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

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Received 6 January 2009; Revised 5 March 2009; Accepted 5 March 2009

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...
terrestrial vegetation since the early Miocene (Pearson and Palmer, 2000) and are facing a future that portends uncertain consequences of ever-increasing [CO₂]. Understanding how plants have and will respond to the rapid change in [CO₂], 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 [CO₂] 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 ≥ 3) large plots (>100 m²) 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 [CO₂] despite acclimation of photosynthetic capacity.**

A first lesson from recent FACE studies of C₃ photosynthetic responses to elevated [CO₂] is that photosynthetic carbon uptake (A) is enhanced by elevated [CO₂] despite acclimation of photosynthetic capacity. Photosynthetic acclimation is most commonly measured as a decreased maximum carboxylation rate of Rubisco (Vₖₘₚₐₓ) and maximum electron transport rate leading to ribulose-1,5-bisphosphate (RuBP) regeneration (Jₘₚₐₓ) (reviewed in Long et al., 2004; Nowak et al., 2004; Ainsworth and Long, 2005; Ainsworth and Rogers, 2007). While early studies of C₃ 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 Vₖₘₚₐₓ and Jₘₚₐₓ, the light-saturated rate of photosynthetic carbon uptake (Aₕₖₚₐₜ) is markedly stimulated in C₃ plants grown at elevated [CO₂] (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 C₃ plants grown at elevated [CO₂]? Using data from FACE experiments, the difference in the magnitude of stimulation of A among species and functional groups was explained by the process that limited A at a given intercellular [CO₂] (Cᵢ) (Ainsworth and Rogers, 2007). The A/Cᵢ response curve, as predicted from the C₃ leaf model of photosynthesis (Farquhar et al., 1980), shows a biphasic response of A to Cᵢ (Fig. 1). As Cᵢ is increased from a minimum concentration, the rate of change in A is great and determined by the activity of Rubisco (Vₖₘₚₐₓ). With a further increase in Cᵢ, there is an inflection to a lower rate of increase in A where RuBP-regeneration capacity (Jₘₚₐₓ) 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 C₄ or legumes) with Rubisco-limited photosynthetic capacity at elevated [CO₂], there is a larger potential for the stimulation of A because elevated [CO₂] 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 [CO₂], showed greater stimulation of A compared to legumes, shrubs, and non-leguminous C₃ crops that were limited by RubP regeneration capacity at elevated [CO₂] (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...
provides a mechanistic basis for the greater than average stimulation in \( A \) observed in trees (46%) and grasses (37%) grown at elevated [CO\(_2\)], compared to shrubs (21%), C\(_3\) 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 [CO\(_2\)] (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 [CO\(_2\)] for a decade (Lu¨ scher et al., 2006), both seasonal (Ainsworth et al., 2003b) and harvest practices (Rogers et al., 1998; I copp et al., 2000; Ainsworth et al., 2003a) played important roles in determining the photosynthetic response of L. perenne and T. repens to elevated [CO\(_2\)]. 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 [CO\(_2\)] (circle symbols in Fig. 1; Ainsworth et al., 2003b). Following the supply functions (grey dashed lines in Fig. 1) from the \( A\slash C\_i \) curves to the y-axis illustrates that \( A \) was stimulated by \( \sim 40\% \) by growth at elevated [CO\(_2\)] 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 \( V_{c\text{,max}} \) and \( J_{\text{max}} \) in elevated [CO\(_2\)] 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 \( A\slash C\_i \) 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, acclimation of photosynthetic capacity to elevated [CO\(_2\)], and therefore degree of stimulation of carbon uptake.

Genetic factors can also play an important role in photosynthetic response to elevated [CO\(_2\)]. This was perhaps most clearly demonstrated with fast-growing Populus trees (poplars) exposed to elevated [CO\(_2\)] in the PopFACE experiment (Scarascia-Mugnozza et al., 2006). Poplars grown for coppice sustained a 55% stimulation in \( A \) at elevated [CO\(_2\)] (Bernacchi et al., 2003) because of their large capacity for starch synthesis and carbon export (Davey et al., 2006). Poplars exported \( >90\% \) of their photosynthetic during the day and stored the rest of the overflow photosynthetic as starch (Davey et al., 2006), which enabled the trees to avoid acclimation of photosynthetic potential, and maintain maximal stimulation of \( A \) at elevated [CO\(_2\)]. 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 [CO\(_2\)] 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 \( V_{c\text{,max}} \) to \( J_{\text{max}} \) (Long et al., 2004; Ainsworth and Long, 2005). However, this potential shift in \( V_{c\text{,max}} \), \( J_{\text{max}} \) is based on data that used the internal [CO\(_2\)] (\( C_i \)) for calculations of photosynthetic parameters, rather than the chloroplast [CO\(_2\)] (\( C_c \)). 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 CO\(_2\) 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 [CO\(_2\)] alters mesophyll conductance? A FACE study of soybean (Glycine max) suggested that mesophyll conductance was not altered by growth at elevated [CO\(_2\)] (Bernacchi et al., 2005); however, Singsaas et al. (2004) found in understory trees that changes in mesophyll conductance at elevated [CO\(_2\)] were species and condition-dependent. Flexas et al. (2008) also reported a large decrease in mesophyll conductance when leaves were exposed to elevated [CO\(_2\)] 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 \( V_{c\text{,max}} \), \( J_{\text{max}} \); 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 [CO\(_2\)] in C\(_3\) 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 [CO₂] anticipated for the middle of this century.

**Lesson 2: photosynthetic nitrogen use efficiency increases at elevated [CO₂]**

In theory, photosynthetic nitrogen use efficiency (PNUE), defined here as the net amount of CO₂ assimilated per unit of leaf N, has the potential to increase in C₃ plants grown at elevated [CO₂] 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 [CO₂] thereby offsetting gains in C acquisition. Results from FACE experiments provide the most confident projections of future changes in PNUE at elevated [CO₂] and have identified potential limitations on plant responsiveness to rising [CO₂].

Theory predicts that a C₃ plant for which photosynthesis is Rubisco limited at both 380 and 580 μmol mol⁻¹ [CO₂] could show a stimulation of photosynthesis by elevated [CO₂] at 25 °C by as much as 40% (Farquhar et al., 1980). A plant where assimilation is limited solely by RubP regeneration at both CO₂ 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 [CO₂] (see Lesson 1 above).

Acclimation to elevated [CO₂] does occur (see Lesson 1), but how much N is conserved at elevated [CO₂] due to Rubisco acclimation? Earlier investigations into the response of plants to rising [CO₂] often reported marked (>50%) reductions in Rubisco content at elevated [CO₂], 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 [CO₂] 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 Vc,max to growth at elevated [CO₂] using FACE technology showed that, on average, Vc,max was reduced by ~10% in plants grown at elevated [CO₂]. The magnitude of this response varied among functional groups (e.g. legumes 7%, crops 17%, grasses 16%, and trees 6%). Table 1 shows that the maximum possible saving in leaf N due to Rubisco acclimation at the elevated [CO₂] typical of current FACE experiments is smaller than anticipated. Even excluding trees that are Rubisco limited at current and elevated [CO₂] and are not hypothesized to show marked acclimation, the mean saving in leaf N is still small, ~3.6%. This estimate assumes that the increase in Vc,max at elevated [CO₂] 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 [CO₂] (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 [CO₂] in FACE experienced a reduction in leaf N content that would impact PNUE? Evidence from meta-analysis suggests that reductions in leaf Narea (leaf N content expressed per unit leaf area) are small, ~4%. Given that reductions in Vc,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 Vc,max at elevated [CO₂] 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 Narea. Comparisons of PNUE at current and elevated [CO₂] are confounded by [CO₂], species, N supply, age, and N storage strategy (Sage and Pearcy, 1987) so a response ratio (PNUE at elevated [CO₂]/PNUE at current [CO₂]) was

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**Table 1. Estimates of the maximum theoretical saving in leaf N due to Rubisco acclimation at the elevated [CO₂] used in FACE experiments**

| Rubisco content* (mg m⁻²) | Maximum potential N saving at elevated [CO₂] (mg m⁻²) | Percentage N saving at elevated [CO₂]* |
|---------------------------|--------------------------------------------------|--------------------------------------|
| 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                                 |

*Rubisco content was calculated from values of Vc,max presented previously (Ainsworth and Rogers, 2007) with the following assumptions: (i) the reduction in Vc,max at elevated [CO₂] was due entirely to a reduction in content, (ii) the kcat 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).
calculated for each observation. Across 15 species it was found that PNUE was increased by 31% ± 3.6% SE (157, \( P < 0.001 \)), consistent with previous studies (Peterson et al., 1999) and the >30% increase in \( A_{\text{sat}} \) and <5% decrease in \( N_{\text{area}} \) 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., 2003, 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 C3 plants grown at elevated \([\text{CO}_2]\). This increase is driven predominantly by enhanced CO2 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 CO2 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 \([\text{CO}_2]\)**

The major function of stomata is to maximize the rate at which CO2 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 \([\text{CO}_2]\)**

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 CO2 assimilation; \( h \) is relative humidity; \([\text{CO}_2]\) is the atmospheric \([\text{CO}_2]\) 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 \([\text{CO}_2]\) results in a decrease in canopy evapotranspiration**

Decreased \( g_s \) for individual leaves in elevated \([\text{CO}_2]\) 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 \( \text{CO}_2 \)-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 $[CO_2]$ 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 $[CO_2]$ (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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ 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 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 (Wullschleger 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ (Leakey et al., 2006a) means that the relationship of $g_s$ to ET would be similar for plants

![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 μmol 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.](http://jxb.oxfordjournals.org)
grown in both ambient and elevated [CO$_2$], a conclusion supported by data from SoyFACE (Bernacchi et al., 2007). There will, in addition, be important regional-scale feedbacks related to elevated [CO$_2$] on water use that are likely to be important in the real world.

_Lesson summary_

That elevated CO$_2$ 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 [CO$_2$]**

Respiration fulfils the vital functions of producing ATP, reducing power and carbon-skeleton intermediates, while consuming O$_2$ and releasing CO$_2$. 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 [CO$_2$] 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 [CO$_2$] 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 [CO$_2$].

Much of the uncertainty surrounding the effects of elevated [CO$_2$] 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 CO$_2$ analysers designed for assessing photosynthesis, to measure the rate of CO$_2$ efflux from a tissue sample. Many early studies used this technique to assess whether there was any instantaneous effect of [CO$_2$] on respiration. On average, doubling the [CO$_2$] around a leaf was reported to inhibit respiratory CO$_2$ 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; Janhke, 2001; Jahnke and Krewitt, 2001). When respiration has been measured as O$_2$ uptake, or when CO$_2$ 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 [CO$_2$] was detected (Amthor et al., 2001; Janhke, 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 [CO$_2$] 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 (Bevers, 1974; Bingham and Farrar, 1988), or by the availability of substrate when respiration is not ADP-limited (Breeze and Elston, 1978; Azco´n-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 [CO$_2$] 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 [CO$_2$] 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 [CO$_2$] (Fig. 3). The number of transcripts reported to have significantly different abundance under ambient and elevated [CO$_2$] 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...
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 [CO2] 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 [CO2] 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 O2 and CO2 as a result of respiratory fluxes. Alternatively, the stimulation of respiration by elevated [CO2] 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 [CO2] favour greater respiration, other changes at elevated [CO2] might be decreasing respiration. One difference between elevated [CO2] and other environmental factors increasing photosynthesis and TNC is that leaf nitrogen (N) per unit leaf area is examined. It has often been suggested that while greater carbohydrate (TNC) content at elevated [CO2] has not been observed to increase, respiratory products (Amthor, 2000). Analysis of the cause of the observed 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 [CO2]. 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 [CO2]. The response is driven by greater gene expression for the respiratory machinery and greater substrate availability. However, increased dark respiration has not been observed in many other species grown with FACE elevated [CO2].

**Lesson 5: stimulation of carbon uptake by elevated [CO2] in C4 plants is indirect and occurs only in situations of drought**

There is a clear mechanistic basis for the stimulation of photosynthesis in C3 plants that is lacking in C4 plants. An increase in the atmospheric [CO2] from the ambient level of 2009 (384 μmol mol⁻¹) to the 550 μmol mol⁻¹ or 700 μmol mol⁻¹ levels conservatively projected for the middle and end of this century, produces, in C3 soybean, a rise in the intercellular [CO2] from approximately 270 μmol mol⁻¹ to 384 μmol mol⁻¹ or 490 μmol 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 [CO2] around Rubisco that accelerates the carboxylation reaction while suppressing the competing oxygenation reaction (Bowes, 1991). In C4 plants, the initial slope of the A/Ci relationship is much steeper and carbon uptake is saturated by CO2 at a lower Ci.

![Fig. 4. Comparing the dependence of photosynthesis (A) on intercellular CO2 concentration (Ci) for C4 maize and C3 soybean. The maize data were fit using the equations for C4 photosynthesis (von Caemmerer, 2000). The soybean data fit according to the C3 leaf biochemical model of photosynthesis of Farquhar et al. (1980). The grey lines illustrate the supply function for CO2, starting at the atmospheric [CO2] and ending at the operating Ci of photosynthesis. This is illustrated for current atmospheric [CO2] (384 μmol mol⁻¹, solid line), elevated [CO2] anticipated for 2050 (550 μmol mol⁻¹, dashed line), and elevated [CO2] anticipated for 2080 (700 μmol mol⁻¹, dashed and dotted line). This figure was redrawn using data from Leakey (2009).](http://jxb.oxfordjournals.org)
(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 [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 [CO$_2$] from 384 mmol mol$^{-1}$ to 550 mmol mol$^{-1}$ or 700 mmol mol$^{-1}$ increases C$_1$ but has no impact on carbon uptake (Fig. 4). Only if the operating $C_i$ of photosynthesis under ambient [CO$_2$] is below the inflexion point of the $A/C_i$ curve or if growth at elevated [CO$_2$] altered the shape of the $A/C_i$ curve could C$_4$ photosynthesis be directly stimulated by increases in the atmospheric [CO$_2$].

While this theory was borne out in many elevated [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 [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 [CO$_2$] on carbon uptake in C$_4$ species in which the data are consistent with elevated [CO$_2$] having no direct effect on carbon uptake in C$_4$ plants. Sorghum was grown at ambient ($\sim$370 mmol mol$^{-1}$) and elevated [CO$_2$] ($\sim$570 mmol mol$^{-1}$) with irrigation or drought stress in Maricopa, AZ. Carbon uptake by the youngest fully expanded leaf from irrigated plots was not enhanced by elevated [CO$_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 mmol mol$^{-1}$) and elevated (475 mmol mol$^{-1}$) [CO$_2$] to be operating at or close to saturating $C_i$ explaining the lack of response of carbon uptake to growth at elevated [CO$_2$] (von Caemmerer et al., 2001). Maize was grown at ambient ($\sim$370 mmol mol$^{-1}$) and elevated [CO$_2$] ($\sim$550 mmol mol$^{-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 [CO$_2$] levels. When there was no drought stress at any stage of the season, maize grown at ambient and elevated [CO$_2$] produced the same final biomass and yield.

The most likely explanation for the apparent difference in effects of elevated [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 [CO$_2$] could reduce the water requirements and alleviate this stress giving the misimpression that elevated [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 [CO$_2$]. In addition, FACE experiments show that elevated [CO$_2$] improves C$_4$ plant water relations and thereby indirectly enhances photosynthesis, growth, and yield by delaying and ameliorating drought stress. Elevated [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 [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 [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 [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 [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 [CO$_2$] is not unique to C$_4$ plants. Decreased stomatal conductance at elevated [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 [CO$_2$] plots relative to outside the plots. However, in the absence of drought stress in 2004 at SoyFACE, elevated [CO$_2$] was estimated to increase leaf temperature by only 0.26 °C and photosynthesis by 0.3 mmol m$^{-2}$ 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 [CO$_2$]-grown plants of 1.47 °C and 1.85 °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 C₄ plants, with reduced water use reported at elevated [CO₂] compared to ambient [CO₂] in chamber-grown maize (–25% to 30%; Samarakoon and Gifford, 1996), Panicum colora-
tum (–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, C₄ photosynthesis is not directly stimulated by elevated [CO₂]. Nevertheless, there is significant potential for increased growth of C₄ plants at elevated [CO₂] to decrease water use and reduce drought stress, leading via this indirect mechanism to greater photosynthesis and yield.

Lesson 6: the [CO₂] ‘fertilization’ effect in FACE studies on crop plants is less than expected

Generally, the increase in C₃ 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 [CO₂]. The results of the FACE experiments show that photosynthesis of crop plants fails to match the theoretical increase that could be obtained at elevated [CO₂] 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 [CO₂] (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 [CO₂] 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 [CO₂] treatments between 450 μmol mol⁻¹ and 550 μmol mol⁻¹, the average increase in Aₙsat was 24% (Fig. 5). Bernacchi et al. (2005) measured Aₙsat every 2 weeks throughout two growing season at SoyFACE and reported a smaller 18% increase in elevated [CO₂]. A meta-analysis of SoyFACE results indicates an average 16% increase in Aₙsat across a variety of soybean cultivars, which is substantially smaller than suggested by chamber studies (Fig. 5). At SoyFACE, the largest stimulation in Aₙsat 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 gₛ was reduced by 21.9% in elevated [CO₂] (Rogers et al., 2004). gₛ under saturating light conditions was reduced on average by 14% at Soy-
FACE (Fig. 5; Ainsworth et al., 2004; Bernacchi et al., 2005). Canopy photosynthesis was stimulated by 59% in soybeans grown under elevated [CO₂] in controlled environ-
ments, with an average elevated [CO₂] treatment of 761 μmol mol⁻¹. Although the SoyFACE treatment was lower (550 μmol 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 gₛ. The database of chamber studies suggests that photo-
synthetic capacity does not change in soybeans grown under elevated [CO₂]. Neither Vₚ₅₅ nor the apparent quantum yield of photosynthesis when measured at a common [CO₂] was altered by growth at elevated [CO₂] (Fig. 5). However, there was a small, yet statistically significant decrease in

\[
\begin{array}{c|c|c|c|c|c}
\text{Change at Elevated [CO₂]} & \text{FACE} & \text{CE} & \text{FACE} & \text{CE} & \text{FACE} \\
\hline
\text{Aₙsat} & (22) & (18) & (7) & (13) & (13) \\
\text{A'} & & & & & \\
\text{gₛ} & & & & & \\
\text{Vₚ₅₅} & & & & & \\
\end{array}
\]

Fig. 5. A comparison of light-saturated photosynthesis (Aₙsat), daily carbon uptake (A’), stomatal conductance (gₛ), and in vivo maximum carboxylation rate (Vₚ₅₅) from a meta-analysis of controlled environment (CE) studies of soybean grown at elevated CO₂ (Ainsworth et al., 2002) and soybeans grown at elevated CO₂ in a Free-Air Carbon dioxide Enrichment (FACE) experiment (Ainsworth et al., 2004; Bernacchi et al., 2005; Rogers et al., 2004). The mean ±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 [CO₂].

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$V_{c,max}$ 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 [CO2] 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 [CO2] 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 [CO2]. A comparison of wheat yields from five different crop models with FACE results showed that the mean response ratio to elevated [CO2] 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 CO2 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 [CO2] 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 [CO2] 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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