

# *Feeding the world: impacts of elevated [CO<sub>2</sub>] on nutrient content of greenhouse grown fruit crops and options for future yield gains*

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## Review Article

# Feeding the world: impacts of elevated [CO<sub>2</sub>] on nutrient content of greenhouse grown fruit crops and options for future yield gains

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## Abstract

Several long-term studies have provided strong support demonstrating that growing crops under elevated [CO<sub>2</sub>] can increase photosynthesis and result in an increase in yield, flavour and nutritional content (including but not limited to Vitamins C, E and pro-vitamin A). In the case of tomato, increases in yield by as much as 80% are observed when plants are cultivated at 1000 ppm [CO<sub>2</sub>], which is consistent with current commercial greenhouse production methods in the tomato fruit industry. These results provide a clear demonstration of the potential for elevating [CO<sub>2</sub>] for improving yield and quality in greenhouse crops. The major focus of this review is to bring together 50 years of observations evaluating the impact of elevated [CO<sub>2</sub>] on fruit yield and fruit nutritional quality. In the final section, we consider the need to engineer improvements to photosynthesis and nitrogen assimilation to allow plants to take greater advantage of elevated CO<sub>2</sub> growth conditions.

## Introduction

Elevated [CO<sub>2</sub>] (e[CO<sub>2</sub>]) has been shown to significantly improved light saturated photosynthetic carbon assimilation rates (A<sub>sat</sub>) by increasing the efficiency of Rubisco CO<sub>2</sub> assimilation (carboxylation) over the alternate RuBP oxygenation (O<sub>2</sub> assimilation), which results in enhanced growth and yield [1, 2] (Figure 1).

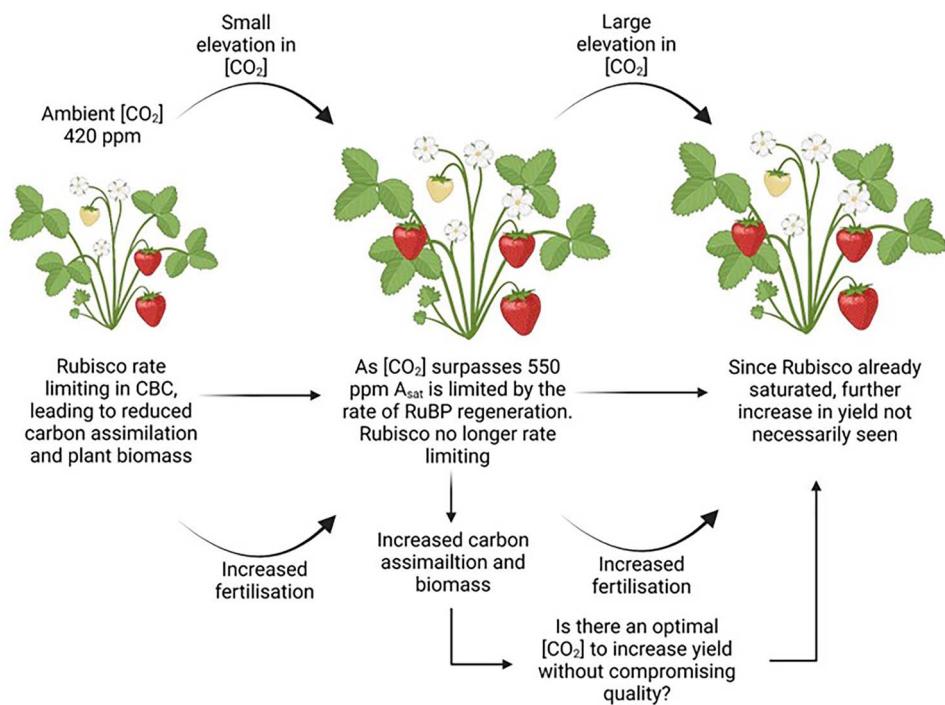
The majority of research evaluating the impact of e[CO<sub>2</sub>] on fruit crop production has been carried out in controlled environment conditions (chambers), polytunnels and commercial greenhouses where crops are grown in e[CO<sub>2</sub>], and focus almost exclusively on soft fruit such as strawberry, tomato and cucumber. Early work in the 1980's suggested that e[CO<sub>2</sub>] increased the average yield of all plants tested by approximately 30%, with optional [CO<sub>2</sub>] concentration for growth and yield in the range of 700 to 900 ppm with concentration in excess of 1000 ppm having a negative impact on plant growth and yield [3–6]. In the case of vegetable crops, much of the work has been carried out in controlled environments, in which elevated [CO<sub>2</sub>] (800–900 ppm) increased lettuce, carrot, and parsley yield by 18%, 19%, and 17%, respectively in greenhouse grown crops. However, the yields of leek, chinese cabbage and celery were not significantly affected by increases in growth [CO<sub>2</sub>] concentration [7]. A meta-analysis of 107 selected articles showed that e[CO<sub>2</sub>] results in an increase in vegetable number (yield) by on average 32% and vegetable mass by 11% [8]. Furthermore, a meta-analysis of 57

articles consisting of 1015 observations found that e[CO<sub>2</sub>] has both positive and negative impacts on vegetable quality. For example, whilst concentrations of fructose (+14.2%), glucose (+13.2%), total soluble sugar (+17.5%), total antioxidant capacity (+59.0%), total phenols (+8.9%), total flavonoids (45.5%), vitamin C (+9.5%), and calcium (+8.2%) increased in the edible part of vegetables, protein (−9.5%), nitrate (−18.0%), magnesium (−9.2%), iron (−16.0%), and zinc (−9.4%) decreased [9]. Moreover, a meta-analysis of legumes found a reduction in zinc and iron (and in non-legumes a reduction in protein) when plants were grown under e[CO<sub>2</sub>] (see Myers et al [10]). In 2018, Zhu et al [11] confirmed these results, and moreover demonstrated that rice grown under e[CO<sub>2</sub>] showed consistent declines in the quantities of vitamins B1, B2, B5, and B9 and, an increase in vitamin E. Finally, studies have shown that grains (wheat, rice, and barley), legumes, and maize-have a 4–10% reduction in iron concentrations of when grown under e[CO<sub>2</sub>] (~550 ppm) [12]. These results shown that e[CO<sub>2</sub>] can positively and negatively impact on legumes, grain and vegetables on a crop-by-crop basis and simultaneously alter quality attributes in the same harvestable material.

The aim of this review is to provide an overview of the current available data of the impact of elevated [CO<sub>2</sub>] on fruiting crops production in commercial growing systems. This paper examines these studies and the long-term implications of e[CO<sub>2</sub>] on the yield and quality of fruit required to feed a growing population.

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**Figure 1.** Schematic representation of elevated  $[CO_2]$  on carbon assimilation. Created with [BioRender.com](https://biorender.com)

In the last section, we discuss the potential for designing crops for these new growing environments and allowing them to take full advantage of the introduced  $CO_2$ , potentially increasing crop yield, reducing costs for commercial producers, and improving quality of the final product providing high nutritional value to consumers.

## Impact of elevated $[CO_2]$ on yield and quality of GREENHOSUE grown crops

### Impact of elevated $[CO_2]$ on solanaceous crops

Commercially, tomato crops are grown in greenhouses with  $e[CO_2]$ , in some cases as high as 2000 ppm. The effects of  $e[CO_2]$  of fruit yield and quality has been extensively studied (Figure 2). Under  $e[CO_2]$ , tomato fruit yield increases ranged from 7% – 125% with  $[CO_2]$  ranged from 450 ppm – 1200 ppm compared with plants grown under  $a[CO_2]$ . An increase in the quantity of non-reducing sugars (glucose and fructose) has been reported [13–17] and fully ripe tomatoes grown in an  $e[CO_2]$  were found to be preferable for consumption in sensory panels [13]. As liking sweetness has been shown to be a universal trait [18], it is possible that this increase in sugar is responsible for preference of the carbon enriched tomato fruits. An increase in vitamin C was also found between most studies [13, 15, 16, 19], potentially improving the health benefit gains from consumption of carbon-enriched grown tomatoes (Table 1). Vitamin C is an important dietary requirement and at high concentrations it has been used as a treatment for cancer, arteriosclerosis, and cardiovascular diseases [20–22]. These results suggest that increasing environmental  $[CO_2]$  could contribute to an increase in Vitamin C improving their nutritional value for the consumer. However, growth at  $e[CO_2]$  does not have the same impact on all species, as another studies in barley reported a significant decrease in Vitamin C content [23] highlighting the species–species response differences to  $e[CO_2]$  and suggesting that high carbon growth environments may not always provide the best outcome for the consumer even though

increases in yield maybe the producers primary concern (see Fenech et al. [24] and references therein).

Similarly, tomato fruit concentration of lycopene and  $\beta$ -carotene (pro-vitamin A) were found to increase in response to  $e[CO_2]$  by as much as 30% and 70% respectively [13]. Rangaswamy et al. [25] reported an increase in carotenoid (+20%) and lycopene (+31%) in the fruits of tomato plants grown at 550 ppm  $[CO_2]$ , however carotenoid content decreased (– 12%) when the concentration was increased to 700 ppm, suggesting that the level of  $CO_2$  enrichment impacts fruit quality and careful consideration is needed to ensure an appropriate balance between levels of  $e[CO_2]$  and final yield. Lycopene is an important phytonutrient, is sold commercially as a dietary supplement, and has been reported to possess anti-cancer properties and can improve cardiovascular health [26, 27].

$\beta$ -carotene is the precursor for Vitamin A, also known as retinol. Vitamin A is an essential micronutrient playing important roles in growth and development, vision [28] and the immune system [29]. More than a third of all pre-school children and a significant number of pregnant women around the world are affected by Vitamin A deficiency, increasing the risk of night blindness and miscarriage [30, 31]. Importantly, most people suffering from a deficiency in Vitamin A show no clinical symptoms resulting in a phenomenon termed “Hidden Hunger” [32]. Production of crops with increased Vitamin A is therefore an important target for improving the diet and health of these at-risk groups; enhanced uptake of carbon may be a useful approach to achieve this. Increases in the Vitamin A precursor  $\beta$ -carotene has been observed in tomato fruit grown under  $e[CO_2]$  of 800–900 ppm, in addition to a 28% increase in vitamin C at ripe stage and an ~8% increase in total soluble solids (Table 1). Zhang et al. [13], suggested that under these growth conditions, improved vitamin A and C and increased carotenoid content may be attainable.

Carotenoids are also the precursors of several flavour and aroma compounds.  $\beta$ -carotene is cleaved by carotenoid cleavage dioxygenases CCD1 and CCD4 [26, 33–35], to form the

Fruit	Effects of elevated $[CO_2]$ on fruit yield and quality
	<ul style="list-style-type: none"> <li>- 7% to 125% increase in fruit yield across all treatments (<math>e[CO_2] = 450\text{ppm} - 1200\text{ppm}</math>)</li> <li>- Greater organoleptic preference.</li> <li>- 16 to 44 % increase in vitamin C (<math>e[CO_2] &lt; 900 \text{ ppm}</math>), 20 to 25% decrease in vitamin C (<math>e[CO_2] = 1000 \text{ ppm}</math>).</li> <li>- 10 to 22% increase in total major sugars (glucose, sucrose, fructose) given sufficient N fertilisation and irrigation across and range of <math>CO_2</math> treatments (<math>e[CO_2] = 550-1000 \text{ ppm}</math>).</li> <li>- Large variation in reported total organic acid content between studies with no detectable trend related to degree of <math>CO_2</math> fertilisation.</li> <li>- 32% increase in lycopene at <math>e[CO_2] = 550 \text{ ppm}</math>, however 3.7 to 25% decrease in lycopene at <math>e[CO_2] = 700 \text{ ppm}</math>.</li> </ul> <p><i>Overall, <math>e[CO_2]</math> of 550 ppm seems optimal for simultaneous increases in yield and quality parameters in tomato</i></p>
	<ul style="list-style-type: none"> <li>- 12.9 % increase in fruit yield 350 ppm and 47.4 % increase in fruit yield 450 ppm.</li> <li>- 18.9 % to 26.6 % increase in yield at 400 and 800 ppm respectively in absence of other treatment.</li> <li>- Yield with irrigation with no significant difference in fruit yield at lowest irrigation.</li> <li>- Little significant effect of increased <math>[CO_2]</math> on fruit inorganic nutrients or colour increased with irrigation and carbon dioxide with a maximum yield increase with both treatments of 264 %.</li> <li>- Fruit yield for <math>e[CO_2]</math> increased</li> </ul> <p><i>Overall, <math>e[CO_2]</math> increases yield, however little work has been carried out on the impacts of <math>e[CO_2]</math> of quality. Increases in yield variable and not observed to be dose dependent.</i></p>
	<ul style="list-style-type: none"> <li>- 53.8 % increase in fruit number per plant at 550ppm <math>e[CO_2]</math>.</li> <li>- Up to 142% increase in fruit yield. 47% – 113% increase in total fruit yield per plant across all <math>CO_2</math> treatments with natural lighting.</li> <li>- Up to ~15% increase in total fruit sugars.</li> <li>- 28% to 61% increase in capsaicin with 2% – 10% decrease in soluble sugars and 13% – 34% decrease in vitamin C in same plants</li> </ul> <p><i>Overall, <math>e[CO_2]</math> increases fruit yield and fruit weight and size. However, increases in yield was variable and accompanied by changes in secondary metabolites (i.e increases in capsaicinoids and decrease in soluble sugars and vitamins).</i></p>
	<ul style="list-style-type: none"> <li>- 1 to 62 % increase in fruit yields across all <math>CO_2</math> treatments (450 ppm – 3000 ppm)</li> <li>- 7 to 35% increase in major sugars (glucose, fructose, sucrose) for <math>e[CO_2] = 650 - 950 \text{ ppm}</math>.</li> <li>- Large variation in effects on total anthocyanin, phenolic, flavonoid and antioxidant content between studies, however 10.2 % increase vitamin C reported at <math>e[CO_2] 650 \text{ ppm}</math>, 13.3% increase reported at <math>e[CO_2] = 950 \text{ ppm}</math>.</li> <li>- Total acid content was reduced with increasing <math>[CO_2]</math>, reducing by ~10% for every 300 ppm increase in <math>e[CO_2]</math>.</li> <li>- Key aroma constituents (esters, furaneol, linalool), increases stepwise with increasing <math>[CO_2]</math> for the conc. tested (350, 650, 950ppm).</li> </ul> <p><i>No specific degree of <math>CO_2</math> fertilisation can be determined as being optimal since the effects of <math>e[CO_2] &gt; 950 \text{ ppm}</math> on fruit quality have not been tested. Greater <math>CO_2</math> fertilisation appears to enhance both yield and quality</i></p>
	<ul style="list-style-type: none"> <li>- <math>CO_2</math> fertilisation (400 ppm – 3000 ppm) sufficient to elevate fresh yields by 14 to 37% with little correlation between yield and degree of fertilisation.</li> <li>- In high nitrogen soil, fresh fruit yield increases of 73% were reported for <math>e[CO_2] = 1200 \text{ ppm}</math>, with greater fruit biomass also reported. This indicates that nitrogen may be a limiting factor on how well cucumber can utilise <math>CO_2</math> fertilisation.</li> <li>- For <math>e[CO_2] = 1200 \text{ ppm}</math> and moderate nitrogen fertilisation, a 73 to 75% increase in fruit fructose and glucose was observed. This was not observed at lower <math>e[CO_2]</math> or at higher N.</li> </ul> <p><i>Optimal concentrations of <math>e[CO_2]</math> and N fertilisation required for optimal cucumber yield and quality</i></p>

**Figure 2.** Effects of elevated  $[CO_2]$  on yield and quality of fruiting crops. Created with [BioRender.com](https://biorender.com)

aromatic apocarotenoid  $\beta$ -ionone, which is important to tomato fruit flavour. Furthermore, lycopene, shown to increase under  $e[CO_2]$  is cleaved by CCD1 to form several important flavour and aroma compounds including 6,10-dimethyl-3,5,9-undecatrien-2-one (pseudoionone [34]), 6-methyl-5-hepten-2-one (MHO [36]; and geranal [37]. MHO has been shown to be an important contributor to tomato fruit flavour [38, 39] and has also been shown to accumulate in tomato fruit with higher lycopene levels [40]. It is therefore apparent that growth in  $e[CO_2]$  can increase a range of key flavour and nutraceutical precursor compounds present in tomato fruit; this phenomenon deserves further study, the optimal levels of  $[CO_2]$  are currently not clear and more work is needed to better understand the relationship between  $CO_2$  assimilation carotenoid content, flavour and overall quality (Table 2).

Similar results have also been found in pepper crops, with yield increase of 12.9% – 370.2% reported when grown at  $e[CO_2]$  of between 450 ppm – 1000 ppm (Table 2) with most other studies reporting yield increases in the range of 12.9% – 47.4% in the absence of other parameters [41–47]. However, it should be noted that growth at ~800 ppm  $e[CO_2]$  was found to reduce sweet pepper total amino acid content by up to 29%, including reductions in the sweet tasting amino acids alanine and glycine, which could be detrimental to the perceived fruit flavour [42]. Yield was also found to vary with different irrigation programmes [41, 48],

nitrogen sources [48], substrate salinity [42, 44] and pruning regimens [46]. Given that previous work in tomato has shown an increase in potential phytonutrients in fruit grown at 550 ppm and a decrease in those grown at 700 ppm, further research is needed to better identify the specific quantity of  $CO_2$  fertilisation necessary for maximally improved yield in solanaceous crops, especially when considering that  $CO_2$  uplift is often accompanied by additional treatments, such as increased nutrient and nitrogen fertilisation (Figure 2).

In chili pepper, yield increases of 43.8% – 142% were reported for  $e[CO_2]$  (in the range of 500 ppm – 1140 ppm). These yield increases were in part attributed to an increase in the size of fruits [49]. However, in controlled environments a 4°C increase in temperature decreased yield, even at  $e[CO_2]$  (750 ppm) [50, 51], indicating that carbon enrichment is not sufficient to rescue yield where glasshouse facilities or growth tunnels experience periods of elevated temperature in an extreme climate change scenario. Carbon-enriched growth was found to increase the capsaicinoid content of fruits, resulting in an increase in Scoville Heat Units (SHU) [49, 52]. This approach therefore has potential for producing hotter varieties of chili, a growing and competitive market. However, at the same time Vitamin C concentration decrease by up to 15.84% [53], reducing potential health benefits gained from growing chili plants under  $e[CO_2]$ . (Table 2).

**Table 1.** Impact of elevated atmospheric  $[CO_2]$  on yield and nutritional quality of tomato

<b>CO<sub>2</sub> Treatment</b>	<b>Additional Treatment(s)</b>	<b>Fruit Yield</b>	<b>Fruit Quality</b>	<b>Ref</b>
510 ppm	N/A	9.9% increase in fruit yield.	N/A	[45]
590 ppm	root drying	Fruit dry weight not significantly affected by $[CO_2]$ across all irrigation treatments.	N/A	[155]
375 ppm – 675 ppm	Ozone treatment 80 nmol mol <sup>-1</sup>	24% increase in fruit yield. 31% decrease in fruit yield when exposed to ozone. Ozone and $CO_2$ treated fruit yields were not significantly different to plants grown in ambient conditions.	N/A	[156]
550 ppm 700 ppm	N/A	54% increase in fruit yield at 550 ppm and 125% increase in fruit yield at 700 ppm.	1.4% – 11.4% decrease in total soluble solids, 27.3% – 31.8% decrease in total acids and 16.1% – 29.0% increase in vitamin C.	[19]
650 ppm 1000 ppm	N/A	+2 °C increase in temperature 18.4% – 21.4% increase in fruit yield due to increased $[CO_2]$ .	10% increase in total sugars, 44% increase in vitamin C, 32% increase in lycopene at $e[CO_2]$ in absence of other treatments. $e[CO_2]$ rescues reduction in quality from increased temperature.	[25]
700 ppm	Doubled N fertilisation	17% increase in fruit yield at 650 ppm and 48% increase in fruit yield at 1000 ppm.	13% – 25% decrease in fruit lycopene content across harvests with $e[CO_2]$ . 9% increase in fruit lycopene content with increased N fertilisation.	[3]
UV-B exposure up to 1,744 kJ m <sup>-2</sup>		38% increase in fruit yield in absence of additional UV-B treatment, up to 46% increase in fruit yield with UV-B treatment.	Up to ~22% increase in soluble sugars, ~24% increase in organic acids, ~40 increase in vitamin C and ~47% increase in lycopene content of fruits grown under $e[CO_2]$ and UV-B treatment.	[15]
700 ppm 900 ppm	N/A	~30% increase in individual fruit weight.	~18% increase in vitamin C. ~Up to 20% reduction in major acids (citric, malic, oxalic). ~45% increase in sugars (glucose, fructose).	[16]
700 ppm 1000 ppm	N/A	32% increase in marketable fruit yield.	N/A	[158]
800 ppm	0–0.5 g N kg <sup>-1</sup> soil. Soil water content 25% – 35%	Across all treatments, –3.3% – 28% increase in total fruit yield.	–17.9% – 11.9% increase in total fruit sugars and –18.9% – 12.7% increase in total fruit acids across all treatments.	[159]
	Salinity treatments at 5–7 ds m <sup>-1</sup>	13% increase in yield in carbon-enriched atmosphere and 31% reduction in marketable fruit yield in increased salinity.	7% increase in total soluble solids. No significant change in citric acid content. Organoleptic qualities of tomatoes grown under increased salinity and $CO_2$ found preferable in sensory trials.	[14]
	100 or 200 mg N kg <sup>-1</sup> soil, 70% irrigation of control and root drying	8% increase in fresh fruit yield with increased $[CO_2]$ .	No significant difference in total sugars, organic acid or fruit firmness for fruits grown in $e[CO_2]$ .	[160]

(Continued)

Table 1. Continued

CO <sub>2</sub> Treatment	Additional Treatment(s)	Fruit Yield	Fruit Quality	Ref
800 ppm – 900 ppm	N/A	N/A	~28% increase in vitamin C at ripe stage, ~8% increase in total soluble solids and no difference in total acids. Marked preference in sensory trials for fruits grown under enriched [CO <sub>2</sub> ].	[13]
900 ppm	N/A	30% increase in marketable fruit yield.	N/A	[161]
	100 $\mu\text{mol s}^{-1} \text{m}^{-2}$ supp lighting	12% – 15% increase in yield under supp lighting, 7% increase in yield in absence of additional treatment	N/A	[162]
1000 ppm	N/A	22% increase in total fruit yield for plants grown in e[CO <sub>2</sub> ].	N/A	[163]
	N/A	30% increase in total fresh fruit yield per plant.	N/A	[164]
		43% increase in total fruit yield.	No significant effect on fruit quality parameters.	[165]
		74.3% – 83.6% increase in tomato fresh weight per plant.	16.1% – 20.9% increase in total sugars, 20.0% – 24.7% decrease in vitamin C, 4.79% – 6.8% decrease in total acids.	[17]
1200 ppm	Salinity up to 4.58 x control	> 40% loss in dry fruit yield at highest salinity treatment completely offset by increased [CO <sub>2</sub> ].	Increased salinity and [CO <sub>2</sub> ] combined increases total sugar and acid content by up to ~30%.	[166]

These reports suggest that the effects of growing crops in e[CO<sub>2</sub>] can have both a positive influence on yield and nutritional quality, however, growth at [CO<sub>2</sub>] levels above what is optimum can negatively impact some quality traits.

### Impact of elevated [CO<sub>2</sub>] on rosaceous crops

Rosaceous crop research in this area has focused primarily on cultivated strawberry with a small number of studies on raspberry and Nashi pear (Table 3). This is likely due to the relatively smaller size and rapid growth of strawberry compared to other commercially important rosaceous fruit species, such as tree fruits, like apple and cherry, and woody stemmed shrub fruits, like raspberry and blackberry. This makes strawberry a convenient plant to study as a rosaceous model. Furthermore, greater production of strawberry fruits would not only increase profits for growers but also decrease costs for consumers, increasing the availability of healthier options. Better access to such products through economic growth is strongly correlated to reduced micronutrient malnutrition or “hidden hunger” [54].

In cultivated strawberry, fresh fruit yield increases ranged from 1.0% – 62.0% in plants grown under atmospheric e[CO<sub>2</sub>] of 450 ppm – 3000 ppm, while dry fruit yield increased by up to 120% (Figure 2; Table 3). This has been directly linked to a 73% increase in assimilation rate of CO<sub>2</sub> in strawberry leaves at optimal e[CO<sub>2</sub>] of 600 ppm [55–60]. Further investigation at a genetic level (through RNA seq analysis) revealed that 150 genes were upregulated in strawberry plants grown in an enriched-carbon atmosphere, with 14 of these being photosynthetic genes [60], suggesting that plants respond to these atmospheric increases by increasing their ability to assimilate the excess carbon.

Additional annual yield increases could be achieved by a two-week reduction in time to fruiting for plants grown in an enriched-carbon atmosphere [58, 61] increasing the field season and the period of productive (fruit) growth. Several fruit quality traits are also improved by growth at e[CO<sub>2</sub>]; increases in reducing sugars, and therefore sweetness index, were reported [62, 63] alongside reductions in organic acids [62]. These increases in sugar-acid ratio is highly favourable for a more pleasant perception of strawberry flavour by the consumer [18] and an increase in key volatile organic compounds, including furaneol, linalool and major esters, was also reported, further enhancing the “strawberry” aroma [62]. Growth in a carbon-enriched atmosphere therefore strongly enhances strawberry flavour and increases vitamin C (an important nutritional compound) by up to 13.3% alongside other antioxidant compounds [64, 65]. Growth in carbon-enriched atmospheres therefore simultaneously improves yield, flavour and health benefits of strawberry fruits, creating enormous potential for strategies involving enhanced photosynthesis of strawberry plants, including genetic manipulation. The greatest reported increase in fresh fruit yield where obtained when [CO<sub>2</sub>] was kept between 600 ppm – 1000 ppm [58], linking greater carbon assimilation to increased fresh fruit yield in strawberry and demonstrating an optimal degree of CO<sub>2</sub> fertilisation for strawberries (Table 3).

### Impact of elevated [CO<sub>2</sub>] on cucurbitaceous crops

Cucumber is the most studied fruit crop of the cucurbitaceae in relation to growth in carbon-enriched atmospheres (Figure 2; Table 4). Improved carbon assimilation rates of up to 99% and 112% have been reported for cucumber and melon respectively when grown in e[CO<sub>2</sub>] [66, 67], demonstrating that growth in e[CO<sub>2</sub>] improves photosynthesis of cucurbitaceous crops.

**Table 2.** Impact of elevated atmospheric  $[CO_2]$  on yield and nutritional quality of other Solanaceous crops

Crop	$CO_2$ Treatment	Additional Treatment(s)	Fruit Yield	Fruit Quality	Ref
Sweet Pepper	350 ppm	N/A	12.9% increase in fruit yield 350 ppm and 47.4% increase in fruit yield 450 ppm.	N/A	[45]
	450 ppm	20 mmol L <sup>-1</sup> NaCl, foliar calcium treatment	18.9% to 26.6% increase in yield at 400 and 800 ppm respectively. Foliar calcium treatment had no impact on yield. e $[CO_2]$ rescued total yield loss from high salinity.	Little significant effect of increased $[CO_2]$ on fruit inorganic nutrients or colour.	[42]
700 ppm	High/low irrigation and N treatments	Fruit yield for e $[CO_2]$ increased with irrigation, with no significant difference in fruit yield at lowest irrigation.	N/A	N/A	[48]
	700 ppm – 750 ppm	N/A	18% – 22% increase in total fruit yield.	N/A	[46]
800 ppm	Nitrogen source and saline treatment (8 and 25 mM NaCl)	8% and 22% increase in marketable fruit yield under salinity stress and unstressed respectively. 23% and 29% maximum increase in daily fruit harvest yield for 2 different nitrogen sources at low salinity.	N/A	N/A	[43,44,168]
	900 ppm	N/A	7% increase in early yielding fruits, no change in total fruit yield.	N/A	[162]
367 ppm – 1000 ppm	Range of irrigation regimens	Yield increased with irrigation and carbon dioxide with a maximum yield increase with both treatments of 264%.	N/A	N/A	[41]
	1000 ppm	N/A	51% – 370% increase in fruit weight per plant.	N/A	[169]
10 000 ppm	N/A	20% increase in fruit yield.	N/A	N/A	[61]
	380 ppm – 750 ppm	+2°C and +4°C temperature elevation	Up to 41.9% increase in fruit diameter under both increased carbon dioxide and increased temperature.	27% – 44% increase in capsaicin across all treatments for 2 cultivars across 2 growth years.	[52]
Chili pepper	380 ppm – 750 ppm	+2°C and +4°C temperature elevation	53.8% increase in fruit number at $[CO_2]$ = 550 ppm and ambient +2°C temperature, 12.3% decrease in fruit number per plant for $[CO_2]$ = 750 ppm and ambient +4°C temperature. Up to ~140% increase in fruit yield per plant for $[CO_2]$ = 550 ppm and ambient +2°C temperature, up to ~36% reduction in fruit yield per plant for $[CO_2]$ = 750 ppm and ambient +4°C temperature.	N/A	[50,51]
	380 ppm – 1140 ppm	N/A	Up to 88.5% increase in number of fruits per plant, up to 13.0% increase in fruit length, up to 15.0% increase in fruit width and up to 14.3% increase in pericarp thickness. Up to 142% increase in fruit yield.	No change in colour of ripe fruits. Up to 28.6% increase in capsaicinoids in ripe fruit.	[49,170]
400 ppm – 900 ppm	Natural light (23 $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> ) and supplementary light (463 $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )		92% – 113% increase in total fruit yield per plant across all $CO_2$ treatments with supplementary lighting relative to ambient control at 400 ppm. 47% – 113% increase in total fruit yield per plant across all $CO_2$ treatments with natural lighting relative to ambient control at 400 ppm.	2% – 10% decrease in soluble sugars. 13% – 34% decrease in vitamin C in e $[CO_2]$ . 61% increase in capsaicin at $[CO_2]$ = 550 ppm, 45% – 61% decrease in capsaicin for $[CO_2]$ > 550 ppm.	[171]

Table 2. Continued

Crop	CO <sub>2</sub> Treatment	Additional Treatment(s)	Fruit Yield	Fruit Quality	Ref
Eggplant	1000 ppm	N/A	43.80% – 59.55% increase in fruit fresh weight per plant across 5 cultivars.	Up to ~15% increase in total fruit sugars. 11.84% – 15.84% decrease in fruit vitamin C, non-significant decrease in fruit titratable acids. Variable effects on inorganic nutrient concentrations. Fruit amino acids and fatty acids mostly reduced.	[53]
	200 ppm – 3000 ppm	N/A	209% increase in fruit fresh weight and 134% increase in fruit dry weight.	N/A	[172]
	1000 ppm	N/A	31% increase in fruit yield across a full year of harvests.	N/A	[169]
	663 ppm	N/A	23.6% increase in fruit yield.	N/A	[45]

In cucumber (*Cucumis sativus*), fruit yield increases for plants grown in enriched-carbon atmospheres ([CO<sub>2</sub>] = 450 ppm – 3000 ppm) ranged between 16.2% and 41% in the absence of other parameters that could alter fruit yield. In high nitrogen supplemented fertilisation, fruit yield was as high as 106% when grown under e[CO<sub>2</sub>] of 800 ppm [68], indicating the potential of increased nitrogen fertilisation alongside [CO<sub>2</sub>] enrichment to unlock the greatest yield increases in cucumber. Interestingly, when grown under e[CO<sub>2</sub>] of 1200 ppm with the addition of high nitrogen fertilisation treatment, studies found a yield increase between 71% – 73% [66, 68], which was lower than the 106% for plants grown at e[CO<sub>2</sub>] of 800 ppm. Concentrations of [CO<sub>2</sub>] above optimal reduced stomatal density, stomatal conductance (gs), the maximum carboxylation rate (V<sub>cmax</sub>) and the maximum photosynthetic electron transport rate (J<sub>max</sub>) [69]. This suggests that an optimal concentration of atmospheric [CO<sub>2</sub>] exists for maximum yield returns and deserves further investigation. There is large variation between studies on how cucumber fruit quality is impacted by carbon-enriched growth. Fructose and glucose were reported to increase by 6% and 12% in one study [68] and by 75% and 73% respectively in another [70]. The inorganic nutrient content of fruits was also reported to decrease in fruits grown in e[CO<sub>2</sub>], however only phosphorus showed a significant reduction in multiple cycles [71]. These data do suggest that e[CO<sub>2</sub>] may enhance fruit flavour and fruit yield at the expense of nutritional value.

### Impact of elevated [CO<sub>2</sub>] on yield and quality of fruiting trees

Sweet clonal cherry (*Prunus avium* L.) plants were grown for 19 months in climate-controlled greenhouses at ambient (1994–358 ppm; 1995–360 ppm) or e[CO<sub>2</sub>] (700 ppm). Elevated [CO<sub>2</sub>] treatment increased photosynthesis and dry matter production, leaf (55%) and stem (61%), after two months at 700 ppm, however, this initial stimulation is not sustained. Photosynthetic rates were less after 10 months of growth than after 2 months of growth, and only small increases in dry mass are still evident after 10-months, suggesting that sweet cherry acclimates to e[CO<sub>2</sub>] due to long-term exposure [72]. Due to the young nature of plants studied compared with fully grown mature trees (deciduous tree 15–32 m in height and with a trunk up to 1.5 m in circumference [73, 74], no information is available to determine the impacts of e[CO<sub>2</sub>] on fruit yield or quality. In Nashi pear, a CO<sub>2</sub>-enriched atmosphere of 700 ppm increased fruit weight, diameter and length along with a 22.5% increase in Brix, (a key measure of sweetness for marketable fruit [75]). However, this also resulted in a reduction in fruit firmness demonstrating that improvements in yield can be nullified by negative impacts on fruit quality (Table 3).

While these studies are limited in, they do indicate the potential of CO<sub>2</sub>-enriched growth for improving photosynthesis, increasing yield and quality of tree crops. However, they also suggest that some crops, especially perennial crops, may become acclimated to higher [CO<sub>2</sub>] and any gains may be lost over time.

### Does increasing carbon assimilation increase environmental tolerances?

The work presented above also suggest that increasing CO<sub>2</sub> uptake could have other benefits. It is notable that growth of fruit crops in carbon enriched atmospheres has a similar effect of protecting against environmental stresses, such as drought and elevated temperature, that may become increasingly common due to climate change as plants genetically engineered to increase carbon assimilation. For example, in melon (*Cucumis melo*), growing plants

Table 3. Impact of elevated atmospheric  $[CO_2]$  on yield and nutritional quality of Rosaceous crops

Crop	$CO_2$ Treatment	Additional Treatment(s)	Fruit Yield	Fruit Quality		Ref
				42% increase in fresh fruit yield in $e[CO_2]$ at high N, 17% increase in fresh fruit yield in $e[CO_2]$ at low N.	N/A	
Strawberry	553 ppm	Nitrate treatment (4 x 10 <sup>-2</sup> - 0 mM)				[173]
	400 ppm, 650 ppm and 900 ppm	Ambient temperature (25°C) and elevated (30°C)	9.9% – 33.4% increase in total fruit yield at ambient temperature for cultivar "Albion", 0.9% – 31.2% decrease in total fruit yield at ambient temperature for cultivar "San Andreas". Elevated $[CO_2]$ rescues yield loss from elevated temperature.	Total fruit polyphenolic content, flavonoid content, monomeric anthocyanin content and antioxidant content increased in correlation with $e[CO_2]$ at both temperatures for multiple cultivars (~9% – ~325% increase overall increase at $[CO_2]$ = 900 ppm).	48%, 21%, 36% and 18% decrease in fruit anthocyanin content, total phenolic content, total flavonoid content and total antioxidant content respectively at $e[CO_2]$ : 29% and 35% increase in fruit fructose and glucose respectively. 43% increase in total sugars.	[56] [65]
	720 ppm	5°C increase in temperature, nitrate treatment (50 mL 0.1% $NH_4NO_3$ twice per week)	~120% increase in total fruit dry weight in $e[CO_2]$ , ~73% increase in total fruit dry weight in $e[CO_2]$ with nitrogen treatment. No significant change in fruit yield for all other treatments individually and in combination.			[63]
	600 ppm – 1000 ppm	N/A	62% increase in total fruit yield in $e[CO_2]$ .	N/A		[58]
	700 ppm – 1000 ppm	N/A	17.6% and 38.5% increase in individual fruit weight at $[CO_2]$ = ambient +300 ppm and $[CO_2]$ = ambient +600 ppm respectively.	7.0% – 25.9% increase in glucose, fructose and sucrose. 5.2% – 47.4% decrease in citric, malic and quinic acids. Stepwise increase in concentration of most key volatile esters and up to 115.0% and 149.6% increase in fruit myrcene and linalool content.		[62]
	700 ppm – 1000 ppm	N/A		13.3% increase in fruit ascorbic acid. Stepwise increase in antioxidant and flavonoid compounds with increasing carbon dioxide.		[64]
	700 ppm – 1000 ppm	N/A	5.4% and 12.7% increase in marketable fruit yield for cultivars "Irvine" and "Handler" respectively.	N/A		[158]
	100 ppm	N/A	47% increase in fruit number per plant, no significant change in individual fruit weight.	N/A		[55]
	200 ppm, 300 ppm, 350 ppm, 3000 ppm	N/A	31%, 43% and 51% increase in fruit yield at 900 ppm, 1500 ppm and 3000 ppm respectively.	N/A		[61]
Raspberry	436 ppm	N/A	12% increase in total berry yield and 5% increase in average individual berry weight.	N/A		[174]
Nashi Pear	700 ppm	Ambient +4°C temperature	16.6% increase in fruit weight with $e[CO_2]$ . Elevated $[CO_2]$ reduces yield loss from increased temperature.	Up to 15% reduction in fruit firmness with $e[CO_2]$ . Up to 22.5% increase in total soluble solids with no significant change in acidity with $e[CO_2]$ .		[75]

**Table 4.** Impact of elevated atmospheric  $[CO_2]$  on yield and nutritional quality of Cucurbitaceus crops

Crop	$CO_2$ Treatment	Additional Treatment(s)	Fruit Yield	Fruit Quality	Ref
Cucumber	400 ppm, 625 ppm, 1200 ppm	2 mmol $L^{-1}$ , 7 mmol $L^{-1}$ , 14 mmol $L^{-1}$ $NO_3^-$	Up to 73% increase in fresh fruit yield for plants grown at highest $[CO_2]$ versus plants grown at lowest $[CO_2]$ at greatest N fertilisation. No significant difference in yield for lower N fertilisation.	75% increase in fruit fructose, 73% increase in glucose at 7 mmol $L^{-1}$ at highest $[CO_2]$ . No significant change in fruit titratable acidity. $\epsilon[CO_2]$ reduced dietary fibre by 13% – 18% across all fertilisation treatments. Up to 84% reduction in fruit nitrogenous compounds in $\epsilon[CO_2]$ across all nitrogen treatments.	[66], [68]
	400 ppm, 800 ppm, 1200 ppm	0.06 g $N kg^{-1}$ soil (low N), 0.24 g $N kg^{-1}$ soil (high N)	31% – 37% increase in fresh fruit yield for $[CO_2]$ = 800 ppm and 1200 ppm at low N. 71% – 108% increase in fresh fruit yield for $[CO_2]$ = 800 ppm and 1200 ppm at high N	Across both nitrogen treatments at $[CO_2]$ = 1200 ppm, fruit fructose was increased by 5% – 6%, fruit glucose was increased by 10% – 12% and starch was increased by 29% – 40%.	[70]
	364 ppm, 620 ppm	N/A	Up to 10.2% increase in individual fruit weight for August production in $\epsilon[CO_2]$	No significant change in fruit dry matter content	[45]
	400–500 ppm	N/A	19% increase in fresh fruit yield at $\epsilon[CO_2]$	N/A	[175]
	600–700 ppm	N/A	20% increase in fresh fruit yield at $\epsilon[CO_2]$	N/A	[176]
	700 ppm	N/A	14.2% – 18.4% increase in fresh fruit yield at $\epsilon[CO_2]$ across two crop cycles.	Overall reduction in fruit inorganic nutrients (N, P, K, Ca, Mg).	[71]
	780 ppm	N/A	35% increase in fresh fruit yield in greenhouse supplemented with $[CO_2]$ versus control greenhouse.	N/A	[177]
	700 ppm – 1000 ppm	N/A	20% – 30% increase in marketable fruit yield across two growing seasons.	N/A	[158]
	900–1000 ppm	0.6°C – 1.8°C cooling	35.4% increase in dry fruit mass in cooled and $\epsilon[CO_2]$ conditions	N/A	[178]
	1000 ppm	N/A	8.9% increase in fruit weight but no significant change in fruit number at $\epsilon[CO_2]$	N/A	[179]
	900 ppm, 1500 ppm, 3500 ppm	N/A	18.4% – 26.3% increase in fresh fruit yield across all $CO_2$ elevations.	N/A	[61]
Melon	400 ppm, 800 ppm, 1200 ppm	0, 25, 50 mmol NaCl	Up to 29% increase in fruit yield in all $\epsilon[CO_2]$ at no additional salinity. Elevated $[CO_2]$ partially rescues yield losses from salinity (by up to 18%) but is insufficient to fully mitigate yield loss.	N/A	[67]
Squash	700–1000 ppm	N/A	13% increase in muskmelon fruit number and 8% increase under $\epsilon[CO_2]$	15% – 19.7% increase in total marketable yield across 2 growing seasons.	[169], [158]

in  $e[\text{CO}_2]$  has been shown to mitigate yield losses from increased salinity [67], and in sweet pepper,  $e[\text{CO}_2]$  of 800 ppm was sufficient to rescue any significant yield loss of total and marketable fruits from salinity stress (20 mmol L<sup>-1</sup> NaCl) [42]. It could be hypothesised that increasing  $\text{CO}_2$  assimilation increases sugar and chlorophyll content triggering salt tolerance. However, it should be noted that these results are not universally translatable. Gray et al. [76] demonstrated in soybean that  $e[\text{CO}_2]$  was insufficient to protect yields from drought conditions triggered by higher temperatures demonstrating that benefits in some crops may not be translatable across all crops of agronomical importance. Furthermore, in tomato plant Zhou et al. [77] showed that plants grown in  $e[\text{CO}_2]$  were more sensitive to combined drought and heat stress;  $e[\text{CO}_2]$  drives  $gs$  and transpiration reducing net photosynthesis and therefore productivity, which is concerning given that greenhouses tend to have elevated temperatures compared to the external environment due to the nature of their construction, glass and metal, and therefore  $e[\text{CO}_2]$  in an enclosed system may negatively impact on yields if water supplies are limiting. This demonstrates that irrigation within greenhouse environments is an essential element and adjusting water regimes to maintain productivity and optimise water-use efficiency.

It is also important to note that it is the increase in atmospheric  $[\text{CO}_2]$  that causes the increase in air temperature (along with associated stresses) by absorbing energy and preventing it from being radiated out into space (see [78, 79]); as such one might view that the cause cannot mitigate its own effects, however, in some crops where both  $[\text{CO}_2]$  and temperature increase simultaneously, yields were maintained compared with data where temperature is increased in the absence of  $e[\text{CO}_2]$  leading to yield loss and these results cannot be ignored, but a better understanding of the impact of cause and effect climate change on crop yields needs to be researched, otherwise, the logic consequences would be further increase amounts of  $[\text{CO}_2]$  in the atmosphere to increase crop tolerance against the effects of ever-increasing temperatures.

Interestingly, some parallels do exist between photosynthetically genetically modified crops and increased tolerance to salinity. In *Arabidopsis*, over-expression of Sedoheptulose-1,7-bisphosphatase (SBPase), which enhances  $\text{CO}_2$  assimilation rates by increasing the regeneration of the Rubisco substrate RuBP [80], enhances salt tolerance through increases in sucrose, starch and chlorophyll content were reported [81]. This suggests that increasing photosynthetic rates, either through increasing the availability of  $[\text{CO}_2]$  for photosynthesis or increasing the plants' ability to assimilate  $[\text{CO}_2]$  under ambient conditions could have a similar protective effect. It would be interesting to explore if increased carbon assimilation rates, through atmospheric manipulation or genetic modification, can have a positive impact on crop resistance to high salt environments and other abiotic stresses in large field trials or commercial greenhouses. There is currently evidence that over-expressing the Calvin-Benson cycle (CBC) enzyme SBPase can increase tolerance to chilling stress in tomato [82] and the expression of the cyanobacterial CBC bifunctional fructose-1,6-bisphosphatases/Sedoheptulose-1,7-bisphosphatase enzyme in soybean prevent yield loss under high temperature [83]. Köhler et al. [83] concluded that the manipulation of  $\text{CO}_2$  uptake could mitigate against the effects of global increases in temperature under  $e[\text{CO}_2]$ . This may be deemed especially important given the expected impact of global climate change. This suggests that increasing carbon assimilation through manipulation of photosynthesis [84, 85] can have similar outputs to improved photosynthesis through growth in an enriched carbon atmosphere and further demonstrates the

viability of this approach for improvement of yield and quality in fruiting crops. This must be studied considering the recent work showing that improved carbon assimilation also results in improved nutrient uptake and an increase in NUE [86].

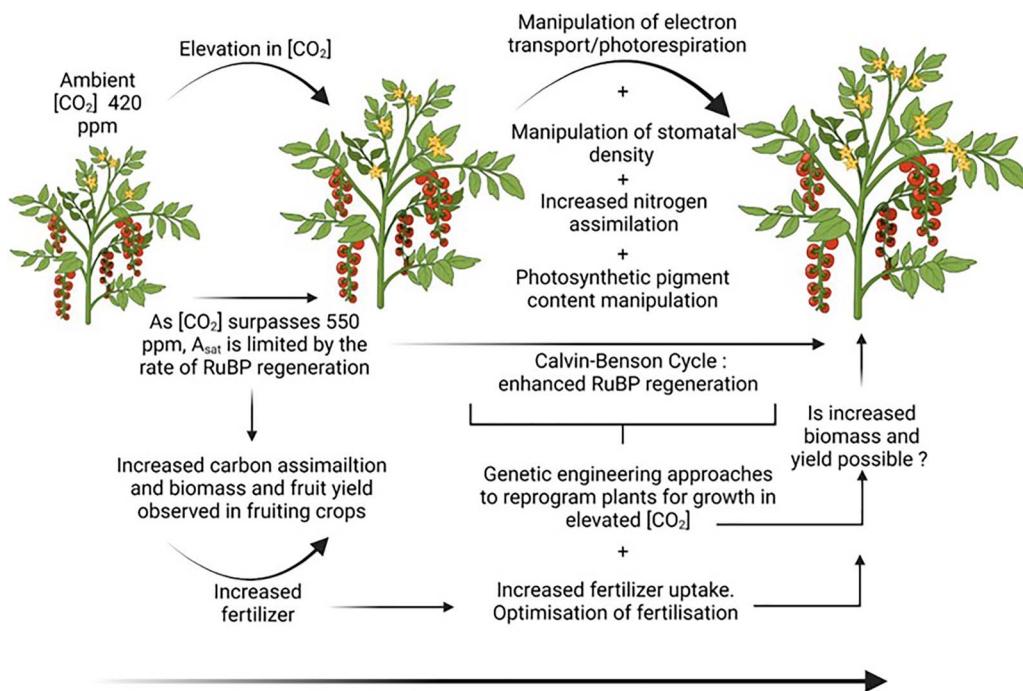
## Future opportunities

As  $[\text{CO}_2]$  surpasses 550 ppm,  $A_{\text{sat}}$  will be limited by the rate of RuBP regeneration rather than Rubisco activity suggesting there is scope to improve plant photosynthesis to increase yield in greenhouse environments where  $\text{CO}_2$  is routinely increased to 1000 ppm or more for short periods of time. These short time-periods are furthermore unpredictable and chaotic given that greenhouses must be vented, due to external environmental conditions, to maintain, as close as possible, optimal growing conditions i.e. temperature and humidity inside the growth facility. Furthermore, the  $[\text{CO}_2]$  dosing capacity must be economically beneficial, especially given the chaotic nature of  $\text{CO}_2$  loss to the environment during periods of venting. As dosing increases, costs go up accordingly determined by the cost of  $\text{CO}_2$ . Moreover, at some point, there is a price limit where the supplemental cost of  $\text{CO}_2$  increases to a point where costs cannot be recovered by the selling price of the product. In the last year,  $\text{CO}_2$  costs have increased for £100 per tonne to as much as £3000 per tonne [87, 88]. Therefore, future options that maximize the ability of the crop to take full benefit of the  $e[\text{CO}_2]$ , or maintaining higher yields when  $\text{CO}_2$  costs are unmanageable become more important.

Araus et al [89], noted that canopy photosynthesis holds a crucial place in a context of yield gains through photosynthetic improvement, which requires additional factors including the availability and uptake of nutrients, such as nitrogen, irrigation, the transport of photoassimilates and sink-source balance. As such, in addition to improving photosynthetic rates via  $\text{CO}_2$  supplementation, the improvement of other plant processes such as N uptake, non-foliar photosynthesis, stomatal function, and rubisco(activase) thermotolerance so that crops are better adapted for growth in  $[\text{CO}_2]$  enriched environments such as greenhouses are discussed below (Figure 3). These works will also need to account for changes to the landscape of greenhouse crop cultivation, such as a move to vertical farming, changes in growth medium from soil to substrates such as coir (derived from coconut husks) or rockwool [90]. It is estimated that more than 50% of strawberry production occurs in substrate rather than soil [91]. Coir is often used as it has been shown to retains water more efficiently than soil, so strawberry plants require less frequent watering improving water use efficiency. Coir also has a high level of aeration, which is ideal for strawberries' whose root systems require a lot of oxygen. More recent developments in hydroponics [92] and aeroponics [93], will impact on irrigation, fertiliser regimes and N uptake.

## Nitrogen use efficiency (NUE)

With regards to fruit quality, this is a complex trait that may not be simply attributed to enhanced carbon assimilation. More research is needed to link increased assimilate, with assimilate distribution and transport, NUE to better understand the sink-source relationship in any given crop, which can vary significantly across varieties and crop types. NUE is determined by yield per unit of available N in the growth medium (i.e. often coir in greenhouse grown crops). Plants with higher NUE may allocate N toward both the photosynthetic complexes (i.e. N is major component of chlorophyll; total N allocated to Rubisco 18.2 ± 6.2% [94];) and/or toward the development of additional sinks. The second definition of NUE



**Figure 3.** Effects of elevated  $\text{CO}_2$  on yield of fruiting crops and a representation of the potential for the manipulation of plant material for further yield increases. Created with BioRender.com

could be described as the efficiency with which N is applied to soils, (through artificial means in greenhouse crops), is taken up by plants and converted to usable products (i.e. biomass, grain yield). This can be manipulated through breeding to identify new varieties with high NUE uptake from selected growing mediums or through engineering nitrogen symbiosis (Figure 3). Recently, scientists reported the engineering nitrogen-fixation into non-legume cereal crops by enabling them to interact with soil bacteria to convert N from the air into ammonia fertiliser [95]. These works could firstly reduce the reliance on commercial synthetic fertilisers and secondly provide alternate sources of N that along with improvements to carbon assimilation, foliar or non-foliar, co-contribute to improving photosynthesis and yields in crops (Figure 3).

A recent review has identified a number of targets in the literature to improve N uptake, assimilation and remobilisation through genetic manipulation (see [96] for review). One of these, the over-expression of the nitrate transporter (NRT2.3) was shown to increase nitrate concentrations in tomato increasing biomass and fruit weight [97]. More recently, the transcription factor DREB1C has been identified as a regulator of NUE by controlling the expression of several important growth-related genes including the rubisco small subunit 3 (RBCS3), nitrate transporters (NRT1.1B, NRT2.4), nitrate reductase (NR2) and the flowering regulator (FTL3). Once over-expressed (OE), OsDREB1C increased the abundance of photosynthetic pigments, plants were shown to have about one-third more chloroplasts, 38% more rubisco and improved photosynthesis and N uptake. The OE of OsDREB1C resulted in a  $>40\%$  increase in grain yield in elite rice varieties and a  $\sim 20\%$  increase in wheat yields, while in *Arabidopsis*, a significant increase in biomass [98]. Many of these identified genes have potential for improving NUE in fruiting crops grown in  $\text{eCO}_2$ . A recent report of a large grain rice cultivar, Akita 63, having a high yield due to an enlarged sink capacity without photosynthesis improvement. However, this work

demonstrated that source capacity was strongly limiting the yield potential under high N fertilization. These authors suggested that enhancing photosynthesis is an important step to further increase yield of current high-yielding cultivars [99]. This work can be extrapolated that engineering NUE and photosynthesis in plants grown at  $\text{eCO}_2$  could provide a step-change in yields in greenhouse cultivated crops.

#### Genetic variation in photosynthetic traits in crops and wild relatives

Methods of improving these traits including breeding, by exploiting the potential of crop wild relatives as a source of new traits, and/or the genetic manipulation/genome editing of specific traits. There is already evidence that substantial genetic variation exists within wild relatives of fruiting crops [100–102], which are now studied as a source of crop improvement in various breeding programs [103]. Further evidence that even in elite material, significant variation is observed in photosynthetic traits. For example,  $V_{\text{max}}$ ,  $J_{\text{max}}$  and  $A_{\text{sat}}$ , indicators of photosynthetic potential, have been shown to vary by as much as 30% in the flag leaves of recent breeding lines of spring and winter wheats [104–106]. Similarly, several quantitative trait loci for photosynthetic efficiency have been identified in elite rice material, including the identification of important transcription factors [107, 108]. This work in wheat and rice is promising, demonstrating the potential for breeding new varieties better adapted to changing growth conditions, however it is unclear if such strategies will work in horticultural crops. In the case of tomato, there is considerable variation within the wild and elite varieties to suggest that such breeding strategies could be used to enhanced yield and quality [109, 110]. See Sharwood et al [111] for review (Figure 3).

In transgenic rice, overproducing Rubisco, increases the biomass production and yield under high N fertilization in paddy fields suggesting that the development of new rice varieties with both high photosynthesis and large sink capacity is essential [99].

Furthermore, genes encoding thermostable variants of Rubisco activase (thermos-Rca) have been identified in wild rice relatives. When over-expressed in domesticated rice, thermos-Rca was sufficient to enhance carbohydrate accumulation and improve yields after periodic exposure to elevated temperatures (+45°C) throughout the vegetative phase [112, 113]. Thermostable Rca have been identified in Thermophilic cyanobacteria, bacteria that thrive in high-temperature environments, making them a potential source of novel genes for engineering crops for growth at higher temperatures [114]. Improving the thermal tolerance of rubisco activase, either through breeding with wild populations or genetic engineering, could aid greenhouse grown crops better tolerate the elevated temperatures that often occur during the growing season (Figure 3).

### Genetic engineering of photosynthetic traits in crops

Increasing the expression of enzymes and/or proteins involved in the regeneration of RuBP, CO<sub>2</sub> transport or chloroplast electron transport have previously been shown to enhance photosynthetic efficiency and increases in yield [84, 85, 115–117]. However, once again, it cannot be ignored that much of this work has focused on non-fruiting crops, such as *Arabidopsis*, tobacco, wheat and rice, (see Simkin et al. [84] for review), grown in controlled conditions, performed in pots, in soil or in the field with controlled irrigation, which is not typical of global agriculture. Furthermore, work carried out in tomato, over-expression of sedoheptulose-1,7-bisphosphatase, involved in RuBP regeneration, did not report on fruit yield [82]. These data indicating that more work is required to understand how these manipulations would impact fruiting crops grown in tightly controlled environments.

One potential target for genetic manipulation is the starch synthesis enzyme adenosine diphosphate glucose pyrophosphorylase (AGPase); increasing AGPase activity has potential to increase starch accumulation for growth. Increased accumulation of starch has been shown to have little negative feedback on photosynthesis [118] and increased AGPase activity in the chloroplast would increase the strength of the transient starch pool, which acts as a sink in the chloroplast. Reduced sink capacity does induce negative feedback on photosynthesis and can limit photosynthesis even in favourable conditions (e.g. elevated [CO<sub>2</sub>] [119]), suggesting that increasing the sink may allow for greater CO<sub>2</sub> assimilation in supplemented [CO<sub>2</sub>] growth environments.

Although genetic manipulation has the potential to further increase yields in crops grown in enriched [CO<sub>2</sub>] environments, allowing them to take better advantage of supplemental CO<sub>2</sub>, increasing net photosynthetic rates and associated yields (Figure 3), it should also be noted that some reports have suggested that increases in yield in genetically enhanced photosynthetic crops are likely not uniquely down to increases in carbon assimilation but a combination of factors; for example improvements in carbon uptake allow for an increase in N assimilation [120]. Furthermore, it has also been reported that such increase in yield from enhanced photosynthetic efficiency critically rely on the availability and uptake of water and nutrients (for review see [121, 122]), therefore, genetic engineering as an approach alone may be limiting if other aspects of crop cultivation, such as irrigation, planting regimes, fertilisation (i.e NUE) and growth media (i.e soil, coir, rockwool), are not taken into account and co-optimised.

### Non-foliar photosynthesis

Leaves are not the only location within the plant where photosynthesis occurs, with evidence of photosynthesis in petioles

and stems [123, 124], and fruit [124] that may provide significant and alternative sources of photo-assimilates essential for optimal yield. Assimilation of atmospheric CO<sub>2</sub> is dependent on the number and behaviour of stomata, and the stems of many plants have stomata distributed along the epidermis [125, 126] and an evaluation of the photosynthetic activity in stems of various plants accounted for up to 4% of the total photosynthetic activity [127]. Furthermore, Hu et al. demonstrated the importance of stem photosynthesis to yield in cotton; maintaining the stem in darkness reduced seed weight by 16% [128] showing the stem provides photoassimilates for plant development and growth.

As previously noted, many fruiting crops produce green fruit containing all the necessary proteins and enzymes to carry out photosynthesis [127, 129, 130] that may provide significant and alternative sources of photoassimilates essential for optimal yield and quality [124]. Tomato fruit photosynthesis contributes to net sugar accumulation and growth and previous work concluded that tomato fruit photosynthesis contributes between 10% and 15% of the total fixed carbon of the fruit [127, 131] [132]. It should be noted that, unlike many crops, cucumber fruit remain green through to maturity, have stomata (suggesting they perform gas exchange to drive photosynthesis), and have a similar surface area to an expanded leaf [130]. It has previously been reported that cucumber fruit had high photosynthetic and respiratory rates [133] and contribute approximately 9.4% of their own carbon requirements [130]. It should be noted that in fruit with stomata, such as cucumber, there are two potential major sources of CO<sub>2</sub>. Firstly, Rubisco assimilates atmospheric [CO<sub>2</sub>] through the stomatal pores, leading to the production of sugars via the CBC and secondly, CO<sub>2</sub> released by mitochondrial respiration is re-fixed (recycling photosynthesis) [125, 134]. Whilst this confirms that photosynthesis occurs in fruits, the extent and importance is not clear. In e[CO<sub>2</sub>], it seems plausible that cucumber fruit photosynthesis may contribute directly to fruit size (and therefore yield by weight) and quality through their ability to directly access carbon in an enriched atmosphere via their stomata (for a review fruit photosynthesis, see [124, 135]). Therefore, increasing carbon capture by non-foliar tissues has the potential to significantly impact yield and combined with an increase N uptake (i.e. slow release fertilizers [136]) to balance the increased carbon uptake, and optimised irrigation regimes has the potential to maximise such yield gains.

### Conclusions

These data show that the yield of fruiting crops benefit from growth in supplemented atmospheres, although, some data suggests that increase in yield can come at the expense of quality traits. It is therefore essential to determine the optimal [CO<sub>2</sub>] concentrations on a crop-by-crop basis, to maximise productivity. An evaluation of fruit quality under these conditions has also been shown to be highly variable between treatments and difference are observed between cultivars with the same treatment suggesting that much more research is required to identify the specific mechanisms behind changes in fruit quality. In the case of soft fruit production in greenhouse environments, it will be important to determine if the quality of fruit harvested early in the season differs from that of fruit harvested later in the season when plants have spent a more significant period of time exposed to e[CO<sub>2</sub>] growth conditions. Cherry for instance, when grown under prolonged periods of e[CO<sub>2</sub>], acclimates to prolonged exposure and initial significant gains in yield observed after two months are less detectable after ten months and are not significantly different to control plants grown at a[CO<sub>2</sub>] [72].

This may in one respect account for differences in nutritional quality observed in fruit grown in similar conditions in different studies (i.e. fruit harvested at different times in the study) where additional fertilizer treatments aren't provided.

Increases in yield associated with e[CO<sub>2</sub>] controlled environments may be about more than additional carbon. Controlled environments also allow the regulation of transpiration (e.g. by controlling vapour pressure deficit) and therefore water uptake and the inclusion of additional fertilisation (specifically N). Breeding new varieties adapted to these growth conditions may also be more amenable given the hostility towards genetically modified crops. A recent review noted that new phenomics, genomics, and bioinformatics tools make it possible to harness the untapped potential of crop genetic resources (including wild relatives) to create combinations of traits to enhance yield in high [CO<sub>2</sub>] controlled environments [137].

Breeding alone may not be sufficient to adapt all varieties, or all crops, to high [CO<sub>2</sub>] growing environments traditionally used in greenhouses. However, over the last several decades, agricultural research has adopted technologies such as genetic engineering and "genome editing" to improve traits in key crops that could be useful in these circumstances [85, 138–140]. These include advances in the tools available to carry out this work, including vectors for multiple gene insertion [141–145] and tissue specific promoters [146–150]. If the promise of these biotechnology programs is to be realized, it will be necessary to address the public perception of genetic modification and genome editing technologies to gain greater acceptance.

Genetic manipulation, may need to go beyond the direct manipulation of carbon assimilation in leaves [84, 180], but focus on the manipulating and control of stomatal function [151, 152], the manipulation of pigments complexes in ripening fruit [153], enhancement of light capture by the leaves through the manipulation of chlorophyll distribution and form [154] and importantly look a methodologies for increasing N uptake via transgenic [96] or traditional means (improved fertilization regimes).

It should also be noted that the introduction of new growing, hydroponics, aquaponics and aeroponics may require further study, to breed and adapt or engineer plants root architecture for these new growth media. In conclusion, greenhouse cultivation offers the opportunity to manipulate growing atmosphere, lights and VPD for improved yields and we can now look at the opportunities to breed and engineer plants specifically optimised for these conditions.

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## Author Contributions

N.H.D and A.J.S drafted and wrote the manuscript with input from T.L, C.A.R and C.W who also edited the final version.

## Conflict of interests

The authors declare no competing interests.

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