Plant responses to simulated Carbon Capture and Storage (CCS) CO_2 pipeline leakage: the effect of soil type.

Janice A. Lake[1, 2*] and Barry H. Lomax[1]

[1] *The School of Biosciences, Division of Agricultural and Environmental Sciences, The University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK.*

[2] *Present address: Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK.*

Running Title

soil type and extreme CO_2

*For correspondence. E-mail Janice.lake@sheffield.ac.uk

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.

The Don Valley Power Project is co-financed by the European Union's European Energy Programme for Recovery
The sole responsibility of this publication lies with the author.
The European Union is not responsible for any use that may be made of the information contained therein.

Keywords Carbon capture and storage, CCS, Crops, Extreme CO_2, Gas exchange, Roots, Soils

1. Introduction

Anthropogenic climate change is driven by the acceleration of the long-term carbon cycle via
51 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
54 (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
57 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".
60 These ambitious goals require the development of multiple mitigation practices and eventually the removal of fossil fuel derived carbon from the energy system.

63 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
66 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
69 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
72 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

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

2. Materials and methods

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 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 21/18ºC day/night; relative humidity 60%. Gas was supplied from either an integral supply (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 (±15) mL min^{-1} to maintain CO_2 and N_2 levels at steady state. Gases were exhausted to atmosphere via a separate manifold to prevent build up within the growth room.

2.2 Soils types

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 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 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 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.

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 (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, 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).

2.3 Soil pH

Soil samples were taken prior to and at the end of each experiment and dried at 40 ± 4°C. A solution of 0.01M calcium chloride dihydrate ($CaCl_2.2H_2O$ analytical grade) was dissolved in 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 electrode and Jenway PHM6 meter, Fisher Scientific, UK) until readings were stable.

2.4 Crop species

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

2.5 Biochemical analyses

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 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 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 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.

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:

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

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

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

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 $CH_3CO_2Na \cdot 3\ H_2O$ 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 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, 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 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).

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

$$A = (A533 - A700)pH\ 1.0 - (A533 - A700)pH\ 4.5 \quad (4)$$

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 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].

252 ## 3.1 Biomass

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 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 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 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 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].

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 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].

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 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 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).

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 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 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 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.

3.3 Plant biochemistry

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

3.4 Chlorophyll

Chlorophyll analysis was undertaken to test for overall plant photosynthetic health, as a 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 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 (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 ($p = 0.005$), 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 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 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).

3.5 Anthocyanin

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

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

For the beetroot anthocyanin content there was no significant difference observed between 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). 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 ($p = <0.000$) [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.

3.6 Phenylalanine lyase (PAL)

PAL analysis was again performed to test for generic plant health as PAL up-regulation is a 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 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 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 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 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 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 yield losses corresponding to 0.0003% ha.

4. Conclusions

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 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. 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

JAL was funded by National Grid, UK and the European Union Energy Programme for Recovery (EEPR) under the COOLTRANS research programme. The European Union is not responsible for any use that may be made of the information contained herein.

References

1. Blackford J, Stahl, H, Bull JM, Berges BJP, Cevatoglu M, Lichtschlag A, Connelly D, James RH, Kita J, Long D, Naylor M, Shitashima K, Smith D, Taylor P, Wright, I, Akhurst M, Chen, BX, Gernon TM, Hauton C, Hayashi M, Kaieda H, Leighton TG, Sato T, Sayer MDJ, Suzumura M, Tait K, Vardy ME, White PR and Widdicombe S. Detection and impacts of leakage from sub-seafloor deep geological carbon dioxide storage. *Nat Clim Change* **4:**1011–1016 (2014).

2 Widdicombe S, McNeill CL, Stahl H, Taylor P, Queiros AM, Nunes J and Tait K. Impact of sub-seabed CO_2 leakage on macrobenthic community structure and diversity. *Int J Greenhouse Gas Control* **38**:182–192 (2015).

3. Tait K, Stahl H and Taylor P, Widdicombe S. Rapid response of the active microbial community to CO_2 exposure from a controlled sub-seabed CO_2 leak in Ardmucknish Bay (Oban, Scotland). *Int J Greenhouse Gas Control* **38:**171–181 (2015).

4. Lessin G, Artioli Y, Queirós AM, Widdicombe S and Blackford JC. Modelling impacts and recovery in benthic communities exposed to localised high CO_2. *Mar. Pollut Bull* **109:**267–280 (2016).

5. Kruger M, Jones, D, Frerichs J, Oppermann, BI, West J, Coombs P, Green K, Barlow T, Lister R, Shaw R, Strutt, M and Moller I. Effects of elevated CO_2 concentrations on the vegetation and microbial populations at a terrestrial CO_2 vent at Laacher See, Germany. *Int J Greenhouse Gas Control* **5**:1093–1098 (2011).

6. Lubben A and Leven C. The Starzach site in Southern Germany: a site with naturally occurring CO_2 emissions recovering from century‐long gas mining as a natural analog for a leaking CCS reservoir. *Environ Earth Sci* **77:** 316 (2018).

7. Noble RRP, Stalker L, Wakelin SA, Pejcic B, Leybourne MI, Hortle AL and Michael K. Biological monitoring for carbon capture and storage – A review and potential future developments. *Int J Greenhouse Gas Control* **10:**520–535 (2012).

8. Schloemer S, Furche M, Dumke I, Poggenburg J, Bahr A, Seeger C, Vidal A and Faber E. A review of continuous soil gas monitoring related to CCS – Technical advances and lessons learned. *Appl Geochem* **30:**148–160 (2013)

9. Moni C and Rasse DP. Detection of simulated leaks from geologically stored CO_2 with ^{13}C monitoring. *Int J Greenhouse Gas Control* **26:** 61–68 (2014)

10. Hao RJ, Wang ZF, Wang WK and Zhao YQ. Change characteristics of chlorophyll content and high spectrum in soybean leaves under the stress of CO_2 leakage. *Bulgarian Chemical Communications* 48: 230–237 (2016).

11. Schacht U and Jenkins, C. Soil gas monitoring of the Otway Project demonstration site in SE Victoria, Australia. *Int J Greenhouse Gas Control* **24:** 14–29 (2014)

12. Sáenz de Mieraa LE, Arroyo P, de Luis Calabuig E and Ansolac G. Effects of varying CO_2 flows on bacterial communities in mesocosms created from two soils. *Int. J. Greenhouse Gas Control* **46:**205–214 (2016).

13. Kim JY, Xuanlin C, He W and Yoo, G. Corn Growth and Development influenced by Potential CO_2 Leakage from Carbon Capture and Storage (CCS) Site. *J of Climate Change Research* **8:**257–264 (2017)

14. Kim JY, He W, Ko D, Chung H and Yoo G Increased N_2O emission by inhibited plant growth in the CO_2 leaked soil environment: Simulation of CO_2 leakage from carbon capture and storage (CCS) site. *Sci of the Total Environ* **607-608:**1278–1285 (2017)

15. Zhou X, Apple ME, Dobeck LM, Cunningham AB and Spangler LH. Observed responses if soil O_2 concentration to leaked CO_2 from an engineered CO_2 leakage
489 experiment. *Int J Greenhouse Gas Control* **16**:116–128. 2013

16. Sharma B, Apple ME, Zhou X, Olson JM, Dorshorst C, Dobeck LM, Cunningham
492 AB and Spangler LH. Physiological responses of dandelion and orchard grass to experimentally released upwelling soil CO_2. *Int J Greenhouse Gas Control* **24:**139–148 (2014).

495

17. Smith KL. Lake JA, Lomax BH and Steven MD. Effects of elevated soil CO_2 concentration on growth and competition in a grass clover mix. *Int J Greenhouse Gas*
498 *Control* **64:**340–348. (2017).

18. Lake JA, Steven MD, Smith KL and Lomax BH. Plant responses to elevated levels in
501 soils: distinct CO_2 and O_2-depletion effects. *Int J Greenhouse Gas Control*. **64:**333–339 (2017).

504 19. Lake JA and Lomax BH. Plant responses to simulated carbon capture and transport leakage: the effect of impurities in the CO_2 gas stream. *Int J Greenhouse Gas Control* **72**, 208–213 (2018).

507

20. Rusin A and Stolecka K. Environmental hazards caused by carbon capture and storage (CCS) technologies. *Polish J of Environ Studies* **22:**205-211 (2013).

510

21. Smith KL, Steven MD, Jones DG, West JM, Coombs P, Green KA, Barlow TS, Breward N, Gwosdz S, Krüger M, Beaubien SE, Annunziatellis A, Graziani S and Lombardi S. Environmental impacts of CO_2 leakage: recent results from the ASGARD facility, UK. *Energy Procedia* **37**, 791–799 (2013).

22. Ni Z, Kim E-D & Chen J. Chlorophyll and starch assays. *Protocol Exchange* (2009) doi:10.1038/nprot.2009.12.

23. Al-Traboulsi M, Sjöegersten S, Colls J, Steven M, Craigon J and Black C. Potential impact of CO_2 leakage from carbon capture and storage (CCS) systems on growth and yield in spring field bean. *Environ Ex Bot* **80**, 43–53. (2012).

24. Al-Traboulsi M, Sjöegersten S, Colls J, Steven M, Craigon J and Black C. Potential impact of CO_2 leakage from carbon capture and storage systems on field bean (*Vicia faba*). *Physiol Plant* **146:** 261–271 (2012)

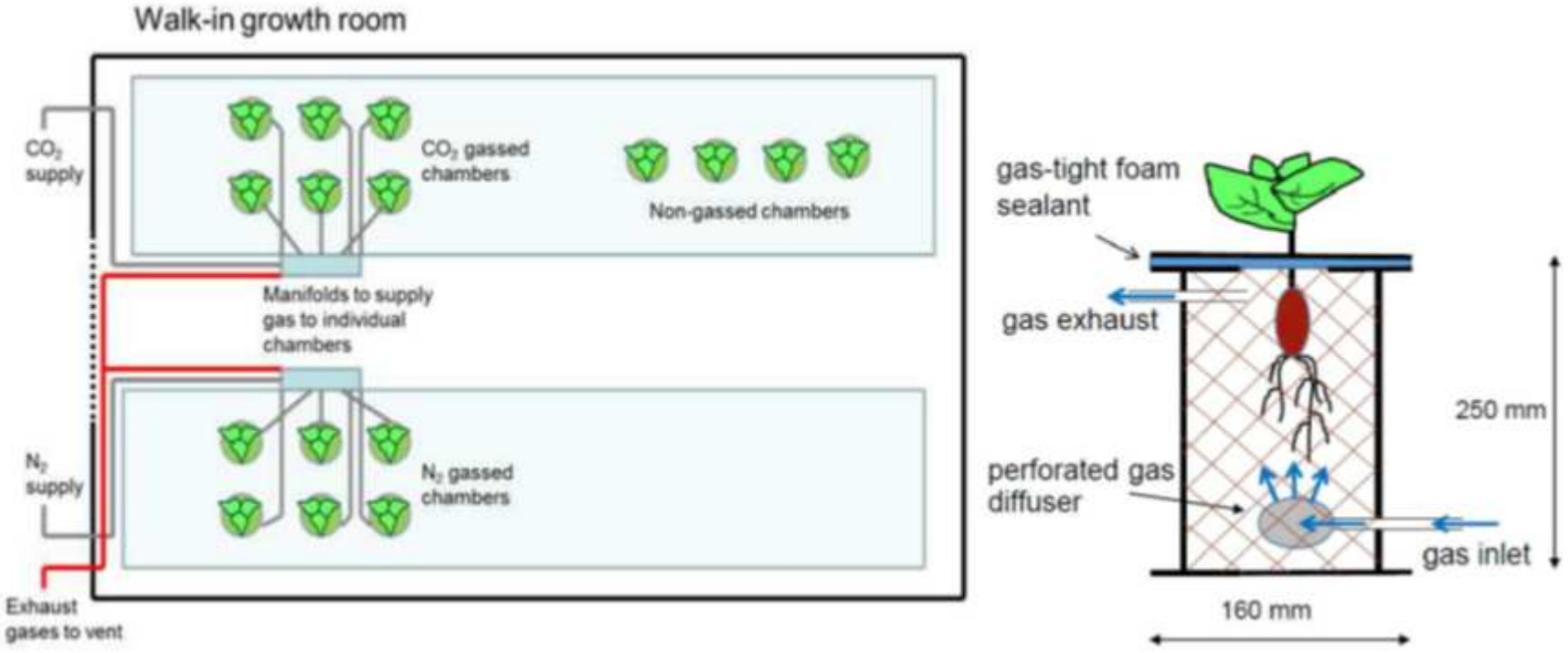
25. Al-Traboulsi M, Sjöegersten S, Colls J, Steven M, Craigon J and Black C. Potential impact of CO_2 leakage from Carbon Capture and Storage (CCS) systems on growth and yield in maize. *Plant Soil* **365:** 267–281 (2013).

26. Schacht U, Regan M, Boreham C, and Sharma S. CO2CRC Otway Project – Soil Gas Baseline and Assurance Monitoring 2007–2010. *Energy Proc* **4:** 3346–3353 (2011).

27. Lake JA, Walker HJ, Cameron DD and Lomax BH. A novel root-to-shoot stomatal response to very high CO_2 levels in the soil: electrical, hydraulic and biochemical signalling. *Physiol Plant* **159:**433–444 (2017).

28. Lake JA, Steven MD, Smith KL and Lomax BH. COOLTRANS - Environmental impact of a hypothetical catastrophic leakage of CO_2 onto the ground surface. *Journal of Pipeline Engineering* **11(3):**239–248 (2012).

Figures



3 **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.

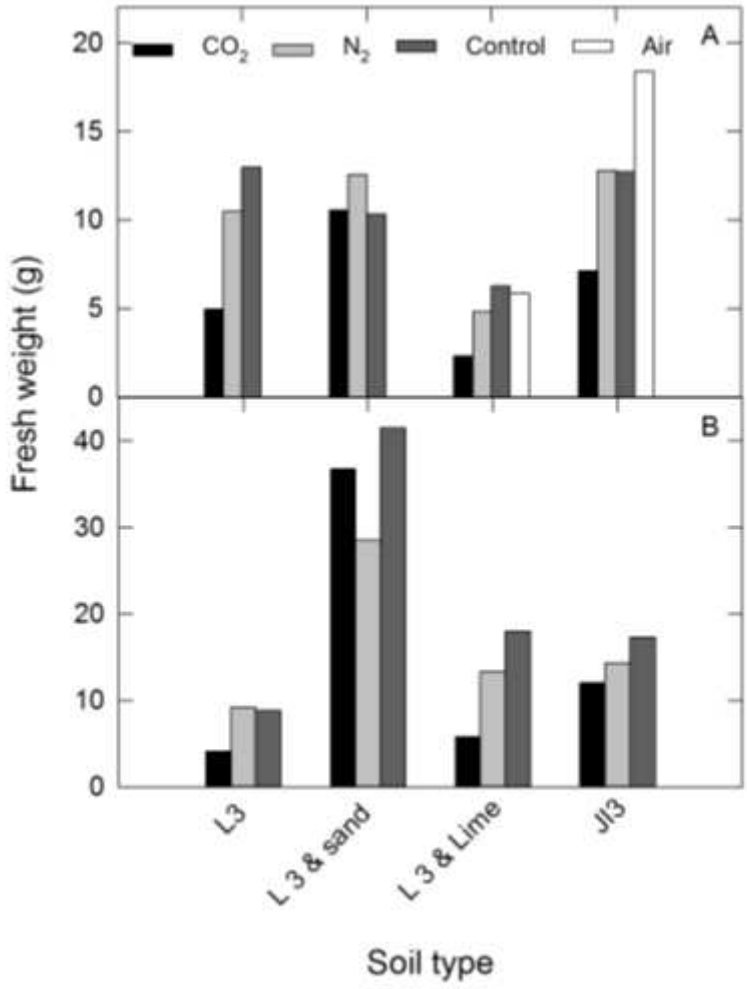


Fig. 2. Fresh weight biomass at harvest across experimental treatment and soils type. (A) Wheat and (B) beetroot. A full statistical break down of results is given in the text. See materials and methods for experimental set up.

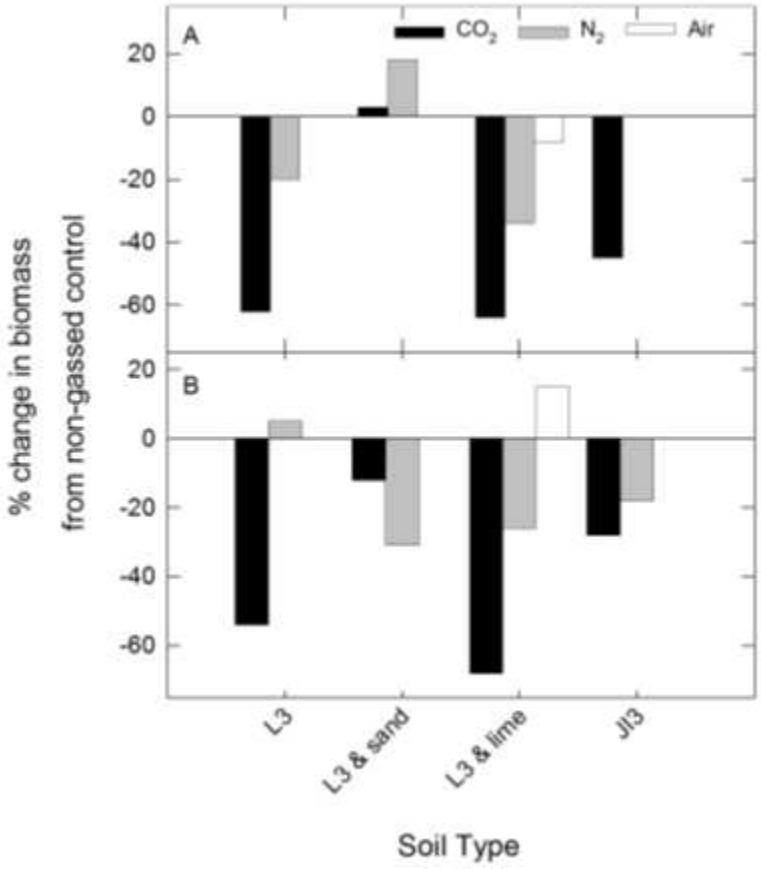
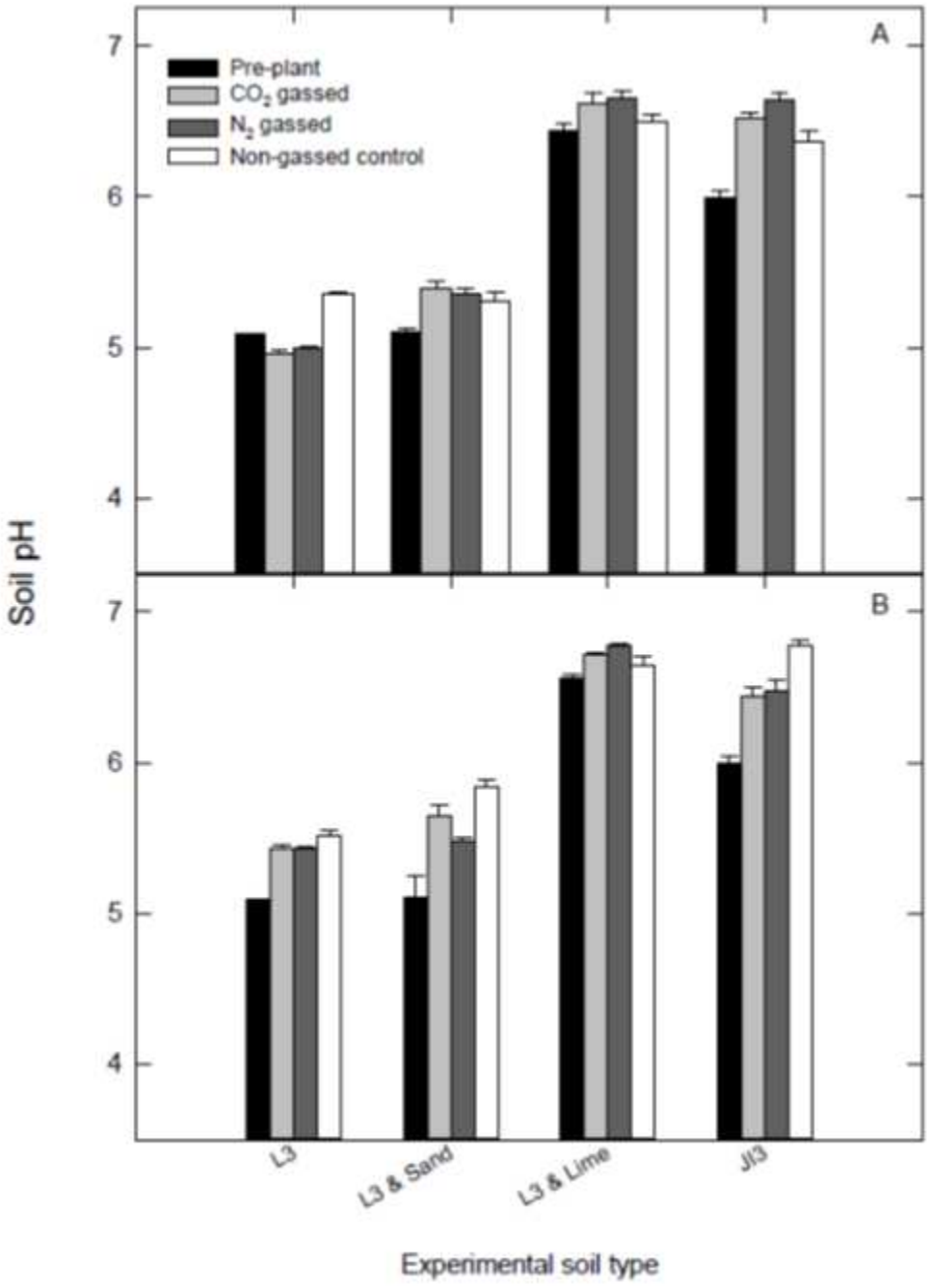


Fig. 3. Percentage change in biomass across experimental treatment and soils type relative to controls. **(**A) Wheat and (B) beetroot. See materials and methods for experimental set up.



3 **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.

6

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).

	Wheat			**Beetroot**		
Soil type	**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.

		Biochemical Compound (non-gassed control only)		
Crop	**Soil type**	**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