

REVIEW PAPER

Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE

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Abstract

Plant responses to the projected future levels of CO₂ were first characterized in short-term experiments lasting days to weeks. However, longer term acclimation responses to elevated CO₂ were subsequently discovered to be very important in determining plant and ecosystem function. Free-Air CO₂ Enrichment (FACE) experiments are the culmination of efforts to assess the impact of elevated CO₂ on plants over multiple seasons and, in the case of crops, over their entire lifetime. FACE has been used to expose vegetation to elevated concentrations of atmospheric CO₂ under completely open-air conditions for nearly two decades. This review describes some of the lessons learned from the long-term investment in these experiments. First, elevated CO₂ stimulates photosynthetic carbon gain and net primary production over the long term despite down-regulation of Rubisco activity. Second, elevated CO₂ improves nitrogen use efficiency and, third, decreases water use at both the leaf and canopy scale. Fourth, elevated CO₂ stimulates dark respiration via a transcriptional reprogramming of metabolism. Fifth, elevated CO₂ does not directly stimulate C₄ photosynthesis, but can indirectly stimulate carbon gain in times and places of drought. Finally, the stimulation of yield by elevated CO₂ in crop species is much smaller than expected. While many of these lessons have been most clearly demonstrated in crop systems, all of the lessons have important implications for natural systems.

Key words: Climate change, elevated CO₂, Free-Air Carbon dioxide Enrichment (FACE), Rubisco.

Introduction

The atmospheric CO₂ concentration ([CO₂]) was stable at ~270 μmol mol⁻¹ for at least the 1000 years prior to the start of the Industrial Revolution. Since that time CO₂ has been accumulating in the global atmosphere at an accelerating pace. Today, in 2009, the [CO₂] is at 384 μmol mol⁻¹, ~40% higher than at any time in the last 20 million years (Pagani *et al.*, 1999; Pearson and Palmer, 2000). By the middle of this century [CO₂] is projected to surpass 550 μmol mol⁻¹ and top 700 μmol mol⁻¹ by the end of the century (Prentice *et al.*, 2001). The recently observed global [CO₂] increase is significantly faster than anticipated by the

Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report (AR4) (Dyson, 2005; Canadell *et al.*, 2007; Hanson *et al.*, 2008). This is considered to be the result of rapidly increasing emissions in China and India from new coal-fired electric plants, alongside limited action by developed economies to constrain their emissions. Despite initial steps taken under the Kyoto Protocol, the world appears to be on a path that is likely to lead to a [CO₂] that exceeds the highest IPCC emissions scenario (A1FI). Thus, both natural and managed ecosystems are currently exposed to an elevated [CO₂] level that has not been experienced by

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terrestrial vegetation since the early Miocene (Pearson and Palmer, 2000) and are facing a future that portends uncertain consequences of ever-increasing $[\text{CO}_2]$. Understanding how plants have and will respond to the rapid change in $[\text{CO}_2]$, together with developing knowledge about their capacity to adapt, is an essential initial step in understanding the full impact that the multiple interacting factors of global change (e.g. drought, temperature, ozone) will have on terrestrial ecosystems. These ecosystems produce services upon which we are dependent for food, fuel, fibre, clean air, and fresh water. An enormous amount has been learned about how plants respond to these projected future levels of $[\text{CO}_2]$ from various sorts of enclosure studies conducted over the past three decades. As scientific understanding advanced and underlying mechanisms were revealed, the need to test findings and hypotheses under truly open-air field conditions became increasingly apparent, leading to the development of a new technology—Free-Air Carbon dioxide Enrichment (FACE) (Lewin *et al.*, 1992, 1994; Hendrey and Miglietta, 2006). Now that there have been 15 major FACE experiments using fully replicated ($n \geq 3$) large plots ($>100 \text{ m}^2$) on different ecosystems in different parts of the world, it is possible to draw a number of important conclusions. Six important lessons about impacts on plant carbon, nitrogen, and water relations that have been learned from FACE experiments are presented and the underlying evidence obtained from these studies is reviewed here.

Lesson 1: carbon uptake is enhanced by elevated $[\text{CO}_2]$ despite acclimation of photosynthetic capacity.

A first lesson from recent FACE studies of C_3 photosynthetic responses to elevated $[\text{CO}_2]$ is that photosynthetic carbon uptake (A) is enhanced by elevated $[\text{CO}_2]$ despite acclimation of photosynthetic capacity. Photosynthetic acclimation is most commonly measured as a decreased maximum carboxylation rate of Rubisco ($V_{c,\text{max}}$) and maximum electron transport rate leading to ribulose-1,5-bisphosphate (RuBP) regeneration (J_{max}) (reviewed in Long *et al.*, 2004; Nowak *et al.*, 2004; Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). While early studies of C_3 plants grown in pots in controlled environments indicated that acclimation of photosynthetic capacity might negate any stimulation in A in some species (reviewed in Arp, 1991; Stitt, 1991; Sage, 1994), more recent evidence from FACE experiments overwhelmingly shows that, despite small decreases in $V_{c,\text{max}}$ and J_{max} , the light-saturated rate of photosynthetic carbon uptake (A_{sat}) is markedly stimulated in C_3 plants grown at elevated $[\text{CO}_2]$ (Ainsworth and Rogers, 2007). However, the evidence from FACE experiments also shows that the degree of stimulation of A varies among species and experimental conditions (Nowak *et al.*, 2004; Ainsworth and Long, 2005).

What factors determine the degree of stimulation of carbon uptake in C_3 plants grown at elevated $[\text{CO}_2]$? Using

data from FACE experiments, the difference in the magnitude of stimulation in A among species and functional groups was explained by the process that limited A at a given intercellular $[\text{CO}_2]$ (C_i) (Ainsworth and Rogers, 2007). The A/C_i response curve, as predicted from the C_3 leaf model of photosynthesis (Farquhar *et al.*, 1980), shows a biphasic response of A to C_i (Fig. 1). As C_i is increased from a minimum concentration, the rate of change in A is great and determined by the activity of Rubisco ($V_{c,\text{max}}$). With a further increase in C_i , there is an inflection to a lower rate of increase in A where RuBP-regeneration capacity (J_{max}) is limiting (Long and Bernacchi, 2003). In species and functional groups (i.e. groups of plants that share functional characteristics with or without phylogenetic relatedness such as C_4 or legumes) with Rubisco-limited photosynthetic capacity at elevated $[\text{CO}_2]$, there is a larger potential for the stimulation of A because elevated $[\text{CO}_2]$ both increases Rubisco carboxylation rates and decreases rates of photorespiration (Long *et al.*, 2004; Ainsworth and Rogers, 2007). Thus, trees and grasses, which were limited by Rubisco capacity at elevated $[\text{CO}_2]$, showed greater stimulation of A compared to legumes, shrubs, and non-leguminous C_3 crops that were limited by RuBP regeneration capacity at elevated $[\text{CO}_2]$ (Ainsworth and Rogers, 2007). When limited by RuBP regeneration capacity, the increase in A resulted almost exclusively from the repression of photorespiration (Long *et al.*, 2004). This explanation

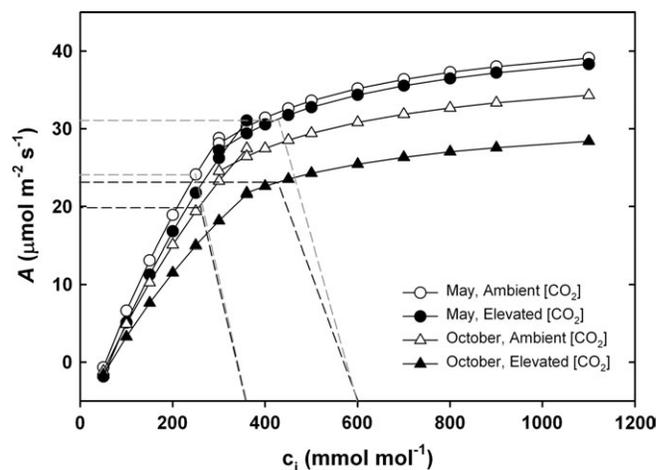


Fig. 1. Average plots of A/C_i response curves for *Trifolium repens* grown at ambient $[\text{CO}_2]$ ($\sim 365 \mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ ($\sim 600 \mu\text{mol mol}^{-1}$) during spring (May 2001) and autumn (October 2000) at the Swiss FACE array in Eschikon, Switzerland (adapted from Ainsworth *et al.*, 2003b and reproduced by kind permission of Oxford University Press). The maximum RuBP-saturated rates of carboxylation *in vivo* ($V_{c,\text{max}}$) were estimated from the initial slopes of each curve, and the maximum *in vivo* rates of electron transport contributing to RuBP regeneration (J_{max}) were estimated using turning points after the inflection. The black dashed lines indicate the supply functions and resultant photosynthetic rates for plants measured in October, while the grey dashed lines indicate the supply function and resultant photosynthetic rates for plants measured in May.

provides a mechanistic basis for the greater than average stimulation in *A* observed in trees (46%) and grasses (37%) grown at elevated [CO₂], compared to shrubs (21%), C₃ crops (13%), and legumes (19%). However, even within functional groups, environmental and genetic factors also influence the magnitude of acclimation of photosynthetic capacity, and the stimulation of *A*.

In general, environmental, experimental, or genetic factors that limit the development of sink strength predispose plants to a greater acclimation of photosynthetic capacity, and lessen the stimulation of *A* by growth at elevated [CO₂] (reviewed in Arp, 1991; Stitt, 1991; Long *et al.*, 2004; Ainsworth and Rogers, 2007). This was demonstrated for plants grown in controlled environments in different-sized pots where sink strength was limited by rooting volume (Arp, 1991; Thomas and Strain, 1991). Field studies have since confirmed that reduced or insufficient sink capacity from environmental, genetic or management practices leads to an increase in foliar carbohydrates, and subsequent down-regulation of photosynthetic capacity (reviewed in Long *et al.*, 2004; Rogers and Ainsworth, 2006; Ainsworth and Rogers, 2007). For example, in a managed *Lolium perenne* and *Trifolium repens* grassland exposed to elevated [CO₂] for a decade (Lüscher *et al.*, 2006), both seasonal (Ainsworth *et al.*, 2003b) and harvest practices (Rogers *et al.*, 1998; Isopp *et al.*, 2000; Ainsworth *et al.*, 2003a) played important roles in determining the photosynthetic response of *L. perenne* and *T. repens* to elevated [CO₂]. In the spring, when day length and radiation were high, growth of *T. repens* swards was rapid, plants were unlikely to be sink-limited, and there was no difference in photosynthetic capacity between plants grown at ambient and elevated [CO₂] (circle symbols in Fig. 1; Ainsworth *et al.*, 2003b). Following the supply functions (grey dashed lines in Fig. 1) from the *A/C_i* curves to the *y*-axis illustrates that *A* was stimulated by ~40% by growth at elevated [CO₂] in the spring. On the other hand, in the fall, *T. repens* sward growth was significantly less than in the spring, and limited by low temperatures and regular nightly frosts. These environmental conditions lead to significant down-regulation of both $V_{c,max}$ and J_{max} in elevated [CO₂] in the autumn (triangle symbols in Fig. 1). Following the supply functions (black dashed lines in Fig. 1) to the *y*-axis for the October *A/C_i* curves shows that stimulation in *A* was essentially halved to only 22% in the autumn (Ainsworth *et al.*, 2003b). In addition to environmental factors altering sink capacity, periodic harvests of above-ground tissue also altered source to sink balance in the Swiss FACE experiment (Lüscher *et al.*, 2006). In *L. perenne*, there was no change in photosynthetic capacity immediately following the removal of source tissue in a periodic harvest (Rogers *et al.*, 1998; Ainsworth *et al.*, 2003a), but significant reductions in $V_{c,max}$ and J_{max} developed approximately 3 weeks after the harvest when leaves were fully expanded and source capacity at elevated [CO₂] outgrew sink capacity (Ainsworth *et al.*, 2003a). The Swiss FACE experiment provided a clear demonstration of how both environmental (season) and experimental (harvest regime) factors altered source-to-sink

balance, acclimation of photosynthetic capacity to elevated [CO₂], and therefore degree of stimulation of carbon uptake.

Genetic factors can also play an important role in photosynthetic response to elevated [CO₂]. This was perhaps most clearly demonstrated with fast-growing *Populus* trees (poplars) exposed to elevated [CO₂] in the PopFACE experiment (Scarascia-Mugnozza *et al.*, 2006). Poplars grown for coppice sustained a 55% stimulation in *A* at elevated [CO₂] (Bernacchi *et al.*, 2003) because of their large capacity for starch synthesis and carbon export (Davey *et al.*, 2006). Poplars exported >90% of their photosynthate during the day and stored the rest of the overflow photosynthate as starch (Davey *et al.*, 2006), which enabled the trees to avoid acclimation of photosynthetic potential, and maintain maximal stimulation of *A* at elevated [CO₂]. In a similar experiment at AspenFACE with the North American plantation species, aspen (*Populus tremuloides*) and birch (*Betula papyrifera*), acclimation of photosynthesis was again not observed in the first years of the experiment, and stimulation of *A* was maximal (Karnosky *et al.*, 2003).

Meta-analyses of plant responses to elevated [CO₂] suggest that when the acclimation of photosynthetic capacity does occur it involves a selective loss of Rubisco compared to proteins involved in light energy conversion, manifest as a decrease in the ratio of $V_{c,max}$ to J_{max} (Long *et al.*, 2004; Ainsworth and Long, 2005). However, this potential shift in $V_{c,max}:J_{max}$ is based on data that used the internal [CO₂] (C_i) for calculations of photosynthetic parameters, rather than the chloroplast [CO₂] (C_c). Therefore, this result would not take account of any change in the limitation to photosynthesis imposed by potential changes in mesophyll conductance (Singsaas *et al.*, 2004), the transfer capacity of CO₂ between the leaf internal air spaces and the site of carboxylation in the chloroplast (Farquhar and Sharkey, 1982; Flexas *et al.*, 2008). Is there evidence that growth at elevated [CO₂] alters mesophyll conductance? A FACE study of soybean (*Glycine max*) suggested that mesophyll conductance was not altered by growth at elevated [CO₂] (Bernacchi *et al.*, 2005); however, Singsaas *et al.* (2004) found in understory trees that changes in mesophyll conductance at elevated [CO₂] were species and condition-dependent. Flexas *et al.* (2008) also reported a large decrease in mesophyll conductance when leaves were exposed to elevated [CO₂] for minutes, although a physical basis for this rapid change is difficult to understand. Therefore, while some evidence strongly supports a clear but modest shift in optimization of photosynthetic metabolism (i.e. a decrease in $V_{c,max}:J_{max}$; Bernacchi *et al.*, 2005), difficulties in accurately measuring and interpreting mesophyll conductance data prevent extrapolating this result to all species and conditions.

Lesson summary

FACE experiments have provided ample evidence that photosynthetic capacity acclimates to elevated [CO₂] in C₃ plants, and the scale of down-regulation varies with genetic

and environmental factors. However, despite acclimation of photosynthetic capacity, carbon gain is markedly greater (19–46%) in plants grown at the $[\text{CO}_2]$ anticipated for the middle of this century.

Lesson 2: photosynthetic nitrogen use efficiency increases at elevated $[\text{CO}_2]$

In theory, photosynthetic nitrogen use efficiency (PNUE), defined here as the net amount of CO_2 assimilated per unit of leaf N, has the potential to increase in C_3 plants grown at elevated $[\text{CO}_2]$ in that photosynthetic acclimation provides an opportunity to optimize the distribution of N to maximize C gain. Alternatively, PNUE could instead be reduced by a non-specific decrease in leaf N content at elevated $[\text{CO}_2]$ thereby offsetting gains in C acquisition. Results from FACE experiments provide the most confident projections of future changes in PNUE at elevated $[\text{CO}_2]$ and have identified potential limitations on plant responsiveness to rising $[\text{CO}_2]$.

Theory predicts that a C_3 plant for which photosynthesis is Rubisco limited at both 380 and 580 $\mu\text{mol mol}^{-1} [\text{CO}_2]$ could show a stimulation of photosynthesis by elevated $[\text{CO}_2]$ at 25 °C by as much as 40% (Farquhar *et al.*, 1980). A plant where assimilation is limited solely by RubP regeneration at both CO_2 concentrations would have a maximum 11% stimulation in photosynthesis under these conditions (Long *et al.*, 2004). Therefore, even in plants where photosynthesis is not limited by Rubisco capacity, PNUE is expected to increase due to the reduced flux into the largely wasteful photorespiratory pathway. The evidence from FACE studies supports previous work conducted in controlled environments and field enclosures and provides overwhelming evidence that photosynthesis in both the short and long term is stimulated by growth at elevated $[\text{CO}_2]$ (see Lesson 1 above).

Acclimation to elevated $[\text{CO}_2]$ does occur (see Lesson 1), but how much N is conserved at elevated $[\text{CO}_2]$ due to Rubisco acclimation? Earlier investigations into the response of plants to rising $[\text{CO}_2]$ often reported marked (>50%) reductions in Rubisco content at elevated $[\text{CO}_2]$, even in naturally rooted plants (Jacob *et al.*, 1995). Rubisco typically accounts for *c.* 25% of leaf N (Sage *et al.*, 1987), and in some cases investment in Rubisco can be as high as 50% (Spreitzer and Salvucci, 2002). Therefore, it was hypothesized that the redistribution of N saved through Rubisco acclimation at elevated $[\text{CO}_2]$ could greatly increase N use efficiency within the leaf, and the plant (Drake *et al.*, 1997). Results from FACE experiments support this trend, but realized that the N savings are much lower than anticipated.

A meta-analysis (Ainsworth and Rogers, 2007) of the response of $V_{c,\text{max}}$ to growth at elevated $[\text{CO}_2]$ using FACE technology showed that, on average, $V_{c,\text{max}}$ was reduced by ~10% in plants grown at elevated $[\text{CO}_2]$. The magnitude of this response varied among functional groups (e.g. legumes 7%, crops 17%, grasses 16%, and trees 6%). Table 1 shows

Table 1. Estimates of the maximum theoretical saving in leaf N due to Rubisco acclimation at the elevated $[\text{CO}_2]$ used in FACE experiments

	Rubisco content ^a (mg m^{-2})		Maximum potential N saving at elevated $[\text{CO}_2]$ (mg m^{-2})	Percentage N saving at elevated $[\text{CO}_2]$ ^b
	Current	Elevated		
Crop	217	177	67	3.9
Tree	153	147	9	0.6
Legume	260	233	45	2.6
Grass	182	150	54	4.4

^a Rubisco content was calculated from values of $V_{c,\text{max}}$ presented previously (Ainsworth and Rogers, 2007) with the following assumptions: (i) the reduction in $V_{c,\text{max}}$ at elevated $[\text{CO}_2]$ was due entirely to a reduction in content, (ii) the k_{cat} of Rubisco = 2.5 s^{-1} (Zhu *et al.*, 1998; Tcherkez *et al.*, 2006), (iii) the molecular mass of Rubisco = 536 kg mol^{-1} (Raines *et al.*, 1991), and (iv) Rubisco is 16.7% N (Steer *et al.*, 1968).

^b Leaf N content at current $[\text{CO}_2]$ was calculated from our database of plant responses to elevated $[\text{CO}_2]$ (Ainsworth and Long, 2005) where crops = 1.72, trees = 1.57, legumes = 1.74, and grasses = 1.22 (g m^{-2}).

that the maximum possible saving in leaf N due to Rubisco acclimation at the elevated $[\text{CO}_2]$ typical of current FACE experiments is smaller than anticipated. Even excluding trees that are Rubisco limited at current and elevated $[\text{CO}_2]$ and are not hypothesized to show marked acclimation, the mean saving in leaf N is still small, ~3.6%. This estimate assumes that the reduction in $V_{c,\text{max}}$ at elevated $[\text{CO}_2]$ is due entirely to a reduction in Rubisco content (Nie *et al.*, 1995; Rogers *et al.*, 1998) with no reduction in Rubisco activation (Socias *et al.*, 1993; Cen and Sage, 2005). These data have shown that the potentially large increases in PNUE at elevated $[\text{CO}_2]$ (Drake *et al.*, 1997) have not been realized in FACE experiments. For a given degree of acclimation, these potential additional future savings in N will be greater for legumes, grasses and crops that invest a greater percentage of their leaf N in Rubisco (25%, 25%, and 21%, respectively) than for trees which only invest 16% of their N in Rubisco (calculated from data in Table 1).

Have plants grown at elevated $[\text{CO}_2]$ in FACE experienced a reduction in leaf N content that would impact PNUE? Evidence from meta-analysis suggests that reductions in leaf N_{area} (leaf N content expressed per unit leaf area) are small, ~4%. Given that reductions in $V_{c,\text{max}}$ in species that acclimate are more than double this and that the reduction in leaf N content associated with Rubisco acclimation could be comparable (~3.6%; Table 1) it suggests that a marked non-specific dilution of leaf N content is not responsible for the reduction in $V_{c,\text{max}}$ at elevated $[\text{CO}_2]$ and is unlikely significantly to impact PNUE.

The mean increase in PNUE was calculated for studies conducted in FACE experiments that reported A_{sat} and leaf N_{area} . Comparisons of PNUE at current and elevated $[\text{CO}_2]$ are confounded by $[\text{CO}_2]$, species, N supply, age, and N storage strategy (Sage and Percy, 1987) so a response ratio (PNUE at elevated $[\text{CO}_2]$ /PNUE at current $[\text{CO}_2]$) was

calculated for each observation. Across 15 species it was found that PNUE was increased by $31\% \pm 3.6\%$ SE (t_{57} , $P < 0.001$), consistent with previous studies (Peterson *et al.*, 1999) and the $>30\%$ increase in A_{sat} and $<5\%$ decrease in N_{area} reported previously for FACE studies (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007).

Matching the increased C supply at elevated $[\text{CO}_2]$ with additional N is key to avoiding sink limitation of A at elevated $[\text{CO}_2]$. Results from FACE have provided clear evidence for the link between acclimation and N supply. Plants growing with a low N supply typically accumulate more foliar carbohydrates and exhibit greater Rubisco acclimation than those grown at high N supply (Ainsworth *et al.*, 2003; Ainsworth and Long, 2005). Plants with a large sink capacity such as well-irrigated and fertilized poplar (Davey *et al.*, 2006) can avoid sink limitation and thus minimize the associated loss of Rubisco when grown at elevated $[\text{CO}_2]$. Legumes have the potential to respond maximally to elevated $[\text{CO}_2]$ because their N-fixing bacteria provide a large C sink where excess C can be traded for N allowing them simultaneously to avoid sink limitation and to increase their N supply (Rogers *et al.*, 2006). This has resulted in improved productivity of legumes at elevated $[\text{CO}_2]$ when compared to non-leguminous plants and the presence of legumes has improved leaf N content, photosynthesis, and in some cases the productivity, of co-occurring non-leguminous plants (Zanetti *et al.*, 1996, 1997; Lee *et al.*, 2003) suggesting that increased A at elevated $[\text{CO}_2]$ in legumes can improve plant, community and ecosystem N acquisition (Prior *et al.*, 2006).

Lesson summary

Results from FACE experiments have shown that PNUE is markedly improved in C_3 plants grown at elevated $[\text{CO}_2]$. This increase is driven predominantly by enhanced CO_2 uptake rather than by the saving and redistribution of leaf N which was found to be smaller than anticipated. However, further improvements in PNUE are anticipated later this century as the atmospheric $[\text{CO}_2]$ surpasses the $\sim 550 \mu\text{mol mol}^{-1}$ level used in the FACE experiments that have been conducted to date.

Lesson 3: water use at both leaf and canopy scales declines at elevated $[\text{CO}_2]$

The undisturbed microenvironment provided by FACE provides a unique opportunity to address the responses of leaf and canopy water use to elevated $[\text{CO}_2]$. Plants control their stomata to regulate the amount of water that is transpired; however, the canopy microclimate will determine the rate at which water is transpired from the stomata. Any enclosure, regardless of construction, will alter the canopy microclimate and thus influence transpiration. FACE allows for a better understanding, relative to enclosure studies, of stomatal responses to elevated $[\text{CO}_2]$, and the ability to measure accurately CO_2 responses of

stomatal conductance, canopy evapotranspiration, and soil moisture is an important asset of FACE studies. In this section, some of the key findings of water use from FACE experiments from the stomatal to the canopy scale will be presented, including evidence supporting a lack of acclimation of stomatal conductance (g_s) to elevated $[\text{CO}_2]$.

Stomatal conductance (g_s) is lower at elevated CO_2

The major function of stomata is to maximize the rate at which CO_2 can diffuse into the leaf for photosynthesis while minimizing the simultaneous loss of water vapour, an optimization that requires continuous regulation. Many factors are known to influence stomata and their response to the environment is often highly predictable (Ball *et al.*, 1987). While it is overwhelmingly evident from both FACE and non-FACE experiments that g_s decreases in elevated $[\text{CO}_2]$ (Curtis and Wang, 1998; Want *et al.*, 1999; Medlyn *et al.*, 2001; Ainsworth *et al.*, 2002; Ort *et al.*, 2006; Ainsworth and Rogers, 2007), these reviews demonstrate a wide degree of variability in the response of g_s to elevated $[\text{CO}_2]$. For example, responses for trees in growth chambers range from a 5% increase in g_s to a 25% decrease (Curtis and Wang, 1998), whereas for FACE experiments the range is a 16–23% decrease (Ainsworth and Rogers, 2007).

Stomata do not acclimate to growth in elevated CO_2

The Ball *et al.* (1987) model, as modified from its original version, predicts g_s as

$$g_s = g_0 + m \frac{Ah}{[\text{CO}_2]}$$

where A is the net rate of photosynthetic CO_2 assimilation; h is relative humidity; $[\text{CO}_2]$ is the atmospheric CO_2 at the leaf surface; g_0 is the y -axis intercept, and m is the slope of the line. The parameters g_0 and m are species-specific and, while evidence from enclosure studies suggests the possibility of acclimation of these variables (Bunce, 2004), FACE work with *Lolium perenne* (Nijs *et al.*, 1997) and soybean (Leakey *et al.*, 2006a) found no acclimation of g_s to $[\text{CO}_2]$. Therefore, the instantaneous decrease in g_s at elevated $[\text{CO}_2]$ is maintained over time in long-term FACE studies.

Elevated CO_2 results in a decrease in canopy evapotranspiration

Decreased g_s for individual leaves in elevated CO_2 may not necessarily translate to a proportional reduction in canopy transpiration. While decreased g_s is consistent with the potential for a decrease in transpiration, many factors in addition to g_s need to be considered. For example, a decrease in g_s is likely to increase leaf temperatures that would, in turn, increase the driving force for transpiration. Additional influences on transpiration include CO_2 -induced changes in leaf and canopy structure that can influence the respective boundary layers. Most modern gas exchange systems provide leaf level measures of transpiration,

however, these values are representative only of the conditions present in the leaf chambers at the time of measurement, which often differ vastly from field conditions. Therefore, actual water use at the leaf level is difficult to obtain. Although chamber-based experiments have been used to determine how increases in $[\text{CO}_2]$ influence canopy-scale evapotranspiration (ET) (Wilson *et al.*, 1999; Hungate *et al.*, 2002; Polley *et al.*, 2008), humidity, radiation, temperature, and coupling to the atmosphere are all altered by chambers such that they may not realistically predict ecosystem responses to $[\text{CO}_2]$ (McLeod and Long, 1999). For example, these 'chamber effects' may offset some, if not all, of the leaf temperature increases associated with a decrease in g_s .

FACE experiments generally preserve the natural coupling between vegetation and the atmosphere and offer the best opportunity to determine how elevated $[\text{CO}_2]$ in future atmospheres will influence ET. Various techniques have been employed to estimate ET at the canopy scale at FACE sites, including micrometeorological, soil moisture, and sap flow measurements. Micrometeorological techniques have been used to assess the impact of elevated $[\text{CO}_2]$ on ET for potato (*Solanum tuberosum*; Magliulo *et al.*, 2003), rice (*Oryza sativa*; Yoshimoto *et al.*, 2005), wheat (*Triticum* spp.; Kimball *et al.*, 1995, 1999; Hunsaker *et al.*, 2000), cotton (*Gossypium* spp.; Hunsaker *et al.*, 1994; Kimball *et al.*, 1994), sorghum (*Sorghum* spp.; Conley *et al.*, 2001; Triggs *et al.*, 2004), and soybean (Bernacchi *et al.*, 2007). With the exceptions of cotton and sorghum grown with low water availability, a consistent decrease in ET ranging from 5% to 20% was observed at elevated $[\text{CO}_2]$ depending on species and measurement location (Fig. 2). The experiments utilizing micrometeorological techniques rely on measuring three of the four major energy fluxes associated with a plant

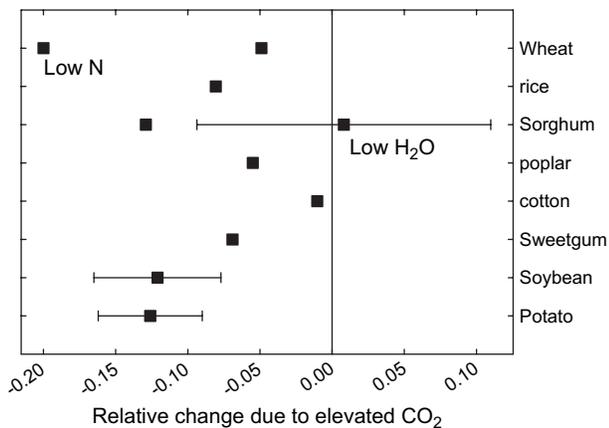


Fig. 2. A summary of results from FACE experiments of change in evapotranspiration as a result of growth in elevated CO_2 of c. $550 \mu\text{mol mol}^{-1}$ relative to control. Data for potato from Magliulo *et al.* (2003), rice from Yoshimoto *et al.* (2005), wheat from Hunsaker *et al.* (2000) and Kimball *et al.* (1995), cotton from Hunsaker *et al.* (1994) and Kimball *et al.* (1994), sorghum from Triggs *et al.* (2004), and soybean from Bernacchi *et al.* (2007). Graph is redrawn from Hatfield *et al.* (2008) and reproduced with permission.

canopy: net radiation, sensible heat flux, and soil heat flux. ET is then solved as the residual energy component not accounted for by those fluxes. While this method has known limitations, as discussed previously (Yoshimoto *et al.*, 2005), these results are consistent with observations made for different tree species grown at FACE sites using sap flow gauges (Wullschlegel and Norby, 2001; Tommasi *et al.*, 2002) and with soil moisture measurements, as discussed below. Despite the general consistency among the experiments listed above, the growth of poplar in elevated CO_2 showed a reduction in g_s for leaf-level measurement at POP/EURO FACE (Bernacchi *et al.*, 2003; Tricker *et al.*, 2008), but sap flow measurements over the similar time periods showed lower whole-plant transpiration in one (Tommasi *et al.*, 2002) and higher whole-plant transpiration in another (Tricker *et al.*, 2008) study using the same poplar experiment. This may suggest that there are exceptions to an otherwise generalized response. It could also suggest that there are other interacting environmental factors in play. For example, as discussed below, decreased ET at elevated $[\text{CO}_2]$ preserves soil moisture, which would allow for greater ET than the controls during the early stages of a drought period, although it is unlikely to be the explanation at the POP/EURO FACE experiment where irrigation was used, presumably removing the opportunity for greater moisture availability in the elevated CO_2 .

The effect of decreased ET at elevated $[\text{CO}_2]$ on the canopy hydrological cycle is an increase in soil moisture, as observed for a range of plants including sorghum (Conley *et al.*, 2001), cotton (Hunsaker *et al.*, 1994), wheat (Hunsaker *et al.*, 1996, 2000), pine forest (Ellsworth, 1999), grass species (Kammann *et al.*, 2005), and maize (Leakey *et al.*, 2006b). A hypothesis surrounding the increase in soil moisture associated with lower ET is that plants will be less susceptible to dry intervals during the growing season. Recently, it was demonstrated that soybean grown in elevated $[\text{CO}_2]$ did not show a decline in ET during a protracted dry period whereas the control plants did (Bernacchi *et al.*, 2007), although it is unlikely that elevated $[\text{CO}_2]$ will alleviate the responses of vegetation to severe drought conditions. Has the increase in CO_2 that plants have experienced thus far already altered evapotranspiration? Current model predictions indicate that runoff from continental interiors has risen as a direct consequence of decreased ET (Betts *et al.* 2007), which is at least partially validated by the results from ET measurements at various FACE sites.

The highly circulated atmosphere associated with chamber-based fumigation techniques makes it difficult to assess how well leaf- and canopy-scale conductances are normally coupled. If these scales of water use are well coupled, then the potential exists to assess ecosystem-level responses from leaf-level data. Data from the SoyFACE experiment show that, when averaged over a growing season, there is strong coupling between changes in stomatal conductance at the leaf level and whole-canopy water use (Fig. 2). Further, the lack of stomatal acclimation to $[\text{CO}_2]$ (Leakey *et al.*, 2006a) means that the relationship of g_s to ET would be similar for plants

grown in both ambient and elevated [CO₂], a conclusion supported by data from SoyFACE (Bernacchi *et al.*, 2007). There will, in addition, be important regional-scale feedback via changes in humidity, as well as other climate feedbacks related to elevated [CO₂] on water use that are likely to be important in the real world.

Lesson summary

That elevated CO₂ reduces g_s has long been well established from a wide range of experiments. FACE experiments extend these findings showing that the decrease g_s is upheld when plants are grown under experimental conditions that allow for the natural coupling of the plants and the atmosphere to be upheld. Most importantly, FACE experiments have shown that the leaf level and canopy level responses are consistent—namely that leaf level decreases in water use scale to the canopy and that the decrease in water use translates to higher soil moisture availability.

Lesson 4: dark respiration is significantly stimulated in soybean leaves grown under elevated [CO₂]

Respiration fulfils the vital functions of producing ATP, reducing power and carbon-skeleton intermediates, while consuming O₂ and releasing CO₂. Respiration is an important determinant of plant carbon balance and crop yield (Amthor, 1989) as well as a key factor controlling the carbon balance of ecosystems (Valentini *et al.*, 2000). At the global scale, the flux of carbon from plant respiration is 5–6-fold greater than anthropogenic emissions (Prentice *et al.*, 2001; Canadell *et al.*, 2007) demonstrating that understanding respiratory responses as growing conditions are altered by climate change is a fundamental issue, with significance from cellular to biogeochemical scales. Although the potential for rising atmospheric [CO₂] to alter plant respiration has been the subject of debate for many years (Ford and Thorne, 1967; Gifford *et al.*, 1985; Farrar and Williams, 1988; Amthor, 1991; Drake *et al.*, 1999), it is still relatively poorly understood (Gonzalez-Meler *et al.*, 2004), particularly by comparison with photosynthesis. There are unique challenges to measuring the respiration of leaves, stems, and roots (Hanson *et al.*, 2000; Davey *et al.*, 2004; Moore *et al.*, 2008) and the relationship between plant productivity and respiration is variable in each of these tissues (Litton *et al.*, 2007). Given that there will probably be tissue-specific responses, as well as the recent evaluation of results from FACE studies into the effects of elevated [CO₂] on soil respiration (King *et al.*, 2004) and stem respiration (Moore *et al.*, 2008), this lesson focuses on the dark respiration responses of leaves grown in elevated [CO₂].

Much of the uncertainty surrounding the effects of elevated [CO₂] on leaf respiration has resulted from the difficulty in measuring the rate of respiration with gas exchange techniques. Until recently, the most common

approach has been to use an open gas-exchange system, incorporating IR CO₂ analysers designed for assessing photosynthesis, to measure the rate of CO₂ efflux from a tissue sample. Many early studies used this technique to assess whether there was any instantaneous effect of [CO₂] on respiration. On average, doubling the [CO₂] around a leaf was reported to inhibit respiratory CO₂ efflux by 15–20% (Amthor, 1997; Curtis and Wang, 1998; Drake *et al.*, 1999). However, it was demonstrated that measurement artefacts mimicked the instantaneous inhibition of respiration when, in fact, none was occurring (Gifford, 1991; Gonzalez-Meler and Siedow, 1999; Jahnke, 2001; Jahnke and Krewitt, 2001). When respiration has been measured as O₂ uptake, or when CO₂ efflux has been measured in a manner that rigorously avoided leaks and diffusion between the chamber and the atmosphere through gaskets or leaves, little or no instantaneous effect of [CO₂] was detected (Amthor *et al.*, 2001; Jahnke, 2001; Jahnke and Krewitt, 2001; Davey *et al.*, 2004). Further details are available in reviews by Gifford (2003) and Gonzalez-Meler *et al.* (2004).

Long-term growth of plants at elevated [CO₂] is also expected to alter the rates of respiration in response to the stimulation of photosynthesis and biomass production. Rates of respiration can be controlled by either the demand for ATP, when respiration is ADP-limited (Beevers, 1974; Bingham and Farrar, 1988), or by the availability of substrate when respiration is not ADP-limited (Breeze and Elston, 1978; Azcón-Bieto and Osmond, 1983). Williams and Farrar (1990) argued that the availability of substrates, primarily carbohydrates, determines the longer term capacity for respiration while the current demand for ATP controls respiratory flux in the shorter term. The mechanism of soybean respiratory responses to growth at elevated [CO₂] has been investigated in two studies that combined molecular, biochemical, and physiological analyses of plants at the SoyFACE experiment in which the findings were consistent with the hypotheses of Williams and Farrar (1990). Ainsworth *et al.* (2006) examined transcript profiles, leaf carbohydrate status, and growth rate of both mature and growing leaves in an early vegetative developmental stage. Leakey *et al.* (2009a) examined transcript profiles, leaf carbohydrate status, rates of photosynthesis and respiration of mature leaves at multiple developmental stages, over two growing seasons. Together, the studies provide evidence that long-term growth at elevated [CO₂] leads to transcriptional reprogramming of metabolism that stimulates respiration. This involves a greater abundance during the day and night of transcripts encoding many enzymes of starch and sugar metabolism, glycolysis, the TCA cycle, and mitochondrial electron transport under elevated [CO₂] (Fig. 3). The number of transcripts reported to have significantly different abundance under ambient and elevated [CO₂] at night (Ainsworth *et al.*, 2006) was smaller than during the day (Leakey *et al.*, 2009a). However, this may reflect the smaller number of dates upon which transcript profiling could be performed on samples collected at night (1 date; Ainsworth *et al.*, 2006) versus during the day (9 dates; Leakey *et al.*, 2009a), rather than a biological

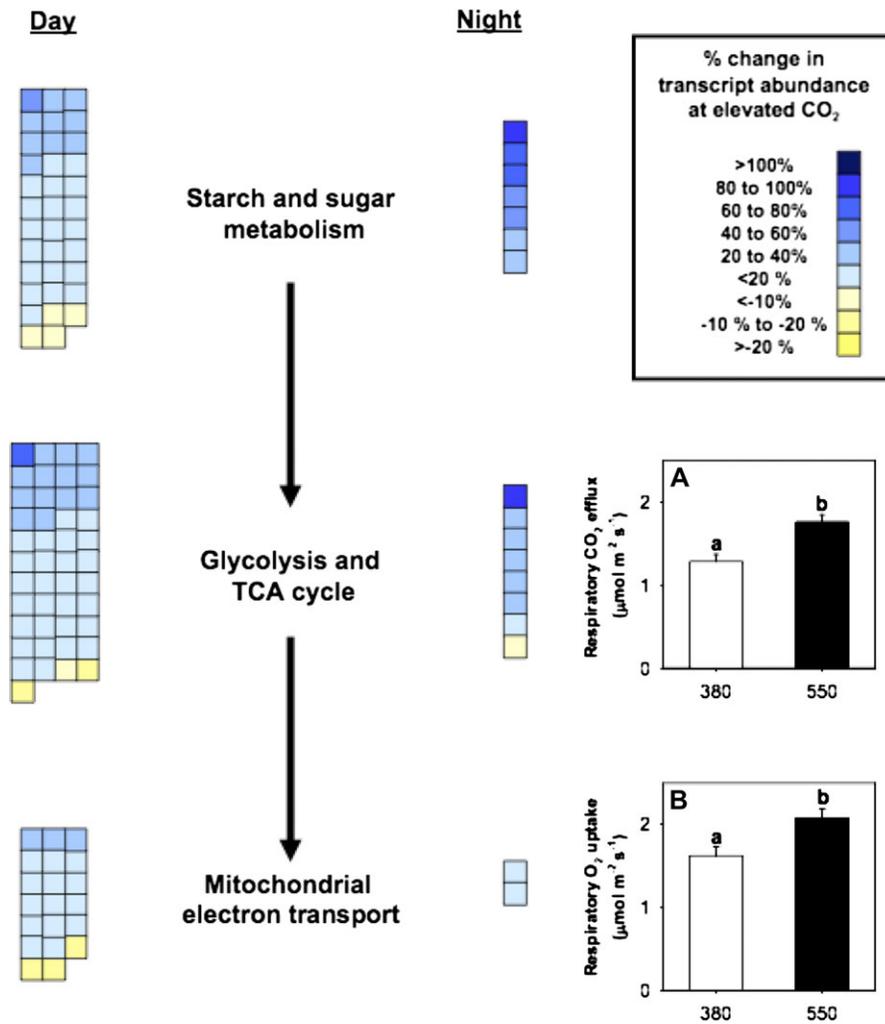


Fig. 3. Graphical representation of transcripts encoding enzymes of carbohydrate metabolism and respiration whose abundance is significantly altered by growth at elevated [CO₂] during the day and night in the leaves of soybean grown at SoyFACE. Each blue or yellow box represents the statistically significant treatment response ($P < 0.05$) of a unique transcript encoding an enzyme or protein structure. Insets show mean treatment values (\pm SE) of the (A) night-time rates of respiratory CO₂ efflux and (B) respiratory O₂ uptake of soybean leaves grown at ambient and elevated [CO₂]. Means sharing a common letter are not statistically different. All data are average responses to elevated [CO₂] (550 $\mu\text{mol mol}^{-1}$) compared to ambient [CO₂] (380 $\mu\text{mol mol}^{-1}$), redrawn from data in Ainsworth *et al.* (2006) and Leakey *et al.* (2008).

phenomenon. The consistency of the results between the two studies is particularly significant given that two different microarray platforms were used for each of the analyses. While changes in transcript abundance do not always relate directly to changes in protein content, activity, and physiology (Leakey *et al.*, 2009b), greater gene expression for the machinery of carbohydrate metabolism and respiration would be necessary to produce the greater number of mitochondria per cell observed in many species, including soybean, under elevated [CO₂] (Griffin *et al.*, 2001).

In mature leaves no longer undergoing growth, greater respiration under elevated [CO₂] was associated with stimulated photosynthesis and increased carbohydrate content during the day and faster carbohydrate utilization at night (Fig. 3; Ainsworth *et al.*, 2006; Leakey *et al.*, 2009a). Respiration was measured using a gas exchange system

designed to avoid the measurement artefacts that can falsely indicate short-term sensitivity of respiration to [CO₂]. With this approach, stimulated respiratory flux was demonstrated as greater CO₂ efflux as well as greater O₂ uptake (Fig. 3). This is consistent with the observation that all other factors which increase diurnal carbon gain and foliar carbohydrate content are also reported to stimulate night-time respiration (Azcón-Bieto and Osmond, 1983; Dewar *et al.*, 1999; Whitehead *et al.*, 2004). The ratio of O₂ uptake to CO₂ efflux (respiratory quotient) was also greater at elevated [CO₂] compared to ambient [CO₂] (Leakey *et al.*, 2009a) indicating that the larger carbohydrate pools resulting from stimulated photosynthesis at elevated [CO₂] were subsequently forming a larger fraction of the substrate pool for respiration. In addition to increasing substrate availability, increased accumulation of non-structural carbohydrate in leaves over the course of the day is likely

to have increased energy demand for mobilization and translocation. This could be significant, since phloem loading accounts, on average, for ~30% of night-time respiratory energy demand in leaves (Bouma *et al.*, 1995; Amthor, 2000). In growing leaves, the increased abundance of transcripts encoding enzymes of carbohydrate metabolism and respiration under elevated $[\text{CO}_2]$ was associated with greater growth (Ainsworth *et al.*, 2006), although rates of respiration were not directly measured.

Increased photosynthesis and total non-structural carbohydrate (TNC) content at elevated $[\text{CO}_2]$ has not been observed to be associated with greater respiration in other FACE studies. No treatment effect on respiration per unit leaf-area was detected in *Populus alba*, *Oryza sativa*, *Acer saccharum*, *Betula papyrifera*, *Liquidambar styraciflua*, or *Pinus taeda* (Hamilton *et al.*, 2001; Tissue *et al.*, 2002; Davey *et al.*, 2004; Xu *et al.*, 2006; Loreto *et al.*, 2007). Detection of treatment effects at the SoyFACE experiment may have been favoured by (i) low environmental and genetic variability of soybean; (ii) greater replication by comparison with other studies ($n=4$ versus $n=3$); and (iii) measurement of respiratory gas exchange with apparatus that avoided leaks and produced large differential concentrations of O_2 and CO_2 as a result of respiratory fluxes. Alternatively, the stimulation of respiration by elevated $[\text{CO}_2]$ may be greater in soybean than other species so far examined. It has often been suggested that while greater photosynthesis and leaf TNC at elevated $[\text{CO}_2]$ favour greater respiration, other changes at elevated $[\text{CO}_2]$ might be decreasing respiration. One difference between elevated $[\text{CO}_2]$ and other environmental factors increasing photosynthesis and TNC is that leaf nitrogen (N) per unit leaf area is commonly and significantly reduced (~4%) in elevated $[\text{CO}_2]$ (see Lesson 2 above; Long *et al.*, 2004). This response is very small, or non-existent in soybean because of its capacity as a legume to fix nitrogen. However, in non-legumes lower N may reduce the leaf level sink for products of respiration, such as in protein turnover, and this has been cited as the cause of lower respiration at elevated $[\text{CO}_2]$, despite greater carbon gain. This explanation assumes that N is a good proxy for leaf protein content (Lambers *et al.*, 1983) and that protein turnover is the primary sink for respiratory products (Amthor, 2000). Analysis of the cause of the decrease in N across the FACE experiments suggests that almost all of the decline in leaf N per unit leaf area could be accounted for by a decrease in Rubisco (Long *et al.*, 2004). In mature leaves there is little or no turnover of Rubisco (Mae *et al.*, 1983; Suzuki *et al.*, 2001). In such a case, decreased N would have little effect on respiration, but further work is needed to test this uncertainty directly. Assuming that the effects of TNC and N upon respiration are not mutually exclusive, the balance of these effects on substrate availability and sink demand within the leaf will determine the response of respiration to long-term growth at elevated $[\text{CO}_2]$. At least in soybean this appears to have the net result of stimulating respiration, but further work is needed to assess the responses of other species and functional groups.

Lesson summary

Dark respiration is strongly stimulated in soybean leaves grown under elevated $[\text{CO}_2]$. The response is driven by greater gene expression for the respiratory machinery and greater substrate availability. However, increased dark respiration has been not been observed in many other species grown with FACE elevated $[\text{CO}_2]$.

Lesson 5: stimulation of carbon uptake by elevated $[\text{CO}_2]$ in C_4 plants is indirect and occurs only in situations of drought

There is a clear mechanistic basis for the stimulation of photosynthesis in C_3 plants that is lacking in C_4 plants. An increase in the atmospheric $[\text{CO}_2]$ from the ambient level of 2009 ($384 \mu\text{mol mol}^{-1}$) to the $550 \mu\text{mol mol}^{-1}$ or $700 \mu\text{mol mol}^{-1}$ levels conservatively projected for the middle and end of this century, produces, in C_3 soybean, a rise in the intercellular $[\text{CO}_2]$ from approximately $270 \mu\text{mol mol}^{-1}$ to $384 \mu\text{mol mol}^{-1}$ or $490 \mu\text{mol mol}^{-1}$ (Fig. 4). The biochemical basis for the resulting stimulation of photosynthesis by 29% under 2050 conditions or 39% under 2100 conditions is a higher $[\text{CO}_2]$ around Rubisco that accelerates the carboxylation reaction while suppressing the competing oxygenation reaction (Bowes, 1991). In C_4 plants, the initial slope of the A/C_i relationship is much steeper and carbon uptake is saturated by CO_2 at a lower C_i

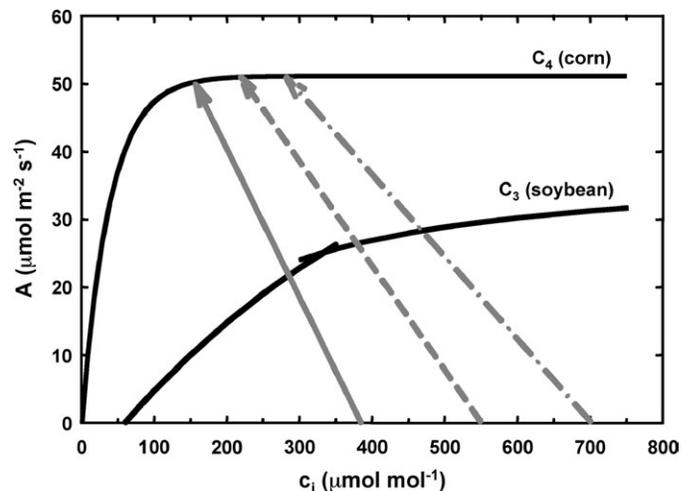


Fig. 4. Comparing the dependence of photosynthesis (A) on intercellular CO_2 concentration (C_i) for C_4 maize and C_3 soybean. The maize data were fit using the equations for C_4 photosynthesis (von Caemmerer, 2000). The soybean data fit according to the C_3 leaf biochemical model of photosynthesis of Farquhar *et al.* (1980). The grey lines illustrate the supply function for CO_2 , starting at the atmospheric $[\text{CO}_2]$ and ending at the operating C_i of photosynthesis. This is illustrated for current atmospheric $[\text{CO}_2]$ ($384 \mu\text{mol mol}^{-1}$, solid line), elevated $[\text{CO}_2]$ anticipated for 2050 ($550 \mu\text{mol mol}^{-1}$, dashed line), and elevated $[\text{CO}_2]$ anticipated for 2080 ($700 \mu\text{mol mol}^{-1}$, dashed and dotted line). This figure was redrawn using data from Leakey (2009).

(Fig. 4) because the primary carboxylase, phosphoenolpyruvate carboxylase (PEPc), has a lower K_m for CO_2 and O_2 is not a competitive substrate and because C_4 plants concentrate CO_2 around Rubisco up to six times ambient in specialized bundle sheath cells. This much greater $[\text{CO}_2]$ effectively saturates the carboxylation reaction and essentially abolishes photorespiration (von Caemmerer and Furbank, 2003). Thus, in C_4 maize plants, an increase in atmospheric $[\text{CO}_2]$ from $384 \mu\text{mol mol}^{-1}$ to $550 \mu\text{mol mol}^{-1}$ or $700 \mu\text{mol mol}^{-1}$ increases C_i but has no impact on carbon uptake (Fig. 4). Only if the operating C_i of photosynthesis under ambient $[\text{CO}_2]$ is below the inflexion point of the A/C_i curve or if growth at elevated $[\text{CO}_2]$ altered the shape of the A/C_i curve could C_4 photosynthesis be directly stimulated by increases in the atmospheric $[\text{CO}_2]$.

While this theory was borne out in many elevated $[\text{CO}_2]$ experiments performed with C_4 plants in growth chambers and other sorts of enclosures (Hocking and Meyer, 1991; Ziska *et al.*, 1991; Samarakoon and Gifford, 1996; Ghannoum *et al.*, 1998, 2000), enhanced carbon uptake under elevated $[\text{CO}_2]$ was observed in other studies on C_4 plants (Knapp *et al.*, 1993; Amthor *et al.*, 1994; Poorter *et al.*, 1996; Wand *et al.*, 1999; Anderson *et al.*, 2001).

Three large-scale FACE experiments have studied the impact of elevated $[\text{CO}_2]$ on carbon uptake in C_4 species in which the data are consistent with elevated $[\text{CO}_2]$ having no direct effect on carbon uptake in C_4 plants. Sorghum was grown at ambient ($\sim 370 \mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ ($\sim 570 \mu\text{mol mol}^{-1}$) with irrigation or drought stress in Maricopa, AZ. Carbon uptake by the youngest fully expanded leaf from irrigated plots was not enhanced by elevated $[\text{CO}_2]$ (Wall *et al.*, 2001). Stimulation of carbon uptake was, however, observed during drought or at midday when short-term water stress developed under high atmospheric vapour pressure deficits and temperatures (Cousins *et al.*, 2002). In the managed grassland FACE experiment in New Zealand, measurements of A/C_i curves showed the C_4 grass *Paspalum dilatatum* growing at both ambient ($360 \mu\text{mol mol}^{-1}$) and elevated ($475 \mu\text{mol mol}^{-1}$) $[\text{CO}_2]$ to be operating at or close to saturating C_i explaining the lack of response of carbon uptake to growth at elevated $[\text{CO}_2]$ (von Caemmerer *et al.*, 2001). Maize was grown at ambient ($\sim 370 \mu\text{mol mol}^{-1}$) and elevated $[\text{CO}_2]$ ($\sim 550 \mu\text{mol mol}^{-1}$) at SoyFACE in Urbana, IL. There was no CO_2 effect on carbon uptake except when the crop was experiencing drought stress (Leakey *et al.*, 2004, 2006b). Measurements on fully expanded leaves showed that the operating C_i was above the inflexion point of the A/C_i curve and that photosynthesis was CO_2 -saturated at both $[\text{CO}_2]$ levels. When there was no drought stress at any stage of the season, maize grown at ambient and elevated $[\text{CO}_2]$ produced the same final biomass and yield.

The most likely explanation for the apparent difference in effects of elevated $[\text{CO}_2]$ on carbon uptake in C_4 plants between FACE experiments and those conducted in enclosure studies is rooting volume. The deep rooting of maize, sorghum, and millet in the field (commonly 1–2 m; Allen *et al.*, 1998; Carcova *et al.*, 2000) provides water from

a much larger soil volume than pots used in some enclosure experiments (e.g. 3.5 l in Ziska and Bunce, 1997; Ziska *et al.*, 1999; 5.0 l in Wong, 1979; Maroco *et al.*, 1999). Even when pots are well watered, there may not be adequate root volume to absorb enough water to meet the requirements of the shoot fully. In this circumstance, growth at elevated $[\text{CO}_2]$ could reduce the water requirements and alleviate this stress giving the misimpression that elevated $[\text{CO}_2]$ directly stimulates carbon gain. Other enclosure studies have avoided this problem by growing plants in open-top chambers (OTCs) with unrestricted rooting depths (Amthor *et al.*, 1994). But under high light conditions, air temperature and vapour pressure deficit within OTCs can be substantially greater than outside the enclosure (Whitehead *et al.*, 1995) promoting water stress in the shoot, particularly around midday.

Overall, FACE results extend the evidence presented by Ghannoum *et al.* (2000) showing that carbon uptake in C_4 plants is not directly stimulated by elevated $[\text{CO}_2]$. In addition, FACE experiments show that elevated $[\text{CO}_2]$ improves C_4 plant water relations and thereby indirectly enhances photosynthesis, growth, and yield by delaying and ameliorating drought stress. Elevated $[\text{CO}_2]$ reduced midday stomatal conductance of FACE-grown sorghum by 32% with irrigation and by 37% under drought stress (Wall *et al.*, 2001). The effect of elevated $[\text{CO}_2]$ on whole plant water use was smaller, but still significant (Conley *et al.*, 2001). Although, under the arid growing conditions in Maricopa, AZ, irrigation was unable to prevent transient drought stress particularly at midday, the effect of elevated $[\text{CO}_2]$ for sorghum under severe drought stress was much stronger, resulting in an improved leaf water potential that translated into 23% greater midday photosynthesis over the two seasons. Maize grown at SoyFACE in a rain-fed experiment allowed comparison between an 'average' year (2002) that included periods of drought stress versus an 'atypical' year (2004) in which the crop experienced no drought stress across the entire season (Leakey *et al.*, 2006). Maize leaf g_s was 23% lower under elevated $[\text{CO}_2]$ in 2002 and 29% lower in 2004. As for sorghum, this translated into a decrease in whole-plant water use (soil moisture under elevated $[\text{CO}_2]$ was up to 31% greater between 5 cm and 25 cm depth, and up to 11% greater between 25 cm and 55 cm depth; Leakey *et al.*, 2006). Of course, this indirect mechanism of enhanced carbon uptake by elevated $[\text{CO}_2]$ is not unique to C_4 plants. Decreased stomatal conductance at elevated $[\text{CO}_2]$ in a C_3 soybean canopy also led to a significant reduction in canopy evapotranspiration (Bernacchi *et al.*, 2007). Decreased stomatal conductance can increase canopy temperatures inside elevated $[\text{CO}_2]$ plots relative to outside the plots. However, in the absence of drought stress in 2004 at SoyFACE, elevated $[\text{CO}_2]$ was estimated to increase leaf temperature by only $0.26 \text{ }^\circ\text{C}$ and photosynthesis by $0.3 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and had no apparent effect on the diurnal course of photosynthesis or end of season biomass (Leakey *et al.*, 2006). Larger average maximum temperature differences between ambient and elevated $[\text{CO}_2]$ -grown plants of $1.47 \text{ }^\circ\text{C}$ and $1.85 \text{ }^\circ\text{C}$ were observed in the well-watered

treatments of sorghum in Maricopa, AZ (Triggs *et al.*, 2004). Enclosure studies have also provided evidence for this indirect mechanism of enhanced carbon uptake by C_4 plants, with reduced water use reported at elevated $[CO_2]$ compared to ambient $[CO_2]$ in chamber-grown maize (-25% to 30% ; Samarakoon and Gifford, 1996), *Panicum coloratum* (-17% ; Seneweera *et al.*, 1998), and *Bouteloua gracilis* (Morgan *et al.*, 1998). In all three of these cases, plant water status under drought conditions was improved, resulting in greater photosynthesis and biomass accumulation.

Lesson summary

FACE experiments establish that, as expected from theory, C_4 photosynthesis is not directly stimulated by elevated $[CO_2]$. Nevertheless, there is significant potential for increased growth of C_4 plants at elevated $[CO_2]$ to decrease water use and reduce drought stress, leading via this indirect mechanism to greater photosynthesis and yield.

Lesson 6: the $[CO_2]$ 'fertilization' effect in FACE studies on crop plants is less than expected

Generally, the increase in C_3 photosynthesis in FACE studies was greater than increases in biomass or yield (Nowak *et al.*, 2004; Ainsworth and Long, 2005), yet the magnitude of the stimulations for crop plant species was lower than predicted from theory and from observations in protected environments (Long *et al.*, 2004, 2006). The average increase in light-saturated photosynthesis in 45 species measured at 11 different FACE studies was 13% , while above-ground production increased by 17% and yield increased by 16% at elevated $[CO_2]$. The results of the FACE experiments show that photosynthesis of crop plants fails to match the theoretical increase that could be obtained at elevated $[CO_2]$ and that a decreased proportion of the increase in photosynthesis is partitioned into the harvestable grain (Long *et al.*, 2006). However, chamber experiments show that germplasm is capable of achieving the theoretical improvement. In addition, FACE studies on woody species showed an average 28% increase in above-ground dry matter production for trees grown under elevated $[CO_2]$ (Ainsworth and Long, 2005) which is much closer to the theoretical expectation than has been seen for crop species. The challenge now is understanding, and in turn overcoming, the factors that prevent our major crops from realizing the full benefit in the farm setting (Boyer, 1982; Zhu *et al.*, 2004, 2007, 2008; Murchie *et al.*, 2008). This will require an integration of research from genomics through physiology to agronomy.

A meta-analysis of 111 elevated $[CO_2]$ chamber studies with soybean (Ainsworth *et al.*, 2002) and results from the SoyFACE experiment (Ainsworth *et al.*, 2004; Rogers *et al.*, 2004; Bernacchi *et al.*, 2005) provide a direct comparison of FACE and chamber studies in a single, agronomically important species. When limiting the database of chamber

studies to those with elevated $[CO_2]$ treatments between $450 \mu\text{mol mol}^{-1}$ and $550 \mu\text{mol mol}^{-1}$, the average increase in A_{sat} was 24% (Fig. 5). Bernacchi *et al.* (2005) measured A_{sat} every 2 weeks throughout two growing seasons at SoyFACE and reported a smaller 18% increase in elevated $[CO_2]$. A meta-analysis of SoyFACE results indicates an average 16% increase in A_{sat} across a variety of soybean cultivars, which is substantially smaller than suggested by chamber studies (Fig. 5). At SoyFACE, the largest stimulation in A_{sat} occurred during grain-filling (Bernacchi *et al.*, 2005) averaging 22% during vegetative growth, 29% during pod-fill and 26% during flowering. Over the course of the first growing season at SoyFACE, midday g_s was reduced by 21.9% in elevated $[CO_2]$ (Rogers *et al.*, 2004). g_s under saturating light conditions was reduced on average by 14% at SoyFACE (Fig. 5; Ainsworth *et al.*, 2004; Bernacchi *et al.*, 2005). Canopy photosynthesis was stimulated by 59% in soybeans grown under elevated $[CO_2]$ in controlled environments, with an average elevated $[CO_2]$ treatment of $761 \mu\text{mol mol}^{-1}$. Although the SoyFACE treatment was lower ($550 \mu\text{mol mol}^{-1}$), the degree of enhancement in diurnal carbon uptake at SoyFACE was considerably lower, only 25% over the course of the first growing season (Rogers *et al.*, 2004), perhaps reflecting differences in reduction of g_s . The database of chamber studies suggests that photosynthetic capacity does not change in soybeans grown under elevated $[CO_2]$. Neither $V_{\text{c,max}}$ nor the apparent quantum yield of photosynthesis when measured at a common $[CO_2]$ was altered by growth at elevated $[CO_2]$ (Fig. 5). However, there was a small, yet statistically significant decrease in

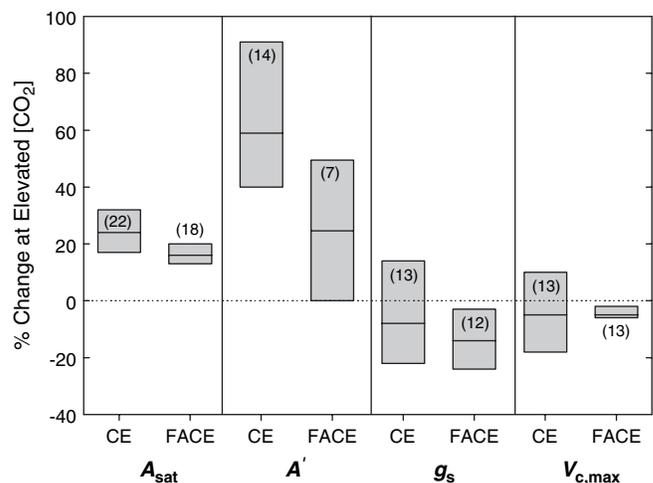


Fig. 5. A comparison of light-saturated photosynthesis (A_{sat}), daily carbon uptake (A'), stomatal conductance (g_s), and *in vivo* maximum carboxylation rate ($V_{\text{c,max}}$) from a meta-analysis of controlled environment (CE) studies of soybean grown at elevated $[CO_2]$ (Ainsworth *et al.*, 2002) and soybeans grown at elevated $[CO_2]$ in a Free-Air Carbon dioxide Enrichment (FACE) experiment (Ainsworth *et al.*, 2004; Bernacchi *et al.*, 2005; Rogers *et al.*, 2004). The mean \pm 95% confidence intervals are shown in the box plots and the sample size for each observation is given in parenthesis. Boxes overlapping 0 indicate no significant change at elevated $[CO_2]$.

$V_{c,max}$ at SoyFACE, which, in turn, drove a decrease in the $V_{c,max}:J_{max}$ inferring a shift in resource investment away from Rubisco (Bernacchi *et al.*, 2005). This change though was insufficient to take full advantage of the theoretical efficiency gain that could be achieved (Long *et al.*, 2004).

More broadly, the stimulation of seed yield in response to growth in elevated $[CO_2]$ is ~50% lower in FACE experiments than in enclosure studies for the world's four most important crops (Long *et al.*, 2006; Ainsworth, 2008; Ainsworth *et al.*, 2008a). With the caveat that FACE experiments have been limited in number and geographical coverage, the much lower elevated $[CO_2]$ fertilization factor on yield observed under agronomically relevant conditions has potentially serious implications for projections of world food supply. Indeed, Parry *et al.* (2004) conclude from their modelling work that the key to forecasting future global food supply is knowing the response of the world's leading grain crops to rising $[CO_2]$. A comparison of wheat yields from five different crop models with FACE results showed that the mean response ratio to elevated $[CO_2]$ was over-estimated by more than a factor of two in the model projections (Ainsworth *et al.*, 2008a). Thus model parameterization and validation with summary data from FACE and non-FACE studies show that the quantitative differences in how crops respond to CO_2 in the field compared to in chambers has important consequences for global food supply projections. Equally FACE has revealed factors operating in the open field situation that were not or cannot be identified by chamber experiments, for example, increased herbivory and performance of herbivore populations (Holton *et al.*, 2003; Hamilton *et al.*, 2004; Zavala *et al.*, 2008). Most important though will be understanding why our major food crops fail to achieve the improved production under elevated $[CO_2]$ that can be achieved in protected environments and by some non-crop species. Overcoming this could deliver a 10–15% increase in crop yields by 2050, an increase that could be critical with an anticipated 3 billion increase in global population coupled with climatic change adverse to crop production. This will require an interplay between fundamental laboratory plant biology research and large-scale genetic/genomic screening in realistic open-air FACE-type facilities (Ainsworth *et al.*, 2008b; Leakey *et al.*, 2009b).

Lesson summary

Controlled laboratory and field chambers have provided an immense database on plant responses to rising $[CO_2]$ and, more importantly, insight into potential mechanisms of response. FACE on the other hand, which allows treatment of plants under field conditions at a realistic scale, has provided an important reality check. It has both shown where hypotheses developed in controlled environments do or do not apply, as well as insights into the mechanisms that may cause the difference. Overwhelmingly, this has shown that data from laboratory and chamber experiments systematically overestimate the yields of the major food crops, yet may underestimate the biomass production of trees.

Improved projection of these hugely important parameterization data for predictive models will require many more FACE experiments, since the large-scale FACE experiments have been conducted at best at just one or two locations in a given ecosystem type.

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