Project title:	Increasing crop yield and resource use efficiency via root-zone CO_2 enrichment
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Project leader:	Ian Dodd
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Key staff:	Estibaliz Leibar-Porcel, PhD student Martin McAinsh, co-supervisor
Location of project:	Lancaster University
Industry Representative:	Philip Morley, British Tomatoes Growers' Association
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DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a three-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

Headlines

- Gaseous CO_2 enrichment (1500 ppm) of the root-zone of aeroponically-grown lettuce increased biomass by up to 19-25%, with variation according to the environmental conditions and lettuce cultivar
- Bicarbonate application (1-5 mM) to hydroponic solutions (which releases CO_2 to the solution) increased shoot growth of lettuce and pepper by 10-20%

Background

Biomass accumulation is the difference between the photosynthesis rate and respiration rate. Greenhouse operators often inject extra CO_2 into the aerial environment to increase photosynthesis and biomass accumulation. However, when the humidity or the temperature is very high, the greenhouse is vented and CO_2 is released into the atmosphere (Figure 1), which is economically wasteful and releases a greenhouse gas to the atmosphere.

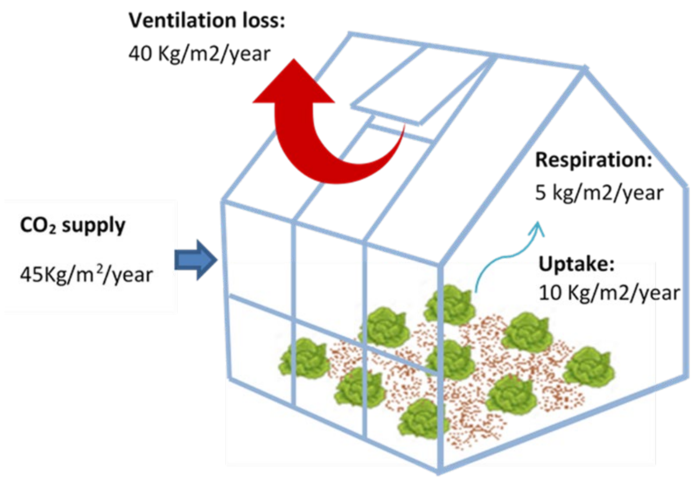

Figure 1. CO_2 balance model. a) General balance model when supplying 45 kg/ (m^2 year). *Modified from Wageningen University & Research, Business Unit Greenhouse Horticulture*

Sources of CO_2 for enrichment include boiler, combined heat, power (CHP), burner exhaust gases, and liquefied pure gas. Flue gases from natural gas boilers are widely used in the UK as a source of CO_2 for enrichment. This practice has high-energy costs of £200,000 per annum for a 5 ha glasshouse (Pratt, 2011). CO_2 is a “greenhouse gas” that contributes to global warming and climate change. Despite the efforts of growers to minimize spending and maximize production through technical improvements, it is necessary to consider other systems such as localized root-zone CO_2 enrichment, to improve crop production while minimising environmental emissions.

This project focused on improving resource use efficiency and the environmental performance of tomato, lettuce and pepper production, by testing whether root-zone CO_2 enrichment of soilless culture systems was beneficial.

Summary

Previous studies have shown that applying either bicarbonate hydroponically at low concentrations (5 mM HCO_3^-) or gaseous CO_2 at high concentrations (2,000-50,000 ppm) to the roots increased growth of some crops such as tomatoes or lettuce. Also, initial studies carried out at Lancaster University by a previous AHDB-funded PhD student indicated that applying 700 ppm CO_2 to the root-zone of semi-aeroponically grown lettuce (without altering the aerial CO_2 concentration) increased biomass by 10%. Therefore, root-zone CO_2 enrichment in greenhouses may provide an alternative technique to increase yield.

Initial studies identified that applying low concentrations of bicarbonate (1-5 mM) to the nutrient solution of hydroponically grown pepper and lettuce increased shoot biomass by 10% compared to those plants that did not receive bicarbonate. In addition, root-zone CO_2 enrichment of aeroponically grown lettuce increased shoot biomass (20%) compared to plants grown without root-zone CO_2 enrichment. However, the response is variable depending on the experimental conditions and the lettuce variety used. Due to time constraints in this project, further work is required to fully understand how other environmental variables (e.g. temperature, light) affect plant responses to root-zone CO_2 enrichment.

Financial Benefits

Developing techniques to more effectively apply CO_2 will decrease the cost of supplying liquefied CO_2 or energy consumption (natural gas boilers) in commercial scale greenhouses.

Action Points

Understand that there are potential alternatives to the current practice of aerial CO_2 enrichment in greenhouses that decrease CO_2 usage and reduce pollution, while maintaining or increasing crop yields.

SCIENCE SECTION

Introduction

Generally, soil CO_2 concentration greatly exceeds that of the atmosphere (400 ppm). Root respiration and microbial respiration, including decomposition of organic material, are major contributors to the soil inorganic carbon pool. Concentrations of CO_2 in the soil vary with depth (Johnson *et al.* 1994, Duenas *et al.* 1995), soil water content (Bouma *et al.* 1997), soil type (Duenas *et al.* 1995) and time of the year (Johnson *et al.* 1994) and range from 2000 to 5000 ppm but may become as great as 200.000 ppm when soils are poorly aerated (De Jong and Shappter, 1972; Norstadt and Porter, 1984).

In most higher plants, leaf stomata are the principal means of gas exchange, including the capture of CO_2. Although some aquatic plants assimilate large amounts of CO_2 from the sediments via roots, terrestrial plants are thought to capture insignificant amounts of CO_2 through their roots. However, the terrestrial plant *Stylites andicola*, which lacks stomata, captures almost all of the CO_2 via its roots (Keeley, Osmond et al. 1984), suggesting that some or perhaps all plants can obtain CO_2 from their roots.

In previous studies, several systems have exposed the roots to different CO_2 concentrations, most of them based on hydroponic and aeroponic systems. Hydroponics is a method where plants are grown without soil using a mixture of water and nutrient salts, called a nutrient solution. Aeroponics is a similar technique except that plant roots are suspended in air and sprayed with nutrient solution. In both systems, studies have applied either carbonate ions (HCO_3-) (Bialczyk, *et al*. 1992, 1994, 2004, 2005; Alhendawi, *et al*.1997; Al Mansouri, *et al.* 2014; Wanek *et al.* 2000; Terraza *et al.* 2012; Yang *et al.* 1994; Siddiqi, *et al.* 2002) or gaseous CO_2 (Gao, *et al.* 1997; Bouma, *et al*. 1997; Cramer and Richard, 1999; Cramer *et al.* 1999; Van der Merwe *et al.* 2000; Cramer *et al.* 2001, Boru, *et al.* 2003; Viktor, *et al.* 2003; Cramer *et al*. 2005; Viktor and Cramer, 2005; He *et al.* 2007; X.Zhao *et al*. 2010; He *et al.* 2010; He *et al.* 2016), with growth increments sometimes reported (Figure 2).

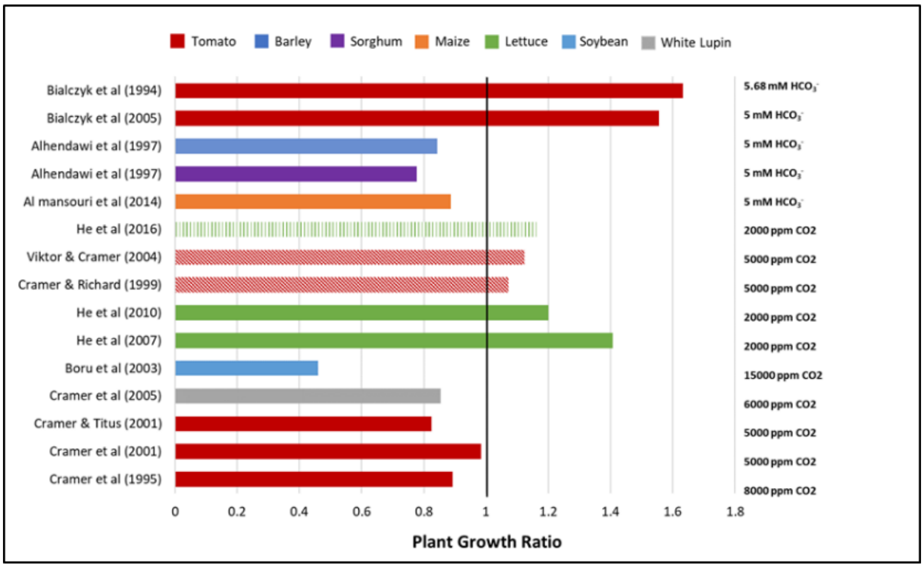

Figure 2. Growth of plants with elevated HCO_3^- and CO_2.
Data are plotted as a ratio of enriched (5-6 mM HCO_3^-) and (2000-15000 ppm CO_2) to control (0 mM HCO_3^-) and (360 ppm CO_2). A value of 1 indicates no response to rootzone CO_2 enrichment

Dissolved inorganic carbon (DIC) effects on nutrients

High aerial [CO_2] typically enhances plants growth rates thus creating greater nutrient demand, especially for N (Rogers *et al*., 2006; Sicher and Bunce, 2008). However, root-zone (RZ) CO_2 enrichment causes variable effects on root and shoot nutrient concentrations. At least part of these changes can be attributed to RZ CO_2 enrichment effects on solution pH and therefore nutrient uptake (Figure 3).

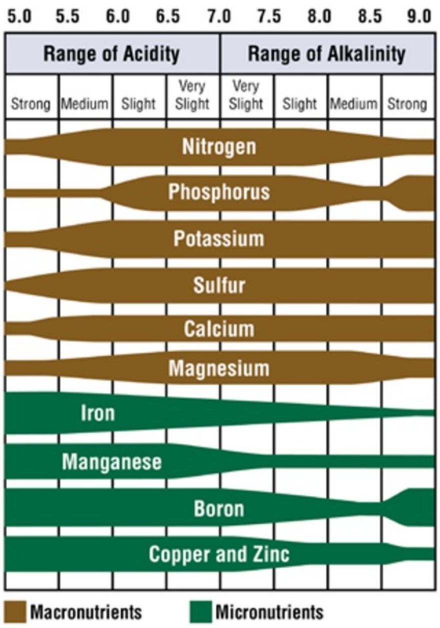

Figure 3. Nutrient availability changes with the pH of mineral soils. Nutrients are most available when the band is wide and less when is narrow. *Source:* Brady and Weil (2007)

In general, increasing the concentration of bicarbonate to 5, 10, 20 mM decreased root uptake of K^+, NO_3^-, Mg^{2+}, S, P and Fe but not Ca^{2+}, in sorghum and maize plants when the pH of the nutrient solution was ~ 8. Decreased nutrient uptake was correlated with a lower shoot and root biomass (Alhendawi *et al*., 1997; Al Mansouri *et al.,* 2014). On the other hand, 5.68 mM bicarbonate increased tomato biomass accumulation, leaf blades and roots N content, K^+ content in all tissues and Ca^{+2} content in roots, shoot and leaf blades, even though P content did not differ from the control (Bialzyck *et al*., 1994). While the variability of some elemental concentrations could be due to the different plant species, nutrient solutions and experimental design used, it seems that bicarbonate application increases Ca^{2+} in all cases. Other studies where RZ CO_2 gas was applied mainly focused on nitrogen metabolism with limited data on other nutrient elements. Tomato plants grown for 60 days at elevated RZ [CO_2] (2500, 5000 and 10000 ppm) had decreased root N, P, K^+, Ca^{2+} and Mg^{2+} concentrations after 60 days, compared to those exposed to RZ ambient CO_2 of 370 ppm (Zhao *et al*., 2010). Since these different types of RZ CO_2 enrichment had quantitatively and qualitatively different effects on various plant nutrients, and these types have not been previously compared in the same experimental facility, Experiment 1 determined nutrient tissue concentrations responses to RZ CO_2 enrichment.

DIC effects on phytohormones

Enoch and Olsen (1993) suggested that CO_2 could act as a plant hormone or at the very least influence plant hormone systems, based on the interaction that ethylene (C_2H_4) has with CO_2 and bicarbonate, as CO_2 can block or promote physiological effects of ethylene (Rothan et al., 1997; Dong *et al.,*1992). Ethylene is a plant hormone involved in different processes such as stimulation of germination (Corbineau *et al.*, 2014), positive regulator of root hair development (Song *et al.*, 2016), negative regulator of root nodulation (Guinel, 2015), promotion, inhibition or induction of organ senescence and abscission, differential cell growth, stress responses and resistance to necrotrophic pathogens (Davies, 2004). In closed environments, elevated C_2H_4 levels can cause shortened height, epinasty, leaf rolling, premature leaf senescence, and sterility (Abeles *et al.*, 1992; Bennet and Hughes, 1972; Morison and Gifford, 1984). Ethylene in the soil can inhibit root growth of various plants (Visser *et al*., 1997, Pierik *et al.*, 1999). However, in some cases high CO_2 concentrations (2-10% CO_2) inhibits the biological activity of ethylene (Sisler and Wood, 1988).

There is limited research focusing on the relationship between elevated ambient [CO_2] (e[CO2]) and plant hormones. Enriching the air environment with CO_2 (700 ppm CO_2)

enhanced plant growth and development of *Arabidopsis thaliana* and also increased foliar ACC (the ethylene precursor), IAA, GA_3 and cytokinin (ZR, DHZR and iPA) concentrations, but significantly reduced the ABA concentration (Teng *et al*., 2006). Contrary to this, e[CO2] (550 ppm) increased the abundance of transcripts of ABA-responsive genes of *A. thaliana* (Li *et al.,* 2006). e[CO_2] also downregulated JA and ethylene signalling, and enhanced SA signalling (DeLucia et al, 2012). e[CO_2] increased root IAA content and ethylene evolution of hydroponic tomato by 26.5% and 100% respectively (Wang *et al*., 2009). Similarly, e[CO_2] significantly increased IAA concentration in tomato roots, promoting root growth and stimulating ethylene production by increasing 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity (Abeles *et al*., 1992; Kende, 1993). Plant response to changes in ethylene signalling and synthesis can vary according to the environmental conditions. While these studies that applied suboptimal ambient [CO_2] may be relevant when applying high RZ CO_2, there have been no studies that specifically studied hormonal responses to RZ CO_2 enrichment. Since variation in hormonal responses may account for the some of the variation in growth response to RZ CO_2 enrichment (Figure 2), Experiment 2 determined hormonal responses to RZ CO_2 enrichment.

Materials and methods

Experiment 1: Bicarbonate and root-zone (RZ) CO_2 effects on lettuce tissue nutrient concentrations.

Aim: Determine the effects of 0,1,20 mM HCO_3- and 1500 ppm RZ CO_2 on leaf tissue nutrient concentrations of lettuce plants.

Experimental procedures:

Seeds of lettuce (*Lactuca sativa* (L.) cv. Sunstar and cv. Antartica), were grown in vermiculite and transferred to hydroponic or aeroponic culture 23 days post germination, after rinsing the roots in water. The controlled environment (CE) room was maintained at 20°C/16°C for 10 days after transferring them to deep flow hydroponics system (DFTS) or to the aeroponic system. The illumination in the CE room was provided by twelve 400 W metal halide lamps (HQI-T 400N, Osram, St Helens, UK) for a 12 h photoperiod (8.00 hrs to 20.00 hrs). The temperature, humidity and CO_2 concentration in the CE room and glasshouse were recorded by Electron II C sensor (HortiMax B.V. Pijnacker, Netherlands).

The growing systems specified in Report 1 and 2 were used to assess nutrient concentrations of plants grown with bicarbonate- and CO_2-enriched root-zones respectively.

For the bicarbonate experiment, the median 4 plants were taken from 0, 1 & 20 mM $NaHCO_3$ treatments and sent to NRM Technologies Ltd. (Bracknell, UK) for C013 Plant Foliar Suite Analysis, incorporating analysis for: total Nitrogen and Sulphur with N:S Ratio and, total; Phosphorus, Potassium, Magnesium, Calcium, Copper, Manganese, Zinc, Iron and Boron.

For the RZ CO_2 experiment, the median 8 plants were taken from each treatment. At harvest lettuce plants had between 10-12 true leaves unfolded initiating cupping stage. The older (10-5) and youngest (4-2) leaves were divided and analysed separately. Macronutrients (Ca, K, Mg, Na, P and S) were analysed via acid microwave digestion followed by ICP-OES. Nitric acid (HNO_3) was used to decompose all organic matter to CO_2. Ball-milled (MM400, Retsch, Haan, Germany) oven dried leaf tissue (0.25 g) was weighed in acid-washed and rinsed reaction vessels. Five mL of 100 % HNO_3 (Aristar grade) was added and left for 15 min in a fume hood until the initial reaction was finished. Vessels were sealed and weighed and then placed in the rotor in a MARS 6 microwave (CEM, Buckingham, UK). Vessels were heated to 200°C over 15 min and then held at 200°C for another 15 min. After cooling down, vessels were weighed again to note weight loss. Samples and blank solutions were then diluted in two steps to first 20% HNO_3 and second to the final concentration of 2% HNO_3 by using MilliQ water. To analyse nutrients, an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, iCAP 6300, Thermo Scientific, Massachusetts, USA) with axial view configuration was used. To validate the digestion, tomato and spinach leaves samples with known nutrient concentrations were run and the recovery detected through the ICP-OES was used to calculate final sample concentration. The element reference standard solutions were prepared daily from 1000 mg L^{-1} stock solutions.

Leaf nitrogen in percentage was analysed using an Elemental Analyser (VARIO- El elemental analyser). Oven-dried leaf tissue samples were wrapped in aluminium capsules and dropped into a furnace held at 905°C onto CuO with a pulse of O_2 and a constant flow of Helium carrier gas. N was converted to gas (N_2) and a pure copper reduction unit after the furnace reduced any conversion of NOx to N_2. N_2 was measured in a TCD (total dissolved carbon) detector positioned at the end of the elemental analyser and peak areas were compared to standards and amounts of N calculated.

Experiment 2: Root-zone CO_2 effects in hormone concentration in lettuce and pepper plants.

Aim: Determine the effects of 1500 ppm RZ CO_2 on hormone concentrations of lettuce and pepper plants grown aeroponically.

Experimental procedures:

Aeroponically grown lettuce and pepper samples were taken, immediately frozen in liquid nitrogen and stored at –20°C before being freeze-dried for 48 h. The samples were then ground and weighed (50 mg) out for an extraction with 0.5 mL extraction buffer (methanol:water 80:20 v/v) for 0.5 h at 4°C. Solids were separated by centrifugation (20 000 *g*, 15 minutes) and re-extracted for 30 minutes at 4ºC in an additional 0.5 ml of the same extraction solution. Pooled supernatants were passed through a Sep-Pak Plus †C_{18} cartridge (SepPak Plus, Waters, USA) to remove interfering lipids and part of the plant pigments and evaporated at 40ºC under vacuum either to near dryness or until organic solvent was removed. The residue was dissolved in a 1 ml methanol/water (20/80, v/v) solution using an ultrasonic bath. The dissolved samples were filtered through 13mm diameter Millex filters with 0.22 μm pore size nylon membrane (Millipore, Bedford, MA, USA). Ten μl of filtrated extract were injected in a U-HPLC-MS system consisting of an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) using a heated electrospray ionization (HESI) interface. Mass spectra were obtained using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). For quantification of the plant hormones, calibration curves were constructed for each analyzed component (1, 10, 50, and 100 μg l^{-1}) and corrected for 10 μg l^{-1} deuterated internal standards. Recovery percentages ranged between 92 and 95%. Samples were analysed by Dr. Alfonso Albacete in CSIC (Murcia, Spain) (Albacete et al., 2008). Five out of the 11 hormones (ACC, tZ, ABA, JA and SA) were detected in both the leaf and root of lettuce and pepper plants.

Statistical analysis

To determine treatment differences, the statistical software SPSS 21.0 (IBM, USA) was used to perform a Student's t-test at the $P < 0.05$ level.

Results

Experiment 1:

Bicarbonate enrichment of the root-zone (1 and 20 mM $NaHCO_3^-$) significantly decreased N (22%-33%), P(13%), K(14%-33%), Zn (20%-52%) and Cu (44%-51%) concentrations, with significantly lower K and Zn concentrations at 20 mM than 1 mM. Furthermore, 20 mM $NaHCO_3^-$ significantly decreased leaf Mn concentration by 28%. In contrast, bicarbonate enrichment of the root-zone (20 mM $NaHCO_3^-$) significantly increased Mg, Fe and B concentrations by 42%, 20% and 42% respectively (Figure 4). Shoot fresh weight was ~20%

higher at 1 mM $NaHCO_3$- and ~ 48% lower at 20 mM compared to control plants (Table 4). While dilution (of a fixed nutrient uptake in a larger plant volume) may account for decreased nutrient concentrations at 1 mM $NaHCO_3^-$, changes in nutrient concentration at 20 mM $NaHCO_{3-}$ likely result from pH-mediated changes in nutrient uptake.

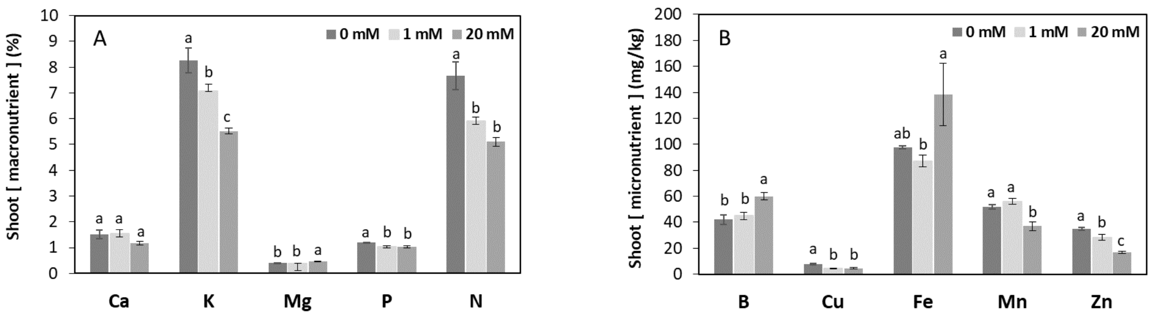

Figure 4. Lettuce shoot macronutrients concentration **(A)**: calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P) and nitrogen (N). Shoot micronutrients concentration **(B)**: boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). Bars are means ± SE of 4 replicates. Different letters above the bars indicate significant differences between treatments (Independent Sample T-test, p-value < 0.05)

Table 4. Shoot fresh weights of lettuce plants grown under 0, 1 and 20mM $NaHCO_3^-$

$NaHCO_3^-$	0 mM	1 mM	20 mM
Shoot fresh weight (g)	28.2 ± 0.3	32.3 ± 1.8	13.3 ± 1.3

In aeroponically grown plants, Ca and K concentrations were lower (40% and 10%) in control young leaves compared to plants exposed to high RZ CO_2, although these differences were not significant. However, in old leaves Ca and K concentrations were similar between treatments. Mg and P did not show any significant treatment differences in both young and old leaves. B, Cu, Fe, Mn concentrations were not significantly different between treatments in young or old leaves, although Fe was ~10% higher in control plants in both young and old tissue. Zn concentration was significantly higher (~ 50%) in control plants in old leaves but was ~50% higher in the younger leaves exposed to elevated RZ CO_2. To place these treatment differences in context, Ca, K and Mg concentrations were higher in old leaves (~80%, ~20% and ~30%) and P levels were higher in younger leaves by ~40% (Figure 5 A&B). Since the magnitude of treatment differences induced by RZ CO_2 were generally smaller than intra-plant differences in nutrient concentrations, it is difficult to argue that plant nutrient relations primarily determined growth.

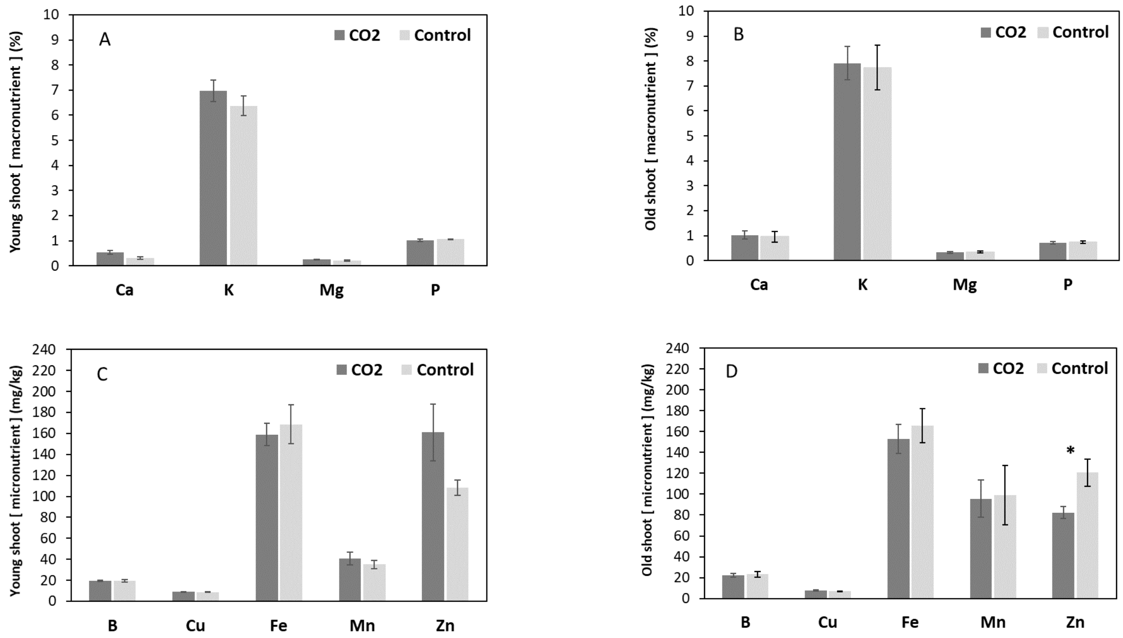

Figure 5. Lettuce young leaves macronutrients concentration **(A)**: calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P). Old leaves macronutrient concentration **(B)**. Young leaves micronutrients concentration **(C)**: boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), Old leaves micronutrients concentration **(D)**. Bars are means ± SE of eight replicates. Asterisks indicate significant differences between treatments (Independent Sample T-test, p-value < 0.05)

Overall, macronutrient and micronutrient concentrations of lettuce shoots did not significantly differ between treatments (Figure 6). However, Ca, K, Mn and Zn levels were higher (~20%, ~10%, 10%, ~10%) in plants exposed to high RZ CO_2 whereas Fe concentration were lower (10%) compared to control plants.

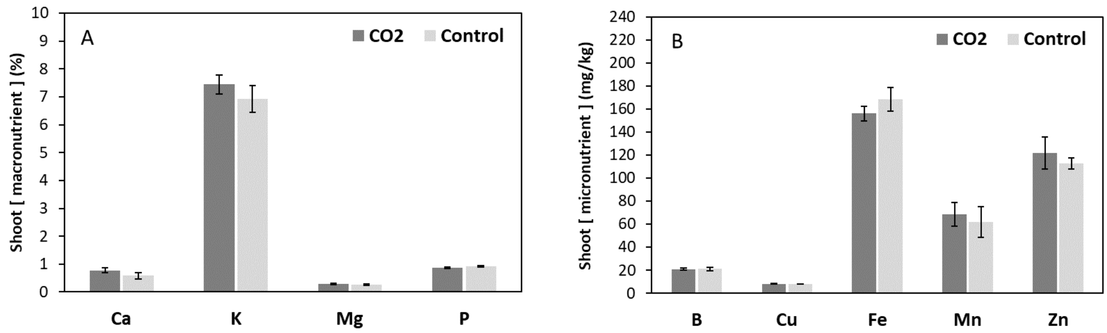

Figure 6. Lettuce shoot macronutrients concentration **(A)**: calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P). Shoot micronutrients concentration **(C)**: boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). Bars are means ± SE of eight replicates. Asterisks indicate significant differences between treatments (Independent Sample T-test, p-value < 0.05)

In plants exposed to elevated RZ CO_2, leaf nitrogen concentration was 5% lower but root nitrogen concentration was 5% higher (Figure 7).

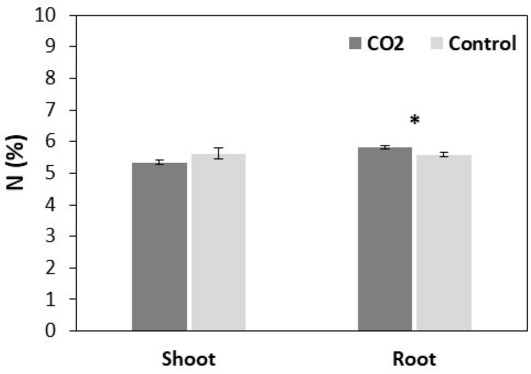

Figure 7. Lettuce shoot and root nitrogen concentration in lettuce plants exposed to high and ambient RZ CO_2. Bars are means ± SE of eight replicates. Asterisks indicate significant differences between treatments (Independent Sample T-test, p-value < 0.05)

Experiment 2:

Compared to control lettuce plants grown aeroponically at ambient root-zone CO_2, RZ CO_2 enrichment had little effect on leaf phytohormone concentrations although jasmonic acid (JA) concentrations significantly increased by 30%. Root phytohormone concentrations did not differ between treatments.

In pepper, RZ CO_2 enrichment decreased leaf *trans*-zeatin (*t*Z) concentrations by 50%, but increased leaf 1-aminocyclopropane-1-carboxylic acid (ACC) by ~60 % (Figure 8). Shoots and roots salicylic acid concentrations showed opposing changes to RZ CO_2 enrichment, with leaf SA concentrations decreasing by 35% while root SA concentrations increased by 50%.

While phytohormone concentrations showed limited responses to RZ CO_2 enrichment, pepper was more responsive than lettuce.

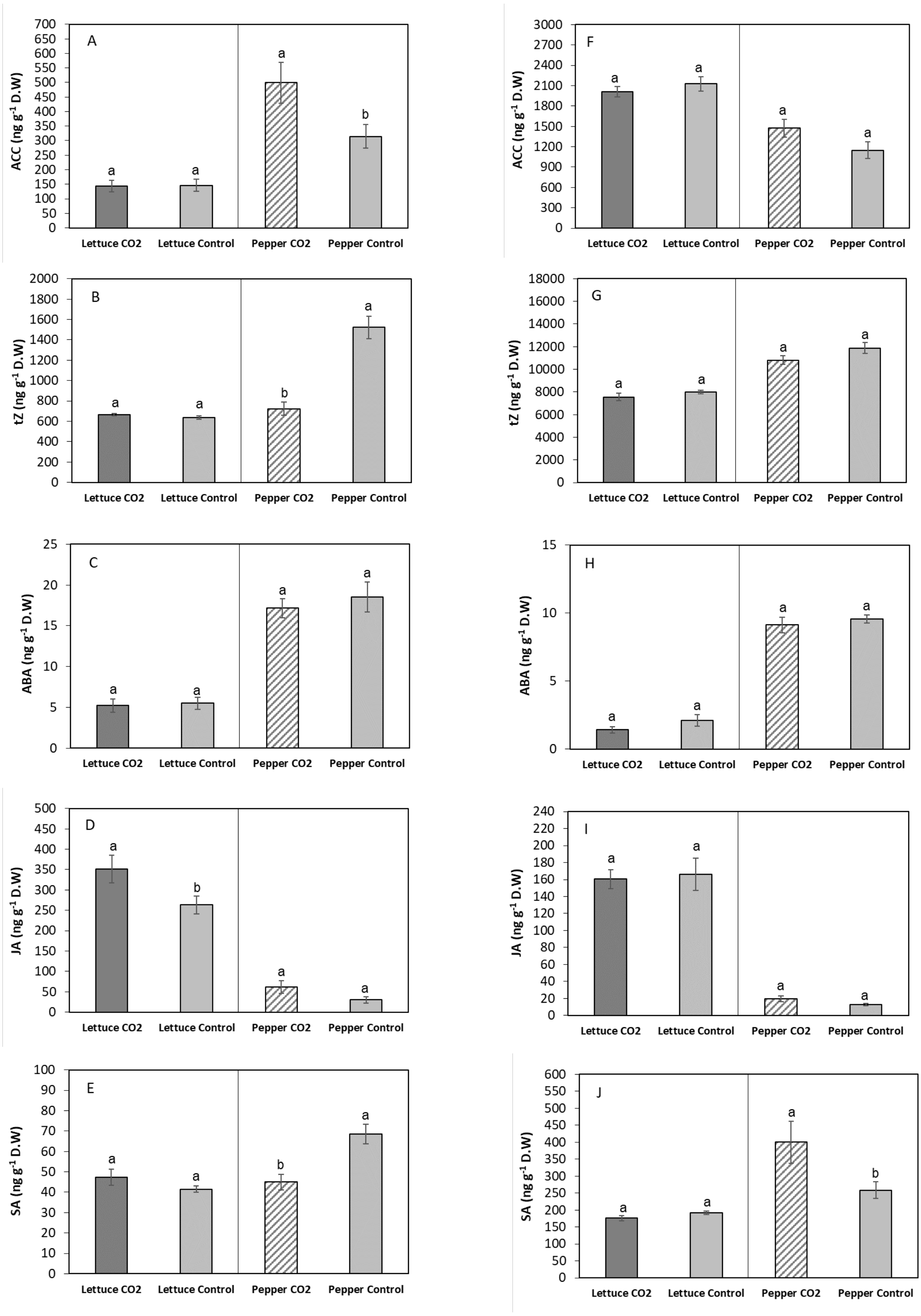

Figure 8. Leaf (A, B, C, D, E) and root (F, G, H, I, J) phytohormone concentrations under high RZ CO_2 and ambient CO_2. Bars are means ± SEM of eight replicates, with different letters indicating significant ($P < 0.05$) differences within a species

Discussion

Many studies have focused on the impact of increasing atmospheric CO_2 on plant metabolism and physiology, however relatively few studies have considered the impact of rhizosphere CO_2 concentrations, even though plant roots are almost certainly exposed to high CO_2 concentrations in the soil. Moreover, past studies are contradictory since some indicated benefits of enriching the roots with CO_2 (Gao *et al.*,1997; Cramer *et al.*,1999; Van der Merwe & Cramer, 2000; Viktor & Cramer, 2003, 2005; He *et al.*, 2007, 2010, 2016), while others showed no significant effect (Cramer *et al.*, 2001; Bouma *et al.*, 1997) and root zone CO_2 enrichment sometimes even limited growth (Cramer *et al.*, 2001, 2005; Boru *et al.*, 2003; X. Zhao *et al.*,2010; Li *et al.*, 2009) (Figure 2). This project investigated the physiological (e.g. growth and development, plant nutrition, plant hormone functions) and metabolic impacts of enriching the root zone with CO_2 concentrations between 700-2000 ppm on tomato, pepper and lettuce, and trying to understand the mechanisms involved.

Bicarbonate enrichment of hydroponic solutions (1 mM and 5 mM HCO_3^-) increased shoot growth of lettuce and pepper plants (Year 1 and 2 reports), as in hydroponically grown rice (Yang *et al.,* 1994) and tomato (Bialczyk *et al.,* 1994, 2005). The right proportions of bicarbonate (5 mM) and N (4 NO_3- : 1 NH_4^+) concentrations in the nutrient solution increase xylem sap concentrations of amides and amino acids, thereby supplying carbon skeletons to NH4+ incorporation and regulating the activity of some enzymes of ammonium metabolism (Bialczyk et al., 2004). However, bicarbonate addition (1 and 20 mM HCO_3^-) decreased shoot N percentage compared to control plants (Figure 4A), suggesting that growth promotion at 1 mM is not due to changes in nitrogen tissue concentrations. At 1 mM $NaHCO_3^-$, the decreases in nutrient concentration were likely a consequence of similar nutrient uptake but greater growth, with shoot weight increasing by 19%. Maintenance of nutrient uptake at 20 mM $NaHCO_3^-$ despite bicarbonate-induced growth inhibition largely accounts for Mg and B accumulation, while Fe accumulation likely reflects increased accumulation due to alkalisation of the root-zone.

Aeroponically grown lettuce did not show significant differences in nutrient concentrations in leaf tissue between RZ CO_2 treatments. High leaf tissue Ca concentrations have been observed when applying bicarbonate and in plants grown in calcareous soils at high pH (Alhendawi *et al.*, 1997, Al Mansouri *et al.,* 2014; Bialzcyk, 1994). Potato plants grown aeroponically under elevated RZ CO_2 (45 000ppm) had higher leaf Ca concentrations (Arteca *et al.,* 1979). The pH in the aeroponic nutrient solution was maintained at around 6 therefore its bicarbonate concentration should not have been high enough to produce the same effects on tissue Ca concentrations as bicarbonate enrichment. Possibly the roots took up carbon as CO_2 gas, with later conversion into bicarbonate in the plant affecting calcium metabolism.

Although Ca concentrations were higher in younger leaves of plants exposed to high RZ CO_2, it is unlikely this was due to Ca redistribution within the plants, as older leaves showed the same Ca concentrations between treatments (Figure 5). Importantly, lettuce tipburn was not observed on either control or RZ CO_2-enriched plants.

Elevated RZ CO_2 increased Zn concentrations in younger leaves (by 50%), but decreased Zn concentrations in older leaves (by 30%) compared to control plants, possibly due to intra-plant redistribution. Zn toxicity occurs when leaf concentrations reach 400–500 mg kg^{-1} of dry mass (Marschner, 1995; Broadley et al., 2007) and although leaf Zn concentrations were higher than expected (80–180 mg kg^{-1} – Figure 5), they are unlikely to inhibit growth.

Although roots were growing in a high CO_2 environment, surprisingly there were more changes in leaf phytohormone concentrations than changes in root phytohormone concentrations (Figure 8). RZ CO_2 enrichment did not change lettuce root phytohormone concentrations, but increased root SA concentrations of pepper while decreasing shoot SA concentrations. These opposing tissue-specific responses may reflect enhanced basipetal transport of SA via the phloem from shoots to roots, but this hypothesis can only be assessed by girdling (phloem removal) the stem (e.g. Castro et al. 2019).

Phytohormonal profiling revealed a solitary difference between aeroponically-grown lettuce plants grown under ambient and elevated rootzone CO_2: increased leaf JA concentrations under elevated RZ CO_2 (Figure 8). Since JA is usually regarded as a growth inhibitor (de Ollas et al. 2018), stimulation of lettuce growth under RZ CO_2 enrichment cannot be attributed to this hormonal difference. Nevertheless, since JA is involved in plant defence responses, the importance of these JA decreases should be investigated with factorial experiments imposing RZ CO_2 enrichment and pest/disease assays.

Although pepper plants grown aeroponically did not show any significant differences in biomass between treatments, phytohormonal profiling showed significant differences with leaf ACC concentrations being significantly higher under elevated RZ CO_2 and leaf tZ and SA concentrations lower compared to control treatment. These hormonal changes suggest that RZ CO_2 enrichment induces a long-distance stress-response in leaves, which will be investigated via transcriptomics (analyses pending). ACC is one of the most important intermediaries in ethylene biosynthesis, and its concentration increases in response to osmotic/ionic stress and other stresses (Albacete et al. 2008). Decreased foliar tZ concentrations occur when nitrogen is depleted in the plant root-zone (Rahayu *et al.* 2005), and may be induced in response to decreased shoot nitrogen concentrations. However, the magnitude of N depletion in lettuce leaves (< 5% - Figure 7) is unlikely to cause significant changes in foliar CK concentrations, suggesting that alternative explanations must be sought to explain the decreased tZ concentration. Decreased foliar SA concentrations in response to RZ CO_2 enrichment may reflect changes in root-shoot communication within the plant (as discussed above). Since high aerial CO_2 levels increase leaf SA levels as a defence strategy (DeLucia et al., 2012), the importance of these SA decreases should be investigated with factorial experiments imposing RZ CO_2 enrichment and pest/disease assays. Further experiments are needed to determine the physiological significance of these hormonal changes.

Since the experiments included in this report indicate that RZ CO_2 enrichment does not cause important changes in crop quality, any future cost-benefit analysis should consider the impacts on crop yields only. Data compiled from reports in previous years demonstrated significant benefits of RZ CO_2 enrichment in lettuce (Figure 9). Whether these yield benefits are commercially attractive depends on upscaling the studies within a commercial-scale facility.

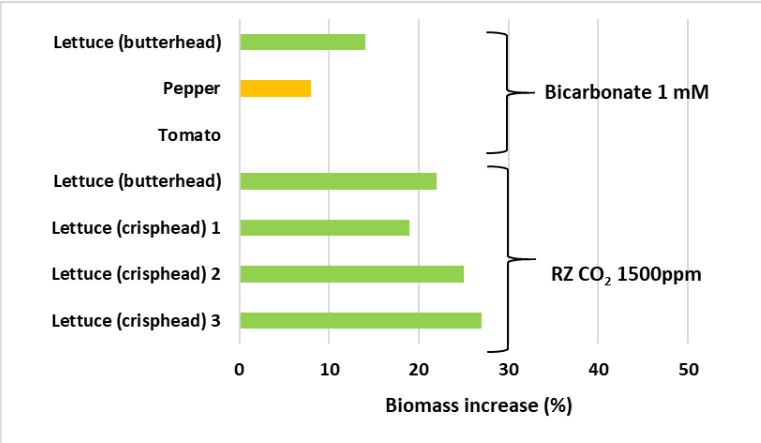

Figure 9. Biomass increase of lettuce, pepper and tomato grown hydroponically with 1 mM RZ bicarbonate and lettuce grown under elevated RZ CO_2 in this project. Different numbers in lettuce (Crisphead) bars, indicate experiment replication within the same variety of plants. Yield enhancement ranges from 8 to 27%

Conclusions

- Bicarbonate enrichment of hydroponics enhanced growth of lettuce and pepper by ~10% at low (< 5 mM) HCO_3^- concentrations
- Applying 1500 ppm root-zone CO_2 to aeroponically grown lettuce plants stimulated growth by 19-25%
- The uptake of DIC through the roots of lettuce plants was demonstrated using $NaH^{13}CO_3$
- 1 mM HCO_3^- did not significantly increase macronutrient and micronutrient concentrations, suggesting that growth promotion was not caused by altered plant nutrition
- Applying 1500 ppm RZ CO_2 to aeroponically grown lettuce did not alter endogenous nutrient concentrations although the difference in concentration between treatments in younger and older leaves can lead to different nutritious values when eating the heart of lettuce or the outer loose leaves
- Although RZ CO_2 enrichment causes variations in some phytohormone concentrations (ACC, tZ, JA and SA), they do not seem to be related to the increased growth of lettuce plants
- Although RZ CO_2 enrichment allows an additional lettuce crop per year (based on a 60 g head weight harvested product), the productivity gains do not appear to justify the additional expense

Knowledge and Technology Transfer

Publications:

Leibar-Porcel, E., McAinsh, M.R., Dodd, I.C. 2019. Root-zone CO_2 enrichment increases biomass accumulation in lettuce and pepper grown hydroponically and aeroponically. *Acta Horticulturae (accepted).*

Leibar-Porcel, E. and Dodd, I.C. 2019. Root-zone CO_2 and bicarbonate enrichment effects in nutrient leaf and root tissue concentrations. (In preparation).

Conferences:

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. The Great British Tomato Conference. Chesford Grange Hotel. 28-29th September 2016.

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. Plant & Crop Science Postgraduate Conference, Lancaster University, 4th October 2016.

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. AHDB Studentship Conference. Stratford Manor. 6-7th November 2017.

Leibar-Porcel, E. Root-zone CO_2 enrichment increases biomass accumulation in lettuce and pepper grown hydroponically and aeroponically. Oral presentation at the XXX. International Horticultural Congress. Istanbul, Turkey. 12-16th August 2018.

Posters:

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. LEC PGR Conference, Lancaster University, 21-22th April 2016.

Leibar-Porcel, E. Increasing crop yield and resource efficiency via root-zone CO_2 enrichment. *2nd* Agriculture and Climate Change Conference, Sitges, 26-28th March 2017.

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