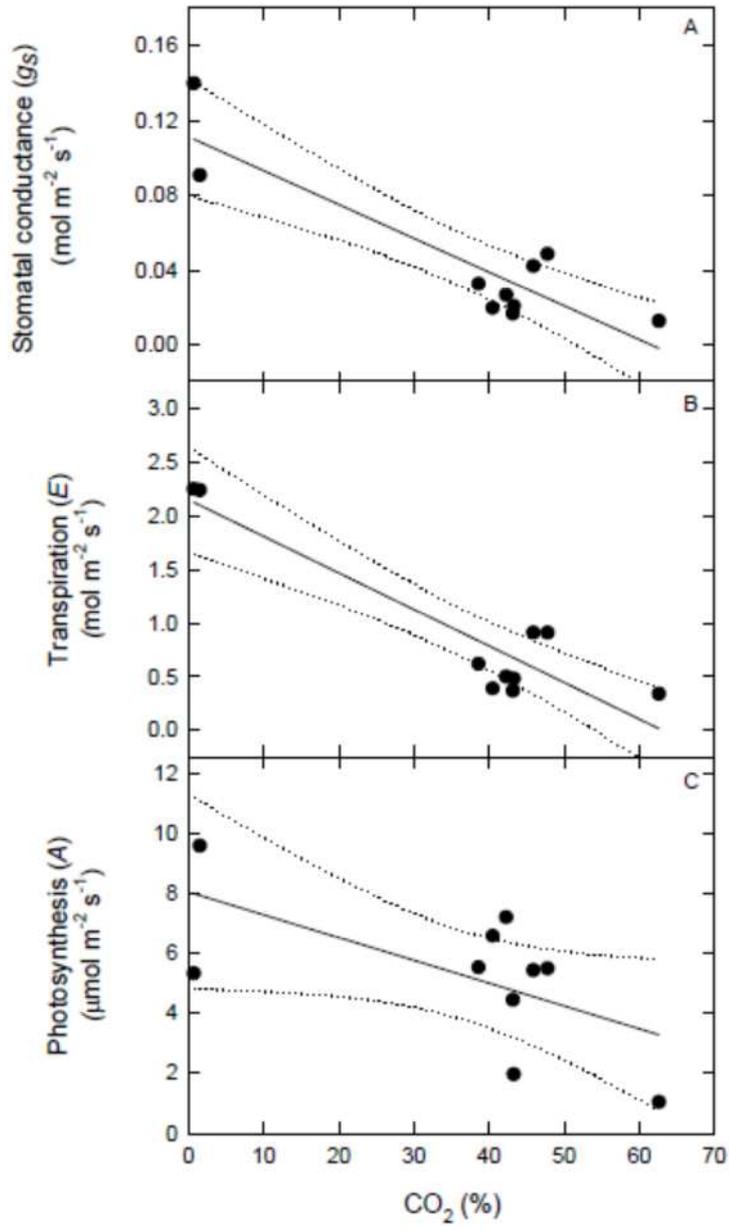


Figure 4

**Table 1.** Range of concentrations of specific impurities (SO<sub>2</sub>, H<sub>2</sub>S) in the CO<sub>2</sub> gas stream from different capture technologies

339	Oxy-fuel combustion			pre-combustion	post-combustion	
	Raw/	double	distillation			
	dehumidified	flashing				
342	CO <sub>2</sub> % v/v	74.8-85	95.8-96.7	99.3-99.4	95-99	99.6-99.8
	SO <sub>2</sub> ppmv	50-100	0-4500	37-50	25	0-61.7
	H <sub>2</sub> S/COS ppmv	0-34000				
345	Adapted from Brown et al 2014; COS = carbonyl sulphide					

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**Table 2.** Mean gas concentrations measured as % CO<sub>2</sub> and % O<sub>2</sub> within the soil chambers.

351	<b>Crop and impurity</b>	<b>CO<sub>2</sub> concentration (%)</b>		<b>O<sub>2</sub> concentration (%)</b>	
		CO <sub>2</sub> gassed	CO <sub>2</sub> + impurity	CO <sub>2</sub> gassed	CO <sub>2</sub> + impurity
	<b>Wheat</b>				
354	SO <sub>2</sub>	42.3 (1.2)	40.5 (0.34)	11.0 (0.36)	12.1 (0.09)
	H <sub>2</sub> S	45.9 (2.08)	47.8 (5.83)	10.5 (0.30)	10.2 (0.38)
357	<b>Beetroot</b>				
	SO <sub>2</sub>	43.2 (2.54)	38.6 (3.93)	11.4 (0.48)	12.3 (2.48)
	H <sub>2</sub> S	43.3 (1.79)	62.6 (3.64)	11.3 (0.34)	7.3 (0.38)
360	[n = 3 for pure CO <sub>2</sub> , 5 for CO <sub>2</sub> + impurity; (SEmean)]				

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375 **Table 3.** Percentage change in biomass and gas exchange parameters from CO<sub>2</sub>-gassed control plants (black outline = H<sub>2</sub>S effect, dashed outline = SO<sub>2</sub> effect). Data from the controls are actual values.

378	Crop and impurity	Biomass		gas exchange parameters		
		fresh weight (g)	dry weight (g)	photosynthetic rate (A)	stomatal conductance (g <sub>s</sub> )	transpiration (E)
<b>Wheat</b>						
381	SO <sub>2</sub> added	+18.6	+6.25	-8.86	-25.19	-22.48
	Control for SO <sub>2</sub> (CO <sub>2</sub> only)	2.57	0.42	7.217	0.027	0.503
	H <sub>2</sub> S added	-1.23	-11.25	+1.12	+16.1	+0.44
384	Control for H <sub>2</sub> S (CO <sub>2</sub> only)	4.88	0.77	5.435	0.042	0.907
<b>Beetroot</b>						
387	SO <sub>2</sub> added	+6.08	+4.61	+24.12	+87.7	+66.8
	Control for SO <sub>2</sub> (CO <sub>2</sub> only)	5.92	0.64	1.972	0.0215	0.476
	H <sub>2</sub> S added	-1.01	-6.81	-47.08	-35.39	-28.67
390	Control for H <sub>2</sub> S (CO <sub>2</sub> only)	17.77	1.9	4.458	0.174	0.374

393 **Table 4.** Mean soil pH [n = 3 for CO<sub>2</sub> only, n = 5 for CO<sub>2</sub> + impurity, letters denote significant difference, see text].

<b>Crop and impurity</b>		<b>soil pH</b>		
		pre-gassed	CO <sub>2</sub> gassed	CO <sub>2</sub> + impurity
<b>Wheat</b>				
	SO <sub>2</sub>	5.23 <sup>a</sup>	5.61 <sup>b</sup>	5.45 <sup>c</sup>
399	H <sub>2</sub> S	5.23 <sup>a</sup>	5.45 <sup>b</sup>	5.49 <sup>b</sup>
<b>Beetroot</b>				
402	SO <sub>2</sub>	5.34 <sup>a</sup>	5.41 <sup>b</sup>	5.61 <sup>b</sup>
	H <sub>2</sub> S	5.34 <sup>a</sup>	5.63 <sup>b</sup>	5.61 <sup>b</sup>

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