

# The response of photosynthesis and stomatal conductance to rising [CO<sub>2</sub>]: mechanisms and environmental interactions

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## ABSTRACT

This review summarizes current understanding of the mechanisms that underlie the response of photosynthesis and stomatal conductance to elevated carbon dioxide concentration ([CO<sub>2</sub>]), and examines how downstream processes and environmental constraints modulate these two fundamental responses. The results from free-air CO<sub>2</sub> enrichment (FACE) experiments were summarized via meta-analysis to quantify the mean responses of stomatal and photosynthetic parameters to elevated [CO<sub>2</sub>]. Elevation of [CO<sub>2</sub>] in FACE experiments reduced stomatal conductance by 22%, yet, this reduction was not associated with a similar change in stomatal density. Elevated [CO<sub>2</sub>] stimulated light-saturated photosynthesis ( $A_{\text{sat}}$ ) in C<sub>3</sub> plants grown in FACE by an average of 31%. However, the magnitude of the increase in  $A_{\text{sat}}$  varied with functional group and environment. Functional groups with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)-limited photosynthesis at elevated [CO<sub>2</sub>] had greater potential for increases in  $A_{\text{sat}}$  than those where photosynthesis became ribulose-1,5-bisphosphate (RubP)-limited at elevated [CO<sub>2</sub>]. Both nitrogen supply and sink capacity modulated the response of photosynthesis to elevated [CO<sub>2</sub>] through their impact on the acclimation of carboxylation capacity. Increased understanding of the molecular and biochemical mechanisms by which plants respond to elevated [CO<sub>2</sub>], and the feedback of environmental factors upon them, will improve our ability to predict ecosystem responses to rising [CO<sub>2</sub>] and increase our potential to adapt crops and managed ecosystems to future atmospheric [CO<sub>2</sub>].

*Key-words:* acclimation; elevated carbon dioxide; free-air CO<sub>2</sub> enrichment (FACE); global change; Rubisco.

## INTRODUCTION

Plants sense and respond to rising carbon dioxide concentration ([CO<sub>2</sub>]) through increased photosynthesis ( $A$ ) and reduced stomatal conductance ( $g_s$ ). All other effects of elevated [CO<sub>2</sub>] on plants and ecosystems are derived from

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these two fundamental responses (Long *et al.* 2004). The effect of elevated [CO<sub>2</sub>] on  $A$  is well characterized, yet the photosynthetic stimulation observed in CO<sub>2</sub> enrichment experiments does not always match theoretical expectations (Long *et al.* 2004; Nowak, Ellsworth & Smith 2004; Ainsworth & Long 2005; Rogers, Ainsworth & Kammann 2006a). Similarly, while  $g_s$  at elevated [CO<sub>2</sub>] is typically reduced, the effect is variable and subject to environmental feedback (Ellsworth 1999; Medlyn *et al.* 2001; Gunderson *et al.* 2002; Wullschlegel, Tschaplinski & Norby 2002; Naumburg *et al.* 2003; Bunce 2004; Herrick, Maherali & Thomas 2004; Marchi *et al.* 2004; Morgan *et al.* 2004; Nowak *et al.* 2004; Leakey *et al.* 2006a).

Most of our fundamental understanding of plant responses to elevated [CO<sub>2</sub>] has come from experiments in controlled environments, greenhouses and open-top chambers. However, because these exposure techniques can alter the environment surrounding the plants (Arp 1991; Long *et al.* 2004, 2006b; Ainsworth & Long 2005; Rogers & Ainsworth 2006), we have restricted the quantitative aspects of this review to results from free-air CO<sub>2</sub> enrichment (FACE) experiments, where plants are grown at elevated [CO<sub>2</sub>] in the field under fully open-air conditions. Here, we outline current understanding of the response of  $A$  and  $g_s$  to elevated [CO<sub>2</sub>], evaluate results from FACE experiments and examine the environmental constraints that impact the primary responses of plants to elevated [CO<sub>2</sub>].

## RESPONSE OF STOMATAL CONDUCTANCE TO ELEVATED [CO<sub>2</sub>]

### Molecular, biochemical and physiological mechanisms of CO<sub>2</sub> sensing and response

CO<sub>2</sub> sensing is an intrinsic property of guard cells, which are thought to respond to the intercellular [CO<sub>2</sub>] ( $c_i$ ) rather than [CO<sub>2</sub>] at the leaf surface (Mott 1988). Guard cell metabolism and signalling have been recently reviewed (Assmann 1999; Hetherington 2001; Hetherington & Woodward 2003; Vavasseur & Raghavendra 2005), so we will only summarize the topic here. Ion and organic solute concentrations mediate the turgor pressure in the guard cells that

determines stomatal aperture. Stomatal closure requires the guard cell membrane potential to be depolarized, i.e. made less negative (Assmann 1999). Electrophysiological studies showed that elevated [CO<sub>2</sub>] increases the activity of outward rectifying K<sup>+</sup> channels, decreases the activity of inward rectifying K<sup>+</sup> channels, enhances S type anion channel activities, stimulates Cl<sup>-</sup> release from guard cells and increases guard cell Ca<sup>2+</sup> concentration (Webb *et al.* 1996; Brearley, Venis & Blatt 1997; Hanstein & Felle 2002; Raschke, Shabahang & Wolf 2003). These changes collectively depolarize the membrane potential of guard cells and cause stomatal closure (Assmann 1993). Therefore, greater depolarization at elevated [CO<sub>2</sub>] will result in a reduced stomatal aperture.

The precise signal transduction pathways that function upstream of the ion channel activities are not as well known (Assmann 1999; Schroeder *et al.* 2001), but it has been argued that a stand-alone, CO<sub>2</sub>-specific signalling pathway in guard cells is unlikely and that guard cell signalling is more likely organized as a network (Hetherington & Woodward 2003). There are multiple potential messengers in the stomatal [CO<sub>2</sub>] response, including cytosolic free calcium concentration ([Ca<sup>2+</sup>]), apoplastic and cytoplasmic pH gradients, ion channels and membrane potential, chloroplastic zeaxanthin levels, photosynthetically derived ATP and protein phosphorylation/dephosphorylation (Assmann 1999; Hetherington & Woodward 2003; Vavasseur & Raghavendra 2005; Hashimoto *et al.* 2006; Messinger, Buckley & Mott 2006; Young *et al.* 2006). Many of these same signals overlap with stomatal responses to abscisic acid and light, supporting the hypothesis that multiple [CO<sub>2</sub>]-sensing mechanisms are employed by guard cells (Hetherington & Woodward 2003; Roelfsema *et al.* 2006). Further, guard cells have both photosynthetic electron transport-dependent and independent mechanisms of response to [CO<sub>2</sub>] (Messinger *et al.* 2006), and calcium-sensitive and -insensitive phases of the response to [CO<sub>2</sub>] (Young *et al.* 2006). The first *Arabidopsis* mutants with impaired guard cell responses to [CO<sub>2</sub>] have recently been identified (Hashimoto *et al.* 2006; Young *et al.* 2006). The *gca2* mutant has impaired reactive oxygen species activation of guard cell Ca<sup>2+</sup>-permeable channels and is insensitive to high [CO<sub>2</sub>] (Pei *et al.* 2000). The mutant lacks the normal decrease in guard cell cytosolic Ca<sup>2+</sup> transients upon transition to high [CO<sub>2</sub>], and is thought to lack priming of Ca<sup>2+</sup> sensors necessary for stomatal closure at elevated [CO<sub>2</sub>] (Young *et al.* 2006). A second set of guard cell CO<sub>2</sub>-sensing mutants was identified by thermography and implicates *HTI* protein kinase as another key molecular regulator of stomatal movements in response to [CO<sub>2</sub>] (Hashimoto *et al.* 2006). This evidence collectively supports the hypothesis that multiple components govern stomatal responses to environmental stimuli and that guard cell signalling is organized as a complex network (Hetherington & Woodward 2003). The recent discovery of two different members of the R2R3-MYB transcription factor family that regulate stomatal opening in response to light, and stomatal closure in response to darkness (Cominelli *et al.* 2005; Liang

*et al.* 2005), suggests that gene expression may be yet another level of regulation (Gray 2005).

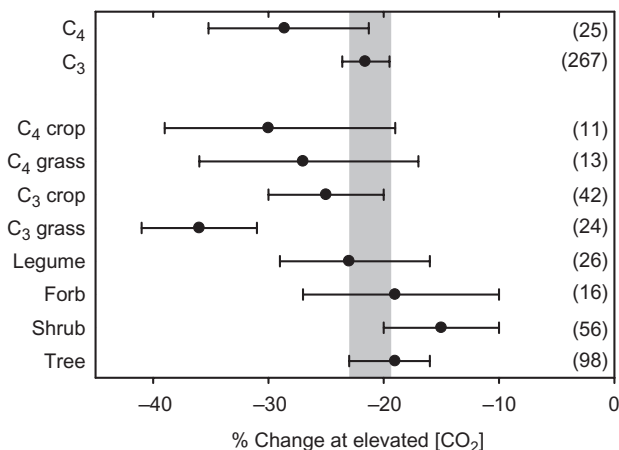
Whether or not photosynthetic processes or metabolites play a direct role in guard cell responses to [CO<sub>2</sub>] is still controversial (Messinger *et al.* 2006); however, there is compelling evidence that the Calvin cycle and photosynthetic electron transport operate in guard cell chloroplasts at similar rates to those in mesophyll cells (Cardon & Berry 1992; Lawson *et al.* 2002, 2003; Zeiger *et al.* 2002). Further, both C<sub>3</sub> and C<sub>4</sub> species show a consistent and similar decrease in g<sub>s</sub> at elevated [CO<sub>2</sub>] (Ainsworth & Long 2005), and guard cell photosynthetic efficiency of both C<sub>3</sub> and C<sub>4</sub> species is sensitive to [O<sub>2</sub>] (Lawson *et al.* 2003). Two hypotheses link guard cell photosynthesis with stomatal responses to [CO<sub>2</sub>]. The first hypothesizes that variation in the concentration of zeaxanthin plays a role in the signal transduction of CO<sub>2</sub> signals in guard cells (Zhu *et al.* 1998). The second hypothesis suggests that photosynthetically derived ATP is shuttled from guard cell chloroplasts to the cytosol, where it drives proton pumping and cation uptake at the plasmalemma (Tominaga, Kinoshita & Shimazaki 2001; Buckley, Mott & Farquhar 2003). Buckley *et al.* (2003) proposed that the guard cell osmotic gradient is proportional to the cytosolic guard cell [ATP], which increases with higher light and decreases with higher [CO<sub>2</sub>]. Both of these hypotheses depend on the balance between photosynthetic electron transport and Calvin cycle activity in the guard cells (Messinger *et al.* 2006). These hypotheses are controversial because studies with antisense plants with suppressed ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) expression showed little difference in stomatal function to wild-type plants, and the correlation between photosynthesis and stomatal conductance breaks down. Therefore, the antisense studies suggest that neither mesophyll nor guard cell photosynthesis is involved in the [CO<sub>2</sub>] response (Stitt *et al.* 1991; von Caemmerer *et al.* 2004). Further, albino *Vicia faba* guard cells that completely lack chlorophyll fluorescence still have a CO<sub>2</sub> response (Roelfsema *et al.* 2006). Thus, the role of photosynthesis in the CO<sub>2</sub> response of guard cells is uncertain.

In the short term, stomatal aperture generally decreases in response to high [CO<sub>2</sub>], as described earlier. In the long term, decreases in g<sub>s</sub> can be caused by changes in stomatal density or stomatal index (the percentage of epidermal cells that are guard cells), as well as stomatal aperture. The *HIC* (high carbon dioxide) gene encodes a putative 3-keto acyl coenzyme A synthase, which is a negative regulator of stomatal development (Gray *et al.* 2000). While many plants decrease stomatal initiation at high [CO<sub>2</sub>] (Woodward & Kelly 1995), mutant *hic* plants increase stomatal density up to 42% in response to high [CO<sub>2</sub>], presumably due to a disruption in the signal transduction pathway responsible for controlling stomatal patterning (Gray *et al.* 2000). There is also evidence that stomatal development in response to [CO<sub>2</sub>] is controlled by long-distance signals from mature leaves (Lake *et al.* 2001). High or low [CO<sub>2</sub>] is detected in mature leaves and signalled to immature leaves, whose stomatal development is altered accordingly (Lake *et al.* 2001).

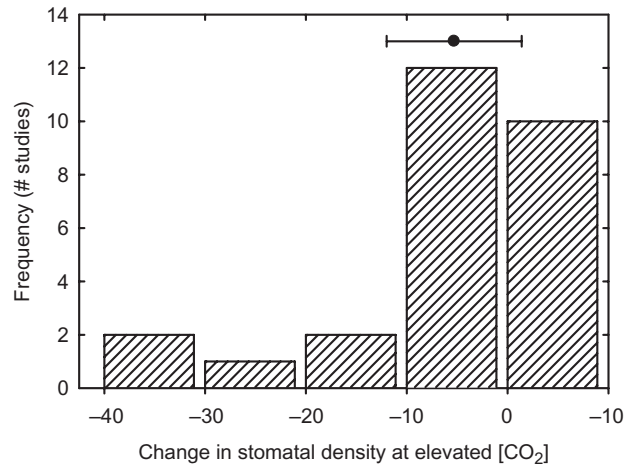
Further work with *Arabidopsis* mutants indicates that abscisic acid, ethylene and jasmonic acid may be involved in the long-distance signalling process, and controls for abaxial and adaxial stomatal responses are independent (Lake, Woodward & Quick 2002).

### The response of $g_s$ to elevated $[\text{CO}_2]$ in FACE

While studies of individual guard cells and *Arabidopsis* mutants are critical in furthering our understanding of the molecular mechanisms of stomatal response to  $[\text{CO}_2]$ , it is equally important to investigate how plants in the field respond to the elevated  $[\text{CO}_2]$  that is anticipated for this century. One of the most consistent responses of plants to elevated atmospheric  $[\text{CO}_2]$  is a decrease in  $g_s$  (reviewed in Wand *et al.* 1999; Medlyn *et al.* 2001; Wullschlegel *et al.* 2002; Long *et al.* 2004; Ainsworth & Long 2005). We updated our database of FACE studies previously used to determine the mean response of  $g_s$  to an elevated  $[\text{CO}_2]$  of ca.  $567 \mu\text{mol mol}^{-1}$  (Supplementary Appendix S1; Long *et al.* 2004; Ainsworth & Long 2005). Averaged across all plant species grown at elevated  $[\text{CO}_2]$  in FACE experiments,  $g_s$  was reduced by 22% (Fig. 1). There was significant variability among functional groups in how  $g_s$  responded to elevated  $[\text{CO}_2]$  (Fig. 1). On average, trees, shrubs and forbs showed a lower percentage decrease in  $g_s$  compared to  $\text{C}_3$  and  $\text{C}_4$  grasses and herbaceous crops, similar to the trend reported previously for herbaceous and woody species (Saxe, Ellsworth & Heath 1998; Nowak *et al.* 2004). However, the results from our analysis show a significant



**Figure 1.** Meta-analysis of the response of stomatal conductance ( $g_s$ ) to elevated  $[\text{CO}_2]$  in free-air  $\text{CO}_2$  enrichment experiments. The ambient and elevated  $[\text{CO}_2]$  for all studies averaged 366 and  $567 \mu\text{mol mol}^{-1}$ , respectively. The grey bar represents the overall mean and 95% confidence interval (CI) of all measurements. The symbol represents the mean response ( $\pm$  95% CI) of  $\text{C}_3$  and  $\text{C}_4$  species, and different functional groups. There was significant between group heterogeneity ( $Q_B$ ) for different functional groups ( $Q_B = 30.03$ ,  $P < 0.01$ ). The degrees of freedom for each measurement are shown in parenthesis. A list of primary references used in this analysis is provided in Supplementary Appendix S1.



**Figure 2.** Histogram of observations from free-air  $\text{CO}_2$  enrichment experiments of the change in stomatal density at elevated  $[\text{CO}_2]$ . The ambient and elevated  $[\text{CO}_2]$  for all studies averaged 363 and  $571 \mu\text{mol mol}^{-1}$ , respectively. The mean response calculated by meta-analysis ( $\pm$  95% confidence interval) is indicated above the histogram. A list of primary references used in this analysis is provided in Supplementary Appendix S2.

decrease in  $g_s$  for all groups. In contrast, the review of Saxe *et al.* (1998) did not find significant decreases in  $g_s$  in trees, particular woody coniferous trees. There are exceptions to the general rule that  $g_s$  declines at elevated  $[\text{CO}_2]$ , even in FACE experiments. In particular, *Pinus taeda* guard cells appear to be insensitive to elevated  $[\text{CO}_2]$  (Ellsworth 1999).

Growth at elevated  $[\text{CO}_2]$  reduces stomatal density in a wide variety of species and in many *Arabidopsis thaliana* ecotypes (Woodward, Lake & Quick 2002; Hetherington & Woodward 2003). However, in FACE experiments, the decrease in  $g_s$  at elevated  $[\text{CO}_2]$  does not appear to be caused by a significant change in stomatal density (Estiarte *et al.* 1994; Bryant, Taylor & Frehner 1998; Reid *et al.* 2003; Marchi *et al.* 2004; Tricker *et al.* 2005). We conducted a meta-analysis of stomatal density responses to elevated  $[\text{CO}_2]$  and found that the average 5% decrease in density was not statistically significant (Fig. 2). The frequency histogram of changes in stomatal density shows relatively few reports of large decreases in stomatal density, while the majority of studies report changes between  $-10$  and  $+10\%$  (Fig. 2). Tricker *et al.* (2005) found that stomatal density of *Poplar × euramericana* decreased in the first 2 years of exposure to elevated  $[\text{CO}_2]$ , but in later years of the experiment, there was no difference in stomatal density in leaves grown at ambient versus elevated  $[\text{CO}_2]$ . Further, Reid *et al.* (2003) report that stomatal density tended to be higher at elevated  $[\text{CO}_2]$  in FACE experiments, although the result was not statistically significant. While there have only been 27 reports of the response of stomatal density to elevated  $[\text{CO}_2]$  in FACE studies, there is a little evidence for a significant decrease in stomatal density (Fig. 2). Therefore, it is likely that changes in stomatal aperture rather than density determine the response of  $g_s$  to elevated  $[\text{CO}_2]$ .

### Acclimation of $g_s$ to elevated [CO<sub>2</sub>]

The response of  $g_s$  to elevated [CO<sub>2</sub>] is a critical parameter for larger scale models of canopy, ecosystem and landscape water flux. Many of these models use the Ball, Woodrow & Berry (1987) model of stomatal conductance solved simultaneously with the Farquhar, von Caemmerer & Berry (1980) model of photosynthesis (and their derivatives), to provide predictions of intact leaf or canopy photosynthesis and transpiration (e.g. Foley *et al.* 1996). The Ball *et al.* (1987) model predicts  $g_s$  on the basis of a linear, empirical relationship:

$$g_s = g_o + m \frac{Ah}{[CO_2]}$$

where  $A$  is the net rate of photosynthesis;  $h$  is the atmospheric relative humidity; [CO<sub>2</sub>] is the atmospheric [CO<sub>2</sub>] at the leaf surface;  $g_o$  is the  $y$ -axis intercept and  $m$  is the slope. Acclimation of  $g_s$  to growth at elevated [CO<sub>2</sub>] would alter the sensitivity of  $g_s$  to [CO<sub>2</sub>],  $A$ , and/or  $h$ , and therefore alter the constants in the equation,  $g_o$  and  $m$ . If  $g_s$  independently acclimates to elevated [CO<sub>2</sub>], then photosynthetic and stomatal models would require re-parameterization at each growth [CO<sub>2</sub>] of interest, significantly complicating the models.

Medlyn *et al.* (2001) investigated the acclimation of  $g_s$  to elevated [CO<sub>2</sub>] in six tree species, and found that in all but one case, there was no change in  $g_o$  or  $m$  with growth [CO<sub>2</sub>]. Only in water-stressed *Phillyrea angustifolia* was acclimation of  $g_s$  detected (Medlyn *et al.* 2001). Further, Gunderson *et al.* (2002) investigated the sensitivity of stomata to elevated [CO<sub>2</sub>] over 3 years in a FACE experiment with *Liquidambar styraciflua* and found no evidence for altered sensitivity to vapour pressure deficit. Acclimation of  $g_s$  to elevated [CO<sub>2</sub>] has been tested in herbaceous species in two FACE studies. In *Lolium perenne* grown at 600  $\mu\text{mol mol}^{-1}$ , there was no evidence of independent acclimation of  $g_s$  to elevated [CO<sub>2</sub>] (Nijs *et al.* 1997). Likewise, there was no evidence for stomatal acclimation in terms of sensitivity to  $A$ ,  $h$  and [CO<sub>2</sub>] in *Glycine max* grown at 550  $\mu\text{mol mol}^{-1}$  (Leakey *et al.* 2006a). While the Ball *et al.* (1987) model predicts that  $g_s$  would be reduced in leaves that significantly down-regulated  $A$  in response to elevated [CO<sub>2</sub>], there is little evidence from FACE that  $g_s$  independently acclimates to elevated [CO<sub>2</sub>].

### Environmental factors alter the response of $g_s$ to elevated [CO<sub>2</sub>]

While the sensitivity of guard cells to environmental factors does not appear to acclimate with growth at elevated [CO<sub>2</sub>], the magnitude of the effect of elevated [CO<sub>2</sub>] on  $g_s$  varies considerably with environmental factors (Medlyn *et al.* 2001; Gunderson *et al.* 2002; Wullschlegel *et al.* 2002; Naumburg *et al.* 2003; Bunce 2004; Herrick *et al.* 2004; Marchi *et al.* 2004; Morgan *et al.* 2004; Nowak *et al.* 2004; Leakey *et al.* 2006a). There is generally a smaller effect of elevated [CO<sub>2</sub>] on  $g_s$  during dry periods (Gunderson *et al.* 2002;

Leakey *et al.* 2004, 2006a,b), Reductions in  $g_s$  at elevated [CO<sub>2</sub>] in *L. styraciflua*, grown at two FACE sites, were smallest when vapour pressure deficit was high and therefore absolute rates of  $g_s$  were low (Gunderson *et al.* 2002; Wullschlegel *et al.* 2002; Herrick *et al.* 2004). These results are consistent with those from *G. max* and *Zea mays*, grown in central Illinois (Leakey *et al.* 2004, 2006a,b) Long dry periods led to greater soil moisture depletion in ambient [CO<sub>2</sub>] compared to elevated [CO<sub>2</sub>] (Leakey, unpublished results). Therefore,  $g_s$  was presumably reduced by drought to a greater extent in ambient [CO<sub>2</sub>] compared to elevated [CO<sub>2</sub>], and the smaller effect of CO<sub>2</sub> on  $g_s$  during dry periods has an indirect origin (Leakey *et al.* 2004, 2006a,b). While the generality of these results may be limited to more mesic ecosystems, it is clear that the indirect effects of CO<sub>2</sub> on plant and soil water relations contribute to reported system-level effects of elevated [CO<sub>2</sub>] (Morgan *et al.* 2004; Nowak *et al.* 2004; Leakey *et al.* 2006a,b). Combining our understanding of molecular controls of guard cell responses to CO<sub>2</sub> with our understanding of the impact of environmental factors will improve our ability to model and predict ecosystem-level carbon and water flux.

## RESPONSE OF PHOTOSYNTHESIS TO ELEVATED [CO<sub>2</sub>]

### Rubisco properties and mechanism

Virtually all of the carbon assimilated by autotrophic organisms has passed through the active site of Rubisco, where ribulose-1,5-bisphosphate (RubP) is combined with CO<sub>2</sub> to yield two molecules of 3-phosphoglyceric acid (3PGA). In addition to its function as a carboxylase, Rubisco also reacts with oxygen to produce one molecule of 3PGA and one molecule of 2-phosphoglycollate (2PG) (Cleland *et al.* 1998). Estimates of the Michaelis–Menten constant ( $K_m$ ) for CO<sub>2</sub> in the carboxylation reaction of Rubisco in higher plants range from 8–34  $\mu\text{M}$  (von Caemmerer & Quick 2000). In C<sub>3</sub> plants, limitations on the diffusion of CO<sub>2</sub> to the active site of Rubisco reduce the [CO<sub>2</sub>] outside the leaf from the current atmospheric [CO<sub>2</sub>] of 380 to ca. 190  $\mu\text{mol mol}^{-1}$ , equivalent to 6.3  $\mu\text{M}$ , at the site of carboxylation (von Caemmerer & Evans 1991; von Caemmerer & Quick 2000; Bernacchi *et al.* 2002). Estimates of the  $K_m$  for O<sub>2</sub> in the oxygenation reaction of Rubisco range from 196 to 810  $\mu\text{M}$  (von Caemmerer & Quick 2000). The intercellular oxygen concentration is ca. 263  $\mu\text{M}$  (von Caemmerer & Quick 2000). Therefore, the higher affinity of Rubisco for CO<sub>2</sub> is offset by the low concentration of CO<sub>2</sub> at the active site and the relatively low affinity for O<sub>2</sub> is compensated for by the relatively high [O<sub>2</sub>] in the stroma. The CO<sub>2</sub> specificity factor for Rubisco is the ratio of the specificity for CO<sub>2</sub> relative to the specificity for O<sub>2</sub>. The mean estimate for the CO<sub>2</sub> specificity of Rubisco is ca. 90 (range = 60–128, von Caemmerer & Quick 2000). Because the ratio of the [CO<sub>2</sub>] : [O<sub>2</sub>] at the active site is ca. 0.024, the relative rate of carboxylation to oxygenation in a C<sub>3</sub> leaf at 25 °C is ca. 2.2. Thus, approximately every third molecule of RubP is consumed in the

oxygenation reaction. The affinity of Rubisco for CO<sub>2</sub>, and the solubility of CO<sub>2</sub> relative to O<sub>2</sub>, both decrease with rising temperature. Therefore as temperature increases, the relative rate of carboxylation to oxygenation is reduced even further and the flux of 2PG into photorespiration increases (Long 1991), e.g. at 35 °C, the relative rate of carboxylation to oxygenation is ca. 1.4.

The 3PGA produced by the oxygenase reaction enters the Calvin cycle, but the 2PG enters the non-essential photorespiratory pathway where 75% of the carbon is recovered and half of a molecule of CO<sub>2</sub> is released for every molecule of 2PG metabolized (Spreitzer 1999; Siedow & Day 2000; Long *et al.* 2006c). At 25 °C, ca. 23% of the carbon fixed by photosynthesis is lost due to photorespiration, and if all of the oxygenation reactions were replaced by carboxylation reactions, CO<sub>2</sub> uptake would be increased by ca. 53%.

Plants with the C<sub>4</sub> pathway avoid photorespiration by a combination of biochemical and anatomical specializations that concentrate CO<sub>2</sub> at the active site of Rubisco (Sage 2004). CO<sub>2</sub> is fixed as HCO<sub>3</sub><sup>-</sup> in the outer mesophyll tissue by phosphoenolpyruvate carboxylase, which lacks an oxygenase function and has a high affinity for its substrate. The C<sub>4</sub> organic acid is then transferred to the bundle sheath cells and decarboxylated to release CO<sub>2</sub>. While exact determination of the [CO<sub>2</sub>] in the bundle sheath is not currently possible (von Caemmerer 2003), recent estimates suggest that the CO<sub>2</sub> concentrating mechanism in C<sub>4</sub> plants raises the [CO<sub>2</sub>] at the active site of Rubisco to between 76 and 126 μM; i.e. 12 to 20 times higher than for C<sub>3</sub> plants (Kiirats *et al.* 2002; von Caemmerer & Furbank 2003). At 25 °C, the estimated CO<sub>2</sub> loss associated with photorespiration in C<sub>4</sub> plants would be less than 2%.

The  $k_{\text{cat}}$  of Rubisco isolated from higher plants ranges from 2.5 to 5.4 s<sup>-1</sup> (Tcherkez, Farquhar & Andrews 2006). For comparison, the turnover number for carbonic anhydrase is ca. 10<sup>5</sup> s<sup>-1</sup> (Heldt 2005). Therefore, despite eliminating photorespiration, C<sub>4</sub> plants are still forced to invest 10–15% of their leaf nitrogen in Rubisco to compensate for its miserable catalytic activity. Because C<sub>3</sub> plants lack a CO<sub>2</sub>-concentrating mechanism, they have lower N use efficiency than C<sub>4</sub> plants and invest even greater amounts of N in Rubisco. In C<sub>3</sub> plants, up to 25% of leaf N can be invested in Rubisco resulting in a stromal Rubisco concentration that can be several fold greater than that of its substrate, CO<sub>2</sub> (Sage, Pearcy & Seemann 1987; Heldt 2005).

By the end of the century, the [CO<sub>2</sub>] at the active site of Rubisco in C<sub>3</sub> plants will have risen from 6.3 to 15 μM (based on the IPPC IS92a emission scenario that predicts an atmospheric [CO<sub>2</sub>] of 750 μmol mol<sup>-1</sup> by 2100, Albritton *et al.* 2001). This will increase the rate and efficiency of photosynthesis in C<sub>3</sub> plants for two reasons. Firstly, Rubisco is substrate limited at current [CO<sub>2</sub>], therefore rising [CO<sub>2</sub>] will increase the rate of the carboxylation reaction. Secondly, an increased [CO<sub>2</sub>] will competitively inhibit the oxygenation reaction of Rubisco and subsequently reduce the CO<sub>2</sub> loss and energy costs associated with the flux of 2PG through the photorespiratory pathway (Long *et al.* 2004). Rising [CO<sub>2</sub>] is not predicted to directly impact C<sub>4</sub> plants

because they avoid photorespiration and are CO<sub>2</sub>-saturated at current [CO<sub>2</sub>]. Therefore, as the [CO<sub>2</sub>] rises, the competitive advantage conferred by C<sub>4</sub> metabolism will be progressively reduced (Sage 2004).

### Molecular control of Rubisco activity and content

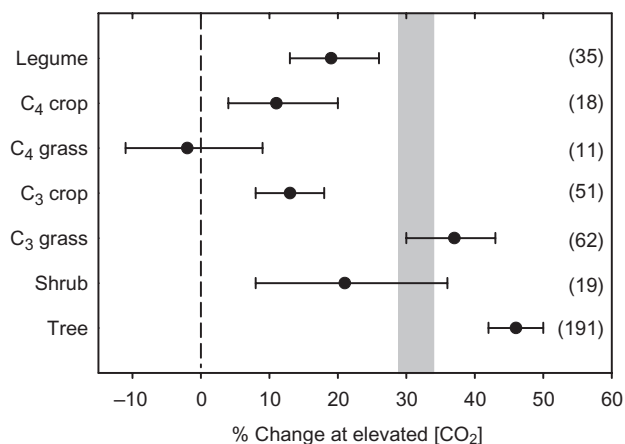
To function, Rubisco must be activated through reversible carbamylation of a lysine residue and binding of Mg<sup>2+</sup> (Cleland *et al.* 1998). Activation of Rubisco is dependent on the catalytic chaperone, Rubisco activase, which promotes the ATP-dependent dissociation of inhibitory sugar phosphates, thereby promoting carbamylation. Regulation of ATP-dependent Rubisco activase activity is not fully understood, but the sensitivity of the activase to the ATP:ADP ratio is clear and there is evidence for the involvement of redox regulation of Rubisco activase in some species (Portis 2003). Rubisco is usually fully active and carbamylated at current [CO<sub>2</sub>] under steady-state high light conditions (von Caemmerer & Quick 2000; Portis 2003). As [CO<sub>2</sub>] increases, carbon fixation increases; there is an increasing demand for ATP (required for RubP regeneration), and control of photosynthesis shifts from being limited by Rubisco to being limited by the capacity for RubP regeneration (Long & Drake 1992; von Caemmerer & Quick 2000). Reductions in the ATP:ADP ratio in the chloroplast then lead to a reduction in activase activity. The resulting reduction in Rubisco activation state would then match the capacity for carboxylation with the capacity for RubP regeneration (von Caemmerer & Quick 2000; Portis 2003; Cen & Sage 2005). Such reductions in Rubisco activation state have been observed at elevated [CO<sub>2</sub>] (Sage, Sharkey & Seemann 1988; Cen & Sage 2005). However, these responses may be more important for the short-term regulation of photosynthesis, rather than acclimation to rising [CO<sub>2</sub>] where reductions in Rubisco activity are well correlated with reductions in Rubisco content (Rolland-Bamford *et al.* 1991; Drake, Gonzalez-Meler & Long 1997; Moore *et al.* 1999; Stitt & Krapp 1999).

Regulation of Rubisco content involves a number of mechanisms that act on transcriptional, post-transcriptional, translational and post-translational events. These mechanisms have been reviewed in depth elsewhere (Moore *et al.* 1999; Stitt & Krapp 1999; Smeeckens 2000; Rolland, Moore & Sheen 2002; Long *et al.* 2004). Succinctly, when the supply of photosynthate from chloroplasts exceeds the capacity for export and utilization by sink tissue, the imbalance in supply and demand is sensed in mesophyll cells by a mechanism that possibly involves hexokinase acting as a flux sensor. The response mechanism initiated by the sugar signal varies among species but appears to target the small subunit of Rubisco through transcriptional or translational control or by interfering with the assembly of the holoenzyme (Long *et al.* 2004). These mechanisms are distinct from non-specific reductions in Rubisco content that can occur when leaf N content is reduced (Makino *et al.* 1997; Sicher & Bunce 1997; Curtis *et al.* 2000; Ellsworth *et al.* 2004).

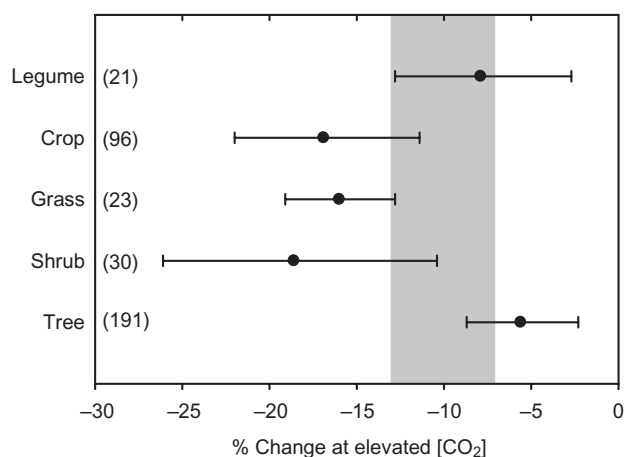
### How does photosynthesis respond to and acclimate to elevated [CO<sub>2</sub>]?

There is no doubt that growth at elevated [CO<sub>2</sub>] stimulates *A* in C<sub>3</sub> plants (Drake *et al.* 1997; Norby *et al.* 1999; Nowak *et al.* 2004; Ainsworth & Long 2005). We conducted a meta-analysis of the response of *A*<sub>sat</sub> to growth at elevated [CO<sub>2</sub>] in FACE experiments based on an updated version of the dataset used by Ainsworth & Long (2005) (Supplementary Appendix S3; Fig. 3). As predicted from the kinetic properties of Rubisco, there was a significant and marked increase in *A*<sub>sat</sub>, but there were significant differences between functional groups of the C<sub>3</sub> species (Fig. 3). Trees showed the largest response to elevated [CO<sub>2</sub>] and shrubs and legumes showed the smallest stimulation (Fig. 3). There was a surprising and significant increase in *A*<sub>sat</sub> in C<sub>4</sub> crops (see further discussion). In C<sub>3</sub> plants, the maximum carboxylation rate (*V*<sub>cm<sub>max</sub></sub>) and the maximum rate of electron transport (*J*<sub>max</sub>) were also significantly reduced at elevated [CO<sub>2</sub>]. The reduction in *V*<sub>cm<sub>max</sub></sub> was approximately double the reduction in *J*<sub>max</sub> (Figs 4 & 5), and the reduction in *V*<sub>cm<sub>max</sub></sub> was smallest in trees and greatest in shrubs, grasses and crops.

The response of C<sub>4</sub> plants to elevated [CO<sub>2</sub>] has been summarized by previous meta-analyses that report an increase in C<sub>4</sub> photosynthesis at elevated [CO<sub>2</sub>] (Wand *et al.* 1999; Ainsworth & Long 2005). A number of possible explanations for this observed response have been discussed in depth elsewhere (Ghannoum *et al.* 2000; Leakey *et al.* 2006b). However, evidence is building to support the hypothesis that the stimulation of C<sub>4</sub> photosynthesis at elevated [CO<sub>2</sub>] is an indirect effect resulting from the



**Figure 3.** The response of light-saturated CO<sub>2</sub> uptake (*A*<sub>sat</sub>) in plants grown at elevated [CO<sub>2</sub>] using free-air CO<sub>2</sub> enrichment technology for different functional groups (legume, C<sub>4</sub> crop, C<sub>4</sub> grass, C<sub>3</sub> crop, C<sub>3</sub> grass, shrub and tree). The ambient and elevated [CO<sub>2</sub>] for all studies averaged 366 and 567 μmol mol<sup>-1</sup>, respectively. The grey bar represents the overall mean and 95% confidence interval (CI) of all measurements. There was significant between group heterogeneity (*Q*<sub>B</sub>) between functional groups (*Q*<sub>B</sub> = 194, *P* < 0.001). Symbols show mean response (± 95% CI) with the degrees of freedom for each functional group given in parentheses. A list of primary references used in this analysis is provided in Supplementary Appendix S3.



**Figure 4.** The response of the maximum carboxylation rate (*V*<sub>cm<sub>max</sub></sub>) in C<sub>3</sub> plants grown at elevated [CO<sub>2</sub>] using free-air CO<sub>2</sub> enrichment technology for different functional groups (legume, crop, grass, shrub and tree). The ambient and elevated [CO<sub>2</sub>] for all studies averaged 365 and 567 μmol mol<sup>-1</sup>, respectively. The grey bar represents the overall mean and 95% confidence interval (CI) of all measurements. There was significant between group heterogeneity (*Q*<sub>B</sub>) between functional groups (*Q*<sub>B</sub> = 56, *P* < 0.001). Symbols show mean response (± 95% CI) with the degrees of freedom for each functional group given in parentheses. A list of primary references used in this analysis is provided in Supplementary Appendix S4.

interaction of water stress with reduced *g*<sub>s</sub> at elevated [CO<sub>2</sub>] (Samarakoon & Gifford 1996; Seneweera, Ghannoum & Conroy 1998; Ghannoum *et al.* 2000). Results from FACE experiments provide additional support for this conclusion. Increases in *A* in sorghum and maize were associated with improved water status or were limited to periods of low rainfall where drought stress was likely ameliorated at elevated [CO<sub>2</sub>] (Conley *et al.* 2001; Wall *et al.* 2001; Leakey *et al.* 2004; Kimball 2006). Further, in a year with no water stress, Leakey *et al.* (2006b) found no increase in *A*, *in vivo* or *in vitro* photosynthetic enzyme activities, biomass or yield. Therefore, it is likely that the mechanism by which *A* is stimulated in C<sub>4</sub> species grown at elevated [CO<sub>2</sub>] occurs through the mitigation of drought stress rather than a direct effect of elevated [CO<sub>2</sub>] on *A*.

### Explanation of the observed response in C<sub>3</sub> plants

Previous reviews of the response of plants to elevated [CO<sub>2</sub>] have also noted that the difference in the magnitude of the stimulation in *A* at elevated [CO<sub>2</sub>] and the occurrence of acclimation appeared to be both growth form and environment specific (Nowak *et al.* 2004; Ainsworth & Long 2005). Using parameters reported in the studies listed in Supplementary Appendices S4 and S5 (Table 1), we reconstructed a representative *A*/*c*<sub>i</sub> curve for each functional group (Fig. 6). As [CO<sub>2</sub>] rises, control of *A*<sub>sat</sub> by Rubisco (*V*<sub>cm<sub>max</sub></sub>) decreases and control by the capacity for RubP regeneration (*J*<sub>max</sub>) increases (Long & Drake 1992; Long *et al.*

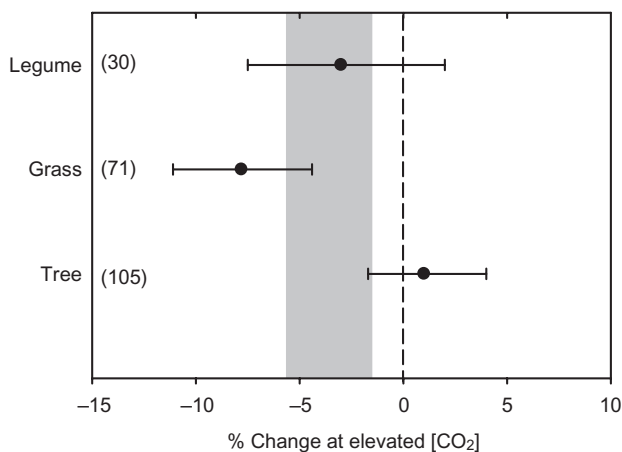
**Table 1.** Parameters used to model photosynthesis (*A*) in Fig. 6

Functional group ( <i>n</i> )	$V_{\text{cmax}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		$J_{\text{max}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	[CO <sub>2</sub> ] ( $\mu\text{mol mol}^{-1}$ )	
	Current [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]	Current [CO <sub>2</sub> ]	Elevated [CO <sub>2</sub> ]		Current	Elevated
Crop (24)	81	66	164	140	1085	363	567
Tree (111)	57	55	124	122	1360	367	567
Legume (31)	97	87	201	191	1195	370	555
Grass (97)	68	56	192	175	964	362	596
Shrub (22)	116	102	228	198	1500	364	550

Mean values for maximum carboxylation rate ( $V_{\text{cmax}}$ ), maximum rate of electron transport ( $J_{\text{max}}$ ) and PPFD for the five functional groups were calculated from the studies listed in Supplementary Appendices S4 and S5. When not reported,  $J_{\text{max}}$  was estimated ( $J_{\text{max}} = 29.1 + 1.64 V_{\text{cmax}}$ ) using the equation provided by Wullschlegel (1993). In all cases photosynthetic photon flux density (PPFD) was described as saturating or close to saturating by the original authors. The atmospheric [CO<sub>2</sub>] ( $c_a$ ) used to plot the supply function at current [CO<sub>2</sub>] was calculated as the [CO<sub>2</sub>] at the mean year of measurement based on the Intergovernmental Panel on Climate Change (IPCC) estimates (Albritton *et al.* 2001) and at elevated [CO<sub>2</sub>] as the mean target concentration for the represented free-air CO<sub>2</sub> enrichment installations (Ainsworth & Long 2005).  $A/c_i$  curves were modelled based on the equations of Farquhar *et al.* (1980), the internal [CO<sub>2</sub>] ( $c_i$ ) was estimated as  $0.7c_a$  (Long *et al.* 2004), temperature = 25 °C, relative humidity = 90%, and dark respiration ( $R_d$ ) at 25 °C was assumed to be  $1.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Long 1991).

2006a). Here we show that the difference in the magnitude of the stimulation in  $A_{\text{sat}}$  observed in response to rising [CO<sub>2</sub>] can be explained by knowledge of what process is limiting  $A$  at a given  $c_i$ . With the exception of grasses, the  $A/c_i$  curve at current [CO<sub>2</sub>] also predicts the likelihood of acclimation at elevated [CO<sub>2</sub>] (Fig. 6).

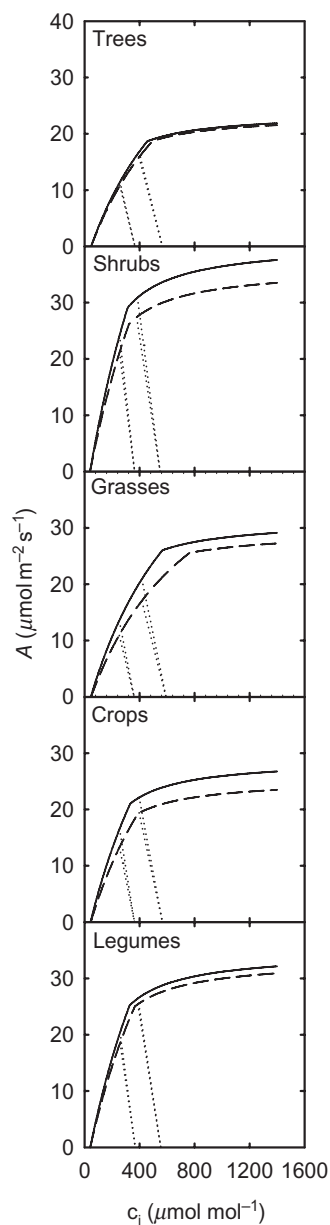
At current [CO<sub>2</sub>],  $A$  is Rubisco limited in all functional groups, as indicated by the supply function intersecting the initial slope of the  $A/c_i$  curve. As expected, no groups have an excess capacity for carboxylation at current [CO<sub>2</sub>] (von Caemmerer & Quick 2000; Rogers & Humphries 2000;



**Figure 5.** The response of the maximum rate of electron transport ( $J_{\text{max}}$ ) in  $C_3$  plants grown at elevated [CO<sub>2</sub>] using free-air CO<sub>2</sub> enrichment technology for different functional groups (legume, grass, tree). The ambient and elevated [CO<sub>2</sub>] for all studies averaged 365 and 567  $\mu\text{mol mol}^{-1}$ , respectively. The grey bar represents the overall mean and 95% confidence interval (CI) of all measurements. There was significant between group heterogeneity ( $Q_B$ ) between functional groups ( $Q_B = 54$ ,  $P < 0.001$ ). Symbols show mean response ( $\pm$  95% CI) with the degrees of freedom for each functional group given in parentheses. A list of primary references used in this analysis is provided in Supplementary Appendix S5.

Parry *et al.* 2003). The operating point at elevated [CO<sub>2</sub>] indicates that without acclimation (solid line Fig. 6),  $A$  in shrubs, legumes and crops would be limited by the capacity to regenerate RubP and leaves would have an excess of Rubisco. However,  $A$  in trees and grasses would still be limited by Rubisco at elevated [CO<sub>2</sub>]. Therefore, trees and grasses have the largest potential for stimulation at elevated [CO<sub>2</sub>] (ca. 50%), because rising [CO<sub>2</sub>] increases carboxylation and reduces photorespiration. In contrast, there is lower potential stimulation of  $A$  in shrubs, legumes and crops (ca. 30%), because as [CO<sub>2</sub>] rises,  $A$  becomes limited by the capacity for RubP regeneration, and further increases in  $A$  with rising [CO<sub>2</sub>] would result only from the repression of photorespiration (Long *et al.* 2004).

All functional groups acclimated to growth at elevated [CO<sub>2</sub>] (Figs 4 & 6), so observed photosynthetic stimulations in acclimated plants were less than the potential maximum stimulations indicated by the modelled  $A/c_i$  response at current [CO<sub>2</sub>]. Shrubs and crops showed ca. 18% reduction in  $V_{\text{cmax}}$  (Fig. 4) and when grown at elevated [CO<sub>2</sub>] (dashed line Fig. 6) were at or close to a co-limitation of  $A$  by carboxylation and RubP regeneration. Legumes showed the same response but Rubisco acclimation was less pronounced. Drake *et al.* (1997) hypothesized that the increased N use efficiency of plants at elevated [CO<sub>2</sub>], coupled to the shift in control of  $A$  away from Rubisco and towards RubP regeneration, would enable plants to reduce Rubisco content at elevated [CO<sub>2</sub>] and optimize their investment in photosynthetic machinery. In a recent review of crop responses to elevated [CO<sub>2</sub>], Long *et al.* (2006a) showed that crops reduced Rubisco activity at elevated [CO<sub>2</sub>] to a greater extent than the capacity for RubP regeneration. The data summarized here also suggest that plants are preferentially reducing their carboxylation capacity relative to RubP regeneration capacity (Figs 3 & 4), and the reconstructed  $A/c_i$  curves suggest that shrubs, crops and legumes are optimizing their resources at elevated [CO<sub>2</sub>] (dashed line in Fig. 6).



**Figure 6.**  $A/c_i$  curves for five functional groups of  $C_3$  plants grown at current [CO<sub>2</sub>] (solid line), and elevated [CO<sub>2</sub>] (dashed line) using free-air CO<sub>2</sub> enrichment technology. The dotted lines indicate the supply function and the point at which these lines meet the  $A/c_i$  curves indicates the operating point at current and elevated [CO<sub>2</sub>]. Values for maximum carboxylation rate ( $V_{\text{cmax}}$ ), maximum rate of electron transport ( $J_{\text{max}}$ ) and photosynthetic photon flux density (PPFD) were taken from the original studies and the means were used to model the response of  $A$  to  $c_i$  (Farquhar *et al.* 1980). The raw values used to fit the  $A/c_i$  curves for each [CO<sub>2</sub>] and functional group are provided in Table 1.

Photosynthesis in trees and grasses was Rubisco limited at both current and elevated [CO<sub>2</sub>] reflecting the ca. 36% lower carboxylation capacity in these groups compared with shrubs, crops and legumes. Because  $A$  is Rubisco limited in trees and grasses at elevated [CO<sub>2</sub>], reductions in Rubisco content will negatively impact carbon acquisition.

Therefore, in the absence of other limitations, no loss of Rubisco activity would be predicted. Trees had the smallest reduction in  $V_{\text{cmax}}$ , consistent with this explanation. However, on average, grasses showed a marked reduction in  $V_{\text{cmax}}$ . Grasses were grown in FACE experiments at different N fertilization regimes and different management practices, and acclimation was dependent on these environmental factors (see further discussion).

### Environmental factors determine the magnitude of the response of photosynthesis to elevated [CO<sub>2</sub>]

Given the large amount of N that plants invest in Rubisco, and its role as the C fixing enzyme, it not surprising that the balance between photosynthate utilization and N status plays a major role in shaping the response of plants to elevated [CO<sub>2</sub>].

#### Nitrogen supply

In plants where photosynthesis becomes RubP limited at elevated [CO<sub>2</sub>] (e.g. crops, legumes and shrubs, Fig. 6), Rubisco will be in excess of requirements. The excess capacity for carboxylation could be reduced through a reduction in the activation state of Rubisco (Cen & Sage 2005). Alternatively, because less Rubisco is required by these plants at elevated [CO<sub>2</sub>], redistribution of the excess N invested in Rubisco could further increase N use efficiency at elevated [CO<sub>2</sub>] without negatively impacting potential C acquisition (Drake *et al.* 1997; Parry *et al.* 2003). However, there is only benefit in reducing the amount of N invested in Rubisco at elevated [CO<sub>2</sub>] when the resources invested in it can be usefully deployed elsewhere (Parry *et al.* 2003). Therefore we would expect greater acclimation in low N conditions than high N conditions. Ainsworth & Long (2005) reported that stimulation in  $A_{\text{sat}}$  at elevated [CO<sub>2</sub>] was 23% lower in plants grown with a low N supply.  $V_{\text{cmax}}$  was reduced at elevated [CO<sub>2</sub>] at both high and low N, but at low N the reduction was 85% greater. The emerging picture from FACE studies is that when acclimation occurs at elevated [CO<sub>2</sub>], it occurs to a greater extent at low N than at high N. This is in agreement with summaries of earlier studies conducted in controlled environments and field enclosures (Drake *et al.* 1997; Moore *et al.* 1999; Stitt & Krapp 1999), and is consistent with current understanding of the mechanism underlying acclimation. When plants are N limited, sink development is restricted, C supply is in excess of demand, and the sugar feedback mechanism outlined earlier can operate to reduce Rubisco content and increase N use efficiency. As N supply increases, the limitation imposed by sink capacity decreases and the sugar linked signal for down-regulating Rubisco content is reduced (Drake *et al.* 1997; Rogers *et al.* 1998; Long *et al.* 2004).

#### Sink strength

Defined here as the capacity to utilize photosynthate, sink strength can be a major constraint on carbon acquisition



(Stitt 1991). A reduced or insufficient sink capacity may be the result of many potentially limiting processes, e.g. N supply (Rogers *et al.* 1998), genetic constraints (Ainsworth *et al.* 2004), temperature (Ainsworth *et al.* 2003b) or developmental changes (Rogers *et al.* 2004; Bernacchi *et al.* 2005; Rogers & Ainsworth 2006). However, the net result is the same, i.e. the appearance of a carbohydrate-derived signal that can lead to the subsequent down-regulation (acclimation) of photosynthetic machinery, principally Rubisco (Stitt & Krapp 1999; Long *et al.* 2004).

FACE experiments have provided field tests of this concept and showed that some plants are better able to cope with excess carbohydrate than others. Davey *et al.* (2006) showed that poplar grown at elevated  $[\text{CO}_2]$  had a large sink capacity. Poplar was able to export >90% of its photosynthate during the day and had a large capacity for the temporary storage of overflow photosynthate as starch (Stitt & Quick 1989; Davey *et al.* 2006). These two traits enabled poplar to maintain high photosynthetic rates at elevated  $[\text{CO}_2]$  and avoid a major source–sink imbalance that could lead to a reduction in the potential for C acquisition. In contrast, *L. perenne* can become extremely sink limited at elevated  $[\text{CO}_2]$  (Rogers & Ainsworth 2006), and reports of large accumulations of carbohydrate which build up in grasses over several days and weeks are common (Fischer *et al.* 1997; Isopp *et al.* 2000; Rogers & Ainsworth 2006). The most likely explanation for the sink limitation observed in *L. perenne* is an insufficient N supply (Fischer *et al.* 1997; Rogers *et al.* 1998). The excess of C and shortage of N may explain why grasses reduced their Rubisco content at elevated  $[\text{CO}_2]$ , despite the negative impact on potential carbon gain (Rogers *et al.* 1998; Ainsworth *et al.* 2003a; Figs 4 & 6).

Legumes grown at elevated  $[\text{CO}_2]$  have an excess of Rubisco, and photosynthesis is limited by the capacity for RubP regeneration (Fig. 6). Therefore, a reduction in carboxylation capacity would be expected. However, legumes can trade photosynthate for reduced forms of N with their bacterial symbionts (Rogers *et al.* 2006b). Therefore, the benefit of an increase in N use efficiency resulting from the reduction of Rubisco content, and the sugar-derived signal required for a reduction in carboxylation capacity would not be expected. It follows that acclimation in legumes likely occurs through reductions in Rubisco activity rather than through a loss of Rubisco protein content, and occurs to maintain the balance between the supply and demand for the products of the light reactions (see earlier discussion and Bernacchi *et al.* 2005; Cen & Sage 2005). Alternatively, other nutrient limitations may also impact N-fixation and sink capacity at elevated  $[\text{CO}_2]$  (Almeida *et al.* 2000; Hungate *et al.* 2004).

## CONCLUSIONS

Rising  $[\text{CO}_2]$  will impact plants and ecosystems through two processes, reduced  $g_s$  and increased  $A$ . Our understanding of the mechanism by which Rubisco responds to short-term increases in  $[\text{CO}_2]$  is well advanced, and our understanding

of the different components of the guard cell-signalling pathway is advancing. However, the  $\text{CO}_2$ -sensing mechanism in guard cells that is responsible for the short-term sensitivity of  $g_s$  to elevated  $[\text{CO}_2]$  is still unknown. Results from FACE studies show that  $g_s$  is consistently decreased in both  $\text{C}_3$  and  $\text{C}_4$  species, yet stomatal density does not significantly change nor does  $g_s$  acclimate to elevated  $[\text{CO}_2]$  independently of  $A$ . Therefore, the short-term change in stomatal aperture likely determines most of the long-term response of  $g_s$  to elevated  $[\text{CO}_2]$ .

Results from FACE studies have demonstrated that magnitude of the stimulation of  $\text{C}_3$  photosynthesis by elevated  $[\text{CO}_2]$  and the potential for photosynthetic acclimation can be understood by examining the  $A/c_i$  response. The key to advancing understanding and being able to predict the responses of plants and ecosystems to rising  $[\text{CO}_2]$  is an improved understanding of downstream limitations, such as an N or micronutrient supply (Hungate *et al.* 2004; Luo *et al.* 2004), and other environmental variables that restrict and modulate the well-characterized primary responses of elevated  $[\text{CO}_2]$  on  $g_s$  and  $A$ . Future FACE research should be focused on making mechanistic advances, and ideally new experiments should include interactions with expected changes in other environmental variables such as water supply and temperature, which are known to modulate the two primary plant responses to elevated  $[\text{CO}_2]$ .

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## SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

**Appendix S1.** References used for the meta-analysis of the response of stomatal conductance ( $g_s$ ) to elevated [CO<sub>2</sub>].

**Appendix S2.** References used for the meta-analysis of the response of stomatal density to elevated [CO<sub>2</sub>].

**Appendix S3.** References used for the meta-analysis of the response of light-saturated photosynthesis ( $A_{sat}$ ) to elevated [CO<sub>2</sub>].

**Appendix S4.** References used for the meta-analysis of the response of the maximum Rubisco carboxylation rate ( $V_{cmax}$ ) to elevated [CO<sub>2</sub>].

**Appendix S5.** References used for the meta-analysis of the response of the maximum rate of electron transport leading to RubP regeneration ( $J_{max}$ ) to elevated [CO<sub>2</sub>].

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