Plant responses to simulated Carbon Capture and Storage (CCS) CO₂ pipeline leakage: the effect of soil type.

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Running Title

soil type and extreme CO₂

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Abstract

Carbon capture and storage (CCS) has been proposed as a bridging technology to enable the transition to an energy system based on renewable sources. Many high CO_2 emitting industries (e.g. power stations) are distant from potential carbon storage sites (such as offshore geological reservoirs) and therefore an infrastructure of CO_2 transportation must be developed to carry the CO_2 to safe storage. As such there is a need to understand the risks involved and the mitigation of potential leaks associated with CCS and dense-phase CO_2 transportation networks. Since 2012 a number of experimental studies have provided a mechanistic understanding of the risks posed to crops as a function of CO_2 leakage from CCS infrastructure. However, what remains largely unresolved is the role played by both soil type and soil structure in mitigating and/or enhancing plant stresses. In this study we provide an experimental framework to evaluate these effects. Wheat and beetroot were grown in four different experimental soils to test the effects of specific spoil attributes (organic, low pH; organic, open structure; organic, limed; loam, neutral pH) on crop performance when exposed to high levels (~40%) of CO_2 in the soil environment. Comparison between treatment and controls and across the soil types reveals little difference in terms of biomass or plant stress chemistry. From a stakeholder perspective these findings suggest that soil type may play only a minor role in mitigating or amplifying plant stress in response to the unlikely event of a CO_2 leak from CCS infrastructure.

1. Introduction

Anthropogenic climate change is driven by the acceleration of the long-term carbon cycle via the combustion of fossil fuel directly transferring carbon from the lithosphere to the atmosphere. Since the dawn of the industrial age this has resulted in atmospheric CO_2 increasing from ~280 ppm in the 1850s to 406 ppm in 2017 (https://www.esrl.noaa.gov/gmd/ccgg/trends/monthly.html). This rise in CO_2 has seen a concomitant increase in global average temperature (http://climate.nasa.gov/vital-signs/global-temperature/). The Paris Agreement in 2015 COP21 climate treatise (ratified November 2016) was designed to limit warming to "… 2°C above pre-industrial levels and pursuing efforts to limit the temperature increase to 1.5°C above pre-industrial levels, recognising that this would significantly reduce the risks and impacts of climate change". These ambitious goals require the development of multiple mitigation practices and eventually the removal of fossil fuel derived carbon from the energy system.

One potential mechanism identified as having a role in delivering these ambitious targets and which has been recognised as a bridging technology for transition from a fossil fuel carbon based energy system to a renewable energy infrastructure is the use of carbon capture and storage (CCS). This mitigation technique essentially allows for energy to be extracted as the carbon is moved from one geological reservoir to another. Many high CO_2 emitting industries (e.g. power stations) in the UK are distant from potential carbon storage sites (such as offshore geological reservoirs) and therefore an infrastructure of CO_2 transportation must be developed to carry the CO_2 to safe storage. As such there is a need to understand the risks involved and the mitigation of potential leaks associated with CCS and dense-phase CO_2 transportation networks into the environment. Whilst risks assessment studies have been undertaken, many have focused on marine benthic studies related to off-shore storage

reservoirs[1-4]. Of those undertaken in terrestrial environments, several have utilised natural

75   CO_2 vent sites, which are not comparable to a sudden or recent release of CO_2, as both the

soil and biological components within have evolved over many years[5, 6]. Specific

experimental systems include outdoor CO_2 gradient studies, which whilst giving a more

78   realistic scenario with comparable CO_2 and O_2 levels of leakage in soils, do not fully replicate

particular scenarios such as soil type and focus largely on leakage detection methods rather

than direct effects on soil or bio-components[7-10]. Studies with the aim of measuring the

81   effects of CO_2 leakage on soils have been undertaken, but have not specifically looked at

different soil types under the same conditions[11], an exception is that of[12] who did investigate

two soil types and the effect of CO_2 on microbial communities in a long-term mesocosm

84   study. The nearest equivalent study system is that of[13, 14] who did specifically measure

vegetation responses, did not investigate different soil types.

87   Recent experimental work has highlighted that the effects of CO_2 leakage on agricultural land

are highly localised[15, 16] as reviewed in[17] (e.g. these effects are also transient with recovery of

vegetation close to complete after 12 months[17] and that this stress is induced by direct CO_2

90   exposure rather than as a function of O_2 depletion[18]. Further, using the system reported here

we have recently demonstrated that the effects of impurities (specifically SO_2 and H_2S)

within the CO_2 gas stream are limited. Within our experimental system there are no additive

93   toxicity effects when comparing plants gassed with a combination of CO_2 and SO_2 or CO_2

and H_2S to control plants exposed just to CO_2[19]

96   However, what remains largely unresolved is the role played by both soil type and structure

in mitigating and/or enhancing reported plant stresses. Closing this knowledge gap is an

important step in the development and deployment of CCS transportation infrastructure as

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99 any potential hazard requires full elucidation. This will aid the decision making process in where and how CCs technologies are deployed[20]. Typically sites suitable for the geological storage of CO_2 are distal to CO_2 emitters, consequently CO_2 pipelines will cross numerous

102 soil types. To address this knowledge gap we build on our experimental protocols[17-19] to test for differences in plant stress/health as a function of soil type when exposed to high soil CO_2 concentrations that simulate CO_2 leakage analogous to the field based experiments conducted

105 at the ASGARD (Artificial Soil Gassing And Response Detection) facility[17, 21].

2. Materials and methods

108 2.1 Experimental setup

Soil chambers were constructed of acrylic plastic with pipe inlets to allow CO_2 gassing of the soil environment exclusively. The experimental system was housed in a controlled

111 environment growth facility (UNIGRO, UK) to standardise all other environmental variables: irradiance was 300 μmol m^{-2} s^{-1} (at plant height), day/night as 12/12 hours; temperature 21/18°C day/night; relative humidity 60%. Gas was supplied from either an integral supply

114 (pure CO_2) or a gas cylinder (N_2) and separated prior to entering each individual soil chamber by 2 flow rate step-down manifolds. Gas was delivered to each individual chamber at a rate of 30 ($\pm$15) mL min^{-1} to maintain CO_2 and N_2 levels at steady state. Gases were exhausted to

117 atmosphere via a separate manifold to prevent build up within the growth room.

2.2 Soils types

120 To simulate a wide variety of soil types (yet maintain standardised growth conditions) a series of commercially available potting media were chosen and/or manipulated to deliver a number of experimental soils. Soil experimental treatments are as follows: (I) Levington's

123 no.3 (L3) compost to represent an organic soil with a low pH; (II) L3 plus sand (25% by

volume), was designed to simulate an organic rich soil with an open structure; (III) L3 plus

lime: organic soil with lime (lime was added to raise the pH by 1 unit) was chosen to see if

126   the addition of lime acted as a potential buffer of CO_2 induced acidity and finally (IV) John

Innes no.3 (JI 3) compost was chosen to simulate a standardised loamy soil with a neutral pH.

We stress that these soils are used as an experimental system. They are not meant to represent

129   actual soil types, but are used as standardised media to determine the specific effects of CO_2

exposure across a range of plausible soil types/ structures and to measure explore these

responses in standardised a consistent experimental setting.

132

To fully elucidate the effect of soil type each experiment consisted of an experimental

treatment and three levels of control: (I) CO_2-gassed soil (the experiment); (II) N_2-gassed soil

135   (O_2-depleted control); and (III) non-gassed soil (natural state control). In all experiments gas

concentrations (CO_2 and O_2) were measured daily using the GEOTECH GA5000 gas

analyser (Geotech, Warwickshire, UK). Each experimental run had the following replication,

138   six chambers were exposed to CO_2, a further six chambers were exposed to N_2 and four

chambers were used as non-gassed chambers (Fig. 1).

141   2.3 Soil pH

Soil samples were taken prior to and at the end of each experiment and dried at $40 \pm 4°C$. A

solution of 0.01M calcium chloride dihydrate ($CaCl_2.2H_2O$ analytical grade) was dissolved in

144   de-ionised water and added to a soil sample to give a final solid to solution ratio of 1:2.5. The

mixture was placed on a magnetic stirrer and stirred for at least 5 minutes. The suspension

was allowed to settle for 15 minutes and measured with a pH electrode (Hanna combination

147   electrode and Jenway PHM6 meter, Fisher Scientific, UK) until readings were stable.

2.4 Crop species

150 In all experiments the crop plants used were spring wheat (*Triticum aestivum* v Tybault - a

monocotyledon, grass) and beetroot (*Beta vulgares* v Pablo F1 - a dicotyledon, vegetable).

The crops were sown and grown within an environmental controlled growth room (details

153 above) for 1 to 2 weeks before being transplanted into the soil chambers. They were then left

to allow sufficient root growth before gassing commenced (approximately 2 weeks later) with

the gassing period lasting for up to 7 days. After that time, plants become pot-bound and

156 performance becomes compromised via physiological changes, making direct comparison

with field data (not pot-bound) problematic. Samples for biochemical analyses were

immediately quenched in liquid nitrogen and stored at -80°C. Biomass (all above ground

159 parts; leaves and stems) were measured as fresh weight (g).

2.5 Biochemical analyses

162 During harvest the plants were sub-sampled for analysis of the key biochemical compounds

that are either necessary for functional integrity or associated with symptoms of stress.

Chlorophyll content was measured following observational discolouration of leaves in field

165 trials[17, 18]. Chlorophyll is a necessary compound for the ability of plants to photosynthesise

efficiently and subsequently grow to produce a crop yield. A decrease in this compound

would suggest that resources are diverted to produce compounds which enable a plant to

168 mitigate stress. Build-up of anthocyanin is indicative of many stresses and is identified via a

red discolouration of leaves and/or stems. In field studies it was observed[17, 18] that some

leaves had turned red; consequently changes in this compound were investigated in this

171 laboratory study. Phenylalanine lyase (PAL) is a compound which mediates the production of

many stress compounds and is a generic indication that plants are suffering from

environmental stress.

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2.6 Chlorophyll analysis

Approximately 300 mg of fresh leaf material was ground in a pestle and mortar in 5 mL 80% acetone (volume to volume (v/v) with distilled water) solution and transferred to a 10 mL universal tube. The tube was covered with aluminum foil, stirred for 30 minutes, and then centrifuged for 15 minutes (at a speed of 3,000 rpm). The supernatant was transferred to a new tube, mixed thoroughly and pipetted into duplicate 1cm path length cuvettes. Absorbance of chlorophyll content was measured using spectrophotometry (Cecil 1100, manufactured by Camlab Ltd, Cambridge, UK) against 80% acetone as a blank.

Chlorophyll concentrations were calculated as follows:

$$Ca \ (mg/g) = [12.7xA663 - 2.69xA645] \times V/1000 \times W \ (Chlorophyll \ a) \qquad (1)$$

$$Cb \ (mg/g) = [22.9xA645 - 4.86xA663] \times V/1000 \times W \ (Chlorophyll \ b) \qquad (2)$$

$$Ca+b \ (mg/g) = [8.02 \times A663 + 20.20xA645] \times V/1000 \times W \ (Chlorophyll \ a+b) \qquad (3)$$

Where A = absorbance wavelength, V = volume of the extract (mL), W = Weight of fresh leaves (g). Content is expressed as mg g^{-1} fresh weight[22].

2.7 Anthocyanin content

Pre flash-frozen plant material was ground in a pestle and mortar in 5mL of 1% HCl in methanol (%v/v) solution to yield 4 x 1 mL samples for duplicate samples at pH 1.0 and pH 4.5. Assays were performed using 0.5mL of sample added to 2.5mL of each of the following buffers: Potassium chloride buffer: 0.025 M, pH 1.0 (1.86 g KCl added to 980 mL of distilled

water in a beaker, pH measured and adjusted to 1.0 with concentrated HCl and made up to 1

L with distilled water); and Sodium acetate buffer, 0.4 M, pH 4.5 (54.43 g $CH_3CO_2Na\cdot3\ H_2O$

201   added to 960 mL distilled water in a beaker, pH adjusted to 4.5 with concentrated HCl, and

made up to 1 L with distilled water). The appropriate dilution factor for the sample was

determined by diluting with potassium chloride buffer, pH 1.0, until the absorbance of the

204   sample at the vis-max is within the linear range of the spectrophotometer (i.e. for most

spectrophotometers the absorbance should be less than 1.2). The final volume was divided by

the initial volume to obtain the dilution factor. In order to not exceed the buffer's capacity,

207   the sample did not exceed 20% of the total volume. Two dilutions of the sample, one with a

potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, were

prepared by diluting each by the previously determined dilution factor. Duplicates of each

210   were pipetted into 1cm path length cuvettes. Dilutions were equilibrated for 15 minutes. Both

are read at 510 and 700nm against a blank of distilled water on a spectrophotometer (Cecil

1100, manufactured by Camlab Ltd, Cambridge UK).

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Anthocyanin content is expressed as mg g^{-1} Gallic Acid equivalent and is calculated as

follows:

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$$A = (A533 - A700)pH\ 1.0 - (A533 - A700)pH\ 4.5 \tag{4}$$

219   2.8 Phenylalanine lyase (PAL)

50 mg of plant material was ground in a pestle and mortar in 2 mL 100mM Tris – HCl buffer

with 12 mM mercaptoethanol (supplied by Fisher, UK), transferred to an eppendorf and

222   centrifuged at a speed of 16,000 rpm for 5 minutes. The sample supernatant was used in the

assay. A 500 µL sample, 450 µL 100mM Tris-HCl (pH 8.8) and50 µL 100mM phenylalanine

was placed in a water bath for one hour at 37^0 C. The reaction was then stopped by the addition of 50 µL 5 M HCl. Change in absorbance was measured on a spectrophotometer (Cary 50 UV-Visible Varian, manufactured by Northstar Scientific, UK) at 290 nm in 1 cm light path cells against blanks containing 50 µL 5 M HCl before the addition of 50 µL 100mM phenylalanine. The amount of PAL present is expressed as nmol *trans*-cinnamic acid gram^{-1} plant tissue hour^{-1}.

2.9 Biomass (shoot and root)

Plants were harvested between days 5 and 7 after gassing commenced. Shoots were taken from each plant, washed and dried at $80^°$C for 2 days. Biomass was measured as fresh and dry weight. Roots were carefully removed from the chambers, washed, patted dry, weighed and dried for 4 days at $50°C$. They were then re-weighed. The beet (storage root) was separated from the lateral roots from the beetroot plants and analysed independently. Beets were dried until the constant dry weight was measured. The wheat roots were measured as dry weight only. All statistical analyses were carried out using Minitab v 12 (USA).

3. Results and discussion

This suite of experiments designed to simulate the unlikely event of a CO_2 leak from CCS infrastructure, set out to test whether established stress responses observed in earlier studies[17,18, 23-25] were alleviated or magnified when crop species were grown in different soils. Mean gas concentrations in both the CO_2 and N_2 gassed chambers show that reductions in O_2 level were comparable both across and between the soil treatments in both crop species (Table 1). N_2 gassed chambers were generally slightly lower in O_2 concentration than the CO_2 chambers. CO_2 and O_2 data are higher than those found in test sites such as the Otway Project in Australia at 10% CO_2 maximum[26] However, they are comparable to values

249   measured in both outdoor field facility ASGARD[17, 18] and the higher CO_2 levels in the

gradient site reported by[9]. Laboratory based systems show similar levels to those reported[12-14].

3.1 Biomass

252   Different soil types influenced the level of biomass decrease in both species (Fig. 2) with a

mean decreases in biomass of ~40% - comparable to field grown counterparts for both crops

255   indicating that biomass (and potentially yield) are affected within the first few days of

exposure to CO_2 in the soil. The N_2-induced O_2 depletion also impacted on biomass, leading

to a ~10% mean decrease. This corroborates evidence that both elevated soil CO_2 and O_2

258   depletion have an effect on vegetation[18], but that soil gassed with CO_2 exerts a greater impact

and is responsible for the majority of the reduced biomass.

261   Specific soil differences reveal that plants growing in L3 had a greater reduction in biomass

than those grown in JI3 when gassed with CO_2. A decrease in biomass, however, is still

evident in JI3, with wheat showing a greater impact than beetroot. The addition of lime

264   ($CaCO_3$) to L3, produced the largest effect in terms of biomass reduction in both species (Fig.

3). This large reduction in biomass with the addition of lime was an unexpected result, as it

was reasoned that liming the soil would provide a buffer against CO_2-induced acidity at the

267   root interface. This finding suggests that acidification of soil pores through the interaction of

CO_2 with water to produce carbonic acid is not a major factor responsible for the observed

reduction in biomass, but rather that the amount of lime may have exceeded that suitable for

270   the crops used in this soil type. The addition of sand to L3 produced an anomalous result

(when compared to the other soil type experiments) as there was no statistically significant

loss in biomass when comparing the CO_2 gassed wheat or the beetroot to their non-gassed

273   control plants (Fig. 3and Table 2). The L3 compost supplemented with sand was used to

simulate a soil with a more open structure. The more open structure of this soil may have

provided a better growing medium for these plants thus they might have been buffered from

276   the stress effects of high concentrations of CO_2 in the root zone. This may be the explanation

for the beetroot as the non-gassed control has the highest biomass (Fig. 3). However this does

not appear to provide an explanation for wheat as there was no increase in biomass in this soil

279   treatment when compared to other non-gassed controls with the exception of L3 and the lime

treatment that shows a reduction in biomass (Fig. 3). It is possible that the open soil structure

could have minimised CO_2/root contact time in this set up. Yet, the similarity in O_2 and CO_2

282   concentrations across soil types (Table 1) suggests this is unlikely and at the moment we are

unable to explain these intriguing findings. In general the analysis of root biomass from both

wheat and beetroot indicates a dramatic reduced root growth of >60% under CO_2 gassed soil

285   when compared to controls. The reduction in root biomass provides a mechanism for the

inability of plants to access sufficient nutrients and water. This response was investigated in

more detail and found to be a whole plant response affecting the water status of the plant[27].

288

The majority of the findings of this short-term study (a reduction in above and below ground

biomass when CO_2 treatments are compared to controls) reflect those conducted on more

291   long-term field trials such as work at the ZERT (Zero Emission Research and Technology)

centre Montana, USA[2] and the ASGARD facility[23-25].

294   ## 3.2 Soil pH

For wheat plants in the L3 experiment with CO_2 and N_2 gassed treatments the soil pH was not

significantly different, but both have a significantly different soil pH when compared to the

297   L3 non-gassed control (p = <0.001) (Fig.4). There is no difference in soil pH between

treatments in L3 and sand. CO_2 gassed soils in the L3 and lime experiment are not

significantly different to N_2 gassed and non-gassed control, but N_2 gassed is significantly

300   higher than control (p = 0.015). CO_2 and N_2 gassed JI3 are not significantly different, but

both are higher than control (p =0.03, p = 0.003 respectively).

303   In beetroot, L3 has the lowest pH (organic acidic soil). Added lime and JI3 have similar

values between pH 6.0 and 7.0 under all treatments. There is no statistical difference between

treatments on pH of L3 or L3 with lime. pH of L3 and sand under N_2 gassing is significantly

306   lower than non-gassed control soil (p = 0.005) but not CO_2 gassed soil. pH of CO_2 and N_2

gassed soil in JI3 are both significantly lower than the non-gassed control soil (p = 0.003, p =

0.012). Both CO_2 and N_2 gassing does have the potential to reduce pH compared to controls

309   in JI3 and with the addition of sand. This suggests that different soil types do interact

differentially with gasses in respect of acidity. Wheat exhibits a different result to beetroot.

Plants are known to exude compounds that stabilise pH levels around the roots. Wheat

312   appears to be more efficient in this process, as the pH levels in gassed plants are higher than

the controls, except in L3, a soil that wheat prefers the least. There is no correlation between

soil pH and biomass in either wheat or beetroot across all experimental soil types.

315

3.3 Plant biochemistry

Biochemical analysis was undertaken to test for plant stress as a function of treatment and to

318   determine if soil type mitigated or amplified the plant stress response. Data shows both

specific treatment effects (Table 3) and differences between soil types (Table 4). Results are

presented by stress compound and subdivided by crop.

321

3.4 Chlorophyll

Chlorophyll analysis was undertaken to test for overall plant photosynthetic health, as a

324 reduction in chlorophyll content would indicate plants reallocating resources from

maintaining photosynthesis to stress mitigation. For wheat chlorophyll content in the L3 soil,

the treatment (CO_2) is not significantly different from either the N_2 (oxygen depletion

327 control) or non- gassed control; in the L3 and sand combination the treatment (CO_2) is

significantly higher than the control, but not significantly different to N_2 control; which is

suggestive of an O_2 depletion effect[18]. In the combined L3 and lime experiment the treatment

330 (CO_2) is not significantly different from either control and this finding is repeated in the JI3

treatment (Table 3). Comparison between soils for a CO_2 effect indicates that the L3

treatment produces statistically lower chlorophyll levels than all other soil types; L3 and sand

333 ($p = 0.005$), L3 and lime ($p = 0.044$) and JI3 ($p = 0.007$) [Student's t-test] in wheat (Table 4).

Analysis of the beetroot chlorophyll data shows that in the L3 experiment chlorophyll

336 concentrations in the CO_2 treatment are not significantly different from either the N_2 control

or non-gassed control and these findings are repeated in the L3 and sand experiment. In the

L3 and lime experiment chlorophyll concentrations in the CO_2 treatment are significantly

339 lower than both controls and in the JI3 experiment there is no statistical difference between

the treatment and control (Table 3). Comparison between soils for a CO_2 effect indicates that

there are no statistical differences in chlorophyll content between the soil types (Table 4).

342

3.5 Anthocyanin

Anthocyanin analysis was undertaken to test for generic plant health as anthocyanin up-

345 regulation is a precursor to numerous plant stresses. For wheat the following was observed

with anthocyanin content: In the L3 and L3 and sand experiment there was no statistical

difference between the CO_2 treatment and the N_2 control or non-gassed control. In the L3 and

348   lime and the JI3 experiment the anthocyanin concentration was significantly lower than both

controls (Table3). Comparison between soil types shows that the anthocyanin content in the

L3 and sand experiment has statistically higher levels of anthocyanin than in the L3 and lime

351   (p = 0.005) and JI (p = <0.0001) experiments [Student's t-test] (Table 4).

For the beetroot anthocyanin content there was no significant difference observed between

354   treatment and either level of control in the L3, the L3 and sand and the L3 and lime

experiments. In the JI3 experiment anthocyanin levels where significantly higher in the CO_2

treatment than the non-gassed control but not different to the N_2 control (Table 3).

357   Comparison between CO_2 treatment and different soil types shows that in the L3 and sand

experiment the anthocyanin content is statistically lower than the JI3 (p = 0.001) and that

anthocyanin content in the L3 and lime experiment is statistically lower than JI3 (p = <0.000)

360   [Student's t-test]. Data indicate that only CO_2-gassed plants grown in JI3 have a higher

anthocyanin content than control plants (p = <0.0001) (Table 4), this could be an indicator of

early onset of stress in this specific treatment when compared to other soil treatments.

363

3.6 Phenylalanine lyase (PAL)

PAL analysis was again performed to test for generic plant health as PAL up-regulation is a

366   precursor to numerous plant stresses. In wheat there were no significant differences in PAL

context between the CO_2 treatment and the controls in any of the soil type experiments (Table

3). Comparisons of PAL data from CO_2 treatments across the soil experiments shows that in

369   the L3 and sand experiment PAL expression is statistically lower than in the L3 and lime soil

(p = 0.04) and the JI3 soil experiments (p = 0.019) [Student's t-test] (Table 4).

372   Analysis of beetroot PAL levels indicates no significant differences between the CO_2

treatment and either set of controls in any of the soil type experiments (Table 3).

Comparisons of PAL data from CO_2 treatments across the soil experiments shows that PAL

375   concentration in the L3 and sand experiment is statistically greater than L3 and lime (p =

0.029), JI3 (p = 0.009) experiments, while L3 and lime is greater than the JI3 experiment (p =

0.001) and in the L3 experiment (p = 0.034) [Student's t-test] (Table 4).

378

Overall, there is little change in stress biochemistry with treatment (CO_2 compared to non-

gassed control). Comparing our pot studies to those of our longer-term field studies[17]

381   indicates leaves change colour (an indication of the up-regulation of stress compounds)

approximately ten days after the initiation of CO_2 treatment[17]. The concentration of CO_2 in

the soil of our pot experiments exceeds that found in our field experiments[17]. Consequently

384   this lack of a stress response can't be explained purely as a function of CO_2 concentration. It

is possible that the more stable environmental conditions in the plant growth room could have

acted as a buffer to the specific CO_2 stress delaying the onset of stress. However, the short

387   duration of these pot experiments may offer an alternative explanation for the lack of an

observed stress. We did find clear differences in non-gassed control plants in different soil

types (Table 5), showing that soil type will influence biochemical composition regardless of

390   the presence of an experimental stress.

The similarity in CO_2 concentration between our field and laboratory data is important as

393   field based experiments to manipulate soil type would be prohibitively expensive. Moving to

a laboratory based system that broadly matches field manipulations allows for the analysis of

more specific soil attributes with adequate experimental replication whilst minimising costs.

396   We have previously demonstrated[18] a similar response to chamber experiments and field

trials undertaken at the ASGARD site in comparable soil types. Consequently we are

confident that the results presented in this lab study are transferable to field situations when

399   soil types are similar to those used in our experimental set up.

Via funding from the National Grid, UK and the European Union Energy Programme for

402   Recovery (EEPR) under the COOLTRANS research programme we have developed an

experimental programme designed to understand the impact on crops of CO_2 leakage from

CCS infrastructure. This programme focussed either on catastrophic failure[28] or small scale

405   leakage[17-19]. Synthesising these findings reveals that although there are noticeable effects on

crops these affects across all experiments are minimal when placed into the context of farm

scale agriculture. For example in field trials where biomass and yield decreased, the area of

408   vegetation that was affected was small, between 0.2 and 0.3 m^2 in spring barley and

grass/clover and ~0.5m^2 for spring oilseed rape and autumn barley. In the context of an

average arable field size in the UK of 12 ha, this represents an area of 0.00006% ha, with

411   yield losses corresponding to 0.0003% ha.

4. Conclusions

414   The loss of biomass is broadly consistent across soil types for both species investigated, the

exception being L3 and sand. From a stakeholder perspective these findings suggest that on

the whole soil type does not amplify plant stress in response to the unlikely event of a CO_2

417   leak from CCS pipelines. Intriguingly our data suggests that plants in a sand rich soil might

be less susceptible to CO_2 induced stress. But the reasons behind this reduction in

susceptibility are currently unknown so these findings should be interpreted with caution.

420   Looking more broadly across our work linked to CO_2 leakage from CCS infrastructure the

impact of crop plants again appears to be localised.

Acknowledgements

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Figures
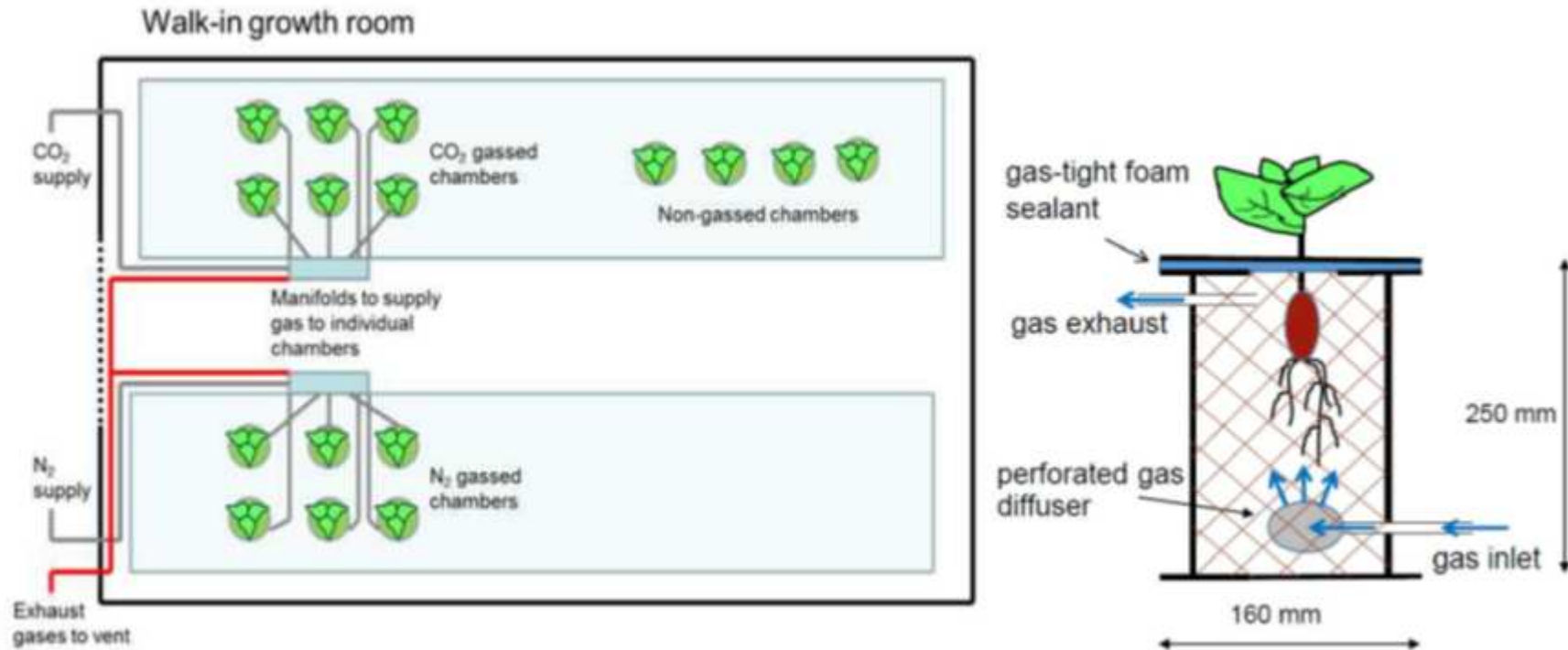
Fig. 1. Schematic representation of the experimental arrangement within a walk-in controlled environment facility and a soil chamber with a beetroot plant. Gases were exhausted to atmosphere via a separate manifold to prevent build up within the growth room.
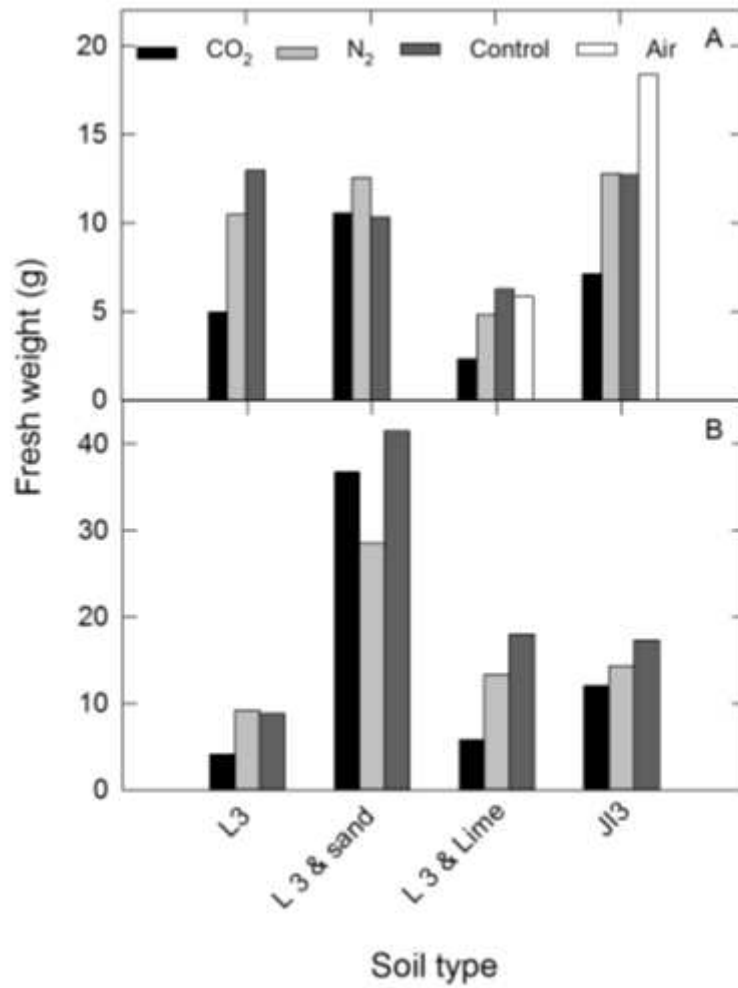
**Fig. 2.** Fresh weight biomass at harvest across experimental treatment and soils type. (A) Wheat and (B) beetroot. A full statistical break down of results is given in the text. See materials and methods for experimental set up.
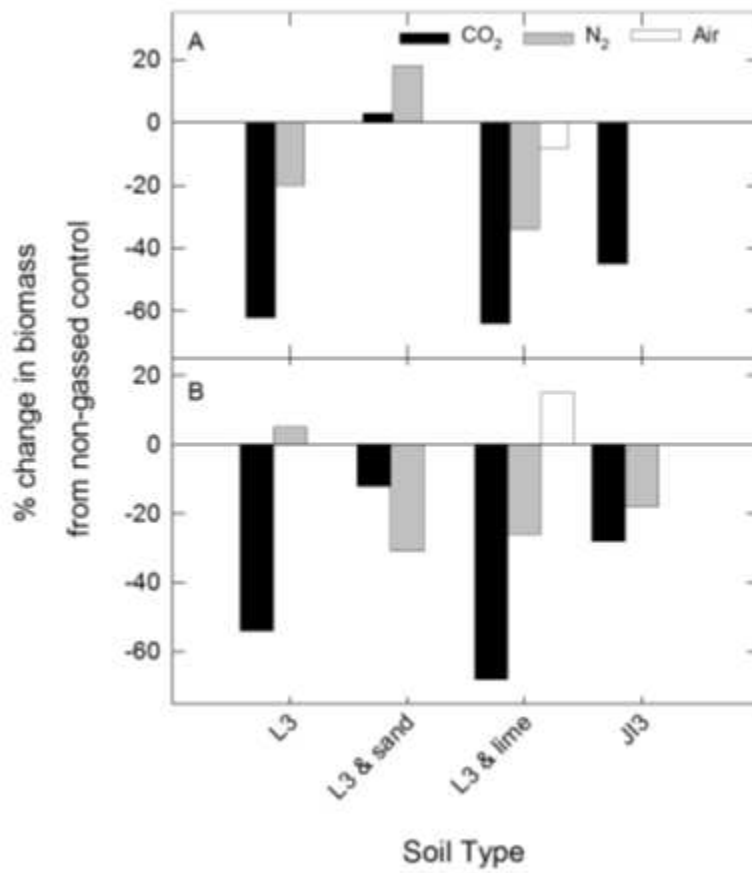
3   **Fig. 3.** Percentage change in biomass across experimental treatment and soils type relative to

controls. **(**A) Wheat and (B) beetroot. See materials and methods for experimental set up.

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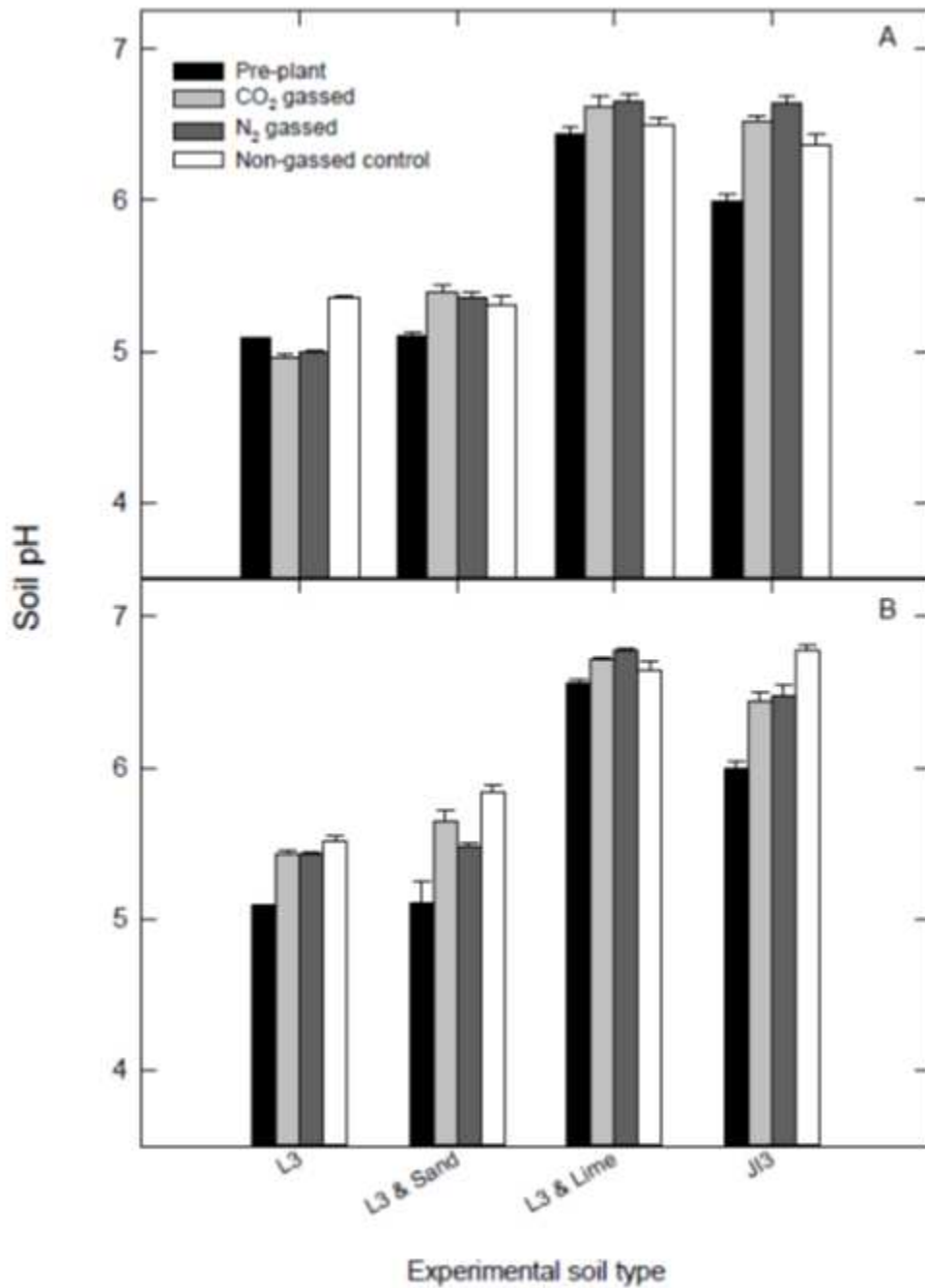
Fig. 4. Comparison in soil pH across the different soil treatments. (A) Wheat and (B) beetroot. A full statistical break down of results is given in the text.

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Tables

Table 1. Mean gas concentrations measured as % CO_2 and % O_2 (v/v) within the soil
chambers.

Crop & soil type	CO_2 concentration (%)		O_2 concentration (%)	
	CO_2 gassed	N_2 gassed	CO_2 gassed	N_2 gassed
Wheat				
L3	48.4 (12.9)	0.15 (0.2)	9.57 (2.7)	8.67 (3.9)
L3 plus sand	40.5 (0.6)	0.14 (0.05)	11.83 (0.09)	9.18 (0.4)
L3 plus lime	42.03 (3.2)	0.24 (0.3)	12.15 (0.4)	10.2 (3.2)
JI3	44.14 (5.9)	0.95 (0.03)	11.48 (1.5)	10.1 (1.7)
Beetroot				
L3	48.0 (4.9)	0.60 (0.5)	8.97 (1.5)	8.76 (1.7)
L3 plus sand	51.9 (4.9)	0.17 (0.02)	8.81 (0.7)	6.74 (0.5)
L3 plus lime	32.0 (17.2)	0.60 (0.4)	13.68 (0.4)	9.04 (1.9)
JI3	32.81 (4.2)	0.59 (0.2)	12.08 (1.4)	12.59 (1.1)

[n = 5; (SEmean)]

Table 2. Percent change in biomass for wheat and beetroot grown in different substrates and gassed with either CO_2, N_2 or air compared to non-gassed (control).

Soil type	Wheat			Beetroot		
	CO_2	N_2	Air	CO_2	N_2	Air
L3	-62	-20	n/a	-54	5	n/a
L3 & sand	+3	+18	n/a	-12	-31	n/a
L3 & lime	-64	-34	-8	-68	-26	15
JI 3	-45	0	n/a	-28	-18	n/a

Table 3. Treatment effects on biochemical compounds associated with stress. Values given are the content of each compound found in CO_2 gassed leaves. Statistical comparison is between treatments (CO_2 gassed) are compared to non-gassed control plants within each soil experiment.

			Soil pH and significance level (difference from control) treatment				
Treatment	**pre-plant**		**CO_2 gassed**	**N_2 gassed**		**non-gassed control**	
Crop	**Soil type**	**pH**	**pH**	*p* value	**pH**	p value	**pH**
Wheat	L3	5.09	4.96	<0.001	4.99	<0.001	5.35
	L3 & sand	5.10	5.39	NS	5.35	NS	5.31
	L3 & lime	6.44	6.62	NS	6.66	0.015	6.5
	JI3	5.99	6.52	0.03	6.64	0.003	6.36
Beetroot	L3	5.09	5.43	NS	5.43	NS	5.51
	L3 & sand	5.10	5.65	NS	5.48	0.005	5.84
	L3 & lime	6.56	6.72	NS	6.77	NS	6.64
	JI3	5.99	6.44	0.003	6.48	0.012	6.78

Table 4. Soil type effects on biochemical compounds associated with stress. Statistical comparisons are between treatments (CO_2 gassed) across each soil type.

Biochemical Compound and significance level

Crop	Soil type	Chlorophyll (mg g^{-1})	p value	Anthocyanin (mg g^{-1} GA equivalent)	p value	PAL (nmol trans-CA g^{-1} hr^{-1})	p value
Wheat	L3	15.84	<0.05	5.56	NS	133483.1	NS
	L3 & sand	22.49	NS	9.84	<0.05	88453.7	<0.05
	L3 & lime	22.15	NS	4.94	NS	72428.7	NS
	JI3	22.16	NS	2.99	NS	118975.4	NS
Beetroot							
	L3	16.92	NS	27.8	NS	75463.3	NS
	L3 & sand	18.06	NS	4.50	0.001	198100.7	NS
	L3 & lime	16.27	NS	4.06	<0.0001	81539.4	NS
	JI3	19.51	NS	10.91	NS	54079.8	<0.05

[mean values of 4 to 6 replicate plants]

Table 5. Comparison of biochemical analysis of non-gassed controls only in all soil types. Influence of soil type alone.

| Crop | Soil type | Biochemical Compound (non-gassed control only) | | |
		Chlorophyll (mg g^{-1})	Anthocyanin (mg g^{-1} GA equivalent)	PAL (nmol trans-CA g^{-1} hr^{-1})
Wheat				
	L3	19.92	10.75	116857.2
	L3 & sand	22.30	10.63	94030.9
	L3 & lime	22.33	10.01	104607.0
	JI3	22.33	8.47	95222.4
Beetroot				
	L3	16.91	17.55	81463.3
	L3 & sand	20.1	3.72	287984.2
	L3 & lime	21.96	7.01	25080.9
	JI3	16.98	3.09	72205.8