MORE EFFICIENT PLANTS: A Consequence of Rising Atmospheric CO2?

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ABSTRACT

The primary effect of the response of plants to rising atmospheric CO₂ (Cₐ) is to increase resource use efficiency. Elevated Cₐ reduces stomatal conductance and transpiration and improves water use efficiency, and at the same time it stimulates higher rates of photosynthesis and increases light-use efficiency. Acclimation of photosynthesis during long-term exposure to elevated Cₐ reduces key enzymes of the photosynthetic carbon reduction cycle, and this increases nutrient use efficiency. Improved soil–water balance, increased carbon uptake in the shade, greater carbon to nitrogen ratio, and reduced nutrient quality for insect and animal grazers are all possibilities that have been observed in field studies of the effects of elevated Cₐ. These effects have major consequences for agriculture and native ecosystems in a world of rising atmospheric Cₐ and climate change.

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INTRODUCTION

Several lines of evidence suggest that terrestrial ecosystems are responding to rising atmospheric carbon dioxide (Cₐ) (39, 80, 116). The terrestrial biosphere responds to this increase solely through the response of plants. Photosynthesis (133) and transpiration (95) have long been known to be sensitive to increase in Cₐ, and it is now evident that respiration will also be affected (85). These three processes appear to be the only mechanisms by which plants and ecosystems can sense and respond directly to rising Cₐ. Understanding how these processes are affected by increase in Cₐ is therefore fundamental to any sound prediction of future response of both natural and agricultural systems to human beings’ influence on the composition of the atmosphere and on the climate system.

Many detailed and thorough reviews identify the long list of changes at the whole plant level to rising Cₐ (e.g. 21, 26, 72, 81), but few focus on these initial steps in perceiving rising Cₐ. Influential ecological discussions appear sometimes to have ignored a physiological understanding. A common view is that the impact of rising Cₐ through stimulation of photosynthesis will be short-lived because other factors, particularly nitrogen, will be limiting in most ecosystems (21, 146, 197). Yet this view may ignore evidence from physiology that elevated Cₐ allows increased efficiency of nitrogen use. Thus the key effect is not removal of a limitation but increase in efficiency. An analysis of the available evidence shows that relative stimulations of plants grown with low N averaged across several studies appear just as large as those for plants grown with high N (130).

In this review, current understanding of the effects of Cₐ on transpiration, photosynthesis, and respiration are examined to help explain why rising Cₐ...
will increase resource-use efficiency and the implications of this increased efficiency. Each topic is introduced with a description of the mechanism by which elevated $C_a$ has its effect, followed by a discussion of acclimation of the process to elevated $C_a$. Acclimation is defined as those physiological changes that occur when plants are grown in elevated $C_a$. We have summarized the most relevant literature to indicate the intensity of the responses for key aspects of each of the three processes we discuss. Current $C_a$ is approximately 36 Pascals (Pa), although in many studies in our survey of the literature $C_a$ was lower than this by as much as 1.5 Pa. Elevated $C_a$ of the studies we reviewed varied considerably, from 55 Pa in the case of the Free Air Carbon Enrichment (FACE) studies to upward of 100 Pa in a few controlled environment studies. In most studies, however, the elevated $C_a$ was approximately 70 Pa, a concentration that is expected sometime during the twenty-first century.

**STOMATA**

In contrast with the effects of $C_a$ on photosynthetic CO$_2$ assimilation ($A$) and respiration, which are mediated by specific molecular targets, the mechanism by which stomata respond to $C_a$ remains unclear (152), although it appears most probable that it is linked to malate synthesis, which is known to regulate anion channels in the guard cell plasma membrane (96). Stomata of most species close with increase in $C_a$ as well as decrease in $A$ and relative humidity (RH). For 41 observations covering 28 species, the average reduction of stomatal conductance was 20% (Table 1; see also 74). A recent analysis of responses in tree seedlings shows that the responses are highly variable, and in some species there is no response to elevated $C_a$ (46). It is not clear, however, whether failure to respond to elevated $C_a$ is due to a genetic trait or to acclimation of stomata to high humidity. For example, stomata of *Xanthium strumarium* grown in a greenhouse in high humidity failed to respond to elevated $C_a$ until given a cycle of chilling stress (62). Reduction of stomatal aperture and conductance ($g_s$) explains the reduction in leaf transpiration observed in plants grown in elevated $C_a$ (151). But does reduced $g_s$ in elevated $C_a$ limit photosynthesis in plants adapted to high $C_a$?

*Stomatal Limitation of Photosynthesis*

Two approaches to making a determination of the limitation of photosynthesis by $g_s$ have been applied (193), and both are based on analysis of the dependence of $A$ on the intercellular CO$_2$ concentration ($C_i$), the $A/C_i$ curve. In plants grown in the present atmosphere, $C_i$ is generally maintained at 0.7 $C_a$. 
even when $C_a$ is varied. In many plants, the value of $A$ at the operational $C_i$ is commonly about 90% of what it would be without the epidermis as a barrier to water loss and CO$_2$ diffusion into the intercellular spaces (i.e. $A$ at $C_i$ is about 0.9$A$ at $C_a$). Here we use $C_i/C_a$ as an index of the limitation of photosynthesis. If $C_i/C_a$ in elevated $C_a$ is less than $C_i/C_a$ in normal ambient $C_a$, then the $g_s$ would have decreased to be more of a limitation to $A$ in elevated than in normal ambient $C_a$. In the literature we examined, mean and range of $C_i/C_a$ were nearly identical for both normal ambient and elevated $C_a$ grown plants in 26 species and 33 observations (Table 1). In six field studies, $C_i/C_a$ was also very close to 0.7 for both treatments (0.73, 0.74 for normal ambient and elevated $C_a$). Thus, although the stomatal conductance is reduced in elevated $C_a$, this by itself does not limit photosynthesis. Similarly, reduced $g_s$ at the leaf level does not necessarily mean that stand transpiration will be lower because there could be a compensatory increase in leaf area index (LAI). But does failure to limit photosynthesis mean that stomata do not acclimate to elevated $C_a$?

**Acclimation of $g_s$ to Elevated $C_a$.**

Because stomatal conductance is mediated by changes in photosynthesis, lower conductance in plants having a reduced photosynthetic capacity is to be expected. There is some evidence that growth in high $C_a$ alters the gain in the feedback loop for regulation of stomatal conductance (195). However, apart
from a single paper (195), there is little evidence that stomata acclimate to elevated $C_a$ independently of acclimation of photosynthesis (65, 133, 193).

ACCLIMATION OF STOMATAL NUMBERS TO ELEVATED $C_a$ An acclimatory decrease in stomatal numbers appears a common but not universal response to growth at elevated $C_a$. In the absence of variation in stomatal dimensions, stomatal density will determine the maximum $g_s$ that a unit area of leaf could attain. One expectation at increased $C_a$ is that fewer stomata are required because the rate of CO$_2$ diffusion into the leaf will be a decreasing limitation to photosynthesis as $C_a$ rises. Reported changes in stomatal density with growth at elevated $C_a$ include increases, decreases, and no change (90, 133). Long-term studies drawing on herbarium material and paleoecological evidence appear more conclusive, showing an inverse relation between variation in $C_a$ and variation in stomatal numbers (22, 23, 239). However, in a detailed study of variation in stomatal density within leaves from a single tree, Poole et al (175) showed that variation within a single tree is of the order found in herbarium specimens covering a 200-year period and previously attributed to the change in $C_a$. The authors further demonstrate that uncertainties in the environment from which palaeobotanical specimens have been sampled could explain the variation attributed to past variation in $C_a$.

RISING $C_a$ AND EVAPOTRANSPIRATION Will reduced leaf transpiration by elevated $C_a$ also lead to reduced stand evapotranspiration (ET)? Whether elevated $C_a$ reduces ET depends on the effects of elevated $C_a$ on leaf area index (LAI) as well as on $g_s$. No savings in water can be expected in canopies where elevated $C_a$ stimulates increase in LAI relatively more than it decreases $g_s$. However, our survey shows that LAI did not increase in any of the long-term field studies of the effects of elevated $C_a$ on crops or native species (Table 1). This survey included studies of wheat (*Triticum aestivum*) and cotton in Arizona where FACE was used to expose the plants to 55 Pa (173) as well as open top chamber studies of native species. Elevated $C_a$ (>68 Pa) reduced ET compared with normal ambient in all the native species including the Maryland wetland (13), Kansas prairie (92), and the California grassland ecosystem (74). In the wetland ecosystem, ET was evaluated for a C$_3$-dominated and a C$_4$-dominated plant community (13). In these two communities, instantaneous values of ET averaged 5.5–6.5 for the C$_3$ and 7.5–8.7 mmol H$_2$O m$^{-2}$s$^{-1}$ for the C$_4$ communities at present ambient $C_a$ but at elevated $C_a$ (68 Pa), ET was reduced 17–22% in the C$_3$ and 28–29% in the C$_4$ community, indicating the relatively greater effect of elevated $C_a$ on $g_s$ in the C$_4$ species. In the prairie ecosystem, cumulative ET over a 34-day period in midsummer was 180 kg m$^{-2}$ at present ambient $C_a$, whereas it was
20% less at elevated $C_a$. In the grassland ecosystem, elevated $C_a$ reduced ET sufficiently that the availability of soil water was increased (74). A four-year study of the responses of native Australian grass to elevated $C_a$ in a phytotron reported higher water content of soils (138).

**STOMATAL CONDUCTANCE AND THE ENERGY BUDGET** Reduced transpiration alters partitioning of energy between latent heat loss and convective exchange, potentially increasing leaf temperature (63). Elevating $C_a$ to 55 Pa consistently decreased $g_s$ and increased canopy temperature of cotton about 1°C (173).

**SUMMARY** Reduced stomatal conductance is expected to be a feature of plants exposed to ever increasing $C_a$. Stomata do not appear to limit photosynthesis with elevation of $C_a$ any more than they do at normal ambient $C_a$, even though $g_s$ is usually decreased. A pattern of decreased $g_s$ coupled with maintenance of a constant $C_i/C_a$ will mean that water use efficiency will rise substantially, and there is evidence that this means increased yield for crops with no additional penalty in water consumption. Elevated $C_a$ does not stimulate increased leaf area index in field studies with both crops and native species. Thus, reduced $g_s$ leads to reduced ET and increased soil water content. However, reduced ET also causes increased warming of the plant canopy and surrounding air. Evidence for acclimation of stomatal development to elevated $C_a$ is conflicting, though there is good evidence for a response of $g_s$ to the acclimation of photosynthesis. The following section examines this acclimation.

**PHOTOSYNTHESIS**

The evidence that elevated $C_a$ stimulates increased photosynthesis is overwhelming. In our survey of 60 experiments, growth in elevated $C_a$ increased photosynthesis 58% compared with the rate for plants grown in normal ambient $C_a$ (Table 2). Acclimation of photosynthesis to elevated $C_a$ clearly reduces photosynthetic capacity but rarely enough to completely compensate for the stimulation of the rate by high $C_a$. This section of the paper reviews the mechanism for the fundamental effects of $C_a$ on photosynthesis and what is known about acclimation to rising $C_a$.

**Direct Effects of Rising $C_a$ on Photosynthesis**

Carbon dioxide has the potential to regulate at a number of points within the photosynthetic apparatus, including binding of Mn on the donor side of photosystem II (119), the quinone binding site on the acceptor side of photosystem II (86), and the activation of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (178). While all these processes show a high affin-
ity for HCO$_3^-$ or CO$_2$ and are saturated at the current $C_a$. Rubisco has a low affinity for CO$_2$ on carboxylation, and this reaction is not saturated at the current $C_a$. Therefore, the carboxylation of Rubisco will respond to rising $C_a$.

Table 2 Acclimation of photosynthesis to elevated $C_a$ determined as the ratio (R) of the value of the attribute for plants grown in elevated to that in normal ambient $C_a$ (R)$^a$

<table>
<thead>
<tr>
<th>Attribute</th>
<th>R</th>
<th>Sp,n</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A at growth $C_a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High N supply</td>
<td>1.57$^b$</td>
<td>8, 10</td>
<td>12, 49, 55, 111, 160, 214, 234, 235</td>
</tr>
<tr>
<td>Low N supply</td>
<td>1.23$^b$</td>
<td>8,10</td>
<td>12, 49, 55, 111, 160, 214, 234, 235</td>
</tr>
<tr>
<td>$A, C_a \leq 35$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large rv</td>
<td>0.93</td>
<td>28, 33</td>
<td>15, 34, 37, 49, 108, 180, 203, 207, 213, 224, 248, 249</td>
</tr>
<tr>
<td>Small rv</td>
<td>0.80$^c$</td>
<td>18, 53</td>
<td>19, 20, 29, 38, 43, 48, 54, 56, 68, 71, 79, 88, 97, 110, 111, 115, 125, 140, 149, 162, 191, 192, 194, 202, 210, 221, 226, 234, 235, 238</td>
</tr>
<tr>
<td>High N supply</td>
<td>0.80$^c$</td>
<td>4, 6</td>
<td>49, 111, 234, 235</td>
</tr>
<tr>
<td>Low N supply</td>
<td>0.61$^b$</td>
<td>4, 6</td>
<td>49, 111, 234, 235</td>
</tr>
<tr>
<td>Starch</td>
<td>2.62$^c$</td>
<td>21, 77</td>
<td>1, 7, 20, 24, 36, 49, 50, 54, 68, 93, 94, 98, 103, 108, 115, 139–141, 144, 155, 156, 170, 171, 177, 196, 203, 210, 215, 221, 228, 235, 243, 244</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.60$^b$</td>
<td>9, 38</td>
<td>1, 7, 24, 50, 68, 82, 93, 94, 98, 103, 104, 139, 141, 144, 156, 169, 177, 203, 221, 229, 243</td>
</tr>
<tr>
<td>Protein</td>
<td>0.86$^b$</td>
<td>11,15</td>
<td>7, 34, 37, 56, 93, 94, 108, 200, 202–204, 220, 229</td>
</tr>
<tr>
<td>[Rubisco]</td>
<td>0.85$^b$</td>
<td>11.8</td>
<td>4–6, 34, 56, 108, 187, 194, 200, 202, 215, 220</td>
</tr>
<tr>
<td>Rubisco activity</td>
<td>0.76$^c$</td>
<td>11,13</td>
<td>4–7, 34, 37, 56, 93, 94, 97, 106, 108, 124, 125, 140, 144, 169, 187, 194, 200, 202, 203, 214, 215, 226, 228, 234, 237, 243</td>
</tr>
<tr>
<td>Leaf [N]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High N supply</td>
<td>0.85$^b$</td>
<td>8, 10</td>
<td>12, 42, 49, 58, 138, 143, 170, 172, 234</td>
</tr>
</tbody>
</table>

$^a$ Rooting volume (rv) is either large (>10 L) or small (<10 L). Other details as in Table 1.
$^b,c$ See table 1.
The kinetic properties of Rubisco appear to explain the short- and many of the long-term responses of photosynthesis to this change in the atmosphere. Rising $C_a$ increases the net rate of CO$_2$ uptake for two reasons. First, Rubisco is not CO$_2$-saturated at the current $C_a$. Second, Rubisco catalyzes the oxygenation of Ribulose-1,5-bisphosphate (RubP), a reaction that is competitively inhibited by CO$_2$ (18). Oxygenation of RubP is the first step of the photosynthetic carbon oxidation or photorespiratory pathway (PCO), which decreases the net efficiency of photosynthesis by 20–50%, depending on temperature (245), by utilizing light energy and by releasing recently assimilated carbon as CO$_2$. CO$_2$ is a competitive inhibitor of the oxygenation reaction, such that a doubling of concentration at Rubisco will roughly halve the rate of oxygenation (131). This second effect on the PCO may be of greater importance, because an increase in net photosynthesis will result regardless of whether photosynthesis is Rubisco- or RubP-limited and regardless of where metabolic control lies. The increase in uptake resulting from suppression of the PCO requires no additional light, water, or nitrogen, making the leaf more efficient with respect to each.

RUBISCO SPECIFICITY Rubisco specificity ($S_r$) is the ratio of carboxylation to oxygenation activity when the concentrations of CO$_2$ and O$_2$ at Rubisco are equal. It determines directly the increase in efficiency of photosynthesis with rising $C_a$. This value is therefore of fundamental importance in predicting the direct responses of plants to rising $C_a$. $S_r$ has been suggested to vary from 88–131 across a range of C$_3$ plants, with an average of about 100 (18). Terrestrial C$_3$ plants show both the highest and a fairly constant $S_r$ in contrast with other photosynthetic groups such as C$_4$ plants and cyanophyta (26, 52, 225).

ELEVATED $C_a$ AND TEMPERATURE As temperature increases, $S_r$ declines dramatically for two reasons: decreased solubility of CO$_2$ relative to O$_2$ and decreased affinity of Rubisco for CO$_2$ relative to O$_2$ (133). About 68% of the decline in $S_r$ is calculated to result from the binding affinity of the protein for CO$_2$ (27, 131). The effect of this decline in $S_r$ with temperature is to produce a progressive increase in the stimulation of photosynthesis by elevated $C_a$ with temperature. The minimum stimulation of RuBP-limited photosynthesis by increasing $C_a$ from 35 to 70 Pa rises from 4% at 10°C to 35% at 30°C. It also follows from this interaction that the temperature optimum ($T_{opt}$) of light-saturated CO$_2$ assimilation ($A_{sat}$) must increase with $C_a$ by 2, 5, and 6°C with increase in $C_a$ to 45, 55, and 65 Pa, respectively (137). The upper temperature at which a positive $A_{sat}$ may be maintained is similarly increased. The change in these
characteristic temperatures underlies the importance of considering rise in $C_a$ not just as a factor that increases photosynthetic rate, but also as one that strongly modifies the response to temperature. Because increasing $C_a$ is predicted to increase leaf temperature, both directly by decreasing latent heat loss and indirectly through radiative forcing of the atmosphere, this interactive effect of $CO_2$ and temperature has profound importance to future photosynthesis. It also suggests a much greater stimulation of photosynthesis in hot versus cold climates (118, 135, 136).

**Acclimation of Photosynthesis to Elevated $C_a$**

There is abundant evidence that in the long term, photosynthesis acclimates to elevated $C_a$, i.e. the photosynthetic properties of leaves developed at elevated $C_a$ differ from those developed at the current $C_a$ (46, 90, 133, 230). The vast majority of studies in our and others’ surveys show a decrease in $A$ of plants grown in elevated $C_a$, relative to controls grown at normal ambient, when both are measured at the current ambient $C_a$ (Table 2; see also 90, 136, 193). Acclimation of photosynthesis is accompanied by higher carbohydrate concentration, lower concentration of soluble proteins and Rubisco, and inhibition of photosynthetic capacity. When there is no rooting-volume limitation, as for example in our survey when the rooting volume exceeded 10L, significant reduction in $A$ caused by growth in elevated $C_a$ is the exception rather than the rule (Table 2, $A$ at $C_a < 35$ Pa) while, exceptionally, an increase in photosynthetic capacity is observed (15, 91).

Two reasons for this acclimation are apparent. First, the plant may be unable to use all the additional carbohydrate that photosynthesis in elevated $C_a$ can provide; therefore a decrease in source activity must result. Second, less Rubisco is required at elevated $C_a$. Our survey shows an average reduction in the amount of Rubisco of 15% in eight studies including 11 species and a reduction in Rubisco activity of about 24% (Table 2). As a protein that can constitute 25% of leaf N, these reductions are a major component of the lower tissue N observed in foliage (15–19%) (Table 2).

**SOURCE/SINK BALANCE** Arp (14) drew attention to the strong correlation between rooting volume and acclimation of photosynthesis of plants in elevated $C_a$. In small pots (i.e. <10 L), $A$ of plants in elevated $C_a$ was less than $A$ of plants in normal ambient $C_a$. In Table 2 we separate the effects of elevated $C_a$ on photosynthesis into the effects of small and large rooting volumes. In our survey of 163 studies, the stimulation of $A$ was about 50% for large rooting volumes and field experiments but reduced by about half of this when the rooting volume is limited (Table 2). When there is no restriction of rooting volume, $A_{sat}$ remains
the same for plants grown in both elevated and ambient \( \text{C}_a \). Similar conclusions are reported for tree seedlings (46). The effect of rooting volume on acclimation is probably confounded with effects of nutrient availability on photosynthesis.

NITROGEN-LIMITATION Other factors, such as available nutrients, also reduce the sink strength. In a small number of studies, reducing the available N had an effect on \( A \) that was the same as the effect of limiting the rooting volume: At high N, the stimulation of \( A \) by elevated \( \text{C}_a \) was about 50\%, but this stimulation dropped to about 25\% when available N was low. Acclimation of photosynthesis to elevated \( \text{C}_a \) has frequently been suggested to be more marked when N supply is limiting (26, 46). Rubisco and large subunit Rubisco RNA (\( RbcS \)) expression in \( \text{Pisum sativum} \) and \( \text{Triticum aestivum} \) were unaffected by growth in elevated \( \text{C}_a \) when N supply was abundant but showed marked decreases in response to elevated \( \text{C}_a \) when N was deficient (158, 185).

For plants such as wheat and pea, which are able to rapidly form additional sinks during early vegetative growth, sink limitation is unlikely, whereas other requirements are not limiting. However, growth of additional sinks would be limited if N supply is limiting. Because less Rubisco is required under elevated \( \text{C}_a \), this redistribution of N would greatly increase the efficiency of N use.

Although acclimation in many early experiments was exaggerated by the artifact of rooting restriction, there is also clear evidence that acclimation can occur in the absence of any rooting restriction (46). In the Maryland wetland ecosystem where open top chambers have been used to study the effects of elevated \( \text{C}_a \) (68 Pa), Rubisco was reduced 30–58\%, and photosynthetic capacity, measured at normal ambient \( \text{C}_a \), was reduced 45–53\% in the sedge (\( \text{Scirpus olneyi} \)) after seven years of exposure (108). Wheat grown with an adequate supply of N and water showed no acclimation of photosynthesis to \( \text{C}_a \) elevated to 55 Pa in FACE until completion of flag leaf development when there was a significant loss of Rubisco followed by other photosynthetic proteins, relative to controls (157).

HOW MUCH RUBISCO IS REQUIRED IN HIGH \( \text{C}_a \)? Rubisco can constitute 25\% of leaf [N] in a \( \text{C}_3 \) leaf (18). Large quantities of this enzyme appear necessary to support light-saturated photosynthesis in present \( \text{C}_a \) (140). Calculations suggest that 35\% of the Rubisco could be lost from the leaf before Rubisco will co-limit photosynthesis when \( \text{C}_a \) is increased to double the current concentration (133). \( \text{Nicotiana tabaccum} \) transformed with antisense \( RbcS \) to produce 13–18\% less Rubisco showed lower rates of carbon gain and growth at the current \( \text{C}_a \) by comparison with the wild type from which they were derived. There was no differ-
ence in C gain or growth when both were grown at 80 Pa C_i (140), providing clear evidence of a decreased requirement for Rubisco at elevated C_i.

Woodrow (238) computed the amount of Rubisco required to maintain constant A as C_i increased from the present level to 100 Pa. At 25°C, the amount of Rubisco needed drops to 59% of present amount at 70 Pa, to 50% at 100 Pa (Figure 1). Because of the strong temperature dependence of S_r, the amount of Rubisco required will also decline strongly with increasing temperature. At 70 Pa and a leaf temperature of 35°C, only 42% of the Rubisco activity required at 35 Pa would be needed to maintain the same rate of photosynthesis. There would be a large need for Rubisco at low temperature, and this requirement changes very little as C_i rises (Figure 1). At 5°C, the requirement for Rubisco to maintain the same rate of photosynthesis at elevated C_i is 89% of that needed at normal ambient.

A wide range of studies have reported decreases in Rubisco content and activity with growth in elevated C_i. In our survey of 18 studies of 12 species, Rubisco was reduced 15% (Table 2). Growth in elevated C_i commonly re-

![Figure 1](image.png)  
*Figure 1* The proportion of Rubisco required to support the same rate of Rubisco-limited photosynthesis at 35 Pa as C_i increases at different leaf temperatures. (After 238.)
sults in decreased photosynthesis relative to controls when measured at the current atmospheric \( C_a \), even though photosynthesis of the elevated grown leaves remains higher when they are measured at their elevated growth \( C_a \). This could be explained by decreased in vivo Rubisco activity. In our survey of 13 studies and 11 species (Table 2), we indicate a reduction of Rubisco activity of 24%. Studies of \textit{Phaseolus vulgaris} (194), \textit{Pinus taeda} (215), and wheat (134) have shown \( A/C_i \) responses that indicate a selective loss of Rubisco activity in vivo without significant loss of capacity for regeneration of RuBP with growth at elevated \( C_a \). A similar conclusion can be drawn from control analysis applied to \textit{Helianthus annuus} (237).

**THE MOLECULAR MECHANISM OF ACCLIMATION** Decrease in Rubisco is commonly correlated with an increase in leaf nonstructural carbohydrates. In our survey we found that sucrose and starch increased 60 and 160% in elevated \( C_a \) (Table 2). Regulation of the expression of photosynthetic genes, via increased soluble carbohydrate concentration, may underlie acclimation to growth in elevated \( C_a \) (Figure 2; 199, 206, 230). Decreased expression of several photosynthetic genes has occurred when sugar concentrations have been increased by directly feeding mature leaves through the transpiration stream (121, 123, 222), by expression of yeast-derived invertase in transgenic tobacco plants that directs the gene product to the cell wall to interrupt export from source leaves (227), and by cooling the petiole to decrease the rate of phloem transport in intact tobacco plants (122). Using chimeric genes created by fusing maize photosynthetic gene promoters with reporter genes, seven promoters including those for the light harvesting subunit (\textit{LhcB}) and \textit{RbcS} were repressed by soluble carbohydrates. The low concentration of glucose required for this repression suggests that other sugars, in particular sucrose and fructose, may be effective via metabolism in the cell to glucose. How might glucose suppress gene expression in the nucleus? Based on glucose signaling in yeasts, a hypothetical scheme whereby hexokinase (HXK) associated with a glucose channel or transporter in the plasmalemma or tonoplast would release an effector in response to glucose has been proposed (Figure 2; 121, 199). The effector would then interact with the promoters of nuclear genes coding for chloroplast components. This system would allow sensing of both an accumulation of sucrose in the vacuole and in the leaf vascular tissue, indicating an imbalance in sink capacity relative to source activity. Repression is blocked by the HXK inhibitor mannoheptulose, providing evidence of the role of HXK in this signal transduction pathway (109). Where carbohydrate repression has been demonstrated it appears to involve both \textit{RbcS}, coding for Rubisco, and genes that will affect capacity for RubP regeneration. Optimum use of resources would require a system that would allow decrease in
Rubisco, without loss of capacity for RubP regeneration. Nie et al (156) showed in wheat that elevated C\textsubscript{a} can result in decreased expression of \textit{RbcS} but not other Calvin cycle of chloroplast membrane genes. This is consistent with Figure 2 because several different promoters are involved that could have different sensitivities to carbohydrate concentrations (199). Is carbohydrate repression consistent with observations of plants grown in elevated C\textsubscript{a}? Although as a general rule Rubisco decreases with growth in elevated C\textsubscript{a} and soluble carbohydrates rise, there are important exceptions (156). This suggests that other possible regulatory elements need to be identified before the mechanisms of acclimation can be fully understood.

ACCLIMATION AND CANOPY PHOTOSYNTHESIS Our analysis of photosynthesis has only concerned the increase in leaf photosynthetic rates that result from
growth in elevated $C_a$. If we consider a crop or natural canopy, carbon gain will only increase with increased leaf photosynthetic rates under elevated $C_a$ in the absence of compensatory decreases in canopy size and architecture. If there is a compensatory decrease in canopy size, then gain at the leaf level might be offset by decrease at the canopy level. In Table 1 we show that for studies carried out in the field, canopy leaf area is not significantly increased or decreased by long-term growth in elevated $C_a$.

Considerable evidence supports the prediction that increase in CO$_2$ uptake will be greater in warm climates (131, 133, 145). Among the long-term experiments in which plants have grown under elevated CO$_2$ for successive seasons, most obvious is that in arctic tundra no sustained increase in net carbon gain was observed (163), whereas in warm temperate climates, e.g. the Maryland wetland ecosystem, stimulation of CO$_2$ uptake was observed for eight successive seasons (60). In two successive FACE experiments on the same site at Maricopa, Arizona, total daily canopy photosynthesis of *Gossypium hirsutum* in the middle of summer was increased by ca 40% in the canopy growing in 55 Pa. In wheat growing on the same site in the cooler temperatures of spring, canopy photosynthesis was increased by ca 10% (173). Relative stimulation of A by a doubled $C_a$ in the evergreen *Pinus taeda* in the field was strongly correlated with seasonal variation in temperature (129).

*Photosynthesis in the Shade*

Photosynthesis is light limited for all leaves for part of the day, and for some leaves, those of the lower canopy, for all of the day. For a crop canopy, light-limited photosynthesis can account for half of total carbon gain, whereas photosynthesis of forest floor species might always be light limited. The initial slope of the response of photosynthesis to light defines the maximum quantum yield or photosynthetic light-use efficiency ($\varphi$) of a leaf and determines the rate of CO$_2$ uptake under strictly light-limiting conditions.

At a given $C_a$, $\varphi$ has been shown to be remarkably constant in C$_3$ terrestrial plants regardless of their taxonomic and ecological origins (158). This may reflect the constancy of the photosynthetic mechanism across C$_3$ species. Even under light-limited conditions net photosynthesis is reduced by the PCO, which consumes absorbed light energy and releases CO$_2$. Inhibition of the PCO by elevated $C_a$ will therefore increase light-limited photosynthesis. This increase may be closely predicted from the kinetic properties of Rubisco (133). Forest floor vegetation commonly exists close to the light compensation point (LCP) of photosynthesis. Any increase in $\varphi$ could therefore result in large increases in net photosynthesis. These predictions are consistent with
recent observations of more than two- to fourfold increases in net carbon gain by leaves of both forest floor herbs (CP Osborne, BG Drake & SP Long, unpublished data) and tree seedlings (126) grown in elevated \( C_a \) in situ. Calculated from the kinetic constants of Rubisco, the maximum quantum yield of photosynthesis at 24°C will increase by 24% when \( C_a \) is doubled. The LCP should decline reciprocally by 20% if mitochondrial respiration remains unchanged. In \textit{S. olneyi} grown and measured in 70 Pa \( C_a \), \( \bar{\varnothing} \) was 20% greater than that of plants grown and measured at 36 Pa, close to theoretical expectation (132). LCP, however, was decreased by 42%, almost double the theoretical expectation. A similar increase in maximum quantum yield was observed in the forest floor herb \textit{Duchesnea indica}, but here LCP decreased by 60% (CP Osborne, BG Drake & SP Long, unpublished data). These greater-than-predicted decreases in LCP could only be explained by a decrease in leaf mitochondrial respiration rate. The next section considers the mechanisms and evidence for such a decrease in respiration rate.

**SUMMARY** Theory and experiments show that in rising \( C_a \), photosynthesis will be stimulated in both light-limited and -saturated conditions and that the stimulation rises with temperature. Optimization theory suggests that substantial decreases in leaf Rubisco content could be sustained under elevated \( C_a \) while maintaining an increased rate of leaf photosynthesis, particularly at higher temperatures. Acclimation decreases Rubisco in response to elevated soluble carbohydrate levels. Higher quantum yield at elevated \( C_a \) reduces the light compensation point. Because of the temperature interaction between Rubisco activity and elevated \( C_a \), we would expect higher rates of photosynthesis in tropical and subtropical species as well as shifts in the C:N for foliage.

**MITOCHONDRIAL RESPIRATION** The earliest reported findings of a direct inhibition of dark respiration by elevated \( C_a \) are those of Mangin from 1896 (quoted in 153), although the 5% level employed far exceeds the doubling of current ambient \( C_a \). It has now been established that the specific rate of dark respiration, measured either by CO\(_2\) efflux or by O\(_2\) uptake, decreases about 20% when the current ambient \( C_a \) is doubled (Table 3, Direct effect; 8, 17, 30, 85, 87, 242). Two different effects of elevated \( C_a \) have been suggested (28): an effect that occurs because of the growth or acclimation of the plant in high \( C_a \) (e.g. 17) and a readily reversible effect (e.g. 9, 28). These two effects are now referred to as the indirect and direct effects of elevated \( C_a \) on respiration. Although the mechanism for the indirect effect is not yet clear, the direct effect appears to be caused by inhibition of the activity of two key enzymes of the mitochondrial electron transport chain, cytochrome \( c \) oxidase (Cytox) and succinate dehydrogenase...
We restrict our comments here to this emerging new direction in CO₂ effects research. For information on other aspects of the interaction of elevated Cₐ and respiration, we refer the reader to the numerous excellent reviews that have recently appeared (8, 16, 30, 70, 153, 176, 242).

**Direct Effect of Elevated Cₐ on Dark Respiration**

There are many reports of a decrease in respiration within minutes of increase in Cₐ (9, 28, 69, 87, 114, 166, 179, 183, 201). Respiration is reduced about 20% for a doubling of the atmospheric Cₐ (Table 3). This effect has been reported for many different kinds of tissues including leaves, roots, stems, and even soil bacteria, suggesting that whatever the basic mechanism, it involves a fundamental aspect of respiration.

**MECHANISM OF DIRECT EFFECT OF Cₐ ON DARK RESPIRATION** A plausible mechanism underlying the direct effect is the inhibition of enzymes of the mitochondrial electron transport system. Experiments with enzymes in vitro showed that elevated Cₐ reduces the activity of both Cytox and succinate dehydrogenase (85, 166, 184a). Under experimental conditions in which Cytox controlled the overall rate of respiration in isolated mitochondria (148), O₂ uptake was inhibited by about 15% (85). Experiments with the enzymes in vitro indicated a direct inhibition by elevated Cₐ on their activity of about 20% for a doubling of the current ambient Cₐ (85; Figure 3). Measurements of O₂ consumption on isolated soybean mitochondria that were fully activated (State 3 conditions, i.e. sufficient ADP) and in which the respiration inhibitor salicylhydroxamic acid (SHAM) was used to inhibit the alternative pathway showed that doubling Cₐ inhibited the cytochrome (Cyt) pathway approximately 10–22% (85). By blocking the Cyt pathway with potassium cyanide (KCN) and using either succinate

**Table 3** Respiration of shoots and leaves in elevated Cₐ

<table>
<thead>
<tr>
<th>Respiration</th>
<th>R</th>
<th>Sp. n</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Effect</td>
<td>0.82a</td>
<td>23, 53</td>
<td>9, 28, 32, 33, 45, 51, 57, 75, 78, 102, 114, 147, 191, 192, 208, 209, 212, 221, 223, 246</td>
</tr>
<tr>
<td>Indirect Effect</td>
<td>0.95</td>
<td>17, 37</td>
<td>17, 28, 32, 57, 103, 117, 120, 147, 154, 191, 192, 203, 221, 231, 246</td>
</tr>
</tbody>
</table>

*a* The direct effect refers to the ratio (R) of rates of dark respiration in the same samples when Cₐ is increased from the current ambient to the elevated level. The indirect effect refers to the ratio of rate of dark respiration of plants grown in elevated to the rate of plants grown in current ambient Cₐ when measured at the same background of Cₐ. Other details as in Table 1.

*b* Significantly different from 1.0 (p < 0.05) by Students’ t test.
or NADH as electron donors, it was shown that the succinate dehydrogenase (SDH) in vivo was also inhibited by doubling $\text{Ca} \ (85)$. The activity of the alternative pathway has been shown to be unaffected directly by changing the level of $\text{Ca} \ (85, 184a)$. What is the specific mechanism for inhibition of Cytox by elevated $\text{Ca} \ ?$ Because the effect is time dependent (85; Figure 3) and appears to be dependent on $\text{CO}_2$ and not $\text{HCO}_3 \ (166)$, one possibility is a carbamylation reaction. The structure of Cytox contains lysine residues (218), necessary for the proposed carbamylation.

Another proposed mechanism for the apparent inhibition of respiration is that elevated $\text{Ca}$ stimulates dark $\text{CO}_2$ fixation (8). Measurements of the respiratory quotient (consumption of $\text{O}_2$/emission of $\text{CO}_2$) show that this is not a viable possibility because reduced $\text{CO}_2$ evolution is balanced by an equal reduction of $\text{O}_2$ uptake in elevated $\text{Ca} \ (184)$.

The possibility that $\text{CO}_2$ inhibition of these enzymes mediates the direct effect of $\text{Ca}$ on respiration in plants is supported by measurements on different types of plant organelles and tissues. Doubling present atmospheric $\text{Ca}$ re-

![Figure 3](image-url)
duced in vivo O₂ uptake by soybean mitochondria, and by extracts from excised shoots of the sedge *S. olneyi* (84, 85). Experiments in which CO₂ efflux was used to measure dark respiration showed that doubling Cₐ reduced respiration in excised shoots removed from the field to the lab and from intact stands in which respiration was determined in the field on the C₃ sedge, *S. olneyi* (58). The importance of this effect for carbon balance of plants and ecosystems is that it apparently occurs at the most fundamental level of organization of the mitochondrial electron transport. Thus all respiring tissues are subject to this effect.

**Acclimation of Respiration to Elevated Cₐ**

Over days to months, the rate of dark respiration of foliage declines. This occurs in parallel with tissue declines in N concentration or protein content that is energetically costly (25), indicating that this decline reflects decreased demand for energy to sustain growth and/or maintenance. Plants grown in elevated Cₐ typically have lower protein and nitrogen concentrations (Table 2). Several reviews indicate the considerable potential for rising Cₐ to reduce respiration through effects on tissue composition (8, 46, 242). We reviewed data on measurements of respiration on leaves of 17 species grown in current ambient and elevated Cₐ. Acclimation of dark respiration was determined by comparison of the rate of CO₂ efflux or O₂ consumption measured on samples of tissue grown in current ambient or elevated Cₐ at a common background Cₐ (Table 3, Indirect effect). In our survey of the literature we found no overall difference between the specific rates of respiration of shoots and leaves grown in elevated or ambient Cₐ (Table 3).

However, some C₃—but not C₄—species do show the effects of acclimation to high Cₐ. Acclimation of the rate of respiration in the C₃ plants, *S. olneyi*, *Lindera benzoin*, and wheat, was due to reduction in activity of enzymatic complexes of the mitochondrial electron transport chain (Cytox and Complex III), which resulted in diminished capacity of tissue respiration (11, 17). Reduction of the activity of these enzymes was not found in the C₄ species *Spartina patens* (11).

**SUMMARY** Exposure of plants to elevated Cₐ usually results in a lower rate of dark respiration. Efflux of CO₂ from stands in the field; from excised leaves, roots, and stems; and from O₂ consumption of isolated mitochondria, suspensions of cells, and pieces of tissues are reduced about 20% for a doubling of current Cₐ. This effect appears to be caused mainly by the direct inhibition of the activity of the respiratory enzymes, cytox, and succinate dehydrogenase by
CO₂. Although acclimation of plants to elevated C₄ has been reported to lower the rate of dark respiration, this correlates with reduced activity of respiratory enzymes.

CONCLUSION

Are plants more efficient when grown in elevated C₄? Different definitions apply to efficiency for water, nitrogen, and light, the three main environmental factors we consider here. However, in each case, greater carbon assimilation per unit of water lost, per unit nitrogen content, or per unit absorbed light is consistently found in plants exposed to elevated C₄.

WATER USE EFFICIENCY Water use efficiency (WUE) means here the ratio of A to T per unit leaf area. Reduced gₛ in elevated C₄ improves WUE by reducing water loss, whether or not photosynthesis is stimulated. In a study of subambient CO₂ effects on oats, mustard, and two cultivars of wheat, WUE increased 40–100% as the ambient CO₂ was increased from about 15 to 35 Pa (174). In a FACE study in wheat, C₄ elevated to 55 Pa increased WUE by 76% and 86% in cotton crops, averaged over two full growing seasons (173). Increased C₄ also increased WUE in both C₃ and C₄ wetland species (13). The greater decrease in stomatal conductance on the upper than on the lower epidermis of leaves in response to elevated C₄ could further decrease WUE under conditions of natural convection (167).

NITROGEN USE EFFICIENCY Rubisco, the primary carboxylase of C₃ photosynthesis, is the most abundant protein in plant leaves and in the biosphere with an estimated 10 kg per capita (10). Acclimation of photosynthesis to a world with higher C₄ will mean that less nitrogen will be needed to meet the requirement for this enzyme, leading to reduction in leaf N concentration and increased C:N (44, 47, 99, 161, 164, 236). By the definition of nitrogen use efficiency (NUE) we apply here, the rate of carbon assimilation per unit of N in the foliage, elevated C₄ clearly increases NUE. Reduction in [N] is not entirely due to dilution but also represents lower concentration of photosynthetic proteins. In our survey, we found that tissue N is reduced 15–20% depending on N availability (Table 2). In a four-year study of a native Australian grass, NUE increased irrespective of the availability of N in the soil, and this was accompanied by accumulation of carbon in the microcosm (138). In a long-term study of a Maryland wetland ecosystem, [N] was reduced an average of 18% in S. olneyi throughout eight years of elevated C₄ exposure (61) during which time the elevated C₄ treatment stimulated net ecosystem production (NEP). However, while reduction in foliage [N]
has the benefit of increasing NUE, it also has the consequence that it may reduce quality or palatability for grazers. The sedge, *S. olneyi*, grown in elevated $C_a$, was less often the target for egg deposition and larval grazing than in those in current ambient $C_a$ (211). Growth in elevated $C_a$ increased phenolics and tannins as well as toughness of the tissues in *Eucalyptus* sp., and the beetle *Chrysophthartus flaveola* fed this material did poorly: The low nutritional status resulted in lower body weight, reduced digestive efficiencies, and increased mortality (127). Protein content of wheat grain was reduced in elevated $C_a$ (99, 100), although it is not clear how this is related to acclimation of photosynthesis and Rubisco to elevated $C_a$.

**LIGHT USE EFFICIENCY** Despite the many studies of plant growth in elevated $C_a$, few have actually analyzed light use efficiency (LUE; dry matter production per unit intercepted light) at the stand level. In a microcosm study of wheat, LUE increased to a maximum at anthesis and declined thereafter (86). Similarly, Pinter et al (173) found that cotton crops grown under FACE at 55 Pa showed a highly significant increase in LUE of 20% and 22% in consecutive years, regardless of whether the crops were grown with full irrigation or only 50% of the optimal water supply.

Even with acclimation of photosynthesis to elevated $C_a$, in the sedge, *S. olneyi*, elevated $C_a$ stimulated ecosystem carbon uptake (60). In four out of five studies of native ecosystems in which NEP was measured by gas exchange, long-term elevated $C_a$ exposure stimulated carbon assimilation (55, 59, 60, 74, 92, 163). The exception was the arctic tussock tundra in which there was no net increase in NEP in response to elevated $C_a$ after three weeks (163). Photosynthesis in the dominant species in this system, *Eriophorum vaginatum*, rapidly adjusted to elevated $C_a$ in controlled environment studies (213). This appears to be one of the few species in which one can demonstrate complete loss of initial increase in photosynthesis resulting from increase in $C_a$.

One of the most important findings of the past ten years of work in elevated $C_a$ is that all but one of the field studies in both crops and native species photosynthesis per unit of ground area was stimulated. Most of the extra carbon from this stimulation must reside in storage tissues such as wood or roots since there is clear evidence that it does not stimulate the increase in foliage. The major consequence of this is that we can expect additional carbon to be accumulated in terrestrial ecosystems as $C_a$ continues to increase.

Improved efficiency generally leads to increased carbon assimilation. Nevertheless, there are a number of consequences that deserve careful study because they may not result in positive outcomes for climate, for yield of
crops, or for plant/insect/animal interactions. Reduced stomatal conductance results in greater WUE and reduced ET, and it may increase soil water content. However, reduced transpiration also alters canopy energy balance and shifts some heat loss from transpiration to convective heat loss. This effect has important consequences for climate. Incorporating a model of stomatal response to elevated Ca into a coupled biosphere-atmosphere model (SiB2-GCM) showed that decreased gs and latent heat transfer will cause a warming of the order of 1–2°C over the continents (198) in addition to warming from the CO2 greenhouse effect. Implicit in this development is that any loss of photosynthetic capacity, through acclimation, would lead to further decreased gs (198). These studies emphasize the need for an improved mechanistic understanding of stomatal response to atmospheric change.

Whereas the effects of CO2 on these separate physiological processes occur via independent mechanisms, there are interactions among all three of them. Acclimation of photosynthesis reduces tissue [N], which may reduce the demand for energy generated by respiration. Reduction of gs improves water balance, which delays the onset of midday water stress and extends the period of most active photosynthesis; reduced ET increases soil water content and leads to increased N mineralization.

There are problems in moving across scales in the interpretation of processes on a global scale based upon effects at the molecular level. Yet the reduction of stomatal conductance, the improvement in the efficiency of photosynthesis, and the inhibition of the activity of respiratory enzymes are primary mechanisms by which terrestrial ecosystems will respond to rising atmospheric carbon dioxide.

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